THE LOCALIZATION OF CALCIUM AND PHOSPHORUS IN HUMAN PLATELETS

R. J. SKAER
Department of Medicine, Hills Road, Cambridge

P. D. PETERS
Cavendish Laboratory, Cambridge

AND J. P. EMMINES
Department of Medicine, Hills Road, Cambridge, England

SUMMARY

Large amounts of calcium and phosphorus have been found by electron-microprobe analysis in the dense bodies of frozen-dried, human platelets. The calcium and phosphorus occurred in a fixed ratio that was similar to that in dicalcium ATP. No other elements were detectable except chlorine. Minute, electron-dense disks were found in the surface membranes of glutaraldehyde-fixed platelets, and are very variable in number in any population of normal platelets. Both dense bodies and membrane granules are absent if platelets are fixed in the absence of calcium, and are also absent from aggregated platelets.

INTRODUCTION

Calcium ions are necessary for blood to clot. They catalyse many of the reactions in the plasma and on the platelet surface (Walsh & Biggs, 1972) that result in the production of thrombin. Calcium ions are also necessary for the platelet release reaction (Grette, 1962; Sneddon, 1972), by which platelets secrete substances such as serotonin (5HT) and ADP (Mills, Robb & Roberts, 1968). These 2 substances are contained together within granules (dense bodies) in the platelets and when released enhance platelet aggregation (Holmsen, Day & Stormorken, 1969). Platelets contain a large but variable amount of calcium (Cousin & Caen, 1964); moreover, when they aggregate up to 88% of this is released (Müller, 1969). That calcium was in granules had already been suspected by Müller (1969), since very little platelet calcium exchanges with extracellular 45Ca. White (1969, 1971) claimed that calcium is localized in the dense bodies, for the time course of release of calcium from platelets coincides with that of the release reaction for dense bodies; he pointed out that platelet dense bodies contain serotonin (Davis & White, 1968) and 'polyanionic acidic mucosubstances', both of which may chelate calcium (Kerby & Taylor, 1961). The evidence is thus indirect.

On the other hand, if the experiments of Pletscher (1969) and Berneis, Pletscher & Da Prada (1969, 1970) are a relevant model for the formation of the granules that contain 5HT, ADP and ATP, only small amounts of calcium should be present in these granules. They found that mixtures of 5HT or other biogenic amines with ATP
form long-chain polymers, especially if a small amount of calcium is present. Larger amounts of calcium cause the break-up of the polymers.

Bygdeman & Stjärne (1971) suggest that calcium is in the platelet membrane since, although calcium is necessary for the release reaction, no significant uptake of $^{45}$Ca from the medium occurred during platelet aggregation.

We have used electron-probe analysis to investigate the composition of platelet granules and in particular to localize calcium and phosphorus. This provides direct evidence that large amounts of both calcium and phosphorus are present in dense bodies.

MATERIALS AND METHODS

Blood was obtained from untreated patients attending a clinic for varicose veins. Only those patients whose platelets exhibited normal aggregation following exposure to ADP were used as donors. Blood was collected in siliconized glassware and processed immediately. No anti-coagulant was used.

Glutaraldehyde fixation

Whole blood was normally fixed in 3% redistilled glutaraldehyde containing 1.25 mM Ca$^{2+}$ and 1.7% sucrose, buffered at pH 7.4 with 0.05 M Hepes (B.D.H.). Fixation was for 2.5 h at 37°C; the mixture was then cooled to 4°C for 12 h and the sedimented red cells and most of the white cells were removed. The platelets were dehydrated in a graded series of ethanol and embedded in Spurr low-viscosity resin. Sections of platelets were occasionally stained for 1 min in a saturated aqueous solution of uranyl acetate brought to pH 1.8-2 with 1 N HCl. This showed up platelet membrane granules and leucocyte micropapillae.

For calcium-free fixation, redistilled glutaraldehyde was always used, since many samples of commercial glutaraldehyde contain calcium (Oschman & Wall, 1972). Whole blood was added to a large volume of 3% glutaraldehyde and 1.7% sucrose, buffered with 0.1 M redistilled collidine (Polysciences). The cells were immediately spun down and resuspended in fresh calcium-free fixative.

