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REVERSIBLE SWELLING EVOKED BY EPINEPHRINE, 8-BROMO-CYCLIC 3′,5′-GUANOSINE MONOPHOSPHATE AND FEEDING IN THE MAST CELLS OF THE RAT

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Abstract— Swelling, shape distortion, and sectorially decreased granule population-density can be observed in rat-mesentery mast cells exposed to epinephrine *in vivo* or *in vitro*. To a lesser degree, such changes are also noted in mast cells of untreated rats. Changes due to epinephrine were quantified both in mesentery spreads and in fixed and photographically magnified isolated rat peritoneal-fluid mast cells. Threshold effectiveness of epinephrine was 0.5 μg/kg *in vivo* (i.v.) and 10^{-8} moles/liter *in vitro*. Rapidity, partly linear log dose–response relationships, and reversibility characterize the responses of mast cells to epinephrine. Within limits, re-exposure to epinephrine of spontaneously deactivated mast cells resulted in renewed change. N^6-2′-*O*-Dibutyril-cyclic 3′,5′-adenosine monophosphate (diBu-cAMP) inhibited, and 8-bromo-cyclic 3′,5′-guanosine monophosphate (8-Br-cGMP) reproduced, the effects of epinephrine; like the catecholamine, neither cyclic nucleotide derivative caused a release of histamine or a degranulation of mast cells in the manner that compound 48/80 was shown to do. A return to normality of mesenteric mast cells of 48/80-treated rats required between 24 and 48 hr; in the epinephrine-treated rat, this return was complete within 60 min. Feeding significantly increased the number of morphologically altered mast cells present in mesenteries of 24 hr-fasted rats. A renewed fast reduced altered mast cell numbers to prefeeding levels. Although mast cell alterations evoked by feeding appeared to be morphologically similar to those caused by epinephrine or 8-Br-cGMP, the catecholamine cannot be the mediator of such changes because phenoxybenzamine, an α-adrenergic blocking agent, prevented the effect of epinephrine but not that of feeding on mast cells.

Mast cells have been studied for several reasons: (a) because of their ubiquitous occurrence on the vicinity of blood vessels and lymphatics in connective tissue [1], which suggests a role in the control of vascular fluid flow; (b) because of their ready and multiple responses to immunological challenge [2, 3] and (c) because of their biochemically subtle, vulnerable and non-specific histamine-releasing machinery, which is an interesting object for the study of exocytotic control [4]. Knowledge accumulated along these lines has strongly linked mast cells with inflammatory and hypersensitivity states [5]. Extending beyond the physiopathological concept of mast cell function, the rather unique intracellular organization of these cells has caused them to be considered as microchromatographically active centers of uptake of undesirable cations and of substrates of apparently sessile, granule-bound intracellular enzymes. These are two conservative forms of cell function that contrast with explosive mast cell granule exteriorization and histamine release [6]. The hypothesis of a non-degranulating, ultrastructurally conservative form of mast cell activity [6] has, however, not yet been subject to experimental confirmation at the pharmacological and biochemical levels. Several years ago we reported on morphological changes observed in rat-mesentery mast cells following exposure of rats to epinephrine or catecholamine-releasing stimuli, such as cold, exercise, or splanchnic nerve stimulation [7, 8]. These changes were characterized by apparent intracellular granule rearrangements, cell swelling, no overt evidence of granule discharge to the outside of the cell, and a considerable difficulty in obtaining evidence of histamine release by affected mast cells. The predominantly α-adrenergic nature of epinephrine action was demonstrated by results showing that α-adrenergic receptor blockers such as phentolamine, dibenamine, and tolazoline were much more efficient in preventing catecholamine-induced changes than were β-receptor blockers such as propranolol or dichloroisopropyl-norepinephrine [7, 8]

Work with epinephrine-treated rat peritoneal-fluid mast cells confirmed the lack of histamine-releasing effects of the catecholamine, but showed that increased tryptic, chymotryptic, and plasma kallikrein activating properties arose in such cells following treatment [9, 10]. Washing did not remove these activities from mast cells, and this was considered evidence that they resulted from the activation or exposure of sessile cell components. In the present report, the action of epinephrine on mast cell morphology has been examined both in cells embedded in their matrix of mesenteric connective tissue and in free mast cells harvested from the peritoneal cavity of the rat. Presented below are results showing the effects on mast cells of various concentrations and times of exposure to the catecholamine. Spontaneous reversal of epinephrine-evoked changes was studied *in vivo* and *in vitro*, and

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its time course was compared with that required for regeneration of normal granular mast cell structure in mesenteries of rats exposed to moderate amounts of compound 48/80. cAMP and, in some instances, cGMP have been implicated as second messengers of the cellular effects of catecholamines and other pharmacological mediators [11–13]. The effects of lipid-soluble, metabolically active [14] derivatives of these nucleotides on morphological change and histamine content of mast cells have therefore been studied and their relationships to 48/80-induced histamine release have been investigated. The fortuitous observation that mesenteric mast cells from 24 hr fasted rats give a relatively greater response to epinephrine than do cells from normally fed animals caused us to investigate whether the simple process of feeding 24 hr fasted rats leads to reversible changes in mast cell morphology reminiscent of those evoked by epinephrine or 8-Br-cGMP.*

MATERIALS AND METHODS

Male, 180–250 g Wistar rats were used. Control or treated animals, anesthetized with Nembutal (80 mg/kg, s.c.), were decapitated and exsanguinated. Unless stated otherwise, all animals had undergone a 24-hr fast, with access to water *ad. lib.* prior to use. Mesenteric spreads were obtained from freshly killed animals by careful dissection, before fixation when they were to be used for incubation and following fixation *in situ* when they were to be examined for the outcome of *in vivo* treatment. *In situ* fixation and staining were accomplished by allowing 5 ml of a solution containing 4% acetic acid, 50% ethanol, 36% water, 10% formaline (v/v) and 0.4% toluidine blue (w/v) to remain for 5 min in the abdominal cavity of the sacrificed animal opened by an approximately 5 cm long medial, longitudinal cut. To complete fixation and staining, after excision mesenteric fragments (windows), contained within their adipose tissue frames, were allowed to remain for an additional 5 min in the staining-fixative solution, after which they were rinsed for 1–2 min with 50% (v/v) ethanol and spread on microscope slides. At least two spreads were collected from each animal. After being allowed to dry for 24 hr at room temperature, each fragment was freed from its adipose frame by dissection, further defatted in xylene for 3 min, allowed to dry at room temperature, and mounted for microscopic examination.

