6/20/56
±

J. Lederberg

McCollum Pratt Symp.

Tues. Beadle Ris | Benzer, Mitchell
Nanney, Rhoades

Wed 9:00 ~~Sp[illegible]~~ Mazia Allfrey Sol | Hewitt, Rollin, Zam

Thurs. 9:00 Hartman Jacob Conrat | Chargaff Markham Chr[illegible]
[illegible]

Fri 9:30 Art K, Ochoa Cohen | Stent, panel.

suggested notes J.J.

1. Bill Mac asked me here after the Fred Symp. where my function was to resist strenuously the idea that geneticists were making any progress at all. If I assume that my function here is to be a "wowser", I hope this won't be taken as a balanced presentation of my own views which are more optimistic & less reasonable than may appear, and I also hope the copy won't read "indefensible" when I read "indefeasible" as happened at Detroit in discussing Dr. Horowitz's paper.

alt - modulator : drab mutation flies & says it plans doing anything
feed back problem of modulator = desc. scales.

(harder to talk about something u know about — eg. feed)
won't comment until could study
but Lp^{+}/Lp^{s} heterozygotes)
{Hp bal
Lp bal linkage}.

Wr. Ochoa for you.

Beadle
①.

1

medicaid .... chromosomes. W/C suggests mechanism

c/o does not cut through genes because they came out
In general different functions of separate and discrete
Quotes pure heterozygotes ↙ (cf. Ds; heterochromatin effects!)

Confusion of classical tests of allelism
① close linkage of related functions ② "complementary alleles"

"M Mitchell phenomenon" a+-/-+b → tetrads c/ following
a+-b a++b A-+B A-+B. ∴ non reciprocal.

? Are complements always found to normal c/o first. markers?

explanations 2:0 mis copying
often not acc. by c/o but there
is a significant correlation.

Assumes c/o is related by requirement for "recombination"
proximity. [Why not c/o as replication phenomenon] seems to leave out this hypothesis

= conversion
"

6-strand c-over? ... markers not c/o. effectively close doubles.

= Lindegren effect
= Lamphere effect

"Recombination may be deviation from randomness of c/o.

oppose Beadle's suggestion: gene = functional unit

Mutational sites = sites
"heteroalleles" = nonalleles
homoalleles = "alleles"

what are means of defining these!

Jacob

Hfr Gal+ × F- Gal- should yield Gal+ HSR
and doesn't

Margin notes:
don't pursue cause means or mech vs struct
knows nothing about genes, only markers.
purpose to leave out word "gene"
define allele & reference to complementarity (a genus!) What do we know about functions

Yanofsky E' sec

B. hyp. mut G — sec may inhibit defect

↓

↓

mutan E ∴ many genes strictly involved in synth each enzyme is direct cosyme

"tenable to suppose that gene acts via RNA as template"
not yet v good evidence. + don't know whether
enzyme "is modified or is absent" : (argument is circular)

also quotes blood Hb's — evidence on allelism not good.
but accepts it!
But Thalassemia is not allelic c sicklemia!

---

quotes Ford Symp. on 1:1 — but indefeasible

"genes always allelic" ∴ believes total spec enzyme cc assoc c single gene!

galactokinase — Contra Su/E

---

eating vs breathing — cor. genes' cytoplasm. + evolution
how do you tell.

Zm? : primary genetic information in all living forms

by NA < DNA / RNA. — where RNA carries information

it is in host cell c DNA, protein. — Ditto for DNA

not necessary to assume c/o is over ... in higher org.

⊃ contains
⊂ is contained in.

(not necessary to assume it is via exchange)

But only when close markers are available can one detect the exceptions! May result either in exchange or non exchange

Ris

Cytochem results ① constancy of DNA - synth only in relation to chr reprod. ② histone also constant ③ protein + RNA - more in active cells, reversible.

EM - polytene in salivaries - fibrils 500Å / 200Å which are hollow.

no other structure. piece consists of NP. cf TMV

fibrils not affected by DNAse.

At prophase, fibrils move apart

Long. differentiation? degree of u-tiny at diplotene!

Mazia - units are 4000Å / 200Å - sites of c/o?
Similar units of RNP are intermediate carriers of inform

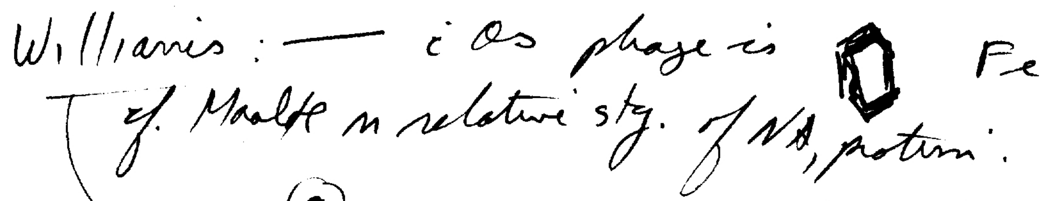
multi-strandedness of chromosome → problems of dl. [illegible] DNA

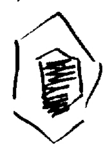
Importance of prophase as the bridge of fibril to chromosome

Bradley asked): Disc 11:30 AM Tues.
Alpert - [Marshak - thinks too much explained away].

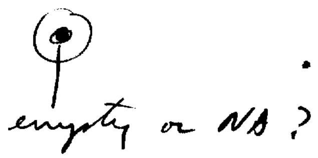
Ris - sequencing c/ Feulgen stain: total amount is small but ~ sperm.
Butler - (Dawson + _); nearly 1 base a al P. Harris 3 nos 6 protein content

Williams: — ¿ Os phage is Fe





of Maaløe n relative stg. of NA, protein.



empty or NA?

matter of fixation: ¿ formalin nor extraction?

Benzer — "gene" is missing — gene-enzyme as a definition. function matter of taste positive test.

applause (!!!)

automotive analogy belonged to Detroit comment that seemingly is a continuous series of entities punctuated by experiments

unit of recombination: [2 existence < map is cm / physical distance

evidence that there is a segment for cistron

T4 (is there a linear cistron?)

trying to exhaust mutations in a segment.

	(K-12)	S = "BB"
r_I	r	r
r_{II}	−	+
r_{III}	+	+

phenotypes

\+ + +

The groups are regional genes.

r_{II} in two cistrons + no exceptions to squares.

A = 4%

B = 2%

stable to inversion

Exceptions: apparent deletions are always linear segments?

can also be crossover suppressor & asynapsis

importance of exhaustive analyses — had its origins possibly in Stadler's study

Anglo Saxon equiv. unit / nit / fit ? bit

Concentrated on deletion class. 1100 to start

150 included + tested in pairs

one loses most pigment, 2 phenotypes (Delmerica)

many singles, rifes o's.

1, not yet "run into the ground".

about 12 sites so far.

importance of terminology

linear alleles

complex loci

confusing

---

overlapping deletes found in some cases. Can be used to subdivide.

---

no exceptions to linearity yet.

mutons + recons = or ?? (nucleotide not excluded.

probably pairing rather than deletions in case of Lac.

S.B.

generate mutants from del x +? screen byproducts
c̄ indicator (screen against + byproducts?)

H.M.

no evidence of -- as complements to ++.

heat shocks < {in plants of} conversion, no effect on rec of exterior markers.

" of conidia > conversion; negligible effect on c/o. to as high as 4%.

~~ABC~~
~~abc~~
~~AbC~~

expects conversion in -X.
(simulation of reciprocal crossover).

non reciprocal crossing over?

Roman confirmed Lindegren on + --- asci and identity of the (-).

