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BACTERIAL GROWTH

The 3rd lecture for 2nd-year students of General Medicine
March 2, 2015

Size of bacteria – revision

Pathogenic bacteria: mainly around 1 – 5 µm
(1 µm = 10^{-3} mm)

Genus *Staphylococcus*: the diameter circa 1 µm

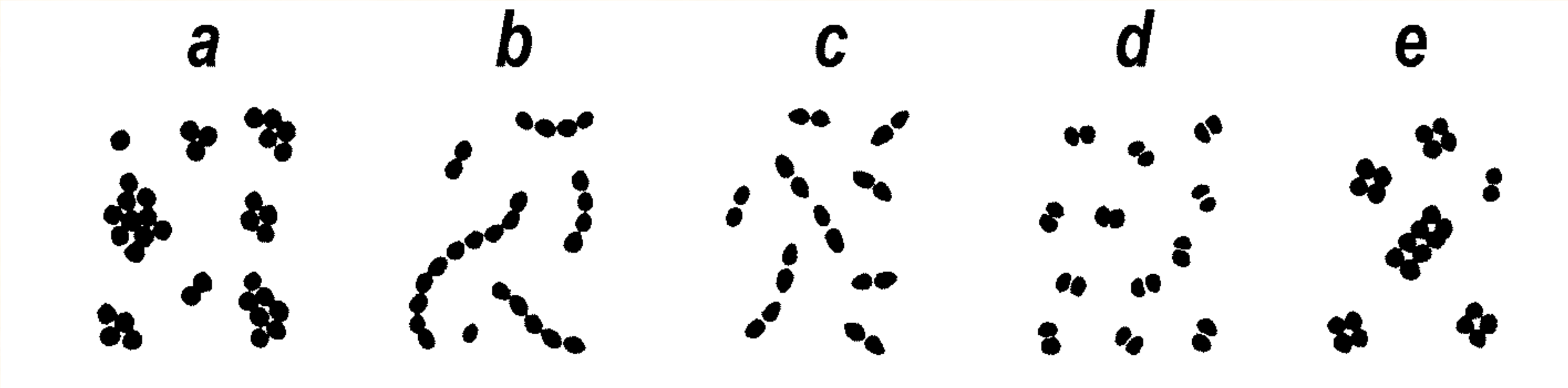
Relatively big: genera *Bacillus* and *Clostridium*
(robust rods around 1 – 2   10 µm)

Relatively long: old cultures of most rods
(fibers up to 50 µm long)

Relatively small: genus *Haemophilus*
(in the sputum approximately 0.3   0.6 µm)

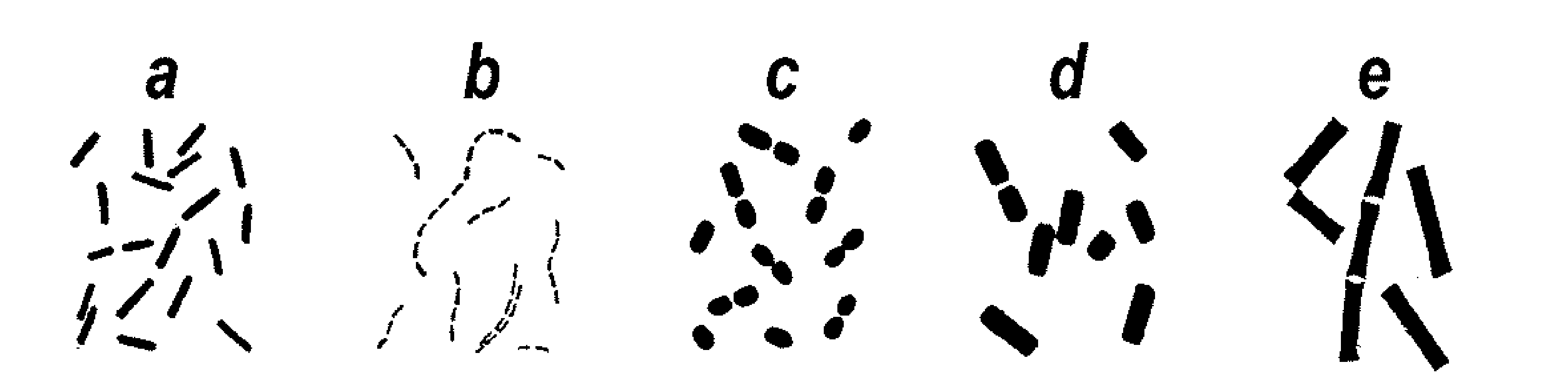
Even smaller:
rickettsiae (circa 0.5 µm)
chlamydiae (elementary bodies circa 0.3 µm)
mycoplasmas (circa 0.2 – 0.25 µm)

Arrangement and shape of cocci – revision



a) in clumps: *Staphylococcus aureus*
b) in chains: *Streptococcus pyogenes*
c) lancet-like diplococci: *Streptococcus pneumoniae*
d) flattened diplococci: *Neisseria gonorrhoeae*
e) cocci in tetrads: *Micrococcus luteus*