For incubation, freshly excised mesenteric fragments were placed in 2 ml of Krebs–Ringer phosphate buffer contained in 10-ml beakers and were allowed to remain in this medium in the presence of test substances for 15 min at 37° under agitation (34 cycles/min) in a Dubnoff metabolic incubator under air. At the end of the incubation period, supernatant fractions were discarded and tissues were fixed, stained, spread on slides, and mounted for microscopic examination as described above.

Morphological alterations were observed under a Zeiss binocular GLF standard microscope under oil, at 800-fold magnification. In every experiment, effects of treatment were determined in at least two mesenteric fragments per animal in each of which three microscopic fields, each containing 100 mast

* Abbreviations: 8-Br-cGMP, 8-bromo-cyclic 3′,5′-guanosine monophosphate; and diBu-cAMP, N^6-2′-*O*-dibutyril-cyclic 3′,5′-adenosine monophosphate.

Table 1. Percentage of altered mast cells, in mesenteric spreads from control and treated rats, counted by four different observers who were uninformed about the nature of the experimental treatment of the material under examination*

Material	Observer							
	M.P.O.A.		I.M.M.		E.L.T.		I.C.F.	
	% Altered mast cells							
	Fragment		Fragment		Fragment		Fragment	
	1	2	1	2	1	2	1	2
Controls	25	45	19	45	32	28	19	23
	35	20	26	39	40	62	26	33
	30	37	38	11	33	38	38	40
	30	34	27.7	22.7	35.0	42.7	27.7	32.0
Averages	32.0		25.2		38.8		29.9	
Treated:	62	56	71	81	77	89	68	66
10 μg/kg	76	59	49	88	91	73	68	59
epinephrine,	84	79	64	59	63	75	72	71
i.v., 10 min	74.0	64.7	61.3	76.0	77.7	79.0	69.3	65.3
Averages	69.4		68.7		78.3		67.3	
Net effect of epinephrine	37.4%		43.5%		39.5%		37.4%	

* Each result refers to counts of not necessarily identical microscopic fields, observed in two mesentery samples (Nos. 1 and 2). None of the examiners [two technicians (I.M.M. and I.C.F.) and two research workers (M.P.O.A. and E.L.T.)] knew about the treatment to which the samples had been submitted previously. Control and treated samples had been obtained from the same animals, i.e. one control and one treated rat.

