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PLATELET PRODUCTION BY MEGACARIOCYTES FOLLOWING INTRA OR EXTRA CYTOPLASMATIC INJECTION OF MEDIATORS

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INTRODUCTION

Platelets (P1) are produced by megacariocytes (M) (WRIGHT J: The origin and nature of the blood platelets: Boston Med.-Surg. J. 1906. **154**. 643-657). but the process by which they are produced is poorly understood. The maintenance of a relatively constant platelet mass. number and volume suggest that P1 production is under a tight regulatory process. On the other hand the theory that P1 are formed by membrane bleb processing. or budding from pseudopods projecting from M into the bone marrow perisinusoidal spaces is no longer tenable. not only because plentiful matter proves that P1 develop in M cytoplasma (THIERY J.P. et M. BESSIS: Mécanisme de la plaquettogénèse. Etude in vitro par la cinematographie: Rév. Hémat. 1956. **11**. 162-178. ZUCKER-FRANKLIN D., P. STAHL & P. HYDE: Megacariocyte Ultrastructure. :Ann. N Y A S 1987. **509**. 25-33). but also because P1 have appreciably constant shape and volume in healthy subjects. In order to clear up the role that either the membrane or the cytosol M play in P1 generation. the effects of intra-or extra-M injection of presumed mediators have been tested.

METHODS

The experiments were carried out on fresh rat's bone marrow M. suspended in Hemagel. at 32 °C. on Petri dishes. in a DAS-LEITZ-DMIL microscope.

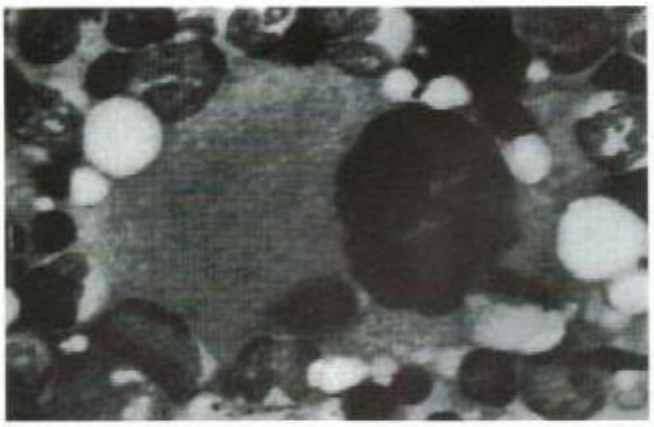
Some substances were injected around the M membrane: others on the contrary directly into the living M. using a finelly glass capillary. through the Eppendorf microinjector 5 2 + 2. properly adjusting the pressure levels.

The tested substances were injected isolated or associated. according to different sequences and proportion.

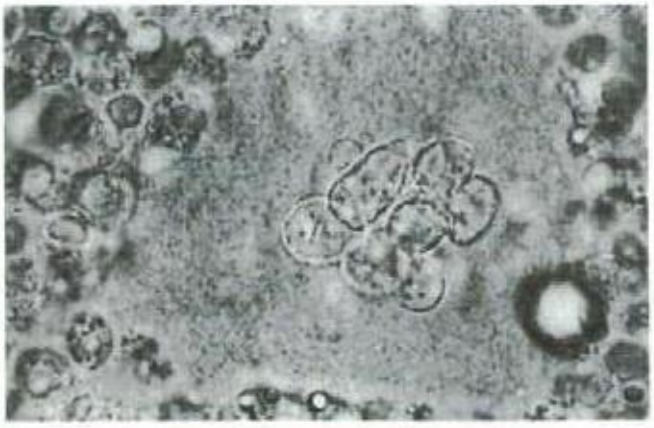
RESULTS

In the cytoplasma of M maturation processes spontaneously occur. that involve biosynthesis and packaging of specific. P1 associated substances (GRANT B.W., L. SOLBERG. W. NICHOLS & K. G. MANN: Proliferation and maturation of human megacariocytes: Ann N Y A S 1987. **509**. 34-40) ("encampment pictures").

The appearance is not necessarily coupled with either normal or elevated blood P1 count. in as much as they can too be met in essential thrombocytopenic patients.