Freeze drying

Whole blood was allowed to stand for 25 min at 4°C in a siliconized, wide-bore glass tube. Plasma from which the red cells had settled was removed and squirted as a very fine jet into isopentane cooled with liquid nitrogen. The powdery slurry of frozen plasma and isopentane was transferred to the cold plate of an Edwards Speedivac-Pearse Model 1 tissue freeze drier and was dried below −65°C for 3 days. The drying temperature was chosen to be below the lowest eutectic point of a calcium salt. This method of freezing and drying produced ice crystals in the plasma, but none could be seen in the platelets, even with high-resolution electron microscopy. Absolute ethanol was added to the resulting powder and the mixture was degassed by regulating the vacuum in the tissue drier. The cells were warmed to room temperature during this stage. The unfixed plasma and platelets were then infiltrated with 2 changes of Spurr low-viscosity resin and embedded in a third change at 60°C. Sections were cut from the blocks either in the normal way and picked up rapidly from the water surface, or on a dry knife edge, stretched mechanically with specially shaped forceps and then placed on carbon-coated grids. Both copper grids and palladium grids were used, coated with carbon and Celloidin. Sections were examined without further treatment in an AEI EM 6B operating at 60 kV and in an AEI EMMA-4 operating at 40 kV.
Aggregated platelets

These were prepared by centrifuging freshly drawn blood, taken without an anticoagulant, in a siliconized centrifuge tube at 250 g for 10 min. The supernatant of platelet-rich plasma was kept in a water bath at 37 °C; to this was added 10^{-5} M ADP to give a final concentration of 1 μM (Hardiart et al. 1970). After 10 min the aggregated platelets were fixed in the normal 3% glutaraldehyde fixative, buffered at pH 7.4 with 0.05 M Hepes and containing either 1.25 or 4 mM calcium. The higher concentration of calcium was to ensure that no platelet calcium was dissolved in the fixative. The fixed cells were cooled to room temperature and left for 6 h; they were then cooled to 4 °C for 12 h. The platelets were dehydrated in a graded series of ethanol and embedded in Spurr low-viscosity resin.

Electron-probe analysis

Specimens were analysed for elemental composition in an AEI EMMA-4 analytical electron microscope. An accelerating voltage of 40 kV was used and the beam current was about 6 nA. The electron beam was focused with a mini-lens to a minimum spot diameter of approximately 150 nm. The analytical equipment consists of 2 diffracting crystal spectrometers. A Keveks Si(Li) energy-dispersive detector has been added to the microscope, and it was this detector which was used in the present study. Its output was displayed on a TV screen in the form of number of pulses recorded against energy, giving peaks in the regions of the elements present in the specimen. Photographs were taken of the TV screen to record spectra, and the data could also be recorded directly on paper with an X-Y plotter. From the TV display, data were recorded within a set window giving total integral counts under a peak (P), and the corrected integral count (P−b), with the count due to background radiation (b) subtracted. When specimens were analysed to determine whether they contained Cu, the specimens were mounted on Pd grids and analysed in a Ti specimen holder.

Calibration

Thin sections of dentine, the mineral composition of which is approximately that of apatite (Ca_{10}P_{6}O_{16}•H_{2}O), were used as a standard to set windows on the Si(Li) detector around the peaks due to radiation emitted by Ca and P. The dentine was also used to find the ratio of the integral counts due to calcium and phosphorus, the elements being present in the atomic ratio P:Ca. A fine dusting on to a carbon-coated grid of dicalcium ATP (Sigma), mol. wt 583.3, whose elemental composition in the anhydrous state is C_{10}H_{12}N_{10}O_{13}P_{3}Ca_{2}, was also used as a standard for measuring the P/Ca peak ratio with the elements present in the atomic ratio P:Ca. The X-ray emission is influenced by the sensitivity of the standard or specimen to beam damage over the period of electron bombardment.

