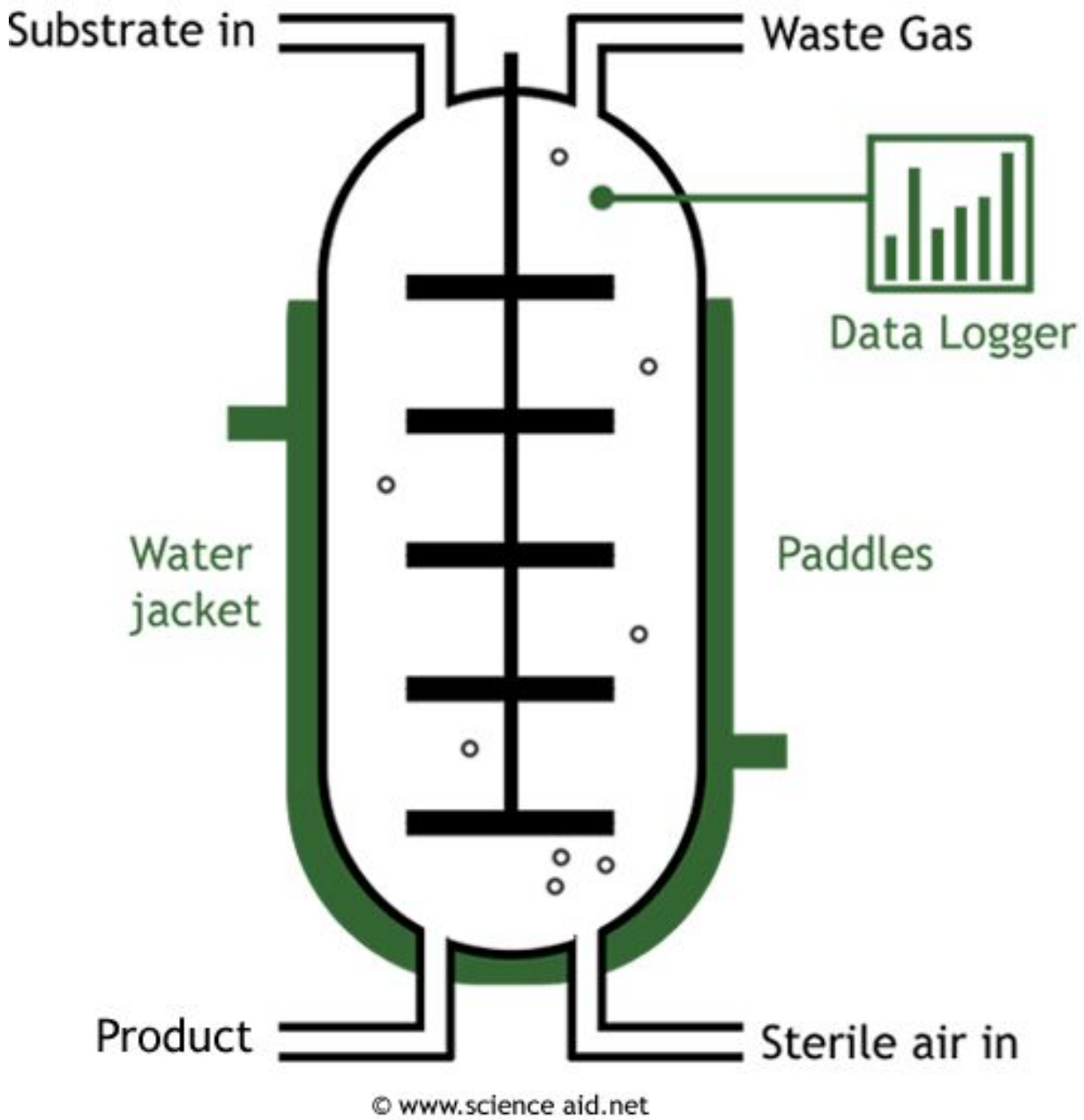

4386d
Lasius
Ameise
Geschlechtstier
mit Flügeln
total


Kidney c.s.
Fly Head


1.Pine Leaf. c.s.
Pine


HomeBeer


Substrate in
Waste Gas
Data Logger
Water jacket
Paddles
Product
Sterile air in
© www.science aid.net

The methods for the preparation of **permanent stains**.

the structure and the working principle of the **fermenter**.

Learning Objectives

1. Describe the **methods for the preparation** of permanent stains.
2. Describe the structure and the working principle of the **fermenter**

Success criteria

1. Knows differences between permanent and temporary preparations.
2. Describes the stages of preparation of permanent preparations.
3. Explains the importance of each stage of the preparation.

1. Know about fermenters. Complete the diagram of ‘typical’ fermenter.
2. Describe arrangement and work principles of fermenter.

Terminology

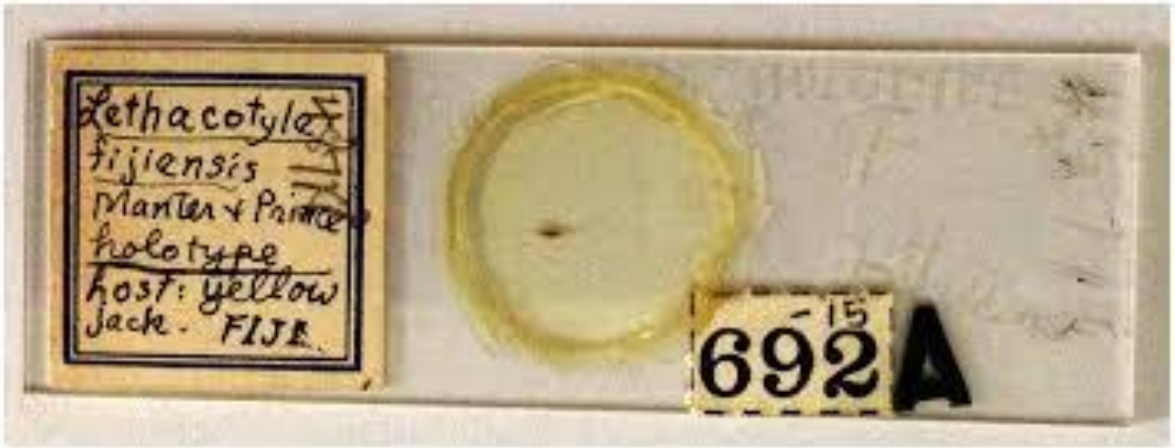
- Fixation, Dehydration, Embedding, Sectioning, Staining/Mounting , Aseptic technique

Describe the methods for the preparation of permanent stains.

-What is a permanent stain?

-How is a permanent stain different than a temporary?

-What are some reasons for / against temporary / permanent?


Lethacotyle
fijiensis
Manter & Prince
holotype
host: yellow
jack. FIJI
692
15
A

Terminology – Permanent Stain

English	Russian
Fixation	Фиксация
Dehydration	Обезвоживание
Embedding	Внедрение/Вставлять
Sectioning	Секционирование
Staining/Mounting	Окрашивание / установка
Aseptic technique	Асептическая техника

Permanent Slide	Temporary Slide
• Stored for a longer duration.	• Cannot be stored for a long duration.
• Cannot be used to observe live specimens	• Can be used to observe live specimens.

The basic steps of a permanent stain (specimen)

1. ***Fixation*** – treatment of tissue with chemical agent.

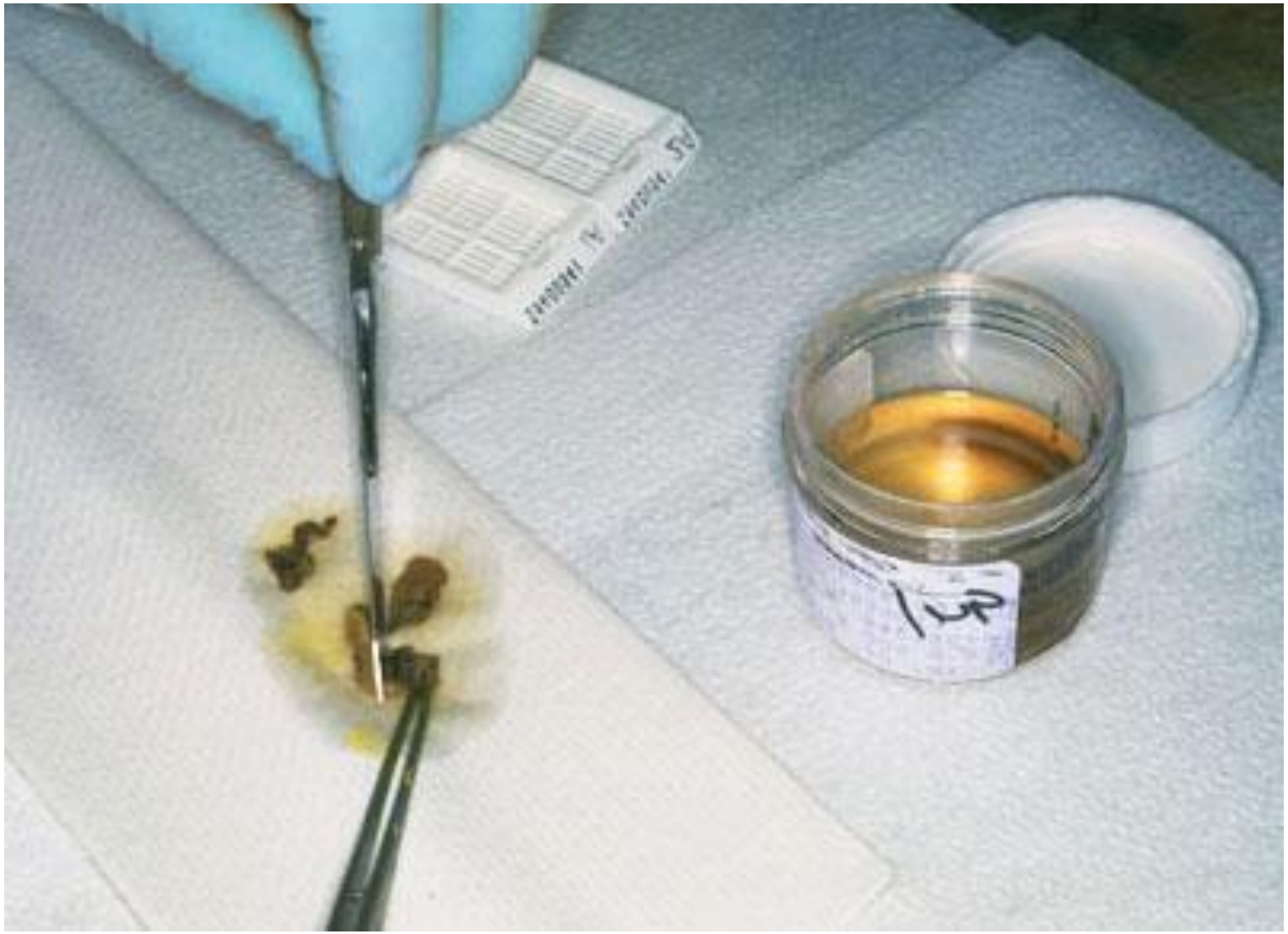
2. ***Dehydration & Clearing*** – removal of water from tissue sample.

3. ***Embedding*** – Infiltration of tissue sample with paraffin.

4. ***Sectioning*** – Cutting tissue sample by section into specific equal increments.

5. ***Mounting and Staining*** – Placing the tissue sample on adhesive glass slides.

FIXATION



1. Fixation

- Any treatment which will preserve cell structure and its biochemical composition in a life like state.
- The chemical used is called a fixative.
- Fixation techniques depends on the type of microscope.
- The fixative should be able to kill the organism quickly, preserve its structure and must enter the specimen well enough to react with all the parts.

