Survey of virally mediated permeability changes

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1. Sendai virus causes permeability changes when added to freshly isolated brain cells (cerebellum or ependymal cells) or to a culture of forebrain cells. 2. Sendai virus causes permeability changes when added to organ cultures of ferret lung or nasal turbinate. Influenza virus causes no permeability changes under these conditions. 3. Rabies virus and vesicular-stomatitis virus, in contrast with Sendai virus, do not cause permeability changes in BHK cells or Lettrée cells. 4. Serum from patients suffering from viral hepatitis does not cause permeability changes in human leucocytes; addition of Sendai virus causes permeability changes. 5. It is concluded that permeability changes accompanying viral entry occur only with certain types of paramyxovirus, but that there is little restriction on cell type. 6. MDBK cells infected with Sendai virus show permeability changes during viral release, similar to those that occur during viral entry. Because these changes do not appear to be restricted to paramyxoviruses, they may have considerable clinical significance.

The nature of the permeability changes caused when viruses such as Newcastle-disease virus or Sendai virus infect cells have been documented (Pasternak & Micklem, 1973, 1974*a,b*; Micklem & Pasternak, 1977; Poste & Pasternak, 1978; Impraim *et al.*, 1980). What is not clear is the specificity of the process with regard to viral type or cell type. In the present paper we show that the process appears to be limited to paramyxoviruses, but that every cell type so far tested responds.

When viruses assemble at the surface of infected cells before being shed or budded off, similar changes may take place (Katzman & Wilson, 1974; Carrasco, 1978), though the nature of this process has not yet been characterized; in this case the permeability change is not restricted to paramyxoviruses, but occurs even with non-enveloped viruses (Schumperli *et al.*, 1978; Contreras & Carrasco, 1979). Since the release of viruses is in many ways more relevant to clinical situations than its entry, it is important to characterize the permeability changes that take place. We show here that the changes resemble those caused by entry of paramyxoviruses in being sensitive to inhibition by Ca^{2+} , but differ in being accompanied by leakage of some intracellular proteins.

Methods and materials

Sendai virus was grown as previously described (Impraim *et al.*, 1980); unless otherwise stated, virus

was of the '3-day' type. Influenza virus strain A/Moscow 1019/65 (H_2N_2), kindly donated by Professor H. Smith (Department of Microbiology, University of Birmingham, Birmingham, U.K.), was grown as described by Kingsman *et al.* (1977*a*). Rabies virus (Flury LEP strain) and vesicular-stomatitis virus (Indiana C strain), kindly donated by Miss J. Crick (Animal Virus Research Institute, Pirbright, Surrey, U.K.), were grown in BHK (baby-hamster kidney) 21 cells and inactivated as follows. A suspension in 30 mM-Hepes/NaOH buffer, pH 7.4, was treated with 0.05% acetyleneimine for 4 h at 37°C, and excess acetyleneimine was destroyed by the addition of 2% (w/v) $\text{Na}_2\text{S}_2\text{O}_3$. Sera from patients diagnosed as being in fulminant hepatic failure secondary to viral hepatitis or to paracetamol overdose, and from patients repeatedly exposed to halothane, all diagnosed as being in Grade 4 hepatic coma (Trey & Davidson, 1970), as well as from control subjects, were kindly donated by Miss L. Poston (Liver Unit, King's College Hospital, London SE5 9RS, U.K.).

Mouse-grown Lettrée cells were obtained as previously described (Impraim *et al.*, 1980); BHK cells and MDBK (bovine kidney) cells (obtained from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.) were propagated in monolayer culture. Cells derived from the forebrain of 1-day-old Furth Wistar rats and suspended in Ham's F10 medium (Flow

Laboratories) supplemented with 10% (v/v) foetal calf serum were kindly donated by Dr. P. Knox (St. George's Hospital Medical School) and used at first sub-culture as described by Knox (1976).

Cerebellar cells were obtained from the brains of 8-day-old rats by the method of Wilkin *et al.* (1976). Ependymal cells from the brains of adult rats were obtained by the method of Manthorpe *et al.* (1977).

Human leucocytes were prepared from whole blood (given by the Regional Blood Transfusion Centre, Tooting, London S.W.17, U.K.) by using Ficoll/Paque (Pharmacia Fine Chemicals, Uppsala, Sweden), washed and suspended in Hepes-buffered saline [150 mM NaCl/5 mM KCl/5 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid / NaOH buffer, pH 7.2].

