An analysis of the evidence for the existence of HIV

Eleni Papadopulos-Eleopulos

Evidence in Chief

IN THE SUPREME COURT

CRIMINAL JURISDICTION ADELAIDE

APPLICATION FOR LEAVE TO APPEAL AGAINST CONVICTION

R V ANDRE CHAD PARENZEE

October 2006

This URL is www.theperthgroup/Parenzee/EPEIsolationSACCourt.pdf

PLEASE NOTE

EPE did not use speaker notes during the court proceedings.

However, with the exception of text marked EXTRA, all information in the speaker notes was provided as testimony

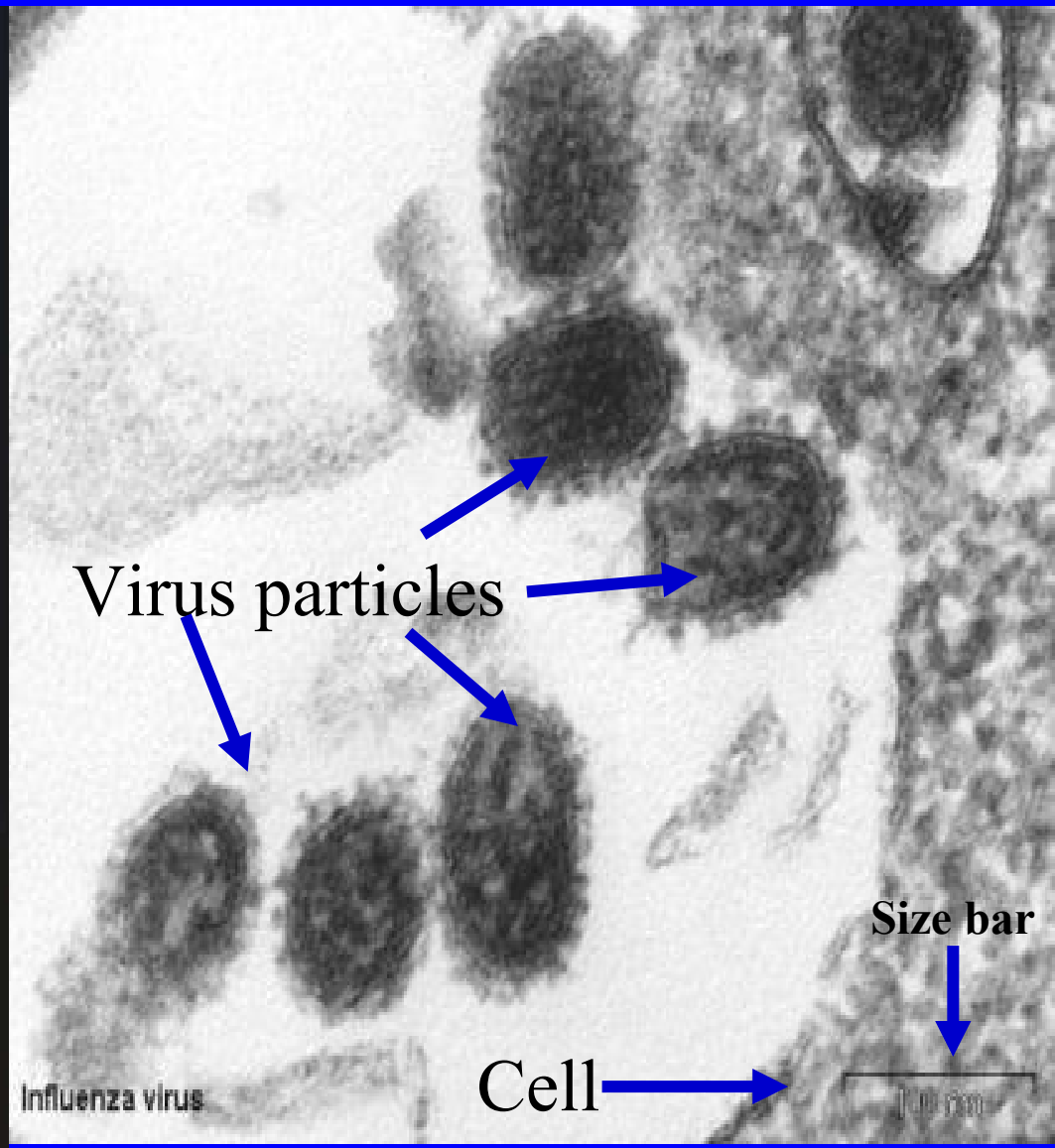
http://www.garlan.org/Cases/Parenzee/

DEFINITIONS

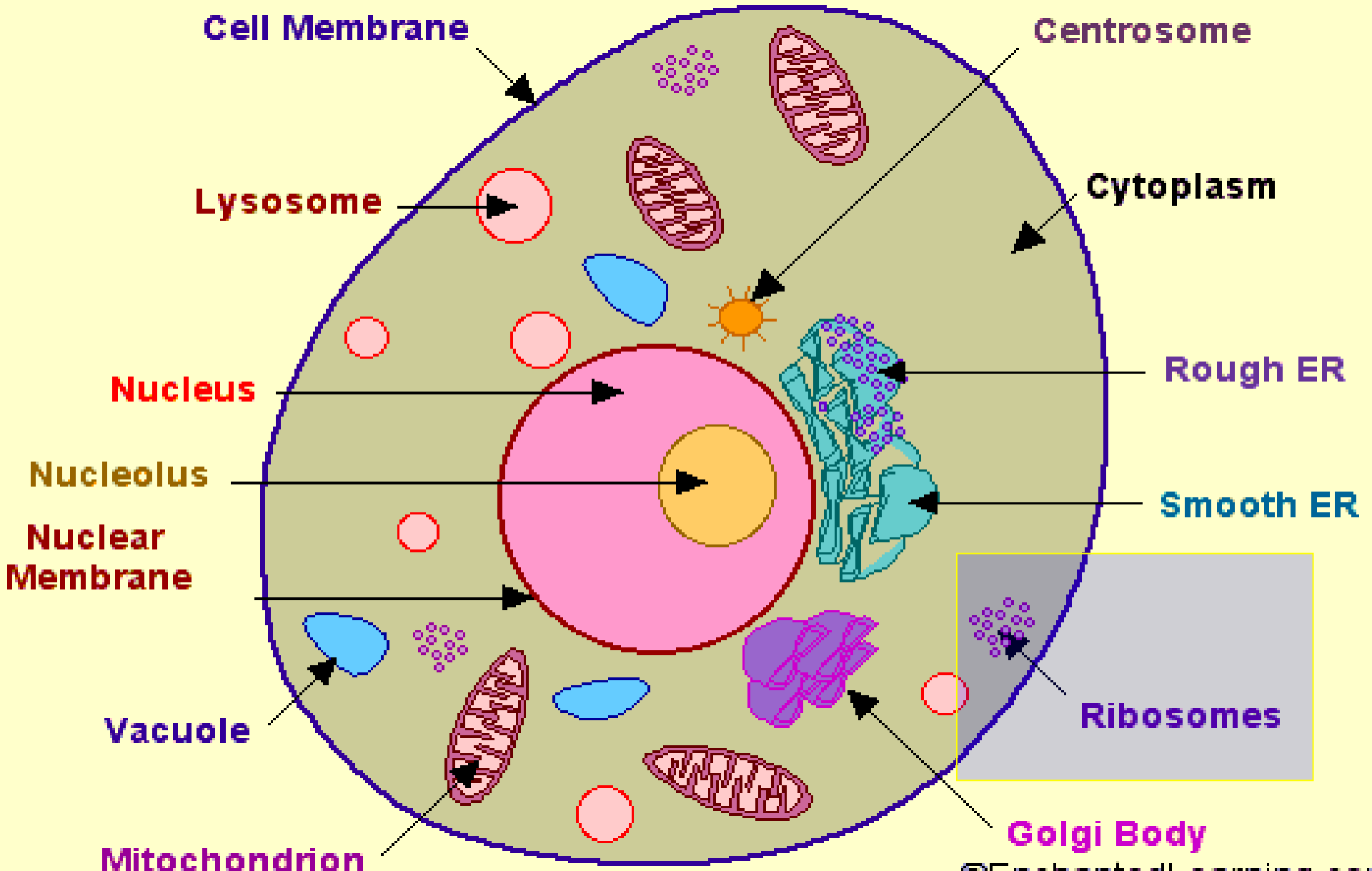
Viruses are microscopic particles also referred to as virions which, by definition means "the intact, fully assembled, infectious particle".

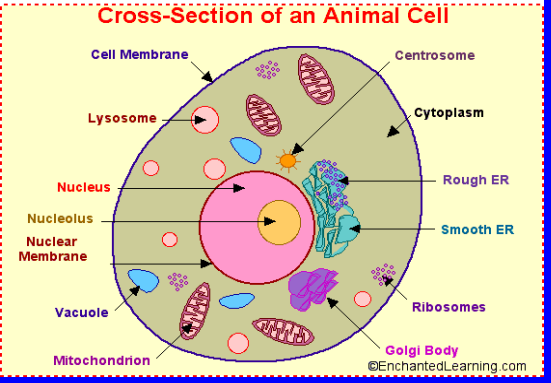
Viruses are too small to be seen with the light microscope. To visualise and study viral particles scientists need the resolving power of electron microscope which is about 200 times greater than the light microscope.


TECNAI


Virus particles
Size bar
Cell
influenza virus

Cross-Section of an Animal Cell



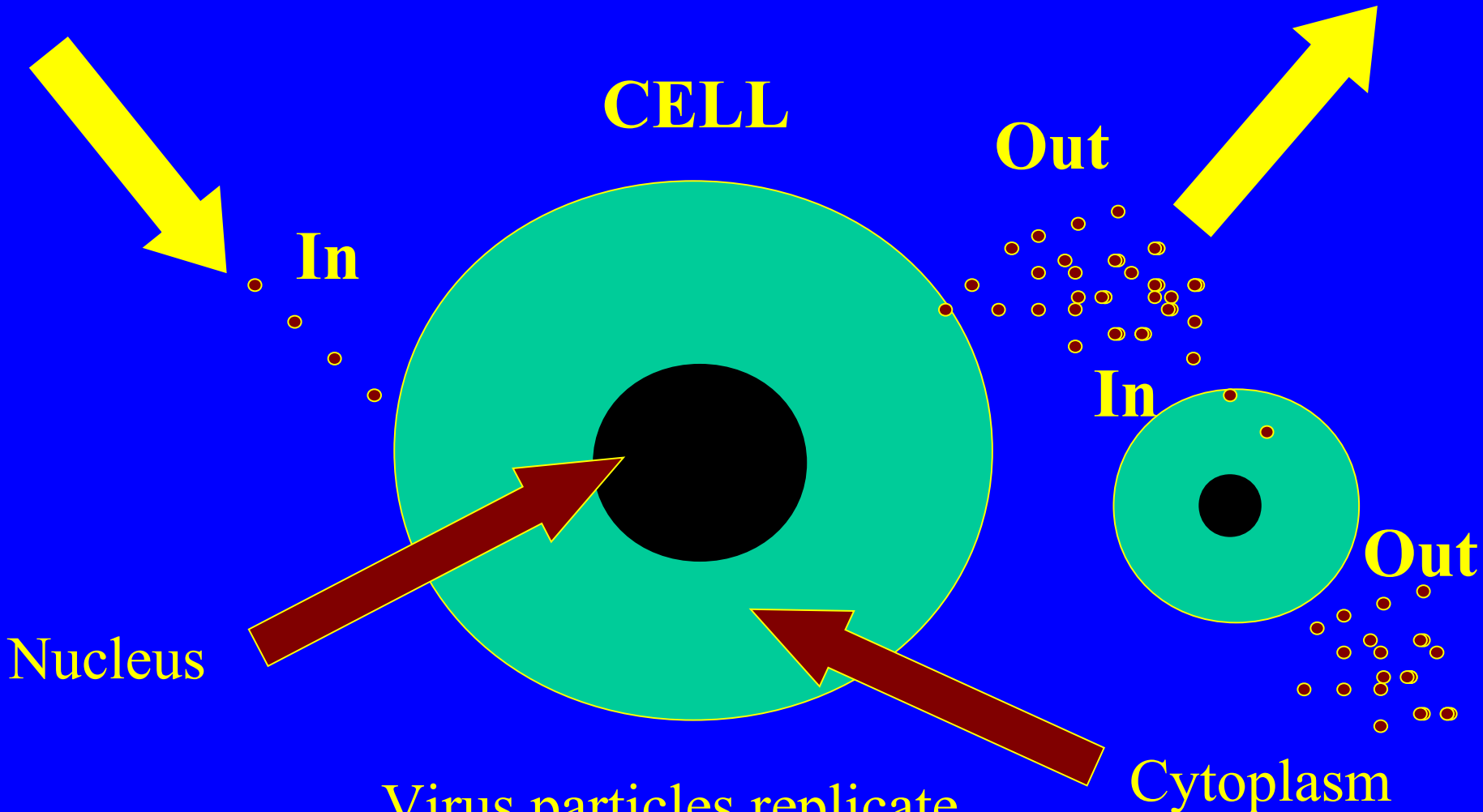


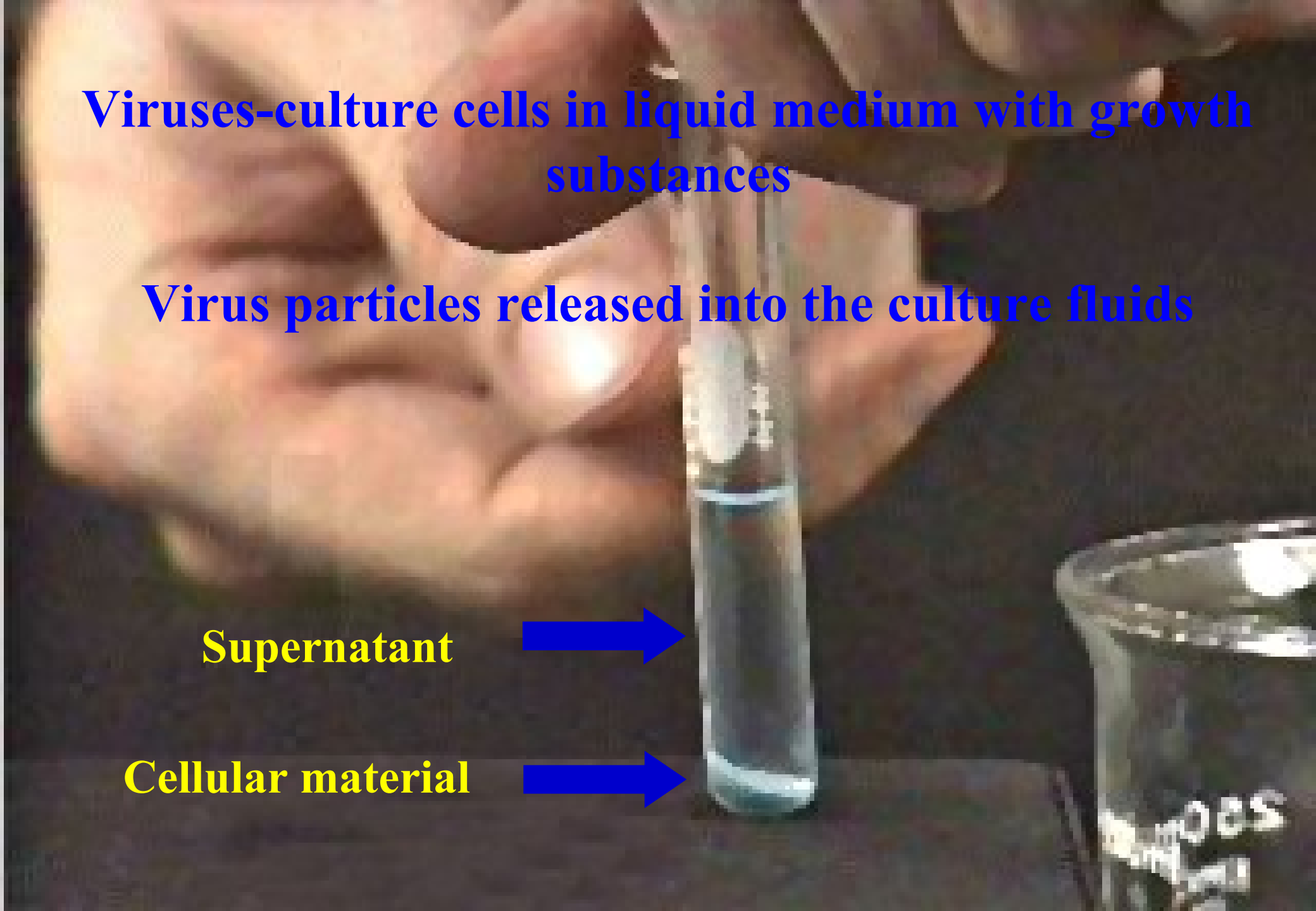
THE CELL

Is the smallest unit of heredity.
Has the machinery to gather raw materials from the environment and from them construct a new cell in its own image

