Ultrastructural Identification of Neoplastic Histiocytes–Monocytes

An Application of a Newly Developed Cytochemical Technique

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We applied a newly developed ultrastructural cytochemical technique utilizing 2-naphthyl thiol acetate (NTA) as a substrate in 3 cases of true histiocytic–monocytic malignancy and 3 controls. The enzyme NTA esterase was identified in both neoplastic and benign histiocytes–monocytes in the form of electron-dense material within the cytoplasm. The NTA esterase varied in size and shape and showed no clear relationship with cytoplasmic organelles. The distribution and pattern of staining were very similar to those of the light-microscopically demonstrated α-naphthyl acetate esterase reaction. The main advantages of this method are: 1) histiocytes–monocytes can be identified in the absence of lysosomes or phagocytosis; 2) unequivocal, simultaneous documentation of both histiocytic esterase and subcellular structure is possible; and 3) both blood and solid tissue specimens can be utilized. Our findings demonstrate the usefulness and applicability of ultrastructurally demonstrated NTA esterase in the study of histiocytic–monocytic malignancies. (Am J Pathol 1982, 106:204–223)

MALIGNANT LYMPHOMAS and leukemias have traditionally been classified on the basis of the light-microscopic appearance and cytochemical findings of the neoplastic cells. Progress in immunology, cytochemistry, and transmission electron microscopy has added further insight into the field of malignant lymphomas, and it is now widely accepted that the majority of the malignant lymphomas designated as "histiocytic" by the Rappaport classification[1] are in fact composed of large transformed lymphoid cells.[2–7] Terms employed for these large-cell lymphomas include *immunoblastic sarcoma*,[3–5,8,9] *centroblastic lymphoma*,[9] and *large cleaved* and *large noncleaved follicular center cell lymphoma*.[3,4] The term histiocytic lymphoma has been excluded from some classifications of non-Hodgkin's lymphomas[8,9] but was retained in the classification of Lukes and Collins.[3–5] A review of the literature on the immunologic identification of so-called histiocytic lymphomas shows that the majority of these tumors are either B- or T-cell lymphomas, and that only a small number are truly histiocytic in nature. In the remainder, surface markers could not be demonstrated with prevailing methods.[3–5,7,10–12] It appears to be difficult to identify true histiocytic or monocytic malignancy on the basis of light-microscopic examination only. The identification of the histiomonocytic origin of neoplastic cells depends on the demonstration of nonspecific esterase in imprints or smears, or on elaborate functional marker studies.[13–19] Transmission electron microscopy has also been employed for the study of histiomonocytic neo-

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plasms.[13,15-17,19,20] It is our purpose in this study to show that true histiocytic-monocytic malignancy can be identified, even in the absence of the usual morphologic markers such as lysosomal granules or phagocytic activity, by an appropriate ultrastructural cytochemical method that was recently developed in our laboratory.[21]

Materials and Methods

In order to illustrate and document the ultrastructural and cytochemical findings in the neoplastic histiomonocytic cells, we obtained material from 2 patients with malignant histiocytosis[1,7,15,17] and from 1 patient with acute monoblastic leukemia. In one of the 2 patients with malignant histiocytosis, the ultrastructural studies were performed on material obtained directly from a fresh lymph node biopsy. In the other, we used an established cell line described below. A reactive lymph node showing lymphangiogram effects, a hyperplastic tonsil, and a malignant lymphoma of the undifferentiated non-Burkitt type (small noncleaved follicular center cell type of Lukes and Collins[3-5]) were used as controls. Details of the materials, diagnoses, and studies performed are listed in Table 1.

Histiocyte Cell Line

We established this cell line by placing mononuclear cells from the heparinized bone marrow of Patient 2 into tissue culture medium RPMI-1640 with 10% fetal calf serum. The cells have been passaged continuously since March 1978 (ie, for 32 months). They grow in a biphasic pattern, both attached to the bottom of the tissue culture flask as well as in suspension. The properties of the attached and the freely floating cells appear to be identical. Cytogenetic studies have demonstrated that the cells are aneuploid, with marker chromosomes. Studies of the cells in soft agar culture studies have shown the capacity of the tumor cells to form individual colonies or clones in that medium.[22]

Light Microscopy

In addition to using the routine stains, we stained touch imprints and blood smears of all specimens and cryostat sections of the fresh-frozen tissue for various cytochemical enzymes as listed in Table 1.

Transmission Electron Microscopy

The procedure was identical to that published previously.[23]

Ultrastructural Cytochemistry

The procedure for ultrastructural cytochemistry was also identical to our previously published method.[21]

I. Fixation

Lymph Node, Jejunum, and Adenoid: The specimens were cut with a surgical blade into 0.1-cm cubes and were placed in a fixative solution of 2.5% glutaraldehyde and 2.0% paraformaldehyde buffered to pH 7.4 with 0.1 M sodium cacodylate. The tissue was kept in the fixative for 4 hours at 4 C. It was then placed in cold 0.1 M cacodylate buffer (pH 7.4) and stored at 4 C.

Blood and Histiocyte Cell Line: Approximately 10 ml of blood was collected in ethylene diamine tetraacetic acid (EDTA). The blood was spun at 1500 rpm for seven minutes, and the serum was removed for preparation of the buffy coat. The buffy coat and the histiocytes were then fixed in a solution of 2.5% glutaraldehyde and 2.0% paraformaldehyde buffered with 0.1 M phosphate (pH 7.4) for 1 hour at 4 C. They were then placed in the same buffer for a minimum of 4 hours at 4 C until used.

II. Incubation

Preparation of Incubation Mediums: 2-Naphthylthiol acetate (NTA) (Polysciences, Inc., Warrington, Pa) was used as the substrate and Fast Blue BB salt (Sigma Chemical Company, St. Louis, Mo) as the coupling agent. The incubation medium was prepared as follows: 1) 6 mg of NTA was dissolved in 1 ml of acetone with constant stirring; 2) 49 ml of 0.1 M phosphate buffer (pH 7.4) was then added, producing a white cloudy solution; 3) 100 mg of Fast Blue BB salt was added last with stirring; 4) the medium was filtered through a Seitz bacteriologic filter and used immediately.

Incubation Procedure: The 0.1 cm cubes of lymph node, jejunum, and adenoid tissue were further minced into smaller pieces and washed three times in cacodylate buffer. Three to four pieces of finely minced tissue were placed in 5 ml of incubation medium and incubated at room temperature for an average of 6 hours. The buffy coat was processed in a similar manner; however, the incubation time was reduced to 2 hours.

After incubation, the specimen was washed twice with 0.1 M cacodylate buffer and placed in 1% unbuffered OsO_4 solution. The specimens were kept in the osmium solution for 6 hours at 37 C.

Table 1—Materials, Diagnoses, and Studies Performed

Patient Age, Sex	Material	Diagnosis	Light-microscopic cytochemistry	Ultrastructure TEM	Ultrastructure NTA
1 36, M	Axillary LN	Malignant histiocytosis	IP, αNA, NASDA, AP	No	No
	Supraclavicular LN*	Malignant histiocytosis	IP, αNA, NASDA, AP NCA, SBB, Px	Yes	Yes
2 61, M	Histiocyte cell line	Malignant histiocytosis	αNA, NASDA, AP, NCA, SBB, Px	Yes	Yes
3 29, M	Blood	Acute monoblastic leukemia	αNA, NASDA, AP, NCA, SBB, Px	Yes	Yes
	Bone marrow	Acute monoblastic leukemia	αNA, AP, NCA, SBB, Px	No	No
Control 1	Obturator LN*	Lymphangiogran effect	αNA, NASDA, AP, NCA	Yes	Yes
Control 2	Adenoid*	Reactive follicular hyperplasia	αNA, NASDA, AP, NCA	Yes	Yes
Control 3	Jejunum	Malignant lymphoma, undifferentiated, non-Burkitt's type	αNA, NASDA, AP, NCA	Yes	Yes

Abbreviations: IP = immunoperoxidase stain for immunoglobulin heavy chains and light chains and muramidase; αNA = α-naphthyl acetate esterase; NASDA = naphthol-AS-D-acetate esterase with and without NaF; AP = acid phosphatase; NCA = naphthol-AS-D-chloroacetate esterase; SBB = Sudan black B; Px = peroxidase; TEM = transmission electron microscopy; NTA = ultrastructural cytochemistry for 2-naphthylthiol acetate esterase.

* Cytochemical examination was performed on both cryostat sections and touch imprints of tumor. In the remainder, the cytochemical examination was done on smears or touch imprints only.

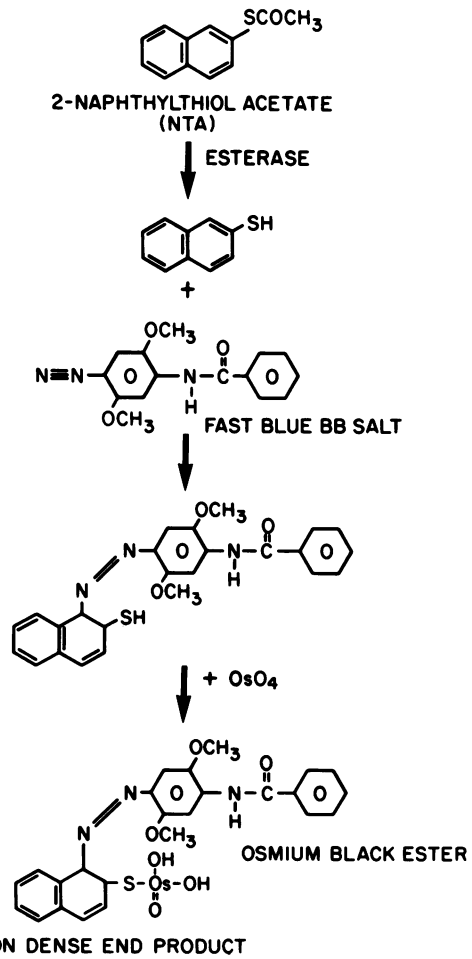

Figure 1—Reaction pathway of the 2-naphthylthiol acetate esterase reaction.

