

Notebook 6

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Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
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INSECT VIRUSES: CHEMISTRY

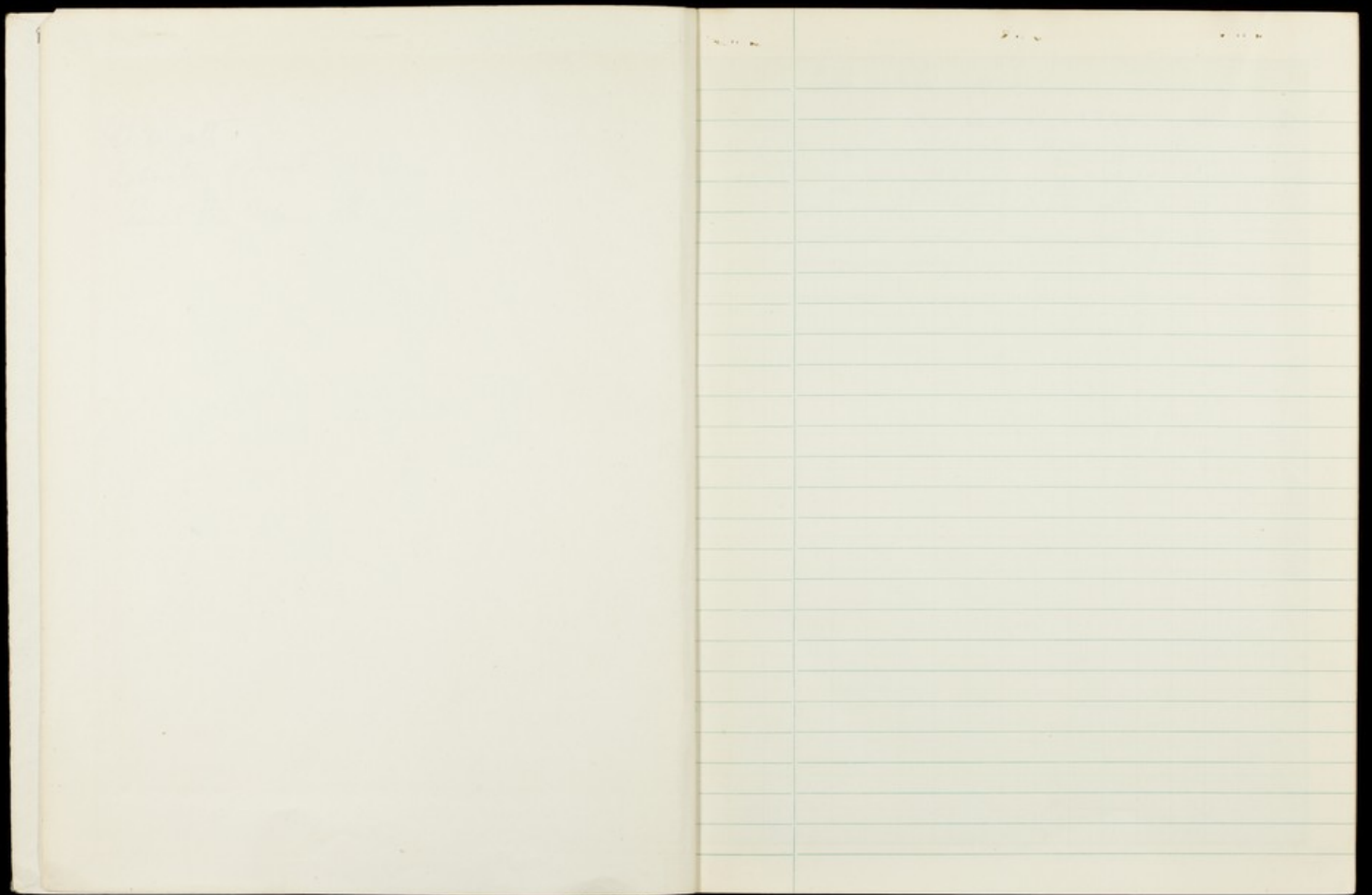
March, 1951. - March 1952.

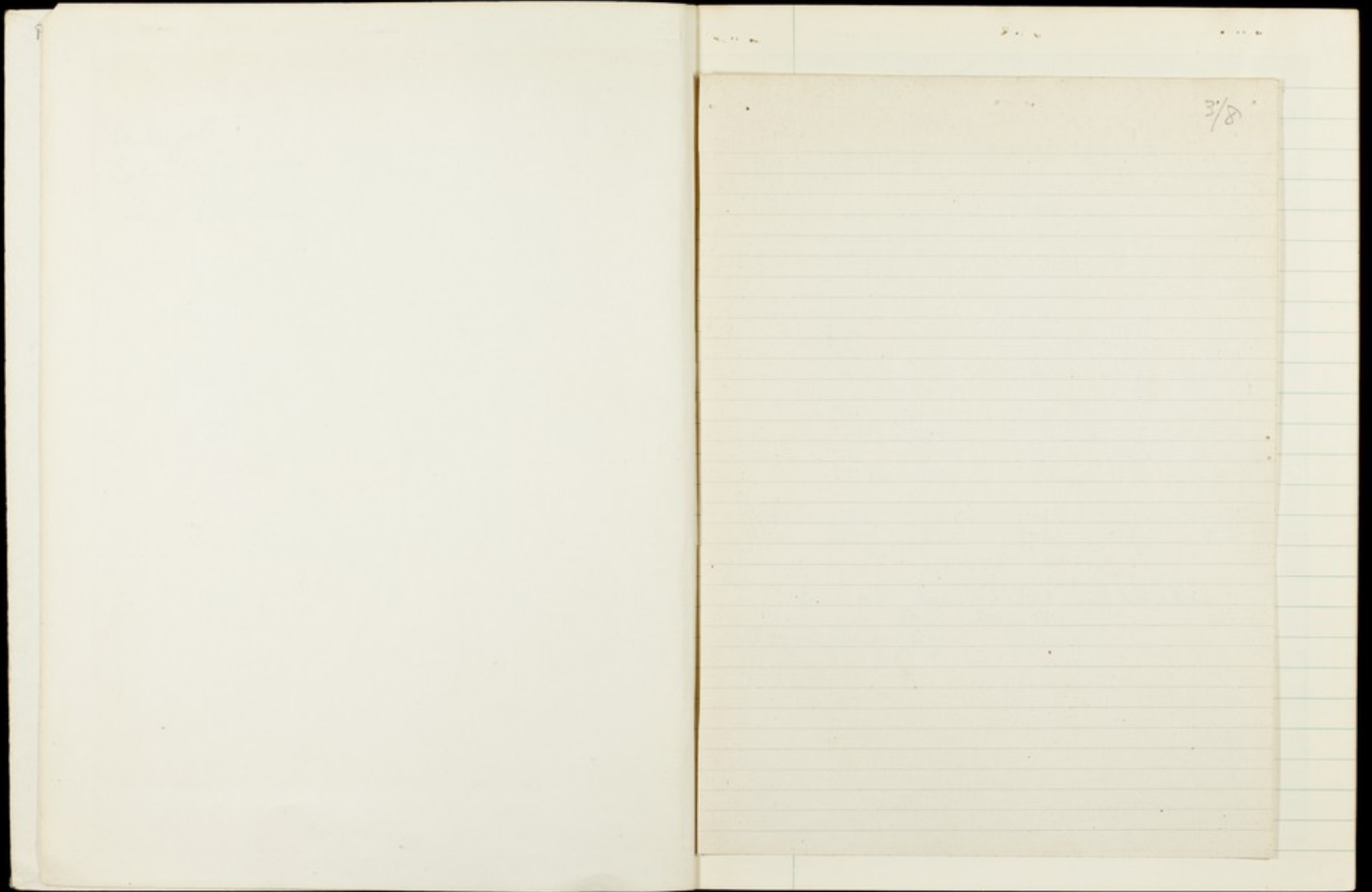
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PP/GRW/AF

A. H. Wyatt
Laboratory of Insect Pathology,
Saint Ste. Marie, Ind.

March, 1951.





0.1M Na₂CO₃ 1.6 ml
 0.1M NaCl 10 ml 10 to am
 water 8.4
 20.0

	A	G	C	T	Total
B ₁	.013	.014	.012	[.027]	
B ₂	.014	.014	.012	.036	
B ₂	.014	.014	.012	.036	
1	.273, .275	.331, .332	.272, .272	.185, .186	
2	.266, .268	.320, .321	.280, .281	.182, .186	
\bar{x}	.270	.326	.286	.185	
$\bar{x} - \bar{B}$.256	.312	.274	.149	
Yield/total	1.97	2.84	2.61	1.87	9.29
Ratios	0.849	1.222	1.123	0.806	4.000

P \approx comp. to total bases = $\frac{3.18}{31} \times \frac{8.35}{1.95} \times \frac{3.98}{3.98} = 10.0$ moles.
 % accounted for = 93%

12-11-51. LdVNA ①

100 mg air dry C₆₀ for 20 ml 0.008 M Na₂CO₃,
 0.05 M NaCl After 50 min. dash ground: mat.
 for dissolved, all dissolving.
 After 2 hrs 20 min. spin lowall 5000 rpm 5 min. →
 brown + white pellet, & large loose brown layer (skin?)
 the latter separated = apt. left. spin 10,000 50 min.
 Brown + white pellet + 20 ml ag. sol. spin 10,000 50 min.
 Pellet still has some brown ↑ 5 ml 0.001 Na₂CO₃ spin
 4000 2 min → ^{sample} pellet = both white & brown, no separation
 of min. from dust. Decant, spin (max. tube) 8000 40 min.
 spin in 0.5 ml ag. sol. transfer to small hydrolysis tube
 spin down 13,000 10 min. decant, & wash off some of brown
 layer, leaving final pellet almost all white.
 3-ii Dry down in tube. Looks like nothing left. Add 8 ul 70%
 H₂O, seal, wash 100° 2 hrs. Add 11.9 ul H₂O.
 Take 2 x 8.35 ul for chromatograms, 1 x 7.95 ul for P.
 Chromatogram spots 7.598 ml 4/10/44. Stand overnight, in
 round bottom test tubes, which mechanically 15 min. stand
 15 min. need., then read whole series again.
 P: 1.95 ul → 3.18 Y

0.1 M Na_2CO_3 6 ml
 0.1 M Na_2O 10 ml
 water 4
 20

Dist.	.60	.65	.57	.58													
A	6	C	T	"Cytidine"	U	"Thymidine"											
260	250	276	260	266	270	270	275	280	282	286	288	290	292	294	296	298	300
B	.026	.029	.024	.037	.04	.020	.09	.028	.03	.028	.03	.039	.039				
\bar{B}	.026	[.036]	.024	.037	.04	.020	.09	.028	.03	.028	.03	.039	.039				
\bar{B}	.026	.029	.024	.037	.04	.020	.09	.028	.03	.028	.03	.039	.039				
1	.212	.126	.105	.127	.128	.119	.125	.126	.127	.128	.129	.130	.131	.132	.133	.134	.135
2	.196	.125	.100	.126	.127	.128	.129	.130	.131	.132	.133	.134	.135	.136	.137	.138	.139
3	.204	.126	.103	.127	.128	.129	.130	.131	.132	.133	.134	.135	.136	.137	.138	.139	.140
5-8	.178	.097	.079	.090	.091	.092	.093	.094	.095	.096	.097	.098	.099	.100	.101	.102	.103
Yield	1.37	0.88	0.75	1.13													
Control	1.37	0.88	0.79	1.29													
Ratio	1.28	0.81	0.73	1.19													

Percent to total = $\frac{1.9}{31} \times \frac{8.35}{1.95} \times \frac{1}{3.98} = 6.6\%$ yield.
 % accounted for = $\frac{4.5}{6.6} = 68\%$

% NA in virus = $0.45 \times 4 \times \frac{21}{8.35} \times \frac{319}{2.7} \times \frac{1}{10} = 5.2\%$

1-iii-51. Cm capsule virus NA ①

100 mg air dry Cm caps. (HNO₃ prep)
 10:45 am ↑ H₂O as usual, then add NaCl + Na₂CO₃ to
 → 0.05 + 0.03 M susp. Sol'n begins to rise, but
 aggregates of caps. break up slowly.
 After 1 1/2 hrs, spin 6000 5 min → dry gray. brown pellet (trsp.)
 sp. spin 13,200 45 min → white pellet to clear brown
 layer over it. Try to wash in 0.5 ml. - great difficulty,
 impossible to decant. ^{adding 10.0 ml of 0.001 M diox. sol'n} spin 15,200 10 min.
 Dark ground: virus aggregates & some frags. Cannot remove frags.
 without losing most of virus. Wash up to 10 ml, spin 13,500
 30 min. susp. in 0.5 ml ag. sol., spin down in small
 hydrospin tube (pt. night) dry over P₂O₅, weigh (0.27 mg)
 add 8.35 ul 70% HClO₄, seal, cook 100° 2 hrs,
 add 11.9 ul ag. sol., take aliquots:
 2 x 8.35 on chromatogram
 2 x 1.95 for P
 → 1.8, 2.0 Y P = 1.9 Y.

Chromatogram poor: spots weak, spread, obvious thymidine spot,
 & v. faint U (?). ↑ 3.98 ml.

Odd results: prep undoubtedly contained some frags,
 but much not enough to account for these ratios! Repeat -
 cleaner fractions.

		0.1M Na ₂ CO ₃	0.1M NaCl	1% NaOH	
Conc. wanted	0.006 M HCl	0.6 ml	5.9	4.4	} HCl in 2 equal vol. for pH.
	0.008	0.8	5.0	4.2	
	0.010	1.0	6.0	4.0	

30 min. 51.

Cpe. Solution Test

Cpe p. prep. in aq. soln, 10 mg/ml. Min. 2 equal vol. Na₂CO₃ - HCl sol'n, to give final conc Na₂CO₃ 0.006, 0.008, 0.010. Day turbidity, storage dishes more quickly, but dead field after 30-45 min, shows most of all dissolved, 0.008 no more than 0.006.
∴ Use 0.006 M Na₂CO₃.

Na_2CO_3 1.2 ml \rightarrow 0.006
 NaCl 10 ml \rightarrow 0.05
 water $\frac{88}{20.0}$

	Yield	Ratio
A	2.46	1.170
G	1.91	0.909
C	1.73	0.824
T	2.31	<u>1.099</u>
	8.41	

$P(\bar{x} - xi - \sigma)$ 3.37 \times \approx 9.14 yields comp. to base
 Recovery = 92.1%

2-iv-51

Cpe V ①

100 mg. air-dry Cpe. \uparrow 20 ml 0.006 M Na_2CO_3 -
 0.05 M. NaCl. After 2 hrs. wash ground above almost all
 dissolved (same bag, same work!). Spin 5000 5 min \rightarrow fine sized
 pellet consisting of solid mixed layer & loose thin layer. Decant,
 leaving most above behind. Spin 10,000 60 min. \rightarrow white &
 brown pellets. Wash in 20 ml ag. dist. Spin 5000 5 min \rightarrow
 small white pellet (no brown). Off spin 10,000 60 min.
 Pellet \uparrow 0.5 ml ag. dist, wash out \approx 0.2 ml, with small trace
 spin down (15,000 10 min.), dry, weigh (= 2.6 mg),
 add 7.7 μ l 70% H_2O_2 , heat 100° (activity about 103).
 2 hrs, add 8.35 μ l ag. dist, take 2 \times 6.52 μ l for
 sp. 1 \times 1.95 μ l for P.
 Spots quite well resolved, tho a little streaky. \uparrow 3.98 ml.

		G ₂₀	A ₂₆	C ₂₇	T ₂₈
Blanks	1	.017	.015	.013	.035
	2	.015	.015	.013	.032
	\bar{x}	.016	.015	.013	.034
Samples	1	.237	.345	.179	.228
	2	.215	.324	.190	.207
	\bar{x}	.226	.335	.195	.218
S-B		.210	.320	.182	.184

5-IV-51.

One capsule - attempt to purify i high sp. g. solns.

Make up 60% sucrose in of dist. η_{inh} is actually purified capsules as follows: Spin 13,000 60 min.

Mill capsules in of dist	60% sucrose	% sucrose	(partic.)	Effect
2	38	57	1.269	All fully around the same: most of caps. have sedimented to give disgusting brownish grey pellets but add. is sediment treated too. See procedure.
4	36	54	1.252	
6	34	51	1.235	
8	32	48	1.219	

Hydrolysis: In 0.05 ml, 140 μ N \equiv 875 μ protein
Total is 5.0 ml \rightarrow 87.5 μ g capsules.

Use: Na_2CO_3 0.1M 3.0 ml \rightarrow 0.02 M
 NaCl 0.1M 8.7 ml \rightarrow 0.01 M
 H_2O up to 17.5 ml

9-iv-51

Con V (2)

Capsules which had been used for sedimentation in previous tests, and treated = chloroform-washed, fairly fine (some "dead bags"), re-washed, but some bound on N.

10.30 am. To susp., add NaCl , Na_2CO_3 to 0.05, 0.02 M, min.
11.10 From turbidity, caps. are obviously not dissolving. Add 1.75 ml 0.1 M $\text{Na}_2\text{CO}_3 \rightarrow$ 0.03 M.

12.11 Still v. turbid. Add 1.75 ml 0.1 M $\text{Na}_2\text{CO}_3 \rightarrow$ 0.04 M.

1 pm Still quite brown-turbid. Dark ground. Most caps. are at least reduced in size. Many bags revealed.

Spin 6000 5 min \rightarrow big gray-brown pellet, some of which from 1000.

Sp. spin 11,000 50 min \rightarrow fine, sized brown gelatinous pellet.

Resp. in 17 ml of det., spin 6000 5 min \rightarrow small gray smudged

Sp. spin 11,000 50 min \rightarrow smaller but still brown-gray pellet.

Resp. in 0.5 ml of det. Sp. spin. Dark ground: various sized particles, incl. a few large. Spin (intensity tube) 4 min

3000, fluff off from small sediment, spin down 10 min 13,000.

Pellet dry weight 3.0 μ g.

Hydrolyse - run chromatogram \rightarrow no NA.

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Capsules did not dissolve properly - chloroform cannot be washed

	Yield/ μ l	Ratio
A	1.66	1.174
G	1.27	0.899
C	1.14	0.806
T	1.58	1.128
	5.65	

$D(8.215) = 2.38 \text{ v} = 6.45 \text{ } \mu\text{mole sample } \mu\text{l}^{-1} \text{ } \mu\text{mole}$
 Recovery = 87.6%

11-iv-51.

