Sage - Spring 2023

Welcome to

Session 4 of

"Genes for Very Smart but Ignorant People"

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Course Outline

1. *Gregor Mendel: How a monk came to discover the rules of inheritance*

2. *Genes and chromosomes - the fly in the ointment (continued)*

4. *Microbiologists discover that genes are made of DNA*

5. *How two amateurs beat the A team to solve the structure of DNA*

6. *The genetic code. Again an obscure team of players beats the pros.*

Grand Summary

I've covered a lot of ground in the first three seminars.

If you're like me, you'll forget most of it.

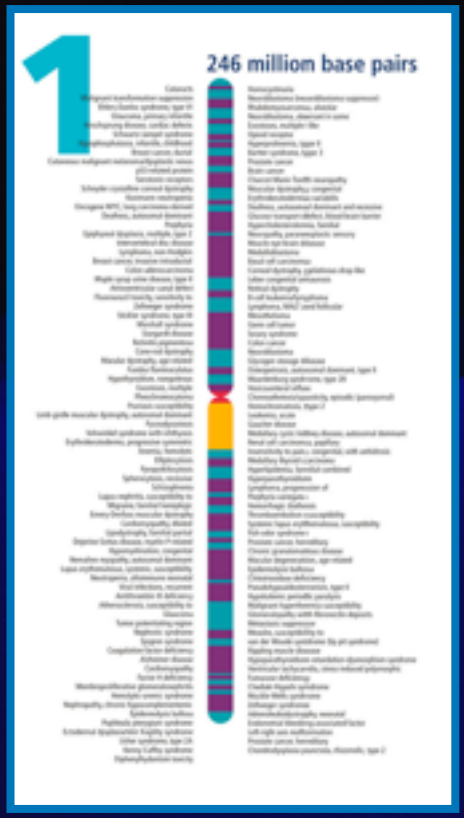
Let me help by telling you the three most important principles to remember.

First

During germ cell formation, one of each pair is randomly apportioned out in sperm and eggs.

Second

Genes are found on chromosomes.



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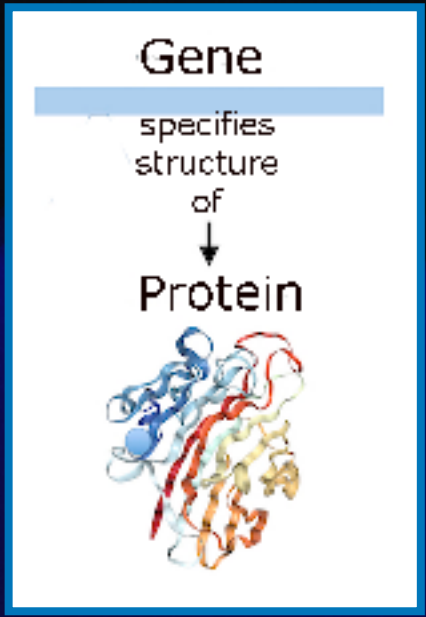
Grand Summary

Third

Genes specify the structure of proteins.

Only indirectly do they specify traits.



Summary

1. Genes come in pairs
2. Chromosomes carry genes
3. Genes ->machines->proteins

I'll add one more today.
Watch out for it!

Grand Summary

Let's move on.

Mendel's and Morgan's work treated genes as theoretical entities.
They had no idea what genes were made of.

The consensus of the scientific community was that genes a were made of proteins.

After all, proteins were known to be the most complex substance in organisms.

Some even acted as chemical machines.

This view was held well into the twentieth century.

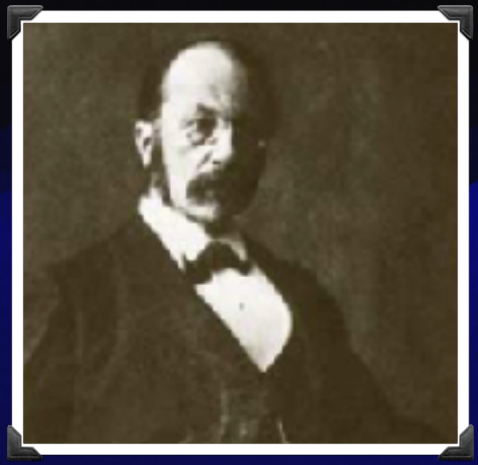
But it was wrong.

Genes are made of DNA.

The story of how DNA was discovered to be the stuff of genes begins in the 19th century with Friedrich Miescher.

Friedrich Miescher and his wife, Maria

Born in 1844 in Basel, Switzerland, Miescher's father and uncle were prominent scientists.



Wilhelm His, Miescher's uncle

At 17, he entered medical school, studying to be an otologist.

This proved impossible, because he had lost some of his hearing due to an infection he had suffered as a child.

Budding ENT doctor

Always interested in discovering the "theoretical foundations of life", he turned to the study of science.

Miescher

He became convinced that chemical analysis of living things, particularly cells, would help him achieve his goal.

Miescher as a young man.

Accordingly, he decided to apprentice in the laboratory of the most prominent biochemist of his day: Felix Hoppe-Seyler.

