

## Notebook 7

### Publication/Creation

27 February 1952 - 13 November 1952

### Persistent URL

<https://wellcomecollection.org/works/aemvnm6a>

### License and attribution

You have permission to make copies of this work under a Creative Commons, Attribution, Non-commercial license.

Non-commercial use includes private study, academic research, teaching, and other activities that are not primarily intended for, or directed towards, commercial advantage or private monetary compensation. See the Legal Code for further information.

Image source should be attributed as specified in the full catalogue record. If no source is given the image should be attributed to Wellcome Collection.



Wellcome Collection  
183 Euston Road  
London NW1 2BE UK  
T +44 (0)20 7611 8722  
E [library@wellcomecollection.org](mailto:library@wellcomecollection.org)  
<https://wellcomecollection.org>

CHEMISTRY

NUCLEIC ACIDS ETC.

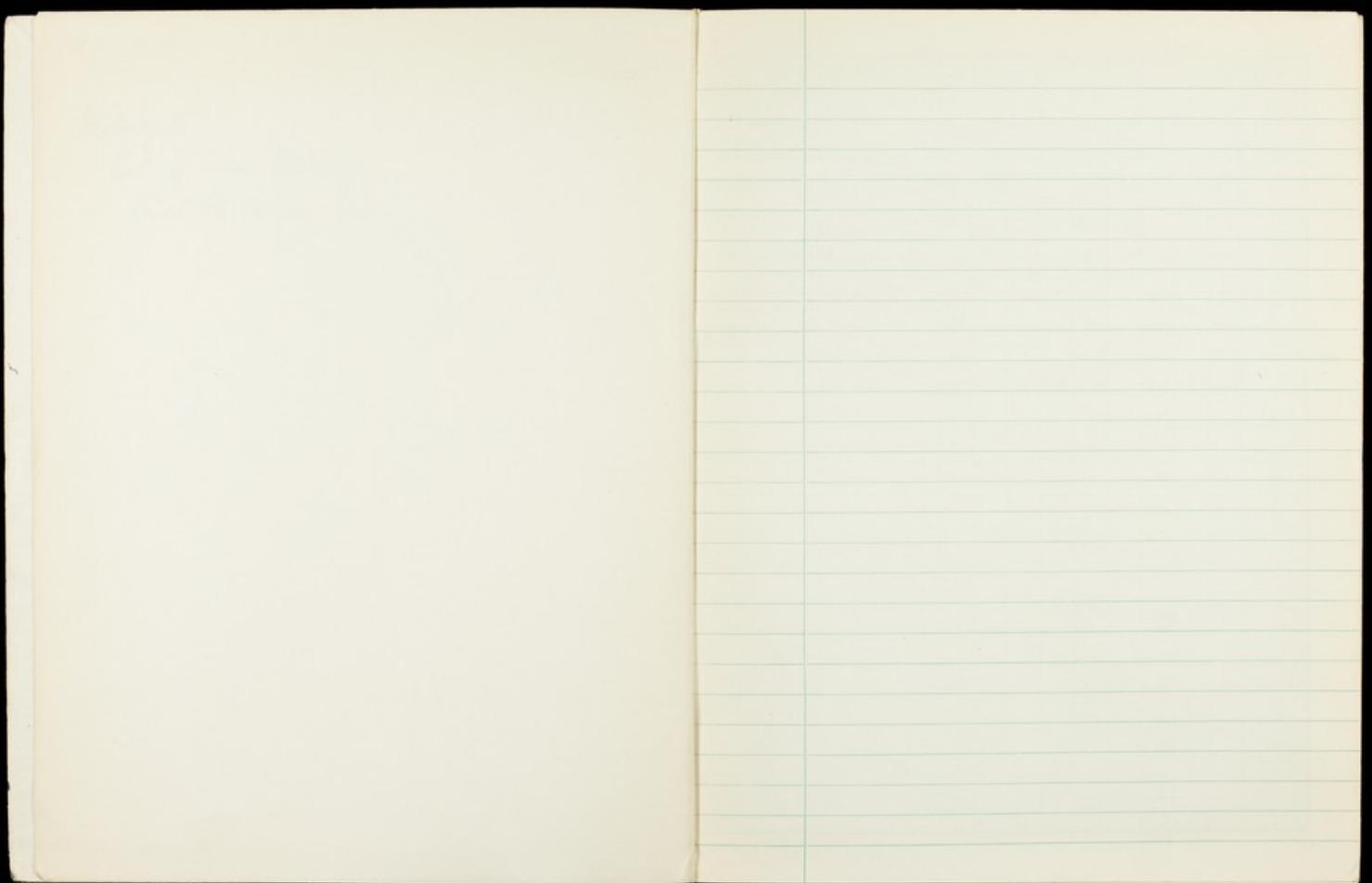
March 1952 — November 1952.

7

PP/CRW/A/6

G. R. Wyatt  
Lab. of Tissue Pathology.  
Sault Ste. Marie, Ont.

mm m



$T_{2r^2-1}$	5' min	10	20	40
	Yankee Ratio 18P	Yankee Ratio 18P	Yankee Ratio 18P	Yankee Ratio 18P
A	2.26	29.8	2.12	29.8 2.41
T	1.74	22.9	2.14	30.1 2.64
G	.24	16.3	1.19	16.7 1.49
C	0.86	11.3	0.85	12.0 0.69
	6.10	10.3	6.3.0	18.6 7.23
P.Y	2.21 { 2.21	2.11 { 2.07	2.44 { 2.42	3.11 { 3.12
Yankee compl. to bases	7.59	7.10	8.31	10.70
R	80.5%	88.8%	87.0%	91.1%

27-ii-52

## Phage DNA 11C074 hydrolysis times

4 samples SSCA  $T_{2r^2-1}$  DNA each 0.5 mg ↑ 0.05 ml  
 88% HCOOM. cook 173-175° 5, 10, 20, 40 min. Daylong,  
 ↑ 25 µl N-HCl. Inc 2x 8.3 µg, 2x 1.95 for P.  
 Chromatogram ③ droffel:  $F_{21}$ , spds. esp. on 10 min °C.

	G	A	$\frac{C}{G}$	$\frac{C}{A}$	T
B	1 .012	.011	.010	.009	.034
	2 [-0.23]	.013	.009	.009	.037
	2̄ .014 (assumed)	.012	.010	.009	.036
S	1 .146	.303	.099	.100	.171
	2 .154	.308		.098	.177
	2̄ .150	.306		.099	.174
	2̄ - B .136	.294		.090	.188
10	1 .148	.288	.026	.098	.206
	2 .145	.288	.06 [1.05]		.206
	2̄ .145	.288		.098	.206
	2̄ - B .131	.276		.089	.170
20	1 .179	.323	.093		.244
	2 .177	.326	.078		.247
	2̄ .178	.325		.081	.246
	2̄ - B .164	.313		.072	.210
40	1 .212	.446	.121		.316
	2 .209	.448	.116		.323
	2̄ .211	.447	.119		.320
	2̄ - B .197	.435	.110		.284

	T2r-2	T4r-3	T6r-4	T6r+
A	2.65 34.1	2.28 34.0	1.89 36.1	2.58 34.1
T	2.86 36.8	2.46 36.7	1.97 36.6	2.72 35.9
G	.54 .19.8	.22 .18.2	.93 .17.3	1.41 .18.6
CX	.073 .9.4	.074 .11.0	.059 .11.0	.086 .11.4
	7.78 .00.1	6.70 99.9	5.38 122.0	7.57 103.2
P, Y	2.55 } 2.56 2.56 }	2.16 } 2.18 2.20 }	1.84 } 1.83 1.81 }	2.45 } 2.44 2.43 }
Yeast concn. per liter	7.46 8.79	7.45 7.48	5.37 6.28	7.46 8.38
R	18.5	89.6	85.8	90.4

5-iii-52

One lot of flagger DNA's.

0.6 mg T4r-3, 0.5 mg each T2r-2, T6r-4, + T6r+ + 50 µl 88% HCO<sub>2</sub> 40 min. By, ↑ 25' at N-HCl, take 2x 7.95 ml P, 2x 9.3 ml Pots.  
All show CX & a weak spot about 1/2 way between it and T, call Y, & cutout.

	G	A	CX 280	T	Density against blank
B	1 .014	.012	.009	.036	265
	2 .013	.014	.010	.043	280 .011
	z .014	.013	.010	.040	270 .017
T2r-2	1 .183	.354	.083	.266	.018
	2 .183	.359	.090	.268	.017
	z .183	.357	.087	.267	.018
	z-B .169	.344	.077	.227	.018
T4r-3	1 .147	.310	.084	.233	.017
	2 .148	.310	.092	.239	.017
	z .149	.310	.088	.236	.018
	z-B .134	.297	.078	.196	.017
T6r-4	1 .118	.257	.069	.196	.020
	2 .114	.258	.075	.198	.019
	z .116	.258	.072	.197	.019
	z-B .102	.245	.062	.157	.020
T6r+	1 .169	.345	.100	.252	.021
	2 .168	.350	.100	.259	.017
	z .169	.349	.106	.256	.019
	z-B .155	.335	.090	.216	.019

10-iii-52

Dicke Tests (Rhona)

Mixture: GSNA 2.5 mg ↑ 25 ml ag. dist + 2 drops N-NaOH.

NaTN (5%) 1 mg ↑ 10 ml ~ + 1 drop ~

ERg 0.6 mg ↑ 6 ml ~ ~

T2r-2 1 mg ↑ 10 ml ~ ~

Results over weekend.

From each, take 2 x 1 ml for Dicke, 2 x 0.4 ml for P.

Results:

	Dicke, E/ml	P, Y	RP/ml	Dicke E/ Y/P
GSNA	240 } 242 244 }	3.10 3.08	3.09 7.73	2884 31.4
NaTN	227 } 239.5 242 }	2.98 3.03	3.005 7.51	2888 31.9
ERg	122 } 129.5 117 }	1.49 1.46	1.475 3.69	8920 22.4
T2r-2	125 } 125 125 }	1.52 1.46	1.49 3.72	8460 33.6

Repeal: added one  
more ERg to same  
soln, + made  
up second T2r-2  
at mg/4 ml

ER <sub>g</sub>		NaTN		
Yields	Ratios	Yields	Ratios	
A	2.69	36.7	2.34	29.0
T	2.40	31.8	2.04	26.3 ← loss because back to back.
G	1.30	17.2	1.88	23.3
C	1.15	15.3	1.705	21.2
MC	[0.01]		0.092	1.14
[U]	[0.11]		[0.075]	
			8.06	
P	2.32	2.56		
Yields comp for bases	2.33	2.56		
	2.37	2.58		
		2.47	2.54	
			58.71	
R	9.8.9	92.5		

P Y  
Yields comp  
for bases  
2.32  
2.33  
2.37  
2.34  
2.56  
2.58  
2.47  
2.54  
58.71

12-iii

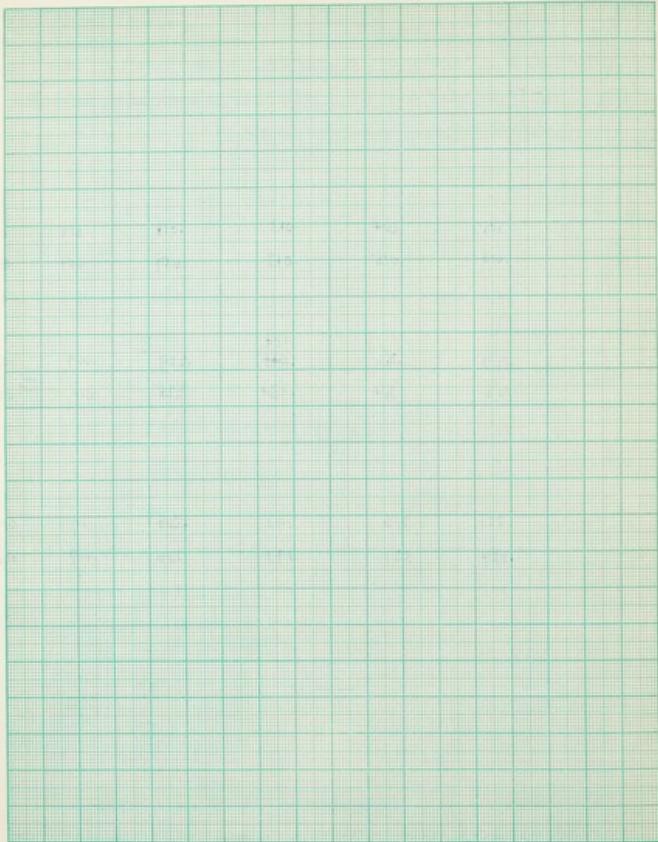
HellO<sub>4</sub> : ER<sub>g</sub> + NaTN

0.5 μg each DNA ↑ 5' end HellO<sub>4</sub>, road 100° 1 hr. (reheated 102° for short time). Add 22 μl H<sub>2</sub>O, ground mixture, take 3 × 1.95 μl for P, 2 × 8.3 for spots.

	G	A	C	T	MC	U
1	.015'	.014	.013	.038	.008	.015'
2	.016	.015'	.013	.037	.009	.014
3	.016	.015'	.013	.038	.009	.016'

ER <sub>g</sub>	1	.159	.266	.144	.229	.009	.025'
	2	.158	.364	.134	.228	.011	.023 .025
	3	.149	.365	.134	.229	.010	.024
	3-B	.143	.350	.121	.191	.001	.009

NaTN	1	.221	.316	.191	.200	.017	.019
	2	.224	.321	.193	.200	.019	.016
	3	.223	.319	.192	.200	.018	.017
	3-B	.207	.304	.179	.162	.009	.002



15-iii-52.

Search for CX in *E. coli* B.

Cohen's lyophilized *E. coli* B 443 mg ↑ 10 ml 1N-NaOH.

27°C. 2 days.

17-iii

Spir 10,000 ↑ mm → big gummy pellet left. clear yellow.

Ppt = HCl (0.6 ml) + EtOH (10 ml) → gooey white ppt.

Thin down, dry, weigh (= 52 mg) into cork tube, add 1.5 ml 88% NaOONa, cook 175° 25 min., dry → tan odd 0.1 ml N-HCl, affy as band on sheet Whatman #3 over ascending in isoprof. HCl 48 hr. Elect band 2. Run spot in  $\text{KNO}_3\text{-Na}_2\text{SO}_4$  → big C spot, faint one in O position.

Elect 3.5 ml N<sub>2</sub>H<sub>4</sub> HCl & swirl slowly against glass.

CX

282°	.0185"	
280	.021	.90
278°	.0215"	
275°	.022	.96
270	.0225"	.91
265°	.022	
260	.022	

19-iii

100 mg *E. coli* whole ↑ 1.5 ml 88% NaOONa, cook 175° 40 min.

Blew up! —||—

22-iv

80 mg *E. coli* whole ↑ 1.0 ml 88% NaOONa 175° 40 min. OK.

Big lysis → much tan. ↑ N-HCl, spreading big slot Whatman #3, run up hill in isoprof. HCl

V foot

For last 45 mg T6r<sup>-2</sup> David "V" eluted, run in Ba(OH)<sub>2</sub>-NH<sub>3</sub>  
→ RF about same as Cx. Elute, re-run as spot in isoprop. 96:4  
→ original (bottom) <sup>& also spots in exterior</sup> Elute spots blank in ag. dest.

Cx (T6r)	pH 7	pH 1	pH 13
175° V31	.208	.010	.012
175° -135°	.290	.015	.013
285° -125°	.280	.024	.023
285° 270°	.023	.023	.059
265° 260°	.060	.027	.068
260°	.0316	.019	.049
280°	.029	.027	.067
240°	.031	.018	
230°	.024	.019	
230°	.040	.024	
230°	.026		
230°	.023	.024	

15-iii

Cx pref. x T6r<sup>-2</sup>

Cohen uses T6r<sup>-2</sup> 55 mg in 2 spectrums.  
In each, 1 ml 88% HCOOH, work 175° 30 min.  
Dry down, each ↑ 0.1 ml N-HCl spread bottom one sheet Whatman #3,  
run H<sub>2</sub> gas according in isoprop.-HCl → 3 main bands  
= G+A (2), Cx, + T. Cut out Cx + elute by running  
water down. Dry down, → black residue, ↑ % NH<sub>3</sub>, run on Whatman #3  
in Ba(OH)<sub>2</sub>-NH<sub>3</sub>. H<sub>2</sub> gas → nice clean-looking band. Start: 150.

17-iii

Further H<sub>2</sub> mg T6r<sup>-2</sup> ↑ 1.0 ml HCOOH work 175° 60 min., dry,  
+ 0.2 ml isoprop.-HCl (dissolves two better than straight HCl) spread a band  
on big sheet Whatman #3, run uphill in isoprop.-HCl. Elute band,

6-iv

run in Ba(OH)<sub>2</sub>-NH<sub>3</sub>. Elute, combine & elute from first 55 mg.,  
concentrate, filter on Whatman #3, 8' on ice, run in Ba(OH)<sub>2</sub>-NH<sub>3</sub>  
(no NH<sub>3</sub>). Elute, dry down → ~~yellow~~ white residue. Weight = 1.4 mg.  
↑ 0.35 ml of ag. dest.

21° 280

192 µl ↑ 4 ml →

0.33 .03+

Glycine, 3.0 mg/ml, 1.95 µl ↑ 4 ml →

.134 .126

17-iii Effect of NaOH on phage DNA  
3.0 mg T<sub>6</sub>φ-2 whole phage + 0.2 ml N-NaOH 27°C

48 hrs.

Centrif. 5 1.02 ml H<sub>2</sub>O → no p.f. Add 0.25 ml 85% → good p.f.  
Spin down, dry 100°, add 0.05 ml 88% HCOOH cool 17° + 0 min.  
Blow up! —+

24-iii

Reheat.

- see phage of 27-iii for analysis.

	T2r-2	T4r-3	T6r-4	T6r+
	Yields, Radio	Yields, Radio	Yields, Radio	Yields, Radio
A	3.02 38.8	1.25 34.4	1.59 33.8	2.08 33.7
T	9.22 36.1	1.194 32.9	1.69 36.1	2.18 35.4
G	1.65 18.5	0.673 18.5	0.946 18.0	1.13 18.3
CX	1.04 11.6	0.504 14.1	0.571 12.2	0.771 12.5
	8.93	3.631	4.697	6.161
P, Y	2.54 2.86 2.88 2.86 2.84 2.86 2.87 2.86	1.12 1.11 1.09 1.11	1.62 1.62 1.61 1.62	2.06 2.06 2.05 2.06
Transfection	98.1	3.81	5.54	7.06
R	90.9 77.70	95.4 %	84.6 %	87.3 %

24.iii

## Plaque DNA's.

(170)

T2r-2 DNA    0.6 mg }  
 T4r-3            0.5 mg } weighed carefully (labeled tube is empty).  
 T6r-4            0.6 mg }  
 T6r+            0.6 mg }

Each + 0.05 ml 88% Acetyl 174° HO min.

Dry. + 25 µl N-Hg, th. 2x8.3 mm, 2x1.95 for P.

	G	A	CX <sub>20</sub>	T	γ (PmU)
B	1 .013	.011	.010	.040	.012 .018* .01
	2 .017	.011	.010	.038	.014 .018 .012
	26 .012	.011	.010	.039	.013 .012* .011*
T2r-2	1 .191	.396	.114	.299	.023 .024 .022
	2 .196	.410	.123	.301	.025 .026 .024
	20 .194	.403	.119	.295	.024 .025 .023
	26-B .182	.392	.109	.256	.011 .012* .011*
T4r-3	1 .087	.174	.062	.131	.014*
	2 .088	.174	.066	.136	.014*
	20 .086	.174	.064	.134	.014*
	26-B .074	.163	.054	.095*	.002*
T6r-4	1 .105	.216	.068	.169	.020
	2 .105	.220	.072	.176	.020
	20 .105	.218	.070	.173	.020
	26-B .093	.207	.060	.134	.008*
T6r+	1 .135	.279	.089	.206	.029
	2 .136	.283	.092	.218	.029
	20 .136	.281	.091	.212	.019
	26-B .124	.270	.081	.173	.006*

	T2C-2	T2r <sup>-1</sup>	T4r <sup>-2</sup>	T6r <sup>+</sup>
	Yeast Ratio	Yeast Ratio	Yeast Ratio	Yeast Ratio
A	2.63 2.63	33.1 33.2	2.01 2.05	1.98 33.0
F	2.87 2.87	36.1 36.5	2.15 2.15	35.6 37.1
G	1.51 1.51	1.345 1.345	2.225 2.225	20.0 39.1
CX	0.94 0.94	11.8 10.0	0.57 0.57	9.05 9.05
	7.95 7.95	100.0 100.0	6.04 6.04	100.1 5.995
P, Y	2.58 2.58	2.00 1.98	2.10 2.07	2.05 2.05
S	8.85 8.85	6.83 6.83	7.17 7.17	7.04 7.04
R	89.9%	92.3%	84.3	88.2
			3	
			n	z

28-iii

## Phage DNA:

T2r<sup>-2</sup> (0.5 μg)  
 T2r<sup>-1</sup> (0.5 μg)  
 T4r<sup>-2</sup> (0.5 μg)  
 T6r<sup>+</sup> (0.6 μg)

Each ↑ 50 μl 88% HCO<sub>2</sub> 17°C 40 min,  
 lay down ↑ 25 μl NaClO tank 2.83 nfb,  
 2x 1.95 for P. (this final was all but fed).