BarVNA ①

Benzole from PBm 80/23₂, virus once sedimented & washed in H₂O & left overnight in fr₅, then sedimented down from in filtered. Wash in ca. 5ml H₂O, wash powder bags. Clarify 4000 4min, spin down 13,000 25 min, wash in 0.5 ml, spin down in hand tube, dry, weigh (= 2.0 mg). Add 7.7 μ l 70% HClO₄, wash 100° 2 hrs, after, add 8.35 μ l H₂O, wash, take 2 x 6.52 μ l chromatogram, 1.95 μ l for P.

Spots a bit weak but well resolved. \uparrow 3.98 ml $\frac{1}{2}$ H₂O.

		G	A	C	T
B	1	0.015 ⁻	0.016	0.015 ⁻	0.038
	2	0.016	0.015	0.014	0.044
	Σ	0.016	0.016	0.015 ⁻	0.041
Spots	1	.157	.236	0.136	0.166
	2	.153 ⁻	.228	0.134	.167
	Σ	.156	.232	.135 ⁻	.167
$\Sigma - B$.140	.216	.120	.126
Y base/spot		7.7	9.0	5.1	8.0

0.1M Na_2CO_3 10.5 ml = 0.03 M
 0.1M NaCl 17.5 ml = 0.03 M
 water 9.0
 35.0 ml.

Yielded Ratios

A	4.71	1.280
G	2.91	0.791
C	2.62	0.712
T	4.48	1.218
	14.72	4.001

P (precip) = 6.81 g = 17.9 yards comp. to bases.
 Recovery = 82.4%

11.9
 5.35
 15.25

8.35
 11.9
 20.25

13-IV-51 CaV (3)

17th my own capsules, treated to no chemicals, but died over silica gel in vac. 1.35 ml 0.03 M H_2CO_3 - 9:50 am.

11:50 0.05 M HCl . Dissolves well, the lump hard up slowly. After 2 hrs., open 5500 5 min \rightarrow red v. large brown flocks. Spd again 11,000 60 min \rightarrow nice, mostly white flocks. Susp. in 2% aq. sol. = some difficulty, add H_2CO_3 \rightarrow 0.001 M., doesn't help much, dil. up to 25 ml. Spd

2:10-2:40 13,000 40 min. Susp. in 0.7 ml of sol., open in small tube 13,000 10 min \rightarrow white flocks + brown stuff at bottom. Separate by filtering, open lower upper part again, dry, weigh (0.48 g). Add 11.9 ml 70% HCl , work 2 hrs 100°, add 16 ml H_2O , min, take 2 x 11.9 for spots, 3:52 after P. Spots 9.394 ml.

		G	A	C	T
B	1	.019	.020	.013	.042
	2	.018	.018	.014	.044
	\bar{x}	.019	.019	.014	.043
S	1	.338	.632	.286	.408
	2	.340	.630	.291	.390
	\bar{x}	.339	.631	.289	.399
S-B		.320	.612	.275	.356
Y base / spot		17.5	25.5	11.6	14.1

0.1 M Na_2CO_3 6.6 ml = 0.03 M
 0.1 M NaCl 11.0 ml = 0.05 M
 water to 22

109 mg Caps.

	Yield/ml	Ratio
A	3.47	1.294
G	2.04	0.761
C	1.45	0.690
T	3.36	1.252
	10.72	

$P(2 \times 15) = 3.60 \times 12.4$ (units comp to base)
 Recovery 86.2%

9.7
 11.3
 21.6

20-IV-51 Con V (4)

9:45 am.

Over specified capsules, no chemical treatment, but not very clean, ↑ 0.03 M Na_2CO_3 0.01 M NaCl

11:45

2 hrs: dark ground: caps. disintegrated, but lots of bugs.

After 6000 5 min → brown filled. Rinse off surface for EM = (2)

After 11,000 60 min → filled mostly white, only small

brown dust. ↑ 15 ml aq. lead, after 13,000 30 min

→ white filled ↑ 0.5 ml, after brown in brown tube, dry, weigh = 3.0 mg.

Add 7.7 ml 70% HClO_4 , cook 100° 2 hrs.

Add 8.35 ml H_2O , mix, low conc, no lead only 1 × 8.35 ml after. = 1.95 for P.

	G	A	C	T
B	.012	.013	.015	.039
S	.236	.464	.209	.306 .236
S-B	.224	.451	.194	.267

0.1 M Na_2CO_3 1.68 ml = 0.008 M 10S mg Pa.
 0.1 M Na_2SO_4 10.5 ml = 0.05 M
 Water up to 21

	Yield	Ratio
A	2.57	0.831
G	3.77	1.218
C	3.53	1.140
T	2.50	0.809
	12.37	

P (2x10) = 4.69 V = 12.7 (mole conc) L hours
 Recovery = 97.3%

20-iv-51
 9:45 am
 11:45

Ld V (2)

Bayliss's polyphosphate from Germany ↑ 0.008 M Na_2CO_3 , 0.05 M Na_2SO_4
 Disolved except for some brown lumps. Dark ground: some
 fragments dist, but for. distilled.
 Spin 6000 5 min → biggest brown & white pellet. (I) Spin off (1000) (2)
 Spin 11,000 60 min → pellet mostly white ↑ 15 ml eq
 1st, spin 13,000 30 min; ↑ 0.5 ml, spin down in bottle tube,
 weigh = 2.0 mg. To recover more resin, take sediment (D)
 ↑ 5 ml eq. dist, spin 5000 3 min: appeared spin. spin 13,000
 20 min → white pellet, wash in 0.5 ml, spin down on top of
 above 2 mg V. Dry. Total = 3.1 mg.
 Add 7.7 ul 70% HClO₄, cook 100° 2 hrs.
 Add 8.35 ul H_2O , take 2 x 6.52 g pellets, ↑ 1.95 for D
 Spin ↑ 3.98 ml.

		G	A	C	T
B	1	.014	.013	.015	.035
	2	.013	.014	.013	.037
	\bar{x}	.014	.014	.014	.035
S	1	.427	.349	.384	.233
	2	.430	.346	.385	.234
	\bar{x}	.429	.348	.385	.234
S-B		.415	.334	.371	.199

21-iv-51

U purification

Cpe U of 2-iv-51

Cm U of 20-iv-51

PBm 80/23 U

} treated identically :-

Spin 13,000 30 min to remove any large amount.
Take to pH 5.6-5.8, by methyl red, by addition of HAc
& NaOH. Spin 11,000 5 min.

	A ₂₆₄	G ₂₅₁	C ₂₇₆	T ₂₆₁	
B	1	.010	.007	.009	.044
	2	.011	.007	.009	.037
	\bar{x}	.011	.007	.009	.041
S	1	.222	.118	.100	.165
	2	.226	.116	.100	.173
	\bar{x}	.224	.117	.100	.169
S-B	.213	.106	.091	.128	
Yundepul	1.64	0.964	0.952	1.61	5.166
Molersato	1.270	0.746	0.736	1.246	3.998

18-V-51. *Modiolus sectifer* - NsV ①

Preliminary sol'n tests on clean Ns ps. - 2 mg ps
 ↑ 0.4 ml 10.006, 0.008, 0.010 M. N₂CO₂ - 0.05 M NaCl
 In all, considerable faunal material remains undissolved, even overnight. Dark ground: unincubated stuff as polished shells, singly & massed - these are mostly complete in outline in 0.006, & increasingly broken up in 0.008 & 0.010, but some seems to have been broken in all. Also, a few apparently intact polychaete shells.

10.10 cm

80 mg Ns ps. ↑ 16 ml 0.008 M N₂CO₂ - 0.05 M NaCl
 After 1 1/2 hrs @ room temp. dark ground: almost all ps. broken up, but fragments of shell remain; some polychaete still look normal, sh. b.
 Further 2 hrs in frag. spin 5500 5' → big brown pellets*
 Deep pellets in 15' and eq. dist. & spin down again 5500 5'.
 Ret. after spin 13,500 40 min. → pellets: black translucent part & brown streak. Combine ↑ 10 ml H₂O (imperfectly).
 spin 13,500 30 min. Pellet ↑ 0.5 ml H₂O, to small tube, spin down, dry, weigh = 1.9 mg NsV.

19-V

*Pellets of undissolved remains dried, transferred to vial = 8.6 mg.
 Transfer not quantitative; orig probably = ca. 12 mg.

21-V

To dry vials add 7.7 μl 72% HClO₄, heat, wash BWS
 2 hrs, add 8.35 μl H₂O, take 2 x 7.7 μl for sp. more for P.

	Bm		Cpe	
	Yield/ml	Ratio	Yield/ml	Ratio
A	2.58	1.152	3.50	1.175
G	2.02	0.903	2.68	0.901
C	1.79	0.800	2.42	0.813
T	2.565	1.146	3.305	1.110
	8.955	4.001	11.905	3.999

P.Y.
Yields correct
to bases

4.43

12.15

4.14

11.35

Height long time!

24-V-31. V Bm ② & V Cpe ②

Two vials to compare NA composition prepared & treated identically: 100 mg fa. + 20 ml 0.006 M Na_2CO_3 -

8:45 a.m.

0.05 M NaCl.

10:15

After 1 1/2 hrs, spin 6000 rpm 5', susp residues in 20 ml aq. dead, spin 6000 5'. Resid. after spin 11,000 50 min. Pellets + ca. 20 ml aq. dead, spin 11,000 60 min. Pellets + 0.5 ml aq. dead, spin down in small tubes.

Bm white - little yellowish layer; Cpe white + small dark brown.

Org: Bm = 3.5 mg; Cpe = 3.2 mg.

30-V

To each add 11.9 μl 70% HClO_4 , cook B&C 2 hrs.

Add 16.7 μl H_2O ($Z=9.35$), take 2 x 11.9 for spots, 1 x 3.52 for P. \uparrow 3.98 ml.

		A	G	C	T
B	1	.033	.022	.017	.058
	2	.030	.025	.020	.059
	\bar{x}	.027	.024	.019	.059
Bm	1	.360	.244	.205	.259
	2	.365	.244	.209	.267
	\bar{x}	.363	.246	.207	.263
$\bar{x} - B$.336	.222	.188	.204	
Cpe	1	.479	.314	.271	.323
	2	.484	.324	.274	.320
	\bar{x}	.482	.319	.273	.322
$\bar{x} - B$.455	.295	.254	.263	

	Pd		Ns	
	Yield/ul	Ratio	Yield/ul	Ratio
A	4.13	0.861	2.38	1.312
G	5.89	1.228	1.49	0.822
C	5.42	1.130	1.26	0.694
T	<u>3.75</u>	<u>0.782</u>	<u>2.125</u>	<u>1.171</u>
	19.19		7.255	

4-VI-51 Pd & Ns²
 9:45 am. 80 mg. for each sort ↑ 16 ml 0.008 M Na₂CO₃ 0.001 M NaCl
 12 M After 2 1/4 hrs. Lab full: Pd: well described; some thin central virus particles. Ns: big aggregated hump of polyhedral osmometer, but most of virus seems to have come out of them.
 Spin 6000 5 min, resins ↑ 15 ml ag. det., spin 5000 5 min. Combined after spin 13,000 30 min → small clear fluid clear pellets. ↑ 15 ml ag. det., spin 11,000 50 min. ↑ small vol. of det. spin 13,000 15 min in small tubes. Dry.
 Weigh Pd = 1.8 mg, Ns = 1.1 mg.
 19-VI. To each add 6.52 μl 70% HClO₄. Cook RWR 100° 2 hrs. Add 7.7 μl H₂O. Transfer whole, in refilling, to paper. Spin 13,980 ul

	A	G	C	T
B ₁	.014	.013	.012	.048
B ₂	.014	.013	.010	.042
B _Σ	.014	.013	.011	.046
Pd	.557	.661	.580	.344
Pd-B	.543	.648	.569	.298
Ns	.324	.177	.143	.215
Ns-B	.310	.164	.132	.169

T4r-8		
Yield/total	Ratio	
A	5.29	1.438
G	3.03	0.824
C	1.46	0.397
T	4.94	1.341
U	[0.13]	[0.035]
	14.72	4.000

19-VI

Phage HA

Calens T4r-8 weighed 2.6 mg \uparrow 11.9 μ l 70% HClO₄
 100' 2 hrs. Add 18 μ l H₂O Carbon residue in solid
 cake, interface = pipetting. Take 3 x 1.95 μ l for P,
 1 x 11.9 for bases (second aliquot lost in pipette).
 Good chromatogram; faint C spot, no visible U.
 Cut out U portion along = other \uparrow 3.98 ml.

	A	G	¹²⁰ C	²⁷⁰ C	²⁸⁰ C	²⁸⁵ C	²⁹⁰ C	²⁹⁵ C	T
B ₁	.016	.015	.011	.017	.017	.016			.045
B ₂	.014	.013	.009	.016	.015	.014			.045
B ₃	.015	.014	.010	.017	.016	.015			.045
T ₄	.703	.377	.146	.163	.167	.026	.026	.026	.438
T ₄ -B	.688	.333	.153	.169	.171	.009	.010	.011	.393

Cytosine spots checked, read directly against blank

	H ₂ HClO ₄		pH 9.5		N ¹⁵ /10 H ₂ O	
215	.204	280 .159	285	.002	260	.009
220	.167	281 .157	280	.022	265	.021
225	.114	282 .155	275	.028	270	.035
230	.064	283 .152	270	.045	275	.047
235	.034	284 .149	269	.046	277	.051
240	.026	285 .146	268	.044	278	.052
245	.023	286 .144	265	.042	280	.050
250	.027	295 .071	260	.034	285	.047
255	.028	303 .032				
260	.028					
265	.029					
270	.036					
275	.034					
280	.036					
285	.038					
290	.039					
295	.039					
300	.039					

(not enough alkali?)

	BSNA		EurTMA		Lysine V	
	Yielded	Ratio	Yielded	Ratio	Yielded	Ratio
A	4.45	1.121	3.61	1.041	3.74	1.060
G	3.72	0.988	3.09	0.892	3.27	0.926
C	3.28	0.827	2.98	0.861	2.90	0.822
T	4.42	1.113	4.17	1.203	4.21	1.191
Total	15.87	3.999	13.85	3.999	14.12	3.999
"U"	0.37	0.098	0.42		0.388	

A	
G	
C	
T	
Total	
"U"	

21-VI-51

DNA - check on Recovery

Weight into small tubes: 3.8 mg German TMA (known!)
 3.8 mg own BSNA
 20.9 mg "synth. V" 15% BSNA, 85% U.P.
 To each add 40 μ l 70% HClO₄.
 Seal, cook BUB 2 hrs. (first 1/2 hrs may have been 2-3' below temp.)

	A	G	C	255	"U" 260	265	T
B ₁	.025	.021	.016	.026	.023	.020	.054
2	.022	.024	.016	.021	.019	.018	.046
\bar{x}	.024	.023	.016	.023	.021	.019	.050
BSNA 1	.579	.407	.346	.033	.031	.029	.352
2	.579	.411	.344	.027	.027	.024	.352
\bar{x}	.578	.409	.345	.030	.029	.027	.352
EurTMA 1	.468	.340	.314	.035	.034	.031	.334
2	.470	.364 $\times \frac{100}{100}$.312	.032	.031	.029	.330
\bar{x}	.469	.340 (?)	.313	.034	.033	.030	.332
Lysine V 1	.468	.349	.299	.029	.027	.025	.321
2	.501	.344 \times	.309	.029	.028	.026	.349
\bar{x}	.486	(.360)	.304	.029	.028	.026	.335

	T6r-7		T6r-6		T4r-7	
	Yielded	Ratios	Yielded	Ratios	Yielded	Ratios
A	7.03 7.03	1.610	3.79	1.622	7.01	1.540
G	4.08	0.955	2.32	0.996	4.46	0.980
C	0.16	0.037	0.10	0.042	0.50	0.110
T	6.19	1.418	3.13	1.300	6.20	1.390
Total	17.46	4.000	9.34	3.948	18.21	4.000
U ^o	0.11	0.025	0.08	0.034	0.16	0.035
P.Y (2000)	3.63 3.68	3.66	3.65 3.27	3.46		
	10.1		11.9			
	96		95.4			

22.vi.

Phages

Weight: T6r-7 30 mg
 T6r-6 30 mg
 T4r-7 3.3 mg. Each + 15 µl 70% HCO₂

Hydr. 2 hrs 100° (actually ca. 103°). Add 20 µl H₂O.
 Centrifuge, but carbon does not sediment well, & infectives =
 floating.

Take: T6r-7: 1 x 11.9 spots, 2 x 1.95 for P
 T6r-6 2 x 8.35 spots, 3 x 1.95 for P
 T4r-7 2 x 11.9 spots, no P.

23.vi Chromotopans being in tank without solvent overnight.
 24.vi Spots appear to have spread by taking up moisture. All solvent.

	A	G	C		U	T				
	270	275	280	285	260	265				
B	1	.027	.027	.016	.015	.013	.023	.021	.019	.059
	2	.028	.030	.014	.012	.011	.026	.023	.020	.042
	\bar{x}	.028	.029	.015	.014	.012	.025	.022	.020	.051
T6r-7		.942	.488	.032	.031	.050	.052	.031	.029	.543
T6r-7-B		.914	.459	.017	.017	.018	.007	.009	.009	.492
T6r-6	1	.572	.281	.026	.025	.023	.030	.029	.027	.297
	2	.529	.287	.024	.023	.022	.017	.026	.024	.303
	\bar{x}	.521	.284	.025	.024	.023	.029	.028	.026	.300
\bar{x} -B		.493	.258	.010	.010	.011	.004	.006	.006	.249
T4r-7	1	.928	.516	.069	.068	.062	.035	.034	.032	.534
	2	.950	.524	.063	.062	.058	.035	.035	.033	.560
	\bar{x}	.939	.520	.066	.066	.060	.035	.035	.033	.547
\bar{x} -B		.911	.491	.057	.052	.048	.010	.013	.013	.496

	T6r-7		① T4r-7		② T4r-7		Mean ratios
	Yield/ml	Ratios	Yield/ml	Ratios	Yield/ml	Ratios	
A	5.86	$\frac{1.607}{0.275}$	6.61	$\frac{1.550}{1.472}$	6.39	1.540	1.545
G	3.47	0.952	3.85	$\frac{.906}{1.419}$	3.82	0.922	0.913
"C"	0.32	0.060	0.97	$\frac{0.228}{0.234}$	0.97	0.234	0.231
T	5.04	1.381	5.63	$\frac{1.220}{1.270}$	5.40	1.302	1.311
Total	14.59	4.000	17.06	$\frac{0.002}{2.474}$	16.58	3.998	4.000
"V"	0.19	0.052	0.18	$\frac{0.036}{0.447}$	0.14	0.034	0.032

	T4r-7		Mean ratios
	Yield/ml	Ratios	
A	7.70	1.553	1.558
G	4.46	0.899	0.903
"C"	0.79	0.160	0.168
T	6.87	1.387	1.372
Total	19.81	3.999	$\frac{4.001}{3.991}$
"V"	0.13	0.026	0.028

25-VI

Phages

Weight, in Pipette tubes made to pointed bottoms:

T4r-7 2.9 mg

T4r-7 3.7 mg

T6r-7 3.0 mg

Add 15 μ l 70% H₂O₂.