Has many organelles
Some organelles or cellular fragments may look like virus particles

VIRUSES ARE MADE IN CELLS




Viruses-culture cells in liquid medium with growth substances
Virus particles released into the culture fluids
Supernatant
Cellular material

Biochemical composition

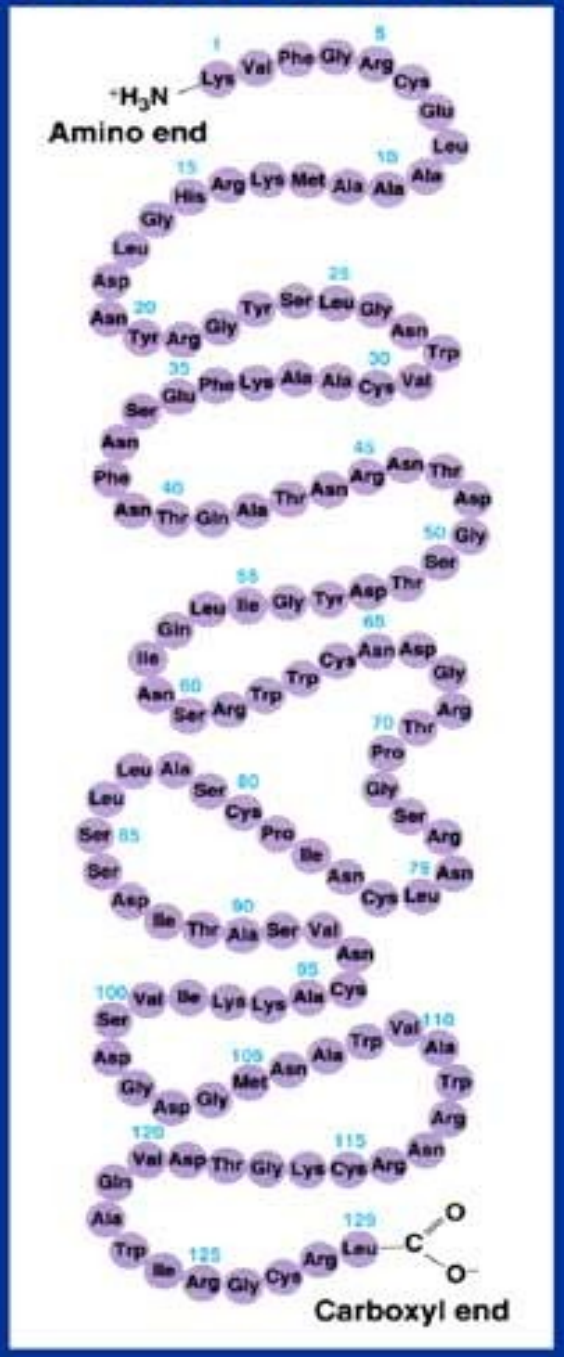
Cells and viruses are made of the same biochemical constituents

The main components of viruses and cells are proteins, RNA and DNA

All cells contain both RNA and DNA
Some viruses contain only RNA

THE PRIMARY STRUCTURE OF A PROTEIN

Proteins are amino acids joined by peptide bonds



Many proteins are enzymes

Some proteins are enzymes

Enzymes are catalysts

A catalyst is a substance that accelerates the rate of a chemical reaction without itself being changed at the end of the chemical reaction.

Enzymes act on reactants to make products

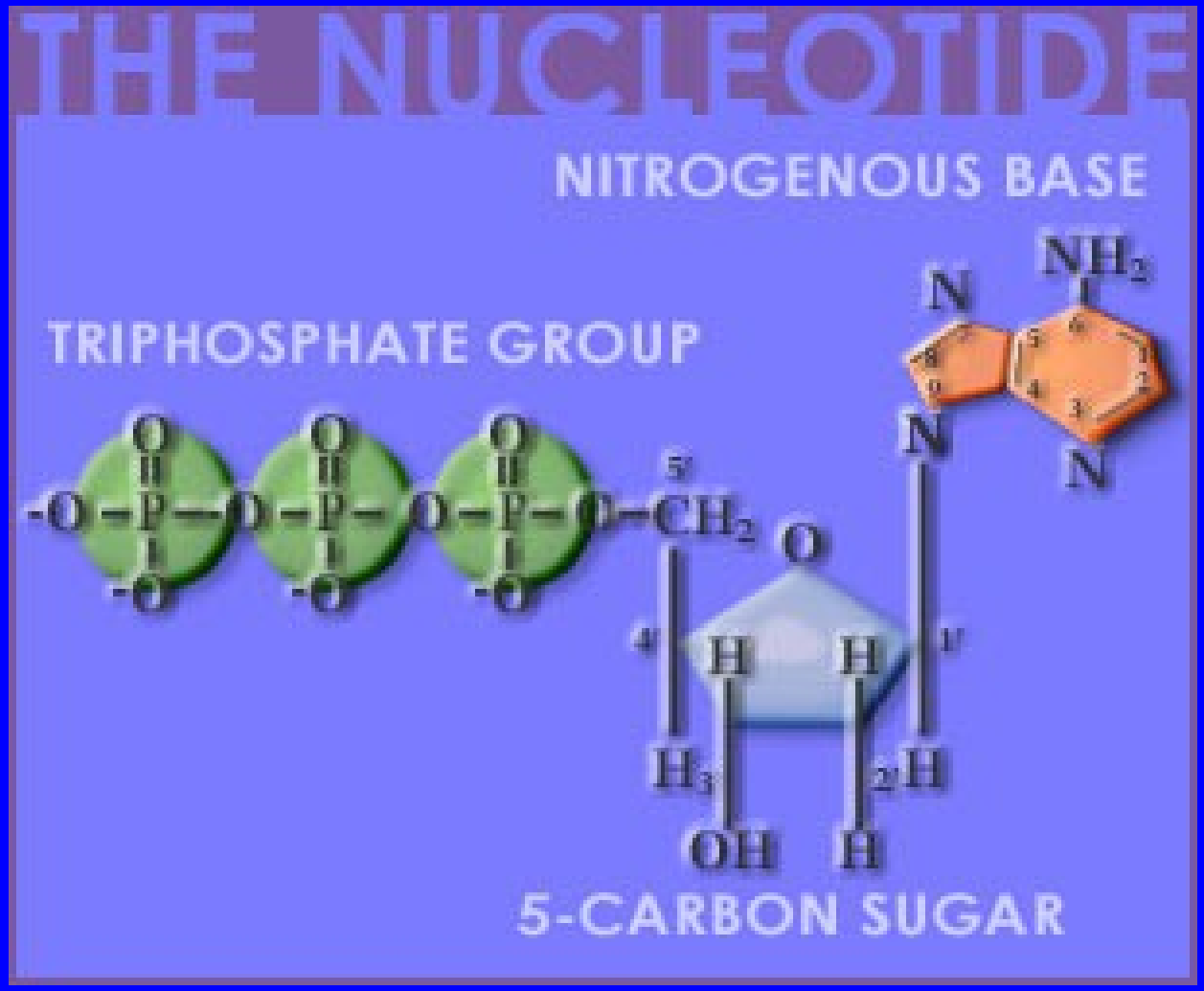
A + B **Enzyme** **X + Y**

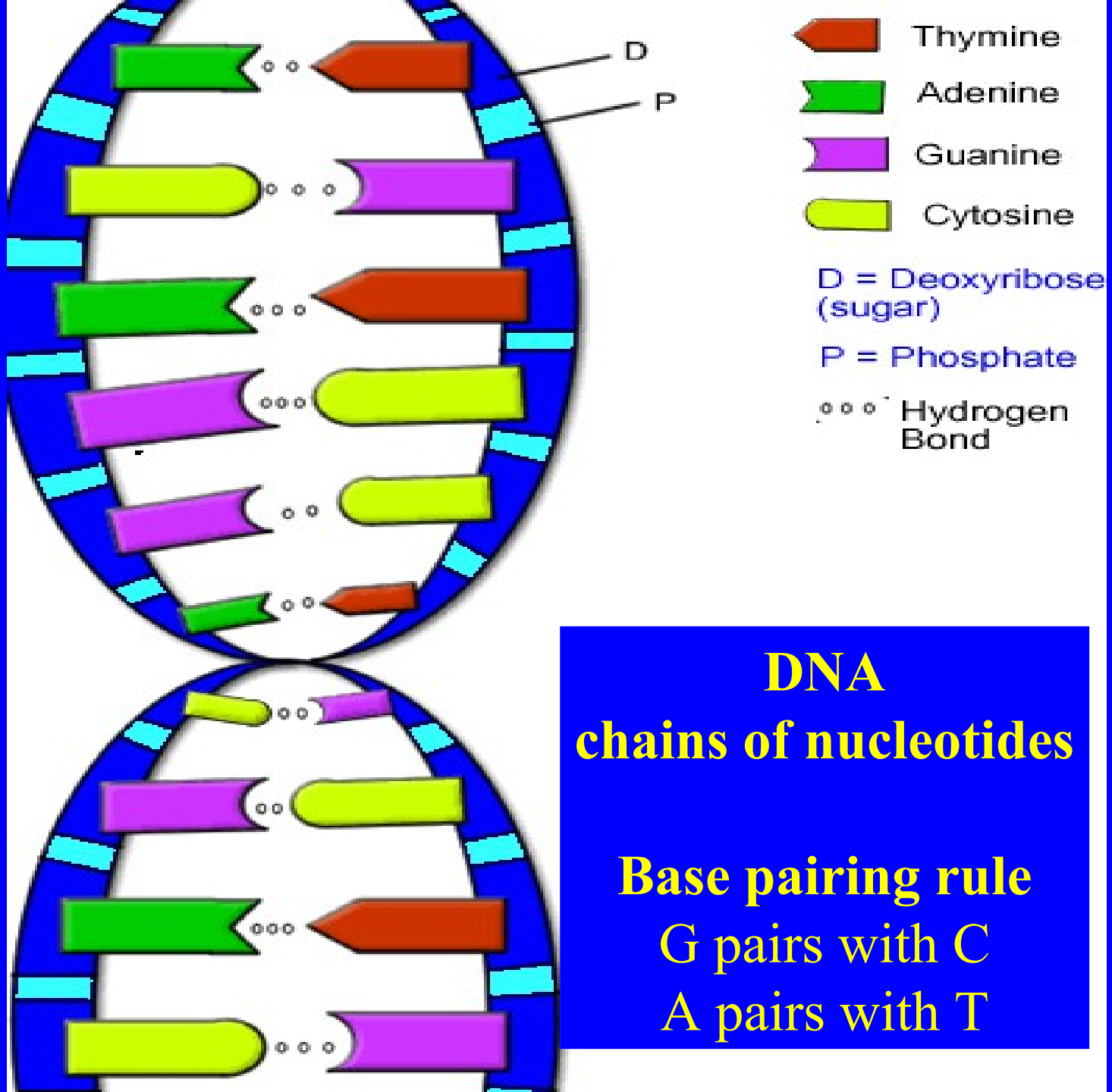
REACTANTS  **PRODUCTS**

Measuring the products detects and measures the enzyme

DNA

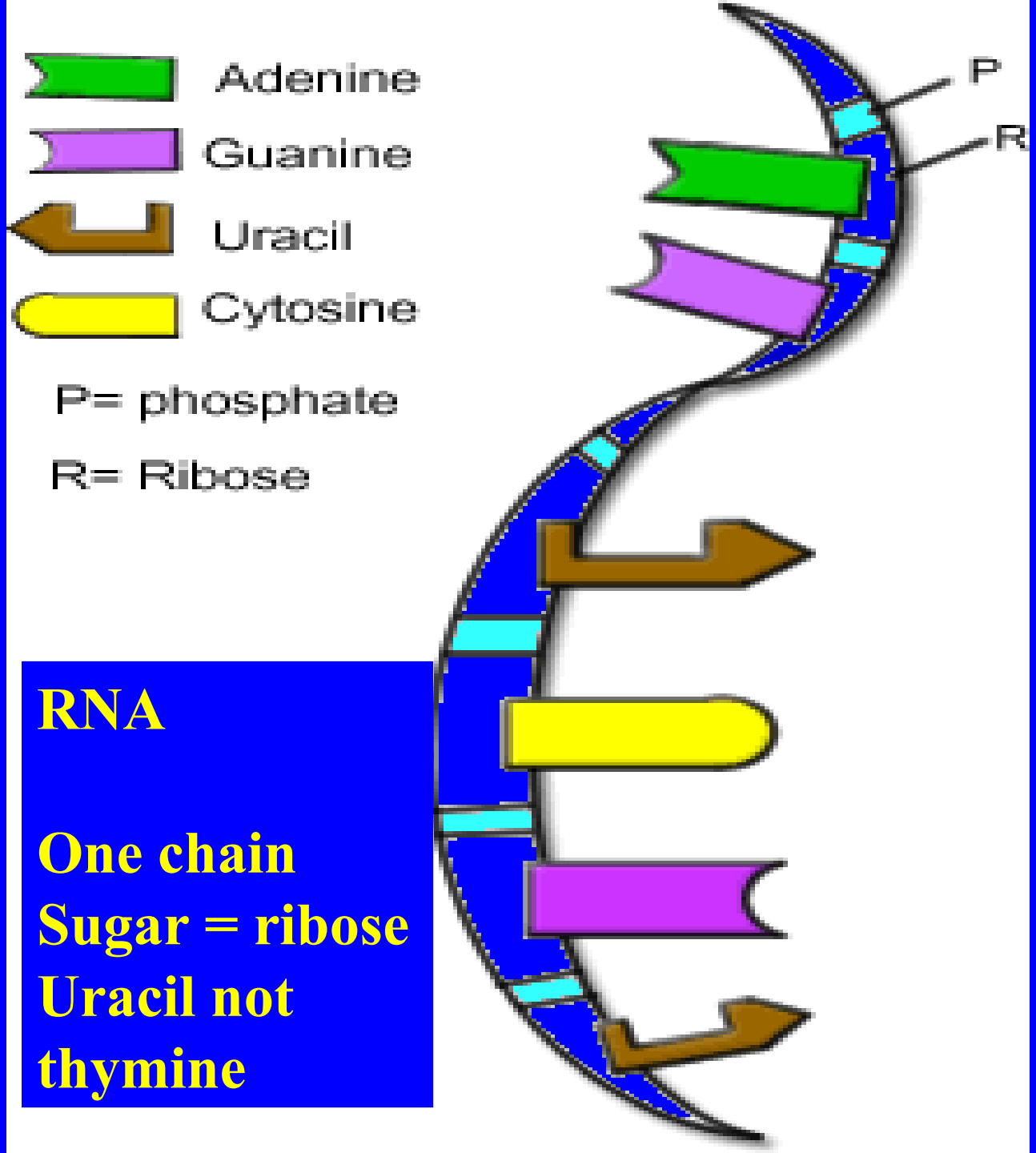
THE NUCLEOTIDE





DNA
chains of nucleotides

Base pairing rule
G pairs with C
A pairs with T



RNA

One chain
Sugar = ribose
Uracil not thymine

Information flow in cells

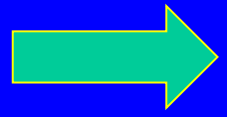
"Biological dogma"
DNA → RNA → proteins ("forwards")

RNA → DNA ("backwards")
"Reverse" transcription

Reversal of information flow first found in certain RNA viruses (oncoviruses) which became known as RETROVIRUSES

Reverse transcription

RT enzyme

RNA + DNA building blocks  DNA

Measure DNA

Proof of existence of a retrovirus

Culture putatively infected cells

Demonstrate:
Retroviral-like particles
Particles contain RT
Such particles produce the same particles (morphology, RNA and proteins [= replication])
Particles can be propagated, that is, they are infectious

To prove it is a new retrovirus
Proteins and RNA must be unique

The discovery of HIV

Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS)

Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet, C. Axler-Blin, C. Vezinet-Brun, F. Rouzioux, C. Rozenbaum, W. Montagnier, L. *Science* 1983;220:868-71.

