The Cytochemical Demonstration of Intracellular Immunoglobulin

In Neoplasms of Lymphoreticular Tissue

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An immunoglobulin–enzyme bridge technique has been employed for the selective localization of immunoglobulin-producing cells in spleen, lymph nodes, and lymphoreticular tumors fixed lightly for optimal immunocytochemistry or processed routinely for surgical diagnosis. Immunostaining for immunoglobulin was encountered consistently in the specially fixed tissues and was observed in some of the surgical specimens in areas where presumably fixation was satisfactory. Many, but not all, of the tumor cells in histiocytic lymphoma (reticulum cell sarcoma) of spleen and of brain stained for IgG, but none evidenced reactivity for IgM or IgA. In Hodgkin's tumors, the prevalence of IgG-reactive stromal immunocytes in or between tumor areas was greatest in the nodular sclerosing form of the disease, abundant in mixed cellular, and least in lymphocyte-predominant and lymphocyte-depleted types. Immunocytes showing reactivity for IgG greatly exceeded those staining for IgM or IgA. Hodgkin's tumor cells immunostained frequently for IgG, infrequently for IgM, not at all for IgA, and in one instance, stained for κ but not for λ light chains. Individual tumor cells failed to stain for more than one type of Ig or light chain. The proportion of Ig reactive *vs.* unstained tumor cells correlated with the prevalence of immunocytes in the different Hodgkin's categories. Reed-Sternberg cells differed in their fine structure in ways which possibly corresponded with the presence or absence of Ig shown at the light microscope level. Tumor cells in Hodgkin's disease and reticulum cell sarcoma also disclosed argyrophilia and strong staining indicative of ribonucleic acid with a Schiff–methylene blue procedure. (Am J Pathol 82:457–478, 1976)

NEOPLASMS OF THE LYMPHORETICULAR TISSUE have been classified according to the location of the primary tumor, state of differentiation of the neoplastic cells, or morphologic characteristics of the tumor cells. Improved understanding of the cell of origin of the various lymphoreticular tumors would afford a more accurate basis for their classification. Lymphocytes from which some of these tumors arise were initially separated morphologically into small, medium, or large cell types. However, the concept has developed of two categories—one the B lymphocyte, functioning in humoral immunity, and the other, the T

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Supported by Grants AM-10956 and AM-11028 from the National Institutes of Health and Training Grant TR-168a from the Veterans Administration.

Accepted for publication November 10, 1975.

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lymphocyte, concerned with cell-mediated immunity. Since B and T lymphocytes and macrophages are often morphologically indistinguishable by routine light microscopy, other methods are required to distinguish them. Attempts at differentiating these cells have depended on their different surface content of immunoglobulins,[1] complement receptors,[2] binding sites for sheep erythrocytes,[3] and immune complex (Fc) receptors,[4] and on differences in their enzymatic content [5,6] and adherence properties.[4] By demonstrating various surface markers on the tumor cells, recent studies have shown that chronic lymphocytic leukemia,[7,8] nodular lymphoma,[9] and Burkitt's lymphoma [10,11,12] are of the B-cell type; whereas Sternberg sarcomas [13] and Sezary's syndrome [14] arise from T cells. *In vitro* immunoglobulin analysis of lymphoid tumors [15] and cultured lymphoid cell lines [10,12,16-19] has also indicated the B cell origin of certain tumors, since immunoglobulin production is a specific property of the B-lymphocyte line. The demonstration of immunoglobulin in or on isolated Hodgkin's tumor cells,[20] or in these cells in tissue sections,[21,22] has afforded evidence for their derivation from B lymphocytes. The present immunocytochemical study undertakes to inquire further into the immunoglobulin synthesis by Hodgkin's tumor cells and to investigate such activity in other lymphoreticular neoplasms.

Materials and Methods

Specimens

The material examined included portions of human lymph nodes and spleens removed for diagnosis or staging during the period of this study. In addition, examples of various specific neoplasms were retrieved from the surgical pathology files at the Medical University of South Carolina and the Laboratory of Pathology of the National Cancer Institute (Table 1). The histopathologic diagnosis on each specimen was established originally by surgical pathologists of the Medical University Hospital or National Cancer Institute and was confirmed through the kind assistance of Dr. A. Kreutner of Surgical Pathology, Medical University Hospital. The cases of Hodgkin disease were separated into four types according to the Rye classification (Table 2).[23]

The specimens retrieved from the surgical files were processed by initial fixation with buffered 10% formalin for undetermined intervals followed by routine dehydration and paraffin embedding. An optimal fixation procedure determined from a preliminary investigation of immunostaining of immunoglobulin in rat spleen and surgical tumor tissue [24] was employed on those specimens obtained during the present study. This fixation entailed a half- to 2-hour immersion at 4 C in a solution containing 0.5% glutaraldehyde and 10% formalin buffered with 2% calcium acetate or a 3-hour immersion in a solution of 4% formaldehyde in 2% calcium acetate. Tissues were then dehydrated routinely and embedded in paraffin.

Immunostaining Procedure

Immunostaining was carried out with an immunoglobulin-enzyme bridge technique which employed antigen-antibody reactions to link specific antibody to the enzymatic

Table 1—Summary of Immunostaining and Basophilia in Lymphoreticular Tumors

		Specimens processed for optimal immunostaining		Specimens retrieved from surgical files		
	Site	Total No.	No. with immuno-reactive tumor cells	Total No.	No. with immuno-reactive tumor cells	Schiff-methylene blue staining of tumor cells
Hodgkin's disease	Lymph node & spleen	10	10	40	15	+++B
Lymphoma						
Histiocytic (reticulum cell sarcoma)	Lymph node & spleen			10	1	+++B
Lymphocytic	Lymph node & spleen			5	0†	
Mixed histiocytic & lymphocytic	Lymph node & spleen			5	0	
Undifferentiated (Burkitt's)	Lymph node & spleen			1	0	
Poorly differentiated	Lymph node & spleen	1	0	5	0†	0
Histiocytic medullary reticulosis	Lymph node & spleen			1	0	
Primary lymphoma (reticulum cell sarcoma)*	Brain			3	2	
Reticulum cell sarcoma	Bone			1	0	
Reticulum cell sarcoma	Thyroid			1	0	

* This specimen was obtained at autopsy, and the others at surgery.
† Plasma cells neighboring tumor cells stained for IgG in 1 case.
B = blue coloration.

marker.[25] This method offers advantages in increased sensitivity and avoidance of the autofluorescence that arises from macrophage pigments and obscures specific staining with the fluorescent antibody procedure. The basic technique was modified by initially exposing rehydrated sections to 3% hydrogen peroxide for 3 to 10 minutes to eliminate the pseudoperoxidase activity of erythrocytes as described elsewhere.[24] After rinsing, the sections were incubated sequentially in: a) heavy chain–specific antiserum prepared in rabbits against human immunoglobulin including IgG, IgM, or IgA (Cappel Laboratories, Inc., Downington, Pennsylvania); b) ammonium sulfate–precipitated goat antiglobulin directed against purified rabbit 7S globulin (Miles Laboratories, Inc., Elkhart, Ind.); c) rabbit antiserum to horseradish peroxidase (HRP); and d) a 1 mg% solution of HRP (Sigma Chemicals, St. Louis, Mo.). Subsequent incubation in the diaminobenzidine hydrogen peroxide medium of Graham and Karnovsky[26] yielded brown to black coloration of immunoglobulin-producing cells. Antisera to human κ and λ light chains kindly provided by Dr. H. Hugh Fudenberg, Medical University of South Carolina, were used in addition as the primary antiserum in staining sections of one of the specimens. Adjacent or serial sections were stained for different immune globulins to evaluate the presence of more than one form of immunoglobulin in a single cell. To minimize back-

