UPTAKE OF COLLOIDAL THORIUM DIOXIDE BY MAST CELLS

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ABSTRACT

Mast cells from the peritoneal cavity of the rat were obtained at various times following *in situ* injection of a colloidal thorium dioxide preparation (Thorotrast). They were prepared for electron microscopy by aldehyde fixation, osmium tetroxide postfixation, and embedding in Epon. Thorotrast was rapidly taken up by mast cells through enhanced or newly elicited surface specializations. It was confined at first to large vesicles which moved to the Golgi area. Subsequently, in a matter of a few hours only, it became associated with progressively more mature granules, including "fully" mature ones. In addition to demonstrating a further phagocytic or pinocytotic activity of mast cells, the findings suggest that mast cell granules share a common membranous investment, and that substances from the tissue environment may theoretically percolate over and interact with the granules. Mast cell function could thus be served primarily by absorptive rather than secretory processes.

INTRODUCTION

Although reports of mast cell phagocytosis have appeared in the early literature, they were not compelling. As early as 1892, Metchnikoff (29) suggested that mast cell granules may represent phagocytized material. This hypothesis was soon abandoned. Uptake of colloidal dyes such as lithium carmine and trypan blue was investigated in several laboratories, with mixed success. Thus Kiyono (25) and Nagayo (33) stressed that mast cells were unable to take up these dyes, while Tsuda (57) reported that they could do so to a limited extent.

Occasional reports of mast cells containing extraneous particulate such as cocci (57) or erythrocytes (6, 7, 33, 58) have appeared, but at least in some of these reports (6, 7, 58) the mast cells may not have been normal. In a detailed assessment of the extant literature up to 1938, Michels (30) concluded that the possibility ". . . that tissue mast cells are capable of extended phagocytosis seems doubtful. . . ." Some thirty years later, a thorough reassessment of the literature led Selye (53) to conclude that ". . . true mast cells possess little if any phagocytic potency. . . ." The problem was complicated by the possibility of confusing with mast cells macrophages that have ingested either metachromatic polysaccharides (21, 22, 23, 52, 53) or shed mast cell granules (8, 23, 54). Less uncertainty arises if one employs electron microscopic techniques. With these, incontrovertible evidence that mast cells can ingest zymosan was recently obtained (42, 43). This paper demonstrates that mast cells also ingest Thorotrast, a particulate substance of much smaller particle size than zymosan. It suggests that phagocytosis may be a common physiological activity of mast cells and that, on this basis, mast cells legitimately may be classified as members of the reticuloendothelial system. Furthermore, the pattern of uptake of the finer particulate allows inferences regarding the ultrastructural organization of mast cells which could have far-reaching implications for yet undetermined mast cell function(s).

Some of these findings have been reported in abstract form (41, 44).

MATERIALS AND METHODS

Sprague-Dawley male rats[1] 31–36 days old and weighing 84–164 g were injected intraperitoneally with 0.2 ml of colloidal thorium dioxide stabilized with dextrin (Thorotrast, Fellows-Testagar, Div. Fellows Medical Mfg. Co., Inc., Detroit, Mich., Lot 14222). At various times thereafter (15 min, 30 min, 1 hr, and 24 hr), animals were anesthetized with ether, exsanguinated by decapitation, and the peritoneal cavity was exposed by a midline incision. Peritoneal fluid, withdrawn with a medicine dropper, was then fixed at room temperature in dilute Karnovsky's fluid (24). The cells were centrifuged into a pellet, washed in cold sucrose-cacodylate buffer solution, and post-fixed for 1 hr at room temperature with 1% osmium tetroxide in Veronal acetate buffer, pH 7.4. After dehydration through ethanol at room temperature, the cells were embedded in Epon by the standard Luft procedure (28). Thin sections were examined (RCA EMU-3G, 100 kv) unstained or after staining with uranium acetate and counterstaining with lead citrate solutions.

As a control, 0.1 ml of Thorotrast was premixed with 1.0 ml of fixative, and this mixture was used to fix peritoneal fluid from an uninjected rat. Further processing was as described above.

RESULTS

Mast cells phagocytized Thorotrast. Uptake commenced rapidly, and, as early as the first sampling a mere 15 min postinjection, some of the particulate was seen in small vacuoles near the cell surface as well as in large vesicles in the vicinity of the Golgi complex. In samples obtained during the first hour after injection, many mast cells displayed enhanced plasmalemmal activity. Whereas almost all peritoneal mast cells of untreated young adult rats are spheroidal, many in the injected rats displayed definite pseudopod-like deformations which gave the cell an irregular outline (Figs. 1 and 5).

In addition, many of the mast cells from injected rats (Fig. 5) displayed richer and more complex membranous outfolding and villosities than was normally seen in untreated rats. Intracytoplasmic invaginations or vacuoles with villous projections were noted often throughout this study. These were seen as early as 15 min after injection (Figs. 1 and 8) and were still unusually common 24 hr later (Fig. 17). They were seen only rarely in mast cells of uninjected animals.

Single particles, as well as aggregates of several particles, occasionally were seen adherent to the outer cell surface during the first hour after injection. These were taken up into the various membranous infoldings described above (Figs. 1 and 5) by what appeared to be hyaloplasmic veil activity. In addition, clublike surface specializations replete with aggregated vesicles (Figs. 2–4) were unusually common. Characteristically, these vesicles contained single or small clumps of several Thorotrast particles (Figs. 2–4). Similar structures were seen only occasionally on the surface of mast cells obtained from untreated control rats.

In the large vesicles, deep within the cytoplasm, the particulate was irregularly clumped and loosely held within the vacuolar confines (Fig. 6). Other materials were often simultaneously present in these vesicles, including amorphous masses of finely granular material and myelin figures. During the first hour after injection accumulations of particulate were seen within normally-occurring large vesicles which contained irregular masses of medium electron opacity (12, 40), vesicles that have been described as aggregating progranules (12). Accumulations of Thorotrast were also noted in association with more condensed and more homogeneous granules thought to be mature or nearly so (Figs. 9 and 10). At a later time (24 hr), Thorotrast was clearly associated with the more mature granules. In these, the particulate was condensed variously into masses capping a granule (most common), in the narrow space between the granule and its membranous investment (less common), or as small cores, or occasionally as irregular channels within the granular substance (relatively rare). These various patterns of Thorotrast deposition are illustrated in Figs. 11–16.

