CYTOLOGY AND COLCHICINE SENSITIVITY OF VIABLE CELLS FROM LYMPH NODES WITH MALIGNANT LYMPHOMA

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Viable cell suspensions were prepared from 31 nodes diagnosed non-Hodgkin's malignant lymphoma, and from 30 non-malignant nodes. The cells were examined and counted by phase contrast microscopy. The suspensions were characterized by the percentage of large cells and by a colchicine-sensitivity index. The finding of more than 6% large cells or the finding of a sensitivity index of more than 30% was considered a positive test for a malignant lymphoma. According to these criteria there were 2 false positives in 30 reactive nodes and one false negative in 31 malignant nodes. Findings on 3 nodes diagnosed angioimmunoblastic lymphadenopathy suggested malignancy. The colchicine-sensitivity index of blood lymphocytes seemed useful for monitoring lymphoma patients for leukemic involvement.

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THE DIAGNOSIS OF LYMPH NODES IS ONE OF THE most difficult problems in histopathology. Even the differentiation of malignant and non-malignant diseases of the nodes may present a problem.[1] Therefore it may be useful to explore new methods that might provide additional diagnostic data. The present study proposes the use of viable cell suspensions as a diagnostic tool in the study of lymph nodes.

Viable cells are an indispensible tool in immunology but are used only infrequently in clinical oncology. In a previous study, viable cells were found useful in characterizing and diagnosing hairy cell leukemia.[8] Viable lymphocytes from the blood of patients with chronic lymphocytic leukemia have been shown to differ from normal lymphocytes both in cytology[9] and in sensitivity to various reagents such as vincristine and colchicine.[7,10]

In earlier work, Schrek *et al.*,[11] studied the viable cells from lymph nodes with malignant lymphoma with respect both to cytology of the viable cells by phase contrast microscopy and to the sensitivity of the cells to reagents. The cytologic studies of 12 lymph nodes diagnosed malignant lymphoma led to the classification of the viable cells into 4 types: 1) CLL cells, 2) small and 3) large lymphosarcoma cells and 4) other large cells.

In the present work, additional malignant and nonmalignant lymph nodes were studied in an attempt to find cytologic characteristics which would aid in the diagnosis of malignant lymphoma.

Recent studies in two independent laboratories have advocated the use of sensitivity to colchicine as a means of differentiating normal and leukemic lymphocyte populations.[10,12] This reagent has therefore been used in the present study to test the sensitivity of cells from malignant and nonmalignant lymph nodes in order to evaluate colchicine-sensitivity as a test for the diagnosis of malignant lymphoma.

MATERIALS AND METHODS

Lymph nodes excised for diagnostic purposes were used for the preparation of cell suspensions and for histologic sections. The pathologic diagnoses were reviewed by one of us (ZM). Most of the malignant lymphomas were sent to Dr. D.

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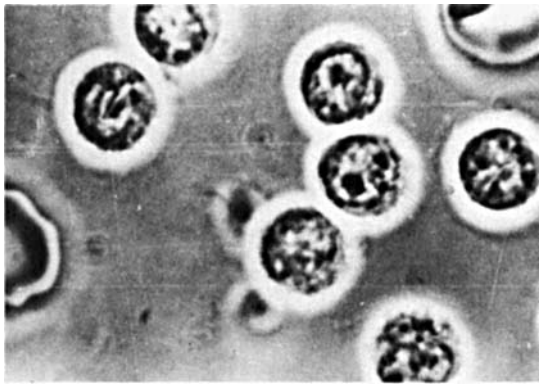


FIG. 1. Small lymphocytes in suspension from a hyperplastic lymph node. The cells are uniform in size with sparse cytoplasm. Two cells have small dark nucleolli (×2000).

Variakojis of the University of Chicago for confirmation of the diagnosis. Thirty lymph nodes were diagnosed as reactive and were considered as controls to 31 lymph nodes diagnosed non-Hodgkins malignant lymphoma.

To prepare cell suspensions, the tissue was chopped up in a test tube with a knife. The isolated cells were washed and suspended in equal parts of fresh human serum and RPMI 1640 medium. The suspensions were incubated in small test tubes. Each tube contained approximately 1,000,000 cells in 1.0 ml suspension.

Counts of viable cells were made by transferring 0.2 ml of a suspension to a chamber composed of two large cover slips separated by a metal disk 0.9 mm in thickness with a central hole 25 mm in diameter. The cells were examined and counted with an inverted phase contrast microscope at a magnification of ×1000. Lymphocytes were considered to be viable if they had distinct morphologic characteristics such as chromatin and nucleolar masses, thin nuclear walls, thin nuclear indentations and anterior pseudopods and posterior uropods. In contrast, round cells with pyknotic, fragmented or lysed nuclei were considered dead. The number of small and large viable cells were counted in an area 10 × 0.4 mm and the percentage of large cells was calculated. The large cells were round or irregular in shape and were 10 μm or more in diameter. The count of large cells did not include macrophages which were characterized by considerable amounts of cytoplasm and many cytoplasmic granules of various types. The viable lymphocytes in triplicate tubes were counted before and after incubation and the percentage of cells surviving incubation was calculated.

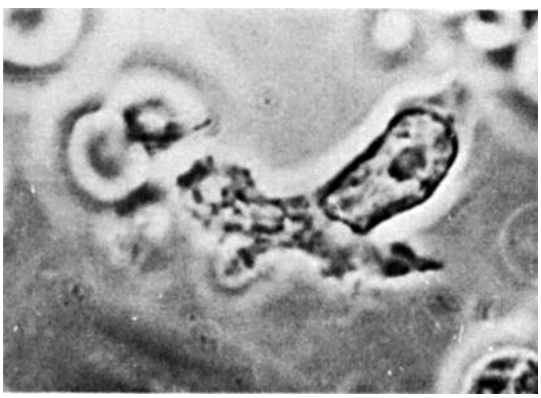


FIG. 2. Large irregular shaped cell from a hyperplastic lymph node. The cell has a hyaline pseudopod and a uropod with small dark mitochondria (×2000).

The effect of colchicine was measured by the per cent cytocidal effect which was defined as 100(1-A/B) where A and B are the number of lymphocytes that survived for a given period of time in suspensions incubated at 37 C with and without a given concentration of colchicine, respectively. The "sensitivity index" was the average cytocidal effect produced by 1.0 and 0.1 μg/ml of colchicine incubated with the cells for 20 hours.

