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STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES

INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III

BY OSWALD T. AVERY, M.D., COLIN M. MACLEOD, M.D., AND
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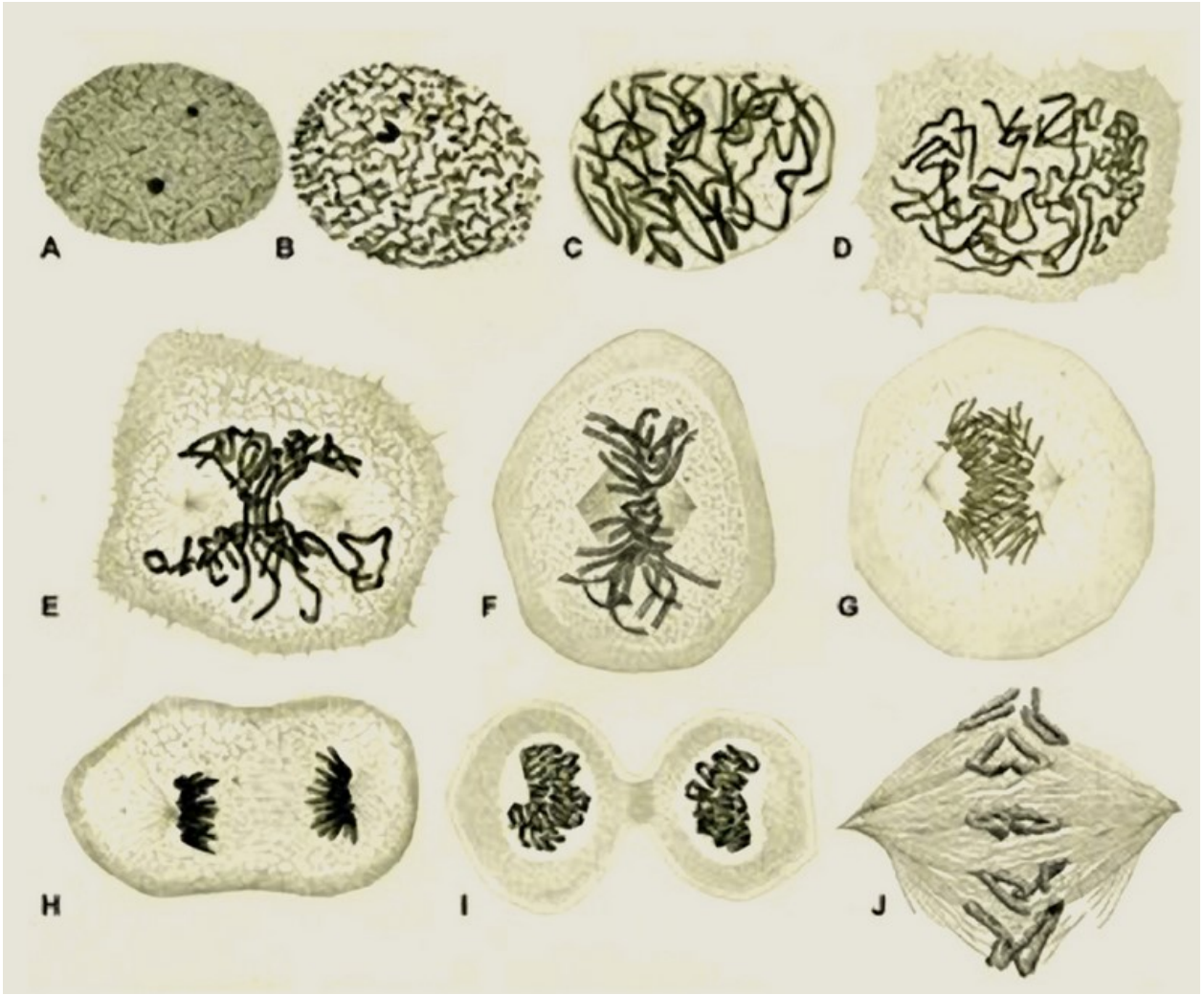
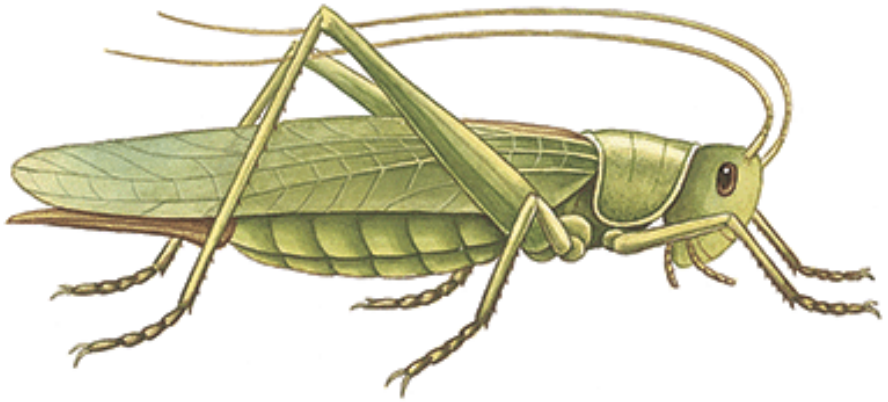
1880s: Walther Flemming Describes Chromosomes