Arrangement and shape of rods I – revision



a) absolute majority of rods: e.g. *Escherichia coli*

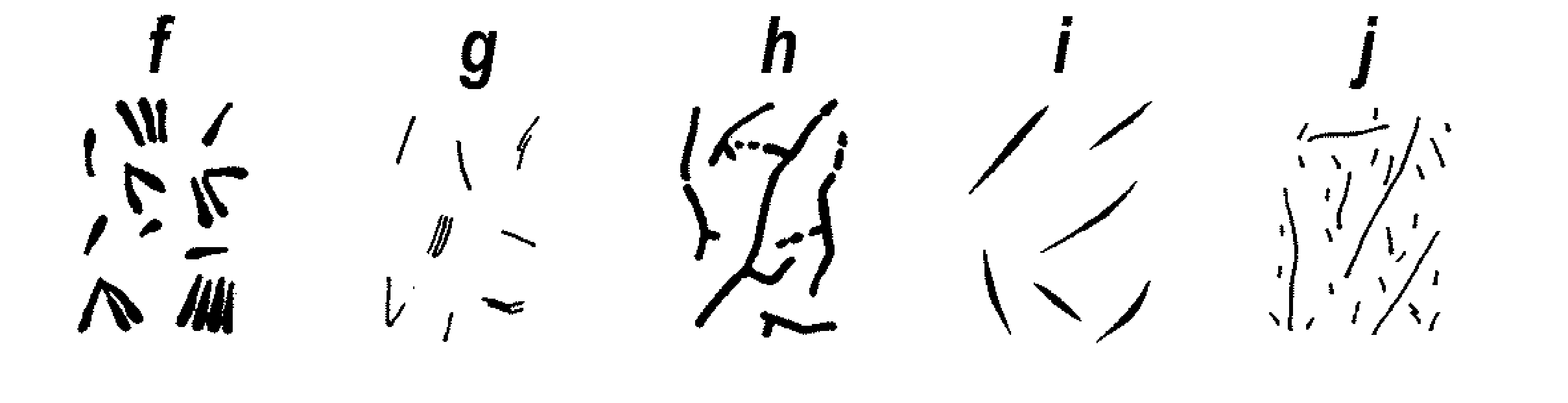
b) delicate streptobacilli: *Haemophilus ducreyi*

c) coccobacilli in pairs or diplobacilli: *Moraxella lacunata*

d) robust rods, rounded ends: *Clostridium perfringens*

e) robust rods, flat up to concave ends, bamboo cane-like chains: *Bacillus anthracis*

Arrangement and shape of rods II – revision



f) club-like in palisades: *Corynebacterium diphtheriae*

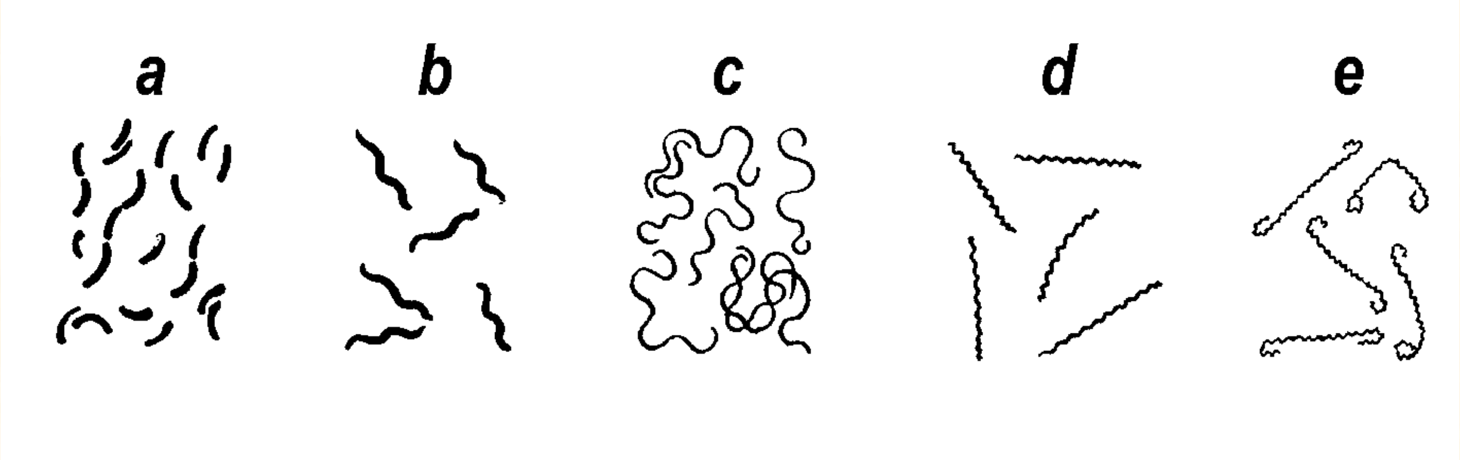
g) slender, in hinted palisades: *Mycobacterium tuberculosis*

