CYTOLOGICAL AND PHARMACOLOGICAL OBSERVATIONS ON THE RELEASE OF HISTAMINE BY MAST CELLS*

By DON W. FAWCETT,‡ M.D.

(From the Department of Anatomy, Harvard Medical School, Boston)

PLATES 24 TO 27

(Received for publication, April 30, 1954)

Of the many theories of mast cell function, the most widely accepted is that they produce heparin. This belief is based principally upon the observation of a close correlation between the number of mast cells in a tissue and the amount of heparin which can be extracted from it (Holmgren and Wilander, 1937; Oliver, Bloom, and Mangieri, 1947). It has recently been shown that an equally good correlation exists between the number of mast cells in a tissue and its histamine content (Riley and West, 1953). The present investigation provides experimental evidence for the presence of histamine in mast cells by demonstrating that the potent histamine liberator, compound 48/80,[1] (Paton, 1951) causes release of mast cell granules, and that it fails to liberate appreciable amounts of histamine from connective tissue previously depleted of mast cells.

The abdominal cavity of the rat is an unusually favorable site for experiments upon mast cells *in vivo* because experimental solutions injected intraperitoneally have access to a very large population of these cells in the serous membranes (Fig. 1) and the effects of chemical agents upon them can be rapidly evaluated in stained spreads of the mesenteries. In a companion study on the regenerative capacity of mast cells (Fawcett, 1954) it was shown that intraperitoneal injection of distilled water resulted in osmotic disruption of the subserous mast cells with dispersal of their granules and cell death, but this treatment caused no enduring damage to other cell types (Fig. 2). Injection of compound 48/80 in isotonic salt solution also led to extensive release of mast granules, but the cells generally survived. It seemed reasonable to believe that if mast cells contained histamine, it would be released from the cells upon intraperitoneal injection either of water or of a solution of compound 48/80 and that the histamine liberated might be detected in samples of fluid re-

* Supported by an institutional grant from the American Cancer Society to Harvard University.

‡ John and Mary R. Markle Scholar in Medical Science.

[1] A product of condensation of *p*-methoxyphenethyl-methylamine with formaldehyde. It is a mixture of dimers and trimers.

covered from the body cavity. This expectation was borne out by experiment, and thus it was possible in this study to quantitate the histamine released and then to examine microscopically the condition of the mast cells in the same animal.

Materials and Methods

Forty female rats of the Hisaw strain weighing 200 to 250 gm., and 12 guinea pigs weighing 300 to 500 gm., were used in the study. Distilled water, Tyrode solution, or Tyrode solution containing varying concentrations of compound 48/80[2] were injected into the peritoneal cavity in 20 ml. amounts. At intervals ranging from 15 to 90 minutes thereafter small samples of fluid were withdrawn from the body cavity and assayed for histamine by the response of an atropinized guinea pig gut. The assays were carried out on a 4 to 5 cm. segment of guinea pig ileum suspended in a 30 ml. muscle chamber kept filled with magnesium-free Tyrode solution containing 1×10^{-7} gm./liter of atropine sulfate. The muscle was attached to a light heart lever for kymographic recording of the contractions. The muscle chamber was constantly aerated by a flow of fine bubbles of air and kept immersed in a constant temperature bath maintained at 37°C. To permit a rough quantitation of the histamine content of the experimental fluids, the contractions of the muscle in response to 0.1, 0.2, 0.3, 0.4, and 0.5 ml. of a 1/100,000 standard solution of histamine diphosphate were recorded at the beginning and at the end of each experiment.

The fact that contractions produced by the experimental solutions were due to histamine was established by the complete suppression of response to test solutions when 0.2 to 0.5 ml. of a 1/1,000,000 solution of the histamine antagonist pyrilamine maleate (neoantergan, Merck)[3] had been added to the immersion fluid.

At the conclusion of the assay of the abdominal fluid for histamine, the animals were killed and the condition of the mast cells was studied in stained preparations of the naturally thin serous membranes of the abdomen. For the morphological observations, mesenteries and mesometria were spread over 11 by 22 mm. coverglasses and cut around the edges with enough margin to overlap slightly onto the back of the glass. These were then fixed by immersion in absolute methyl alcohol in Columbia coplin jars and stained by the May-Grünwald-Giemsa stain (Jacobson and Webb, 1952) or by pinacyanol erythrosinate (Bensley, 1952).

Fluids introduced intraperitoneally are quite rapidly absorbed and therefore a relatively large volume (20 ml.) was used in order to prolong the period of contact with the serous membranes, to increase the surface area exposed, and to facilitate subsequent withdrawal of samples for assay. In each experiment the three solutions were injected into three rats. Samples of fluid recovered from rats injected with Tyrode solution alone caused little or no contraction of the guinea pig ileum whereas the fluid (0.1 to 0.4 ml.) from rats which received Tyrode solution containing 300 μg. 48/80 caused a strong contraction (Figs. 3, 7, and 8). Fluid withdrawn from the abdomen of rats injected intraperitoneally with distilled water also produced a marked contraction. The substance in these fluids responsible for the smooth muscle contraction was almost certainly histamine for its effect upon the atropinized guinea pig ileum was completely suppressed by addition of an histamine antagonist to the fluid bathing the muscle (0.5 ml. of neoantergan 1/1,000,000).

