Degranulating Activity in Hybrid Cells Derived from Rat Peritoneal Mast Cells and Ehrlich Ascites Tumor Cells

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Summary. Fusion of rat mast cells and Ehrlich ascites tumor cells was mediated by HVJ. Compound 48/80-induced degranulation occurred in the fused cells formed from two mast cells and one tumor cell, but not in the fused cells from one mast cell and two or more tumor cells.

Numerous biochemical and pharmacological studies have been made on the mechanism of histamine release from mast cells, but the details have not been fully elucidated. This is especially true regarding its correlation with the function of the cell membrane, there being hardly any work other than morphological studies[2,3]. The present studies were undertaken in order to see the effect of changes in the composition of cell membrane, and in the conformation of the membrane on the histamine-releasing ability of mast cells. For this purpose, the techniques for somatic cell hybridization[4,5], which are supplying valuable information for biological research, including genetics, was used.

Material and methods. Mast cells were isolated from the rat peritoneal fluid by the gum arabic density gradient centrifugation[6] and washed twice with phosphate-buffered saline (PBS) (154 m*M* NaCl, 2.7 m*M* KCl, 0.9 m*M* $CaCl_2$ and 6.7 m*M* KH_2PO_4–Na_2HPO_4, pH 7.2). Ehrlich ascites tumor cells (ETC) were harvested from the abdomen of *ddD* mice. The hemagglutinating virus of Japan (HVJ)-z strain was propagated in embryonated eggs[7]. Hybrid cells of the mast cell and ETC were obtained as follows; ca. 5×10^5 each of mast cells and ETC suspended in 0.9 ml of PBS in a centrifuge tube was mixed 0.1 ml of 10,000 HAU of HVJ. After incubation at 2 °C for 5 min, the mixture was centrifuged at 40 *g* for 2 min and 0.5 ml of the supernatant was removed with a pipette and discarded, and the residual mixture was incubated at 37 °C for 20 min with shaking in a water-bath. In the case of fused cells consisting of only mast cells, ETC was not included.

Results and discussion. Figure 1 shows the fusion of mast cells. Most of them were fusions of 2–3 cells, and those of more than 5 cells were rare. Addition of the histamine releaser, compound 48/80 (1 μg/ml) to these fused cells resulted in the extrusion of granules from fused cells, as in the case of unfused cells (Figure 1b). Figure 2 shows the fused cell (ME cell) of mast cell and ETC, in which characteristic granules of mast cells were seen under the phase-contrast microscope. When these ME cells were stained with Toluidine Blue, metachromasia of mast cell granules was observed in one side of fused cells, but such metachromasia was not observed in ETC.

When compound 48/80 was added to ME cells, degranulation occurred in the fused cells formed from 2 mast cells and 1 ETC (Figure 3a) but not in the fused cell formed from 1 mast cell and 1 ETC, or even if it did occur, degranulation was not so marked. Degranulation was

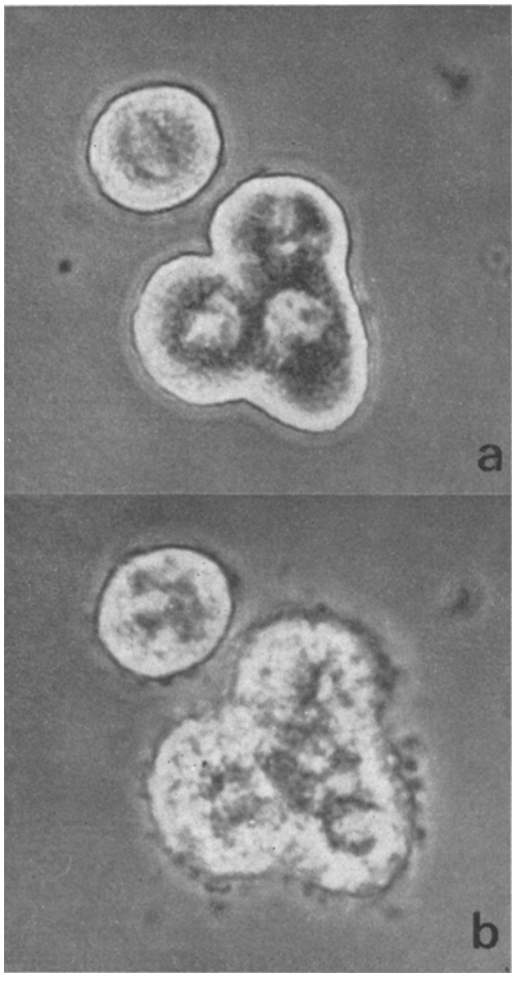

Fig. 1. Degranulation from fused mast cells. ×1140. a) Fused mast cells after 20 min of incubation at 37 °C. b) Degranulation by compound 48/80 (1 μg/ml).

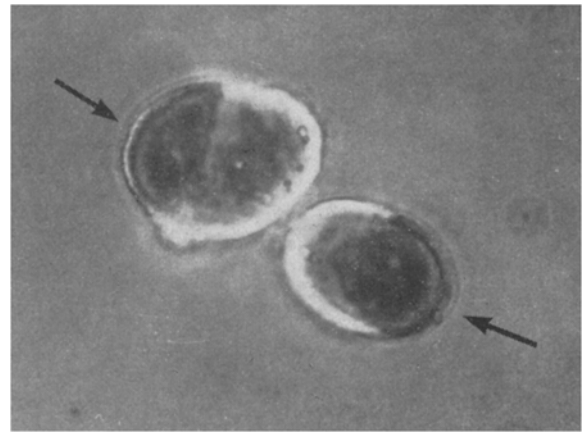

Fig. 2. Fusion of a mast cell and ETC. ×960. Toluidin Blue was added after fusion. Metachromasia (arrow) appears in one area of the fused cell.

