J. Physiol. (1959) 147, 425–436

EFFECT OF ANTIGEN AND OCTYLAMINE ON MAST CELLS AND HISTAMINE CONTENT OF SENSITIZED GUINEA-PIG TISSUES

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(*Received* 25 *July* 1958)

It has been widely accepted that mammalian mast cells contain not only heparin but also histamine (Riley & West, 1953). In rats (Benditt, Wong, Arase & Roeper, 1955; Bhattacharya & Lewis, 1956) and in mice (Sjoerdsma, Waalkes & Weissbach, 1957) these cells may also contain 5-hydroxytryptamine. Histamine release induced by histamine liberators is usually associated with damage to mast cells (Riley, 1953; Mota, Beraldo & Junqueira, 1953; Fawcett, 1954).

Mast cell alterations induced by the antigen–antibody reaction in the guinea-pig were described by Mota & Vugman (1956). By making biopsies before and after the injection of antigen into sensitized guinea-pigs, these authors demonstrated a parallel decrease in the mast cell population and in the histamine content of the guinea-pig lung in anaphylaxis. This work was carried out in the intact guinea-pig and the occurrence of oedema during anaphylactic shock may have accounted for the reduction in the mast cell population and in the histamine content of the lung. It was decided therefore to carry out corresponding experiments with isolated tissue particles *in vitro*, under conditions in which oedema formation was excluded. A further aim of this work was to discover whether the various procedures used by Mongar & Schild (1957*a*, *b*, *c*) for inhibiting histamine release in anaphylaxis would also inhibit mast cell alterations induced by antigen. The action of octylamine on mast cells has also been studied. A brief report of this work has already been published (Mota, 1958).

METHODS

Guinea-pigs weighing about 300 g were sensitized with a 2% solution of egg albumin in 0·5% aqueous phenol, of which 1 ml. was administered subcutaneously and 1 ml. intraperitoneally. The animals were used from three weeks after sensitization. Evidence of sensitization was

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provided by testing whether the tissues released histamine in the presence of the specific antigen. Lung tissue was chopped with a McIlwain tissue chopper (McIlwain & Buddle, 1953) into rods of 0·8 × 0·8 mm cross-section. A series of uniform samples were prepared as described by Mongar & Schild (1953) and incubated at 37° C in 10 ml. beakers for 15 min with 0·9 ml. of the required concentration of inhibitor in Tyrode solution. The inhibitors used were: 0·001 M iodoacetate, 0·005 or 0·01 M phenol, and 0·01 % versene (sodium edetate). After this treatment either 0·1 ml. of Tyrode solution containing 1 mg of antigen or 0·2 mg of octylamine was added and the tissue incubated for a further period of 15 min.

The mesentery was dissected out and cut into several small pieces which were dipped into cold Tyrode solution. Three or four of these pieces were used as a sample and incubated under conditions already described for the lung samples. The mesentery is a suitable preparation for the study of cytological details and the description of the mast cell alterations presented here is based chiefly on observations in the mesentery.

From each tissue several samples were prepared, some being used for mast cell counts and others for measuring either the amount of histamine released or the amount of histamine remaining inside the tissue. For the bioassay on the guinea-pig ileum of released histamine the solution was removed with a filter pipette from the lung particles or pieces of mesentery and diluted as required. The histamine content of the tissue was determined after heating the tissue in Tyrode solution in a boiling water-bath for 5 min. The histamine content of guinea-pig lungs was about 15 μg/g and that of mesentery 10 μg/g. For microscopical observation of the mast cells, the chopped lung tissue was fixed with a solution of lead subacetate in acidified alcohol as previously described (Mota & Vugman, 1956), and frozen sections 50 μ thick were stained with 0·1 % toluidine blue in acidified aqueous solution (pH about 4). The number of mast cells was determined in 100 randomly chosen microscopical fields with the aid of a square-ruled ocular micrometer, the magnification being × 250. The mesentery was fixed in the same fixative, examined as a whole-mount preparation and its mast cells counted in 30 microscopical fields, the magnification being × 80. In mounting the mesentery care was taken not to fold or stretch the tissue.