h) branched, fragmented: *Nocardia asteroides*

i) spindle-like: *Fusobacterium fusiforme*

j) minute, pleomorphic: *Haemophilus influenzae*

Curved and spiral rods – revision



a) curved rods, crescent-shaped: *Vibrio cholerae*

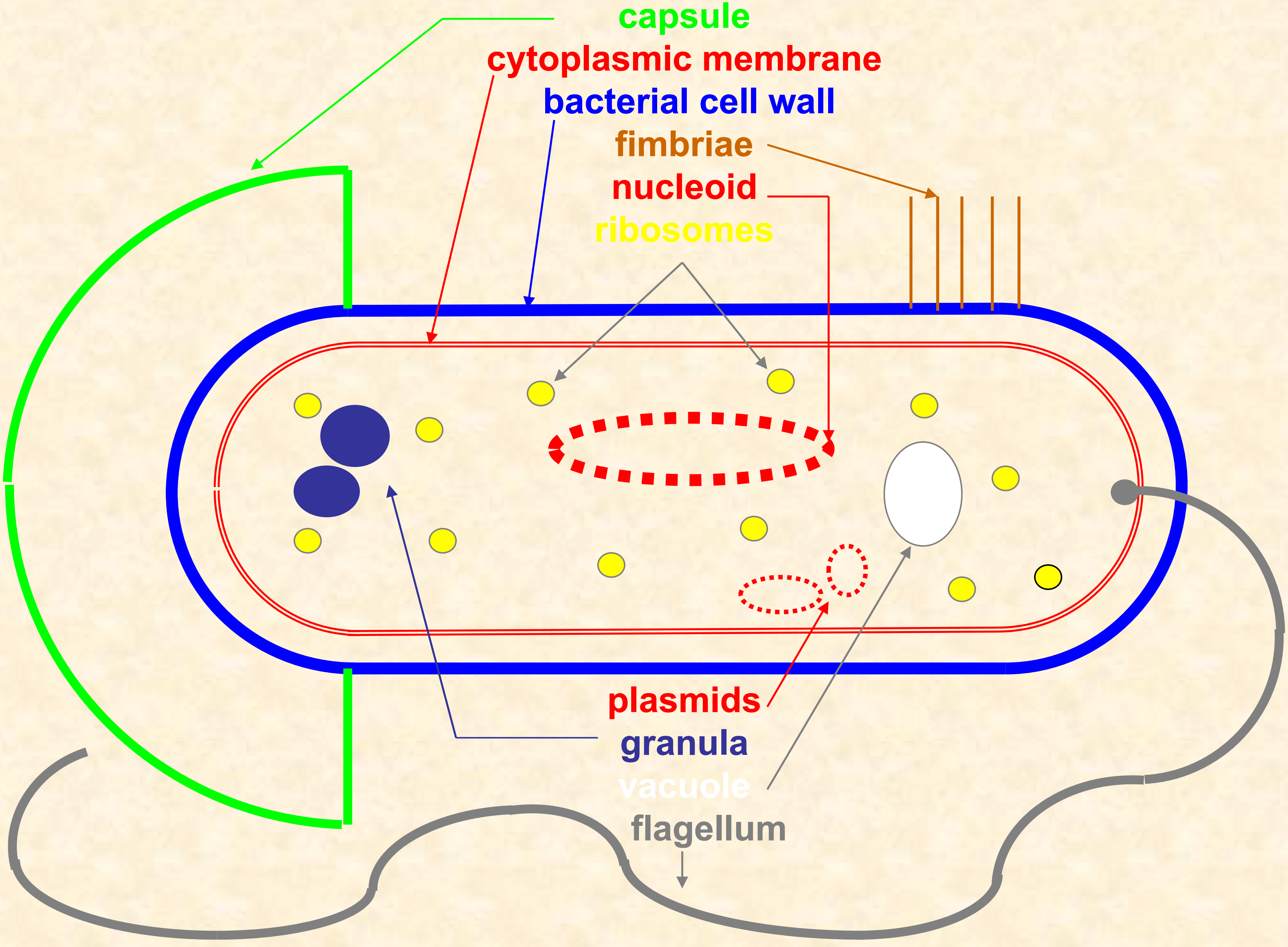
b) thick spirals: *Spirillum minus*

c) uneven spirals: *Borrelia recurrentis*

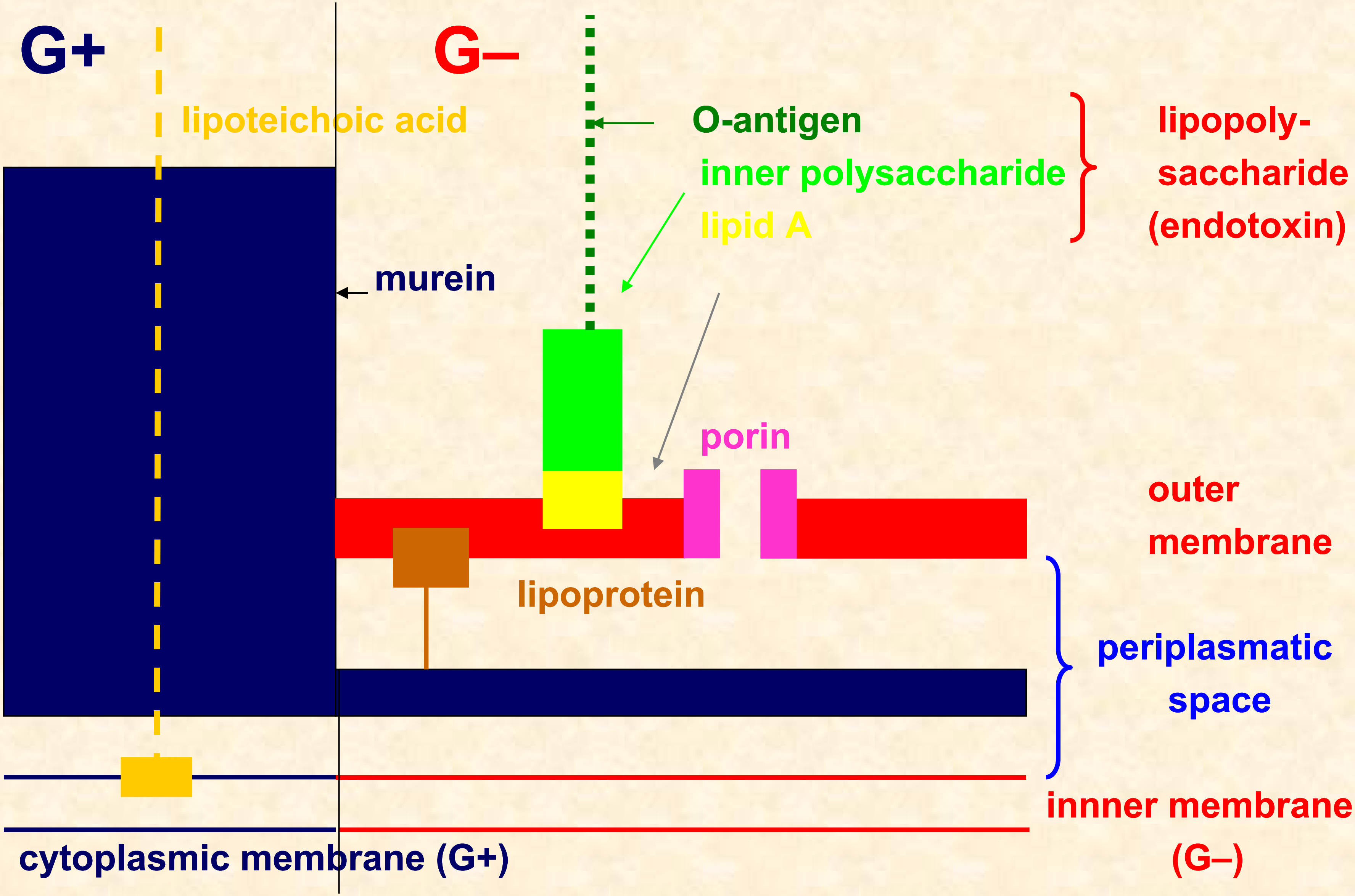
d) delicate, regular spirals: *Treponema pallidum*