Hoppe-Seyler

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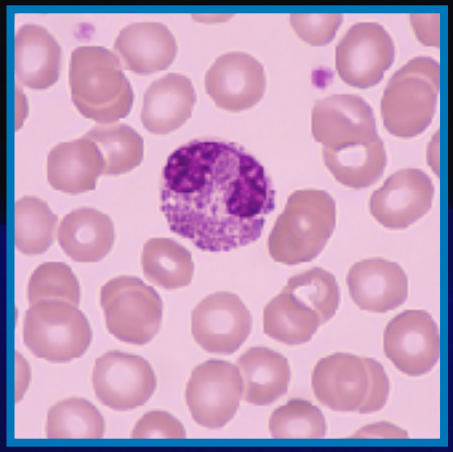
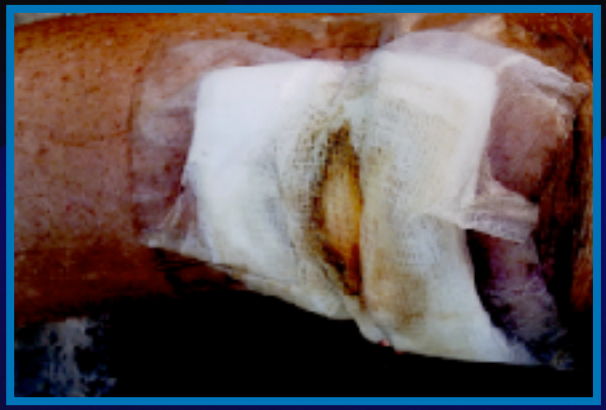
He travelled to Tübingen, Germany, to work in Hoppe-Seyler's laboratory (located in a castle!).

Hoppe-Seyler assigned him the problem of studying the proteins in white blood cells.



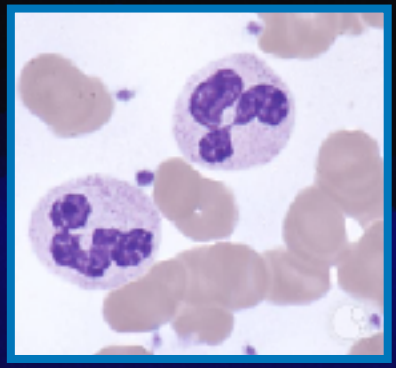
He needed to find a rich source of white blood cells.

He found it in pus that he isolated from bandages of patients with infections.



Miescher discovered a substance in these pus cells that wasn't a protein, but was abundant and high in phosphorous.

He called this chemical 'nuclein' because he thought it resided in the cell nucleus.

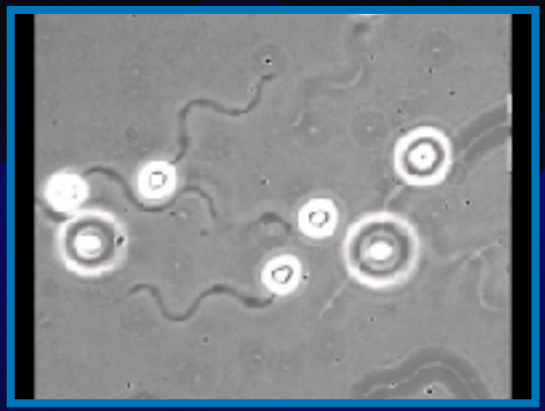


Neutrophil

At first, Hoppe-Seyler didn't believe him and repeated Miescher's experiments.

He was able to confirm Miescher's results.

Miescher showed that nuclein was found in the nucleus by isolating it from salmon sperm which are mostly nuclei.



Miescher didn't know it at the time, but nuclein was impure DNA.

He was a perfectionist and wouldn't speculate what the function of this stuff was.

He died in 1895 from tuberculosis.

For a while, investigating nuclein (DNA) fell out of favor.

However, a remarkable scientist took up the task some decades later.

Phoebus Levene

Born in Russia in 1869, Levene obtained his medical degree from the Imperial Medical Academy of St. Petersburg in 1892.

In 1891, he and his family moved to the United States because of increased antisemitism in the Soviet Union.

They arrived on July 4, 1891.

Levene hadn't completed his degree at the time, and returned to the USSR soon after immigrating to finish his studies at the Imperial Medical Academy of St. Petersburg.

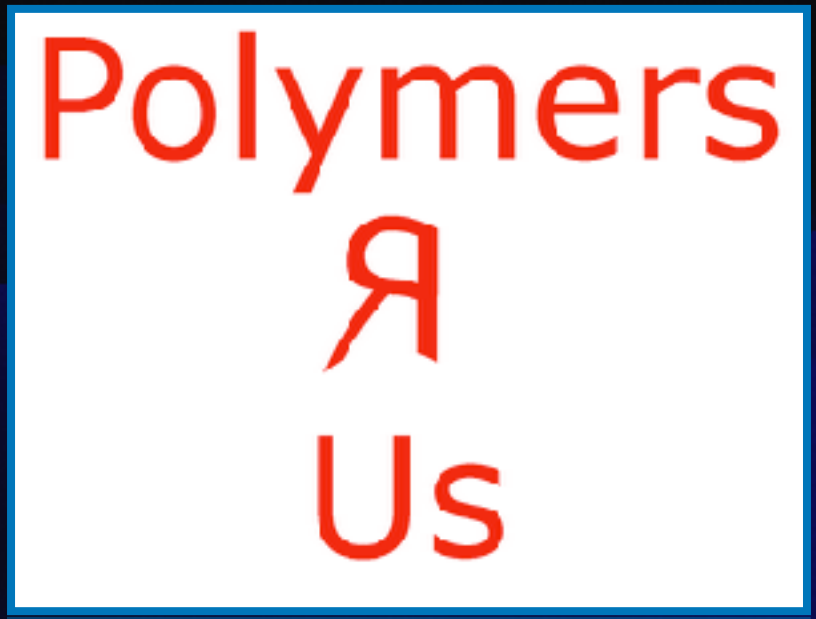
He immediately returned to the US in 1892 and began to practice medicine on the lower East Side of Manhattan.

While practicing, he began to study biochemistry at Columbia University and other schools over the next decade.

Eventually, he joined the Rockefeller Institute and became the head of its biochemistry unit.