III. Dehydration and Embedding

After the capturing step with OsO_4 was completed, the specimen was dehydrated through a graded series of alcohol (50–100%) and passed twice through 100% propylene oxide. Finally, the specimen was placed in a one-to-one mixture of propylene oxide and Spurr's Epon for 8 hours. The tissue was then embedded in Spurr's Epon. One-micron sections and thin (650 Å) sections were prepared on an LKB Ultramicrotome. One-micron sections were stained with 1% aqueous toluidine blue solution.

The reaction pathway of the enzyme reaction described above is shown in Figure 1.

IV. After Staining and Examination

On all specimens, half of the thin sections thus prepared were stained with uranyl acetate and lead citrate. The remainder were examined unstained with a Philips 301 electron microscope.

Results

Light-Microscopic Observations

Malignant Histiocytosis

Both axillary and supraclavicular lymph nodes from Patient 1 showed a proliferation of neoplastic cells with a striking predilection for the lymph node sinuses (Figure 2a). The neoplastic cells ranged from 16 to 40 μ in diameter and had an abundant, lightly eosinophilic to amphophilic cytoplasm. The nuclei

were relatively small, and nucleoli generally were not prominent. Mitoses were frequent (Figure 2b). In imprint preparations, some of the nuclei were folded or lobated (Figure 2c). Erythrophagocytosis by the neoplastic cells was not evident in these sections. Methyl green pyronin stains showed varying degrees of cytoplasmic pyroninophilia. Immunoperoxidase (IP) stain for lysozyme was positive for neoplastic cells. Cytochemical findings included sodium fluoride-sensitive, α-naphthyl acetate (αNA) and naphthol AS-D acetate (NASDA) esterase positivity. Acid phosphatase (AP) was equally positive. On the other hand, the neoplastic cells were negative for naphthol AS-D chloroacetate (NCA) esterase, Sudan black B (SBB), and peroxidase (Px) (Table 2).

In Patient 2 with malignant histiocytosis, the histiocytic cell line was derived from the bone marrow. The bone marrow biopsy was normocellular. Several discrete foci of malignant cells were detected. The malignant cells were present in loosely cohesive groups, with individual cells having approximately the size of megakaryoctyes. Their abundant cytoplasm was amphophilic. A minority of the cells contained intact erythrocytes or erythrocyte fragments. Their large nuclei were pleomorphic and had one or more prominent nucleoli.

The cellular bone marrow aspirate contained adequate numbers of megakaryocytes. The myeloid-to-erythroid ratio was approximately 2.5:1, with normal maturation of both series. There was a slight increase in mature plasma cells. The most noteworthy finding was the presence of discrete groups of 3 to 20 or more tumor cells that had abundant basophilic, often vacuolated, cytoplasm. Phagocytized platelets and/or erythrocytes were found in a minority of the tumor cells (Figure 3). They had one and occasionally two or more large nuclei with prominent basophilic nucleoli. Mitotic figures were frequent.

The cytocentrifuge preparation of the cell line established from this bone marrow revealed a pure culture of neoplastic cells. These were very similar in appearance to the neoplastic cells noted in the bone marrow and were characterized by abundant basophilic, often vacuolated cytoplasms. The Golgi apparatus was prominent. These cells were often binucleated or multinucleated.

Cytochemical findings on this cell line were identical to those of Patient 1, except that the NASDA reaction was only of moderate intensity (Table 2).

Acute Monoblastic Leukemia

The bone marrow of Patient 3 was almost totally replaced by blast cells having moderate to abundant pale blue cytoplasm and round to ovoid nuclei. Some

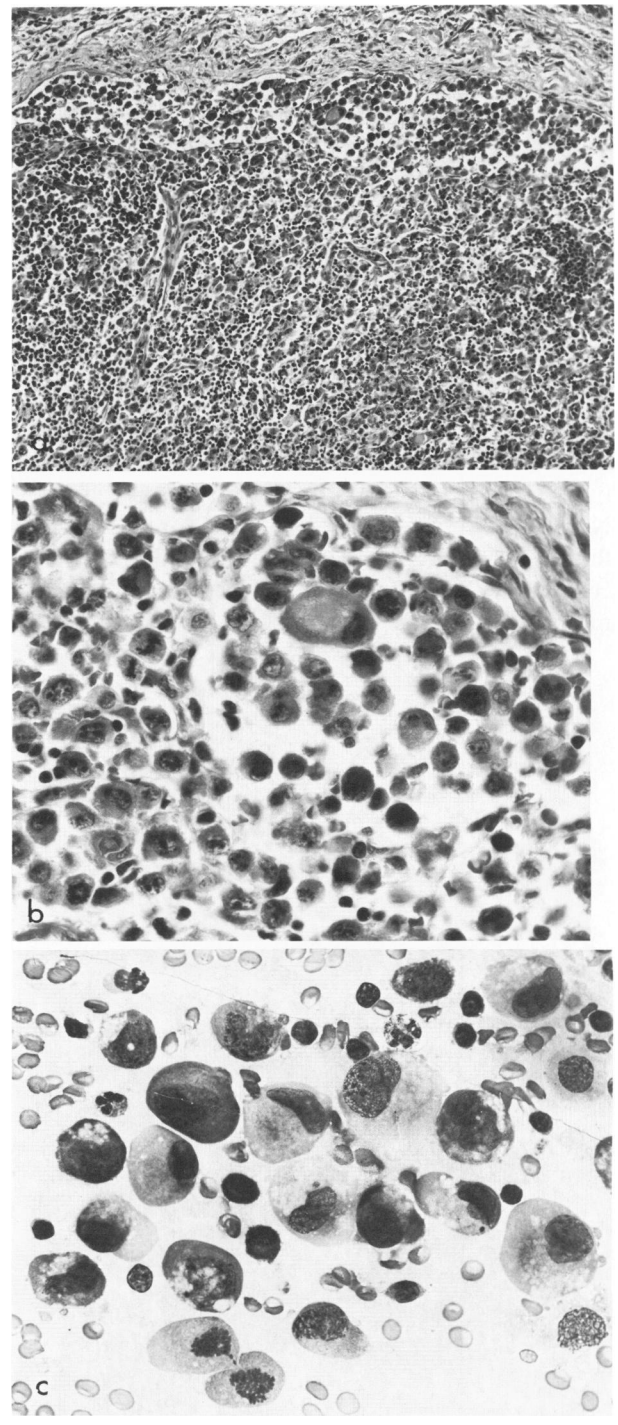

Figure 2—Supraclavicular lymph node involved by malignant histiocytosis; Patient 1. **a**—Proliferation of neoplastic cells in the lymph node sinus. (H&E, ×120) **b**—Higher magnification showing the details of the neoplastic cells. (H&E, ×480) **c**—The touch imprint preparation. Note nuclear folding and lobation as in monocytes. (MGG, ×480) (With a photographic reduction of 34%)

Table 2—Materials, Diagnoses, and Cytochemical Findings

Patient Age, Sex	Material	Diagnosis	Light-microscopic cytochemistry αNA	NASDA	NASDA-NaF	AP	NCA	SBB	Px	Ultrastructure NTA
1 36, M	Axillary LN	MH	+ + +	+ + +	+	+ + +	0	0	0	0
	Supraclavicular LN*	MH	+ + + †	+ + +	+	+ + +	–	–	–	+ + +
2 61, M	Histiocyte cell line	MH	+ + + †	+ +	+	+ + +	0	0	0	+ +
3 37, M	Blood	AMobL	+ + +	+ + +	+	+ + +	+ +	–	–	+ + +
	Bone marrow	AMobL	+ + +	+ + +	+	+ + +	+ +	–	–	0

Abbreviations: αNA = α-naphthyl acetate esterase; NASDA = naphthol-AS-D-acetate esterase with and without NaF; AP = acid phosphatase; NCA = naphthol-AS-D-chloroacetate esterase; SBB = Sudan black B; Px = peroxidase; NTA = ultrastructural cytochemistry for 2-naphthylthiol acetate esterase. 0 = not tested; – = negative reaction; + = weak reaction; + + = moderate reaction; + + + = strong reaction. Degree of reaction is based on neoplastic cells.

* Cytochemistry was performed on both cryostat sections and touch imprints of tumor. In the remainder, the cytochemistry was done on smears or touch imprints only.

† Completely inhibited by NaF.

of the nuclei were folded and lobated. Cytochemically, these cells showed strong positivity for αNA and NASDA esterases, both of which were sodium-fluoride-sensitive. The cytoplasm of the neoplastic cells also had abundant AP. The cytoplasm was moderately positive for NCA esterase, whereas a negative reaction was noted with SBB and Px. Most of the white blood cells in circulation had identical morphologic and cytochemical findings (Table 2).

