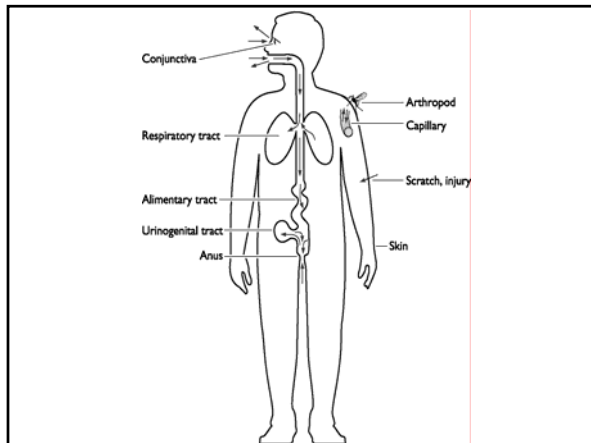
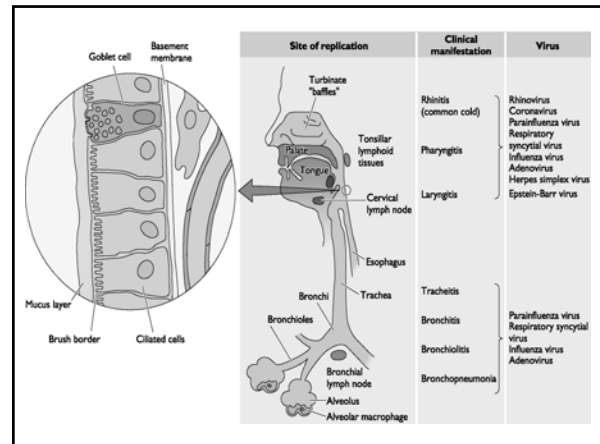


## Viral Pathogenesis

- **Pathogenesis:** the process by which one organism causes disease in another
- Two components of viral disease:
  - Effects of virus replication on the host
  - Effects of host response on virus and the host
- The goal of studies on pathogenesis is to identify the viral and host genes that influence the production of

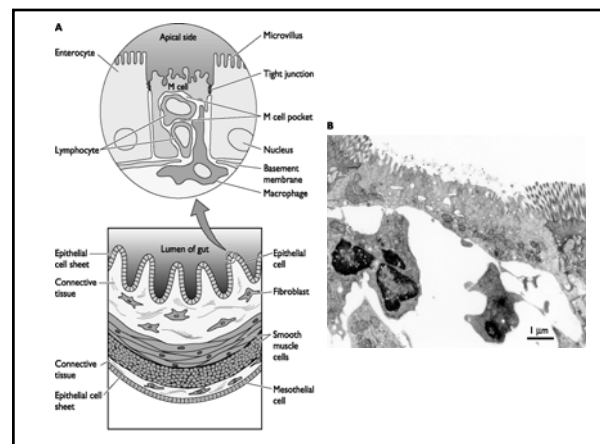


## Alimentary tract

- Eating, drinking, social activities introduce viruses into the alimentary tract
- Designed to mix, digest, absorb food, so contents are always in motion; good opportunities for virus-cell interactions
- Extremely hostile environment: stomach is acidic, intestine is alkaline; presence of digestive enzymes, bile detergents, mucus, antibodies, phagocytic cells
- Viruses have evolved to infect are resistant: enteroviruses; reovirus (require proteases); enteric coronavirus (enveloped!)

## Respiratory tract

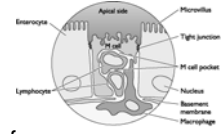
- Most common route of viral entry
- Absorptive area of lung: 140 m<sup>2</sup>; ventilation rate 6 L/min
- Barriers to infection: swallowing; ciliary action from lower tract; macrophages in alveoli (no cilia or mucus); IgA
- Viruses enter by aerosolized droplets from cough or sneeze, or contact with saliva
- Large droplets lodge in nose; smaller in airways or alveoli



