The Cytology, Histology and Prevalence of Cell Types in Canine Lymphoma Classified According to the National Cancer Institute Working Formulation

R.F. Carter, V.E.O. Valli and J.H. Lumsden*

ABSTRACT

No significance has been shown yet between the cytological subtypes of canine lymphoma and clinical behaviour. This paper describes and illustrates the cytological and histological criteria for application of the National Cancer Institute Working Formulation classification system, a scheme with demonstrated prognostic capability for human non-Hodgkin's lymphomas, to a series of 285 canine lymphomas. The Working Formulation can be used without difficulty for canine lymphomas. Low grade follicular tumors were found to be much less common, and high grade, aggressive tumors much more common than these cell types in humans. Low grade tumors tend to have low mitotic rates and high grade tumors tend to have high mitotic rates. There may be an association between hypercalcemia and lymphoblastic cell type. A review of available literature data for canine lymphomas suggests that prognostic extrapolation of clinical behaviour based on human lymphoma data may be possible. These results suggest that there may be strong similarities of morphology and behaviour between human non-Hodgkin's lymphomas and canine lymphomas.

Key words: Lymphoma, canine, cytology, histology, classification.

RÉSUMÉ

Nous ignorons toujours la possibilité d'une relation entre les variétés cytologiques du lymphome canin et leur comportement clinique. Le présent article décrit et illustre, à partir de 285 de ces lymphomes, les critères cytologiques et histologiques propres à l'application du système de classification de la formulation de travail de l'Institut National du Cancer, une façon de procéder dont on connaît la valeur pronostique pour les lymphomes humains autres que celui de Hodgkin. On peut appliquer, sans difficulté, la formulation précitée aux lymphomes canins. Les tumeurs folliculaires et peu malignes s'avérèrent beaucoup moins fréquentes que leurs correspondantes, chez les humains, contrairement à celles qui étaient plus malignes et plus agressives. Les tumeurs peu malignes affichaient ordinairement peu de mitoses, contrairement aux plus malignes. Il existe peut-être une relation entre l'hypercalcémie et le type de cellules lymphoblastiques. Une revue de la littérature disponible sur les lymphomes canins suggère la possibilité d'une extrapolation pronostique de leur comportement clinique, en se basant sur les données relatives au lymphome humain. Les résultats de l'expérience permettent de penser à la possibilité de fortes ressemblances de morphologie et de comportement entre les lymphomes humains autres que celui de Hodgkin et les lymphomes canins.

Mots clés: lymphome, chiens, cytologie, histologie, classification.

INTRODUCTION

Current clinical management of multicentric canine lymphoma is unsatisfying because of unpredictable variation in survival time and short average survival time. These factors make counselling difficult when advising owners about the benefits of treatment. Attempts to predict response to therapy have primarily studied possible correlations to clinical stage and histological cell types. Clinical stage at presentation is known to affect both complete remission rate and overall survival time (1). However, despite repeated consideration in the past (2,3,4,5,6,7,8), no strong correlation has been shown between histological classification of the diversity of cell types which occur and either remission rate or survival. Several recent studies have either completely ignored histology (1,9), or have used simplistic classification schemes (10). A recent phenotypical study (8) has shown a correlation between attainment of complete remission and absence of a membrane marker characteristic of activated T-lymphocytes.

We remain hopeful that correlations between cell types and clinical behaviour exist for canine lymphoma. In humans, such correlations have been known for at least 20 years, and there are numerous classification schemes (11,12). Canine lymphomas have numerous similarities with human non-Hodgkin's lymphomas, and it has not yet adequately been shown that application of a clinically relevant human lymphoma classification scheme to canine lymphomas is without prognostic value.

This paper describes the histological

*Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Reprint requests to Dr. V.E.O. Valli.

Supported in part by the Macdonald-Stewart Foundation and the Medical Research Council of Canada.

Submitted May 1, 1985.

and cytological criteria for our application of the National Cancer Institute Working Formulation (11) to canine lymphomas, and the distribution of cell types found in a retrospective random survey of 285 cases. The Working Formulation describes ten basic cell types which are divided into three grades of clinical behaviour: low, intermediate, and high. Low grade tumors have the best five year survival rate, while high grade tumors have the worst. The Working Foundation was chosen as the classification scheme for this study because it represents a concensus of experts, has excellent descriptions of cell types and demonstrated prognostic capabilities, and can be used as a cross reference for all other major terminologies for non-Hodgkin's lymphoma cell types.

MATERIALS AND METHODS

One hundred and ninety-one specimens were compiled from those available at the provincial Veterinary Laboratory Services diagnostic facility (VLS), Guelph, Ontario and 94 specimens from the Ontario Veterinary College (OVC), University of Guelph. The VLS specimens consisted only of histological preparations from biopsies; both cytological and histological preparations were available from the OVC cases. The histological specimens were fixed in formalin and embedded in paraffin, and routine 4 and 6 μm sections were stained with hematoxylin and eosin. For cytology, air-dried fine needle aspirations or imprints were stained with the Ames Hema-Tek (Wright's) staining instrument. Very cellular samples were processed twice so that nucleolar and chromatin patterns could be seen clearly. Wet-fixed cytological specimens were sprayed with Pro-Fixx (Lerner Laboratories, New Haven, Connecticut) and stained by the Papanicaloau method. The Osbaldiston method (13) was used to demonstrate alpha-naphthyl acetate esterase activity on air-dried cytological preparations. Cells which contained a single "dot" reaction or multifocal small dot reaction were considered to be T lymphocytes. The mitotic rate was estimated in histological specimens by scanning four or five fields at 40x power and counting mitotic figures. A low mitotic index was defined as 0 to 2 mitoses per field, medium as 3 to 5 per field, and high as 6 or more per field. Correlations of mitotic rate with cell type were evaluated statistically by the Chi-square (χ^2) test. The nuclear size of lymphocytes was estimated by comparison to the diameter of undistorted red blood cells present in the specimen under consideration. All high power photomicrographs shown are enlarged to the same magnification to facilitate size comparisons between specimen preparation methods and cell types.

RESULTS

A. CRITERIA FOR CLASSIFICATION

The morphological criteria used for this study were derived from the NCI Working Formulation in the course of pilot studies on the classification of canine lymphoma. The following describes the criteria used for designation according to architecture, mitotic rate and cell type. All figures except Fig. 1 are from canine tumors. The prevalence of each cell type and observed mitotic rates are shown in Table I, along with data from the reference NCI series of human lymphomas (11).

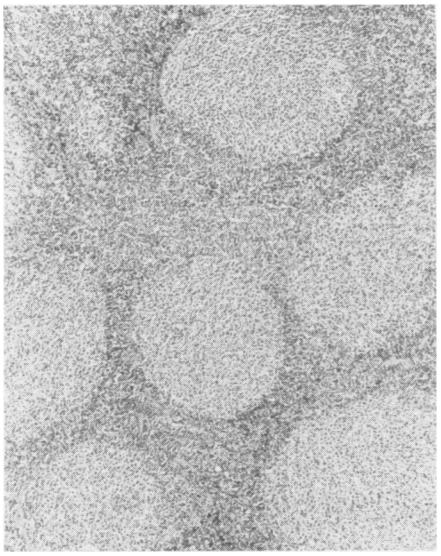


Fig. 1. Section from human lymphoma demonstrating pronounced circular areas of neoplastic expansion characteristic of a follicular lymphoma. All such tumors are of B-cell origin. H & E. X32.

