INDUCTION OF CELLULAR MOVEMENTS IN MAST CELLS BY COLCHICINE TREATMENT

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The free peritoneal fluid cells of the rat (eosinophils, mast cells, monocytic and lymphoid elements) are normally spheroidal. When allowed to settle on a solid substrate, such as a microscope slide, all but the mast cells display ameboid movements. After administration of colchicine to the animals, the morphology of the free cells collected has become altered in a characteristic manner which involves displacement of the nucleus to the periphery and pronounced anisodiametry of the cytoplasm. The elongated cytoplasmic mass then displays nodal constrictions. Although all the cellular types in the peritoneal fluid are similarly affected (9), the response is most strikingly evidenced by the mast cell, which thus affords an excellent test object for detailed study of this morphological effect. The higher the dose, the greater the percentage of affected cells and the greater the departure from normal morphology (for review, see reference 10).

In young rats, the spheroidal shape of the free peritoneal mast cell and the central location of its nucleus are readily visualized in both living (Fig. 1) and in fixed and stained (Fig. 2) preparations. In randomly oriented cells of wet-fixed preparations, the nuclear morphology can be seen to advantage: the nucleus is consistently spherical or nearly so (less than 2% are overtly nonspherical). Time-lapse cinematography (1 frame per sec) of normal mast cells in vitro (11, 12) shows that they do not display ectoplasmic deformations. Neither stubby pseudopods nor hyaloplasmic veils are seen. Only in a minority of cells do the crowded cytoplasmic granules exhibit slight saltatory movement, but it remains to be established that this is a normal attribute of the undamaged cell.

On the other hand, microscope observation of fixed and stained colchicine-affected mast cells not only demonstrates the over-all cytomorphic changes mentioned, but also suggests that the nucleus is subjected to deforming pressures directed from the main cytoplasmic mass, as evidenced by its often hemispherical appearance and by its orientation, in which the collapsed side consistently faces and abuts on the granular portion of the cytoplasm (Fig. 3). Time-lapse cinematography of living colchicine-affected cells reveals that they radically depart from their normal behavior. Indeed, from the granule-free face of the nucleus that protrudes from the cell, stubby or broad cytoplasmic extensions, large and actively waving protuberances, or hyaloplasmic veils are repeatedly formed and withdrawn by every affected cell (Fig. 4). In addition, the deformed granular cytoplasmic mass is seen to exhibit a continued and extensive churning activity which is

evidenced by local streaming of the specific cytoplasmic granules. This causes the affected cells to twist and deform, in striking contrast to their placid normal counterparts. The normally ameboid eosinophilic granulocytes and macrophages continue to move about, albeit in a pattern that is less directed than that shown by similar cells from untreated animals.

The effect of colchicine is rapid: it is seen within minutes after subcutaneous injection and almost immediately upon intraperitoneal injection of the drug. At optimal dosages, as many as 98% of the mast cells are affected. It is a reversible effect; normal looking cells are recovered if sampling is delayed for several hours after the animal has received an injection of the drug (the exact interval before recognition of the effect as well as of the recovery depends on drug dosage administered), and their concentration is then still within the normal range. Several weeks are required for the reappearance of mast cells after their experimental eradication from the peritoneal cavity of the rat (1), and it is therefore unlikely that new, normal cells could replace the affected ones in a matter of only a few hours.

When mast cells from untreated animals are observed for prolonged periods in vitro, none of the changes described herein are seen before cell death and lysis, and therefore the cytoplasmic movements of colchicine-affected mast cells are not agonal ones. Nor do these movements represent a response to a physicochemical property of the drug, such as surface activity, for instance, since the response is highly specific (9) and since low doses are effective (4×10^{-6} M is optimal for subcutaneous administration in 1-month-old rats). It seems more likely that a specific biochemical lesion is involved.