Even where Thorotrast was intimately apposed to the granules or dispersed through their substance, there was no evidence of alteration in the appearance of the granules. Lysed granules were irregularly seen in both intact and treated animals and, in treated rats, did not appear to be related to Thorotrast accumulations. The proportion of mast cells containing Thorotrast increased with time, and most mast cell profiles contained some

[1] Rats were obtained from the Holtzman Company, Madison, Wis., and from the Charles River Breeding Laboratories, North Wilmington, Mass. There was no apparent difference in the mast cell behavior of these two substrains.

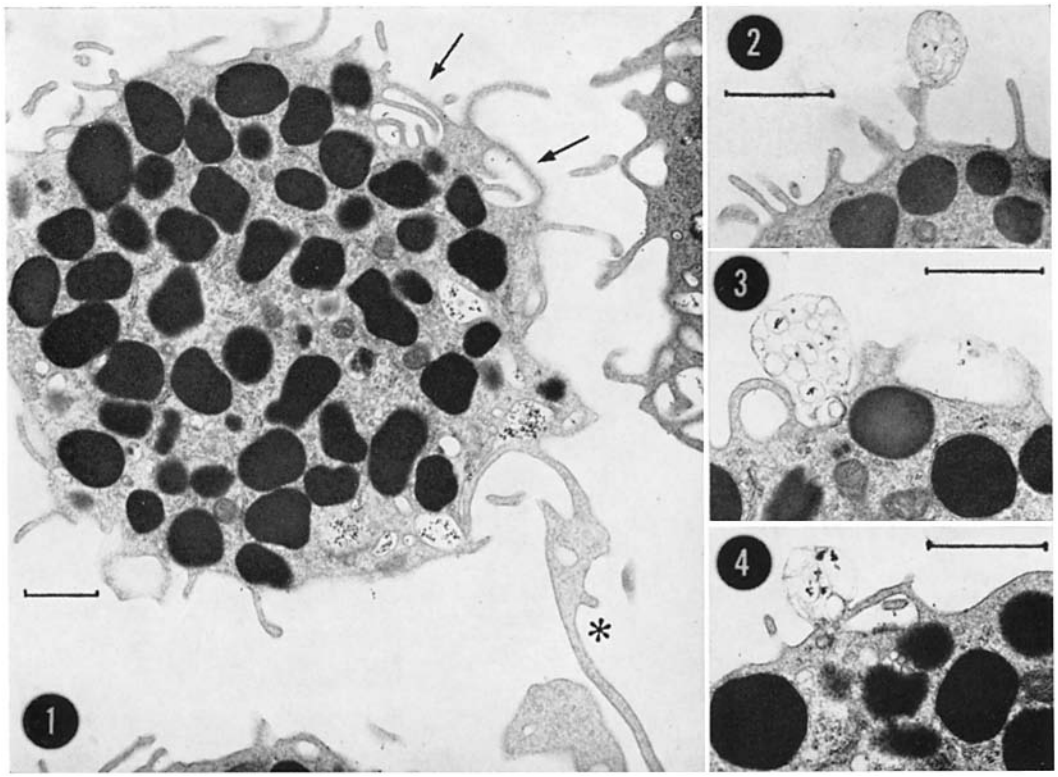


FIGURE 1 Rat peritoneal fluid mast cell 15 min after Thorotrast injection. Enhanced plasmalemmal activity is evident in the form of surface invaginations (arrows) and hyaloplasmic veils (*). Single particles of Thorotrast are sequestered by the invaginating channels. Larger accumulations of particulate are seen in numerous vacuoles which are near the more active surface areas of the cell. Doubly stained with uranyl acetate and lead citrate solutions. × 9,400, approximately.

Scale marks on all electron micrographs equal 1 μ.

FIGURES 2–4 Free surfaces of three mast cells 15 min after injection, showing club-shaped cytoplasmic specializations. These club-shaped structures are very similar to the ones described by Bloom and Häegermark (see Fig. 16 of reference 5). The characteristic feature of these structures is a cluster of small vesicles reminiscent of foam, many of which contain individual or very few particles of Thorotrast. These specializations are in continuity with the cell body through either a tenuous stalk (Fig. 2) or a short broad neck (Figs. 3 and 4). It should be noted that the "microvilli" on the mast cell surface often represent thin ruffles or folds whose leading edges may coalesce with the plasmalemma to form pinocytotic vesicles (Fig. 3, at left of clublike structure) which act to entrap Thorotrast particles (Fig. 3, at right of clublike structure). Double stain. Approximate magnifications: Fig. 2, × 14,100; Fig. 3, × 15,450; Fig. 4, × 10,200.

at 24 hr. Mast cells were plentiful at this time. Similarly, the amount of Thorotrast in individual mast cells increased with time after injection. In some animals, free Thorotrast was abundant in the spaces between the loosely centrifuged cells (Fig. 1), and some was still found there 24 hr after injection (Fig. 17). Thorotrast was taken up by macrophages and by eosinophils, cell types that are normal constituents of rat peritoneal fluid. The macrophages were extremely phagocytic, much more so than the mast cells. In the macrophages the Thorotrast was also initially taken up by pinocytotic and small phagocytic vacuoles, but as these vacuoles coalesced to form larger phagocytic vacuoles during the early stages of uptake, the particulate showed a strong predilection for the vacuolar membrane, leaving a particulate-free area at the center of the vacuole (Fig. 7). This