1902: Boveri–Sutton Chromosome Theory

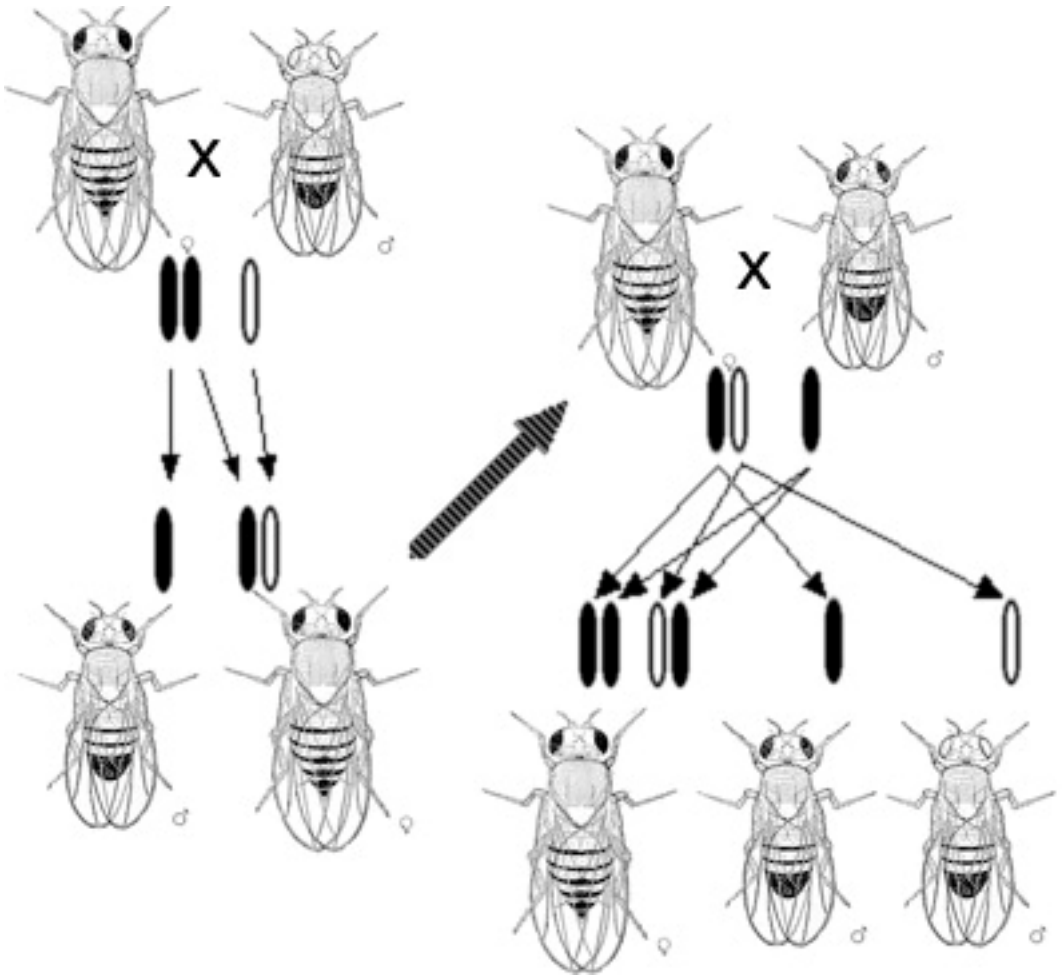


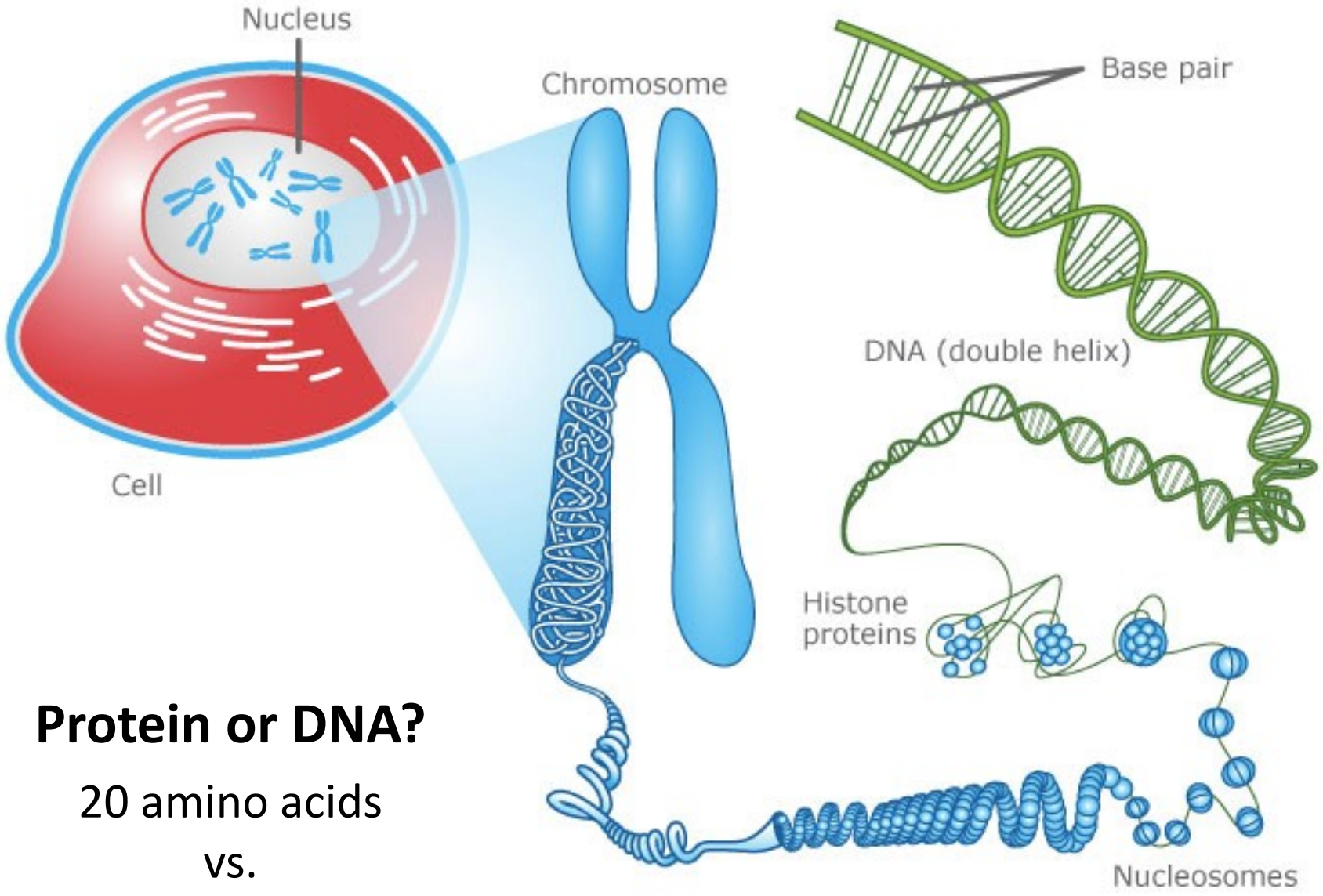
Boveri
Correct number of chromosomes must be present for normal embryonic development

Sutton
Matched pairs of paternal and maternal chromosomes separate during meiosis

“May constitute the physical basis of the Mendelian law of heredity”.

1910s: Thomas Hunt Morgan Experimentally Proves Chromosome Theory





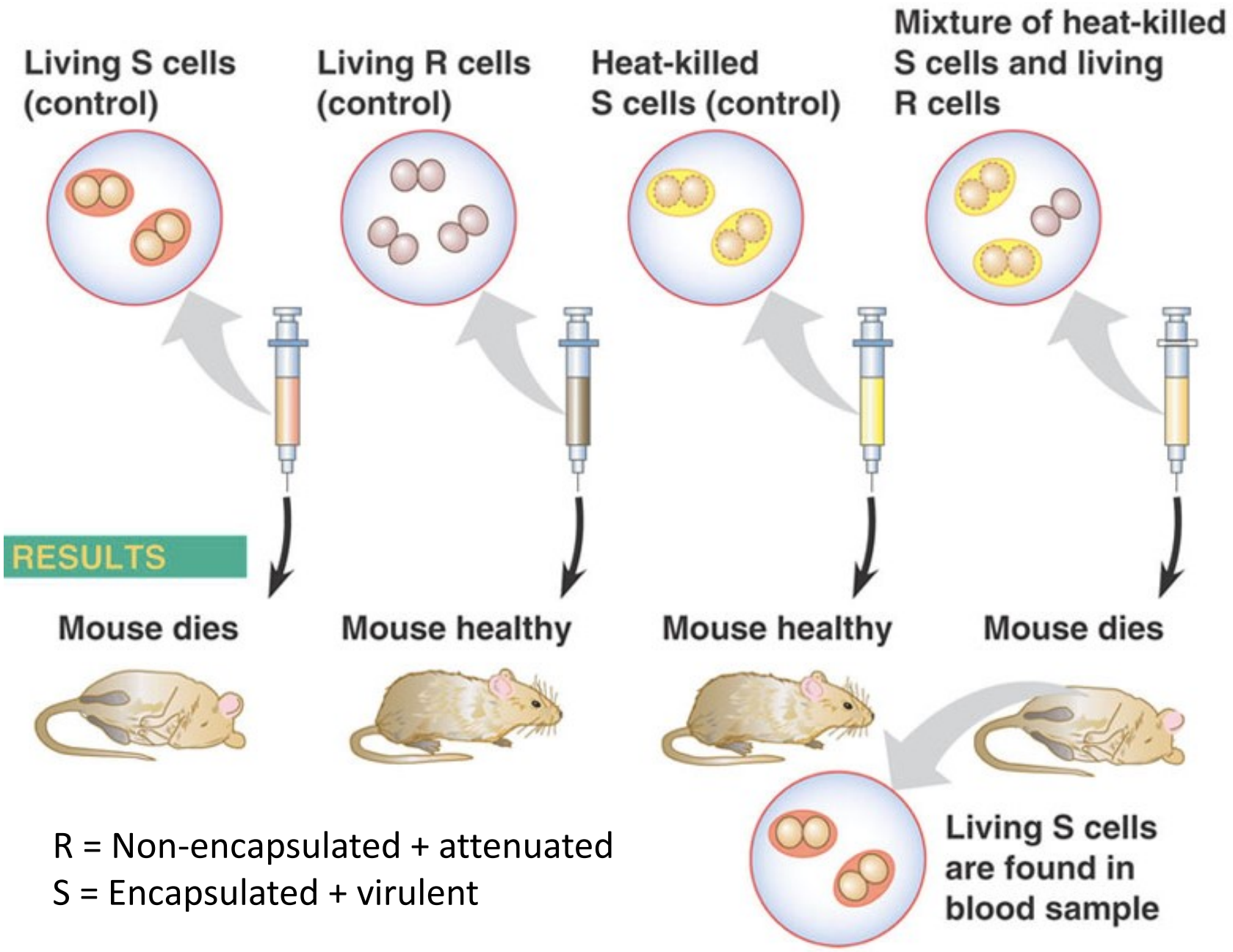
Protein or DNA?

20 amino acids

vs.