These observations are of special interest in several respects. First, since interphase and postmitotic cells are involved in the morphological response to colchicine, they underscore the drug's paramitotic effects, effects shared by several other c-mitostatic substances and their derivatives such as colcemid, podophyllotoxin, and vinblastine (10). These paramitotic effects have been emphasized repeatedly (9, 10) since they were first observed in mast cells (4, 7, 14), and their attendant ultrastructural features have more recently been described for HeLa cells in tissue culture (17). Second, they attest to the rapid interaction of colchicine and cytological receptor sites, an interaction much more rapid, in fact, than that suggested by Taylor on the basis of a complex experimental approach (18). Thirdly, they indicate clearly that development of static constriction rings squeezing the passive, remaining cytoplasm into a nodular mass, a hypothesis previously advanced on the basis of fixed preparations (15), does not adequately define the underlying mechanism, although sol $\rightleftharpoons$ gel changes, albeit of a more transient nature, remain implicated. The constriction rings are definitely not static structures, and a more widespread cytoplasmic involvement than was at first surmised is indicated. Finally, they perhaps bear on the question of mast cell migration, an often debated topic for which no convincing direct evidence has so far been available. Mast cells can mold themselves intimately to underlying connective tissue fibers, implying an ability to modulate their shape. Nevertheless, the morphological response to colchicine is of an altogether different order of magnitude, suggesting that, under some as yet undiscovered physiological stimuli, a rapid redistribution of mast cells would not be impossible.

A similar activating effect of vinblastine on protoplasmic streaming has been recently reported for cultured HeLa cells exposed to the drug for 2 hr (2). On the other hand, colchicine inhibition of leukocyte ameboid movement in vitro also has been reported, but much higher concentrations of the drug were used (5). Leukocyte diapedesis in vivo and migration in vitro are both inhibited by colchicine (3, 6).

Colchicine is known to affect the mitotic spindle. For various derivatives of colchicine, this mitostatic effect quantitatively parallels the morphological effects on nonmitosing mast cells (9, 13). The present experiments suggest the possibility that spindle protein(s) also may be involved in the paramitotic effects of the drug, as already suggested in previous studies on mast cells (8–10, 13, 15). Since colchicine disorganizes both microtubules and spindle, one would expect a rounding effect on cells rather than the asphericity elicited in peritoneal fluid cells. The reasons for this unanticipated finding remain to be explained.

Streaming of cytoplasmic granules has also been demonstrated by cinematography of mast cells obtained from peritoneal transudates evoked by particulate substances such as quartz (16). No ectoplasmic pseudopodial activity was reported, but the phase optics used in that study are not so

suitable for this purpose as the Nomarski optics used in this study.

