Transfer of Glycolipid between Membranes of Tissue Culture Cells, Using Dansylcerebroside as a Model

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Donor cells, which had incorporated dansylcerebroside in their membranes, could further transfer this glycolipid to monolayers of acceptor cells. The ease of transfer varied among acceptor cells, BHK cells being the best and MDBK cells the poorest acceptors of the cells tested.

The process of transfer seemed to be mediated by lipids rather than by proteins of the membranes. The mode of attachment between donors and acceptors, such as classified as loose contact, tight adhesion or binding by lectin, did not significantly influence the extent of glycolipid transfer. However, modification of plasma membranes by infection of acceptor cells with myxoviruses resulted in enhancement of glycolipid transfer in some cases.

Various factors have been evaluated with respect to dynamics of cellular membranes.

Introduction

We have recently reported [1] that cerebroside, a normally occurring glycolipid, can be derivatized to form a dansylated compound which fluoresces intensely. It was observed that this dansylcerebroside could be incorporated into membranes of tissue culture cells and erythrocytes. After an equilibration period of several hours dansylcerebroside was present in membranes of cells as patches and distribution of dansylcerebroside in the membranes was seen to be altered by infection with myxoviruses. Since glycolipids are antigens [2] and receptors of macromolecules [3] such as tetanus toxin and cholera toxin, it is of great interest to see whether the incorporated glycolipid can be further transferred into other membranes. A proof of such transfer would indicate direct exchange of biologically active constituents between closely apposing membranes. Dansylcerebroside is a convenient tool for transfer studies, since it is rapidly incorporated into membranes and its intense fluorescence permits a quick evaluation of the events occurring. The present report is an account of such experiments.

Material and Methods

Dansylcerebroside was prepared as described previously [1].

Cell cultures: BHK, L, HeLa, Vero, chick fibroblast, rabbit kidney and MDBK cells were grown in plastic Petri dishes in reinforced Eagle's medium or Dulbecco's medium with 10% calf serum accordding to Choppin [4].

Donor cells: Monolayers of chick fibroblasts were labeled with dansylcerebroside as described before [1]. In general, the labeling period was 5 to 6 hours. After the monolayers were washed several times with isotonic saline, they were dissociated with a calcium free phosphate buffer (PBS) containing 0.02% of EDTA and 0.2% of trypsin. Dissociated cells were then washed by centrifugation and resuspension in PBS. To stabilize cells the suspensions were treated with glutaraldehyde (0.1%) for 30 min, after which period cells were washed three times with PBS in the same way. Donor cells were finally suspended in PBS in a concentration of approximately 10^8 cells per milliliter.

Acceptor cells: Monolayers of cells listed above were used as acceptors. Monolayers were infected with myxoviruses or treated with Concanavalin A or fixed with glutaraldehyde or used as such. Adsorption of Concanavalin A on monolayers was achieved by incubation of monolayers with 5 ml of PBS containing 200 μg of Concanavalin A for 30 min. Glutaraldehyde fixation was accomplished in a PBS solution containing 0.1% of glutaraldehyde in 30 min. Infection of monolayers with fowl plague virus, strain Rostock or Newcastle disease virus, strain Italien was performed in the usual manner.

Incubation of donor cells and acceptor cells for transfer studies

Monolayers of cells, which were untreated or modified in the various ways as described above, were overlayed with 4 ml of serum free Eagle's

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medium. 0.1 ml of the above described suspension of donor cells containing approximately 10^7 cells were added to each plate and the mixture was incubated at 37 °C for 6 hours or 12 hours. After the incubation period, the media were decanted and monolayers were washed five times with PBS and observed under an UV-microscope (Zeiss).

Results and Discussion

Transfer of lipids between biological membranes may be considered as a consequence of the dynamic state of the system. Of the membrane lipids, phospholipids have been reported to rapidly exchange between membrane structures and a series of transfer proteins have been shown to mediate the exchange process [5]. In tissue culture systems, Peterson and Rubin [6] also reported a rapid exchange of phospholipids among cells in contact. Although glycolipids are important components of membranes, their transferability has not been studied. In contrast to phospholipids which are ubiquitous structural components, glycolipids are present specifically in plasma membranes [7] and are biologically active as antigens [2] and receptors [8]. It is therefore important to see whether glycolipids can also be transferred between membranes.