	G <sub>250</sub>	A <sub>260</sub>	CX <sub>260</sub>	T <sub>26r</sub>	Y <sub>26r</sub>
B	1 2 2	.016 .016 .016	.015 .015 .015	.010 .012 .011	.089 .029 .034
	1	.181	.349	.108	.264 .024
	2	.183	.365	.112	.259 .025
2 - R	2 2 2 - R	.192 .166 .149	.357 .342 .272	.110 .099 .060	.262 .228 .183
	1	.161	.289	.071	.249 .023
	2	.166	.285	.071	.215 .022
T2r <sup>-1</sup>	2 2 2 - R	.164 .164 .149	.287 .287 .272	.071 .071 .060	.217 .217 .183
	1	.148	.276	.082	.209 .018
	2	.148	.276	.082	.201 .018
T4r <sup>-2</sup>	2 2 2 - R	.148 .148 .132	.276 .276 .261	.082 .082 .071	.205 .205 .171
	1	.148	.271	.071	.244 .022
	2	.148	.272	.074	.207 .022
T6r <sup>+</sup>	2 2 2 - R	.148 .148 .132	.272 .272 .257	.073 .073 .062	.211 .211 .177

	T4r-3	T4r <sup>r</sup> -2	T6r-4	T6r <sup>r</sup> -NaOH phage
A	1.94	33.6	2.44	34.1
T	2.06	35.7	2.45	34.4
G	1.027	17.8	1.336	18.7
CX	0.782	13.0	0.905	12.7
SMT9	100.9		7.131	20.0
P, Y	1.90	2.31	2.32	2.32
	1.93	2.31	2.30	2.30
Bromophenol blue	6.59		7.96	
R	87.8	89.5	85.8	81.6%

27-III-52	Plaque DNA's				
	$T4r-3$ 0.7 mg $T4r^r-2$ 0.6 mg $T6r-4$ 0.6 mg $T6r^r$ whole phage + NaOH				
	each + 50 µl 88% H <sub>2</sub> O <sub>2</sub> , 175° 40 min.				
	{ }				
	G	A	C <sub>250</sub>	T	Y <sub>165</sub>
B	1	.020	.016	.014	.013
	2	.020	.016	.014	.013
	2	.020	.016	.014	.013
T4r-3	1	.131	.265	.091	.092
	2	.134	.270	.094	.212
	2	.133	.268	.093	.209
	2-B	.113	.252	.079	.164
T4r <sup>r</sup> -2	1	.165	.331	.106	.241
	2	.167	.334	.112	.239
	2	.167	.333	.109	.240
	2-B	.147	.317	.095	.195
T6r-4	1	.143	.319	.104	.238
	2	.144	.315	.105	.242
	2	.144	.317	.105	.240
	2-B	.124	.301	.091	.195
T6r <sup>r</sup>	1	.435	1.07	.164	.163
NaOH	2	.433	1.04	.165	.163
	2	.445	1.055	.163	.163
	2-B	.425	1.039	.149	.138

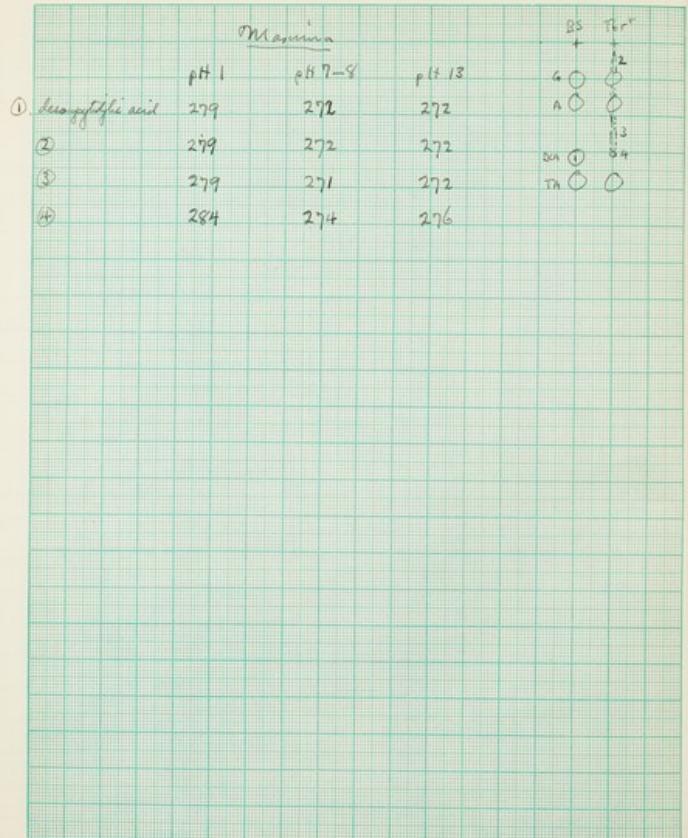
	T2r-2	T4r-3	T4r+2	T6r+
A	2.15° 33.8	3.295° 32.4	3.74° 33.3	3.675° 33.4
T	2.235° 35.0	3.72° 37.8	4.27° 37.6	4.04° 36.8
G	1.19° 18.7	1.78° 18.1	2.21° 19.4	2.055° 18.7
LX	0.80° 12.5°	1.05° 10.7°	1.16° 9.7°	1.22° 11.1°
	6.365° 100.0	9.845° 102.0	11.37° 100.0	10.99° 104.0
P.Y	2.11° { 2.13 2.15° { 2.13	3.21° { 3.24 3.27° { 3.24	3.85° { 3.84 3.76° { 3.84	3.57° { 3.49 3.46° { 3.49
mag. to base	7.31	11.11	13.06	11.96
R	87.1	88.4°	87.2	91.7

2-11-52

### Phage DNA's.

T<sub>2r-2</sub> 0.6 mg } each ↑ 50 µl 88% HCOOH 175° 40 min  
T<sub>4r-3</sub> 0.9 mg }  
T<sub>4r-2</sub> 0.7 mg }  
T<sub>6r+</sub> 0.7 mg }

	G	A	$\alpha_{250}$	T	$\gamma_{250}$
1	.017	.022	.015	.010	.016
2	.017	.020	.014	.007	.016
$\bar{x}$	.017	.021	.015	.011	
T2+2					
1	.146	.297	—	.229	.024
2	.150	.302	.299	.236	.024
$\bar{x}$	.148	.306		.239	
T2-3					
1	.181	.279	.084	.177	
2	.212	.46	.126	.346	.040
$\bar{x}$	.214	.452	.124	.348	.046
T4+3					
1	.213	.449	.125	.347	
2	.213	.449	.125	.347	
$\bar{x}$	.213	.449	.125	.347	
T2-3					
1	.196	.428	.110	.296	
2	.218	.471	.124	.393	.042
$\bar{x}$	.218	.471	.124	.393	
T4+2					
1	.261	.513	.120	.392	.036
2	.261	.513	.120	.392	
$\bar{x}$	.260	.512	.120	.391	
T-3					
1	.243	.491	.117	.340	
2	.243	.491	.117	.340	
$\bar{x}$	.243	.491	.117	.340	
T6+					
1	.243	.498	.142	.269	.040
2	.242	.498	.143	.274	.044
$\bar{x}$	.243	.498	.143	.272	
$\bar{x}$ -3					
1	.226	.477	.128	.321	
2	.226	.477	.128	.321	
$\bar{x}$	.226	.477	.128	.321	



8-11-52:

CX nucleotide

1.0 mg BSNA } cool to 0.25 ml ag. heat, add 0.05 ml  
1.1 mg T6<sup>r7</sup> DNA } 0.1% Drierite in 0.04 M NaSO<sub>4</sub>. Adjust pH

12 M.

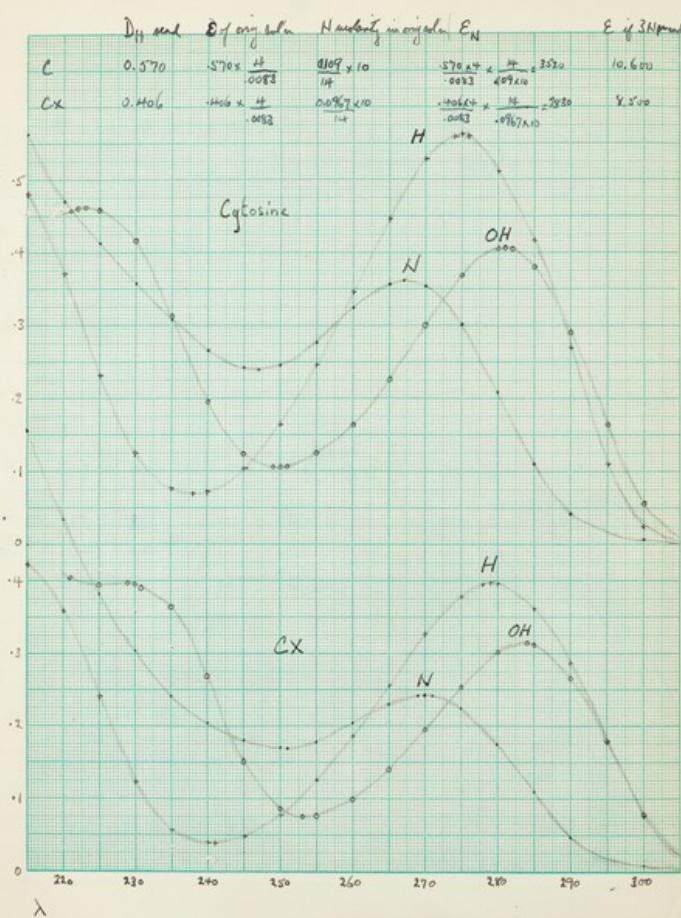
a few µl 0.1 N NaOH, & keep at 7-8 °C for initial addition NaOH.  
After 3 hrs., we further release of acid obtained. 3½ hrs., adjust to  
pH ≈ 8.6 & NaOH, add 0.1 ml Staphine (1:10,000) re-adjust to 8.5-9.0  
immediately at 27° 2 hrs. Then take base, adjust to 8.5-9.0 any base,  
keep at room temp. 5 hrs, then to fix overnight.

Env. to degrees. ↑ 20 µl N-HO, warm to 80° 2 min. Transfer to  
paper, rinsing 2-3 gels H<sub>2</sub>O. Run in 10% acryl. (DCG)

BSNA → 4 good spots = G, A, deoxyguanosine acid, thymidine acid  
T6<sup>r7</sup> → good G, A, & thymidine spots, v. weak DCA, & check both conjugates

	①	②	③					
7	1	13	7	1	13	7-2	1	13
265' 725'								
270 .729	.750		.140	.147	.133	.0587	.092	.088
271 .728	.746		.141		.134			
271' 713	1.085	.732	.139	.144	.131	.085	.101	.079
275 1.075								
276 + 279 1.09								
282 1.06								
285 1.01								

	④			
7	1	1	13	13
265				
270 .078			.069	.075
273 .081			.073	.079
274 .0815			.075	.079
275 .081	.0875		.075	.079
276 .075	.0785	.078	.066	.077
278 .075	.078	.078		
282 .075	.078	.078		
285 .077	.0865	.086		
286 .075	.075	.075		



7-11

C: N: 267.5 - 0.90 = .320  
 H: 267.5 - 0.90 = .298  
 CX UV Vtcs  
 CX prof (w, 15-iii) swpd to hydrogen, ↑ 0.1 and H<sub>2</sub>O.  
 Photon paper: 2 × 83 µl for UV }  
 { some in isopropt 91% →  
 | × 2.1 } for N } some in isopropt 91% →  
 | × 42.2 } some influence of lower RF.

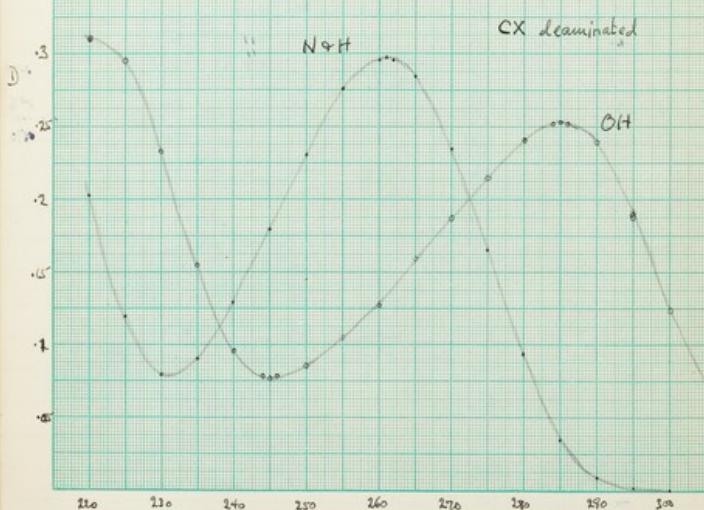
Identical controls to cytosine. Elute one in H<sub>2</sub>O, one in N<sub>2</sub>O/H<sub>2</sub>O. Read directly against blanks.

λ	pH 7.0			pH 1.0			pH 14.0		
	C	CX	E	C	CX	E	C	CX	E
310	0.02	0.07	0.03	0.08	0.03	0.08	0.06	0.08	0.06
304	0.07	0.08	0.24	0.76	0.24	0.76	0.26	0.78	0.26
295	0.18	0.20	0.10	0.79	0.10	0.79	1.67	1.79	1.67
290	0.41	0.47	0.20	2.97	0.20	2.97	2.91	2.66	2.91
285	1.10	1.09	0.67	3.62	1.2	3.61	3.12	2.14	3.12
280	2.09	1.74	1.22	3.96	4.11	3.96	1.82	4.45	2.02
275	2.75	2.24	2.16	3.47	4.11	3.47	2.13	4.08	2.14
270	3.00	2.24	2.13	3.83	4.18	3.83	2.69	4.46	2.64
269	2.54	2.72	2.29	3.27	3.00	3.27	2.00	3.48	1.95
267	2.69	2.41	2.41	3.00	3.27	3.00	1.87	3.69	1.87
265	1.61	2.30	0.47	2.86	2.26	2.86	1.39	3.90	1.39
260	3.24	2.03	2.46	1.86	1.64	1.86	0.99	2.21	0.99
255	2.77	1.77	2.46	1.26	1.24	1.26	2.52	0.77	2.52
251	2.45	1.69	1.64	0.78	1.06	0.78	2.51	0.67	0.67
250	2.45	1.69	1.64	0.78	1.06	0.78	2.49	0.65	0.65
247	2.40	1.80	1.04	0.49	1.23	1.04	1.52	1.52	1.52
245	2.42	1.80	1.04	0.49	1.23	1.04	1.52	1.52	1.52
241	2.66	2.44	0.73	0.40	1.93	0.40	2.69	2.69	2.69
240	2.66	2.44	0.70	0.40	1.93	0.40	2.67	2.67	2.67
238	3.48	2.41	0.77	0.57	3.13	0.57	2.21	1.98	1.98
230	3.57	3.03	1.23	1.23	4.07	1.23	2.29	2.98	2.98
225	4.13	3.82	2.22	2.41	4.59	2.22	2.21	4.60	2.21
220	4.70	4.84	3.71	3.59	4.59	3.71	4.04	4.04	4.04
215	5.63	6.02	4.60	4.24	4.24	4.60			

→ C: D<sub>275</sub> pH 1 = 5.67, 5.73 = 5.70

CX: D<sub>279</sub> pH 1 = 4.01, 4.11 = 4.06

	Maximum			Minimum		
	pH 1	pH 7	pH 13	pH 1	pH 7	pH 13
CX	279	270	284, 289	241	251	253
Lysine	275	267	281, 293	238	247	250
CX deaminated	261	261	285	231	231	245
Uracil	289	289	283	230	230	242



Grid.

### CX deamination

Residue of CX prep. ( $\approx 20 \mu\text{l}$ ) dried, + 35  $\mu\text{l}$  2M NaOH + 7  $\mu\text{l}$  glacial HAc, to 27° overnight.

Transfer whole to paper, drying on spot. Run in  $\text{Pb(OH)}_2 \cdot \text{NH}_3$ .  
→ single big spot,  $\approx$  RF slightly greater than CX. Dark in  $\text{H}_2\text{O}$ .

CX deam		U	
250	pH 7 .000	1 .002	38-14 45% loading in $\text{N}_2$ - heat again water
255	.002	0	7
260	.009	.005	1
265	.035	.029	13
270	.093	.084	.124
275	.166	.117	.192
280	.272	.241	.241
285	.235	.227	.315
290	.276	.239	.278
295	.294	.277	.302
300	.296	.277	.322
305	.296	.277	.420
310	.296	.277	.424
315	.296	.277	.424
320	.296	.277	.424
325	.296	.277	.424
330	.296	.277	.424
335	.296	.277	.424
340	.296	.277	.424
345	.296	.277	.424
350	.296	.277	.424
355	.296	.277	.424
360	.296	.277	.424
365	.296	.277	.424
370	.296	.277	.424
375	.296	.277	.424
380	.296	.277	.424
385	.296	.277	.424
390	.296	.277	.424
395	.296	.277	.424
400	.296	.277	.424
405	.296	.277	.424
410	.296	.277	.424
415	.296	.277	.424
420	.296	.277	.424
425	.296	.277	.424
430	.296	.277	.424
435	.296	.277	.424
440	.296	.277	.424
445	.296	.277	.424
450	.296	.277	.424
455	.296	.277	.424
460	.296	.277	.424
465	.296	.277	.424
470	.296	.277	.424
475	.296	.277	.424
480	.296	.277	.424
485	.296	.277	.424
490	.296	.277	.424
495	.296	.277	.424
500	.296	.277	.424
505	.296	.277	.424
510	.296	.277	.424
515	.296	.277	.424
520	.296	.277	.424
525	.296	.277	.424
530	.296	.277	.424
535	.296	.277	.424
540	.296	.277	.424
545	.296	.277	.424
550	.296	.277	.424
555	.296	.277	.424
560	.296	.277	.424
565	.296	.277	.424
570	.296	.277	.424
575	.296	.277	.424
580	.296	.277	.424
585	.296	.277	.424
590	.296	.277	.424
595	.296	.277	.424
600	.296	.277	.424
605	.296	.277	.424
610	.296	.277	.424
615	.296	.277	.424
620	.296	.277	.424
625	.296	.277	.424
630	.296	.277	.424
635	.296	.277	.424
640	.296	.277	.424
645	.296	.277	.424
650	.296	.277	.424
655	.296	.277	.424
660	.296	.277	.424
665	.296	.277	.424
670	.296	.277	.424
675	.296	.277	.424
680	.296	.277	.424
685	.296	.277	.424
690	.296	.277	.424
695	.296	.277	.424
700	.296	.277	.424
705	.296	.277	.424
710	.296	.277	.424
715	.296	.277	.424
720	.296	.277	.424
725	.296	.277	.424
730	.296	.277	.424
735	.296	.277	.424
740	.296	.277	.424
745	.296	.277	.424
750	.296	.277	.424
755	.296	.277	.424
760	.296	.277	.424
765	.296	.277	.424
770	.296	.277	.424
775	.296	.277	.424
780	.296	.277	.424
785	.296	.277	.424
790	.296	.277	.424
795	.296	.277	.424
800	.296	.277	.424
805	.296	.277	.424
810	.296	.277	.424
815	.296	.277	.424
820	.296	.277	.424
825	.296	.277	.424
830	.296	.277	.424
835	.296	.277	.424
840	.296	.277	.424
845	.296	.277	.424
850	.296	.277	.424
855	.296	.277	.424
860	.296	.277	.424
865	.296	.277	.424
870	.296	.277	.424
875	.296	.277	.424
880	.296	.277	.424
885	.296	.277	.424
890	.296	.277	.424
895	.296	.277	.424
900	.296	.277	.424
905	.296	.277	.424
910	.296	.277	.424
915	.296	.277	.424
920	.296	.277	.424
925	.296	.277	.424
930	.296	.277	.424
935	.296	.277	.424
940	.296	.277	.424
945	.296	.277	.424
950	.296	.277	.424
955	.296	.277	.424
960	.296	.277	.424
965	.296	.277	.424
970	.296	.277	.424
975	.296	.277	.424
980	.296	.277	.424
985	.296	.277	.424
990	.296	.277	.424
995	.296	.277	.424
1000	.296	.277	.424

30-IV-52

UV shift of CX in alkali.

Spots of CX (recryst) & C off neutralized naph.-HCl chromatogram  
↑ 4 ml of alk. along = blank. Add  $K_2HPO_4$  to 0.01 M = pH 8.85

i-81

	148	245 nm	1/2 hrs	24 hrs	44 hrs	1/2 days	8 days	(H <sub>2</sub> O)	1/2 days
265	.553	.562		.604	.659	.678			
262.5	.556	.564		.614	.654	.687			
260	.544	.549		.607	.656	.672			
257.5				.586	.529				
255									
252.5									
250									
247.5									
245									

C 290

265	.662	.663	.670	.669	.672
262.5	.669	.670	.671	.683	.686
260	.650	.651	.669	.671	.685
257.5				.640	.687
255				.599	.667
252.5					
250					
247.5					
245					

CX 290

265	.662	.663	.670	.669	.672
262.5	.669	.670	.671	.683	.686
260	.650	.651	.669	.671	.685
257.5				.640	.687
255				.599	.667
252.5					
250					
247.5					
245					

6-V-52.

## 5-071 wail & Barbium and

Made up 10 g/ml in water (+ 0.04 ml  $\frac{M}{10}$   $K_2HPO_4$ ),  $\frac{M}{10}$   $H_2O_2$ , +  $\frac{1}{2}$  NaOH.