Sol did mention Lindegren

6
glossary
policy

Nanney - definitions series at: prob. of specificity
discrepancy gametes [illegible].

general "homeostatic principle".
of mutation s.l. - any change s.s. - via se, ? chance - genes.
"hereditary modification" is broader.

cytoplasmic = extranuclear chromosomal.
① ② steady state concept
[illegible] concept
most observations on sexual heredity fit ①

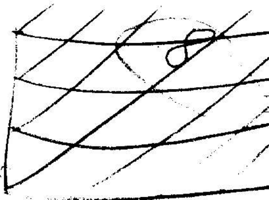
but not satisf. for differentiation he says. But gross partitioning not hold up. ∴ suggested that cytoplasm ⊃ cellular heredity.
there are works (Briggs & King, etc.). But: ⓐ speed ⓑ direction in time ⓒ regular predictable pattern ⓓ integration ⓔ
most data are ambiguous.

why not apply steady state concept to the nucleus

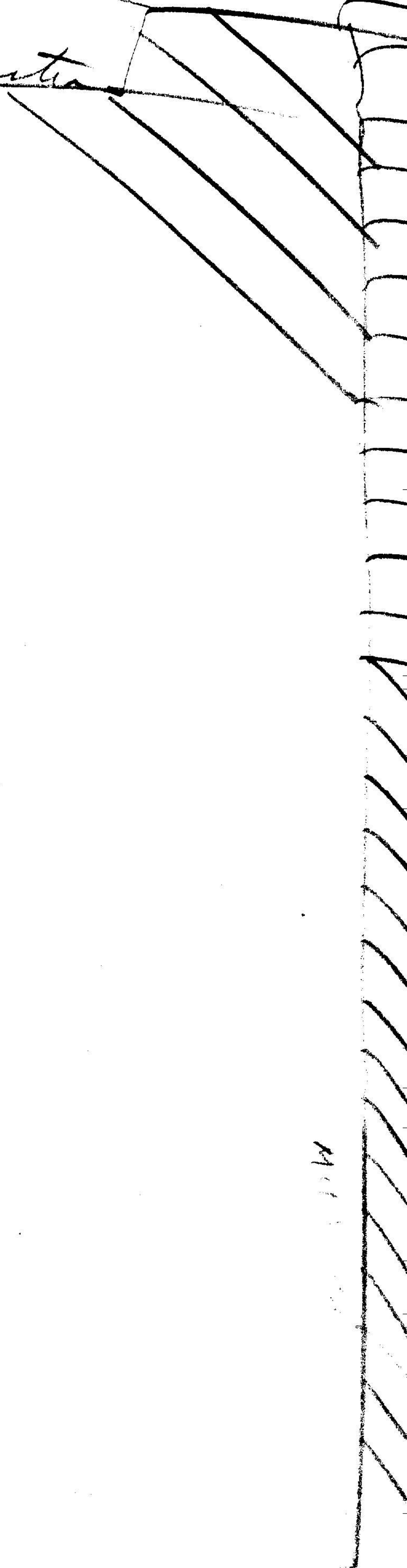
Rhoades - point mutations
extragenic; minute dfs; rearrangements c/ PE; PE: c/o
c/o of components of a "compound locus".
intragenic: "true gene mutation"

Some [illegible] seem [illegible]! But correct the typo!
need a dictionary; [illegible] glossaries into paper

Send Berger Tox. repts.



7 – Rhoades – not semantic — [illegible]
why not a whole key – [illegible]



Dan -
look for such conjugates in flagella?

implant cysteine in vivo to induce entire [illegible]
cytochem evidence of -SH accumul.

Mazia

spindles.

Digitonin as solubilyzer - sea urchin eggs.
did not work in KCl solution!

ATP - M/100 (natural level = 5×10^{-3}).

98% protein: but not RNP as formerly thought.
- 10% of total cell protein

nucleotide of spindle found not RNA - no pyrimidine. May be ADP or ATP conjugate, i.e. nucleotide-protein (= actin)
back to Rapkine!

Multipolar mitosis - centers may influence orientation of chains - binding agent may be diffusing from them.

Core extract + crystals of cysteine give model asters ?and spindle.

Where does this protein come from?
soluble proteins in s.u. eggs by u/c. technique
- a component disappears during cleavage division.

Hoffman-Berling: glycerol extr + ATP:
elongation of spindle & shortening of chromosomal fibres.

mammal nuclei Allfrey
Hepenus. .25M sucrose $CaCl_2$ - low speed blender + differentl.
25% contamination c whole cells. Study AA incorporation.

chloramphenicol, FPA had little or no effect on alanine incorporation.
Others had no effect on M - uptake.

certis one abolished uptake!!! 5-6-Cl Bz Rib

interfer'd effects. inhib of uptake 11 effects mononus.
Na^+ is required contra K^+!!...

opt sucrose is .25M   10% deviation → 30% inhibition!
secular variations from animal to animal   (hormonal?)

F&T., heating, treating in blender destroy activity. Old nuclei do not
last overnight.
Specificity:
a) of d, l-alanine* ~~dat~~ flooded with unlabelled d, -l. etc
b) labelled val. nuclei do not dose*.
"not exchange" — not proven unless substantial
replacement had occurred! ~~not satisf~~ realized by Vassar. reply.

30 mg nuclei .6 γ alanine* / hour.
∴ replacement is 8 γ NP/hour
DNA removal with crystalline, phosphatases (many platinum)
cells are unaffected.

Radioautography is homogenous — close closely examined
"from whole slide" — we spot/spot autography.

Supplementary DNA. (DNAse not removed at first
but this not so successful)
but 4X increase by "adding DNA" ~~Specificity of DNA?~~
add "denatured DNA" — just as effective
"apurinic acid" had some activity.
"deoxyribose phosphate
DNAse treated — core
dialysate } both fractions +
RNA also +

RNA mononucleotides etc. ur. q.

dinucleotides: AA, AG, AU, GC, u.q.

Sol) template hypothesis — "modern theology" → DNA RNA protein

now? either DNA or RNA

azathymine ?

Blocks DNA synthesis UV, azathymine, thymineless stages — no effect on enz. synthesis

Blocks RNA synthesis — uracil-mutants; analogues. inhibits enzyme synth.

Chantrenne: RNAS accelerates when catalase is induced

Volkin — T2 inf cells — spec RNAS?

? Is RNA a template? Need a subst. expt.

"I would have preferred to be ingenious than courageous" (Gale) ingenuity no longer paying off.

cell parts expts:

Gale: —RNA fractured cells, restore by intact RNA. species-sp!

Sol's lab: needed steps: activated a.a. needed as preliminaries. + would take fools to expect a water-soluble preps to work. thought to use protoplasts

Properties:

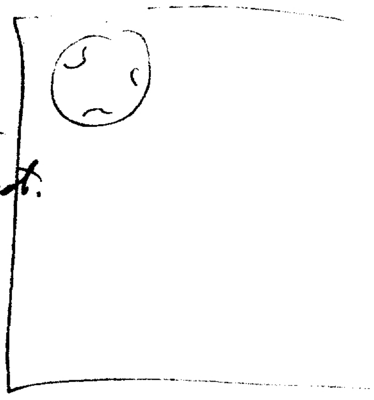
osmotic conditions

dissolved by lipase; collected all DNA = amt RNA some basic prot.

what is membrane? dimensions?

1 per protoplast

"fairly good" quant recovery



Properties: enzyme synthesis under right conditions.

Enzymes had little effect as a rule, but a few preps did slow effects. Age matters. All protoplasts are unresolvable ≥ 30 mins u.q. Resolve while making

(Is this in Ford paper?)

> 99% DNA removed. no effect on enzyme synth and some stimulation.

∴ might make "strong statement".

But acid soluble DNA is still present (60% remains!)
System was also very labile. ∴ studied broken protoplasts.
Many methods tried: importance of initial stabilizing medium.
sucrose, phosphate e.g.; used a K, succinate system
then osmotic stable.

1:3 substrate vacuoles!
1:5 " matrix!

(900 uM/mg P/hour.
what is enzyme activity?)

Using pellets phospholipids are designated.
dil ~~[illegible]~~ c 4.2-4.7-fold. (from what?)

resuspended loses 85% of DNA

RM = reading mixture. Substrate stabilizes in a phosph HDP
lose DNA.

(what are "substrates" morphologically??)

again washed RNA causes factor fractivity
and soluble NA now absent.

∴ Substrates s/DNA make Enzyme
— RNA do not " ".

Is RNA being resynth. in induction medium:
from 4 – 118 γ/10ml.
<1 – 44.
40 min lag + linear resynthesis.
What is "RNA" – acid pptable Dische reaction
hydrolysis → UV absorpt. exp.

