- Pembuktian DNA adalah materi genetik yang berperan dalam kehidupan

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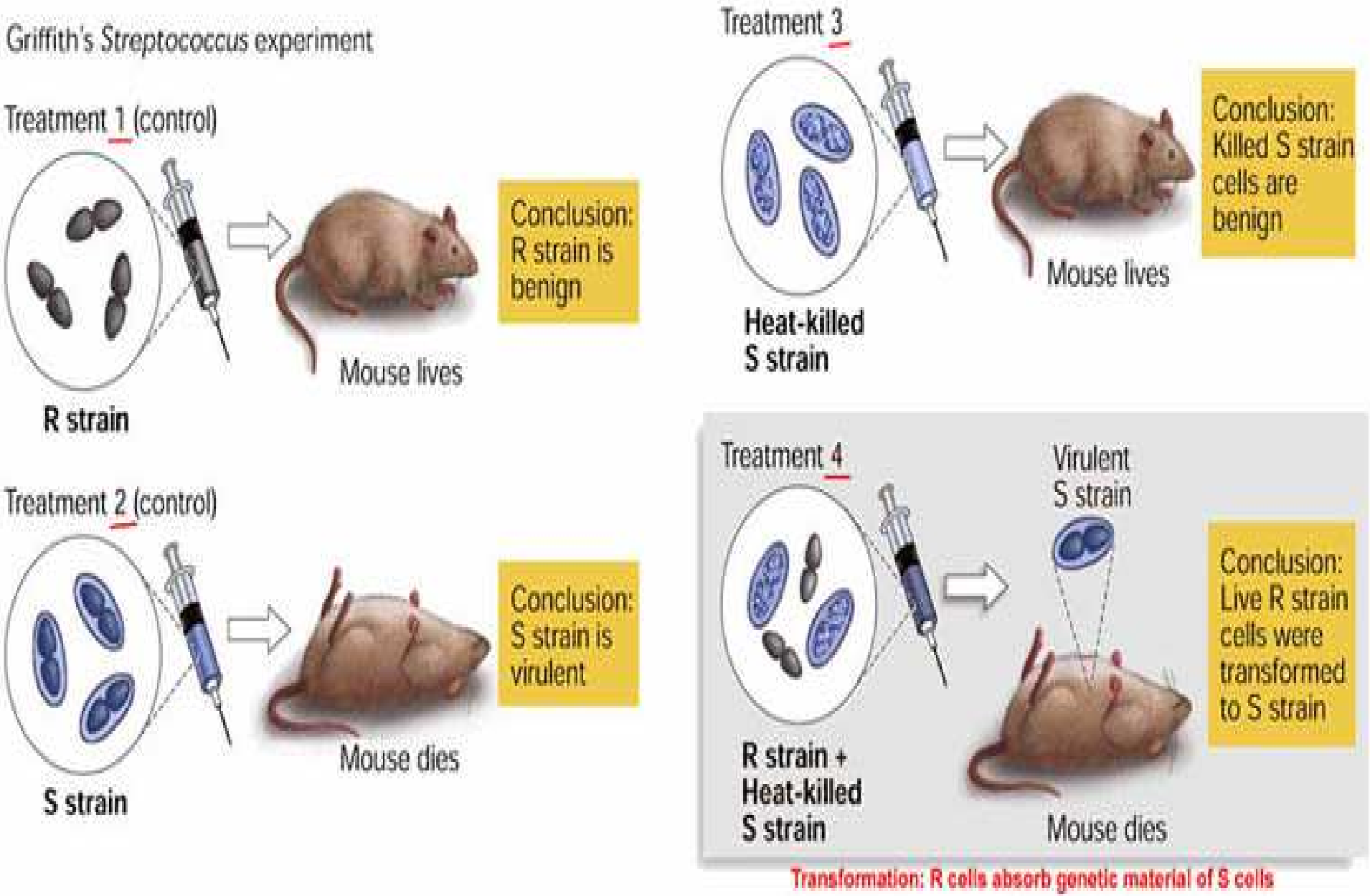


