

# **PRIMARY CELL CULTURING**

BY

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# CELL CULTURE

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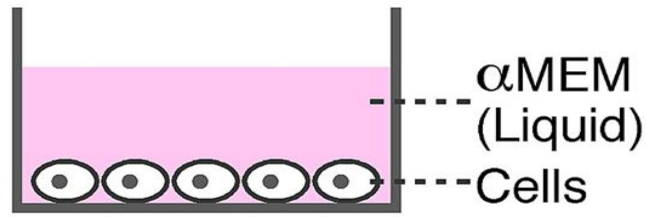
- ✘ Cell culture is the process by which cells are grown under controlled conditions, generally outside the native environment. The cells of human interest are introduced after isolating from living tissue, they can be maintained under controlled conditions. The conditions may vary for each cell type, but it consist of suitable vessel with certain medium that supplies the essential nutrients (amino acid ,carbohydrates, vitamins &minerals) growth factors, hormones and gases.(CO<sub>2</sub>, O<sub>2</sub>)

# CELL CULTURE(ATTACHED CELL)

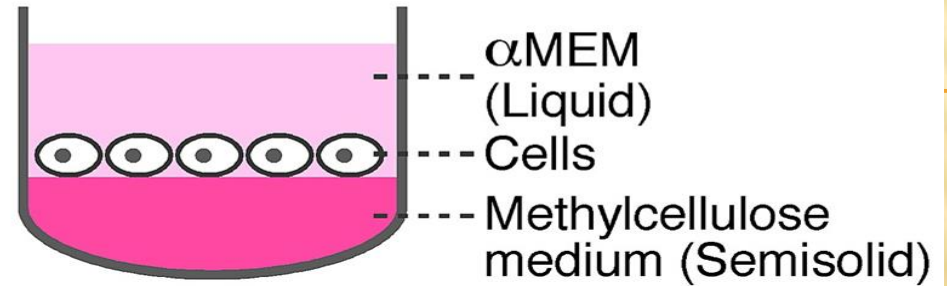
Below are few steps for culturing adherent cells:-

1. Assess cultures
2. Remove the culture spent
3. Wash cells with PBS without calcium and magnesium.  
Repeat if required
4. Pipette trypsin/EDTA onto cell monolayer, rotate flask and decant excess.
5. Incubate for 2-10 minutes.
6. Examine cells.
7. Re-suspend cells in fresh medium.
8. Transfer cells into fresh , warmed , new media.
9. Incubate as required.
10. Repeat as necessary.