Table 2—Prevalence of IgG-Positive Tumor Cells and Immunocytes in Each Histologic Type of Hodgkin's Disease

Type	Tumor cells: Average no. of stained tumor cells/high power field for each patient					Tumor cells: Average for type	Abundance of immunocytes stained for IgG
Lymphocyte predominant	3	4	—	—	—	3.5	+
Nodular sclerosing	10.0	6.2	9.0	9.7	10.5	9.1	++++
Mixed cellularity	6.4	6.0	3.0	8.1	8.1	6.3	++
Lymphocyte depleted	0.6	—	—	—	—	0.6	+

ground interference, the antisera were diluted in phosphate-buffered saline as follows: primary antiserum, 1:10; goat antiserum to rabbit immunoglobulin, 1:5; and antiserum to HRP, 1:5.

Control sections were prepared from each tissue block by employing nonimmune rabbit serum as the first step of the immunoglobulin-peroxidase bridge or omitting the primary antiserum to immunoglobulin. The specificity of the primary antiserum was determined by absorbing equal volumes of the antiserum for 3 days at 4 C with 0.5 mg/ml of purified human IgG or IgM (Cappel Laboratories, Inc.) in serial dilutions and employing the mixture as the first step of the immunoglobulin–enzyme bridge.

Reference Stains

The immunostained sections were compared with sections stained by reference procedures for identifying the cell type. This comparison was facilitated by simultaneously viewing the same field stained by two methods in a Nikon comparison microscope. A section adjacent or serial to each immunostained section was stained by hematoxylin and eosin or a recent modification [27] of a Schiff-methylene blue technique [28] or both. The latter method permitted staining of routine paraffin sections by employing Bouin's fluid for a Feulgen-type hydrolysis prior to staining with Schiff reagent and then methylene blue. This sequence differentiated redstained deoxyribonucleic acid (DNA) from turquoise-colored ribonucleic acid (RNA) and identified cells active in protein synthesis by their blue coloration. To facilitate further the identification of immunoglobulin-containing cells, some immunostained sections were counterstained with hematoxylin or, less preferably, the Feulgen method for providing nuclear detail. Sections of many of the blocks were also stained with the Black and Ansley ammoniacal silver method,[29] which has been shown to stain tumor cells in Hodgkin's lymphoma.[30]

Ultrastructure

Minced portions of lymph node containing Hodgkin's tumor were fixed in 3% glutaraldehyde buffered with 0.1 M cacodylate, rinsed in buffer, postfixed for 1 hour in 2% osmium tetroxide, dehydrated, and embedded in Epon. Part of some lymph nodes was fixed by the optimal procedure for immunostaining, dehydrated to absolute ethanol, rehydrated, sectioned in the cryostat and stained for IgG with the immunoglobulin–peroxidase bridge. Minced lymph nodes were also fixed in a 10% formalin–2% calcium acetate solution for 24 to 48 hours, rinsed for 2 hours in phosphate-buffered saline, and stained with ammoniacal silver.[29] These cytochemically processed specimens were then rinsed, postfixed for 1 hour in 2% osmium tetroxide, dehydrated, and embedded in Epon. Thin sections of the morphologic specimens were stained with a

uranyl acetate and lead citrate sequence and viewed with an Hitachi HS-8 electron microscope, but thin sections of the cytochemical preparations were examined without further staining.

Results

Histiocytic Lymphoma

Areas showing histiocytic lymphoma (reticulum cell sarcoma) in hematoxylin and eosin–stained sections (Figure 1) disclosed immunostaining for IgG in tumor cells (Figures 2 to 4) of adjacent sections. One of ten routinely processed tumors of spleen and lymph node and the two available routinely processed tumors originating in brain (Figure 4) revealed such staining (Table 1). A large portion, but not all, of the tumor cells stained for immunoglobulin in the three positive specimens. None of the tumors revealed cells reactive for IgM or IgA. Tumors of this type were not available for optimal fixation for immunocytochemistry. The specimens lacking immunostained tumor cells also failed to show reactive immunocytes, but the specimens with reactive tumor cells also disclosed occasional stained immunocytes. In addition, the tumor cells stained a deep blue, indicative of abundant cytoplasmic RNA with the Schiff–methylene blue method. Many also stained strongly with the ammoniacal silver method (Figures 5 and 6).

A poorly differentiated histiocytic lymphoma revealed no immunoreactivity for IgG or IgM in tumor cells despite optimal processing for immunostaining of immunoglobulin (Table 1). This lack of reactivity of neoplastic cells contrasted with the strong reactivity for IgG and IgM in sparse immunocytes in or near tumor areas. Notably, the tumor cells in this lymphoma differed from those in the above histiocytic lymphomas in lacking blue staining indicative of RNA in adjacent sections stained with the Schiff–methylene blue sequence.

Hodgkin's Disease

Hematoxylin and eosin–stained sections of lymph nodes and spleens from patients with Hodgkin's disease disclosed nodular or diffuse obliteration of normal architecture by varying proportions of Reed-Sternberg cells, uninuclear Hodgkin's cells, lymphocytes, plasma cells, eosinophils, and histiocytic cells. Large uninucleate or multinucleate cells disclosed prominent nucleoli with a surrounding nuclear space which gave them a classic "owl's eye" appearance. The specimens from the patients with Hodgkin's disease included one or more examples of each histologic type of Hodgkin's tumor recognized in the Rye classification (Table 2).

Immunostaining selectively revealed immunocytes with strong reac-

tivity for immunoglobulin in many of these specimens. The stained immunocytes varied from very numerous in the nodular sclerosing variety (Figure 7) and abundant in the mixed cellular type to infrequent or rare in the lymphocyte-depleted (Figure 8) and lymphocyte-predominant (Figure 10) types of Hodgkin's disease (Table 2). The stained immunocytes were characteristically clustered around nodules in the nodular sclerosing type and scattered throughout the mixed cellular type of lesion. Most of the immunocytes contained IgG. A relatively few plasma cells distributed like those containing IgG disclosed content of IgM or IgA.

Immunostaining for IgG also selectively visualized large uninuclear and binuclear cells in the neoplastic regions of the lymph nodes and spleens (Figures 7 to 10). Comparison of hematoxylin and eosin–stained sections with immunostained serial sections at high magnification and counterstaining immunostained sections (Figure 11) served to identify many of the positively stained cells as Reed-Sternberg or Hodgkin's cells. The intensity of staining varied from light tan to dark black and bore no apparent relation to the proximity to plasma cells or areas of necrosis. In counterstained sections, IgG was clearly evident within the cytoplasm of giant cells (Figure 12) and of tumor cells in mitosis (Figure 13) in Hodgkin's disease.

