Ministry of Health, Republic of Belarus

Institution of Education

“Grodno State Medical University”

Department of Microbiology, Virology and Immunology named after S.I.Gelberg

VIROLOGY

Training appliance for students of the Department for International Students

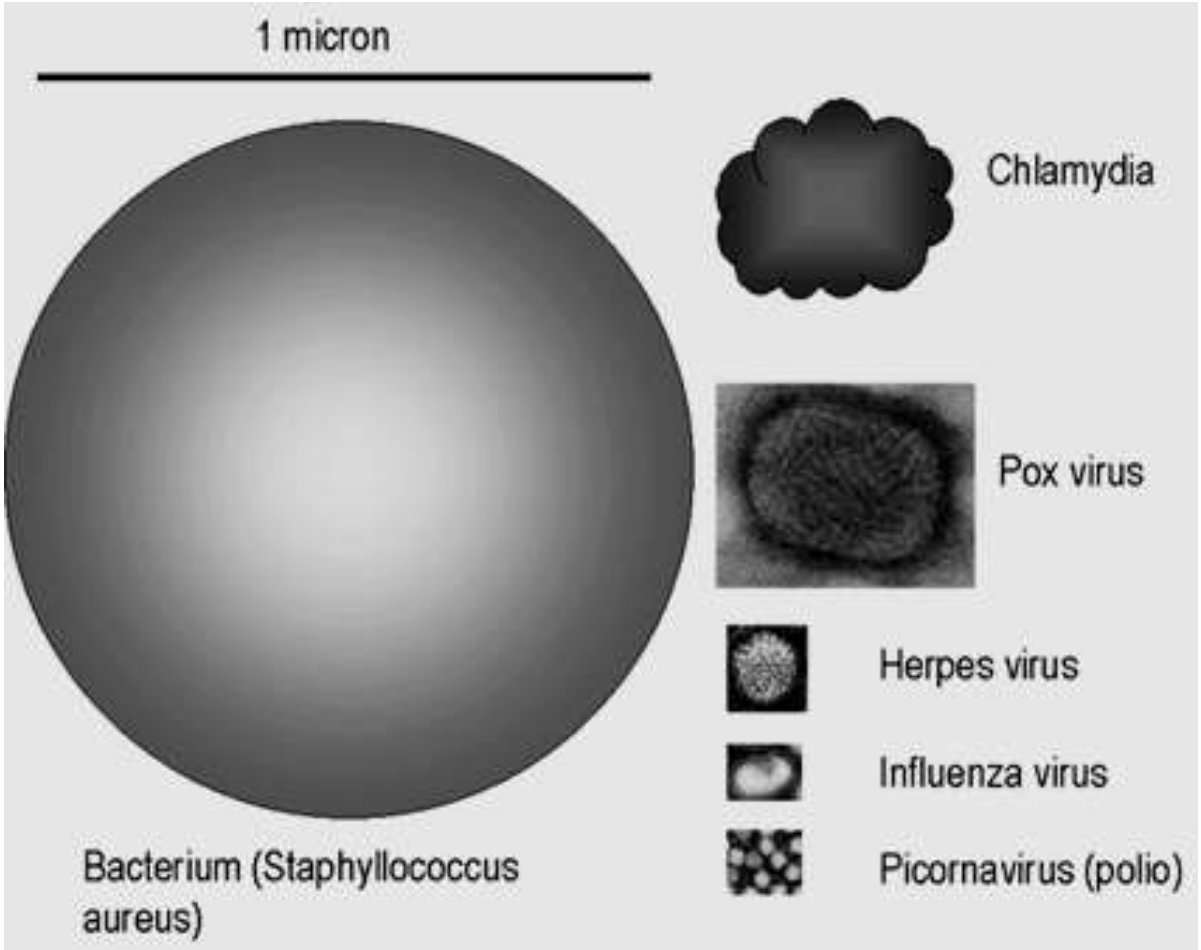
Theme N29

Discovery of viruses

Tobacco mosaic virus –
the first virus discovered by
Dmitry Ivanovsky – in 1892.

Dmitry Ivanosky
(1864 – 1920)

Relative sizes of viruses and bacteria



Main peculiarities of viruses

- ⇨ viruses are small, subcellular agents that are unable to multiply outside a host cell
- ⇨ the assembled mature virus (called virion) consist of a nucleic acid (either DNA or RNA) associated with proteins encoded by the nucleic acid
- ⇨ many viruses encode a few structural proteins = components of virion and enzymes that participates in the replication of the viral genome
- ⇨ viruses don't possess
  - ✓ cellular composition
  - ✓ own metabolism: unable to synthesise protein and to generate energy
  - ✓ own ribosomes or other organelles
- ⇨ viral nucleic acid can be integrated into the DNA of the infected cell and to replicate synchronously with the DNA of the host cell
- ⇨ the various virion components are synthesised separately within the host cell and then assembled to form progeny particles – ***disjunctive way of multiplication***

Structure of virions (fully assembled infectious viruses)

simple (<60 capsomeres)

complex (>60 capsomeres)

naked

nucleic acid + capsid → (2, 3 or 5 polypeptides = capsomere)

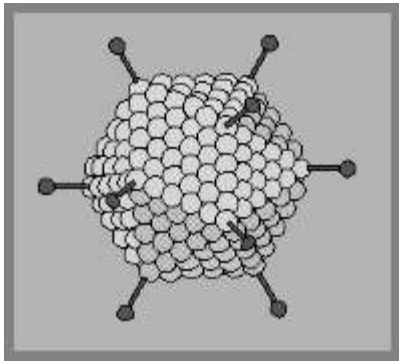
nucleocapsid

helical symmetry

coated

symmetry of virion

+ envelope

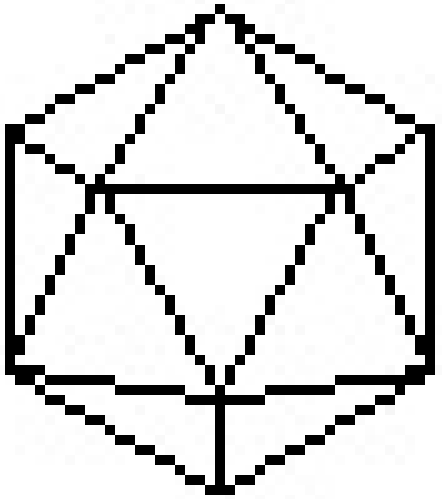


cubic symmetry

Structure of virions

- All viruses contain a nucleic acid genome and a protective protein coat (called the capsid).
- The nucleic acid could be:
  - RNA or DNA
  - single-stranded or double-stranded,
  - non-segmented or segmented,
  - linear or circular
  - negative or positive.
- The nucleic acid genome plus the protective protein coat is called the nucleocapsid which may have icosahedral, helical or complex symmetry.

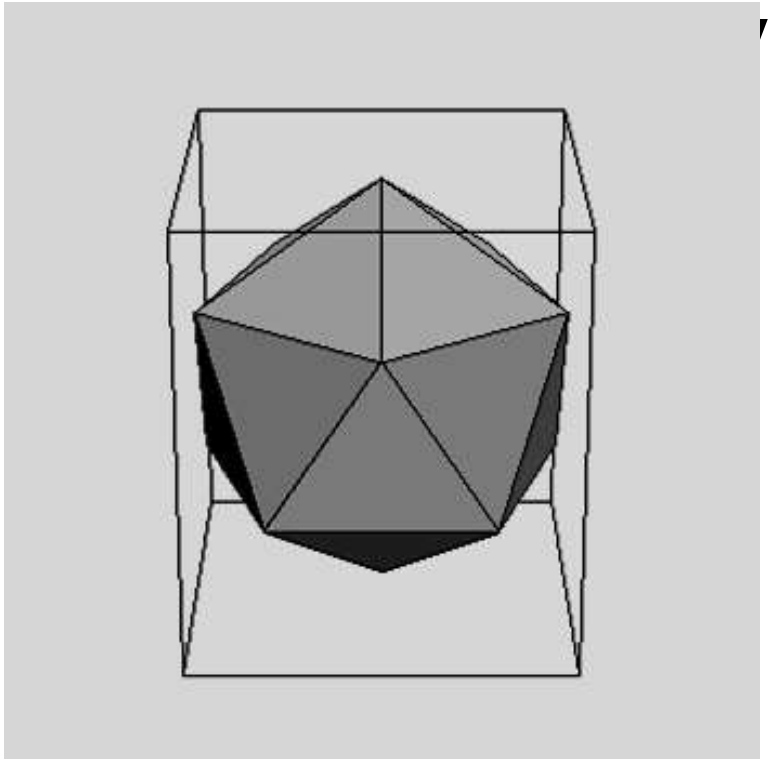
⇨ The virion may also have a lipid bilayer membrane (or envelope) that is acquired from the host cell (usually after budding of the virus from the host cell).

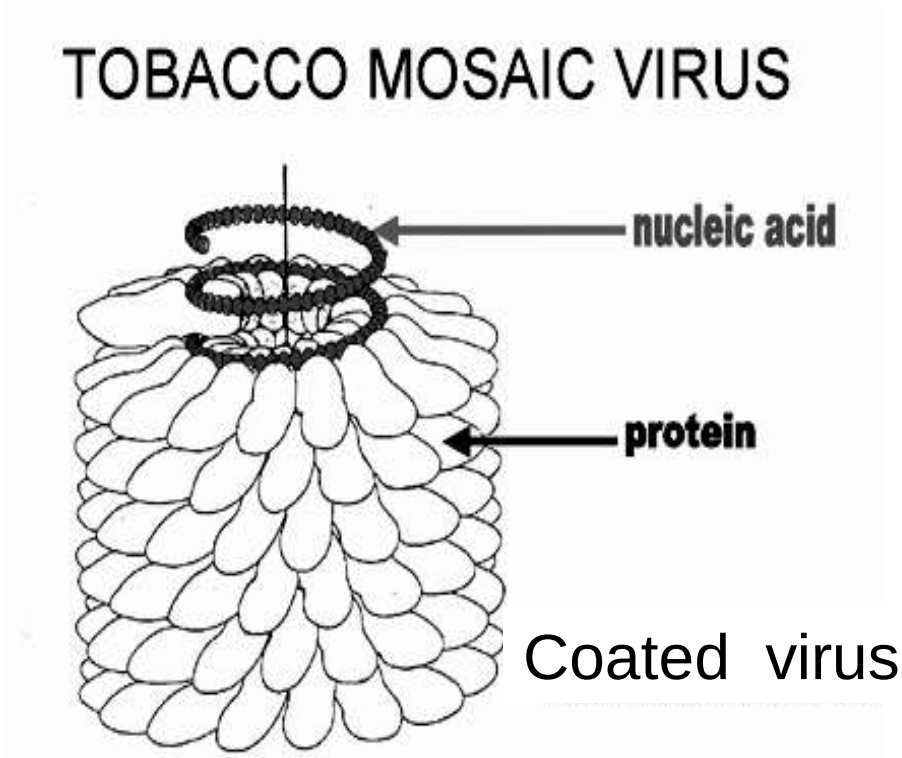


Naked virus

Coated virus

Icosahedral and helical symmetry

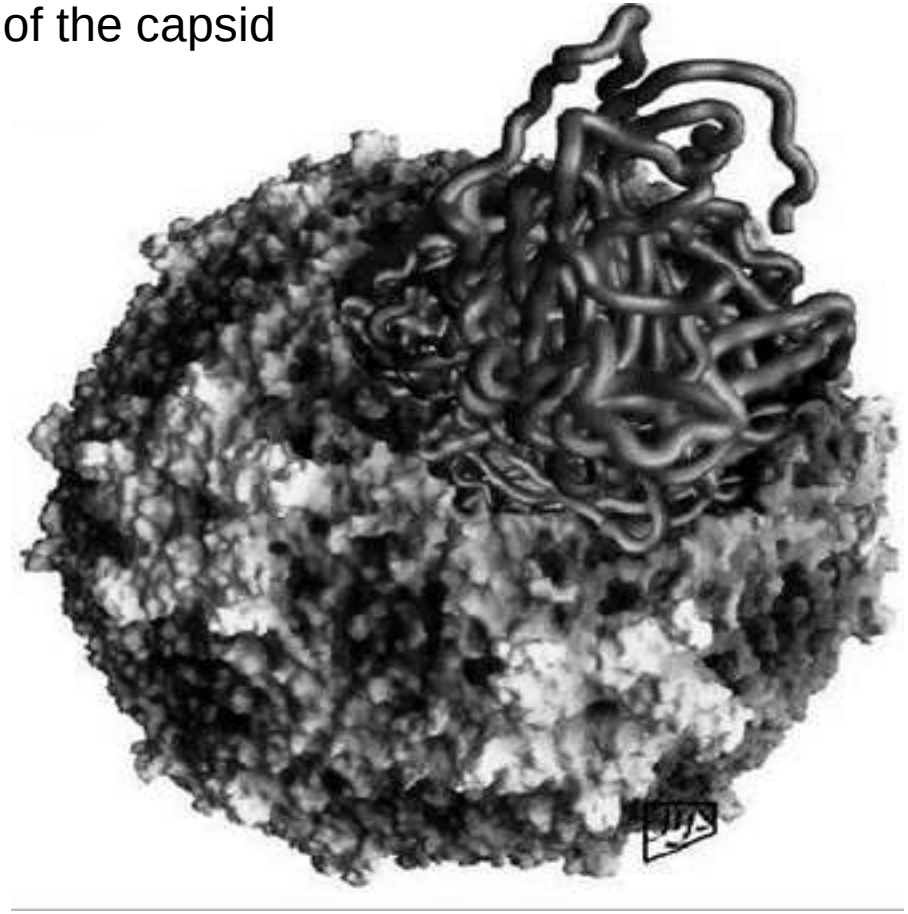




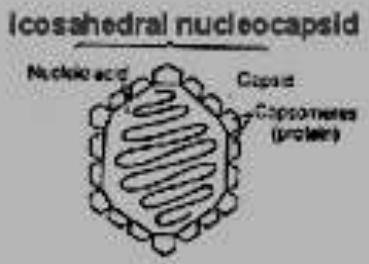
Coated virus

Structure of virion

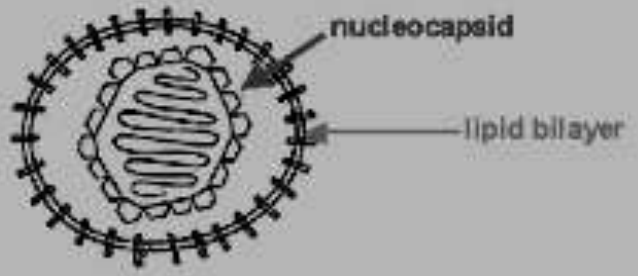
nucleic acid is packaged
inside of the capsid



5 BASIC TYPES OF VIRAL SYMMETRY

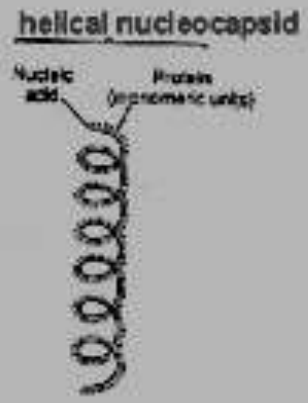


ICOSAHEDRAL

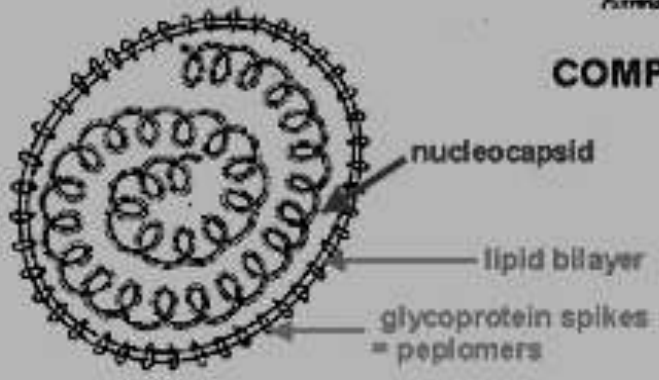


ENVELOPED ICOSAHEDRAL

COMPLEX



HELICAL

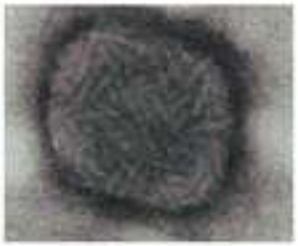


ENVELOPED HELICAL

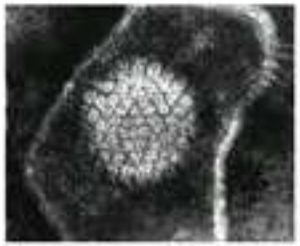
CLASSIFICATION OF VARUSES

Families of DNA-containing viruses (-viridae)		
ssDNA	dsDNA	
naked	naked	enveloped
Parvo- **Circino-**	**Adeno-** **Papilloma-** **Polyoma-**	**Pox-**(complex coats) **Herpes-** **Hepadna-**

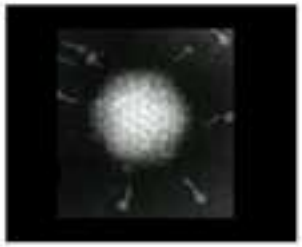
DNA viruses



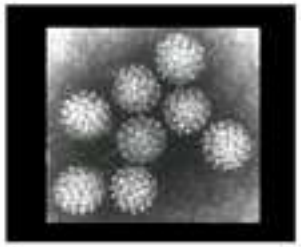
Poxviridae



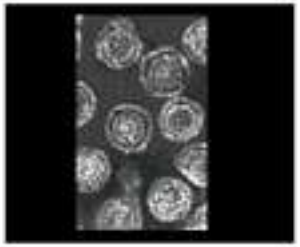
Herpesviridae



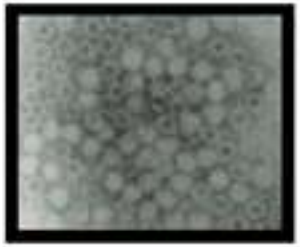
Adenoviridae



Papovaviridae
human papilloma



Hepadnaviridae



Parvoviridae

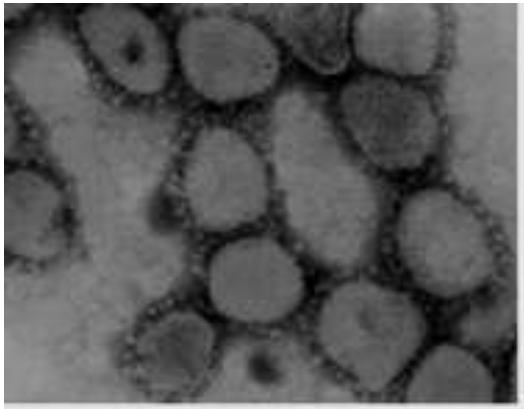
DNA Viruses

100 nanometers

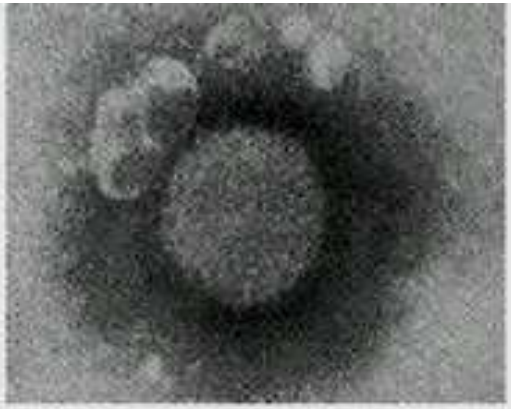
CLASSIFICATION OF VIRUSES

<table>
<tr><th colspan="5">Families of RNA-containing viruses(-viridae)</th></tr>
<tr><td colspan="4">ss</td><td>ds</td></tr>
<tr><td>naked</td><td colspan="3">enveloped</td><td>naked</td></tr>
<tr><td>+</td><td>+</td><td colspan="2">“–”(negative-sense strand)</td><td>+/–</td></tr>
<tr><td rowspan="2">Picorna-
Calici-</td><td rowspan="2">Retro-
Toga-
Flavi-
Corona-</td><td>nonsegmented</td><td>segmented</td><td>segmented</td></tr>
<tr><td>Paramyxo-
Rhabdo-
Filo-</td><td>Orthomyxo-
Bunya-
Arena-</td><td>Reo-</td></tr>
</table>

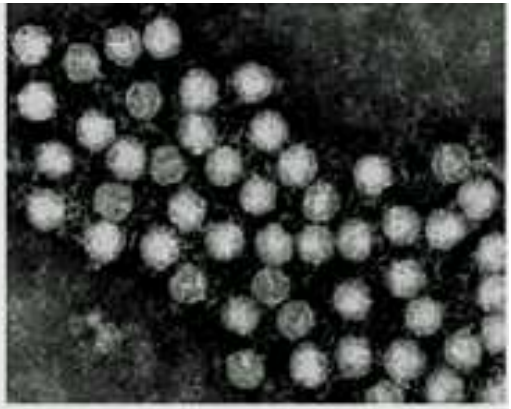
RNA viruses



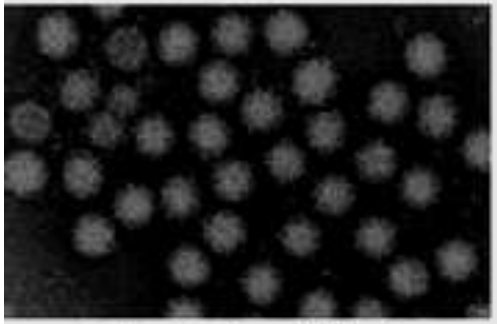
Coronaviridae (NS+)



Arenaviridae (S, ambi)



Picornaviridae (NS+)



Calciviridae (NS+)

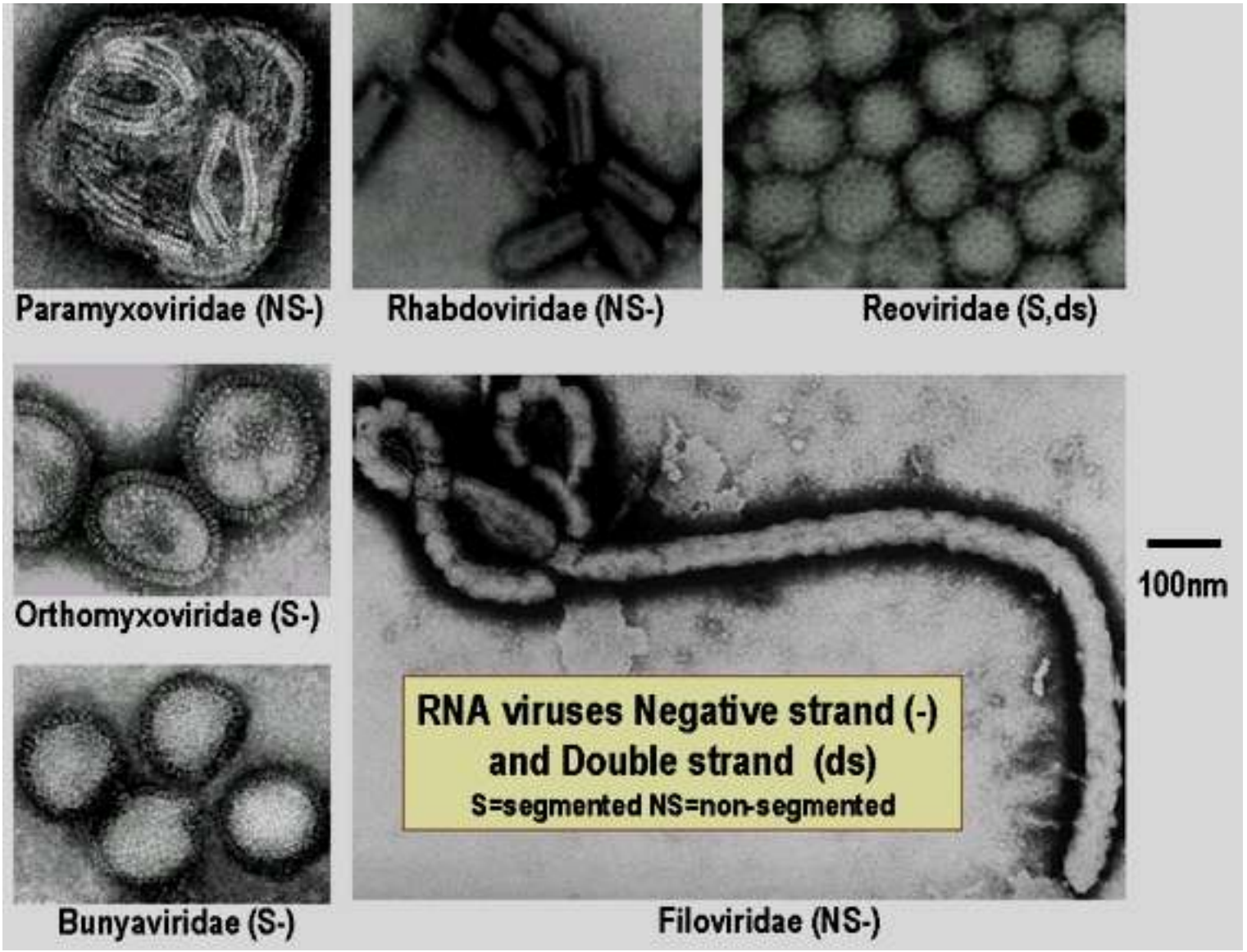
RNA viruses Positive strand (+)

S=segmented NS=non-segmented

Ambi: part + and part -

100nm

RNA viruses


Paramyxoviridae (NS-)
Rhabdoviridae (NS-)
Reoviridae (S,ds)
Orthomyxoviridae (S-)
Bunyaviridae (S-)
Filoviridae (NS-)
RNA viruses Negative strand (-)
and Double strand (ds)
S=segmented NS=non-segmented
100nm

Existing forms of viruses

entire (extracellular) infectious unit - **virion**

contains

- ✓ a molecule of **nucleic acid** (ss or ds DNA or RNA)
- ✓ **capsid –** protein shell
- ✓ in some viruses - additional covering, called the **envelope**

intracellular

(**virus**)

- ✓ presented only by **nucleic acid**

Tissue tropism of viruses

Viral affinity for specific body tissues (tropism) is determined by

(1)cell receptors necessary for attachment of virus,

(2)cell transcription factors that recognize viral promoters and enhancer sequences,

(3)ability of the cell to support virus replication,

(4)cell deproteinisation factors (enzymes)

Other small infectious agents resembling viruses

Virus contains nucleic acid (DNA or RNA) and protein, but there are other infectious agents which contain:

1. only nucleic acid – ***viroids (small - less than 400 nucleotides, single stranded, circular RNAs).***
2. only protein – ***prions (small, proteinaceous particles and there is controversy as to whether they contain any nucleic acid, but if there is any, there is very little, and not enough to code for protein)***.

Steps of multiplication of the viruses in the infected cell (productive infection)

1. Attachment
2. Penetration and uncoating (loss of proteins)
3. Synthesis of the viral
   - early and late proteins
   - replication of viral genome
4. Assembly of virions (maturation)
5. Release of mature virions from the host cell

The mechanisms involved in the processes of penetration of the virions into the cell

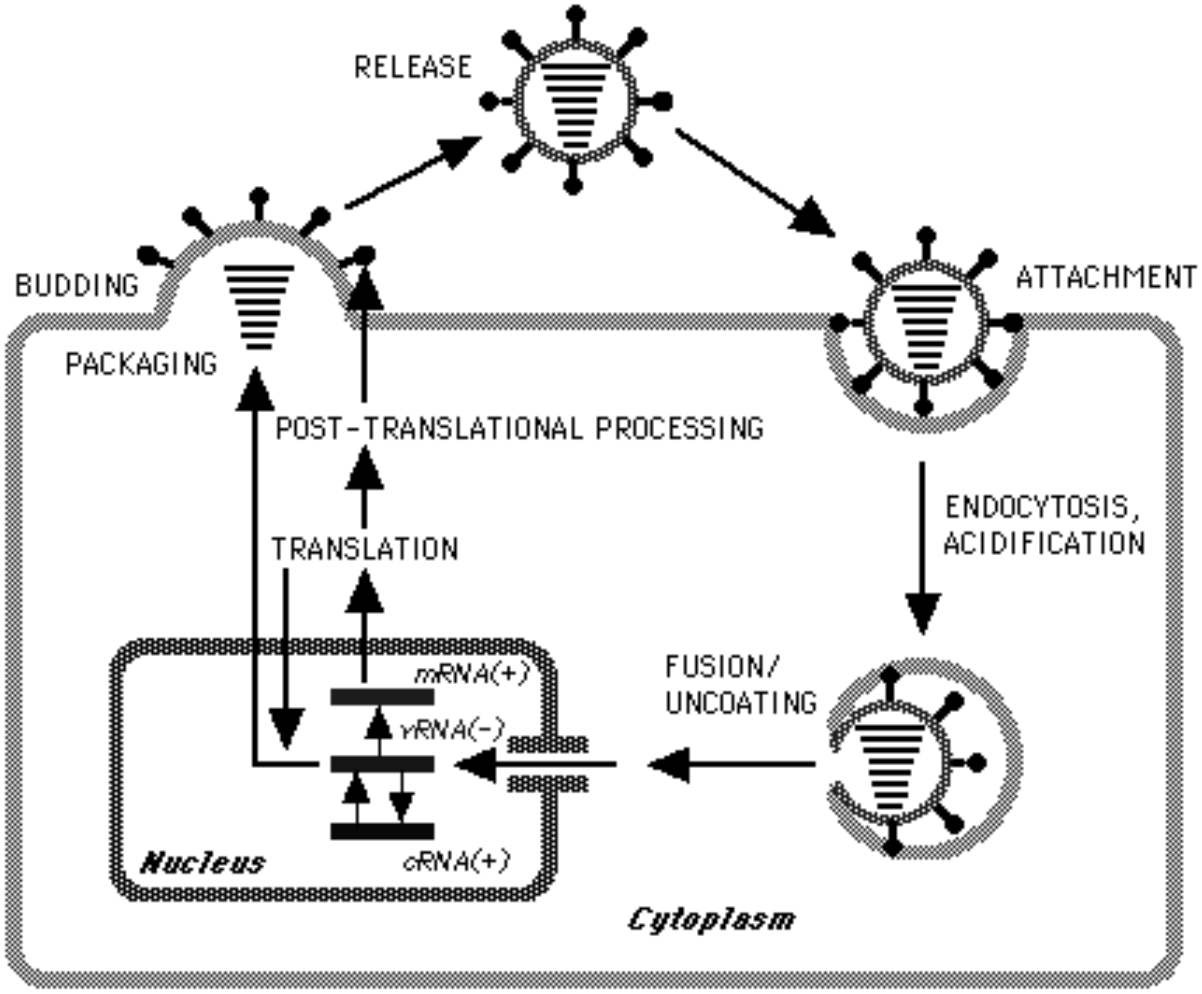
Penetration is an energy-dependent step. It involves one of three mechanisms:

(a) translocation of the virion across the plasma membrane,

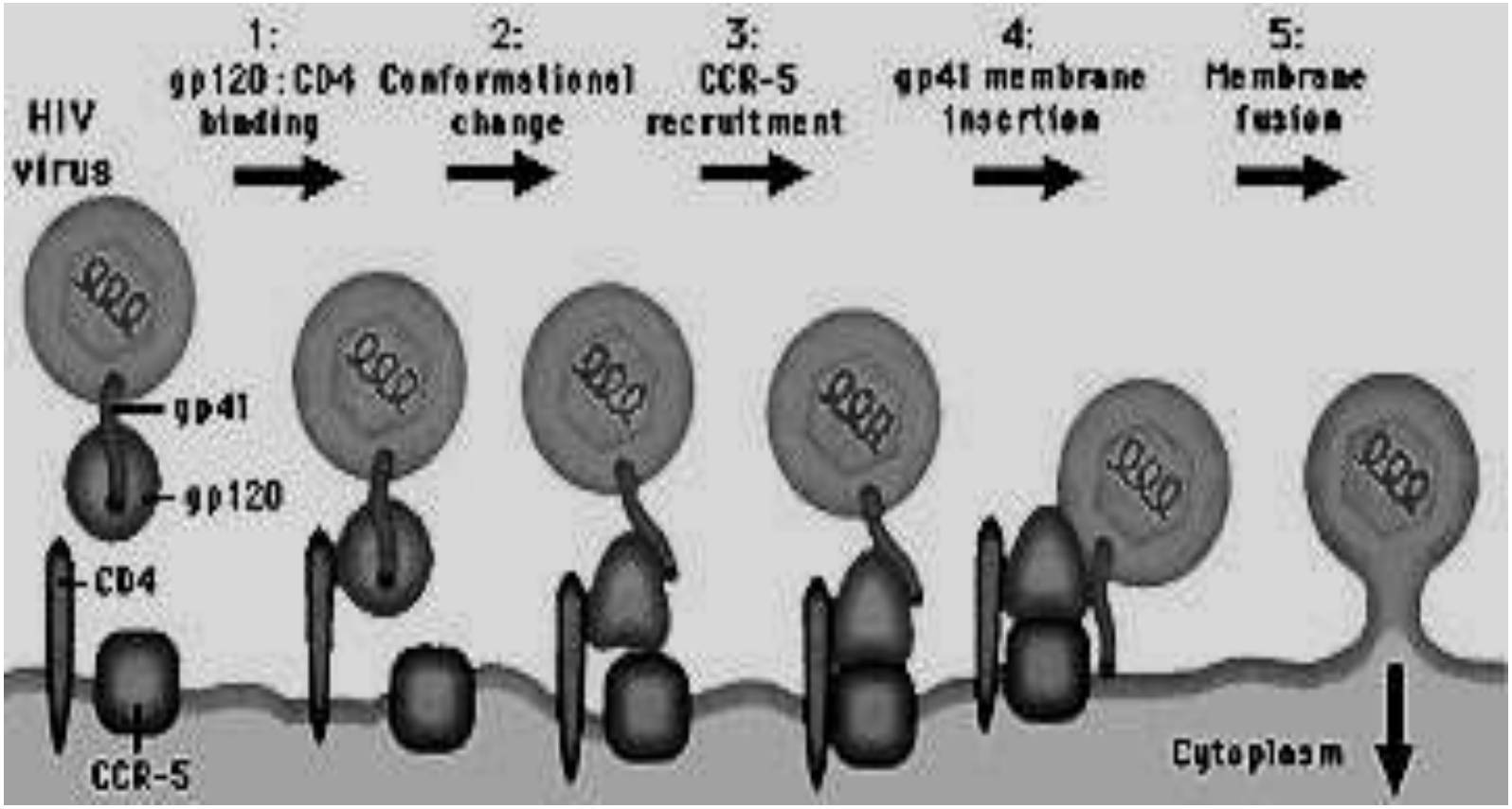
(b) endocytosis of the virus particle resulting in accumulation of virions inside cytoplasmic vacuoles

(c) fusion of the cellular membrane with the virion envelope

Endocytosis (example with the influenza virus)



Fusion of the cellular membrane with the virion envelope (example with HIV)



The ways of transcription of viral genome

DNA	RNA		
	“-” negative-sense strand	+strand	Retro-
DNA ⇓ mRNA ⇓ protein	RNA ⇓ mRNA ⇓ protein	RNA ⇓ protein	RNA ⇓ DNA ⇓ RNA ⇓ protein

The ways of the egress (release) of viruses from the infected cell

naked

↓

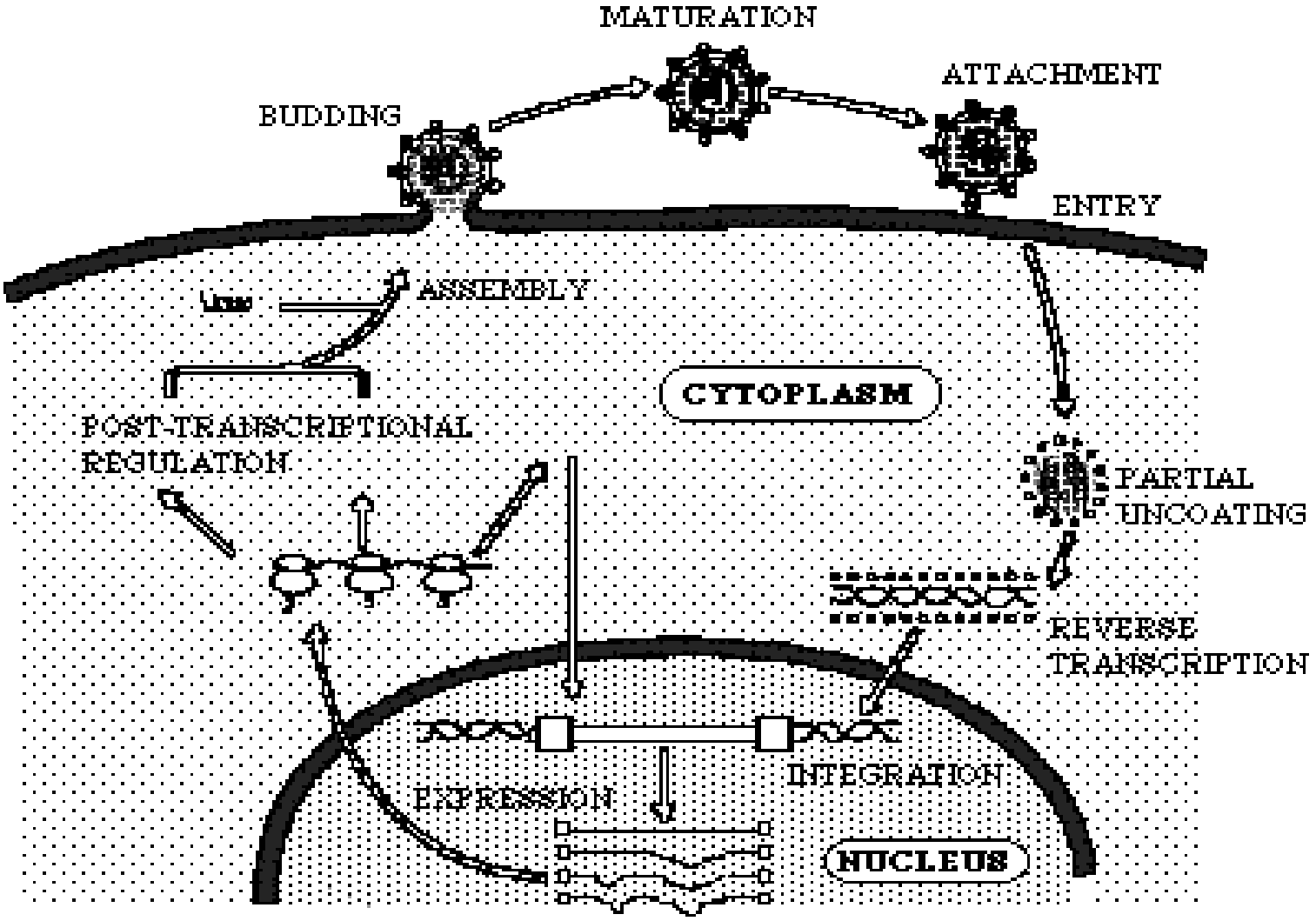
disintegration of the infected cell after release of the virion

enveloped

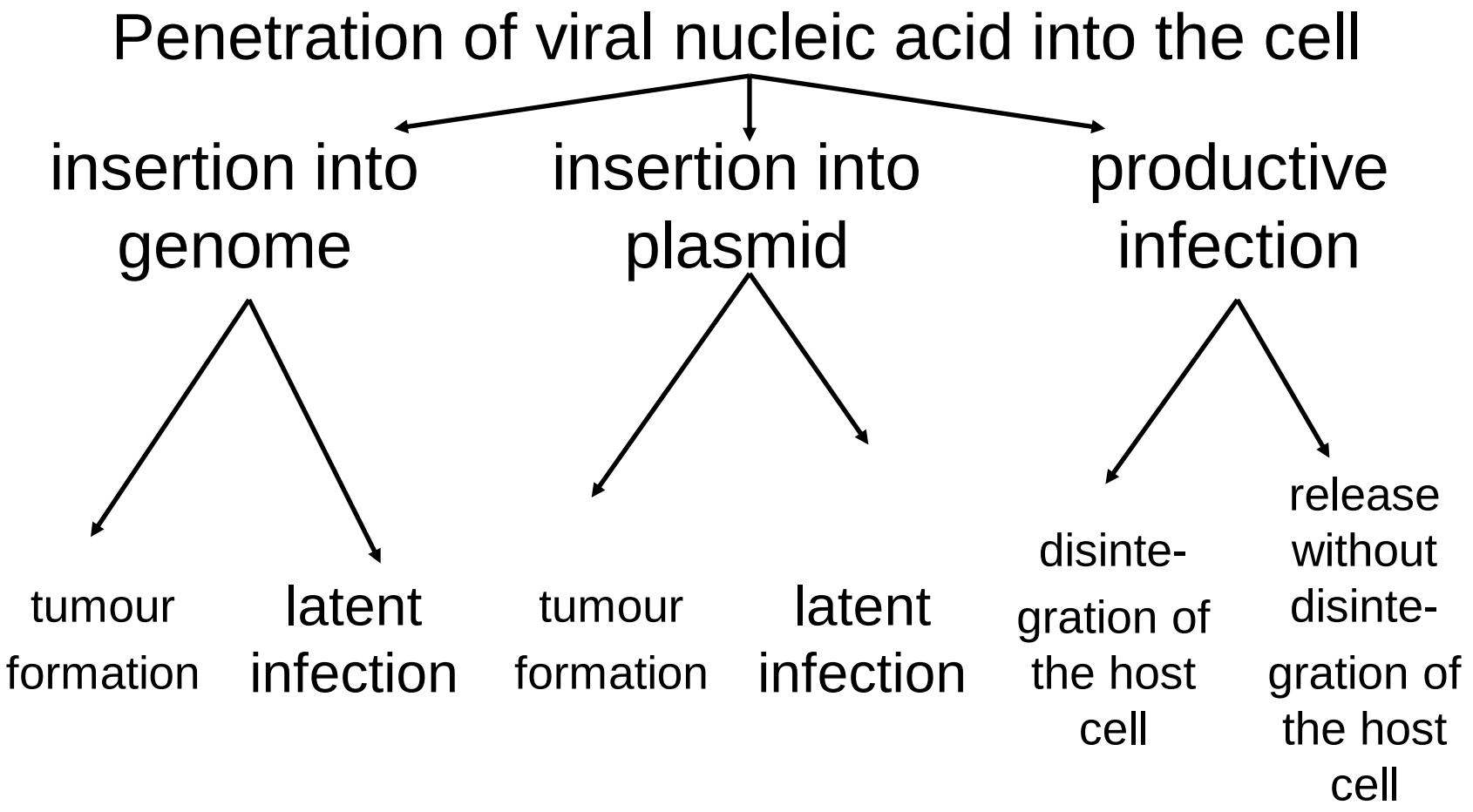
↓

virion is "extruded" or "buds" into the extracellular environment and does not cause the disintegration of the infected cell