ARCHITECTURE

The basic pattern of nodal infiltra-

TABLE I. Prevalence of Cell Types in a Survey of 285 Cases of Canine Lymphoma

	Cell Type	Mitotic Rate			Total			Human (11)
		Low	Medium	High	#		%	%
Low Grade	Diff Small Lymphocytic	12	2	0	14		4.9	3.6
	Foll Small Cleaved	0	0	0	0	15	0.0 5.3	22.5
	Foll Mixed	0	1	0	1		0.4	7.7
Intermediate Grade	Foll Large	0	0	1	1		0.4	3.8
	Diff Small Cleaved	15	2	0	17		5.9	6.9
	Diff Mixed	2	3	1	6	81	2.1 28.4	6.7
	Diff Large	3	17	37	57		20.0	19.7
High Grade	Immunoblastic	2	28	41	71		24.9	7.9
	Lymphoblastic	2	13	34	49	189	17.2 66.3	4.2
	Small Noncleaved	1	23	45	69		24.2	5.0
	Total #	37	89	159	285		—	1104
	Total %	13.0	31.2	55.8	—		100	100

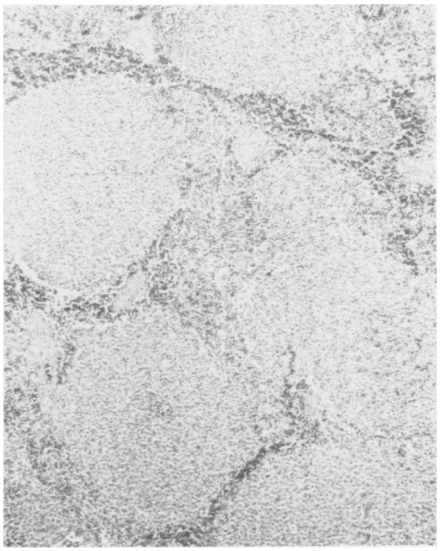


Fig. 2. Section from canine lymphoma showing follicular architecture similar to that of Fig. 1. High endothelial venules, a feature of paracortical (thymus-dependent) areas, are only found in interfollicular areas. H & E. X32.

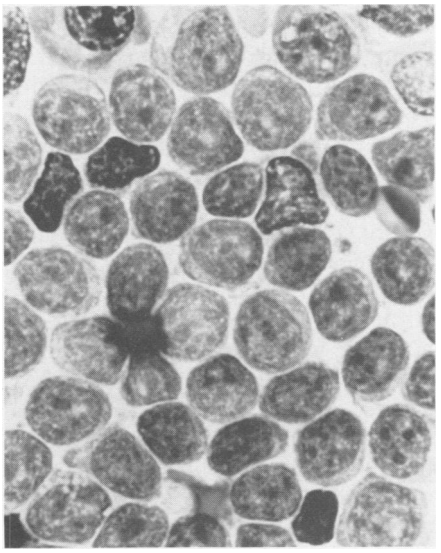


Fig. 3. Air-dried imprint of canine lymphoma: DSL type consistent with CLL. Note the largely uniform chromatin pattern, inapparent nucleoli, small round nuclei and small amount of cytoplasm. Wright's. X695.

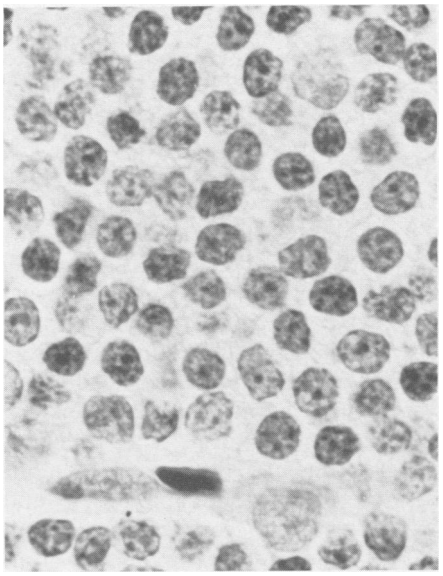


Fig. 4. Section from same tumor shown in Fig. 3: DSL, consistent with CLL. Note the uniform and nonmalignant appearance of the cells. H & E. X695.

tion is either follicular or diffuse. In humans, follicular lymphomas are relatively common (Fig. 1). In the present series of cases, tumors which truly reflected a follicular appearance similar to that of human cases were rare (Fig. 2). By far the majority of canine lymphomas exhibit a diffuse pattern of proliferation which in completely affected nodes totally effaces normal nodal architecture. In less affected nodes the medulla may have only some filling of the medullary cords with normal sinuses evident; large cell types may preferentially seed to the cortex while small cell types may fill the medullary cords, causing sinus compression.

MITOTIC RATE

An actual count of mitotic figures is necessary so that the mitotic index can be accurately assessed. The presence of numerous macrophages, causing a "starry sky effect", has been used as an indicator of high mitotic rate. This may be misleading, for if there is considerable necrosis evident, numerous macrophages may be present while the actual mitotic rate can be low. In cytological specimens, mitotic figures are characteristically fewer and harder to detect, so that the finding of two or three mitoses in a 40x field would indicate a very high mitotic rate.

CELL TYPE

Low grade

Diffuse Small Lymphocytic — DSL (Figs. 3-6) — The architecture is diffuse, the mitotic index is low and mitoses may not be observed. The nuclei are about the same size as a canine erythrocyte (Fig. 4) and uniformly round with an even periphery. The chromatin pattern is

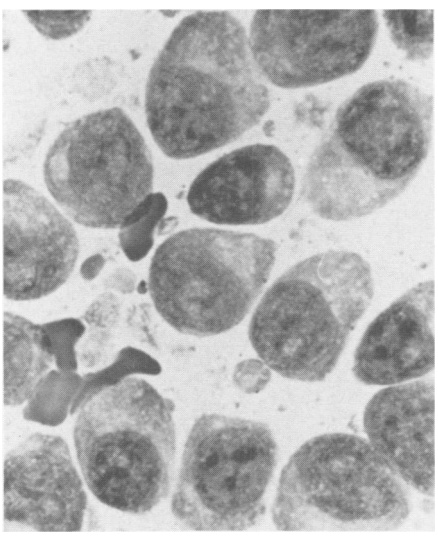


Fig. 5. Air-dried imprint from canine lymphoma: DSL of plasmacytoid differentiation. There is a resemblance to both benign plasma cells and immunoblastic lymphoma (see Fig. 15). Diagnosis by cytology is difficult; in this case histological sections demonstrated a diffuse homogeneous population of small cells with low mitotic rate and plasmacytoid differentiation. Wright's. X695.

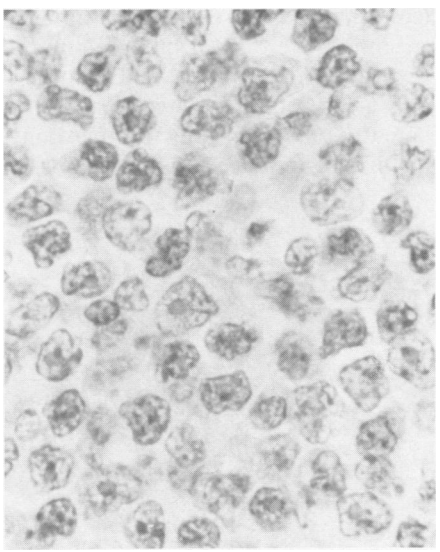


Fig. 6. Section from canine lymphoma: DSL of intermediate differentiation. The mitotic rate was low. Note the increased nuclear detail, greater irregularity of the nuclear periphery and increased prominence of nucleoli compared to Fig. 4. This cell type must also be distinguished from the DSC, LB and SNC types. H & E. X695.

like that of cortical thymocytes but coarsely aggregated and with more internal nuclear detail. Nucleoli are absent or there may be one small, usually central nucleolus. The cytoplasm is usually scant and poorly staining, although in Wright's stained cytological preparations, it may be quite basophilic. The distinguishing feature is that the cells resemble normal, "well-differentiated" small lymphocytes, and that it is only their homogeneity, hypercellularity and architectural pattern which allows the diagnosis to be made. Recognition of this cell type is especially difficult from fine needle aspiration smears. In humans the coexistence of small lymphocytic lymphoma and chronic lymphocytic leukemia is a common finding, and a similar association was found in the present series.

Two variants of DSL exist in human lymphomas and these have also been recognized in our series. The term "DSL-plasmacytoid" (Fig. 5) refers to cases where the nuclei are similar to the above description but eccentrically placed in cytoplasms which are more abundant, predominantly amphophilic in staining and have Golgi zones. Cells indistinguishable from plasma cells may be found. A second variant is termed "DSL-intermediate type" (Fig. 6). Not described in the Working Formulation, it is a recently recognized subtype of intermediate differentiation frequently included in the DSL category. The nuclei of these cells may be up to 1.5 red blood cells in diameter, with more irregularity of the nuclear membrane, and typically a more prominent central nucleolus. Mitoses are evident but infrequent.

