"The Replication of DNA"

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The Replication of DNA

The following ideas were suggested by two facts ~~suggested~~ established by Dr. A. Kornberg and communicated to me privately. ~~They are~~ In the polymerase system studied by Kornberg and his colleagues it is found that:

(1) the DNA of the virus ϕx 174, supposed to be single-stranded, acts as a good primer. The product appears to be normal two-strand DNA

(2) Some specimens of DNA when used with some specimens of the polymerase give hardly any synthesis unless the DNA is lightly attacked by ~~DNA~~ DNase.

On the basis of these facts I was led to consider ~~the~~ in detail what might be happening in the synthesis of DNA in the test-tube.

PROGRAM

October 17, 1958

5th All-University Virology Conference

Berkeley, Friday and Saturday, October 31 - November 1, 1958

Friday, October 31

Registration--outside the Board Room, State of California Department of Public Health Building, 2151 Berkeley Way, from 8:30 - 12:00 A.M.

Chairman, First Session - T. T. Crocker, Department of Medicine (SF)

9:15 - 9:35 — M. E. McClain, A. J. Hackett and T. D. Dixon
Naval Biological Laboratory (B)
"Mixed infections with polioviruses, types 2 and 3: phenotypic mixing analyzed by single cell virus yields"

9:45 - 10:05 — H. Newton and R. E. Bevis
Naval Biological Laboratory (B)
"Preliminary biophysical data on certain animal viruses following purification by a simplified technique"

10:15 - 10:30 — W. L. Bostick
Department of Pathology (SF)
"Current avenues of virus study on Hodgkin's disease"

10:40 - 11:00 — Intermission

11:00 - 11:10 — L. C. Raggi
Department of Veterinary Medicine (D)
"A macroscopic plate-test for Newcastle disease"

11:15 - 11:25 — R. L. Russell, C. J. Jann and S. Froman
Department of Bacteriology (LA)
"Phage-host relationships in lysogenic mycobacteria"

11:30 - 11:40 — S. Shadomy, S. Froman and M. J. Pickett
Departments of Bacteriology and Infectious Diseases (LA)
"Cytological studies on phage-host interactions in mycobacteria. II. Phase contrast studies of bacteriophage lysis in mycobacteria"

11:45 - 11:55 — H. J. Shadomy and O. A. Plunkett
Departments of Botany and Bacteriology (LA)
"Preliminary studies on the identification of atypical Nocardia species through application of bacteriophage"

12:05 - 12:15 — L. V. Crawford
Virus Laboratory (B)
"DNA breakdown after phage T5 infection in E. coli"

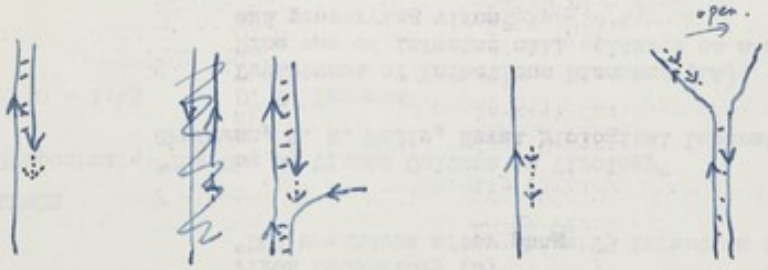
LUNCH

Symposium - "The Use of Tissue Culture in Virology"
Chairman, S. H. Madin, Naval Biological Laboratory (B)

1:30 - 1:45 — D. T. Imagawa
Department of Infectious Diseases (LA)
"The use of infected cell cultures as a method of shipping and preserving virus"

1:55 - 2:10 — M. E. McClain
Naval Biological Laboratory (B)
"Tissue culture as related to problems of assay and isolation of mammalian viruses"

Situations, for which rates are required:



ditto.

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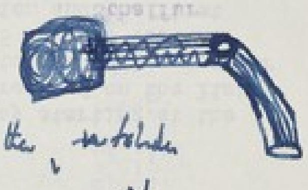
Chains folding back on themselves.

① is folding? 2 items

4 items.

② sequence to prevent any folding back?

must state minimum length.



if — then, unfolded

- - - - -A- - - - - - - . . . A'- -

- - - - A'- - - - - - - . . . A - -

October 1, 1958

Virus Laboratory Staff

Revised Wednesday Noon Speakers
Room 411 BV Lab
Beginning September 24

Two persons will speak each Wednesday starting at the top of the list. As a person's name is reached on the list, it will be that person's responsibility to see that a speaker is provided. Each speaker is allotted 15 minutes for the presentation plus 10 minutes for discussion and the first speaker will begin at 12:20 p.m.

Doctors Alava
Bonhoeffer
Bové
Bradish
Christensen
Clark
Crawford
Dixon
Froenkel-Conrat
Gan
Gish
Gordon
Haschemeyer
Kaper
Knight
Makepeace
Mattern
Moring-Claesson

Doctors Olsen
Pardee
Pratt
Raacke
Ramachandran
Ramel
Reddi
Rubin
Schachman
Schaffer
Steere
Stent
Thomas
Tsugita
Uchida
Williams
Wollman

A 1.19
U 1.05
G 1.01
C 0.74+

purine = 2.20
pyr = 1.79+) 45%

% Adenine
calc

Pyr. Ap. Cp.	.45 x .19 ≏ .09 –		.07
Pyr Ap. Up.	.45 x .25	.11	.08
Pyr. A. G. C.	.45 x .25 x .19 ~ .02		.185

claims no PyrGC !!!
lots of AGC !!!

looks wrong!

how defined?

80 γ/mg = 80/1000 ≃ 8%

8/3 × 4/1.2

no UGC
CGC
but lots of AGC

A G C

A C U G
A C G U

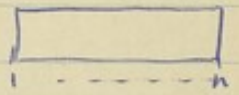
A G U G . C
G

only way!

CGC

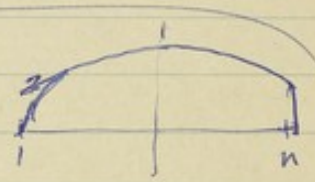
cutting function

we have taken probability function is



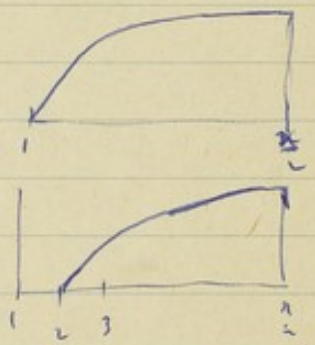
i.e. equal probability.

now consider a function of the form



we assume this form for (i.e monotonic increase from ends to middle, max at middle) for all n.

better - a function like this:



cohort is the same for all n

i.e. for all lengths a

car near an end is less likely

than one nearer the middle. or

Consider a specific example

take $n = 2$

then for $n = 3$ no need to specify.

①	2 : 2	
②	1 : 1 : 2	

1	2
2	1
	4 2
2	4 3

$n = 4$ assume 2 : 2 = pair 1.
1 : 3 pair $\frac{1}{2} \times 2$

1	2
4	1
4	4
12	12
12	12
	36
36	36
68	101

$n = 5$ assume 1 : 4 -- $\frac{1}{2}$
2 : 3 -- 1

$n = 6$ assume 1 : 5 -- $\frac{1}{2}$
2 : 4 -- 1
3 : 3 -- 1

$\frac{1}{2}$ 1. 1. 1. 3 ①
1 1 1. 1. 2. 2. ②

Then for $n = 6$

④ $\frac{1}{2}$ 1 : 5 → $\frac{1}{2}$ 1 : 1 : 4 → 1 1 : 1 : 2 : 3 ⑥ → 1 : 2 : 1 : 2 ⑥

⑱ 1 1 2 : 4 → $\frac{1}{2}$ 2. 1. 3. → 2 : 1 : 1 : 2 ⑥
1 1 2 . 2 . 2 ⑫

⑱ 1 1 3 : 3 → 1 : 2 : 1 : 2 ⑱

Problem

a chain of n links can be cut anywhere, at random; and is so cut repeatedly; ~~it~~ except that a diner cannot be cut into two monomers. one ends up with a mixture of ~~them~~ monomers and ~~by~~ diners. what are the relative ~~amount~~ amounts?

Answer: equal numbers of monomers and diners.

Proof: by induction

consider $n=3$ then → $1+2$ = equal amounts

consider $n=4$ then → $1:3$, $2:2$, $3:1$ → $1:1:2$, 2.2, $2.1.1$ = equal amounts

consider $n=5$ then → $1:4$, $2:3$ = $(1+2)+(1+2)+3|3+4|4$, $3:2$, $4:1$

↓ ↓ these go to equal amounts

assume true for all $n \leq$ ~~n~~ $n-1$ $(n \geq 6)$

consider n ∴ then divide to $n-1:1$, $n-2:2$, $n-3:3$, ⋮, $3:(n-3)$, $2:n-2$, $1:n-1$ } for to equal 1's and 2's and numbers between $(n-1)$ and 3 but all these give equal number ∴ = all give equal numbers.

Thus since true for $n=5$, is true for all n.

P.To

General theorem

polygons of n units $(n > m)$

can angulate, respectively, but a length of m units cannot be cut.

Ans: equal numbers of units, $1, 2 \ldots m$.

Proof.

Consider a length of $m+1$.

Then equally by which ever it is cut.

ie equal numbers, $1, 2 \ldots m$

$m : 1$

$(m-1) : 2$

$(m-2) : 3$

$\vdots \quad \vdots$

$2 : m-1$

$1 : m$

Now consider any length $m+2$.

cuts.

$m+1 : 1$

$m : 2$

$\vdots \quad \vdots$

ie. $(m+1) + (m+1) +$ equal numbers $\ldots$ $2 : m$

but $(m+1) \rightarrow$ equal numbers $1 : m+1$

$\therefore$ give equal numbers.

Ind. for $m+3$, etc, by induction. Q.E.D.

Replication of DNA : Kornberg's system

@2a

AUG 4 1959 . (1)

Assume ① replication only when (one) chain broken i.e. not from ends

② replication is one (chemical) direction only

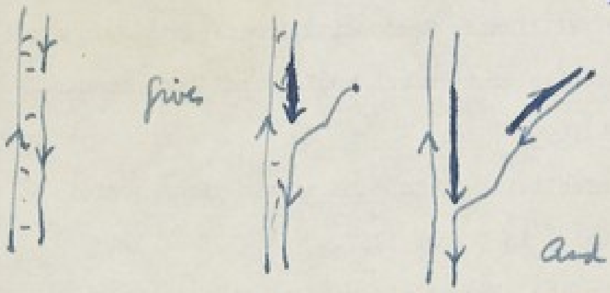
③ ~~?~~ discarded single strands can act as templates for synthesis. (assume for the moment that "primed breaks" can't be joined)

~~to~~ Thus we assume that there are certain number of breaks ~~and that~~ where replication starts : we want to know MW ~~of~~ (etc) of product for a given distribution of breaks.

Gen Ltr
says only
not less than
by enzymes

Query : does it matter in kis system whether a 3' or a 5' phosphate (or no phosphate) exists at the point of break?

One break



gives ... and eventually

i.e. one copy of original MW
one copy of MW of size of broken chain beyond the break.

Alexander, H., and G. Leidy: Determination of inherited traits of H. influenzae by desoxyribonucleic acid fractions isolated from type-specific cells. J. Exp. Med., 93:345, 1951.

Stanley, W. M.: Biochemistry and biophysics of viruses. In Handbuch der Virusforschung. (R. Doerr and C. Hallauer, eds.) Julius Springer. Berlin. 1938.

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Markham, R., and K. M. Smith: Studies on the virus of turnip yellow mosaic. Parasitology, 39:330, 1949.

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Fraenkel-Conrat, H.: Effect of pyrophosphate on reconstitution of TMV. American Chemical Society Abstracts, 131st Natl. Meeting, April, 1957.

Sue

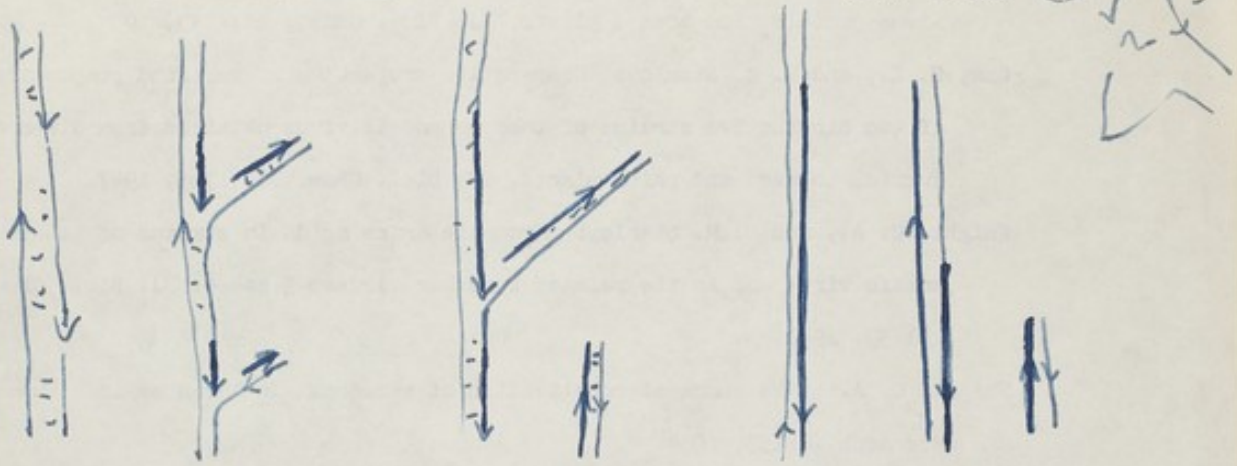
TH-3-1622

Sue

Ervin

Two breaks in one chain

AUG 4 1959 (2)



And we can then generalize to n breaks in one chain

(1) n+1 product molecules

(2) Mw of each. from the break (near end or the) to the far end.

(3) Less material : all new except

(a) one of the unbroken chain

(b) the fragment of the old chain : the first of them stays with the unbroken chain : the others is on each on the end of the product molecule : only how the last fragment is joined to a new material.

total amount of replication : (work out)

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Loring, H. S., and W. M. Stanley: Isolation of crystalline tobacco mosaic virus protein from tomato plants. J. Biol. Chem., 117:733, 1937.

Gaw, H. Z., and W. M. Stanley: Comparative properties of purified preparations of two distinctive strains of tobacco mosaic virus obtained from diseased Turkish tobacco and phlox plants. J. Biol. Chem., 167:765, 1947.

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Stanley, W. M.: Isolation of a crystalline protein possessing the properties of tobacco-mosaic virus. Science, 81:644, 1935.

Bawden, F. C., N. W. Pirie, J. D. Bernal and I. Fankuchen: Liquid crystalline substances from virus-infected plants. Nature, 138:1051, 1936.

