A personal journey through coronavirus evolution

The Sir Arnold Theiler Memorial Lecture

Let me introduce myself

My name is Marian (♂)

Horzinek (ž),

I am a Dutchman by choice…

…a Pole by birth (1936)

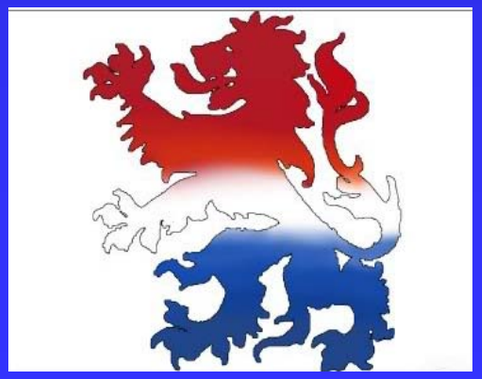
became a German by annexation (1939)

lived in the “German Democratic Republic” (1945)

studied in the Federal Republic of Germany (1951)

worked in Venezuela (1967)

and finally emigrated to the Netherlands (1971)



I graduated in veterinary medicine,

but (fortunately for the animals) never worked as an animal doctor -

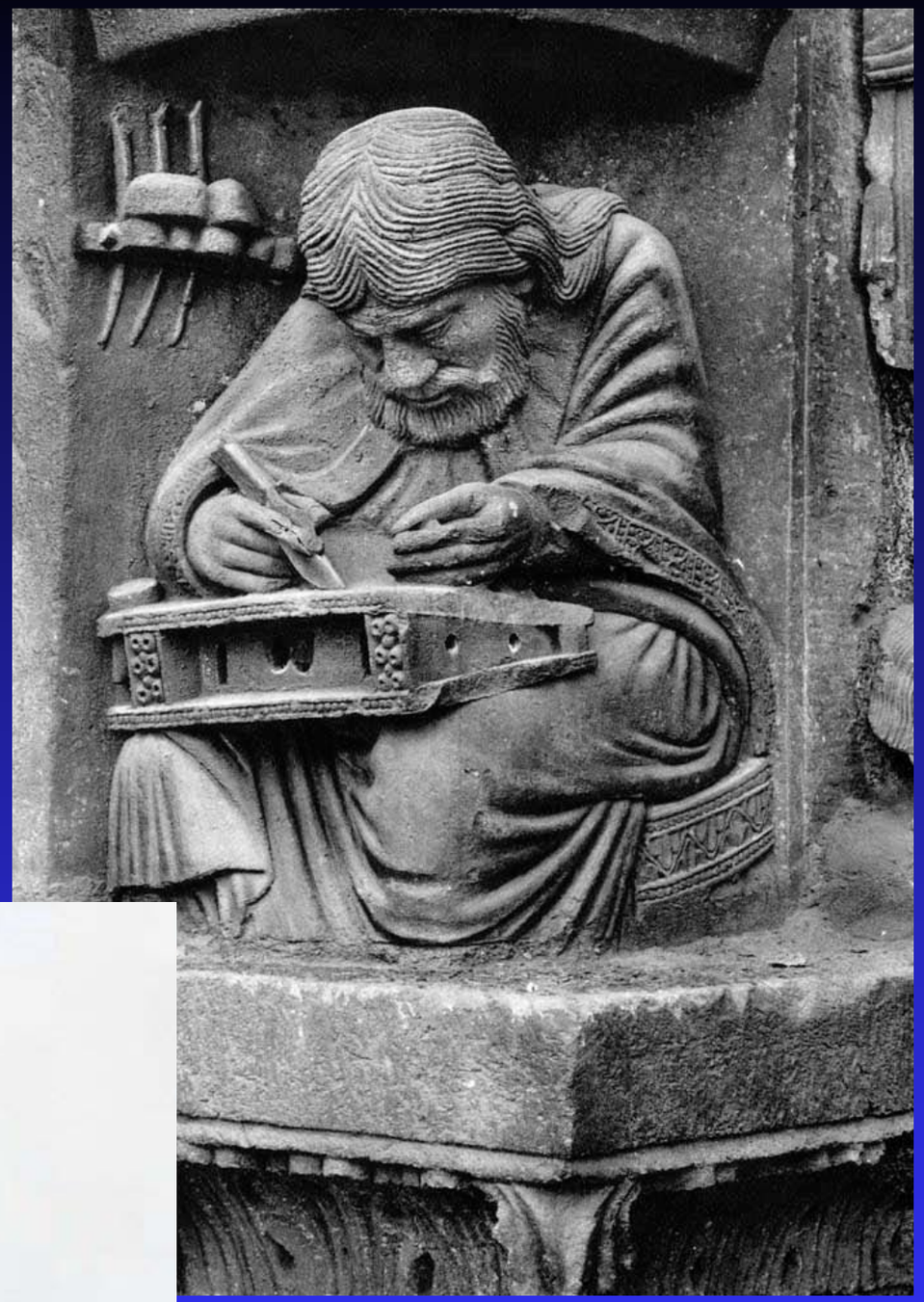
I went into science

and became a virologist

…but my professional evolution continued: from a laboratory worker,
I became a writer and speaker (oratory)

Virology laboratory,

Veterinary University Vienna/Austria, 2010



Pythagoras

Chartres, XII century

In Utrecht, it all started with a disease: Feline Infectious Peritonitis (FIP)

which is fatal in most (clinical) cases
its biology was poorly understood
prevention (still) is difficult

It is an enigmatic disease:

a sporadic fatal viral condition is a contradiction in terms

antibodies are of no benefit for the cat

they may even precipitate disease, causing the 'early death' phenomenon

Clinical signs

extended abdomen

undulating, unresponsive fever

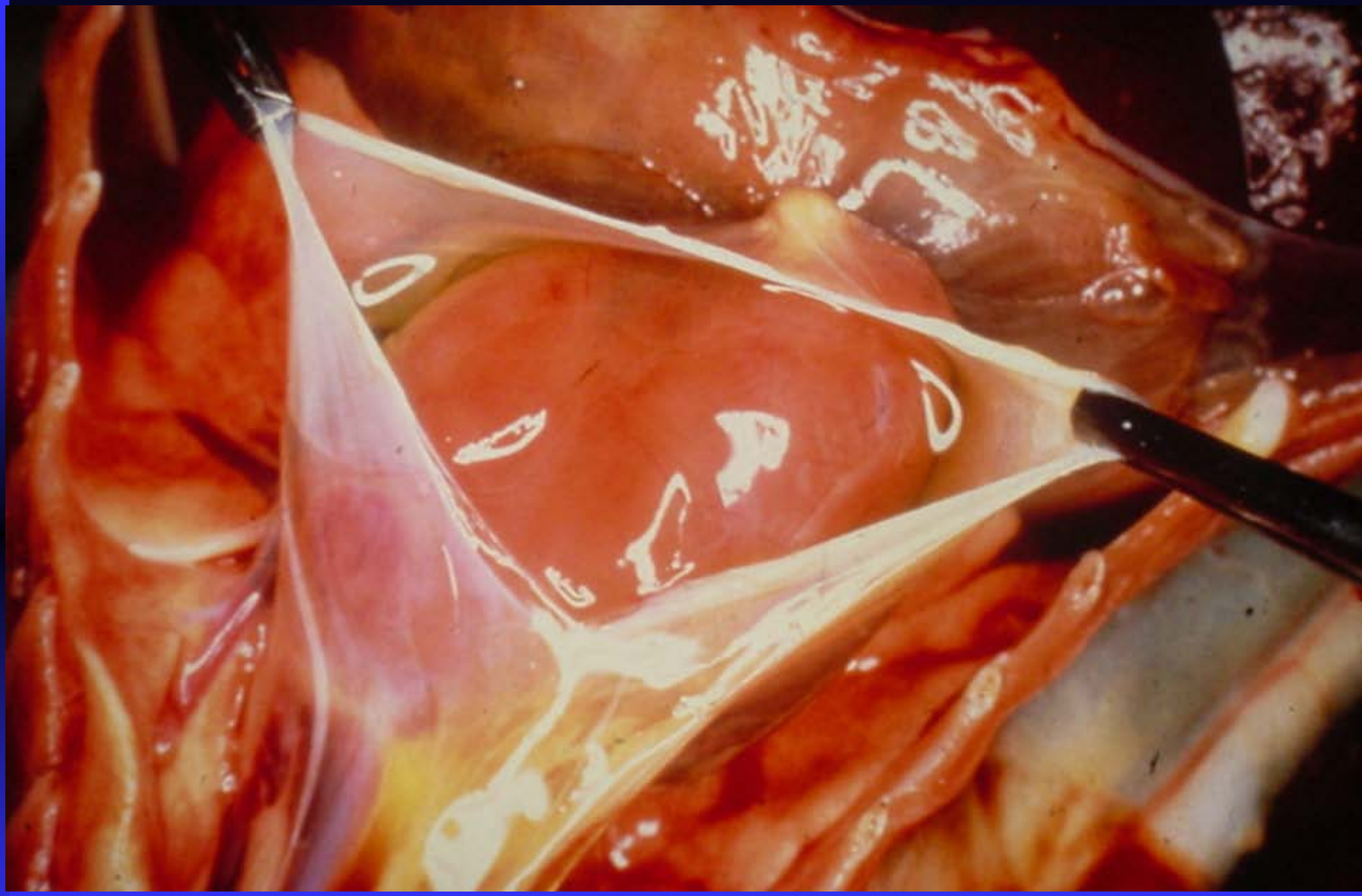
anorexia, emaciation

malaise

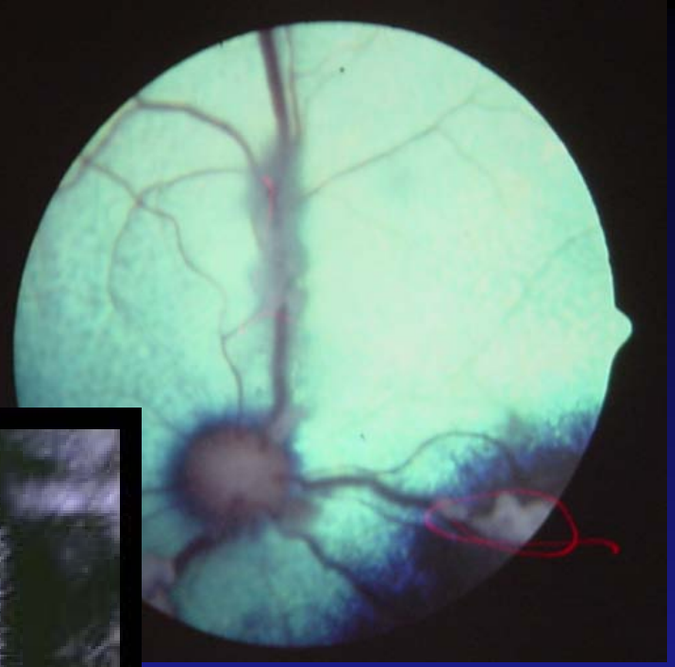
ocular/neurologic symptoms, icterus

wet form: polyserositis with effusions

dry form: disseminated pyogranulomas

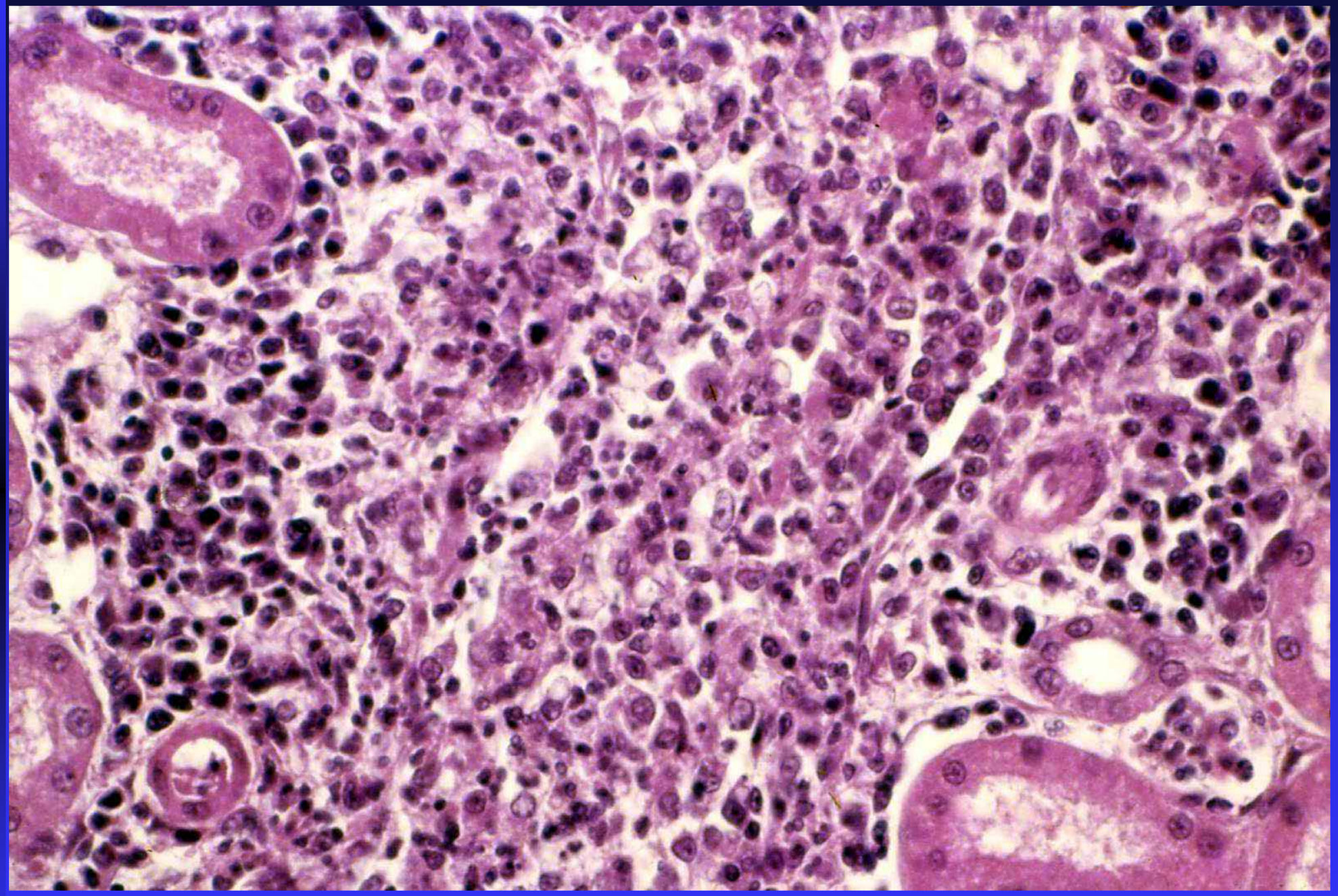


dry



FIP

wet



The discovery (1977): FIP is caused by a coronavirus

Zbl. Vet. Med. B, 24, 398—405 (1977)
© 1977 Verlag Paul Parey, Berlin und Hamburg
ISSN 0514—7166/ASTM-Coden: ZVRBA2