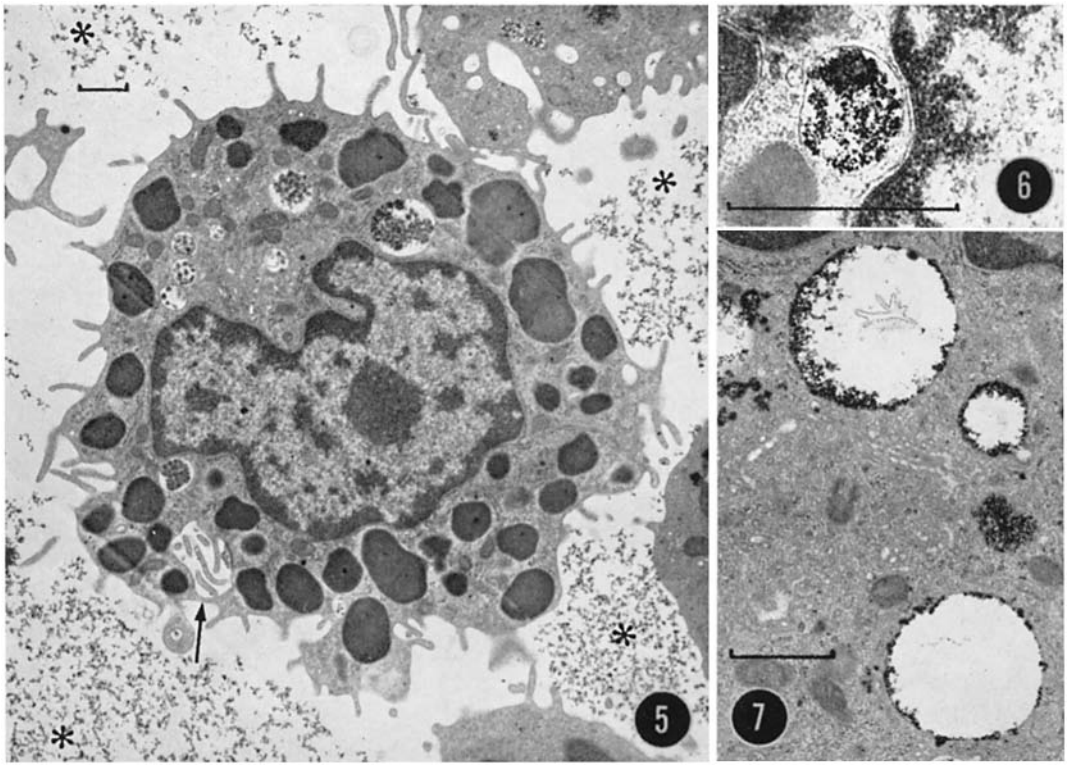


FIGURE 5 Peritoneal fluid mast cell 1 hr after Thorotrast injection. The outline of this cell is far more irregular than is normally seen in mast cells of untreated animals. Note that granules, mitochondria, and rough-surfaced endoplasmic reticulum are represented in the two pseudopod-like processes at lower left. Surface invaginations are present (arrow). There are several vacuoles containing Thorotrast in this micrograph, but these are not readily evident at this low magnification. Much Thorotrast is present in the extracellular space (*). Double stain. × 6,600, approximately.

FIGURE 6 Large Thorotrast-containing vacuole in a mast cell 1 hr after injection. The Thorotrast is loosely held in the lumen and shows no predilection for the vacuolar membrane. Some amorphous material of unknown nature is also present in the vacuole. Double stain. × 30,600, approximately.

FIGURE 7 Peritoneal macrophage vacuoles containing Thorotrast, 30 min after injection. Note definite association of the particulate with the vacuolar membrane. Contrast with Fig. 6. Double stain. × 13,500, approximately.

contrasted with the loose manner in which Thorotrast was held at this time within mast cell vesicles (Fig. 6). Eosinophilic leukocytes were less phagocytic than the mast cells, and the particulate within their phagocytic vacuoles assumed the pattern encountered in mast cells rather than that seen in macrophages. No Thorotrast particles were seen within the specific eosinophilic cytoplasmic granules.

Temporally speaking, a definite progression was observed. Thus, gross deformation of the mast cell was seen mainly during the first hour. Other types of surface activity, initiated early, were maintained throughout. Particulate, first seen in these surface specializations as well as in large vesicles, soon was seen within the vesicles associated with early stages of progranule aggregation, then within those vesicles representing later stages (all within the first hour). Finally, the particulate was located in the "mature" granules at 24 hr.

No Thorotrast was ever noted within the saccules of the Golgi apparatus, within the various

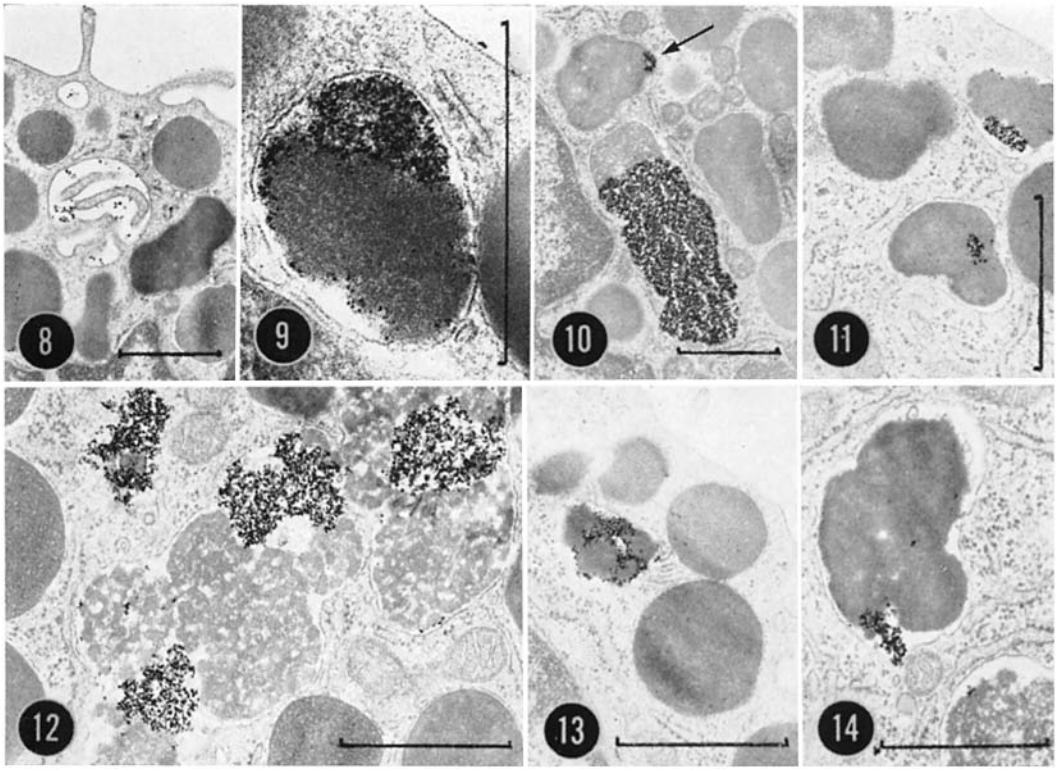


FIGURE 8 Peritoneal mast cell 15 min after injection. Small pinocytotic vacuoles containing Thorotrast are seen just below the plasmalemma. A larger area with thorium represents either a curving invagination of the cell surface forming an intracytoplasmic canaliculus or a phagocytic vacuole whose limiting membrane is still thrown into hyaloplasmic veils or microvilli. Double stain. × 13,100, approximately.