Examined other capacities & get considerable
resynth. of ~~RN~~ RNA DNA + some protein
resyntheses. 5-6 x increase in all components
make more DNA than RNA, prot.
10 x. arrested by culture.
but RNAse-treated are inactive.
~~RNA~~
[nucleoside diphosphates]
Put back RNA. — Ochoa substrates restore EF ability.
enzyme stops effect.
∴ not specificity.

Ochoa: ~~[illegible]~~

But not all RNA
was removed.
>50% removal e.g.
how much till disphosph.
DNA is nonsense DNA.

if so, is
the template only
protein
"what is RNA" —

Did you say – RNA cells can
be restored by nucleoside diph.
do they make enzyme?

poss. synthesis of [illegible]?
What are substrates made of?
relate to [illegible] –
restoration by NA small fragments
of low specificity.

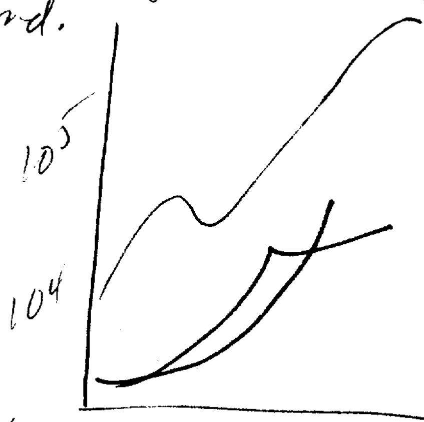
Hannett

Radiation effects on TP. Target theory may apply (S^R → x S^S).

1. Quantitation problems. Reactants a → x b. b is competent only about 15 mins. a is impure, containing unknown ineffective molecules. Synchronous development of competence in exptl. cultures (Method II) went from 0 to 10^5 to 100 / 10^6 bacteria. Later waves may also occur. Can add DNA from start and get higher assays. Linear assay possible with ②.

Relationship of competence to division cycle.

Thomas found × eff. depends on number of competent bacteria. Limiting reaction evidently bimolecular. Had to study kinetics at high DNA, plateaus are found.



log log plates

What are reasons for nonlinearity? heterogeneity of DNA? Extra DNA gave 2 ⇐ gives early plateau, decreased by props of unrelated particles. 1st plateau is f(total DNA). Break due to multisorption. At high multi., inhibition is partly reversed, may be max. of effective sorptions.

1st plateau is region where doubles begin to occur detectably.

[Must be sensitive + resistant particles

?- genic imbalance? - transformed cell may be killed or nontransformable.

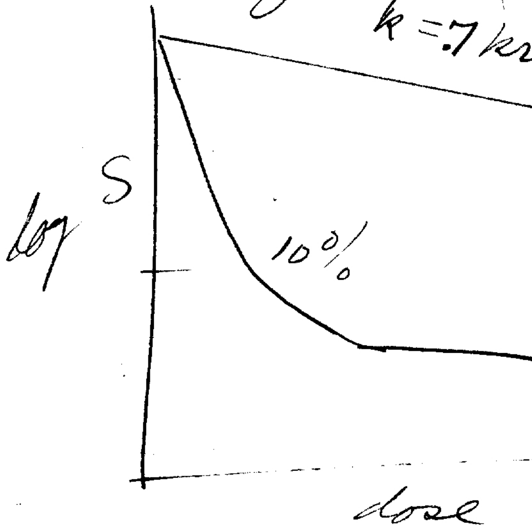
Extra DNA in phage may have a similar function]

[polyspermy?]

Irradiated DNA –

~~los~~ linear assay at 3-4% survival.
~~native DNA~~ mass of DNA determines linear region.
indirect effects problem: – irradiated in hagen state, where protective substances have no effect, & sens. volumes are smallest. i.e. sens to indirect effects.

Kinetics of inactivation



[Marmur + Fluke (2 ✱ 6× 10⁶]

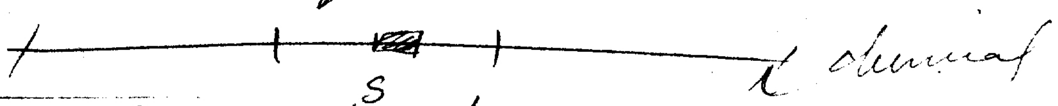
only 1/10 app. chem. m.w.

(radiat) radar < centrar. ? Residual activity possibilities.
Surviving DNA not heterogeneous in classes.

Distinctive particles? Fractionate DNA < high gravity high T.P. / low gravity low act.
no difference.

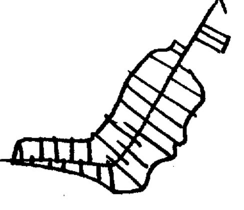
urea no effect

more sensitive units are present in some preparations.
why are small aggregates > sensitive
postulates a sensitive region for S^r locus (guess 3×10⁴ mol.)
target prevents incorporation.



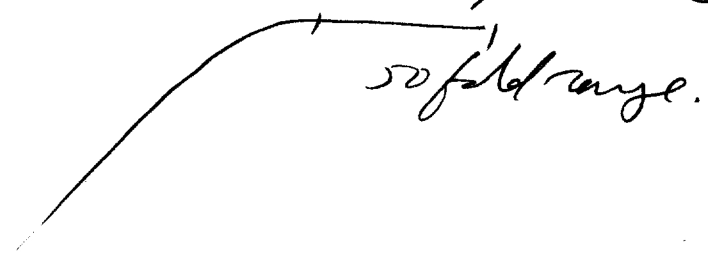
c chemical

Aggregates: ? energy transfer or cross-links which inactivate.



Hotchkiss: assume × hypothesis.
Quantitative factors
Capsule system – originally by dilution. Examinations: sediment too soon or too late n.g. Inoculum, antibody must be specific.
If sediment is resuspended, may let reveal addl. ×'s.
pres. matter of selective conditions. etc. So moved to drug resistance.

Rollin doesn't see plateaus — level of plateau depends on proportion of S^{r}/S^{s} DNA.



put H's on word
DNA/cell
DNA/nucleus ×
max.
"molecules"/DNA/cell.

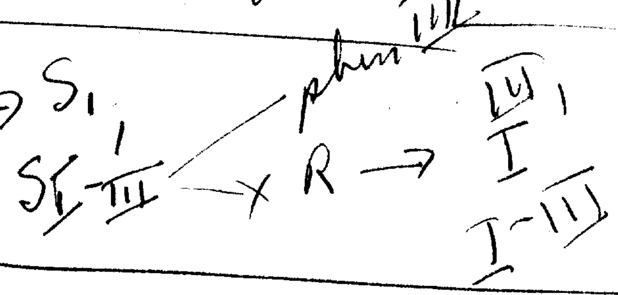
Benedict – chromatography of DNA
anion exch.

Ecteola Cellulose –
Variety of fractions tested
least eluable DNA is most active
2M NaCl MNH$_3$. ∴ heterogeneous

In any expts, can get initial plateau?

sp. separation?

Austrian: SI → × SIII, → S,



H. Taylor – Quant. assay.

DNA is not exhaustive!

What is being measured in essays?
∴ not a stoichiometry + assumptions of a time slice of a bimolecular reaction needs kinetic justification

Zamenhof: The transforming principle.
① test for activity of DNA. assn physical analyses.
② composition – modified by Bu[illegible]
Effect of BU uptake: sp. mut. rate not changed, cells still alive
But do get two colony types, incl. "pinpoint" colonies, but these → large by mutation. + can establish pure lines.
~10% of such mutations.
large colonies also gave 2 colony types. "Unstabilized" the strains, every colony started to be altered.
③ Nature of heterogeneity?
④ No fractionation so far.
⑤ auxiliary system.

[+ in synthetic medium?

2×10^{-9} γ/cell per nucleus = 5× content of cell.

1 cell has 250 molecules. @? groups of characters not DNA ⓑ 1 mol > 1 determinant (i.e. linkage).

attempt to decrease size of DNA → inact.

