VIRUS INFECTION OF POLARIZED EPITHELIAL CELLS

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I. INTRODUCTION

The evolution of higher eukaryotes is marked by the development of defined extracellular environments. The formation of these compartments was mediated by the evolution of cells specialized to form an epithelial layer that serves to enclose and isolate a space from the surrounding milieu. Epithelial cells therefore represent one of the earliest and fundamental cell types. The observation that the trophectoderm, an epithelial cell sheet that lines the blastocoel, is the first differentiated tissue to form during embryogenesis (Fleming and Johnson, 1988) is consistent with the notion that epithelial cells fulfill a primary developmental role. As a consequence of their involvement in the definition of extracellular compartments, epithelial cell layers

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line all the body cavities of higher eukaryotes and therefore represent the primary barrier to infection of vertebrate hosts by microorganisms. A number of established cell lines derived from epithelial tissues have retained many of the differentiated properties of the tissue of origin, and provide useful model systems for studies of the interaction of microorganisms with epithelial cell layers.

Early observations of the interaction of viruses with epithelial cells were carried out by Murphy and Bang (1952), who studied the infection of the egg chorioallantoic membrane by influenza virus. They observed that the release of progeny virus particles occurred exclusively by budding at the free cell surface. Thus, release of the virus was found to be polarized, occurring at only one side of this epithelial cell layer. More recently, Rodriguez-Boulan and Sabatini (1978) reported the polarized budding of several enveloped viruses from either the apical or the basolateral surfaces of epithelial cells in culture. Subsequently, a great deal of interest has developed in studies of virus-infected epithelial cells. Studies of the intracellular transport and surface expression of viral proteins have provided important information about the mechanisms by which membrane proteins are targeted to specific plasma membrane domains of epithelial cells. In addition, the restriction of virus entry or release to specific membrane domains has significant implications for the pathogenesis of viral infections. The present article will focus largely on this aspect of the interaction of viruses with epithelial cells. The role of specific pathways of virus entry and release in the pathogenesis of viral infection will be examined together with the mechanisms utilized by viruses to circumvent the epithelial barrier.

II. PROPERTIES OF EPITHELIAL CELLS AND TISSUES

A. General Properties of Polarized Epithelial Cells

A cell is described as polarized if it can be divided into morphological or functional subdivisions. Such subdivisions are created as a result of cellular compartmentalization and the specific directional transport of cellular components. The degree of polarization in epithelial cells is more striking than that exhibited by most other cell types, because epithelial cells are organized as tissues that in general have a coherent polarity. A polarized epithelial cell (Fig. 1) is divided into several domains, but for the purposes of this discussion particular attention will be given to the plasma membrane domains and the mechanisms that define them. The basal membrane, which faces the serosal compart-
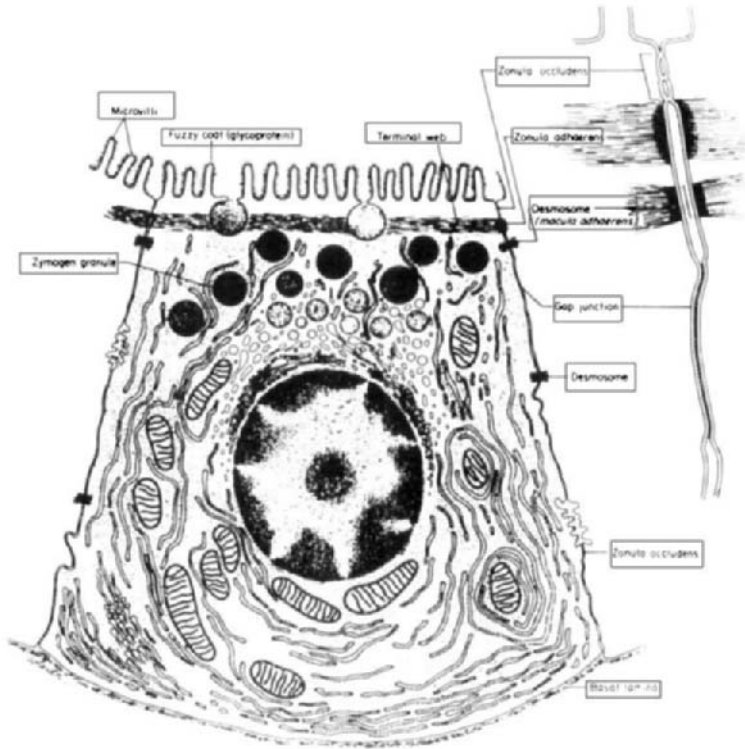
FIG. 1. Features of a typical polarized epithelial cell as seen with the electron microscope. A magnification of the junctional complex is included. (Drawing from Hay, 1973.)

ment (generally the internal environment), interacts with the underlying cells and basal lamina. This membrane is contiguous with the lateral plasma membrane, which contains a number of specialized components for cell-to-cell interactions. Both the basal and lateral cell surfaces are generally considered to be a single domain for purposes of localization studies because membrane proteins or lipids may move freely between them. In contrast the apical, or mucosal, surface is a separate and frequently specialized domain that forms a free surface and generally does not contact adherent cells or extracellular components other than coating substances. The polypeptide and lipid compositions of the apical surface are distinct from those of the basolateral domain. These differences, which are essential for epithelial cell function, are maintained by both vectorial transport and the imposition of a barrier at the apical/basolateral interface that prevents the targeted membrane components from mixing within the

lateral plane of the membrane (for recent reviews, see Rodriguez-Boulan and Nelson, 1989; Hubbard and Stieger, 1989; Cereijido *et al.*, 1989; Compans and Srinivas, 1991). The apical and basolateral plasma membrane domains are defined and separated as a consequence of the close apposition of neighboring cells that, with few exceptions, are tightly adherent to one another. Adherence is mediated in part by an area of specialized attachment structures normally located at the apical–lateral margin. Originally identified by light microscopy as a region of intense staining at the apical periphery and termed the terminal bar, this junctional complex forms a band that surrounds each cell, creating a barrier that excludes diffusible substances. The junctional complex is now known to be composed of three structural components: the zonula occludens, the zonula adherens, and the macula adherens.

The zonula occludens, or tight junction, forms the apical edge of the lateral surface and defines the boundary between the apical and lateral membrane domains. This structure is thought to function primarily as a barrier of limited permeability between the serosal and luminal spaces. However, it is misleading to suggest that the zonula occludens is absolutely impermeable. The degree of permeability varies among epithelial cell types (Gonzalez-Mariscal *et al.*, 1989), the diffusible molecules, and the extracellular environment. Estimates have also been made of the ability of epithelia to prevent the movement of molecules with defined hydrodynamic radii. For example, the human intestinal epithelial cell line T84 is able to exclude more effectively the passage of inulin, which has a radius of 1.5 nm, than mannitol, which has a radius of 0.36 nm (Madara and Dharmsathaphorn, 1985). The restriction on the movement of diffusible substances imposed by the zonula occludens is mediated by the extremely close contact of neighboring cell plasma membranes that by electron microscopy appear to fuse, resulting in the exclusion of intercellular fluid. The extent of these membrane contacts has been equated with the ability of distinct epithelia to maintain a permeability barrier. Highly impermeable epithelia, such as intestinal or urinary bladder epithelia, have extensive contacts whereas epithelia, such as some kidney tubule epithelia, with fewer membrane contacts appear less able to exclude diffusible substances (Claude and Goodenough, 1973; Claude, 1978). Freeze-fracture studies have revealed that the points of apparent membrane fusion are composed of linear arrays of filaments, of variable complexity, that traverse the apposing membranes (Farquhar and Palade, 1963; Martinez-Palomo and Erlij, 1975). There is some controversy over whether these filaments represent complexes of lipid or protein. Although the structures resemble cylindrical, inverted lipid micelles in some respects (Kachar and Reese, 1982; Pinto da Silva and Kachar, 1982),

available evidence indicates that proteins are both intimately involved in the formation and function of tight junctions (Griepp et al., 1983; Behrens et al., 1985) and are localized to these specific regions (Imhoff et al., 1983; Fey et al., 1984; Stevenson et al., 1986). The tight junction appears to be highly susceptible to virus-induced cytopathogy, because infection of various epithelial cells with either enveloped or nonenveloped viruses results in a reduction in transepithelial resistance and increased permeability to macromolecules, which in many cases occurs prior to the onset of a visible cytopathic effect (Lopez-Vancell et al., 1984; Svensson et al., 1991; S. Tucker, unpublished observations).

In addition to its role as a permeability barrier for extracellular materials there is considerable evidence to suggest that the zonula occludens functions as a "fence" restricting the passage of plasma membrane components. Disruption of cell-to-cell contacts results in the redistribution of polarized enzyme markers (Ziomek et al., 1980). The apical and basolateral plasma membranes have distinct lipid compositions that become equalized on disruption of the zonula occludens (van Meer and Simons, 1982; 1986). Plasma membrane protein polarization correlates with the formation of the tight junction (Balcarova-Stander et al., 1984; Herzlinger and Ojakian, 1984); and the boundary of apical and basolateral plasma membrane protein markers has been localized precisely to the zonula occludens. This apparent restriction in the mobility of plasma membrane constituents imposed by the zonula occludens is confined to the outer leaflet of the lipid bilayer (Dragsten et al., 1981; van Meer and Simons, 1986). However, the expression of a polarized phenotype is not absolutely dependent on the integrity of the zonula occludens because polarized budding of enveloped viruses (Basak et al., 1983; Rodriguez-Boulan et al., 1983) and the polarized expression of plasma membrane markers (Ziomek and Johnson, 1980; Johnson and Ziomek, 1981; Vegas-Salas et al., 1987) have been observed in epithelial cells that lack the appropriate cell contacts necessary for the formation of a contiguous zonula occludens.

The zonula adherens, or belt desmosome, characteristically lies just below the zonula occludens and comprises a region 200–500 nm wide, which surrounds the cell where neighboring plasma membranes are separated by a uniform space of 15–20 nm. This space presumably contains materials that mediate cell adhesion, considered to be the primary function of the zonula adherens. The cytoplasmic face of the plasma membrane within this region is lined with a moderately electron-dense structure from which 6-nm actin microfilaments project into the cytoplasm. These filaments are connected to a belt, known as the terminal web, which extends across the breadth of the cell and is connected to the cellular actin network (Hull and Staehelin, 1979;

Tilney, 1983). There is evidence that the terminal web is contractile and may be responsible for cell motility, as exemplified by the contractile nature of the intestinal epithelial cell brush border (Burgess, 1982). The tension imposed by the contractile ring is countered by the adhesive properties of cellular adhesion molecules, such as uvomorulin, which also localize to the zonula adherens (Boller et al., 1985).

The macula adherens, or desmosome, is also involved in mediating cell-to-cell contact of epithelial and other cell types. In several types of epithelial cells these structures are numerous and at different levels within the lateral membrane below the zonula adherens. In contrast to the latter component, which is continuous, the macula adherens consists of a series of disk-shaped spots arranged in a row around the cell periphery. Each spot represents an area where the plasma membranes of neighboring cells are thought to be linked across a gap of 30 nm, providing a strong adhesive contact (Grinnell, 1978). The importance of this contact as a means of ensuring the integrity of the epithelium is exemplified by the high number of desmosomes in tissues subject to mechanical stress, such as the epidermal epithelium (McNutt and Weinstein, 1973). An adjacent cytoplasmic plaque containing a variety of proteins has been described (Geiger et al., 1983; Franke et al., 1981; Mueller and Franke, 1983; Gorbsky et al., 1985) from which bundles of tonofilaments emanate. These connect to a network of intermediate filaments, characteristic of epithelial cells, which encase the nucleus and extend to hemidesmosomes in the basal membrane (Fey et al., 1984). Hemidesmosomes, which are morphologically similar to half desmosomes, are found at the basal surface of some epithelial cells and mediate attachment to the basal lamina (Gipson et al., 1983; Steinberg et al., 1987). The basal lamina, also known as the basement membrane, is a specialized form of extracellular matrix composed of proteoglycans (largely heparan sulfate proteoglycans), type IV collagen, and laminin, which are secreted by many epithelial cell types (Kleinman et al., 1981; Timple and Martin, 1982). The basal lamina is generally apparent as a continuous layer of electron-dense material, 50–100 nm wide, underlying the epithelial cell sheet. The gap junction is an additional cell-to-cell contact point present in epithelial and other cell types. Gap junctions are unlikely to provide physical support but instead serve to form channels that allow the passage of metabolites and ions between cells (Hertzberg and Gilula, 1979; Lowenstein, 1981; Hertzberg and Skibbens, 1984).

The surfaces of epithelial cells also exhibit a number of characteristic features that are consistent with their specialized function. Most epithelial cells have projections, termed microvilli, that emanate from the apical plasma membrane and serve to increase the available

cell surface area. This function is exemplified in epithelial cells that specialize in fluid transport. Epithelial cells of intestinal or kidney tubule origin, for example, exhibit such densely packed arrays of tall microvilli that they may be readily detected by light microscopy. These arrays are termed striated and brush borders, respectively. Other epithelial cell types may have smaller, more irregular microvilli. Microvilli contain a core of actin filaments that interact with the terminal web. The filaments provide support for the microvilli and may serve to mediate the limited motility of microvilli apparent in some tissues (Burgess, 1982). Certain epithelial cells, such as those found lining the trachea, bronchi, or oviducts, are ciliated. Cilia are distributed across the apical surface of these cells and, by their coordinated movement, serve to facilitate the flow of mucous across epithelial surfaces or the movement of fluids and other substances through ducts and tubular organs. Some epithelial cells also exhibit a series of extensive folds in their lateral and/or basal plasma membrane surfaces that are involved in fluid transport.

B. Epithelial Tissues

Although various epithelial cells exhibit some common features, epithelial tissues are composed of cells with distinct and varied morphologies. Epithelia can be divided into at least eight different types on the basis of morphological and functional attributes (Fig. 2). These types may be grouped into two broad categories: simple epithelia, which consist of a single layer of cells, and complex or stratified epithelia, which are composed of several cell layers. Whereas all cells in the first category remain in contact with the basal lamina, the cells that form the superficial layers of stratified epithelia have no contact with the basal lamina. In addition, certain epithelia have been given specific names. The epithelial cells lining the vascular system form the endothelium and the cells that line the thoracic, pericardial, and abdominal cavities are termed the mesothelium. Both of these epithelia are generally considered to belong to the simple squamous group (see below).