However, a portion of the neoplastic cells ranging from 10 to 70%, according to the type of Hodgkin's disease, lacked immunoreactivity in the positively stained specimens (Figure 14). The number of IgG-containing tumor cells per high power field was counted for all slides which exhibited optimal immunostaining (Table 2). The density of stained tumor cells appeared greatest in the nodular sclerosing group (Figure 9) of the Rye classification [23] and somewhat less in the mixed cellular type. The specimens from the lymphocyte-predominant group (Figure 10) and from the 1 patient with lymphocyte-depleted Hodgkin's disease (Figure 8) disclosed sparse immunostained cells.

Serial sections from one optimally fixed tumor stained for κ and λ light chains revealed staining for κ chains in many tumor cells (Figure 15) but showed no staining for λ chains. A very few tumor cells revealed immunostaining indicative of IgM (Figure 16) in the cases studied and none evidenced content of IgA. Comparison of serial sections stained for IgG and IgM failed to afford evidence for both immunoglobulins within the same cell.

In control sections of Hodgkin's tumors and other tumors stained with normal rabbit serum, as the first step of the bridge, or with anti-IgG serum absorbed with purified IgG only the eosinophils with intrinsic peroxidase showed diaminobenzidine staining.

The Schiff–methylene blue method stained the nucleoli of the uninuclear and binuclear tumor cells turquoise. The cytoplasm of tumor cells ranged from intense turquoise to unstained. On comparison with immunostained sections, staining of the cytoplasm in the Schiff–methylene blue sequence appeared to correlate with IgG content. However, positively immunostained cells could not be selected on Schiff–methylene blue stains alone. This stain also highlighted plasma cells, as previously shown in hyperplastic lymph nodes.[24]

The ammoniacal silver procedure stained nuclei of most cells black but yielded yellow coloration of nuclei and nucleoli in tumor cells. This method also stained the cytoplasm black in plasma cells and some tumor cells (Figure 20). Comparison with immunostained serial sections failed to establish a correlation between IgG-containing tumor cells and those stained with the silver method.

Ultrastructure

The tumor cells possessed large nuclei with rather scant condensed chromatin and large nucleoli. Both the mononuclear and the Reed Sternberg cells could be divided into two general tumor cell populations on the basis of cytoplasmic components. One cell type (Figure 17) was characterized by an abundance of organelles—including mitochondria, cytoplasmic dense bodies, and microfilaments—and a prominent Golgi apparatus. The second cell type (Figure 18) possessed numerous free polyribosomes but had few mitochondria or other organelles and contained only sparse profiles of collapsed endoplasmic reticulum with a few attached ribosomes. Both varieties of tumor cells were seen in lymphocyte-predominant and mixed cellular types of Hodgkin's disease.

Lymph nodes stained with the ammoniacal silver technique revealed electron-opaque silver deposits in the heterochromatin region of nuclei and in cisternae of the rough endoplasmic reticulum of plasma cells (Figure 19). In lymph nodes processed for ultrastructural immunostaining, plasma cells exhibited moderate electron opacity indicative of IgG in the cisternae of the granular reticulum. Tumor cells stained by these methods have not as yet been encountered.

Discussion

Histiocytic Lymphoma

Demonstration of cytoplasmic IgG in tumor cells of histiocytic lymphoma (reticulum cell sarcoma) in the spleen provides evidence for the origin of these cells from the B lymphocytic series which normally

accounts for all IgG production. The immunostaining thus extends earlier biochemical observations of immunoglobulin production by reticulum cell sarcoma in relating this tumor to B lymphocytes.[15] Mixed lymphocytic–histiocytic lymphoma has previously been considered to originate from B cells on the basis of the presence of surface markers for B lymphocytes on these tumor cells.[9] Moreover, the staining of these lymphoma cells for IgM by immunofluorescence and the ultrastructural demonstration in these tumor cells of cytoplasmic crystals that appeared to correspond with the immunofluorescent material further indicated a B-cell derivation for mixed lymphocytic–histiocytic lymphomas.[31] On the other hand, properties such as absence of surface immunoglobulins and presence of hydrolase-rich lysosomes in tumor cells growing in culture have indicated a relationship of some diffuse histiocytic lymphomas to histiocytes.[32] Future studies employing immunostaining and hydrolase cytochemistry on optimally fixed specimens possibly will clarify whether the presently designated category of histiocytic lymphomas actually comprises two types of tumor—one derived from lymphocytes that is lymphoid in nature and the other derived from bone marrow monocytes that is histiocytic in character. The varied ultrastructure of histiocytic lymphoma cells supports the possibility of heterogeneity within this category.[33]

The observed IgG formation in tumor cells of reticulum cell sarcoma in the brain provides a significant characteristic linking this tumor to the histiocytic lymphomas in other organs. The morphologic characteristics also link these tumors to histiocytic lymphomas elsewhere and indicate they are primary lymphomas of the central nervous system.[34] However, cytogenesis of this tumor from B lymphocytes as suggested by such a property has not been previously considered. These tumors have generally been regarded as deriving from the histiocytic type of cell in brain, the microglia, or from their presumed precursors, the hypothetical reticulum cell.[35] From the present findings, the term *reticulum cell sarcoma* seems inappropriate for these brain tumors.

A lymphoid origin of this primary brain tumor cannot be readily explained since the central nervous system is not known normally to harbor a fixed population of lymphoid cells. Perhaps cells in brain with unrestricted polyblastic mesenchymal potential, as previously postulated,[36] or the perivascular lymphocytes that frequently infiltrate the Virchow-Robin space in various pathologic conditions of the brain could account for development of lymphoid neoplasms in this site. The apparent perivascular site of origin of these tumors [35] seems consistent with genesis from a polypotential stem cell or vascular cuff cell. The increased

occurrence of these tumors in patients with renal transplant appears compatible with a lymphoid cytogenesis, since the lymphocytic system is the target of immunosuppressive measures applied to these patients.[37]

The argyrophilia held in common between the various reticulum cell sarcomas and Hodgkin's tumor cells links these types of tumor cell on another basis. Although this staining property has not been explained chemically, the argyrophilia in these different tumor cells might be attributed to the content of IgG common to all. Unfortunately, however, staining for IgG did not correlate with argyrophilia in serial sections stained by these methods. Blue cytoplasmic staining with the Schiff–methylene blue method, on the other hand, correlated precisely with immunoglobulin content of the tumor cells. This method, presumably visualizing the cytoplasmic RNA that produces the immunoglobulin in the tumor cells, provides a convenient means of localizing immunoglobulin-forming tumor cells. Such staining would offer a means of histologically distinguishing histiocytic lymphomas of lymphoid origin from those of truly histiocytic nature as previously postulated, since the latter would not be expected to stain.

Comparison of the ultrastructure with immunoglobulin formation to assess the mechanism of immunoglobulin production in the tumor cells was not possible in the histiocytic lymphomas available for study. However, illustrations available in the literature show abundant free polysomes and scant granular reticulum in these cells,[38] as is seen in Hodgkin's tumor cells. Lacking the granular reticulum required for synthesis of protein for export, the histiocytic lymphoma cells possibly synthesize the immunoglobulin on unbound ribosomes and retain it unsecreted in the cytoplasm, as probably occurs also in the Hodgkin's tumor cells.