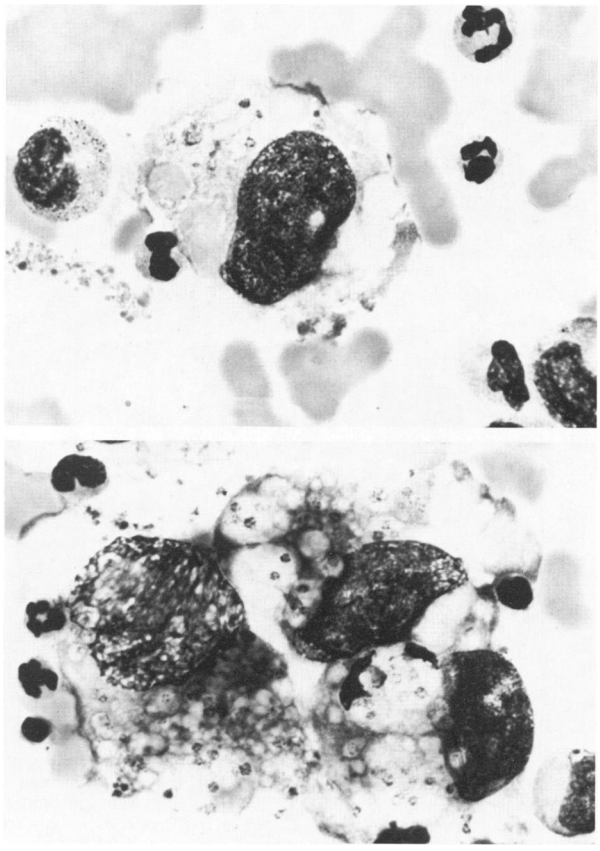

Figure 3—Aspirate of bone marrow involved by malignant histiocytosis from Patient 2. Phagocytized platelets and erythrocytes are present in neoplastic histiocytes. (MGG, ×860) (With a photographic reduction of 5%)

Controls

An obturator lymph node (Control 1) showed a lymphangiogram effect. The adenoids (Control 2) showed only reactive follicular hyperplasia, without any other significant pathologic findings. Histiocytes of the lymph node sinuses in Control 1 and germinal centers in Control 2 showed strong sodium-fluoride-sensitive αNA and NASDA positivity. They were also positive for AP, whereas NCA was negative. A small number of lymphocytes in the paracortical area of the obturator lymph node and some of the lymphocytes in touch imprints of the reactive adenoid showed rare dot-like positivity for αNA esterase.

Sections of the jejunum (Control 3) showed a diffuse, transmural infiltrate of intermediate-sized neoplastic cells that were characterized by noncleaved nuclei often having multiple, small nucleoli. There was a small to moderate amount of amphophilic to basophilic cytoplasm. Mitoses were numerous, and many tingible body macrophages were evident among the neoplastic cells. Special stains for αNA esterase, AP, and cytoplasmic immunoglobulin by IP were all negative. There was a weak reaction for NASDA esterase that was resistant to prior treatment with sodium fluoride. Special stains for NCA, SBB, and Px were also negative.

Ultrastructural Observations

The following observations are based on both regular transmission electron-microscopic (TEM) and ultrastructural NTA cytochemical examination. As mentioned in Materials and Methods, half the thin

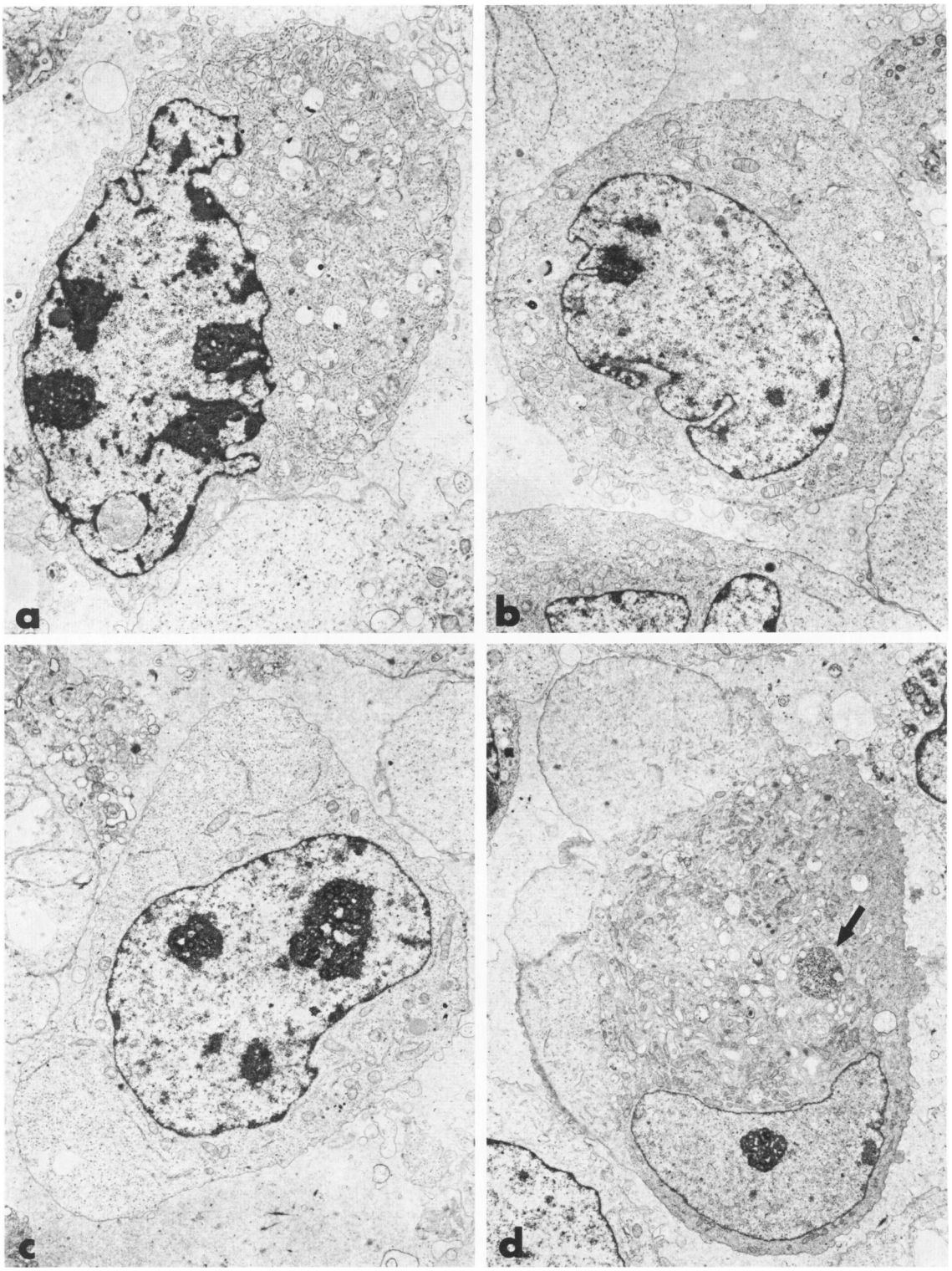

Figure 4—Representative neoplastic cells from supraclavicular lymph node involved by malignant histiocytosis. Patient 1. The cell shows (**a**) many swollen mitochondria with ruptured cristae; these are much more numerous than in cells **b**, **c**, and **d**. The nuclei and cytoplasm of these neoplastic cells show considerable variation in size, shape, and contour. None of these cells is characterized by readily demonstrable lysosomes, although a few membrane-bound dense bodies are present in cells **c** and **d**. Cell **d**, in addition, shows a large dense body, probably a lysosome (*arrow*), and a peculiar cytoplasmic "bleb" (see text). (TEM, uranyl acetate and lead citrate; **a** and **c**, ×3700; **b**, ×4100; **d**, ×3400)

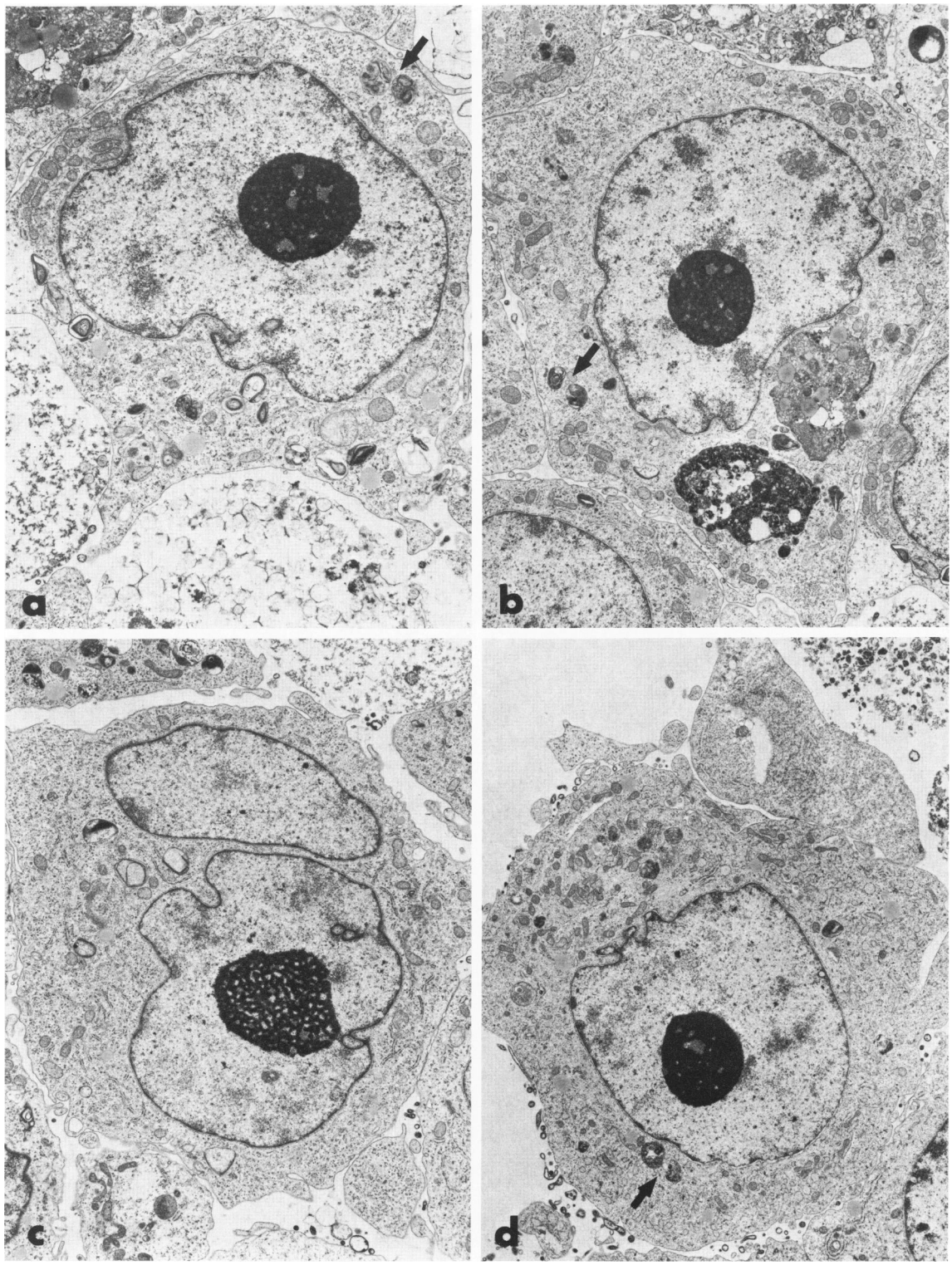

Figure 5—Histiocytic cell line derived from bone marrow of Patient 2. These representative cultured neoplastic cells reveal scattered lysosomes (*arrow*) and phagocytized material (**b**). All four cells (**a–d**) contain lipid droplets and a prominent nucleolus. The one illustrated in cell **c** is particularly atypical and shows a reticulated nucleolonema. (TEM, uranyl acetate and lead citrate; **a**, ×6500; **b** and **c**, ×6000; **d**, ×4500)

sections were stained with uranylacetate and lead citrate, whereas the remainder were examined unstained for the demonstration of NTA esterase. The poststaining with uranylacetate and lead citrate made nuclear cytoplasmic details more clear and did not interfere with the interpretation of the positive reaction.