Simple epithelia are divided into squamous, cuboidal, and columnar cell types. A simple squamous epithelial cell has a flattened morphology and is arranged in close association with its neighbors. Such cells may be isolated from the alveolar lining in the lung, the renal glomerulus, and some renal collecting tubules. As the name suggests, cuboidal epithelial cells have an approximately cubic morphology. These cell types are involved in secretion and absorption processes and may be found lining tissues of the renal tubular system, among others.
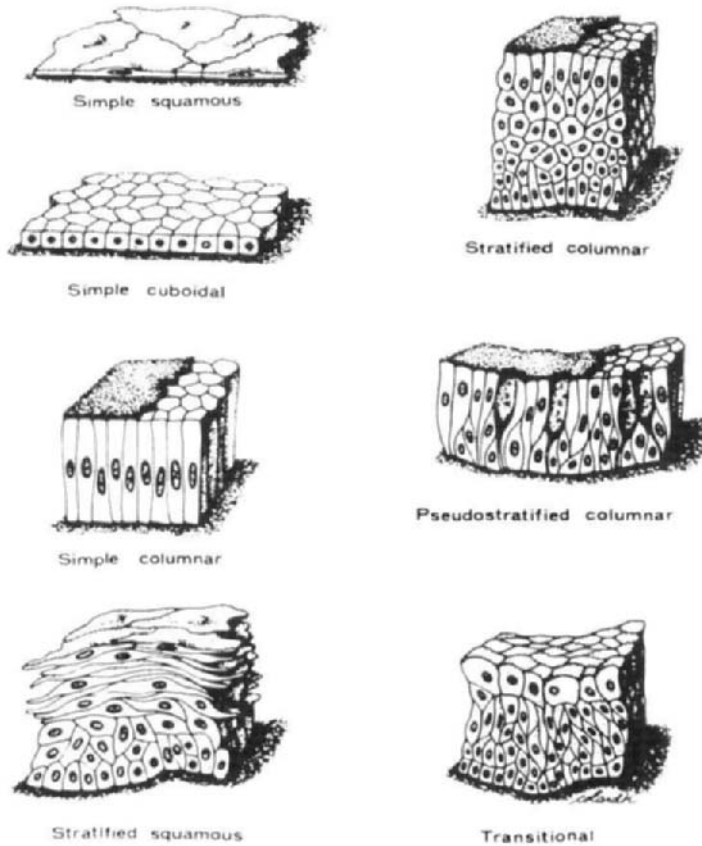
FIG. 2. The arrangement and shape of epithelial cells in the principal types of epithelial tissues. (Drawing from Bloom and Fawcett, 1975.)

In contrast, columnar epithelia are composed of tall, rectangular cells that frequently exhibit marked polarization of subcellular components. Cells lining the villus of the small intestine and Fallopian tube are examples of this type.

Complex epithelia are grouped into four categories: transitional, stratified squamous, stratified cuboidal and columnar, and pseudostratified. Transitional epithelia are composed of large surface cells that project into the lumen and smaller basal cells that interdigitate with their larger neighbors. This cell type lines the bladder, the urinary tract, upper urethra, and ureters. Stratified squamous epithelia are composed of cells at various stages of differentiation arranged in multiple layers. The proliferating cells are located in the

basal layer and are the least differentiated. As the cells migrate to the surface of the epithelium they undergo differentiation, becoming terminally differentiated in the uppermost layer. Cells of this type form the largest organ of the body, the skin, and are also found lining the mouth, esophagus, vagina, and cornea. The latter types are generally covered in fluid and contain little keratin. In contrast the epidermal cells, which are exposed to the air, become keratinized and anuclear. Interestingly, injury of a nonkeratinized stratified squamous epithelium frequently results in the synthesis of keratin and the development of a keratinized phenotype. Surfaces covered by stratified cuboidal and columnar epithelial cells are less widely distributed but may be found in the sweat gland and intermediate zones of the pharynx, larynx, and conjuctiva, and female urethra. The last cell type of this group is the pseudostratified columnar epithelial cell. The term *pseudostratified* is used because both the differentiated surface cells and the additional layer of basal cells maintain their attachment to the basal substratum. These cells are located in regions such as the trachea, large bronchi, endocervical canal, and vas deferens.

C. Polarized Epithelial Cells in Culture

The growth of epithelial cells in culture has provided a powerful tool for investigators interested in the mechanisms of cell polarization and the regulation of epithelial transport processes. Renal physiologists initiated these studies using the Madin–Darby canine kidney (MDCK) cell line in the late 1960s, following the observations of Leighton *et al.* (1969) that this cell line exhibits structural and functional features of native epithelia. MDCK cells have since become the most widely studied and most extensively characterized epithelial cell line. Many other continuous cell lines have subsequently become available, some of which are briefly described in Table I. Each of these cell lines exhibits features that, to varying degrees, correspond to those of the tissue of origin. In general, the cultured cells form organized epithelial layers when grown to confluence; these layers exhibit junctional complexes and defined apical (facing the culture media) and basolateral (facing the culture dish) domains. The culture of epithelial cells on porous supports (Michalopoulos and Pitot, 1976; Misfeldt *et al.*, 1976; Cereijido *et al.*, 1978) was a significant advance in the field because this enabled an epithelium to be established under conditions that more closely resemble those prevalent *in vivo*. Epithelia grown on porous supports show evidence of increased differentiation in comparison to epithelial cells grown on conventional solid surfaces (Shannon and Pitelka, 1981; van Meer and Simons, 1982; Handler *et al.*, 1984) and

TABLE I

SOME ESTABLISHED EPITHELIAL CELL LINES
EXHIBITING CHARACTERISTICS OF DIFFERENTIATED TISSUES

Cell line	Species	Tissue source	Refs.
A6	*Xenopus laevis*	Kidney distal tubule or collecting duct	Rafferty (1969); Perkins and Handler (1981)
TB-M TB-6c	*Bufo marinus*	Urinary bladder	Handler *et al.* (1979)
OK	Opossum	Kidney proximal tubule	Koyama *et al.* (1978); Malmström *et al.* (1987)
M-mTAL-IC M-mTAL-IP	Mouse	Kidney medullary thick ascending limb	Valentich and Stokols (1986a,b)
MME	Mouse	Mammary epithelium	Damsky *et al.* (1981)
GRB-MAL	Rabbit	Kidney medullary thick ascending limb	Burg *et al.* (1982); Green *et al.* (1985)
GRB-PAP 1	Rabbit	Kidney inner medullary epithelium	Bagnasco *et al.* (1987)
MDCK	Dog	Kidney distal tubule or collecting duct	Madin and Darby (1958)
MDBK	Bovine	Kidney epithelium	Madin and Darby (1958); Ishizuka *et al.* (1978)
LLC-PK1	Pig	Kidney proximal tubule	Hull *et al.* (1976); Perantoni and Berman (1979)
JTC-12	Monkey	Kidney proximal tubule	Takaoka *et al.* (1962); Takuwa and Ogata (1985)
Vero C1008	Monkey	Kidney epithelium	Srinivas *et al.* (1986)
Caco-2	Human	Colon adenocarcinoma	Fogh *et al.* (1977)
HT29	Human	Colon adenocarcinoma	Fogh *et al.* (1977)
T84	Human	Colon adenocarcinoma	Murakami and Masui (1980)

the investigator is provided with ready access to both the apical and basolateral surfaces. As a result of the comparative simplicity of these systems, much of the data generated on the interactions of viruses with epithelial surfaces are derived from studies using continuous cell lines. For this reason some of the well-characterized epithelial cell lines will be described in more detail.

The MDCK cell line was derived from the kidney of a female cocker spaniel (Madin and Darby, 1958) and exhibits several features characteristic of renal epithelium, including brush borders, junctional complexes, and defined lateral spaces (Misfeldt *et al.*, 1976; Cereijido *et al.*,

1978, 1980). The tight junctions are functional and resist the passage of ions, causing a significant transepithelial resistance and the establishment of a measurable ionic gradient (Misfeldt et al., 1976; Cereijido et al., 1978). Endogenous plasma membrane polypeptides are expressed in a polarized fashion (Richardson and Simmons, 1979) and an asymmetric distribution of enzyme activities consistent with native epithelial activity has been demonstrated (Simons and Fuller, 1985; Gstraunthaler, 1988). The cell line retains additional characteristics of a differentiated renal epithelium, including appropriate hormone responsiveness (Ishizuka et al., 1978; Rindler et al., 1979) and the expression of mineral corticoid-binding protein (Ludens et al., 1978). Based on these and other observations it has been proposed that MDCK cells most closely resemble the epithelium found lining the renal collecting duct (Rindler et al., 1979; Barker and Simmons, 1981). At least two strains of MDCK cells have been isolated from the original cell line. These differ on the basis of morphology (Barker and Simmons, 1981; Valentich, 1981), transepithelial resistance (Barker and Simmons, 1981; Richardson et al., 1981), distribution of glycosphingolipids (Hansson et al., 1986; Nichols et al., 1986), metabolism of arachidonic acid (Lewis and Spector, 1981), and the polarity of at least one plasma membrane glycoprotein (Ojakian, 1987). A bovine kidney cell line (MDBK) that also forms polarized monolayers has also been widely used in studies of virus infection (Madin and Darby, 1958).

Numerous cell lines derived from human adenocarcinomas have been described (Fogh, 1975; Fogh et al., 1977). Although in most cases these exhibit limited differentiation, there are exceptions. Three cell lines of this type, HT29, Caco-2, and T84, are arguably the most well characterized. HT29 cells exhibit a highly differentiated phenotype when grown under specific nutrient conditions. Cells resembling terminally differentiated enterocytes and goblet cells may be distinguished when glucose is omitted or substituted for galactose, inosine, or uridine (Pinto et al., 1982; Wice et al., 1985; Zweibaum et al., 1985). Several clones and subclones of the parental cell line have been isolated, resulting in cell populations that express only a single differentiated phenotype, either enterocyte or goblet cell, under appropriate growth conditions (Huet et al., 1987). HT29 cells express four brush border enzymes, aminopeptidase N, dipeptidyl peptidase IV, alkaline phosphatase, and sucrose isomaltase, which are typical of cells derived from the fetal colon (Pinto et al., 1982; Zweibaum et al., 1983, 1984, 1985).

Unlike HT29 cells, the Caco-2 and T84 cell lines spontaneously form monolayers of polarized epithelial cells that develop a high trans-

epithelial resistance (Pinto *et al.*, 1983; Dharmsathaphorn *et al.*, 1984, 1985). Caco-2 cells exhibit apical microvilli, transport water and ions to the basolateral surface, form domes on impermeable substrates, and express several enzyme activities typical of normal small intestine absorptive enterocytes (Pinto *et al.*, 1983). Because the membrane hydrolases expressed by Caco-2 cells resemble those found in fetal tissues, it has been proposed that they are most similar to normal human fetus enterocytes that transiently express these activities at approximately the 15-week stage (Hauri *et al.*, 1985). A comparison of the properties of Caco-2 cells and epithelial cells of the normal small intestine suggests that they most closely resemble cells lining colonic crypts (Grasset *et al.*, 1984). In contrast, T84 cells do not express similar brush border enzyme activities and lack well-developed cilia. Because T84 cells resemble crypt cells on the basis of morphology, electrical resistance, and ionic transport properties, it has been proposed that they are derived from committed cells of this type (Madara and Dharmsathaphorn, 1985). Both T84 and Caco-2 cells synthesize and secrete basal lamina components (Madara *et al.*, 1987).

III. Virus Entry and Release from Model Epithelial Cell Lines

A. Entry

Virus infection is mediated by binding of a viral attachment protein on the surface of the virion to a component of the plasma membrane. The latter molecule is defined as the host cell receptor if, subsequent to binding, infection of the cell occurs (Tardieu *et al.*, 1982). The presence or absence of the host cell receptor molecule(s) therefore determines whether the virus may gain entry into the cell and establish an infection. Indeed, the differential expression of host cell receptors is a major determinant of viral host range and tissue tropism (Holland, 1961; Lonberg-Holm and Phillipson, 1974; Crowell and Landau, 1979; Paulson, 1985; Mims, 1986). In the context of virus infection of epithelial cells, the polarity of host cell receptor distribution defines the domain from which infection may be mediated. Thus vesicular stomatitis virus (VSV) entry is restricted to the basolateral surface of MDCK cells (Fuller *et al.*, 1984), whereas simian virus 40 (SV40) infection occurs only following binding to the apical plasma membrane domain (Clayson and Compans, 1988). A summary of the data obtained to date concerning virus entry into polarized epithelial cells is provided in Table II. In most cases studied, the characteristics of entry appear to be similar for various epithelial cell types. It is apparent that

TABLE II

THE POLARITY OF VIRUS ENTRY AND RELEASE FROM POLARIZED EPITHELIAL CELLS[a]

Virus	Epithelial cell type	Polarity of entry[a]	Polarity of release	Refs.
Orthomyxovirus				
Influenza	MDCK	Nonpolar	Apical	Rodriguez-Boulan and Sabatini (1978); Fuller et al. (1984)
	Caco-2	ND	Apical	Rindler and Traber (1988)
	Mouse mammary	ND	Apical	Roth et al. (1983b)
	Vero C1008	ND	Apical	Basak et al. (1983); Srinivas et al. (1986)
Paramyxovirus				
Sendai	MDCK	ND	Apical	Rodriguez-Boulan and Sabatini (1978); Tashiro et al. (1990a,b)
	Mouse bronchial[b]	ND	Apical	Tashiro et al. (1990a,b)
	Mouse olfactory[b]	ND	Apical	Lundh et al. (1987)
Sendai F1-R	MDCK	ND	Nonpolar	Tashiro et al. (1990a,b)
SV5	MDCK	ND	Apical	Rodriguez-Boulan and Sabatini (1978)
Rhabdovirus				
Vesicular stomatitis	MDCK	Basolateral	Basolateral	Fuller et al. (1984); Rodriguez-Boulan and Sabatini (1978)
	Vero C1008	ND	Basolateral	Srinivas et al. (1986)
	Caco-2	ND	Basolateral	Rindler and Traber (1988)
	Mouse olfactory[b]	ND	Basolateral	Lundh et al. (1987)
	Mouse mammary	ND	Basolateral	Roth et al. (1983b)
Alphavirus				
Semliki Forest	MDCK	Basolateral	Basolateral	Fuller et al. (1985b)
Retrovirus				
Friend MuLV	Mouse mammary	ND	Basolateral	Roth et al. (1983b)
Moloney MuLV	Mouse mammary	ND	Basolateral	Roth et al. (1983b)
Kirsten MuLV	Mouse mammary	ND	Basolateral	Roth et al. (1983b)
Rauscher MuLV	Mouse mammary	ND	Basolateral	Roth et al. (1983b)
REV-A	MDCK	ND	Basolateral	Roth et al. (1983b)