There he investigated the chemistry of the nucleic acids (DNA and RNA) among other subjects.

At this point, I'm going to stop discussing Levene's biography and present the fourth major point that I hope you'll take home from these seminars.


Polymers
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Us

Questions

What's a polymer?

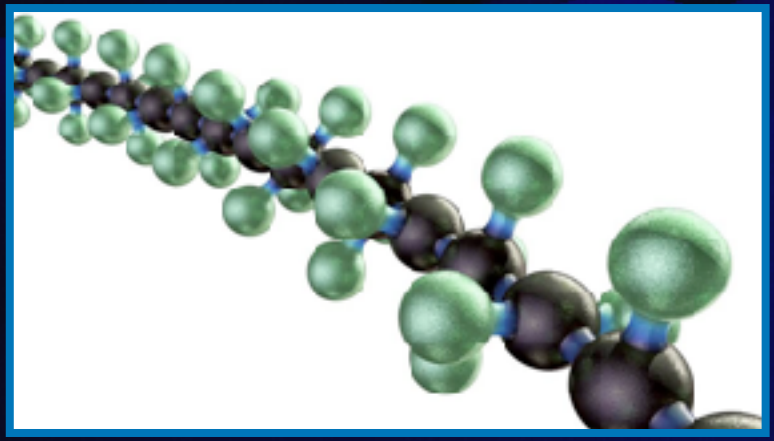
Why are polymers so important?

Polymers are chemicals.

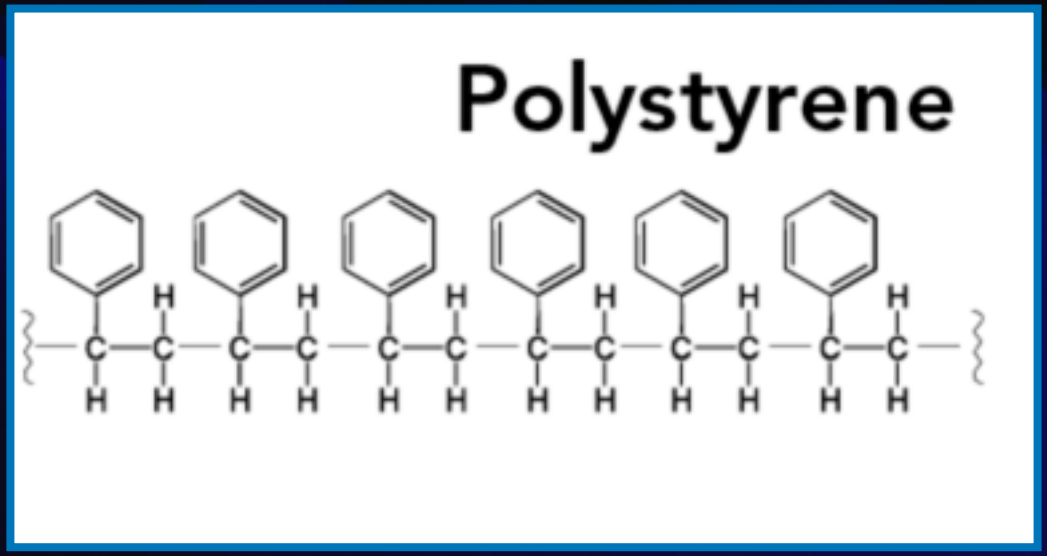
Big chemicals.

They're formed from little chemicals that are linked together in long chains.

Like this

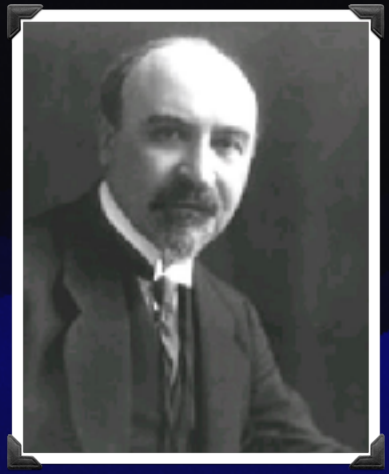


Or this



Artificial polymers are a relatively recent invention.

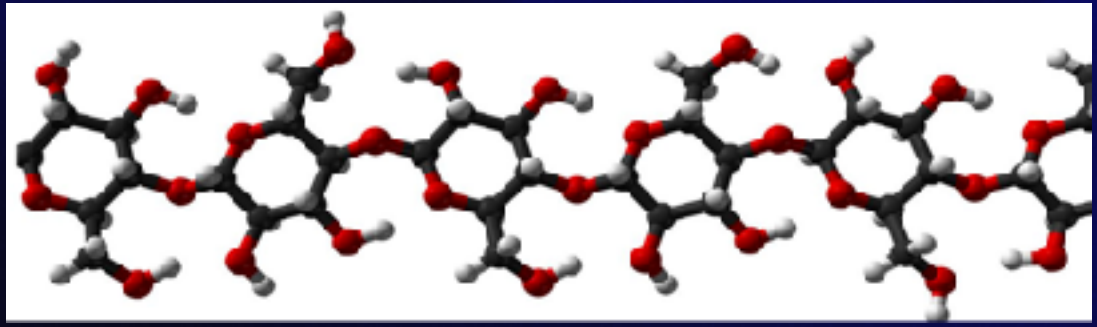
The first one, bakelite, was patented in 1909 by Leo Baekeland.



