Phage Compilations

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11 March 1952

Dear Dr. Wyatt,

This past weekend Dr. Cohen brought to my attention your very interesting work with phage. Certainly your findings, particullarly those related to cytosine, have evoked a great deal of interest on my part. In an effort to reconcile your data with our reported cytidylic acid , the following points should be raised. 1. Because the virus was considered to be pure DNA, and since the amounts of material in the radioactive studies was limited, only paper separations were used in isolation of the purine bases and pyrimidine nucleotides. (Ina ll bacterial and tissue work resin purifications were used in addition to insure complete separation of RNA and DNA components.)
2. A hydrolysate of virus DNA run on paper in the tert butyl alcohol solvent gives bands compaprable to those produced by thymidylic acid, desoxycytidylic acid, adenine and guanine from known DNA preparations.
3. The"cytidylic" acid band, after elution, gives a spectrophotometric curve consistent with that of desoxycytidylic acid - max 278 - with some flattening toward 276 as has been noted with DNA cyt. RNA cytidylic gives a sharp peak at 278.
4. Since cytosine could run to the cytidylic acid region of the paper in that solvent , it is possible that our so-called "cytidylic acid" could have hydrolyzed under our conditions, producing your "c" substance which in turn could go to the cyt region - giving the max 278 which you describe. In such a case neither Rf values or spectrophotometric curves would alert one to the anomolous situation which your work has brought forward.

Your work, however, did bring back to mind one analysis which I did long ago when I first embarked on chromatographic separations and virus studies at the Univ of Pa. The crudeness and confusion in the methods in those early days took the blame for many aberrations in results, and rightly so. However, one peculiar result was never checked and indeed never occurred to me again until Seympur mentioned your findings. Essentially th matter was this. Having run some cytidylic acid from Tor φ on paper and having obtained agreeable Rf and curve spectrophotometrically - a companion band was eluted and the eluate put on a Dowex resin column. Nothing of significance was eluted with water or .002 N acid. (RNA cyt is eluted with .002 N). However, when .01 N acid was put on (which normally elutes desoxycytidylic with max of 278) material was eluted with max of 282 and giving total extinction of .350 in 10 cc. In addition material was eluted with stronger acid with max of 274 and giving ext of .245 in 10cc. These of course mixed would give a max in region of 278 and thereby be condidered cytidylic acid on paper studies alone. This, if all true, - and many

more exps would be necessary to consider it so - presents an amazing series of coincidences , the existence and significance of which completely escaped me until your letter propmpted a perusal of data and notes collected early in the game.

It is true that in general the nucleotides have a spectro peak 3-4 mu above that of the free base; and if what we have on the resin is the nucleotide of your "c" substance then a max of 282 is consistent at least with a base max of 278 as you report it.

Of course it is to be remembered that the material, whatever it is, becomes radioactive in the presence of labeled host pyrimidines and in accordance with what one might expect if the labeled host cytosine were transferred. This of course raised the question, as Seymour has emphasized, of the existence of such material in the bacteria. In the past resin studies with bacteria or tissue have not revealed such a substance, although we have had in all DNA prep a substance coming off the resin column with 0.1 HCl with max of 274. We do not know what this is, unless possible it is a dinucleotide of thymidylic and cytidylic. If indeed the virus contains a substance not present in the bacteria , but which is closely related by transfer and radioactive studies to bacterial cytosine, we then have before us another extremely unusual coincidence.

In the past year my duties as a medical officer have kept me busy, but I now find myself in a postion with facilities for looking further into this matter at least at the nucleotide level. If such a course of action is agreeable to you and if I can get material to study I shall plan to investigate this matter further.

Certainly the possibliety of a qualitative difference between host and virus is a tremendous stimulus to more work. Also I shall follow with the greatest of interest your very fruitful studies with phage.

Sincerely yours,

Larry Weed.

Lawrence L. Weed

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AMSCS
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PHAGE INFORMATION SERVICE

No. 6

Phage meeting, Cold Spring Harbor, Long Island

August 20 - 22, 1951

(Appended summaries of papers in press by various authors)

NB These abstracts do not constitute a publication. They are distributed to the participants of the discussion and a very few others only. They should be treated as a private communication, and should be quoted as such only after obtaining the consent of the respective author.

