Notebook 5

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G.W.

NUCLEIC ACIDS (3)

Mar 1950 – Feb. 51.

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DNA ex bull sperm.

24-V-50. 4 ml bull semen, sperm spun off. discard plasma. Susp. in 10 ml 1:1 EtOH-ether 2 hrs to de-fat. Spin off. Susp. in 5 ml water, add fresh papain + KCN, leave overnight at 37°.

25-V. Add 5 ml 2 M NaCl, shake up well, leave 4 hrs at room temp. Spin off undissolved, susp. in 1N. NaOH to check completeness of extraction.

Supernat. ppt c HAc + EtOH → stringy ppt. + ~~5~~ ml 1M. NaCl + NaOH → pH 9. Sevag twice. 2nd time → almost no gel.

Dil 1:100 for Beckman

230	.052
240	.036
250	.038
260	.044
270	.038

Total N.A. < .044 x .040 x 100 = 0.1 mg.

Discard.

26-V. After 20 hrs. at 37°, mostly dissolved. Spin clear, ppt supernat c HAc + EtOH. → big ppt. Combine c ppt from sperm from 8 ml fresh semen, extracted directly c NaOH. Combined ppts dissolved in 1M NaCl + some

6
8 28-V. NaOH (turbid). Sevag 10 x (!) - still some gel.
~~ppt~~ ppt c HAc + EtOH, wash c EtOH, ether, let air-dry.
Weight 23.1 mgs.

27.v.50. <u>Muscle Adenylic acid</u> .

4.5mg/ml (not dried) ~~in~~ weak NH_3 dil 1:400 → pH 7, 11.2 [illegible]

290	·002		
280	·069		
270	·298		
265	·406		
261	·435		·448
260	·436		·450
259	·433		·460
255	·389		
250	·290		
240	·081		
235	·044		
		233 ·042	·109
230	·049		·091
		228	·086
225	·099		·093
220	·267		
	cells not matched		matched cells

E_{260} = ·450 × 400 × $\frac{347.}{4.5}$ = 13,900

	Analysed	Ratios
A	6.12	1.244
G	3.81	0.776
C	3.67	0.748
T	5.85	1.190
MC	0.245	0.050
	19.69	4.008
Corr. P	21.3	

Accounted for: 92%

Ratios re-calculated to allow for MC = 0.07

	A	G	C	T	MC
8·V	1.269	0.778	0.714	1.190	
11·V	1.204	0.781	0.763	1.182	
26·V	1.237	0.770	0.742	1.181	
$\bar{x}$	1.237	0.776	0.740	1.178	0.07
	±.019	±.003	±.014	±.004	

26·V. Echinus DNA 2nd hydrolysis.

8.6 mgs air-dry NA hydr. HCOOH 175° 40'. ↑0.4 ml 1N HCl.

3 × 18.0 μl spots, 5 at same time, 2 × 18.0 μl for P.

2 cm. cell, [illegible] blank

P_1	·302	} ·296 = 33 γP
P_2	·290	
20 γP	·287	– ~~[illegible]~~
30 γP	·266	

$= \frac{.033}{8.6} \times \frac{.4}{.018} = 8.6\%$ [illegible]

	A	G	C	T	MC 275	MC 283	MC 290
1	·792	·481	·381	·460	·020	·022	·018
2	·801	·434	·389	·463	·024	·026	·023
3	·798	·419	·389	·469	·020	·023	·020
$\bar{x}$	·797	·428 ·009 ·419	·386	·464		·024	

C + MC in usual way. Completeness of elution checked by [illegible] eluted band, & adenine marker run, clear of MC.

	MC in 5 ml 275	283	290	C in 10 ml 275
1	·085	·097	·095	·975
2	·077	·090	·078	·975
$\bar{x}$		·0935		·975

Molar ratio of MC $= \frac{.0935}{.975 \times 2} \times \frac{102}{.95} \times \frac{1}{.745} = 0.069$

26-V-50. ε_p of YNA + DNA after NaOH treatment.

20 ml of each YNA + BSNA solution of 24-V pipetted into specimen tubes. Add to each 0·2 ml 40% NaOH, leave at 37° 18 hrs.

27-V. Add to each 0·1 5ml HAc, mix, make dilution ·5 4 2 5.

Total vol. = 2·31

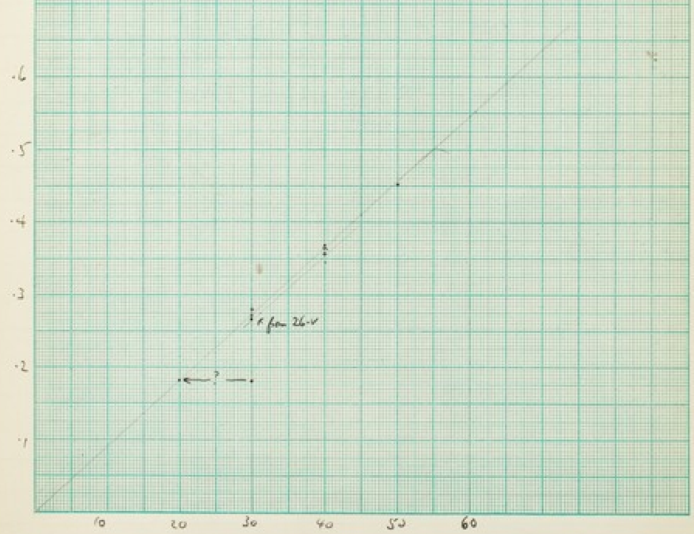
	YNA	BSNA
262	·975	·760
260	·980	·776
258	·960	·775
230	·519	.369

$\varepsilon_{p_{260}}$ – YNA = $·980 \times \frac{775}{·087} \times \frac{2·31}{2} = 10,100$

BSNA = $·776 \times \frac{775}{·082} \times \frac{2·31}{2} = 8,450.$

	Residual	Ratios
A	6.94	1.150
G	5.57	0.914
C	4.98	0.825
T	6.43	1.065
MC	0.276	0.046
	24.17	4.000
Comp P.	27.4	

P. accounted for = 88



28·V. <u>Analysis of Bull sperm DNA</u>.

13.3 mg (air-dry) hydr., ↑ 0.4 ml 1N.HCl.

3 × 18.0 µl spots, & 2 × 18.0 µl for P.

30 γ	[.182]	2 mm cell 608 filter
40 γ	.369	
50 γ	.451	
P_1	.389	} .405 = 44.5 γ
P_2	.422	

$P = \frac{44.5}{13.3} \times \frac{.4}{.018} = 7.4\%$ of wt. mean

$P = \frac{42.5}{13.3} \times \frac{.4}{.018} = 7.1\%$

	A	G	C	T	Past-T "thymidine"	MC 275	282	290
P_{250} was .025	.881	.616	[.571] big cell	.494	.007	.025	.025	.025
	.920	.613	.524	.496	.004	.021	.030	.027
	.902	.612	.524	.528	0	.022	.033	.031
	.901	.614 −.008 .606	.524	.506 −.004 .510	.004	.030	.029	.027

250 .012
255 .009
260 .008
265 .007
270 .006
275 .004

240 .045

Avg .027

Re-reading old (Beckman) cells

A	G	C	T	MC_{282}
.934	.619	[.614]	.498	026
.925	.601	.521	.493	027
.908	.598	.525	.528	034
.922	.606 +.007 .613	.522	.506	029

Eluted "thymidine":

250	.034	240	.024
260	.056	35	.030.
265	.042		
270	.039		

Tube 9.4347
+NA .4437
9.0 mgs.

