Lymph Node Reactivity. III. Lymphomas and Allied Diseases

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MARSHALL has reported on the use of ammoniacal silver staining to clarify the basic physiology of the reticular tissues. With this technic he has visualized silver staining cells in the sinusoids and pulp of lymph nodes. He has termed such cells metalophils and has described variations in their numbers and appearance in association with different functional and pathologic states. His technic involves the use of unmounted frozen sections cut at 20 to 25 μ or unmounted paraffin sections cut at 15 to 20 μ. Due to the thickness of the sections, the detailed cytologic features are obscured, and subtle variations in the metalophils are lost. In fact, Marshall discusses at some length the justification for considering the staining as indicative of particular cells rather than artifactual silver deposits.

Recently we have found that ammoniacal silver staining could be applied to routine 5 μ paraffin sections, provided that the deparaffinized sections were repeatedly washed in distilled water before and after exposure to the ammoniacal silver solution. The well washed sections were then developed in 3 per cent formalin, dehydrated, cleared in xylol and mounted in the conventional fashion. With this procedure, distinct and discrete staining of the cytoplasm of the metalophil cells was readily apparent. The nuclei of these cells were unstained and thus clearly visualized within the black stained cytoplasm (fig. 1). Furthermore, such detailed features of the cytoplasmic staining as vacuolization, fragmentation and variations in the intensity of staining of individual cells were readily discernible.

We have reported on the staining of lymph nodes reacting to foreign protein injections, infectious diseases and the presence of spontaneous mammary carcinoma. It was found that lymph nodes reacting to foreign protein injections and infectious diseases were usually characterized by a previously undescribed type of ammoniacal silver staining. In such instances, the nuclei of the lymphoid cells were stained so that the sections appeared as if they had been stained with iron-hematoxylin. Such nuclear staining of lymphoid cells was also found in the developing lymph nodes of human fetuses.

Since the present modification of the ammoniacal silver staining technic was capable of visualizing reactive changes in both the reticular and lymphoid cells, it seemed pertinent to evaluate the staining characteristics of neoplastic proliferations of lymphoid and reticular cells.
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Fig. 1.—Femoral lymph node removed in course of vein ligation, no histopathologic abnormality. Note the distinct staining of the cytoplasm of the reticular cells of pulp and sinusoids. The lymphoid cells are unstained. (In figures 1 to 4 all sections were stained with ammoniacal silver.)

MATERIAL AND METHODS

In the present study, duplicate 5 μ paraffin sections were prepared from formalin-fixed (10 per cent neutral formalin) lymphomatous nodes: Hodgkin's disease, 12 cases; lymphosarcoma, 13 cases; giant follicular lymphoblastoma, 5 cases; acute leukemia, 5 cases; and chronic lymphatic leukemia, 3 cases. These specimens were obtained by excisional biopsy, except for the acute leukemic nodes, which were obtained at autopsy. In three of the lymphosarcoma cases, skin metastases were also available for study.

Duplicate sections were stained with H&E and ammoniacal silver. The ammoniacal silver staining was performed according to our previously published technic.¹

RESULTS

Hodgkin's disease.—Marshall has described the presence of three types of cells in Hodgkin's granuloma as judged by his silver staining technic: (1) unstained cells, which he considers to be primitive reticular cells; (2) atypical forms of metalophils; (3) elongated, faintly stained, fibroblastic types of cells. Our findings are in agreement with these observations. However, we would
interpret the atypical metalophils as degenerating reticular cells which have been overgrown by the Hodgkin's tissue. Analogous cells have also been seen in lymph nodes involved by metastatic carcinoma.

Proliferating fibroblasts have been shown by Marshall, and also seen by us to possess variable degrees of cytoplasmic metalophilia. Such cells may be found in association with Hodgkin's granuloma. However, it should be emphasized that many areas of Hodgkin's tissue lacked any cells which stained as metalophils. Nor did the proliferating lymphoid cells of Hodgkin's disease exhibit nuclear staining with the silver technic.

In Hodgkin's sarcoma, most of the cells were unstained. Scattered cells having similar morphology exhibited variable degrees of cytoplasmic staining. These findings correspond to Marshall's observations. However, it should be emphasized that the metalophil staining never approached the intensity found in normal or reactive pulp, or sinusoidal reticular cells of non-neoplastic lymph nodes.

**Lymphosarcomas of lymphoid cell type.**—Under this heading we have included five cases of small and large cell types of lymphosarcoma as distinguished from the varieties of reticulum cell lymphosarcoma. Reactive proliferations of lymphoid cells draining inflammatory foci usually exhibit nuclear staining with ammoniacal silver. Such staining has been observed in nodes with and without the formation of secondary follicles and plasma cell aggregates. The findings in lymphosarcoma were in contrast to such staining of reactive proliferations of lymphoid cells.

The lymphosarcomatous nodes were characterized by proliferations of cells which were essentially unstained by ammoniacal silver, either in cytoplasm or nucleus. Degenerating, faintly stained pulp metalophils were noted in some areas and appeared to be analogous to similar cells seen in nodes involved with Hodgkin's disease or metastatic carcinoma.

**Reticulum cell sarcoma.**—This group of eight cases included those lymphosarcomas characterized by proliferations of large monocytoid cells showing varying degrees of cellular anaplasia. In three of these cases, skin metastases were available for study in addition to the involved lymph nodes. This type of neoplasm provides an excellent comparison with normal and reactive reticular cells. Such non-neoplastic reticular cell proliferations are intensely metalophilic.