e) very fine spirals with bent ends: *Leptospira icterohaemorrhagiae*

Outline of bacterial cell – revision

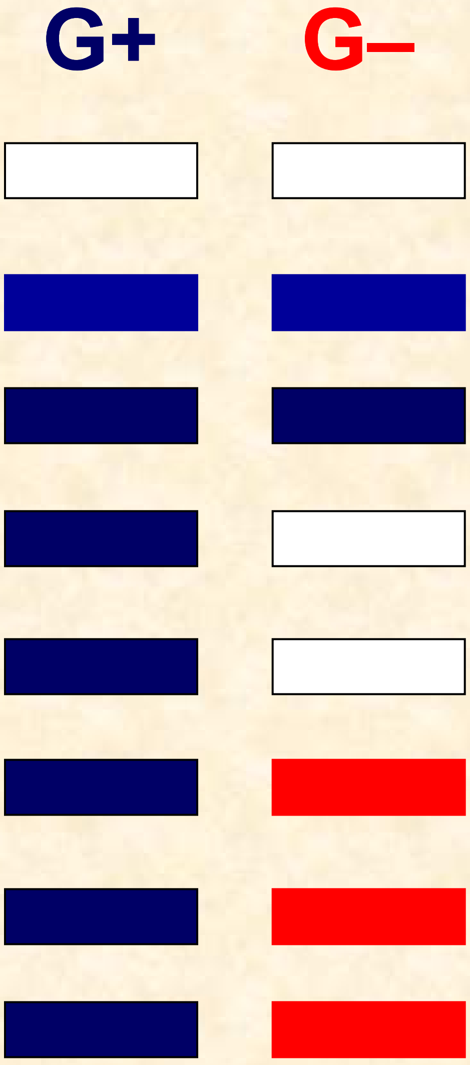


Bacterial cell wall – revision



Gram staining – revision

1. Fixation by flame 3 times
2. Gram stain 20 s
3. Lugol solution 20 s
4. Alcohol max. 20 s
5. Aqua fontis rinse
6. Safranin 1 min
7. Aqua fontis rinse
8. Drying



Sensitivity to antibiotics – revision

Effect mostly on Gram-positives:	Effect mostly on Gram-negatives:
beta-lactams (penicillin, oxacillin = methicillin)	aminoglycosides (gentamicin)
macrolides (erytromycin)	monobactams (aztreonam)
lincosamides (lincomycin)	polypeptides (colistin)
glycopeptides (vancomycin)	3rd gen. cephalosporins (cephtriaxon)

Resistance to the environment – an addition

Gram-positives

They endure well drying up and higher salt concentrations

→ and so we find them:

- on skin (staphs, propionibacteria)
- in soil (clostridia, bacilli, nocardiae, moulds)

Gram-negatives

They endure well the effect of toxic substances and extremes of pH

→ and so we find them:

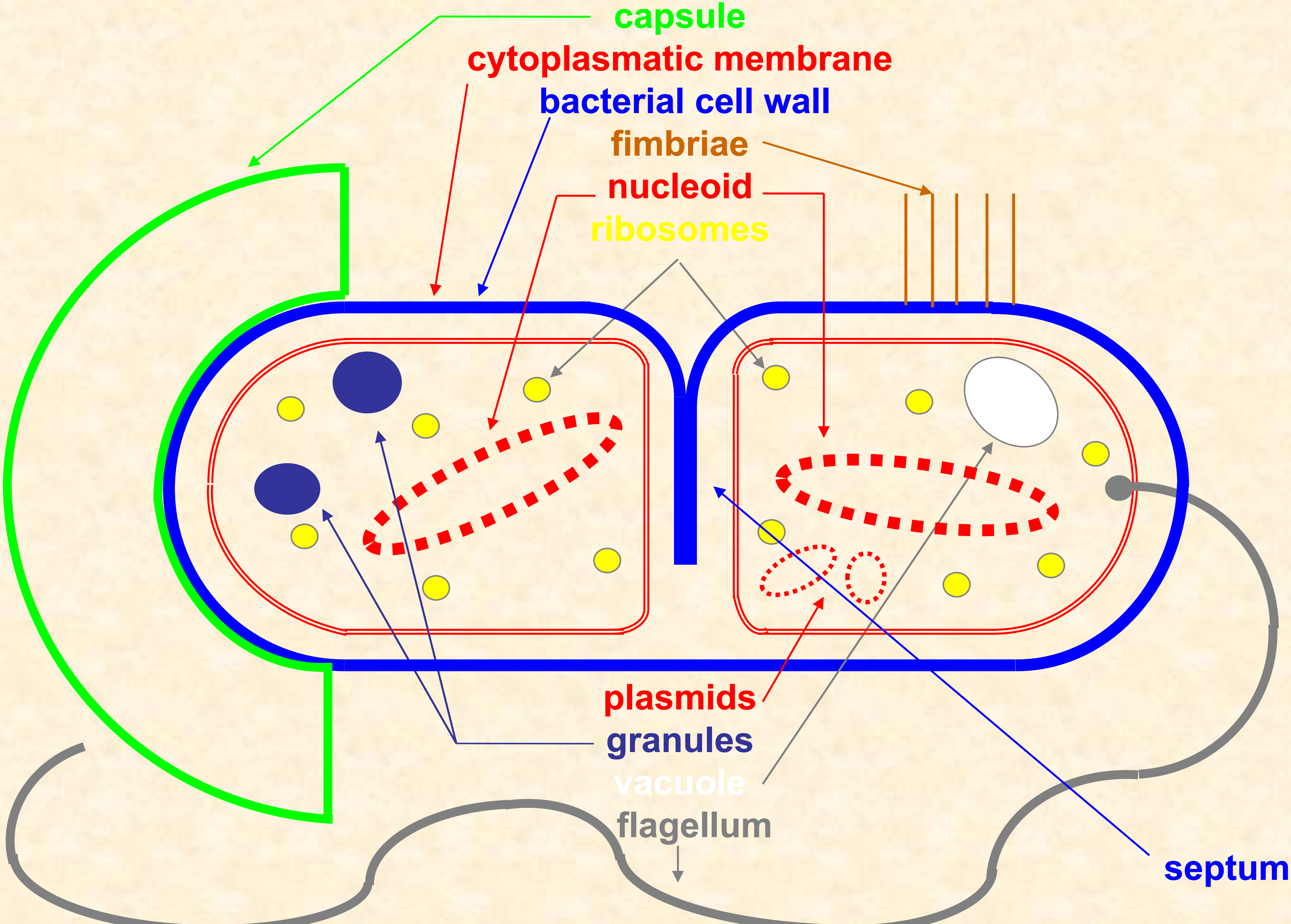
- above all in moist places (enterobacteriae, pseudomonads, other non-fermenting rods, vibria)

Growth cycle of bacteria

Bacteria reproduce by binary fission

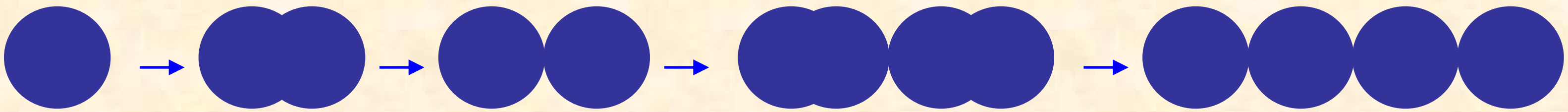
- Period I (initiation): the cell grows, inside it proteins initiating the next step accumulate
- Period C (chromosome replication): the chromosome diverges from one spot in both directions opposite one another
- Period D (division):
  - supply of macromolecules is created
  - cytoplasmatic membrane inserts between the replicated chromosomes and separates them
  - cell wall grows into the cell at a particular spot and forms a septum that ultimately divides the maternal cell into two daughter cells