The present observations encourage examination of routine surgical specimens for immunocytochemical localization of Ig, although only a small proportion of the specimens yielded reactive cells. Since tissues fixed by a predetermined optimal procedure stained well in all instances, it appears the three reactive histiocytic lymphomas fortuitously were not over exposed to fixative. This view agrees with the present experience in which brain and relatively large tissue blocks of spleen yielded more immunoreactive areas than did lymph nodes. The more favorable results with brain and spleen presumably depend on greater gradients of fixative action in large or impenetrable tissue blocks.

Hodgkin's Disease

The increased numbers of IgG and IgM-producing immunocytes scattered throughout the red pulp and clustered around arterioles in spleens

from patients with Hodgkin's disease could account for the synthesis of IgG found biochemically in such specimens. [39] Lymph nodes and spleens with the different types of Hodgkin's lesions varied markedly in the number of immunoglobulin-containing cells, from virtually none in cases of lymphocyte-depleted Hodgkin cases to a great abundance in the nodular sclerosis type. The sparsity of stained immunocytes in the lymphocyte-depleted category thus corresponds with the absence of plasma cell precursors and depletion of the humoral immune system in these lesions. The dearth of stained immunocytes in the lymphocyte-predominant group indicates the numerous lymphocytes infiltrating these lesions either fail to transform into plasma cells or consist almost entirely of the thymus-dependent variety. Such a consideration concurs with the observation that the majority of lymphocytes within the Hodgkin's tumor nodules and in uninvolved spleens removed at laparotomy from these patients possess surface markers of T cells.[40-42] A predominance of T-cell hyperplasia in these lesions would appear, however, to disagree with the current view of impaired T-lymphocyte function in Hodgkin's disease. The tremendous proliferation of plasma cells in the nodular sclerosis lesion, on the other hand, reflects either a different type of lymphocytic infiltrate or different capacity of a B-cell exudate to transform into plasma cells. Possibly, antigenic stimulation differs in this type of lesion. The identity of the antigenic determinant for the immunoglobulin formed by the numerous immunocytes in nodular sclerosing and in mixed-cell Hodgkin's lesions, remains a question central to an understanding of the role of these cells in the tumor. Antibodies against lymphocytes have been detected in a spleen from a patient with Hodgkin's disease, and this finding has suggested that immunocytes mediate an immune response against tumor cells derived from these lymphocytes.[43]

Additionally, the immunostaining clearly demonstrated abundant IgG in cells identified as Hodgkin's tumor cells and Reed-Sternberg cells, thus extending previous immunofluorescent evidence for immunoglobulin in or on isolated Reed-Sternberg cells [20] and immunostaining of these cells in sections.[21,22] Immunoglobulin production by tumor cells was found consistently in all properly fixed cases. Knowledge of the antigen against which the immunoglobulin in the Hodgkin's tumor cells is directed compared with the antigen determining the immunoglobulin in neighboring immunocytes could possibly clarify whether a clonal relationship exists between the immunocytes and neoplastically transformed cells in the tumor. Such knowledge possibly would elucidate the tumorigenic agent in Hodgkin's disease if the immunoglobulin in tumor cells and that in neighboring immunocytes share a common anti-

genic determinant, since such an antigenic agent conceivably could play a part in the neoplastic transformation. The staining of the tumor cells in 1 patient for κ and not for λ light chains indicates the monoclonal nature of the antibody production in these cells. By highlighting the tumor cells, the immunostaining aided in the diagnosis, as no other conditions studied, except the histiocytic lymphomas mentioned earlier, gave similar results. However, specimens of infectious mononucleosis and hydantoin lymphadenitis were not available for evaluation of the stain's diagnostic potential in distinguishing between the Reed-Sternberg-like cells of the latter diseases and those of Hodgkin's disease.

Immunoglobulin production by tumor cells differed for the histologic classes of Hodgkin's disease as shown in the cell counts (Table 2). The density of stained tumor cells depended both on the numbers of other cell types present and on the proportion of stained to unstained tumor cells. The latter ratio could not be accurately ascertained for the different forms of Hodgkin's disease, however. Why some tumor cells stained strongly and near neighbors were unstained remains unexplained, but it presumably can not be attributed to differences in fixation of cells at such close proximity and in optimally reactive specimens. The tumor cells also revealed differences in their fine structure, appearing to fall into two general types. Although ultrastructural immunostaining of these cells has not been accomplished, it is assumed that the cell type with abundant polysomes at the ultrastructural level corresponded with the immunoreactive type of cell at the light microscope level. The ultrastructure of the tumor cells presumed to lack immunoreactivity resembled a histiocytic more than a lymphocytic cell type, however. The differences in immunoreactivity and fine structure among the Hodgkin's tumor cells could theoretically be ascribed to differences in the cell of origin or the degree of differentiation or to differences in the maturation of the cell or the stage of its activity in a functional cycle.

Since none of the Hodgkin's tumor cells possessed abundant granular reticulum, the IgG in their cytoplasm is apparently formed on free ribosomes and released into the cytoplasm where it may remain for want of the export mechanism by which protein synthesized by granular reticulum is normally secreted. Some neoplastic cell lines obtained from Burkitt's lymphomas have also been shown to produce—but not secrete—IgG although other lines have both synthetic and secretory capacity.[11]

Historically, the Reed-Sternberg cell was thought to derive from the theoretically widespread "reticulum cell."[33,44,45] However, Dorfman [5] concluded, after extensive histochemical studies, that Reed-Sternberg cells were probably derived from cells other than histiocytes. Ultrastructural

studies [44] suggested a transition from the reticulum cell to the Reed-Sternberg cell. Studies on Reed-Sternberg-like cells in infectious mononuculeosis indicated that these binucleated cells were derived from the lymphoblast.[26] The demonstration of antibodies against peripheral blood lymphocytes in spleens from patients with Hodgkin's disease [39] has led to the view that the neoplastic cell is a transformed T cell against which B cells produce antibodies.[43,47] The correlation of a predominance of tumor cells lacking immunoreactivity with a dearth of stained immunocytes in the present study seems consistent with a T-cell origin of unreactive tumor cells. However, cell lines from Hodgkin's tumor fail to stain with fluorescent antithymocyte serum,[48] and studies with the sheep erythrocyte-rosetting technique provided no evidence that the Hodgkin's tumor cells contain the typical T-cell binding sites for sheep erythrocytes.[41] The present and earlier [20-22] immunostaining for IgG point rather to cytogenesis of Hodgkin's tumor cells from the B-lymphocyte series. Such staining properties offer a means of classifying lymphomas on a functional basis.

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Acknowledgments

The authors are grateful to Dr. C. W. Berard for making available specimens from the files of the National Cancer Institute and to B. J. Hall, J. Farrington, and K. S. Beaufort for skilled technical and secretarial assistance. The authors are indebted to Drs. G. R. Hennigar and A. Kreutner, Jr., for their generous advice and consultation.

