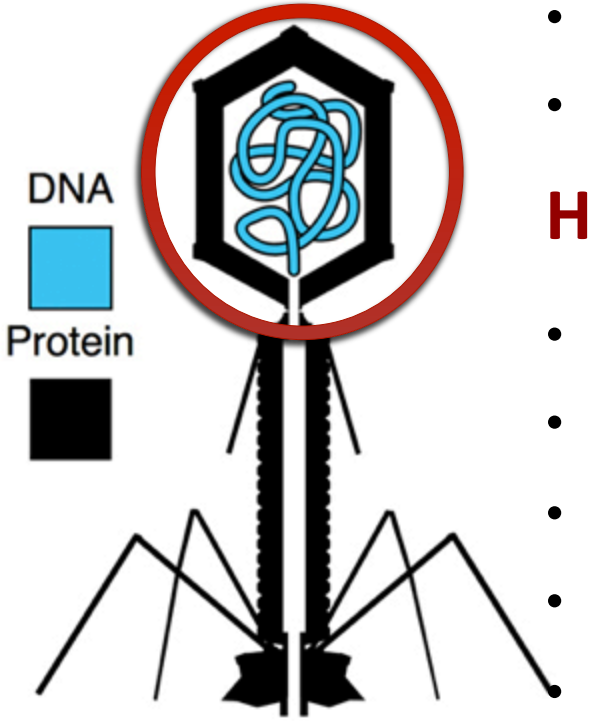
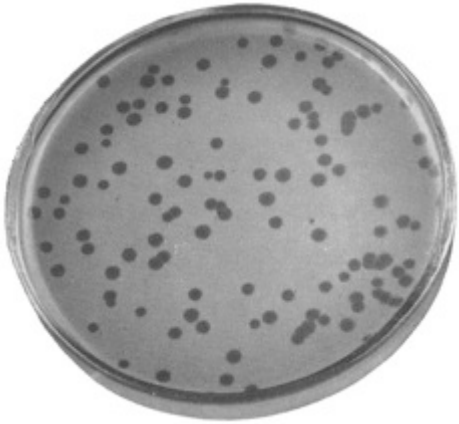
Independent Functions of Viral Protein and Nucleic Acid Growth of Bacteriophage

By A.D. Hershey and Martha Chase

Things you should know…



- **Phage is short for Bacteriophage (“bacteria eater”)**
- **Phages are viruses**



Hershey and Chase

- **Protein vs. DNA**
- **Knew components of phages**
- **Phages replicate within their host cells**
- **Progeny inherits genetic material**
- **Use of radioactive labeling**

http://en.wikipedia.org/wiki/Enterobacteria_phage_T2

http://www.mun.ca/biology/scarr/4241smc_Ecoli_&_T2_bacteriophage.html

Radioactive labeling

^{35}S

- Incorporates into Amino Acids
- Proteins contain Sulfur (Cysteine and Methionine)
- Not present in DNA

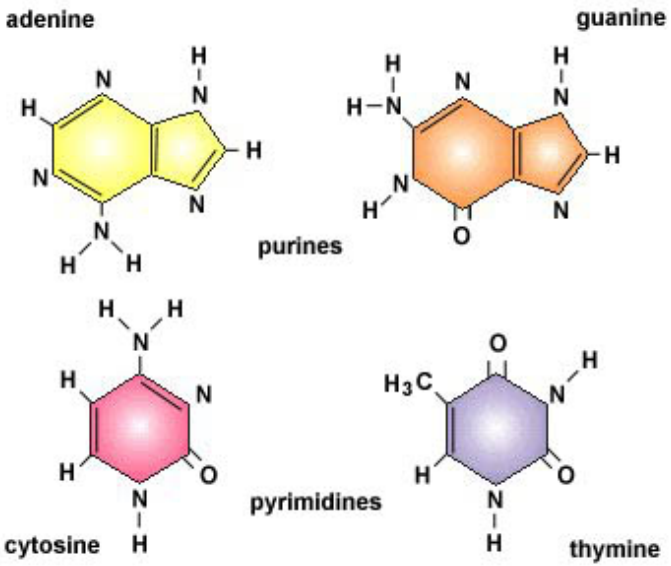
^{32}P

- Incorporates into DNA
- DNA contains Phosphorus
- None of the 20 Amino acids contain Phosphorus

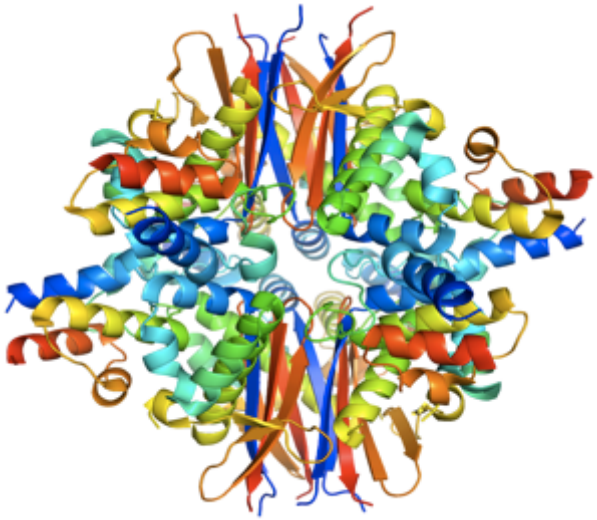
Protein vs. DNA as Genetic Material

Protein

- 20 amino acids
- Sperm contain protein



http://applejacksgirl.blogspot.ca/2012/04/nucleotides.html



http://csgid.org/cake/deposits/index/page:18

DNA

- 4 nucleotides
- Sperm do not contain histones (which are present in DNA)