---

[2] Compound 48/80 was supplied through the generosity of Dr. Edwin de Beer of the Wellcome Laboratories, Tuckahoe, New York.

[3] Pyrilamine maleate (neoantergan) was furnished by Merck and Co., Inc., of Rahway, New Jersey.

OBSERVATIONS

After a preliminary experiment had demonstrated that histamine could easily be detected in the abdominal fluid following intraperitoneal injection of water or compound 48/80, five additional experiments (15 rats) were made comparing water, Tyrode solution alone, and 48/80 in Tyrode solution with respect to the amount of histamine released by each and their effect upon the mast cells.

Although substantial amounts of histamine were released both by water and 48/80, the rate of release with the two agents differed. 15 to 20 minutes after injection of these fluids the concentration of histamine was usually greater in abdominal fluid of the animal which received water than in the one receiving 48/80 in Tyrode solution, but after 30 minutes the situation was usually reversed, the concentration having fallen off rapidly in the water-injected animal while it was maintained at a fairly high level in the animal injected with 48/80 (Fig. 8). Thus it appeared that with water there had been a rapid and transient liberation of histamine followed by its speedy absorption or degradation, while the release of histamine by 48/80 was more gradual in its onset and continued over a longer period of time. Detectable concentrations of histamine were still present an hour and a half or even 2 hours after injection of 48/80.

Microscopic examination of the mesenteries 2 to 3 hours after injection of the experimental solutions disclosed that Tyrode solution had had very little effect upon the cells except perhaps to cause them to round up slightly. Cells with extruded granules were found occasionally but the great majority of the mast cells were intact and entirely normal in appearance (Fig. 5). In animals which had received water, on the other hand, nearly all of the mast cells were completely disrupted, and the location of each was marked only by a cluster of mast granules lying free in the tissue spaces (Fig. 4). Although intact mast cells were very seldom observed the other cellular elements of the connective tissue showed only slight damage from their exposure to water. Spreads of mesentery from rats which received Tyrode solution containing 48/80 (300 μg.) showed very extensive liberation of mast granules. Indeed, the release of granules from some mast cells seemed as complete as though they had been osmotically disrupted with water. The majority of mast cells however, appeared to have given up only half to two-thirds of their granules. These cells, although greatly reduced in size, nevertheless appeared to be intact and viable. Each was distinguishable as a fairly sharply circumscribed aggregation of intensely stained granules in the midst of an area of paler-staining dispersed granules (Fig. 6). The fate of these cells became clear later when the injected fluid had been absorbed and the extracellular mast granules were phagocytized by macrophages. Thus, in mesenteries examined at 24 to 36 hours after injection of 48/80, it was apparent that the majority of the mast cells had

survived after liberation of a large proportion of their granules. At the same interval, after injection of water the serous membranes were virtually devoid of mast cells. Intraperitoneal injection of the histamine antagonist pyrilamine maleate (neoantergan) a short time before the injection of 48/80 did not prevent the release of mast cell granules. This is in accord with current opinion that histamine antagonists do not suppress histamine release but in some manner prevent it from penetrating the membranes of cells normally sensitive to its action (Dale, 1954).

Thus, both water and isotonic solution of 48/80 caused release of mast cell granules, and liberation of histamine. Inasmuch as neither of these fluids produced conspicuous cytological changes in other cell types, it seemed highly probable that the mast cells were the principal source of the histamine detected.

Further evidence for this was obtained by additional experiments wherein compound 48/80 in physiological salt solution was injected intraperitoneally into pairs of rats, one member of each pair being normal, and the other having had the mast cells in the lining of its peritoneal cavity destroyed 7 to 10 days earlier by injection of water. In each case the microscopic appearance of the mesenteries in the two rats was the same except for the absence of mast cells in one. (Figs. 11 and 12). Although both animals received the same dose of compound 48/80 (300 μg. in 20 ml. Tyrode solution) the animal with the normal complement of mast cells released into the abdominal fluid a considerable quantity of histamine (125 to 130 μg.) while the rat which lacked peritoneal mast cells released none or only trace amounts. Three such experiments were carried out with consistent results (Figs. 9 and 10.

Thus, although histamine may be present in fibroblasts, mesothelial cells, monocytes, macrophages, and eosinophils it does not appear to be released from these cell types by the chemical histamine liberator 48/80 in sufficient quantity to be detected by the assay procedure employed here.

If liberation of histamine by 48/80 were simply the result of a damaging effect of the compound upon the delicate plasma membrane of the mast cell, then it would be reasonable to expect that the lowest concentration of 48/80 which would lyse the mast cell membrane would produce a maximal release of histamine. This point was explored in two experiments designed to show whether a series of increasing doses of 48/80 would result in a graded morphological and pharmacological response.