For mast cell counts on the ileum and uterus the fixative was injected under pressure into the lumen. The organs were then ligated at both ends and immersed in the fixative for 20–24 hr. Frozen sections 50 μ thick were prepared and stained with toluidine blue. Mast cell alterations were assessed either by calculating the percentage of partially degranulated cells, as compared with an untreated control, or by counting the total number of metachromatic granule-containing cells before and after treatment.

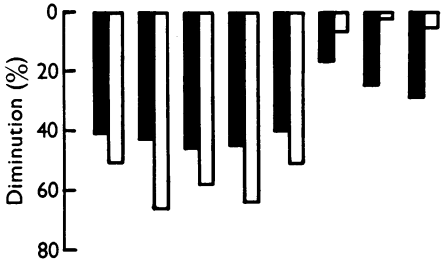
RESULTS

Effect of antigen–antibody reaction on mast cell morphology

In the guinea-pig lung the mast cells take the form of round, oval, elongated or spindle-shaped cells crowded with metachromatic granules. They are distributed in the walls of the alveoli, and are concentrated around the bronchioles (Pl. 1, fig. *A*). Their number (range 7–35 cells per field) varies in different individuals. In the mesentery the mast cells are found throughout the tissue, although they follow especially the blood vessels. They are uniform in their metachromasia and morphology, taking oval or elongated shapes (Pl. 2, fig. *E*). Their number varies (range 11–30 cells per field) in different animals.

When pieces of chopped lung tissue or mesentery from a sensitized guinea-pig are incubated at 37° C with antigen in Tyrode solution, damage to the mast cells occurs, as is shown by disappearance of their metachromatic material and a consequent reduction in the number of stainable mast cells (Pl. 1, fig. *B*). The antigen-induced alterations are characterized by a disappearance

of the mast cell granules (degranulation) without any morphological evidence of their fate. No granules are found outside the cells and those remaining inside the cells keep their usual aspect (Pl. 2, fig. *F*). No such alterations of mast cells were seen when non-sensitized tissue was incubated with antigen. Even in tissues which have been in contact with antigen for only a few seconds the mast cells are partially deprived of their granules; no other recognizable alterations are seen. It seems that the granules are subjected to a quick dissolution or lysis either inside or outside the cells.



Text-fig. 1. Diminution of histamine content (■) and mast cell number (□) of chopped sensitized guinea-pig lung in anaphylaxis.

Effect of antigen–antibody reaction on mast cell number and histamine content of guinea-pig lung and mesentery

Lung

The effect of antigen on mast cell number and histamine content of chopped sensitized lung tissue in eight separate experiments is shown in Text-fig. 1. As a result of the anaphylactic reaction there is a diminution in both mast cell population and histamine content of the lung and there is a correlation between histamine release and mast cell reduction. Thus, in five experiments in which a substantial histamine release occurred there was also a substantial reduction in the mast cell number, whereas in the three experiments in which histamine release was less than 30% there was only a small decrease in mast cell number although many mast cells were degranulated.

Mesentery

Preliminary experiments showed that histamine is released by antigen from sensitized mesentery, although the amount released is usually too small to permit an accurate quantitative assay. On the other hand, the release of histamine in the control experiments was negligible. When sensitized mesentery is incubated with antigen the number of cells which can be recognized as mast cells becomes reduced. The results of incubation of pieces of mesentery with antigen are shown in Table 1. When these results were analysed by the *t* test the difference was found to be significant ($P < 0{\cdot}005$). In those cases in which there was no decrease in the mast cell number,

qualitative alterations represented by partial degranulation of the mast cells were nevertheless seen, the number of degranulated mast cells in these cases varying from 15 to 68%.

Effects of inhibitors of anaphylaxis on mast cell alterations induced by antigen–antibody reaction

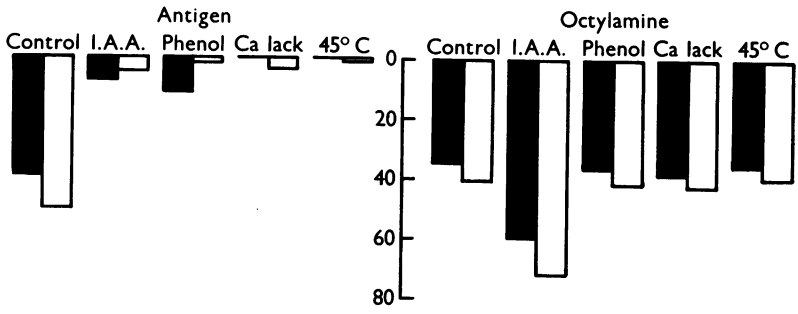
Experiments with lung tissue

The experiments with lung tissue are summarized in Text-fig. 2.

