Principles of clinical virology

Structure and pathogenesis

Viruses in May 2006

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Viral Disease

- Oldest recorded disease (Rabies, polio)
- Modern epidemics/pandemics – HIV-AIDS, HCV, SARS, Avian Influenza
- Impact on - humans
  - animals
  - plants
  - evolution

1. What is a virus
   - Characteristics
   - Structure
   - Replication
   - Definitions
2. How viruses cause disease
   - Molecular principles
   - Disease pathogenesis
3. Diagnosis of viral illness
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A virus is a molecular genetic parasite that uses cellular systems for its own replication

[Villarreal, 2005]

Viruses

- This intimate relationship between the virus and the cell causes several important effects:
  - Viruses are not killed by antibiotics
  - Antivirals often damage the cell
  - Viruses can persist in cells either replicating (HIV) or resting – latent (HSV)

Viruses

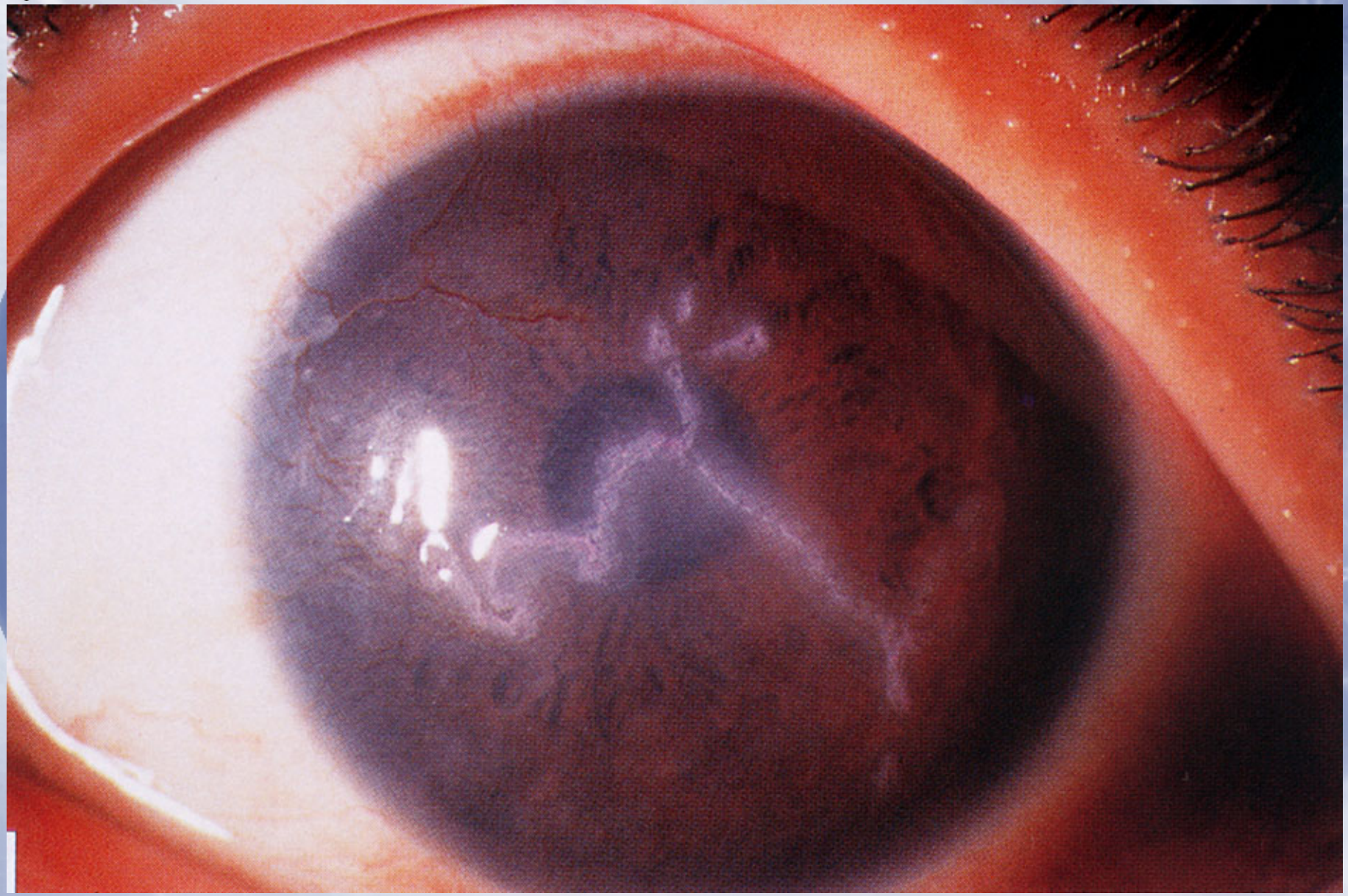
- Viruses are the simplest organisms, containing DNA or RNA, but not both.
- Prions are similar, but distinct, only contain protein. Original concept was a growing organism like a virus or a human had to contain either DNA or RNA (virus) or both (humans). Prions are the only known exception

Viruses have life

- Can be killed
- Can become extinct
- Undergo Darwinian selection
- Subject to evolutionary biology