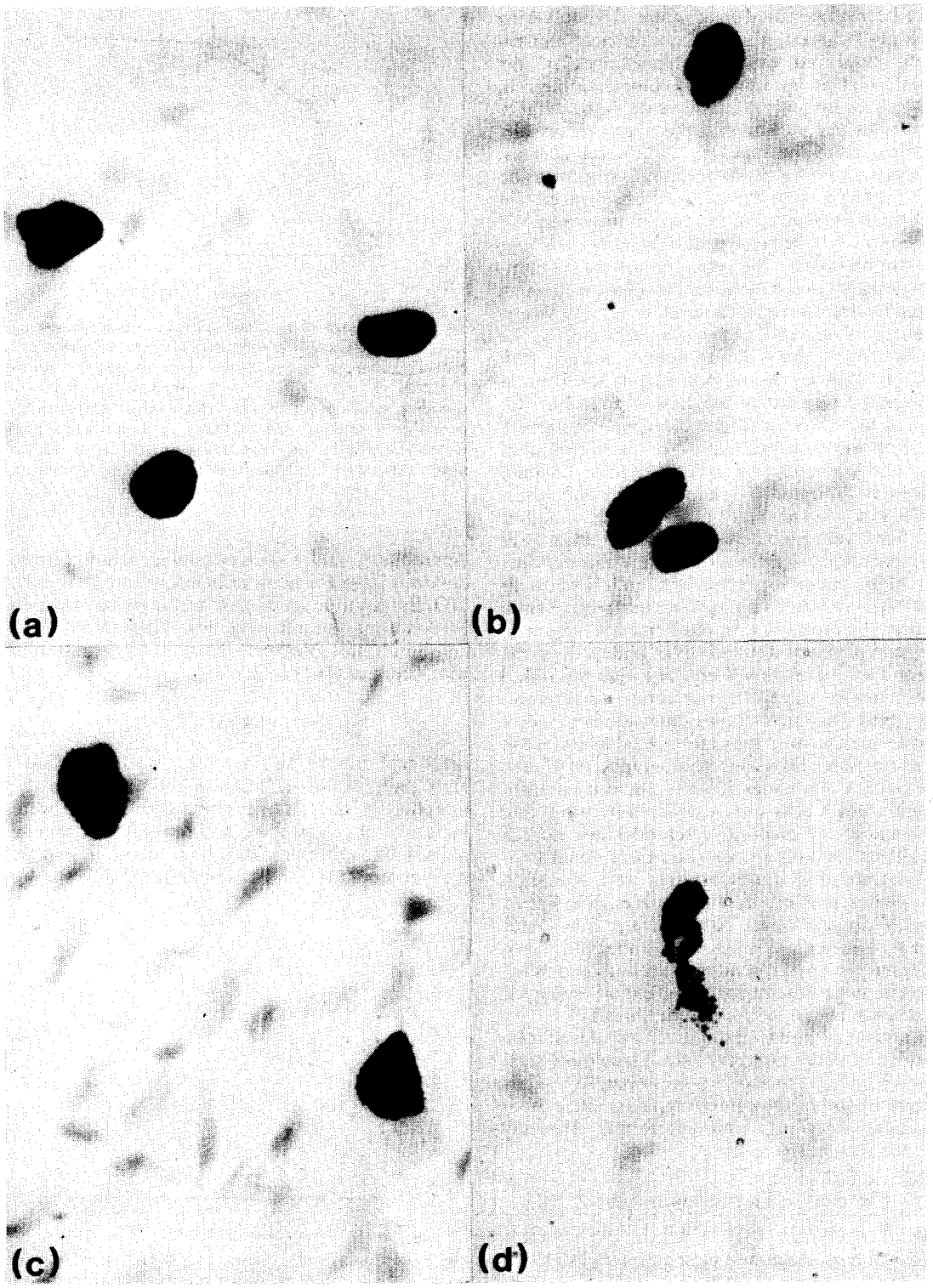

Fig. 1. Microscopic appearance of rat-mesentery mast cells following incubation in (A) buffer, (B) 2.7×10^{-6} M epinephrine, (C) 1×10^{-4} M 8-Br-cGMP, or (D) in the presence of 1 μg/ml of compound 48/80. Toluidine blue stain was used, and the magnification was 800-fold. The discrete changes in contour and shape observed in mast cells of epinephrine- or 8-Br-cGMP-treated tissue contrast with the clear cut picture of degranulation evoked by compound 48/80.

cells, were examined; at least four animals supplied tissues for the test of each experimental treatment.

Mast cells were classified as morphologically altered if they presented one or more of the following signs: (a) localized or generalized granule redistribution evidenced by the disappearance of the smooth, regular, oval or round contour of the cell; (b) localized or generalized decrease in the number of granules per unit area of the mast cell, usually accompanied by the appearance of the cell nucleus; (c) appearance of granule-filled areas of protrusion of the cytoplasm of the cell. Although a certain subjectivity is involved in this type of measurement, the results could be confirmed in blind tests by four different observers. The results of a typical experiment designed to test the reliability of the examining procedure are shown in Table 1.

Peritoneal-fluid cells were collected by flushing the rat abdominal cavity during gentle massage with 10 ml of buffer for 30 sec, centrifuging for 5 min at 900 g, and resuspending the cells in adequate volumes of buffer. Suspensions containing 5–10 × 10^4 mast cells were incubated at 37° in air in a Dubnoff metabolic incubator at a shaking cycle of 125/min. Cells were collected by centrifugation and resuspended in a small amount (usually 0.2 ml) of buffer. Thin films were prepared by spreading aliquots of this suspension on microscope slides and allowing them to dry under a stream of moderately warm air for 30–40 min. After staining for 3 min with Giemsa solution, the excess of this solution was diluted with an equal volume of distilled water while still on the slide and left for another 8 min. The slide was rinsed under running water, dried at room temperature, and prepared for microscopic examination by placing the cells under cover slips and embedding them in Canada balsam. Following photography on Kodak Panatomic-X film under 200-fold microscopic magnification, mast cells were examined by projecting the image of the diapositive reproduction of each microscopic field on a screen at an additional 16.5-fold magnification. Cell diameters were measured to the nearest mm; in untreated mast cells they varied between 10 and 30 mm. Alternatively, cells under cover slips were directly examined under the microscope; the percentage of altered cells was quantified using the same criteria for classification employed for the examination of mesenteric spreads.

Histamine in the supernatant of incubates of mesentery and in HCl extracts of this tissue or of sedimented peritoneal-fluid cells was estimated by bioassay on the atropinized guinea pig ileum. Release of the amine occurring in mesentery was estimated using the relationships,

$$\% \text{ Release histamine} = \frac{\text{histamine in supernatant fraction} \times 100}{\text{histamine in supernatant fraction} + \text{histamine in tissue}}$$

Release in peritoneal-fluid mast cells was estimated using the formula,

$$\% \text{ Histamine released} = 100 - \frac{\text{histamine in sediment after incubation}}{\text{histamine in sediment prior to incubation}} \times 100$$

Drugs. *l*-Epinephrine, compound 48/80, diBu-cAMP, 8-Br-cGMP, toluidine blue, histamine diphosphate, and Giemsa staining solution were obtained from the Sigma Chemical Co., St. Louis, MO. Epinephrine solutions (1 mg/ml) were prepared by dissolving the amine in just sufficient 1 M HCl and diluting this solution to the desired concentration with saline at pH 5–6.

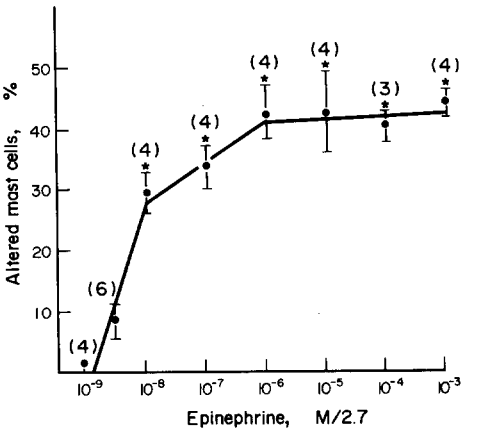

Fig. 2. Percentage of mast cells of rat mesentery showing morphological alterations following incubation with various concentrations of epinephrine. Percentage alterations observed in controls have been subtracted from corresponding values observed after treatment. Figures within parentheses refer to the numbers of experiments performed. Vertical bars represent standard errors of the mean. An asterisk (*) indicates a statistically significant ($P < 0.05$) effect of epinephrine.