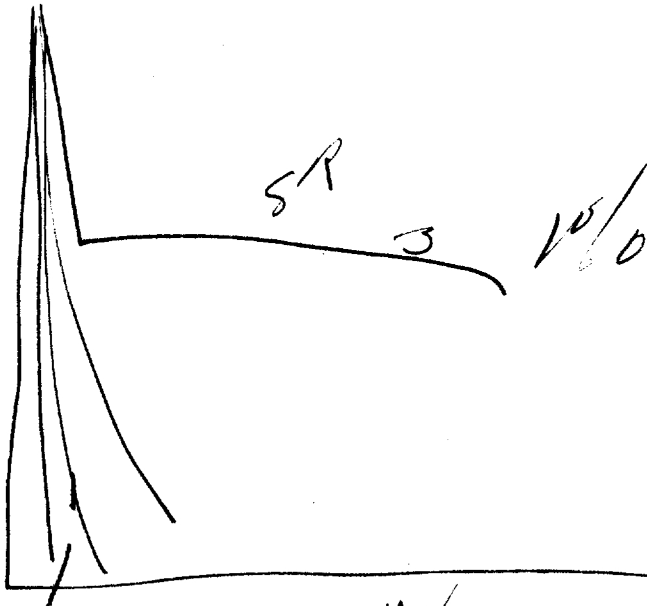
(synergisms problem

10^{-4} γ DNAse destroys 100γ/ml DNA in 20 minutes
drying, dialysis also ", act disappears by prechopping.
deamination " 5.2%, NH_2–
Dialysis: loss of heat stability + desoxyribonucleity.
M. [illegible]:
~~diaminopurine~~ Me₂SO₄ reactions c̄ guanine. also esterifies PO_4^{3}.
diaminopurine – much?

[assay methods for these procedures

∴ DNA is target of strong mutagens

UV treated, heat treated all unstabilized.



UV

1 capsule c

3 = SR

2 capsuled

4. SR from type 6.

differences

1 + 2 similar

assay systems for DNA

Bring up again.

mutant broth + DNA competent

but cells' genes is a protein medium

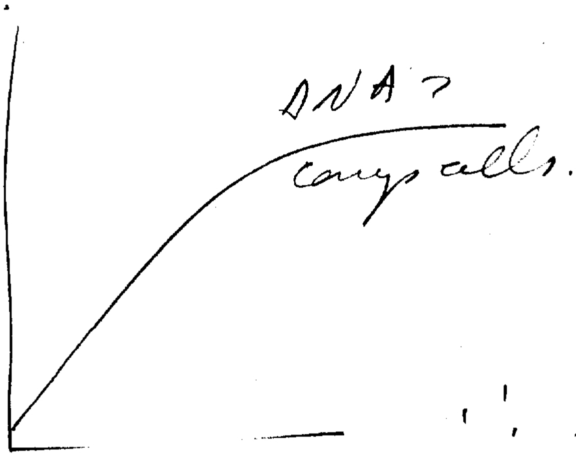
pn. Hem. coli? Neisseria

importance of

A6 pm W. — Bell incorporated after cessation of growth + no increase in DNA/cell. ∴ exchange without resynthesis of DNA

Goodgal: ADNA 300 molecules/nucleus ~ 1 cell's worth

(is uptake homogeneous? yes!) [illegible] 0 - 90% range



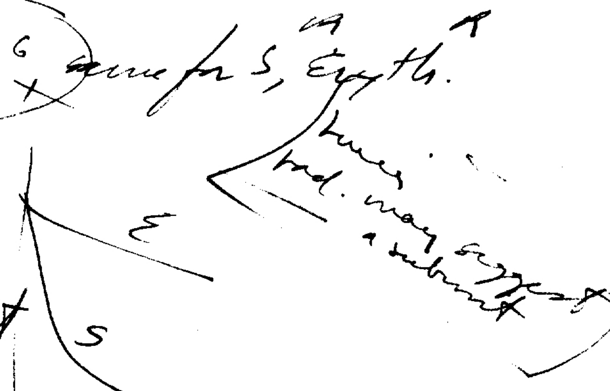
Sd cells do not x —
do not x DNA.

∴ 1 gene per cell.

~~II~~ Assumed M = 6×10^6 (i.e., $10^{-17} N_A$)

~~coeff.~~ cent - ~~diff~~ values = (5.5×10^6) same for S, Erythr.

No separation obtained or up by UV



Goodgal coli enzyme + ATP + Mg + light restored UV'd TP from 3-30%

says Hemophilus, pneumoc. to not photo-reactivate per se.

Luria - ? as i phage. — no.

Bendich Theulas early fractions have M ~ 400,00, even oligonucleotides (pairing minimum)

Leidy virus specific (à la Schaeffer)

10^{-6} 10^{-7} 10^{-3}

induced influenzae paraninfluenzae suis

S^R → x

homo hetero ratio is constant for a given pop. + end of strain of donor.

of Salmonella x coli x Shigella

"Crossing-over"

15 sems in heterozygotes

4 steps in histidine

v. occ. recurrence. possibilities for reversion.

also differs in recomb frequencies!

some mutants give confusing overlaps. histidine.

Kalckar

Only 2-point in

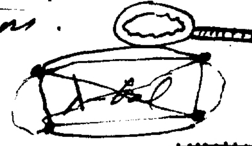
only comment is that detailed data have yet to be published & therefore actual comments not yet available. There are difficulties in analyzing esp. 2-point tests

Non-aggression pact

Hewitt - general review of label vols in re DNA effects

<u>Hartman</u> : — classifies recombination by vector
<u>DNA</u>, <u>phage</u>, <u>cell conjugation</u>.

a. DNA b. Transduction; lysogeny c. Cell conjugation.



Types: generalized vs. prophage-linked.
stable vs. abortive

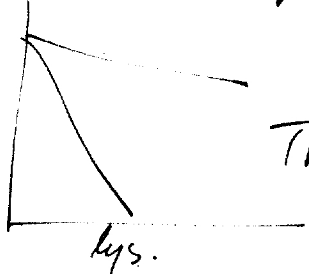
Induced lysogenics are → in Salmonella! generalized.

(S^R → not transduced in TMD?)

- Zinder etc. Expts.
Britain's hyp. — attachment specificity?

Jacob: gal by heterologous phage → ly^+ : induction can be lethal
metabolic state of recipients: need phenomic expression: or effect of host - background on eff. →

chemistry of exogenotes — analogy; no direct evidence.
P32 decay :



∴ exog ≪ prophage.

This is Salmonella.

at least several gene loci long. — "20 – 30 loci" long? (not sites?)

incorporation step — possibilities: "unilinear" Fla^+ → Fla^-.

Jacob: eclipse to lwoff. cyclic behavior
temperate quality.

prophage = phage mat in noninfectious state.
replication coordinated c/ bact. div.
expression: → phage + immunity.
induction UV → → phage.
etc.
immunity usually specific for related
superinf. phage does not interact genetically except on induction.

?chemistry of prophage — Hershey & Chase
injected DNA, but no data on whether info is transferred.

How many prophage/cell. Double infection, markers in output
have input ratio. Induced bacteria superinfected behave as if 3
per bacterium. ~ 3 nuclei.

Jacob — also incompatibility between related prophages, usually no mixed lysogenics for +, mutants of same phage.

E. coli K-12 L+T. EML-λ^+. can cross $\lambda^+ \times \lambda^s$.
1st crypts by L's + E.W.

clones "never segregate Xyl, Mal, etc.!

14 phages s/ cross-immunity. 7 are inducible A
7 are noninducible. B

A are ~~closest to λ.~~ between Gal and R
all show zygotic induction, degree varies in same order as time of entrance.

B: all are closest to R — most are linked to

T L Az Tl Lac Gal λ, Xyl Mal S

no zygotic induction c these phages.
each phage has fixed site. two general regions.

one of the B's ⟶ x

3 prophages can be ⟶ x by one other.

---

phage crosses among A's.
3 c mutants

specific immunity site = localization-determining sites, near C1-2

---

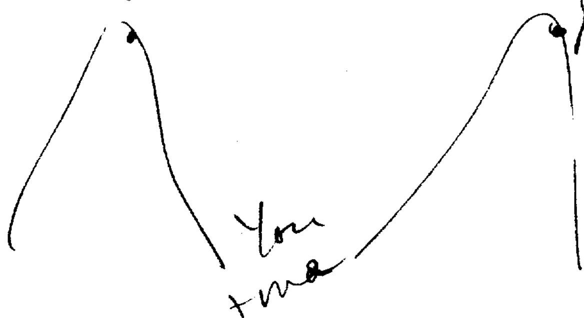
carcinogens: depth toxin.