Types of fixation

- Physical fixation
  - Specimen subjected to low temperature treatment (cryo-fixation)
  - Specimen subjected to high temperature (boiling/microwave)
- Chemical fixation
  - small specimens are immersed in the fixative (immersion fixation) like formalin.
  - in the case of some whole organs such as a lungs or brain the fixative is perfused through the circulatory system (perfusion fixation)

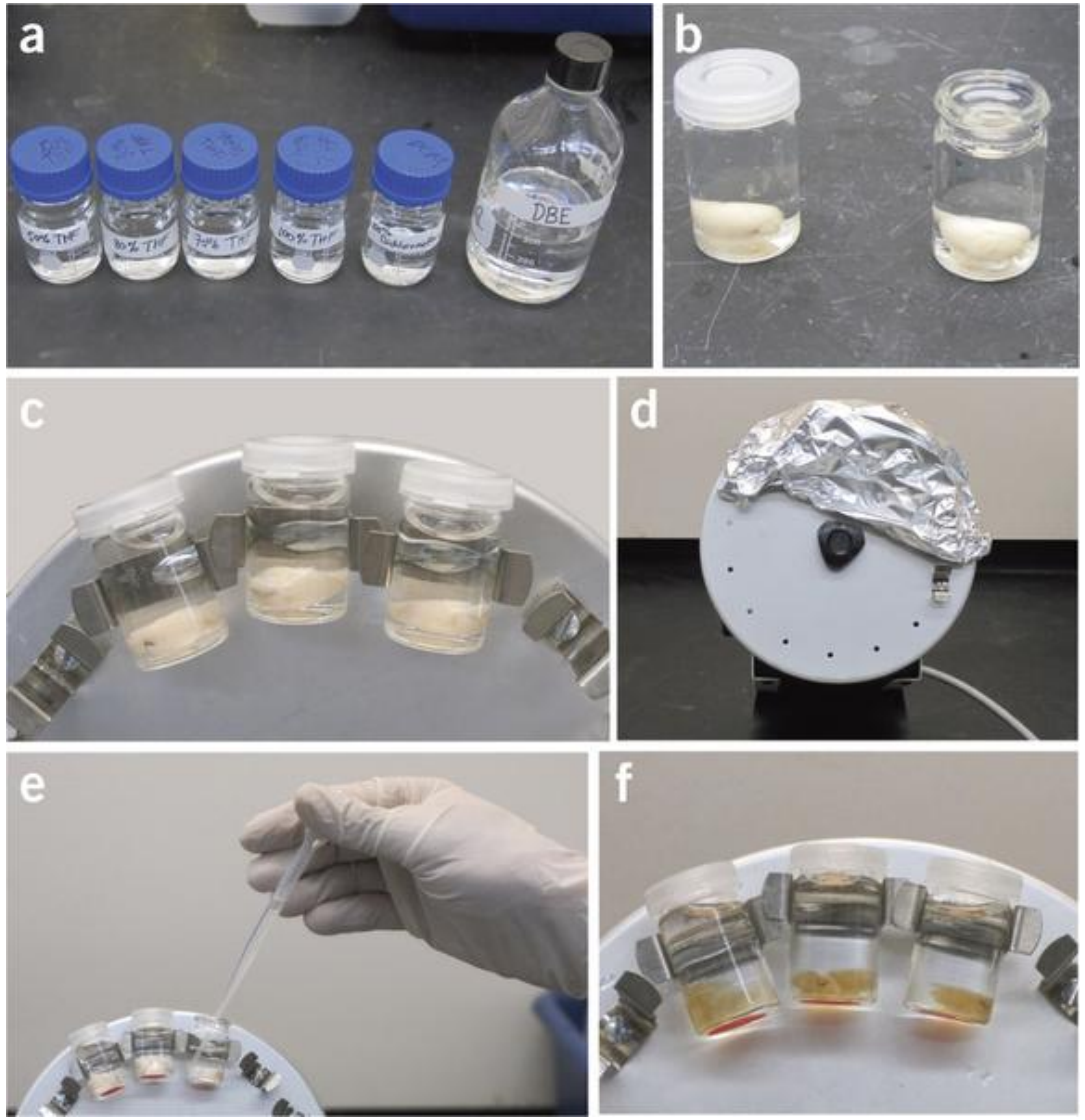

shutterstock


shutterstock
www.shutterstock.com · 171447704


www.shutterstock.com · 189737723


shutterstock
IMAGE ID: 135683750

DEHYDRATION & CLEARING



2. Washing:

The excess fixative agent is washed or rinsed in clean water.

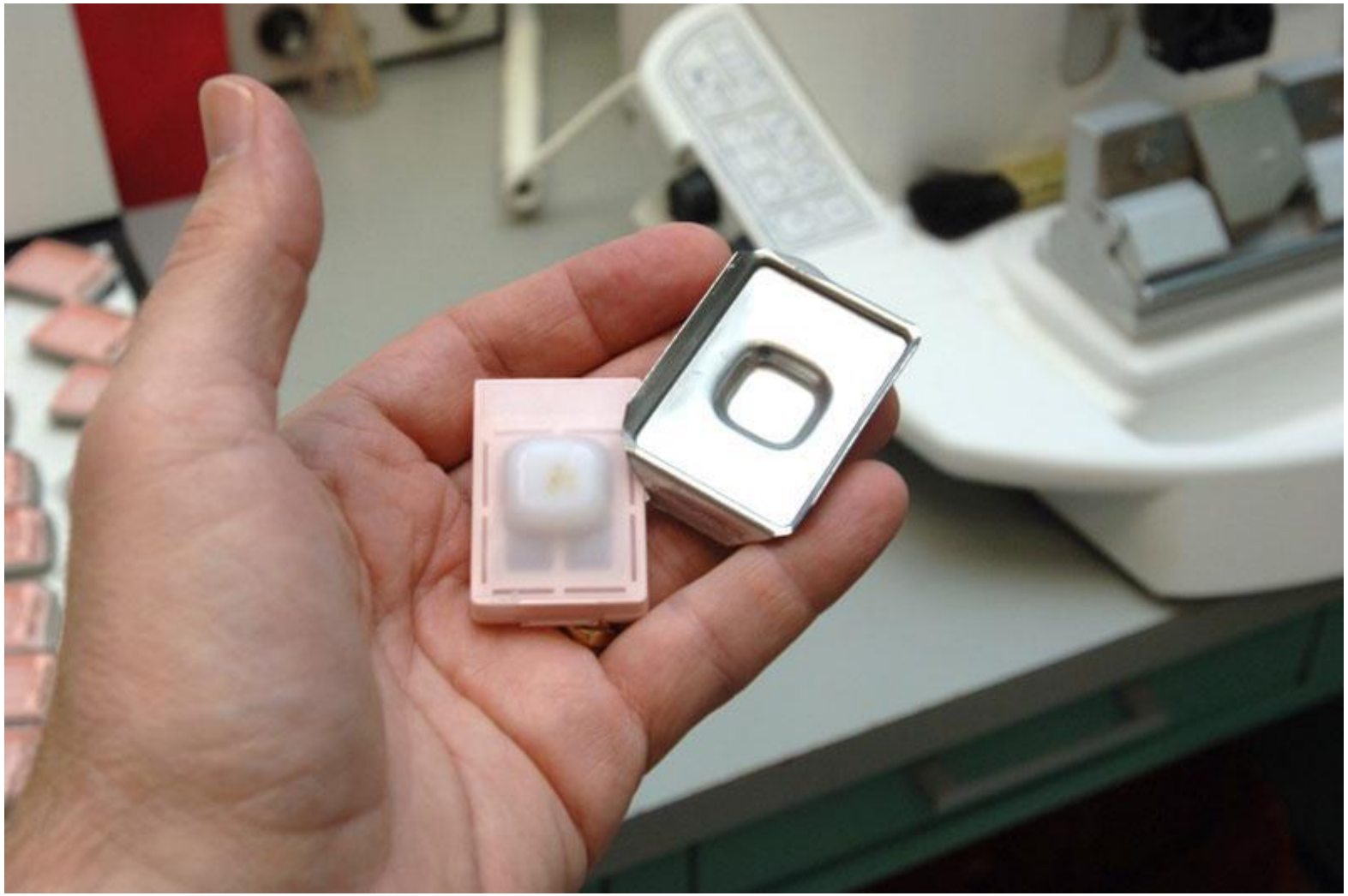
3. Dehydration:

- Removal of water from the specimen.
- Necessary when the specimen is mounted on non water based medium.
- done by placing the specimen in successively higher concentrations of ethanol or acetone.
- The potential problems of dehydration are shrinkage of the specimen, plasmolysis, and removal of soluble components from the specimen.

4. Clearing

- Clearing is the process of placing the specimen in xylene to prepare it for embedding.
- Ethanol used for dehydration and wax for embedding are immiscible.
- An intermediate solvent miscible with both ethanol and wax is used. This solvent will displace the ethanol in the tissue, which in turn will be displaced by molten paraffin wax.
- This stage in the process is called "clearing" and the reagent used is called a "clearing agent".
- Common clearing agent: xylene.

