ABSTRACT

STUDIES OF FOUR ENZYME SYSTEMS IN CANINE NEOPLASIA

by Walter Fred Loeb

Activities of lactic dehydrogenase, phosphohexose isomerase, leucyl aminopeptidase, and alkaline phosphatase were determined in the sera of 60 dogs with naturally occurring neoplastic disease, and in 56 out of 89 neoplasms which these dogs had. Additional determinations of the same enzymes in serum were performed following biopsy or excision of the neoplasms. The results obtained were statistically analyzed relative to the gross and microscopic morphologic characteristics and the behavior of the neoplasms.

No general pattern of biochemical uniformity was noted among the neoplasms. Some biochemical similarity was noted between tumors and their tissues of origin. In the absence of the marker enzyme, alkaline phosphatase, this was not sufficient to relate the tumor to the parent tissue in the absence of other information. Tumor tissue-enzyme activity per unit of weight was less for all 4 enzymes in larger tumors than in smaller ones.

The mean value of tissue lactic dehydrogenase was significantly higher in malignant tumors than in benign ones. The correlation between tumor-tissue lactic dehydrogenase and serum lactic dehydrogenase activity suggested that the increased serum lactic dehydrogenase was due to increased synthesis of the enzyme by the tumor.

A positive correlation was observed in both tumor tissue and serum between the activities of lactic dehydrogenase and phosphohexose isomerase activities. This correlation appeared to be related to the functions of these 2 enzymes in glycolysis.

A high activity of tumor-tissue phosphohexose isomerase as herein determined was found to be closely correlated with the property of invasiveness in the tumors. Tumors could be classified in decreasing order of tumor-tissue phosphohexose isomerase activity as malignant epithelial, malignant mesenchymal, benign epithelial, and benign mesenchymal. The value of the application and interpretation of this observation has been suggested.

Alkaline phosphatase was the only enzyme considered in this study which fulfilled the criteria of a marker enzyme. This enzyme was present in significantly higher activity in osteogenic sarcomas, including metastases to soft tissue, than in other neoplasms, including other tumors metastatic to bone.

Serum lactic dehydrogenase activity decreased as a function of increasing age. It was significantly increased by the presence of hemolysis as determined by serum hemoglobin measurements. The mean serum lactic dehydrogenase value was significantly elevated above normal and similar in value for dogs with benign and dogs with malignant tumors. However, many individuals in both groups had values in the normal range.

Like serum lactic dehydrogenase, serum phosphohexose isomerase increased in the presence of hemolysis. The mean serum phosphohexose isomerase activity was significantly elevated in dogs with malignant neoplastic disease, but the mean value in dogs with benign neoplastic disease was less than in the healthy control group.

The mean serum leucyl aminopeptidase value was significantly elevated among dogs with benign neoplastic disease, but not among those with malignant neoplastic disease. A negative correlation was

obtained between this activity and several parameters of malignancy. A possible mechanism for this relationship has been postulated.

The mean serum alkaline phosphatase activity was significantly higher in dogs with malignant neoplastic disease and slightly higher in dogs with benign neoplastic disease than in the healthy controls. Again, this activity was subject to marked variance.

Postoperative serum enzymatic activities were not significantly lower in dogs from which neoplasms had been excised than in dogs in which they were biopsied. It is postulated that the mean postoperative interval of 4.7 days was insufficient for the inactivation or excretion of enzymes released by the tumor and by the surgical procedure.

STUDIES OF FOUR ENZYME SYSTEMS IN CANINE NEOPLASIA

By

Walter Fred Loeb

A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Pathology

1965

ACKNOWLEDGEMENTS

In the course of any study such as this, one becomes indebted for assistance and advice to a greater number of friends, colleagues, and associates than it is possible to acknowledge individually. Thus, the author extends his sincere gratitude to all who made this study possible.

Individual recognition, however, must be extended for the extensive efforts of those without whose help this study could not have been performed. Dr. L. A. Nagode has been my associate and collaborator in the study of which this thesis represents a major segment. The guidance and advice of Dr. C. R. Cole made the initiation of this study possible. The counsel of Dr. C. C. Morrill, who served as the author's adviser during the conclusion of the segment of the study contained in this thesis, was sincerely appreciated. Dr. W. J. Frajola has served as consultant in the area of enzyme biochemistry. The suggestions of Dr. J. E. Hunter have proven invaluable in the analysis of the data. Most of the neoplastic tissues for analysis were provided by Dr. G. P. Wilson.

Materials for histopathologic study were obtained from the library of the Department of Pathology, Ohio State University. Technical assistance in this study was rendered by Miss E. P. Henry and Mrs. S. K. Young.

Finally, no man is an island. Thus, without the cooperation and assistance of the author's wife, Lore, this study could not have been performed.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
OBJECTIVES	4
REVIEW OF LITERATURE	6
The History of Enzymology and of Enzyme Studies in Oncology	6
The Synthesis of Enzymes	9
Intracellular Enzyme Systems	12
The Balance of Intracellular and Extracellular Enzymatic Activity	13
Alterations in Enzymatic Activity Related to Neoplasia	16
Biochemical Interactions Affecting Observed Enzymatic Activity	19
MATERIALS AND METHODS	21
Selection of Experimental Material	21
Collection, Preparation, and Preservation of Specimens	22
Procedures for Evaluation and Description of Cases	23
Procedures for the Determination of Enzymatic Activity and Associated Biochemical Measurements	25
RESULTS	28
Protocols of Cases	28
Neoplasms of Epithelial Origin	28
Neoplasms of Mesenchymal Origin	81
Analysis of Data	154
Tabulation of Data	154
Mean Values and Standard Deviations	160

Page

Tests of Correlation 161

Tests of Hypotheses. 165

DISCUSSION 173

SUMMARY AND CONCLUSIONS. 185

REFERENCES 188

LIST OF TABLES

Table		Page
1	Enzymatic activities relating to Tumor 1	30
2	Enzymatic activities relating to Tumor 2	31
3	Enzymatic activities relating to Tumor 3	33
4	Enzymatic activities relating to Tumor 4	34
5	Enzymatic activities relating to Tumor 5	35
6	Enzymatic activities relating to Tumor 6	38
7	Enzymatic activities relating to Tumor 7	41
8	Enzymatic activities relating to Tumor 8	42
9	Enzymatic activities relating to Tumor 9	44
10	Enzymatic activities relating to Tumor 10	44
11	Enzymatic activities relating to Tumor 11	45
12	Enzymatic activities relating to Tumor 12	47
13	Enzymatic activities relating to Tumor 13	49
14	Enzymatic activities relating to Tumor 14	51
15	Enzymatic activities relating to Tumor 15	53
16	Enzymatic activities relating to Tumor 16	55
17	Enzymatic activities relating to Tumor 17	56
18	Host hematologic data relating to Tumor 18	57
19	Enzymatic activities relating to Tumor 18	58
20	Enzymatic activities relating to Tumor 19	59
21	Enzymatic activities relating to Tumor 20	60
22	Enzymatic activities relating to Tumor 22	63
23	Enzymatic activities relating to Tumor 23	63

Table		Page
24	Enzymatic activities relating to Tumor 25	67
25	Enzymatic activities relating to Tumor 26	68
26	Enzymatic activities relating to Tumor 27	69
27	Enzymatic activities relating to Tumor 28	70
28	Enzymatic activities relating to Tumor 29	71
29	Enzymatic activities relating to Tumor 30	73
30	Enzymatic activities relating to Tumor 31	75
31	Enzymatic activities relating to Tumor 32	75
32	Enzymatic activities relating to Tumor 33	76
33	Enzymatic activities relating to Tumor 34	77
34	Enzymatic activities relating to Tumor 35	78
35	Enzymatic activities relating to Tumor 36	79
36	Enzymatic activities relating to Tumor 37	79
37	Enzymatic activities relating to Tumor 38	81
38	Host hematologic data relating to Tumor 39.	82
39	Enzymatic activities relating to Tumor 39	84
40	Enzymatic activities relating to Tumor 40	87
41	Enzymatic activities relating to Tumor 41	89
42	Host hematologic data relating to Tumor 42.	90
43	Enzymatic activities relating to Tumor 42	92
44	Enzymatic activities relating to Tumor 43	93
45	Enzymatic activities relating to Tumor 44	94
46	Enzymatic activities relating to Tumor 45	96
47	Enzymatic activities relating to Tumor 46	98
48	Enzymatic activities relating to Tumor 47	100
49	Enzymatic activities relating to Tumor 48	101
50	Enzymatic activities relating to Tumor 49	101

Table		Page
51	Enzymatic activities relating to Tumor 50	103
52	Enzymatic activities relating to Tumor 51	105
53	Enzymatic activities relating to Tumor 52	106
54	Enzymatic activities relating to Tumors 53 and 73 . . .	108
55	Enzymatic activities relating to Tumor 54	109
56	Enzymatic activities relating to Tumor 55	109
57	Enzymatic activities relating to Tumor 56	110
58	Enzymatic activities relating to Tumor 57	111
59	Enzymatic activities relating to Tumor 58	112
60	Enzymatic activities relating to Tumor 59	113
61	Enzymatic activities relating to Tumor 60	113
62	Enzymatic activities relating to Tumor 61	113
63	Enzymatic activities relating to Tumor 62	116
64	Enzymatic activities relating to Tumor 63	119
65	Enzymatic activities relating to Tumor 64	120
66	Enzymatic activities relating to Tumor 65	120
67	Enzymatic activities relating to Tumor 66	121
68	Enzymatic activities relating to Tumor 67	122
69	Host hematologic data relating to Tumor 68.	125
70	Enzymatic activities relating to Tumor 68	126
71	Enzymatic activities relating to Tumor 69	127
72	Enzymatic activities relating to Tumor 70	129
73	Enzymatic activities relating to Tumor 71	132
74	Enzymatic activities relating to Tumor 72	132
75	Enzymatic activities relating to Tumor 74	133
76	Enzymatic activities relating to Tumor 75	135
77	Enzymatic activities relating to Tumor 76	137
78	Enzymatic activities relating to Tumor 77	139

Table		Page
79	Enzymatic activities relating to Tumor 78	142
80	Enzymatic activities relating to Tumor 79	144
81	Enzymatic activities relating to Tumor 80	145
82	Enzymatic activities relating to Tumor 81	146
83	Enzymatic activities relating to Tumor 82	148
84	Enzymatic activities relating to Tumor 83	149
85	Enzymatic activities relating to Tumor 84	149
86	Enzymatic activities relating to Tumor 85	150
87	Enzymatic activities relating to Tumor 86	151
88	Enzymatic activities relating to Tumor 87	152
89	Enzymatic activities relating to Tumor 88	153
90	Enzymatic activities relating to Tumor 89	153
91	A summary of identifying, morphologic, and behavioral tumor data.	154
92	Mean values and standard deviations for serum and tissue enzyme activity.	160
93	Results of significant tests of simple linear correlation	161
94	Means and standard deviations for serum enzyme activities.	165
95	Mean postoperative serum enzyme activities.	166
96	Mean values and standard deviations for enzymatic activities in benign and malignant neoplasms.	167
97	F statistics for comparison of benign epithelial, malignant epithelial, benign mesenchymal, and malignant mesenchymal tumors	169
98	F values testing equality of the means among four types of tumors	171
99	Enzymatic activities in normal and neoplastic lymph nodes	175

LIST OF FIGURES

Figure		Page
1	Tumor 67, diagnosed as a rhabdomyosarcoma, (A) in which a vessel contains an embolus of Tumor 30, a bronchial adenocarcinoma, (B). Hematoxylin and eosin. x87.5	123
2	Embolus of Tumor 30 lying within the vessel passing through Tumor 67. Hematoxylin and eosin. x87.5	123
3	Tumor 67. Note strap cells. Hematoxylin and eosin. x875	124
4	Embolus of Tumor 30 lying within the vessel passing through Tumor 67. Hematoxylin and eosin. x875	124

INTRODUCTION

Neoplasia may be defined as an autonomous proliferation of cells serving its host no beneficial function. The obvious implication that the proliferation continues until the affected cells are excised or destroyed or until the death of the host occurs, either due to neoplasia or other causes, is, in fact, generally valid.

Neoplastic disease has been recognized since antiquity, not only in man and other animals, but in plants as well. Currently it is one of the leading causes of death in man, a significant cause of disease and death in those species of animals regularly falling under scientific scrutiny, and one of the costliest of all medical problems.

From the dawn of recorded medical history until the beginning of the nineteenth century, little progress was made in the study of neoplastic disease. Medical knowledge of oncology was limited to the clinical and gross pathologic aspects of the disease and records were fraught with misdiagnosis. From the beginning of the nineteenth century to the middle of the twentieth, effort in the study of neoplasia was concentrated on the natural history of the disease and on the microscopic structure of neoplasms. Relating the neoplastic cell to its cell of origin resulted in the scheme for the classification of neoplasms in use currently. Attempts were made to evaluate the rate of growth of the neoplasm and assess its actual or potential effects upon its host.

While the studies of oncology conducted during the past one-and-one-half centuries have been primarily morphologic in nature, the

second decade of the twentieth century witnessed the beginning of biochemical studies in oncology, a trend which has increased at a rate seemingly without limit during the past 15 years. Broadly, these biochemical investigations fall into three categories: (1) the etiology of neoplastic disease, (2) the biochemical nature of the cancer cell and of its effects on its host and (3) the biochemistry of cancer therapy. It is with the second of these areas that this study is concerned.

Although many biochemical routes for the study of the cancer cell have been pursued, none would appear potentially more fruitful than studies of the activities of enzyme systems. This potential is based on a rational evaluation of the biologic position and nature of enzyme systems and is borne out by the studies of other investigators. Enzymes are proteins having a specific catalytic function and are determined qualitatively and, in part, quantitatively by the genetic constitution of the cell. All metabolic functions are subject to enzymatic regulation; conversely, metabolism is the summation of all enzymatic activity. At the investigatory level, too, this approach is facilitated by the sensitivity of enzymatic measurements. Because enzymatic activity is measured by the alteration in the rate of the catalyzed reaction rather than the biochemical quantitation of the enzyme protein itself, measurements of enzyme systems are second in sensitivity only to radioisotope studies.

There are three approaches to the study of the relationship of enzyme systems to tissue, viz., biochemical, histochemical and cytochemical. In the biochemical approach, quantitative measurements are made relating the activity of a single enzyme system to a specific

quantity of tissue or body fluid. In the histochemical approach, the presence or absence of an enzyme and its location within a tissue are studied, using a staining technique which is specific for the enzyme system under consideration, and examining the tissue with the light microscope. In the cytochemical approach, a suitable method of enzyme staining is utilized, followed by electron microscopy. In this manner, the location of the enzyme system within the cell may be related to the cell's ultrastructure. The biochemical approach is used in this study.

OBJECTIVES

The objectives of this study can be subdivided into two groups, the specific, short-term objectives which, it is hoped, will be fulfilled by this study, and the more general, long-term objectives to which this study may make a small contribution.

The specific, short-term objectives are:

1. To determine the tissue activity of lactic dehydrogenase, phosphohexose isomerase, leucyl aminopeptidase, and alkaline phosphatase in a representative group of canine neoplasms.

2. To determine the serum activity of the same enzyme systems in dogs having the above neoplasms.

3. To determine the serum activity of the same enzyme systems on the above dogs after the excision of the neoplasms.

4. To determine whether neoplasms <u>per se</u>, specific types of neoplasms, or specific variables associated with neoplasia, such as necrosis, inflammation, or invasiveness, produce characteristic alterations in the tissue enzyme levels.

5. To determine whether any of the factors enumerated in 4 (above) characteristically influence the serum enzyme activity.

6. To determine whether the data compiled by other workers in studies on man and the rat can be extrapolated across species lines to the dog.

The more general, long-term objectives of this study are:

1. To add to the body of knowledge on the biochemistry of neoplasia.

2. To provide data which can be used in comparing neoplasms with their normal parent cells, in order to determine to what degree neoplastic cells show biochemical similarity to their parent tissue.

3. To better define biochemical techniques which may serve as an aid in the diagnosis of neoplasia and specific neoplasms.

REVIEW OF LITERATURE

The History of Enzymology and of Enzyme Studies in Oncology

Enzymology is a science of comparatively recent development. It is doubtful whether the findings and theories of workers prior to the eighteenth century could be considered to have any direct bearing on the science of enzymology as it is known today. Fabroni, in 1787, defined fermentation as the decomposition of one substance by another substance. In 1789, Lavoisier published a balance sheet accounting for the carbon, hydrogen and oxygen from sugar undergoing fermentation to alcohol, carbon dioxide and acetic acid. Schwann, in 1837, attributed putrefaction to the presence of living bacteria. This view was shared by Pasteur, who held that fermentation was inseparable from living cells. The obligatory association of enzymatic function with living cells was retained until 1897 when Edward Buchner extracted zymase from yeast and showed that this cell-free liquid was able to ferment sugar to alcohol and carbon dioxide. Although earlier workers had observed reactions in which enzymatic function was demonstrated in a system free of living cells, the enzymatic components of these systems went unrecognized.

The term "enzyme" (literally "in yeast") was coined by Willy Kuhne in 1878, and it was his suggestion that it replace the former designation "ferment" as a term for such "unorganized" (more properly, extracellular) "ferments" as pepsin, ptyalin, and emulsin. The schema of designating an enzyme by the name of the substrate followed by the suffix "-ase" was introduced by Duclaus in 1883 (Dixon and Webb, 1964; Sumner and Myrback, 1950).

The chemical nature of enzymes was first revealed in 1926 by Sumner, who crystallized urease from jack-bean meal, and demonstrated it to be a globulin. Subsequent studies have proven that all enzymes are protein in nature, having a prosthetic group in which the enzymatic activity resides and which may be integrally associated with the protein molecule or separable from it.

Studies leading to the purification and crystallization of enzymes were conducted by Wilstätter from 1922 to 1928. Today over 600 enzymes have been purified, and over 100 are available in crystalline form.

The concept of enzyme specificity was developed by Emil Fischer, who regarded the relationship between enzyme and substrate as analogous to that between key and lock.

Although individual observations by early workers gave consideration to the alterations of enzymatic activities of higher animals in health and disease, most of their studies were focussed on reactions of industrial, rather than medical, significance (Sumner and Myrback, 1950).

In 1912, Otto Cohnheim, in a volume entitled "Enzymes", published a compilation of his lectures in physiologic chemistry. He stated that although, in the future, the synthesis of enzymes by chemists might be possible, for the present, enzymes were regarded as exclusively the products of cells, a statement which remains generally true today, more than 50 years later. This book dealt extensively with enzymes involved in digestion and, to a limited degree, with those associated with cell metabolism; but no reference was made to alterations of enzymes in disease (Cohnheim, 1912).

Studies leading to the diagnostic application of enzyme analysis were first conducted by Wohlgemuth who, in 1908, observed that ligation of the pancreatic duct produced an increase in the activity of amylase in the serum and urine. Stocks, in 1915, brought the findings of this study to fruition by demonstrating that elevated levels of amylase were present in the serum of individuals with pancreatic disease and that the highest levels are obtained in acute pancreatitis (Bodansky, 1961).

The systematic approach to the study of neoplasia via the study of enzymatic alterations was initiated by Otto Warburg (1930). Warburg's principal objective was the study of cell metabolism, and he concerned himself with the enzymes only insofar as they were essential to his primary target. Nevertheless, the organized interest in the study of enzymatic activities in normal and neoplastic tissues very clearly originated with his investigations. Warburg published his findings in an extensive series of papers beginning in 1923, and summarized his work to 1930 in a book entitled "Metabolism of Tumors". Most of his studies were performed in manometric chambers which he, himself, designed and which are still in limited use today. Warburg observed the relatively low respiratory quotient in neoplasms, as contrasted with normal tissues, and their high rate of glycolysis with the formation of lactate, especially in an anaerobic system. He noted that in this respect neoplasms resembled embryonic tissues more closely than they did normal adult tissues. These findings are still held valid today, despite the development of much more sophisticated instruments and methods. From these findings, Warburg concluded that carcinogenic agents act by impairing cell respiration. Under these

circumstances, the cell must either die or adapt to the new state of impaired respiration. Warburg theorized that, in the course of adjusting to rapid anaerobic glycolysis, the cell becomes neoplastic. This theory of carcinogenesis was never widely accepted and tended to be disproved by later investigations.

A period of relative inactivity on the application of enzymatic study to neoplastic disease followed the investigations of Warburg. Renewed interest in this field of study took place only after Wroblewski's adaptation (1955) of the method of analysis of lactic dehydrogenase according to Kubowitz and Ott (1943) and other similar advances in the methodology of enzyme analysis, yielding procedures which combined increased accuracy with greater technical simplicity. Since 1955, a tremendous interest in this approach to neoplasia has been demonstrated. This is evident in the review by Douglas (1963), whose bibliography contains over 250 papers of significance, written on the relationship of some facet of enzymology to neoplasia in the years 1955 to 1962.

The Synthesis of Enzymes

Information regarding the mechanism of enzyme synthesis is available from several types of studies. The most specific information is available from studies of bacteria or other unicellular organisms or from cell-free fractions of bacteria. Such information must withstand the hazards of extrapolation to forms of life very different from those from which it was obtained. Present knowledge, however, does not suggest specific contraindications to such extrapolation. A second source of information is the study of enzyme systems in higher, multicellular animals, especially utilizing the comparative approach. The

principal shortcomings in this type of study are the complexity and multiplicity of interacting reactions in such systems, which may hinder the development of specific conclusions. Finally, in higher animals we are presented with a tool for the study of enzyme synthesis in the form of spontaneous hereditary molecular diseases or "inborn errors of metabolism", diseases in which there is a defect in one or more enzyme systems.

Monod (1956) and others have shown that the synthesis of enzymes by the cell involves the complete de novo formation from amino acids. This has been confirmed both by the inability of cells, deprived of even single amino acids but provided with other potential precursors, to synthesize enzyme and by radioactive tracer incorporation studies. In addition to the amino-acid building blocks, ribose nucleic acid and a substance to serve as an inducer are required for enzyme synthesis. The ultimate substrate of the enzyme may serve as inducer, though studies by various workers including Buttin (1956, cited by Monod, 1956) have shown that chemical substances closely related to the substrate may, in some instances, serve more effectively than the substrate itself, and lend themselves to clearer distinction in in vitro studies. Depletion of RNA in centrifugally fractionated, intact-cell-free, ultrasonic cell lysates terminated enzyme synthesis. A source of energy is also required. In the cell-free system described, Gale (1956) utilized adenosine triphosphate and hexose diphosphate as the energy source. Gale also noted the necessity of the system's containing a purine-pyrimidine mixture, a finding confirmed by the studies of Marmur (1955) and, from this, deduced that not only the presence but also the concurrent synthesis of RNA is essential to enzyme formation.

Concurrent synthesis of RNA does not, however, appear to be essential for the formation of all enzymes.

Gale et al. (1956), as well as Marmur et al. (1955), also investigated the necessity of desoxyribose nucleic acid in the synthesis of enzymes using cell-free systems similar to those described before. When additional DNA was added to such a system, it did not alter the rate of enzyme synthesis. However, when the system was depleted of nucleic acid while still containing the other components, enzyme synthesis terminated but was restored upon the addition of DNA. In addition to proving the necessity of DNA in the enzyme synthesis system, Gale inferred that the determination of the specific enzymes which the cell is able to produce may lie in the DNA.

The studies of enzyme synthesis, which can be considered a special form of protein synthesis, suggest the necessity of a template for the formation of the enzyme. Spiegelman (1956) rejected the theory of a simple nucleic-acid template or one resulting from the attachment of inducer to an incomplete nucleic-acid template on the basis of enzyme synthesis kinetics, which do not substantiate such a mechanism. Rather, he postulated that templates may be in an active, convertible, or inactive form. The inactive templates are capable of no function except the genetic transmission of the ability to synthesize a given enzyme. The mechanism for the activation of inactive templates was not clearly indicated. Spiegelman did, however, indicate that, even in the presence of inducer, inactive templates were only rarely converted to active templates. The convertible templates could be activated by the addition of inducer. The active synthesis of enzymes occurred at the active templates only. His studies tended to indicate that RNA but not DNA could serve as the template.

The genetic nature of the ability or inability to synthesize specific enzymes, in higher animals, is demonstrated by an examination of species and even breed variation and by the study of individuals having "inborn errors of metabolism" related to an inability to synthesize specific enzymes. Cornelius et al. (1959) have shown that while hepatic necrosis results in the liberation of a high activity of glutamic pyruvic transaminase into the serum in the canine species, this is not true for the bovine or equine species. Evidence for breed differences is given by Friedman and Byers (1948, cited by Knox, 1960), who reported the high uric-acid excretion in the Dalmatian dog in contrast with other breeds of dogs to be due to a deficiency of uricase. The hereditary nature of many diseases due to enzyme-synthesis defects in man and other higher animals is well substantiated (Knox, 1960). These include such diseases as phenylketonuria, porphyria, and glycogen storage disease.

Intracellular Enzyme Systems

With the exception of very limited in vitro studies utilizing cell fractions such as those described above, all enzymes produced to date are the products of living cells. From a physiologic view, however, it is possible to classify enzymes according to their ultimate site of activity as extracellular enzymes or intracellular enzymes. Most of the enzymes of animal origin studied early in the history of enzymology were of the former class, usually having functions related to digestion. These enzymes are synthesized in high activity only by highly specialized glandular cells and are then secreted from the cell, either as active enzymes or their precursors to function outside of the cell of origin. The intracellular enzymes, in contrast, are synthesized within a given cell for its own metabolic use. A vast number

of such intracellular enzymes is recognized; virtually every metabolic chemical reaction is under the control of one or more such enzymes. It is well accepted that marked differences in the level of activity of such enzymes exist among the various tissues (Greenstein, 1956; Douglas, 1963; Nagode et al., 1965).. A great portion of the enzymologic study of neoplasia has been directed toward a search for a specific enzyme which is absent from normal cells and present in cancerous ones. Tentative reports of such an enzyme have ultimately been disproven (Weinhouse, 1960). Thus it appears that the differences among normal cell types, and those between normal and neoplastic cells, with respect to enzymatic activity are quantitative and generally not qualitative.

Three of the four enzyme systems investigated in this study clearly fall into the category of intracellular enzymes. These are lactic dehydrogenase, phosphohexose isomerase, and leucyl aminopeptidase. Regarding the fourth, there is some difference of opinion as to whether the alkaline phosphatase of the osteoblast also exerts an extracellular function in the mineralization of osteoid (Majno et al., 1951).

Intracellular enzymes may be localized in the mitochondria, associated with other submicroscopic particles such as microsomes, or appear to be free within the cytoplasm, or the so-called soluble fraction (Holter, 1952; Kuff and Hogeboom, 1956). The enzymes under consideration in this study are considered as cytoplasmic enzymes, though alkaline phosphatase may be in the form of a lipoprotein complex.

The Balance of Intracellular and Extracellular Enzymatic Activity

As has been indicated above, the enzymes under consideration in this study are synthesized by the cell for functions within the cell.

Nevertheless, some activity of each of these enzymes may be found in the serum of all individuals. Among healthy dogs, this activity varies within a relatively narrow range (Loeb and Nagode, 1965). This is in contrast with some of the other intracellular enzymes, such as those located in the mitochondria which are absent from the sera of normal individuals. The passage of enzymes from cells to serum appears to be via the intercellular fluids into which the enzymes are liberated by limited passage through intact cell membranes or by the death and lysis of individual cells (necrobiosis), thence via the lymphatics to the general circulation. Three factors influence the balance between intracellular and extracellular enzymatic activity (Bodansky, 1961). These are (1) the rate of synthesis of the enzyme, (2) the permeability or continuity of the cell membrane allowing the enzyme to pass into the extracellular fluids, and (3) alterations in the pattern of excretion or degradation of the enzyme. Factors affecting the rate of synthesis of enzymes will be given further consideration in the following pages. Suffice it at this point to simply indicate that the potential amount of an enzyme liberated from a mass of tissue cannot exceed that which the tissue is capable of synthesizing. In many of the early studies relating to the diagnostic application of serum levels of intracellular-enzyme activity, the accelerated passage of these enzymes through the cell membrane of injured or necrotic cells was stressed (Wroblewski, 1956; Bodansky et al., 1959). Not only cell death, but even reversible cell injury may affect the cell membrane in such a way as to increase enzymatic loss. Warburg and Hiepler (1956) found that aldolase passed from ascites-tumor cells into the ascitic fluid and that such passage was increased in anaerobiasis; Wu (1959) observed

that glycolytic enzymes as well as enzymes of the pentose phosphate cycle leaked from ascites-tumor cells into the surrounding medium. Alkaline pH increased the rate of leakage, but did not affect the rate of leakage of all enzymes uniformly. This phenomenon is termed "selective increase in cell membrane permeability" (Bodansky, 1961). Consideration must also be given to instances in which extracellular membrane-like structures may influence the passage of intracellular enzymes into the serum. Guttman (1942), as well as Fishman, has shown that while serum acid phosphatase is generally elevated in prostatic carcinoma with metastasis, it may not be elevated when the carcinoma is limited within the prostatic capsule (Fishman, 1956).

The influence of alterations in the route of excretion or degradation is best indicated by several examples of systems in which discrete information is available. The activity of alkaline phosphatase in hepatic parenchymal cells is very low. The alkaline phosphatase present in the serum of normal individuals appears to be primarily of osteoblastic and intestinal origin. This enzyme is transferred from the serum into the bile canaliculi by the action of the hepatic parenchymal cells, whence it is excreted into the lumen of the intestine. Disease of the hepatic parenchymal cells does not, as a rule, influence the activity of alkaline phosphatase in the serum. Naturally occurring or experimental occlusion of the common bile duct or naturally occurring occlusion of the intrahepatic bile ducts, however, results in massive elevation of this activity in the serum, apparently due to an inability to excrete the enzyme via the normal pathway (Sherlock and Walshe, 1947, cited by Bodansky, 1961). Such mechanisms are not applicable to all enzyme systems. White (1960) studied the rate of disappearance of

lactic dehydrogenase and isocitric dehydrogenase injected into normal dogs. Following the establishment of standard values relating to the rate of disappearance of these two enzymes, the study was repeated following nephrectomy, hepatectomy, and splenectomy of the same animals. These experimental procedures did not alter the rate of decrease in the serum enzymatic activity. From this White concluded that the enzymes under consideration were inactivated or denatured, rather than excreted, and that this function did not necessarily occur in the liver, kidneys, or spleen.

Alterations in Enzymatic Activity Related to Neoplasia

Greenstein, in 1956, reported the results of an extensive study in which he measured the activity of ten enzyme systems in normal tissues of mice and in neoplasms arising from these tissues. From his findings he concluded that, in general, neoplasms constituted a far more homogeneous group with respect to enzymatic activity than did normal tissues and had little similarity to the parent tissue. From similar data, Weinhouse (1960) concluded that hepatomas bear little biochemical resemblance to normal liver cells. Both of these workers thus noted that the neoplastic cell is enzymatically devoid of such enzymatic capacities as characterize cellular specificity and, rather, is adapted to its limited function of survival and reproduction. Certain very marked exceptions to this rule are recognized. Greenstein observed that in the osteogenic sarcoma, in comparison to normal bone, an increase rather than a decrease in alkaline phosphatase may be noted. This observation has been often confirmed, as may be noted by the histochemical studies cited by Wachstein (1962). Similarly, the high acid phosphatase of the prostatic carcinoma has been previously indicated.

These observations by Greenstein and by Weinhouse have been contradicted by the observations of Bär, Schmidt, and Schmidt (1963). From a study of 19 enzyme systems in 33 tumors and normal tissues, and from a limited number of isozyme separation studies, Bär *et al.* concluded that although similar tumors differ from one another as do normal tissues, a remarkable similarity exists between neoplasms and their normal cell of origin. Deviations in enzymatic activity between tumors and their tissues of origin corresponded to observed alterations in metabolism. Similar findings were reported by Pitot (1960, cited by Busch, 1962).

Although both Weinhouse and Greenstein stated that the determined enzymatic activities represented the maximal potential enzymatic activity of the tissue under consideration rather than an actual representation of *in vivo* function, it is possible that this discrepancy between the studies cited above lies in the factors which may alter the relationship between the quantity of enzyme protein in a system and the observed activity (Weinhouse, 1960).

Although mechanisms which may cause a neoplasm to differ in enzymatic activity from its parent tissue have been postulated, scientific proof of these hypotheses has not been forthcoming. Two hypotheses appear to have distinct potential merit. The genetic control of enzyme synthesis has been discussed before and is summarized by Fishman (1959). On a cellular basis, as Busch (1962) demonstrates, neoplasia can be considered a genetic disease. Both phenotypic characteristics and chromosomal analyses support this premise. The ability of the tumor cell to reproduce itself, with the maintenance of such properties as atypical mitosis (division into more than two daughter cells),

invasiveness, decreased intercellular cohesiveness and resistance to anoxia, serve as phenotypic characters of neoplasia, while chromosomal studies of some neoplasms have revealed aberrations characterized by either alteration of chromosome number or alteration of the morphology of one or more chromosomes in comparison with the parent tissue. In view of this characterization of the tumor cell, it is not difficult to accept the hypothesis that alterations in enzymatic activity observed in neoplastic cells are genetically occasioned and, perhaps, the result of alterations in the tumor cell's DNA.

A different hypothesis is formulated by Pitot and Heidelberger (1963), based initially on the studies of Jacob and Monod (1961). Jacob and Monod demonstrated that, in addition to the inducer, discussed earlier, the synthesis of some enzymes is under the regulation of a substance termed "repressor", which may be the end-product of the enzymatic reaction and which is capable of affecting cessation or marked reduction in the rate of enzyme synthesis. In other enzyme systems, however, a substance similar to the repressor does not act on the rate of production of the enzyme, but rather combines with the enzyme molecule itself to inhibit its catalytic function. This inhibition, termed allosteric inhibition, may function much in the manner of a feedback, or servomechanism. Furthermore, it is shown that chemical treatment can alter the enzyme in such a way as to retain its enzymatic function but render it refractory to allosteric inhibition. Although Pitot and Heidelberger extend their hypothesis further, using it to develop a theory of carcinogenesis, it is not necessary to do so in order to explain differences in enzymatic activity between normal and neoplastic cells. Thus, alterations in the quantity of

inducer, repressor, or allosteric regulator, or chemical alterations of the enzyme making it refractory to allosteric regulator might alter the enzymatic activity of the neoplastic cell.

Fishman (1959) discussed several other mechanisms by which the enzymatic activity of the neoplastic cell may be altered: none of these, however, is as free from conflict with observed data as the two mechanisms considered above. These include dietary influence, hormonal effect, and a consideration of the enzymatic characteristics of functional tumors.

Biochemical Interactions Affecting Observed Enzymatic Activity

The measurement of enzymatic activity is performed in order to obtain an index of the quantity of enzyme protein present. The relationship between enzymatic activity and the quantity of enzyme can be linear only if all other components of the enzyme system are optimal. This includes the pH, concentration of coenzymes and activators, and the absence of inhibitors. Weinhouse (1960) stated,

> "In the past, enzymes were reported to be low or missing in tumors, only to have discovered that a coenzyme was lost in preparing the enzyme."