Cohen, S. S. - The laboratory has been engaged in the following: -

1. Weed, L. L. and Cohen, S. S. - Pyrimidines from host nucleic acid are transferred to form virus DNA.; however only early virus obtained host material. The last 3/4 of intracellular virus received none of the host pyrimidine. This is in contradiction of the report from Chicago that each virus particle receives the same contribution from host DNA.

Host pyrimidines were labelled by growth of B in C^{14}-orotic acid. Each pyrimidine nucleotide was isolated and its label estimated. $T6r^+$ and T6r were produced in aliquots of the same labelled host. Intracellular $T6r^+$ was isolated during the latent period by means of CN^- lysis. The pyrimidine nucleotides of the virus preparations were isolated. Early $T6r^+$ and T6r were far more heavily labelled than late T6r. The extent of labelling of virus was inversely proportional to the DNA synthesis corresponding to the virus isolated.

2. Growth of B on gluconate is almost as good as on glucose. However, $T2r^+$ and DNA synthesis on gluconate were poor, as compared to these functions on glucose. Experiments with C_1-labelled gluconate have shown that this substance was metabolized in growth or $T2r^+$ infection via the oxidative pathway through pentose phosphate. This pathway can not maintain a stimulated DNA synthesis in virus infection.

In experiments with C_1-labelled glucose, the oxidative pathway in growing B accounted for 16% (minimum) to 37% (maximum) of the glucose used, the remainder presumably passing through the Meyerhof pathway. These figures do not represent variability in the data but the two extreme interpretations of highly reproducible data. On infection the use of the oxidative pathway was greatly reduced and glucose was shunted into the Meyerhof scheme. The pivotal point of the P and nucleic acid shunts observed in comparing growth and virus infection appears to be in the utilization of glucose-6-phosphate. Also our experiments suggest that in these systems the main source of ribose for RNA is the oxidative pathway whereas the major source of desoxyribose for DNA is the Meyerhof scheme.

3. Lanning, M. and Scott, D. B. M. are continuing work on various aspects of the oxidative pathway. E. coli strain B has now been shown to be basically similar to yeast with respect to the presence of the enzymes and intermediates of the oxidative pathway. In addition various E. coli mutants have been demonstrated to contain adaptive D-arabinokinase and D-ribokinase.

$\frac{31}{14} = 2.214$

	P/N wt. ratio	N/P atomic ratio		DNA bases/P (moles/g. atom)
T2r+	0.316	$\frac{1}{0.316} \times \frac{31}{14} = 0.701$	7.0	.99
T2r	0.318	0.696	7.0	.95
T4r+	0.319	0.694	6.9	.96
T4r	0.329	0.673	6.7	.94
T6r+	0.306	0.724	7.2	.99
T6r	0.329	0.673	6.7	.88
T6r+ DNA		3.85		
Hershey r / Barlow T2	0.324	6.83		

14
43
2)57
29

T6r+ DNA Mean of 10 analyses Sept. 8 — Dec. 9, 1952.

	A	T	G	OC	% D recovery
Total	325.6	325.2	183.4	167.3	991.3
Mean	32.6	32.5	18.3	16.7	99.1
Corr. for total	32.5	32.5	18.3	16.7	
Corr for HMC loss	32.3	32.2	18.2	17.3	99.8

	Corr. for total		Corr. for HMC loss 4%	
3256	− 5	= 3251	3251 − 22	= 3229
3252	5	3247	3247 − 22	= 3225
1834	3	1831	1831 − 12	= 1819
1673	2	1676	1739 − 12	= 1727
10015		10000	10068	10000

Recovery of 5-hydroxymethylcytosine subjected to hydrolytic conditions in formic acid in the presence of thymus DNA.