Follicular Small Cleaved — FSC — There were no examples of this tumor in the present series. Tumors of a pseudofollicular architecture and the appropriate cell type, small and cleaved, were included with the diffuse small cleaved cell type.

Follicular Mixed — FM — The architecture is follicular and the mitotic rate is low. The cells in the proliferation centres are similar to those of diffuse mixed lymphomas (q.v.; Figs. 9-11); that is, there is a definite coexistence of small cleaved nuclei and large nuclei.

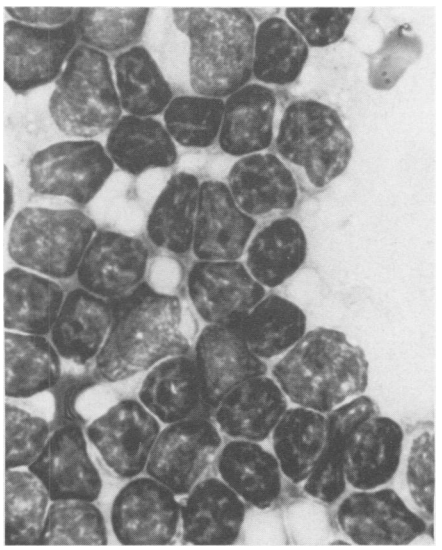


Fig. 7. Air-dried imprint from canine lymphoma: DSC. Although the nuclei are extensively and deeply cleft by linear invaginations, there is little evidence of this in air-dried preparations. They are small and very hyperchromatic with a coarse retiform chromatin pattern. Wright's. X695.

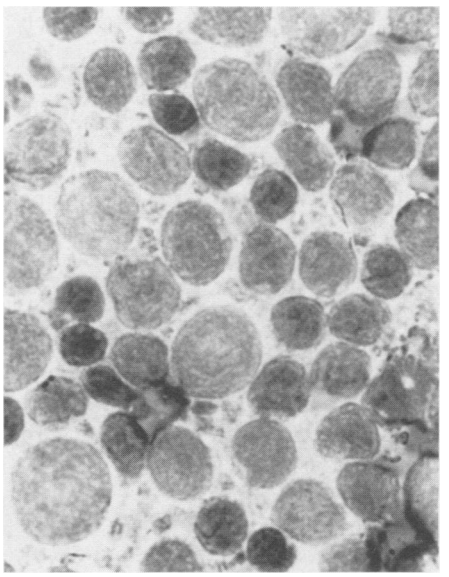


Fig. 9. Air-dried imprint from a rectal mass diagnosed as canine lymphoma: DM. The small cells have densely stained cleaved nuclei, while the large cells have a cribriform chromatin pattern and variable numbers of nucleoli. Wright's. X695.

Intermediate Grade

Follicular Large — FL — The architecture is follicular and the mitotic rate is high in the follicles. The proliferating cells are similar to those of diffuse large tumors (q.v.; Figs. 12-14), and have a predominance of large nuclei at least two red blood cells in diameter with multiple nucleoli.

Diffuse Small Cleaved — DSC (Figs. 7 and 8) — The architecture is diffuse

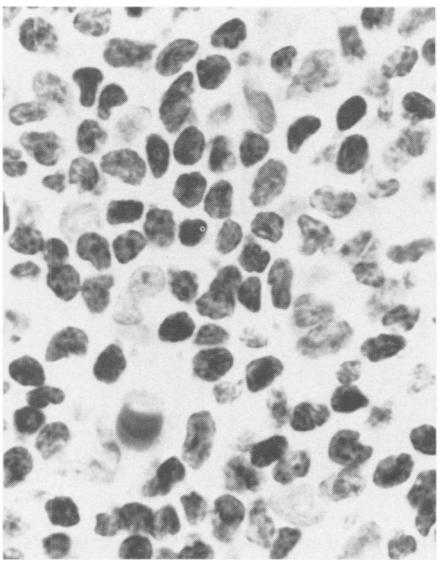


Fig. 8. Section from canine lymphoma: DSC. Note the dense and angular appearance of the nuclei, which is due to deep linear clefts. H & E. X695.

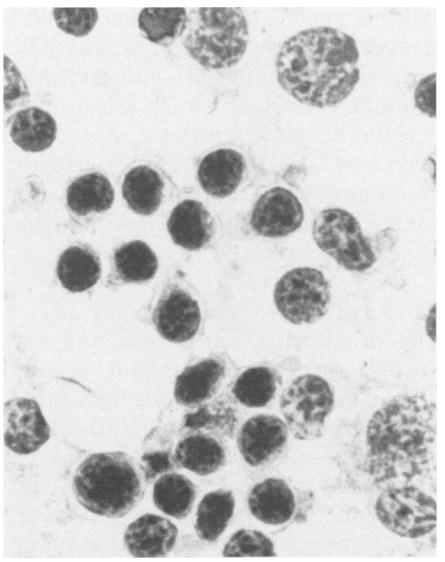


Fig. 10. Wet-fixed imprint from canine lymphoma: DM. Note the contrast between the small cleaved and large noncleaved nuclei. Paps. X695.

and the mitotic rate is low, or rarely medium to high. The nuclei are the same size as a red blood cell or smaller, with deep linear indentations of the nuclear membrane which give them an irregular angular look on low power histology. In cytological specimens, the deep clefts are more evident in wet-fixed than in air-dried preparations, where the only clue may be shallow indentations in the nuclear periphery (Figs. 7 and 10). The nuclei are typically dense and hyperchromatic, with a coarse heterochromatin pattern obscuring one or two small nucleoli. The cytoplasm is usually scant.

Diffuse Mixed — DM (Figs. 9-11) — The architecture is diffuse and the mitotic rate is variable but usually low or medium. There must be two distinctly different cell populations: small cleaved, and large noncleaved or cleaved. If more than one-third of the cells present in a representative field are large, then the tumor is classified diffuse large. The small cells are similar to those of diffuse small cleaved, i.e. small, densely stained and angular in outline. The large nuclei may be cleaved or noncleaved; in the present series nearly all were noncleaved. Noncleaved nuclei are two or more red blood cells in diameter, round to oblong in shape, and may have irregularities of nuclear outline but do not have the deep linear and folded indentations characteristic of cleaved nuclei. Both noncleaved and cleaved large nuclei have a vesicular, open chromatin pattern and multiple, prominent nucleoli which are usually peripheral in the nucleus. The cytoplasm of the large cells is usually abundant but faintly stained with somewhat indistinct borders in histological specimens.

Diffuse Large — DL (Figs. 12-14) — The architecture is diffuse and the mitotic rate is variable but usually high. The nuclei are almost invariably noncleaved, and easily recognizable as large; they are usually two or more red blood cells in diameter, very open and vesicular, with multiple prominent, usually peripheral nucleoli impinging on the nuclear membrane that are especially apparent in wet-fixed Papanicolaou cytology preparations. The nucleoli can be as large as red blood cells and frequently five or six in number. The cytoplasm varies from scant to abundant, but if most cells have single prominent central nucleoli and are plasmacytic in appearance the tumor is an immunoblastic lymphoma.

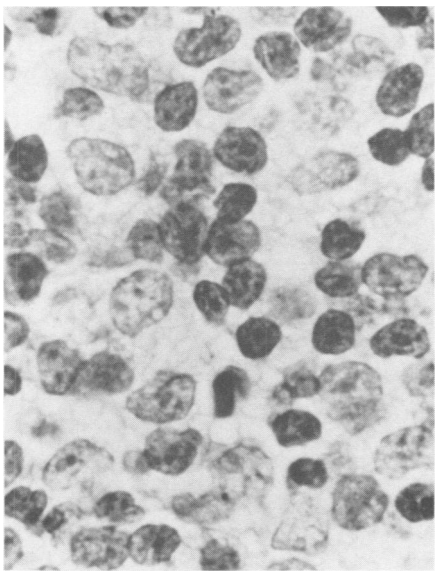


Fig. 11. Section from canine lymphoma: DM. This specimen demonstrates the coexistence of two distinct sizes of nuclei. Deep cleavage planes can be seen in the small nuclei. H & E. X695.