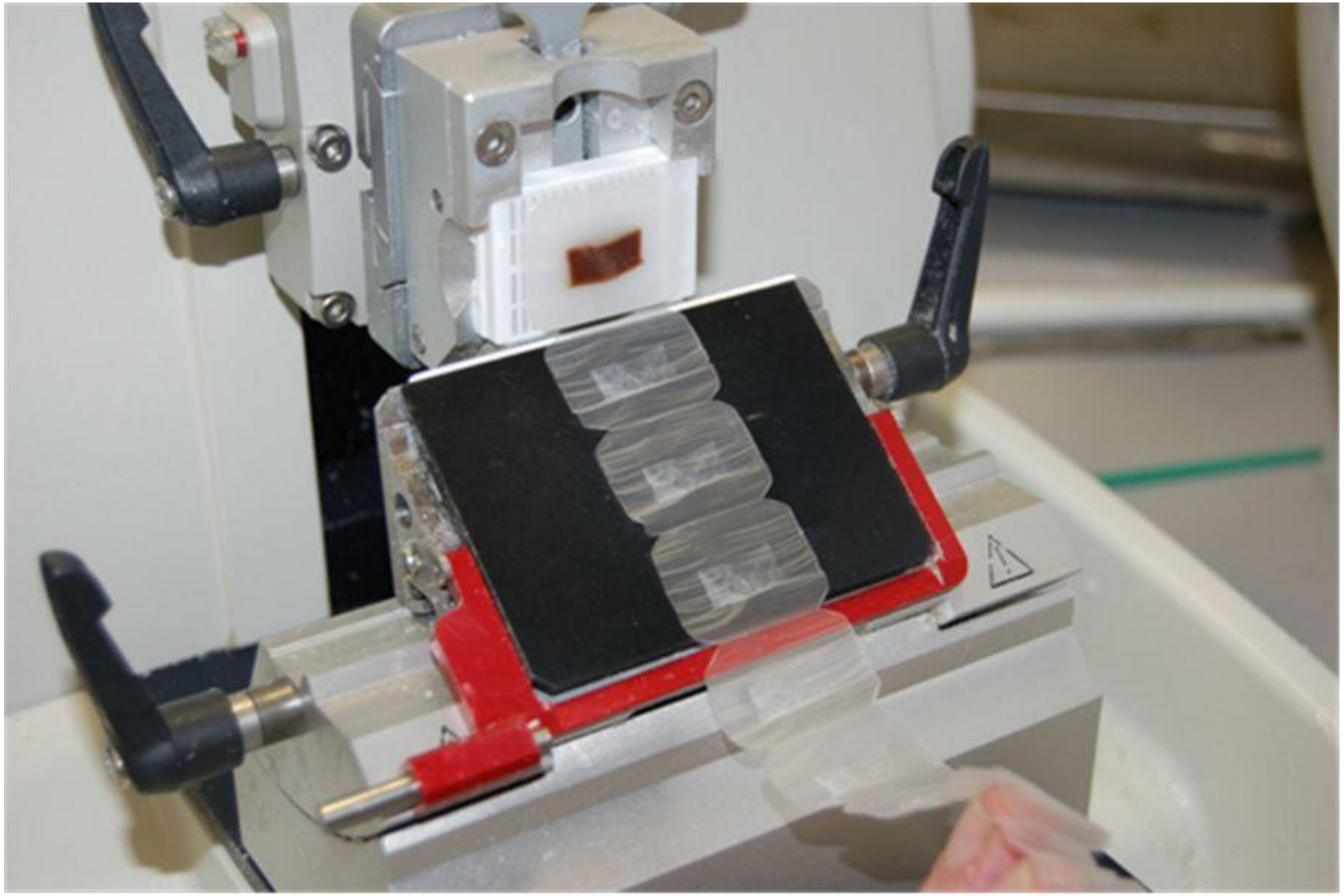
EMBEDDING



5. Embedding/blocking out

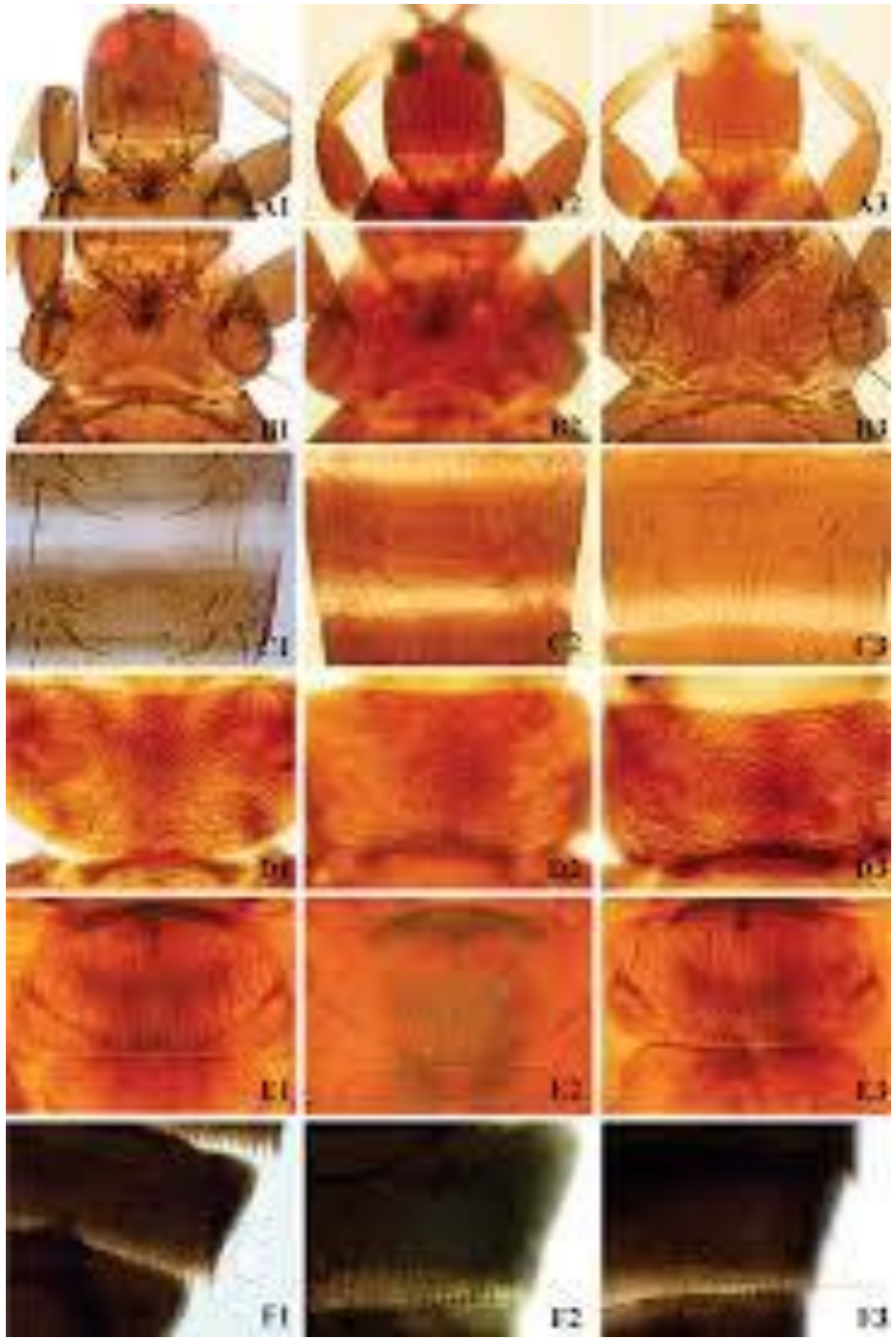
- Preparing the specimen to be set and sectioned (cut into thin slices) is called embedding.
- It involves soaking the material with molten wax and allowing it to cool and set.(
- This allows accurate cross and longitudinal sections to be prepared.
- Selection of embedding material used depends on the orientation of the section and the type of microscope used.

SECTIONING

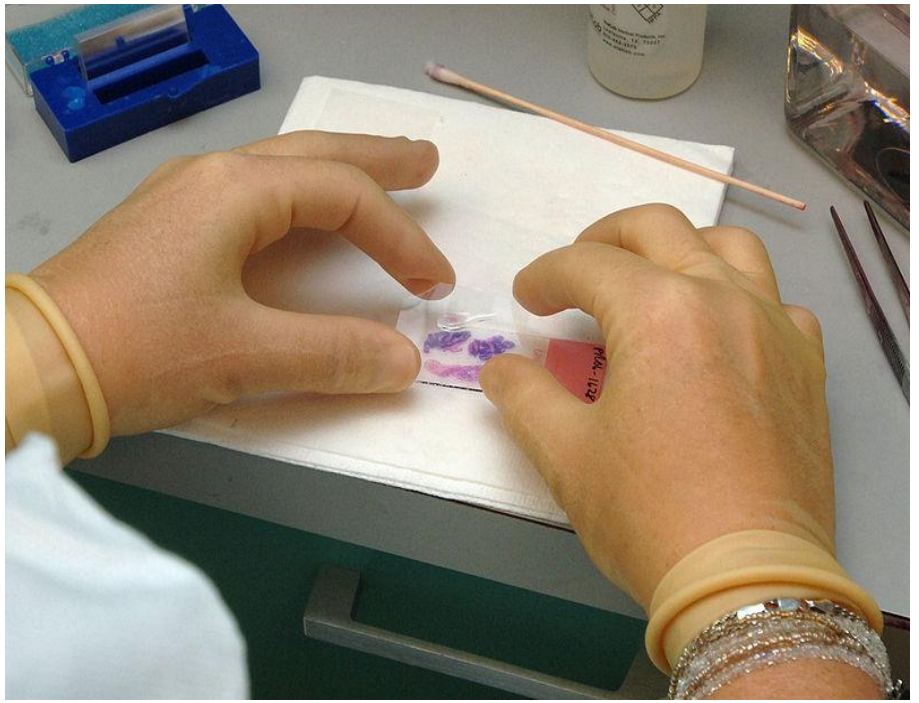


6. Sectioning

- Cutting of the specimen into very thin slices is called sectioning.
- The specimen has to be very thin to allow the light to pass through.
- It is done using a razor or microtome



MOUNTING AND STAINING



7. Staining

- Cell staining is a technique that can be used to enhance visualization of the cell or certain cellular components under a microscope.
- Most stains can be used on fixed, or non-living cells, while only some can be used on living cells; some stains can be used on either living or non-living cells.
- The specimen is immersed in the solution of the stain/dye for few minutes and excess dye is rinsed off using clean water.

COMMONLY USED STAINS

1. **Hematoxylin** -Specialized stains that differentiate the fibrous components of the extracellular matrix.

2. **Eosin** – Stains that differentiate between acidic and basic cellular components.

3. **Toluidine Blue** - Metallic salts that precipitate on tissue forming metal deposits.

8. Mounting

- It is the placing of the sample to a glass slide for observation and analysis.
- The mounting medium holds the specimens in place between the cover slip and the slide, preventing contact with air.
- In case of liquid mounting medium the four sides of the cover slip has to be sealed.
- Common mounting medium: Canada balsam, euparol, glycerol, clear nail polish, etc.

HISTOCHEMISTRY

1. Periodic Acid Schiff (PAS) - Is a staining method used to detect polysaccharides (e.g. glycogen, glycoproteins, glycolipids and mucins in tissues.

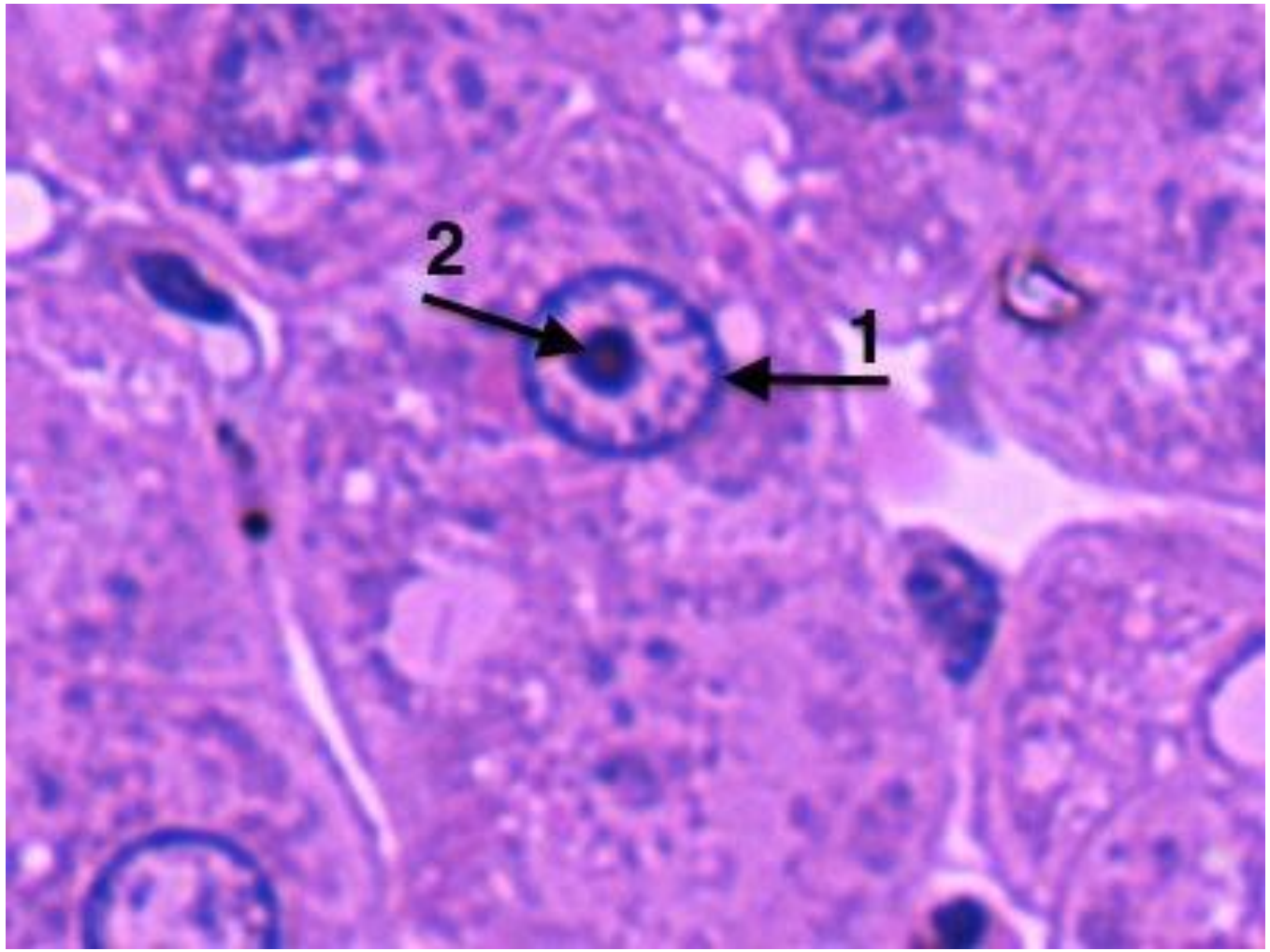
2. Feulgen Reaction - Is used to identify chromosomal material or DNA in cell specimens.

3. Gomori-Takamatsu - a method for localizing the alkaline phosphatase enzyme.

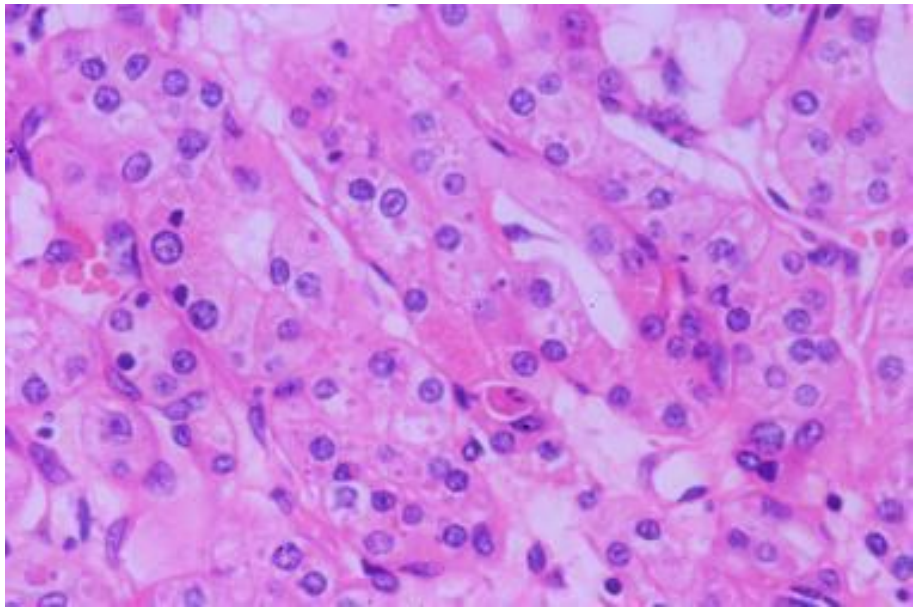
4. Mordant - Is a substance used to set dyes on tissue sections by forming a coordination complex with the dye which then attaches to the tissue. It may be used for intensifying stains in cell or tissue preparations.