RESULTS

Epinephrine, as well as the lipid soluble cGMP analogue [14] 8-Br-cGMP, evoked changes in the morphology of mesenteric mast cells that were indicative of cell swelling and intracellular granule redistribution, but produced no evidence of granule ejection from the cell. These changes in the cells are

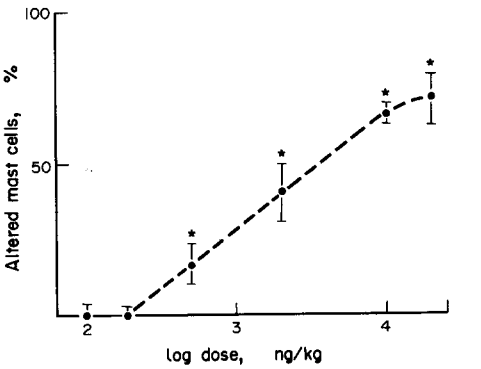

Fig. 3. Percentages of mesenteric mast cells showing morphological alterations following intravenous injections of epinephrine into rats. Percentage alterations observed in mesentery of untreated control animals have been subtracted from corresponding averages in tissues from treated animals. Each result is the average of three to five experiments. Vertical bars represent standard errors of the mean. An asterisk (*) indicates a statistically significant ($P < 0.05$) effect of epinephrine.

Table 2. Time course of development of morphological alterations observed *in vivo* and *in vitro* in rat-mesentery mast cells following exposure to epinephrine*

Treatment	Time interval (min)	N	% Mast cells showing alterations Controls†	Treated
Epinephrine (2.7×10^{-6} M, 37°)	1	3	27 ± 1	52 ± 4‡
	5	3	29 ± 2	55 ± 5‡
	10	11	34 ± 3	57 ± 5‡
	25	3	34 ± 8	41 ± 6
Epinephrine (10 μg/kg, i.v.)	1	3	23 ± 3	65 ± 3‡
	5	3		80 ± 1
	10	6	28 ± 2	82 ± 3‡
	60	6	29 ± 5	19 ± 6

* Values are means ± S.E.; N = number of experiments performed, each employing a different animal.

† Controls were incubated in buffer or obtained from saline-injected animals.

‡ Statistically significant ($P < 0.05$) effect of epinephrine.

illustrated in Fig. 1. The appearance of epinephrine- and 8-Br-cGMP-treated mesenteric mast cells contrasted strongly with that of the compound 48/80-treated cell in which swelling, if present, was totally overcast by a cloud of ejected granules indicative of the well-known phenomenon of exocytosis [4]. Figure 2 shows the percentage of mesenteric mast cells that presented morphological alterations following incubation with various concentrations of epinephrine. The effect of the catecholamine appeared to be exponentially related to concentrations between the 10^{-8} and 10^{-6} M levels; above these concentrations the effectiveness of epinephrine increased little by raising further its concentration in the medium. The epinephrine-treated rat exhibited morphologically altered mesenteric mast cells that apparently were identical to those observed following *in vitro* treatment. Figure 3 shows the log dose–response relationship characterizing this action of epinephrine.

Mast cell changes following exposure to epinephrine occurred quickly. Table 2 shows that, within 1 min following either *in vitro* or *in vivo* treatment, nearly maximum percentages of cells were morphologically altered. The effect persisted for at least another 10 min, but it was no longer detectable in animals killed 60 min after intravenous administration of the catecholamine. This finding, of a reversal of the effect of epinephrine, was examined in more detail. Figure 4 shows that 10 min after the injection of epinephrine, increased numbers of morphologically deformed mast cells were observed in the mesentery; 60 min after treatment the number of such cells had decreased to pretreatment levels, but the number increased again following a second injection of the catecholamine. Sixty minutes after this renewed treatment, the number of altered mast cells had decreased; however, it only reached control levels in animals examined after 120 min. It appears that mast cell alterations evoked by epinephrine are reversible and can, within limits, be evoked by renewed injection of the catecholamine.

In vitro experiments had clearly shown that alterations following exposure of mesenteric mast cells to epinephrine differ from those evoked by compound 48/80 [7]. Figure 5 shows that, in contrast to the rapid reversal of the epinephrine effect, the typical degranulation that characterized the effect of compound 48/80 on mast cells persisted for at least 24 hr; it had subsided after 2 days, but it only disappeared fully 4 days following treatment.

It had been noted that the effects of epinephrine on mast cells reached a level of increased statistical significance in rats that, prior to treatment, had undergone a 24-hr period without solid food.

To better understand this result, we have studied the effect on the morphology of mesenteric mast

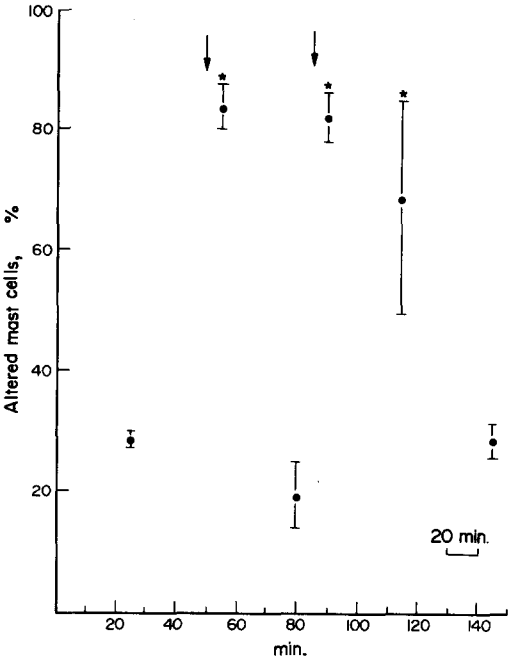

Fig. 4. Reversal of morphological alterations evoked by intravenously administered epinephrine in rat mesentery mast cells. Arrows indicate the moment of injection of 10 μg/kg of epinephrine. Experimental points refer to the moment of killing of groups of rats that had been submitted to all preceding steps of treatment. There were seven rats in each group. An asterisk (*) indicates a statistically significant ($P < 0.05$) effect of epinephrine. Vertical bars represent standard errors of the mean.

