# INDUCTION OF MEMBRANE FUSION BY POLYSIALOGANGLIOSIDES

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## 1. Introduction

Recently reported studies on the behaviour of gangliosides and neutral glycolipids at the air–water interface revealed that the complexity of the polar head group, and specially the presence of one or more sialic acids, greatly influences their surface properties [1].

Di- and tri-sialogangliosides, but not neutral glycolipids or monosialogangliosides, showed interactions in mixed monolayers with phosphatidylcholine (in preparation) similar to those shown by lipids that are able to induce fusion between biological membranes [2,3]. In the present work it is shown that di- and tri-sialogangliosides induce membrane fusion in chicken erythrocytes.

## 2. Materials and methods

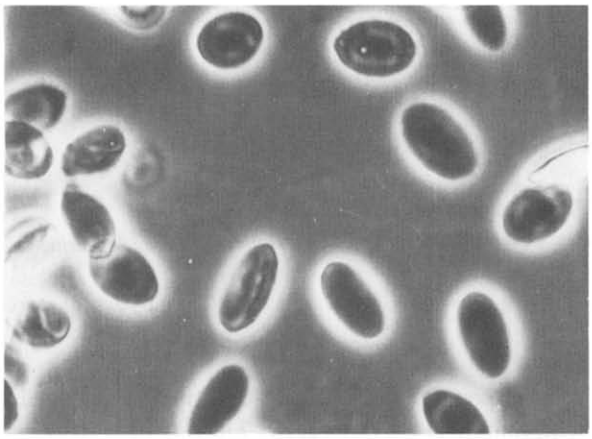
The glycosphingolipids were obtained and purified as in [1]. Glyceryl mono-oleate (99% pure) was from Sigma Chem. Co. (London SW6). Induction of membrane fusion was carried out in a system similar to that in [4]. Briefly, washed chicken erythrocytes (approx. $10^8$ cells/ml) were incubated at 37°C in a basal medium consisting of a modified Eagle's salt solution containing Dextran 60 C (80 mg/ml) buffered with sodium cacodylate, at pH 7.4. Any lipid to be tested was ultrasonically dispersed in the Eagle's medium and added to the medium containing the erythrocytes at final conc. 100 μg/ml. At different periods time samples of the incubated cells were examined by phase-contrast microscopy.

*Abbreviations:* Cer, Ceramide (*N*-acyl-sphingoid); GlcCer, Glcβ1→1 Cer; $Gg_4Cer$, Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer; $GM_3$, NeuGcα2→3Galβ1→4Glcβ1→1Cer; $GD_3$, NeuAcα2→8NeuAcα2→3Galβ1→4Glcβ1→1Cer; $GM_1$, Galβ1→3GalNAcβ1→4Gal(3←2αNeuAc)β1→4Glcβ1→1Cer; $GD_{1a}$, NeuAcα2→3Galβ1→3GalNAcβ1→4Gal(3←2αNeuAc) β1→4Glcβ1→1Cer; $GT_1$, NeuAcα2→3Galβ1→3GalNAcβ1→4Gal(3←2αNeuAc8←2αNeuAc)β1→4Glcβ1→1Cer; abbreviations according to [8] for neutral sphingolipids and [9] for gangliosides

## 3. Results

Erythrocytes incubated in the basal medium remained oval and mononucleated for more than 4 h (fig.1a). When the fusogenic lipid glyceryl mono-oleate [4] was added to the basal medium the cells became rounded within 10 min (fig.1b) and after 20–30 min bi- and multinucleated cells were seen, indicating extensive membrane fusion (fig.1c). Cells incubated for up to 4 h in the basal medium containing Cer, GlcCer, $Gg_4Cer$, $GM_1$ or $GM_3$ did not show changes with respect to the cells incubated in absence of added lipids.

Addition of the polysialogangliosides $GD_3$, $GD_{1a}$ or $GT_1$ to the incubation medium brought about changes in the morphology of the cells. Between 20–60 min incubation the cells were crenated (fig.1d). This phenomenon was more noticeable upon addition of $GD_3$ than when $GD_{1a}$ or $GT_1$ were added. In the erythrocytes incubated with $GD_3$ the crenation gradually disappeared after 1.5 h and rounded and oddly-shaped cells with vesicles that where pinched off by fusion of the cell membrane appeared (fig.1e). With $GD_{1a}$ and $GT_1$ similar changes to those described for $GD_3$ were observed except that no pinching off of the membranes were seen. Bi- and multinucleated cells appeared after 2–3.5 h incubation



