VACUOLATION IN NORMAL MAST CELLS AND IN MAST CELLS TREATED WITH PROTAMINE SULFATE [1]

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TEN FIGURES

Paff and Bloom ('49) described several methods by which metachromatic material contained within tumor mast cells growing in vitro could be discharged into the surrounding medium. One method involved the progressive appearance of vacuoles which, after increasing in size, were observed to pass out of the cell.

Because the problem may have some relationship to the elaboration of metachromatic material it was decided to extend our studies. The present work is based on the study of normal living mast cells of the rat and the question of vacuolation as it occurs in untreated and protamine sulfate treated animals. We have included an observation on the solubility of mast cell granules of the rat in water.

PREPARATION OF MATERIAL

Three procedures were followed in preparing material for the study of living mast cells. In each case every reasonable precaution was taken to maintain isotonic conditions. In the first instance we worked with the opened abdomen of rats anaesthetized with nembutal. The mesentery was laid over a raised portion of a lucite platform fitted to the mechanical stage of a binocular microscope. The mesentery was bathed with Tyrode solution and was observed with the oil immersion objective without an intervening coverslip.

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In a second procedure "embroidery hoops" made from polyethylene tubing were used. A rat was etherized and a loop of gut with its mesentery was rapidly removed by placing two hemostats close together at the base of the loop and cutting gut and mesentery between the hemostats. The mesentery was then laid over the smaller hoop and the larger hoop was fitted over it. The gut and mesentery outside the hoops were discarded and a trace of Tyrode solution was dropped on the mesentery held by the hoops. The preparation was then laid on a coverslip and sealed in a hollow ground slide (fig. 1). Critical examination under oil immersion is possible if fat-free areas of mesentery are chosen for study. Four or 5 of these preparations can be set up in 5 minutes.

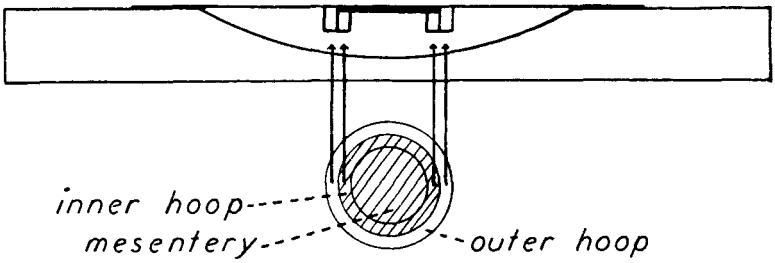

Fig. 1 "Embroidery hoop" technique for mounting living mesentery for oil immersion observation. See text for explanation.

The third procedure permitted us to study mast cells under phase contrast microscopy. Peritoneal fluid from anaesthetized rats was drawn into fine, blunt tipped glass tubing and placed on a 40 X 50 mm coverslip and covered by a second coverslip $\frac{7}{8}$ inch square. This was then inverted over a previously prepared depression chamber consisting of a metal plate with a central hole [2] over which a 40 X 50 mm coverslip had been sealed and which contained a damp washer-shaped piece of filter paper and a drop of high viscosity immersion oil (fig. 2). This type of preparation can be set up in a few seconds.

[2] Obtained from Wyble Engineering Development Corporation, Silver Spring, Md.

THE MAST CELL GRANULE OF THE RAT AND WATER

The mast cell granule of the rat is insoluble in water. This conclusion is based on the fact that if one takes from a live rat a loop of gut with its mesentery clamped in a hemostat and places it immediately in running water for 24 hours, the mast cell granules remain and can be stained with methylene blue (two drops of 1% watery solution of methylene blue in 10 cm^3 of water). Unfortunately it is quite impossible to extend this treatment of the mesentery beyond 24 hours because of the rapidity with which the mesentery macerates in water. However, if such a preparation is suspended in a large beaker of water to which antibiotics have been added, washing may continue for several days without dissolution