	Yield	Ratio	Mean of 2 analyses & MC estimations
A	4.92	1.159	1.15
G	3.78	0.888	0.89
C	3.55	0.834	0.83
T	4.72	1.107	1.09
MC	0.163	0.038	0.052
	17.08		

Comp P. $\frac{.0265}{21} \cdot \frac{1}{5} = 18.4$ % accounted for = 93

By orig. anal'n, 3~~24~~ " 91.5

$\frac{31.2}{31} \times \frac{1.8}{1} \times \frac{.018}{.35} \cdot \frac{1}{5} = 18.7$

29.V. **Bull sperm DNA. 2nd analysis.**

Remainder of prep (9.0 mg air dry) ↑ 2.0 ml 0.5 N NH_4OH.
Take 2 × 0.1 ml for P, returning remainder to bomb tube & dry down.
(Loss pipette = probably ≯ 1%). ↑ 0.35 ml
3× 18.0 μl on paper + 2 × 18 μl for P.

Allens P: 30 γ .280
40 γ .364
0.1 ml orig .289, .281 } .285 = 31.2 γP = $\frac{.0312}{9.0} \times \frac{2}{.1}$ = 6.95 %
18 μl hydr .256, .261 } .258 = 28.5 γP = $\frac{.0285}{9.0} \times \frac{.35}{.018} \times \frac{2}{1.8}$ = 6.85 %

A	G	C	T	Bud-T 265	MC 275	MC 283	MC 290
.624	.408	.368	.361	0	.017	.019	.016
.626	.412	.373	.389	−.004	.013	.014	.013
.668	.426	.382	.375	−.010	.013	.014	.013
.639	.415, .008, .407	.374	.375		.014	.016	.014

C + MC – usual way – 2 separate analyses
C ~10 MC ~ 2

	C	MC 275	MC 283	MC 290
1	1.66	.124	.131	.113
2	1.68	.131	.136	.126
$\bar{x}$	1.67		.133	

Amt. C in appl. = $\frac{1.67}{.95}$. 10 = 17.6 γ

Ratio = $\frac{.133}{2 \times 1.67} \times \frac{1.02}{.95} \times \frac{1}{.83}$ = 0.0516

	C	MC 275	MC 283	MC 290
1	1.03	.079	.085	.074
2	1.03	.072	.080	.069
$\bar{x}$	1.03		.082	

Ratio = $\frac{.082}{2.06} \times \frac{1.02}{.95} \times \frac{1}{.83}$ = 0.0516

	BSNA		Bl. TNA	
	Yields/ml	Ratios	Yields/ml	Ratios
A	8.01	1.098	6.40	1.121
G	6.35	0.871	5.04	0.884
C	6.20	0.851	4.85	0.851
T	8.29	1.136	6.30	1.103
MC	0.31	0.043	0.21	0.037
Total	29.16	3.999	22.80	3.996
Corresp P	33.22		24.85	
P accounted for	87.6 %		91.7 %	

2.VI.50. Check P recovery in BSNA + TNA by doing P on hydrolysates.

Old hydrolysates: "BSNA-RNase" & "TNA Bailey 10mg / 0.4ml"

Pipette 0.01 ml aliquots of P & onto paper at same time.

30 γ P		·271	
40 γ P		·356	
g	BSNA	·479	} ·464 = 51.5 γP
h	"	·450	
j	TNA	·343	} ·346 = 38.5 γP
i	"	·350	

		A	G	C	T	MC
BS	1	1.04	·697	·651	·669	·032
	2	1.04	·693	·655	·644	·029
	x̄	1.04	·695	·653	·657	·030
			·003			
			·698			
T	1	·840	·554	·509	·504	·023
	2	·824	·548	·511	·495	·019
	x̄	·832	·551	·510	·500	·021
			·003			
			·554			

	μmol/gel ×10⁻²	Ratios
A	5.21	1.148
G	4.06	0.895
C	3.87	0.854
T	4.91	1.083
MC	0.12	0.026
	18.17	4.006
corrn?	20.06	

Pare counted for = 91%

6-VI-50. Effect of re-precipitation on BSNA P. (Lysin)

150 mgs air dry BSNA ↑ 10 ml 0.1 N. NaOH, acidify to pH 4 ± glac. HAc., add 1 vol. EtOH. Ppts. ± diffusely. Add little $MgSO_4$ → good ppts. Spin down, re-dissolve in 10 ml 0.1 N. NaOH, & repeat 8 times, then ppts. twice from H Hall without $MgSO_4$. (Split after penultimate ppton – examine ppt, dissolve, filter, re ppt.) Wash = 60%, 90% EtOH, abs., ether. Total wt. 47.9 mgs.

Take 17.1 mg, hydr., ↑ 0.7 ml N-HCl. 3 spots on paper, 3 for P.

30 γ P ·268
40 γ ·344
·018 μM ·273
" ·273
" [·33]
} ·275 = 31 γP (32%)

A	G	C	T	271	MC 282	280
·684	·460	·404	·400	·009	·012	·010
·673	·445	·410	·386	·006	·009	·009
·679	·448	·407	·383	·011	·014	·013
·677	·451 / ·003 / ·446	·407	·390		·012	

Bottle wt 7.5834

+2 ml fr. susp., dried @ 110° 3 hrs. 7.6511

677 Total = $\frac{67.7 \times 101}{2}$ = 3.42 g.

11-VI-50. Big LdV prep.

2 bottles (supposedly = ~~2.98~~ 3.36 g.) Geal Ld polyhedra (kept frozen in saline 11 mos.), thawed, spun down, susp. in aq. dest., spun down, susp. in 101 ml aq. dest. Pipette out 2.0 ml for dry wt.

12-VI. Remaining 99 ml, dil. to 326 ml., & add 170 ml 0.9% NaCl

12:30 pm. + 4.0 ml 1M Na_2CO_3 → 500 ml 0.008 M Na_2CO_3 0.05 M NaCl

3.20. 3 hrs. @ room temp.

Spin 6 min @ 140 v. Sorvall (6000? rpm), decant from loose

(over) ← brown sediment (+ small white sediment), spin 1 hr. @ 190 v. (9600?)

Virus pellets are rather brown. Supernat. is still opalescent — re-spin together 1 hr. Combine pellets, susp. in 70 ml aq. dest., spin 6000 5 min., decant from small brown pellet.