Malignant Histiocytosis

On routine TEM examination, the neoplastic cells from Patient 1 (Figure 4) were characterized by polymorphism, with great variations in the number, type, and distribution of cytoplasmic organelles. Some of the mitochondria were markedly swollen, with ruptured cristae (Figure 4a). Some of the cells had a high content of cytoplasmic fat (not illustrated). There were considerable variations in ribosomal content. Cytoplasmic outlines were irregular, with frequent bulbous or villous projections. Of particular interest was the presence of peculiar cytoplasmic "blebs," which had electron-lucent cytoplasm with few or absent cytoplasmic organelles (Figure 4d). No phagocytic activity by the neoplastic cells was noted. The nuclei were polymorphous and had 1–4 nucleoli, which varied in size and shape. Multivesicular bodies were rarely observed in the neoplastic cells. Other forms of membrane-bound dense bodies were absent in most of them; if present, they were few in number. The TEM findings in the true histiocytic cell line from Patient 2 were similar. However, lysosomes were observed more frequently (Figure 5), and rare phagocytic activity was noted (Figure 5b). Nucleoli tended to be somewhat more prominent.

In ultrastructural cytochemistry studies, NTA esterase was found in most of the neoplastic cells (Figure 6) from both Patients 1 and 2. In 1-μ sections, the positivity was characterized by granular black precipitates of varying sizes and shapes in the cytoplasm. Most of the reaction product was unrelated to the cytoplasmic organelles such as the endoplasmic reticulum, mitochondria, or membrane-bound dense bodies (Figures 7 and 8). The reaction product was more electron-dense than cytoplasmic fat (Figure 8). The reaction product, however, could be demonstrated at the peripheral margins in some of the multivesicular bodies. The quantity of the reaction product was less in the neoplastic histiocytes than in the benign macrophages. Nevertheless, they were much more abundant than the membrane-bound dense bodies, which were sparse, especially in the material from Patient 1. In the neoplastic cells, the end products were heavily concentrated in the electron-dense portion of the cytoplasm; the electron-lucent "blebs" mentioned previously contained very few or no end products (Figure 6). Rare lymphocytes and plasma cells among the neoplastic cells showed a few scattered dotlike granular reaction products.

Acute Monoblastic Leukemia

Neoplastic monoblasts in the blood from Patient 3 showed scattered membrane-bound dense granules in the cytoplasm, some with crystalline linear densities (Figure 9). They also had smooth endoplasmic reticulum and mitochondria. Intracytoplasmic fibrils were frequently observed, especially around nuclei.

In ultrastructural cytochemistry studies, the neoplastic monoblasts showed a high cytoplasmic NTA-esterase content (Figure 10), which was inhibited entirely by the addition of sodium fluoride. The end products were randomly scattered throughout the cytoplasm, without any relationship to the membrane-bound dense core granules, although some end products were seen overlying such dense bodies (Figure 10c and d). As in benign and neoplastic histiocytes, the end products varied in size and shape. The number of end products noted by ultrastructural cytochemical examination far exceeded the number of membrane-bound dense granules identified by TEM examination.

The results of the ultrastructural NTA study on the materials from these 3 patients are listed in Table 2.

Controls

The obturator lymph node from Control 1 showed a lymphangiogram effect on light-microscopic examination. Ultrastructurally, the cytoplasm of the histiocytes in the sinuses was almost completely filled with NTA esterase granules (Figure 11). In contrast, most of the lymphocytes in the adjacent paracortical areas were devoid of such granules. Only a small number of lymphocytes showed a positive reaction and usually contained a single, round, electron-dense end product.

The reactive follicles of the hyperplastic adenoid from Control 2 showed similar results (Figure 12). The findings in the macrophages of the germinal centers were identical to those in the histiocytes in Control 1. In contrast, the lymphocytes in these germinal centers were devoid of NTA esterase (Figures 12 and 13).

Most of the neoplastic cells in the tumor of the jejunum from Control 3 were characterized by round to ovoid nuclei with slight nuclear indentations. Most nuclei had one nucleolus, but a few had multiple nucleoli. They had a small amount of cytoplasm characterized by a high content of ribosomes, few strands of rough endoplasmic reticulum, and

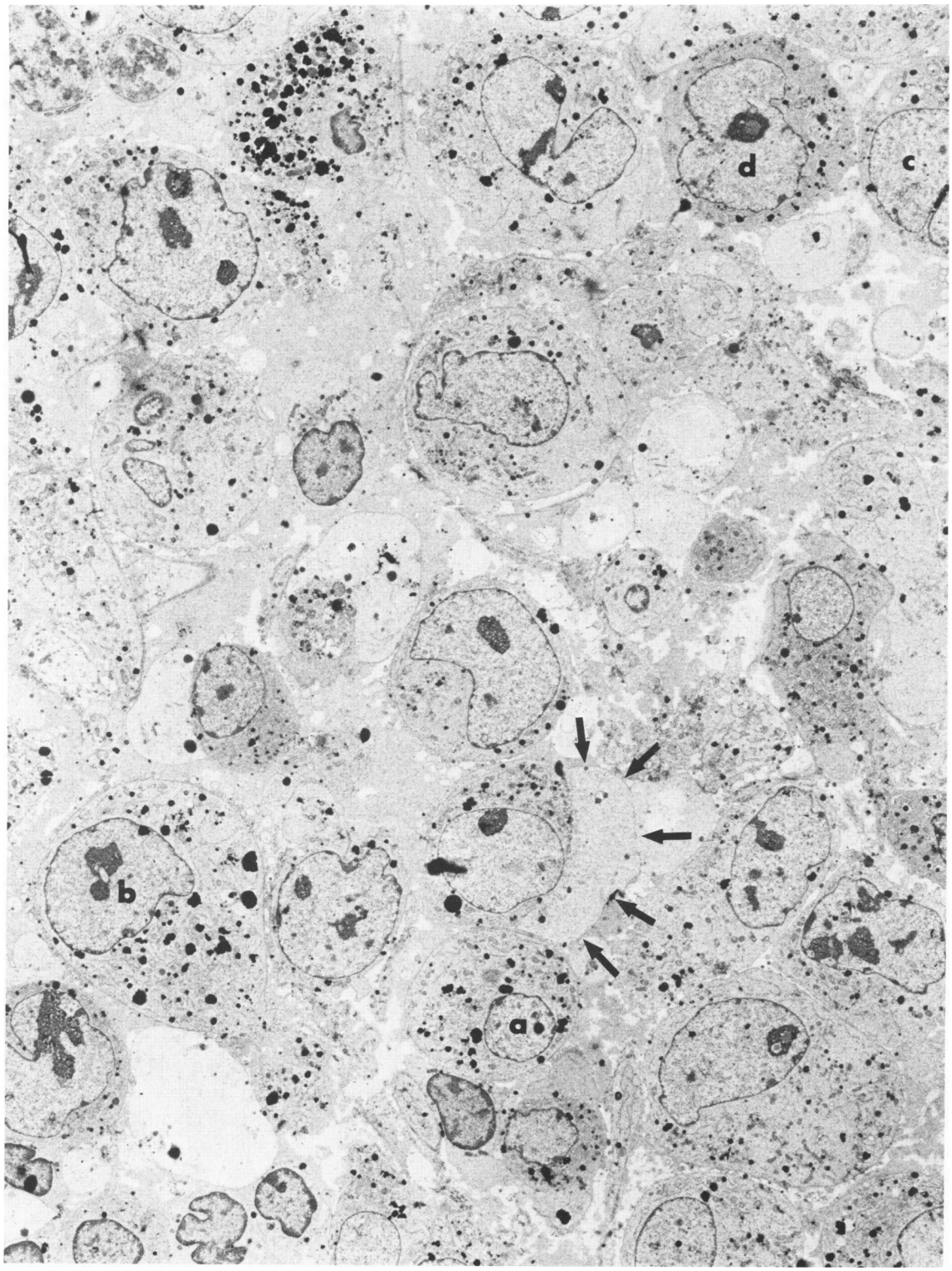

Figure 6—Supraclavicular lymph node involved by malignant histiocytosis; Patient 1. Ultrastructural cytochemistry for NTA esterase applied to the lymph node illustrated in Figure 2. All of the neoplastic cells contain electron-dense reaction products representing NTA esterase. The cytoplasmic "bleb" shows little NTA esterase, compared with the rest of the cytoplasm (*arrows*). Cells **a–d** are shown at higher magnification in Figure 7. (NTA, uranyl acetate and lead citrate, ×2000)

