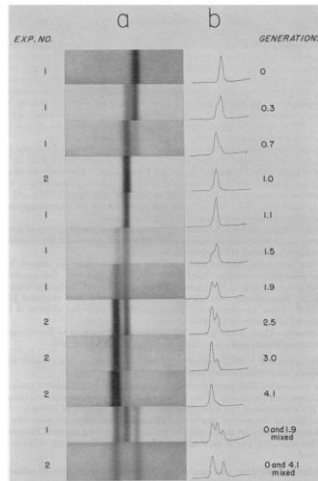# Semi-conservative replication of DNA - The experiment of Meselson & Stahl

"Timing, hard work & Serendipity"

1958

2

INTRODUCTION - BIOCHEMISTRY
THE PRECURSORS

3

The Structure of the Hereditary Material

An account of the investigations which have led to the formulation of an understandable structure for DNA. The chemical reactions of this material within the nucleus govern the process of reproduction
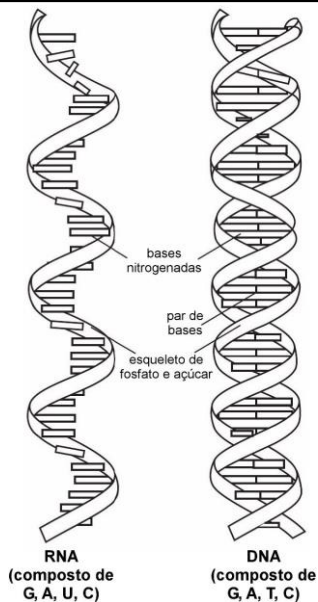
by F. H. C. Crick

If we knew the monomers from which nature makes DNA, RNA and protein, we might be able to carry out very spectacular experiments in the test tube. Be that as it may, we now have for the first time a well-defined model for DNA and for a possible replication process, and this in itself should make it easier to devise crucial experiments.

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4

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)

RNA (composto de G, A, U, C)
DNA (composto de G, A, T, C)
bases nitrogenadas
par de bases
esqueleto de fosfato e açúcar

- 1909 the word gene is introduced by Johannsen (1857-1927)

-1941 Beadle (1903-1989) & Tatum (1909-1975) -> 1 gene:1enzyme.
-> Nobel prize 1958

-1944 Oswald Avery (1877-1955) – DNA is the genetic material -> Never received a Nobel prize.

-1952 Hershey (1908-1997) & Chase (1927-2003) – Hershey Nobel 1969

-1953 Watson (1928-) & Crick (1916-2004) – model of DNA double helix -> Nobel prize 1962

-1958 Crick – "Central Dogma"

5

The central dogma according to Francis Crick

"... once 'information' has passed into protein *it cannot get out again*."

6

The central dogma: an oversimplification?

"The central dogma, enunciated by Crick in 1958 and the keystone of molecular biology ever since, is likely to prove a considerable over-simplification."

NATURE VOL. 227 AUGUST 8 1970   561

Central Dogma of Molecular Biology

by
FRANCIS CRICK
MRC Laboratory of Molecular Biology,
Hills Road,
Cambridge CB2 2QH

The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred from protein to either protein or nucleic acid.

"The central dogma, enunciated by Crick in 1958 and the keystone of molecular biology ever since, is likely to prove a considerable over-simplification."

THIS quotation is taken from the beginning of an unsigned article[1] headed "Central dogma reversed", recounting the very important work of Dr Howard Temin[2] and others[3] showing that an RNA tumour virus can use viral RNA as a template for DNA synthesis.
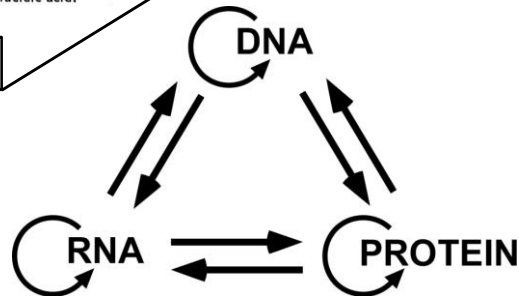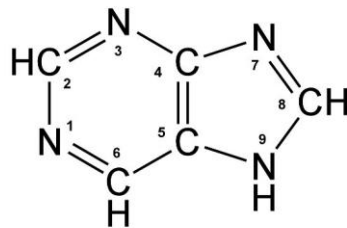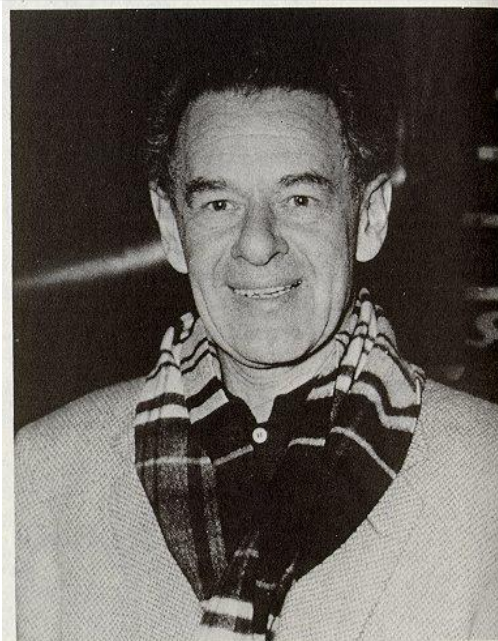

7

Bases that make up the nucleic acids
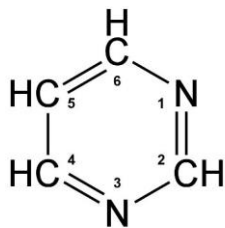
PYRIMIDINE   PURINE