2 lbs on B.W.B. open, add 25 μ l H₂O

26 VI: Min in little stirring rod, centrifuge on International, tube 3 x 1.96" for P. 2 x 11.9 for appts (in 4r 54r would not fill pipette second time).

	A	G	C	270	275	280	285	260	261	T
B ₁	.021	.017	.015	.014	.013	.024	.022	.021	.021	.065
2	.024	.022	.018	.017	.016	.021	.020	.019	.019	.050
2̄	.023	.020	.017	.016	.015	.023	.021	.020	.020	.058

T6r-7 (1)	.791	.401	.039	.040	.034	.033	.032	.449
HK (2)	.798	.402	.039	.038	.039	.039	.449	
̄	.795	.402	.039	.039	.036	.036	.449	
T6r-7-B	.762	.382	.023	.024	.015	.015	.401	
T4r-7 (1)	.882	.407	.107	.118	.120	.031	.586	
"-B	.859	.424	.102	.105	.010	.449		
T4r-7 (2)	.853	.419	.118	.117	.033	.487		
"-B	.830	.420	.102	.102	.011	.429		
T4r-7 (1)	1.02	.510	.099	.100	.031	.604		
"-B	1.00	.490	.088	.088	.010	.546		
T4r-7 (1)	.755	.380	.082	.082	.030	.449		
"-B	.732	.360	.066	.067	.008	.389		

3-11-51.

Identification of spot in C position

All 'C' tubes from freeze analysis of 26-11 & all T tubes filtered, laid down, ↑ small vol. H₂O, run in PonOH-NH_3 .

'C' → spot = RF slightly less than G.

T → big T spot, ↑ faint streak of low RF.

Then support unlabeled spots put out, eluted by dropping, laid down, ↑ 0.1 ml 86% HCOOH, higher 175° 2 hrs. Dry down, ↑ in v. small vol. H₂O, transfer whole to paper, run in PonOH-NH_3 → spot still in G position!

6-11

Elute directly on to fresh paper, run in PonOH-HCOOH (10%, 86%)

9-11

→ spot still in G position!

Elute to check UV spectrum, in eq. solvent; read directly against standard

pH 7.5		pH 1		pH 13	
245	.009	240	.001	245	.009
250	.008 ←	245	.0015	250	.005
255	.009	250	.0035	255	.005
265	.0125	2		275	.016
270	.0135 ← P	275	.0225	280	.018
275	.012	280	.024 ← P	285	.019 ← P
		285	.021	290	.016

Substance is unchanged by HCOOH hydrolysis, and is not cysteine, because of spectrum in alkali

UV spectra		OH	N	H
Alkuaon	Peak	285	270	280
	Langh	250	250	245
Cytosine	Peak	279	267	275
	Langh	250	247	238
Cytidine	Peak	272	271	280
	Langh	251	250	241

30-IV-51. "Cytosine" ex flage.
 Effects of C position of 2 runs of Tet r⁻⁷ of 24-01
 dried down, ↑ aq. leat., run in Sandell-NH₄. Small spot
 in G (!) position, more or almost none in C position.
 Out out, along i spots from TONIK NA, ↑ 4 ml aq. leat.,
 take pH to 8 i 2 drops 1% NaHCO₃, read directly against corrected
 blanks.

pH 8.

Tet spot in G position	G ex TONIK NA	T4 C position	C ex TONIK NA
290 .004	280 .063		
280 .011	275 .068 P		
275 .013	270 .067		270 .089
270 .014 P		265 -.005	265 .092
265 .013	250 .053		260 .085
260 .011	245 .055 P		
255 .010	240 .078		
250 .0081 T	235 .049 T		
245 .0095			
240 .011			
230 .017			
225 .021			

pH 1
 (by adding
 1 drop
 conc. HCl)

T4 spot in G position
295 .011
290 .017
285 .022
280 .024 P
275 .023
270 .019
265 .015
260 .011
255 .009
250 .005
245 .004
240 .005
235 .006
230 .010
225 .016

pH 13, by adding 2 drops 40% NaOH.

295 .012
290 .016
285 .019 ← P
280 .017
275 .015
270 .012
265 .009
260 .006
255 .0045
250 .0035
245 .007
240 .013
235 .019
230 .021
225 .020

	G	A	C		U	T
			275	280	260	
Blank 1	.029	.031	.020	.018	.031	.063
2	.023	.026	.017	.015	.025	.057
\bar{x}	.026	.029	.019	.017	.028	.060
T4r-7 1	.378	.673	.057	.055	.039	.374
2	.370	.661	.057	.055	.033	.365
\bar{x}	.374	.667	.057	.055	.036	.370
$\bar{x}-B$.348	.638	.028	.028	.008	.310
T4r-8 1	.316	.569	.059	.056	.027	.295
2	.301	.528	.051	.048	.027	.252
\bar{x}	.309	.547	.055	.052	.022	.264
$\bar{x}-B$.283	.520	.026	.025	.004	.204
	T4r-7		T4r-8			
	Yield/total Ratio		Yield/total Ratio			
A	4.91	1.590	4.00	1.689		
G	3.16	1.023	2.575	1.027		
"C"	0.362	0.117	0.343	0.145		
T	3.90	1.264	2.565	1.083		
Total	12.332	3.994	9.465			
through U	0.10	0.032	0.085	0.021		

4-VII-51

Phage Schmidt's Flammanor fractionation

Weigh: T4r-7 - 3.3 mg

T4r-8 - 2.8 mg with small portion of end tubes

Add 0.15 ml $N H_2O$ to each. Allow Phase in 26-28" incubator 4×10^4

5:41

9 am. Stir up flocculent material

2 pm 32 hrs. Add 10 μ l H_2O (check pH and to water part)

0.2 ml 95% EtOH, spin. Stir up ppt in 0.3 ml undiluted 95% EtOH & spin. Dry.

Weigh: T4r-7 1.5 mg

T4r-8 1.1 mg assuming weight of tubes unchanged

Add 15 μ l 70% HClO₄. 90% 100° 2 hrs. Add 25 μ l H_2O .

Spin. Take 2 x 11.9 μ l spots, 4 x 1.95 μ l P.

Chromatograms show "C" spots, perhaps residual but still hardly found, v. faint spot between C & U (not end end), and biggest spot just above G.

"Pre-G" read through against blanks 1 & 2 -

	T4r-7 ①	②	T4r-8 ①	②
225				
230	.045			
235	.034			
240	.033	.033	.026	
245	.039		.021	
250	.049		.029	
255	.059			
260	.068			
265	.071			
270	.072		.054	
275	.072	.071	.055	.046
280	.071		.054	
285	.064			
290	.056			
295	.049			
300	.038			
305	.015			
310	.004			

	Cpe		NBC-RNA		Cpe + RNA	
	Yield/total	Ratio	Yield/total	Yield/total	Yield/total	\bar{x}
A	2.855	1.236	576	1.073	3.30	
G	2.09	0.907	648	1.207	2.67	
C	1.96	0.850	4.485	0.836	2.315	
U	[0.27]		4.69	0.874	0.645	
T	2.325	1.009	[0.10]	[0.019]	2.19	
	9.330	4.002	21.415	4.000		

Hydrolysis too fast!

Cpe + RNA cont'd.

13-VII-51. All tubes hydro. in 10 μ l 72% HClO₄ 100° 2 hrs.
Add 15 μ l H₂O. Min, after (no admittance of carbon in run containing Cpe), take 2 x 8.35 μ l each, T 3 or 4 x 195 for P.

17-VII.

Final set

		G	A	C	T	U	C	T
B	1	.031	.029	.016	.015	.032		.044
	2	.031	.025	.016	.015	.031		.034
	\bar{x}	.031	.027	.016	.015	.034		.049
Cpe	1	.262	.394	.221	.205	.049		.224
	2	.260	.401	.223	.207	.060		.243
	\bar{x}	.261	.398	.222	.206	.055		.237
\bar{x} -B		.230	.371	.206	.191	.021		.185
NBC-RNA	1	.740	.777	.483	.450	.399		.050
	2	.748	.774	.490	.457	.408		.064
	\bar{x}	.744	.776	.487	.454	.404		.057
\bar{x} -B		.713	.749	.471	.439	.370		.058
Cpe + RNA	1	.327	.454	.256	.240	.083		.217
	2	.323	.457	.264	.244	.086		.229
	\bar{x}	.325	.456	.259	.242	.085		.223
\bar{x} -B		.294	.429	.243	.227	.057		.174

	Cpe-NaOH		Cpe-RNA-NaOH(D)		Cpe-RNA-NaOH(2)	
	Yield	Ratio	Yield	Ratio	Yield	Ratio
A	2.33	1.42	2.62	1.38	2.145	1.49
G	1.79	1.09	2.04	1.08	1.62	1.12
C	1.54	0.94	1.74	0.92	1.315	0.91
T	0.92	0.57	1.17	0.62	0.69	0.48
U	[0.14]	0.085	[0.165]	0.087	[0.125]	0.087
	6.58		7.57		5.77	

Cpe RNA con'd.

200. second set.

	G	A	C	U	T
B ₁ 11	.015	.016	.013	.255	.260
2	.015	.015	.013	.022	.021
\bar{x}	.015	.016	.013	.019	.019
Cpe-NaOH 11	.211	.316	.174	.031	.029
2	.212	.322	.176	.032	.030
\bar{x}	.212	.319	.175	.032	.030
\bar{x} -B	.197	.303	.162	.011	.010
Cpe-RNA-NaOH 2					
1	.238	.359	.196	.034	.031
2	.240	.355	.196	.035	.032
\bar{x}	.239	.357	.196	.035	.032
\bar{x} -B	.224	.341	.183	.014	.018
Cpe-RNA-NaOH 2					
1	.198	.296	.153	.031	.029
2	.193	.294	.149	.031	.029
\bar{x}	.193	.295	.151	.031	.029
\bar{x} -B	.178	.279	.138	.010	.010

T4r-8 - NaOH

	Yield/pt	Rates
A	4.83	1.65
G	3.16	1.08
C	0.29	0.10
T	3.72	1.17
V	[0.09]	[0.03]
	11.70	4.00

19.VII

Phage S & T treatment (2)

3.5 mg T4r-8 + 0.1 ml H-NaOH 27° 48 hrs.

21.VII

Ppt = 0.01 ml H₂O + 0.1 ml ESBM, spin down, wash ppt twice: 0.1 ml and 5% ESBM, dry down ppt & washings. In each add 10 µl 70% HCl, cook 100° 2 hrs., add 15 µl H₂O. Take, from ppt 2 x 8.35 µl (could not get any for P), 5 µl from ppt. 2 x 16.7 µl.

Chromatograms show v. little C, no U, but faint "Pre-G" & "Pre-U" spots. Spt. shows practically nothing! - had used ppt. & a faint T? spot.

		G	A	275 ^c	280	"U"	T
B	1	.017	.016	.010	.010	.023	.052
	2	.012	.015	.009	.008	.024	.053
	\bar{x}	.015	.015	.010	.009	.024	.053
T4r-8 NaOH	1	.309	.640	.040	.038	.029	.324
	2	.366	.644	.040	.038	.032	.326
	\bar{x}	.363	.642	.040	.038	.031	.326
\bar{x}	- B	.348	.627	.030	.029	.007	.272

Read directly against own standards.

Pre-G	Pre-U	"Pre-U"	
		①	②
250	.045	.230	.018
260	.053	.244	.011
263	.054	.280	.019
265	.054	.260	.033
267	.054	.265	.041
270	.054	.270	.048
275	.050	.275	.051
		.280	.044
		.290	.018

22 Could be decay of "Pre-U" !!

Spis.	N/ml	Volume	Protein/ml	Total Protein ml
			Initial	
1	111 222	117 ml	0.70	82 mg
2	14 24		1.39 mg	
3	5.6 H-2	50	0.087	9.7
4	5.01 10.3		2.17 mg	
5			0.035	1.7
			0.070	1.6
			0.032	2.0
			0.064	
			Assume	

Upe Purification (Mason)

17-23-41-51

U from 500 mg Upe ⁽¹²⁻⁴⁻⁵¹⁾ from 13,000 60 min, ppt
 by taking to pH 5.8 (methyl red) + HCl, as described in
 0.1 M. NaHSO₃ r.n. ppt. to total of 4 times. Bridge against
 eq. dist. in 5' change. → pH 7. for begins pfling, but
 took to pH 5.8 to make sure. Spin down, dry.

T4r-7 RNase		T6r-7-NaOH		
Yards	Ratio	Yards	Ratio	
A	5.13	1.61	3.22	1.57
G	3.16	1.00	2.16	1.05
C	0.18	0.057	0.20	0.097
T	4.23	1.33	2.64	1.38
[U]	[0.63]	[0.041]	[0.11]	[0.024]
	12.70	4.00	8.22	4.00

25-11-51.

Plugs - ① Removal of "c" by RNase
 ② Effect of NaOH on flange and containing "c"

- ① T4r-7 2.9 mg + 0.2 ml ag. sol. containing RNase 0.1 mg/ml
- ② T6r-7 2.4 mg + 0.1 ml 1N-NaOH.

12 M.

Incubate 27° 48 hrs.

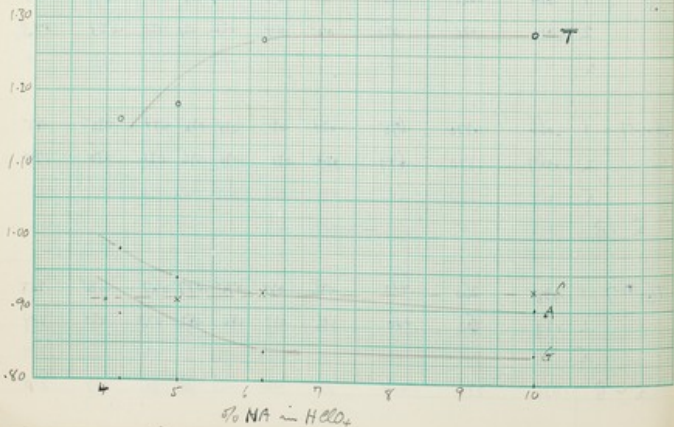
27th.

~~Plugs~~ ① Thin down, wash once in acid 50% EtOH, dry.
 ② Ppt. by first HCl, but → fine non-sedimentable ppt., so use EtOH, Re-precipitate in 1% H₂O₂ = re-ppt. Dry.
 Add to each: 15 µl 70% HClO₄, cook 100° 2 hrs, add 15 µl H₂O. Lick 2 x 8.35 for spots, from ② only, 2 x 1.95 for P.

HYDROLYSIS
 TEMP. 700 1100 !!

	Px-G	G	A	C		Px-U			U	T ₂₆₅	
	265	270	280	275	280	270	275	280	260	265	
B	1	.014	.025	.021	.015	.04	.019	.018	.07	.027	.044
	2	.011	.021	.021	.012	.04	.022	.024	.020	.024	.043
	\bar{x}	.013	.023	.021	.015	.04	.021	.020	.019	.026	.044
T4r-7	1	.066	.370	.675	.034	.031	.038	.038	.034	.035	.386
	2	.069	.370	.679	.034	.031	.036	.036	.032	.036	.374
	\bar{x}	.068	.370	.687	.034	.031	.037	.037	.033	.036	.380
	$\bar{x} - B$.055	.347	.666	.019	.017	.016	.017	.014	.010	.336
T6r-7	1	.038	.260	.440	.036	.034	.074	.077	.067	.036	.258
	2	.039	.262	.440	.036	.033	.080	.082	.073	.034	.249
	\bar{x}	.039	.261	.440	.036	.034	.078	.080	.070	.035	.254
	$\bar{x} - B$.026	.238	.419	.021	.020	.057	.060	.051	.009	.210

	① 10%		② 6.2%		③ 5%		④ 4.2%	
	Yards	Ratios	Yards	Ratios	Yards	Ratios	Yards	Ratios
A	0.977 0.90	1.054	0.92	1.36	0.94	2.26	0.98	
G	0.909	0.84	0.963	0.84	1.31	0.91	2.05	0.89
C	1.000	1.925	1.057	0.92	1.32	0.91	2.10	0.91
T	1.382	1.28	1.489	1.27	1.71	1.18	2.66	1.16
	4.268	3.94	4.833	3.95	5.90	3.94	9.07	



25-10-51

Effect of conc. in H₂O₂ during hydrolysis, with protein.

4 tubes, each cont. weight 2.5 mg ^{100% protein} + 10 mg bovine serum albumin (protein)

All ① 25 μl H₂O₂ (NA = 10%, protein 2%), 46 μl pH 7.5 buffer

② 40 - (NA = 6.2%, protein 2%) - 60

③ 50 - (NA = 5%, protein 2%) - 40

④ 60 - (NA = 4.2%, protein 2%) - 40

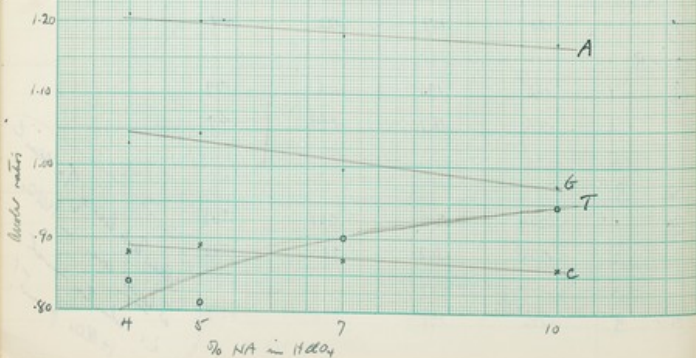
100° 2 hr. 8.35 μl pH 7.5 buffer. Well washed, over at end of ③ - 2.

		G	A	C	T
B	1	.016	.014	.011	.040
	2	.013	.014	.011	.036
	\bar{x}	.014	.014	.011	.038
①	1	.118	.146	.120	.154
	2	.109	.136	.112	.142
	\bar{x}	.114	.141	.116	.148
$\bar{x} - B$.100	.127	.105	.110
②		.120	.151	.122	.154
② - B		.106	.137	.111	.116
③	1	.163	.195	.156	.179
	2	.152	.187	.144	.169
	\bar{x}	.158	.191	.150	.174
$\bar{x} - B$.144	.177	.139	.136
④	1	.244	.310	.231	.253
	2	.235	.303	.230	.246
	\bar{x}	.240	.307	.231	.250
$\bar{x} - B$.226	.293	.220	.212

Effect of the conc. of the protein
at lower conc. in H₂O₂
i.e. when too much
H₂O₂ present!