13-VI. Spin 10,000 1 hr. → brownish-gray pellet. Susp. in 4 ml aq. dest., freeze.

14-VI Dry from frozen state. Yield, dried, 40 mg.

3.36 g pr. → 40 mg virus

Yield = 1.2%

12.VI.50. "Skin" prep.

Sediment from 11/12 of virus prep. (1 tube broke) ↑ 75 ml aq. dest. Leave in frig.

13.VI. Spin 5000 2 min. → pellet largish, part brown, part whitish (milk-mottly bugs)

22 Supernat spin 8000 10 min. (Ecco) Not sedimented. Spin 16,000 10 min. Mostly skins now - some virus, v. few bugs. (susp in water) (Ecco) Spin 8000 4 min, + decant from small pellet which is mostly bugs + small solid particles. Supernat, spin 16,000 20 min. Not all sedimented, re-spin. 16,000 30 min, decant as much as possible. Suspend skins in remaining water (ca 4 ml) freeze.

14.VI. Dry from frozen state. Yield, dried 40 mg.

13.vi. <u>Poly. prot.</u> ex prep. of 12-vi.

Twice spun for ? mins, still slightly opalescent. Ppt. c̄ HAc pH 5·8 (approx.), spin off, dissolve in 200 ml 0·05 M. Na_2CO_3, → clear. Dialyse against running water overnight, then aq. dist. 2 days.

15·vi. pH 8. still in sol'n. Spin 11,000 70 mins.
Ppt. at pH 5·8. Spin down, susp. in little water, freeze-dry.

	LdV	Skins	
Bottle MT	7.7174	7.6772	
+ stuff	.7575	.7168	Total wt = 39.6 mg. Some kept.
		.6978	
+ stuff dried 110° 3 hrs.	.7565	.6972	
Dry wt.	39.1 mg.	20.0 mg.	
Add:	5.0 ml N-NaOH	5.0 ml 0.1 N-NaOH	
		+ 0.05 ml 10 N-NaOH	
	= 7.82 mg/ml	~~= 3.96 mg/ml~~	
		↑ 10 ml = 2.0 mg/ml	

.7
.6
.5
.4
.3
.2
.1

5 10 15 20 30 40 45

16-VI. Analysis of LdV & skins.

39.1 mg. LdV (prep. 14-VI) ↑ 5.0 ml 1N-NaOH, leave overnight @ 37°.

20.0 mg. "skin prep." ↑ 5.0 ml 0.1N-NaOH, does not dissolve, then add 0.05 ml 10 N-NaOH, but still does not dissolve. Test samples of dry "skins": does not dissolve in 6N. HCl, (hot or cold) cold glac. HAc., cold 10 N. H_2SO_4, but does in boiling 10 N. H_2SO_4. So, to susp. in N-NaOH add 4 ml conc. H_2SO_4, heat till dissolved, then make up to 10.0 ml.

17-VI. LdV not perfectly dissolved; also note possible NH_3 loss by NaOH @ 37°.

Kjeldahls: Acid: 474 γ N/ml (Hutchinson) = 480

A	blank > .02 < .04			
D	"	.020		
a	1 ml skins	.585		
d	"	.595	.592	$= \frac{.592 \times .474}{2.0} = 13.6\%$ N + 3% = 14.0%
		.595		
P_4	"			
k	0.5 ml LdV	1.245		
i	"	1.240	1.24	$= \frac{1.22 \times .474}{.5 \times 7.82} = 11.6$ % N – must be loss by incubation in NaOH. +3% = 12.0
l	"	1.240		

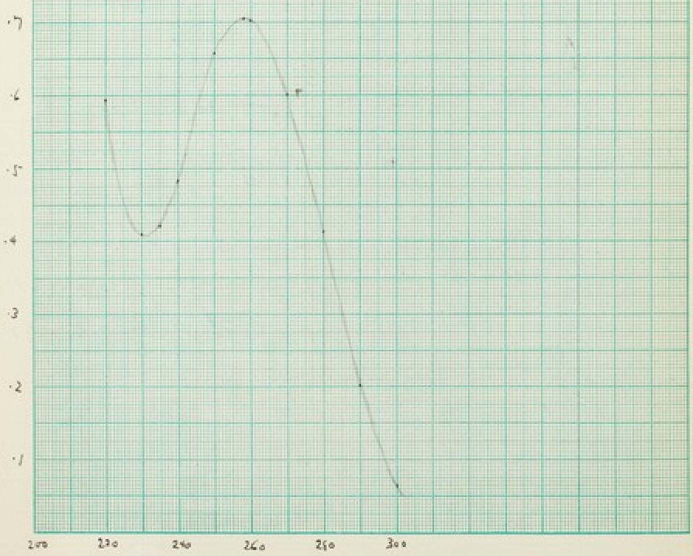
Allen's P. 608 filter, 4 cm. cells.

P_2	0.25 ml LdV (+0.8 ml H_2SO_4)	.744	.747	$= 43\gamma P = \frac{43}{.25} \times \frac{100}{7.82} = 2.2\%$ P !
P_3	"	.750		
P_1	2.0 ml skins (in 15 N. H_2SO_4)	.119	.124	$= 7\gamma P = \frac{7}{2} \times \frac{10}{2} = 0.175\%$ P
j	"	.129		
5 γ P	(+0.8 ml H_2SO_4)	.095		
15 γ P	"	.256		
30 γ P	"	.527		
45 γ P				

Bomb tube

L, H.A.	moles × 10[2]	Ratios
A	5.47	0.826
G	7.97	1.203
C	7.71	1.164
T	5.33	0.805
Total	26.48	[illegible]
Corresp. P	29.7	

% P accounted for : 89.3



17-vi. LdVNA.

Soln of LdV in N-NaOH remaining from P & N estimation — (3 ml = 24 mgs) acidified c HAc & add 1 vol. EtOH, leave overnight in frig.

19.vi. Spin down big ppt, ↑ alkaline M-NaCl, Sevag twice → no denat. gel.. ppt. c HAc + EtOH. spin down, wash c 90% EtOH, dry c ether.

After letting ether evaporate, ~~weigh into bomb tube without further drying.~~ too little to weigh.

Dissolve in ca. 1.5 ml M-NaCl, take 0.05 ↑ 5 for Beckman, ppt. remainder in bomb tube.

Beckman:

300	·064	258	·705
290	·201	250	·657
280	·414	240	·493
270	·608	235	·421
260	·702	230	·410
		220	·594

$\frac{D_{258}}{D_{235}}$ = 1.72

Total NA ≏ ·705 × ~~·035~~ 40 × 1.5 × 100 = ~~3.7~~ 4.2 mg. Recovery of 16% of 10 $\frac{42}{24\times16}$ = 110%!

Hydrolyse c HCOOH, ↑ 0.2 ml. 3 × 18 µl to paper, 2 × 18 µl for P.