The *in vitro* studies on the cells of the lymph nodes and the histologic diagnosis were made independently by different individuals.

Photomicrographs of viable cells in suspension were all taken with an inverted phase contrast microscope (×2000). The suspension was in a slide chamber 0.9 mm in thickness to avoid compression of cells.

RESULTS

Reactive Lymph Nodes

Histology: The routine histologic study of 30 lymph nodes indicated hyperplastic changes. Some of these nodes had characteristic irregularly shaped and sized follicles with large cells in the germinal centers and rims of small lymphocytes. Other nodes showed sinus histiocytosis or paracortical nodular hyperplasia. The hyperplastic nodes may be considered as controls for the work on malignant lymphoma. The nodes were obtained from the cervical, axillary, inguinal and abdominal regions. The underlying conditions leading to the lymph node biopsy included suspected or known inflammatory or malignant disease.

Cytology of viable cells: The viable cells in the suspensions of the control nodes were studied by

phase contrast microscopy at a magnification of ×1000.

The viable cells in the suspensions from the hyperplastic nodes were nearly all small (6-7 μm, Fig. 1) and were similar to the small lymphocytes from normal blood. The nuclei were round or slightly irregular with thin nuclear walls, fine gray chromatin granules and small poorly visualized nucleoli.

Some cells of the hyperplastic nodes were large (10 μm) with large nuclei and dark prominent nucleoli (Figs. 2–3). These large cells with nuceloli resemble malignant cells but they were seen in small numbers in nearly all the nonmalignant nodes. These cells are presumably reactive lymphoblasts derived from the germinal centers.

A second type of large cell has been previously described and called "flagellated" cell,[8] because of the multiple thin elongated cytoplasmic processes (Fig. 4). The nuclei of these cells varied in appearance and were either small and crescentic without nucleoli or were large and oval with prominent nucleoli. The cytoplasm was moderate in amount and sometimes had multiple clear vacuoles. The nature of these cells is not known.

A third type of large cells were macrophages which spread out and flattened themselves on the glass slide. The cells had small nuclei and considerable cytoplasm usually with moderate numbers of small and large granules of various types.

Five hundred cells were counted for each node to determine the number of large cells and mitotic cells. Macrophages were not included in the count. In 29 of 30 reactive nodes, less than 5% of the cells were large, 10 μm or more in diameter. The suspension of one node had 12% large cells. This node was obtained from a patient CL with arthritis. Histology showed pronounced germinal center hyperplasia and sinus histiocytosis. A rare mitotic figure was seen in the freshly prepared suspensions of only 2 reactive nodes.

Colchicine-sensitivity: Previous work with peripheral blood cells showed a distinct difference in the sensitivity of normal and leukemic blood lymphocytes to the cytocidal action of colchicine.[10] A sensitivity index was used to measure sensitivity and was defined as the mean cytocidal effect produced by 1.0 and 0.1 μg/ml of colchicine in 20 hours of incubation at 37 C. The index for lymphocytes from 16 normal persons was 0 to 15%, *i.e.*, with 15% or less of normal lymphocytes were killed by a small dose of colchicine in 20 hours. In contrast, the indices were

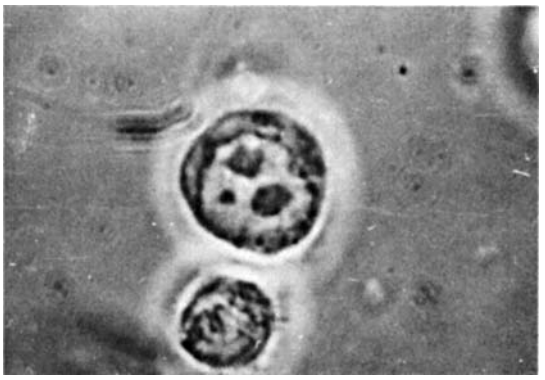


FIG. 3. Large cell from a hyperplastic node. The cell has a large nucleus with two large dark nucleoli (×2000).

61 to 98% for blood lymphocytes from 23 of 25 patients with chronic lymphocytic leukemia. The lymphocytes from 21 of 22 reactive lymph nodes had sensitivity indices from 3 to 29%. The index for only one node was very high (70%). The suspension of this node had 0% large cells. The histology of the node showed an increased number of immunoblasts. The node of patient CL, described previously, with 12% large cells had a colchicine sensitivity index of 27.0%.

Malignant Lymphoma, Histiocytic Type, Diffuse

Histology: The nodes of 9 patients were diagnosed as malignant lymphoma, histiocytic type according to Rappaport's classification.[6] The histology of these nodes showed a uniform pattern of large cells called histiocytes by Rappaport. Lukes and Collins considered these cells to be transformed lymphocytes and therefore called them large lymphoid cells.[5] Only a few of these lymphomas are "true histiocytic" as seen

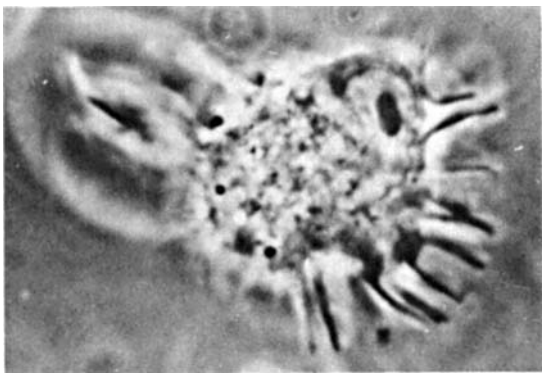


FIG. 4. Large flagellated cell with many thin cytoplasmic processes, an oval nucleus and a large dark nucleolus. The cell is from a hyperplastic node (×2000).

