Leukemia occurring in treated Hodgkin's disease: Two neoplasms or one?

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The origin of the malignant cell in Hodgkin's disease and the increased incidence of second malignancies in patients with Hodgkin's disease remain unsolved problems.[1-4] The results of cytochemical, immunomicroscopic, immunologic, and ultrastructural studies of the neoplastic cells[5-17] have failed to identify their origin, but suggest either a histiocytic or B-lymphocytic derivation. Similar studies in acute leukemias[18, 19] arising in patients with Hodgkin's disease have suggested differences between the leukemic cells and the cells of Hodgkin's disease. We report a case of Hodgkin's disease in which an apparent second neoplasm developed. Sequential studies of the neoplastic cells suggest that radical changes occurred in the cell type during evolution of the disease. Such changes may reflect treatment-mediated or naturally occurring evolution of the neoplastic cell lines of Hodgkin's disease, rather than a second neoplasm originating from a different cell line.

Clinical summary

A 44-year-old white male was well until September 1974 when progressive shortness of breath developed. A diagnosis of nodular sclerosing Hodgkin's disease, Stage I, involving a left scalene lymph node was made at another hospital (*Fig. 1*). The patient received 4000 rads to the right and left

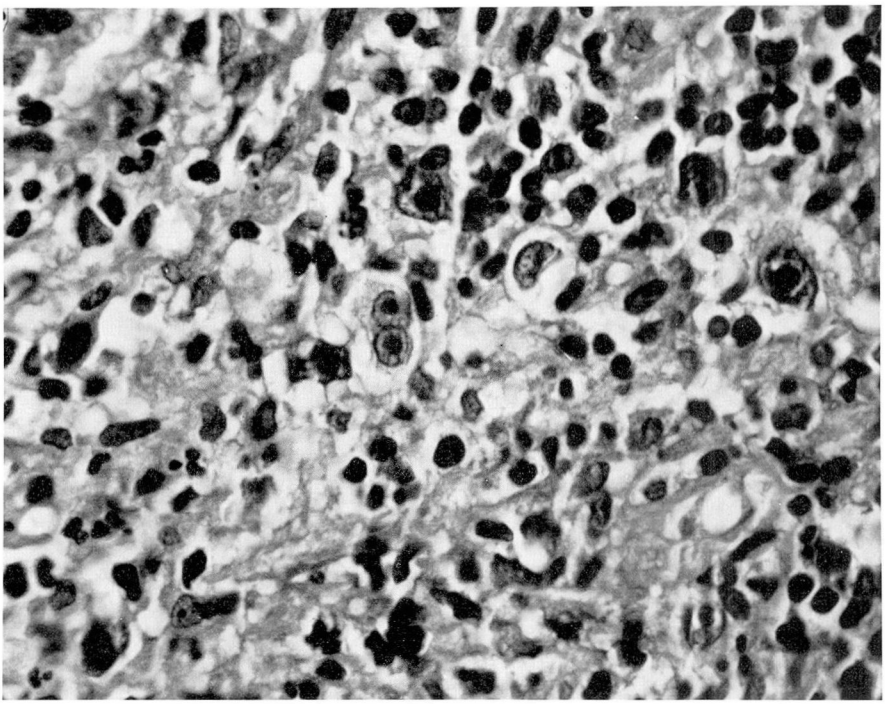

Fig. 1. Left scalene lymph node containing a polymorphous infiltrate with atypical lacunar cells and occasional Reed-Sternberg cells (hematoxylin and eosin stain, ×640)

supraclavicular lymph node areas. He was referred to the Cleveland Clinic 8 months later for consideration of chemotherapy. At this time there was evidence of radiation pneumonitis. The blood count showed hemoglobin, 8.7 g/dl and white blood cell count (WBC), 8.3 × 10^9/1 with a normal differential count. A biopsy specimen of the left posterior iliac crest revealed a marrow of normal cellularity containing small foci of undifferentiated cells suggesting the possibility of acute monocytic leukemia; the remainder of the biopsy specimen appeared normal. He was treated with MOPP (nitrogen mustard, vincristine, prednisone, and procarbazine). Five weeks later the WBC was 3.4 × 10^9/1 with a normal differential count; hemoglobin level, 7.9 g/dl; hematocrit, 21.9%; and platelet count, 165 × 10^9/1.