TABLE 1. Mast cell counts* in sensitized guinea-pig mesentery incubated with Tyrode solution or Tyrode plus antigen

Guinea-pig	Mast cells in Tyrode	Mast cells in T + antigen	Decrease (%)
1	30	25	16
2	17	7	58
3	26	16	38
4	21	21	0
5	27	21	22
6	27	11	59
7	17	3	82
8	28	28	0
9	20	10	50
10	18	6	66
11	11	11	0

* Means of 30 microscopical fields at a magnification of × 80.



Text-fig. 2. Effect of various inhibitors on histamine release (■) and mast cell decrease (□) in chopped sensitized guinea-pig lung by antigen or by octylamine. Figures represent percentage diminution.

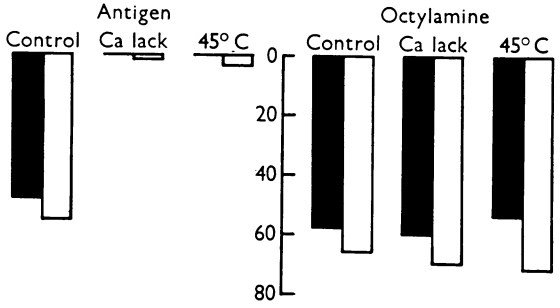
Iodoacetate or phenol. When sensitized lung particles were incubated with antigen in the presence of iodoacetate (0·001 M) or phenol (0·005 M), the damage to mast cells and the histamine release by antigen were greatly reduced.

Calcium lack. When the sensitized lung particles were incubated in calcium-free Tyrode solution containing versene there was a total inhibition of mast cell damage and of histamine release by antigen, but when calcium ions were added subsequently both histamine release and mast cell disruption occurred.

Previous heating of the tissue. When sensitized lung particles were kept at 45° C for 15 min and subsequently incubated with antigen at 37° C there was no mast cell damage or histamine release. Heating at 45° C itself produced no visible mast cell damage.

Experiments with mesentery

The effects of previous heating of this tissue at 45° C for 15 min and of the absence of calcium on mast cell destruction and histamine release are shown in Text-fig. 3. As in the lung, both these treatments inhibited mast cell damage by antigen. When iodoacetate or phenol was used the amount of histamine released from the mesentery by antigen was too small to be detected and no decrease in mast cell number occurred, but qualitative alterations were present.



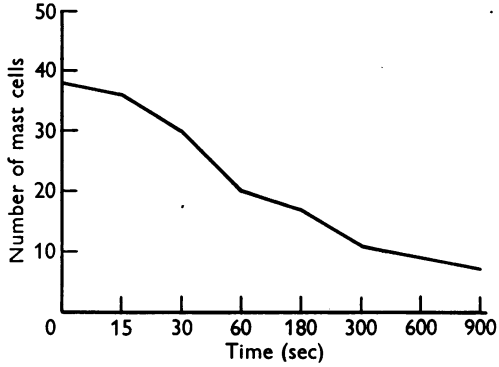
Text-fig. 3. Effect of previous heating of the tissue to 45° C and the absence of calcium on histamine release (■) and mast cell decrease (□) induced by antigen or by octylamine in sensitized guinea-pig mesentery. Figures represent percentage diminution.

Effect of phenol on desensitization

Some experiments were then carried out to see if phenol would inhibit desensitization as well as mast cell destruction. A preliminary test was made to see whether phenol denatures antibody. Sensitized mesentery was incubated for 15 min with 10 mM phenol in Tyrode solution and then transferred to Tyrode solution containing no phenol. Addition of antigen now produced the usual degranulation of mast cells, showing that antibody was not denatured. The next test was whether phenol prevents desensitization. When antigen was added in the presence of 10 mM phenol there was no mast cell damage, as has already been shown. If now the mesentery was washed in 10 mM phenol to remove antigen, then in Tyrode solution to remove phenol, and a second dose was added, this also failed to produce mast cell damage. Thus the mast cell had been desensitized without becoming degranulated.

