

## Unusual Inclusions Occurring in the Blasts of Four Patients With Acute Leukemia and Down's Syndrome

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**The blast cells from four patients with acute leukemia and Down's syndrome were found to contain unusual inclusions which manifested similar morphologic and cytochemical characteristics. Ultrastructure studies of the blasts from one of these patients revealed the inclusions to be single membrane-bound structures containing electron-dense amorphous debris, membranous substance, and virus-like particles.**

**I**T IS NOW WELL established that children with Down's syndrome have an increased incidence of acute leukemia; the several studies supporting this conclusion have been reviewed by Rosner and Lee.<sup>1</sup> These authors also found acute lymphoblastic leukemia to be the most common form of acute leukemia occurring in children with Down's syndrome, dispelling the commonly held impression of the predominance of acute myeloblastic leukemia in mongoloid children. In addition to the increased incidence of acute leukemia in Down's syndrome, there have been several reports of severe myeloid leukemoid reactions and "transient myeloid leukemia" occurring in newborn mongoloids.<sup>2,3</sup>

Blood and bone marrow specimens from seven patients with mongolism and acute leukemia have been studied in this laboratory in the last 5 yr. In three of these patients, two newborns and one 2-yr-old, the leukemic process was diagnosed as acute myelogenous leukemia. In the other four patients, ranging in age from 2 to 9 yr, the leukemic process was diagnosed as acute lymphoblastic leukemia. The blasts in the four patients diagnosed as acute lymphoblastic leukemia were characterized by prominent inclusions which have been noted only rarely in the leukemic cells of nonmongoloid individuals. The morphologic, ultrastructural, and cytochemical studies of these inclusions constitute the basis of this report.

### MATERIALS AND METHODS

Three of the four patients were admitted to the pediatric service of the University of Minnesota Hospital. Material on the fourth patient was referred from another institution. All specimens on which observations are reported were obtained prior to chemotherapy.

Peripheral blood and bone marrow smears on all four patients were stained by a Wright-Giemsa technique. In cases 1 and 2, blood and bone marrow smears were also subjected to peroxidase, Sudan Black B, toluidine blue O, periodic acid-Schiff, methyl green pyronin, and oil red O reactions. The smears from case 3 were stained with Sudan Black B in addition to the routine Romanovsky stains. Smears from case 4 were stained with peroxidase and Sudan Black B in addition to the Wright Giemsa stain. Ultrastructural studies were performed on the bone marrow specimen from case 2.

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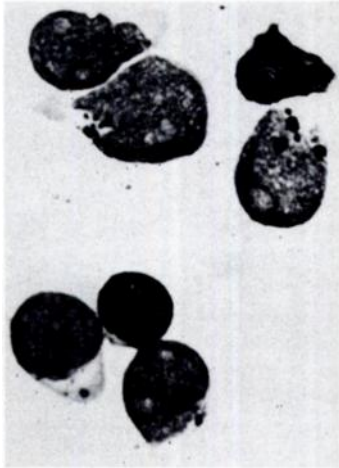
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**Fig. 1. Bone marrow smear from case 1 showing blasts with inclusions. Wright-Giemsa.  $\times 1000$**

All of the four patients have died. Postmortem examinations were performed on three of the four, and evidence of organ infiltration by leukemic cells was present in these three patients. Permission for postmortem examination on the fourth patient was not obtained.

#### *Electron Microscopy*

Bone marrow aspiration particles from case 2 were processed for electron microscopy by standard techniques. Immediately following aspiration, the bone marrow particles were placed in a modified Karnovsky fixative<sup>4</sup> of 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate-HCl buffer with 0.5 mg/ml calcium chloride at pH 7.3 and 4°C. The specimen was fixed for 12 hr and then washed for 24 hr at 4°C in 0.05 M sodium cacodylate-HCl buffer with 0.2 M sucrose at pH 7.2. Postfixation was carried out at 0°C for 1½ hr in 1% OsO<sub>4</sub> buffered with veronal acetate at pH 7.4,<sup>5</sup> followed by dehydration in a graded series of ethanol with two changes of propylene oxide. The specimen was embedded in Maraglas (the Marlette Co., Division of Allied Products Corp., Long Island City, N.Y.) and polymerized at 52°C for 48 hr. Silver sections were cut with a diamond knife on a Reichert OMU2 ultramicrotome, picked up on uncoated 300-mesh grids, and double stained with uranyl acetate (20 min) and lead citrate (4 min).<sup>6</sup> The sections were examined with a RCA-3G electron microscope.

