The Biochemistry of Virus-Induced Cell Fusion

CHANGES IN MEMBRANE INTEGRITY

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1. Phospholipids prelabelled with [^{14}C]acetate, [^{32}P]phosphate, [^3H]- or [^{14}C]-choline or [^3H]inositol are not significantly degraded during fusion of Lettrée cells mediated by Sendai virus, nor are carbohydrates prelabelled with [^3H]fucose, [^{14}C]galactose or [^3H]glucosamine. Less than 1 nmol of lysophosphatidylcholine/ 10^7 cells is formed during fusion. Diethyl *p*-nitrophenyl phosphate, which inhibits phospholipase A by more than 95%, has no effect on fusion. It is concluded that none of the events leading to cell fusion is accompanied by significant turnover of phospholipids or other membrane components. 2. Intracellular K^+ leaks out during virally mediated cell fusion; the loss is not as extensive as that of intracellularly accumulated choline or deoxyglucose. Movement of Ca^{2+} into or out of cells could not be detected. 3. At concentrations of Lettrée cells insufficient to be agglutinated by virus, intracellularly accumulated choline and deoxyglucose leak out. Agglutination caused by concanavalin A does not result in leakage of intracellular metabolites. 4. P815Y cells, which agglutinate but do not fuse in the presence of virus, show leakage of intracellularly accumulated metabolites. The extent of leakage does not alter during the G_1 and S periods of the cell cycle. 5. Leakage is inhibited by Ca^{2+} , but is unaffected by EDTA. 6. It is concluded that the interaction of Sendai virus with mammalian cells causes a weakening of membrane integrity so that intracellular metabolites leak out. Such destabilization may facilitate viral entry and is therefore an interesting system for further biochemical studies.

Cell fusion caused by Sendai virus is a useful model for investigating membrane fusion in general. The suggestion that some kind of membrane destabilization precedes fusion (Poste & Allison, 1971) seems borne out by the fact that a leakage of intracellular metabolites accompanies virally mediated cell fusion (Pasternak & Micklem, 1973). The mechanism by which this is achieved is not known. Lysophospholipids are detergents capable of causing cell fusion (Ahkong *et al.*, 1972) and it has been proposed (Poole *et al.*, 1970) that a temporary increase in lysophosphatidylcholine generated from phosphatidylcholine by the action of phospholipase A may be involved. Certainly high concentrations of Ca^{2+} , which activate endogenous phospholipase A, can cause erythrocytes to fuse (Toister & Loyter, 1973). On the other hand Elsbach *et al.* (1969) detected no phospholipase A-mediated turnover of phosphatidylcholine during fusion of baby-hamster kidney cells by SV5 virus. Another possibility is that membrane destabilization is brought about by a phospholipase C type of degradation, which affects phosphatidyl-inositol rather than phosphatidylcholine. We have investigated both possibilities by following the fate of suitably prelabelled phospholipids during viral fusion of Lettrée cells. At the same time we have extended our initial observations on leakage of

phosphorylated intermediates (Pasternak & Micklem, 1973) to include ionic changes.

A second aim was to confirm that membrane destabilization is a discrete event (Pasternak & Micklem, 1973) in cellular fusion. We have done this with Lettrée cells at concentrations too low to agglutinate, and with P815Y cells, which do not fuse but show leakage of intracellular metabolites. The latter observation has enabled us to extend some of our studies on topographical changes during the cell cycle of P815Y cells (Pasternak *et al.*, 1971; Graham *et al.*, 1973; Sumner *et al.*, 1973).

Experimental

Materials

Lettrée cells passaged in Swiss white mice and Sendai virus [grown in 11-day-old hen eggs and purified by centrifugation at 700g for 10 min and then at 20000g for 30 min and suspended in Hanks (1948) medium without glucose] u.v.- and heat-inactivated as described by Pasternak & Micklem (1973), were obtained by courtesy of Professor H. Harris. P815Y cells were grown in culture (Warmesley & Pasternak, 1970).

