rate of hemoglobin LR is about 2-fold slower than that of hemoglobin A similar to the case of hemoglobin Wood. These studies suggest that in spite of slower reduction of hemoglobin LR, cells containing such hemoglobin are not more susceptible to the oxidizing effect of nitrites. However, this does not guarantee that under physiological conditions when part of the hemoglobin circulates in the deoxy form this may not happen.

The conclusion is that although the carriers of those high oxygen affinity mutant hemoglobins which are stabilized in the R conformation might develop methemoglobinemia after drug ingestion (due to slow reduction rate), they might be as well more sensitive to the oxidizing effect of nitrites than carriers of normal hemoglobin although this has not been tested in vivo.

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Platelet heterogeneity and dense tubular system changes on activation

R. Yarom, G. Lijovetzky and Y. Havivi

Department of Pathology, Hebrew University, Hadassah Medical School, POB 1172, Jerusalem (Israel), 3 April 1980

Summary. Platelets impregnated with heavy metals appeared as 3 distinct morphological types: 'reticular' cells with a polygonal dense tubular network and stained granules, dark metallophilic cells, and pale metallophobic cells with microvesicles and non-staining granules. On stimulation, type 1 cells decreased while type 3 cells increased, suggesting that with activation dense tubules break up into microvesicles and granules become metallophobic. In the type 2 cells a different functional mechanism may exist.

The dense tubular system (DTS) of platelets is considered to be the pivotal structure in cell activation. Its functions are concerned with enzyme and prostaglandin metabolism and also, in analogy to muscle sarcoplasmic reticulum, with control of intracellular calcium fluxes[1-5].

The evidence for this is largely circumstantial. While various microanalytical and histochemical methods have succeeded in demonstrating calcium stores in dense bodies, nucleoids of alpha granules, plasmalemma and its extensions[6-11], they have so far failed to detect DTS-related calcium. In fact, not even the proper structure of the DTS has been described.

The technique of heavy metal impregnation has been employed in several cell types to study the endoplasmic reticulum[12,13]. It has been applied here to platelets with the hope that it will reveal the dense tubules at rest and following stimulation.

Materials and methods. The cases studied comprised 14 healthy volunteers, 9 females and 5 males. 4 cases were 15–19 years old, 8 were 20–30 and 2 were over 40. The blood was collected by venipuncture into 0.1 volume of 3.8% trisodium citrate. Platelet rich plasma (PRP) was separated by standing for 1 h at 37 °C. It was fixed with 0.2% glutaraldehyde in 0.1 M cacodylate buffer for 30 min at 37 °C, ph 7.4. The fixed PRP was then centrifuged at 300 × g for 15 min and the pellet was further fixed for 30 min in 2% glutaraldehyde in the above buffer. The pellet was next treated with a 5% solution of uranyl acetate at a pH of 3.5 for 1 h at 37 °C. After quick rinses in water a double solution of lead and copper citrate was added and this was followed by overnight postfixation in osmium tetroxide at 4 °C. The pellet was then dehydrated in graded alcohols, cut into small pieces and embedded in araldite.

In several cases the uranyl acetate solutions were adjusted to pH's of 1, 5.5, and 7[13]. Sections 0.1–0.5 µm thick were cut onto formvar coated grids which were examined unstained with a Philips 400 electron microscope using accelerating voltages of 60–100 kV.

Platelet type distribution at rest and after stimulation

	Type 1 cells (%)	Type 2 cells (%)	Type 3 cells (%)
Resting cells (14 cases)	36 ± 18	29 ± 12	35 ± 20
Activated cells (10 cases)*	7 ± 5**	22 ± 15	66 ± 12**

Cumulative results from all the cases, average ± SD. * 60 and/or 120 sec after 1 U/ml thrombin and 2 µM ADP added to PRP at 37 °C. ** Statistically significant (Student's t-test) $p < 0.001$.