Rat's bone marrow, freshly removed M, stained with M G M method



Fresh Megacariocytes from rat's femora bone marrow

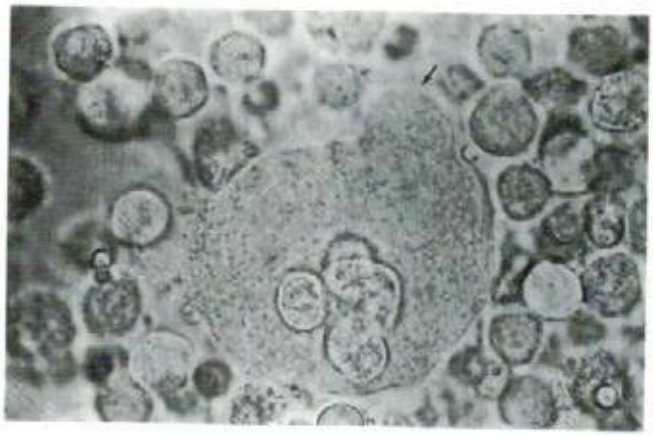
The membrane shows an unbroken, smoth and even border, that is distorted in a tract by a big protrusion, however without any breaking of the membrane continuity (A).

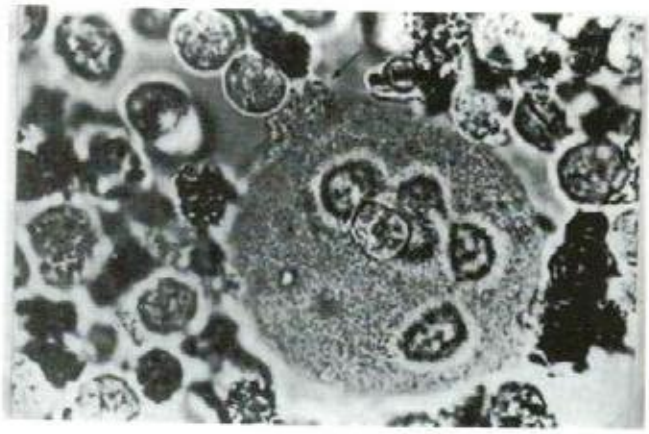
In two other not distant tracts of A and B the membrane seems to fade away and to allow the outlet of many more or less delineated committed membrane systems, through the breakage.

These changes of the membrane are the result of the local injection of Melatonin or Adenosin with glass capillary of Eppendorf microinjector.

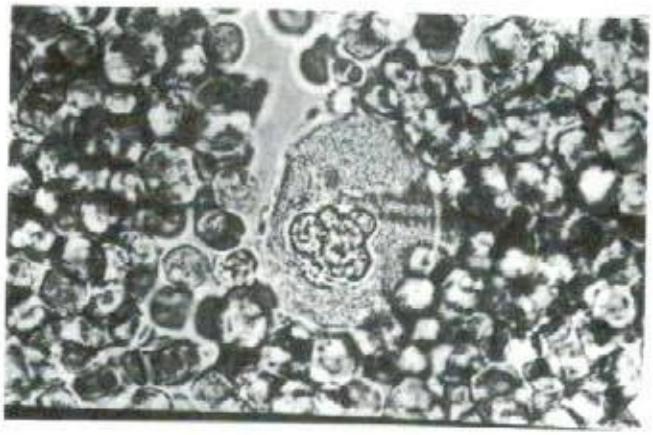
Indeed Adenosine improves M viability and recovery, according to SCHICK P.K. & B. SCHICK (Methods for studying the Biochemistry of recognizable Megacariocytes. In: COLMAN R.W. & J. BRYAN SMITH Ed. A.L. Inc. N Y 19-31) a clear expression of the role that M play in the metabolism and storage of purines (LEVINE R.F. & H.K. WEBSTER: Purine metabolism in megakaryocytes and platelets: Throm. Haemost. 1981, **6**, 22-29; MILLER J.L.: Characterization of the megakaryocyte secretory response: Studies of continuously monitored release of endogenous ATP Blood 1983, **61**, 967-972).