[$^3\text{H}_2$]Inositol (2 Ci/mmol), [$\text{Me-}^3\text{H}$]choline chloride (1 Ci/mmol), [$\text{Me-}^{14}\text{C}$]choline chloride (60 mCi/mmol), sodium [$\text{U-}^{14}\text{C}$]acetate (57 mCi/mmol) and [^{32}P]orthophosphoric acid (96 Ci/mg of P) were obtained from The Radiochemical Centre, Amersham Bucks., U.K. For certain experiments unlabelled isotope was added as indicated in the legends. Concanavalin A was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Methods

Cells were incubated with u.v.-inactivated (intact) or with u.v.-inactivated heat-killed virus and sampled as described by Pasternak & Micklem (1973). Fusion index, assessed microscopically, is defined as:

$$\frac{\text{Number of cells participating in fusion}}{\text{Total number of cells}}$$

Agglutination index is defined as follows: + + + +, >95% of cells agglutinated; +, 20–50% of cells agglutinated; –, <5% of cells agglutinated.

Phospholipids in cell pellets were extracted with chloroform-methanol (2:1, v/v), except for [^{14}C]acetate-labelled cells, which were extracted with 5% (w/v) trichloroacetic acid (Pasternak & Micklem, 1973). In cells prelabelled with [^3H]glucosamine or [^{14}C]galactose, approx. 80% of the label was present in the chloroform-methanol-insoluble pellets, and [^3H]fucose was distributed as follows: 20% in insoluble pellet, 35% in chloroform-methanol lower layer, 45% in upper layer.

Leak-out of intracellular components was measured by centrifuging samples and assaying the supernatant medium and pellet as described by Pasternak & Micklem (1973). Leakage is expressed as:

$$\frac{\text{Amount (d.p.m. or mol) appearing in medium}}{\text{Amount in medium} + \text{amount in cell pellet}} \times 100$$

Some 80% of [^{14}C]choline taken up by Lettrée or P815Y cells during incubation for 30 min at 37°C is in the form of water-soluble products.

For determination of cation changes, cells were washed four times in 50 mM-sodium phosphate-buffered iso-osmotic saline (pH 7.2; K^+ - and Ca^{2+} -free) and incubated with virus previously dialysed in the same medium overnight. K^+ and Ca^{2+} in the medium or the cell pellet [extracted with 70% (w/v) HClO_4] were determined in a Pye SP.90 atomic absorption spectrophotometer by courtesy of Dr. R. J. P. Williams.

For determination of leakage during the cell cycle, P815Y cells were separated by zonal centrifugation (Warmsley & Pasternak, 1970) into ten successive fractions (each with 5×10^6 cells/ml) which were exposed to [^3H]thymidine (2 $\mu\text{Ci}/\text{ml}$) and [^{14}C]choline (0.16 $\mu\text{Ci}/\text{ml}$) for 30 min at 37°C, washed and

then incubated at (5×10^6 cells/ml) with intact or heat-killed virus (16 haemagglutinin units/ml). The ^3H content of the cell pellet and the cell size indicate position in the cell cycle. Intact virus caused no difference in leakout of ^3H (less than 0.04% of the total in the cells, more than 95% of which is trichloroacetic acid-insoluble).

Results

Lack of turnover during fusion

Synthesis of phospholipids during fusion is difficult to assess because of changes in the concentration of the intracellular precursors such as phosphorylcholine (Pasternak & Micklem, 1973). Degradation of prelabelled phospholipids was therefore investigated. Phospholipids were labelled by injecting ascites-bearing mice with [^{14}C]acetate, [^{32}P]phosphate, [$\text{Me-}^3\text{H}$] or [$\text{Me-}^{14}\text{C}$]choline and [^3H]inositol. In no case was a significant difference between fusing and non-fusing cells detected (Fig. 1).