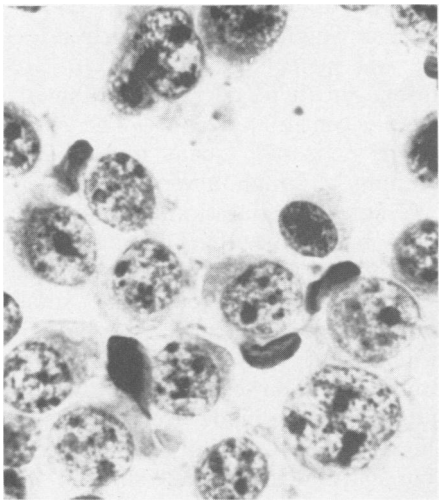


Fig. 13. Wet-fixed imprint of same tumor shown in Fig. 12: DL noncleaved. Nucleoli which may be difficult to detect in Wright's stained material are immediately obvious in wet-fixed Paps preparations. This specimen demonstrates a wide range in nuclear size, common in DL tumors. Paps. X695.

High Grade

Immunoblastic — IB (Figs. 15-18) — The architecture is diffuse and the mitotic rate is usually high. In human lymphomas, this cell type has the largest nuclei of all lymphomas. In the

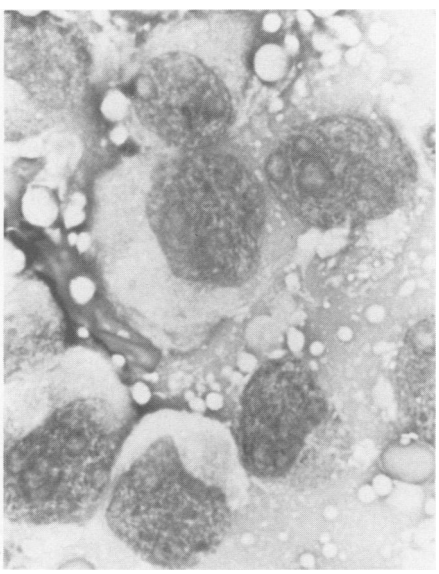


Fig. 12. Air-dried imprint from canine lymphoma: DL noncleaved. Note the large size of nuclei, multiple prominent nucleoli and abundant cytoplasm. Such tumors may be noncleaved or cleaved, but the latter are rare. Wright's. X695.

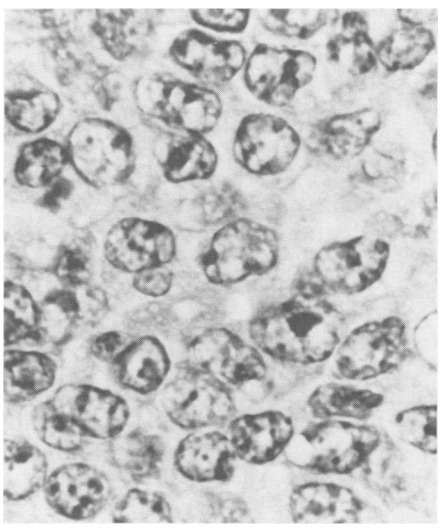


Fig. 14. Section from canine lymphoma: DL noncleaved. Most nuclei should have multiple peripheral nucleoli. In some cases numerous cells have single nucleoli and are more consistent with the IB cell type; classification is then primarily based upon other characteristics such as cytoplasmic differentiation. H & E. X695.

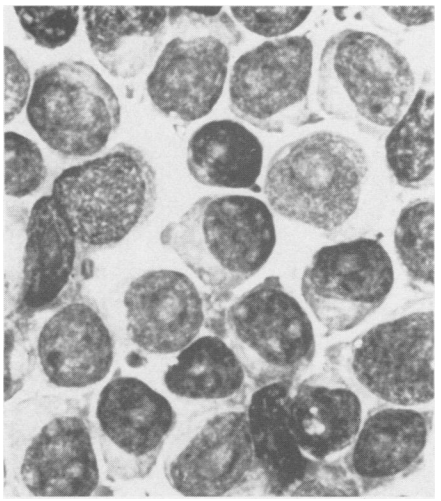


Fig. 15. Air-dried imprint from canine lymphoma: IB with plasmacytoid differentiation. Prominent large single central nucleoli in cells with plasmacytoid cytoplasm and a high mitotic rate are reliable criteria. The nuclear diameter is less than that of comparable human tumors. Wright's. X695.

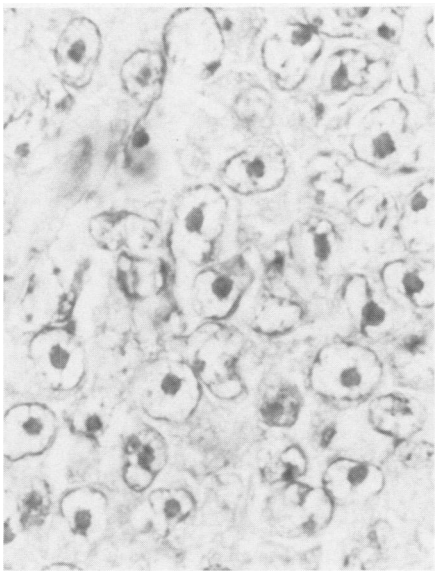


Fig. 17. Section from canine lymphoma: IB. The size of the nuclei is similar to that of comparable human lymphomas, but uncommonly large for canine IB. H & E. X695.

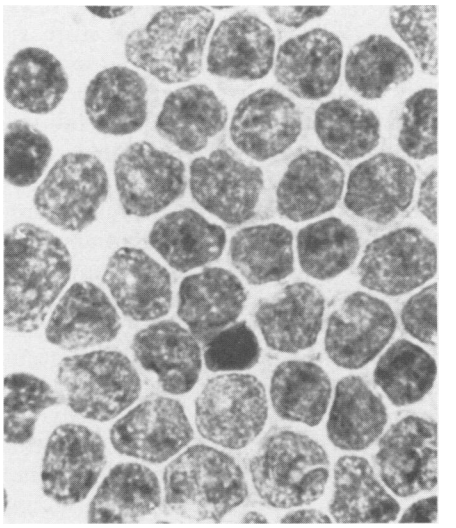


Fig. 19. Air-dried imprint from canine lymphoma: convoluted LB. Note the absence of nucleoli in nuclei which have fine to coarse cribriform chromatin and very irregular and indented nuclear peripheries. Cytoplasm is almost undetectable. Wright's. X695.

present series a minority of cases fit the classic criteria of very large, vesicular nuclei with prominent usually single central large nucleoli and plasmacytoid or clear cell type cytoplasm (Fig. 17). However, numerous cases fit the nuclear criteria except for size (Fig. 18); that is, the nuclei are about 1.5 to 2 red blood cells in diameter, the nuclear membrane is generally round to oblong, the chromatin pattern open and vesicular, numerous nuclei have prominent large central nucleoli, and there is a high mitotic rate. The cytoplasm is variable but frequently amphophilic or basophilic, and prominent Golgi zones may be especially evident in air-dried Wright's stained preparations. These tumors fit best in the immunoblastic category. Although plasmacytic type differentiation was common in this category in general, no clear cell type immunoblastic lymphomas were found. In the rare case where a gammopathy is present, then the tumor is termed a secretory immunoblastic lymphoma.

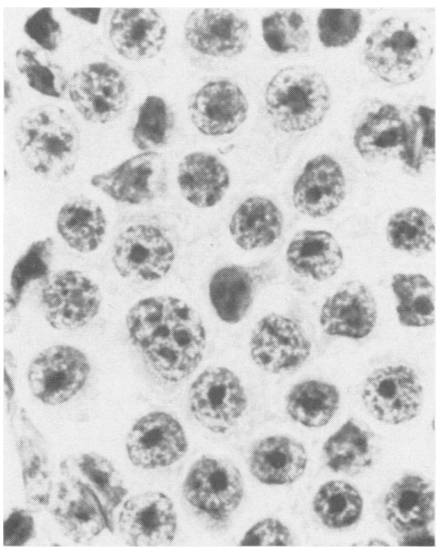


Fig. 16. Wet-fixed imprint from canine lymphoma: IB. Note that although nuclei with single and multiple nucleoli are both present, the latter are much less common. Paps. X695.