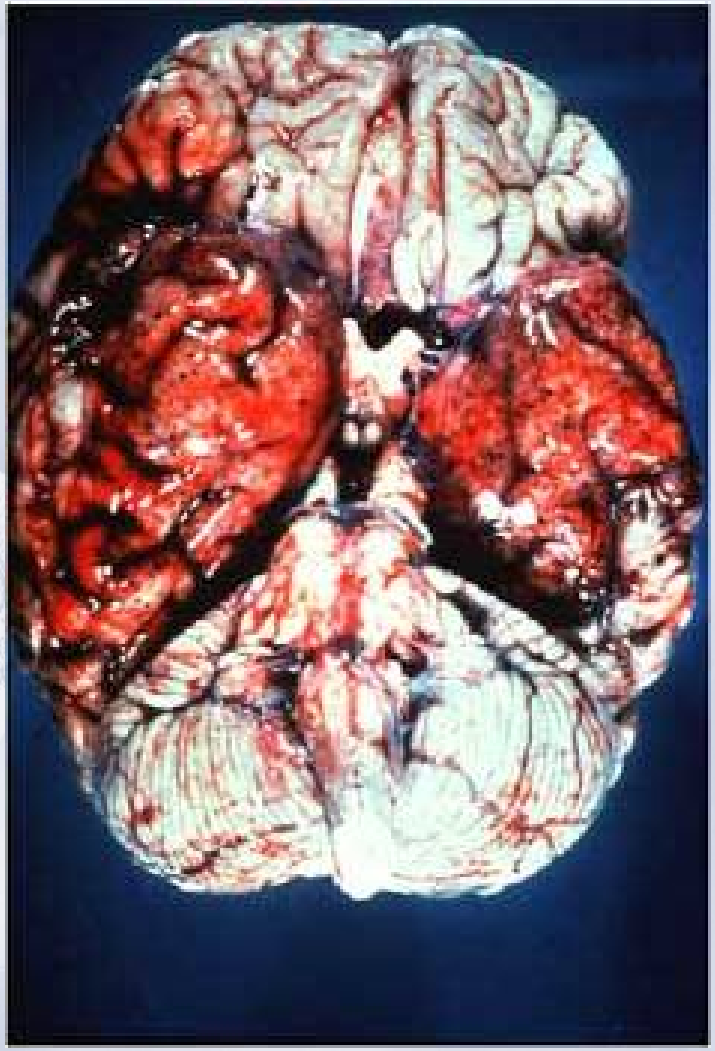
But viruses

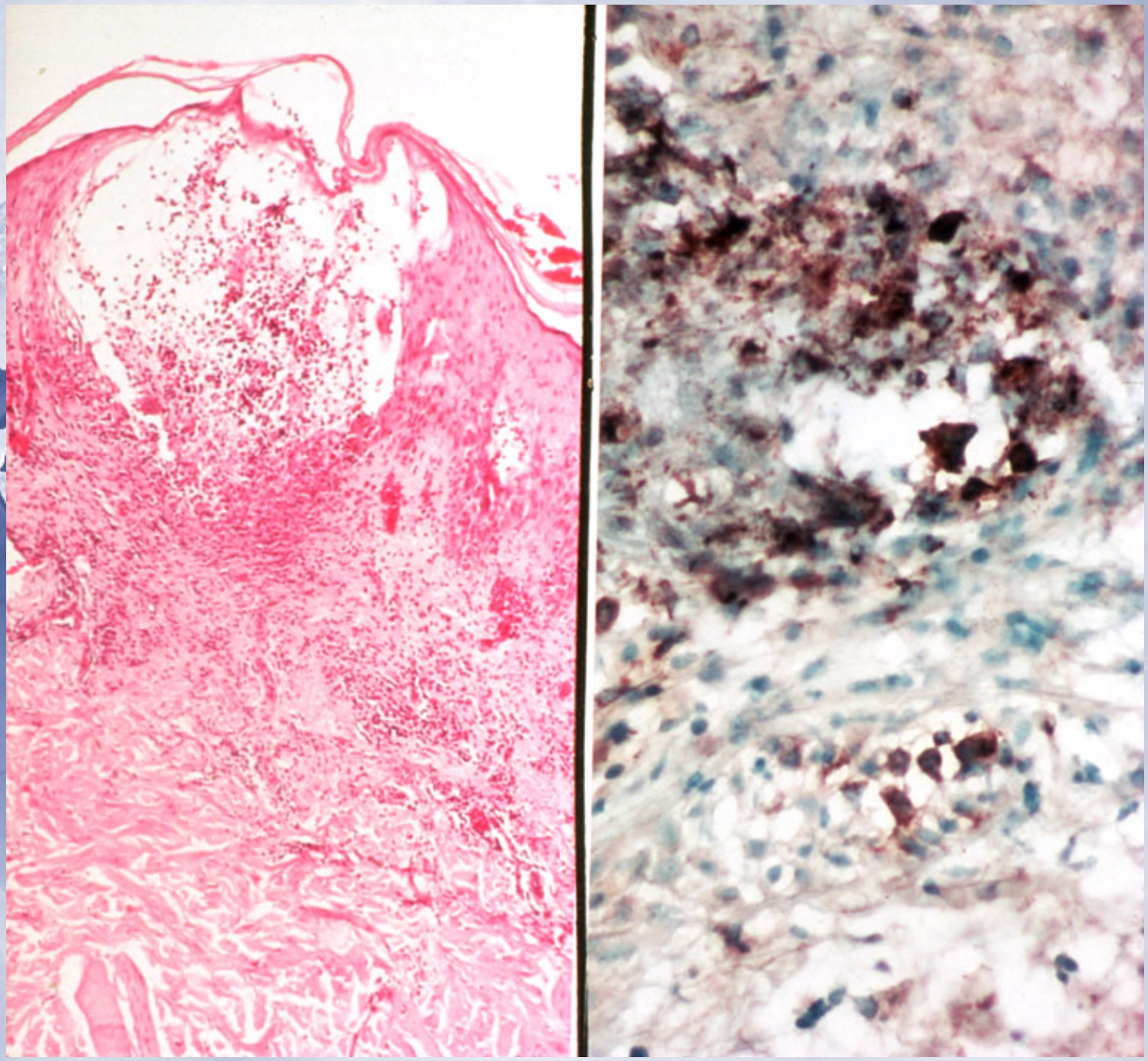
- Have no sexual exchange process
- Species is defined by its lineage
- Species is a class that occupies a replicating lineage and occupies an ecological niche