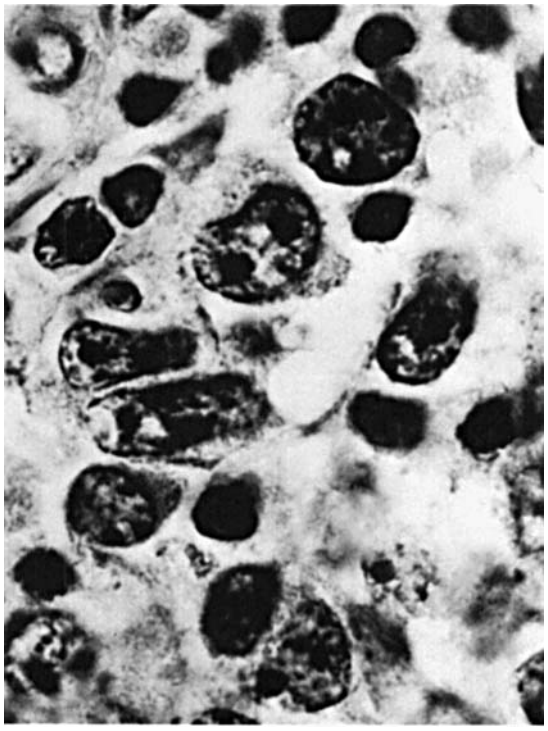


FIG. 5. Lymph node section of patient OR with histocytic lymphoma, diffuse with sclerosis. A predominance of large cells, some with pleomorphic nuclear configurations are seen enmeshed with collagen fibrils. Compare with cells in Fig. 8 (H & E ×1200).

by phagocytic activity or by nonspecific esterase activity.

The histology of these nodes varied. Some showed monotonous large uniform cells with uniform nuclei, prominent nucleoli and scanty cytoplasm. Other nodes had a rather pleomorphic cell population, often with irregular shapes, plasmacytoid features, highly MGP positive cytoplasm and occasional multinucleated cells (Fig. 5).

Cytology: The suspensions from the histiocytic lymphomas were characterized by many large cells, 10 μm or more in diameter (Figs. 6–9). These large cells could be readily differentiated from the small lymphocytes. The large cells comprised 7 to 90% of the viable cells in the suspensions (Table 1). The percentages of large cells in the suspensions from the histiocytic lymphomas were significantly greater than those for the reactive nodes. The large cells had sparse cytoplasm, and large irregularly shaped and sometimes multiple dark nucleoli. Some cells had a few small refractive, apparently lipid, granules. In 3 of the freshly prepared suspensions, a few cells were seen in mitotic division and many mitoses were seen in the suspensions incubated at 37 C for 1 day. The small cells in the suspensions were similar in cytology to normal small lymphocytes.

Colchicine sensitivity: The cells of 2 of the nodes diagnosed histiocytic lymphoma (CN and TC) had colchicine sensitivity indices of 0% (Table 1). These 2 nodes had many large cells (50 and 86%). In these nodes both the small and the large cells were highly resistant to the cytocidal effect of colchicine. The histology of these nodes showed abundant large cells, some with prominent eosinophilic cytoplasm. Neither of these nodes showed phagocytosis by the tumor cells in histologic sections. Further histologic and electron microscopic studies are in progress.

The sensitivity indices for the nodes of 5 other patients with histiocytic lymphoma were 37 to 97%. The cells of these nodes were much more sensitive to colchicine than the cells of the reac-

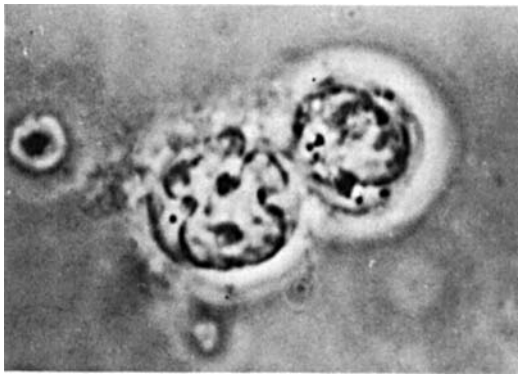


FIG. 6. Large cells from node of patient TC, diagnosed histiocytic lymphoma, diffuse. The cells have large irregular shaped nuclei (×2000).

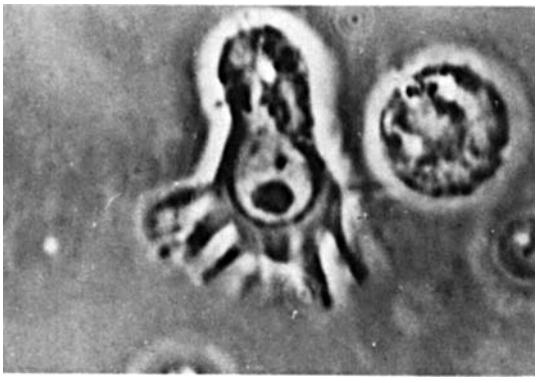


FIG. 7. Large cell from a node of patient CR with the diagnosis of histiocytic lymphoma. The cell has an anterior hyaline pseudopod with thin cytoplasmic processes and a thick uropod (×2000).

tive nodes. The percentage of large cells in these nodes was 8 to 90%. There was no significant correlation between the number of large cells and the sensitivity indices.

Two suspensions (OR and BK) had sufficient numbers of large and small cells to permit calculation of the sensitivity of the small and large cells. In both cases, the large but not the small cells were highly sensitive to the cytocidal action of colchicine.

The findings on patient SR are of particular interest. A biopsy of a lymph node from the left axilla (SR-1) on July 16 was found on histologic examination to have sheets of anaplastic tumor cells with striking variation in size and shape of the cells and nuclei. Possibilities of anaplastic carcinoma, amelanotic melanoma, anaplastic seminoma and histiocytic lymphoma were considered. Electron microscopy did not aid in the elucidation of the origin of the tumor cells. The histologic diagnosis at the time was anaplastic malignant tumor, unclassified.

A suspension from this node had small lymphocytes and 7% large cells which did not appear to be similar to carcinoma or melanoma cells observed previously in suspensions from lymph nodes with metastatic tumor (unpublished observations). Not enough cells were available to permit a colchicine-sensitivity test.

On September 22, a node removed from the right axilla (SR-2) was diagnosed reticulo endothelial hyperplasia. On September 30, another left axiallary node (SR-3) showed massive replacement of the node with large sheets of large cells. There were many pleomorphic and giant cells some of which appeared similar to Reed-Sternberg cells. Electron microscopic studies showed absence of desmosomes and tonofilaments. A diagnosis was made of malignant lymphoma histiocytic, pleomorphic cell type. It should be noted that the suspensions from the nodes of both right and left axilla (SR-2 and SR-3) had high percentages of large cells (8 and 20% respectively) and high sensitivity indices (45 and 39%) in spite of the differences in the histologic diagnoses.

