tritiated thymidine. The proportion of labeled cells was low prior to and for the first 2 days following initiation of inflammation. Beginning on the 3rd day the proportion of labeled cells gradually increased reaching a maximum on or about the 7th day in the non-sensitized mice. In animals previously sensitized to the Diphtheria toxoid, the increase in labeled cells occurred more rapidly, reaching a maximum on or about 5th day of inflammation. However, number of cells capable of DNA synthesis in both groups appeared to be identical. The origin of inflammatory mononuclear cells was discussed. Only a small proportion of the cells originate by division of cells suspended in the inflammatory exudate. The majority of cells, both granulocytes and mononuclear cells, migrate into the inflammatory area from the blood vessels and possibly from adjoining tissues.

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In vitro Histamine Release from Rat Mast Cells by Chemical and Physical Agents.* (26302)

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A variety of simple chemical compounds capable of *in vivo* and *in vitro* histamine release from cells has been investigated during the last 13 years(1). Most studies, particularly with compound 48/80, have utilized disruption and degranulation of tissue mast cells as an index of histamine release(1-6). Observations on mast cell degranulation may not be adequate for quantitative study of 48/80 and histamine release; indeed, histamine release may occur without degranulation(7). Furthermore, there is almost no quantitative information concerning other chemical releasers. Accordingly, we made direct measurements of histamine released after addition of various concentrations of compound 48/80 to rat peritoneal mast cells, and compared these effects with those of sev-

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eral other substances including 1935L, a substituted butylamine(8).

Method and materials. A. *Source of mast cells.* The method of Padawer and Gordon, modified slightly, was used to obtain suspensions of intact rat peritoneal mast cells(9). Sixteen ml of a modified Tyrode's solution (no glucose) was warmed to 37°C and injected into the peritoneal cavity of male Sprague-Dawley rats weighing from 250 to 400 g. The abdomen of each animal was gently massaged for about one minute after injection. The animal was then exsanguinated rapidly by decapitation. The abdomen was opened and 10 to 12 ml of fluid withdrawn into a 20 ml siliconed‡ syringe with an 18 gauge needle. A 10 ml aliquot was added immediately to 20 ml of modified Tyrode's solution in a siliconed beaker kept at 37°C. The resulting 30 ml suspension of mast cells was termed the reference cell suspension, and will be referred to as such below. We found that the mast cell suspensions could be handled easily without addition of any anticoagulant.

B. *Determination of mast cell concentration.* Mast cells were counted by mixing one part of 0.1% dimethyltoluthionine chloride (toluidine blue) with 19 parts of the reference cell suspension in a standard white-cell counting pipette. The mixture was shaken vigorously and total number of nucleated cells was then counted in a hemocytometer with a counting chamber depth of 0.2 mm. All cells staining metachromatically with toluidine blue were assumed to be mast cells. These were easily recognizable because of their large size and typical intracellular granules.

C. *Release of histamine by compound 48/80 and other substances.* Two ml aliquots were taken from the reference cell suspension and placed in separate 12 ml siliconed centrifuge tubes. After the samples were warmed to 37°C various amounts of 48/80‖ in 0.05 ml volumes were added to different aliquots to provide the final concentrations indicated in *Table I.* The solutions were mixed by gentle tapping of each tube 10-12 times. Each animal provided a volume of reference suspension adequate for at least 6 determinations (each one being run in duplicate).

After 45 minutes incubation at 37°C and gentle agitation every 10 minutes, the suspensions were centrifuged at 350 g for 15 minutes to separate the cells. One ml of each supernatant solution was mixed with an equal volume of 10% trichloroacetic acid (TCA) and allowed to stand for 20 minutes at room temperature. The samples were then centrifuged at 1100 g for 8 minutes and one ml of the resulting 5% TCA supernatant solution used for histamine analysis.

Other agents, 1935L,¶ 4-4′-diamidinostilbene isethionate (Stilbamidine), protamine sulphate, and n-octylamine were likewise introduced in 0.05 ml volumes to give the concentrations shown in Table I. Other substances were tested at the single concentration indicated in Table II. The effects on pH of each concenrtation of each substance were also noted. D. *Effect of mast cell dilution on release of histamine by 48/80.* The reference cell suspension was diluted with an equal volume of modified Tyrode's solution and aliquots were subjected to 3 low concentrations of 48/80. The fraction of histamine released was compared with that produced from undiluted suspensions by these concentrations. E. *Effects of physical changes on release of histamine by compound 48/80.* Different aliquots of the reference cell suspension were brought to 4°, 20°, and 40°C. After 15 minutes, 0.05 ml of 48/80 was added to each (final concentration 10^{-6}M) and the same temperature maintained. After 20 minutes all samples were centrifuged at 6°C to separate the cells and the supernatant solutions were prepared for histamine assay as before. Investigation of osmotic effects was accomplished by diluting aliquots of reference suspension with one and 2 volumes of distilled water and measuring extracellular

‡ All glassware used in contact with mast cells was freshly siliconed with Desicote® obtained from Beckman Instrument Co., Fullerton, Calif.