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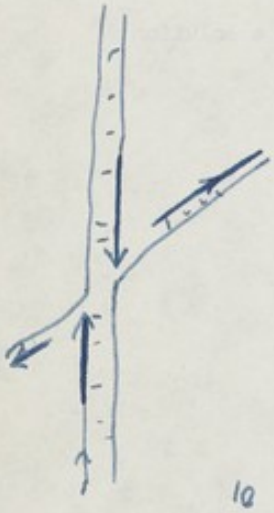
one break at each chain

AUG 4 1959 (?)

case A : before pairing ie.

two pieces :



to pair

ie two molecules :

ie two molecules : pair those of the two pieces beyond the breaks.

pieces before the breaks stay attached and have new material added.

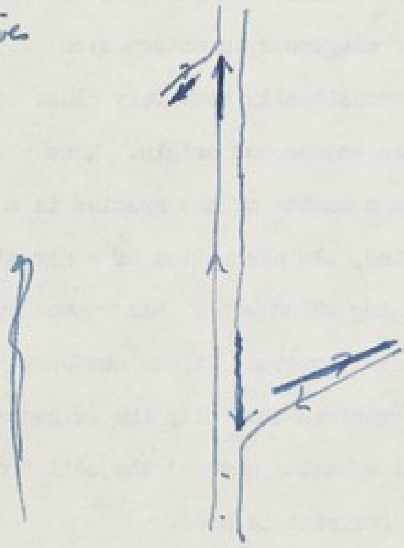
total replication. 2 x 2

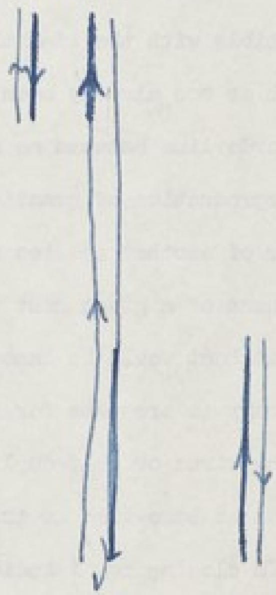
highly specific polypeptide. It provides a much more sound experimental as well as theoretical base for contemplating the existence of the myriads of living things on earth. It is a discovery that is affecting the thinking and the course of events in virus research and all that virus research is concerned with, such as genetics, infectious disease, cancer and life itself. Eventually chemists should be able to synthesize a small polynucleotide specifically arranged, hence one may now dare to think of synthesizing in the biochemical laboratory a structure possessing genetic continuity and of all the tremendous implications of such an accomplishment. Studies of this nature and related studies could easily lead directly to the heart of the cancer problem and provide a solution to a situation which today appears so very perplexing.

Case B: "other pairings" i.e.

AUG 4 1959 (4)

This gives





i.e. three molecules.

total replicate < ×2

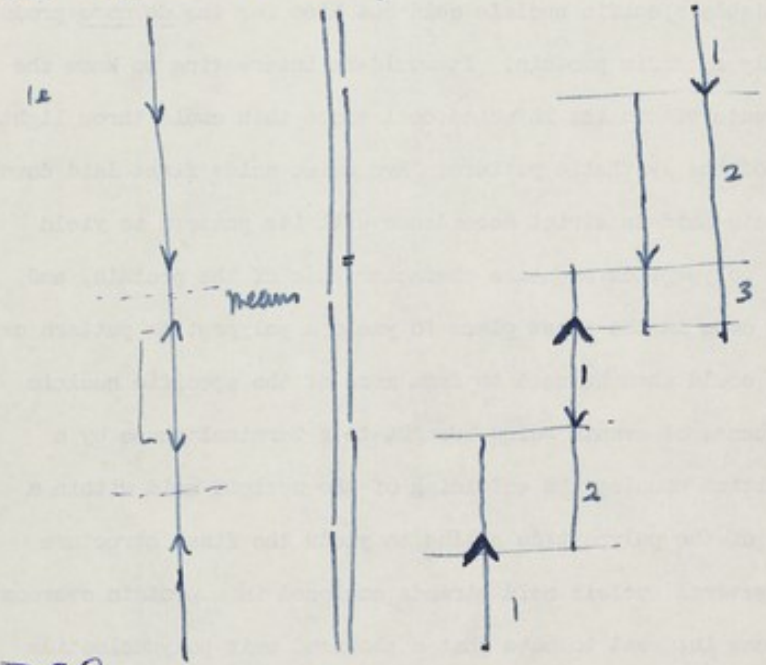
questions of "self" or of "not self" and of "essentiality" are of the utmost importance. Fortunately we now have many powerful devices for characterizing biological entities, hence the demonstration of the existence of a "foreign" or of a "non-essential" component of a cell is far easier than it was a few years ago. Even so Knight (1946) and Beard and associates (Eckert, Sharp, Beard, Green and Beard, 1955) have found purified preparations of influenza and of myeloblastic leukosis viruses, respectively, to retain antigenic components characteristic of the host in which the virus was grown. In time it may prove possible to separate active nucleic acids from these preparations. I would hope that in order to avoid utter confusion the experimental results will continue to be compatible with the idea that viruses are exogenous in nature even though as has already been indicated we occasionally come very close to the borderline between an exogenous and an endogenous origin. Thus while the reproduction of genetic material from a member of one species in a member of another species might be accepted, the aberration of a normal component of a given host to yield something which would then reproduce in that host would be incompatible. So far, however, it has not been necessary to trespass for in every case examined carefully the evidence for the virus or self-duplicating mechanism having entered the cell from without at some time in the immediate or far past is good.

In closing may I indicate that I believe that the finding of Fraenkel-Conrat and of Gierer and Schramm that virus activity may reside in a nucleic acid represents a discovery of the first magnitude, for it means that a polynucleotide of comprehendible structural complexity can carry the code or informational pattern not only for the production of more of the same polynucleotide but also for the de novo production of a

Rules for writing down products for multiple breaks.

AUG 4 1959 (5)

① draw breaks, putting arrows to show direction (this allows one line to be used)



→ 2(c)

② join together any arrows ⇕ in this situation (as above)
ie to give a "pair"

③ draw products : one for each arrow, or "pair" of arrows.
beginning of chain is either the end or the two low arrows in the same direction
end of chain is near arrow in the same direction.

P.S. 2(c) add arrows at either end if the low arrows or either end points there ends

tobacco mosaic virus nucleic acid results in the production of the normal TMV nucleoprotein containing no histidine or methionine whereas inoculation of the nucleic acid from the ribgrass strain results in the production of a serologically distinct nucleoprotein containing these two amino acids. The nucleic acid obviously provides the pattern not only for the production of more highly specific nucleic acid but also for the de novo production of a highly specific protein. It would be interesting to know the sequence of events within the infected cell since this could throw light on the nature of the synthetic pattern. Are amino acids first laid down along the nucleic acid in strict accordance with its pattern to yield eventually the polypeptide sequence characteristic of the protein, and, if so, is this done in the first place to yield a polypeptide pattern or template which could then be used to form more of the specific nucleic acid--this sequence of events being brought to a terminal phase by a metabolic situation causing the enfolding of the nucleic acid within a super assembly of the polypeptide chains to yield the final structure consisting of several nucleic acid strands enclosed in a protein overcoat? It may be of some interest to note that a thousand unit polynucleotide linear chain having the same base composition as TMV nucleic acid could exist in about 10^{590} different arrangements. This number is so large that it is practically incomprehensible. Even a one hundred unit chain of this composition could exist in about 10^{57} different arrangements, and this number is about the same as the total number of electrons on and within the earth and vastly larger than the total of all of the living things on earth. It is obvious that insofar as the possibility of coding information relative to nucleic acid and protein synthesis is concerned, nature has provided a tremendous safety factor if a nucleic acid of

AUG 4 1959 (6)

one break

then → →

gives → "→

+ → ∴ average is $\times 1\frac{1}{2}$

two breaks (and ← ←)

results → → → gives → → → $\times 2$ (double ends)

or → ← gives → ← $\times 1\frac{1}{2}$

or ← ↔ → gives ← → ↔ $\times 1\frac{1}{2}$

∴ aver. $\times 1\frac{3}{4}$

Let distances go from 0 to 1. (x from one end,)

if one lesion is at x, then average is

and other lesion at y. $= 1 + (1-x) + (1-y)$

$3-(x+y)$

So $\bar{x} = \frac{1}{2}$, $\bar{y} = \frac{1}{2}$ ∴ $= 2$

add these two cases. ∴ sum has average of $\times 1\frac{1}{2}$

→ MW

average no. of pieces $= \frac{11}{4} = 2\frac{3}{4}$

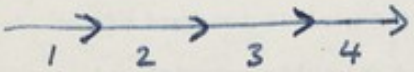
$\frac{7}{4} \times \frac{4}{11} = \frac{7}{11}$ c .64

treatment of tobacco mosaic virus with phenol. In this case, however, the results, which have been confirmed in our laboratory, indicated that the molecular weight of the nucleic acid was around one million instead of the 300,000 or so obtained when detergent was used. It would appear that the use of phenol causes the TMV nucleic acid to come out in essentially one piece whereas detergent causes this unit to break down into about 10 or 12 smaller, yet infectious units. Both groups have found that the virus activity of the nucleic acid preparations, in marked contrast to that of the intact tobacco mosaic virus nucleoprotein rods 15 by 300 mμ in size, was very sensitive to the enzyme ribonuclease and to storage in salt solutions; it was not readily sedimented in the centrifuge and it was not affected by antiserum to tobacco mosaic virus (Fraenkel-Conrat, 1957; Schramm, 1957).

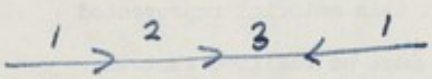
Recently in our laboratory nucleic acid preparations have been obtained possessing potential virus activity as demonstrated by reconstitution with protein equivalent to about one half of that originally present in the tobacco mosaic virus nucleoprotein used as starting material (Fraenkel-Conrat, 1957). One may, therefore, reach the very important conclusion that the intact virus nucleoprotein rod 15 by 300 mμ in size and 50 million in molecular weight is not absolutely necessary but that all of the stored up information of this structure is actually contained within a ribonucleic acid molecule of around 300,000 molecular weight and hence containing about 1000 nucleotides. This nucleic acid may be prepared sufficiently pure and of sufficiently high biological activity to insure that the activity must be due to nucleic acid structure and not to traces of nucleoprotein or of peptides. The activity may be demonstrated quite readily on direct inoculation of susceptible hosts with the nucleic acid preparation although because of the instability of

three breaks

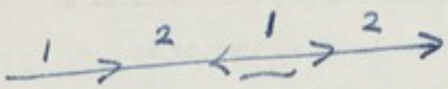
AUG 4 1959 (7)



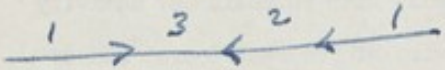
average = ~~1~~ $\frac{10}{4}$ $\frac{15}{6}$



average = $\frac{7}{4}$ $\frac{12}{6}$



= $\frac{6}{4}$ $\frac{9}{6}$



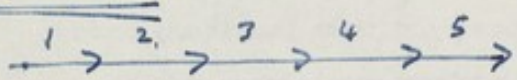
= $\frac{7}{4}$ $\frac{12}{6}$

$\frac{48}{24} = 2$

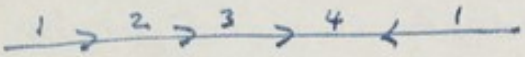
plus see still all reversed.
('See ~~over~~ next sheet)

aver = $\frac{30}{16}$ = ~~1 7/8~~

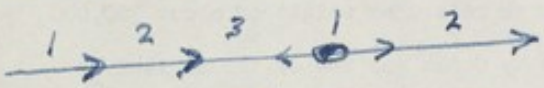
four breaks



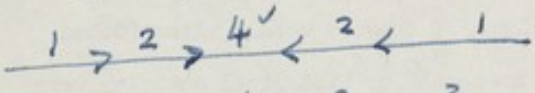
av. $\frac{15}{5}$



av. $\frac{11}{5}$

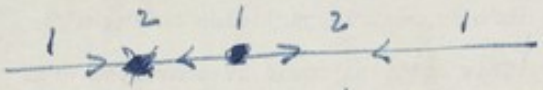


~~9~~ 45

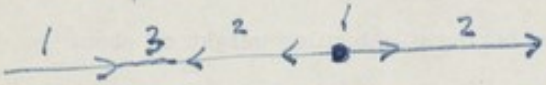


10/45

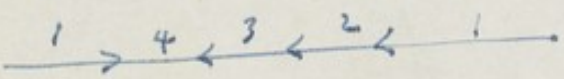
9/45



7/45



~~9~~ 45



11/45

total no. of pieces.

pieces	
8	
4	
20	
18	
16	
81	
1	

~~81~~ 81 / 15 x 8 = 81/40

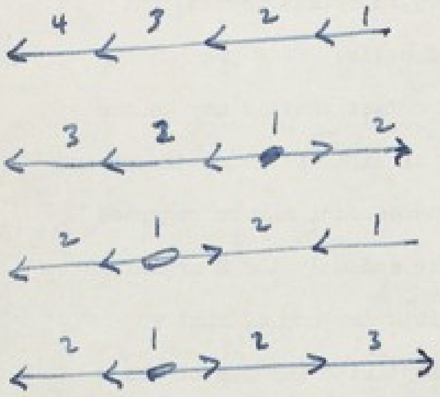
+ eight more (See next sheet)

ghosts from the infected bacteria the sulfur was found with the phage ghosts whereas the phosphorus was found within the infected bacterial cells. From this it was inferred that the material in the head of the phage, presumably deoxyribonucleic acid, and only this material had been introduced into the bacteria and hence that this material represented the actual infecting element. However, it must be realized that the introduction of some protein was not and still has not been excluded so that this experiment is still not on the same level as that of the transforming principle. This is especially so since all attempts to separate the deoxyribonucleic acid chemically with retention of activity have failed as yet. Therefore, the discovery of Fraenkel-Conrat and of Gierer and Schramm that preparations of the nucleic acid of tobacco mosaic virus can carry virus activity is a most important one, since this is the first time that this has been achieved with a regularly accepted virus.

In view of the task which I have accepted to consider the potential significance of nucleic acids in malignancy, I think that it may prove useful to examine this experiment with tobacco mosaic virus and the nature and properties of the biologically active nucleic acid. The first isolation of ribonucleic acid of high molecular weight of about 300,000 from tobacco mosaic virus was made by Cohen and the writer in 1942 by very short heat treatment of a solution of tobacco mosaic virus (Cohen and Stanley, 1942). Unfortunately no tests for biological activity were made on this material. The nucleic acid was found to be highly asymmetric and to decompose spontaneously to form asymmetric particles having a molecular weight of about 60,000. This material could be converted with cold 5 percent alkali to particles having a molecular weight of about

BUT reversed sets not necessarily the same. check

check for three breaks



: 10 $\frac{15}{6}$

: 8 $\frac{11}{6}$

: 6 $\frac{9}{6}$

: 8 $\frac{11}{6}$

32 ~~30~~ $\frac{46}{24}$ ~~[illegible]~~ !