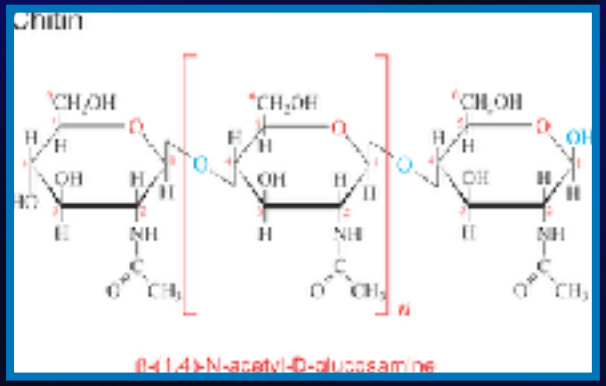
Since then, many others have been developed.

But Mother Nature had invented polymers a billion years before Baekeland.

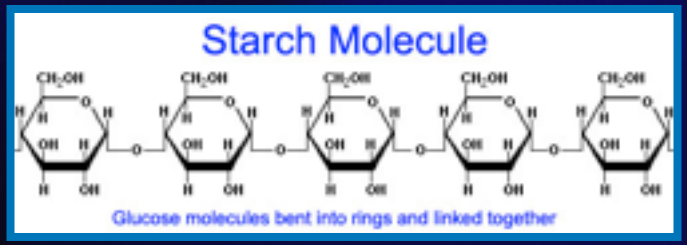
The most abundant biomolecule on earth, cellulose, is a polymer.



The most abundant biomolecule in animals, chitin, is a polymer.



Starch and glycogen are polymers.



However, there are three other natural polymers, DNA, RNA, and proteins, that are subtly different from these others.

How different?

They consist of more than one monomer.

DNA and RNA are polymers with four different monomers called A, C, G, and T (or U).

That's what Levene discovered.

Proteins carry 20 different kinds of monomers.

a,c,d,e,f

g,h,i,k,l

m,n,p,q,r

s,t,v,w,y

These three polymers are found only in living things.

They are the chemicals that make life possible.

We'll discuss them more in subsequent seminars.

Back to Levene.

Despite publishing more than 700 articles in his lifetime, many on DNA and RNA, he made a fundamental mistake.

He thought that the four monomers of DNA, A,C,G and T, repeated in a regular pattern.

ACGT ACGT ACGT ACGT ACGT ACGT ACGT ACGT ACGT ACGT ACGT ACGT

Because of this regularity, DNA couldn't possibly be the stuff of heredity: it was too simple.

He was wrong!

The next step that impacted DNA as the stuff of genes, occurred in England, just after the first world war.

Fred Griffith was an extremely shy, retiring, reclusive microbiologist who earned his medical degree in 1901.

He worked for the British government in the Pathology Laboratory in the Ministry of Health.

He worked on the bacterium Streptococcus pneumonia, one of the organisms responsible for pneumonia. He published little, but had a reputation for accuracy.

His goal was to develop an antiserum against the disease.

But the bacterium had many genetic strains, many of which were resistant to the antisera against other strains.

In 1928, he mixed killed bacteria with live ones.

The results were unexpected.

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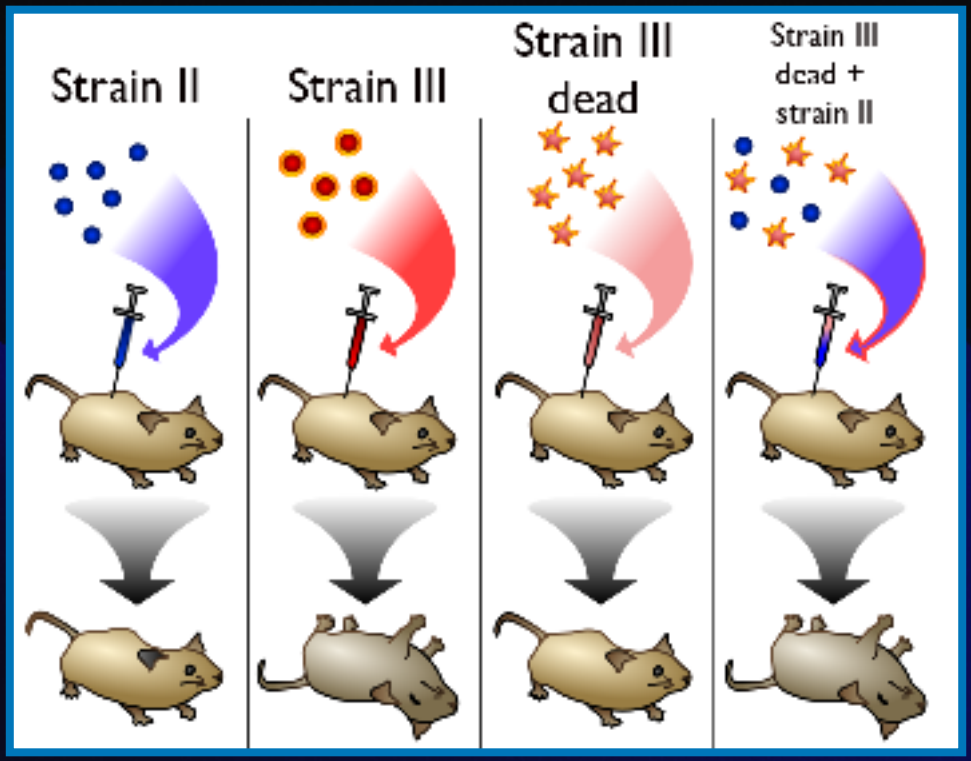
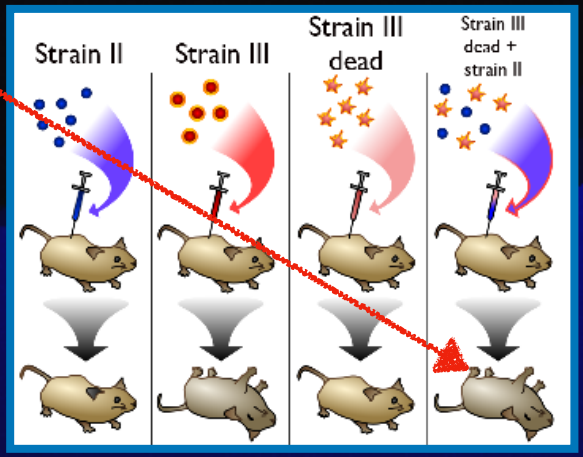


These mice were found to have been killed by strain III bacteria.