HSE - MRI

HSE





Structure

- Viruses are:
  - not cells
  - dependent upon the cell they infect. Inside cells they can replicate, outside cells they can be transmitted, but cannot replicate (grow)
  - contain DNA or RNA but not both
  - can grow from only virus DNA or RNA inside a cell
  - sometimes viruses integrate their nucleic acid into the host cell genome

Virion Architecture

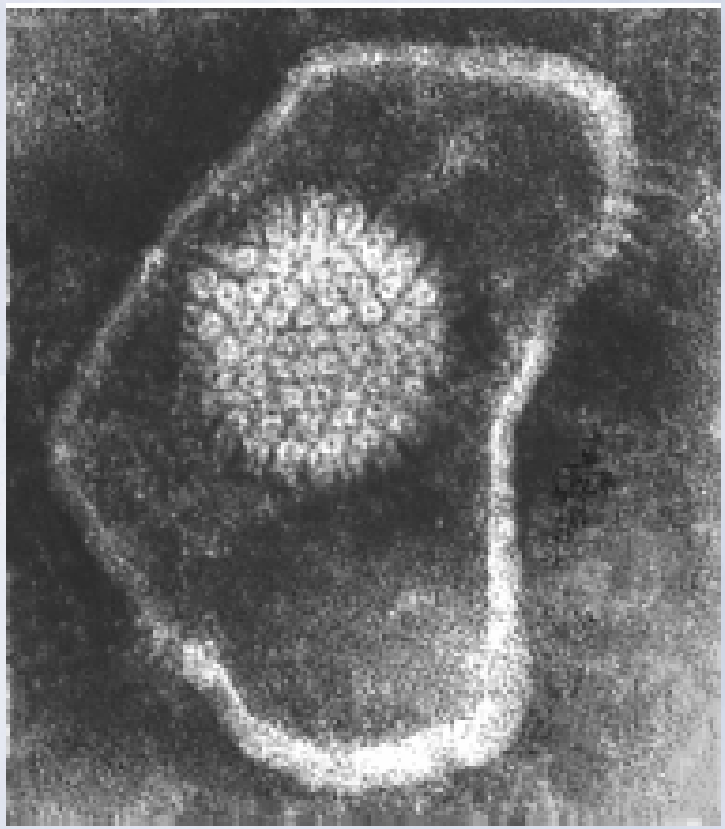
Architecture of virions regardless of host is based on two simple themes:

Sphere – normally in the form icosahedron (cubical)

- **Best way of producing a shell of equivalently bonded identical structures**
- **Minimum free energy state**
- **Strong structure that can enclose a maximal volume**

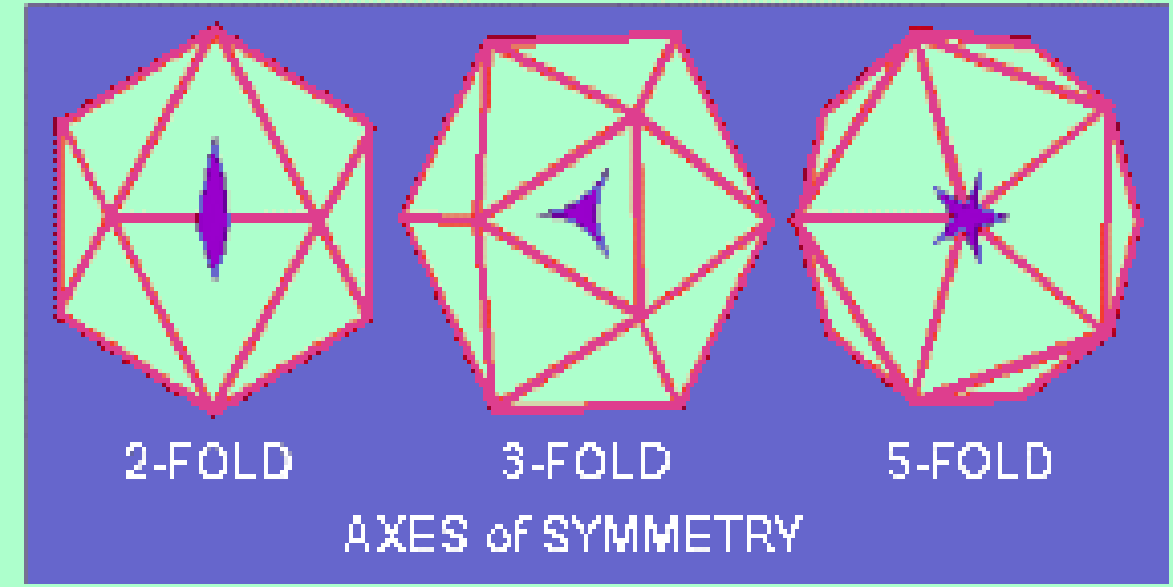
Helix – cylindrical shape (spiral staircase)

HSV-2 EM



Virion Architecture – icosahedron

An ICOSAHEDRON is composed of 20 facets, each an equilateral triangle, and 12 vertices (corners)