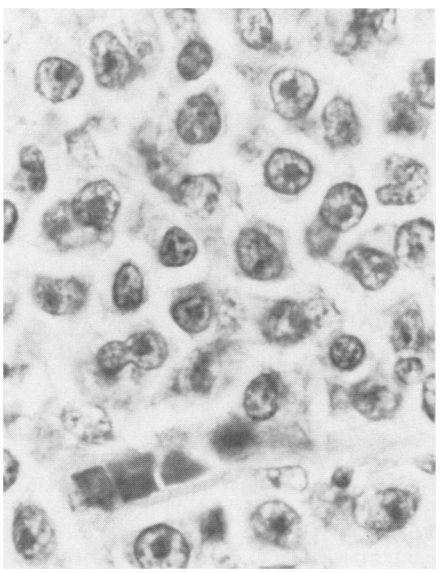


Fig. 18. Section from canine lymphoma: IB. An example of the more common size range for canine IB lymphomas. H & E. X695.

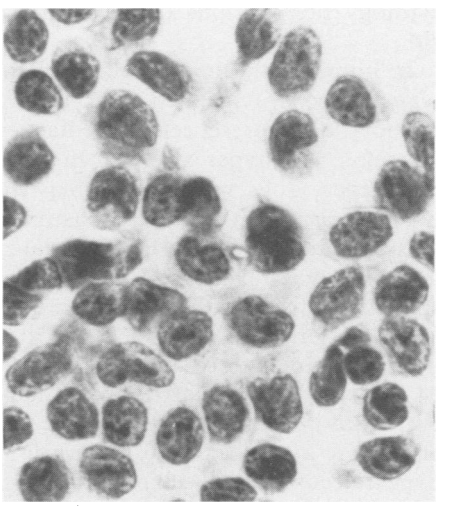


Fig. 20. Wet-fixed imprint from canine lymphoma: convoluted LB. Densely stained areas in the nuclei are heterochromatin aggregations, not nucleoli. The extremely irregular outlines of the nuclei are very evident in wet-fixed preparations. Paps. X695.

Lymphoblastic Lymphoma — LB (Figs. 19-22) — The architecture is diffuse, and the mitotic rate is usually very high (up to 35 per field). The mitotic nuclei are frequently faintly stained in both histological and cytological specimens and must be looked for carefully. The nuclei are about 1.5 red blood cells in diameter, round, oval, or irregular in shape, and distinguished by a uniform chromatin pattern which obscures any nucleoli. The nuclei are frequently dusky grey in hematoxylin and eosin histological preparations, while in air-dried Wright's specimens, the chromatin pattern is hyperchromatic and fine cribriform to coarse cribriform in pattern. Nucleoli are either not present or multiple, small and not prominent. The nuclear shape can be either nonconvoluted or convoluted, with the former more common (about 60%). Convoluted lymphoblastic tumors have prominent infolding and irregularities of the nuclear membrane, generally visible only at high magnification. Convoluted lymphoblastic lymphomas are distinguished from diffuse small cleaved tumors by their larger nuclear size, lighter and more homogeneous staining pattern, inobvious nucleoli, irregularities of nuclear membrane as opposed to deep linear clefts, and generally much higher mitotic rate. Cytoplasm in the lymphoblastic cell types is scant and lightly stained and does not form a complete ring around the nuclei in cytology preparations (Figs. 19 and 20).

In humans, the lymphoblastic cell type has been associated with T-cell phenotype, the presence of a mediastinal mass and hypercalcemia. In our experience, dogs with lymphoblastic lymphomas often have mediastinal masses, and when the appropriate material was available, frequently stained positively with alpha-naphthyl acetate esterase in either a single or multifocal dot pattern (Fig. 22). Out of 14 cases of lymphoblastic lymphoma with a known chemical profile, 12 or 86% were hypercalcemic. However, hypercalcemia and T-cell positivity by ANAE staining are not always coexistent and are not restricted to lymphoblastic lymphomas; occasionally both have been found independently and together in diffuse small cleaved, diffuse large and small noncleaved canine lymphomas.

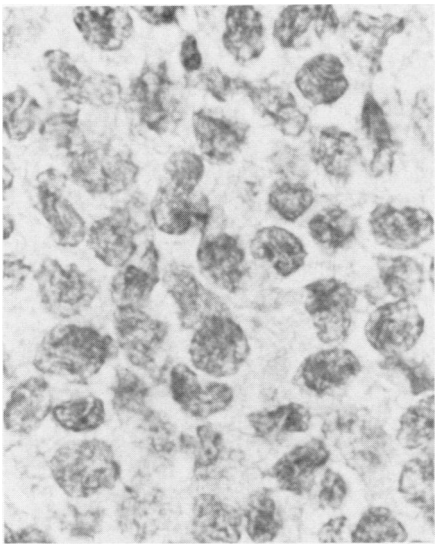


Fig. 21. Section from same specimen as Fig. 20: convoluted LB. The nuclei have no nucleoli and the margins are so irregular that they are indistinct. Nonconvoluted LB lymphomas have a similar homogenous chromatin staining pattern in cells of uniform round to oval shape. H & E. X695.

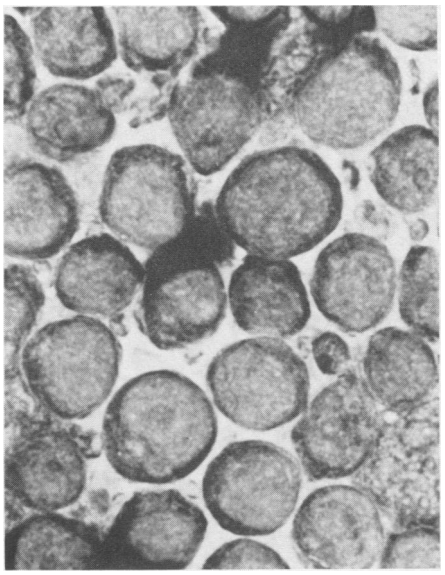


Fig. 23. Air-dried imprint from canine lymphoma: SNC of non-Burkitt's type. These nuclei are at the upper limit of diameter for this cell type. Multiple nucleoli are present in each nucleus but are difficult to discern. Note the complete rings of cytoplasm. Wright's. X695.

Small Noncleaved — SNC (Figs. 23-26) — The architecture is diffuse and the mitotic rate is usually high. The Working Formulation describes two cell types: Burkitt's type and non-Burkitt's type. In the non-Burkitt's type of SNC lymphoma, the nuclei are larger than those of benign small lymphocytes but not the obviously large nuclei of diffuse large tumor type. They average about 1 to 1.5 red

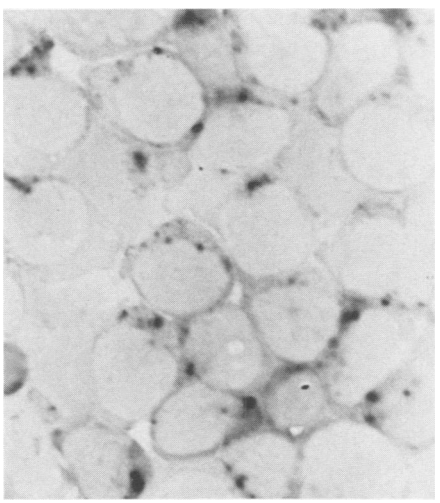


Fig. 22. Air-dried imprint from same specimen as Figs. 20 and 21: convoluted LB. When stained for alpha-naphthyl acetate esterase activity, many LB lymphomas are positive in either a focal, multifocal or mixed (as in this case) pattern, indicating their T-cell nature. ANAE and methyl green, X695.

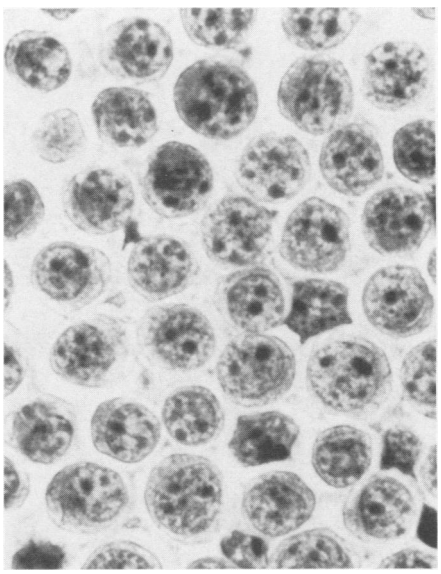


Fig. 24. Wet-fixed imprint from same specimen as Fig. 23: SNC of non-Burkitt's type. The multiple nucleoli and noncleaved nature of the nuclei are obvious. Paps. X695.