Independent Functions of Viral Protein and Nucleic Acid Growth of Bacteriophage

Martha Chase →

← Alfred Hershey

http://www.dnalc.org/view/16406-Gallery-18-Alfred-Hershey-and-Martha-Chase-1953.html

- Hershey and Chase wanted to know:

Which one is the genetic material ?

Consider This…

- This experiment has been called a **“dirty experiment.”**
- Can you think of some reasons why ?

Experiment 1

“The Chemical Morphology of Resting Phage Particles”

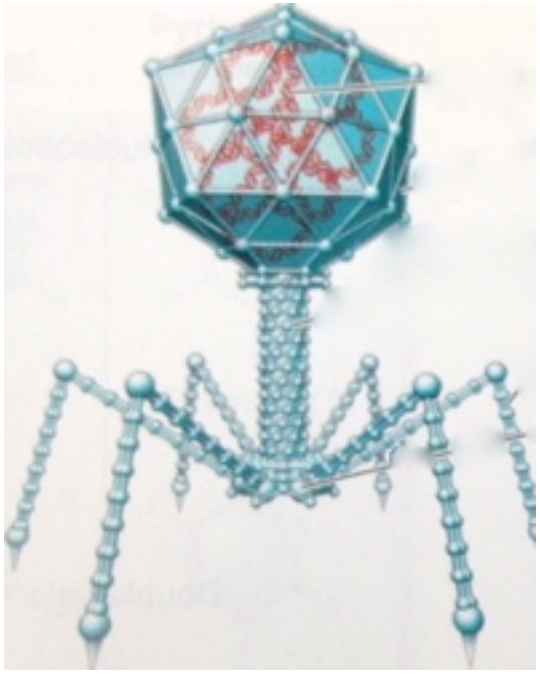
Background

Osmotic Shock:

- Bacteriophage T2 is inactivated by suspending the particles in high concentrations of NaCl, and rapidly diluting the suspension with water.
- The inactivated phage was visible in electron micrographs as tad-pole-shaped "ghosts."
- Believed that DNA was released into the solution during this process.

Materials and Methods

- They plasmolyzed the labeled T2 by suspending the phage in NaCl at room temperature, and rapidly poured into the suspension of distilled water.
- Then the plasmolyzed phage, with less than 2% survivors, was analyzed for phosphorus and sulfur.



Biology, Brooker et al.

Results

Composition of Ghosts and Solution of Plasmolyzed Phage

Per cent of isotope]	Whole phage labeled with		Plasmolyzed phage labeled with	
	P^{32}	S^{35}	P^{32}	S^{35}
Acid-soluble	—	—	1	—
Acid-soluble after treatment with DNase	1	1	80	1
Adsorbed to sensitive bacteria	85	90	2	90
Precipitated by antiphage	90	99	5	97

- Plasmolysis separated phage T2 into ghosts containing nearly all the S and a solution containing nearly all the DNA of the intact particles.
- The ghosts contained the principal antigens of the phage particle detectable by their antiserum. The DNA was released as the free acid, or possibly linked to sulfur-free substances.
- The ghosts were specifically adsorbed to phage-susceptible bacteria and the DNA was not.

Results

Composition of Ghosts and Solution of Plasmolyzed Phage

Per cent of isotope]	Whole phage labeled with		Plasmolyzed phage labeled with	
	P^{32}	S^{35}	P^{32}	S^{35}
Acid-soluble	—	—	1	—
Acid-soluble after treatment with DNase	1	1	80	1
Adsorbed to sensitive bacteria	85	90	2	90
Precipitated by antiphage	90	99	5	97

- The ghosts represent protein coats that surround the DNA of the intact particles, react with antiserum, protect the DNA from DNase, and carry the organ of attachment to bacteria.
- The effects were from osmotic shock, because phage suspended in salt and diluted slowly is not inactivated, and its DNA is not exposed to DNase.

