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THE IMPRINT TECHNIQUE AS AN ADJUNCT IN THE STUDY OF LYMPH NODE NEOPLASMS

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The diseases affecting lymph nodes are many and varied, their diagnosis by routine histologic methods is often difficult. Added to this is the field of the so-called "malignant lymphomas" are the marked variations in classifications schemes which have made this subject unnecessarily confusing. It is interesting that in spite of the marked variability in taxonomy of these diseases almost all workers in the field agree that there are but three major cell groups affected. The chasm often seen between the diagnoses of some clinical pathologists and anatomical pathologists on the same individual is indeed alarming and disturbing. Further, the variegated neoplastic diseases of lymph nodes often assert their presence in many different fields of medicine, e.g., the abdominal mass often palpated in a surgical clinic; the cutaneous manifestations in dermatological practices; the generalized or focal lymphadenopathy in any practice or clinic, etc., and as a result many clinical and distributional classifications have added to the problem of diagnosis.

The classification based on cytological type has been shown to be remarkable constant and appears to correlate with clinical pictures as well.[1] The probability of the development of a leukemia can also be roughly prognosticated when the diagnosis according to cell type is made.[2]

However, in much of the routine material submitted to the pathologist the cytologic details needed for such a diagnosis is not available. The same problem is faced when he is given only sectioned material from a bone marrow biopsy to diagnose various blood dyscrasias. So too, is the solution similar in each case. While the use of smears or imprints is commonplace in hematology, it is not as often used in helping the pathologist in the study of lymph node neoplasms. The purpose of this paper is to show how the use of lymph node imprints or smears can be used as an effective supplementary aid in the diagnosis, by cell type, of the lymphomas.

The term lymphoma (lymphosarcoma, lymphoblastoma) is the diagnosis used for all presently considered neoplastic diseases of lymph nodes when the only tissue examined is a lymph node. If bone marrow and/or blood shows the presence of neoplastic elements with or without a tumor of the lymph nodes the diagnosis is then termed lymphocytic leukemia (or if reticulum cells are involved primarily, leukemic reticulo-endotheliosis or monocytic leukemia of Shilling).

BRIEF HISTORICAL BACKGROUND:

The use of the imprint or smear technic on tissues other than blood or bone marrow is not new. Paul Ehrlich[3] used this technic in studying hepatic tissue in

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1883. Much of the emphasis on the use of the freshly air dried imprints or smears in the study of lymph nodes and other hematopoietic tissues in this country can be credited to Downey and Kirshbaum[4], later Sundberg and Downey[5]. The role of the lymphoid cells in mycosis fungoides was aptly demonstrated by Berman[6] using this method. Sundberg[7] offered the most plausible explanation of lymphocytogenesis in man by this technique. The important difference of nuclear structure vs. nuclear pattern of lymphoid cells was elucidated by Jones[8]. Rebuck[9] was one of the first to show morphological resemblance of pathologic reticulum cells to the Reed-Sternberg cells seen in Hodgkin's disease. The latter work also showed by the effective aid of imprints, the similarity of the cells of the reticulum cell type of lymphoma and the so-called Hodgkin's sarcoma. This was confirmed, by the same means, by Berman[2] who also aided our understanding of the relation of the "lymphomas" to leukemia.

The dry imprint or smear technique, valuable as it may be, should never be relied upon solely for pathologic diagnoses in tumors in lymph nodes. It is restated at this point that its virtue is as a supplementary procedure to the routine microscopic examination of sectioned material of the lymphoid organs. To this must be added, where feasible, the history, physical examination and laboratory data for complete evaluation of the patient.

CLASSIFICATION:

As mentioned previously, the classification in lymphoid neoplasms is indeed confusing and complex with many varied schemes offered [1, 2, 10, 11, 12]. Most oncologists have agreed that the most constant morphological factor is the cell type. Since there are but three basic morphologically distinguishable groups of cells, theoretically there should be but three groups of neoplasms. However, the occurrence of mixtures of cells plus physiological variants and varied clinical pictures requires a more complex scheme. The following is offered as a possible classification of these diseases.