	① 4%		② 5%		③ 7%		④ 10%	
	Yields	Ratio	Yields	Ratio	Yields	Ratio	Yields	Ratio
A	2.88	1.21	3.12	1.20	4.88	1.18	5.06	1.17
G	2.45	1.03	2.71	1.045	4.10	0.995	4.21	0.975
C	2.10	0.88	2.31	0.89	3.58	0.87	3.70	0.86
T	2.00	0.84	2.09	0.81	3.72	0.90	4.07	0.945
	9.43		10.28		16.28		17.04	

P, Y 2.68 } 2.58
 2.47 }
 Yields of
 or lower 8.90 10.06
 % recovery 10.5% 10.2%



28-11-11. Effect of $HClO_4$ conc. during hydrolysis - BSKA without protein.
 into 4 tubes weigh 3 mg each BSKA.

① Add 75 μ l 70% $HClO_4$ \rightarrow 4% NA

② 60 5%

③ 43 7%

④ 30 10%

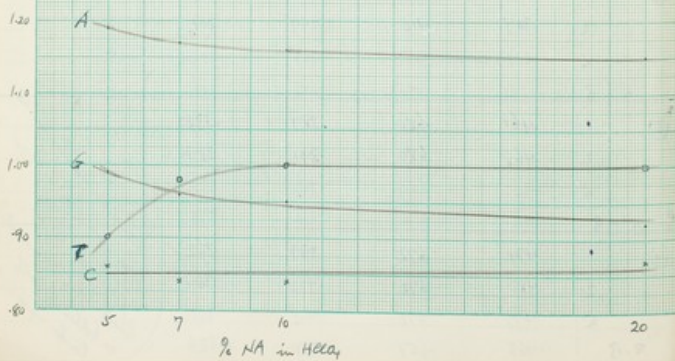
Cook 100° 2 hrs. Add to each 50 μ l H_2O . Take 2.5, 3.5, 1.3, 1.4 μ l P

		G	A	C	T
B	1	.014	.014	.012	.040
	2	.014	.015	.013	.038
	\bar{x}	.014	.015	.013	.039
①	1	.294	.401	.236	.202
	2	.274	.379	.231	.194
	\bar{x}	.284	.390	.234	.198
$\bar{x} - B$.270	.375	.221	.159
②	1	.314	.428	.262	.208
	2	.309	.410	.249	.201
	\bar{x}	.312	.419	.266	.205
$\bar{x} - B$.298	.405	.248	.166
③	1	.461	.650	.387	.335
	2	.468	.650	.391	.334
	\bar{x}	.465	.650	.389	.335
$\bar{x} - B$.451	.635	.376	.296
④	1	.474	.672	.399	.362
	2	.480	.672	.405	.364
	\bar{x}	.477	.672	.402	.363
$\bar{x} - B$.463	.657	.389	.324

Purity as marked to show
 from counting at 110°
 check oil bath
 on bottom of bath
 temp. up to 110°

	① 5%		② 7%		③ 10%		④ 20%	
	Yields	Ratios	Yields	Ratios	Yields	Ratios	Yields	Ratios
A	3.66	$\frac{1.17}{.32}$	3.83	1.17	3.73	1.16	3.54	1.15
G	3.05	$\frac{0.99}{.32}$	3.14	0.96	3.05	0.95	2.88	0.92
C	2.64	0.86	2.76	0.84	2.70	0.84	2.71	0.87
T	2.77	0.90	3.22	0.98	3.21	1.00	3.13	1.00
	12.12	3.94	12.95	3.95	12.69	3.92	12.30	3.94

P ₁ Y	2.45	3.18	3.70	3.75
	3.30	3.44	3.25	3.65
	2.98	2.99	3.15	3.73
Yields sum or twice (x 2.34)	9.8	11.1	11.65	12.8
"% remaining"	12.4%	11.7%	10.9%	9.6%



31-11-51.

Effect of $HClO_4$ conc. during hydrolysis - BSHNA, accurate temperature
 into each of 4 tubes, 3 runs over BSHNA.

- ① 60 μ l $HClO_4$ \rightarrow 5% NA. After hydrolysis, 40 μ l H_2O
- ② 43 " \rightarrow 7% " 57 2 hrs at 100°
- ③ 30 " \rightarrow 10% " 70 well controlled
- ④ $\frac{15}{20}$ " \rightarrow $\frac{20}{15}$ % " 85 (5 10°)

	G	A	C	T
B 1	.014	.014	.013	.013
2	.014	.014	.014	.014
\bar{x}	.014	.014	.014	.014
① 1	.349	.480	.286	.261
2	.351	.488	.296	.262
\bar{x}	.350	.489	.291	.262
$\bar{x} - B$.336	.475	.277	.250
② 1	.360	.513	.305	.293
2	.358	.508	.302	.302
\bar{x}	.359	.511	.304	.298
$\bar{x} - B$.345	.497	.290	.286
③ 1	.348	.495	.297	.292
2	.352	.502	.299	.301
\bar{x}	.350	.499	.298	.297
$\bar{x} - B$.336	.485	.284	.285
4 1	.332	.479	.298	.290
2	.330	.478	.299	.292
\bar{x}	.331	.479	.299	.291
$\bar{x} - B$.317	.465	.285	.279

	T6r-6 RPhase		T4r-7 RPhase	
	Yields	Ratios	Yields	Ratios
A	4.19	1.619	4.33	1.627
G	2.36	0.912	2.45	0.938
C	0.11	0.042	0.14	0.054
T	3.70	1.430	3.52	1.347
U	[0.09]	[0.035]	[0.11]	[0.042]
	10.36	4.003	10.44	3.996

P. 6-ix T4r-7 RPhase 3.45 } 3.49 / P = 0.1127 yields
3.52 }
Conc. base = $0.1044 \times 3.98 \times 1.95 = 0.898$ yields
% recovery = 86.8
Yields P conc. to base = 12.0 86.8%

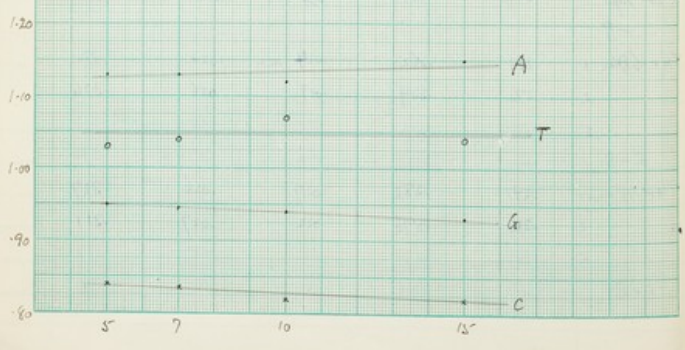
T6r-6 RPhase 3.22 } 3.51
3.70 } Yields conc. to base = $3.46 \times 3.81 = 12.1$ 85.3%

1-VIII-51. Phase + RPhase
T6r-6 2.5" avg. } To each, 0.2 ml RPhase 0.1 mg/ml in water.
T4r-7 2.7" avg. } Incubate 27° 24 hrs. Then stand in paper until
3:00 spin down (Lowell), draw off apt., small tubes = 0.2 ml portions
of each, spinning down. Dry down orig. apt. & first wash in
small tubes. Dry virus residues.
4:00 To residue tubes add 10 µl MeOH; to apt. tubes add 5 µl. Look 100x 2 hrs.
Add to residue tubes 20 µl H₂O; to apt. ¹⁰µl H₂O. 82 µl apt. 1.95 µl?

	G	A	C	U	T
B	1 .016 .017	.016	.010	.018	.036
	2 .024 .023	.016	.009	.017	.042
\bar{x}	.020	.016	.010	.018	.039
F6r-6 apt	.232	.415	.055	.027	.218
-B					
T4r-7 apt	.042	.064	.019	.015	.066
-B					
F6r-6 RPhase	1 .283	.562	.024	.024	.330
	2 .276	.539	.019	.026	.336
\bar{x}	.280	.561	.022	.025	.333
\bar{x} -B	.260	.545	.012	.007	.294
T4r-7 RPhase	1 .291	.582	.027	.026	.327
	2 .286	.576	.022	.027	.311
\bar{x}	.289	.579	.025	.027	.319
\bar{x} -B	.269	.563	.015	.009	.280

	① 5%		② 7%		③ 10%		④ 15%	
	Units	Ratio	Units	Ratio	Units	Ratio	Units	Ratio
A	3.65	1.13	3.81	1.13	3.60	1.12	3.48	1.15
G	3.08	0.95	3.19	0.965	3.03	0.94	2.82	0.92
C	2.92	0.84	2.82	0.835	2.65	0.82	2.44	0.82
T	3.32	1.03	3.51	1.04	3.44	1.07	3.16	1.04
	12.77	3.95	13.33	3.95	12.72	3.95	11.94	3.97

P, Y	3.44 } 3.38 3.32 }	2.83 } 3.35 3.86 }	3.65 } 3.60 3.55 }	4.00 } 3.66 3.32 }
Yield comp. to base (100%)	11.6	11.6	12.4	12.6
Yield comp. to P	110	115	103	95



2-VII-51 Effect of HCO_3^- concn BSNA + protein.
 Total of 4 tubes, weigh 3 mg BSNA + 12 mg bovine serum albumin.
 Add: ① 60 μ l HCO_3^- \rightarrow 5% NA. All 40 at 10%
 (amount diff in each tube)
 ② 43 7 57
 ③ 30 10 70 look 100' (across) 2hr.
 ④ 20 15 80 look 82' for 1.95 for P.

	G	A	C	T
B	1 .012	.014	.012	.048
2 .013	.012	.012	.044	
\bar{x} .013	.013	.012	.046	
①	1 .351	.484	.298	.312
2 .353	.490	.298	.307	
\bar{x} .352	.487	.298	.310	
$\bar{x} - B$.339	.474	.286	.264
②	1 .365	.508	.310	.329
2 .363	.508	.306	.321	
\bar{x} .364	.508	.308	.325	
$\bar{x} - B$.351	.495	.296	.279
③	1 .349	.481	.291	.319
2 .343	.479	.290	.304	
\bar{x} .346	.480	.290	.312	
$\bar{x} - B$.333	.467	.278	.266
④	1 .324	.454	.274	.304
2 .321	.450	.271	.290	
\bar{x} .323	.452	.273	.297	
$\bar{x} - B$.310	.439	.261	.251

3/11/51

Effect of HClO₂ hydrolysis on thymine

In each of 2 60 ml vol. flasks weigh 20.0 mg HClO₂ thymine (not dried).
Add 4 ml 70% HClO₂. Then worked 100% 2 hrs., other not. Both
↑ to 10 ml = of each, 8.2 μl spots on paper.

Both hydrolysis & methyls → V spots, not well resolved from T, but put out.

		V	T
B	1	.014	.026
	2	.012	.022
	\bar{x}	.013	.024

		V	T
Hydroly.	1	.034	.241
	2	.033	.242
	\bar{x}	.034	.242
	$\bar{x} - B$.021	.218

		V	T
Unhydroly	1	.032	.254
	2	.031	.244
	\bar{x}	.032	.249
	$\bar{x} - B$.019	.225

Apparent loss from 2 hrs at 100°
 $= \frac{7}{225} = 3.1\%$

0.1 M Na₂CO₃ 1.0 ml → 0.006
 0.1 M NaCl 8.0 ml → 0.08
 water 7.0 ml
 16.0 ml

	Cpe		T6r-6	
	Units	Ratio	Units	Ratio
A	3.99	1.241	4.19 2.07	1.646
G	2.80	0.874	2.45	0.963
C	2.56	0.779	0.40	0.039
T	3.54	1.102	3.44	1.350
U			[0.11]	[0.043]
	12.83	3.996	10.18	3.998

DISREGARD!

4-11-57 T6r-6
 VCpe (for demonstration)

80 mg PCpe ↑ 16 ml alkali 9.00 am.
 11 am 2 hrs. Spin 5000 5 min, residue re-susp. 20 ml water, spin
 5000 5 min. Centrifuge after spin 12,000 30 min. Ppt + 0.5 ml,
 spin down 15 min 12,000 in bank tube. Dry. Hyd. 5 ml HCl, 100° 2 hrs.
 Cold ca. 7 ml H₂O. Tolu 1 x 8.2 ml of pt. = 1.95 for 1?

T6r-6. Weigh 1.0 mg. Hyd. 5 ml HCl, 100° 2 hrs.
 Cold ca. 7 ml H₂O. Tolu 1 x 8.2 ml of pt.

		G	A	C	U	T
B	1	.018	.018	.018	.018	.037
from separate 2		.012	.016	.011	.016	.031
that of paper 2		.013	.017	.011	.017	.034
T6r-6		.282	.562	.021	.026	.307
		.269	.545	.010	.009	.273
Cpe		.110	.523	.021		.315
Witt	(Pkt of 275)		.548		(Pkt 200 = 321)	
		.274		.274		.315
		.321		.536		
-B		.308	.579	.263		.281

$$\text{Factor} = \frac{1}{31} \times \frac{8.3}{1.95} \times \frac{1}{3.98} \times 10^2 = 3.46$$

	Bm		Cpc	
	Yields	Ratio	Yields	Ratio
A	2.045	1.186	2.98	1.211
G	1.545	0.896	2.17	0.883
C	1.41	0.817	1.96	0.797
T	1.90	1.101	2.72	1.107
	6.90	4.000	9.83	3.998

Control P

P: Bm	Yields	Ratio
2.05	2.10	2.23
2.15		
2.50		

Cpc	Yields	Ratio
3.40	3.41	3.21
3.42		
2.80		

Yields comp. to basis

2.10	7.26	95%
2.23	7.7	89.5%

$$\frac{3.41}{31} \times \frac{8.3}{1.95} \times \frac{1}{3.98} \times 10^2 = 11.8 \quad 83.2\%$$

$$3.21 \approx 11.1 \quad 88.5\%$$

2-111

V Bm - V Cpc (Mann)

Vin from 100 mg each kind Pa. prepared by Kuen (Dissolved 3 hrs. at room temp + 3 hrs in frig.).

V Bm = 3.8 mg (looks good)

V Cpc = 4.3 mg (a bit brown)

In each, 10 µl 70% HClO₄, with 100' 2 hrs. Add 15 µl H₂O (?)

Take 2 x 8.3 µl of sol. = 3 x 1.95 for P. Blank also run from 8.3 µl 35% (1:1) HClO₄

		G	A	C	T
B	1	.008	.009	.007	.038
	2	.010	.009	.007	.034
	\bar{x}	.009	.009	.007	.036
HClO ₄ B	1	.009	.009	.008	.035
	2	.008	.009	.006	.034
	\bar{x}	.009	.009	.007	.035
Bm	1	.179	.276	.154	.185
	2	.179	.273	.155	.189
	\bar{x}	.179	.275	.155	.187
$\bar{x} - B$.170	.266	.148	.151
Cpc	1	.245	.398	.211	.249
	2	.250	.396	.214	.254
	\bar{x}	.248	.397	.213	.252
$\bar{x} - B$.239	.388	.206	.216

M _a			
	Yield	Ratio	
A	2.97	1.313	1.181
G	2.24	1.089	0.908
C	2.01	0.899	.900
T	2.78	1.230	1.106
	10.04	4.5	3.995

P	3.13	} 3.20 Y	
	3.30		
	3.18		

Yield correct for loss = 3.20 x 3.46 = 11.1 91% accounted for.

11-11-51

VMa ① (Kason)

100 mg. PMA + 20 ml 0.03 M. H₂CO₃ - 0.05 M NaCl.
 After 2 hrs, spin 4000 5 min, re-susp. sediment & spin 4000
 5 min. SpG. spin 11,000 30 min. Wash 5 water, re-spin 11,000
 30 min. 10.5 ml, spin down in small tube. Let brown; wash
 some of brownest stuff off top. Dry = 3.6 mg.
 Add 10 μl 70% HClO₄, wait 2 hrs 100°, add 15 μl H₂O, take
 8.2 μl spG, 1.95 for P. Small spot in Upton.

	G	A	C	T	255	260	265
B	1	0.012	.012	0.009	.032	.013	.016
	2	0.012	.012	.009	.037	.014	.013
	Σ	.012	.012	.009	.035	.014	.012
M _a	1	0.256	.391	.216	.280	.029	.028
	2	0.269	.406	.223	.261	.024	.037
	Σ	.263	.398	.220	.252	.032	.031
	Σ - B	.251	.386	.211	.221	.018	.018

$\frac{1}{10} \text{Na}_2\text{CO}_3$ in 8 ml
 $\frac{1}{10} \text{NaCl}$ 8.0 ml
 H_2O 3.2 ml
 16.0

	Yards	Ratio
A	3.18 3.28	1.151 0.844
G	2.44	6.737
C	2.25	0.815 0.680
T	3.17	1.149
	11.24 11.04	

P. 3.22 } 3.24 X
 3.26 }

Involvement to base = $3.22 \times 3.46 = 11.2$ % accounted for = 98.7

26-ix-51

VMA (2)

9.15 am 80 mg PMA + 16 ml 0.03 M Na_2CO_3 0.05 M NaCl

11.15. 2 hrs. Spin 5000 5 min. Long brown pellet + 15 ml of liquid, spin 5000 5 min. Combined apts. spin 11,000 40 min. Pellet white + some brown + ca. 20 ml of liquid. spin 10,000 30 min.

Spin down in small tube, dry, weight = 3.6 mg.

+ 10 μl 70% HClO_4 , into 100° 2 hrs, cool, add 15 μl H_2O , tube (fragile)
 2 x 1.95 μl for P. Then 2 x 9.3 for apts. Small apts in 4:1 ratio.

		G	A	C	T	U		
B	1	.019	.016	.014	.038	.255	.260	.265
	2	.016	.017	.015	.042	.015	.014	.012
	\bar{x}	.018	.017	.015	.039	.016	.015	.013
VMA	1	.289	.430	.252	.289	.032	.030	.028
	2	.283	.432	.250	.293	.028	.022	.029
	\bar{x}	.286	.431	.251	.291	.022	.031	.029
	$\bar{x}-B$.268	.414	.236	.252	.017	.016	.016

2.1 M Na_2CO_3 2.4 ml $\rightarrow 0.006 \text{ M}$
 0.1 M Na_2CO_3 2.0 ml $\rightarrow 0.05 \text{ M}$
 water 17.6
 40

P. clean 2.08 }
 2.13 } 2.12 Y. Yields comp. to base = 2.12 x 3.46 = 7.34
 2.15 }

Brown 3.04 }
 3.09 } 3.07 Y. Yields comp. to base = 3.07 x 3.46 = 10.60

1-x-51. Cpe - effect of RNAase.