[Illustrations follow]

Legends for Figures

Figure 1—A nodule composed of histiocytic lymphoma (reticulum cell sarcoma) cells replaces normal architecture of the spleen in a specimen from the surgical files. (H&E, × 90)

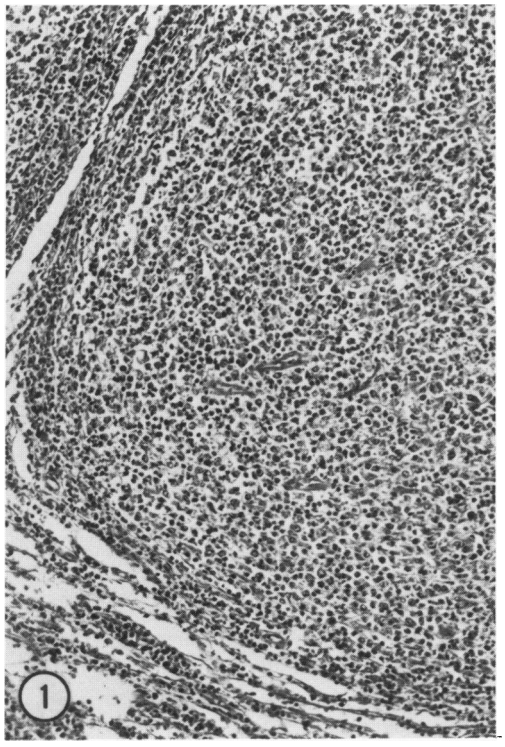
Figure 2—Section adjacent to that in Figure 1. Histiocytic lymphoma cells replace normal splenic tissue. Selective dark immunostaining identifies IgG within a majority of the tumor cells (*arrows*). (Immunostained for IgG and counterstained with hematoxylin, × 110)

Figure 3—Like Figure 2; tumor cells with prominent nucleoli show dark staining of cytoplasm demonstrative of IgG (× 500).

Figure 4—An area composed of histiocytic lymphoma cells (reticulum cell sarcoma) from the brain reveals darkly stained tumor cells similar to those in the splenic tumor (compare with Figure 2) (Routine surgical specimen immunostained for IgG, and counterstained with hematoxylin × 240).

Figure 5—Section adjacent to that in Figure 1. Histiocytic lymphoma in the spleen exhibits argyrophilic cells (*arrows*) which resemble immunoreactive tumor cells in Figure 2. (Ammoniacal silver stain, × 90)

Figure 6—An area in a reticulum cell sarcoma of the brain also displays argyrophilic tumor cells. (Ammoniacal silver stain, × 90)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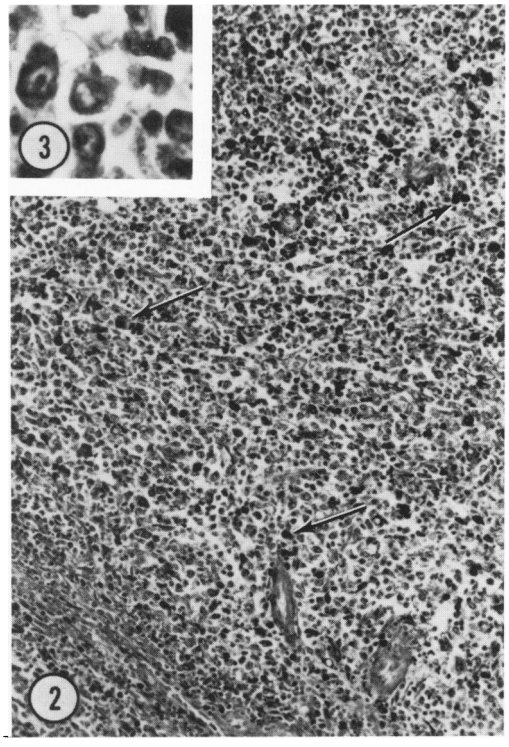
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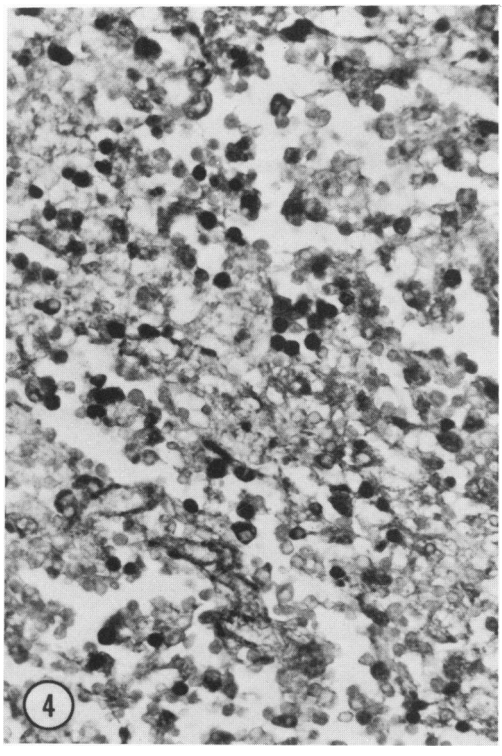
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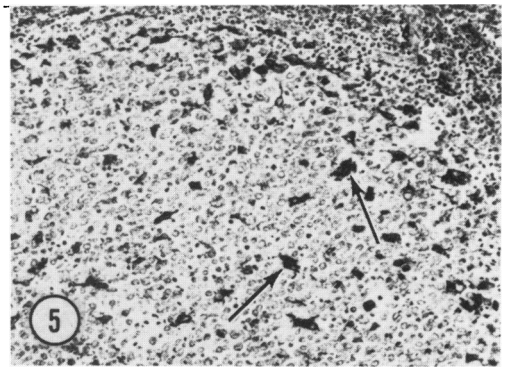
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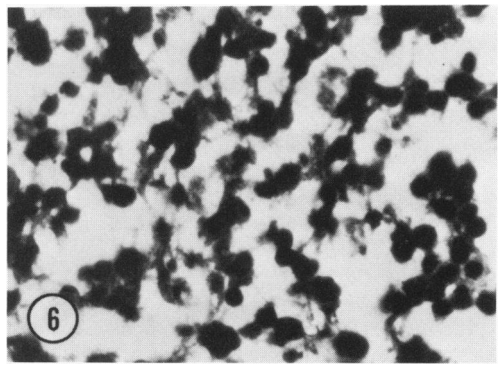
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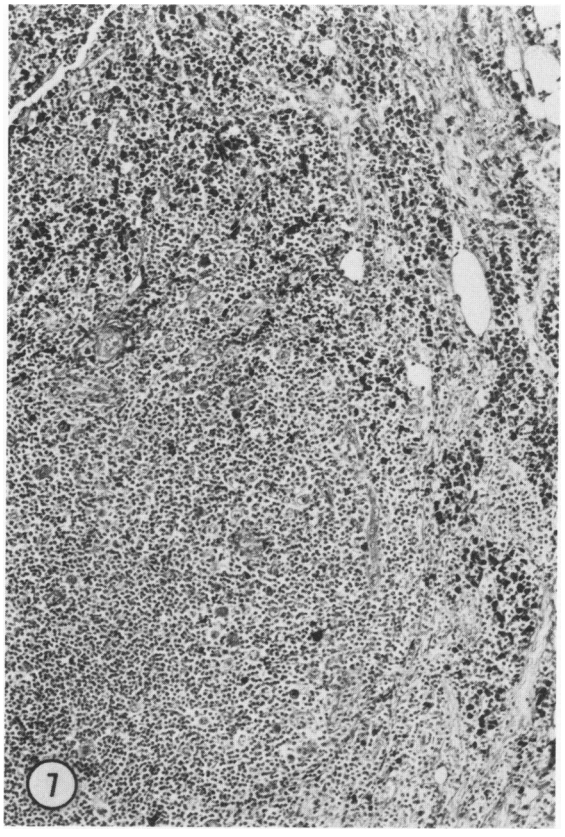
Figure 7—A neoplastic area (lower left) in a lymph node involved with nodular sclerosing Hodgkin's disease reveals immunoglobulin-positive tumor cells and is bordered by stroma heavily infiltrated with stained immunocytes (Routine surgical specimen immunostained for IgG and counterstained with hematoxylin, × 80).

