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# QUANTITATIVE DETERMINATION OF MAST CELL FRAGMENTATION BY COMPOUND 48/80

BY

STATA NORTON

*From the Wellcome Research Laboratories, Tuckahoe, New York*

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Compound 48/80 as reported by Baltzly, Buck, de Beer, and Webb (1949) is a polymer of *N*-methylhomoanisylamine and formaldehyde. Although similar LD 50's have been obtained with different batches of Compound 48/80 (Dews, Wnuck, Fanelli, Light, Tornaben, Norton, Ellis, and de Beer, 1953), it was felt desirable to have an assay method which might be more closely related to the histamine-liberating property of 48/80.

Compound 48/80 was described as a histamine liberator by Feldberg and Paton (1951) and Paton (1951). Recently, Mota, Beraldo and Junqueira, (1953) and Fawcett (1954) have shown the *in vivo* fragmentation of mast cells by 48/80. A relatively simple technique for *in vitro* fragmentation of the mast cells in pieces of rat mesentery was also used by Mota. I felt this latter method could be adapted to quantitative determination of mast cell fragmentation. The method described here is based on the observation that at low concentrations of 48/80 the proportion of cells fragmented depends on the concentration of 48/80.

## METHODS

Adult male albino CF1 rats were killed with ether. Pieces of mesentery with connecting lobes of fat and blood vessels were rapidly dissected out and placed in Ringer-Locke solution (NaCl 0.9%, KCl 0.042%, $CaCl_2$ 0.024%, $NaHCO_3$ 0.015%, and dextrose 0.1%). From 10 to 15 pieces were obtained from each rat. This procedure required about 5 min. The pieces were then placed in solutions of 48/80 in Ringer-Locke for 30 ± 1 min. The pieces of mesentery were removed to a 4% formaldehyde solution containing 0.1% toluidine blue for 20 to 30 min. and then transferred through acetone (two changes) and xylene (two changes) and mounted on slides. Before mounting, excess pieces of fat were trimmed from the edges of the mesentery.

All solutions were at room temperature. Usually two pieces of mesentery were used in each concentration of drug. From these pieces five microscope fields were selected at random under 100 × magnification from widely separated areas of the mesentery. In each field, using 430 × magnification, the first 10 mast cells were examined, starting from the left-hand side of the field and proceeding clockwise. For each drug concentration 50 cells were counted and the percentage of disrupted cells was obtained. Each cell was considered either "disrupted" or "not disrupted." The term "disrupted" was selected instead of "fragmented," since granules were found around many cells which did not appear to be in fragments. Fawcett (1954) has also reported this phenomenon. The sole criterion for calling a cell "disrupted" was the presence of granules outside the cell. Many cells which did not show extrusion of granules appeared swollen at low concentrations of 48/80.

For evaluating the percentage disruption the labels of the slides were covered and the slides were counted in random sequence.

## RESULTS

*Comparison of Different Batches of 48/80.*— Dose-response curves for the percentage disruption of mast cells were obtained for different batches of 48/80 with 0.2, 0.4, and either 0.1 or 0.8 μg./ml. of 48/80. Control mesentery with Ringer-Locke solution alone was run with each experiment. Seven batches of 48/80 were tested. The percentage disruption at each concentration is given in Table I.

TABLE I

DISRUPTION OF MAST CELLS BY DIFFERENT BATCHES OF 48/80

| Batch No. | Expt. No. | Percentage Disruption: Concn. of 48/80 (μg./ml.) 0 | 0·1 | 0·2 | 0·4 | 0·8 | ED 50 (μg./ml.) | Fiducial Limits (P=0·95) |
|---|---|---|---|---|---|---|---|---|
| NS 280 | 1 | 10 | | 28 | 76 | 92 | 0·27 | ·23–·32 |
| | 2 | 6 | | 26 | 68 | 90 | 0·30 | ·26–·35 |
| | 3 | 8 | | 54 | 66 | 86 | 0·20 | ·12–·31 |
| | 4 | 2 | 28 | 68 | 76 | | 0·16 | ·12–·19 |
| | 5 | 6 | 12 | 48 | 70 | | 0·22 | ·19–·26 |
| AE 92 | 3 | | | 56 | 82 | 86 | 0·13 | ·07–·26 |
| AE 2 | 1 | | | 40 | 88 | 100 | 0·23 | ·20–·26 |
| | 2 | | | 4 | 68 | 100 | 0·33 | ·28–·39 |
| GD 78 | 3 | | | 40 | 64 | 72 | 0·26 | ·19–·35 |
| VI 64 | 1 | | | 44 | 54 | 62 | 0·30 | ·16–·56 |
| VI 53 | 3 | | | 6 | 28 | 90 | 0·47 | ·42–·53 |
| VI 65 | 3 | | | 12 | 36 | 64 | 0·57 | ·45–·70 |

The ED 50 values (50% disruption of mast cells) were calculated by probit analysis, following the method of Bliss as described by Goulden (1952). The calculated ED 50's and fiducial limits are given in the last two columns of Table I. Batch NS 280 was selected as the standard. The ED 50's for two batches (VI 53 and VI 65) were outside the range found for NS 280.