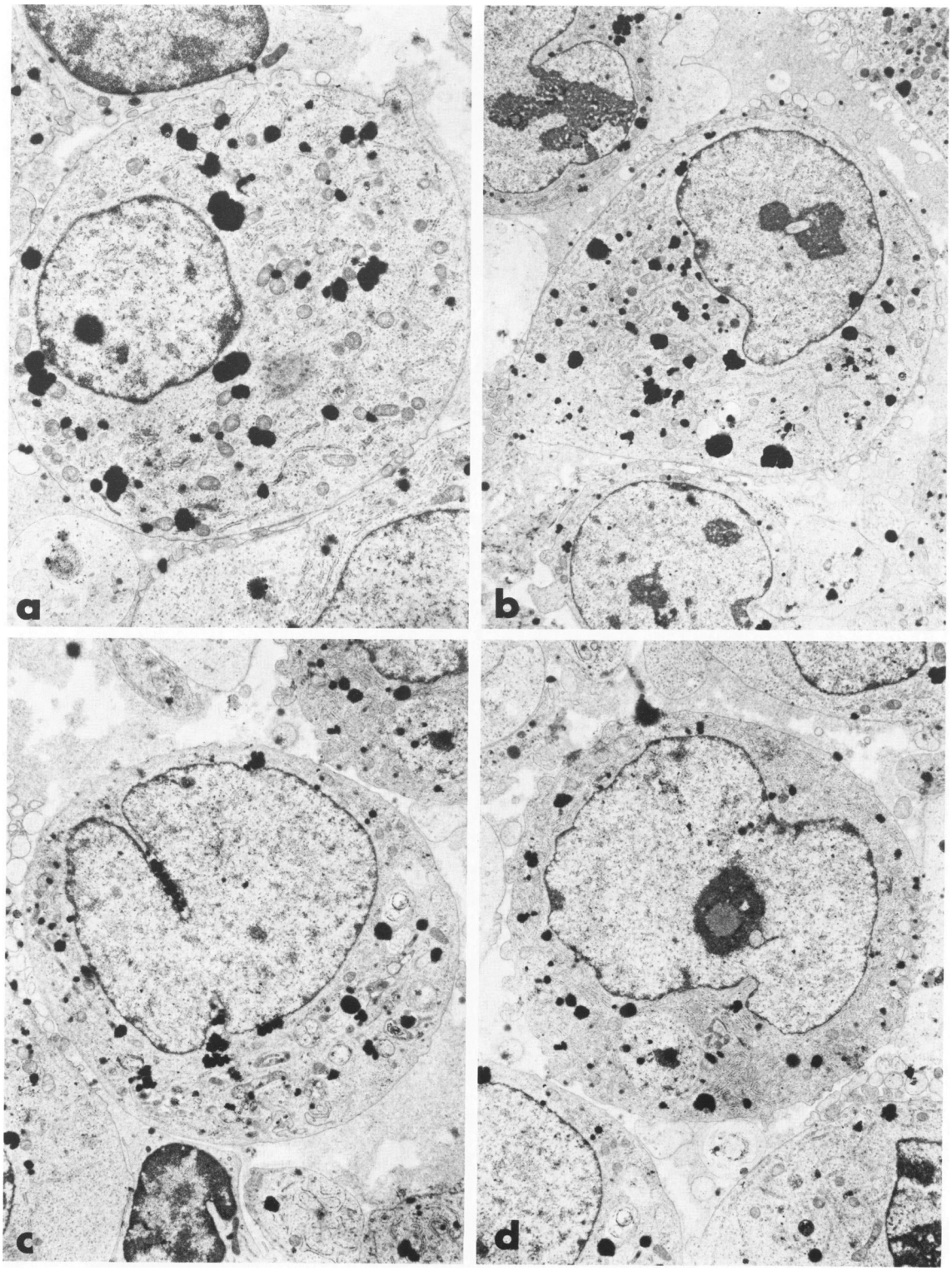

Figure 7—Representative neoplastic cells from Figure 6 at higher magnification (Patient 1). Distribution of NTA esterase, represented by electron-dense irregular clumps, is random without any relationship to cytoplasmic organelles (NTA, uranyl acetate and lead citrate; **a**, **c**, and **d**, ×4100; **b**, ×3000)

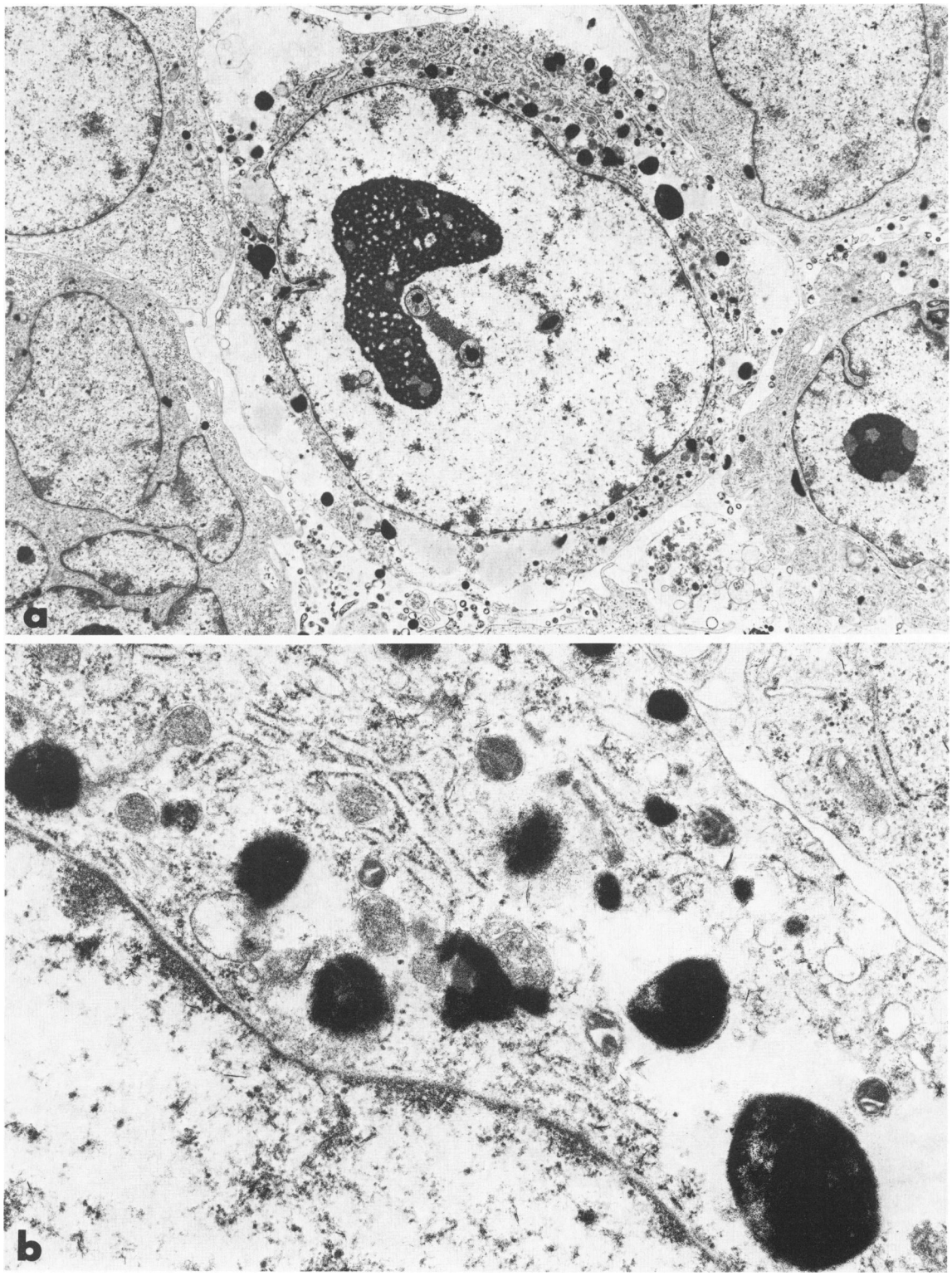

Figure 8—Histiocytic cell line derived from bone marrow of Patient 2. **a**—Representative neoplastic histiocyte showing a large amount of electron-dense NTA esterase. (NTA, uranyl acetate and lead citrate, ×5000) **b**—Detail of the reaction products. (×30,000)