Experiment 2

“Sensitization of Phage DNA to DNase by Adsorption to Bacteria”

Purpose

- As seen from the previous experiment, the structure of the resting phage particle suggests that there is a possibility that multiplication of virus is preceded by the alteration/ removal of the protective coats of the particles.
- This change might be expected to be shown as a sensitization of the phage DNA to DNase.
- Two ways:
  - Heat Killing
  - Alternate Freezing and Thawing with Formaldehyde Fixation

Materials and Methods

- Intact T2 phages (some have undergone plasmolysis by osmotic shock) were labeled with P and S isotopes and analyzed for these isotopes in the following methods:

1. Exposing the phages to trichloroacetic acid
2. Exposing the phages to trichloroacetic acid and treatment with DNase
3. Adsorbance of the phages to live bacteria
4. Exposing phage solution to an antibody specific to a protein on the phage coat

Results (Heat Killing)

Sensitization of Phage DNA to DNase by Adsorption to Bacteria

Phage adsorbed to	Phage labeled with	Non-sedimentable isotope, *per cent*	
		After DNase	No DNase
Live bacteria	S^{35}	2	1
" "	P^{32}	8	7
Bacteria heated before infection	S^{35}	15	11
" " " "	P^{32}	76	13
Bacteria heated after infection	S^{35}	12	14
" " " "	P^{32}	66	23
Heated unadsorbed phage: acid-soluble P^{32} 70°	P^{32}	5	
80°	P^{32}	13	
90°	P^{32}	81	
100°	P^{32}	88	

- Minimal non-sedimentable isotope was found when phage directly infected the bacterial cell
- When the cell was heat-killed before and after infection, a fair amount of non-sedimental P^{32} was found when no DNase was added
- Below 80°C, small amounts of isotope were found but the amount of non-sedimentable isotope increased drastically when the temperature raised above 90°C

Results
(Freezing &Thawing, Formaldehyde Fixation)

Sensitization of Intracellular Phage to DNase by Freezing, Thawing, and Fixation with Formaldehyde

	Unadsorbed phage frozen, thawed, fixed	Infected cells frozen, thawed, fixed	Infected cells fixed only
Low speed sediment fraction			
Total P^{32}	—	71	86
Acid-soluble	—	0	0.5
Acid-soluble after DNase	—	59	28
Low speed supernatant fraction			
Total P^{32}	—	29	14
Acid-soluble	1	0.8	0.4
Acid-soluble after DNase	11	21	5.5

- The cell membrane can be made permeable to DNase under conditions that do not permit the escape of intracellular ^{32}P or most of the cell contents.
- Even if cells lyse by freezing and thawing, most of the ^{32}P derived from phage and the mature phage progeny remains inside the cell membranes.
- The intracellular ^{32}P derived from phage is mostly freed during spontaneous lysis with phage liberation.
- Supernatent fraction and labile DNA

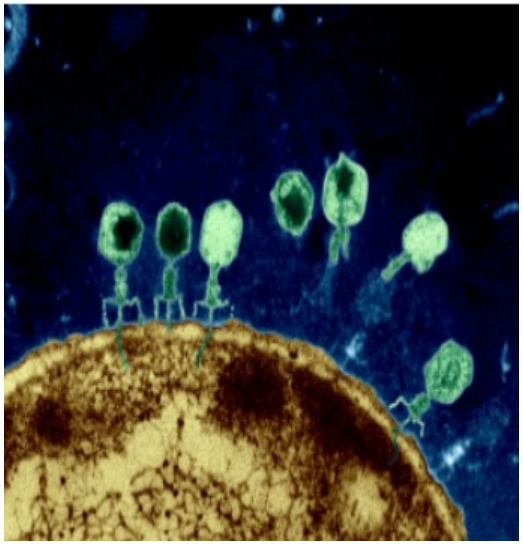
Discussion

- When DNA is inside a phage protein coat or inside an intact cell, it is not sensitive to DNase. When the DNA is separated from its coat or injected into a heat-killed cell (heat-killed either before or after injection), it becomes sensitive to DNase.
- Freezing and thawing disrupts the cell, making the DNA sensitive to DNase.
- Therefore, by interpreting the results, it seems that the phage protein coat plays a role in protecting the DNA from chemical modifications or damage.

Experiment 3

“Liberation of DNA from Phage Particles by Adsorption to Bacterial Fragments ”

Methods: Part 1



http://ehp.niehs.nih.gov/121-a48/

- Bacterial debris was created by infecting bacterial cells with four T2 phages each
- Cells transferred to salt-poor broth (37°C), then aerated, HCN added, incubated
- Debris from lysed cells washed by centrifugation (1700 G) then resuspended in adsorption medium.
- The mixtures were then centrifuged for 15 minutes (2200 G)

Results: Part 1

TABLE IV

Release of DNA from Phage Adsorbed to Bacterial Debris

	Phage labeled with	
	S^{35}	P^{32}
Sediment fraction		
Surviving phage	16	22
Total isotope	87	55
Acid-soluble isotope	0	2
Acid-soluble after DNase	2	29
Supernatant fraction		
Surviving phage	5	5
Total isotope	13	45
Acid-soluble isotope	0.8	0.5
Acid-soluble after DNase	0.8	39