Organ cultures of ferret lung and nasal turbinates were prepared as described by Kingsman *et al.* (1977a,b).

For leakage experiments, cells or organ cultures were incubated with [3 H]choline (The Radiochemical Centre, Amersham, Bucks., U.K.), washed and re-incubated with or without virus as previously described (Pasternak & Micklem, 1973, 1974a). For uptake experiments, cells were exposed to radioactive substrates (The Radiochemical Centre), then incubated, and uptake was assessed by spinning through oil in the case of suspension cells (Impraim *et al.*, 1979) and by washing rapidly four times with Hepes-buffered saline in the case of monolayer cells. All incubations were in Hepes-buffered saline. The uptake data quoted in Fig. 5 were calculated in terms of cellular DNA (measured as described by Hinegardner, 1971).

Haemagglutination was measured by the method of Martin (1978). Lactate dehydrogenase activity was measured by the method of Hohorst (1963); at least 95% of control or infected cells excluded Trypan Blue.

Results and discussion

Survey of viruses and cell types

Paramyxoviruses have been implicated in a number of neurological disorders (see, e.g., Prineas, 1972; Norrby *et al.*, 1974; Raine *et al.*, 1974; Illis, 1975), and it is therefore important to test the sensitivity of brain cells to Sendai virus. Fig. 1(a) shows that cells freshly isolated from rat cerebellum show virally mediated leakage of [3 H]choline-derived metabolites. Note that inhibition by Ca^{2+} , though significant, is not extensive. Ependymal cells, which may be a site at which viruses become concentrated in brain (Herndon *et al.*, 1974; Wolinsky *et al.*, 1974; Bleier *et al.*, 1975; Walker *et al.*, 1975), were also tested. A suspension of ependymal cells was incubated with [3 H]choline, washed and tested for leakage: during a 15 min incubation, 33% of

Fig. 1. Effect of Sendai virus on leakage from pre-labelled brain cells

Brain cells were isolated, incubated with [3 H]choline washed and re-incubated at 37°C with or without Sendai virus (500 haemagglutination units/ml) as described in the Methods and materials section. (a) Suspension of cells from rat cerebellum (5 mg of cell protein/ml). ♦, Control; ○, Sendai virus present; ●, Sendai virus plus 3 mM- CaCl_2 present. (b) Culture of fore-brain cells (10^6 cells/35 mm-diameter dish) in 0.5 ml. ♦, Control; ○, Sendai virus present; ●, Sendai virus plus 3 mM- CaCl_2 present.

^3H -labelled metabolites appeared in the medium without virus and 54% in the presence of Sendai virus (200 haemagglutination units/ml; approx. 10^{-4} haemagglutination units per cell); the rather high rate of leakage in control cells may reflect a relatively small conversion of [3 H]choline into phosphoryl[3 H]choline, as well as some cell damage occurring during the isolation procedure. Insufficient cells were recovered to allow further tests, and the observed stimulation of leakage by Sendai virus should be regarded as a preliminary value only.

A culture of cells derived from the forebrain of newborn rats, consisting largely of astrocytes, was found to be sensitive to Sendai virus. In this case leakage from control cells is low, and the effect of Sendai virus, as well as the inhibitory action of Ca^{2+} , is clear-cut (Fig. 1*b*). The nature of the ^3H -labelled metabolites appearing in the medium was shown by paper electrophoresis to be predominantly phosphoryl ^3H choline, as with Lettrée cells (Pasternak & Micklem, 1973; Impraïm *et al.*, 1980).

Organ cultures of ferret respiratory tissue have proved a useful model *in vitro* for influenza-virus infection, since they show many of the characteristics observed *in vivo* (Kingsman *et al.*, 1977*a,b*). Their use here has two advantages. First, it extends the nature of sensitive cells to a situation more akin to that *in vivo*; secondly, it allows one to assess the action of the closely related orthomyxoviruses, particularly the clinically important influenza virus.

Fig. 2. Effect of Sendai virus and influenza virus on leakage from prelabelled organ cultures

Organ cultures of ferret lung and nasal turbinate were incubated with ^3H choline, washed and re-incubated in a final volume of 0.5 ml at 37°C with or without Sendai virus (1000 haemagglutination units/ml) or influenza virus (1000 haemagglutination units/ml) as described in the Methods and materials section. (a) Lung (ten pieces randomly chosen). ♦, Control; ○, Sendai virus present; ●, Sendai virus plus 3 mM- CaCl_2 present; ■, influenza virus present; ■, influenza virus plus 3 mM- CaCl_2 present. (b) Nasal turbinate (ten pieces randomly chosen). ♦, Control; ○, Sendai virus present; ●, Sendai virus plus 3 mM- CaCl_2 present.

