Protamine-like Property of Compounds 48/80 and Stilbamidine and Their Action on Mast Cells.* (20384)

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Compound 48/80, obtained from the condensation of p-methoxyphenethyl-methylamine with formaldehyde(1), is the most active histamine liberator so far described(2). Stilbamidine, a trypanocidal diamine(3) was also observed to be a histamine liberator(4). The present paper deals with the effect of these compounds on tissue mast cells and their action on the anti-coagulant property of heparin and allied substances.

Material and methods. The effect of these compounds on the mast cells of male Wistar rats (weighing 200-300 g) was studied both *in vivo* and *in vitro*. In the experiments *in vivo* the animals under light ether anesthesia were injected intravenously with 5 mg per kg of each of the studied compounds, diluted in 1 ml saline (0.9% NaCl). Both compounds produced a severe histamine shock with death of the animal in 5 to 8 minutes. Ten rats injected with one ml of saline and sacrificed with ether were used as controls for similar groups injected with Compound 48/80 or Stilbamidine. To avoid artifacts due to common histologic technic, mesentery mast cells were fixed by aortic perfusion. Ten minutes later the abdominal cavity was opened and the mesentery fixed for 12 hours more with Bouin's fluid or Holmgren and Wilander's lead acetate(5) modified by addition of 10% formaldehyde. The same results were obtained with either fixative. Mast cells were observed after staining with 1% toluidine blue or unstained with phase contrast microscopy. In all rats injected with Compounds 48/80 and Stilbamidine, coagulation time was determined by the Lee-White method(6). The experiments *in vitro* were performed by immersing fragments of mesentery into saline containing different concentrations (from 1/1000 to 1/20000) of either Compound 48/80 or Stilbamidine, for 5 minutes at room temperature (25°C). Controls were immersed in saline for the same period. These fragments were then fixed and stained by transferring them to a 10% formaldehyde solution containing 1% toluidine blue. To observe the protamine-like action of Compound 48/80 and Stilbamidine, different quantities of these substances were dissolved in saline and mixed with whole blood containing heparin (Vitrum), Liquoid (Roche) or cellulose sulphate and the coagulation time was determined.

Results. In the experiments *in vivo,* the injection of Compound 48/80 or Stilbamidine always produced bursting of mesentery mast cells with extrusion of granules (Fig. 1). This was more evident and always present in perivascularly located mast cells. No change could be observed in the coagulation time of the injected animals.

Both Compound 48/80 and Stilbamidine promoted a generalized disruption and granule liberation of mesentery mast cells *in vitro,* with no preference for perivascular cells. Heparin when previously added to solutions of both compounds inhibited the disruption of mast cells. Due mainly to traction of the mesentery during dissection, bursting of mast cells could be occasionally observed in the controls, but this was easily recognized as an artifact. The bursting effect with Compound 48/80 could be observed under the phase contrast microscope as a rapid bubbling of the cell surface during a few seconds.

Tables I and II show the results obtained in a typical experiment studying the anti-heparin effect of Compound 48/80 and Stilbamidine. Inspection of these tables demonstrates an anti-heparin effect of these compounds, and with higher concentration an increase in the coagulation time as well.

Discussion. The alteration of mast cells in peptone and anaphylactic shock has been related to heparin liberation(7,8). Although

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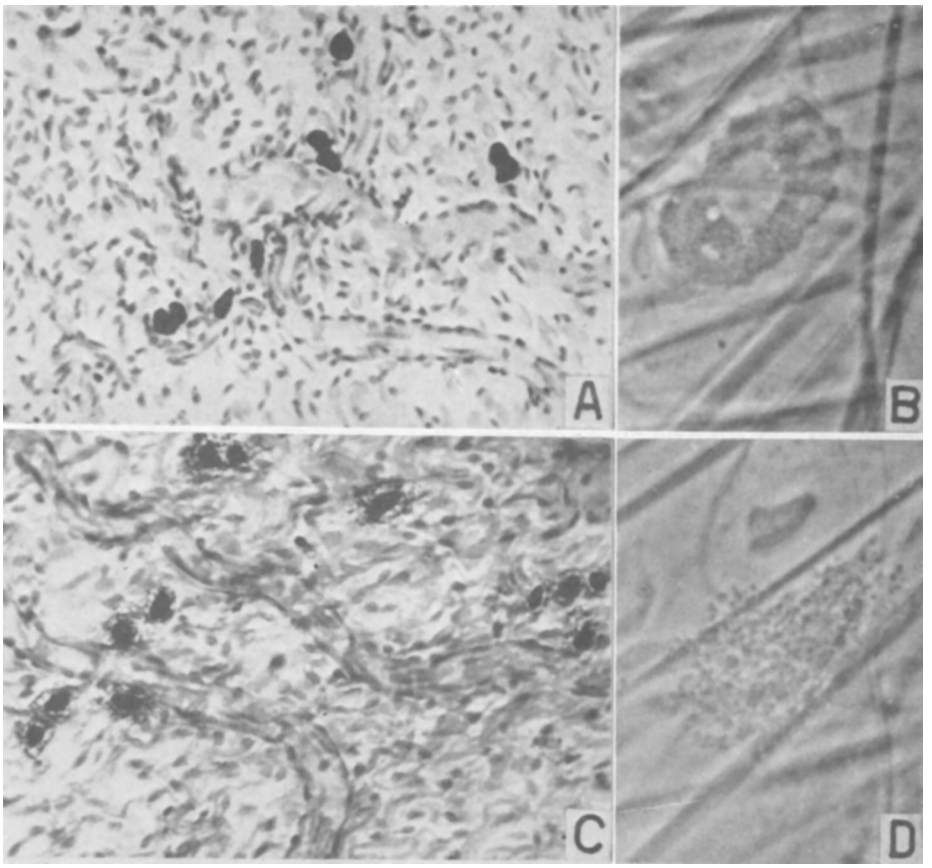