Glycolipid can be transferred between membranes

The experiments necessitated the use of morphologically distinct membranes which could donate and accept the fluorescent glycolipid. This requirement was met by employing the dansylcerebroside labeled, EDTA-trypsin dissociated chick fibroblasts as donors and monolayers of various tissue culture cells as acceptors. Donor cells were fixed with glutaraldehyde in order to preserve their round morphology for ready recognition (Fig. 1 a). Monolayers of BHK, L, HeLa, chick fibroblasts, rabbit kidney, Vero and MDBK cells were used as acceptors. Transfer of the glycolipid from donor to acceptor cells was evaluated under a UV-microscope, and spreading of fluorescence in the monolayers was interpreted as evidence of glycolipid transfer.

In general, although transfer of glycolipid from the donor to the acceptors became visible after one hour of incubation, the evaluation was made after 6 hours and 12 hours of incubation. It was observed that of the cells tested BHK cells accepted dansylcerebroside most readily, whereas the lipid was transferred to MDBK cells with difficulty. After the indicated incubation periods BHK cells which surrounded the donor cells fluoresced intensely (Fig. 1 b, c, d), whereas only limited transfer of the lipid occurred with MDBK cells (Fig. 1 e). Other cells tested showed intermediate acceptor properties. The transferred glycolipid was present in the acceptor membranes not homogeneously but as patches (Fig. 1 b, c, d, and Fig. 2 a) similar to those observed in the case of direct incorporation of glycolipid from the medium [1].

The extent of glycolipid transfer is not altered by changing the mode of attachment between cells but may be enhanced by modifying the membrane structure with viruses

Since the incorporated dansylcerebroside can no longer be dissolved into the aqueous medium [1], the transfer of it between membranes must depend on contact between acceptors and donors. It was thought therefore that the extent of lipid transfer might be influenced by altering the mode of attachment between cells or by modifying the membrane structure.

The mode of contact or attachment between cells can be altered by binding agents such as Concanavalin A [8] and modification of the plasma membranes can be brought about by infection of acceptor cells with myxoviruses which bud at the plasma membranes. It was found that, even in the absence of Concanavalin A or viruses, donor cells could eventually adhere tightly to acceptor cells after 5 – 6 hours of incubation. In contrast, addition of Concanavalin A or infection of cells with myxoviruses resulted in immediate tight binding between acceptor cells and donor cells. In all cases, although the mechanism of binding was different, attachment of donor cells to acceptor cells was so tight that they could not be separated by rinsing the monolayers with PBS.

It was observed that with BHK cells which very readily accepted dansylcerebroside from the donor cell (Fig. 1 b), the use of Concanavalin A (Fig. 1 c) or infection with myxoviruses (Fig. 1 d) did not visibly result in further enhancement of glycolipid transfer. With MDBK cells, to which transfer of glycolipid occurred with difficulty, modification of the monolayers by infection with fowl plague virus or Newcastle disease virus caused noticeable