Numbering of atoms in the nitrogenous bases

8

- 1953 -
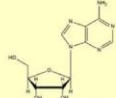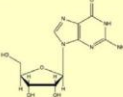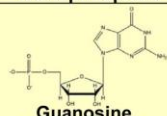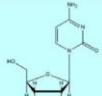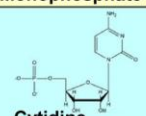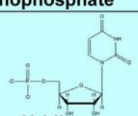
**Fritz Lipmann
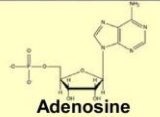(1899-1986)**

NPW 1953

9

Adenosine Triphosphate (ATP)
(Lipmann, 1937)

10

A nucleoside

ADENOSINE

11

RNA Mononucleotide components

	BASE	NUCLEOSIDE	NUCLEOTIDE
PURINES	Adenine	Adenosine	Adenosine Monophosphate
PURINES	Guanine	Guanosine	Guanosine Monophosphate
PYRIMIDINES	Cytosine	Cytidine	Cytidine Monophosphate
PYRIMIDINES	Uracil	Uridine	Uridine Monophosphate

12

RNA and some nucleotides

RIBOTHYMIDILIC ACID

RNA
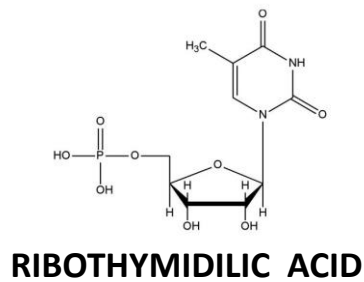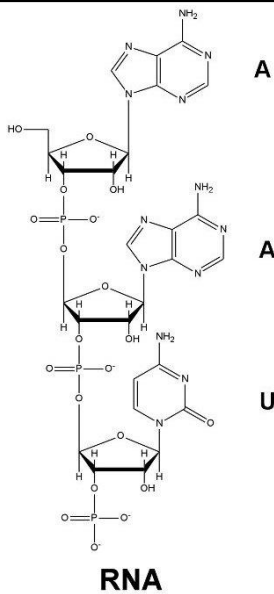
13

RNA and some nucleotides

A

A

U
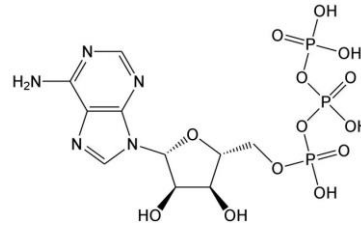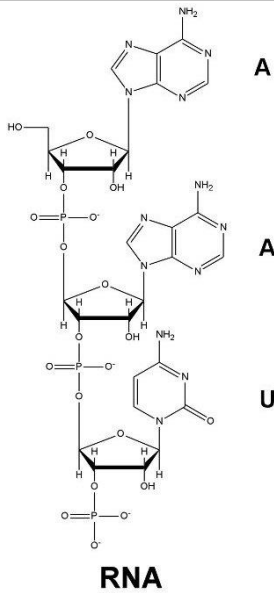
ATP (adenosine triphosphate)

RNA

14

RNA and some nucleotides

A

A

U
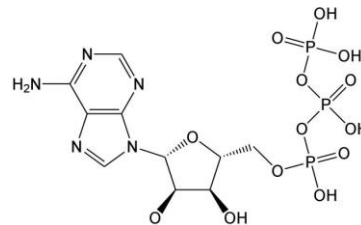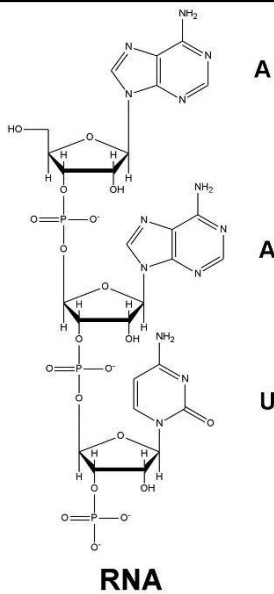
dATP (2′-deoxyadenosine triphosphate)

RNA

15

RNA and some nucleotides
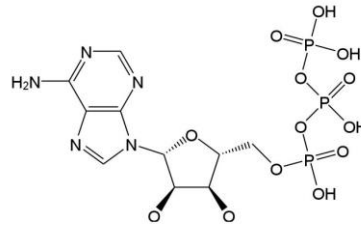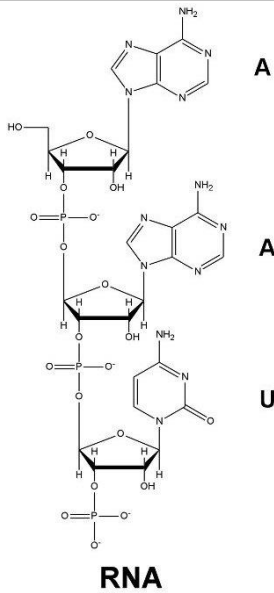
ddATP (dideoxyadenosine triphosphate)

RNA

16

Polyribonucleotides are degraded by alkali
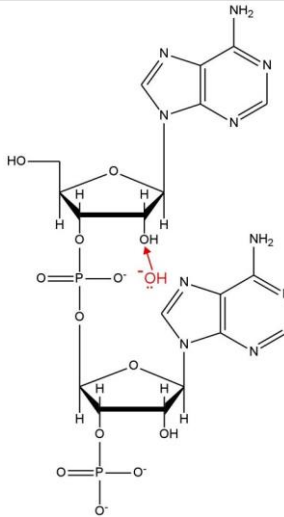
17

Polyribonucleotides are degraded by alkali
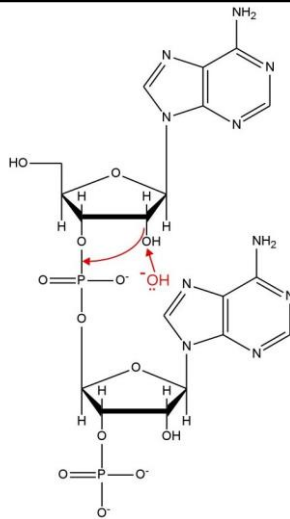
18