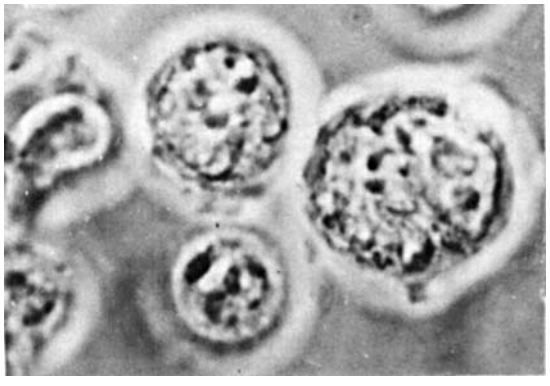


FIG. 8. Large cells from a node of patient OR diagnosed histiocytic lymphoma. The nuclei are large, irregular shaped with gray chromatin and nucleolar masses (×2000).

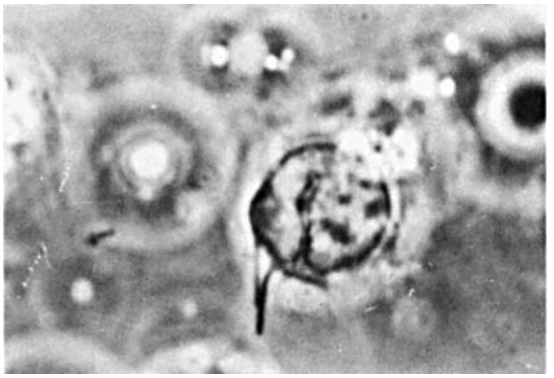


FIG. 9. Large cell from node of patient MT, diagnosed histiocytic lymphoma. The nucleus has a thin deep indentation (×2000).

Malignant Lymphoma, Lymphocytic Type, Diffuse

Histology: Lymph nodes from 11 patients had the diagnosis of malignant lymphoma, diffuse, well differentiated or intermediate degree of differentiation. The nodes had a monotonous replacement of the lymphoid architecture by ma-

TABLE 1. The Percentages of Large Cells and the Colchicine-Sensitivity Indices of 11 Lymph Nodes From 9 Patients with the Histologic Diagnoses of Malignant Lymphoma, Histiocytic Type, Diffuse (Rappaport's Classification)

	Mitoses	% Large cells	Sensitivity Index All cells	Large cells	Small cells
CN	+	50	0		
TC	+	86	0		
OR	+	33	37	62	29
SR-1	0	7	–		
SR-2*	0	8	45		
SR-3	+	20	39		
BK	+	69	59	88	29
CR	+	90	97		
MT	0	17	–		
ST	0	15	–		
RI	0	46	–		

* Diagnosed reactive hyperplasia.

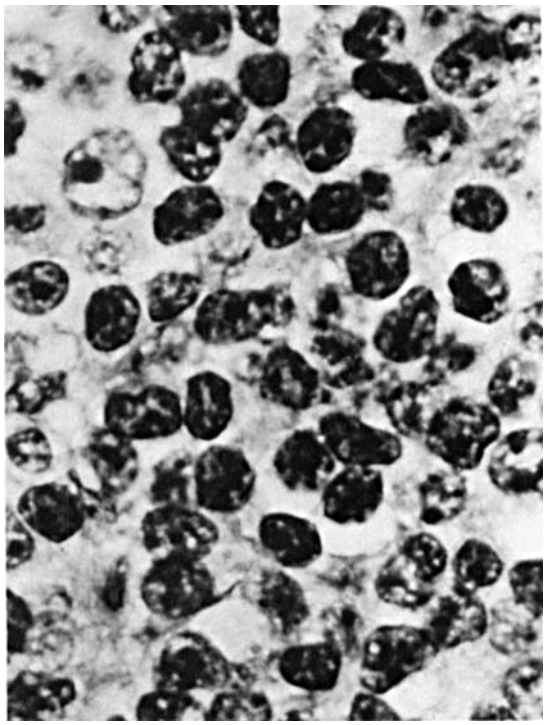


FIG. 10. Lymph node section of patient AR who had chronic lymphocytic leukemia. Scattered among the well differentiated lymphocytes are some blast-like cells with prominent nucleoli. No mitoses are seen. Compare with Fig. 11 (H & E ×1200).

ture, small lymphocytes (well differentiated) (Fig. 10) or by lymphocytes with somewhat prominent nucleoli and less mature nuclear features (intermediate differentiation). In both instances, larger lymphoblasts in variable numbers were scattered diffusely between the small

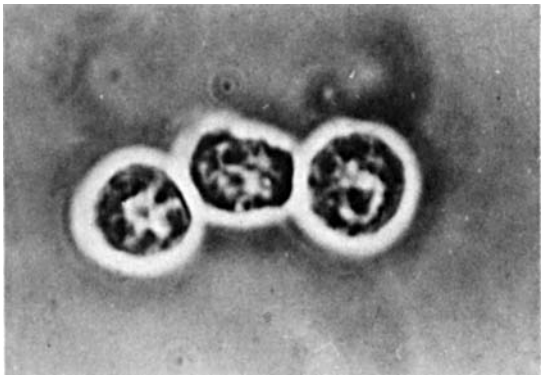


FIG. 11. Small lymphocytes from node of patient AR with malignant lymphoma, lymphocytic type, diffuse. One cell has a dark nucleolus and another cell has a few marginated triangular shaped chromatin masses (×2000).

TABLE 2. Data on 11 Lymph Nodes with the Histologic Diagnoses of Malignant Lymphoma, Lymphocytic, Diffuse

	% Large cells	Sensitivity Index All cells	Large cells	Small cells
SD	4	34		
MK	6	41		
SW	5	48		
SV	0	52		
HN	2	62		
KC	2	71		
CH	10	74		
ZL	0	81		
MZ	13	82	95	58
KN	4	85	94	80
AR	0	93		

lymphocytes or in small clusters. These clusters, "regenerating foci" occasionally showed mitotic figures. One lymph node, diagnosed as malignant lymphoma, lymphocytic, diffuse, poorly differentiated (patient MK) had irregularly shaped convoluted nuclei and many mitoses.