AUG 4 1959 (8)

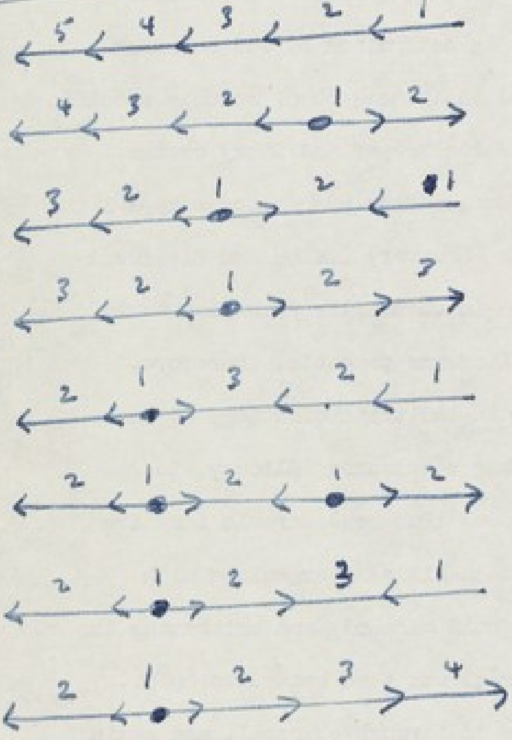
total no of pieces (= active arrows) = 13 + ~~15~~ 15 = 28

average no of pieces = $\frac{28}{8}$ = $3\frac{1}{2}$

average is $\frac{31}{16}$

no. av. AvW = ~~[illegible]~~

four breaks



15
12
9
11
9
8
9
12

85 !!

$\frac{85}{40}$

pieces

~~34~~
32
36
68

23
15
16
16
15
85

average no of pieces is $\frac{68}{16} = \frac{17}{4} = 4\frac{1}{4}$

∴ average is $\frac{83}{40}$

no. av AvW = ~~[illegible]~~ 49

organisms that is normally absent from R type cells, but that when added to such cells induces their conversion into the same type of S organisms from which the factor was derived, with the very important result that more of the factor is produced in the induced S cells. This phenomenon is virus-like, and it is because of this and the fact that it may become important from the standpoint of the chemistry of viruses that a discussion is included here. The various type-specific pneumococci may be regarded as cells infected with different 'virus' strains and only the R organisms as healthy. The R organisms may be converted into any one of what we refer to as type-specific organisms by 'infection' with any one of the different 'viruses'. By appropriate treatment it is again possible to free the pneumococci of 'virus' and secure the healthy R type. It is of interest, therefore, to examine the nature of this factor or 'virus'. The type-specificity of the pneumococcus is determined by its capsular polysaccharide, hence it might be assumed that the type of soluble specific substance or polysaccharide isolated by Heidelberger and Avery or the acetyl derivative isolated by Avery and Goebel from pneumococcus type 1 might be responsible for this conversion. However, Dawson and Sia found that the specific capsular polysaccharide in chemically pure form would not induce the transformation in type. It seems probable, therefore, that, if the polysaccharide plays a role in the transformation, it does so only when in combination with some other substance. Alloway, in attempting to purify the active agent, found that considerable inactive material could be removed by dissolving heat-killed S organisms with sodium desoxycholate, precipitating with cold alcohol, and extracting the precipitate with salt solution. The extract was then heated to 60°C., centrifuged, the supernatant liquid filtered through charcoal, and again

AUG 6 1959 (1)

The time course of MW (assume that single chain acts independently as a primer)

one break (assume rate limited by breaks ie excess enzyme) ($x < 1$)

then will eventually give MW $= M_0$ and $M_0 x$

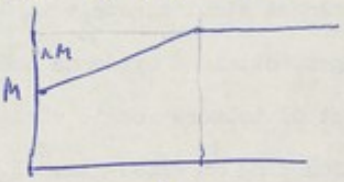
Let v = appropriate velocity constant. $V = \frac{\text{mol. wt}}{\text{time}}$ (for one break per molecule)

Then total MW ~~$M(1 + vt)$~~ up to $vt = M_0 x$

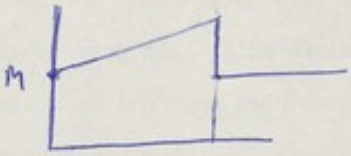
$= M_0 + vt$

this is actually the MW as used in the case.

$\therefore$ total MW proc. total MW



mean MW $=$



Now for a population, where x takes all values equally between 0 and 1, we get:

Consider a time $t' = \frac{M_0 x'}{v}$:

Then all molecules with $x < x'$ will have finished

$x > x'$ will have synthesized $M = vt'$

Thus total MW. $= M_0 + \int_0^{x'} M_0 x \, dx + (x' - x') vt'$

$= M_0 + \frac{1}{2} M_0 x'^2 + (1 - x') M_0 x'$

$= M_0 (1 + x' - \frac{1}{2} x'^2)$

years ago. There has never been a time in the past which is the equal of the present with respect to rich opportunities to explore experimentally the possibility that human cancer is wholly or in part due to the activities of viruses.

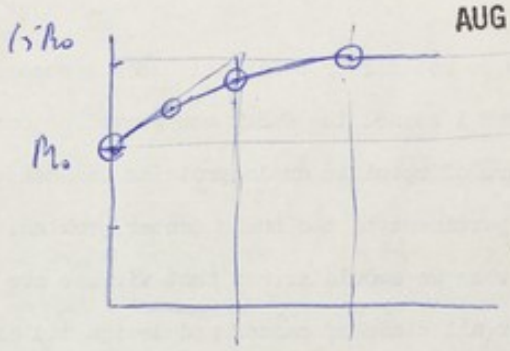
Following the isolation of tobacco mosaic virus in the form of a crystallizable nucleoprotein (Stanley, 1935; Bawden, Pirie, Bernal and Fankuchen, 1936), many viruses were characterized as nucleoproteins or as more complex structures having added carbohydrate and lipid and in some cases a complex morphology involving among other things limiting membranes (Stanley, 1947; Bawden, 1950; Luria, 1953). For a long time viruses were regarded as structures at least as complex as a nucleoprotein. Recently, however, my colleague, Fraenkel-Conrat (1956), at the Berkeley Virus Laboratory and almost simultaneously in Germany, Gierer and Schramm (1956) of Tubingen, obtained evidence that nucleic acid obtained by special treatment of tobacco mosaic virus possessed virus activity. This result, astounding at first glance, served to bring into sharp focus the role of nucleic acid in virus activity and, because of my earlier remarks, also the possible role of nucleic acid in malignancy. Actually, the idea that nucleic acid might possess virus activity is not new since genes have sometimes been regarded as nucleic acid and the actions of genes and viruses have long been regarded as being similar in quality. Then the transforming principles of the pneumococcus (Avery, MacLeod and McCarty, 1944) and of Hemophilus influenzae (Alexander and Leidy, 1951) have been found to be deoxyribonucleic acids. It may be of some interest that twenty years ago, before the discovery of the chemical nature of the transforming principle, I was inclined to regard this activity as virus-like (Stanley, 1938) for in 1937 I wrote: "It is obvious that there is a factor which may be obtained from any one of the S type of

AUG 6 1959 (2)

1½-

1.500
.125
1.375

1.2500
.003



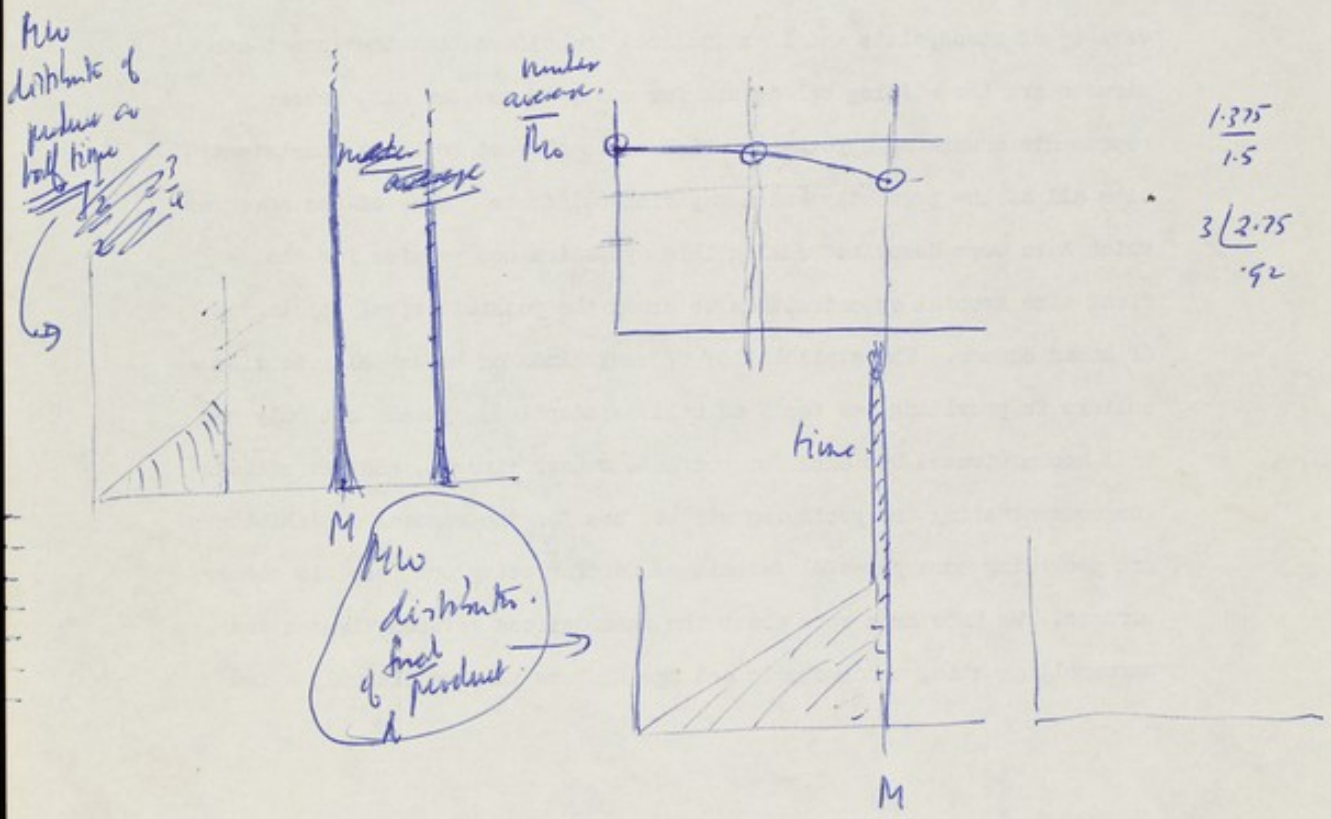
MW

number-average mol weight

time. (enzyme is excess)

$$= M_0(2-x')M_0x'$$

$$= \frac{M_0(1+x'-x'^2) + \frac{1}{2}M_0x'^2}{1+x'} = \frac{M_0(1+x'-\frac{1}{2}x'^2)}{1+x'}$$



1.375
1.5

3 | 2.75
.92

generally do not differ strikingly as one goes from one species to another and I regard the fact, now proved beyond contention, that viruses in the form of specific nucleoproteins can cause cancer in animals to be directly pertinent to the human cancer problem. I believe that the time has come when we should assume that viruses are responsible for most, if not for all kinds of cancer and design and execute our experiments accordingly. Cancer is basically a problem in growth and there is no reason to believe the growth of most human cells is different basically from the growth of most animal cells. Acceptance of the viral etiology of human cancer as a working hypothesis will involve a marked change in attitude on the part of many investigators, but this will be necessary if the right approach and the right design of experimentation are to result. What we do in the laboratory depends in large measure upon what we think. Previous speakers have discussed viral-cancer relationships from a variety of standpoints and I am inclined to believe that the idea that viruses are the etiological agents for most, if not for all, cancer represents a working hypothesis which comes closest to being consistent with all of the presently known experimental facts. Many of the advances which have been described during this symposium now provide for the first time special opportunities to study the possible viral etiology of human cancer. The availability of many kinds of human cells in tissue culture is providing new test, as well as adaptive, systems not only for well known viruses but also for possible cancer viruses, and new methods for concentrating and purifying viruses and for the removal of inhibitors are providing more powerful techniques for ferreting out possible cancer viruses. We know much more about the interactions between viruses and hormonal, genetic, carcinogenic and age factors than we did only a few

AUG 6 1959 (3)

no of final product molecules for n breaks on all molecules distributed in position on each molecule at random

assuming rules of page (5), 4 Aug.

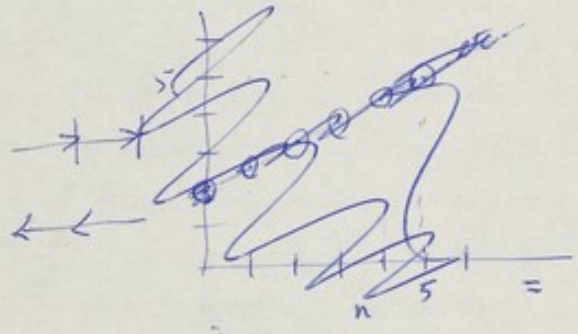
Then no of products = no of initial arrows
= "effective arrows" plus no. of extra arrows added at the ends
minus no. of pairs of arrows i.e. (←→)

we have 2^n arrows, all equally probable: to get sum of effective arrows we note:

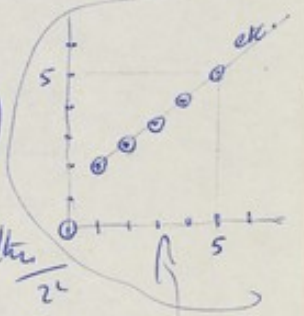
$2^n . n$ $\quad n \geq 1$

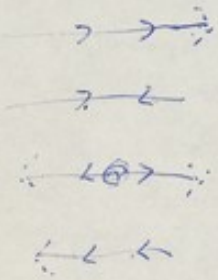
$+ \; 2 . 2^n . \frac{1}{2}$ $\quad n \geq 1$

$- \; 2^n \frac{(n-1)}{4}$ $\quad n \geq 1$



$= 2^n \left(n + 1 - \frac{n-1}{4} \right)$





$\therefore$ mean no. of products = $\frac{\text{sum}}{2^n}$

$\frac{11}{4} = 2\frac{3}{4}$

$= \left(n+1 - \frac{n-1}{4} \right) = \frac{3}{4} n + 1\frac{1}{4}$ $\quad n \geq 1$

n	0	1	2	3	4	5	6	-	-
mean no. of final products	0	2	$2\frac{3}{4}$ ✓	$3\frac{1}{2}$	$4\frac{1}{4}$	5	$5\frac{3}{4}$	-	-

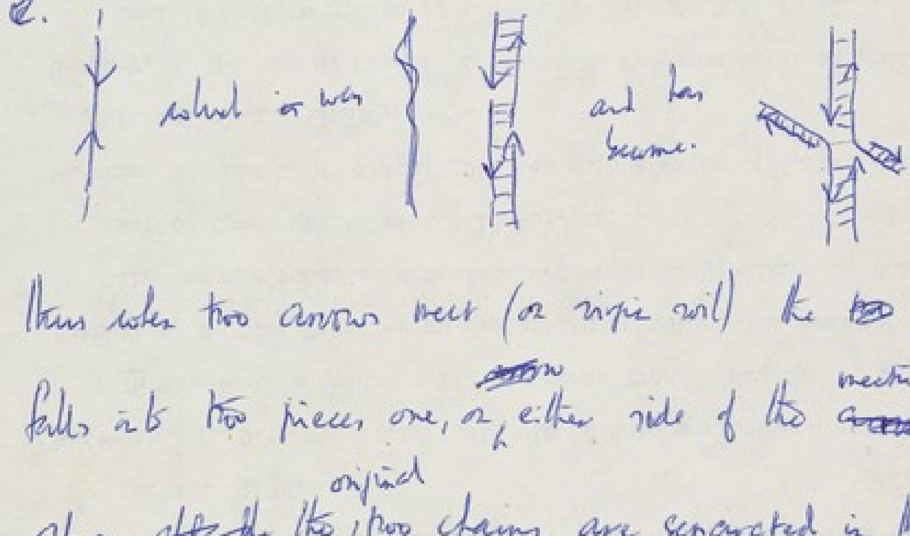
to yield new strains and that growth in different hosts or under different conditions may result in a newly formed mutant strain growing out preferentially and despite the general plasticity of viruses, it must be concluded that a given virus preparation may consist of a nucleoprotein of definite and specific composition. At the same time it must be realized that all virus preparations are somewhat impure in that by the very nature of their production they probably contain greater or lesser amounts of mutants. The actual mass of such mutants in a properly prepared virus sample is probably infinitesimally small, say 0.01 percent or less, and hence of no real concern from an analytical standpoint.