Fig. 1. Phospholipid turnover during virally mediated cell fusion

Mice (30 g) bearing Lettrée cells were injected with [^{14}C]acetate (30–50 μCi), [^{32}P]phosphate (2–60 μCi), [^3H]choline (100 μCi), [^{14}C]choline (10 μCi) or [^3H]inositol (50–100 μCi) 4–113 h before removal of ascites fluid. Cells were washed and incubated (7×10^6 – $1.4 \times 10^7/\text{ml}$) with intact (open symbols) or heat-killed (closed symbols) virus (1600–2700 haemagglutinin units/ml) at room temperature or 37°C. Fusion indices with intact virus varied between 0.10 and 0.30. At times indicated, cells were centrifuged and phospholipids assayed as described in the Experimental section. The Figure represents the composite results from 13 experiments.

Inositol labelling tends to increase somewhat during incubation (with or without virus), whereas acetate-labelled material decreases; each change probably reflects a certain amount of turnover, the direction of change being determined by precursor pools, incorporation pathways and other factors.

The data of Fig. 1 refer to total phospholipids, of which only some 10–20% could be in the surface membrane. Changes of less than 1 or 2% of total phospholipids are unlikely to be detected, so that as much as 10% of the surface phospholipids could be involved in metabolic alteration. This is unlikely, however, since less than 2% of phospholipid in cells prelabelled by exchange with exogenous [^{32}P]-phosphatidylcholine or [^{32}P]-phosphatidylinositol (Quinn, 1973) is degraded.

Cells prelabelled with sugars such as [^3H]fucose, [^{14}C]galactose or [^3H]glucosamine, which are incorporated predominantly into surface membrane, showed no detectable turnover (less than 1% in each case) in the presence or absence of virus. Neither is any turnover of protein (prelabelled with [^{14}C]valine or [^{35}S]methionine) apparent.

The possible participation of lysophosphatidylcholine in fusion was investigated in two ways. First, the amount of [^3H]- or [^{32}P]-lysophosphatidylcholine formed during incubation of [^3H]choline- or [^{32}P]-phosphorus-labelled cells was measured by t.l.c.; less than 0.5% of total phospholipid was recovered as lysophosphatidylcholine at the end of 30 min in the presence or absence of intact virus. This corresponds to less than 1 nmol/ 10^7 cells, i.e. an intracellular concentration of less than 100 μM . Secondly, the effect of diethyl *p*-nitrophenyl phosphate (E600), a compound known to inhibit phospholipase A (Meldolesi *et al.*, 1971), on fusion was assessed. Fusion index was unaffected by 0.05% diethyl *p*-nitrophenyl phosphate (0.18 without, 0.16 with diethyl *p*-nitrophenyl phosphate). At that concentration, diethyl *p*-nitrophenyl phosphate completely inhibits the hydrolysis of [^{32}P]phosphatidylcholine by freeze-thawed Lettrée cells (97% degraded to lysophosphatidylcholine and water-soluble products without diethyl *p*-nitrophenyl phosphate, 3% degraded with diethyl *p*-nitrophenyl phosphate). After 30 min of incubation at 37°C, the cell homogenate without diethyl *p*-nitrophenyl phosphate had become completely solubilized; that with diethyl *p*-nitrophenyl phosphate was intact.

It has been shown that the increase in phospholipid turnover that accompanies the serum-mediated growth stimulation of static cells (Cunningham, 1972; Pasternak, 1972) is preceded by a release of intact phospholipid molecules (Knox & Pasternak, 1973, 1974; Pasternak & Knox, 1973; Ristow *et al.*, 1973). Since removal of surface phospholipids might be an alternative to destabilization by turnover, this possibility was tested. No loss of phospholipid,

protein or carbohydrate-containing components could be detected.