Division of bacterial cell

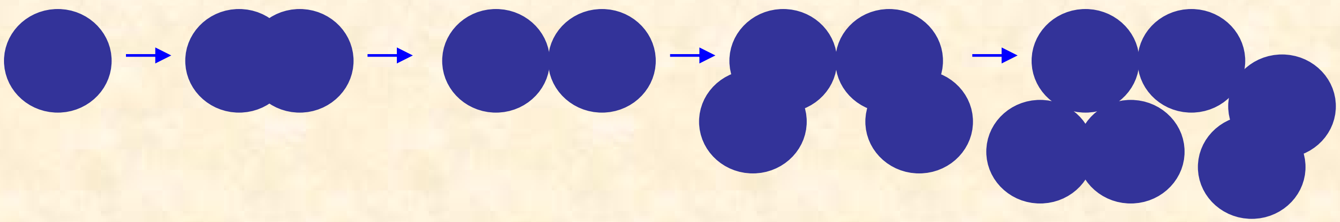


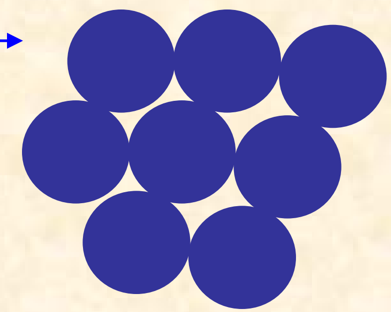
Division & arrangement of cocci

Cocci, dividing in one plane: **streptococci** ➡ <u>chains</u>



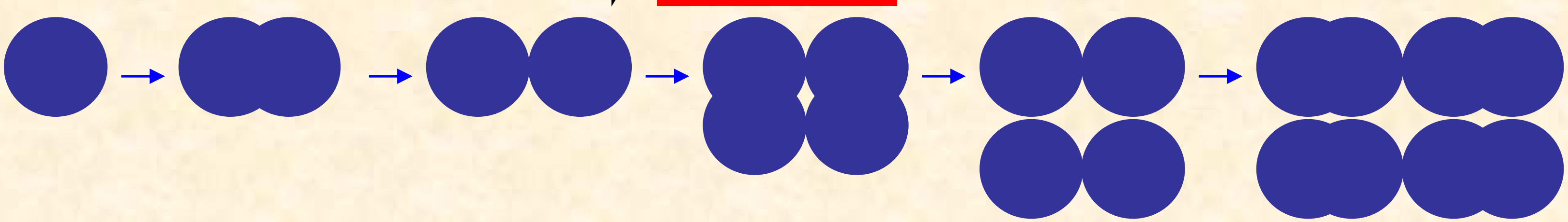
Cocci, in different planes: **staphylococci** ➡ <u>clumps</u>





Cocci, in two perpendicular planes:

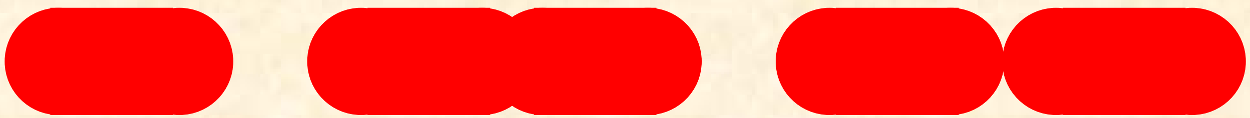
micrococci ➡ <u>tetrads</u>

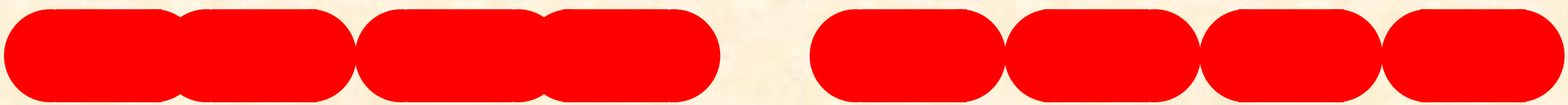


Notice that after dividing <u>cocci touch each other!</u>

Division and arrangement of rods

Rods, transverse division: majority (chains of rods)

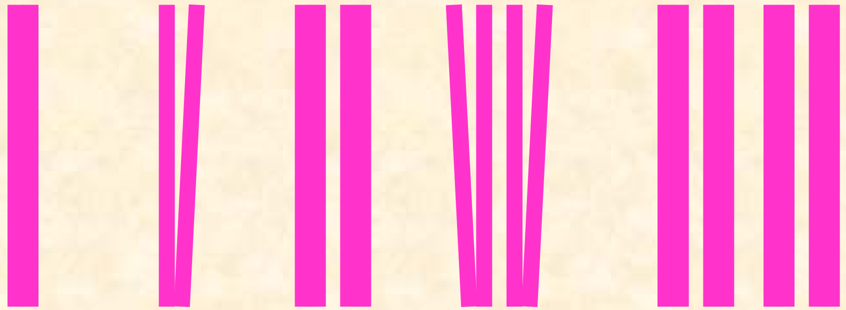




Rods, lengthwise division: mycobacteria

corynebacteria

(arrangement in palisades)



Generation time

Generation time = duration of the growth cycle =
= duplication time = duration of doubling the number of bacteria

Generation time of bacteria: on average cca 30 min

Escherichia coli

under ideal conditions 20 min

Mycobacterium tuberculosis

approximately 12 hrs

Since during each generation time the number of bacteria doubles, bacteria multiply by geometric progression

Geometric progression – I

Number of bacteria by generation time 0.5 hour

time (hrs)	number	time (hrs)	number
0	$2^0=1$	4	$2^8=256$
0.5	$2^1=2$	4.5	$2^9=512$
1	$2^2=4$	5	$2^{10}=1024$
1.5	$2^3=8$	5.5	$2^{11}=2048$
2	$2^4=16$	6	$2^{12}=4096$
2.5	$2^5=32$	12	$2^{24} \approx 10^7$
3	$2^6=64$	18	$2^{36} \approx 10^{11}$
3.5	$2^7=128$	24	$2^{48} \approx 10^{14}$

Geometric progression – II

If the generation time is 30 min, after 24 hrs theoretically one cell gives origin to 2^{48} = 2.8 10^{14} cells, actually it is by approximately 5 orders less (i.e. around 10^{9} cells)

10^{9} bacteria is such an amount that it is visible even by the naked eye:

Liquid medium (broth) becomes 1. cloudy or 2. a sediment appears at the bottom or 3. a pellicle is seen at the top

On a solid medium (agar) a bacterial colony forms

What is a bacterial colony?

- Bacterial colony = a form on the surface of the agar, containing mutually touching cells, cca 10^9 living and cca 10^5 already dead

- Appearance of the colony depends apart from other things on the
  1. microbial species (e.g. on the size of its cells)
  2. sort of culture medium (e.g. on the amount of its nutrients)
  3. distance among colonies (the higher distance, the larger and more typical the colony)

Features of a bacterial colony

Bacterial colony can have up to 10 features:

1. Size – usually around 1-2 mm
2. Shape – round, oval, irregular, lobular etc.
3. Profile – flat, convex, dish-shaped etc.
4. Margins – straight, fibrous, with projections etc.
5. Surface – smooth & glossy, matt, rough, wrinkled
6. Transparency – transparent, nontransparent
7. Colour – colourless, pigmented (yellowish etc.)
8. Changes in vicinity – pigmentation, haemolysis
9. Consistency – sticky, mucous, crumbly, rooted
10. Smell – foul, pungent, of jasmin, sperm, fruit etc.