TABLE II   (*Continued*)

Virus	Epithelial cell type	Polarity of entry[a]	Polarity of release	Refs.
RD114	MDCK	ND	Basolateral	Roth *et al.* (1983b)
HIV-1	Vero C1008	ND	Basolateral	Owens *et al.* (1991)
HIV-1	HT29-D4	Nonpolar	Basolateral	Fantini *et al.* (1991a,b)
Poxvirus				
Vaccinia	MDCK	Basolateral	ND	Rodriguez *et al.* (1991)
Vaccinia	Choroid plexus[b]	ND	Basolateral	Kristensson *et al.* (1984)
Herpesvirus				
Herpes simplex	MDCK	Nonpolar	ND	Sears *et al.* (1991)
	MDBK	ND	Basolateral	Srinivas *et al.* (1986)
Bunyavirus				
Punta Toro	Vero C1008	ND	Basolateral	Chen *et al.* (1991)
Rift Valley fever	Rat hepatocytes[c]	ND	Basolateral	Anderson and Smith (1987)
Rotavirus				
Rhesus rotavirus	MDCK	Nonpolar	ND	Svensson *et al.* (1991)
Reovirus				
Reovirus type 1	Intestinal cells[b]	Basolateral	ND	Rubin (1987)
	Caco-2	Nonpolar	ND	
Enterovirus				
Poliovirus	Vero C1008	Nonpolar	Nonpolar	Tucker *et al.* (1992a)
	Caco-2	Nonpolar	Apical	
Parvovirus				
Canine parvovirus	MDCK	Basolateral	ND	Basak and Compans (1989)
Papovavirus				
SV40	MDCK	Apical	ND	Clayson and Compans (1988)
	Vero C1008	Apical	Apical	Clayson *et al.* (1989)
	African green monkey kidney[c]	Apical	Apical	

[a] A summary of some of the data currently available. ND, No data.

[b] Native epithelia.

[c] Primary tissue culture.

some viruses exhibit a marked restriction of binding/entry to a specific membrane domain, whereas others exhibit little preference. Because some viruses may bind to more than a single host cell receptor molecule the possibility exists that infection by viruses in the latter category is mediated by binding to a different receptor population on each membrane domain. Indeed, this appears to be the mechanism by which herpes simplex viruses enter epithelial cells via either surface. Evidence indicates that the infectious route of a glycoprotein C (gC)-deficient herpes simplex virus 1 (HSV-1) mutant is exclusively polarized to the basolateral surface of MDCK cells (Sears *et al.*, 1991). Because wild-type HSV-1 was able to infect MDCK cells following adsorption to either the apical or basolateral surfaces, it was concluded that HSV-1 infection is mediated by two different host cell receptor molecules; one expressed on the apical domain that must interact with gC to mediate infection, and a second located on the basolateral surface that binds to viral attachment proteins other than gC (Sears *et al.*, 1991). This example also illustrates the potential advantages of epithelial cells in the identification of host cell receptors.

As an alternative to the utilization of different receptor molecules, nonpolarized entry may be mediated by binding to a single receptor molecule expressed on both surfaces at sufficient density to mediate virus infection. Influenza virus is therefore able to bind at both the apical and basolateral domains because membrane glycoproteins on both surfaces contain sialic acid residues, which serve as receptors for the viral hemagglutinin. In studies of poliovirus (Tucker *et al.*, 1992a), which binds to an immunoglobulin-like host cell receptor (Mendelsohn *et al.*, 1989), binding to both the apical and basolateral surfaces of Caco-2 and Vero C1008 epithelial cell lines was inhibited by prior incubation with a monoclonal antibody (D171) that is known to block the poliovirus cellular receptor (Nobis *et al.*, 1985). Because adsorption to either surface resulted in infection, the simplest interpretation of these data is that the level of poliovirus host cell receptor expression on both the apical and basolateral surfaces is sufficient to mediate virus entry from either domain.

B. Release

In 1978 Rodriguez-Boulan and Sabatini reported that enveloped RNA viruses are released from MDCK cells in a polar fashion; VSV budded predominantly from the basolateral plasma membrane whereas Sendai and influenza virions were released exclusively from the apical domain (see Fig. 3). Type C retroviruses were also observed to be shed preferentially from the basolateral membrane of polarized epi-
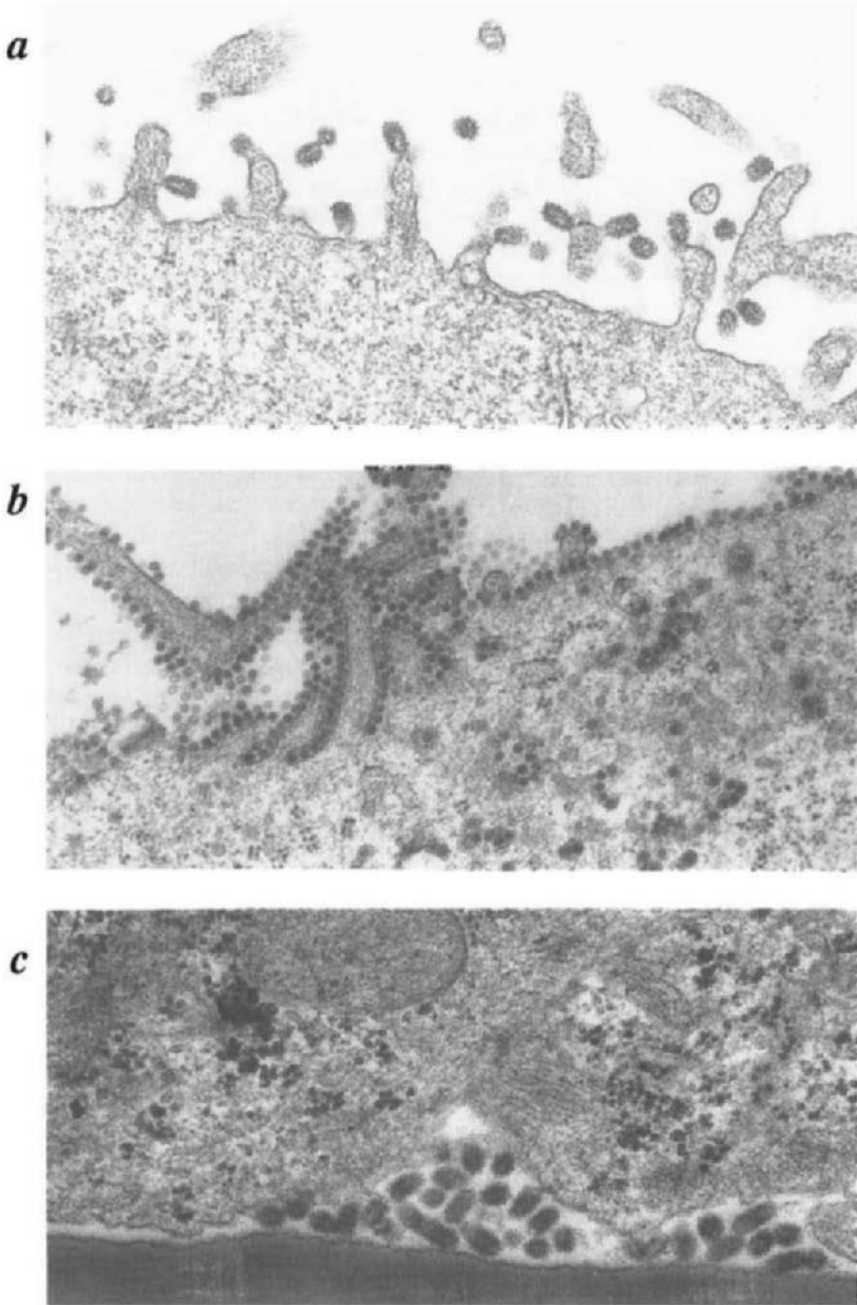
FIG. 3.   Polarized release of viruses from epithelial cells in tissue culture. (a) Influenza virus (strain WSN) release from the apical surface of MDCK cells at 8 hr postinfection (magnification, ×35,000). (b) Association of SV40 virions with the apical plasma membrane of polarized African green monkey kidney epithelial cells at 48 hr postinfec-

thelial cells (Roth et al., 1983b). More detailed analysis revealed that the envelope glycoproteins of these viruses accumulated on the same membrane domain from which budding occurs (Rodriguez-Boulan and Pendergast, 1980; Roth et al., 1983a,b). The mechanism of virus assembly (reviewed by Stephens and Compans, 1988) is of relevance to studies concerned with the process by which viruses may bud asymmetrically from infected epithelial cells. The glycoproteins of enveloped viruses that assemble at the plasma membrane are directionally transported to the surface from which virus buds when expressed in the absence of other virus-specific polypeptides (Roth et al., 1983a; Jones et al., 1985; Stephens et al., 1986). These observations have led to the hypothesis that the site of insertion of the envelope glycoprotein(s) determines the site of viral assembly. A report on the assembly of retrovirus particles in polarized epithelial cells supports this hypothesis (Owens et al., 1991). Infection of epithelial cells with a recombinant vaccinia virus expressing the HIV-1 Gag (core) polyprotein in the absence of the envelope glycoprotein resulted in the assembly and release of HIV-like particles in approximately equivalent amounts from both apical and basolateral domains. In contrast, coexpression of gag and env genes resulted in targeting of 94–97% of the particles to the basolateral domain. Because the envelope glycoprotein exhibited an almost exclusive distribution to this domain when expressed from a recombinant vector (Owens and Compans, 1989), it is reasonable to conclude that the envelope glycoprotein is the only targeted component, and that its interaction with gag defines the domain from which virus is selectively released. It is not clear whether a similar situation exists with other viruses. For example, a VSV temperature-sensitive mutant (ts045) that exhibited a defect in the transport of its envelope glycoprotein, G, at the restrictive temperature still budded from the basolateral domain (Bergmann and Fusco, 1988). In this case preferential transport of the M protein to the basolateral plasma membrane was reported, suggesting that M protein may be targeted similarly to G. However, the spikeless particles produced by the ts045 mutant are known to contain the membrane anchor region of the G protein (Matsikko and Simons, 1986), and this may play an essential role in determining the maturation site of the virus particles produced by this mutant.

Several enveloped viruses that assemble at intracellular membranes are also asymmetrically released from polarized epithelial cells.

tion (magnification ×35,000). (c) Vesicular stomatitis virus budding from the basolateral plasma membrane of Caco-2 cells at 8 hr postinfection (magnification, ×44,600).

Herpes simplex viruses (HSV) 1 and 2, which assemble and bud at the inner nuclear membrane (Darlington and Moss, 1968), are preferentially released at the basolateral plasma membrane (Srinivas *et al.*, 1986). Because herpesviruses bud into the lumen of the nuclear envelope, which is continuous with the endoplasmic reticulum, the virions are presumably transported by the same mechanism responsible for the delivery of soluble, secreted polypeptides by these cells. The expression of soluble, truncated variants of membrane-bound glycoproteins has resulted in a nonpolar pattern of release from MDCK cells that is thought to be the result of a default or "bulk-flow" transport pathway in this cell type (Stephens and Compans, 1986; Gonzalez *et al.*, 1987). Similar conclusions were drawn following the expression of exogenous secreted polypeptides in MDCK cells (Kondor-Koch *et al.*, 1985; Gottlieb *et al.*, 1986) or treatment of cells with NH_4Cl (Caplan *et al.*, 1987). The basolateral targeting of HSV therefore implies that the virions express targeting signals recognized in a luminal context, or that an interaction occurs between virions and a targeted membrane-bound factor(s). In this respect the virions may resemble endogenous secretory products that are released in a directional fashion. Bunyaviruses and coronaviruses, which are also assembled at intracellular membranes, use a similar transport pathway. Most bunyavirus assembly occurs in the Golgi complex and budding occurs at smooth-surfaced membranes in this region (Murphy *et al.*, 1973; Smith and Pifat, 1982), after which virus is transported to the cell surface. Punta Toro virus, a member of the sandfly fever group of bunyaviruses, was released virtually exclusively from the basolateral surface of polarized epithelial cells following assembly in the Golgi complex (Chen *et al.*, 1991). Immunoelectron microscopic analysis of hepatocytes infected with another bunyavirus, Rift Valley fever virus, also revealed an apparent selective release from the basolateral domain (Anderson and Smith, 1987). Because in the latter example direct virus budding through the basolateral plasma membrane was also sometimes observed, at least one of the components of Rift Valley fever virus contains the appropriate signals to direct vectorial transport prior to virion assembly.

The vectorial transport and release of nonenveloped viruses has been less widely investigated. However, studies using SV40 and poliovirus have indicated that nonenveloped viruses may also be targeted for release at a particular plasma membrane domain. Simian virus 40 is a nonenveloped virus that is assembled in the nucleus of infected cells. An almost exclusive release of SV40 from the apical surface of polarized Vero C1008 and primary African green monkey kidney epithelial cells has been observed (Fig. 3; Clayson *et al.*, 1989). It was also noted that treatment of infected cells with the sodium

ionophore monensin, which serves as an inhibitor of vesicular transport, resulted in the inhibition of SV40 release but had no effect on virus-specific protein synthesis or the assembly of infectious virus. Because extensive virus release was observed prior to detectable cell lysis, the vectorial transport and release of SV40 may therefore be mediated by a vesicular transport mechanism in these cell types. Consistent with this hypothesis, cytoplasmic virions have been observed to be enclosed within membranous vesicular compartments during the period of virus release (Clayson et al., 1989). Because the SV40 receptor is expressed on the apical surface (Clayson and Compans, 1988) it is possible that targeting of progeny virions to this domain is mediated by intracellular association with receptor molecules. Poliovirus assembles in the cytoplasm and virions have been detected by electron microscopy in several forms; free within the cytoplasm, tightly packed in crystalline arrays, and within membrane-enclosed bodies (Dales et al., 1965; Dunnebacke et al., 1969). Poliovirus was predominantly released from the apical domain of Caco-2 intestinal cells (Tucker et al., 1992b). Poliovirus release is generally considered to be mediated by cellular lysis, but several apparently nonlytic processes have also been described (Dunnebacke et al., 1969; Bienz et al., 1973). Although the mechanism of vectorial release from Caco-2 cells is currently unclear, it is conceivable that the targeting of poliovirus-containing vesicles or cytoplasmic aggregates to the apical plasma membrane may be involved. In this respect the processes involved may be similar to those carried out by specialized secretory epithelial cells that package their secretory products into membrane-enclosed granules prior to secretion at a particular membrane domain (Burgess and Kelly, 1987). Interestingly, monensin had little or no effect on the release of poliovirus from Caco-2 or Vero C1008 cells (Tucker et al., 1992b), suggesting that more than a single mechanism may be involved in the vectorial release of nonenveloped viruses.

