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Key aspects of melatonin physiology: Thirty years of research

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Submitted: March 11, 2006 *Accepted: May 30, 2006*

Key words: **melatonin; thrombocytogenesis; platelet aggregation; ion channels; tumour**

Neuroendocrinol Lett 2006; **27**(4):425–432 **PMID:** 16892002 NEL270406A08 ©Neuroendocrinology Letters **www.nel.edu**

Abstract

Numerous studies of melatonin, by now widely acknowledged as a circadian rhythm-affecting neurohormone, also describe its anti-oxidant, anti-cytotoxic or immune-modulating activity. While emphasizing the multifunctional aspect of melatonin action, this review presents the results of our thirty years of research, which point to the following conclusions: melatonin is capable of promoting platelet production by megakaryocytes, of acting on the latter's ion channels by way of the outward currents, and of performing a physiological anti-aggregation function thus lengthening platelet life span. Melatonin can be transported everywhere by platelets and, thanks to its lipophilicity, can cross cellular membranes easily, thus regulating blood-tissue exchanges and ensuring an improved haematic crasis. It interacts with endothelial cells by regulating their release of both relaxing-factor and contracting-factor, and with platelets by affecting their discharge of dense-body components. Finally, platelets could behave as mobile and itinerant serotonergic and/or melatonergic elements, a function comparable to the release of neurotransmitters by neurons of the central nervous system. This dynamism in melatonin physiology could prove to be a key in approaching tumour aetiopathogenesis.

Abbreviations

MLT	– melatonin
MEG	– megakaryocyte(s)
PLT	– platelet(s)
5-HT	– serotonin
NAT	– N-acetyltransferase
Ac.CoA	– acetyl coenzyme A
HIOMT	– hydroxyindole-O-methyltransferase
DMS	– demarcation membrane system
2,3-DPG	– 2,3-diphospho-D-glycerate
SAD	– seasonal affective disorder
EDRF	– endothelium-derived relaxing factor
EDCF	– endothelium-derived contracting factor
PDGF	– platelet-derived growth factor
APUD	– amine precursor uptake decarboxylation

Introduction

While an ever-increasing number of papers and reviews are devoted to the disputed oncostatic effects of melatonin (MLT) [59, 2, 74, 46], we think it opportune, after researching the field for over thirty years, to summarize the results of our studies with particular regard to megakaryocytes (MEG), platelets (PLT) and MLT.

The multidisciplinary quality of MLT action (to adopt Dr. Beatrice Guardiola's definition at the 1994 Congress of the Society for Neuroscience) appears to be fully warranted. The ubiquity of the

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substance, its neuroactivity and its uptake can indeed influence in many ways the physiology of the nervous system, and particularly the serotonergic system [Di Bella[6]] [29, 81, 100]. A discussion of MLT action limited to its effects on circadian rhythms or its anti-oxidant and anti-cytotoxic activities is probably too narrow, whereas it may not be unreasonable to speculate on its effects on all peripheral organs within its direct reach via the platelets, which can store MLT, circulate it everywhere via the blood vessels and, hence, use it in the metabolism of central nervous system neurotransmitters [85, 1, 13, 78].

Melatonin Synthesis

MLT is synthesized in several organs, including the pineal gland, retina, Harder's glands, gastro-enteric mucous membrane, MEG and PLT, at rates and in quantities, modalities and conditions that vary in relation to the respective organ, the latter's function and relationship with other organs, the temperature and the availability of the precursor amino acid, tryptophan.

The essential amino acid L-tryptophan is hydroxylated and decarboxylated to 5-hydroxytryptamine or serotonin (5-HT); the latter is at first acetylated by N-acetyltransferase (NAT) by transfer of an acetyl group from acetyl coenzyme A (Ac.CoA), then methylated by hydroxyindole-O-methyltransferase (HIOMT) to N-acetyl-5-methoxytryptamine or MLT, with the methyl group at position 5 probably provided by S-adenosylmethionine [80]; MLT then binds at position 6 to sulphates or glycuronates in the liver and is eventually discarded in the saliva and urine [51, 56]. MLT can, lastly, be reconverted into its precursor N-acetylserotonin, presumably in the retina [102] and PLT [54].

MLT is circulated throughout the organism by plasma, in which it is suspended, or by platelets, in which it accumulates due to a hydrogen bond with adenosine [17]. This allows platelets to regulate the melatonin concentration in the surrounding environment.