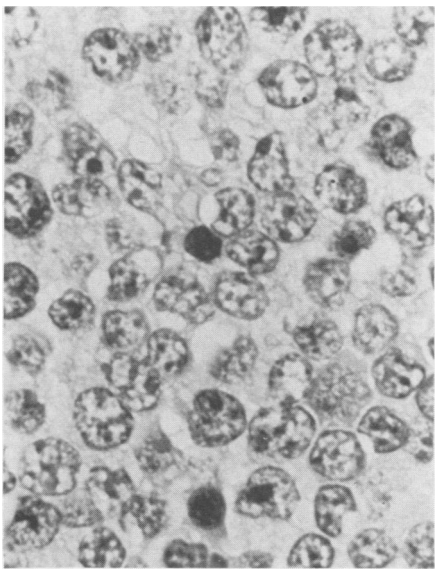


Fig. 25. Section from canine lymphoma: SNC of non-Burkitt's type. Note that although small, the nuclei are very transformed with multiple small and large nucleoli in a vesicular chromatin pattern. H & E. X695.

blood cells in diameter. The nuclei are uniformly round to ovoid, and the membrane is focally thickened, or sometimes slightly irregular but definitely not cleaved. The heterochromatin is coarse, peripheralized and hyperchromatic, the euchromatin is clear and there are usually single or multiple prominent nucleoli. We have included in this cell type cases where the nuclei are small with a single large central nucleolus somewhat obscured by chromatin. These cells could be immunoblastic but are small and do fit the criteria described by the Working Formulation for small noncleaved type. The cytoplasm is more commonly pale and small but occasionally amphophilic and abundant; it always forms at least a complete ring around the nucleus.

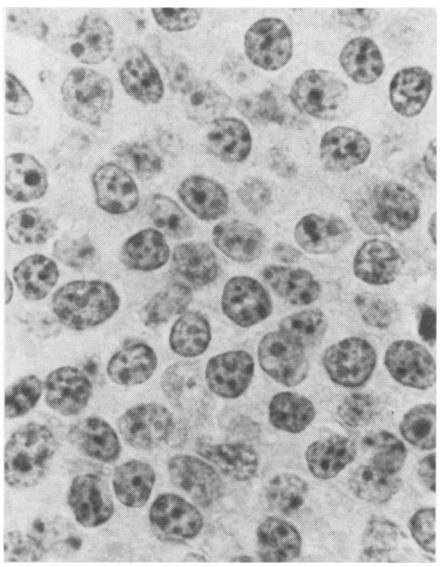


Fig. 26. Section from canine lymphoma: SNC of Burkitt's type appearance. The multiple small nucleoli in a homogeneous population of round small nuclei are very similar to Burkitt's type lymphoma. However, the morphology is not exactly comparable in this case because the cytoplasms are not facetted with squared off corners due to crowding. H & E. X695.

Two cases with the appearance of a Burkitt's type lymphoma have been seen (Fig. 26). The nuclei are monotonously similar and round with vesicular nuclei and multiple small nucleoli. The cells have distinct boundaries and typically are squared off due to separations of cell membranes. These tumors are identified by their extreme homogeneity of appearance.

B. PREVALENCE OF CELL TYPES

The results of classification of a random survey of 285 cases are summarized in Table I, where cell type is compared with mitotic rate.

The common cell types for dogs are immunoblastic (24.9%), small noncleaved (24.2%), diffuse large (20%) and lymphoblastic (17.2%). Less common are the diffuse small cleaved (5.9%), diffuse small lymphocytic (4.9%) and diffuse mixed (2.1%) types. Follicular mixed and follicular large tumors were rare (0.4% each), and no follicular small cleaved lymphomas were found. The relative proportions of low, intermediate and high grade tumors are 5.3%, 28.4% and 66.3% respectively. If the diffuse large tumors are included, the four most aggressive cell types in human lymphomas comprise fully 86% of canine lymphomas.

Mitotic rate and cell type are correlated ($P < 0.005$). For example, most diffuse small lymphocytic lymphomas had low or absent mitotic indices; the two tumors which had slightly higher rates of mitoses were both of the intermediate type of differentiation, which allows more heterogeneity and transformation of nuclear appearance. No low grade tumors have a high mitotic rate. In the intermediate category, most diffuse small cleaved tumors have a low mitotic rate (15 of 17 or 85%), whereas most of the diffuse large tumors have a medium or high index (54 of 57 or 95%). In the high grade tumors, 64% (121 of 189) had a high mitotic rate, and less than 3% (3 of 189) had a low mitotic rate. Thus it would appear that estimation of mitotic rate alone is a good approximation of tumor grade. A medium or high mitotic index is much more frequently found in the four most aggressive cell types,and the opposite is also true; aggressive cell types rarely have a low mitotic rate.

DISCUSSION

The first purpose of this work was to determine if the Working Formulation could be applied to canine lymphomas. In general, few adaptations of descriptions were required and most tumors fitted easily into a described category.

There were, however, exceptions. The major one is that tumors which we have called immunoblastic lymphomas frequently consist of cells with nuclei which are decidedly smaller than those of human immunoblastic lymphomas. This has been observed previously (8), and could be considered a significant problem given the number of "immunoblastic" lymphomas which are found. These cells could be termed "partially transformed" or given some other similar term and a new category created, but this would defeat the purpose of using an established classification. Instead, we emphasize the similarities these tumors have with the classic immunoblastic cell type (architecture, mitotic rate, nuclear and nucleolar appearance, and frequently cytoplasmic differentiation), and suggest that the sole difference of smaller nuclear diameter is less important. We thus classify immunoblastic lymphomas primarily by nuclear morphology, and subtype them as plasmacytoid (common) or clear cell type (rare) if such differentiation is present. In the clear cell type the nuclei appear widely separated by abundant pale staining cytoplasm, and in the past have often been found in classic "multiple myeloma" cases.

Canine lymphoblastic lymphomas appear to be a little more variable in morphology than their human counterparts. Occasionally dogs with mediastinal masses and hypercalcemia will have tumors with a largely cribriform chromatin pattern and finely dispersed heterochromatin, but also have one or two small nucleoli which are more prominent than would be expected. Given that these tumors are often also ANAE-positive (unpublished data) and therefore likely T-cell types, they fit best as lymphoblastic lymphomas. Our finding that hypercalcemia frequently coexists with lymphoblastic lymphomna (12 out of 14 cases with known serum biochemical values) is contrary to earlier findings for canine lymphoma (6,14), but consistent with human tumors.