RESULTS

Electron microscopy

Two components of platelets were found to be electron-dense in material that had been fixed in glutaraldehyde containing 1.25 mM calcium: platelet dense bodies (White, 1970) (Fig. 1), and some previously undescribed, dense granules in the platelet membrane (Fig. 2). The platelet dense bodies are spheres, approximately 100 nm in diameter (though often smaller), which in both glutaraldehyde-fixed and also frozen-dried platelets are almost uniformly electron-dense. The frequency of occurrence of the platelet dense bodies was similar to that found in human platelets by White (1970), i.e. approximately 8 per whole platelet, provided that calcium ions were included in the fixative. If calcium was rigorously excluded from the fixative, no dense bodies were seen.
The membrane granules are small plates or disks 8–10 nm high and approximately 15 nm in diameter. The frequency of occurrence of these membrane granules is very variable within any given sample of platelets; often there are none in a platelet profile (Fig. 1), sometimes 2 or 3, and very occasionally more than 10 (Fig. 2). The membrane granules occur principally on the plasma membrane, but also on the membranes lining the channels of the open canalicular system of the platelet (Fig. 6). Like the platelet dense bodies, the membrane granules are completely absent if calcium is excluded from the fixative. Membrane granules could not be seen in frozen-dried platelets (Fig. 5). The frozen-dried platelets in our preparations are usually surrounded by a coat that is slightly electron-dense and is probably produced by extracellular ice crystals. These could cause the deposition of plasma salts on to the platelet membrane and thus obscure the membrane granules. When the acidified uranyl stain devised by Clawson & Good (1971) was used to stain sections of glutaraldehyde-fixed platelets, the membrane granules stain intensely (Fig. 6), as do the micropapillae of leucocytes (Fig. 4).

No platelet dense bodies or membrane granules have been seen in the aggregated platelets, even when 4 mM calcium was present in the fixative. Platelets have not been examined at the first (reversible) phase of aggregation to determine which, if any, of the dense components are present then.

**Electron-probe analysis**

This has been confined to the platelet dense bodies in material that had been frozen dried. Platelets fixed in glutaraldehyde containing 1.25 mM calcium gave such variable results with electron-probe analysis that they were not used. The membrane granules were too small and sparse, unfortunately, to give a significant reading.

A spectrum obtained with the beam incident on the dentine standard is shown in Fig. 7; at 1970 eV is the phosphorus peak and at 3670 eV is the much larger calcium peak. The peak (P) and peak minus background (P−b) data for calcium and phosphorus are given in Table 1, together with the P:Ca peak count ratios.

Dense bodies from frozen-dried platelets show large and unambiguous peaks at wavelengths corresponding to phosphorus and calcium (Figs. 8, 9). All other peaks can be shown, by moving the microbeam off the platelet granule, to be due to emission from the embedding medium, the grid or the instrument (Fig. 10). Thus the small peak at 1760 eV is due to silicon, which may originate in the Si (Li) detector itself, or may be contamination arising in the microscope; the peak at 2260 eV is due to sulphur contamination, possibly arising from the pump oils; and the peak at 2600 eV is at least partly due to chloride in the embedding medium (van Steveninck, van Steveninck, Hall & Peters, 1974). The large peaks at 8040, 8900 and 890 eV are the Kα, Kβ and L emissions respectively from copper in the grid and instrument. There were no other significant peaks.

In this study an estimate of the quantities of P and Ca in the deposits was not possible, since the diameter of the dense bodies was smaller than that of the electron probe: quantitation depends upon the probe spot being entirely incident on the structure being analysed. When, however, the signals from dense bodies in frozen-
Calcium in platelets
dried platelets that had been cut on a water surface (Fig. 8) were compared with those from sections that had been cut dry (Fig. 11), there was no significant change in the P:Ca ratio (Table 1). There was no difference in the total number of peaks present, and the electron density of the platelet dense bodies prepared in the 2 ways was the same. Thus our results show that there does not appear to be a significant loss of material from platelet dense bodies when sections of plastic-embedded material are cut on to a water surface.

