# Human Polymorphonuclear Leukocytes: Demonstration of Microtubules and Effect of Colchicine

Abstract. *Microtubules are demonstrable in mature human polymorphonuclear leukocytes, even after prolonged incubation in vitro. These organelles are not seen after treatment with colchicine, which has various functional effects on these cells but does not inhibit phagocytosis.*

Since glutaraldehyde was introduced as a fixative for electron-microscopic studies (*1*), characteristic microtubular elements have been found in an increasingly wide variety of plant and animal cells. Microtubules may be important in the mechanics of cellular motion generally, and in the stabilization of cellular form (*2*); they have been implicated in the development of protoplasmic gel strength (*3*). These organelles are prominent in the mitotic spindle (*4*), where the centriolar regions seem to function as at least one set of orienting centers. Treatment with colchicine results in dissolution and contraction of the spindle (*5*).

Microtubules have not previously been described in mature polymorphonuclear leukocytes (PMN's) (*6*), perhaps because of the apparent sensitivity of microtubules to the low temperature and relatively high *p*H used in most previous methods of fixation of PMN's for electron-microscopic study. The PMN is the predominant cell type in the fluid of inflamed joints during acute attacks of gout, for which colchicine is a time-honored and efficient therapeutic agent. Treatment of PMN's with colchicine in vitro has various functional consequences—interference with locomotion (*7*), with chemotaxis (*8*), with adhesiveness (*9*), with lysosomal degranulation (*10, 11*), and with the metabolic burst during phagocytosis (*10, 12*) [but not with phagocytosis per se (*10*)]—all of which may be important for the anti-inflammatory effect of colchicine in acute gouty arthritis.

Leukocytes of human blood were incubated at 37.5°C in a 12 percent serum Krebs-Ringer phosphate buffer for 1 hour with or without colchicine, and for a second hour with or without live $\alpha$-hemolytic streptococci. The cells in suspension were then fixed at room temperature in 2 percent glutaraldehyde buffered to about *p*H 6.0 and were treated with $OsO_4$, imbedded in Maraglas, stained with lead salts, and examined in an Elmiskop I electron microscope. This experiment was repeated three times, and in each instance sections of several hundred cells were examined.

In untreated granulocytes, whether resting or phagocytizing, microtubules approximately 250 Å in diameter were seen (Fig. 1). Centrioles were also present, often with microtubules radiating from them. In resting and phagocytizing cells treated with colchicine ($2.5 \times 10^{-5}M$ to $2.5 \times 10^{-4}M$), centrioles could still be found, but microtubules could not (Fig. 2).

This ultrastructural alteration by colchicine has not yet been definitively related to the functional effects of the drug on PMN's. However, the observations are consistent with the thesis (*13*) that interference with protoplasmic gelation is the common basis for the ac-