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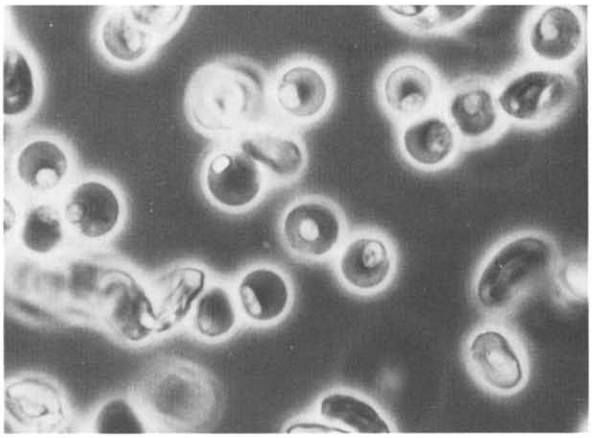

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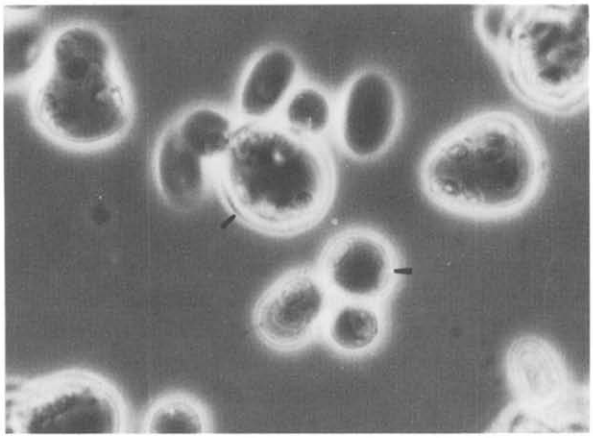

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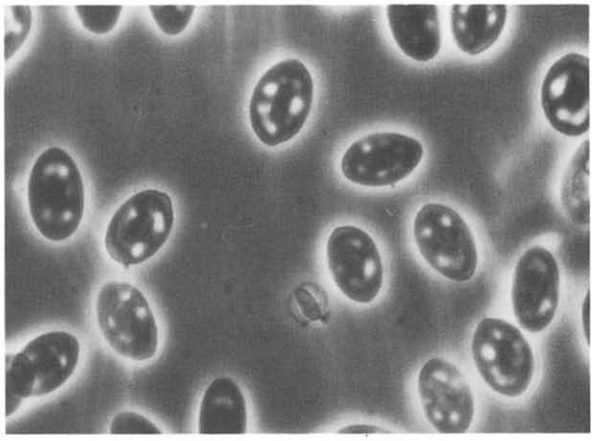

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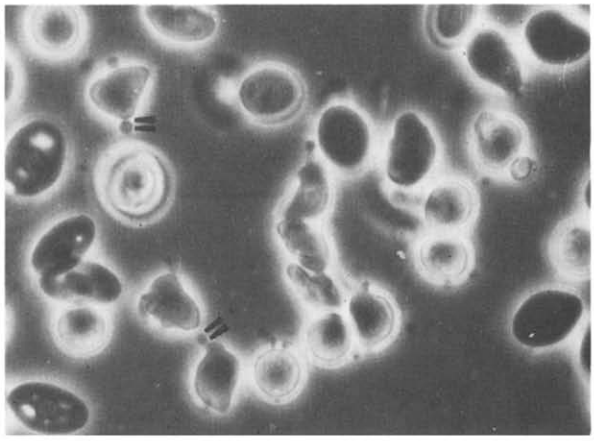

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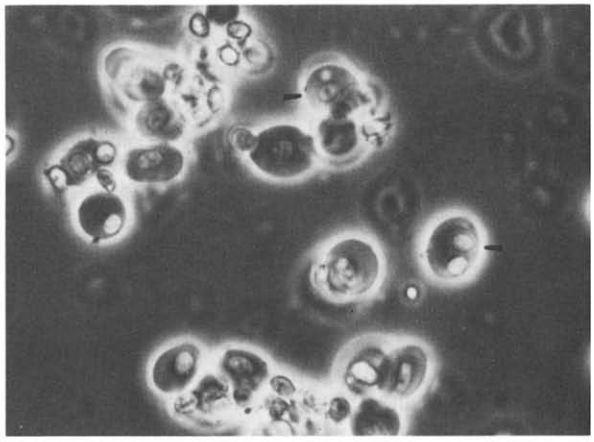