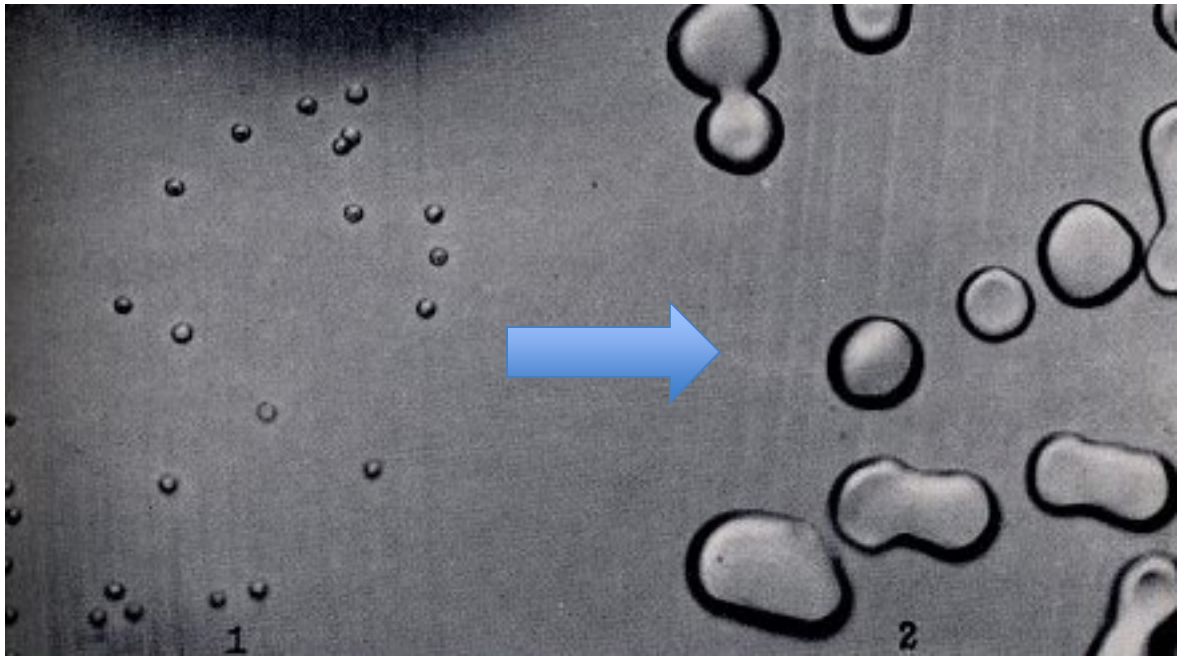
4 nucleotides

1928: Frederick Griffith discovers transformation



R = Non-encapsulated + attenuated

S = Encapsulated + virulent

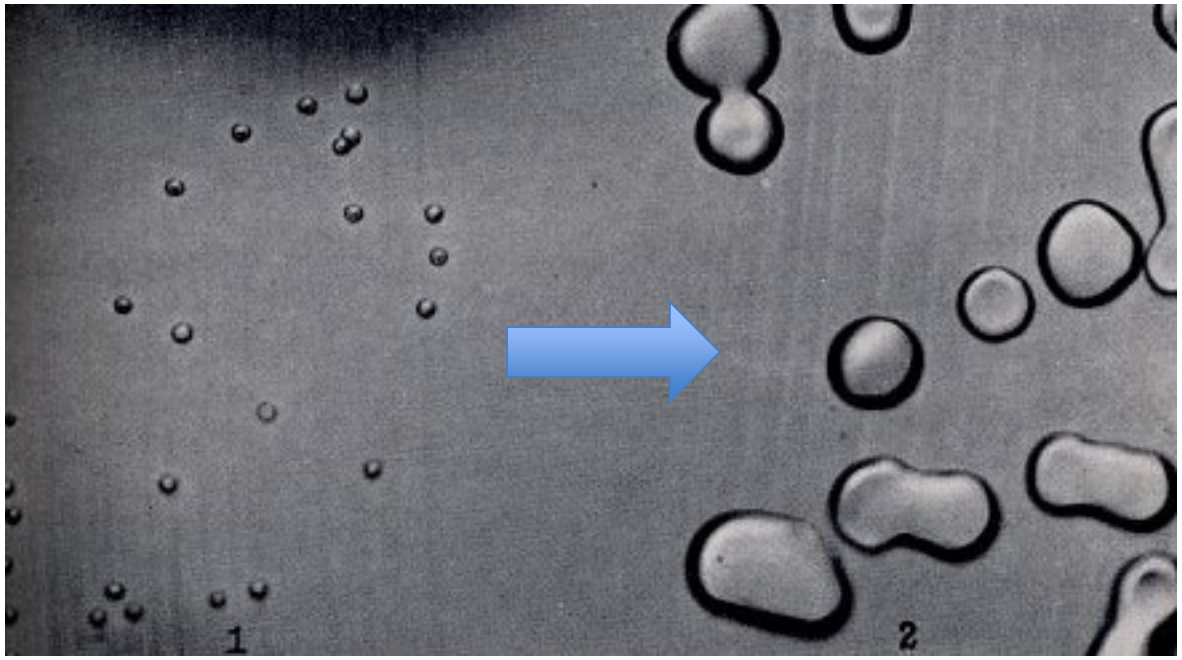


R variant R36A
Pneumococcus Type II

R variant R36A
After addition of “transforming principle”
Pneumococcus Type III S

Transformed R variants gain:

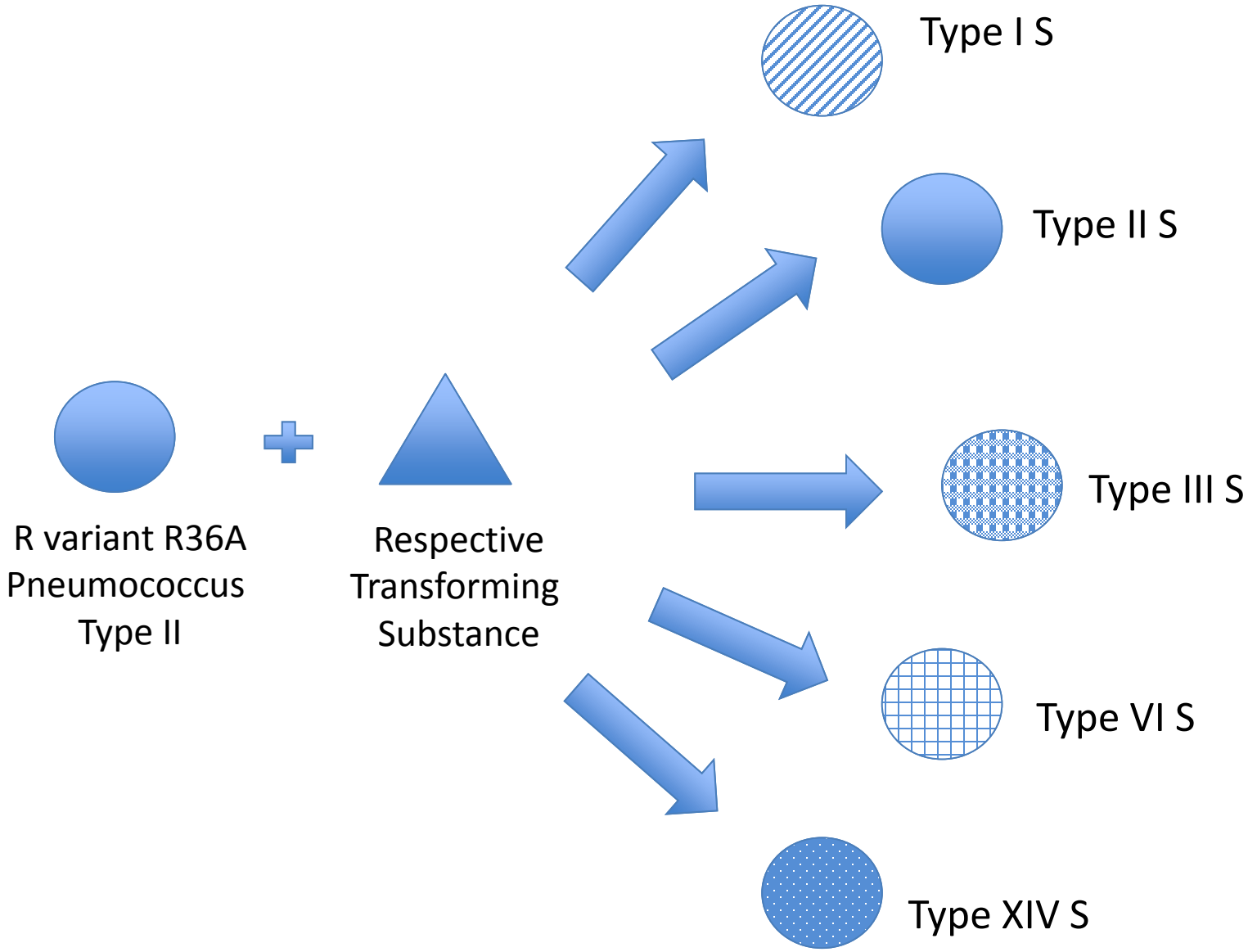
Polysaccharide capsule
Type specificity
Ability to produce infection



R variant R36A
Pneumococcus **Type II**

R variant R36A
After addition of “transforming principle”
Pneumococcus **Type III** S

Spontaneous transformation of R variants back to S variants of same type had been observed, but never transformation to different **type**


Type I S
Type II S
Type III S
Type VI S
Type XIV S
R variant R36A
Pneumococcus
Type II
Respective
Transforming
Substance

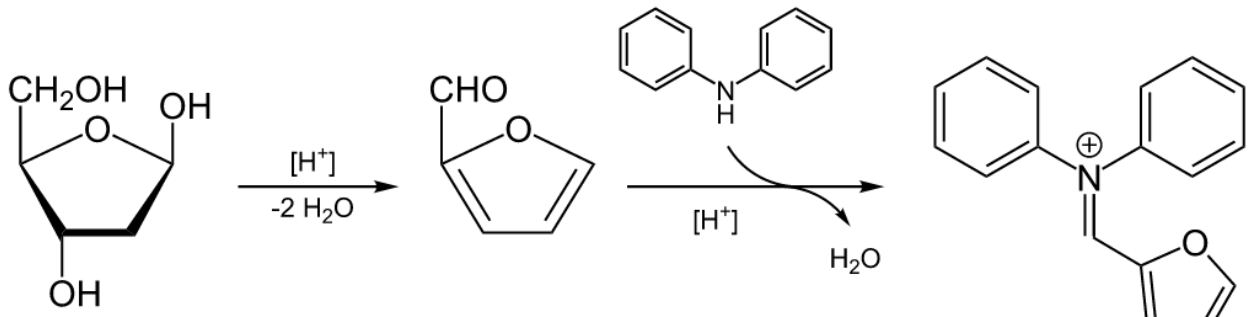
“The crude extract (type III) is full of capsular polysaccharide,” Avery wrote to his brother Roy in 1943, “ C (somatic) carbohydrate, nucleoproteins, free nucleic acids of both the yeast [RNA] and thymus [DNA] types, lipids and other cell constituents.