Chemotherapy was continued. In March 1976 the WBC was 6.7 × 10^9/1 with a normal differential count, but the hemoglobin level was 5.3 g/dl. A bone marrow aspirate revealed complete replacement with primitive cells having pleomorphic folded nuclei suggestive of histiocytic lymphoma or monocytic leukemia (*Fig. 2*); similar cells were present in a bone marrow biopsy performed at the same time (*Fig. 3*). The patient was readmitted to the hospital one month later with a temperature of 39 C and severe acute respiratory distress. Pneumonia with a mixed gram-negative and gram-positive bacterial flora was present, and his course was marked by deteriorating respiratory function. He died 9 days after admission. At no time during the course of his illness were malignant cells identified in the peripheral blood.

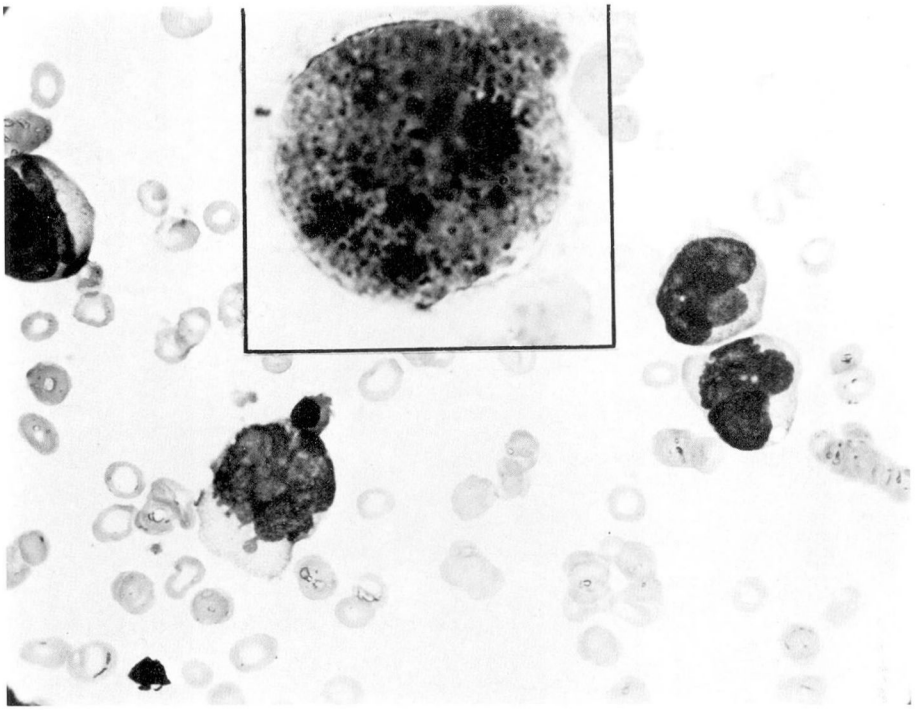

Fig. 2. Bone marrow aspirate specimen obtained concomitantly with the biopsy illustrated in *Figure 3.* Malignant cells are characterized by folded nuclei and prominent nucleolation (Wright's stain, ×640). Individual neoplastic cells demonstrate NaF-resistant alpha naphthyl acetate esterase positivity (inset, ×1600).

Autopsy findings

At autopsy, each pleural cavity contained approximately 500 ml of straw-colored fluid. The lungs were congested and weighed 1630 g. Multiple firm white nodules 0.5 to 1.5 cm in diameter were scattered throughout the visceral pleura. A firm, centrally cavitating gray-white mass measuring 7 x 5 x 2 cm was present below the right clavicle. A firm gray-white spherical nodule was present in the left posterior eighth intercostal space. The liver and spleen were enlarged and congested, but no neoplasm was identified grossly or microscopically in these organs.

Peribronchial, mediastinal, periaortic, and peripancreatic lymph nodes were enlarged with fleshy gray-white nodules grossly visible within them. Numerous round gray-white nodules measuring 0.5 cm in maximum diameter were scattered diffusely throughout the gastric mucosa. The gastrointestinal tract was otherwise normal. A small subdural hematoma was present over the left frontal lobe.