C. Mechanisms of Protein Sorting in Epithelial Cells

Viral glycoproteins have been extensively used as model systems to investigate the mechanism of protein sorting in polarized epithelial cells. This area of research has been the subject of a number of other reviews (Roth, 1989; Simons and Wandinger-Ness, 1990; Compans and Srinivas, 1991) and will be summarized briefly here. Studies have focused on the identification of sorting signals for polarized transport, as well as the intracellular events in the sorting of proteins destined for distinct plasma membrane domains. Because identical patterns of polarized expression were observed on expression of viral membrane

glycoproteins from cDNA clones in MDCK and other polarized epithelial cell lines, it was concluded that sorting signals are an intrinsic property of the glycoproteins and not dependant on other virion components, nor were they specific to MDCK cells (Roth *et al.*, 1983a; Jones *et al.*, 1985; Stephens *et al.*, 1986). These sorting signals are thought to be defined by a polypeptide sequence because viruses continued to bud asymmetrically in the presence of glycosylation inhibitors (Roth *et al.*, 1979; Green *et al.*, 1981). However, no conserved amino acid sequence motifs have been identified in polypeptides sorted to the same domain, and similarly sorted proteins lack obvious tertiary or quaternary structural similarities. Attempts to identify glycoprotein sorting signals by the expression of deletion mutants or chimeric molecules have met with mixed success (reviewed by Roth, 1989; Simons and Wandinger-Ness, 1990; Compans and Srinivas, 1991; Hopkins, 1991). Recent evidence has, however, identified two types of structures that may function as signals for polarized transport of membrane glycoproteins. Proteins linked to membranes by a glycosyl-phosphotidylinositol (GPI) anchor are localized on apical membranes, and introduction of a GPI anchor is sufficient to redirect proteins from the basolateral to the apical surface (Brown *et al.*, 1989; Lisanti *et al.*, 1989). Thus, the GPI anchor may serve as a signal for apical transport. Another set of studies with mutant glycoproteins has indicated that an amino acid sequence thought to form a hydrogen-bonded loop, or β turn, in the cytoplasmic domain, which may also function as an internalization signal and in some cases involves a tyrosine residue, may serve as a signal for basolateral targeting (Lazarovits and Roth, 1988; Vega and Strominger, 1989; Ktiskakis *et al.*, 1990; Brewer and Roth, 1991; Hopkins, 1991; Hunziker *et al.*, 1991).

Biochemical and immunoelectron microscopic studies of MDCK cells doubly infected with VSV and influenza virus have provided evidence that their respective glycoproteins are separated and consigned to specific transport vesicles following, or coincident with, exit from the Golgi complex (Rindler *et al.*, 1984; Fuller *et al.*, 1985a). It has also been demonstrated that glycoprotein transport in MDCK cells involves direct insertion into the target plasma membrane domain without a transient appearance in the opposing membrane (Matlin and Simons, 1984; Misek *et al.*, 1984; Pfeiffer *et al.*, 1985; Rindler *et al.*, 1985), and it appears that glycoprotein sorting occurs at a terminal, or immediately post-Golgi compartment. Additional indirect evidence suggests that the Golgi complex is involved. Lysosomotrophic agents that raise the pH of several intracellular compartments, including the Golgi complex, resulted in missorting of secreted proteins normally released at the basolateral surface (Caplan *et al.*, 1987). No effect was observed

on an antigen secreted at the apical surface or on membrane-bound polypeptides destined for the apical or basolateral compartments (Caplan *et al.*, 1986; Matlin, 1986). Treatment of VSV or influenza virus-infected MDCK cells with the sodium ionophore monensin, which is thought to interfere with the exit of proteins from the Golgi complex, resulted in complete inhibition of VSV G transport whereas influenza hemagglutinin (HA) was expressed on the apical membrane and incorporated into infectious virus (Alonso and Compans, 1981; Alonso-Caplen and Compans, 1983). These results are consistent with the observation that low extracellular pH in the apical domain had no effect on the polarity of influenza virus glycoprotein expression (Daniels-Holgate and Edwardson, 1989). Vesicular structures apparently responsible for the transport of influenza HA from the Golgi complex to the plasma membrane have also been described (Rodriguez Boulan *et al.*, 1984). However, the transport pathway found in kidney cells differs from that of epithelial cells derived from other organs, such as liver and intestine, in which basolateral-to-apical transport routes have been observed (Hauri *et al.*, 1979; Quaroni *et al.*, 1979, 1980; Massey *et al.*, 1987; Bartles *et al.*, 1987). These cell types also exhibit a basolateral default pathway for foreign secreted proteins (Rindler and Traber, 1988; Bartles and Hubbard, 1988), which is in contrast to the nonpolarized secretion of foreign polypeptides expressed in MDCK cells (Konder-Koch *et al.*, 1985; Gottlieb *et al.*, 1986; Stephens and Compans, 1986). However, influenza HA is reported to be expressed on the apical surface of Caco-2 cells and the vectorial release of VSV from the basolateral surface and influenza virus from the apical surface of this cell type has been observed (Rindler and Traber, 1988; Tucker *et al.*, 1992b,c). Whether the sorting of other viruses and viral proteins exhibits significant tissue-specific differences has not been determined.

D. The Significance of in Vitro Observations on Virus Polarity to Viral Pathogenesis

The finding of polarized entry and release of viruses from epithelial cells *in vitro* has led to increasing interest in the importance of such processes within the infected organism. The polarized expression of receptor molecules has obvious significance for viruses that infect epithelial cells. If the receptor molecule is localized to the basolateral surface, it is evident that the barrier presented by the epithelium is more significant than if the receptor is expressed on the apical surface or is nonpolarized. In addition, because a virus that is specifically released from the apical surface of an epithelial cell is targeted to the

lumen and away from underlying tissues, the resulting infection should have an increased likelihood of being localized to the epithelial surface. Conversely, basolateral release should favor the establishment of a systemic infection. Although such simple generalizations are not applicable to many virus infections, in some cases correlations of these types have been observed. Thus parainfluenza viruses, which at least in humans establish a localized infection of the respiratory epithelial surface, are released by budding through the apical plasma membrane of several different epithelial cell types (Rodriguez-Boulan and Sabatini, 1978; R. Ray and R. Compans, unpublished). The hypothesis has been elegantly tested in a series of experiments carried out by Tashiro *et al.* These authors observed that structural changes within the fusion glycoprotein (F) were responsible for alterations in the type of infection established by a host range mutant of Sendai virus (F1-R) in mice (Tashiro *et al.*, 1988). Wild-type Sendai virus (a parainfluenza virus) was found to be exclusively pneumotropic while the F1-R mutant established a pantropic infection. Further studies revealed that whereas the wild-type virus budded from the apical surface of the bronchial epithelium, F1-R virions were observed to bud through both the apical and basolateral domains (Tashiro *et al.*, 1990a). This altered polarity of virus budding was also reported to occur following infection of MDCK cells and was shown to correlate with the polarity of viral glycoprotein expression in this cell type (Tashiro *et al.*, 1990a,b). The authors therefore conclude that the differential budding of Sendai virus from the bronchial epithelium is a primary determinant for organ tropism in mice (Tashiro *et al.*, 1992).

Many of the viruses that infect epithelial cells have significant cytopathic effects. Because epithelial polarity is lost as a consequence of cytopathology (Fuller *et al.*, 1984; Lopez-Vancell *et al.*, 1984; Srinivas *et al.*, 1986; S. Tucker, unpublished observations), the vectorial release of some virus types from infected epithelial cells observed in tissue culture may have reduced significance for disease pathogenesis. Influenza virus subtypes that are highly virulent in birds are representative of such viruses. Influenza viruses of the H7 and H5 subtypes elicit a systemic disease in certain avian species that frequently involves infection of the central nervous system in addition to other organs (Nestorowicz *et al.*, 1987). In these cases the barrier presented by the respiratory epithelium is clearly breached. However, electron microscopic analysis of MDCK epithelial cell monolayers infected with these viruses revealed that both the virulent and nonvirulent subtypes were predominantly released from the apical surface (Basak *et al.*, 1983). If it is assumed that a similar polarity of release occurs from infected avian epithelial cells, then these data are in apparent conflict

with the hypothesis that vectorial virus release is of pathogenic significance. However, other factors are known to be involved as determinants of virulence. The virulence of H5 and H7 subtypes has been correlated with the ability of their HA molecules to undergo cleavage (reviewed by Murphy and Webster, 1990). Cleavage of the HA molecule has been equated with the ability of influenza virus to infect cells; influenza virus grown under conditions such that HA cleavage does not occur exhibits low infectivity, which can be restored by trypsin-mediated cleavage of the HA. The HA molecules of highly virulent strains are cleaved in various cell cultures in the absence of exogenous protease. The virulent H5 and H7 subtypes are therefore highly infectious and capable of rapid spread both *in vivo* and *in vitro*. There is a similar correlation between virulence and glycoprotein cleavage for paramyxoviruses, which are also released from the apical surface of polarized epithelial cells. *In vitro* studies have indicated a requirement for F glycoprotein cleavage to mediate cell-to-cell spread (Choppin and Scheid, 1980; Nagai *et al.*, 1976; Rott, 1979) and virus strains that readily undergo cleavage have enhanced virulence in birds (Garten *et al.*, 1980; Nagai *et al.*, 1976, 1979; Rott, 1979). Because representatives of both of these virus types are released from the apical (or luminal) surface, preferential release into the luminal compartment may not be sufficient to restrict the spread of viruses that are both highly infectious and cytopathic.

IV. VIRAL INFECTIONS OF EPITHELIAL TISSUES

A. The Role of Epithelial Cells in the Establishment of Local or Systemic Viral Infections

Some viruses, notably papillomaviruses, rhinoviruses, rotaviruses, influenza, parainfluenza, and coronaviruses, establish a localized infection largely within the epithelial cells near the region of initial entry. Although the factors that inhibit virus spread and subsequent systemic infection are not clearly elucidated in all cases, it is apparent that the interaction between the virus and the target epithelial cells is of some importance. Epithelial cells fulfill a unique position, acting both as barriers to passive diffusion and as a system for selective vectorial transport. Viruses that replicate in epithelial tissues are subject to several factors characteristic of this specialized cell type. The two most significant factors are the ability of these cells to transport polypeptides and lipids to specific plasma membrane domains, and the presence of a barrier to intercellular diffusion defined by the tight

junction. Thus a virus particle is unable to simply diffuse through an epithelial cell sheet by passing between neighboring cells. Consequently to gain access to the underlying tissues a virus must utilize one of three predominant routes of transepithelial transport. These are (1) direct infection of the epithelial cell via the apical surface, followed by basolateral release (or cell destruction), (2) specific or nonspecific transcytosis, which may not involve infection of the transporter cell, or (3) advantageous utilization of an epithelial breach mediated, for example, by physical trauma. Examples of viruses that utilize each of these processes are given in the following sections. Direct infection of an epithelial cell requires that the virus be able to initiate infection after adsorption onto the apical surface. Examples of viruses that are able to infect epithelial cells in this fashion include influenza, parainfluenza, herpes simplex virus, poliovirus, and SV40. In the event that the host cell receptor molecule is expressed exclusively on the basolateral surface, infection may occur only following transport across the tight junction-imposed barrier. Thus the epithelium presents a more significant barrier to infection in this case, which must occur subsequent to transepithelial transport. The routes of infectious entry into MDCK cells by VSV and vaccinia virus provides examples of this restriction.

Some epithelial tissues contain specialized epithelial cells, known as M cells (see Section IV,C), which carry out a function related to immune surveillance of the mucosal surface. Microorganisms and macromolecules are transcytosed by this cell type in order that they may be exposed to underlying lymphoid cells. Clearly such a function may be utilized by viruses to gain access to cell types susceptible to infection. Reovirus, poliovirus, and human immunodeficiency virus are examples of viruses that may utilize this portal of entry. Physical trauma is less clearly defined. Several virus infections are thought to occur following exposure of susceptible tissues as a result of mechanical injury. Rabies virus, hepatitis B, papillomaviruses, and poxviruses are examples that fall into this category.

As discussed above, enveloped viruses and some nonenveloped viruses are directionally released at either the apical or basal domains prior to cell lysis. Apart from being of interest to investigators examining the mechanism underlying cellular polarity, the vectorial release of viruses has obvious pathological implications. A virus that is preferentially shed into the luminal (apical) compartment is clearly less likely to establish a systemic infection than a virus that is released from the serosal (basolateral) surface. Although not apparent in all cases, this hypothesis may be relevant to many virus infections.

As a consequence of the protective function fulfilled by epithelial

cells, epithelia are under continual mechanical stress, resulting in the loss of cells from the epithelial surface (known as desquamation). Most epithelia are therefore in a state of perpetual replacement and represent some of the most actively replicating tissues of the adult body. This characteristic appears to be of some importance to certain virus types that exhibit a preference for actively dividing cells. Thus several parvoviruses are characteristically restricted to the intestinal epithelium and rarely elicit a more systemic infection in adult organisms.