Leakage of cations during fusion

Since it has been suggested (Poste & Allison, 1971) that membrane destabilization is accompanied by an increase in cation permeability, leakage of K^+ during cell fusion was measured. In confirmation of an earlier report (Klemperer, 1960), addition of virus induced loss of K^+ . Some 20% of the internal K^+ leaks out (Fig. 2), compared with 50–80% of the choline or deoxyglucose (mainly as phosphorylated products; Pasternak & Micklem, 1973). Little K^+ leaks out at 20°C; at 0°C K^+ leaks out irrespective of the presence of virus. Such differences presumably reflect the mechanisms by which high internal concentrations are achieved. For K^+ , an 'entry-pump' is superimposed on passive leakage; experiments with $^{42}\text{K}^+$ might delineate the site of action of virus. If $^{42}\text{K}^+$ is added to baby-hamster kidney cells 15 min after Sendai virus, an increased entry is observed and this may reflect a repair of previously depleted cells (Fuchs & Giberman, 1973). For phosphorylated compounds, the driving force is a kinase-catalysed phosphorylation of compounds taken up in unphosphorylated form; the action of virus is most likely to be a stimulation of passive leakage (Pasternak & Micklem, 1973). Note that concanavalin A, which agglutinates Lettrée cells as effectively as virus, does not cause leakage of K^+ (or of phosphorylated compounds, see below).

During fusion in such Ca^{2+} -free media (total external Ca^{2+} due to virus and cells 10 μM), no movement of Ca^{2+} into or out of cells is detectable

Fig. 2. Leakage of intracellular K^+ during virally mediated cell fusion

Lettrée cells were washed and incubated ($10^7/\text{ml}$) with dialysed virus (800 haemagglutinin units/ml) at 37°C as described in the Experimental section; at the times indicated cells were spun and the supernatant medium and pellet assayed as described. ●, Intact virus; ○, heat-killed virus.

(less than $\pm 10\%$ change in internal Ca^{2+}). In the presence of 1 mM-Ca^{2+} , the intracellular Ca^{2+} concentration remains at $\leq 10\text{ }\mu\text{M}$, irrespective of the presence or absence of virus; in this case, the possibility that Ca^{2+} does enter cells but is removed again during the washing procedure cannot be dismissed.

Leakage without fusion

When virus is added to cells at low density (e.g. $1.6 \times 10^5/\text{ml}$ compared with $1.6 \times 10^6/\text{ml}$) agglutination does not occur; leakage of [^{14}C]choline, on the

other hand, is unaffected (Table 1); the same is true for deoxy[^3H]glucose. Decreasing the concentration of virus decreases leakage and agglutination; both are abolished by heat treatment of the virus. Similarly P815Y cells, which are agglutinated by Sendai virus but do not fuse, show leakage of intracellularly accumulated choline or deoxyglucose. P815Y cells are generally 'leakier' than Lettrée cells (Table 1). Again, virus added to P815Y cells at concentrations too low to agglutinate causes cell leakage. Thus it is clear that leakage is an event separate from agglutination or fusion.

Table 1. *Leakage of intracellular [^{14}C]choline*

Lettrée or P815Y cells ($10^8/\text{ml}$) were incubated with [^{14}C]choline (0.5 and $3\text{ }\mu\text{Ci}/\text{ml}$ respectively) for 30 min at 37°C , washed three times and re-incubated at 37°C with virus or concanavalin A. After 30 min (Lettrée cells) or 20 min (P815Y cells) cells were centrifuged, and radioactivity in supernatant and pellet was assayed as described in the Experimental section.

Lettrée cells							
Addition Virus (haemagglutinin units/ml)	1.6×10 ⁶ /ml		1.6×10 ⁵ /ml		Addition Virus (haem- agglutinin units/ml)	P815Y cells (1.1×10 ⁷ /ml)	
	Leakage (%)	Agglut. index	Leakage (%)	Agglut. index		Leakage (%)	Agglut. index
0*	8	—	5	—	0*	26	—
1600	60	++++	75	—	1000	90	++++
160	40	++++	73	—	10	37	+
16	10	+	25	—			
1.6	8	—	11	—			
Concanavalin A (mg/ml)					Concanavalin A (mg/ml)		
2	4	++++	8	—	2	28	++++
* Heat-killed							

* Heat-killed

Table 2. *Leakage of intracellular [^{14}C] choline at different stages of the cell cycle*