Microscopic examination of lymph nodes, the intercostal mass, gastric mucosa, pancreas, and thyroid gland revealed a nodular infiltrate consisting of small lymphocytes, mature plasma cells, mature histiocytes, and occasional Reed-Sternberg cells diagnostic of nodular sclerosing Hodgkin's disease. This morphologic picture was virtually identical to that seen in the original scalene lymph node biopsy. A morphologically different neoplasm was identified in the bone marrow and in the pleural nodules. The infiltrate in these tissues con-

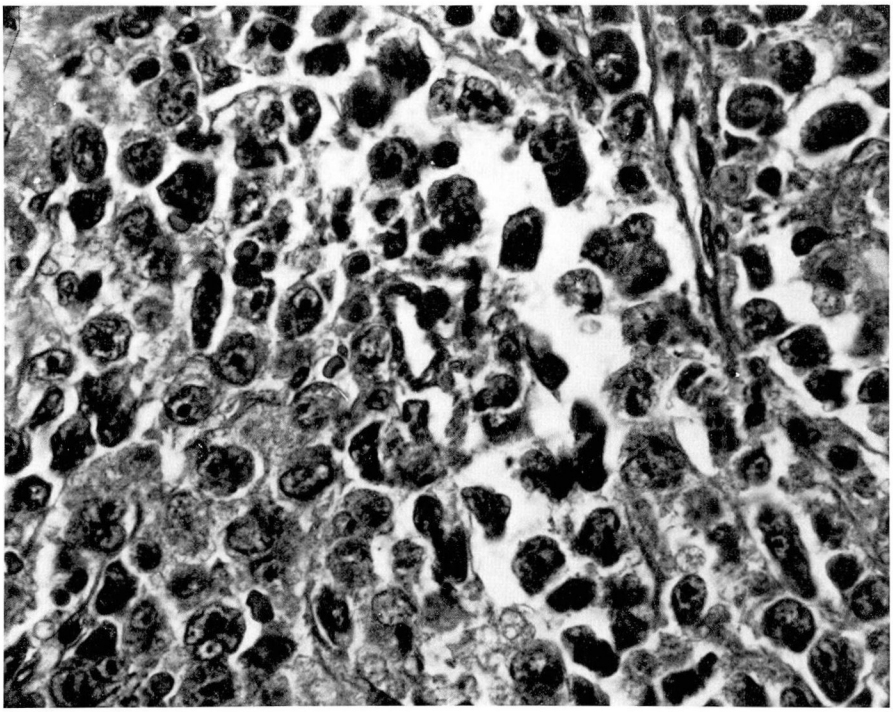

Fig. 3. Marrow biopsy demonstrating infiltration by cytologically malignant mononuclear cells with irregular folded nuclei (hematoxylin and eosin stain, ×640).

sisted of large pleomorphic mononuclear cells with vesicular nuclei and prominent basophilic nucleoli; binucleate and multinucleate variants were also found, but typical Reed-Sternberg cells were not identified.

Materials and methods

Tissues fixed in buffered formalin and in Zenker's solution were stained with hematoxylin and eosin, and methyl-green pyronine. Air-dried bone marrow aspirate preparations were stained with Wright's stain, periodic acid-Schiff (PAS) stain and alpha naphthyl acetate esterase with and without fluoride inhibition.[20, 21]

Cell suspensions were prepared at autopsy from a lymph node showing Hodgkin's disease and from the bone marrow containing the morphologically different neoplasm using a Ficoll-Hypaque gradient. These cells were washed with Hank's solution without calcium and magnesium (Grand Island Biological Company), and consisted principally of viable, cytologically malignant forms. Surface markers were evaluated using techniques previously described.[22] Tissue immunomicroscopy was performed for cytoplasmic immunoglobulins (CIg) using unlabeled[5] peroxidase-antiperoxidase (PAP) and direct techniques,[23] and for cytoplasmic muramidase (CM)[24] using the PAP technique. Controls consisted of the substitution of normal goat or rabbit serum for the primary or conjugated antibody without changes in subsequent steps of the procedures.