Polyribonucleotides are degraded by alkali
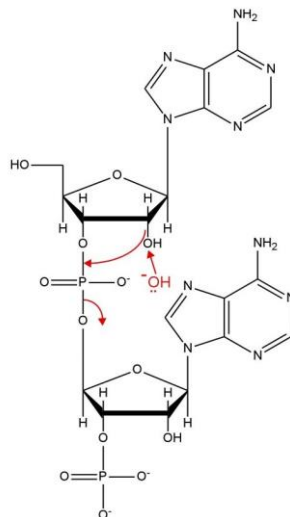
19

Polyribonucleotides are degraded by alkali
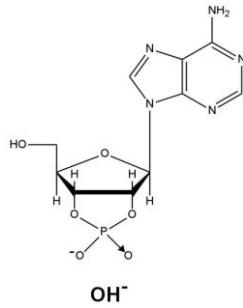
20

Polyribonucleotides are degraded by alkali
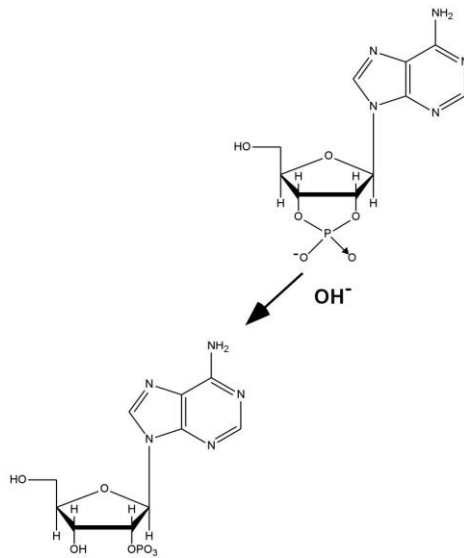
21

Polyribonucleotides are degraded by alkali

22
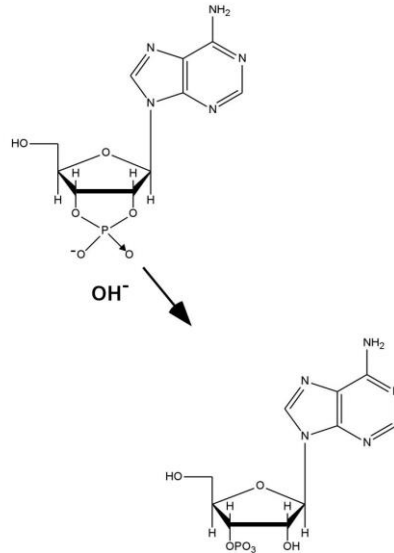
EXPERIENTIA

Chemical Specificity of Nucleic Acids and Mechanism
of their Enzymatic Degradation[1]
By ERWIN CHARGAFF[2], New York, N.Y.

Erwin Chargaff
(1905-2002)

$A + G = T + C$

or

$$\frac{A + G}{T + C} = 1$$

$A = T$

$G = C$
thus,

$A + C = G + T$

23

Chargaff 1950

Table IV [5]

Composition of two microbial desoxyribonucleic acids.

Constituent	Yeast		Avian tubercle bacilli
	Prep. 1	Prep. 2	
Adenine	0·24	0·30	0·12
Guanine	0·14	0·18	0·28
Cytosine	0·13	0·15	0·26
Thymine	0·25	0·29	0·11
Recovery	0·76	0·92	0·77

Table III [2]

Composition of desoxypentose nucleic acid of man (in moles of nitrogenous constituent per mole of P).

| Constituent	Sperm		Thymus	Liver	
	Prep. 1	Prep. 2		Normal	Carcinoma
Adenine . . .	0·29	0·27	0·28	0·27	0·27
Guanine . . .	0·18	0·17	0·19	0·19	0·18
Cytosine . . .	0·18	0·18	0·16		0·15
Thymine . . .	0·31	0·30	0·28		0·27
Recovery . . .	0·96	0·92	0·91		0·87

Chargaff E (1950) *Experientia* **6:**201-240

24

---

most students no longer study nature; they test models.

Erwin Chargaff

One of the obnoxious dogmas to which it has given rise - the so-called Central Dogma: DNA makes RNA; RNA makes proteins
-is no longer valid. (I had never accepted it, as shown in
lectures I gave in 1957 in Moscow and in 1958 in Vienna)
But the fact that dogmas could be handed down from the
mountains shows that science had changed disastrously.

Erwin Chargaff

25

I have a vivid recollection of a 1956 discussion with two distinguished biochemists, X and Y, about the significance of the Watson-Crick structure of DNA. I was not making much of an impression. Finally I asked X, ..."Do you believe that the Watson-Crick structure is essentially correct?" The amazing answer: "Yes, I think it is correct, but I don't think it has anything to do with replication."

George Beadle, 1966

26

How DNA duplicates, according to Max Delbrück

ON THE REPLICATION OF DESOXYRIBONUCLEIC ACID (DNA)

BY M. DELBRÜCK

KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated May 18, 1954

The discoveries of Hershey and Chase[1] concerning the role of DNA in transmitting genetic information in phage and of Watson and Crick[2] concerning the structure of DNA have brought the problem of the replication of DNA into focus.   The structure proposed by Watson and Crick consists of two polynucleotide chains wound helically around a common axis, tied together by hydrogen bonds between the

Delbrück M (1954) Proc. *Natl. Acad. Sci. USA* **40:**783-787

27

How DNA duplicates, according to Max Delbrück
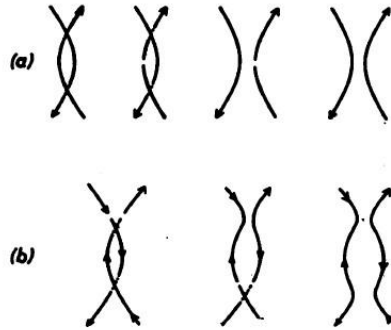
FIG. 2.—Two methods of resolving an interlock between two chains: *a*, By breaking one chain and slipping the other through the gap. *b*, By breaking both chains at each overlap and rejoining them crisscross. *Two pairs of breaks* are needed to resolve one interlock.