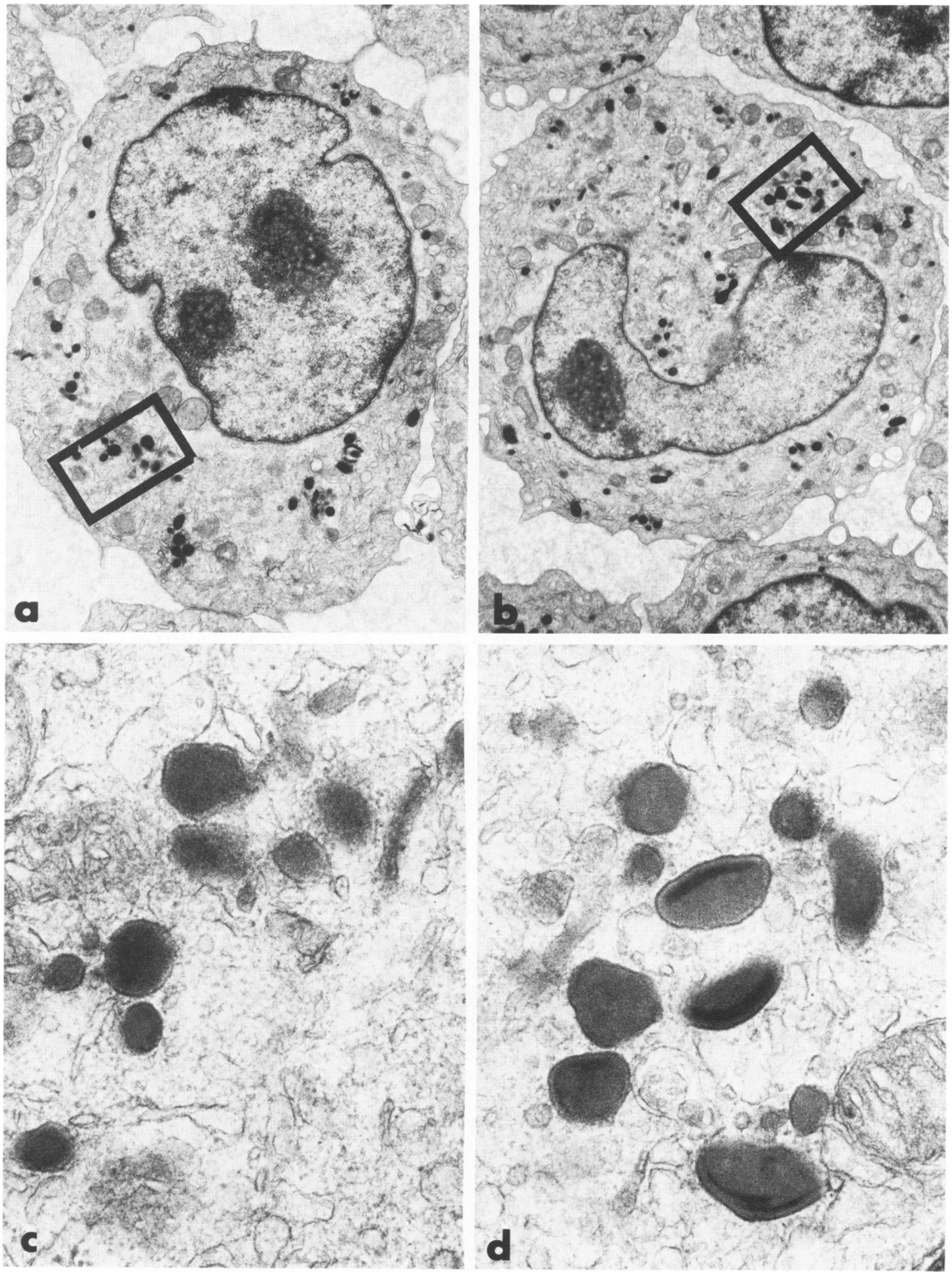

Figure 9—Acute monoblastic leukemia; peripheral blood from Patient 3. **a** and **b**—Two representative monoblasts showing abundant cytoplasm, scattered mitochondria, and membrane-bound dense azurophilic granules (TEM, uranyl acetate and lead citrate; **a**, ×9200; **b**, ×7300) **c** and **d**—Higher magnification showing details of the granules in rectangles of **a** and **b**. Note crystalline linear densities in **d** (TEM, uranyl acetate and lead citrate, ×55,000)

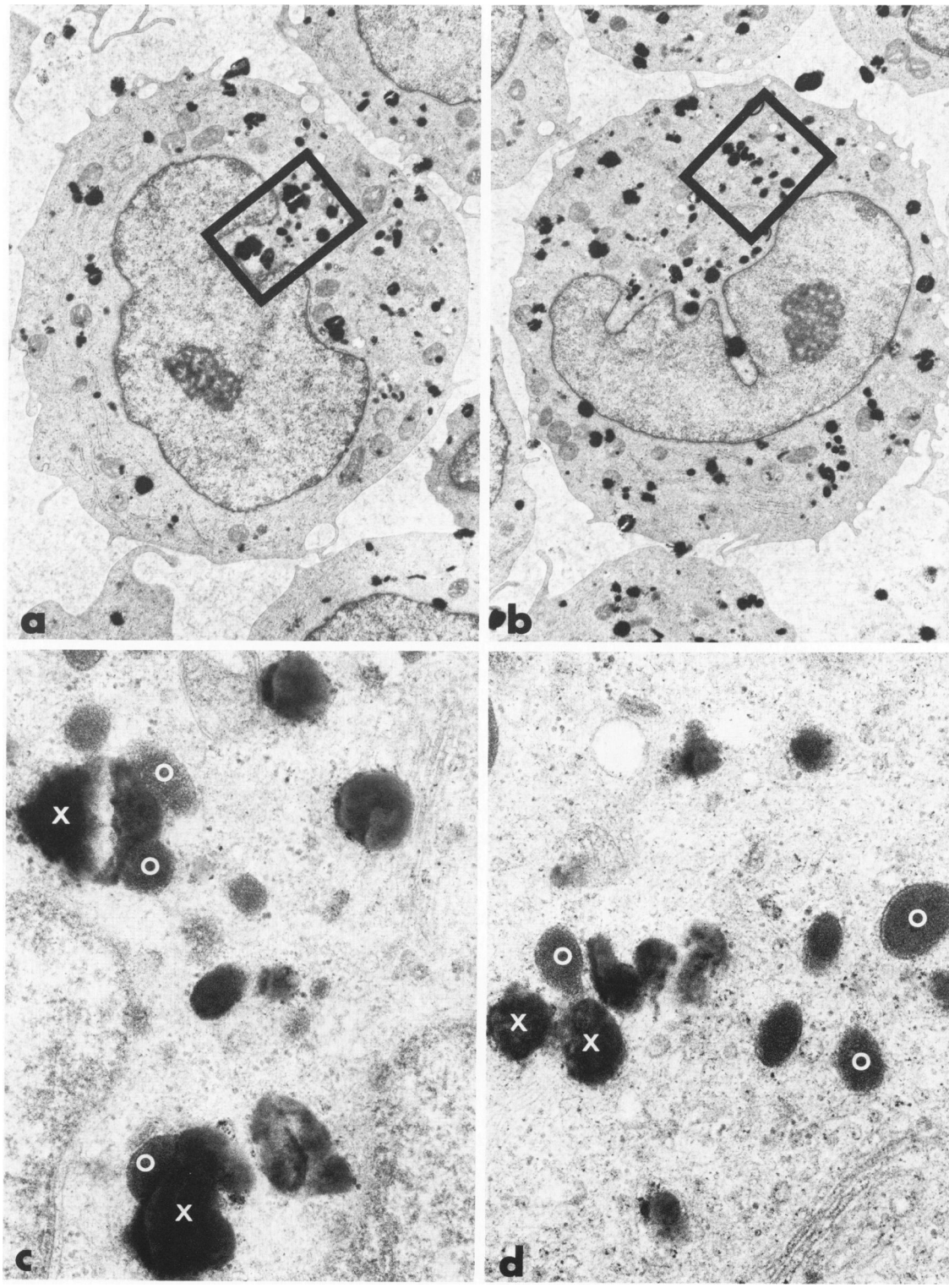

Figure 10—Acute monoblastic leukemia; peripheral blood from Patient 3. **a** and **b**—Two representative monoblasts showing high content of NTA esterase. Note their random distribution (NTA, uranyl acetate and lead citrate, × 7300) **c** and **d**—Details of the granules. Reaction products (*X*) usually are not related but are occasionally superimposed on membrane-bound azurophilic granules (*circles*). (NTA, uranyl acetate and lead citrate, × 55,000)

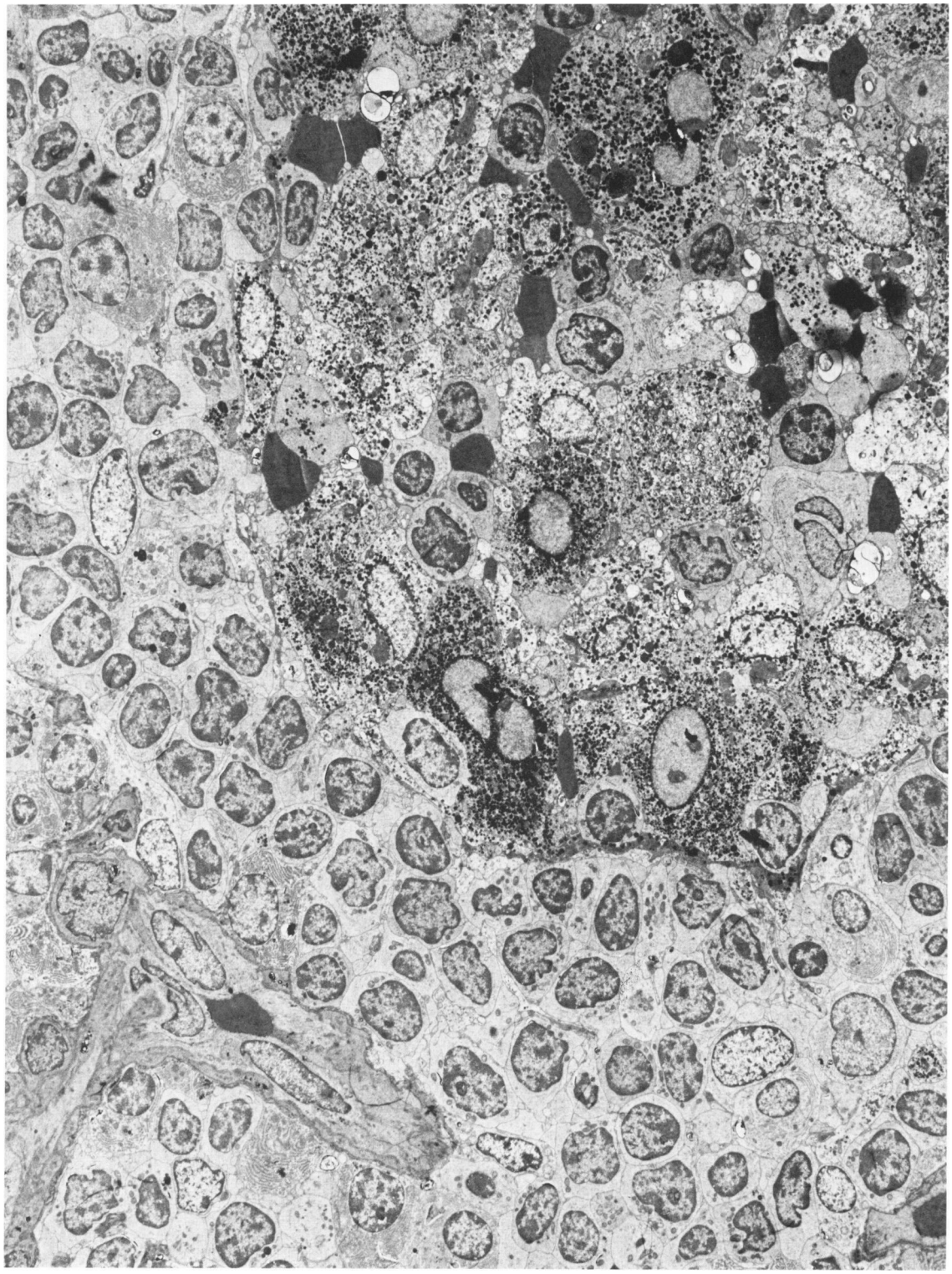

Figure 11—Obturator lymph node from Control 1. The lymph node sinus is filled with histiocytes that are loaded with NTA esterase granules (NTA, uranyl acetate and lead citrate, ×2000)