From the Institute of Virology
Director Prof. Dr. M. C. Horzinek

of the State University Utrecht
and the Central Veterinary Institute, Virology Department,
Director Prof. Dr. J. G. van Bekkum

Lelystad, The Netherlands

Feline Infectious Peritonitis Virus

By

Marian C. Horzinek, Albert D. M. E. Osterhaus and Daniel [illegible]

With 6 figures and 3 tables

(Received for publication October 15, 1976)

Introduction

Feline infectious peritonitis (FIP) was described as a
entity only ten years ago (9); it is a variably progressive, u
tion affecting domestic and wild Felidae, characterized b
depression and ascites. Prominent pathologic changes ar
peritonitis, mesothelial hyperplasia and focal necrosis in
(for literature see 5, 8). The infectious nature of the disea
epidemiologic observations; evidence for its viral etiolog
mission experiments with filtrates passing through 200
(10). In thin-sections through histiocytes, macrophages
from pathologic lesions virus particles were observed el
(7, 8); their etiologic rôle was established recently
experiments of virus grown in cat peritoneal cell cu
dy was undertaken to show by density gradient
microscopy that the virus found in
for classification of coro
suspensions of th

Zbl. Vet. Med. B, 24, 835—841 (1977)
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ISSN 0514—7166/ASTM-Coden: ZVRBA2

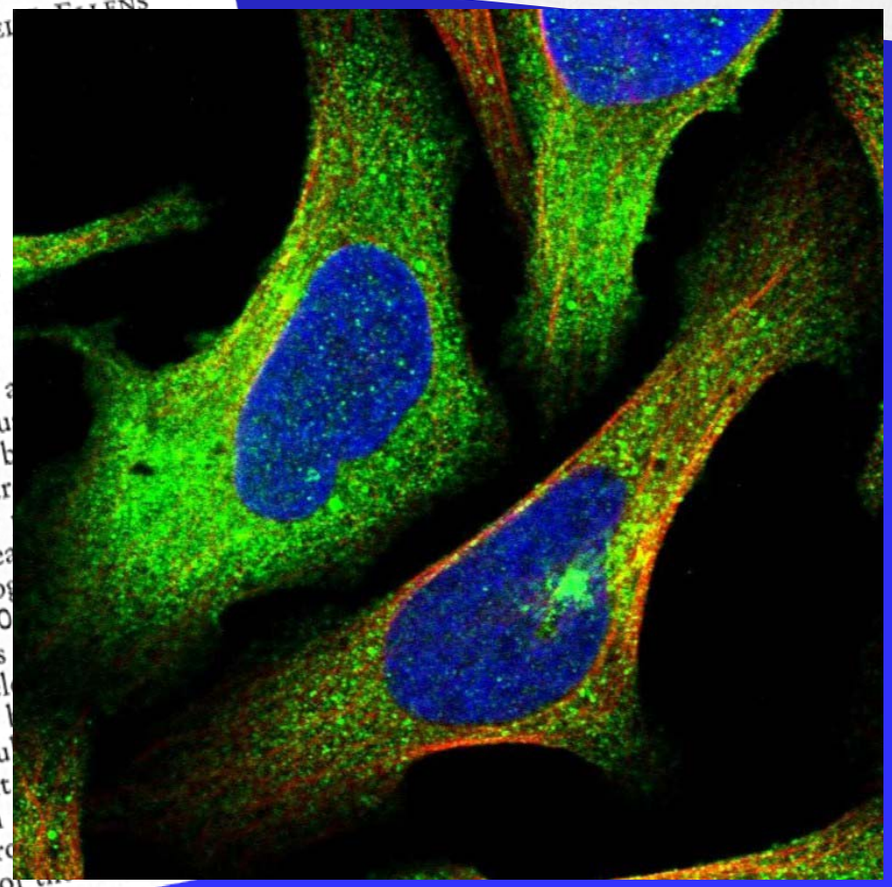
From the Institute of Virology
State University Utrecht, The Netherlands
Director: Prof. Dr. M. C. Horzinek

and the Institute for Research on Animal Diseases
Compton, Newbury, Berkshire, Great Britain
Director: J. M. Payne, B. Sc., Ph. D., M. R. C. V. S.

Seroepidemiology of Feline Infectious Peritonitis Virus Infections Using Transmissible Gastroenteritis Virus as Antigen

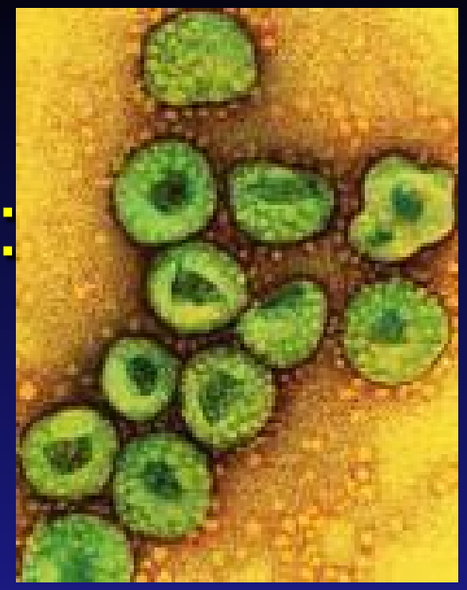
By

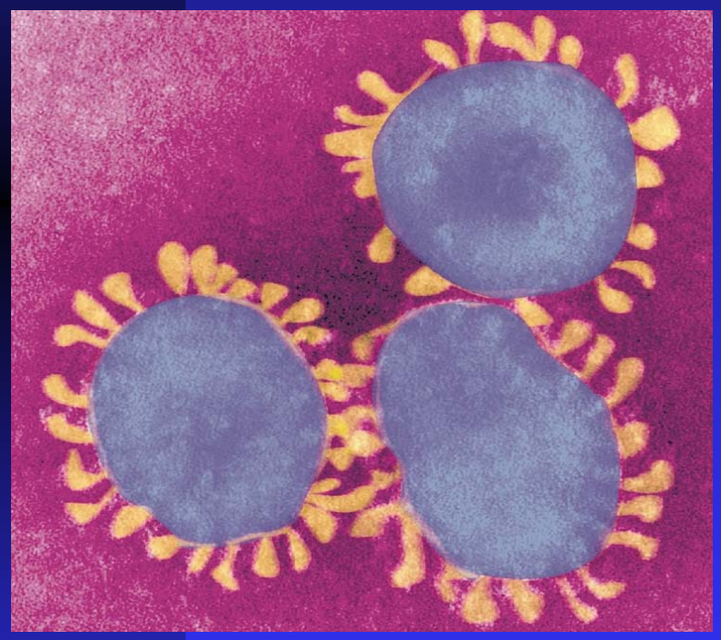
Albert D. M. E. Osterhaus, Marian C. Horzinek and Debby J. Reynolds

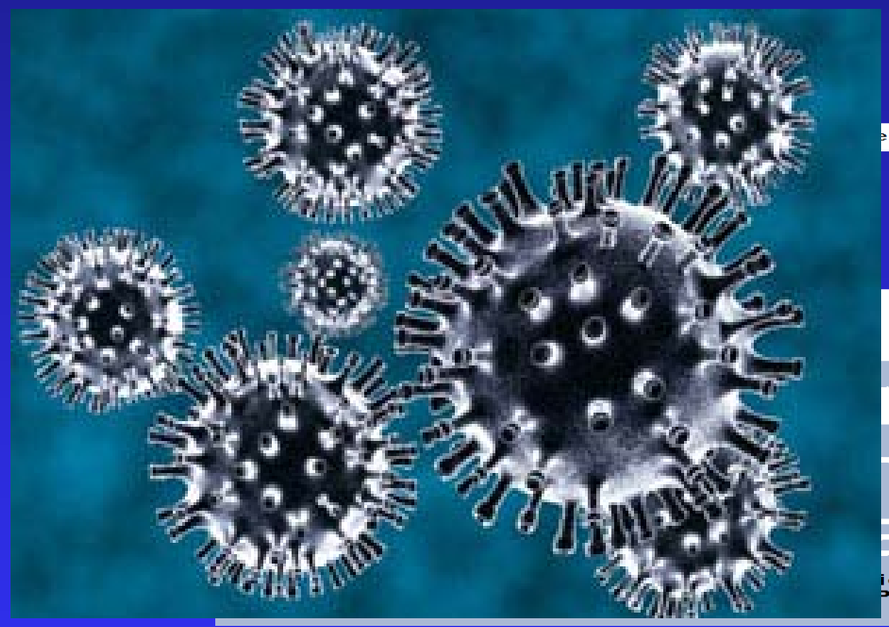


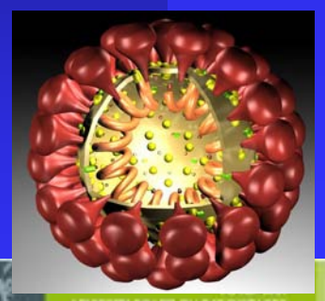
The result of this discovery was threefold:

1. we started to work on feline viruses
2. we focused on coronaviruses
3. we became fascinated by viral evolution

