Alterations of Platelet Ultrastructure in Patients with Carcinoid Syndrome

James G. White, MD, and Richard B. Davis, MD*

SEROTONIN (5-HYDROXYTRYPTAMINE; 5-HT) in blood is transported almost exclusively by platelets,[1] and most of it is concentrated in a particular organelle, the dense body.[2-4] The number of dense bodies correlates with platelet 5-HT content.[3,5] Human platelet 5-HT content is less than in most animal species, and dense bodies are considered to be rare in human platelets.[3,5]

Platelets from patients with the carcinoid syndrome have higher concentrations of 5-HT, and have recently been reported to contain 50–60 times more dense bodies than normal in thin sections.[5] However, one study of carcinoid platelets [6] suggested an association between elevated levels of serotonin and dilated channels, without mentioning dense bodies.

The present study has examined platelets from 4 patients with the carcinoid syndrome. All patients had known hepatic metastases and elevated urinary 5-hydroxyindoleacetic acid excreation. Dilated channels were more common in carcinoid platelets than in normal cells. Dense bodies were also more prominent. The distinctive feature of carcinoid platelets examined in this study, however, was the frequent occurrence of unusual organelles which have not been seen previously in normal platelets.

Materials and Methods

The techniques we used for collection of blood in 0.1 volume of 3.8% trisodium citrate, separation of citrated platelet-rich plasma (C-PRP), maintenance of control C-PRP at 37° C or aggregation by adenosine diphosphate and thrombin, and preparation of platelet samples and whole mounts for electron microscopy have been described recently.[7-10]

From the Department of Pediatrics, University of Minnesota Medical Center, Minneapolis, Minn 55455.

Supported by grants from the Minnesota Division of the American Cancer Society, the Minnesota Heart Association, Grant no. CA–08832, HE–11880, AI–05153 and HE–7150, US Public Health Service and the Cardiovascular Clinical Research Program Project.

Accepted for publication May 29, 1969.

Address for reprint requests: Dr. White, Department of Pediatrics, University of Minnesota Medical Center, Box 284 Mayo Memorial Building, Minneapolis, Minn 55455.

* USPHS Research Career Development Recipient 5–K3–HE–14, 919–08. Present address: Division of Hematology, University of Nebraska Medical Center, Omaha, Neb.

Counting Method

Thin sections of normal and carcinoid platelets were photographed at low magnification in order to include large numbers of cells and prevent selection. The number of dense bodies was determined in platelets cut in cross section or parallel to the plane of the circumferential band of microtubules. Portions of platelets not containing organelles were not counted. One hundred platelets were evaluated in a sample from each of 5 normal donors and 4 patients with carcinoid syndrome. The mean number of dense bodies per platelet in whole mounts was determined in 25 consecutively photographed platelets from each of 3 normal donors and 3 patients with carcinoid.

Results

The mean number of dense bodies per platelet found in sections from 5 normal donors ranged from 1.1 to 1.6 (Fig 1). In the 4 patients with carcinoid syndrome the mean was 1.4, 1.5, 1.6, and 1.8. The significance of the difference between the average mean of the normals (1.4) and the carcinoids (1.6) is not certain because of the small number of cells counted and the inherent errors in the method. Errors include the difficulty in identifying dense bodies accurately at low magnification, the tendency of opaque organelles to drop out of unsupported thin sections, and the marked variation in size, shape, and substructure of opaque organelles. The carcinoid platelet 5-HT concentrations correlated poorly with the number of dense bodies (Table 1).

Determination of dense bodies in unstained, whole mounted platelets obviated the problems normally found in evaluating thin sections, but presented other difficulties (Fig 2). Since only inherently opaque organelles were apparent in the electron microscope, platelets without dense bodies could not be counted. However, staining the same grids with phosphotungstic acid indicated that normal and carcinoid platelets rarely occurred without opaque organelles. Summation of the results from normal and carcinoid platelets revealed an average of 8.3 and 8.7 dense bodies per cell respectively. However, the variation in individual cells was great. As few as 2 dense bodies, and as many as 18, were observed in single normal and carcinoid platelets.