This paper has been cited over 4000 times since publication

ISOLATION

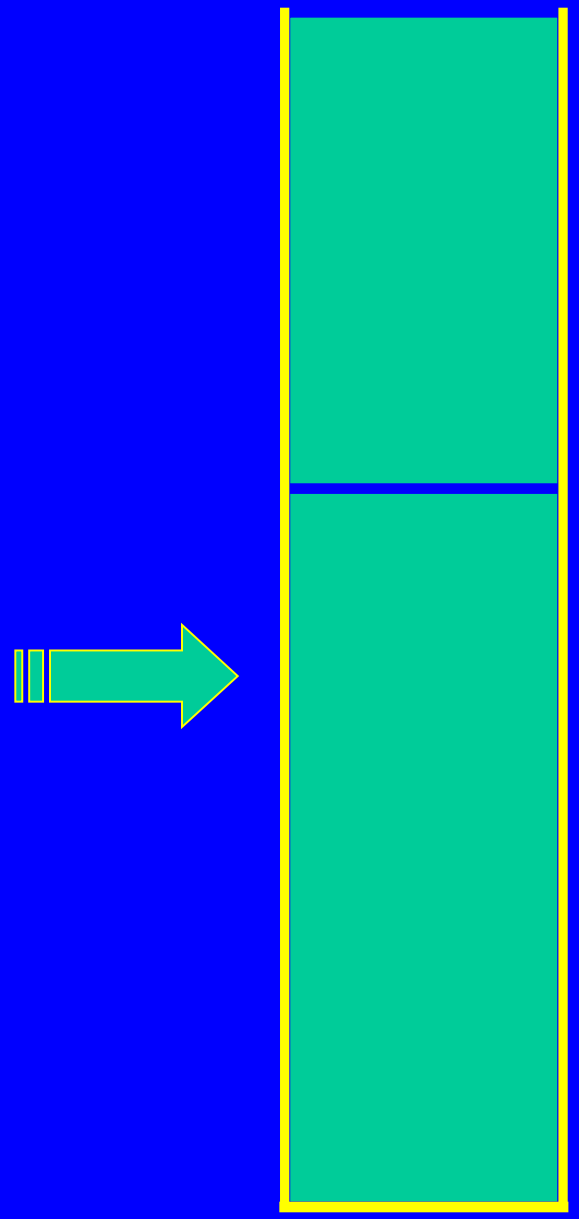
From Latin "*insulatis*" - made into an island

To isolate = "place apart or alone…separate (a substance) from a mixture"

The Australian Concise Oxford Dictionary 3rd edition

Montagnier- first experiment

T lymphocytes* BRU
PHA
Growth factors
Interferon antibodies
Nutrients
Antibiotics



RT activity
at 15 days

= "Virus production"
= Virus detection

No EM
No control

*Lymphocytes - one type of white blood cell present in blood and lymph nodes

Reverse transcription is not specific to retroviruses

Harold Varmus – Retrovirologist and 1989 Nobel Laureate

"reverse transcription is hardly unique to retroviruses;
it is now recognized as a widespread phenomenon in
eukaryotic cells"

(eukaryotic = cell with a nucleus = human cells)

"evidence has made it clear that reverse transcription takes place…in the uninfected cells of yeast, insects and mammals"

Varmus H. Retroviruses. *Science* 1988; 240:1427-35.
Varmus H. Reverse Transcription. *Scientific American* 1987; 257:48-54.

Reverse transcription is not specific to retroviruses

40% of human DNA is reverse transcribed

Hepatitis B virus contains RT
Caulimoviruses, Myxobacteria and *E. coli*. contain RT

PHA on its own induces reverse transcription (Gallo, 1972)

General public *Shares Magazine*

RT is not unique to retroviruses

Barre-Sinoussi and Chermann on RT activity in 1973

1973- "This enzymatic activity can be explained by the presence of some virus particles in these regions, and since similar polymerase activity has been found in normal cells, may be mainly ascribed to the cellular enzyme"*

Montagnier interview 1997
Reverse transcriptase is:

1. "truly specific for retroviruses"
2. "is the enzyme characteristic of retroviruses"

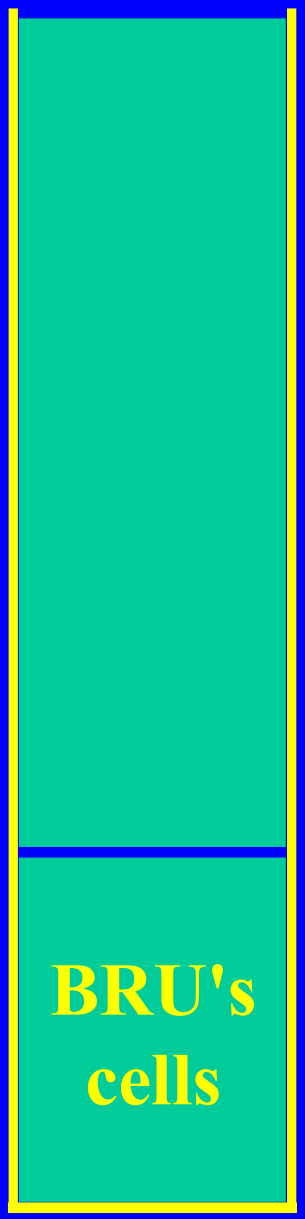
*Sinoussi F, Mendiola L, Chermann JC. Purification and partial differentiation of the particles of murine sarcoma virus (M. MSV) according to their sedimentation rates in sucrose density gradients. Spectra 1973;4:237-243

Hair is a characteristic of humans

Not every animal with hair is human

Montagnier- second experiment

PHA
Growth factors
Interferon
Antibodies
Nutrients
Antibiotics



+ lymphocytes from a healthy blood donor

RT activity = "Propagation" and "isolation"

No EM
No control

Montagnier's conclusion

The finding of RT activity in:

BRU's cell culture ≡ retrovirus detection

BRU's cells culture + healthy donor cells ≡ virus isolation and propagation (transmission)

Montagnier and Gallo – RT activity ≡ retrovirus

"The specimen [portion of a lymph node] was minced, put into tissue culture and analysed for reverse transcriptase. After two weeks of culture, reverse-transcriptase activity was detected in the culture medium. A retrovirus was present but which one?"

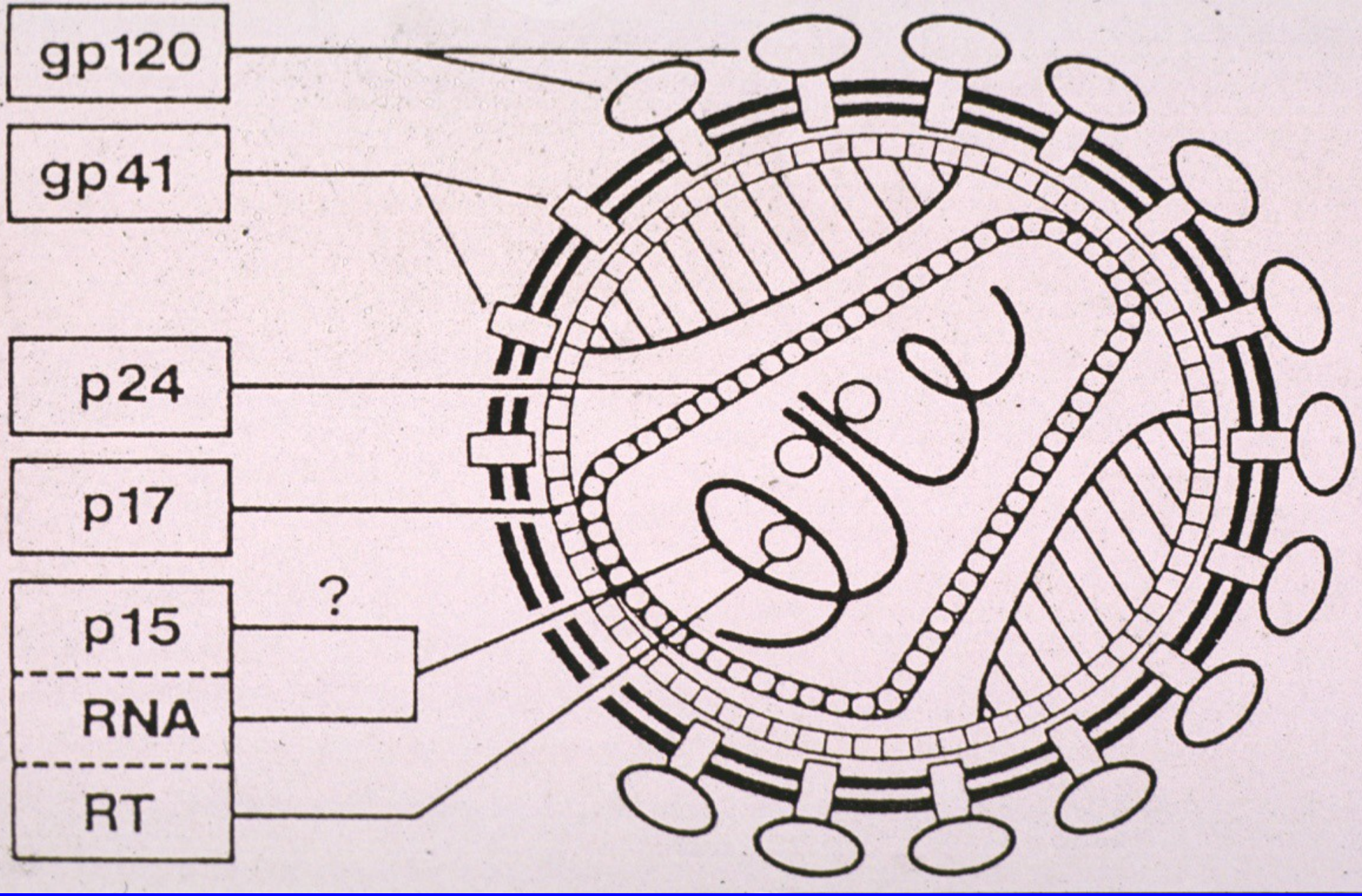
Gallo RC, Montagnier L. AIDS in 1988. *Scientific American* 1988;259:24-32

Hominid taxonomy

Virus taxonomy—also Families, Genera, Species

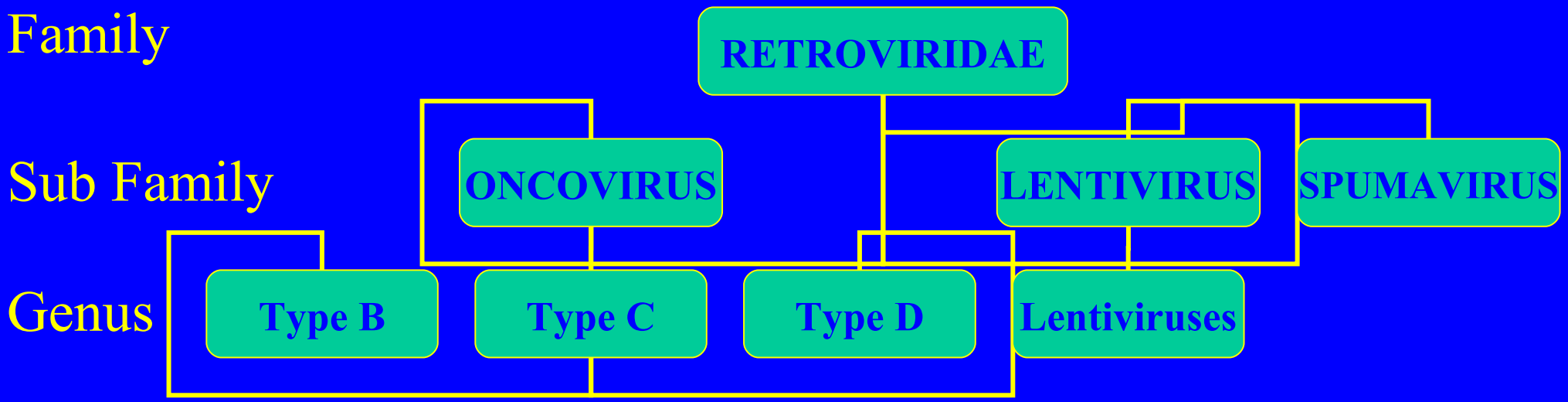
By definition particles belonging to the Family of retroviruses are:
"enveloped viruses with a diameter of 100-120 nm budding at cellular membranes. Cell released virions [individual virus particles] contain condensed inner bodies (cores) and are studded with projections (spikes, knobs)"

Gelderblom, H. R. et al. *Micron Microscopica* **19**, 41-60 (1988).



Retroviral particle diameter = 100-120 nm

Retroviral taxonomy



Montagnier- third experiment

PHA
Growth factors
Interferon
Antibodies
Nutrients
Antibiotics

Supernatant from the second culture
+ umbilical cord lymphocytes

RT activity

Electron microscopy → virus-like particles in the culture and on the cells

No control

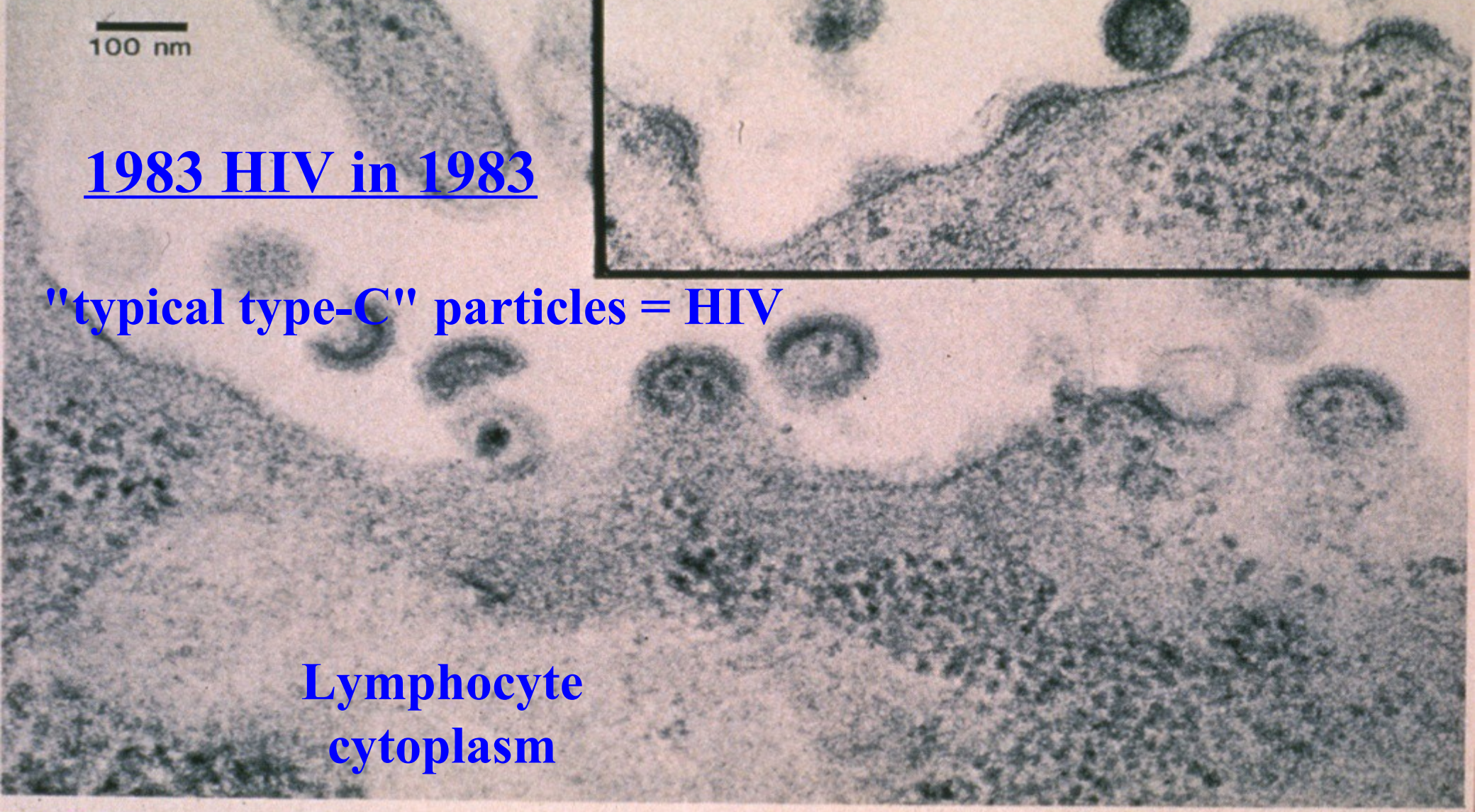


Fig. 2. Electron microscopy of thin sections of virus-producing cord lymphocytes. The inset shows various stages of particle budding at the cell surface.