Expt. No.	µl. HCOOH / mg. DNA	Conditions of heating	Yields from Thymus DNA		Recovery of added hydroxymethylcytosine	
			Adenine Mols./mol.P	Cytosine Mols./mol.P	Mols./mol.P	Hydrolyzed / Unhydrolyzed
1	780	100° 2 min.	0.260	-	0.146	-
		165° 30 min.	0.252	0.191	0.112	0.77
		170° 30 min.	0.251	0.192	0.106	0.73
		175° 30 min.	0.247	0.189	0.099	0.68
		175° 30 min*	0.249	0.186	0.066	0.45
2	83	100° 2 min.	0.256	-	0.175	-
		175° 30 min.	0.252	0.186	0.134	0.77
		178° 30 min*	0.258	0.193	0.108	0.62
3	259	100° 2 min.	0.256	-	0.215	-
		175° 30 min.	0.256	0.192	0.187	0.87
4	357	100° 2 min.	0.244	-	0.206	-
		175° 30 min.	0.255	0.187	0.195	0.95
5	600	100° 2 min.	0.246	-	0.330	-
		175° 30 min.	0.255	0.196	0.318	0.965

Tables.

1. Ultraviolet absorption data on OC.

2. Recovery of OC from hydrolysis.

(3. Max. uracil content of phages.)

4. Composition of phage DNA's

5. Composition from whole phages

(6. Max Cx values in coli + TNA.)

Bacteriophage compilations

	A	G	C	T	Max U	% P recovery
T4r-2. 19-VI	1.438	0.824	0.397	1.341	0.035⁻	
4-VII [NaOH]	1.689	1.087	0.145⁻	1.083	.021	
21-VII	1.65⁻	1.08	0.10	1.17	.03	
(NaOH 1-XI. HCOOH.	1.32	0.72	0.49	1.47		86
T4r-7 26-VI	1.545⁻	0.913	0.231	1.311	0.032	
4-VII [NaOH]	1.590	1.023	0.117	1.264	0.032	
25-VII. RNase	1.61	1.00	0.057	1.33	0.041	
31-X H_2O	1.54	0.89	0.129	1.45⁻	0.037	81.6
T4r⁺-7 22-VI	1.540	0.980	0.110	1.370	0.036⁻	
26-VI	1.558	0.903	0.168	1.372	0.027	
1-VIII RNase	1.657	0.938	0.054	1.347	0.042	[85.3]
5-XI. HCOOH	1.32	0.78	0.60	1.44		90
T4r⁺-6 13-X	1.575⁻	0.896	0.086	1.443	~~0.036~~	82.5⁻ ✓
26-X RNase	1.56	0.92	0.089	1.43	0.036⁻	76 ✓
26-X H_2O	1.59	0.93	0.076	1.41	0.015⁻	87 ✓
T6r-6 22-VI	1.622	0.994	0.042	1.340	0.034	[78]
4-VIII	1.646	0.963	0.039	1.350	0.043	
1-VIII RNase	1.619	0.912	0.042	1.430	0.035⁻	[86.8]
30-X	1.576	0.876	0.149	1.428	0.037	[69.4] ✓
5-XI. HCOOH	1.35	0.90	0.49	1.47		~~76~~
T6r⁺-6 13-X	1.521	0.871	0.231	1.376		75.8 ✓
30-X	1.521	0.866	0.230	1.381	0.093	71.9 ✓
T6r⁺-7 26-VI	1.610	0.935⁻	0.037	1.418	0.025⁻	[96]
	1.607	0.952	0.060	1.381	0.052	
25-VI NaOH	1.57	1.05⁻	0.097	1.28	0.054	
1-XI HCOOH	1.34	0.72	0.43	1.50		90.2