In the following sections the role of native epithelia in the pathogenesis of viral infections will be considered. The body has four predominant epithelial surfaces that are most significant for viral infection. These are the skin, the alimentary canal, the respiratory canal, and the urogenitary tract. Some viruses also infect the conjunctival epithelium. Because the epithelium may vary significantly in each of these regions, a brief description of the epithelial surfaces involved will be provided prior to discussion of the viral infections manifest within them. For more detailed information several publications may be consulted (Leeson *et al.*, 1988; Ross and Romrell, 1989).

B. The Skin

The skin forms the external surface of the body and is composed of two predominant layers: the dermis and the epidermis. The latter is composed of stratified squamous epithelial cells and overlies the connective tissue of the dermis. The epidermis is divided into several layers of morpholically distinct cell types. The deepest layer, which is adjacent to the basal lamina, contains dividing cells and is termed the stratum basale or stratum germinatum. Daughter cells move from this layer upward through the stratum spinosum, the stratum granulosum, the stratum lucidum (which is found only in the thick epidermal layers of the feet and hands), and finally the stratum corneum. The epidermis contains four cell types: keratinocytes, melanocytes, Langerhans cells, and Merkel cells.

The keratinocytes are the most numerous cell type and provide both structural integrity and maintain the physical barrier of the skin. Following replication these cells move from the stratum basale and into the stratum spinosum. At this stage the cells exhibit numerous desmosomes, which are responsible for the extremely strong cellular attachments typifying this cell type. In addition the cells contain large filamentous bodies termed tonofibrils and synthesize a glycolipid that is packaged into lamellar bodies. These tonofibrils are converted to keratin after passage through the stratum granulosum. Coincident with keratinization the cells become anucleate, organelles are lost, and

the outermost cells synthesize an intracellular network of involucrin that reinforces the cytoplasmic surface of the plasma membrane. The lamellar bodies fuse with the plasma membrane just prior to this stage, releasing the glycolipid that forms a water barrier. Because the surface of the skin is constantly subjected to mechanical stress and abrasion, the surface-differentiated cells are rapidly lost and subsequently replaced by underlying cells. The whole process from synthesis to desquamation takes between 2 and 4 weeks in humans.

The other cell types found in the epidermis carry out specialized functions. Melanocytes, which are located largely in the stratum basale, produce a melanin, the primary skin pigment. They have a rounded morphology with long dendritic processes that extend throughout the stratum basale and spinosum. Melanocytes are not structurally integrated into the epithelium to the same extent as keratinocytes because they do not form desmosomal attachments to neighboring cells. However, cellular exchange does occur because the melanocytes transfer melanin directly to the keratinocytes via their dendritic processes. The Langerhans' cell is located in the stratum spinosum and functions as an antigen-presenting cell. This cell type also possesses dendritic processes and does not form desmosomal contacts with surrounding keratinocytes. The Merkel cell, which is located in the stratum basale, is associated with nerve endings in the epidermis and functions as a mechanoreceptor. In contrast to the other specialized cell types described above, Merkel cells make contact with neighboring keratinocytes by means of desmosomes. There are also numerous free nerve endings that extend into the stratum granulosum. In addition, certain specialized cells are sequestered in areas of the epidermis and dermis to form secretory glands. The epithelial cells layers may be fewer in these regions and of different morphology. For example, the cells lining eccrine sweat gland ducts are arranged in two layers and are of the stratified cuboidal type.

Because the outer layers of the skin are largely composed of dead keratinized cells that cannot support virus replication, a virus must breach this barrier and gain access to the underlying tissues. The close cellular association and strength of intercellular contacts between keratinocytes ensures that such access is limited and generally occurs as a result of physical injury to the outer layers. The type of injuries most commonly associated with viral infection include minor traumas, such as accidental cuts or abrasions, injection, or the bite of vertebrate or arthropod vectors. Examples of viral infections mediated by these routes are given below.

Several viruses take advantage of fortuitous lesions in the epidermal barrier. Papillomaviruses, together with hepatitis B and members

of the poxvirus and herpesvirus families, are known to infect via minor epidermal traumas. Most papillomavirus infections are confined to epithelial cells in the local area of the lesion and in the majority of cases a specific tropism for squamous epithelial cells is exhibited. In this example tissue restriction appears to be a consequence of restriction of viral replicative functions. Although papillomavirus DNA and early mRNA have been detected in epithelial cells of the stratum basale (Stoler and Broker, 1986; Schneider *et al.*, 1987), expression of late genes and the assembly of virus has been detected only in the terminally differentiated keratinocyte (Jenson *et al.*, 1980; Amtmann and Sauer, 1982). Papillomavirus release is thus polarized to the upper layers of the epidermal epithelia because maturation is restricted to the cell type localized at this position. Infection is initiated in cells, or a single cell (Murray *et al.*, 1971), of the stratum basale and results in a proliferative cell response without apparent damage to the basal lamina. The proliferating infected cells appear to undergo the same sequence of events involved in uninfected cell differentiation, although some morphological changes associated with the formation of a wart are evident in infected cells of the stratum spinosum and stratum granulosum (Shah and Howley, 1990). Papillomavirus infection therefore represents an example of polarized infection and release at the level of the epithelial tissue rather than a particular epithelial cell layer. Although the establishment of a localized infection in this case appears to be a consequence of the growth requirements of the virus, clearly it is the particular characteristics of the epidermal epithelia that fulfill these requirements and define the spread of infection.

Poxviruses that infect via epidermal trauma, such as vaccinia or ectromelia, generally undergo a period of initial replication at or near the site of inoculation. There is evidence that infection is initiated in cells of the dermis (Fenner, 1949; Roberts, 1962, 1964). The progeny viruses invade the associated lymphatic system, leading to a viremia that may be brief and only rarely leads to secondary lesions, as in the case of vaccinia and cowpox, or results in the infection of numerous additional tissues and organs as has been described for ectromelia, variola, rabbitpox, and monkeypox (Fenner, 1990). Epithelial cells do not, therefore, play a significant role in the entry process except as the initial barrier to infection. However, dissemination of poxviruses is mediated by the formation of epidermal lesions. These lesions form as a consequence of viral invasion of the epidermis, probably mediated by infiltration of infected macrophages, leading to infection of cells in the stratum basale and stratum spinosum. Infected cells in the middle epidermal layers become enlarged and vacuolated, which, together

with the increase in proliferation of infected basal cells, leads to a local thickening of the epidermis. A characteristic pustule containing poly-morphonuclear leukocytes and the remains of ruptured virus-infected cells subsequently forms. Eventually the pustule dries and the sur-rounding epithelial cells grow under the lesion, resulting in the recre-ation of an intact epidermis and the formation of a scab of degenerated cellular material. Regeneration of the skin epithelium may be en-hanced by the localized release of a virus-specific growth factor that has mitogenic properties (Brown *et al.*, 1985; Twardzik *et al.*, 1985) and stimulates cell proliferation (Schultz *et al.*, 1987). Evidence indicates that the cellular receptor for vaccinia virus is localized to the basolateral surface of cultured MDCK cells (Rodriguez *et al.*, 1991). Although a similar receptor localization in the basal cell layer of epi-dermal epithelial cells has not been demonstrated, such a distribution would be consistent with infection of this and overlying cell types during the formation of the epidermal lesions described above. Be-cause infection of the host organism is mediated by rupture of the epithelial layer, which may expose the basolateral surfaces of epider-mal epithelial cells or underlying dermal tissue, receptor polarization is less likely to be of significance at this stage. However, a tropism toward the basolateral surface presumably would encourage invasion of the surrounding tissues and subsequent uptake by macrophages, which are thought to be the means by which the virus is transported to the circulatory system (Fenner, 1990). Vaccinia virus is also reported to bud preferentially from the basal surface of ependymal and choroid plexus epithelial cells following intracerebral inoculation of the mouse (Kristensson *et al.*, 1984). Directional budding of this nature from epidermal epithelia may be important for invasion of the dermis dur-ing the initial stages of infection.

Herpes simplex viruses, which infect the host through skin abra-sions, initiate a localized infection resulting in a characteristic skin lesion. Infected epidermal cells, which are generally located in the middle layers of the epithelium, increase markedly in size, their nuclei degenerate, and neighboring cells fuse to form giant cells. Cell lysis follows and vesicular fluid containing progeny virus, together with other cellular components, accumulates between the epidermis and dermis. Healing is associated with the infiltration of inflammatory cells into the vesicular fluid, which becomes pustular prior to the for-mation of a scab. During this initial stage of replication, neural inva-sion occurs and the virus is transported to the dorsal root ganglia where an infection is established leading to latency. Appropriate stim-uli lead to recurrence of the disease and a skin lesion similar to that produced as a result of the primary infection. Herpesviruses bud from

the inner nuclear membrane and are transported to the cell surface in a vesicular structure. Studies using monoclonal antibodies have revealed that the five major HSV-1 glycoproteins are transported to the basolateral surface of virus-infected Vero C1008 cells (a polarized monkey kidney epithelial cell line), mouse mammary epithelial cells, and Madin–Darby bovine kidney cells (Srinivas et al., 1986). At least one HSV-1 glycoprotein was found to be similarly polarized when expressed from a vaccinia virus recombinant. Srinivas et al. (1986) also observed a basolateral accumulation of extracellular HSV-1 and HSV-2 particles by electron microscopic analysis of infected epithelial cells, prior to the onset of gross cytopathic effects. Other studies suggest that the initial binding event that occurs prior to HSV-1 and HSV-2 infection of the host cell involves recognition of heparan sulfate proteoglycans (Wudunn and Spear, 1989; Shieh et al., 1992). These molecules are an important component of the extracellular matrix and are therefore secreted from the basolateral surface of many epithelial cells (Konder-Koch et al., 1985; Gottlieb et al., 1986; Caplan et al., 1987). In addition, heparan sulfate may intercalate into the plasma membrane (Kjellen et al., 1981; Rapraeger and Bernfield, 1985). The membrane-associated form also predominantly localizes to the basolateral surface, where it is involved in cell–cell and cell–substratum interactions (Cole et al., 1985, 1986; Cole and Glaser, 1986; Laterra et al., 1983). Although the latter observations suggest that an affinity for heparan sulfate should favor the basolateral route of infection the vectorial secretion of receptor molecules could conceivably interfere with infection via this route. The integral membrane-bound form of heparan sulfate proteoglycan is thought to be anchored via a GPI moiety (Ishihara et al., 1987; Carey and Evans, 1989). The presence of such an apical targeting signal (see Section III,C) is consistent with reports that heparan sulfate proteoglycans are also expressed on the apical plasma membrane and are secreted into the luminal space by some cell types (Tang et al., 1987; Carson et al., 1988; Sun et al., 1989). Indeed, evidence indicates that HSV-1 entry can occur following binding to either the basolateral or apical surface of MDCK cells. Infection via the apical surface in this case is probably mediated by a different viral attachment protein than that involved in virus entry through the basolateral plasma membrane (Sears et al., 1991). Because physical disruption of the keratinized layer is a necessity for viral infection of the epidermis, whether the virus receptors on the underlying epithelial cells are nonpolarized or expressed on the basolateral surface is of reduced significance for the initiation of infection. Nevertheless, the expression of viral receptors and the release of herpes simplex viruses from the basolateral surface is consistent with the

pathological changes associated with the establishment of a systemic infection and the formation of the epidermal lesion, which may occur in a fashion similar to that outlined for poxviruses above.

Transmission of hepatitis B virus may occur by several mechanisms, including the use of shared toiletry items, such as toothbrushes, bath towels, bath brushes, and razors (Braconier and Nordenfelt, 1972; Mitch et al., 1974; Mosley, 1975), which are likely to result in minor skin trauma. However, unlike the examples described above, it is unclear whether the epidermal epithelium has a significant role in viral pathogenesis except as the initial barrier to infection. Inoculation of infectious material into subcutaneous tissues as a result of hypodermic needle injections or similar invasive procedures is another important route of infection for hepatitis B (Hollinger, 1990a), in addition to certain retroviruses (Friedland and Klein, 1987), filoviruses (Pattyn, 1978), and herpesviruses (Gerber et al., 1969; Alford and Britt, 1990). Hepatitis C and hepatitis D viruses are also thought to be transmitted in this fashion (Hollinger, 1990b; Reyes and Baroudy, 1991). Because the epidermal epithelium is effectively breached as a consequence of this infectious route its primary protective function is abrogated and of little further consequence to viral replication. Similarly, the bite of a vertebrate vector frequently involves extensive rupture of the epithelial barrier and deep inoculation into underlying tissues. Animal bites are the primary route of transmission of rabies virus, a rhabdovirus (McKendrick, 1940), and herpesvirus simiae (Tyler and Fields, 1990). In the case of rabies there appears to be an initial period of localized replication and virus may be detected in striated muscle cells in the area of inoculation (Murphy et al., 1973; Charlton and Casey, 1979) prior to invasion of the peripheral nervous system.

Transmission by arthropod vectors accounts for the final method by which viruses breach the epidermal barrier. All alphaviruses and flaviviruses and some poxviruses, orbiviruses, and bunyaviruses are transmitted by this route. Transmission may either be purely mechanical and not involve replication in the arthropod or replication may occur within the vector. Interestingly, in the latter case the virus must overcome additional epithelial barriers in the insect host. Flaviviruses multiply in the epithelium of the insect mesenteron, or midgut, following ingestion of virus-contaminated blood. Replication is associated with invasion of the organism, leading to secondary infection of additional tissues prior to the infection of the salivary glands. In some cases amplification in other tissues does not occur before infection of the salivary gland epithelium. Release of virus from the salivary gland epithelium into the saliva results in the transmission of virus to the vertebrate circulatory system and completes the cycle (Hardy,

1988; Turell, 1988). Infection of the insect does not appear to have obvious pathogenic effects although some alphaviruses are reported to induce cytopathic changes in salivary and midgut epithelium (Mims *et al.*, 1966; Weaver *et al.*, 1988) that may be associated with breach of the epithelial barrier.

C. The Alimentary Canal

The alimentary canal may be divided into several regions, including the oral cavity, the pharynx, the esophagus, the stomach, the small intestine, and the large intestine. In addition the digestive system is associated with a number of glandular organs, such as the liver and pancreas. Each portion of the alimentary canal has specific functions that are reflected in the various epithelial surfaces exhibited throughout.