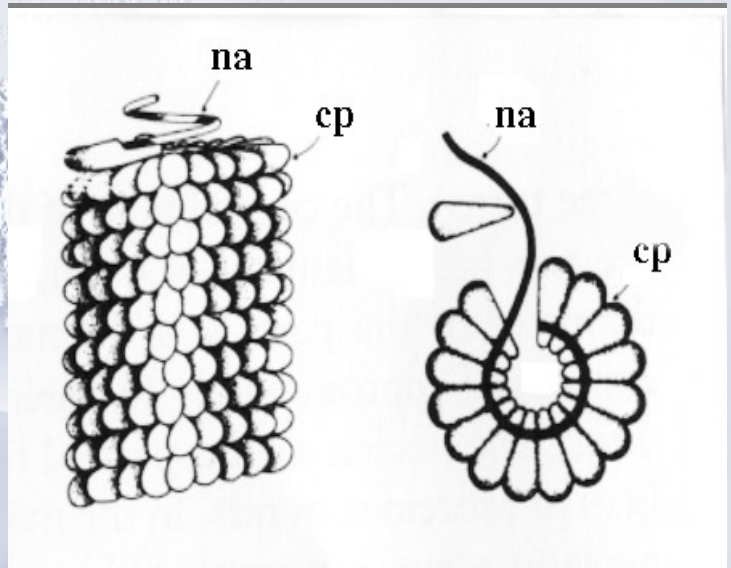


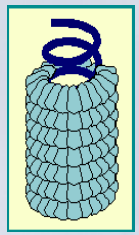
Helical viral structure

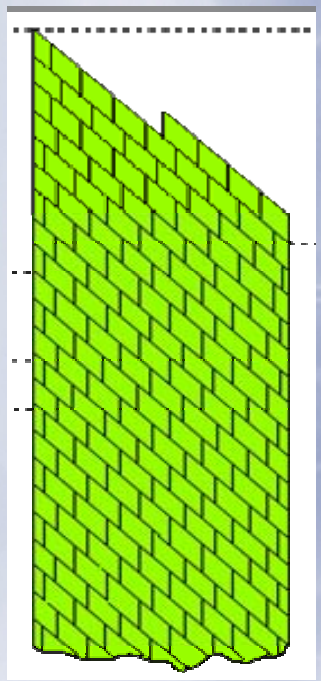
Several RNA viruses undergo self assembly as a cylindrical nucleocapsid. *(hollow tube)*

The viral RNA forms a spiral within the capsid structure

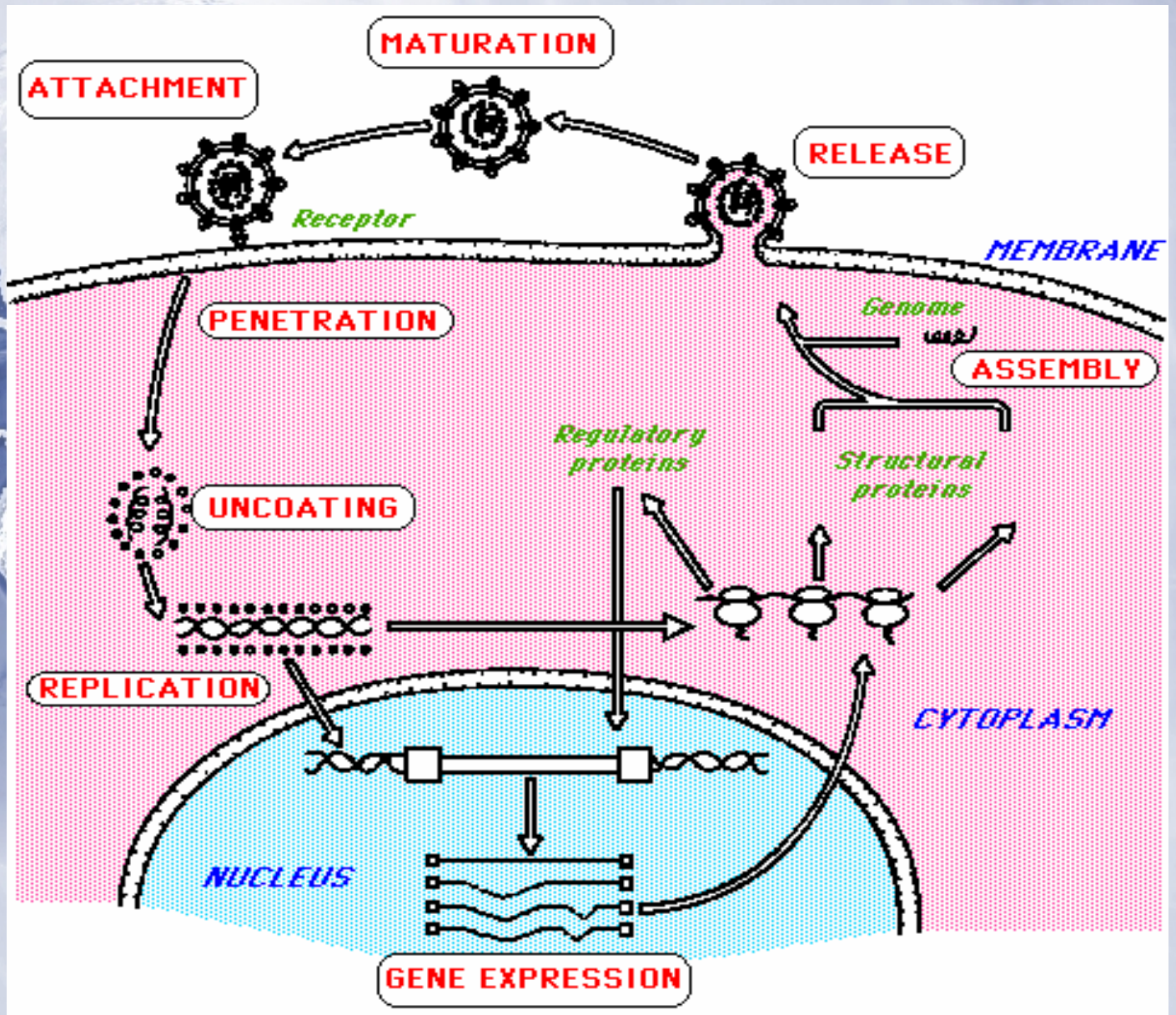
Each capsomer consists of a single protein