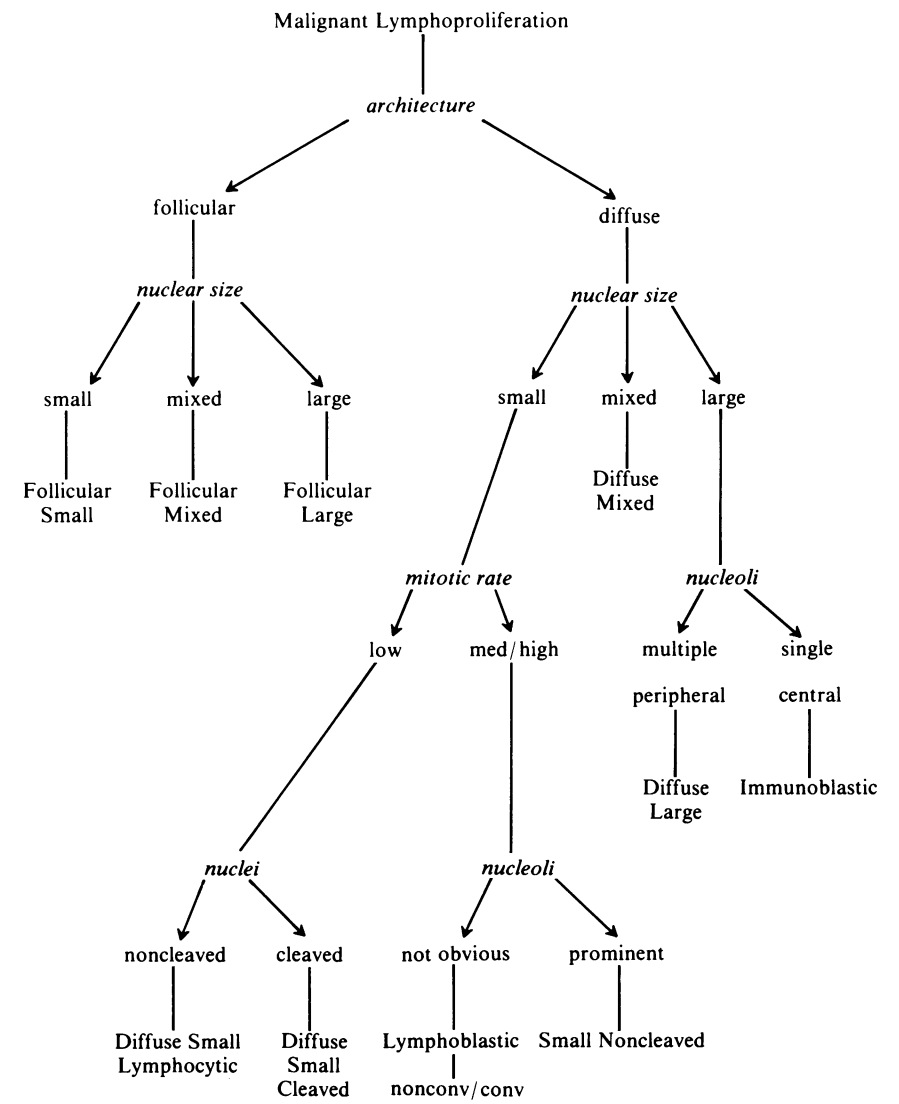
A third adaptation is required for small noncleaved lymphomas. Only two tumors out of 285 total (69 small noncleaved) had a nuclear homogeneity similar to the classic Burkitt's type morphology. The other 67 small noncleaved cases were therefore of non-Burkitt's type. Included in this category were a diverse group of small cells which did not fit well into other categories and which appeared aggressive. There were two major cell types, both of which were common. One cell type has a vesicular nucleus with peripheralized chromatin pattern and multiple prominent small nucleoli in a small irregular or rounded nucleus. The other has a single nucleolus somewhat obscured by a fine chromatin pattern in a small round nucleus; these nuclei are not "immunoblastic" because they are quite small (1 to 1.5 red blood cell in diameter) and the nucleolus, while present, is not prominent.

To be classified as a follicular lymphoma, the node had to have more or less evident circular neoplastic centers of expansion distributed throughout most of the node which did not contain numerous high endothelial venules and which were primarily demarcated by a cuff of lymphocytes rather than sclerotic bands of tissue. The few canine tumors which have a follicular appearance are usually "pseudofollicular" paracortical expansions of cells which contain numerous high endothelial venules and have compressed any residual benign follicles between them. True follicular tumors are considered to be B-cell, germinal center-derived lymphomas. The pseudofollicular tumors are frequently more consistent with a T-cell origin. Difficulties of interpretation of nodularity have been described previously for canine lymphomas (2,15,16).

Specimen preparation is an important factor. Accurate classification requires histological specimens from nodal excisions that have minimal autolysis, are well-fixed and have been taken before any attempted treatment. Tru-cut biopsies of nodes may be sufficient, but are much harder to interpret due to crush artifact, variation in angle of penetration into the node, and failure to demonstrate the architecture of the node due to small sample size. While histological specimens are the definitive samples, cytology can provide a rapid indication of cell type. Since almost all canine lymphomas are diffuse tumors, architecture is rarely a consideration. Several types may be difficult to distinguish on air-dried preparations, such as diffuse large, immunoblastic, small noncleaved and lymphoblastic; in these cases, wet-fixed preparations are very helpful because of their excellent nucleolar detail.

The process of classification and allocation into various tumor types is

TABLE II. A Basic Algorithm for Canine Lymphoma Classification



very dependent upon training and experience. We are without the long history of classification and clinical correlation available to human pathologists. Few recent detailed studies of the morphology of canine lymphomas exist (2,5,8,15,16). In general, our results appear to be similar to those previously reported, but it is evident that there may be marked differences of interpretation. For example, we found that follicular tumors are rare (2 out of 284); similar distributions have been reported by Squire *et al* (2) in 1973 (1 out of 100), and Appelbaum *et al* (8) in 1984 (1 out of 40), while Holmberg *et al* (15) reported 9 out of 23, and Valli *et al* (16) reported 6 out of 72. Even greater differences can be shown for cell types. Our distribution is markedly different from that of Appelbaum *et al* (8) even though these workers also used the Working Formulation. However, geographic variation in morphology has been reported for human non-Hodgkin's lymphomas (11,17), and it is possible that a similar variation in canine lymphomas could account for these differences. Our own reproducibility upon second viewing is good although not perfect, and when our interpretations are compared to those of experts in human lymphoma classification there is general agreement. Any difficulties are usually due to too literal an interpretation of the descriptions of the Working Formulation. A basic alogrithm of classification is provided (Table II).

The mitotic index is a useful estimate of implied tumor grade. We found a strong correlation between mitotic index and cell type, in that low grade tumors usually have a low mitotic index, and high grade tumors usually have a medium or high mitotic index. This finding could be predicted from the criteria of the Working Formulation, but we emphasize that mitotic rate is less important than morphology in classification.

In summary, the available classification criteria identified by the Working Formulation adequately fit the diversity of cell types exhibited by canine lymphomas. Few tumors are genuinely unclassifiable. The distribution of cell types, when compared to those of humans (Table I), demonstrates that canine lymphomas have a much different relative frequency of tumor types. Follicular tumors are much less common, and high grade, aggressive tumors are much more common in general than in humans. In particular, the follicular small cleaved, immunoblastic, lymphoblastic and small noncleaved cell types exhibit notably different frequencies. The Working Formulation is easily applied, reproducible, translatable to other schemes and clinically relevant (11,18,19,20,21,22,23,24). When applied to canine lymphomas, the first three benefits at least are evident.

In humans, histological classification of tumor type by the Working Formulation is the best single prognosticator available to the clinician (11,22,23). The Working Formulation predicts overall survival; the designation of low, intermediate or high grade is based upon the increasingly poor rates of five year survival determined for each cell type. It does not predict the attainment of complete remission, remission time, appearance or severity of organ involvement or other factors. However, in some cases classification according to the Working Formulation can predict response to therapy protocol. For example, patients with low grade tumors have the best prognosis in terms of survival in spite of being essentially untreatable by current protocols; no difference in survival time was observed whether such patients were treated or not in one series (21). Low grade tumors may exhibit spontaneous regressions, and their mitotic rate is so low that they are unaffected by chemotherapy (11,19,21,24). Such tumors have been considered benign proliferations subject only to immunoregulatory influences (25). People with low grade tumors die because of an inevitable slow accumulation of nonfunctional lymphoid mass. In comparison, higher grade tumors respond well to aggressive chemotherapeutic regimens (e.g. 12,24), and paradoxically, cures are possible (19). In the majority of cases, however, the aggressive nature of the tumor results in shorter survival despite attainment of remissions. People with high grade tumors die because of rapid proliferation of autonomous neoplastic cells which eventually overcome an initial susceptibility to therapy. Long term survival in high grade tumors only occurs with aggressive initial therapy.