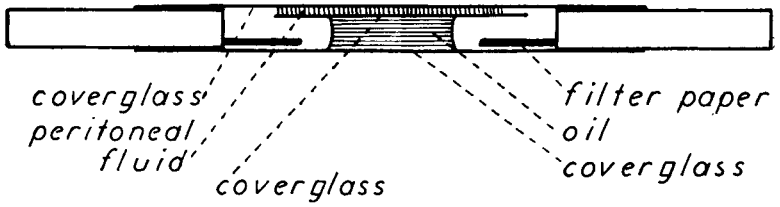

Fig. 2 Technique for preparing peritoneal fluid for examination by phase contrast microscopy. Total thickness of preparation is 1.25 mm. See text for explanation.

of the granules. Fresh, undried connective tissue spreads from rats, placed immediately in plain water at room temperature overnight, then refrigerated in water for one month still show great numbers of mast cell granules (fig. 4).

The question of mast cell granule solubility is important because the unqualified belief that mast cell granules are soluble in water has carried with it the corollary that if a metachromatic halo is seen surrounding a mast cell, it is there because of poor technique involving the use of water (Devitt et al., '54). We believe that if any investigator finds that normal mast cells granules of the rat are soluble in water, then he has, by some technical insult, previously changed the composition of these granules.

Although we have demonstrated that the mast cell granule of the normal rat will not dissolve in water, it must not be inferred that water has no effect upon the cell itself. Living mast cells literally explode when in contact with water.

VACUOLES IN LIVING, UNSTAINED MAST CELLS OF NORMAL RATS

In the mesentery of normal rats it is difficult to demonstrate vacuoles unless they are of fairly large size. Figure 5 illustrates this. At first glance there appears to be only one large vacuole (upper right). As is often the case however, a smaller vacuole may be seen at the lower left. This observation is more easily verified in mast cells from the peritoneal fluid because it is possible to study these cells under phase microscopy. Ordinary light did not show any vacuoles in the cell shown in figure 6. By phase contrast it became immediately evident that this cell contained not only the three vacuoles which lie in the optical plane pictured, but others at different planes. A more advanced stage of this type of activity of the mast cell can be seen in figures 7 and 8 in which a cell is pictured at two different optical planes. In figure 7 several distinct vacuoles appear centrally within the cell while 4 or 5 notched areas appear at the periphery. Critical study reveals that these notched areas are occupied by peripherally located vacuoles (fig. 8).

MAST CELLS IN RATS TREATED WITH PROTAMINE SULFATE

The effect of protamine sulfate upon the mast cell can be sudden and drastic or unhurried and prolonged depending upon the method of introducing it. If one cm^3 of a 1% solution of protamine sulfate [3] is injected into the peritoneal cavity of an adult rat, every mast cell in the mesentery is ruptured within 15 to 30 minutes. If the injection of protamine sulfate is subcutaneous (1 mg per 10 mg of rat) a considerably dif-

[3] Each cm^3 of material contained 10 mg of protamine sulfate in normal salt solution, with 0.25% phenol as preservative (Eli Lilly and Co., Indianapolis, Ind.).

ferent effect becomes evident in a few cells after 24 hours and in progressively more cells if additional injections are given at intervals of a day.

Thus figures 9 and 10 show cells found in the mesentery of rats given 4 subcutaneous injections of protamine sulfate and killed two hours after the last injection. In these animals most of the mesenteric mast cells were vacuolated to varying degrees. As is evident the vacuoles range in size from structures of debatable significance, i.e., are they small vacuoles or refractive halos around granules, to large reservoirs of material with a bordering array of discrete granules. Of special interest to us have been the vacuoles to be seen at the periphery of the cells (fig. 8). One of these is pictured in figure 10 (compare with figs. 7 and 8).