‖ Obtained through the generosity of the late Dr. J. S. deBeer, Burroughs, Wellcome and Co., Tuckahoe, N. Y.

¶ Kindly supplied by Dr. B. N. Halpern, Hosp. Broussais, Paris, France.

TABLE I. The Chemical Releasers of Histamine.

Molar conc. of releaser	48/80 N*	48/80 Mean† ± S.D.	1935L N	1935L Mean ± S.D.	Protamine N	Protamine Mean ± S.D.	Stilbamidine N	Stilbamidine Mean ± S.D.	Octylamine N	Octylamine Mean ± S.D.
10^{-7}	5	.05 ± .02			2	.15				
2×10^{-7}	10	.32 ± .12	2	.01	3	.29 ± .19				
4×10^{-7}	8	.58 ± .07	2	.07						
10^{-6}	28	.76 ± .10			3	.66 ± .05	1	.07		
2×10^{-6}	3	.82 ± .12	4	.48 ± .07			2	.05		
5×10^{-6}			8	.49 ± .15	3	.74 ± .07				
10^{-5}	8	.92 ± .10	4	.55 ± .09	3	.88 ± .03	3	.16 ± .07	1	.08 pH 8.28
2×10^{-5}			4	.70 ± .07	2	1.0				
5×10^{-5}	6	.94 ± .07					3	.45 ± .16	1	.08 pH 8.3
10^{-4}	4	1.0 ± .10	10	.73 ± .07			3	.58 ± .18		
2×10^{-4}	3	1.0 ± .10					3	.77 ± .06	1	.78 pH 8.4
5×10^{-4}			6	.78 ± .08			4	.85 ± .12	2	.80 pH 8.5
1.6×10^{-3}									3	1.07 ± .06 pH 8.9

* N = No. of duplicate determinations.

† Fraction of available histamine released.

histamine as before. F. *Control samples.* In conjunction with each study, duplicate 2 ml aliquots were taken from the reference cell suspension and carried through the various procedures without addition of releaser or inhibitor. Also, total available histamine (cellular and free) of the reference suspension used for each study was measured by adding 1 ml of 10% TCA to duplicate uncentrifuged 1 ml aliquots. These preparations were mixed and centrifuged as above and the supernatant solutions, being rich in histamine, were diluted 5-fold. One ml aliquots were then used for histamine analysis. G. *Measurement of histamine.* All of the final solutions (containing 5% TCA) from the above experiments were buffered with 1/10 volume of 4M sodium acetate and histamine content measured chemically by the method of Lowry *et al.*(10). Each group of samples was analyzed along with duplicate standard solutions containing 0.25 μg of histamine base. All solutions were prepared using triple glass-distilled water and all glassware was meticulously cleaned and rinsed with triple distilled water before use.

Results. A. *Cell counts and histamine values.* A differential count of the reference suspensions from 74 rats (mean weight of 312 g) yielded 48 ± 21 mast cells per cubic mm, 5.8% of the total nucleated cells. Red cells were rarely seen. These reference suspensions contained 1.38 ± 0.51 μg/ml of intracellular histamine and 0.06 ± 0.02 μg/ml of free histamine. Calculating histamine per mast cell for each animal separately, we obtained the value of 29 ± 10 μg of histamine/10^6 mast cells, assuming that the other nucleated cells contained no histamine(11). Because of the biological variation, all data on histamine released have been expressed in terms of the fraction of available cellular histamine released rather than in absolute values.

B. *Histamine releasers.* A significant amount of histamine was released by 2×10^{-7}M 48/80 (Table I). Histamine release increased greatly with 48/80 concentrations up to 10^{-6}M. The release of histamine by 48/80 was found to be independent of mast cell concentration; the proportion of histamine released from diluted cellular suspensions was identical to that from the standard reference suspension after incubation with 10^{-7}, 2×10^{-7} or 4×10^{-7}M 48/80.

Compound 1935L was also an effective histamine releaser but less potent than 48/80 (Table I). Concentrations of 4×10^{-7}M and below released no appreciable histamine. A small increment (to 10^{-6}M) resulted in a marked rise in amount of histamine released but higher concentrations did not release as much "available" histamine as 48/80.

Both protamine sulfate and n-octylamine

TABLE II. Other Releasers of Histamine.

Substance	Concentration	Release*
Parachloromercuribenzoate	10^{-3}M	.88
Formaldehyde	10^{-3}M	.40
5-hydroxytryptamine	3×10^{-5}M	.39
Phenol	10^{-2}M	.16
Tannic acid	10^{-2}M	.52
Ammonium oxalate	10^{-2}M	.31
Ethanol	35%	1.00
Methanol	50%	1.00

* Fraction of total available histamine released.

released more histamine than 1935L, but higher concentrations of stilbamidine were required and it exhibited a less abrupt rise in percentage of histamine in relation to increases in concentration. Other substances treated for their histamine releasing capacities are listed in Table II. Although we first used parachloromercuribenzoate as a potential inhibitor of histamine release, it released significant amounts of histamine at 10^{-3}M concentration so was not studied further. Both d-tubocurarine and toluidine blue produced high blank readings on histamine assay, so could not be studied.