Virus replication



Classification

- Standard levels
  - Order ~virales
  - Family ~viridae
  - Subfamily ~virinae
  - Genus ~virus
  - Species

Classification

- Classification is based on:
  - Genomic makeup e.g: Caliciviruses
  - Virion structure – EM appearance e.g: HV
  - Replication strategy
  - Virion antigenicity e.g: adenoviruses, serological distinction MVE / JE / WNV
  - Virion chemical characteristics, stability
  - Diseases caused in the host e.g: hepatitis

Definitions

Capsid	Protein coat surrounding viral DNA or RNA. Made up of smaller subunits (capsomers) that self-assemble into symmetrical helices (all of which are enveloped) or icosaherons (with cubical symmetry)
Envelope	Lipoprotein surrounding the nucleocapsid
Ligand	Receptor binding molecule of a virus
Light microscope	Level of resolution is 200 nm, most viruses are <200 nm diameter

Definitions

Prion	Infectious protein, containing no DNA or RNA
Replication	Growth
Virion	Infectious viral particle
Viroid	Simplest virus-like organism, with infectious RNA and no protein coat
Virus	Infectious particle consisting of DNA or RNA, ± protein coat (capsid), ± lipid coat (envelope) cannot be seen with a light microscope of resolution 200 nm

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Viruses as a molecule

- Most viruses
  - 10 – 20 genes
  - Genomes 5,000 – 25,000 bp
- ICTVdb
  - 3,600 species
  - 30,000 strains + subtypes

Viruses as a molecule

- ssRNA – most diverse Noro, HCV, HIV
- dsDNA – Adeno, CMV, HSV, Variola
- dsRNA – Rota
- ssDNA – least diverse PVB19

Biological characteristics of acute and persistent virus life strategies

- Acute virus life strategy
  - No persistence in individual host
  - Often disease associated
  - High mutation rates (RNA viruses)
  - Virus replicates in more than one species
  - Virus does not show coevolution with host
  - Transmission is horizontal
  - Highly dependent on host population structure
  - Seldom evolves to persistence


1


0 cm 1 2 3 4 5

Biological characteristics of acute and persistent virus life strategies

- Persistent virus life strategy
  - Persistent in individual host
  - Seldom causes acute disease; often inapparent
  - Genetically stable
  - Virus is highly species specific
  - Virus often shows coevolution with host
  - Transmission is often from parent to offspring (vertical) or through sexual contact
  - Less dependent on host population structure
  - Often the source of emerging acute disease in new host species

Viral Syndromes

- Adenopathy and glandular fever
- Arthritis
- Carditis
- Chronic Fatigue Syndrome
- Congenital and perinatal disease
- Exanthemata and skin disease
- Eye disease
- Gastroenteritis

Viral Syndromes

- Haemorrhagic fevers
- Hepatitis
- Immunocompromised infections
- Neurological disease
  - encephalitis and meningitis
- Pancreatitis and diabetes
- Respiratory disease
- Sexually Transmitted Infections (STD, STI)

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Types of diagnostic test

- Open (Cytology, EM, Culture)

Vs Closed NAT, LA, Solid phase ELISA
Specific serology

- Viral specific (NAT)

Vs Host response (Ab, Microarray)

- Highly specific (Adenovirus PCR)

Vs Genus wide (Picornavirus PCR)

Diagnostic Methods

- Serology - retrospective
- Ag
  - Protein based
  - IFA (Respiratory)
  - WB (HIV)
  - Protein function (HIV-RT)
- Culture – some viruses non-cultivable
- Molecular
  - Virion nucleic acid
  - HIV RNA, HCV RNA
  - CMV DNA
- Emerging Microarray different formats, HPLC, Protein amplification

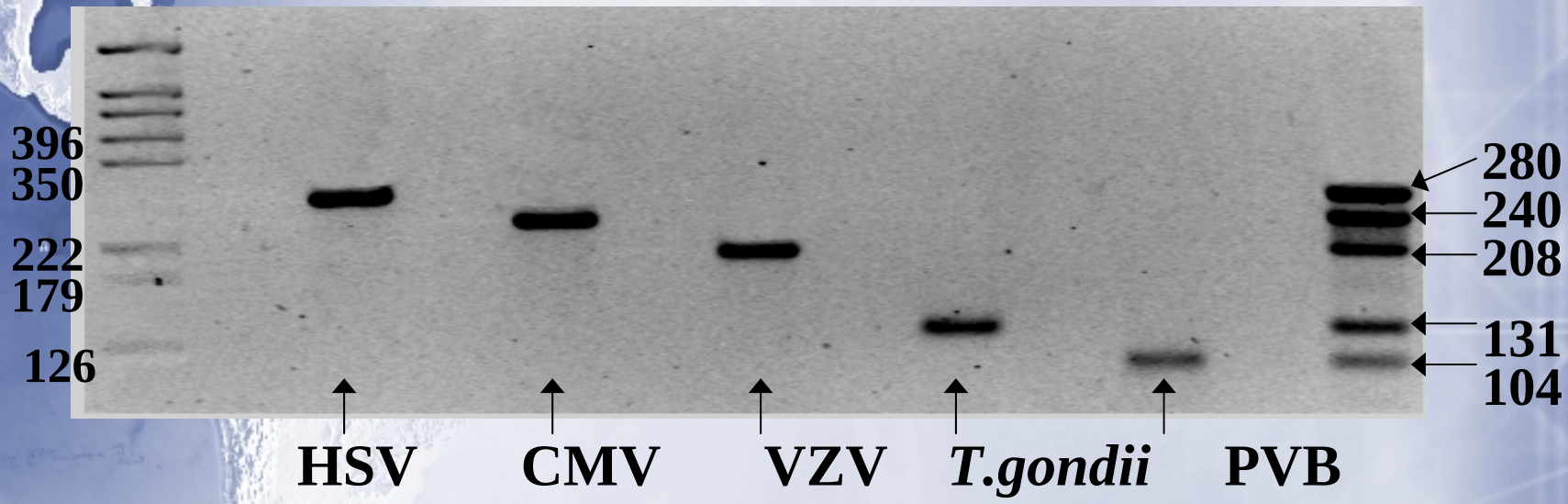
Serology

- ELISA, IFA, CFT
- Total (EV, HAV, HCV, HW, Influenza)
- Igm (Adeno, HSV)
- IgG (CMV, Mumps, Measles, PVB19 Rubella)
- Complex (HBV, EBV)
- No use (Rota, Noro, Variola)