All methods used in this study bear their originators' claim of linearity as evidence for the optimal state of all associated factors. However, Nagode (1965) has shown that many normal canine tissues fail to demonstrate a linear phosphohexose isomerase activity in the dilutions used in this study. In fact, in some determinations, the quantity of fructose-6-phosphate (the measured end-product) decreased in the last two-thirds of the reaction period. He found that this nonlinearity of demonstrable activity could be overcome by preparing higher dilutions of the tissue. It was his conclusion that this was

not due to a decrease in the concentration of substrate (Michaelis phenomenon), since this occurred in an activity range in which, according to Bodansky (1954), the reaction was linear. This was not observed with sera, including sera of high activity.

Zondag (1963) has shown that the storage of tissue homogenates at -20° C. does not prevent loss of the slowly migrating isozymes of lactic dehydrogenase, apparently by reversible oxidation. Nagode (1965) demonstrated that this form of denaturation occurred even though the homogenates were frozen in liquid nitrogen (-196° C.) and stored at -70° C.

The extraction of enzymes from a suitable tissue source may be accomplished by rupturing the cell membranes in the presence of a suitable buffered solvent. Of the enzymes considered in this study, all but alkaline phosphatase may be present as a lipoprotein complex, dissociable by fat solvent or detergent (Schmidt and Schmidt, 1963; Dixon and Webb, 1964).

MATERIALS AND METHODS

Selection of Experimental Material

The animals which were investigated in this study, and from which the tumors under consideration were obtained, were dogs which were presented to the Ohio State University Veterinary Clinic for treatment between September, 1963, and April, 1964. All were selected on the basis of an admitting or provisional diagnosis of neoplastic disease. Dogs which had had previous chemotherapy or radiation therapy for the neoplasm were excluded, but those which had had previous attempted surgical excision of neoplasms were not excluded if they met the other requirements. Some of the animals initially studied were ultimately excluded for the following reasons: (1) if the lesion clinically regarded as a neoplasm did not prove to be so histologically, (2) if no histopathologic examination of the lesion clinically regarded as a neoplasm was performed, (3) if adequate follow-up evaluation of the case was not possible.

Controls for the study were obtained in the following manners:

1. Serum enzyme activities were determined in healthy dogs under a variety of conditions, simulating the conditions of the dogs with neoplastic disease under study (Loeb and Nagode, 1965).

2. Enzyme activities of a variety of normal tissues obtained from healthy dogs were determined (Nagode, 1965; Nagode, Frajola, and Loeb, 1965).

3. Serum enzyme activity determinations were done postoperatively in dogs with neoplastic disease, both in dogs from which the tumor had

been successfully excised and in dogs in which only a biopsy of the tumor had been performed. These dogs had, in every way, been treated in the same manner as those from which the tumors had been excised and thus constituted, for the purpose of this study, a series of sham operations.

Collection, Preparation, and Preservation of Specimens

Blood samples were taken from all dogs having an admitting or provisional diagnosis of neoplastic disease on the day of admission prior to physical examination. Each sample was taken by jugular puncture using a 19-gauge disposable needle and a silicone-coated syringe. It was transferred from the syringe into a silicone-coated test tube and placed in an ice bath for the period of coagulation and clot retraction. It was then centrifuged for 10 to 15 minutes at 1600xG. The clear supernatant serum was aspirated using a syringe equipped with a 24- or 26-gauge needle. A series of aliquot vials was prepared by immersing them in liquid nitrogen. The vials were removed from the liquid nitrogen, and an aliquot (0.2 to 0.8 ml.) of the serum was sprayed into the vial, displacing the nitrogen and freezing immediately. The vials were then placed in a freezer at -70° C. and were stoppered after all the nitrogen had had sufficient time to boil off.

Neoplastic tissue for analysis was collected at the time of surgical removal. One or more grossly homogeneous pieces of tumor, free from visible areas of necrosis, were selected, when possible approximating a 1-cm. cube. Tissue for histopathologic examination was taken immediately adjacent to the specimen for enzyme analysis. The tissue for enzyme analysis was immediately placed into a 0.154 M.

solution of potassium chloride buffered with potassium bicarbonate to pH 7.0, in an ice bath. The tissue was weighed, scissors-minced, and homogenized in a Potter-Elvehjem homogenizer with a teflon pestle. Clearance between pestle and tube was 0.10 to 0.15 mm. Pestle speed was 1,750 R.P.M. The homogenizing tube was kept in an ice bath. During the homogenization, the tissue was diluted to a 10% suspension on a weight/volume basis with the 0.154 M. potassium chloride diluent. Following the homogenization, the homogenate was centrifuged at 1600 x G. for 10 minutes in order to separate particulate matter such as collagen fibers and intact nuclei from the soluble fraction. The supernate was spray-frozen in liquid nitrogen and stored in the same manner as the serum.

Postoperative sera were collected at intervals as indicated in the case protocols. They were collected, processed and stored in the same manner as the preoperative sera.

Procedures for Evaluation and Description of Cases

Thorough clinical examinations were performed and recorded for all animals. These included history, physical examination and, where indicated, appropriate laboratory studies. Following the excision of the neoplasm, a gross description of the neoplastic tissue was prepared. Histologic sections were prepared from all excised tissues, examined, and described. Because of the association by other workers of alterations in enzymatic activity with such parameters as necrosis, inflammation and anaplasia, these were especially noted in the histopathologic examination of tissues. The postoperative course of each animal was noted, and recorded where significant. Complete necropsy was performed on each animal which died or was euthanatized during the course of the study.

Measurements of enzymatic activity were performed on all specimens for enzyme analysis as described in the following section. In the Report of the Commission on Enzymes of the International Union of Biochemistry (1961) the use of a standard international unit is recommended. This is defined as that amount which will catalyze the transformation of 1 micromole of substrate per minute. All values in this study are recorded in international units. The same report also recommended the use of systematic rather than trivial names for enzyme systems. To date, this recommendation has not generally been followed by workers in the field of medical enzymology; thus, to introduce it here would appear to create more confusion than it would alleviate. Trivial names will be used throughout this study; however, the systematic names have been indicated in the description of the technique of analysis for individual enzyme systems, as are the constants for the conversion from trivial to international units. In accord with the recommendation of the International Union of Pure and Applied Chemistry (Mather, 1965), activities in serum are expressed as international units per liter, and those in tumor extracts as international units per kilogram of wet weight or per gram of tissue protein, so that the vast majority of activities are expressed in not less than 1 nor more than 3 whole digits.

The term "specific activity" (Sp.A.) is the enzymatic activity in international units per gram of tumor tissue protein. Concentrations of hemoglobin are indicated in grams per liter or kilogram.

The terms tumor and neoplasm are used synonymously throughout this study.

Procedures for the Determination of Enzymatic Activity and Associated Biochemical Measurements

Lactic dehydrogenase (LDH; systematic name: L-Lactate: NAD Oxidoreductase) was determined by the method of Wroblewski and LaDue (1955) at a wavelength of 340 millimicrons at 25° C. Measurements were made in a Beckman DU spectrophotometer equipped with a Gilford programming and recording system and were controlled with Enzatrol ® (enzyme control, Dade Reagents, Inc., Miami Beach, Florida). Activity was determined in units/ml. or units/gm., as defined by Wroblewski and LaDue and converted to international units/liter or international units/kilogram by multiplying by a factor of 0.482.

Phosphohexose isomerase (PHI), also called glucosephosphate isomerase (systematic name: D-glucose-6-phosphate Ketol-isomerase), was determined by the method of Bodansky (1954) at 37° C. Measurements of the fructose-resorcinol complex formed were made in a Beckman DU spectrophotometer at a wavelength of 490 millimicrons. Calibration curves were prepared with each lot of analyses. Activity was determined as defined by Bodansky and was converted to international units by multiplying by a factor of 4.63.

Leucyl aminopeptidase (LAP), also termed leucine amino peptidase, has not been given a systematic name (Report of the Commission on Enzymes, 1961). This enzyme was determined by the method of Goldbarg and Rutenburg (1958) as modified by Fox (1960). Reagents were prepared as follows: the substrate was prepared by dissolving l-leucyl-beta-naphthylamide in water at a concentration of 0.00137 M. (0.4 mg./ml.). This was mixed with an equal volume of 0.2 M. phosphate buffer pH 7.0 to yield a solution of pH 7.1. The acetate buffer used was a 1 M.

buffer having a pH of 4.5 to 4.8 and containing 10% Tween 80. A fresh aqueous solution of fast red B salt, 2.5 mg./ml., was prepared immediately prior to use. The naphthylamine standard solution was prepared by dissolving 5 mg. of beta-naphthylamine in 2 ml. of absolute ethanol and diluting with water to 100 ml. A standard curve was then prepared by diluting this solution with water so that 0.75 ml. of the final dilutions contained 5, 10, 15, 20, 25, and 30 micrograms of beta-naphthylamine, respectively. The enzymatic activity was determined as follows: 3.0 ml. of substrate, 0.1 ml. of a 1 in 5 dilution of serum or tumor extract, and 0.65 ml. of water were incubated in a 37° C. water bath for 1 hour. The tubes were then placed in ice water, 0.5 ml. of the acetate buffer and 0.25 ml. of the fast red B salt solution added and the contents of the tubes mixed. Blanks were prepared by mixing similar amounts of the same reagents and serum, in the following order: substrate, water, acetate buffer, serum, fast red B salt solution. The standardization curve, prepared with each group of analyses, contained 0.75 ml. of standard, diluted as above, 1.5 ml. 0.2M phosphate buffer pH 7.0, 1.5 ml. distilled water, 0.5 ml. acetate buffer, and 0.25 ml. fast red B salt solution. Blanks and standards were not incubated. All reactions were read 5 to 15 minutes after addition of the fast red B salt solution, in a Beckman DU spectrophotometer at 530 millimicrons. Activity was calculated in units, as prescribed by Fox, defined as that enzymatic activity which will liberate 1 microgram of naphthylamine/ml. of serum at 37° C. in 1 hour. Conversion to international units was performed by multiplication by 0.1164.

Alkaline Phosphatase (Alk.Ptase; systematic name: Orthophosphoric Monoester Phosphohydrolase) was determined by a micro-modification of

the method of Bodansky as modified by Roe and Whitmore (1938). Reagents used were as prescribed, except in 1/5 volume. The procedure was standardized with inorganic phosphorus standard and controlled with Enzatrol. Incubation was at 37° C. Blanks were prepared by incubating tubes of substrate and, after incubation, adding trichloracetic acid, followed by serum. Separation of the supernate from the precipitated proteins was accomplished by centrifugation, after which the released inorganic phosphorus was measured by the technique of Fiske and Subbarrow. Reactions were read in a Coleman Junior spectrophotometer. Enzyme activities were converted from Bodansky units/100 ml. to international units/liter by multiplying by 5.38.

Hemoglobin determinations were performed on sera and on tissue extracts by a calibrated micro-modification of the cyanmethemoglobin method, utilizing 2.0 ml. of cyanmethemoglobin reagent and 0.2 ml. of serum or tissue extract. Calibration was performed both with cyanmethemoglobin standard and with canine erythrocyte lysates in which the hemoglobin had been determined by oxygen uptake, using a Van Slyke volumetric blood-gas apparatus. Hemoglobin concentration was read on a Coleman Junior spectrophotometer at a wavelength of 540 millimicrons (Frankel and Reitman, 1963).

Protein concentrations were determined on tissue extracts by a micro-modification of the biuret procedure (Caraway, 1960). Total protein concentration was measured at a wavelength of 540 millimicrons on the Coleman Junior spectrophotometer.

RESULTS

Protocols of Cases

Neoplasms of Epithelial Origin

Tumor 1 was obtained from a male Welsh Terrier, 9 years of age, which was initially presented on August 5, 1961, for "warts and lumps all over body". These lesions had been present for over a year. At this admission surgery consisted of orchiectomy of a cryptorchid testis, excision of a benign calcifying epithelioma from the shoulder and a Meibomian-gland adenoma from the eyelid. Histopathologic examination of the cryptorchid testis revealed the presence of a seminoma. On February 26, 1962, a keratoacanthoma was excised from the left midthoracic wall. On June 4, 1962, 5 neoplasms involving the skin were excised. Histopathologic examination revealed that 3 were keratoacanthomas and the others sebaceous adenomas. On August 31, 1963, 1 keratoacanthoma and 2 sebaceous adenomas were excised. On January 28, 1964, the dog was again presented for the excision of 3 neoplasms involving the skin. Physical examination at this time revealed no other significant abnormalities. Hemogram revealed: hemoglobin 15.05 gm.; packed cell volume, 43%; leukocytes, 14,800/cu. mm.; segmented neutrophils, 85%; nonsegmented neutrophils, 1%; lymphocytes, 6%; eosinophils, 3%; monocytes, 5%. Urinalysis revealed a specific gravity of 1.006 and the presence of 100 mg. of albumin per 100 ml. Remaining findings disclosed no additional abnormalities. Masses were excised from the dorsal midline in the anterior lumbar region, from the right lateral

aspect of the dorsal lumbar region, and from the lateral surface of the right forefoot. The anterior lumbar neoplasm measured 3.2 x 1.7 x 0.6 cm. and was irregular, firm, and white with no capsule evident. Histopathologic examination revealed a mass partially covered by keratinized stratified squamous epithelium with adnexa. The covering epithelium was interrupted by a crater or depression, the surface of which was partially ulcerated, partially covered by keratin and neutrophils. The tumor mass arose irregularly from the basal-cell layer and matured outward, polarized like normal epidermis. A few keratin pearls were present in the mass. At the site of ulceration, the mass was infiltrated by neutrophils. Histopathologically, this neoplasm was diagnosed keratoacanthoma. The mass from the right lateral aspect of the dorsal lumbar skin measured 3.0 x 2.1 x 1.0 cm. and was soft, gray, and encapsulated. Histopathologically, it resembled the previous mass, except that there was no communication with the exterior by ulceration of the covering epithelium. A capsule was present. Adjacent to the capsule was an area of necrosis containing cholesterol clefts and areas of mineralization. This mass was also diagnosed a keratoacanthoma. The lesion excised from the right forefoot is considered in this study as Tumor 35.

On Feburuary 25, 1964, additional neoplasms were excised from the midcervical skin 2 cm. to the left of the midline and from the posterolateral surface of the right hind leg 2 cm. proximal to the tarsus. A portion of the first of these 2 neoplasms was collected and extracted for enzyme analysis. This neoplasm measured 2.5 x 1.7 x 0.7 cm. Histopathologically it was identical to the keratoacanthomas described above. The surface was ulcerated and a marked neutrophilic

infiltrate was present. The other neoplasm was also a keratoacanthoma of similar size. Centrally, it formed a keratin-filled cyst. Enzymatic activities in the serum and tumor tissue are recorded in TABLE 1.

TABLE 1. Enzymatic activities relating to Tumor 1.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk Ptase.	Alk Ptase. Sp.A.
Serum	1/27	0.48	14		116		20		328	
Serum	2/24	1.53	214		306		11		440	
Tumor	2/25	22.0	3543	46	11112	144	136	1.7	59	0.8
Serum	2/27	1.93	366		333		13		112	

Tumor 2 was obtained from a spayed female German Shepherd, 6 years of age. This dog was presented for numerous tumors of the skin on Februrary 9, 1964. Hemogram revealed: hemoglobin, 19.4 gm./100 ml.; packed cell volume, 57%; erythrocyte sedimentation rate, 8 mm./60 minutes, in contrast with an expected value of 0 mm./60 minutes; leukocytes, 8200 per cu. mm.; segmented neutrophils, 68%; lymphocytes, 24%; eosinophils, 8%. On February 10, 7 white, firm, skin-covered masses were surgically excised. These measured from 0.5 x 0.6 x 0.4 cm. to 2.6 x 1.5 x 1.0 cm. in size. Histopathologic examination revealed that the mass excised from the left shoulder was covered by intact skin with adnexa. The mass consisted of several lobules separated by dense connective tissue. The largest of these lobules was a cyst-like structure of proliferating stratified squamous epithelium. Centrally, and multifocally within the wall, there was maturation to keratin pearls. The smaller lobules were cystlike structures completely

filled with a proteinaceous, eosinophilic debris, focally mineralized. The walls were infiltrated by histiocytes, macrophages, foreign-body giant cells, fibroblasts, and contained remnants of glandular epithelium. The lesion was diagnosed histopathologically as a keratoacanthoma and foreign body granuloma arising in the wall of a dermoid cyst. Lesions excised from the right shoulder and from the dorsum of the neck were also identified as keratoacanthomas. The lesion from the dorsum of the neck contained, within its capsule, cholesterol clefts and a minimal diffuse foreign-body granuloma. Lesions excised from the left shoulder, the right dorsum of the calvarium, and the right side of the neck consisted of masses covered by skin with normal adnexa. The masses consisted of cysts having walls of stratified squamous epithelium, maturing centrally to fill the cavity of the cyst with keratin. In some areas in the wall of these epidermal inclusion cysts, there was thickening and proliferation of the epithelium, suggesting the possibility of a transformation of this non-neoplastic lesion to early keratoacanthoma. The seventh section of surgically excised tissue revealed no definitive lesion on histopathologic examination.

Enzymatic activities in the serum are recorded in TABLE 2.

TABLE 2. Enzymatic activities relating to Tumor 2.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/10	1.11	43	88	16	2.7
Serum	2/15	0.89	41	97	16	7.0

Tumor 3 was obtained from a castrated male Chow-type dog, $10\frac{1}{2}$ years of age. This dog was initially presented for veterinary attention on October 30, 1963, because of alternating periods of diarrhea and constipation. Physical examination at that time revealed an enlarged prostate and bilateral nodularity of the testes. An orchiectomy was performed. Histopathologic examination of the testes disclosed bilateral interstitial-cell tumors. The dog was again presented on March 11, 1964, for excision of 7 small tumors from the skin of the back. These were surgically excised, and measured from 0.3 x 0.6 x 0.8 cm. to 2.8 x 1.3 x 1.1 cm. Histopathologic examination of all masses except 1 revealed similar findings. They were covered by stratified squamous epithelium with adnexa. In the dermis, extending in some areas to the epidermis, were nests of stratified squamous epithelium, maturing inward to form keratin pearls. Around or between these structures were other epithelial cells in nests or strands. In some areas, the cells contained or surrounded vacuoles. The tumor cells were of moderate size, with oval nuclei stippled with chromatin and with prominent nucleoli. The cytoplasm was eosinophilic and joined the cytoplasm of adjacent cells by intercellular bridges. Occasional mitoses were present. All of these lesions were diagnosed as keratoacanthomas. The remaining tumor is listed in this study as Tumor 60. Serum-enzyme activities, determined prior to surgery, are recorded in TABLE 3. On June 9, 1964, the dog was again presented for removal of 4 additional keratoacanthomas, histopathologically identical to those previously excised.

TABLE 3. Enzymatic activities relating to Tumor 3.

Specimen	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	0.6	29	116	29	0.5

Tumor 4 was obtained from an 11-year-old spayed female Boxer. This dog was presented on December 13, 1963, for multiple skin neoplasms. Physical examination failed to disclose additional abnormalities. Five neoplasms were excised surgically on December 14. Tumor 4 was excised from the third left mammary gland. The remaining neoplasms are considered in this study as Tumors 53 and 73. Tumor 4 measured 6.5 x 6.0 x 2.5 cm. and was firm and white. Grossly, it appeared encapsulated and did not appear to involve the dermis. Microscopically it consisted partially of dense fibrous connective tissue diffusely infiltrated by mastocytes (see also Tumor 53). In the center of this fibrous tissue was a nodule 0.5 x 1.0 cm. composed of stratified epithelium maturing centrally to form keratin pearls. Adjacent to this structure was a cluster of epithelial cells showing no evidence of maturation. These cells had large oval nuclei with extremely prominent, sometimes multiple nucleoli, and violet-staining cytoplasm. Many of the cells were irregular in shape and very large in size. Mitoses were evident. This neoplasm was diagnosed as a squamous cell carcinoma occurring within a mastocytoma. Surgery for the excision of additional neoplasms was performed on December 31, 1963, on January 2, 1964, and on January 8, 1964. Serum enzyme activities are recorded in TABLE 4.

TABLE 4. Enzymatic activities relating to Tumor 4.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	12/13	0.69	24	83	40	79.5
Serum	12/26	0.80	111	125	37	16.6
Serum	1/3	1.04	48	111	36	0
Serum	1/27	0.97	67	97	32	7.5
Serum	3/14	2.11	410	268	39	20.4
Serum	5/14	0.69	183	148	17	4.3

Tumor 5 was obtained from a 9-year-old spayed female Poodle. This dog was presented on January 3, 1964, with a history of having had an ovariohysterectomy and excision of mammary neoplasms elsewhere in July 1962 and excision of additional mammary neoplasms in July 1963. Masses were present in the fourth and fifth right mammary glands. The fifth right mammary gland was ulcerated. A biopsy of the fifth right mammary gland was performed on January 3, 1964, and additional biopsies were performed on the fourth and fifth right mammary glands on January 15. Following these procedures, the neoplasm was treated by x-irradiation. On March 20, 1964, the third, fourth, and fifth right mammary glands were excised. The tissue excised on January 3 was a light gray- to tan-colored mass, 1.1 x 0.7 x 0.4 cm., covered by integument. Histopathologically, the surface was seen to be covered by ulcerated and focally inflamed epidermis, and dermis with adnexa. In the dermis was a highly lobulated, highly cellular tumor composed of nests of cells having moderate-sized round to oval nuclei and scanty, pale eosinophilic cytoplasm. These nests were enclosed in a thin fibrous stroma. This neoplasm was diagnosed as a basal-cell tumor.

The biopsy on January 15 yielded 4 soft white masses of tissue measuring 1.8 x 1.3 x 0.7 cm., 1.6 x 1.9 x 0.2 cm., 1.4 x 0.8 x 2.0 cm., and 1.4 x 0.7 x 0.2 cm., respectively. The microscopic structure of these tissues was similar to that described above, except that the aggregation of cells into nests was not as distinct and the connective tissue separating the cell nests was finer and more fibrillar. These sections confirmed the diagnosis of basal-cell tumor. The mammary glands excised on March 23, 1964, comprised 5 fragments of firm tan tissue. Histopathologically they resembled the above description, except that the cell nests were small, the cells pale-staining, and the stroma dense and abundant with an over-all hyaline appearance. Enzymatic activities of serum and tumor tissue extracts are recorded in TABLE 5.

TABLE 5. Enzymatic activities relating to Tumor 5.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	1/3	0.64	58		93		13		17.7	
Biopsy (tumor)	1/3	3.8	3760		10464		136		1514.	
Serum	1/7	0.72	77		222		12		23.6	
Serum	1/9	0.45	53		102		19		20.9	
Serum	1/11	0.56	53		97		16		26.3	
Serum	1/21	1.15	82		111		24		7.5	
Serum	1/30	0.41	29		102		20		31.1	
Serum	2/11	0.88	24		222		20			
Serum	3/20	0.80	77				23			
Tumor	3/20	0.0	1350	34	1250	31	358	8.96	306.1	
Serum	3/23	1.82	77		222		31		8.1	

Tumor 6 was obtained from a 12-year-old spayed female Cocker Spaniel. The dog was presented on January 30, 1964, with the history that a tumor had been excised from the right ear but had recurred. The dog had been deaf for the past 5 years. The history indicated that the dog had had nephritis over the past 4 years. Physical examination revealed an obese dog with bilateral juvenile cataracts. Neoplasms were present on the pinna of the right ear and ventral to the anus. Small "wart-like" growths were present multifocally on the skin of the head and left shoulder. Auscultation of the thorax disclosed a slight diastolic heart murmur.

The hemogram revealed: hemoglobin, 13.8 grams/100 ml.; packed cell volume, 30%; erythrocyte sedimentation rate, 26 mm./60 minutes, compared to an expected value of 25 mm./60 minutes; leukocytes, 15,950/cu. mm.; segmented neutrophils, 73%; lymphocytes, 26%; monocytes, 1%. The urinalysis yielded no abnormal findings.

Euthanasia and necropsy were performed on February 2, 1964. Examination of the integumentary system revealed a firm pedunculated pink mass arising from the left upper gingiva posterior to the canine tooth, a mass 4.5 x 5.0 x 1.0 cm. on the lateral surface of the pinna of the right ear, a round, ulcerated, firm growth 1.0 cm. in diameter ventral to the anus (see Tumor 23), and 7 nodules ranging from 0.2 to 1.0 cm. in diameter located in the skin of the head, right shoulder, left shoulder, and right thoracic wall (see Tumor 33). The right intermediate lobe of the lung contained a grossly necrotic-appearing tissue mass 3.0 x 3.0 x 2.5 cm. The left diaphragmatic lobe of the lung contained a firm, white, round mass 2.0 cm. in diameter (see Tumor 28). The surface of the kidneys was rough and marked by scars

of healed infarcts. The renal capsule peeled off with difficulty. The pancreas had a nodular appearance. The liver had an irregularly nodular appearance (see Tumor 10). The gallbladder contained a firm white rough mass measuring 1.5 x 1.5 x 2.0 cm. within its wall (see Tumor 65).

Histopathologic examination of the lesion from the mouth revealed that it was fibroplastic inflammatory tissue with an ulcerated surface. The right retropharyngeal and right submandibular lymph nodes revealed lymphoid hyperplasia. Hemosiderosis was also noted in the right submandibular lymph node. Sections of the kidney revealed mild, diffuse, chronic, interstitial nephritis with mineralized streaks extending into the medulla. Sections of the neoplasm from the left ear revealed a mass covered by ulcerated stratified squamous epithelium. The mass was composed predominantly of basal cells having oval nuclei with the long axes perpendicular to the basement membrane. The nuclei were rather vesicular, each with 1 or more prominent nucleoli. The cytoplasm was scanty and eosinophilic. In many areas, these cells were arranged in 2 parallel rows, in others they formed round, duct-like spaces in which the epithelium matured to a single layer or 2 of squamous epithelium. Some of these structures had empty lumina, while the lumina of others contained a keratin-like substance. On the periphery of the mass were cells with lipid-filled cytoplasm, arranged in small to large clusters. The neoplasm was diagnosed as a basal-cell tumor with differentiation to sebaceous cells and hair follicles. Enzymatic activity in the serum of this dog, and in this neoplasm, are recorded in TABLE 6.

TABLE 6. Enzymatic activities relating to Tumor 6.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	1.06	72		157		27		94.5	
Basal Cell Tumor	5.2	241	11.5	93	4	102	4.8	4350.7	207.3

Tumor 7 was obtained from a 13-year-old mixed-breed female dog. This dog was presented on October 21, 1963, with a history of having undergone parturition 7 months earlier. Following parturition, tumors had developed in the posterior left and last right mammary glands. The dog's general health was reported to be good, except for urinary incontinence. Other abnormalities revealed by physical examination were limited to 2 pigmented nodules, 1 located in the skin at the inner canthus of each eye. Hemogram revealed: hemoglobin 15.55 gm./100 ml.; packed cell volume, 45%; erythrocyte sedimentation rate, 23 mm./60 minutes, compared to an expected value of 5 mm./60 minutes; leukocytes, 7000/cu. mm.; segmented neutrophils, 89%; lymphocytes, 10%; eosinophils, 1%.

On October 23, 1963, an ovariohysterectomy was performed, and third, fourth, and fifth mammary glands were excised, as were the lesions at the inner canthi of the eyes (see Tumor 88). The excised mammary tissue measured 22.0 x 6.0 x 6.0 cm. It was hard and tan-colored and included nipples of the third, fourth, and fifth mammary glands. It contained a cystic cavity, 7.0 cm. in its greatest diameter, and filled with fluid. Microscopic examination revealed that the section was covered on 1 surface by stratified squamous epithelium, thin and lightly keratinized with sparse adnexa. The neoplasm was a highly

cellular mass which enclosed several clumps of mammary alveoli lined by a single layer of cuboidal epithelium and filled with eosinophilic secretion. The bulk of the mass was composed of anaplastic, pleomorphic, primitive-looking cells. The nuclei varied from vesicular to hyperchromatic. The vesicular nuclei contained prominent nucleoli. The cytoplasm was scanty and eosinophilic with indistinct borders. These cells were usually randomly arranged, but occasionally appeared to form ducts. The pre-existing mammary ducts present in the underlying tissue were filled with similar tumor cells, and centrally with necrotic detritus. Masses of the same type of cells filled the lumina of blood and lymphatic vessels present. This lesion was diagnosed as mammary duct carcinoma. The left ovary measured 2.0 x 1.0 x 0.6 cm. Microscopic examination revealed that most of the section consisted of normal functional ovarian tissue with numerous follicles in various stages of development and numerous corpora lutea. On the surface of the ovary was a cystic space lined by simple squamous epithelium and surrounded by delicate fibrous connective tissue. This structure was identified as a parovarian cyst. Within the ovary, in an atretic corpus luteum, was a group of cells loosely and irregularly arranged singly and in small clumps and nests. These cells had large polyhedral nuclei with prominent nucleoli and scanty cytoplasm. This lesion was identified as a metastasis of the mammary duct carcinoma. The left ovary measured 0.7 x 0.6 x 1.0 cm. and was histologically normal.

Following surgery, the dog made a good recovery. On December 7, 1963, the dog was returned for evaluation and further therapy. At this time she was weak and partially anorectic. She was unable to climb stairs and did not use the right hind leg except for balance. Thoracic

radiographs revealed scattered pulmonary metastatic nodules. Pelvic radiographs revealed demineralization of the femoral head, separation of the femoral capital epiphysis, and expansion of the femoral neck. Hematologic values were: hemoglobin, 14.15 gm./100 ml.; packed cell volume, 44%; erythrocyte sedimentation rate, 32 mm./60 minutes, compared with an expected value of 6 mm./60 minutes; leukocytes, 9200/cu. mm.; segmented neutrophils, 84%; nonsegmented neutrophils, 3%; lymphocytes, 10%; eosinophils, 1%; and monocytes, 1%.

Euthanasia and necropsy were performed on December 13, 1963. Gross lesions observed at necropsy were: a gray-white, firm mass which measured 4 x 3 x 2 cm. in the right mid flank between the internal and external oblique muscles; a mass in the femoral neck measured 2 x 2 x 2 cm. and was related to a comminuted pathologic fracture; 2 masses in the wall of the right ventricle extended from the epicardium to the endocardium, measuring 1.5 x 1.5 x 1.0 and 1.5 x 1.0 x 0.7 cm., respectively; a mass in the interventricular septum measured 2.0 x 0.7 x 0.7 cm.; a mass in the bronchial lymph node was 3.0 x 1.5 x 1.0 cm.; multifocal masses ranging from 0.1 to 0.5 cm. in diameter were scattered throughout the lung; the left axillary lymph node contained a mass measuring 1.5 x 0.5 x 1.0 cm.; masses measuring 3.0 x 1.5 x 1.5 cm. were present in both iliac lymph nodes; the right thyroid measured 3.0 x 1.0 x 0.5 cm., while the left measured 2.0 x 2.7 x 0.5 cm.; 1 of the right parathyroids measured 0.5 x 0.7 x 1.0 cm.; all three of these structures were firm and gray-white, suggesting neoplastic infiltration; the right fourth and fifth mammary glands grossly appeared neoplastic and jointly measured 8.5 x 4.0 x 6.5 cm.; 2 masses were present in the wall of the vagina, 1 in the dorsolateral aspect measuring 3.0 x 3.0 x 3.0 cm. and the 1 in the ventral wall measuring 1.5 x 1.5 x 1.0 cm.

(see Tumor 66). Examination of the liver disclosed a pattern of large irregular nodules, suggesting nodular regeneration. There was cortical hyperplasia of the adrenal glands. The kidneys were shrunken and fibrotic. Microscopically, the masses described above in the lymph nodes, abdominal musculature, myocardium, lung, thyroid, parathyroid, femur, and mammary gland had, in all sections, a structure similar to the mammary duct carcinoma described. Individual sections varied only in the degree of duct formation. All tumors were invasive and destroyed the adjacent tissue. Histopathologic examination of adrenal gland and liver confirmed the gross diagnoses. Enzymatic activities in the serum and tumor extract of this dog are recorded in TABLE 7.

TABLE 7. Enzymatic activities relating to Tumor 7.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	10/23	0.64	29		116		13		12.9	
Tumor	10/23	1.9	2704	87	14538	468	182	5.88	75.2	2.4
Serum	10/26	1.82	175				16		21.5	
Serum	12/7	0.64	54		74		20		7.0	

Tumor 8 was obtained from a 5-year-old spayed female Great Dane. This dog was presented on February 9, 1964, with lesions grossly considered neoplastic on the right upper lip, below the right ear, on the fourth digit of the left hind leg, and involving the third mammary gland on the left side. On February 10, the mammary neoplasm and the lesion from the digit were excised. The mammary neoplasm was a mass 8 x 7 x 3 cm., tan in color, and firm in consistency. Microscopically it

consisted of highly cellular solid aggregates of cells surrounded by stroma. Many of these aggregates were within mammary ducts, arising from the lining epithelium and filling the duct. The cells had hyperchromatic round or oval nuclei, nucleoli of moderate prominence, and eosinophilic cytoplasm without distinct borders. Mitoses were moderately numerous. Dilated lymphatics were present, some containing tumor emboli. A diagnosis of mammary duct carcinoma was made. Histopathologic examination of the lesion excised from the foot revealed chronic ulcerative dermatitis with fibrosis of the subcutis. On February 25, the lesion beneath the ear was excised (see Tumor 58). At subsequent surgery, the left axillary lymph node, left popliteal lymph node, and the lesion from the left lower eyelid were excised. Histopathologic examination revealed metastasis of the mammary duct carcinoma to the left axillary lymph node, lymphoid hyperplasia of the left popliteal lymph node, and fibrosis of the skin beneath the eye. Serum and tumor extract enzyme activities for this case are given in TABLE 8.

TABLE 8. Enzymatic activities relating to Tumor 8.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/10	0.60	34	106	14	49.4
Tumor	3/10		72	11343	475	123.5
Serum	2/14	0.45	29	97	15	57.5

Tumor 9 was obtained from a 12-year-old female Cocker Spaniel. This dog was presented on January 20, 1964, for surgical removal of mammary tumors and the enlarged right inguinal lymph node and for ovariohysterectomy. Physical examination failed to reveal additional

abnormalities. Surgery was performed on January 21. The right third, fourth, and fifth mammary glands were removed en masse. The fourth gland contained a gray, firm nodule 6.5 x 4.0 x 3.5 cm. in size, without evidence of capsule. Both ovaries were enlarged (see Tumor 37), and the cavity of the uterus contained a creamy yellow exudate. Microscopically the mammary neoplasm was seen to consist predominantly of ductular structures 1 or more layers thick formed by the neoplastic epithelial cells. The attempts at duct formation were surrounded by collagenous connective tissue containing some foci of lymphocytes. Some of the ducts were filled with necrotic detritus and neutrophils. The neoplastic epithelial cells were rather uniform, with large oval nuclei, small nucleoli, and a moderate amount of eosinophilic cytoplasm. Focally, proliferation of the tumor cells into the duct lumen was evident. Additional sections revealed the multifocal nature of this neoplasm. Areas of myoepithelial proliferation, and a single focus of papillary adenocarcinoma, were also present. The mammary neoplasm was diagnosed as a mammary duct carcinoma. Histologic examination of the excised inguinal lymph node revealed a depletion of lymphocytes and a proliferation of reticulum cells. Several emboli of tumor cells were present in the marginal sinus. These nests were composed of cells with irregular pale nuclei and abundant eosinophilic cytoplasm. There was no evidence of attachment of further proliferation of these emboli. Evaluation 2 months after surgery failed to reveal evidence of recurrence or metastasis. Serum enzyme activities are tabulated in TABLE 9.