Melatonin and Thrombocytogenesis

The following intuitions, listed in their chronological order, led us to associate MLT with thrombocytogenesis.

1969. Because stimulation of the rat habenular-epiphyseal system induces a transient yet significant increase in PLT count [29, 28] without any concurrent significant modification in red or white blood cell count [27], and because MLT is synthesized in the epiphysis [1, 57], ascribing the PLT count modification to MLT action was deemed to be not unreasonable [85, 21, 61]. The above necessarily implied the existence of two important conditions: bone marrow permeability to MLT and MEG responsiveness to the latter [Di Bella[4]] [54, 104, 16].

1977. As a result, the decision was made to add MLT in vitro to a suspension of rat bone marrow [40, 38]. Megakaryocytes produce platelets from Behnke's demarcation membrane system (DMS) [4], with different modalities at the peripheral compared with the perinuclear area. Thrombocytogenesis is affected by microtubular systems and actomyosin networks, whose activation depends on the serotonin concentration [71], which in turn depends on MLT synthesis and such chemical mediators as adrenalin and acetylcholine [Di Bella[1]] [25]. The methylated and/or acetylated compounds required for PLT production might be provided to MEG by bone marrow cholinergic nerve fibres and transferred to 5-HT by red blood cell acetylcholinesterase enzyme [35, 32]. In vitro, megakaryocyte cytoplasmic lumps were seen to detach from the mother cell and result in platelets [50, 37]; the latter would be formed by the coalescence, lengthening and bending of the DMS [4, 66]. The DMS, which forms cytoplasmic lumps first and then platelets, descends deep into the open microtubular system and enables the transfer of substances from the outside towards the alpha granules and dense bodies [75, 101, 67, 103], the latter being plentiful in PLT where they can form 5-HT deposits [69, 20, 98]. Platelets, moreover, possess an acetyl-CoA carboxylase activity, as well as the NAT and HIOMT enzymes required for 5-HT metabolism [68, 64, 70], hence their potential ability to synthesize melatonin [29, 54, 14].

1979. Melatonin and/or ADP addition to a Wistar rat fresh bone marrow suspension led to the collection of numerous images that indicated that thrombocytogenesis was occurring (Fig.1, Fig.2) [39, 24]. One might argue that this accelerated and/or enhanced thrombocytogenic effect could be plausible if indeed MLT had an influence on MEG similar to that of 5-HT on platelets [105] and if the megakaryocyte membrane microtubular actomyosin system could be rapidly activated [71].

1980. The study of MEG fluorescence (510 nm), after MLT addition to the suspension medium, demonstrated that NAT and/or HIOMT inhibitors must be present for truly fluorescent images to appear [Gualano[8]]. When the NAT inhibitor 4-(1-naphthylvinyl)pyridine is present, melatonin can actually promote noticeable PLT formation over the whole MEG membrane; a less remarkable effect, albeit of the same nature, occurs with the HIOMT inhibitor S-adenosyl-L-cysteine [83].

1984. Thrombocytogenesis was observed with adult rat megakaryocytes even with i.v. melatonin administration (1 mg MLT/0.1 ml anhydrous ethanol 95°, 10% sol.) [Rossi[9]]. Scanning electron microscopy of MEG behaviour in the presence of MLT has vielded images comparable to those obtained with the optical microscope and confirmed MLT's influence on membrane integrity and on thrombocytogenesis [84].

1999. Melatonin, which does not react with the megakaryocyte's external membrane or with its DMS, probably does reach the nucleus, where it may exert a

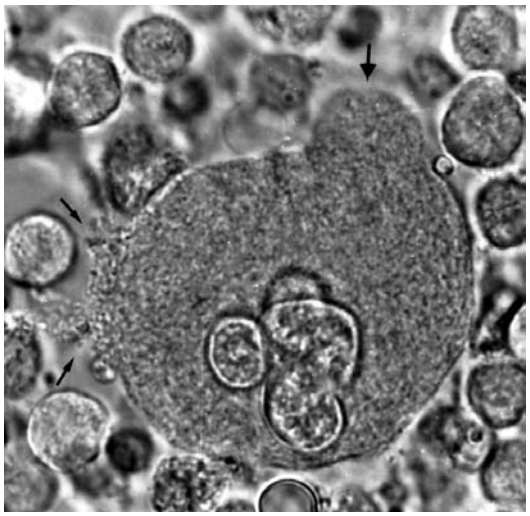

Fig.1: Megakaryocyte in melatonin and ADP solution (1200x). The upper arrow points to a discernible granulated cytoplasmic lump. Melatonin causes a progressive increase of cytoplasm granulations. On the left, the megakaryocyte membrane becomes thinner, until it disappears and the release of platelets is achived.