## Urogenital tract

- Protected by mucus, low pH
- Minute abrasions from sexual activity may allow viruses to enter
- Some viruses produce local lesions (HPV)
- Some viruses spread from urogenital tract (HIV, HSV)

## Viral Spread

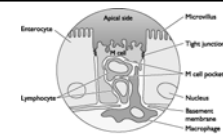


- After replication at the site of entry, viruses may remain localized: virus spreads within the epithelium and is contained by tissue structure and immune system
- Some viruses spread beyond the primary site: **disseminated**; if many organs are infected, **systemic**
- Physical and immune barriers must be breached

## Eye

- Sclera and conjunctiva are route of entry
- Every few seconds eyelid passes over sclera, washing away foreign particles; little opportunity for infection
- Infection usually occurs after injury: grit, ophthalmologic procedures, improperly sanitized swimming pools
- Localized infection: conjunctivitis
- Disseminated infection: EV70 spread to CNS
- HSV-1 can infect cornea, blindness may result, virus spread to sensory ganglia

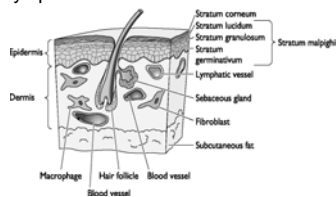
## Viral Spread



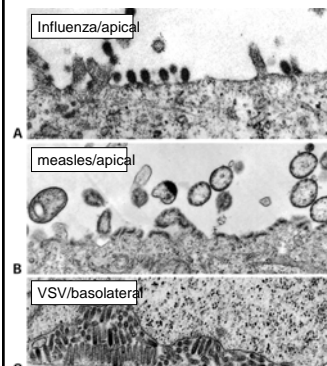
- Below the epithelium is the basement membrane; integrity can be compromised by epithelial inflammation and destruction
- Below basement membrane are subepithelial tissues, where virus encounters tissue fluids, lymphatic system, and phagocytes; all play roles in clearing and spreading infection
- Role of directional release of virus from

## Skin

- Outer layer of dead, keratinized cells cannot support viral infection; entry usually occurs by breaks or punctures
- Skin abrasions; insect or animal bites; needle punctures
- Epidermis is devoid of blood or lymphatics; local replication
- Dermis and sub-dermal tissues are highly vascularized; infection may spread

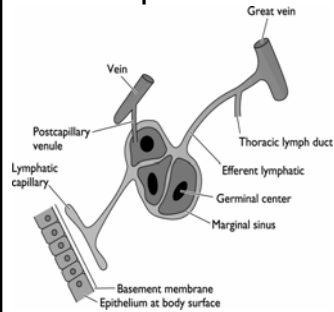


## Viral Spread



- Apical release facilitates virus dispersal; virus usually does not invade underlying tissues
- Basolateral release provides access to underlying tissues and may facilitate systemic spread
- Sendai virus: apical release from respiratory tract, local infection; mutant that is released from both apical and basal surfaces causes disseminated infection

## Hematogenous Spread

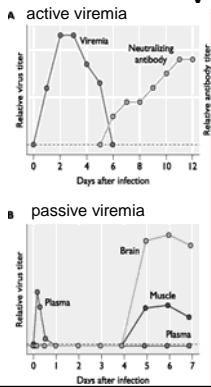


- Viruses that produce disseminated infection often do so by entering the blood
- Viruses may enter blood directly through capillaries, by replicating in endothelial cells, or through vector bite
- Virus in the extracellular fluids is taken up by lymphatic capillaries, which are more permeable than circulatory capillaries, then spread to blood
- Once in blood, virus has access to almost every tissue
- In lymph nodes, viruses encounter lymphocytes and other immune cells, and may replicate in them; may also spread infection to distant tissues
- Other viruses spread freely in the blood