In the study of vacuoles a peculiar thing became evident. Almost invariably it could be predicted that within each discrete vacuole there could be found, one, not two or more, just one, granule busily exhibiting brownian movement. These do not show in many of the vacuoles illustrated because of the length of exposure time required to photograph the cells (figs. 3 and 9).

ON THE FORMATION OF VACUOLES

Vacuole formation in mast cells involves the dissolution of a few granules with the production of a small spherical mass of liquid material. Rarely we observed a surprising phenomenon i.e. the formation of a fairly large vacuole from what appeared to be irregular, branching, granule free tracts lying at different planes within the cytoplasm. These tracts are probably identical with the so-called canals observed by Lehner ('24) in living mast cells. In figure 3, one of these granule free tracts or canals is diagrammatically represented. We first saw the phenomenon in a mesenteric mast cell under direct observation following the addition of a drop of methylene blue stain (concentration of two drops of a 1% watery solution of dye to 10 cm^3 of Tyrode solution). In this

case the reaction was instantaneous. We dismissed it as an artefact caused by some unknown quirk of technique. Later however, we had the good fortune to obtain a cinephotomicrographic record of it in a living unstained cell. Here the process occurred at a much slower rate. A granule free tract or "canal" retracted its extensions toward what appeared to be a focal point and assumed the shape of a spherical vacuole.

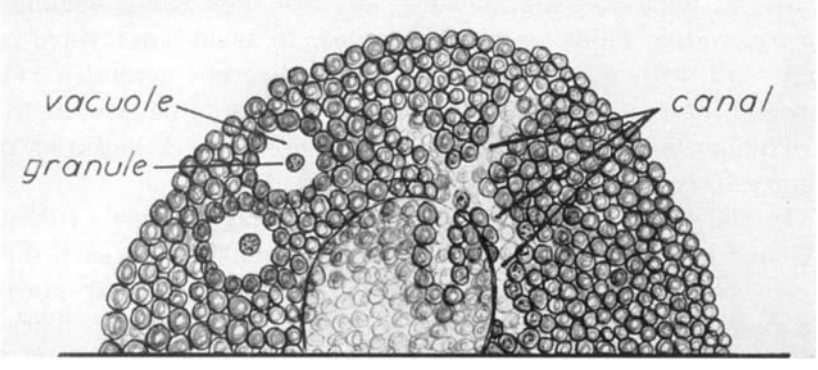

Fig. 3 Diagrammatic representation of a portion of a mast cell showing two vacuoles with one granule in each vacuole. Also represented is a granule free tract or canal.

DISCUSSION

We agree with Compton ('52) that normal mast cells rarely show vacuoles when fixed and stained. It is our belief, however, that technical procedures involving shrinkage, death, and staining of cells may mask or even destroy, many of the vacuoles which can be seen in living mesenteric mast cells. Moreover the recognition of vacuoles in living mast cells is greatly facilitated where it is possible to employ phase contrast microscopy. Perhaps mast cells are never normal unless observed in the living state, in a natural habitat.

After determining the occurrence of vacuoles in normal untreated mast cells a substance was sought which would stimulate mast cells to increase their supposed secretory activity and thus possibly lead to vacuole formation. Protamine

sulfate was chosen and the rationale underlying its selection was this: 1st, mast cells are believed by many investigators to be the source of heparin or a precursor; 2nd, protamine sulfate supposedly acts by neutralizing the negative charge of heparin (Beckman, '52); 3rd, if heparin is of functional importance in body economy, then maintenance of a favorable level might be expected; 4th, if the heparin level is seriously disturbed downwards by injection of protamine sulfate, then the cells which produce heparin can logically be expected to increase the tempo of their activity. Whatever the validity of this reasoning there was no doubt that, following the subcutaneous injections of protamine sulfate, the great majority of mesenteric mast cells became vacuolated. This effect was probably enhanced by the presence of the phenol used as a preservative for the protamine sulfate (Sylven, '48). We are pursuing studies at the present time which lead us to believe that the mast cell is in some respects, e.g. movement, the most sluggish inhabitant of the connective tissue, but in other respects, e.g. ease of rupture, the most sensitive. As still another example of the sensitivity of mast cells we suggest the ease with which vacuoles are produced.