Try to find in that complex mixture the active principle.. Try to isolate and chemically identify the particular [transforming] substance....

Some job--full of heartaches and heartbreaks. But at last perhaps we have it....”

Clues on Transforming Principle Identity

- Store in salt solution @ 2-4°C for 3 months
- Rapidly loses activity in water
- Inactivated at pH 5 or below
- Dische diphenylamine reaction positive



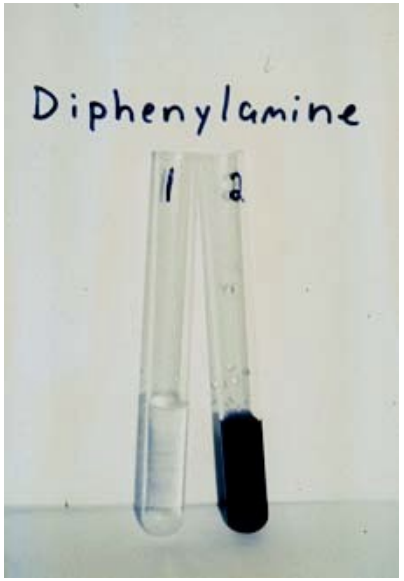


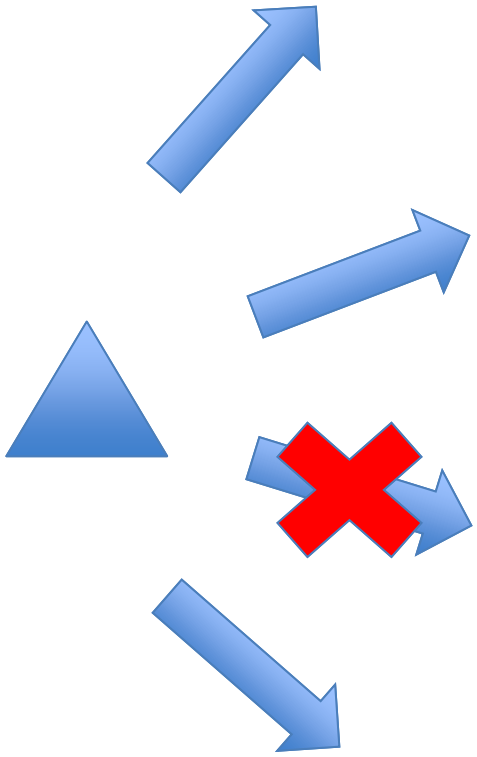
TABLE I

Elementary Chemical Analysis of Purified Preparations of the Transforming Substance

Preparation No.	Carbon	Hydrogen	Nitrogen	Phosphorus	N/P ratio
	per cent	*per cent*	*per cent*	*per cent*	
37	34.27	3.89	14.21	8.57	1.66
38B	—	—	15.93	9.09	1.75
42	35.50	3.76	15.36	9.04	1.69
44	—	—	13.40	8.45	1.58
Theory for sodium desoxyribonucleate.....	34.20	3.21	15.32	9.05	1.69

Combinations that retain transforming principle

Repeated alcohol and ether extraction



Ribonuclease treatment

Enzymes which depolymerize deoxyribonucleic acid

Trypsin and chymotrypsin digestion

TABLE II

The Inactivation of Transforming Principle by Crude Enzyme Preparations

Crude enzyme preparations	Enzymatic activity			
	Phosphatase	Tributyrin esterase	Depolymerase for desoxyribonucleate	Inactivation of transforming principle
Dog intestinal mucosa	+	+	+	+
Rabbit bone phosphatase	+	+	−	−
Swine kidney "	+	−	−	−
Pneumococcus autolysates	−	+	+	+
Normal dog and rabbit serum	+	+	+	+

TABLE III

Differential Heat Inactivation of Enzymes in Dog and Rabbit Serum Which Destroy the Transforming Substance

	Heat treatment of serum	Dilution*	Triplicate tests 1 Diffuse growth	1 Colony form	2 Diffuse growth	2 Colony form	3 Diffuse growth	3 Colony form
Dog serum	Unheated	Undiluted	–	R only	–	R only	–	R only
		1:5	–	R "	–	R "	–	R "
		1:25	–	R "	–	R "	–	R "
	60°C. for 30 min.	Undiluted	+	SIII	+	SIII	+	SIII
		1:5	+	SIII	+	SIII	+	SIII
		1:25	+	SIII	+	SIII	+	SIII
	65°C. for 30 min.	Undiluted	+	SIII	+	SIII	+	SIII
		1:5	+	SIII	+	SIII	+	SIII
		1:25	+	SIII	+	SIII	+	SIII
Rabbit serum	Unheated	Undiluted	–	R only	–	R only	–	R only
		1:5	–	R "	–	R "	–	R "
		1:25	–	R "	–	R "	–	R "
	60°C. for 30 min.	Undiluted	–	R only	–	R only	–	R only
		1:5	–	R "	–	R "	–	R "
		1:25	–	R "	–	R "	–	R "
	65°C. for 30 min.	Undiluted	+	SIII	+	SIII	+	SIII
		1:5	+	SIII	+	SIII	+	SIII
		1:25	+	SIII	+	SIII	+	SIII
Control (no serum)	None	Undiluted	+	SIII	+	SIII	+	SIII
		1:5	+	SIII	+	SIII	+	SIII
		1:25	+	SIII	+	SIII	+	SIII

* Dilution of the digest mixture of serum and transforming substance.