The replication cycle of HIV



Results of the effect of viral genome on the infected cell



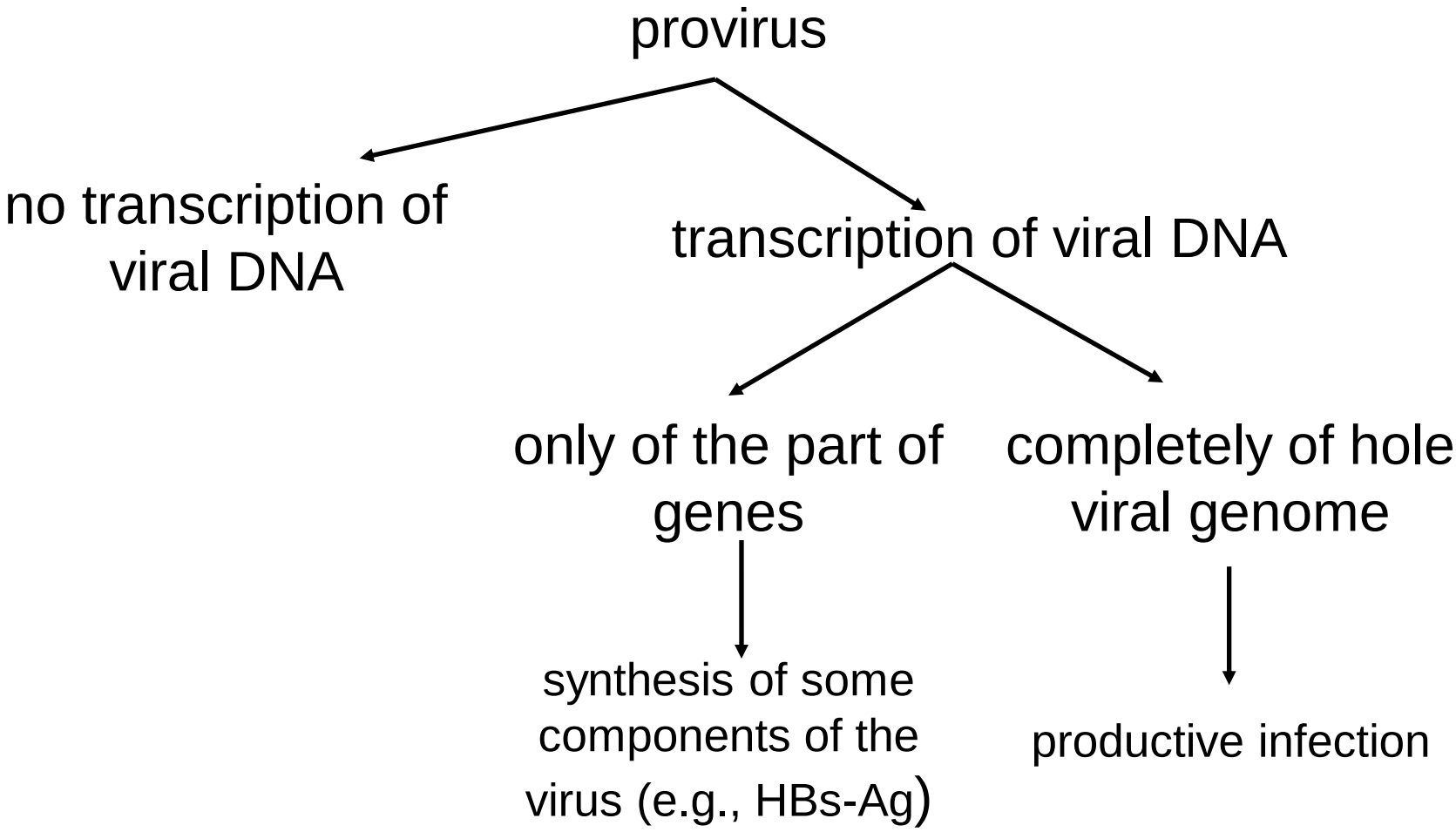
Pathological processes induced by viruses in the infected cell

1. Infectious diseases = viral infections
2. Oncogenic transformation of the infected cell (tumour-inducing effect)

Main characteristics of viral infections

1. Integration of viral DNA into host cell DNA (typical for integral infection)
2. Blood-stream dissemination (viremia) is typical for most of the virus (excluding viruses which are disseminated by nerves)
3. Defects of immune cells caused by viruses $\Rightarrow$ immune pathological disorders
4. Latent viral infections (inapparent infection when virus stays in the infected cell for many years without any signs of the disease)

Variants of the integration of viral DNA into host cell DNA



Virus-mediated effects on the infected cells and macroorganism

Infected cells:

- **Cytopathic effect** – cell defects (down to its death)
- **Immune-mediated defects** – autoimmune reactions

Infected macroorganism:

- **Immunotropic effect** – defects in immune cells
- **Tolerogenic effect** – induction of immune tolerance
- **Oncogenic effect** – induction of oncogenic transformation
- **Teratogenic effect** – foetus defects

General scheme of pathogenesis of viral infections

implantation in macroorganism

⇓

propagation in the portal of entry

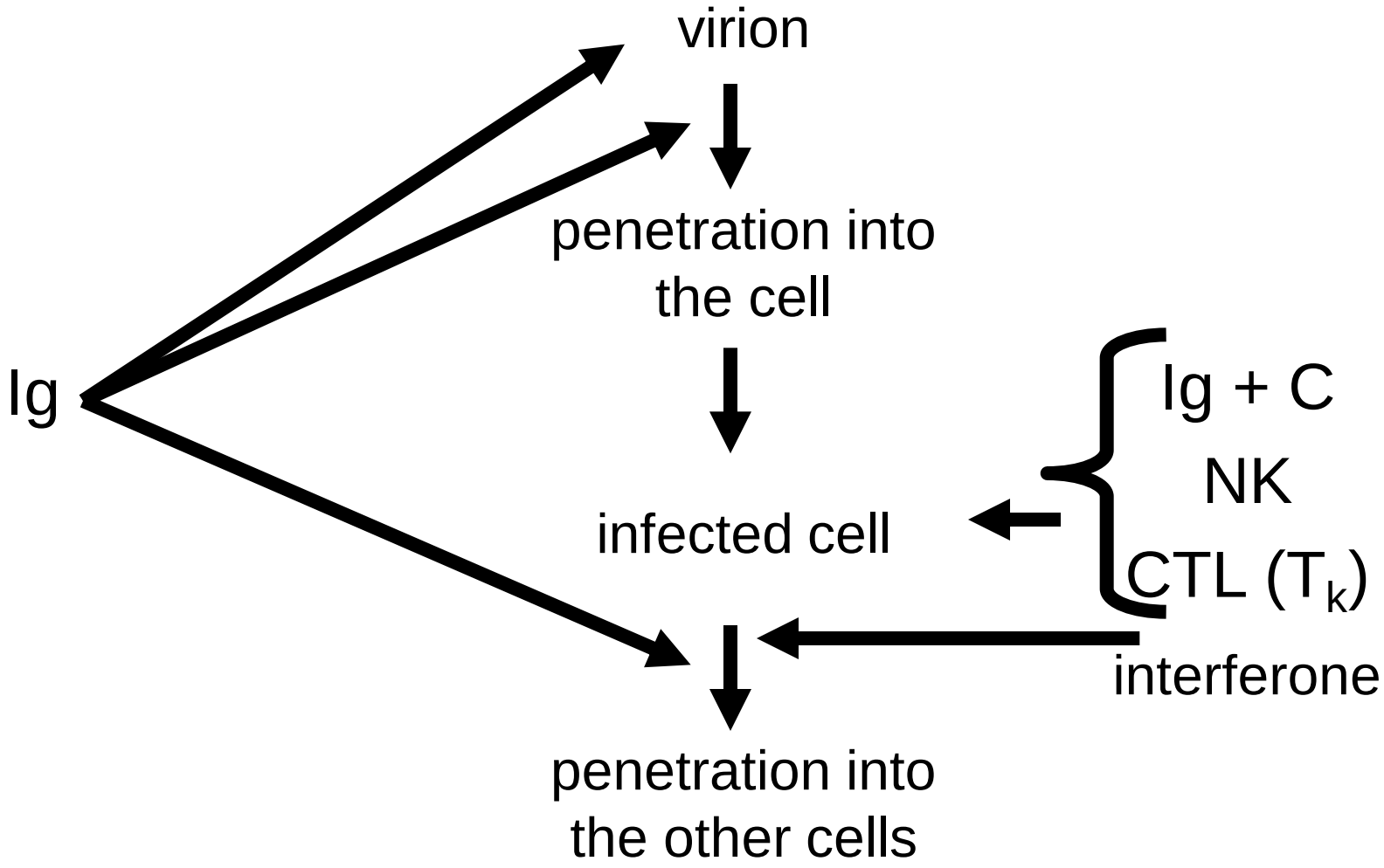
⇓

dissemination in the bloodstream

⇓

multiplication in target organs

Viral activation of immunity



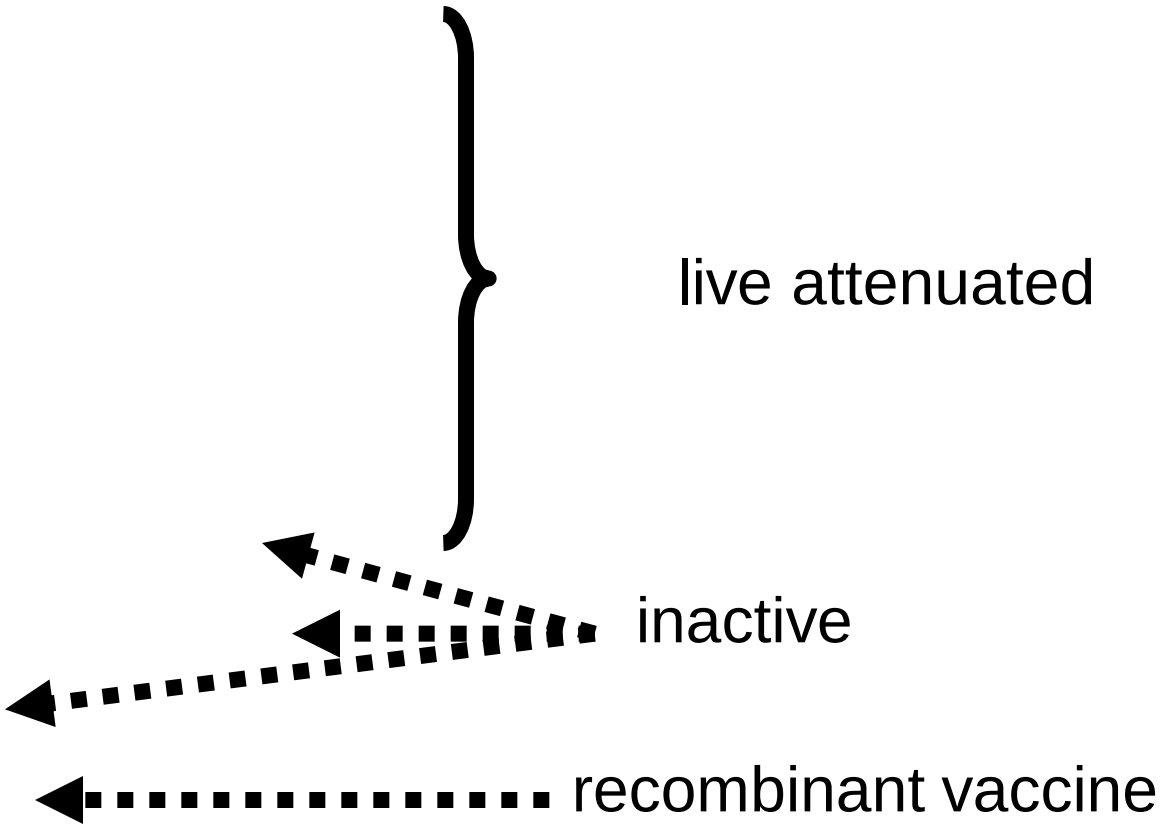
Antiviral agents

1. Drugs which effect directly on virus
2. Immune modulators
3. Preparations used for treatment of clinical symptoms caused by viral infections
4. Symptomatic preparation

Immunologic prophylaxis of viral infections (virus vaccines)

Vaccines effective against:

- Yellow fever
- Pox
- Rabies
- Measles
- Rubella
- Mumps
- Poliomyelitis
- encephalitis
- Hepatitis A
- Hepatitis B



Immune therapy of viral infections

- immunoglobulins
- interferon
- inductors of synthesis of interferon

Methods of diagnostics of viral infections

1. Cytological
2. Virological
3. Serological
4. Molecular – genetic

Virological method of diagnostics

Virus isolation techniques

- Embryonated eggs
- Cell culture
- Animal inoculation

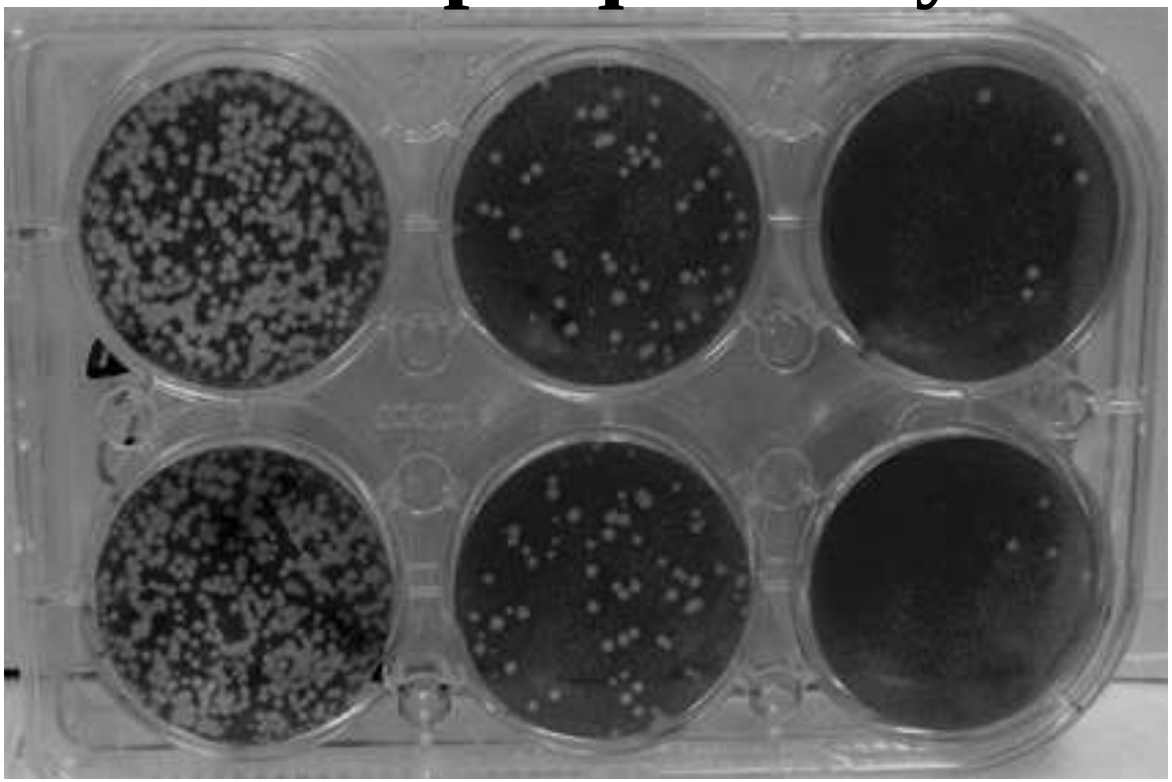
⇓

revealing the virus
(indication)

⇓

determination of the type of the virus
(identification)

A plaque assay



Steps:

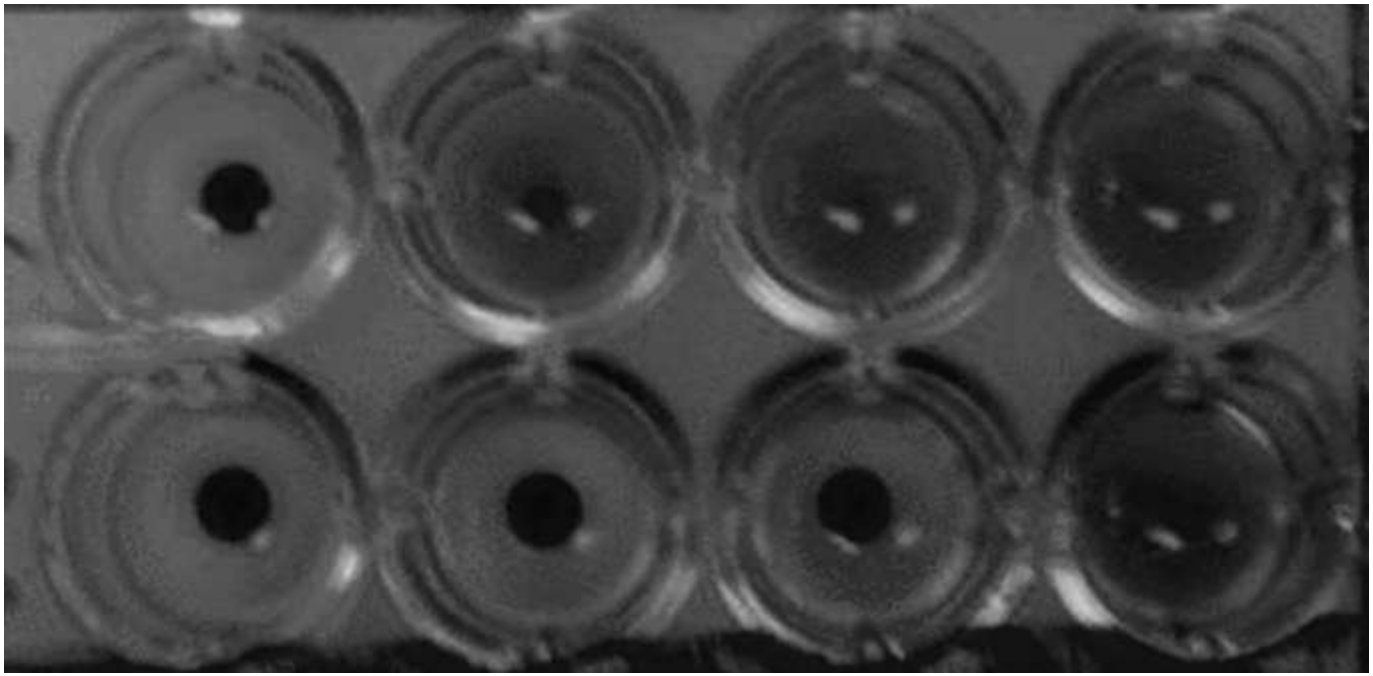
- serial dilutions of virus have been plated on monolayer cultures of cells,
- the cells are stained after a period of time in which a virus infects surrounding cells,
- the white areas show areas of the culture in which the cells have been killed,
- each "plaque" is the result of the presence of one original infectious virus particle.

Serologic diagnostics and immunologic detection of viral infections

Quantitative tests (the growth of titre of antibodies detected in ”paired sera”)

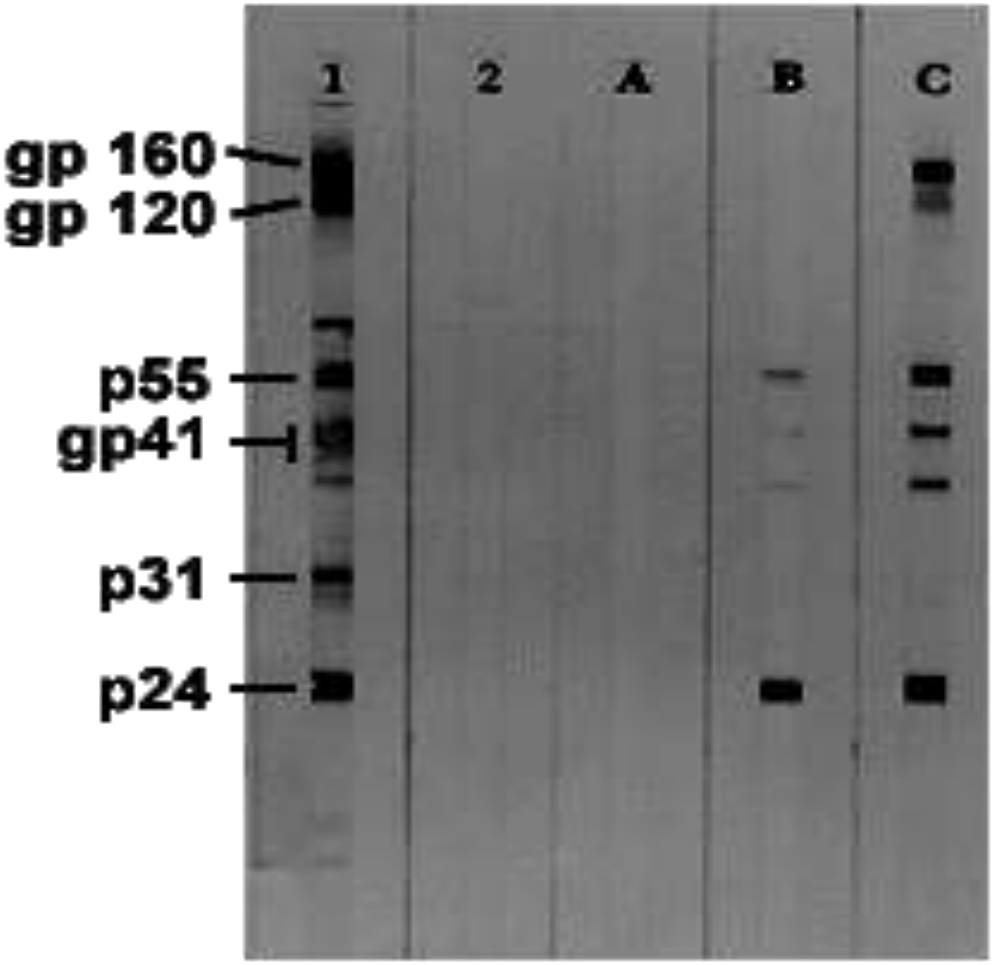
- the neutralisation tests (hemagglutination inhibition (HI) tests, mixed hemadsorption test)
- complement fixation tests
- precipitation tests (immunodiffusion test)
- RIHA
- immune fluorescence test,
- ELISA,
- radioimmunoassay method

CF reaction in microtitre plates

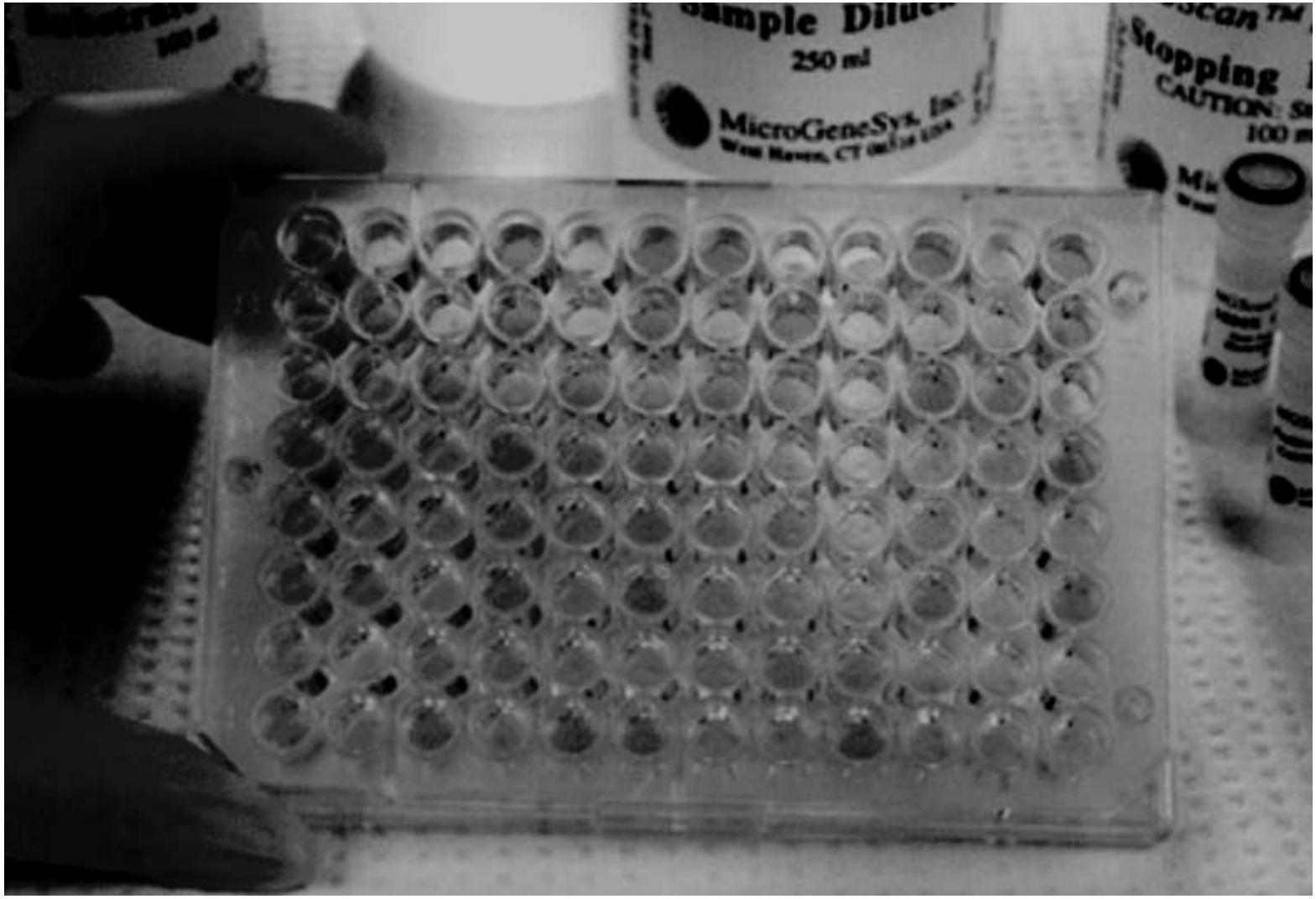


Double diffusion technique (by Ouchterlony)

Immunoblotting



ELISA



Orthomyxoviridae. Paramyxoviridae. Coronaviridae. Rubivirus

Theme N30

Orthomyxoviridae: classification

- Orthomyxoviridae (family)
  - Influenzavirus A (genus)
    - influenza virus A
  - Influenzavirus B

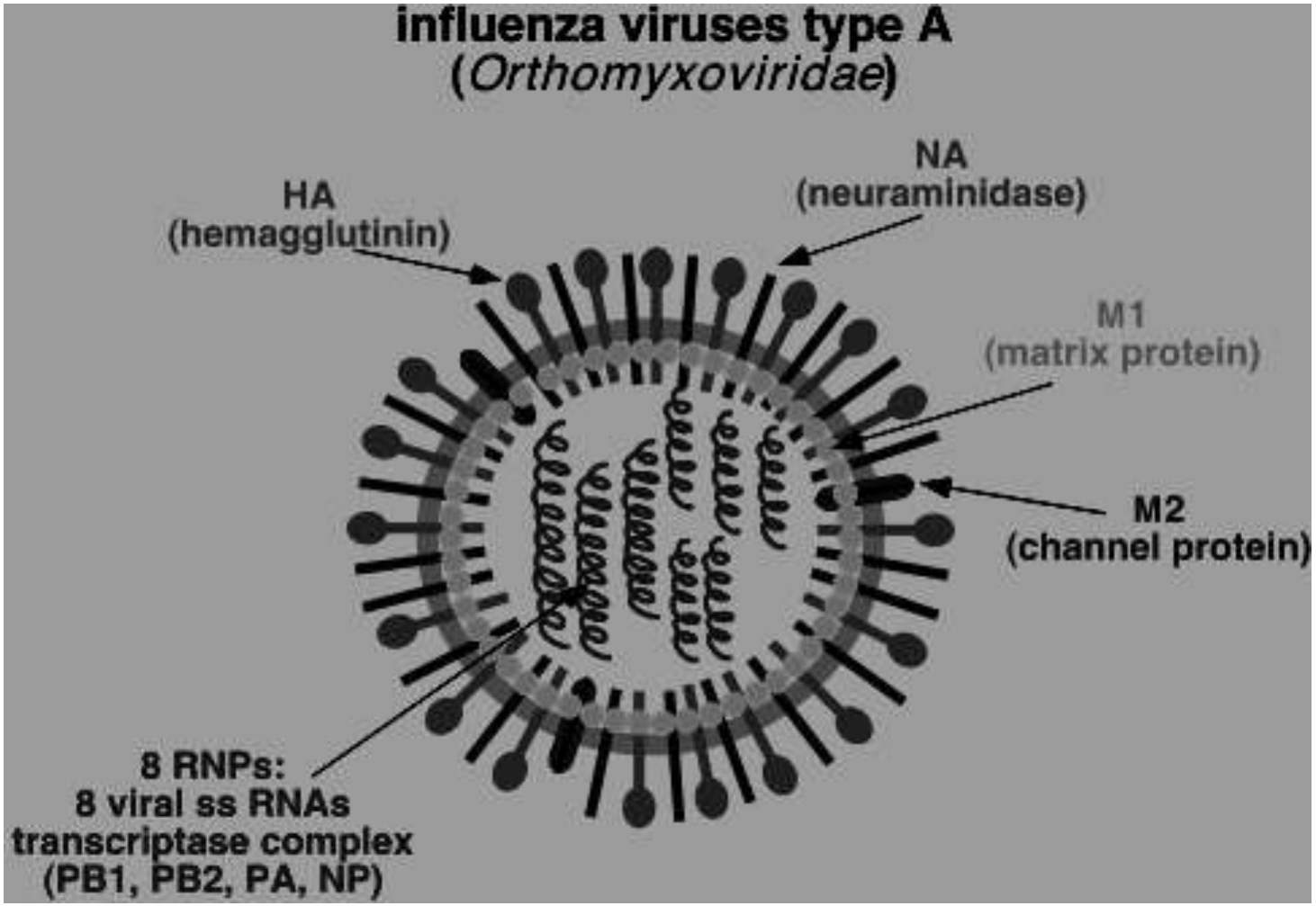
    influenza virus B
  - Influenzavirus C
    - influenza virus C

Orthomyxoviridae: main characteristics of the family

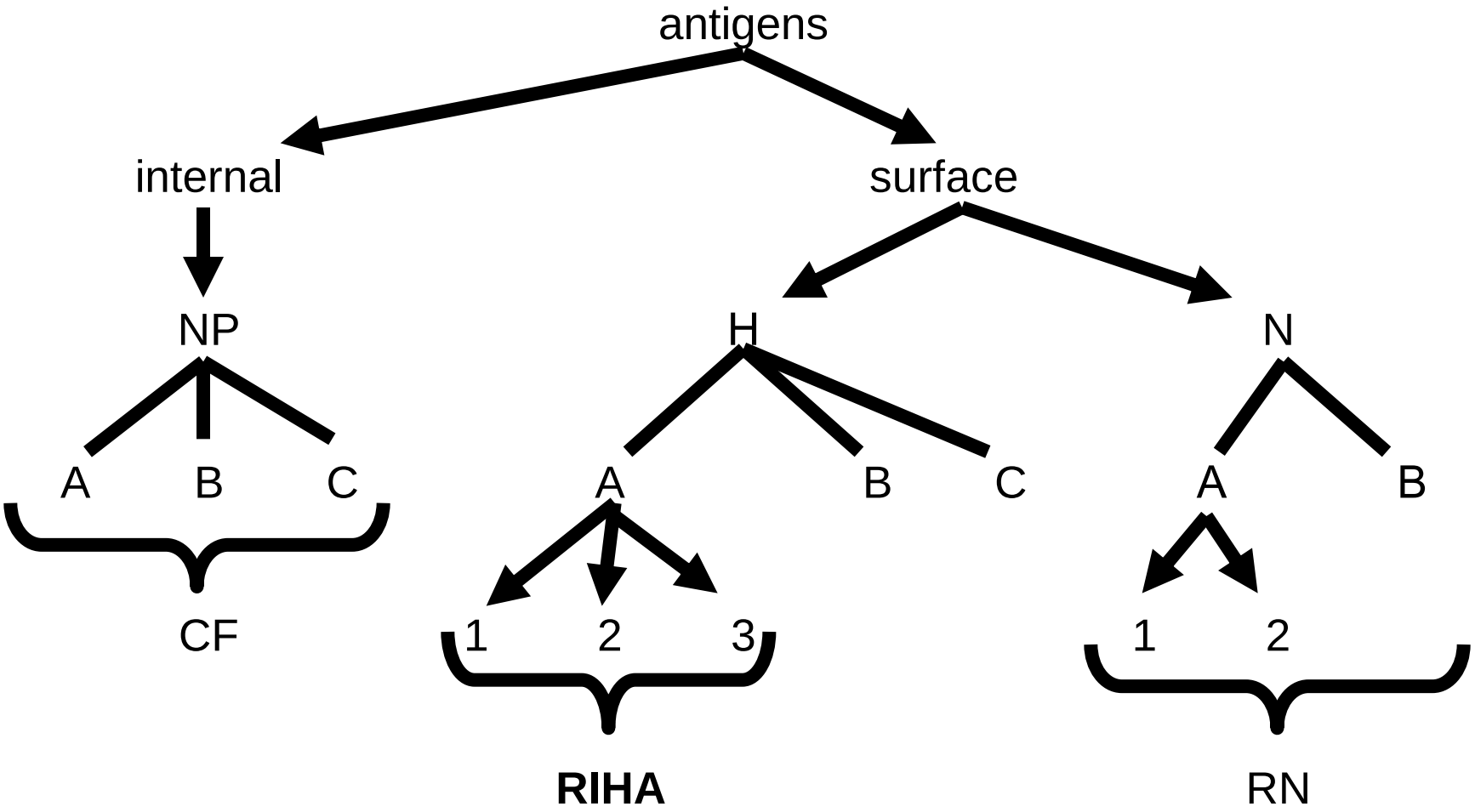
- helical symmetry of nucleocapsid,
- formation of inclusion bodies in cytoplasm,
- one of the stages of replication occurs in nucleus (processing of nucleoprotein).

Orthomyxoviridae: structure of virion

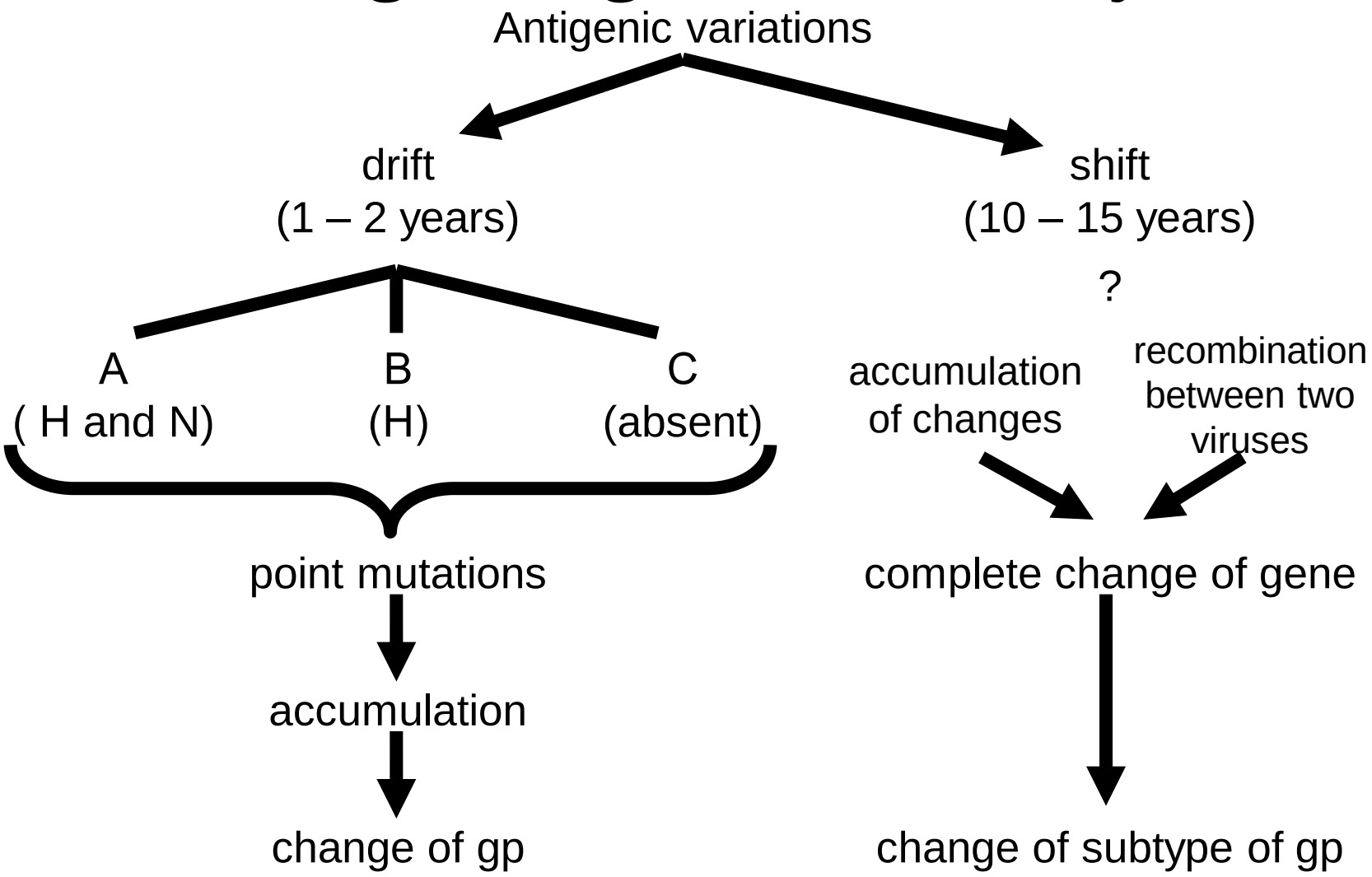
- spherical or filamentous enveloped particles,
- 80 to 120 nm in diameter,
- the nucleocapsid consists of a nucleoprotein and a multipartite genome of single-stranded negative-sense (“-”chain) RNA in seven (influenza virus C) or eight segments (influenza viruses A and B),
- the nucleocapsid associates with the matrix protein (M),
- the envelope (a lipid bilayer) carries two types of surface glycoprotein spikes: a hemagglutinin and a neuraminidase.

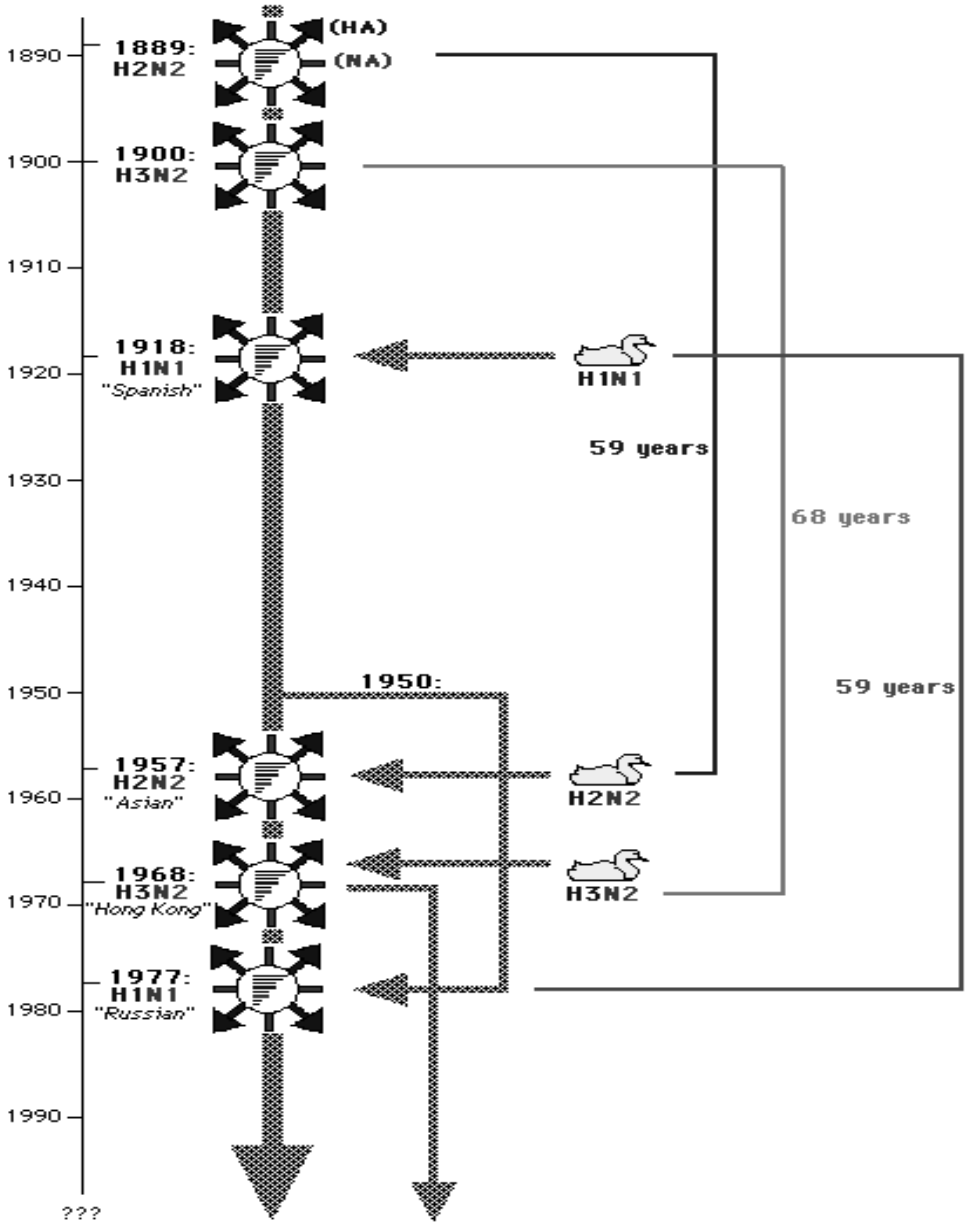

influenza viruses type A
(*Orthomyxoviridae*)
HA
(hemagglutinin)
NA
(neuraminidase)
M1
(matrix protein)
M2
(channel protein)
8 RNPs:
8 viral ss RNAs
transcriptase complex
(PB1, PB2, PA, NP)

Human orthomyxoviridae: antigenic structure



Human orthomyxoviridae: variation of antigenic structure and the consequences of high antigenic variability




1890
1900
1910
1920
1930
1940
1950
1960
1970
1980
1990
???
1889:
H2N2
(HA)
(NA)
1900:
H3N2
1918:
H1N1
"Spanish"
H1N1
59 years
68 years
59 years
1950:
1957:
H2N2
"Asian"
H2N2
1968:
H3N2
"Hong Kong"
H3N2
1977:
H1N1
"Russian"

February 2008, 369 people have been infected as a result of close and direct contact with infected birds. 234 of these have subsequently died.

Bird flu virus (serotype H5N1)

- Avian influenza is an infectious disease of birds caused by type A strains of the influenza virus.
- Humans came down with the bird flu in Hong Kong in 1997, when the H5N1 strain of influenza virus type A infected 18 humans, 6 of whom died.
- Genetic studies showed the virus jumped directly from birds to humans, and caused severe illness with high mortality.
- Virus mutates rapidly and acquires genes from viruses infecting other animal species.

Bird flu virus (serotype H5N1)

- Patients developed symptoms of fever, sore throat, cough and, in the fatal cases, viral pneumonia following by severe respiratory distress.
- The more humans get infected, the greater is probability that people can become infected with both human and bird flu strains.
- Humans could then serve as a "mixing vessel" for a new type of virus that could easily be transmitted from person to person. Such an event would mark the start of an influenza pandemic.

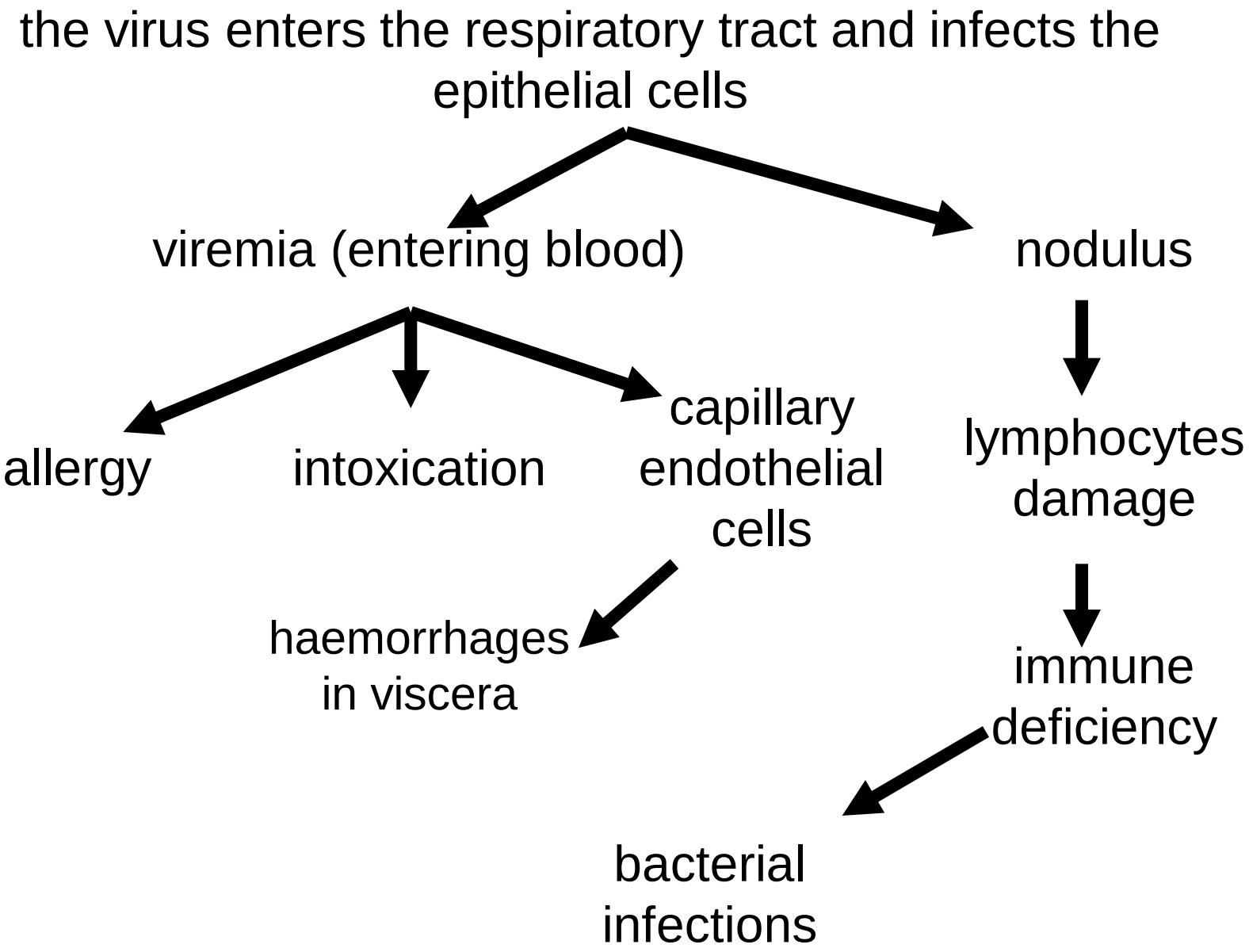

Betreten der

Influenza virus C

The virus possesses some different features in comparison with A and B:

- it contains only seven RNA segments,
- its surface spikes are presented by only a single type of surface glycoprotein – hemagglutinin,
- the spikes are arranged on the surface of the virus producing hexagonal type of symmetry,
- the virus use different receptors for attachment at the membrane of host cell.