### Tables 1. Results of electron-probe analyses

<table>
<thead>
<tr>
<th></th>
<th>Phosphorus</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>P-b</td>
</tr>
<tr>
<td>Dentine</td>
<td>14288</td>
<td>13153</td>
</tr>
<tr>
<td></td>
<td>7072</td>
<td>6143</td>
</tr>
<tr>
<td>Dicalcium ATP</td>
<td>5798</td>
<td>5256</td>
</tr>
<tr>
<td></td>
<td>7172</td>
<td>6780</td>
</tr>
<tr>
<td>Dense body</td>
<td>18392</td>
<td>10063</td>
</tr>
<tr>
<td></td>
<td>7702</td>
<td>6012</td>
</tr>
<tr>
<td></td>
<td>5231</td>
<td>3801</td>
</tr>
<tr>
<td></td>
<td>4459</td>
<td>2541</td>
</tr>
<tr>
<td></td>
<td>3436</td>
<td>1426</td>
</tr>
<tr>
<td></td>
<td>6207</td>
<td>4707</td>
</tr>
<tr>
<td></td>
<td>3088</td>
<td>1403</td>
</tr>
<tr>
<td></td>
<td>2425</td>
<td>1426</td>
</tr>
<tr>
<td></td>
<td>1630</td>
<td>1051</td>
</tr>
<tr>
<td></td>
<td>1913</td>
<td>1267</td>
</tr>
<tr>
<td></td>
<td>4402</td>
<td>3473</td>
</tr>
<tr>
<td></td>
<td>12784</td>
<td>7143</td>
</tr>
<tr>
<td></td>
<td>2549</td>
<td>1141</td>
</tr>
<tr>
<td></td>
<td>2539</td>
<td>1380</td>
</tr>
<tr>
<td></td>
<td>1815</td>
<td>722</td>
</tr>
<tr>
<td></td>
<td>3821</td>
<td>2872</td>
</tr>
<tr>
<td></td>
<td>2739</td>
<td>1554</td>
</tr>
<tr>
<td></td>
<td>4334</td>
<td>2910</td>
</tr>
</tbody>
</table>

Mean P:Ca ratio for dense bodies is 1.15 (s.e. = ±0.04, n = 18).
* Sections cut and mounted dry.

In order to test whether the platelet dense bodies themselves contained copper or zinc or other elements whose emission was likely to be swamped by that from copper in the grid, sections were mounted on palladium grids held in a titanium holder. No significant quantities of copper or zinc were detected (Figs. 13, 14). There is a suggestion of a slight peak at approximately 8000 eV (Fig. 13); this is probably due to very slight emission from copper in the electron microscope, for it is not affected by moving the electron beam off the dense body and on to the platelet cytoplasm (Fig. 14).

The ratio between emission peaks of phosphorus and calcium in platelet dense bodies was measured (Table 1) and gave a mean P:Ca ratio of 1.15 (s.e. = ±0.04, n = 18). An almost identical ratio between these peaks was found in dicalcium ATP (Fig. 15); 2 measurements of this ratio gave values of 1.04 and 1.15.
When the microprobe spot was moved on to parts of platelets free from dense bodies, no calcium and almost no phosphorus signals were obtained (Fig. 14); since dense bodies are the only component of platelets that contain detectable quantities of calcium, most of the large calcium content of platelets must be in these bodies.

DISCUSSION

Composition of dense bodies

The electron density of platelet dense bodies must be due to calcium and phosphorus, since no other elements were detected in them with the electron microprobe. Fixation in glutaraldehyde with calcium results in dense bodies with a more homogeneous dense appearance than does fixation in osmium or glutaraldehyde and osmium. This is probably due to decalcification of the dense bodies by osmium. Decalcification will occur unless the osmium is saturated with calcium (Oschman, Hall, Peters & Wall, 1974). Fixation in the absence of calcium results in loss of calcium from the dense bodies; even in the presence of 1-25 mM Ca^{2+} there is a large variation in P:Ca ratio of platelet dense bodies. Tissues can take up calcium during fixation (Schoenberg, Goodford, Wolowyk & Wooton, 1973), so one might suspect that the density of dense bodies in platelets fixed in glutaraldehyde and calcium might be due to calcium taken up from the fixative. This does not seem to be the case, since dense bodies in sections of frozen-dried, unfixed platelets also have a homogeneous dense appearance. White (1970) showed that dense bodies are homogeneous in whole mounts of unfixed platelets.

The quantity of calcium in dense bodies cannot at present be calculated. The dense bodies in sectioned material are too small for the entire microprobe spot to be contained within a dense body, so accurate quantitation by microprobe analysis cannot be used. The quantity of calcium must be large, however, since the dense bodies are so electron-dense and the microprobe shows this can only be due to calcium and phosphorus, and that only dense bodies in platelets give a significant reading for calcium. Thus the majority of the total calcium content of platelets (1.23–3.9 mg/g dry wt., Cousin & Caen, 1964) must be in the dense bodies. One cannot use the known ATP and ADP content of dense bodies (Mills & Thomas, 1969; Holmsen, Day & Storm, 1969) to calculate their calcium content from the P:Ca ratio we have measured (Table 1), for we do not know that all the phosphorus peak is due to adenine nucleotides.