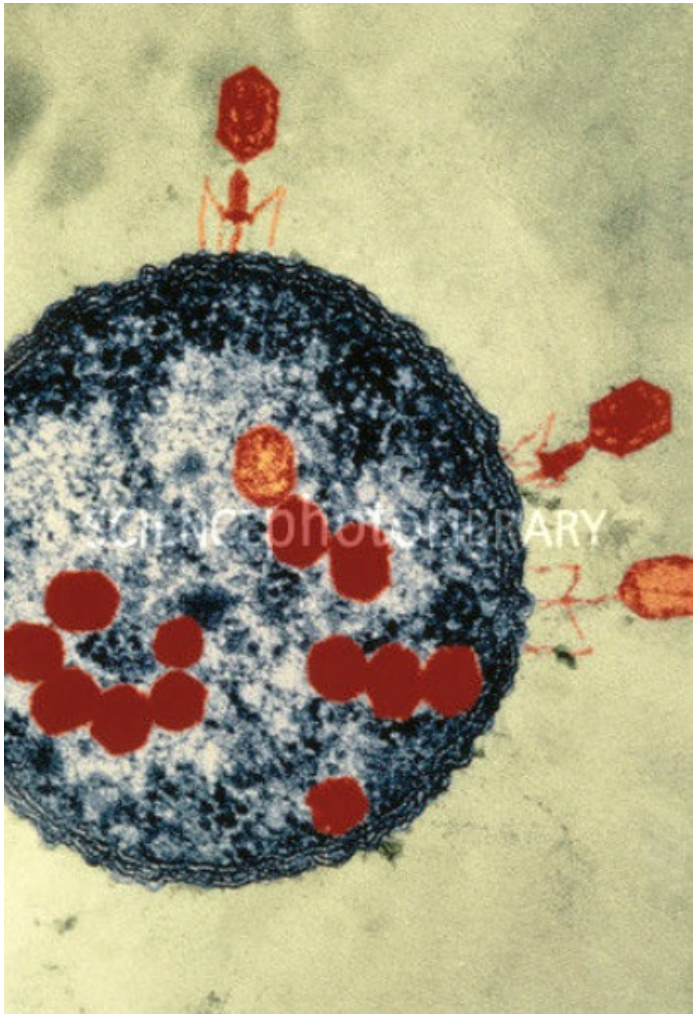
Figure adapted from original source material

- After centrifugation at 1700 G the supernatant consisted mostly of collapsed and fragmented cell membrane
- ^{35}S found mostly in sediment (inactivation)
- ^{32}P almost equally distributed in sediment and supernatant

Part 2

- B strain of bacteria lysed by either large amounts of T2 or T4 (UV-killed)
- Tested in the same way with ^{32}P labeled T2 and T4

- Bacterial debris that had been saturated with UV-killed T2 adsorbs T4 better than T2
- Debris saturated with T4 adsorbs T2 better than T4
- Some adsorbed phage not inactivated, some DNA of inactivated phage not released from debris



http://www.sciencephoto.com/media/249812/enlarge

Summary Points

http://biology.clc.uc.edu/fankhauser/Labs/Genetics/DNA_Isolation/Thymus_DNA.htm

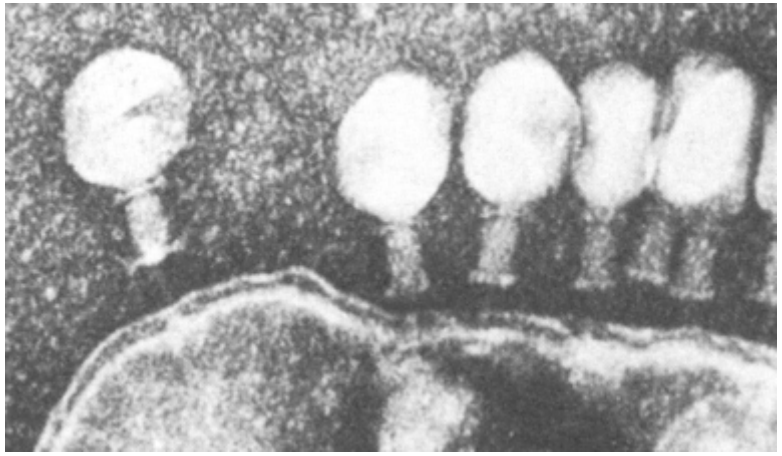
- 5% of phage particles in infective form and unadsorbed (13% of total sulfur)
- 80% of phage adsorbed to debris
- Most of ^{35}S of the inactivated phage and surviving phage found in the sediment
- Supernatant fraction contains 40% of phage DNA (sensitive to DNAse) and DNA from unadsorbed surviving phage.
- Most of the DNA that was sedimented was either surviving phage or DNA sensitive to DNAse
- Some of the cell receptors for T2 are different than those for T4

Experiment 4

“Removal of Phage Coats from Infected Bacteria”

Background

- Anderson (1951) obtained an electron micrograph showing that phage T2 attaches to bacteria via it's tail
- Hershey and Chase thought… if the phage attachment to bacteria is preserved during infection, it should be easy to break off "empty" phage membranes, leaving phage DNA inside the infected bacteria.