Influenza: pathogenesis



Influenza: immunity

Immunity lasts for many years but it is strongly specific and recurrent cases of disease could be caused by antigenically different strains

Immune response presented by

antibodies (humoral):

- interferon
- Ig
- IgAS – in upper respiratory

secretions: against H -

neutralisation of the virus

against-N –

prevent distribution of the virus

immune cells (cell-mediated):

- NK
- macrophages
- T_k

Influenza: immune- and chemoprophylaxis

Immune prophylaxis

- vaccines
  - live
  - killed (inactivated)
  - chemical
- immunoglobulin
- interferon

Chemoprophylaxis

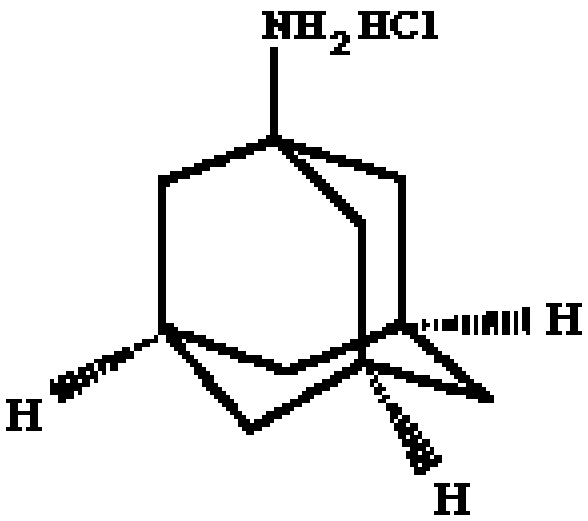
- Rimantadine:

  effective against influenza A

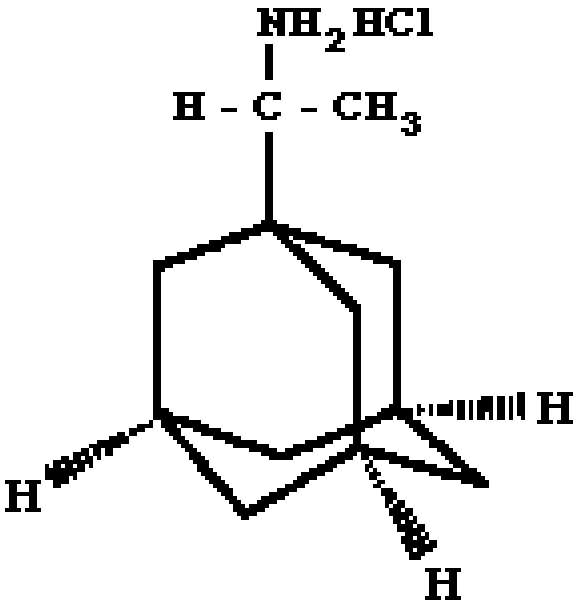
  in early stage of the disease (blocks penetration of influenza A into cells)

Rimantadine: chemical structure

Amantadine:



Rimantadine:



Influenza: laboratory diagnostics

1. Express method: rapid detection of the virus
   – Immune fluorescence reaction: detection of Ag in epithelial cells
2. Virological method (recovery of virus) (first days of the disease)

specimens (throat washing)

⇓

inoculation of embryonated egg

⇓

HA reaction with amniotic or allantoic fluids – indication (revealing of the virus)

⇓

CF– determination of the type of virus

RIHA (inhibition of hemagglutination) – subtype determination

3. Serology
   – antibodies: revealing of increasing in their titre by repeating the serological reactions (CF and RIHA) after 8-14 days (paired sera)

Paramyxoviridae: classification

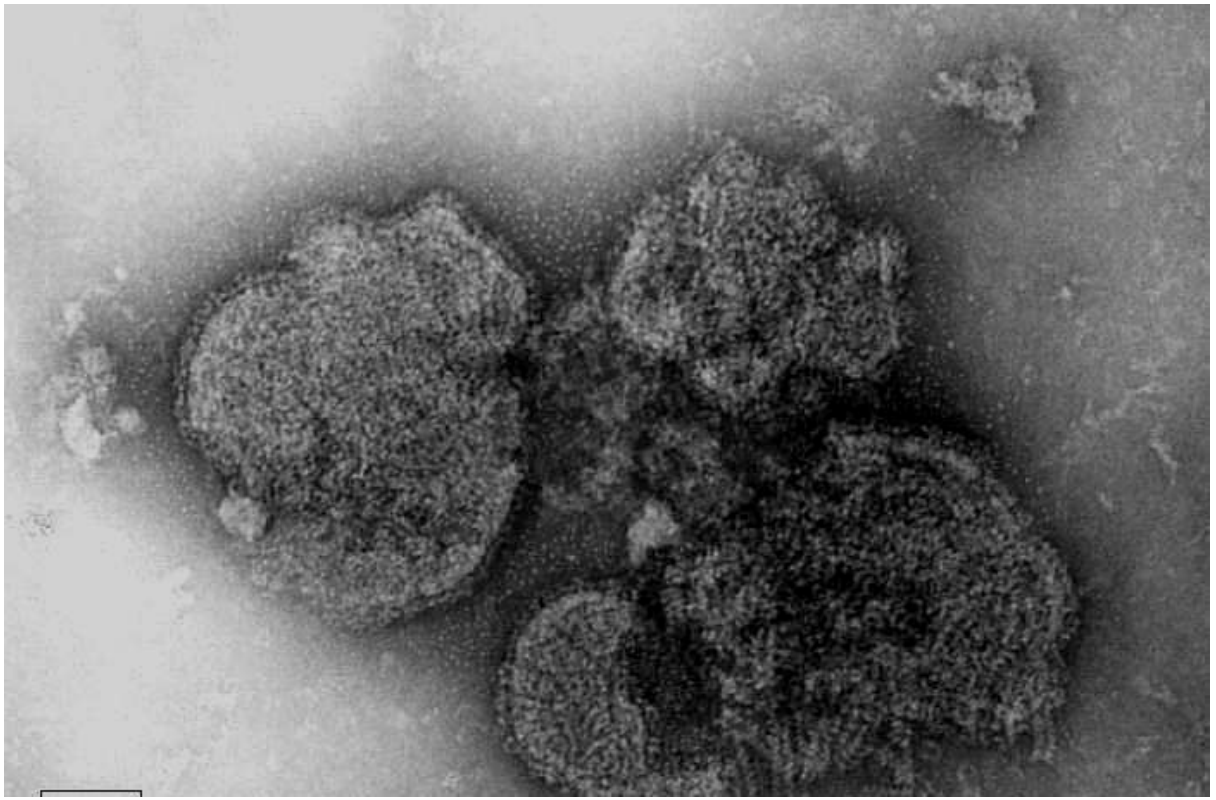
Paramyxoviridae

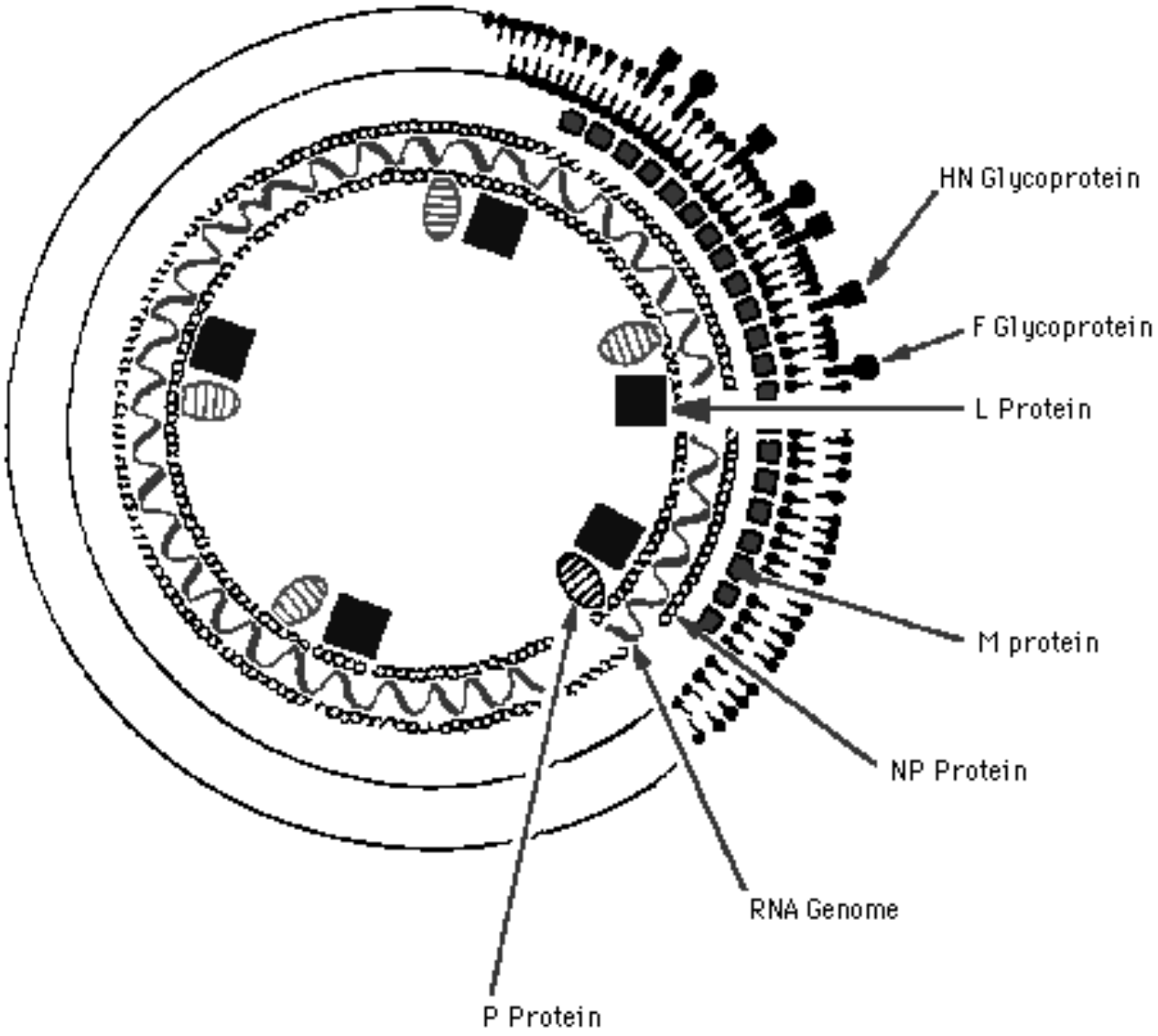
- Paramyxovirus
  - parainfluenza viruses
  - mumps virus
- Morbillivirus
  - measles virus
- Pneumovirus
  - respiratory syncytial virus

Paramyxoviridae: structure of virion

- enveloped particles 150 to 300 nm in diameter,
- the tube-like, helically symmetrical nucleocapsid,
- monopartite, single-stranded, negative-sense RNA genome,
- the nucleocapsid associates with the matrix protein (M),
- virus contains a double-layered lipid envelope,
- the spikes on the envelope contain two glycoproteins (presented by hemagglutinating and neuraminidase activities - HN) , a viral attachment protein, and a fusion protein.

Paramyxoviridae



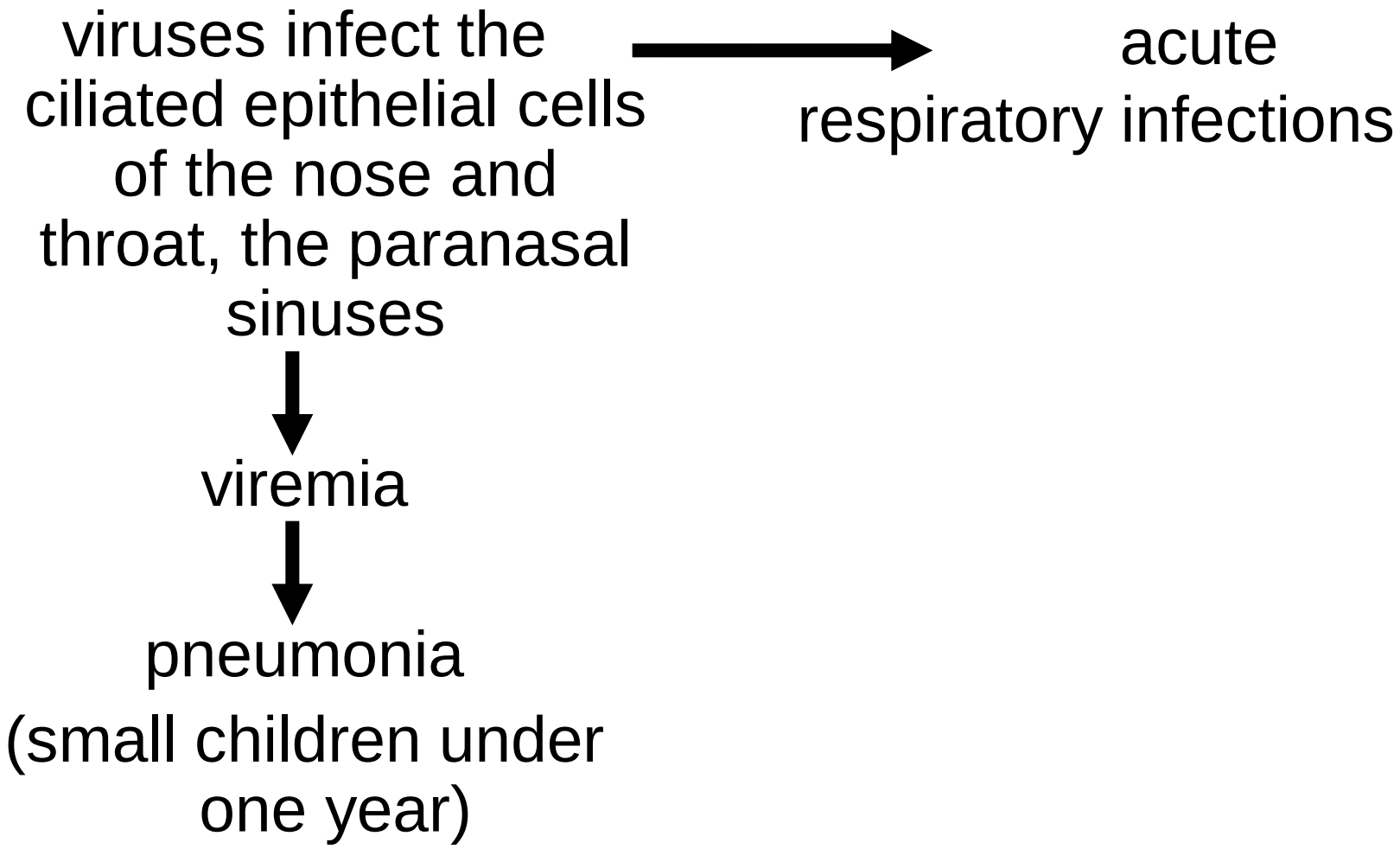

HN Glycoprotein
F Glycoprotein
L Protein
M protein
NP Protein
RNA Genome
P Protein

Parainfluenza viruse: main characteristics

- 5 serological types (predominating type is human parainfluenza virus-3)
- hemagglutinating activity
- cytopathic effect

} the properties are dependent on serotype

Parainfluenza: pathogenesis



Mumps virus : main characteristics

- unstable outside the human organism
- presented by only one serotype
- when inoculated into embryonated egg (amniotic sac) its pathogenicity is reduced (this method of attenuation was used to prepare the live virus vaccine)
  embryonated egg

Mumps : pathogenesis

multiplication in epithelial cells of

- nose and throat
- parotid glands

⇓

generalisation by viremia

⇓

entry into organs:

- testes
- ovaries
- pancreas
- thyroid
- brain (meningitis)

Mumps: specific prophylaxis

Immunisation by a live attenuated vaccines:

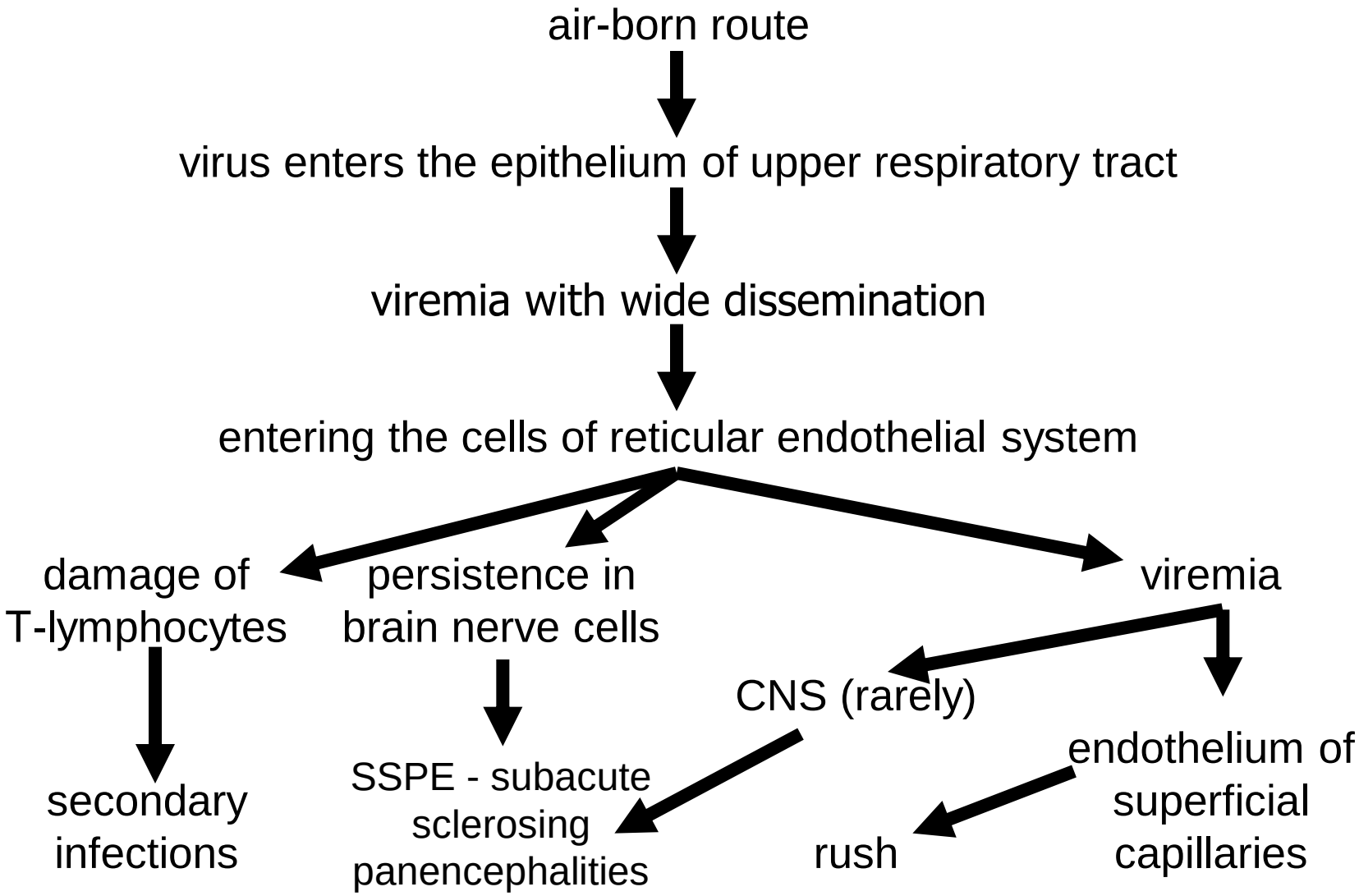
1. single vaccine (L-3, Smorodintsev vaccine)
2. combination of two live vaccines (mumps+ measles)
3. combination of three live vaccines (mumps+ measles + rubella - MMR)

Recommended for all children at 12 to 18 months of age.

Measles virus: main characteristics

- lost neuraminidase,
- H and F glycoprotein spikes contain the hemagglutination (H), the hemolytic and cell fusion (F) activities,
- Ig produced against H and F cause immune-mediated cytotoxic effect on the infected cells,
- virus is unstable outside the human organism (preventive measures including disinfection of inanimate environment are not necessary).

Measles: pathogenesis



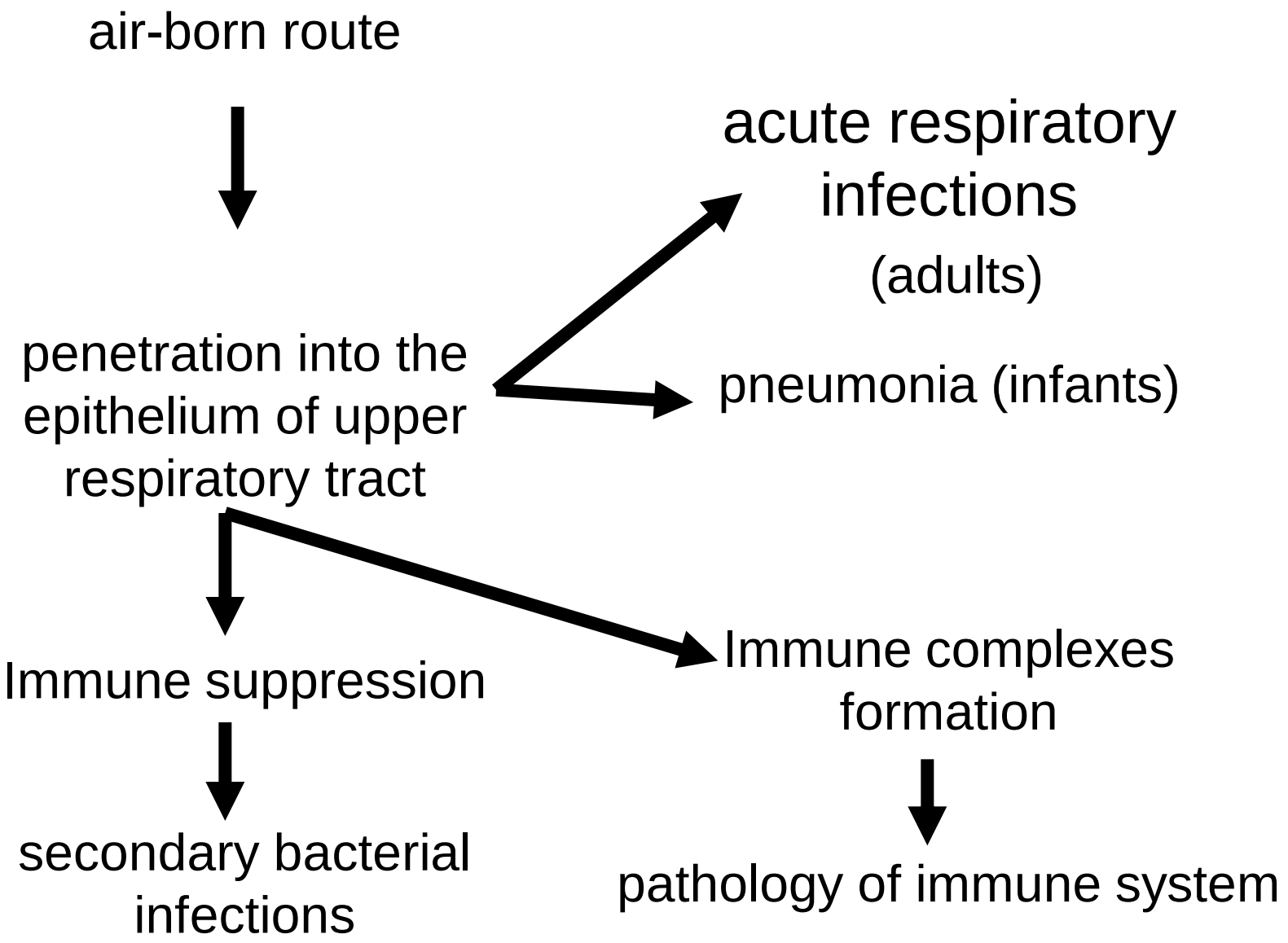
Measles: immune prophylaxis

- Live attenuated vaccine (L-16, Smorodintsev and Chumakov), recommended for all children under one year of age.
- Passive prophylaxis with measles immunoglobulin is used to prevent disease in susceptible, exposed individuals.

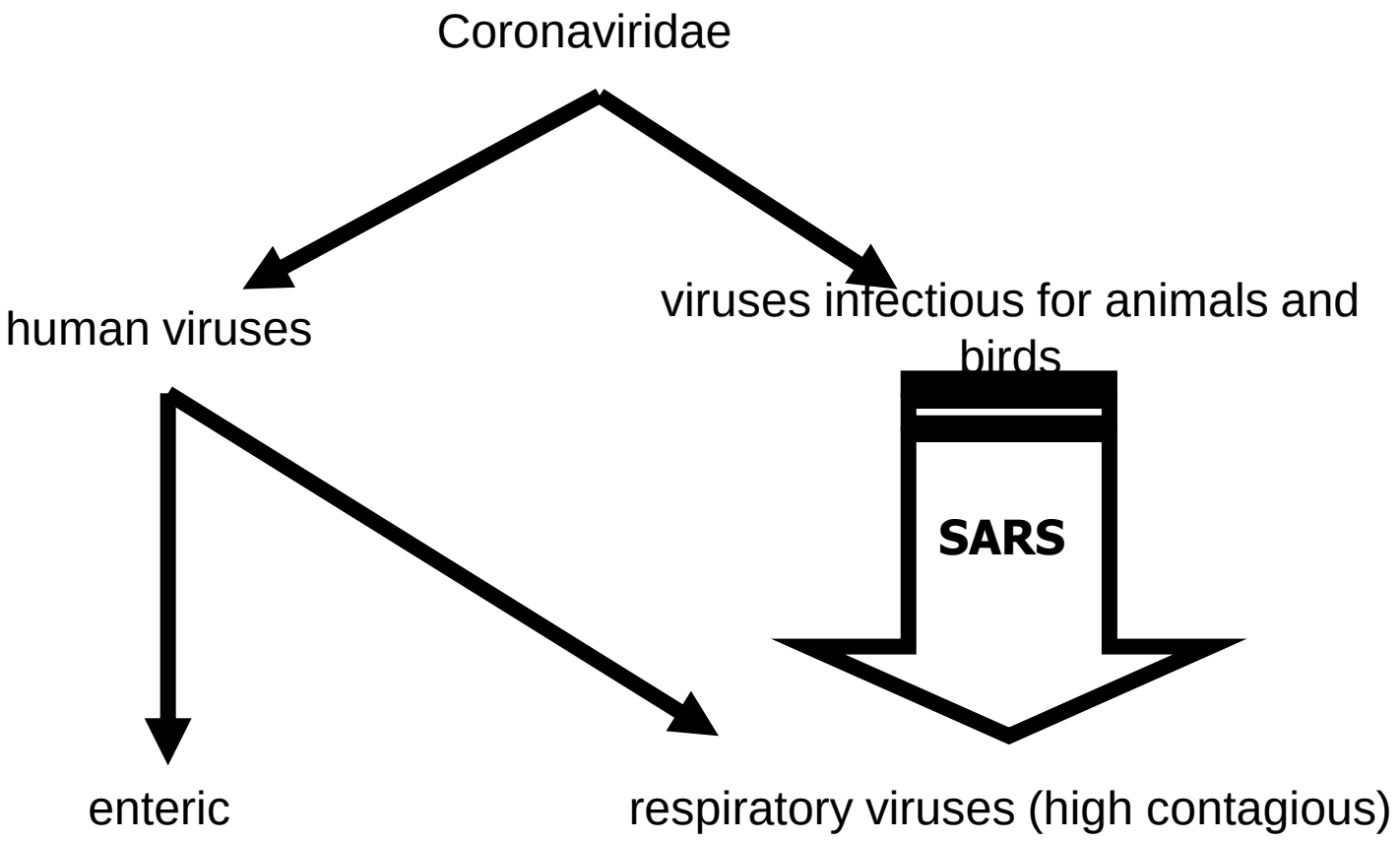
Respiratory syncytial virus (RSV): main characteristics

- the virus is classified in a genus Pneumovirus,
- produces a characteristic syncytial effect (formation of pseudo giant cells),
- virus has neither hemagglutinin nor neuraminidase activity,
- the spikes on the envelope contain glycoproteins:
  - G – attachment of virus
  - F – fusion of cell membranes (penetration) and formation of syncytia
- virus is presented by two serotypes (detected in neutralisation reaction - NR)

Pathogenesis of RSV-infection



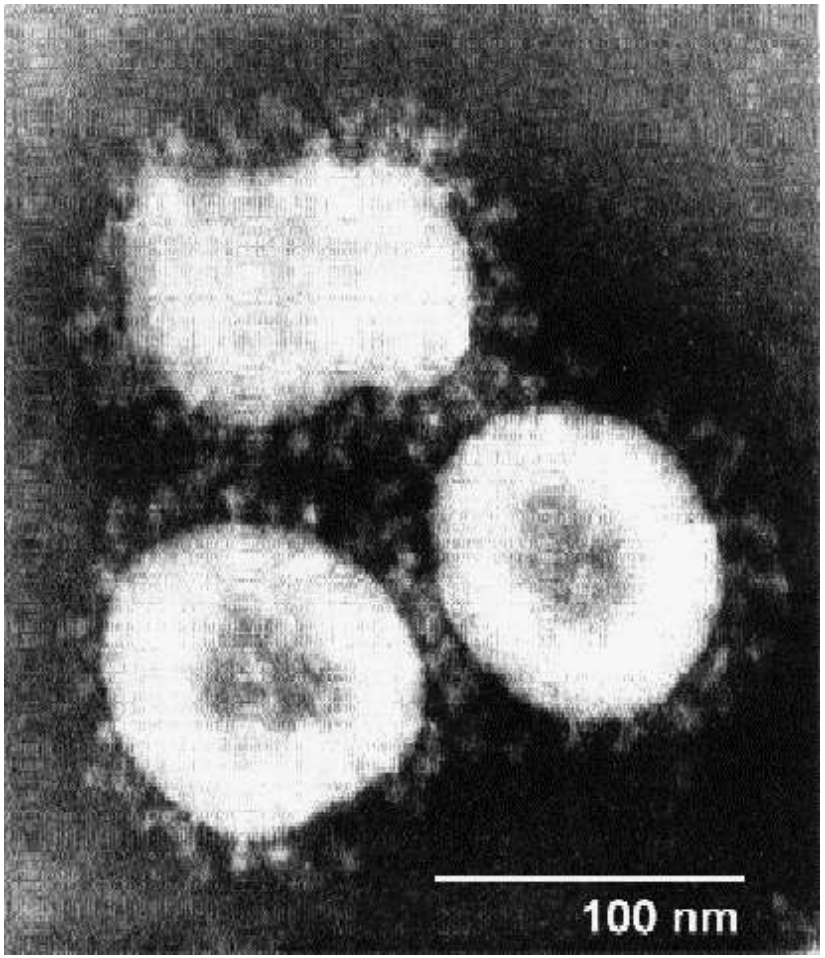
Coronaviruses : classification

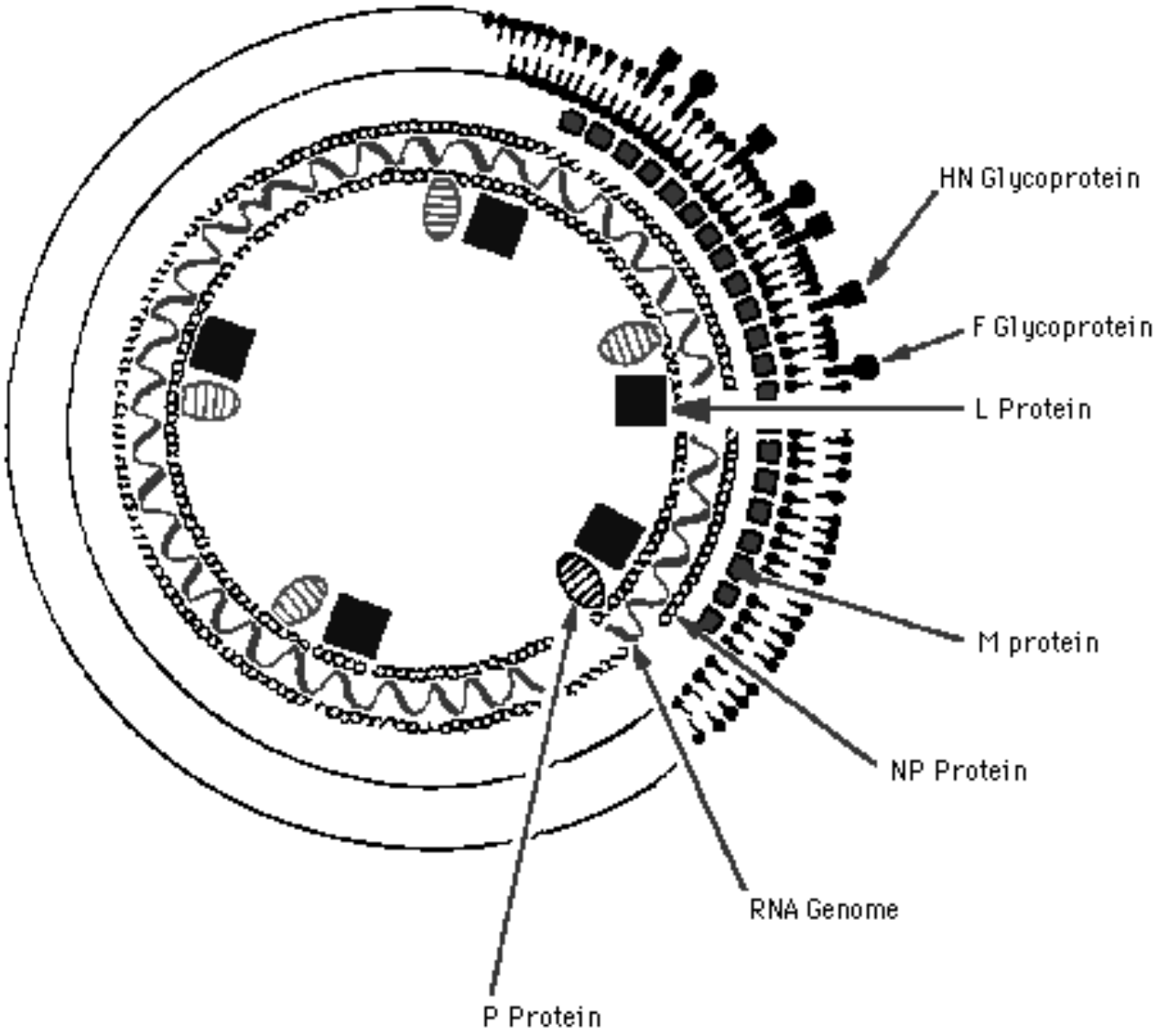


Coronaviruses: main characteristics

- spherical enveloped particles,
- single-stranded (positive-sense) RNA,
- matrix protein associated with nucleoprotein,
- the envelope bears club-shaped glycoproteins (spikes) responsible for attachment to the host cell,
- the spikes are sticking out of their surfaces (due to a large glycoprotein), leading to their name (corona = crown)
- viruses are presented in 4 serological groups (NR) – cross-reactivity between antigens of the groups is absent,
- Almost impossible to cultivate in cell culture.

Coronaviridae




HN Glycoprotein
F Glycoprotein
L Protein
M protein
NP Protein
RNA Genome
P Protein

Coronaviruses:pathology of diseases

Respiratory disease

- asymptomatic infections,
- acute respiratory infections,
- bronchitis,
- pneumonia (young children under 2 years of age),
- SARS – severe adult respiratory syndrome.

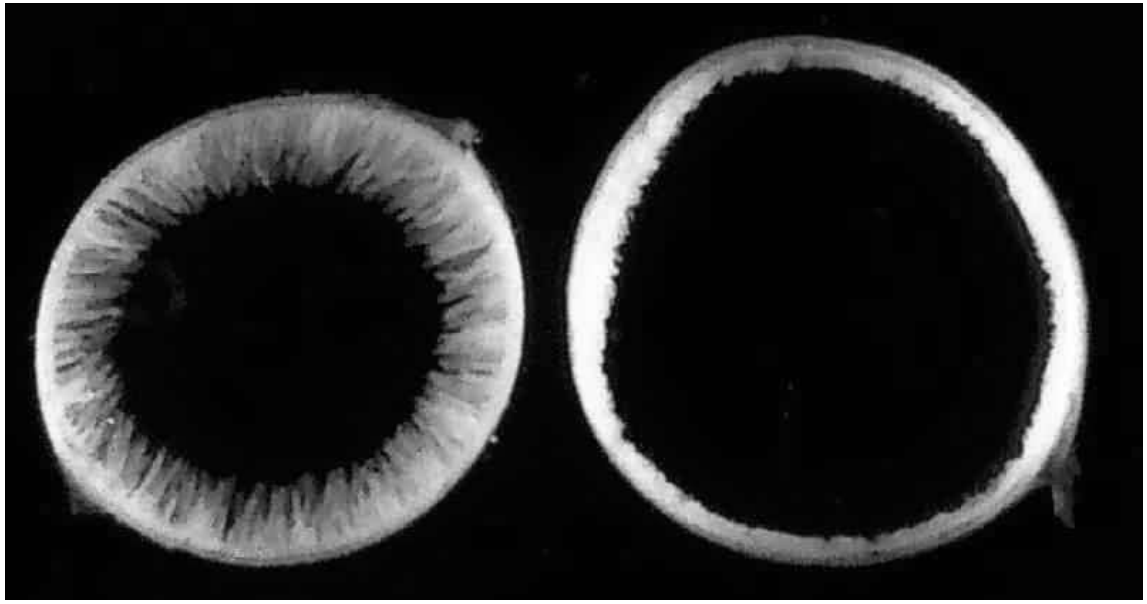
Enteric disease

- gastroenteritis (children)

SARS

- In late 2002, a new syndrome was observed in southern China.
- This disease, has now been reported in Asia, North America, and Europe.
- It was named severe adult respiratory syndrome (SARS).
- The disease is characterized by a fever above 38 degrees, headache, general malaise and aches.
- After a few days (or a week), the patient may develop a dry non-productive cough and dyspnea.
- Respiratory distress leads to death in 3-30% of cases.
- The initial outbreak of SARS peaked in April 2003. By that time, there had been about 8,000 cases worldwide and 775 deaths.

Enteritis: affected intestine



in normal state

Rubivirus: classification

- Togaviridae (family)
  - Rubivirus
    - **rubella virus**

Rubivirus: structure of virion and main properties

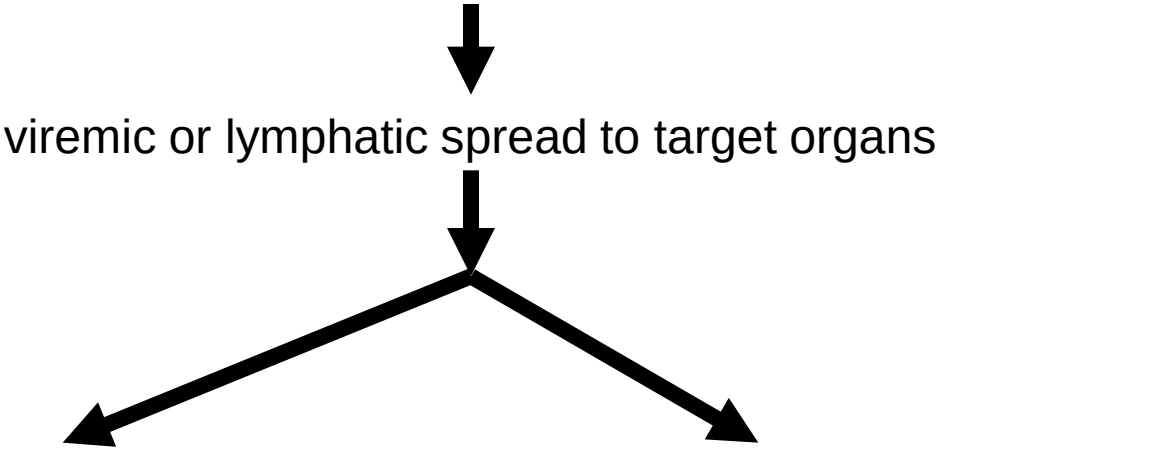
- spherical particles, 40- to 80-nm,
- positive-sense, single-stranded RNA,
- spike-like, hemagglutinin-containing surface projections,
- an electron-dense 30 to 35 nm core is surrounded by a lipoprotein envelope,
- virus is presented by one serotype,
- possesses haemolysins,
- could be cultivated in cell culture but doesn’t cause cytopathic effect.

Pathogenesis of rubella

transmission occurs via:

- direct contact
- droplet way

virus multiplies in cells of the respiratory tract
extends to local lymph nodes
(lymphadenopathy)



viremic or lymphatic spread to target organs

rush

vertical (transplacental) infection

Rubella: specific prophylaxis

Combination of three live vaccines (mumps+ measles + rubella - MMR).

Recommended for all children at 12 to 18 months of age.

Retroviridae

Theme N31

Retroviruses: classification

Retroviridae (family) includes genera:

- Alpharetrovirus
- Betaretrovirus
- Gammaretrovirus
- Deltaretrovirus
- Epsilonretrovirus

} Oncogenic RNA-containing viruses

- Spumavirus – virus infects humans and monkey and produces foamy appearance in the cell culture.
- Lentivirus includes:
  1. Visna virus – infects sheep (slow virus disease).
  2. HIV (HIV-1, HIV-2) – human immune deficiency virus.