P: 30 γ ·275
40 γ ·351
NA ·411
NA ·418 } ·414 = 46 γ P

A	G	C	T	U 255	U 260	U 265
·707	·876	·806	·414	·021	·019	·017
·715	·876	·815	·426	·018	·015	·010
·705	·876	·815	·429	·033	·030	·026
·710	·876	·812	·423		·021	= 22 γ C

	Polyphot	Skins.
Bomb tube HT	5.9514	6.0155
+ protein	9776	.0194
	26·2 mg (Recrystd. Ammo-dog)	3.9 mg (Recrystd.)

19-VI. Amino-acids in skins & poly. prot.

Ld p. skin prep. & polyphot., weighed, + 6 N. HCl, hyde. 175° 30'. Dry down, + water to make ca. 5% sol'n. 18 pl spots on big sheets, run in phenol-NH$_3$-KCN first, then in BuOH-Acetic.

Chromatograms identical:

Aspartic	++	blurred by salt effect.
Glutamic	++	
Serine	+	
Glycine	++	
Cystine	+	
Threonine	++	
Alanine	+++	
Valine	+++	
Leucine	+++	
Tyrosine	++	
Phenylalanine	++	
Histidine	++	
Lysine	++	
Arginine	++	
Proline	++	

19-VI-50. Ld DNA

Ld leaves + a few purples. Some in soups look dead of anim, but most seemed healthy. Smears of 15 cf random: 1 only shows polyhedra. Thick: ether, weigh (474 g.), blending blend in beaker in total 1200 ml 0.60 NaCl 0.05 Na citrate, spin 2400 ~~20~~ min.

Re-susp in 0.15 M NaCl-citrate + spin 2400 20 min.

Susp in 0.9% NaCl to 250 ml, add 250 ml 2 M. NaCl. — somewhat viscous, but not very.

20 Spin clear, (~~black~~) ppt into 3 l. water. Small flaky ppt. spin off, dissolve in M NaCl, re-ppt by pouring into water. Dissolve in M NaCl + little NaOH, layer 3 x (1st: big brown gel; 2nd + 3rd: v. little gel). Acidify + HAc, add 1 vol EtOH. Only minute ppt! Spin down, ↑ 3 ml water + little NaOH.

24. Add 1/10 vol. 10 N-NaOH, leave overnight at 37°.

25. Ppt + HAc-EtOH.

26. Spin down small ppt. Dried.

- 2

Bottle	7.8303 (2g)			7.4533
+ poly prot, not dried	.9216		+ skins	.4648
" dried 110° 2hr	.9124		after drying	.4649
+ 1½ hrs	.9121			
Protein	81.8 mg			11.5

	Poly prot. A	B polyhedra (brown prot)
For N: Kjeldahl flask HT dried + aluminium rack	32.9348	31.0934
+ undried prot.	.9839	.1328
After drying 110° 4 hrs (cooled over H_2SO_4)	.9802	.1296
	454 mg	362 mg
% water removed	8.1 %	8.8

	j. polyprot.	i. polyhedra
For P: Flask + rack	29.0730	29.7235
+ undried prot.	.1640	.7638
Dried	.1549	.7600
Actual wt.	81.9 mg	36.5 mg
% water removed	11.1 %	10.4

	g. polyprot.	d. polyhedra
Repeat N: Flask + rack	30.4404	32.9225
+ undried prot.	.5376	.9703
Dried	.5277	.9651
Actual wt.	87.3 mg	42.6 mg
% water removed	11.3 %	12.2

Analysis of poly prot. & whole polyhedra. & repeat on skins.

Skins

11.5 mg dissolved in 1 ml 10 N H_2SO_4 by heat. Dil. to 10.0 ml. (possible some lost in making up).

a. blank 0.03

d - 2.0 ml skin soln .645 }
g - " " .645 } .645 - .025 = .62 = $\frac{.62}{2} \times .474$ (.460) $\times \frac{100}{11.5}$ = 12.9 % N

Add 3.0 % for brucite = 13.3

Oven-dried polyhedra & poly prot. would not dissolve, even in 10 N H_2SO_4 at 150°. Dried, & weigh some directly into Kjeldahl flasks, incinerate, dil to 100 ml, distil 5 ml aliquots.

Blanks, 0.014, 0.012, 0.010 = 0.012

Not dry { A, poly prot. 0.677, .686, .677 = .680 - .01 = $.67 \times .474$ (.460) $\times \frac{20}{45.4} \times 100$ = 14.0 % N. (+3% = 14.5; +3% brucite = 14.9)
D, polyhedra (brownfed!) .564, .550, .570 = .56 = $.55 \times .474$ (.460) $\times \frac{20}{36.2} \times 100$ = 14.5 % N. (+3% = 14.9; +3% more = 15.3)

P.

Poly prot. 81.9 mg → 61.5 γ P P = $\frac{0.0615 \times 100}{81.9}$ = 0.075 % P

Polyhedra 36.5 mg → 83 γ P = $\frac{.083 \times 100}{36.5}$ = 0.227 % P

Repeat N. - same way - dil to 100 ml, 5 ml aliquots

D: blank. .010, .010

g - polyprot 1.265, 1.275, 1.265 = 1.268 - .01 = $1.258 \times .474$ (.460) $\times \frac{20}{87.3} \times 100$ = 13.8 % (+3% = 14.1)

d - polyhedra 0.64, .654, .640 = .645 - .01 = $.635 \times .474$ (.460) $\times \frac{20}{42.6} \times 100$ = 14.2 % (+3% = 14.6)

Sintered glass filter dried	9.1460
+ skins	.1586
" dried	.1576
	11.6 mg.
After fat extraction	9.1575

24-VI-50. Fat extraction from skins

Small sintered glass filter washed c water, EtOH, ether, dried in oven. Weigh. Weigh in skins, dry at 110° ½ hrs. weigh. Put in suction flask, add ca. 4 ml 60-80° petrol ether, let stand 20 mins., suck thro, repeat c second 4 ml.

Dry in oven & weigh.

Loss ≯ 0.1 mg in 11.6 mg i.e. < 1%.

∴ No fat.

Carbohydrate in skins

3.2 mg. skins hyd. in 0.1 ml 2% HNO_3 100° 60'. Spot on paper gives faint pink c aniline phthalate, no color c $AgNO_3$-NH_3. Chromatogram run in pyridine - EtAc - H_2O, with ca. ⅔ of hydrolysate (≡ 2 mg skins) → faint clear rose-pink spot (aniline) at level of ribose (just beyond xylose). < 5γ sugar ≡ < 0.25% of skins. TNA similarly hyd. & run → faint brownish spot just behind xylose — not same.

N.A. in skins 5 ml sol'n in N-H_2SO_4 neutralized c $Ba(OH)_2$, filter, hydrolyze, part all in one spot.