Table 1. Relationship of 5-HT Levels and Number of Dense Bodies in Platelets of Patients with Carcinoid Syndrome

Patient	Platelet 5-HT ($\mu g/10^9$ platelets)	Avg. no. of dense bodies/platelet
1	2.96	1.4
2	5.1	1.8
3	5.4	1.5
4	6.4	1.6
Normal	0.8–1	1.4(Mean)

Despite the failure of methods used in this study to reveal marked differences in the number of dense bodies found in normal and carcinoid platelets, there were notable variations in fine structure. Platelets from our 4 patients were irregular in form and contained a relatively high proportion of dilated canaliculi when compared with normal platelets, even though collected and prepared by methods which preserve discoid shape in over 90% of the cells (Fig 3, 5, 7, 8). Thus, the alterations in surface contour and swelling of channels of the canalicular system appear related to the high concentrations of platelet serotonin. These findings agree with those of Crawford *et al.*[6] The dense bodies in carcinoid platelets were more prominent than in normal cells (Fig 3). They were generally larger, more complex, and more fragmented than those observed in control platelets. In addition, a wide variety of alterations were evident in granules adjacent to, fused with, or in stages of transformation to dense bodies. Many of these granules revealed a highly organized internal structure (Fig 4–6). Complex arrays of fine lines were arranged in parallel or intersecting patterns resembling tire treads (Fig 4). Other granules contained peculiar lattices composed of tubules in cross section or in other arrangements indicating a highly organized substructure.

An unusual organelle present in many carcinoid platelets consisted of irregular masses of roughly parallel filaments enclosed within a membrane (Fig 5, 6). The filamentous masses were frequently fused to portions of dense bodies, or granules, or to both. Some filamentous masses were quite large, involving sizable areas of the hyaloplasm (Fig 7, 8). The swollen membrane-bound areas often contained debris resembling substance from the hyaloplasm as well as from granules (Fig 8).

Discussion

The methods used in the present study to determine the number of dense bodies in normal and carcinoid platelets are not precise. However, it is reasonable to suggest that there are considerably more dense bodies in normal human platelets than previously reported.[3,5] The lower numbers determined in previous investigations may be related to the difficulties described, or to differences in sample preparation. Other workers used chilled samples of citrated or EDTA platelet-rich plasma fixed in cold glutaraldehyde-osmium to determine numbers of platelet dense bodies. These methods preserve dense bodies, but cause platelets to lose their disc shape and become irregularly swollen.[7,11] As a result, the organelles are more widely dispersed in the cells. Sections through the

altered cells would be less likely to include dense bodies than compact platelet discs.

In spite of these considerations, the number of dense bodies per sectioned normal platelet observed in the present study is difficult to reconcile with previously reported findings. Tranzer *et al*[3] found dense bodies to be rare in human platelets, and May *et al*[5] found only 16 dense bodies/1000 cells. In the present investigation many examples of normal platelets with four or more opaque organelles were encountered.

Although there were a few more opaque organelles in carcinoid cells than in normal cells, their size and complexity were more prominent features than the increase in number. The alterations in platelet shape, dilated canalicular system, and unusual organelles were also more characteristic of carcinoid platelets. Elongated granules with a periodic substructure have been observed in human platelets,[12] and occur regularly in the cells of some animal species.[13] However, the type of substructural arrangements observed in granules from carcinoid platelets were unlike any reported previously.

It is not known whether the highly organized structure in the granules of carcinoid platelets is related to an aberration of development in the megakaryocyte or whether it results from exposure of the cells to high concentrations of 5-HT in the circulation. Since relatively few granules were involved, the latter possibility seems more likely.

Uptake of low concentrations of 5-HT by platelets in vitro involves an active transport mechanism.[14] At concentrations of 1–2 μg/ml in plasma the 5-HT uptake of normal platelets reaches a steady state, and further increases in 5-HT content depend on the initial concentration in the cells.[15] Serotonin-laden platelets from patients with carcinoid syndrome do not take up additional amounts of amine until the concentration in incubation mixtures exceeds 3–6 μg/ml. Since the active transport mechanisms are saturated, uptake at high concentrations in vitro by carcinoid platelets appears to take place by diffusion. A similar mechanism has been postulated to operate in vivo when platelets are exposed to locally high levels of 5-HT liberated by carcinoid tumors. It can be speculated that transfer of 5-HT from hyaloplasm to granules may cause changes in substructure which do not occur when the normal transport mechanism is involved.