The epithelial lining of the oral cavity may be divided into three regions in accordance with associated functions. These are masticatory, lining, and specialized epithelia. The masticatory epithelium lines the hard palate and gingiva and consists of stratified squamous epithelial cells. These are generally keratinized although there are areas of parakeratinized (the cells of the stratum corneum retain their nuclei) and nonkeratinized epithelia. The overall structure of this epithelium is similar to that of the skin although there are some specialized connective structures at the interface between the basal surface and underlying connective tissue that serve to anchor the epithelium, creating a relatively immobile surface. The epithelial cells of the gingiva also secrete basal lamina components onto the surface of the tooth to ensure an adherent surface. Lining epithelium, which is less rigid than the masticatory epithelium and of similar structure to the skin except that little keratinization is evident, is found on the lips, cheeks, alveolar mucosa, the undersurface of the tongue, and the soft palate. Specialized epithelial cells cover the tongue, the lymphatic tissue associated with the tonsils, and comprise the salivary glands. In the region of the tonsils the stratified squamous epithelium is extensively invaded by lymphocytes. The surface of the tongue is lined with both keratinized and nonkeratinized stratified squamous epithelium arranged into a surface of ridges and grooves that contain specialized epithelial cells of different morphology within taste buds and serous secreting glands. The undersurface epithelium is similar except that it is thinner and generally nonkeratinized.

The salivary glands are divided into the major and minor salivary glands, which are both derived from the oral epithelium. The basic unit of a salivary gland, known as a salivon, is composed of secretory

acinar cells, duct cells, and a basal layer of contractile myoepithelial cells. The secretory cells are divided into serous cells and mucous cells, which discharge their contents into the duct network. All salivary ducts contain a region termed the excretory duct. In small ducts the epithelial lining is composed of pseudostratified cells interspersed with goblet cells. As the ducts increase in size the epithelium becomes stratified columnar and finally stratified squamous at the terminus of the duct near the oral epithelium. Some ducts also contain regions of simple cuboidal epithelium and simple columnar epithelium termed intercalated and striated, respectively. Secretory IgA, which is transported by the salivary gland epithelium, forms 19% of saliva by dry weight (Jenkins, 1978). Other components of significance for viruses that utilize the alimentary canal as a portal of entry include lysozyme (22%), urea (20%), IgG (1.4%), and IgM (0.2%).

The pharynx includes regions that are exposed to food particles in the early stages of maceration and therefore likely to be significantly abraded. These regions are lined with nonkeratinized stratified squamous epithelial cells. Other regions that are less likely to be damaged are covered with a pseudostratified ciliated columnar epithelium containing goblet cells. Mucous glands are present at the junction with the esophagus and several lymph nodes are contained within the pharynx.

Several herpesvirus infections are characteristically manifest in the oral cavity. The oropharynx is considered to be the main portal of entry of Epstein–Barr virus (EBV), which initiates an infection in numerous epithelial tissues including cells lining salivary glands, the tongue, the oral cavity, and pharynx (Sixbey et al., 1984; Wolf et al., 1984; Greenspan et al., 1985). The epithelium of the salivary gland is thought to be a lifelong reservoir of the virus and probably remains in a state of chronic productive infection (Yao et al., 1985) leading to continual desquamation and release of infected cells and virus in salivary secretions (Wolf et al., 1984). Immunofluorescence studies using monoclonal antibodies to the cellular receptor have revealed that EBV receptor expression is restricted to less differentiated epithelial cell types in the basal layers of stratified squamous epithelia (Young et al., 1986; Sixbey et al., 1987). Receptor-positive cells should thus become exposed as a consequence of minor epithelial trauma, a common occurrence in oropharyngeal epithelia. Active virus replication appears to be restricted to the epithelial cells of the upper layers that have undergone terminal differentiation and are in the process of desquamation (Sixbey et al., 1983). In this respect EBV infections resemble those of papillomaviruses described above. Unlike papillomaviruses, however, EBV release is not solely polarized to the apical surface of the epithelia; following the establishment of an epithelial infection the virus

is transmitted to B cells located in tissues underlying the infected epithelia. Cytomegaloviruses also characteristically infect epithelial cells lining salivary ducts. Although underlying cell types do not frequently appear infected, the virus infection is invasive and leads to a systemic disease that involves a variety of organs (Alford and Britt, 1990). Herpes simplex virus infections of the oropharyngeal epithelium exhibit a histopathology similar to that of infections of the skin (described above), except that vesicular lesions rupture more readily and are therefore less apparent.

The human esophagus is lined with nonkeratinized squamous epithelial cells, although keratinization is found in some animals. The stomach is covered with a largely impermeable, simple columnar epithelium and is arranged in a series of folds. Each epithelial cell secretes mucus that, together with that produced from mucus-secreting glands, serves to protect the epithelium from acid or proteolytic damage. Three types of gland are found in the stomach lining: fundic glands, cardiac glands, and pyloric glands. Fundic glands are the most numerous and contain several specialized epithelial cells that secrete mucus, pepsinogen, and produce hydrochloric acid. Cardiac glands and pyloric glands are largely responsible for the secretion of mucus. The surface of the small intestine is extensively folded and exhibits projections, or villi, that serve to increase the surface area. The epithelium is simple columnar and contains five cell types: enterocytes, goblet cells, amine precursor uptake decarboxylase (APUD) cells, Paneth cells, and undifferentiated cells. The enterocytes are tall columnar cells with a basal offset nucleus and numerous microvilli. These are located largely on the villi and surface of the small intestine although some may be found in intestinal glands. Goblet cells are most numerous on the villi where they function, as in other epithelia, to secrete mucus. Amine precursor uptake decarboxylase cells have an endocrine function and are generally not exposed at the apical surface. Paneth cells are located in intestinal glands and are thought to be responsible for the regulation of the microbial flora mediated by the secretion of lysosyme and phagocytosis of certain bacteria. The predominant cell type found lining the large intestine is a simple columnar absorptive epithelial cell that resembles the enterocyte of the small intestine. Because most nutrients are absorbed by the small intestine, these cells largely specialize in the uptake of water. Goblet cells are also numerous and their mucous secretions serve to lubricate the contents of the large intestine. Simple columnar epithelium line the upper portion of the anal canal near the junction with the large intestine. This epithelium changes to stratified columnar and finally a stratified squamous epithelium forms that is continuous with the skin. There are several glands lined with stratified columnar epithelial cells and numerous goblet cells throughout.

Membranous or microfold epithelial cells, which are known as M cells, constitute a specialized group of epithelial cells that overlay subepithelial lymphoid tissue in the gut (gut-associated lymphatic tissue, or GALT) and respiratory tracts (bronchial-associated lymphoid tissue, or BALT). In the gastrointestinal tract these areas are known as Peyer's patches and appear as domes predominantly in the epithelium of the small intestine (Cornes, 1965). The epithelial surface of Peyer's patches consists of enterocytes and goblet cells interspersed with M cells, which maintain tight junctions and desmosomal contacts with adjacent cells but are morphologically distinct from their neighbors when analyzed by electron microscopy. Their apical surface is characterized by short, irregular microvilli and extensive vesiculation of the apical cytoplasm. The basolateral plasma membrane of the M cell surrounds one or more intrusive cells, which are most commonly lymphocytes (Owen, 1977), lymphoblasts (Bhalla and Owen, 1982), or macrophages (Atsushi, 1977; Abe and Ito, 1978; Owen et al., 1982b, 1983). It has been reported that lymphocytes may also migrate through the M cell epithelial layer and into the intestinal lumen (Smith and Peacock, 1982; Owen et al., 1982a). The M cell provides a sampling mechanism by which antigens contained within the lumen are exposed to the immunological system. Macromolecules and microorganisms may be endocytosed, transported to the basolateral surface, and there released into the extracellular space surrounding the lymphoid cells. Studies using horseradish peroxidase, native and cationized ferritin, India ink, and lectins have shown that macromolecular transcytosis is efficient, nonspecific, and confined to the M cell population (Bockman and Cooper, 1973; Fournier et al., 1977; Owen, 1977; Myrvik et al., 1979; Neutra et al., 1982). Several types of prokaryotic microorganisms are endocytosed and transported in a similar fashion. However, specificity is evident at this level because numerous microorganisms do not penetrate the epithelial barrier by this route (Wolf and Bye, 1984). The M cell therefore represents an obvious potential portal of entry for viruses that initiate infection from the gastrointestinal and respiratory tracts. Indeed, a series of elegant studies has demonstrated that reoviruses adhere to the surface of M cells, are endocytosed, transported in vesicles to the basolateral membrane, released into the extracellular space, and finally adhere to the surface of underlying mononuclear cells (Wolf et al., 1981, 1983, 1987; Bass et al., 1988). M cell-mediated transepithelial transport may also be utilized by other viruses. High titers of poliovirus have been recovered from gut-associated lymphoid tissue during the initial stages of infection (Bodian, 1956; Sabin, 1956) and poliovirus appears to be endocytosed by M cells (Sicinski et al., 1990). Because IgA appears to selectively bind to, and be endocytosed by M cells it is possible that

luminal IgA–virus complexes may be involved in facilitating M cell-mediated transcytosis of gut pathogens (Weltzin *et al.*, 1989). The enterocytes surrounding Peyer's patches may also be more susceptible than other gut epithelial cells to virus infection as a consequence of the reduced number of goblet cells and therefore mucous secretions in this region (Owen and Nemanic, 1978). The M cell glycocalyx, which is a diffuse layer composed largely of glycosaminoglycans overlying the apical plasma membrane of mucosal epithelia, is also thought to be less elaborate than that associated with absorptive cells (Inman and Cantey, 1983). Because these cell surface constituents are thought to fulfill a barrier function in the gastrointestinal tract and other mucosal surfaces (Lopez-Vidriero, 1989), a localized reduction should facilitate microorganism–epithelial cell interactions.

Viruses that utilize the gastrointestinal tract as a portal of entry must be able to withstand the seemingly harsh environment associated with the digestive process. Accordingly, viruses that produce enteric infections are typically stable to reduced pH, proteolytic enzymes, and bile salts. Because bile salts solubilize lipids, enveloped viruses do not, with the notable exception of coronaviruses, generally initiate infection via the gastrointestinal route. Perversely, the proteolytic enzymes secreted into the lumen frequently enhance viral infectivity. Protease enhancement is thought to be mediated by specific cleavage of viral outer capsid proteins (Clark *et al.*, 1981; Estes *et al.*, 1981; Espejo *et al.*, 1981; Storz *et al.*, 1981; Holmes, 1990), indicating that these viruses have evolved mechanisms to exploit a potentially degradative process. Virus infection may be localized to the epithelial surface or result in a more extensive infection of the host. Viruses that initiate infections in the former category include some parvoviruses, some adenoviruses, coronaviruses, caliciviruses, and reoviruses. Viruses that produce a systemic infection include enteroviruses, some parvoviruses, and some adenoviruses. Examples of both categories are considered below. Virus infection of epithelial tissues lining the gut may also occur without apparent exposure of the virus to the digestive process. Thus human and simian immunodeficiency viruses are transmitted as a consequence of exposure of the rectal epithelium to virus (see Section IV,E). Avian influenza viruses also establish an infection of the gut epithelium in ducks that can be mediated by ingestion or rectal administration of virus (Webster *et al.*, 1978). Although the avian influenza viruses exhibit an increased stability at reduced pH in comparison to human influenza viruses, they are inactivated at the pH of the duck gizzard (Webster *et al.*, 1978). Ingested virus is therefore likely to be at least partially protected during passage through the duck digestive system; perhaps by association with food particles and a relatively rapid transport through the degradative compartments.

Parvovirus replication is intimately associated with replication of the host cell. Because replication appears restricted to cells that are in specific mitotic phases (Tattersall, 1972), infection is most often manifest in tissues, such as the intestinal epithelium, that contain large populations of actively dividing cells located in the crypts. Several parvoviruses produce an enteric disease that is associated with extensive damage of the intestinal epithelium (Cooper *et al.*, 1979; Pattison, 1990). The breakdown of the epithelial barrier is exemplified by the characteristic symptoms of diarrhea and rapid dehydration in these cases. It is thought that subsequent spread to other tissues and organs is determined by the ability of the virus to replicate at these sites. This in turn appears dependent on levels of mitotic activity and cellular differentiation in the potential targets. Thus the results of parvovirus infection may be severe in developing embryos or newborns, whereas in adults the infection is frequently asymptomatic unless cellular division is initiated, for example, as a consequence of wounding (Tattersall, 1978; Pattison, 1990). The mechanism by which infection of the intestinal epithelium is established is not clear. Basak and Compans (1989) have demonstrated that a canine parvovirus receptor capable of mediating viral endocytosis is expressed in MDCK cells and is restricted to the basolateral surface of this cell type. Although the distribution in intestinal epithelial cells has not been determined, such a localization would be consistent with a requirement for virus transport across the epithelia, to gain access to the basolateral surface, prior to infection.

Coronavirus infection of the gastrointestinal tract is characterized by infection of absorptive and crypt epithelial cells with consequent atrophy of the villi. The specific cells infected, the site of infection within the gastrointestinal tract, and the severity of the disease vary between virus strains (Siddell *et al.*, 1983). A loss of absorptive capacity has been correlated with infection (Doughri and Storz, 1977) and the infected epithelial cells are reported to exhibit cytoplasmic vesiculation and desquamation (Baker *et al.*, 1982; Rousset *et al.*, 1984; Rettig and Altshuler, 1985). It has been reported that the receptor for the murine coronavirus mouse hepatitis virus (MHV) is a member of the carcinoembryonic antigen (CEA) glycoprotein family (Williams *et al.*, 1991). Carcinoembryonic antigen has been localized to the apical plasma membrane surface of differentiated intestinal and bile duct epithelial cells in both native tissues and continuous cell lines (Gerber and Thung, 1978; Sugiyama *et al.*, 1988; Shirota *et al.*, 1988; Lisanti *et al.*, 1990; Baghdiguian *et al.*, 1991). A similar distribution of MHV and other coronavirus receptors would be consistent with infectious entry at the apical surface of the intestinal epithelium. Indeed, aminopep-

tidase N, which is expressed on the apical surface of intestinal and respiratory epithelial cells, has recently been identified as the receptor for two coronaviruses (transmissible gastroenteritis virus and human coronavirus 229E) that are serologically unrelated to MHV (Delmas *et al.*, 1992; Yeager *et al*, 1992). Studies on the tissue distribution of the MHV receptor suggest that subsequent spread of the virus from the site of entry is at least partly dependent on differential receptor expression. Thus the liver, small intestine, and colon of susceptible mice express the highest levels of receptor and are major targets of the virus during the course of infection (Boyle *et al.*, 1987; Williams *et al.*, 1990, 1991). However, tissue tropism and the pathogenesis of disease are both host and virus strain specific, indicating that receptor distribution is not solely responsible for viral spread, which is dependent on numerous additional factors (Siddell *et al.*, 1983; Holmes, 1990). Interestingly, CEA is reported to be shed into the apical media following stimulation of an intestinal epithelial cell line with interferon γ (Baghdiguian *et al.*, 1991). The signal-mediated release of a cell surface receptor constitutes a potential antiviral response and provides a possible mechanism for the reported inhibitory effect of interferon γ on MHV and transmissible gastroenteritis virus replication in epithelial cells and macrophages (Charley *et al.*, 1988; Lucchiari *et al.*, 1991).