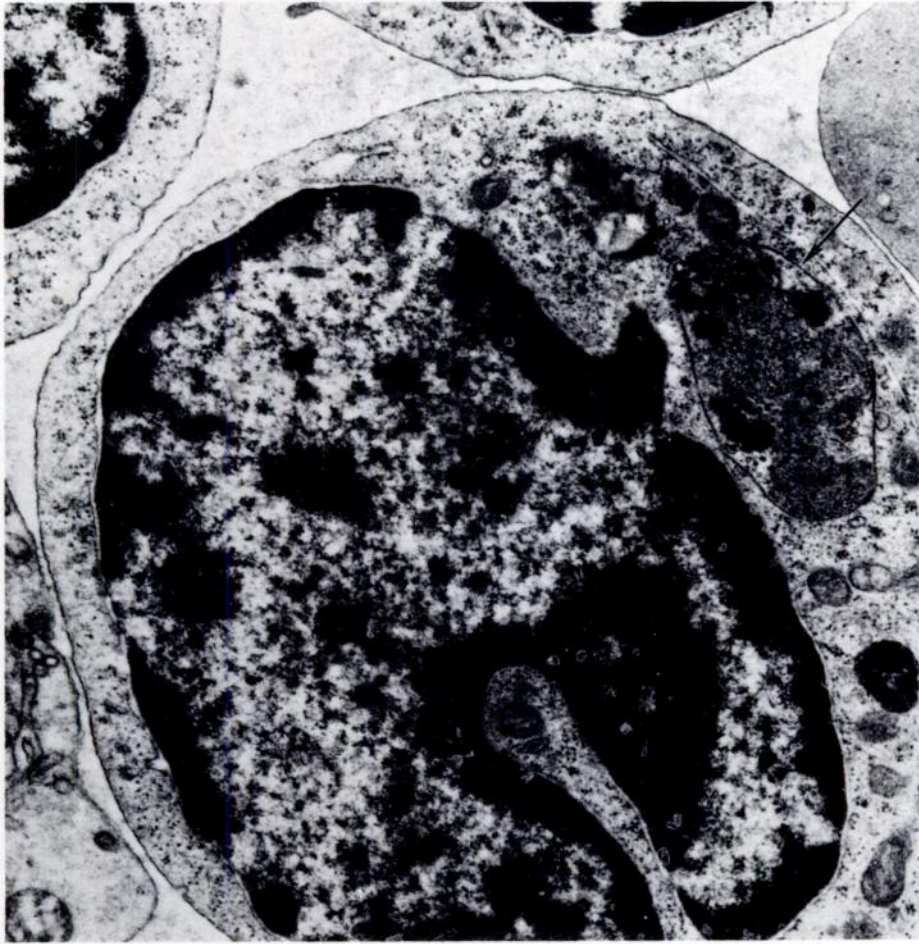
### RESULTS

#### *Light Microscopy*

In routine Wright-Giemsa-stained preparations, a high per cent of the blasts in the peripheral blood and bone marrow smears of the four patients exhibited prominent basophilic inclusions (Fig. 1). The number of inclusions per cell varied considerably; cells completely devoid of inclusions as well as cells with a small number were noted. The majority of cells, however, contained numerous

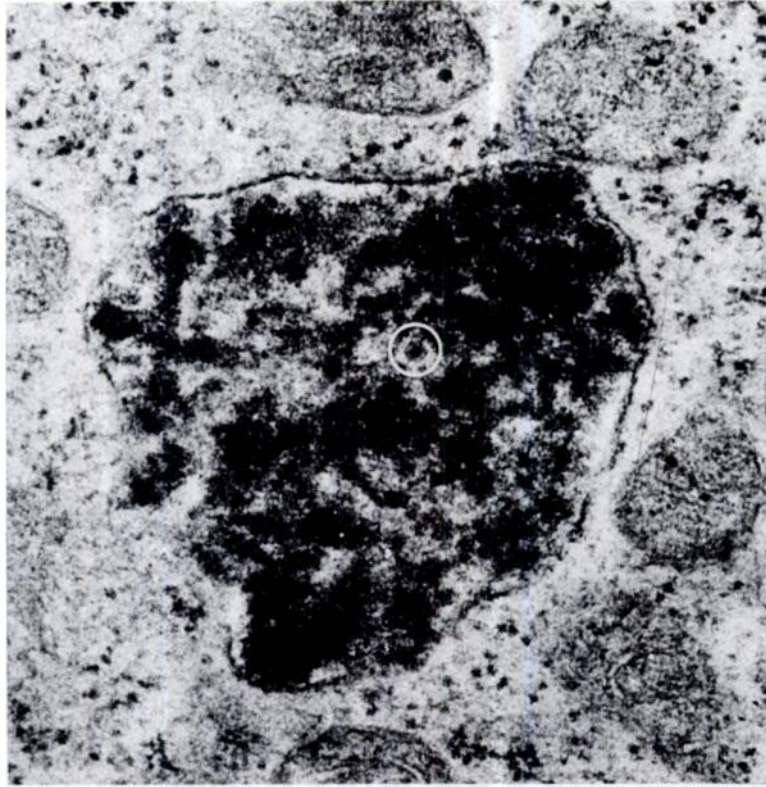


**Fig. 2. Blast in peripheral blood smear from case 4 showing several inclusions overlying the nucleus. Wright-Giemsa.  $\times 1000$ .**



**Fig. 3.** Electron micrograph of bone marrow lymphoblast showing large inclusion body (arrow) bound by a single membrane, and containing numerous doughnut-shaped particles.  $\times 30,100$ .