Results

Results of comparative studies performed on the two morphologically distinct neoplasms are summarized in the

Table. The tumor cells in the original lymph node biopsy and in autopsy tissue showing Hodgkin's disease were strongly reactive with methyl-green pyronine and contained polyclonal cytoplasmic immunoglobulin identified by both PAP and direct techniques (*Fig. 4A and B*). The cytoplasmic staining for CIg in these cells, although strong, was somewhat less intense and delayed compared to the staining observed in morphologically typical plasma cells in the tissue adjacent to the neoplastic cells. CM was not identified in neoplastic cells in the original or residual Hodgkin's disease. Similar results have previously been reported in Hodgkin's disease.[5, 9]

The neoplastic cells in the bone marrow biopsy obtained in March 1976 were devoid of significant pyroninophilia and contained no cytoplasmic immunoglobulins (*Fig. 5A*), but CM was present. These findings suggest a histiocytic origin.[24, 25] The marrow aspirate performed simultaneously contained cells showing strongly positive cytoplasmic staining for alpha naphthyl acetate esterase; the strength of this reaction was not inhibited by sodium fluoride[21] (*Fig. 2, inset*). These findings also suggest a histiocytic origin. No significant staining with PAS was observed.

The suspension of Hodgkin's tumor cells obtained at autopsy did not form a significant number (less than 5%) of spontaneous rosettes with sheep red blood cells (Srbc). However, 78% of the cells formed rosettes with Srbc coated with antibody and complement (IgM-EAC) (*Fig. 5B*) reflecting a preponderance of complement receptors on the Hodgkin's tumor cells. The malignant cells aspirated from the bone marrow at autopsy formed 9% spontaneous Srbc rosettes and 15% IgMEAC rosettes.

Discussion

The initial concept of either a T-cell or true histiocytic origin for Hodgkin's disease was based on a variety of indirect evidence.[7] Some investigators have suggested that the malignant cells of Hodgkin's disease are of B-cell origin, since they are characterized by cytoplasmic immunoglobulin, receptors for complement components, prominent pyroninophilia in the cytoplasm, many polyribosomes, and a varying amount of cytoplasmic microfibrils.[8–14, 26] However, tissue culture studies of spleen involved by Hodgkin's disease that have shown cell lines morphologically acceptable as Reed-Sternberg cells or their mononuclear variants have met the cri-

Table. Summary of comparative studies

	NSHD	Patient's original neoplasm	Histiocytic malignancies	Patient's subsequent neoplasm
Cytochemistry				
Pyronine	+	+	+	+
α-NAE	V	ND	+	+
Immunocytochemistry				
CIg	+	+	NWE	–
CM	V	–	+	+
Surface markers				
SIg	V	ND	V	ND
IgMEAC	+	+	V	–

NSHD = nodular sclerosing Hodgkin's disease; αNAE = alpha naphthyl acetate esterase; CIg = cytoplasmic immunoglobulin; CM = cytoplasmic muramidase; SIg = surface immunoglobulin; IgMEAC = immunoadherence study using sheep red blood cells coated with rabbit IgM and guinea pig complement; V = variable; ND = not done; NWE = not well established.