Adherent condition

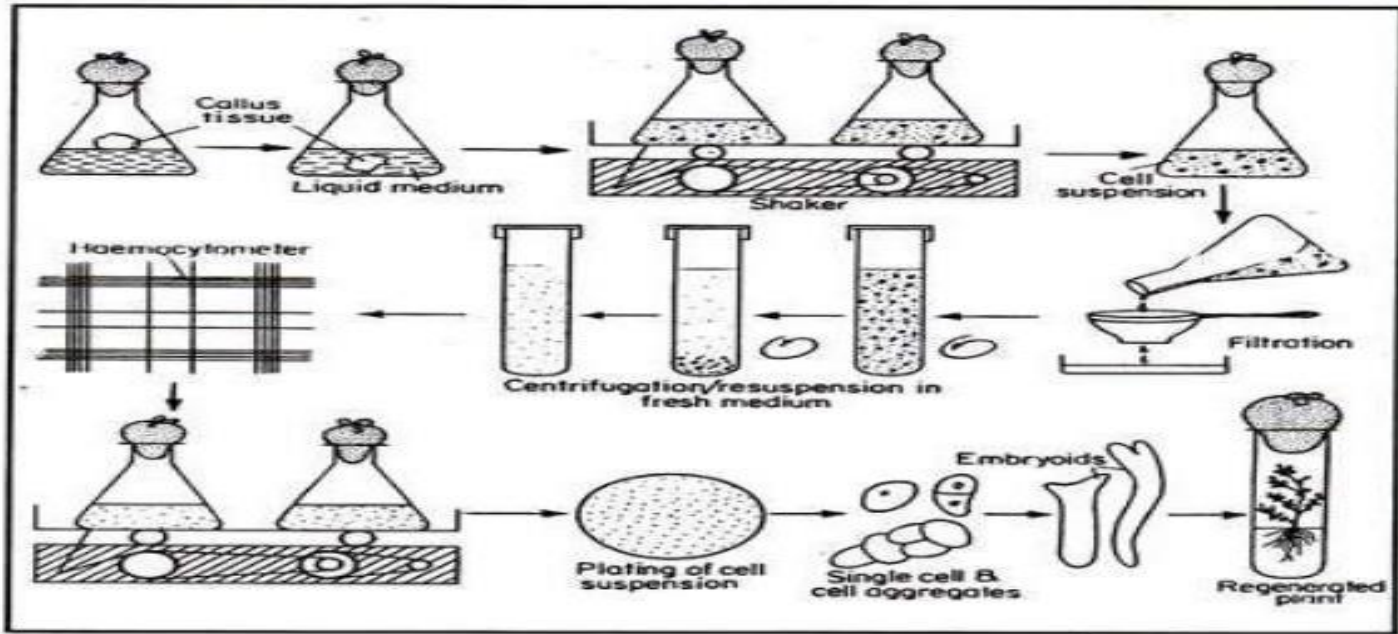


Non-adherent condition



## SUSPENSION CELLS

**A SUSPENSION CELL OR ANCHORAGE-INDEPENDENT CELLS, REFERS TO A CELL THAT CAN BE GROWN BY FLOATING IN THE CELL CULTURE MEDIUM. WHEN OBSERVED UNDER MICROSCOPE, WE CAN TYPICALLY SEE THAT RESEMBLE TINY DOTS MOVING RANDOMLY IN THE LIQUID SOLUTION.**

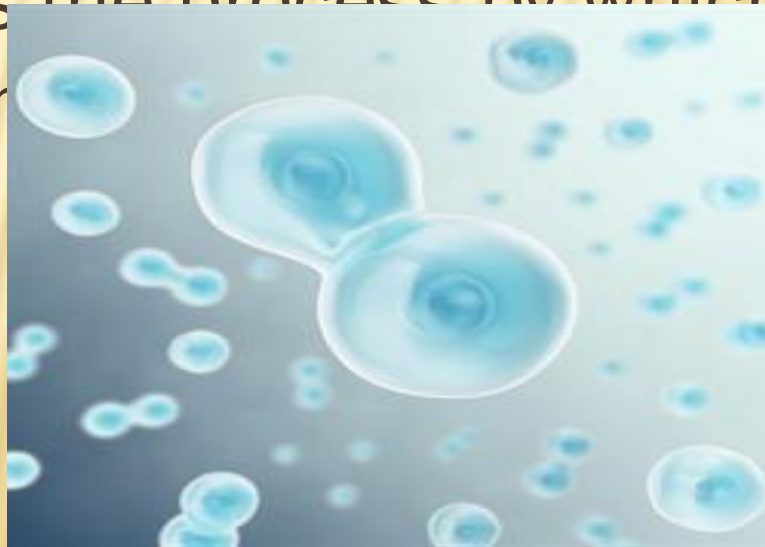


Ways to culture suspension culture.

# CELL GROWTH

Cell growth refers to cell proliferation, the increase in number of cells that is the result of repeated cell division. We can also say that cell growth also refers to enlargement of cell volume, which can occur in the absence of cell division.

Cell growth is the process by which cells accumulate mass and increase their physical size.



# PHASES OF CELL GROWTH

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- ❑ Lag phase
- ❑ Log phase or exponential phase
- ❑ Stationary phase
- ❑ Death phase

The cell growth can be portrait on growth curve. Growth curve is the graphical representation of how a particular quantity increases over time. Growth curve determines the type of growth pattern –be it linear, exponential or cubic.

**THE BACTERIAL GROWTH CURVE REPRESENTS THE  
NUMBER OF LIVE CELLS IN A BACTERIAL POPULATION  
OVER A PERIOD OF TIME:-**

**LAG PHASE :-** This is the initial phase. It is marked by cellular activity but not growth. Small group of cells are placed in a nutrient rich medium. The cells increase in size but no cell division occurs.

**LOG PHASE (EXPONENTIAL) :-** After lag phase, bacterial cells enter the log phase. This phase shows the division of cells by binary fission. Metabolic activity is high as DNA, RNA, cell wall. In this growth phase, antibiotics & disinfectants are most effective.