Retroviruses: main characteristics

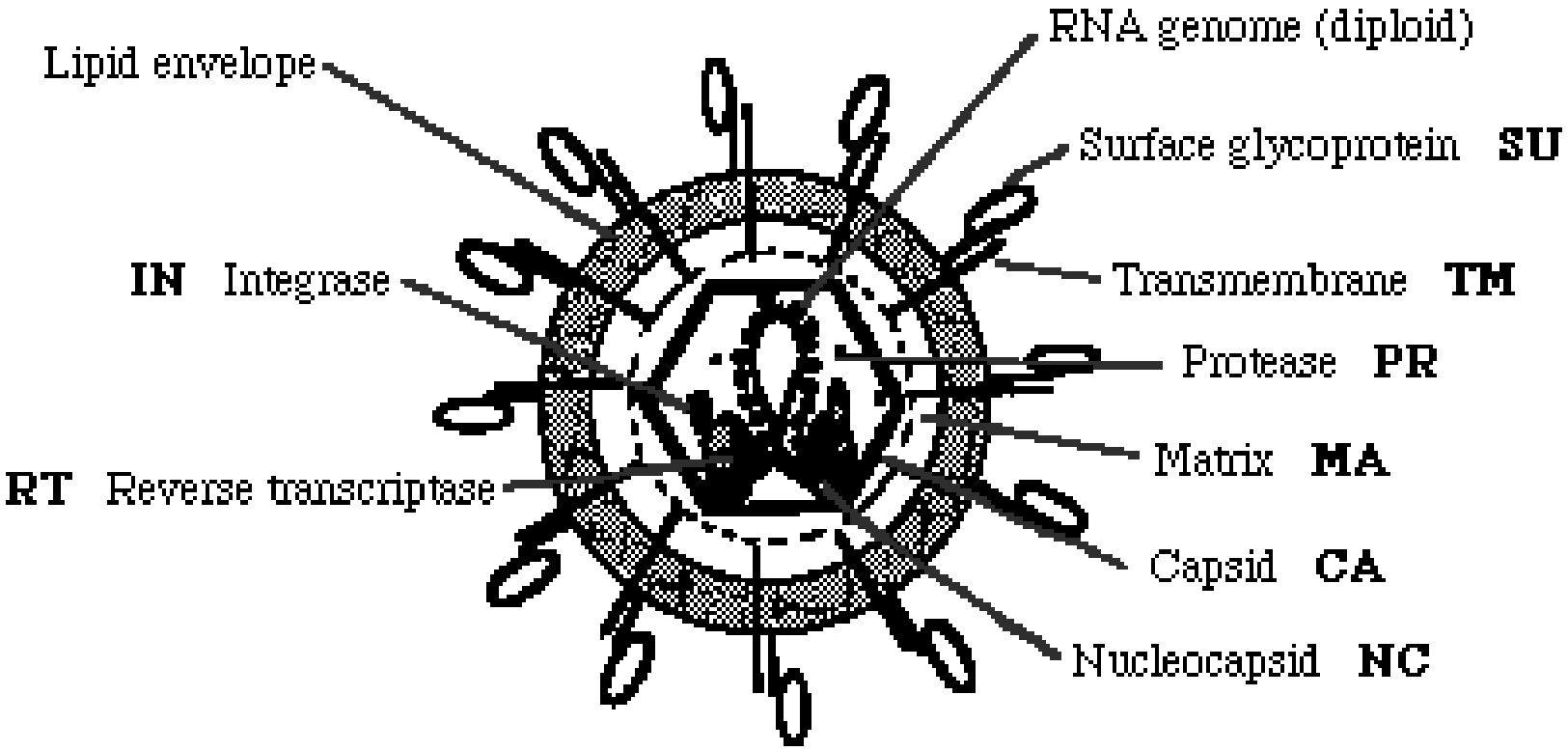
- They are the only viruses which are truly diploid (RNA of the viruses presented by two absolutely identical strands).
- They are the only RNA viruses whose genome is produced by cellular transcriptional machinery (without any participation by a virus-encoded polymerase).
- They are the only viruses whose genome requires a specific cellular RNA (tRNA) for replication.
- They are the only (+)sense RNA viruses whose genome does **not** serve directly as mRNA immediately after infection.

Retroviruses: main characteristics of virion structure

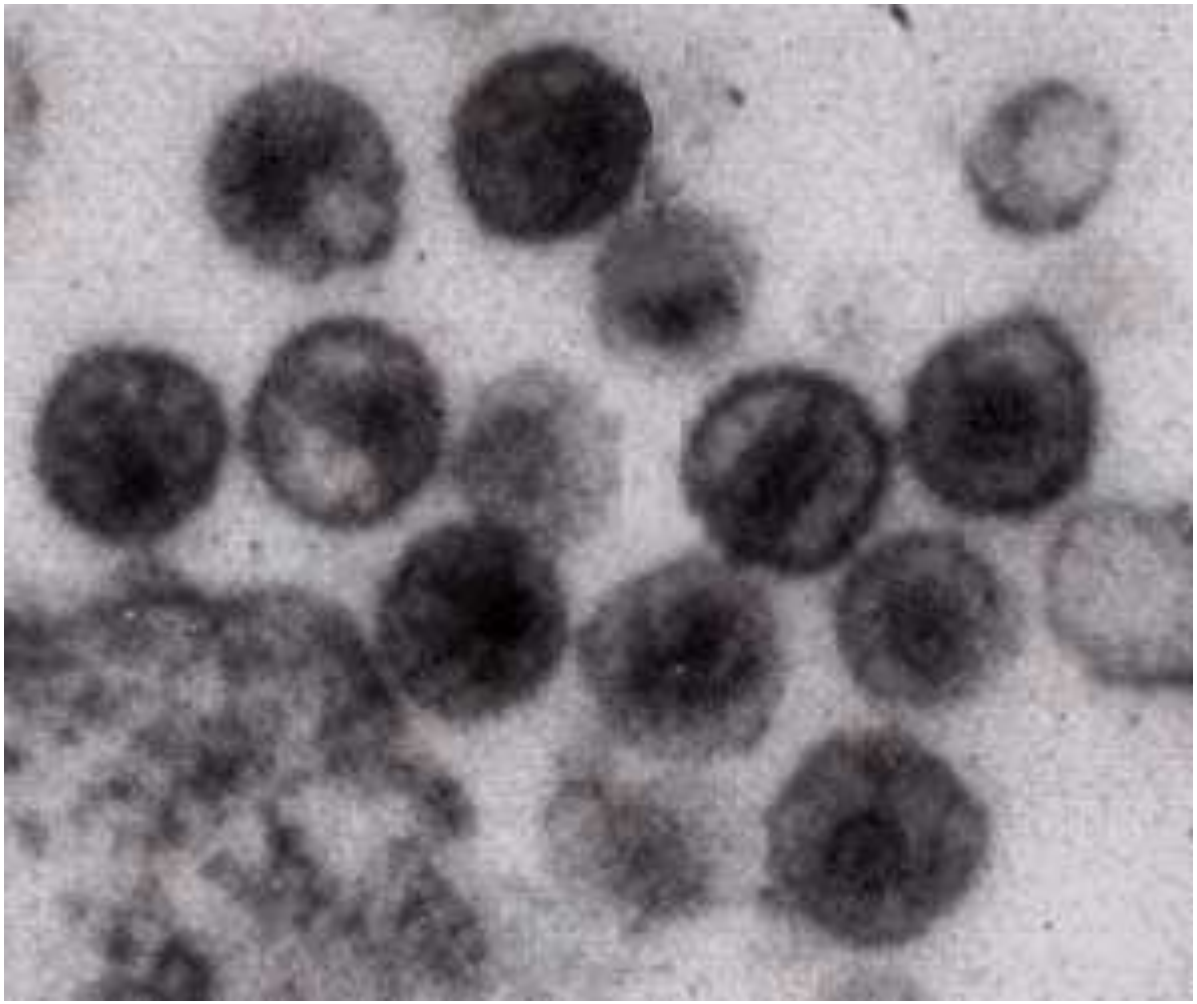
- spherical particles, l00 nm in diameter,
- contain an external lipid bilayer/glycoprotein envelope covering an internal nuclearprotein core,
- the core contains several copies of reverse transcriptase (the enzyme that transcribes RNA to DNA) bound to two identical single-stranded RNA molecules,
- the RNA codes internal core proteins (gag), external envelope proteins (env), reverse transcriptase (polymerase) (pol), and regulatory proteins.

A model of HIV

A model of HIV



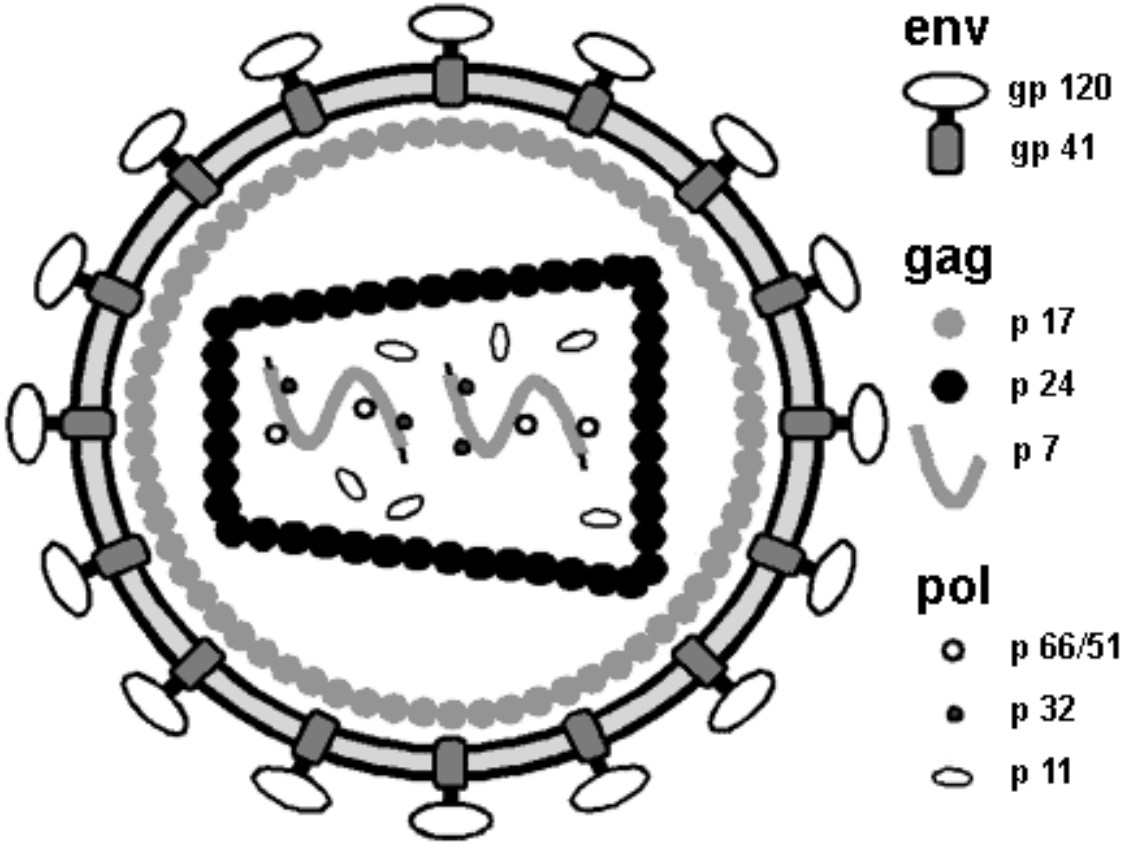
HIV (electron microscopy photo)



HIV: proteins

- **Spikes**:
  - **gp120** -the outer envelope glycoproteins (**SU**), responsible for receptor binding, linked by disulphide bonds to:
  - **gp41** - the trans-membrane glycoprotein (**TM**), holds the SU protein in the envelope, responsible for membrane fusion
- **The core**:
  - inside of the lipid membrane is the **matrix (MA)** protein,
  - inside of the capsid is the **core** (this is usually a conical, electron-dense structure) = RNA genome +NC protein + RT (reverse transcriptase) +IN (integrase),
  - the **capsid proteins (CA)**, which is *believed* to be icosahedral are the most abundant proteins in the particle (~33% total weight)
  - **Enzymes:**
    - RT -reverse transcriptase
    - protease
    - IN - integrase (=endonuclease)

A model of HIV: structure of genome



HIV: structure and function of genome

A. Structural genes:

- gag (group-specific antigens) ⇒encode core proteins
- pol (polymerase) ⇒encode enzymes of virion
- env (envelope) ⇒encode envelope glycoprotein spikes (the major antigens of the virus)

Gene order in all retroviruses is invariant:

5' - gag - pol - env - 3'

HIV: structure and function of genome

Б. Regulatory genes

- tat (trans-activator of transcription) $\Rightarrow$ activates synthesis of viral RNA on the basis of DNA-transcript
- rev (regulator of expression of viral proteins) $\Rightarrow$ activates transport of RNA-transcript to cytoplasm for translation – synthesis of viral proteins (transition from latent form of the infection to productive form takes place)
- vif (factor of the infectiveness of virus) $\Rightarrow$ activates budding of the virus from infected cell

HIV: structure and function of genome

- nef (negative expression factor) $\Rightarrow$ causes transition to latent form of infection
- vpu (viral protein u)
- vpr (viral protein r)

} causes decrease of expression of the cell CD4

} activate replication of provirus DNA

[HIV-2 contains vpx instead of vpu as in SIV]

HIV: structure and function of genome

C. Long terminal repeats (LTR)

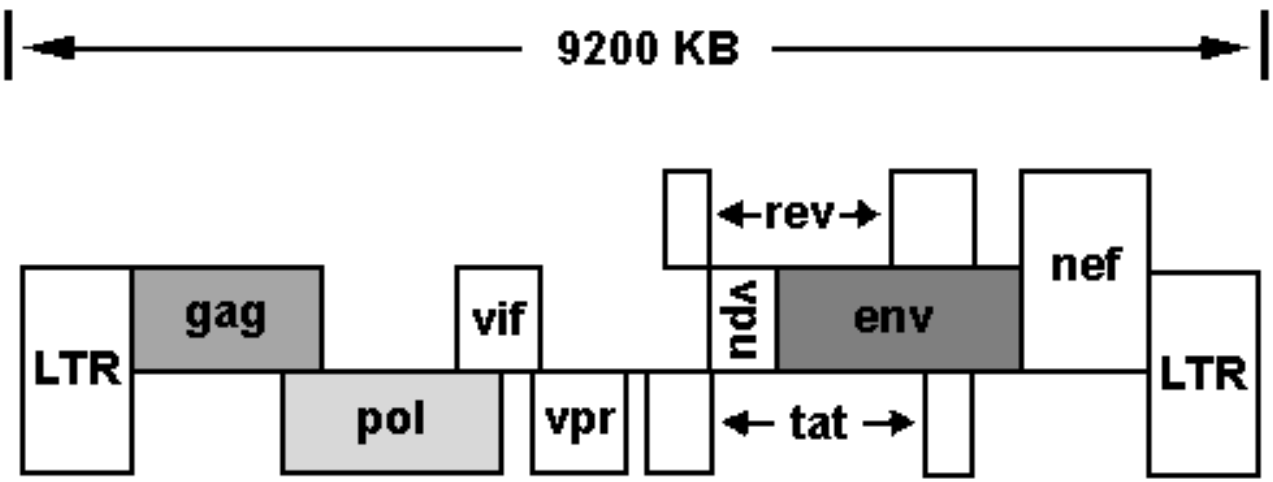
– The ends of the LTRs consist of inverted repeats of 4-6 bp. These are brought together to form a cleavage site for integrase. This molecule is then inserted into the host cell DNA.

The functions of LTR:

– integration of virus into genome of infected cell

– regulation of the expression of genes of provirus

A model of HIV: structure of genome



HIV-1

HIV: antigenic types

Two antigenic types are distinguished by antibody reactivity to envelope glycoproteins:

- HIV-1 – main infectious agent that produces AIDS in humans
- HIV-2 – is endemic in Western Africa (it is rarely found in other regions) – it differs from HIV-1 because HIV-2 contains gp125 (instead of gp120) and gene vpx (instead of vpu)

Tropism of HIV to human cells (phenotypes of HIV)

Receptors for binding with gp120 are presented by:

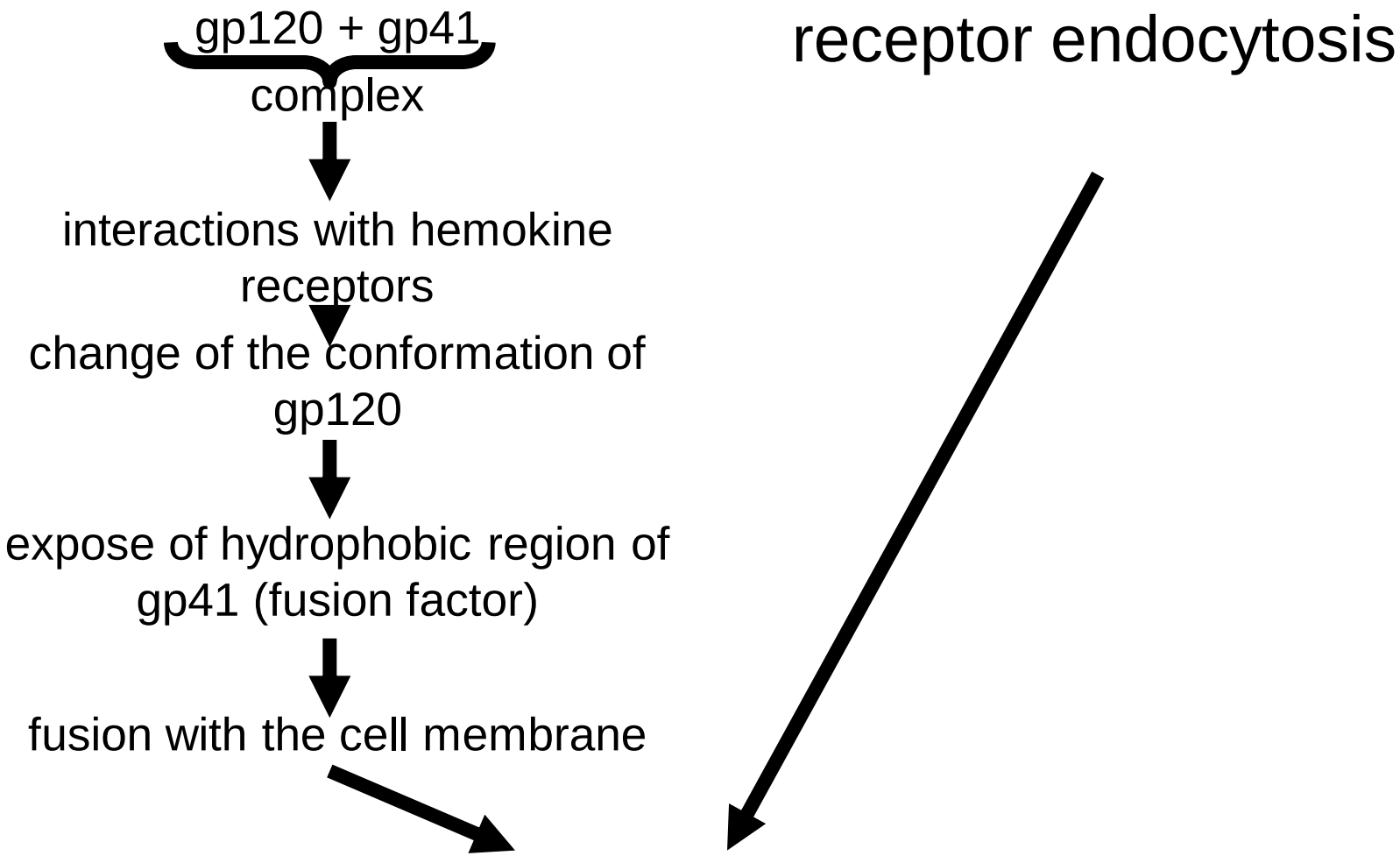
- CD4 (T-lymphocytes)
- other receptors are the receptors to hemokines

By tropism to the human cells HIV could be:

- macrophage tropic (slow/low) – phenotype M
- T-lymphotropic (“rapid/high”) – phenotype T
- viruses which display both types of tropism

Progress in development of HIV infection is dependent on the change of phenotype from M- to T.

Life cycle of HIV in T4-lymphocytes (integrative infection)



Life cycle of HIV in T4-lymphocytes (integrative infection – continuation)

↓

reverse transcription of viral RNA

↓ RT-activity

ds DNA

↓

penetration into the cell nucleus

↓ integrase activity

integration into DNA of T-lymphocyte (provirus)

↓

Life cycle of HIV in T4-lymphocytes (activation of the virus)

derepression

↓

initiation of LTR transcription

↓ tat

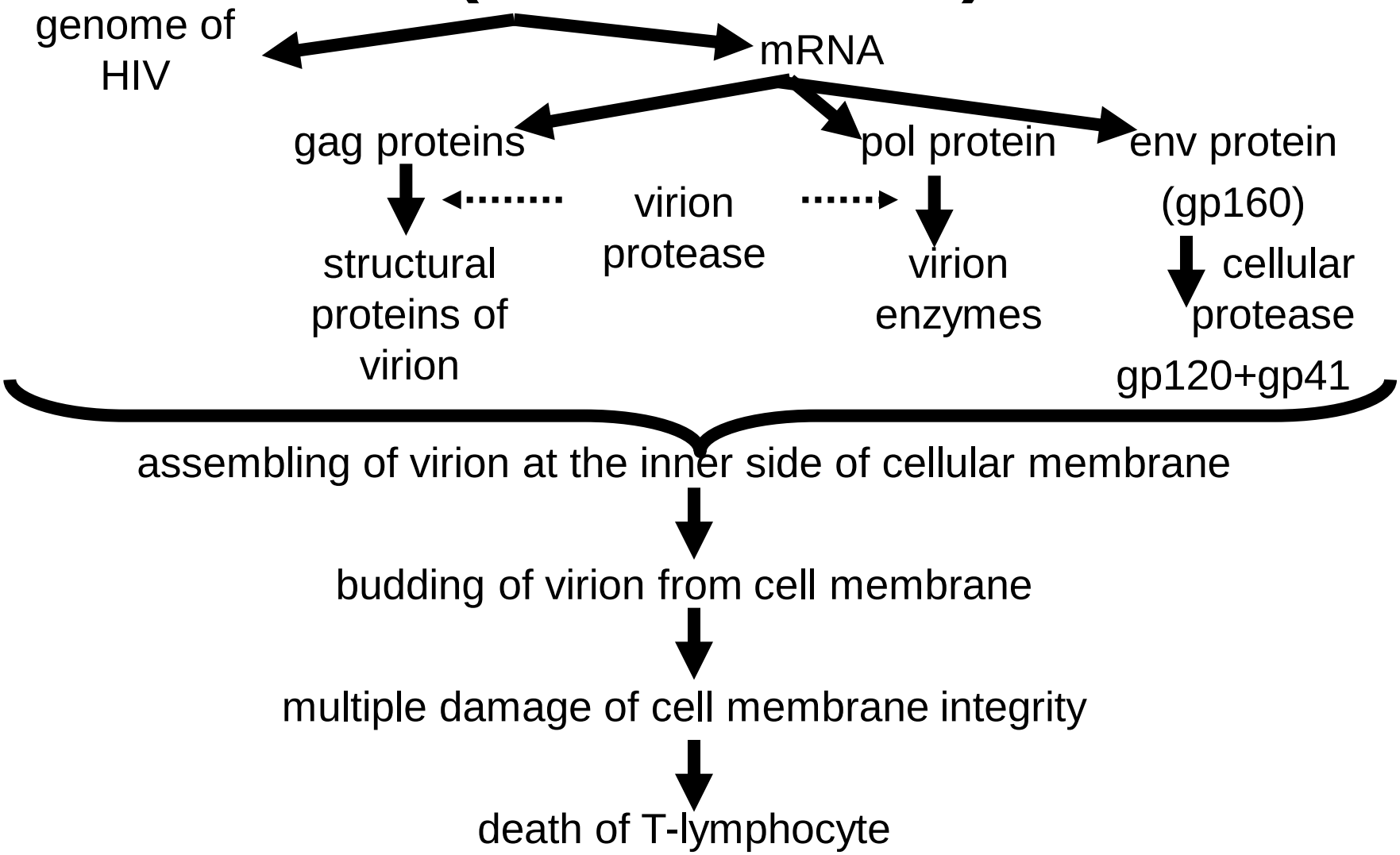
↓ cellular RNA-polymerase

RNA-transcript

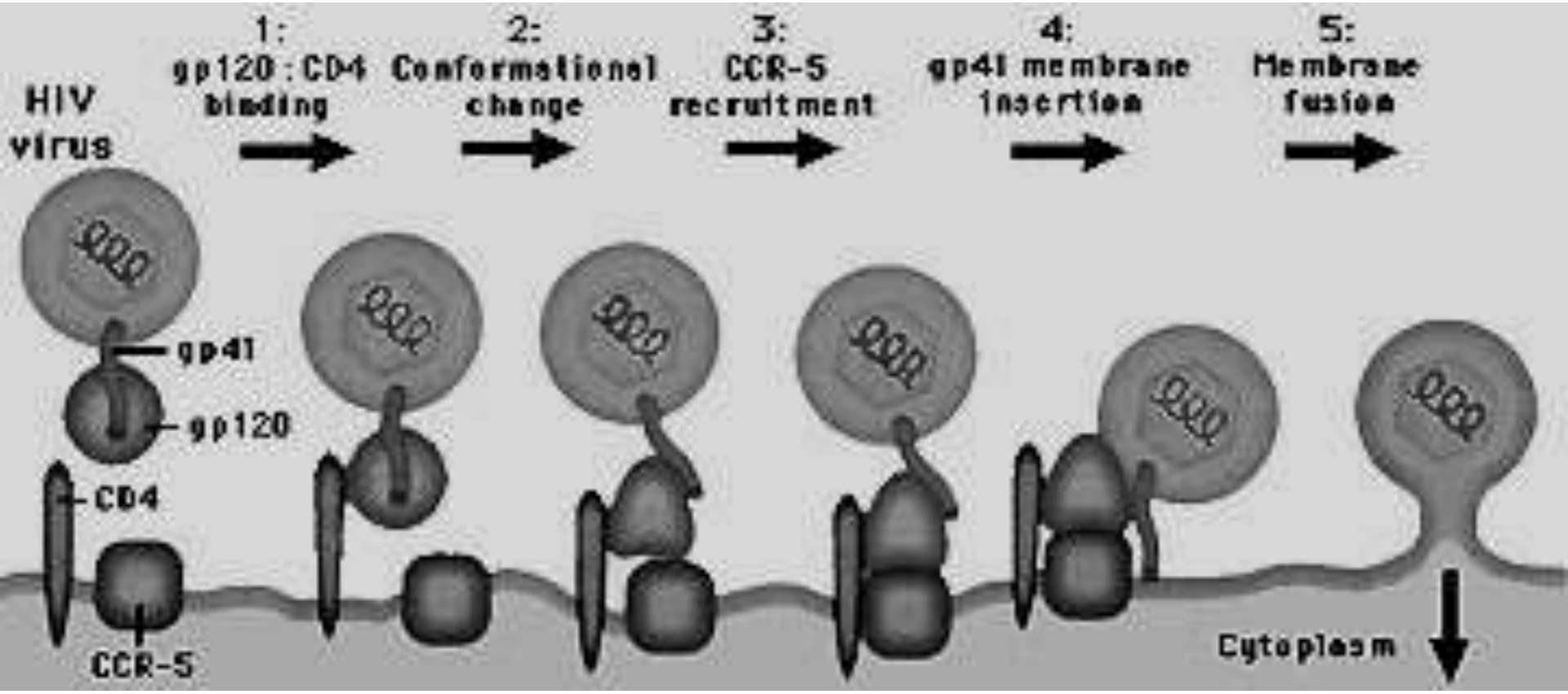
↓ rev

transfer of RNA-transcript into cytoplasm of T-lymphocyte

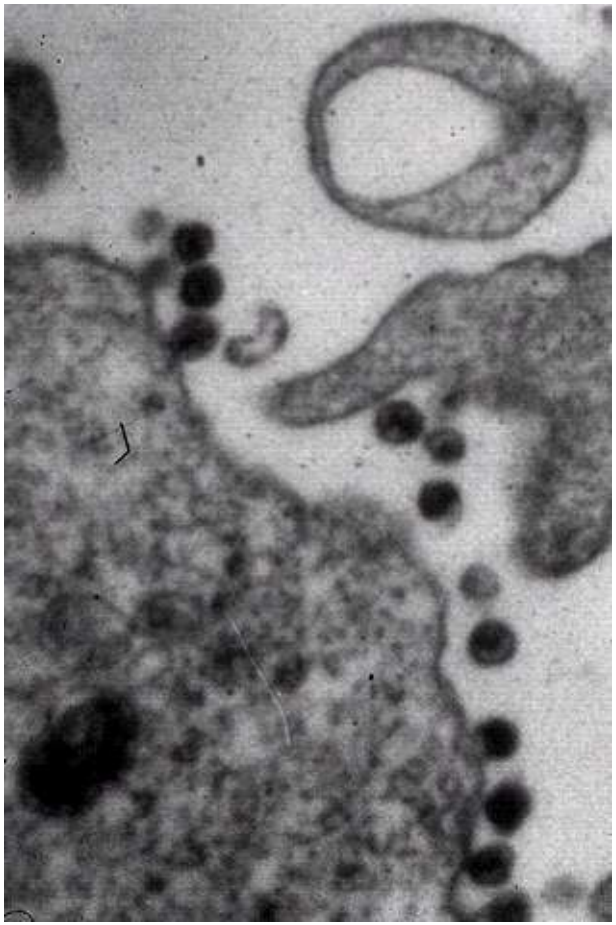
Life cycle of HIV in T4-lymphocytes (main scheme)



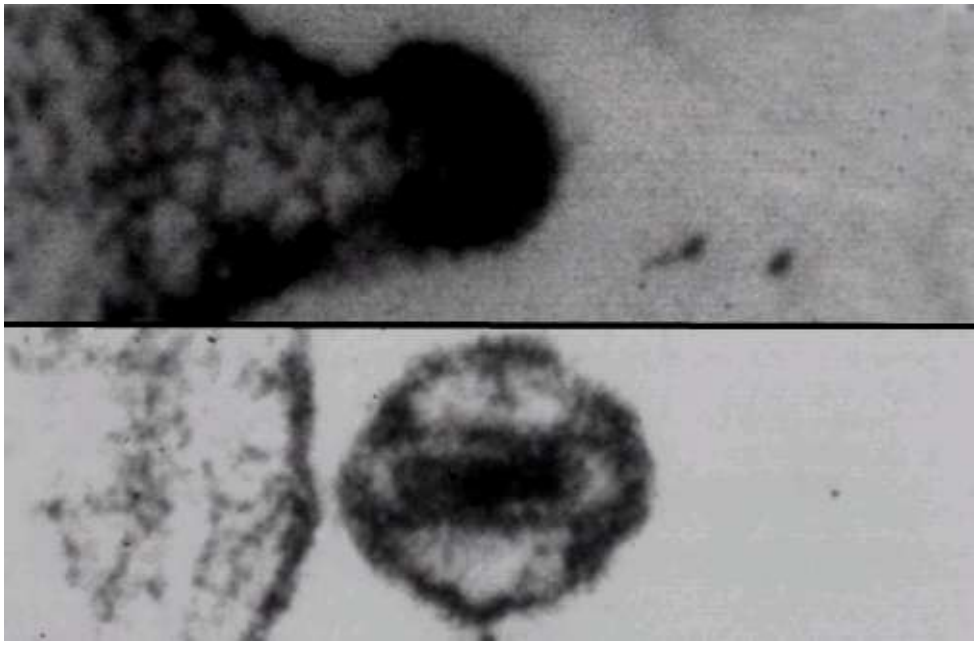
A scheme of HIV penetration into T_h



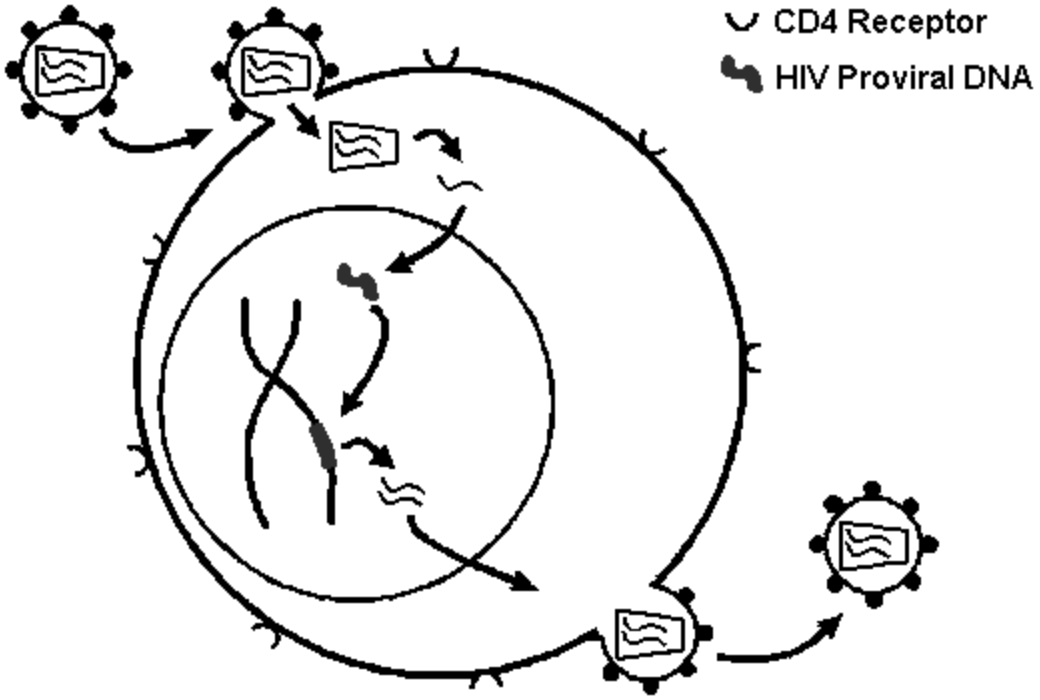
Attachment of the HIV to the cell



Budding of HIV



Main scheme of HIV replication



HIV: epidemiology

1. Sexual contact (85-90 %):
   - 70% - bisexual contacts
   - 5-10% - homosexual contacts
2. Exposure to contaminated blood or blood products
   - 3-5% - blood transfusion recipients (haemophiliacs)
   - 10% - intravenous drug users (mostly drag-abused humans)
3. Perinatal
   - transplacental during the last months of pregnancy
   - infants born to infected mothers
   - with mother's milk to newborn

Humans belonging to high risk groups

- homosexual partners
- intravenous drug users
- prostitutes (both sexes)
- humans who frequently change sexual partners
- haemophiliacs

Contamination of biological fluids by HIV

- Contain concentration of virus particles which is enough to cause disease (approximately 10 000 virions)
  - blood
  - sperm
  - vaginal secretion
  - cervical secretion
  - breast milk
  - liquor
- Contain concentration of virus particles which is not enough to cause disease
  - saliva
  - urea
  - tears

Pathogenesis of HIV infection

contaminated material

↓

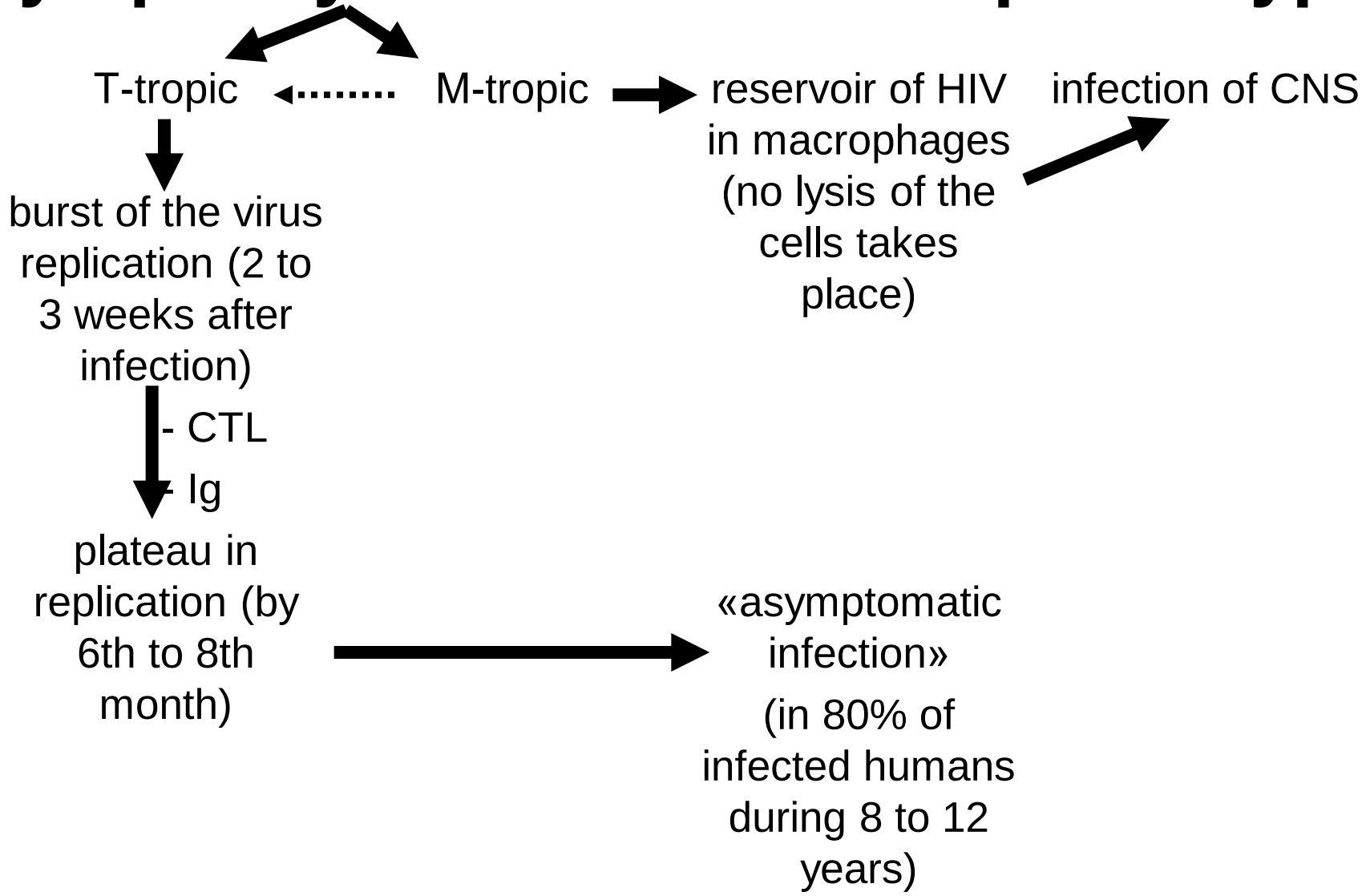
- Mucous membranes of human genitalia
- Mucous membranes of rectum (straight intestine)
- Blood
- Through placenta

↓

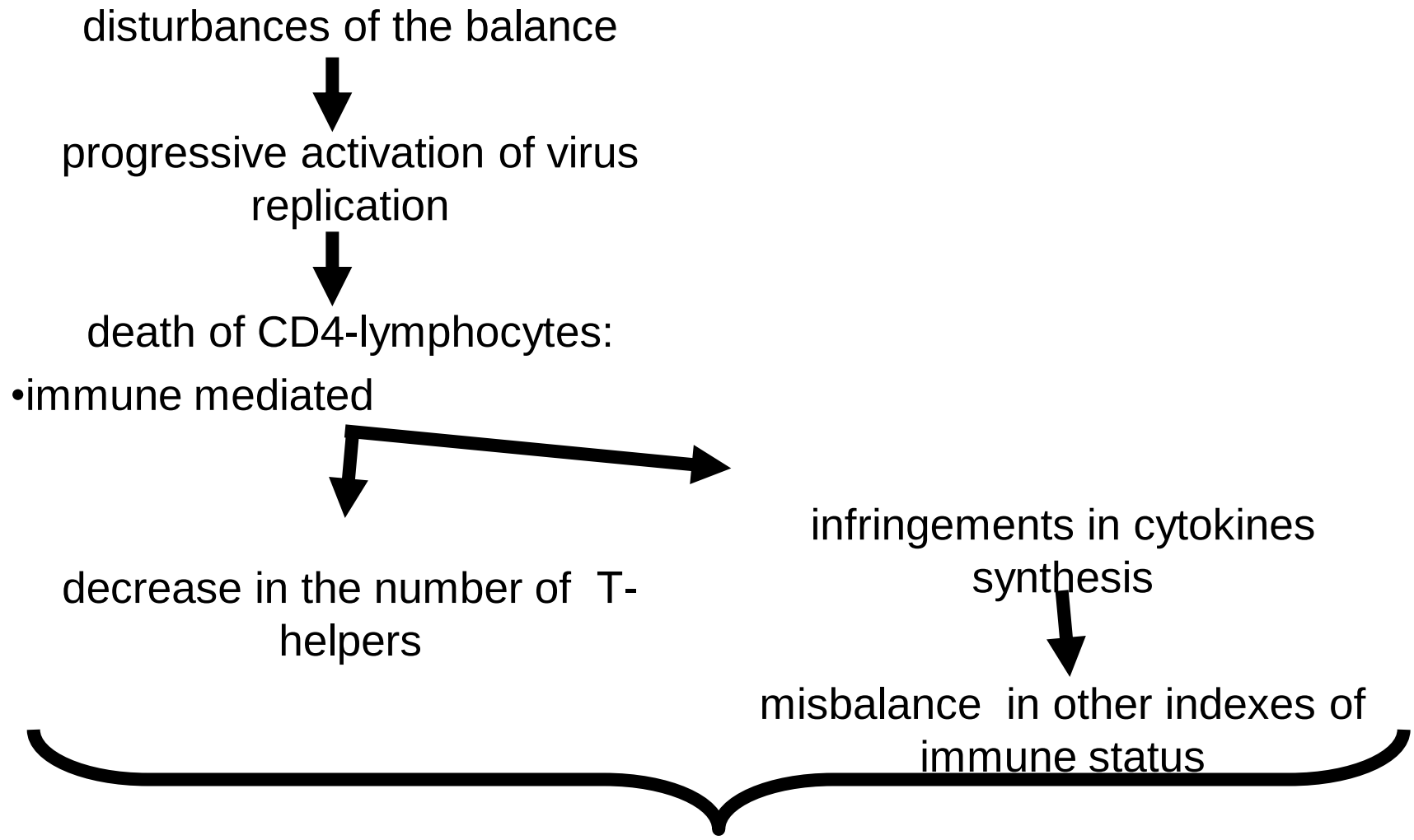
infection of the target cells

- CD4 - containing
  - T-helpers
  - monocytes/macrophages
- cells which don’t contain CD4
  - astrocytes
  - endothelial cells
  - epithelial cells

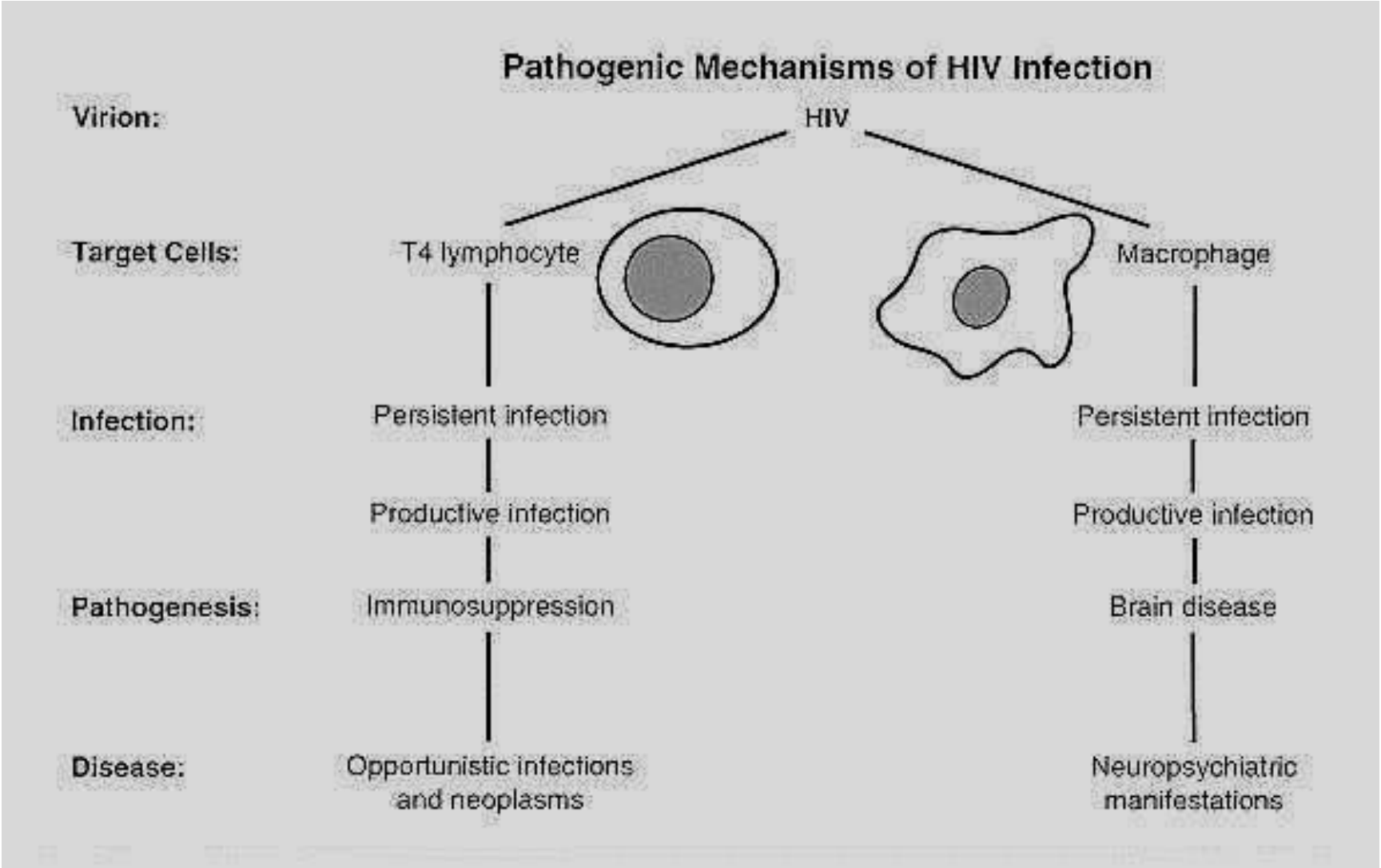
Pathogenesis of HIV infection in T-lymphocytes with different phenotype