Myers Lerner.

F-Conrat
8/15 becomes mixed from (mixed RNA)-protein.
none mixed from mixed virus.

Schramm

L. Pauling



host modification in chemical terms.

any pure clones in TMV —

Vollam asked
any effort to stabilize RNA with normal plant proteins
answered in course.

Is the ~~prophage~~ bacterium

Lwoff — an ensemble of prophages?

Buy Ch+ Dav. | Are there conservative exchange reactions?

Doty — plan of mountains.

adv. of section is to show the clouds.

Chargaff: language difficulties — dictionaries lacking
u coil ≠ mol helix

MC, HMC
N-Me-Adenine.

base composition: 4 bases in RNA — AGUC
4 " DNA AGUT

plant viruses may be atypical. In animal RNA closer association c/ protein.

This is the fundamental question & cancels synopsis

"NA can carry information" >>> Is protein unimportant?
pres. as nucleotide sequence. Any finite sequence represented more than once. Is message carried many times?

maybe other than 3-5 linkages; i.e. as folds.
not excluded that NA is large cycle

$\frac{A+T}{G+C}$ varies from 0.4 - 2.0
Tb sea urchin

6 amino = 6 bits

But A ≈ T
G ≈ C $\frac{A+T}{G+C}$ → 1.

Meaning of regularities.

—Chargaff— unitary complements well preserved.

now: sequential analyses:

Crudely Pu–Py– relations in trinucleotides.

can fractionate to small extent DNA's for different G/A ratios:

cleanest material genetic content of T2?

~~Of~~ Is 5HC/C random. ~~No~~ occurs fractionally.

RNA. — no regularities, but no pure purines at first.

crude nucleoproteins: the relation A+C = G+U was preserved, ~~i.e.~~, but no others.

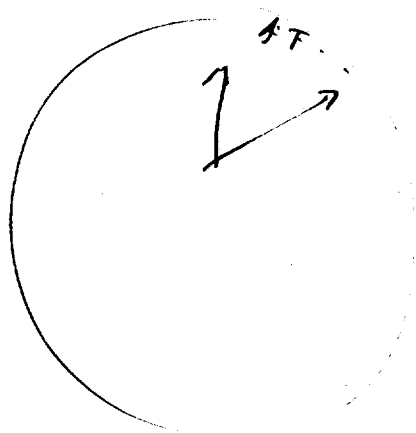
Smith. — no good evidence on branching of RNA.

Sequence problems.

Whitfield — remove phosph

oxidise sugar

remove purine ... experimental pure material.



Chargaff
5BC

Ochoa
Pasi 1

Smith —

agag uptake in B. cereus cells.
not homogeneously. cyclic end turnover faster.

Crick 5MC does not go in at random. Agree that naive repl. mechanism
is ruled out & maybe unit of agglomeration as not nucleotide but
a oligonucleotide unit.
5MC is preferred next to guanine. Suggests repl principle.

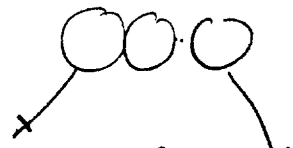
~~6 amino~~ - N- methyl adenine, replaces thymine =X
He suggests that XT replaces TT pairs. but gives tremendous
(flexibility, too much.)
Chargaff complains

Toulouse & Zam.

Ochoa — whole cell RNA & enzyme RNA.

Bendich — recorded deviations from 1:1

Crick: must have Pur: Pyr (large: small)



X-ray picks out the regularities. and for given conn, G-C
A T fit best.

maybe some unpaired chains in some preps.

If all 4 bases are in any chain, only W/C model so far fits
the X ray.

[felicitous

Not yet central professional judgment on X-ray concordance but no more conscientiousness yet.

coiling is paranemic not plectonemic. No sales of model yet, at least

(Why read SF if you can read PNAS!)

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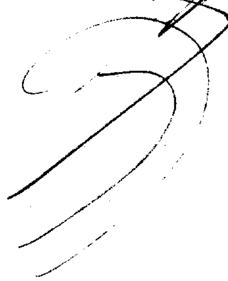
nucleoprotamine: prot fills in the "minor" groove.

1 side chain per 3½ Å: not slight up.



as 3d chains, basic groups matching P.

non polar spacings loops of 2 or more

∴ non polar AA should occur in pairs.

much analytic detail fits that.

---

nucleohistone

Kalckar: what he's publishing

Need concentration of analytical work on biol significant material!

[illegible] factory?

JA

4AGUC.

synth poly RNA, \ or AU

all gave = bad photos

poly A - good clear photo

cand RNA ~ DNA s[illegible] regular base pairing

except A:A

Largest issues are

esp. on organisms based on feasibility of manipulation methods; but [illegible] like eggs.

1. Availability of test systems for DNA, RNA.
2. Concentration of analytic effort on the assay systems.

specificity absolutely essential.

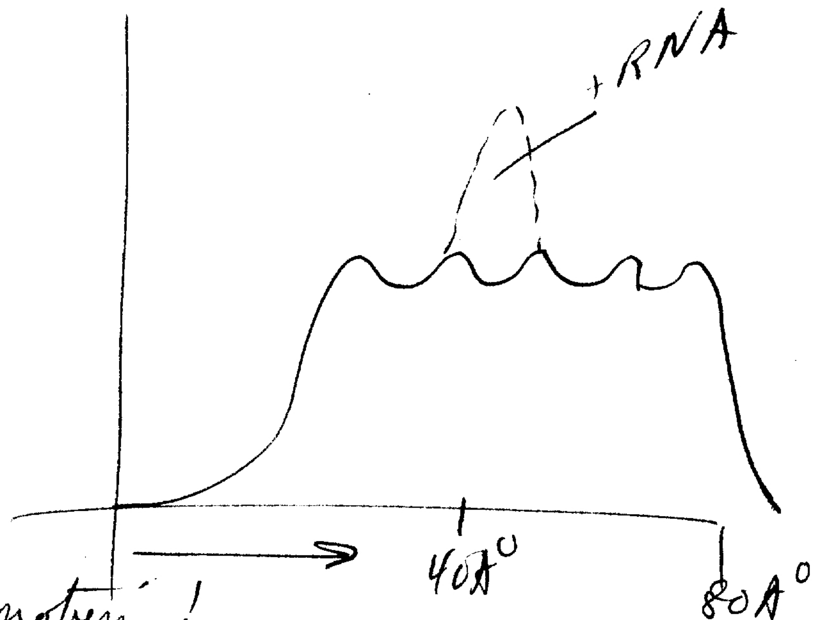
phage
Transformations (now [illegible] like to call them DNA-mediated)

Rich — mixing poly A poly U interact at 1: ratio
as measured by specific absorption. as well as sedimentation.

possibility of base pairs as well as others.

Franklin:

density diagrams
of TMV
and TMV.

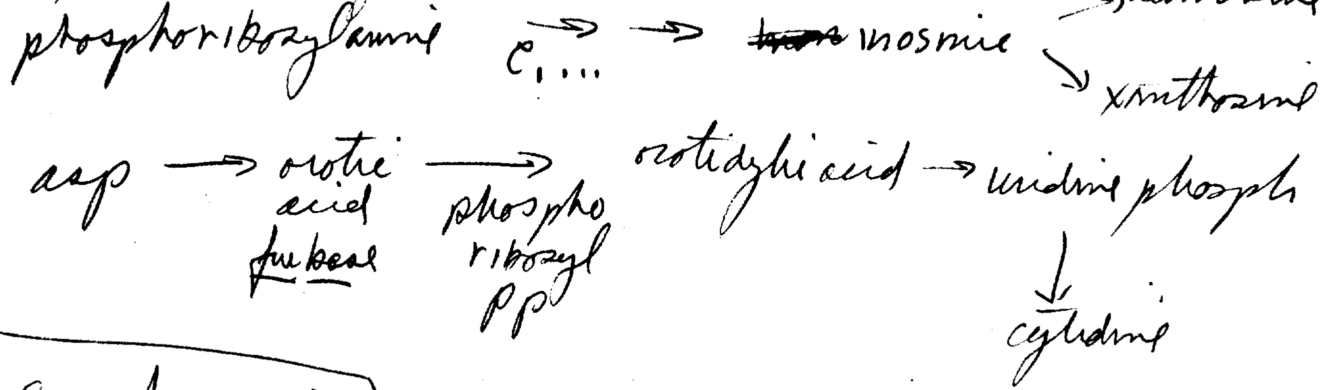


∴ not core but RNA is
wound into peripheral protein!