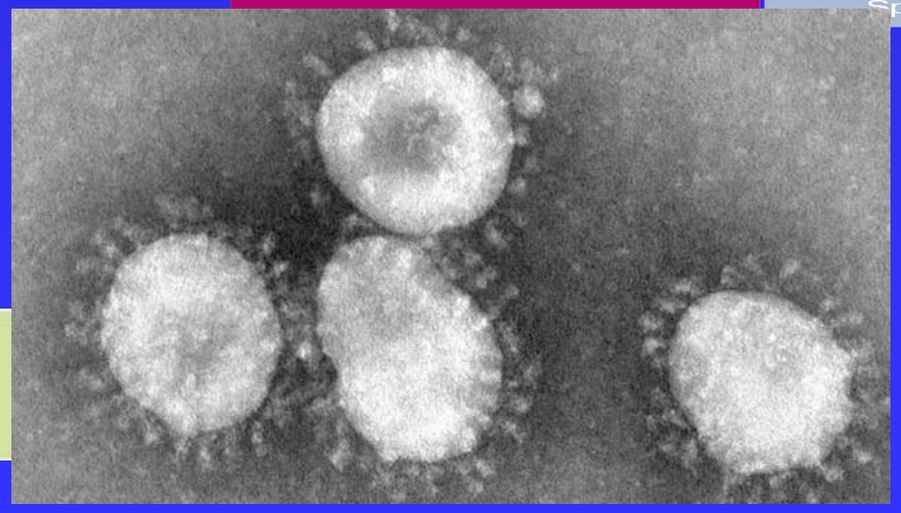




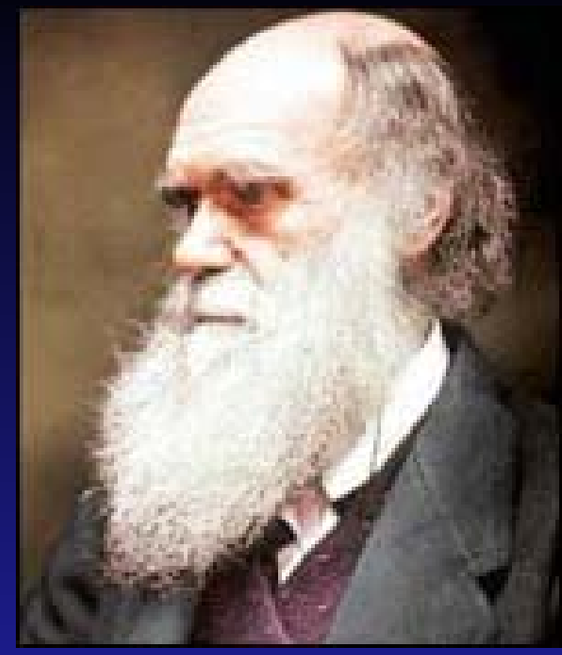




ADVISORY BOARD ON CAT DISEASES
ABCD



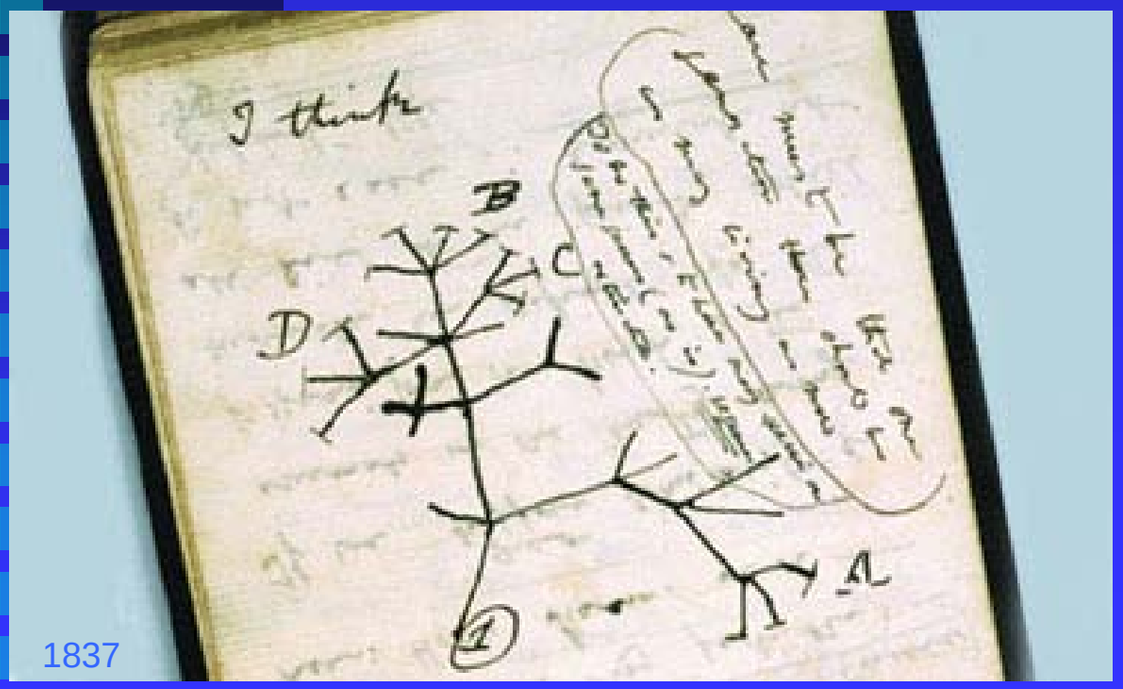
following the Darwinian adage:



“…nothing in biology makes sense except in the light of evolution…”

Theodosius Dobzhansky

(1900-1975)



I should like to entertain you about coronavirus evolution

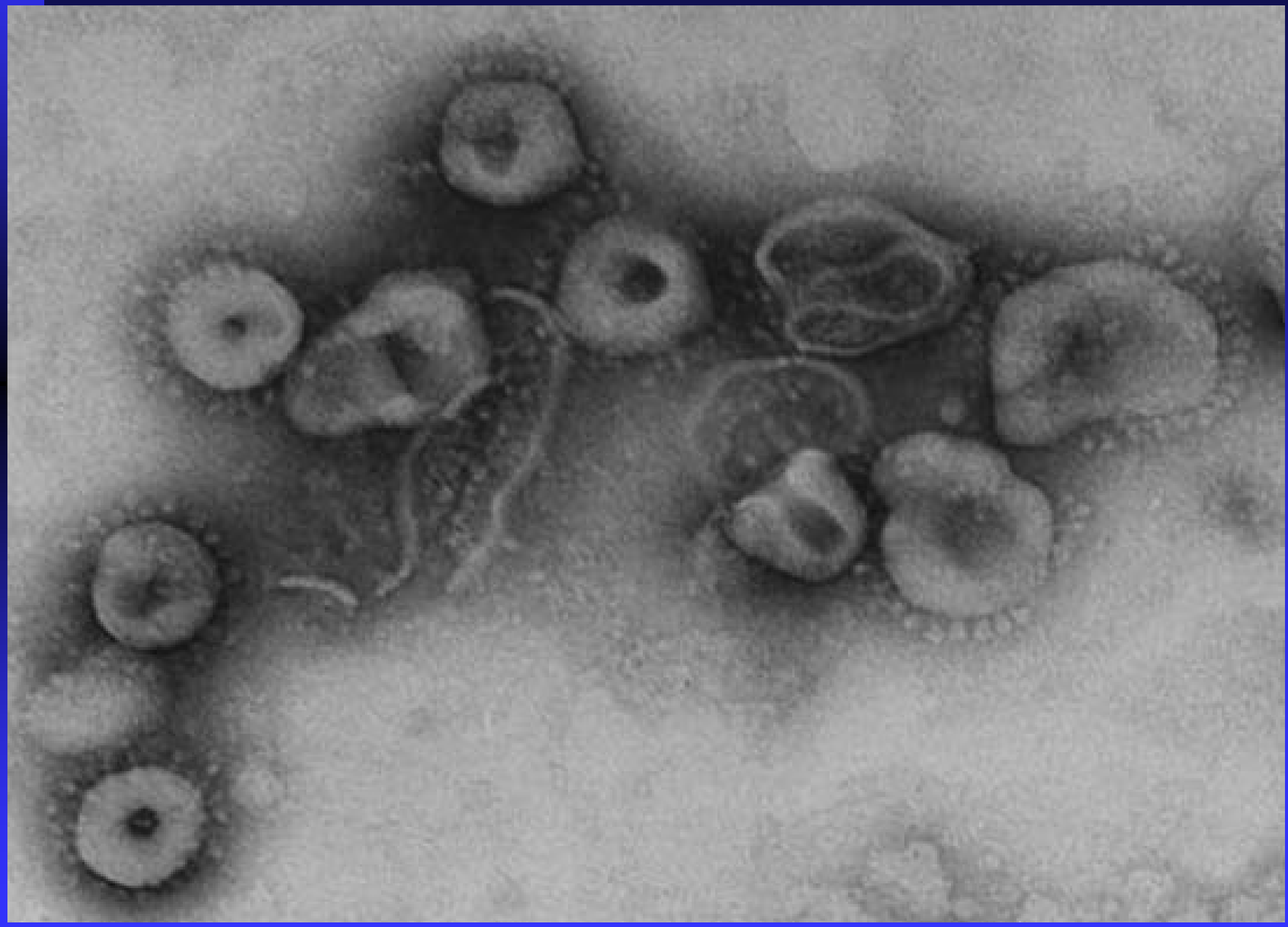
as it leads to new* diseases

in individual animals: pathogenesis (FIP)

in the field: epidemiology (TGEV/PEDV; SARS; MERS)

*new in the sense: hitherto unknown to science

Why coronaviruses?



Because

they are the largest enveloped, positive-stranded RNA viruses

with the largest viral RNA known to science,

and thus: the highest probability of making genetic mistakes (errors - mutations)

without a proof-reading mechanism to correct them

Mutation frequencies

10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1}

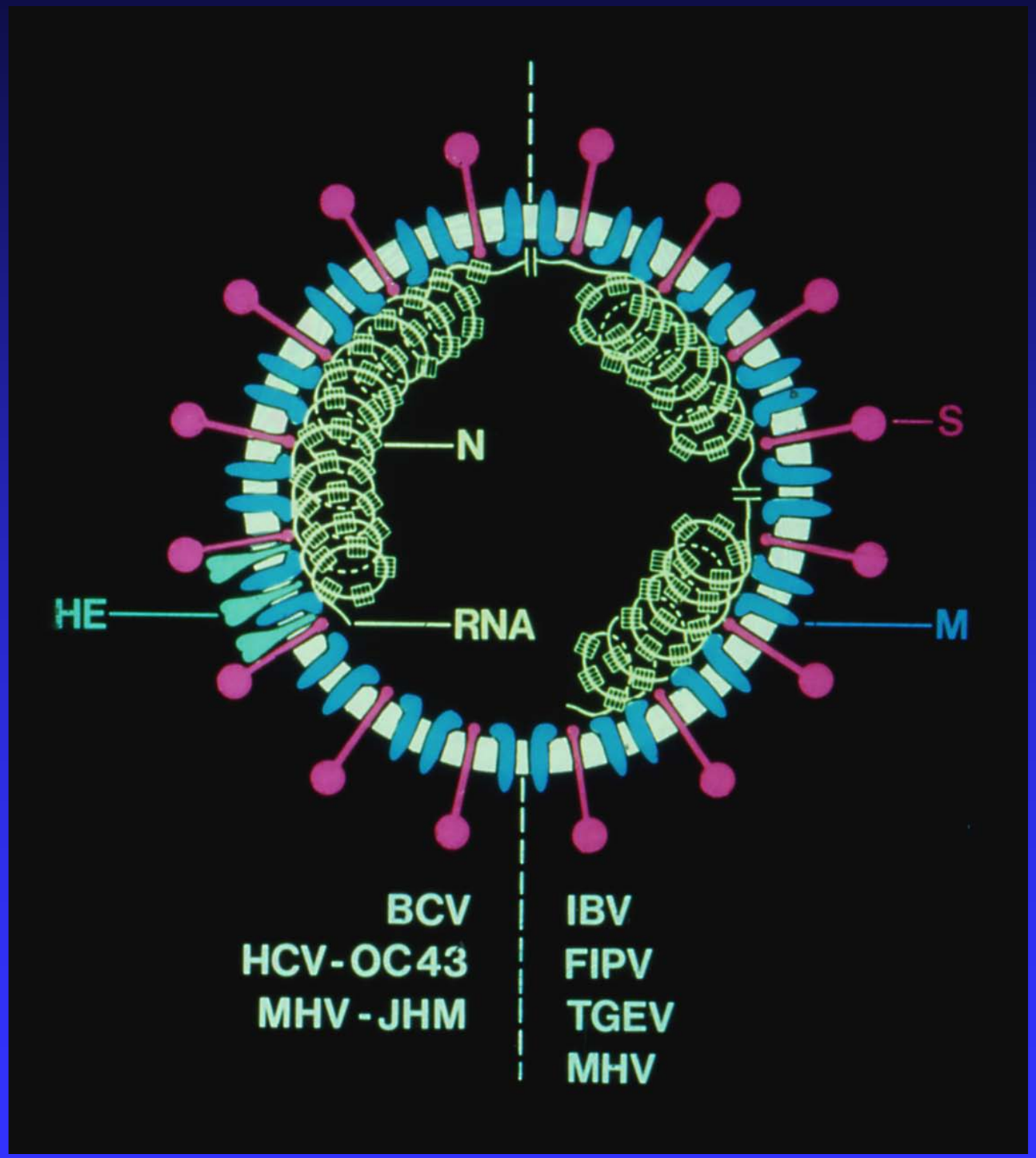
Cellular DNA

RNA virus genomes

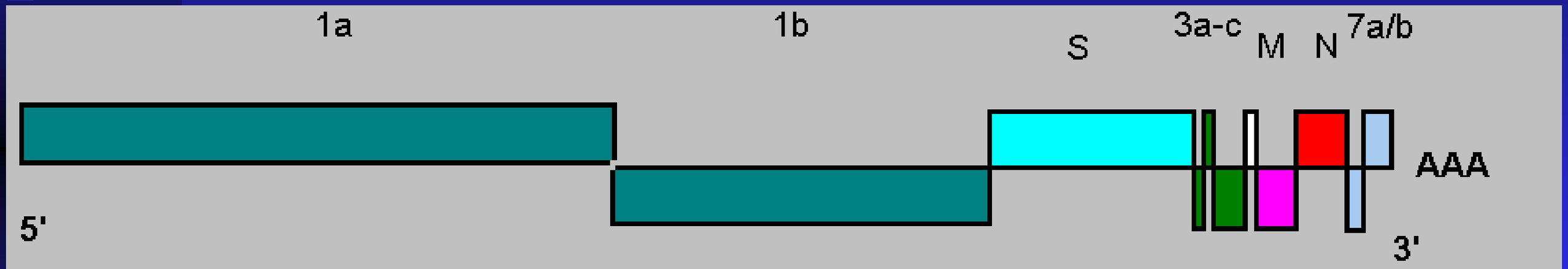
Suppression of proofreading/repair activities

Low fidelity polymerases

Anatomy of the coronavirion



The genome of a feline coronavirus



Genes:

S – spike

M – membrane

N – nucleocapsid

1a/b – polymerase

3a-c - nonstructural

7a/b - nonstructural

Evolutionary “behaviour” of coronaviruses

Occupation of new ecological niches through change in tropism (deletions; point mutations; recombinations)

TGEV – gut to lung

FIPV – enterocyte to macrophage

SARS-CoV: “species jumping” civet to human

MERV-CoV: “species jumping” bat to camel to human

Deletions:

Transmissible Gastroenteritis Virus (TGEV) of swine is found in feces of pigs ≤ 8 wk after recovery

but has been isolated from lungs > 3 mo p.i. - meaning virus persistence

1984: a “new” respiratory coronavirus was identified in pigs in Belgium,

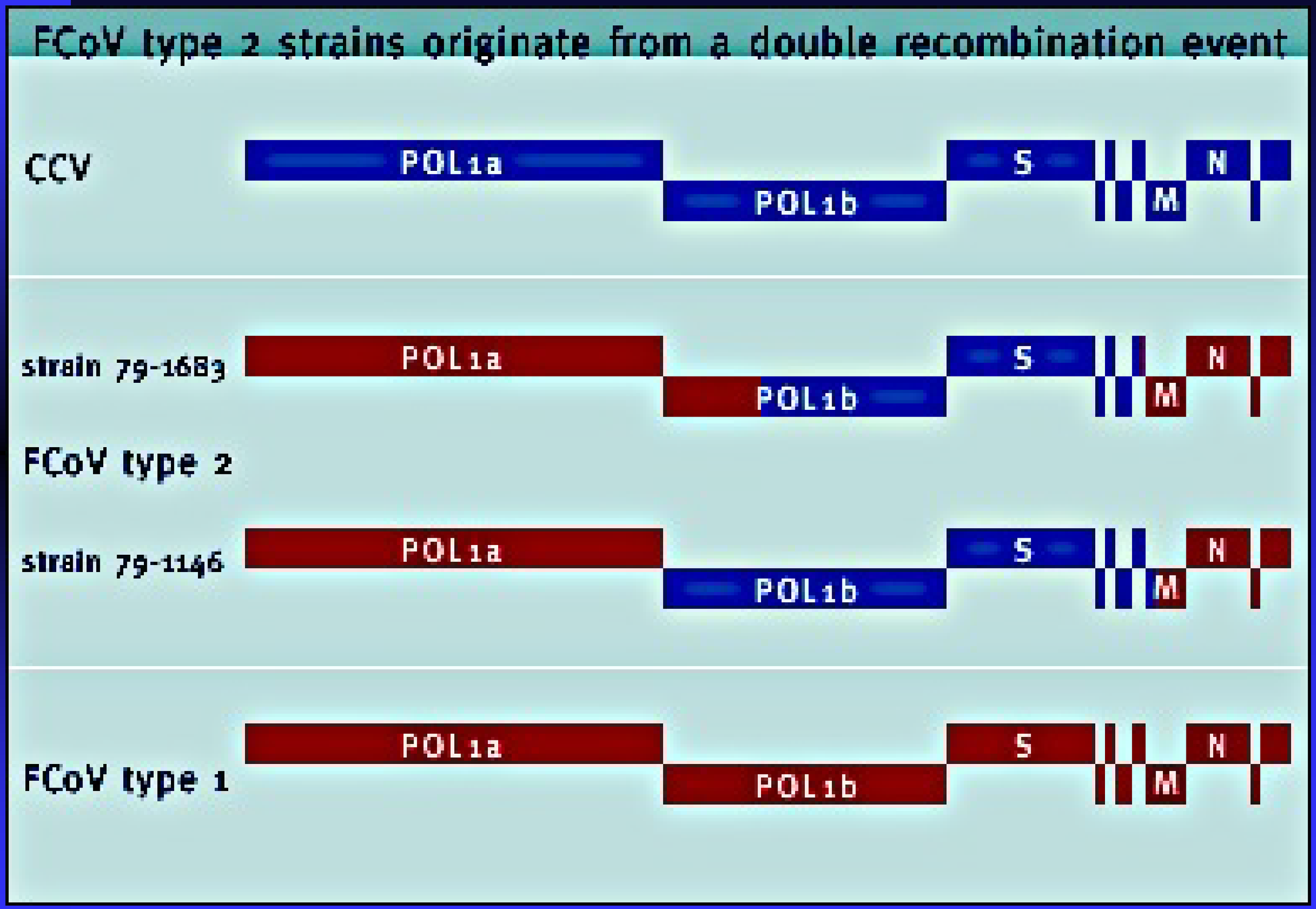
with ≈700nt deletions in the S gene, but conservation of neutralisation-relevant epitopes

The respiratory variant has displaced the enteropathogenic parent virus in all pig populations thereby acting as a “natural vaccine”

Interspecies transmission and genomic recombination:

CCV = canine coronavirus

FCoV = feline coronavirus


FCoV type 2 strains originate from a double recombination event
CCV
POL1a
POL1b
S
M
N
strain 79-1683
POL1a
POL1b
S
M
N
FCoV type 2
strain 79-1146
POL1a
POL1b
S
M
N
FCoV type 1
POL1a
POL1b
S
M
N

Feline (enteric) coronavirus infections

are widespread - seropositive cats in:

catteries: >90%

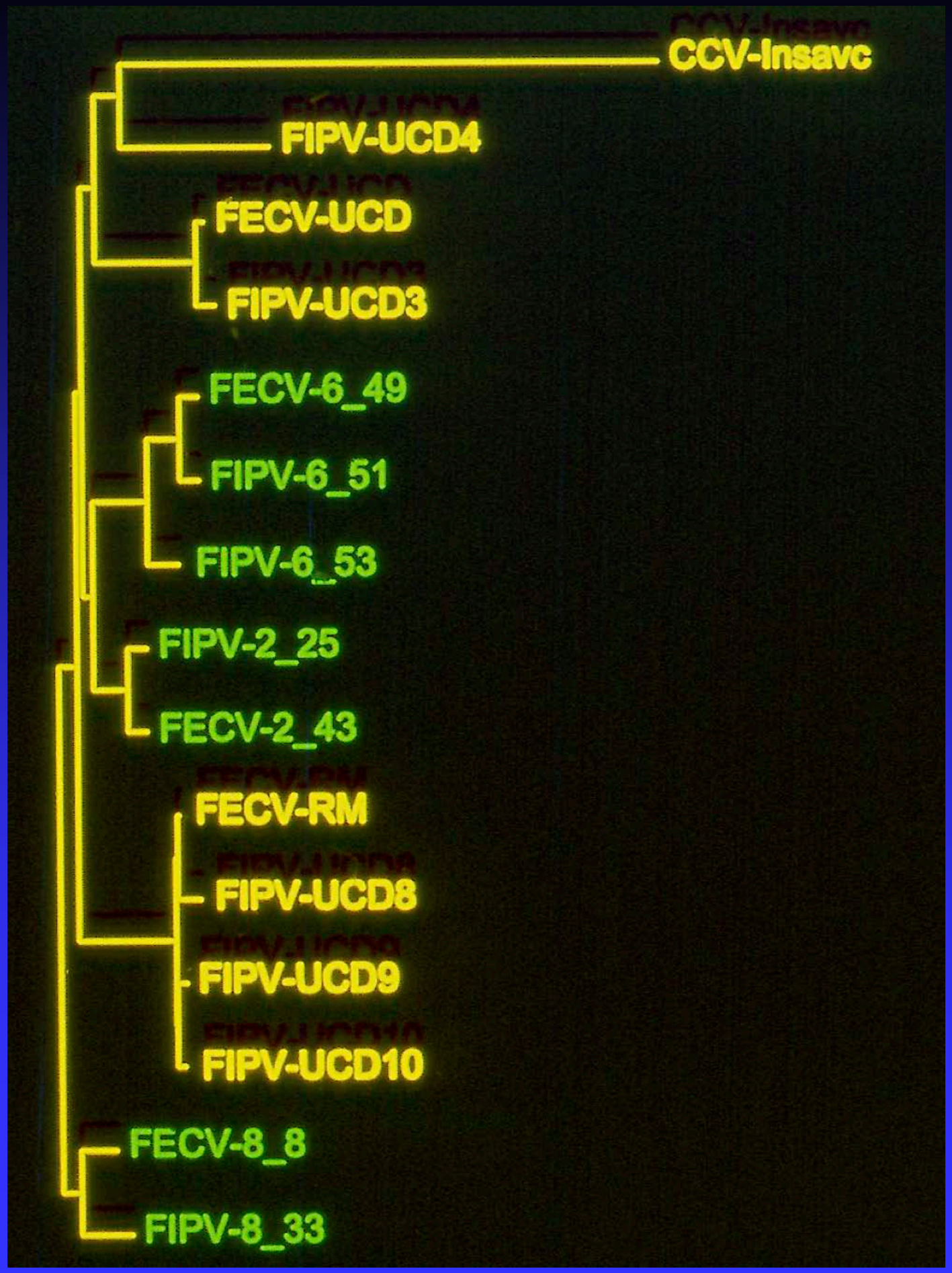
single-cat households: <25%

cause FIP only rarely:

in 1 – 5% of the seropositive cats

in the young and the very old

The close phylogenetic relationships between FECV/FIPV pairs in isolates from kitten litter mates



Peritonitis-causing feline coronaviruses

are *in vivo* mutants occurring in individual, persistently infected cats

e.g. when cell-mediated immunity is suppressed (such as under “crowding” stress, after FeLV- or FIV- infections)

arise stochastically, under conditions that allow expansion of the so-called “quasispecies cloud”

Manfred Eigen:
1967 Nobel Prize in Chemistry

Statistical geometry in sequence space: A method of quantitative comparative sequence analysis