Pathogenesis of HIV infection in T-lymphocytes: activation of the infection



Pathogenesis of HIV infection: mechanisms



Pathogenesis of HIV infection in T-lymphocytes: effect on the immune system status

↓

malfunction of immune system activity

↓

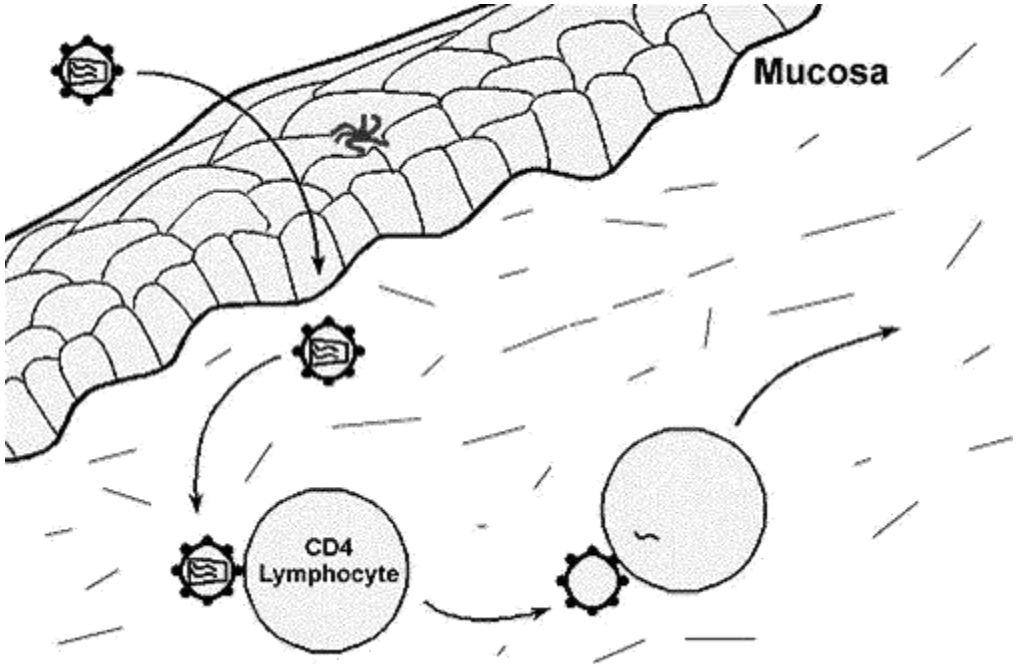
+ AIDS associated diseases

- tumours
- infections

↓

death of patient

Penetration of HIV through the mucous membrane into T-lymphocytes



HIV: stages of the disease

1. Asymptomatic infection (HIV-infection)
   - Acute infection with symptoms that may include:
     - fever
     - intoxication
     - sweats
     - lymphadenopathy
     - myalgia or arthralgia
     - nausea, etc
   - Asymptomatic infection (seraconvertion, could continue for years)
   - Persisting generalised lymphadenopathy (PGL)

2. Acquired immune deficiency syndrome (AIDS), characterized by progressive immune deficiency accompanied by:
   - neurologic abnormalities (abscesses, meningitis , encephalitis, etc.)
   - Pneumocystis carinii pneumonia
   - chronic diarrhoea, weight loss
   - neoplasms (Kaposi sarcoma and others)
3. Terminal stage of AIDS characterised by fatal decrease of the function of immune system
   - critical weight loss
   - adynamia
   - AIDS-dementia complex including progressive dementia and peripheral neuropathy

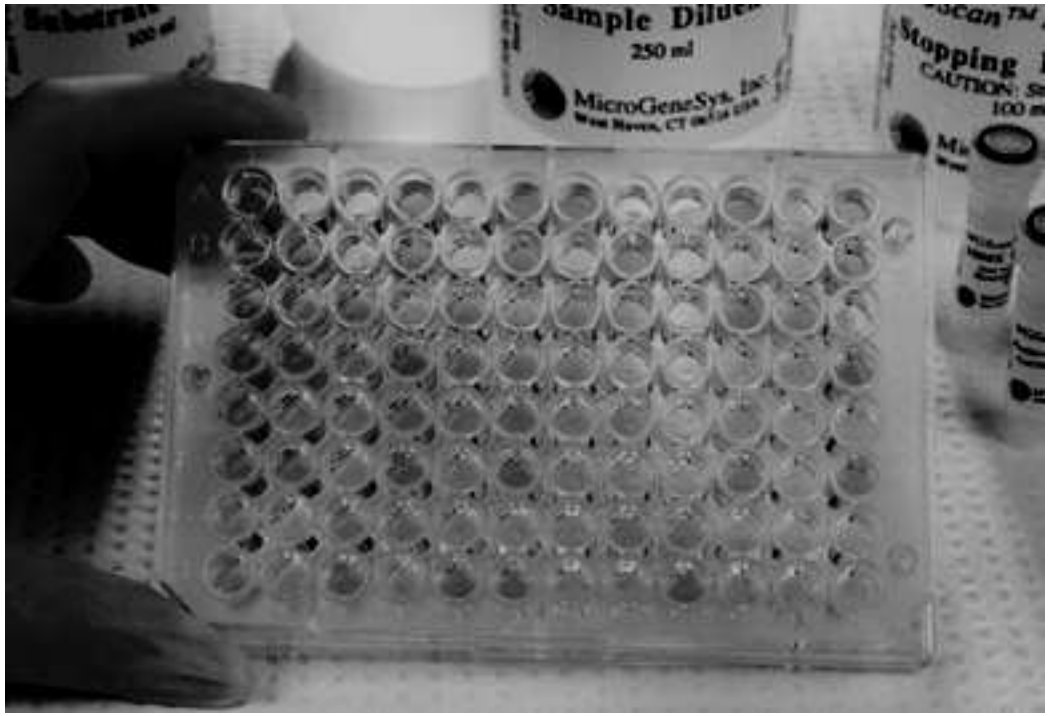
AIDS-associated diseases

1. *P carinii* pneumonia
2. MAC (Mycobacterium avium intracellularae complex)-infection
3. Cytomegalovirus and herpes virus infections
4. Candida infections (especially oral cavity infections)

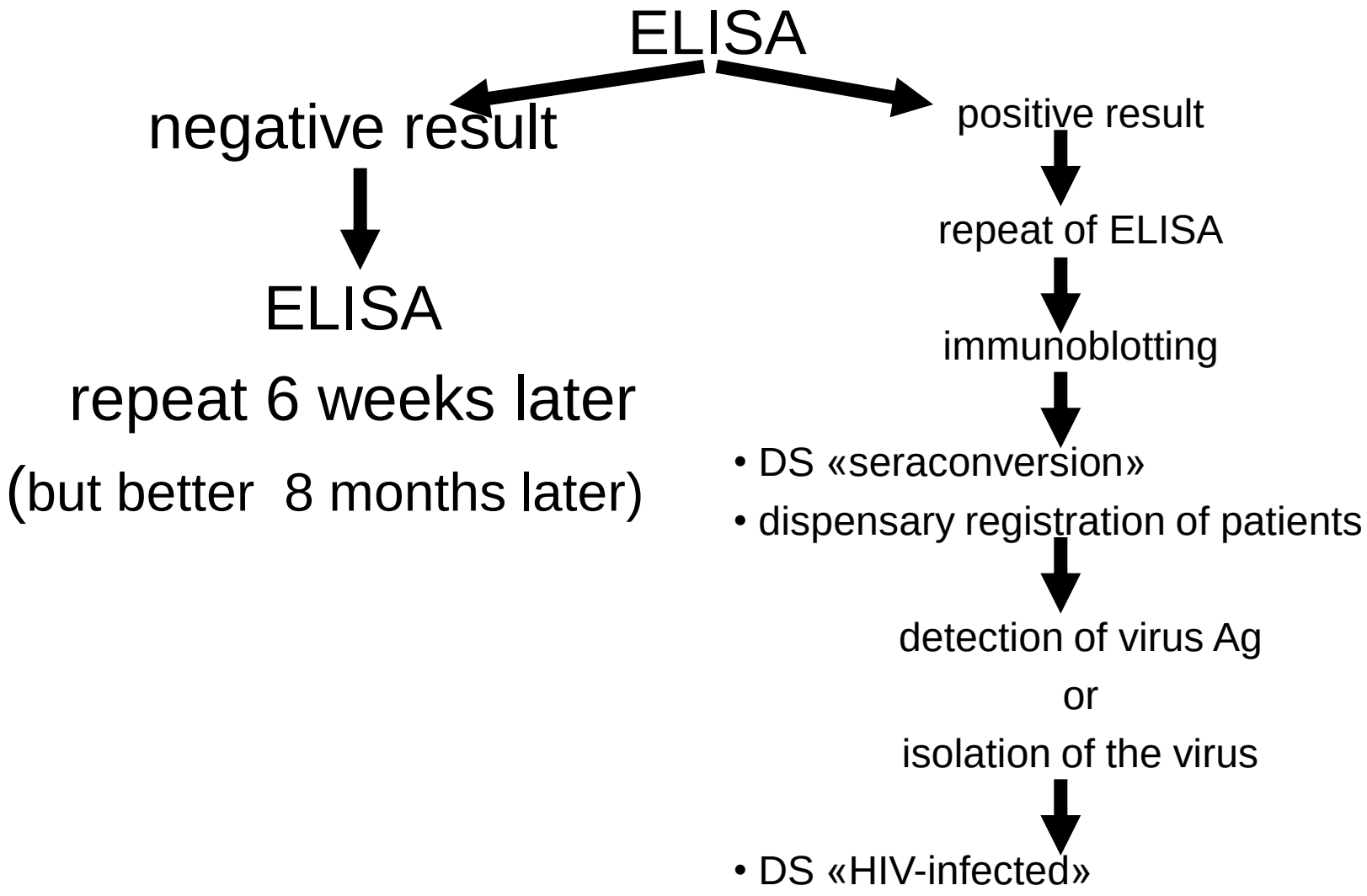
HIV: laboratory diagnostics

1. Revealing of specific antibodies
   - ELISA – summarising number of the antibodies (Ig)
     - classic (microtitre plates) tests
     - express tests (10-15 min)
   - immunoblotting – revealing of Ig specific against definite proteins of HIV
2. Revealing of virus antigens
   - ELISA
   - immunoblotting
3. Detection of virus genetic material
   - PCR – revealing of provirus DNA in mononuclear cells
   - Reverse PCR – revealing of virus RNA in blood
     - level of virus replication («virus load» - about 20-50 copies/ml)
     - control of the effectiveness of the therapy
4. Isolation of the virus
   - cultivation of the virus

ELISA



Procedure of testing in high risk groups to reveal HIV-infected humans



Picornaviridae.
Caliciviridae.
Theme N32

Picornaviruses: classification

- Picornaviridae
  - Enterovirus
  - Aphtovirus
  - Rhinovirus
  - Hepatovirus (Hepatitis A virus)

Picornaviruses: main characteristics

- the picornavirus virion is an icosahedral,
- nonenveloped , very small (22 to 30 nm) particle,
- the capsid proteins encase a sense RNA strand
- replication occurs in cytoplasm
- the way of translation:

RNA

⇩

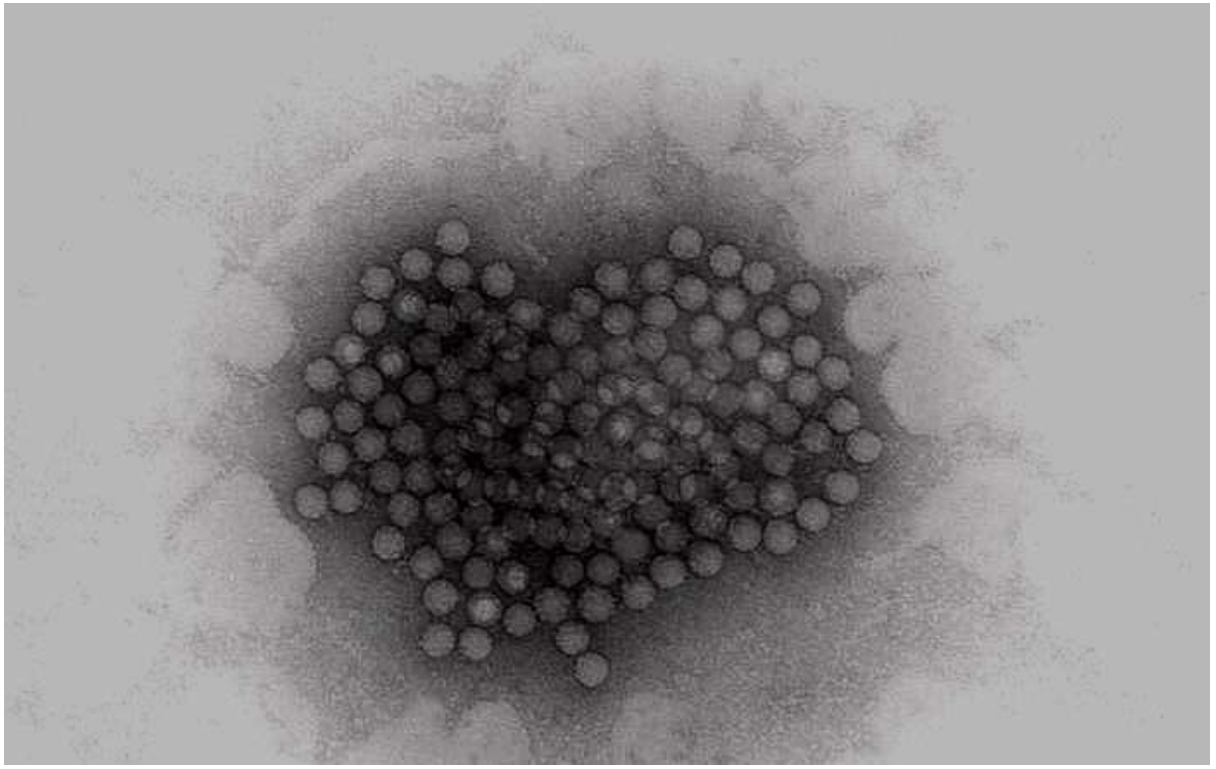
synthesis of one big polypeptide

⇩

cutting of the polypeptide into separate specific proteins

- release from the infected cell ⇨ lysis of the cell

Picornaviridae



Enteroviruses: classification

1. Human polioviruses (3 serological types)
2. Human coxsackieviruses– 30 serotypes
   - group A – 24 serotypes
   - group B – 6 serotypes
3. Human echoviruses (ECHO) – 34 serotypes
4. Enteroviruses: serotypes 68-71 are infectious for humans :
   - 70 – acute hemorrhagic conjunctivitis
   - 71 – poliomyelitis-like infection (meningitis and encephalitis)

Enteroviruses: cultivation

cell culture (excluding coxsackieviruses A)

⇓

- cytopathic effect
- negative colonies formation

Enteroviruses: : characteristics of the infections

- Picornaviruses are found worldwide
- produce mostly infections of gastro-intestinal tract
- could get a mass character
- healthy carriage is highly distributed in human populations
- disease occurs more frequently in children under 12 years of age

Infections caused by Enteroviruses: pathogenesis

the virus infects and replicates in the epithelium and regional lymphoid tissues:
nasopharyngeal
small intestine (viruses are stable at pH 3.0)

⇓

blood (viremia)

⇓

secondary target organs – the type of organ is dependent on :

- type of Enterovirus
- immune status of human

CNS could be affected by:

- human polioviruses
- Enteroviruses which cause poliomyelitis-like infection
- meningitis-producing viruses
- encephalitis-producing viruses

Polioviruses : main characteristics

- Possess common antigen (CF)
- Presented by three serotypes (NR)
- Attachment of the virus occurs at the cellular lipoprotein receptors
- Penetration – by endocytosis
- Release of the virus causes disintegration of the infected cell (release is accompanying by liberation of hundreds of virions)

Poliomyelitis: pathogenesis

poliovirus

⇓

penetrates to the portals of the infection

– oropharyngeal

⇓

replication of poliovirus occurs in:

- epithelial cells
  - oropharyngeal ⇨ transmission in the case of epidemic
  - intestinal mucosa ⇨ main route of transmission
- lymph nodes
  - the tonsils
  - Peyer's patches of the intestine

⇓

viremia

Poliomyelitis: pathogenesis

⇓

immune complex formation

⇓

increase of the permeability of hematoencephalitic barrier

⇓

virus spreads along axons of peripheral nerves to

- the brain
- the spinal cord

⇓

Poliomyelitis: pathogenesis

⇓

secondary reproduction of virus

- neurons of the spinal cord
- neurons of the brain

⇓

- degenerative (frequently irreversible) changes
- crystal-like inclusions in cytoplasm of neurons

⇓

- paresis
- flaccid paralysis

Clinical forms of poliomyelitis

1. Paralytic poliomyelitis (1% of all cases)
   - spinal poliomyelitis (the spinal cord)
   - bulbar poliomyelitis (cranial nerve nuclei or medullary centers are involved)
   - spinal-bulbar form of poliomyelitis
2. Nonparalytic poliomyelitis (aseptic meningitis) (1% of all cases)
3. Abortive poliomyelitis
4. Inapparent poliomyelitis

Poliomyelitis: immunity

- Humoral immune response
- type-specific (antibodies neutralising virus)
- continues for all life

Poliomyelitis: laboratory diagnostics

faeces, pharyngeal swabs, blood, brain, nodulus

⇓

cell culture

⇓

cytopathic effect

⇓

CF – poliovirus

NR – type of virus

⇓

ELISA, gel diffusion test , PCR

to differentiate from the vaccine strain

serum, liquor

⇓

increase (by 2-3rd week) Ig titer

Poliomyelitis: immune prophylaxis

- the Salk-type inactivated poliovirus vaccine (IPV) consists of a mixture of three poliovirus serotypes grown in monkey kidney cell cultures and made non-infectious by Formalin treatment
- immunoglobulin (human)

Human coxsackieviruses : disease in humans

- herpangina presents as small, scattered oral vesicles in the posterior oropharynx, tonsils
- fatal myocarditis in adults and neonates associated with focal necrosis
- meningoencephalitis
- pericarditis
- poliomyelitis-like infections
- acute respiratory infections
- acute intestinal infections

immunity is type-specific and lasts for a lifetime

Echoviruses: disease in humans

The echoviruses (enteric, cytopathic, human, and orphan viruses) are associated with various disorders involving:

- CNS
- muscles
- fever + rush
- in some cases:
  - gastroenteric infections
  - acute respiratory infections
  - occasional conjunctivitis

immunity is type-specific but the duration of the immune defence is different

Aphtovirus: disease in humans

- The virus causes outbreaks of hand-foot-mouth disease with or without encephalitis (highly infectious disease of cattle, sheep, pigs) may be transmitted to humans.
- In human the disease characterised by fever, salivation and vesiculation of the mucous membranes of the oropharynx and of the skin of the palms, soles, fingers.

Rhinovirus group: characteristics of the genus

- 115 serotypes (NR)
- Rhinoviruses cause mainly respiratory infections including the common cold accompanying with redness and swelling of the nasal and nasopharyngeal mucosa.
- Immunity is type specific.

CALICIVIRIDAE: main characteristics

- Small, spherical, non-enveloped,
- **ss RNA**
- "Calix" means "cup" – cup-shaped structures on capsid.
- REPLICATION occurs in cytoplasm.

CALICIVIRIDAE: classification

- **NORWALK AGENT (virus)**: the name has its origin from little village in Ohio.
  - Major cause of gastroenteritis.
  - Short incubation period.
  - Infection more common in adults.
  - TRANSMISSION: faecal-oral.
  - TREATMENT: none.
  - IMMUNITY: induced immunity is poor.
- **HEPATITIS E virus** (described in the lecture on Hepatitis viruses)

Arbo- and roboviruses. Rhabdoviridae. Reoviridae.

Theme N33

Ecologic grouping of arbo- and roboviruses: main characteristics

Arboviruses

Arthropods serve as vectors, transmitting viruses by their bites:

arthropod born viruses

Roboviruses

Vertebrate are hosts and sources of the infection – infection is transmitted without involvement of arthropods: rodent born viruses

comparatively mild disease

•fevers of undifferentiated type (systemic fever states)

severe disease characterised by high mortality

• hemorrhagic fevers

• encephalitis

Man-Arthropod-Man Cycle

Ecologic grouping of arbo- and roboviruses: composition of the group

Main families:

- Togaviridae - family includes genus Alphavirus and genus Rubivirus
- Flaviviridae
- Bunyaviridae
- Arenaviridae
- Filoviridae
- Reoviridae

Togaviridae (Alphavirus): antigenic groups

Viruses belonging to the genus Alphavirus are classified on the basis of antigenic properties (in IHA test):

1. Venezuelan and western equine encephalitis.
2. Eastern equine encephalitis.
3. Semliki forest encephalitis.

Togaviridae (Alphavirus): multiplication

- continues for 4 – 8 hours
- occurs in cytoplasm
- release of the virions occurs by budding from cytoplasm membrane of the infected cells which later die.

Togaviridae (Alphavirus): human disease

1. Mild disease with (Semliki forest encephalitis)
2. Low mortality
   - Venezuelan encephalitis.
3. Average mortality
   - Western equine encephalitis
4. High mortality
   - Eastern equine encephalitis

Flaviviruses: classification

- **Flavivirus** – arboviruses which produce disease in humans and animals
- **Pestivirus** – viruses which produce diarrhoea in caws and pigs
- **Hepacivirus** – hepatitis C virus

Flaviviruses: antigenic groups

The antigenic groups determined in inhibition of hemagglutination reaction (IHR)

- The tick-borne complex viruses (produce hemorrhagic fever or encephalitis)
- Japanese encephalitis virus
- Dengue fever virus
- Yellow fever virus

Flaviviruses: multiplication

- occur in cytoplasm
- takes over 20 hours
- virions are released by budding from endoplasmic reticulum (after budding exocytose of the virion from the cell occurs) – the cell stays viable

The tick-borne encephalitis: pathogenesis

- tick bite
- unpasteurised caw or goat milk

⇓

viremia

⇓

primary multiplication

- lymphocytes
- hepatocytes
- cells of spleen
- endothelial cells of blood vessels

⇓

generalisation

- spreading with blood
- spreading with lymph

⇓

secondary multiplication

- neurones of spinal cord and brain

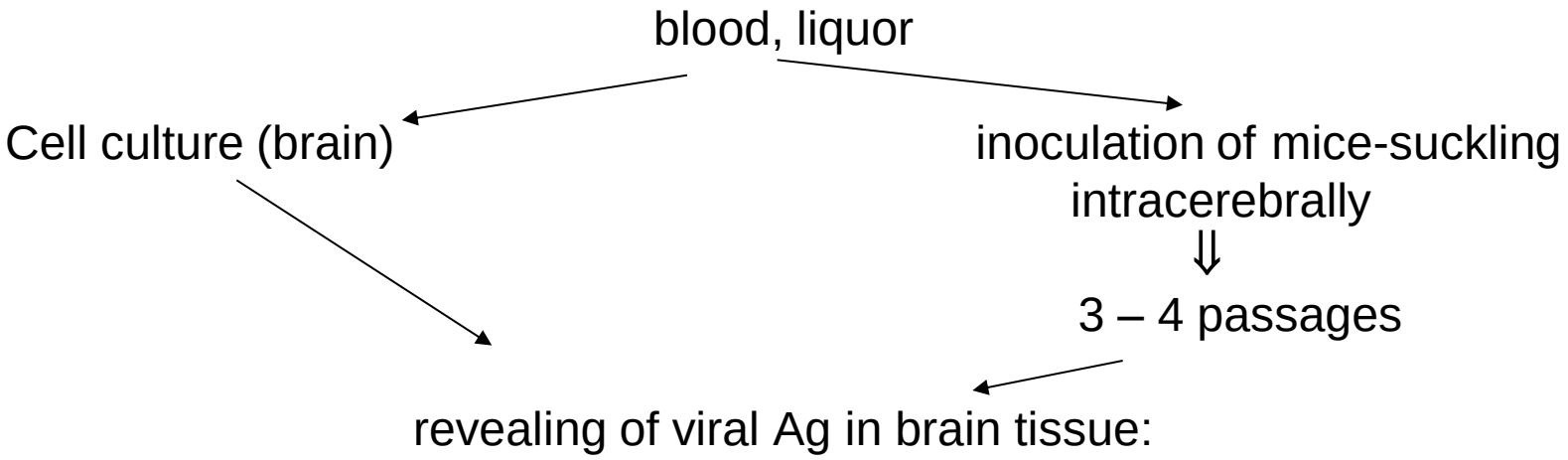
Tick-borne encephalitis: clinical forms

1. Feverish
2. Meningeal
3. Focal (the most severe – superior paraplegia)

In 1 – 3 % of cases chronic-progressive clinical course.

Tick-borne encephalitis: laboratory diagnostics

1. Rapid diagnostics:
   - revealing of Ag in blood (IHA test, ELISA)
   - revealing of viral genome in blood (PCR)
2. Virological method



   - IHA test
   - CF test
   - NR and PCR (the most specific tests – help to reveal the virus)
3. Serology
   - paired sera (IHA test –one week after inoculation, CF – 2 weeks after inoculation, NR – one month after inoculation)

Tick-borne encephalitis: **specific prophylaxis**

- Inactivated vaccine (formalin treated).
- Specific immunoglobulins

Japanese encephalitis

- In clinical cases, a life-threatening encephalitis occurs.
- It is transmitted by mosquitoes (human could be infected only in the pestholes of the disease).
- Inactivated vaccine is used for specific prophylaxis.

Dengue fever

Reservoir

1. humans
2. monkeys

Vectors

mosquitoes

Virus

4 antigenic types

Infects human in tropical countries

Symptoms: fever, arthralgia, rush. In serious cases – Dengue Shock Syndrome – a form of hemorrhagic fever.

Yellow fever

Virus

- 1 antigenic type
- Infects humans in tropical countries
- Target organs: liver (causes jaundice), kidney and myocardium
- Live vaccine – it is necessary vaccine for immunisation of people travelling to the endemic regions

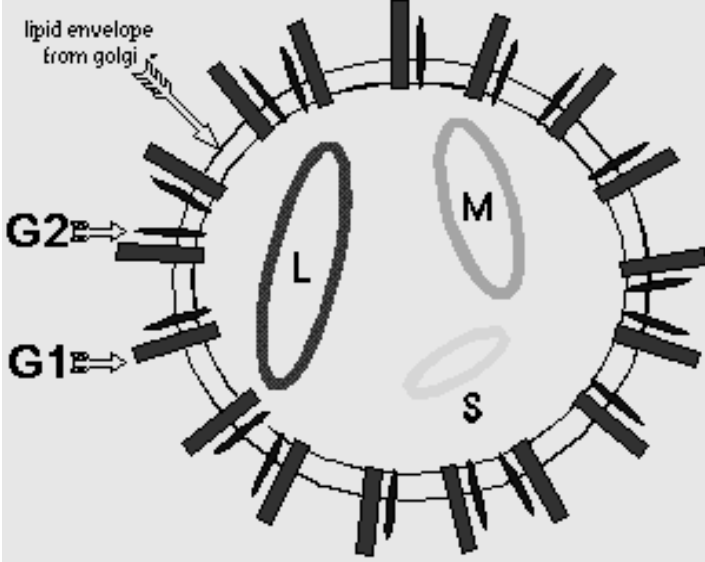
Global Distribution of Yellow Fever, 1996

Areas endemic with yellow fever

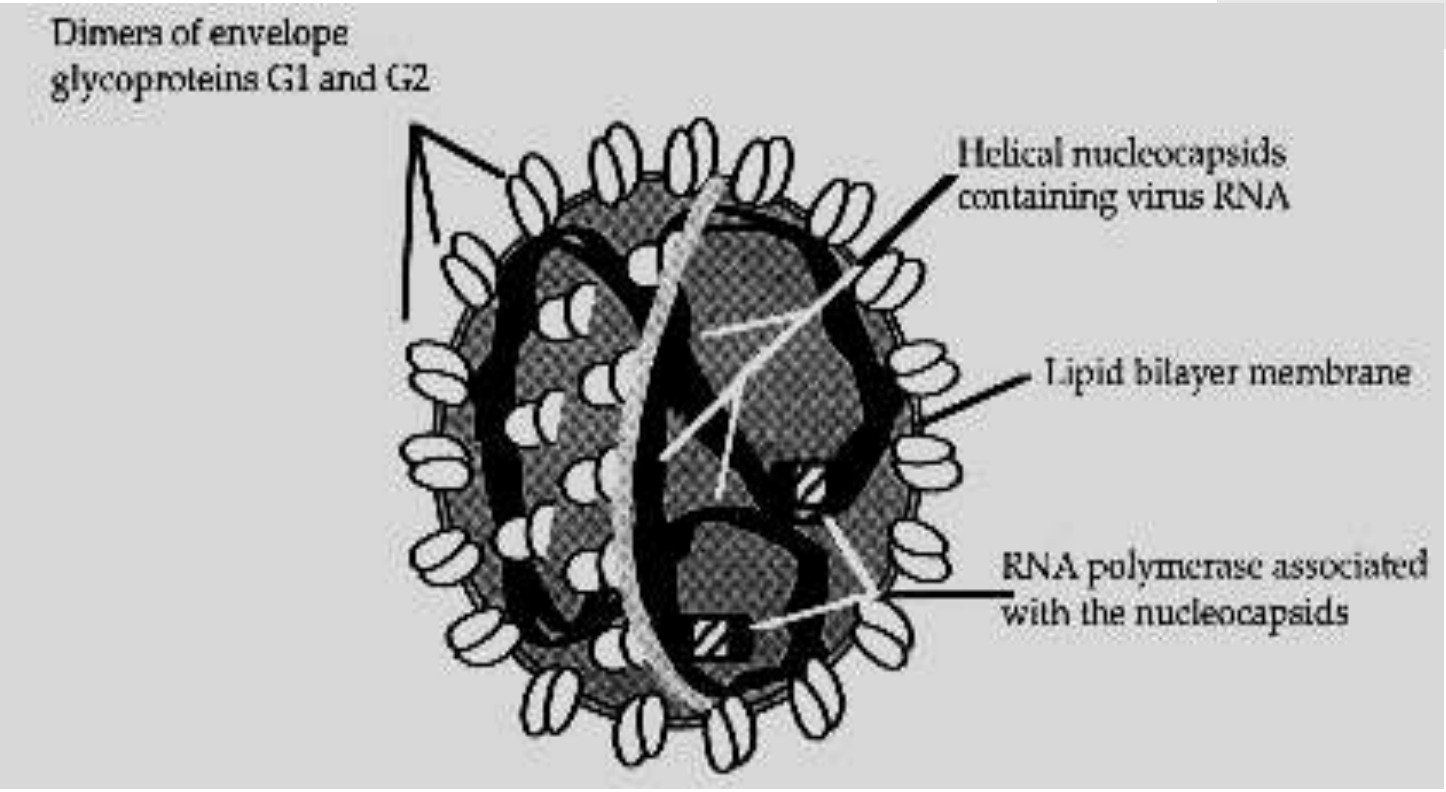
Bunyaviruses: classification

Bunyaviridae (family) includes the next genera:

1. **Bunyavirus** – produces mosquito encephalitis;
2. **Phlebovirus** – causes mosquito fevers;
3. **Nairovirus** (Nairobivirus) – virus producing Crimean-Congo hemorrhagic fever (the disease is transmitted by ixodes ticks);
4. **Hantavirus** – produces hemorrhagic fever with renal syndrome (robovirus).



Bunyavirus particle



Bunyaviruses: main characteristics

- Bunyaviruses are spherical, enveloped particles 90 to 100 nm in diameter.
- They contain three segments of antisense single-stranded RNA.
- Nucleocapsid is presented by spiral type of symmetry.
- Bunyaviruses replicate in the cytoplasm.
- They mature by budding into vesicles at the Golgi apparatus.
- The viruses are liberated from the cell by plasma membrane disruption and by fusion of intracellular vacuoles with the plasma membrane.

Arenaviruses: main characteristics

- Arenaviruses are oval enveloped particles with a diameter of 110 to 130 nm.
- The viral envelope carries club-shaped surface projections about 10 nm long.
- During morphogenesis, sandy-appearing granules are found within the unstructured interior of nascent viruses.
- These particles appear to contain aggregates of host ribosomal RNAs and they give arenaviruses their name: arena is Latin for "sand.“
- The viral RNA comes in five distinct segments. The viruses are divided into two antigenic groups:
  1. Old World viruses
     - virus producing lymphocytic choriomeningitis
     - Lassa virus
  2. New World viruses
     - Junin virus
     - Machupo virus
- Arenaviruses are roboviruses.
- These viruses produce severe diseases (hemorrhagic fevers) with high mortality (up to 70%).

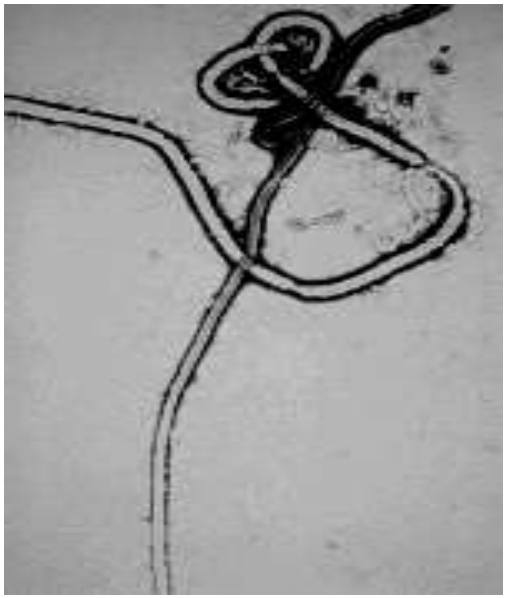
Filoviruses: general concepts

Filoviridae

- Filovirus:

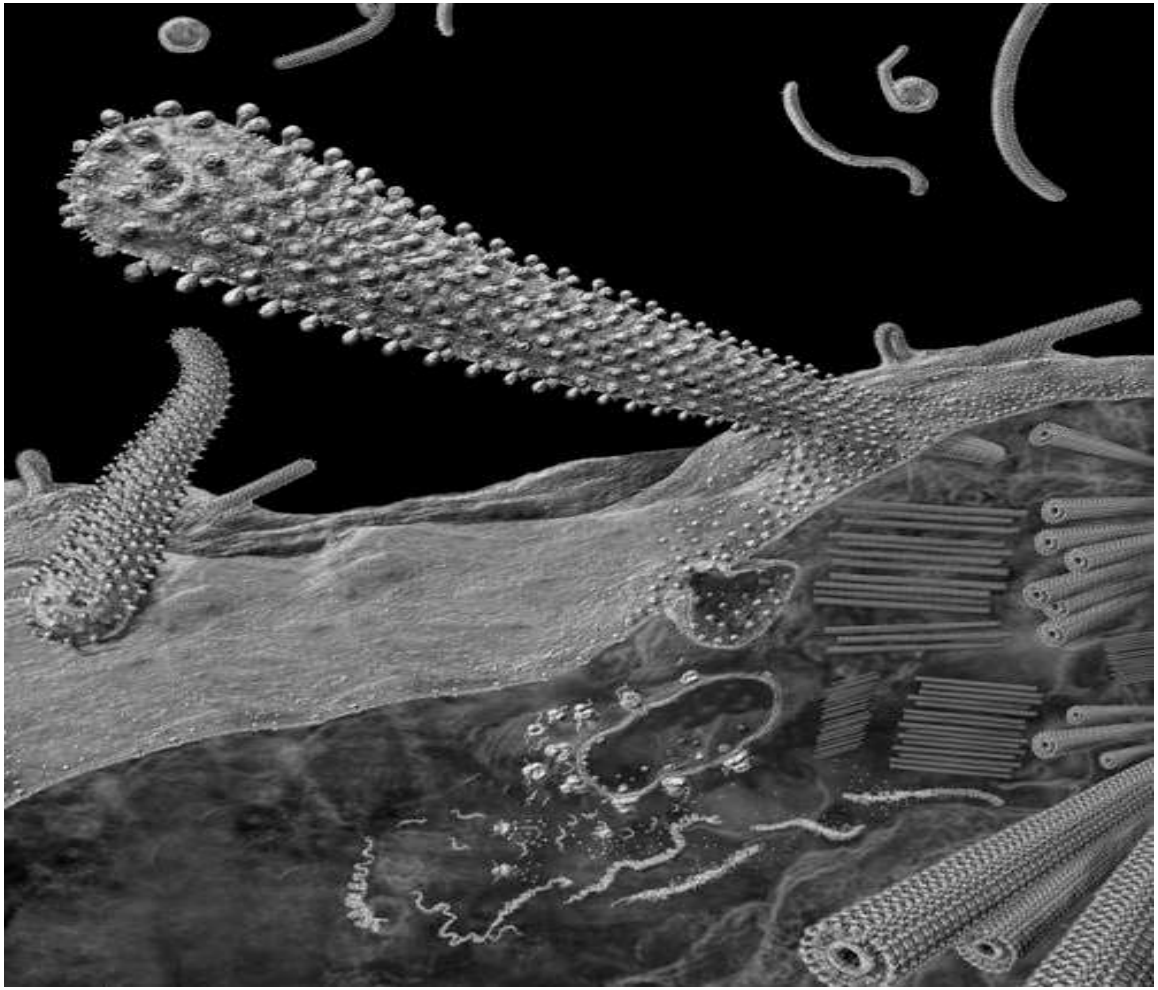
single genus separated into two sero-/genotypes

- Marburg virus
- Ebola virus



- spiral symmetry,
- they are filamentous, enveloped particles,
- negative-sense, single-stranded RNA genome,
- endemic in many countries of the central African region,
- cause outbreaks seem of a zoonotic nature (monkey are the source of the infection),
- cause a severe, often fatal hemorrhagic fever in human and non-human primates that has appeared sporadically since its initial recognition in 1976.

Ebola virus



Rhabdoviruses : classification

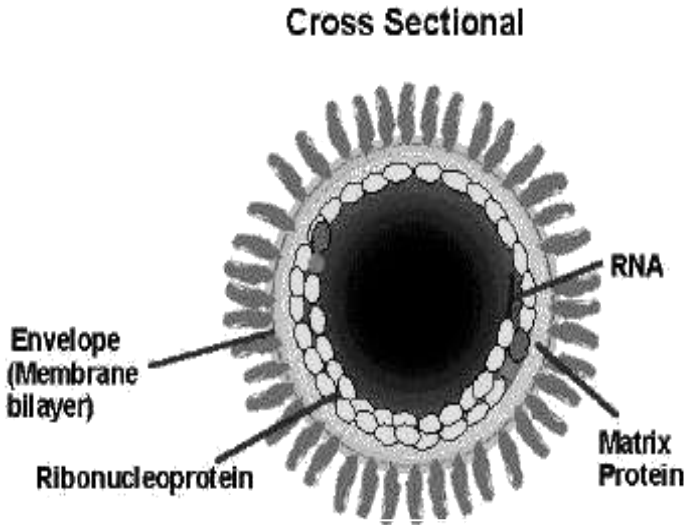
Rhabdoviridae (family) includes the next genera:

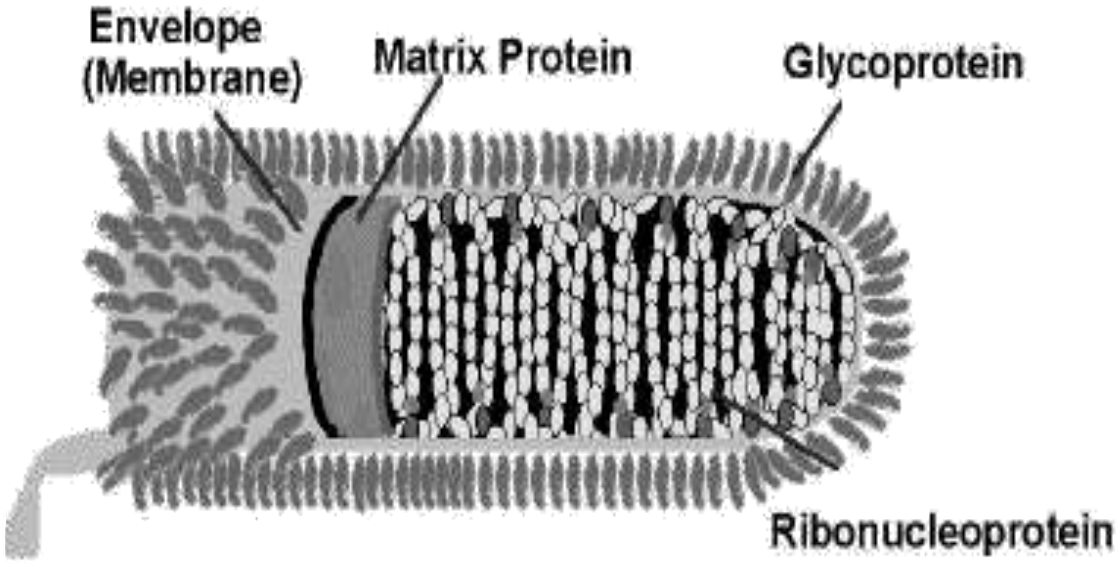
- Vesiculovirus (virus produces vesicular stomatithis, it is arbovirus),
- Lyssavirus (rabies virus).

Rabies virus: main characteristics

- Rabies virus is a rod- or bullet-shaped, enveloped virus measuring approximately 60 nm x 180 nm;
- the envelop covered with trans-membrane glycoprotein spikes;
- it possesses single-stranded, negative-sense, non-segmented RNA;
- the virus is unstable outside the host cell;
- multiplication of the virus occurs in cytoplasm of infected cells , viral proteins together with the viral RNA aggregate in the cytoplasm of virus-infected neurons and compose Negri bodies.

Rabies virus





Rabies: pathogenesis of the disease

virus is most commonly transmitted through the bite of an infected mammal

⇓

virus replicates in:

- muscle tissue
- connective tissue

⇓

virus enters the peripheral nervous system via the neuromuscular junctions, and moves rapidly centripetally to the central nervous system

⇓

it replicates in brain and causes degenerative damage of neurons

⇓

the virus then begins to pass centrifugally to many tissues and organs, such as the salivary glands

Rabies: stages in the course of the disease

Five general stages of rabies are recognized in humans:

- incubation (duration is from 7 days up to the year),
- prodrome (1 to 3 days),
- acute neurological period(2 to 3 days, rarely up to 6 days),
- coma,
- death (or, very rarely, recovery).

The whole duration of the disease is from 5 to 14 days.

Rabies: laboratory diagnostics

1. Revealing of Negri bodies in brain biopsy (could not be applied for all biological variants of the virus).
2. The detection of rabies antigen (direct immunofluorescence testing in skin biopsies).
3. Detection of rabies virus-neutralizing antibody, as typically performed by the rapid fluorescent focus inhibition test (RFFIT), in the serum of unvaccinated individuals.
4. The isolation of virus:

saliva specimens are used for culture of virus

⇓

- inoculation of white mice or rabbits intracerebrally
- inoculation of hamsters intravenously

⇓

use the brain of died animal

⇓

to detect Negri bodies and viral Ag

Rabies: modern methods of prophylaxis

- Animal rabies is prevented by vaccinating susceptible species, particularly dogs and cats.
- Human rabies is best prevented by avoiding exposures to the disease.
- Pre-exposure immunization may be offered to persons at high risk, such as veterinarians, animal handlers, etc.
- When an exposure is suspected, post-exposure prophylaxis should be initiated promptly with use of human rabies immune globulin (HRIG) and rabies vaccine.

Rabies vaccines are two cell culture products currently licensed in the United States which include:

- rabies vaccine adsorbed (RVA)
- the human diploid cell vaccine (HDCV)

Post-exposure treatment will abort the infection, but there is no cure for clinical disease.

Reoviruses : classifictaion

Reoviridae (family) includes the next genera:

- Orthoreovirus is a causative agents of respiratory and intestinal tract infections.
- Rotavirus causes enteritis in human infants and young children.
- Orbivirus is arbovirus and can cause mild fevers in human.