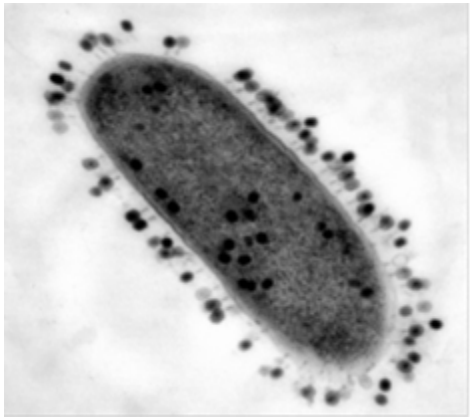
Methods: Part 1

- Bacteria were infected with either ^{32}P or ^{35}S labelled phages
- Unadsorbed material was removed with centrifugation
- Resuspended, then the suspension was spun in Waring blender (10 000 RPM), cooled in ice water
- Antiphage serum titration (to measure # of bacteria capable of reproducing phages)
- These samples then centrifuged to measure proportion of isotope released by bacteria

http://www.cutleryandmore.com/waring-pro/stainless-steel-professional-blender-p17870

Results: Part 1

- Phage do not adsorb well to cells when agitated
- At higher rates of infection there was considerable amounts of ^{35}S that elutes spontaneously, however the elution of ^{32}P was not affected by multiplicity of infection



http://varietyofrna.wikispaces.com/Hershey+and+Chase

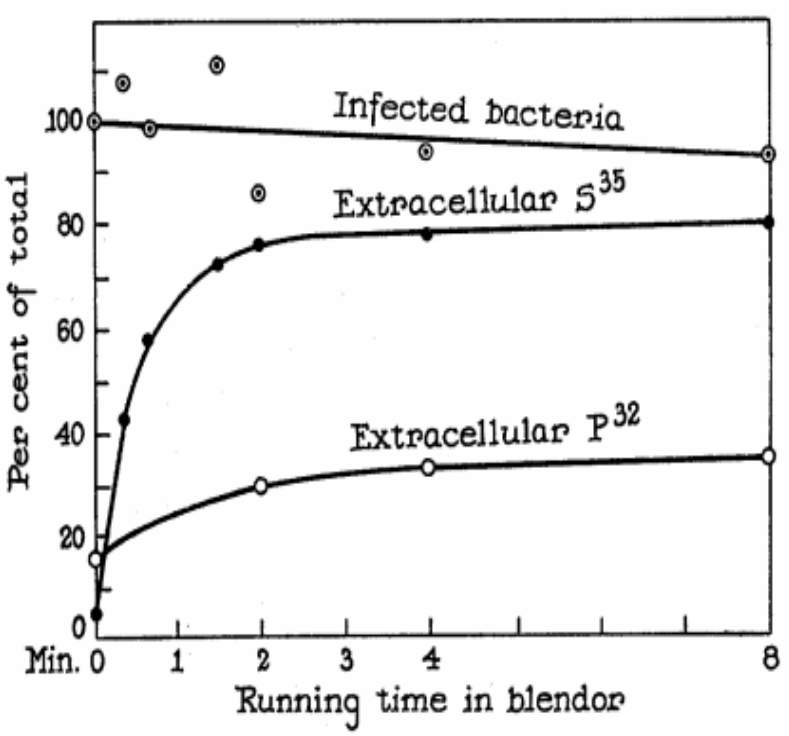


Figure adapted from original source material

Part 2

- Release of ^{32}P from cells was slightly less than ^{35}S when infection rate was low and blending had not begun
- Almost half of the ^{35}S isotope had already eluted at t = 0 when the multiplicity of infection was 6.0
- Agitation causes significant increases in the amount of ^{35}S eluted
- Elution of ^{32}P was consistent regardless of multiplicity of infection

TABLE V

Effect of Multiplicity of Infection on Elution of Phage Membranes from Infected Bacteria

Running time in blendor	Multiplicity of infection	P^{32}-labeled phage		S^{35}-labeled phage	
		Isotope eluted	Infected bacteria surviving	Isotope eluted	Infected bacteria surviving
min.		*per cent*	*per cent*	*per cent*	*per cent*
0	0.6	10	120	16	101
2.5	0.6	21	82	81	78
0	6.0	13	89	46	90
2.5	6.0	24	86	82	85

Figure adapted from original source material

Summary Points

- Violent agitation can strip 75-80% of ^{35}S phage material
  - This material must be at cell surface
- Properties of ^{35}S-labeled material consists of more or less intact phage membranes
- Violent agitation releases only 21-35% of ^{32}P-labeled material
  - This material must be inside cell
- This violent agitation does not inactivate the phage progeny
- During infection, most of the sulfur remains at the cell surface and does not take part in the multiplication of intracellular phage. Most of the phage DNA enters cell soon after phage adsorption to the bacteria.