Whether application of the Working Formulation to canine tumors has clinical relevance, i.e. prognostic capability, remains to be shown. Our own initial findings show promising examples where low grade tumor types had poor responses to therapy but long term survival, whereas high grade tumor types had complete but short remissions. We have not, however, achieved any of the predicted cures of high grade tumor types. There are other indications that histological classification can explain some clinical observations peculiar to canine lymphomas. The large portion of high grade tumor types with high mitotic rates might explain the rapid clinical progression of canine lymphomas compared to humans. In most studies of treated cases, a variable but low proportion of cases (5 to 20%), have significantly longer survival than the mean (1,2,7,9). These cases may correspond to the low proportion of cases which are not high grade tumors. When combining chemotherapy and immunotherapy, Weller *et al* (4,5) only included those cases which had a complete response to initial chemotherapy initially attempted; none of 56 cases were indicated to have a diffuse small lymphocytic morphology, and only two were apparently of intermediate or low grade cell type. By requiring an initial complete response to chemotherapy for inclusion in their project, Weller *et al* (4) may have actually screened out any dogs with the best natural prognosis. Furthermore, when Calvert and Leifer (7) studied dogs which were resistant to cyclophosphamide, vincristine, methotrexate and prednisone (COMP) and then treated with doxorubicin, three cases were classified as types consistent with diffuse small lymphocytic; all three were nonresponders to either COMP or doxorubicin and their survival times were relatively long (210, 240 and 330 days). The longest surviving dog (390 days) had a described cell type consistent with an intermediate grade tumor and only achieved a partial clinical response.

Conclusive proof that the Working Formulation has prognostic capability in canine lymphomas will be

difficult to obtain. In humans, the Formulation only predicts survival, which in dogs is usually an elective endpoint that is variably determined by owners. Only a large series of cases with relatively uniform treatment and consistent client consultations will demonstrate whether or not this procedure can be justified.

It may well be that application of the Working Formulation or other similar histological criteria will predict clinical behavior and identify those canine tumor types where current treatment protocols are beneficial. Any correlation of a particular cell type or types with clinical behaviour would be desirable, whether or not it occurs as predicted by extrapolation from human lymphocytes. Eventually, histological classification may aid in the design of protocols specifically adapted to cell type to enhance survival. Histological classification is, at the very least, an important first step in the development of any phenotypic system designed to generate prognostic capability in canine lymphomas.

ACKNOWLEDGMENTS

This study was made possible by the cooperation of laboratory head Dr. G. Thomson and the staff of Veterinary Laboratory Services (OMAF) at Guelph, Ontario. Consultations with Drs. U. Sarmiento, I. Dardick and G. Frizzera are gratefully acknowledged. Excellent assistance was provided by S. Brown. This study was funded by the Macdonald-Stewart Foundation of Montreal and the Medical Research Council of Canada.

REFERENCES

1. **COTTER SM.** Treatment of lymphoma and leukemia with cyclophosphamide, vincristine and prednisone. I. Treatment of dogs. J Am Anim Hosp Assoc 1983; 19: 159-165.
2. **SQUIRE RA, BUSH M, MELBY EC, NEELEY LM, YARBROUGH B.** Clinical and pathologic study of canine lymphoma: clinical staging, cell classification and therapy. J Natl Cancer Inst 1973; 41: 566-574.
3. **MADEWELL BR.** Chemotherapy for canine lymphoma. Am J Vet Res 1975; 36: 1525-1528.
4. **WELLER RE, THEILEN GH, MADEWELL BR, CROW SE, BENJAMIN E, VILLALOBOS A.** Chemoimmunotherapy for canine lymphosarcoma: a prospective evaluation of specific and nonspecific immunomodulation. Am J Vet Res 1980; 41: 516-521.
5. **WELLER RE, HOLMBERG CA, THEILEN GH, MADEWELL BR.** Histologic classification as a prognostic criterion for canine lymphosarcoma. Am J Vet Res 1980; 41: 1310-1314.
6. **WELLER RE, HOLMBERG CA, THEILEN GH, MADEWELL BR.** Canine lymphosarcoma and hypercalcemia: clinical, laboratory and pathologic evaluation of twenty-four cases. J Small Anim Pract 1982; 23: 649-658.
7. **CALVERT CA, LEIFER CE.** Doxorubicin for treatment of canine lymphosarcoma after development of resistance to combination chemotherapy. J Am Vet Med Assoc 1981; 179: 1011-1012.
8. **APPELBAUM FR, SALE GE, STORB R, CHARRIER K, DEEG HJ, GRAHAM E, WULFF JC.** Phenotyping of canine lymphoma with monoclonal antibodies directed at cell surface antigens: classification, morphology, clinical presentation and response to chemotherapy. Haematol Oncol 1984; 2: 151-168.
9. **MACEWEN EG, BROWN NO, PATNAIK AK, HAYES AA, PASSE S.** Cyclic combination chemotherapy of canine lymphosarcoma. J Am Vet Med Assoc 1981; 178: 1178-1181.
10. **GRAY KN, RAULSTON GL, GLEISER CA, JARDINE JH.** Histologic classification as an indication of therapeutic response in malignant lymphoma of dogs. J Am Vet Med Assoc 1984; 814-817.
11. **NON-HODGKIN'S LYMPHOMA PATHOLOGIC CLASSIFICATION PROJECT.** National Cancer Institute sponsored study of classifications of non-Hodgkins lymphomas. Cancer 1982; 49: 2112-2135.
12. **TINDLE BH.** Teaching monograph: malignant lymphomas. Am J Pathol 1984; 116: 115-174.
13. **OSBALDISTON GW, SULLIVAN RJ, FOX A.** Cytochemical demonstration of esterases in peripheral blood leukocytes. Am J Vet Res 1978; 39: 683-685.
14. **MEUTEN DJ, KOCIBA GJ, CAPEN CC, CHEW DJ, SEGRE GV, LEVINE L, TASHJIAN AH, VOELKEL EF, NAGODE LA.** Hypercalcemia in dogs with lymphosarcoma. Biochemical, ultrastructural and histomorphometric investigations. Lab Invest 1983; 49: 553-562.
15. **HOLMBERG CA, MANNING JS, OSBURN BI.** Canine malignant lymphomas: comparison of morphologic and immunologic parameters. J Natl Cancer Inst 1976; 56: 125-135.
16. **VALLI VE, McSHERRY BJ, DUNHAM BM, JACOBS RM, LUMSDEN JH.** Histocytology of lymphoid tumors in the dog, cat and cow. Vet Pathol 1981; 18: 494-512.
17. **APAJA-SARKKINNEN M, ALA-VAIKKO M.** The reproducibilty of the Lukes-Collins classification of non-Hodgkin's lymphomas in a retrospective classification. Eur J Cancer Clin Oncol 1984; 20: 735-741.
18. **MOIR DH.** War of the words: classification of the non-Hodgkin's lymphomas. Pathology 1983; 15: 359-360.
19. **GAYNOR ER, ULTMANN JE.** Non-Hodgkin's lymphoma: management strategies. N Engl J Med 1984; 311: 1506-1508.
20. **HONEGGER HP, CAVALLI F.** Current status and perspectives in the treatment of non-Hodgkin's lymphomas. Eur J Cancer Clin Oncol 1984; 20: 305-314.
21. **HORNING SJ, ROSENBERG SA.** The natural history of initially untreated low-grade non-Hodgkin's lymphomas. N Engl J Med 1984; 311: 1471-1475.
22. **MONFARDINI S, RILKE F, VALAGUSSA P, BAJETTA E, CANETTA R, BUZZONI R, GIARDINI R, VIVIANI S.** A clinicopathologic study in advanced non-Hodgkin's lymphomas treated with sequential non-Cross-resistant regimens: comparison of the Working Formulation with the Rappaport and Kiel classifications. Eur J Cancer Clin Oncol 1984; 20: 609-617.
23. **PERCY C, O'CONOR G, GLOECKLER RIES L, JAFFE ES.** Non-Hodgkin's lymphomas. Application of the International Classification of Diseases for Oncology (ICD-O) to the Working Formulation. Cancer 1984; 54: 1435-1438.
24. **SMITH BR, WEINBERG DS, ROBERT NJ, TOWLE M, LUTHER E, PINKUS GS, AULT KA.** Circulating monoclonal B-lymphocytes in non-Hodgkin's lymphoma. N Engl J Med 1984; 311: 1476-1481.
25. **JAFFE ES.** Hypothesis: Follicular lymphomas - are they benign tumors of the lymphoid system? In: Vitetta ES, Fox CF, eds. B and T cell tumours. New York: Academic Press Inc., 1982: 91-96.