A		G		C		"U"		T	
255	.108	245	.058	270	.131	255	.148	260	.192
260	.112	250	.061	275	.137	260	.158	265	.194
265	.113	255	.059	280	.129	265	.179	270	.189

G = .0055 γmol/ml in eluate ≡ 0.0185 [×5] γmols total N.A.-P = 0.092 = 0.29 γP

2.9 γ P in 5.8 mg skins = 0.05% P

2.9×100 / 5800

Single spots from BuOH-NH3 chromatogram

			Nucleotide	Ratios
A	255	·114		
	260	·124	0·935	1.48
	265	·124		
G	245	·033		
	250	·034	0·31	0.48
	255	·032		
C	270	·058		
	275	·064	0·61	0.95
	280	·060		
U	255	·052		
	260	·056	0·71	1·10
	265	·049		
			2·585	4·01
T	265	–·003		

γmols of nucleotide per mg. virus $= \frac{2\cdot6}{150} \times \frac{5}{9\cdot8} = 0\cdot013$

Mean nucleotide M = 330.

% NA in virus $= 0\cdot013 \times \frac{330}{10} = 0\cdot4\%$!

3/4 in PO4 j, P4
3/4 in NaCl h, i.

Mumps.

24-VI-50. 275 ml infected allantoic fluid spun 10 min. on MSE to clarify, then on Sorvall in 6½ tubes top speed 1 hour. Supernat is clear. Susp. 3 tubes' pellets in 13.5 ml M/100 PO4 buffer pH 7. + 3½ tubes' pellets in 14 ml 0.9% NaCl. Leave in frig.

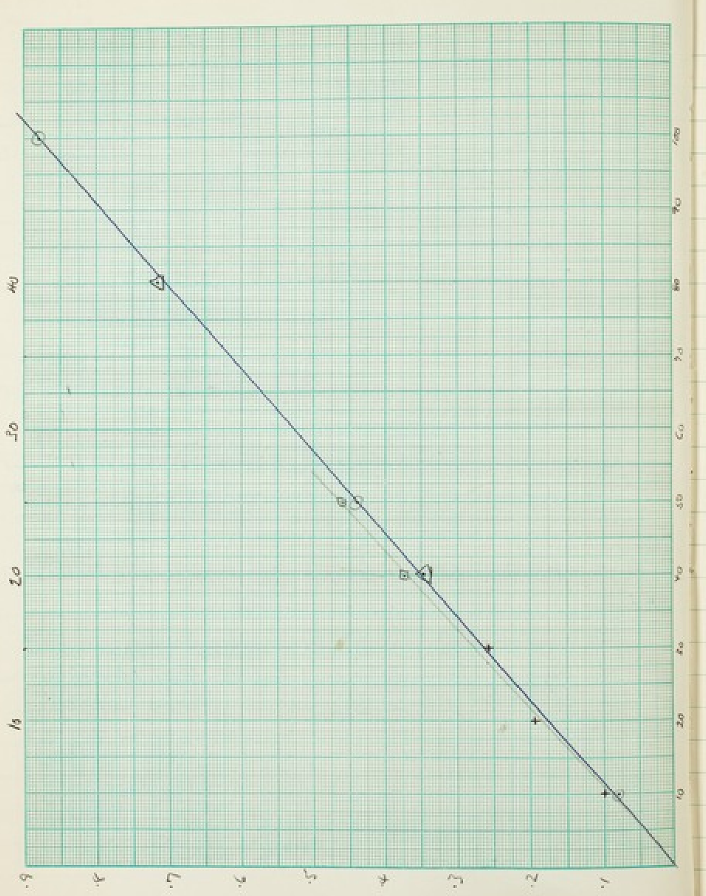
26.VI Spin both on Sorvall 6000 2 min → v. small pellet. Decant, spin 9600 75'. Susp (more difficult, but no difference in buffer + saline) in 5 ml buffer + NaCl respectively.
Finter took 0.2 ml from each for haemagglutinin titre.
Kjeldahls:

j 0.5 ml PO4 buffer sol'n 0.165 }
P4 " ·170 } ·168 – ·020 = ·148 × ·474 × 2 = 0·148 mg N/ml
Total N = 5 × ·148 = 0·70
Total virus = 7.0 mgs
h 0.5 ml saline sol'n 0.205 }
i " 0.195 } ·200 – ·030 = ·170 × ·474 × 2 = 0.168 mg N/ml
Total N = 5 × ·168 = 0·805
(assume 10% N) Total virus = 8.0 mgs.

Amt. remaining in each = 5 – 1.2 = 3.8 ml

27-VI. P. 1.0 ml from NaCl sol'n for P. (Pi) → 15·6 γP = $\frac{·0156 \times 100}{1·61}$ = 0.97% P

Remainder (3.8 ml in PO4 [5.3 mg.] + 2.8 ml in NaCl [4.5]) [= 9.8 mg total] pptd: 4 drops N-HCl + 1 ml EtOH. Spin down, dry at 100°, add 0.05 ml 72% HClO4, cook 100° 60 min. Transfer all to one spot on paper (spread rather badly). → heavy brown streak + faint spots, appear to be G, A, C, & U. Elute each separately from strip, placing 1st 3 drops as spots on paper, run in BuOH-NH3.



27.VI.50. P estimations

I cm cells 608 filter. Incineration blank.

10 γ P	·081	
50 γ	·440	
100 γ	·880	
Shat's TMV	·600	} 68.2 γ
"	·600	
Polyphos	·731	= 83 γ
Poly. pot.	·54	= 61.5 γ
Ammps.	·137	= 15.6 γ

1-VII-50. Hem. cells 608 filter. Reagent blank] Repeat. Clean glassware Incineration blank (A)

10 γ	·28	20 γ		·347
30 γ	·625	40 γ		·715
Lcl V (R)	·88			·359 = 20.5 γ
" (A)	·496			
LdVNA (i)	·85			·541 } ·555 = 31.5 γ
LdVNA (R)	·89			·57 }
Supernat. ring (turbid)	·404		2.5 ml	·223 = 12.5 γ By difference, 10 γ = 4 γ/ml
Supernat. total 2.5 ml	·74	(a)	2.5 ml	·300 = 17 γ = 6.8 γ/ml
" "	·88	(R)	1 ml	·115 = 6.5 γ } 6.7 γ/ml

Smith & Markham published ratios.		mean $HClO_4$ ratios (3 analyses incl. July 26)
A	1.03 ± ·01	1.04 ± 0.010
G	1.25 ± ·01	1.22 ± 0.004
C	0.80 ± ·02	0.82 ± 0.008
U	0.93 ± ·04	0.92 ± 0.014

28-VI-50. YNA, purified

12 N $HClO_4$ 100° 1 h.

1 spot, [illegible] HCl.

		γ mols/ml	Ratio
A	1.06	8.16	1.033
G	1.06	9.65	1.223
C	·675	6.41	0.813
U	·579	7.35	0.931
		31.57	4.000

5-VII-50. Same hydrolysate (left open to evap.). 2 pipette-fuls for P, 1 on paper.

Allen's P: 40γ ·374
50γ ·460
P_1 ·259
P_2 ·257 } ·258 = 28γ P = 0.181 γ mols/ml eluate.