HISTO-CYTOLOGICAL CLASSIFICATION OF NEOPLASTIC LESIONS OF LYMPH NODES

(Malignant Lymphomas)

I. Reticulum Cell Type
(Rarely Leukemic)

II. Lymphoblastic Cell Type
(38-50% Leukemic)

III. Lymphocytic Type
(48-80% Leukemic)

Mixed

I. Hodgkin's Type
   A. Sarcoma (Morph. indistinguishable from Reticulum cell type)
   B. Granuloma
   C. Para Granuloma

II. Giant Follicular Lymphoma.

No originality for the above table is claimed by the author. However, it is believed to be based on most of the available knowledge to date.

The predominant cell type will determine the position in the so-called "pure" tumors of lymph nodes as represented to the left of the midline above. The incidence of leukemia shows a progressive increase as we go down the list. Also the size of the cells decrease in the same order, viz., reticulum cell > lymphoblast > lymphocyte (in addition, this in general terms, parallels normal lymphocytogenesis). It is believed that the smaller cells are less likely to be trapped in the tissues and therefore gain access to the blood stream in large numbers. The reticulum cell type and all members of the mixed group are rarely leukemic. The lymphoblastic group often is accompanied by leukemia, up to 50% according to Berman[2]. The older term leukosarcoma was often used to designate this complication. Lymphocytic cell type usually shows a leukemic picture in the course of the disease. In Jackson and Parker's[10] series of 303 cases of lymphocytoma and lymphoblastoma 263 patients had a manifest leukemia.

In the mixed types the order listed roughly parallels the life span or prognosis. Though there are occassional exceptions, the sarcomatous variety of Hodgkin's disease is invariably fatal in two years, in one series[10]; the granulomatous generally leads to death in one to three years. The paragranulomatous variety has a comparatively good prognosis, 55% being alive and symptom free for five years or more, while 18% of the same series survived a fifteen year period.

In the Hodgkin's group, in the table, the interesting progression of the disease from the paragranuloma to the granuloma and the latter to the sarcoma is an accepted view. The reverse order is never seen. The giant-follicular lymphoma is an intermediate form of tumor.

METHOD:

The unfixed specimen, immediately following its removal, is sectioned with a sharp knife. The cut surface is touched lightly against a scrupulously cleaned slide. This is air-dried rapidly and subsequently treated as a bone marrow aspirate or peripheral blood smear, usually being stained by some Romanowsky dye. The procedure commonly used at the Henry Ford Hospital is the Strumia modification of the May-Gruenwald-Giemsa stain.[13]

If the dried imprints can not be used immediately they should be fixed in absolute methyl alcohol. Also, when the time element is important, they can be satisfactorily stained in 40 secs. using a solution of 0.5% Toluidine Blue 0 in 20% alcohol, to which 1% acetic acid is added.

Best cytological detail is retained if the imprint is made within two hours of its removal from the body. Immediately following the contact or touch preparation, the lymph node is then placed in a fixative and routine 5μ sections are cut and stained with hematoxylin and eosin or other useful dyes.

Histological examination will ascertain whether or not a neoplastic lesion is present because this is the *only* method of studying the topography of the lesions, invasiveness, loss of normal architecture, etc.

OBSERVATIONS:

The simultaneous study of lymph node sections and imprints requires some working knowledge of lymphocytogenesis. It is truly beyond the scope of this paper to either discuss the many theories in detail or present numerous transitional phases of this process, according to any one school of thought. Bloom[16] and Sundberg[7] adequately discuss these theories. In lymph nodes the developmental maturation series, in general terms, of reticulum cell → lymphoblast → mature lymphocyte would cause little alarm in most circles. This method of production of lymphocytes through a series of *different* cell types is termed *heteroplastic lymphocytogenesis*. In tumors of lymph nodes there is chiefly a homeoplastic process, most of the mitoses found in *one* cell type. The characteristics of the individual cells in imprints and sections will be described briefly in a simplified form.