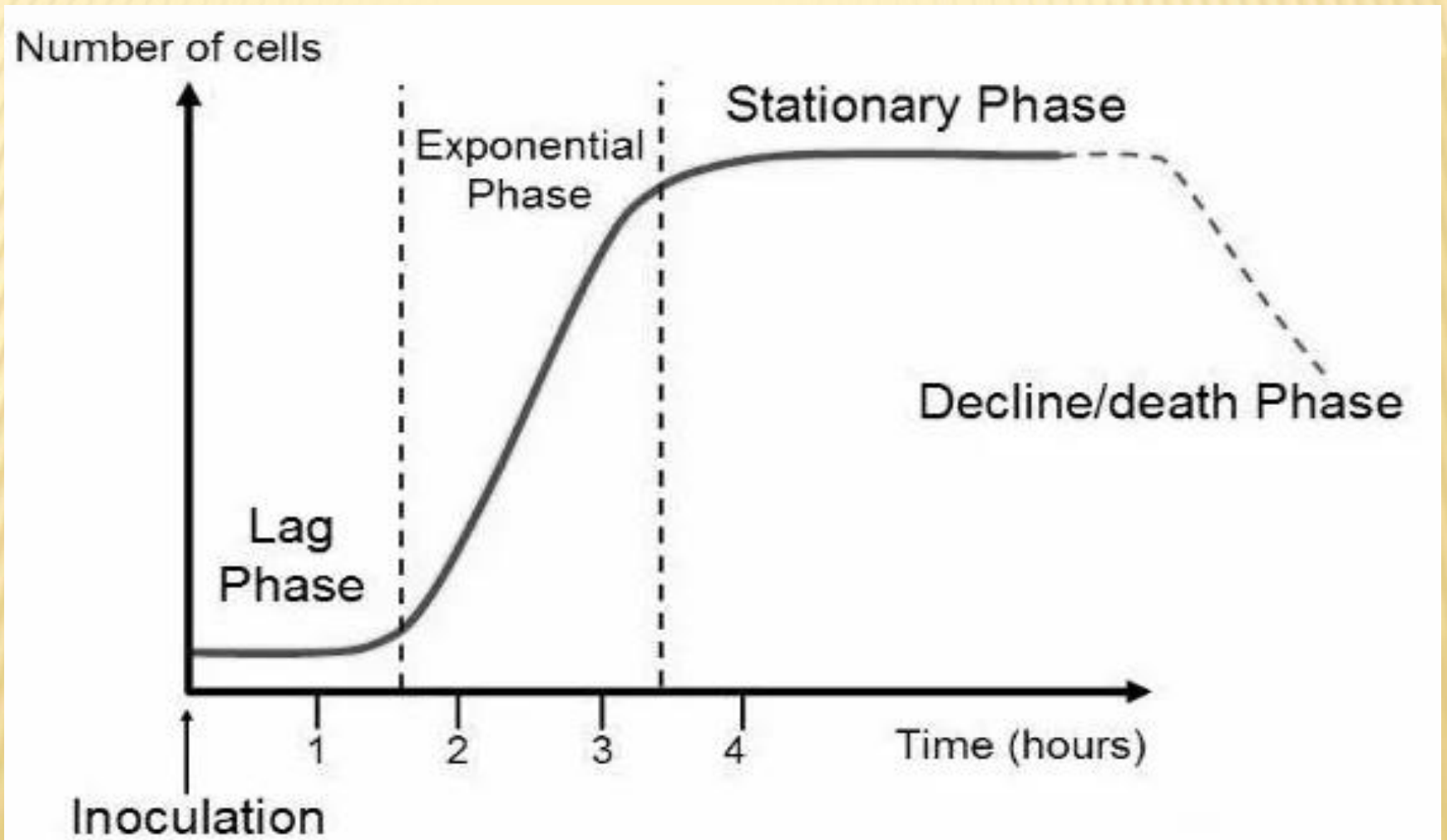


## STATIONARY PHASE :- THE POPULATION GROWTH THAT IS

experienced in the above phase begins to decline as the nutrients become depleted and waste products start to accumulate. In this phase number of cells dividing = number of dying cells. This results in overall population growth.

**DEATH PHASE :-** The availability of nutrients become less, the number of dying cells continues to rise. The no. of living cells decreases exponentially & the population growth experience a sharp decline.

BELOW PICTURE DEPICT THE GROWTH CURVE.



Calculation in vitro:-multiplication rate and cell doubling time.

$$T_2 = \frac{\Delta t}{\log_2\left(\frac{\Delta N}{N_0} + 1\right)}$$

**N** IN THE EQUATION  $N_0$  IS THE NUMBER OF CELLS AT THE BEGINNING OF OBSERVATION AND  $\Delta N$  IS THE INCREASE IN THE NUMBER OF CELLS DURING THE PERIOD OF TIME OF THE LENGTH  $\Delta t$ .