TABLE 9. Enzymatic activities relating to Tumor 9.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	1/21	0.80	125	241	23	9.1
Serum	1/23	0.26	39	93	11	12.9
Serum	3/26	1.72	29	255	15	8.6

Tumor 10 was obtained from the same dog as Tumor 6, q.v. for the history and physical findings. A solitary pale nodule on the surface of the liver could be distinguished from the remaining nodular hepatic parenchyma. Histopathologic examination of this nodule revealed a mass covered by Glisson's capsule. Microscopically the mass was composed of fronds having a connective tissue stroma and covered by a single layer of mature-appearing columnar epithelial cells, resembling those lining the bile ducts. These cells had normally polarized, basally located hyperchromatic nuclei and abundant eosinophilic cytoplasm. This lesion was diagnosed as a bile duct papilloma. Enzymatic activity in the serum and this neoplasm are recorded in TABLE 10.

TABLE 10. Enzymatic activities relating to Tumor 10.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	1.06	72		157		27		94.5	
Tumor	6.0	2073	57	93	3	295	8	4134.9	114.4

Tumor 11 was obtained from a Chihuahua-Manchester terrier cross female dog, 15 years of age. This dog was presented on January 30, 1964, with a history of hematuria of 6 months' duration. Microbiologic examination of the urine yielded a pure culture of Proteus. A cytologic study of the urine revealed neoplastic epithelial cells with hyperchromatic nuclei and large nucleoli. Elongated cell forms with basally located nuclei were present. A cytologic diagnosis of transitional cell carcinoma was made. At laparotomy, a dorsal segment of the bladder wall was excised. The excised tissue was 1.1 x 1.7 x 0.9 cm., irregular, gray, and firm. Microscopic examination revealed a mass of smooth muscle covered on 1 surface by proliferating neoplastic epithelium. The neoplasm was arranged in papillary convolutions. It was composed of primitive, though rather uniform, cells with large nuclei, prominent nucleoli, and eosinophilic cytoplasm. Some of the cells were vacuolated. Mitoses, including atypical forms, were numerous. Many lymphatics contained tumor emboli. A few foci of lymphocytes and superficial hemorrhage were noted. A diagnosis of transitional cell carcinoma was made. Enzymatic activity values, as determined in this tumor and in the serum of this dog, are recorded in TABLE 11.

TABLE 11. Enzymatic activities relating to Tumor 11.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.48	48		139		14		12.9	
Tumor	8.0	35668	519	11899	173	58	0.9	145.0	2.1

Tumor 12 was obtained from a Collie-type female mongrel dog 13 years of age. This dog was presented for veterinary attention in 1956 because of a tumor on the left foreleg. Several attempts were made to resect the tumor, but each was followed by recurrence. In 1959, the attending veterinarian submitted a section of this tumor for histopathologic examination (see Tumor 63). On July 13, 1962, the dog was referred to the Ohio State University Veterinary Clinic for recurrence of a mass on the posterior aspect of the left foreleg halfway between the carpus and the radio-humeral joint. At this time, pulmonary radiographs revealed a 3-cm. nodule in the left diaphragmatic lobe. Physical examination disclosed a soft, fluctuating subcutaneous mass in the ventral thoracic area which, largely on the basis of its consistency, was considered to be a lipoma. A biopsy of the lesion involving the left foreleg was performed, following which x-ray irradiation therapy was applied to the left foreleg. Doses totaling 3500 r were administered between July 13 and August 17, 1962. On September 29, 1962, another biopsy of the left foreleg was performed. The left foreleg was amputated at the humero-scapular joint on October 13, 1962. At the same time, the soft subcutaneous mass described above was excised. Histopathologic examination confirmed the diagnosis of lipoma. At this time, radiographs revealed some increase in the size of the pulmonary nodule. On January 5, 1963, a 2-cm. nodule was palpated in the posterior costo-chondral region. On December 19, 1963, radiographs disclosed that the pulmonary nodule described above was now 5 cm. in diameter and that a second nodule, 2.5 cm. in diameter, was present in the mediastinum or the right apical area. An enlargement of the left superior cervical region was also noted, and was thought to involve the

salivary gland. On March 21, 1964, the left mandibular salivary gland and left mandibular lymph node were excised. The mandibular salivary gland was rounded, brown, and firm, measuring 3.0 x 2.0 x 1.5 cm. A portion appeared grossly normal, while 1 border appeared rough and irregular. Histopathologic examination revealed that a portion of the tissue consisted of normal mucous salivary gland. Adjacent to the normal tissue was a duct within which there was a neoplastic proliferation of the neoplasm. Near the origin, the cells were still fairly mature, and attempted to form ducts. Further removed, the cells lay in lobules with only indistinct attempts at organoid formation. The cells retained their cuboidal appearance with moderate-sized nuclei containing large coarse chromatin granules. The cytoplasm was scanty and violet-staining or clear. There were areas of necrosis and, at 1 border, an area of mineralization: this neoplasm was diagnosed as a salivary-duct carcinoma.

On November 22, 1964, the dog was presented with the owner's complaint of a staggering gait and in markedly deteriorated condition: euthanasia was performed. Necropsy findings are described with Tumor 63. No recurrence or metastasis of the salivary duct carcinoma were found. Enzyme activities in the serum of this dog and in the extract of the salivary duct carcinoma are recorded in TABLE 12.

TABLE 12. Enzymatic activities relating to Tumor 12.

Specimen	Date	Hb	PHI	LAP	LAP Sp.A.	Alk. Ptase.
Serum	3/20/64	0.8	125	41		50.5
Tumor	3/21/64	1.4		473	15.8	
Serum	3/27/64		130			78.9

Tumor 13 was obtained from a 7-year-old spayed female Boxer. This dog was presented on January 23, 1964, for swelling of the proximal third of the right humerus, which had been present for 2 weeks. The right foreleg was now carried in a semiflexed condition. There was no history of trauma. A hemogram revealed: hemoglobin, 18.75 gm./100 ml.; packed cell volume, 57%; leukocytes, 7,600/cu. mm.; segmented neutrophils, 75%; lymphocytes, 25%. A biopsy of the lesion made at this time failed to yield a tissue specimen adequate for diagnosis. On February 20, 1964, amputation at the humero-scapular joint and resection of the distal 5 cm. of the scapula were performed. Grossly, the proximal end of the humerus was expanded into a 10 x 12 x 16-cm., irregular mass. On microscopic examination, sections revealed a neoplasm involving the underlying bone, bone marrow, and skeletal muscle. Scattered randomly about, in many areas eroding the bone, were areas of tumor tissue composed of highly anaplastic pleomorphic cells having large, hyperchromatic nuclei and scanty irregular eosinophilic cytoplasm. Tumor giant cells were scattered throughout. The cells were arranged in rows, but no further pattern was evident, nor was there evidence of any line of cellular maturation or any cellular secretion. Mitoses, including irregular forms, were numerous. The underlying bone showed foci of necrosis. This tumor was diagnosed an undifferentiated carcinoma. Four months postoperatively, there was no evidence of metastasis, recurrence, or indication of the site of origin. Enzymatic activity values from the serum of this dog and from the tumor extract are recorded in TABLE 13.

TABLE 13. Enzymatic activities relating to Tumor 13.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	1/23	0.72	63		79		42		28.5	
Serum	2/11	0.72	96		139		32		33.8	
Serum	2/19						47		67.1	
Tumor	2/20	3.7	22654	515	45837	1042			995.9	21.5
Serum	2/28	1.27	362		199		36		40.3	

Tumor 14 was obtained from a 12-year-old male Boxer. This dog was presented on December 4, 1963, with a history of polydypsia, polyuria, and partial blindness, of 3 months' duration. The owner noted that the dog had had intermittent anorexia, coughing, retching, and posterior paralysis. On admission, the dog was weak, depressed and emaciated, but responded to stimuli. Ophthalmoscopic examination revealed distended fundic veins. Urinalysis showed the urine to be cloudy, with specific gravity of 1.011, and moderately positive for occult blood. Leukocytes were very numerous. The hemogram revealed: hemoglobin, 13.0 gm./100 ml.; packed cell volume, 38%; erythrocyte sedimentation rate, 29 mm./60 minutes, compared to an expected value of 12 mm./60 minutes; leukocytes, 46,500/cu. mm.; segmented neutrophils, 88%; nonsegmented neutrophils, 5%; lymphocytes, 6%; and monocytes, 1%. The blood urea nitrogen concentration was 38.5 mg./100 ml. Electroencephalographic examination revealed changes interpreted as indicative of a deep-seated space-occupying mass, possibly a tumor of the thalamus. Neurologic examination revealed exaggerated toe-pinch reflex and crossed extensor reflex, anisocoria, and absence of patellar reflexes

and left pupillary reflex. Examination of cerebrospinal fluid revealed: erythrocytes, 3160/cu. mm., of which 97.5% were crenated; 20 lymphocytes per cu. mm.; protein, 35 mg./100 ml.; glucose, 102 mg./100 ml.; and chlorides, 148 meq./l. On December 6 the dog became convulsant, had episodes of howling, and lapsed into coma. Death followed on December 8.

At necropsy, gross examination of the heart disclosed dilatation and bilateral endocarditis of the atrioventricular valves. The kidneys had infarcts of various duration. The bladder contained pus-laden urine, and there were abscesses in the prostate. Gross examination of the brain revealed a mass in the medial wall of the left ventricle, 2.0 x 1.5 x 1.5 cm. in size, gray to white, with central necrosis. The mass was firmer than the surrounding brain tissue, and the left ventricle was dilated, with asymmetry obvious upon frontal section.

Microscopic examination of the liver revealed congestion and periportal fibrosis and hyalinization. Sections of the gray matter of the diencephalon, thalamus, hippocampus, and adjacent areas of cerebral cortex revealed vacuolation and neuronal degeneration. The number of glial cells appeared to be increased diffusely. A focus of necrosis in the diencephalon, suggesting an infarct, was surrounded by a pleomorphic aggregate of small cells with hyperchromatic nuclei. Much of the tissue in all areas indicated above was diffusely infiltrated by small, pleomorphic cells with hyperchromatic nuclei and scanty eosinophilic cytoplasm. Multinucleated tumor cells were also present. The tumor was widely invasive, destroying the underlying architecture. In the cerebral cortex, the cells appeared better differentiated into neoplastic astrocytes. Here, too, many tumor giant cells were present. In sections of cerebral cortex more remote to the tumor mass, similar

aggregates of pleomorphic neoplastic cells were present perivascularly. This neoplasm was identified as a glioblastoma multiforme, also designated astrocytoma, grade 4. Enzymatic activity, as determined in the serum of this dog and in the tumor, are recorded in TABLE 14.

TABLE 14. Enzymatic activities relating to Tumor 14.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	0.64	101		505		35		73.0	
Tumor	1.9	3133	285	11667	1060	35	3.2	80.5	7.5

Tumor 15 was obtained from an 8-year-old female English Setter. This dog was presented on September 3, 1963, with the history that she had undergone parturition 1½ years earlier and had been anestrous since. The right posterior mammary glands grossly appeared neoplastic. A hemogram revealed: hemoglobin, 13.0 gm./100 ml.; packed cell volume, 39%; leukocytes, 21,000/cu. mm.; segmented neutrophils, 71%; nonsegmented neutrophils, 1%; lymphocytes, 25%; eosinophils, 2%; and monocytes, 1%. An ovariohysterectomy was performed, and the neoplastic mass in the mammary glands excised. Grossly, the excised mammary gland measured 5 x 5 x 7 cm. The right ovary contained a follicular cyst and a cystic cavity. The left ovary contained a follicular cyst. Microscopic examination of the myometrium revealed dilated myometrial glands. The right ovary contained a small neoplasm composed of acini lined by a single layer of regular cuboidal epithelial cells, having clear cytoplasm. This was diagnosed as a serous ovarian cystadenoma. The left

ovary was microscopically normal. The mammary neoplasm was covered by skin with adnexa. The glandular mammary elements showed neoplastic proliferation into islets of alveoli, many of which contained an eosinophilic secretion. Focally there was metaplasia of the connective tissue to mucoid, chondroid, and osteoid connective tissue and to bone. In several small areas the epithelial component was more anaplastic and arranged in a papillary to villous pattern several cells thick on a connective tissue base. This neoplasm was diagnosed as a mixed mammary tumor with areas of mammary adenocarcinoma.

On October 14, the dog was again presented for excision of additional neoplasms from the right fourth and fifth mammary glands. At this time the hemogram revealed: hemoglobin, 14.6 gm./100 ml.; packed cell volume, 44%; leukocytes, 13,200/cu. mm.; segmented neutrophils, 60%; nonsegmented neutrophils, 4%; lymphocytes, 30%; eosinophils, 5%; and monocytes, 1%. At this time, complete excision of the fourth and fifth mammary glands from the right side was performed. The gross specimen submitted consisted of the fourth and fifth mammary glands, excised en masse. It measured 17 x 8 x 1.5 cm., was tan in color, firm, and encapsulated. Microscopic examination revealed a neoplasm very similar to that described above. The epithelial component was arranged in a more papillary, less alveolar pattern, and was focally highly cellular and slightly anaplastic. One section contained a large section of chondroid connective tissue and bone. The diagnosis of this neoplasm was also mixed mammary tumor containing areas of mammary adenocarcinoma. The enzymatic activities, as determined in the serum of this dog and in the extract of the tumor, are recorded in TABLE 15.

TABLE 15. Enzymatic activities relating to Tumor 15.

Specimen	Date	Hb	LDH	LDH Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	10/14	1.94	170		23		18.8	
Tumor	10/14	0	833	40	306	15	473	20.4
Serum	10/16		51		18		27.9	

Tumor 16 was obtained from a 12-year-old female Beagle. This dog was presented on March 5, 1963, for an inguinal herniorrhaphy, ovariohysterectomy, and excision of mammary neoplasms. Hemogram at that time disclosed no abnormalities. Surgery was performed as indicated. Examination of the excised uterus revealed endometrial cysts. The ovaries contained multiple follicular cysts. Histopathologic diagnosis of the mammary neoplasm was mixed mammary tumor.

The dog was again presented on December 12, 1963, because of several mammary tumors which, the owner indicated, were increasing in size perceptibly. One of these was ulcerated. Hemogram at this time indicated: hemoglobin, 19.4 gm./100 ml.; packed cell volume, 63%; leukocytes, 9500/cu. mm.; segmented neutrophils, 82%; nonsegmented neutrophils, 1%; lymphocytes, 12%; eosinophils, 1%; and monocytes, 4%. The third, fourth, and fifth left mammary glands and the third right mammary gland were excised together with the left inguinal lymph node. The excised mammary neoplasms measured 3.5 x 4.0 x 1.8, 3.5 x 2.0 x 0.5, 3.0 x 2.5 x 3.5, 4.0 x 0.5 x 3.0, and 2.5 x 2.5 x 1.5 cm., respectively. The third left mammary gland was cystic, and the fifth left mammary gland was ulcerated. All neoplasms were firm and tan and appeared encapsulated. Microscopically, all sections of the

left mammary chain were essentially similar. They disclosed a glandular appearing mass covered by skin with normal adnexa. Peripherally, normal mammary alveoli and ducts were present. Beneath these were encapsulated glandular masses having a regular papillary structure, interspersed with dense collagen. In the centers of these alveoli was an eosinophilic secretion. Focally, the interstitium showed calcification. Beneath these nodules was a highly cellular, highly vascular neoplasm of essentially similar pattern, but with papillary aggregates several cells thick, and with some of the acinar lumina filled with cells similar to those lining it. Within the neoplasm were foci of neutrophils, lymphocytes, and macrophages. In some areas the mesenchymal component showed metaplasia to cartilage and myxoid tissue. Focally, the surface was ulcerated with neutrophilia. The sections of the third right mammary gland were somewhat similar, except that they did not show any criteria of malignancy. The left inguinal lymph node revealed lymphoid hyperplasia, and phagocytosis of hemosiderin. Within the marginal sinus was a single metastatic focus of mammary adenocarcinoma. This neoplasm was diagnosed as a mixed mammary tumor containing areas of mammary adenocarcinoma. Enzymatic activities, as determined in the serum of this dog and in the extract of the neoplasm, are recorded in TABLE 16.

TABLE 16. Enzymatic activities relating to Tumor 16.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	12/12	4.33	337		671		15		8.1	
Tumor	12/12	3.3	482	9	35882	644	116	2.2	247.0	4.6
Serum	12/18	0.93	53		120		19		30.6	
Serum	12/23	1.93	145		375		19		15.0	

Tumor 17 was obtained from a female English Bulldog, 8 years of age. This dog was presented on January 24, 1964, because of dermatitis which had been present for the past 5 years, and mammary tumors which were palpable in second, third, fourth, and fifth mammary glands and the fourth and fifth left mammary glands. On January 27, an ovario-hysterectomy was performed, and the fourth and fifth right mammary glands, together with the right inguinal lymph node, were excised. On gross examination, the uterus and ovaries appeared normal. The mammary glands, excised en masse, measured 15.0 x 5.8 x 2.8 cm. Between the nipples of the fourth and fifth glands was a firm white mass measuring 1.8 x 1.8 x 1.7 cm. The remaining tissue was irregularly nodular.

Microscopic examination of both ovaries revealed well developed, functional-appearing ovaries containing large corpora lutea. A section of the uterus revealed a hyperplastic endometrium. Sections of the mammary glands revealed essentially normal mammary tissue with functional alveoli filled with eosinophilic secretion. This degree of mammary nodularity and function was regarded as compatible with the stage of metestrus, as evidenced by ovarian and uterine morphology. The nodule grossly described above was, to a great degree, composed of

proliferating fibrous connective tissue and myoepithelial cells. Focally, the bluish tinctorial characteristic of chondroitin sulfate suggested cartilagenous metaplasia. Islands of mammary alveolar tissue showed evidence of neoplastic proliferation characterized by increased cellularity, piling up, and incomplete alveoli. No tumor cells were detected in sections of the inguinal lymph node. This neoplasm was diagnosed as a mixed mammary tumor. Enzymatic activities, as determined in the serum of this dog and in the neoplasm, are recorded in TABLE 17.

TABLE 17. Enzymatic activities relating to Tumor 17.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	1/24	1.36	530		111		28		21.5	
Tumor	1/27	1.5	482	10	9353	187	363	7.3	5316.3	106.3
Serum	1/31	0.26	31		15		20		40.8	

Tumor 18 was obtained from an 11-year-old female Pointer. This dog was presented for veterinary attention on October 19, 1963, because of a swollen left forefoot and a mammary tumor. Conservative treatment failed to produce improvement, and the dog was referred to the Ohio State University Veterinary Clinic on November 15, 1963. Physical examination on admission revealed a 40-pound bitch, well nourished and well developed. The left foreleg was swollen to about twice the normal size from the carpus distally. The swollen area felt hot, and palpation elicited evidence of pain. The left supraspinatus and infraspinatus muscles were atrophic. Multiple mammary tumors were present.

Radiographs of the thorax revealed multiple large nodules in the lungs. Radiographs of the foot revealed areas of osseous proliferation and areas of osteolysis. Hematologic findings are recorded in TABLE 18.

TABLE 18. Host hematologic data relating to Tumor 18.

Date	Hb	PCV (%)	Leuk./ cu.mm.	Seg.Neut. (%)	Nonseg. Neut.(%)	Lymph. (%)	Eosin. (%)	Mono. (%)
11/15	11.95	35	16,000	74	1	17	3	3
11/18	11.25	34	10,750	81	3	13	2	1
11/20	12.25	38	18,600	83	4	10	2	1

Euthanasia and necropsy were performed on November 20, 1963. Examination of the integumentary system revealed a neoplasm measuring 2.0 x 2.0 x 2.5 cm. in the second left mammary gland and 2 neoplasms measuring 2.0 x 1.7 x 1.7 and 1.5 x 1.5 x 1.5 cm., respectively, in the fifth left mammary gland. These tumors were hard, but not gritty, and grayish-white with central necrosis. They appeared grossly to be well encapsulated. The bones of the metacarpus were soft and were surrounded by both bony and soft-tissue proliferation. A cyst 1.5 cm. in diameter was located over the metacarpus. Post-mortem radiographs of the left forefoot revealed osteolysis of the metacarpal bones with pathologic fractures and subperiosteal proliferation (see Tumor 67). The dog's anal glands were swollen, with a purulent exudate. Multiple nodules were found throughout the lungs; these varied from 0.1 to 5 cm. in diameter. They were white to gray in color, firm, and grossly resembled the mammary tumor. The right cardiac lobe was almost entirely replaced by neoplastic tissue, while the other lobes were less extensively involved (Tumor 30). There were 2 healed infarcts in the cortex of the right kidney. A solitary nodule, 1 cm. in diameter, was present in the liver.

Microscopic examination of the kidneys revealed congestion, chronic interstitial nephritis, and infarcts. The liver contained regenerative nodules. Some of the portal veins contained tumor emboli. These emboli were morphologically identical to the neoplasm described in this study as Tumor 30, q.v. The tumor in the second left mammary gland consisted of lobules of cells lying between fibrous septa. Areas of necrosis were evident. The individual cells were pleomorphic with the cytoplasm containing 1 or more vacuoles and the hyperchromatic nuclei eccentrically located. In some areas there was neoplastic intraductular proliferation. There was no distinct alveolar formation by the tumor cells. This tumor was diagnosed as a mammary adenocarcinoma. Sections of the remaining mammary tumors revealed a neoplastic admixture of epithelial and connective tissue. The epithelial component consisted of apocrine gland alveoli presenting a rather mature appearance. These were composed of a single or double layer of cells lined up in circular arrangement inward from a basement membrane, with apices protruding into the lumina. The lumina contained varying amounts of eosinophilic secretion. The connective tissue component consisted of fibrous, myxoid, and chondroid areas. Sections of the axillary lymph node revealed congestion and lymphoid hyperplasia. These neoplasms were diagnosed as mixed mammary tumors. Enzymatic activities, as determined in the serum of this dog and in this neoplasm, are recorded in TABLE 19.

TABLE 19. Enzymatic activities relating to Tumor 18.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.45	22		97		16		16.1	
Tumor	1.1	3509	251	4491	321	58	4.1	537.0	38.1

Tumor 19 was obtained from a 13-year-old spayed female Poodle. This dog, which had had an epidermal inclusion cyst excised 12 years earlier, was presented for tumors in the first left and third right mammary glands which had been noted to be growing slowly over the past 18 months. The masses were excised on February 25, 1964. The excised left mammary neoplasm measured 4.0 x 4.0 x 2.4 cm., was irregularly round, cream-colored, and bony. The right mammary neoplasm measured 2.1 x 1.8 x 1.0 cm., was round, encapsulated, yellow, and firm. Microscopically, the former consisted predominantly of mature-appearing bone, cartilage, and fibrous connective tissue. There were occasional duct-like structures lined with stratified low cuboidal epithelium. Some of these structures were filled with homogeneous eosinophilic material or granular cell detritus. The right mammary neoplasm was covered by intact epithelium. Some apparently normal mammary alveoli were present. Most of the tumor was composed of benign glandular epithelial cells attempting to form alveoli between dense fibrous stroma and connective tissue components of mature-appearing bone and cartilage. In 1 area, the cells lining several ducts showed anaplasia, large nuclei with prominent nucleoli, some necrosis, and an irregular pattern of proliferation. A lymphatic vessel present within an active lymph nodule contained a tumor embolus. The former tumor was diagnosed as a mixed mammary tumor, and the latter a mammary duct carcinoma arising from a mixed mammary tumor. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 20.

TABLE 20. Enzymatic activities relating to Tumor 19.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/25	1.32		162	19	12.4
Serum	3/1	0.56	275	213	22	29.0

Tumor 20 was obtained from a 6½-year-old male Blue Tick Hound. This dog was presented for veterinary attention 8 months prior to admission for a mass measuring about 3.5 x 3.5 x 5 cm. located on the lateral surface of the prepuce. This mass was excised, but recurred in 6 months. At that time, the mass, 8 cm. in diameter, was again excised. On February 24, 1964, the dog was referred to the Ohio State University Veterinary Clinic with a large ulcerated neoplasm 10.0 x 8.0 x 4.5 cm. in the perianal area. The scrotum was edematous. Hematologic examination at this time revealed: hemoglobin, 6.4 gm./100 ml.; erythrocytes, 2.5 million/cu. mm.; packed cell volume, 20%; erythrocyte sedimentation rate, 42 mm./60 minutes compared to an expected value of 45 mm./60 minutes; leukocytes, 18,650/cu. mm.; segmented neutrophils, 87%; nonsegmented neutrophils, 1%; lymphocytes, 11%; and eosinophils, 1%. A biopsy was performed (see Tumor 57), together with bilateral orchiectomy. On microscopic examination, 1 testis was found to contain a nodule 0.5 cm. in diameter, consisting of proliferating interstitial cells, surrounding and obliterating the tubules. This was diagnosed as an interstitial cell adenoma. The other testis was histologically normal. Six days after surgery, euthanasia and necropsy were performed. Significant necropsy findings were limited to the neoplasm in the perianal region (Tumor 57, q.v.). The serum enzyme activities of this dog are recorded in TABLE 21.

TABLE 21. Enzymatic activities relating to Tumor 20.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/24	0.48	188	389	22	9.7
Serum	3/3	1.01	400	569	19	1.1

Tumor 21 was obtained from a male Newfoundland dog 8 years of age. This dog had had surgery for anal gland disease 3 months prior to admission, and was presented on March 25, 1964, because of nodular enlargements around the anus. Two perianal masses were excised (see Tumor 24) and a bilateral orchiectomy performed. The right testis was 6.0 x 3.5 x 3.0 cm., with a dark brown nodule 0.5 cm. in diameter located beneath the capsule. The left testis measured 6.0 x 4.0 x 3.0 cm. and was grossly normal. Microscopic examination of the right testis revealed an excessive clear space between the tubules. The tubules evinced minimal spermatogenesis. Many of the tubules contained neutrophils. Along 1 capsular surface was a nodule composed of cells with small, round, hyperchromatic, eccentrically located nuclei and eosinophilic cytoplasm. These cells appeared to be arranged in nests and tubules. This nodule was diagnosed as an interstitial cell adenoma. The right testis showed aberrations of the tubular architecture similar to that described above. Enzymatic activities determined in the serum of this dog prior to surgery were phosphohexose isomerase 125 international units and alkaline phosphatase 5.9 international units per liter of serum.

Tumor 22 was obtained from a male Cocker Spaniel 8 years of age. This dog was presented on November 5, 1963, because of an ulcerated mass which had been present for about 6 months at the base of the tail. Physical examination disclosed no further abnormalities. Hemogram revealed: hemoglobin, 10.95 gm./100 ml.; packed cell volume, 34%; erythrocyte sedimentation rate, 36 mm./60 minutes, contrasted with an expected value of 17 mm./60 minutes; leukocytes, 13,300/cu. mm.; segmented neutrophils, 77%; nonsegmented neutrophils, 2%; lymphocytes,

17%; and eosinophils, 4%. Excision of the neoplasm and bilateral orchiectomy were followed by radiation therapy, in which total doses of 3000 r were administered to the tumor site.

The excised mass measured 5 x 5 x 7 cm., was rounded, firm, dark in color, and had an ulcerated surface. On gross and microscopic examination the testes and epididymides revealed no abnormalities. Sections of the tumor revealed a mass partially covered by skin with sparse adnexa. The mass was composed of lobules resembling holocrine glands separated by fibrous stroma showing evidence of active proliferation. Cytologically, the tumor consisted of an admixture of a hepatoid cell type having a rounded vesicular nucleus, prominent nucleolus and abundant eosinophilic cytoplasm, and a reserve cell type having an oval hyperchromatic nucleus and scanty cytoplasm. In most areas, the hepatoid cell type predominated, but in some, the reserve cells were more numerous. There were small foci of necrosis and neutrophilia. Tumor emboli were present within the lymphatics.

On July 24, 1964, the dog was presented for re-evaluation. A neoplasm was present in the right ventral perianal region. This mass was soft and nonencapsulated, and measured 0.8 cm. in diameter. It was excised, and histopathologic examination revealed a predominantly regular appearance similar to that described above. There were areas of necrosis, chromatolysis, and hyalinization, and a few papillary areas showing anaplasia, suggesting malignancy. Both of these neoplasms were diagnosed as circumanal gland adenocarcinomas. Enzymatic activities, as determined in the serum of this dog and in the first of the neoplasms excised, are recorded in TABLE 22.

TABLE 22. Enzymatic activities relating to Tumor 22.

Specimen	Date	Hb	LDH	LDH Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	11/5	1.06	281		23		.7.5	
Tumor	11/5	1.1	0	0	44	0.38	12189.9	105.8
Serum	11/7	0.37	62		22		2.7	

Tumor 23 was obtained from the same dog as Tumor 6, q.v. for history and physical findings. Tumor 23 was a round, ulcerated, firm mass, 1.0 cm. in diameter, located ventral to the anus. Microscopically it consisted of hepatoid cells with round nuclei and abundant eosinophilic cytoplasm, arranged in lobules separated by fibrous connective tissue. It was diagnosed as a circumanal gland adenoma. Enzyme activities, as determined in the serum of this dog, are recorded in TABLE 23.

TABLE 23. Enzymatic activities relating to Tumor 23.

Specimen	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	1.06	72	157	27	94.5

Tumor 24 was obtained from the same dog as Tumor 21, q v. for history and physical findings. Tumor 24 consisted of 2 perianal masses 1.0 x 1.0 x 0.4 cm. and 0.9 x 0.6 x 0.4 cm., respectively, which were grossly tan in color, soft, and irregular in outline. On microscopic examination the masses were found to be covered by skin with adnexa. They consisted of masses of hepatoid cells with small,

round, hyperchromatic nuclei eccentrically located, and abundant, eosinophilic cytoplasm. An occasional duct-like structure filled with keratinized cells was interspersed among the holocrine glands. This neoplasm was diagnosed as circumanal gland adenoma. Enzymatic activity determinations in the serum of this dog revealed activities of 125 units of phosphohexose isomerase and 5.9 units of alkaline phosphatase per liter. The tumor extract evinced an activity of 2593 units of phosphohexose isomerase per kilogram of tumor tissue.

Tumor 25 was obtained from a male Great Dane 3 years of age. This dog first showed signs related to the lesion under study on November 10, 1963, when the owner noted that the dog had epistaxis. The epistaxis recurred on November 13, and the dog was presented to a veterinarian, who cauterized a blood vessel in the left nasal cavity and packed the nostril. Parenteral and oral medication was administered for the purpose of improving coagulation. Following this episode, the owner noted that the dog had sneezing spells which stopped when the dog's wool blanket was removed. Antihistamine therapy was prescribed for this condition. On December 8, 1963, the dog had an episode of sneezing and epistaxis which was treated with a nasal pack and injectable antibiotics. The pack was removed on December 13, at which time a thick nasal exudate was noted. Treatment with antibiotics and antihistamines was continued. On December 30, radiographs of the nasal cavity disclosed no abnormalities, and the conservative therapy was continued. On January 9, 1964, the owner noted that the dog was losing weight. At this time, the fourth upper premolar teeth were extracted, and adrenocorticotrophic hormone was added to the therapeutic regimen. During the following

month, drainage of the nasal cavity and application of proteolytic enzymes were performed. A swelling developed over the left nasal area, and pressure on this swelling caused sanguineous exudation from the left nostril. On February 24, 1964, the dog was presented to the Ohio State University Veterinary Clinic. Physical examination revealed the dog to be emaciated. There were 2 fistulous tracts from the firm swelling over the left nostril, from which a mucopurulent to sanguineous fluid exuded. A hemogram at the time of admission revealed: hemoglobin, 8.8 gm./100 ml.; packed cell volume, 27%; erythrocytes, 3,310,000/cu. mm.; leukocytes, 20,000/cu. mm.; segmented neutrophils, 83%; non-segmented neutrophils, 3%; lymphocytes, 10%; and eosinophils, 4%. Microbiologic cultures from the nostrils yielded Staphylococcus, Escherichia coli, and beta-hemolytic Streptococcus. A biopsy of the nasal swelling was performed on February 28, 1964. The biopsy yielded several fragments of tissue, soft and dark red, ranging from a size of a few millimeters' diameter to 1.9 x 1.1 x 0.8 cm. In the areas where necrosis and hemorrhage predominated, microscopic examination revealed a loose fibrous connective tissue, large areas of which were necrotic. The tissue was vascular, congested, and hemorrhagic. Scattered about singly and in small nests were primitive cells with large chromatin-rich nuclei and a moderate amount of basophilic cytoplasm. There appeared to be desquamated cells and implants from the better preserved areas of the tumor. In other areas, the mass appeared less necrotic or friable and was covered by tall columnar, pseudostratified epithelium. The stroma was a loose connective tissue of fibroblasts, fibrocytes, and collagen fibers, but principally an edematous to mucoid interstitium. In this interstitium were islands of epithelial

cells which varied from distinct attempts at mucous-gland formation to undifferentiated nests. These cells were very pleomorphic with scanty cytoplasm and large, irregularly round nuclei varying from vesicular to hyperchromatic. Nucleoli were generally not evident, but mitotic figures were numerous. The epithelial component of this mass distinctly constituted a malignant neoplasm; the mesenchymal component, while showing evidence of active proliferation, appeared reactively hyperplastic and regular in arrangement. This neoplasm was diagnosed as a mucoepidermoid carcinoma.

Radiation therapy was administered over the period from March 7 to March 21, together with continued conservative antibiotic and supportive therapy.

On May 23, 1964, euthanasia and necropsy were performed. At necropsy, cross section of the left nostril revealed a mass 8 x 6 x 4 cm., having an opening on the dorsal surface, from which a clear viscous fluid exuded. The left nasal cavity was greatly dilated with obliteration of the turbinates. The bones of the lateral and dorsal walls were markedly thickened. The right nasal cavity was smaller than normal due to displacement of the septum; however, the turbinates were intact. Other gross lesions observed at necropsy were (1) an abscess 0.5 x 0.5 x 2.0 cm. in the belly of the right semimembranosus muscle, probably the result of an injection, and (2) cystitis with the presence of magnesium ammonium phosphate calculi. Microscopic examination of the nasal neoplasm disclosed a structure identical to the biopsy described above.

Enzymatic activities, as determined in the serum of this dog and in the tumor extract, are recorded in TABLE 24.

TABLE 24. Enzymatic activities relating to Tumor 25.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	2/28	0.81	434		236		22		88	
Tumor	2/28	4.0	17352	479	1111	31	192	5.4	38	1.1
Serum	3/7	0.72	364		255		16		22	
Serum	3/14	0.80	147		134		18		5.9	
Serum	3/21	0.40	77		93		18		11.7	
Serum	5/22	0.20	241		278		17		46	

Tumor 26 was obtained from a male Springer Spaniel 16 years of age. The owner had considered this dog to be in good health until 12 days prior to presentation, when the dog developed a deep cough. The attending veterinarian noted (1) the absence of lung sounds over the right cardiac lobe and (2) generally harsh, rough lung sounds, with râles and friction sounds. Radiographs revealed a large thoracic mass. The erythrocyte sedimentation rate was 55 mm./60 minutes.