Cytology: The cells from the lymph nodes with lymphocytic lymphoma were nearly all small lymphocytes (Fig. 11) with a few abnormal features, such as irregularity in shape, a few small refractile lipid granules, increase in the number of observable small dark nucleoli, and some marginated chromatin masses. The number of large cells were few (less than 5%, Table 2) except for three nodes with 6, 10 and 13% large cells (one, MK, poorly differentiated and the

TABLE 3. Data on 6 Lymph Nodes of 5 patients with the Histologic Diagnoses of Malignant Lymphoma, Mixed Type, Lymphocytic and Histiocytic, Nodular and 3 Nodes Diagnosed Malignant Lymphoma, Lymphocytic, Poorly Differentiated, Nodular

	% Large cells	Sensitivity Index All cells	Large cells	Small cells
ML, mixed type, nodular				
HR-1	40*	25		
HR-2	73*	39	59	30
ZD	3	47		
YG	58	–		
DR	19	51	82	47
BU	61	41	59	6
ML, lymphocytic poorly differentiated, nodular				
BR	3	27		
FN	3	60		
WS	6	35		

* After 1 day incubation.

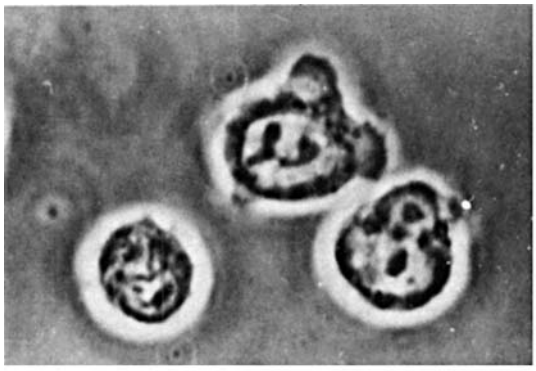


FIG. 12. Cells from node of patient BU diagnosed malignant lymphoma, mixed type, nodular. Two cells are large with large multiple irregularly dark nucleoli (×2000).

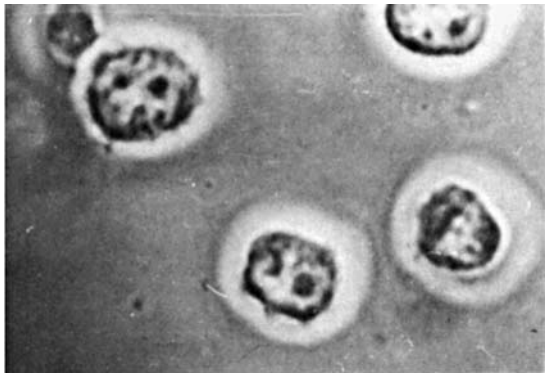


FIG. 14. Small lymphocytes from the node of patient FN with the diagnosis of malignant lymphoma, lymphocytic, poorly differentiated, nodular. The lymphocytes have dark nucleoli (×20000).

others, CH and MZ, well differentiated). No mitoses were seen in the freshly prepared or incubated suspensions, in contrast to the mitoses in suspensions from histiocytic lymphomas.

Colchicine sensitivity: The sensitivity indices for 8 of the 11 nodes were very high (52 to 93%). The average index for the 11 nodes was 66% and was significantly higher than that for the reactive nodes. Both the small and large cells from the node of patients MZ and KN were sensitive to colchicine although the large cells were more sensitive. The small lymphocytes of the nodes of these patients had higher sensitivity indices (58 and 80%) than the small lymphocytes of the histiocytic lymphomas of patients OR and BK (29%, Table 1).

Malignant Lymphoma, Mixed Type, Nodular

Histology: Six lymph nodes from 5 patients had the histologic diagnosis of malignant lymphoma, mixed type, nodular (Table 3). The nodular pattern consisted of follicles of rather uniform size. On higher magnification there was a mixture of large and small lymphoid cells.

Cytology: Suspensions from nodes subsequently diagnosed malignant lymphoma, mixed type, had some large cells usually about 10 μm in diameter (Fig. 12). Suspensions from 2 nodes of one patient (HR) had relatively few large cells. Both nodes were diagnosed malignant lymphoma, mixed type, nodular. On incubation of the suspensions for one day, the suspension of both nodes had many large cells (40 and 73%). Apparently, the small lymphocytes of the nodes had transformed into the large cells on incubation, although no mitogen had been added.

Colchicine sensitivity: The sensitivity indices for 4 of 5 nodes were moderately high (39–51%) and in one node (BU) only the large cells were sensitive (59%).

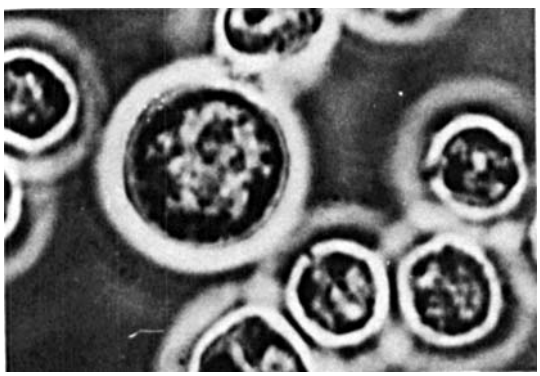


FIG. 13. Large and small cells from node of patient WS, diagnosed malignant lymphoma, lymphocytic type, poorly differentiated, nodular. The large cell has sparse cytoplasm and a large, irregularly shaped nucleous (×2000).

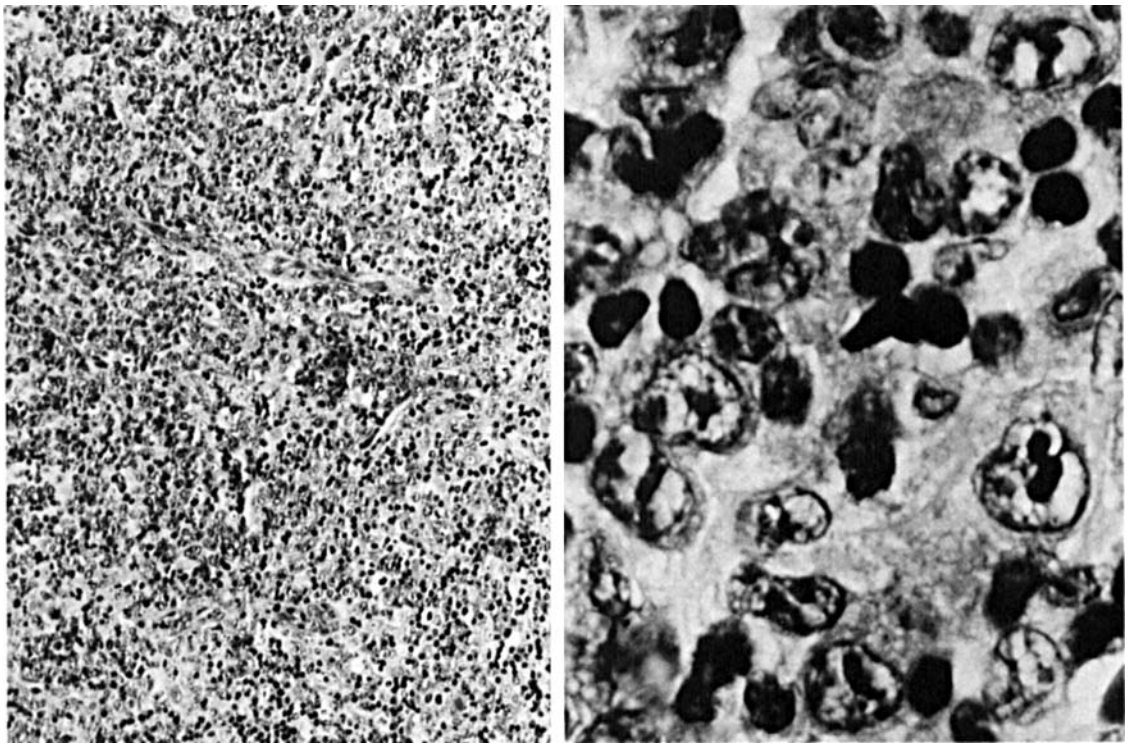
Malignant Lymphoma, Poorly Differentiated, Nodular