The unusual organelles containing masses of irregularly parallel filaments do not appear to originate primarily from granules. Membrane-enclosed bundles of fibers strongly resemble the microfilaments which form the matrix of platelet hyaloplasm.[8] Exposure of carcinoid platelets to high concentrations of 5-HT in vivo appears to cause irregular shapes

and alterations in the canalicular system. Distortion of the tortuous channels may result in sequestration of areas of the hyaloplasm. Alternatively, diffusion of large amounts of 5-HT into platelet hyaloplasm may stimulate the cell to wall-off the 5-HT within a membrane. If these speculations are correct, the membrane-bound masses of filaments may represent sequestered areas of hyaloplasm resembling autophagic vacuoles.[16,17]

In conclusion, the major anomalies observed in carcinoid platelets were irregular shape, dilation of the channel system, and presence of unusual organelles. Dense bodies were increased over the number found in normal cells, but not to a marked degree. The apparent discrepancy from observations made by others is probably due to the larger number of dense bodies found in normal platelets in the present study. The unusual organelles observed in carcinoid platelets may result from exposure to high concentrations of 5-HT. Exposure to large concentrations of 5-HT over prolonged periods in the bone marrow and blood of patients with the carcinoid syndrome may conceivably produce toxic effects on membrane systems and other structural components of platelets, as well as an increased uptake of the amine into dense bodies.

Summary

The high concentration of serotonin transported by platelets in patients with carcinoid syndrome has been associated previously with alterations in cell shape, dilatation of the canalicular system, and a marked increase in 5-HT rich dense bodies. In platelets from 4 patients with carcinoid syndrome the number of dense bodies in thin sections and in whole mounts was slightly in excess of normal. Alterations in contour and swelling of canaliculi were frequent. The most striking feature of carcinoid platelets was the presence of unusual hyaloplasmic organelles not observed previously in normal or pathologic platelets. The substructure of platelet granules revealed complex arrangements of parallel or intersecting lines. Other organelles consisted of masses of roughly parallel filaments enclosed by unit membranes. The presence of the unusual organelles appears related to the increased content of serotonin; possible mechanisms of origin are discussed.

References

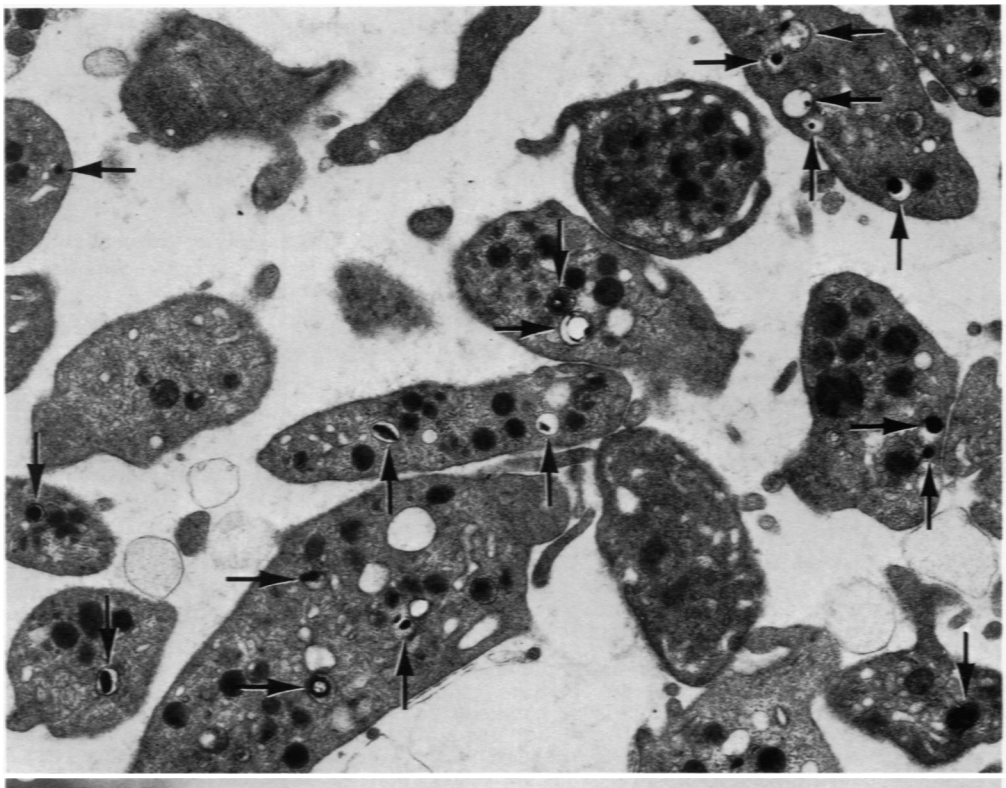
1. Rand, M., and Reid, G. Source of serotonin in serum. *Nature (London) 168*: 385, 1951.
2. Solatunturi, E., and Paasonen, M. K. Intracellular distribution of monoamine oxidase, 5-hydroxytryptamine and histamine in blood platelets of rabbit. *Ann Med Exp Fenn 44*:427–430, 1966.