12-1-62

Isolation of cytosine from DNA - test procedure from CX  
200 mg NBC DNA + 200 mg bovine serum albumen, in two tubes, each = 2 ml HCO<sub>3</sub><sup>-</sup> (4 ml total) cook 75° (180 on therm.)  
for 30 min. Open, allow to dry in oven. ↑ to 1 ml HCO<sub>3</sub><sup>-</sup>.  
Distribute as bandson 2 sheets What. #3: ~ 1 ml on sheet 1,  
~ 0.6 ml on sheet 2. (~ 125 mg DNA + 75 mg reagent).  
Run descending in isoprop.-HCl.  
~ 40 hrs.

W.V

	pH	7.8	I	13
6-Me ureid		261	261	276

12-V-52.

6-CH<sub>3</sub> ureid

Sample from Cohen 1.2 mg + 1 ml ag. dest. dil. 1:100 in  
 1% NaCl, 1% NaOH, + water + K<sub>2</sub>HPO<sub>4</sub> to .005 M.

	pH	~8	I	13	12 Friedlin	12 Krantz pH 13
305'		.002			.013	
300		.007			.078	
295'		.024	.002	.277		
290		.049	.009	.512		
285'		.102	.018	.660		
280		.256	.226	.710	.724	.1.216
				.715	.730	.282 / .290
				.716	.731	.282 / .290
275'		.529	.326	.74	.731	.1.216
270		.778	.386	.704	.79	.1.073
265'	265	.950	.950	1.02	.618	
	264	.949	.949	1.02		
260		1.00	1.00	1.07	.587	
255'		1.00				
250		.921	.995	.480		
245'		.777	.826	.371		
240		.638	.623	.281		
235'		.408	.424	.250		
230		.273	.285	.329		
225'		.214	.220	.371		
220		.138	.159	.485		
215'		.649	.688			
212		.751	.808			

$$\text{Coli DNA: } CX = \frac{0.0014}{1.24} \times 100 = 0.3 \text{ mole \% of C, or } \frac{0.3 \times 7.6}{100} = 0.023\% \text{ of Coli DNA.}$$

$$T_{6(r^*)}: C = \frac{0.0085}{10.5} \times 111 \times \frac{100}{145} \times \frac{20}{1000} = 0.004\% \text{ of phage, or } 0.01\% \text{ of phage DNA.}$$

$$\text{BSVA: } MC = \frac{2.04}{5 \times 743} \times \frac{1.05}{0.98} \times 100 = 5.9 \text{ mole \% of C. (See figure, 6.4)}$$

$$CX = \frac{0.0085}{5 \times 743} \times 100 = 0.23 \text{ mole \% of C, or } 0.014\% \text{ of BSVA.}$$

$$\begin{aligned} \text{Whole colo: } CX &= \frac{0.078}{10.2} \times \frac{1.05}{100} \times \frac{125}{33} (\text{allowing } \frac{4}{20} \text{ for } C) \\ &= \frac{0.078}{10.5} \times 125 \times \frac{4}{20} \times \frac{1.05}{100} = 0.0014 + 32\% = 0.0019\% \text{ of colo.} \end{aligned}$$

	Max. values for CX in DNA + poli, & C in T <sub>6(r*)</sub> .		
	Readings against blank cell correction; ambient 0.001 at 274.		
Old chromatograms	270	275	280
	273	278	280
MC	280	282	284
	270	275	276
line by CX			
80 mg whole colo	2.75	.022	.028
		.022	

Coli const  
DNA (NaOH)

45 mg T<sub>6(r\*)</sub>

Final  
190 mg whole colo

Kling BSVA

Whole colo "CX" fraction combined (80 + 190 mg) concentrated in vials,  
run in reading T<sub>6(r\*)</sub> along C trough. Could see nothing in  
C trough, but elute appropriate bands - read directly against blank.

CX marker	Coli CX position	band above CX	band below CX
270	.142	.071	.265 .022
275	.163	.076	.265 .022
278	.169	.077	.271 .024
280	.169 .170	.219 .077 .078	.280 .055
283	.164	.073	.275 .061

similar to original  
not CX.

16-V-52.

Big CX prep.

20.8 g. Blafler Bohne "T6 concentrate" + 1000 ml 0.15 M NaCl. Shake. Add 360 g. water, shake, warming, until dissolved.  $\rightarrow$  pH  $\approx$  6. Add 25 ml 0.2 M NaOH  $\rightarrow$  pH  $\approx$  8.5. Leave room temp overnight.

17-V-52.

Visionite has dropped off standing. Settling slowly on timer ( $\approx$  8??) until no more gel formed. Is opt. add  $\approx$  1 vol. 8% NaOH +  $\approx$  1/2 vol 8% NH<sub>4</sub>Cl  $\rightarrow$  pH 5. Ppt. forms, fine cloudy at first, then aggregating. Spin down  $\rightarrow$  white fluffy pellet. Yellowish alcohol opt = (C). Pellet  $\uparrow$  100 ml water + few drops 1% NaOH  $\rightarrow$  turbid somewhat viscous soln.

Aliquots for Beckman:

Dilute protein Alcohol opt  
Optical density (C) 1.10  
 $\text{Optical density } \textcircled{C} : 1.10$

Pellet (C) in  
10 ml 1:100

260	1.10	1.93
260	0.130	2.03
225	1.18	1.98
240	0.71	1.12
231	0.68	0.90
230	0.54	0.90

$$\begin{aligned}\text{Total dilution: } & 1.16 \times 0.04 \times 10 \times 2500 \\ & 0.12 \times 0.04 \times 10 \\ & \approx 1.16 \text{ g.} \quad 2.03 \times 0.04 \times 10 \times 100 \\ & = 810 \text{ mg.}\end{aligned}$$

To 100 ml in (C) in 100 ml add 7 ml H<sub>2</sub>SO<sub>4</sub>  $\rightarrow$  pH 6.6. Warm to  $\approx$  30°. Does not dissolve. Leave at 40° <sup>overnight</sup> (C). After 8 hrs, mostly dissolved, transfer to stopper jar, + dilute against 1.025 N NaOH (11.6) at ~~37°~~ 12°. Change to 2000 ml eq. dil. (C).

18-V.

Buchanan readings				
Stuff	Diluting HCl	Dilute water	"Aqueous acid"	Benzaldehyde
Solution	1:10	1:10	1:1000	1:1000
Total vol.	1070 ml	1800 ml	196 ml	10
270			.560	.552
270	1:10	0.164	271 .576 .589	.608
269	1:22	0.176	269 .589 .599	.612
262	1:24		268 .565	
260	1:24	0.176	.572	.580
259	1:24			
258	1:24			
255	1:24	0.171		.526
254	1:24			
250	1:12	0.182		.460
246				.428
Total 666	1.24 x 10 x 1000 x 0.002	0.176 x 10 x 1000 x 0.002	0.59 x 10 x 10 x 0.002	0.61 x 10 x 10 x 0.002
bars (mm) x 15 ml				
$\rightarrow D = 1.0$	= 190 mg	= 48 mg	= 170 mg	= 9.2 mg
1.260				= 41.9 mg
Barrels	①	②	③	
280	.371	.332		
276	.374			
275	.369	.363		
270	.334	.373	479	
266		.384	.580	
260	.332	.377	.520	
260	.354	.346	427	
240	.415			

19-V-52

Cx prep. cont'd.

Still some undissolved material in dilution v. Spin off ffl in 196 ml. Dl.  $\uparrow$  10 ml  $\frac{1}{2}$  NaOH + ③

APA soln. ③ neutralized  $\approx$  2 ml 0.2 M NaOH, then wrap on water bath to dryness  $\rightarrow$  brown tar.

20-V

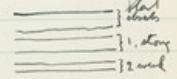
Take 2 ml NaOH, transfer to 2 open tubes, min = 1 meassn. Look 165° KBr  
Porous, del.  $\downarrow$  H<sub>2</sub>O ( $\rightarrow$  brown ppt) add activated charcoal, warm, filter  $\rightarrow$  brown soln. Evap.  $\downarrow$  to remove H<sub>2</sub>O,  $\uparrow$   $\approx$  75° and H<sub>2</sub>O, neutralize  $\downarrow$  add Ba(OH)<sub>2</sub>  $\rightarrow$  white ppt. ( $\text{Ba}_2(\text{PO}_4)_2$ ?). Filter.

Filter out brown, wrap. P.W.D. to  $\approx$  12 ml. Add  $\approx$  8 ml 96% formic acid.

No ffl. Evap. to  $\approx$  3 ml, spread on 2 sheets Whatman 3.  $\frac{1}{2}$  sheet

26-V

Labeled bands:

Elute little squares  $\uparrow$   $\frac{1}{2}$  H<sub>2</sub>O for tank

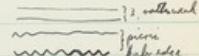
Elute bands.

Eluate of band ① del 1:2 : 40 ml H<sub>2</sub>O

275 1.88

279 1.88

283 1.80



$$\text{Total} = 1.88 \times 125 \times \frac{1}{2} \times \frac{1}{1000} \approx 2.4 \text{ mg}$$

		$\ell$ (BaP <sub>4</sub> off)	i dissolved	Dissolved water	8:6
		1:50	1:50	1:50	
240	2.14'		0.66	2.61	1.04 - .93 .40 -.17 .02
250	1.86	0.92	0.37	2.67	.79 .66 .31 .24 .03
260	.55'	0.61'	0.32	2.54	.62 .50 .26 .11 .02
270	1.18	0.49	0.27'	2.36	.47 .39 .20 .09 .03
275			0.50		
Rounds from chromatogram of R:		40 drops remaining until dryness "Off bottom" - "Used ppt portion" 1:25			
255'	.071		270	.091	.089
260	.080		265'	.098	
270	.080		260	<b>0.099</b>	.097
270'	.078		255'	.091'	
270	.069	.109	250	.087	.087
265'		.124	240	.066	
260		.125'	230	.061'	
255'		.118			
	X	A			
			<u>Total 164 (40 drops 0.10) x</u> <u>0.099 x 25 x 0.04 x 350 = 34 mg</u>		

19-V.

Cx ppt cont'd.

Alcohollic ppt (R) from 17-V methylequi (little NaOH), evap on BEWB to ~ 500 ml (so even just remains in soln). Add ~ 50 ml satd Ba(OH)<sub>2</sub> → milky ppt. Stand over night. Off top try to hydrolyze. Blurred ppt. still milky. Boil, let cool, & spin again → more ppt. Ppt washed once in water, by 240 1.96 → attempt to hydrolyze: blurred.

Ppt (R) 1:20 245' 2.17 Total NaOH ~  $2 \times 0.015 \times 20 \times 500 = 300$  mg.

Evap under vac., & try to distill off urea. Not successful. Solub residue ↑ water: does not dissolve. Stand several days. Filter; filtrate = (i). Wash residue: hot water. Washing = (j). (i) gets ppt. on standing couple of days. Dishes against 5 lots ag. lead. Urethane not yet complete, but enough to test. (i). 1:50

6-V.

$\alpha$  fib.

6-VI

Some protein filtered off from water-chloroform-alcohol mixture. Filter washed. Protein allowed to dry overnight, then + 100 ml 0.1 N-NaOH + gray turbid emulsion. Spin 9000 5 min.

Before spinning 1/50 After 9000 5 min 100%  
still turbid after base  
boiling 1/50

220	1.04	0.99	0.97
260	1.11	1.07	1.03
285	1.10	1.16	1.07
320	1.30	1.29	1.14
240	1.62		

21-VI

Precipitate of watered out filters by alcohol from over-night collected + dialyzed against running water, then 0.03 M HCl at 32°, clear water. Same = non-affected sol'n from Prof. Dr.

Dialysate (1)	Unadjusted but washed		Lysis:	
	water	water	Dry (2)	Turbid
HCl				
24 hours	1/2 hour	1/2 hour		
3 hours	2 hours	2 hours		
260	.475	.117	.066	.246
270	.610	.153	.0584	.291
265	.642	.147	.067	.287
260	.649	.160	.067	.281
255	.615	.154		
250	.849	.139	.076	.238
Total base	.649 - .015 = .634	.16 x .38 = .608	.99 x .045 x .576 = .79 x .95 x .320	.79 x .95 x .320
> 29 mg	26.7 mg		-2.35	+3.2
→ 45 mg Ca			5.5 → 2 mg Ca	

27-V

4-methyl-cystosine

From Duschek. Dissolved at arbitrary conc. in water, made up in 1/10 HCl, 1% NaOH, + water + PdC 11.7%.

pH 7.5	1	13
205	0.04	0.08
210		
225	0.74	0.52
240	0.14	1.24
255		
260	3.40	2.18
265	1.89	4.20
270	2.70	
275	2.74	
280	4.04	2.44
285	2.84	2.31
290	2.77	
295	2.62	
300	1.49	
305	1.71	
310	1.54	0.96
315	1.54	0.96
320	1.45	0.92
325	1.45	0.92
330	0.69	1.07
340	1.67	0.46
345	1.67	0.46
350	2.61	2.78
355		
360	3.07	2.36
365	3.40	2.18 3.25
370	3.70	3.51

	T2r <sup>+</sup> -5	T6r-6	T6r <sup>+</sup> -2
A	5.17 33.6 33.3	4.31 33.5 33.2	4.01 33.6 33.3
T	5.48 35.6 35.2	5.00 38.9 38.4	4.10 34.4 34.0
G	2.78 18.1 17.9	1.94 15.1 14.9	2.15 18.0 17.8
CX	(45.94) 2.19 1.96 13.7 13.7	1.76 12.6 13.5 13.01 12.06	1.80 13.9 14.9
	(15.56) 15.39 100.0 100.1	12.87 100.0 100.0	14.92 100.0
P	5.00 { 4.99 4.97 } 4.56 { 4.56 4.56 }	3.94 { 3.94 3.81 }	
Concn	17.1	15.6	12.96
R	94.0	89.4	93.0

28-V Whole flages in HCOOH to check G:CX ratios.

T2r<sup>+</sup>-5 (new fresh) } 1.5 my each ↑ 0.15 ml HCOOH, cook  
T6r-6 ( ~ ) } 175° (stirred at 175°) - 30 min.  
T6r<sup>+</sup>-2 (old ) }

	G	A	CX <sub>200</sub>	T
B	1 .029	.024	.019	.089
	2 .037	.025	.017	.046
	3 .033	.025	.018	.051

T2r <sup>+</sup> -5	1 .336	.689	.223	.486
	2 .342	.704	.225	.487
	3 .339	.697	.224	.487
	2-3 .306	.672	.206	.436

T6r-6	1 .226	.560	.180	.432
	2 .236	.609	.196	.466
	3 .246	.585	.188	.449
	2-3 .213	.560	.170	.398

T6r <sup>+</sup> -2	1 .266	.542	.191	.378
	2 .274	.550	.193	.376
	3 .270	.546	.192	.377
	2-3 .237	.521	.174	.326

Radiation readings on dialysate undiluted.

	(1)	(2)	(3)	(4)	(5) Centrifug at 10,000 rev/min
Shatov	0.025 M HCl	0.025 M HCl	0.025 M HCl	H <sub>2</sub> O	
Shatov	100 ml	75 ml	75 ml	75 ml	80 ml
Dialysis time	5 hrs	18 hrs	22 hrs	5 hrs	10 hrs
D. 215	.069	.290	.125	.010	.940
210	.084	.371	.154	.010	1.406
265	.094	.416	.198	.011	1.298
260	.096	.426	.193	.010	1.189
265	.092	.427	.192	.009	.970
250	.088	.388	.129	.009	.712
Total hours					
of 4 (first 2 = 10)	140 Y	470 Y	180 Y	12 Y	195 Y

28.V

Reparation of whole phage.

10 mg T6+6 ↑ 5 ml ag. sol., pour into dialysis sac, add in 100 ml 0.025 N-HCl (pH 1.6), incubate at 37°, 3 changes HCl for total 45 hrs. Then remove from sac, washing out 5 ml ag. sol., & spin. Pellet dry. Transfer to lyophilic tube, add 0.2 ml H<sub>2</sub>O, rock 15° (not 10) 30 min.

Chromatograms got phenol.

Rhoad's data on Shatov-Dobrin filtrate.

	% P	% DNA	repeat DNA
13-1-52	Prof(1) "T6 concentrate"	1.92	19.1
	Prof(2) "T6 <sup>c</sup> "	2.05	17.0

Dialyses			Büchner bars ("APM")						
①	②	③	①	④	⑤	⑥	⑦	⑧	
0.025 M HCl	HCl	water	full 24 hr	additional water	water	water	water	water	
2000 ml	2000 ml	x 250 ml	not in	25° and	25° and	25° and	25° and	25° and	
7 hrs.	16 hrs.	4 hrs.	20 ml	25° and	25° and	25° and	25° and	25° and	
1:5	1:5	1:5	1:25	1:25	1:1	1:1	1:1	1:1	
275	0.069	.37	.185	1.08	0.52	.48	1.37	1.02	
220		.49		1.23	0.60	.60	1.21	1.06	
265	0.100	.55	.184	1.29	0.62	.69	1.33	1.05	
260	-1.02	.56	.187	1.21	0.60	.69	1.16	0.98	
235	-1.03	.56	.187	1.11	0.54	.69	1.06	0.90	
230	-0.92	.51	.144	1.01	0.52	.635	0.99	0.71	
Total HCl losses	0.425 x 2000	-0.425 x 2000	-0.17 x 25 x 250	1.27 x 25 x 250	-62 x 25 x 250	69 x 250	12 x 250	11-11	
of 0.25 M NaOH	x 250 ml	x 250 ml	x 250 ml	x 250 ml	x 250 ml	x 250 ml	x 250 ml		
	= 15 mg	= 84 mg	= 29 mg	= 157	2.57	= 91	= 225		
Protein	15 + 84 + 29 + 21 = 159								
Proteinure									

10-VI

## Second Cr prep.

Shaffer Robbins Mead batch: 7.0 g ↑ 300 ml 0.15 M NaOH  
 0.01 M Na citrate. Add 108 g urea. Dissolve, leave at room  
 temp. 2 hrs. Warming blend, swirling. Forgot to adjust pH  
 (=6); add ~1 ml NaOH → pH ~8.5. Swirl 3x (incomplete; still  
 → gel). Re-emulsify gel in 100 ml 36% urea, spin, to further remove  
 aqueous phase. Dialyze overnight & running water.

Dialyze against 0.025 N-HCl at 35°, as on off page.

Evap. until vol. (b.p. ~30°) to total vol. 250 ml, & dialyze overnight  
 against water.

Evap until urea, to degrees. Dissolve in 20 ml 88% HCOOH, cool under ice  
 to ~3 ml, add ~3 ml. Pipette into 4 tubes, seal, heat 165° 15 min.,  
 spin on 8 sheets Whatman #3, run 4 ml in 10% H<sub>2</sub>-NH<sub>3</sub> (without NH<sub>3</sub> for weight),  
 then weigh. HCl. Also filter paper = 10 ml in centrifuge. HCl.  
 Filter paper: not well resolved, but clear spot in 4 ml:

	A	B	C	T
243	2.43	2.28*	2.65	2.66
260	0.713	2.9	.930	.918
275			2.52	2.58
			2.57	2.65 - 1.88

Total Cr =  $\frac{0.93}{1.00} \times \frac{4}{125} \times \frac{6.90}{10} = 27 \text{ mg.}$

11-11

Cr elutes from what HCl paper:

Elutes ①  
 Elutes ②  
 17 ml ex 4 steps - 1 ml

1:100  
 2.83 2.46 0.13  
 2.79 2.64 0.14  
 2.75 2.41 0.13

Total:  $\frac{1.6}{17} \times \frac{125}{100} \times \frac{4}{125} = 0.13$

21 mg.

Eluted Cr concentrated in urea, off

to 2 sheets Whatman #3, run in 10% H<sub>2</sub>-NH<sub>3</sub>

4.45 ml

el. 4.45 ml

From 0.1 M HCl paper run 4 steps:

Elutes sample

A B C

2.63 .210 .266

2.59 .217 .272

2.75 .204 .264

.73 .264 .264

.60 .073 .362

.55 .084 .337

2.50 .492 .492

.45 .45 .45

fftr washing

(1) fftr	(2) washing	(3) fftr + water
2.24 ml	2.24 ml	~1 ml
2.61 ml	~1 ml	~1 ml
283 .253	.021	
278 .262	.023	.009
275 .242	.023	
262 .131		.011
240 .048	.012	.013
Total $\frac{.262 \times 125 \times 4}{10.5} = 12.45$		
$\frac{.048 \times 125}{10.5} = 0.92$		
		$\frac{4 \times 3}{1000} = 0.012$

(9-V.

Prom-NH<sub>2</sub> paper bands "A" eluted.

First 14 ml (+ stripe) Second 15 ml

$\frac{1.44}{1.44}$

283 1.57 1.59  
279 1.63 0.194  
275 1.60 0.214

270 .220  
266 .199

$$\text{Total Cx} = \frac{1.6}{10.5} \times 125 \times \frac{4}{1000} \times \frac{14}{0.1} = 0.7 \text{ mg.}$$

25-VI

Prom elute wash in was. to dryness, add fftr elute, lyogarn, dry overnight in desiccator. Dissolve in ~ 0.2 ml H<sub>2</sub>O. Very acid. Neutralize: 0.2 N-KOH (tubes ~ 1 ml) → brown flocculent fpt. Spin down, wash fpt, spin down.

10-VII

After several m - fftr, solid material still dry, brown.