Preparations of lung and nasal turbinates that have been pre-incubated with ^3H choline show virally mediated leakage of ^3H -labelled metabolites when treated with Sendai virus; influenza virus is ineffective (Fig. 2), which confirms an earlier result obtained with monkey kidney cells (Poste & Pasternak, 1978) and is in accord with the inability of influenza virus to mediate virus-cell fusion (Maeda *et al.*, 1977); freeze-thawing of influenza virus (see Wyke *et al.*, 1980) does not render it active. Sendai-virus-mediated leakage is inhibited by Ca^{2+} , though (as with freshly isolated cerebellar cells) not to the same extent as with cultured cells; in other words conditions exist under which cells, in the presence of physiological extracellular concentrations of Ca^{2+} , are made 'leaky' by virus (see Impraïm *et al.*, 1980).

Two rhabdoviruses, rabies virus and vesicular-stomatitis virus, were tested for their ability to cause permeability changes in BHK cells in monolayer, the cell type in which these viruses are routinely grown. Fig. 3 shows that neither rabies virus nor vesicular-stomatitis virus causes any leakage from prelabelled BHK cells under conditions in which Sendai virus is effective. Because of the possibility that the preparations of rabies virus and vesicular-stomatitis virus contain inhibitory compounds such as Ca^{2+} (which effectively prevents Sendai-virus-mediated leakage from BHK cells, as shown in Fig. 3), the effect of adding these two virus preparations to

Fig. 3. Effect of rhabdoviruses on leakage from prelabelled BHK cells

BHK cells (10^6 cells/35 mm-diameter dish) were incubated with ^3H choline, washed and re-incubated in a final volume of 0.5 ml at 37°C with or without Sendai virus (80 haemagglutination units/ml), rabies virus (6×10^6 mouse ID_{50} units/ml) or vesicular-stomatitis virus (6×10^9 mouse ID_{50} units/ml) as described in the Methods and materials section. ♦, Control; ○, Sendai virus present; ●, Sendai virus plus 3 mM- CaCl_2 present; ▲, rabies virus present; △, rabies virus plus Sendai virus present; ■, vesicular-stomatitis virus present; □, vesicular-stomatitis plus Sendai virus present.

Fig. 4. Effect of rhabdoviruses on leakage from pre-labelled Lettrée cells

Lettrée cells were incubated with [^3H]choline, washed and re-incubated ($2 \times 10^7/\text{ml}$) at 37°C with or without Sendai virus (80 haemagglutination units/ml), rabies virus (6×10^6 mouse ID_{50} units/ml) or vesicular-stomatitis virus (6×10^9 mouse ID_{50} units/ml) as described in the Methods and materials section. (a) $\diamond$, Control; $\circ$, Sendai virus present; $\bullet$, Sendai virus plus 3 mM- CaCl_2 present; $\blacktriangle$, rabies virus present; $\triangle$, rabies virus plus Sendai virus present; $\blacksquare$, vesicular-stomatitis virus present; $\square$, vesicular-stomatitis virus plus Sendai virus present. (b) $\diamond$, Control; $\circ$, Sendai virus present; $\bullet$, acetyleneimine-treated Sendai virus present; $\diamond$, dialysed rabies virus present; $\blacktriangle$, freeze-thawed rabies virus present; $\triangle$, freeze-thawed rabies virus plus Sendai virus present; $\blacksquare$, freeze-thawed vesicular-stomatitis virus present; $\square$, freeze-thawed vesicular-stomatitis virus plus Sendai virus present.

Sendai virus was tested: Fig. 3 shows that this does not significantly impair Sendai-virus-mediated leakage.

Mouse-grown Lettrée cells in suspension offer some technical advantages as a test system for virally mediated leakage, and, since rhabdoviruses infect mice, the effect of the two rhabdoviruses on Lettrée cells was assessed. Fig. 4(a) shows that neither rabies virus nor vesicular-stomatitis virus causes leakage. Because of the possibility that the treatment used to inactivate the viruses (the use of live viruses was not allowed by the Ministry of Agriculture, Food and Fisheries) might affect the capacity to cause virally mediated permeability changes, Sendai virus was subjected to the same inactivation procedure (incubation with acetyleneimine as described in the Methods and materials section). Although there is a slight decrease in the activity of acetyleneimine-treated Sendai virus, it does not account for the complete inactivity of the rhabdovirus preparations; moreover, dialysis of acetyleneimine-treated Sendai virus restores it to full activity, whereas dialysis of the rabies preparation is without effect (Fig. 4b).