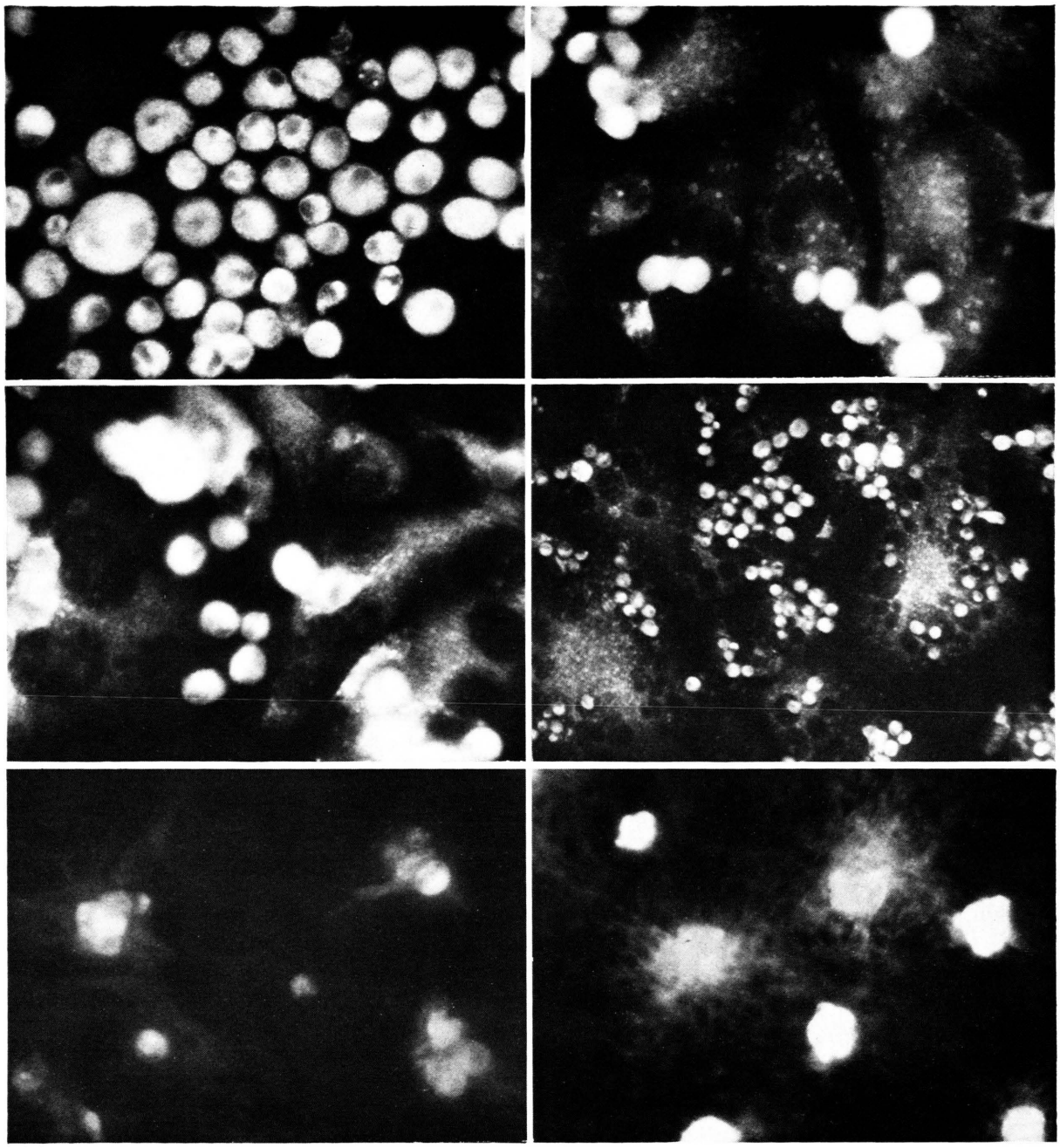

Fig. 1. Transfer of dansylcerebroside from donors to monolayers of acceptor cells. a: donor chick fibroblasts, × 2400. b: transfer from donors (round) to monolayers of untreated BHK cells, × 2400. c: transfer from donors (round) to Concanavalin A treated BHK cells, × 2400. d: transfer from donors to monolayer of BHK cells, which had been infected with Newcastle disease virus, showing in addition fusion of the monolayer, × 1200. e: transfer from donors (round, aggregated) to monolayer of MDBK cells, × 2400. f: transfer from donors (round, aggregated) to monolayers of MDBK cells which had been infected with Newcastle disease virus, showing enhanced transfer of the glycolipid by infection with virus, × 1200.