**NOTE THAT SINCE EACH DIVISION INCREASES THE NUMBER OF CELLS BY ADDING 1,  $\Delta N$  IS ALSO THE NUMBER OF CELL DIVISIONS DURING THE SAME PERIOD.**

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Cell counting and phases of cell cycle:-

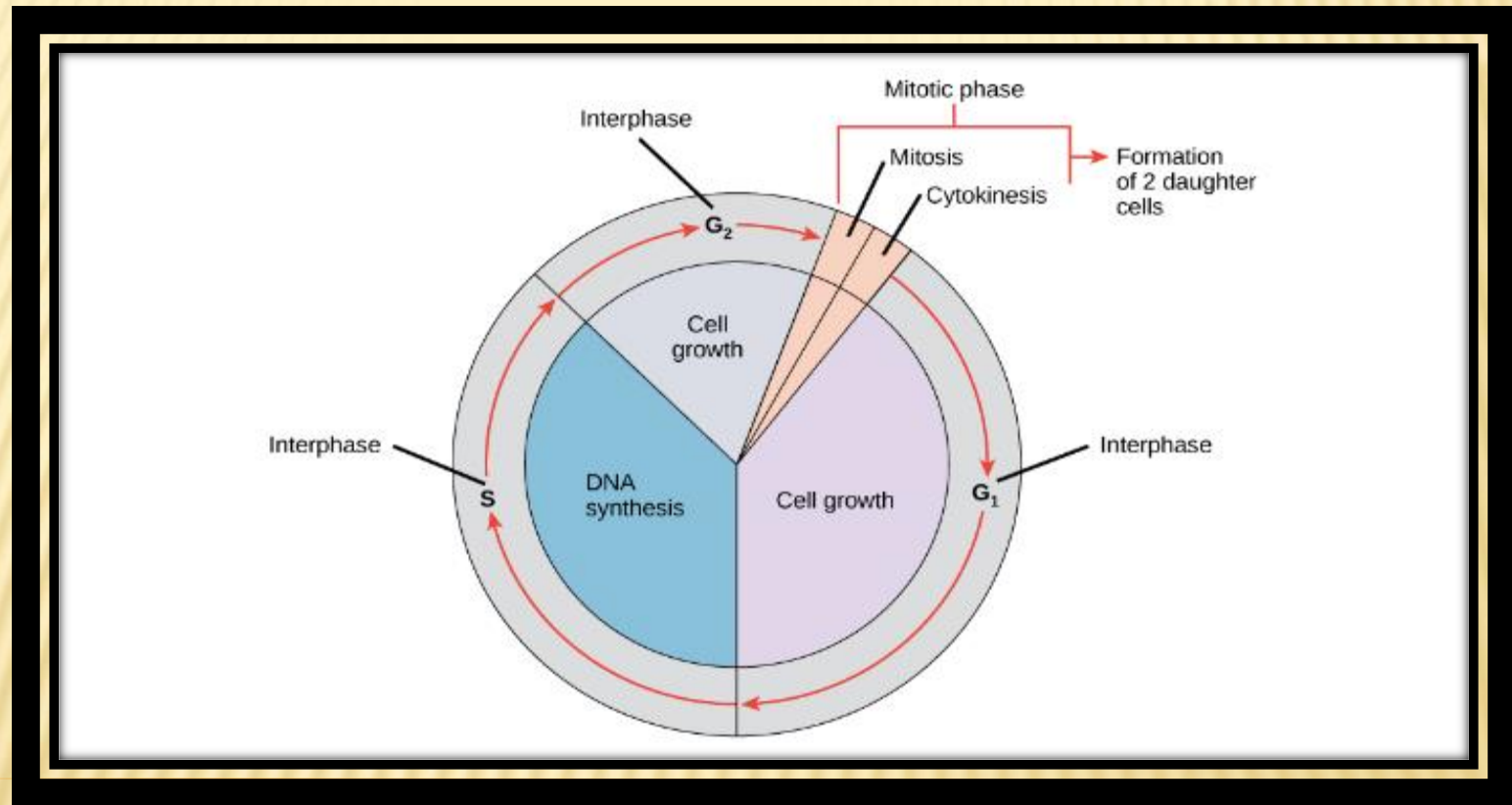
**IN EUKARYOTIC CELLS, OR CELLS WITH A NUCLEUS, THE STAGES OF THE CELL CYCLE ARE DIVIDED INTO TWO MAJOR PHASES: INTERPHASE AND THE MITOTIC (M) PHASE.**