Effect of low temperature on mast cell destruction by antigen–antibody reaction

To test the effect of low temperature, pieces of sensitized mesentery were incubated with antigen at 15° C for 15 min; some of the pieces were then immediately transferred to fixative and others to antigen solution at 37° C, kept there for 15 min, and fixed afterwards. Microscopical observation of the tissue showed that mast cell degranulation was absent in the pieces of mesentery that had been fixed immediately after contact with antigen at 15° C, but present in those that were fixed after warming to 37° C. However, the diminution in mast cell number in the pieces of mesentery that had been in contact with antigen at 15° C and were subsequently brought to 37° C was smaller than in control pieces from the same mesentery in which antigen was added in the first instance at 37° C. These experiments show that some desensitization occurs even at 15° C.



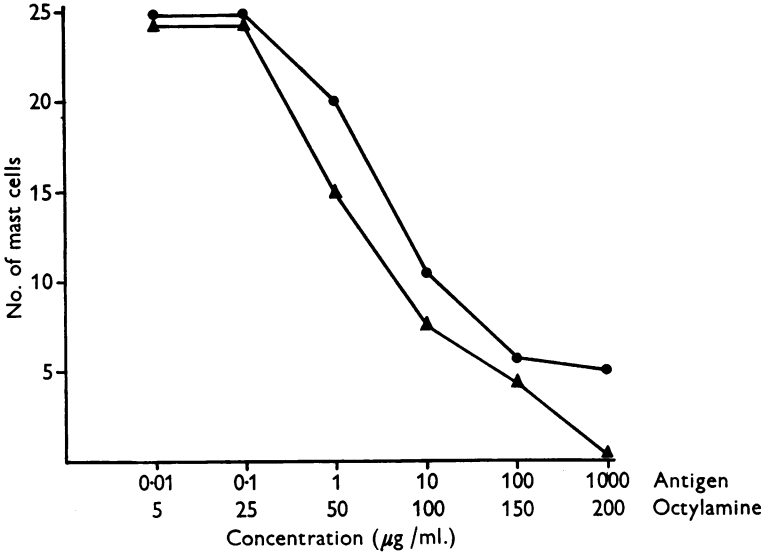
Text-fig. 4. Effect of time of incubation with antigen on the number of mast cells in sensitized guinea-pig mesentery. Note irregular abscissa scale.

Effect of time of incubation with antigen on mast cell degranulation and histamine release

Pieces of sensitized mesentery were incubated with antigen for periods of 15 sec up to 15 min. The results are shown in Text-fig. 4. A significant decrease (50–70%) in mast cell number was detected as early as 1 min after incubation and degranulation was seen to occur after about 15 sec incubation. To test whether histamine release also occurs within 15 sec, pieces of mesentery were incubated for 15 sec in 1 ml. of Tyrode solution and afterwards transferred to 1 ml. of a 0·1% solution of antigen in Tyrode for the same period. Both control and antigen solutions in which the mesentery was incubated were then tested for histamine content. In each of seven experiments a detectable amount of histamine was released after 15 sec contact of the tissue with antigen.

Effect of concentration of antigen on mast cells

To see if mast cell alterations increased with the concentration of antigen, pieces of sensitized mesentery were incubated with different concentrations of antigen for 15 min at 37° C and afterwards fixed. The results of these experiments are summarized in Text-fig. 5, which shows that the decrease in mast cell number is related to the concentration of antigen. Antigen is active in very low concentrations of the order of 10^{-6}.



Text-fig. 5. Effect of different concentrations of antigen (●—●) and octylamine (▲—▲) on mast cell number in sensitized mesentery. Semi-log. scale for antigen.