28

How DNA duplicates, according to Max Delbrück
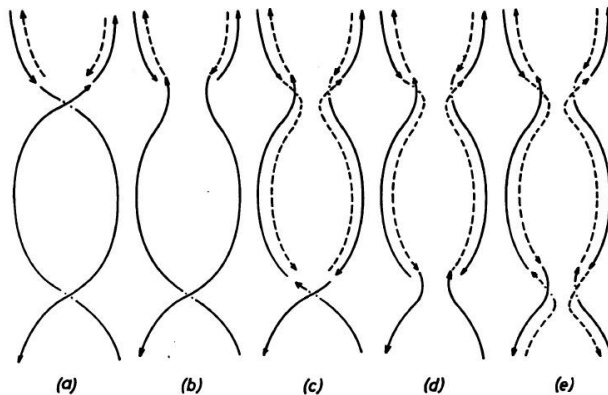
FIG. 3.—Resolution of an interlock in a replicating duplex by breaking both old chains at each half-turn of the helix and rejoining the lower terminals of the breaks to the open ends of equal polarity of the new chains. Lateral view. *a*, Location of first pair of breaks. *b*, Rejoining of lower terminals of breaks. *c*, Location of second pair of breaks. *d*, Rejoining of lower terminals.

Parental chains are represented by solid lines; new chains, by dashed lines. At the overlaps the lower chains are dotted.

29

How DNA duplicates, according to Max Delbrück

"If a labeled duplex replicates repeatedly at the expense of an unlabeled pool, then according to this model, the label will be statistically equally distributed to the daughter-duplexes at each sucessive replication."

30

How DNA duplicates, according to Max Delbrück

(a)

(b)

"Distribution of labeled parental chains to daughter-duplexes, with breaks and rejoins as postulated in the theory here presented"

31

Precursor (7 months before M&S !!!)

THE ORGANIZATION AND DUPLICATION OF CHROMOSOMES AS REVEALED BY AUTORADIOGRAPHIC STUDIES USING TRITIUM-LABELED THYMIDINE

BY J. HERBERT TAYLOR,* PHILIP S. WOODS, AND WALTER L. HUGHES

DEPARTMENT OF BOTANY, COLUMBIA UNIVERSITY; BIOLOGY DEPARTMENT AND MEDICAL DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY

Communicated by Franz Schrader, October 26, 1956

32

Precursor (7 months before M&S !!!)

| Duplication with labeled thymidine | 1st c-metaphase after labeling | Duplication without labeled thymidine | 2nd c-metaphase after labeling |
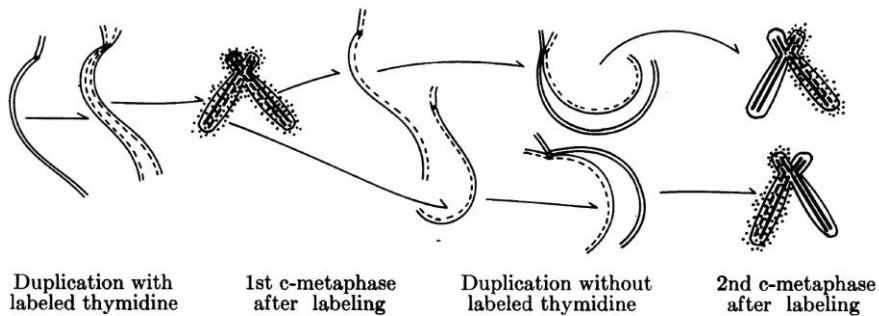
FIG. 3.—Diagrammatic representation of proposed organization and mode of replication which would produce the result seen in the autoradiographs. The two units necessary to explain the results are shown, although these were not resolved by microscopic examination. Solid lines represent nonlabeled units, while those in dashed lines are labeled. The dots represent grains in the autoradiographs.

It is immediately apparent that this pattern of replication is analogous to the replicating scheme proposed for DNA by Watson and Crick.[6] We cannot be sure,

33

16

Precursor (7 months before M&S !!!)

Summary.—Tritium-labeled thymidine was prepared and used for labeling chromosomes during their duplication. Analysis of autoradiographs showed that both daughter chromosomes resulting from duplication in the presence of labeled thymidine appeared equally and uniformly labeled. After an ensuing duplication in the absence of the labeled DNA precursor, the label appeared in only one of each two chromatids (daughter chromosomes). These findings indicate that DNA is synthesized as a unit which extends throughout the length of the chromosome. The units remain intact through succeeding replications and nuclear divisions, except for occasional chromatid exchanges. Each chromosome is composed of two such units, probably complementary to each other. After each replication the four resulting units separate, so that each daughter chromosome always contains an "original" and a "new" unit. To explain the results, a model with two complementary units and a scheme of replication analogous to the Watson-Crick model of DNA is proposed.