In figure 1 we have an imprint of a relatively normal lymph node. The arrow points to a reticulum cell which has a typical nuclear chromatin pattern, a nucleolus and a large amount of cytoplasm. The latter is subject to considerable variation. The average size of these cells is 15-25μ. The irregular network of variable-sized nuclear particles making up the chromatin, along with the irregular, clear interstices (parachromatin) make this an easy cell to identify. Its counterpart in sectioned material is shown in figure 3, which is taken from a reticulum cell type of lymphoma. In figure 4, is an imprint of the same node. Though these are neoplastic cells, the basic similarity to the same type cell in figure 1, is apparent.

The large cell in figure 2 represents a lymphoblast as seen in normal node imprint. The average size of these cells is 10-18μ, they often have a narrow band of cytoplasm. The nucleus is round or oval and their chromatin pattern is finely stippled or "reticulated." They may have 0-5 nucleoli. Its malignant counterpart is illustrated in a section of a lymphoblastic lymphoma (two of the marked cells are in mitosis) figure 5. The imprint in figure 6 is taken from the same node and the large marked cells are neoplastic lymphoblasts. As can be seen from figure 5 the predominant cell type is difficult to evaluate in sections.

The mature lymphocytes are familiar, to all who have studied air dried blood smears and hardly need any description here. Figure 7 is a section from a lymphocytic type of lymphoma, while figure 8 is an imprint of the same node.

Hodgkin's sarcoma is illustrated in figure 9 with its prominent tumor giant cell (Reed-Sternberg, Greenfield) and pathologic reticulum cells. The marked cell in figure 10 is a smaller giant cell, as shown in an imprint from the same lymph node.

DISCUSSION:

The morphological diagnosis of neoplastic lymph node lesions using histologic and cytologic methods should be more accurate than study of sectioned material alone. The diagnoses of the clinical pathologist and anatomical pathologist on a

given patient can be made on the observation of the same cells under similar conditions. The use of imprints as a supplementary aid offers to the surgical pathologist neoplastic cells of a given lymph node in a state which allows more complete examination of cellular morphology. It is estimated that cells shrink 11-20% following fixation in 10% formaldehyde, hence the marked differences in cell sizes in the same cells when compared with the imprint. Since there are three cell types in lymphomas, the usual histological grading of degrees of differentiation (as done in carcinomas) is not possible and the imprint study offers a more accurate definition of the predominant cell type.

So too, metastatic tumors of lymph nodes occasionally offer a difficult problem in deciding whether the lesion is primary or secondary. This is especially true of transitional cell carcinomas and unpigmented melanomas.[15] If the large marked cells in figure 11 are compared with those of figure 3 they appear quite similar; yet the latter is a section from a reticulum cell lymphoma while figure 11 is a section of a lymph node invaded by metastatic transitional cell carcinoma. On imprint from the same node figure 12, the foreign neoplastic cell can be readily differentiated. The misinterpretation of a lymphoma (especially reticulum cell type) as a carcinoma can also be avoided in some cases, if this technique is used.

In many instances the surgeon requires a diagnosis on some lymphatic tissue while he is operating. Good rapid fresh frozen sections of this material are at times difficult to obtain due to the soft consistency of lymphoid tissue. The lymphoma represented in figures 3 and 4 was diagnosed using both sections and imprints when the surgeon was confronted with a retroperitoneal mass near the pancreas. His first biopsy was histologically pancreatic tissue with no evidence of malignancy. The second biopsy of the mass on frozen section appeared neoplastic. Imprints of this mass showed sheets of reticulum cells.

In infections mononucleosis, histological distinction from a lymphoma can be almost impossible. The imprints will show leukocytoid lymphocytes in abundance and a heteroplastic rather than homeoplastic lymphocytogenesis.

Mycosis fungoides[6] often a difficult histologic problem, as well as Hodgkin's Disease[15] with cutaneous involvement can be resolved in some instances by the aid of imprints of skin lesions.

The use of cellular preparations obtained by needle biopsy of lymph nodes, or imprints without histological examination is dangerous. In giant follicular lymphoma the predominant cell type may be any of three major groups and a grave error in subsequent prognosis could be made. Likewise imprints from nodes in Boeck's Sarcoid and severe inflammations, may show hyperplasia of one cell type while Hodgkin's Disease may give varied types.