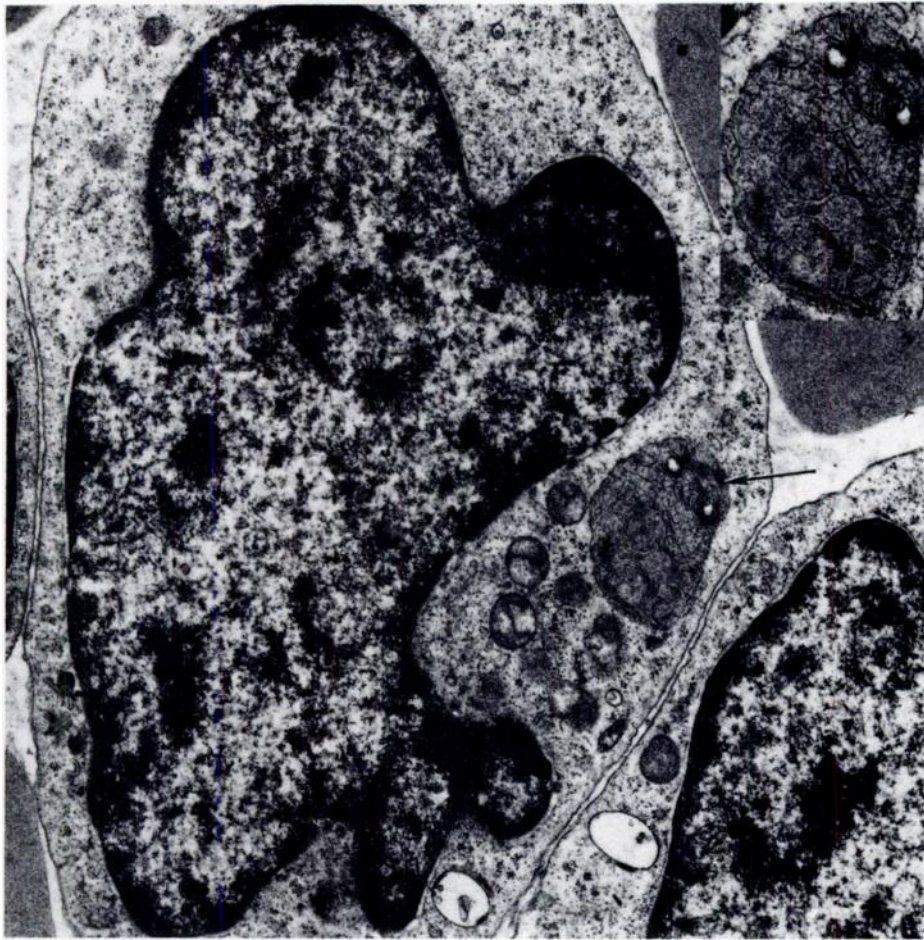
inclusions which were found overlying the nucleus as well as in the cytoplasm (Fig. 2). The inclusions varied markedly in size and occasionally exhibited a tendency to aggregation. In Wright-Giemsa-stained smears the inclusions exhibited a striking similarity to basophil granules. In those cases in which special cytochemical techniques could be utilized, the staining results were similar. The blasts were uniformly peroxidase and Sudan Black B negative. The inclusions manifested varying degrees of positivity with the periodic acid-Schiff reaction, with most staining moderately positive. A majority of the inclusions exhibited some degree of metachromasia with the toluidine blue O stain; no staining reaction was obtained with oil red O. Most of the inclusions were pyrophilic with the methyl green pyronin stain. Nuclear budding was noted in occasional blasts, and the resulting nuclear fragments appeared in the cytoplasm as large homogeneous structures which resembled aggregate inclusions.



**Fig. 4.** High-magnification view of an inclusion body showing a particle which has a dense nucleoid (circle).  $\times 63,500$ .