34

# THE CHARACTERS
AND
THE PROBLEM

35

Matthew Meselson
Born: 24 de maio de 1930, em Denver, CO
PhD Advisor (1957): Linus Pauling
PhD at: California Institute of Technology (CalTech)
PhD thesis on: (1) Ultracentrifugation; (2) Crystalography

Franklin W. Stahl
Born: 8 de outubro de 1929 em Boston, MA
PhD Advisor (1956): August H. Doermann
PhD at: University of Rochester
PhD thesis on: Genetics of Bacteriophage T4

36

https://ibiology.org

https://youtu.be/V2evjmkur7k?si=ISj7865Wvc9E9RSE

Meselson & Stahl interview -> https://youtu.be/7-tnuAqEp9g?si=A1p9If3TODSBwAOt

Semi-conservative replication cartoon -> https://youtu.be/8jPK3S9S8rg?si=VWUB5R96kUgTLLgv

DNA replication – 3D animation -> https://youtu.be/UpNZws-G8HQ?si=UZlnpXUqbdOYdBmI

Stephen Bell – Replication fork -> https://youtu.be/TweBOe3DlfY?si=hMVd-DCUMEgR3OO7

37

Matt Meselon and Frank Stahl in 1996 under the tree in Woods Hole where they decided the famous experiment 42 years earlier
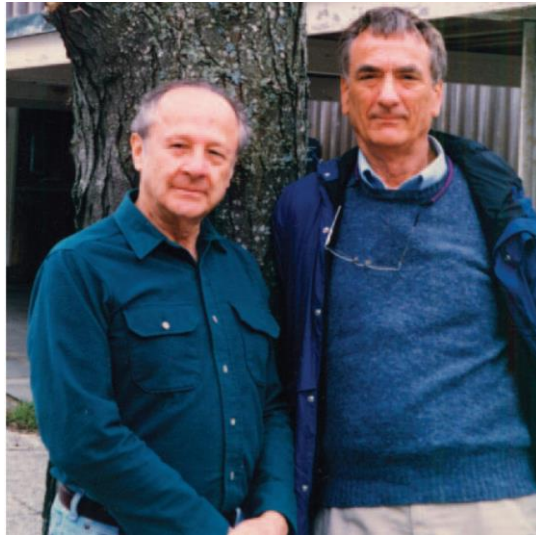
Hanawalt, PNAS **101:**17889-17894, 2004

38

THEORETICAL
AND
METHODOLOGICAL BASES

39

The problem of macromolecule transport

DIFFUSION is the transport of matter in a mixture caused by concentration gradients

STOKES-EINSTEIN-SUTHERLAND EQUATION (1905)

$$D = \frac{k_B T}{6\pi\,\eta\,r}$$

D = diffusion coeficient
k_B = Boltzman constant
T = absolute temperature
η = Dynamic viscosity
r = radius of the spherical particle

Constante dos Gases (R)
Número de Avogadro (N)

SEDIMENTATION is the transport of matter in a mixture due to a external field, in particular due to gravity force or to centrifugal force.

40

THEODOR SVEDBERG [1884-1971]

- When 15 years old constructs a Marconi transmitter
- When 16 years old begins the studies at the University of Uppsala
- First paper published when he was 17 years old
- PhD at age 19

Chemistry Nobel Prize in 1926, age 42

Main interest: colloidal solutions

Helped the foundation of LKB that later fused with Pharmacia and today is part of GE Lifesciences

Married four times, had 12 children and published 229 papers (the last one when aged 81) and 13 books!

41

THE ULTRA-CENTRIFUGE, A NEW INSTRUMENT FOR THE DETERMINATION OF SIZE AND DISTRIBUTION OF SIZE OF PARTICLE IN AMICROSCOPIC COLLOIDS

BY THE SVEDBERG AND HERMAN RINDE

"The new centrifuge constructed by us allows the determination of particles that cannot be made visible in the ultramicroscope. In analogy with the naming of the ultra-microscope and ultra-filtration apparatus **we propose the name ultra-centrifuge** for this apparatus."

42

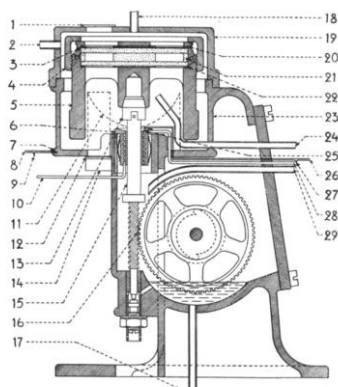# Svedberg's "ultracentrifuge" attained a maximum of 5000 *g*

Fig. 1.
1, Upper window; 2, Hydrogen outlet; 3, Ebonite plate; 4, Lid of rotor; 5, Rotor; 6, Water-cooled spring bearing; 7–9, Thermocouple; 10, Water outlet; 11, Copper screen; 12, Lower window; 13, Carrying cone; 14, Reflecting prism; 15, Shaft of rotor; 16, Toothed wheel; 17, Oil outlet; 18, Hydrogen inlet; 19, Lid; 20, Rubber plate; 21, Cell; 22, Rubber plate; 23, Casing of centrifuge; 24, Hydrogen inlet; 25–27, Thermocouple; 28, Water inlet; 29, Oil inlet.
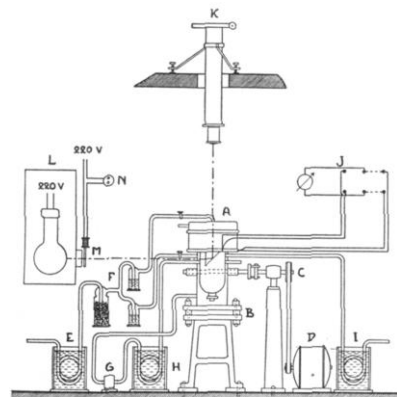
Fig. 2.
A, Centrifuge; B, Stand; C, Pulley; D, Motor; E, Cooling spiral for hydrogen current; F, Valves; G, Oil circulation pump; H, Cooling spiral for oil current; I, Cooling spiral for water current; J, Galvanometer and key; K, Camera; L, Lamp; M, Shutter; N, Switch for shutter.