FIG. 1. Mast cells of rat mesentery. A and C toluidine blue stained (×400). B and D phase contrast (×1300). A and B from control animal. C and D compound 48/80 injected animal.

these results were confirmed in the dog, in the rat the disruption of mast cells evoked by either anaphylactic, peptone shock(9), Compound 48/80 or Stilbamidine, does not seem to be accompanied by heparin liberation if judged by blood coagulation time. In the case of the last 2 substances this phenomenon might be related to their anti-heparin effect. However the fact that in peptone and anaphylactic shock no substance with anti-heparin effect exists, might suggest that heparin in the rat mast cell is bound in a more stable linkage than in the dog mast cell. It is interesting to point out here the difficulty met with in the histologic fixation of the dog mast cell as compared to the rat mast cell.

The striking positive correlation observed between the mast cell content of a tissue and its concentration of histamine(10) suggests that mast cells contain histamine. Riley(11), also observed that Stilbamidine disrupts mast cells *in vivo*. The fact that mast cell disruption occurs in conditions in which histamine liberation is observed, as in anaphylactic and peptone shock(7-9), coupled with our observations that Compound 48/80 and Stilbamidine cause the break-up of mast cells *in vivo* and *in vitro*, lend support to the hypothesis that histamine arises from mast cells. If this is true, mast cells probably would be one of, but not the only source of tissue histamine, since it is known that histamine may be obtained *in vitro* from tissue free of mast cells such as blood(12). The way histamine may be bound to the mast cell's cytoplasm and its relation to heparin, constitutes a challenging problem.

The observations that Compound 48/80 or Stilbamidine increase coagulation time and the fact that these compounds counteract the anti-

TABLE I. Action of Compound 48/80 on Anti-Coagulant Property of Heparin, Liquoid and Cellulose Sulphate.

C. 48/80, μg/ml final vol	Coagulation time of blood: + heparin + C. 48/80	+ Liquoid + C. 48/80	+ cellulose sulphate + C. 48/80
0	>20 hr	>20 hr	>20 hr
20	7'00"	4'30"	>20 hr
40	3'45"	5'00"	>20 hr
60	5'30"	5'10"	20'10"
80	5'50"	7'10"	8'20"
100	18'10"	12'30"	7'50"
200	27'20"	18'20"	8'10"
500	>20 hr	40'15"	>20 hr

Blood plus heparin 5 U/ml. Blood plus Liquoid or cellulose sulphate 100 μg/ml. 0.5 ml of rat's blood was added to each test tube. The final vol (1 ml) was completed with saline.

TABLE II. Action of Stilbamidine on Anti-Coagulant Property of Heparin, Liquoid and Cellulose Sulphate.

Stilbamidine, μg/ml of final vol	Coagulation time of blood: +heparin +Stilbamidine	+Liquoid +Stilbamidine	+cellulose sulphate +Stilbamidine
0	>20 hr	>20 hr	>20 hr
20	9'50"	40'00"	>20 hr
40	9'50"	10'00"	20'10"
60	13'30"	8'10"	14'30"
80	20'40"	15'00"	15'35"
100	30'50"	40'20"	30'10"
200	>20 hr	>20 hr	>20 hr
500	>20 hr	>20 hr	>20 hr

Blood plus heparin 5 U/ml. Blood plus Liquoid or cellulose sulphate 100 μg/ml. 0.5 ml of rat's blood was added to each test tube. The final vol (1 ml) was completed with saline.

coagulant action of heparin and allied substances, strongly suggest a protamine-like action of these compounds. The addition of Compound 48/80 or Stilbamidine to a solution of heparin, Liquoid or cellulose sulphate, causes a precipitate(9) and one might suppose that these substances act like protamines with the formation of insoluble complexes which remove heparin from the system.

Summary. Compound 48/80 and Stilbamidine produce both *in vivo* and *in vitro* bursting of rat's mast cells. This phenomenon was observed with phase contrast microscopy as a rapid bubbling of the cell surface. These compounds also possess a protamine-like effect. The possible significance of these results is discussed.

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