"^{32}P goes in, ^{35}S stays out"

- **Upon infection, most of the phage sulfur (^{35}S labeled AA's) stays on the surface of the subject bacteria, and most of the phage DNA (^{32}P labeled), enters the infected cell.**

Experiment 5

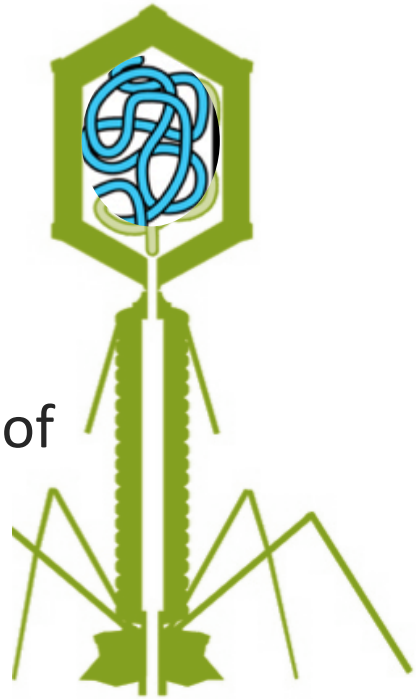
“Transfer of Sulfur and Phosphorus from Parental Phage to Progeny”

Background

- Most of the S-containing phage protein takes no part in multiplication of phage, and does not enter the bacteria cell.
- Expect that little or no sulfur should be transferred from parental phage to progeny

Materials and Methods

- Grow *E. Coli,* sediment them, then re-suspend them in adsorption medium.
- Infect bacteria with ^{35}S-labeled phage T2.
- Add HCN and UV-killed phage (lysing phage)
- Centrifuge and observe phage and ^{35}S distribution of different fractions
- Repeat experiment using ^{32}P-labeled phages



Results

TABLE VI

Per Cent Distributions of Phage and S^{35} among Centrifugally Separated Fractions of Lysates after Infection with S^{35}-Labeled T2

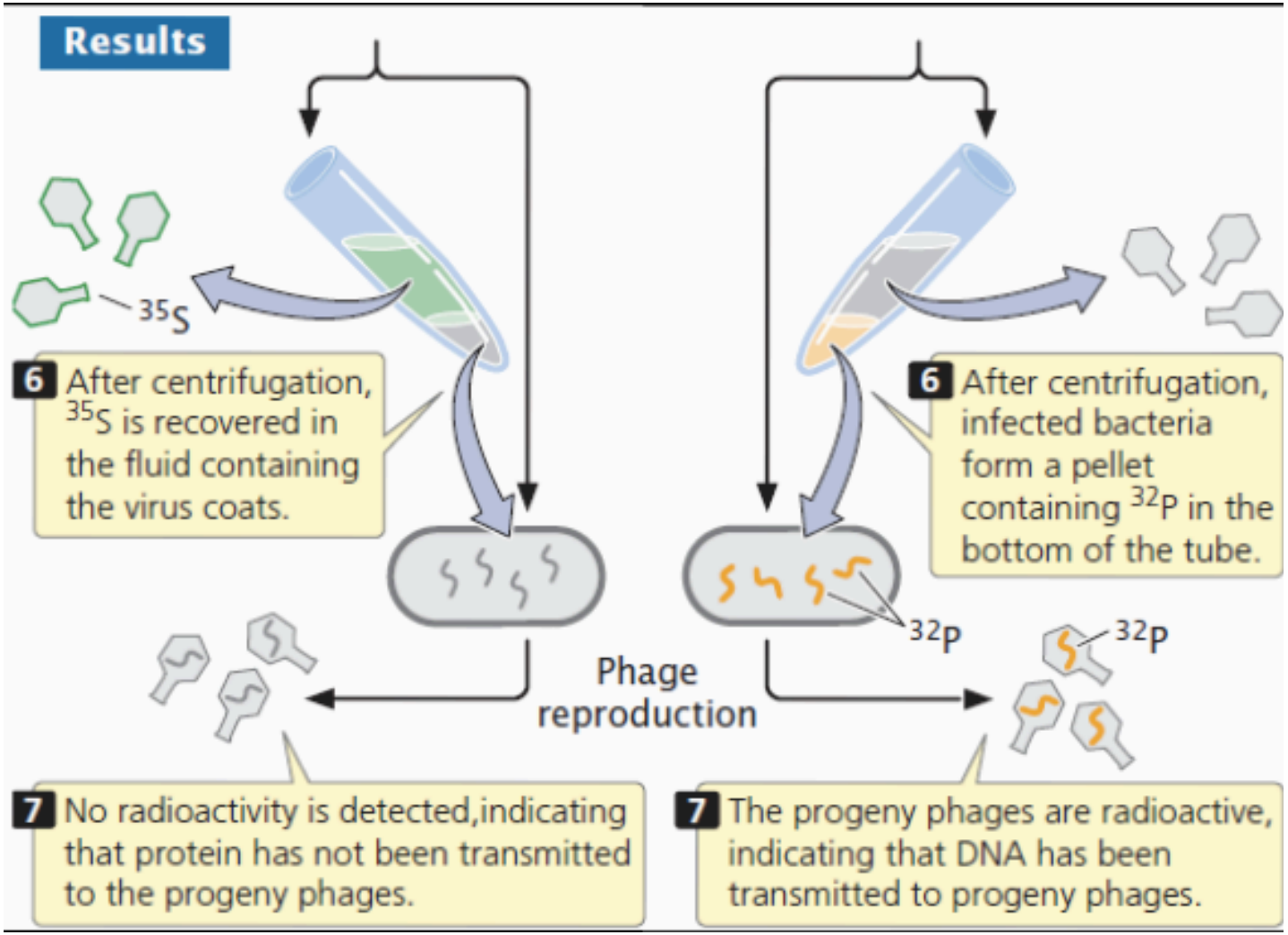
Fraction	Lysis at $t = 0$ S^{35}	Lysis at $t = 10$ S^{35}	Maximal yield	
			S^{35}	Phage
1st low speed sediment	79	81	82	19
2nd " " "	2.4	2.1	2.8	14
High speed "	8.6	6.9	7.1	61
" " supernatant	10	10	7.5	7.0
Recovery	100	100	96	100