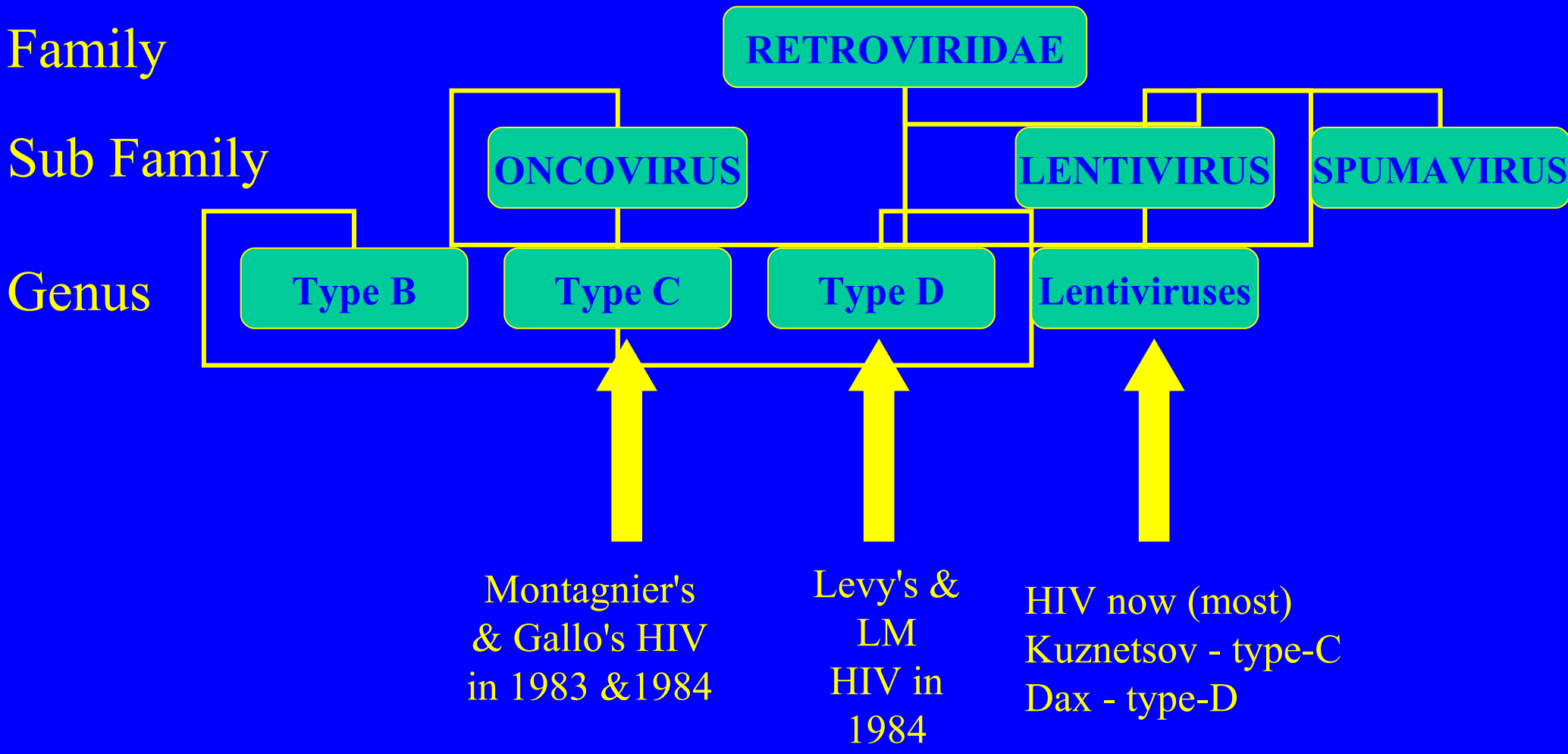
Gallo 1984 - HIV = also a type-C particle

1983 HIV in 1988

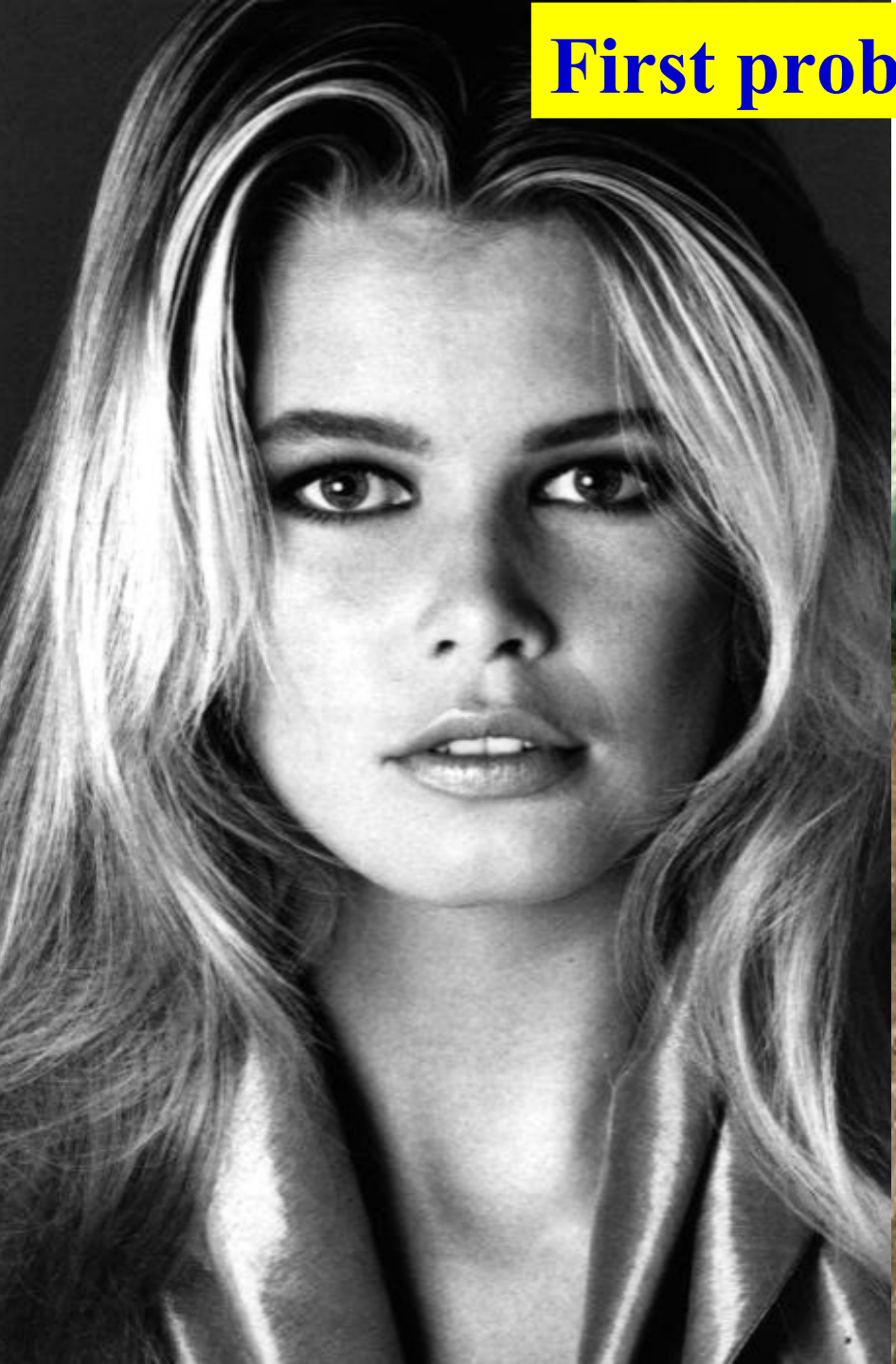
"Electron micrographs of the new virus were different from those of HTLV-I [type-C particles] and resembled those of a retrovirus of horses [lentivirus particles]"

Gallo RC, Montagnier L. AIDS in 1988. *Scientific American* 1988;259:24-32

HIV taxonomy



First problem with particles



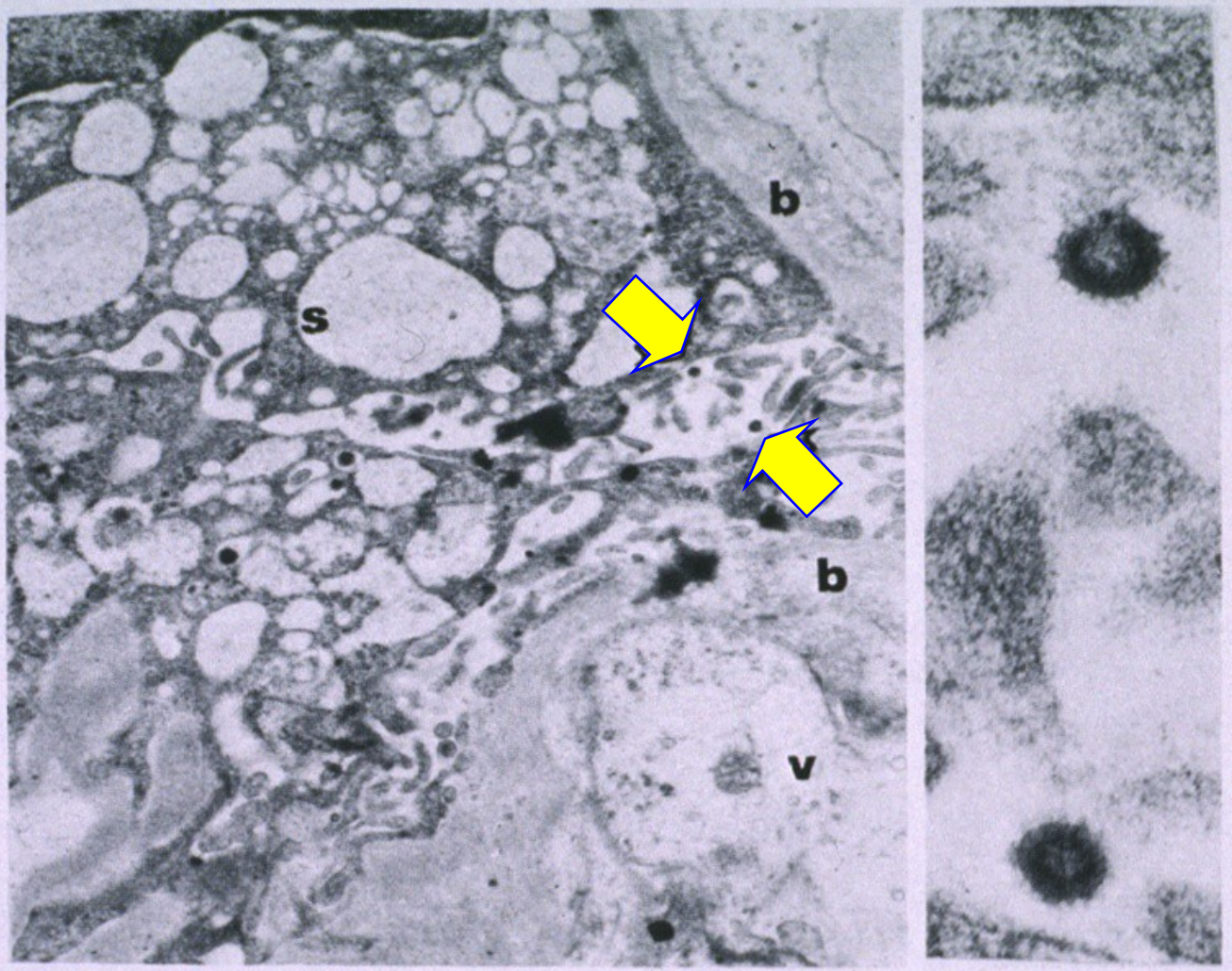
Second problem with particles

Type-C particles ubiquitous- reason a biological mystery

Fish, snakes, worms, pheasant, quail, partridge, turkey, tree-mice, … tapeworms, insects, mammals

1970s frequently in human leukaemia tissues and cultured embryonic cells

In the majority of human placentas (umbilical cord lymphocytes are obtained from placentas)

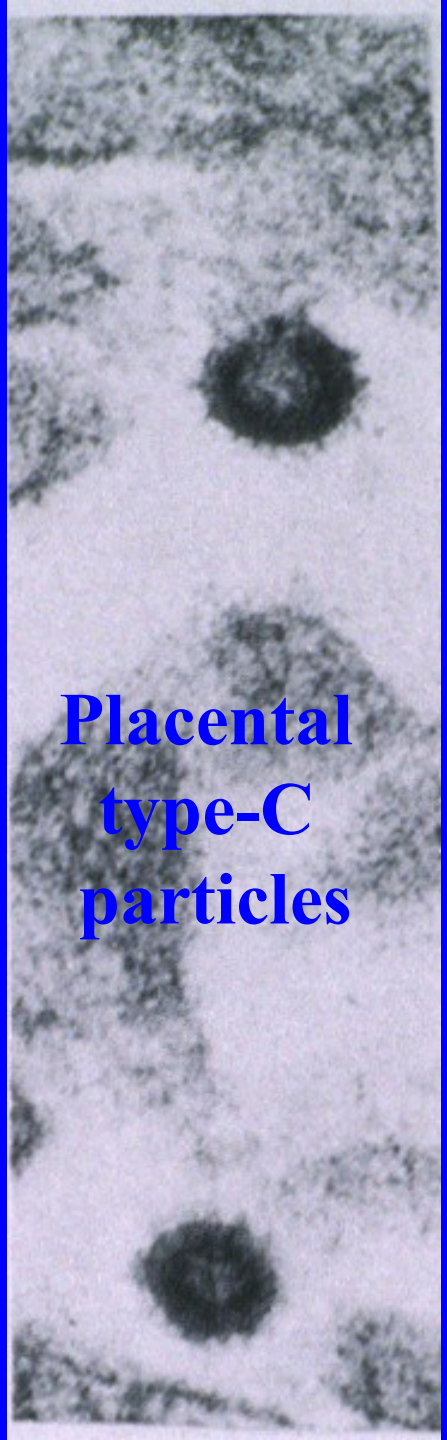


Type C particles

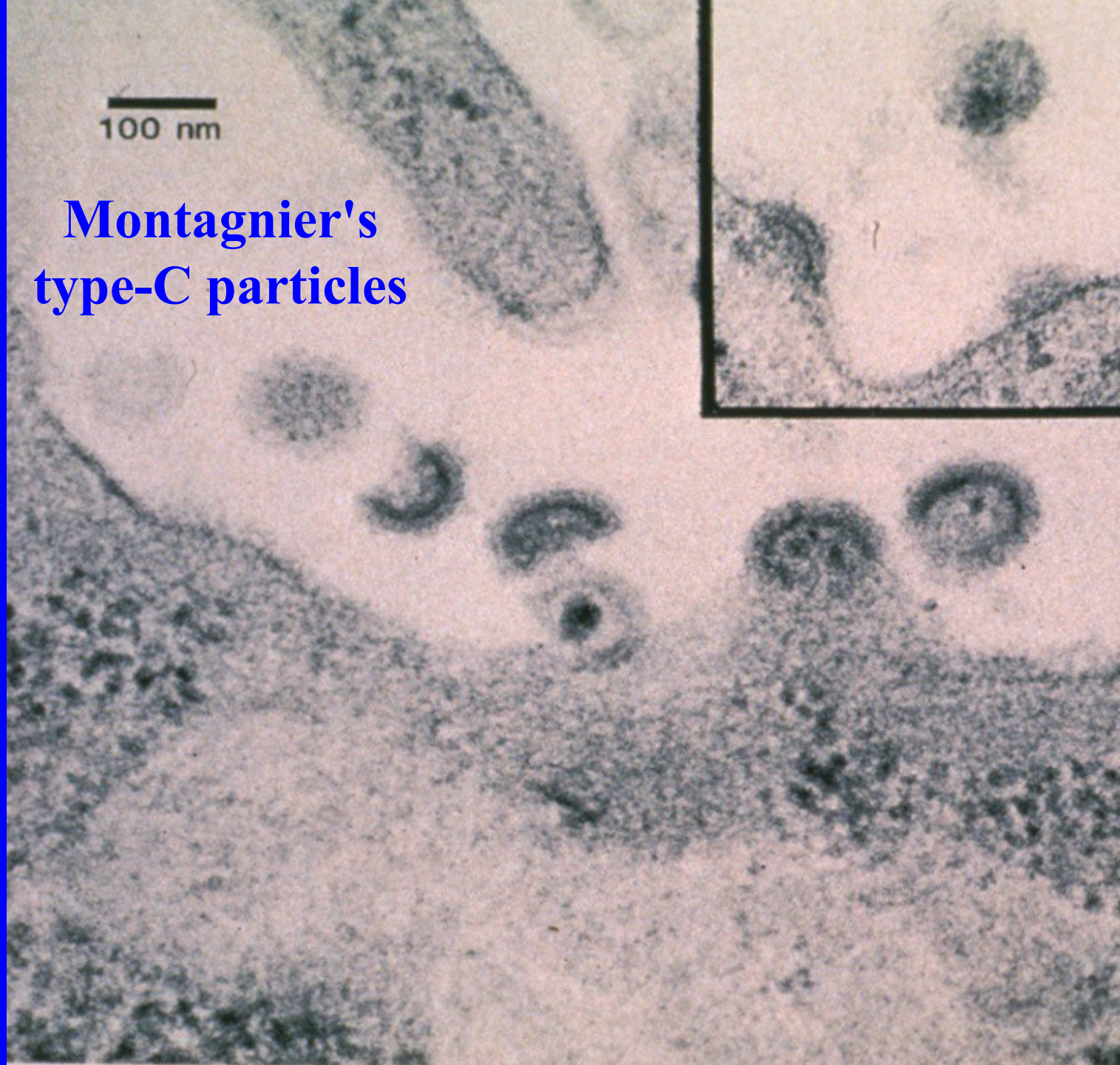
are found in the majority of normal human placentas

Fig. 4. Extracellular virions. A complete and a budding virion located near the basal lamina *(b)* (x 9,000). The two virions are seen enlarged at the *right* (x 90,000)

Panem S. C Type Virus Expression in the Placenta. Curr Top Pathol 1979;66:175-189