43

A NEW METHOD FOR THE DETERMINATION OF THE MOLECULAR WEIGHT OF THE PROTEINS

By THE SVEDBERG AND ROBIN FÅHRAEUS

44

The Svedberg equation

$$M = \frac{RT}{D\,(1 - \bar{v}\rho)}\ \frac{dr/dt}{\omega^2 r}$$

M = molecular mass

D = diffusion coefficient

R = gas constant ($8{,}3143 \times 10^7$ ergs/grau mol)

T = absolute temperature (in K)

r = distance from the rotation center to the center of the band

ω = angular velocity

ρ = solvent density

v = protein partial specific volume

45

The Svedberg equation

$$\frac{dr/dt}{\omega^2 r} = s = \text{sedimentation constant}$$

$$M = \frac{RTs}{D\,(1 - \bar{\nu}\rho)}$$

s is measured in reciprocal seconds

s basic unit 10^{-13} s, is called "*one Svedberg (S)*"

46

Analytical Ultracentrifuge Beckman/Spinco –
(Scientific American, 1951)

SPINCO = **Sp**ecialized **In**struments **Co**rp.

47

Pannel of an analytical ultracentrifuge in the 1950s

ROTOR

48

SPINCO Model E Analytical Ultracentrifuge

49

4/15/2024

The rotor and its parts

Gray, Scient. Amer. **184(6):**42-51, 1951

50

The rotor and its parts

approx. 18 cm

ROTOR
CENTER

ROTOR
EDGE

Analytical ultracentrifuge
rotor

Cell where the sample is applied

Gray, Scient. Amer. **184(6):**42-51, 1951

51

Observing the behavior of two proteins

ROTOR CENTER

TOP
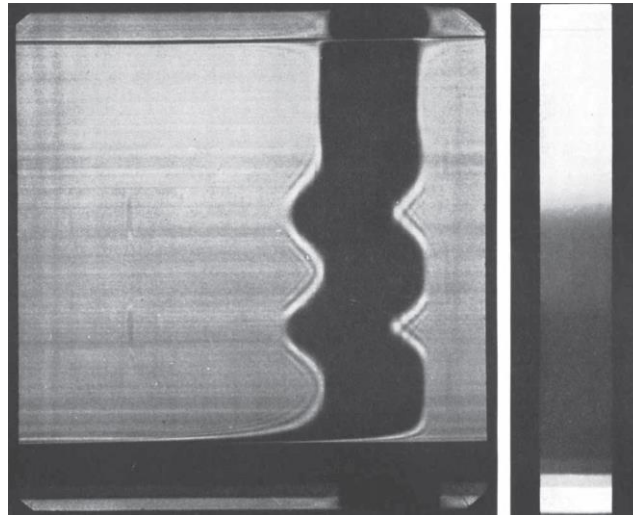
BOTTOM

ROTOR EDGE

Photography of the Refraction pattern   Absorption

52

Ultracentrifugation of *Limulus polyphemus* hemocyanin

ROTOR CENTRE
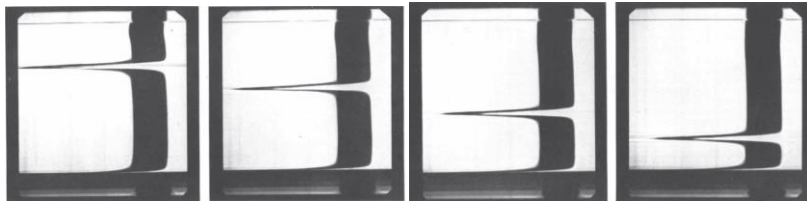
ROTOR EDGE   1:00 h   1:30 h   2:00 h   2:30 h

Centrifugation time

53

Jerome Vinograd and Mathew Meselson and the analytical ultracentrifuge *Spinco Model E* ser. #168
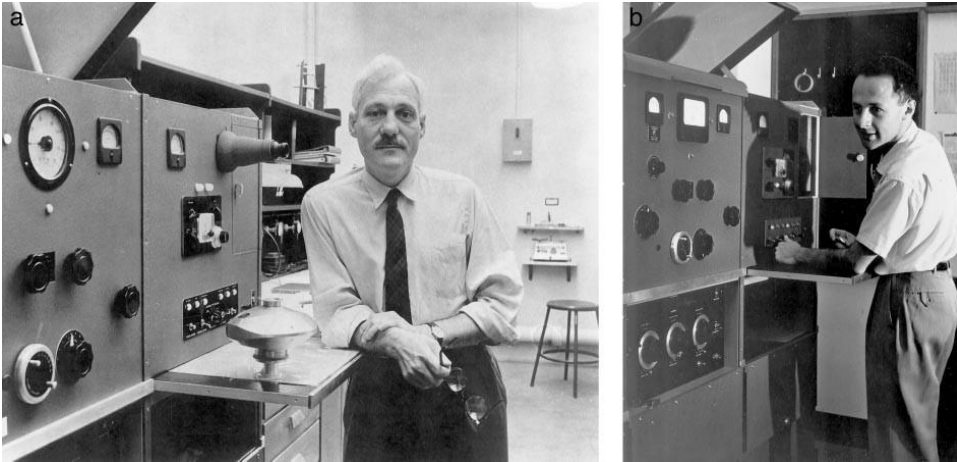
Hanawalt, PNAS **101:**17889-17894, 2004