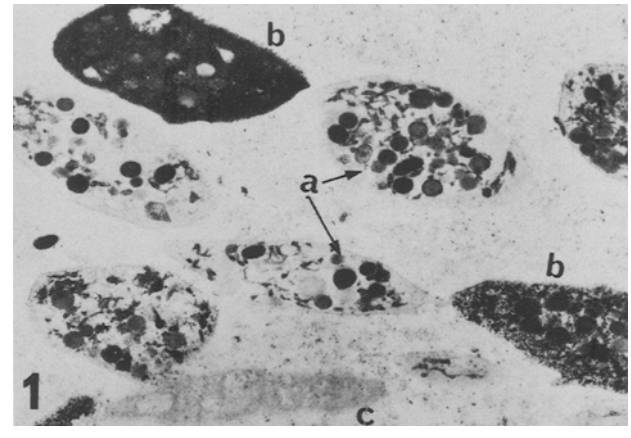

Fig. 1. Platelets impregnated with heavy metals, showing heterogeneous staining. Type 1 cells (a) have a reticular dense tubular system and dark alpha granules. Type 2 cells (b) are very electron opaque. A type 3 cell (c) is very pale. × 6000.

The proportions of the 3 cell types in each case were calculated from counts of several hundred cells observed on the EM screen. In order to ensure fair sampling of the material, different grids and sections from several blocks were used.

The platelets were also photographed at magnifications between 5500 and 33,000 times. Measurements of surface areas were done by point counting of enlarged prints. The values were converted to logarithms for statistical comparisons[14]. Tubular and microvesicular diameters were measured on projections enlarged half to 1 million times. The exact dimensions were calculated with the aid of replica gratings photographed and projected together with the specimens.

The metal impregnations were also performed on platelets fixed 60 and 120 sec after the addition to PRP of ADP (2 μM final concentration) and thrombin (1 unit/ml).

Results. The heavy metal impregnation at pH 3.5 produced platelets of 3 types: a) 'reticular cells' with an electron dense polygonal tubular network and very dark granules (figures 1 and 2), b) 'dark, metallophilic cells' with a finely granular, electron-dense cytoplasm and indistinct internal structure; c) 'microvesicular, metallophobic cells' with pallor of granules and cytoplasm (figure 3).

The 1st type, the reticular cells, were sometimes large and oval shaped. They contained a variable number of homogeneous, dark alpha granules (average = 12 ± 6/cell) and mitochondria of finely granular texture. The distinctive feature of these cells was a network of anastamosing tubules traversing the cell in various directions. No definite connections between this reticulum and other intracellular structures could be seen. The tubules varied in diameter from 20 to 70 nm and seemed to contain fine granular material. The cytoplasm of the reticular cells looked rather empty. Occasionally structures resembling surface-connected canaliculi and sacs or Golgi apparatus with nearby saccules could be recognized.

The 2nd type of cell had a cytoplasm so electron-dense that it was difficult to recognize organelles. In some less dense cells, granules of variable depth of staining, often surrounded by a fine double membrane, could be seen. Their average number per cell was like that in type 1 cells, 12 ± 5. Empty canaliculi and sacculi related to the surface-connected system were often present but no definite dense tubular network was discernible.

The 3rd type, the metallophobic cells, were sometimes irregular in shape and often had processes. Their plasma membrane was usually surrounded by a granular electron dense coat. The alpha granules and mitochondria were visible in outline but did not stain. The cytoplasm contained numerous microvesicles 30–40 nm in diameter. These pale platelets seemed to be 'sticky', because wherever 2 or more cells adhered, at least 1 of them was a type 3 cell. Microtubules and microfilaments were not visible with the techniques employed here.

The proportions of the 3 cell types were roughly one third of each, but there was much case-to-case variability, especially in the type 3 cells (table). The 4 teenage donors seemed to have more type 1 cells, and no difference was noted between males and females. Although all the cell types varied in size, the average log-area of type 2 was significantly smaller than that of type 1 in each case and in cumulative averages ($p < 0.001$).