Previous speakers have shown quite conclusively that some malignancies may be caused by viruses, hence it follows directly that nucleoproteins of specific composition have great significance in malignancy and that insofar as nucleoproteins are concerned the word "potential" in the title should be removed. However, it may be well to point out that the significance of this relationship with respect to malignancies of man is badly in need of experimental support. Only two little studied and benign virus induced tumors of man are known and viruses have not as yet been seriously implicated in human cancer as generally experienced. This may be due to the fact that few really serious attempts to demonstrate experimentally the presence of a virus in human cancer have been made and to a general reluctance to accept viruses as being of etiological importance in cancers of man. I must say that I continue to be amazed at the willingness of so many investigators to accept viruses as etiological agents for animal cancers and their unwillingness to consider them of potential etiological importance in cancers of man. It should be recognized that cancer is a biological and not a uniquely human problem. Basic biological phenomena

Proof of rules of page ⑤ 4 August

Let arrows move to represent growing point.

~~Hence (?) understood too~~ We assume all arrows move at equal rates. Thus no two arrows in the same direction can overtake each other. Thus consider what happens when two arrows meet (no arrows having traversed the ground before) This must come from the situation



Thus when two arrows meet (or ripple wil) the ~~...~~ molecule falls into two pieces one, on either side of the ~~arrow~~ meeting place.

also ~~attach~~ the original two chains are separated in the region between two arrows pointing at each other.

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Double choice Schemes

(1) 18 Aug 59

One fault

It seems that if we have the situation

this can go

but then could go valley with chance ½

and then goes

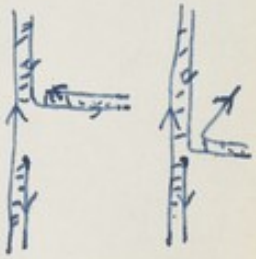
finally.

in this case the fault is preserved.

This a 50% chance of preserving the fault.

1st ½(½) + ½(½)

2nd



October 1, 1958

Virus Laboratory Staff

Revised Wednesday Noon Speakers
Room 411 BV Lab
Beginning September 24

Two persons will speak each Wednesday starting at the top of the list. As a person's name is reached on the list, it will be that person's responsibility to see that a speaker is provided. Each speaker is allotted 15 minutes for the presentation plus 10 minutes for discussion and the first speaker will begin at 12:20 p.m.

Doctors	Alava	Doctors	Olsen
	Bonhoeffer		Pardee
	Bové		Pratt
	Bradish		Raacke
	Christensen		Ramachandran
	Clark		Ramel
	Crawford		Reddi
	Dixon		Rubin
	Fraenkel-Conrat		Schachman
	Gan		Schaffer
	Gish		Steere
	Gordon		Stent
	Haschemeyer		Thomas
	Kaper		Tsugita
	Knight		Uchida
	Makepeace		Williams
	Mattern		Wollman
	Moring-Claesson		

Let faults be x times on each end. (neglecting the "log increase") ② 18 Aug 59

1st round of replication — increase each time — amount with fault.

$\frac{1}{2}(1-x) + \frac{1}{2}(x)$ — $\frac{1}{2}$

$= \frac{1}{2}$

$\frac{1}{2}\cdot\frac{1}{2}$ — $\frac{1}{2}\cdot\frac{1}{2}$

etc

Then total amount is $1 + \frac{1}{2} + \frac{1}{4} + \frac{1}{8} \ldots = 2$

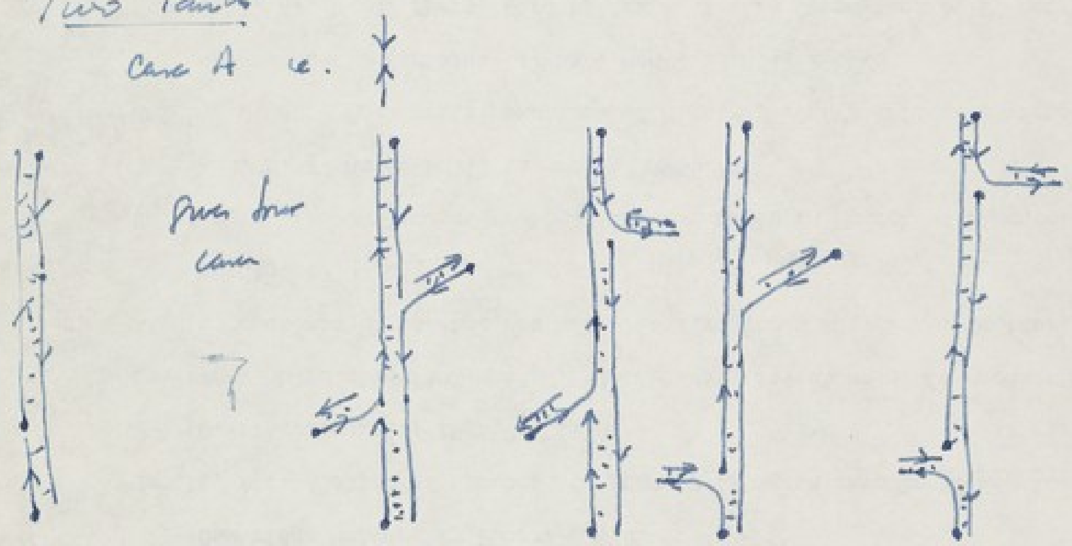
We start with 1 complete molecule

1 ~~½ two~~ unwinding of molecule in the new part.

no of ie spreading molecules $= \times 3$

Two Faults

Case A ie.



gives four cases

Fraenkel-Conrat, H., and B. Singer: Virus reconstitution. II. Combination of protein and nucleic acid from different strains. Biochim. et Biophys. Acta, in press. 1957.

Lwoff, A.: The nature of phage reproduction. In The Nature of Virus Multiplication. (Sir Paul Fildes and W. E. Van Heyningen, eds.) Cambridge University Press. 1952.

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Lwoff, A., L. Siminovitch, and N. Kjeldgaard: Induction de la production de bactériophages chez une bacterie lysogène. Ann. Inst. Pasteur, 79:815, 1950.

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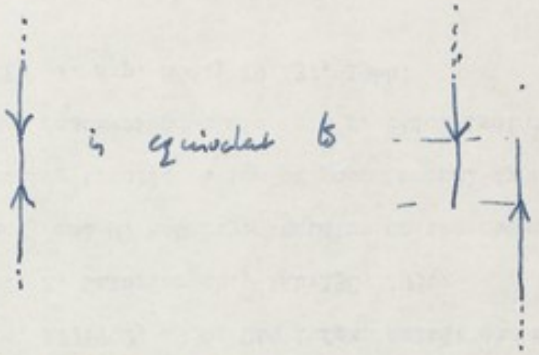
Re Proof of Rules (cont)

Thus the situation  is equivalent to

in every respect except that

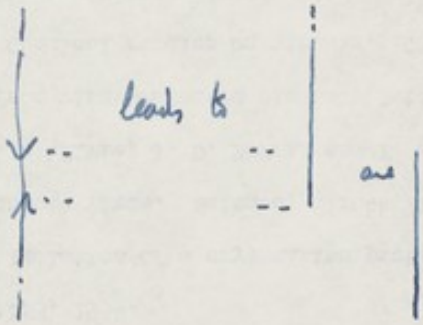
in many respects

has to be written

since it leads to

and

another rule



leads to

are

no matter which follows other ?

Thus the rule is : when two arrows pass together, separate the chains at the midpoint but don't add arrow to the end: merely lengthen the chains

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Bawden, F. C., N. W. Pirie, J. D. Bernal and I. Fankuchen: Liquid crystalline substances from virus-infected plants. Nature, 138:1051, 1936.

Stanley, W. M.: Chemical studies on viruses. Chem. and Eng. News, 25:3786, 1947.

Bawden, F. C.: Plant viruses and virus diseases. Chronica Botanica Company. Waltham. 1950.

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Gierer, A., and G. Schramm: Infectivity of ribonucleic acid from tobacco mosaic virus. Nature, 177:702, 1956; Die infektiosität der nucleinsäure aus tabakmosaikvirus. Z. Naturforsch., 11b:138, 1956.

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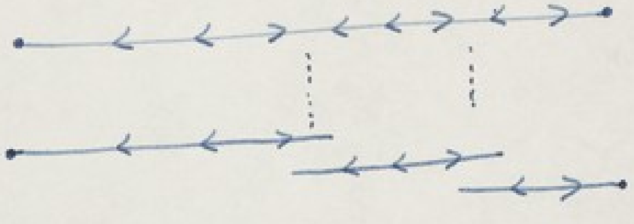
Rules (cont)

Thus any sequence of arrows is ~~left~~ "broken" at the meeting points

ie. 

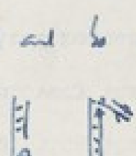
into pieces into which no arrows will meet.

As any arrow moving along separates the two original chains, and (so far) we have no means of re-uniting them. Hence Conversely if no arrow passes over them then the two original chains remain together. Thus between regions  the chains never separate: we write these as 

Neurons

Theorem: chains only separate when
(a) arrows meet
(b) an arrow reaches the (true) end

Since arrows cannot re-attach there are no other cases except ordinary growth of arrows. (b) and (a) and (b) can be proved by

Examples: (a) we have done. (b): ie.  then new arrow coming along has the same structure

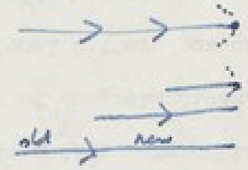
This also gives justification for arrows putting effective arrows on the true ends.

highly specific polypeptide. It provides a much more sound experimental as well as theoretical base for contemplating the existence of the myriads of living things on earth. It is a discovery that is affecting the thinking and the course of events in virus research and all that virus research is concerned with, such as genetics, infectious disease, cancer and life itself. Eventually chemists should be able to synthesize a small polynucleotide specifically arranged, hence one may now dare to think of synthesizing in the biochemical laboratory a structure possessing genetic continuity and of all the tremendous implications of such an accomplishment. Studies of this nature and related studies could easily lead directly to the heart of the cancer problem and provide a solution to a situation which today appears so very perplexing.

Rules (cont)

Length of chains

in situation



its clear that chain lengths are (similar in relation to chain)

(Note that using this convention arrow separates old from new. on chain going ~~→~~ this → way.

This, together with our "staying together" rule, essential process length of all chains).

Thus, in brief symbolism.

① each product has one "effective" arrow ie → or ↔ or →

② ~~arrow~~ chain length terminates in front of arrow at ~~the~~ free end of chain or at first opposite arrow

③ chain length terminates behind arrow at free end of chain or first arrow of same direction.

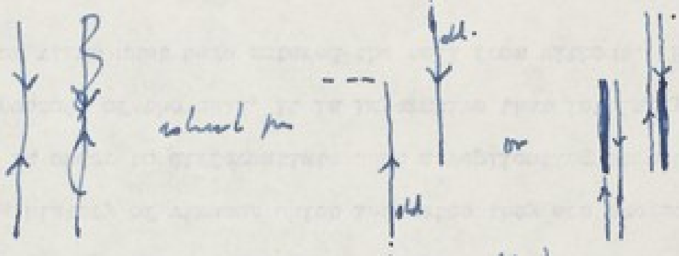
④ arrows always separate "old" from "new" region in own chain. "old" is behind arrow. ~~Is~~ also true if ↔ this situation. What is about other chain in this → situation?

questions of "self" or of "not self" and of "essentiality" are of the utmost importance. Fortunately we now have many powerful devices for characterizing biological entities, hence the demonstration of the existence of a "foreign" or of a "non-essential" component of a cell is far easier than it was a few years ago. Even so Knight (1946) and Beard and associates (Eckert, Sharp, Beard, Green and Beard, 1955) have found purified preparations of influenza and of myeloblastic leukosis viruses, respectively, to retain antigenic components characteristic of the host in which the virus was grown. In time it may prove possible to separate active nucleic acids from these preparations. I would hope that in order to avoid utter confusion the experimental results will continue to be compatible with the idea that viruses are exogenous in nature even though as has already been indicated we occasionally come very close to the borderline between an exogenous and an endogenous origin. Thus while the reproduction of genetic material from a member of one species in a member of another species might be accepted, the aberration of a normal component of a given host to yield something which would then reproduce in that host would be incompatible. So far, however, it has not been necessary to trespass for in every case examined carefully the evidence for the virus or self-duplicating mechanism having entered the cell from without at some time in the immediate or far past is good.

In closing may I indicate that I believe that the finding of Fraenkel-Conrat and of Gierer and Schramm that virus activity may reside in a nucleic acid represents a discovery of the first magnitude, for it means that a polynucleotide of comprehendible structural complexity can carry the code or informational pattern not only for the production of more of the same polynucleotide but also for the de novo production of a

easiest way is to consider distribution of "old" chain.