Comparison with octylamine effect

When pieces of guinea-pig lung or mesentery are incubated with octylamine in Tyrode solution at 37° C, damage to the mast cells occurs, as is shown by reduction in the number of these cells. As with antigen–antibody reaction this reduction is proportional to the concentration of the substance and in the concentrations of 0·2 mg/ml. octylamine is sometimes more effective than antigen (see Text-fig. 5). Thus both the antigen–antibody reaction and octylamine produce conspicuous alterations of the same cell. However, cytologically the alterations induced by octylamine are different from those induced by antigen. The first visible change induced by octylamine is an apparent loss of the granule limit, as if a diffusion of the metachromatic material from the granules had taken place (Pl. 2, fig. *G*). When this phenomenon progresses the cell looks like a metachromatic blot, in which some basophilic granules can sometimes be noticed. In the final stages these metachromatic blots present an irregular contour and a faint metachromasia and the cell is apparently in a

state of dissolution. It is interesting that the morphological difference between the mast cell damage induced by the antigen–antibody reaction and by octylamine is a very sharp one. Thus, while the antigen-induced mast cell alterations are characterized by a total or partial loss of the granules without fusion of the metachromatic material, the octylamine-induced mast cell alterations are characterized by an apparent fusion of the metachromatic material without any loss of granules. Furthermore, when the effect of the several inhibitors of anaphylaxis on mast cell destruction by octylamine was tried, the results showed that neither histamine release nor mast cell destruction by octylamine was inhibited by any of the inhibitors which had been found effective in anaphylaxis (see Text-figs. 2, 3).

Passive sensitization of mast cells. Since isolated tissues can be sensitized by incubation with antibodies *in vitro* (Dale, 1913) it seemed likely that mast cells could also be sensitized in this way. To test this possibility guinea-pig mesentery was incubated for half an hour with rabbit anti-ovalbumin serum, washed in Tyrode solution and then incubated with antigen. Under these conditions similar alterations of mast cells were produced as in mesentery of the actively sensitized guinea-pig. Similar experiments were done with guinea-pig uterus and ileum. Two horns of the uterus or two segments of the ileum were used. One piece of each organ was incubated for half an hour with rabbit anti-ovalbumin serum diluted in a ratio of 1:5 with Tyrode solution and the other was used as a control and incubated in plain Tyrode solution. The pieces were then suspended in oxygenated Tyrode solution and 15 min later the antigen (ovalbumin 1 mg/ml.) was added. Only the organs which had been in contact with the antiserum showed an anaphylactic contraction whereas the controls gave no reaction. After 30 min contact with antigen the pieces were removed and fixed. On microscopical inspection both sensitized uterus and ileum showed typical degranulation of the mast cells present in the mucosa and amongst the muscle fibres, whilst the control pieces showed no visible mast cell alterations (Pl. 1, figs. *C* and *D*).

DISCUSSION

The mast cell alterations produced *in vitro* by the antigen–antibody reaction are identical with those previously described in the intact animal; both conditions are characterized by a disappearance of mast cell granules. It is known that mast cell granules are of a lipidic-proteic nature (Hedborn & Snellman, 1955) and their quick disappearance induced by antigen in sensitized tissues may be mediated through lysis induced by activation of an enzyme as suggested by Rocha e Silva (1944). This is also suggested by the dependence of this phenomenon on temperature and its inhibition by metabolic inhibitors.

The morphological alterations induced by octylamine suggest that this base

combines directly with the metachromatic material in the granules, and in so doing produces a complete disorganization of the granular structure with subsequent histamine release. A similar mechanism of action was postulated by MacIntosh (1956), based on experiments with intracellular particles.

It is interesting to emphasize here that the morphological difference between the mast cell damage, induced by the antigen–antibody reaction and by octylamine, might also reflect a difference in the mechanism of action of these agents on the mast cell, although both produce histamine release. This agrees with the fact that iodoacetate, phenol, calcium lack or heating to 45° C, although producing a partial or total inhibition of mast cell destruction by antigen in sensitized tissues, do not block the mast cell destruction by octylamine. These results are also in agreement with the conclusion by Mongar & Schild (1957*a*, *b*) that in the guinea-pig the histamine-releasing mechanism of the antigen–antibody reaction is different from that of octylamine. Nevertheless, the fact that both antigen–antibody reaction and octylamine produce alterations of mast cells shows that at least part of the histamine released by these two agents originates from the same source. Other histamine releasers may act on the guinea-pig mast cells in a similar way to octylamine; thus we have observed that compound 48/80 (Baltzly, Buck, de Beer & Webb, 1949) *in vitro* induces mast cell alterations similar to those produced by octylamine, although this compound was effective only in much higher concentration than octylamine.