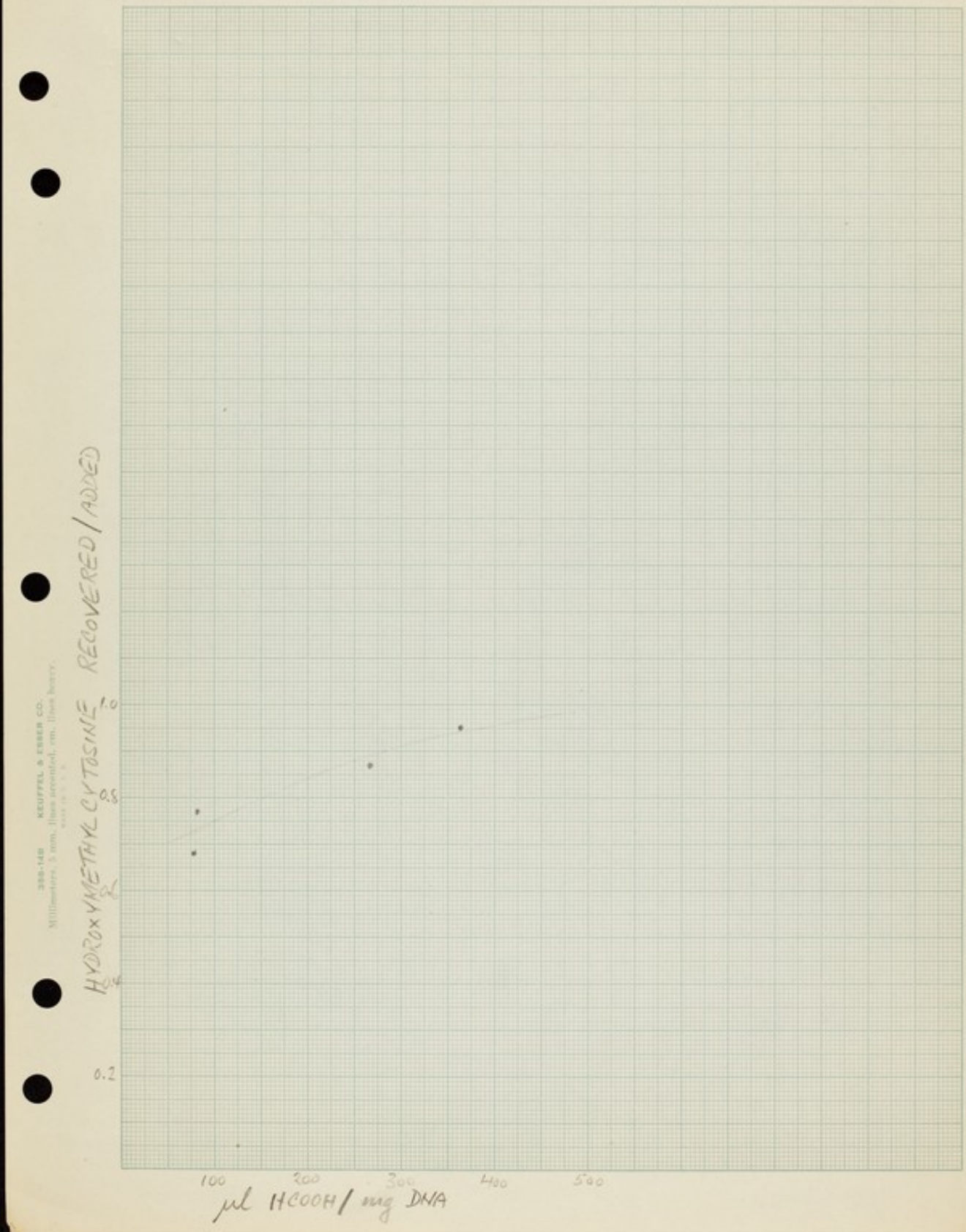
% Virus in $HClO_4$	% P accounted for
-	93
40	[97.3]
28	
24	90.6
26	87.6
29	
38	89.5
34	92.1
27	
43	88.5
	[86.4]
32	68
~~40~~	82.4
39	86.2
25	
17	
36	91
36	98.7
30	89.1

