The Dense Bodies of Human Platelets: Inherent Electron Opacity of the Serotonin Storage Particles

By James G. White

Serotonin (5-hydroxytryptamine, 5-HT) in blood is transported almost exclusively by circulating platelets. Early biochemical and pharmacologic studies demonstrated that most of the 5-HT was found in granules present in platelet hyaloplasm. Recently, several laboratories have suggested that platelet serotonin is concentrated within a specific type of platelet organelle, the dense body (Fig. 1). The relationship between serotonin and dense bodies has been confirmed by ultrastructural autoradiography, and by chemical determinations on pure fractions of dense bodies obtained from rabbit platelets.

The cytochemical basis for localizing 5-HT to specific platelet organelles at the ultrastructural level has been related to a reaction between serotonin and metal containing oxidizing agents used to fix the cells for study in the electron microscope. Platelets exposed initially to osmic acid rarely contained dense bodies. However, if the cells were fixed in glutaraldehyde before exposure to osmic acid, a small population of electron dense particles remained in the hyaloplasm. The opacity of the dense bodies was significantly greater than other granules in the hyaloplasm, suggesting an affinity for osmium. In vitro experiments corroborated the reaction between serotonin and the metallic fixative. Aqueous solutions of 5-HT yielded a dense precipitate in the test tube when combined with glutaraldehyde and osmic acid.

A similar pattern was observed with potassium dichromate was used at low pH to fix platelets. Dense bodies were not preserved when the agent was used alone, but remained if the cells were fixed in glutaraldehyde before exposure to dichromate at pH 4.1 (Fig. 2). Aqueous solutions of 5-HT, glutaraldehyde and potassium dichromate also resulted in dark precipitates when mixed together in vitro.

The method for cytochemical demonstration of 5-HT in platelets evolved from technics used initially to demonstrate chromaffin granules, and to distinguish between epinephrine and norepinephrine containing particles. Glutaraldehyde is believed to form an insoluble complex with unsubstituted...
Fig. 1.—Platelet from sample of citrate platelet rich plasma (C-PRP) fixed initially in glutaraldehyde at 37°C., then in osmic acid at 4°C. and embedded in Epon 812. The blood for this sample was obtained from a child with no measurable platelet or plasma fibrinogen. The discoid shape is supported by the circumferential band of microtubules (T). A fenestrated canalicular system (C.S.), glycogen particles, and moderately opaque granules (G) are present in the hyaloplasm. In addition, two dense bodies (D.B.) are also present. The opaque substance in dense bodies is often contracted away from the enclosing membrane, suggesting that the dark organelles are vacuoles rather than granules. Mag. × 22,400.

catecholamines and indole amines. Free amino groups of these compounds appear to react with the aldehyde to form a Schiff base. The insoluble agents may act on metal containing fixatives by reducing them, or by forming metal complexes. Either mechanism of interaction would result in formation of an electron dense material. The reaction has been discussed in detail in the

Fig. 2.—Cells from C-PRP fixed initially in glutaraldehyde at 37°C., then incubated in potassium dichromate at pH 4.1 for 24 hours before embedding in Epon. Platelets are damaged by dichromate, but dense bodies (D.B.) are well preserved. Mag. × 25,700.
literature,\textsuperscript{14,15} and the evidence strongly supports the concept that electron density of platelet granules containing 5-HT and catecholamines is due to a selective deposition of metallic fixatives within the particles.\textsuperscript{6,8,11}

In 1966 Bull described the ultrastructure of platelets prepared for study in the electron microscope by the negative stain whole mount technic.\textsuperscript{16} He observed a number of electron opaque bodies on the cells which appeared to be imbedded in a coat lying on the exterior surface of the platelet membrane. Subsequent investigations in this laboratory have attempted to define the origin of the dense bodies observed in the extraneous coat of whole mounted platelets.\textsuperscript{17,18} Results of these investigations have indicated that dense bodies are not present on the cell exterior (Fig. 3A, 3B). Rather, they are located in the platelet hyaloplasm among the electron lucent granules. Apparent location of dense bodies in the coat covering the cell membrane as suggested by Bull\textsuperscript{16} is the result of partial extrusion of dense bodies during platelet spreading on grid surfaces.

The finding that dense bodies, formerly thought to lie exterior to the platelet surface, were actually located within the confines of the hyaloplasm immediately raised the possibility that the opaque particles might be similar to dense bodies observed in thin sections of glutaraldehyde-osmium fixed, plastic imbedded platelets. Subsequent evaluation has shown that dense bodies in whole mounted platelets are identical to the hyaloplasmic dense bodies of thin sectioned cells.\textsuperscript{18}

An interesting point raised by Bull in his report\textsuperscript{16} appears to have escaped general recognition. The dense bodies he associated with the exterior coat were electron dense \textit{whether or not} the cells were subsequently stained with phosphotungstic acid. This observation was most intriguing, particularly in view of the fact that the dense bodies in whole mount platelets and serotonin-rich dense bodies in plastic imbedded cells are one and the same.