As shown above with BHK cells (Fig. 3), neither rabies virus nor vesicular-stomatitis virus preparations contain inhibitors that prevent the action of Sendai virus when mixed before addition to Lettrée cells (Fig. 4a). Since it is known that '1-day' Sendai virus, which does not elicit permeability changes (Impraim *et al.*, 1979; Knutton & Pasternak, 1979), can be rendered active by freeze-thawing (Wyke *et al.*, 1980), rabies virus and vesicular-stomatitis virus were freeze-thawed ten times and re-tested. Neither type of freeze-thawed virus was active; such preparations do not affect the ability of Sendai virus to cause leakage (Fig. 4b). It may be concluded that, under the conditions described, neither rabies virus nor vesicular-stomatitis virus causes leakage of the kind shown by Sendai virus.

Cells modified by Sendai virus show permeability changes other than leakage of phosphorylated metabolites: passive permeability to molecules such as sugars, choline and amino acids, and to cations such as Na^+ or K^+ , is also increased (Poste & Pasternak, 1978; Impraim *et al.*, 1980). Since any 'pores' required for passage of such molecules would be smaller than those required for passage of phosphorylated metabolites, the possibility that rhabdoviruses generate small 'pores' was tested. Uptake of choline at high concentration, and uptake of 2-aminoisobutyrate (in Na^+ medium) at low concentration, were measured: a change in the uptake of choline indicates a change in simple diffusion of choline; a change in the uptake of 2-aminoisobutyrate indicates a change in simple diffusion of Na^+ , to which 2-aminoisobutyrate transport is linked (see Impraim *et al.*, 1980). By

measuring the uptake of [³H]choline and 2-amino-[¹⁴C]isobutyrate simultaneously, an effect of virus can be assessed with some precision, since the cellular ³H increases at the same time as the cellular ¹⁴C decreases, i.e. the ³H/¹⁴C ratio in cells is a sensitive measure of viral action (Poste & Pasternak, 1978). Such an effect, and its reversal by Ca²⁺ is illustrated in the case of Sendai virus in Table 1. Neither rabies virus nor vesicular-stomatitis virus shows a significant change. As with virally mediated leakage of phosphorylated metabolites (Figs. 3 and 4), addition of rabies virus or vesicular-stomatitis virus does not affect the activity of Sendai virus. Sendai virus that had been inactivated in the same way as the rhabdoviruses shows full activity (Table 1). It can be concluded that, under the conditions of the experiments, neither rabies virus nor vesicular-stomatitis virus causes virally mediated permeability changes of the kind elicited by Sendai virus. Of course, the possibility that insufficient virus was used cannot be discounted: assuming that the inactivation treatment causes no loss in the ability to modify the cell membrane, the dose in the case of rabies virus corresponded to 0.1–0.4 mouse ID₅₀ unit per cell, and in the case of vesicular-stomatitis virus to 100–400 mouse ID₅₀ units per cell; note that the number of virus particles added is approximately the same in each case, since rabies virus has a much lower ID₅₀/virus particle ratio than does vesicular-stomatitis virus (J. Crick, personal communication).

During viral and other types of hepatitis, the serum of patients has been shown to cause ion

changes in leucocytes: the Na⁺ content increases, whereas K⁺ concentrations fall somewhat (Alam *et al.*, 1977); the causative agent has not so far been identified, but may be related to chemotactically active peptides of the type described by Showell *et al.* (1976). Since such ion changes are typical of those elicited by paramyxoviruses (Poste & Pasternak, 1978; Impraim *et al.*, 1980), it seemed worthwhile to test whether serum from hepatitis patients causes leakage of phosphorylated metabolites and other compounds. None of the sera tested caused leakage of ³H-labelled metabolites from prelabelled human leucocytes; addition of Sendai virus resulted in leakage. It is likely that the ion changes caused by hepatitis serum are due to a faulty Na⁺+K⁺-stimulated ATPase (Alam *et al.*, 1978), and these results therefore demonstrate that, just as Sendai-virus-mediated permeability changes do not affect the Na⁺+K⁺-stimulated ATPase (Poste & Pasternak, 1978), so a faulty Na⁺+K⁺-stimulated ATPase does not necessarily result in a permeability change.