The lymph nodes of 3 patients (BR, FN, WS) had the histologic diagnosis of malignant lym-

TABLE 4. Data on 4 Lymph Nodes of 2 Patients with the Histologic Diagnosis of Angioimmunoblastic Lymphadenopathy

	% Large cells	Sensitivity Index All cells	Large cells	Small cells
KZ-1*	3	25		
KZ-2	34	54	91	25
CT-1	19	38		
CT-2	27	39	62	34

* Diagnosed reactive hyperplasia.



FIGS. 15–16. Axillary lymph node section of patient KZ with angioimmunoblastic lymphadenopathy. Low power photomicrograph (Fig. 15, left) shows prominence of post capillary venules and a pleomorphic cellular reaction composed of large immunoblasts, plasma cells and many cells in mitosis. High power photomicrograph (Fig. 16, right) shows numerous large immunoblasts with prominent nucleoli, plasma cells and small lymphocytes. Compare with Fig. 17 H & E Fig. 15 ×400; Fig. 16 ×1200).

phoma, lymphocytic, poorly differentiated, nodular (Table 3). The uniform nodular pattern consisted predominantly of small lymphoid cells with clefted, angulated nuclei. A variable but low number of large lymphoid cells were present in all of these lymph nodes.

These lymphomas are believed to originate from the germinal center cells as was shown by recent immunologic and electron microscopic studies.[3,4,5] Cell suspensions from these nodes had relatively few large cells (3–6%, Figs. 13, 14). The sensitivity indices varied from 27–60%.

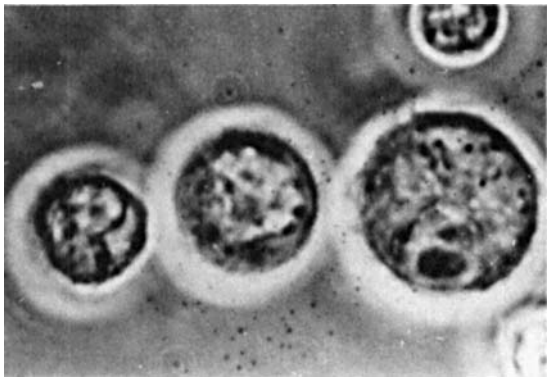


FIG. 17. Variable sized cells from node of patient KZ. The node was diagnosed angioimmunoblastic lymphadenopathy (×2000).

Angioimmunoblastic Lymphadenopathy

Two patients had the histologic diagnosis of angioimmunoblastic lymphadenopathy (Table 4). The architecture of these nodes was effaced by a diffuse proliferation of abundant large immunoblasts (Figs. 15, 16), plasma cells, arborizing post capillary vessels and variable amounts of PAS positive perivascular "sludgy" material.[2]

Two nodal biopsies were done on each of the two patients. Three of the 4 nodes were diagnosed angioimmunoblastic lymphadenopathy and one node was called reactive. The 3 positive nodes had high percentages of large cells (19-34%, Fig. 17) and high sensitivity indices (38–54%). In addition, the suspension of one of the nodes had several cells in mitotic division in the suspensions on first examination and after incubation for one day.

TABLE 5. *In vitro* Studies on Blood Cells of Patients with Malignant Lymphoma or with Angioimmunoblastic Lymphadenopathy (AILD)

Type of lymphoma and patient	% Large cells	Sensitivity Index All cells	Sensitivity Index Large cells	Sensitivity Index Small cells	Absolute lymphocyte count/mm³
Histiocytic, diffuse					
MT	85	94	96	23	3,000
Lymphocytic, diffuse					
KC	0	24			250
SV	0	47			16,000
ZL	0	70			7,700
KN	1	74			6,000
MZ	0	87			4,600
CH	0	88			
HN	0	89			33,000
AR	0	95			12,000
Mixed, nodular					
BU	80	58			1,400
Lymphocytic, nodular, poorly differentiated					
BR	0	10			1,400
WS	0	18			
FN	0	58			2,100
AILD					
KZ	0	26			4,200
CL	0	40			

Of particular interest are the differences in the findings on the 2 nodes of KZ. An inguinal node (KZ-1) had the histologic findings of reactive hyperplasia with plasma cells and eosinophiles, while an axillary node (KZ-2) had the typical histologic finding of angioimmunoblastic lymphadenopathy. The reactive node from the inguinal area gave viable cell findings which were in the normal range while the other node gave abnormal high findings, with 34% large cells (Fig. 17) and a sensitivity index of 54% (Table 4).

Blood Cells

In vitro studies were also done on viable blood cells of patients known or suspected to have leukemic involvement and of a number of other patients. The work on blood cells was done shortly before or after the lymph node biopsies. Most of the patients had no significant prior treatment. The data are summarized in Table 5.

The blood cell suspensions from 2 patients (MT, BU) had many large lymphoid cells, 8 μm or more in diameter (Figs. 18, 19). MT had a nodal diagnosis of histiocytic lymphoma, diffuse and BU had nodular lymphoma, mixed type.