A thoracotomy was performed, and the right cardiac lobe excised. Multiple nodules were found scattered throughout the lungs. The surgical procedure was terminated by euthanasia. At necropsy, lesions were limited to the thoracic lesions described and a subcutaneous nodule in the skin of the left elbow. The right cardiac lobe contained 3 grossly visible nodules, 6.5 x 8.0 x 5.0 cm., 8.0 x 6.0 x 5.0 cm., and 3.5 x 1.5 x 2.5 cm., respectively. These nodules were firm, cream-colored, and merged imperceptibly with the pulmonary parenchyma. The remainder of the lung contained numerous nodules ranging from 0.1 cm. in diameter to 9 x 9 x 5 cm. Microscopically, all pulmonary neoplasms were similar. They were made up of cuboidal tumor cells lined up on a basement

membrane with focal piling-up. Rows of tumor cells were separated by fibrous stroma. Focally, there were duct-like structures formed by the tumor, some filled with tumor cells. In other areas the tumor cells formed dense masses. The cells had round, oval, or reniform nuclei, with reticular chromatin and prominent, sometimes multiple, nucleoli. The moderately abundant cytoplasm was eosinophilic or clear. Clusters of tumor cells were seen in vessels. Mitoses, including atypical forms, were numerous. Among the well preserved areas of tumor described were foci of necrosis containing cholesterol clefts. This tumor was diagnosed as a bronchiolar adenocarcinoma. The nodule in the subcutis of the left elbow was an epidermal inclusion cyst. Enzymatic activities, as determined in the serum of this dog and in the extract of the bronchiolar adenocarcinoma, are recorded in TABLE 25.

TABLE 25. Enzymatic activities relating to Tumor 26.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.41	77		213		12		5.4	
Tumor	4.8	5302	155	5695	167	225	6.6	7732.8	226.1

Tumor 27 was obtained from a spayed female Boxer 12 years of age. This dog, which had been used as a seeing-eye dog, was presented on January 25, 1965, with a history of rapid weight loss and cough. Pulmonary radiographs revealed a large irregular mass in the left diaphragmatic lobe, 5 x 6 cm., located just within the rib cage. In addition, a few miliary calcific densities and a diffuse infiltrate

were radiographically evident. On January 28, a left diaphragmatic lobectomy was performed. A left diaphragmatic lobe contained dorsally a soft pink-gray mass 7.5 x 6.5 x 3.2 cm. in size, oval, and not encapsulated. Microscopically it consisted of columnar epithelium in a single layer on a connective tissue base. The cells had centrally located oval nuclei, polarized with the cell, and a moderate amount of eosinophilic cytoplasm. Some of the nuclei contained distinct nucleoli. Focally there was marked necrosis of the neoplasm, with mineralization and the formation of cholesterol clefts. A few neutrophils were present in the necrotic areas. This neoplasm was diagnosed as a bronchiolar adenocarcinoma.

Euthanasia and necropsy were performed on January 31, 1964. At necropsy the apical and cardiac lobes of the lung were consolidated into a neoplastic mass 10 x 6 x 2.5 cm. This mass and the remaining left lung were adhered to the sternum and to the pericardium over the left atrium and ventricle. The thoracic cavity contained 250 ml. of dark red, serosanguineous fluid. The spleen contained approximately 20 nodules, 0.5 to 1.0 cm. in diameter and raised above the surface. Microscopically these neoplasms were similar to that described above. Enzymatic activities, as determined in the serum and tumor extract of this dog, are recorded in TABLE 26.

TABLE 26. Enzymatic activities relating to Tumor 27.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.48	67		69		42		144	
Tumor	5.9	96	1.7	12871	230	6402	114	5155	92

Tumor 28 was obtained from the same dog as Tumor 6, q.v. for history and physical findings. This tumor consisted of a nodule in the right intermediate lobe of the lung measuring 3.0 x 3.0 x 2.5 cm., necrotic in the center and a firm, white, round nodule 2.0 cm. in diameter in the left diaphragmatic lobe of the lung. Microscopically both of these neoplasms were similar, except that the lesion in the right diaphragmatic lobe was more highly cellular, and necrosis in this mass was more extensive. Sections of both revealed masses subdivided into many lobules. These were composed of fibrous stroma covered by a single layer of cuboidal to low columnar epithelium. In a few areas, these cells presented a pseudostratified appearance. Within the masses were areas of necrosis and cholesterol clefts. The sections of the mass in the left diaphragmatic lobe revealed that it lay in the wall of a large bronchiole. A transition from normal to neoplastic epithelium was evident. These neoplasms were diagnosed as bronchiolar adenomas, and were considered to be separate primary tumors. Enzymatic activities, as determined in the serum of this dog and in the extract of the bronchiolar adenoma, are recorded in TABLE 27.

TABLE 27. Enzymatic activities relating to Tumor 28.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	1.06	72		157		27		94.5	
Tumor	2.4	217	11.5	232	14	29	1.8	1246.	78

Tumor 29 was obtained from a male, mixed-breed, terrier-type dog 12 years of age. This dog was initially presented on August 27, 1962,

because of a mass ventral to the anus. This mass, measuring 2.0 x 2.0 x 1.5 cm., was excised. Histopathologic examination disclosed that it was a melanoma with foci of suppuration. On March 28, 1963, a recurrence of the melanoma, in the same location, was resected. On April 3, 1963, a bilateral orchiectomy was performed. Histopathologic examination revealed no abnormalities in the testes. On August 19, 1963, another recurrence of the melanoma was excised. At this time, histopathologic examination disclosed invasion of the melanoma into the rectum. At the same time, a sebaceous-gland adenoma was resected from the right thigh.

On December 30, 1963, the dog was presented for euthanasia and necropsy. An ulcerated mass, measuring 3 x 2.5 cm., was present below the anus. Necropsy revealed the above neoplasm with metastases to the liver (Tumor 87). In addition, a solitary nodule 5.0 cm. in diameter was present in the lung. This consisted of regularly arranged cuboidal to low columnar epithelial cells in a single layer on a base of connective tissue. No criteria of malignancy were noted in this lesion. This tumor was diagnosed as a bronchiolar adenoma. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 28.

TABLE 28. Enzymatic activities relating to Tumor 29.

Hb	LDH	PHI	LAP	Alk. Ptase.
0.56	43	125	13	18

Tumor 30 was obtained from the same dog as Tumor 18, q.v. for history and physical findings. This tumor consisted of multiple nodules in the lungs ranging from 0.1 to 5.0 cm. in diameter. In addition, metastases of this tumor were present in the mediastinal and bronchial lymph nodes and within 1 section of the tumor of the carpus (Tumor 67). Emboli of this tumor were present in the liver within portal veins, but no gross neoplasms or foci of extravascular invasion were evident. Microscopically, the tumor showed a considerable amount of variation in maturity, but transitional stages between all cell types were present. In the most mature areas, the tumor consisted of a single layer of tall columnar, epithelial cells arranged on a basement membrane covering a papillary fibrous connective tissue stroma. There were large spaces formed by the tumor. Many of the epithelial cells were mucous cells, and some of the spaces formed by the tumor were filled with mucus. In less well differentiated areas, the tumor formed only a vague papillary pattern, or anaplastic cells arranged in nests. These cells had abundant violet-staining cytoplasm, with indistinct borders, large oval nuclei, and prominent nucleoli. Even these undifferentiated areas revealed attempts at tubule or lumen formation. Mitoses and invasion of vessels constituted prominent features of the neoplasm. A few microabscesses were present. In areas of lung adjacent to the neoplastic mass, clusters of tumor cells filled many of the alveoli, forming a "carcinomatous pneumonia". Within the tumor mass, foci of necrosis and mineralization were present. The metastases to the mediastinal and bronchial lymph nodes replaced much of the nodal architecture. The cells formed nests in vessels and within the lymph-node parenchyma. The cells appeared primitive and anaplastic, very similar to those described

in the less differentiated areas of the pulmonary neoplasm. Many plasma cells were present in the connective tissue surrounding the neoplasm. The metastasis to the foot and the emboli in the liver were cytologically similar to the lymph-node metastases. This neoplasm was diagnosed as a bronchial adenocarcinoma. Enzymatic activities, as determined in the serum of this dog and in the pulmonary neoplasm, are recorded in TABLE 29.

TABLE 29. Enzymatic activities relating to Tumor 30.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.45	22		97		16		16.1	
Tumor	1.1	16301	776	8288	382	194	9.3	177.	8.6

Tumor 31 was obtained from a 7-year-old female Beagle. This dog was presented because of bilateral enlargement of the posterior mammary glands. The glands were hot, firm, and pendulous. The swelling had been present for 3 weeks prior to admission, and had enlarged rapidly. An ovariohysterectomy was performed, and the mammary swellings treated with antibiotics systemically and the application of heat locally, after which the swelling and heat decreased. Histopathologic examination of the ovaries and uterus revealed no significant abnormalities. The dog was again presented 6 weeks later, on March 13, 1964, very depressed and lethargic. The mammary masses were hot with ulcerated and fluctuating areas. There was an enlargement lateral to the left side of the vulva and anus. Biopsies of this mass and of the mammary tumors were performed on March 17, 1964. Microscopically, the tissues obtained at

biopsy constituted a mass covered by stratified squamous epithelium with areas of ulceration and hemorrhage. Some adnexa were present, but generally they had been replaced by the tumor mass lying in the dermis and subcutis. There were vast areas of necrosis and hemorrhage within the tumor. The tumor cells were arranged into solid masses, lobules, and papilliform projections which invaded, Medusa-like, into the adjacent tissues. The tumor-cell nuclei were large and round, vesicular with marginated chromatin and 1, or often more, prominent nucleoli. The cytoplasm was eosinophilic, with indistinct cell boundaries, and contained lipoid vacuoles. A loose fibrillar stroma lay between the lobules. Sections of the mammary tumors were identical, microscopically, to sections of the mass lateral to the vulva and anus. The mass was diagnosed as an undifferentiated, holocrine-gland adenocarcinoma, possibly of circumanal gland or sebaceous gland origin.

Euthanasia and necropsy were performed on March 19, 1964. At necropsy, the posterior section of each mammary chain measured 20 x 4 x 8 cm. The skin overlying the neoplasms was nodular and ulcerated. The neoplasms contained areas of caseation and suppuration. There was generalized enlargement of the superficial lymph nodes. The lungs revealed generalized anthracosis. A nodule measuring 0.2 x 0.2 x 0.3 cm. was present in the right apical lobe beneath the pleura. Sections of the mammary neoplasms and those lateral to the vulva and anus were identical to those described above. Microscopic examination of the lung revealed anthracosis, atelectasis, and congestion. The grossly noted nodule microscopically resembled the above described neoplasms. A metastasis of this neoplasm was also present in the right sublumbar lymph node. Enzymatic activities, as determined in the serum of this

dog and in the extract of the tumor are recorded in TABLE 30.

TABLE 30. Enzymatic activities relating to Tumor 31.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/13	0.97	125	287	18	2.7
Serum	3/18	0.64	89	171	20	22.
Tumor	3/19		53020			

Tumor 32 was obtained from the same dog as Tumor 1, q.v. for history and physical findings. Tumor 32 was excised on January 28, 1964, from the lateral surface of the right forefoot. Grossly, it was an irregular, nodular, firm, white mass which measured 0.8 x 0.7 x 0.7 cm. On microscopic examination the neoplasm was seen to be located in the dermis and covered by stratified squamous epithelium. The mass was composed of cells containing lipid vacuoles and arranged in uniform holocrine lobules. Around this mass were pigment-laden melanophores. This mass was diagnosed as a sebaceous gland adenoma. Enzymatic activities in the serum of this dog before and after excision of the neoplasm are recorded in TABLE 31.

TABLE 31. Enzymatic activities relating to Tumor 32.

Specimen	Date	Hb	LDH	PHI	LAP	Alk.Ptase.
Serum	1/27	0.48	14	116	20	328
Serum	2/24	1.53	214	306	11	440
Serum	2/27	1.93	366	333	13	112

Tumor 33 was obtained from the same dog as Tumor 6, q.v. for history and physical findings. At necropsy, this dog was found to have multiple neoplasms of the skin. These consisted of a mass on the inner surface of the pinna of the right ear measuring 4.5 x 5.0 x 1.0 cm. and superficially ulcerated, 3 masses approximately spherical located on the face about the eyes, measuring 0.8 cm., 1.0 cm., and 0.5 cm. in diameter, respectively, 2 masses dorsal to the right scapula, measuring 0.6 cm. and 0.2 cm. in diameter, respectively, and 2 masses dorsal to the left scapula, both measuring 0.6 cm. in diameter. These masses were all gray to white in color on cut section, rather firm, and slightly greasy. Microscopically all consisted of clusters of rather mature, regular lipid-laden cells lying in the dermis, and, in the larger masses, extending into the subcutis. None showed any criteria of malignancy. All of these nodules were diagnosed as sebaceous-gland adenomas. Serum enzyme activities, as determined in this dog, are recorded in TABLE 32.

TABLE 32. Enzymatic activities relating to Tumor 33.

Specimen	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	1.06	72	157	27	94.5

Tumor 34 was obtained from a 7-year-old female Boxer. This dog was presented on February 18, 1964, for the excision of multiple skin neoplasms. The history indicated that the dog had polydypsia and, on physical examination, the dog was noted to have a poor hair coat and an ulcer on the right cornea. Small neoplasms were excised

from the left thigh, stifle, medial surface of the right foreleg, humeral midshaft, ventral thorax (2 masses) and medial surface of the right hock. These are considered in this study as Tumor 54. A small mass was excised from the ventral abdomen (Tumor 61) and another, measuring about 0.6 cm. in diameter, was excised from the medial surface of the right elbow. This last lesion was firm and white. On microscopic section, it was seen to be covered by stratified squamous epithelium. The neoplastic cells, located in the dermis, were filled with lipid, and were arranged in regular nodules. This neoplasm was diagnosed as a sebaceous-gland adenoma.

On April 22, 1964, additional nodules were excised from the scalp and left flank (Tumor 72), from the right carpus (Tumor 89). Four further nodules excised proved to be areas of dermal fibrosis and hyperkeratosis without any evidence of neoplasia. Enzymatic activities determined in the serum of this dog are recorded in TABLE 33.

TABLE 33. Enzymatic activities relating to Tumor 34.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/18	1.28	251	231	39	26.
Serum	4/22	1.19	255	153	44	8.1

Tumor 35 was obtained from the same dog as Tumor 19, q.v. for history and physical findings. Tumor 35 was a small mass on the margin of the lower right eyelid, which was excised at the same time as Tumor 19, on February 15, 1964. It measured 0.4 x 0.5 x 0.2 cm., was irregular in shape, yellow in color, and friable. Microscopically

It consisted of regular lobules of lipid-filled cells lying in the dermis. This neoplasm was diagnosed as a sebaceous-gland adenoma. Enzyme activities, as determined in the serum of this dog, are recorded in TABLE 34.

TABLE 34. Enzymatic activities relating to Tumor 35.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/25	1.32		162	19	12.4
Serum	3/1	0.56	275	213	22	29.0

Tumor 36 was obtained from an 8-year-old female Boston Terrier. This dog was presented on February 25, 1964, because of a tumor on the medial side of the left hind leg, a tumor on top of the skull, and a tumor on the right foreleg at the level of the dew claw. The total leukocyte count was 5,600/cu. mm., with a differential leukocyte count of 71% segmented neutrophils, 14% lymphocytes, 3% eosinophils, and 7% monocytes. At surgery, 2 nodules were excised from the left hind leg. These are considered in this study as Tumor 55. The nodule from the right forefoot measured 0.8 x 0.7 x 0.5 cm. and was pink and firm. The nodule excised from the skull was 0.9 x 0.6 x 0.3 cm. in size, pink and firm. Microscopically, both nodules were similar. They lay in the dermis, covered by stratified squamous epithelium. They consisted of lobules of lipid-filled cells having a mature appearance, similar to the morphology of sebaceous glands, and were diagnosed as sebaceous-gland adenomas. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 35.

TABLE 35. Enzymatic activities relating to Tumor 36.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/25	1.27	82	153	18	10.2
Serum	3/3		111		15	29.0

Tumor 37 was obtained from the same dog as Tumor 9, q.v. for history and physical findings. An ovariohysterectomy was performed on this dog on January 21, 1964. Both ovaries were found to be enlarged, and the lumen of the uterus contained a creamy yellow exudate. Microscopic examination of the left ovary revealed multiple, large, follicular cysts and a neoplasm consisting of simple cuboidal cells in papillary arrangement, surrounding spaces. These papilliform convolutions had a central fibrous base and were separated by abundant fibrous stroma. The cells had large, round nuclei and scanty cytoplasm. The right ovary was essentially similar except that, instead of a single mass, the neoplasm appeared multifocal. These neoplasms were diagnosed as papillary adenomas of the ovaries. Examination of the uterus revealed a suppurative endometritis. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 36.

TABLE 36. Enzymatic activities relating to Tumor 37.

Specimen	Date	Hb	LDH	PHI	LAP	Alk.Ptase.
Serum	1/21	0.80	125	241	23	9.1
Serum	1/25	0.26	39	93	11	12.9
Serum	3/26	1.72	29	255	15	8.6

Tumor 38 was obtained from a male Golden Retriever 10 years of age. This dog was presented on February 21, 1964, because of a nodule in the skin dorsal to the point of the left shoulder, and a small protuberance of tissue on the left border of the tongue opposite the

canine tooth. The referring veterinarian also requested that an orchiectomy be performed. Surgery was performed as indicated. The mass excised from the tongue measured 0.8 x 0.7 x 0.3 cm. and was tan and firm. The mass excised from the shoulder is considered in this study as Tumor 51. The testes measured 3.8 x 2.8 x 2.2 cm. and appeared grossly normal. On microscopic examination, the mass from the tongue was seen to be covered by stratified squamous epithelium without adnexa. The covering mucosa was locally ulcerated, with neutrophilic infiltration around the site of ulceration. The mass consisted of cells with clear cytoplasm and small hyperchromatic nuclei, eccentrically located. The cells were generally arranged in lobules. This neoplasm was diagnosed as an acinic tumor originating from a minor salivary gland. Histopathologic examination of the testes revealed tubular atrophy. A nodule was excised from the lateral surface of the left foreleg just distal to the humero-radial joint on June 10, 1964. Histopathologic examination disclosed only dermal fibrosis, without the presence of neoplastic cells. Recurrences of the neoplasm involving the shoulder were treated on several occasions by excision and by x-irradiation. On November 6, 1964, euthanasia and necropsy were performed. A more detailed description of the course of illness and therapy and necropsy findings are presented with Tumor 51, to which they are pertinent. There was no recurrence or metastasis of the acinic-cell tumor. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 37.

TABLE 37. Enzymatic activities relating to Tumor 38.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/24	1.01	116	185	27	0
Serum	2/27	2.2	202	407	27	7.0

Neoplasms of Mesenchymal Origin

Tumor 39 was obtained from a male Poodle 8 years of age. This dog was referred to the Ohio State University Veterinary Clinic on November 22, 1963, with a history of bronchitis and pharyngitis 4 months prior to admission. This had responded to the antibiotic therapy administered by the attending veterinarian. On November 1, the left eye was noted to be inflamed and was treated with antibiotic ointment and hot packs. On November 4, generalized enlargement of the lymph nodes was first noted. The attending veterinarian made a tentative diagnosis of malignant lymphoma and referred the dog for admission as noted. Physical examination at the time of admission revealed mucopurulent exudate in the right eye. The left eye was covered by the enlarged membrana nictitans. All superficial lymph nodes were enlarged, as were the tonsils. Upon auscultation, lung sounds were found to be normal. Hematologic values during the hospital course are recorded in TABLE 38.

TABLE 38. Host hematologic data relating to Tumor 39.

Date	Hb gm./ 100 ml.	PCV (%)	Platelets /cu. mm.	Leuk. /cu.mm.	Seg. Neut. (%)	Nonseg. Neut. (%)	Lymph. (%)	Eosin. (%)	Mono. (%)
11/22	15.05	47.5		12,700	72		15	13	
11/27	15.5	47.5	90,000	20,000	78	2	11	7	2
12/4	10.0	33.		45,200	88		10	1	1

On November 23, a biopsy of the popliteal lymph node was performed, and a tonsil excised. The popliteal lymph node was bilobed and had an over-all size of 4.0 x 1.6 x 2.0 cm. The tonsil was firm and tan, with focal areas of hemorrhage. It measured 4.5 x 2.2 x 1.6 cm. Microscopic examination of the tonsil revealed a neoplastic mass consisting of small round cells with pale round nuclei, containing clumps of chromatin. The cells had a scanty to moderate amount of basophilic cytoplasm; they were randomly arranged. One section revealed remnants of the underlying mucous glands with lymphoblastic invasion. Occasional mitotic figures were seen. In the popliteal lymph node, the germinal centers were indistinct and the marginal sinus was obliterated. There were foci of hemorrhage. Occasional cells similar to those described above invaded the capsule. These lesions confirmed the diagnosis of malignant lymphoma. Thoracic radiographs on November 25 showed no hilar lymph node enlargement or evidence of pulmonary neoplasia. Progressive depression continued throughout the hospital course. On December 5, 1963, euthanasia and necropsy were performed. At necropsy, all lymph nodes were found to be greatly enlarged, grayish-white in color and slightly softer than normal. The left mandibular node

measured 5 x 2 x 1 cm., while the right measured 2 x 1 x 0.5 cm. The spleen appeared slightly but diffusely swollen. It contained 3 nodules measuring 1 to 2.5 cm. in diameter. The mucosa of the urinary bladder had several raised, small, hemorrhagic areas. In addition, the wall of the bladder was diffusely thickened.

On microscopic examination, the various lymph nodes were found to be similar to the node secured at biopsy and described above. The architecture of much of the membrana nictitans was obliterated and replaced by an infiltrate of neoplastic lymphoblasts and lymphocytes. A section of femoral bone marrow disclosed a relatively sparse (grade 2) cellularity with hemosiderin-filled macrophages present. No lymphomatous infiltration of the bone marrow was noted. The splenic architecture was generally preserved. The white pulp was hypercellular but, in the absence of lesions in other lymphoid organs, it would not have been possible to determine whether this was due to hyperplasia or neoplasia. The submucosa and, focally, the base of the mucosa of the gastric fundus were infiltrated by mature-appearing lymphocytes. The urinary bladder was diffusely edematous and had foci of hemorrhage on the mucosal surface. The cortex of the right adrenal gland contained a well differentiated, encapsulated, cortical adenoma. Necropsy findings confirmed the diagnosis of malignant lymphoma. Enzymatic activities, as determined in the serum of this dog and in extracts of the neoplastic lymph nodes collected at necropsy, are recorded in TABLE 39.

TABLE 39. Enzymatic activities relating to Tumor 39.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	11/22	1.06	34		74		31		3.2	
Serum	11/26	0.80	70		213		27		15.6	
Tumor	12/5	4.8	1253	21	11899	202	480	8.15	247.	4.3
Tumor	12/5	5.6	1350	21	3889	60	262	4.07	1079.	16.6

Tumor 40 was obtained from a 5-year-old female Terrier. This dog was presented for veterinary attention on May 10, 1963, because of a cough and vomiting. These were attributed to tonsillitis and appeared to respond to therapy. On August 30, 1963, swelling of the face and neck were noted. On September 14, 1963, all superficial lymph nodes were found to be enlarged. The tonsils were enlarged and protruded from their crypts. On September 16, a bilateral tonsillectomy and excision of the right popliteal lymph node were performed. Grossly, the popliteal lymph node was soft, friable and grayish-tan, measuring 2.9 x 1.5 x 1.5 cm. The tonsils were pink in color, with a prominent follicular pattern and superficial hemorrhages. Microscopically, the lymph node was a highly cellular mass. The cells appeared randomly arranged, obliterating the underlying follicular architecture, filling the marginal sinus, and invading the capsule. The tumor cells appeared to be of lymphogenous origin, with large, pale nuclei and rather abundant, basophilic cytoplasm. In the tonsils, the germinal centers and the mucous glands remained intact. There was a diffuse infiltrate of cells like those described above. Focal areas of hemorrhage were present. These histopathologic findings confirmed the tentative

diagnosis of malignant lymphoma, lymphoblastic type. Therapy was initiated using Leukeran® (brand of chlorambucil, Burroughs Wellcome & Co.) orally at a dose of 1.3 mg. daily for periods of 2 weeks separated by intervals of 2 weeks without this medication. Frequent hematologic examinations were performed throughout the hospital course. The hemoglobin and packed cell volume were normal at all times. The leukocyte count vacillated, during this period, between 11,600 and 31,000/cu. mm., with lymphocytes constituting 18 to 51% of the total leukocytes. However, no atypical or neoplastic lymphocytes were observed among the circulating leukocytes, nor did a frankly leukemic hematologic pattern develop at any time. Platelets varied from 92,000 to 221,000/cu. mm. A urinalysis performed on October 7, 1963, revealed a pH of 8, 100 mg./100 ml. albumin, and numerous erythrocytes, leukocytes and phosphate crystals on microscopic examination. On November 5, hematuria was first noted. This occurred intermittently thereafter throughout the hospital course. Radiographic studies during the period of hospitalization revealed a mass in the anterior mediastinal space, infiltration of the lung fields, splenomegaly, and the presence of cystic calculi.

Euthanasia and necropsy were performed on January 5, 1964. External examination at necropsy revealed an emaciated, dehydrated-appearing cadaver. Mucopurulent exudate was present in both eyes. All superficial lymph nodes appeared enlarged. On the dorsal aspect of the posterior portion of the tongue was a nodule measuring 3 x 2 x 2 cm. The mesenteric lymph nodes could be palpated through the abdominal wall. A hard mass, 1.5 cm. in diameter, was palpable in the urinary bladder. Grossly, the lungs were heavier and firmer than normal. The

spleen was enlarged and slightly congested. A nodule at the anterior border was 2.0 x 3.5 x 3.5 cm. in size. White streaks traversed the kidneys paralleling the nephrons. A calculus, 1.0 cm. in diameter, and several small calculi, approximately 0.1 cm. in diameter, were present in the urinary bladder. All lymph nodes were enlarged.

Microscopic examination of the tongue revealed that the nodule grossly described was lymphoblastic. Sections of the spleen revealed massive lymphoblastic infiltration with obliteration of the underlying splenic architecture. Within the lung, many of the alveoli were enlarged into solid neoplastic masses by infiltration and proliferation of lymphoblasts. Other alveolar septa were diffusely infiltrated and thickened. In the liver, neoplastic cells similar to those described formed multifocal discrete masses, but especially surrounded the portal triads. Bilirubin-filled macrophages were also seen throughout the liver. The bone marrow was of a moderate (grade 3) cellularity, and had a normal distribution of hematopoietic cells. No lymphoblastic infiltration was evident. Hemosiderin-filled macrophages were present in the bone marrow. Sections of the lymph nodes were similar, on histopathologic examination, to those obtained by biopsy and previously described. Necropsy findings confirmed the diagnosis of malignant lymphoma, lymphoblastic type. Enzymatic activities, as determined in the serum of this dog throughout the hospital course and in the extract of a neoplastic lymph node, are recorded in TABLE 40.

TABLE 40. Enzymatic activities relating to Tumor 40.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	10/12	1.06	347		389		18			
Serum	10/30	0.72	77		134		21			
Serum	11/8	0.69	48		46		15		73.6	
Serum	11/20	1.11	67		116		18		48.3	
Serum	12/7	0.69	67		106		23		162.7	
Tumor	12/19	3.7	14219	241	8982	152	407	6.9	26.9	0.4
Serum	12/20	1.19	34		116		24		143.9	
Serum	1/5	1.01	36				17			

Tumor 41 was obtained from a male Boston Terrier 8 years of age. This dog was presented by referral on March 6, 1964, with a tentative diagnosis of malignant lymphoma. Physical examination revealed enlargement of all lymph nodes and of the tonsils. On the day of admission, a tonsil and a popliteal lymph node were excised. The tonsil measured 2.0 x 1.2 x 0.6 cm., was oval in shape, pink, and soft. The popliteal lymph node was 2.4 x 2.0 x 1.6 cm., red, and soft in texture. Microscopic examination of the section of tonsil revealed a covering of intact mucous membrane overlying the mucous glands. The remainder of the section consisted of a disorganized-appearing mass of cells having round to oval hyperchromatic nuclei and poorly demarcated cytoplasm. Occasional mitoses were present. In the popliteal lymph node, there was a loss of normal architecture, which was replaced by an erratically arranged mass of cells with round or oval hyperchromatic nuclei with prominent nucleoli. The cells had abundant pale-blue-staining cytoplasm

with indistinct cytoplasmic boundaries. Numerous mitoses, including atypical forms, were present. The cells filled the marginal sinus and invaded the capsule extensively. These findings confirmed the diagnosis of malignant lymphoma, lymphoblastic type.

Frequent hematologic examinations were performed during the hospital course. Hemoglobin and packed cell volume values decreased from initial normal values to terminal values of 8.8 gm./100 ml. and 29%, respectively. Leukocyte counts varied from 11,700 to 32,550/cu. mm., with 77 to 97% neutrophils. At no time throughout the course of illness were hematologic findings indicative of leukemia noted. Platelet counts ranged from 150,000 to 288,000/cu. mm. Prednisolone was administered from March 19 to April 1. During the remainder of the course, x-irradiation was administered to enlarged nodes, and supportive therapy was given as required. Death occurred on May 2, 1964.

At necropsy, all lymph nodes were noted to be enlarged. There was an abscess in the area of the right prescapular lymph node. The right testis was retained in the inguinal canal. Chronic fibrous valvular endocarditis was noted in both the mitral and tricuspid valves. The lungs were congested with areas of consolidation and some nodularity. Microscopically, foci of amyloidosis were noted in the spleen, where there was a depletion of lymphocytes and no evidence of neoplastic involvement. Multiple sections of lymph nodes revealed marked variations, ranging from findings characteristic of malignant lymphoma, as described in the section obtained at biopsy, to atrophy of lymphoid elements, coagulation necrosis, and replacement by collagen and hyaline, with hyalinization of vessel walls. All intermediate

stages were seen as well, and areas of extreme variation were seen adjacent to one another in individual sections. The liver had lesions of fatty metamorphosis of the large-droplet type and with a centrolobular distribution. The lungs contained areas of anthracosis and congestion. No lesions of malignant lymphoma were found in liver or lungs. Despite the response to therapy, sufficient foci of neoplasia were found to confirm the diagnosis of malignant lymphoma. Enzymatic activities, as determined in the serum and in an extract of a neoplastic lymph node, are recorded in TABLE 41.

TABLE 41. Enzymatic activities relating to Tumor 41.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	3/6	0.8	149				19			
Tumor	3/6	4.0	22654	468	11112	229	518	11	75.2	1.6
Serum	4/1		87		125		16		1804.	
Serum	4/14	1.32	1109		431		20		1042.	
Serum	4/28	0.26	164		162		17		1353.	

Tumor 42 was obtained from a spayed, female, mongrel terrier $6\frac{1}{2}$ years of age. This dog was presented on March 26, 1964, because of generalized enlargement of the superficial lymph nodes. A biopsy of the popliteal lymph node was performed. The lymph node obtained by biopsy was an irregular mass 2.5 x 1.3 x 1.3 cm., brownish in color, and soft. Microscopic examination revealed that the follicular architecture of the node was almost entirely lost, though some adipose cells were still present. The predominant cell type had a large, pale

nucleus, prominent nucleolus, and a small amount of violet-staining cytoplasm. These cells were present in dense, random-appearing aggregations. A moderate number of mitotic figures was present. This neoplasm was diagnosed as malignant lymphoma, reticulum cell type. Hematologic findings during the course of illness are recorded in TABLE 42.

TABLE 42. Host hematologic data relating to Tumor 42.

Date	Hb gm./ 100 ml.	PCV (%)	Platelets /cu. mm.	Leuk. /cu.mm.	Seg. Neut. (%)	Nonseg. Neut. (%)	Lymph. (%)
4/7	12.6	38	136,000	23,900	79	5	14
4/14	7.95	23	98,000	30,000	84	6	10
4/21	4.6	16	153,000	56,800	82	13	3

Minor cell types, when evident, were not present in excessive numbers.

On April 8, Leukeran® therapy was initiated at a level of 3 mg./day. Because of the depression of erythrocytic and platelet values, this therapy was discontinued on April 15, and prednisolone, in a dose of 30 mg./day was begun. On April 20, respirations became labored and abdominal in type. Death occurred on April 22.

At necropsy, the pleural cavity contained 20 ml. of transudate. The lungs were edematous. The free edges of the mitral valve were thickened and roughened, and the valve appeared insufficient. The abdominal cavity contained 75 ml. of transudate. The lymph nodes were hemorrhagic and appeared swollen and moist. The bone marrow was gelatinous and gray. Microscopic examination of the lymph nodes

revealed a loss of follicular architecture. The capsules were invaded by neoplastic lymphocytes. The nodes were only moderately cellular. The majority of cells present were mature-appearing lymphocytes. Hemosiderin-filled macrophages were also present. Large spaces separated the structures, indicating edema. Examination of sections of the mitral valve revealed areas of necrosis, fibrin, and bacterial colonies. The hepatic architecture was intact. Many hemosiderin-filled macrophages were scattered throughout. The parenchymal cells were vacuolated, indicating a diffuse pattern of fatty metamorphosis. The portal triads were surrounded and interspersed by a somewhat increased number of lymphocytes. In the spleen, a loss of structural differentiation between the red and white pulp was evident. Lymphocytes, diffusely distributed, replaced the pre-existing architecture. Numerous hemosiderin-filled macrophages were present. There were foci of extramedullary hematopoiesis. The bone marrow was slightly more cellular than normal, but the normal components were present. In addition, there were foci of lymphocytes. Hemosiderin-filled macrophages were scattered throughout. These lesions confirmed the previous diagnosis of malignant lymphoma, as modified by the therapy. Terminally, a septicemia developed from the pre-existing endocarditis, to which the dog was unable to make the normal inflammatory response because of the malignant lymphoma and therapy. The enzymatic activities, as determined in the serum and in a neoplastic lymph node from this dog, are recorded in TABLE 43.

TABLE 43. Enzymatic activities relating to Tumor 42.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	3/26	0.48			125		19		5.4	
Tumor	3/27	3.8	19280	438	9539	217	480	11	69.8	1.6
Serum	4/7	0.48			259		19		6.4	

Tumor 43 was obtained from a 10-year-old spayed female Dalmatian. This dog was presented because of enlargement of the mandibular, anterior cervical, retropharyngeal, prescapular and popliteal lymph nodes. The nodes had been enlarged for 2 years, but increased rapidly in size during the 6 months prior to admission. The left tonsil, left popliteal lymph node, and left mandibular lymph node were excised. The tonsil measured 2 x 1 x 1 cm. It was tan, with brown areas on the cut surface. An ulcer, 0.2 cm. in diameter, was present on 1 surface. Microscopically, the morphologic distinction between the lymphoid areas and the mucous glands was preserved. Several small round epithelial structures were seen in the lymphoid area, with epithelial maturation centrally. These appeared to be cross sections of crypts. These areas were invaded, and partially obliterated, by lymphocytes. The lymphoid areas were hypercellular and, in some areas, the follicular architecture was indistinct. The predominant cell type was the small, mature lymphocyte. In the popliteal lymph node, there was an excessive separation of structures, suggesting the presence of edema. The architecture of the marginal sinus and the germinal centers was otherwise intact in some areas; in others, there was a hypercellularity of small mature lymphocytes, obliterating the underlying architecture

and massively invading the capsule. Sections of the mandibular lymph node were essentially similar to those of the popliteal node, except that no edema was noted, and hypercellularity, loss of architecture, and invasion of the capsule were more prominent features. This neoplasm was diagnosed as a malignant lymphoma, lymphocytic type. Enzyme activities in the serum of this dog are recorded in TABLE 44.