Figure 8—An area in a lymph node invaded by lymphocyte-depleted Hodgkin's cells reveals a few immunostained (*arrow*) and many unstained tumor cells and sparse reactive small cells presumed to be immunocytes (Routine surgical specimen immunostained for IgG and counterstained with hematoxylin, × 110)

Figure 9—Nodular sclerosing Hodgkin's tumor in a lymph node contains very numerous immunoreactive tumor cells, some of which are multinucleate, as well as unreactive tumor cells (*arrow*) (Routine surgical specimen immunostained for IgG and counterstained with hematoxylin, × 120).

Figure 10—Very few immunostained tumor cells (*arrows*) or immunocytes are evident in this lymph node involved with the lymphocyte-predominant type of Hodgkin's tumor (Processed for optimal immunostaining; stained for IgG and counterstained with feulgen, × 120).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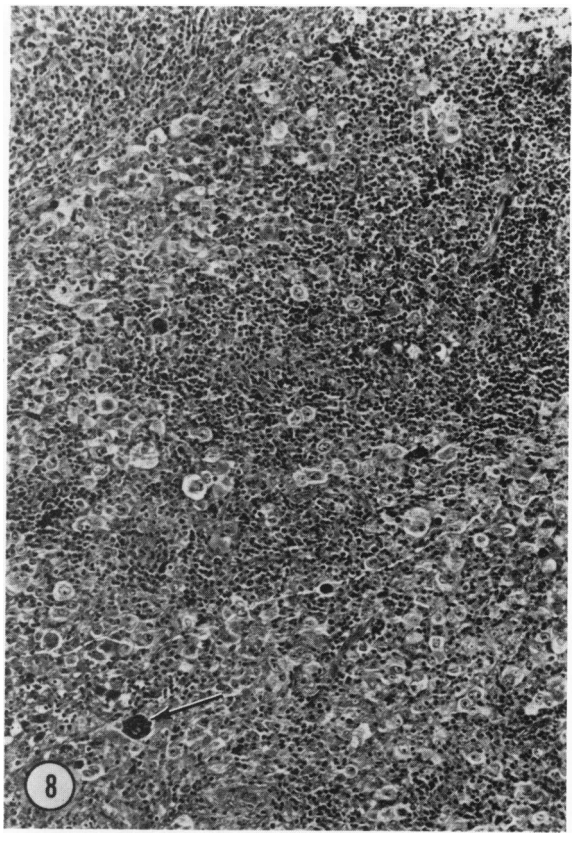
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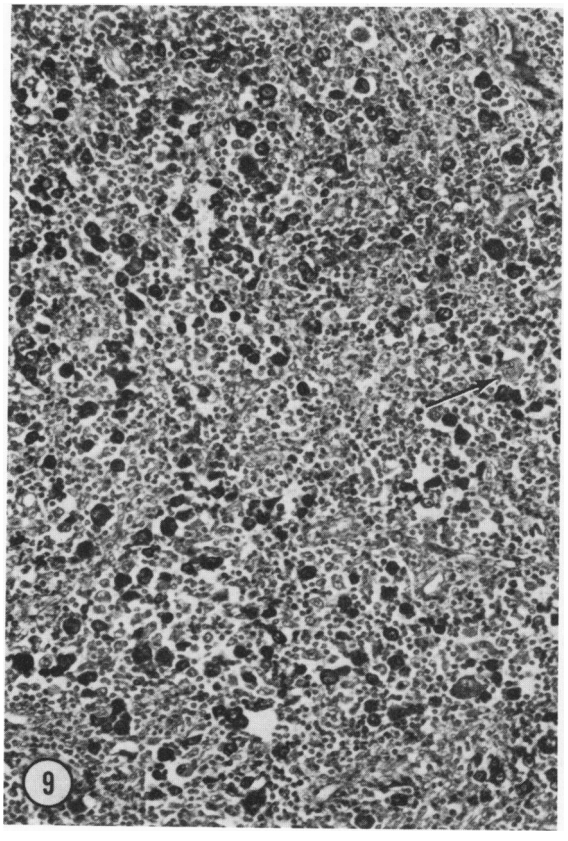
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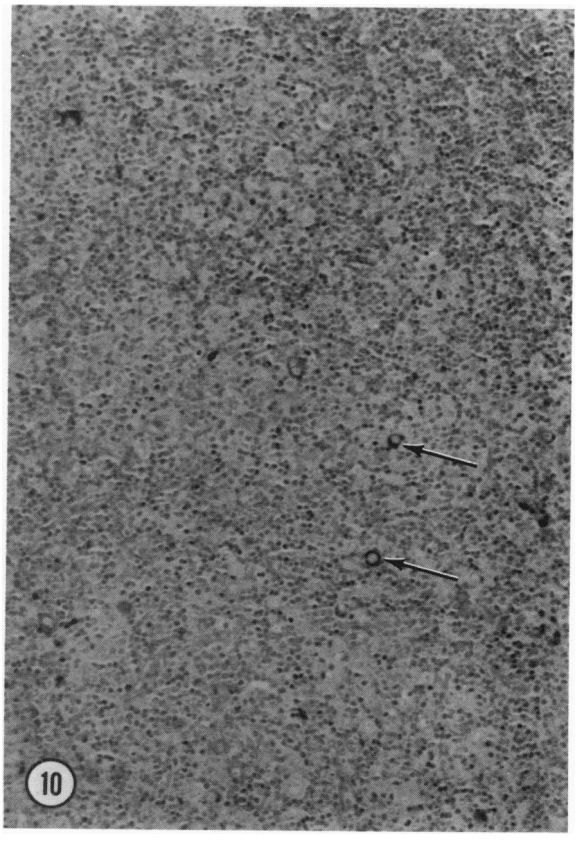
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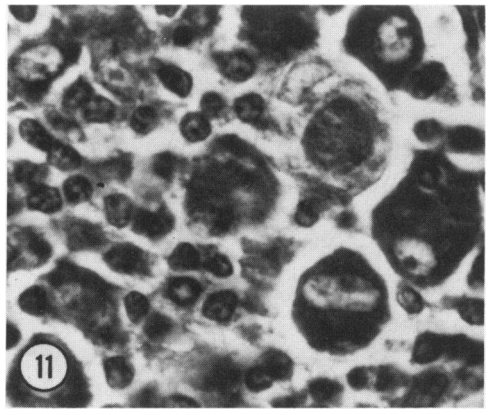
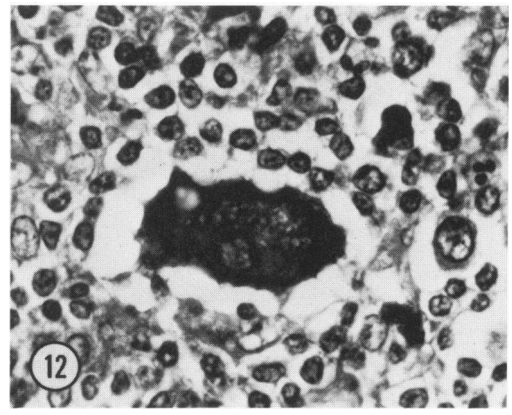
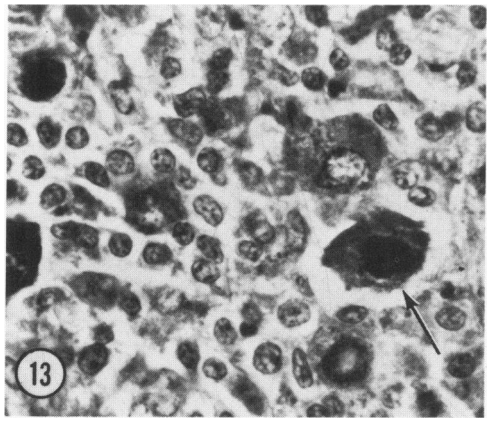
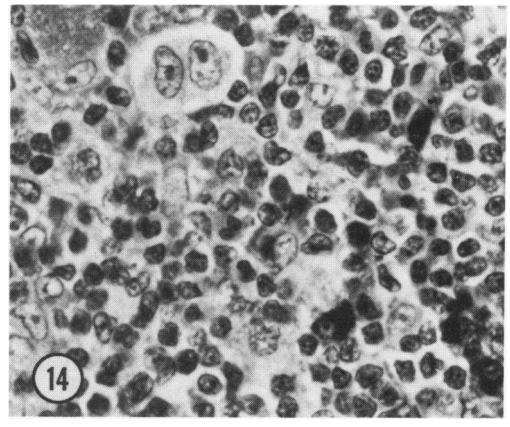
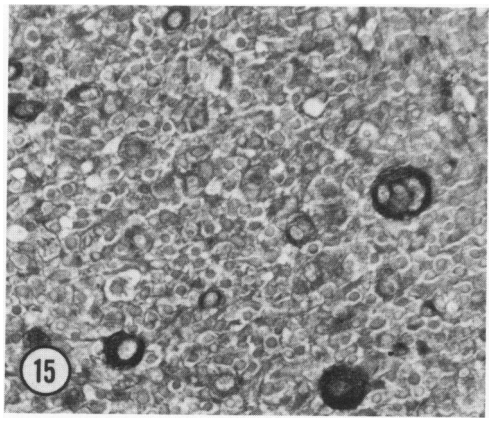