Reoviruses: main characteristics

- Reoviruses are naked viruses.
- The viruses possess 2 distinct capsid shells.
- The nucleocapsid is presented by icosahedral type of symmetry.
- The double-stranded reovirus RNA exists as a collection of 10 -11 discrete segments.

Rotaviruses: main characteristics

- *Rotavirus* is a genus in the family Reoviridae.
- They are 70-nm-diameter wheel-shaped particles consist of a double-layered icosahedral capsid and contain 11 segments of double-stranded RNA.
- The viruses cause enteric disease with symptoms characterized by:

  diarrhoea,

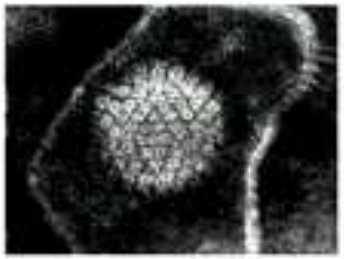
  vomiting,

  abdominal discomfort,

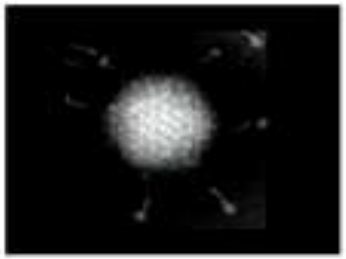
  fever.
- The virus affects mainly infants and young children. Diarrhoea ranges from mild to severe and can cause fatal dehydration.
- Virus could be revealed in faeces with use of immune electron microscopy

DNA viruses
Theme N34

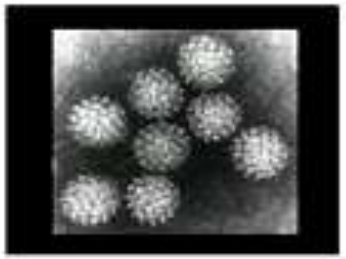
Poxviridae



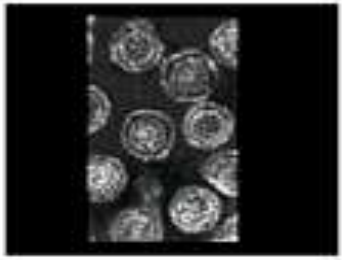
Herpesviridae



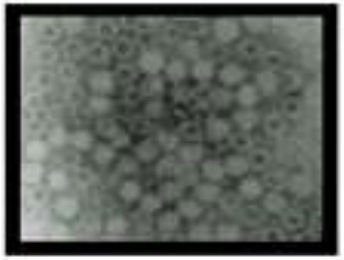
Adenoviridae



Papovaviridae
human papilloma



Hepadnaviridae



Parvoviridae

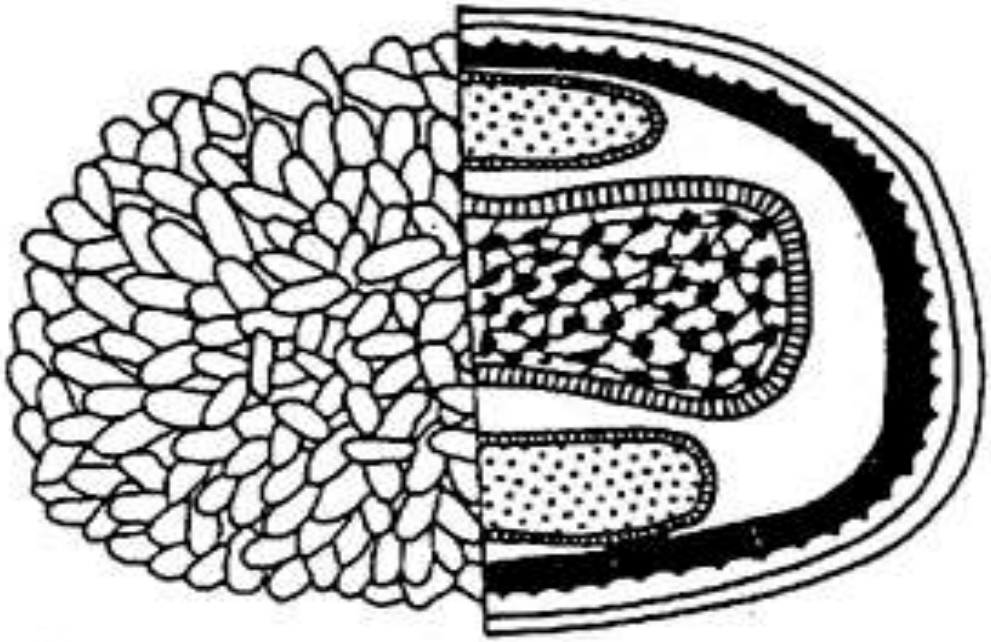
DNA Viruses

100 nanometers

Poxviruses: main characteristics

- Poxvirus virions are large (240 nm by 300 nm) and brick shaped.
- Internally, virions have a dumbbell-shaped core and two lateral bodies.
- The envelop is covered with short surface tubules 10 nm wide.
- The genome consists of one molecule of double-stranded DNA .

COMPLEX SYMMETRY



POXVIRUS FAMILY

Poxviruses: classification

Poxviridae

- Entomopoxvirinae
  - arthropods viruses
- Chordopoxvirinae
  - smallpox (variola major)
  - monkeypox
  - cowpox
  - vaccinia poxvirus

  } **Orthopoxvirus**

  - molluscum contagiosum – **Molluscipoxvirus**

The history of development of the methods of immune prophylaxis of smallpox and worldwide smallpox eradication

- Variolation, the introduction into the skin of variola virus obtained from mild cases of smallpox, has been used centuries ago by Chinese.
- 1798 – introduction of living vaccinia virus into the skin dates from Jenner's work, published in 1798.
- 1967 – a worldwide Smallpox Eradication Program sponsored by WHO has been pursued with remarkable success.
- 1976 – all virus livestock reserves were accumulated in special laboratories.
- 1977 – the last cases of the disease in Somalia.
- 1980-ths – the routine vaccination of infants and children has been discontinued.

XX-XXI centuries – the danger of «outbreaks» of the disease:

- **biological terrorism**
- thawing of snow in the regions of permafrost

Herpesvirus family: classification

Herpesviridae

- ***Alphaherpesvirinae***

  Simplexvirus
  - Herpes simplex virus 1 (HSV-1)
  - Herpes simplex virus 2 (HSV-2)

  Varicellovirus
  - varicella-zoster virus (VZV)

- ***Betaherpesvirinae***

  Cytomegalovirus
  - CMV - cytomegalovirus

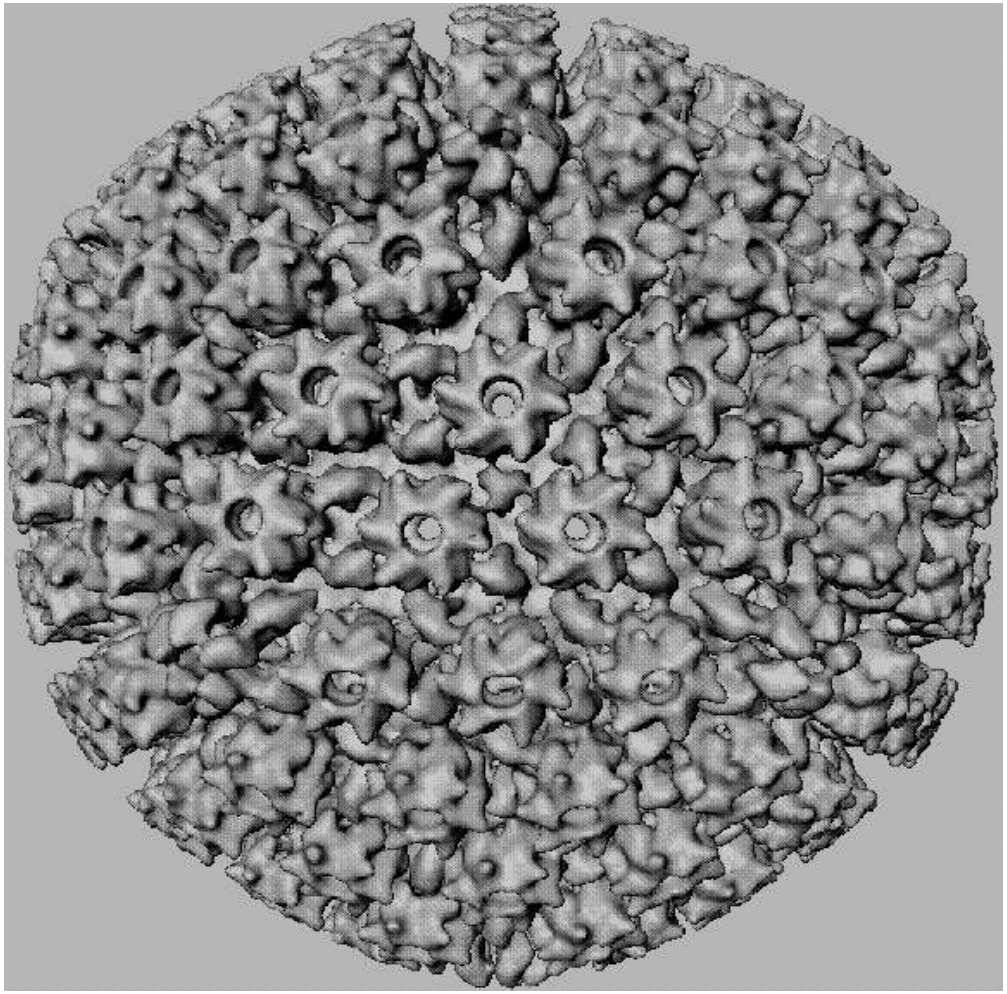
  Roseolovirus
  - HHV-6
  - HHV-7

- ***Gammaherpesvirinae***

  Lymphocryptovirus
  - Epstein-Barr virus (EPV)

  Rhadinovirus
  - HHV- 8

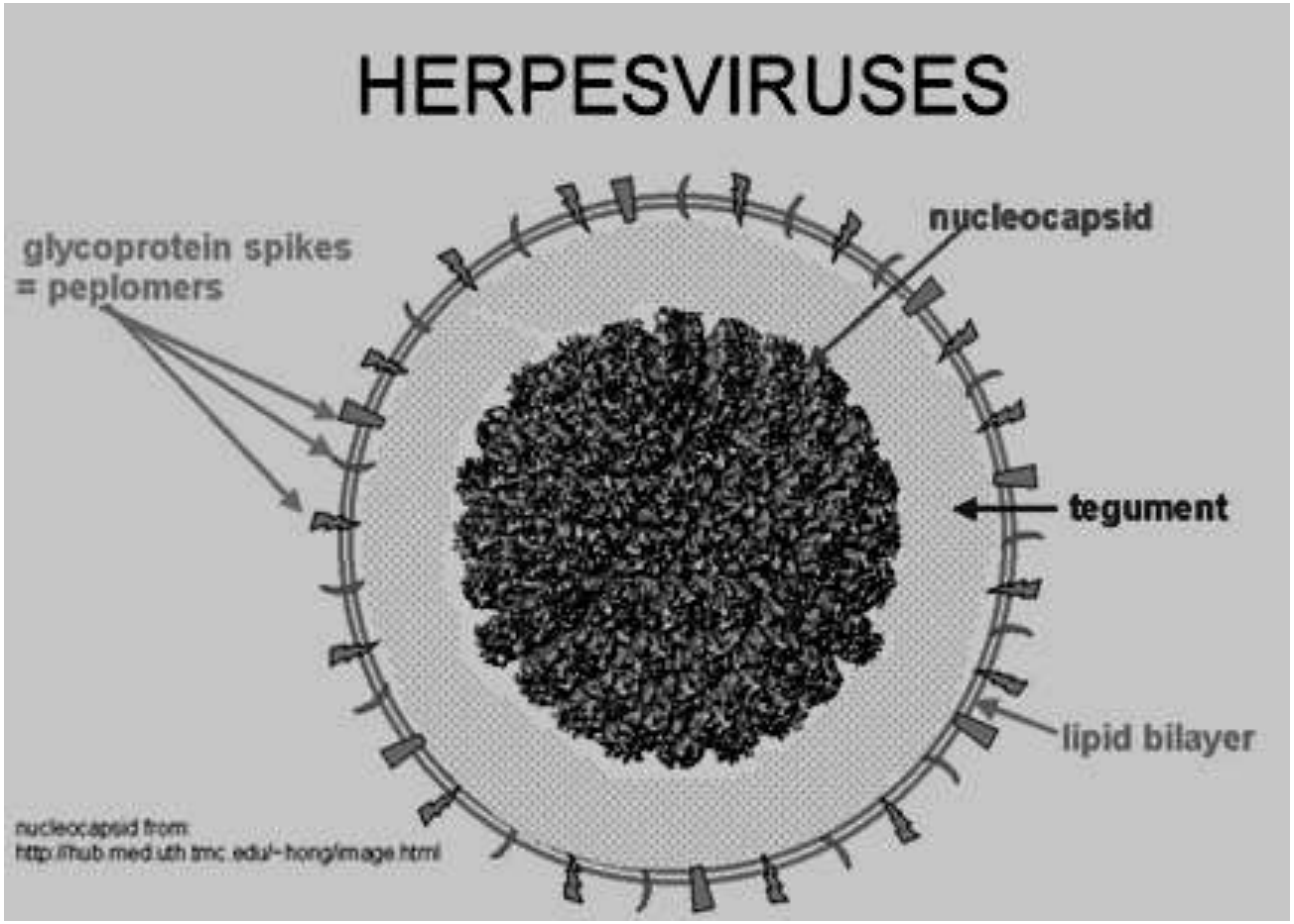
HSV 1



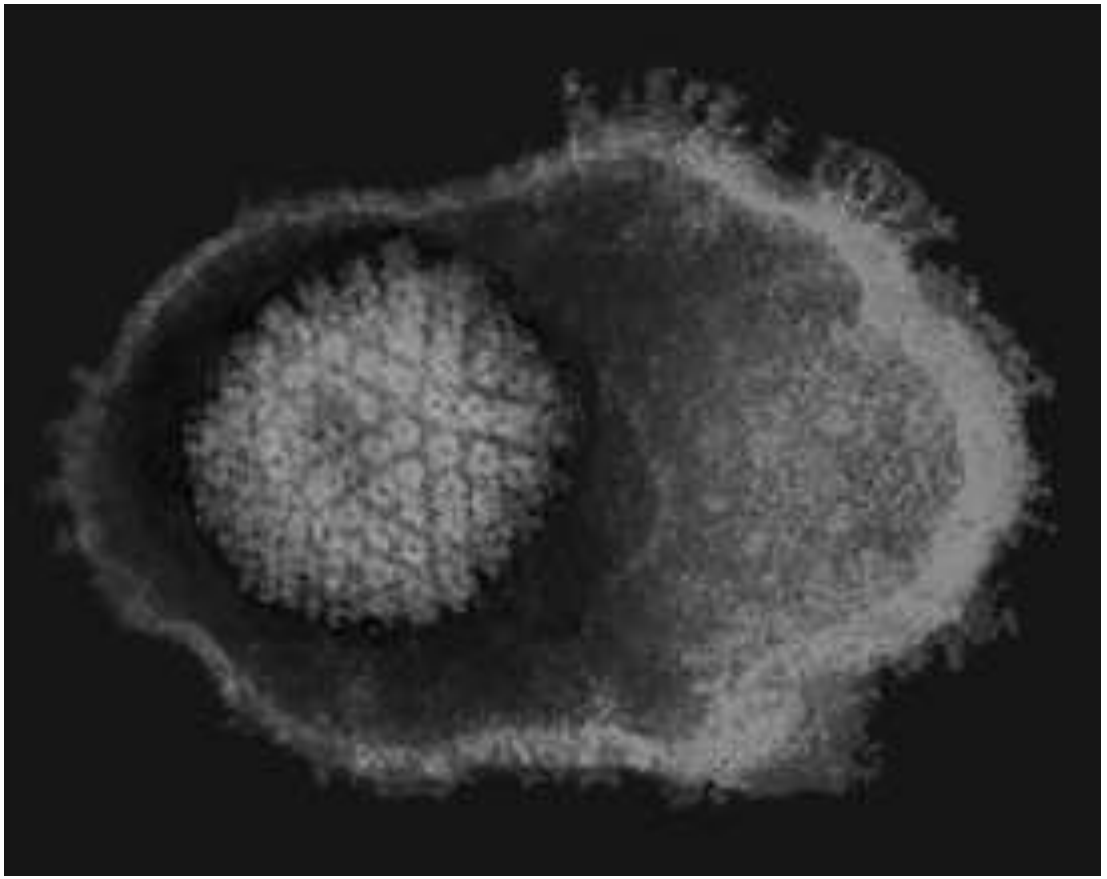
Herpesvirus family (Herpetoviridae): main characteristics

- double-stranded DNA,
- icosahedral capsid contains 162 capsomeres,
- the enveloped virus,
- spikes on the surface of the envelop are probably derived from the inner membrane of the infected cell,
- viral infection causes accumulation of inclusion bodies in the infected cell.

Herpesvirus family: structure of virion



Herpesvirus: electron microscopy photo



Herpesviruses: replication

virus enters the cell by pinocytosis
(or fusion with the cell membrane)
⇓
DNA becomes uncoated and is transported to the nucleus
⇓
final loss of proteins
⇓
replication of viral DNA in the nucleus
⇓
synthesis of viral structural proteins in cytoplasm
⇓
selective transport of viral proteins to the nucleus and assembling with DNA into nucleocapsid
⇓
maturation of virion by budding of nucleocapsid through the inner nuclear membrane
⇓
release of enveloped particles into cytoplasm and leaving the cell by exocytosis

Infections caused by Herpesviruses

Characterised by:

- tropism to many organs,
- persistence of virus in the infected cell (chronic or latent infections with periodical relapses),
- integrative infections,
- strong immune suppression,
- accumulation of immune complexes (allergy),
- transplacental infections (intrawomb and neonatal pathology),
- link with malignant diseases,
- practically all the human population is infected by Herpesviruses.

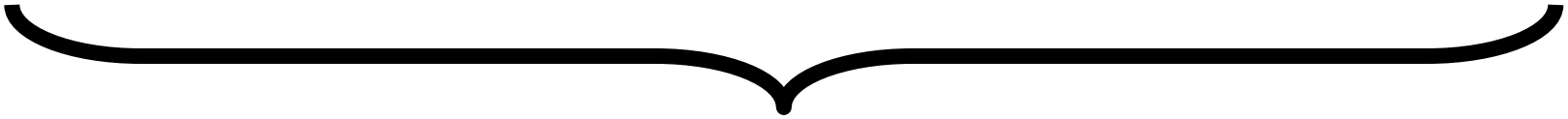
HSV: the role in human disease

HSV-1

- herpes labiales
- keratoconjunctivitis
- encephalitis
- disseminated herpes

HSV-2

- herpes henitales
- mendingoencephalitis
- herpes of newborn



wound herpes

Herpesviruses: laboratory diagnostics of the infections caused by HSV

1. **Rapid diagnostics**
   - Intranuclear acidophilic inclusion bodies – Zank probe

smear-print of vesicles (herpetic lesions)

⇓

Romanovsky - Giemsa stain

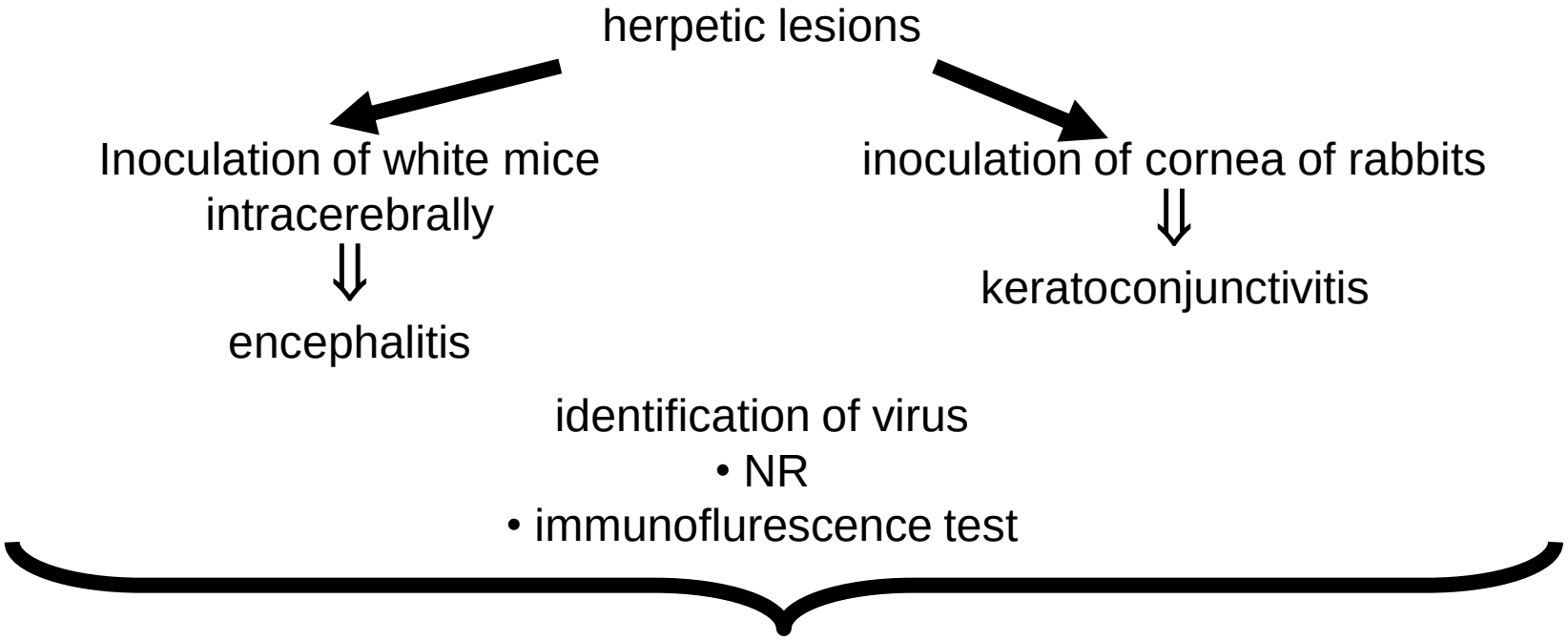
⇓

giant multinucleated cells + nuclear inclusion bodies

   - immunofluorescence test with use of brain biopsy (encephalitis diagnostics)

Herpesviruses: laboratory diagnostics of the infections caused by HSV

2. **Recovery (isolation) of virus**



Herpesviruses: laboratory diagnostics of the infections caused by HSV

3. **Serology**
   – antibodies are measured quantitatively by
     - CF
     - immunofluorescence test
     - ELISA

   Only increase of antibody titre is essential for establishing of a diagnostics, the presence of antibodies in a single sample of serum is of a little value since the majority of adults have antibodies in their blood at all times.

Chickenpox: pathogenesis

Ways of transmission of the infection:

- air-born
- contact

⇓

multiplication in the portals: epithelial cells of mucous membranes of the respiratory tract ⇓

lymphatic vessels

⇓

virus circulates in blood

⇓

replication in the epithelial cells of skin and in mucous membranes

- vesicular eruption of the skin and mucous membranes

disruption of vesicles

⇓

transmission of the virus

transplacental infection by maternal virus

(occurs in the first trimester of pregnancy)

⇓

congenital defects in infants

Herpes Zoster: pathogenesis

chickenpox (acute disease)

⇓

persistence of virus in dorsal nerve roots and sensory ganglia

⇓

activation of the infection (years later)

⇓

flare-up of the virus along posterior root fibres

⇓

zoster vesicles appear on the skin of the body

Cytomegalovirus

- Multiplication of the virus is slow (1 to 2 weeks).
- Virus produces insufficient cytopathic effect upon the infected cell.
- The virus possesses cytotropism to the cells of:
  - salivary glands
  - kidney

} virus causes appearance of big inclusion bodies in the nucleus of the infected cell

Cytomegalovirus: human disease

1. Prenatal infection in children
2. Latent infection in adults

⇓

Could be activated in the next cases:

- pregnancy
- repeating hemotransfusions
- immune deficiency

Epstein-Barr Virus and Nasopharyngeal Carcinoma (NPC)

- The virus is an antigenically distinct member of Herpesvirus family.
- Virus causes proliferation of B-lymphocytes.
- The virus is a causative agent of the next diseases:
  - infectious mononucleosis,
  - lymphoproliferative diseases and lymphomas of CNS,
  - as an oncogenic agent found in ***Burkitt's lymphoma*** (the tumour of the jaw in African children and young adults) and ***nasopharyngeal carcinoma*** (common in males of Chinese origin).

HHV-6: the role in human disease

- It is believed is a causative agent of B-cell lymphoma.
- The virus is found in roseola (false rubella) in children.
- It is possible the virus is a causative agent of the chronic tiredness syndrome.

HHV-8: the role in human disease

HHV-8 – Kaposi's Sarcoma Herpes Virus

- HHV-8 infects lymphocytes and epithelial/endothelial cells and is the causative agent of Kaposi's sarcoma.
- HHV-8 has been found to be associated with oral lesions and neoplasms in HIV-infected patients.

Adenoviruses: classification

Adenoviridae (family).

Genera:

- Aviadenovirus – viruses which infect birds
- Mastadenovirus – viruses which infect mammals:
  - 47 human adenovirus serotypes are classified (IHR)

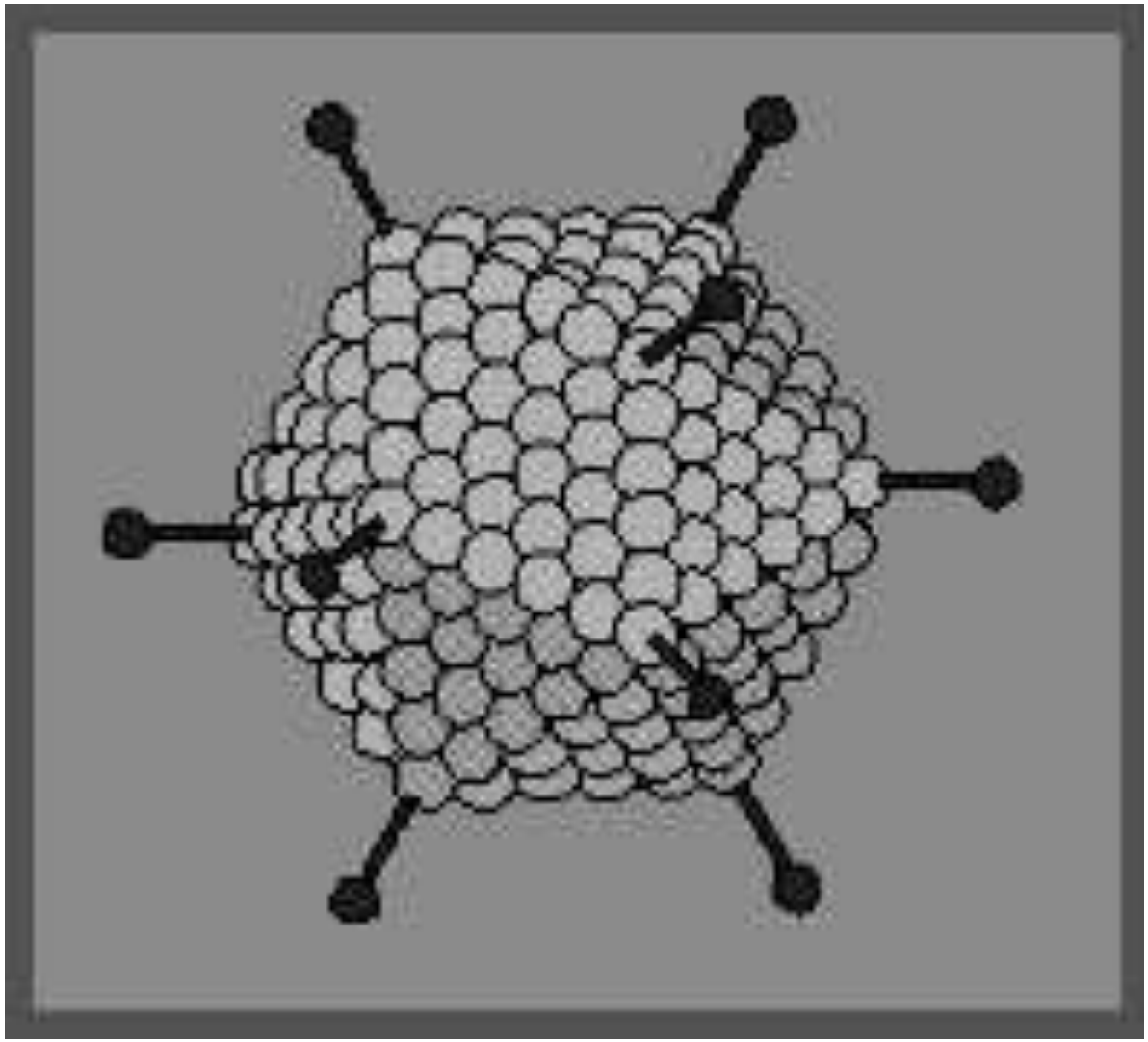
Adenoviruses: main characteristics

- The virion is nonenveloped.
- The icosahedral capsid (70 to 100 nm) is made up of 252 capsomeres.
- A nucleoprotein core contains the linear, double-stranded DNA genome.
- The 12 vertices of the icosahedron are occupied by units called pentons, each of which has a slender projection called a fibre.

Viral antigens:

  - group-specific (nucleocapsid) – revealed in CF
  - type-specific (fibres=hemagglutinins) – IHA test, NR
- Some adenovirus types are oncogenic in newborn rodents (human oncogenesis has not been found but may nevertheless occur).

Model of Adenovirus virion structure



Adenoviruses: multiplication

the virion enters host cells by attaching the fibres to the cytoplasm membrane

⇓

it is engulfed into the cytoplasm in a membrane-bound vesicle (viropexis)

⇓

the viral DNA is gradually uncoated in the cytoplasm and enters the nucleus of the cell where it is finally uncoated

⇓

the viral DNA is transcribed and replicates in the nucleus

⇓

viral mRNA transported into the cytoplasm where it is translated by polysomes into viral proteins

⇓

the proteins return to the nucleus, where new virions self-assemble (the mass of newly synthesized virus particles can assume crystal-like arrangements)

⇓

the virions are released from the nucleus and then from the cell

- result is destruction of the cell

Adenovirus infections: pathogenesis

Infection is usually transmitted in droplets of respiratory or ocular secretions

⇓

multiplication at the portals

- mucous epithelium of
  - respiratory tract
  - intestine
  - conjunctiva
- lymphoid tissue of
  - tonsils
  - mesenterial lymph nodes

entering of the virus into the blood

⇓

endothelial cells of blood vessels

⇓

inflammation of mucous membranes

⇓

fibrinose inflammation, necrosis

Adenovirus infections: clinical forms

- acute respiratory disease,
- pharyngoconjunctivitis with fever,
- pneumonia (children and elderly people),
- keratoconjunctivitis,
- gastroenteritis,
- rarely occurring infections:
  - meningoencephalitis
  - hemorrhagic cystitis

Adenovirus infections: laboratory diagnostics

1. Revealing of Ag in the epithelial cells of the respiratory tract (immunofluorescence) and faeces (immunoelectron microscopy).
2. Recovery of virus:

   throat and nose swabs, pharyngeal washings, conjunctiva secretions, faeces (stool)

   ⇓

   cell culture

   ⇓

   CF

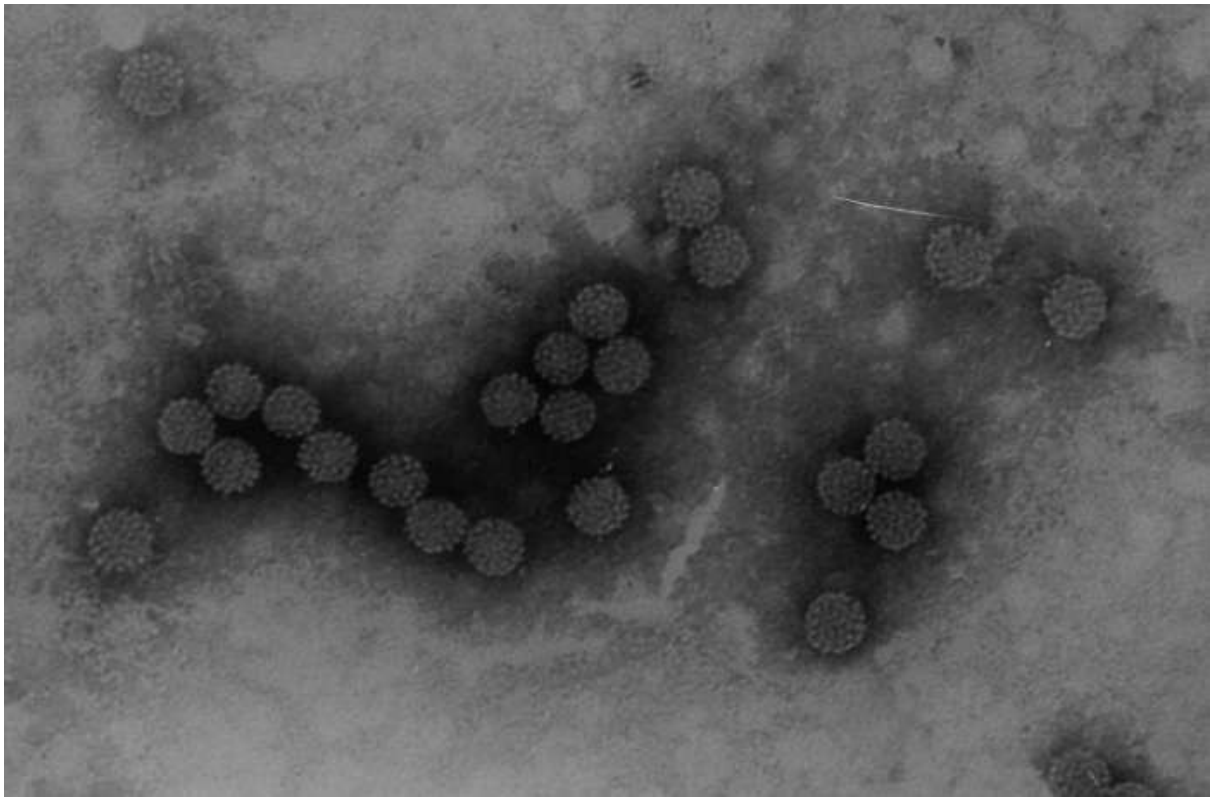
   ⇓

   NR, IHA
3. Revealing of a rise in antibody titres (CF, IHA, NR)

Papilloma- and polyomaviridae: general concepts

- Papilloma and polyomaviruses are small, nonenveloped, icosahedral viruses.
- They contain circular, double-stranded DNA.
- The capsid is made up of 72 capsomeres.
- They are belonging to the two different families: Papillomaviridae and Polyomaviridae:
  - Papillomaviridae
    - associated with a variety of benign papillomatous lesions of the skin and squamous mucosa
    - they also certainly cause human neoplasms (cervical cancer)
  - Polyomaviridae
    - **BK** virus is often activated in renal transplant patients and in others who have received immunosuppressive agents and causes nephropathy.
    - Recently, BK viral DNA has been associated with human prostate cancer.
    - **JC** virus is presumed to cause progressive multifocal leukoencephalopathy,
    - viruses which can induce tumours only by experimental inoculation of certain newborn animals.

Papovaviruses: *electron microscopy photo*



Parvoviruses: their role in human disease

- The viruses are non-enveloped, icosahedral particles.
- Plus and minus DNA strands are packaged into separate virions in approximately equal proportion.
- Capsid is made up of 32 capsomeres.
- They are highly resistant outside the host cell: viral infectivity is resistant to ether, high temperature, chloroform, deoxyribonuclease (DNase) and ribonuclease (RNase) treatment.

Genera:

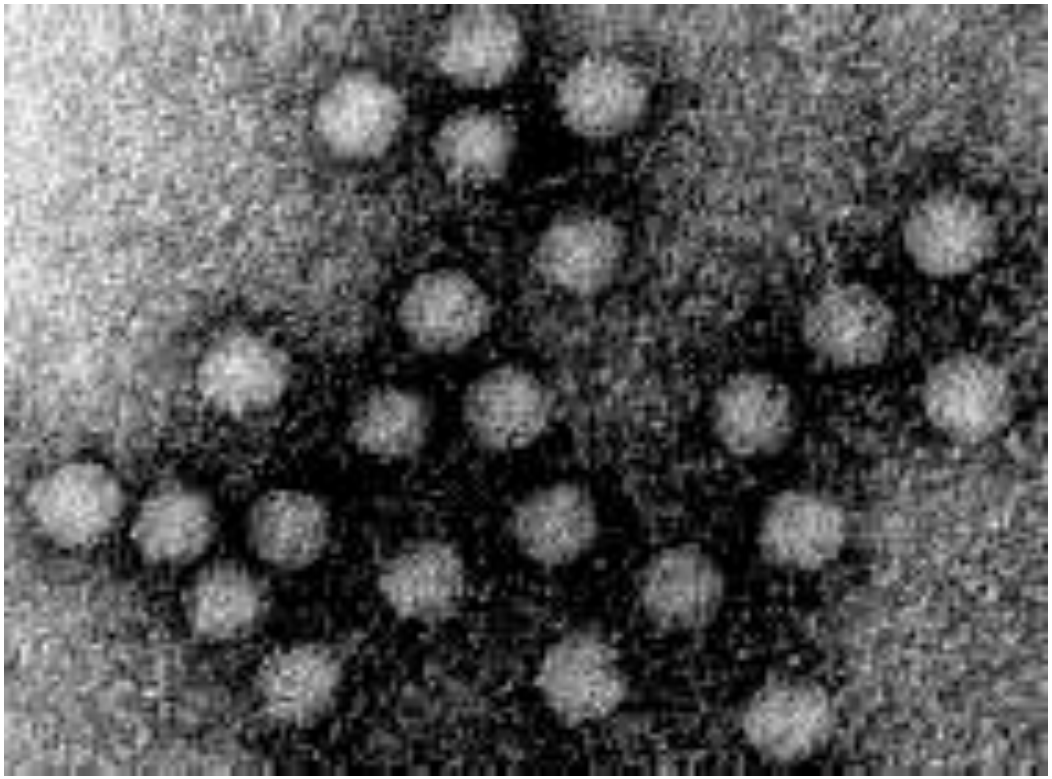
- ***Erythrovirus***

  B-19 virus

  - the virus replicates in erythroid precursor cells and produces aplastic crisis in predisposed individuals with underlying haemolytic anaemia or immunodeficiency;
  - erythema infectiosum is found worldwide; outbreaks occur predominantly in spring, mainly in school children and young adults, with peaks of activity at 4- to 5-year intervals.

- ***Dependovirus*** – defective viruses associated with adenoviruses.

Parvoviruses: *electron microscopy photo*



Hepatitis viruses. Oncogenic viruses. *Theme N35*

Viral hepatitis: classification

Hepatitis	Classification		Epidemiological group
	Family (-viridae)	**Genus (-virus)**	
A	Picorna-	Hepato-	Enteric
B	Hepadna-	**Orthohepadna-**	Parenteral
C	Flavi-	Hepaci-	Parenteral
D	–	–	Parenteral
E	Calici-	Group of hepatitis E –like viruses	Enteric
F	?	?	Enteric?
G	Flavi-	Hepaci-	Parenteral
TTV	**Circino-**	**–**	Parenteral

HAV: morphology of the virion and antigenic structure

Hepatitis A virus (HAV) is spread by the faecal-oral route, person-to-person contact; and under conditions of poor sanitation and overcrowding is the cause of infectious or epidemic hepatitis

- it is a small, unenveloped symmetrical RNA virus which shares many of the characteristics of the picornaviruses' family,
- the antigenic structure is identical to other picornaviruses, but:
  - it is presented by only one serotype

HAV: main characteristics (differences in comparison with other picornaviruses)

- it resistant to unfavourable conditions of surroundings outside the human organism,
- virus is sensitive to formalin and UV-irradiation,
- it is destroyed after 5 min of boiling,
- isolation of virus in tissue culture requires prolonged adaptation and it is, therefore, not suitable for diagnostics,
- it is non-cytopathic when grown in cell culture and practically couldn’t be revealed in cultural liquid.

Hepatitis A: pathogenesis

Hepatitis A virus enters the body by ingestion and intestinal infection

virus multiplies in:

- epithelial cells of small intestine
- regional nodulus

⇓

it spreads by the bloodstream

⇓

it is getting to the liver, a target organ,

⇓

disintegration of hepatocytes (necrosis of parenchymatous cells) is a result of:

  - multiplication of the virus
  - induction of IFN synthesis → activation of NK cells

⇓

- jaundice development (10% of all cases)
- increase elevation of serum aminotransferases

⇓

virus particles are detectable in bile

⇓

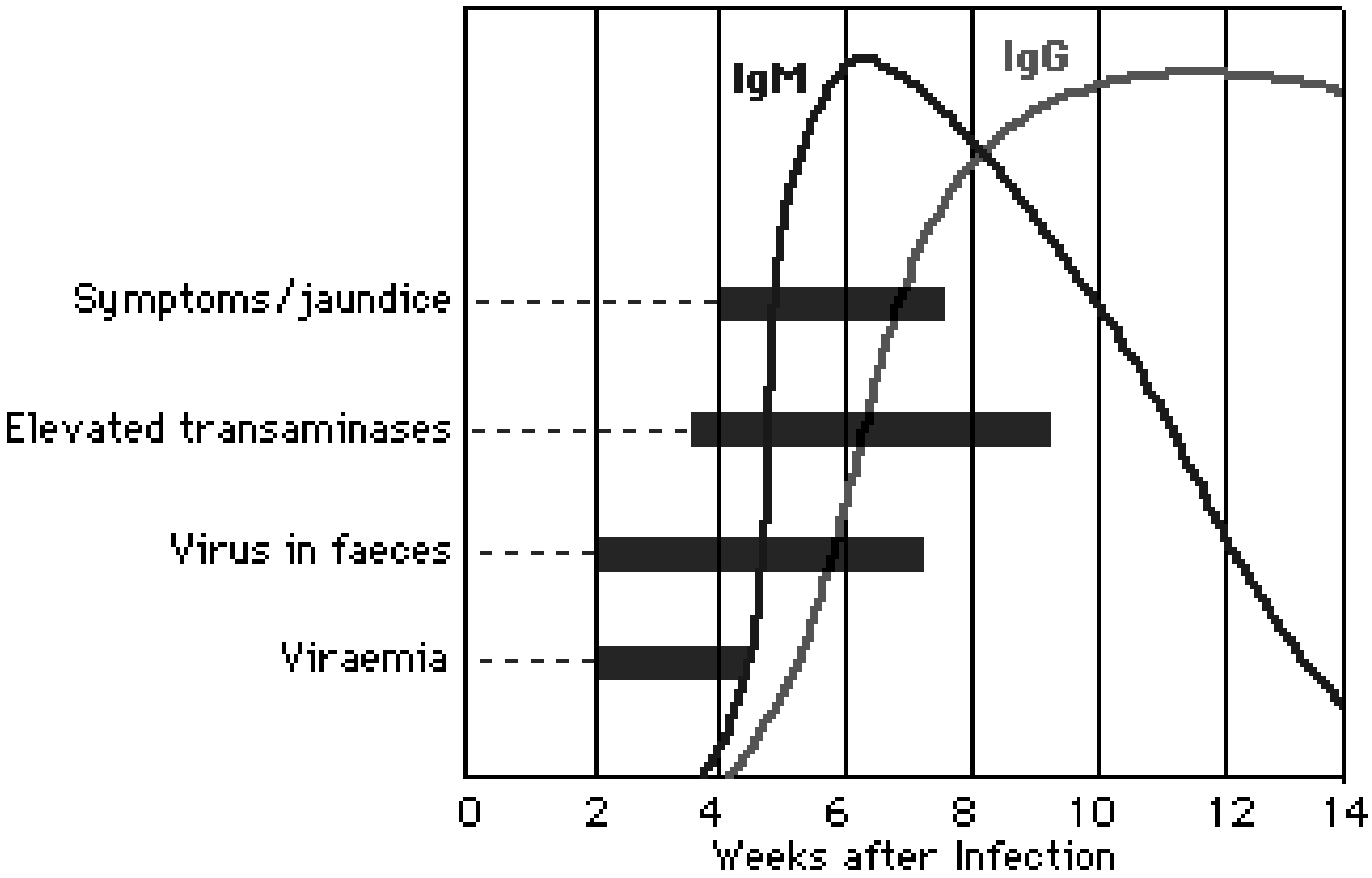
virus particles are found in faeces

Hepatitis A: variants of the outcome of the disease

- complete recovery (usually),
- no evidence of progression to chronic liver disease has been found
- in 8 to 12 weeks after clinical recovery necrotic parenchymatous cells of liver are replaced by new ones.

