

## Separation of DNA by Centrifugation.

- \* The detailed description of Meselson and Stahl experiment are as follows which proves that DNA replication is of semiconservative type.
- i) Meselson and Stahl did their experiments in bacteria E.coli.
- ii) They develop E.coli for many generation in a medium

containing heavy isotope of Nitrogen  $^{15}\text{N}$ . The medium was  $^{15}\text{NH}_4\text{Cl}$  because of source of nitrogen. The DNA of bacterial cell was labelled with  $^{15}\text{N}$ .

The heavy DNA molecule can be distinguished from the normal DNA by centrifugation in a cesium chloride ( $\text{CsCl}$ ) density gradient.

- iii) The bacterial cells with  $^{15}\text{N}$  were then transferred to a medium containing normal nitrogen means  $\text{NH}_4\text{Cl}$ .
- iv) After certain times the process of replication starts after this, the samples were taken and DNA was isolated by high speed and then densities were evaluated.

The place where DNA settled down gave its density gradient.

## Result :-

After the bacterial cells had multiplied ones after 20 minutes because it takes the same time for multiplication then after 20 minutes the density of DNA was intermediate between heavier  $^{15}\text{N}$  strand acts as template and made new complementary  $^{14}\text{N}$  strand from the raw material available. Now each DNA molecule had one  $^{15}\text{N}$  strand and  $^{14}\text{N}$  strand hence the  $^{14}\text{N}$  strand had the density midway between  $^{14}\text{N}$  and  $^{15}\text{N}$ .

After second replication means after 40 minutes DNA extract from the culture shows that the density of one half of the DNA was between  $^{14}\text{N}$  and  $^{15}\text{N}$  identical to the one obtained during first replication And other half way

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was in  $^{14}\text{N}$  density region  
are exchanged because it  
does not contain any new  
heavy nitrogen or  $^{15}\text{N}$ .  
Hence it confirms that  
replication is of semi  
conservative type.