FIGURE 9 Peritoneal mast cell 1 hr after Thorotrast injection. Thorotrast in contact with substance of developing granule. Note that the particulate is loosely associated with the condensing granular matrix, some scattered loosely at its periphery and much apposed as a large mass in a rather restricted area. The limiting membrane is not associated with particulate. Uranyl acetate-lead citrate stain. × 45,000, approximately.

FIGURE 10 Peritoneal mast cell 30 min after Thorotrast injection. Localized accumulations of particulate are seen in two granules deep within the cytoplasm. Both granules are in an advanced stage of condensation. The small accumulation (arrow) might suggest that the material is channeled into the granular space through a narrow inlet. The large mass of Thorotrast appears to consist of three merging segments. There is no membrane between the granule and its area of contact with the particulate. Double stain. Approximately × 13,500.

FIGURE 11 Fairly compacted mast cell granules 24 hr after Thorotrast administration. Small nests of particulate appear embedded in the granule substance. Double stain. × 22,500, approximately.

FIGURE 12 Peritoneal mast cell 24 hr after Thorotrast injection. Cluster of developing granules in an early stage of maturation when condensing progranules can still be discerned. Not only are these developing granules in relative proximity to each other, but several seem to be merging. Thorotrast is becoming embedded in the condensing granular substance not only as large masses but also throughout as individual particles. Note that there is no Thorotrast in the extragranular areas. Free Thorotrast was still present in the peritoneal fluid of this animal at 24 hr (same rat as Fig. 17). Uranyl acetate-lead citrate stain. × 22,500, approximately.

FIGURE 13 Complex pattern of Thorotrast deposition within the granular matrix of a peritoneal mast cell 24 hr after injection. This pattern could arise from coalescence of smaller granules already coated with Thorotrast. Uranyl acetate-lead citrate stain. × 22,500, approximately.

FIGURE 14 Nearly mature mast cell granule 24 hr after Thorotrast injection. The lobed outline of this granule suggests that it may have arisen from fusion of several smaller ones (cf. Fig. 13). A small accumulation of Thorotrast is present under the delimiting membrane. Uranyl acetate-lead citrate stain. × 22,500, approximately.

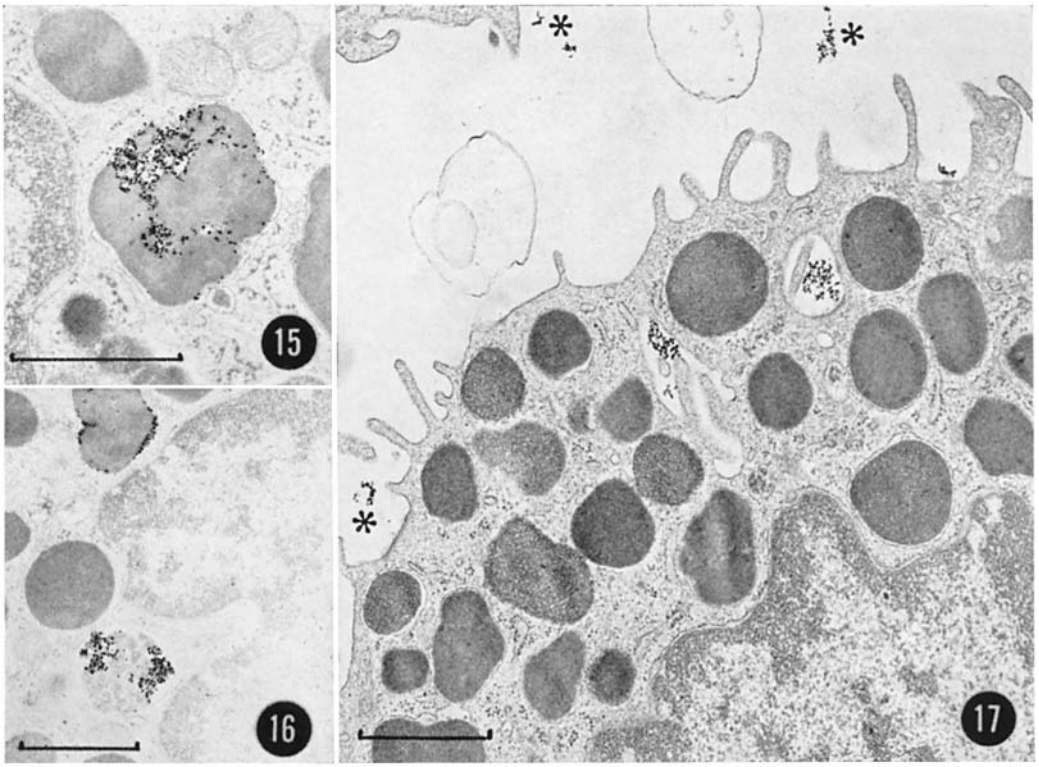


FIGURE 15 Complex Thorotrast deposition pattern in a mature mast cell granule at 24 hr after injection. Some of the Thorotrast-laden areas do not display the same matrix density as the particulate-free areas, a fact that lends support to the granule growth concept suggested by Figs. 13 and 14. Uranyl acetate-lead citrate stains. Approximately × 22,500.

FIGURE 16 Peritoneal mast cell 24 hr after Thorotrast injection. A "young" granule (bottom) displays loosely aggregated Thorotrast masses that penetrate the open granular matrix. A more mature granule (top) is surrounded by a thin deposit of Thorotrast particles restricted to the narrow gap between the granule and its enveloping membrane. The dense granular matrix is not penetrated by the particulate. More mature granules evidently remain accessible to the ingested material. Double stain. Approximately × 15,600.

FIGURE 17 Peritoneal mast cell displaying continued surface invagination at 24 hr after Thorotrast injection. Small clumps of Thorotrast (*) are still seen outside the cell. Their size, often similar to that of material found in the phagocytic channels and vesicles, suggests they may be taken up as such and thus that the localized accumulations of particulate seen in the previous figures may be in part artifactitious. Doubly stained. Approximately × 17,100.

vesicles that crowd the Golgi internum, within the cisternae of the endoplasmic reticulum, within mitochondria, or within the nucleus.

Addition of Thorotrast to the fixative before use was without effect. The cells displayed their normal shape with no increase in surface activity; no Thorotrast was seen in any of the cells, and only an occasional particle was seen sticking to a cell surface.