Same conditions deactivate transforming principle and depolymerase

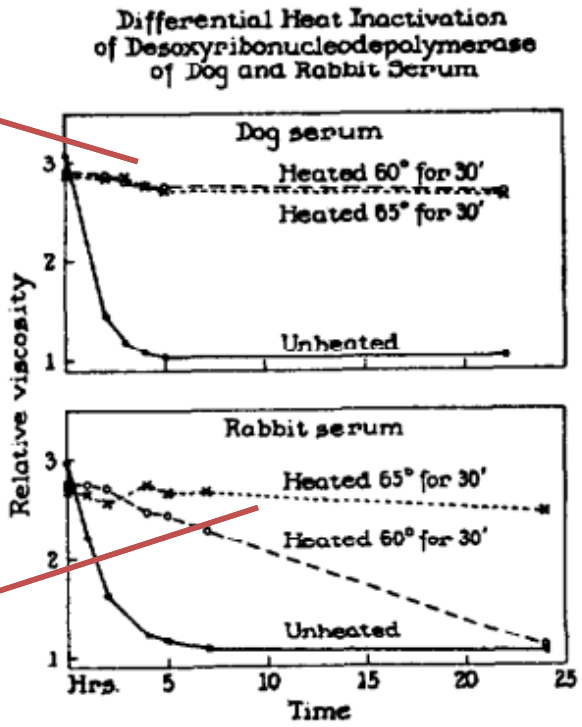


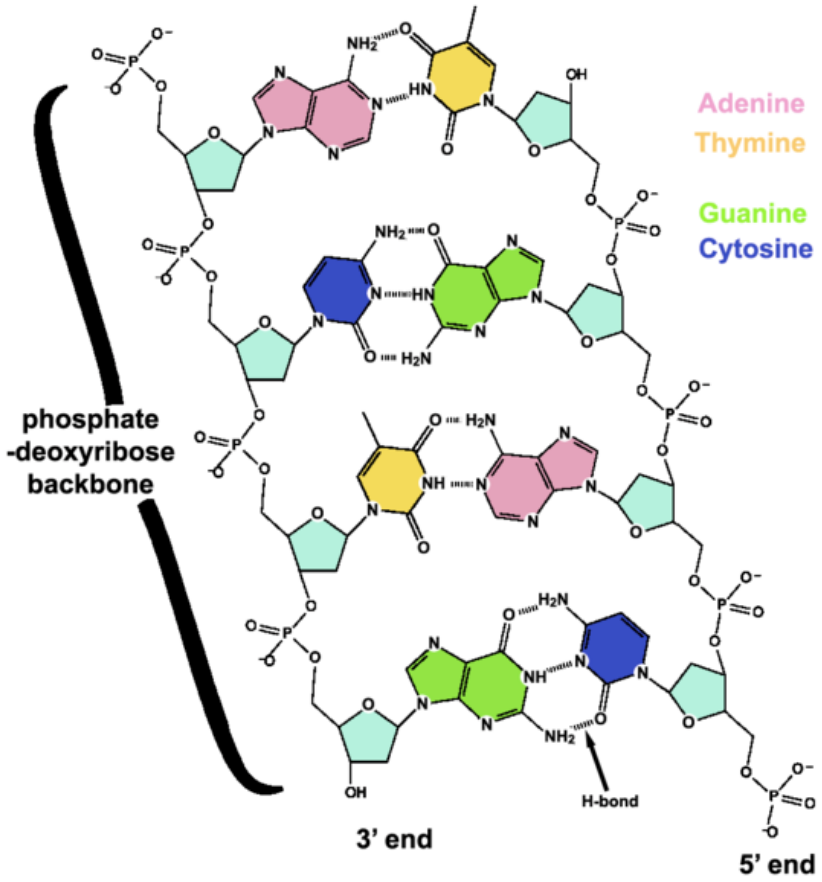
CHART 1

TABLE IV

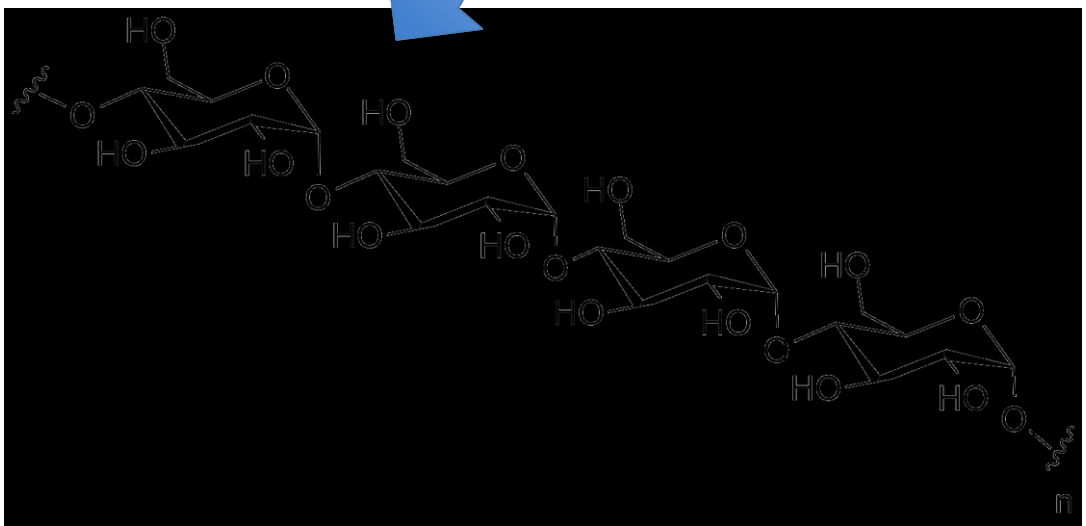
Titration of Transforming Activity of Preparation 44

Transforming principle Preparation 44*		Quadruplicate tests							
		1		2		3		4	
Dilution	Amount added	Diffuse growth	Colony form	Diffuse growth	Colony form	Diffuse growth	Colony form	Diffuse growth	Colony form
	μg.								
10^{-2}	1.0	+	SIII	+	SIII	+	SIII	+	SIII
$10^{-2.5}$	0.3	+	SIII	+	SIII	+	SIII	+	SIII
10^{-3}	0.1	+	SIII	+	SIII	+	SIII	+	SIII
$10^{-3.5}$	0.03	+	SIII	+	SIII	+	SIII	+	SIII
10^{-4}	0.01	+	SIII	+	SIII	+	SIII	+	SIII
$10^{-4.5}$	0.003	−	R only	+	SIII	−	R only	+	SIII
10^{-5}	0.001	−	R "	−	R only	−	R "	−	R only
Control	None	−	R "	−	R "	−	R "	−	R "

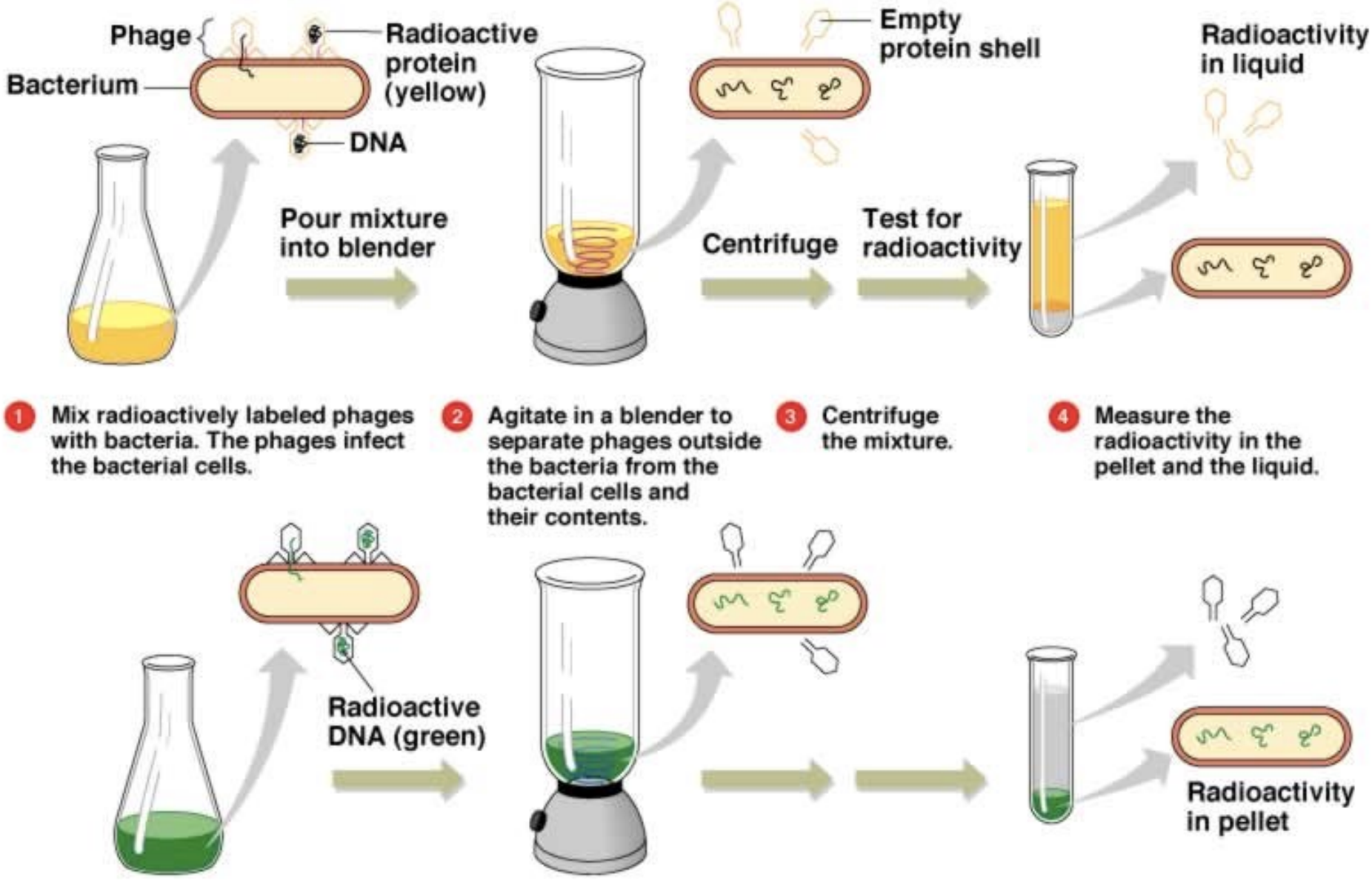
* Solution from which dilutions were made contained 0.5 mg. per cc. of purified material. 0.2 cc. of each dilution added to quadruplicate tubes containing 2.0 cc. of standard serum broth. 0.05 cc. of a 10^{-4} dilution of a blood broth culture of R36A is added to each tube.



"Equally striking is the fact that the substance evoking the reaction and the capsular substance produced in response to it are chemically distinct, each belonging to a wholly different class of chemical compounds."