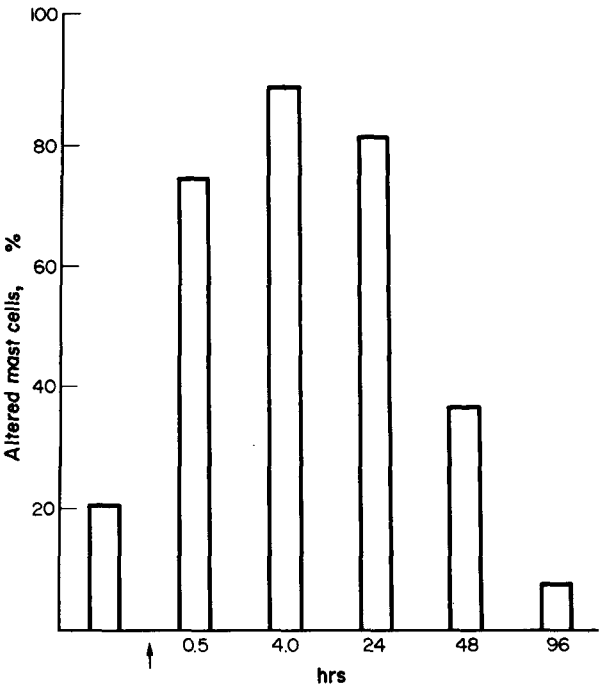

Fig. 5. Reversal of degranulation evoked in the mast cells of rat mesentery by intravenous administration (arrow) of 50 μg/kg of compound 48/80. Results are averages of two experiments.

cells, of subjecting rats to alternate feeding and fasting periods. The results shown in Fig. 6 reveal that, when normally fed rats were fasted for 24 hr the number of mesenteric mast cells with evidence of altered morphology was reduced significantly. One hour after a period of feeding *ad. lib.* a statistically significant return to increased levels of altered mast cells was observed. This effect persisted during the first 4 hr of a renewed fast but progressively decreased thereafter; after 12 hr of fasting, the percentage of altered mast cells had dropped to near prefeeding values, which they reached within the following 12 hr of fasting. Endogenous epinephrine was probably not responsible for the mast cell alterations evoked by feeding. Figure 7 shows that phenoxybenzamine-treated rats that had become

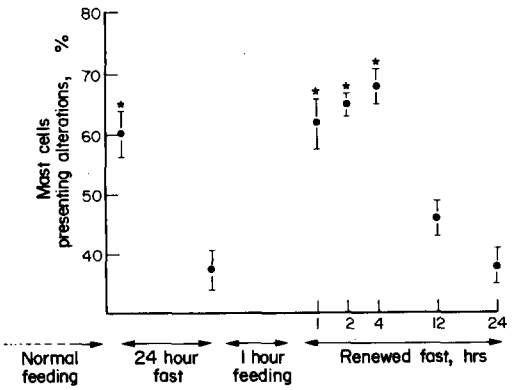

Fig. 6. Morphological alterations of rat-mesentery mast cells accompanying a feeding–fasting–feeding sequence. Experimental points refer to the moment of killing of groups of eight rats, each of which had been subjected to all preceding steps of treatments. Vertical bars refer to standard errors of the mean. An asterisk (*) indicates a statistically significant ($P < 0.05$) effect of feeding.

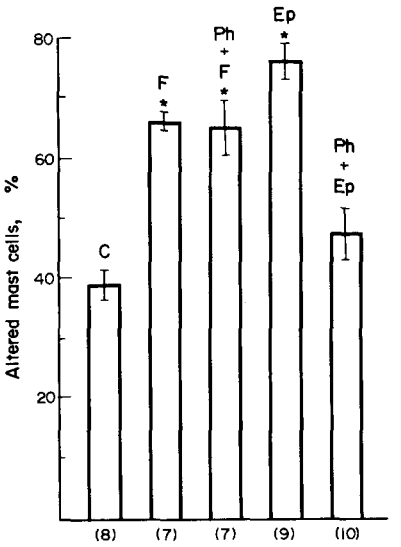

Fig. 7. Influence of phenoxybenzamine (Ph) on the effect of feeding (F) and of epinephrine (Ep) on mesenteric mast cells of fasted rats. Ph (15 mg/kg) was administered to 24 hr-fasted rats either 3 hr prior to a 1 hr period of exposure to food and a 1 hr rest period, or 3 hr prior to an i.v. injection with 10 μg/kg of Ep. C refers to values from control rats subjected prior to killing, to a 24 hr fast with free access to water. Each bar refers to averaged values presented with their standard errors of the mean. Figures within parentheses indicate the numbers of animals employed. An asterisk (*) indicates a statistically significant ($P < 0.05$) effect of epinephrine or of feeding.