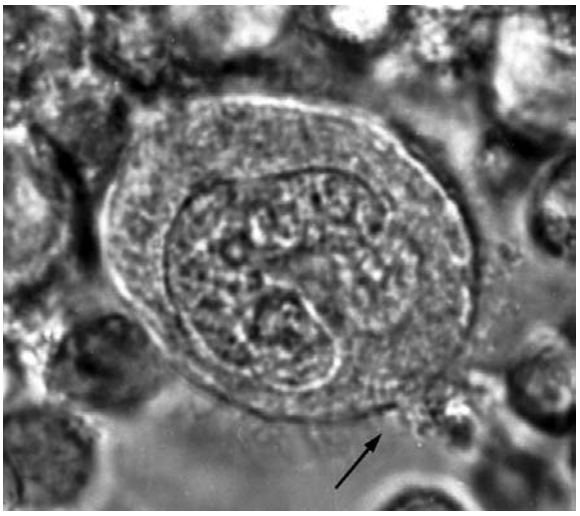

Fig.2: Megakaryocyte in melatonin and ADP solution (1600x). The arrow evidences a gap in cell membrane, whence a platelet is formed and expelled. This megakaryocyte is at a perceptibly low maturation stage.

β-cytochalasin-like effect both inhibiting the endoreduplication process and enhancing nuclear polyploidy [36, 23]. Certain images where the fluorescence is confined to the MEG cytoplasm or nucleus, in keeping with the presence or absence of thrombocytogenesis, seem to validate this hypothesis.

From all of the above, one could infer the following: (a) the occurrence of melatonin demethylation and deacetylation reactions on the megakaryocyte membrane [80, 54, 84, 94]; (b) the existence of contractile systems both on MEG and on PLT membrane [71, 12, 76]; (c) the activation of these systems by MLT [84, 6]; (d) the coalescence of DMS by virtue of MLT with resulting new PLT production; (e) that only the spots on the MEG membrane where MLT could attain an adequate concentration might be fit to discharge platelets; and (f) that the balancing of the chemical mediator energetic network would promote the contraction of MEG membrane filaments thus inducing PLT expulsion [Di Bella[2]] [95, 19].

Melatonin and Platelet Aggregation

1976. In view of the favourable effects on blood and tissue oxygenation after melatonin treatment, we investigated whether an increase in red blood cell 2,3-diphospho-D-glycerate (2,3-DPG) occurred [5, 26]. In comparison with control animals, MLT treatment seemed to induce, with a 150-minute time lag, a significant decrease in intra-erythrocyte 2,3-DPG; this decrement could in part explain the enhanced oxygenation [88, 86].

1979. Given all the premises outlined above, the study of MLT's influence on PLT aggregation was deemed to be conducive to a better understanding of the complex physiology of PLTs [Di Bella[5]] [52, 31].

MLT, at pharmacological concentration (4.0 μM) in human or rat plasma, can, partially, disaggregate a PLT suspension (platelet-rich plasma) caused to aggregate by addition of ADP (2.5 μM), or, if administered beforehand, block the ADP-release reaction in platelets, probably through its binding with PLT tubulin [12, 72], thus rendering the event reversible (Fig.3) and prolonging the life span of those platelets. It is likely that, within PLTs, MLT can coexist with 5-HT, so that the concentration of the two indolic compounds, and one's transformation into the other [98], may in part depend on the platelet shape change that precedes platelet adherence and aggregation as well as certain functional PLT disorders [3, 34]. Melatonin would thus prove to be the most inherently physiological of PLT anti-aggregants, enabling platelets to reach the blood vessel endothelia and to gradually free the substances contained therein [Rossi[10]] [97, 96].

Melatonin and Ion Channels

2002. After observing abnormal development of platelet microtubular systems in numerous haematopathies [91] as well as variations in the outward K+ currents which are normally present over the megakaryocyte membrane [49, 82], in relation to cell maturation [47], a study of MLT's influence on MEG membrane ion channels was initiated.