Since vacuoles can be demonstrated in untreated normal cells and since their increased formation can be stimulated with ease, the question arises as to what part they play in the economy of the cell. Several ideas suggest themselves: 1st, vacuoles represent stages in a degenerative process which may lead to death of the cells; 2nd, they represent stores of toxic material which had entered the cell and which the cell has diluted and isolated at least temporarily as vacuoles; 3rd, they are stores of secretory material. We have undertaken studies which, we hope, will answer this question.

As to what disposition is made of the vacuoles by the cells in which they are found, perhaps a clue lies in an observation made on tumor mast cells growing in tissue culture. There we occasionally observed vacuoles floating from tumor cells into the surrounding medium. It was suggested that this might well be one of the methods of discharging secreted material

(Paff and Bloom, '49). In the present study we have not observed vacuoles being discharged from the cytoplasm in either the control or protamine treated animals, but we believe that this is a possibility (figs. 7, 8, and 10).

SUMMARY AND CONCLUSIONS

Mast cell granules can be demonstrated in unfixed rat mesentery immersed in water for days. Fresh undried connective tissue spreads placed in water show mast cell granules at the end of a month. Mast cell granules of the rat are not soluble in water.

Critical study of living mesentery, and of living cells from peritoneal fluid, reveals that the cytoplasm in mast cells of normal rats contains vacuoles. These vacuoles are of various sizes and are best seen by phase contrast microscopy in the living state. They are more numerous in normal untreated mast cells than has been recognized.

Protamine sulfate injected into the peritoneal cavity of a normal rat ruptures mesenteric mast cells within 15 to 30 minutes. When the drug is injected subcutaneously into normal rats, it is posible to create an environment in which the cytoplasm of as many as 9 of every 10 mast cells in the mesentery becomes vacuolated. Although passage of vacuoles from the normal mast cell into the surrounding tissue was not observed, it is our belief that this may occur.

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PLATES

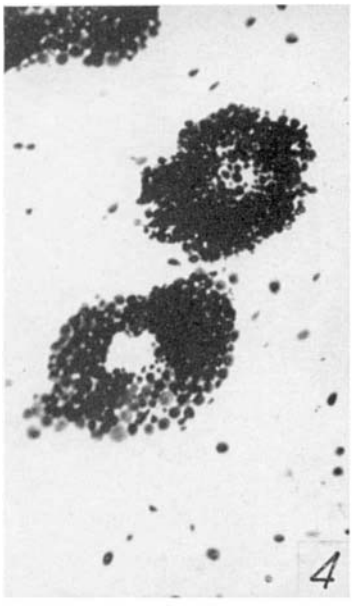
PLATE 1

EXPLANATION OF FIGURES

4 Mast cells in rat connective tissue spread. Cells were immersed in distilled water for one month and then stained with a dilute watery solution of methylene blue. × 1200.

5 Living, normal mast cell in mesentery of rat. Note large vacuole to the right of the nucleus and the small indistinct vacuole to the lower left of the nucleus. Unstained. × 3360.