"Cx" + 0.75 ml 1:500 fftr, agitate 1:300

280 0.97 1.06
279 0.97 1.06
275 0.93 1.02
240 0.104 1.43

Total Cx

$$\frac{0.97 \times 125 \times 0.75 \times 500}{10.5} = 6.9 \text{ mg.}$$

$$\frac{1.06 \times 125 \times 0.9 \times 500}{10.5} = 9.1 \text{ mg.}$$

Total = 16 mg.

Dilution analysis on  $T_{65^{\circ}}$  DNA.

5.9 mg DNA, laid overnight in vials over silica gel at  $25^{\circ}$  and water = 236  $\mu$ l vol

Substance	Sample size	$\gamma$ found	$\gamma/\mu\text{d}$	% of weight
DNA (deoxy DNA standard)	0.5	90.6 87.5 88.6 81.8 79.4	179 161.6	75.9 68.5
P	0.2	3.09 3.12 3.14	10.56	6.61
N	2.0	54.4 54.3 54.2	27.2	11.5

$$N/P = 1.74$$

#### Dilution:

	(1) HCl	(2) HCl	(3) water	(4) water	(5) water
900 ml	1000 $\mu\text{l}$	1300 ml	50 ml		
$25^{\circ}$ 16 hrs	32 $^{\circ}$ 6 hrs	20 $^{\circ}$ 16 hrs.	18 $^{\circ}$ 4 hrs	14 $^{\circ}$ HCl 2 hrs	absolute and water
1:4	1:4	1:4	1:1	1:1	
250	-528				2.6
270	-932				3.?
265 <sup>1.07</sup>					2.96
26.0	2.02	-1.64	.096	2.82	2.74
25.5	1.10			2.44	2.41
250	1.01	2.02	-1.55	0.92	
					Total dilution
					$3.0 \times 0.015 \times 500$
					$= 22.5$
					(approximate)

16-VI-52.

#### Third prep.

4.5 g. Blaupur & Dolive prep. (2)  $\rightarrow$  250 ml 0.15 M NaCl 0.01 M Na acetate. Add 90 g. silica, dissolve, add  $\sim 4$  ml 0.2 N NaOH  $\rightarrow$  pH 8.5.

Emulsify, leave 3 hrs., emulsify in living min. (this night), spin.

Living 3x  $\rightarrow$  1. little gel. Gel +  $\sim$  150 ml 36% sucrose + living 3x.

First aqueous phase second extract from

265	0.885	1.140
260	.94	1.155
255	.92	1.149

Add few drops HCl to pH  $\sim$  5. Pour both extracts into 1000 ml 95% EtOH. Leave overnight at  $4^{\circ}\text{C}$ . Spin down ppt.

17-VI

Absolute water ppt.

265	6.67	1.1
260	.70	
255	.682	
252 <sup>1.07</sup>	.684	
250	.596	
255	.594	

Total NA =  $0.70 \times 0.04 \times 1200 = 34$  mg. Recover by concentrating in water + bridging away the sucrose.

DNA ppt. water (tunis = 70% EtOH, over water 95% + absolute). Dry overnight over silica gel in water. Weigh: 66.5 mg.

19-VI.

500 mg  $T_{65^{\circ}}$  DNA  $\uparrow$  50 ml water  $\rightarrow$  slightly opalescent solution. Dilute in mechanical stirring agamer 1000 ml 0.03 M HCl at  $88.9^{\circ}\text{F}$  ( $32^{\circ}\text{C}$ ). 4 hr.

20-VI

10 cm Change HCl. 4.50 pm. change to water to room temp.

26-VI

After all changes, waf. dilute in wa. to 5 ml, freeze dry.

3-VII

Dry wt = 235 mg.

220 mg  $\uparrow$  1.5 ml 88% (EtOH), heat  $165^{\circ}$  20 min. Spread on one sheet

What's 3, over in isoprop. HCl  $\rightarrow$  Port G (weak), G (weak) A (mod strong),

CX (strong), - T (v. strong). Elute Port G + CX.

Last changes water (S) also weak, hydrolyzed, room. Similar hydrolyzed bands (over)

Run T6<sup>r</sup> DNA

A	7.06	33.5	$P = \frac{6.59}{6.62} / 6.59 = 22.6$ nucleotides to bases
T	7.68	36.4	
G	3.72	17.6	
CX	2.65	12.55	
	24.11		

CX pref (cont'd) from earlier HPLC prep.

CX dilution 1:200 H2O

280 .452  
278 .462  
275 .481

$$\text{Total CX} = \frac{0.46 \times 125 \times 10}{10.5} = 11 \text{ mg}$$

Concentrate apply to paper for Pontos. Concentrate some also collected remnants.

Elute with EtOH + water acetone.

CX pref CX dilution 1:200 2% H2O dilution 1:100

260 .500

275 .586

278 .588

275 .494

278 .494

$$Cx = \frac{0.46 \times 200 \times 12.5 \times 10}{10.5} = 265 - 118$$

$$\frac{10.5}{10} \times \frac{12.5}{10} \times (-1.2) = 32$$

10-VII Combine all CX from 2% H2O should be 27 mg in ~2 ml. Spin off over

methanol. Add 60 mg fine acid dissolved in 1 ml hot water →

HPLC eluent (EtOH + water). Spin down HPLC.

Add 3 ml water warm to make partially re-dissolve HPLC. Leave in free-surface layer. Spin down HPLC.

Dissolve HPLC in 5 ml 0.2 M Na2CO3 by morning. Return to 3-5 ml portion. Dissolve

in EtOH. Spin down HPLC (x 4 x 4 ml). Still a little brown. Recently

spin down HPLC. Spin down HPLC. Spin down HPLC more almost clear

solutes. Leave in frig over weekend.

11-VII A few big stalks in frig!! Concentrate to ~ 0.5 ml, stand 3 hrs in frig after

Wt > 0.4 ml H2O : warning (soln slightly turbid). Leave in frig overnight.

18-VI

Demonstration chromatogram

- ① BSNA 1.0 mg + 10 µl 70% HCOOH 100° 1 hr. Add 15 µl H2O.
- ② T6<sup>r</sup> DNA (even) 1.0 mg " "
- ③ T6<sup>r</sup> DNA (even) 1.0 mg + 50 µl 88% HCOOH 170° 30 min. Dig. + 25 µl H2O.

20-VI.

Load pts. ① → no CX, but pre-G

② → CX, some pre-G & Y (pre-V).

Cut and relate ②.

	G	A	CX <sub>278</sub>	T	Pre-G	Y
①	.020	.018	.015	.017		
2	.021	.021	.016	.048		
3	.021	.020	.015	.050		
T6 <sup>r</sup> DNA	.480	.935	.294	.660		
(Blurred)	2.30	.768	.238	.540		
2	.351	.939	.291	.660		
2	.430	.937	.293	.660		
2-B	.409	.919	.278	.610		

15-VII

Spin down HPLC. Dig. Transfer to small tube, dry overnight. Use while N/10 = 21.5 mg.

Residue in big tube + 0.25 ml 1/10 H2O. 2 x 0.05 ml for HPLC (1-3% EtOH solution)

2 x 0.016 ml and 1/10 of food. N/10 = 0.016 mg

2 x 0.016 ml and for chromatograms. N/10 = 0.016 mg

0.016 ml + 4.0 ml 1/10 H2O. N/10 = 0.016 mg

2.30 .896 .899

2.79 .897 .899

2.78 .897 .896

D<sub>278</sub> = 8.4

N/10 of my solution = 0.899 × 4.0 = 21.5

0.016

2.42 .197 .199

2.41 .197 .198

2.40 .198 .199

2.39 .192 .193

E<sub>N</sub> = 21.5 ×  $\frac{1.41}{0.016}$  = 337.0

0.016

$\Sigma$  of 3 N/10 = 10.100. (over)

4-VII-52

Purification through precipitate: test & cytosine.

20 mg cytosine + 2 ml warm water. Add 2 ml sat'd picric acid  $\rightarrow$  big fpt. Leave overnight in frig.

7-VII

Spin down. Discard off top. Add 5 ml of. 6M + 0.1 ml 9N-H<sub>2</sub>SO<sub>4</sub>. Dissolve on warming. Extract picric acid in 4-3 ml portions toluene. Then 2-3 ml toluene ether. Neutralize = Ba(OH)<sub>2</sub>.

8-VII

Evap. aqueous soln to ~ spin down BaSO<sub>4</sub>. Evap. off. to ~ 2 ml. Is yellow (got some picric acid from rubber stopper). Add 0.2 ml 0.4M H<sub>2</sub>SO<sub>4</sub>, re-extract in toluene + ether.  $\alpha$ -neutralize,  $\alpha$ -spin. Evap to ~ 0.5 ml. Stand in frig.

Cx prep. cont'd.

16-VII 2.5 mg 2x dialyzed Ktols + 0.5 ml H<sub>2</sub>O, warm to dissolve. pH  $\approx$  6.5. Add  $\approx$  5  $\mu$ l cold sat'd Ba(OH)<sub>2</sub> ( $\approx$  0.3 mg Ba(OH)<sub>2</sub>)  $\rightarrow$  pH  $\approx$  7.5.

Bubble thru ~~the~~ exhaled air. Spin, keeping heat ( $\approx$  80°), filter off from small bit residue in hot pipette. Leave in frig overnight.

17-VII

Spin down off liquor, dry Ktols. = 15.4 mg. 3x dialyzed.

22-VII

Recovery of old fractions.

Old fractions phage DNA hydrolyzed, run in acryl. -96%, then banding NH<sub>3</sub> on washed paper. Elute  $\uparrow$  13 ml

in acryl.	2.8	.204	.765	Total C <sub>6</sub> = $\frac{.40}{10.0} \times 155 \times \frac{13}{100} \times 50 = 32.5$ mg.
1.50	2.79	.204	.765	
			.402	
2.75	.204			
3.1	.204 (?)			
2.70	.204			
2.65	.202			

$\epsilon_N$ :

$$N = \frac{0.0468 \times 4}{0.8} \times 100 = 23.4$$

pH 1. Dif. eng. soln. =  $0.629 \times 5.3 = 11.11$

N formal = 0.0468 mg/ml  $\epsilon_N = 11.11 \times \frac{14.01}{0.0468} = 3,330$

of 3 N formal,  $\epsilon = 9,990$

$$\epsilon_{241} = \frac{0.990}{0.629} \times 10,000 = 1220$$

pH 7.4  $\epsilon_{269} = \frac{3.72}{0.629} \times 10,000 = 5,920$

$$\epsilon_{281} = 4160$$

pH 13.  $\epsilon_{283} = \frac{4.87}{0.629} \times 10,000 = 7,750$   $\epsilon_{284} = 1940$

Marc

Acetin

	mpm	$\epsilon$		mpm	$\epsilon$
pH 7.4	269.5	5,920		251	4160
pH 1	279.5	9,990		241	1220
pH 13	283.5	7,750		254	1940

17-VII-52.

Cx UV Ktris.

3x stabilized Cx 0.8 mg ↑ 4 ml ag. sol.

2x 1.0 ml for N; for UV, 0.3 ml factors ↑ 5.3 ml, by adding 5.0 ml solvent.

pH 1, % NaP		pH 7.4, 0.01 M PbI buffer		pH 13, 0.1 N-NaOH	
(1)	(2)	310	318	310	318
310	0.92 0.06	0.01	0.42	0.10	0.16
301	0.72 0.04	.003	0.45	0.27	0.58
300	1.81 1.20	0.05	2.08	1.22	1.90
296	4.37 2.84	0.9	2.29	2.78	4.33
290	6.92 4.50	0.62	0.97	4.14	6.06
285	8.32 5.75	1.49	2.44	6.63	9.27
280	9.60 6.28	.626	2.64	4.05	7.52
279	9.50 6.20	2.27	3.70	4.92	7.30
278	9.47 6.27	2.24	3.43	5.26	7.30
275	9.30 6.00	2.71	2.76	5.66	7.30
270	8.02 5.21	2.70	2.72	5.71	7.06
269	7.92 5.20	2.69	2.70	5.66	7.06
268	7.89 4.12	2.68	2.66	5.95	7.41
265	6.39 4.12	2.64	4.32	1.57	2.04
260	4.66 3.03	2.74	4.21	1.24	1.93
255	3.17 2.06	2.51	3.62	4.02	5.23
250	2.66 1.34	2.62	3.02	1.41	2.26
245	1.27 0.89	2.80	4.30	2.47	3.85
242	1.20 0.78	2.76	4.96	4.32	6.74
241	1.18 0.78	0.06	2.22	4.96	6.74
240	1.11 0.79				
235	1.94 1.13	3.76	5.77	5.60	9.26
230	3.49 2.27	4.68	7.18	2.31	6.23
225	6.51 4.23	5.66	9.00	6.58	9.26
220	9.44 6.27	7.30	11.20	6.46	10.20
215	11.55 7.81	8.95	13.72		

$\frac{D_{229}}{D_{241}} = 9.2$

18.VII.52 Melting points:

5-methyl-uracil (Doherty)  
Darkens 270-280. Melts (brown) 289° (Aleur of 2 thymine  
(2° different, however.)  
(reverse: melts 270°).

Barbitone acid (M.R.C.)  
Effervesces at 250. Does not liquify by 265. (Should be 261 d.)

L-aspartic acid - melting up to 293

Cx - Put into bath at 240. Melts immediately; Turns foaming  
orange. Then reddish brown up to 280. Does not melt by 320.

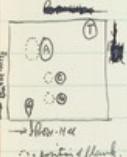
	A <sub>260</sub>			C <sub>218</sub>			C <sub>X209</sub>				
	Blank	Spot	A.	B.	S.	A.	B.	S.	A.	B.	S.
a 1	37	661	624				23	293	270		
2											
b 1	50	1093	1043	1038	035	672	637	636	31	389	358
2	59	1092	1033		039	674	635		37	389	352
c 1	48	1060	1012	1019	032	660	628	630	30	358	328
2	52	1077	1025		023	664	631		31	362	331
d 1	41	102	1061	1044	029	670	641	647	29	350	321
2	46	1071	1026		030	683	653		31	356	325
e 1	42	1053	1089	1013	020	646	616	611	26	234	209
2	42	1055	1013		025	634	606		22	226	204
P for 1.95 μl	Yield/conc.	Inches (x2.43)	A	C	Cx (E=0.01)	P					
a	5.33	5.39	18.48	4.80	0.260		2.70	0.146			
b	9.24	9.24	31.65	7.98	.252	6.06	0.91	3.56	.112	77	
c	9.05	9.11	31.22	7.84	.251	6.00	.92	3.30	.106	73	
d	9.52	9.51	32.60	8.04	.247	6.16	.89	3.23	.099	69	
e	9.16	9.12	31.27	7.79	.249	5.82	.186	2.07	.066	45	

23-M-52.

## Destruction of Cx in hydrolysis.

0.6 mg crystals Cx } ↑ 0.8 ml 88% HCOOH.  
102 mg GSNA }↓ into each of 5 tubes, 0.15 ml. | a. 100° 2 min. Control  
heat as follows: | b. 165° 30 min  
c. 170° 30 min  
d. 175° 30 min  
e. + 2.0 mg Stannous aluminum. 175° 20 min.

brief to dryness; partial vol., heating heat. Control (a) lost, so we heat 205 ml of mixture, ↑ 15°. Others, ↑ 30 ml N-HCl. 2 x 8 gel spots, 2 x 1.95 for P. Chromatograms paper 17" agree. Spot 3" from edge. Run further in 90% EtOH, long, run in 10% H-NH<sub>2</sub>. Point: good separation. Cx and control A, C, & Cx, along: blank forced from each paper.



○: point of blank.

a  
b  
c  
d  
e

31-VII-52

Repeat estimation of OC in hydrolysate.

0.4 mg stols "Cx" }  
6.0 mg BSNA }  
0.15 ml fatone sealed in tubes treated:

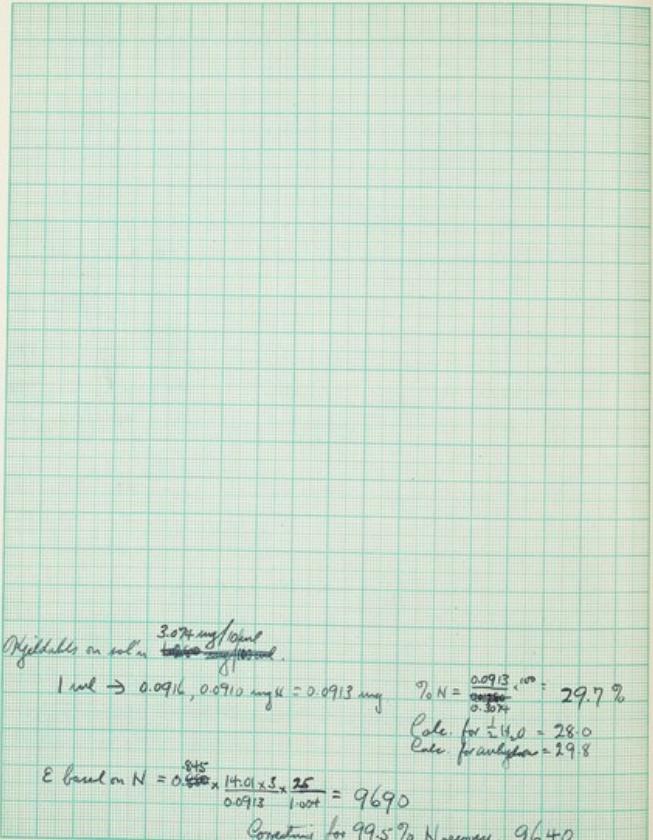
(a)  $100^\circ$  2 min.  
(b)  $175^\circ$  30 min.

(c) + 1.8 mg bromosulfophenyl  $175^\circ$  30 min.

Bry donor, avoiding heat,  $\uparrow$  30 µl N-MeI, take 2  $\times$  8.2 mlots, 2  $\times$  195 for P.  
Run 2 dimensionally as before. 24 hrs in P-114, 20 hrs in PdH-NH<sub>2</sub>.

	A <sub>160</sub> B, S, Δ, Δ <sub>x</sub>	C <sub>275</sub> B, S, Δ, Δ <sub>x</sub>	OC <sub>279</sub> B, S, Δ, Δ <sub>x</sub>
a 1	49 1020 971 } 984		[51] 529 474 } 501
2	42 1040 998 }		25 542 507 }
b 1	46 1030 984 } 973	21 611 580 } 593	34 420 386 } 392
2	49 1030 1001 }	24 640 606 }	31 429 398 }
c 1	56 1070 1016 } 1024	34 649 615 } 619	33 350 317 } 318
2	89 1120 1031 }	36 659 623 }	36 354 318 }

	A PdH, 95% 86 } 8.65	C PdH, 95% 86 } 8.65	OC (Ex 960) 57.20 0.175 [1.02]
a	0.256	0.256	
b	0.252	5.65 0.186	4.07 0.184 7.66
c	0.258	5.90 0.193	3.30 0.108 61



12-11-52

Extinction coefficients of Synthetic OC

3.074 mg synth. OC, dried in vac over silica gel, weighed;  
weigh balance onto small cup. Add 10 ml  $\text{H}_2\text{O}$ , heat, dissolve.  
Dilute 1:0 ml portions to 25 ml.

$$\text{M for } \frac{1}{2} \text{ H}_2\text{O} = 150.3$$

% HCl	0.01 M $\text{P}_4\text{O}_{10}$ buffer	% NaOH
281 .840	271 .495	285 .658
280 .845	270 .497	284 .661
279 .844	269 .497	283 .661
278 .840	268 .494	282 .658
243 .109	232 .355	255 .658
242 .107	251 .354	254 .658
241 .107	251 .354	253 .66
240 .109	250 .355	

	Amax	E	Amin	E
pH 1	279.5	$845 \times \frac{141.3}{3.074} \times \frac{25}{1.001} = 9700$ Assume $= 10,290 - 9,680$	241.5	1300 1230
pH 7	269.5	6060 5.710	251	4310 4060
pH 13	283.5	8080 7590	254	2010 1890

Fresh soln, 1.280 mg  $\uparrow$  100 ml % HCl.

$$260 .879 \quad E = 0.880 \times \frac{141.3}{1.280} \times \frac{25}{1.001} = 10,330 - 9,720$$

$$279 .880$$

$$242 .107 \quad E = 1260 \quad \frac{C_{\text{max}}}{C_{\text{min}}} = 8.21$$

$$241 .107$$

	TNA		$T6r^+ DNA$		$T6r^+ APA$	
	Yields	Ratio	Yields	Ratio	(Molar)	(Molar) and P.
A	3.89	28.2	3.88	32.8	31.6	0.91
T	3.85	27.9	4.08	33.9	33.2	5.14
G	3.08	22.8	2.06	17.1	16.8	0.59
C	2.82	20.5	2.00	16.6	16.3	2.67
OC	0.143	1.06	—	—	—	16.8
	13.78		12.02	99.9	97.9	
P	4.24	4.24	3.68	3.68	4.67	4.63
Conc & %	14.5%		12.28		15.89	
R	94.6%		97.9%			

18-VII-52.

#### Misc. Analyses

DNA's new Thymus DNA 0.8 mg

Clear  $T6r^+$  DNA 0.8 mg each  $\pm 0.15$  and 88% T6CFA, work

Clear  $T6r^+$  APA 0.7 mg  $17k^2$  30 min. Dry down,  $\uparrow$

25 pl H2O, take 2mL spots,  $2 \times 1.95$  for P.