In each of these experiments five rats received intraperitoneally 20 ml. of Tyrode solution containing respectively 0, 20, 100, 500, and 1000 μg. of 48/80. The fluids were then assayed for histamine after 30 minutes by withdrawing the same volume of fluid (0.3 ml.) from the abdomen of each of the five animals and testing these samples in sequence on the same smooth muscle preparation. The height of the resulting excursions on the kymograph thus reflected the relative amount of histamine released in the five animals. The kymographic record of one of these experiments is presented in Fig. 13. In this instance a small amount of histamine was released in response to injection of Tyrode solution alone (rat A). A progressively greater

amount, however, was released with each increment in dosage of 48/80 (rats B, C, D, and E). The abdominal fluids were compared again after an hour, using a larger volume (0.6 ml.) to offset the anticipated decline in histamine concentration and a graded response was again obtained. Microscopic examination of the mesenteries 3 hours after the beginning of the experiment revealed that there had also been a graded effect upon the morphology of the mast cells (Fig. 14). After Tyrode solution alone, occasional cells were found to have released a few granules but most of them were normal (A). 20 μg. of 48/80 caused release of a few granules from most of the mast cells (B) and with 100 μg. a moderate number of granules were given up by nearly all of the mast cells (C). Very extensive release of granules was produced by 500 μg. involving all but a few of the mast cells (D), and finally, 1000 μg. appeared to cause total disruption of most of the mast cells with wide dispersal of the granules (E).

Therefore, compound 48/80 is effective over a wide range of doses most of which do not lead to destruction of the cell and its effect appears to be more subtle, more selective for the mast cell, and entirely different from water in its mechanism of action.

DISCUSSION

Riley (1953) reported that the chemical histamine liberators stilbamidine and *d*-tubocurarine, administered intravenously to rats, had a damaging effect upon the mast cells and diminished to some extent the amount of histamine extractable from the tissues. In the dosage required to produce these results, however, both stilbamidine and *d*-tubocurarine were fatal in from 1 to 3 minutes. Therefore little time was available for cytological observation on the mast cells and there was danger of confusing specific effects of these pharmacological agents with agonal changes accompanying death of the animal.

The design of the present experiments has proven more favorable for cytological and pharmacological observations relating histamine to mast cells. The peritoneal cavity of the rat was an ideal site for exposing *in vivo* a large population of mast cells to the action of a chemical histamine liberator and the potent compound used, compound 48/80, was highly selective in its action and effective in small amounts which had no apparent toxic effect upon the rat. Thus both the immediate and delayed effects of the agent upon the mast cells could be studied microscopically and the quantity of histamine actually released could be estimated instead of the amount of extractable histamine remaining in the tissue.

Histamine has been detected in every mammalian tissue studied (Goodman and Gilman, 1941) including several which contain very few mast cells or none at all. However, the fact that compound 48/80 failed to release detectable quantities of histamine when injected into the peritoneal cavity of a rat previously depleted of mast cells, would seem to indicate that the histamine released by this compound in the normal rat is derived principally, if not exclusively, from the tissue mast cells. The seemingly selective effect of 48/80 upon mast cells may afford an explanation for the observation of Feld-

berg and Talesnik (1953) that repeated daily injections of 48/80 caused proportionally a greater depletion of the histamine content of the skin than of the gut. The gastro-intestinal mucosa is rich in histamine without being abundantly supplied with mast cells whereas it is likely that most of the histamine content of the skin resides in the large population of mast cells in the dermis and subcutaneous areolar tissue.

The quantity of histamine liberated by the mast cells under the conditions described here is noteworthy. On several occasions the concentration found after injection of 48/80 corresponded to the addition of 100 to 180 μg. histamine to the 20 ml. of fluid injected into the peritoneal cavity. It is probable that considerably more was actually released than was reflected by the histamine concentration in the fluid at any one time for one must assume that histamine is constantly being absorbed from the body cavity into the blood stream and quite possibly it is also being degraded at an appreciable rate by local histaminase activity. 100 to 180 μg. is indeed an impressive amount when it is recalled that ox pleura, which is one of the richest normal tissue sources, contains approximately 200 μg. of extractable histamine per gram wet weight (Riley and West, 1953). Since the aggregate weight of all the mast cells in the serous membranes of the rat would be a very small fraction of a gram, it follows that the concentration of histamine in the mast cells themselves must be extraordinarily high.

Because tissues contain an amount of extractable histamine which would be toxic if it were liberated, it is believed to be present normally in a bound or inactive form and compounds which liberate histamine are thought to have the property of freeing it from chemical combination with a tissue constituent. In view of the rough parallelism observed here between the number of mast granules released and the amount of histamine liberated, it is tempting to speculate that the histamine in mast cells is associated with their granules. However, inasmuch as simple disruption of the cells with water suffices to release an appreciable quantity of histamine, it appears that it is either very loosely bound to the granules or else the mast cell cytoplasm contains a certain amount of unbound histamine which is set free synchronously with the release of mast granules.