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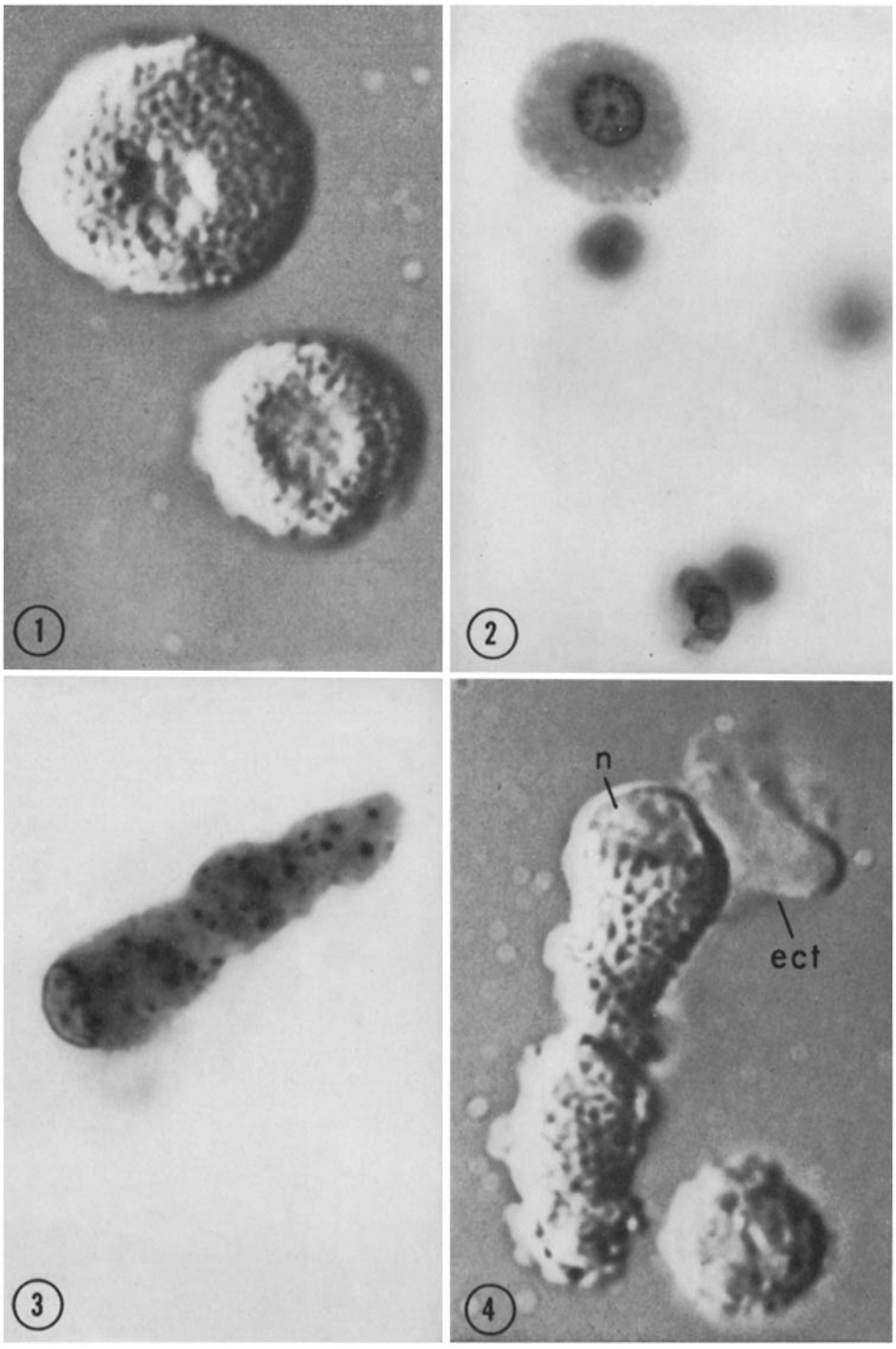
FIGURE 1 Normal rat mast cells in whole, unmodified peritoneal fluid, as visualized by contrast interference microscopy (Nomarski system) shortly after withdrawal from the cavity. The cells are spheroidal, and their nucleus is visualized at the center of the granular cytoplasm. No ectoplasmic projections are seen. Enlarged frame from movie sequence. × 2700.

FIGURE 2 Normal rat mast cell. Peritoneal fluid was wet-fixed with Carnoy's solution and then stained with Grenacher's alum carmine and Bismarck Brown. The spherical nucleus is clearly visualized in its central location. The specific granules outline the cytoplasm which itself remains unstained. × 2000.

FIGURE 3 Fixed and stained peritoneal fluid mast cell from rat injected subcutaneously with colchicine. The cytoplasm has become elongated and shows nodal constrictions. The nucleus, seen at one pole of the cell, shows a normally curved border on one side where there are no cytoplasmic granules (at left on the illustration) but a collapsed outline on the opposite side where the cytoplasmic granules are present and presumably pushed (or pushing?) against it. × 2000.

FIGURE 4 Living mast cell from colchicine-treated rat. The elongation of the cytoplasm and its nodal constrictions are evident, as is the nucleus at one pole (*n*). A prominent ectoplasmic extension (*ect*) is also seen. It is devoid of granules and, in the motion picture sequence, it is seen to undulate and flow rapidly, changing its shape constantly at a rate at least as rapid as normally seen in pseudopods of moving leukocytes. The small cell at lower right is a macrophage. Enlarged motion picture frame. × 2700.



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