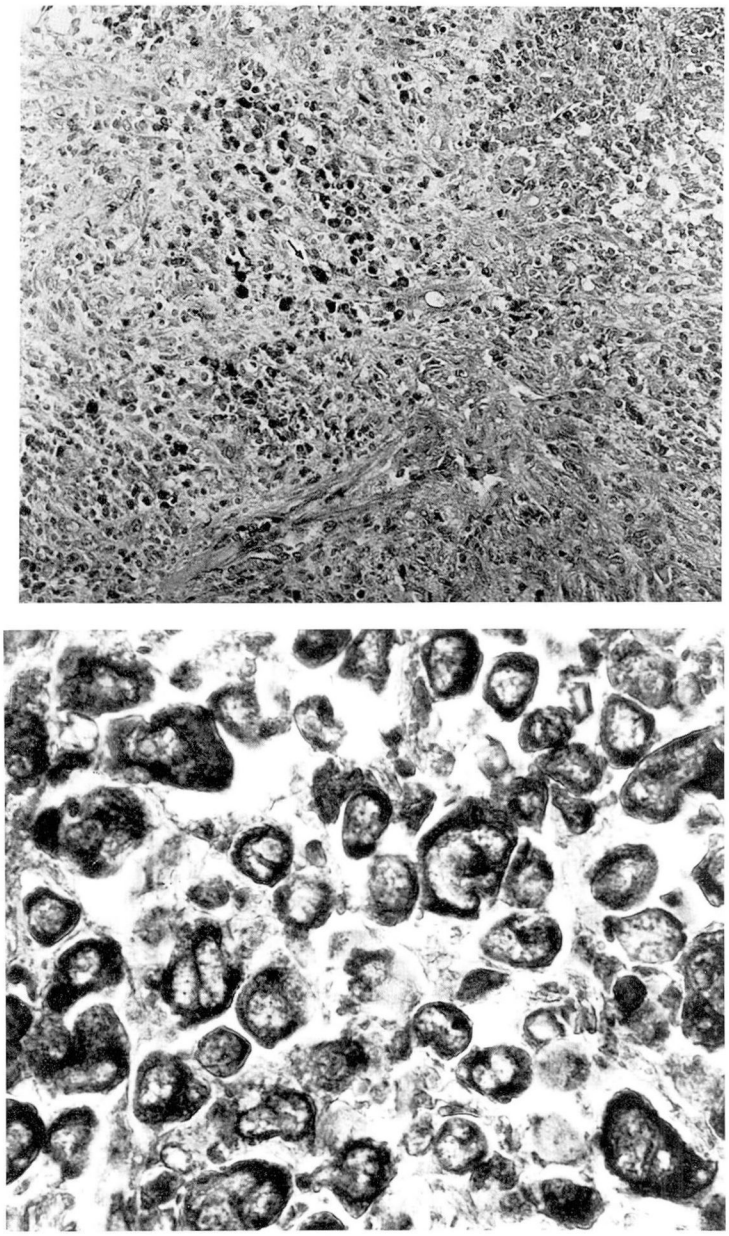

Fig. 4. A, (*top*) Comparative immunomicroscopy of the original node involved with Hodgkin's disease. The malignant mononuclear cells of the original neoplasm contain cytoplasmic immunoglobulin, identified as black staining of the cytoplasm of these cells (×160). **B,** (*bottom*) Cytoplasmic immunoglobulin present within the residual Hodgkin's tumor cells (direct technique using anti-IgG peroxidase conjugate, ×640).

teria for histiocytes.[15, 16] The CIg and SIg present in the malignant cells of Hodgkin's disease are not endogenous and probably are a consequence of phagocytic activity.[17] Other investigators have suggested that the presence of CIg in Reed-Sternberg cells is the result of a defective cell membrane.[27] Both