The nature of our experiments required the pooling of data obtained by study of many animals; thus the ranges of values in Table I represent mainly the degree of biological variability. Large numbers of determinations were carried out at certain concentrations of releaser, particularly 48/80 (Table I). Most of these were control values for further studies concerning the inhibition of histamine release, to be reported later. The effects of pH change were not studied *per se*. None of the agents used altered the pH of the system significantly except for tannic acid (pH of 6.6) and octylamine (Table I).

As expected, several physical procedures caused release of histamine. Both freeze-thawing and heating to 62°C released cellular histamine completely. When an equal volume of distilled water was added to a mast cell suspension no histamine release occurred. However, addition of 2 volumes of water released 60% of the total histamine.

At 4°C, 10^{-6}M 48/80 released insignificant amounts of histamine. Histamine release by 48/80 occurred to about the same degree at 20°C, and 40°C as at the usual temperature of 37°C. However, as the temperature was increased above 40°C spontaneous release of histamine (control values) increased significantly.

Discussion. Our finding that 5.8% of the total number of peritoneal cells were mast cells is comparable to the 3.5% found by Padawer(12) and 5.2% by Lagunoff and Benditt(11). The histamine content, 29 μg/10^6 mast cells is surprisingly close to that reported by others(11,13).

Among the agents studied, compound 48/80 was the most effective histamine releaser on a molar basis; a concentration of 10^{-6}M released a majority of cellular histamine. Our observations with 48/80 are similar to those reported by Norton, who used degranulation of mast cells as an index of histamine release (4). Accordingly, histamine release and mast cell degranulation by 48/80 seem to be comparable quantitatively as well as qualitatively. There is little comparable quantitative information concerning the degranulation effects of the other agents studied.

Small concentrations of the more effective substances released a majority of available histamine, yet large increments were necessary to release the remainder. Indeed, large concentrations of 1935L and stilbamidine were not capable of releasing all "available" histamine. Some mast cells may have been more resistant to release than others; mast cells do not comprise a uniform population, varying in size, staining reactions, and sensitivity to drugs(4,5). Certain "mast" cells might be totally resistant to the effects of 1935L and stilbamidine. Octylamine was relatively weak, and a high concentration was required to produce total histamine release. Although the pH was high, it was not high enough to induce spontaneous histamine release(14,15). Mongar has demonstrated that only the non-ionized form of octylamine is active; this requires an alkaline pH, since the pKa of octylamine is 10.8(14). Thus, histamine release required a concentration of 2×10^{-4}M of total octylamine but the non-ionized form had a concentration of only 7.9×10^{-7} at pH of 8.4. Thus, this base is active in the same range as 48/80.

Protamine was also potent in terms of

molar concentration. However, this large polypeptide has a molecular weight of 5600; the concentrations used would be meaningful only if there is just one histamine-releasing site on each molecule.

The proportion of histamine released was dependent on the concentration of 48/80 but independent of the concentration of mast cells (and thus, the amount of available cellular histamine). The number of molecules of histamine released by one molecule of 48/80 thus would be proportional to the amount of cellular histamine. With 4×10^{-7}M 48/80, 58% of the intracellular histamine was released or about 0.8 μg of base (7.2×10^{-3} μmoles) from 1 ml of reference cell suspension. Thus, 1 molecule of 48/80 would release up to 18 molecules of histamine, and might have released more from more concentrated suspensions.

Summary. Release of histamine from suspensions of rat peritoneal mast cells under various conditions has been measured directly by a microchemical technic. Compound 48/80 released histamine in a concentration as low as 2×10^{-7}M and was the most potent substance investigated. Several other substances released histamine; the effects of compound 1935L, protamine, n-octylamine and stilbamidine were evaluated at several concentrations and compared with those of 48/80. Stilbamidine was considerably less potent than the others. As expected, certain physical changes released histamine, and low temperatures prevented 48/80 histamine release. Qualitative variations in response of these mast cells to different releasers suggested that mechanisms of histamine release vary considerably among the chemical agents now being investigated.

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Acute Effects of Guanethidine on Myocardial Contractility and Catecholamine Levels.*† (26303)

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Recent pharmacological reports describe guanethidine, [2-(octahydro-1-azocinyl)-ethyl]-guanidine sulfate, as a potent antihypertensive agent(1,2). An immediate period of adrenergic-like stimulation—manifested by hypertension, increased cardiac output, and positive chronotropism—exists after the agent is acutely administered to dogs(3). The objects of this study were to determine the initial changes in heart contractility and in plasma and tissue catechola-

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† Preliminary report in: *Symposium on Guanethidine*, Ciba Pharmaceutical Products, Inc., 1960, p75.

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