Rough (R) avirulen, Smooth (S) virulen

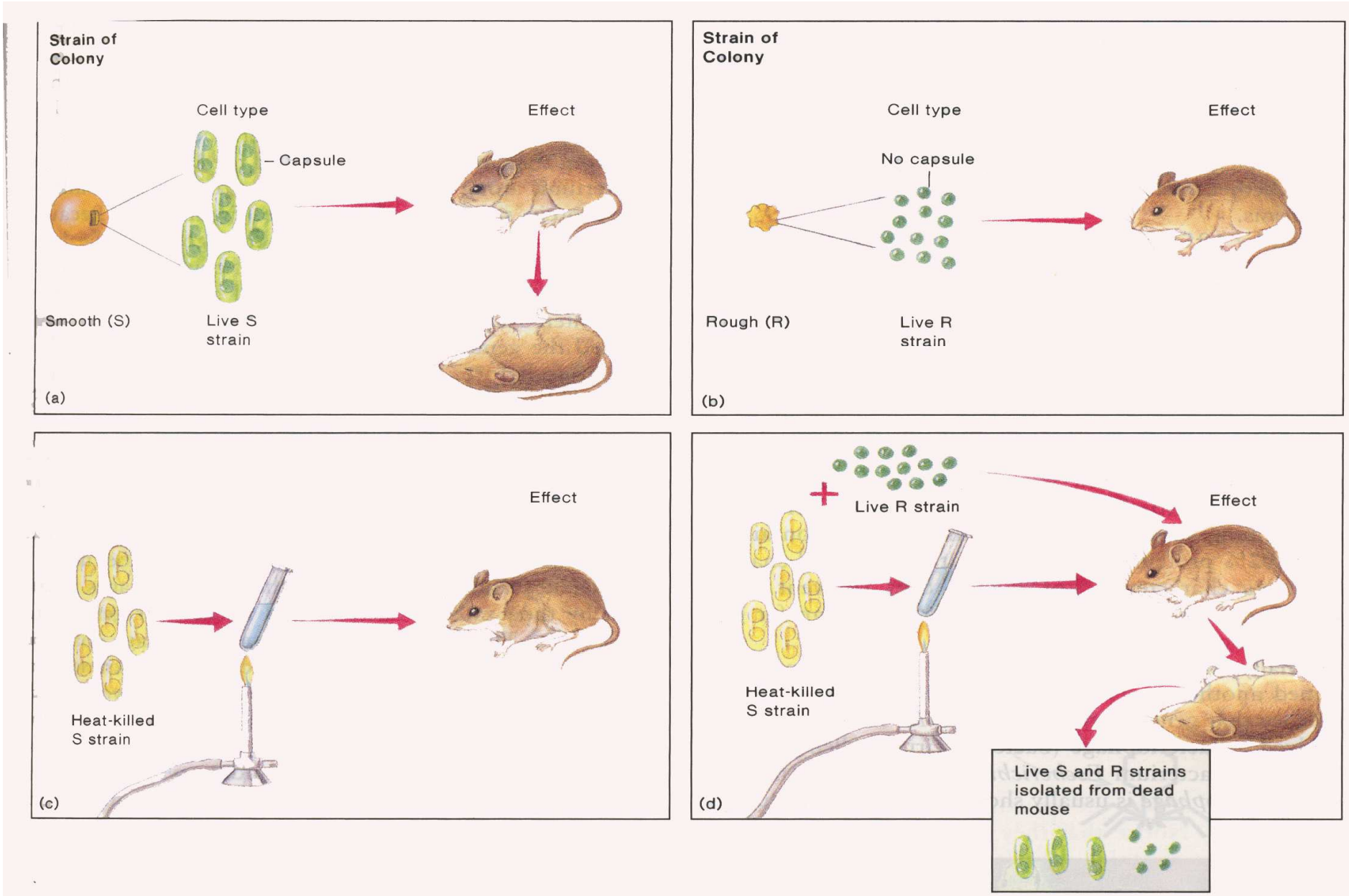