**EXPERIMENTAL OBSERVATIONS**

In order to clarify the point, drops of citrate platelet rich plasma were placed on carbon stabilized, formvar coated grids, and excess plasma removed from the edge with filter paper.\textsuperscript{19} No stains of any kind were applied to the grids. Examination of the unstained whole mounts in the electron microscope confirmed Bull's findings (Fig. 4A, 4B). Though the outlines of platelets could barely be discerned, the dense bodies stood out sharply. The degree of electron opacity varied, but there was no question that unstained dense bodies were identical to dense bodies in stained whole mounts and those in the hyaloplasm of sectioned platelets.

Additional studies were carried out to define the nature of the inherent opacity of platelet dense bodies. Samples of citrate-platelet rich plasma obtained by methods previously described\textsuperscript{20} were fixed in glutaraldehyde alone. The cells \textit{were not} subsequently fixed in osmic acid or dichromate. Instead, the glutaraldehyde fixed platelets were embedded directly in water-soluble methacrylate. Thin sections obtained from the plastic blocks \textit{were not} stained with uranyl acetate or lead citrate before study. Typical dense bodies, similar in number, size and distribution to those observed in thin sections of glutaraldehyde-osmium fixed cells, were evident in the unstained platelets fixed in glutaraldehyde alone (Fig. 5).

The possibilities that dense bodies might represent droplets of neutral fat or accumulations of platelet fibrinogen were also explored. A 1:10 dilution of intravenous Lipomul*
Fig. 3A.—Whole mounted platelet. The C-PRP was mixed with an equal volume of distilled water before mounting. Cells were negatively stained with phosphotungstic acid. A portion of a swollen platelet is shown. The surface membrane (S) is a pale line on the left side of the illustration. Membranes (M) avulsed from granules (G) and the ribbon-like components of the canalicular system are also electron lucent. Granules (G) free of their enclosing membranes are somewhat opaque, but their substructure can easily be identified. Electron opaque dense bodies (D.B.) are present within the hyaloplasm and outside the ruptured cell wall. Mag. × 23,900.

Fig. 3B.—Negatively stained, whole mounted platelet from a sample prepared in the same manner as the cell shown in the previous illustration. Hyaloplasmic granules (G) with membranes intact appear electron lucent. Dense bodies (D.B.) are also enclosed by membranes in the hyaloplasm, but the opaque particles outside the cells appear stripped of their pale covering. Mag. × 25,700.

in distilled water was prepared, and single drops placed on coated grids. No stain was utilized. Many of the fine droplets of neutral fat were similar in size to dense bodies, but were not electron opaque (Fig. 6). Platelets from a child with absence of measurable plasma or platelet fibrinogen were also studied. Samples of her C-PRP were fixed in glutaraldehyde-osmium and embedded in Epon 812. Thin sections of her platelets contained typical hyaloplasmic dense bodies (Fig. 1).
Fig. 4A.—Whole mount of undiluted, unstained C-PRP appear as vague densities on the grid surface. The dense bodies are clearly apparent, however, and their size, shape and distribution are identical to dense bodies in stained whole mounts or in doubly fixed, sectioned platelets. Opacity of dense bodies varies considerably, just as it does in sectioned cells. Mag. × 25,700.

Fig. 4B.—Another platelet prepared by the whole mount technic, but not stained with phosphotungstic acid. The variable opacity of the unstained dense bodies is apparent. One of the dark particles has a less dense halo strongly resembling the contracted particles in stained materia (see Fig. 1,2). Dense bodies with long tails are common in whole mount preparations. Mag. × 29,400.

Discussion

The evidence provided by these studies suggests that dense bodies present in platelet hyaloplasm are not primarily dependent upon osmic acid or potassium dichromate for their opacity to the electron beam. Particles of similar size, shape, density and distribution were evident in whole mounted platelets, negatively stained or not, and in thin sections of plastic embedded platelets.
Fig. 5.—Platelet from a sample of C-PRP fixed in glutaraldehyde and imbedded in water-soluble methacrylate without being exposed to oxmic acid or potassium dichromate. Thin sections were not stained to enhance contrast. The hyaloplasm has the same vague density as in unstained whole mount preparations. Dense bodies (D.B.) stand out sharply, however. Their size, shape, distribution and opacity are identical to dense bodies in doubly fixed, sectioned platelets. Mag. × 29,400.

