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The effects of hypotonicity on the degranulating action of Compound 48/80 on mast cells

SIR,—The range of compounds releasing histamine and producing degranulation of mast cells is so wide and chemically diverse that it has proved difficult to find a unified concept which will explain their mechanism of action. Uvnäs & Antonsson (1963) have suggested that the degranulation process may be initiated by different chemical reactions and that the main differences in action of the compounds lie in their activity in this initial "triggering process." The nature of this process and of the final common pathway for these reactions remains in doubt.

As a result of a study of the action of Compound 48/80 in hypotonic solutions on rat mesenteric mast cells, Norton (1954) has postulated that Compound 48/80 produces degranulation of mast cells by increasing the permeability of the outer cell membrane to extracellular ions and that the concentration of ions within the cell leads to osmotic rupture and the release of granules. Furthermore, Asboe-Hansen (1964) has suggested that one function of the mast cell mucopolysaccharides is to absorb an excess of tissue water and that degranulation, with the release of these mucopolysaccharides, occurs in response to such an excess. Against the view that degranulation is produced by osmotic rupture of the cell, it has been shown by cinephotomicrography and electron microscopy (Horsfield, 1965a,b) that degranulation is an active process and does not involve dissolution of the external cell membrane. In addition, the degranulation produced by the application of chemical reagents takes up to 20 min before completion whereas osmotic rupture following the application of distilled water is complete within a few seconds. In view of these various findings the effects of hypotonic solutions on the degranulating action of Compound 48/80 have been re-examined.

In these *in vitro* experiments biopsies of rat mesentery were taken using a metal spring clip with opposing loops at one end. The biopsies, still in the clips, were placed in test solutions for 20 min at room temperature. The specimens were then fixed in methanol for 30 min and the discs of mesentery put on slides and a few drops of 0·1% toluidine blue in 50% methanol applied. The preparations were ringed with petroleum jelly and cover slips applied. 300 mast cells were counted in each preparation and the % degranulated cells recorded. Experiments were made in duplicate and average values plotted.

In the control series the biopsies were placed in various concentrations of saline and in the test series 0·1 μg/ml of Compound 48/80 was added to each solution. Since Högberg & Uvnäs (1960) have shown that calcium ions are necessary for degranulation with Compound 48/80, 1 mmol/100 ml of calcium chloride was added to the saline.

The results for the control series of experiments are shown in Fig. 1A. It is evident that significant degranulation does not occur until the tonicity of the test solution falls below one half that of an isotonic solution. Therefore above this level no correction need be applied in this system for degranulation due to the hypotonicity of the test solution. The results of the test series of experiments are shown in Fig. 1B. It can be seen that when Compound 48/80 is present

the % of mast cells degranulated steadily increases as the test solution becomes more hypotonic over the range 1·0–0·5 × isotonic.

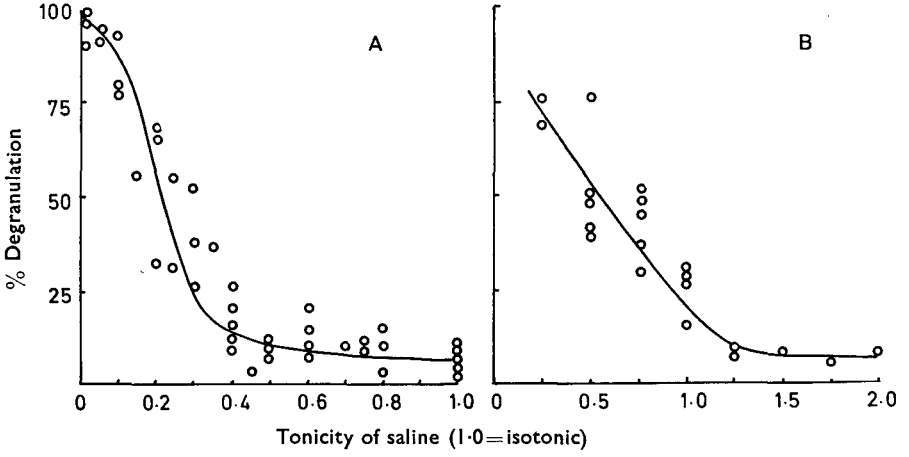

FIG. 1 The effect on rat mesenteric mast cells of hypotonic solutions of saline (A) and Compound 48/80 (B), 0·1 μg/ml, in the presence of varying tonicities of saline. In A degranulation is not increased until the tonicity reaches 0·4. Over the range 0·5—1·0 there is no significant difference between the amount of degranulation produced at each point. In B over the range 0·5—1·0 the amount of degranulation increases as the tonicity of test solution falls.

It can be concluded from these experiments that for degranulation to occur solely because of an excess of tissue water the tonicity of the extra-cellular fluid must fall to about half of normal (which seems unlikely under physiological conditions) and that lowering the tonicity, and thus the extracellular ion concentration, increases the degranulating action of Compound 48/80. It seems unlikely therefore that Compound 48/80 produces its effect by concentrating extracellular ions within the cell and thus producing osmotic rupture.

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