The occurrence of extracellular granules in the neighborhood of mast cells has usually been interpreted as an artifact of specimen preparation or a degenerative phenomenon. Several investigators however, have interpreted this as evidence of secretory activity (Nakajima, 1928; Asboe-Hansen, 1950; Kelsall and Crabb, 1954). The observations recorded here lend some support to the latter view. For example, injection of 48/80 in a moderate dose which caused release of many granules but permitted survival of the cells resulted in maintenance of a higher concentration of histamine in the abdominal fluid for a longer time than was the case after injection of water which completely

destroyed the mast cells and released all their granules. This points to the possibility that the living mast cell actively participates in the release of histamine after treatment with 48/80. The occurrence of a graded response to a graded dose of the histamine liberator and the rapid recovery of the partially degranulated cells are also consistent with the thesis that liberation of mast cell granules after 48/80 may be an exaggeration of normal secretory activity rather than a manifestation of non-specific cell damage. While an adequate understanding of the mechanism of action of compound 48/80 awaits further pharmacological study, its selective action on mast cells and its absence of toxic side effects make it an extremely valuable tool for studies on the histophysiology of the mast cell and the role of histamine in the connective tissues.

SUMMARY

Experimental solutions known to affect mast cells or to cause liberation of histamine from the tissue were introduced into the peritoneal cavity of rats. Samples of the peritoneal fluid were withdrawn at intervals afterward and assayed for histamine and the condition of the mast cells was subsequently ascertained by microscopic examination of stained spreads of the mesenteries.

Intraperitoneal injection of distilled water caused osmotic disruption of the mast cells and the appearance of an appreciable amount of histamine in the peritoneal fluid.

Injection of Tyrode solution alone was not particularly damaging to the mast cells and little or no histamine was released.

Injection of Tyrode solution containing compound 48/80 resulted in extensive release of granules from mast cells and the appearance of large amounts of histamine in the fluid. Solution of 48/80 failed however to cause histamine release when injected into rats whose subserosal mast cells had previously been destroyed.

A series of increasing doses of compound 48/80 had a graded morphological effect upon mast cells and resulted in a graded increase in the amount of histamine that appeared in the peritoneal fluid. It is unlikely therefore that this compound acts by simply lysing the plasma membrane.

It is concluded that mast cells in the rat are extraordinarily rich in histamine which is liberated under conditions which cause mast cells to release their granules. The histamine set free by the potent histamine liberator, compound 48/80, appears to come principally from the tissue mast cells.

BIBLIOGRAPHY

Asboe-Hanson, G., *Ann. Rheumat. Dis.*, 1950, **9,** 149; *Proc. Soc. Exp. Biol. and Med.*, 1952, **80,** 677.

Bensley, S., *Stain Technol.*, 1952, **27,** 269.

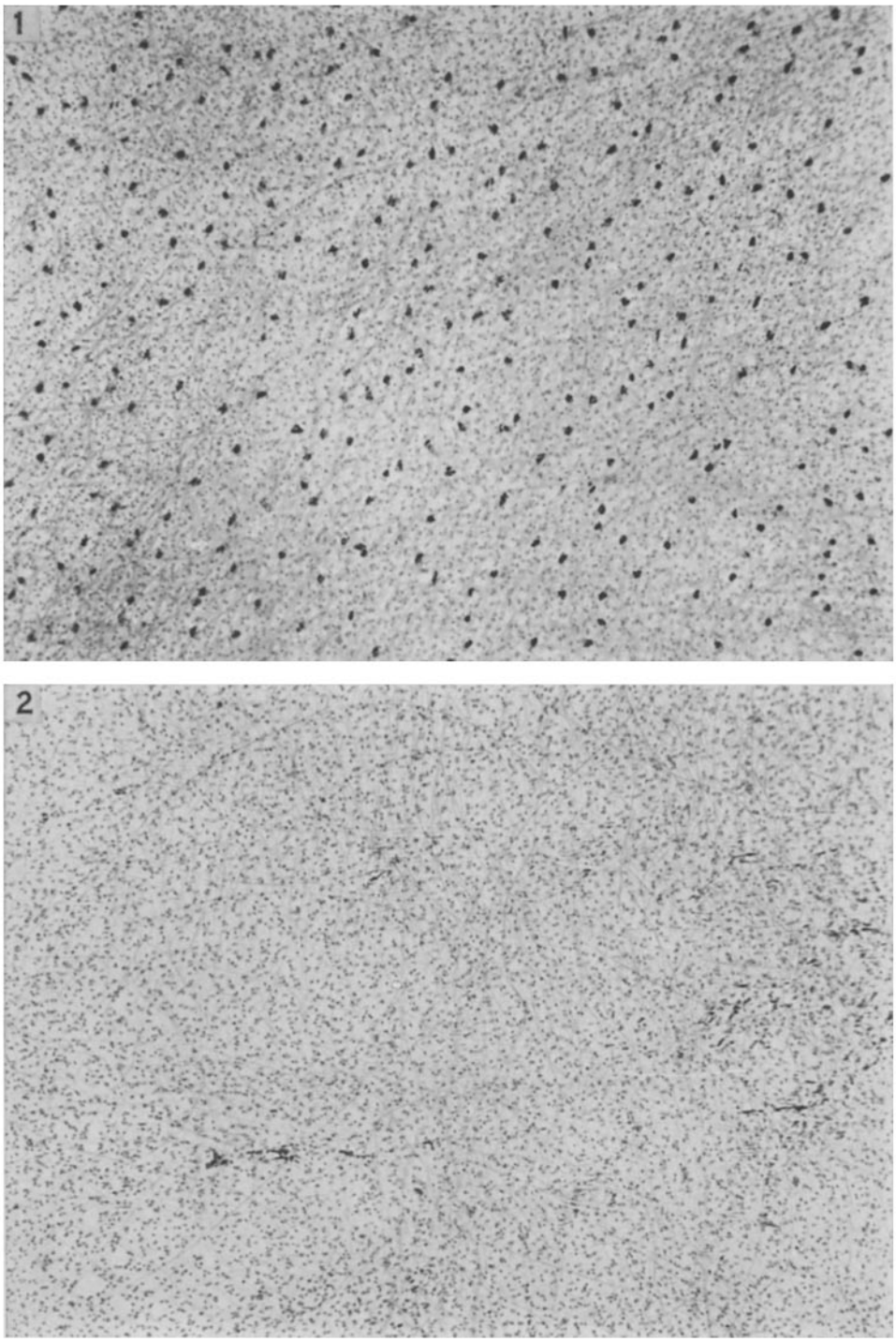
Dale, H., *J. Laryngol. and Otol.*, 1954, **68,** 28.
Fawcett, D. W., *Anat. Rec.*, 1954, in press.
Feldberg, W., and Talesnik, J., *J. Physiol.*, 1953, **120,** 550.
Goodman, L., and Gilman, A., The Pharmacological Basis of Therapeutics, The Macmillan Co., New York, 1941.
Holmgren, H., and Wilander, O., *Z. mikr-anat. Forsch.*, 1937, **42,** 242.
Jacobson, W., and Webb, M., *Exp. Cell. Research*, 1952, **3,** 163.
Kelsall, M. A., and Crabb, E. D., *Anat. Rec.*, 1954, **118,** 393.
Nakajima, Y., *Tr. Jap. Path. Soc.*, 1928, **18,** 150.
Oliver, J., Bloom, F., and Mangieri, C., *J. Exp. Med.*, 1947, **86,** 107.
Paton, W. D. M., *Brit. J. Pharmacol.*, 1951, **6,** 499.
Riley, J. F., *J. Path. and Bact.*, 1953, **50,** 471.
Riley, J. F., and West, G. B., *J. Physiol.*, 1953, **120,** 528.