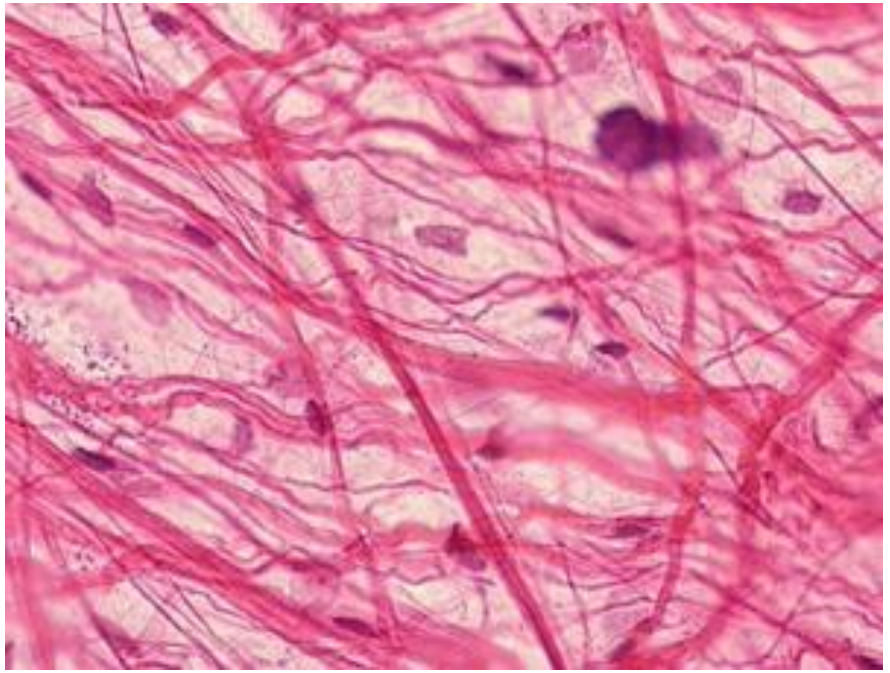
5. Counterstain - Is a stain with colour contrasting to the principal stain, making the stained structure more easily visible.