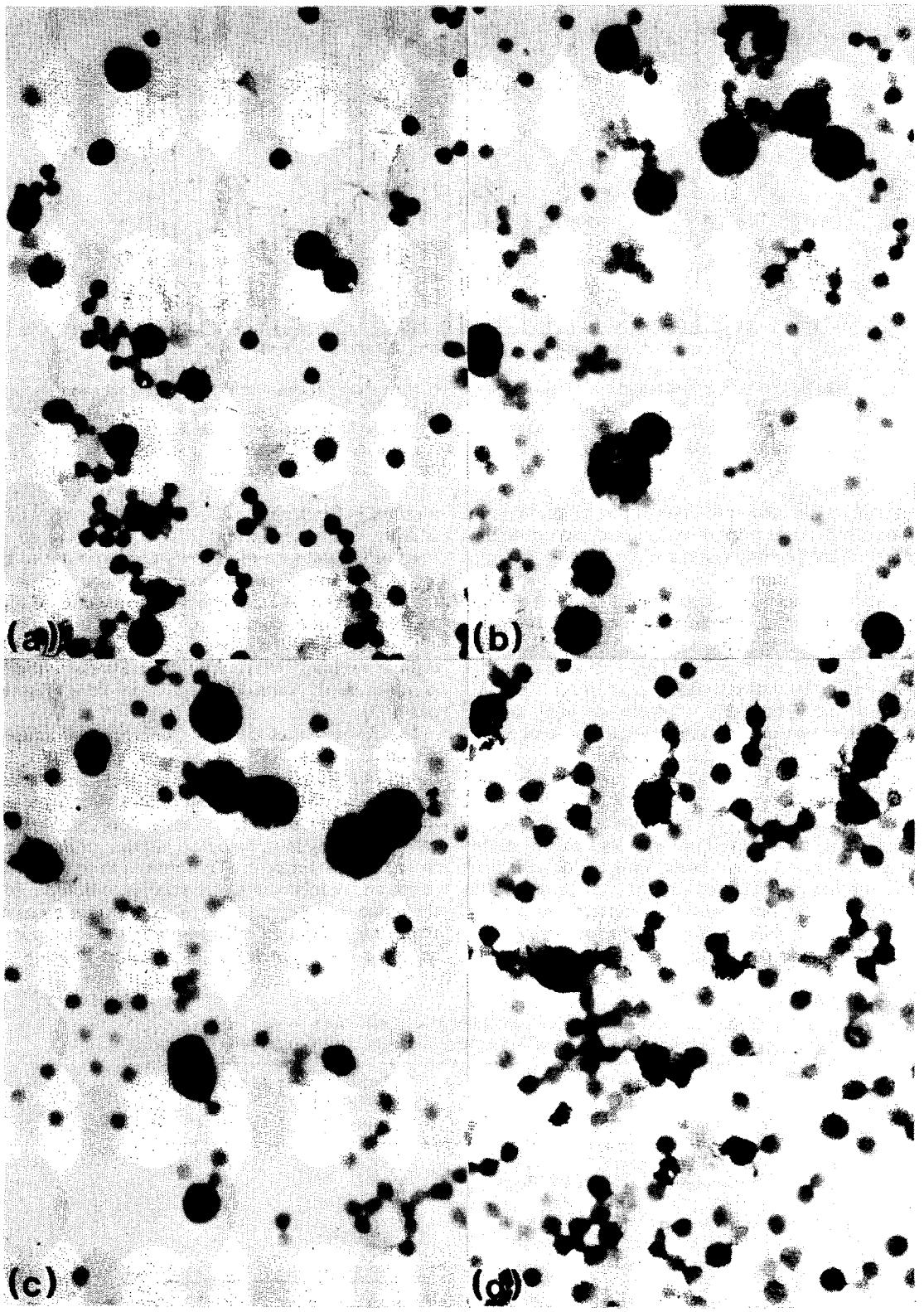

Fig. 8. Microscopic appearance of peritoneal-fluid mast cells following incubation in (A) buffer, (B) 2.7×10^{-6} M epinephrine, (C) 1×10^{-4} M 8-Br-cGMP, or (D) in the presence of 1 μg/ml of compound 48/80. Giemsa stain was used and the magnification was 750-fold. By not being limited in their expansion, isolated mast cells show changes in size and density of granules evoked by epinephrine of 8-Br-cGMP more clearly than do rat mesentery cells.

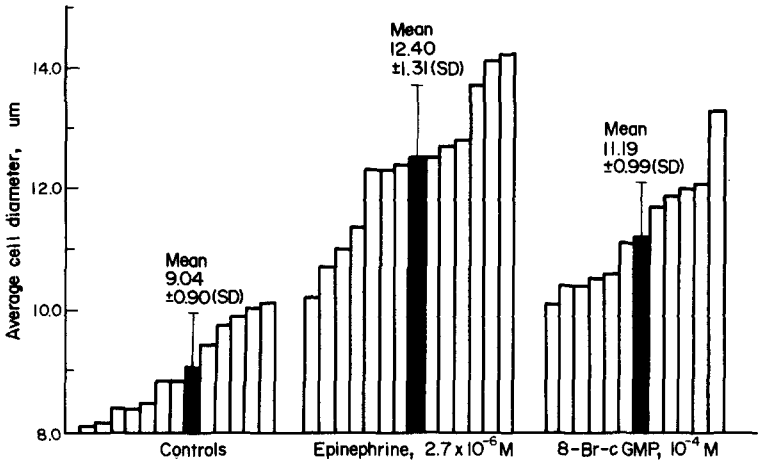

Fig. 9. Averages of horizontal and vertical diameters of peritoneal-fluid mast cells following incubation in buffer, 2.7×10^{-6} M epiniphrine, or 1×10^{-4} M 8-Br-cGMP. Diameters were obtained from 16.5-fold enlarged projections of 200-fold magnified photomicrographs of microscopic fields. Each column represents the average of measurements taken from six to eight cells. Dark column are the mean ± standard deviation (S.D.) values corresponding to each treatment.

resistant to the action of epinephrine on mesenteric mast cells were as responsive as untreated rats to the effect of feeding on mast cells.