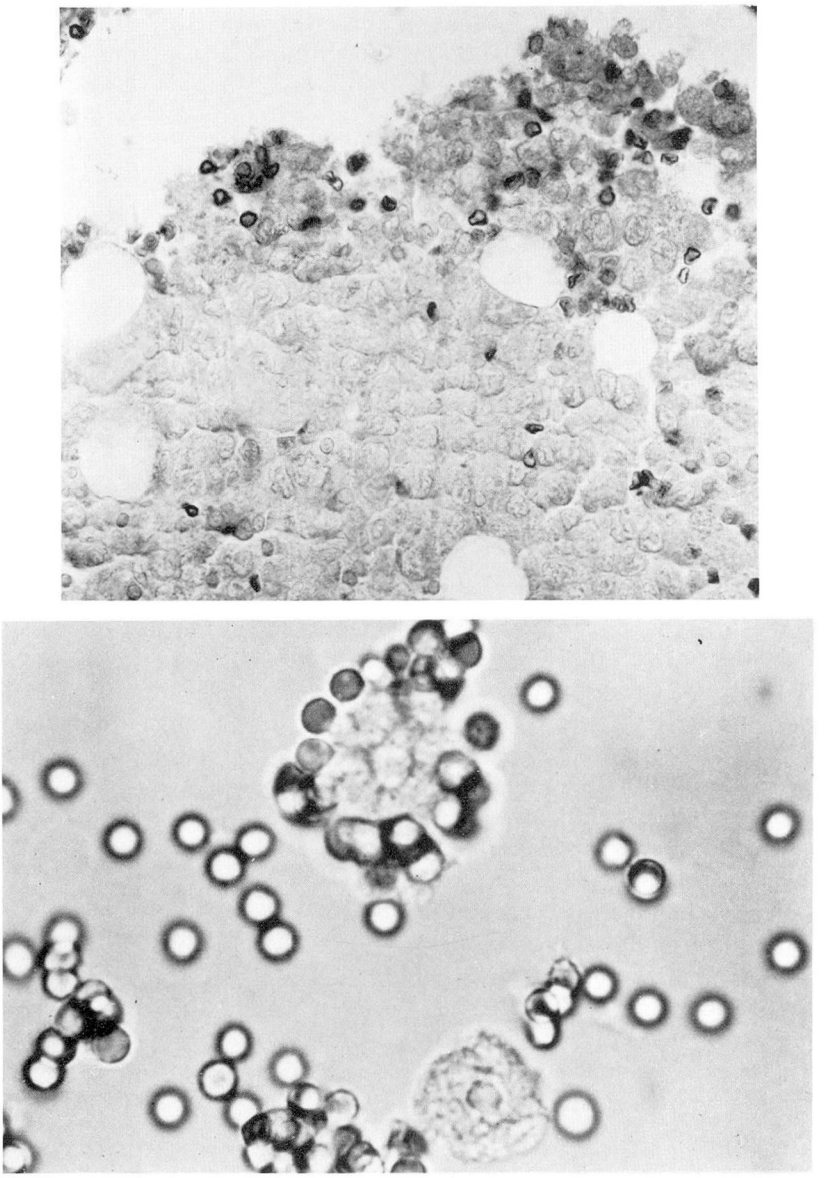

Fig. 5. A, (*top*) The tumor cells in the bone marrow do not have cytoplasmic immunoglobulin; red blood cells are stained due to the pseudoperoxidase activity of hemoglobin (×400). **B,** (*bottom*) IgMEAC rosette formed by a nucleolated tumor cell in the cell suspension prepared from residual Hodgkin's disease at biopsy (×640).

these findings suggest an exogenous origin for the CIg in the malignant cells of Hodgkin's disease.

The cytochemical and immunomicroscopic marker studies of the neoplastic cells from various sites in this case do not resolve the question of the ultimate origin of the malignant cell in Hodgkin's disease. However, they do suggest that the malignant mononuclear cells in the scalene lymph node were different from those in the bone marrow. Differences

were also demonstrable between different sites of tumor in the autopsy specimens. Cytoplasmic immunoglobulin was detected in the original and residual Hodgkin's disease tissue, but not in the bone marrow biopsy specimen; the reverse was true for cytoplasmic muramidase. The neoplastic cells in the bone marrow were positive for fluoride-resistant alpha naphthyl acetate esterase, a cytochemical marker expressed by phagocytic histiocytes and epitheloid cells.[20, 21, 28] Results reported for this enzyme in Reed-Sternberg cells in touch preparations[20, 28] and in tissue culture[15, 16] have been variable. Differences in the distribution of CIg and SIg in fresh cell suspensions from Hodgkin's disease have been documented previously.[17] However, we are unaware of any previous demonstration of the sequential variability in cytochemical and immunologic parameters seen in this patient.

We propose that our observations, as well as those of others,[15–17] indicate that the evolution of treated Hodgkin's disease may involve a change in the cytochemical and immunologic characteristics of the neoplasm, possibly reflecting a selective effect of treatment on varying cell types within the original neoplasm. Myelomonocytic leukemia is a known complication in patients who have received intensive chemotherapy and radiotherapy; heretofore such leukemias have been considered different neoplasms induced by therapy. This study suggests that development of leukemias with monocytic characteristics in patients with Hodgkin's disease may reflect a naturally occurring or therapy-induced overgrowth of a histiocytic component which may form part of the original neoplastic population in Hodgkin's disease. Further sequential surface marker and cytochemical studies are in progress in this laboratory and in other institutions to determine if indeed such a phenomenon does occur.

Summary

Investigators have suggested either a B-lymphocytic or a histiocytic origin for the malignant cell in Hodgkin's disease. The results of cytologic, cytochemical, and immunologic studies of two morphologically different neoplasms encountered sequentially in a patient with nodular sclerosing Hodgkin's disease are reported. These results could suggest either B-lymphocytic or histiocytic origin for cytologically malignant cells in different sites. However, these results probably reflect differential expression of properties possessed by all Reed-Sternberg cells. These differences may have been induced by therapy. Thus, monocytic leukemia arising in a post-therapeutic setting may represent an overgrowth of one component of a single neoplasm rather than a new malignancy.

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