Placental type-C particles



Montagnier's type-C particles

Third problem with particles

Retroviral particles are non-specific

That is

Retroviral particles do not equal a retrovirus

For example, cellular fragments and organelles may look like RVPs

Fourth problem with particles

Endogenous retroviruses

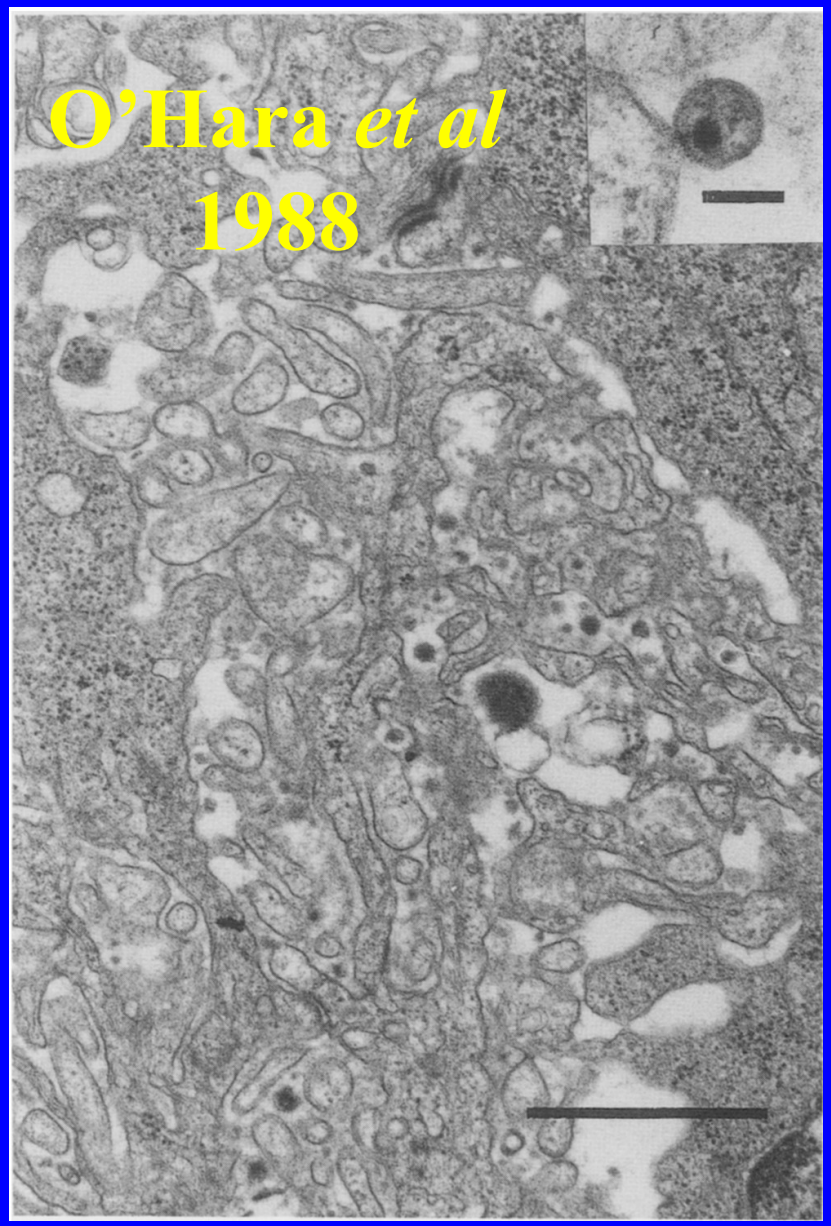
Cells may produce retroviruses spontaneously
Yield accelerated by culture conditions
Up to a million fold

Retrovirologist George Todaro: "…the failure to isolate endogenous viruses from certain species may reflect the limitation of *in vitro* cocultivation techniques"

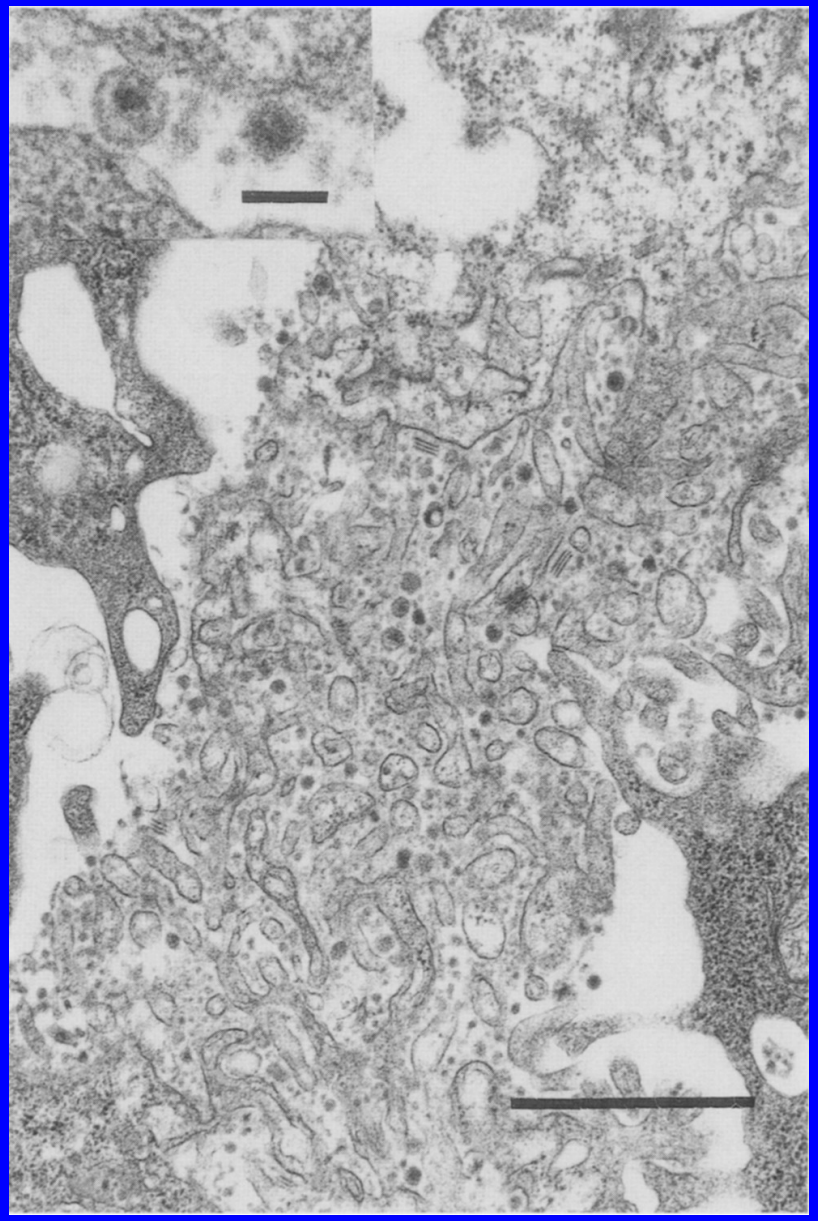
Statement by Professor Martyn French

"…the first demonstration of a virus with retrovirus features that was subsequently shown to be HIV was reported by Dr. John Armstrong and colleagues from Royal Perth Hospital in 1984"*

*Armstrong JA, Horne R. Follicular dendritic cells and virus-like particles in AIDS-related lymphadenopathy. *Lancet* 1984;ii:370-372 (emphasis added)



18/20 AIDS related



13/15 non-AIDS related

Particles do not equal HIV

"The presence of such particles does not, by themselves indicate infection by HIV".

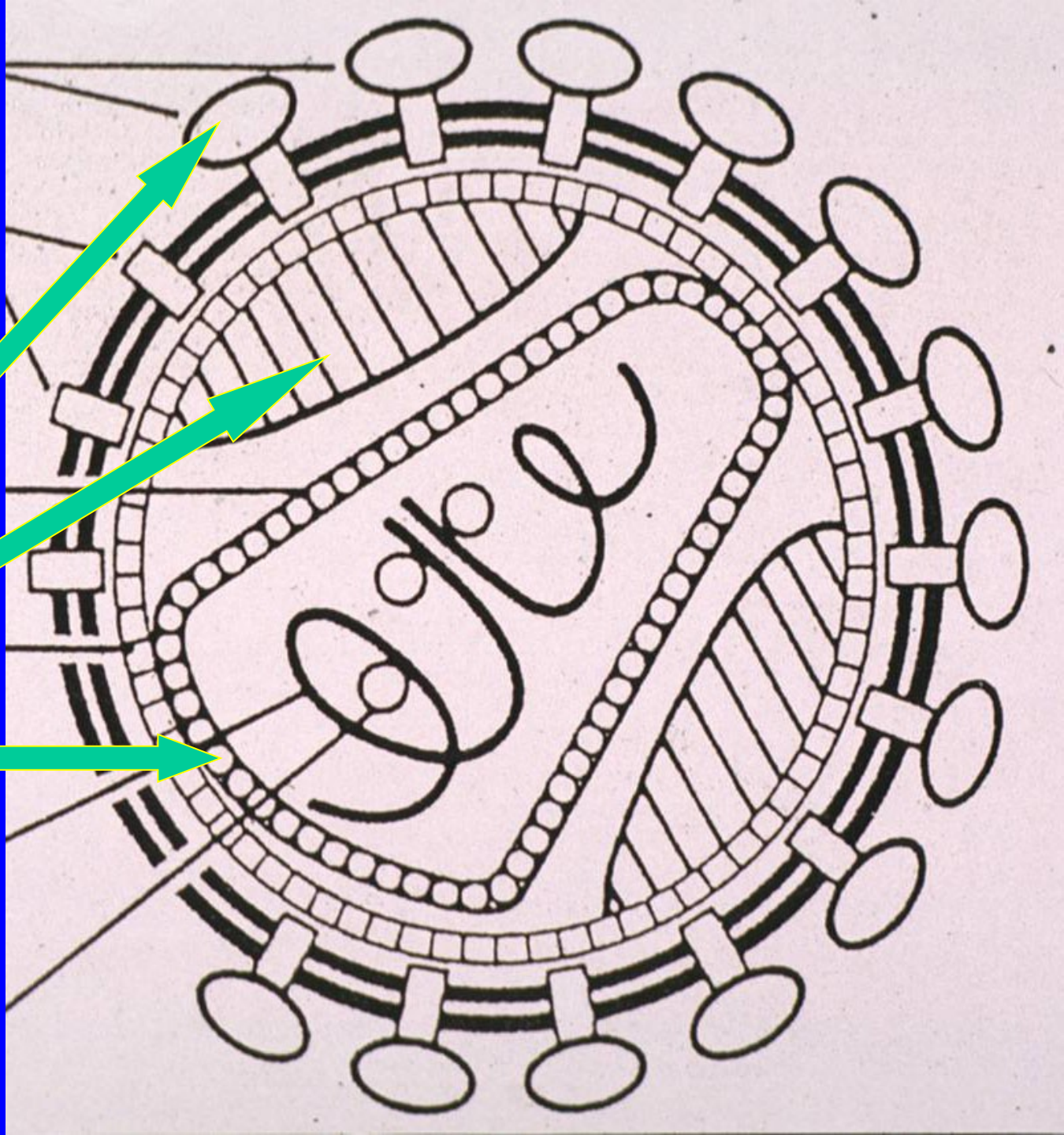
O'Hara CJ, Groopman JE, Federman M. The ultrastructural and immunohistochemical demonstration of viral particles in lymph nodes from human immunodeficiency virus-related and non-human immunodeficiency virus-related lymphadenopathy syndromes. Hum Pathol 1988;19:545-9

Gelderblom model of HIV

Knobs on surface

Lateral body

Cone shaped core



Diameter 100-120 nm

Fifth problem with particles

Particles observed in cell cultures containing tissue from AIDS patients have many different morphologies

Variation in size 65-250 nm (HIV=100-120 nm)

- 65-90 nm with knobs
- 100-120 nm without knobs
- > 120 nm without knobs

Conical cores

Tubular cores

More than one core

Tubular and conic cores

No cores

http://theperthgroup.com/CONTINUUM/Weiss1.doc

Sixth problem with particles

Virus-like particles can be found in all cells used for the study of HIV particles even when the cells are not "infected" with HIV

Seventh problem with particles

How many knobs on HIV?

Montagnier: "Particles of HIV are shaped like little spheres, each with roughly eighty rounded projections shaped like pegs [knobs]"

Constantine *et al*: there are "72 knobs or spikes of the external envelope of HIV"

KNOBS

are

"critical"

"crucial"

for infection to take place

KNOBS?

Knobs are said to be made up of the HIV gp120 protein

Hans Gelderblom and his colleagues have estimated that immediately after being released from the cell membrane "HIV particles" possess an average of 0.5 knob per particle which are rapidly lost, but also pointed out that "it was possible that structures resembling knobs might be observed even when there was no gp120 [knobs] present, i.e. false positives".

Layne SP *et al*. Factors underlying spontaneous inactivation and susceptibility to neutralization of human immunodeficiency virus. Virol 1992;189:695-714

KNOBS?

Knobs are said to be made up of the HIV gp120 protein

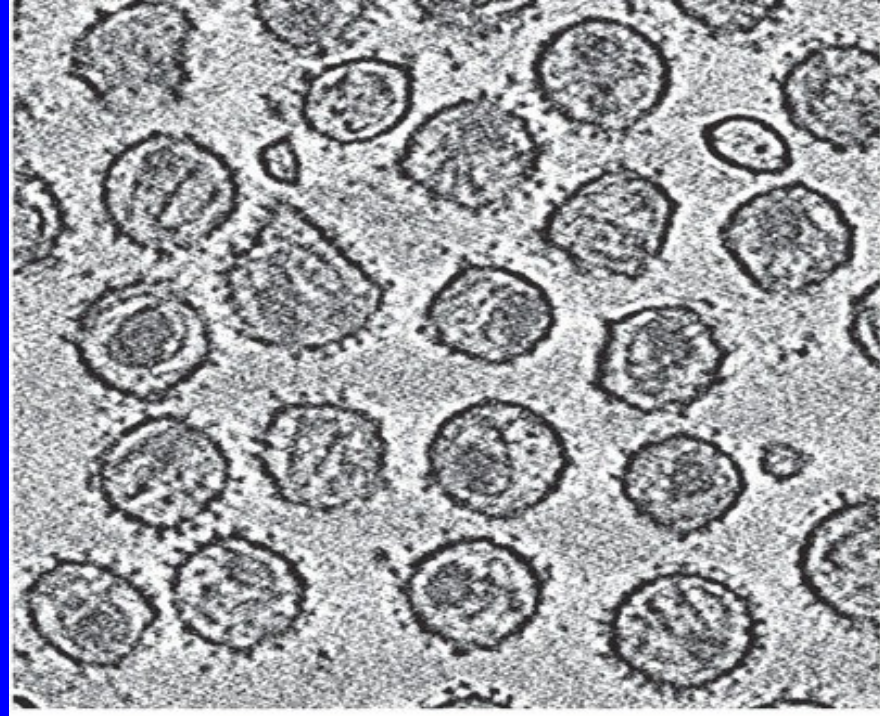
"The clusters of gp120 do not form spikes on the surface of HIV as is commonly described in the literature"

"We suggest that the spikes [knobs] observed by negative-staining electron microscopy may be an artifact of the penetration of heavy metal stain between envelope proteins"

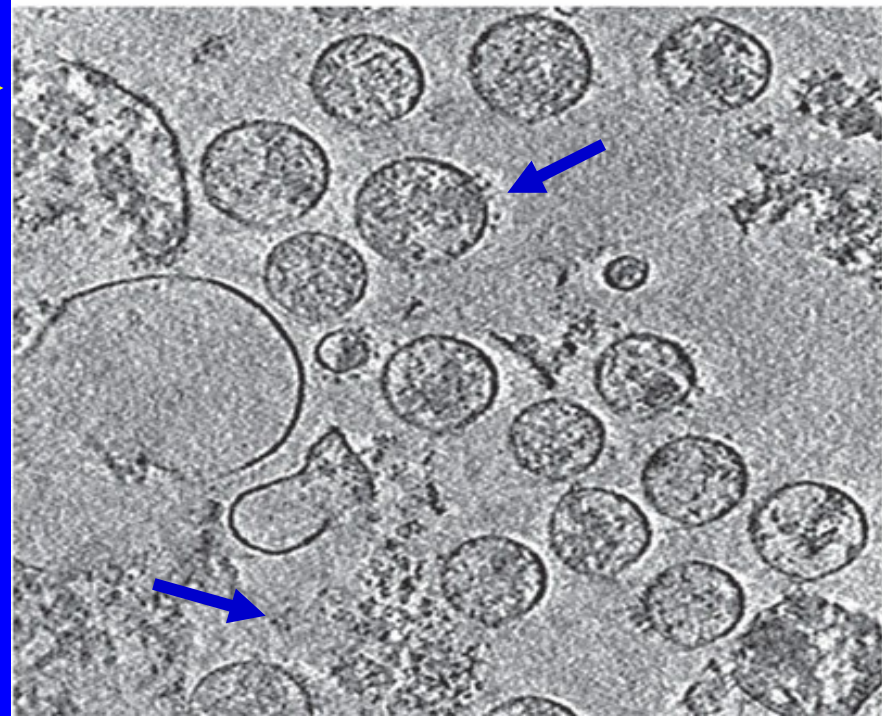
Kuznetsov YG *et al*. Atomic force microscopy investigation of human immunodeficiency virus (HIV) and HIV-infected lymphocytes. J Virol 2003;77:11896-909

SIV

(a monkey retrovirus)



HIV



Knobs "putative"

putative = supposedly

Zhu P et al. Distribution and three-dimensional structure of AIDS virus envelope spikes. *Nature* 2006; 441:847-52.