3. TRANZER, J. P., DA PRADA, M., and PLETCHER, A. Ultrastructural localization of 5-hydroxytryptamine in blood platelets. *Nature (London) 212*:1574–1575, 1966.
4. DAVIS, R. B., and WHITE, J. G. Localization of 5-hydroxytryptamine in blood platelets: an autoradiographic and ultrastructural study. *Brit J Haemat 15*: 93–99, 1968.
5. MAY, B., BAK, I. J., BÖHLE, E., and HASSLER, R. Electron microscopical and biochemical studies on the serotonin granules in carcinoid syndrome. *Life Sci* 7:785–800, 1968.
6. CRAWFORD, N., SUTTON, M., and HORSFIELD, G. I. Platelets in the carcinoid syndrome: A chemical and ultrastructural investigation. *Brit J Haemat 13*: 181–188, 1967.
7. WHITE, J. G., and KRIVIT, W. An ultrastructural basis for the shape changes induced in platelets by chilling. *Blood 30*:625–635, 1967.
8. WHITE, J. G. Fine structural alterations induced in platelets by adenosine diphosphate. *Blood 31*:604–622, 1968.
9. WHITE, J. G. The dense bodies of human platelets: Origin of serotonin storage particles from platelet granules. *Amer J Path* 53:791–808, 1968.
10. WHITE, J. G. The origin of dense bodies in the surface coat of negatively stained platelets. *Scand J Haemat* 5:371-382, 1968.
11. WHITE, J. G. Effects of ethylenediamine tetracetic acid (EDTA) on platelet structure. *Scand J Haemat* 5:241–254, 1968.
12. WHITE, J. G., and KRIVIT, W. The ultrastructural localization and release of platelet lipids. *Blood 27*:167–186, 1966.
13. STEHBENS, W. E., and BISCOE, T. J. The ultrastructure of early platelet aggregation in vivo. *Amer J Path 50*:219–244, 1967.
14. BORN, G. V. R., and GILSON, R. E. Studies on the uptake of 5-hydroxytryptamine by blood platelets. *J Physiol (London) 146*:472–491, 1959.
15. CRAWFORD, N. Serotonin absorption by normal and "carcinoid" platelets. *Clin Chim Acta 18*:297–307, 1967.
16. WHITE, J. G. The Chediak-Higashi syndrome: cytoplasmic sequestration in circulating leukocytes. *Blood 29*:435–451, 1967.
17. SWIFT, H., and HRUBAN, Z. Focal degradation as a biological process. *Fed Proc 23*:1026–1037, 1964.