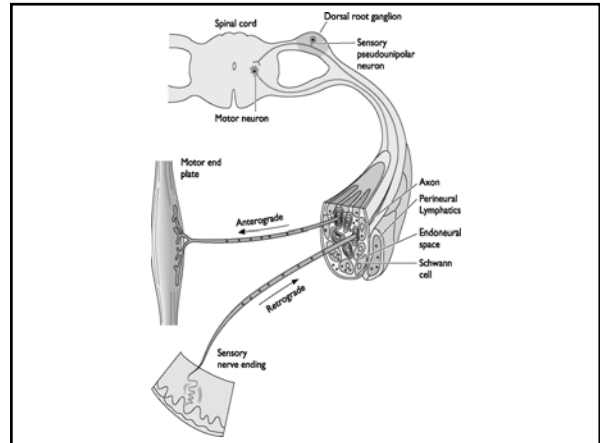
## Neural spread

- Many viruses spread from primary site of infection by entering local nerve endings
- For some viruses (rabies, alpha herpesviruses) neural spread is definitive characteristic of pathogenesis
- For other viruses (poliovirus, reovirus) invasion of the CNS is an infrequent diversion from normal replication and host defense

## Viremia

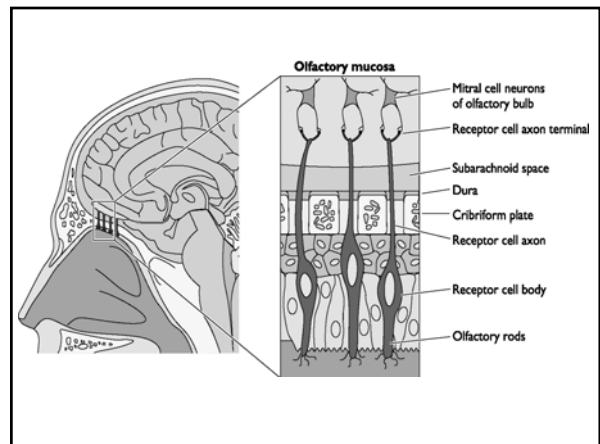
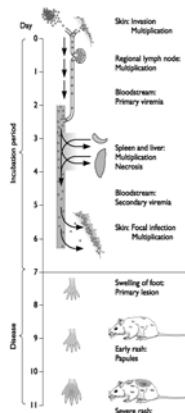


- Presence of infectious virus in the blood
- Active viremia: results from virus replication
- Passive viremia: results from virus introduced into the blood without replication
- Diagnostic value
- Practical problems (blood supply)



## Pathogenesis of mousepox

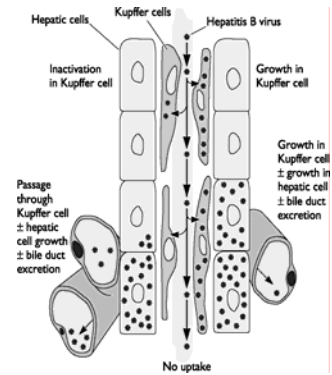
- Frank Fenner
- First to demonstrate how disseminated viral infections develop from local multiplication to primary and secondary viremia to target organs



## Viral spread to the central nervous system

Pathway	Viruses
Neural	Poliovirus, yellow fever virus, mouse hepatitis virus, Venezuelan encephalitis virus, rabies virus, reovirus (type 3 only; type 1 spread by viremia), herpes simplex virus types 1 and 2, pseudorabies virus
Olfactory	Poliovirus (experimental), herpes simplex virus, coronavirus
Hematogenous	Poliovirus, coxsackievirus, arenavirus, mumps virus, measles virus, herpes simplex virus, cytomegalovirus