			Ratio
A	·591	4.55	1.061
G	·570	5.19	1.210
C	·379	3.60	0.840
U	·299	3.80	0.887
		17.14	3.998

P accounted for = 95%.

	Glass	Porcelain
Dish	20.8415	22.80[illegible]1
+ semi-dry LdV	.8774	
	35.9	

29-VI-50. LdV prep.

Remaining bottle (20 ml) + half (13.5 ml) of Geck polyhedra (≃ 3.0 g.) mixed with 3.2 ml 1M. Na_2CO_3 + 100 ml 0.9% NaCl + water to 320 ml → Na_2CO_3 0.01 M, NaCl

10.45 a.m. 0.05 M. polyhedra 9.5 mg/ml.

12.45. Mostly dissolved. Spin 6 min Servall 140 v. (6000 rpm), decant from large brown pellet, spin 9600 65' → good blue-white pellets, each c brown streak. Susp. in 50 ml aq. dest., spin 9600 60'. Wash brownish layer from surface of pellet - combine as "dirty virus" prep. (0.2 ml for ultramicrographs) for H.A. Clean virus ↑ 10 ml aq. dest., spin 5000 2 min., decant from small pellet, spin 9600 45' (small tube) → big clean bluish-white pellet. ↑ ca. 2 ml aq. dest., freeze-dry. Yield 36 mg. clean virus, + ca. 30 mg. virus containing some skins c muck.

Skins

Susp. in aq. dest., spin 5000 3 min to remove bugs, decant, spin 9600 3 min. to sediment skins - decant supernat for more virus recovery. Pour skins from top of pellet, leaving whitish residue of aggregated virus rz. Spin down on Ecco (16,000 15') twice, c short spin (10,000 3') between to remove more bugs.

Dark ground: still much virus. Try to filter thru #50 paper.

30.VI.50. Ultracentrifuge run.
Virus in M/50 borate pH 7.5 M/50 KCl.

Speeds taken after reference.
Temp. 18.8°

1000 revs in 18.8 sec.
1000 Revs in 18.4 secs.

Exp. 5. 11.21 am.
1000 Revs in 18.5 secs.

6. 11.25.
1000 Revs in 17.8 secs.

7. 11.29.
1000 Revs. in 18.0.

8. 11.34.
1000 . 18.2

9.

10.

Sintered glass filter	9.1462	
+ undried virus	.1797	Semi-dry virus = 33.5 mg.
Dried 2½ hrs at 110°	.1775	
Dry virus	31.3 mg.	
After fat extraction	.1773	

Bottle	7.8308 (9 corrected to 8)
+ dried LdV	.8446
Virus	13.8 mg.

Tube	9.7463
+ dry LdV	9.7493
∴ Virus	3.0 mg.

30-VI-50. Analysis of new LdV

Fat extraction.

Weigh whole of freeze-dried clean virus into sintered glass filter, dry in oven, weigh. Extract c 2 3 ml portions of 60-80 petrol ether, letting each stand 30 min before sucking thro. Dry 1 hr at 110°, weigh. ≯ 0.6 % fat.

—‖—

N + P

30-VI. 13.8 mg. dry virus weighed into specimen tube. Add 0.2 ml 10 N H_2SO_4, heat 5 180° several hours. Does not all dissolve.

3-VII. Add 0.4 ml 10 N H_2SO_4, heat again. Does not dissolve perfectly, but rinse quantitatively into 10 ml flask & try to keep suspended while taking aliquots.

P:
V 2.0 ml
P_2 " } 48 γP = 1.74 % P

N A blank overlap. assume .026 (blank) +3% = 14.8%
Z 2.0 ml 0.855
K " .850 } .853 - .026 = .827 = $\frac{.827 \times .1490 \times 100}{2 \times 13.8}$ = 14.4 % N.

Carbohydrate

3 mg dry virus hydr (0.05 ml 2% HNO_3) sealed 100° 1 hr. Ca. 20 μl on paper (≡ 1.2 mg) → v. faint brownish spot about level of glucose, (the spots not resolved — could be pentose!). Max carbohydrate 2 γ = $\frac{2}{1000}$ = 0.2 %.

	Yield	Ratio
A	4.05	0.879
G	5.64	1.223
C	5.44	1.180
T	3.30	0.716
	18.43	3.998
Comp. P	20.3	

P accounted for = 91 %

LdVNA ③

29-VI. Freshly prepared LdV, dialysis fraction containing some skin + much (guessed 15-20 mg V) in 4.5 ml, mixed c 0.5 ml 40% NaOH. → v. viscous. Leave overnight @ 37°. Less viscous. Saving twice → not much gel. Pptd. c HCl + EtOH. Redissolve in M-NaCl + little NaOH, saving once → more gel. Pptd, ↑ in weak NaOH (turbid + brown!) Vol = 1.7 ml

1:100	260	~~0.900~~ .878	258	.88
	250	~~.83~~ .805	265	.815
	240	.618	270	.73
	235	.550	280	.514
	232	.537	290	.269
	230	.540	300	.106
	220	.736	310	.074

$\frac{D_{258}}{D_{232}} = 1.64$

Pipette equally into 2 tubes, dry, set one aside. To other add 0.05 ml (~3 mg) 12N HClO₄ hydr. 100° 60', dil. c 0.1 ml aq. dest., spin., pipette 3 × 18 µl spots + 2 aliquots for P.

P – 3 } 31.5 γP

Total virus used = $\frac{31.5}{1500} \times 2 \times \frac{.15}{.018} \times \frac{100}{1.8} = 29$ mg.

2-VII Beckman cells, 6214 as blank.

	A	G	C	T
1	.547	.626	.568	.261
2	.521	.620	.569	.264
3	.511	.627	.579	.261
x̄	.526	.624	.572	.262
		– .005		
		.619		

Tube 9.3597
+ dry LdV .3678
Virus 8.1 mg. + 0.05 ml 12N HClO₄, then + 0.07 ml aq. dist.

	Nucleotide	Ratio	γ polydeoxy-nucleotide ml
A	2.50	0.860	7.83
G	3.46	1.189	11.38
C	3.24	1.127	9.48
T	2.39	0.821	7.26
	11.63	3.997	35.95
Corr. of P	13.2		P accounted for = 88 %

% of NA in virus = $\frac{35.95}{1000} \times 5 \times \frac{0.12}{0.018} \times \frac{100}{8.1} \times 1.03 = 15.2$ %

30-VI-50. LdV hyd. whole for NA.

8.1 mg. dry pure LdV hyd. 12 N. HClO₄ 100° 60', dil. c aq. dist., pipette 18 µl - 3 spots on paper + 2 aliquots for P.

l = 20.5 γ P
P - A

P as % of dry virus = $20.5 \times \frac{0.12}{.018} \times \frac{103}{100} \times \frac{100}{8100} = 1.74$ %

2-VII. Same blank as for standard NA. Cell 6214 as blank.

