Meselson, Matthew & Stahl, Franklin W. (1958) The replication of DNA in *Escherichia coli*. *Proc. Nat'l Sci. USA* **44:**671-682.

1. Why Meselson and Stahl do not quote Avery's work?

2. What are the theoretical bases of the method developed by Meselson, Stahl e Vinograd (1957) (ref. 10)?

3. What is the strategy used in this paper to analyze the different densities of DNA?

4. What did the authors mean by "The concentration and pressure gradients..." (p. 673)? What is "buoyant density" and how does it differ from the density (ρ) or partial specific volume ($\nu=1/\rho$) of a molecule?

5. Why DNAs labelled with ^{15}N and ^{14}N have different densities? What does mean the superscript 15 and 14? Is ^{15}N radioactive? Why?

6. What is the difference between isopicnic centrifugation and sedimentation rate centrifugation?

7. How did the authors calculate the molecular mass of DNA in the cesium gradient?

8. Why they did not grow the bacteria in Lysogeny-Broth (LB) medium [erroneously named Luria-Bertani]?

9. Observe carefully Figure 3 (page 674). (A) Why did they make colony count and cell count? (B) Why does the stationary part of the curve of Exp # 1 contain only points of microscopic cell count (∇)? (C) What would you expect from the colony count in this phase of the growth curve? (D) Why add ribosides along with ^{14}NH$_4$Cl?

10. Why instead of adding an excess of ^{14}NH$_4$Cl to the medium containing ^{15}NH$_4$Cl they did not centrifuge out the cells and resuspend them in new medium containing only ^{14}NH$_4$Cl?

11. If the *E. coli* culture is not synchronized, it is expected that by the time they add excess ^{14}NH$_4$Cl there are DNA molecules at different stages of DNA duplication. So why a generation later the DNA band has exactly half the density of the DNA band originated from a culture that has been grown for several generations in ^{15}NH$_4$Cl?

12. What does M & S mean by sedimentation equilibrium (page 676) (compare with your answer to question 6)?

13. Knowing that RCF (relative centrifugal force) is given by $RCF = (1,118 \: x \: 10^{-5})rpm^2 \: x \: r$ calculate the radial distance (r in cm) from the rotor center to the middle of the centrifugation cell used in the experiments of M&S (data in page 676).

14. In Figure 4 how would be the CsCl gradient (increasing or decreasing from left to right)?

15. What are the three main conclusions of M & S experiments (Figure 5)? Which one is important to definitively discard the model proposed by Delbrück?

16. Why do the authors always refer to DNA *subunits* and not DNA *strands*?

17. Why did the DNA utilized in the experiments, which was not purified by two cycles of preparative centrifugation in CsCl gradients, had the same density of DNA so purified?
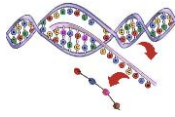
18. Comment and discuss figures 7 e 8.

19. It was later shown that the content of GC of a DNA molecule is proportional to its floating density. Empirically this is shown by the relation below:

$$GCfraction = \frac{r - 1,660\,g/cm^3}{0,098}$$ (Schildkraut et al., *J. Mol. Biol.* **4**:430, 1962). What is the quantity of GC (in %) in the genome of *E. coli* calculated by this relation? Does this value coincide with the more precise data obtained by genome sequencing? What is the % discrepancy?

20. In what contrast the denaturation of DNA and proteins? Why?

21. Why denatured DNA has a higher floating density than native one in CsCl gradients? What happens when we add an intercalating agent to DNA submitted to CsCl gradient ultracentrifugation?

22. Why Figure 9B is the key to the experiment of M & S and could be used by them to prove the model of Watson & Crick was correct, but was not so discussed? [see your answer to question 16]