For reference purposes, mast cells from an uninjected control animal are shown in Fig. 19.

DISCUSSION

The amount of Thorotrast injected for the present studies (0.2 ml) was large in that it was equivalent to the amount of peritoneal fluid normally present in the rat peritoneal cavity (35, 45). This amount could be expected to overload the extremely active

competition from the many macrophages present in peritoneal fluid, and it insured that particulate remained available to the mast cells for some time.

It has been reported that Thorotrast can be carried into cells during and subsequent to fixation with osmium tetroxide solutions (56, in Discussion). The controls used in the present study demonstrate that this was not the case for Karnovsky's fixative, nor did it occur during the post-fixation step with osmium tetroxide. Thus localization of Thorotrast within mast cells was demonstrably not artifactitious, and the presence of extracellular Thorotrast, even in the large amounts found in the earlier samples, could not have contributed to a post mortem intracellular localization of particulate.

The value of the present findings is twofold. First, they reinforce the possibility that uptake of particulate materials by mast cells, already demonstrated with zymosan (42, 43), may have broad physiological significance. Second, the pattern and the dynamics of both uptake and distribution of the ingested Thorotrast within the mast cell bear directly on the nature of anatomical and physiological subcellular compartments and on the interconnections between these compartments. The findings also reveal some ultrastructural features that are not readily inferred from thin sections. These points will be discussed in turn.

Mast Cells and Phagocytosis

Phagocytosis is a process of broad significance in the mammalian organism. Indeed, in addition to the scavenger function of phagocytes, these cells have been linked to an intimate involvement with the antigen–antibody reaction. Both fixed macrophages and wandering neutrophilic leukocytes take up antigens directly, while lymphocytes (20) and eosinophils may do so as well, the latter showing a particular affinity for antigen–antibody complexes (27). The recent demonstration (42, 43) that mast cells can take up zymosan is thus of particular interest since it invites the speculation that mast cells too may participate in, or be affected by, antigen–antibody processes. Their possible involvement in these reactions has been suggested by a number of observations including (*a*) the degranulation of mast cells, and ensuing histamine shock, in rats challenged with pertussis vaccine (31), (*b*) the development of "mast cell-lytic antibodies" in response to presentation of new antigens and the resultant "reverse anaphylaxis" demonstrable with both rat (32, 47) and mouse (48) mast cells in vitro, (*c*) the reported differentiation of lymphatic tissue cells into mast cell-like elements (18, 19) in tissue culture, and (*d*) the development of mast cell nests ("clones?") in the thymus of a mouse strain afflicted with a form of autoimmune disease (9).

Attempted phagocytosis of glucan particles by normal mast cells in vitro has been reported (14, 15, 51) on the basis of time-lapse cinematographic records.[2] In the course of light microscopic studies on leukocyte mobilization in vivo (16; Fruhman, G. J. Personal communication), it was noted incidentally that mast cells became intimately associated with zymosan, a particulate closely related to glucan, but the techniques used did not afford the resolution required to establish conclusively whether ingestion of the particles had occurred. When these studies were taken up again with electron microscopic techniques which are amply adequate for the purpose, it was shown that zymosan is indeed ingested by mast cells (42, 43), thus establishing conclusively that mast cells are capable of phagocytosis. Satisfactory fixation is a prerequisite to adequate observation of this phenomenon, a goal not achievable with mast cells at the time of the earlier literature reports but more readily met recently (12, 40). The ultrastructure of these cells is exceptionally difficult to retain with most of the standard techniques.

The relatively scant uptake[3] of particulate by mast cells, as compared to macrophages, is of interest since it suggests that, for both zymosan and colloidal thorium dioxide, mast cells play a secondary role, at least as far as bulk removal is concerned. Bulk removal is only one of several important concomitants of phagocytosis, however,

[2] I thank Drs. Thomas F. Dougherty and Gottlieb Schneebeli, Department of Anatomy, University of Utah School of Medicine, Salt Lake City, for the opportunity to review their excellent time-lapse motion pictures. In the opinion of this investigator, the cinematographic records are open to alternate interpretation.

[3] It is fully appreciated that pino- and phagocytosis cannot always be sharply distinguished from each other and that they may reflect one aspect of the general metabolism and self-maintenance of the living cell. For this reason, the terms pinocytosis and phagocytosis are often used in tandem throughout this paper. In this context, it remains for further studies to delineate the physiological significance of the data presented.

and therefore the modest participation of mast cells needs not signify that their role in the over-all antigen–antibody reaction is secondary; they may be concerned with some other, special aspect of the process. Eosinophils, it will be recalled, took up even less Thorotrast than did mast cells.

Thorotrast has been used previously by Rowley as a tool to investigate the relationship of mast cells to vascular permeability (50). In those studies, mention was made of the fact that ". . . particles of Thorotrast . . . were sometimes adherent to mast cell granules which were dispersed in surrounding edematous connective tissue. . . ."[4], but the possibility that mast cells phagocytized the Thorotrast was not even suggested. Significant differences in availability of the particulate to the tissue mast cells in Rowley's experiments as compared to peritoneal fluid mast cells in the present study could no doubt account for the discrepancy. This fact points once more to the advantages of peritoneal fluid as a site for the study of mast cell function (35). Despite the enormous differences in particle size between zymosan and Thorotrast, there may be a common factor between them that renders them subject to ingestion by mast cells. Zymosan is essentially the polysaccharide residue of yeast cell walls. The colloidal thorium dioxide preparation used for this study is stabilized with dextrin, a polysaccharide which most probably remains adsorbed onto the particles after injection. Thus, polysaccharide could be a prerequisite to mast cell involvement in the phagocytic reaction. A suggestion that mast cells arise from a precursor type by phagocytosis of ground substance polysaccharides has been advanced by Burton (10), although the data he presented were not sufficient to establish this point convincingly. The present findings are part of a broader program in which various particulates are being screened. Preliminary experiments suggest that polysaccharide substances may enhance uptake of foreign particulates by mast cells, but that they are neither essential nor the only substances that can do so. The complex interactions of several factors (adhesion, particle charge and size, induction of pinocytosis and cellular movements, fixation of complement and other molecular species, etc.) suggest that further interpretation of the findings may be premature. However, the preliminary data alluded to above already establish that Thorotrast is taken up by virtue of some definite affinity, rather