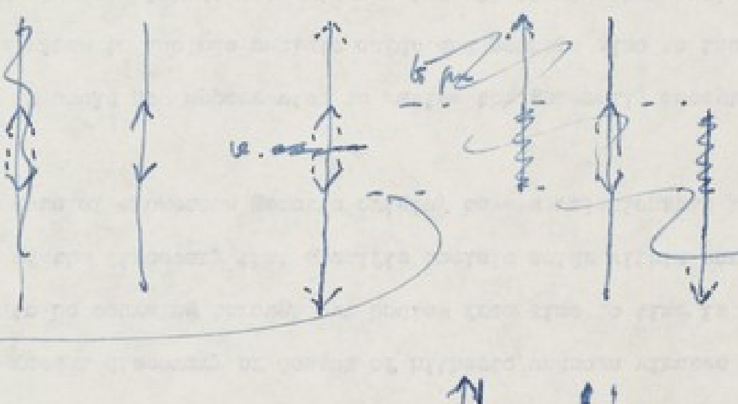
Consider case



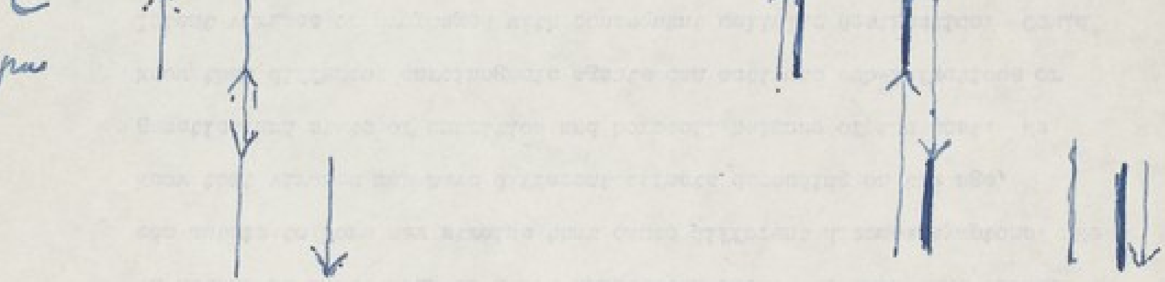
rule is old chain stays behind (hinge or bar) arrow till it reaches one
an arrow of same sign, or a free end
(this is obvious).

all other chains are new

Variation



to pass



✓ S.M.

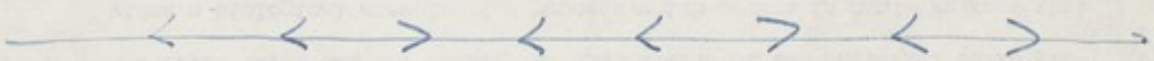
if all arrows same sign, other old chain stays with the two hind-most arrows.

years ago and we do not know what many of these are doing there (Huebner, 1956). We know that viruses can persist in their host for generations, in either an infectious or a non-infectious form. We know that viruses can mutate to form new strains that cause different disease symptoms. We know that viruses may have different effects depending on the age, genetics and state of nutrition and hormonal balance of the host. We know that different carcinogenic agents can activate sub-infectious or latent viruses or prophages with consequent cellular destruction. Could it be that the recent discovery of dozens of hitherto unknown viruses of man which seem to be coursing through our bodies from time to time is but the forerunner of the discovery that specific nucleic acids within our cells, perhaps even of exogenous genetic origin, have a relationship to our cancers?

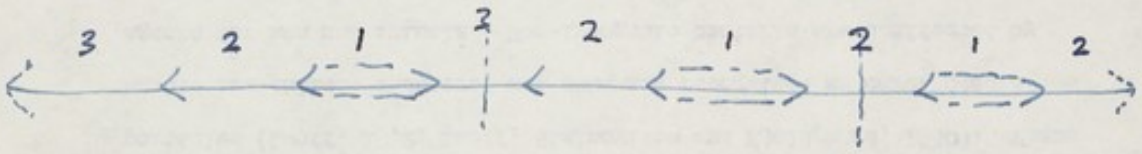
Certainly it would now appear wise to revise the generally accepted definition of a virus to include nucleic acids and perhaps also to include replicating structures which do not evidence infectivity in the usual sense because normally they are duplicated only once or a minimal number of times during each cell division and may never leave the cell during many generations. Such a viral nucleic acid might temporarily appear to be a part of, or associated with, the genetic apparatus of the cell, but be subject to chemical or physical stimulation or shock which could cause it to mature, increase greatly its rate of replication, perhaps mutate, but in any case to separate and act as an independent functional unit. Because of the long history of viruses which indicates they are exogenous in nature and in order to differentiate such a replicating structure from the normal components of the cell, it is imperative that initially the structure or virus must have entered the cell from without. Here the

exercise

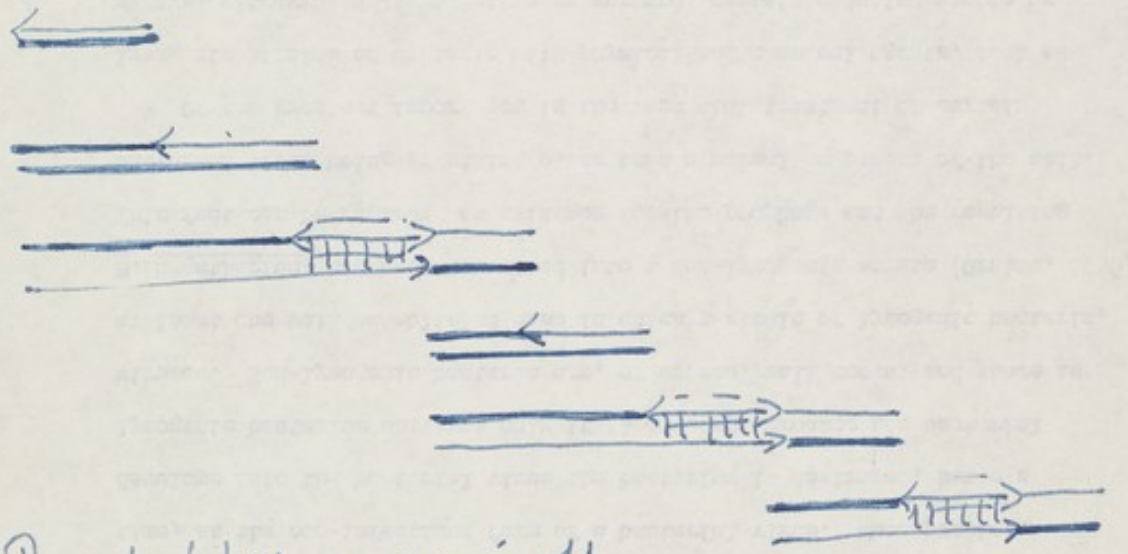
write down in detail the product of



we mate them



products are



Then only <u>behind</u> an arrow is old

the rest is new

<u>unless</u> all arrows to one vertex

then hindmost arrow gets other chain.

easiest way is to put shortest arrow at the <u>front</u>

end of every chain with no <u>arrow</u>.

the potentiality to produce a bacterial virus is perpetuated in lysogenic bacteria. Prophage is non-pathogenic and non-infectious in the normal sense, but since it is multiplied at least once with each cell division, it may be regarded as infectious in the sense that genes or chromosomes are infectious. In other words, the prophage might be considered as a temporary part of the genetic apparatus of the cell and, at the same time, as the non-infectious form of a bacterial virus. When prophage develops into the bacterial virus the bacterium is destroyed, hence a lysogenic bacterium survives only if it does not produce the bacterial viruses. Non-lysogenic bacteria are, of course, well known, and there is at least one well established case in which a strain of lysogenic bacteria, B. megatherium, has been converted into a non-lysogenic strain (Gratia, 1936). This fact can be regarded as evidence for the prophage and the resulting bacterial virus being something other than a normal component of the cell.

Of the greatest importance is the fact that treatment of certain lysogenic strains of bacteria with physical and chemical agents, such as x-rays, ultraviolet light, nitrogen mustard, certain reducing agents or iron chelating agents, results, after a maturation period, in the lysis of the bacterial cells and the release of large amounts of bacterial virus particles (Lwoff, 1952; Lwoff, Siminovitch and Kjeldgaard, 1950). These agents are called "inducers" and some are recognized as carcinogenic agents for man and animals. Non-lysogenic bacteria are unaffected by these "inducers." Is it possible that this activation of a prophage by certain chemical or physical agents with development into a fully infectious bacterial virus and the consequent destruction of the bacterial cells provides a biological example of a process which occurs in man? We know that man has coursing through his body many viruses which were unknown a few

These rules are

① pass dummy arrow
   ⓐ at end of any line if last arrow at that end points to that end
   ⓑ if no arrow at all points to that end.

② join all arrows separated ⟷ including dummy at end.

③ each product has one "effective" arrow: it starts at same base arrow & same sign behind and finishes at base arrow & opposite sign ahead

   e.g. same → off
   (and adj)

④ old material always behind all the arrows, even at dummy.
   all other material new.

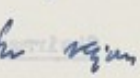
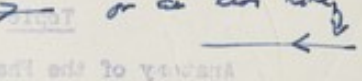
⑤ separations occur
   ⓐ when arrows meet
   ~~ⓑ when an arrow~~ including overtaking a dummy arrow
   (note case ⓐ never separates)

overcoat must insure that these different nucleic acid molecules enter the same cell and that they should have equal opportunity to initiate the replicative processes. Here again nature has provided wonderful tools with which to explore these very interesting possibilities.

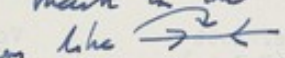
The viral nucleoproteins and nucleic acids I have discussed so far are characterized by their possession of regular virus activity which is readily demonstrable and is not complicated by extraneous factors. We know that many animal and plant tumors are caused by such viruses. However, there are other animal and plant tumors as well as most of the malignancies of man for which no such etiological agent has been found. As I have already indicated I believe that the many new kinds of animal and human cells which are now being grown in tissue culture, as well as the new methods for purifying and concentrating viruses are providing unusual new opportunities, which, when fully exploited experimentally, may result in the demonstration that many of these malignancies of animals, man and plants actually have viruses as their causative agents. Failure to find a virus in a given malignancy need not necessarily mean the absence of a virus for we now have sufficient information to warrant our being ever alert to the possibility of a somewhat different kind of virus activity existing in such cases.

For the purposes of the present discussion I should like to direct your attention to the persistence of a bacterial virus in the form of a prophage in lysogenic strains of bacteria and suggest that this might provide a most significant experimental model for the process that I have in mind (Lwoff, 1952). Lysogenic bacteria perpetuate, in what may be considered a hereditary manner, the property of being able to produce a bacterial virus. The term prophage is used to describe the form in which

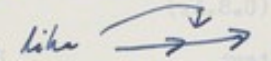
Rules for amount of replication

Secondly enters two signal chains stating together the factor is ×1. a) for region like  or an exit like 

Rule is: mark in the ×1 regions.

ⓐ For region like  ~~consider any region~~: then score is

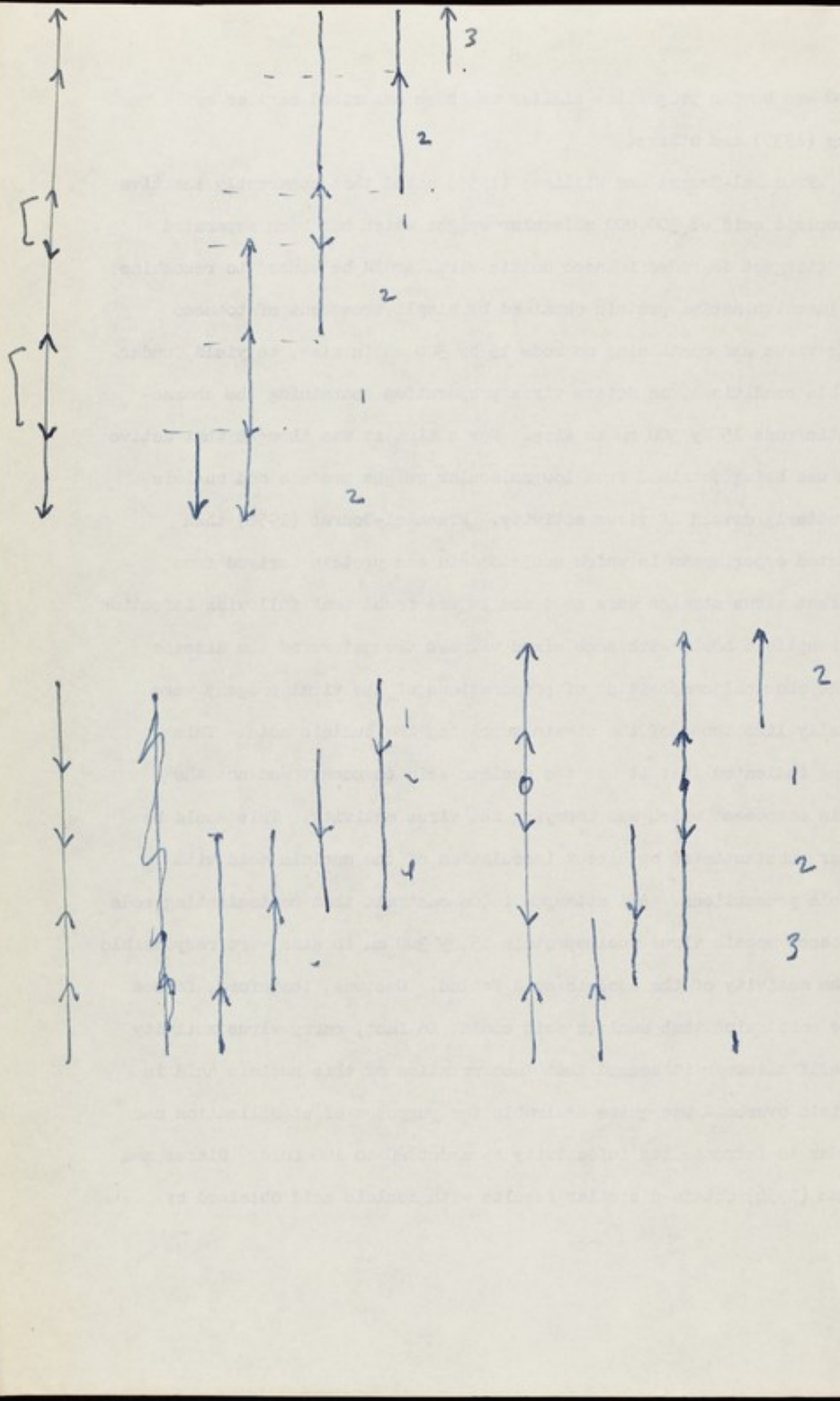
1 plus total number of arrows pointing towards the region from the one region on either side (or on one side, if only one)

ⓑ For region like  count for the "1" for which the same is one plus arrow.

Post is more or less done: there are all the arrows which exit from the new region.