EXPLANATION OF PLATES

PLATE 24

FIG. 1. Mesentery from a normal rat at low magnification illustrating the abundance and uniform distribution of mast cells. May-Grünwald-Giemsa stain. × 40.

FIG. 2. Mesentery of a rat which, 5 days earlier, had received an intraperitoneal injection of 20 ml. of distilled water. Mast cells are entirely lacking but in other respects the mesentery appears normal. May-Grünwald-Giemsa stain. × 40.



(Fawcett: Release of histamine by mast cells)

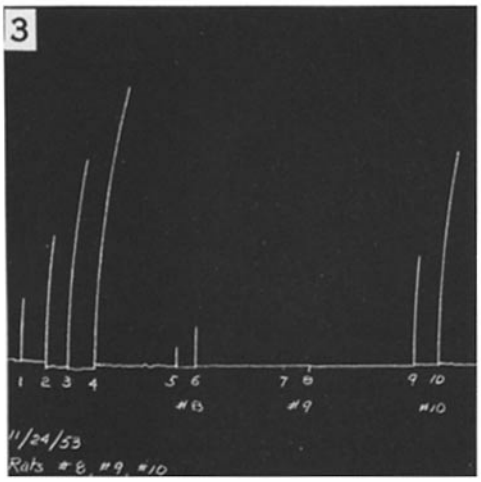
PLATE 25

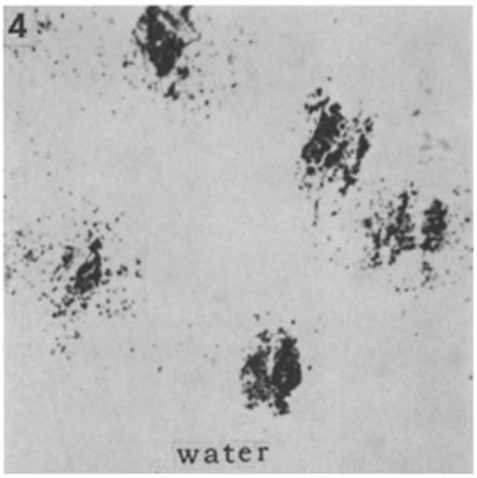
FIG. 3. Kymographic record of an experiment comparing the effects of intraperitoneal injection of water, Tyrode solution and compound 48/80 in Tyrode solution. Excursions 1 to 4 record contractions of the atropinized guinea pig ileum to 0.1, 0.2, 0.3, and 0.4 ml. of a standard solution of histamine 1/100,000. Excursions 5 and 6 are the response to 0.2 and 0.4 ml. of abdominal fluid from a rat (No. 8) which had been injected intraperitoneally with water. No contraction was produced (7 and 8) by the same volumes of fluid from the animal (No. 9) injected with Tyrode solution. Fluid from the animal (No. 10) which received compound 48/80 in Tyrode solution produced moderately strong contractions (9 and 10).