- probably single strand if fully ordered., not ev. RNA.

Low: { many helices.
globular proteins any here?

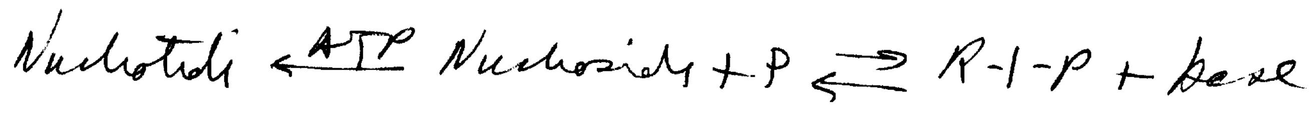
Art Kornberg . jigsaw puzzles
– ingenuity, bolstering courage, unlikelihood.
Enzymatic synthesis of nucleotides :



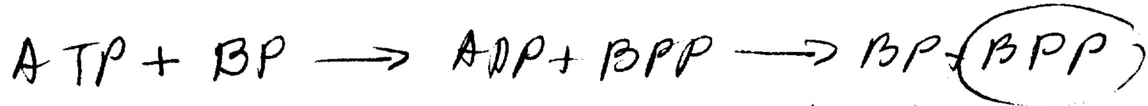
i.e. no free purines or pyrimidines

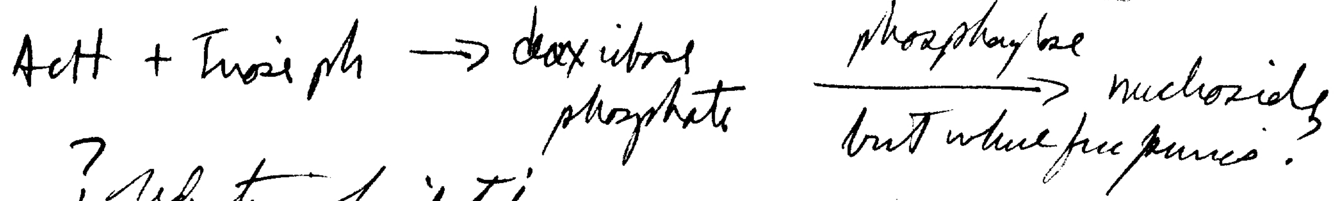
O → uridine typical
L. arabinosus works through uracil → uridine phosph.

"salvage pathways" HX, G, A or U $\underset{PRPP}{\rightleftarrows}$ nucleotide

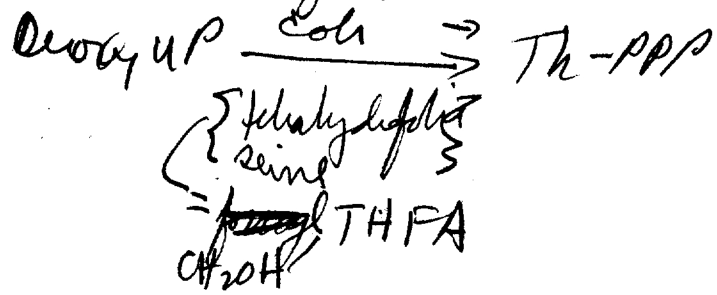


Nucleotide –P –PP } needed for further synthesis ; Kalckar reaction :

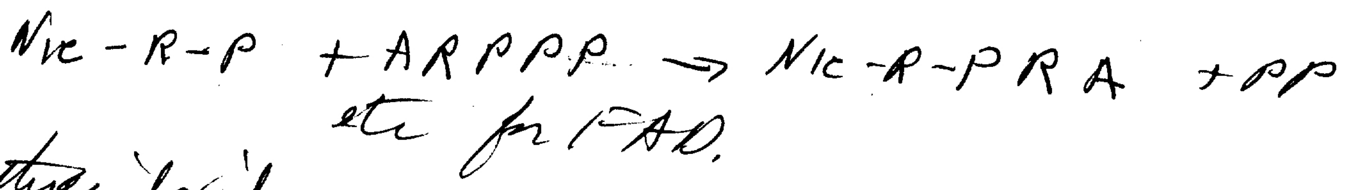




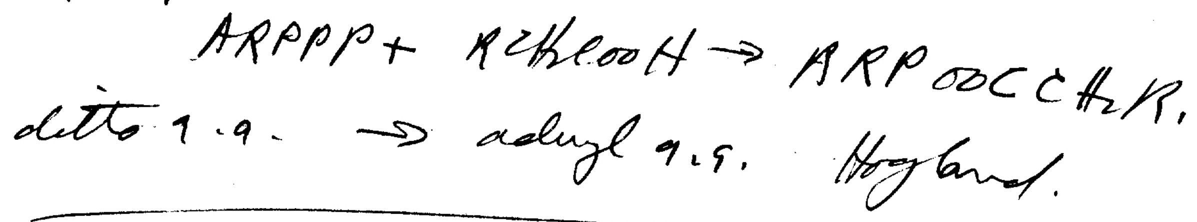
? reduction of ribotides..



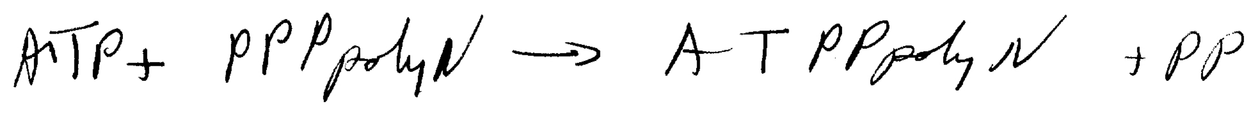
of coenzyme synthesis
nucleotidyl-residue reaction.



fattyacid oxid.

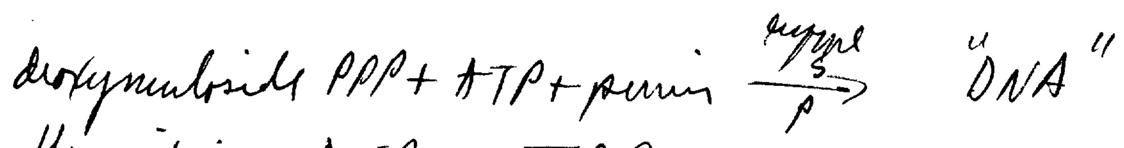


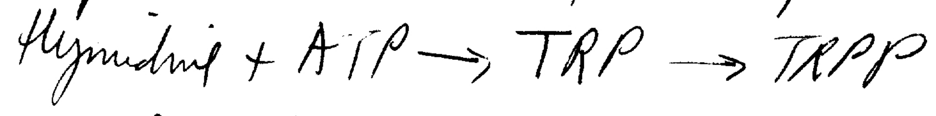
? polynucleotide



C14 ATP → NA. Then Azotobacter Ochoa / Littauer purified a coli enzyme
~~nucl~~ NRPP → "RNA".

DNA:

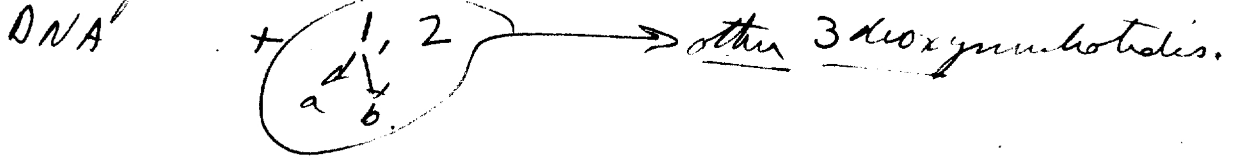




TDP as effective as TTP.
mixtures of base TP more active than single. All 4 together do react
somewhat of added primer (about 1%).

primer

DNA - primer ~~heated~~ rich in
crude coli fraction ↑, rich in DNA. Recently purified. Broken down to nucleotide fraction + dialyzed.



now need all four components

ATP not needed. Since enzyme probably phosphorylase not needed.