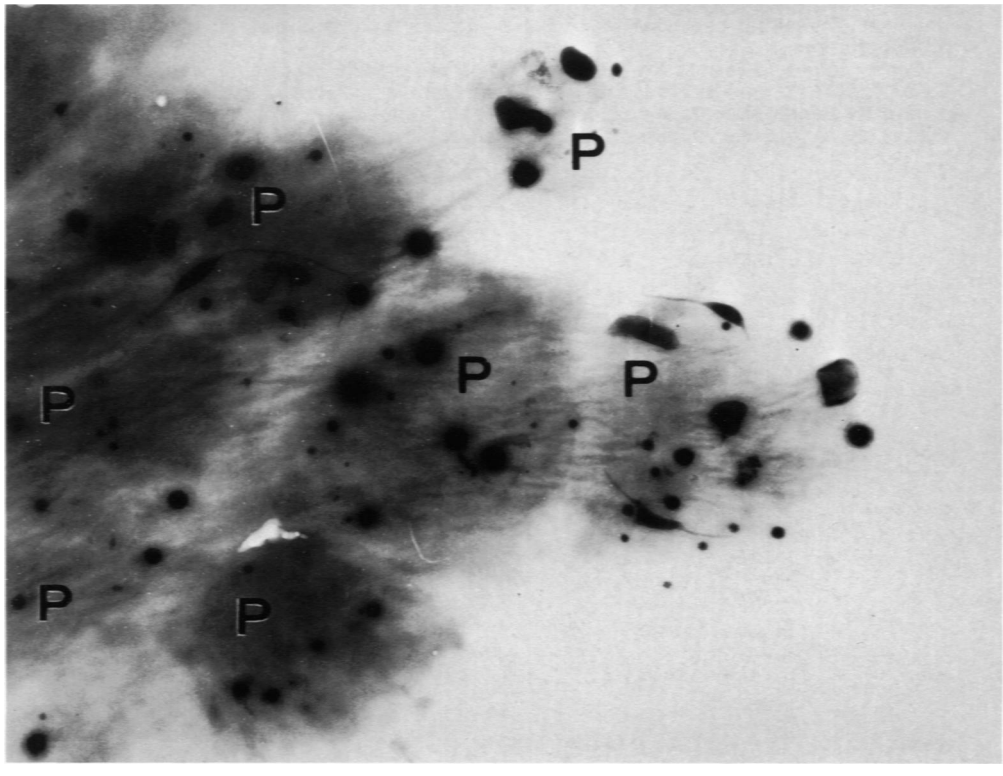
Legends for Figures

Fig 1. Platelets from sample of normal citrated platelet-rich plasma (C-PRP) fixed in glutaraldehyde then osmic acid before imbedding in Epon 812. Thin sections through cells reveal characteristic morphologic features, including a variable number of extremely opaque organelles; 18 dense bodies are indicated by *arrows* in 12 platelets. Portions of additional dense bodies are evident when cells are viewed under a magnifying glass. × 10,200.

Fig 2. Whole mount of platelets from normal sample of C-PRP. Cells were air dried on formvar grid and examined without staining. Portions of seven platelets *(P)* are evident. Over 60 inherently opaque bodies can be identified in the spread cells. × 10,500.



