



FIGURE 1 Unstained section of platelets demonstrating dense 5-HT granules. $\times 21,000$.

FIGURE 2 Above section after microincineration at 450°C for 15 min. Dense granules remain unchanged, suggesting presence of inorganic material. $\times 21,000$.

tification a focusing crystal detector was used. A 100-s counting time was found to be suitable for this analysis. The beam size of this instrument is just under $2,000 \text{ \AA}$, and when the beam was centered over one of the 5-HT granules, an average count of 120 was obtained for calcium, and an average count of 1,800 was obtained for phosphorus. Areas of the platelet cytoplasm of comparable size and free of the 5-HT granules were used as controls. These control areas gave counts of zero for calcium and an average of 1,200 for phosphorus. Interpretation of these counts can be confusing. The zero count for calcium does not mean that there is no calcium in that part of the platelet cytoplasm; it simply means that there is much less calcium present there than in the 5-HT granule. The minimum amount of calcium detectable by X-ray spectroscopy is

approximately 10^{-16} g ; therefore, we can conclude from these counts that considerably more calcium is present in the 5-HT granules than in a comparable area of the platelet cytoplasm.

DISCUSSION

In our experience, if the 5-HT granule is apparent, then calcium can be proved to be present by the above described techniques. The functional significance of the calcium and how it is associated with the 5-HT in these granules is not known, but it may act to increase the storage of 5-HT by forming osmotically stable, high molecular weight aggregates of amines and nucleotides combined with bivalent cations (6). These investigators (6) reported that the concentration of calcium is at least 50 times higher in isolated dense granules than in intact platelets.

The above described techniques would be of interest in studying other amine-containing granules such as chromaffin granules of the adrenal medulla or mast cell granules to determine if they also contain high concentrations of bivalent metals.

SUMMARY

By using electron microscopy combined with microincineration and X-ray spectroscopy, calcium has been proved to be present in the 5-HT granules of platelets. The functional significance of this calcium is unknown, but it may act to increase the storage of 5-HT.

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