Were they brought back from the dead?

No. it looked like strain II bacteria were somehow transformed into strain III's.



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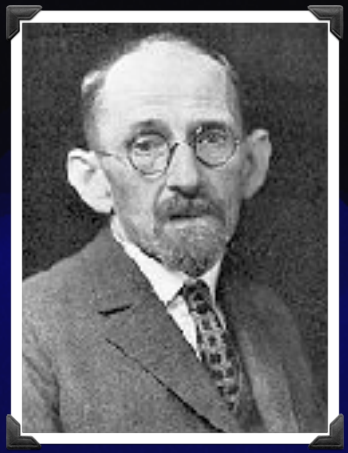
He never really followed up on this observation. But although originally greeted with skepticism, his experiments were repeated in two major laboratories.

Griffith was to die tragically in London in 1941 in the blitz.

A prominent microbiologist, Fred Neufeld, was visiting his lab at the time Griffith was conducting his experiments.

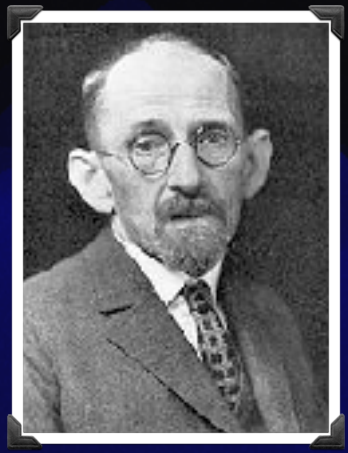


Fred Neufeld

Neufeld was the Director of the Koch Institute in Berlin.

Neufeld was the discover of the various Pneumococcal strains

He went back to his laboratory and successfully repeated Griffith's studies.



Fred Neufeld

Neufeld was later dismissed from the Koch Institute by the Nazi's, and died in Berlin in 1945 of starvation.

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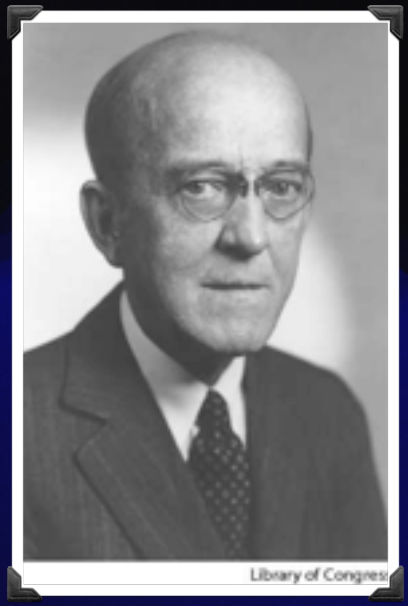
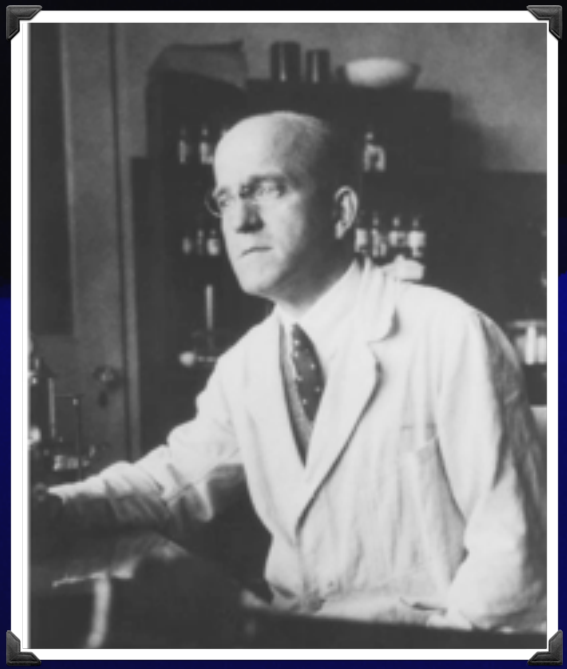
Meanwhile, at the Rockefeller Institute in New York, Oswald Avery, recognizing that Griffith's work indicated that something in the dead bacteria was causing a hereditary change, asked: What was this substance?



Oswald Avery

Avery was born in Halifax, Canada in 1877, the son of a Baptist minister.
The family moved to New York City when Avery was 10.
He went to Colgate University intending to become a clergyman like his father.