Geometric progression – III

Consequences will become evident by the quantitative examination of urine:

From the external orifice of urethra bacteria can be flushed into urine up to the concentration of 10^3/ml

= a mere contamination (in cystitis the urine contains >10^5 bacteria/ml, i.e. >10^5 CFU, colony forming units)

In 1 µl of this urine there will be 1 bacterium (1 CFU)

→ in this case from 1 µl only 1 colony will appear

The result of the examination will be:

10^3 CFU/ml = probably contamination

However, it applies only when the urine is processed immediately

But what if the urine takes several hours to get into the laboratory in the hot summer?

Geometric progression – IV

Urine is a good culture medium, bacteria multiply in it even during the transportation

At the generation time of 30 min:

After 2 hrs: from 1000 cells → 16,000 cells
from 1 μl of urine 16 colonies will grow

The result:

10^4 CFU/ml = suspect finding

After 4 hrs: from 1000 cells → 256 000 cells
from 1 μl of urine 256 colonies will grow

The result:

$>10^5$ CFU/ml = positive finding (of course a false one!)

→ <u>the urine must be processed up to 2 hrs</u> after the sampling <u>or placed in refrigerator at 4 C</u>

Microbial growth curve – I

The result 10^9 cells/24 hrs applies for the <u>stationary culture</u>, in which nutrients are consumed and products of metabolism accumulate

⇨ **the speed of multiplication changes depending on time**

⇨ **growth phases exist that can be depicted by the growth curve**

Microbial growth curve – II

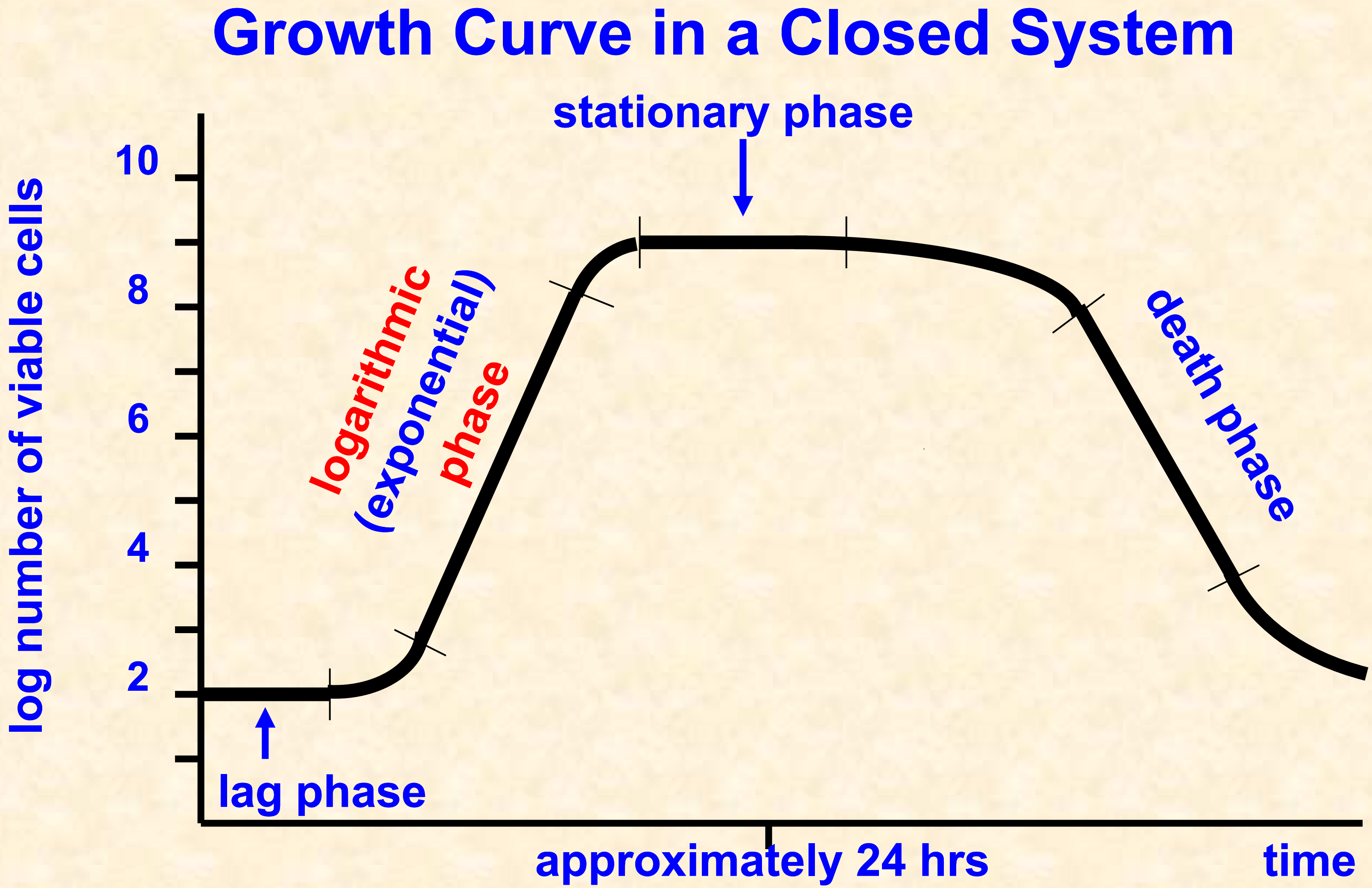
Growth curve depicts the number of viable cells in the logarithmic scale, depending on the age of culture

<u>Growth phases</u>

1. lag phase
2. log (exponential) phase
3. stationary phase
4. death phase

There are gradual transitions between the phases

Microbial growth curve – III



What is a logarithm?