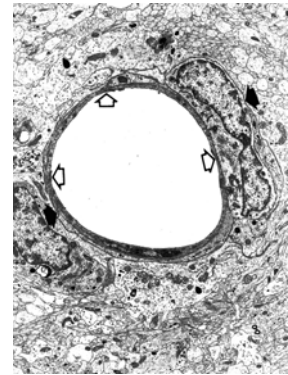
## Tissue invasion: Liver



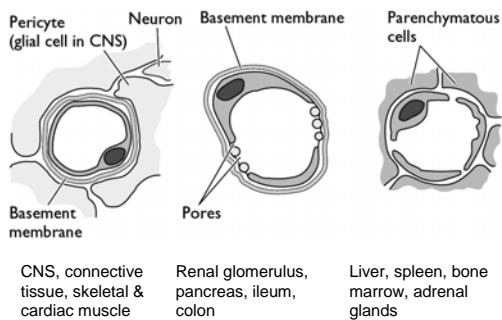
## Infections of the CNS

- A neurotropic virus can infect neural cells; infection may occur by neural or hematogenous spread from a peripheral site
- A neuroinvasive virus can enter the CNS after infection of a peripheral site
- A neurovirulent virus can cause disease of nervous tissue
- HSV: low neuroinvasiveness, high neurovirulence
- Mumps: high neuroinvasiveness, low neurovirulence
- Rabies: high neuroinvasiveness, high neurovirulence

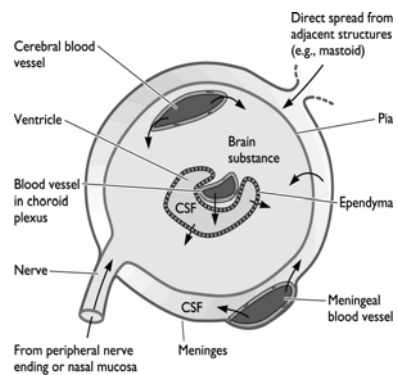
## Tissue invasion: blood-brain junction



## Tissue invasion



## Tissue invasion: CNS



## Tissue Tropism

- The spectrum of tissues infected by a virus
  - e.g. an enteric virus replicates in the gut and not in the lung; a neurotropic virus replicates in cells of the nervous system and not in hematopoietic cells
- The tropism of some viruses is limited; other viruses are pantropic, e.g. can replicate in many organs
- What are the determinants of viral tropism?

## Viral virulence

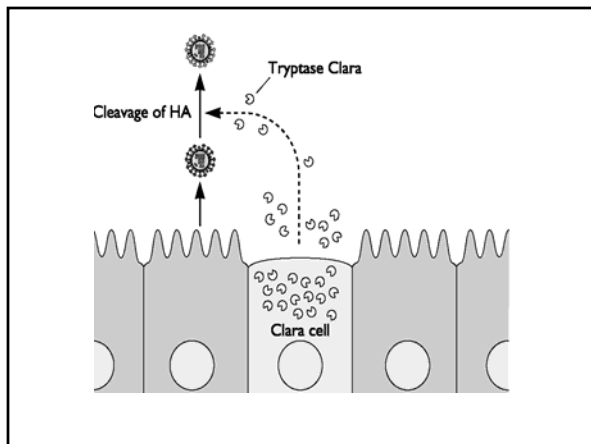
- The capacity of a virus to cause disease in an infected host
- A virulent virus causes significant disease, while an avirulent or attenuated virus causes reduced or no disease
- Virulence can be quantitated:
  - LD<sub>50</sub> (Lethal Dose 50%; amount of virus needed to kill 50% of infected animals)
  - The mean time to death
  - The mean time to appearance of symptoms
  - Measurement of fever, or weight loss
  - measurement of pathological lesions (poliovirus); reduction in blood CD4+ lymphocytes (HIV-1)

## Determinants of Tissue Tropism

- Cell receptors for viruses
  - e.g. HIV 1&CD4; EBV & CR2 but not poliovirus or influenza virus
- Cellular proteins that regulate viral transcription
  - e.g. JC papovavirus replicates in oligodendrocytes because the viral enhancer is active only in this cell type
- Cell proteases
  - e.g. cleavage of influenza virus HA by serine proteases

## What makes viruses virulent?

- A major goal of virology is to identify viral and host genes that determine virulence
- Virulence genes are usually identified by mutation: deletion or disruption of one of these genes results in a virus that causes reduced or no disease in a specified system
- Viral genes affecting virulence fall into four classes:
  - Those that affect the ability of the virus to replicate
  - Those that modify the host's defense mechanisms
  - Those that enable the virus to spread in the host
  - Those which have intrinsic cell killing effects