Further advantages of the method not mentioned above are its relative simplicity and its value as a second diagnostic approach on the same biopsy material. It is inexpensive and does not interfere with subsequent use of the excised tissue for histologic study.

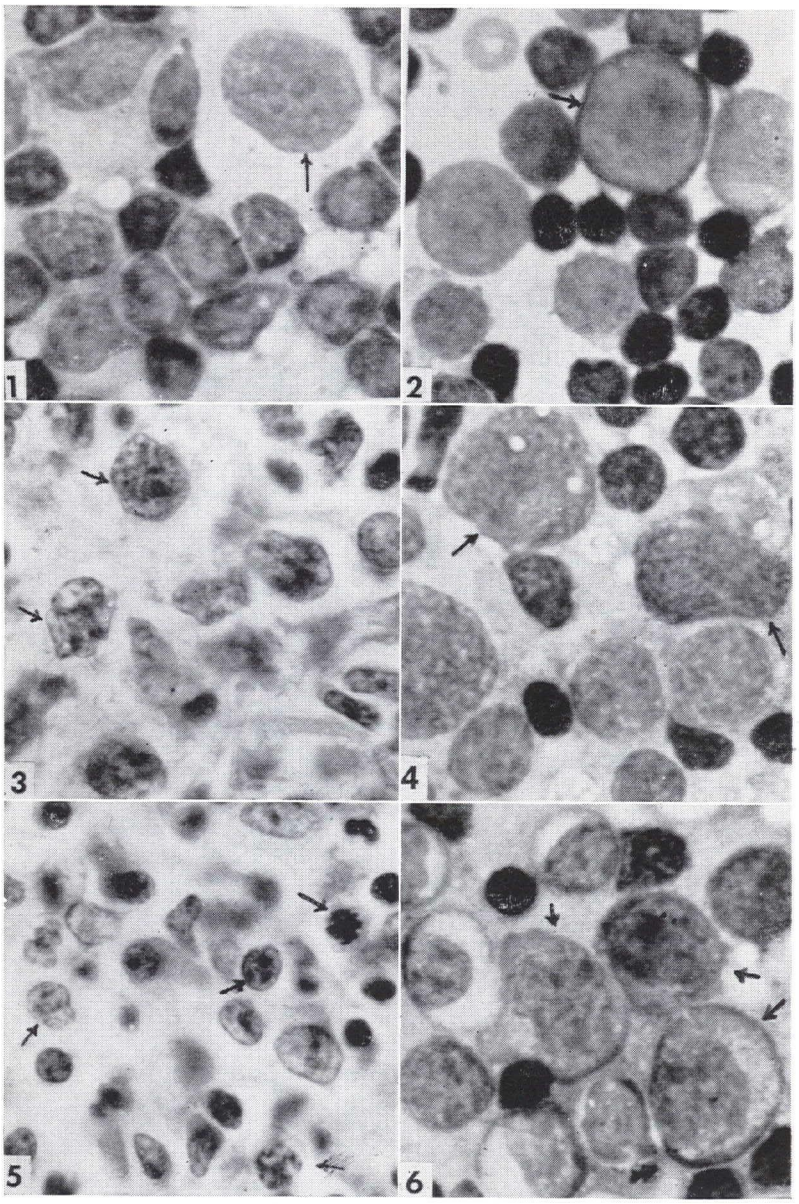

PLATE I

All figures at same magnification 1150 x.
All imprints stained with May-Grunewald-Giemsa stain.
All sections stained with H & E.

Fig. 1—Imprint of normal lymph node. Large cell (↑) in upper right is normal reticulum cell.

Fig. 2—Imprint of normal lymph node. Large cell (↑) in upper center is a lymphoblast and smaller darker cells in center are lymphocytes.

Fig. 3—Section of lymph node, lymphoma, recticulum cell type. Large cells (↑) are reticulum cells.

Fig. 4—Imprint of same lymph node shown in figure 3. Large cells (↑) with vacuoles are reticulum cells.

Fig. 5—Section of lymph node, lymphoma, lymphoblastic cell type. Marked cells (↑) are lymphoblasts, two of which are in mitoses.