1

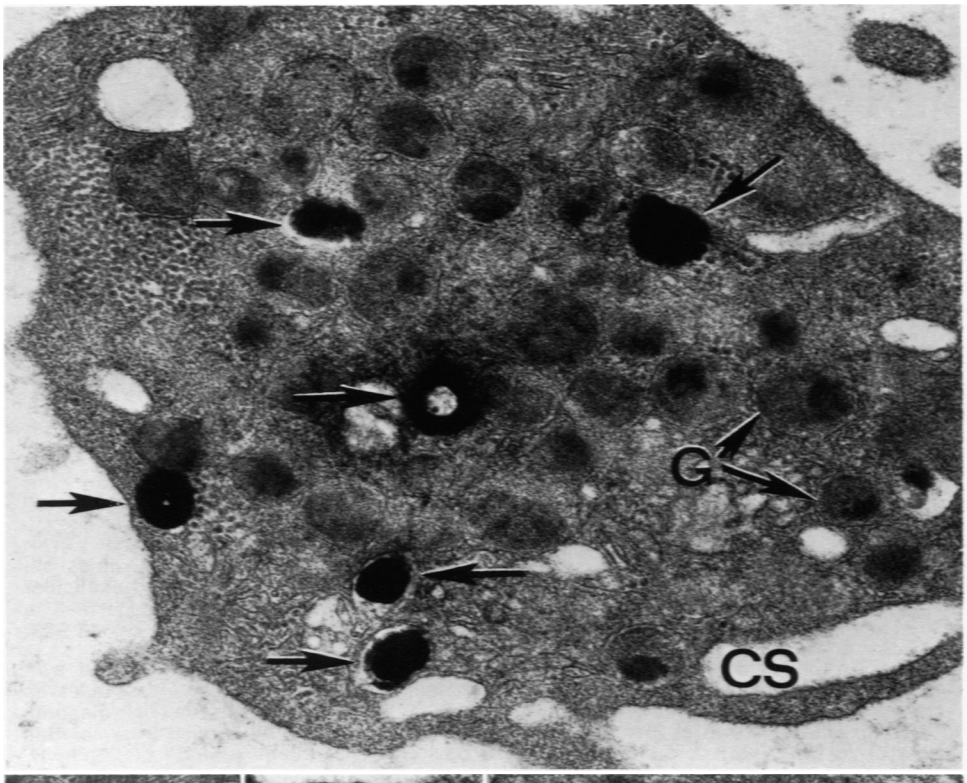


2

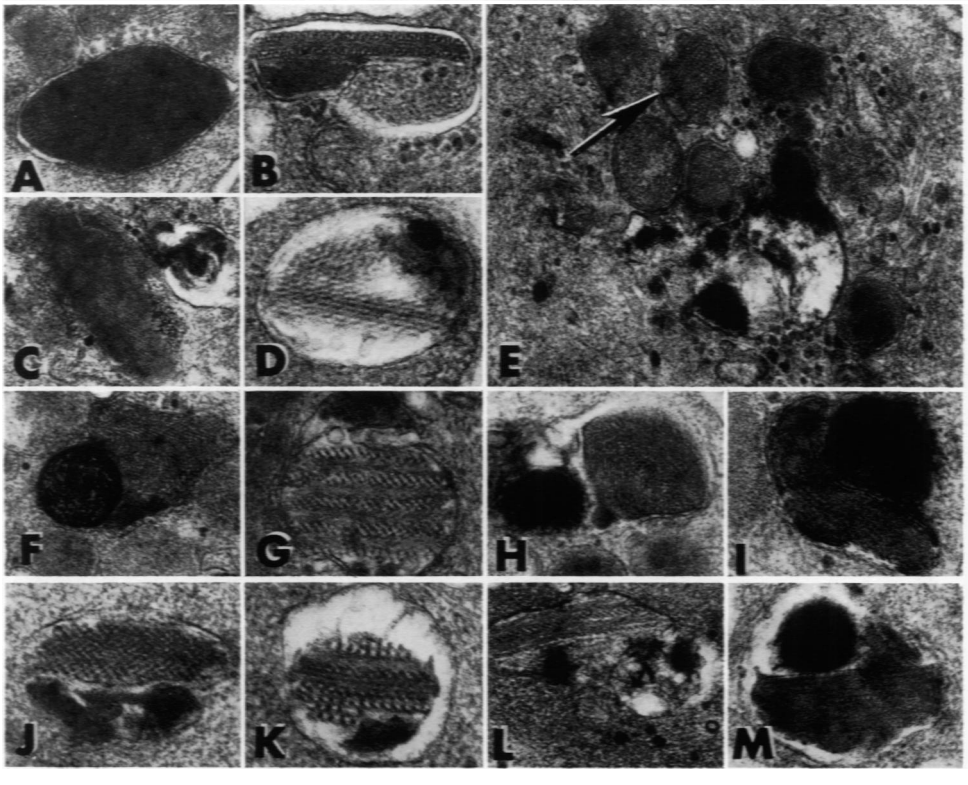
Fig 3. Platelet from patient with carcinoid syndrome. Most platelets in samples obtained from patients had irregular surfaces and dilated channels of the canalicular system (*CS*). Granules (*G*) and other structural elements are usually normal in appearance. Six dense bodies (*arrows*) are evident in this single cell. × 43,900.

Fig 4. Examples of granules present in platelets of 4 patients with carcinoid syndrome. The organization of substructure within the membrane-bound particles is extremely complex and variable. Fine parallel lines are usually intersected by diagonal patterns, yielding the appearance of tire treads. Granules in **A**, **B**, and **C** reveal this unusual arrangement. Patterns of interlaced diagonal lines are evident in **D**, **E**, **F**, **H**, and **J**, and compact groups of circular profiles are apparent in **K**. Particles in **G** and **L** manifest alternating dense bars and diagonal lines; and examples **H**, **I**, and **M** reveal other variations.

Dense bodies in various stages of formation are closely associated with granules in **C**, **D**, **F**, **H**, **I**, **L**, and **M**. Portions of normal granule matrix are retained in **D**, **F**, **G**, **J**, and **K**. Dense appearance of enclosing membrane in **I** and **L** has been observed previously in granules transforming to dense bodies.