Virus	Growth in cell culture	Effect on mice	Virulence phenotype
Wild type			Neurovirulent
Mutation leading to a general defect in replication			Attenuated
Mutation in a gene specifically required for virulence			Attenuated

## Genes that modify the host's defense mechanisms

- Virokines (secreted proteins that mimic cytokines, growth factors, or similar extracellular immune regulators) and viroceptors (homologs of host receptors or cell surface immune molecules)
- Mimic normal cellular molecules critical to host defense
  - sabotage the body's innate and adaptive defenses
  - Not required for growth in cell culture
  - Most have been found in large DNA viruses (pox, herpes, adenovirus)
- Examples:
  - Soluble cytokine receptor - bind cytokines, block action
  - Proteins that bind key proteins in complement cascade
  - Proteins that affect MHC-1 antigen presentation

## Mechanisms of cell injury by viruses

- Non cytolytic viruses: disease usually a consequence of the immune response:

Proposed mechanism	Virus	
CD8 <sup>+</sup> T cell mediated	Coxsackievirus B	
	Lymphocytic choriomeningitis virus	
	Sin Nombre virus	
	Human immunodeficiency virus type 1	
	Hepatitis B virus	
CD4 <sup>+</sup> T cell mediated		
	Th1	Theiler's virus
		Mouse coronavirus
		Semliki Forest virus
		Measles virus
Th2		
		Visna virus
		Herpes simplex virus
		Respiratory syncytial virus
	Antibody mediated	Dengue virus
	Feline infectious peritonitis virus	

## Toxic viral proteins

- NSP4 nonstructural glycoprotein of rotaviruses: a viral enterotoxin
- When expressed in cells, causes increase in intracellular calcium.
- When fed to young mice, causes diarrhea by potentiating chloride secretion. Thus, NSP4 triggers a signal transduction pathway in intestinal mucosa

## Mechanisms of cell injury by viruses

- Lesions associated with **CD8<sup>+</sup> T cells**: myocarditis caused by coxsackievirus B
- Hypothesis: tissue damage due to cytotoxicity of CD8<sup>+</sup> T cells; perforin knockout mice develop less severe disease
- CD8<sup>+</sup> T cells may also release proteins that recruit inflammatory cells which elaborate proinflammatory cytokines

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## How do viruses injure cells?

- Infection of cultured cells by cytolytic viruses: cytopathic effects
- Many viruses cause inhibition of host protein and RNA synthesis, which leads to loss of membrane integrity, leakage of enzymes from lysosomes, cytoplasmic degradation
- Syncytium formation by enveloped viruses (parainfluenza, HIV)
- Virus infection can induce *apoptosis* (programmed cell death)

## Lesions associated with B cells: Dengue

- Caused by Dengue virus, transmitted mainly by bites of *Aedes aegypti* mosquitoes
- Endemic in the Caribbean, Central and South America, Africa and Southeast Asia
- 50 million infections/year
- Primary infection is usually asymptomatic, but may result in standard symptoms of virus infection: *acute febrile illness with severe headache, back and limb pain and rash. Severe aches and pains in the bones.*
  - Normally self-limiting, patients recover in 7-10 days

## Dengue Fever

- In 1/14,000 *primary* infections, people get **Dengue Hemorrhagic Fever (DHF)**, a life threatening disease.
- Patients produce antibodies to virus, but there are four serotypes, and no cross-protection
- Non-protective antibodies can **enhance** the infection of peripheral blood monocytes by **F<sub>c</sub>-receptor mediated uptake** of antibody coated virus particles. Infected macrophages release cytokines, causing severe symptoms
- After *secondary* dengue infections, (i.e. infections of people with antibody to Dengue virus), the incidence of DHF 1/90.

## Cell injury associated with free radicals

- Superoxide ( $O_2^-$ ) and nitric oxide (NO) are produced during the inflammatory response
- NO is made by nitric oxide synthase, an interferon-inducible enzyme
- Low concentrations of NO have a protective effect, high concentrations cause tissue damage by reacting with  $O_2^-$  to form peroxynitrite, which is much more reactive than either radical