	A	G	C	T
1	.328	.382	.340	.189
2	.317	.371	.332	.188
3	.331	.389	.362	.192
$\bar{x}$	.325	.381	.345	.190

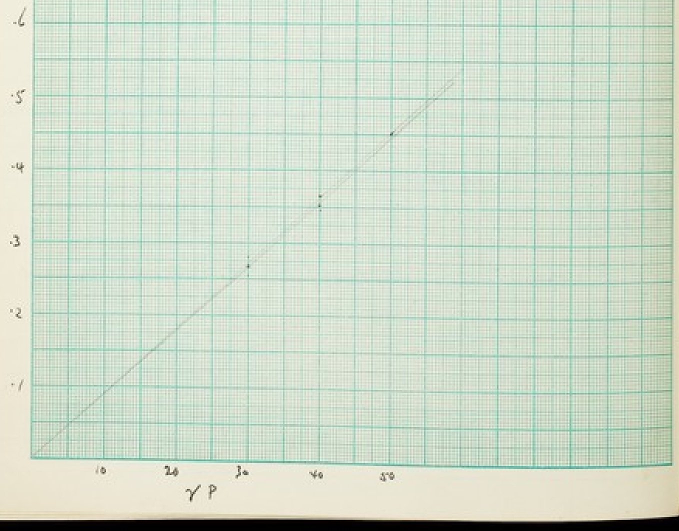
Total γ moles of base per mg. virus = $0.116 \times 5 \times \frac{.12}{.018} \times \frac{1.03}{8.1} =$ ~~0.478~~ 0.492

Assuming straight polynucleotide c average M = 309

% NA in virus = $0.478 \times \frac{309}{1000} \times 100 =$ ~~14.8~~ 15.2 %

Theoret. % P in NA with the bases in the ratios found = $\frac{31 \times 100}{309} = 10.0$

	Polyphelon a	Poly prot. P_3
Flask + rack	33.0701	33.5651
+ air dry protein	.0986	.6624
Dried	.0967	.6537
Actual wt.	26.3 mg.	88.6 mg.
Water loss	8.4 %	9.8 %



3-VII-50. P in home-grown polyphelon & Geal poly prot.

Poly prot. from bottle marked "Some pptd; in 0.01 M. Na_2CO_3; 4-VIII-49; Geal". pptated c HCl pH 5.8, filter off on paper, wash c water, let dry on paper. Scrape off. (Some cellulose contam.)

Polyphelon from home-grown but purified by Sylvia ca. 3 wks ago.

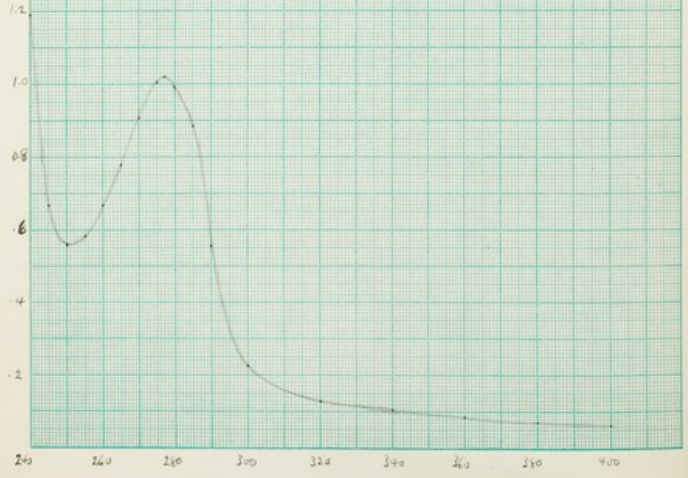
Incinerate = 0.5 ml conc. H_2SO_4 + few drops HNO_3

2 cm cell, 608 filter.

40 γ	.365		
50 γ	.450		
Polyphelon	.541	= 60 γP	= 0.23 %
Polyprot	.448	= 49.5 γP	= 0.056 %
Virus V	.431	.435 = 48 γP	=
" P_2	.438		

Blank
+ dimboy 11

13. H310
.4457
14.7 mg



UV spectrum of bd poly prot.

Same poly prot as on first page for P determination 14.7 mg ≡ 13.2 mg/dry.

↑ water + NaOH → pH 7 to 20 ml.

400 mμ	.061	0.66 mg/ml.
380	.072	
360	.086	
340	.103	
320	.128	
300	.224	
290	.363	
285	.885	
280	.992	
277	1.02	
275	1.005	
270	.964	
265	.779	
260	.668	
255	.381	
252	.363	
250	.380	
248	.371	
245	.469	
240	1.19	

Kjeldahls on 5-M.C.

HCl = 0.480 mg N/ml

Z	Blank	·020			
V	0.985 mg	·629	·609	$\frac{\cdot 609 \times \cdot 480}{\cdot 985}$	= 29.7 %
W	1.306 mg	·824		$\frac{\cdot 804 \times \cdot 480}{1\cdot 306}$	= 29.6 %

Theoretical composition

M = 125			
N =	3 × 14	= 42	= 33.6 %
C =	5 × 12	= 60	= 48.0 %
H =		7	= 5.6 %
O =		16	12.8 %
		125	100.0

22-VII-50.

Analysis by Chem. Dept. on last 8 mg of purified 5-MC

C	44.8
H	6.52 %
N	31.7 %

5-VII-50. 5-methyl-cytosine.

M.p.

Purified synthetic 5-M.C.

ca. 247° - begins to turn brown.

ca. 256° - melts

270-271 - melts c̄ decomposition.

Big crystals of partially purified synth.

240 - begins to brown.

264 - decomposing badly.

265-272. melting c̄ decomp.

UV spectrum.

Sample of purified synth 5-M.C 4.3 mg/ml in water (now buggy)

dil. 0.1 → 50 ml water.

Sample taken to pH 11 (by indicator) c̄ NaOH.

		274	·404		
		273	·405		
275	·402	272	·405		
280	·352	270	·403		
285	·261	260	·304		
290	·164	25?	·261	262	·252
		250	·255		
		240	·342		

pH 12.

270	·293
275	·314
280	·310

pH 13

280	·359
288	·391
290	·366

Tube + ~~NA~~ LdV (air-dry, but in air 10 days) 9.4409 − .4133 = 2.4 mg.

	Extract γ mols/ml		Residue γ mols/ml	Total γ mols/ml	Ratio	γ poly deoxynucleotide /ml
A	1.73	0.89	~~0.13~~	1.73 (~~1.86~~)	~~0.90~~ 0.83	5.41
G	2.44	1.25		2.44	~~1.19~~ 1.17	8.03
C	2.19	1.12	0.24	2.43 (~~2.19~~)	~~1.06~~ 1.17	7.02
T	1.45	0.74	0.29	1.74	~~0.86~~ 0.83	5.29
	7.81	4.00		8.34 (~~8.23~~)	3.99	25.75

~~[illegible]~~ % of pyrimidines not extracted = .53 / ~~[illegible]~~ 4.17 = ~~[illegible]~~ % ~~14.6~~ % 12.7

γ mols of total base / mg. virus

$= 0.08\,34\,(\text{~~23~~}) \times \frac{.049}{.018} \times 5 \times \frac{1}{2.4}$ = ~~0.187~~ ~~0.466~~ ~~0.472~~ 0.475 (×1.008) .477

Assuming average deoxynucleotide wt = ~~215, − H₂O~~ = 309.