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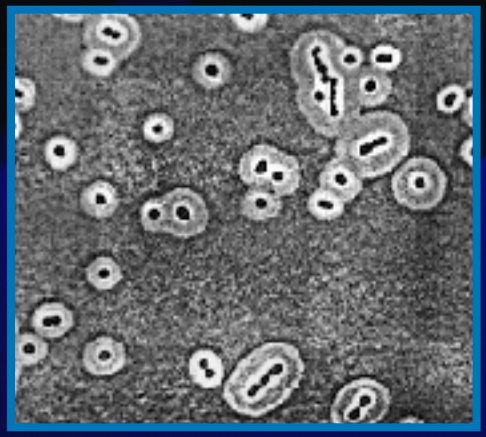
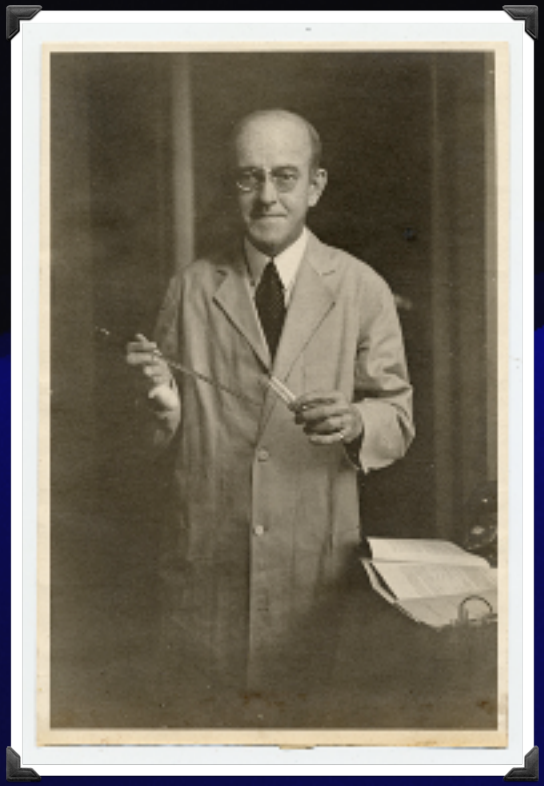
For reasons that aren't clear, he switched his interest to medicine, attended Columbia, and graduated with a medical degree in 1900.

He practiced medicine for awhile, but quickly became interested in microbiology.

He headed a laboratory at the Rockefeller that had an impressive string of discoveries to its credit.



After some members of his laboratory repeated Griffith's work, he decided to purify the substance that caused this hereditary change.



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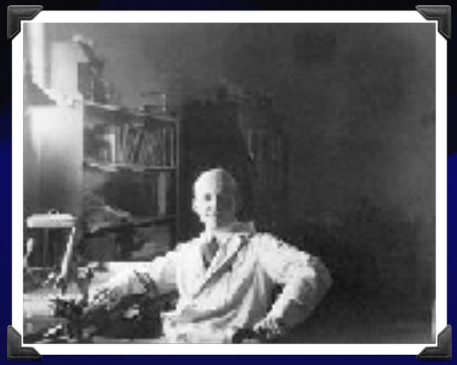
Avery had two insights that others missed.

1. This substance was the hereditary material (he called it "transforming principle")
2. Griffith had developed an "assay" for it, thereby allowing its detection.

His first assay was primitive and difficult (it involved killing mice). In time they developed better ways of measuring transforming principle.

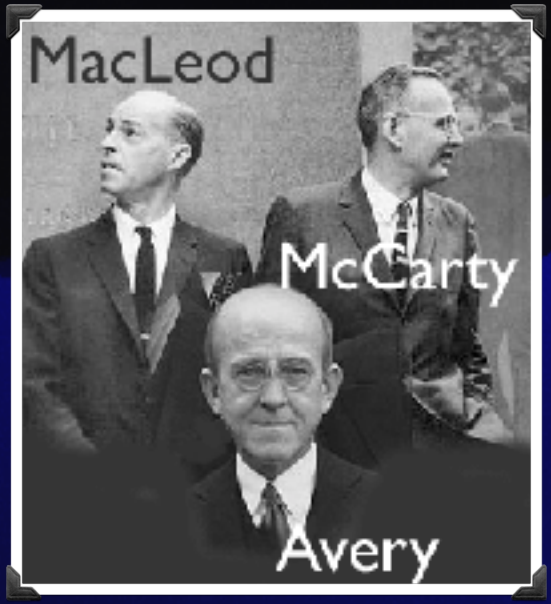


Once you can figure out the quantity of a substance in a mixture, you can try to purify it, so that you can analyze it properly.

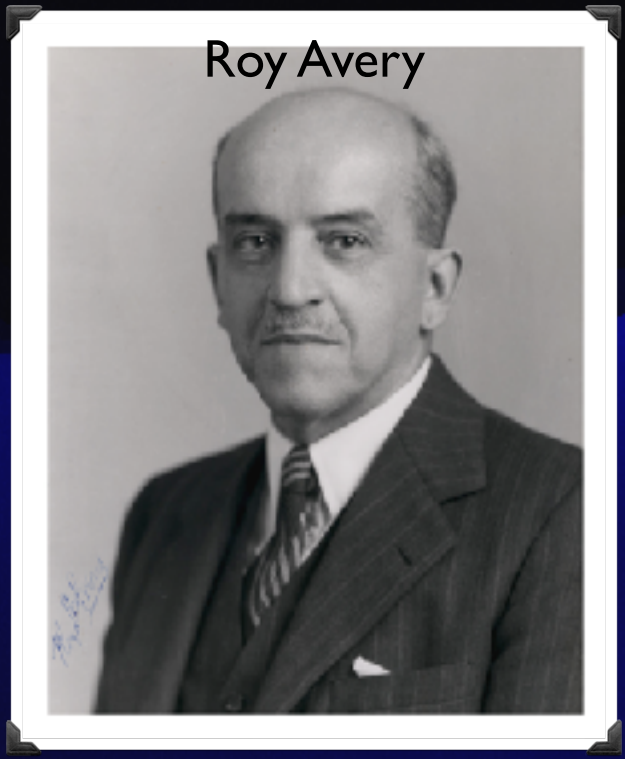
Avery's goal was to purify the "transforming principle" in order to figure out what it was.

He enlisted the aid of several associates.

It was a long and tedious process. The assay didn't always work.



In 1943, he wrote a remarkable letter to his brother Roy (also a microbiologist) describing what he had learned.



The letter captures the way that scientists think, but rarely express to outsiders.

I'll present an annotated version in the next few slides.