#### *Electron Microscopy*

By electron microscopy, the predominant cell type in the bone marrow of case 2 appeared to be lymphocytic, and virtually all of the cells contained cytoplasmic inclusion bodies. The inclusions varied from 0.5 to 2.0  $\mu$  in diameter and most often appeared as single membrane-bound lyzomelike structures (Fig. 3) containing particles which were round, approximately 400  $\text{\AA}$  in diameter, with an electron-dense outer membrane and an electron-lucent core. Very rarely, particles were observed which had a dense nucleoid and resembled mature viruslike particles (Fig. 4). Occasionally the inclusion-limiting membrane was broken or missing, and the particles were free in the cytoplasm. Within many of the inclusions, mixed with the particles, was an electron-dense material and unusual whorled membranes in a fingerprintlike configuration. In some instances the entire inclusion was filled with these structures, which resembled collections of mitochondrial membranes (Fig. 5). Frequently, the inclusions were found lying close to the nuclear envelope which in a few cells was focally dilated and contained fragments of cytoplasm.



**Fig. 5.** Electron micrograph of a bone marrow lymphoblast showing a large inclusion body (arrow) filled with whorled membranes.  $\times 22,600$ . The insert is a higher magnification view of the same inclusion, showing the membranous configuration.  $\times 36,000$ .

#### DISCUSSION

The hematologic abnormalities which are observed with increased frequency in Down's syndrome vary from acute leukemia to severe myeloid leukemoid reactions which at times are morphologically indistinguishable from acute myelogenous leukemia. Prior to the review of Rosner and Lee which found acute lymphoblastic leukemia as the predominant type of acute leukemia in mongloid children exclusive of the newborn period, it was apparently widely assumed that acute myeloblastic leukemia was the more common type of acute leukemia in children with Down's syndrome. The reason for this misconception is not clear but may be related to the occurrence of myeloid leukemoid reactions and the preponderance of acute myeloblastic leukemia in newborn mongo-

loids. The several reports of Down's syndrome and acute leukemia contain very few details of the morphologic characteristics of the blasts; cases of promyelocytic leukemia have been noted, and Thompson et al. found rare blasts with basophilic granules in their patient with acute leukemia and mongolism.<sup>7</sup> Although there has been some confusion about which cytologic type of acute leukemia is most frequent in Down's syndrome, there is little evidence in previous reports to suggest anything unusual about the cytology of the leukemic cells.

The inclusions in the blasts in the blood and marrow specimens from the four children with acute leukemia and Down's syndrome in this series manifested similar morphologic characteristics, and in those instances in which cytochemical techniques could be utilized, the staining reactions were similar. Inclusions of the type noted in these four patients are an unusual occurrence in the blast cells of acute leukemia in nonmongoloid individuals. Sun et al. reported similar morphologic and ultrastructural findings in the blast cells of one nonmongoloid individual with acute lymphoblastic leukemia.<sup>8</sup>

Ultrastructural studies of the blasts from one of the present patients show the majority of the inclusions to be single membrane-bound lyzozomelike structures containing an electron-dense material, whorled membranous structures, and viral-like particles. The inclusions containing the electron-dense amorphous material and membranelike structures bear some resemblance to some of the inclusions which have been observed in the Chediak-Higashi syndrome.<sup>9</sup>

The nature of the cytoplasmic and extracellular particles which are found in specimens from leukemic patients is unclear.<sup>10</sup> Although the term "virallike" has at times been used to describe them, conclusive evidence of a viral origin is lacking. It is possible, at least in some instances, that they represent an abnormal secretory product of the cell. The ultrastructural characteristics of the lesions in the present patient bear no resemblance to the crystalline inclusions which have been observed in chronic lymphatic leukemia<sup>11</sup> or to the tubular aggregates which have been noted in lymphocytes and other cells in a diverse group of disease processes including lupus erythematosus.<sup>12-14</sup> Several observers have attributed a viral origin to the latter inclusions, but this possibility has been discounted by others.<sup>15</sup>

The inclusions occur in the blasts of a relatively high per cent of children with Down's syndrome and acute leukemia in this series. The morphologic studies of the leukemic cells in these four patients suggest the possibility that the blast cells in some mongoloids with acute leukemia may have features which, although not necessarily unique to patients with Down's syndrome, are distinctly uncommon in other patients with acute leukemia. Although the inclusions in the blasts from these four patients share similar light-microscopic and cytochemical features, it cannot be stated with certainty that they would be ultrastructurally identical. The present series admittedly is a small group of patients; a much larger number of patients with Down's syndrome and acute leukemia will have to be studied to obtain a more accurate reflection of the occurrence of this finding.

The possibility that the inclusions are primarily related to Down's syndrome

with no relationship to the leukemic process must be considered. However, case 2 gained a 1-yr complete remission on vincristine and prednisone. During this period, blood and bone marrow smears were studied by light microscopy, and no inclusions could be detected in the normal hematopoietic cells.

The relationship of these inclusions to the leukemic process and Down's syndrome must remain a matter of speculation. Considering the occurrence of the lesions in a population at such a high risk for acute leukemia, it would appear worthwhile to carefully study the leukemic cells in children with Down's syndrome and acute leukemia in an attempt to establish the incidence of these inclusions and elucidate what role, if any, they play in the leukemic process.

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