Serotonin Storage Organelles in Human Megakaryocytes

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Serotonin (5-hydroxytryptamine, 5-HT) in circulating blood is confined almost exclusively within the platelets.1–3 It is concentrated in specialized organelles of the platelet referred to as dense bodies because of their inherent opacity to electrons.4–8 Platelets appear to take up serotonin after entering the circulation since very little biochemically detectable 5-HT is present in the bone marrow. Also, Tranzer et al, whose investigations helped establish that platelet dense bodies are serotonin storage granules, were unable to find the characteristic opaque organelles in megakaryocytes.9 However, Tanaka and his colleagues,10 utilizing the sensitive fluorescence technic of Falck and co-workers, demonstrated that the small amount of serotonin in human and rabbit bone marrow was concentrated in mature megakaryocytes. This observation appears to conflict with the electron microscopic investigations, which failed to detect dense serotonin storage organelles in megakaryocytes.9 Evidence accumulated by many laboratories indicates that the number of dense bodies present in platelets is proportional to their serotonin concentration, and even cells with minute amounts of this amine contain some of the characteristic organelles.5–7,11 If megakaryocytes contain amounts of 5-HT detectable by fluorescence,10 then dense bodies should be present in them or the concept that serotonin is confined to opaque organelles should be revised.12 The present study resolves this problem by demonstrating the regular presence of dense bodies in mature megakaryocytes.

Materials and Methods

Bone marrow for this study was obtained from 2 adults and 2 children. One of the adults was a normal volunteer, and the other had chronic thrombocytopenic purpura. One of the children had acute thrombocytopenic purpura, and bone marrow from the second child was obtained during the course of a workup for mild

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anemia. Methods used in this laboratory for the preparation of blood cells for study in the electron microscope have been described previously. Bone marrow was processed in a similar manner. A heparinized syringe was used to aspirate 2 ml of marrow fluid which was transferred immediately to an equal volume of 0.1% glutaraldehyde in White's saline, pH 7.3. After gentle mixing, the tube was centrifuged to obtain a buffy coat. The leukocyte-rich layer was removed and processed through a standard fixation and embedding procedure. The red cell mass left in the tube was decanted into a Petri dish containing three times its volume of 0.1% glutaraldehyde fixative. Small particles or "units" of marrow tissue were picked out with a Wintrobe pipet and transferred to a tube containing 3% glutaraldehyde in White's saline. The particles were then fixed and embedded in the same manner as the buffy coat. Thick sections of the plastic-embedded samples were examined in the light microscope to locate megakaryocytes, and thin sections were then obtained from suitable areas. The thin sections were stained with uranyl acetate and lead citrate, and studied in a Philips 200 electron microscope.

Results
Megakaryocytes observed in the marrow samples of all 4 patients revealed morphologic features similar to those described previously by others. Of particular interest for the present study were the large megakaryocytes in which the demarcation membrane system dividing the cytoplasm into individual platelets was well developed (Fig 1A). The cytoplasm of the massive cells contained granules, the Golgi system of vacuoles and vesicles, microtubules, microfilaments, elements of rough endoplasmic reticulum and polyribosomes. In addition, megakaryocytes at this stage of development revealed a small number of electron-opaque granules (Fig 1B-F). The dark bodies were identical in appearance to the serotonin storage organelles observed in platelets from peripheral blood (Fig 1D). Contracted, spherical masses of opaque substance were eccentrically located within a unit membrane, and separated from it by a clear space (Fig 1E). The number of dense bodies in megakaryocytes varied considerably from cell to cell. However, they were always present in cells in which demarcation was advanced, and absent from megakaryocytes in which demarcation had not occurred. Megakaryocytes containing dense bodies were observed in the bone marrow of all 4 individuals studied.

Discussion
Results of the present study indicate that dense bodies identical to the serotonin storage organelles of peripheral blood platelets do occur in megakaryocytes. The opaque organelles were not found in young megakaryocytes, but were readily detected in mature cells in which the demarcation system of membranes had sequestered the cytoplasm into individual platelet units. This finding is in agreement with
the observations of Tanaka et al, who detected fluorescence indicative of serotonin only in mature, platelet-forming megakaryocytes.10

The finding that dense bodies are limited to mature, platelet-forming megakaryocytes is intriguing. Behnke has demonstrated that the demarcation membrane system develops primarily by infolding of the megakaryocyte surface membrane.14 Extensive interiorization of the surface membrane not only sequesters areas of the cytoplasm into platelet units, but also provides an open pathway for substances outside the megakaryocyte to penetrate into the deepest recesses of the cell. The demarcation membrane system of the megakaryocyte is similar in this regard to the open channel system tunneling into the hyaloplasm of circulating blood platelets, and in fact they are one and the same.17 Previous experiments have shown that the open channel system of the platelet acts as a conduit permitting plasma-borne particles to enter deeply into the cell and be transferred to intact granules.18 It was suggested that this route might facilitate concentration of serotonin in specific platelet granules closely associated with the channels.12,18

Serotonin may not be taken up by megakaryocytes until the changes in the surface membrane associated with formation of the demarcation system take place. After this event, the giant cell may be as efficient as its progeny in the uptake and concentration of 5-HT, but may be limited by the paucity of 5-HT. It will be of interest to see if megakaryocytes from patients with carcinoid syndrome contain increased numbers of dense bodies and whether normal megakaryocytes incubated in vitro with serotonin can take up and concentrate the amine by forming opaque organelles. These possibilities are currently being explored.

Summary

Serotonin in the circulation is confined almost exclusively to electron-opaque organelles within blood platelets. Small amounts of this amine have also been detected in bone marrow, where it is found concentrated in the megakaryocytes. The present study demonstrates the regular presence of dense bodies identical to the serotonin storage organelles of circulating platelets in the cytoplasm of mature megakaryocytes from human marrow.

References


[ Illustrations follow ]
**Fig 1A**—Thin section of a megakaryocyte from a unit of human bone marrow. Formation of the demarcation system of membranes (arrow) is at an advanced stage in this cell. Electron-opaque organelles resembling dense bodies of circulating platelets were regularly present in mature megakaryocytes of this type, but were not found in young megakaryocytes without a system of demarcation membranes (× 3750).

**Fig 1B**—Two dense bodies (arrow), identical in size and appearance to the serotonin storage organelles of blood platelets, are evident in the cytoplasm of a megakaryocyte from normal adult human bone marrow (× 15,400).

**Fig 1C**—Four opaque organelles (arrow) identical to platelet dense bodies are present close to a nuclear lobe (N) deep within the cytoplasm of this megakaryocyte from a child with mild anemia (× 15,800).

**Fig 1D**—Platelet from a sample of normal human citrated platelet-rich plasma. The opaque serotonin storage organelles (arrow) in these cells are shown for comparison with the dense bodies observed in megakaryocytes (× 10,600).

**Fig 1E**—Megakaryocyte from the bone marrow of an adult with chronic thrombocytopenic purpura. Over ten dense bodies (arrow) similar to those observed in platelets are apparent in this area of the cell cytoplasm (× 14,800).

**Fig 1F**—Two dense bodies (arrow) in a megakaryocyte from a child with acute thrombocytopenic purpura are shown at high magnification. The morphology of these organelles is identical to that of platelet serotonin storage organelles (× 54,700).
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