Fig. 6.—Droplets of neutral fat prepared by the whole mount method, but not stained with phosphotungstic acid. The particles are similar in size to dense bodies, but do not impede transmission of the electron beam. Mag. × 36,800.

fixed in glutaraldehyde alone. Exposure of glutaraldehyde-fixed platelets to metal containing oxidizing agents may enhance the innate opacity of the particles, but neither osmium nor dichromate is essential for demonstration of the amine rich dense bodies of platelets.

The inherent opacity of platelet dense bodies does not appear to be caused by the usual chemical components associated with platelet organelles. Phospholipid membranes enclosing platelet granules and dense bodies do not inhibit transmission of electrons (Fig. 3A, 3B). The phospholipoprotein rich ma-
trix of platelet granules is more opaque than the enclosing membrane, but passage of electrons is sufficient to permit visualization of particle substructure (Fig. 3A). Dense bodies could result from condensation of fat droplets ingested by platelets or accumulations of platelet fibrinogen. However, neutral fat is not inherently electron dense (Fig. 6), and afibrinogenemic cells contain the usual number of dense bodies (Fig. 1).

Exposure to reserpine or tyramine in vitro eliminates most of the dense bodies from platelets. Cells obtained from animals treated with reserpine are essentially devoid of dense bodies, but incubation of reserpinized platelets with serotonin in vitro restores opaque particles to the hyaloplasm. Serotonin, however, is not innately electron dense. Drops of 1 Molar solutions of 5-HT dried on grid surfaces do not produce dense deposits, and platelets aggregated by serotonin are not coated by dark precipitates. Though concentration of serotonin in platelet dense bodies causes the particles to become opaque, it is not the amine per se which inhibits transmission of the electron beam.

A possible explanation for the inherent opacity of platelet dense bodies has been suggested in previous biochemical studies. The capacity of catecholamines and serotonin to chelate heavy metals is well known, and the investigation of Kerby and Taylor provided evidence that chelation is important for the uptake of serotonin by platelets in plasma systems. Their findings suggested that serotonin formed a complex with calcium and a plasma protein which facilitated transport into platelets. Concentration of calcium in dense bodies along with serotonin may lead to precipitation of chelates or formation of calcium salts. The possibility that zinc, iron, copper or other trace metals are present in dense bodies, however, has not been ruled out. Basic principles of electron optics suggest that innate opacity of particles in biologic tissue is most likely due to a nucleation of heavy metals.

The present study has been concerned with the inherent opacity of platelet dense bodies. Though the exact cause of opacity has not been determined, these findings may have application to other tissues. The cytochemical method for demonstrating catecholamines and indole amines at the fine structural level is based on a specific reaction of amines with metal containing fixatives. Platelet dense bodies containing serotonin and catecholamines are opaque without staining or exposure to metallic fixatives. The results described raise doubts concerning the essential requirement of strong oxidizing agents such as osmium or dichromate for demonstrating amine containing particles. Preparation of other tissues rich in serotonin or norepinephrine in glutaraldehyde alone may reveal a similar inherent density of specific granules. This possibility is under investigation.

**SUMMARY**

Platelets fixed in glutaraldehyde and then in osmic acid or potassium dichromate contain dense bodies which are known to be rich in serotonin. Opacity of the particles is believed to be due to a highly specific reaction between glutaraldehyde, serotonin and osmic acid. The present study has examined
dense bodies of platelets in negatively stained whole mount preparations, unstained whole mounts, and glutaraldehyde fixed, plastic embedded cells not exposed to osmium or dichromate. The results demonstrate that dense bodies in platelet hyaloplasm are innately electron opaque. Osmic acid and potassium dichromate may deposit in dense bodies, but the metal containing oxidizing fixatives are not the primary cause of opacity to the electron beam. It is suggested that the inherent electron opacity may be due to a nucleation of heavy metals in the matrix of dense bodies.

SUMMARIO IN INTERLINGUA

Plachettas fixate in glutaraldehyda e postea in acido os'mic o dichromato de kalium contine dense corpores que es cognoscitamente nc in serotonina. Es opinate que le opacitate de iste particulas es le effecto de un altemente specific reaction inter glutaraldehyda, serotonina, e acido osmic. Le presente studio ha examine le dense corpores del plachettas in negativemente tincturate preparationes a montage integre, in non-tincturate montages integre, e in cellulas includite in plastico post fixation a glutaraldehyda, non exponite a osmium o dichromato. Le resultatos demonstra que dense corpores in le hyaloplasm del plachettas es innatemente opac pro electrones. Acido osmic e dichromato de kalium pote deponer se in le dense corpores, sed le fixativos oxidatori a contento de metallo non es le causa primari del opacitate pro le fasce de electrones. Es suggestionate que le inherente opacitate pro electrones es possibilemente le effecto de un nucleation de metallos pesante in le matrice del dense corpores.

REFERENCES


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