[4] Rowley's experiments also demonstrated that the dextrin used as a carrier for the colloid itself caused mast cell degranulation. This did not appear to be the case in our studies. Indeed, very few of the peritoneal mast cells harvested showed cytological damage at either the light or the electron microscopic level. Several observations suggest that this is a complex situation which presently defies rational explanation. Indeed, considerably more cytological damage to mast cells resulted when equivalent amounts of the particular dextrin preparation (lyophylized, refined, pyrogen-free dextrin, lot P-7038, courtesy Fellows-Testagar, Div. Fellows Medical Mfg. Co., Inc., Detroit, Mich.) used by the makers of Thorotrast was injected intraperitoneally as for the experimental animals. Extensive uptake of metachromatic granules by peritoneal macrophages was then also noted. Perhaps adsorption of dextrin onto the colloid reduces availability of free dextrin to nontoxic levels. This could explain both our results and those of Rowley, despite differences in experimental procedures. Incidentally, at 1 hr after i.p. injection of dextrin, neither cellular shape nor degree of surface activation (which are both altered most at 1 hr after Thorotrast injection) departed significantly from that seen in uninjected control animals. The strain of rats used may determine mast cell susceptibility since this is the case for histamine release by dextran (Goth, A. 1967. *Adv. Pharmacol.* **5**:47). Similarly, organ specificity may exist (Goth, A. 1967). In tissues, mast cell degranulation may be secondary to vascular injury. Lastly, Rowley fixed his material in Caulfield's osmium tetroxide formulation. In our hands, primary fixation with osmium tetroxide solutions has always led to extensive swelling of the mast cell granules and to significant disruption of intergranular cytological integrity. Thus, Rowley's findings cannot fairly be contrasted to ours.

It seems unlikely that the pattern of distribution of Thorotrast could represent formation of continuities between the external milieu and the intracellular perigranular compartment, related to the activation of a hypothetical granule-release mechanism. Indeed, in damaged mast cells where granule release is seen to occur, the granules involved (both intra- and extracellular) are typically swollen, and their appearance in the electron micrographs contrasts sharply with the normal. Furthermore, initial uptake is *not* related to the granules but rather is related to a system of vacuoles which become associated with the Golgi area before any intimate relationship with the granules occurs. What little degranulation is seen occurs early, well before the peak of Thorotrast-granule contact; signs of histamine release (hyperemia of snout, paws, and ears, respiratory embarrassment, tearing, etc.) occurred within 2–5 min after an intrajugular injection of 0.4 ml dextrin.

than by being passively carried into the cell by the constant pinocytotic activity; several substances of similar or smaller particle size that are readily taken up by macrophages are apparently excluded by the mast cells.

Digestion of ingested particulate by phagocytic cells generally involves lysosomal activity. In neutrophilic and eosinophilic leukocytes, the specific granules are lysosomes which fuse with the phagocytic vacuoles and are destroyed in the process. Acid phosphatase is generally demonstrable in phagosomes. This is in sharp contrast with the present observations on mast cells. Obviously, one cannot expect thorium dioxide to be "digested," and the polysaccharide carrier was not visualized by the techniques used, and thus no direct comparisons to leukocytes are possible. However, Thorotrast became intimately associated with the granules, but the granules were not destroyed thereby. In previous experiments, zymosan was found to be rapidly digested by macrophages, whereas it was still morphologically unchanged several days after ingestion by mast cells (42). Lastly, although acid phosphatase activity has been detected in normal mast cell granules by Gomori's lead method (13), these findings are open to question because only some intergranular sites are revealed by dye-coupling methods (Padawer, J. Unpublished observations). Nor does acid phosphatase activity appear to increase in mast cells after zymosan ingestion. The absence of Thorotrast from the substance of eosinophil granules is similarly a differentiating feature. It thus is most likely that mast cell granules are not lysosomes.

The clublike surface specializations (Figs. 2–4) are of some interest. Structures that are almost certainly identical have been described as "tubulovesicular formations" by Bloom and Häegermark (5) for cells subjected to an artificial medium. Because we have occasionally encountered these structures in cells from untreated animals, it would appear that they are neither artifactitious nor agonal ones. Bloom and Häegermark were unable to relate these formations to their experimental conditions and offered no suggestion as to their functional significance. The present experiments clearly point to a role in absorption processes. In addition to being more commonly encountered in injected animals, many of the small vesicles comprising these structures were associated with small deposits of Thorotrast. This suggests that they are perhaps concerned with the earliest stages of uptake, such as initial binding. More than likely, they could represent micropinocytotic vacuoles being channeled towards the cell body or being crowded together by retraction of a hyaloplasmic veil. In the absence of serial sections and three-dimentional reconstructions, it seems idle to speculate further as to their origin.

Ultrastructural Implications

The pattern in which ingested Thorotrast became distributed within the mast cell allows a number of inferences regarding the ultrastructural organization of the cell. Since some free Thorotrast was still found 24 hr after injection, it explains why early signs of uptake, namely surface and pinocytotic activity as well as small macrophagic vacuoles, were still seen at this time. In effect, the pattern of uptake and distribution within the cell was "smeared" by the continued availability of the foreign material. This should not detract from the fact that transfer of Thorotrast from the cell surface to the "almost mature" granules was remarkably fast. Indeed, particulate thorium was seen in the vicinity of the Golgi apparatus within 15 min and within the more mature progranules within a mere 30 min. Within a few more hours, Thorotrast had invaded the substance of the mature granules.

These observations bear on the nature of the membrane that invests the mast cell granules. The nature of this membrane and, indeed, even whether such a membrane existed were much debated in the earlier mast cell literature. Proper fixation for ultrastructural studies of mast cells, a prerequisite to settling this question, had proved most difficult until recently. Convincing evidence that normal rat mast cell granules are membrane-bounded, so long in forthcoming, has now been obtained in several laboratories (5, 12, 17, 40). The unconventional behavior of this membrane had led Benditt (1, 2), among others, to suggest ". . . that the membrane is not associated directly with the granule but is part of the cytoplasm," a statement of no little insight when one considers that it was made prior to the time when entirely satisfactory fixation was achieved. This concept would appear at variance with a recent scheme of granule formation (see Table I of reference 12). The concept is still retained by others (5, 55). All investigators, to date, have apparently thought of the membrane in reference to individual granules.