Exposure to epinephrine of the isolated mast cells present in rinsings of the peritoneal cavity of the rat resulted in remarkable effects on cell morphology. The photomicrographs of Fig. 8 reproduce the appearance of peritoneal fluid mast cells exposed for 5 min at 37° to buffer (panel A), or to 2.7×10^{-6} M epinephrine (panel B). Notable swelling and a diminished packing of intracellular granules were shown by the epinephrine-treated cells. These changes resemble closely those appearing in epinephrine-treated mesenteric mast cells, differing from the latter probably only because structural tissue around the mast cell prevents its free expansion in mesentery. A quantitative view of the effect of epinephrine is assembled in Fig. 9. It presents the averages of the perpendicular diameters, measured under appropriate photomicrographic magnification, of a large population of control and treated mast cells. Epinephrine caused a 37.6 per cent statistically highly significant ($P < 0.001$), increase in mast cell diameters; other cells appeared not to be affected. 8-Br-cGMP produced the same morphological effects as the catecholamine (panel C of Fig. 8 and third set of columns of Fig. 9).

Compound 48/80 treatment apparently led to little cellular swelling but typically caused the ejection of granules to the outside of the mast cells (Fig. 8, panel D).

The reversible character of epinephrine-induced mesenteric mast cell alterations could also be observed *in vitro* in peritoneal-fluid cell suspensions. Table 3 shows that, when such cells were exposed to epinephrine for 1 min, the percentage of mast cells presenting morphological alterations increased from 15 to 68 per cent. Withdrawal of the catecholamine and re-incubation of the cells in buffer reduced the number of altered cells to 28 per cent, controls remaining unaltered; re-exposure of the cells to epinephrine for 1 min led to a renewed increase of

Table 3. Reversal of the morphological alterations observed in rat peritoneal-fluid mast cells following incubation with epinephrine*

Treatment	% Mast cells presenting morphological alterations	
	Controls†	Treated
Epinephrine (1 min)	15 ± 4	68 ± 13‡
Epinephrine (1 min), C–W–Rs,§ buffer (30 min)	18 ± 6	28 ± 9
Epinephrine (1 min), C–W–Rs, buffer (30 min), C–W–Rs, epinephrine (1 min)	12 ± 3	60 ± 6‡

* Values are means ± S.E. Treatment conditions: epinephrine, 2.7×10^{-6} M, 37°.

† Controls were incubated in the absence of epinephrine.

‡ Statistically significant ($P < 0.05$) effect of epinephrine.

§ Centrifugation (C), washing (W), and resuspension (Rs) in a volume of buffer identical to that employed in the immediately preceding step. Results refer to averages of six experiments.

Table 4. Histamine release and morphological changes in rat mast cells following incubation with epinephrine, 8-Br-cGMP, or compound 48/80 alone or in the presence of diBu-cAMP*

Incubation†	% Mesentric mast cells presenting morphological alterations	% Histamine released From mesentery	% Histamine released From peritoneal-fluid cell suspensions
Controls	29 ± 2 (7)	5.6 ± 3.2 (3)	4.5 ± 0.3 (6)
Epinephrine (2.7×10^{-6} M)	56 ± 6‡ (7)		6.3 ± 0.8 (6)
diBu-cAMP (10^{-4} M)	30 ± 4 (7)	6.1 ± 2.4 (3)	5.8 ± 0.4 (3)
diBu-cAMP + epinephrine	30 ± 3§ (7)		
8-Br-cGMP (10^{-4} M)	51 ± 5‡ (7)	4.1 ± 1.7 (3)	6.5 ± 0.6 (3)
8-Br-cGMP + epinephrine	65 ± 4‡ (5)		
diBu-cAMP + 8-Br-cGMP	31 ± 3§ (5)		5.1 ± 1.3 (3)
48/80 (1 μg/ml)			55 ± 9 (6)‖
48/80 + diBu-cAMP (10^{-4} M)			58 ± 9 (3)‖
48/80 + 8-Br-cGMP (10^{-4} M)			53 ± 10 (3)‖

* Figures in parentheses refer to the numbers of experiments performed. Values are means ± standard errors. The effects of epinephrine alone or of 8-Br-cGMP alone did not differ significantly. Epinephrine did, however, slightly enhance the effectiveness of 8-Br-cGMP ($P = 0.05$).

† Cell suspension or mesenteric fragments were incubated for 10 min at 37°.

‡ $P < 0.005$ that such a change from control level would occur by chance (Student's *t*-test).

§ $P < 0.001$ that such a change from epinephrine or 8-Br-cGMP-evoked level would occur by chance.

‖ $P < 0.001$ that such a change from control level would occur by chance.

altered mast cells equaling 60 per cent; the percentage of altered cells in controls again remained unaltered.

Table 4 shows that 8-Br-cGMP reproduced, but did not enhance, the effect of epinephrine on mesenteric mast cell morphology. diBu-cAMP, a functional analogue of cAMP [14], in contrast, fully inhibited the actions of both epinephrine and the cGMP derivative. Neither of the cyclic nucleotide derivatives released histamine from rat mesentery or rat peritoneal-fluid mast cells or affected the activity of compound 48/80 on the latter.

DISCUSSION

Structural changes observed, under the light microscope, and classified as reversible swelling and intracellular granule displacement occurred in rat mast cells following exposure to epinephrine or 8-Br-cGMP. These changes were not accompanied by the release of histamine. When related to the effects of epinephrine or 8-Br-cGMP on mast cell enzymes [9] and on mast cell evoked plasma kallikrein activation [15], they provide a better understanding of the biochemical, structural and functional aspects of what may be termed non-histamine releasing mast cell activity.