P815Y cells were separated by zonal centrifugation, incubated with [^3H]thymidine and [^{14}C]choline, washed and re-incubated in the presence of virus at 20°C as described in the Experimental section. [^3H]Thymidine incorporated refers to ^3H in trichloroacetic acid-insoluble material; [^{14}C]choline accumulated refers to ^{14}C in trichloroacetic acid-soluble material, each at the start of the re-incubation. Values of ^{14}C leakage in the absence of virus have been subtracted for the calculation of initial rate of ^{14}C leakage (measured over 15 min) and for calculation of total ^{14}C lost by 40 min. Note that the even-numbered fractions were re-incubated approx. 1 h after the odd-numbered ones.

Fraction no.	[^3H]Thymidine incorporated (d.p.m./ 10^4 cells)	Approx. position	[^{14}C]Choline accumulated (d.p.m./ 10^4 cells)	Initial rate of ^{14}C leakage (d.p.m./ 10^4 cells per min)	Total ^{14}C lost by 40 min (d.p.m./ 10^4 cells)
1	920	G ₁	20	—	3.0
2	1400	G ₁	20	0.10	4.2
3	2900	G ₁	20	0.12	3.0
4	3700	G ₁	15	0.10	1.5
5	10700	S	21	0.31	4.0
6	11700	S	26	0.44	6.0
7	14400	S	28	0.65	8.1
8	10000	S	21	0.34	4.0
9	14600	S	33	0.59	7.3
10	10900	S	25	0.40	5.2

Table 1 also shows that concanavalin A, which agglutinates Lettrée or P815Y cells at concentrations above 10^6 cells/ml, does not cause leakage. Leakage is therefore an event caused specifically by the binding of virus to cells.

Leakage during the cell cycle

P815Y cells, separated by zonal centrifugation according to their position in the cell cycle (Warmesley & Pasternak, 1970), were incubated with [3 H]-thymidine (to confirm the position indicated by cell volume) and [14 C]choline. G_2 phase, which is a very short period in P815Y cells (Bergeron *et al.*, 1970), was not effectively separated from S phase in this experiment (Table 2). Progressively more 14 C is accumulated in water-soluble metabolites between G_1 and S phases. This is presumably due to a number of reasons, such as an increase in volume or an increase in transport or phosphorylating enzymes. As noted previously (Bergeron *et al.*, 1970; Warmesley & Pasternak, 1970), incorporation into phospholipids increases gradually between G_1 and S phases (from 10 to 20% of total intracellular 14 C in this instance). Initial rate of leak-out likewise increases between G_1 and S phases, though the total amount lost, relative to that accumulated, remains approximately the same (Table 2); by 40 min, an average of some 25% of intracellular [14 C]choline is lost in the

presence of virus, compared with 5% in its absence.

Since susceptibility to immune (Pasternak *et al.*, 1971) or non-immune (Graham *et al.*, 1973; Sumner *et al.*, 1973) lysis is significantly lessened in mid-interphase cells, it is clear that leakage and lysis reflect different biochemical events at the cell surface (Pasternak *et al.*, 1974). The apparent similarity between immune cytolysis and sensitivity to viral fusion (Holmes *et al.*, 1969) is therefore unlikely to be due to an early alteration common to the two processes.

Effect of Ca^{2+} on leakage by Lettrée cells

In view of an inhibitory action of EDTA on cell fusion, the effect of EDTA and Ca^{2+} on leakage of intracellular metabolites was studied. Paradoxically, EDTA proved to be without inhibitory effect, whereas Ca^{2+} decreases leakage; both the rate and the extent of leakage are inhibited (Table 3). The slight stimulation by EDTA is presumably caused by removal of trace amounts of Ca^{2+} . In the absence of virus, neither EDTA nor Ca^{2+} significantly affects leakage of accumulated deoxy[3 H]glucose; leakage of accumulated [14 C]choline is inhibited by Ca^{2+} . Note that leakage of deoxy[3 H]glucose in the absence of virus is more extensive than that of [14 C]choline; this may reflect a less effective phosphorylation of deoxyglucose than of choline (Pasternak & Micklem,