The change in MEG membrane outward K+ current intensity, studied by means of the patch-clamp technique [41] and recorded with an Axopatch amplifier (Axon instruments model 1D, U.S.A.), was found to be directly affected by the melatonin concentration

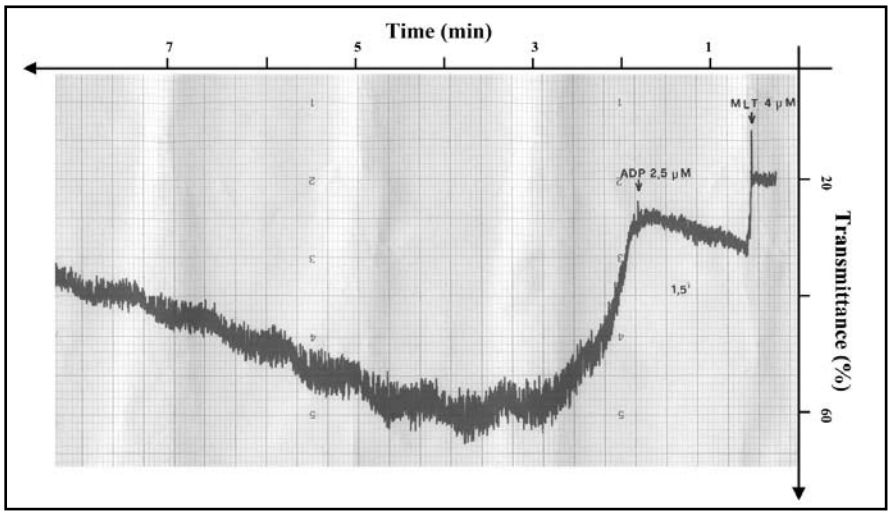

Fig.3: Platelet aggregation. Melatonin (4.0 µM) added 1.5 minutes prior to ADP (2.5 µM) succeeds in blocking the «release reaction», thus reversing the platelet aggregation process (sheet flow rate: 2.5 cm/min).

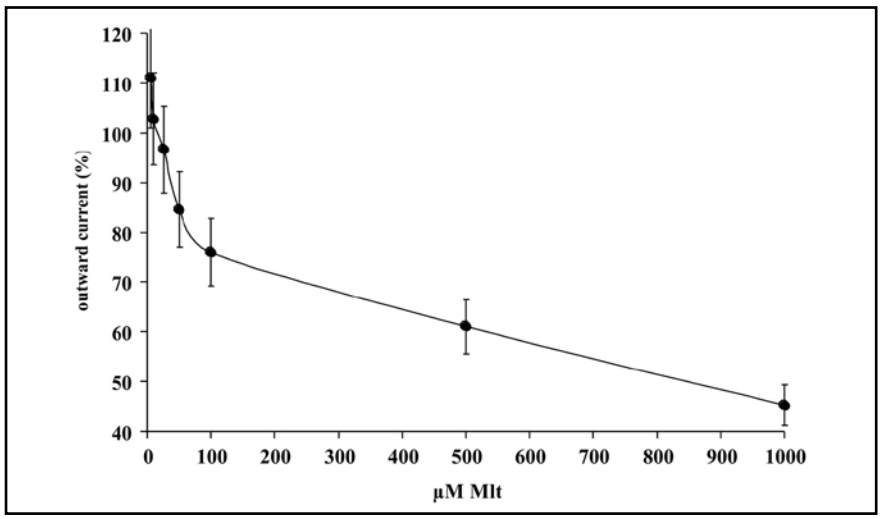

Fig.4: Outward K+ current and melatonin concentration. The curve shows the outward K+ current behaviour, after 2.0 minutes of perfusion, when different melatonin concentrations (5, 10, 25, 50, 100, 500, 1000 µM) were used (mean ± SD; n=5). From 25 µM upwards, the higher the melatonin concentration, the more the outward current decreases. The decline in current-voltage (I/V) is reversible up to 500 µM melatonin concentration; it becomes irreversible when concentration reaches or exceeds 1000 µM.

perfused in a fresh, Wistar rat bone marrow suspension (Fig.4). The clampage was performed on whole-cell at –60 mV and voltage protocols (the membrane was depolarized in 10 mV steps from –60 to +50 mV; 180 ms pulse duration) were monitored by an oscilloscope. After membrane breakage, the mean resting potential (V0) ranged from –17 to –65 mV (–35±15; n=50) and the input resistance measured 0.6 to 2.6 GΩ (1.6±1.2; n=50) [22, 90, 44].