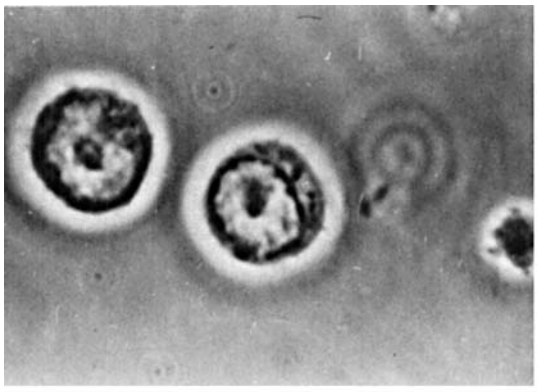


FIG. 18. Large lymphoid cells from the blood of patient MT with the histologic diagnosis of malignant lymphoma, histiocytic type, diffuse. The cells have large nuclei, moderate amounts of cytoplasm and large nucleoli (×2000).

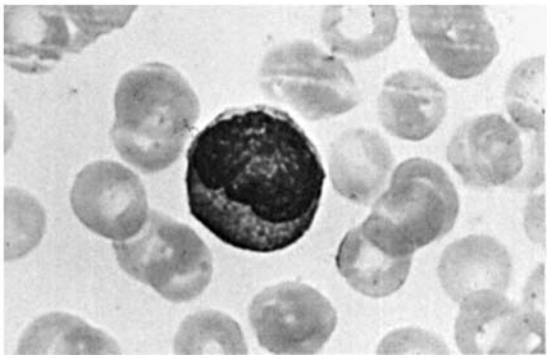


FIG. 19. Large histiocytic type cells with indented or lobulated nuclei in peripheral blood of patient MT with diffuse histiocytic lymphoma (Giemsa ×1200).

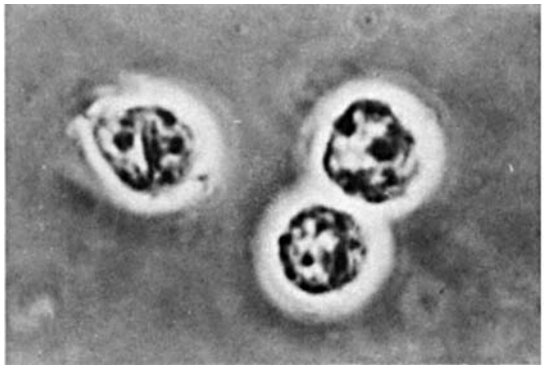


FIG. 20. Small lymphocytes from the blood of patient AR with lymphocytic lymphoma, well differentiated, diffuse. The cells have marginated chromatin masses and one cell has a deep nuclear indentation (×2000).

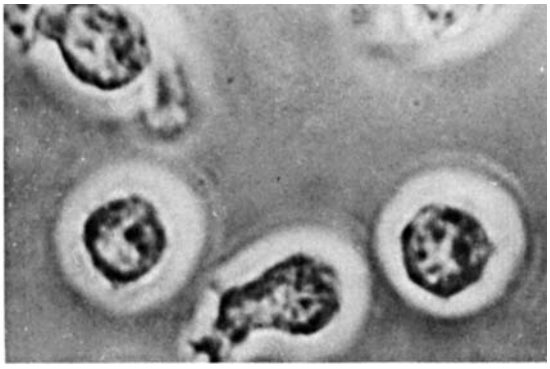


FIG. 22. Small round and ameboid lymphocytes from the blood of patient FN with malignant lymphoma, lymphocytic, poorly differentiated, nodular. The cells had a colchicine sensitivity index of 58% and are therefore considered leukemic (×2000).

The large cells from the blood of these patients were highly sensitive to colchicine but the small lymphocytes of patient MT had only slight sensitivity.

The small lymphocytes from the blood of 7 of 8 patients with malignant lymphoma, lymphocytic type, diffuse, had high colchicine sensitivity indices (47–95%). It should be noted that most of these patients had only moderate lymphocytosis. The blood lymphocytes were uniformly small sized and had characteristic marginated triangular chromatin masses or phase microscopy (Fig. 20), and clumped chromatin in blood smears.

The patients with angioimmunoblastic lymphadenopathy did not have lymphocytosis but their blood lymphocytes had moderately elevated sensitivity indices (26 and 40%) which were higher than the indices for normal blood lymphocytes (0–15%). Both patients had large, plasmacytoid lymphocytes in smears of their peripheral blood, and in blood cell suspensions (Fig. 21).

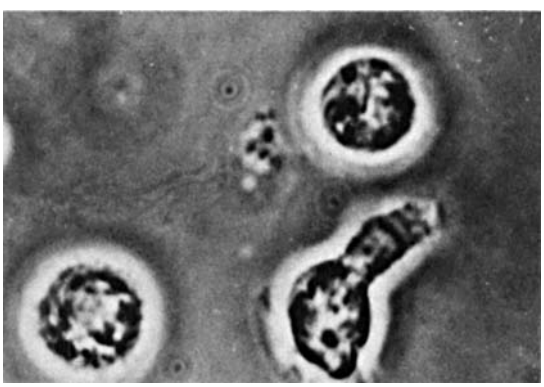


FIG. 21. Atypical lymphocytes from the blood of patient KZ with angioimmunoblastic lympadenopathy. The two round lymphocytes have sparse cytoplasm and marginated chromatin masses (×2000).

It should be noted that 3 patients (MT, BU, FN) had normal absolute counts (1400–3000/mm^3). The cytology of 2 of the patients (MT, BU) were abnormal (Figs. 18, 19) and the third patient (FN) appeared to have normal lymphocytes according to blood films and phase microscopy (Fig. 22). The blood lymphocytes of all 3 patients were sensitive to colchicine with indices of 94, 58 and 58%, respectively. The high indices were considered to be an indication of leukemic involvement.

DISCUSSION

Diagnostic Features of Viable Cells

Viable cell suspensions from lymph nodes were studied to determine factors that might differentiate malignant and nonmalignant lymph nodes. Factors that were studied included percentage of large cells and the sensitivity of the cells to colchicine as measured by the sensitivity index. The results are summarized in Table 6 which divides each of these factors into three groups: 1) an approximately normal range to include nearly all the data for the control reactive nodes, 2) a high abnormal group, arbitrarily defined, and 3) an intermediate range. The table indicates that both factors are useful as diagnostic criteria of malignancy.