Molecular testing

- Rapid, sensitive, costly (initially)
- Amplified
  - Target (PCR, LCR, NASBA, TMA)
  - Signal (bDNA)
- Non amplified
  - Probe based (ISH, Hybrid Capture)

Multiplex PCR



Ag testing

- Existing technique (Respiratory)
- Initial testing (p24)
- Only available (Prion)
- Dependent upon
  - many host cells in specimen
  - operator

Tests – emerging techniques

- New arrays
  - microarray
  - optical bead arrays
  - protein nanoarrays
- Redevelopment of existing methods
  - quantitation
  - multiplex
  - recombinant targets
  - combined NA-protein
  - interpretation

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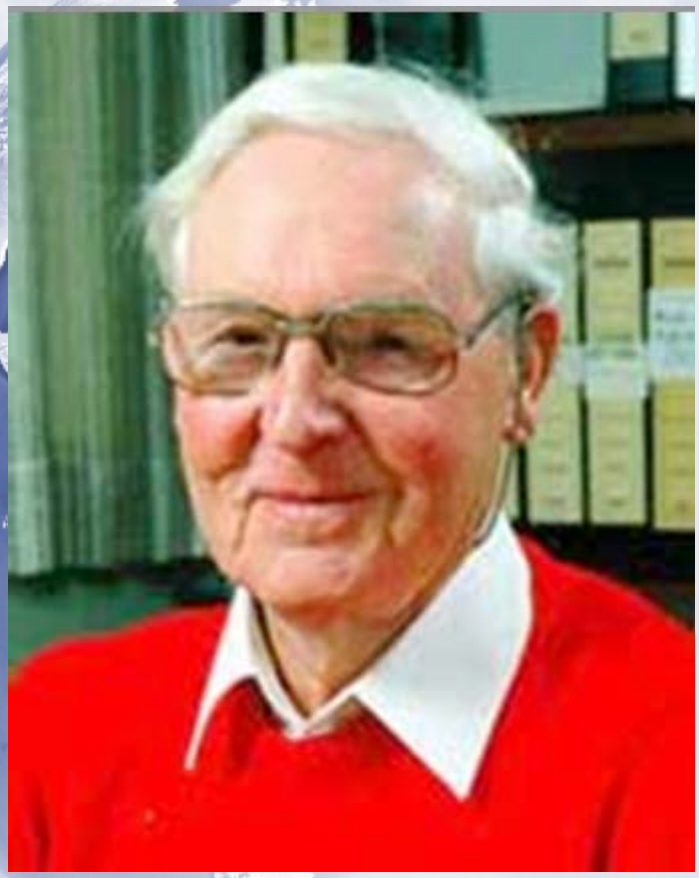
Epidemics

- Measles
  - Plague of Athens (436 B.C.) described a distemper-like epidemic with high mortality
  - Epidemics - Rome, China AD165, AD251
  - Considered a normal process of development
  - All adults survivors of childhood infection in Europe