6 Living, normal mast cell in peritoneal fluid. Under ordinary light no vacuoles were visible. Under phase contrast note three small vacuoles in line. Unstained. Phase contrast. × 2670.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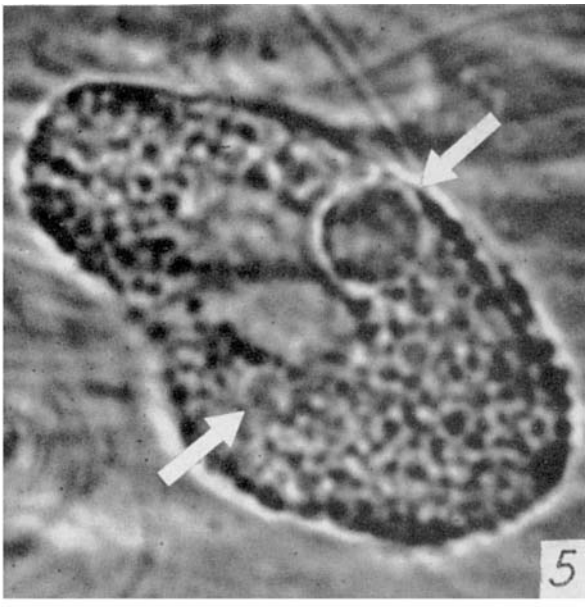
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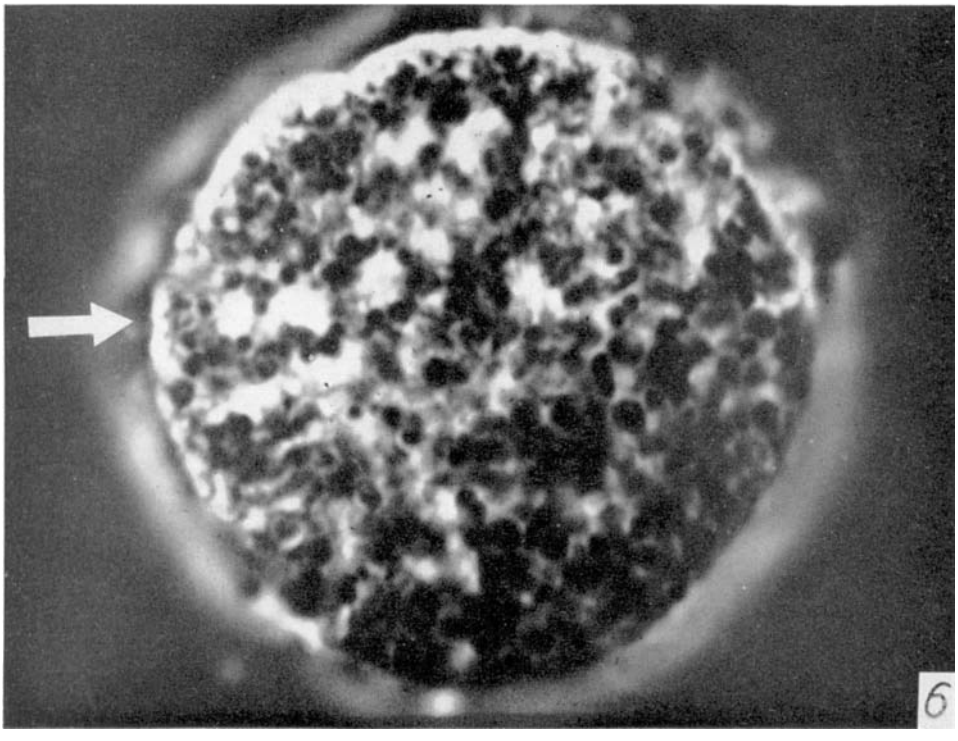
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PLATE 2