Infection with S^{35}-labeled T2, 0.8 particles per bacterium. Lysing phage UV-killed *h* mutant of T2. Phage yields per infected bacterium: <0.1 after lysis at $t = 0$; 0.12 at $t = 10$; maximal yield 29. Recovery of S^{35} means per cent of adsorbed input recovered in the four fractions; recovery of phage means per cent of total phage yield (by plaque count before fractionation) recovered by titration of fractions.

Discussion

- Almost all sulfur removed after 1st centrifuge
- The ^{35}S distribution is the same in early lysates without a progeny, as it is for late lysates that do contain a progeny
- Shows that little or no ^{35}S is contained in the mature progeny
- Phosphorus is transferred from parental phage to progeny (30%)
- Supports the expectation that sulfur does not get passed from parental phage to progeny

Summary


Results
^{35}S
6 After centrifugation, ^{35}S is recovered in the fluid containing the virus coats.
6 After centrifugation, infected bacteria form a pellet containing ^{32}P in the bottom of the tube.
^{32}P
^{32}P
Phage reproduction
7 No radioactivity is detected, indicating that protein has not been transmitted to the progeny phages.
7 The progeny phages are radioactive, indicating that DNA has been transmitted to progeny phages.

Experiment 6

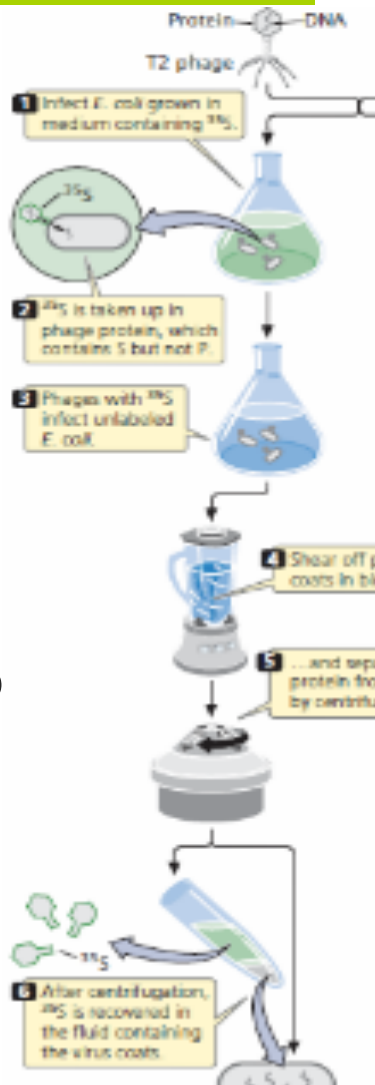
“A Progeny of ^{35}S-Labeled Phage Nearly Free from the Parental Label”

Purpose

- To prove that the obligatory transfer of parental sulfur to offspring is less than 1 per cent
- An extension on the last experiment
- Provides extra proof and support

Material and Methods

- Sensitive *E.Coli* infected with five ^{35}S-labeled phage T2 per bacterium
- Agitate one sample in Waring blendor. Leave other sample un-agitated
- Centrifuge both
- Lyse cells (HCN and lysing phages)
- Add NaCl to avoid elution of labeled phages attached to debris
- Fractionate and assay lysates



Results

TABLE VIII

Lysates of Bacteria Infected with S^{35}-Labeled T2 and Stripped in the Waring Blendor

Per cent of adsorbed S^{35} or of phage yield:	Cells stripped		Cells not stripped	
	S^{35}	Phage	S^{35}	Phage
Eluted in blendor fluid	86	—	39	—
1st low-speed sediment	3.8	9.3	31	13
2nd " " "	(0.2)	11	2.7	11
High-speed "	(0.7)	58	9.4	89
" " supernatant	(2.0)	1.1	(1.7)	1.6
Recovery	93	79	84	115

All the input bacteria were recovered in assays of infected cells made during the latent period of both cultures. The phage yields were 270 (stripped cells) and 200 per bacterium, assayed before fractionation. Figures in parentheses were obtained from counting rates close to background.

Results

- Stripping of cells reduces ^{35}S content of all fractions proportionately (ie. loss of ^{35}S in blended sample comes from the agitation)
- The ^{35}S content of the fraction containing most of the phage progeny is reduced from nearly 10 per cent, to less than 1 per cent.