Epidemics

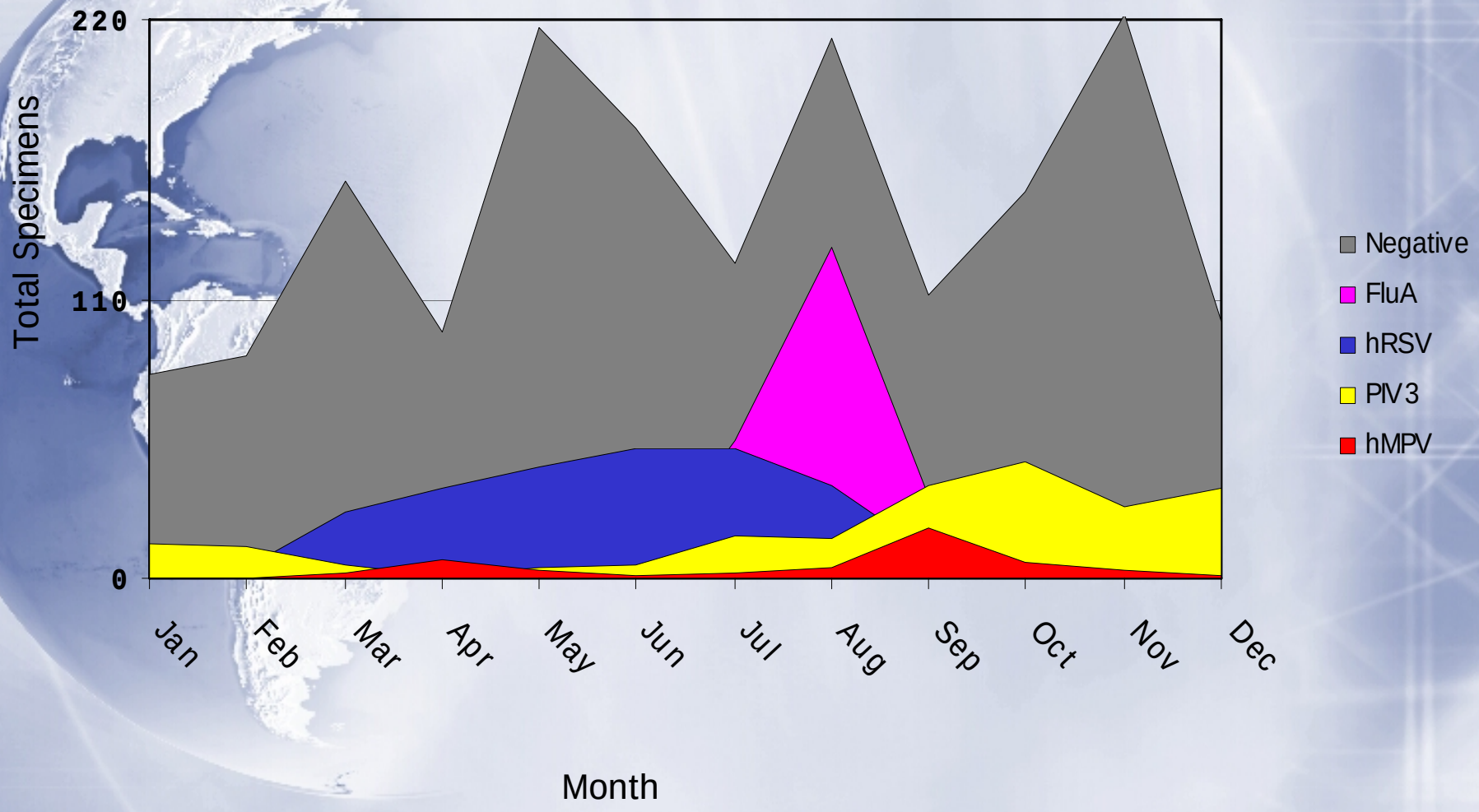
- Smallpox
  - Earliest accounts from India, in Sanskrit medical text, China, 1122 B.C.
  - Entered Europe via Islamic North African expansion to Spain; epidemics in Syria (A.D.302) and Mecca (A.D. 569)
  - Reintroduced to Europe via crusaders
  - Disease milder limited to children (Spain in the 1400s)

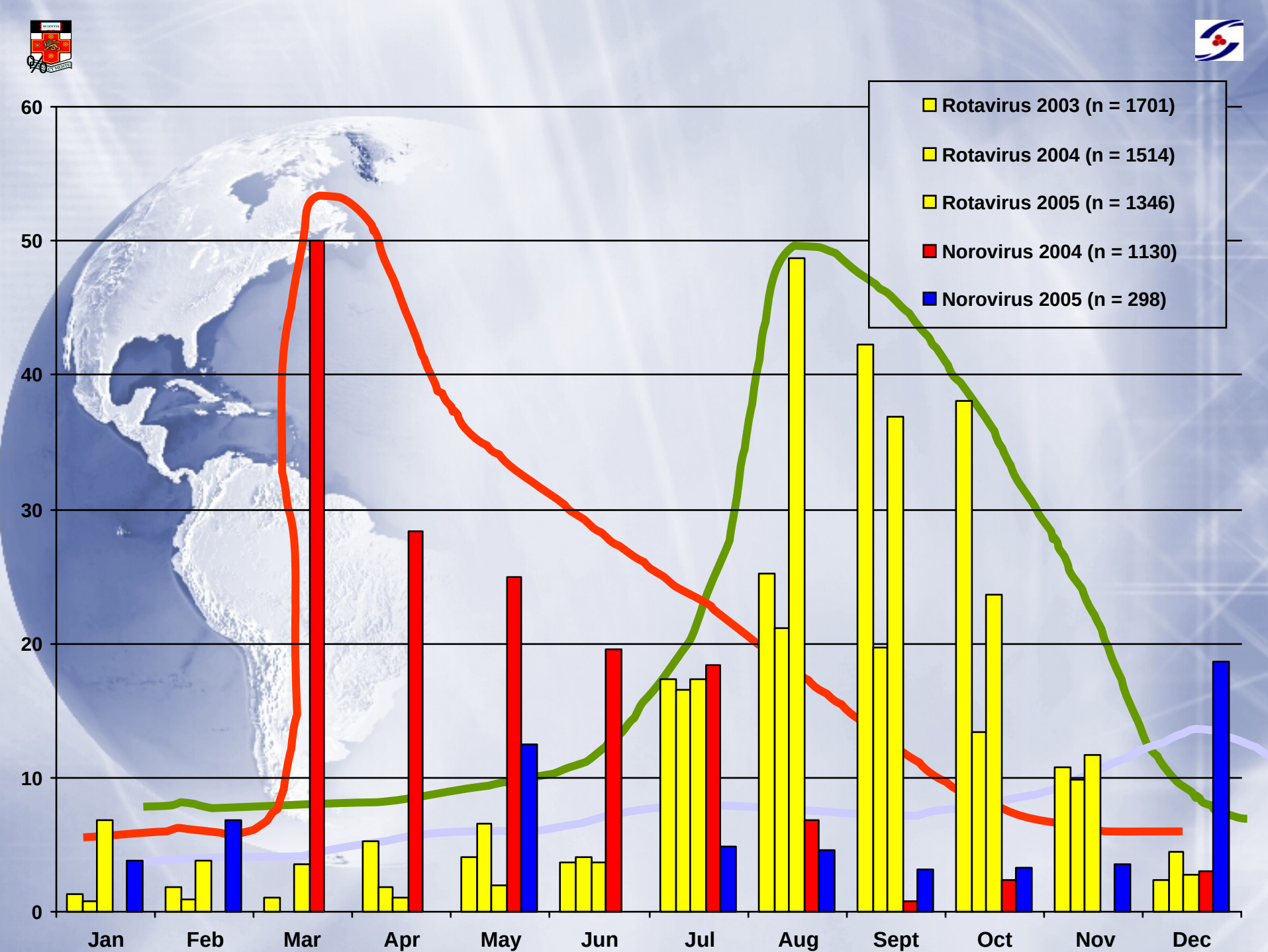


- Poxviruses
  - Variola (smallpox)
  - Myxoma
  - Ectromelia (mousepox)
- Infectious diseases
  - Malaria
  - TB
- Viral taxonomy
- Microbiology history