1952: Hershey-Chase Experiment



MUTATIONS OF BACTERIA FROM VIRUS SENSITIVITY TO VIRUS RESISTANCE[1,2]

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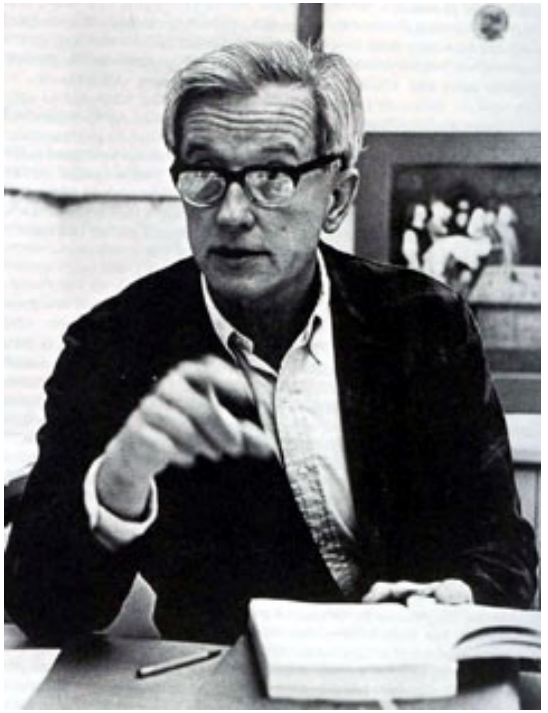
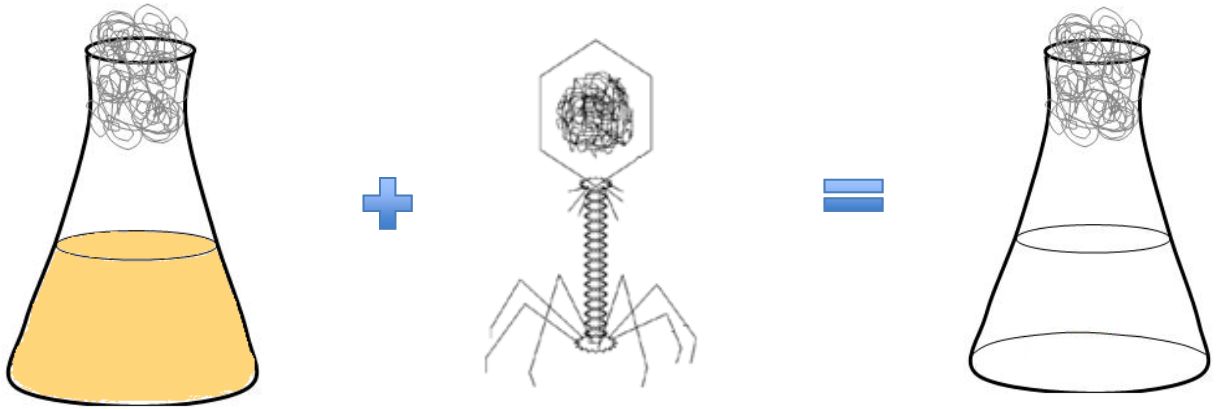
Received May 29, 1943

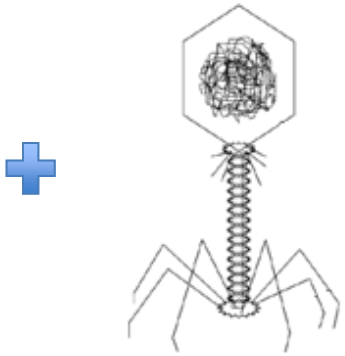
Salvador Luria

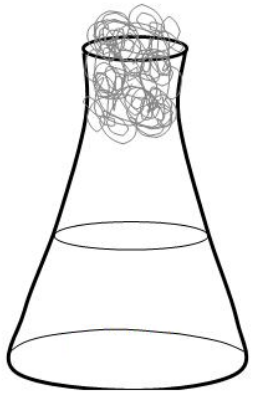


Max Delbrück



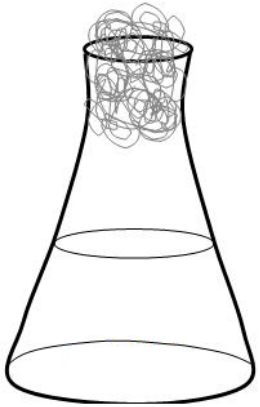
Virus sensitivity

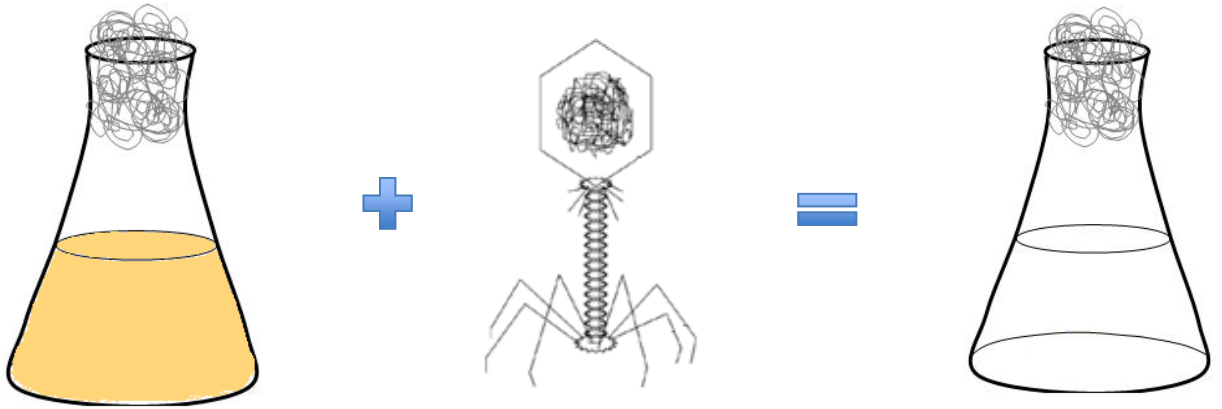




Virus sensitivity

Incubate cleared culture for an hour or two





Virus sensitivity

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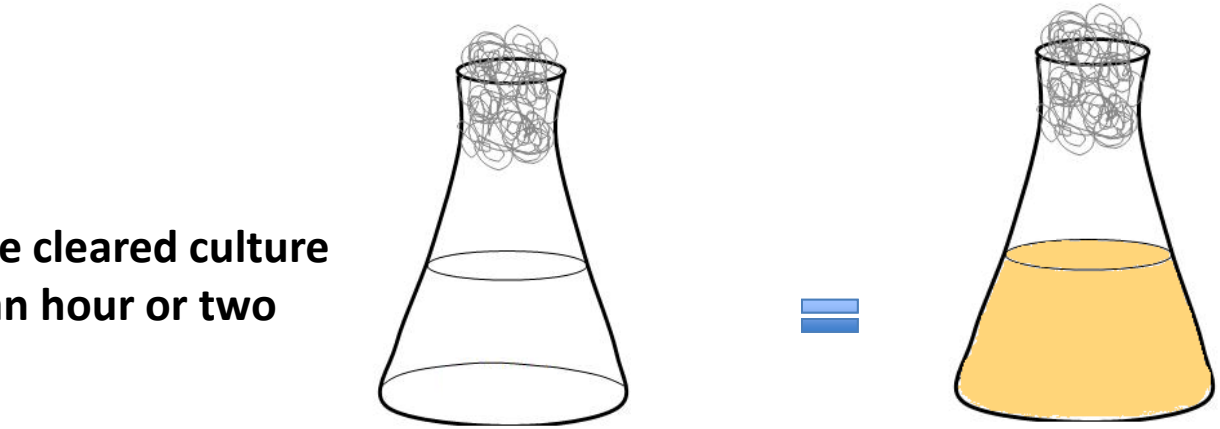
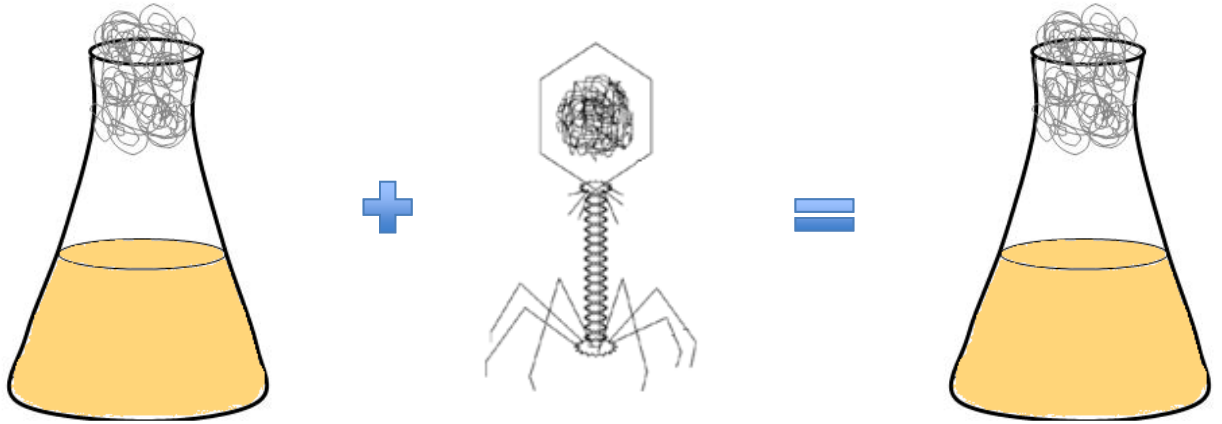