May 13, 1943

Dear Roy,

...

You will recall that Griffith in London, some 15 years ago described a technique whereby he could change one specific type into another...

For the past two years, first with MacLeod and now with Dr. McCarty -- I have been trying to find out what is the chemical nature of the substance in the bacterial extract which induces this specific change...

The crude extract ... is full of ... polysaccharide, ... carbohydrate, nucleoproteins [DNA and RNA bound to proteins], free nucleic acids of both the yeast [the old name for RNA] and thymus type [an old name for DNA], lipids [fats] and other cell constituents. Try to find in that complex mixture the active principle!

Try to isolate and chemically identify the particular substance that will by itself [accomplish this change]. Some job -- full of heartache and heart breaks. But at last perhaps we have it...

The active substance is not digested with crystallin trypsin or chymotrypsin [enzymes that digest proteins]. It does not lose activity when treated with crystalline Ribonuclease [RNase, an enzyme that digests RNA] ...

When extracts, treated and purified to this extent, but still containing traces of protein... are further fractionated by the dropwise addition of absolute ethyl alcohol, an interesting thing occurs. When alcohol reaches a concentration of about 9/10 by volume there separates out a fibrous substance which on stirring the mixture wraps itself about the glass rod like thread on a spool -- and the other impurities stay behind ...

The fibrous material is redissolved and the process repeated several times. In short, this substance is highly reactive and on elementary analysis [a determination of the elements in the substance] conforms very closely to the theoretical values of pure desoxyribose nucleic acid (thymus type [again, the old name for DNA. It's now called deoxyribonucleic acid]. Who would have guessed it? ...

We have isolated highly purified substance of which as little as 0.02 of a microgram [that's 20 billionths of a gram] is active in inducing transformation. In the reaction mixture ... this represents a dilution of 1 part in a hundred million -- potent stuff that -- and highly specific. This does not leave much room for impurities -- but the evidence is not good enough yet.

If we are right -- and of course that's not yet proven, then it means that nucleic acids are not merely structurally important but functionally active substances in determining the biochemical activities and specific characteristics of cells --

... and that by means of a known chemical substance it is possible to induce predictable and hereditary changes in cells. This is something that has long been the dream of geneticists...

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So there's the story Roy -- right or wrong it's been good fun and lots of work... I'm so tired and sleepy I'm afraid I have not made this very clear -- but I want you to know -- and sure you will see that I cannot well leave this problem until we've got convincing evidence...

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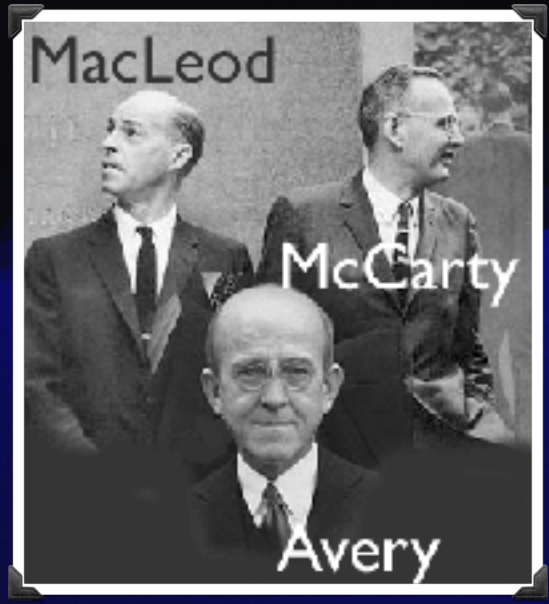
Avery, Macleod and McCarty published their paper in 1944.

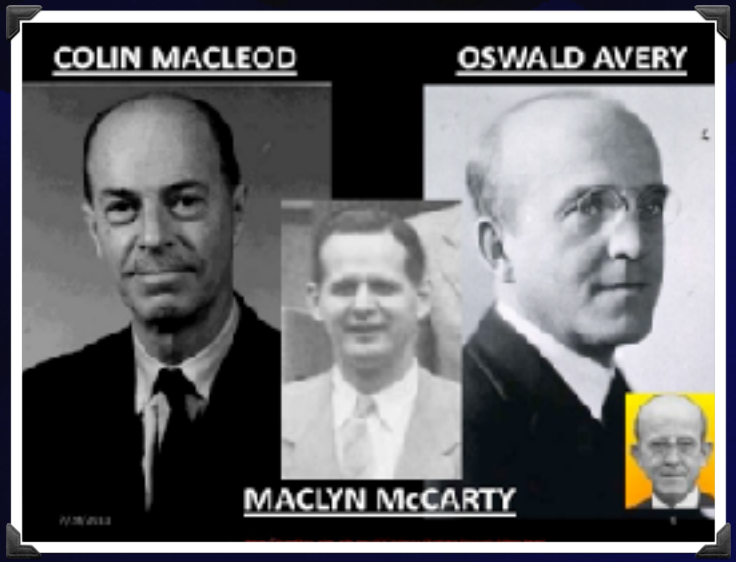
His work was criticized (as he expected).

Interestingly, the scientific community didn't accept the fact that DNA was the hereditary material for almost another decade.