An occasional pore linking two or three mature granules has been noted in thin sections of normal peritoneal fluid mast cells in the course of our own studies (Padawer, J. unpublished observations). This finding and the presence of Thorotrast within the mature granules are consistent with the hypothesis that the membranous investments of vesicles near the Golgi externum, of progranules at various stages of condensation, and even of the mature granules are continuous with each other, and that they maintain a series of patent channels that interconnect these various stages of maturation, at least for several clustered subpopulations within any given cell. The suggestion that many mast cell granules are invested by a common membranous sac has been advanced previously (41, 44). It is of course possible that the connecting pores are transient features that arise or disappear more or less randomly. Because of the orderly transit of substance along the maturational stages and a definite tendency for Thorotrast-containing granules to be near each other, it is the feeling of this investigator that the pores are probably permanent features.

Combs (12) has also recorded examples of continuities between rough endoplasmic reticulum cisternae and developing granules in mast cells of embryo and neonate rats. The fact that, in this study, no Thorotrast was found in such rough-surfaced endoplasmic reticulum cisternae suggests that they do not receive contributions from pinocytotic vesicles, and that they somehow can maintain a unidirectional flow of substance within their confines. A similar statement can be made for the small vesicles of the Golgi internum and for the peripheral Golgi saccules.

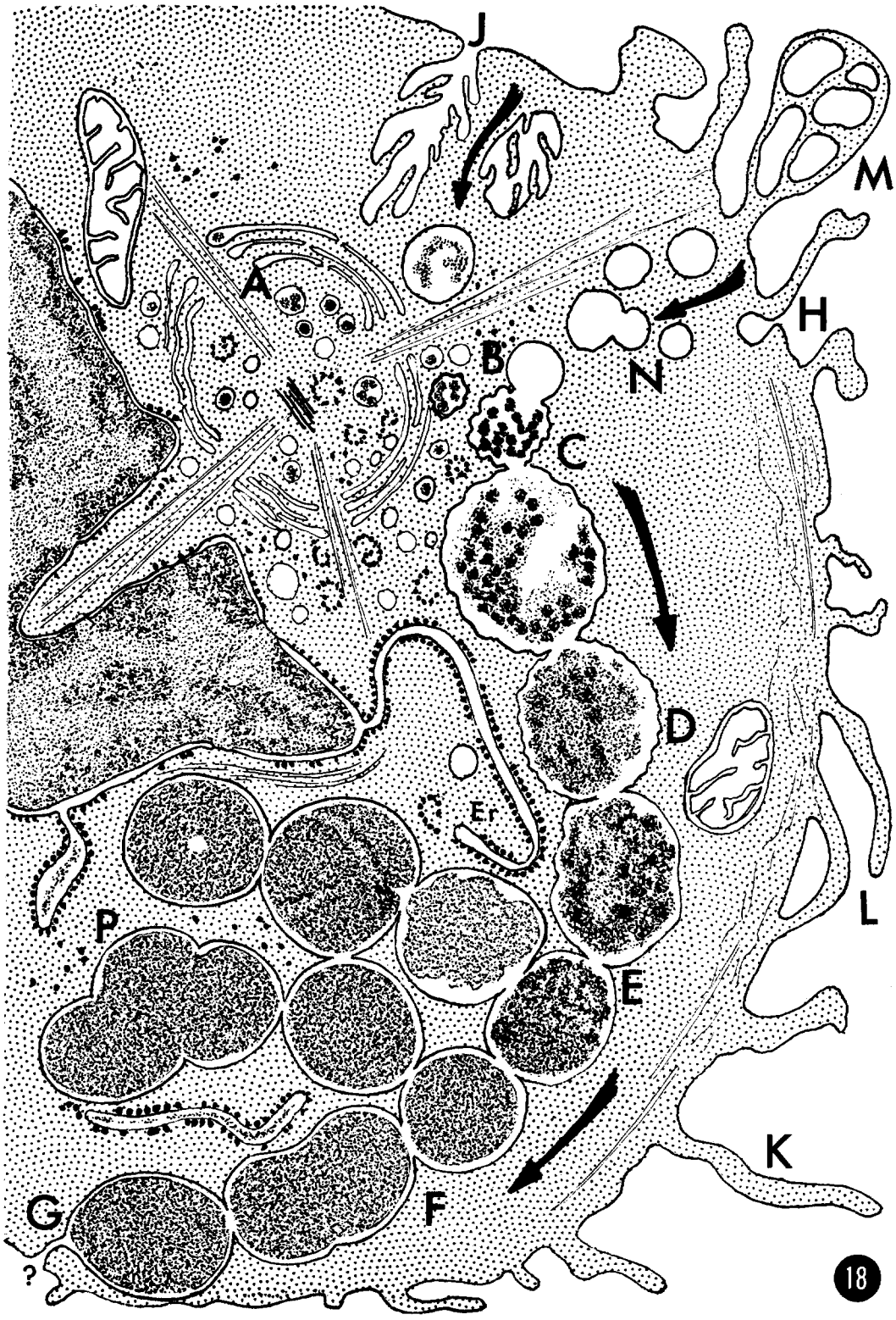
The membranous-complex interpretation advanced herein would be consistent with two other observations, namely (a) that isolated mast cell granules have no perigranular membranes (26) and (*b*) that, in rapidly lysing mast cells, contiguous granules are affected as lysis proceeds in a rapidly propagated wave through large cytoplasmic areas, often sparing other areas altogether (37, 38). It is therefore suggested that, far from

FIGURE 18 Postulated ultrastructural organization of a mast cell of the adult rat. This semidiagrammatic composite is based on present and unpublished experimental data. The fusion of progranules and their compaction into mature granules (*A–F*) is more or less in accordance with the one proposed by Combs for rat embryonic and neonate tissue mast cells (12), but the interconnections between the granules are shown here as permanent channels resulting from the existence of a common membranous sac that invests a cluster of granules. Although only one cluster is shown here, several may exist in any given cell, each with its origin near the Golgi area. The common sac is shown here beginning at stage *C* because no experimental evidence is as yet available to suggest otherwise, but neither is there evidence to rule out that it is continuous with the vesicles and saccules of the Golgi apparatus.

Progranules vesicles (*A*) formed in the Golgi internum area (and perhaps in the Golgi externum as well?) fuse to form larger and larger vesicles (*B*, *C*). The contents progressively condense (*D–F*) and receive additional substance, presumably from ergastoplasmic origin (*Er*); but it is not determined how early, or how late, in the maturation sequence this ergastoplasmic contribution may carry, or how far down the chain of maturing granules direct channels may be established to link ergastoplasmic and granular sac lumina.