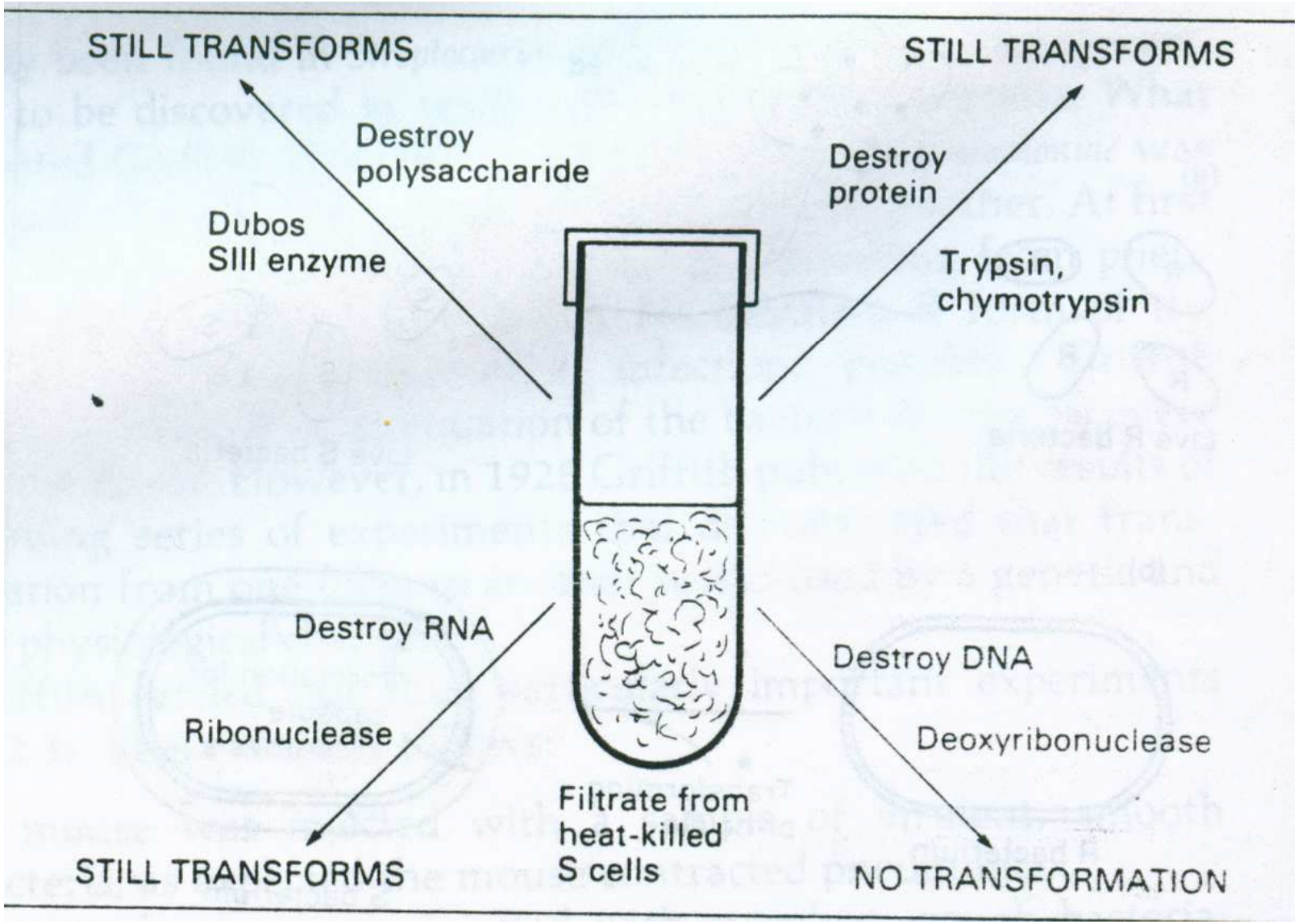