**DURING *INTERPHASE*, THE CELL GROWS AND MAKES A COPY OF ITS DNA.**

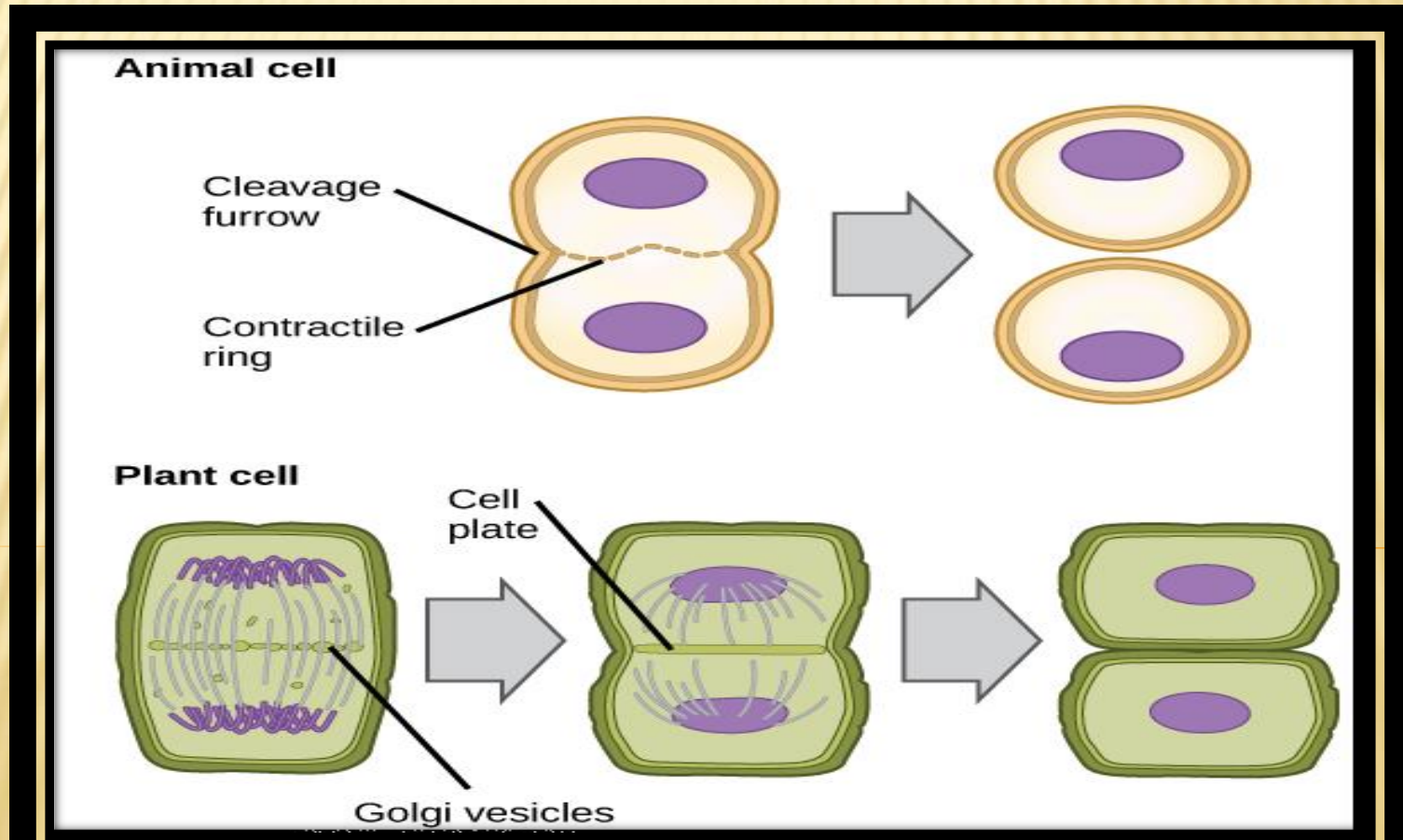
**DURING THE *MITOTIC (M) PHASE*, THE CELL SEPARATES ITS DNA INTO TWO SETS AND DIVIDES ITS CYTOPLASM, FORMING TWO NEW CELLS.**

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# PHASES OF CELL CYCLE:-



# CYTOKINESIS:-



# REFERENCES

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