TABLE 44. Enzymatic activities relating to Tumor 43.

Hb	LDH	PHI	LAP	Alk. Ptase.
0.89	70	79	19	1.1

Tumor 44 was obtained from a 7-year-old spayed female Collie. This dog was presented because of a lesion diagnosed as dermatitis on the dorsum of the nose. Under the inflamed skin were 2 hard subcutaneous nodules measuring 1.0 x 0.5 x 0.25 cm. and 1.25 x 0.5 x 0.25 cm., respectively. The nasal passages were occluded by swelling from the lesions. Radiographs revealed no further lesions. Therapy with antihistamines failed to produce improvement. Six months later, on November 5, 1963, the dog was presented for excision of neoplasms on the dorsum of the nose and over the right carpal joint. Firm white masses measuring 1.5 x 0.5 x 0.3 cm. and 1.3 x 0.4 x 0.3 cm., respectively, were excised. Sections of both neoplasms were similar and revealed intact epidermis and dermis with adnexa. The dermis and the subcutis were infiltrated by small round cells having no regular arrangement demonstrable. By hematoxylin and eosin stain, the cells had round hyperchromatic nuclei and clear or pale eosinophilic cytoplasm.

Wilder's reticulum stain revealed a network of reticulum fibers, which separated the individual tumor cells. This lesion was diagnosed as a reticulum-cell sarcoma of the skin. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 45.

TABLE 45. Enzymatic activities relating to Tumor 44.

Date	Hb	LDH	PHI	LAP	Alk.Ptase.
11/21/63	0.52	29	51	24	10.7
1/17/64	1.06	34	106	19	6.4
4/23/64	1.15	121	185	18	16.6

Tumor 45 was obtained from a male Chow 12 years of age. This dog was presented because of an enlargement which had been present for 4 months at the distal end of the right radius. The dog had been lame on the affected foreleg for a week prior to presentation. In addition to the above findings, physical examination revealed enlargement of the right axillary and prescapular lymph nodes. Radiographs revealed a periosteal reaction but no involvement of the bone itself. The hemoglobin concentration and the packed cell volume were within the normal ranges. The total leukocyte count was 22,500/cu. mm., with 91% segmented neutrophils, 2% nonsegmented neutrophils, 6% lymphocytes, and 1% monocytes. The erythrocyte sedimentation rate was 41 mm/60 minutes, contrasted with an expected value of 10.5 mm./60 minutes. The urinalysis revealed the presence of 30 mg. of albumin per 100 ml. and occasional granular casts. The remaining results of the urinalysis were within normal limits.

Euthanasia was performed, and necropsy revealed no significant lesions other than those indicated above, except that the liver was diffusely and coarsely nodular. The neoplastic mass described clinically was gray to brown in color, soft, nonencapsulated, and measured 13.5 x 7.5 x 2.8 cm. Microscopically, it was found to be covered by lightly keratinized epithelium, with adnexa, which was focally ulcerated. Some of the hair follicles were slightly cystic and, in the ulcerated areas, there was coagulation necrosis with neutrophilic infiltration. Mast cells were present in the dermis in somewhat excessive numbers. In the subcutis were areas of fibrosis and of necrosis with neutrophilic infiltration, areas of fibroplasia characterized by an admixture of active young fibroblasts with occasional mast cells, and areas of dense aggregates of round cells having abundant gray-staining cytoplasm. By the use of Giemsa stain, these cells were seen to contain numerous metachromatic granules in their cytoplasm. Many of the lymphatics were filled with mast cells, singly and in clusters. The neoplasm was diagnosed as a mastocytoma. Because of frequent lack of correlation between histologic appearance and clinical behavior, benign-appearing mastocytomas may often prove to be pre-malignant. Therefore, the diagnosis "mastocytoma" has been used without a qualifying adjective. The morphologic and behavioral properties associated with benign or malignant classification have been individually described for each mastocytoma in this study. Sections of the axillary and inguinal lymph node were found to contain metastases of the mastocytoma. Histopathologic examination of the liver revealed the presence of regenerative nodules. Enzymatic activities, as determined in the serum and in the neoplasm of this dog at the time of necropsy, are recorded in TABLE 46.

TABLE 46. Enzymatic activities relating to Tumor 45.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.6	125		361		15		61	
Tumor	5.2	530	8	4167	634	494	7.6	113	1.6

Tumor 46 was obtained from a spayed female Cocker Spaniel 11 years of age. This dog was presented on February 18, 1964, because of a neoplasm on the left forefoot. This neoplasm had been present for 6 months and had increased in size rapidly in the few weeks just prior to presentation. Radiographic examination revealed no bone lesions. The hemogram revealed: hemoglobin, 12.25 gm./100 ml.; packed cell volume, 37%; total leukocytes, 10,900/cu. mm.; segmented neutrophils, 90%; nonsegmented neutrophils, 2%; and lymphocytes, 8%. The erythrocyte sedimentation rate was not accelerated. The mass was excised, following which x-irradiation therapy was administered to the site of excision. The excised mass was approximately spherical, measuring 7.5 x 7.0 x 6.5 cm., and was tan and firm. Microscopic examination revealed a highly cellular mass covered by intact epidermis with adnexa. The mass was located in the dermis and subcutis. It consisted of large, immature-appearing, round cells densely aggregated between fibrous septa, and invading them. In the fibrous septa were many blood and lymph vessels, some of which were filled with tumor cells. The cells had very large nuclei, marginated chromatin, prominent nucleoli, and a moderate amount of violet-stained cytoplasm. Mitoses, including atypical forms, were numerous. There were areas of necrosis with neutrophilic infiltration. Giemsa's stain disclosed the presence of

numerous metachromatic granules in the cytoplasm of the tumor cells. The neoplasm was diagnosed as a mastocytoma.

The dog was readmitted on April 13, 1964. Two large ulcers were present on the dorsal surface of the left forepaw, and the left prescapular lymph node was enlarged. The hemogram revealed: hemoglobin, 14.6 gm./100 ml.; packed cell volume, 44%; total leukocytes, 6340/cu. mm.; segmented neutrophils, 83%; lymphocytes, 14%; eosinophils, 2%; and monocytes, 1%. The erythrocyte sedimentation rate was 30 mm./60 minutes, in contrast with the expected sedimentation rate of 6 mm./60 minutes. Biopsies of the forepaw and the prescapular lymph node were performed. The tissue excised from the paw measured 1.7 x 1.0 x 0.3 cm. It was flattened, tan, and firm with an ulcerated surface. Microscopic examination revealed an ulcerated epidermal surface infiltrated by neutrophils. Beneath this was a dense aggregation of mastocytes. The only remnants of adnexa present were some broken hair shafts and a few dilated sweat glands. The excised prescapular lymph node was 2.0 x 1.7 x 1.0 cm. in size, round, white, and soft. Histopathologic examination revealed only the stroma to be intact. The cell population of the node consisted exclusively of mastocytes. No lymphocytes were evident. This neoplasm was diagnosed as a mastocytoma with lymph node metastasis.

The dog died 10 hours after surgery. At necropsy, the lungs were found to be congested. Petechiae were present on the surface of the kidneys. The liver was fatty and had a spongy consistency. Multiple hematocysts were present in the spleen. Multiple small calculi were present in the bladder. Microscopically, sections of the liver revealed fatty metamorphosis, having a midzonal to centrolobular distribution. There were irregular areas of necrosis and

nodular regeneration. The spleen was hypocellular and fibrotic and contained hematocysts. The lung was congested and contained foci of anthracosis. Multiple sections of the lesion involving the forepaw and lymph node were as described above. Some, in addition, contained eosinophils diffusely distributed throughout the neoplasm. Enzymatic activities, as determined in the serum and tumors of this dog, are recorded in TABLE 47.

TABLE 47. Enzymatic activities relating to Tumor 46.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	2/18	1.06	251		255		11		69	
Tumor	2/20	6.9	2314	96	9539	191	218	4.4	553	11.1
Serum	2/22	1.76	1350		491		20		22	
Serum	4/13	0.8			139				10	
Tumor	4/13		72300						161	

Tumor 47 was obtained from a male Boxer 6 years of age. This dog was presented on March 12, 1964, because of a large mass on the ventro-lateral aspect of the thorax at the level of the eighth and ninth costo-chondral junctions. Neoplasms had been excised from this site by the referring veterinarian 5 months and a month prior to admission and had recurred. The hemogram revealed: hemoglobin, 18.2 gm./100 ml.; packed cell volume, 52%; leukocytes, 11,300/cu. mm.; segmented neutrophils, 77%; nonsegmented neutrophils, 2%; lymphocytes, 19%; and eosinophils, 2%. The neoplasm was surgically excised. The tissue obtained at surgery was a section of skin having a nodular, tumorous

subcutis. The tumorous aggregate measured 12 x 8 x 1 cm., was non-encapsulated, yellow, and soft. Microscopically, it was found to be a multilocular mass with dermal adnexa on 1 surface. The cells were pleomorphic and in random arrangement or in cords. The cells had clear-to-violet-stained cytoplasm and large, oval vesicular nuclei with prominent nucleoli. The lobules were separated by dense fibrous bands which were infiltrated by similar cells. Mitoses were very numerous. Focally, the neoplasms contained areas of necrosis with neutrophilia and abundant neocapillaries. This neoplasm was diagnosed as a mastocytoma.

On May 21, 1964, 3 additional subcutaneous nodules were excised from the skin of the abdomen. Histopathologic examination revealed that these, also, were mastocytomas. Nodules removed at the same time from the medial surface of the right thigh and from the gingiva were found to be non-neoplastic. Additional mastocytomas were excised on October 26 and 28 and on November 9, 1964. The last of these was histopathologically considered to be invasive. Bone marrow which was aspirated on November 9 was interpreted as being hyperplastic, with selective depression of the granulocytic elements. Enzymatic activities, as determined in the serum and tumor tissue of this dog, are recorded in TABLE 48.

TABLE 48. Enzymatic activities relating to Tumor 47.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	3/12	1.19	198		125		22		20	
Tumor	3/12	6.4	62660	1144	10603	194	495	9.0	242.	4.3
Serum	3/14	0.72			93				14.	
Serum	5/7	0.89			130				6.4	
Serum	5/21	1.28			106				4.3	

Tumor 48 was obtained from a spayed, female, mongrel terrier 13 years of age. This dog was presented because of a swelling of the left foreleg which had been present for 2 months. The owner noted that the swelling appeared to increase and regress in size. A soft, tan, irregular mass, parallel to the radius and ulna, was excised. It was 12 cm. long and 3.5 x 1.5 cm. in the smaller dimensions. Microscopic examination revealed a fragmented piece of skeletal muscle, bone, and connective tissue. These tissus were embedded in, and infiltrated by, cells randomly arranged and in loose rows. The cells had abundant violet-stained cytoplasm and round, oval, or reniform nuclei with stippled chromatin. Occasional mitoses were present. This neoplasm was diagnosed as a mastocytoma. Enzymatic activities, as determined in the serum and tumor of this dog, are recorded in TABLE 49.

TABLE 49. Enzymatic activities relating to Tumor 48.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	3/20	0.56	104		125		14		15.	
Tumor	3/25	3.8	18280	410	13242	61	268	5.7	107.	2.1
Serum	3/27	0.56			130				1.7	

Tumor 49 was obtained from a 10-year-old female Scottish Terrier. This dog was presented on March 19, 1964, because of a mass in the left axilla. Hemoglobin level and packed cell volume fell within the normal range. Total leukocytes were 14,100/cu. mm., with 70% segmented neutrophils, 3% nonsegmented neutrophils, 19% lymphocytes, 6% eosinophils, and 2% monocytes. A rounded, tan, soft mass, 2.0 x 2.0 x 1.2 cm., was excised from the left axillary space. Microscopic examination revealed a piece of very lightly keratinized epidermis with adnexa. In the dermis and subcutis was a diffuse aggregation of uniform round cells with abundant violet-stained cytoplasm and round nuclei with a stippled chromatin pattern. The cells tended to be arranged in rows. The neoplasm was diagnosed as a mastocytoma. Enzymatic activities, as determined in the serum and in the tumor of this dog, are recorded in TABLE 50.

TABLE 50. Enzymatic activities relating to Tumor 49.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	3/19	1.53	142		162		28		34	
Tumor	3/20	1.93	6748	207			299	9.1	430	13.4
Serum	3/23						25		7	

Tumor 50 was obtained from a male Boston Terrier, 9 years of age. This dog was initially presented on February 16, 1962, because of a mass postero-medial to the right stifle joint, which had increased noticeably shortly prior to presentation, and a small nodule on the left side of the scrotum which had not undergone any noticeable change in size. The lesion near the stifle was excised and, on histopathologic examination, was found to be a mastocytoma. On October 31, 1962, bilateral orchiectomy was performed. Histopathologic examination of the testes revealed no abnormality in the right testis, and tubular atrophy in the left. On December 30, 1963, the dog was presented because of 3 nodules in the wall of the scrotum. These nodules were excised. One nodule in the scrotum was located, grossly, in the dermis. It was spherical, 1.0 cm. in diameter, and white in color. The remaining 2 nodules were 1.5 x 1.0 x 1.0 cm. and 1.5 x 0.7 x 0.3 cm., respectively, in size. The last of these is considered in this study as Tumor 74. Histopathologic examination of the first 2 of the scrotal nodules revealed similar findings. These were covered by lightly keratinized, pigmented epithelium with adnexa. Under the epidermis was a collagenous mass traversed by bundles of smooth muscle. Within the collagen were multiple nodules of round cells with pale round nuclei and abundant, gray-staining cytoplasm. Giemsa's stain confirmed the identity of these cells as mastocytes. These lesions were diagnosed as mastocytomas. Enzymatic activities, as determined in the serum and tumor of this dog, are recorded in TABLE 51.

TABLE 51. Enzymatic activities relating to Tumor 50.

Specimen	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	0.52	43	65	22	8.6
Tumor		48	0	44	75.2

Tumor 51 was obtained from the same dog as Tumor 38, q.v. for history and physical findings. On February 24, 1964, a mass was excised from the skin dorsal to the left shoulder. This mass was round, firm, cream-colored, and measured 2.0 x 1.8 x 1.2 cm. Microscopically, it was found to be covered by thin stratified squamous epithelium with adnexa. The tumor was located in the subcutis, and consisted of round cells individually located or in chains. These cells had round nuclei and scanty violet-staining cytoplasm. Numerous eosinophils were scattered throughout. The tumor cells invaded the underlying musculature. The mass was diagnosed as a mastocytoma.

On July 2, 1964, a nodule appeared on the left shoulder, which was clinically diagnosed as a recurrence of the mastocytoma and was treated with x-irradiation, being given a total dose of 5000 r. On September 2, a mass, supposedly of 2 days' duration, was excised from the shoulder anterior to the scapula. This mass measured 9.0 x 3.5 x 2.0 cm., was white and firm, and appeared encapsulated. Microscopic examination revealed the capsule and a few foci of lymphocytes as remnants of the underlying architecture of the prescapular lymph node. The remainder of the section consisted of a dense aggregate of mastocytes as seen in the previously described neoplasm. On October 26, 1964, the dog was again presented with a history of vomiting,

anorexia, and posterior weakness. The hemogram at this time revealed: hemoglobin, 11.95 gm./100 ml.; packed cell volume, 33%; erythrocyte sedimentation rate, 33 mm./60 minutes, contrasted with an expected rate of 19 mm./60 minutes. Total leukocytes were 7750, with 61% segmented neutrophils and 39% lymphocytes. On November 5, an aspiration of femoral bone marrow was performed. Smears and sections of the bone marrow revealed that the predominant cell type present was the neoplastic mastocyte. These constituted 70% of all cells present. The remaining bone marrow elements were scarce, but morphologically normal. A hemogram at this time revealed: hemoglobin, 8.45 gm./100 ml.; packed cell volume, 25%; erythrocytes, 3.2 million/cu. mm.; leukocytes, 29,550/cu. mm.; segmented neutrophils, 72%; nonsegmented neutrophils, 1%; lymphocytes, 23%; and malignant mastocytes, 4%. Euthanasia and necropsy were performed on November 6, 1964. At necropsy, the spleen was found to be enlarged and purple in color, with many raised nodules. Three duodenal ulcers were present. No other significant lesions were noted. Microscopically, the left axillary lymph node was entirely replaced by mastocytoma which penetrated the capsule and invaded the surrounding tissue. Areas of hemorrhage and hemosiderosis were also present. The hepatic sinusoids contained numerous mastocytes. Small nodular aggregates of mastocytes were also scattered through the liver. The remaining hepatic architecture appeared normal. Some of the renal tubules contained albuminous casts. Otherwise, the renal architecture appeared normal. However, Giemsa's stain revealed the presence of numerous mastocytes singly within vessels, especially within the glomerular capillaries. The final diagnosis was mastocytoma with metastases to lymph nodes and

bone marrow, terminating in mast cell leukemia. Enzymatic activities determined in the serum and tumor tissue of this dog are recorded in TABLE 52.

TABLE 52. Enzymatic activities relating to Tumor 51.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	2/24	1.01	116		185		27		0	
Tumor	2/24	4.5			2778	49	153	2.7	32	0.5
Serum	2/27	2.2	202		407		27		7	

Tumor 52 was obtained from a male Scottish Terrier 6 months of age. This dog was presented on March 12, 1964, because of multiple nodules present in the skin of the ventral abdomen. The skin over the nodules appeared hyperemic. The nodules were circumscribed, elevated and hard. A similar nodule had been excised from the lateral surface of the right foreleg 2 months previously. Within 2 weeks following this excision, the nodule recurred and additional nodules appeared on the abdomen as indicated. The hemogram on the day of admission revealed: hemoglobin, 15.5 gm./100 ml.; packed cell volume, 45%; leukocytes, 15,950/cu. mm.; segmented neutrophils, 69%; lymphocytes, 31%; erythrocyte sedimentation rate, 5 mm./60 minutes, identical to the expected value. A section of skin measuring 2.9 x 0.9 x 0.7 was surgically excised. This contained 2 round gray nodules, each about 1.0 cm. in the greatest diameter. Microscopic examination revealed sections of skin with discrete, nonencapsulated but circumscribed nodules in the dermis. These consisted of densely aggregated cells randomly arranged. These cells

had large round or oval nuclei, which were rather hyperchromatic and stippled with chromatin, and abundant pale-violet-staining cytoplasm.. Mitoses were focally quite numerous. Giemsa's stain revealed the presence of cytoplasmic mast granules. These nodules were diagnosed as mastocytomas. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 53.

TABLE 53. Enzymatic activities relating to Tumor 52.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	3/12	1.58	171		38	17.2
Serum	3/27	0.69	48	120	22	12.9

Tumor 53 was obtained from the same dog as Tumor 4, q.v. for history and physical findings. Five large neoplasms were excised from various sites of the skin of this dog on December 14, 1963. A mass from the right perineal region measured 2.0 x 2.2 x 1.2 cm. It was covered by highly pigmented, hairless skin. Microscopic sections revealed a mass covered by stratified squamous epithelium. Over the mass, the epithelium was in part ulcerated and infiltrated by neutrophils. No intact adnexa were present over the mass, though they were present in the periphery of the section. The mass was composed predominantly of round cells in the dermis and subcutis, randomly arranged and in rows. The cells had round, pale nuclei with nucleoli and abundant, granular, gray-violet-staining cytoplasm. Eosinophils and some neutrophils were scattered throughout the tumor, as were areas of hemorrhage. Within the tumor were cystic hair follicles

and a large epidermal inclusion cyst. The neoplasm was diagnosed as a mastocytoma. A mass excised from the left perineal region was 9.0 x 7.5 x 3.0 cm. in size. It was firm and white, covered by skin, and appeared encapsulated. Microscopically, the mass was composed predominantly of collagenous connective tissue throughout which numerous mastocytes were scattered. This lesion was also diagnosed as a mastocytoma. The mass excised from the left gluteal region was 6.5 x 6.0 x 3.0 cm. in size. It was very firm, tan, and appeared encapsulated. Microscopically, it was similar to the previous neoplasm. Within the mass were multiple foci formed by dense aggregations of mastocytes. The largest of these was 0.1 cm. in diameter. The diagnosis of this mass was the same as the preceding. The mass from the left gluteal region is considered in this study as Tumor 73. The mass from the third left mammary gland was 6.5 x 6.0 x 2.5 cm. in size, firm, and white, and appeared encapsulated. It did not appear attached to the epidermis, on gross examination. Microscopically, it was found to be composed predominantly of fibrous connective tissue infiltrated by mastocytes, similar to the neoplasms described above. Within this section of the mass lay a nodule 0.5 x 1.0 cm. This nodule is described as Tumor 4. The mass was diagnosed as a squamous carcinoma within a mastocytoma. Additional sections of the same mass were similar but failed to contain cross sections of the squamous carcinoma. On January 2, 1964, 7 small nodules were excised from the skin, the largest of these measuring 0.3 cm. in its greatest diameter. Microscopically, 2 of these proved to be mastocytomas, while the remainder were chronic inflammatory lesions. On January 8, 3 additional small skin lesions were excised. One of these proved to

be a mastocytoma, the second an epidermal inclusion cyst, while the third was a chronic inflammatory lesion. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 54.

TABLE 54. Enzymatic activities relating to Tumors 53 and 73.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	12/13/63	0.69	24	83	40	80.
Serum	12/26/63	0.80	111	125	37	16.6
Serum	1/3/64	1.04	48	111	36	0.
Serum	1/27/64	0.97	67	97	32	7.5
Serum	3/14/64	2.11	410	268	39	20.4
Serum	5/14/64	0.69	183	148	17	4.3

Tumor 54 was obtained from the same dog as Tumor 34, q.v. for history and physical findings. On February 18, 1964, tumors were excised from the left thigh, left stifle, medial surface of right foreleg at the humeral midshaft, medial surface of the right hock, and 2 tumors from the skin of the ventral thorax. All of these nodules were small, measuring from 0.1 x 0.1 x 0.1 cm. to 1.8 x 1.5 x 0.4 cm. Microscopically, all revealed infiltration of the dermis with masses of mastocytes among which occasional eosinophils were seen. All of these tumors were diagnosed as mastocytomas. Two months later, when several additional primary neoplasms of the skin were excised, none proved to be new or recurrent mastocytomas. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 55.

TABLE 55. Enzymatic activities relating to Tumor 54.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/18	1.28	251	231	39	25.8
Serum	4/22	1.19	255	153	44	8.1

Tumor 55 was obtained from the same dog as Tumor 36, q.v. for history and physical findings. On February 25, 1964, 2 nodules were excised from the left hind leg of this dog. They measured 1.1 x 1.0 x 0.3 cm. and 1.8 x 1.5 x 0.8 cm., respectively, and were irregular, soft, and white. Grossly the larger of the 2 appeared encapsulated. Microscopic examination revealed that both had a similar morphology. In the smaller, the neoplastic infiltration involved predominantly the dermis, in the larger, the subcutis. The neoplastic cells were arranged randomly or in chains. The cells were round with abundant gray-staining cytoplasm and large, round, hyperchromatic nuclei with some margination of chromatin. In the larger mass, groups of cells were separated by bands of collagen. These neoplasms were diagnosed as mastocytomas. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 56.

TABLE 56. Enzymatic activities relating to Tumor 55.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/25	1.27	82	153	18	10.2
Serum	3/3		111		15	29.

Tumor 56 was obtained from a 6½-year-old male crossbred Collie-type dog. This dog was presented because of a mass in the right lumbar area which had been present for 2 months and which had enlarged noticeably during the 2 weeks prior to admission. The mass was surgically excised and submitted in 3 fragments having a combined size of 2.0 x 1.5 x 1.5 cm. The tissue was firm, glistening, and white without evidence of a capsule. Microscopically it was composed of plump cells suggesting active fibroblasts. The tumor cells had large chromatin-rich nuclei, many of which contained nucleoli. Focally there were multinucleated tumor giant cells. The tumor was rather cellular with areas of hemorrhage and deposits of eosinophilic proteinaceous material. It was diagnosed as a fibrosarcoma. Enzymatic activities, as determined in the serum of this dog and in the tumor, are recorded in TABLE 57.

TABLE 57. Enzymatic activities relating to Tumor 56.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.97	53		97		20		18.8	
Tumor	3.3	1109	21	9167	161	243	4.5	59.1	1.1

Tumor 57 was obtained from the same dog as Tumor 20, q.v. for history and physical findings. This mass on the lateral surface of the prepuce measured 3.5 x 3.5 x 5.0 cm. was first excised by the attending veterinarian 8 months prior to presentation. It recurred and, 6 months after the first excision, a mass 8 cm. in diameter was excised from the same site. At the time of presentation, an ulcerated

neoplasm 10.0 x 8.0 x 4.5 cm. was present in the perianal area. A biopsy of this neoplasm was performed on February 27, 1964. The tissue obtained at biopsy was irregular, pink, and soft. Microscopic examination revealed a tumor mass covered by skin with adnexa. Circumanal glands were present in the subcutis superficial to the tumor. The tumor was composed of plump, fusiform cells arranged irregularly and in bundles. The cells had oval hyperchromatic nuclei and eosinophilic, fibrillar cytoplasm. Mitotic figures, including atypical forms, were very numerous. Focally, there were areas of necrosis and neutrophilia. This tumor was diagnosed as a fibrosarcoma.

Euthanasia and necropsy were performed on March 3, 1964. At necropsy, no significant lesions were found except the neoplasm to the left of, and ventral to, the anus. This was white and firm and measured 10 x 8 x 5 cm. The skin covering it was focally ulcerated and gross foci of necrosis were identifiable within the tumor mass. Microscopically, it was similar to the tissue obtained at biopsy. Necropsy findings confirmed the biopsy diagnosis. Enzymatic activities, as determined in the serum of this dog and in the tumor, are recorded in TABLE 58.

TABLE 58. Enzymatic activities relating to Tumor 57.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	2/24	0.48	188		389		22		9.7	
Tumor	2/27	6.9	17352	434	4676	117	357	8.9	177.	4.3
Tumor	3/3	15.6	3278	91	93	3	160	4.4	54.	1.6
Serum	3/3	1.01	400		569		19		1.1	

Tumor 58 was obtained from the same dog as Tumor 8, q.v. for history and physical findings. On February 25, 1964, a mass, located in the subcutaneous tissue ventral to the right ear, was excised. Grossly this mass was rounded, white, firm and appeared encapsulated. It measured 3.0 x 2.5 x 2.0 cm. Microscopically it was found to consist of a dense aggregate of collagenous fibers and a small number of mature fibrocytes. Some bundles of the underlying musculature lay adjacent to the tumor. This mass was diagnosed as a fibroma durum. Enzymatic activities, as determined in the serum of this dog and in the tumor, are recorded in TABLE 59.

TABLE 59. Enzymatic activities relating to Tumor 58.

Specimen	Date	HB	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	2/10	0.6	34		106		14		49.	
Serum	2/14	0.45	29		97		15		58.	
Tumor	2/25	6.4	771	12	4074	63	29	0.47	16.1	0.3

Tumor 59 was obtained from a female Boston Terrier 10 years of age. This dog was initially presented on January 23, 1961, because of a large soft mass which had been present in the right perineal region for 2 years and was enlarging slowly. The mass was excised and diagnosed grossly and histopathologically as a lipoma. On November 6, 1963, the dog was returned because of a pendulous neoplasm to the right of, and ventral to, the anus (Tumor 86) and a small wart-like mass on the skin of the left side of the thorax near the eighth rib. This nodule was excised. Microscopic examination revealed a mass of

collagenous fibers and fibrocytes lying in the dermis covered by intact epidermis. The lesion was diagnosed as a fibroma. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 60.

TABLE 60. Enzymatic activities relating to Tumor 59.

Specimen	Date	Hb	LDH	LAP	Alk. Ptase.
Serum	11/6	0.81	44	25	26.3
Serum	11/8	0.64	92	22	54.2

Tumor 60 was obtained from the same dog as Tumor 3, q.v. for history and physical findings. On March 11, 1964, a small nodule was excised from the skin of the back of this dog. Histopathologic examination revealed a mass of collagen and mature fibrocytes, irregularly arranged, lying in the dermis and covered by intact epidermis. This lesion was diagnosed as a fibroma. Enzymatic activities, as recorded in the serum of this dog prior to surgery, are recorded in TABLE 61.

TABLE 61. Enzymatic activities relating to Tumor 60.

Hb	LDH	PHI	LAP	Alk. Ptase.
0.60	29	116	29	0.5

Tumor 61 was obtained from the same dog as Tumor 34, q.v. for history and physical findings. Tumor 61 was excised from the skin of the ventral abdomen on February 18, 1964. Microscopic sections of this small nodule revealed a normal epidermis overlying a dense, collagenous, hypercellular mass, in which mature fibrocytes were the mature cell type. This mass was diagnosed as a fibroma. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 62.

TABLE 62. Enzymatic activities relating to Tumor 61.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	2/18	1.28	251	231	39	25.8
Serum	4/22	1.19	255	153	44	8.1

Tumor 62 was obtained from a spayed, female, crossbred, Beagle-type dog 12 years of age. This dog was presented to the attending veterinarian on December 10, 1963, because of a mass which had been present for a month on the oral surface of the left lip. The mass was excised, but recurred a month later. It was noted to be very sensitive and in proximity to the facial nerve. It was again excised, and submitted for microscopic examination. The mass was firm, white, spherical, and appeared encapsulated. Microscopically, it was found to be composed of long fusiform cells. The nuclei were markedly elongated and many appeared curled or wavy. The cytoplasm was eosinophilic, but not abundant. The cells lay parallel in bundles which swirled and had a wavy arrangement. The tumor invaded muscle and remnants of a few muscle bundles were scattered among the tumor cells. Focal aggregates of lymphocytes were present. In the periphery, tumor emboli were present in the lumen of lymphatic vessels. One surface of the tumor was covered by skin and adnexa. Masson's trichrome stain indicated a high content of collagen within the tumor. It was diagnosed as a neurofibrosarcoma. On January 13, 1964, the dog was referred to the Ohio State University Veterinary Clinic. Abnormal physical findings were limited to several nodules which were present on the upper lip. Hemogram at this time revealed: hemoglobin, 16.55 gm./100 ml.; packed cell volume, 47%; erythrocyte sedimentation rate, 54 mm./60 minutes, contrasted with an expected value of 3 mm./60 minutes. Total leukocyte count was 6650/cu. mm., with 79% segmented neutrophils, 20% lymphocytes, and 1% monocytes. Thoracic radiographs revealed no pulmonary lesions. On January 15 a biopsy of the neoplasm was taken. The tissue obtained at biopsy was firm and light brown, and measured 1.8 x 1.5 x 1.9 cm. Microscopically it was identical to the previously excised tumor.

X-irradiation therapy was administered from February 14 until March 13. On May 6, physical examination revealed hard, swollen mandibular lymph nodes. A fluctuant mass was palpated in the cervical region anterior to the manubrium sterni. The mouth had a foul odor, and necrosis of the surface of the tumorous upper lip was evident. There was depigmentation of the lip about the tumor. A hemogram at this time revealed: hemoglobin, 16 gm./100 ml.; packed cell volume, 46%; erythrocyte sedimentation rate, 36 mm./60 minutes, contrasted with an expected value of 4 mm./60 minutes; total leukocytes, 11,250/cu. mm.; segmented neutrophils, 72%; nonsegmented neutrophils, 1%; lymphocytes, 21%; eosinophils, 5%; and monocytes, 1%.

On May 16, 1964, euthanasia and necropsy were performed. At necropsy, bilateral senile cataracts were noted. The mandibular lymph nodes were round, firm, and white, with small dark areas scattered throughout. They measured 2.5 x 2.0 x 2.0 cm. The retropharyngeal lymph nodes were congested and 1.0 x 1.0 x 3.0 cm. in size. The fluctuant mass in the anterior thoracic area was identified as adipose tissue. The lungs were anthracotic. Microscopically, a section of the skin from the left maxillary region was found to be covered by parakeratotic epithelium with deep rete pegs and without adnexa. Some melanocytes were present immediately under the epithelium. The epithelium was in part ulcerated. Below the epithelium was a zone of fibrosis and necrosis in which were many necrotic neutrophils. No viable tumor cells were evident. This lesion was regarded as radiation necrosis. In an adjacent area, thickening of the vessel walls, especially of the media, was noted. Thrombosis of some of the vessels had occurred. A marked histiocytic and lymphocytic infiltration was present. In an area of coagu-

lation necrosis, a swirling pattern was detected in the necrotic cells. Most of the architecture of the left maxillary lymph node was replaced by metastatic neurofibrosarcoma, having a microscopic appearance similar to the primary tumor. Examination of the lungs and bronchial lymph node revealed only anthracosis. Examination of the right mandibular and left and right pharyngeal lymph nodes revealed only lymphocytic hyperplasia. The spleen was congested, had hyperplasia of the reticulum cells, and contained hemosiderin-filled macrophages. Enzymatic activities, as determined in the serum and in the tumor of this dog, are recorded in TABLE 63.

TABLE 63. Enzymatic activities relating to Tumor 62.

Specimen	Date	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	1/14	0.45	24	42	24	65
Tumor	1/15	2.2	5013	28428	43	32
Serum	1/17	0.52	92	125	19	7

Tumor 63 was obtained from the same dog as Tumor 12, q.v. for history and physical findings. A neoplasm was first excised from the left foreleg of this dog by the attending veterinarian in 1956. This recurred and was re-excised several times. Neoplastic tissue excised by the attending veterinarian on April 29, 1959, was an ovoid, lobulated, soft mass, which measured 3.0 x 2.5 x 2.0 cm. Microscopically, it was found to be covered by thinly keratinized skin with adnexa. It was composed of large masses of cells which were occasionally interrupted by fibrous connective tissue or areas

of necrosis. The tumor cells had nuclei which varied from round to fusiform. Although in some areas the tumor cells appeared to lie parallel to one another, no organoid formation was evident. The cells had eosinophilic cytoplasm with indistinct cytoplasmic boundaries. The round nuclei were vesicular, with prominent nucleoli. As the nuclei became more elongated, they also became more hyperchromatic. Mitotic figures were extremely numerous.