200 mg Pcp + ca. 15' ml of dist. cond. 0.5' mg RNAase.
 Brought to room temp. (to remove any surface RNA). Spin down
 fa., then + 40 ml 0.006 M Na_2CO_3 - 0.05 M NaCl

After 1 hr, checked. Spin 5000 5 min. Ppt + 20 ml of dist.
 spin 5000 5'. Spin both ppt. (separately) 11,000 45' min. pellets
 combined + 20 ml of dist. Wash brown, so spin 4000 3 min to
 clean. Spn spin 11,000 45' min. Pellet has no material
 bottom, but brown layer on top. Add 1 ml H_2O , let stand few min,
 the brown layer falls off, leaving pure green-white virus. Separate, sup.
 clean virus + membrane fraction separately, spin down in small tubes today.

Weigh: clean virus = 2.2 mg, ^{membrane} fraction = 3.7

Incub. 10 μl 70% H_2SO_4 , cook 100° 2 hrs, add 15 μl H_2O to 8.2, ppt 1:1988
 "Clean" shows practically no ppt in U fraction; state this along; other bases
 for measure of mem. fossils U. "Brown" shows distinct ppt; state this as
 paper to run in B-201-414.

	Cpe clean		Cpe base		Mean ratio
	Yield/mol	Ratio	Yield/mol	Ratio	
A	1.961	1.220	2.776	1.229	1.326
G	1.418	0.888	1.991	0.882	0.883
C	1.248	0.776	1.780	0.789	0.783
T	1.797	1.118	2.490	1.102	1.110
	6.424	3.997	9.037	4.002	4.001
Group P	7.34		10.60		
% Polymethyl	87.6		85.2		86.4
Group U	0.063				

Cpe contd.

	G	A	C	"U" ₂₆₀	T
B 1	.016	.014	.010	.014	.030
2	.015	.015	.010	.012	.027
\bar{x}	.016	.015	.010	.013	.029
clean 1	.172	.266	.142	.019	.170
2	.172	.273	.140	.017	.173
\bar{x}	.172	.270	.141	.018	.172
$\bar{x} - \beta$.156	.255	.131	.005	.143
Base 1	.238	.376	.198		.226
2	.231	.376	.196		.227
\bar{x}	.235	.376	.197		.227
$\bar{x} - \beta$.219	.361	.187		.198

	① BSNA 1 hr.			② BSNA 2 hrs.			③ + prot. 1 hr.			④ + prot. 2 hrs.		
	Yields	Ratio	H	Yields	Ratio	H	Yields	Ratio	H	Yields	Ratio	H
A	3.83	1.133	19.1	3.96	1.186	19.5	3.68	1.115		3.53	1.100	
G	3.145	0.922	15.2	3.19	0.938	15.9	3.075	0.922		2.80	0.926	
C	2.79	0.826	8.4	2.865	0.843	8.6	2.74	0.831		2.53	0.826	
T	3.56	1.054	7.1	3.47	1.020	6.95	3.53	1.070		3.27	1.006	
	13.325	3.945	49.8	13.225	3.947	50.95	13.025	3.948		11.93	3.972	
P ₁ Y	3.45	} 3.62		4.31	} 4.25 (damped)		3.52	} 3.49		3.32	} 3.31	
	3.71			4.19			3.50			3.24		
	3.66			3.80			3.46			3.32		
	3.67			3.75			3.46			3.27		
Yields conv. to base	72.51		18.98			12.08		11.44				
% of P present for by base	106		103			108		104				
N ₁ Y	71.5		72.2	71.9								
Yields conv. to base	50.4		50.7									
% recovery	98.8		100									

5-x-51

Re-check BSNA + BSNA + prot. recovery.
 Weigh: ① 3.0 mg BSNA for 1 hr 100° hydrolysis.
 ② 3.0 mg " " 2 hr " "
 ③ 3.0 mg " + 12 mg capb. borine succinimide for 1 hr.
 ④ 3.0 mg " + 12 mg " " " " 2 hr.
 Each ↑ 30 μl HClO₄, cook 1 + 2 hrs. sep., add to each 70 μl H₂O, min., spin, take 8.3 spots, 1.95 for P, 25.1 for H.

		G	A	C	T
B	1	.020	.020	.014	.043
	2	.016	.016	.015	.047
	\bar{x}	.018	.018	.015	.045
F	1	.365	.512	.308	.327
	2	.362	.519	.308	.328
	\bar{x}	.364	.516	.308	.328
	$\bar{x} - B$.346	.498	.293	.283
2	1	.367	.531	.316	.321
	2	.370	.516	.316	.320
	\bar{x}	.369	.524	.316	.321
	$\bar{x} - B$.351	.506	.301	.276
3	1	.354	.499	.300	.327
	2	.357	.493	.306	.324
	\bar{x}	.356	.496	.305	.326
	$\bar{x} - B$.338	.478	.289	.281
4	1	.325	.450	.280	.308
	2	.327	.451	.282	.301
	\bar{x}	.326	.451	.281	.305
	$\bar{x} - B$.308	.433	.266	.260

Yields H

	BSNA ①	BSNA ②
A	19.14	19.50
G	15.71	15.94
C	8.37	8.60
T	7.12	6.94
	50.34	50.98

Correct N found. $\frac{71.5 \times 8.3 \times 1}{14 \times 211 \times 3.94} = 50.5$

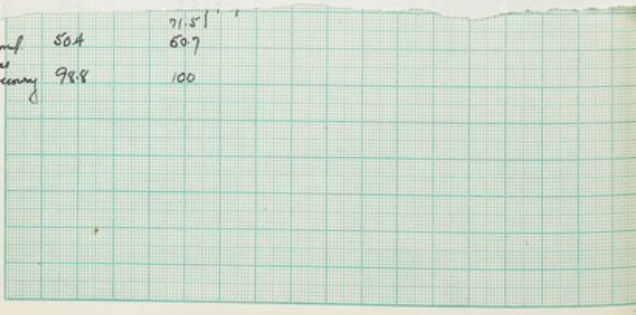
% of N accounted for by bases 99.6

50.4
71.5
50.7
98.8
100

50X-51 Re-check BSNA + BSNA + prot. recovery

Weight: ① 3.0 mg BSNA for 1 hr 100' hydrolysis
 ② 3.0 mg " " 2 hr "
 ③ 3.0 mg " + 12 mg capd. bovine serum albumen for 1 hr.
 ④ 3.0 mg " + 12 mg " " " " 2 hr.
 Each ↑ 30 μl HClO₄, cook 15-2 hrs. inf., add to each 70 μl H₂O, mix, spin, take 8.3 spots, 1.95 for P, 21.1 for H.

		G	A	C	T
B	1	.020	.020	.014	.043
	2	.016	.016	.015	.047
	\bar{x}	.018	.018	.015	.045
1	1	.365	.512	.308	.327
	2	.362	.519	.308	.328
	\bar{x}	.364	.516	.308	.328
$\bar{x} - B$.346	.498	.293	.283
2	1	.367	.531	.316	.321
	2	.370	.516	.316	.320
	\bar{x}	.369	.524	.316	.321
$\bar{x} - B$.351	.506	.301	.276
3	1	.354	.499	.300	.327
	2	.357	.498	.306	.324
	\bar{x}	.356	.496	.303	.326
$\bar{x} - B$.338	.478	.288	.281
4	1	.325	.450	.280	.308
	2	.327	.451	.282	.301
	\bar{x}	.326	.451	.281	.305
$\bar{x} - B$.308	.433	.266	.260



	T4r ⁻⁶			T6r ⁻⁶		
	Yields	Ratios	Old/undP	Yields	Ratios	Old/undP
A	3.87	1.575		3.45	1.521	
G	2.20	0.896		1.993	0.871	
C	0.21	0.086		0.524	0.231	
T	3.575	1.443		3.120	1.376	
	9.825	4.000		9.067	3.999	

P, Y (cm)
 3.46
 3.43
 3.44
 3.46

Yield, \bar{y}
 11.90
 82.5

3.46

11.96

75.8

13-x-51. Phage, untreated for Precip.
 Phage (left in fridge - mixed?) analyzed: T4r⁻⁶ 1.7 mg
 T6r⁻⁶ 1.9 mg
 End \uparrow 10 μ l 70% HCl, cook 100° 1 hour only.
 Add to T4r⁻⁶, 20 μ l H₂O. Lube 2 x 8.3 μ l, r⁺ + x 1.95 for P
 T6r⁻⁶, 15 μ l H₂O Lube " " 1 only 1.95 for P.
 [Before filtering, grind up carbon resin, & pipette from suspension.]

		G	A	C	T
B	1	.013	.012	.009	.046
	2	.012	.015	.007	.059
	\bar{x}	.013	.014	.008	.053

		G	A	C	T
T4r ⁻⁶	1	.251	.574	.031	.328
	2	.259	.519	.029	.321
	\bar{x}	.255	.547	.030	.325
	$\bar{x} - B$.242	.508	.022	.282

		G	A	C	T
T6r ⁻⁶	1	.229	.462	.064	.294
	2	.231	.462	.062	.307
	\bar{x}	.230	.462	.063	.301
	$\bar{x} - B$.217	.448	.055	.248

③ BSHA + pad. 1 hr		④ 2 hrs.	
Yards	Rates	Yards	Rates
A	3.80 1.113	3.98	1.117
G	3.175 0.981	3.335	0.936
C	2.865 0.840	3.01	0.846
T	3.625 1.064	3.75	1.052
	13.475 3.948	14.075	3.951

P, Y (corr.)

3.87
3.84 3.83
3.77
3.82

3.87
3.87 3.94
4.07

①	②
5.4 } #92	4.69 } #58
#72	4.06

big increase over figures of 5-x probably indicates Pin carbon.

Y. Yards corr. to base 13.92
% of P recovered for by base 96.9

14.33
98.1

1/2
2

3rx-51.

BSNA of 5-x, 40-run for Recovery when coke is included.
Remaining factors of same hydrocarbon ground of well glass rod.
depleted while fresh samples filtered out (with same efficiency).
From (1) & (2) for P only; from (3) & (4), 1.95 for P and 8.3 eff. P

	G	A	C	T
B	1 } .017	.017	.011	.038
	2 } <u>.017</u>	.017	.011	.041
\bar{x}	.017	.017	.011	.040
BSNA ③	1	.364	.507	.321
	2	.368	.514	.336
\bar{x}	.366	.511	.312	.329
$\bar{x} - B$.349	.494	.301	.289
BSNA ④	1	.384	.531	.330
	2	.383	.536	.346
\bar{x}	.384	.534	.327	.338
$\bar{x} - B$.367	.517	.316	.298

	① NA 1 hr		② NA 2 hrs		③ +prot 1 hr		④ +prot 2 hrs	
	Yards	Ratio	Yards	Ratio	Yards	Ratio	Yards	Ratio
A	4.04	1.143	20.2	1.12	1.144	20.6	3.89	1.129
G	3.29	0.933	16.4	3.24	0.928	16.7	3.155	0.916
C	2.98	0.844	8.94	2.96	0.822	8.88	2.90	0.842
T	3.62	1.024	7.24	3.80	1.055	7.61	3.64	1.061
	13.93	3.944	52.78	14.22	3.949	63.99	13.605	3.948

P, Y (mm)	4.13	4.25	3.91	3.94
	4.27	4.28	4.06	4.06
	4.23	4.24	3.91	4.09
	4.20	4.18	3.82	3.72

Yards	14.59	14.59	13.54	13.79
% accounted for	96.6%	97.5%	100.4%	101.2%

N, Y	78.61	79.1	78.4	79.5
	78.2	79.9		

Yards	55.3	56.1
% accounted for	95.4%	95.9%

20 x 50

BSNA repeated again for P & N.

① 3 mg BSNA

↑ 39 μM 70% H₂O, 100° 1 hr, + Pap 11.0

② - + 2 mg protein - 2 lbs

③ - + 12 mg protein - 1 lb (unstable) 2 lbs

④ - + 12 mg protein -

8.3 spots, 1.95 for P, 2.11 for N.

		G	A	C	T
B	1	.020	.018	.013	.049
	2	.018	.016	.013	.039
	\bar{x}	.019	.017	.013	.044
	1	.373	.534	.321	.334
	2	.389	.550	.320	.329
	\bar{x}	.381	.542	.326	.332
	$\bar{x} - B$.362	.525	.313	.288
	2	.394	.561	.331	.354
	2	.378	.542	.316	.337
	2	.386	.552	.324	.346
	$\bar{x} - B$.367	.535	.311	.302
	3	.363	.520	.316	.335
	2	.369	.526	.319	.338
	\bar{x}	.366	.523	.318	.335
	$\bar{x} - B$.347	.506	.305	.291
	4	.359	.538	.323	.351
	2	.361	.546	.328	.349
	\bar{x}	.360	.542	.326	.350
	$\bar{x} - B$.341	.525	.313	.306

T4r-6

	RNAse		DNAse		H ₂ O	
	Yields	Ratio	yields	Ratio	yields	Ratio
A	1.66	1.56	0.25	1.47	1.40	1.59
G	0.98	0.92	0.18	1.06	0.82	$\frac{93}{0.898}$
C	0.095	0.089	0.019	0.11	0.67	0.076
U	[0.028]	[0.036]	[0.000]		[0.013]	[0.015]
T	1.52	1.43	0.23	1.35	1.24	1.41
	4.253	3.999	0.679	3.99	3.527	4.006
P, Y	1.62		0.25 } 0.23 0.20 }		1.17	
Yields comparison	560		0.79		4.04	
	76.2%		86%		87%	

26-x-51

Phage: Effect of RNAse, DNAse, & H₂O

Into each of 3 tubes, weigh 1 mg T4r-6. Add (1) 0.2 ml RNAse 0.1 mg/ml, (2) 0.2 ml DNAse 0.1 mg/ml, MgSO₄ 0.005 M, (3) 0.2 ml H₂O. Leave overnight at 27°

27-x Phage has remained on surface, largely not melted. Virus well - red, & leave overnight at 27°

28-x In frig.

29-x Spin down, leave off ph., wash once in 0.2 ml 50% EtOH. Dry, wt? Weigh RNAse: 0.8 mg; DNAse 0.4 mg; H₂O 0.5 mg To end, Spil HCl, wash 100° 1 hr, add 20 ul 1% to tube 83 after 1 hr 1.99% P

		G	A	C	U	T
B	1	[0.36]	.010	.006	.014	.029
	2	.009	.009	.007	.014	.029
	\bar{x}	.010	.010	.007	.014	.029
T4r-6 RNAse	1	.116	.223	.06	.015	.149
	2	.120	.229	.018	.019	.151
	\bar{x}	.118	.226	.017	.017	.150
	$\bar{x} - \bar{B}$.108	.216	.010	.003	.121
T4r-6 DNAse	1	.050	.043	.009	.013	.046
	2	.029	.040	.009	.015	.048
	\bar{x}	.030	.042	.009	.014	.047
	$\bar{x} - \bar{B}$.020	.032	.002	.000	.018
T4r-6 H ₂ O	1	.078	.193	.013	.014	.125
	2	.102	.191	.015	.016	.131
	\bar{x}	.100	.192	.014	.015	.128
	$\bar{x} - \bar{B}$.090	.182	.007	.001	.099

	T6r-6		T6r*-6	
	Yards	Ratio	Yards	Ratio
A	5.34	1.546	2.285	1.521
G	3.02	0.876	1.30	0.866
C	0.515	0.149	0.345	0.230
V	[0.127]	[0.037]	[0.139]	0.093
T	4.98	1.428	2.075	1.381
	13.805	3.999	6.005	
P.Y	5.74 5.76 5.78		2.37 2.42 2.47	
(underway to base)	19.9		8.35	
% of P. accounted for	69.4%		71.9%	

30x. Phages: repeat for Precarity
 Wt: T6r-6: 1.8 mg; T6r*-6: 1.6 mg
 In cond. 10 µl 70% H₂O. Cook 100° 1 hr. All 18-20 µl H₂O.
 Take 2x 8.3 µl spurs 1.95 for P.

		G	A	"C"	"V"	T
B	1	.013	.019	.016	.020	.052
	2	.012	.015	.015	.017	.042
	\bar{x}	.013	.017	.016	.019	.047
T6r-6	1	.344	.704	.072	.029	1.440
	2	.346	.718	.067	.029	1.438
	\bar{x}	.345	.711	.070	.029	1.439
	$\bar{x}-B$.332	.694	.054	.010	.392
T6r*-6	1	.157	.316	.055	.019	.217
	2	.155	.311	.049	.030	.206
	\bar{x}	.156	.314	.052	.030	.212
	$\bar{x}-B$.143	.297	.036	.011	.165

	Yards	Rebar
A	4.18	1.540 0.578
G	2.38	0.478
C	0.35	0.129
V	[0.10]	[0.037]
T	3.94	1.451
	10.85	3.998

P.Y
 $\left. \begin{array}{l} 3.94 \\ 3.83 \\ 3.81 \\ 3.80 \end{array} \right\} 3.85 = 13.3 \text{ corr. to base Recovery} = 81.6\%$

31-x - Plage - effect of insolation in water.

T4r-7: 1.9 mag ↑ 0.2 and eq. dist. 27° overnight.

1-xi - Spin horn, Wash, prod. 50%, then 95% EFF. By, weight 1.7 mag.
 Add 10 pl H₂O, wash 100° 1 hr, add 20 pl H₂O, take 9.3 gts, 1.8° P.

	G	A	C_{N-40}	U	T	
B	1	.011	.012	.009	.016	.039
	2	.010	.012	.009	-	.035
	\bar{x}	.014	.012	.009	.016	.037

	T4r-7	1	2	\bar{x}	$\bar{x}-\beta$		
	1	.270	.551	.046	.047	.024	.348
	2	.275	.558	.046	.047	.024	.351
	\bar{x}	.273	.555	.046	.047	.024	.350
	$\bar{x}-\beta$.262	.543	.037	.038	.018	.318

Corrected for

$E_{\text{corr}} = 9650$
 index

	T4r-8			T6r-7		
	Yields	Ratio		Yields	Ratio	
A	2.88	1.32	32.6	3.74	1.34	33.1
G	1.537	0.72	17.7	2.02	0.72	17.9
C	1.160 1.048	0.49	13.4	1.335 1.20	0.43	11.8
T	3.14	1.47	36.2	4.50	1.50	37.2
	8.667 8.558	4.00	99.9	11.295 11.16	3.99	100.0

P, Y

2.89		2.62	
2.87	2.68	3.57	3.61
2.89		3.63	

Yields
 comp. to base
 % of P
 uncorrected

9.94	12.4
8.667 87.3	90.2 91.1

1x1-51 Phenyl: HCOOH hydrolysis.