This close interplay between melatonin, platelets and ion channels, by virtue of the correlation between cationic membrane charge and PLT production, may therefore suggest a novel approach to the study of thrombocytogenesis. Moreover, one could infer that the most inherently physiological role of the pineal gland is not limited to MLT incretion, but that, by virtue of the electrophysiological mechanisms linking MLT and outward currents, it may well extend to regulating every single cell activity. Melatonin and its metabolism might indeed represent the main neurovegetative integration system.

Melatonin and Tumours

Melatonin is universally acknowledged to influence the metabolic processes connected to circadian sleep/wake rhythms influenced by the light/dark cycle, while the latter in turn are linked to seasonal affective disorder (SAD) and other behavioural disorders [73]. However, these well-known and amply studied mechanisms would be of little value if one seeks to explore MLT function from an oncological perspective. MLT

action in malignancies is neither unique nor identical in every case; the key points that, in our opinion, best characterize and explain this action may be summarized as follows:

(1) Direct action on thrombocytogenesis and indirect repercussion on haematopoiesis as a whole. Nutritional disorders with malabsorption of essential aminoacids are often accompanied with malignancies. Shortage or lack of 5-HT, NAT and HIOMT cause either a decrease in PLT production or PLT malfunction; a substantial number of cancer patients suffer from anaemia, leucopoenia and thrombocytopoenia. Melatonin administration in neoplastic patients can normalize their haematic crasis and restore their serotonin system functions [Di Bella[3], Gualano[7]] [28, 63, 7].

(2) Interaction with endothelial cells and exchanges with the basal membrane, prompting discharge of endothelium-derived relaxing factor (EDRF) or endothelium-derived contracting factor (EDCF) and release of platelet-derived growth factor (PDGF). The normal number and functionality of platelets are essential for efficient blood circulation and for the normal interactions with endothelial cells . MLT's anti-aggregating and thrombocytogenic action provides for all this, preserving the integrity of the PLT membrane, increasing platelet life span and regulating the release of the compounds contained within the dense bodies, including PDGF, towards the underlying endothelium [86, 15, 42, 8]. The latter assumes, with regard to EDRF and EDCF, an endocrine role influenced in turn by melatonin, which would thus also affect capillary permeability [65, 33].

(3) Platelet behaviour is comparable to that of serotonergic neurotransmitters. Platelets, with their content of 5-HT, norepinephrine, acetylcholine, epinephrine, MLT, NAT and HIOMT as well as AMP, ADP and ATP, might be regarded as mobile, itinerant, multifactorial units of a plastic and ubiquitous APUD (amine precursor uptake decarboxylation) system [Di Bella[4]] [77, 53]. Platelets would thus behave from time to time as serotonergic, melatonergic and dopaminergic vehicles in accordance with the peculiar local circumstances and/or the nature of the cells with which they interact. This typical, intrinsic task of discharging 5-HT or MLT could be plausibly likened to the release of neurotransmitters on the part of central nervous system neurons. The sleep/wake cycle might modulate these cellular exchanges, as was demonstrated by partially or totally removing the sleep phases with c-fos (immediate-early gene) protein involvement; likewise, the MLT rhythmicity might influence the functional state of the suprachiasmatic nucleus [92, 45, 89]. Given that 5-HT can promote c-fos protein expression in rat cerebral cortex [9, 58, 93], one could postulate the opening of a new chapter in the study of central nervous system physiology in which melatonin, no longer seen as a static molecule but in all its chemical dynamism, would play a complex role culminating in its interference in tumour aetiopathogenesis [21, 18, 79].

In many chemotherapy protocols, the oncostatic agent is often complemented by other substances that enhance its effect; hence our conviction that, although melatonin alone is unable to heal any tumour, it is extremely arduous to treat cancer successfully without making use of it [Di Bella[6]] [11, 60, 48].