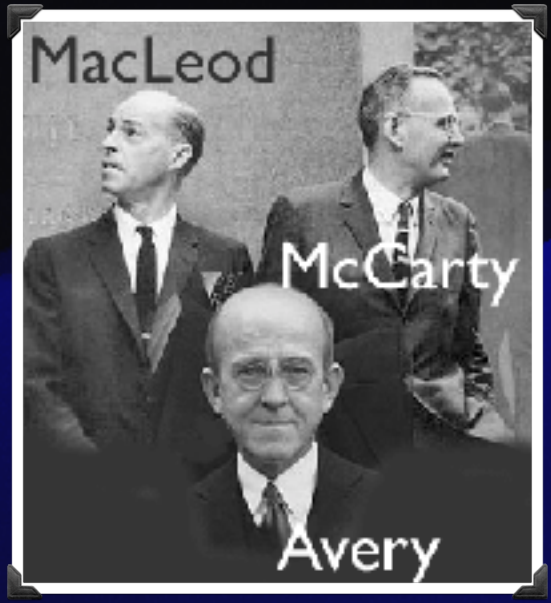
Some thought that a tiny amount of protein contaminated Avery's DNA preps, and it was the cause of transformation.

Others thought that transformation was a phenomenon peculiar to microbes.

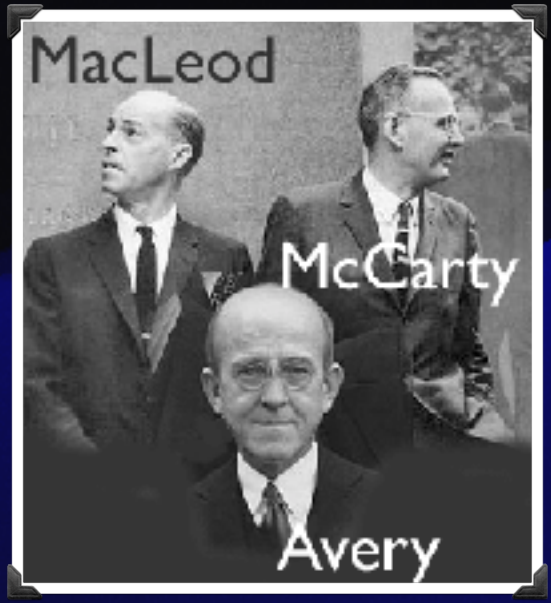


Virtually no one know anything of the size and complexity of DNA.

Most scientists thought that DNA was a repetitive substance (because of Levene).

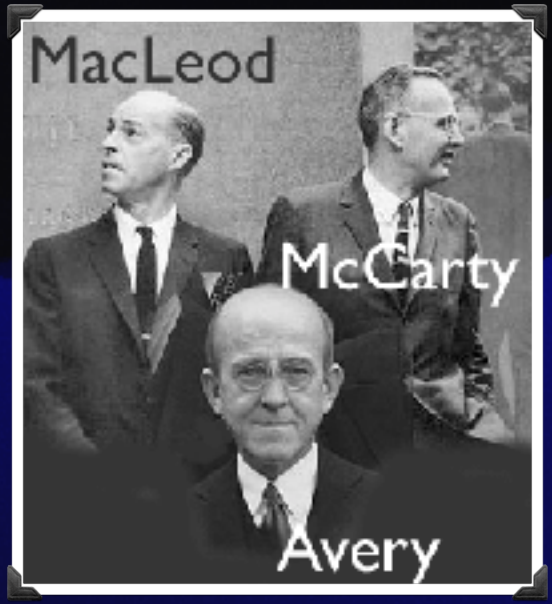


Or how it could pass from one generation to another, and how it specified the structure of proteins.

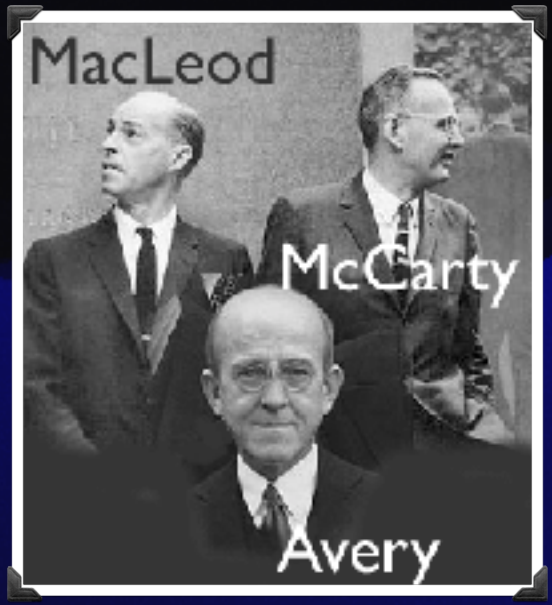


Avery retired shortly after the publication of his classic paper. He moved to Nashville to be with his brother and his family.

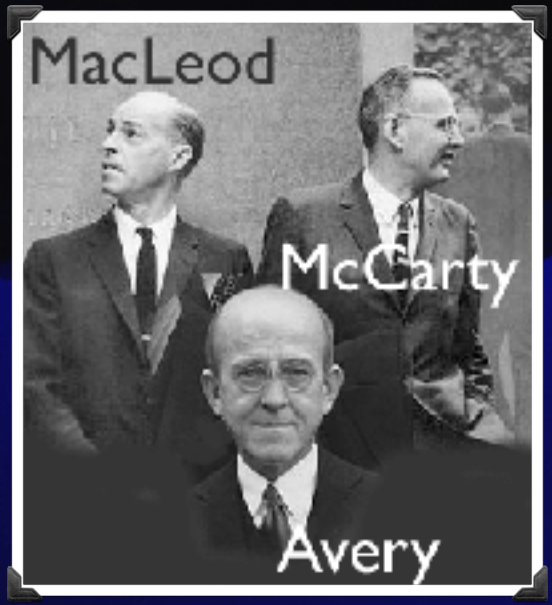
He died in 1955.



While accorded many honors in his lifetime, he missed the greatest award, the Nobel Prize.



But his paper made an impression on a number of his colleagues.



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And it led to Watson and Crick's search for the structure of DNA, which we'll take up next time...