54
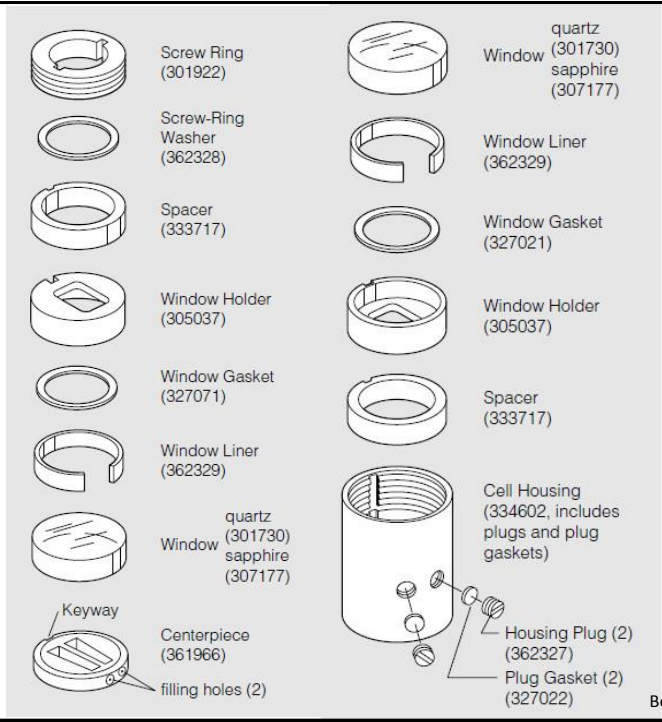
Mounting the cell of a modern analytical ultracentrifuge rotor

Beckman-Coulter™ LXL/A-TB-003F, 2001

55

Filling the the two chambered standard cell

Solvent

Sample+Solvent

Cell end with
Screw Ring

Filling with hand
pipette

Filling with
syringe
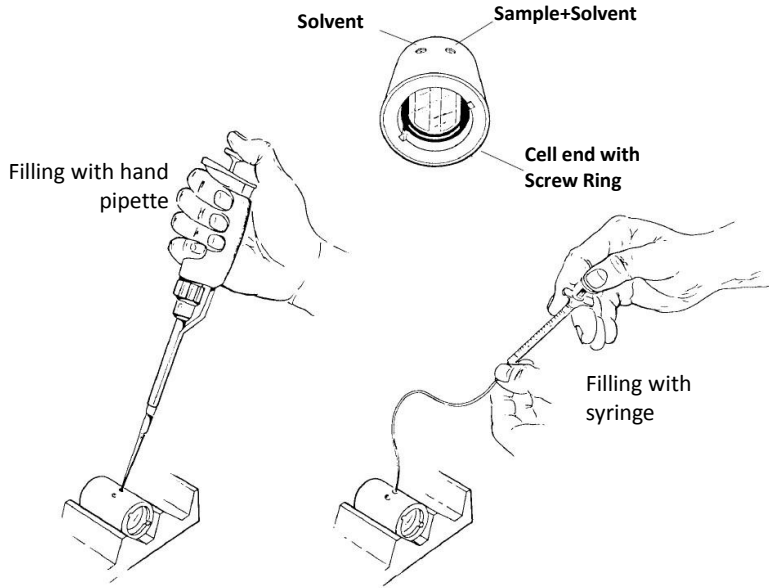
Beckman-Coulter™ LXL/A-TB-003F, 2001

56

A modern analytical ultracentrifuge

ProteomeLab XL-A or XL-I Instrument and Data System Space Requirements

$\frac{cm}{in.}$

$\frac{15.2}{6}$

$\frac{67.3}{26.5}$

$\frac{94.0}{37}$

$\frac{5.1}{2}$

$\frac{5.1}{2}$
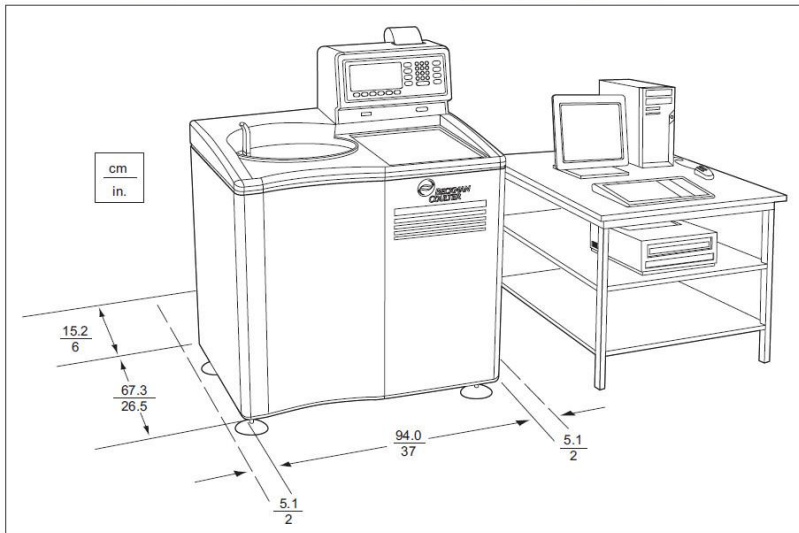
Beckman-Coulter™ PN LXLAI-IM-10AB 2014

57

Analytical Ultracentrifuge Beckman-Coulter Model XL-1

Planken KL (2008) – PhD Thesis - ISBN 978-90-393-4798-0

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UV absorption measurement system In a modern analytical ultracentrifuge
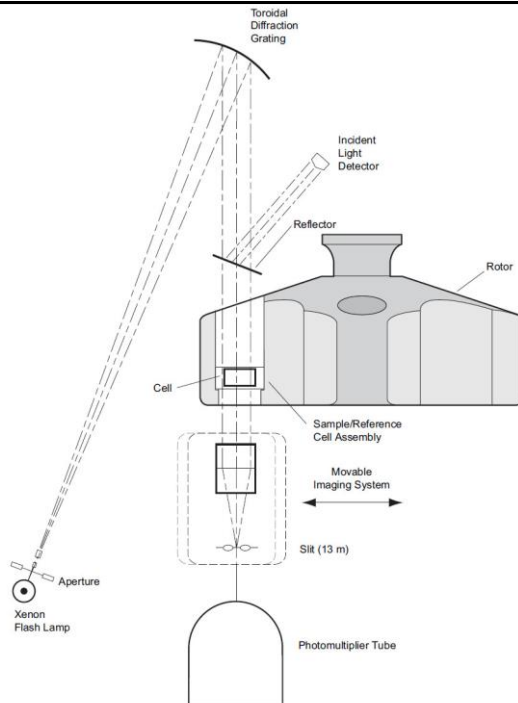
Beckman-Coulter™ PN LXLAI-IM-10AB 2014