On July 13, 1962, the dog was referred to the Ohio State University Veterinary Clinic because of regrowth of the mass on the posterior aspect of the left foreleg halfway between the carpus and radio-humeral joint. Pulmonary radiographs revealed a nodule 3 cm. in diameter in the left diaphragmatic lobe of the lung. Physical examination revealed a soft fluctuant mass in the ventral thoracic area which was diagnosed by palpation as a lipoma. The spherical mass was excised from the left foreleg and found to be 1.5 cm. in diameter, mottled red and white, soft in consistency, and grossly appearing encapsulated. Microscopically it was found to be partly covered by thin, lightly keratinized epithelium with adnexa, which was focally ulcerated. In the center of the mass were relatively acellular areas of necrosis and edema. The tumor cells were fusiform with fibrillar eosinophilic cytoplasm and oval nuclei whose axes paralleled the cytoplasm. Some of the nuclei were vesicular with prominent nucleoli, while others were hyperchromatic. The cells lay parallel to one another, principally in whorls. Masson's trichrome stain revealed the presence of thin collagen strands between the tumor cells. This tumor was diagnosed as a neurofibrosarcoma. Following the excision of this tumor, x-irradiation therapy was applied to the left foreleg. A total of 3500 r was administered

between July 13 and August 17, 1962. On September 29, another biopsy of the left foreleg was performed. Two irregular fragments of tissue were obtained. One was 2.0 x 0.9 x 0.5 cm., firm and yellow to brown in color, while the other was 2.0 x 1.0 x 0.7 cm., tan in color, and soft. Microscopically, these were found to consist of necrotic connective tissue, highly vascular, with areas of hemorrhage and thrombosis. Individual viable-appearing cells with vesicular nuclei and prominent nucleoli were present, as were foci of lymphocytic infiltration. Because of the findings of this biopsy, the left foreleg was amputated at the humero-scapular joint on October 13, 1962. At the same time, the soft subcutaneous mass described above was also excised. The specimen obtained by amputation consisted of a foreleg with an area of ulceration 7.0 x 4.0 x 0.5 cm. in size, deep on the midvolar aspect of the radius and ulna. Microscopic examination revealed sections of ulcerated skin with underlying muscle. Large areas of radiation necrosis were evident. No viable-appearing tumor cells could be recognized. The subcutaneous mass was 1 x 2 x 1 cm., soft, and yellowish-white. Microscopic examination revealed that it consisted entirely of mature adipose cells and confirmed the diagnosis of lipoma.

On January 5, 1963, a nodule 2 cm. in diameter was palpated in the posterior costo-chondral region. On December 14, 1963, thoracic radiographs disclosed that the pulmonary nodule described above was now 5 cm. in diameter. A second nodule 2.5 cm. in diameter was present in the mediastinum or right apical area. On March 21, 1964, the tumor in the left superior cervical area, considered in this study as Tumor 12, was excised. On November 22, 1964, the dog

was presented with a history of a staggering gait and in a markedly deteriorated condition; euthanasia was performed. Neoplasms which were microscopically found to be composed of whorls of fusiform cells similar to the biopsy described above were found in the right infraspinatus muscle, the second left mammary gland, and the left diaphragmatic, right apical, intermediate, and the right cardiac lobes of the lung. The liver was grossly irregular due to the presence of regenerative nodules. Hemorrhagic enteritis was present in the small intestine. The mitral valve had lesions of chronic valvular endocarditis. Enzymatic activities, as determined in the serum of this dog, are recorded in TABLE 64.

TABLE 64. Enzymatic activities relating to Tumor 63.

Specimen	Date	Hb	PHI	LAP	Alk. Ptase.
Serum	3/20	0.8	125	41	51
Serum	3/27		130		79

Tumor 64 was obtained from a 6-year-old female Boxer. This dog was presented on February 7, 1964, because of weakness of the hind legs. Rectal palpation revealed a firm, nodular mass in the dorsal wall of the rectum. The mass was excised and found to be 3.2 x 2.2 x 1.8 cm. in size, irregularly lobular, firm and cream-colored. Microscopically, it was found to consist of a mass of fusiform cells arranged in interlacing bundles. The cells had small, dark, oval nuclei and abundant cytoplasm. Masson's trichrome stain revealed a moderate amount of collagen. This tumor was diagnosed as a leiomyofibroma. Enzymatic activities, as determined in the serum of this dog

and in the tumor, are recorded in TABLE 65.

TABLE 65. Enzymatic activities relating to Tumor 64.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	2/5	0.32	43		88		16		9.7	
Tumor	2/7	1.5	24100	502	10001	208	1513	32	53.7	1.1
Serum	2/11	0.56	46		120		36		24.2	

Tumor 65 was obtained from the same dog as Tumor 6, q.v. for history and physical findings. Tumor 65 was a firm, white, rough mass 1.5 x 1.5 x 2.0 cm., located in the wall of the gallbladder. Microscopic examination revealed a mass covered by gallbladder epithelium with lymphocytic infiltration. Under the epithelium was a mass of interlacing bundles of smooth muscle in orderly arrangement. The nuclei were fusiform, but tended to be flattened on 1 side. The cytoplasm, the boundaries of which were indistinct, was eosinophilic, abundant, and fusiform. This neoplasm was diagnosed as a leiomyoma. Enzymatic activities, as determined in the serum of this dog and in the tumor, are recorded in TABLE 66.

TABLE 66. Enzymatic activities relating to Tumor 65.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	1.06	72		157		27		94.5	
Tumor	10.1	916	64	463	32	243	17	172.	11.8

Tumor 66 was obtained from the same dog as Tumor 7, q.v. for history and physical findings. Tumor 66 consisted of 2 nodules found in the wall of the vagina at necropsy, 1 located in the dorsolateral wall and measuring 3 x 3 x 3 cm., and the other located in the ventral wall and measuring 1.5 x 1.5 x 1.0 cm. Microscopically, both were similar and consisted of interlacing bundles of fusiform cells, having elongated nuclei flattened on 1 side and abundant eosinophilic cytoplasm. These were diagnosed as leiomyomas. Enzymatic activities, as determined in the serum of this dog prior to necropsy, are recorded in TABLE 67.

TABLE 67. Enzymatic activities relating to Tumor 66.

Hb	LDH	PHI	LAP	Alk.Ptase.
0.64	54	74	20	18.8

Tumor 67 was obtained from the same dog as Tumor 18, q.v. for history and physical findings. Tumor 67 involved the left forefoot, which was swollen to about twice the normal size from the carpus distally. Radiographs revealed areas of osseous proliferation and areas of lysis. At necropsy, the bones of the metacarpus were found to be soft and were surrounded by both bony and soft-tissue proliferation. A cyst 1.5 cm. in diameter was located over the metacarpus. Post-mortem radiographs revealed osteolysis of the metacarpal bones with pathologic fractures and subperiosteal proliferation. Microscopically the mass was found to consist of a very pleomorphic aggregate of cells having no definitive pattern of arrangement. The

individual cells were elongated with violet-stained cytoplasm and with very large hyperchromatic nuclei whose long axes paralleled the long axes of the cells. Among the tumor cells were numerous strap cells, while other cells were not so well differentiated. No cross-striations could be identified with certainty in the tumor cells, but some of them had multiple nuclei in tandem. In 1 section of the tumor, a vessel contained an embolus of the bronchial adenocarcinoma described as Tumor 30. Adjacent to the tumor was an area of fibrosis with remnants of skeletal muscle cells throughout it. Many of these muscle cells appeared to make abortive attempts at regeneration, as seen by multiple nuclei. This tumor was diagnosed as a rhabdomyosarcoma; however, not all consultants to whom this tissue was shown concurred in this diagnosis. Sections of the tumor are illustrated in Figures 1 to 4. Enzymatic activities, as determined in the serum of this dog and in the tumor, are recorded in TABLE 68.

TABLE 68. Enzymatic activities relating to Tumor 67.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	0.45	22		97		16		16.1	
Tumor	2.8			6019	334	262	14.6	263.	14.5

Tumor 68 was obtained from a spayed female Boxer 7 years of age. This dog was presented because of posterior paresis and arthritis which had progressed over the 4 months prior to admission. The dog was incontinent of urine. Physical examination revealed a mass in the pelvic canal on the right side. Edema was present in both hind legs.

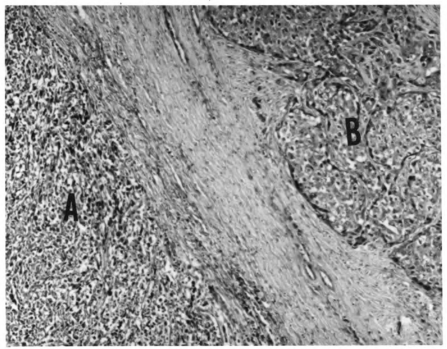

Figure 1. Tumor 67, diagnosed as a rhabdomyosarcoma, (A) in which a vessel contains an embolus of Tumor 30, a bronchial adenocarcinoma, (B). Hematoxylin and eosin. x87.5.

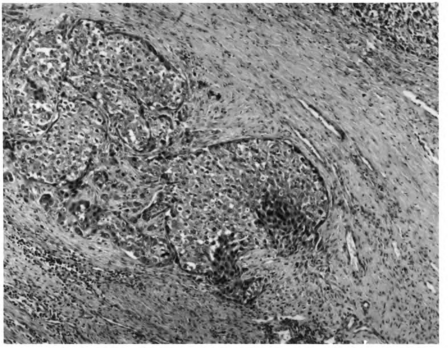

Figure 2. Embolus of Tumor 30 lying within the vessel passing through Tumor 67. Hematoxylin and eosin. x87.5.

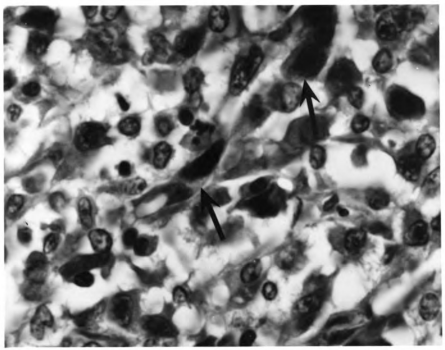

Figure 3. Tumor 67. Note strap cells. Hematoxylin and eosin. x875.

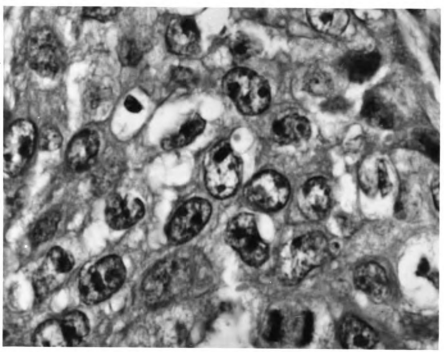

Figure 4. Embolus of Tumor 30 lying within the vessel passing through Tumor 67. Hematoxylin and eosin. x875.

Hemograms performed during the hospital course are recorded in TABLE 69.

TABLE 69. Host hematologic data relating to Tumor 68.

Date	Hb gm./ 100 ml.	PCV (%)	Leuk. /cu. mm.	Seg. Neut. (%)	Nonseg. Neut. (%)	Lymph. (%)	Eosin. (%)	Mono. (%)
10/29/63	15.5	46	13,000	79	2	16	1	2
10/31/63	12.25	39	6,000	79	2	17	1	1

On October 29, the erythrocyte sedimentation rate was 15 mm./60 minutes, contrasted with an expected value of 4 mm./60 minutes.

On October 31, an exploratory laparotomy was performed which was terminated by euthanasia. At necropsy, 2 masses were found in the left diaphragmatic lobe of the lung. One of these was approximately spherical, 2 cm. in diameter, and umbilicate, while the other was 4 x 2 x 2 cm. Small calcific nodules (ca. 0.1 cm. in diameter) were distributed throughout the lungs. A dark, hemorrhagic mass, 5.0 x 2.0 x 4.0 cm. and located adjacent to the right iliac shaft, compressed the adjacent vessels. Microscopic sections of the tumor revealed invasion of skeletal muscle by a hemorrhagic, necrotic neoplasm. Where the tumor cells were intact, they were plump to fusiform with round nuclei and large nucleoli. The tumor cells interlaced to form spaces filled with blood cells. Neutrophils were scattered throughout. Tumor cells forming structures as above were present in the iliac veins, the inguinal lymph node, and the 2 masses in the lung grossly described. Within the pulmonary metastases, vascular invasion by the tumor could be seen. Sections of the liver revealed congestion.

Sections of the kidneys revealed minimal chronic interstitial nephritis. The tumor was diagnosed as a hemangiosarcoma. Enzymatic activities, as determined in the serum of this dog and in the tumor, are recorded in TABLE 70.

TABLE 70. Enzymatic activities relating to Tumor 68.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.52	81				31		9.1	
Tumor	51.2	3133	261	463	38	155	12.9	1181.	98

Tumor 69 was obtained from a male German Shepherd 13 years of age. This dog was presented on January 8, 1964, because of an inability to urinate without catheterization during the preceding 5 weeks. An orchiectomy, performed at the onset of the dysuria, failed to influence the condition. Hematuria was present. The total leukocyte count was 18,250/cu. mm., with 94% segmented neutrophils and 6% lymphocytes. Palpation revealed a tumor of the penis. A penectomy was performed. One centimeter anterior to the distal end of the os penis and paralleling it on the ventrolateral aspect was an irregularly ovoid, tapered mass 1.7 x 3.0 x 5.0 cm. which was firm and black to red in color. At low magnification of the penis in cross section, the outline was found to be distorted by a tumor mass occupying the ventrolateral aspect and invading the remaining tissue. The urethra was compressed by necrotic tissue and the os penis partially destroyed. There was very extensive hemorrhage and necrosis. The mass was made up of plump to fusiform cells lying in dense clumps or outlining slits or

spaces, many of which were filled with blood. The cells were of moderate size, with nuclei that had marginated chromatin. Some of the cells had large nucleoli. A moderate number of mitotic figures was present. Emboli of the tumor were present in vessels. The tumor was diagnosed as a hemangiosarcoma.

Euthanasia and necropsy were performed on February 10, 1964. At necropsy, hemangiosarcomas were found in the lung, the spleen, and the renal cortex. Sections of the liver revealed midzonal fatty metamorphosis and diffuse bile stasis. Enzymatic activities, as determined in the serum of this dog and in the neoplasm, are recorded in TABLE 71.

TABLE 71. Enzymatic activities relating to Tumor 69.

Specimen	Date	Hb	LDH	PHI	LAP	Alk.Ptase.
Serum	1/8	0.97	63	93	20	8.1
Tumor	1/8	2.8	1518	1019	369	16.1
Serum	1/17	1.81	34	46	17	31.7

Tumor 70 was obtained from a male German Shepherd 9 years of age. This dog was presented because of a cough, hemorrhage from the penis and rectum, and weight loss. The onset of the present illness was considered to be 3 months prior to admission, at which time the dog was struck by an automobile, and after which he was noted to move stiffly. Two weeks prior to admission, hemorrhage from the penis and anus were noted and treated by the attending veterinarian. One week prior to admission, cough and anorexia were noted. At that time, the

attending veterinarian excised a tumor from beneath the anus. Physical examination on admission revealed a mass in the region of the spleen. Radiographs confirmed the presence of this mass and disclosed the presence of multiple pulmonary nodules. A hemogram revealed: hemoglobin, 8.8 gm./100 ml.; packed cell volume, 24%; erythrocytes, 3,670,000/cu. mm.; total leukocytes, 14,600/cu. mm., with 85% segmented neutrophils, 2% nonsegmented neutrophils, 10% lymphocytes, and 3% monocytes. Urinalysis revealed 100 mg. of albumin/100 ml., bile and occult blood, strongly positive; and, microscopically, very numerous erythrocytes. Euthanasia and necropsy were performed. All lobes of the lung contained multiple soft nodules ranging from 0.1 to 2.0 cm. in diameter. These nodules appeared to be filled with blood. Soft, dark red nodules 0.5 to 1.5 cm. in diameter were scattered over the surface of the pericardial sac. On the anterior surface of the quadrate lobe of the liver, 3 cm. ventral to the dorsal border, were 3 soft, dark-red nodules, 0.5 to 1.0 cm. in diameter. A similar mass, 1.5 cm. in diameter, was present on the lateral surface of the right lateral lobe. Many other similar nodules were present throughout the liver parenchyma. Many similar nodules, 0.1 to 1.5 cm. in diameter, were present in the mesentery and omentum, as were 4 large nodules, 4 to 6 cm. in diameter. A blood clot was present in the posterior abdominal cavity, together with 650 ml. of a deep red, serosanguineous fluid. The kidneys contained infarcts. The spleen contained 9 raised, dark-red nodules 0.5 to 1.0 cm. in diameter. A dark red-black mass, 4 x 3 x 3 cm., surrounded the prostate. In the prostatic parenchyma was a nodule 0.5 cm. in diameter and dark red in color. Numerous dark-red nodules were present throughout the brain.

Microscopically, sections of all dark-red nodules described above were similar and consisted of proliferating plump to fusiform endothelial cells surrounding blood-filled cavities. There was invasion by these endothelial cells into the adjacent tissues. Focally, within the tumors, were areas of necrosis. In the kidneys, albuminous casts and calcific deposits were present within the tubules. The neoplasms were diagnosed as hemangiosarcomas. Enzymatic activities, as determined in the serum of this dog and in 2 of the tumors, are recorded in TABLE 72.

TABLE 72. Enzymatic activities relating to Tumor 70.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.64	164		278		17		3.8	
Tumor	92.	169		9816		44		10.7	
Tumor	78.4	1253	13	10603	114	87	0.94	32.2	0.3

Tumor 71 was obtained from a male Springer Spaniel 12 years of age. The pertinent history indicated that a tumorous nodule had been excised from the left lower eyelid at the palpebral fissure in 1961. In 1963, a diagnosis of testicular tumors was made, and a bilateral orchiectomy performed.

On February 18, 1964, the animal was presented because of the development of dyspnea. Firm nodules were present on both sides of the trachea in the region of the thyroid. Radiographs revealed that the lungs were nodular in appearance and that the cardiac silhouette was enlarged. Electroencephalographic studies did not detect further

abnormalities. A urinalysis performed February 19 was within normal limits, except for the presence of 30 mg. albumin per 100 ml. and a strongly positive reaction for bile. A hemogram revealed: hemoglobin, 18.2 gm./100 ml.; packed cell volume, 52%; leukocytes, 5200/cu. mm.; segmented neutrophils, 82%; lymphocytes, 14%; and monocytes, 4%. A biopsy was performed on the left tumorous mass on the ventral surface of the neck. The tissue obtained by this biopsy was submitted in several pieces, 1 measuring 5.5 x 2.5 x 2.0 cm., 2 approximately spherical and 1 cm. in diameter, and 2 approximately spherical and 3 cm. in diameter. The masses were irregular in shape, soft, pink, and nonencapsulated. Microscopic examination revealed no remnants of normal organ structure. The tissue was composed of alternately cellular and necrotic areas. In the cellular areas nuclei appeared densely packed and little cytoplasm was evident. The individual cells were fusiform with round to elongated vesicular nuclei and prominent nucleoli. The cells in all but the most cellular areas lay in a circular arrangement, surrounding spaces. Some of these spaces appeared to be empty, others were filled with a uniform, proteinaceous eosinophilic substance (serum), while still others were filled with erythrocytes. Occasional neutrophils lay within the tumor. Interspersed among these highly cellular areas were areas of coagulation necrosis, fibrin, and hemorrhage. This tumor was diagnosed as hemangiosarcoma.

Euthanasia and necropsy were performed on February 29, 1964. At necropsy a tumorous mass 9 x 5 x 4 cm. was present in the subcutis of the right side of the neck near, but not attached to, the thyroid. The mass was reddish-black in color and firm in consistency. A firm

nodule of 2.5 x 2.0 x 0.5 cm. was present at the thoracic inlet ventral to the trachea. Two similar masses were present in the subcutaneous tissue of the right prescapular area. These were firm in consistency, reddish-black in color, and measured 1.0 x 1.0 x 1.0 cm. and 2.0 x 2.0 x 1.0 cm., respectively. Two similar nodules, 2.5 x 0.5 x 2.0 and 1.0 x 1.0 x 1.0 cm., respectively, were present in the abdominal cavity attached to the omentum while another, 3.5 x 2.5 x 3.0 cm., was attached to the parietal peritoneum of the right lateral abdominal wall. The lungs contained many nodules 0.2 to 0.4 cm. in diameter, well circumscribed with black centers and pale peripheries. Multiple smaller white focal areas were also disseminated throughout the lungs, as were lesions of anthracosis. The entire spleen was replaced by confluent nodules ranging from 1 cm. in diameter to 6 x 5 x 5 cm. These were firm in consistency and, on cut surface, had a reddish-black color. Nodules, 4 x 3 x 3 and 6 x 3 x 3 cm., respectively, were present in the right lateral and right central lobes of the liver. The parenchyma of the left lateral lobe of the liver contained 2 nodules each, 4 cm. in diameter, which were not elevated above the surface of the liver. There was cystic hyperplasia of the mucosa of the gallbladder. Microscopic examination of various organs revealed that the dark red nodules were hemangiosarcomas similar in structure to the tumor obtained at biopsy and previously described. The small white nodules in the lung were nematode larva granulomas. Enzymatic activities, as determined in the serum of this dog and in tumor extracts, are recorded in TABLE 73.

TABLE 73. Enzymatic activities relating to Tumor 71.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	2/18	0.69	125		231		19		28.	
Tumor	2/21	25.6	5061	67	7176	95	211	2.79	48.3	0.5
Serum	2/29	2.05	434		324		22		25.	
Tumor	2/29	11.4	46590	561	8797	106	495	5.94	107.	1.3

Tumor 72 was obtained from the same dog as Tumor 34, q.v. for history and physical findings. Tumor 72 consisted of 2 nodules, 1 from the scalp and the other from the flank, which were excised on April 22, 1964. Both of these nodules were covered by skin with adnexa and were composed of fusiform cells in regular arrangement outlining blood-filled channels. No characteristics of malignancy were noted. These tumors were diagnosed as hemangiomas. Enzymatic activities, as determined in the serum of this dog prior to the excision of the neoplasms, and in the tumor extract, are recorded in TABLE 74.

TABLE 74. Enzymatic activities relating to Tumor 72.

Specimen	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	1.19	255	153	44	8.1
Tumor	16.	8194	5324	44	0

Tumor 73 was obtained from the same dog as Tumor 4, q.v. for history and physical findings. Tumor 73 was excised from the left

gluteal region on December 14, 1963. It was 7.2 x 2.0 x 0.6 cm. in size, and covered by scaly epidermis. Microscopically, the neoplasm was found to lie within the dermis. It consisted of rather large, flattened, fusiform endothelial cells lining channels filled with blood. This tumor was diagnosed as a hemangioma. Enzymatic activities in the serum of this dog are recorded in TABLE 54, which follows the protocol of Tumor 53.

Tumor 74 was obtained from the same dog as Tumor 50, q.v. for history and physical findings. Tumor 74 was excised from the skin of the scrotum. It measured 1.5 x 0.7 x 0.3 cm. Microscopically, it was found to be covered by lightly keratinized, pigmented epidermis with adnexa. In the dermis was an aggregation of fusiform endothelial cells which surrounded blood-filled spaces. This tumor was diagnosed as a hemangioma. Enzymatic activities, as determined in the serum of this dog prior to surgery, are recorded in TABLE 75.

TABLE 75. Enzymatic activities relating to Tumor 74.

Hb	LDH	PHI	LAP	Alk. Ptase.
0.52	43	65	22	8.6

Tumor 75 was obtained from a 6-year-old spayed female Dalmatian. This dog was presented because of lameness which had been present in the left hind leg for 6 weeks prior to admission. There was no history of trauma. Conservative treatment had been administered by the attending veterinarian without improvement. Physical examination revealed atrophy

of the muscles of the left hind leg. The dog used the leg in walking but knuckled when weight was placed on it. Radiographs demonstrated a large calcified mass involving the ilium. A large, soft mass was demonstrable in the sublumbar area extending to the pelvic inlet. The mass may have involved the seventh lumbar vertebra. Thoracic radiographs demonstrated no evidence of pulmonary metastasis. On October 18, 1963, a biopsy of the sublumbar mass was performed. The tissue obtained by this punch biopsy consisted of 3 cylindrical specimens, each 0.5 cm. in diameter, and, respectively, 1.2, 0.7, and 0.6 cm. long. These were tan to white and of bony consistency. Microscopic examination of these tissue fragments revealed spicules of bone, in part intact and in part undergoing osteolysis. Between the spicules were cells which varied from fusiform to stellate. The nuclei of these cells were hyperchromatic and tended to assume the same shape as the cell itself. Surrounding this structure were areas of myxoid connective tissue, areas of cartilage, and areas of osteoid. On October 24, it was noted that the pelvic mass occluded the rectum. The dog's general condition continued to deteriorate. A hemogram on October 30, 1963, revealed: hemoglobin, 11.6 gm./100 ml.; packed cell volume, 34%; leukocytes, 36,300/cu. mm.; segmented neutrophils, 56%; nonsegmented neutrophils, 17%; lymphocytes, 18%; and monocytes, 9%.

Euthanasia and necropsy were performed on October 30, 1963. A hard granular gray-black nodule 0.4 cm. in diameter was present in the lobe of the lung. Anthracosis and foci of atelectasis were seen throughout the lungs. The colon was compressed by the pelvic mass, as was the urinary bladder. The neoplastic mass appeared to originate from the left wing of the ilium and project dorsally, filling the

area between the wing of the ilium and the coxofemoral joint. The mass projected medially and anteriorly into the abdominal cavity, nearly occluding the pelvic inlet. It was 5.5 x 3 x 2 cm. in size. The ventral surface of the tumor was crossed by the external iliac vein. The mass was of fibrous consistency with fluctuant areas in the anterior part. Microscopically, it consisted of cells similar to those described in the biopsy, which varied from the appearance of fusiform fibroblasts to that of stellate osteoblasts. In some areas, the osteoblasts were widely separated by clear spaces which resulted in a myxoid appearance. Mineralized spicules and granules were also present. Focally, there were areas of cartilage. Interspersed areas of bone spicules and myxoid tissue mimicked the appearance of cancellous bone. The nodules in the lungs were surrounded by a fibrous capsule. The contents of this capsule included mineralized granules and cholesterol clefts. Sections of the lung removed from these nodules revealed only anthracosis. The neoplasm was diagnosed as an osteogenic sarcoma. Enzymatic activities, as determined in the serum of this dog and in the tumor, are recorded in TABLE 76.

TABLE 76. Enzymatic activities relating to Tumor 75.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	10/18	1.86	18				21		96.6	
Tumor	10/18		154	10	3148	197	204	12.8	7625.	476.3
Tumor	10/30	0	4820	643	8288	1107	247	33.	18688.	2492.

Tumor 76 was obtained from a male Irish Wolfhound 6 years of age. This dog was presented on September 14, 1963, because of a swelling of the midshaft of the right radius and ulna. The dog had been lame for 3 months prior to presentation, and the swelling had appeared 1 month after the onset of lameness. Radiographs revealed an osteolytic lesion which was interpreted as an osteogenic sarcoma. Two biopsies were performed using a trephine. Microscopically the tissue obtained consisted predominantly of normal bone. Focally there were areas of necrosis. The marrow spaces were filled with blood, probably due to the trauma of the biopsy. Much of the marrow cavity was filled with cells which seemed to be of 2 distinct types. Some of these were stellate cells with long, fine, fibrillar processes, while others were fusiform cells with spindle-shaped eosinophilic cytoplasm and small, hyperchromatic nuclei lying in bundles. A hemogram on November 29, 1963, revealed: hemoglobin, 19.4 gm./100 ml.; packed cell volume, 55.5%; erythrocyte sedimentation rate, 0 mm./60 minutes, equal to the expected value; leukocytes, 7850/cu. mm.; segmented neutrophils, 65%; nonsegmented neutrophils, 1%; lymphocytes, 22%; eosinophils, 9%; and monocytes, 3%. Euthanasia and necropsy were performed on January 11, 1964. At necropsy, the area of the midshaft of the radius and ulna was found to be enlarged by a mass which was 38.5 cm. long and 19.5 x 11.0 cm. in cross section. Grossly, the mass felt fibrous. A soft area, 8.0 cm. in diameter, protruded from the skin. The marrow space was filled with fibrous tissue. No other significant lesions were noted. Microscopically the mass was found to be highly cellular. It was predominantly composed of fusiform cells suggesting plump fibroblasts. Focally, there were areas of

necrotic bone, presumably from the underlying tissue rather than the tumor. Rarely, there were microabscesses within the tumor. Focally, the tumor formed discrete areas of cartilage and osteoid. The predominant tumor cells had pale-staining oval nuclei, small but prominent nucleoli, and fusiform eosinophilic cytoplasm. Numerous mitotic figures were present. This neoplasm was diagnosed as an osteogenic sarcoma. Enzymatic activities, as determined in the serum and in the tumor tissue of this dog, are recorded in TABLE 77.

TABLE 77. Enzymatic activities relating to Tumor 76.

Specimen	Date	Hb	LDH	PHI	LAP	Alk.Ptase.
Serum	11/29/63	2.05	63	93	14	29.
Serum	1/11/64	1.15	29	102	16	13.2
Tumor	1/11/64	1.5	1952	8982	238	3491.

Tumor 77 was obtained from a male Doberman Pinscher 3 years of age. This dog was presented on January 2, 1964, because of an enlargement of the distal left radius and ulna, and lameness of the left foreleg. Both of these signs had been present for 2 weeks prior to admission. Surgical excision of the distal half of the ulna was performed. The tissue obtained by this procedure was a firm white fusiform mass involving the distal end of the ulna and elevating the periosteum. The mass was 5.0 x 3.0 x 1.5 cm. Microscopic examination revealed spicules of mature bone radiating from the epiphyseal cartilage plate. Between these spicules lay a neoplastic tissue of stellate and fusiform cells and an eosinophilic matrix of osteoid. Fragments

of bone spicules lay within the tumor suggesting destruction of pre-existing bone by the tumor. There were areas of hemorrhage and necrosis. This tumor was diagnosed as an osteogenic sarcoma. On February 18, 1964, radiographs demonstrated recurrence of the tumor in the original site. Euthanasia and necropsy were performed. At necropsy a mass of tumor tissue 5 cm. in diameter and 13 cm. in length was present distal to the stump of the ulna adjacent to the radius at the site of the previous neoplasm. The left axillary lymph node was 5 times the size of the right axillary lymph node, and measured 7 x 5 x 3 cm. It was grayish-white, firm, and gritty. The lungs contained multiple firm nodules randomly scattered. These nodules ranged to 1.0 cm. in diameter. A nodule, 2 cm. in diameter, was present at the tip of the spleen. Microscopic examination of the neoplasm at the primary site revealed an architecture similar to the primary neoplasm described above. However, there was abundant cartilage and osteoid between the tumor cells. The tumor was not highly cellular. There were large areas of necrosis present. The axillary lymph node revealed only a small portion of the cortex including marginal sinus and germinal centers intact. In these areas, lymphoid hyperplasia, hemorrhage, and hemosiderosis were evident. The remainder of the architecture was replaced by a mass of neoplastic cells similar to the primary neoplasm, but with the formation of abundant osteoid and tumor bone. The nodules in the lungs and spleen were histologically similar to the tumor metastatic to the lymph node. These findings confirmed the diagnosis of osteogenic sarcoma. Enzymatic activities, as determined in the serum of this dog and in the tumor tissue, are recorded in TABLE 78.

TABLE 78. Enzymatic activities relating to Tumor 77.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Tumor	1/2	2.2	193	5	1574	39	291	7.3	9236	231
Serum	1/5	0.45	82		477		16		11.3	
Serum	1/20	0.72	43		162		16		8.6	
Serum	1/25	0.48	24		93		16		15.0	
Serum	2/1	0.72	34		46		19		12.4	
Serum	2/15	2.2	202		343		15		15.6	
Tumor from lung	2/18	5.6	17352	244	23335	329	1660	23.4	39738.	560

Tumor 78 was obtained from a male Boxer 12 years of age. This dog was referred to the Ohio State University Veterinary Clinic because of tumorous growths of the testes and of the left hock. These had been present for 6 months and had not been treated. A small mass was also present in the area of the eleventh rib near the ventral midline. The dog's general health was considered to be good. A hemogram revealed: hemoglobin, 15.05 gm./100 ml.; packed cell volume, 42%; leukocytes, 8200/cu. mm.; segmented neutrophils, 80%; nonsegmented neutrophils, 1%; lymphocytes, 17%; eosinophils, 1%; and monocytes, 1%. The erythrocyte sedimentation rate was not accelerated. On August 23, 1963, a bilateral orchiectomy with ablation of the scrotum and excision of the lesion on the hock were performed. Histopathologic examination revealed that the lesion from the hock was an epidermal inclusion cyst. One testis contained a Sertoli-cell tumor, while an interstitial-cell tumor was present in the other. Seminiferous-cell hyperplasia was noted in both testes. A mastocytoma was present in the

skin of the scrotum. The dog was returned on December 11, 1963, because of a lesion on the thigh. Radiographs at this time revealed a solitary nodule 1.0 cm. in diameter in the right diaphragmatic lobe of the lung.

The dog was again presented on January 21, 1964, because of the mass on the left ventral side of the thorax. The previous week the dog had had an episode of acute posterior paralysis with incontinence of urine, and prolapse of the membrana nictitans. These signs had regressed spontaneously. Hemogram at this time revealed: hemoglobin, 13.8 gm./100 ml.; packed cell volume, 40%; leukocytes, 10,600/cu. mm.; segmented neutrophils, 76%; lymphocytes, 23%; and eosinophils, 1%. Radiographs revealed multiple nodular densities 1.0 to 3.0 cm. in diameter throughout the lungs.

Euthanasia and necropsy were performed on January 23, 1964. At necropsy a mass 3 x 3 x 4 cm. involving the eleventh rib was present 1 cm. to the left of the ventral midline. The mass was continuous with the marrow cavity of the rib, 1 cm. from the costo-chondral junction. The center of the mass was brown and necrotic. The thyroids contained raised, blue-black areas, 0.2 cm. in diameter. The lungs contained multiple dark-red nodules 0.2 to 2.0 cm. in diameter. These nodules were firm and were centrally necrotic and hemorrhagic. Contraction and lesions of chronic interstitial nephritis were noted in the kidneys. Multiple lesions, 0.2 cm. in diameter and depressed below the surface, were present in the liver. The caudate and right central lobes each contained a circular lesion 1 cm. in diameter, while smaller lesions were present in the other lobes. A moderate internal hydrocephalus was present. Microscopically the section

from the eleventh rib revealed filling of the marrow cavity with neoplastic cells which also surrounded all but 1 aspect of the cortex. The tumor cells were polyhedral to stellate with pleomorphic, rather vesicular nuclei and prominent nucleoli. The cytoplasm was lightly eosinophilic and, in some areas, abundant enough to suggest osteoid formation. The tumor cells formed large lobules separated by stroma. There was a central area of necrosis. Mitotic figures were numerous. Multiple sections of the lungs revealed that the neoplastic masses had an outer zone of primitive, pleomorphic mesenchymal cells. These cells varied from round to fusiform to polyhedral. In some areas they lay in a fine stroma. No regular arrangement was evident. The cells had large, chromatin-rich, round, oval, or irregular nuclei, prominent nucleoli, and a violet-stained cytoplasm with indistinct cell boundaries. Mitotic figures were extremely numerous. Some giant cells, identified as osteoclasts, were present. Neutrophils were scattered about diffusely. Centrally, there was a large area of hemorrhage and necrosis. The nodules in the thyroid were microscopically similar to those in the lung. The hepatic lesions were identified microscopically as regenerative nodules. Hemosiderosis and congestion of the liver were also noted. Sections of the kidneys revealed glomerulitis, with fibrous adhesions of the glomerular tufts. The neoplasms were diagnosed as osteogenic sarcomas. Enzymatic activities, as determined in the serum of the dog prior to necropsy, and in tumor tissue obtained from the lung at necropsy, are recorded in TABLE 79.