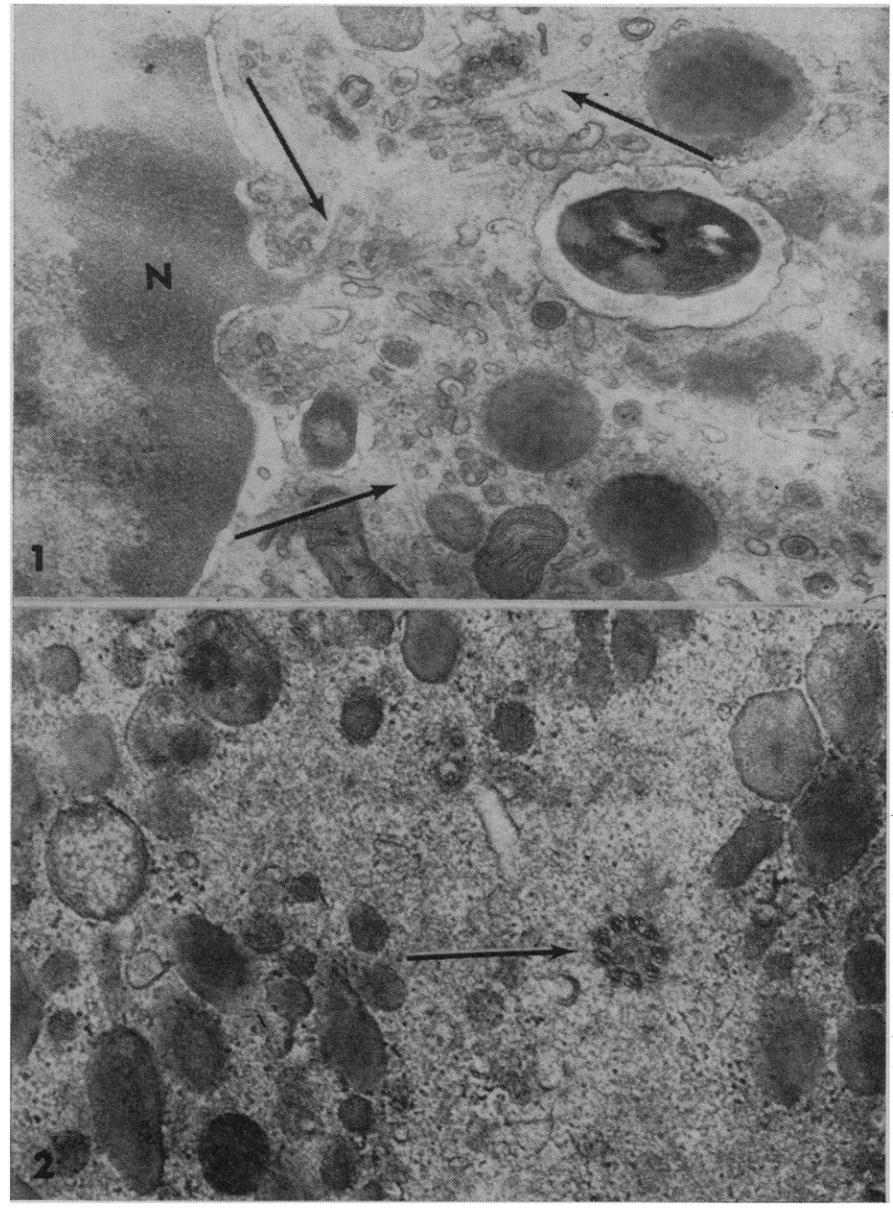


Fig. 1. Human polymorphonuclear leukocyte after incubation with streptococci; one of the latter (*S*) is present in a digestive vacuole. Several of the numerous microtubules visible are marked by arrows. *N*, Part of the nucleus (× 35,000). Fig. 2. Human polymorphonuclear leukocyte after incubation in a medium containing $2.5 \times 10^{-4}$ mole of colchicine per liter. Microtubules were not identifiable, but centrioles were present. A cross section of one of the latter is marked by an arrow. Note the neutrophilic granules in both illustrations (× 32,000).

tion of colchicine both as an arrester of metaphase in dividing cells and as an anti-inflammatory agent in acute gouty arthritis.

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## Calcareous Septa Formed in Snail Shells by Larvae of Snail-Killing Flies

Abstract. *Larvae of 13 species of* Pherbellia *and* Colobaea *that feed in exposed aquatic snails uitilize a product of the Malpighian tubules before they pupate to form a plate-like structure within the shell or to reinforce the anterior end of the puparium. The substance is partly calcium carbonate, and carbonic anhydrase may be involved in its production.*

A few insects are known to utilize calcium carbonate collected in their Malpighian tubules. A cercopid, *Ptyelus*, makes a spiral shell of calcium carbonate; larvae of *Cerambyx* beetles form a calcareous plate to close their burrows; cuticle of stratiomyid larvae is reinforced by deposits of lime; chorion of eggs of phasmids is composed partly of lime derived from the Malpighian tubules; and the inner surface of the puparium of the celery fly, *Euleia* (=*Acidia*) *heraclei* (L.), is strengthened with a hard layer of lime (*1*). The puparium of the face fly, *Musca autumnalis* De Geer, also is strengthened with calcium carbonate (*2*).