Wt: T4r-8 1.4 mg
 T6r-7 1.7 mg

Add 0.15 ml 88% HCOOH. Cook 175° 35 min. Open, dry down,
 ↑ 25 μ l N-Me. Take 8.3 spots + 1.95 for P.

	G	A	C	T	
B	1	.008	.010	.009	.031
	2	.010	.013	.009	.034
\bar{x}		.009	.012	.009	.033

T4r-8	1	.174	.576	.116	.118	.280
	2	.181	.384	.121	.127	.284
\bar{x}		.178	.380	.119	.121	.283
$\bar{x}-B$.169	.368	.110	.112	.250

T6r-7	1	.229	.499	.133	.136	.368
	2	.232	.497	.127	.129	.366
\bar{x}		.231	.498	.130	.138	.367
$\bar{x}-B$.222	.486	.126	.129	.354

Corr.
5-11-51

	T4r-7		T6r-6			
	Yield	Ratio	Yield	Ratio		
A	3.60	1.32	32.6	4.37	1.35	33.3
G	2.01	0.78	18.2	2.26	0.70	17.2
C	1.52	0.56	13.75	1.76	0.49	13.4
T	3.92	1.44	35.5	4.76	1.47	36.2
	11.05		13.15			
	10.89	3.99	100.05	12.97		100.1

P.Y

$\left. \begin{array}{l} 4.90 \\ 4.78 \end{array} \right\} 4.84$
 17.0
 (91.4)

$\left. \begin{array}{l} 3.48 \\ 3.52 \end{array} \right\} 3.50$
 12.1
 (77.5)

5-xi-51.

Phase: HCOOH hydrolysis.

T4r-7: 1.3 mg.

T6r-6: 1.4 mg.

↑ 0.16 and 88% HCOOH 175° 30 min. Day. ↑ 22 ml N-H₂, 8346, 1996P.

		G	A	C	T	
B	1	.008	.010	.275	.280	.022
	2	.008	.009	.007	.007	.034
	\bar{x}	.008	.010	.007	.007	.033
T4r-7	1	.226	.475	.150	.154	.342
	2	.232	.481	.149	.153	.348
	\bar{x}	.229	.479	.150	.154	.345
$\bar{x}-B$.221	.469	.143	.147	.312
T6r-6	1	.255	.582	.172	.176	.408
	2	.258	.574	.173	.177	.414
	\bar{x}	.257	.578	.173	.177	.411
$\bar{x}-B$.249	.568	.166	.170	.378

K_2CO_3 0.1M 3.6 ml \rightarrow 0.03 M
 KCl 0.1M 6.0 ml \rightarrow 0.05 M
 water to 12 ml

	Yards	Ratios
A	1.03	1.188
G	0.773	0.892
C	0.701	0.809
T	0.962	1.100
	3.466	4.00

P.Y

$\left. \begin{matrix} 1.24 \\ 1.19 \\ 1.20 \\ 1.02 \\ 1.00 \\ 1.11 \end{matrix} \right\} \begin{matrix} 1.22 \\ 1.14 \\ 1.05 \end{matrix} \begin{matrix} = 4.22 \\ = 3.98 \text{ rounds} \\ \end{matrix} \quad 87.8\% \text{ energy}$

13-XI-51

VMd.

10-45m

1.20

14m

60 mg PMd (not yet quite dry) into tube along routes \rightarrow PMd.
 Add 12 ml 0.03 M K_2CO_3 0.05 M. KCl . Disturb slowly.
 Dark felds ($2\frac{1}{2}$ L): many large barbed spines; also, considerable no. of spines not fully developed. Thick skins. 3 hrs, spin 5000 5 min \rightarrow long broad felds, \uparrow 12 ml of dist. spin 5000 5 min. Original felds. Both after spin (small tubes) 11,000 30 min \rightarrow felds with some brown tint.
 Centrifuge \uparrow 12 ml of dist. spin to clear 2500 2 min. Small amount left spin 11,000 30 min. Felds mostly white, small brown which removed by rinsing from surface; remainder \uparrow 0.4 ml of dist. spin down in broad tube. Weight = 1.6 mg. \uparrow 6 ml HClO₄ 100° 2 hrs.
 Add 25 ml H₂O 8.3 spines, \uparrow 6 (!) \times 1.95 for P.

		G	A	C	T
B	1	.005	.005	.005	.031
	2	.005	.005	.004	.026
	\bar{x}	.005	.005	.0045	.0285
VMd	1	.089	.138	.078	.104
	2	.091	.140	.078	.106
	\bar{x}	.090	.139	.078	.105
	$\bar{x} - B$.085	.134	.0735	.0765

K₂CO₃ 7.5
KCl 12.5
water 62.5

	VMA		VMd	
	Yields	Ratios	Yields	Ratios
A	1.554	1.176	1.391	1.159
G	1.191	0.904	1.046	0.871
C	1.067	0.808	0.972	0.810
T	1.471	1.113	1.395	1.161
	5.283	4.001	4.804	4.001

P, Y

1.59	1.81
1.59	1.62
1.95	1.72

Yields comparison

5.92	5.95
85.1%	80.7%

14-11-51

VMA & VMd.

9.45 60 mg each PMd & PMA ↑ 12 ml 0.03M K₂CO₃ - 0.05M KCl.
1.30 3 1/2 hrs. spin (small paper tubes) 5000 5 min, decant. prep. ppt in
2.05-2.45 12 ml eq. sol., spin again 5000 5 min. Prod. ppt spin 11,000 30 min.
ppt ↑ 12 ml eq. sol., spin 3500 2 1/2 min to clean, spin 11,000
30 min. Wash some brown from surface, ↑ drop of sol., spin soon in bottle.
15.20 Dry, weigh. Ma = 1.8 mg, Md = 1.4 mg.
↑ Gyl HCl, cool 100° 2 hrs, add 20 μl eq. sol., boil 83 of sol., r 2 or 3 x 1/2 hr?

		G	A	C	T
B	1	.004	.004	.002	.022
	2	.005	.005	.004	.021
	\bar{x}	.005	.005	.003	.026
Ma	1	.132	.205	.114	.108
	2	.139	.208	.115	.109
	\bar{x}	.136	.207	.115	.113
$\bar{x} - B$.131	.202	.112	.117
Md	1	.121	.186	.103	.106
	2	.129	.186	.106	.103
	\bar{x}	.120	.186	.105	.137
$\bar{x} - B$.115	.181	.102	.111

K_2CO_3 4.8 ml \rightarrow 0.03 M 0.96 \rightarrow 0.008
 KCl 8 6
 water to 16 water to 12

	MId		Pd	
	Yields	Ratio	Yields	Ratio
A	1.531	1.160	0.947	0.851
G	1.145	0.869	1.344	1.208
C	1.076	0.816	1.257	1.129
T	1.521	1.152	0.906	0.814
	5.273	3.997	4.464	4.002

P, Y	1.737	1.437	} 1.42
	1.70	1.41	
	1.67		
Yield comp to base	5.89	4.92	
% recovery	89.6 %	90.6 %	

P1

15-xi-51. VMId & VPd.

80 mg PMid \uparrow 16 ml 0.08 M K_2CO_3 - 0.05 M KCl

12:15 am. 60 mg PPd \uparrow 12 ml 0.08 M K_2CO_3 - 0.05 M KCl.

3 1/2 hrs. Spin 5000 5 min, filter \uparrow 12 ml of sol., spin 5000 5 min.
 After spin 11,000 30 min, filter \uparrow 12 ml of sol., spin 3500 2 1/2 min,
 discard filter, spin 11,000 30 min. Wash brown membrane layer
 from surface, leaving very white crins. \uparrow 2 drops of sol., spin down in
 bomb tube. Dry.

16-xi. MId = 1.8 mg. Pd = 1.2 mg. MId \uparrow 6 μ l H₂O, Pd \uparrow 5 μ l.
 100° 2 hrs. MId was not all used by H₂O, 1 check, & put back as 1.05 mg.
 Add 20 μ l H₂O. Take 8.3 after, & 2 x 3 x 1.95 for P.

		G	A	C	T
B	1	.006	.004	.004	.020
	2	.005	.004	.003	.024
	\bar{x}	.006	.004	.004	.022
Md	1	.130	.201	.116	.162
	2	.134	.205	.117	.145
	\bar{x}	.132	.203	.117	.143
	$\bar{x} - \bar{y}$.126	.199	.113	.121
Pd	1	.153	.127	.135	.092
	2	.154	.127	.137	.101
	\bar{x}	.154	.127	.136	.094
	$\bar{x} - \bar{y}$.148	.123	.132	.072

(first had most stock from paper: amount 0.096)

15-xi

"C" ex flags.

"C" tubes from H4054 hydrolysis of 1-xi & 5-xi control, laid down
 pd on paper run in ParD11-NH₃. Good spots in cysteine-guanine
 position, none in cytosine position. Elute, dry down in small tube, seal:
 0.15 ml H4054, work 175° 1 1/2 hrs, dry down, 7 1/10 HCl, put all on paper.
 Control: elute cysteine washer from paper & hydrolyze identically. Run in ParD11-09.
 Control is all converted to cytosine; "C" ex flags is unwashed!!

Elute, along: blank, in 4 ml 1/10 HCl (by mistake for water), stand against
 blank. Neutral & alk. spots not read - too much alkali used to
 neutralize.

HCl.

300	.018
290	.064
285	.084
280	.074
278	.0745
275	.073
270	.084
260	.056
250	.029
245	.021
240	.018
235	.020
230	.034
220	.090
215	.108
213	.111

K₂CO₃ 0.4 ml
 K₂SO₄ 2.5 ml
 water 5'

	Yields	Ratios
A	1.377	0.986
G	1.501	1.074
C	1.381	0.989
T	1.332	0.954
	5.591	4.003

$$\frac{A}{T} = 1.03$$

$$\frac{G}{C} = 1.09$$

$$\frac{A+T}{G+C} = 0.941$$

18-21.

VLm.

VLm 35' (14, 21, 44). Remainder of patch weighed into centrifuge tube = 22.4 mg. Dry 25 mg when tube dried out.

9:30 am. ↑ 5' and 0.008 M K₂CO₃ 0.05 M KCl.

11:45 2 1/2 hrs. books all dissolved. Spin 5000 4 min. discard ^(length) sediment.

↑ 5' and eq. dist. Spin 5000 4 min. Combined after spin 11,000 30 min.

Pellet + 5' and eq. dist. Spin 11,000 45 min. Pellet + top water.

19-21 spin down in small tube, dry. = 0.7 mg.

↑ 3 pul HCl, work 100' 2 hrs. Add 5 pul H₂O, 2 min.

Transfer whole to one spot.

		G	A	C	T
B	1	.010	.007	.009	.029
	2	.008	.007	.004	.027
	\bar{x}	.009	.007	.007	.028
VLm		.174	.186	.152 ²⁵⁰ ₁₀₀	.134
VLm - B		.165	.179	.145	.106

	CCF		PCF	
	Yrnds	Ratio	Yrnds	Ratio
A	1.909	1.310	0.808	0.985
G	1.072	0.736	0.862	1.075
C	0.954	0.656	0.800	0.975
T	1.886	1.296	0.792	0.966
	5.821	3.999	3.232	4.001

20-11-57

PCF and CCF.

60 mg PCF-4 ↑ 12 ml 0.03 M K_2CO_3 - 0.05 M KCl
 10 am 75 mg CCF-1, prepared by self but obviously dirty, ↑ 15 ml ^{0.02M NaCl} and 0.05M NaCl.
 3 1/2 hrs. Caps. completely dissolved (v. brown), nearly even sediment.
 A: still some undissolved remnants.
 After 5500 s/min C → small pellet; P → big. Pellet ↑ 15 ml 1%
 spin down again. After spin 11,000 45 min. Pellets: some brown.
 P: add fast ml 0.025 M $CaCl_2$. Will not resuspend. Add water to 13 ml;
 still don't resuspend. C: add fast ml water. Resuspends well. Add ^{0.025M} $CaCl_2$;
 appears to increase turbidity - aggregation? Spin 11,000 30 min.
 → sticky ppt. all down side of tube. Stir up in water. Spin down again.
 Clear resuspend. ↑ small volume water (hump) transfer to small tubes.
 spin down again. Dry

21-11-57 Weigh: PCF 1.4 mg ↑ 5 ml HClO₄ + 20 H₂O
 • CCF 2.4 ↑ 8 ml + 18 H₂O

(Dry after ^{spin} washing, looks as if labels reversed ???) 83 spots, 195 for P.

		P	C	T	
B	1	.004	.004	.006	.024
	2	.006	.005	.006	.024
	\bar{x}	.005	.005	.006	.024
CCF	1	.119	.248	.103	.171
	2	.127	.258	.109	.176
	\bar{x}	.123	.253	.106	.174
	$\bar{x} - B$.118	.248	.100	.150
PCF	1	.101	.110	.090	.088
	2	.103	.110	.090	.085
	\bar{x}	.102	.110	.090	.087
	$\bar{x} - B$.098	.105	.084	.063

0.1M. K_2CO_3 12 ml = 0.03 M
 0.1M. KCl 20 ml = 0.05 M
 water 40

	P_s		PCF	
	Yield	Ratio	Yield	Ratio
A	1.5374	1.068	1.192	1.000
G	1.419	0.974	1.273	1.068
C	1.352	0.929	1.161	0.975
T	1.497	1.027	1.143	0.960
	5.822	3.998	4.769	4.003

P.Y.	1.987	1.99	1.64	1.60
	2.04		1.88	
	1.95		1.59	

Yield as shown	6.69	5.54
90% P accounted for	87.1	86.2

26-XI-51

VPCF and VP_s

80 mg each PCF.4 and PP_s (fully purified by self)
 10.10 ↑ 16 ml 0.03 M K_2CO_3 0.05 M KCl .
 1.10. P_s dissolves slowly, but after 35 hrs almost completely; Cf leaves some ^{undissolved residue}
 After 5000 s min → big brown residue in both Reaction + water, after
 again 5000 s min. Combined after 11,000 45 min.
 Pellets: Cf small brown top layer, P_s big dark brown layer. Add in 2 ml
 of each, then 5 ml 0.0001 M K_2CO_3 . Dissolve slowly, → stronger + some K_2CO_3 .
 Not full emulsified, but after 12,000 30 min. Then ↑ 0.2 ml H_2O K_2CO_3
 (compare quite well). 5 spin down in hydrophobic tubes. Dry at 100°. Weigh.
 $P_s = 1.9$ mg, PCF = 1.5 mg. In each, 6 ml H_2O , each 100° 2 hrs,
 then repeat H_2O . r take 8.3 ml after, 3x 1.9% ml for P.

		G	A	C	T
B	1	[0.012]	.006	.004	.028
	2	.005	.005	.004	.021
	\bar{x}	.007	.006	.004	.025
P_s	1	.165	.211	.145	.146
	2	.161	.204	.146	.141
	\bar{x}	.163	.208	.146	.144
$\bar{x}-B$.156	.202	.142	.119
PCF	1	^{2.45} ^{2.02} ^{2.04} 1.73	.162	.126	.114
	2	^{2.04} ^{2.17} ^{1.91} 1.45-1.47	.159	.125	.118
	\bar{x}		.161	.126	.116
$\bar{x}-B$.140	.155	.122	.091

0.1M K_2CO_3 7.5 \rightarrow 0.03
 0.1M KCl 2.5 \rightarrow 0.05
 water to 25

	P_s		PCF	
	Yield	Ratio	Yield	Ratio
A	1.361	26.6	1.454	24.9
T	$\frac{1.320}{1.255}$	25.7	$\frac{1.376}{1.526}$	23.9
G	1.288	24.5	1.546	26.4
C	$\frac{1.190}{5.126}$	$\frac{23.2}{100.0}$	$\frac{1.448}{5.844}$	$\frac{24.8}{100.0}$

30.01.

$VP_s = VECF$

9.30 am

60 mg each \uparrow 12 ml 0.03 M K_2CO_3 - 0.05 M KCl .
 Stand 4 hrs. Spin 5390 5 min. \rightarrow big vesicles, \uparrow in water, spin again 5390
 5 min. Spin 11,000 45 min \rightarrow brown & white pellet (small dark brown P_s).
 \uparrow 1 ml $\frac{1}{10,000}$ K_2CO_3 (not too diffused, will be filtered), wash w/ 0.12 ml
 = eq. dist. spin 11,000 45 min. Wash some brown off P_s . \uparrow 1 ml $\frac{1}{10,000}$ K_2CO_3 ,
 spin down in small tube, lay in cassette.

3-211

Wash $P_s = 1.5$ mg, PCF = 1.1. Wash \uparrow 5 μ l 70% HClO₄, wash

9.40-11.40

100' 2 hrs. Add 15 μ l eq. dist. take 2x 8.3 for spots, use P.

		G	A	C	T
B	1	.010	0.009	.006	.026
	2	.009	0.007	.006	.024
	\bar{x}	.010	.008	.006	.025
P_s	1	.146	.184	.130	.129
	2	.149	.186	.131	.131
	\bar{x}	.148	.185	.131	.130
$\bar{x} - B$.138	.177	.125	.105
PCF	1	.181	.199	.159	.138
	2	.178	.195	.156	.134
	\bar{x}	.180	.197	.158	.136
$\bar{x} - B$.170	.189	.152	.111

BSNA		NaTN		T2r ⁻¹	
Yards	Ratio	Yards	Ratio	Yards	Ratio
A	5.29 28.0	5.46 28.1	5.41	34.1	
T	5.20 27.5	5.44 28.0	5.20	32.7	
G	4.17 22.1	4.19 21.6	3.02	19.0	
C	3.94 20.8	4.10 21.8	2.88	14.3	
MC	0.31 1.6	0.22 1.1			
	18.91 100.0	19.41 99.9	15.91	100.1	
P.Y	5.397 5.35 5.301	5.56 5.48 5.59	4.86 4.96		
Transfer of base 70% uncorrected for 102	18.4	18.9	16.8		
		103	95		

16-i-52 Cohen's NA's.