Swelling, caused by the catecholamine, in some of a population of mesenteric mast cells *in vivo*, disappeared within 60 min after injection but rapidly reappeared upon renewed treatment with the catecholamine. Reversal of the effects of this second exposure was not as fast as that following the first exposure. This may be due to partial saturation of catecholamine uptake mechanisms in the pretreated animal, leading to a greater proportion of the free amine in the extracellular pool [16]. Washing and re-incubation in fresh medium of peritoneal-fluid mast cells that had been exposed to epinephrine also led to disappearance of signs of enhanced morphological alteration; re-incubation of washed cells with the catecholamine produced renewed appearance of altered cells. This ready reversibility may be physiologically meaningful since, in contrast to the relatively long period required for regranulation after compound 48/80 treatment, it may occur without the expenditure of temporally extended and biochemically expensive efforts.

The controlled synthesis and degradation of the ubiquitous second messengers of cellular activity, cAMP and cGMP [11–13], probably represent routine events required by cells to maintain functional integrity. Mast cells demonstrated negative or positive sensitivity to swelling and granule displacement following exposure to active derivatives of these nucleotides. The biochemical mechanism by which nucleotides may control rat mast cell morphology remains unexplained. It is relevant to note, however, that two other expressions of epinephrine-induced mast cell activity—namely, the generation and/or exposure of trypsin and chymotrypsin-like activity [9], and the generation and/or exposure of the ability to affect the kininogen-depleting system of rat plasma [10, 15]—can be controlled by exogenous cGMP and cAMP derivatives in the same manner as mast cell deformation. Like epinephrine, 8-Br-cGMP and diBu-cAMP were not active in mast cell histamine release.

Granule segregation, similar to that evoked by epinephrine, occurs after exposure of mast cells to colchicine [17]. This drug has strong effects on microtubules, a part of the cytoskeletal system [18]. This system seems to be under the functional control of cAmp and cGMP [19, 20]. It would be of considerable interest to examine the extent to which colchicine and cytochalasin B, (an inhibitor of cell myofibril (actin) alignment [18]) mimic or inhibit the

effects of epinephrine or cyclic nucleotides on mast cells.

Although mast cells are abundant in the mesentery of the rat and other species [1], their functions at this site have not been clarified [21, 22]. The observation that mesenteric mast cell changes, identical apparently to those evoked by intravenously administered epinephrine, occur in the feeding rat and are reversed by a short-term 12-hr fast suggest that these changes are related to a physiologically meaningful form of mast cell activity. It is, however, premature in our view to speculate on which microenvironmental changes occurring during digestion are directly responsible for the mast cell alterations evoked by feeding a fasted rat. Lack of an effect of phenoxybenzamine, a potent inhibitor of catecholamine effects on mast cells, shows that epinephrine cannot be responsible. This is not surprising, for mast cell changes of the type described can be evoked in isolated mesentery, not only by catecholamines but also by such diverse stimuli as 8-Br-cGMP, 2,4-dinitrophenol [23], carbamylcholine [24], and even incubation in glucose-free Krebs-Ringer phosphate (unpublished observations). Like epinephrine, two of these stimuli, 8-Br-cGMP [15] and carbamyl choline [25] have been shown to cause cell-mediated kininogen depletion and kinin release in the rat. Neither 2,4-dinitrophenol nor glucose deficiency has yet been investigated in this respect. Knowledge about changes in circulating kininogen during a fast–feed–fast cycle in the rat has not been forthcoming either. Nevertheless, should they occur, involvement of the kinin system in digestion may be expected. Such a possibility receives some support from observations showing the kallikrein, the enzyme mediator of kinin release, enhances sodium and amino acid transport across the wall of the isolated rat jejunum [26]. *In vivo*, this effect could be mediated by kinin, perhaps released by enzymes on mast cells localized near vessels composing the mesenteric intestinal circulation [27]. The ability of bradykinin to increase venous permeability is well known [28]. Mast cell deformation evoked by catecholamines may well have functional importance in processes other than digestion. Earlier results [8] had shown that sudden exposure of a rat to a cold environment caused a rapid increase in the percentage of mesenteric mast cells that exhibited altered morphology. The inhibition of this change in animals pretreated with phentolamine, an α-type adrenergic blocking agent that also inhibits the effects of epinephrine or norepinephrine on mast cells [7, 8], suggests that one, or both, of these substances could be a mediator of the changes in mast cell appearance observed in cold-stressed rats. Similar reasoning applies to alterations in mast cell appearance that have been observed following physical exercise [8] which, like the digestive process or cold adaptation, may require rapid adjustment of blood flow and of vascular permeability. Mast cells are strategically located near or within vessel walls [27, 29]; the possibility of their participation, in a rapidly reversible manner, in microvascular control via kinin release suggests a solution to part of what has become known as the "riddle of the mast cell".

It is of special interest to reflect on how far views [6] on the presence of an intracellular, insoluble, enzymatically active matrix, over which the mast cell percolates extracellular substrate, go toward explaining a possible functional significance of the cell activated by epinephrine, cGMP, or other substances. Previously, one of us has shown that following such activation neither tryptic/chymotryptic esterase [9] nor heparin, believed to interact with kininogen-depleting system [15], has to leave the mast cell in order to interact with substrates or other reactants. This behavior, the only one able to account for a rapidly repetitive, open–close type of cell activity, may be the link between reversible swelling (admittance of extracellular fluid and its contents) of mast cells and their role as non-histamine releasing, catalytic centers involved in partly kinin-mediated microcirculatory control.

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