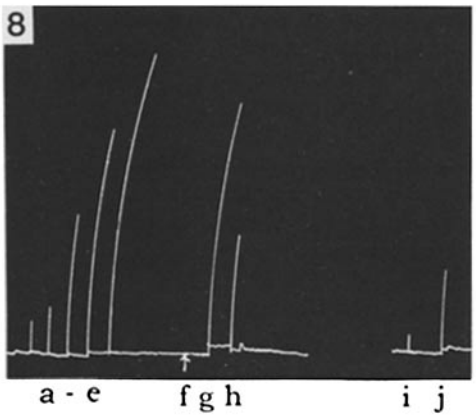
FIG. 4 to 6. Cytological condition of the mast cells of the 3 animals involved in the experiment depicted in Fig. 3. After water (rat 8) the cells were completely disrupted. After Tyrode solution (rat 9) they were intact. After 48/80 (rat 10) many granules were released but most of the mast cells remained viable.

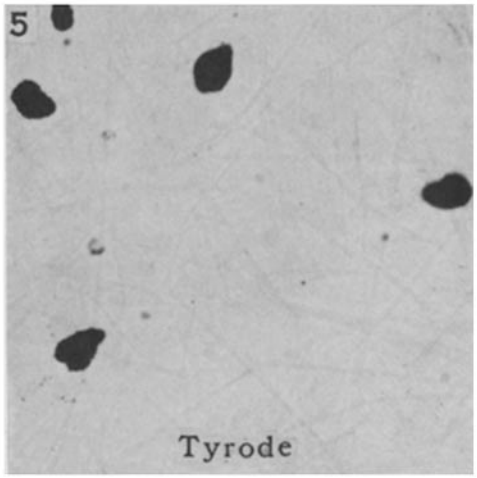
FIG. 7. Kymographic record of a similar experiment. Excursions 1 to 5 represent contractions in response to 0.1, 0.2, 0.3, 0.4, 0.5 ml. of histamine solution 1/100,000. 6 and 7 in this instance show very slight response with 0.2 and 0.4 ml. of fluid from the abdomen of a rat injected with Tyrode solution. 8 and 9 are contractions produced by the same volume of fluid from the abdomen of a rat injected with compound 48/80. 10 and 11 are the results with samples of fluid from a rat injected with water.

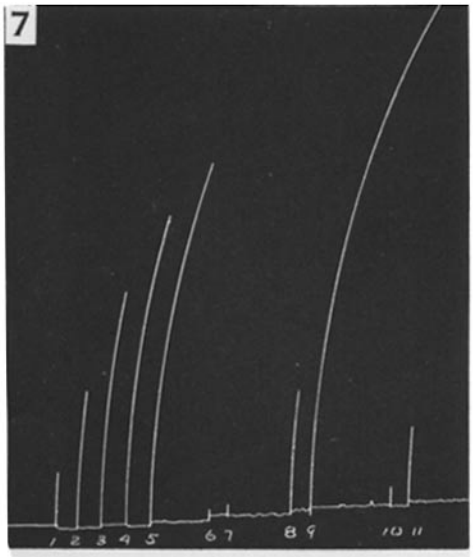
FIG. 8. Record of an experiment demonstrating that the release of histamine by water occurs more rapidly and is more transient than that produced by 48/80. Excursions (*a*) to (*e*) are contractions induced by 0.1 to 0.5 ml. of 1/500,000 histamine solution. 15 minutes after intraperitoneal injection of the experimental fluids, no contraction (*f*) was produced by a 0.1 ml. sample of fluid from the rat injected with Tyrode solution. A sample of the same volume from the water-injected rat produced a response (*g*) greater than that from the rat which received 48/80 (*h*). After 1 hour the concentration of histamine in the abdominal fluid from the water-injected animal (*i*) was lower than in the rat given 48/80 (*j*).

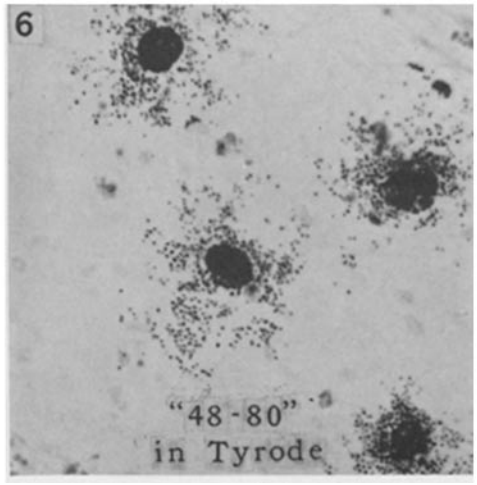












(Fawcett: Release of histamine by mast cells)