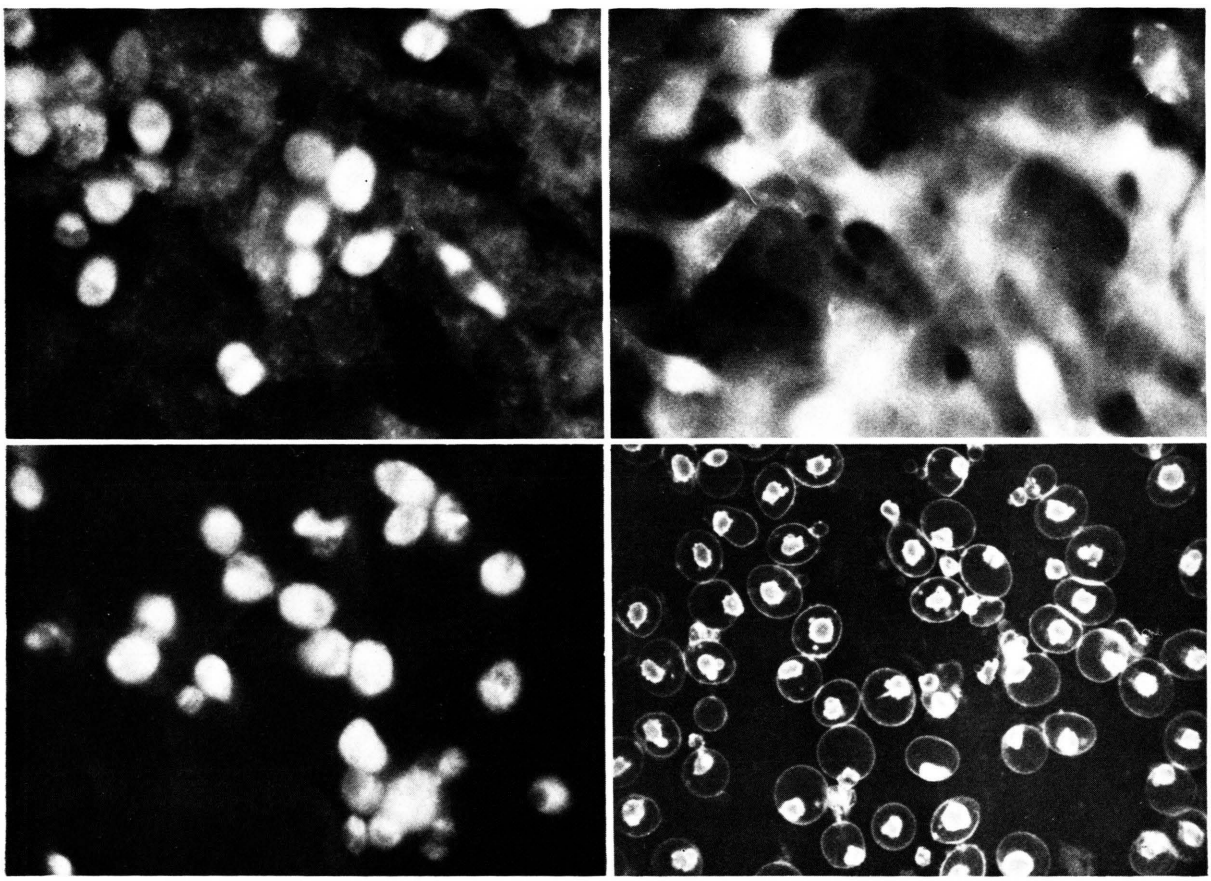

Fig. 2. Transfer of dansylcerebroside from donors to acceptor membranes. a: transfer from donors (round) to monolayer of BHK cells, showing patch formation of dansylcerebroside in the monolayer. b: transfer from donors (removed by washing) to monolayer of glutaraldehyde fixed MDBK cells, showing homogeneous distribution of the transferred glycolipid and absence of donor cells on the monolayer, c: no transfer of dansylcerebroside from donors (round) to monolayer (dark invisible) of BHK cells, which had been extracted with ethanol to remove lipids. d: Chick erythrocytes, showing labeling of both cytoplasmic and nuclear membranes.

increase in the transfer of glycolipid (Fig. 1 e *vs* f). As in the case of BHK cells, Concanavalin A did not seem to affect the transferability of glycolipid from donor to acceptor cells.

Transfer of glycolipid occurs independent of proteins

To test whether protein constituents of the membranes were involved in the glycolipid transfer, acceptor monolayers were treated with glutaraldehyde to denature proteins. As described earlier, donor membranes had already been fixed with glutaraldehyde. Donor and acceptor cells were then brougth together and incubated for 6 or 12 hours. It was found that glutaraldehyde completely abolished the adhesiveness of both cells, *i. e.* after the incubation periods, donor cell could be totally removed from the monolayers by rinsing with PBS. However, despite the abolishment of adhesion between cells after glutaraldehyde treatment, transfer of glycolipid still occurred extensively (Fig. 2 b as compared to 2 a). It is concluded, therefore, that neither functional proteins nor tight adhesion is necessary for the transfer process. In comparison, it should be mentioned that a series of exchange proteins have been reported to participate in the transfer of phospholipids between membranes. It is interesting to note that the glycolipid, which was transferred to the glutaraldehyde fixed monolayers, did not form patches (Fig. 2 b) as observed with native monolayers (Fig. 1 b, c, d, Fig. 2 a). This suggests that patch formation may be the result of a higher order of membrane mobility than the transfer process.

It can be concluded that loose contact between membranes alone cause transfer of glycolipid between membranes. It is thought that occasional contact or collision might account for the labeling of nuclear membranes of chick erythrocytes after incubation with dansylcerebroside [1]. Fig. 2 d shows many nuclei of erythrocytes in direct contact with the cytoplasmic membrane, suggesting that nuclei of erythrocytes are labeled by transfer of glycolipid from the cytoplasmic membrane.

Transfer of glycolipid occurs directly between lipids of the membranes

Participation of membrane lipids in the transfer of glycolipid was checked by extraction of lipids from the monolayers with ethanol. It was found that removal of lipids from the acceptors resulted in complete hindrance of the transfer of glycolipid between membranes (Fig. 2 c). In contrast to glutaraldehyde, ethanol did not change the adhesiveness between cells, *i. e.*, donor cells were firmly attached to ethanol treated monolayers after several hours of incubation. It is therefore concluded that membrane lipid is the primary factor involved in the glycolipid transfer.

To summarize, the present work indicates that dansylcerebroside as a model of glycolipid can be transferred between membranes. Various factors which involve in the process have been evaluated. Further experiments are being carried out to see whether the phenomenon observed with dansylcerebroside is of general biological significance.

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