*Mechanism of Disruption of Mast Cells.*—Mota *et al.* (1953) observed the disruption of mast cells in 48/80 solutions using phase-contrast microscopy, and described the process as "bubbling." In mast cells vitally stained with neutral red I have observed a similar phenomenon caused by 48/80. When a piece of mesentery is placed in a solution of 48/80 the cell swells and becomes balloon-like, sometimes twisting and turning as it swells, possibly in response to the mechanical forces of the surrounding tissue.

Since living cells swell before they fragment, and since stained intact cells appear swollen, it was assumed that at least one of the effects of 48/80 was to increase the osmotic pressure within the mast cell and hence cause an inflow of water. This increase in osmotic pressure might come about in several ways:

(1) The mast cells might preferentially concentrate 48/80.

(2) A substance which was previously not available for osmotic effect might be liberated or formed in the cell.

(3) The cell might take up or become more permeable to ions present in the extracellular fluid.

An experiment was designed to obtain evidence regarding these possibilities. If the third mechanism listed above is correct, it would follow that diluting the extracellular fluid and reducing the concentrations of extracellular ions would decrease the effectiveness of 48/80. Such a procedure would tend also to cause mast cells to swell in hypotonic solutions. The effect of 48/80 would, however, be expected to be enhanced by hypotonic solutions if the first or second possibility were true.

Table II gives the results of two experiments in which concentrations of 0.05, 0.1, 0.2 and 0.4 μg./ml. of 48/80 were used in combination with undiluted Ringer-Locke, and with Ringer-Locke diluted by 1/4 and 1/2 with distilled water.

In Fig. 1 the percentage of disruption observed in the controls was subtracted from each reading for that experiment. The values for the two experiments were averaged.

TABLE II

PERCENTAGE DISRUPTION OF MAST CELLS BY 48/80 IN ISOTONIC AND HYPOTONIC SOLUTIONS

| Concn. of 48/80 (μg./ml.) | Expt. | Dilution of Ringer-Locke | | |
|---|---|---|---|---|
| | | Undiluted | 1 Part Water, 3 Parts Ringer-Locke | 1 Part Water, 1 Part Ringer-Locke |
| 0 | 1 | 6 | 8 | 48 |
| | 2 | 2 | 28 | 66 |
| 0·05 | 1 | 6 | 2 | 88 |
| | 2 | 8 | 18 | 58 |
| 0·1 | 1 | 12 | 14 | 68 |
| | 2 | 28 | 38 | 98 |
| 0·2 | 1 | 48 | 26 | 54 |
| | 2 | 68 | 32 | 82 |
| 0·4 | 1 | 70 | 22 | 38 |
| | 2 | 76 | 54 | 60 |

From the data in Table II and the curves in Fig. 1, it appears that dilution of Ringer-Locke solution inhibits the disruption of mast cells by

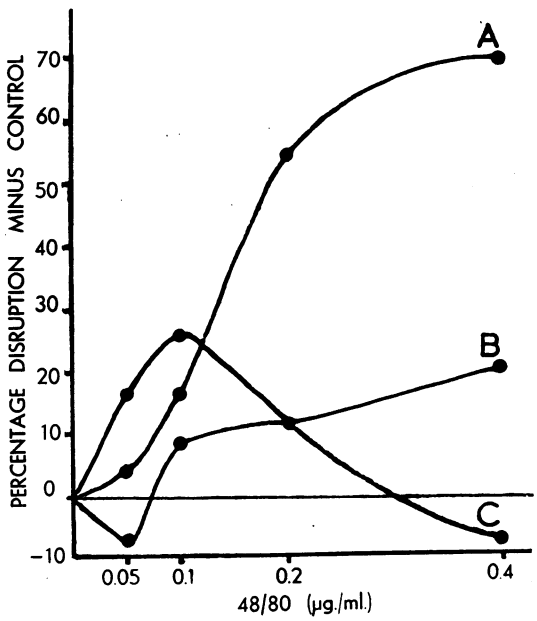


FIG. 1.—Effect of diluting Ringer-Locke solution on disruption of mast cells by 48/80. A=undiluted. B=3 parts Ringer-Locke, 1 part distilled $H_2O$. C=1 part Ringer-Locke, 1 part distilled $H_2O$.

48/80. The evidence, therefore, is in favour of the possibility that 48/80 may make the cell more permeable to extracellular ions and thereby cause disruption of the cell. The evidence is, of course, indirect and no explanation is advanced for the particular shape of the curves in hypotonic solutions. The shapes of these curves were, however, fairly similar in both experiments.