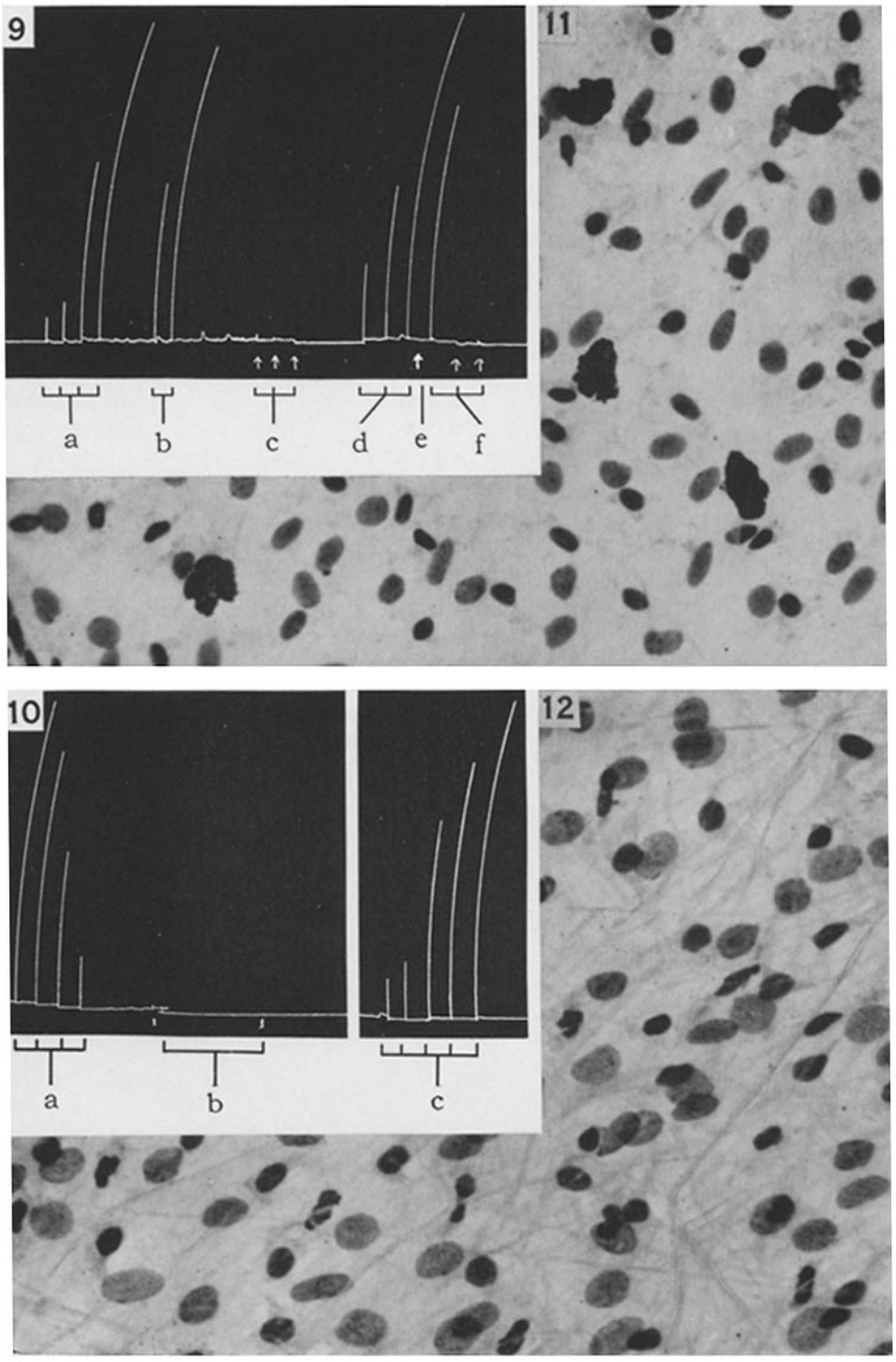
PLATE 26

FIG. 9. Kymograph record of an experiment demonstrating that intraperitoneal injection of compound 48/80 causes a marked release of histamine in a normal rat but fails to liberate histamine in a rat lacking peritoneal mast cells. (*a*) Contractions produced by 0.1, 0.2, 0.3, 0.4 ml. of 1/100,000 dilution of histamine. (*b*) Response to 0.4 and 0.6 ml. of abdominal fluid from a normal rat 20 minutes after injection of 300 μg. 48/80 in 20 ml. Tyrode solution. (*c*) Lack of response to 0.4, 0.6, 0.8 ml. of fluid from a rat which had had its mast cells destroyed 10 days earlier. (*d*) Response to 0.6, 0.8, and 1.0 ml. of fluid from normal rat 60 minutes after receiving 48/80. (*e*) Histamine antagonist neoantergan 0.5 ml. 1/1,000,000 introduced into muscle chamber. (*f*) Action of 1.0 ml. of the abdominal fluid on the muscle was first partially then completely blocked.

FIG. 10. Record of a similar experiment. (*a*) Contractions caused by 0.8, 0.6, 0.4, and 0.2 ml. of abdominal fluid from a normal rat 30 minutes after injection of 300 μg. 48/80. (*b*) No response to 0.8 and 0.6 ml. of abdominal fluid from rat whose subserous mast cells were destroyed 7 days earlier. (*c*) Contractions produced by 0.1, 0.2, 0.3, 0.4, 0.5 ml. of histamine solution 1/100,000.

FIG. 11. Appearance of the mesentery of a normal rat for comparison with Fig. 12. Several mast cells are present. May-Grünwald-Giemsa stain. × 300.

FIG. 12. Mesentery of a rat which had received 20 ml. of water intraperitoneally 10 days earlier. Mesothelial cells and fibroblasts are normal in appearance but mast cells are absent. May-Grünwald-Giemsa stain. × 300.