Now they net synthesis: 30-50% increase.

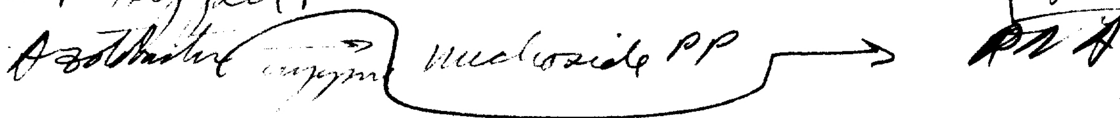
Thy RPPP is precursor.

Chargaff analysis:
coli }
Herpes } +
T2 ½

which specificity of primer DNA is primer

could biol activity increase if pure DNA were used?

Ochoa + Heppel,
Azotobacter enzyme



"polynucleotidephosphorylase"

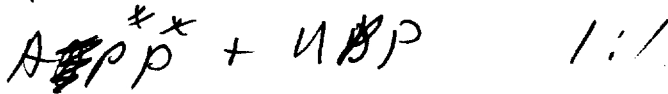
has made ADP → poly A, G, U, C etc. also

AU, AGUC

Potter had shown the other phosphates in cells.

? structure + nature of polynucleotide — structure w. is RNA (Rich)

poly AU.



[illegible] AU_n about 1:1

<u>products of RNAse.</u> [phosphodiester attacks pyr nucl. ∴ yields pyr ends.]

U 38 AU .35 AAU .20

AAAU 4.9 AAAAU 2.1

50% of X in UX sequence is A,

takes advantage of P* transfer from A to U at splitting: further sites spaced A, U, in polymer.

? Sequence is "<u>random</u>".

[meaning of <u>random</u>. random like polystyrene bottle.]

? Intracellular route. Means of <u>orientation</u>? Ks is however very low. Km 10^{-2}; these conc < intracellular. Maybe local sites of NDP formation. The enzyme is unspecific — e.g. poly HX. also unspecific in phosphorolysis: also bacterial RNA, viruses etc. rates vary. dihydrouridine DP not active as 5BrCh DP. also inactive on ribose-5-pp

Now general path is clear, but specificity is still dubious. (try virus RNA

Mechanism: primer? Most preps <u>may</u> have some nucleotide. possibly not.

~~[illegible]~~ NMP as start of chain? ✓ also

But AMP + UDP → no A in endgroups.

? one or several enzymes.

Is RNA specific exc. in plant viruses

Change of [illegible] spec.

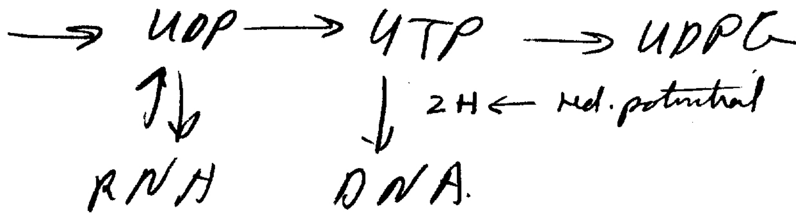
Potter: ATP → DNA ADP → RNA.

growth control. Thus ATP inhibits RNA formation:

contra biol stability of DNA.

/ irreversibility of Hornby rx Solubilized.

pomegranate labelled RNA does exchange activity } no net
liver " " does not } change



Hornby contrasts:

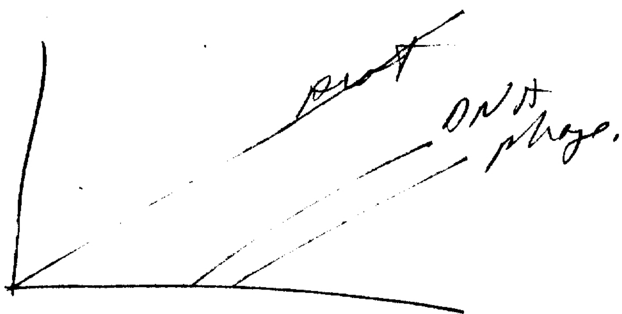
	RNA purine phosph. inhibits	DNA but not inhib.
Reversibility	+	(−)
Km	10^{-2}	10^{-5}
diversity of N	1	4
primer	− ?	+
Extent	∞	> 1½
specificity	...	ATP not usable.

Cohen – correlatable systems. point strongly made.

RNA synthesis only as energy state?

reduces almost to E coli and phage

T evens: HMC and GHMC
1. may be unwise to extrapolate
2. inf cells stop new enzyme synth. – do ghosts?
3.

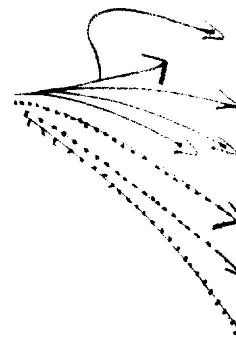


– Stent: transfer DNA inf to protein as 1/several posn.

early S35 does not migrate in T4 strain.
? repair mechanisms
5MT inhibits DNA synth. only if added at 0 lysing.
if added later, → DNA s/ further protein synth.
Rate of DNA synth ∝ time of add Chlo.

? role of protein: inform or logistic support.

some small RNA is also turned over



In B growing, thymine, thymidine ⇸ DNA.

pres. thymidylic acid.

Infected B. thymidine → DNA.

(consistent c/ Hornberg scheme)

ribose nucleotides → deoxyribotide

thymidine analogues won't inhibit B.
do inhibit virus synthesis

Do thyminuric. in wild L15?

Amino uracil → methyl purine incorp.

Br uracil inserts itself!

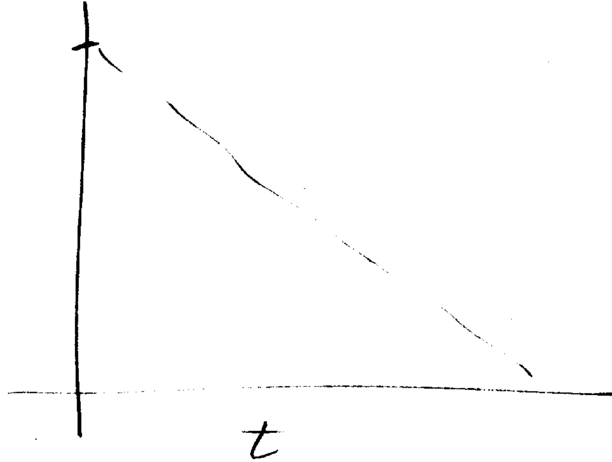
Nov + Sz. mutrate in df t; adenosine vs. aluminesh.
∴ metabolic control of genetic material
Zamenhof Bell effect – "exchange in DNA"
Adelberg $T^- h^- \to T^+ h^+$ 100× contest in deficient medium.
control of nucleotide pool a key to mutagenesis.

turnover in
nucleolus rather
than whole RNA
only fraction was ...
Vollsin
shape of pulse
curves

Vollsin spec. in granule fractions. In pulse expt
showed RNA, not DNA turnover

ask Stent ~~Seymour~~:

What's the suicide curve of T2 grown on hot bacteria, cold medium or reverse. (i.e., what is distribution of the bacterial DNA in progeny phage. ?)



Stent: rotation —
unwinding by breaks + reunions about every 5th.
separation c/replication → dispersive replication:

other schemes are conservative. keep base pairing.

c/o affects dispersion depending on mechanism.

how does protamine dissociate?
C-O can still be a copy mechanism.

does this mean second step is still semi dispersive? has 4 understand 2d expt.

1 : 200 :
par₁ → prog₁
grow hot phage₁ → prog₁

50% conservation.

what is RNA DNA?
mountains
✓Potter

W + M rabbe prog₁ → prog₂ still 50%
→ prog.

Hershey thinks just muffins.

Cy: individual autoradiography
GS: P_{32} inactivation method prog₁ – inactivation assay, as ability to ~~measure detect ability~~ to transfer P_{32} to progeny.

Cy: } big pieces Gunther { ½ is distributed to 10–20% into
½ small pieces.

i.e., same dispersion of parental atoms.

of Cy: Both methods on prog₂ < Gunther same fraction of big, small pieces

bacterial nuclei — hot cells at 1 division
assumes nucleus is unit — all cells divid.