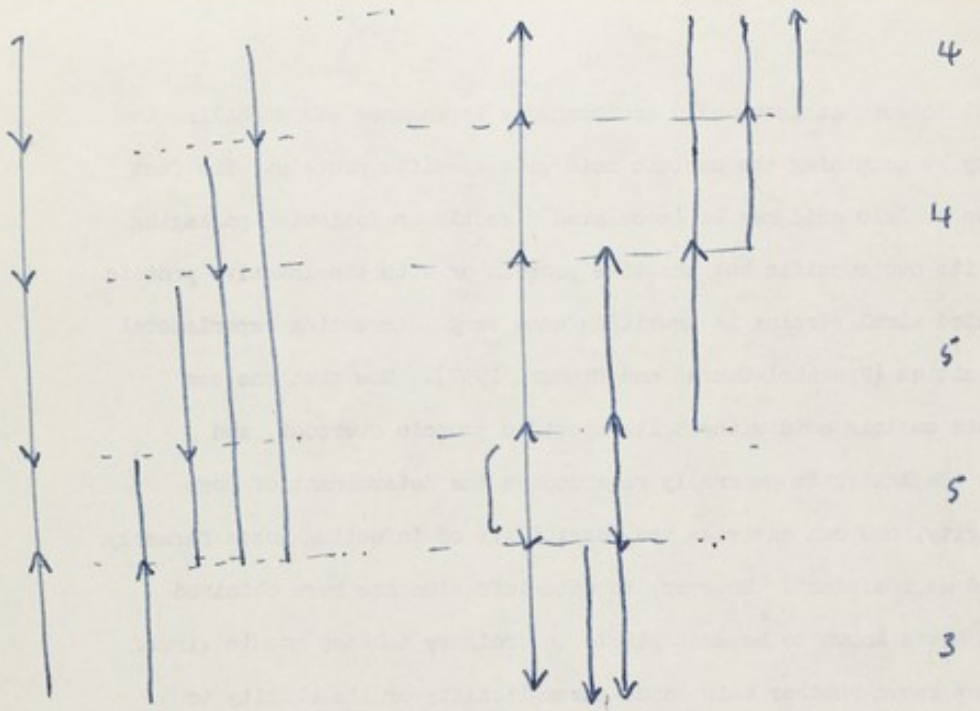
discussion of a given topic under the leadership of a chairman, after a mise-au-point of the subject had been delivered by a rapporteur, according to this program:

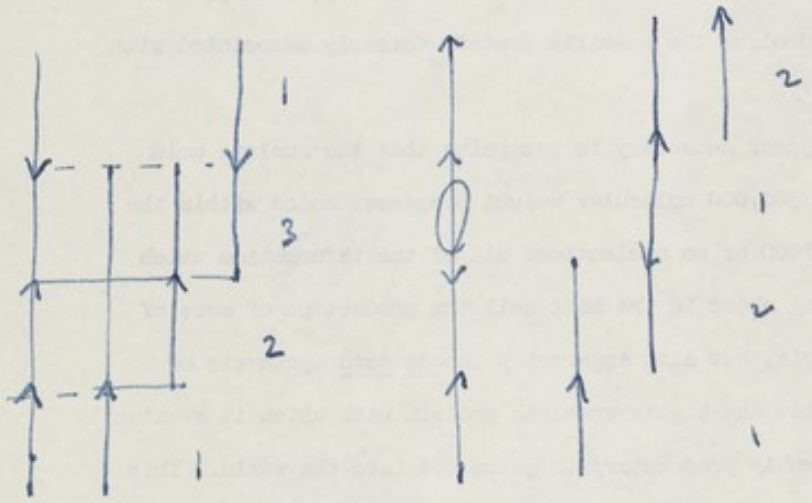
Topic	Chairman	Rapporteur
Anatomy of the Phage Particle	Kellenberger (Switzerland)	Brenner (Gt. Britain)
Metabolism of Infected Bacteria	Herriott (U.S.A.)	Volkin (U.S.A.)
Synthesis of Phage Constituents	Haalge (Denmark)	Streisinger (U.S.A.)
Kinetics of Recombination	Doermann (U.S.A.)	Bresch (Germany)
The Elementary Recombination Act	Levinthal (U.S.A.)	Edgar (U.S.A.)
Genetic Fine Structure	Stent (U.S.A.)	Benzer (U.S.A.)
Replication	Crick (Gt. Britain)	Stahl (U.S.A.)
Prophage and Chromosome, Transduction	R.S. Anderson (Gt. Britain)	J. Lederberg (U.S.A.)
Phage Conversion	Stocker (Gt. Britain)	Groman (U.S.A.)
Bacteriocines	Ivanovics (Hungary)	Fredericq (Belgium)


3
2
1
2
1
2
1
2
4
2
1
0
1
2
3
1

15,000 and having properties similar to those described earlier by Loring (1939) and others.

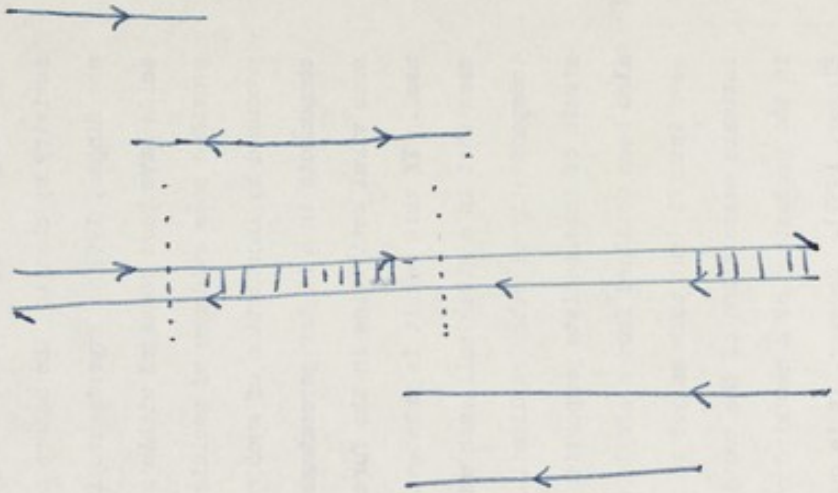
Fraenkel-Conrat and Williams (1955) noted that apparently inactive ribonucleic acid of 300,000 molecular weight which had been separated from detergent degraded tobacco mosaic virus could be caused to recombine with inactive native protein obtained by alkali treatment of tobacco mosaic virus and containing no rods 15 by 300 mμ in size, to yield, under suitable conditions, an active virus preparation containing the characteristic rods 15 by 300 mμ in size. For a time it was thought that active virus was being obtained from low molecular weight protein and nucleic acid utterly devoid of virus activity. Fraenkel-Conrat (1956) then conducted experiments in which nucleic acid and protein derived from different virus strains were used and it was found that following infection of susceptible hosts with such mixed viruses the nature of the disease and the chemical composition of preparations of the viral progeny were generally like those of the strain supplying the nucleic acid. This finding indicated that it was the nucleic acid component and not the protein component which was carrying the virus activity. This could be further substantiated by direct inoculation of the nucleic acid with suitable precautions. All attempts to demonstrate that contaminating rods of tobacco mosaic virus nucleoprotein 15 by 300 mμ in size were responsible for the activity of the nucleic acid failed. One was, therefore, forced to the conclusion that nucleic acid could, in fact, carry virus activity by itself although it seemed that incorporation of this nucleic acid in a protein overcoat was quite desirable for purposes of stabilization and in order to increase its infectivity by about 20 to 100 fold. Gierer and Schramm (1956) obtained similar results with nucleic acid obtained by


4
4
5
5
3


1
3
2
1
2
1
2
1

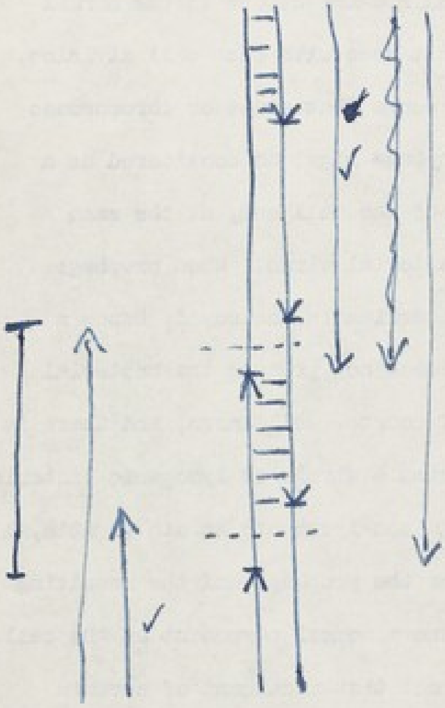
the nucleic acid it is usually advantageous to enhance and stabilize the activity by combining the nucleic acid with specific protein. The fact that the nucleic acid may be inoculated directly or following packaging within its own specific but inactive protein or with the inactive protein of related viral strains is providing some very interesting experimental opportunities (Fraenkel-Conrat and Singer, 1957). Now that one can inoculate nucleic acid without its specific protein overcoat, and because the latter is generally regarded as the determinant of host specificity, one can envisage the possibility of infecting hosts formerly regarded as resistant. However, to date infection has been obtained only in hosts known to be susceptible to ordinary tobacco mosaic virus. It is not known whether this means susceptibility or the ability to bring about duplication of the infecting structure is or is not dependent upon the presence within the host cell of a certain pattern of enzymatic activities which, in addition to their synthetic ability, also possess the potential to hydrolyze the specific protein formerly associated with the nucleic acid.

It would now appear necessary to recognize that the nucleic acid structure of around 300,000 molecular weight possesses coded within the arrangement of its 1000 or so nucleotides all of the information which is necessary to bring about in the host cell the production of more of this same nucleic acid, but also apparently the de novo synthesis of its own characteristic and highly specific protein with which it eventually coats itself, presumably preparatory to going out into the world. This is, to say the least, a quite wonderful and highly significant course of events. It provides for the first time a direct relationship between specific nucleic acid and specific protein synthesis. Inoculation of



molecular weight 300,000 continues to represent the smallest biologically active molecule. Considerations such as those just mentioned might cause one to wonder if considerably smaller nucleic acids possessing virus activity may not eventually be discovered. Truly nature has provided us with some fascinating experimental material, and with patience and intelligence it should prove possible to unravel these interrelationships to the welfare of mankind.

Still another interesting approach is provided by the possibility of packaging viral nucleic acid within specific proteins. So far, it has not proved possible to change the host specificity of a given viral activity by changing the nature of the protein overcoat (Fraenkel-Conrat and Singer, 1957). Experiments in which mixtures of different viral nucleic acids have been packaged within a given protein overcoat have, however, provided some evidence of nucleic acid interaction. Individual lesions produced by inoculation of such material seem to have both genetic components in a higher percentage than when artificial mixtures of the same viral activities in the form of the intact viral nucleoproteins were used. If this result is substantiated in further experiments, it could mean that in a given cell each nucleic acid evokes its own replication independently of other replicating molecules. In one case a new viral strain of intermediate properties was obtained but occasional mutants have also been obtained from a single nucleic acid infection, and there has been insufficient work as yet to warrant the conclusion that the forced intimate association of the two different nucleic acids actually resulted in the production of a new kind of nucleic acid (Fraenkel-Conrat and Singer, 1957). However, it is quite obvious that the packaging of two or more viral strains in the form of nucleic acids within a single protein

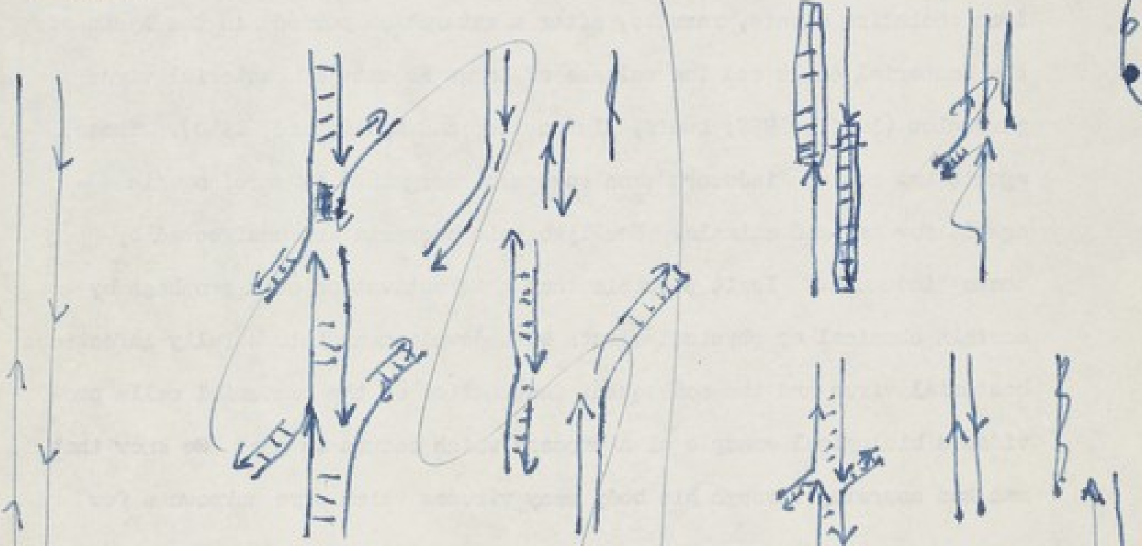


① pour in arrow
② decide where chains part due to arrows
③ decide if any pieces stay together.

④ draw products : rule is that an arrow progresses until it meets start of one or the opposite of chain : and so comes to stop an end. unless pieces stay together.

When two arrows meet
then chains part.
When the chains part

check.



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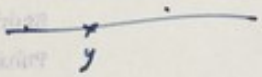
let x = first

second = y

Consider case where $x > y$

we have $\int_0^x dy \int_y^1 dx$

$$\int_0^1 \left(\frac{1}{2} - \frac{y^2}{2}\right) dy = \left[\frac{y}{2} - \frac{y^3}{6}\right]_0^1 = \frac{1}{2} - \frac{1}{6} = \frac{1}{3}$$



$$y + \frac{(1-y)}{2} = \frac{1+y}{2}$$

$$y = \frac{1}{2} = \frac{1+\frac{1}{2}}{2} = \frac{3}{4}$$ ✓✓

October 1, 1958

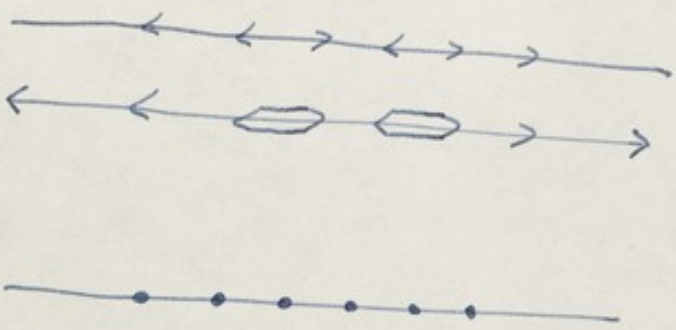
Virus Laboratory Staff

Revised Wednesday Noon Speakers
Room 411 BV Lab
Beginning September 24

Two persons will speak each Wednesday starting at the top of the list. As a person's name is reached on the list, it will be that person's responsibility to see that a speaker is provided. Each speaker is allotted 15 minutes for the presentation plus 10 minutes for discussion and the first speaker will begin at 12:20 p.m.