Elute in 4 ml H2O.

	G	A	$272^{\circ}C$	$299^{\circ}C$	T	$260^{\circ}C$	$282^{\circ}C$	$297^{\circ}C$
B	1	.017	.017	.017	.016	.060	.018	.017
	2	.019	.018	.019	.016	.070	.018	.018
	2	.018	.018	.018	.016	.065	.018	.017
TNA	1	.356	.524	.312	.202	.360	.021	.021
	2	.354	.524	.312	.200	.382	.022	.022
	2	.356	.524	.314	.209	.371	.022	.022
	2 - B	.338	.506	.296	.193	.306	.014	.014
$T6r^+ DNA$	1	.246	.321	.202	.209	.374		
	2	.242	.525	.204	.209	.400		
	2	.245	.523	.203	.209	.389		
	2 - B	.227	.505	.193	.193	.324		
$T6r^+ APA$	1	.085	.129	.274	.274	.459		
	2	.081	.132	.273	.273	.486		
	2	.083	.130	.274	.274	.473		
	2 - B	.065	.118	.258	.258	.408		

	T2NA(a)		T2NA(b)		T2r+S phage		T6r-6 phage	
	Yield	Ratio	Yield	Ratio	Yield	Ratio	Yield	Ratio
A	2.52	33.4	1.975	32.0	5.24	32.9	3.07	32.8
T	2.59	34.3	1.95	32.5	5.62	35.3	3.53	37.9
G	1.345	17.8	1.10	18.4	2.755	17.3	1.40	14.9
OC	1.10	14.6	0.974	16.2	2.31	14.5	1.36	14.5
	7.555	10.1	5.999	10.1	15.925	10.0	9.38	10.1
P.Y	2.447	2.46	1.78	1.75	4.527	4.54	3.05	3.02
	2.48		1.72		4.86		3.00	
Growth	8.44		6.00		15.60		10.40	
R =	89.6%		100%		[102%]		90.1%	

19-VIII

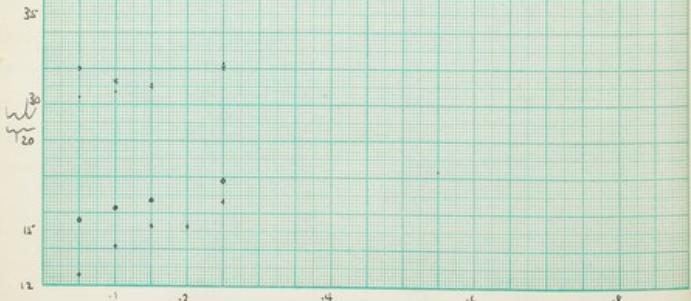
## Phage re-checks

- (a) T2r-2 DNA 0.6 mg ↑ 0.05 ml HCOOH  
 (b) " " 0.6 mg ↑ 0.15 ml HCOOH 17.5° 30 min.  
 T2r+S phage 1.5 mg ↑ 0.15 ml HCOOH  
 T6r-6 phage 1.5 mg ↑ 0.15 ml HCOOH

	G	A	OC <sub>279</sub>	T
B	1 .017	.014	.012	.047
	2 .017	.016	.014	.049
	2̄ .017	.015	.013	.048
T2NA(a)	1 .164	.342	.118	.280
	2 .166	.344	.120	.288
	2̄ .165	.343	.119	.284
	2̄-3 .148	.328	.106	.206
T2NA(b)	1 .136	.271	.106	.199
	2 .139	.272	.108	.207
	2̄ .138	.272	.107	.203
	2̄-3 .121	.257	.094	.153
T2r+S	1 .321	.699	.234	.494
	2 .319	.693	.227	.496
	2̄ .320	.696	.236	.495
	2̄-3 .303	.681	.223	.447
T6r-6	1 .169	.416	.142	.329
	2 .173	.412	.146	.330
	2̄ .171	.414	.144	.330
	2̄-3 .154	.399	.131	.282

	a	b	c	d
	micro photo	micro photo	micro photo	micro photo
A	3.89	3.36	3.02	4.06
T	4.09	3.33	3.27	4.14
G	2.00	1.72	1.55	2.16
OC	1.615	1.39	1.25	1.875
(E. coli)	[0.342]	[0.266]	[0.190]	[0.189]
	11.895	10.00	8.99	12.235
	91.6	12.056	98.1	12.27
				98.0

P, (	3.60	3.62	3.76	3.70
corrected to 3.60	3.64	3.72	3.83	3.80
R	12.52	12.94	13.35	13.23
R of ratio of sample to standard	9.016	9.18	9.32	9.11
% P (calculated)	6.57	6.69	6.72	6.67



26-VII

Plunge DNA: effect of HCOOH volume.

Weigh out even T6r<sup>+</sup> DNA; using micro-balances.

- (a) 0.71 mg ↑ 0.05 ml 8% HCOOH Cook 17k<sup>o</sup> 35 min. (30 mm)  
 (b) 0.73 0.10  
 (c) 0.71 0.15  
 (d) 0.70 0.25  
 Dry. ↑ 25 µl N-HCl.  
 195° for P, 83 for P<sub>0</sub>.

Noted that less known is found in hydrolysis with bigger volumes.

	G	A	OC279	T	260	264
G	1 0.32	0.25	0.18	0.5*	0.24	0.21
	2 0.32	0.26	0.20	0.56	0.22	0.20
	3 0.32	0.26	0.19	0.55	0.23	0.21
(a)	1 2.39	5.31	1.73	3.73	0.52	0.12
	2 2.45	5.31	1.76	3.86	0.46	0.14
	3 2.52	5.31	1.75	3.80	0.48	
	2-B 2.20	5.05	1.56	3.25	0.27	
(b)	1 2.79	5.57	2.03	3.79	0.45	0.11
	2 2.62	5.48	1.97	3.65	0.48	0.10
	3 2.70	5.53	2.00	3.84	0.42	
	2-B 2.38	5.27	1.81	3.29	0.21	
(c)	1 2.73	5.48	2.06	3.66	0.37	
	2 2.61	5.40	2.09	3.76	0.34	
	3 2.67	5.44	2.08	3.71	0.36	
	2-B 2.35	5.18	1.89	3.16	0.15	
(d)	1 2.83	5.50	2.20	3.69	0.32	
	2 2.67	5.44	2.18	3.74	0.31	
	3 2.75	5.47	2.19	3.72	0.32	
	2-B 2.43	5.21	2.00	3.17	0.11	

Rhodis analysis on *Spiriginea* "T7".

Lolm No.	Conc.	P	Protein % of wt.	DNA	264 260
1 (R.E.)	10.0 mg $\pm$ 10 ml	50.8	5.08	40.4 Y	45.0 $\pm$ 0.96
2 (G.W.)	50.0 mg $\pm$ 10 ml	21.8	4.36	177.6 Y	3.56 $\pm$ 0.98

"C" Band from unopif. T7 eluted with ammonium formate 1M-Hg along marker  $\rightarrow$  big C spot little if any OC. Etch

OC	Blank	Std	A	C	B	S	A
202	.014	.024	.010				
274	.0145	.026	.011	225	.026	0.915	0.889
275	.018	.028	.010				

$$OC = \frac{0.011 \times 10^5}{0.899 \times 97} = 1.3\%$$

Printed Ratios

A	1.92	202.6
T	2.20	203.4
G	1.22	16.8
C	1.90	26.2
	7.84	99.9

$$D_r = \frac{1.92}{1.22} = 1.58$$

$$R = 95\%$$

27-VII-52.

T7. prep. recd. 26-mm from Spirigen.

32 mg  $\pm$  1.0 ml 88% HCOOH. Cook 175° 30 min. Dry  $\uparrow$  0.2 ml N-HCl. Add on 2.83  $\mu$ l peps (1.95 for P)  $\pm$  8° band. Spots exceedingly weak. Discard.

Solution of NA.

1.0 g. "T7"  $\pm$  50 ml 0.2 N-NaCl + 18 g. sucrose. Stand 2 hrs. pH = 7.0. Swung 4 x  $\rightarrow$  small gel at first, then weak brownish gel. Stab some on last swing. Add 100 ml 95% EtOH  $\rightarrow$  large HCl.  $\rightarrow$  fine flocs. Spin down. Wash = 30 ml EtOH, spin down. Dry. Weighs 48 mg.

5.0 mg  $\pm$  1.0 ml HCOOH, cook 175° 45' min. (extended 30). Dry  $\uparrow$  0.15 ml. Take 16.6  $\mu$ l peps, 1.95 for P, + barbitone paper.

Read directly against blends:

G <sub>260</sub>	A <sub>260</sub>	"C"	T <sub>260</sub>	Print
1.166 $\mu$ l only.	.134	.250	.175	260 .61+
			270 .69	265 .92
			275 .66	270 1.13
			275 .69	275 1.26
			273 .69	273 1.28
			272 .69	272 1.63
			271 .69	271 1.63
			270 .69	270 1.63
			269 .69	269 1.63
			268 .69	268 1.63
			267 .69	267 1.63
			266 .69	266 1.63
			265 .69	265 1.63
			264 .69	264 1.63
			263 .69	263 1.63
			262 .69	262 1.63
			261 .69	261 1.63
			260 .69	260 1.63

From here on,  $E_{260}$  at pH 1 for OC = 9700.

Difference to G (C)      Difference to A (G)

Weighted      Weighted      Weighted      Weighted

A 4.09 32.6 32.8      B 4.18 32.4 32.8      C 3.84 32.2 32.8

T 4.04 32.2 31.9      B 4.12 31.9 33.0      C 3.84 32.0 32.8

G 2.26 18.0 17.9      B 2.37 18.35 18.9      C 2.21 18.5 18.5

OC 2.13 17.0 16.9      B 2.24 17.35 17.8      C 2.06 17.28 16.2

12.52 99.8 98.2      B 12.71 100.0 102.6      C 11.93 105.2

P Y  
Conc.  
Pbels  
x 340

12.64 12.76

12.48 12.59

12.43 12.55

12.24 11.34

R 9.80 9.82

10.25 10.26

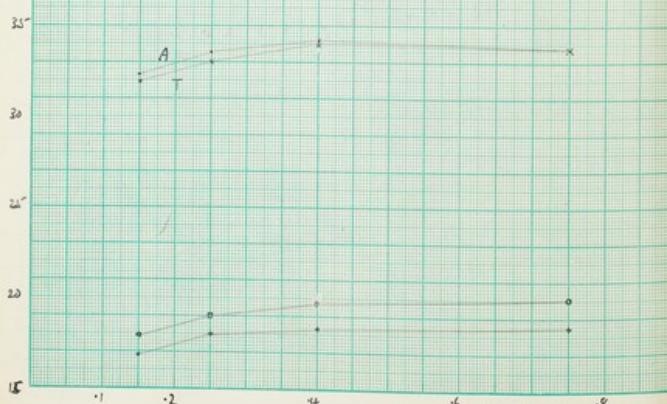
10.49 10.51

T D 6.34

6.51

6.40

5.71



28-VIII-52.

Further amounts of HCOOH.

Draw TGA+ DNA, weighed in unres. balance.

(a) 0.74 mg  $\uparrow$  0.15 ml HCOOH

(b) 0.71 mg 0.26 ml \*

(c) 0.72 mg 0.280 0.75 ml \*

(d) 0.73 mg 0.280 0.40 ml \*

175° 30 min. Day  $\uparrow$  25 pl HCl

8.3 for spds, 1.95 for?

Read sample 1+2 in cells -09 + -17 simultaneously against air blank.

G  $\frac{abs}{abs}$  A  $\frac{abs}{abs}$  OC  $\frac{abs}{abs}$  T  $\frac{abs}{abs}$  Residual<sub>1</sub>

B 1 1.51 0.63 0.85 0.61 1.12 2.65 0.47

2 1.41 0.63 0.96 0.62 1.03 2.50 0.62

$\bar{x}$  1.36 0.91 0.62 0.62 1.08 2.40 0.60

C 1 1.36 0.62 0.69 0.69 1.41 2.45 0.98

2 1.39 0.64 0.69 0.69 1.42 2.45 0.98

$\bar{x}$  1.38 0.63 0.69 0.69 1.42 2.45 0.98

$\bar{x}-\bar{x}$  1.249 0.532 0.207 0.207 0.321 2.45 0.98

B 1 1.34 0.63 0.69 0.69 1.41 2.45 0.98

2 1.40 0.63 0.79 0.79 1.43 2.45 0.98

$\bar{x}$  1.39 0.64 0.74 0.74 1.43 2.45 0.98

$\bar{x}-\bar{x}$  1.261 0.543 0.218 0.218 0.328 2.45 0.98

C 1 1.36 0.62 0.61 0.61 1.41 2.45 0.98

2 1.39 0.64 0.63 0.63 1.40 2.45 0.98

$\bar{x}$  1.37 0.63 0.62 0.62 1.42 2.45 0.98

$\bar{x}-\bar{x}$  1.243 0.499 0.200 0.200 0.344 2.45 0.98

D 1 1.34 0.62 0.69 0.69 1.43 2.45 0.98

2 1.42 0.63 0.79 0.79 1.43 2.45 0.98

$\bar{x}$  1.40 0.64 0.76 0.76 1.44 2.45 0.98

$\bar{x}-\bar{x}$  1.274 0.545 0.223 0.223 0.333 2.45 0.98

$$\begin{aligned}
 T_7 & M \text{ by biological test} = 246 \times 10^6 \text{ (Pulham 1950)} = 10^{-15.4} \text{ grams} \\
 M \text{ by S-cd} & = 31 \times 10^6 \\
 \text{Infectious unit} & = 10^{-16.5} \text{ g of } N \text{ (Gulyas et al 1950)} = 10^{-16.5} \text{ g}
 \end{aligned}$$

$$\begin{aligned}
 \text{If } M = 31 \times 10^6, \text{ (U.S. fathead minnow)} \\
 & \frac{10^{15.4}}{6 \times 10^{15.5}} \times 31 \times 10^6 = 5 \times 10^3 = 0.05 \text{ g} \\
 & = 5\% \text{ of wt.}
 \end{aligned}$$

Sigizuris "T7 lot #1" recd 14-x-52.

14-x-52. Rhom's analysis: P = 3.36%  
D NA = 4.0%.

120 mg hydrolyzed (2 tubes, each 60 mg  $\uparrow$  2.0 ml HCOOH) spread on  
big sheet (lot #1). Elute C band + run  $\approx$  40 hrs in  $\text{BnNH}_2\text{-NH}_3$ .

Directly against blank: C in 4 ml OC in 4 ml.  
275 .104 (1) 275 .061  
279 .054

4-ix

5-ix

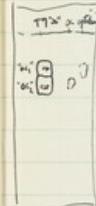
T7 re-examined for C & OC

(continued 3)

18 mg T7 NA  $\uparrow$  1.5 ml HCOOH  $175^\circ$  40 min.  
Cap to  $\approx$  0.1 ml, spread on one fine pipet.

Elute C band, conc., run in  $\text{BnNH}_2\text{-NH}_3$

$\rightarrow$  big C spot, + faint crescent-shaped spot on  
some background streak in OC position. Elute OC  
position into 0.1 ml pipette, conc. on paper, conc in  
 $\text{BnNH}_2\text{-NH}_3$  again.



$\rightarrow$  Read directly against blanks in 0.1N-HCl.

C in 4 ml	OC in 4 ml	OC <sub>2</sub> in 4 ml
270	.033	

275	.218	275	.026	275	.027
279	.209	279	.033	279	.021

Max OC =  $\frac{0.021 \times 100}{2.18 \times 2} = 0.5\% \text{ of C.}$

22-ix

50 mg whole crude T7 in 2 tubes each 25 mg in 1.5 ml HCOOH  
 $175^\circ$  30 min, spread in 8" band on Whatman #3, run in 10% HCl.  
 $\rightarrow$  C spot, no OC. Elute:

C in 4 ml	OC in 4 ml
275	.012
279	.011

Max. OC = 2% of C

	(a) BSNA	(b) Chm T6 <sup>+</sup> DNA	(c) SSC T6 <sup>+</sup> DNA	(d) SSC T2 <sup>+</sup> DNA
(mole Ratio, undiluted)				
A	3.51	2.80	3.86	3.22
T	3.45	2.75	3.86	3.22
G	2.84	2.66	2.20	18.35
C	2.57	2.05	2.07	17.3
nc	12.37	9.86	11.99	100.05
P.Y	1.23	1.38	3.49	3.47
	3.60	3.62	3.49	3.13
	2.63	3.45	3.15	2.00
Virus complex	12.40	11.89	10.72	6.86
R	101%	101%	102%	102%
%P	7.94	6.68	5.94	4.17

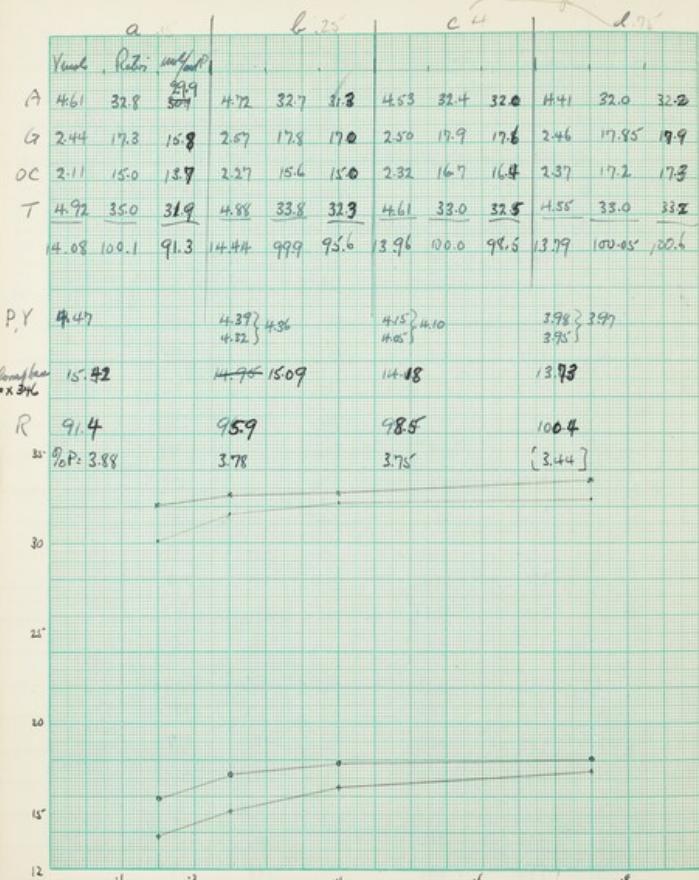
8-IX-52

Misc DNAs &amp; increased vol. HCOOH.

- a. Chm BSNA 0.58 mg.  
 b. Chm T6<sup>+</sup> DNA 0.66 mg. }  
 c. SSC T6<sup>+</sup> DNA 0.67 mg. }  
 d. SSC T2<sup>+</sup> DNA 0.61 mg. }

Read samples 102 simultaneously in sets of 17 against air blank.

	G	A	C + OC	T
B	1 .94	.64	.050	.048
	2 .94	.62	.050	.048
	<u><math>\bar{x}</math></u> .94	.63	.050	.048
A	1 .407	.521	.320	.306
	2 .407	.577	.320	.306
	<u><math>\bar{x}</math></u> .407	.519	.320	.306
	<u><math>\bar{x}</math></u> - B 313	.456	.270	.274
B	1 337	.565	.244	.249
	2 335	.562	.243	.248
	<u><math>\bar{x}</math></u> 336	.564	.249	.249
	<u><math>\bar{x}</math></u> - B 242	.501	.201	.207
C	1 315	.530	.212	.218
	2 316	.529	.214	.220
	<u><math>\bar{x}</math></u> 316	.530	.219	.219
	<u><math>\bar{x}</math></u> - B 222	.467	.171	.288
D	1 233	.359	.166	.165
	2 232	.357	.165	.165
	<u><math>\bar{x}</math></u> 233	.358	.166	.164
	<u><math>\bar{x}</math></u> - B 139	.295	.118	.119



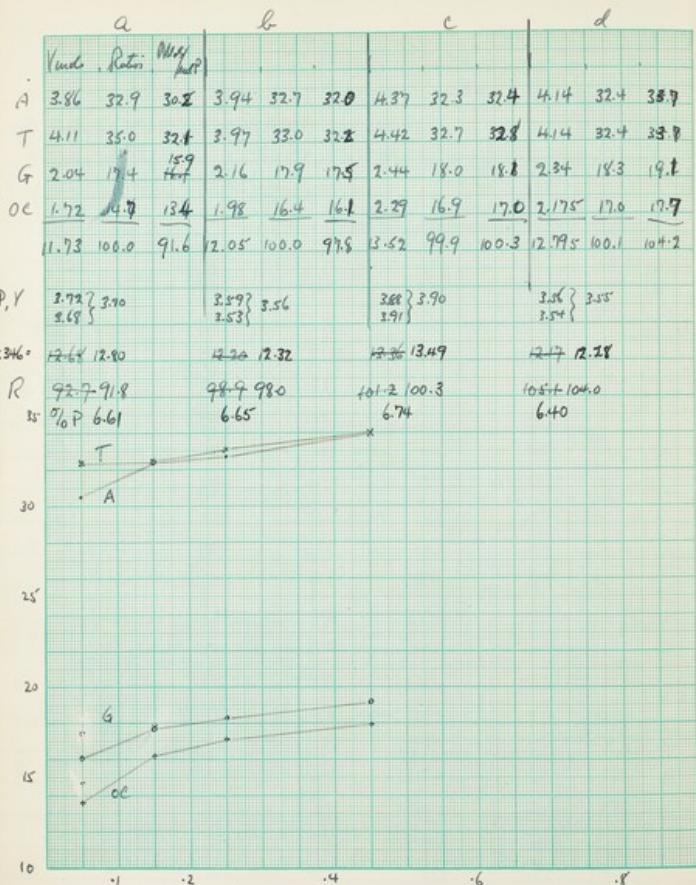
10-14-52

Effect of vol. of HCO<sub>3</sub>H on whole phage.