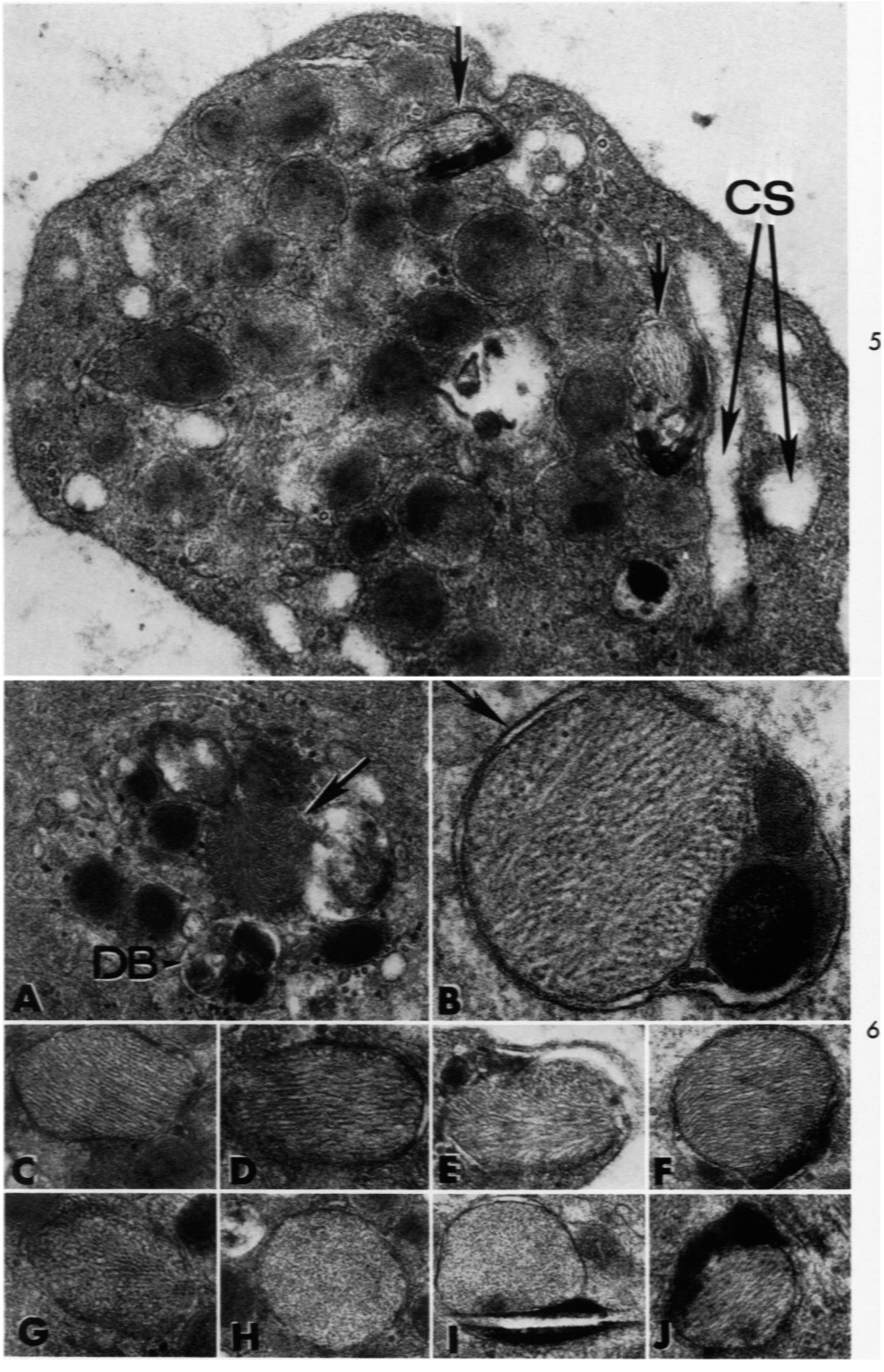
3



4

Fig 5. Platelet manifesting irregular shape, dilated canalicular system (*CS*), and two unusual membrane-bound organelles (*arrow*), from a patient with carcinoid syndrome. Matrix of the organelles consists of irregular parallel lines. Material having the opacity of dense bodies is present at periphery of each organelle. × 41,500.

Fig 6. Other examples of the unusual organelles from 4 patients with carcinoid syndrome. The platelet in **A** had been exposed to adenosine diphosphate 3 min prior to fixation. Mass of filamentous material (*arrow*) is present in central area, and may have fused with another organelle. In **B**, roughly parallel filaments of organelle matrix are cut in various planes; matrix is enclosed by two membranes (*arrow*). Outer layer also encloses a particle resembling a dense body and granules fused with the filamentous mass. Similar double-unit membranes enclose organelles in **C-J**. Granule or dense body material is associated with organelles in **E**, **F**, **I**, and **J**. This type of organelle has the highest frequency of the unusual particles observed in carcinoid platelets.