Doctors Alava
Bonhoeffer
Bové
Bradish
Christensen
Clark
Crawford
Dixon
Fraenkel-Conrat
Gan
Gish
Gordon
Haschemeyer
Kaper
Knight
Makepeace
Mattern
Moring-Claesson

Doctors Olsen
Pardee
Pratt
Raacke
Ramachandran
Ramel
Reddi
Rubin
Schachman
Schaffer
Steere
Stent
Thomas
Tsugita
Uchida
Williams
Wollman



$= 6$

< <
> >

$(2\times2)\times(2\times2)\times(2\times2) = 64$

$4 \times 4 \times 4 =$

$16 \times 4 =$

64×6 $\quad$ $2^n \times n$

$+64$ $\quad$ $+2^n$

Retrait $\quad \frac{5\times64}{4} = 5\times16 \quad \frac{(n-1)2^n}{4}$

n	n+1	n-1	∴
2	3	1	$2\frac{3}{4}$
3	4	2	$3\frac{1}{2}$
4	5	3	$4\frac{1}{4}$
5	6	4	5
6	7	5	

$$= \frac{2^n}{2^n}\left[n+1 - \frac{(n-1)}{4}\right]$$

$$= n+1 - \frac{(n-1)}{4} = \frac{3}{4}(n+1) + \frac{1}{2}$$

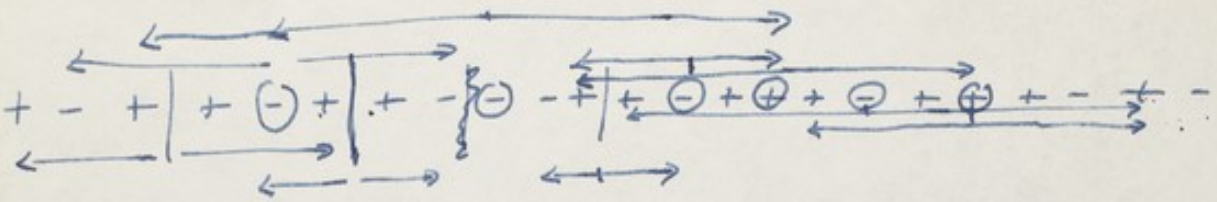
$$= \frac{3}{4}n + 1\frac{1}{4} \checkmark$$

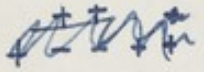
$\Delta = \frac{3}{4}$

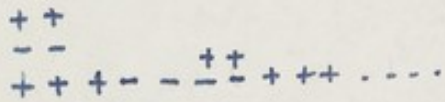
THE POTENTIAL SIGNIFICANCE OF NUCLEIC ACIDS AND NUCLEOPROTEINS OF SPECIFIC COMPOSITION IN MALIGNANCY

Wendell M. Stanley, Virus Laboratory
University of California, Berkeley, California

The subject which has been assigned to me is the potential significance of nucleic acids and nucleoproteins of specific composition in malignancy. The work from our laboratory over the years as well as contributions from other laboratories has provided sound experimental evidence that viruses may be composed of nucleoproteins of definite and specific composition. It was shown quite early in our laboratory that tobacco mosaic virus isolated from infected turkish tobacco and from infected tomato plants was identical in all properties examined (Loring and Stanley, 1937). Later the same situation was found to hold in the case of virus isolated from turkish tobacco and phlox plants (Gay and Stanley, 1947), a finding of special importance because of the fact that the normal proteins of turkish tobacco and phlox show no serological relationship. It is interesting and significant that the same highly complex and specific viral nucleoprotein can be produced in two manufacturing plants, the normal contents of which are serologically unrelated. It has also been demonstrated in our laboratory that different strains of tobacco mosaic virus consist of nucleoproteins which may differ in a reproducible manner in their amino acid composition (Knight and Stanley, 1941; Knight, 1945). For example, ordinary tobacco mosaic virus contains no histidine or methionine whereas the ribgrass strain contains 0.6 percent histidine and 1.0 percent methionine. As a result it is easy to distinguish between these two strains in the chemical laboratory merely by making a colorimetric histidine determination. However, despite the fact that viruses do contain







Then the problem of four lines might be easier because fewer overlaps!

Now how this result should be applied.

For a $2n$ unit. $\left(\frac{1}{4}\right)^n \times \frac{3}{4}$ $\therefore$ n and $= 2n\left(\frac{1}{4}\right)^n \times \left(\frac{3}{4}\right)$

$= 6n\left(\frac{1}{4}\right)^{n+1} = \frac{3}{2}n\left(\frac{1}{4}\right)^n$

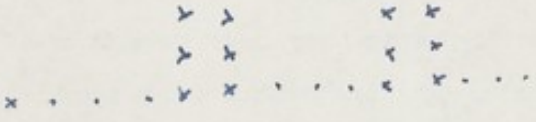
For $n \geq 2$, then becomes.

$\frac{3}{16} + \frac{9}{128} + \frac{3}{128} + \cdots\cdots$

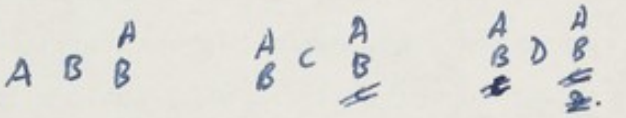
·18
·08
·02
·28

highly specific polypeptide. It provides a much more sound experimental as well as theoretical base for contemplating the existence of the myriads of living things on earth. It is a discovery that is affecting the thinking and the course of events in virus research and all that virus research is concerned with, such as genetics, infectious disease, cancer and life itself. Eventually chemists should be able to synthesize a small polynucleotide specifically arranged, hence one may now dare to think of synthesizing in the biochemical laboratory a structure possessing genetic continuity and of all the tremendous implications of such an accomplishment. Studies of this nature and related studies could easily lead directly to the heart of the cancer problem and provide a solution to a situation which today appears so very perplexing.

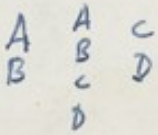
How to make a square to give a regular fold?

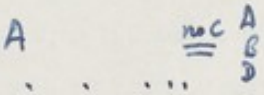


$2 \times 4 \times 2 = 16$

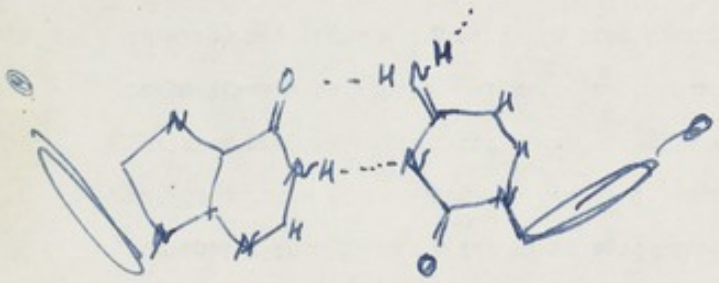


(10)





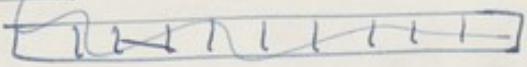
(1)



questions of "self" or of "not self" and of "essentiality" are of the utmost importance. Fortunately we now have many powerful devices for characterizing biological entities, hence the demonstration of the existence of a "foreign" or of a "non-essential" component of a cell is far easier than it was a few years ago. Even so Knight (1946) and Beard and associates (Eckert, Sharp, Beard, Green and Beard, 1955) have found purified preparations of influenza and of myeloblastic leukosis viruses, respectively, to retain antigenic components characteristic of the host in which the virus was grown. In time it may prove possible to separate active nucleic acids from these preparations. I would hope that in order to avoid utter confusion the experimental results will continue to be compatible with the idea that viruses are exogenous in nature even though as has already been indicated we occasionally come very close to the borderline between an exogenous and an endogenous origin. Thus while the reproduction of genetic material from a member of one species in a member of another species might be accepted, the aberration of a normal component of a given host to yield something which would then reproduce in that host would be incompatible. So far, however, it has not been necessary to trespass for in every case examined carefully the evidence for the virus or self-duplicating mechanism having entered the cell from without at some time in the immediate or far past is good.

In closing may I indicate that I believe that the finding of Fraenkel-Conrat and of Gierer and Schramm that virus activity may reside in a nucleic acid represents a discovery of the first magnitude, for it means that a polynucleotide of comprehendible structural complexity can carry the code or informational pattern not only for the production of more of the same polynucleotide but also for the de novo production of a

distance problem



chance for a $2n$ unit is

$$\left(\frac{1}{2}\right)^n \times \frac{1}{2}$$

Then amount is $2n \times \left(\frac{1}{2}\right)^{n+1} = n\left(\frac{1}{2}\right)^n$

Then for $n \geq 2$, we get total wanted in loops is

$$2 \cdot \frac{1}{4} + 3 \cdot \frac{1}{8} + 4 \cdot \frac{1}{16}$$

can be right!

This includes overlaps

conditions for a run to stop.

① L or R incompatible. $= \frac{1}{2}$ chance

② does stop it
L L. starts, R goes up to $2n$
chance is $\left(\frac{1}{2}\right)^{n+1}$
$\therefore \left(1 - \left(\frac{1}{2}\right)^{n+1}\right)$

③ ditto goes up 4
$\left(\frac{1}{2}\right)^{n+1}$
$\therefore \left(1 - \left(\frac{1}{2}\right)^{n+1}\right)$
etc)

Then the problem: essentially one of estimating the overlaps.

④ ditto ETC ditto to other side

Prob. ditto mixed sides.

Alexander, H., and G. Leidy: Determination of inherited traits of H. influenzae by desoxyribonucleic acid fractions isolated from type-specific cells. J. Exp. Med., 93:345, 1951.

Stanley, W. M.: Biochemistry and biophysics of viruses. In Handbuch der Virusforschung. (R. Doerr and C. Hallauer, eds.) Julius Springer. Berlin. 1938.

Hotchkiss, R. D.: The biological role of the desoxypentose nucleic acids. In The Nucleic Acids, v II. Academic Press. New York. 1955.

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Markham, R., and K. M. Smith: Studies on the virus of turnip yellow mosaic. Parasitology, 39:330, 1949.

Hershey, A. D., and M. Chase: Independent function of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol., 36:39, 1952.

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Fraenkel-Conrat, H., and R. C. Williams: Reconstitution of active tobacco mosaic virus from its inactive protein and nucleic acid components. Proc. Natl. Acad. Sci., 41:690, 1955.

Fraenkel-Conrat, H. L. The infectivity of tobacco mosaic virus nucleic acid. Ann. N. Y. Acad. Sci., in press. 1957.

Schramm, G.: Investigations on the ribonucleic acid of tobacco mosaic virus. Ann. N. Y. Acad. Sci., in press, 1957.

Fraenkel-Conrat, H.: Effect of pyrophosphate on reconstitution of TMV. American Chemical Society Abstracts, 131st Natl. Meeting, April, 1957.

m

Three Rods breaks

Consider here case where all answers are rational. 1 → 2 → 3 → 4 ...

Let parameters be x y & z $(0 \le .. \le 1)$

Then density total is $1 + (1-x) + (1-y) + (1-z)$

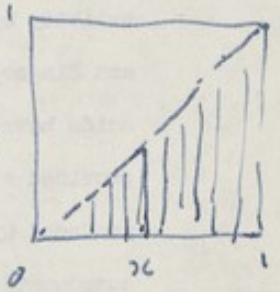
$\therefore$ average is $4 - (x+y+z) = 2\frac{1}{2}$; as expected.

random distribution between 0 & 1 for all variables

What is average value of x, given $x > y$?

represent graphically.

$\frac{1}{2} = \int_0^1 1.x\,dx = \left[\frac{x^2}{2}\right]_0^1 = \frac{1}{2}$ for $\frac{1}{2}$ of x



for $x > y$

$\frac{1}{4} = \int_0^1 x.x.dx = \left[\frac{x^3}{3}\right]_0^1 = \frac{1}{3}$

What is average value of $(x-y)$, given $x > y$?

→ here this is for all cases where $x > y$ is half the cases

Thus answer to the average value of these cases = $\frac{\frac{1}{3}}{\frac{1}{2}} = \frac{2}{3}$

What is average value of $(x-y)$ given $x > y$?

here, what is average value of y given $x > y$:

$\int_0^1 y(1-y)\,dy = \left[\frac{y^2}{2} - \frac{y^3}{3}\right]_0^1 = \frac{1}{6}$

as this is only half the values of y . Average = $\frac{1}{3}$

$\int_0^1 (1-y)\,dy = \left[y - \frac{y^2}{2}\right]_0^1 = \frac{1}{2}$

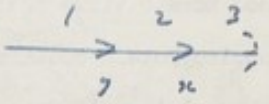
$\therefore \overline{x-y} = \bar{x} - \bar{y} = \frac{2}{3} - \frac{1}{3} = \frac{1}{3}$

for $x - y > 0$

molecular weight 300,000 continues to represent the smallest biologically active molecule. Considerations such as those just mentioned might cause one to wonder if considerably smaller nucleic acids possessing virus activity may not eventually be discovered. Truly nature has provided us with some fascinating experimental material, and with patience and intelligence it should prove possible to unravel these interrelationships to the welfare of mankind.

Still another interesting approach is provided by the possibility of packaging viral nucleic acid within specific proteins. So far, it has not proved possible to change the host specificity of a given viral activity by changing the nature of the protein overcoat (Fraenkel-Conrat and Singer, 1957). Experiments in which mixtures of different viral nucleic acids have been packaged within a given protein overcoat have, however, provided some evidence of nucleic acid interaction. Individual lesions produced by inoculation of such material seem to have both genetic components in a higher percentage than when artificial mixtures of the same viral activities in the form of the intact viral nucleoproteins were used. If this result is substantiated in further experiments, it could mean that in a given cell each nucleic acid evokes its own replication independently of other replicating molecules. In one case a new viral strain of intermediate properties was obtained but occasional mutants have also been obtained from a single nucleic acid infection, and there has been insufficient work as yet to warrant the conclusion that the forced intimate association of the two different nucleic acids actually resulted in the production of a new kind of nucleic acid (Fraenkel-Conrat and Singer, 1957). However, it is quite obvious that the packaging of two or more viral strains in the form of nucleic acids within a single protein

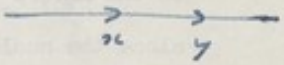
apply method to these breaks cases



Let $x > y$: Then we want $\overline{y} + 2\overline{(x-y)} + 3\overline{(1-x)}$

$= \frac{1}{3} + 2\frac{1}{3} + 3 \cdot \frac{1}{3} = 2$

for $y > x$ answer is $\frac{1}{3}$.



we want obtuse the same

$\therefore$ answer = $\underline{\underline{2}}$

Then general problem is

given n variables $x_1, x_2, x_3, \ldots x_n$

such that $x_1 \leq x_2 \leq x_3 \ldots \leq x_m$ and $0 \leq$ all $x \leq 1$

and also all x are equally distributed in these intervals, since only these cases are considered:

Then problem is what is $\overline{x_n - x_{n-1}}$?

we assume the answer is $\frac{1}{n}$: Prob make circle

and then circle symmetry.