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HOW THEY DID IT

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I

EQUILIBRIUM SEDIMENTATION OF MACROMOLECULES IN DENSITY GRADIENTS

WITH APPLICATION TO THE STUDY OF DEOXYRIBONUCLEIC ACID

II

THE CRYSTAL STRUCTURE OF N,N'-DIMETHYL MALONAMIDE

Thesis by

Matthew Meselson

In Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1957

**PhD thesis of
Matthew Meselson
1957**

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ACKNOWLEDGMENTS

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PNAS, **43:**581-588, 1957

EQUILIBRIUM SEDIMENTATION OF MACROMOLECULES IN DENSITY GRADIENTS*

By Matthew Meselson,† Franklin W. Stahl,‡ and Jerome Vinograd

GATES AND CRELLIN LABORATORIES OF CHEMISTRY§ AND NORMAN W. CHURCH LABORATORY OF CHEMICAL BIOLOGY

CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

Communicated by Linus Pauling, May 27, 1957

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Resumo do método desenvolvido

The centrifugal field tends to drive the
macromolecules into the region where the sum
of the forces acting on a given molecule is zero.
(The effective density of the macromolecular
material is here defined as the density of the
solution in this region.). This concentrating
tendency is opposed by Brownian motion, with
the result that at equilibrium the
macromolecules are distributed with respect to
concentration in a band of width inversely
related to their molecular weight.

Meselson et al., *PNAS* **43:**581-588, 1957

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Ultracentrifugação de DNA de T7

2,1h

4,3h

6,4h

8,5h

9,6h

Equilíbrio

In a CsCl solution at 20 °C with initial density
of 1,720 g/cm^3 a DNA initial concentration of
3 µg/mL

In a *Spinco Model E* Analytical
Ultracentrifuge at 31.410 rpm

The time required for the concentration
of a species to be at 1% of the equilibrium
value of the center of a Gaussian with two
standard deviations can be estimated as:

$$t^* = \frac{S^2}{D}\left(\ln\frac{L}{S} + 1,26\right)$$

$$L \gg \sigma$$

L = Length of the liquid column
σ = standard deviation at
    equilibrium
D = diffusion constant

Meselson et al., PNAS 43:581-588, 1957

Meselson e Nazarian (1963), in: J.W. Williams (ed.) *Ultracentrifugal Analysis*, pp.131-142

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THE REBUTTALS

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Vol. 1, No. 3 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS Sept. 1959

THE SUBUNIT OF DEOXYRIBONUCLEIC ACID*   —— 1919 - 2013

Liebe F. Cavalieri, Barbara Hatch Rosenberg and Joan F. Deutsch

Sloan-Kettering Institute for Cancer Research,
Sloan-Kettering Division of Cornell University Medical College
New York, New York

Received September 8, 1959

The DNA molecules of E. coli B have been shown by Meselson and Stahl (1958) to be composed of two subunits, one of which is parental and the other, newly synthesized. Similar results have been obtained at the chromosomal level by Taylor, Woods and Hughes (1957). It is obviously essential to know the nature of the molecular subunit in considering possible mechanisms of replication at both levels.

Experiments carried out in this laboratory show that the subunit consists of two strands, rather than the single polynucleotide chain necessitated by the Watson-Crick (1953) replication hypothesis. Molecular-weight measurements by the light-scattering method confirm the results obtained by Meselson and Stahl, who used the method of equilibrium sedimentation in a density gradient, that the molecular weight of E. coli DNA is reduced to half by heating in cesium chloride. However, the DNA used by them has been found to be an aggregate held together by protein links; this type of linkage differs from that occurring between the two subunits.

> Experiments carried out in this laboratory show that the subunit consists of two strands, rather than the single polynucleotide chain necessitated by the Watson-Crick (1953) replication hypothesis.

Cavalieri et al., *BBRC* **1:**124-129, 1959

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DNA isolated from E. coli B grown under the identical conditions used by Meselson and Stahl and deproteinized by the Duponol method had a molecular weight of 11 x 10⁶ (light scattering) and, when centrifuged in CsCl, formed a band identical with that obtained by Meselson and Stahl. The molecular weight dropped to 5.6 x 10⁶ on heating to 100° in CsCl, and the band width increased. Heating in the absence of CsCl did not alter the molecular weight.

When the 11 x 10⁶ DNA was either treated with chymotrypsin, or shaken with a chloroform-octanol mixture, its molecular weight dropped to 2.4 ± 0.2 x 10⁶ while its length actually increased. Repeated treatments

Cavalieri et al., *BBRC* **1**:124-129, 1959

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- Knowing that a deoxynucleotide has a mean molecular mass of 324.5 Da, what is the size in base pairs of a molecule with 11×10^6 Da

$$\frac{11x10^6\,Da}{2x324.5Da} = \frac{11x10^6\,Da}{649Da} = 1.7x10^4\,pb = 170kbp$$

- Knowing that the genome of *E. coli* has 4.6×10^6 base pairs, how many fragments of 11×10^6 Da exist in the genome?

$$\frac{4.6x10^6}{1.7x10^4} = 271\,fragments$$

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The *E. coli* DNA is a dimer with two double helices!

We deduce that the unaggregated, unit DNA molecule of E. coli is actually a dimer composed of two double helices, laterally bonded together; that each double helix is conserved intact during cell division; and that the bonds holding the dimer together are ruptured by heating in CsCl, as well as by some part of the replication cycle in the cell. The dimer bonds are clearly

Cavalieri et al., *BBRC* **1:**124-129, 1959

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