HEMATOXYLIN

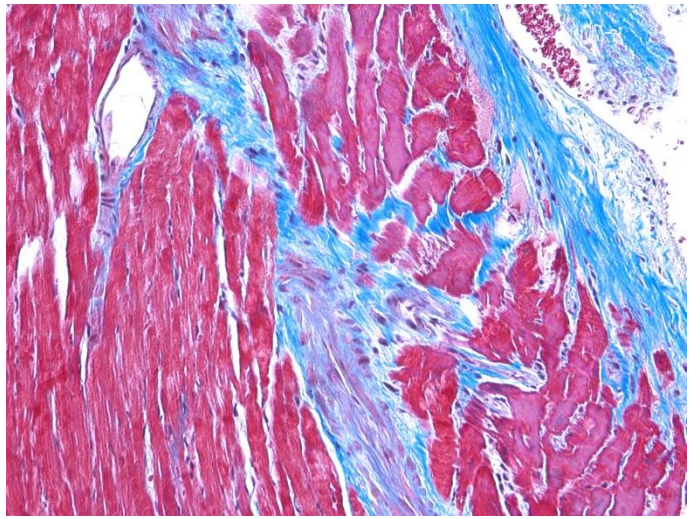


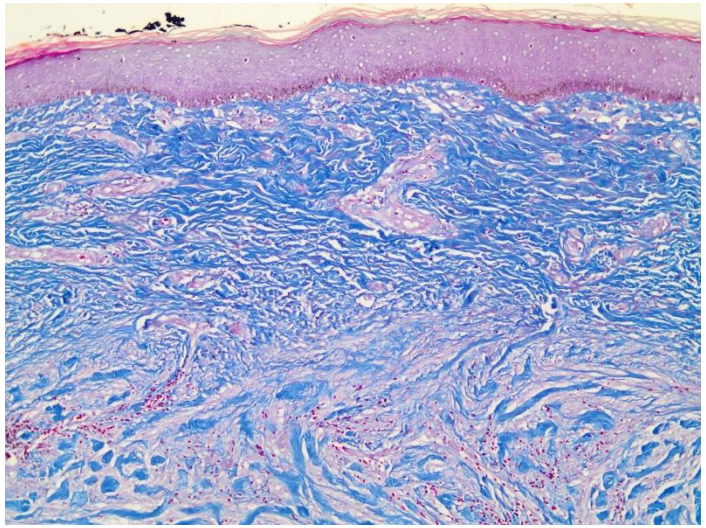
EOSIN

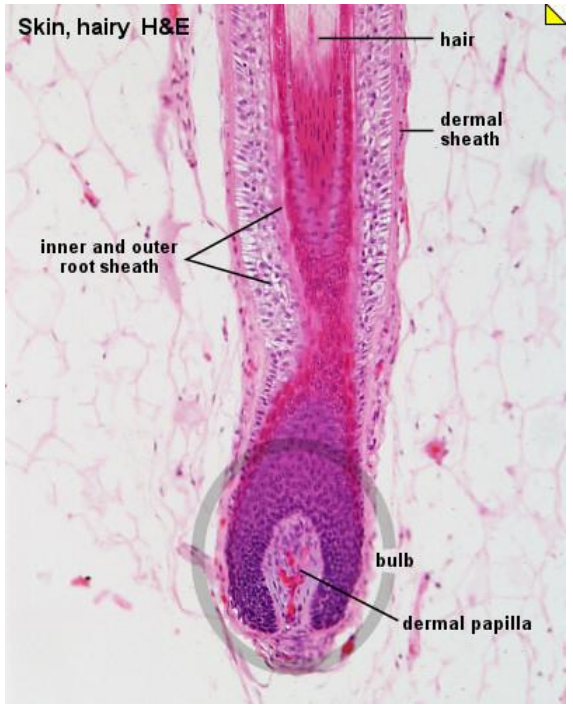


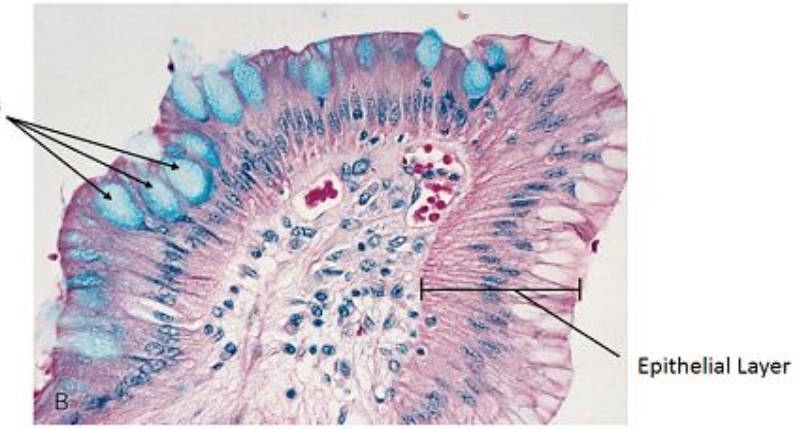


MASSON'S TRICHOME



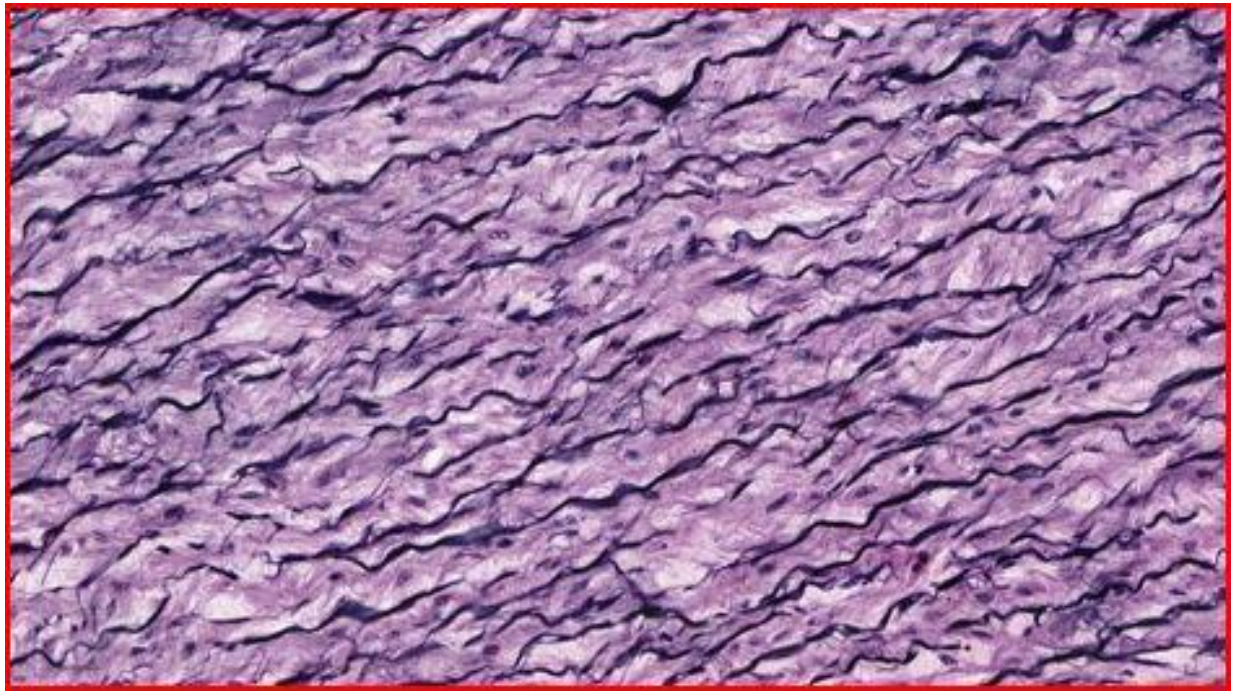




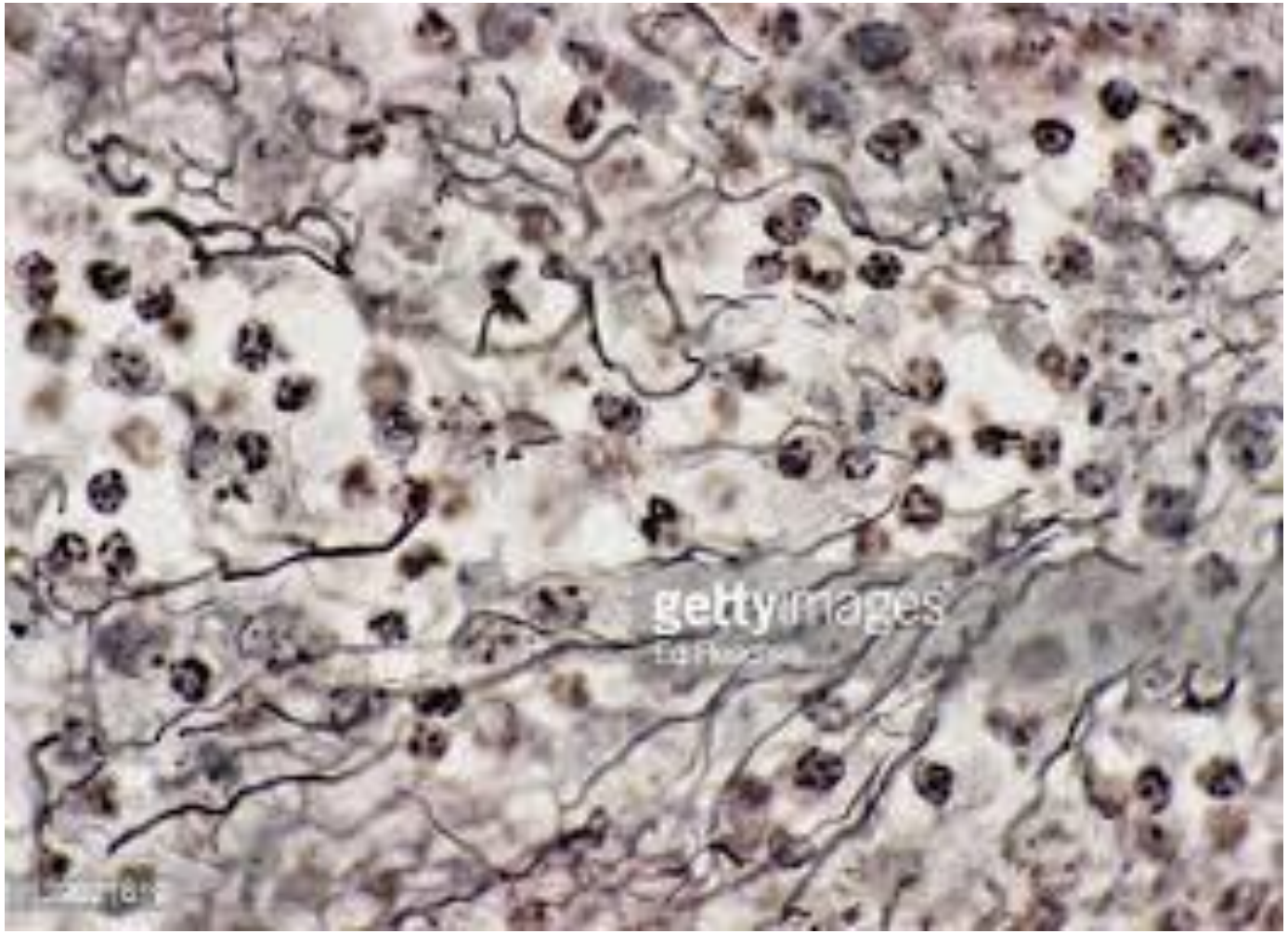


ORCEIN'S ELASTIC STAIN

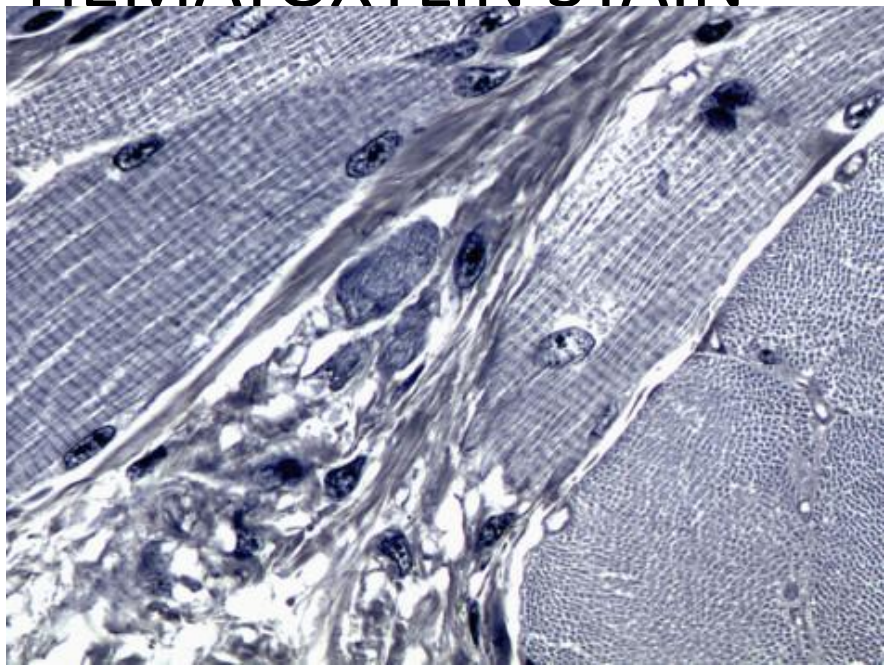
WEIGERT'S ELASTIC STAIN

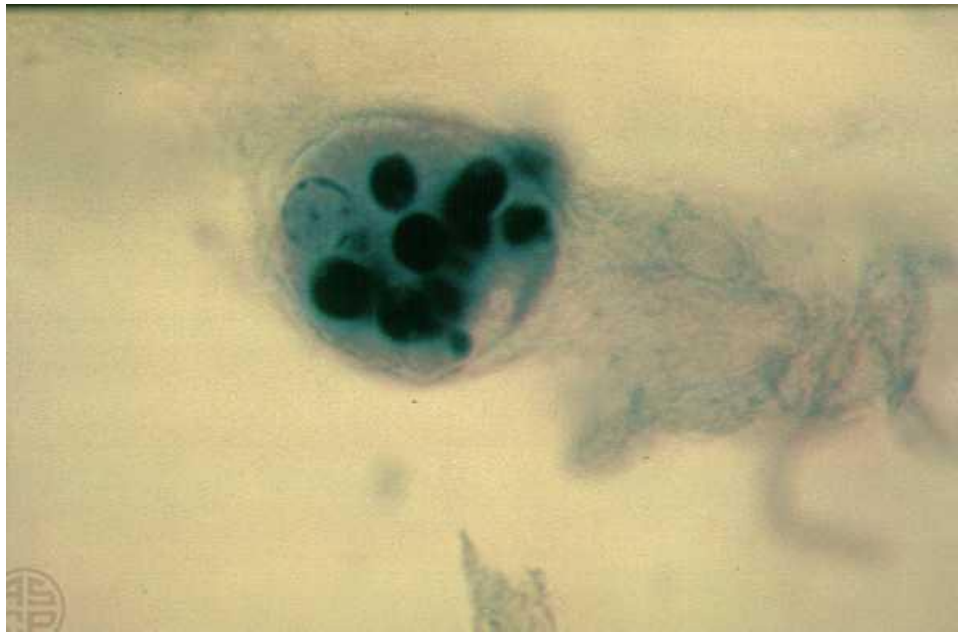


SILVER STAIN

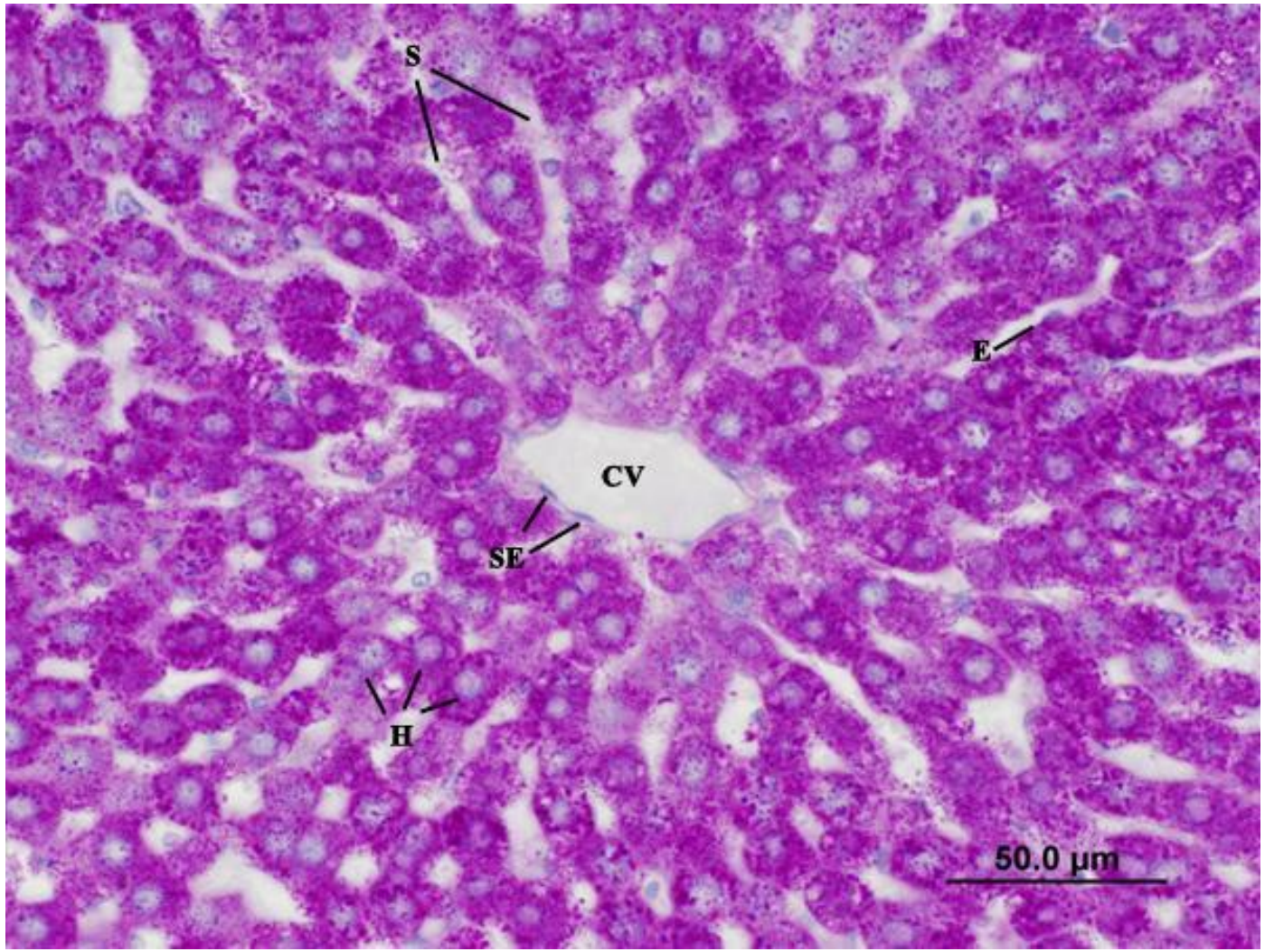


IRON HEMATOXYLIN STAIN

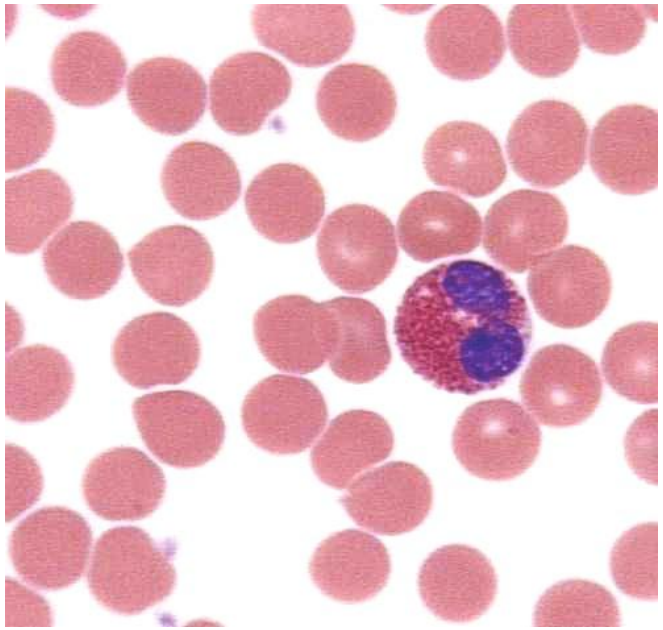


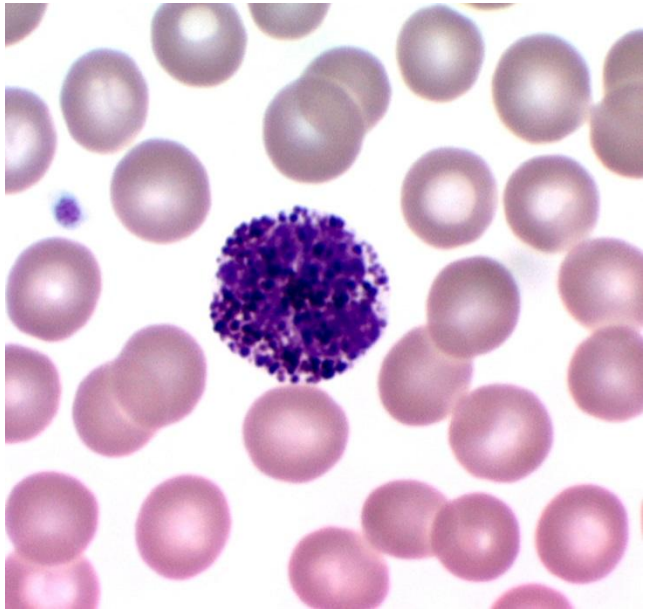


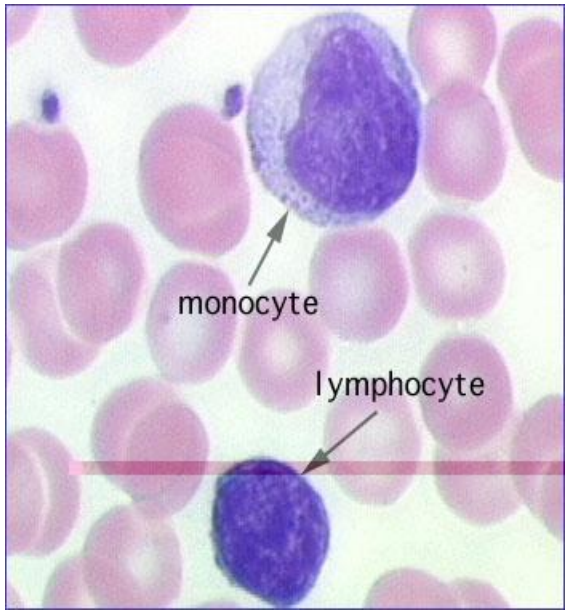
PERIODIC ACID-SCHIFF



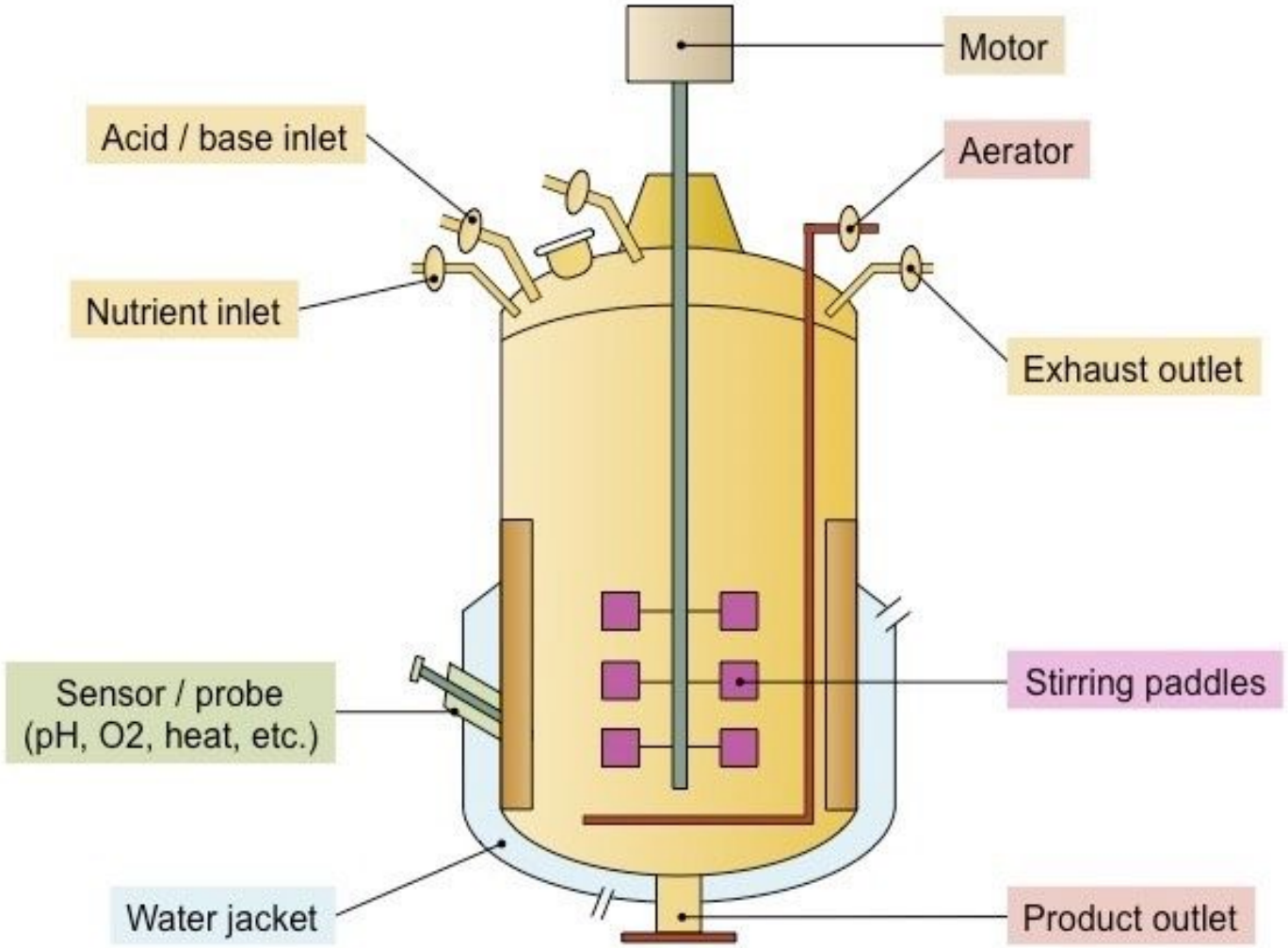
WRIGHT AND GIEMSA STAINS



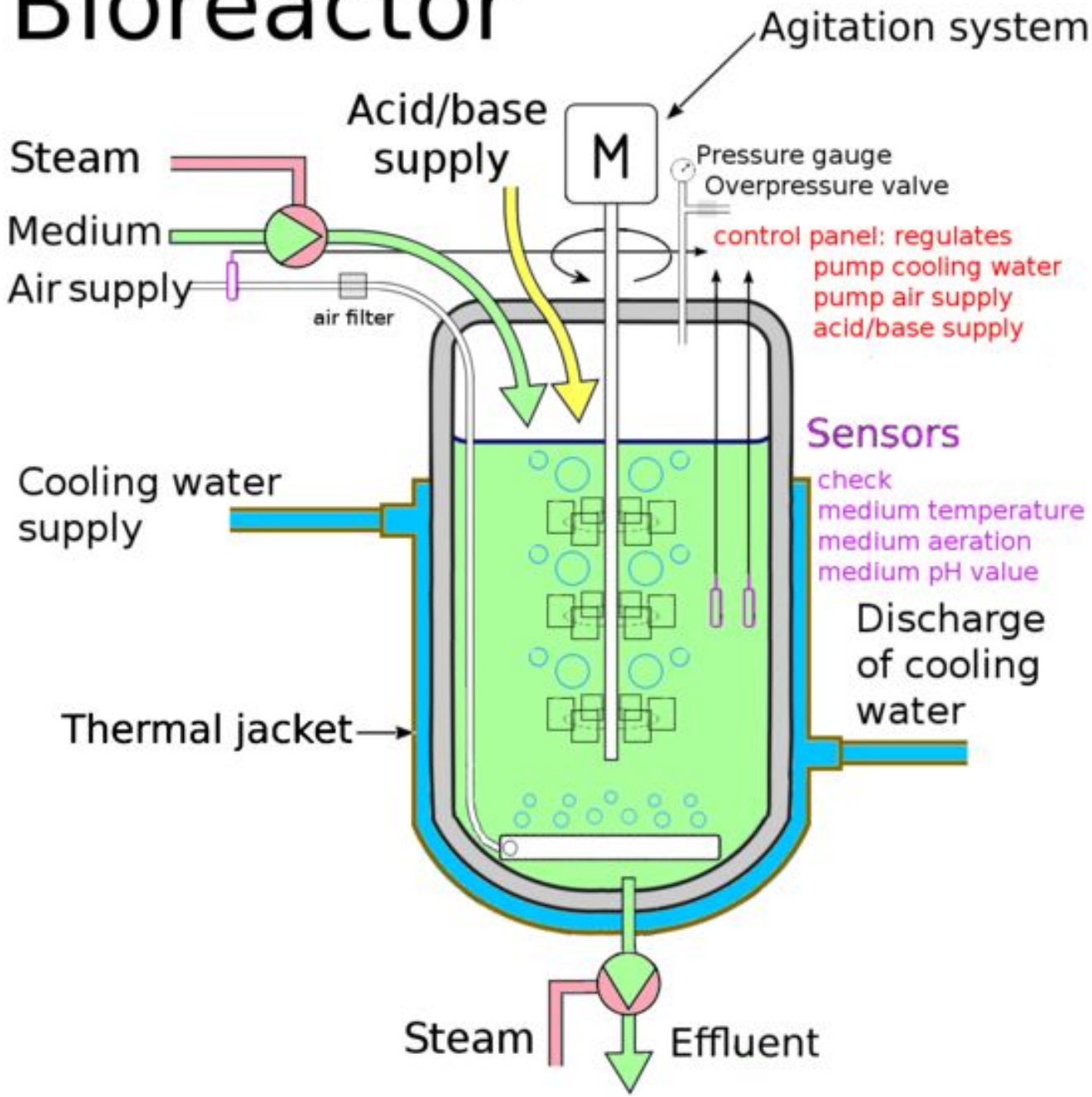




4. Describe the structure and the working principle of the **fermenter**



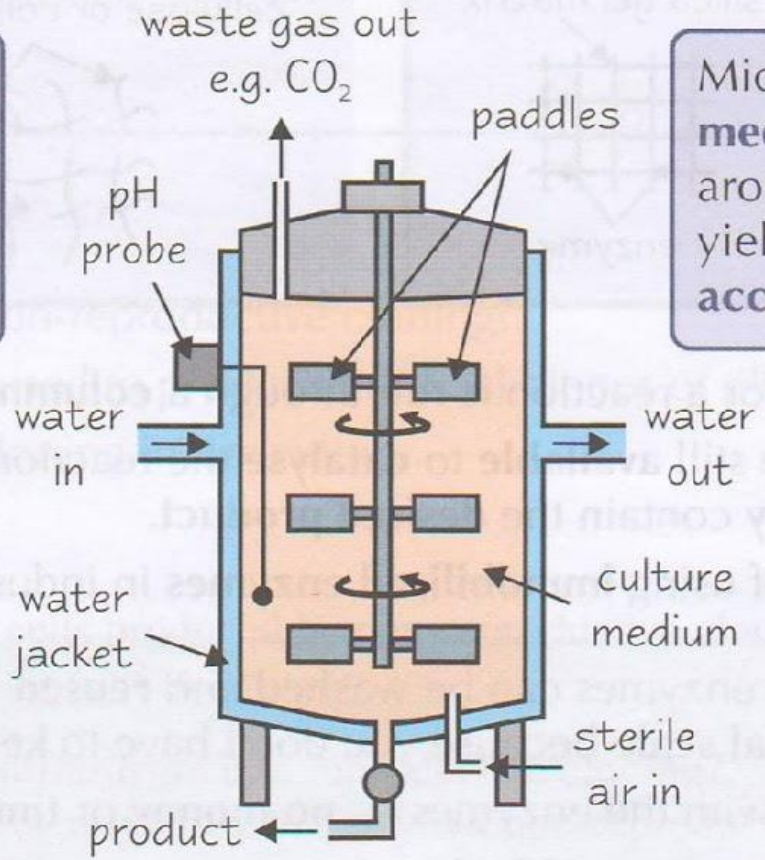
Bioreactor


Agitation system
Acid/base supply
M
Steam
Medium
Air supply
air filter
Pressure gauge
Overpressure valve
control panel: regulates
pump cooling water
pump air supply
acid/base supply
Sensors
check
medium temperature
medium aeration
medium pH value
Cooling water supply
Discharge of cooling water
Thermal jacket
Steam
Effluent

Microorganisms are Grown in Fermentation Vessels

Cultures of microorganisms are grown in **large containers** called **fermentation vessels**. The **conditions** inside the fermentation vessels are kept at the **optimum for growth** — this **maximises** the **yield** of **desirable products** from the microorganisms. Here's a bit about how they work:

The **pH** is **monitored** and kept at the **optimum level**. This **increases** the product yield because **enzymes** can work **efficiently**, so the **rate of reaction** is kept as **high** as possible.