In the equation

$$10^3 = 1000$$

10 is a base, 3 is an exponent

The exponent (*3*) = logarithm of the number *1000* (at the base *10*)

Logarithms at the base *10* = common logarithms

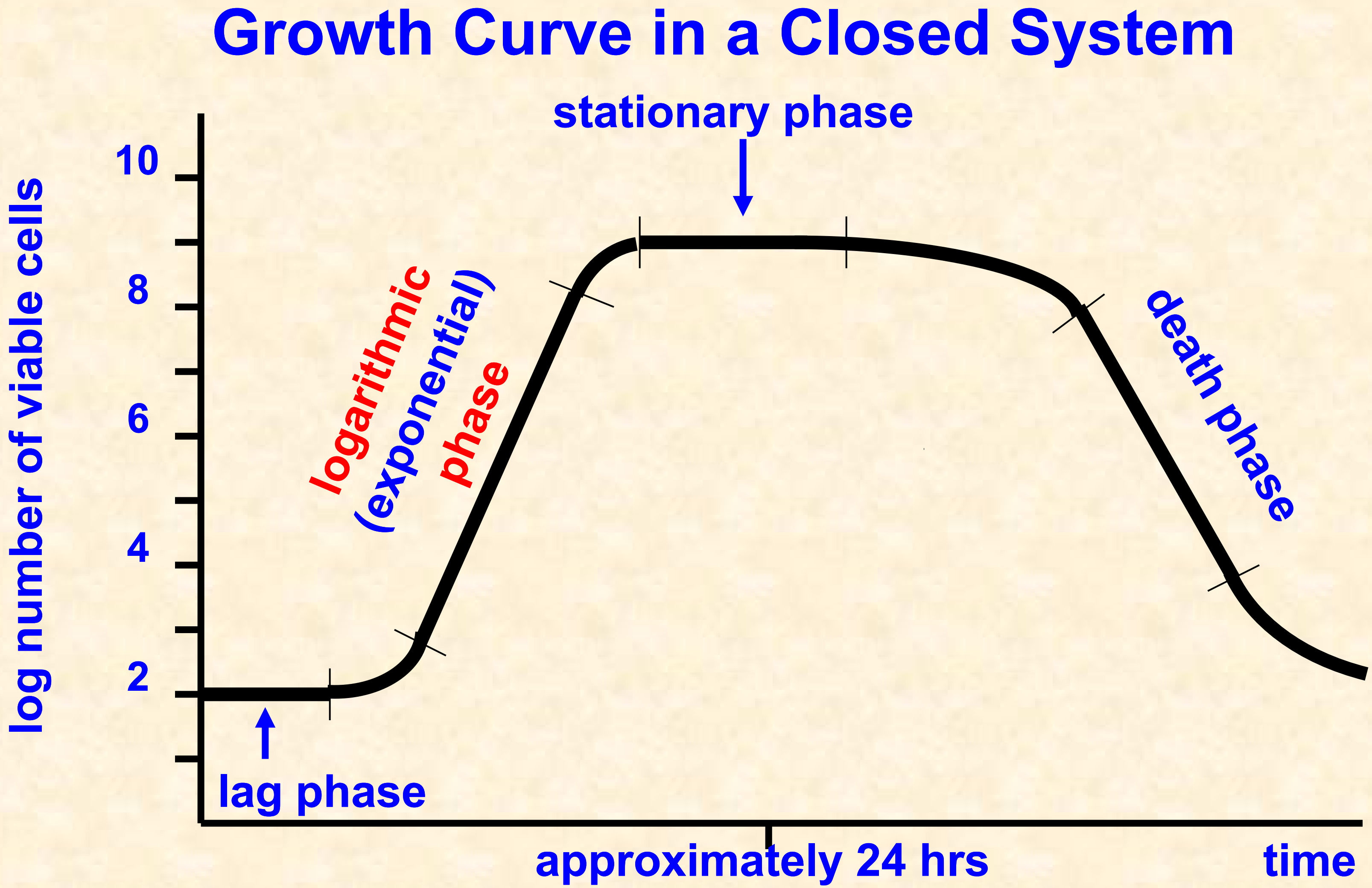
In general:

Logarithm of the number $\underline{a}$ is an exponent (*e*) to the power of which the base (B) is raised so that it equals the number $\underline{a}$

Therefore: if $\underline{a} = B^e$, then $\log_B \underline{a} = e$

Example: if $\underline{a} = 1000 = 10^3$ (and $B = 10$), then $\log \underline{a} = 3$

Microbial growth curve – III



Microbial growth curve – IV

Lag phase: microbes grow, but do not divide

Logarithmic phase: cells divide at a constant speed (generation time is constant);
relation between the number of the living cells and the time is **exponential**

Stationary phase: the number of cells is stable

Death phase: sometimes it proceeds according to the exponential curve

Continuous culture

The culture is continually supplied with nutrients and simultaneously disposed of the products of metabolism as well as the reproduced cells

Culture vessels are called fermentors

Used in industry for the production of microbial mass, but mostly for the production of various substances (organic acids, antibiotics, enzymes, vitamins etc.)

Recommended reading material

Paul de Kruif: Microbe Hunters

Paul de Kruif: Men against Death

Axel Munthe: The Story of San Michele

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Thank you for your attention