- Prime Minister's Prize (2002)
- Royal Society Copley medal (1996)
- Japan Prize for Preventive Medicine (1988)

VIRAL INFECTIONS CHILDREN

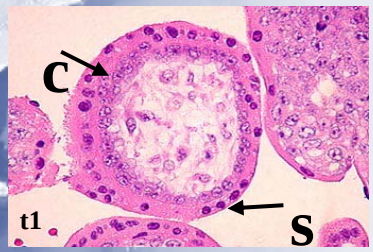


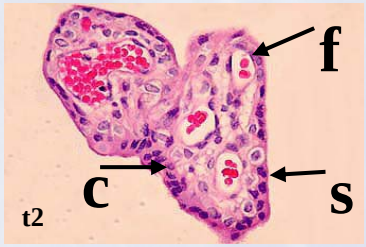

Rotavirus 2003 (n = 1701)
Rotavirus 2004 (n = 1514)
Rotavirus 2005 (n = 1346)
Norovirus 2004 (n = 1130)
Norovirus 2005 (n = 298)
60
50
40
30
20
10
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec

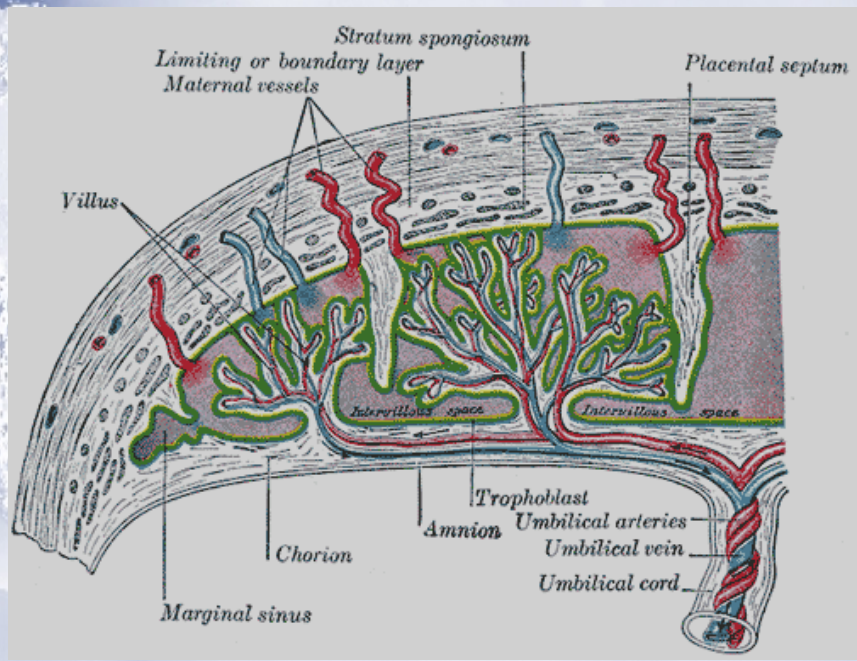
Congenital CMV Infection

- Hepatosplenomegaly
- Jaundice
- Microcephaly
- Prematurity
- Chorioretinitis
- Petechiae
- Mental retardation
- Hearing loss

Normal (uninfected) placental tissue







Blood Supply testing

- Mainly viral
- Ignores persistent non-threatening viruses
- Constantly changing
- Role of emerging viruses

Agents of risk – known / tested

- HB_sAg - Sept 1970
  - Abbott PRISM
- HCVAb - Feb 1990
  - Abbott PRISM
- HIV 1/2 Ab - April 1985
  - Abbott PRISM
- HTLV I/II Ab - Jan 1993
  - Abbott PRISM

- HCV RNA - June 2000
  - Pooled 24, TMA Chiron
  - Pooled 16, April 2005
- HIV 1 RNA - June 2000
  - Pooled 24, TMA Chiron
  - Pooled 16, April 2005
- CMV - selected

Agents of risk – known / not tested

- CMV
- GB-C virus - unknown
- HHV8
  - few donors
  - theoretical KS
- Prions vCJD
  - few donors
  - transmissible encephalopathy
- SARS coronavirus - few donors
- TT virus – 80-95% of donors
- SEN-V
  - 2% of donors
  - types A-H
  - hepatitis
- WNV
  - few donors
  - 3 week deferral process
  - encephalitis

Agents of risk – known / emerging risk

- Dengue - deferral procedures for at-risk
- Prions - vCJD [Llewellyn, 2004]
  - exclusion
- SARS coronavirus
- WNV
- Unknown

[Chamberland, 2001]

HEPATITIS VIRUS	ACUTE HEPATITIS	CHRONIC HEPATITIS	FULMINANT HEPATITIS	CIRRHOSIS	HCC
A	+	0	+	-	-
B	+	5-10%	+	+	+
C	+	75%	+	+	+
D	+	<5% 50%	+	+	+
E	+	0	+	-	-
G	+	20%	-	-	-
TTV	+	60%	-	-	-

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Some Emerging Issues

- New respiratory virus – SARS
  - hMPV
  - Associations with chronic conditions
- Transfusion – HCV
  - Non A non B non C
  - New viruses (GBV, TTV, Sen V)
- Old viruses re-emerging
  - Smallpox
- Zoonoses
  - SARS
  - Rabies
  - Arenaviruses
  - Hantaviruses