TABLE 79. Enzymatic activities relating to Tumor 78.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.64	92		162		17		22.6	
Tumor	3.7	13014	420	787	25	611	19.8	12190.	393

Tumor 79 was obtained from a 13-year-old spayed female Scottish Terrier. This dog was presented because of progressive lameness of the right hind leg. Lameness and apparent pain when moving the leg had first been noted 2 years prior to admission. Radiographs revealed a radiolucent mass attached to the lumbar vertebrae. A hemogram revealed: hemoglobin, 17.05 gm./100 ml.; packed cell volume, 58%; leukocytes, 8950/cu. mm.; segmented neutrophils, 54%; lymphocytes, 45%; monocytes, 1%. Euthanasia and necropsy were performed.

At necropsy a fatty, soft, white mass measuring 6.0 x 2.5 x 2.0 cm. was found posterior to the xyphoid cartilage. A similar mass 9.0 x 9.0 x 1.5 was present in the subcutis over the thorax posterior to the left elbow. The other similar masses were present on the lateral surfaces of the thorax at the ninth to twelfth rib, under the external abdominal oblique muscle. These measured 7.0 x 9.0 x 1.5 cm. and 3.5 x 3.5 x 1.3 cm., respectively. These tumors are considered in this study as Tumor 85. A number of the ribs appeared to be thickened, and softer than normal. A large mass was present in the right paralumbar fossa attached to the vertebrae and extending to the wing and shaft of the right ilium. This mass was 13.0 x 8.0 x 7.5 cm. in size. A similar but independent mass over the right ilium measured 5.0 x 4.0 x 2.5 cm. Eight firm, white nodules

were present in the liver. The largest of these was 4 x 4 x 3 cm. A small, white nodule was present in the adrenal medulla. Microscopically, the masses attached to the right ilium were found to be composed of fusiform cells with hyperchromatic nuclei and abundant eosinophilic cytoplasm. In some areas, an eosinophilic matrix identified as osteoid was present between these cells. Within the matrix were small cells in lacunae. Areas of necrosis were present. Multinucleated giant cells identified as osteoclasts were present. Focally, plates of mature bone, presumably from the underlying structure, were present. However, tumor cells were seen between the plates of bone. Occasional hemosiderin-filled macrophages were present. This neoplasm was diagnosed as an osteogenic sarcoma. Sections of the enlarged ribs revealed that the marrow cavity and, in some instances, the cortex were invaded by a neoplasm of similar morphology. The nodules in the liver were similar, but less well differentiated. No regular arrangement of the cells, formation of osteoid, or osteoclasts were apparent. Sections of the lungs revealed anthracosis and small mineralized fibrogranulomas without evident etiologic agent. No microscopic lesions were seen in sections of the stomach and of the kidneys. Enzymatic activities, as determined in the serum of this dog and in tumor tissue from the primary site and hepatic metastasis, are recorded in TABLE 80.

TABLE 80. Enzymatic activities relating to Tumor 79.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	11/22	1.15	82		139		42		1613.	
Tumor (primary)	12/5	0.	386	20	7964	419	138	7.3	17023.	896.
Tumor (hepatic)	12/5	3.7	530	11	4630	96	233	4.8	26313.	548.
Serum	12/5	2.05	130		194		36		1901.	

Tumor 80 was obtained from a spayed female Saint Bernard 7 years of age. This dog was presented on November 12, 1963, because of an enlargement of the distal right radius and lameness in the right foreleg, both of which had been present for 3 weeks prior to admission. A hemogram revealed: hemoglobin, 11.6 gm./100 ml.; packed cell volume, 38%; leukocytes, 9400/cu. mm.; segmented neutrophils, 85%; nonsegmented neutrophils, 1%; lymphocytes, 9%; eosinophils, 3%; and monocytes, 2%. The erythrocyte sedimentation rate was not accelerated. A punch biopsy of the enlargement of the radius was performed. This yielded 2 pieces of bone; 1 was 1.3 cm. long and 0.3 cm. in diameter, and the other 1.5 cm. long and 0.6 cm. in diameter. Microscopic examination of these specimens revealed mature bone with adjacent tendon and muscle. There were small areas of necrosis evident. At the periosteum adjacent to the tendon, the osteoblasts appeared to be proliferating and more active than normal. However, there was no well-defined evidence of neoplasia. The dog made an uneventful recovery from the biopsy and was discharged 4 days later. Two days later the dog was unexpectedly found dead. At necropsy, a gastric torsion was evident.

The distal end of the right radius was enlarged to 8.0 x 7.0 x 4.0 cm., with erosion of the cortex and the formation of new bone. The medullary cavity was filled with neoplastic tissue and was colored a mottled red and gray. Microscopic examination revealed bone with adjacent muscle. The bone appeared more spiculate than normal, with proliferation beyond the cortical periosteal line. The marrow cavity was filled by a rather undifferentiated-appearing mass of polyhedral cells which focally underwent a transition to fibroblast-like forms and, in other areas, formed osteoid. Spicules of the underlying mature bone transversed the tumor. Fragments of the same tumor were seen in the soft tissue adjacent to the radius, and tumor emboli were present in some of the vessels. The tumor was diagnosed as an osteogenic sarcoma. Lymphocytic hyperplasia was seen in the right axillary lymph node. The lungs were congested and contained foci of mineralization. Enzymatic activities in the serum of this dog prior to biopsy are recorded in TABLE 81.

TABLE 81. Enzymatic activities relating to Tumor 80.

Specimen	LDH	PHI	LAP	Alk. Ptase.
Serum	9	116	17	16

Tumor 81 was obtained from an 11-year-old female Boxer. This dog had a tumor excised from the lower jaw by the attending veterinarian on March 13, 1964. The tumor was spherical, 4 cm. in diameter, white, firm, and appeared encapsulated. Microscopic examination revealed a mass covered by ulcerated mucosa with inflammatory cells

in the submucosa. Thrombosis of several vessels in the submucosa was noted. The mass was composed predominantly of fusiform cells arranged in sheets, bundles and whorls. The cells had large hyperchromatic nuclei and eosinophilic cytoplasmic processes. In some areas the cells were polyhedral, embedded in abundant osteoid. Mitotic figures, including atypical forms, were numerous. The tumor contained numerous small areas of bone. These, however, were not associated with the areas of osteoid and suggested osteolysis of the underlying bone rather than osteogenesis by the tumor. A very few osteoclasts were also present. This tumor was diagnosed as an osteogenic sarcoma. Enzymatic activities, as determined in the serum of this dog at intervals following the excision of this tumor, are recorded in TABLE 82.

TABLE 82. Enzymatic activities relating to Tumor 81.

Specimen	Date	Hb	LDH	PHI	Alk. Ptase.
Serum	3/20		188		37.6
Serum	3/30			213	20.9
Serum	4/6	0.56		93	5.4
Serum	5/15	0.56		144	7.0

Tumor 82 was obtained from a 5-year-old spayed female mixed-breed Collie-type dog. This dog was initially presented on May 23, 1963, because of a mass on the left side of the rib cage, which had been present for 6 months prior to admission. Radiographs revealed destruction of the twelfth rib. There was no evidence of metastasis.

At surgery, attachment to, or invasion of, the diaphragm by the tumor was noted. The mass surgically excised was white, opaque, and cystic. It measured 11 x 14 x 9 cm., appeared encapsulated, and was generally very firm except for the cystic area, which was soft. Microscopically the capsule consisted of a few thick collagenous fibers. The mass was composed of pale, irregularly basophilic material (chondroitin sulfate) containing lacunae in which mature-appearing chondrocytes were seen. Some of these were randomly arranged, others in rows.

On October 23, 1963, the dog was returned because of enlargement of the abdomen and vomiting. Ascitic fluid was aspirated and found to contain tissue fragments. Microscopically these consisted of immature cartilage having a pale matrix suggesting mucoid connective tissue. Many of the chondrocytes, which were irregularly arranged, were lightly eosinophilic and necrotic. A few were polyhedral, had hyperchromatic nuclei, and appeared immature. Hematologic findings at this time fell within the normal ranges. Euthanasia and necropsy were performed on October 24, 1963.

At necropsy, a neoplasm 30 x 25 x 12 cm. in size was found in the abdomen. It was lobulated, yellow to white, firm, and friable, and attached only to the omentum and the mesentery. The abdominal cavity contained 250 ml. of serosanguineous fluid. Two similar, but smaller, masses in the left lateral wall of the abdomen, just posterior to the diaphragm, measured 8 x 6 x 4 cm. and 8 x 6 x 3 cm., respectively. One of these had invaded the diaphragm. A similar neoplasm on the thoracic aspect of the diaphragm measured 5 x 4 x 3 cm. Two neoplasms filled the mediastinal space. The 1 to the left was 18 x 12 x 10 cm. in size, while the 1 to the right measured 7 x 6 x 5 cm.

Microscopically, these tumors were similar in structure to the fragments of tissue described above. They consisted of rather well differentiated chondrocytes embedded in lacunae within an irregularly pale basophilic matrix. Invasiveness of the tumor, especially into skeletal muscle, was evident. This tumor was diagnosed as a chondrosarcoma. Enzymatic activities, as determined in the serum of this dog prior to necropsy, and in the tumor, are recorded in TABLE 83.

TABLE 83. Enzymatic activities relating to Tumor 82.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.72	72		213		15		7.5	
Tumor	3/3	0	0	1296	13	0	0	0	0

Tumor 83 was obtained from a 10-year-old male Beagle. This dog was presented because of 2 masses, 1 located in the right scapular area and the other in the left flank. Hemogram revealed: hemoglobin, 19.4 gm./100 ml.; packed cell volume, 56%; leukocytes, 10,600/cu. mm.; segmented neutrophils, 75%; nonsegmented neutrophils, 2%; lymphocytes, 20%; and monocytes, 3%. On November 7, 1963, the masses were surgically excised. Both masses were white in color, soft, and contained within a thin capsule. The mass near the scapula was located under the latissimus dorsi and measured 5 x 5 x 8 cm. The mass in the flank measured 10 x 12 x 2.5 cm. All sections of these tumors consisted predominantly or entirely of regular, mature-appearing adipose cells without any evidence of anaplasia. One section of the tumor under the

latissimus dorsi contained a small focus of mineralization. Both of these tumors were diagnosed as lipomas. Enzymatic activities, as determined in the serum of this dog and in the tumor, are recorded in TABLE 84.

TABLE 84. Enzymatic activities relating to Tumor 83.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	11/7	0.69	22				29		43.0	
Tumor	11/7	0	178	5	0	0	0	0	0	0
Serum	11/10	0.89	54		65		29		60.1	

Tumor 84 was obtained from a 13-year-old spayed female Collie. This dog was presented because of an enlargement on the left side of the thorax extending from the second to the tenth rib. The mass had been present for 3 months prior to admission. The mass was excised, and was found to be encapsulated, irregularly oval in shape, tan in color, and fatty in consistency. Microscopically it was found to consist of a mass of mature-appearing adipose tissue surrounded by a delicate connective tissue capsule. The mass was diagnosed as a lipoma. Enzymatic activities, as determined in the serum of this dog prior to surgery, and in the tumor, are recorded in TABLE 85.

TABLE 85. Enzymatic activities relating to Tumor 84.

Specimen	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk. Ptase. Sp.A.
Serum	0.69	43		102				3.8	
Tumor	1.9	5	1.6	555	185	0	0	0	0

Tumor 85 was obtained from the same dog as Tumor 79, q.v. for history and physical findings. Tumor 85 consisted of 4 masses. All were soft, white, and fatty in consistency. A mass immediately posterior to the xyphoid cartilage measured 6.0 x 2.5 x 2.0 cm. A mass in the subcutis over the thorax, posterior to the left elbow, measured 9.0 x 9.0 x 1.5 cm. Two masses on the lateral surface of the thorax from the ninth to twelfth rib under the external abdominal oblique muscle measured 7.0 x 9.0 x 1.5 cm. and 3.5 x 3.5 x 1.3 cm., respectively. Microscopically, all of these tumors consisted of mature adipose cells. Occasional foci of lymphocytes were evident within the tumors. At the junction of the tumor with the adjacent skeletal muscle, the tumor was seen to compress but not invade the muscle. All of these tumors were diagnosed as lipomas. Enzymatic activities in the serum of this dog and in these tumors are recorded in TABLE 86.

TABLE 86. Enzymatic activities relating to Tumor 85.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	11/22	1.15	82		139		42		1613	
Tumor	12/5	0	0	0	509	93	22	3.9	113	20.4
Tumor	12/5	1.5	0	0	231	93	0	0	48	19.3
Serum	12/5	2.05	130		194		36		1901	

Tumor 86 was obtained from the same dog as Tumor 59, q.v. for history and physical findings. Tumor 86 was a pendulous mass to the right of, and ventral to, the anus. It was excised on November 6, 1963.

Grossly, it was elongated and tapered, measuring 14 cm. in length, 6.5 cm. in diameter at the free end, and 4 cm. at the attached end. It was soft in consistency and white in color. Microscopic examination revealed that the covering epidermis was highly pigmented and slightly hyperkeratotic. Fibrous thickening of the dermis covered the mass, which consisted of regular-appearing mature adipose cells with no evidence of anaplasia. The tumor was diagnosed as a lipoma. Enzymatic activities in the serum of this dog and in the tumor are recorded in TABLE 87.

TABLE 87. Enzymatic activities relating to Tumor 86.

Specimen	Date	Hb	LDH	LDH Sp.A.	PHI	PHI Sp.A.	LAP	LAP Sp.A.	Alk. Ptase.	Alk.Ptase. Sp.A.
Serum	11/6	0.81	44				25		26	
Tumor	11/6	6.4	1195	25	741	15	67	1.3	0	0
Serum	11/8	0.64	92				22		54	

Tumor 87 was obtained from the same dog as Tumor 29, q.v. for history and physical findings. At necropsy, an ulcerated mass measuring 3.0 x 2.5 x 2.5 cm. was present below the anus. Sections from this mass were covered by skin with adnexa, smooth muscle, and numerous hyperplastic circumanal glands. The tumor cells were very anaplastic and pleomorphic and varied from round to fibrillar to stellate. A marked variation in the melanin content of the cells was seen from area to area; some areas were unpigmented, while in other areas the cytoplasm of the cells was densely packed with melanin granules. Foci of neutrophilia were seen within the tumor. At the periphery of the tumor, the

invasiveness of the tumor cells into the surrounding tissue was evident. This neoplasm was diagnosed as a melanoma. A similar melanoma was present in the section of eyelid, invading and destroying the Meibomian glands. Metastases of melanoma, having a similar histologic structure, were present in the liver. Enzymatic activities, as determined in the serum and in the tumor of this dog, are recorded in TABLE 88.

TABLE 88. Enzymatic activities relating to Tumor 87.

Specimen	Hb	LDH	PHI	LAP	Alk. Ptase.
Serum	0.56	43	125	13	18
Tumor	6.0	627	11112	155	1010

Tumor 88 was obtained from the same dog as Tumor 7, q.v. for history and physical findings. Tumor 88 consisted of 2 masses, excised from the skin at the lateral canthi of the eyes on October 23, 1963. These masses were firm and pigmented. The 1 from the left side measured 0.5 x 0.4 x 0.2 cm., while the 1 from the right side measured 0.5 x 0.5 x 0.3 cm. Microscopically, they were found to be covered by thin, keratinized epidermis. The adnexa were present, but atrophic. The dermis was densely packed with melanin-filled melanocytes. In 1 section, an area of dermal fibrosis was also present. These tumors were diagnosed as nevi. Enzymatic activities present in the serum of this dog are recorded in TABLE 89.

TABLE 89. Enzymatic activities relating to Tumor 88.

Specimen	Date	Hb	LDH	PHI	LAP	Alk.Ptase.
Serum	10/23	0.64	29	116	13	12.9
Serum	10/26	1.82	175		16	21.5

Tumor 89 was obtained from the same dog as Tumor 34, q.v. for history and physical findings. Tumor 89 was excised from the distal end of the right carpus on April 22, 1964. It was a small nodule which, microscopically, was found to consist of an aggregate of mature-appearing, melanin-filled melanocytes in the dermis, covered by stratified squamous epithelium with adnexa. This tumor was diagnosed as a nevus. Enzymatic activities, as determined in the serum of this dog prior to the excision of the nevus are recorded in TABLE 90.

TABLE 90. Enzymatic activities relating to Tumor 89.

Specimen	Hb	LDH	PHI	LAP	Alk.Ptase.
Serum	1.19	255	153	44	8.1

Analysis of Data

Tabulation of Data

In order to facilitate the recording of tumor-host identifying data and morphologic and behavioral characteristics of the neoplasms on Hollerith computer cards in Fortran as well as the use of these variables in statistical analysis, this information was converted to numerical values, which are recorded in TABLE 91. The same information, except the diagnosis, relating to each tumor was punched onto Hollerith computer cards, together with all results of enzymatic analyses pertinent to that tumor, both for analysis and data retrieval.

TABLE 91. A summary of identifying, morphologic, and behavioral tumor data.

Tumor No.	Sex	Age	Diag.	Mult.	Size	Necrosis	Inflammation	Anaplasia	Invasiveness	Metastasis	Excision	Tumor Diagnosis
1	1	9	1	2	11	1	2	0	0	0	1	Keratoacanthoma
2	3	6	1	1	15	1	1	0	0	0	1	Keratoacanthoma
3	2	11	1	2	11	0	0	0	0	0	1	Keratoacanthoma
4	3	11	2	3	1	0	0	2	1	0	1	Squamous Carcinoma
5	3	9	3	1	72	0	1	0	1	0	0	Basal Cell Tumor
6	3	12	3	6	23	2	1	1	1	0	-	Basal Cell Tumor
7	4	13	4	3	792	2	1	3	1	1	1	Mammary Duct Carcinoma
8	3	5	4	2	176	1	0	3	1	1	1	Mammary Duct Carcinoma
9	4	12	4	2	91	1	1	1	1	1	1	Mammary Duct Carcinoma

Tumor No.	Sex	Age	Diag.	Mult.	Size	Necrosis	Inflammation	Anaplasia	Invasiveness	Metastasis	Excision	Tumor Diagnosis
10	3	12	5	6	1	0	0	0	0	0	-	Bile Duct Papilloma
11	4	15	6	1	2	1	1	2	1	0	-	Transitional Cell Carcinoma
12	4	13	7	2	9	2	1	2	1	0	1	Salivary Duct Carcinoma
13	3	7	8	1	1920	3	1	4	1	1	1	Undifferentiated Carcinoma
14	1	12	9	1	5	3	2	4	1	0	-	Glioblastome Multiforme
15	4	8	10	1	204	0	0	1	1	0	1	Mixed Mammary Adeno-carcinoma
16	4	12	10	1	65	1	2	0	1	1	1	Mixed Mammary Adeno-carcinoma
17	4	8	10	1	6	0	0	1	0	0	1	Mixed Mammary Tumor
18	4	11	10	3	19	2	1	3	0	0	-	Mixed Mammary Tumor
19	3	13	10	2	44	1	0	1	1	0	1	Mixed Mammary Tumor (malignant)
20	1	7	11	2	0	0	0	0	0	0	-	Interstitial Cell Adenoma
21	1	8	11	2	0	0	0	0	0	0	-	Interstitial Cell Adenoma
22	1	8	12	1	175	2	2	2	1	0	1	Circumanal Gland Adenocarcinoma
23	3	12	13	6	1	0	0	0	0	0	-	Circumanal Gland Adenoma
24	1	8	13	2	1	0	0	0	0	0	-	Circumanal Gland Adenoma
25	1	3	14	1	192	4	1	3	1	0	0	Mucoepidermoid Carcinoma
26	1	16	15	1	1000	4	1	2	1	0	-	Bronchiolar Adeno-carcinoma

Tumor No.	Sex	Age	Diag.	Mult.	Size	Necrosis	Inflammation	Anaplasia	Invasiveness	Metastasis	Excision	Tumor Diagnosis
27	3	12	15	1	300	3	1	1	1	1	-	Bronchiolar Adeno-carcinoma
28	3	12	15	6	29	2	1	1	0	0	-	Bronchiolar Adenoma
29	1	12	15	2	125	0	0	0	0	0	-	Bronchiolar Adenoma
30	4	11	16	3	1000	2	1	3	1	1	-	Bronchial Adeno-carcinoma
31	4	7	17	1	640	4	4	3	1	1	0	Undifferentiated Adenocarcinoma
32	1	9	18	2	0	0	0	0	0	0	1	Sebaceous Gland Adenoma
33	3	12	18	6	25	0	0	0	0	0	-	Sebaceous Gland Adenoma
34	4	7	18	5	0	0	0	0	0	0	1	Sebaceous Gland Adenoma
35	3	13	18	2	0	0	0	0	0	0	1	Sebaceous Gland Adenoma
36	4	8	18	2	1	0	0	0	0	0	1	Sebaceous Gland Adenoma
37	4	12	19	2	2	0	0	0	0	0	1	Ovarian Papillary Adenoma
38	1	10	20	2	0	0	1	0	0	0	1	Acinic Cell Tumor
39	1	8	21	1	1500	0	0	0	1	1	0	Malignant Lymphoma
40	4	5	21	1	150	0	0	2	1	1	0	Malignant Lymphoma
41	1	8	21	1	150	0	0	2	1	1	0	Malignant Lymphoma
42	3	7	21	1	40	0	0	1	1	1	0	Malignant Lymphoma
43	3	10	21	1	20	0	0	0	1	1	-	Malignant Lymphoma
44	3	7	22	1	2	0	1	0	0	0	1	Reticulum Cell Sarcoma
45	1	12	23	1	280	3	3	2	1	1	-	Mastocytoma
46	3	11	23	1	350	2	2	2	1	1	1	Mastocytoma

Tumor No.	Sex	Age	Diag.	Mult.	Size	Necrosis	Inflammation	Anaplasia	Invasiveness	Metastasis	Excision	Tumor Diagnosis
47	1	6	23	1	96	2	2	2	1	0	1	Mastocytoma
48	3	13	23	1	60	1	0	1	1	0	1	Mastocytoma
49	4	10	23	1	5	0	0	0	0	0	1	Mastocytoma
50	1	9	23	2	2	0	0	0	0	0	-	Mastocytoma
51	1	10	23	2	4	0	0	1	1	1	1	Mastocytoma
52	1	1	23	1	2	0	0	1	0	0	1	Mastocytoma
53	3	11	23	3	294	0	1	0	1	0	1	Mastocytoma
54	4	7	23	5	6	0	0	0	0	0	1	Mastocytoma
55	4	8	23	2	3	0	0	0	0	0	1	Mastocytoma
56	1	7	24	1	5	1	0	1	1	0	-	Fibrosarcoma
57	1	7	24	2	360	1	1	3	1	0	0	Fibrosarcoma
58	3	5	25	2	15	0	0	0	0	0	-	Fibroma
59	4	10	25	2	1	0	0	0	0	0	1	Fibroma
60	2	11	25	2	1	0	0	0	0	0	-	Fibroma
61	4	7	25	5	4	0	0	0	0	0	1	Fibroma
62	3	12	26	1	32	0	0	1	1	1	0	Neurofibrosarcoma
63	4	13	26	2	112	2	1	3	1	1	-	Neurofibrosarcoma
64	4	6	27	1	12	0	0	0	0	0	1	Leiomyofibroma
65	3	12	28	6	5	0	0	0	0	0	-	Leiomyoma
66	4	13	28	3	29	0	0	0	0	0	-	Leiomyoma
67	4	11	29	3	128	2	1	3	1	0	-	Rhabdomyosarcoma
68	3	7	30	1	64	4	1	0	1	1	-	Hemangiosarcoma
69	1	13	30	1	25	4	2	3	1	1	0	Hemangiosarcoma

Tumor No.	Sex	Age	Diag.	Mult.	Size	Necrosis	Inflammation	Anaplasia	Invasiveness	Metastasis	Excision	Tumor Diagnosis
70	1	9	30	1	180	1	0	1	1	1	-	Hemangiosarcoma
71	1	12	30	1	1010	3	1	2	1	1	0	Hemangiosarcoma
72	4	7	31	5	1	0	0	0	0	0	-	Hemangioma
73	3	11	31	3	10	0	0	0	0	0	1	Hemangioma
74	1	9	31	2	1	0	0	0	0	0	-	Hemangioma
75	3	6	32	1	30	1	0	2	1	0	-	Osteogenic Sarcoma
76	1	6	32	1	7600	2	1	1	1	0	0	Osteogenic Sarcoma
77	1	3	32	1	450	2	0	2	1	1	0	Osteogenic Sarcoma
78	2	12	32	1	86	3	1	4	1	1	-	Osteogenic Sarcoma
79	3	13	32	2	800	3	0	4	1	1	-	Osteogenic Sarcoma
80	3	7	32	1	230	1	0	2	1	0	-	Osteogenic Sarcoma
81	4	11	32	1	64	1	1	3	1	0	1	Osteogenic Sarcoma
82	3	5	33	1	11,400	1	0	0	1	1	-	Chondrosarcoma
83	1	10	34	1	500	0	0	0	0	0	1	Lipoma
84	3	13	34	1	560	0	0	0	0	0	-	Lipoma
85	3	13	34	2	260	0	0	0	0	0	-	Lipoma
86	4	10	34	2	350	0	0	0	0	0	1	Lipoma
87	1	12	35	2	25	2	2	4	1	1	-	Melanoma
88	4	13	36	3	1	0	0	0	0	0	1	Nevus
89	4	7	36	5	1	0	0	0	0	0	-	Nevus

Key: Sex: 1-male, intact, 2-male, castrated, 3-female, spayed, 4-female, intact.

TABLE 91--concluded

Age: in years, to nearest year.

Diag.: Consecutive number assigned to tumor diagnoses.

Mult.: Number of different types of neoplasms harbored by host of this tumor.

Size: Mass of tumor, including multiple primaries and metastases of this diagnosis, at time of enzyme assay, in cubic centimeters. Volume less than 0.5 cubic centimeters is indicated 0.

Necrosis, Inflammation, Anaplasia: graded 0 to 4. 0-none, 1-minimal, 2-slight, 3-moderate, 4-marked.

Invasiveness, Metastasis: 1-present, 0-absent.

Excision: - no surgical procedure or no postsurgical serum enzyme assays. 0-biopsy, 1-excision.

Mean Values and Standard Deviations

Mean values and standard deviations for the activities of the enzymes in the serum of dogs prior to surgery were determined, as were similar values for the activities of the enzymes in tumor tissue and the specific activity of the enzymes. These values are tabulated in TABLE 92.

TABLE 92. Mean values and standard deviations for serum and tissue enzyme activity

		LDH	PHI	LAP	Alk.Ptase.
Tumor (i.u./kg.)	Mean	9,131	8070	372	2265
	S.D.	13,750	8667	883	5299
Tumor Sp.A. (i.u./gm. protein)	Mean	197	213	9.43	118
	S.D.	255	263	16.6	388
Serum (i.u./l.)	Mean	108	173	22.24	60
	S.D.	107	125	8.97	212

It may be seen in the above table that, for the enzymes and specimens tabulated, with the exception of serum phosphohexose isomerase and serum leucyl aminopeptidase, the value of 1 standard deviation approaches or exceeds the value of the mean. This confirms the impression conveyed by the raw data, that values of these enzyme activities, for which the standard deviation is very large with respect to the mean, are subject to great variance among the specimens, and that the distribution of these values does not resemble a normal distribution curve, but rather that the values form a distribution skewed to the right.

Tests of Correlation

Correlation coefficients were determined between each of the numerical variables listed in TABLE 91 (except that tabulated in the column headed, "Excision") and each enzyme activity, whether in serum prior to tumor excision or in tumor tissue. Correlation coefficients were also determined between all possible combinations of preoperative serum enzyme activity determinations and tumor tissue enzyme activity determinations. Because of the skewed distribution of the enzyme activity values demonstrated in the previous section, an additional set of variables was generated by taking the logarithm of each enzyme activity value and testing it against the other variables in a manner similar to tests performed on the raw enzyme activity values, above. This operation both served to distribute the transformed variables more normally and to make possible the detection of non-linear relationships between the variables. Relationships, whose correlation coefficients had sufficiently large absolute values to be significantly different from zero at the 5% level, and their actual levels are recorded in TABLE 93.

TABLE 93. Results of significant tests of simple linear correlation.

Variable	Variable	Correlation Coefficient	Level of Significance (Less than:)
Log of Tissue LDH	Size	-.474361	0.5%
Tissue PHI	Invasiveness	.343727	5.0%
Log of Tissue PHI	Invasiveness	.441783	0.5%
Tissue PHI	Multiplicity	-.389195	2.5%

Variable	Variable	Correlation Coefficient	Level of Significance (Less than:)
Log of Tissue PHI	Multiplicity	-.522100	0.5%
Log of Tissue LAP	Size	-.647794	0.5%
Log of Tissue LDH	Log of Tissue PHI	.399340	2.5%
Log of Tissue LDH	Log of Tissue LAP	.532582	0.5%
Log of Tissue LAP	Log of Tissue Alk. Ptase.	.473662	0.5%
Serum LDH	Age	-.343283	0.5%
Log of Serum LDH	Age	-.255933	5.0%
Log of Serum LDH	Tissue LDH	.318231	5.0%
Serum PHI	Inflammation	.328350	1.0%
Serum PHI	Serum LDH	.384271	0.5%
Log of Serum PHI	Serum LDH	.412875	0.5%
Log of Serum PHI	Log of Serum LDH	.592584	0.5%
Serum LAP	Multiplicity	.386953	0.5%
Log of Serum LAP	Multiplicity	.370197	0.5%
Serum LAP	Inflammation	-.270032	2.5%
Log of Serum LAP	Inflammation	-.289993	2.5%
Log of Serum LAP	Metastasis	-.255738	5.0%
Log of Serum LAP	Invasiveness	-.241023	5.0%
Serum LAP	Serum Alk. Ptase.	.302687	2.5%
Log of Serum LAP	Serum Alk. Ptase	.276627	2.5%
Log of Serum LAP	Log of Serum Alk. Ptase.	.330047	1.0%
Serum LAP	Log of Serum Alk. Ptase.	.362122	0.5%

Simple linear correlation coefficients were determined for the relationships between the enzymatic activities and the concentrations of hemoglobin in the respective sera or tissue extracts. These correlation coefficients were not significantly different from zero at the 5% level except for the relationship between serum lactic dehydrogenase and hemoglobin, which yielded a coefficient of .344414, significant at less than the 2.5% level, and that between serum phosphohexose isomerase and hemoglobin, which yielded a correlation coefficient of .424162, significant at less than the 0.5% level.

Tests of multiple correlation and regression were performed using the values from TABLE 91 for size, necrosis, inflammation, anaplasia, invasiveness, and metastasis as the independent variables and the serum or tissue enzyme activity as the dependent variable. The regression equations so obtained which have a correlation coefficient significantly above zero at the 5% level or below are given below, with the standard error given in parentheses for each regression coefficient.

The tumor tissue phosphohexose isomerase activity in i.u./kg. =

5097. - 1.2 (volume of the tumor in cubic centimeters) - 2849. (variable
(1901.) (0.6) (1291.)

for necrosis) + 2264. (variable for inflammation) - 986. (variable for
(1470.) (1094.)

anaplasia) + 8641. (variable for invasiveness) + 3494. (variable for
(2911.) (2480.)

metastasis). The enzyme activity value obtained from this regression equation has a standard deviation of 6036. The correlation coefficient is .6474, which differs from zero at less than the 1.0% level.

The tumor tissue alkaline phosphatase activity in i.u./kg. =

1284. - .5 (volume of the tumor in cubic centimeters) + 2360. (variable
(1534.) (.5) (1041.)

for necrosis) - 3820. (variable for inflammation) + 247. (variable
(1186.) (883.)

for anaplasia) - 557. (variable for invasiveness) + 2275. (variable
(2349.) (2001.)

for metastasis). The enzyme activity value obtained from this regression equation has a standard deviation of 4870. The correlation coefficient is .5773, which differs from zero at the 5% level of significance.

The common logarithm of the tumor tissue lactic dehydrogenase

activity in i.u./kg. = 2.7483 - .0003 (volume of the tumor in cubic
(.2843) (.0001)

centimeters) - .0962 (variable for necrosis) + .1176 (variable for
(.2152) (.2451)

inflammation) + .1654 (variable for anaplasia) + .7525 (variable
(.1825) (.4854)

for invasiveness) - .3331 (variable for metastasis). The logarithm
(.4134)

of the enzyme activity value obtained from this regression equation has a standard deviation of 0.91568. The correlation coefficient is .5883, which differs from zero at the 5% level of significance.

The common logarithm of the tumor tissue leucyl aminopeptidase

activity in i.u./kg. = 1.9923 - .0003 (volume of the tumor in cubic
(.3767) (.00005)

centimeters) + .1852 (variable for necrosis) - .1213 (variable for
(.1151) (.1312)

inflammation) - .1009 (variable for anaplasia) + .3611 (variable
(.0976) (.2597)

for invasiveness) + .1455 (variable for metastasis). The logarithm
(.2213)

of the enzyme activity value obtained from this regression equation has a standard deviation of 0.37672, and a correlation coefficient of .6779, which differs from zero at the 0.5% level of significance.

Tests of Hypotheses

For each enzyme considered in this study, the mean and standard deviation of serum activity levels was determined for dogs with benign neoplasms and for dogs with malignant neoplasms. Means and standard deviations for values of the same enzymes were determined for healthy dogs (Loeb and Nagode, 1965). These values are given in TABLE 94.

TABLE 94. Means and standard deviations for serum enzyme activities.

	LDH		PHI		LAP		Alk.Ptase.	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Healthy	50.61	6.294	138.9	29.12	17.15	3.277	12.4	17.50
Benign Tumors	128.21	168.21	101.9	36.53	25.37	2.398	18.2	13.45
Malignant Tumors	126.28	103.75	212.98	153.2	20.25	8.38	28.5	32.28

For serum lactic dehydrogenase, the hypothesis that the mean serum enzyme activity in healthy dogs equals the mean serum activity value in dogs with neoplastic disease was tested. This test yielded a value of $t = 4.051$ with 39 degrees of freedom, which is greater than the value for $t_{0.005}$; therefore, the null hypothesis is rejected at the 0.5% level of significance.

For serum phosphohexose isomerase, the hypothesis that the mean serum enzyme activity in healthy dogs equals the mean serum enzyme activity in dogs with malignant neoplastic disease was tested. This test yielded a value of $t = 2.38$ with 30 degrees of freedom, which is

greater than $t_{0.025}$; therefore, the null hypothesis was rejected at the 2.5% level of significance.

For leucyl aminopeptidase, the hypothesis that the mean serum enzyme activity in healthy dogs equals the mean serum enzyme activity in dogs with neoplastic disease was tested. This test yielded a value of $t = 1.739$ with 38 degrees of freedom, which exceeds $t_{0.05}$; therefore, the null hypothesis was rejected at the 5.0% level of significance.

For alkaline phosphatase, the hypothesis that the mean serum enzyme activity in healthy dogs equals the mean serum enzyme activity of dogs with malignant neoplastic disease was tested. This test yielded a value of $t = 2.00$ with 38 degrees of freedom, which exceeded the value for $t_{0.05}$; therefore, the null hypothesis was rejected at the 5% level of significance.

For each enzyme considered in this study, mean postoperative values of activity in the serum were determined separately for those dogs from which the neoplasms had been excised, and for those from which only biopsies of the tumors had been taken. These mean values are tabulated in TABLE 95.

TABLE 95. Mean postoperative serum enzyme activities.