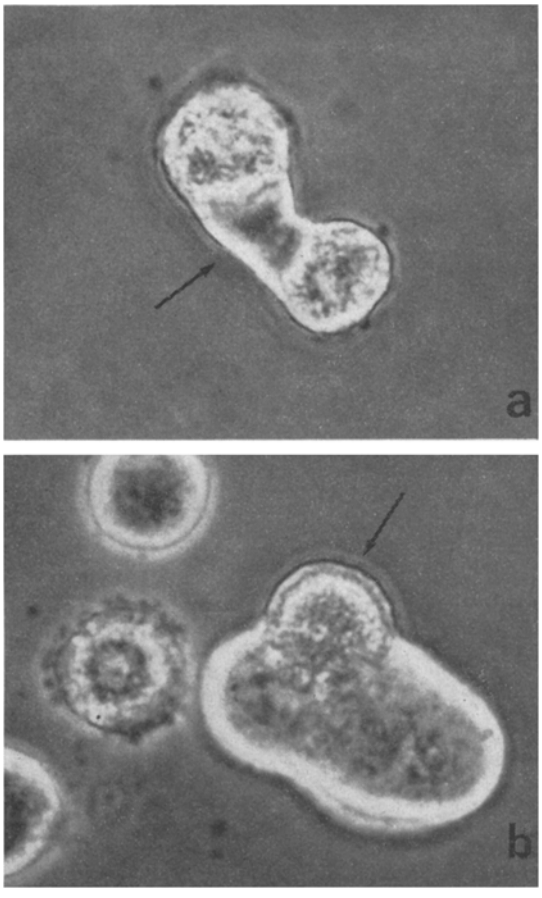

Fig. 3. Reactivity of ME cell to compound 48/80. a) Degranulation from a hybrid cell derived from 2 mast cells and 1 ETC (arrow). b) No degranulation from a hybrid cell formed from 1 mast cell (arrow) and 3 ETC. Degranulation from an adjacent mast cell was observed.

entirely absent in fused cells formed from 1 mast cell and 2 or more ETC. Disappearance of this degranulating activity was seen 10 min after incubation at 37 °C when fusion was still not completed.

These observations indicate that the reactivity of the mast cells to a histamine releaser changed according to the change in the membrane composition of mast cell. In the fusion of cells by virus, it has been reported that the membrane lipids of fusing cells undergo intermixing within 5 min[8], so it is considered that a membrane lipid bilayer of a new composition is formed in the ME cells. We have found the compound 48/80 binds specifically with acidic phospholipids[9], such as phosphatidylserine, phosphatidylinositol and phosphatidic acid; it was presumed that the binding site of this histamine releaser on the mast cell would be the acidic phospholipids in its membrane. It seems possible that the specific composition of the membrane lipids, including acidic phospholipids, in the mast cells is forming a receptor for compound 48/80. Based on this view, disappearance of the degranulating activity by the fusion of mast cells with ETC is probably due to the dissociation of the receptor of compound 48/80 by changes in the composition and distribution of membrane lipids in mast cells, although it is considered that loss of degranulating activity may also be attributed to mixing of cytoplasmic factors.

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[2] B. Uvnäs, Fedn Proc. *33*, 2172 (1974).
[3] D. Lugunoff, J. Cell Biol. *57*, 252 (1973).
[4] H. Harris, *Cell Fusion* (Clarendon Press, Oxford 1970).
[5] B. Ephrussi, *Hybridization of Somatic Cells* (Princeton Univ. Press, Princeton 1972).
[6] K. Sugiyama, Jap. J. Pharmac. *21*, 209 (1971).
[7] Y. Okada, Expl Cell Res. *26*, 98 (1962).
[8] T. Maeda, A. Asano, K. Ohki, Y. Okada and S. Ohnishi, Biochemistry *14*, 3736 (1975).
[9] K. Sugiyama and H. Yamasaki, Jap. J. Pharmac. suppl. *26*, 125 (1976).

The Circadian Rhythm of Oviposition in *Drosophila melanogaster:* A Genetic Latitudinal Cline in Wild Populations

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Summary. Under laboratory conditions with a photoperiod of 12 h at a light intensity of about 1100 lux, *Drosophila melanogaster* strains of different latitudinal origin showed significant differences in oviposition rhythm. These genetic differences follow a cline and may have an adaptative value.

Behavioural genetic variations are probably of great importance in evolutionary processes[2,3]. Such changes may be significant for the initiation of sexual isolation and also for the diversification into ecological niches.

In *Drosophila,* oviposition rhythm is a convenient trait for characterizing one behavioural aspect of species or populations[4]. Oviposition recordings can be easily made on an hourly basis and give highly reproducible results: the average curve, typical of a given strain, is reproduced almost exactly in successive and independent experiments[5].

Previous works[6,7] have demonstrated that *D. melanogaster* wild populations exhibit genetic latitudinal clines for certain biometrical traits (adult fresh weight and female ovariole number) and for a physiological trait, alcohol tolerance. Since behavioural differences are probably more directly linked to fitness, we decided to

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[2] Th. Dobzhansky, *Genetic of the Evolutionary Process* (Columbia University Press, New York 1970).
[3] P. A. Parsons, *Behavioral and Ecological Genetics* (Clarendon Press, Oxford 1973).
[4] R. Allemand, C. r. Acad. Sci., Paris *282*, 85 (1976).
[5] R. Allemand, Thèse de spécialité, Univ. Claude Bernard, Lyon (1975).
[6] J. David and C. Bocquet, Nature, Lond. *257*, 588 (1975).
[7] J. David and C. Bocquet, Experientia *31*, 164 (1975).