**Interaction of phospholipid vesicles with cultured mammalian cells**

To delineate some of the relationships between membrane composition and the functional states of mammalian cells, we are exploring the possibility of producing controlled membrane changes in the plasma membrane of cultured cells, using artificially generated lipid vesicles. Although other groups have reported the use of lipid dispersions for producing cellular modifications, the underlying molecular mechanisms by which these effects are produced have not been determined. We present here preliminary findings on the mechanism of interaction of chemically and physically well-defined phospholipid vesicles with cultured mammalian cells.

Typical data obtained when Chinese hamster V79 cells were incubated with vesicles produced from radiolabeled egg yolk lecithin are given in Fig. 1. Unilamellar (F-II) vesicles were more efficient in the transfer of exogenous phospholipid to the cells than multilamellar (F-I) vesicles. In both cases a considerable amount of phospholipid became associated with the cells in a relatively short time. The amount of lipid taken up depended on the physical state of the lipids comprising the vesicles, being substantially greater below the gel–liquid crystalline phase transition temperature. Between 20% and 30% of the labeled phospholipid taken up by vesicle-treated cells appeared in their plasma membranes. This lipid was not degraded, but remained as lecithin. The remaining radioactivity was distributed in intracellular fractions in which considerable degradation was observed. No effect on cell viability could be detected for short periods of incubation (up to 6 h), and normal cell growth resumed when the vesicle suspension was replaced by fresh medium. Prolonged incubation of sparse cultures (~10⁵ cells cm⁻²), however, resulted in considerable cell death as determined by dye exclusion tests. This deleterious effect of lipid vesicle treatment agrees with our observation that sparse cultures take up about an order of magnitude more phospholipid than those approaching confluency.

Figure 2a shows a transmission electron micrograph obtained from a thin section through a preparation of V79 cells incubated with a suspension of F-I vesicles. A large vesicle, about 7,000 Å in diameter, with the characteristic multilamellar appearance of this vesicle fraction, is seen in direct contact with the plasma membrane of the cell. Figure 2b shows a preparation of unilamellar vesicles, about 500 Å in diameter, incubated with V79 cells pretreated with cationised ferritin (0.5 mg ml⁻¹ for 5 min at 25°C). There are numerous vesicles, both attached to the surface membrane and apparently fused with the cell. Although there were no essential differences in the amount of phospholipid taken up in the presence or absence of cationised ferritin, the fate of the applied unilamellar vesicle suspension could not be traced by electron microscopy without this marker.

**Table 1** Uptake by Chinese hamster V79 cells of ¹⁴C-dioleyl lecithin vesicles containing ³H-inulin

| Multilamellar vesicles | ¹⁴C-dioleyl lecithin c.p.m. | ³H-inulin c.p.m. | Ratio (¹⁴C c.p.m./³H c.p.m.) | Expected |
|-------------------------|-----------------------------|-----------------|-----------------------------|----------|
| 2°C                     | 126                         | 370             | 0.41                        | 0.41     |
| 37°C                    | 108                         | 220             | 0.44                        | 0.44     |

| Unilamellar vesicles    | ²⁵°C                        | ³⁵°C            | Expected                    |
|-------------------------|-----------------------------|-----------------|-----------------------------|----------|
| 2°C                     | 800                         | 2.000           | 0.44                        | 0.44     |
| 37°C                    | 5.2                         | 1.000           | 0.44                        | 0.44     |

* Doubly labelled vesicles were prepared by cosonation of ¹⁴C-dioleyl lecithin and ³H-inulin. The vesicle fractions containing the trapped marker were subsequently separated from free inulin by chromatography on Sepharose 4B. No leakage of the trapped marker could be detected during this experiment.

† Protocol as in Fig. 1; 1 h incubation.
phospholipid from the applied vesicles is adsorbed to the cell surface, resulting in a net transfer of lipid to the cell.

If fusion is involved in the interaction of phospholipid vesicles with cultured cells, then the aqueous contents of such vesicles should be released into the cytoplasm of vesicle-treated cells after each fusion event. To test this possibility, vesicles comprised of \(^{14}C\)-dioleoyl lecithin and containing \(\text{H}^2\)-inulin as a marker for the internal aqueous volume were used in an uptake experiment (Table 1). If all the phospholipid enters the cell by fusion, the ratio of phospholipid to inulin radioactivity found associated with vesicle-treated cells should be that of the applied vesicle suspension. Table 1 shows the amounts of \(^{14}C\)-dioleoyl lecithin and \(\text{H}^2\)-inulin found associated with cells treated with either unilamellar or unilamellar vesicles together with the observed and expected ratios of c.p.m. of \(^{14}C\) to tritium. The observed ratios were calculated from the \(^{14}C\)-lecithin and \(\text{H}^2\)-inulin taken up by the cells for each treatment, whereas the expected values were derived directly from the measured ratios of the applied vesicle fractions. In each case the amount of the trapped marker, \(\text{H}^2\)-inulin, which became cell-associated was not proportional to the amount of phospholipid taken up. For example, for multi-lamellar vesicles at \(37^\circ\) C, the observed ratio of phospholipid c.p.m. to inulin c.p.m. was 3.4, compared with an expected value of 0.41. If the trapped marker can become associated with the cell only as the result of fusion, we calculate that at \(2^\circ\) C, only small amounts of the observed phospholipid uptake (<2% for multilamellar and ~10% for unilamellar vesicles) can be accounted for by fusion, whereas at \(37^\circ\) C, more significant fractions of the lipid uptake (~10% for multilamellar and ~50% for unilamellar vesicles) could enter the cells by such a mechanism. The remaining lipid would have to be taken up by some other pathway such as phospholipid exchange or adsorption. Experiments are in progress to test these possibilities. Since the cultures were pretreated with glutaraldehyde, demonstrated that the association of the trapped marker with vesicle-treated cells was not significantly inhibited, thus confirming the supposition that vesicle uptake is not an active process.

To demonstrate unequivocally that a fusion process is involved in the overall mechanism of uptake of unilamellar vesicles by cultured cells, we require in addition to the uptake of phospholipid and trapped marker, that the following criteria be met. First, the trapped marker must be truly soluble in the internal aqueous space of the vesicle. The use of lipid vesicles bearing a net charge to entrap soluble markers of opposite charge raises the question of whether such markers are bound or associated with the wall of the lipid vesicle. If such an interaction exists, the bound marker might enter the cell by some internalisation mechanism and not by fusion. Second, the marker once associated with the cell must be demonstrated to be free within the cytoplasm of the cell and not compartmentalised either in the lysosomal apparatus or in some other membrane-bound vesicular body. Although we believe that the first condition has been fulfilled in our studies, we have no sufficient proof that the second criterion has been met. Thus we can only suggest vesicle-cell fusion as an explanation for our observations. Nevertheless, these studies should provide a useful basis for achieving modification of the surface properties of mammalian cells.

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Yeast mitochondrial RNA
does not contain poly(A)

Poly(A) is a universal constituent of eukaryotic messenger RNAs with the exception of histone mRNA. Recently Pennman\(^3\) and Attardi\(^4\) have demonstrated that poly(A) was also present in mitochondrial RNA from animal cells. This finding was of interest to us for two reasons. First, it suggested a simple means of isolating mitochondrial mRNAs and, second, it suggested that the similarity between prokaryotes and mitochondria might not be as close as previously suggested. We therefore looked for poly(A)-containing RNA (poly(A)-RNA) in the simple eukaryote Saccharomyces carlsbergensis.