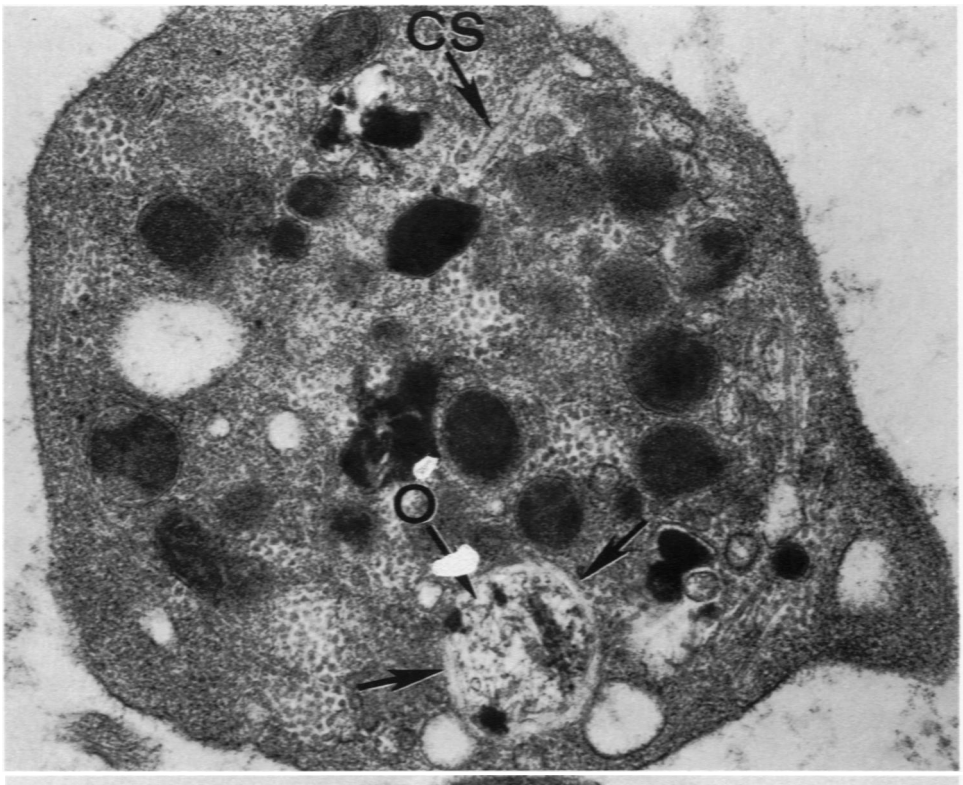


5

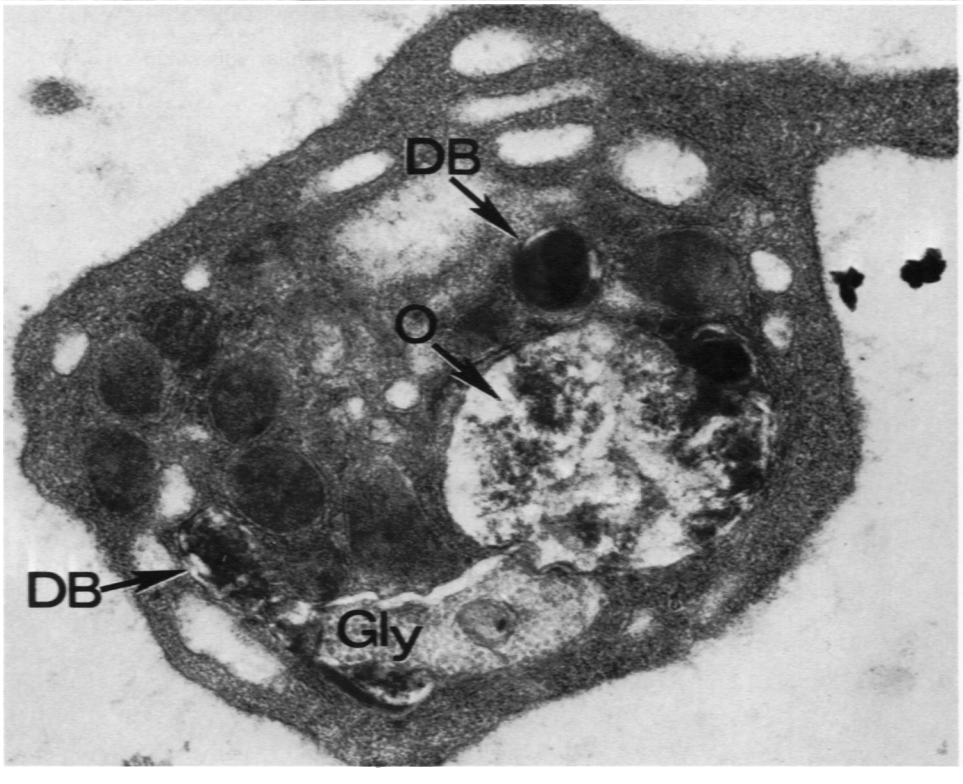
6

Fig 7. Carcinoid platelet in this illustration has a swollen organelle (*O*) containing fragments of opaque substance and debris resembling hyaloplasmic matrix. A channel (*arrow*) similar in appearance to elements of the canalicular system (*CS*) encircles the organelle. × 41,500.

Fig 8. Large cavity is present in central area of this carcinoid platelet. The organelle (*O*) contains debris resembling hyaloplasm, granules, and dense bodies. A dense body is fused to it superior surface. Another dense body (*DB*), adjacent to the organelle, is encircled by concentric lamellae. Dense body (*DB*) near the mass of glycogen (*Gly*) has a long tail-like extension resembling granular substance. × 42,000.



7



8