Hepatitis A: laboratory diagnostics

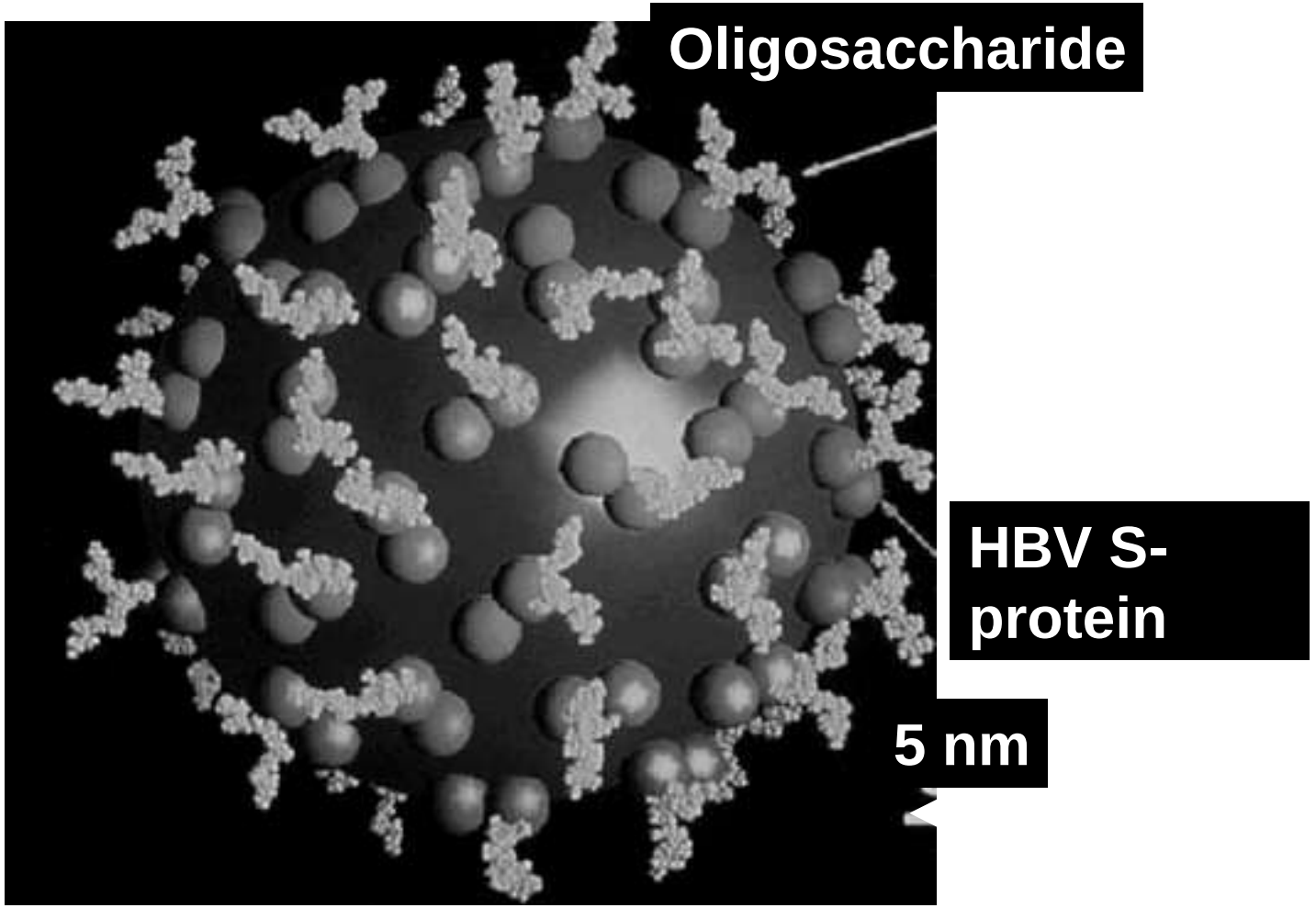
1. Revealing of HAV in faeces (immune electron microscopy).
2. Revealing of HAV genome in feaces, water, food (PCR).
3. Revealing of HAV-Ag in faeces (ELISA, RIA - radioimmunoassay).
4. Revealing of IgM in serum (ELISA, RIA).


IgM
IgG
Symptoms/jaundice
Elevated transaminases
Virus in faeces
Viraemia
0
2
4
6
8
10
12
14
Weeks after Infection

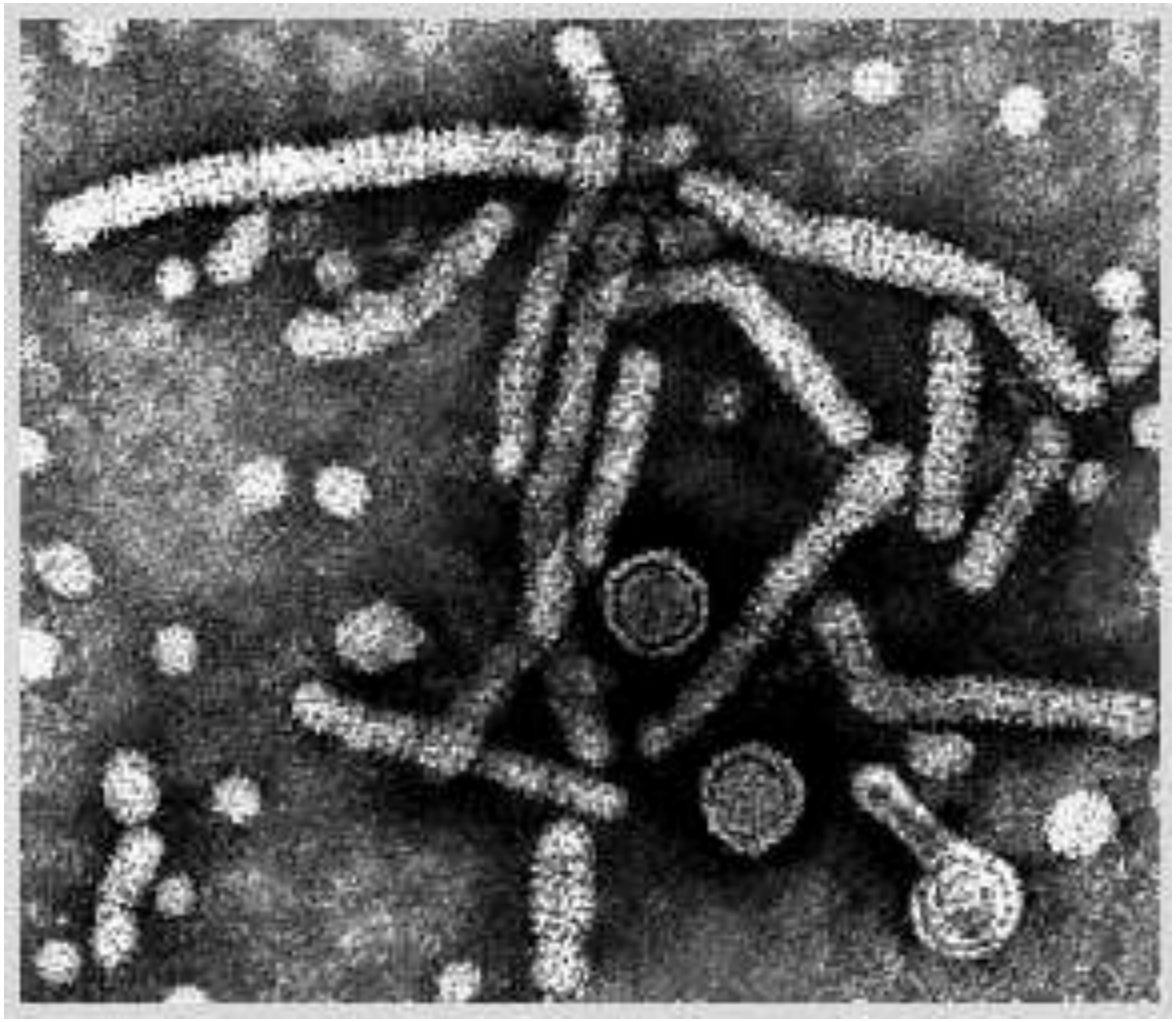
HBV: morphology of the virion

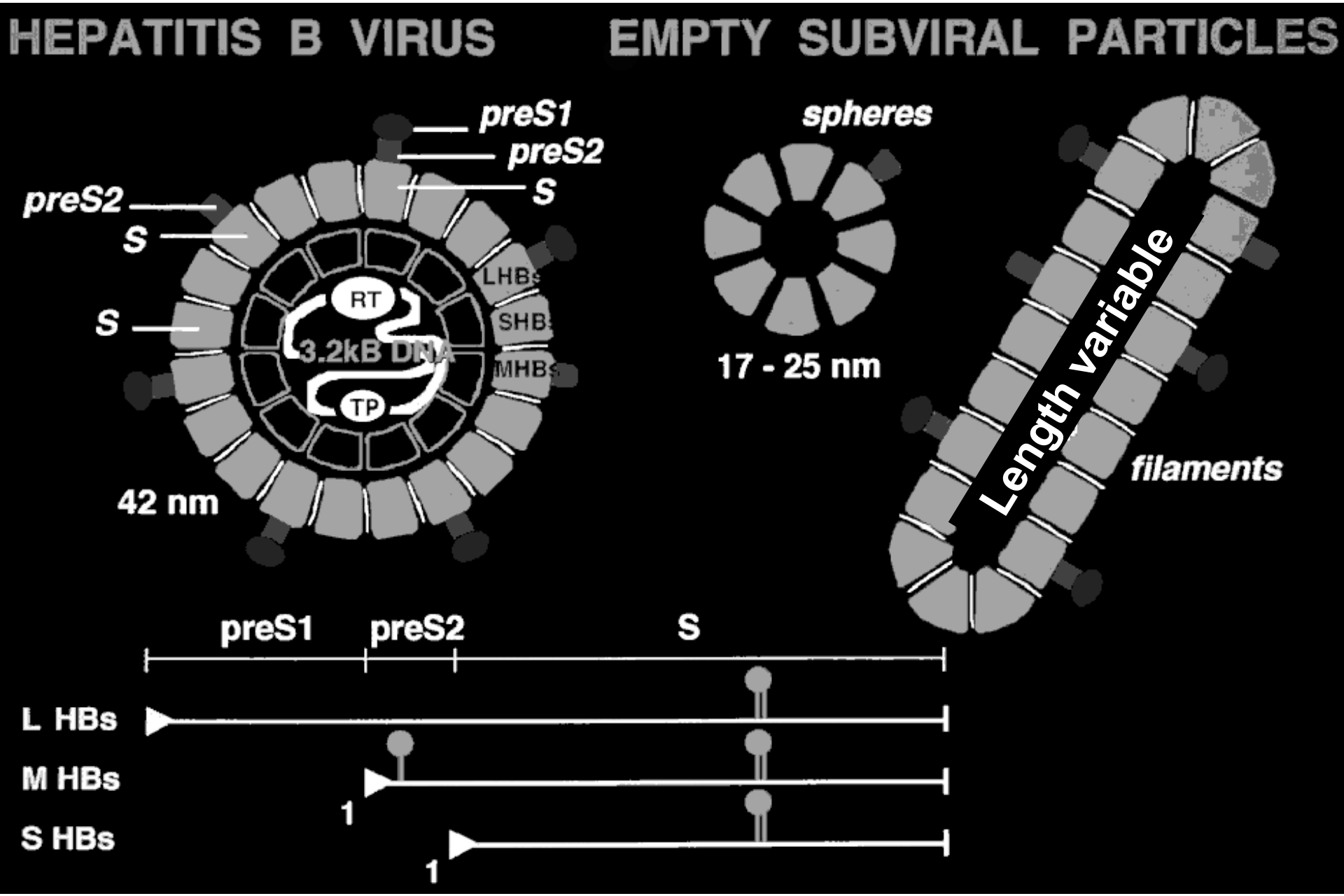
- the hepatitis B virion is a 42-nm particle comprising an electron-dense core (nucleocapsid),
- the core presented by icosahedral symmetry and is surrounded by an outer envelope composed of the surface protein embedded in lipid envelope derived from the host cell,
- the viral genome has an unusual structure and is composed of two linear strands of DNA held in a circular configuration,
- one of the strands is incomplete at the 3' end (about 15 to 60% of the length) and it is associated with a DNA polymerase molecule which is able to complete that strand .

Hepatitis B: virion structure



Hepatitis B: electron microscopy photo

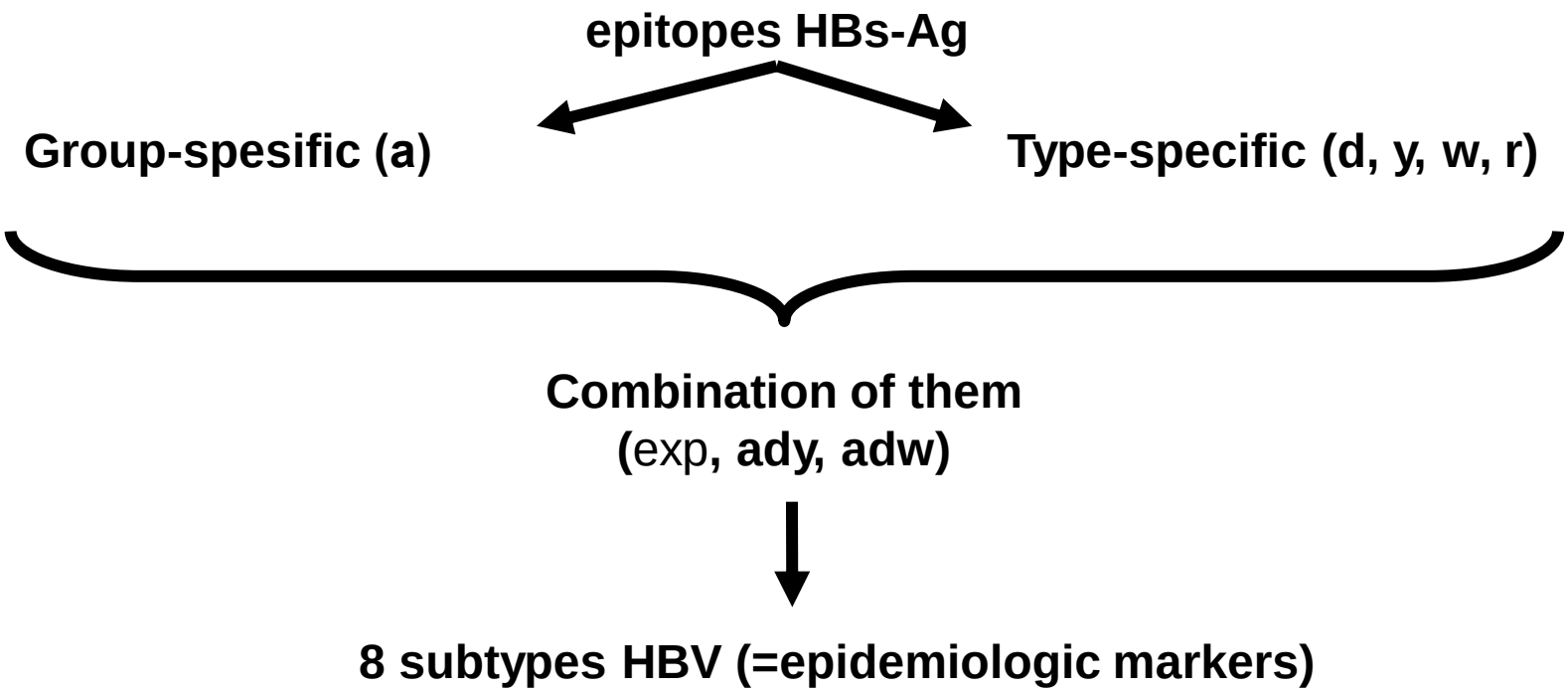


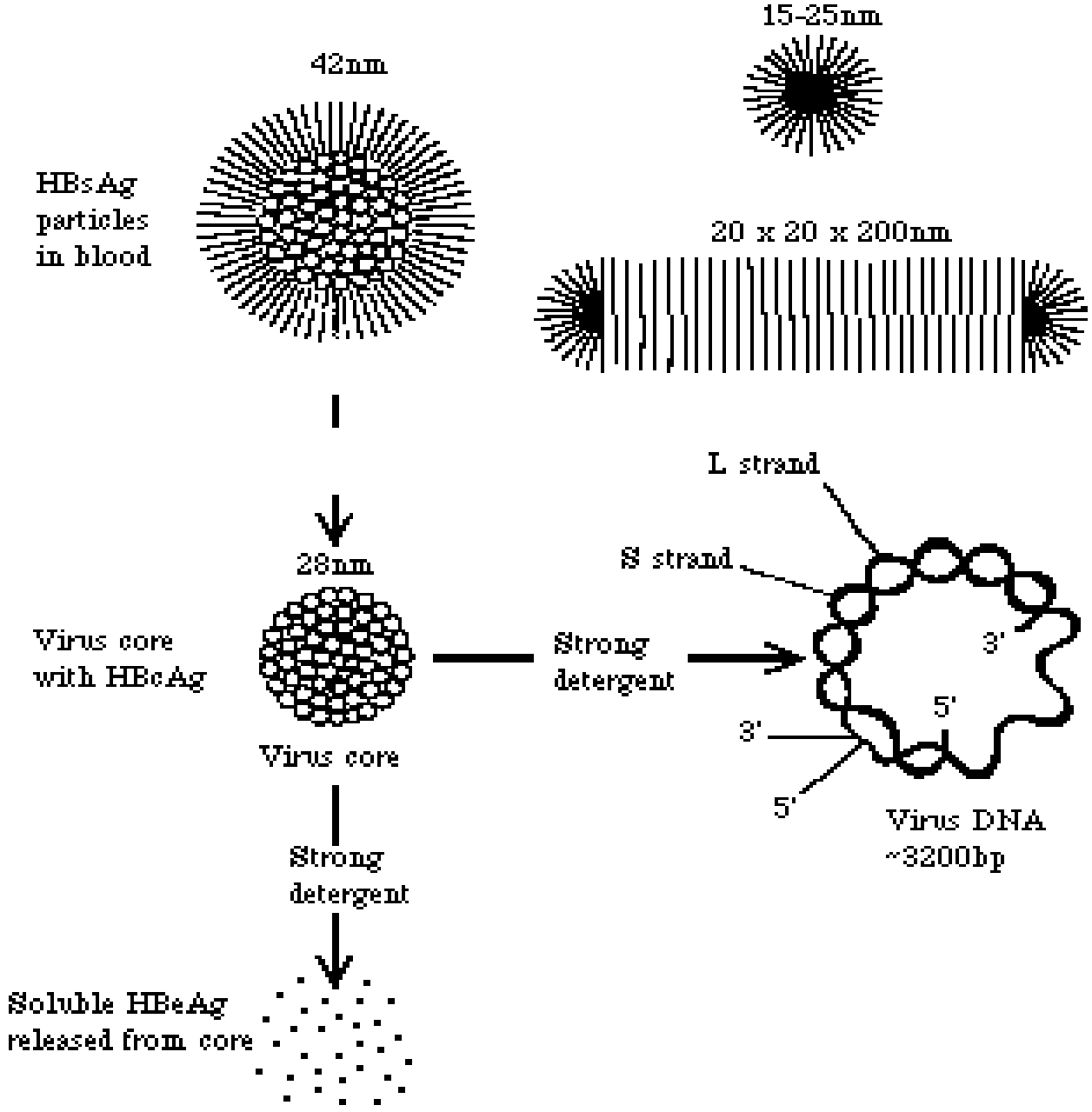

HEPATITIS B VIRUS
EMPTY SUBVIRAL PARTICLES
preS1
preS2
S
preS2
S
S
LHBs
SHBs
MHBs
RT
3.2kB DNA
TP
42 nm
spheres
17 - 25 nm
Length variable
filaments
preS1
preS2
S
L HBs
M HBs
S HBs
1
1

HBV: antigenic structure

Antigen	Localisation in the virion	Chemical composition	Localisation in the human organism	Immuno-genicity
HBs (surface antigen)	envelop	glycoprotein + lipid	membrane of hepatocyte, blood	+
HBc (core antigen)	capsid	nucleo-protein	nucleus of hepatocyte	+
HBe (soluble antigen)	Ag is secreted by infected hepatocytes	protein	blood	+
HBx	Not studied yet. Possibly involved into oncogenic transformation of hepatocytes.			–

Subtypes of HBV




15-25nm
42nm
HBsAg
particles
in blood
20 x 20 x 200nm
28nm
Virus core
with HBcAg
Virus core
Strong
detergent
L strand
S strand
3'
5'
3'
5'
Virus DNA
~3200bp
Strong
detergent
Soluble HBeAg
released from core

Hepatitis B (serum hepatitis): the ways of spreading of the infection

Infectivity is especially related to blood and the epidemiologic propensities of this infection include:

- infection by inadequately sterilized syringes and instruments,
- transmission by unscreened blood transfusion and blood products,
- sexual contact,
- perinatal transmission of hepatitis B infection from mother to child,
- there are more than one million Americans with chronic hepatitis B; up to one quarter of these patients will die of some form of liver disease.

Hepatitis B: pathogenesis

the place of the first virus multiplication is unknown

⇓

virus is found in hepatocytes (about 2 weeks after infection)

⇓

multiplication of the virus in hepatocytes

⇓

expression of virus antigens **(HBsAg)** on the membrane of the infected hepatocytes

⇓

antibody and cell-mediated (CTL) immune responses cause damaging effect on hepatocytes by direct cytopathic changes

⇓

complications of the disease:

- liver cirrhosis (up to a quarter of patients within five years)
- liver carcinoma (up to one quarter of patients with liver cirrhosis develop hepatocellular carcinoma)

Hepatitis B: different forms of pathogenesis

The character of pathogenesis is dependent on:

- the character of development of the infection\interaction of HBV with hepatocytes (active or integrative infection),
- the character of the immune response,
- immunopathological processes,
- cytopathic effect of HBV on T-helper cells,
- cytopathic effect of HBV on macrophages.

clinical forms of the disease:

- acute
- subacute
- chronic
- persistent
- fulminant (massive hepatocellular necrosis could be seen in 1-3% of patients)

Hepatitis B: laboratory diagnostics

1. Presence of virus antigens (tissue biopsy, serum) – ELISA, RIA, passive HA.
2. Presence of Ig specific to virus antigens (acute infection – by the presence of IgM against HBcAg in serum) – ELISA, RIA and passive HA.
3. Presence of virus DNA (in blood plasma) – PCR.

TABLE 70-2 Interpretation of Results of Serologic Tests for Hepatitis B

			Anti-HBc			
HBsAg	HBeAg	Anti-HBe	IgM	IgG	Anti-HBs	Interpretation
+	+	−	−	−	−	Incubation period
+	+	−	+	+	−	Acute hepatitis B or persistent carrier state
+	+	−	−	+	−	Persistent carrier state
+	−	+	+/−	+	−	Persistent carrier state
−	−	+	+/−	+	+	Convalescence
−	−	−	−	+	+	Recovery
−	−	−	+	−	−	Infection with hepatitis B virus without detectable HBsAg
−	−	−	−	+	−	Recovery with loss of detectable anti-HBs
−	−	−	−	−	+	Immunization without infection, repeated exposure to antigen without infection, or recovery from infection with loss of detectable anti-HBc

Hepatitis B: specific prophylaxis

Immunization against hepatitis B is now recognized as a high priority in preventive medicine in all countries.

As a result of the currently available excellent vaccine, the number of acute hepatitis B infections has been falling.

Strategy for immunization is universal vaccination of infants and adolescents to control the transmission of this infection.

1. Vaccine:
   - recombinant vaccines produced by the expression of HBsAg in yeast cells,
   - administration of a course of vaccine with the first dose immediately after birth,
   - helps to control hepatitis D
2. Specific immunoglobulin.

Hepatitis B: nonspecific prophylaxis

- To use of disposable needles and syringes
- To autoclave medical instruments between use.
- To control donor blood to prevent post-transfusional hepatitis.
- To use condoms to prevent sexual way of transmission of the disease.

Distinctive properties of HCV

- Hepatitis C virus (HCV), is an enveloped single-stranded RNA virus.
- It appears to be distantly related (possibly in its evolution) to flaviviruses, although hepatitis C is not transmitted by arthropod vectors.
- Worldwide, there are approximately 200 million HCV carriers.

Hepatitis C: main characteristics of the disease

- The disease is asymptomatic in many cases.
- HCV enters the bloodstream and infects hepatocytes. The virus usually does not kill the host cell and thus can set up a persistent infection leading to chronic infection.
- Hepatitis C virus (HCV) is associated with chronic liver disease.
- The disease is associated with:
  - cirrhosis
  - primary liver cancer

Hepatitis D: main characteristics of the virus

- defective hepatitis delta virus (HDV),
- it is small (36 нм), single-stranded RNA virus,
- it cannot code for its own surface protein and to produce more virus particles it needs a helper virus: the helper is HBV,
- HDV is coated with HBsAg which is needed for release from the host hepatocyte and for entry in the next round of infection,
- HDV itself seems to be cytopathic and HDV-Ag may be directly cytotoxic: produces direct cytotopathic effect to hepatocytes.
- HDV is usually transmitted via similar means to HBV.

Hepatitis D (delta hepatitis): main characteristics of the disease

Two forms of delta hepatitis infection are known:

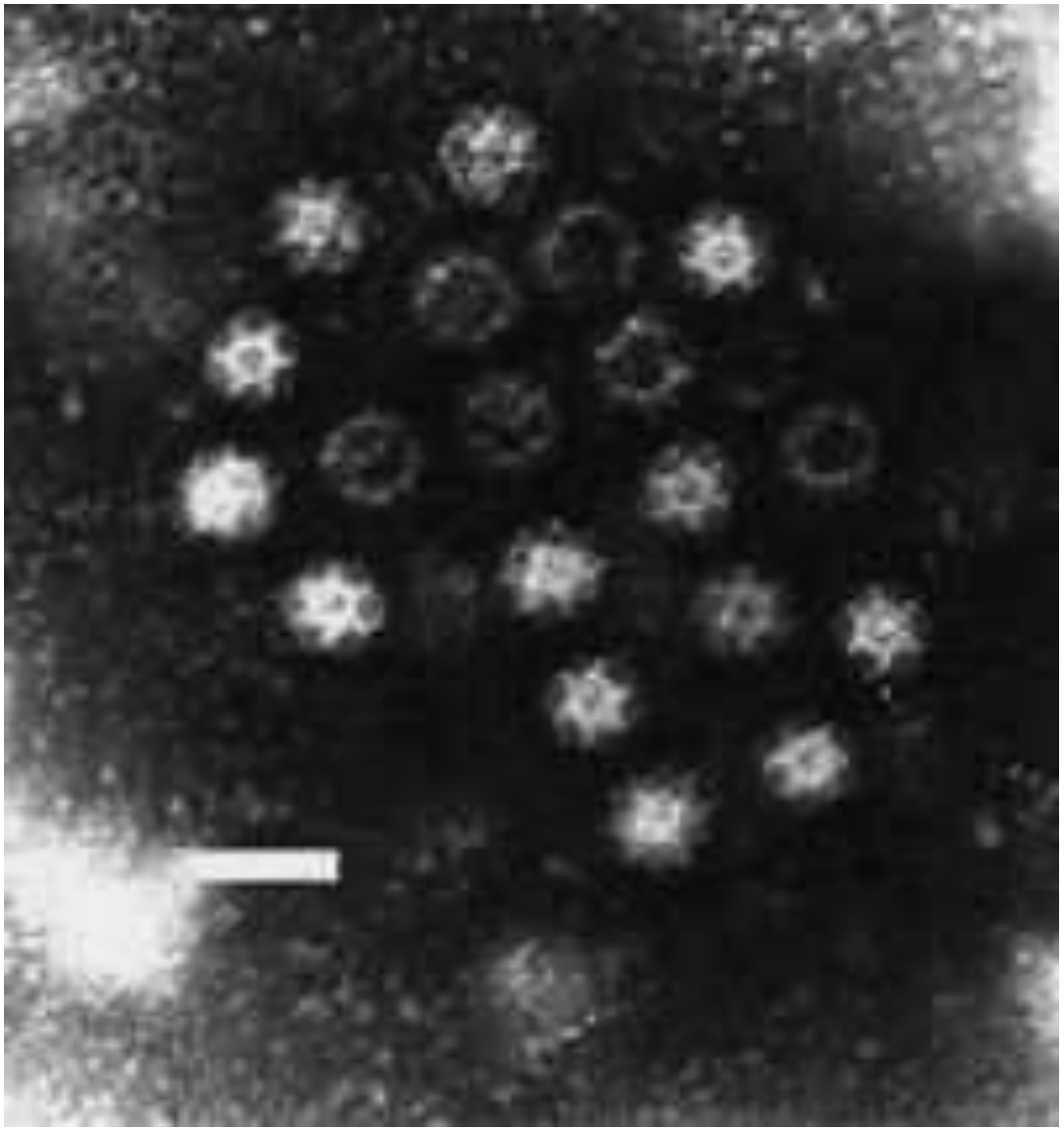
- in the first, a susceptible individual is co-infected with HBV and HDV, often leading to a more severe form of acute hepatitis caused by HBV;
- the second, an individual chronically infected with HBV becomes super-infected with HDV: this event may cause a second episode of clinical hepatitis and accelerate the course of the chronic liver disease.

Hepatitis E: main characteristics of the virus

- hepatitis E virus (HEV), the cause of enterically-transmitted non-A, non-B hepatitis;
- it is non-enveloped, single-stranded RNA virus, which shares many biophysical and biochemical features with caliciviruses.

Hepatitis E: main characteristics of the disease

- the infection is enterically transmitted, mainly with contaminated water;
- outbreaks involving tens of thousands of cases of acute hepatitis have been documented in the former USSR, Southeast Asia, Northern Africa, Mexico and previously in India.
- the average incubation period is slightly longer than for hepatitis A,
- the disease is mostly not severe but the highest attack rates are found in some young adults,
- high mortality rates of up to 20% have been reported in women during pregnancy (especially in the third trimester).



Hepatitis F

- practically it is not studied yet,
- it is known that the HFV infection is probably transmitted by faecal-oral route.

Hepatitis G: main characteristics of the virus

- HGV is a flavivirus ,
- defective virus (doesn’t encode the synthesis of own capsid proteins),
- to form the capsid virus uses proteins of unknown viruses or cellular proteins,
- HGV can produce mild acute infection and most infected people then go on to a persistent infection,
- transmission is via blood contamination such as transfusion or intravenous drug use,
- diagnostics is by reveling of antiviral antibodies or the detection of viral RNA by PCR.

TTV

- TTV - transfusion transmitted virus.
- The cases of TTV infection are high among HIV-infected patients.
- The main risk to be infected is parenteral risk of exposure: a higher prevalence of TTV infection observed among patients with hemophilia.
- TTV is also transmitted through sexual routes.

Laboratory diagnostics:

- Revealing of virus DNA – in PCR.
- Revealing of specific antibodies.

Oncogenic viruses. Slow infections.

Modern theory of oncogenic transformation: proto-oncogenes

c-oncs or proto-oncogenes are cellular genes which fulfill normal functions

⇓

governing signal transduction, cell proliferation, differentiation and synthesis of *protein kinase* (enzyme that catalyzes phosphorylation of amino acids)

⇓

all this processes involved into the cell growth and differentiation

Modern theory of oncogenic transformation: the mechanisms of viral cancerogenesis

Highly oncogenic viruses are defective: a portion of viral genome is replaced with host DNA (onc-genes):

v-onc+ viruses

(contain the transduced cellular proto-oncogene within the viral genome)

⇓

insertion of the virus into the genome of the cell

⇓

introduction of viral onc-gene into the cellular genome

⇓

expression of the viral onc-gene in the cell

Non-defective: don't contain cellular proto-oncogene

v-onc⁻viruses

⇓

insertion of the virus into the genome of the cell

⇓

use of virus promoter by the cellular genes

⇓

change of the expression of cellular genome without integration of v-onc-genes

⇓

disturbances in proliferation and differentiation of the host cell

Modern theory of oncogenic transformation: results of the transformation

Results of the transformation:

- disturbance of the protein kinase activity
- loss of the specificity of protein kinase
- functional membrane changes: loss of large molecular weight glycoproteins from the cell surface
- lack of the contact inhibition between cells

⇓

disregulation of the growth and differentiation of the cells

⇓

transformation of the normal cell into tumour cell

Modern theory of oncogenic transformation: future of the tumour cell

- In normal state – the tumour cell should be destroyed by immune response of human immune system.
- In conditions of immune deficiency – the tumour cell stays live and process of tumour growth is started.

Modern theory of oncogenic transformation: factors which promote oncogenic transformation of the cells

1. Amplification (activation) of the cell gene by virus promoter.
2. Mutations (as a result of the action of physical and chemical mutagens).
3. Integration of the cell gene into the virus genome (formation of v-onc+ viruses)

Oncogenic viruses: classification

DNA-viruses

- Papillomaviridae
- Polyomaviridae
- Herpesviridae
- Adenoviridae
- Poxviridae
- Hepadnaviridae

RNA-viruses

- Retroviridae
  - Alpharetrovirus
  - Betaretrovirus
  - Gammaretrovirus
  - Deltaretrovirus
  - Epsilonretrovirus

Retrotransposons

RNA of oncornaviruses

⇓

entering the host cell

⇓

synthesis of DNA-transcript

⇓

integration of the transcript into the cellular genome

⇓

change of the localisation in the genome (=transposition)

Slow infections. Clinical Microbiology

Theme N32

Slow infections: main characteristics

1. They have a prolonged incubation period lasting months or years.
2. The infections characterised by slow and irreversible progress in the course of the disease.
3. The results are unusual - damage found in tissues and organs.
4. The diseases usually cause irreversible degenerative changes of CNS.
5. Lethal outcome is usually inevitable.

Slow infections: scrapie

A disease of sheep which were imported to Iceland

many years after their arrival their disease has been discovered by

damage in lungs damage in CNS

The disease was characterized by

- a prolonged incubation period
- slow but constant (<u>without remissions</u>) increase in the appearance of the symptoms
- lethal outcome

Disease has been classified as *«slow infection»* (Burn Sygurdson, 1954)

Slow infections: history of scrapie

- The first reports about disease – mid XVIII century.
- The reports about transmission of the infection with brain tissue – the end of XIX century.

Slow infections: kuru

1957– New Guinea, Papua Gajdusek and Zigas discovered disease **kuru** in aborigines living on the islands.

Many similarities with scrapie were found:

- clinical symptoms (disturbances of coordination)
- typical damage found in organs (involved only CNS)

↓

Conclusion: *slow infections are typical for humans*

Slow viral infections: first described cases in humans and animals

- 1960– **visna** (progressive pneumonia of sheep) infectious agent is oncornavirus from subfamily Lentivirinae
- SSPE –**subacute sclerosing panencephalities** (known from 1933) – pathogen is variant of measles virus

Numerous viruses

normally cause acute diseases

under curtain conditions they can produce slow infections

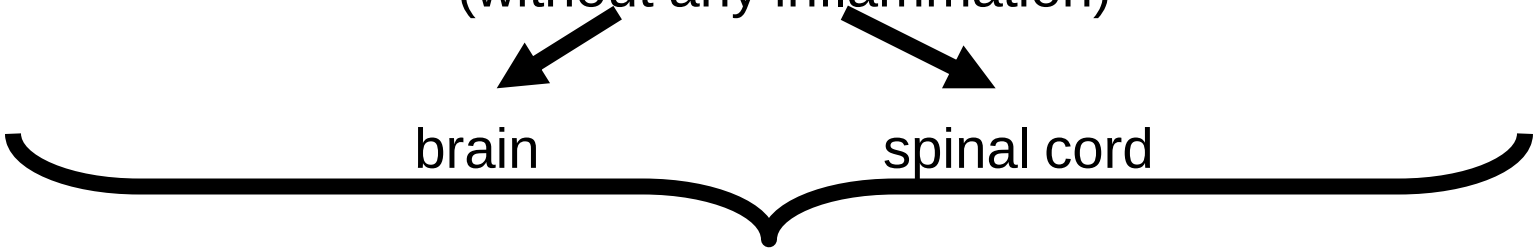
Slow viral infections: viruses which can produce disease in humnas

- Measles virus - subacute sclerosing panencephalities (SSPE)
- Rubella virus
- Herpes virus
- Tick-bourn encephalitis virus
- JC virus: progressive multifocal leukoencephalopathy (PML)
- Old World Arena viruses - lymphocytic choriomeningitis (LCM)
- Rabies virus
- Influenza virus
- AIDS
- others

Slow infections caused by prions (prions disease)

Primary degenerative changes of the tissue of

(without any inflammation)



progressive vacuolations and damage of the cell bodies of neurons, astrocytes and oligodendrocytes

extensive astroglial, proliferation and spongiform change = **status spongiosis of grey matter**

accompanied by:

- presence of amyloidal plaques in the brain,
- extensive astroglial hypertrophy and proliferation.

Transmissive spongiform encephalopathy (TSE)

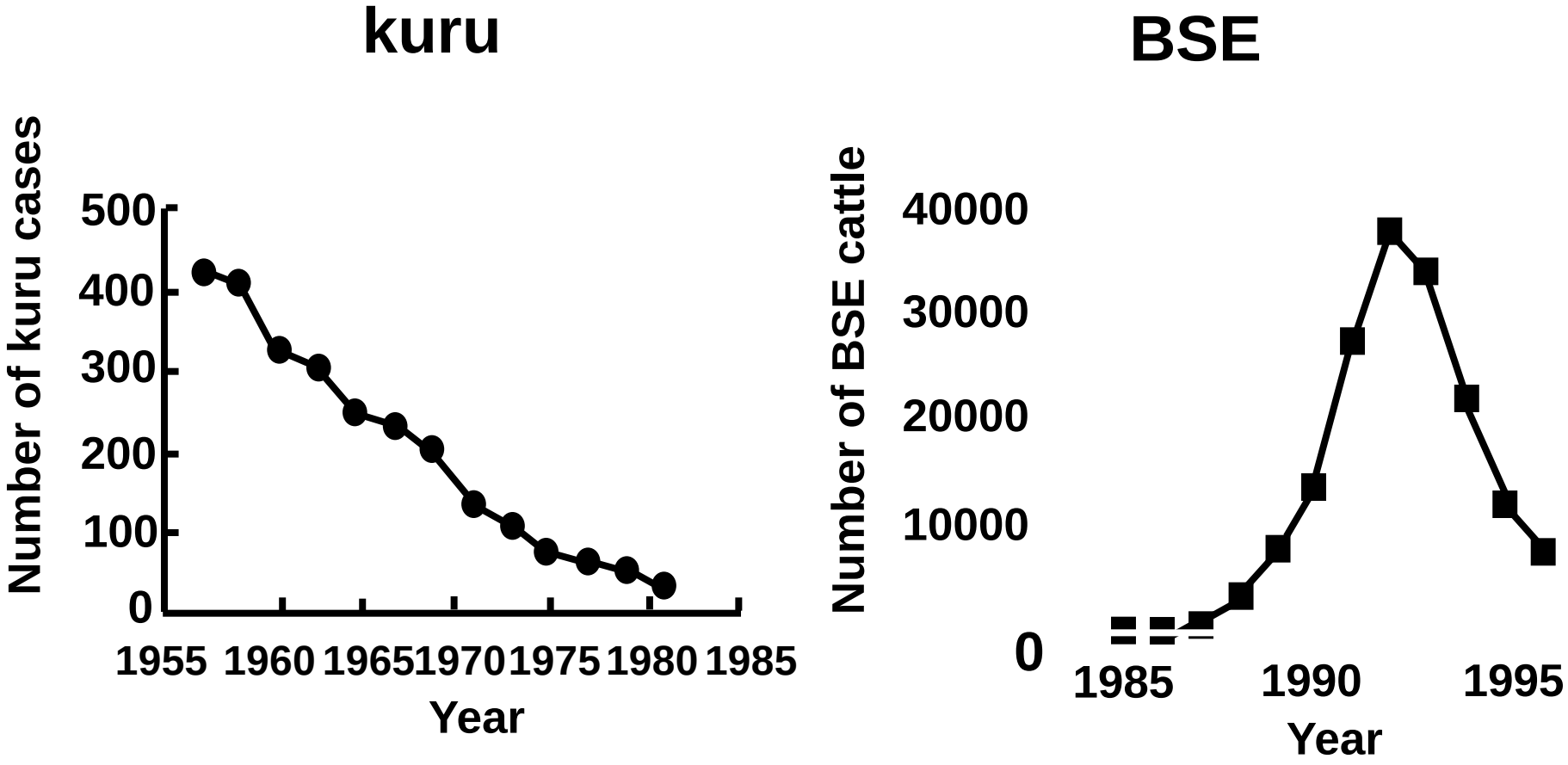
In general, the pathology is CNS disease which is characterised as:

- noninflammatory,
- transmissible,

cerebral amyloidosis including:

- **status spongiosis -** slowly progressive vacuolation of neurons of the grey matter and astrocytes,
- **astrocytic gliosis**,
- neuronal dropout,
- periodic acid-Schiff-positive amyloidal plaques appearance.