The very short time in which detectable morphological alterations of mast cells and histamine release can be produced in the mesentery can be explained by the thinness of this tissue and the high velocity of the antigen–antibody reaction (Mayer & Heidelberger, 1942). The finding that in the presence of phenol desensitization may occur without mast cell degranulation suggests that the antigen–antibody reaction itself is not responsible for degranulation but that this is a consequence of some later reaction. This is also indicated by the fact that a partial desensitization occurs when sensitized tissue contacts antigen in the cold.

It was postulated by Schild (1956) that when a mast cell disintegrates during the anaphylactic reaction it creates a histamine gradient in its surroundings, and that the degree of muscular contraction will then depend on the number of plain muscle cells reached by effective concentrations of histamine. Our results, which suggest that mast cells release histamine in anaphylaxis, together with the finding that in the guinea-pig lung, ileum and uterus these cells are in close proximity to the smooth muscle fibres, fit in well with this hypothesis. This concept implies that the 'intrinsic histamine' of Dale might originate from the mast cells, although it does not exclude the possibility that some of the released histamine comes from the muscle cells themselves. In a wider sense 'intrinsic histamine' could be defined as histamine

liberated from, or in close proximity to, the responsive structure so that the distinction between extrinsic and intrinsic histamine would not be a sharp one.

In relation to the hypothesis that histamine released in anaphylaxis may originate from mast cells, it is interesting to observe that mast cells are in close association with the responsive structures in the so-called shock organ, as, for example, in bronchial muscle of the guinea-pig lung and in the muscular layer of the veins in dog liver (Mota, Ferri & Junqueira, 1956).

The correlation found between mast cell destruction and histamine release suggests that histamine released in anaphylaxis originates from mast cells, although it does not prove that these cells are the only source of released histamine. This correlation, together with the finding of other workers (Armitage, Herxheimer & Rose, 1952; Weissbach, Waalkes & Udenfriend, 1957) that substances other than histamine do not fulfil a major role in anaphylaxis in the guinea-pig, indicates that mast cells play a very important part in the anaphylactic reaction of this species. The finding that mast cell alterations occur in anaphylaxis in the dog (Jaques & Waters, 1941), the rat (Mota, 1957), and the mouse (Carter, Higginbotham & Dougherty, 1957) and in active and passive anaphylaxis in the guinea-pig, suggests that these cells might be particularly concerned with the uptake of antibodies: this problem is being investigated.

SUMMARY

1. Antigen (ovalbumin) produces mast cell alterations in sensitized guinea-pig lung, ileum, uterus and mesentery. These alterations are characterized by a disappearance of mast cell granules and a reduction in the total number of these cells.

2. The mast cell alterations occur simultaneously with histamine release and both processes are inhibited by iodoacetate, phenol, calcium lack, previous heating of the tissue to 45° C and cooling to 15° C.

3. The mast cell damage, induced by antigen in sensitized tissues, does not appear to be a direct result of the antigen–antibody union but rather a consequence of some later reaction.

4. Octylamine produces mast cell alterations that are morphologically different from those induced by antigen, and are characterized by an apparent diffusion of the metachromatic material from the granules.

5. The mast cell alterations induced by octylamine are also accompanied by histamine release but they are not inhibited by the various inhibitors of anaphylaxis.

6. It is suggested that most of the histamine released in anaphylaxis comes from the mast cells and that these cells may be concerned with the uptake of antibodies.

The author is greatly indebted to Dr H. O. Schild for his valuable advice and helpful suggestions during this work.

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EXPLANATION OF PLATES

PLATE 1

(Toluidine blue stain.)

A. Mast cells in sensitized guinea-pig lung incubated with Tyrode solution, approx. ×110. Note numerous mast cells distributed through the pulmonary tissue and concentrated around the bronchioles.

B. Mast cells in sensitized guinea-pig lung after contact with ovalbumin, approx. ×110. Note diminution in mast cell number.

C. Mast cells in non-sensitized guinea-pig ileum after contact with ovalbumin, approx. ×160. Note mast cells crowded with granules in the mucosa and muscular layer.

D. Mast cells in sensitized guinea-pig ileum after contact with ovalbumin, approx. ×160. Note degranulated mast cells in the mucosa and in the muscle layer.