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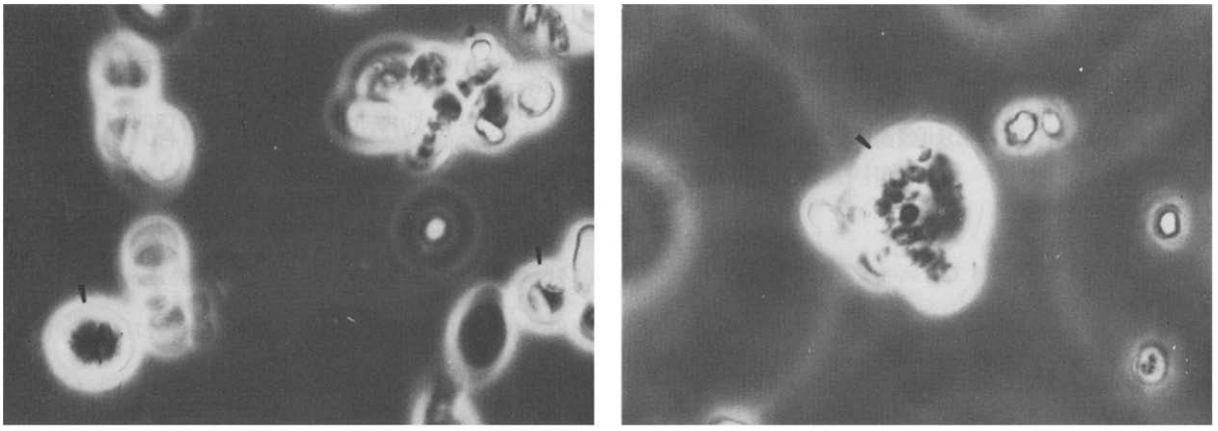

g h

Fig.1. Morphological changes during ganglioside-induced fusion. The erythrocytes were incubated in the following conditions: (a) in absence of added lipids. In presence of: (b) glyceryl mono-oleate, 10 min incubation; (c) glyceryl mono-oleate, 20 min incubation; (d) $GD_3$, 60 min incubation; (e) $GD_3$, 1.67 h incubation; (f) $GD_3$, 3.33 h incubation; (g) $GD_{1a}$, 3.33 h incubation; (h) $GT_1$, 2 h incubation. Phase-contrast microscopy: × 576. As with most fusogenic lipids [4] gangliosides capable of inducing membrane fusion also increased cell lysis. Single arrows show bi- or multinucleated cells, double arrows indicate membrane pinching off.

with $GD_3$ (fig.1f) or $GD_{1a}$ (fig.1g) or $GT_1$ (fig.1h). In the modified Eagle's medium used for incubation the concentration of $Ca^{2+}$ was 1.8 mM. In a case in which 3.3 mM $Na_2EDTA$ was present in the medium, the addition of glyceryl mono-oleate or $GD_3$ induced the appearance of rounded cells or of crenated or oddly-shaped cells, respectively, but neither membrane pinching off nor multinucleated cells were found. These observations indicate that as with other fusogenic agents [4–6] free $Ca^{2+}$ is required for the ganglioside-induced fusion process.

## 4. Discussion

Lipids having a liquid-expanded type behaviour in monolayers and that exhibit characteristic changes of the molecular packing and decrease of the surface potential in mixed monolayers with phosphatidyl-choline are also capable of inducing membrane fusion. Conversely, lipids chemically similar to those but not showing these type of interactions are ineffective as fusogenics [2,3]. On this basis it was surmised that a reduction of the electrostatic field perpendicular to the surface of the lipid bilayer induced by a lipid or a water-soluble substance is a key event for the initial increase of the membrane permeability and aggregation that leads to membrane fusion [6,7].

Unpublished results from this laboratory show that the polysialogangliosides $GD_3$, $GD_{1a}$ and $GT_1$ that induced fusion of the erythrocyte membrane behaved as other fusogenic lipids in mixed monolayers with phospholipids whereas the non-fusogenic neutral glycosphingolipids and the monosialogangliosides $GM_1$ and $GM_3$ did not. This suggests that similar molecular mechanisms to those involved in the action of other fusogenic agents are operating in the poly-sialoganglioside-induced fusion.

The high concentration of gangliosides in nerve endings where the plasma membrane fuses with that of the synaptic vesicles previous to the release of neurotransmitter emphasizes the interest of extending the studies of the fusogenic capability of gangliosides to membranes of the nervous system.

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## References

[1] Maggio, B., Cumar, F. A. and Caputto, R. (1978) Biochem. J. 171, 559–565.
[2] Maggio, B. and Lucy, J. A. (1975) Biochem. J. 149, 597–608.
[3] Maggio, B. and Lucy, J. A. (1976) Biochem. J. 155, 353–364.
[4] Ahkong, Q. F., Fisher, D., Tampion, W. and Lucy, J. A. (1973) Biochem. J. 136, 147–155.
[5] Ahkong, Q. F., Tampion, W. and Lucy, J. A. (1975) Nature 256, 208–209.
[6] Maggio, B., Ahkong, Q. F. and Lucy, J. A. (1976) Biochem. J. 158, 647–650.
[7] Maggio, B. and Lucy, J. A. (1977) Adv. Exp. Med. Biol. 83, 225–231.
[8] IUPAC–IUB Nomenclature of lipids. (1977) Lipids 12, 455–468.
[9] Svennerholm, L. (1963) J. Neurochem. 10, 613–623.