Disappearance of kuru and the BSE epidemics



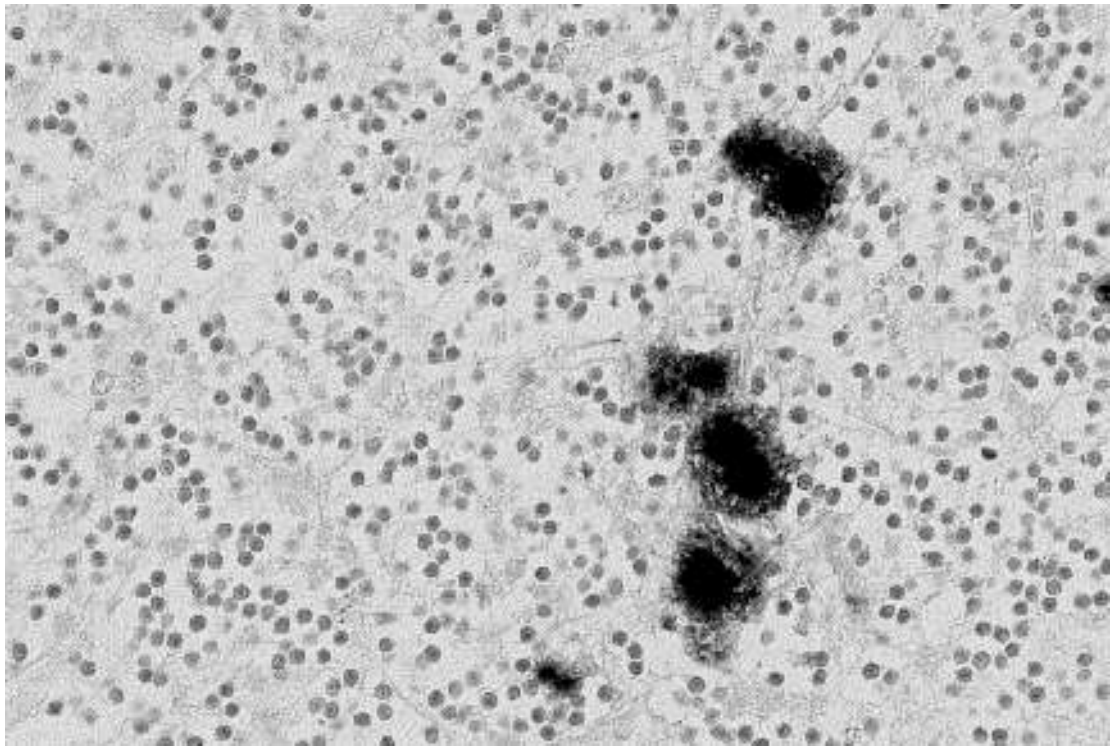
Prion diseases: main characteristics

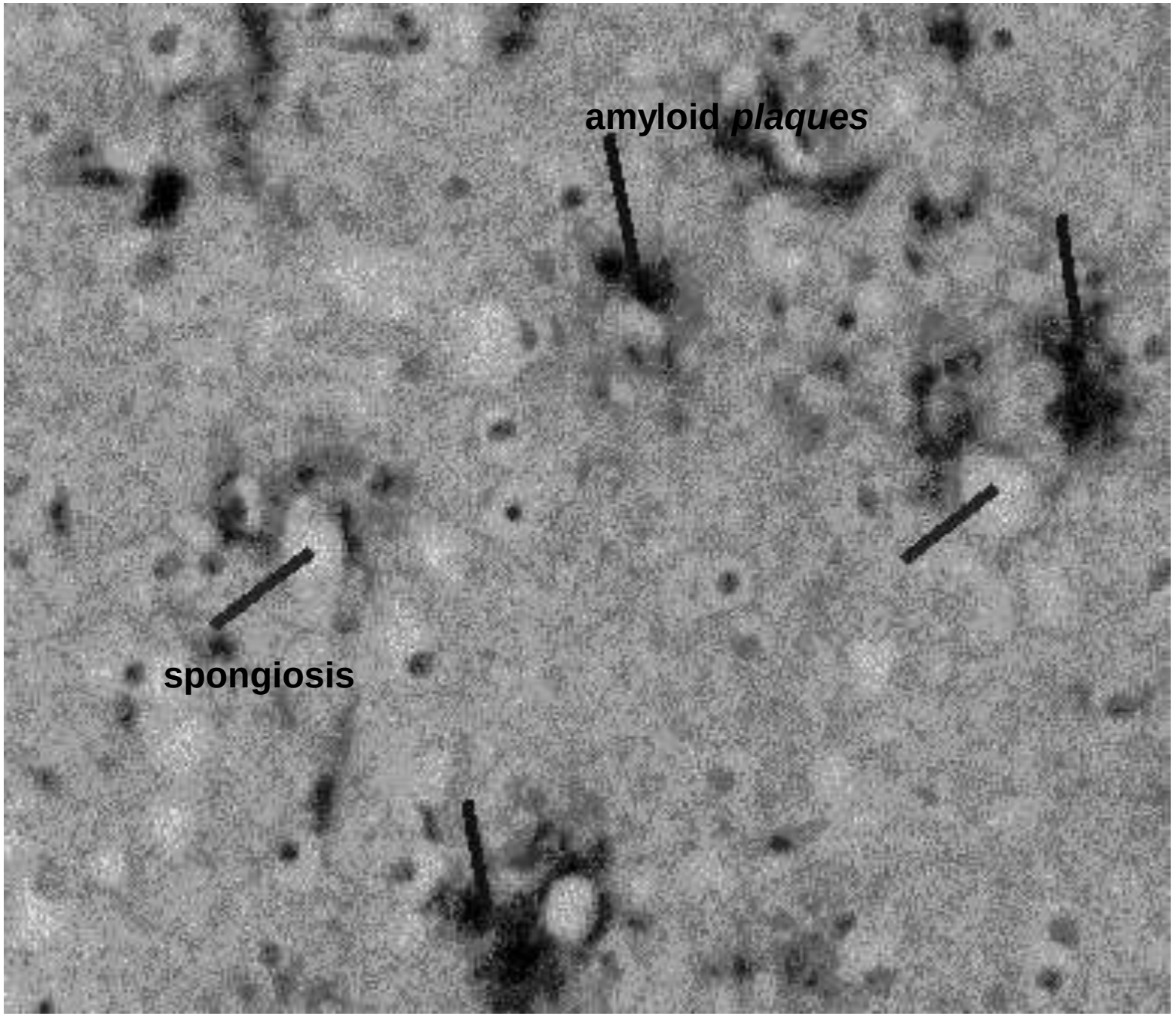
1. No detectable antibodies or cell-mediated immunity found in prion disease.
2. Amyloid plaques appearance in brain tissue.
3. Generalised hypertrophy of astrocytes.
4. Pronounced spongioform degeneration.

The typical clinical symptoms of progressive degenerative changes of the central nervous system in human prion disease

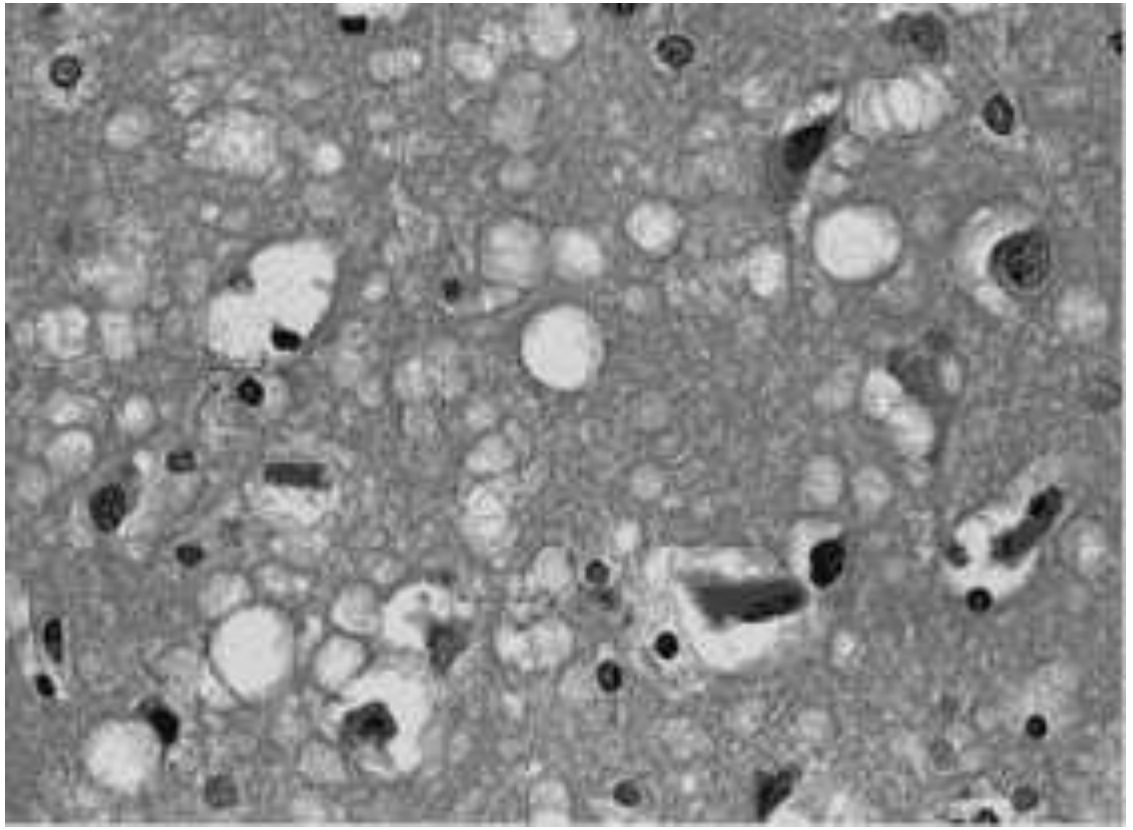
1. Disturbances in sensor activity
   - loss or change of sensitivity
   - loosing of some senses
   - amnesia
2. Motor dysfunction
   - ataxia
   - adynamia
   - atrophy of muscle activity (including the muscles of the respiratory tract)
3. Psychological dysfunction
   - depression
   - sleepiness
   - aggressiveness
   - dementia

Creutzfeldt-Jakob disease (C-J). Amyloid plaques (with antibodies to PrP-sc.) X 400.

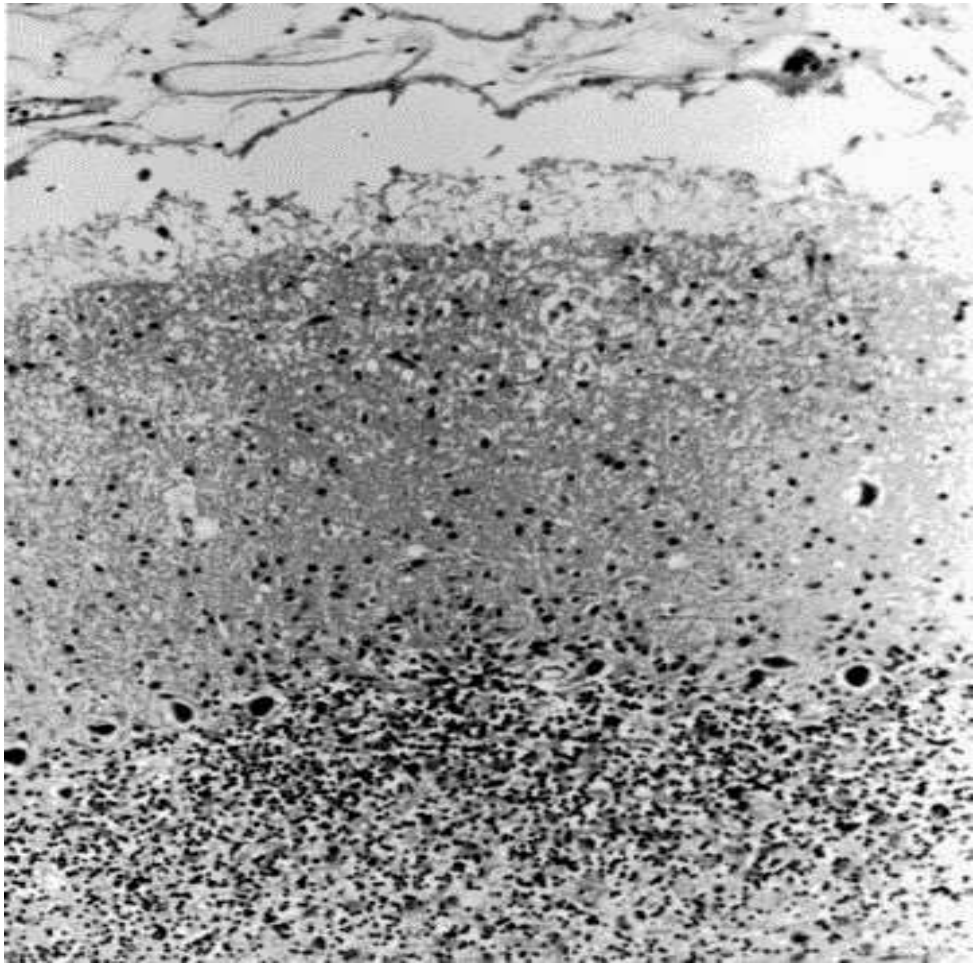



amyloid *plaques*
spongiosis

Creutzfeldt-Jakob disease: spongiosis in brain.



Creutzfeldt-Jakob disease: spongiosis and gliosis in brain.



Prusiner's discovery of prions: experimental model

1982

sheep infected with scrapie

↓

isolation of the brain

↓

inoculation of hamsters

↓

accumulation of the brain of the infected animals

↓

isolation of low molecular weight protein (uncontaminated with nucleic acid)

Photo : University of California, San Francisco

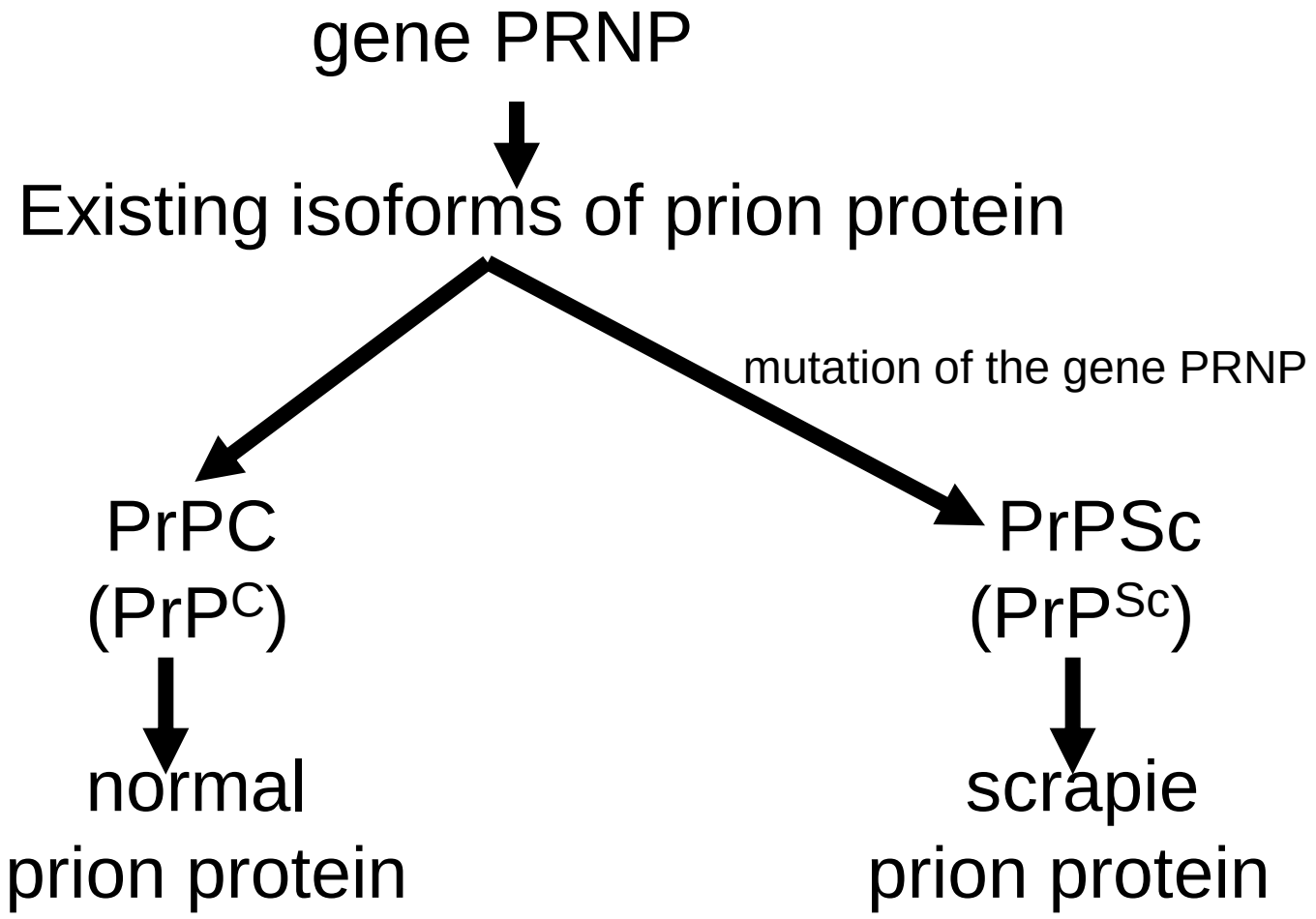
Stanley Prusiner, Prix Nobel 1997

Stanley Prusiner visiting the Oxford University

Prions: the meaning of the term

prion = proteinaceous infectious (particle)

Prions: two isoforms



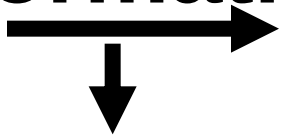
PrPC
(Prion Protein of Cell)

- Normal cellular protein
- Found in all mammals
- The protein is encoded in 20th chromosome
- It is mostly expressed in neurons, glial cells and in some other tissues
- It participates in:
  1. transmission of nervous impulses
  2. regulation of the daily rhythms

PrPSc
(scrapie infectious prion protein)

- Infectious form of prion (first found in sheep infected with scrapie).
- Isolated from humans and animals having prion diseases.

The mechanism of PrPC transformation to PrPSc



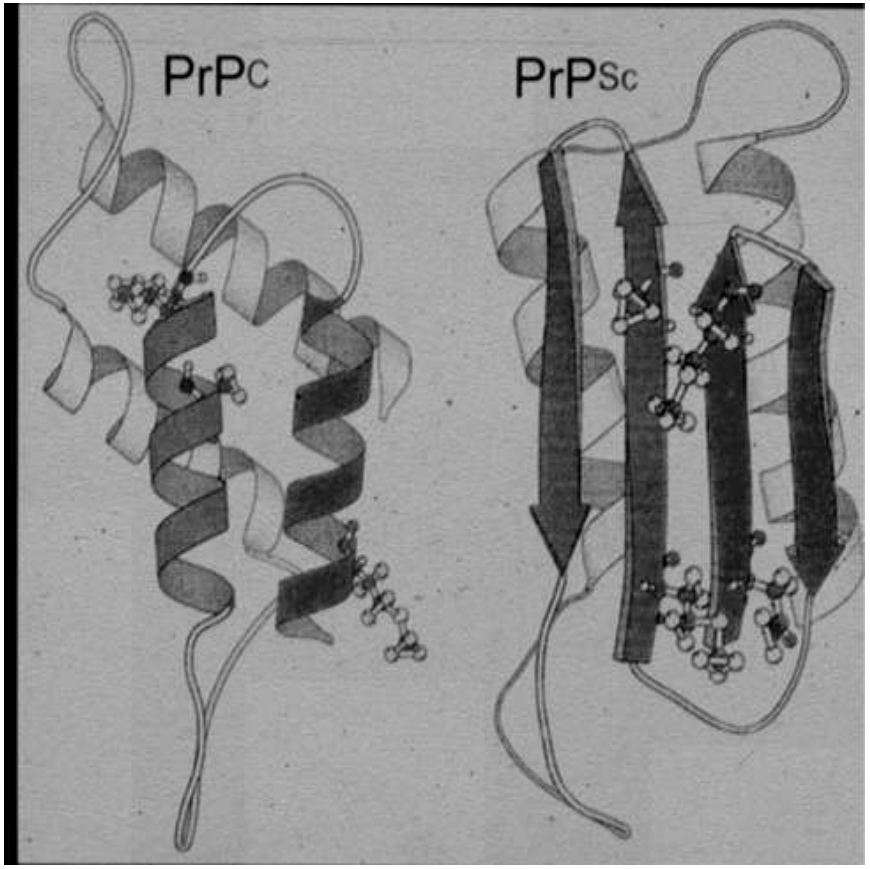
the transformation occurs in posttranslational stage
(DNA⇒**m** RNA⇒protein⇒infectious protein)

the reason of the transformation is
significant conformational rearrangement of normal prion protein molecules

modification of the host prion protein into *insoluble amyloid fibrils*

the fibrils appear as rods revealed by electron microscopy
or could be seen as crystalline deposits in the form of extracellular amyloid plaques by applying light microscopy

Proposed conformational differences between PrP^{c} and PrP^{Sc}

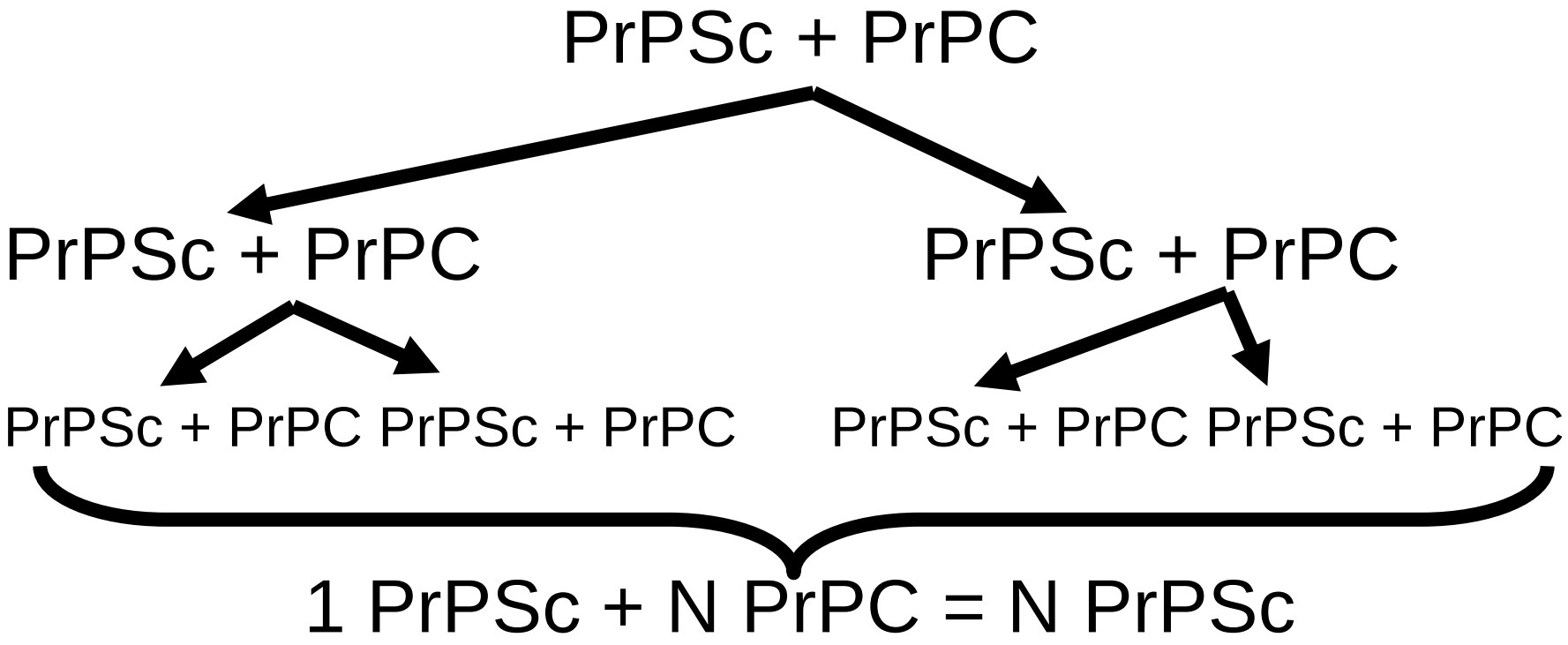


The principal differences between PrSc and normal isoform (PrPC)

- infectiveness
- unique physical and chemical properties of the PrPsc:

1. resistant to high temperature (even to boiling)
2. resistant to 70% ethanol and formaldehyde
3. stays viable in the tissues fixed by 10% solution of formaldehyde
4. not sensitive to the effect of nucleases and other reagents which inactivate nucleic acids
5. sensitive to the effect of the reagents which cause denaturation or modification of proteins

The process of the accumulation of PrPSc



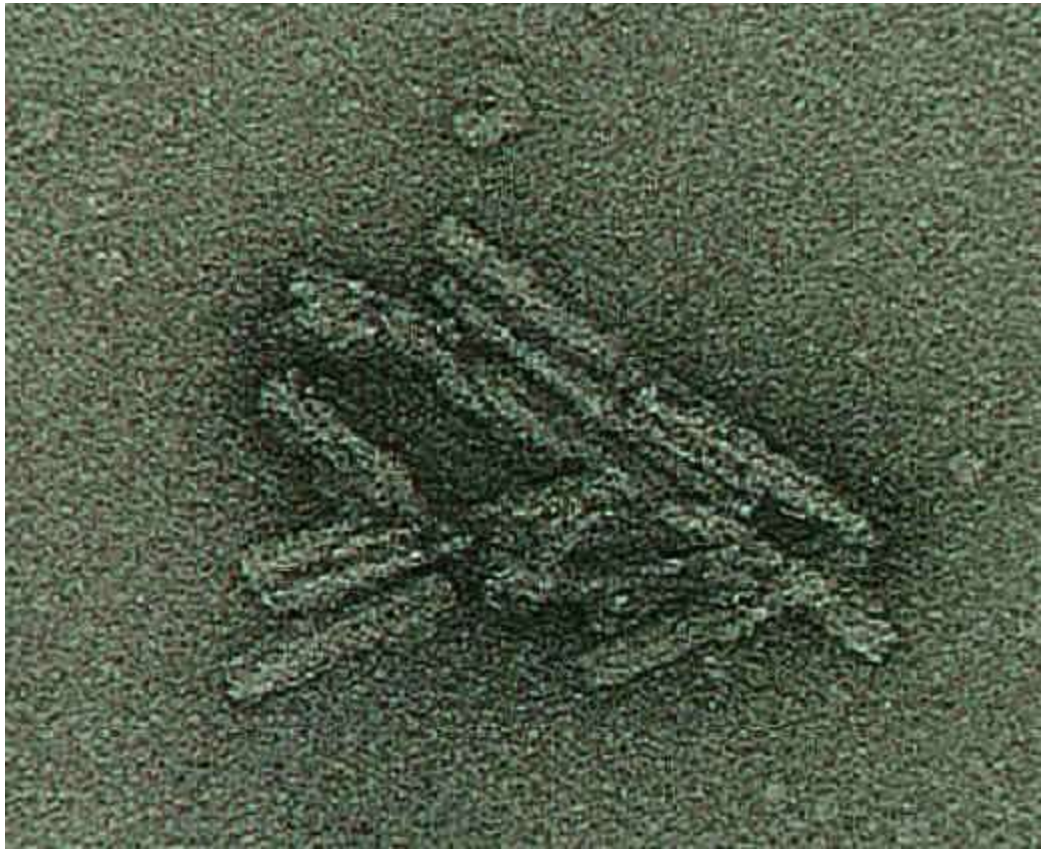
Pathogenesis of prion infections

- PrPSc is localised intracellular, polymerises and produces appearance of rod-like structures
  - intracellular localisation and resistance to the effect of intracellular proteases causes lack of any immunogenic properties by prions (no immune response)
- Penetration of the infectious prion into the neurons causes⇒ change of the structure of the normal PrPC and as a result⇒ accumulation of new infectious PrPSc molecules in the neurons leading to ⇒ degeneration of the nervous tissue

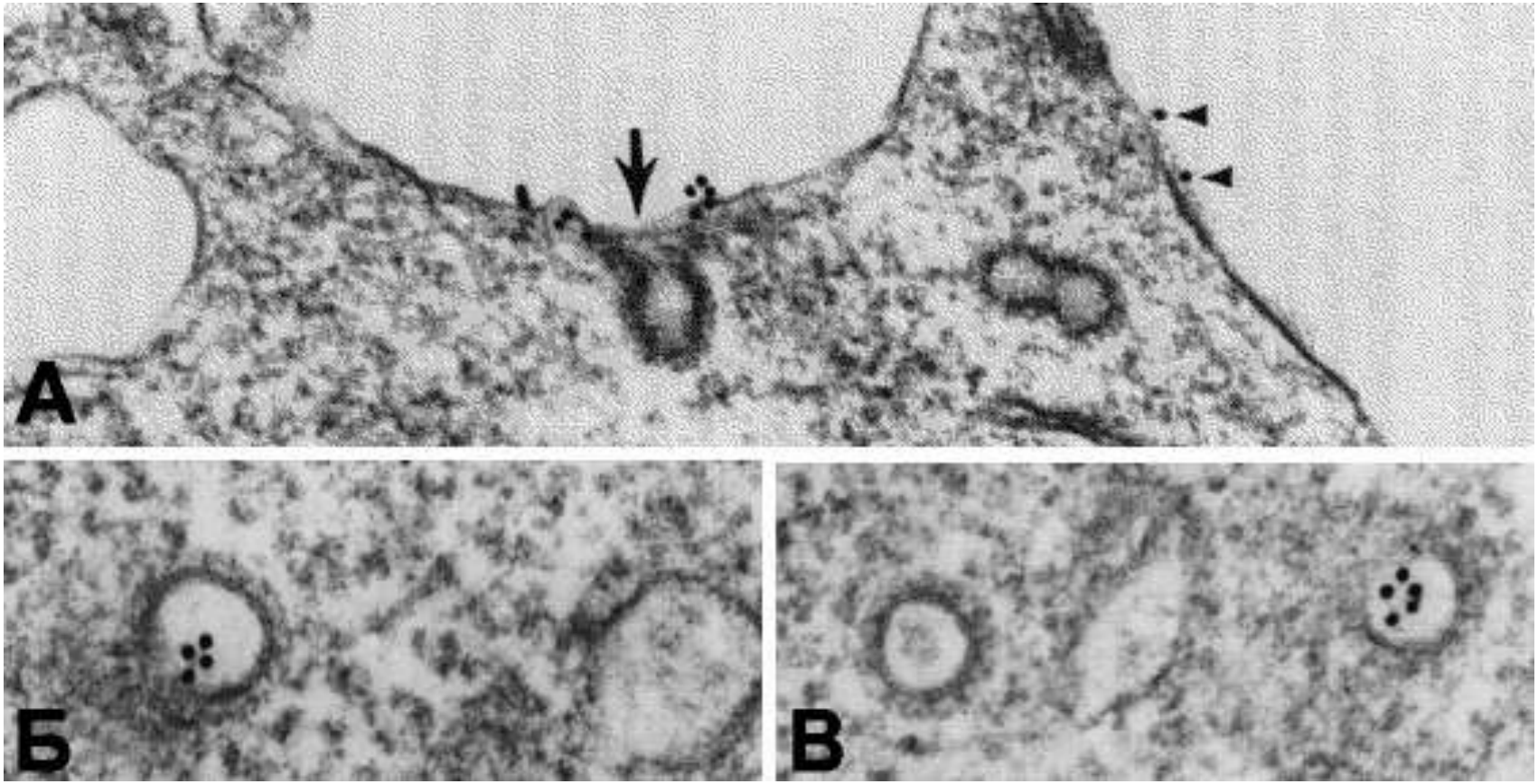
Laboratory diagnostics of prion disease

- Revealing of prion protein and amiloid tissue in brain (histological and immunochemical tests)
- Revealing of special markers of prion damage in brain (ELISA, monoclonal antibodies, etc).
- Revealing of mutated PRNP gene in PCR.

PrPSc - rod-like structures



Creutzfeldt-Jakob disease: endocytosis of PrPsc in neurons (А). Б и В – PrPsc localised inside of the embosoms. Electron photograph (immune gold stain) of PrPsc. X 100 000



The frequency of the prion disease in human population

1 : 1 000 000

The sickness rate is higher in:

- Slovakia
- Israel
- Chile

- spongiosis
- amyloidosis
- gliosis

} the difference in the intensity }

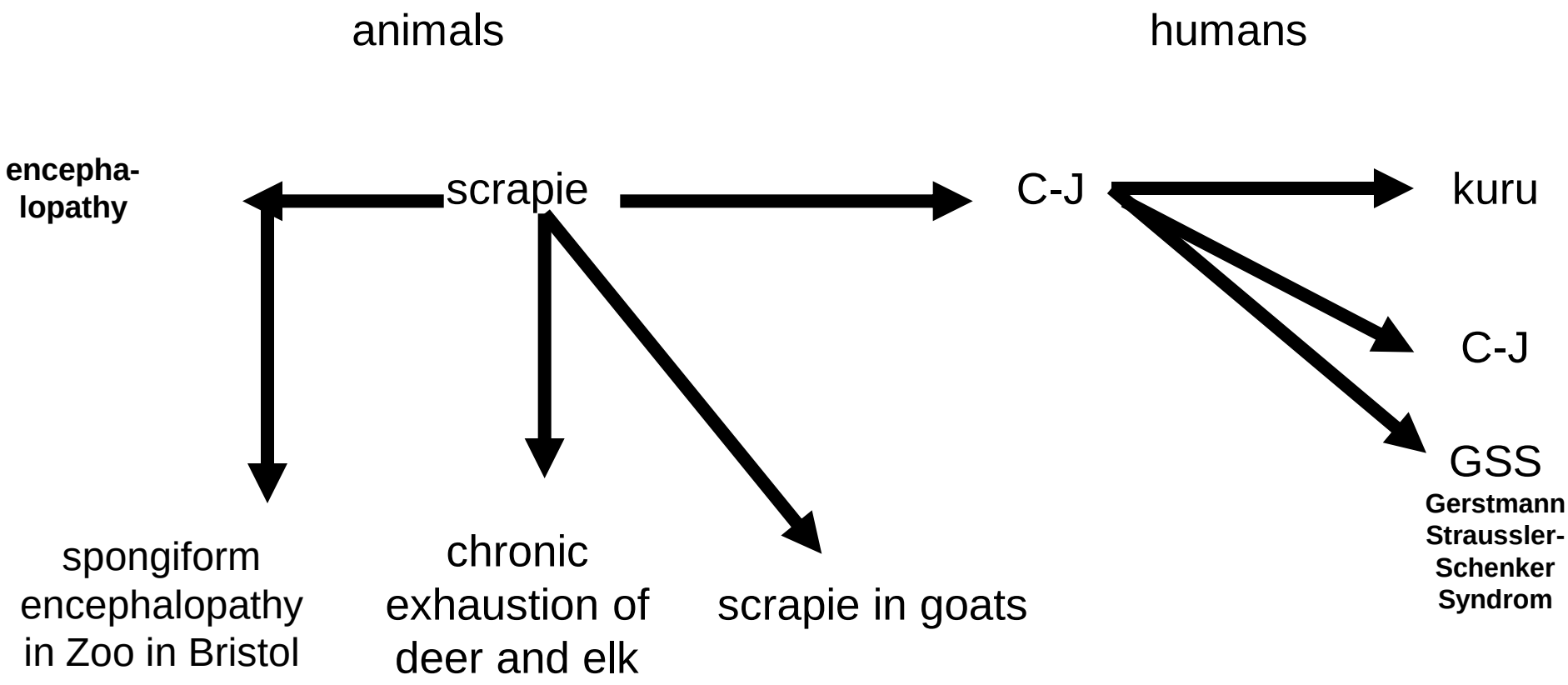
clinical forms of C-J disease

The clinical forms of Creutzfeldt-Jakob disease

In 100 % of the humans having this disease:

- 85 % - sporadic disease (usually in elderly people of 40-69 years old)
- 10-15 % - the patients having family history of the disease (inherited disease)
- < 1-5 % - infectious disease (when the typical exogenous source of the infection could be registered including iatrogenic transmission

The schematic picture of the history of spongiform encephalopathy by Gajdusek



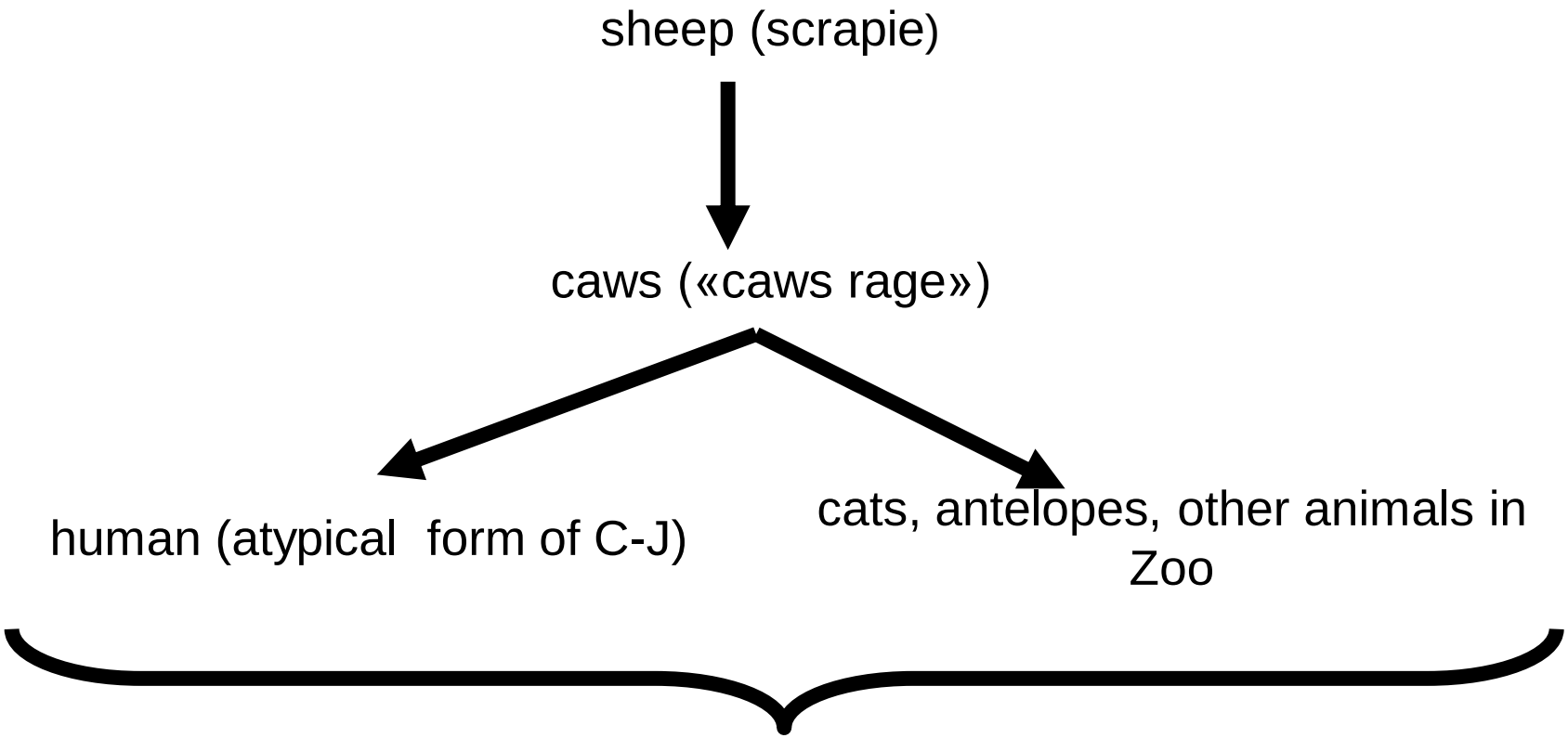
Factors of iatrogenic transmission of C-J disease

- needles
- electrodes for implantation
- instruments for dentist manipulations
- transplantation of cornea
- transplantation of other tissues
- blood and blood preparations used for transfusion

Professional risk of getting the prion disease

- neuropathologists
- neurosurgeons
- other surgeons
- tattoo and piercing- makers
- sexual ways of transmission (micro-traumas)

New variant of C-J disease (the end of XX century)



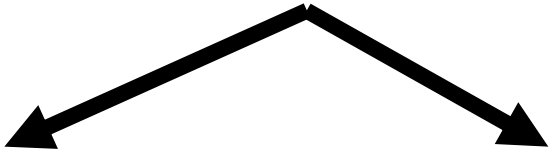
prions easily enter through the gastrointestinal tract

The most significant discoveries in Microbiology

- ***Levenhook*** (the end of XVII century) – discovery of bacteria
- ***Dmitry Ivanovsky*** (1892) – discovery of viruses
- ***Prusiner*** (1982) – discovery of prions

The hypothesis of aging as a prion disease

accumulation of degenerative changes in brain



in the course of prion disease = in aging

The hypothesis of aging as a prion disease

Hypothetic protein

(~ prion)

↓

process of conformational changes in normal proteins

↓

aging

Clinical microbiology

Clinical microbiology: definition of the term

It is a division of medical microbiology which includes the study of:

- infectious diseases which occur in hospitals where the patients are getting treatment in the case of non-infectious diseases,
- these infections called hospital-acquired (iatrogenic or nosocomial) ones.

Clinical microbiology: main aspects of the study

The main aspects are:

- study of hospital-acquired infections:
  - their aetiology
  - pathogenesis
  - specificity of the immune response
- development and applying of the methods which include:
  - laboratory diagnostics
  - specific treatment
  - prophylaxis

Clinical microbiology: methods

- The methods which are used by clinical microbiology include usual methods characteristic for medical microbiology but the main method is:
  - ***the method of growing of bacterial culture and the quantitative aspect of the method (the number of the bacterial cells in the specimens is especially important)***

Clinical microbiology: subjects of the study

- common opportunistic pathogens (microorganisms)
- hospital environment

Opportunistic microorganisms: the main groups

1. Gram-positive cocci (staphylococci and pneumococci)
2. Gram-negative enteric rods
3. Pseudomonades
4. Fungi Candida
5. Fungi of the genus Pneumocystis

Opportunistic microorganisms : main characteristics

1. Ecological grouping
   - free-living microorganisms
   - the patient's indigenous micro-flora
2. Conditions for the realisation of the pathogenicity by opportunistic microorganisms:
   - high adaptive capacities in microbial population
   - production of endotoxin
   - production of toxic enzymes
3. Characteristics of the opportunistic population:
   - heterogeneity
   - changeability
4. Specificity of the methods applied for the diagnosis of nosocomial infections:
   - it is necessary to analyse many different strains even if they are belonging to the same species
   - it is necessary to pay special attention to high resistant strains (bacteria resistant to drugs, antiseptics and disinfectants).

Opportunistic infections: definition of the term

- The infections caused by opportunistic pathogens (more frequently not by one representative of opportunistic micro-flora but by the associations of the several ones).

Opportunistic infections: their appearance

- These infections can occur everywhere.

Opportunistic infections: conditions for their development

1. High inocula of pathogens.
2. Debilitation of hospitalised patients.
3. Infection by more virulent pathogens: unusually high virulent opportunistic microorganisms.

Opportunistic infections: specific features

- Tropism to many organs.
- Clinical symptoms usually dependent on the site of localisation of the infectious process: symptomatology may be vague (unclear) or atypical.
- Opportunistic diseases often are getting:
  - chronic
  - generalised
  - could result in septicopyemia
- Problems for the therapy:
  - drug resistance of wide spectrum
  - low resistance of human organism to the infection (immune compromised patients)
- The infections may be endogenous (caused by own indigenous microorganisms).
- The hospital – acquired infections are dominant among the opportunistic ones.

Opportunistic infections : laboratory diagnosis

Rapid diagnosis of opportunistic infections is important to a favourable prognosis for the patient (early start of the treatment).

Laboratory diagnosis includes:

- Aetiology - isolation and identification of the opportunistic pathogen, that is especially important for the diagnostics in the cases when the pathogen has been isolated:
  - in high titre,
  - it demonstrates unusually high virulence,
  - it has been isolated from unusual site in human organism.
- The status of the immune response of the patient should be taken into consideration.
- Epidemiological aspects should be considered such as:
  - the source of the infection
  - factors of the transmission of the infection.

Hospital-acquired infections: definition of the term

- Infections which develop in patients due to medical manipulations for example, invasive procedures (intravenous cannulation, urinary cauterisation, surgery, etc) which are carried out:
  - in hospital
  - in ambulatory
  - at home.

Hospital-acquired infections: the reasons for their spreading

- The widespread and frequent use of therapeutic and prophylactic antimicrobial agents (drugs) provide selective pressure for the proliferation of drug-resistant microorganisms.
- Increase of the number of invasive methods of therapy and medical tests:
  - diagnostics that is accompanied by breakdown of physical barriers such as skin and mucous membranes,
  - surgery,
  - use of the immune suppressive drugs,
  - aging of the human population, survival of immune compromised persons,
  - high frequency of non-infectious underlying chronic diseases.
- Changes which occur in the hospital environment:
  - larger inocula of opportunistic pathogens which are circulating in hospital surroundings,
  - increase in the number of visits the hospitals by patients.

Hospital-acquired infections: aetiology

- Opportunistic microorganisms.
- Highly pathogenic microorganisms:
  - Hepatitis B virus
  - AIDS
  - Influenza virus
  - Viruses which cause acute respiratory and enteric infections
  - Salmonellae and Escherichiae in children
  - Adenoviruses (especially conjunctivitis)
  - Herpes and Cytomegalovirus infections
  - Chlamydia and Mycoplasma (urethritis)
  - Fungi producing dermatomycosises

Hospital-acquired infections: clinical ecological variants of pathogens

The pathogens found in the hospital surroundings are characterised by the next properties:

- highly resistant to numerous antimicrobial agents,
- highly resistant to antiseptics and disinfectants,
- highly resistant to the factors of innate immunity of the human organism.

Hospital-acquired infections : condition for their development

- Infection which is developing due to the medical manipulations

+

- Debilitation of hospitalised patients: their susceptibility to the infection

Hospital-acquired infections: specificity of pathogenesis

- Clinical manifestation and the composition of pathogens are due to the site where the invasive diagnostics or corrective, and maintenance procedures where applied.

Hospital-acquired infections: specificity of the immune response

- Usually patients are debilitated or immune compromised.
- Even in normal the immune response developed against opportunistic microorganisms is lower then one formed against high pathogenic microbes as the majority of opportunistic pathogens is belonging to human normal micro-flora.
- In the course of the disease the immune deficiency manifestations are increased in debilitated patients (the results are generalisation of the infectious process or development of chronic disease).

Hospital-acquired infections: diagnosis

The diagnostics includes:

investigation of the possible sources of the infectious agents:

- patient
- medical personnel and other patients
- hospital surroundings as they are possible factors of transmission of the pathogens

The diagnosis ***"hospital-acquired infection"*** could be stated when:

- hospital variant of pathogen has been isolated from the patient (even if the source of the infections and the factors of transmission where not found)
- when infectious process appeared as a result of the contacts with hospital personnel after passing the period of time equal to the incubation period (it takes 2 to 3 days in the case of opportunistic infections).

Hospital-acquired infections: prophylaxis

- Examination of patients and hospital personnel in connection with possible carriage of the agents of hospital infections.
- Examination of hospital living areas and therapeutic preparations to reveal their possible contamination with hospital opportunistic and iatrogenic pathogens.
- Maintaining a clean and disinfected environment.
- Treatment of patients, physicians and other hospital personnel who are shown to be carriers of potential pathogens by applying specific chemotherapy to eliminate the carrier state (to cure the patients and personnel completely).