Is nucleus one unit
Are cells 1 nucleus?
B.?
[Maxie]

Nozzi

Obligate mating for replication:

Ey: can measure 15 c/p month.

star size does not change from peg. → pg 2
semiconservative replication: 2 → 3.

Ksbey precipitation?
stars are in h^+ fraction & 90%.
Total activity? →

---

problem of bi helix — may be segments of open loops.

Baltimore Symposium Notes.

[Bridges] genes as units of co; no co in genes. (Private discussion: are there discontinuities e.g. in Drosophila data). Review Muller + Raffel.

Benzer made most of the necessary replies.

"Mary Mitchell phenomenon" — glad someone mentioned Lindegren; for a while it sounded like the new edition of the Soviet Encyclopedia on Stalin.

"classical crossing over" — implication of breakage model — possibility of copy-choice still remains.

" not necessary to assume c/o is ever intragenic.

[cf. Detroit Symp. — Science as series of conferences punctuated by short recesses in the lab.] — so many farm tours.

[Ris] — emphasized binding from chromosome fibril to chromosome.

[Benzer] — "Advanced Genetics for an Elementary Student." — main objection as a many times damned neologist: simple Anglo Saxon terms are preferable. around our lab, 'find rit, mit and pfit (Scit), ralt malt and pfalt.

Serious semantic problem & necessity of prefatory glossaries since rit defns. too often abandoned.

automotive analogy belonged to Detroit.

meaning of cit, e.g. in McClintock's material.

Comment on content: possible relative reliability of asymptotic enumeration and mapping. discontinuity in Drosophila data? cf Muller + Raffel.

[Ranney] — steady state concept in nucleus. — B+K; L+1.

[Rhoades] — why not go whole hog — or is Goldschmidt too close too?

not a semantic question: only way to satisfy "point mutation" is nucleotide substitution.

[Hartman] Reliability of 2-point data? 3-pt. better but few results yet reviewed critically by geneticists. Questions both on the enzymes & on mapping.

[Jacob] — no comment now would take all day. Lewis's red. ad absurdum: whole genome as ensemble of prophages

F. Commoner — (a) no pure clones in TMV (b) stabilize RNA c plant proteins — converse of Volkin? (c) host modification of fn

(Call Dr. Cedrian)

(10 AM Sat. Bwy — 6:50 P.M.)

[2 — #29]

[Chargaff] — nucleic vs gene.

suggestive comments

Wednesday.

[Mazia] flagella as promising contractile units

is there cytochemical evidence of SH accumulation? Implant cysteine?

[Allfrey] "Exchange reaction" — whatever that means: is there any real evidence for this, if so this might be more interesting than synthesis. Did he answer Mazia's objection? ?

DNA removal inhibited uptake — but pieces of RNA, DNA worked. Any evidence here of DNA resynthesis à la Sol?

RNA removal studied? did he mention?

[Sol] Review specificity of RNA removal
morphology of altered, inactive Stoolsates?

[Harriett - Pollins - Zamenhof] — relationship of competence waves to fission cycle

importance of clarifying quantitative aspects for evaluating L. Bendich results e.g.

discrepancy in kinetics illustrates problem. What are units of activity.

role of synapsis if, after all uni multitile subst. is source of mutation.

auxiliary conditions in Hemophilus? Clarify growth in defined media.

Species interrelations — have some examples in Salmonella, which may be connected with phage adaptation. The datum is usually

$A \to \times B$	1	$1 < 3 < 4$
$B_A \to \times A$	2	
$B_A \to \times B$	3	$3 > 1$ may select a more compatible segment
$B \to \times B$	4	recombination best explanation.

Segregation only a transient condition.

Zamenhof may be implying a dis-integrated system of DNA "molecules", — this is what Hamill had in mind calling genetic recombination.

Biol. function of "DNA" as emphasized here. Review other situations < Boivin, Demerec

v.. few papers had anything to do w/ chemistry of heredity. / a sense [illegible] may not [illegible]
have to say what anyone does - [illegible] approach.

Most genetic discussions were matters of definition; Dr. Benzer's remarks were especially refreshing. We are looking for discontinuities, either of the molecular order which we must assume as the ultimate chemical unit if DNA is accepted as the basic hereditary material. This is not the occasion to review the evidence for this thesis; several authors have cautioned that it may ultimately be not the whole story but at the present time, nothing else is in the picture (except RNA/virus).

Re Benzer - his euphonious terms have the objection only of being Greek derivatives; in our lab we had tried bit, nit and pfit; muton and cistron are fated for a longer life. At this point would adopt Dr. Lederthal's suggestion; we should have absolutely no discussion of terminology, but in a mathematical sense should insist that each author start by defining his terms. Our next papers in ~~Gould~~ Curtis's starts with a glossary & until we seriously adopt a really useful dictionary, this may not be so outlandish an idea.

Of course we may also need existence theorems. One of Benzer's contributions is that cistrons do exist as recon segments. — ~~Del. relativity of extraction~~

Drosophila has not necessarily been pushed to extremes: Hartman + Benzer's exhaustive analysis of a segment had its ancestry in Muller + Raffel's analysis of the scute - achaete region, a job that itself needs extension. Del. relativity of exhaustive enumeration + 2-point tests in any material.

On Dr. Rhoades' talk, ~~Sol~~ Dr Spiegelman asked whether this was a semantic ~~[illegible]~~ problem; of course Rhoades was asking the material question, are any mutations substitutions of nucleotides, which are the closest possible approximations to point mutations. — Sign of McClintock for pos. effect

Benzer to a point of Chargaff's query, which is really an old question in the relationship of mutons to genes? Conceivably a muton is a single nucleotide substitution, but it would be a meaningless case if desoxyribonucleic acid were a transforming agent. The meaning of the code is not in the letters or syllables, but the words and sentences. The specificity of transforming agents can best be visualized in terms of synaptic abilities; it would be worth knowing if ^unknown oligonucleotides were mutagenic in the pneumococcus.

(Sl. over)

[illegible] Meeting reminiscent of the Detroit Symposium - where Benzer would have been even more topical - seems to remind me that the sc. life these days is a lineal sequence of conferences punctuated by short spits in the laboratory.
& informational exchange = recons

somewhat platitudinous

Gale's material here and Dr. Spiegelman's here had emphasized the ease of this ingenious frontal attack (have to watch out for such words. at Detroit indefeasible became indefensible.) What does this attack mean?

① We biologists need all the help we can get from the chemists & physicists; disappointing that the most advantage is not taken of biologically active systems e.g. in the phys. chem. chemistry of pneumococcal rather than calf thymus DNA. Agreed this may need same [illegible] but would be worth.

(2) We biologists have to be more courageous about doing the impossible in bioassay — Dr Spiegelman was not denying he had been making gold.

~~E.G. Mazia's model expt c cysteine should not be too hard to duplicate in ... The RNA systems have turned out to be astonishingly ...~~

The transformists have reported encouraging progress in the quantitation of the two presently available systems; ~~the further discrepancies are somewhat disturbing, ... so in the catalytic activity of DNA which is not ensured ... I would at least ask ...~~

There are good reasons for wanting to find simpler systems, though no doubt Dr. Spiegelman could make protoplasts in Hemophilus or pneumococcus if he put his brain at it. The two systems now in T[r] are relatively uncongenial both from the viewpoint of availability of genetic markers + physiological complexity & there is little detailed analysis of their specific ... . Reports of DNA-mediated transfer in ... have appeared from time to time but no reproducible recipes are now in print. As the crucial problem may well be the <u>penetration</u> of DNA, we may be more optimistic about the use of protoplasts, such as Dr. Spiegelman has described. Unfortunately, our trials <u>so far</u> with penicillin-induced protoplasts of E coli have been entirely unrewarding.

The direct approach at the replacement of cellular organelles also has its place in the justification of models; e.g., Dr Mazia assures me it would be feasible to implant cysteine <u>in ovo</u> to try to set up ... in the centr...

I would like to take the privilege of asking some questions to clear up some points on which I was confused.

Would Dr. Sp. clarify the specificity of RNA substitution in his protoplast system? This was reminiscent of Dr. Allfrey's ~~...~~ denucleated nuclei. Any chance of DNA resynthesis here?