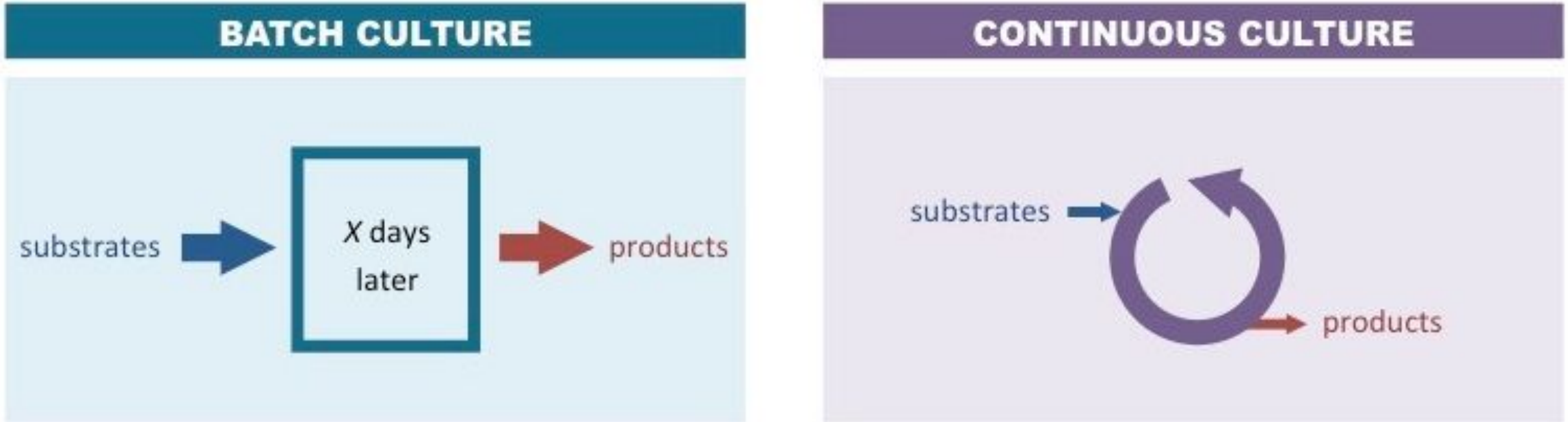
Microorganisms are kept in **contact** with **fresh medium** by **paddles** that **circulate** the medium around the vessel. This **increases** the product yield because microorganisms can **always access** the **nutrients** needed for **growth**.

The **temperature** is kept at the **optimum level** by a **water jacket** that surrounds the vessel. This **increases** the product yield because **enzymes** can work **efficiently**, so the **rate of reaction** is kept as **high** as possible.

The volume of **oxygen** is kept at the **optimum level** for **respiration** by pumping in sterile air when needed. This **increases** the product yield because microorganisms can always **respire** to provide the **energy** for **growth**.

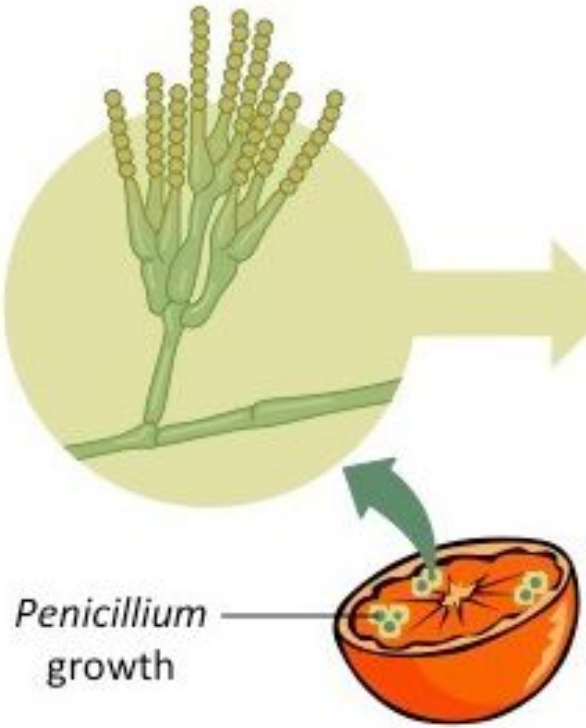
Vessels are **sterilised** between uses with **superheated steam** to kill any **unwanted organisms**. This **increases** the product yield because the microorganisms **aren't competing** with other organisms.

Batch Culture Versus Continuous Culture

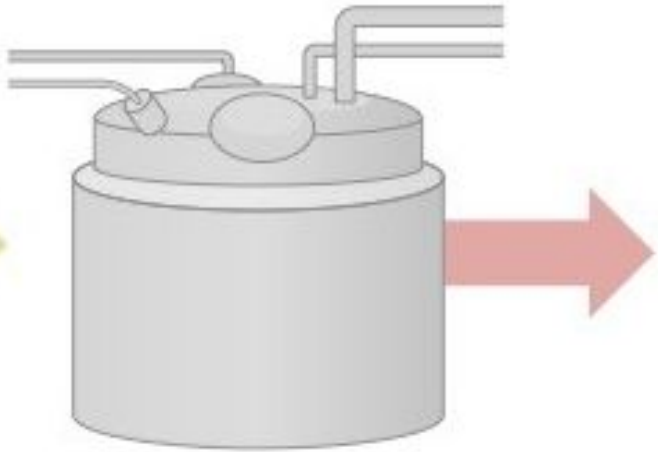


Production of Penicillin via Batch Fermentation

1. *Penicillium* mold produces the antibiotic penicillin

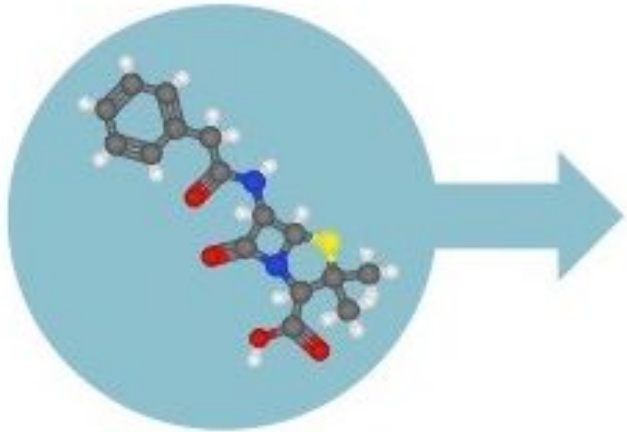


2. Scientists grow mold in deep batch fermenters by adding sugar and other key ingredients



Fermentation tank

3. Scientists separate the penicillin from the mold



Penicillin molecule

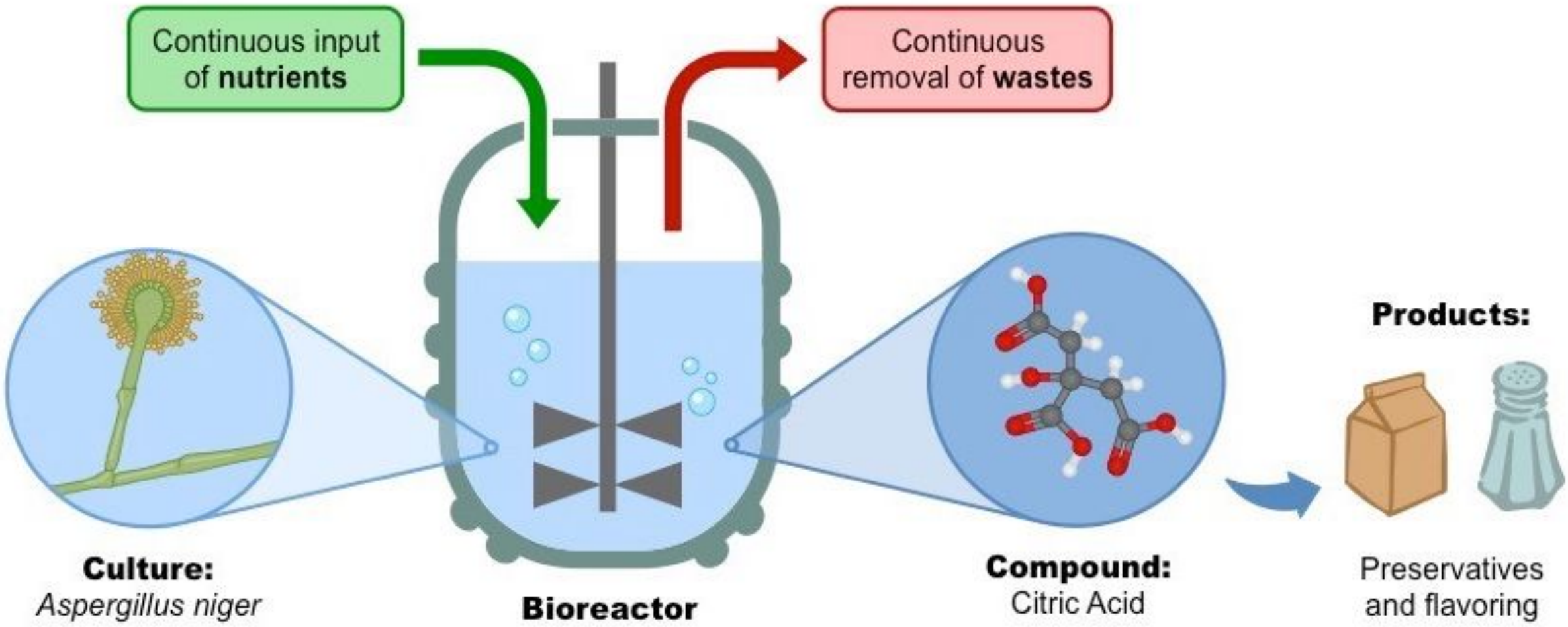
4. Penicillin is purified for use as an antibiotic medicine

Antibiotic medicine

Batch Culture

- Fermentation is carried out in a closed fermenter, with nothing added or removed during the process (except venting of gas)
- Microorganisms and nutrients are left for a set period of time, during which the nutrient stock is depleted
- The advantage of a batch culture is that the fermenter can be used for different reactions with each separate use
- A disadvantage of a batch culture is that it results in significant periods of idle time between use, resulting in higher costs

Production of Citric Acid via Continuous Fermentation



Continuous Culture

- Fermentation is carried out in an open fermenter, with nutrients added and product removed at a steady rate throughout
- This results in a continuous reaction with no idle time, reducing labour costs and increasing product yields
- A disadvantage of continuous culture is that there is a higher risk of contamination due to the constant adjustments
- Continuous fermentation is feasible only when the inoculated cells are genetically stable

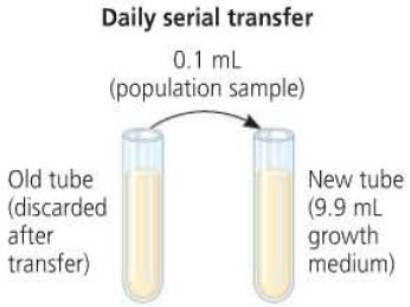
Bacteria Dividing

- http://www.cellsalive.com/ecoli.htm

Figure 27.10 Inquiry

Can prokaryotes evolve rapidly in response to environmental change?

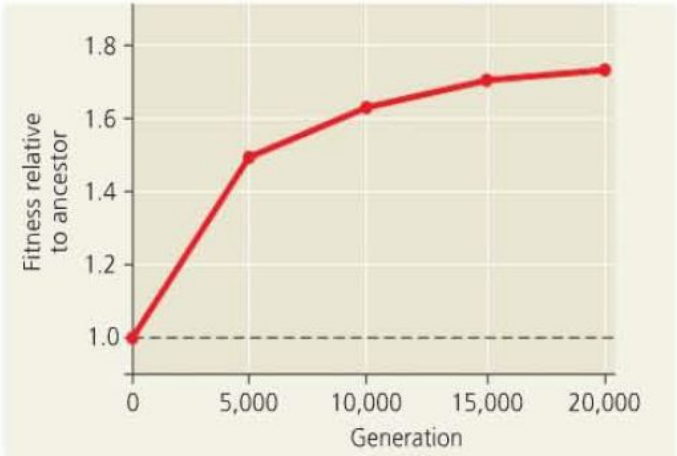
EXPERIMENT Vaughn Cooper and Richard Lenski, of Michigan State University, tested the ability of *E. coli* populations to adapt to a new environment. They established 12 populations, each founded by a single cell from an asexual strain of *E. coli*, and followed these populations for 20,000 generations (3,000 days). To maintain a continual supply of resources, each day the researchers performed a *serial transfer*: They transferred 0.1 mL of each population to a new tube containing 9.9 mL of fresh growth medium. The growth medium used throughout the experiment represented a challenging environment that contained only low levels of glucose and other resources needed for growth.



Samples were periodically removed from the 12 populations and grown in competition with the common ancestor in the experimental (low-glucose) environment.