- (a) T6<sup>r+2</sup> phage 1.50 mlg.  $\uparrow$  0.15 ml HCO<sub>3</sub>H  
 (b) " " 1.50  $\uparrow$  0.25 ml " 17.5° 30 min. O<sub>2</sub>  
 (c) T6<sup>r+2</sup> 0.7 } 1.46  $\uparrow$  0.40 ml "  $\uparrow$  0.024 ml. Toluene  
 (d) T3<sup>r+5</sup> 0.8 } 1.50  $\uparrow$  0.75 ml " 8.3 after 5.95 for P.

Read 2 Sa line against air blank:

	G	A	OC	T
R	1 .094	.062	.047	.086
	2 .095	.067	.047	.091
	3 .095	.065	.047	.089
a	1 362	665	252	480
	2 364	663	251	479
	3 363	664	252	480
	2-3 268	599	205	391
b	1 376	678	267	475
	2 380	680	267	479
	3 378	679	267	477
	2-3 283	614	220	388
c	1 366	648	272	454
	2 374	660	271	458
	3 370	654	272	456
	2-3 275	589	225	367
d	1 363	637	278	451
	2 368	639	276	451
	3 366	638	277	451
	2-3 271	573	230	362



15-i-52.

Repetit effect of HCOOH mol. on phage DNA.

(a)	0.71	my own T6 <sup>r+</sup> DNA ↑ 0.05 mol 88% HCOOH		
(b)	0.68	~	0.15	~
(c)	0.735	~	0.25	~
(d)	0.705	~	0.40	~

175° 30 min.  
Ag. 125 ph.  
8.3 for E, 1.95 for P?

	G	A	OC	T
B	1 .091	.059	.164	.081
	2 <u>.095</u>	<u>.061</u>	<u>.050</u>	<u>.082</u>
	<u>.093</u>	<u>.060</u>	<u>.050</u>	<u>.082</u>
a	1 316	560	219	409
	2 318	563	215	409
	<u>317</u>	<u>562</u>	<u>217</u>	<u>409</u>
z-B	224	502	167	327
f	1 329	568	244	400
	2 331	574	240	395
	<u>330</u>	<u>571</u>	<u>242</u>	<u>398</u>
z-B	237	511	192	316
e	1 356	625	272	436
	2 365	630	272	430
	<u>361</u>	<u>628</u>	<u>272</u>	<u>433</u>
z-B	268	568	222	351
d	1 348	595	258	411
	2 352	599	264	411
	<u>360</u>	<u>597</u>	<u>261</u>	<u>411</u>
z-B	257	537	211	329

$0.1 \text{ M } \text{Na}_2\text{CO}_3$  9.6 ml  $\rightarrow 0.008 \text{ M}$ ,  
 $0.1 \text{ M NaCl}$  60.0 ml  $\rightarrow 0.05 \text{ M}$ .  
 water 50.4  
120.0

16-ix-52. Check insect wings for OC.  
 9.45 ml. 606 mg Pape  $\uparrow$  120 ml 0.008 M  $\text{Na}_2\text{CO}_3$  - 0.05 M NaCl.  
 see Cp 1/1

Hydrolysis 4.0 mg dried wings  $\uparrow$  1.0 ml  
 4.0 " " "  $\uparrow$  1.0 ml }  $175^\circ$  30 min.  
 2.6 mg clean "  $\uparrow$  0.75 ml } combine, dry, wrap.

To  $\sim 0.1$  ml, spread on paper.

18-ix Elute "C" band in  $\sim 0.5$  ml, w.b. to layers.  $\uparrow$  1st wash.

apply to fiber. resin in  $\text{Ba}(\text{OH})_2 \cdot \text{NH}_3$ .  
 20-ix Long C spots, only faint streak in OC position. Elute.  
 read directly against B.

	OC in 4 ml	C in 8 ml
284	.029	280 .712
279	.037	275 .761
275	.040	270 .720
270	.037	

$$\text{All max OC} = \frac{0.037 \times 100}{2 \times 0.761} = 2.4 \%$$

27-ix Check for P loss in DNA under hydrolytic conditions.

6.3 mg. Shear's DNA + 2.0 ml H<sub>2</sub>O. Add ~10 µl HCOOH - heat to 100° to dephosphorylate. Pipette 0.2 ml samples into 6 tubes (5 mm) or 1.0 mm (5 mm). Dry in oven.

(a)	Large tube. Control, unheated.		↑ 1.0 ml H <sub>2</sub> O
(b)	"	↑ 0.05 ml HCOOH, heat 175° 30 min.	↑ 1.0 ml H <sub>2</sub> O
(c)	"	↑ 0.15 "	"
(d)	"	0.40 "	"
(e)	"	0.75 "	"
(f)	Small tube	0.40 "	0.9 "

From each, 2 x 0.1 ml for P.

P, mean of 2 dilutions: (a) 4.91 Y

(b) 4.91

(c) 4.93

(d) 4.91

(e) 4.94

(f)  $5.47 \times \frac{9}{10} = 4.93$ .

No difference at all!

17-ix

Where does the P go?

Check loss of P during evaporation of formic acid - Into each of 4 pyrex tubes pipette 0.05 ml N 0.05 M. KH<sub>2</sub>PO<sub>4</sub> ( $\approx 0.075$  mg P). Dry in oven.

Add: (a) nothing  
 (b) 0.05 ml " " " "  
 (c) 0.15 ml " " " "  
 (d) 0.5 ml " " " "  
 ↑ 1.0 ml 1 N-HCl.

Take 0.05 ml samples for P.

P, Y	a	2.95	2.98
	b	2.86	2.92
	c	2.90	2.92
	d	2.87	2.90

- difference small, if significant

28-ix Into each of 4 tubes, 0.05 ml ~ 0.05 M. KH<sub>2</sub>PO<sub>4</sub>, dry in oven.  
 In. diam. 5 mm. (a) No hydrolysis. Small tube.

$$P = \frac{4.41}{4.40} r^2 4.41$$

(b) ↑ 0.05 ml HCOOH, 175° 30 min.

$$4.59 \quad 4.59$$

(c) ↑ 0.50 ml " " "

$$4.51 \quad 4.51$$

In. diam. 9 mm. (d) Big tube, 10.5 ml HCOOH, 175° 30 min.

$$\frac{4.50}{4.43} \quad 4.47$$

Evap. to dryness, ↑ 1.0 ml H<sub>2</sub>O, take 0.05 ml sample for P.

	(a) BSNA	(b) Rhomis	(c) Worthington	(d) Schuchardt
Yield Ratio	1	1	1	1
A	4.34 27.8	4.43 28.1	3.44 28.1	4.12 26.3
T	4.25 27.8	4.32 27.4	3.31 27.1	4.50 28.7
G	3.44 22.5	3.58 22.7	2.90 22.9	3.40 21.7
C	3.17 20.8	3.24 20.5	2.535 20.7	3.42 21.8
MC	0.173 1.18	0.214 1.4	0.143 1.2	0.245 1.6
N <sub>273</sub> 100.0	100.0	100.0	100.0	100.0
P,Y	44.36 { 44.40 44.44 } 45.58	45.56 { 45.58 45.60 } 45.58	3.64 { 3.67 3.70 } 3.67	4.81 { 4.83 4.88 } 4.83
Comptd Ratios	15.09	15.70	12.59	16.61
R	101.0	100.5	97.3	94.5
% P	7.21	7.45		

19-12-12

## DNAs for Osische standard.

- (a) Ocean DNA 1.55 mg } Equal + 0.5 ml 8% HCOOH, 175° 30 min.  
 (b) Rhomis Egg DNA 1.56 } Digested ↑ 0.05 and N-HCl.  
 (c) Worthington DNA 1.51 } Take: 2x8.3 mg  
 (d) Schuchardt DNA 1.54 } 2x1.95 mg  
 2x244 N

	G	A	C <sub>273</sub>	MC	273	269	T
B	1 .109	.073	.059	.052	.051	.050	.104
	2 .102	.069	.057	.050	.048	.049	.090
	2̄ .106	.071	.058	.051	.050	.049	.097
a	1 487	626	392	.069	.069	.066	.446
	2 483	618	390	.065	.064	.062	.424
	2̄ 485	622	391	.067	.067	.064	.435
b 2̄ - B	379	551	333	.016	.017	.014	.338
	1 498	648	401	.072	.072	.069	.446
	2 501	646	395	.069	.069	.066	.436
2̄	500	647	398	.071	.071	.068	.441
	2̄ - B 394	576	340	.020	.021	.018	.344
c	1 415	519	324	.067	.066	.064	.362
	2 412	517	324	.063	.062	.060	.357
	2̄ 414	518	324	.065	.064	.062	.360
2̄ - B	308	447	266	.014	.014	.013	.263
	1 410	606	419	.071	.074	.072	.458
	2 410	607	414	.074	.073	.070	.451
2̄	480	607	417	.075	.074	.071	.455
	2̄ - B 374	536	359	.024	.024	.022	.358

Cytosine-H<sub>2</sub>O has M = 129.2  
N = 32.5 %.

29-ix.

Cytosine extinction coefficient.

100 mg Dried cytosine 2 x re-crystallized from water  
→ 60 mg. Dried overnight in vac over silica gel at room temp.  
weigh out 20.9 mg. into 25 ml volumetric fl.

For E, dilute 1.0 ml + 100 ml H<sub>2</sub>O.

	(1)	(2)	
276	.676	.674	
275	.676	.674	
274	.674	.673	$0.675 \times \frac{2}{2} = 0.675$
239	.086	.086	
238	.085	.085	
237	.086	.086	

$$E_{275}, \text{ assuming } M = 129.2, = 0.675 \times 129.2 \times \frac{100 \times 25}{20.9} = 10,420$$

Molar absorptivity: N formal =  $\frac{0.2674}{0.2660} \left\{ \frac{0.2669}{0.2674} \right\}$  mg.

$$E_{275}, \text{ based on N} = 0.675 \times 100 \times \frac{3 \times 14.01}{0.267} = 10,620.$$

	a	b	c	d
	Fauro, Rubin.	Fauro, Rubin.		
A	3.98 2.67	27.25 24.55	3.70 2.615	3.87 2.615
T	4.34	29.7	4.02	29.7
G	3.16	21.6	2.90	21.4
C	2.94	20.1	2.76	20.4
	14.42	98.6	13.38	98.8
Others for HCl + 3%	18.60		13.84	18.39
P	3.94 3.96	3.95 3.48	3.77 3.73	3.75 3.96
	13.54	11.92	12.85	13.54
R	105	114	112	112

30-ix-52

Effect of HCOOH vol. on TNA + albumin.Chlor's TNA (17 mg) + bovine serum albumin (86 mg wet by 379 mg tannic)  
ground together. 4.0 mg protein weighed into 4 tubes (0.875 mm tubes).Add (a) 0.25 ml 81% HCOOH  
(b) 0.50  
(c) 0.75  
(d) 1.0Look 17<sup>o</sup> 30 min. Dry down (parted  
over, < 60°) ↑ 25 µl H-Hg, take 2 x 83  
spins, 2 x 1.95 for? Elute 4 hrs.

	G	A	C	T
B	1 .107	.064	.055"	.087
	2 .107	.066	.056"	.086
	z .107	.065	.055"	.085
a	1 454	583	365'	431
	2 454	579	362	428
	z 454	581	364	430
	z - 3 347	516	309	345'
b	1 429	546	350	410
	2 428'	544	340	400
	z 426	545"	345"	405
	z - 3 319	498	290	320
c	1 450	570	366	432
	2 443	566	357	426
	z 447	568	362	429
	z - 3 340	503	307	344
d	1 465	598	386	458
	2 463	598	376	448
	z 464	598	381	451
	z - 3 357	633	326	366

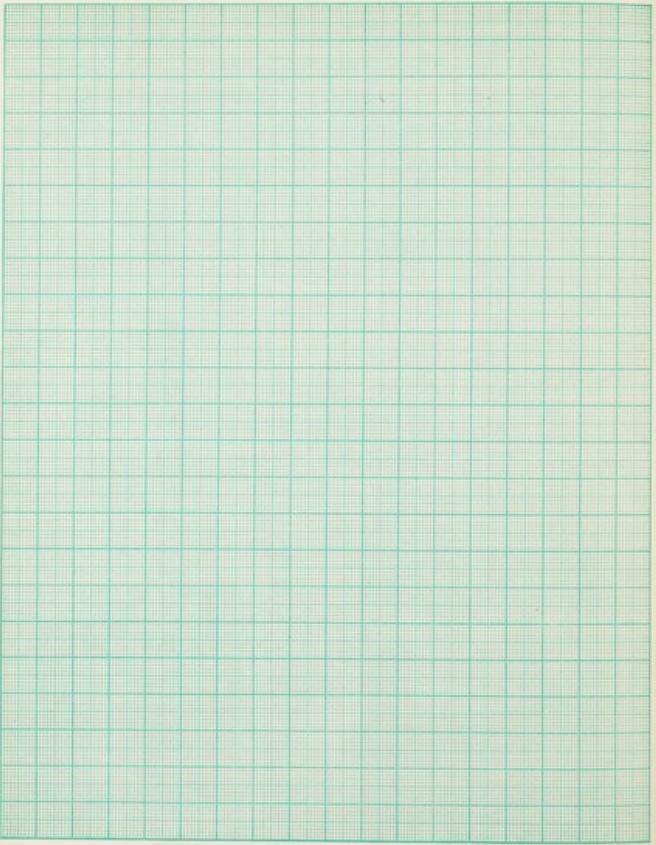
	a	b	c	d
Yield, Pfu/ml	1	1	1	1
A	3.31	31.9	44.45	32.0
T	3.51	33.8	4.61	33.2
G	1.79	17.2	2.475	17.8
OC	1.78	17.1	2.37	17.0
	0.39	100.0	13.905	100.0
P	3.26 { 3.27	14.04 { 14.04	3.10 { 3.13	3.19 { 3.21
x346	3.28	4.03	3.15	3.23
R	11.31	13.97	10.83	11.10
P%	91.9	99.6	97.1	96.7
	2.73	3.44	5.60	5.74

1-1-52.

## Phages &amp; Phage DNA's

- (a) T6r-6 phage 1.56 mg ↑ 0.5 ml HCOOH } 10x76' mm. Pipet tubes.  
 (b) T2r<sup>-5</sup> - 1.53 - - } 175" 30 mm. Obj.  
 (c) SSC T6r<sup>-</sup> DNA 0.71 - 0.25 ml. } ↑ 25μl, 8.3' spores.  
 (d) T2r<sup>-1</sup> DNA 0.71 - - - 1.95 for?

	G	A	OC 279	T
R	1 .096	.060	.052	.079
	2 .094	.060	.051	.078
	2 .095	.060	.052	.079
a	1 292	489	226	365'
	2 292	491	224	351
	2 292	490	225'	358
	2 - 3 197	430	173	279
b	1 368	689	284	449
	2 368	639	279	439
	2 367	639	282	444
	2 - 3 272	579	230	365'
c	1 320	505'	190	372
	2 318	501	184	368
	2 319	503	187	370
	2 - 3 224	443	135	291
d	1 327	514	186	383
	2 320	516	184	375'
	2 324	515'	185'	379
	2 - 3 229	488'	133	300



3-x-52

Error introduced by proteins.

- (a) 3.9 mg PPD 0.2 + 0.5 ml H2O + 172° 30 min  
(b) 3.9 mg Bov. se. alb. " "

Dry. + 25 µl, mix 8:3 µl apof. in isoprop.-HCl, along c. DNA marker.  
(a) above + faint spots in base position. Do not cut out.  
(b) Cut out areas corresponding to spots. & read against black.

G<sub>260</sub> . A<sub>260</sub> . C<sub>270</sub> . T<sub>265</sub> .  
0.004 . 0.018 . 0.010 . 0.037

	a	b	c	d								
Yields Radio $\frac{\text{Wt}}{\text{wt P}}$												
A	4.07	32.5	30.5	4.24	32.8	31.3	4.22	32.1	28.9	3.46	32.6	25.2
T	4.24	33.9	31.8	4.31	33.3	31.8	4.65	36.4	31.8	4.31	41.9	31.4
G	2.24	19.9	16.8	2.32	17.9	17.1	2.26	17.7	15.5	1.97	19.1	14.3
OC	1.96	15.7	14.7	2.08	16.1	15.4	1.64	12.8	11.2	0.56	5.4	4.1
	2.51			2.95			2.77			10.30		
P, Y	3.86	3.86		3.96	3.92		4.29	4.23		4.01	3.98	
X 346	13.35			13.55			14.62			13.76		
R	93.8			95.6			87.4			75.0		
% P	6.63			6.73			7.16			6.94		6.81

H-x-52.

#### Effect of atmosphere over HCOOH.

App 10x75 mm (a) 0.74 mgm Tbr<sup>32</sup>DNA + 0.1 ml HCOOH N<sub>2</sub> atmosphere 175° 30 min.  
tubes: (A) 0.74 - - - HCOOH vapor - By perf. vac. (b) 0.75 - - - air ↑ 25 µl H-HOAc  
(c) 0.75 - - - vacuum (water) 1.95 µl, 8.3 µl  
(d) 0.75 - - - vacuum (vacufuge) 1.95 µl, 8.3 µl

Difficult to get several 8.3 µl fifthful, so base calc on no. 1. Stand overnight

	G	A	OC	T
B	1 107	.065	.059	.087
	2 104	.076	.026	.093
	2 106	.071	.058	.090
a	1 351	699	250	427
	Perf. 353	601	246	+27
	2 322	548	224	389
	2 352	600	248	427
	2-3 246	529	190	337
b	1 363	624	263	433
	2 358	619	256	432
	2 361	622	260	433
	2-3 255	551	202	343
c	1 358	620	221	466
	2 351	617	212	454
	2 353	619	217	460
	2-3 249	548	159	370
d	1 324	523	116	440
	2 322	519	108	426
	2 323	521	112	433
	2-3 217	450	054	343

	a	b	c	d							
Yards	Ratios (wt/wt)										
A	4.60	32.5	30.0	4.94	32.4	30.9	5.27	31.9	31.4	4.53	32.7
T	4.89	34.5	31.9	5.26	34.5	32.9	5.50	33.2	32.9	4.51	32.6
G	2.40	16.9	15.6	2.67	17.5	16.7	2.96	17.9	17.6	2.515	18.4
OC	2.24	16.1	14.9	2.37	15.4	14.8	2.81	17.0	16.7	2.28	16.5
	14.17			92.4	15.24		96.3	16.54		98.4	13.865
P, Y	4.123 +28 15.34	4.44 4.52 15.34	4.57 4.52 15.83	4.55 4.60 16.00	4.78 4.79 16.09	4.63 4.63 16.80	3.49 3.32 4.19 4.19	3.41 3.41 4.19 4.19	* Phorbol esters * Extra marks done and done		
R	92	95 98'	103	99			98	97.6			
% P	3.87		4.06		4.13			7.02			
		$\bar{x}_c = 64.02$									

16x

Effect of vol. of HCOOH in HCOOH vapor atmosphere.

Yards made from:  
Pipette tubing (6mm)  
Int. dia.

- (a) 1.48 ml SSC T2<sup>+</sup> phage  $\uparrow$  0.15 ml 9% HCOOH  
 (b) 1.47 ml -  $\uparrow$  0.25 ml -  
 (c) 1.52 ml -  $\uparrow$  0.50 ml -  
 (d) 0.74 ml own T6<sup>+</sup> DNA  $\uparrow$  0.25 ml -

HA for elution delivered from broken filter = 3.98 ml. Allow 1% error. Factors 348

G, A, OC, T

G	1	.94	.062	.052	.90
	2	.095	.062	.051	.086
	3	.095	.062	.052	.088
(a)	1	360	65.9	272	478
	2	357	65.9	274	474
	3	359	65.9	273	477
	2-3	264	597	221	389
(b)	1	389	701	260	507
	2	389	706	284	504
	3	389	704	282	506
	2-3	294	642	230	418
(c)	1	420	746	326	527
	2	422	748	323	522
	3	421	747	325	525
	2-3	326	685	273	437
(d)	1	372	651	284	466
	2	377	650	282	448
	3	375	651	283	447
	2-3	280	589	221	369

	a	b	c	d
Yanole, Rubin				
A	2.93	32.4	3.88	32.6
T	2.97	32.8	3.82	32.4
G	1.64	18.1	2.13	18.05
OC	1.504	16.6	2.00	16.95
	9.044	99.9	11.80	100.0
P.Y	2.73	2.73	3.52	3.52
X346	9.45	12.18	14.68	11.80
96	97	97	99	99
%P	4.89	6.13	7.50	6.28

21-X-52.  
One spot test  
10.6 mm.

