Quadrant II – Transcript and Related Materials

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Notes

1.Frederick Griffith (Discovery of Transforming Principle)
Streptococcus pneumoniae (Pneumococcus bacteria - the bacterium that causes pneumonia bacterium that causes pneumonia is of two types) in 1928,
i) **Smooth 'S' type:** It is the virulent form of bacteria. The virulence is due to the presence of capsular gelatinous Polysaccharide coating which contains O-antigens through which it recognizes the cells it infects. ii) **Rough 'R' type:** It is a mutant variety and the nonvirulent form of bacteria because it lacks the capsular gelatinous polysaccharide coating

Experiment:
Griffith in his experiment with pneumococcus took four groups of mice which were treated differently.To one group, he injected live S-type → Mice develop pneumonia and die. To the second group, he injected live R-type → Mice live. To the third group heat inactivated (i.e. bacteria killed by heat treatment) virulent S-type bacteria → Mice live. (Griffith found that heating kills the bacteria). To the fourth group a mixture of heat killed smooth bacteria and live rough bacteria were injected → Mice die.

Observations
He saw different observations and the following results were obtained.

1. Neither heat inactivated virulent form nor do the rough forms kill the mice.
2. But the live S-type and mixture of both kills the mice.
3. Not only did the mice injected with the heat-killed S strain + R strain die, but Griffith also recovered live S strain bacteria from these dead mice.
   Post mortem analysis of blood of infected mice of fourth group showed live smooth forms. Frederick Griffith observed a miraculous transformation in this bacterium.

When you grow this bacterium on a culture plate, some produce shiny colonies (denoted as 'S') and some produce rough colonies (denoted as 'R').
The S strain bacteria have a polysaccharide coat which gives rise to smooth, shiny

colonies. The R strain lacks this coat and hence, it gives rough colonies. Also, the S strain is virulent and causes pneumonia; while the R strain is non-virulent. It is a mutant variety and the nonvirulent form of bacteria because it lacks the capsular gelatinous polysaccharide coating.

Moreover, the transformed ones give rise to virulent ones showing that the transformation was permanent.

Conclusions

He concluded that this was because the non-virulent (the R strain/form) had somehow been 'transformed' by some components of the dead virulent form (the heat-killed S strain) which was then called the 'transforming Principle'.

This he argued was due to the transfer of a 'transforming principle'from the S strain to the R strain, which made the R strain virulent.

Although significant, his observations did not identify the biochemical nature of the transforming principle.

2. ALFRED D. HERSHEY & M MARTHA CHASE ON BACTERIOPHAGE T_2 (ISOTOPIC STUDIES ON BACTERIAL VIRUS T_2 - 1952): Blender Experiment

Hersey & Chase used and worked with bacteriophages T2 which is a DNA virus –viruses infect bacteria. A single particle of phage T_2 consists of DNA encased in a protein shell. The DNA is the only phosphorus containing substance in the phage particle where as the proteins of the shell which contain the amino acids methionine and cysteine have the only sulphur atoms.

Phage T_2 contains genes that allow it to replicate in *E.coli.* A bacteriophage attaches and delivers its genetic material into a bacterial cell, where it generates more virus particles. Since, the phage is composed of DNA and protein only, its genes must be made of one of these substance. To function as genes, one of these substances has to enter into *E.coli*. By utilizing this thought experiment performed.

Labelling – In their experiment, they grew bacteriophage T_2 on *E.coli* in a medium containing radioactive isotopes ^{32}P and ^{35}S. The phage DNA was made radioactive by growing infected bacteria on a medium containing radioactive phosphate (^{32}P). This labelled the phage capsid which contains no phosphorus with ^{35}S and it's DNA which contain no sulphur with ^{32}P. Since phage proteins do not contain phosphorus, only DNA would be labelled. Similarly, phage proteins were labelled with the help of ^{35}S. Since DNA does not contain sulphur, only protein would be labelled with ^{35}S. Such differential labelling would enable one to distinguish between DNA and proteins of the phage with out performing any chemical tests.

Viruses – grown on radioactive phosphorus have radioactive DNA but not protein since DNA contains phosphorus but protein does not. Contrarily, viruses grown on radioactive sulphur have radioactive protein but not DNA since DNA does not contain sulfur.

Infection – These radioactive labeled phages were added to an unlabelled culture of E.Coli and allowed to infect the bacteria for sufficient time for the phages to infect the bacterial cells.