Components from the tissue fluids are taken up by a variety of transient surface specializations such as pinocytotic vacuoles (*H*), deep invaginations (*J*), large or small hyaloplasmic veils (*K*, *L*), or complex vesicular specializations (*M*) and are confined to vacuoles (*N*) which are channeled toward the outer Golgi area where they fuse with aggregating progranule vesicles (*C*). Fusion of mature granules may also occur (*P*) which indicates that some plasticity is retained. The linkage of granule chains is complex, involving cross-connections between branches of the chain. The terminal point of the chain has yet to be determined with certainty, but it seems likely to be at the cell surface (*G?*).

This scheme, although in substantial agreement with a number of experimental observations, including all those in this paper, raises several theoretical questions for which, admittedly, compelling evidence is still lacking. It thus must be regarded as an only partially established working hypothesis.


J
M
A
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Er
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?
18

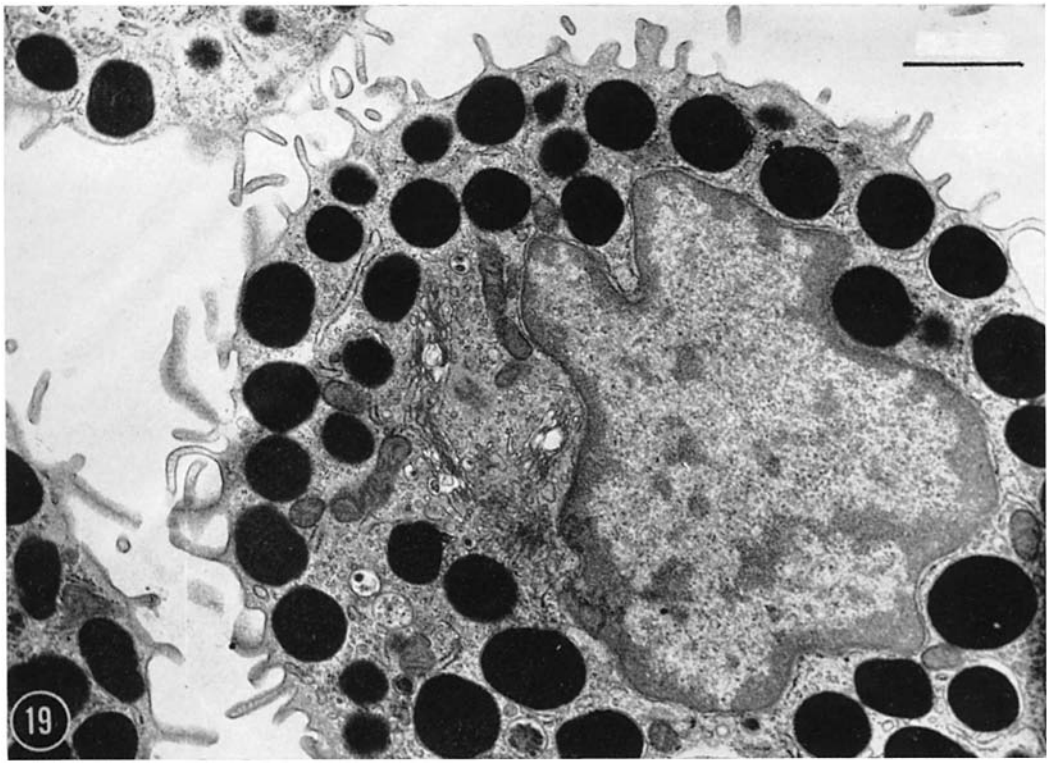

FIGURE 19 Portions of three mast cells from the peritoneal cavity of a 34 day old rat (uninjected control). Technical procedures were as for the other groups of this study. Note the well-developed Golgi apparatus, the centriole cut tangentially, the typical mitochondria, the scattered ergastoplasmic profiles, and the free ribosomal clusters. In some areas where the plane of sectioning is propitious, a membrane is seen to envelop the granules. Attention is drawn to the generally spherical shape of the cell. The plasmalemmal folds seen here are typical of those encountered in peritoneal fluid mast cells of untreated control rats. (This print was somewhat overexposed to bring out the cell boundaries more clearly). Compare with Figs. 1 and 5. Double stain. × 15,300, approximately.

being ephemeral, the postulated interconnections between mast cell granules are permanent features. This scheme is presented semidiagrammatically in Fig. 18. In this way, granular substance could continue to accrue throughout the life of the cell in a way that could result in increased granular size as well as in over-all cellular size, as indeed is found to be the case as the animal ages (11, 46, 49).

The concept proposed herein might also explain the interesting observations of Thon and Uvnäs which led these authors to postulate that degranulation and histamine release are two consecutive steps in the response of rat mast cells to Compound 48/80 (The Wellcome Research Laboratories, Tuckahoe, N. Y.) (55). It is not inconceivable that a contractile protein system in mast cells (34) might be associated with the membranous sacs that confine the granules. Such a mechanism would afford the mechanical force needed to effect the saltatory movements of granules often localized to only a part of the cytoplasm (36, 37), as well as the force for the cytoplasmic streaming of mast cell granules elicited by colchicine (39), for intracellular transport of Thorotrast or other materials taken up by mast cells by means of a peristaltic-like action and, if violent enough, for the actual extrusion of granules into the extracellular compartment, e.g. in response to Compound 48/80.

The apparent labeling of only a portion of the mast cell population and of only a few granules among the several hundreds present in any given mast cell is partly a function of the sampling problems inherent in electron microscopic observation

of thin sections. However, it is felt that sampling problems can only partly account for the observations which probably reflect a real nonrandomness in the actual distribution of particulate. If so, this would suggest that there may be several subpopulations of granules within each mast cell, each set of granules being segregated into its own common membranous investment. Only one or two such subpopulations may be in functional continuity with the Golgi externum at any one time, and this anatomical continuity may be subject to intracellular control. Further work is needed to evaluate this and other possibilities. It would follow from this novel concept of mast cell ultrastructure that these cells may be intrinsically absorptive rather than secretory.

One is tempted to wonder whether there is a constant percolation of the granules that allows them to perform an as yet undefined function, but such a hypothetical percolate and its possible avenue(s) of exit remain to be demonstrated. The probable long life of mast cells (3, 4, 38), possibly equal to the life span of the animal itself, invites further speculations as to functions that also might be served if the granules were to remain in dynamic equilibrium with some factor(s) of the tissue environment or if they were to retain indefinitely small amounts of substances gleaned from the tissue fluids.

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