Discussion

Melatonin is widely known as a neurohormone produced by the pineal gland that mainly affects circadian rhythms. We believe that melatonin is a molecule exerting multiple physiological effects, the details of which have not been fully clarified yet. Nevertheless, the following considerations may serve the purpose of shedding more light on melatonin's range of action.

Melatonin synthesis is not restricted to the pineal gland but also takes place in megakaryocytes and platelets, whenever the NAT and HIOMT enzymes are present [28, 39, 84, 19]. Megakaryocytes form platelets from the DMS, although modalities differ at the peripheral compared with the perinuclear area. This thrombocytogenesis is governed by the microtubular system and by the actomyosin network, whose activation depends on the 5-HT concentration, which in turn is dependent on melatonin synthesis [25, 39, 12, 72].

Melatonin, transported by the platelets, is capable of reaching all regions of the body and, by virtue of its lipophilicity, of crossing all membranes. It can thus regulate blood tissue exchanges with attendant effects on tissue tropism, ensure an improved haematic crasis, perform the most intrinsically physiological anti-aggregative activity [Di Bella[5]] [86, 52]. Consequently, it can both lengthen platelet life span and affect PDGF, EDRF and EDCF release [Di Bella[2]] [42, 65, 33]. Somehow similar to a release of neurotransmitters, the discharge of melatonin by platelets, through melatonin's action on ion channels via the outward K+ currents, can direct interfere with cell physiology [Di Bella[4]] [22, 30, 99].

It was not possible for the authors of this study to conclusively demonstrate the influence of melatonin on tumour growth. However, several peer-reviewed papers have provided plausible glimpses into the mechanisms implicated in this correlation [79, 43, 10, 62]. Given that we deem the ubiquity of melatonin-carrying-platelets as described above to be inherently relevant to the need of targeting tumours wherever they occur, we believe that further studies are warranted to improve our understanding of this latter aspect of melatonin's action.

From a clinical perspective, numerous are the studies documenting the antioxidant and oncostatic action of melatonin [74, 46, 7], or summarizing the evidence

regarding its potential use in cancer treatment [100, 11, 48]. On the basis of the available literature, one may sensibly propose that, besides being possibly helpful in the treatment of both neurovegetative [73] and thrombocytic disorders [Di Bella[3]] [21, 63], melatonin could also prove valuable for the prevention/therapy of several types of tumours [18, 87, 55].

Conclusions

We believe that melatonin metabolism could represent the crucial means by which neurovegetative integration is achieved. As a multifactorial molecule, melatonin could indeed provide the ultimate gateway to a better comprehension of the aetiophatogenesis of tumours and to the implementation of a truly biological and preventive neoplastic therapy. Although several studies are already available, we deem that further research is needed.

Acknowledgment

This review is published posthumously, in memory of Prof. Luigi Di Bella, a man of science.

Footnotes

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A P P E N D I X (online only):

The following photographic sequence reveals the appearance of a platelet-like granulation as a result of intracytoplasmic microinjection of a melatonin-adenosin solution. Adenosin significantly enhances melatonin's hydro-solubility [Di Bella[1]] [17]. The images cover a mere twenty minute timeframe and the resulting neo-granulation, as observed in fluorescent light (510nm), is always much more luminous than the surrounding megakaryocyte's cytoplasm [Gualano[8]] [22].

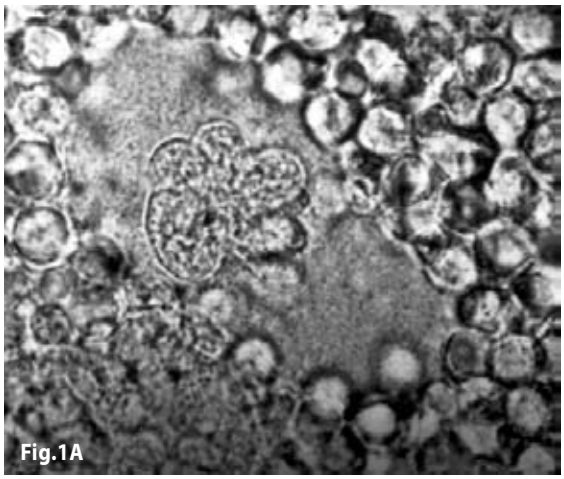

Fig.1A: Megakaryocyte in culture solution (1200x). Cytoplasm looks homogeneously granular.

