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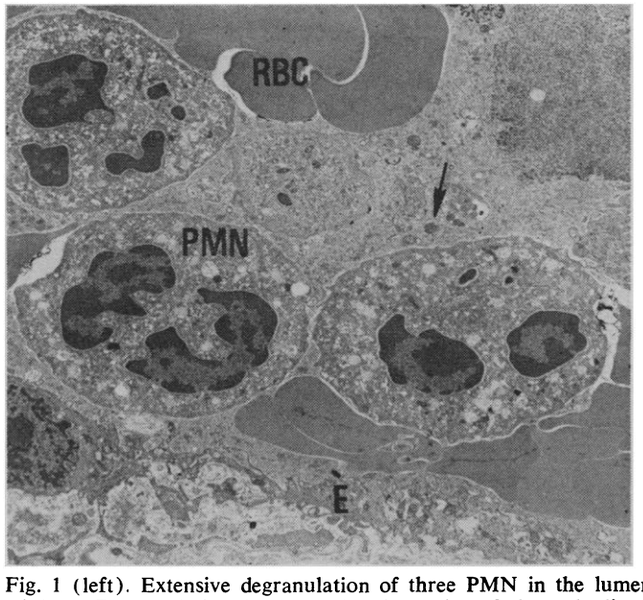
Intravascular Degranulation of Neutrophils: An Important Factor in Inflammation?

Abstract. *Polymorphonuclear leukocytes are degranulated in the lumens of vessels in synovial membrane in humans with various types of inflammatory arthritis and in dogs with synovitis induced by urate crystals. This degranulation, accompanied by the release of lysosomal enzymes and vasoactive materials, may be an important part of the mechanism resulting in vascular injury.*

In studies with electron microscopy of venules in synovial membrane in inflammatory conditions we have noted both degranulation (Fig. 1) and occasional fragmentation of polymorphonuclear leukocytes (PMN) in the lumens. Such intravascular degranulation, not previously reported in human disease, has been found in gouty arthritis, ulcerative colitis, serum sickness, rheumatoid arthritis, and undiagnosed acute arthritis. Degranulation has been seen in vessels where there is no other evidence of vascular injury but has most commonly been associated with changes in the adjacent vessel wall. Vascular alterations accompanying such degranulations include endothelial necrosis; gaps between endothelial cells; fibrin infiltration of the vessel wall and deposition of PMN, erythrocytes, and cell debris; and electron-dense deposits between endothelium and pericytes. Not all PMN were degranulated and most other cells in the specimens showed preservation of structure. Identical preparative techniques (*1*) have shown no PMN degranulation in degenerative arthritis or in normal animal joints but intraluminal PMN are less common in these conditions.

In order to study the intraluminal degranulation we produced an experimental synovitis in dogs by injection of sodium urate crystals. Sterile (pyrogen-free) crystals of monosodium urate in physiologic saline (4 ml) (*2*) were injected into one knee joint of each of ten mongrel dogs (who weighed between 18 and 25 kg) while the dogs were under sodium pentobarbital anesthesia. Tissue samples from each knee injected with urate were examined at various intervals with both light and electron microscopy. After 3 minutes and after 10 minutes, the synovia injected with urate crystals appeared normal, but



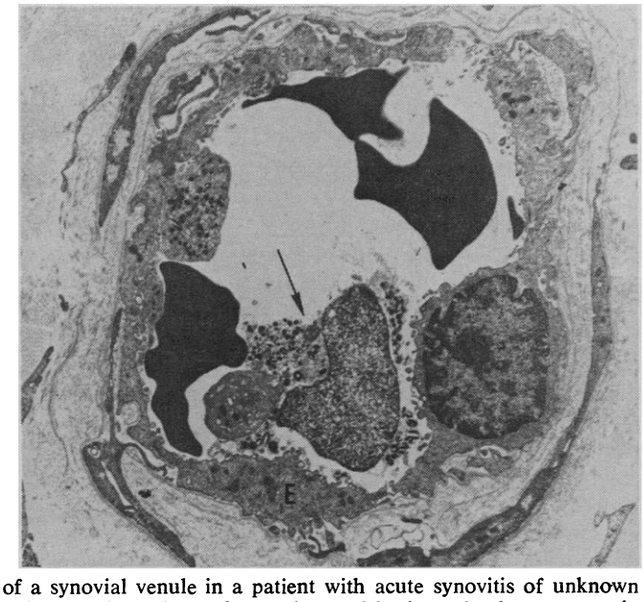


Fig. 1 (left). Extensive degranulation of three PMN in the lumen of a synovial venule in a patient with acute synovitis of unknown etiology. Degranulation is shown by the rarity of dense bodies, by increased numbers of vacuoles, and by irregular lucent areas in the cytoplasm of the PMN. Free cellular organelles present in the lumen, including mitochondria (arrow), are evidence of cell fragmentation; *RBC*, erythrocytes; *E*, venular endothelium (× 3000). Fig. 2 (right). Fragmentation of PMN (arrow) in lumen of an intact venule in dog, 30 minutes after injection of urate crystals into the knee. The PMN cell membrane has been lost and dense bodies lie free in the lumen; *E*, venular endothelium (× 6000).

after 30 minutes, the PMN were present at the margins of synovial venules. At this time numerous PMN were fragmented and had released dense bodies (Fig 2) and many had lost dense bodies but were otherwise intact. By 3½ hours, specimens exhibited much less degranulation. No degranulation was seen in controls injected with saline. In four dogs injected with urate crystals each week for 4 weeks and again 3½ hours before being killed, a chronic synovitis was produced and intraluminal degranulation of PMN was still very prominent 3½ hours after the last injection.