Norwalk virus is the prototype strain of a group of poorly characterized viruses assigned to the calicivirus family (Kapikian and Chanock, 1990). Infection with Norwalk virus manifests as a gastrointestinal illness associated with histopathological changes in the epithelium of the proximal small intestine (Agus *et al.*, 1973; Schreiber *et al.*, 1973, 1974; Dolin *et al.*, 1975). Electron microscopic analyses have revealed that infected epithelial cells remain intact but exhibit shortened microvilli. No virus particles were observed in the infected epithelial cells. Because similar histopathology was apparent during asymptomatic infection (Meeroff *et al.*, 1980; Schreiber *et al.*, 1973, 1974), these changes may not be responsible for the disease symptoms. However, some epithelial cell functions are affected by infection with these viruses because alterations in transport processes and the levels of brush border enzyme activities have been described (Blacklow *et al.*, 1972; Schreiber *et al.*, 1973; Agus *et al.*, 1973). Electron microscopic analyses of intestinal biopsy samples have failed to reveal virus particles, and it is uncertain whether these viruses exhibit vectorial release or infect epithelial cells via a particular plasma membrane domain.

In contrast, the infectious route of reoviruses has been relatively well characterized and may provide a model for the pathogenesis of other viral infections associated with the gastrointestinal tract (Sharpe and Fields, 1985). Following ingestion, the reovirus particle

undergoes proteolytic cleavage mediated by a host protease in the lumen of the gastrointestinal tract (Bodkin *et al.*, 1989). An infection is subsequently established in epithelial cells, predominantly in the ileum in the case of reovirus type 1 (Rubin *et al.*, 1985; Wolf *et al.*, 1987), or throughout the small intestine and colon in the case of reovirus type 3 (Rubin *et al.*, 1986). Studies involving the oral inoculation of mice with high doses of virus have revealed an apparent specificity for M cells of the Peyer's patch (discussed above). Virus binding to the apical surface of M cells was followed by endocytosis and apparent transcellular to the basolateral surface (Wolf *et al.*, 1981, 1983). The M cell population was also observed to decline shortly after inoculation, corresponding to virus infection of this cell type, which appears to precede infection of other intestinal epithelial cells (Bass *et al.*, 1988). Once released from the basolateral surface, presumably mediated by fusion of the virus-containing vesicles with the plasma membrane (Wolf *et al.*, 1983), the virus establishes an infection in the adjacent epithelial cells and subsequently spreads to other sites, probably via the lymphatic system and blood stream (Kauffman *et al.*, 1983). Infection of the enterocytes adjacent to the M cells is thought to be mediated by virus binding to their basolateral surface (Rubin *et al.*, 1985; Rubin, 1987; Bass *et al.*, 1988). Although virus has been observed to bind to the apical surface of a minority of enterocytes (Wolf *et al.*, 1983) and has been shown to be endocytosed by Caco-2 cells following adsorption to the apical plasma membrane (Ambler and Mackay, 1991), entry may not occur at this surface *in vivo* and a preferential binding to the basolateral surface of intestinal epithelial cells has been demonstrated (Rubin, 1987; Weiner *et al.*, 1988). Thus the infectious entry route of reoviruses provides an example of epithelial barrier circumvention via specific transcellular transport.

Poliovirus is considered to be the most important enterovirus that infects humans. As in all enterovirus infections, poliovirus infection is mediated by ingestion of the virus. Shortly after ingestion virus may be recovered from lymphoid tissues, suggesting that these are the sites of primary replication. Within 4 days of ingestion the highest titers of virus were found to be predominantly associated with the tonsils and to a greater extent Peyer's patches (Bodian, 1955, 1956). A viremia has been detected in some cases although it is not always evident (Bodian, 1955, 1956; Sabin, 1956). Occasionally infection with poliovirus results in invasion of the central nervous system, probably via the blood (Bodian, 1956). Infection of neurons leads to transport to the anterior horn of the spinal cord and is associated with significant pathological lesions. The probability of neural invasion appears to be increased by physical trauma, such as tonsillectomy, which may result in exposure

of neurons to the virus (Melnick, 1990). In the later stages of infection virus may be recovered from the feces, which is the predominant means by which dissemination occurs. Poliovirus infection of the gastrointestinal epithelium results in lesions of the Peyer's patches and evidence suggests that poliovirus may be endocytosed by M cells of human Peyer's patches (Sicinski *et al.*, 1990). Although not readily apparent from this study, the possibility exists that transepithelial transport of poliovirus occurs via M cells in the same fashion as described for reovirus (see above). Alternatively M cells, or the surrounding enterocytes, may become infected directly. *In vitro* studies using a human intestinal epithelial cell line (Caco-2) have revealed that poliovirus infection can be mediated by binding to either the apical or basal plasma membrane, probably via the same polypeptide receptor (Tucker *et al.*, 1992a). It may not, therefore, be necessary to invoke M cell transcytosis to provide a mechanism for poliovirus invasion of the gut epithelium; direct infection of susceptible epithelial cells via the apical surface may also be involved. However, the release of poliovirus from Caco-2 cells exhibits a marked polarization to the apical domain (Tucker *et al.*, 1992b). It therefore appears that infection of enterocytes may not result in the invasion of underlying tissues. In addition, the glycocalyx is likely to present a significant barrier to infection of enterocytes in the gut. The most likely scenario for infection via the alimentary canal based on the information currently available is as follows: ingested poliovirus is adsorbed to the surface of Peyer's patch M cells, which are subsequently infected and/or transport the virus to the underlying lymphoid tissue. A localized infection of cells in the vicinity of the Peyer's patch is initiated, followed by a viremia leading to infection of other target organs and tissues, such as the central nervous system, brown fat, and somatic lymph nodes (Bodian, 1955, 1956). Release of virus into the feces may be mediated by the movement of infected lymphocytes from lymphoid tissues into the lumen of the gut (Bodian, 1956) and/or infection of nonlymphoid gut epithelial cells (Sabin, 1956), resulting in vectorial transport and release of virions from their apical surface (Tucker *et al.*, 1992b). In the latter case, because the virus receptor is expressed on both the apical and the basolateral surfaces of intestinal epithelial cells (Tucker *et al.*, 1992a), exposure of the virus to the basolateral surface via hematogenous distribution may mediate infection and subsequent vectorial release.

D. The Respiratory System

The epithelium of the respiratory tract carries out a number of specialized functions and is composed of several different cell types. Air

passing into the nasal cavity first flows through the vestibule, which is lined with stratified squamous epithelial cells continuous with the skin of the face. The air subsequently enters the olfactory chamber, which is lined with three predominant cell types: (1) olfactory cells, which are neurons, (2) sustentacular cells, which maintain junctional complexes with the olfactory cells and provide them with physical and metabolic support, and (3) basal cells, which provide a partial sheath for the olfactory cell's axon and are not exposed at the apical surface. An epithelial cell known as the brush cell, which forms synaptic contacts with nerve fibers and is specialized as a sensation detector, may also be found in the olfactory chamber. The olfactory epithelium is bathed in secretory products that originate in the glands of Bowman located in the underlying connective tissue. The olfactory chamber leads to the respiratory segment of the nasal cavity. The epithelium in this region is largely pseudostratified columnar, contains numerous cilia, and is composed of three main cell types in addition to the less numerous brush cells. Although in many cases there is extensive variation in the epithelium, which may range from simple cuboidal to stratified squamous in some areas, the predominant epithelial cells are ciliated cells, goblet cells, and basal cells. The mucous secretions of goblet cells and submucosal glands cover almost the entire luminal surface of the upper respiratory tract and are continually propelled toward the pharynx by the coordinated movement of cilia.

The nasal cavity leads to the pharynx and in turn to the larynx, which is lined with a pseudostratified columnar epithelium containing ciliated cells, goblet cells, and basal cells. The surface of the larynx contains several areas of stratified squamous epithelial cells. The epithelium in the primary bronchi is also composed of ciliated pseudostratified columnar cells. Two other epithelial cells may be found in the tracheobronchial epithelium: brush cells, which are thought to have a sensory function, and dense core granule cells, which also associate with neurons and may be important in reflexive regulatory responses of the airway or vascular system. The epithelium continues without apparent change into the smaller tubes of the bronchioles. Toward the end of the bronchioles goblet cells are lost from the epithelium and an epithelial cell type known as the Clara cell becomes numerous. These cells are nonciliated and specialize in the secretion of surfactants. Changes within the epithelial surface continue into the portion of the bronchioles that carry out gaseous exchange. In this region the epithelium is simple cuboidal or columnar and ciliated cells are progressively lost until Clara cells become the predominant cell type further along the respiratory bronchioles. The epithelium of the respiratory bronchioles is interrupted by alveoli, which are lined with

three epithelial cell types; sparsely distributed brush cells, alveolar type I cells, and alveolar type II cells, which secrete surfactant. Alveolar type I cells have a squamous morphology and are the most numerous cell type; they form an exceedingly thin surface separating the serosal and luminal compartments, which facilitates gaseous exchange.

Virus infection of the respiratory epithelium is generally mediated by the inhalation of virus-containing small droplets, or aerosols, which are frequently generated as a consequence of coughing or sneezing. If these particles are of sufficiently small size to pass through the nasal filters they may be drawn a significant distance into the respiratory tract (Knight et al., 1985; Lippmann et al., 1980). Once inhaled several additional host defense systems are encountered. These include humoral and cellular immune mechanisms, mucous secretions, and the relatively low temperature maintained in the upper respiratory tract. In addition, the concerted action of ciliated epithelial cells results in the physical removal of material from the epithelial surface. The importance of the latter mechanism is illustrated by an increased susceptibility to viral infection following the inhibition of mucociliary transport (Bang et al., 1966). Irrespective of these apparent barriers a large number of different viruses are able to initiate an infection via this portal of entry. These viruses include orthomyxoviruses (influenza), paramyxoviruses (parainfluenza, respiratory syncytial, mumps, and measles), herpesviruses (Epstein Barr, herpes simplex, cytomegalovirus, and varicella) picornaviruses (rhinoviruses and enteroviruses), coronaviruses, adenoviruses, togaviruses (rubella), papovaviruses (JC and BK), bunyaviruses (Hantaan), and arenaviruses (lymphocytic choriomeningitis) (White and Fenner, 1986). The pathogenesis of several specific examples illustrative of viral infections of the respiratory tract will be considered below.

Influenza virus causes what is arguably the most important respiratory viral infection of humans. Influenza A viruses are thought to be transmitted by aerosol (Alford et al., 1966; Douglas, 1975; Moser et al., 1979) and establish an infection in the respiratory tract epithelial cells. Although damage is generally confined to the epithelium lining the upper respiratory tract, the most significant pathological changes occur in the epithelia of the lower respiratory tract. The infected columnar epithelial cells exhibit vacuolation and a reduction in the number of cilia. Desquamation follows, causing exposure of the basement membrane, which becomes thickened and hyalinized in some places, and in other regions leaving a single cell layer overlying the basement membrane. Viral antigens have been shown to be predominantly restricted to the mononuclear cells and epithelial cells lining

the airway with less detected in the basal cell layers (Mulder and Hers, 1979). Following this early destruction phase the remaining epithelial cells of the basal layer begin to divide and regenerate the epithelia.

Influenza infections of humans do not usually result in extensive viremia (Kilbourne, 1959; Minuse and Willis, 1962), although a small amount of virus may be found in the blood shortly after infection (1–3 days) (Stanley and Jackson, 1966; Khakpour and Saidi, 1969). Although infection is generally confined to the respiratory epithelium, viral antigens have also been detected in cells and secretions derived from the conjunctiva of infected individuals (Tateno and Kitamoto, 1965). The virus is known to be able to replicate in conjunctival tissues (Murphy et al., 1983) and has been isolated from this source in at least one case (Webster et al., 1981); however, little is known of the relevance of this site of infection during normal pathogenesis. Influenza virus has also been isolated from a variety of tissues in patients who contracted severe pneumonia following infection with influenza virus (Kaji, 1958; Roberts and Roberts, 1976).

There are some differences in the pathology of influenza virus infections in host species other than humans. In avian species and seals certain influenza A subtypes cause an acute disease, which in contrast to the human illness is associated with invasion and systemic spread of the virus (reviewed by Murphy and Webster, 1990). At least for viruses that infect avian species there appears to be a correlation between the ability of the HA glycoprotein to undergo cleavage and virulence (Murphy and Webster, 1990). In ducks the majority of avian strains establish an infection of the epithelial cells lining the intestinal tract that does not require prior infection of the respiratory tract (Webster et al., 1978). In this case transmission is thought to occur following ingestion of virus-contaminated water.

The influenza virus receptor, sialic acid, is widely distributed and is apparently expressed on both the apical and basolateral surfaces of MDCK cells (Fuller et al., 1984). Apical receptor expression should facilitate infection of the respiratory epithelium and is consistent with the pathology described above. Interestingly, Fuller et al. (1984) observed that influenza virus infection was virtually exclusively mediated by binding to the apical surface of MDCK cells grown in the presence of serum. However, MDCK cells grown in serum-free medium were susceptible to influenza virus infection following application of virus to either surface. Because the latter cells remained polarized by other criteria, it was proposed that sialic acid contained within serum bound to the filter support was responsible for the inhibition of influenza virus binding to the basal surface. Virus that is exposed to the basolateral environment underlying native epithelia may be simi-

larly inhibited by serum-derived sialic acid, suggesting that a selective pressure against further invasion of the epithelial cell layer might operate *in vivo* as the result of such a mechanism (Fuller *et al.*, 1984). Moreover, influenza virus is known to mature predominantly at the apical surface of a variety of epithelial cells (see Table II). Influenza virus entry and release may therefore be largely restricted to the apical surface of epithelial cells. Such a restriction should favor the establishment of the type of localized infection observed during influenza infection of humans.