### Plaque DNA analyses.

- (a) 0.71 mg T2<sup>r2</sup> DNA ↑ 0.25 ml 18% HCOOH, 175° 30 min.
- (b) 0.73 mg T2<sup>r1</sup> (②) JNA } Dye ↑ 25 µl. 8.3 spots, 19% for P.
- (c) 0.72 mg T2<sup>r1</sup> (③) JNA }
- (d) 0.69 mg T6<sup>r1</sup> (SSC) DNA Good chromatograms. Elect in 4.50 and overnight

	G	A	OC	T
B	1 102	.065'	.056	.085'
	2 104	.069	.056	.095'
	z 103	.067	.056	.090
a	1 279	449	263	323
	2 287	447	260	328
	z 283	448	262	326
z-B	1 180	381	146	236
	2 335'	566	250	393
	z 338	567	250	394
f	1 337	567	250	394
	2 234	500	194	304
	z 287	599	230	366
c	1 389	663	287	434
	2 391	669	285'	437
	z 390	666	286	436
z-B	1 287	599	230	366
	2 336	567	244	389
	z 331	567	236	390
d	1 334	567	241	390
	2 231	500	185'	300
	z 231	500	185'	300

(a) PBm 80/34

BSNA + all

	Yards	Ratio	Yards	Ratio
A	1.54	29.8	3.55	28.0
T	1.535	29.2	3.51	27.7
G	1.12	21.3	2.86	22.6
C	1.06	20.2	2.60	21.3
[Mc]			0.16	1.33
	5.255		12.68	
f				
x P/V	1.98 2.05 2.11	2.05	3.70 3.76 3.81	2.76
x 246	7.10		13.00	
% R	74%		97.5%	

$$\text{Avg wt.} = 5.0 \text{ mg in } 42.7 \mu\text{l} = 0.117 \text{ mg/\mu l}$$

$$P = 2.05 Y \text{ in } 1.95 \mu\text{l} = 1.05 Y/\mu\text{l} \quad P = 0.90\%$$

$$N = \frac{63.2}{64.0} \times 1.63 \text{ in } 3.70 \mu\text{l} = 1.63 Y/\mu\text{l} \quad N = 13.9\%$$

$$N/P = 15.5$$

$$\text{DNA in virus} = 9.0 \times \frac{74}{100} = 6.7\%$$

22-x

## PBm V. Check Recovery.

(a) 5-10 mg PBm 80/34<sub>2</sub> (dissolve P<sub>2</sub>O<sub>7</sub> in water) } each  $\uparrow$  20  $\mu\text{l}$  HCl<sub>aq.</sub>  
 (b) 5.0 mg BSNA + albumin (15:85) } dilute 100<sup>1/2</sup> (QWS) 1 $\frac{1}{2}$   
 hrs, shaking occasionally to wash down tube walls. Open, add  
 20  $\mu\text{l}$  aq. dest., mix, grinding up residue  $\geq$  red. Vol  $= (20+20) \times 0.916 + 3.7$   
 Take:  $2 \times 8.3 \mu\text{l}$  spots

 $3 \times 1.95 \mu\text{l}$  for P (filtering efficient)

 $2 \times 3.70 \mu\text{l}$  for N (2  $\times$  1.95  $\mu\text{l}$  filter, filtering efficient; PBm only).

G . A . C . T

B	1	.103	.071	.062	.095
	2	.098	.068	.057	.090
	3	.101	.070	.066	.093

a (PBm)	1	.224	.270	.174	.218
	2	.224	.270	.168	.212
	3	.224	.270	.171	.215
	4-8	.123	.200	.111	.122

b (BS)	1	.417	.531	.385	.374
	2	.413	.531	.380	.369
	3	.415	.531	.383	.372
	4-8	.314	.461	.273	.279

	A	C	OC
P for 1.92 ml. x 3.46	1.625	1.625	1.625
7.93	27.62	6.74	0.244
8.28	8.25	28.55	7.26
8.21	8.25	28.55	0.255
			5.34
			5.55
			0.195
			9.48

24-X-52

Check recovery of OC cooked in presence of dsDNA under new conditions.

5.6 mg dsDNA } ↑ 2.0 ml HCOOH. Take portions each  
0.5 mg each OC }

0.5 ml sealed off in Open Glass Tubes.

Heat: (a) 100° 2 min.

(b) 175° 30 min.

Dry down (got up to ~ 80°; (a) got some reddish in it).

↑ 25 µl. Take 1.95 for P, 8.3 apd over 24 hrs in  
icebox. HCl, 26 hrs in HCl-NaCl. Elute OC, C, rA, i-blanks  
as on 23.VII.A<sub>260</sub>, C<sub>278</sub>, OC<sub>259</sub>.

(a)	B	1	.137	.074
		2	.112	.076
		<u>z</u>	<u>.125</u>	<u>.075</u>

Spot	1	1.025	.628
	2	0.976	.628
	<u>z</u>	<u>1.000</u>	<u>.628</u>

S-8		0.875	.553
-----	--	-------	------

(b)	B	1	.112	.075	.076
		2	.125	.077	.075
		<u>z</u>	<u>.119</u>	<u>.076</u>	<u>.076</u>

Spot	1	1.060	.630	.613
	2	1.065	.641	.617
	<u>z</u>	<u>1.063</u>	<u>.636</u>	<u>.615</u>

S-3		0.944	.560	.539
-----	--	-------	------	------

	A (Bm)		G (85)	
	Y-mols	Ratios	Y-mols	Ratios
A	1.64	29.0	2.91	28.0 27.6
T	1.72	30.4	2.87	27.6 27.3
G	1.145	20.2	2.35	22.6 22.3
C	1.16	20.5	2.25	21.0 21.4
	5.665	100.1	10.38	99.9 98.6
			MC	1.3
				99.9
D, Y	2.53	2.51	3.23	2.22
x	2.49		3.21	
x324	8.14		10.43	
R	69.6 %		99.4 %	

Dry wt. =  $3.475 \text{ mg} \times 25 \times 0.976 + 25 = 26.9 \text{ mg} = 0.129 \text{ mg/ml}$   
 $P = 2.51 \text{ Y in } 2.11 \mu\text{l} = 1.19 \text{ Y/ml} = 0.92\%$   
 DNA recovered =  $9.2 \times 69.6\% = 6.4\% \text{ of virus.}$

28x

PBM V. Check Recovery (2)

(a) 3.5 mg PBM 80/34<sub>2</sub> } each 1 20 µl 70% HCHO. Glycerin  
 (b) 2.5 mg 85% A-albumin mixture } BBR 10° 2 hrs. Add 15 µl of  
 dextr. Take 2x 0.4 mlots, 2x 2.11 for P. (new pipette)

	G	A	B	T
B	1	91	59	51
	2	94	60	51
	$\bar{x}$	93	60	51

(a)	1	215	274	171	213
	2	223	272	175	227
	$\bar{x}$	219	273	173	220
	$\bar{x}-\bar{B}$	126	218	122	137

(b)	1	350	438	286	305
	2	351	438	288	317
	$\bar{x}$	351	438	287	311
	$\bar{x}-\bar{B}$	258	378	236	228

	a	b	c	d
Yield, Protein (Weight %)				
A	4.62	32.8	3.72	32.8
T	4.65	33.0	3.76	33.1
G	2.56	18.15	2.06	18.2
C, OC	2.27	16.1	1.80	15.9
[MC]				
	14.10	100.0	11.34	100.0
P, V	5.17 5.26	3.64 3.64	4.28 4.30	4.29 4.71
X, S2+	16.92	11.79	13.90	15.90
R	83.4%	96.4	96.5	99.8
% P	4.46 4.16	3.09 2.88	2.47 6.94	8.72 8.10

1-XI-52.

Bacteriophages  
6 mm.

## Phage and DNA's.

- (a) T6<sup>r</sup>-b phage (prof. rec'd. 3Lx52) 1.52 mg 1.52  
 (b) T6<sup>r</sup>-n ~ ~ 1.53 1.53  
 (c) T6<sup>r</sup>+DNA (own) 0.73 0.73  
 (d) BSNA 0.715 0.715

Each ↑ 0.5 ml HCOOH,  
17A<sup>r</sup> 30 min.Dyldom 1.25 ml. Inha  
8.4 ml. + 2.11 for P.

	G	A	C <sub>275'</sub>	OC <sub>279</sub>	T
B	1 121	77	60	99	
	2 121	79	63	102	
	2 121	78	62	101	
a	1 463	680	283	470	
	2 403	677	281	472	
	2 403	679	282	471	
S-3	282	601	220	370	
b	1 346	568	235	401	
	2 348	553	238	399	
	2 347	561	237	400	
S-3	226	483	175	299	
c	1 392	638	277	447	
	2 398	642	277	446	
	2 395	642	277	447	
S-3	274	564	215	346	
d	1 508	658	405	446	
	2 522	670	413	450	
	2 515	664	409	448	
S-3	394	586	347	347	

	a	b	c	d								
Yields	Ratios	Wt/P										
A	3.17	27.7	26.45	3.28	28.0	27.4	3.27	28.0	27.3	3.32	28.0	27.4
T	3.16	27.6	26.35	3.13	26.7	26.1	3.06	26.2	25.5	3.12	26.3	25.8
G	2.55	22.3	21.3	2.67	22.8	22.3	2.71	23.2	22.6	2.745	23.2	22.7
C	2.41	21.8*	20.1	2.47	21.1	20.6	2.48	21.2	20.7	2.505	21.2	20.7
(MC)												
	11.29	98.7	95.5	11.55	98.6	97.7	11.52	98.6	97.4	11.69	98.7	97.9
P,Y	3.72	3.70	3.67	3.18	3.70	3.72	3.71	3.70	3.68	3.68	3.74	3.74
X324	11.98		11.98		11.98		12.11					
R	95.6		97.8		97.5		97.9					
70P	8.21		7.64									

3-XI-52

Recovery from DSNA in vol. HCOOH

3.5 my DSNA  $\uparrow$  0.5 ml HCOOH. Into each of 4 tubes (1 per 6 mm) add 0.1 ml ( $\sim$  0.7 my No).

① 10 glug

Add: (a) 0 ml HCOOH  $\rightarrow$  0.1 ml }  
(b) 0.2 ml -  $\rightarrow$  0.3 }  
(c) 0.4 ml -  $\rightarrow$  0.5 }  
(d) 0.6 ml -  $\rightarrow$  0.7 }  
Each, with 175° 30 min. by oven.(a) is brownish,  $\uparrow$  30 ml HCl.

Lk 2.11 for? 8.4 photo.

	G	A	C	T
B	1 104	76	57	109
	2 114	96	59	139
	2 109	86	58	124
Q	1 399	497	310	364
	2 388	499	312	386
	2 389	498	311	375
Z-B	280	412	253	251
	1 404	508	317	363
	2 402	515	316	383
Z	403	512	317	373
	294	426	259	249
	1 407	508	317	364
C	2 407	514	318	370
	2 407	511	318	367
	2 298	425	260	243
D	1 415	517	324	377
	2 406	517	318	367
	2 411	517	321	372
Z-B	302	431	263	248

	A	C	OC
P for 2 ml vol.	x 3.2 +	1.1 ml added, 1 ml 3.2 ml P. Vials for 1 ml each. 1 ml added, each vial 0.9 ml.	
a 7.21 7.15	23.16	5.92	0.25±6

7.09

b 7.14 7.20

7.25

23.32

5.98

0.256

4.48

0.92

4.35

0.187

86.9

29-xi

OC recovery cooked in presence of DSNA

From soln of OC + DSNA in HCOOH made up 24-ml. Take 2 x 0.3 ml. (all I could get).

(a) 150° 2 min

(b) 175° 30 min.

By lower. ↑ 25 µl. Take 8.4 µl, 2.11 for? Run in ring 90°. Then  $\text{NaBH}_4 \cdot \text{NH}_3$ . Spots blanks as before.

	A	C	OC
a B 1	109	67	70
2	112	72	73
2̄	111		72
S 1	875		550
2	887		557
2̄	881		554
S-B	770		482

h	B	1	97	67	70
	2	109	72	69	
	2̄	103	70	70	
S	1	881	540	494	
	2	880	539	490	
	2̄	881	540	492	
S-B		778	470	422	

	a	b	c	d
<i>Yeast</i> , <i>Rutin</i> , <i>Allyl P</i>				
A	4.06	3.88	4.01	3.85
T	4.26	3.44	4.03	3.25
G	2.19	1.77	2.29	1.85
OC	1.875	1.51	2.02	1.64
"Y"	(0.39)	(0.64)	(0.64)	(0.72)
	2.385	100.0	2.35	100.0
P, Y	4.25	4.20	3.97	4.00
	4.14	4.01	4.11	4.00
L321	13.60	12.99	13.10	12.96
R	91.2	95.7	94.4	94.4
% P		6.76		

6-xi.

Preserving from  $\text{Ter}^+$  DNA & solo HCOOH (again!)

3.5 mg  $\text{Ter}^+$  DNA (even)  $\uparrow$  0.5 ml HCOOH. Dilute further in  
+ tube: (A) add 0 = 0.1 ml  $\rightarrow$  175° 30 min (new silicon in  
 •  
 60 °C  
 40 °C  
 20 °C  
 10 °C  
 + tube:  
 (B) add 0.2  $\rightarrow$  0.3  
 (C) add 0.4  $\rightarrow$  0.5  
 (D) add 0.6  $\rightarrow$  0.7  
 } ball. Dry  $\uparrow$  25 µl. Take 84  
 parts, 2.11 for P.

	G	A	OC <sub>209</sub>	T	"Y" (P <sub>204</sub> )
B	91	60	50	81	80 66 59 56
	92	58	49	81	78 61 58 52
	92	59	50	81	78 64 59 54
a	332	586	233	412	100 89 82 77
	334	586	231	428	113 99 91 84
	333	586	232	420	107 94 87 81
	32-3	527	182	339	29 30 30 27
b	344	586	248	406	77 70
	345	584	247	411	76 70
	345	585	248	409	77 70
	32-3	526	198	328	13 13
c	341	580	244	409	78 68
	346	580	248	409	78 71
	344	580	246	409	77 70
	32-3	521	196	328	13 13
d	338	571	247	400	76 69
	342	574	247	404	79 72
	340	573	247	402	78 71
	32-3	514	197	321	14 14

	a	b	c	d
<i>Yards Rate MPH</i>				
A	5.24	32.0	4.11	32.3
T	5.51	33.7	4.29	33.8
G	2.96	18.1	2.30	18.1
OC	2.65	16.2	2.02	15.9
	6.36	100.0	12.72	100.1
P, Y	4.76 4.80	4.78 3.94	3.89 3.87 3.84	3.80 3.85 3.87
A-346	16.52	13.39	11.41	13.38
R	99.0	98.1	98.6	98.0
70P	4.09	3.35	2.86	3.31

7-1-52.

Phases

- (a) 1.52 avg T<sub>4+7</sub> of 22.4 ↑ 0.6 sec HCO<sub>3</sub>  
 (b) 1.50 avg T<sub>4+7</sub> of 31.4 ↑ - 175° 30 min. Avg. + 21 sec.  
 (c) 1.50 avg T<sub>4+8</sub> of 31.4 ↑ - Take 8.4 sec, 3+1.97 for P.  
 (d) 1.52 avg T<sub>4+7</sub> of 31.4 ↑ 0.65 sec HCO<sub>3</sub>

	G	A	OC	T
B	1 92 2 [92] 2 92	60 60 60	57 49 50	80 81 81
a	1 416 2 420 2 418 2-3 326	742 740 741 681	310 304 307 257	519 519 519 438
b	1 345 2 345 2 345 2-3 253	593 594 594 534	247 244 246 196	424 419 422 341
c	1 307 2 303 2 305 2-3 213	560 563 562 442	220 220 220 170	370 361 366 285
d	1 356 2 360 2 358 2-3 266	614 613 614 554	254 252 253 203	426 428 427 346

	a	b	c	d
<i>Virus</i> : Rautin Methyl P				
A	4.20	32.5	1.881	32.4
T	4.24	32.9	1.320	32.2
G	2.36	18.3	0.764	18.6
OC	2.11	16.35*	0.691	16.9
	12.91	100.05*	9.106	100.1
			1552	100.05*
P/V	44.47	47.13	1.32	1.32
	41.8	13.38	1.32	1.32
x 324	12.24	4.28	15.59	17.29
R	96.6	96.0	99.6	87.9
% P	7.27	—	3.96	4.25

13-xi-52. Phages & DNA's.

General Procedure: (a) T2<sup>r+3</sup> DNA 0.67 mg. (b) T2<sup>r-2</sup> DNA 0.71 mg. (c) T2<sup>r+5</sup> of 22-v phage 1.47 mg. (d) T6<sup>r-6</sup> of 31-xi phage 1.52 mg. All r 0.5' and 1400 rpm. Cook 170° 30 min. Dry. ↑ 2.5μl, 100.8-4μg/ml 2.11 for P

	G	A	OC	T
B	1 97 2 95 3 96 2.3 260	62 60 61 546	53 52 53 205	87 80 84 337
a	1 356 2 356 3 356 2.3 260	603 610 607 546	259 257 258 205	423 419 421 337
b	1 180 2 178 3 179 2.3 84	237 231 234 173	121 119 120 67	193 185 189 105
c	1 404 2 403 3 404 2.3 308	705' 710 708 647	309 304 307 254	493 488 491 407
d	1 393 2 391 3 392 2.3 296	696 697 697 636	299 295' 297 244	492 485' 489 405'

10 XII '52 Chromatogram

(a)

Fuel 2.484.730 4.783 14.787 4.782

MT 2.678 2.6% 2.693 2.683

Dif. 2.102 2.087 2.09+ 2.099

Labeled & to low from separation.

(d)

6.124.589 24.587 35.564 24.584

16.910 16.900 16.876 16.865

2.678 7.687 7.688 7.699

7.69+ 7.71 µl

29-x-52.

Micro-pipette calibration.

Adenosine  $\text{PO}_4$  (6 mg/ml) delivered on filter paper & diluted in 4 ml of  $\text{H}_2\text{O}$ .  
Read at 260.

Blank	(a)	(b)	(c)	(d)	(e)
paper.	new	commercial	8.40	new	new
52	<u>2.68</u>	2.46	8.82	8.16	9.95
53	2.61	2.49	8.82	8.19	-
52	<u>2.64</u>	2.49	8.82	8.18	9.97
52	2.63	2.47	8.82	8.18	9.96
2-8	2.11	1.95	8.30	7.66	9.44
Volume based on (c) = 8.40 (gravimetric)	<u>2.13</u>	<u>1.97</u>	[8.40]	7.78	9.55

12-1:

Repeat from same soln., but delivering 2 pipetfuls from 1.97+ 2.11.

B	(a)	(b)	(c)	B	x4	x4
55'	4.67	4.44	8.93	55'	<u>1.618</u>	8.94
54	4.66	4.32	8.91	55'	<u>8.36</u>	8.88
54	<u>4.66</u>	<u>4.47</u>	<u>8.92</u>	-	-	-
54	4.66	4.41	8.92	55'	8.91	8.92
2-8	4.12	3.87	8.38	7.81	8.36	8.37
Per filling	2.06	1.94	-	1.95	2.09	-
Vol blankatio	<u>2.06</u>	<u>1.94</u>	-	<u>1.96</u>	<u>2.10</u>	-

$\text{P}^32$

BSNA	8-x	7.94
	19-x	7.21
	1-x	8.10
	2-x	7.64

Additional Preliminary

Date 9/8

- - -

Base ratios	Conditions A, T, G, C, $\text{P}^32$					101
	BSNA	8-x 0.95 und Hecm	28.0	27.5	22.6	20.5
9-x 0.95 und	27.8	27.8	22.5	20.8	101	
1-x 0.9 und	27.9	27.5	22.5	20.8	99.8	
3-x 0.9 und	28.0	26.2	23.2	21.2	97.5	
					1-A	5.74
						100
		27.9	27.25	22.9	20.8	[13]

Plaque T4 DNA

1-x 5.60  
4-x 6.81

Phage DNAs: analyses in improved conditions (0.7 mg  $\text{A}$ , 0.25 ml Hecm).