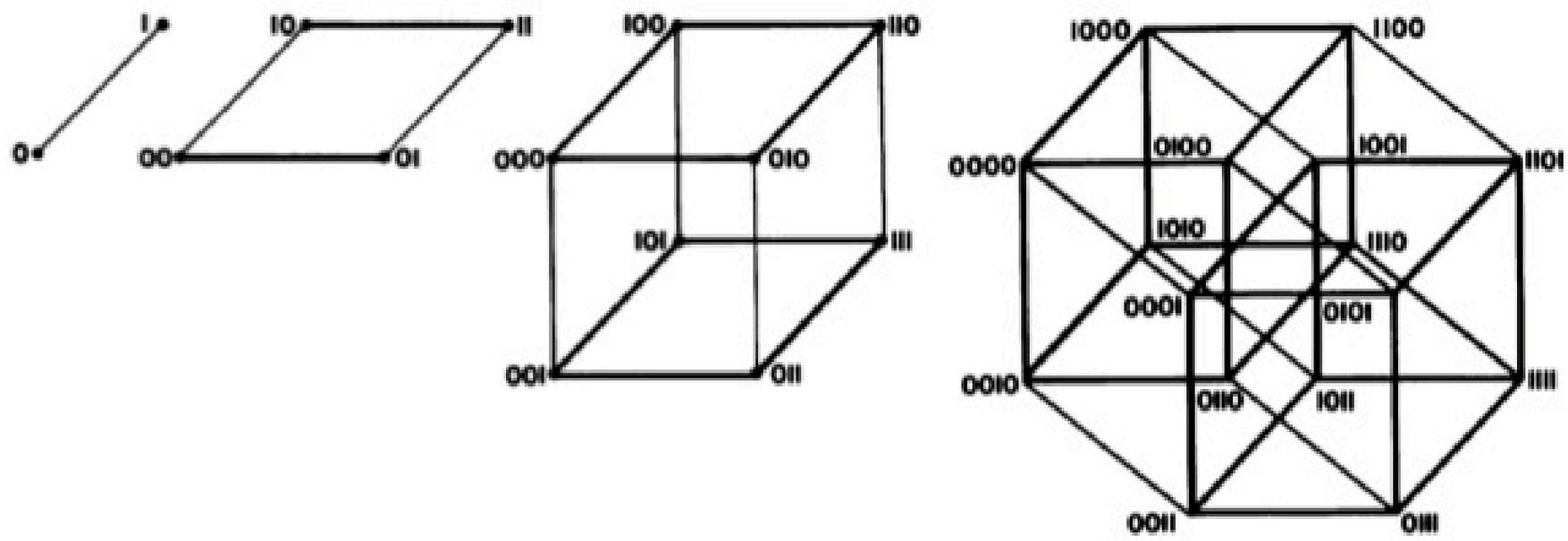
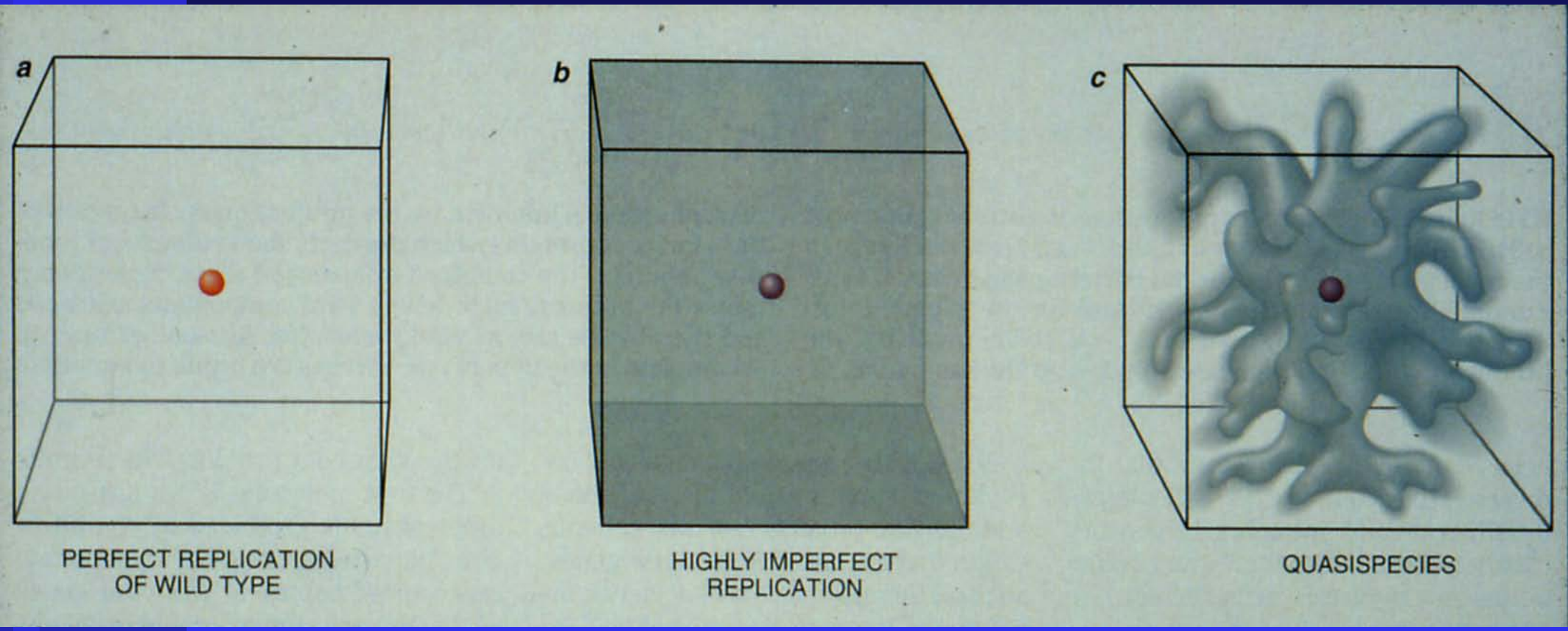
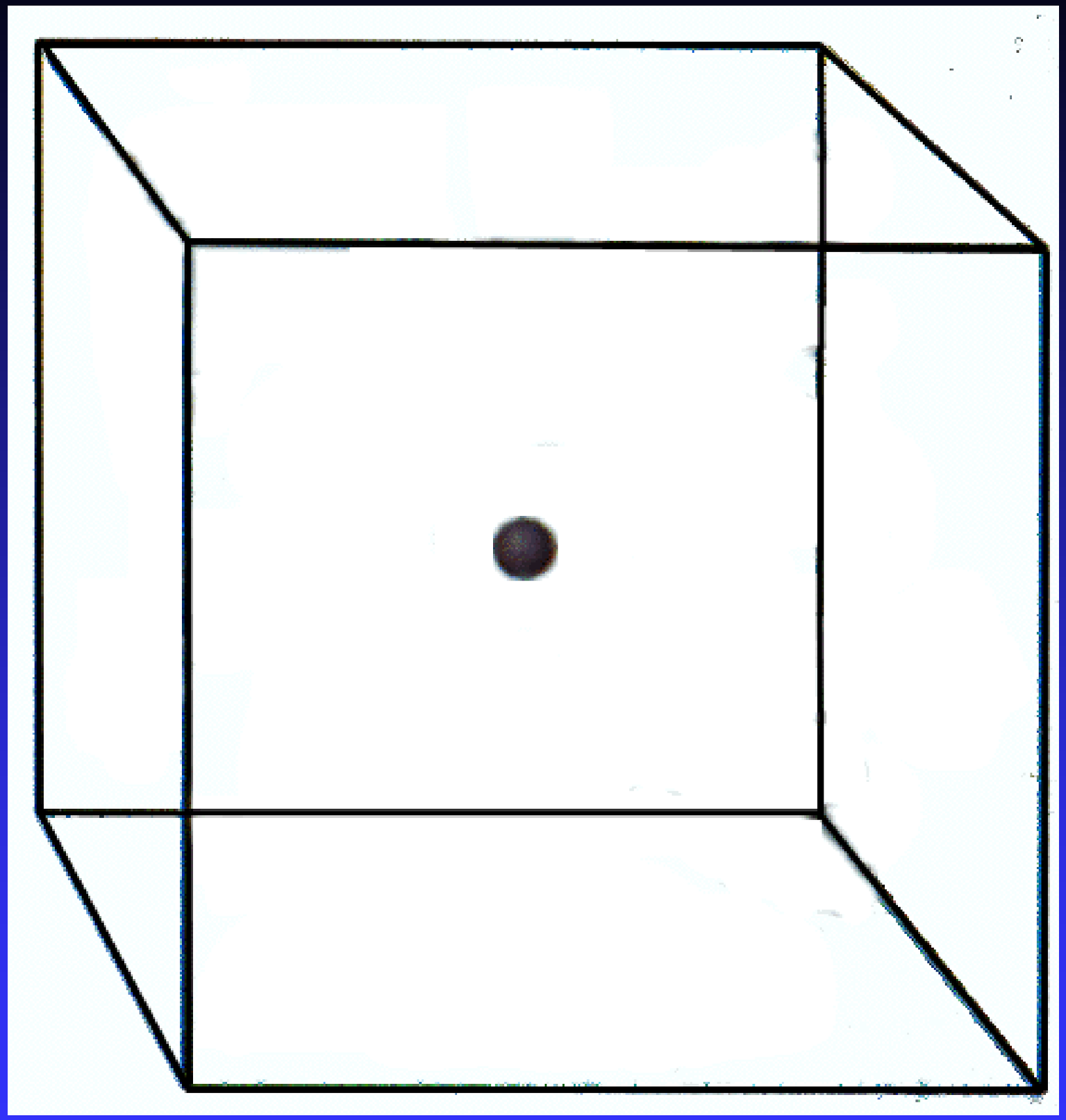
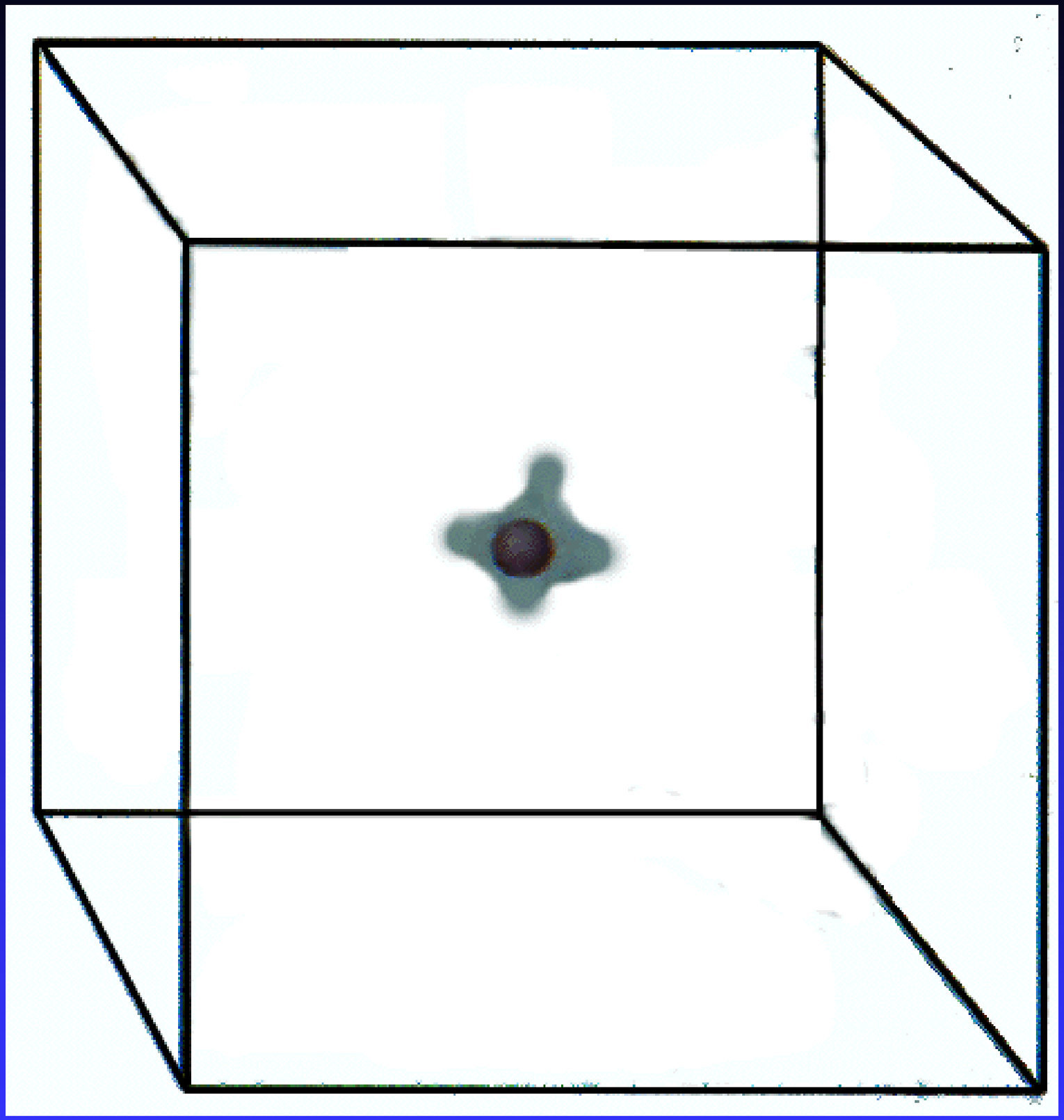


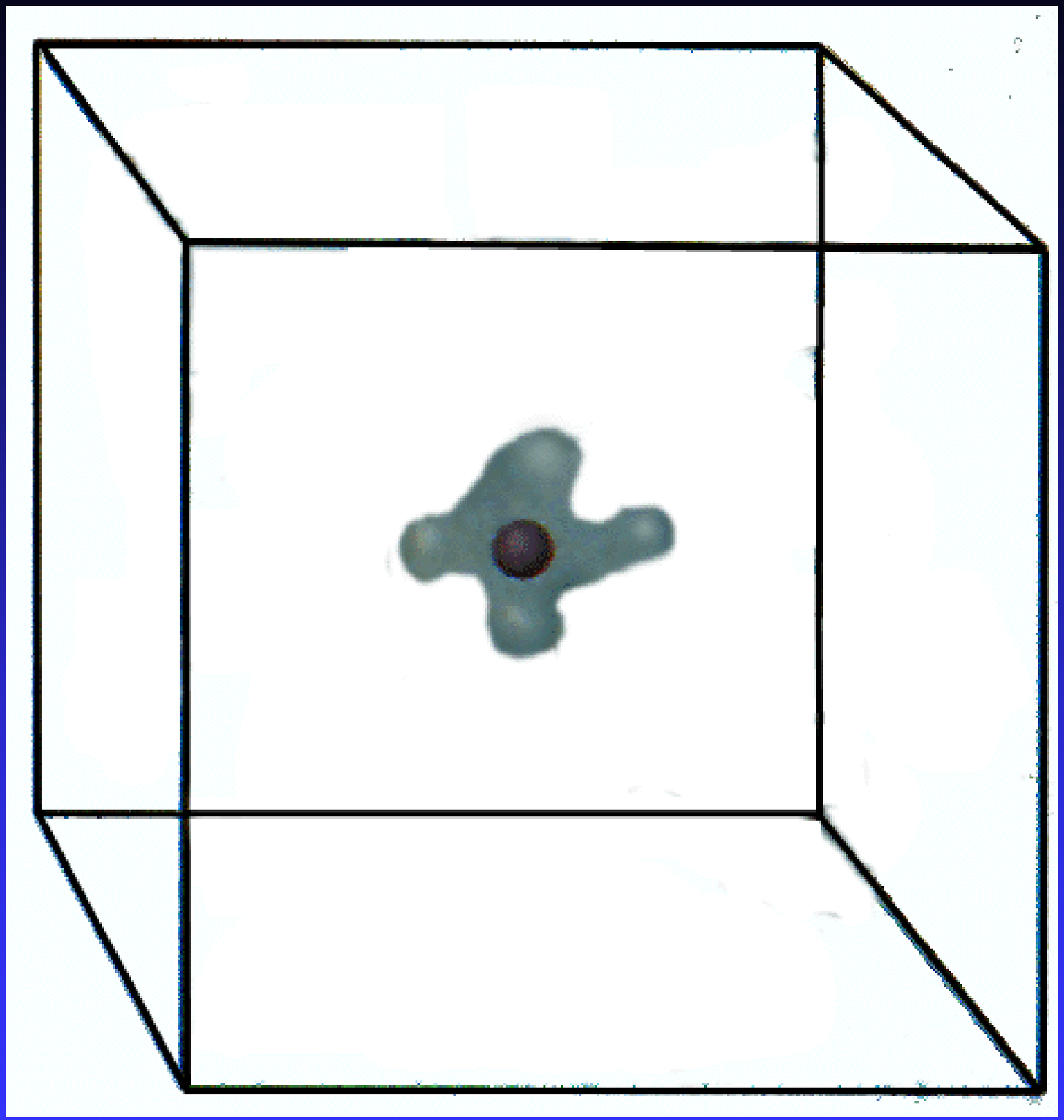
FIG. 3. The iterative buildup of sequence space, starting with one position. Each additional position requires a doubling of the former diagram and to connect corresponding points in both diagrams (which represent nearest neighbors). The final hypercube of dimension ν contains as subspaces $\binom{\nu}{k}2^{\nu-k}$ hypercubes of dimension k.