In contrast, the cells of the reticulum cell sarcomas had minimal or no metalophilia (fig. 2). In some of the cells, silver-positive cytoplasmic granules were noted, but in no instance were such cells stained with the intensity of the reticular cells of normal or stimulated lymph nodes. Variable numbers of normal appearing and degenerating metalophils were also noted among the sarcoma cells. It seems that sarcomatous reticular cells possess far less metalophilia than normal reticular cells.

**Giant follicular lymphoblastoma.**—Five cases of this type of lymphoma were studied. The silver staining produced a picture which was similar to that of the previously considered types of lymphoma: viz., the majority of the cells were unstained. Scattered, poorly stained, degenerating metalophil
cells were noted in the "follicular" and pulp areas. In one of the cases, there appeared to be an area of remaining uninvolved nodal tissue. Here the sinusoidal metalophils were well stained.

Acute leukemia.—Axillary lymph nodes were obtained at autopsy from five cases of acute leukemia. In three of these cases 6-mercaptopurine and corticoids had been used, which may possibly have altered the staining reaction. However, as judged by the H&E sections, there seemed to be no evidence of cytotoxicity of the leukemic cells. There was no appreciable silver staining of the leukemic cells per se. Background metalophil cells were present, and stained with minimal to moderate intensity. In one case which had succumbed to a superimposed varicella infection, the reticular cells were well demonstrated by the silver stain. (fig. 3).

Chronic lymphatic leukemia.—The cases of chronic lymphatic leukemia had similar appearing lymph nodes in the H&E preparations. However, after silver staining, two of the cases showed no appreciable staining, whereas one showed a distinct nuclear type of staining (fig. 4). In two additional cases of chronic lymphatic leukemia, we studied the staining of sections of
Fig. 3.—Axillary lymph node involved by cells of acute leukemia. Hypertrophied and degenerating metachromatized cells are present in the pulp and sinusoidal regions. The leukemic cells are essentially unstained.

marrow aspirates. In both instances the nuclei of the leukemic cells stained distinctly with ammonical silver.

COMMENTS

The technic employed has allowed a detailed evaluation of the silver staining of the cells of various types of lymphoma and allied diseases. The reticular cells of Hodgkin’s disease, reticulum cell sarcoma and follicular lymphoma were characterized by a loss or marked decrease in metachromasia as compared to reticular cells of normal or reactive lymph nodes. The lymphoid cells of Hodgkin’s disease, lymphosarcoma and follicular lymphoma were not stained by ammoniacal silver. This lack of nuclear staining was similar to the findings in adult control nodes but in contrast to the nuclear staining of proliferating lymphoid cells in fetal lymph nodes and the nuclear staining of lymphoid cells in nodes reacting to inflammatory disease.

It would appear from these data that neoplastic proliferations of lymphoid and reticular cells are associated with a decreased ability to stain with am-
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Fig. 4.—Replacement of axillary lymph node by cells of chronic lymphatic leukemia. Note the distinct nuclear staining of the leukemic lymphocytes.

Moniacal silver, whereas non-neoplastic reactivity tends to be associated with an increased silver staining.

In 17 of the Hodgkin's disease and lymphosarcoma cases, measurements had been made of the in vitro dehydrogenase activity; it was found to be equal to, or greater than, such activity in control lymph nodes. Thus, the diminished silver staining of the neoplastic nodes was not due to diminished metabolic activity of such nodes.

In view of the limited number of cases of leukemia in the present study, comments as to the observed findings must be limited. It is noteworthy, however, that the staining characteristics of the acute leukemia cases' tissues were similar to those of the malignant lymphomatous tissues. However, one of the three cases of chronic lymphatic leukemia had distinct nuclear staining. Nuclear staining was also observed in sections of bone marrow aspirates from two additional cases of chronic lymphatic leukemia.

The observed diminution or loss of silver staining ability of the lymphomatous, lymphoid and reticular cells as compared to the staining of analogous control or hyperplastic cells may be of some general importance, since we
have observed similar differences between the silver staining of malignant epithelial neoplasms and hyperplastic epithelial tissues.

While the biochemical basis of ammoniacal silver staining is not known, it seems to possess empirical value in the study of lymph node reactivity. The present study suggests that it may also contribute to the study of malignant neoplasia in lymph nodes.

**Summary**

A study was made of the ammoniacal silver staining of the cells of Hodgkin’s disease, lymphosarcoma, follicular lymphoma, acute leukemia and chronic leukemia as seen in routine 5 μ paraffin sections. With the exception of chronic lymphatic leukemia, the cells of lymphomas and allied diseases exhibited minimal staining. These data were compared with the ammoniacal silver staining of normal lymph nodes and the enhanced staining of non-neoplastic proliferations in developing and reactive lymph nodes.

**Summary in Interlingua**

Esseva studiate le tincturation per argento ammoniacal de cellulas de morbo de Hodgkin, lymphosarcoma, lymphoma follicular, leucemia acute, e leucemia chronic, omnes in sectiones routinari a paraffin de 5 μ. Con le exception de chronic leucemia lymphatic, le cellulas de lymphomas e morbos affin exhibiva grados minimal de tincturation. Iste datos esseva comparete con le tincturation per argento ammoniacal de normal nodos lymphatic e con le tincturation intensificate de proliferationes non-neoplastic in nodos lymphatic evolvente e reactive.

**References**

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