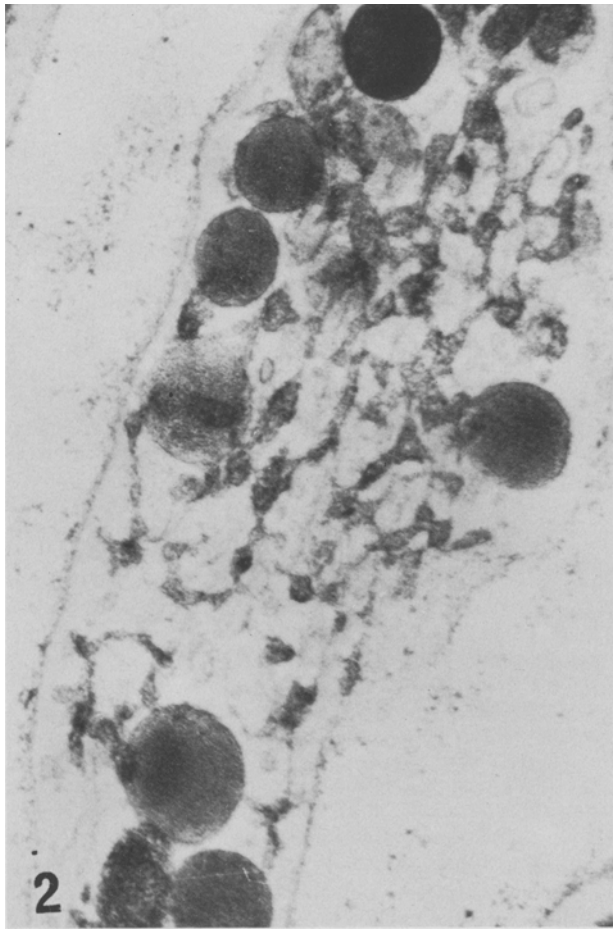

Fig. 2. Type 1, reticular cell at high magnification, showing the anastomosing polygonal network of the dense tubular system. 0.25 μm thick, unstained section, ×60,000; photographed at 100 kV.

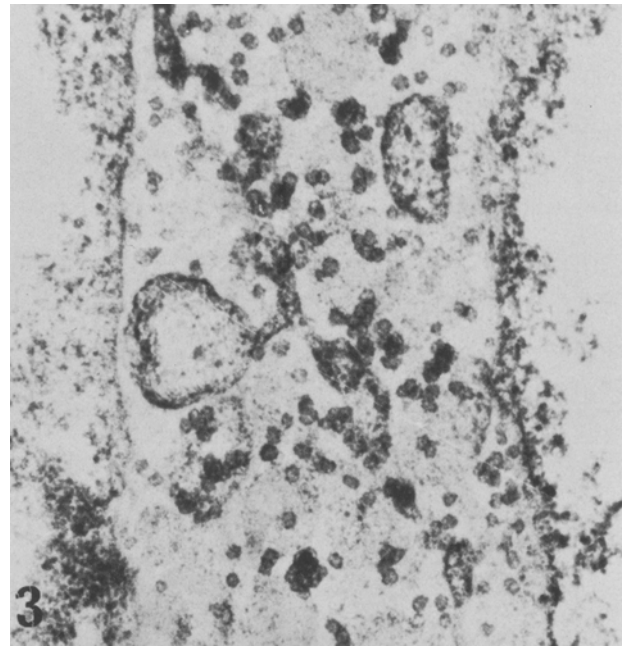

Fig. 3. A type 3 cell at high magnification, showing numerous microvesicles throughout the cytoplasm and unstained alpha granules. Some surface-connected elements are visible. The cell membrane has a granular outer coat. 0.25 μm thick, unstained section, ×65,000; photographed at 100 kV.

After stimulation of the platelets by ADP and thrombin a marked change occurred in cell appearance and distribution (table). After 60 sec, there was centration of organelles (obvious in type 1 cells mainly), while after 120 sec the granules as well as the cytoplasm of type 1 cells became pale and the DTS broke up into microvesicles. In this way the reticular cells decreased markedly while the type 3 pale cells increased accordingly. The metallophilic type 2 cells, although not changing much in percentage, became a little paler. Their granules as well as the cytoplasm showed decreased electron opacity and the double membrane around many granules appeared to be interrupted.