The hyperplastic nodes had few large lymphoid cells (defined as 10 μm or more in diameter). In contrast, the suspensions of many of the malignant nodes had a moderate or consid-

TABLE 6. Summary of Data on Nonmalignant Lymph Nodes and on Nodes with Various Types of Malignant Lymphoma and Angioimmunoblastic Lymphadenopathy (AILD)

	Reactive	Lymph node diagnosis Malignant lymphoma				AILD
		Histiocytic	Lymphocytic	Nodular	Total	
Percentage large cells						
0–5	29	0	8	3	11	0
6–14	1	2	3	1	6	0
15+	0	9	0	3	12	3
Total	30	11	11	7	29	3
Colchicine Sensitivity Index %						
0–30	21	2	0	2	4	0
31–50	0	3	3	4	10	2
51+	1	2	8	2	12	1
Total	22	7	11	8	26	3

erable number of large cells, some of which were characterized by abnormal features such as large dark nucleoli, small cytoplasmic lipid granules and mitotic figures. As would be expected from the histologic sections, the large cells were particularly numerous (15% or more) in suspensions from histiocytic lymphoma.

The determination of the percentage of large cells was made about 2 hours after excision of a node. If we use 6% or more as a criterion of a malignant lymphoma, 18 of 29 malignant nodes would have been accurately diagnosed by this criterion alone (Table 6) and only one false positive would have been made in a patient who had arthritis and prominent germinal center hyperplasia.

The present study was designed to determine whether colchicine sensitivity of nodal lymphocytes would have any diagnostic value in differentiating malignant and hyperplastic nodes. The cells of all but one hyperplastic node had sensitivity indices of less than 30%. In contrast, 22 of 26 malignant nodes had indices greater than 30%. Although the number of nodes tested was small, the differences in the sensitivity of malignant and hyperplastic nodes is statistically significant. Therefore, an elevated index could be considered presumptive evidence of malignancy. It should be noted that this test also is a rapid one and takes less than 24 hours. Although an index greater than 30 would be a strong indication of malignancy, a low index would not rule out malignancy.

From Table 6, we might consider the finding either of 6% or more large cells or of a colchicine sensitivity index of 31% or more as a positive test for malignant lymphoma. According to this criterion, a false negative was obtained only in 1 of 30 lymph nodes with the histologic diagnosis of malignant lymphoma (BR, Table 3). A false positive was obtained in 2 of 30 reactive nodes. One of these nodes had 12% large cells and the other had a sensitivity index of 70%. The sensitivity index of 70% was obtained for a hyperplastic axillary node of a patient 80-years-old who had a confirmed clinical history of cancer of the prostate and cancer of the breast. After the nodal biopsy, the patient was found to have multiple myeloma involving bone. We do not know whether there was any relationship between the high sensitivity index and the multiple tumors of this patient.

Another discrepancy between the *in vitro* findings and the histologic diagnosis was observed in patient SR (Table 1). One of his axillary nodes, SR-2, was diagnosed as reactive but the in vitro findings were positive (8% large cells and 45% index). However, 2 nodes of the contralateral axilla of this patient were diagnosed histiocytic lymphoma. Therefore the *in vitro* findings on node SR-2 are not necessarily a false positive but may represent subclinical involvement, not identifiable by routine microscopy even on review.

Another indication of the diagnostic possibilities of viable cell suspensions was exemplified by the node SR-1 (Table 1) which was originally diagnosed anaplastic malignant tumor, unclassified. The anaplastic tumor cells in this node could not be classified by histologic or electron microscopic studies. The appearance of the living cells in a suspension from this node was not consistent with carcinoma or melanoma cells observed previously in metastatic nodes. We believe we can differentiate in viable cell suspensions between histiocytic lymphoma cells and anaplastic cancer cells although the sensitivity of epithelial cells to colchicine has not yet been tested. In preliminary studies, both normal and leukemic granulocytes and monocytes were resistant to colchicine.

A combination of the cytologic findings and the colchicine sensitivity of the nodal lymphocytes in nearly all cases gave a rapid and accurate indication of the malignancy of a node. Perhaps further studies on viable cells would also aid in the classification of the malignant lymph nodes.

Large Cells

The large cells from 5 histiocytic lymphomas were highly sensitivie to colchicine. This finding would suggest that the large cells are malignant. The malignancy is also indicated by the ability of the cells to invade the blood and produce leukemia. The sensitivity of the large cells from 5 of 7 histiocytic lymphomas to colchicine possibly indicated the lymphoid character of the cells since only leukemic lymphocytes have so far been found to be colchicine sensitive. Perhaps the colchicine resistant cells of 2 histiocytic lymphomas are "true" histiocytes as defined by Lukes' classification.

Small Cells

The small lymphocytes from histiocytic lymphoma were resistant to colchicine. The finding suggests that these cells are not malignant. In contrast, the small cells in diffuse and nodular lymphocytic lymphomas were considered to be malignant as they were usually highly sensitive to colchicine. In 3 patients, (2 with the histologic diagnosis of malignant lymphoma, lymphocytic, diffuse, well differentiated and 1 with intermediate differentiation), the small cells invaded or entered the blood, possibly due to the preservation of the normal property of the mature lymphocyte.

Angioimmunoblastic Lymphadenopathy

In view of the questionable nature of this disease, the viable cell studies on three nodes from 2 patients with this diagnosis are of particular interest. All 3 nodes had viable cell characteristics similar to those of malignant lymphoma including high percentages of large cells (immunoblasts), high sensitivity to colchicine and increased numbers of mitoses. The findings would indicate that this lymphadenopathy, at least in our 2 patients may be considered as a malignant disease. In one patient, bone marrow and liver biopsies showed involvement by a similar process as in the lymph node. Both patients died of intervening infection.

Leukemic Blood Cells

Six of the 31 patients with malignant lymphoma had lymphocytosis, usually moderate in amount (4600 or more lymphocytes/mm^3). The blood lymphocytes of these 6 patients had elevated colchicine-sensitivity indices, as would be expected from the studies on chronic lymphocytic leukemia by Thomson and Robinson[12] and Schrek *et al.*[10] High sensitivity indices were also obtained for blood lymphocytes from 3 lymphoma patients with normal absolute counts (less than 4000/mm^3). These 3 patients must also be considered to have leukemic involvement which was also indicated by many large lymphoid blood cells in 2 of the patients (MT, BU). The findings show that the colchicine-sensitivity index of blood lymphocytes is a helpful tool for monitoring the lymphoma patient for leukemic involvement.

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