Nature of permeability changes accompanying viral release

Near-confluent cultures of MDBK cells in monolayer were infected with '1-day' Sendai virus, and plates sampled at intervals thereafter. The use of '1-day' Sendai virus, which is infective but does not elicit permeability changes (Knutton & Pasternak, 1979; Impraim *et al.*, 1979), ensures that any changes that occur are not due to the initial infection; because the virus that appears in the

Table 1. *Effect of rhabdoviruses on uptake of [³H]choline and 2-amino[¹⁴C]isobutyrate*

Lettrée cells (2 × 10⁷/ml) were exposed to 15 mM-[³H]choline and 1 μM-2-amino[¹⁴C]isobutyrate, in the presence of the viral preparations with or without 4 mM-CaCl₂, and incubated at 37°C. Uptake was assessed at various time intervals as described in the Methods and materials section; the ratios quoted are means of three determinations. Approx. 1.8% and 2.7% of [³H]choline in the medium were taken up in 15 min by control and Sendai-virus-treated cells respectively; the intracellular water compartment of control Lettrée cells at 2 × 10⁷/ml is approx. 1–2%, and that of virally swollen cells approx. 2–3%, suggesting that the (passive) uptake of choline has reached equilibrium. The uptake of 2-amino[¹⁴C]isobutyrate was 33% and 11% by control and virally treated cells respectively.

Virus preparation	³ H/ ¹⁴ C ratio in cells	
	–Ca ²⁺	+Ca ²⁺
None (control)	0.47	—
Sendai virus	2.13	0.66
Acetyleneimine-treated Sendai virus	3.12	—
Rabies virus (dialysed)	0.68	0.55
Rabies virus and Sendai virus	2.00	—
Freeze–thawed rabies virus	0.53	0.40
Freeze–thawed rabies and Sendai virus	1.77	—
Vesicular-stomatitis virus	0.59	0.49
Vesicular-stomatitis virus and Sendai virus	1.56	—
Freeze–thawed vesicular-stomatitis virus	0.55	0.44
Freeze–thawed vesicular-stomatitis virus and Sendai virus	1.94	—

Fig. 5. *Permeability changes during viral release*

Near-confluent MDBK cells on 35 mm-diameter dishes were infected with '1-day' Sendai virus (20 haemagglutination units/dish) for 1 h at 37°C, and maintained in Eagle's minimal essential medium containing Earle's salts (Flow Laboratories), supplemented with 0.5% (v/v) foetal calf serum, at 37°C. At the times indicated, the medium was removed and tested for the presence of virus (haemagglutination assay) and lactate dehydrogenase, and the residual cells were exposed to [U-¹⁴C]α-methyl glucoside in HEPES-buffered saline for 20 min at 37°C and uptake was measured, as described in the Methods and materials section. A second series of cultures was 'mock-infected' with HEPES-buffered saline and treated as above ('control' cells). ▲, Uptake of [U-¹⁴C]α-methyl glucoside (expressed as % of that taken up by control cells); ■, lactate dehydrogenase released [cumulative values, expressed as % in medium, compared with total; the corresponding % release by control cells (approx. 5% by 50 h) has been subtracted in each case]; ♦, virus released (cumulative values, expressed in haemagglutination units/ml).

Table 2. *Effect of Ca²⁺ on permeability changes during viral release*

MDBK cells were infected as described in the legend to Fig. 5. At the times stated, cultures were washed, re-incubated in HEPES-buffered saline and uptake of [U-¹⁴C]α-methyl glucoside (amount taken up in 20 min) and release of lactate dehydrogenase (amount released in 2 h) measured as described in the Methods and materials section.

Time of sampling after infection (h)	Uptake of [¹⁴ C] α-methyl glucoside (% of that by non-infected cells)		Time of sampling after infection (h)	Release of lactate dehydrogenase (%, expressed as in Fig. 5)	
	-Ca ²⁺	+4 mM-Ca ²⁺		-Ca ²⁺	+4 mM-Ca ²⁺
2	170	80	0	3	3
8.5	225	100	18	26	17
20.5	940	280	28	52	24
25	890	290			