Aph	Date	Conditions	A	T	G	C	Precip.	
			8-x	32.4	32.1	18.1	17.4	
T2 r-2	21-x 13-x	0.5 und Hecm, small sample	32.4	32.8	18.1	16.6	96 +89	
			32.4	32.2	18.6	16.9	96.0 -	
			32.4	32.4	18.7	17.0		
T2 r+ (dd)	21-x 13-x	0.5 und Hecm	32.6	32.4	18.05	16.95	97 7.50	
			32.5	32.9	18.3	16.25	96.6 7.27	
T2 r+-1 (d)	21-x		32.5	32.4	18.4	16.7	97 6.13	
			32.5	32.6	18.35	16.7		
T4r								
T4r <sup>2</sup>								
T6r								
T6r <sup>2</sup> (sc)	8-x 21-x		32.7	32.9	18.4	16.0	102 5.94	
			33.1	32.4	18.1	16.4	98.6 6.38	
T6r <sup>2</sup> (sm)	9-xii 6-xi 21-VIII 28-mm 8-x 15-x 16-x 16-x	0.5 und 0.5 und 21-x 32.7 32.4 32.2 32.2 32.7 32.7 32.7 32.7	32.1 32.6 32.7 31.9 32.2 32.2 32.2 32.7 32.7 32.7	32.7 32.5 32.4 31.9 32.2 32.2 32.2 32.7 32.7 32.7	18.4 18.5 19.0 18.35 18.35 17.3 17.3 18.0 18.0 18.0	16.8 16.4 16.9 17.35 17.35 10.1 10.1 16.9 16.9 16.9	98.8 95.7 98.2 102.6 6.51 10.1 6.68 100.3 6.76 98.6	95.7 6.51 98.2 102.6 6.51 10.1 6.68 100.3 6.76 98.6
	Hecm, und 0.5 und Hecm	21-x 32.4	32.5	32.6	18.6	16.6	98.3 6.94	

Calculation of P based on weight:

$$V/d = 25 \times 0.976 + 1.50 \times 0.7 = 24.4 + 1.1 = 25.5 \mu\text{l}$$

$$\%P = \frac{P}{W} \times \frac{25.5 \times 1000}{1000} = \frac{P}{W} \times 1300$$

$$\text{or } \frac{P}{W} \times \frac{25.5 \times 1000}{1000} = \frac{P}{W} \times 1.213$$

$$V/d = 24.4 + 0.7 \times 0.7 = 24.9 \mu\text{l}$$

$$\%P = \frac{P}{W} \times 24.9 = \frac{P}{W} \times 1.270$$

$$\text{or } \frac{P}{W} \times \frac{24.9}{2.10} = \frac{P}{W} \times 1.185$$

Calculation of P based on weight:

$$V/d = 25 \times 0.976 + 1.50 \times 0.7 = 24.4 + 1.1 = 25.5 \mu\text{l}$$

$$\%P = \frac{P}{W} \times \frac{25.5 \times 1000}{1000} = \frac{P}{W} \times 1300$$

$$\text{or } \frac{P}{W} \times \frac{25.5 \times 1000}{1000} = \frac{P}{W} \times 1.213$$

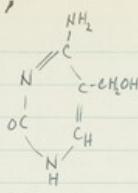
$$V/d = 24.4 + 0.7 \times 0.7 = 24.9 \mu\text{l}$$

$$\%P = \frac{P}{W} \times 24.9 = \frac{P}{W} \times 1.270$$

$$\text{or } \frac{P}{W} \times \frac{24.9}{2.10} = \frac{P}{W} \times 1.185$$

Whole phage: analysis in improved conditions. 1.5 ml phage + 0.5 ml 4M NaCl

Preparation	Date	Conditions	A	T	G	OC	Purity	$\%P$
			32.4	33.0	17.8	16.8	97.9	3.50
T2r-3 (1-1-53)	12-1-53		32.6	33.8	17.4	16.2	97.1	3.86
	12-1-53		32.1	33.4	17.6	17.0	92.4	
	6-11		32.1	33.4	17.6	16.9	96.5	
	12-11		32.3	33.4	17.6	16.7	95.3	
T2r <sup>+</sup> -5 (22-v)	1-1-X	big tube	32.0	33.2	17.8	17.0	99.6	3.44
	1-1-X	small tube; 12000 rpm	31.9	33.2	17.9	17.0	99	4.02
	7-11		32.0	33.7	18.1	16.2	99.0	4.09
	8-11		32.1	33.0	18.05	16.9	99.6	3.9%
			32.0	33.3	18.0	16.8		
T4r-7 (21-x)	7-11		32.3	33.8	18.1	16.9	95.1	3.35
	(=)		32.3	33.2	17.9	16.6	93.8	3.49
	22-11							
T4r-8 (21-x)	7-11		31.9	33.6	18.1	16.4	93.6	2.96
			32.2	33.7	18.0	16.3		
T4r <sup>+</sup> -7 (21-x)	7-11	blunt end caps 7.75 μl	32.5	33.2	18.45	15.9	98.0	3.31
	(=)		32.1	33.1	18.0	16.7	94.4	3.09
	6-11		32.2	33.0	18.4	16.3	96.2	3.35
			32.0	33.1	18.3	16.2		
T5r-6 (fusopref)	1-X	big tube	31.9	33.8	17.2	17.1	91.9	2.73
	1-X	small tube	32.8	33.0	18.15	16.1	83.4	4.16
	(21-x)		32.2	33.5	17.7	16.6	87.9	4.25
T5r <sup>+</sup> -2	10-1X	0.4 ml in big tube	32.2	33.0	17.9	16.95	99.4	3.75
	1-X	small tube	32.8	33.1	18.2	15.9	96.4	2.99
T6r <sup>+</sup> -1 (1-1-53)	12-1-53		32.7	33.4	18.15	15.65	99.3	3.36
	12-1-53		32.7	34.1	17.3	16.0	99.4	3.22
	12-11		32.7	33.4	17.6	16.8	97.9	
			32.5	33.11	17.8	16.2		



5'-methyl-cytosine

576

$\text{f}_{\text{m}}$

$\text{H}_2\text{O}$

M	C	H	O	N
---	---	---	---	---

Cytosine  $\text{C}_4\text{H}_5\text{ON}_3$  111.2 43.2 4.53 14.4 37.8

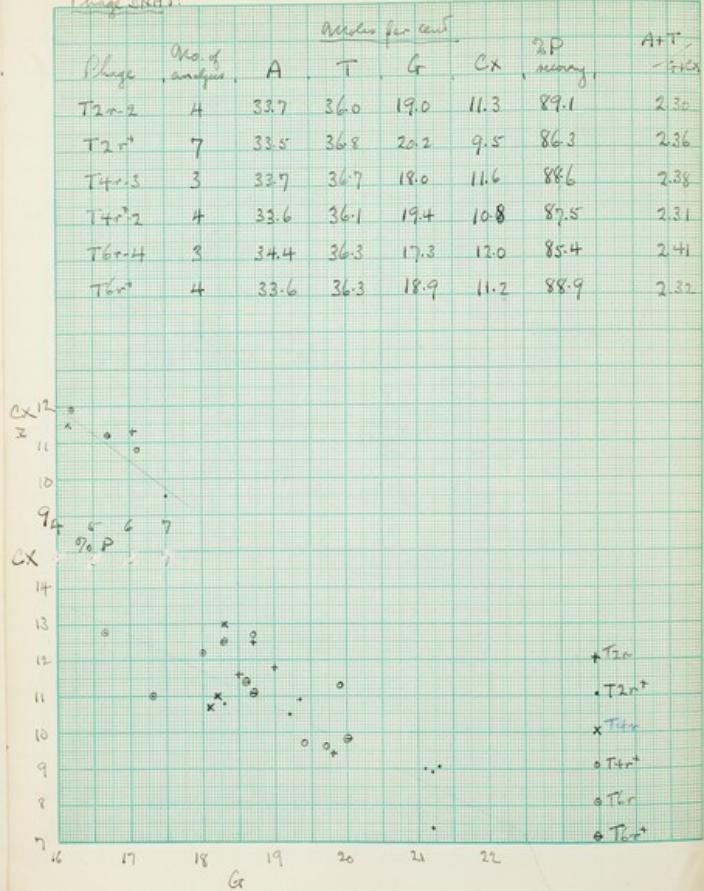
5'-methyl  
cytosine  $\text{C}_5\text{H}_7\text{ON}_3$  125.3 48.0 5.64 12.8 33.6

5'-methyl  
cytosine  $\text{C}_5\text{H}_7\text{ON}_3$  141.3 42.6 5.00 22.7 29.8 26

Cytosine- $\text{H}_2\text{O}$   $\text{C}_4\text{H}_4\text{O}_2\text{N}_3$  129.2

32.5

Phage DNA's:



Phage DNAs: HCOH hydrolysis: Molar per cent.

	A	T	G	Cx	P molar
T2r-2	5-ii	34.1	36.8	19.8	9.4
	17 <sup>o</sup> 24 <sup>o</sup> m	33.8	36.1	18.5	11.6 ←
	17 <sup>o</sup> 25 <sup>o</sup> m	33.1	36.1	19.0	11.8 ←
T2r <sup>+</sup> ③	5-ii	33.9	35.0	18.7	12.5 ←
T2r <sup>+</sup> 2-1	21-1	32.2	37.6	21.1	9.0 ←
	1-ii	33.5	36.3	19.3	10.9 ←
	4-ii	33.7	36.6	19.2	10.5 ←
T4r-3	5-ii	33.8	37.6	21.2	7.4 ←
	27-ii	34.3	36.6	18.3	10.8 ←
	17 <sup>o</sup> 23 <sup>o</sup> m	33.2	36.3	21.3	9.05 ←
					91.3
T4r <sup>+</sup> 2	4-ii	34.0	36.7	18.2	11.0 ←
	17 <sup>o</sup> 24 <sup>o</sup> m	34.4	32.9	18.5	11.1 ←
	17 <sup>o</sup> 27 <sup>o</sup> m	33.6	35.7	17.8	12.0 ←
	2-iv	33.4	37.8	18.1	10.7 ←
T6r-4	5-ii	33.8	36.9	19.7	9.6 ←
	17 <sup>o</sup> 25 <sup>o</sup> m	33.2	35.6	19.9	11.3 ←
	17 <sup>o</sup> 27 <sup>o</sup> m	34.2	34.4	18.7	12.7 ←
	2-iv	33.3	37.6	19.4	9.7 ←
T6r <sup>+</sup>	5-ii	35.1	36.6	17.3	11.0 ←
	17 <sup>o</sup> 24 <sup>o</sup> m	33.8	36.1	18.0	12.2 ←
	17 <sup>o</sup> 27 <sup>o</sup> m	34.3	36.2	16.6	12.8 ←
T6r <sup>+</sup>	5-ii	34.1	35.9	18.6	11.4 ←
	17 <sup>o</sup> 24 <sup>o</sup> m	33.7	35.4	18.3	12.5 ←
	17 <sup>o</sup> 26 <sup>o</sup> m	33.0	37.1	20.0	9.8 ←
	2-iv	33.4	36.8	18.7	11.1 ←
					91.7

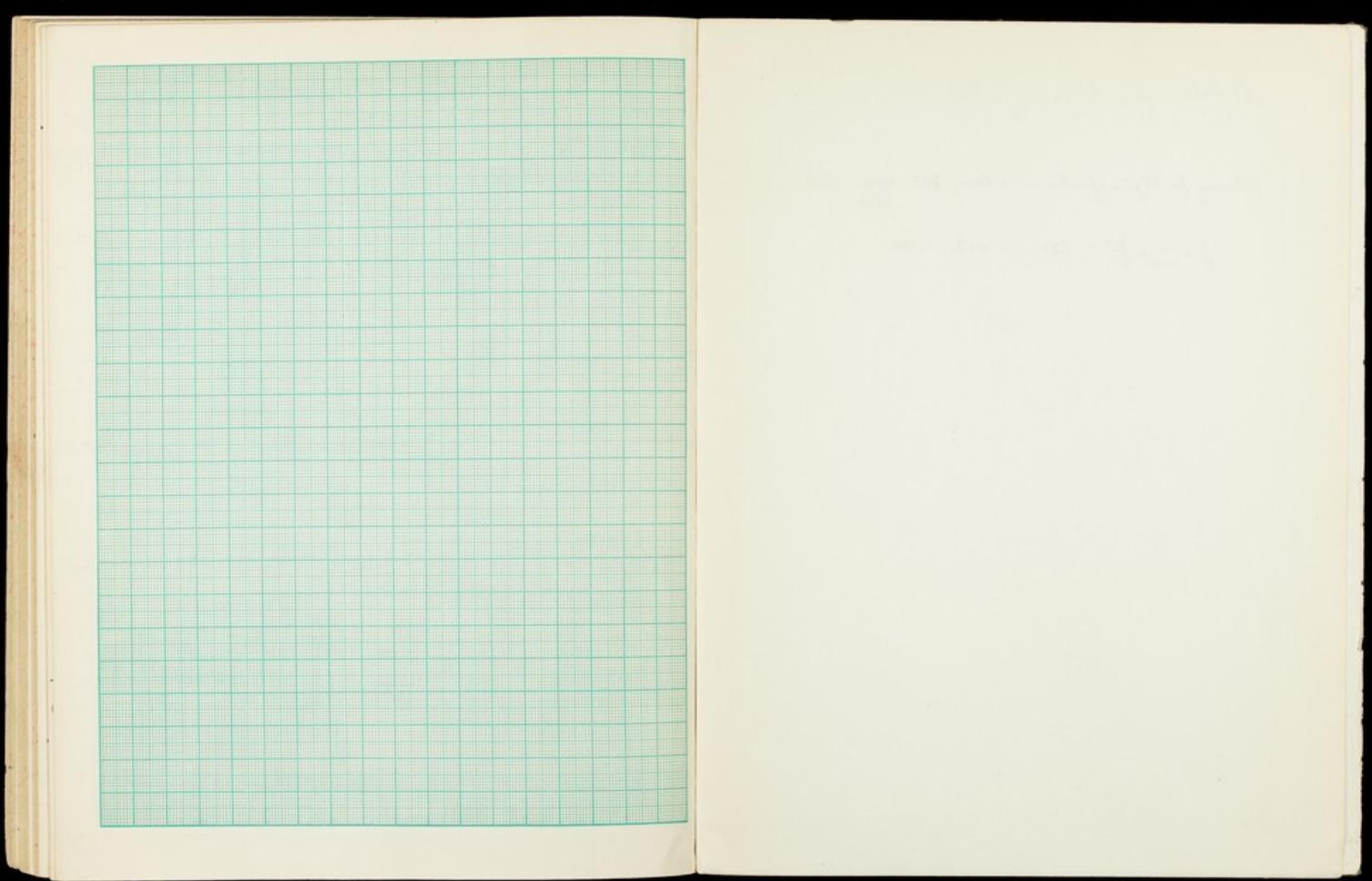
Phage DNA's HCOOH hydrolysis.

	Date	A	T	G	OC	%R
T2 <sup>r-2</sup>	28.viii.52 and 19.viii.52	33.4	34.3	17.8	146	89.6
	19.viii.52	33.0	32.5	18.4	16.2	100

T6<sup>r+</sup>  
(open)

Whole phages, HCOOH hydrolysis.

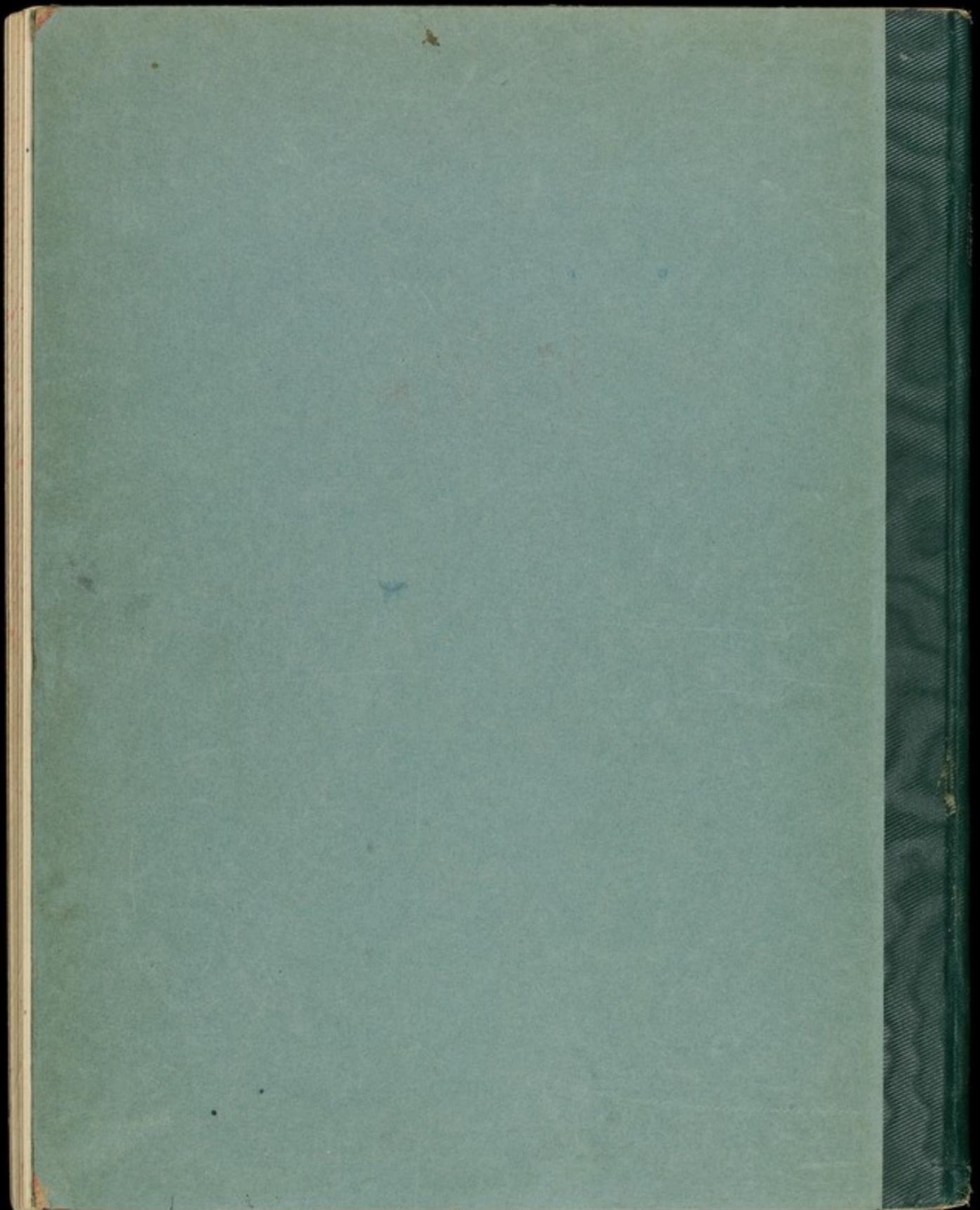
Phage	Date	A	T	G	OC	% P recovery
T2 <sup>r-2</sup>	28.viii.52	33.3	35.2	17.9	13.7	91.0
	19.viii.52	32.9	35.3	17.3	14.5	[102]
T4 <sup>r-2</sup>	5.xi.51	32.6	36.2	17.7	13.4	87.3
T4 <sup>r+</sup>	5.xi.51	32.6	35.5	18.2	13.7	91.4 (?)
T6 <sup>r-6</sup>	5.xi.51	33.3	36.2	17.2	13.4	77.5 (?)
T6 <sup>r-6</sup>	28.viii.52	33.2	38.4	14.9	12.5	83.4
	19.viii.52	32.8	37.9	14.9	14.5	90.1
T6 <sup>r+</sup>	5.xi.51	33.1	37.2	17.9	11.8	91.1
T6 <sup>r-2</sup>	28.viii.52	33.3	34.0	17.8	14.9	93.1



$$P \text{ factor } \frac{1}{31} \times \frac{8.3}{4.00} \times \frac{1}{1.95} = 343$$

Allowing for 28 µl evaporation in 18 hours,  $\frac{343 \times 4.000}{3.972} = 346$

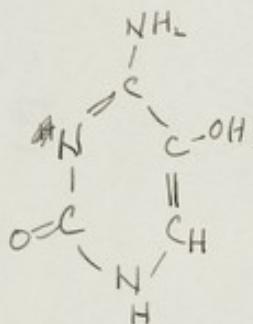
$$\frac{1}{31} \times \frac{1}{4.00} \times \frac{8.4}{2.11} = 321 \quad + 1\% = 324$$





ROYAL YORK HOTEL  
TORONTO

Canadian Pacific Hotels



Guanine and CX ratios in phage DNA.

	21-i	1-ii	4-ii	5-ii	27-ii	5-iii	170° 24-iii	170° 25-iii	170° 27-iii	2-iv
T <sub>2r</sub>	G					19.8	18.5 <sup>-</sup>	19.0		18.7 <sup>-</sup>
	CX					9.4 <sub>26</sub>	11.6 <sub>29</sub>	11.8 <sub>26</sub>		12.3 <sup>-</sup> <sub>21</sub>
T <sub>2r<sup>+</sup></sub>	G	21.2	19.3	19.2	21.1, 21.2	18.3		21.3		
	CX	8.9 <sub>18</sub>	10.9 <sub>32</sub>	10.5 <sub>23</sub>	9.0, 7.4 <sub>27, 22</sub>	10.8 <sub>21</sub>		9.05 <sub>20</sub>		
T <sub>4r</sub>	G					18.2		17.8	18.1	
	CX					11.0 <sub>22</sub>		13.0 <sub>19</sub>	10.7 <sub>32</sub>	
T <sub>4r<sup>+</sup></sub>	G		19.7				19.9	18.7	19.4	
	CX		9.6 <sub>29</sub>				11.3 <sub>21</sub>	12.7 <sub>23</sub>	9.7 <sub>28</sub>	
T <sub>6r</sub>	G					17.3	18.0		16.65 <sup>-</sup>	
	CX					11.0 <sub>18</sub>	12.2 <sub>16</sub>		12.8 <sub>21</sub>	
T <sub>6r<sup>+</sup></sub>	G					18.6	18.3	20.0		18.7
	CX					11.4 <sub>24</sub>	12.5 <sub>21</sub>	9.8 <sub>26</sub>		11.1 <sub>25</sub>



ROYAL YORK HOTEL  
TORONTO

Canadian Pacific Hotels

w

Phage DNAs - P content from base estimations.

	21-i	1-ii	4-ii	5-ii	27-ii	5-iii	24-iii	25-iii	27-iii	2-iv	3-iv
T2 <sup>r</sup>						6.6	6.6	6.7		4.6	6.1
T2 <sup>r+</sup>	3.9	8.6	8.5	7.9, [4.8]	8.0			5.2			
T4 <sup>r</sup>						4.7			3.6	4.7	4.4
T4 <sup>r+</sup>				7.5				5.4	5.0	7.1	6.2
T6 <sup>r</sup>						4.8	3.5		5.0		4.4
T6 <sup>r+</sup>						6.3	4.5	4.4		6.5	5.4

163