$x_n \; dx_1 dx_2 \ldots dx_n$

$dx_1 dx_2 \ldots dx_n$

tobacco mosaic virus nucleic acid results in the production of the normal TMV nucleoprotein containing no histidine or methionine whereas inoculation of the nucleic acid from the ribgrass strain results in the production of a serologically distinct nucleoprotein containing these two amino acids. The nucleic acid obviously provides the pattern not only for the production of more highly specific nucleic acid but also for the de novo production of a highly specific protein. It would be interesting to know the sequence of events within the infected cell since this could throw light on the nature of the synthetic pattern. Are amino acids first laid down along the nucleic acid in strict accordance with its pattern to yield eventually the polypeptide sequence characteristic of the protein, and, if so, is this done in the first place to yield a polypeptide pattern or template which could then be used to form more of the specific nucleic acid--this sequence of events being brought to a terminal phase by a metabolic situation causing the enfolding of the nucleic acid within a super assembly of the polypeptide chains to yield the final structure consisting of several nucleic acid strands enclosed in a protein overcoat? It may be of some interest to note that a thousand unit polynucleotide linear chain having the same base composition as TMV nucleic acid could exist in about 10^{590} different arrangements. This number is so large that it is practically incomprehensible. Even a one hundred unit chain of this composition could exist in about 10^{57} different arrangements, and this number is about the same as the total number of electrons on and within the earth and vastly larger than the total of all of the living things on earth. It is obvious that insofar as the possibility of coding information relative to nucleic acid and protein synthesis is concerned, nature has provided a tremendous safety factor if a nucleic acid of

$9 + 12 = \frac{21}{12}$

$42 + 20 = \frac{62}{72}$

~~$21 + 10 = \frac{31}{72}$~~

$136 \text{ 怪} \quad 30 = \frac{166}{80}$

=

20 4=

42 6.7

$\frac{166}{124}$

42

$1\frac{1}{2} \quad 1\frac{3}{4} \quad 1\frac{15}{16} \quad 2\frac{3}{40}$

$\frac{1}{4} \quad \frac{3}{16} \quad \frac{11}{80}$

$\times \frac{3}{4} \quad \times$

$- \times \frac{1}{4} \quad - \times \frac{1}{20}$

$\frac{1}{16} + \frac{3}{40}$

$\frac{5+6}{80} = \frac{11}{80}$

n	1	2	3	4	5
total mean.	$1\frac{1}{2}$	$1\frac{7}{4}$	$1\frac{15}{16}$	$2\frac{3}{40}$	
no. of pieces.	2	$2\frac{3}{4}$	$3\frac{1}{2}$	$4\frac{1}{4}$	5
no. av. new of pieces	0.75	$\frac{7}{11}$ $= .636$	$\frac{31}{56}$	$\frac{83}{170}$ $\sim .49$	

no av. new.

$\frac{85}{170}$

$\frac{3}{2}$

$\frac{83}{17 \times 10}$

SCALE OF 64

	0	1	2	3	4	5	6	7	8	9
0	00000	00064	00128	00192	00256	00320	00384	00448	00512	00576
1	00640	00704	00768	00832	00896	00960	01024	01088	01152	01216
2	01280	01344	01408	01472	01536	01600	01664	01728	01792	01856
3	01920	01984	02048	02112	02176	02240	02304	02368	02432	02496
4	02560	02624	02688	02752	02816	02880	02944	03008	03072	03136
5	03200	03264	03328	03392	03456	03520	03584	03648	03712	03776
6	03840	03904	03968	04032	04096	04160	04224	04288	04352	04416
7	04480	04544	04608	04672	04736	04800	04864	04928	04992	05056
8	05120	05184	05248	05312	05376	05440	05504	05568	05632	05696
9	05760	05824	05888	05952	06016	06080	06144	06208	06272	06336
10	06400	06464	06528	06592	06656	06720	06784	06848	06912	06976
11	07040	07104	07168	07232	07296	07360	07424	07488	07552	07616
12	07680	07744	07808	07872	07936	08000	08064	08128	08192	08256
13	08320	08384	08448	08512	08576	08640	08704	08768	08832	08896
14	08960	09024	09088	09152	09216	09280	09344	09408	09472	09536
15	09600	09664	09728	09792	09856	09920	09984	10048	10112	10176
16	10240	10304	10368	10432	10496	10560	10624	10688	10752	10816
17	10880	10944	11008	11072	11136	11200	11264	11328	11392	11456
18	11520	11584	11648	11712	11776	11840	11904	11968	12032	12096
19	12160	12224	12288	12352	12416	12480	12544	12608	12672	12736
20	12800	12864	12928	12992	13056	13120	13184	13248	13312	13376
21	13440	13504	13568	13632	13696	13760	13824	13888	13952	14016
22	14080	14144	14208	14272	14336	14400	14464	14528	14592	14656
23	14720	14784	14848	14912	14976	15040	15104	15168	15232	15296
24	15360	15424	15488	15552	15616	15680	15744	15808	15872	15936
25	16000	16064	16128	16192	16256	16320	16384	16448	16512	16576
26	16640	16704	16768	16832	16896	16960	17024	17088	17152	17216
27	17280	17344	17408	17472	17536	17600	17664	17728	17792	17856
28	17920	17984	18048	18112	18176	18240	18304	18368	18432	18496
29	18560	18624	18688	18752	18816	18880	18944	19008	19072	19136
30	19200	19264	19328	19392	19456	19520	19584	19648	19712	19776
31	19840	19904	19968	20032	20096	20160	20224	20288	20352	20416
32	20480	20544	20608	20672	20736	20800	20864	20928	20992	21056
33	21120	21184	21248	21312	21376	21440	21504	21568	21632	21696
34	21760	21824	21888	21952	22016	22080	22144	22208	22272	22336
35	22400	22464	22528	22592	22656	22720	22784	22848	22912	22976
36	23040	23104	23168	23232	23296	23360	23424	23488	23552	23616
37	23680	23744	23808	23872	23936	24000	24064	24128	24192	24256
38	24320	24384	24448	24512	24576	24640	24704	24768	24832	24896
39	24960	25024	25088	25152	25216	25280	25344	25408	25472	25536
40	25600	25664	25728	25792	25856	25920	25984	26048	26112	26176
41	26240	26304	26368	26432	26496	26560	26624	26688	26752	26816
42	26880	26944	27008	27072	27136	27200	27264	27328	27392	27456
43	27520	27584	27648	27712	27776	27840	27904	27968	28032	28096
44	28160	28224	28288	28352	28416	28480	28544	28608	28672	28736
45	28800	28864	28928	28992	29056	29120	29184	29248	29312	29376
46	29440	29504	29568	29632	29696	29760	29824	29888	29952	30016
47	30080	30144	30208	30272	30336	30400	30464	30528	30592	30656
48	30720	30784	30848	30912	30976	31040	31104	31168	31232	31296
49	31360	31424	31488	31552	31616	31680	31744	31808	31872	31936

Consider a process of putting in breaks & so show

that after each the break all replicate [illegible] to complete before

the new break occurs.

Then each break doubles the number of pieces. (×2)

and multiplies total mass by (1½)

∴ we get $1+\frac{1}{4} = 2\frac{1}{2}$ $4\frac{?}{4}$

total breaks	1	~~[illegible]~~	[illegible]	?	~~No [illegible]~~ ~~breaks [illegible]~~
total mass ~~no. of pieces~~	$1\frac{1}{2}$	$\frac{9}{4}$	$\frac{27}{16}$	$\frac{81}{32}$	~~[illegible]~~ $\frac{3}{2}$
total no. of pieces.	2	4	8	16	
no. av. M.W.	.75	$\frac{9}{16}$	$\frac{27}{128}$	$\frac{81}{512}$	

~ .16

I ..

i.e. ~~no. average~~ MW is ~~lower~~ for same no. of breaks.

if put in proportionally.

~~Now distribution is very peculiar because [illegible] always have~~

~~the original molecular size remaining plus a lot of~~

smaller bits.

October 1, 1958

Virus Laboratory Staff

Revised Wednesday Noon Speakers
Room 411 BV Lab
Beginning September 24

Two persons will speak each Wednesday starting at the top of the list. As a person's name is reached on the list, it will be that person's responsibility to see that a speaker is provided. Each speaker is allotted 15 minutes for the presentation plus 10 minutes for discussion and the first speaker will begin at 12:20 p.m.

Doctors Alava
Bonhoeffer
Bové
Bradish
Christensen
Clark
Crawford
Dixon
Fraenkel-Conrat
Gan
Gish
Gordon
Haschemeyer
Kaper
Knight
Makepeace
Mattern
Moring-Claesson

Doctors Olsen
Pardee
Pratt
Raacke
Ramachandran
Ramel
Reddi
Rubin
Schachman
Schaffer
Steere
Stent
Thomas
Tsugita
Uchida
Williams
Wollman

① DNA makes RNA

ⓐ very fast
ⓑ with a control mechanism which shuts it off when enough RNA is made.
- control mechanism must depend on
ratio of $\frac{RNA}{DNA}$ since if more DNA we get bigger rate

② RNA makes protein

this we can reduce the rate of protein synthesis to zero if we have the c^+ gene and no inducer.
i.e. if we have a repressor gene which is not reversed by the inducer.

thus when the "air complexes" are destroyed they must be inhibited after a delay (+ after protein synthesis?) by the c gene or its products.

c^+ is ⓐ dominant
ⓑ has delayed expression

What happens if inducer is added after synthesis has stopped by (delayed) action of c^+?

Consider cell in two extreme states

(a) rich medium (b) poor medium, i.e. contain minimum nutr. for growth.

in latter all small mol. which can pass cell.

What conditions rate of protein synthesis?

we ask "P" all the protein apparatus needed to make all proteins.
which are needed in a rich medium.

call M all the protein apparatus needed in addition in case (b)

And assume the rate of protein synthesis is $\propto P$

then "growth rate" $\propto \frac{P}{P+M}$ ← P+M = total protein

[ignore for the moment the effect which will depend on the actual size of the cell.]

then protein for manufacturing amino acids which are used only for
protein synthesis fall into P
proteins for energy supply are $\propto$ to P+M
protein for making lipids (assuming cell membrane contains mol. P+M)
are $\propto P+M$

[re size: in a rich medium we need less "processors" relatively, thus
cell can be bigger i.e. have smaller surface/volume ratio]

RNA content (ribosomal or sol.) is $\propto P$ This assumes RNA is not used directly to make molecules which can be dispensed with in poor medium

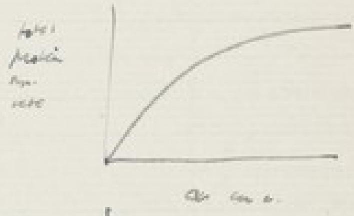
expand to include nucleation:

Let P_0 = protein part of ~~apparatus needed to make protein (not RNA) from~~ ribosomes
~~amino acids and nucleotides etc.~~

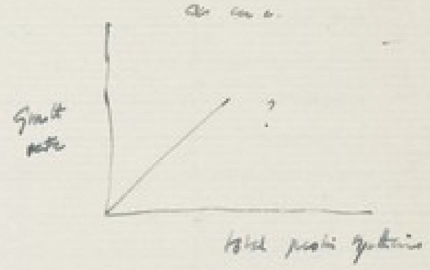
P_1 = protein part of apparatus rest of P i.e. $P = P_0 + P_1$

Start again:

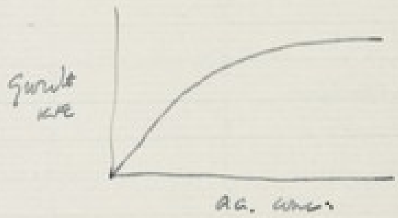
if enzymes were missing, and we supplied a amino ac., then we shall get a curve of rate of total protein synthesis for supply of the ac.



and we should have a curve for growth rate against (regardless of climate, time) for various total protein synthesis of the kind.



Thus assume we have same curve.



growth rate $\propto \frac{(P+M)}{(P+M)+m} \times$ total. prot. syn.

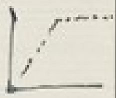
$\propto \frac{(P+M)}{(P+M)+m} \times f$ (ac. conc.)

* assume f(ac.conc.) $\propto$ $\left(\frac{Ca}{Ca+k}\right)$

no because complete max saturation.

Also total protein synthesis $\propto m.V \propto m.V_0\left(\frac{c}{c+k}\right)$

c = conc of Ca present.



$\therefore$ growth rate $\propto \frac{(P+M)}{(P+M)+m} \times m\,V_0\left(\frac{c}{c+k}\right)$

and flux will be $\propto$ M. V. (change)

Then $v_0 \left(\frac{c}{c+k}\right) \propto \frac{P+M}{M}$

we assume that M will be adjusted to give maximum "growth rate" i.e. minimum doubling time.

It is still always hard to take M as small as possible ???

? ? . ?

Assume case where nutrient uptake is growth limiting: (as c also rate)

as we need a curve.

$\left(\frac{M+P}{P}\right)$ total protein/cell ??

growth rate

now consider an operon engage

as c is very low the cell will increase the protein and decrease other proteins till a stable, low growth rate is achieved

Then $v_0 \left(\frac{c}{c+k}\right)$ M protein size $\propto$ rate. $\propto$ P

when present cell has produced a large amount of enzyme.

when increase in total protein will reduce growth rate more than increase rate increases it.

how rate be then depend upon?

if only then spread ac. then rate will be if nothing else changes.

rate

conc. of limit ac

??

The advantage of this is that it helps to fix ideas.

Thus we assume that under optimal conditions total protein synthesis is proportional to amount of ribosomes

This implies an proportional amount (i.e. prop. to ribosomes)

of ② – microsomal RNA
③ and probably of
– soluble RNA
– enzymes for making both RNA's
– activating enzymes
–

? ? ?

Rate v temperature

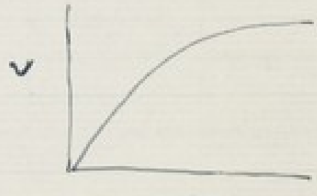
If we observe that ratio $\frac{RNA}{mass}$ stays constant with temperature.

then a sufficient condition for this is that

$\frac{P}{P+M}$ = const i.e. that the "average" temp. coeff of P and of M are the same?

Can we work out where a Michaelis curve of protein will work.

Consider a case where only one protein is altered, to which in one step makes say amino ac. and from some precursor.



$$v = V_0 \left(\frac{c}{c+K}\right)$$

first let conc. of precursor be very high

then $v = V_0$

we need flux of aa $\propto P+M$

Shaffner T = generation time $\approx t_0 \cdot \frac{T+m}{T}$

$$\frac{dT}{dm} = \frac{t_0}{T}$$

Let us suppose m is a 5% increase

the approx. generation time will increase by 5% assuming total apparatus remains constant.

~~[illegible] and some [illegible] is~~

~~but because of this other proteins are needed in lesser amounts~~

~~but apparatus will have to increase to do this~~

(generation time $\propto \frac{P+M}{P}$)

is there a problem in parallel reactions?

consider complete synchronized supply of 'activated' aa to be constant

except for one whose concentration is varied how does rate of protein

synthesis vary with conc. of this odd amino acid?

supply will settle down so that $\frac{\text{flux}}{\text{conc}}$ = flux of odd aa.

Generation time generation time $\propto$ ~~[illegible] (total protein)~~ ?

$\propto \left(\frac{\text{total protein}}{\text{total RNA}}\right)$?