The chief function of the M Membrane is to dissolve at several sites and to control the platelet outlet. P1 have a different biochemical composition from M membrane, so that anti-P antibodies do not react with M.





Fresh Rat's M. following extracellular injection of Melatonin or Adenosin



Our discovery of the curative effect of Melatonin in essential Thrombocytopenia sanctions the primary role of Melatonin, Adenosin, and other serotonin derivates in the control of P1 outlet from M cytoplasma.

P1 are certainly built in M cytoplasma, which employs the principles that can cross the membrane.

One such substance is serotonin, which cannot be synthesized by M (TOCANTINS L.M./Serotonin: Haematological aspects. Progr.Hemat. 1 8 6 2, Vol. II, 206, Grune & Stratton) and reaches very high concentration in blood P1 (DA PRADA M., J. C. RICHARDS & R. KETTLER: Amino storage organelles in Platelets. In: GORDON J.L. Ed.: Platelets in Biology and Pathology, 1981, Vol. II, Elsevier/North Holland Amsterdam, pgg. 1 0 7-1 4 5), where it can enter by passive diffusion (HUGMES F. B. & B. B. BRODIR: The mecanism of serotonin and cathecolamine uptake by platelets: J. Pharmacol. Exp. Ther 1959, 127, 96) or by an active transport mechanism.

Epinephrine and norepinephrine are also taken yp by an active but different transport mechanism (WEISSBAC H., D. BOGDANSKI & S. UNDERFRIEND: Binding of serotonin and other amines by blood platelets. Arch. Biochem. 1958, **73**, 492).

A 3rd neurotransmitter, which can mediate bone marrow nerve ending secretion to haemopoietic cells is AcChol (CALVO W.: The innervation of the bone marrow in laboratory animals. Amer J. Anat. 1968, **123**, 315; CALVO W. 1 J. FORTEZA-VILA: On the development of bone marrow innervation in newborn rats as studied with silver impregnation and electron microscopy. (Amer J., Anat, 1969, **126**, 155 KUNZ A. & C.A. RICHINS: Innervation of the bone marrow. J. Comp. Neurol. 1945, **213**, 227).

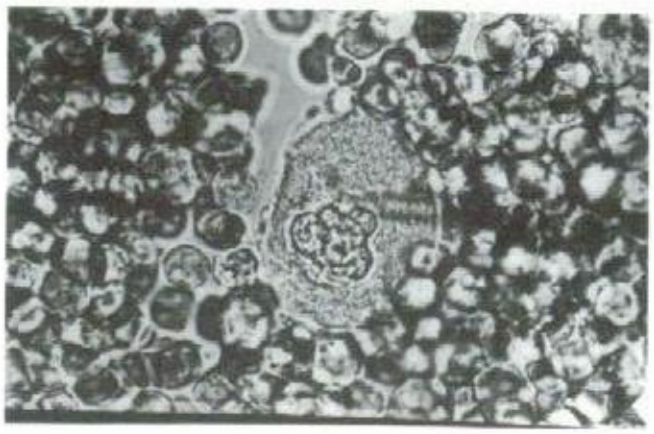
The physiological meaning of the bone marrow cholinergic innervation (McDONALD T.P.: Regulation of Megakaryocytopoiesis by thrombopoietin. ANN.N.Y.A.S. 1987, **509**, 1-24) is made clear by the following reports: 1) murine M. secrete AcChol-esterase; 2) AcChol-esterase inhibitors (neostigmine) stimulate cells that form M Colonies; 3) Neostigmine elevates the % of AcChol-esterase positive cells: 4) Injection of AcChol-esterase reduces the % of a AcCholesterase-positive cells of murine bone marrow.

AcChol-esterase produced by M inhibits megakaryocytopoiesis in rodents.

This mechanism further illustrates the intimate intercourse between thrombopoietin and megakaryocytopoiesis.

The foregoing epitomes have suggested the experimental attempts that we have followed of injecting into M the most likely mediators that could cross the M membrane, i.e. Serotonin, Epinephirine and AcChol. The substances have been injected through Eppendorf microinjector.