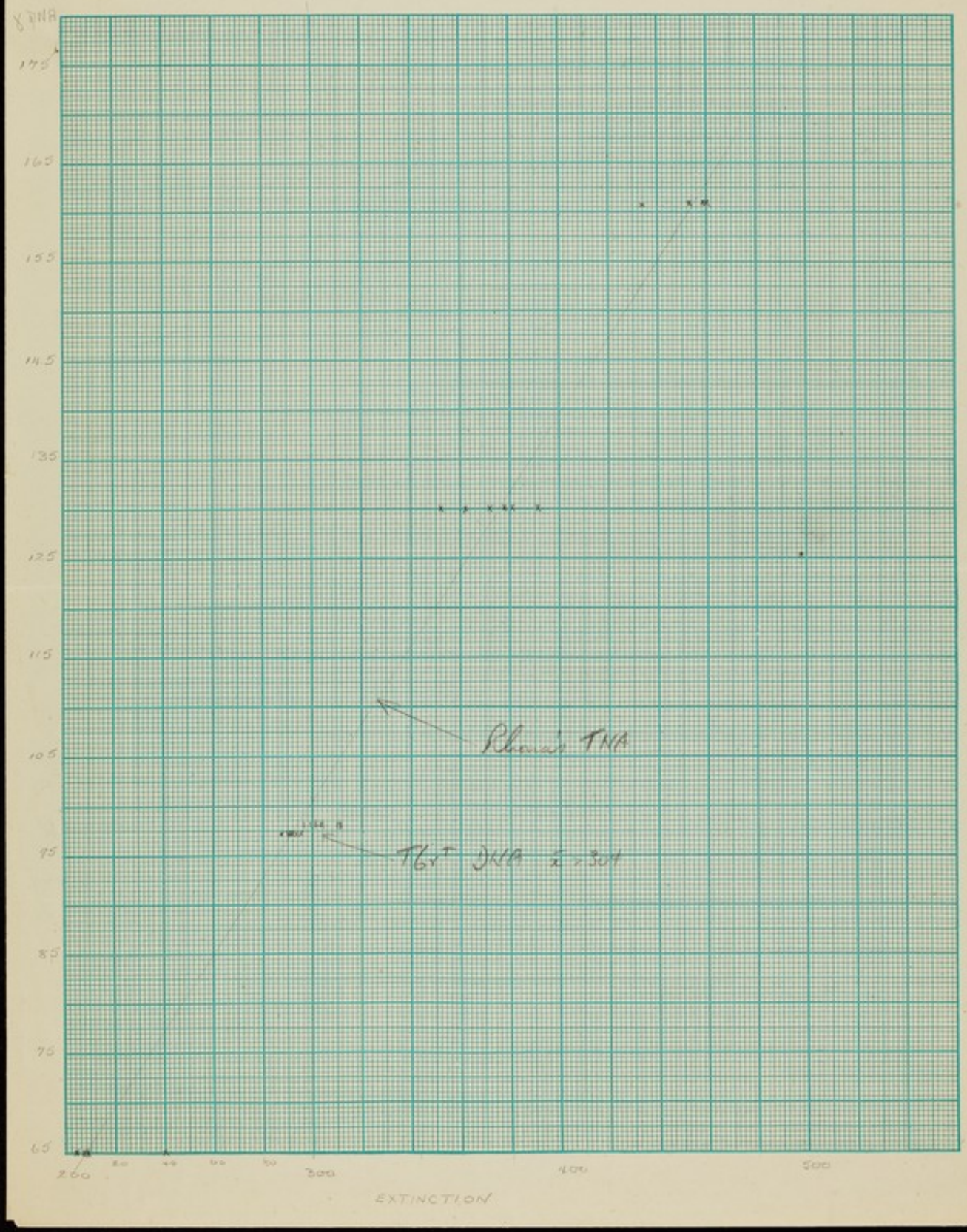
Bacteriophage compilations

		A	T	G	C	MAX U	? P
T4r-8	19-UI	35.9	36.6	20.6	9.9	0.88	
	4-UIII NaOH	42.1	27.0	27.1	3.6		
	21-UII NaOH	41.25	29.1	27.0	2.7		
	1-XI HCOOH	33.0	36.8	18.0	12.2		
T4r-7	26-UI	38.6	32.8	22.8	5.8	0.80	
	4-UII NaOH	39.8	31.6	25.6	2.8		
	25-UII RNase	40.3	33.1	25.0	1.4		
	31-X H_2O	38.5	36.3	22.1	3.2	0.92	
$T4r^+$-7	22-UI	38.5	34.1	24.5	2.7	0.68	
	26-UI	38.8	34.4	22.6	4.2	0.88	
	1-UIII RNase	41.4	33.6	23.4	1.35		—
	5-XI HCOOH	33.0	36.0	18.3	12.5		
$T4r^+$-6	13-XI	39.4	36.2	22.4	2.1		
	26-X RNase	39.0	35.6	23.0	2.2		
	26-X H_2O	39.8	35.2	23.4	1.9	0.38	
T6r-6	22-UI	40.6	33.4	24.8	1.5	0.85	
	4-UIII	41.1	33.7	24.0	.9	1.0	—
	1-UIII RNase	40.3	35.7	22.8	1.5		
	30-X	40.5	35.5	20.9	3.7	0.93	
	5-XI-HCOOH	33.8	36.8	17.5	12.2		
T6r-6	13-X	38.0	34.4	21.8	5.8		—
	30-X	38.0	34.6	21.6	5.7	2.3	
$T6r^+$-7	26-UI	40.2	35.4	23.4	.9	0.63	
		40.1	34.6	23.8	1.5	1.3	
	25-UII NaOH	39.4	32.0	26.3	2.1		
	1-XI HCOOH	33.5	37.5	18.0	10.75		

Table . Maximum possible uracil content of bacteriophages, estimated ~~by absorption~~ after hydrolysis in perchloric acid.

	No. of analyses	Molar ratio of maximum possible uracil to thymine
T4 r	3	0.024
T4 r+	3	0.019
T6 r	3	0.028
T6 r+	3	0.028


HYDROXYMETHYL CYTOSINE RECOVERED / ADDED
1.0
0.8
0.6
0.4
0.2
100
200
300
400
500
μl HCOOH / mg DNA


175
165
155
145
135
125
115
105
95
85
75
65
300
400
500
EXTINCTION

0·1	0·2	0·3	0·4	0·5
113	240	293	372	459
114	207	295	381	460
120	204	290	391	+453
120	208	292	378	434
		290	352 ×	
		287	362	