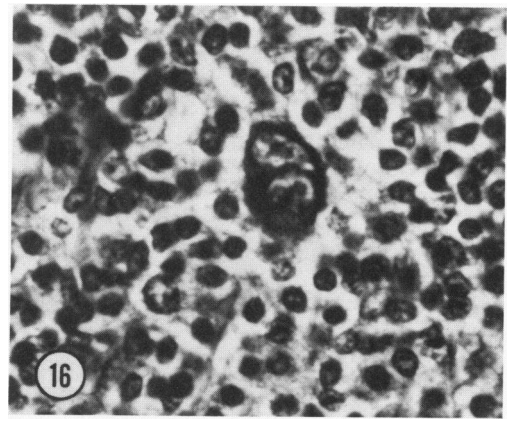


Figure 11—Hodgkin's tumor cells disclose abundant intracellular IgG (Immunostained for IgG with hematoxylin counterstain, × 500). **Figure 12**—A multinucleate giant cell surrounded by lymphocytes in a Hodgkin's tumor shows immunoreactivity (Immunostained for IgG with hematoxylin counterstain, × 500). **Figure 13**—Rare Hodgkin's tumor cells in mitosis were immunoglobulin positive as illustrated here (*arrow*) (Immunostained for IgG with hematoxylin counterstain, × 500). **Figure 14**—Tumor cells are not immunoreactive in this area from a lymph node from a patient with Hodgkin's disease optimally fixed for immunostaining. Neighboring immunocytes are stained, however. (Immunostained for IgG and counterstained with hematoxylin, × 500) **Figure 15**—Tumor cells evidence content of κ light chains. Serial sections immunostained for κ and λ light chains showed only staining for κ chains. (Immunostained for λ light chains, × 240) **Figure 16**—A classic Reed-Sternberg cell shows intense staining for IgM. Only a few tumor cells demonstrated such reactivity, however. (Immunostained for IgM with hematoxylin counterstain, × 500)