Summary of RT and particles

- RT not specific
- No agreement of particle taxonomy
- Particles, even with RT activity, not proof of isolation (Gallo, 1976)
- RV particles may appear in any culture, infected or non-infected
- Knobs fundamental to the definition of a retrovirus – absence of knobs
- Knobs absolutely necessary for infectivity – no infectivity – cannot be transmitted

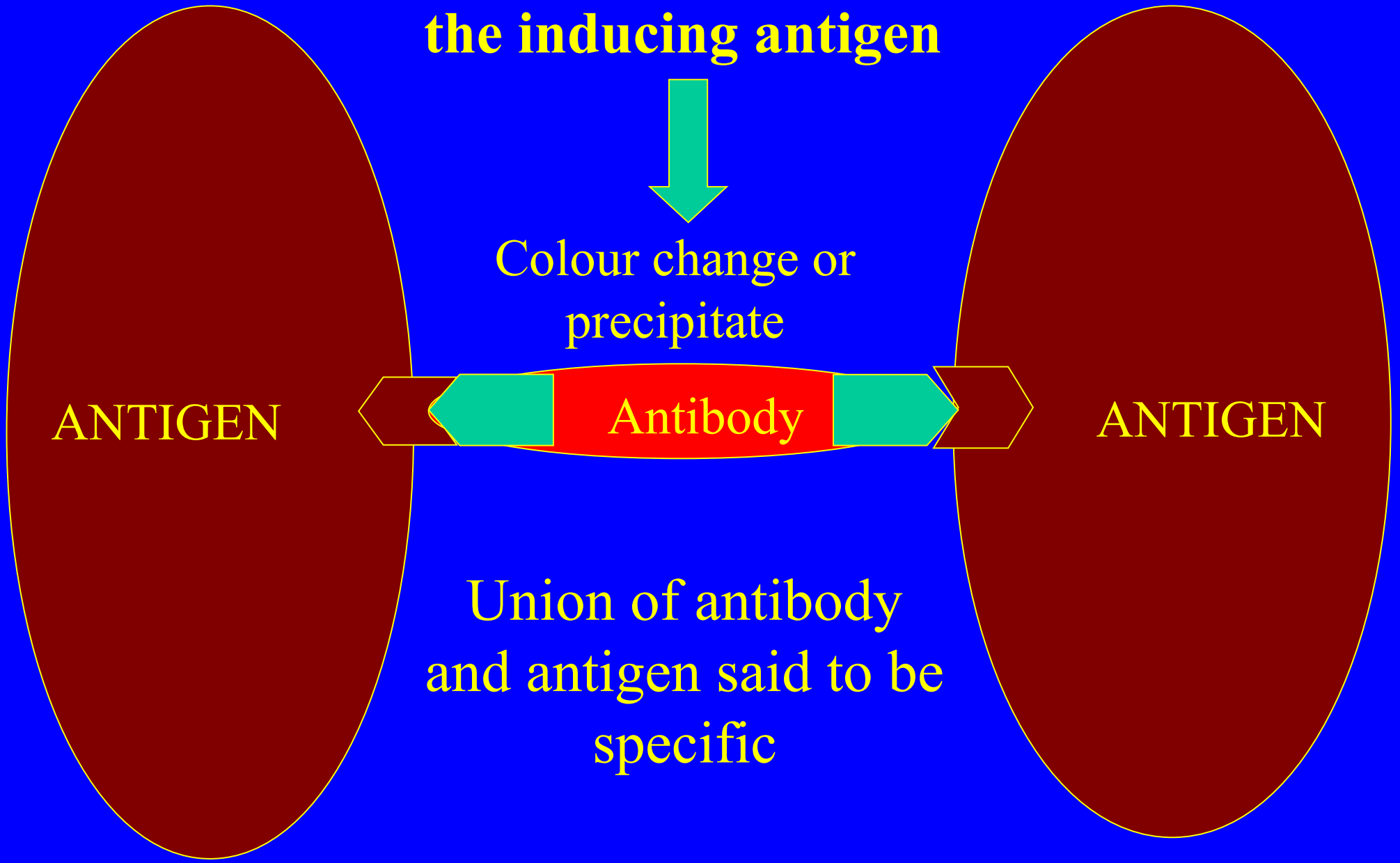
Gallo RC *et al*. Some evidence for infectious type-C virus in humans. In: Balimore D, Huang AS, Fox CF, editors. Animal Virology. New York: Academic Press Inc.; 1976. p. 385-405.

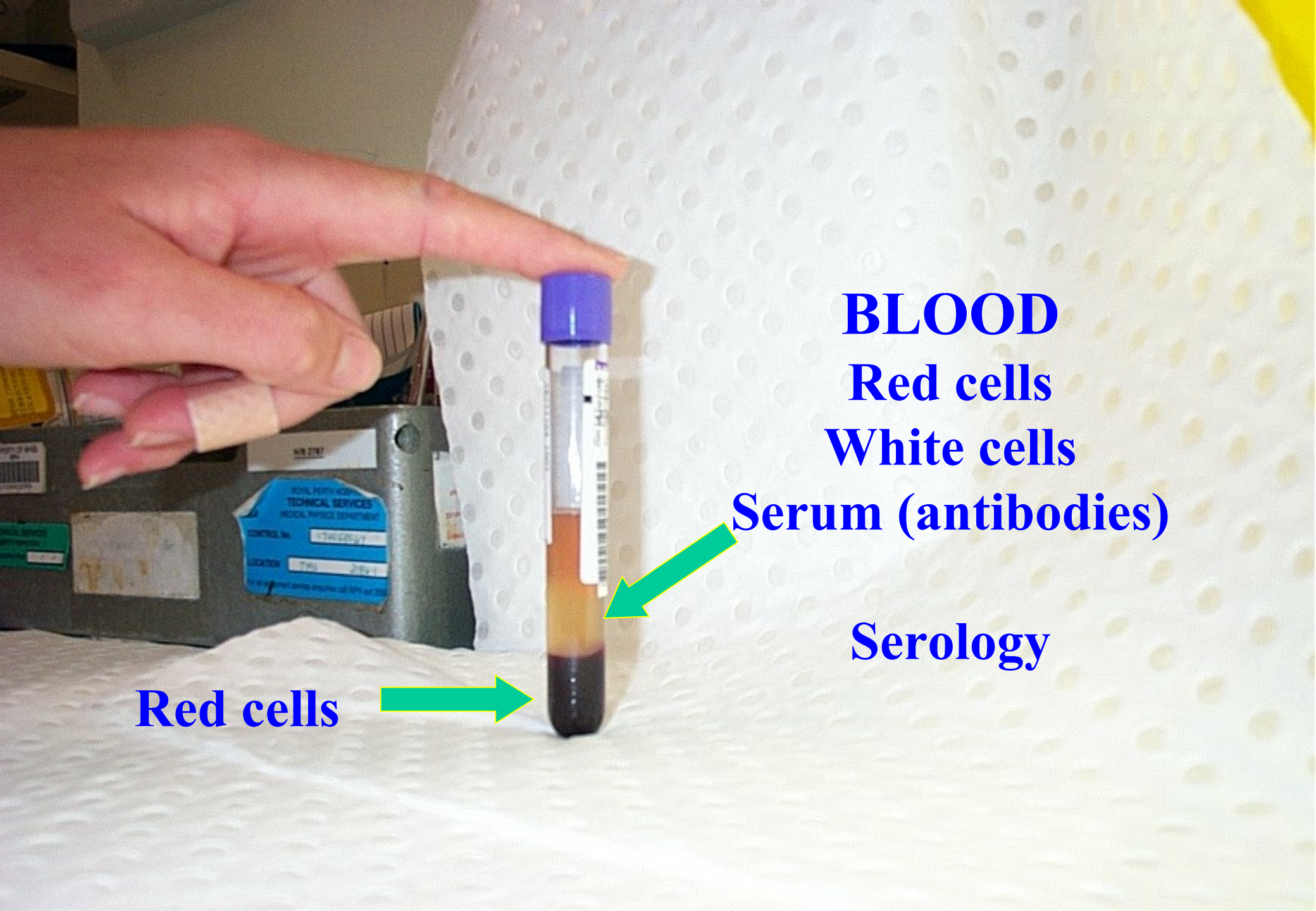
Antibodies and antigens

ANTIBODIES: The immune system responds to the presence of foreign material such as the proteins of bacteria and viruses by producing the proteins known as antibodies

ANTIGEN: Any substance that induces the formation of antibodies (from ANTIbody GENerating).

Antibodies react with the inducing antigen




BLOOD
Red cells
White cells
Serum (antibodies)
Serology
Red cells

Antibodies are not monogamous

"an antibody molecule made following the injection of one antigen frequently can combine also with a second antigen of a related or similar shape…In other words, the antibody cross-reacts with the second antigen"

Nossal GJV. Antibodies and Immunity. Harmondsworth, UK: Penguin Books Ltd, 1971: page 36

Antibodies are polyreactive

"The immunological community was shocked to find that antibodies would be polyreactive in binding to multiple antigens that were complex and ostensibly unrelated to one another"

This means:

1. Known antigens cannot identify antibodies

2. Known antibodies cannot identify antigens

Marchalonis JJ *et al.* Journal of Molecular Recognition 2001; 14:110-

The HIV proteins and antibodies

Montagnier claimed that the particles observed in the umbilical cord lymphocytes had proteins that were not present in the cells or in other retroviruses…

Thus…

the proteins are retroviral

and…

the retrovirus is new

Proof for the existence of HIV proteins

By definition viral proteins are those proteins found in viral particles

Cannot work with one viral particle
Need a mass of particles separated from all other sources of proteins
Virus particles must be separated from cellular material
Particles must be purified
Montagnier agrees

Luc Montagnier Pasteur Institute interview July 1997

Tahi: "But there comes a point when one must do the characterisation of the virus. This means: what are the proteins of which it's composed?"

Montagnier: *"That's it. So then analysis of the proteins of the virus demands mass production and purification. It is necessary to do that"*

Djamel Tahi. Videotaped Interview with Luc Montagnier. Pasteur Institute July 18th 1997. Continuum 1998;5:30-34.

DENSITY GRADIENT CENTRIFUGATION

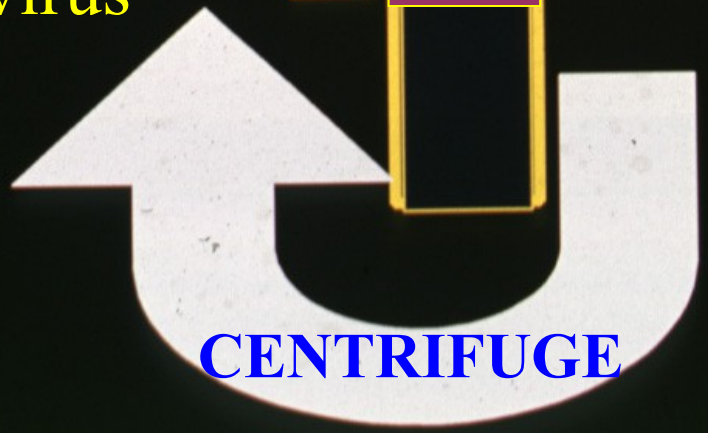
Long and well established laboratory procedure for purifying retroviral particles based on their characteristic density of 1.16 g/ml in sucrose solution

RETROVIRAL ISOLATION

Sample

1.16 g/ml density band = "purified virus"

BAND IN U/CENTRIFUGE
CONFIRM WITH EM
CONFIRM RT
PROVE PURITY



CENTRIFUGE

RVP BAND AT 1.16 GM/ML

Montagnier's one HIV protein

Found three proteins that reacted with antibodies

p24, p45, p80

p41/45 = cellular actin
p80 not discussed but not HIV
p24 = HIV

No reaction with HTLV-I (another human retrovirus)

Only p24 is HIV

No "one protein" viruses

Gallo 1984

Similar experiments to Montagnier

Similar findings

Use of H9 cell line in place of umbilicar lymphocytes

No EM of "purified virus"