SSC NaTN and 4A 0.9 mg (ex-dimenter)

T2r⁻¹ DNA 1.1 mg (-)

sum BSNA 1.0 mg (air-dry)

3.28 In each, 10 µl 70% HClO₄, work 100' 1 hr. Add 15 µl H₂O. Take 2x 8.2 spots, 2x 19x for P (mass all)

T2r⁻¹ shows considerable spots in C position

	G	A	27.5	280	215	MC	T
B	1	.017	.013	.012		.008	.046
	2	.018	.016	.012	.012	.011	.052
\bar{x}		.018	.015	.012		.010	.049
BSNA	1	.475	.694	.419	.388	.038	.457
	2	.480	.710	.430	.425	.041	.469
\bar{x}		.477	.702	.425		.040	.463
$\bar{x} - \beta$.459	.687	.413		.030	.444
NaTN	1	.478	.723	.442	.410	.030	.480
	2	.480	.726	.441	.424	.034	.481
\bar{x}		.479	.725	.442		.032	.481
$\bar{x} - \beta$.461	.710	.430		.022	.462
T2r ⁻¹	1	.349	.714	.246	.262-230	.015	.463
	2	.351	.724	.243	.249	.016	.463
\bar{x}		.350	.719	.245	.251	.016	.463
$\bar{x} - \beta$.332	.704	.233	.239	.006	.444

	NaTN		T2r-1	
	Yield	Ratio	Yield	Ratio
A	4.71 4.81	28.0	1.67 1.95	32.2
T	3.69	22.0	1.10	21.2
G	3.69	22.0	1.10	21.2
C	3.37	20.1	0.46	8.9
MC	0.21	1.3		
[U]	0.25			
	16.99	100.0	51.8	
P, Y	5.11 5.11 5.05	5.09	1.82 1.80	r.61
Yield	17.5		6.2	
Percentage	94%		84%	

SSC

2-1-52

HCOOH hydrolysis

NaTN 0.8 mg

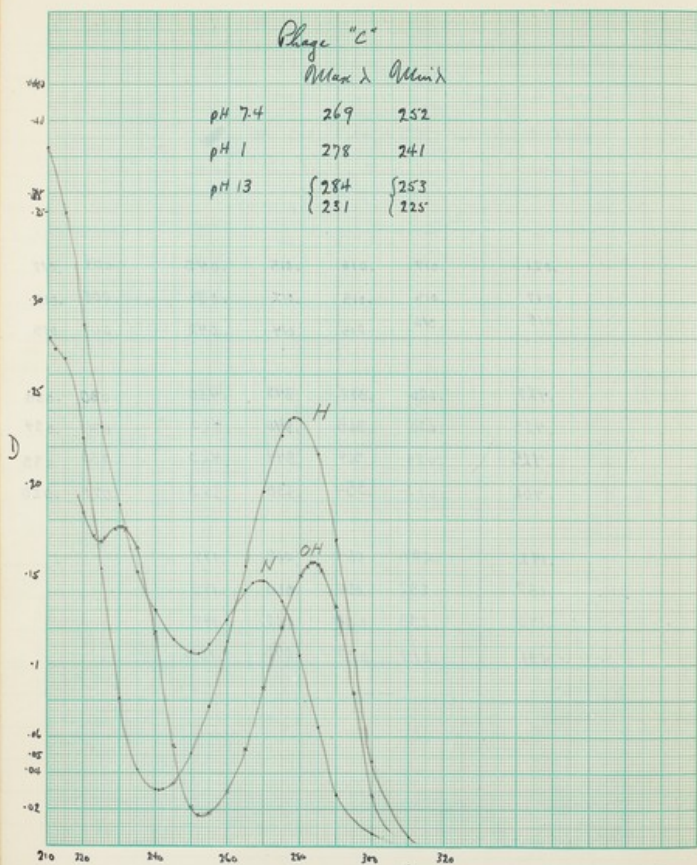
T2r-1 0.6 mg

1.05

Each ↑ 0.1 ml 88% HCOOH 175° 40 min

Dry down, ↑ 25 μl N-HCl. Take 2-53 after, 2 or 3 x 1.95 for 1?

B		G		A		C		T	MC	U
		275	250	275	250	275	250			
1		.021		.017		.016	.015	.045	.009	.017
2		.017		.015		.013	.012	.038	.008	.012
	\bar{x}	.019		.016		.015	.014	.042	.009	.015
NaTN	1	.427		.626		.372	.348	.430	.030	.035
	2	.423		.630		.365	.340	.420	.030	.034
	\bar{x}	.425		.628		.369	.344	.425	.030	.035
	$\bar{x} - \beta$.406		.612		.354	.330	.383	.021	.020
T2r-1	1	.142		.234		.064	.063	.199		
	2	.137		.232		.059	.060	.195		
	\bar{x}	.140		.233		.062	.062	.197		
	$\bar{x} - \beta$.121		.217		.047	.048	.155		



SSC

Phase "c"

21-11-52 Combined "c" elution of T2 & T4, which have $D_{200} > D_{270}$. Lysis done, put on paper, run in DMSO-NH_3 . Index position = G of BSA marker. C is mixture ER₂C₁ run B treated like usual C fraction.

*Pr. G has for G R₂ in DMSO-NH_3 too.

22-1 Elute phase "c" a few times in water.

23-1 Take to pH 7.4 by adding 0.1 ml 0.5M phosphate buffer.

"c"

cell number	pH 7.4	pH 1	pH 13
310		.005	-.012
300	-.007	.047	+0.028
295		.108	.084
290	-.028	.169	.132
285			.158
280	.065	.216	.188
275		.238	.185
270	.105	.238	.197
265	.135	.226	.120
260		.195	.087
255	.176	.194	.058
250	.171	.154	.030
245	.125	.113	.018
240	.111	.097	.018
235	.106	.081	.017
230	.107	.051	.021
225		.034	.055
220	.114	.031	.118
215	.150	.042	.165
210	.151	.051	.180
205	.138	.053	.175
200	.231	.153	.178
195	.223	.225	.168
190	.228	.225	.151
185	.215	.349	.212
180	.395	.210	.240

Decomposition

25-i-52

Small spots of sytserini and sytserini eluted from paper into small tubes.
Dry down. Add to each 20 μ l 1 M NaNO_2 and 5 μ l glacial HCl . Leave at
 27° 4 hrs. Dry down. \uparrow 10 μ l of each, spot on paper, run in solvent. HCl .
 \rightarrow 2 spots each, about 50% decomposition.

28-i-52

Drops sytserini dried down in 2 tubes, add: (1) 20 μ l 2 M NaNO_2 + 5 μ l HCl
(2) 10 μ l - - - + 2.5 μ l HCl .

10-15

Incubate 40° 6 hrs. Concentrate, spot on paper, run in solvent. HCl .

29-i

10- μ l sample ca. 50% converted to U. } RF_2 increased over marker C,
20- μ l - - - 75% - - - } presumably because of self,
but spots compared good.

T2+1: HCCO₂

	15 min.		30 min.		60 min.		120 min.	
	Yields	Ratios	Yields	Ratios	Yields	Ratios	Yields	Ratios
A	3.37	36.4	3.54	3.64	3.81	36.8	3.46	37.8
T	3.18	34.4	3.38	34.4	3.50	33.8	2.98	32.5
G	1.95	21.1	2.08	21.2	2.28	22.0	2.05	22.4
"C"	0.44	5.2	0.44	4.5	0.35	3.4	0.29	3.2
Ph-G (assume C=100%)	0.27	2.9	0.37	3.8	0.43	4.1	0.38	4.1
	9.25	100.0	9.81	100.0	10.37	100.1	9.16	100.0
P, Y	3.07	3.02	3.05	3.11	3.46		3.20	
(Yields comp. to base)	2.96		3.16					
% recovery	10.40		10.71		11.92		11.03	
	89%		92%		87%		88%	

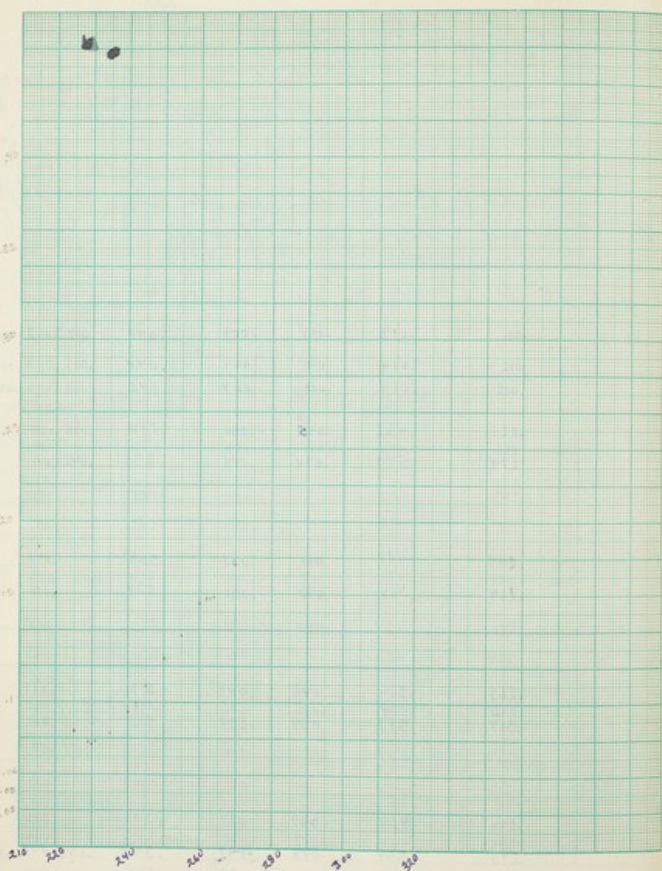
SSC.

28-i-52. Phage DNA: rate of hydrolysis = HCCO₂
 0.7 mg T2+1 DNA + 7 µl 70% HCCO₂ 100° 15 min.
 0.6 mg " " " " " " 30 min.
 0.7 " " " " " " 60 min.
 0.6 " " " " " " 120 min.

29-i. !! Very weak "C" spots in all; big "G" spots.

Index stand for 2 1/2 hrs after adding HCCO₂ before testing. (Note: not for spots - probably splitting because of reaction!)

		G	A	C		T	Ph-G
				275	260		270
B	1	.011	.013	.009	.008	.040	.007 .007 .006
	2	.012	.014	.010	.009	.044	.009 .008 .007
	\bar{x}	.012	.014	.010	.009	.042	.008 .008 .007
15	1	.227	.452	.056	.056	.295	.035 .035 .034
	2 corr.	.226	.452	.061	.062	.295	.034 .034 .034
	2	.227	.459	.074	.075	.358	.042 .041 .041
	\bar{x}	.227	.452		.059	.295	.035
	$\bar{x}-B$.215	.438		.050	.253	.027
30	1	.244	.477	.051	.052	.305	.042
	2 corr.	.238	.473	.057	.058	.316	.047
	2	.209	.416	.050	.051	.278	.041
	\bar{x}	.241	.475	.054	.055	.311	.045
	$\bar{x}-B$.229	.461		.046	.269	.037
60	1	.263	.509	.045	.045	.319	.049
	2 corr.	.262	.509	.046	.046	.323	.052
	2	.307	.597	.054	.054	.376	.061
	\bar{x}	.263	.509		.046	.320	.051
	$\bar{x}-B$.251	.495		.037	.278	.043
120	1	.238	.468	.037	.037	.275	.046
	2 corr.	.238	.459	.039	.040	.282	.046
	2	.290	.559	.048	.049	.343	.055 .056 .054
	\bar{x}	.238	.464		.039	.279	.046
	$\bar{x}-B$.226	.450		.030	.237	.038



30-1-52. Prec. G. eluted from T2⁺-1, conc'd, run in Sandon-HCl, eluted -
M/100 phosphate pH 7.4

	PH 7.4	PH 1	PH 13
320			.021
310	.009	.009	.024
305	.011	.014	
300	.015	.026	.047
295	.022	.043	.068
290	.034	.069	.097
285	.054	.090	.101
280	.080	.105	.114
275	.111	.122	.129
270	.136	.136	.141
265	.157	.159	.156
260	.177	.175	.172
255	.195	.193	.192
250	.137	.121	.125
245	.124	.118	.112
240	.109	.092	.104
235	.083	.062	.114
230	.077	.062	.134
225	.084	.079	.174
220	.115	.107	.233
215	.166	.131	

	20 min			40 min			60 min		
	Yards	Plates	OD ₆₀₀ /ml	Yards	Plates		Yards	Plates	
A	2.92	34.1	22.1	3.33	33.5	29.2	2.16	33.7	28.2
T	3.08	35.9	29.6	3.61	36.3	31.7	2.35	36.7	30.7
G	1.62	18.9	15.6	1.92	19.3	16.9	1.29	20.2	16.4
\bar{x}	2.95	11.1	9.1	1.09	10.9	9.6	0.60	9.4	7.8
	8.57	100.0	82.4	9.95	100.0	87.4	6.40	100.0	83.5
P, Y	2.97			3.37			2.21		
	2.96	3.01		3.32	3.31		2.22	2.22	
	3.11			3.38					
Control lines	10.4			11.4			7.66		
% recovery	83%			87%			84%		

1-11-52 Phage DNA: hydrolysis = HCOOH ^{25 mg}
 3 samples T2 r⁻¹ DNA each \uparrow 0.05 ml 88% HCOOH, cook
 175° 20, 40, & 60 min. Dry down, \uparrow 25 μ l N-HCl, take
 2 x 8.3 spots, & 3 x 1.95 for P. Pipettes easily; messy all.

		G	A	275° C	280	T
B	1	.017	.015	.011	.010	.040
	2	.016	.016	.012	.011	.038
	\bar{x}	.017	.016	.012	.011	.039
20	1	.201	.387	.104	.105	.278
	2	.201	.404	.114	.116	.290
	\bar{x}	.201	.396	.109	.111	.284
	$\bar{x}-S$.184	.380	.097	.100	.245
40	1	.229	.443	.117	.119	.321
	2	.226	.455	.125	.127	.331
	\bar{x}	.228	.449	.121	.125	.326
	$\bar{x}-S$.211	.433	.109	.114	.287
60	1	.158	.292	.070	.071	.219
	2	.161	.302	.075	.076	.232
	\bar{x}	.159	.297	.073	.074	.226
	$\bar{x}-S$.142	.281	.061	.063	.187

	T2r-1		T4r-2		ERyC _n		NaTN	
	Yield	Ratio	Yield	Ratio	Yield	Ratio	Yield	Ratio
A	3.25	33.7	3.01	33.8	4.12	35.2	5.39	29.0
T	3.53	36.6	3.38	36.9	3.97	33.9	5.54	28.8
G	1.85	19.2	1.75	19.7	1.85	15.8	4.21	21.9
C	1.01	10.5	0.85	9.6	1.77	15.1	3.95	20.0
MC					[>0.05]		0.24	1.2
	9.64	100.0	8.89	100.0	11.71	100.0	19.23	99.9
PY	3.24 3.28	3.26	2.91 2.89	2.90	3.54 3.53	3.54	5.76 6.64	5.70
Yield	11.2		9.98		12.2		19.6	
R	86%		89%		96%		98%	

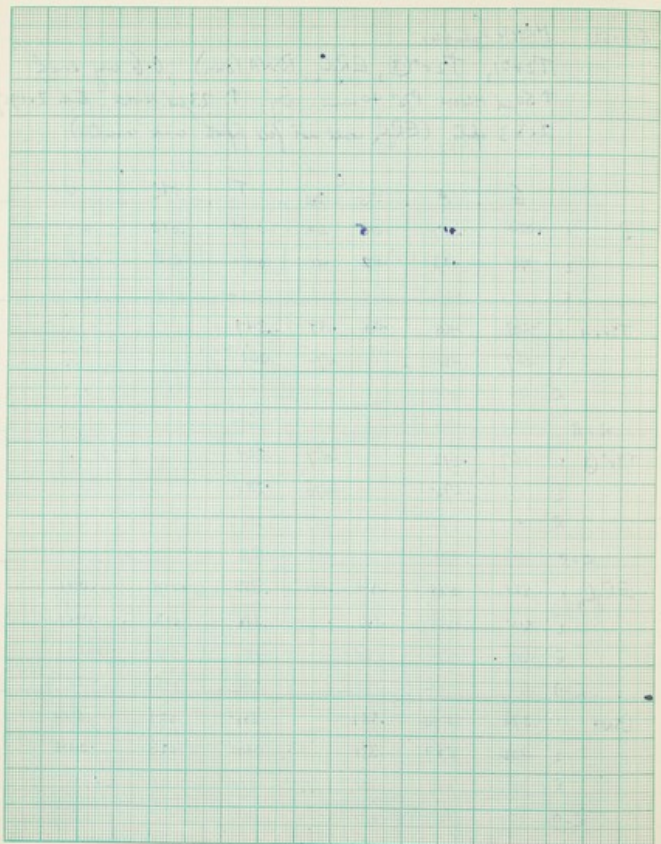
4-ii-52 Phyco. DIA. HCOOH.

T2r-1 0.5' ang
 T4r-2 0.5' ang
 ERyC_n 0.7' ang
 NaTN(SiO) 0.7' ang

each ↑ 50 μl 88% HCOOH 175° 40 min,
 lay down, ↑ 23 μl N-HCl. take 2x
 8.3 apks, 2 x 1.95 for P. Glass all.