Table 3. *Effect of Ca^{2+} on leakage of intracellular deoxy[3 H]glucose and [14 C]choline*

Lettrée cells were incubated for 30 min at 37°C as follows: Expts. 1 and 2, 5×10^7 cells/ml, deoxy[3 H]glucose (12.5 μ Ci/ml; 0.2 mM) and [14 C]choline (1.25 μ Ci/ml; 0.02 mM); Expts. 3 and 4, 8×10^7 cells/ml; deoxy[3 H]glucose (10 μ Ci/ml; 0.2 mM) and [14 C]choline (1 μ Ci/ml; 0.017 mM). Cells were washed and re-incubated in the presence of virus at 22°C as described in the Experimental section. Values of 3 H and 14 C leakage in the absence of virus have been subtracted for the calculation of rate of leakage (after a lag of 0–10 min) and for the calculation of per cent lost by 40 min. The totals accumulated (d.p.m./ 10^4 cells) in cell pellets at the start of the re-incubation were as follows: Expt. 1, 5000 (3 H) and 84 (14 C); Expt. 2, 2800 (3 H) and 70 (14 C); Expt. 3, 2900 (3 H) and 120 (14 C); Expt. 4, 2400 (3 H) and 94 (14 C).

Expt. no.	Cells	Virus (haemagglutinin units/ml)	Addition	Rate of leakage (d.p.m./ 10^4 cells per min)		Leakage by 40 min (%)	
				3 H	14 C	3 H	14 C
1	1.9×10^5 /ml	130	—	120	1.4	36	43
	1.9×10^5 /ml	130	1 mM- Ca^{2+}	35	0.5	18	14
	1.9×10^5 /ml	130	10 mM-EDTA	160	2.8	55	70
2	4.4×10^5 /ml	180	—	110	1.4	70	65
	4.4×10^5 /ml	180	0.01 mM- Ca^{2+}	120	1.2	62	42
	4.4×10^5 /ml	180	0.1 mM- Ca^{2+}	85	0.91	40	34
	4.4×10^5 /ml	180	1 mM- Ca^{2+}	66	0.35	38	10
3	2.7×10^5 /ml	110	—	140	5.4	39	51
	2.7×10^5 /ml	110	1 mM- Ca^{2+}	36	0.79	33	6
	2.7×10^5 /ml	530	—	56	3.1	26	45
	2.7×10^5 /ml	530	1 mM- Ca^{2+}	29	0.95	30	9
4	1.1×10^7 /ml	110	—	47	2.0	38	44
	1.1×10^7 /ml	110	1 mM- Ca^{2+}	16	1.1	10	12
	1.1×10^7 /ml	530	—	27	1.4	30	34
	1.1×10^7 /ml	530	1 mM- Ca^{2+}	19	1.2	12	12

1973). Inhibition of intracellular leakage by Ca^{2+} has also been demonstrated in the case of virally mediated loss of K^+ (Klemperer, 1960).

Table 3 shows that the inhibition by 1 mM-Ca^{2+} is relatively independent of virus concentration and of cell concentration or agglutination (Expts. 1–3, no agglutination; expt. 4, cells agglutinated). A combination of high cell density and high virus concentration (Expt. 4) shows the least effect; this may explain why cell fusion, which occurs only under such conditions, is not inhibited by Ca^{2+} . Since Ca^{2+} entry is not promoted by virus (see above), the inhibitory effect on leakage is presumably on the outside of the cell. The extent to which this is by a direct effect on cell permeability (Rasmussen, 1970), rather than by interference with viral attachment (at low concentration of cells and virus), remains to be established. What is clear is that the effect of Ca^{2+} and EDTA, like that of cytochalasin B (Pasternak & Micklem, 1973), separates virally mediated membrane destabilization from subsequent cellular fusion.