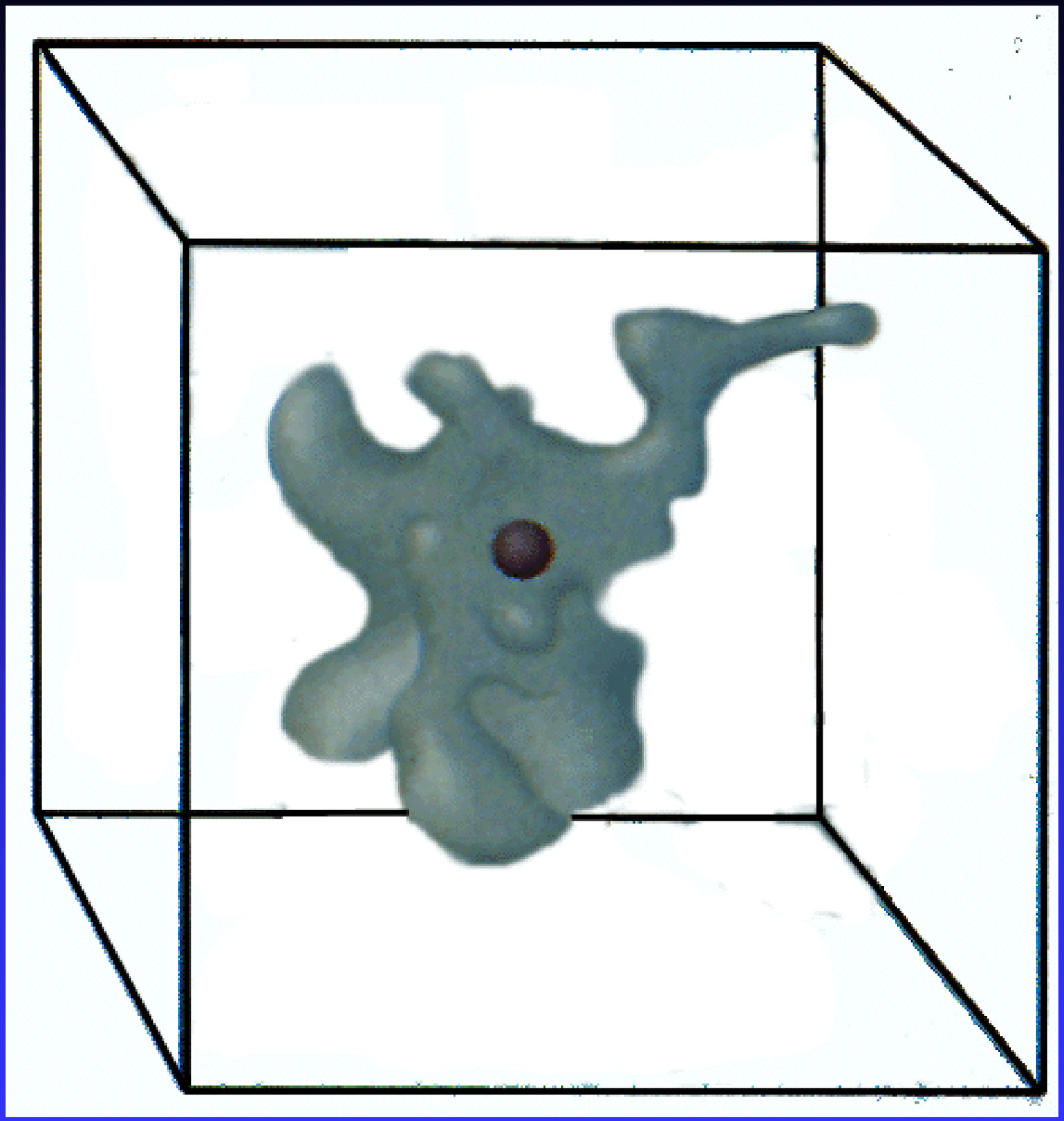
The ‘quasispecies’ concept

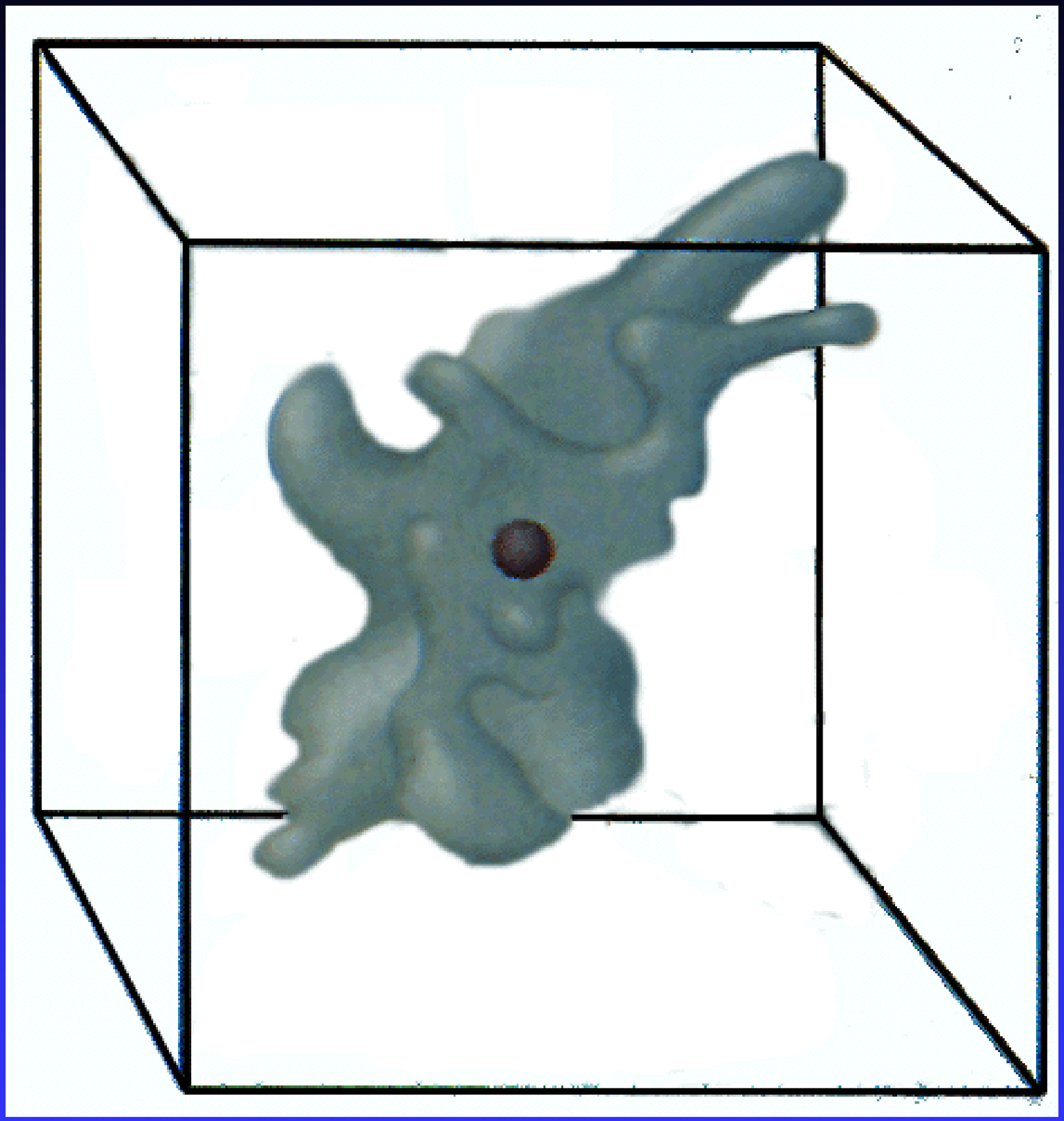


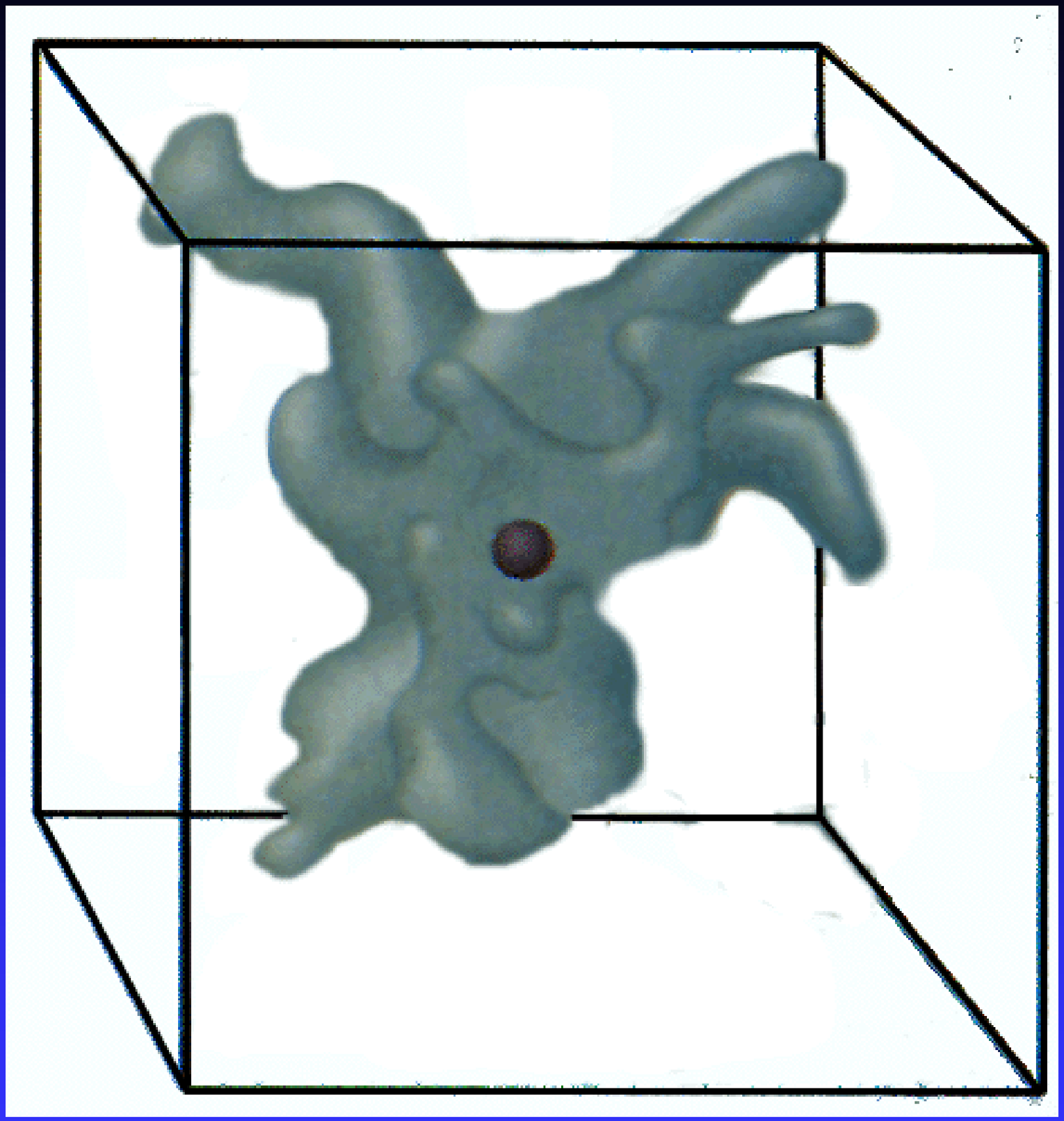


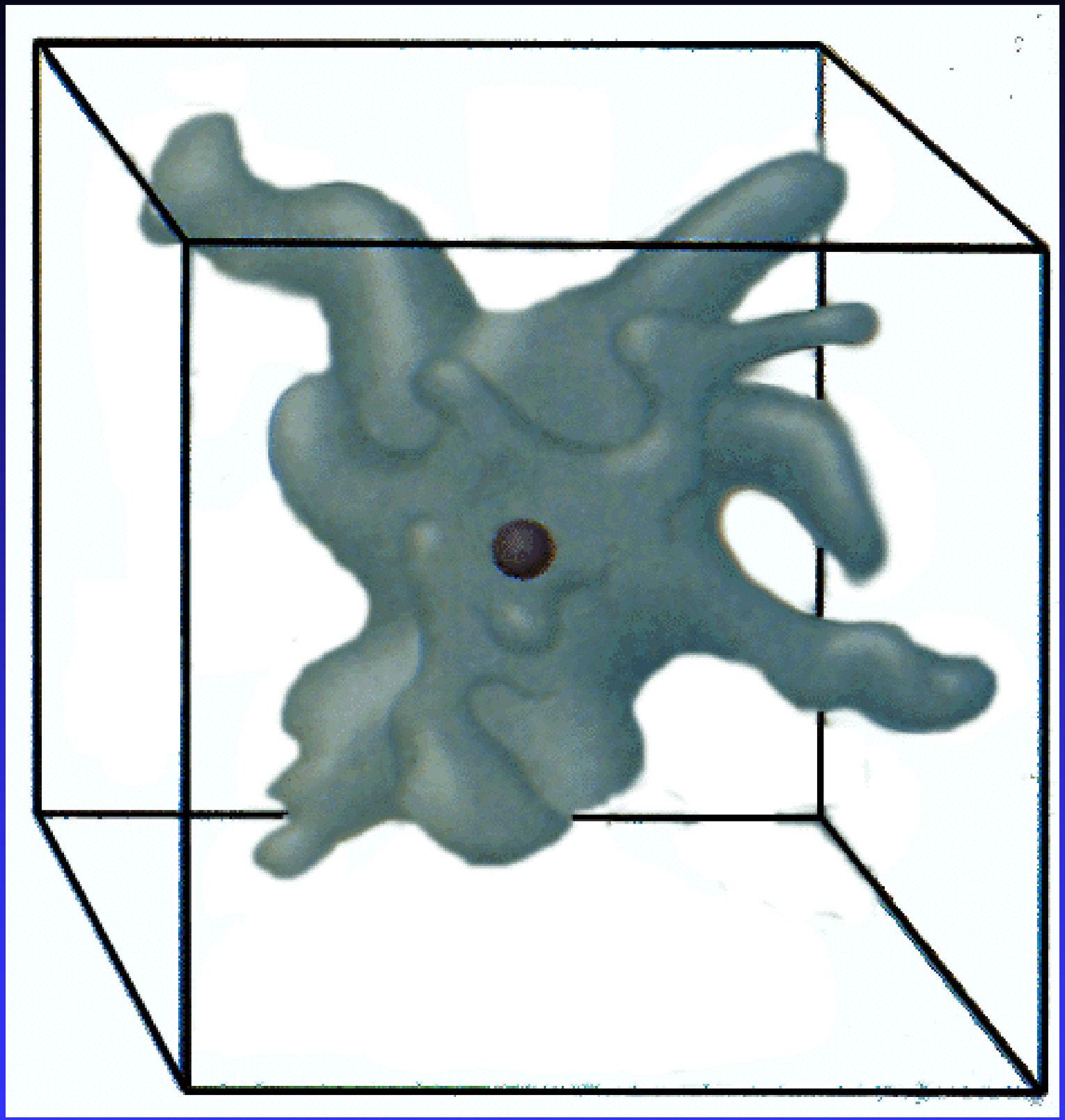


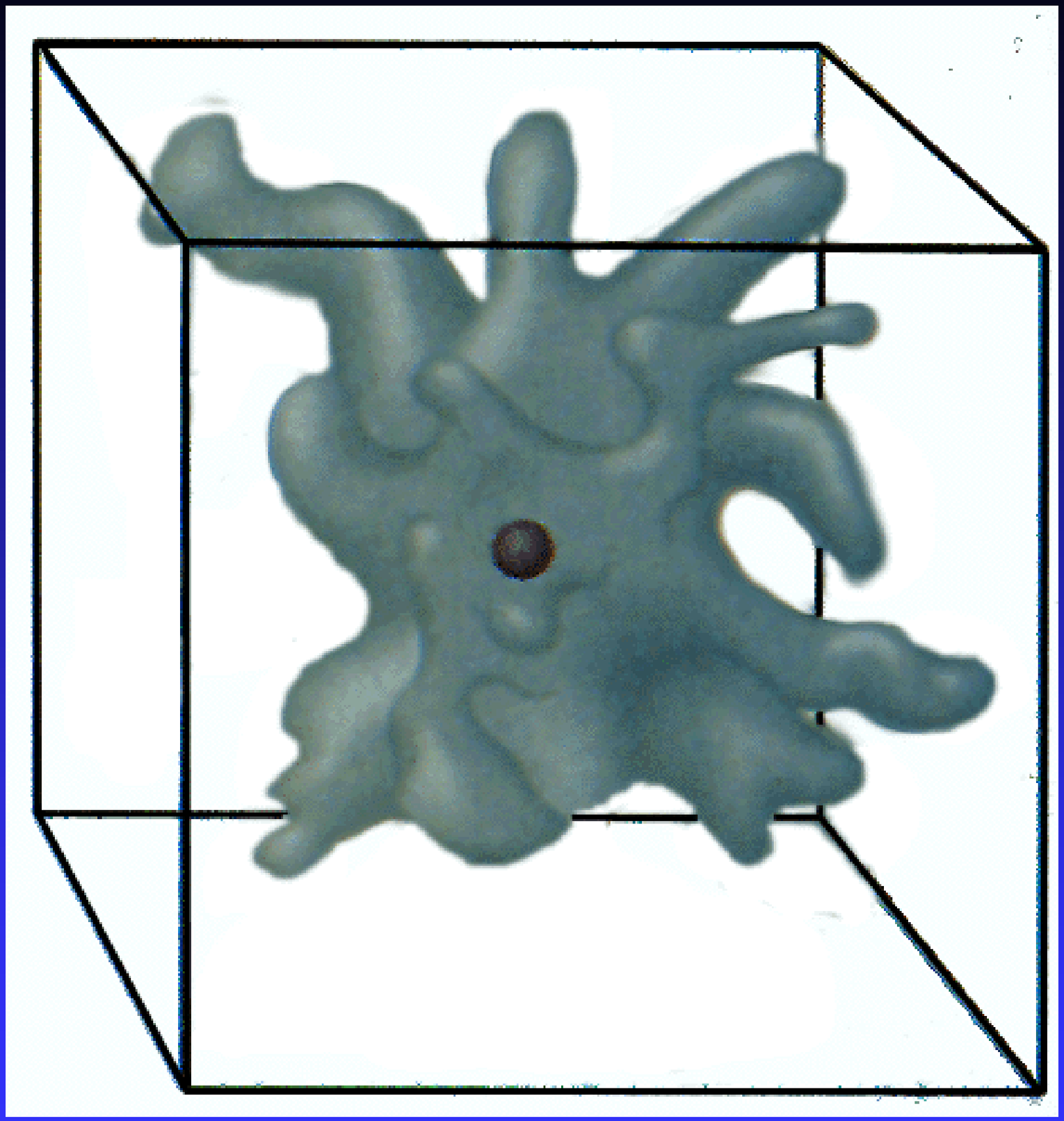


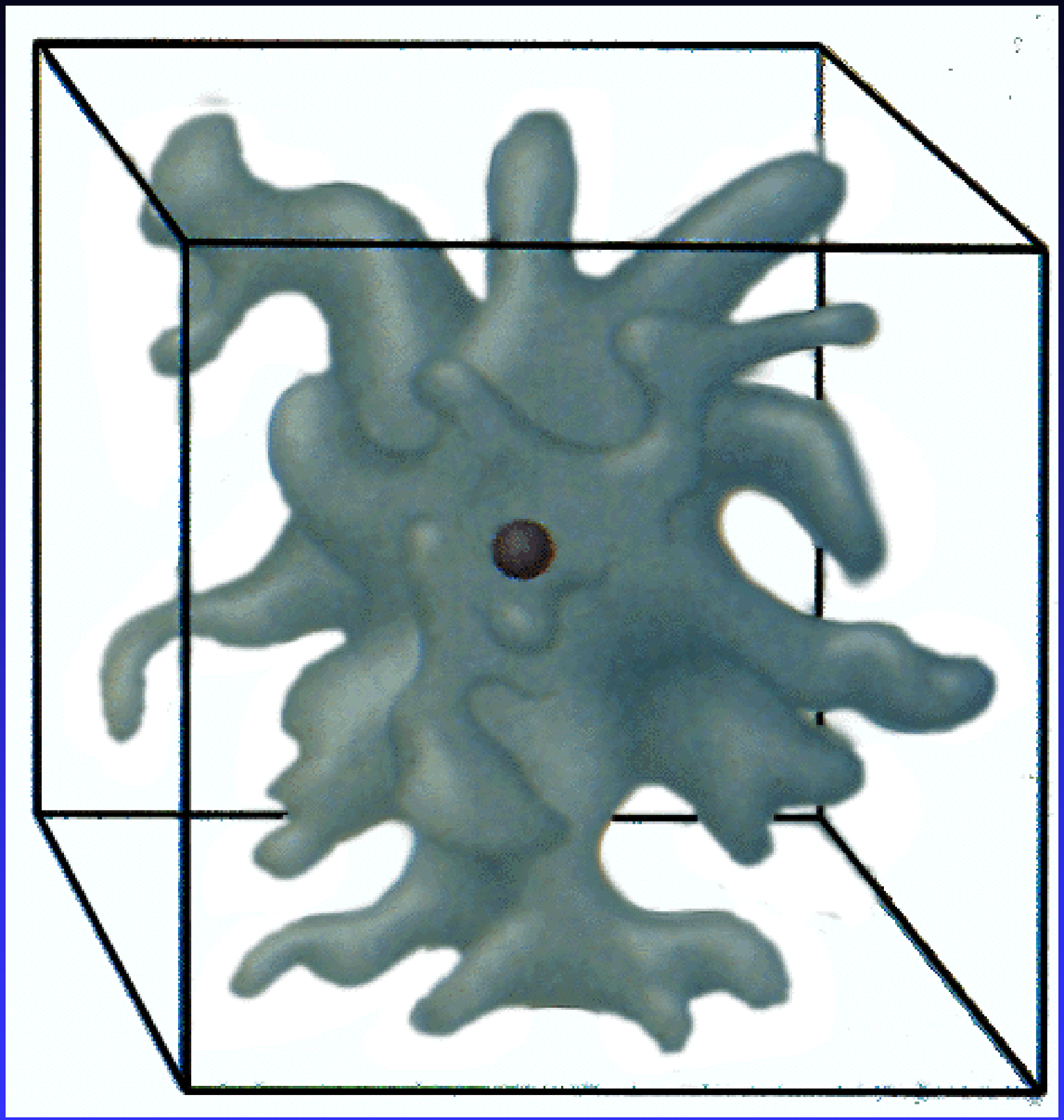


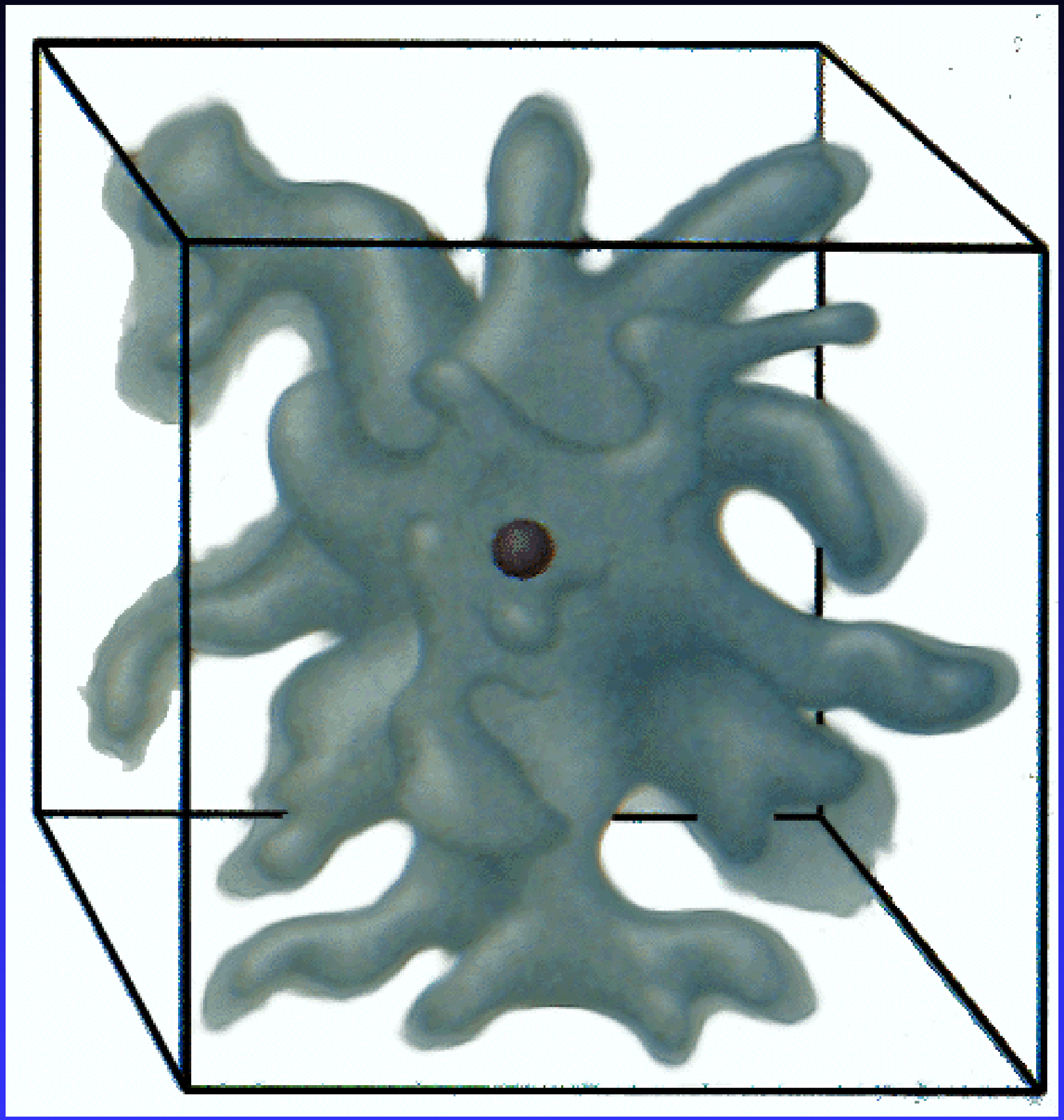




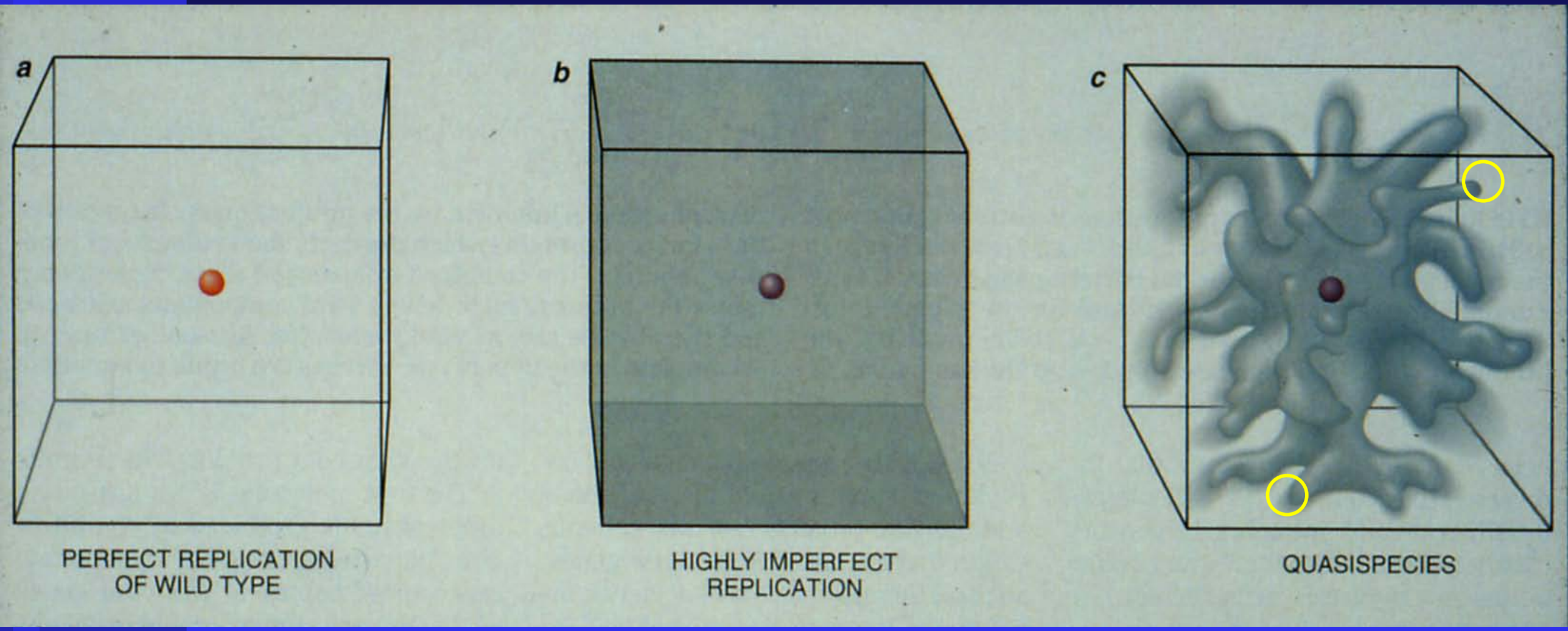








The ‘quasispecies’ concept

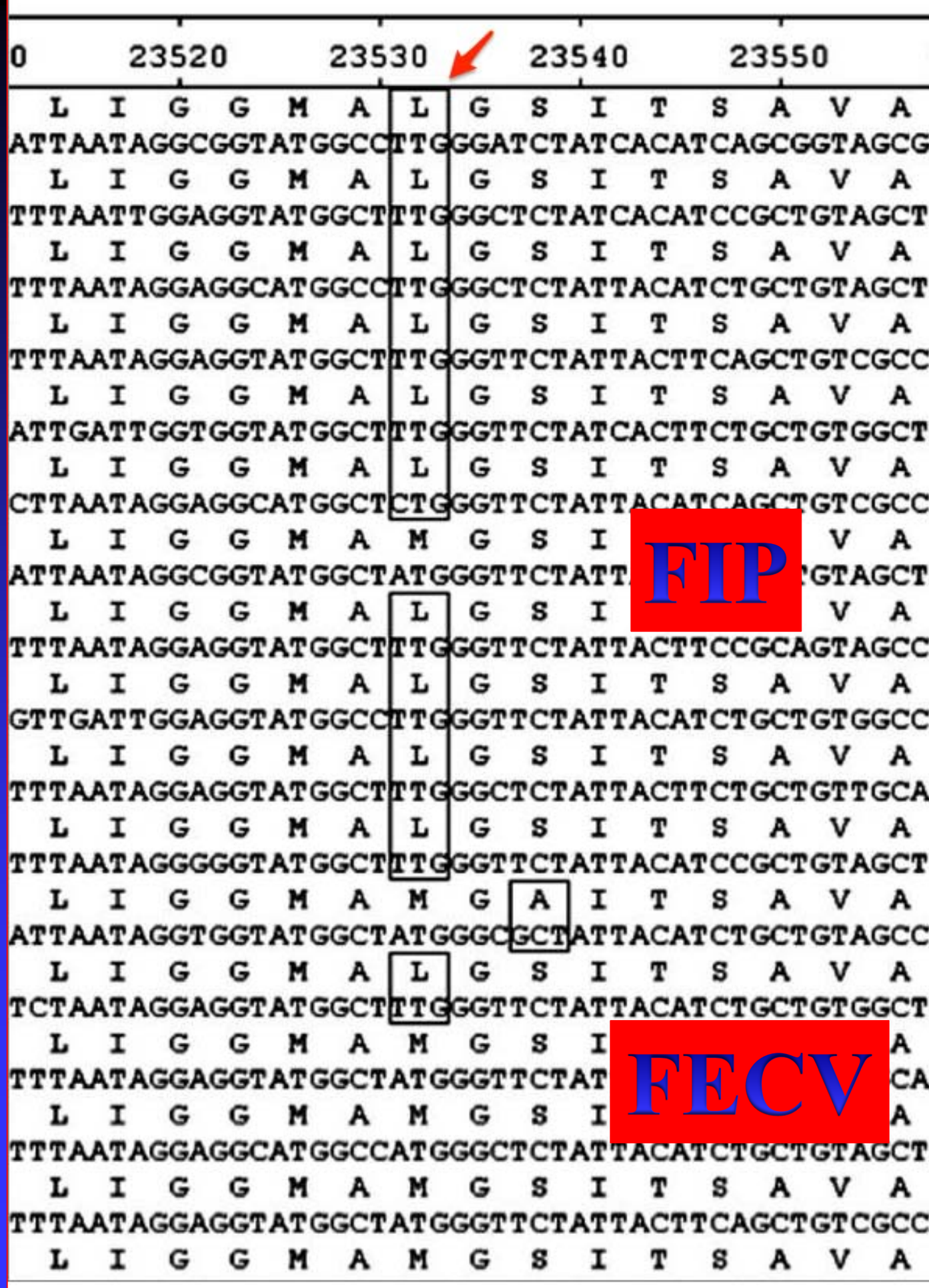


○ = FIP variants

Crucial for the FECV – FIPV transition:

The A at nucleotide 23531 was 100% conserved in all 183 FECVs in our collection.

Of the 118 FIPVs, 96 (81.4%) had a T and 12 (10.2%) a C at this position; in both cases, this changes the methionine (M) occurring at position 1058 in the FECV S protein into a leucine (L) in FIPV (i.e., mutation M1058L).



Viruses have crossed the host species barrier

time and again,

and will forever…

Interspecies transmission

severe acute respiratory syndrome (SARS): palm civet – to man

middle East respiratory syndrome (MERS): African bats, camelids – to man

bat coronavirus: Leschenault's rousettes (*Rousettus leschenaulti*, fruit bats Megachiroptera) - to Pomona leaf-nosed bats (*Hipposideros pomona*, insectivorous, Microchiroptera)

SARS – the first human ‘killer’ coronavirus

Severe Acute Respiratory Syndrome (SARS)