As a result of the intra-M injection of the mixture of Serotonin + Epinephrine + AcChol a radical change in M cytoplasma aspect appears, pronounced, especially along the membrane outline, where the distribution of the mediators could change most lively.



Fresh Rat's M, prepared from bone marrow femora, suspended in hemagel

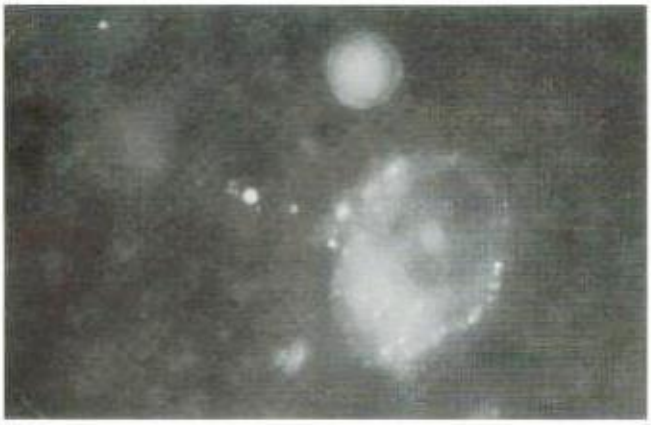
Before and after intracytoplasmatic injection of Serotonina + Epinephrine + AcCholine

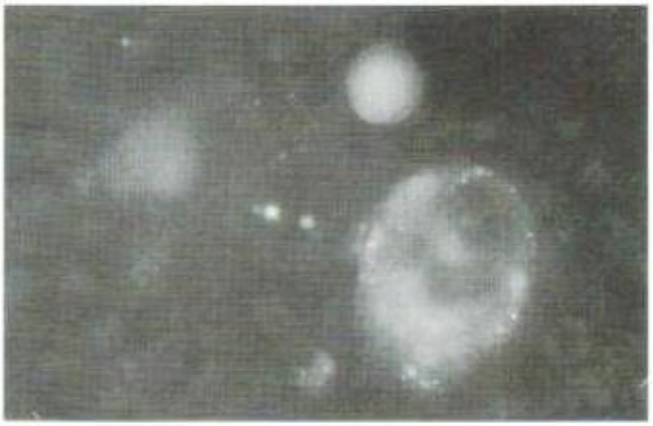
The mechanism to concentrate and actively transfer the mediators from the membrane to interior of M could draw valid advantage from the presence of enzymes such as N A T or H I O M T. Both enzymes have been found in M as well as in P1 since 1981 by LEMAITRE & Al. (2 nd Colloquium of the E P S G, Giessen, 42).

The microinjection of the NAT inhibitor -4 (1 -naphtylvinil pyridine H C1- is followed by a copious formation of P1, whereas the injection of the HIOMT - inhibitor S-Adenosyl - cysteine. H20 seems having no influence.

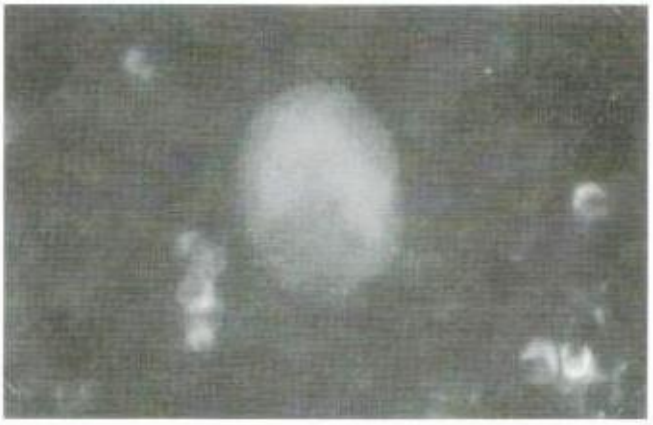
CONCLUSIONS

P1 production by M is a very complex, multivectorial phenomenon, in which both nucleus (ploidy) and citoplasma (committed membrane system), both membrane and surrounding medium play autonomous, although well cohordinated functions, all finalistically directed to build a constant and sufficient number of regular and functional Platelets.





After intracytoplasmatic injection of NAT inhibitor



After intracytoplasmatic injection of HIOMT inhibitor