Discussion

Our observations on the lack of turnover of any phospholipid or carbohydrate-containing compound during Sendai-virus-mediated cell fusion of Lettrée cells (e.g. Fig. 1) confirm and extend previous data on the lack of involvement of lysophosphatidylcholine in the fusion of baby-hamster kidney cells by SV5 virus (Elsbach *et al.*, 1969) or of rabbit kidney cells by herpes simplex virus (Falke *et al.*, 1967). Whether variations in gross lipid composition do (Klenk & Choppin, 1970 *a,b*) or do not (Poste *et al.*, 1972) affect susceptibility of different cells to fusion is another matter. Of course the number of molecules involved may be rather small (cf. Lachmann *et al.*, 1973) and one cannot therefore discount the participation of turnover entirely. For example an estimated change of less than $100\mu\text{M}$ in the intracellular concentration of lysophosphatidylcholine should be compared with values of approx. $20\mu\text{M}$ of external lysophosphatidylcholine (Ahkong *et al.*, 1972) or approx. $100\text{--}400\mu\text{M}$ of other lipids (Ahkong *et al.*, 1973) which are sufficient to cause fusion of various types of cells. In any case if deacylation by phospholipase A is rapidly followed by reacylation, local fluctuations of lysophosphatidylcholine might escape detection. Alternatively the lysophosphatidylcholine might be derived from the virus (Barbanti-Brodano *et al.*, 1971), which would not conflict with the present results. Such a situation appears to exist in chromaffin granules, in which a permanently high concentration of lysophosphatidylcholine is observed (Blaschko *et al.*, 1967). Whether this represents a prerequisite for subsequent fusion with the cell membrane in this

particular (da Prada *et al.*, 1972) case or whether it is unconnected with membrane fusion is not known. What is clear is that a content of lysophosphatidylcholine as high as 17% of total phospholipid (Blaschko *et al.*, 1967) does not prevent chromaffin granules from existing as intact organelles. Another point to note is that addition of lysophosphatidylcholine to rat muscle cells actually prevents their fusion (Reporter & Norris, 1973).

The fact that leakage of intracellular metabolites occurs in the absence of cellular fusion or agglutination (e.g. Table 1) confirms our suggestions, based on the use of cytochalasin B (Pasternak & Micklem, 1973), that leakage represents a destabilization event independent of subsequent fusion. It also makes the system a suitable one for biochemical studies.

The correlation between membrane changes and an altered permeability towards certain compounds (Pasternak & Micklem, 1973) is of obvious relevance to an understanding of membrane function in general. In addition the changes represent the earliest documented event after the attachment of virus to the cell surface, and may therefore have a bearing on the mechanism by which viruses enter cells. Since Sendai is a membrane-coated virus, fusion between virus and the plasma membrane may be part of the process (Morgan & Howe, 1968). It should be noted that many infectious viruses do not have lipid envelopes and some of these are also able to cause cell fusion (Poste, 1972). The next stage will be to examine the generality of the leakage phenomenon, and to determine the nature of the temperature-dependent (Pasternak & Micklem, 1973) event.

Many investigators have suggested the participation of some sort of enzyme activity in viral attack (see Poste, 1972), but our results are equally compatible with a non-enzymic modification of membrane structure. What is clear is that the modification is a specific one: treatment of cells with trypsin or phospholipase C, for example, does not cause leakage (K. J. Micklem & C. A. Pasternak, unpublished work), nor does the binding of concanavalin A (Table 1); the fact that concanavalin A agglutinates Lettrée or P815Y cells without causing leakage confirms that leakage is not an artifact (Pasternak & Micklem, 1973) caused by cell clumping. Another difference between the interaction of concanavalin A and virus with membrane receptors is that agglutination by virus occurs at 0°C , whereas agglutination by concanavalin A (in contrast with that caused by some other lectins) is a temperature-dependent process (Inbar *et al.*, 1971). Whatever the mechanism of lectin-mediated agglutination (e.g. Nicolson, 1974), it is obviously not the same as that accompanying viral modification of the cell surface.

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