Pupating larvae of several species of snail-killing flies (Diptera: Sciomyzidae) utilize a product of their Malpighian tubules to form a plate-like partition, the "septum," in shells of their food snails (Figs. 1–3) or simply to coat the anterior end of the puparium with a layer of the material. The occurrence, formation, morphology, chemical composition, and possible involvement of an enzyme are discussed here.

Although larvae of various Sciomyzidae feed on aquatic and terrestrial snails, snail eggs, slugs, and fingernail clams, septa are produced only by a few species that routinely feed on and pupate within the shells of exposed aquatic snails (Lymnaeidae, Physidae, Planorbidae). The phenomenon was studied first during rearings of *Pherbellia dorsata* (Zetterstedt) (*3*). Later it was observed during rearings of 11 other species of *Pherbellia* [*P. argyra* Verbeke, *P. griseola* (Fallén), *P. idahoensis* Steyskal, *P. nana* (Fallén), *P. obtusa* (Fallén), *P. quadrata* Steyskal, *P. seticoxa* Steyskal, *P. similis* (Cresson), *P. trabeculata* (Loew), *P. trivittata* (Cresson), and *P. vitalis* (Cresson) (*4*)] and *Colobaea americana* Steyskal.

Production of septum material is not correlated strictly with the taxonomic affinities of the species concerned, and the feature apparently has evolved several times (*4*). All of the 12 species of *Pherbellia* that have been studied do not belong to the same species groups (which are based on morphological characters), and some other members of these groups do not produce septum material. Seven of the producing species are Nearctic, two are Palearctic, and *P. argyra, P. griseola,* and *P. nana* are Holarctic in distribution. Of the four reared species of the Holarctic genus *Colobaea,* only the Nearctic *C. americana* produces septa. Species that produce septa overwinter as pupae in habitats that become flooded during spring. The integument of the puparia of some species that form septa is characteristically thinner and weaker than the integument of species that form puparia outside of shells.

The septum or coating material is produced in the Malpighian tubules. These tubules are white, extend almost the length of the body, and are easily visible through the transparent integument during the entire larval period. They become enlarged several days before formation of the puparium, and their color often changes to light pink or yellow a few hours before excretion of the septum material.

Formation of complete septa requires 1 to 2 hours. The larva shortens, thickens, and extends its anterior spiracles (as do pupating larvae of other reared Sciomyzidae) just before elimination of the milky material through its anus. A rather large amount of this liquid is squeezed anteriorly between the shell and larva by peristaltic movements of the body wall. By the time this material reaches the anterior end of the larva it has a frothy texture. The hardening material is then formed into the plate-like structure by movements of the anterior end of the larva. These movements consist primarily of a side-to-side waving and occur most frequently at the margin, where in most species a hole eventually develops in the septum. It is not known whether any other substances, such as salivary gland secretions, are added to the septum material after it reaches the anterior end of the larva.

The calcareous material dries and hardens quickly on exposure to air, which indicates that moisture conditions within the shell are important for construction of well-formed septa. Species that optimally produce complete septa in slightly damp containers formed imperfect septa when rearing containers were wet. Pupating larvae of a few species (*P. idahoensis, P. nana,* and *P. quadrata*) do not form complete septa even in drier containers. They produce only a rim of the material on the inner wall of the shell or a thick coating on the anterior end of the puparium, or both. Although puparia of these and other species whose larvae occasionally leave the shell to pupate become encrusted with septum material, the anterior and posterior spiracles remain clean. This is probably due to a hydrofuge substance secreted by glands around the spiracular openings.

Septa are formed in discoidal (such as *Helisoma*) as well as pyramidal (such as *Lymnaea*) shells. Septa and puparia of some species (for example, *P. seticoxa*) have been found in both types of shells in nature. When the shell of the food snail is several times larger than the puparium, the larva pupates