RESULTS The fitness of the experimental populations, as measured by the rate at which each population grew, increased rapidly for the first 5,000 generations (two years) and more

RESULTS The fitness of the experimental populations, as measured by the rate at which each population grew, increased rapidly for the first 5,000 generations (two years) and more slowly for the next 15,000 generations. The graph below shows the averages for the 12 populations.



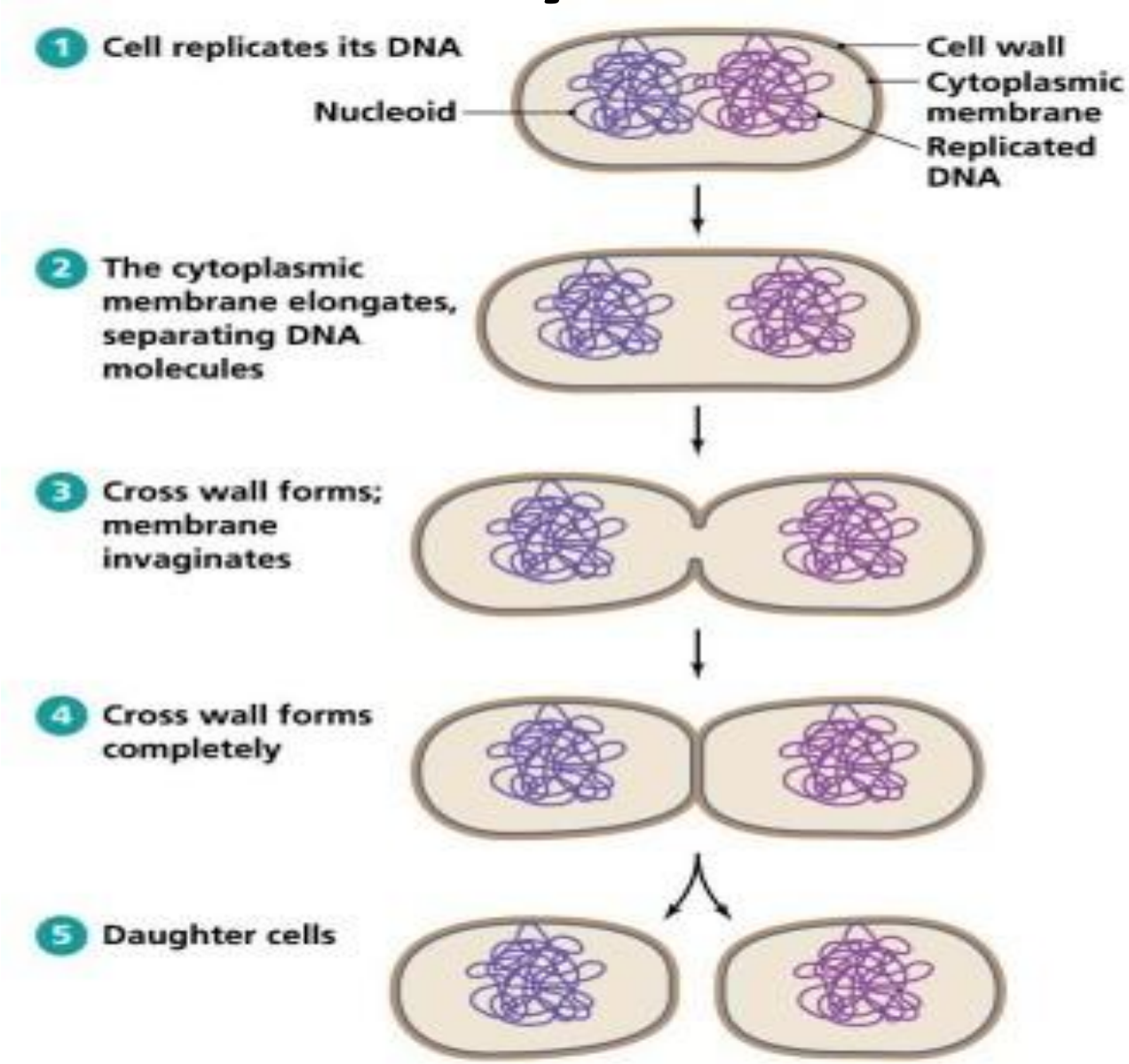
CONCLUSION Asexual populations of *E. coli* continued to accumulate beneficial mutations for 20,000 generations, allowing rapid evolution of improved performance in their new environment.

SOURCE V. S. Cooper and R. E. Lenski, The population genetics of ecological specialization in evolving *Escherichia coli* populations, *Nature* 407:736–739 (2000).

WHAT IF? Suggest possible functions of the genes whose sequence or expression was altered as the experimental populations evolved in the low-glucose environment.

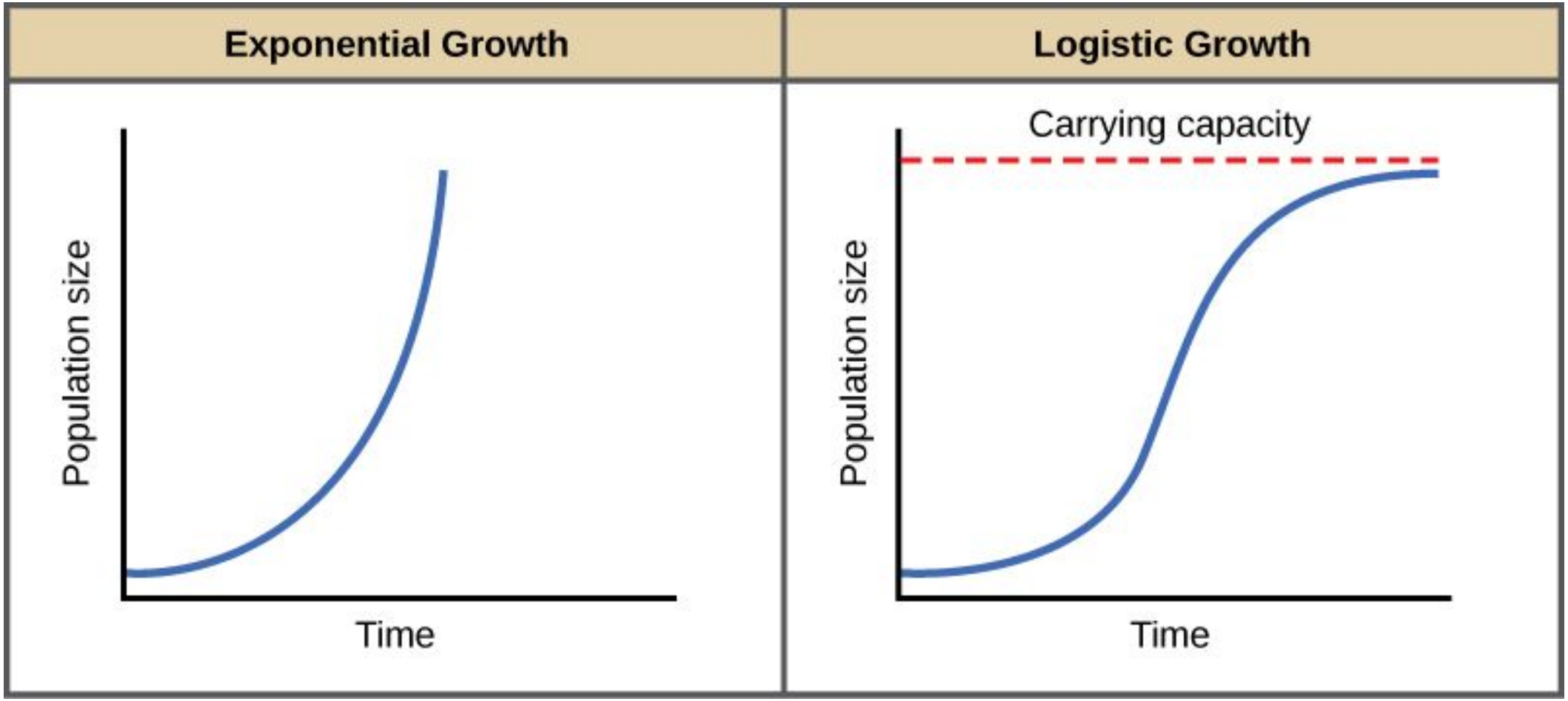
Asexual Reproduction : Binary Fission

Do you remember?



Exponential or J-shaped

Logarithmic or Sigmoid



Microorganisms Standard Growth Curves

Exponential or J-shaped

1. It occurs when the resources are abundant.

2. Population passes well beyond the carrying capacity of the ecosystem.

3. A stationary or steady phase is seldom achieved.

4. Population crashes ultimately due to mass mortality.

5. It has two phases, lag and log.

6. It occurs in fewer organisms, e.g., Lemmings, algal bloom.

Logarithmic or Sigmoid

1. It occurs when the resources are limited.

2. Population seldom grows beyond the carrying capacity of ecosystem

3. A stationary or steady phase is reached.

4. Population seldom crashes.

1. **Answer the questions about growth curves.**

2. On the blank area of your Microbiology metabolism worksheet answer the following:

   "With respect to oxygen, explain the main forms of metabolism in microorganisms".

Complete the following table for the two types of growth curves:

growth curve	shows unlimited, unchecked growth	growth limited by extrinsic or intrinsic factors	shape of curve (S or J)	shows carrying capacity for a population.	typical of short term or long term growth
exponential	xxxx		J		short term
logistic		xxxx	S	xxxx	long term

Directions: For each of the following scenarios circle whether the population growth would best be represented by a logistic or exponential growth curve.

a. a strep bacterium invades your throat and reproduces for 4 hours **exponential**

b. the flea population on a rat is monitored for 5 weeks with flea powder added **logistic**

c. loggerhead turtle populations are tracked for 5 years in the Atlantic **logistic**

d. a lucky yeast cell falls into your glass of grape juice and reproduces for 10 hours **exponential**

e. bull frog population in a local pond is monitored for 3 seasons **logistic**

There are **Two Main Culture Methods — Batch** and **Continuous**

1) **Batch culture** is where microorganisms are grown in **individual batches** in a fermentation vessel — when one culture **ends** it's **removed** and then a **different batch** of microorganisms is grown in the vessel.

2) **Continuous culture** is where microorganisms are **continually grown** in a fermentation vessel **without stopping**.

3) Here are some of the **differences** between batch culture and continuous culture:

Batch Culture	Continuous Culture
A fixed volume of growth medium (nutrients) is added to the fermentation vessel at the start of the culture and no more is added. The culture is a closed system.	Growth medium flows through the vessel at a steady rate so there's a constant supply of fresh nutrients. The culture is an open system.
Each culture goes through the lag, exponential and stationary growth phases.	The culture goes through the lag phase but is then kept at the exponential growth phase.
The product is harvested once, during the stationary phase.	The product is continuously taken out of the fermentation vessel at a steady rate.
The product yield is relatively low — stopping the reaction and sterilising the vessel between fermentations means there's a period when no product is being harvested.	The product yield is relatively high — microorganisms are constantly growing at an exponential rate.
If contamination occurs it only affects one batch. It's not very expensive to discard the contaminated batch and start a new one.	If the culture is contaminated the whole lot has to be discarded — this is very expensive when the cultures are done on an industrial scale.
Used when you want to produce secondary metabolites.	Usually used when you want primary metabolites or the microorganisms themselves as the desired product.

Asepsis is Important when Culturing Microorganisms

1) **Asepsis** is the practice of **preventing contamination** of cultures by **unwanted microorganisms.**
2) It's important when culturing microorganisms because contamination can **affect** the **growth** of the microorganism that you're **interested in.**
3) Contaminated cultures in **laboratory experiments** give **inaccurate results.**
4) Contamination on an **industrial scale** can be **very costly** because **entire cultures** may have to be **thrown away.**
5) A number of **aseptic techniques** can be used when working with microorganisms:

- **Work surfaces** are **regularly disinfected** to minimise contamination.
- **Gloves should be worn** and **long hair** is **tied back** to prevent it from falling into anything.
- The **instruments** used to **transfer** cultures are **sterilised before** and **after** each use, e.g. **inoculation loops** (small wire loops) are **heated** using a **Bunsen burner** to **kill** any microorganisms on them.
- In laboratories, the **necks** of **culture containers** are **briefly flamed** before they're **opened** or **closed** — this causes **air** to **move out** of the container, **preventing** unwanted microorganisms from **falling in.**
- **Lids** are **held over** open containers after they're removed, instead of putting them on a work surface. This **prevents** unwanted microorganisms from **falling** onto the culture.

- https://www.youtube.com/watch?v=YX_b02KYN9g