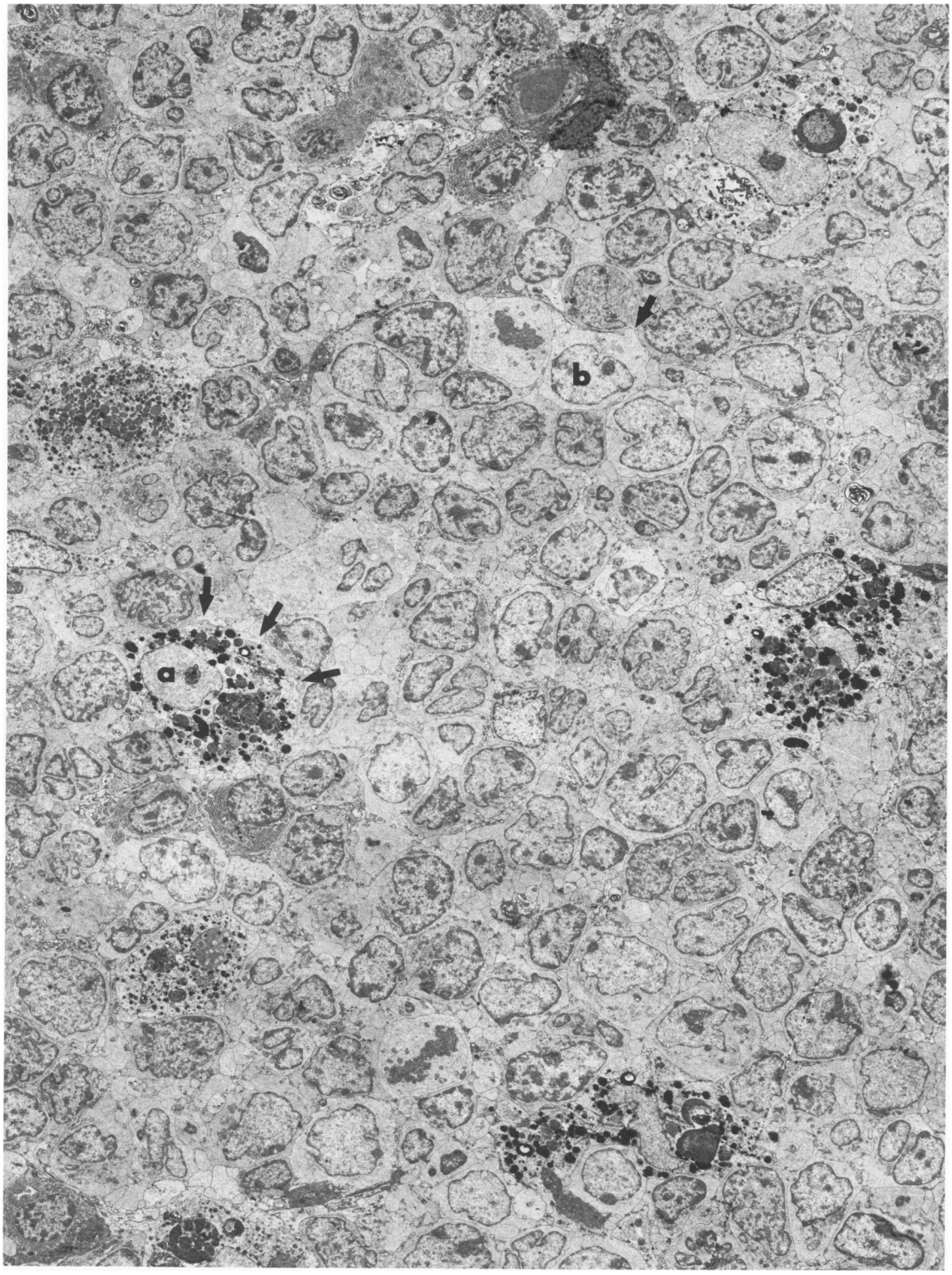

Figure 12—Reactive follicle of the hyperplastic adenoid from Control 2. Macrophages of this germinal center (*arrows*) contain a larger number of NTA esterase granules, whereas lymphocytes are devoid of them. A macrophage (**a**) and a transformed lymphocyte (**b**) are shown at higher magnification in Figure 13. (NTA, uranyl acetate and lead citrate, ×1700)

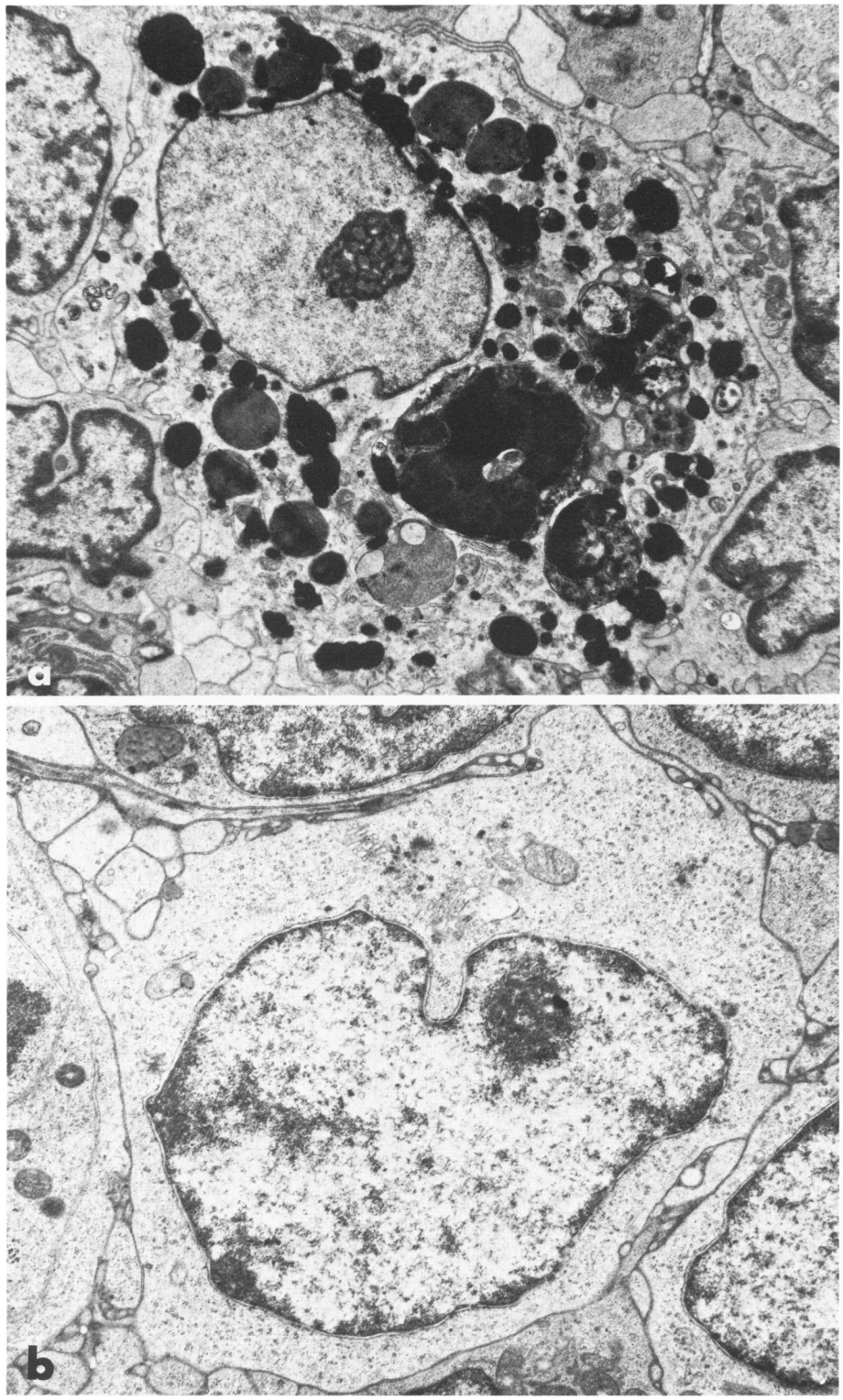

Figure 13—Reactive follicle of the hyperplastic adenoid from Control 2. **a**—Macrophage with electron-dense NTA esterase granules. These granules are more electron-opaque than the cytoplasmic lipid. (NTA, uranyl acetate and lead citrate, ×7000) **b**—A transformed lymphocyte with no NTA esterase granules (NTA, uranyl acetate and lead citrate, ×11,600)

variably dilated mitrochondria, usually localized to one side of the cytoplasm.

Scattered macrophages contained nuclear debris and lipid droplets. The cytoplasm of these macrophages was characterized by randomly dispersed smooth vesicles, membrane-bound dense core granules, and a small number of mitochondria.

In ultrastructural cytochemistry studies, most of the neoplastic cells were devoid of any end products (Figure 14a). Rarely, neoplastic cells contained some electron-dense end product, but only inside the mitochondria (Figure 14b). In contrast, the scattered macrophages contained numerous granular reaction products throughout the cytoplasm; these were not limited to mitochondria (Figure 14c).