ORDER OF THE CENTERS FOR DISEASE CONTROL AND PREVENTION, DEPARTMENT OF HEALTH AND HUMAN SERVICES

Notice of Embargo of Civets

ACTION: Notice of embargo of civets (Family: Viverridae).

SUMMARY: According to published scientific articles, Severe Acute Respiratory Syndrome (SARS)-like virus has been isolated from civets (Family: Viverridae) captured in areas of China where the 2002-2003 SARS outbreak originated. Shipments of civet are being imported into the United States and further distributed. CDC is banning the importation of all civets immediately and until further notice. CDC is taking this action to prevent the importation and spread of SARS, a communicable disease.

DATE: This embargo is effective on January 13, 2004, and will remain in effect until further notice.

SARS

- What Everyone Should Know
- What's New
- Travel

Common palm civet

Paradoxurus hermaphroditus

Viruses are “the mistletoe on the tree of life”

They have co-evolved with their hosts and continue to do so.

Each milliliter of ocean water contains several million virus particles – a global total of 10^{30} virions!
If lined up end to end, they would stretch 200 million light years into space…

Wikipedia (condensed)
Sir Arnold Theiler (1867 – 1936)

is the father of veterinary science in South Africa – studied in Zurich, and in 1891 started practicing as a veterinarian,