Also type-C particles in the culture

GALLO 1984

p24

p41 –most specific but for Montagnier p41/p45 = actin

Subsequently several more p7, p17/18, p31/32, p39, p51, p55, p66, p120, p160

Electron micrograph (EM) of a density gradient purified retrovirus

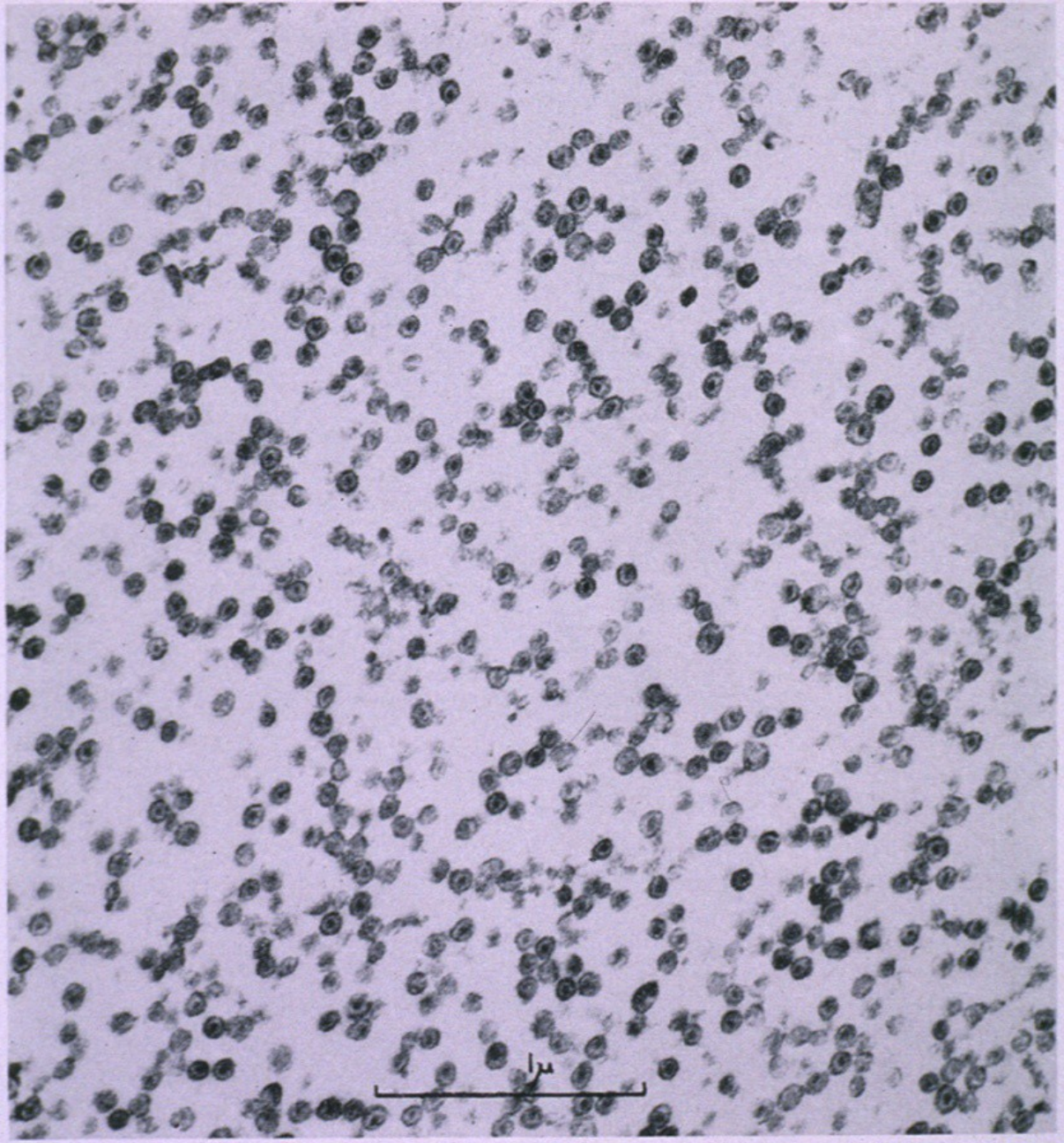


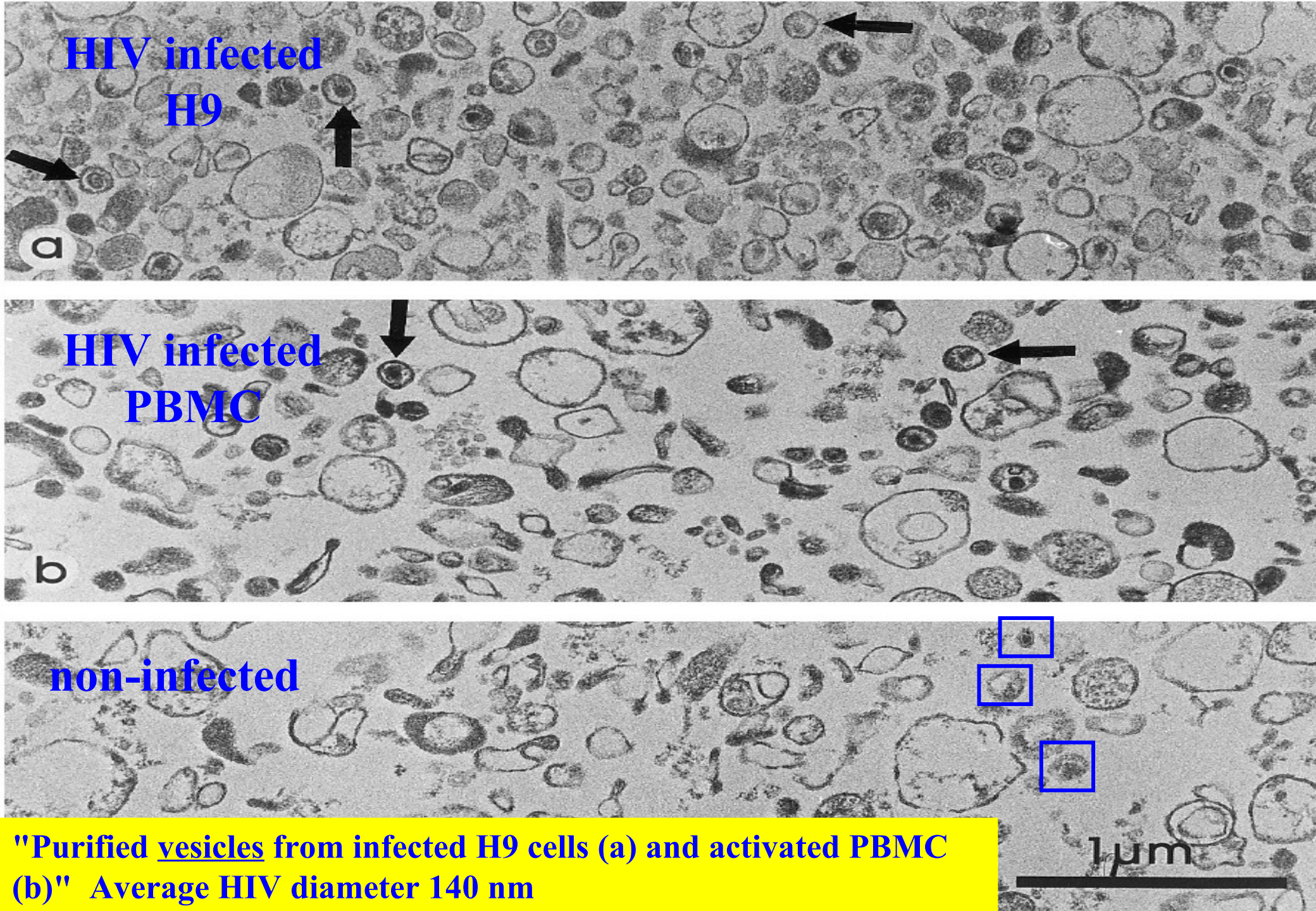
FIG. 1. Electron micrograph of preparation of Rous sarcoma virus. Fixed in potassium permanganate, sectioned and the section stained in uranyl acetate. The electron micrograph was taken with a Siemens Elmiscop II at an instrumental magnification of 15,000 times. Actual magnification of figure: × 30,000.

Crawford LV *et al*. The properties of Rous sarcoma virus purified by density gradient centrifugation. Virology 1961; 13:227-232

HIV has not been purified

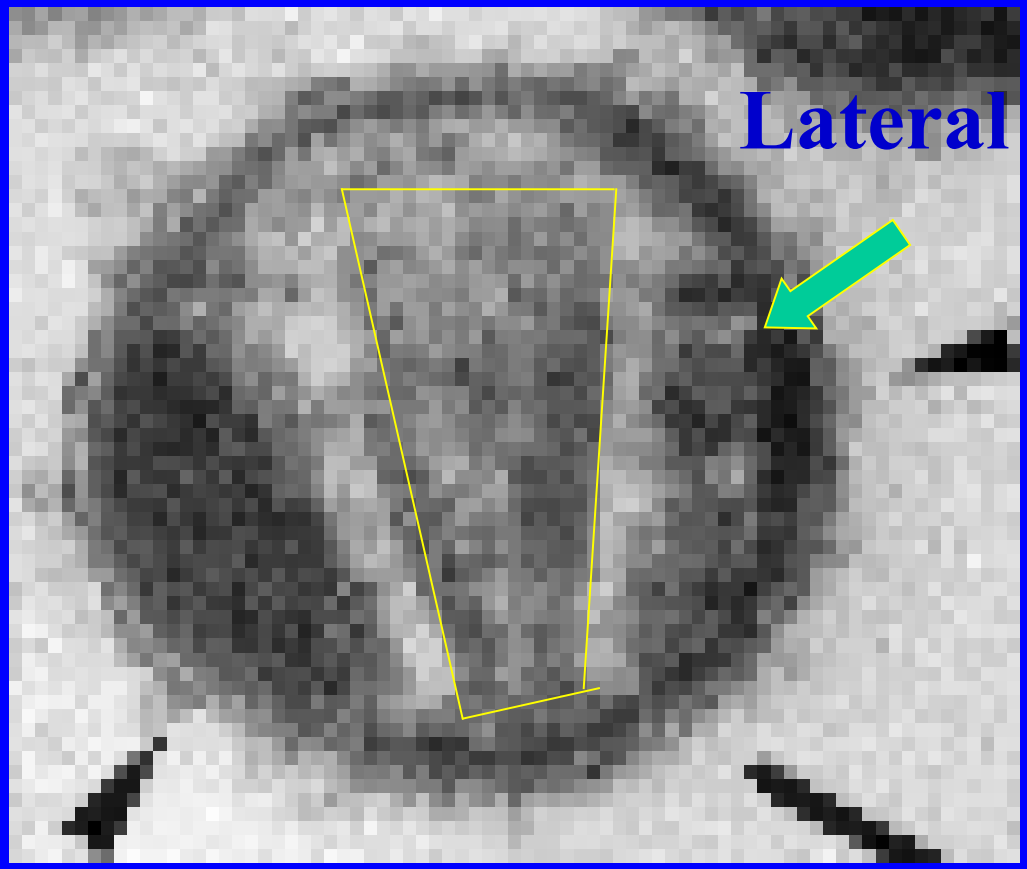
"Virus to be used for biochemical and serological analyses or as an immunogen [antigen] is frequently prepared by centrifugation through sucrose gradients", and that in none of the studies "has the purity of the virus preparation been verified"

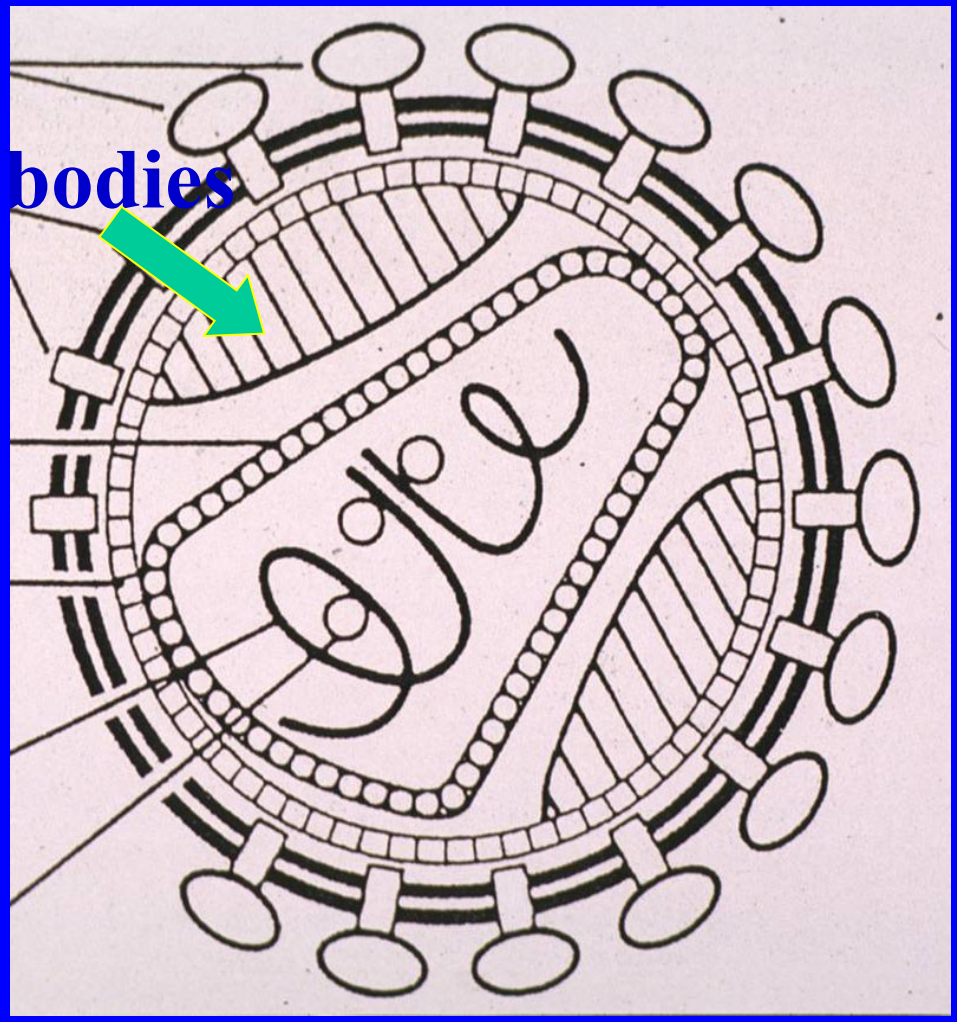
Gluschankof P *et al*. Cell membrane vesicles are a major contaminant of gradient-enriched human immunodeficiency virus type-1 preparations. *Virology* 1997;230:125-133

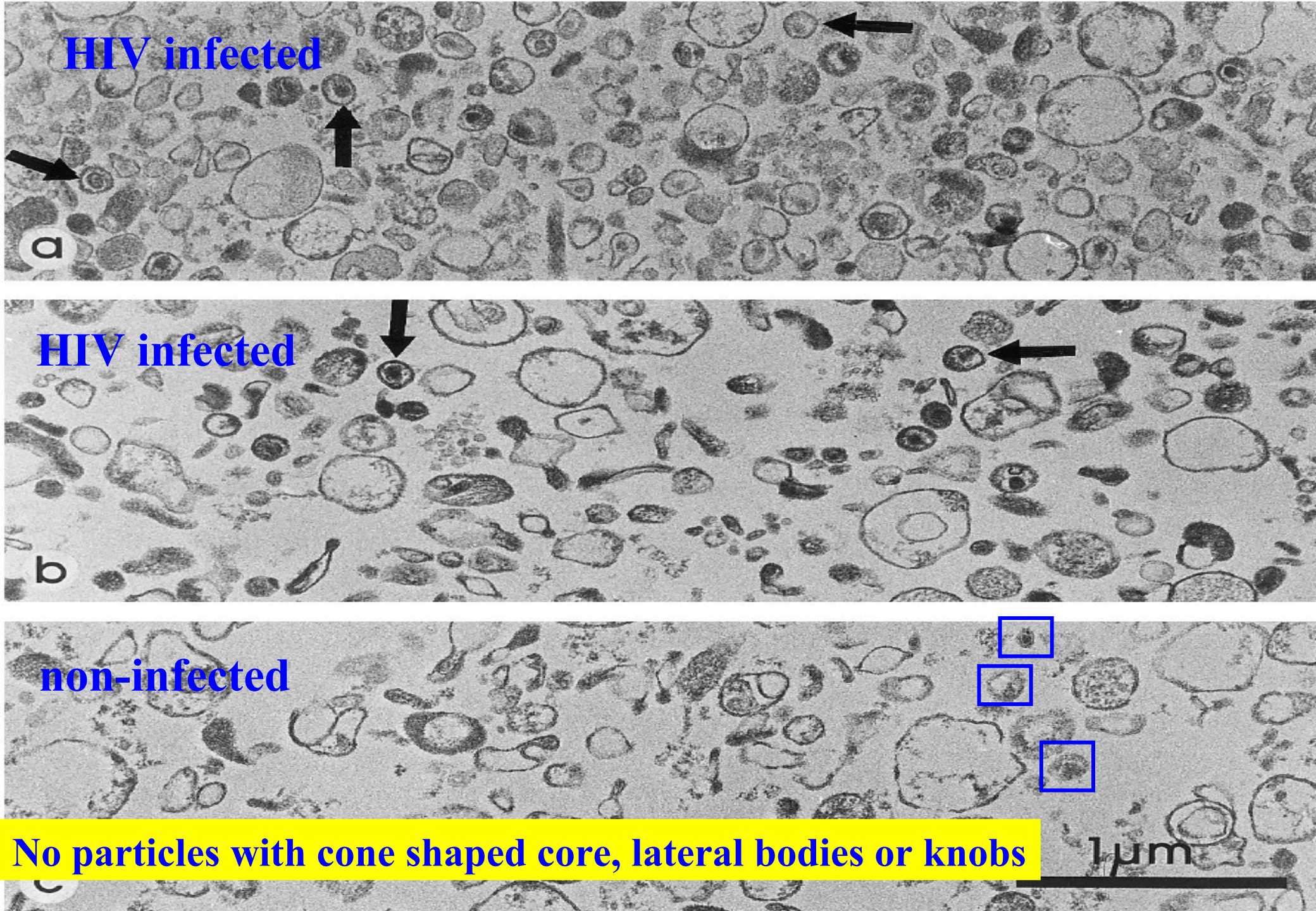


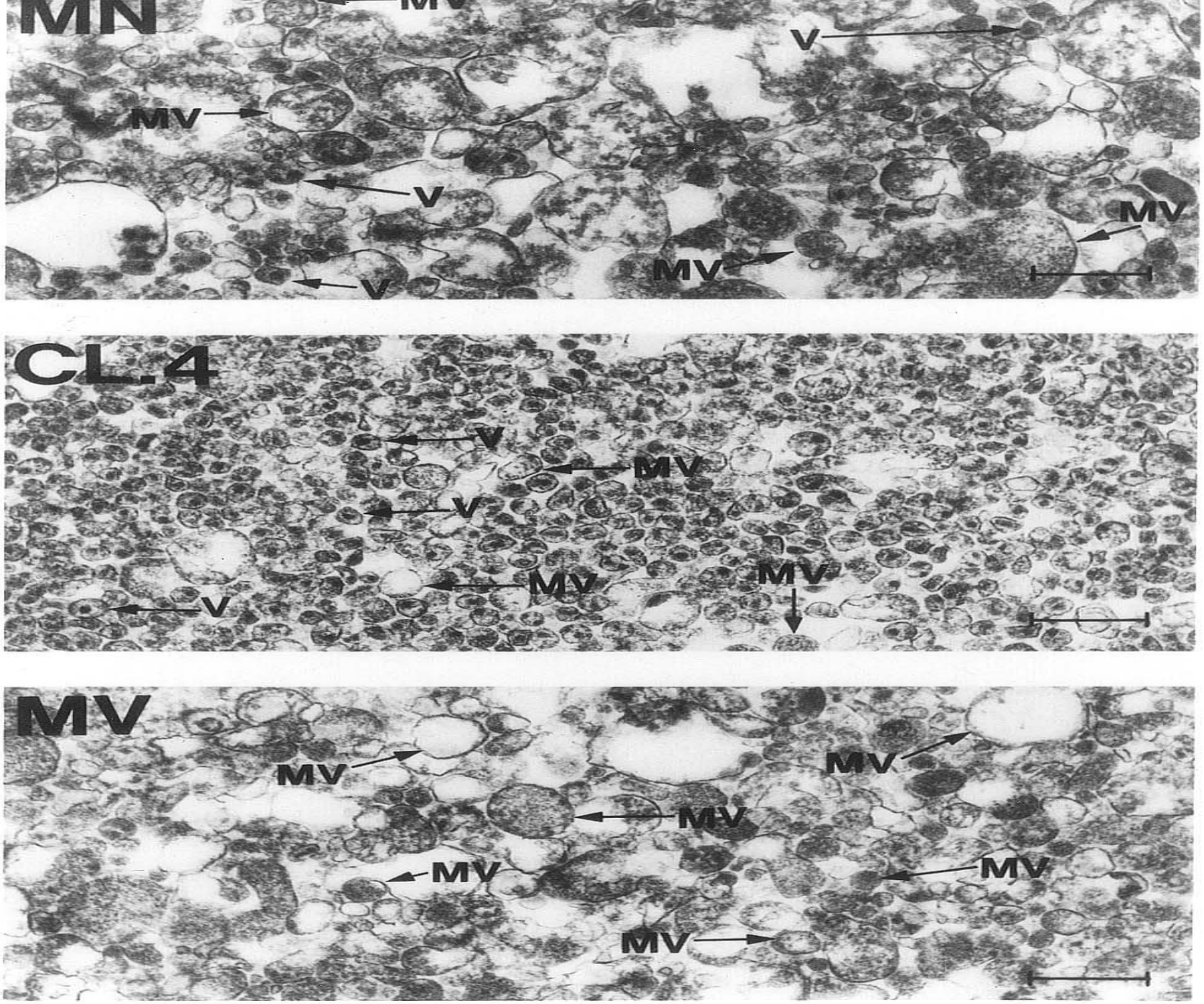
"Purified vesicles from infected H9 cells (a) and activated PBMC (b)" Average HIV diameter 140 nm