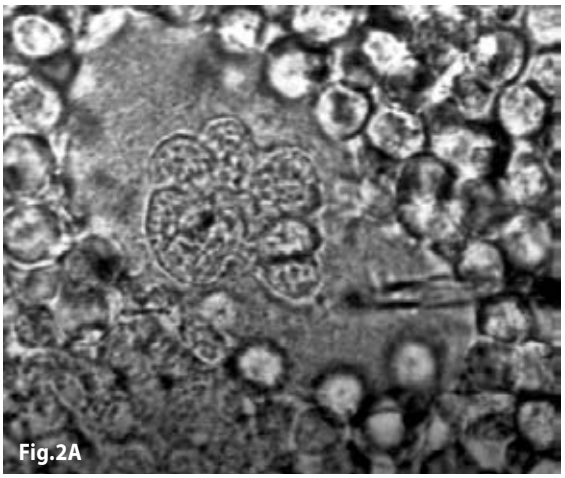

Fig.2A: Megakaryocyte and intracytoplasmic microinjection (1200x). Using Eppendorf 5242 microinjector, an intracytoplasmic injection of melatonin-adenosin (2/9, 5mg/200µl) is performed.

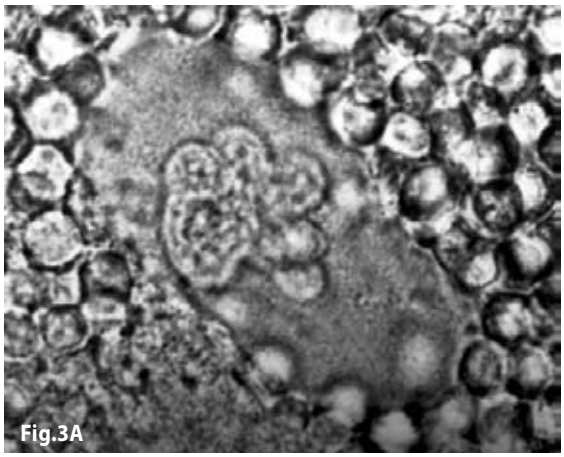

Fig.3A: Megakaryocyte after one minute (1200x). After only one minute, within the gap caused by the needle, one small granule becomes visible.

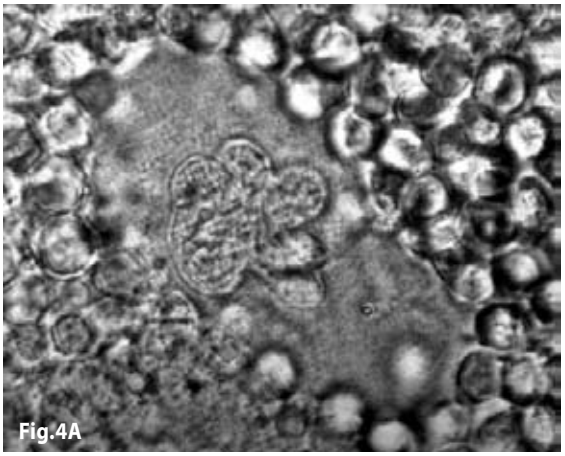

Fig.4A: Same after six minutes (1200x). Six minutes after the injection, the neo-granulation is noticeable.

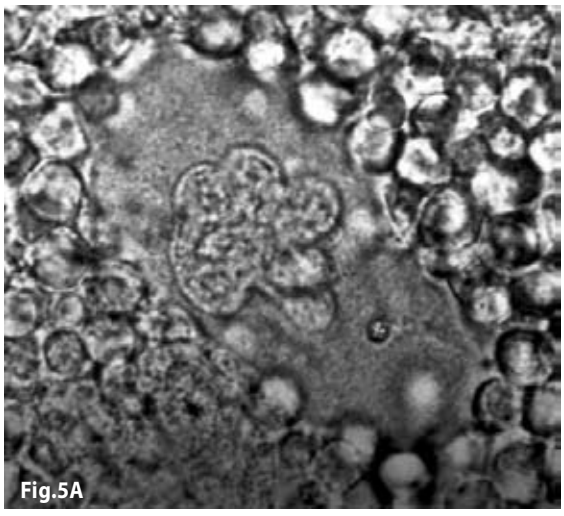

Fig.5A: Same after twenty minutes (1200x). After twenty minutes, the neo-granulation has attained substantial dimensions.