medium is non-infective [it has haemagglutination activity but not fusion activity (Scheid & Choppin, 1974; Ishida & Homma, 1978) and does not elicit permeability changes (K. A. Foster & C. A. Pasternak, unpublished work)], permeability changes can be unequivocally ascribed to membrane alterations that take place during viral assembly and/or release. Fig. 5 shows that there is a dramatic increase in the capacity of cells to transport α-methyl glucoside, a compound that enters cells by simple diffusion; whereas virus production continues to increase throughout the test period, permeability towards α-methyl glucoside begins to return to normal after about 30 h. Table 2 shows that, in common with the increase in permeability towards α-methyl glucoside accompanying viral entry (Pasternak & Micklem, 1974b; Imprim *et al.*, 1980), the increase preceding viral release is

inhibited by Ca²⁺. Unlike the situation during viral entry (Poste & Pasternak, 1978), lactate dehydrogenase leaks out of cells into the medium during viral release; such an effect has previously been reported during release of virus by chick-embryo fibroblasts (Gilbert, 1963; Cassells, 1973; Katzman & Wilson, 1974) and other cells (Gilbert, 1963). Note that considerably more lactate dehydrogenase is released when infected cells are incubated in HEPES-buffered saline (Table 2) than when incubated in Eagle's medium (Flow Laboratories) containing 0.5% (v/v) serum (Fig. 5). Interestingly, this leakage of lactate dehydrogenase can be prevented by Ca²⁺ (Table 2). Changes in the permeability of cells to phosphorylated compounds during viral infection of cells have been documented by Carrasco (1978) and Contreras & Carrasco (1979), who have suggested them to be due to the assembly and clustering of

viral proteins at the cell surface. These authors have envisaged the use of normally non-permeant inhibitors of protein synthesis to enter infected cells selectively, and thus to inhibit viral protein synthesis. Although it is by no means clear that cells are still synthesizing viral proteins at the time that their surface membranes become modified through the presence of such proteins, the possibility of selectively killing virally modified cells is certainly an interesting one.

General discussion and conclusions

The results presented here, taken together with previous work (Poste & Pasternak, 1978), show that the permeability changes caused by Sendai virus and Newcastle-disease virus have little cell-specificity. This is not surprising, since it is known that the receptor for these viruses is wide-spread: this is a sialic acid-containing grouping present either as glycoprotein (Bachi *et al.*, 1977) or as glycolipid (Haywood, 1974), and such compounds are known to be present at the surface of most, if not all, animal cells.

On the other hand the specificity with regard to viral type is precise. Only two types of paramyxovirus, Sendai virus and Newcastle-disease virus (Poste & Pasternak, 1978), have been shown to elicit permeability changes, and even certain preparations of Sendai virus, such as '1-day' virus, are inactive (Knutton & Pasternak, 1979; Impraim *et al.*, 1979; Wyke *et al.*, 1980). A closely related (Gething *et al.*, 1978) orthomyxovirus, namely influenza virus, does not cause the changes (Poste & Pasternak, 1978; also Fig. 2), nor do other enveloped viruses such as cytomegalovirus (Poste & Pasternak, 1978), rabies virus or vesicular-stomatitis virus (Table 1 and Figs. 3 and 4), or non-enveloped viruses such as adenovirus (Poste & Pasternak, 1978). The requirements for causing permeability changes appear to be (i) the possession of a 'leaky' viral envelope (see Shimizu *et al.*, 1976) and (ii) fusion of this with the plasma membrane of cells (Wyke *et al.*, 1980). Permeability changes accompanying viral attack are therefore likely to occur clinically to a limited extent only.

In contrast, permeability changes accompanying viral release appear to be much more widespread. Carrasco (1978) and Contreras & Carrasco (1979) have shown that cells become permeable to nucleotides and other compounds during maturation of a number of viruses, including enveloped (Semliki Forest virus) and non-enveloped (encephalomyocarditis virus, mengovirus and simian virus 40) types. Although macromolecules such as lactate dehydrogenase also leak out of cells during viral proliferation, in contrast with the altered permeability accompanying viral entry (Pasternak & Micklem, 1973; Poste & Pasternak, 1978), the two types of permeability change share an important feature: each is inhibited by Ca^{2+} (compare Fig. 1 and Table 1 with Table 2). Some of the membrane changes that occur may be diagrammatically represented as shown in Scheme 1; note the similarity to Fig. 10 of Impraim *et al.* (1980). Since the possibility exists that viral release itself may be modulated by Ca^{2+} , further characterization of surface changes during viral maturation is an important topic for further study.

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Scheme 1. Membrane changes in infected cells

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