Discussion

- Shows that ^{35}S remains on the outside of the bacteria
- Shows that the bulk of ^{35}S appearing in all lysate fractions comes from the remains of the coats of parental phage particles
- Confirms that less than <1% of sulfur is transferred from parental phage to progeny

Experiment 7

“Properties of Phage Inactivated by Formaldehyde”

Experiment 7

- Phages were warmed and treated with formaldehyde
- The formaldehyde inactivated the phage resulting in an inability of the phage to inject DNA into the cells
- The results suggest that the bacterial components required for attachment are not so easily altered as the act itself was still observed.
- However, this suggests that the attachment mechanism of phages to DNA is comprised of phage components that can be easily altered.
- The nature of the interaction, however, is unknown.

Discussion

- Upon attachment, most of the phage DNA entered cell whereas only 20% of protein did the same.
- The other 80% of the protein stayed as a residue on the outside of the cell, and plays no further role in infection.
- Of the remaining 20%, very little, if any, is passed on to progeny of the viruses.
- However, it had been shown that the phosphorus and adenine derived from DNA of infecting virus, was transferred to progeny.

The Defining Statement

“ We infer that sulfur-containing protein has no function in phage multiplication, and that DNA has some function.”

-Hershey and Chase, p. 54

Further Questions from Hershey and Chase

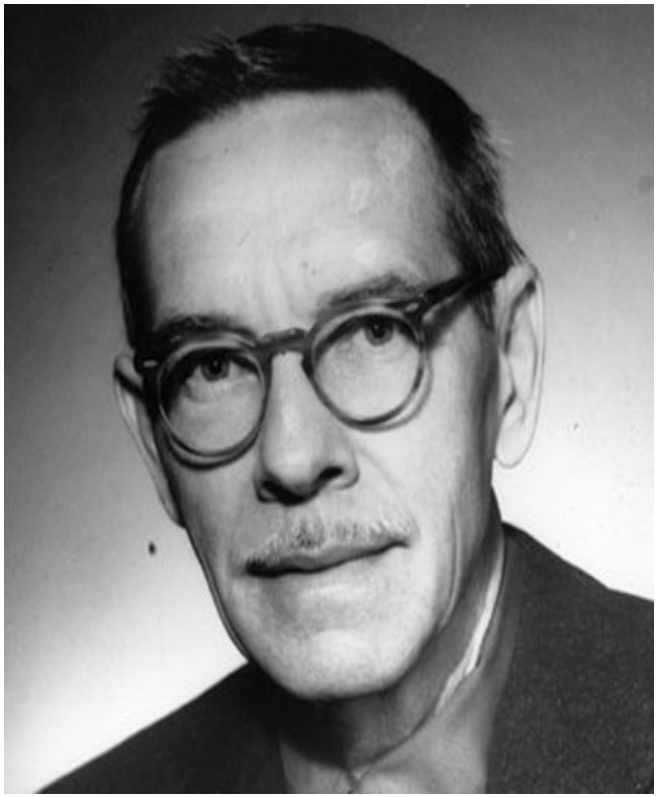
- Is there another sulfur-free material from the phage that enters the cell?
- Is this material then transferable?
- What is the manner of the transfer of phosphorus, or this unknown alternative substance?

Importance of these experiments

- Crucial to note that Hershey & Chase did **not** state that DNA is the hereditary material, but **did** state that protein was **not** the hereditary material of cells.
- Upon the conclusion of these experiments, the role and mechanisms of DNA was still undefined. These issues would be resolved by future scientists.

Alfred Day Hershey

December 4, 1908- May 22,1997



http://www.nobelprize.org/nobel_prizes/medicine/laureates/1969/hershey-bio.html

- Born in Owosso, Michigan
- B. Sc. (Chemistry) and Ph. D. from Michigan State University
- Honorary D. Sc. from University of Chicago
- Taught at Washington University and Carnegie Institution of Washington, Cold Spring Harbor
- Married with one son
- A member of several Societies and Academies
- Kimber Genetics Award of the National Academy of Sciences
- M.D.h.c. from Michigan State University
- Nobel Prize in Physiology or Medicine

Martha Chase

November 30, 1927- August 8, 2003

http://en.wikipedia.org/wiki/Martha_Chase

- Born in Cleveland, Ohio
- B. Sc. From The College of Wooster
- Ph. D. from The University of Southern California
- Lab assistant to Hershey at Cold Spring Harbor
- Married and divorced scientist Richard Epstein
- Suffered from dementia for decades
- Best known for her work in discovering DNA as the genetic material

Nobel Prize

- Alfred Hershey, along with Max Delbrück and Salvador Luria, was awarded the Nobel Prize in Physiology or Medicine in 1969 for their discoveries in “the replication mechanism and the genetic structure of viruses.”
- Martha Chase, however, was not given this award along with Hershey, despite being co-author of the definitive paper on the nature of the hereditary material.

The End !

Presented By:

Daria Snow

Emily Fitzgerald

Maggie Hickey

Sean Kennedy

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