3·95
3·88
7·83
3·91

1·96
1·99

18

19·7
5
43
98·5

1·98
1·94
1·96
1·99
37
1·97

1·96
5
3
180

3°

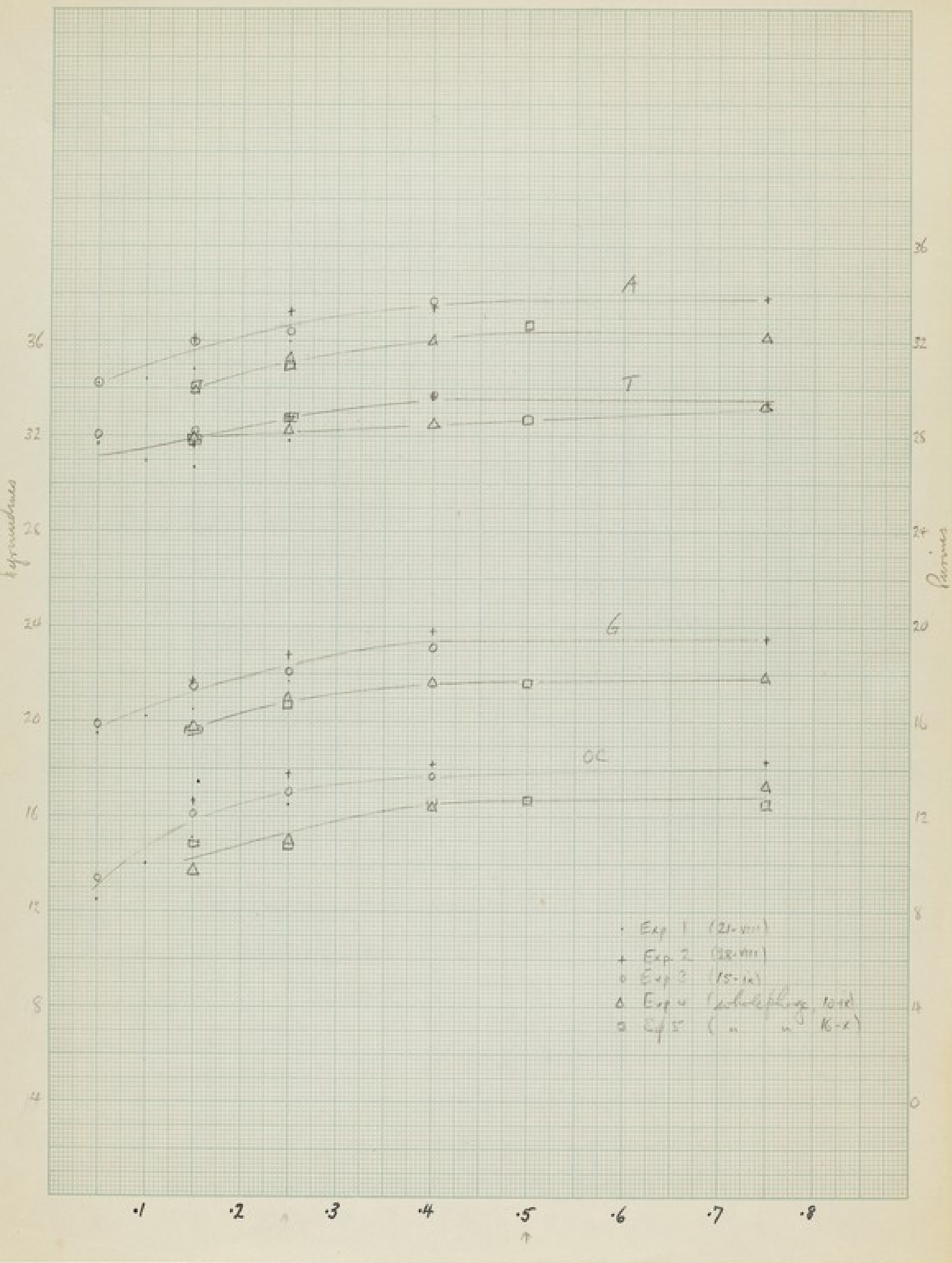
200 – 500· 475 15/300 20· 300· 5· 300/15

301	65 –	165.
303		
311	232	75.
311	227	73
310 +	309	105
296.	311	105·8
299	261	86·2
304	260	85·8
22	230	74·3
2435	226.	72·6.
304		

3·95
3·88
3·92
1·96

301
290

14·4
14·1
20·2
20·4
18·6
18·6
14·3
14·0



Phages

19-VI-51 UV spectrum of "C": λ_{max} pH 1 = 279 λ_{min} pH 1 = 242

λ_{max} pH 9.5 = 269

λ_{max} pH 13 = 278

3-VII-51 "C" has same R_F after cooking 2 hrs in 88% HCOOH at 117°.
R_F same as G in both BuOH-NH_3 and BuOH-HCOOH.

19-VII T4r-8 after NaOH fractionation shows "Pre-G".

26-X T4r⁺-6 DNase does remove most of HA.

acids, Expt. Cell. Res. Suppl. 2, in press.

Weed's data, from letter to Cohen 11 May 52.

"Cytidylic acid" region from virus phage chromatograms was run on columns → 2 peaks:

① 0.01 N-HCl → peak ≈ mass of 282
② 0.1 N-HCl → peak = mass of 274

Exp. 1. The 2 eluates were evaporated to dryness in HCl, on assumption that some hydrolysis would occur, & re-run on paper.
282-substance → 2 components = peaks 278 & 282. Presumably Cx and its nucleotide.
278 fraction has ratio $\frac{D_{279}}{D_{262}} = 1.88$

274-substance → 2 components = peaks 278 & 278-280 (diffuse)
278 fraction has "ratio" 1.54.

Exp. 2. The 2 column eluate fractions were evaporated down, first in 88% formic acid & cooked 2 hours in autoclave at 15 lbs.
Complete destruction of all UV-absorbing substances!