Most investigators of experimental inflammation have not reported such intraluminal degranulation (*3*). However, in systemic anaphylaxis (produced by injection of immune aggregates) in the rabbit, Movat *et al.* (*4*) noted that intraluminal PMN phagocytized the immune aggregates and also that they gradually degranulate. This suggested that the rise in serum cathepsins and acid proteases in anaphylaxis might be a result, at least partly, of an intraluminal release from the PMN's (*5*). Fragmentation of PMN has been observed in pulmonary capillaries after injection of endotoxin which produces the generalized Schwartzman reaction (*6*); we saw some similar disruptions of PMN's in synovial vessels. The relation of this fragmentation to the more common form of degranulation of otherwise intact cells in not clear. Peripheral blood with bacterial infection (*7*) did not have PMN with decreased numbers of granules but the PMN did degranulate in vitro more quickly than did controls where no bacterial infection was present. Degranulated PMN in vessels located at sites of inflammation may not survive to be reflected in any large numbers in the peripheral circulation.

The variety of diseases which are accompanied by intraluminal degranulation suggests that this occurrence is not a specific one, but we propose that intraluminal release of PMN granules that contain histamine, kinins, and other mediators of inflammation (*8*) may be an important mechanism in vascular injury in many diseases and at many sites.

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Synaptic Transmission Depressed by Colchicine Blockade of Axoplasmic Flow

Abstract. *Colchicine, which inhibits axoplasmic transport and induces organelle alterations in nerve terminals, was injected intraocularly in pigeons. Electrical stimulation of the optic nerve yielded normal evoked potentials in retinotectal fibers, whereas postsynaptic responses recorded in the tectum were reduced. Postsynaptic depression suggests a deficit of synaptic transmission, presumably dependent on colchicine interference with migrating material.*

Material that is involved in rapid axonal flow and is synthesized in the neuronal perikarion migrates at speeds greater than 40 mm/day and reaches the presynaptic nerve terminals (*1*). Ganglion cells of the retina provide a good experimental preparation for axonal flow studies (*2*), particularly in birds whose optic fibers are generally considered to be completely crossed (*3*). A few hours after intraocular injection of radioactive amino acids, labeled proteins can be detected in the optic nerve terminals (*4*). The role of this migrating material in the presynaptic terminals is unknown, although investigations on adrenergic neurons suggest its involvement in catecholamine metabolism and in formation of dense core vesicles (*5*). Interference with the supply of this fast flow material to nerve endings may shed light on synaptic as well as axonal functions.

Colchicine has been shown to interfere with axoplasmic transport, especially with the rapid flow processes (*6*). When injected intraocularly in the pigeon, colchicine inhibits the appearance in the optic tectum of labeled proteins after introducton of [^{3}H]leucine in the vitreous humor 24 hours earlier. This effect induced by colchicine is evident within 1 day, reaches a maximum at 4 days, and disappears after a few weeks (*7*). Moreover, intraocular injection of colchicine in the pigeon causes reversible alterations in the ultrastructure of optic nerve terminals (*8*); within a week synaptic vesicles enlarge, fibrils aggregate, mitochondria swell, and glycogen granules accumulate. The first ultrastructural alterations are evident 4 days after colchicine injection and disappear gradually by 4 to 6 weeks. These changes are strikingly similar to those observed after retinal ablation, except that colchicine-induced effects are reversible (*9*).

These observations suggested that the rapid phases of axoplasmic transport

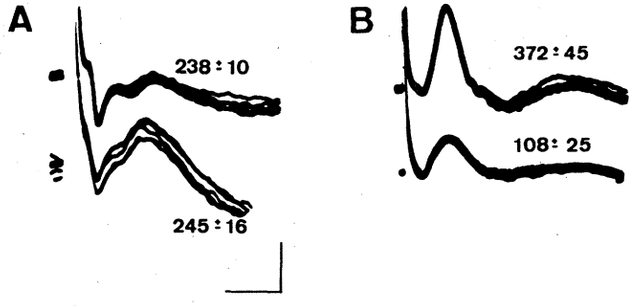

Fig. 1. (A) Optic tract responses and (B) tectal responses (depth, 500 μm) to optic nerve stimulation 3 days after injection of colchicine (100 μg) in the vitreous humor of one eye. Stimulus intensity, 1.0 ma; in both (A) and (B), upper trace from control side, lower trace from colchicine-treated side. Numbers indicate mean amplitude (μv) ± S.D. ($n = 10$); vertical calibration, 200 μv; horizontal calibration, 5 msec; positivity upward.