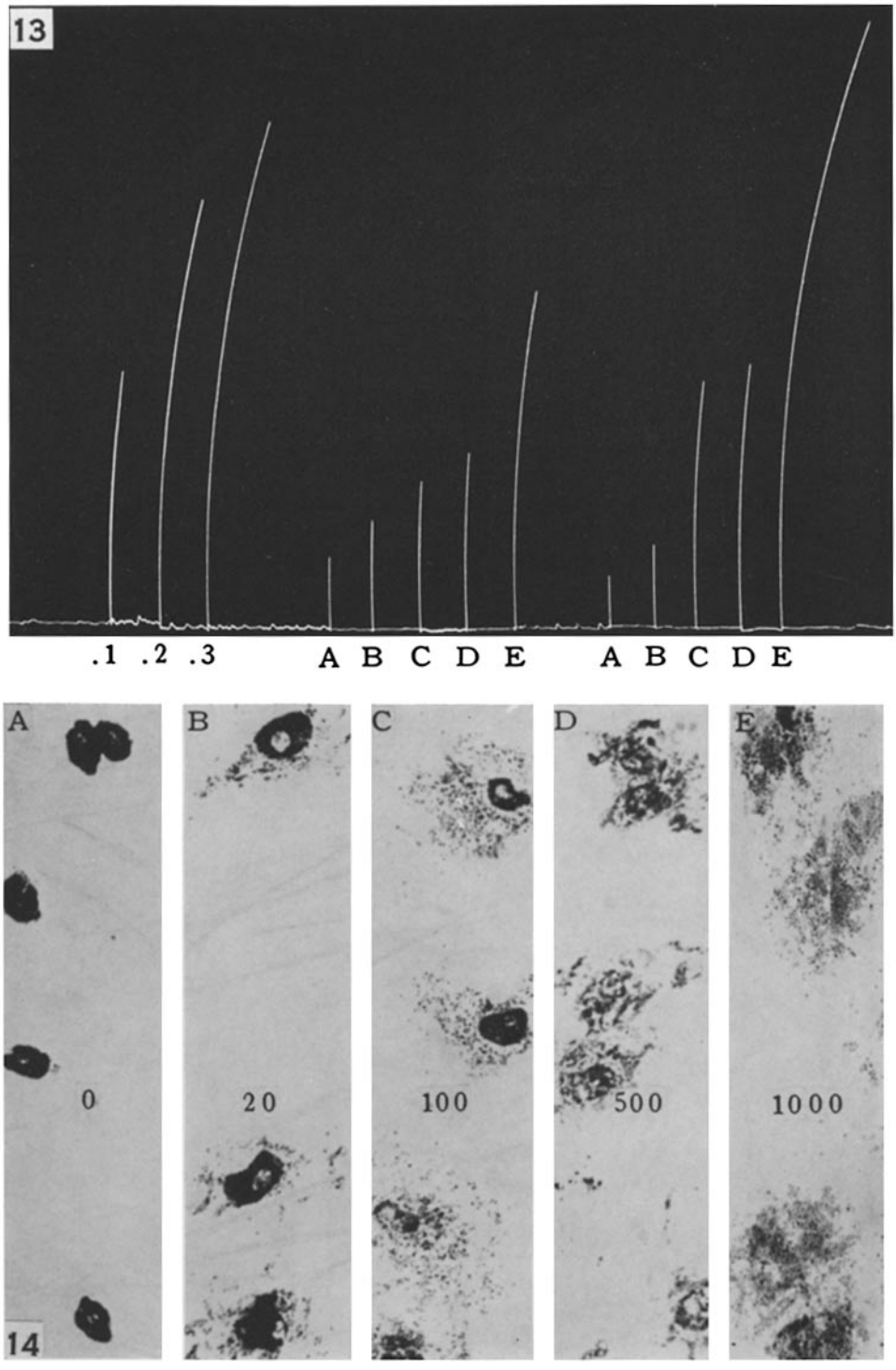


(Fawcett: Release of histamine by mast cells)

PLATE 27

FIG. 13. Kymographic record of an experiment demonstrating a graded pharmacological response to a graded dose of compound 48/80. The first three excursions on the record represent contractions in response to 0.1 to 0.3 ml. of histamine solution 1/100,000. Five animals A to E received intraperitoneal injections of 20 ml. of Tyrode solution containing respectively 0, 20, 100, 500, and 1000 μg. of 48/80. The set of excursions labelled A to E represent contractions of the guinea pig ileum to 0.3 ml. of fluid recovered from each of the 5 animals 30 minutes after injection. An increasing concentration of histamine with increasing dosage is clearly demonstrated. The final set of excursions represents responses to 0.6 ml. of fluid from each animal 60 minutes after injection of 48/80.

FIG. 14. Photomicrographs showing the condition of the mesenteric mast cells in the 5 animals whose histamine release was recorded in Fig. 13. There is a graded morphological response in the mast cells ranging from release of occasional granules in the rat receiving Tyrode solution alone to discharge of nearly all of the granules in the one which received 1000 μg. of 48/80.



(Fawcett: Release of histamine by mast cells)