Blending - After this time, as the infection progressed, the bacterial cells were agitated violently and this culture was spun for few minutes in a warring blender at 10,000rpm. This was designed to remove the protein coats of the infecting viruses from the surface of the

bacteria. This treatment subjected the phage infected cells to a very strong sharing force which rupture the connection between viruses and bacteria, the viral coats were removed from the bacteria by blending.

Centrifugation The resulting suspension was centrifuged at a speed sufficient to separate the viral particles from the bacteria. Bacteria to the bottom of the tube while the phage ghosts (empty phage shells) remained in the supernatant.
The centrifuged fractions were then analyzed for the distribution of radioactive substances ^{32}P and ^{35}S.

Conclusion
In 1952, Hershey & Chase conclusively proved that DNA (not protein) is the genetic material

3. Oswald T. Avery, Colin MacLeod & Maclym McCarty Experiment , 1944 (Isolation and Establishing the transforming principle)

Avery, MacLeod, and McCarty, together set out to determine the biochemical nature of the 'transforming principle' identified by Griffith. These people purified DNA, RNA, and proteins from the heat-killed S strain and determined which macromolecule converted the R strain into the S strain.

Experiment:
In their experiments, cell free form of DNA from the virulent form of *Pneumococcus* (the heat-killed S-form) were extracted through the process of centrifugation, heating and homogenization and injected into the colony of non-virulent (R-type).
After sometimes, when these colonies were injected into group of mice they found to cause pneumonia.
Similarly the cell free extract, first treated with RNase and a portion mixed with colony of R type and then injected to group of mice.
To the remaining RNase treated cell free extract, Protease added and a portion mixed with colony of R type and then injected to group of mice.

Observation
The transforming principle which was identified as DNA transforms the non-virulent bacteria into virulent form of *Pneumococcus*. The results are as follows:
1) Mixture of cell free extract and R-type cause pneumonia and mouse dead.
2) Addition of RNase did not affect the phenomena of transformation of the R strain into the virulent one which again neglects the possibility of RNA being the transformation factor.
3) Addition of Proteases like Trypsin and Chymotrypsin again did not affect the transformation of the R strain into the virulent one showing that the transforming principle was not a protein.
4) Addition of DNase (which specifically destroys DNA) to the transforming principle extract abolish transformation, showing that the DNA is the active genetic factor. Finally, treatment with DNAses inhibited the transformation of the R strain.

Conclusions
Finally, direct physical-chemical analysis also showed the purified transforming substance to be DNA. They concluded that the genetic material is not protein or RNA, but it is DNA.The

authors after more than 10 years of intense investigation conclusively proved that the transforming principle was indeed the DNA.

4. Fraenkel-Conrat's experiment

In some viruses, RNA is the genetic material.

The tobacco mosaic virus that infects tobacco plants consists only of RNA and protein. The single, long RNA molecule is packaged within a rod like structure formed by over two thousand copies of a single protein. No DNA is present in tobacco mosaic virus particles.

In 1955, H. Fraenkel Conrat and R.Williams showed that a virus can be separated in vitro into its component parts and reconstituted as a viable virus. This finding led to experiments by Fraenkel Conrat and B. Singer who reconstituted Tobacco Mosaic Virus (TMV) with parts from different strains.

In this experiment, they used two different types of RNA viruses, namely TMV (Tobacco Mosaic Virus) and HRV (Holmes rib-grass virus). Plantago virus since this virus was isolated from *Plantago lanceolata*. Proteins of these two viruses differ in having different frequencies and sequences of amino acids and give different symptoms. On leaves of a particular variety of tobacco, TMV produces mottling of leaves while HRV produces distinct ring patterns.

They created hybrid viruses consisting of the protein coat from masked (HR) strain of TMV and an RNA core from the common TMV. He infected tobacco plants with hybrid viruses.

In their experiments they used proteins as well as RNAs obtained from different strains and produced different recombinant strains.

They sprayed reconstituted hybrid virus on to tobacco leaf or were rubbed (mixed with abrasive) them separately on the leaves of tobacco plants to cause infection and observed the characteristics symptoms developed.

Observation

The infection that developed exhibited the symptoms of the disease typical of the strain from which the RNA was taken (and not the protein)

The tobacco mosaic virus produced during the process of infection was of the type associated with the RNA, not with the protein.

Conclusions

This proved that specificity of virus proteins was determined by RNA alone and that proteins carried no genetic information.

Thus it was concluded that nucleic acid (RNA) that was shown to be the genetic material.