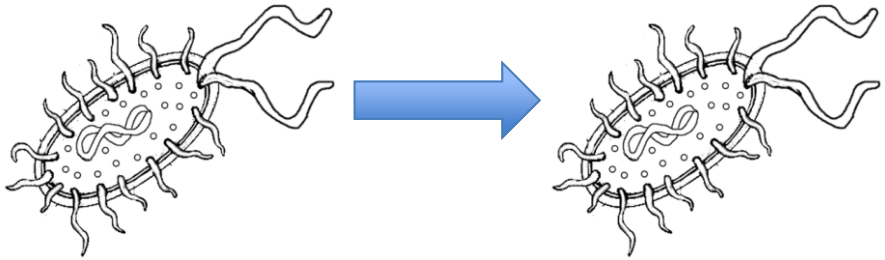
Virus resistance

Theories on resistance

- D'Herelle (1926) – Virus induced resistant variants by direct action

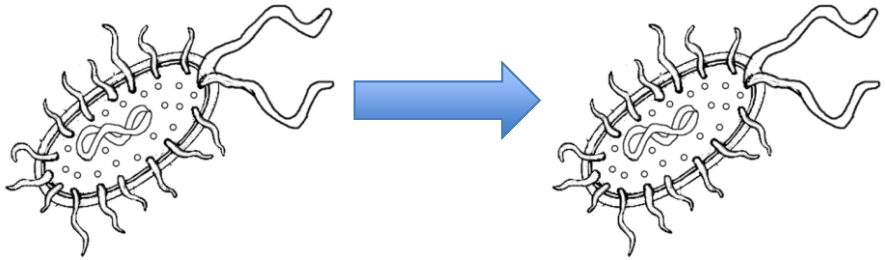
- Gratia (1921) & Burnet (1929) – Resistant variants produced by mutation in culture prior to virus addition

A. Hypothesis of mutation to immunity



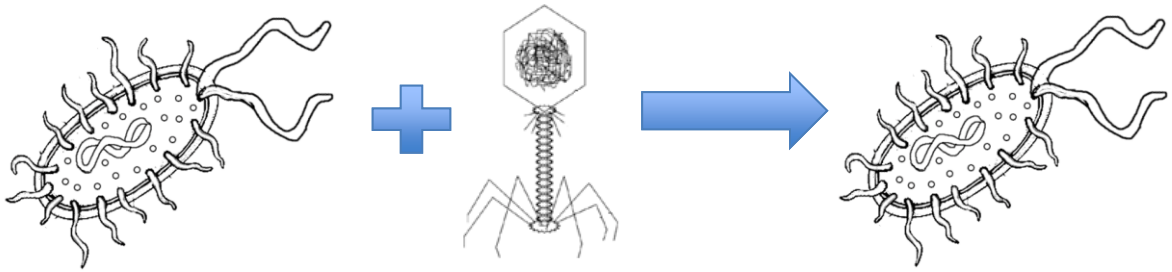
- Mutation occurs independent of phage
- Never interacts with phage

A. Hypothesis of mutation to immunity



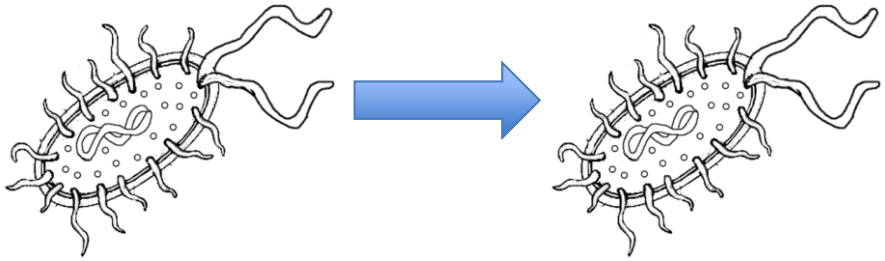
- Mutation occurs independent of phage
- Never interacts with phage

B1. Hypothesis of acquired immunity of hereditarily predisposed individuals



- Mutation occurs independent of phage
- Interact w/ phage but survive

A. Hypothesis of mutation to immunity



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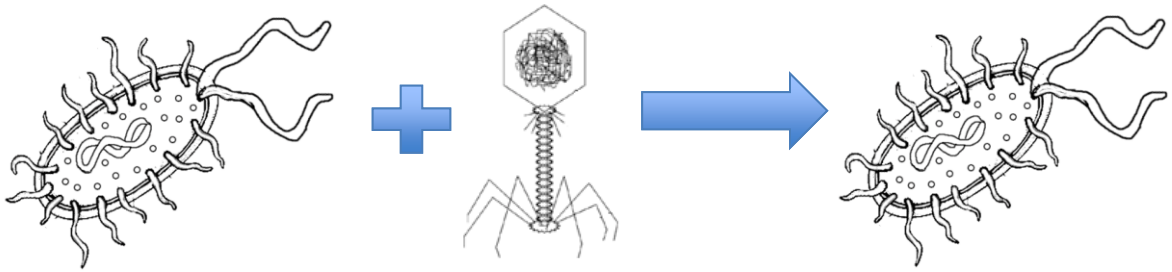
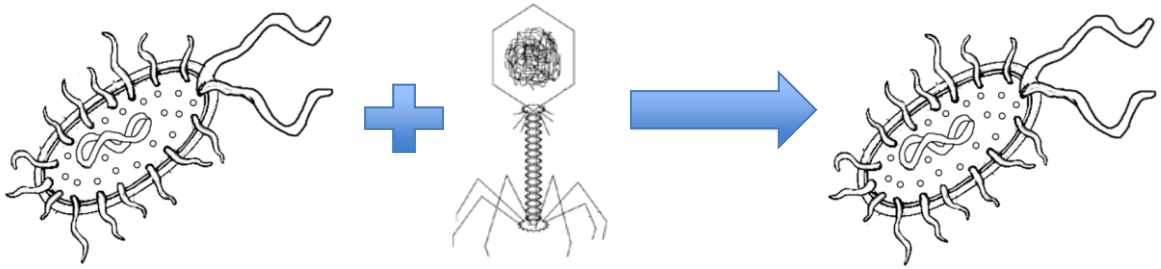


- Mutation occurs independent of phage
- Interact w/ phage but survive

B2. Hypothesis of acquired immunity – hereditary after infection



- Predisposed to survive due to random physiological variation
- Interact w/ phage but survive
- Offspring hereditarily immune

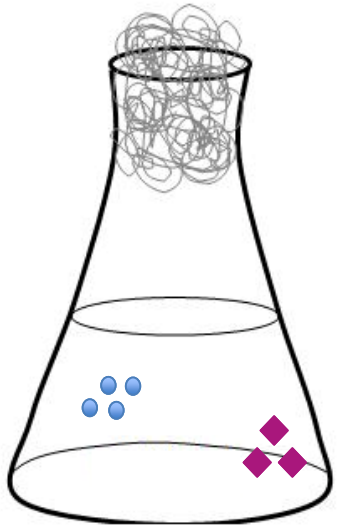
1. **First Hypothesis (mutation)**

   Finite Probability to mutate from “sensitive” to “resistant”

   All offspring will be resistant

   Survivors will be clones of resistant bacteria of various sizes

   % resistant increases over time – new mutations appear



All offspring resistant

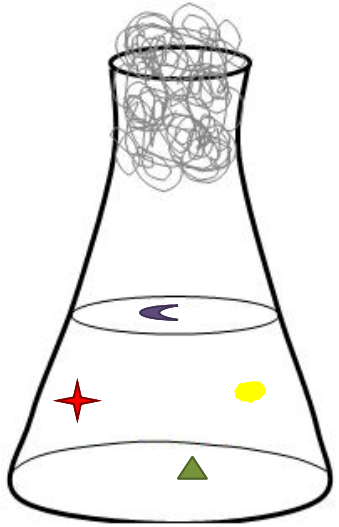
2. **Second Hypothesis (acquired hereditary immunity)**