Lentiviral morphology






HIV infected
a
HIV infected
b
non-infected
No particles with cone shaped core, lateral bodies or knobs
1µm

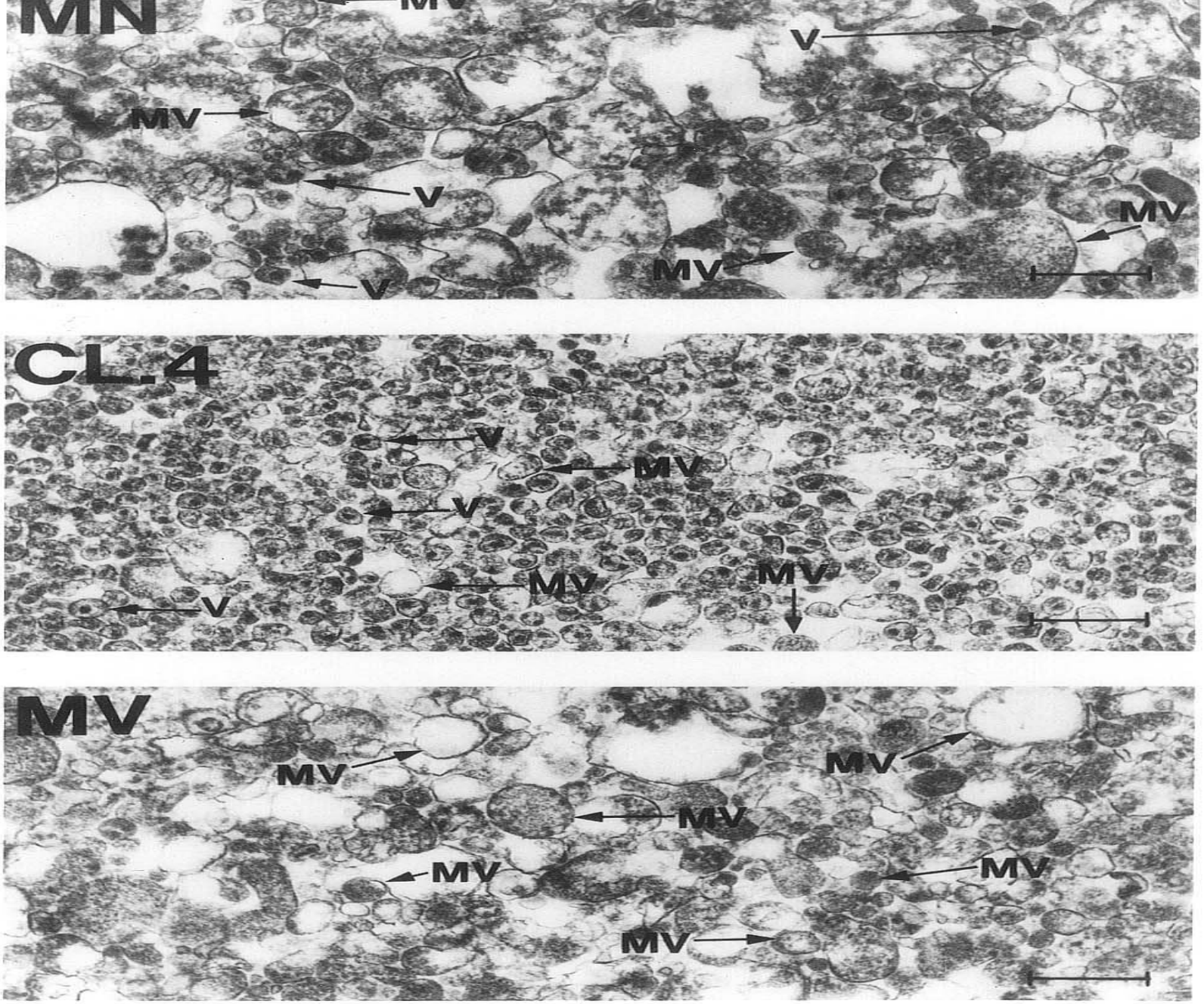


Bess, J. W et al *Virol.* **230**, 134-144 (1997).

MV= microvesicles = cellular fragments

Average HIV particle diameter 234 nm

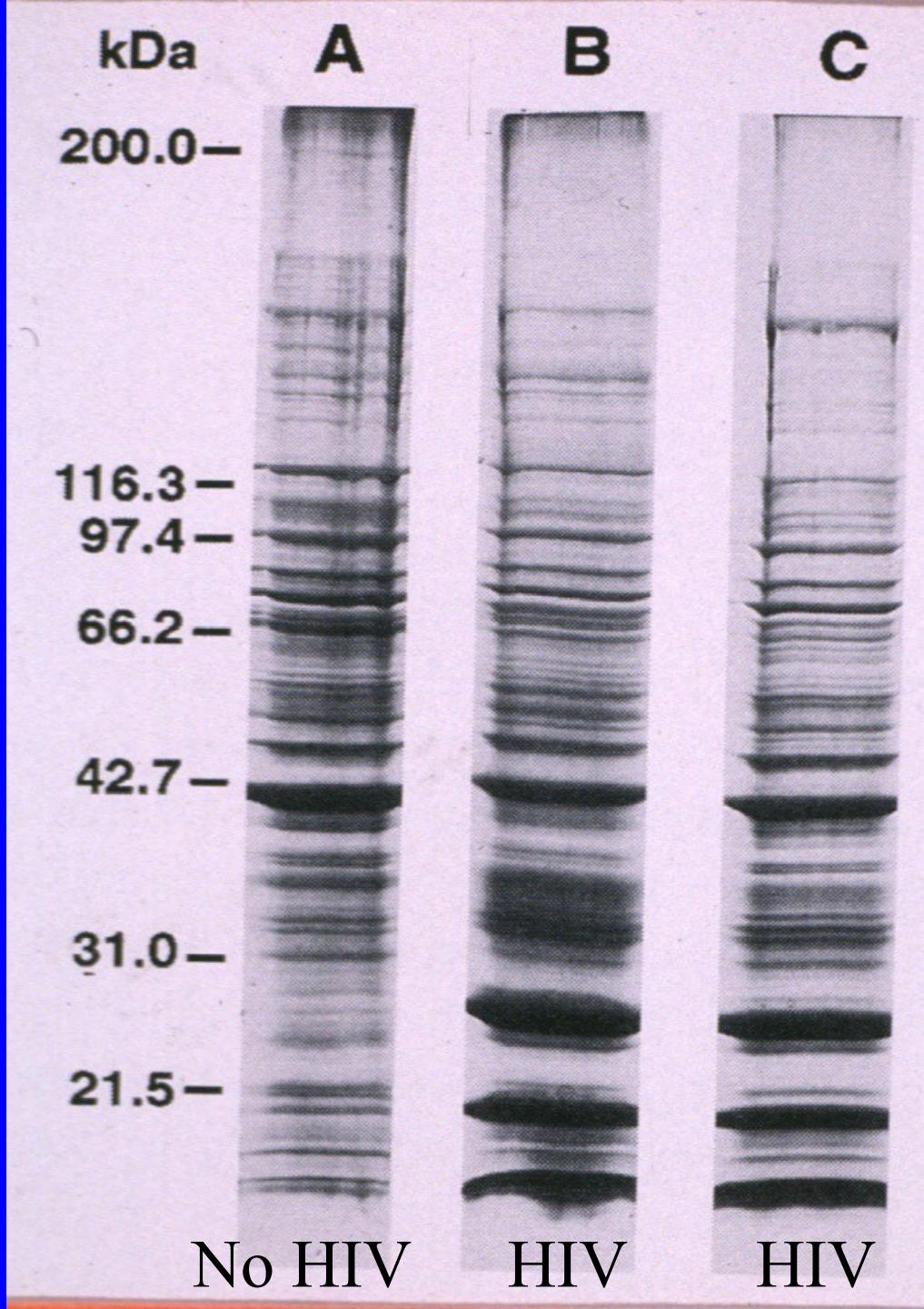
None less than 160 nm



No particles with cone shaped core, lateral bodies or knobs

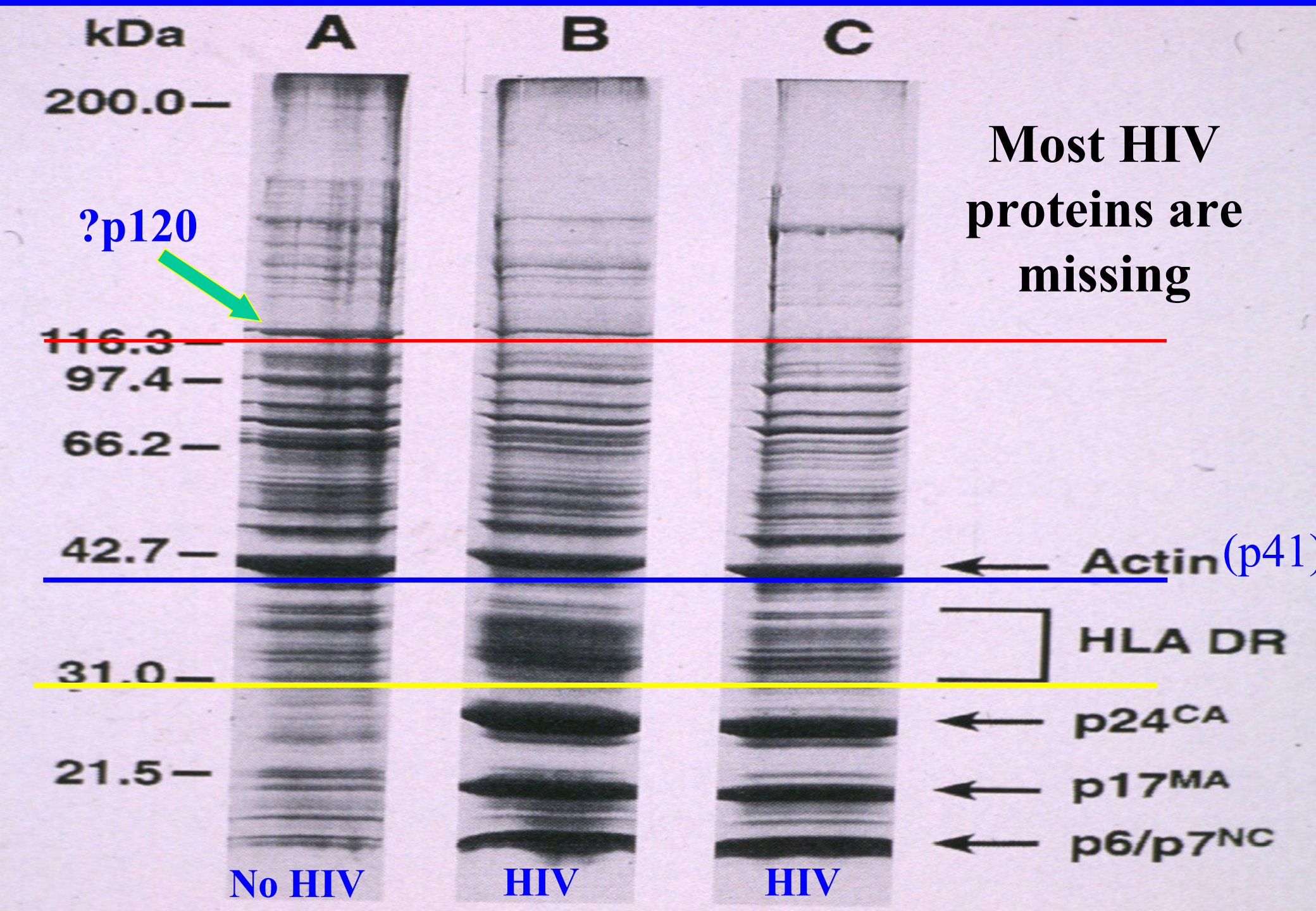
A: proteins from non-infected material

B and C: proteins from "purified virus"



Bess correspondence

"We agree that you can come to the conclusion from gel electrophoresis patterns that there are only quantitative differences between HIV and microvesicles" (the uninfected material).


kDa
A
B
C
200.0
116.3
97.4
66.2
42.7
31.0
21.5
?p120
Most HIV proteins are missing
Actin (p41)
HLA DR
p24CA
p17MA
p6/p7NC
No HIV
HIV
HIV

Bess correspondence

"Several bands are labeled as either Actin, HLA DR, p24CA, p17MA, or p6/p7NC and you are wondering how we determined the identity of these…these labels were added when one of the reviewers asked for them. He felt it would help orient readers when looking at the figure - the reviewer is correct. We did not determine the identities of the bands in this particular gel".

The HIV proteins

The gp120 and gp160 proteins integer subunits (trimers [X3] and tetramers [X4]) of gp41*

Constantine and Schupbach agree with Pinter

Constantine – "Other viral bands appear to be cell associated with the most common being in the molecular weight range of 70K, 51-55K (HLA DR)…"

Henderson** p32 chemical analysis → cellular protein HLA DR

*Pinter A *et al*. Oligomeric structure of gp41, the transmembrane protein of human immunodeficiency virus type 1. J Virol 1989;63:2674-9

**Henderson LE et al. Direct Identification of Class II Histocompatibility DR Proteins in Preparations of Human T-Cell Lymphotropic Virus Type III. J Virol 1987;61:629-632

Montagnier interview Pasteur Institute July 1997

Montagnier : "*We found some particles but they did not have the morphology typical of retroviruses*"

Montagnier: *I repeat we did not purify"*

Montagnier: *"I don't know if he [Gallo] really purified. I don't believe so*"

Djamel Tahi. Videotaped Interview with Luc Montagnier. Pasteur Institute July 18th 1997. *Continuum* 1998;5:30-34.

Montagnier interview Pasteur Institute July 1997

Tahi: Do EM pictures from the purification exist?

Montagnier: *Yes. Of course*

Tahi: Have they been published?

Montagnier: *I couldn't tell you…we have some somewhere but it is not of interest, not of any interest*

Djamel Tahi. Videotaped Interview with Luc Montagnier. Pasteur Institute July 18th 1997. *Continuum* 1998;5:30-34.

Gallo to Perth Group -- email correspondence

September 2003

"Montagnier subsequently published many EM pictures of purified HIV particles, as, of course, we did in our first papers. You have no need of worry. The evidence is obvious and overwhelming"

Charles Dauget

Electron microscopist for Montagnier group

Interviewed in Paris, December 2005

"Purified virus" = "Cellular debris"

No virus particles

"The most specific HIV protein p24"

At present HIV experts claim there are approximately twelve proteins unique to HIV

There is no evidence that proves this claim

The evidence is that the HIV proteins are cellular proteins

SUMMARY

Viruses are particles

Each type of virus particle has unique morphological characteristics

Even today no agreement exists as to what are the morphological characteristics of the particles said to be HIV

No HIV particle has all the morphological characteristics of retroviruses.

Knobs are fundamental to the definition of a retrovirus – No knobs on the HIV particles

Retrovirus-like particles may appear in any culture infected or not infected

SUMMARY

Viruses are infectious particles (transmissible)

Particles, even with RT are not proof they are viruses (Gallo, 1976)

Knobs absolutely necessary for infectivity – No knobs on the HIV particle

The only evidence for transmission and "isolation"– RT activity in consecutive cultures

RT not specific – It may be detected in hundreds of consecutive cultures even if not infected

Gallo RC *et al*. Some evidence for infectious type-C virus in humans. In: Balimore D, Huang AS, Fox CF, editors. Animal Virology. New York: Academic Press Inc.; 1976. p. 385-405.

SUMMARY

HIV proteins

Each virus contains unique proteins

Purification absolutely necessary to prove their existence

No proof for HIV purification

The evidence is that the HIV proteins are cellular proteins

CONCLUSION

No proof for the existence of unique HIV particles

No proof for HIV transmission

No proof for the existence of unique HIV proteins

No proof for the existence of a unique human retrovirus

EXTRA

Antibody promiscuity and diagnostic serology

Another inconvenient truth?

Proof of the existence of a retrovirus requires purification of the retroviral particles

http://theperthgroup.com/LATEST/PGRevisitHIVExistence.pdf

END