developed a vaccine against rinderpest (eradicated in 2011) during the Anglo-Boer War of 1899-1902.

was the first Director of the Onderstepoort Veterinary Research Institute and

first Dean of the University of Pretoria Faculty of Veterinary Science (1920).

His son Max Theiler (1899-1972), was awarded the Nobel Prize in Physiology and Medicine (1951) for the development of a Yellow Fever vaccine.

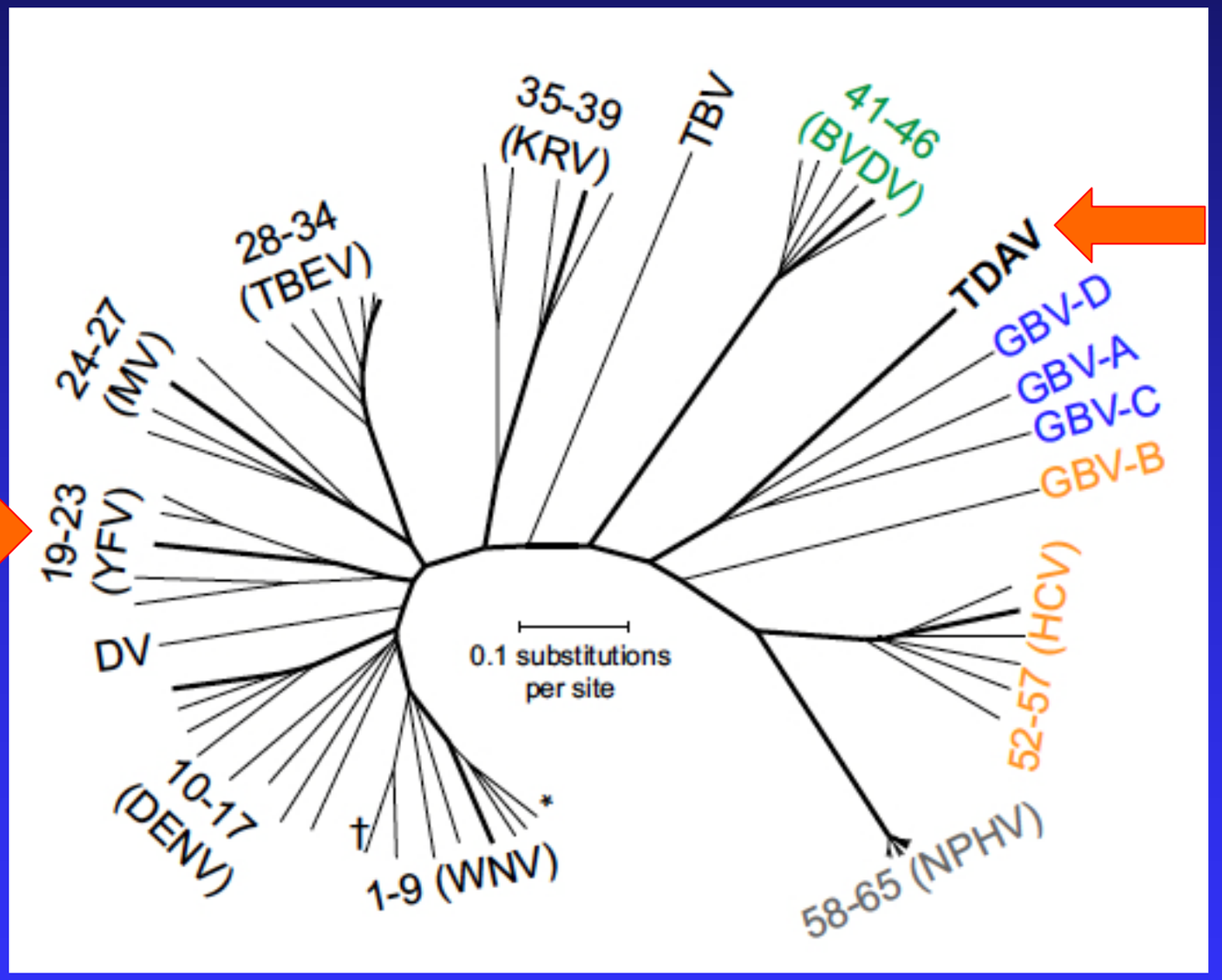
Arnold Theiler (1919) Acute liver-atrophy and parenchymatous hepatitis in horses. The Fifth and Sixth Reports of the Director of Veterinary Research, April, 1918. Department of Agriculture, Union of South Africa (The Government Printing and Stationery Office, Pretoria, Union of South Africa), pp. 7–164.

…and then, about a century later:

Sanjay Chandriania, Peter Skewes-Coxa, Weidong Zhonga, Donald E. Ganema, Thomas J. Divers, Anita J. Van Blaricum, Bud C. Tennant, Amy L. Kistler (2013) Identification of a previously undescribed divergent virus from the *Flaviviridae* family in an outbreak of equine serum hepatitis

A *flavivirus* story:

son Max
(Nobel 1951)



father Arnold
(1919)

…it runs in the family…

A personal paraphrase:

"…nothing in virology makes sense except in the light of evolution…"

Marian C. Horzinek
(1936-2018)

…also of its history – and the role of its protagonists

The End