*Rate of Action of 48/80 on Mast Cells.*—If pieces of mesentery are allowed to remain in Ringer-Locke solution the number of cells spontaneously releasing granules increases with time. Table III gives the results of an experiment varying the time

TABLE III

PERCENTAGE DISRUPTION OF MAST CELLS AFTER VARIOUS LENGTHS OF EXPOSURE TO 48/80

| Concn. of 48/80 (μg./ml.) | Time of Exposure (Min.) | | | |
|---|---|---|---|---|
| | 1 | 15 | 30 | 60 |
| 0 | — | 0 | 2 | — |
| | — | — | 8 | 4 |
| 0·05 | — | 8 | 8 | — |
| 0·1 | — | 36 | 28 | — |
| 0·2 | 0 | 50 | 68 | — |
| | — | — | 54 | 54 |
| 0·4 | 2 | — | 66 | 82 |
| | 40 | 44 | 76 | 90 |
| 0·8 | 16 | — | 86 | 98 |

of exposure to 48/80. At a concentration of 0.2 μg./ml. disruption increased markedly during the first 15 min. of exposure to 48/80. After this there is little increase in the number of disrupted cells. At higher concentrations (0.4 and 0.8 μg./ml.), the rate of disruption is rapid up to 30 min. and decreases between 30 and 60 min. These changes in rate are shown in Fig. 2.

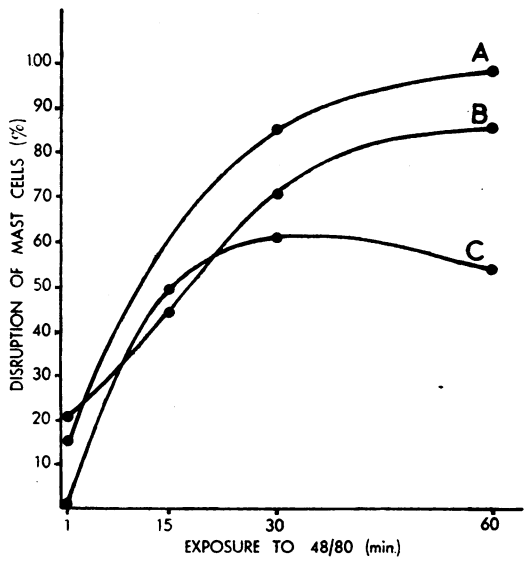


FIG. 2.—Rate of action of 48/80 on mast cells. A=0·8 μg. 48/80/ml. B=0·4 μg. 48/80/ml. C=0·2 μg. 48/80/ml.

A standard immersion period of 30 min. was, therefore, chosen for these experiments.

## DISCUSSION

Using the method described here for measuring the effect of compounds on mast cells, sigmoid dose-response curves are obtained when the concentration of 48/80 in Ringer-Locke is plotted against the percentage disruption (Fig. 1). An ED 50 can be calculated for such curves. With this method, it is possible to compare quantitatively the effectiveness of compounds which disrupt mast cells. This method also makes it possible to obtain quantitative evaluations of compounds which block this action.

The error of the ED 50's, in the experiments with the best distribution of data in Table I, is somewhat less than 20%. Presumably the error of the method could be decreased by increasing the number of points establishing the dose-response curves.

Although 30 min. was selected as the time allowed for the action of solutions of 48/80 it is possible that somewhat less error would have been obtained if the time had been increased to 60 min. This would appear to be closer to the time required for the maximum effect of 48/80 at concentrations above 0.2 μg./ml. The increased time needed with higher concentrations of 48/80 may be dependent on the time needed to achieve equilibrium, since higher concentrations would take longer to reach equilibrium under conditions such as exist with passage across a semi-permeable membrane.

The dissimilarity of the shapes of the curves in Fig. 1 may be postulated to reflect two processes acting to disrupt the mast cells. One process, previously discussed, would be that 48/80 makes the cells more permeable to extracellular ions. This process would be responsible for all of the disruption produced by concentration of 48/80 above 0.1 μg./ml. However, at concentrations of 0.1 and 0.5 μg./ml., another process might be operating to produce some disruption. This would account for the fragmentation observed in these low concentrations of 48/80 in diluted Ringer-Locke. Such a process could be either of the possibilities mentioned previously as alternatives to the effect of 48/80 on permeability to extracellular ions. Further studies are in progress, to obtain more data regarding these hypotheses.

## SUMMARY

1. A quantitative method for evaluation or disruption of mast cells by compound 48/80 is described. With this method, ED 50 values can be calculated by probit analysis and used to determine differences in various batches of 48/80.

2. Preliminary experiments to study the method by which 48/80 disrupts mast cells support the hypothesis that, in solutions of 48/80, the mast cells become more permeable to extracellular ions.

3. The time required for maximum effect of solutions of 48/80 on mast cells varies between 30 and 60 min., depending on the concentration of 48/80.

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