It would seem that the presence of large amounts of calcium in dense bodies might militate against the theories of Berneis, Pletscher & Da Prada (1969, 1970) on the forces that influence the formation of dense bodies and other granules rich in both biogenic amines and ATP. These authors regarded the polymerization of ATP with serotonin as a significant feature in the formation of dense bodies. In their experiments a small amount of divalent cation increased the degree of polymerization; larger amounts broke up the polymers. They themselves found, by flame photometry, large amounts of magnesium and a little calcium in isolated dense bodies of rabbit platelets, but did not discuss the matter further (Berneis, Da Prada & Pletscher,
Calcium in platelets 685

1969). If the calcium in dense bodies were found to be non-ionized, however, it might not influence the polymers in the way that ionized calcium does. Wallach, Surgenor & Steele (1958) found that more than 80% of the calcium in platelets went into the lipid fraction when stored platelets were extracted with chloroform and methanol. In view of the rapid and extensive disintegration of stored platelets found by numerous authors (Wolf, 1967) it is as well to bear in mind that some of the analyses reported by Wallach et al. were on platelets that had been stored for up to 18 months at 4 °C. Robblee, Shepro, Blamarich & Towle (1973) fixed platelets that had been transferred to a calcium-free medium in glutaraldehyde containing pyroantimonate. There were no pyroantimonate deposits and therefore supposedly no ionized calcium in normal unaggregated platelets. The calcium in dense bodies may well be stored, therefore, in a non-ionized form.

While it is likely that most of the large phosphorus peak we obtained from dense bodies is due to ATP and ADP, some of the phosphorus signal could be from phospholipid, possibly in the membrane bounding the dense body (White, 1970). This phospholipid may correspond to platelet factor 3, since (1) clotting activity due to this factor appears at the same time as the platelet release reaction (Hardisty & Hutton, 1966; White, 1971); (2) there is a condition (Hermansky-Pudlak syndrome) in which there are few dense bodies and reduced levels of platelet factor 3 (White, 1972; White, Edson, Desnick & Witkop, 1971); and (3) platelet factor 3 activity is associated with the membrane of aggregated platelets (Born, 1972), possibly due to fusion of the granule membrane with the plasma membrane. Lipid has been isolated from platelet granules (Marcus, Ullman & Safier, 1969), but it is possible that most of it came not from the dense bodies but from another sort of granule-containing myelin figures (White & Krivit, 1966).

Chlorine may occur in platelet dense bodies as well as in the embedding medium. This could be checked with air-dried platelets.

No potassium emission was obtained from platelet dense bodies. Although Zieve, Gamble & Jackson (1964) found some bound potassium in platelets, and potassium is released during aggregation (Holmsen, Day & Stormorken, 1969) and enhances the release reaction (Born & Cross, 1964), it is unlikely that platelet dense bodies contain potassium. It is more likely that potassium is released from the cytoplasm, for its release can be independent of the release of serotonin – a substance that does occur in dense bodies (Buckingham & Maynert, 1964).

No zinc was detected in platelet dense bodies, although zinc is released from platelets during blood clotting (Foley et al. 1968). It is probable that the amount present in platelets (0.2–0.45 μg/10⁹ platelets) should be detectable with the microprobe if all this zinc were present in dense bodies. As Foley et al. (1968) point out, however, zinc is a component of several enzymes present in platelets. The release of zinc from platelets may well be a relatively slow leakage through the membranes of the aggregated platelets.
Membrane granules

Dense membrane granules have been seen in several, very different cell types: intestinal cells (Oschman & Wall, 1972), erythrocytes (Oschman & Wall, 1973), squid axons (Oschman et al. 1974), and leucocytes (Clawson & Good, 1971). As with platelet membrane granules, these dense granules are visible only if calcium ions are present in the fixative. In the case of the platelet granules it is tempting to regard them as membrane-bound sites of calcium. Since, however, they have been seen so far only in fixed material we do not know whether these granules represent sites where calcium or other electron-dense material is bound to the membrane in the living cell, or whether they are calcium deposits resulting from fixation in a fixative containing calcium. As pointed out by Bygdeman & Stjärne (1971), the existence of calcium deposits in the membrane of the living platelet could explain the fact that while the release reaction is dependent on calcium, no significant uptake of $^{46}$Ca is detectable on aggregation. The disappearance of the granules from the membranes of aggregated platelets is not incompatible with this view. Until we know more about the composition of the granules and whether they are produced by fixation, further speculation on their function and the reasons for their very different numbers on different platelets is pointless.