Figure 2.2 Griffith's transformation experiments. **(a)** Virulent strain S *S. pneumoniae* bacteria kill their host; **(b)** avirulent strain R bacteria cannot infect successfully, so the mouse survives; **(c)** strain S bacteria that are heat-killed can no longer infect; **(d)** a mixture of strain R and heat-killed strain S bacteria kills the mouse. The killed virulent (S) bacteria have transformed the avirulent (R) bacteria to virulent (S).

Avery, McLeod &McCarty

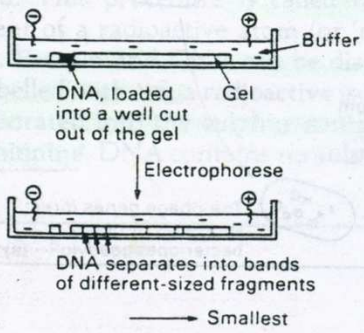


Electrophoresis, ultracentrifugation and ultraviolet spectroscopy

These three techniques, which were in their infancy when used by Avery, MacLeod and McCarty to analyse the transforming principle, are now important methods regularly employed in research projects concerning DNA.

Electrophoresis is defined as the movement of charged molecules in an electric field. DNA molecules, like proteins and many other biological compounds, carry an electric charge, negative in the case of DNA. Consequently, when DNA molecules are placed in an electrical field they will migrate towards the positive pole. In an aqueous solution the rate of migration of a molecule depends on two factors: its shape and its electrical charge, meaning that most DNA molecules will migrate at the same speed in an aqueous electrophoresis system. Avery's group subjected the purified transforming principle to electrophoresis and demonstrated that its migration rate is the same as that of a sample of pure DNA.

Nowadays, electrophoresis of DNA is usually carried out in a gel made of agarose, polyacrylamide or a mixture of the two. In a gel, the migration rate of a macromolecule is influenced by a third factor, its size. This is because the gel comprises a complex network of pores through which the molecules must travel to reach the electrode. The smaller the molecule, the faster it can migrate through the gel. Gel electrophoresis will therefore separate DNA molecules according to size.

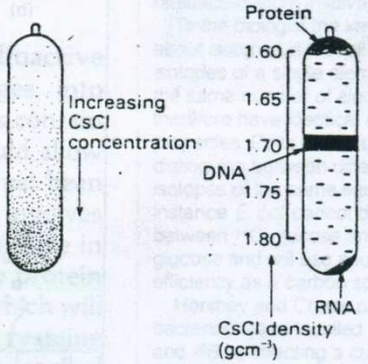


Gel electrophoresis is also routinely used to separate proteins of different molecular masses.

Ultracentrifugation. The ultracentrifuge, invented by Svedberg in 1925, allows samples to be subjected to intense centrifugal forces, up to several hundred thousand x*g*. Cells, cell components and macromolecules sediment during ultracentrifugation at a rate dependent on their size, shape, density and molecular weight. Avery showed that the transforming principle sedimented in the ultracentrifuge at a rate similar to that of pure DNA.

Two versions of ultracentrifugation are now important in studying DNA. The first is called **velocity sedimentation analysis**, a procedure that involves measuring the rate at which a macromolecule or particle sediments through a dense solution, often of sucrose, whilst subjected to a high centrifugal force. The rate of sedimentation is a measure of the size of the molecule or particle (although shape and density also influence the rate), and is expressed as a sedimentation coefficient (see section 6.1.1).

The second technique is called **density gradient centrifugation**. A density gradient is produced by centrifuging a dense solution (usually of caesium chloride), as a high centrifugal force will pull the caesium and chloride ions towards the bottom of the tube. Their downward migration will be counterbalanced by diffusion, so a concentration gradient will be set up, with the CsCl density greater towards the bottom of the tube. Macromolecules present in the CsCl solution when it is centrifuged will form bands at distinct points in the gradient, the exact position depending on the buoyant density of the macromolecule.



DNA has a buoyant density of about 1.7 g cm^{-3}, and will therefore migrate to the point in the gradient where the CsCl density is also 1.7 g cm^{-3}. Proteins have much lower and RNA somewhat higher buoyant densities.

Ultraviolet spectroscopy. Spectroscopy involves analysis of substances by the spectra they produce. The spectrum of light emitted or absorbed by a substance is characteristic of the substance. For example, DNA strongly absorbs ultraviolet radiation with a wavelength of 260 nm; proteins on the other hand have a strong absorbance at 280 nm. Avery showed that the ultraviolet absorbance spectrum of the transforming principle is the same as that for pure DNA.

Ultraviolet spectroscopy is often used to check that samples of DNA obtained from living cells are pure and do not contain protein or other contaminants. As the amount of ultraviolet radiation absorbed by a solution of DNA is directly proportional to the amount of DNA in the solution, the technique can also be used to determine the concentration of DNA in a sample.

Hershey &Chase