Discussion

The importance of accurate cytologic classification of neoplasms of the hematopoietic system has been clearly established. When the proliferative cellular component reveals the "morphologic" features of the presumed cell of origin, it is customary to classify such a lesion according to the cytologic type of the parent cell. Frequently, however, abnormally proliferating cells fail to reveal a clearly recognizable morphologic similarity to their nonneoplastic counterparts, and one may have to depend on methods other than light microscopy to identify their nature. Cytochemistry, electron microscopy, surface markers, biochemistry, tissue culture, and cytogenetics have been employed for this purpose.[12,24-29] Of these methods, cytochemistry has been particularly helpful, especially in the classification of acute leukemias. Among many cytochemical enzymes, nonspecific esterases have been utilized for distinguishing of histiocytes and monocytes from granulocytes.[30] According to recent reports, nonspecific esterase with α-naphthyl acetate as a substrate can also be utilized for identification of subsets of T cells.[31-34] In view of the current controversies and difficulties in the classification of large-cell lymphomas on the basis of conventional morphologic criteria alone, our efforts have been directed toward characterizing these large cell lymphomas on the basis of cytochemistry and ultrastructure in addition to light-microscopic examination. The method which we recently developed[21] and applied for this series has been added to the armamentarium for diagnostic hematopathology. The method not only makes it possible to identify neoplastic cells of the histiomonocytic series, but, at the same time, provides us with the ultrastructural details of these cells. Whereas ordinary transmission electron microscopy depends on customary features such as lysosomes and phagocytosis for identification of histiocytes and monocytes, the main advantage of the method described here is that it identifies these cells even in the absence of the characteristic transmission EM features, as demonstrated in the case of malignant histiocytosis (MH) in Patient 1. Furthermore, nonhistiocytic malignant cells may reveal phagocytic activity.[35] As mentioned in our previous paper on the ultrastructural demonstration of nonspecific esterase,[21] there have been other methods for demonstrating cytoplasmic esterase activity.[36,37] Ours differs from these in providing 1) better preservation of cytologic details at the ultrastructural level and 2) a specificity of NTA esterase that is similar or identical to that of α-naphthyl acetate esterase. The only other method attempting to identify monocytes by ultrastructural cytochemistry was recently described by Bozdech and Bainton,[38] who were able to characterize circulating monocytes by demonstrating α-naphthyl butyrate esterase on the plasma membrane. Whether this method is applicable to solid tissue has not yet been established.

The specificity and sensitivity of NTA esterase have been tested previously[21] and have been confirmed in our present work. As expected, the reaction product was most abundant in the benign histiocytes. All of the lymphocytes in the germinal centers were negative for NTA esterase. The same was true for most, but not all, lymphoid cells in the paracortical areas. The reaction products in lymphoid cells, however, appeared most often in the form of single dotlike granules. The granulocytes were negative. This specificity of the NTA esterase reaction is very similar to that of α-naphthyl acetate esterase as seen with the light microscope.[30-33] As for the staining of lymphocytes in the paracortical areas, we were not able to establish whether only certain subsets of T cells are stained, since immunologic parameters were not investigated simultaneously.

In both malignant histiocytosis and acute monoblastic leukemia, the NTA esterase was not clearly related to any membrane-bound dense-core granules, although, in some of the cells, the end product appeared to be superimposed on the electron-dense granules. The distribution of NTA esterase was random, and the amount of the end product was much larger than could be accounted for by dense-core granules.

The procedure described may be helpful in the following diagnostic situations:

1) It can identify malignant histiocytosis as a clinicopathologic entity distinct and different from malignant lymphoma. This has become particularly important since Osborne et al.[39] recently reported that

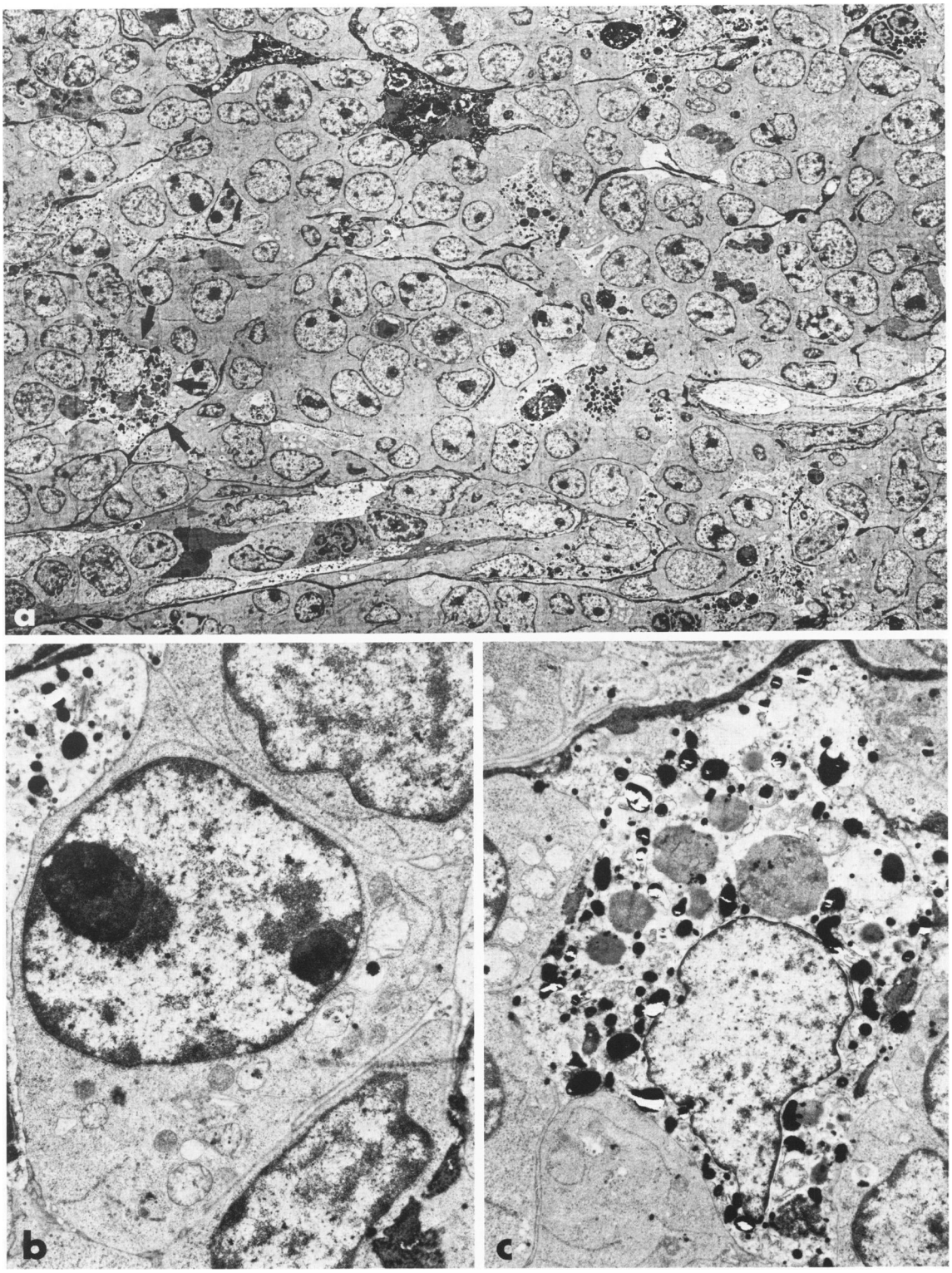

Figure 14—Diffuse lymphoma, undifferentiated, non-Burkitt's type, of jejunum from Control 3. **a**—Most of the neoplastic cells are devoid of NTA esterase. A few scattered macrophages, in contrast, show a high content of the end product (*arrows*). (NTA, uranyl acetate and lead citrate, × 1200) **b**—Rare neoplastic lymphoid cells show dotlike positivity inside what appears to be a swollen mitochondrion. (NTA, uranyl acetate and lead citrate, × 8000) **c**—One of the macrophages shown in Figure 14a. NTA esterase shows greater electron-opacity than do the lipids or other electron-opaque materials in the cytoplasm. (NTA, uranyl acetate and lead citrate, × 5400)

malignant lymphomas of the large lymphoid type may have a pattern of infiltration in lymph nodes identical to that previously considered as diagnostic of malignant histiocytosis.

2) It may serve to identify the existence of a true histiocytic lymphoma and help us to distinguish it from malignant lymphoma of the large lymphoid type, and to establish true histiocytic lymphoma as a tumor entity that starts as a localized lesion and is distinct and different from malignant histiocytosis. The latter is a leukemia-like disorder, usually disseminated at the time of its clinical inception.

3) It may help to establish the monoblastic nature of an unclassifiable ("undifferentiated") leukemia.

As mentioned above, other methods are available with which it might be possible to achieve similar goals. They include functional studies, the immunoperoxidase reaction for lysozyme, and cytochemical studies at the light-microscopic level. Functional studies require cell suspension, and the validity of their results requires either a uniform tumor cell population or, in cases of polymorphous proliferations, morphologic recognition of the cells in question. The immunoperoxidase method for the demonstration of lysozymes for identifying a true histiocytic malignant tumor has not been reliable in our hands when applied to paraffin sections. Although light-microscopic cytochemical studies are simpler and are excellent for tissue imprints and marrow films, they do not provide a clear image of morphologic detail and cytochemical reaction simultaneously, since most of these procedures have to be applied to frozen sections of solid tumors. The advantage of the proposed method is that it provides crisp, unequivocal documentation of both the presence of esterase, characteristic of histiocytes, and of subcellular structure for both fluid and solid specimens. Furthermore, this procedure may be helpful in demonstrating heretofore unknown features of early differentiation of monocytes and histiocytes, thereby making accurate recognition of their malignancies possible.

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