Bunyaviruses of the Hantavirus genus are exceptional in that the predominant route of transmission to humans is by aerosolized rodent excreta (Smorodintsev *et al.*, 1959; Lee *et al.*, 1982) and not by insect vectors. Infection is manifest as either a respiratory illness in mild cases, or an acute hemorrhagic fever in its more severe form. In the latter case, the most severe lesions are found in the kidneys and predominantly within the renal tubule epithelium (Oliver and Mac-Dowell, 1957). Although it is not known whether Hantaviruses exhibit a polarity of release from respiratory epithelium, studies on two other bunyaviruses have revealed a basolateral preference in two different epithelial cell types (Anderson and Smith, 1987; Chen *et al.*, 1991). Targeting of infectious Hantaan virions to the basolateral surface of the respiratory epithelium would be consistent with the establishment of such a systemic infection.

The primary site of replication of rhinoviruses is the epithelial surface of the nasal mucosa (Douglas *et al.*, 1968). Immunolocalization studies have demonstrated a tropism for columnar epithelial cells in this region (Turner *et al.*, 1982, 1984). Although histological changes of the infected epithelium were not apparent in some studies (Hamory *et al.*, 1977; Turner and Gwaltney, 1984; Winther *et al.*, 1984), others have observed the progressive desquamation of ciliated cells (Reed and Boyde, 1972), which appear to be shed into the nasal secretions of infected individuals (Turner *et al.*, 1982). The host cell receptor for rhinoviruses has been identified as intercellular adhesion molecule 1 (ICAM-1) (Greve *et al.*, 1989). Because ICAM-1 is widely distributed it is thought to be unlikely to have an important role in the determination of tissue tropism. However, ICAM-1 is restricted to the luminal surface of the lung epithelium (Albelda, 1991), which may be of some significance to the pathogenesis of infection. Because the receptor is expressed on the apical surface, transepithelial transport is presumably not required for infection, suggesting that there is little selective pressure for the virus to further invade the mucosal surface. Although no information is available on the polarity of rhinovirus entry or release from epithelial cells, another picornavirus, poliovirus, appears to

be released from the apical surface of specific epithelial cells (see Section III,B). In the event that rhinoviruses were to follow a similar pathway, virus replication may be restricted to the epithelial surface as a consequence of directional release into the luminal compartment. Other factors that undoubtedly contribute to the pathogenesis of rhinovirus infections include the sensitivity of these viruses to reduced pH (Hamparian, 1979; Jackson and Muldoon, 1973; Gwaltney *et al.*, 1966) and their adaptation for optimal growth at 33°C, the temperature of the upper respiratory tract (Couch, 1990).

E. The Genitourinary Tract

The genitourinary tract is an important portal of entry for several viruses, including papillomaviruses, HIV, and herpes simplex. In general transmission occurs as a result of sexual activity. The penile urethra is lined with pseudostratified columnar epithelial cells for most of its length but near its opening the surface becomes covered with stratified squamous epithelial cells that are contiguous with the skin. The female urethra exhibits a similar epithelial cell distribution. The vagina and external female genitalia are also lined with stratified squamous epithelial cells. Infection of other epithelial surfaces by papilloma and herpes simplex has been considered in the preceding sections. Apart from the presence of cervical mucus, vaginal secretions, and urine, which may have antiviral function, the type of epithelial surface involved in infection of the genitourinary tract is similar to that described in the previous examples and the pathogenic processes are comparable. For this reason HIV will be considered as a specific example of the viruses that utilize this portal of entry.

Human immunodeficiency virus infection may occur via a number of routes, which include transfusion of blood or blood products and sexual activity, although the latter appears to be the most predominant means by which the virus is currently transmitted (Quinn, 1989; Friedland and Klein, 1987). Heterosexual or homosexual activities may mediate transmission and the virus has been isolated from semen and female genital secretions (Ho *et al.*, 1984; Zagury *et al.*, 1984; Vogt *et al.*, 1986, 1987; Wofsy *et al.*, 1986). Although both activities are expected to produce physical lesions in the genital epithelia, there is considerable evidence that HIV is able to penetrate an intact mucosal surface. Thus virus transmission has been documented as a result of nontraumatic artificial insemination using infected semen (Stewart *et al.*, 1985), and vaginal transmission to a chimpanzee has been reported (Fultz *et al.*, 1986). Experimental transmission of cell-free simian immunodeficiency virus (SIV) to both male and female rhesus macaques

has been demonstrated under conditions that are unlikely to result in damage of the urethral or vaginal epithelium (Miller *et al.*, 1989). Because HIV appears capable of replication in various epithelial cell types, both in cell culture and *in vivo* (Adachi *et al.*, 1987; Nelson *et al.*, 1988; Mathijs *et al.*, 1988; Cohen *et al.*, 1989; Moyer *et al.*, 1990), the possibility exists that infection of epithelial cells within the genital mucosa results in virus transmission. The HIV virions appear to be preferentially released from the basolateral surface of epithelial cells (Fantini *et al.*, 1991a; Owens *et al.*, 1991). Such directional release may be significant in the context of urogenitary transmission because infection of cells underlying the epithelial surface should be facilitated. The HIV receptor has been localized to the basolateral surface of the human adenocarcinoma cell line HT29 D4 (Rabenandras-ana *et al.*, 1990; Fantini *et al.*, 1991b), which suggests that transepithelial transport may be required prior to infection. Indeed, based on the observation that much higher doses of SIV are required to mediate infection following inoculation onto undamaged genital epithelia than are required by intravenous inoculation, Miller *et al.* (1989) conclude that genital epithelia do act as a barrier to virus infection. It is likely that the genital epithelia are a significant barrier to HIV because many individuals do not become infected despite repeated exposure to the virus at this site (Kim *et al.*, 1988). However Fantini *et al.* (1991b) have reported that differentiated HT29 D4 cells are susceptible to infection with HIV-1, albeit at a low level, following adsorption to either the basolateral or apical plasma membrane domains. Because these authors also demonstrated, by immunofluorescence staining, that CD4 remained localized to the basolateral surface and reported that anti-CD4 monoclonal antibodies did not inhibit HIV replication, infection of this cell type may be mediated by a CD4-independent mechanism. Although CD4-independent infection of several cell types has been described and an alternate receptor identified for at least one cell type (Harouse *et al.*, 1991), it is not yet clear whether a similar, nonpolarized infectious route is involved in HIV replication within epithelial tissues *in vivo*. Human immunodeficiency virus infection of epithelial cells may also be facilitated by contact with infected monocytes. Such physical contact is reported to induce the rapid assembly and release of virions from the monocyte into an enclosed space between the two cell types (Bourinbaiar and Phillips, 1991).

Reports suggest that Langerhans' cells, which are present within the urogenitary stratified squamous epithelium, and related dendritic cells are susceptible to HIV infection *in vivo* and *in vitro* (Tshachler *et al.*, 1987; Langhoff *et al.*, 1991; Zambruno *et al.*, 1991). Langerhans'

cells are specialized antigen-presenting cells that migrate through the epithelium to the regional lymph nodes on binding to antigen. This cell type therefore represents an important potential mechanism by which HIV, or other viruses, may circumvent the epithelial barrier (Braathen, 1988). Interestingly, studies with transgenic mice have indicated that the HIV long terminal repeat is preferentially expressed in Langerhans' cells (Leonard *et al.*, 1989), suggesting that additional cell-specific factors may be involved in targeting HIV replication to this cell type. A similar cell-mediated portal of entry has been proposed for infections resulting from rectal HIV exposure. Amerongen *et al.* (1991) observed that HIV-1 virions adhered to the luminal membranes of M cells contained within explanted rabbit and mouse Peyer's patches, were endocytosed, and apparently transcytosed to the basal intraepithelial space. These authors did not observe virus entry into adjacent enterocytes. Because M cells are reported to be numerous in the rectal epithelium (O'Leary and Sweeny, 1986), it is feasible that virus infection by this route results from M cell-mediated transcytosis.

F. Virus Infection of Other Polarized Cell Types

Neuronal cells exhibit a distinct polarity of membrane protein distribution. The cellular processes of the neural cell, or neurites, are divided into two functional categories: the axon and the dendrites. Dendrites generally act as signal receptors whereas the axon is typically involved in signal transmission. To carry out these distinct roles, a neural cell exhibits a polarized distribution of functionally significant membrane polypeptides between dendrites and axons (Angelides *et al.*, 1988; Jones *et al.*, 1989). Evidence indicates that the mechanism of polypeptide sorting utilized by neuronal cells may be similar to that involved in the targeting of epithelial cell plasma membrane polypeptides. The infection of cultured rat hippocampal neurons with VSV or influenza virus revealed a marked polarization of the respective viral envelope glycoproteins (Dotti and Simons, 1990; Dotti *et al.*, 1991). VSV G was confined to the dendrites whereas influenza HA was predominantly detected in the axon. In addition to providing insight into the mechanism of polypeptide sorting in neuronal cells, these observations have interesting implications for viral pathogenesis. A number of viruses are neurotropic and in some cases an exclusive dependency on neural spread from the site of inoculation is exhibited. Although it has yet to be established that such viral transport within a neuron is equivalent to vectorial release from epithelial cells, in the light of the observations described above it is evident that the transport mechanisms involved in neuronal spread may be similar to those involved in

the polarized targeting of viruses in epithelial cells. Thus herpes simplex virus, for example, which is released from the basolateral surface of epithelial cells may express appropriate targeting signals to direct a dendritic localization. Indeed, a retrograde axonal transport of viral capsids has been demonstrated to occur subsequent to HSV infection of sensory neurons (Baringer and Swoveland, 1973; Bastion et al., 1972; Stevens, 1975; Stevens et al., 1975; Cook and Stevens, 1973). In this example the virus becomes localized to the cell body dorsal root ganglia, where replication occurs and latency is established (reviewed by Whitley, 1990).

Viral latency and the establishment of chronic persistent infections are characteristic of several diverse virus groups (reviewed by Ahmed and Stevens, 1990). Although other cell types are more commonly associated with viral persistence, epithelial cells may play a role in the maintenance of a persistent infection and in some cases be involved in the process of virus shedding from the chronically infected host. Persistently infected cells often exhibit a reduced expression of viral proteins on the cell surface, which is thought to be important for the evasion of antibody–complement-mediated cell lysis (Oldstone and Buchmeier, 1982; Roizman and Sears, 1987; Francis and Southern, 1988). The polarized expression of viral proteins on the apical domain of epithelial cells, which may not be subject to significant immune surveillance, should therefore fulfill a similar purpose and may be important for the maintenance of persistent infections involving epithelial tissues. In addition, several viral infections are typified by a prolonged period of virus shedding into the saliva and/or urine and are likely to involve the infection of polarized epithelial cells. Two examples of viruses (Epstein–Barr virus and cytomegalovirus) that initiate long-term infections of salivary gland epithelial cells and utilize their secretory product as a means of transmission have been discussed above.

Although the kidney is not a significant portal of entry for viruses, this organ is often central to the process of virus shedding from the host and may remain chronically infected for extended periods. Thus SV40 establishes a persistent infection of the kidneys of its natural host, the rhesus monkey, without apparent pathological consequences (Sweet and Hilleman, 1960). Some polyomaviruses, such as JC and BK viruses, establish similar infections of humans and have been isolated from the urine of individuals under conditions that may result in reactivation of a latent virus (Borgatti et al., 1979; Coleman et al., 1977, 1980; Hogan et al., 1980). Although polyomavirus infections are not necessarily limited to the kidney, virus-contaminated urine is generally considered to be the primary source of infectious virus, which is

probably transmitted by the oral or respiratory route (Walker and Frisque, 1986; Yoshike and Takemoto, 1986). Observations of polarized monkey kidney epithelial cells infected with SV40 *in vitro* revealed an almost exclusive release of virus from the apical surface prior to cell lysis (Clayson *et al.*, 1989). Although these observations have not yet been extended to other nonenveloped viruses or to *in vivo* infections, preferential release from the apical surface of epithelial cells lining the kidney tubules would be consistent with the presence of virus within the urine and, therefore, may play an important role in virus release from the host organism.

Another example of a virus group that may utilize the kidney and salivary epithelium to facilitate virus shedding is the arenaviruses. Arenaviruses characteristically initiate persistent infections that are typically localized to the cells of the reticuloendothelial and lymphoid systems; lymphocytic choriomeningitis virus (LCMV), the prototype arenavirus, has been demonstrated in the thymus, lymph node, spleen, and blood lymphocytes of the persistently infected mouse (Popescu *et al.*, 1979). Arenavirus infection of congenital, newborn, or (with some exceptions) mature hosts does not normally result in significant disease and the viremic period is comparatively short (Johnson and Webb, 1973; Rawls *et al.*, 1981). However, virus is characteristically detected in the saliva and urine of infected organisms. Lymphocytic choriomeningitis virus is preferentially released from the apical surface of monkey kidney epithelial cells *in vitro* (C. Stephenson, personal communication), and it seems likely that vectorial release from kidney or salivary gland epithelial cells may be involved in virus shedding from the host organism.

V. Conclusions

The demonstration that virus entry and release is often restricted to specific plasma membrane domains of polarized epithelial cells has stimulated a great deal of interest concerning the cell biology of virus infection and its relationship to viral pathogenesis. Polarized epithelial cells in culture, which can be grown on permeable supports, provide excellent systems for investigating the events in virus entry and release at the cellular level, and much information is being obtained using such systems. Much remains to be learned about the precise routes by which many viruses traverse the epithelial barrier to initiate their natural infection processes, although important information has been obtained in some systems. Another area of great interest for future investigation is the process of virus entry and release from other polarized cell types, including neuronal cells. In addition to

providing new insights into our understanding of viral pathogenesis, it is anticipated that increased knowledge concerning the cell biology of viral infection will also provide novel insights that will be useful for development of new approaches for the control of viral diseases.

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