Handwritten: All above table in MC fraction ERy - NaTN after in U fraction; Phyco. apks /

	G	A	275		T	275		283		280	
			280	280		280	280	280	280		
B	1	.024	.020	.011	.010	.041					
	2	.024	.020	.012	.012	.034					
	\bar{x}	.024	.020	.012	.011	.038					
T2r-1	1	.224	.426	.112	.114	.319	.011	.010			.660 .65 .68
	2	.231	.448	.119	.109	.319		.008			.621 .62 .62
	\bar{x}	.228	.442	.116	.117	.319					
	$\bar{x} - B$.204	.422	.104	.106	.281		.009			
T4r-2	1	.216	.407	.096	.097	.300	.012	.011			.660 .67 .66
	2	.216	.414	.102	.103	.298		.011			.619 .62 .63
	\bar{x}	.216	.411	.099	.100	.299		.011			
	$\bar{x} - B$.192	.391	.087	.089	.261					
ERyC _n	1	.226	.531	.174	.181	.353	.006	.005			.645 .65 .64
	2	.239	.560	.202		.355		.005			.630
	\bar{x}	.228	.535	.198		.354		.005			.630
	$\bar{x} - B$.204	.535	.186		.316					
NaTN	1	.480	.711	.410		.474	.021	.024			.631 .63 .63
	2	.484	.729	.421		.482	.022	.024			.622 .62 .62
	\bar{x}	.487	.720	.416		.478					
	$\bar{x} - B$.463	.700	.404		.440		.024			.631



12-ii-52

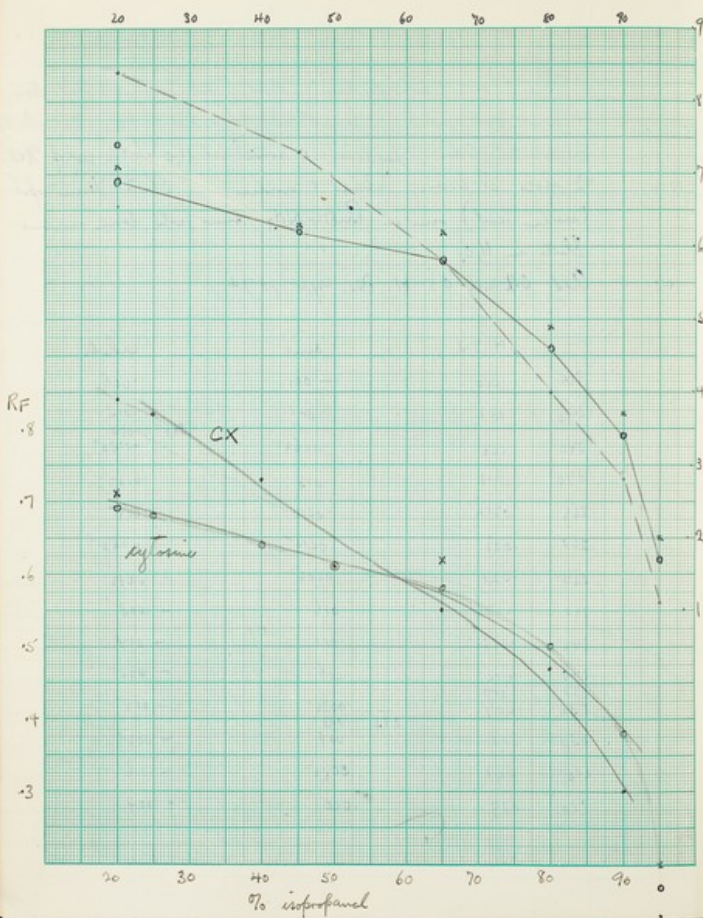
X deamination

Take 3 small tubes dry down: (1) eluted X spot, (2) small amt cysteine, (3) small amt cysteine. In each, add 25 μ l 2M NH_4OH , + 5 μ l glycine buffer to 27° 5 hrs., dry down. + small vol. H_2O , spin in centrifuge 2 spots, at levels of cysteine + methionine. Elute "deam" spot (methionine level) run in SDS-PAGE, same faster than methionine. Elute in H_2O .

18:

Add 0.2 vol 0.5 M PO_4 buffer pH 7.4.

	pH 7.4	acid	alkali
300	.000	-.001	.006
290	.001	.001	.013
280	.008	.0065	.014
270	.019	.014	.0125
265	.0215	.0210	.0105
260	.022	.0215	.008
255	.021	.0205	.0055
250	.0175	.016	.003
245	.0135	.011	.001
240	.010	.006	.001
237	.007	.0035	.001
235	.009	.003	.001
230	.011	.004	.001
225	.021	.0045	.001
220	.029	.020	.009



RF's.

A.P.	Phase X	Cytosine	Cytosine	Cytosine
25%	0.82	0.68	0.74	0.76
Isoprof. 40%	0.73	0.64	0.67	0.59
Isoprof. 50%	0.61	0.61	0.64	0.62
" 65%	0.55	0.58	0.58	0.49
" 80%	0.47	0.50	0.48	0.28
" 90%	0.30	0.38	0.31	0.09

Isoprof. 65% NH ₃	0.58	0.55	0.56	0.32
Isoprof. 65% neutral	0.55	0.58	0.58	0.40
Isoprof. 65% HCl	0.62	0.64	0.63	0.24
Isoprof. 65% HCl	0.48	0.48	0.50	0.61

Run H₂O-NH₃ 0.07 0.17

Repeats 5-12	CX	CX	C	S-MC	S-MC	H-MC
Apr 01 + 20%	.84	1.22	.69	.71	1.03	.72
45%	.73	1.18	.62	.63	1.02	.66
65%	.58	1.00	.58	.62	1.07	.66
80%	.40	.87	.46	.49	1.07	.52
90%	.28	.82	.34	.37	1.09	.41
95%	.11	.65	.17	.20	1.18	.25

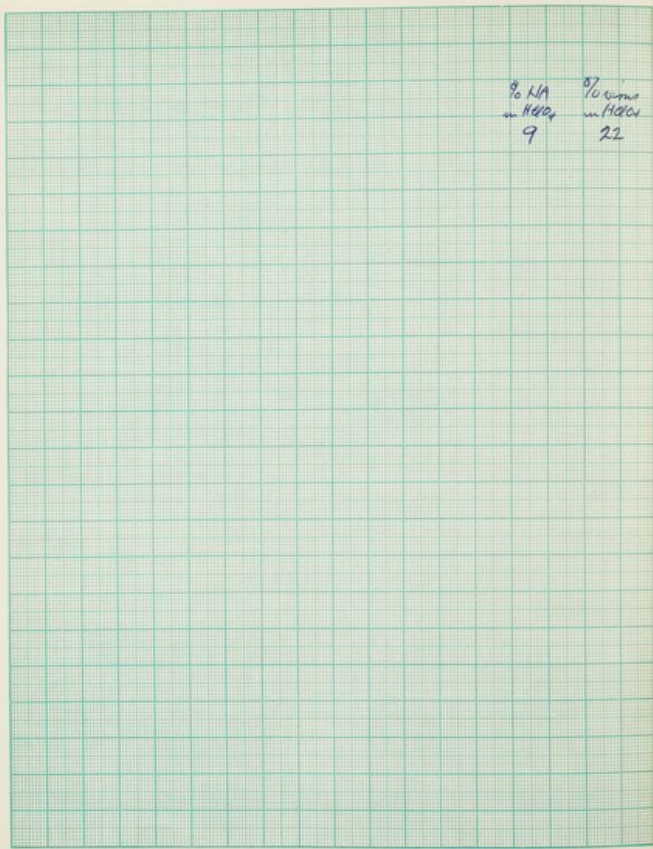
22-6-51 Recalibration of micro-pipettes

Stock sol'n: Ad. 50 μ v 6mg/ml in $\frac{1}{10}$ HCl.

Standards: 0.5 ml (pipetted) + 200 ml $\frac{1}{10}$ HCl.

Pipettes emptied onto filter paper & dried overnight, along with blanks.

	"1.95"	"?	"82"	"11.9"	0.5 + 200	
1	.010	201	.592	.832	1.186	0.979
2	.009	202	.592	.832	1.191	0.981
3	.011	204	.592	.830	1.190	
4	.008	202	.591	.830	1.193	
\bar{x}	.0095	202.2	.592	.831	1.190	0.980
$\bar{x} - s$		192.7	.582	.821	1.180	
Volume, μ l					1.180	
		<u>1.951</u>	<u>5.90</u>	<u>8.32</u>	<u>11.96</u>	

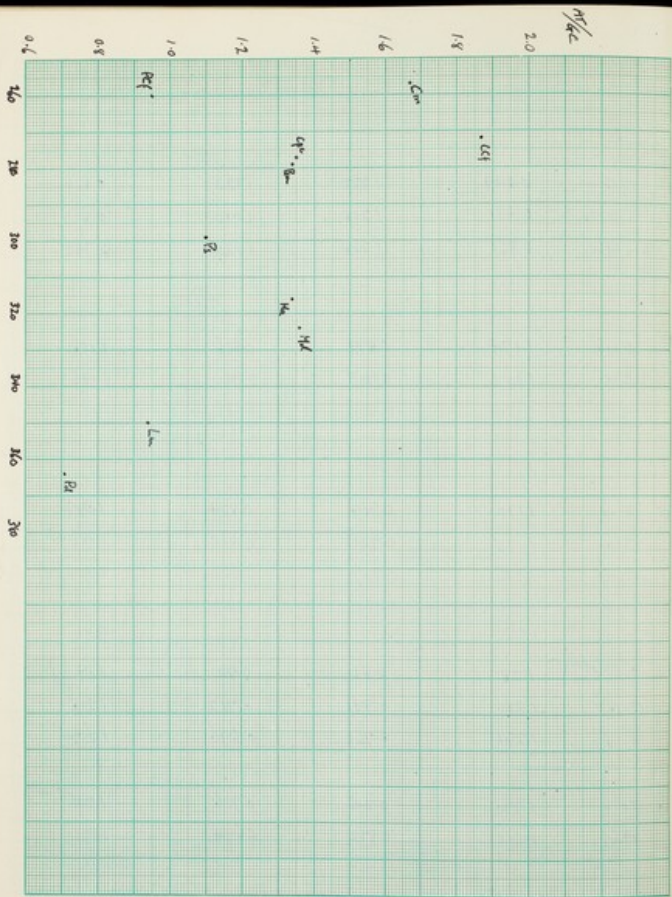


% NA
in H2O
9

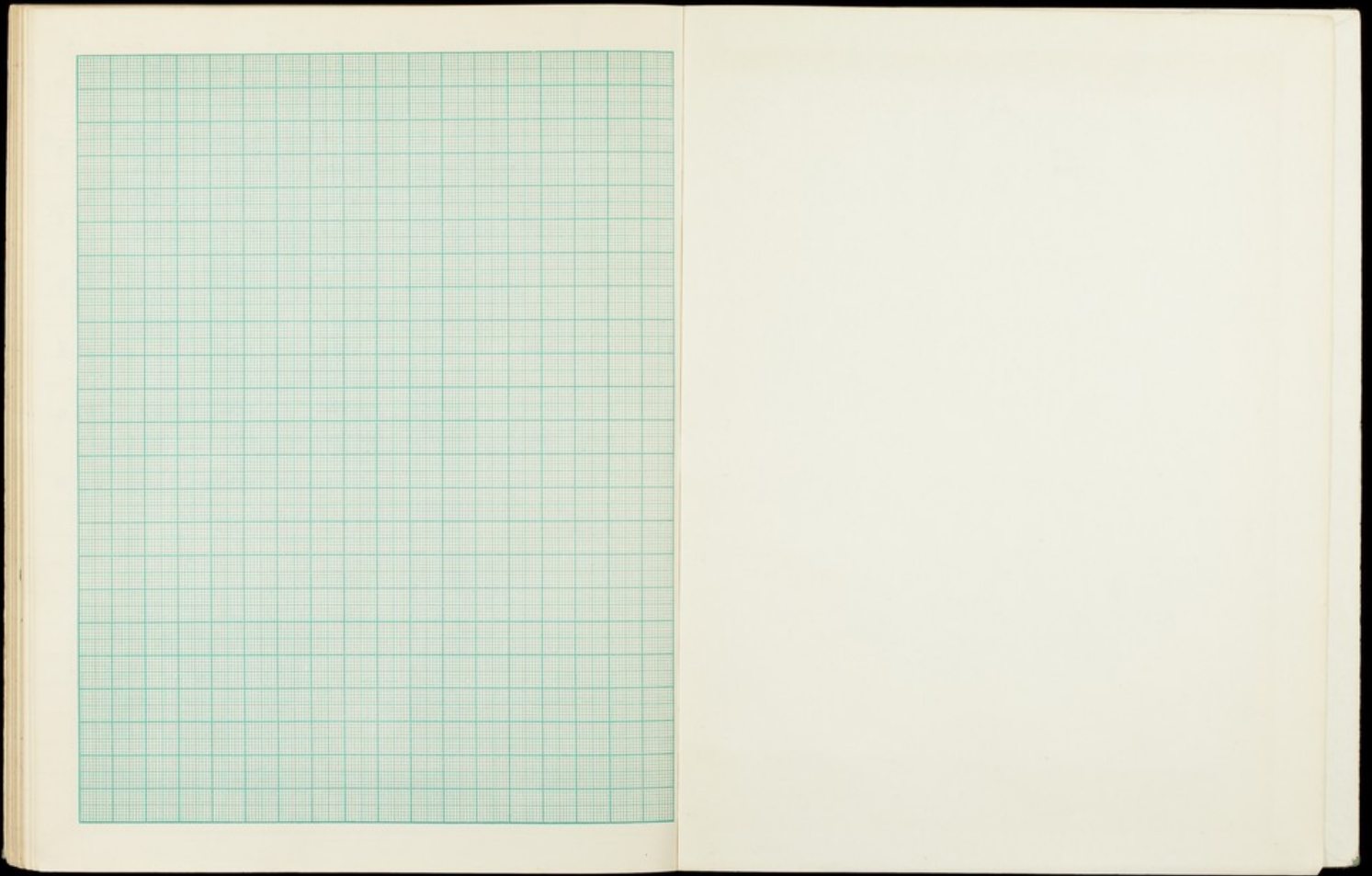
% virus
in H2O
22

Insect Virus NA's - Correlation

	A	G	C	T
Ld. con. 12-ii	0.849	1.222	1.123	0.806
GHB 20-iv	0.831	1.218	1.140	0.809
Ban 19-vi	0.861	1.228	1.130	0.782
Qan 15-x	0.851	1.208	1.129	0.817
Bm 11-iv	1.174	0.899	0.806	1.118
2-vi	1.152	0.903	0.800	1.146
3-viii	1.186	0.896	0.817	1.101
Cpc 2-iv	1.170	0.909	0.824	1.099
2-vi	1.175	0.901	0.813	1.110
[13-vi	1.236	0.907	0.850	1.009] too hot!
3-viii	1.211	0.883	0.797	1.107
4-x	1.225	0.883	0.783	1.110
Cm 1-iii ^{from 14}	1.28	0.81	0.78	1.19]
13-iv	1.280	0.791	0.712	1.218
20-iv	1.294	0.761	0.690	1.252
N6 20-v	1.270	0.746	0.736	1.246
19-vi	1.312	0.822	0.694	1.171
MA 11-viii	1.181	0.908	0.800	1.106
28-ix	1.151	0.884	0.815	1.149
14-xi	1.176	0.904	0.808	1.113

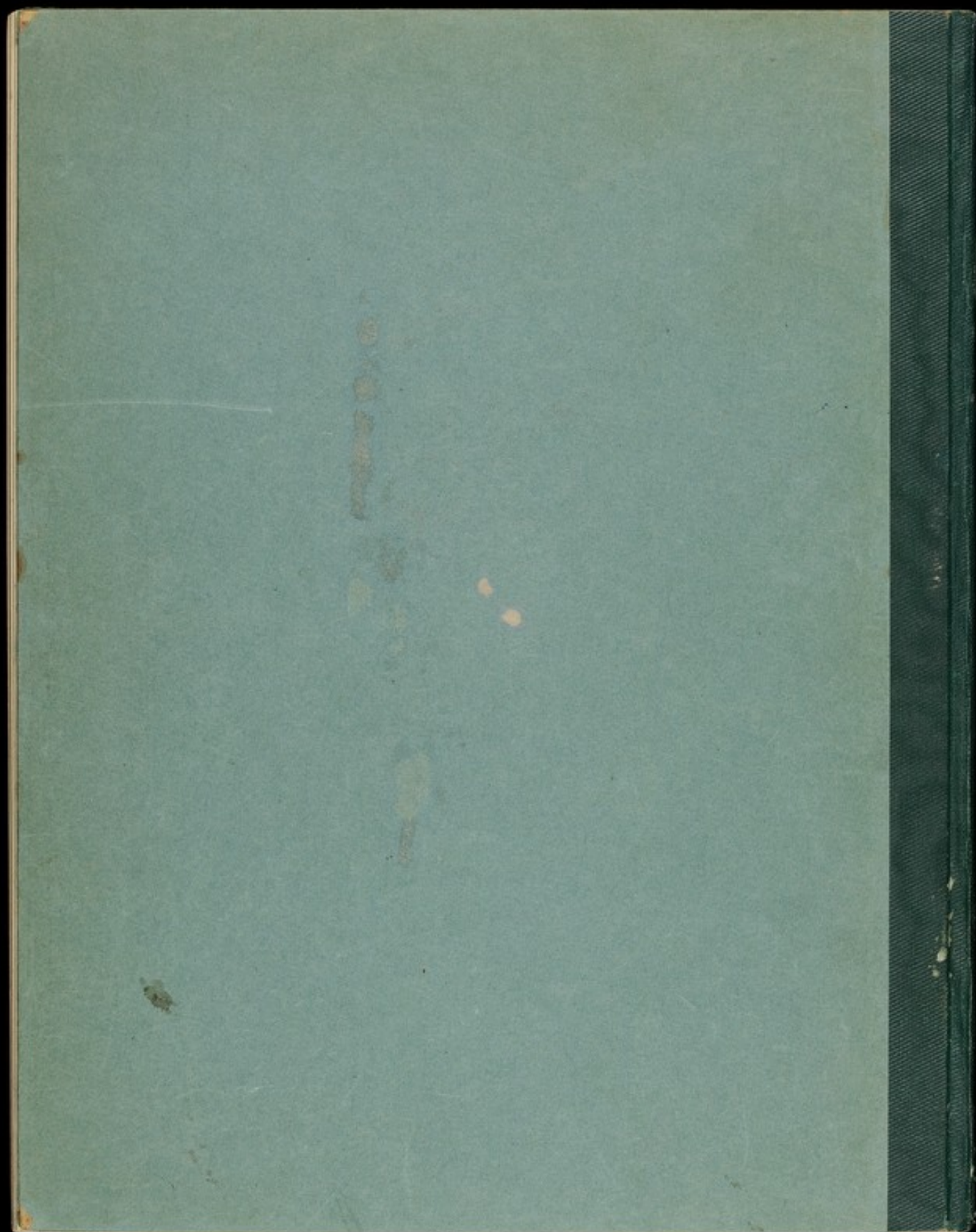


	A	G	C	T	% Pig	No. animals
<u>Md</u> 13-xi	1.188	0.892	0.809	1.110	87.8	27
14-xi	1.159	0.871	0.810	1.161	80.7	28
15-xi	1.160	0.869	0.816	1.182	89.6	30
<u>Lm</u> 18-xi	0.986	1.074	0.989	0.954		23
<u>PCF</u> 20-xi	0.985	1.075	0.975	0.966		28
26-xi	1.000	1.068	0.975	0.960	86.2	25
<u>CCF</u> 20-xi	1.311	0.736	0.656	1.296		30
<u>Ps</u> 26-xi	1.068	0.974	0.929	1.027	87.1	31



$$N \text{ factor} = \frac{1}{14} \times \frac{8.3}{21.1} \times \frac{1}{3.98} = 706$$

$$P \text{ factor} = \frac{1}{31} \times \frac{8.3}{1.95} \times \frac{1}{5.98} = 345$$



100

10.3.52 12.3.52 14.3.52 19.3.52 24.3.52 31.4.52

BSNA	31.3	32.0		31.7			32.1
NaTN	31.8	31.6	30.8	31.0	28.2	28.9	32.6
FRg	32.4	23.5	31.0				
T2r-2	33.6	31.0	25.3				32.1
T6r+			31.3				28.8
T4r-3				41.3	38.2	40.3	
T6r-4				35.6	38.2	36.0	60.8

Book

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