   Finite Probability for any to survive viral attack

   Survival confers immunity & offspring will be resistant

   Survivors will be random

   % resistant should be static

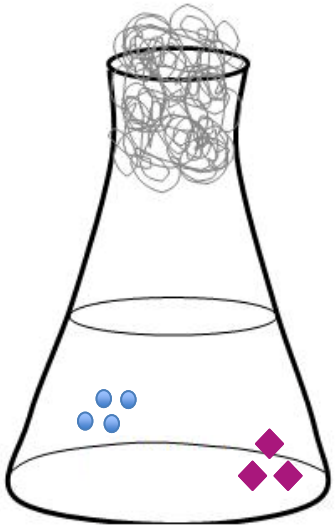


Offspring resistant after phage encounter

Mutation hypothesis

Distribution of resistant bacteria has long tail of high numbers of resistant bacteria

Predicts variance larger than average

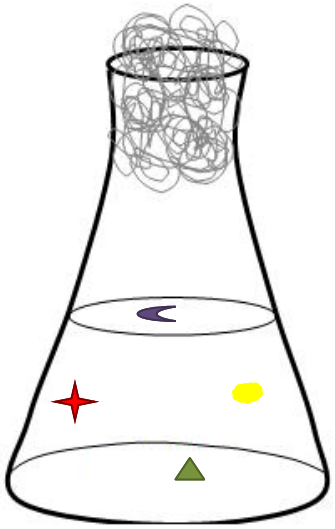


All offspring resistant

Acquired hypothesis

Random, so described by Poisson's law

Predicts variance equal to average



Offspring resistant after phage encounter

number of resistant bacteria

number of bacteria at the time of observation

$$r = aN_t \ln (N_t Ca).$$

mutation rate

group of C similar cultures

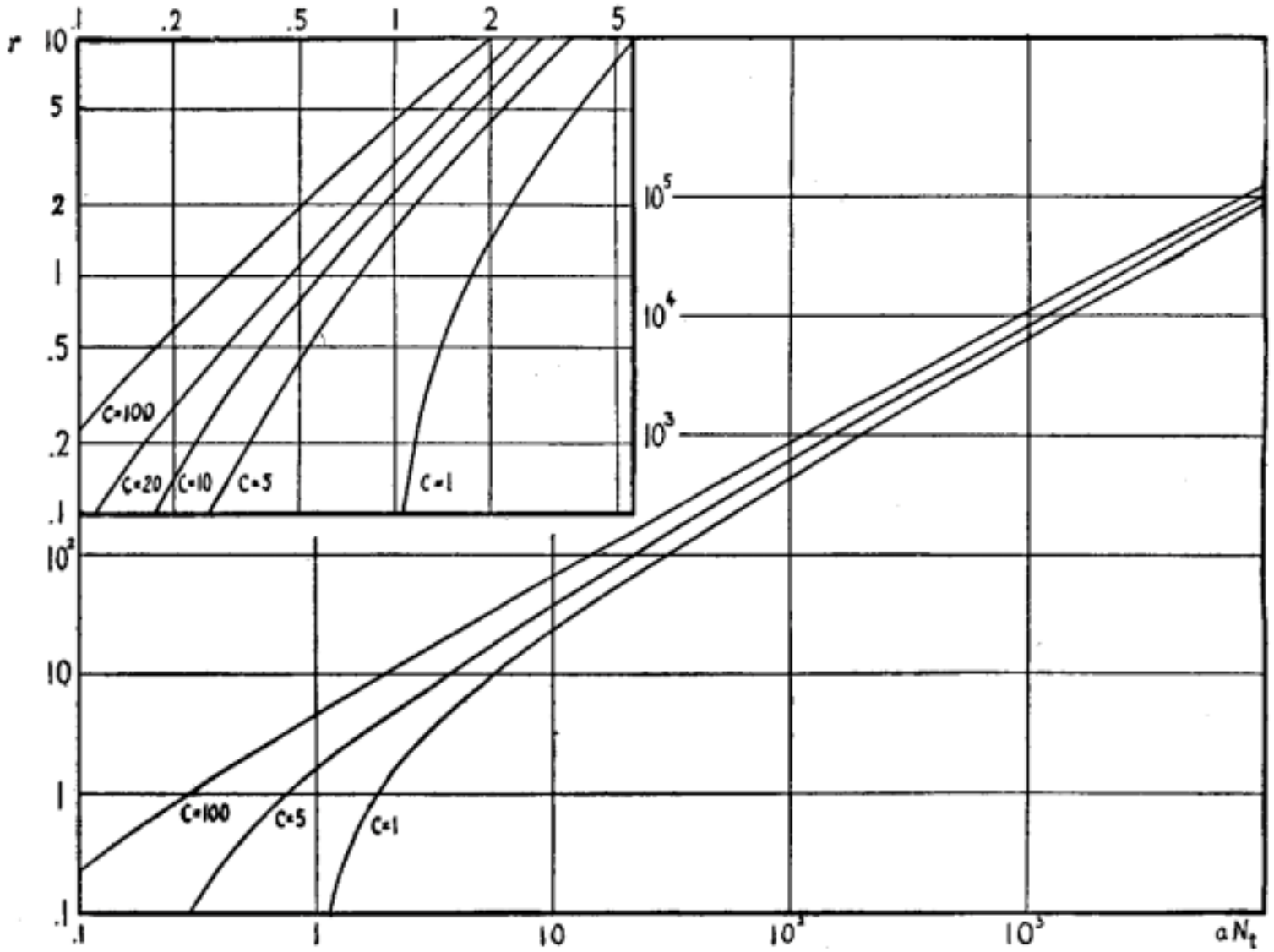


TABLE 1

The number of resistant bacteria in different samples from the same culture.

SAMPLE NO.	EXP. NO. 10a RESISTANT COLONIES	EXP. NO. 11a RESISTANT COLONIES	EXP. NO. 3 RESISTANT COLONIES
1	14	46	4
2	15	56	2
3	13	52	2
4	21	48	1
5	15	65	5
6	14	44	2
7	26	49	4
8	16	51	2
9	20	56	4
10	13	47	7
mean	16.7	51.4	3.3
variance	15	27	3.8
χ^2	9	5.3	12
P	.4	.8	.2

TABLE 2

The number of resistant bacteria in series of similar cultures.

EXPERIMENT NO.	1	10	11	15	16	17	21a	21b
Number of cultures	9	8	10	10	20	12	19	5
Volume of cultures, cc	10.0	10.0	10.0	10.0	.2*	.2*	.2	10.0
Volume of samples, cc	.05	.05	.05	.05	.08	.08	.05	.05
Culture No.								
1	10	29	30	6	1	1	0	38
2	18	41	10	5	0	0	0	28
3	125	17	40	10	3	0	0	35
4	10	20	45	8	0	7	0	107
5	14	31	183	24	0	0	8	13
6	27	30	12	13	5	303	1	
7	3	7	173	165	0	0	0	
8	17	17	23	15	5	0	1	
9	17		57	6	0	3	0	
10			51	10	6	48	15	
11					107	1	0	
12					0	4	0	
13					0		19	
14					0		0	
15					1		0	
16					0		17	
17					0		11	
18					64		0	
19					0		0	
20					35			
Average per sample	26.8	23.8	62	26.2	11.35	30	3.8	48.2
Variance (corrected for sampling)	1217	84	3498	2178	694	6620	40.8	1171
Average per culture	5360	4760	12400	5240	28.4	75	15.1	8440
Bacteria per culture	3.4×10^{10}	4×10^{10}	4×10^{10}	2.9×10^{10}	5.6×10^{8}	5×10^{8}	1.1×10^{8}	3.2×10^{10}
Mutation rate	1.8×10^{-8}	1.4×10^{-8}	4.1×10^{-8}	2.1×10^{-8}	1.1×10^{-6}	3.0×10^{-8}	3.3×10^{-8}	3.0×10^{-8}
Standard deviation / Average (exp.)	1.3	.39	.95	1.8	2.3	2.7	1.7	.71
Standard deviation / Average (calc.)	.35	.33	.33	.37	.94	.67	1.04	.26

* Cultures in synthetic medium.

Experimental values larger than calculated b/c mutations can occur before time 0

$$m = a(N_t - N_0)$$

m = # of mutations
a = mutation rate

TABLE 4

Values of mutation rate from different experiments.

EXPERIMENT NO.	NUMBER OF CULTURES	VOLUME OF CULTURES	MUTATION RATE
		cc	*Mutations per bacterium per time unit*
1	9	10.0	1.8×10^{-8}
10	8	10.0	1.4×10^{-8}
11	10	10.0	4.1×10^{-8}
15	10	10.0	2.1×10^{-8}
16	20	.2*	1.1×10^{-8}
17	12	.2*	3.0×10^{-8}
21a	19	.2	3.3×10^{-8}
21b	5	10.0	3.0×10^{-8}
22	100	.2*	2.3×10^{-8}
23	87	.2*	2.4×10^{-8}
Average			2.45×10^{-8}

* Cultures in synthetic medium.