One reason for discussing this paper is that it illustrates a fundamental strategy for studying the fate of molecules (although, with a special twist): the pulse-chase experiment. As traditionally implemented with radioactive precursors, the pulse allows you to label a cohort of molecules, and the chase allows you to follow the fate of that cohort without the distraction of subsequently synthesized molecules. If needed, the pulse part of the experiment also provides the opportunity to look at a synchronous cohort in an asynchronous process. By labeling with a short pulse, one can examine molecules that were created in a temporally narrow window of time and thus effectively start out synchronous with one another.

This paper also provides a lesson in the difference between population-based assays, which measure the average properties of a population, and single cell/molecule assays. Most molecular biological and biochemical assays are population-based. They are most useful when the population is uniform so that the average properties can be assigned to individual cells or molecules within the population. The few single cell/molecule assays that readily come to mind are microscopy, FACS, and mass spectrometry. In these techniques single cells or molecules pass single file by a detector (in the case of microscopy that detector is usually a person, although computers are getting better at it), which measure some key parameters of these cells or molecules. These assays are most useful when you can look at many cells or molecules so that you can tell whether your individual observations are representative of one or more populations.

Finally, a little primer on the technique of equilibrium centrifugation, which was accidentally “invented” by Meselson, Stahl, and Vinograd and was first published in their paper preceding this one. During equilibrium centrifugation a density gradient is established in the centrifuge chamber, from lowest density at the top to highest density at the bottom, by the redistribution of a small solute molecule, in this case CsCl. This gradient is established in response to the increasing centrifugal force experienced as one moves down the chamber further away from the rotor axis (the tube is spinning horizontally, bottom outward). Macromolecules introduced into this gradient will move to the position in the chamber where their buoyant density equals the density of the solute gradient. At this point, an equilibrium is reached where the upward buoyant force from the displaced solute solution is equal to the downward centrifugal force on the macromolecule. Any molecule above its equilibrium point in the lighter density solution will sink toward that point; any molecule below its equilibrium point in the heavier density solution will rise toward that point. Hence, the distribution of molecules should be stable indefinitely once the solute gradient has been established and all the macromolecules have converged to their equilibrium points. To visualize the position of DNA, Meselson and Stahl took advantage of the fact that DNA absorbs UV at specific wavelengths (same principle you use to “spec” your DNA to figure out its concentration). Where there is a lot of DNA, less UV will get through so the area with DNA looks dark. Because the rotor is spinning around and the camera is stationary (usually positioned above or below the plane where the tube is spinning), the UV illumination is strobed so that it flashes briefly only when the chamber passes by the camera.
1) What is the key question of molecular fate that Meselson and Stahl wish to address? Do they need a population-based assay or a single molecule assay to address this question?

2) How is the density labeling used by Meselson and Stahl to monitor the fate of parental DNA different from the traditional radiolabeling approach?

3) Meselson and Stahl place a huge population of DNA molecules in a single analytical centrifuge tube and use a detector that can only “see” large populations of molecules. Is their assay a population-based assay or a single molecule assay? How does it manage to address the question they are asking?

5) Could the authors have used a radiolabeling approach to address their question of molecular fate? If not, why not? If so, how would they assay the radiolabeled DNA and what should they expect to see?

6) In this day and age, could Meselson and Stahl have used a mass spectrometer in their density labeling experiment?

7) The model shown in Figure 6 depicts what Meselson and Stahl think is happening to a single nonreplicating molecule if you look at it one generation then two generations later. However, the cells they actually examined were asynchronous, with many released into the light isotopic media while their chromosomes were in the midst of replication. What would the density of these chromosomes be exactly one and two generations later? (Draw out a replicating circular E. coli chromosome and its progeny during the experiment). How does this prediction match with their results in Figure 4 and how do you explain any discrepancies?

8) Supposed Meselson and Stahl had told you that the data in Figure 4 were from a synchronized experiment, where the light isotope chase was begun a short period before all the cells started to replicate their DNA. Would the data in Figure 4 now perfectly match the Watson-Crick prediction expected for density labeling of E. coli chromosomes? If not, how do you explain the data that was obtained?

9) If you ask many scientists what they know or remember of the Meselson Stahl experiment, they will say “replicated DNA becomes HL”. Is this the key result of this experiment?

10) Why are the author’s so cautious about what they consider an inherited subunit of E. coli DNA? Can you propose a scenario where the Watson-Crick structure of DS DNA is correct, but a subunit of inheritance does not correspond to a single strand?
11) Meselson and Stahl state that their results disprove the DNA duplication scheme of Delbruck (PNAS 40:783, 1954) shown below:

![Diagram of DNA replication](image)

Figure Legend: Resolution of an interlock in a replicating duplex by breaking both old chains at each half-turn of the helix and rejoining the lower terminals of the breaks to the open ends of equal polarity of the new chains. Lateral view. A, Location of first pair of breaks. B, Rejoining of lower terminals of breaks. C, Location of second pair of breaks. D, Rejoining of lower terminals. Parental chains are represented by solid lines; new chains, by dashed lines. At the overlaps the lower chains are dotted.

Delbruck seemingly harebrained scheme was trying to solve the topological problem of replicating two DNA strands that are interwound around each other. We now know this topological problem is solved by enzymes called topoisomerases. It is clear that this model would account for hybrid density HL DNA after one round of replication, but would it also account for the conservation of HL DNA after the second round?

12) Meselson and Stahl look at boiled DNA to rule out the Delbruck model. What is their rationale for doing this? We now know that boiling separates the two DNA strands, but at that time, when people weren’t even sure what the structure of DNA was, the effect of boiling was not clear (in fact a renown Harvard biochemist, Paul Doty, “showed” that boiling the salmon sperm DNA did not change its molecular weight). What evidence did the authors have that at least for E. coli DNA boiling did what they wanted it to do, and how do they argue against the Delbruck model?

13) What does footnote/reference 14 mean? What is the logic behind this conclusion?