We are most grateful to Professor F. G. J. Hayhoe, Dr D’A Kok and Dr H. le B. Skaer for reading and criticizing this paper, and to Dr J. L. Oschman for predicting the existence of electron-dense membrane granules in platelets and commenting on the manuscript. We thank Dr D’A Kok for the samples of normal blood, and Miss S. Tomlin and Mrs P. Montgomery for checking that the platelet aggregation in these samples was normal. We thank Dr T. A. Hall for preliminary discussions about the microprobe analysis. R.J.S. was supported by the Leukaemia Research Fund. Funds for the instrumentation and research were provided by the British Science Research Council.

NOTE ADDED IN PROOF

After this paper went to press a paper by Martin, J. H., Carson, F. L. & Race, G. J. ‘Calcium-containing platelet granules’ appeared in J. Cell Biol. (1974) 60, 775–777. These authors found both calcium and phosphorus in platelet dense bodies but the ratio between the two, with peak counts for P 15 times as many as the peak counts for Ca, is very different from the ratio we find. This could be because they used platelets fixed in osmium so that the dense bodies were largely decalcified. However, they do not indicate how their ratios relate to the composition of the calcium-phosphorus compound.

REFERENCES

Calcium in platelets


R. J. Skaer, P. D. Peters and J. P. Emmines


*Received 20 December 1973*

Fig. 1. Section of platelet fixed in glutaraldehyde containing 1.25 mM Ca\(^{2+}\) showing a platelet dense body. The dense body is unusually close to the cell membrane in this platelet. \(\times 45\,000\).

Fig. 2. Section of platelet fixed in glutaraldehyde containing 1.25 mM Ca\(^{2+}\) showing many dense membrane granules. \(\times 45\,000\).

Fig. 3. Section of platelet fixed in glutaraldehyde containing 1.25 mM Ca\(^{2+}\) showing a typically placed dense body. Two small dense membrane granules are visible in the adjacent plasma membrane. \(\times 75\,000\).

Fig. 4. Section of monocyte fixed in glutaraldehyde containing 1.25 mM Ca\(^{2+}\) stained with acidic uranyl acetate showing dome-shaped electron-dense micropapillae. \(\times 75\,000\).

Fig. 5. Section of frozen-dried platelet containing 2 large and 2 small dense bodies. The large dense body at lower right is typical, that above is a shape occasionally seen, especially if the platelet is starting the release reaction. The 2 small dense bodies resemble developing dense bodies described by White (1970). Other platelet granules are only slightly more electron-dense than the cytoplasm. No ice-crystal artifacts are present. \(\times 28\,000\).

Fig. 6. Section of platelet fixed in glutaraldehyde containing 1.25 mM Ca\(^{2+}\) stained with acidic uranyl acetate showing many electron-dense membrane granules in the plasma membrane and the membrane of the open canalicular system. \(\times 96\,000\).
Calcium in platelets
Fig. 7. Emission spectrum from section of dentine used as a standard. Cu grid.

Fig. 8. Emission spectrum from a platelet dense body in a thick section of a frozen-dried platelet cut on a water surface in the normal way. Cu grid.

Fig. 9. Wide-scale emission spectrum of a platelet dense body prepared as in Fig. 8. Cu grid.
Fig. 10. As Fig. 9 but with the analyser spot moved off the platelet. Cu grid.

Fig. 11. Emission spectrum from a dense body in a frozen-dried platelet sectioned on a dry knife edge and stretched mechanically. Cu grid.

Fig. 12. Emission spectrum from a platelet dense body prepared as in Fig. 8. Pd grid.
Fig. 13. Wide-scale emission spectrum from a dense body in a frozen-dried platelet sectioned on a dry knife and stretched mechanically. Pd grid.

Fig. 14. As Fig. 13 but with the analyser spot moved on to a part of the platelet free from dense bodies. Pd grid.

Fig. 15. Emission spectrum from dicalcium ATP powder. Cu grid.