Fig. 6—Imprint of same lymph node shown in figure 5. Marked cells (↑) are lymphoblasts.

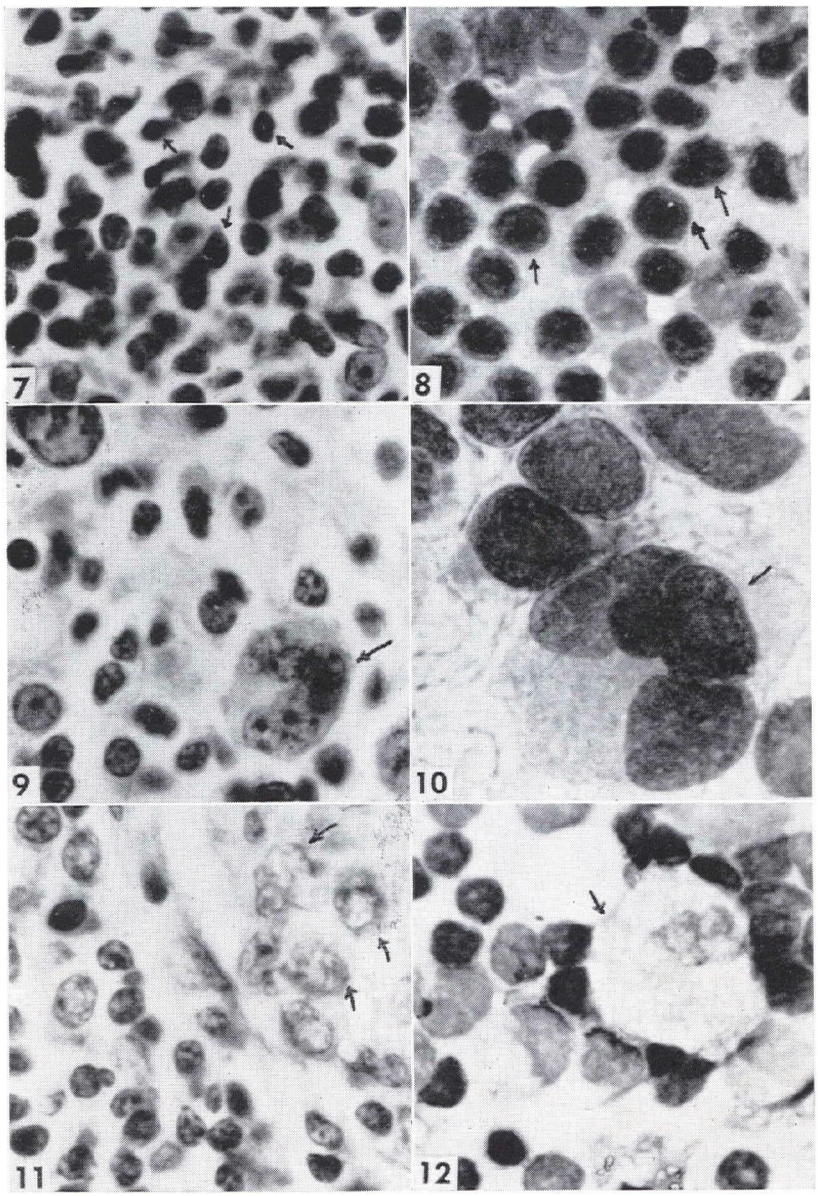

PLATE II

Fig. 7—Section of lymph node, lymphoma, lymphocytic cell type. Numerous small dark cells are lymphocytes, some of these are marked (↟).

Fig. 8—Imprint of same lymph node shown in figure 7, showing numerous lymphocytes.

Fig. 9—Section of lymph node from Hodgkin's Sarcoma showing prominent bizarre giant cell.

Fig. 10—Imprint of same lymph node showing in figure 9, showing a similar but smaller tumor giant cell.

Fig. 11—Section of cervical lymph node showing metastatic cells from a transitional cell carcinoma of nasopharynx. Note similarity of marked cells (↟) to reticulum cells (fig. 3).

Fig. 12—Imprint of same node shown in figure 11. Marked cell (↟) is metastatic carcinoma cell.

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