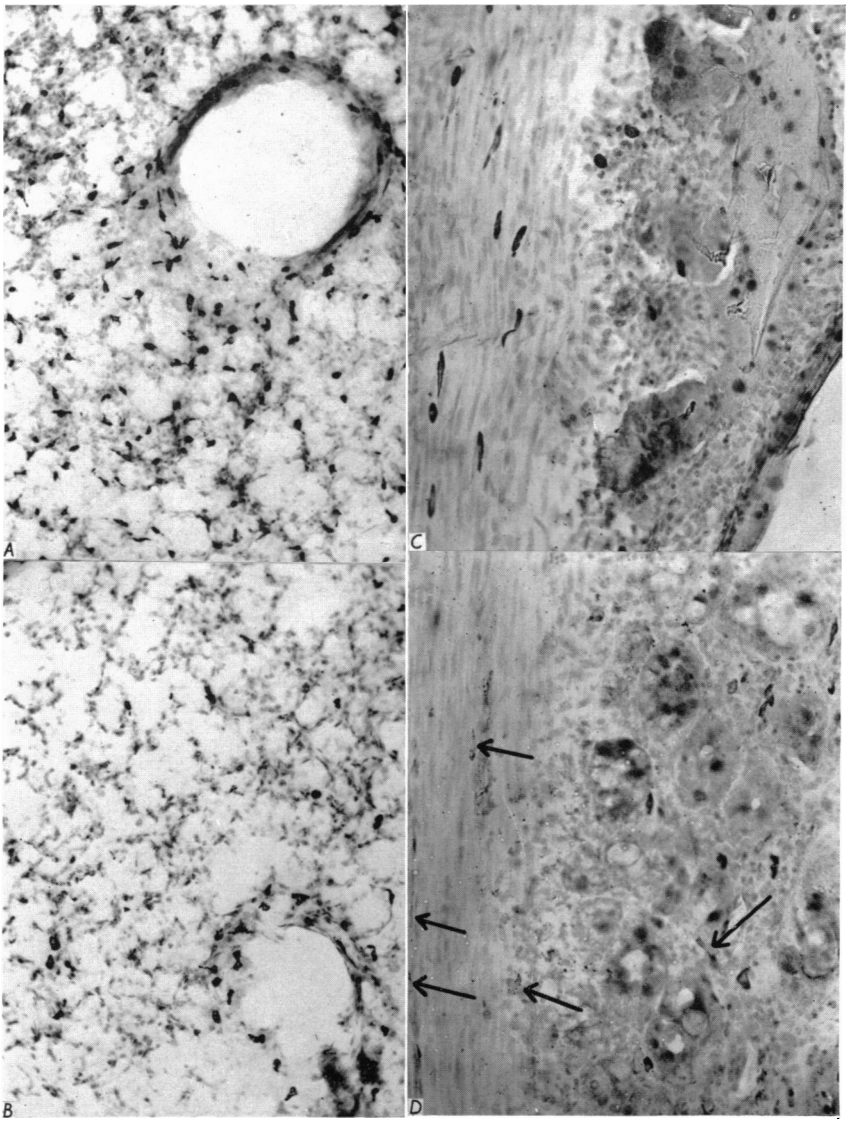
PLATE 2

(Toluidine blue stain, approx. ×1000.)

E. Mast cell in sensitized guinea-pig mesentery incubated with Tyrode solution. Note cell crowded with granules.

F. Mast cell in sensitized guinea-pig mesentery incubated with ovalbumin. Note that the cell has lost most of its granules.

G. Mast cell in sensitized guinea-pig mesentery incubated with octylamine. Note the aspect of fusion of the metachromatic material.



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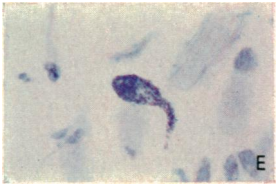
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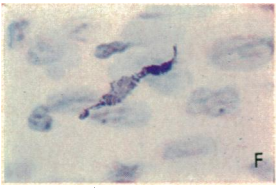
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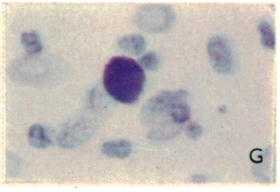
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