	LDH	PHI	LAP	Alk. Ptase.
Excision Group	181	173	19.73	27.22
Biopsy Group	153	195	23.05	18.45

For lactic dehydrogenase and alkaline phosphatase, examination of the above values indicates that the values for the excision group exceed those for the biopsy group. Thus, under the conditions of this study, excision of the neoplasm did not reduce the serum enzyme activity. For phosphohexose isomerase and for leucyl aminopeptidase, the hypothesis that the mean serum activity for the excision group equals that for the biopsy group was tested. These tests yielded values of $t = 0.54$ with 35 degrees of freedom and $t = 1.03$ with 42 degrees of freedom, respectively. Since neither of these values exceeds $t_{0.05}$, the null hypothesis with respect to these 2 enzymes cannot be rejected.

Mean values and standard deviations were determined for tissue activities of each enzyme in benign neoplasms and in malignant neoplasms. Because of the high values of alkaline phosphatase activity in osteogenic sarcomas, the mean value and standard deviation for alkaline phosphatase in malignant neoplasms excluding osteogenic sarcomas was also determined. These values are tabulated in TABLE 96.

TABLE 96. Mean values and standard deviations for enzymatic activities in benign and malignant neoplasms.

		LDH	PHI	LAP	Alk. Ptase.
Benign Neoplasms	Mean	3691	2736	208.4	484.2
	S.D.	6684	3866	407.1	1108.2
All Malignant Neoplasms	Mean	10543	9964	394.0	2593.1
	S.D.	15072	9107	987.9	5094.9
Malignant Neoplasms Excluding Osteogenic Sarcomas	Mean	---	---	---	1291.2
	S.D.	---	---	---	2528.6

For each enzyme, the hypothesis that the mean value for tissue activity in benign neoplasms equals that in malignant neoplasms was tested. For lactic dehydrogenase, a value of t = 2.25 with 46 degrees of freedom was obtained. Since this value exceeds $t_{0.025}$, the hypothesis was rejected at the 2.5% level. For phosphohexose isomerase, a value of t = 3.00 with 46 degrees of freedom was obtained. Since this exceeded $t_{0.005}$, the null hypothesis was rejected at the 0.5% level. For leucyl aminopeptidase, a value of t = 0.848 with 46 degrees of freedom was obtained. Since this value was less than the value for $t_{0.05}$, the null hypothesis was not rejected. For alkaline phosphatase, a value of t = 2.47 with 46 degrees of freedom was obtained. Since this exceeded the value for $t_{0.01}$, the null hypothesis was rejected at the 1.0% level. However, when values for malignant neoplasms excluding osteogenic sarcomas were tested against values for benign neoplasms, a value of t = 1.66 with 41 degrees of freedom was obtained. Since this was less than $t_{0.05}$, the null hypothesis was not rejected.

Values of tumor tissue-enzyme activity, specific activity, and preoperative serum-enzyme activity were subdivided into 4 classes, determined by the origin and malignancy of the tumor. These classes were termed (1) epithelial, benign, (2) epithelial, malignant, (3) mesenchymal, benign, and (4) mesenchymal, malignant. The values so classified were tested by a 1-way analysis of variance technique, modified for unequal sample sizes, against the hypothesis that the means of the 4 populations were equal. Values for the F statistic so obtained are given in TABLE 97, together with the appropriate number of degrees of freedom.

TABLE 97. F statistics for comparison of benign epithelial, malignant epithelial, benign mesenchymal, and malignant mesenchymal tumors.

	LDH	PHI	LAP	Alk.Ptase.
Tissue	0.98 3,48	7.98 3,47	0.61 3,49	0.65 3,49
Specific Activity	1.42 3,39	2.99 3,38	0.56 3,41	0.77 3,41
Serum	2.34 3,35	1.19 3,29	1.73 3,35	0.79 3,34

Comparison of these data to a table of values of F revealed that the F statistics here tabulated were less than the critical values for $F_{0.05}$, except for those in the tissue and in the specific activity tests for phosphohexose isomerase, which exceeded the critical values for $F_{0.005}$, and $F_{0.05}$, respectively. According to these tests, the null hypothesis that the mean values for the enzymatic activity for each of the groups were equal could be rejected except for the tissue activity of phosphohexose isomerase, for which it was rejected at the 0.5% level of significance, and for the tissue specific activity of phosphohexose isomerase, for which it was rejected at the 5.0% level of significance.

Mean values were determined for phosphohexose isomerase activity in neoplastic tissues as tested in TABLE 97. These were 2394 i.u./kg. for benign mesenchymal tumors, 5477 i.u./kg. for benign epithelial tumors, 8130 i.u./kg. for malignant mesenchymal tumors, and 14473 i.u./kg. for malignant epithelial tumors. Differences between these values were tested by Duncan's multiple range test. The difference

between the value for benign mesenchymal tumors and that for malignant epithelial tumors was found to be significant at the 1% level. The differences between the values for benign epithelial tumors and malignant epithelial tumors and those between malignant mesenchymal tumors and malignant epithelial tumors were found to be significant at the 5% level. The differences between the remaining pairs of values were not statistically significant.

The mean values for the specific activity of phosphohexose isomerase were found to be: 85.28 i.u./gm. of tissue protein in benign mesenchymal tumors, 133.34 i.u./gm. of tissue protein in benign epithelial tumors, 198.16 i.u./gm. of tissue protein in malignant mesenchymal tumors, and 422.72 i.u./gm. of tissue protein in malignant epithelial tumors. Differences between these values were tested by Duncan's multiple range test. The difference between the value for malignant epithelial neoplasms and benign mesenchymal neoplasms was found to be significant at the 1% level, while that between malignant epithelial neoplasms and benign epithelial neoplasms was found to be significant at the 5% level. The differences between the remaining pairs of values were not statistically significant.

Tissue enzyme-activity levels, tissue specific-activity levels, and preoperative serum-enzyme activities were classified according to the tumor diagnosis. In order to keep the number of observations in each class statistically valid, this test was restricted to 4 types of tumors: (1) mixed mammary tumor (benign and malignant), (2) malignant lymphoma, (3) mastocytoma, and (4) osteogenic sarcoma. One-way analyses of variance were performed on these sets of data, testing the hypothesis for each enzyme and for each group of observations, that

the mean enzyme-activity value for all 4 types of tumors tested was equal. Values for F so obtained are given in TABLE 98, with the appropriate number of degrees of freedom.

TABLE 98. F values testing equality of the means among four types of tumors.

	LDH	PHI	LAP	Alk.Ptase.
Tumor tissue	1.6 3,15	0.67 3,14	1.94 3,16	17.56 3,17
Tumor Sp.A.	0.67 3,13	0.91 3,12	3.67 3,14	4.63 3,14
Serum	2.78 3,21	0.41 3,20	0.61 3,23	1.09 3,21

Comparison of these data to a table of values of F revealed that the F statistics here tabulated were less than the critical values for $F_{0.05}$ except for the value for the specific activity of leucyl aminopeptidase, which exceeded the value for $F_{0.05}$, the specific activity of alkaline phosphatase, which exceeded the value for $F_{0.025}$, and the tissue activity value of alkaline phosphatase, which exceeded the value for $F_{0.005}$. Therefore, the null hypotheses that the enzymatic activities among the 4 tumors were equal were not rejected, except in case of (1) the tumor specific activity of leucyl aminopeptidase for which the hypothesis was rejected at the 5% level of significance, (2) the tumor specific activity of alkaline phosphatase for which the hypothesis was rejected at the 2.5% level of significance, and (3) the tumor activity of alkaline phosphatase, for which the hypothesis was rejected at the 0.5% level of significance.

The analysis of variance was repeated for the tissue activity of alkaline phosphatase with the osteogenic sarcomas excluded. This yielded a value of $F = 2.13_{2.13}$ which is not significant at the 5% level.

DISCUSSION

Before it is possible to make any rational evaluation of the significance of enzymatic alterations in neoplastic disease, the causes of neoplasia and the mechanisms of tumorigenesis must first be considered. As there are many defects in the current knowledge of both of these facets, the interpretation of segments of data obtained in this study may remain obscure. Many widely diverse types of agents are known to be carcinogenic. These include radiation, a multitude of chemical agents, and certain viruses. Genetic studies suggest an inheritable susceptibility to neoplastic disease. In the vast majority of naturally occurring neoplasms, the inducing agent or agents escape recognition. The mechanisms by which such a variety of agents appear to induce neoplastic transformation may be equally varied (Potter, 1964). Most widely accepted is the concept that any of the above agents, and presumably many others, may produce an injury to the deoxyribonucleic acid of the cell, thereby altering the cell's genetic constitution. It then follows that if the cell's genetically regulated characteristics with regard to the control of the rate of reproduction are altered, other biochemical characteristics of the cell (i.e., the rate of enzyme synthesis) may also be altered. This mechanism concept is subject to less conflict in the light of existing data than are others. However, this may be due rather to the generality and vagueness of the concept than to its accuracy.

By applying the theories of Jacob and Monod to the above mechanism, Potter (1964) postulated that alteration of the genetic constitution

of the cell did not directly affect enzyme synthesis mechanisms but genetically deleted the inhibition of these systems by the feedback mechanism. He theorized that if this inhibition of feedback applied to the synthesis of DNA, the abnormal proliferation of the cancer cell was explained. In contrast to this, Monod and Jacob (1961) theorized that carcinogenesis need not involve the genetic constitution of the cell, but that the injury inducing neoplasia could act directly by blocking the feedback inhibition of enzyme synthesis, and from this concluded that the process of neoplastic transformation was, in some instances, reversible.

Both the mechanism postulated by Potter and that postulated by Monod and Jacob would give rise to the implication that the synthesis of enzymes related to cell replication and to energy metabolism would be greater in the neoplastic than in the normal cell. This would tend to support the hypothesis of biochemical uniformity of tumor tissue as formulated by Greenstein (1956). Greenstein's study in rats revealed a much greater uniformity among various types of induced tumors than among a group of normal tissues. From these data, Greenstein was unable to detect similarities between tumors and their tissues of origin as determined by morphologic criteria and, from this, suggested that the histologic classification of neoplasms was of limited value. In his evaluation of this study, however, Potter (1964) noted the diversities in enzyme patterns among the neoplasms studied by Greenstein and commented on the great behavioral and morphologic deviation between the tumor cells and their cells of origin. He suggested that the biochemical investigation of tumors might be more fruitful if minimally deviating neoplasms were compared to the cell of origin.

The wide variation among the activities in each enzyme system in this study failed to support Greenstein's hypothesis. Furthermore, tumors having low enzymatic activities were either thought to be in conflict with the mechanisms postulated by Potter and by Jacob and Monod, or to have cell walls of increased permeability, resulting in loss of enzyme from the tumor at a rate more rapid than the rate of synthesis.

In contrast with Greenstein's hypothesis of the biochemical uniformity of tumor tissues, Bär et al. (1963) showed that in a series of naturally occurring human neoplasms, the enzyme patterns of the neoplastic tissues resembled those of the parent tissues. Observations in this study yielded only a limited amount of data immediately applicable to such comparison, as many neoplasms arose from a cell type not readily accessible in quantities suitable for biochemical study. A comparison of enzymatic activities in normal lymph nodes (Nagode, 1965) and in neoplastic lymph nodes is recorded in TABLE 99.

TABLE 99. Enzymatic activities in normal and neoplastic lymph nodes.

Tissue		LDH	LAP	Alk. Ptase.
Normal Lymph Node	Mean	24,000	300	300
	S.D.	10,000	100	800
Malignant Lymphoma	Mean	14,363	443	198
	S.D.	32,468	65	326

For each enzyme system illustrated in the table, the difference between the means is not significant. However, in the absence of

marker enzymes, that is, enzymes present in a single tissue or a very limited number of tissues in high activity, and persisting in neoplasms of these tissues, it is unlikely that an individual neoplastic tissue could be identified by a knowledge of its enzyme pattern and those of normal tissues.

Simple and multiple correlation analyses of data in this study have shown a negative correlation between each of the 4 enzymes measured in the tumor tissues and the tumor volume. This is undoubtedly due to the impaired blood supply to the center of large tumors, possibly resulting in decreased enzyme synthesis due to decreased oxygen, amino acid, or energy supply. However, a more probable explanation in harmony with current theories of enzyme synthesis is the inhibition of intracellular enzyme synthesis by a local decrease in the concentration of substrate serving as inducer and/or a local increase of end-product serving as inhibitor.

The mean value of tissue lactic dehydrogenase was found to be significantly higher in the malignant tumors than in the benign ones. This again lent support to Warburg's hypothesis that anaerobic glycolysis is more rapid in malignant neoplastic tissues which, in this respect, resemble embryonic tissue. Again, however, variability within both of these groups was so great as to render the interpretation of a single value impossible.

A significant positive correlation existed between the activity of lactic dehydrogenase in the tumors and the logarithm of the activity of lactic dehydrogenase in the sera. This suggested that the elevated activity of lactic dehydrogenase in the sera of individuals with tumors rich in lactic dehydrogenase was due, at least in part, to

increased lactic dehydrogenase synthesis in the tumor and not to increased tumor cell membrane permeability to lactic dehydrogenase or to muscle cell necrosis, as postulated by some investigators (Bierman et al., 1957; White, 1960).

A positive correlation existed between the logarithm of the tumor tissue lactic dehydrogenase and the logarithm of the tumor tissue phosphohexose isomerase. This finding, together with the similarity in mean activity and mean specific activity between these 2 enzymes, was explained by the fact that each catalyzes a reaction in the metabolism of glucose for energy release. The correlation between the logarithm of tumor tissue lactic dehydrogenase and tumor tissue leucyl aminopeptidase was not so readily explainable, since each is influenced by different factors. If, as Greenstein postulated, the cancer cell were indeed "streamlined" for its limited functions by a loss of all nonessential systems, an inverse relationship between these 2 enzymes would have been expected. The positive correlation, then, can only have indicated a general suitability of the tumor to enzyme synthesis as indicated by such parameters as small size, rather than a functional relationship. Furthermore, in the regression equations for the logarithms of lactic dehydrogenase and leucyl aminopeptidase activity, the large α term with respect to the sizes of the regression coefficients showed that the greatest portion of the activities of both enzymes remained unexplained by the variables considered in the regression equation.

The lack of significance of the values obtained in the analyses of variance for tissue lactic dehydrogenase both among 4 general categories of tumors and among 4 specific types of tumors suggested that no value can be attributed to lactic dehydrogenase as a marker enzyme.

The observation by Nagode (1965) that the technique for phosphohexose isomerase determination used in this study did not yield linear results with tissue extracts and, in fact, that in some analyses the concentration of the end-product, fructose-6-phosphate, decreased during the incubation period, cast some doubt on the numerical validity of the tissue phosphohexose isomerase levels recorded. No doubt could exist, however, as to the correlation between the activity of tissue phosphohexose isomerase or the logarithm of tissue phosphohexose isomerase and the variable for tumor invasiveness. This correlation was further substantiated by the high regression coefficient for invasiveness in the multiple regression equation for phosphohexose isomerase and by the analysis of variance coupled with Duncan's multiple range test by which it was shown that malignant epithelial neoplasms contained a significantly higher activity of phosphohexose isomerase than did malignant mesenchymal neoplasms, benign epithelial neoplasms, or benign mesenchymal neoplasms. The conflict created by Nagode's observation could be resolved by indicating that the test for phosphohexose isomerase as performed in this study failed to distinguish between a high activity of phosphohexose isomerase and a low activity of the phosphohexose isomerase inhibitor or fructose-degrading substance described by Nagode. Hereinafter, apparent high levels of phosphohexose isomerase in tissue by the method used in this study have been indicated as high activities of phosphohexose isomerase in the full knowledge that they may, in fact, have represented low activity of an interfering mechanism.

The high significance of the F statistic in the analysis of variance for the difference in phosphohexose isomerase activity among

malignant epithelial, malignant mesenchymal, benign epithelial, and benign mesenchymal tumors, coupled with the further analysis of these data by Duncan's multiple range test, suggested that the determination of this enzymatic activity in the tissue might serve as an aid, either as a measure of invasive potential in tumors of identifiable histologic type or as an index of epithelial or mesenchymal origin in highly anaplastic tumors. The finding that anaplasia per se did not markedly influence the activity of this enzyme, as demonstrated in the multiple regression equation for phosphohexose isomerase, further strengthened this concept. The association between phosphohexose isomerase and invasiveness was also noted when the specific activity was considered. Here, however, the effect was diluted as revealed by the lower F value.

The negative correlation between phosphohexose isomerase and multiplicity of neoplasms was readily explained when it was noted that multiplicity could be regarded as a parameter of "benignness" of the neoplasm, both in concept, since malignant neoplasms tended to cause the animals' death, thus not resulting in multiplicity, and based on statistical evidence. Thus, conceptually, multiplicity could have been regarded as having a significance converse to invasiveness.

The logarithm of the tumor-tissue leucyl aminopeptidase activity was positively correlated with the logarithm of the tumor-tissue alkaline phosphatase activity. This association between the activities of these 2 enzymes was close enough that the specific activity of the leucyl aminopeptidase yielded a significant F value in the analysis of variance test among the 4 types of tumor tested.

Among the enzymes considered in this study, only alkaline phosphatase had the characteristics of a marker enzyme as described by Potter (1964). In the analysis of variance testing the hypothesis that mixed mammary tumors, mastocytomas, malignant lymphomas, and osteogenic sarcomas had the same mean activity of alkaline phosphatase, the resulting F statistic was sufficiently large to permit rejection of the null hypothesis at less than the 0.5% level of significance. When the test was repeated with osteogenic sarcomas excluded, the null hypothesis was accepted. This demonstrated the specificity of alkaline phosphatase for the osteogenic sarcoma. Examination of the raw data revealed not only this close association, but some important ramifications of it as well. Metastases of osteogenic sarcomas to the liver and to the lung also contained an extremely high activity of alkaline phosphatase, while tumors of bone other than osteogenic sarcomas did not. (See, for example, Tumors 13 and 67.) No alkaline phosphatase activity was demonstrated in a chondrosarcoma. These findings further confirmed the specific relationship between osteogenic sarcoma and high alkaline phosphatase activity, and served to prove that the elevated activity was related to the neoplastic osteoblasts and not to reaction by adjacent pre-existing bone.

The serum lactic dehydrogenase values had a negative correlation with ages of the dogs, suggesting that mean values decrease with age, similar to the findings in man by Bierman *et al.* (1957). This observation tended to lend added significance to the difference in mean values between serum lactic dehydrogenase values in healthy dogs and those with neoplastic disease, since the group with neoplastic disease had a higher mean age.

The mean values of serum lactic-dehydrogenase activity for dogs with benign tumors and for those with malignant tumors was almost identical. Both showed a large variance, which suggested that serum lactic dehydrogenase may be a valuable technique for testing a population of dogs as a general parameter of neoplastic disease, but of very little value in the individual dog.

A viral agent, Riley's lactate dehydrogenase elevating agent, which had been found in association with transmissible murine tumors, was studied by Yaffe (1962) and by Georgii (1964). This agent produced a marked elevation of serum lactic dehydrogenase in infected mice. Yaffe found that it was not the oncogenic agent, but possibly a passenger virus. While it was interesting to speculate that this agent or a similar one may produce elevated serum lactic dehydrogenase in some animals with neoplastic disease, it was not possible to accept the hypothesis that all elevation of serum enzymes related to neoplastic disease is the result of such an agent.

From their studies of serum lactic dehydrogenase in children, Maciejewski and Bobinski (1965) concluded that the activity of this enzyme is elevated in almost all children with malignant neoplastic disease, and in all children with disseminated neoplastic disease. If this observation is indeed correct, this study clearly shows that it cannot be extrapolated across species lines to the dog. For selected examples of dogs with disseminated malignant neoplasms and low serum lactic dehydrogenase values, Tumors 30 and 87 may be reviewed.

As in the tissues, a high correlation was obtained between the serum lactic dehydrogenase activity and the serum phosphohexose isomerase activity. The highest correlation obtained was between

the logarithms of the 2 values, suggesting that the relationship is a non-linear one.

The mean serum phosphohexose isomerase activity was significantly higher in dogs with malignant neoplastic disease than in healthy ones. However, in dogs with benign neoplastic disease it was lower than in the healthy group. Conceptually, this appeared to be in agreement with the high correlation observed between tumor tissue phosphohexose isomerase and invasiveness. A positive correlation was also observed between serum phosphohexose isomerase and inflammation as a parameter of the tumor.

A significant positive correlation was obtained between serum hemoglobin and both serum lactic dehydrogenase and serum phosphohexose isomerase. This supported other observations (Loeb and Nagode, 1965) concerning the high activity of lactic dehydrogenase and phosphohexose isomerase in the canine erythrocyte, and the value of serum hemoglobin as a parameter of both the *in vivo* and *in vitro* lysis of erythrocytes. For this reason, hemolyzed serum should not be used for the determination of these enzymes, and, in order to achieve the highest validity of interpretation, serum hemoglobin determination should be performed and interpreted in conjunction with measurements of serum lactic dehydrogenase and serum phosphohexose isomerase activity.

Serum leucyl aminopeptidase activity and its logarithm showed a positive correlation with the parameter of "benignness", viz., multiplicity, and a negative correlation with several parameters of malignancy, viz., inflammation, invasiveness, and metastasis. This negative association was further confirmed by the observation that the mean serum activity of leucyl aminopeptidase in dogs with malignant neoplastic disease was not significantly higher than in normal

dogs, while that in dogs with benign neoplastic disease was. Loeb and Nagode have demonstrated that the canine erythrocyte contains an inhibitor of leucyl aminopeptidase; thus, when a hemolysate is added to serum of known leucyl aminopeptidase activity, the resultant mixture has a lower activity than did the raw serum. Therefore, it was possible to postulate that a similar inhibitor may be released from malignant neoplasms, resulting in the observed inhibition of leucyl aminopeptidase. As in the tissue, a positive association was noted between the activity of serum leucyl aminopeptidase and alkaline phosphatase.

The mean serum alkaline-phosphatase activity was higher in dogs with benign neoplasia than in healthy dogs, and significantly higher in dogs with malignant neoplastic disease. However, as with the other enzymes studied, the variance was so great as to make the interpretation of a single value questionable.

A high activity of serum alkaline phosphatase was noted in some of the dogs with malignant lymphoma. This was found to be associated with periportal neoplastic proliferation of lymphogenous cells, and is in agreement with the observation that serum alkaline phosphatase is elevated in intrahepatic and extrahepatic obstruction of the biliary tract (Cornelius, 1963).

The level of activity of all 4 enzymes in the serum was compared postoperatively between the group of dogs from which the neoplasms had been surgically excised and the group in which they had been biopsied. As it was assumed that the presence of the tumor resulted in the presence of the increased activity of the enzyme in the serum, it followed that postoperative values were expected to be lower in the

"excision" group than in the "biopsy" group. However, comparison revealed that values for serum lactic dehydrogenase and alkaline phosphatase were lower in the "biopsy" group, and values for the other 2 enzymes did not differ significantly between the 2 groups. Several investigators have shown that, following a single insult in which enzymes are released into the serum, such as a myocardial infarct, the elevated level of enzyme activity will persist for up to 12 days, depending on the enzyme and its route of excretion or inactivation (White, 1960). Mean postoperative enzyme analyses in this study were performed 4.7 days following surgery, at which time not only the enzymes present as a characteristic of the neoplasm, but also those liberated by surgery, still appeared to be present. Individual protocols tended to suggest that, at a longer interval following excision of the neoplasm or following response to other types of therapy, the serum enzyme levels returned to normal.

SUMMARY AND CONCLUSIONS

Activities of lactic dehydrogenase, phosphohexose isomerase, leucyl aminopeptidase, and alkaline phosphatase were determined in the sera of 60 dogs with naturally occurring neoplastic disease, and in 56 out of 89 neoplasms which these dogs had. Additional determinations of the same enzymes in serum were performed following biopsy or excision of the neoplasms. The results obtained were statistically analyzed relative to the gross and microscopic morphologic characteristics and the behavior of the neoplasms.

No general pattern of biochemical uniformity was noted among the neoplasms. Some biochemical similarity was noted between tumors and their tissues of origin. In the absence of the marker enzyme, alkaline phosphatase, this was not sufficient to relate the tumor to the parent tissue in the absence of other information. Tumor tissue-enzyme activity per unit of weight was less for all 4 enzymes in larger tumors than in smaller ones.

The mean value of tissue lactic dehydrogenase was significantly higher in malignant tumors than in benign ones. The correlation between tumor-tissue lactic dehydrogenase and serum lactic dehydrogenase activity suggested that the increased serum lactic dehydrogenase was due to increased synthesis of the enzyme by the tumor.

A positive correlation was observed in both tumor tissue and serum between the activities of lactic dehydrogenase and phosphohexose isomerase activities. This correlation appeared to be related to the functions of these 2 enzymes in glycolysis.

A high activity of tumor-tissue phosphohexose isomerase as herein determined was found to be closely correlated with the property of invasiveness in the tumors. Tumors could be classified in decreasing order of tumor-tissue phosphohexose isomerase activity as malignant epithelial, malignant mesenchymal, benign epithelial, and benign mesenchymal. The value of the application and interpretation of this observation has been suggested.

Alkaline phosphatase was the only enzyme considered in this study which fulfilled the criteria of a marker enzyme. This enzyme was present in significantly higher activity in osteogenic sarcomas, including metastases to soft tissue, than in other neoplasms, including other tumors metastatic to bone.

Serum lactic dehydrogenase activity decreased as a function of increasing age. It was significantly increased by the presence of hemolysis as determined by serum hemoglobin measurements. The mean serum lactic dehydrogenase value was significantly elevated above normal and similar in value for dogs with benign and dogs with malignant tumors. However, many individuals in both groups had values in the normal range.

Like serum lactic dehydrogenase, serum phosphohexose isomerase increased by the presence of hemolysis. The mean serum phosphohexose isomerase activity was significantly elevated in dogs with malignant neoplastic disease, but the mean value in dogs with benign neoplastic disease was less than in the healthy control group.

The mean serum leucyl aminopeptidase value was significantly elevated among dogs with benign neoplastic disease, but not among those with malignant neoplastic disease. A negative correlation was

obtained between this activity and several parameters of malignancy. A possible mechanism for this relationship has been postulated.

The mean serum alkaline-phosphatase activity was significantly higher in dogs with malignant neoplastic disease and slightly higher in dogs with benign neoplastic disease than in the healthy controls. Again, this activity was subject to marked variance.

Postoperative serum enzymatic activities were not significantly lower in dogs from which neoplasms had been excised than in dogs in which they were biopsied. It is postulated that the mean postoperative interval of 4.7 days was insufficient for the inactivation or excretion of enzymes released by the tumor and by the surgical procedure.

REFERENCES

Bär, U., Schmidt, E., and Schmidt, F. W.: Enzym-Muster und Isozyme menschlicher Tumoren. Klin. Wschr., 41, (1963): 977-988.

Bierman, H. R., Hill, B. R., Reinhardt, L., and Emory, E.: Correlation of Serum Lactic Dehydrogenase Activity with Clinical Status of Patients with Cancer, Lymphomas, and Leukemias. Cancer Res., 17, (1957): 660-667.

Bodansky, O.: Serum Phosphohexose Isomerase in Cancer. I. Method of Determination and Establishment of Range of Normal Values. Cancer, 7, (1954): 1191-1199.

_____: Summary of Panel Discussion on Clinical Significance of Enzymes in Blood. N. Y. Acad. of Sci. Annals, 75, (1959): 380-384.

_____: Blood Enzymes in Cancer and Other Diseases. In Advances in Cancer Research Vol. 6 (Haddow and Weinhouse, ed.) Academic Press, New York, N. Y., (1961): 2-80.

Busch, H.: An Introduction to the Biochemistry of the Cancer Cell. Academic Press, New York, N. Y., (1962).

Caraway, W. T.: Microchemical Methods for Blood Analysis. Charles C. Thomas Co., Springfield, Ill., (1960).

Cohnheim, O.: Enzymes. John Wiley and Sons, New York, N. Y., (1912).

Cornelius, C. E.: Liver Function. In Clinical Biochemistry of Domestic Animals. (Cornelius and Kaneko, ed.), Academic Press, New York, N. Y., (1963): 225-302.

Cornelius, C. E., Bishop, J., Switzer, J., and Rhode, E. A.: Serum and Tissue Transminase Activities in Domestic Animals. Cornell Vet., 49, (1959): 116-126.

Dixon, M. and Webb, E.: Enzymes. 2nd ed. Academic Press, New York, N. Y., (1964).

Douglas, W. R.: Relationships of Enzymology to Cancer - A Review. Brit. J. Cancer, 17, (1963): 415-445.

Fishman, W. H., Bonner, C. D., and Homberger, F.: Serum "Prostatic" Acid Phosphatase and Cancer of the Prostate. N. Eng. J. Med., 255, (1956): 925-933.

Fox, T.: Serum Levels of Enzymes in Dogs Subjected to Vibration. Master's Thesis, The Ohio State University, 1960.

Frankel, S. and Reitman, S.: Gradwohl's Clinical Laboratory Methods and Diagnosis. 6th ed. C. V. Mosby Co., St. Louis, Mo., (1963).

Gale, E. F.: Nucleic Acids and Enzyme Synthesis. In Enzymes: Units of Biological Structure and Function. (Gaebler, ed.) Academic Press, New York, N. Y., (1956): 49-66.

Georgii, A.: Über das Lactatedehydrogenase-Erhöhende Virus in Laboratoriumsgeschwülsten. Klin. Wschr., 42, (1964): 559-563.

Goldbarg, J. A., and Rutenberg, A. M.: Colorimetric Determination of Leucine Aminopeptidase in Urine and Serum of Normal Subjects and Patients with Cancer and Other Diseases. Cancer 11, (1958): 283-291.

Greenstein, J. P.: Some Biochemical Characteristics of Morphologically Separable Cancers. Cancer Res., 16, (1956): 641-653.

Gutman, A. B.: Serum Acid Phosphatase in Patients with Carcinoma of the Prostate Gland. J.A.M.A., 120, (1942): 1112-1116.

Holter, H.: Localization of Enzymes in Cytoplasm. In Advances in Enzymology. Vol. 13. Interscience Publishers, Inc., New York, N. Y., (1952): 1-20.

Jacob, F. and Monod, J.: On the Regulation of Gene Activity. Cold Spring Harbor Symposium for Quantitative Biology, 26, (1961): 193-209.

Knox, W. E.: The Hereditary Molecular Diseases. In Enzymes in Health and Disease. (Greenberg and Harper, ed.), Charles C. Thomas Co., Springfield, Ill., (1960): 104-121.

Kubowitz, F. and Ott, P.: Isolierung und Kristallisation eines Gärungsfermentes aus Tumoren. Biochem. Ztschr., 314, (1943): 94-117.

Kuff, E. L. and Hogeboom, G. H.: Sedimentation and Biochemical Characteristics of Cytoplasmic Particles. In Enzymes: Units of Biological Structure and Function. (Gaebler, ed.), Academic Press, New York, N. Y., (1956): 235-251.

Loeb, W. F. and Nagode, L. A.: The Distribution of Enzymes in Canine Serum and Erythrocytes. Manuscript in preparation, (1965).

Maciejewski, A. and Bobinski, H.: Serum Lactic Dehydrogenase and Ribonuclease in the Diagnosis of Malignant Tumors in Children. Arch. Dis. Childh., 40, (1965): 183-185.

Majno, G. and Rouiller, C.: Die alkalische Phosphotase in der Biologie des Knochengewebes. Virchows Arch. Path. Anat., 321, (1951): 1-61.

Marmur, J. and Hotchkiss, R. D.: Mannitol Metabolism, A Transferable Property of Pneumococcus. J. Biol. Chem., 214, (1955): 383-396.

Mather, A.: Standardization of Nomenclature in Clinical Chemistry. Clin. Chem., 11, (1965): 348-353.

Monod, J.: Remarks on the Mechanism of Enzyme Induction. In Enzymes: Units of Biological Structure and Function. (Gaebler, ed.), Academic Press, New York, N. Y., (1956): 7-28.

Monod, J. and Jacob, F.: General Conclusions: Teleonomic Mechanisms in Cellular Metabolism, Growth, and Differentiation. Cold Spring Harbor Symposium on Quantitative Biology, 26, (1961): 389-401.

Nagode, L. A.: Enzymatic Activities in Normal Canine Tissues. Master's Thesis, The Ohio State University, (1965).

Nagode, L. A., Frajola, W. J., and Loeb, W. F.: Enzymes in Normal Canine Tissues. In Press, (1965).

Pitot, H. C. and Heidelberger, C.: Metabolic Regulatory Circuits and Carcinogenesis. Cancer Res., 23, (1963): 1694-1700.

Potter, V. R.: Biochemical Studies on Minimal Deviation Hepatomas. In Cellular Control Mechanisms and Cancer. (Emmelot and Mülbock, ed.), Elsevier Publishing Co., New York, N. Y., (1964): 190-210.

Report of the Commission on Enzymes of the International Union of Biochemistry. International Union of Biochemistry Symposium Series, Vol. 20. Pergamon Press, New York, N. Y., (1961).

Roe, J. H. and Whitmore, E. R.: Clinicopathologic Application of Serum Phosphatase Determinations, with Special Reference to Lesions of the Bones. Am. J. Clin. Path., 8, (1938): 233-254.

Schmidt, E. and Schmidt, F. W.: Untersuchungen über die Alterung von Enzymen in vitro. IV. Mitteilung: Zur Methodik von Enzym-Bestimmungen in Menschlichen Organ-Extrakten und im Serum. Enzym. Biol. et Clin., 3, (1963): 80-86.

Spiegelman, S.: On the Nature of the Enzyme Forming System. In Enzymes: Units of Biological Structure and Function. (Gaebler, ed.), Academic Press, New York, N. Y., (1956): 67-89.

Sumner, J. B. and Myrback, K.: The Enzymes. Vol. 1 Part 1. Academic Press, New York, N. Y., (1950).

Warburg, O.: Metabolism of Tumors. Arnold Constable, London, England, (1930).

Warburg, O. and Hiepler, E.: Versuche mit Ascites-Tumorzellen. Z. Naturforsch., 7b, (1952): 193-194.

Wachstein, M.: Histochemistry of Enzymes in Tumors. In Handbuch der Histochemie. (Graumann and Neumann, ed.), Vol. 7, Part 2, Gustav Fischer Verlag, Stuttgart, Germany, (1962): 73-153.

Weinhouse, S.: Enzyme Systems in Tumor Cells. In Enzymes in Health and Disease. (Greenberg and Harper, ed.), Charles C. Thomas Co., Springfield, Ill., (1960): 191-211.

White, L. P.: Advances in the Diagnostic Use of Enzymes. In Enzymes in Health and Disease. (Greenberg and Harper, ed.), Charles C. Thomas Co., Springfield, Ill., (1960): 331-358.

Wroblewski, F.: The Significance of Serum Glutamic Oxaloacetic Transaminase in Experimental and Clinical Studies. Trans. N. Y. Acad. Sci., 18, (1956): 444-450.

Wroblewski, F. and LaDue, J. S.: Lactic Dehydrogenase Activity in Blood. Proc. Soc. Exp. Biol. and Med., 90, (1955): 210-213.

Wu, R.: Leakage of Enzymes from Ascites Tumor Cells. Cancer Res., 19, (1959): 1217-1222.

Yaffe, D.: The Distribution and In Vitro Propagation of an Agent Causing High Plasma Lactic Dehydrogenase Activity. Cancer Res., 22, (1962): 573-580.

Zondag, H. A.: L. D. H. Isozymes - Lability at Low Temperatures. Science, 142, (1963): 965-966.