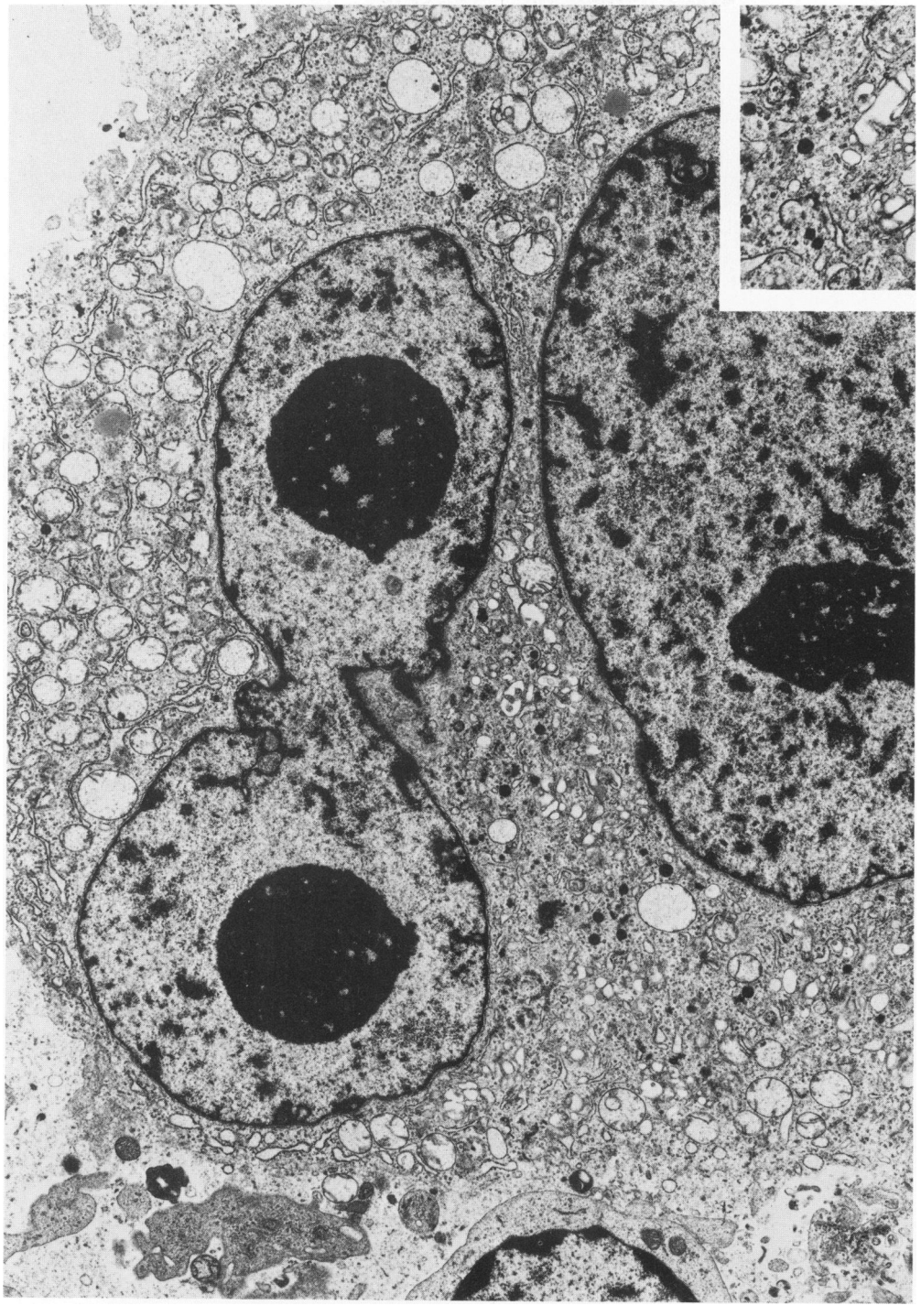
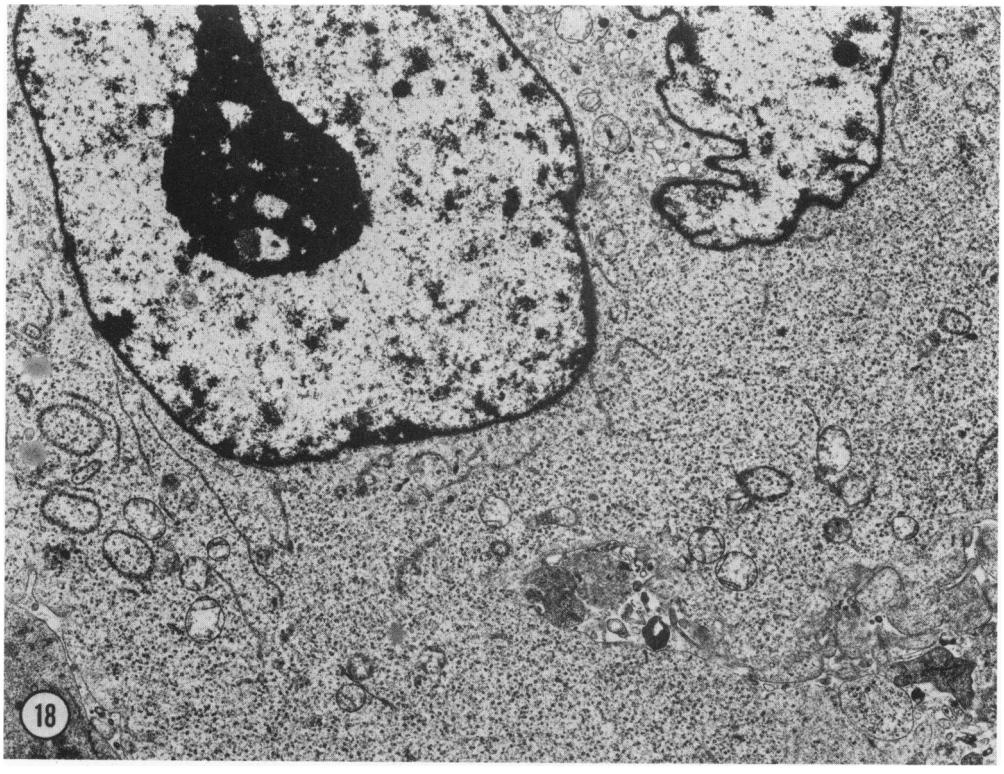


Figure 17—A Reed-Sternberg cell in a lymph node from a patient with Hodgkin's disease discloses nuclei with largely dispersed chromatin, infoldings of the nuclear envelope, and prominent nucleoli. The cytoplasm contains abundant mitochondria, small cytoplasmic dense bodies, and sparse granular reticulum. This type tumor cell possibly corresponds with the immunonegative cells (Uranyl acetate and lead citrate, × 9000)



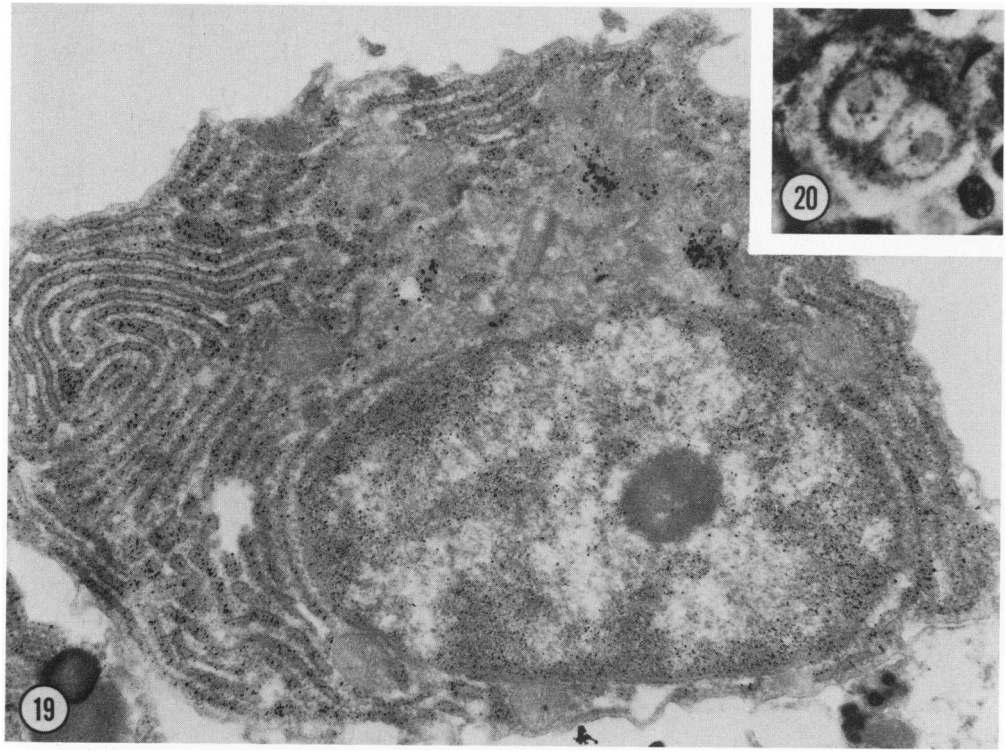


Figure 18—Another Reed-Sternberg cell in a lymph node from a patient with Hodgkin's disease differs from that in Figure 17 in that it contains very abundant free polysomes and scant mitochondria and other organelles. Presumably, this type of cell contains the IgG demonstrable with immunostaining. The immunoglobulin is apparently formed on the free ribosomes since the cell possesses little rough endoplasmic reticulum. (Thin section, and uranyl acetate and lead citrate, × 6000) **Figure 19**—A plasma cell in a normal lymph node displays selective silver deposits within the rough endoplasmic reticulum and variable staining of the nuclear heterochromatin. (Ammoniacal silver, × 24,000) **Figure 20**—The cytoplasm of Reed-Sternberg cells and plasma cells stains black with the ammoniacal silver method (× 300).