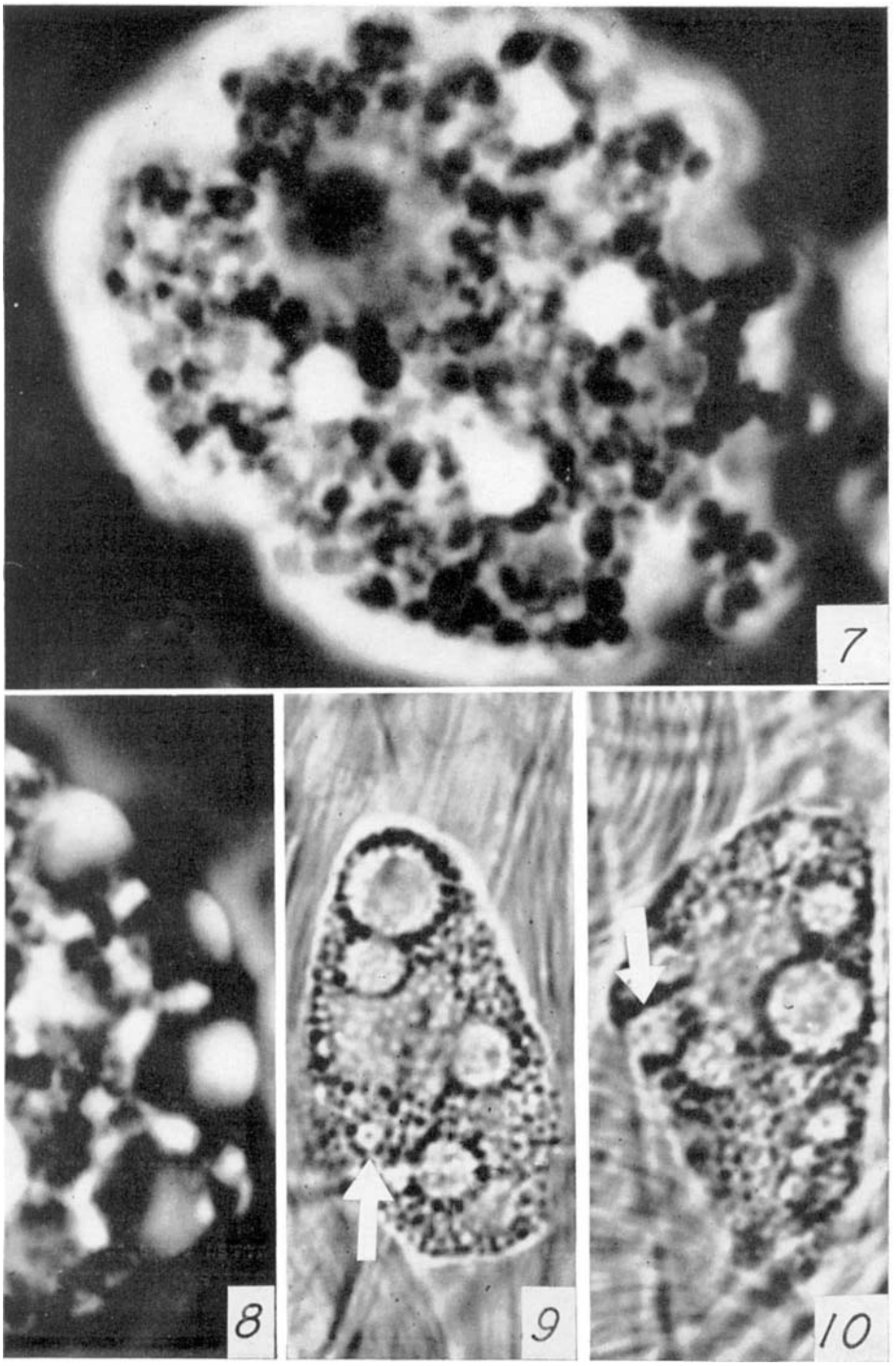
EXPLANATION OF FIGURES

7 Living normal mast cell found in peritoneal fluid. Note vacuoles outlined at periphery. Unstained. Phase contrast. × 4500.

8 Same cell as in figure 7 at a different optical plane. Note silhouettes of 4 vacuoles lying at periphery. Phase contrast. × 4500.

9 Living mast cell in mesentery of a rat injected subcutaneously with protamine sulfate (1 mg per 10 gm of body weight each day for 4 days). Note vacuoles and single granule in small vacuole off arrow. Unstained. × 2500.

10 Living mast cell in mesentery (same rat as fig. 9). Note vacuole at periphery off arrow. Compare with figures 7 and 8. Unstained. × 2500.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