Heavy metal impregnations performed at pH's of 1, 5.5, or 7 failed to stain any intracellular structures.

Discussion. Heavy metal impregnation is thought to cause electrostatic interaction between the ionized groups of macromolecules and the metal of the ionized metallic salt[12]. The metals thus behave as cations which in suitable conditions may replace calcium in different loci.

The electron density of the heavy metals and the thickness of the sections allow the visualization of the polygonal tubular network traversing the cell, which is not apparent in routinely prepared ultrathin sections of platelets.

In other cells, metal impregnation at a pH of 3.5 is known to stain mitochondria and endoplasmic reticulum[12,13]. The platelet DTS which originates from the endoplasmic reticulum of the parent megakaryocyte[5,15] appears to retain the same staining properties. It is not clear why not all the cells exhibit the reticular network. Perhaps the natural heterogeneity of platelet structure is responsible.

The view that differences in platelet size, density, metabolic function and reaction to stimuli are caused by aging[16], is now modified by the belief that heterogeneity may be acquired during platelet production[14,17]. It is related to the parent megakaryocyte ploidy and is retained to a large extent throughout the platelet's life span. With the method used here, platelet subgrouping can be given additional, morphological expression.

The changes in structural and metal-binding characteristics of activated platelets are noteworthy. A similar change from tubules to vesicles has been observed in a study of nerve endings with heavy metal impregnation[12].

Perhaps, as in nerve endings, in platelets too this change is part of a mechanism for the release of substances from the DTS.

The metallophilic cytoplasm, indistinct DTS and only moderate changes in staining characteristics after activation, suggest that the type 2 cells may have a functional mechanism differing from type 1 cells.

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Localisation ultrastructurale de l'œstradiol et du moxestrol dans les cellules gonadotropes du rat par immunocytologie après cryo-ultramicrotomie: caractérisation de la spécificité hormonale[1]

Ultrastructural localization of estradiol and moxestrol in the gonadotropic cells of rat by immunocytology after cryo-ultramicrotomy: evidence for hormonal specificity

G. Morel, J.P. Raynaud et P.M. Dubois

Laboratoire d'Histologie et Embryologie, Faculté de Médecine Lyon-Sud B.P. 12, F-69600 Oullins (France), et Centre de Recherche Roussel-Uclaf, F-93230 Romainville (France), 10 avril 1980

Summary. Estradiol (E_2) was specifically localized by immunocytochemistry in the cytoplasm and in the nucleus of the gonadotropic cells. The immunocytochemical reaction was not observed after injection of moxestrol, but it was not modified by injection of testosterone, progesterone, or dexamethasone. These data suggest that E_2 might be bound to a high-affinity binding-site which could also have a hormonal specificity.

La localisation d'un stéroïde par immunocytochimie ultrastructurale après cryo-ultramicrotomie est possible dans un tissu hétérogène tel que l'hypophyse[2,3]. Les variations des hormones sexuelles après gonadectomie[4,5] et le déplacement de ces hormones par des agonistes et antagonistes[5] font penser que ces molécules sont fixées à un site de liaison. Dans le but de caractériser la spécificité de cette liaison nous étudions la spécificité hormonale.

Matériels et méthodes. Des rats femelles de souche Wistar (IFFA-Credo, France) ont été réparties en 2 groupes par lots de 2 à 3 annimaux. Les animaux du premier groupe sont des rattes de 17 jours, qui reçoivent toutes une injection de 1 mg d'œstradiol (Sigma) (E_2). Le premier des 13 lots sert de témoin, les 12 autres lots subissent une autre injection de doses croissantes (0,5; 1; 5 et 10 mg/rat) soit de progestérone (Sigma) (P), de testostérone (Sigma) (T) ou de