γ of NA / mg virus = 0.472 × 309 = 145

% NA = ~~[illegible]~~ 14.6 %.

% NA in virus by potential nucleotide wts = $\frac{25.75}{1000} \times 5 \times \frac{.049}{.018} \times \frac{100}{2.4}$ = 14.6 %.

17-VII-50. **Extraction of NA from LdV c̄ TCA**

To 2.4 mg semi-dry LdV add 0.5 ml. 7% TCA, heat on W.B. 90° 15'. Spin, decant, wash sediment c̄ 0.5 ml 5% TCA, spin. Dry down supernate, & dry sediment. Hydrolyse both identically: add 0.018 ml 72% HClO₄, heat 100° 60', cool, add 0.031 ml water. Vol. should = 0.049 ml. Spin. Put 2 × 10 μl spots from each on paper, run in isoprop. HCl.

18-VII. Extract chromatogram → good spots, no background absorption; residue → faint spot of A

		A	G	C	T
Extract	1	~~.236~~ .224	~~.284~~ .270	.225	~~.126~~ .115
	2	.224	.264	.236	.112
	x̄	.224	.267	.230	.114

HT cells

250	260	~~270~~ 265	275
.004	.012	.011	.010
.000	−.002	−.002	−.001

Residue		A 260	A 265	C 270	C 275	C 280	T 260	T 265	T 270
	1	~~.024~~ .017	~~.018~~	.029	.026		~~.029~~ .022	~~.034~~ .023	~~.030~~ .020
	2	~~.016~~	~~.014~~	.026	.024		.024	.023	.016
	x̄	.017			.025			.023	

17-VII-50. **Influenza**

Two preps. by Lister: B8 - red cell eluate in 4.3 ml

B12 - 2-cycle centrifuged. in 8.6 ml.

in Ca. Ringer.

Kjeldahl: +32 ~~+10%~~ =

h	0.3 ml B8	.076	$\cdot 078 - \cdot 017 = \frac{\cdot 061 \times \cdot 480}{\cdot 3}$ =	~~108~~ 101 ~~96~~ γN/ml
l	"	.080		
P_2	0.6 ml B12	.165	$\cdot 165 - \cdot 017 = \frac{\cdot 148 \times \cdot 480}{\cdot 6}$ =	~~130~~ 122 ~~118~~ γN/ml
P_3	"	.165		
P_6	Blank	.016		
X	"	.017		
W	2 ml B7 (unfused)		$\cdot 081 - \cdot 017 = \frac{\cdot 064 \times \cdot 480}{2}$ =	~~17.0~~ 15.9 ~~15.4~~ γN/ml

P. Allen. 4 mm cell. 608 filter. Ion. blank.

	5 γ	.100		
	10 γ	.194	See 27-VI for curve.	
	15 γ	.262		
Z	0.4 ml B8	.183	= 10 γP	= 25 γ P/ml
P_1	0.8 ml B12.	.249	= 27.5 γP	= 34 γ P/ml

Assuming N = 10% of dry wt.

B_8 - dry wt. = ~~1.08~~ 1.08 ~~0.98~~ mg/ml   Total = ~~4.5~~ 4.2 ~~4.1~~ mg.

P = ~~2.4~~ 2.5 ~~2.6~~ %

B12 - dry wt. = ~~1.30~~ 1.22 ~~1.18~~ mg/ml   Total = ~~11.2~~ 1.05 ~~10.2~~ mg.

P = ~~2.6~~ 2.8 ~~2.9~~ %

B8.	2.7 ml	=	~~2.6~~ 2.9 mg	2.7
B12	6.0 ml	=	~~7.1~~ 7.8 mg	7.3
	8.7 ml		9.7 mg ~~10.7~~	10.0

18-VII-50. **Influenza NA**

Combine remainder of 2 preps → 9.7 mg. in 8.7 ml. Add 1 ml 5% TCA, leave in frig 5 min. Coagulates. Spin 20 min 3500, decant. (Supt. still faintly opalescent.) Susp. pellet in 5 ml 3:1 alcohol: ether, leave 30 min at room temp., spin 15 min 3500. Dry pellet briefly in oven, add 0.5 ml 5% TCA, put in W.B. 90° 15'. Spin, decant, dry down supt., wash residue c̄ 0.5 ml 5% TCA, spin, add supt. & dry down.

Add 12 μl 72% $HClO_4$, heat 100° 60'. add 12 μl H_2O, spin. Some carbon is sedimented, but supernat is still brown. 18 μl spot on paper.

	λ	E	γ mols/ml eluate × 10²	Corrected for incomplete extraction (add 14.5%)	Molar ratios
A	265	·236	1·82	1·82	
G	250	·190	1·73	1·73	
C	275	·128	1·22	1·40 ~~1·39~~	
U	255 / 260 / 265	·121 / ·123 / ·112	1·56	1·79 ~~2·00~~	
T	260 / 265	·035 / ·049	0·62	0·71 ~~0·80~~	
				~~7·92~~ 7·45	
? between C & U	270	·035			
	260	·016			
	280	·028			
	285	·024			
	275	·027			

γ mols total bases/mg. virus = ~~·0792~~ ·0745 × 5 × $\frac{24}{18}$ × $\frac{1}{10·0}$ = ~~·0544~~ 0.0496

Assuming mean nucleotide wt = ~~309~~ 320, % NA in virus = $\frac{·0496 \times 320}{10}$ (~~·054 × 309~~) = 1.6 %.

By ratio U : T, this is 1.05% RNA

0.~~45~~55% DNA.

	BSNA	YNA
Tube + NA	9.8412	9.5970
Tube	.8622	.6122
NA	21.0 mg.	15.2 mg

		μmoles/ml ×10²	Ratios
BSNA -	A	6.93	~~1.069~~ 1.096
	G	5.61	~~0.865~~ 0.886
	C	5.36	~~0.827~~ 0.846
	T	7.19	~~1.204~~ 1.137
	MC	0.24	~~0.037~~ 0.038
	Total	25.33 ~~25.95~~	4.003

Corresp P = $\frac{39}{31} \times \frac{1}{5}$ = 25.2 Accounted for = 101%.

YNA	A	5.67	1.028
	G	6.73	1.219
	C	4.52	0.820
	U	5.14	0.931
	Total	22.06	

Corresp. P = $\frac{38}{31} \times \frac{1}{5}$ = 24.5 Accounted for = 92%

25-VII-50 P recovery in YNA BSNA hydr. c̄ 12 N. $HClO_4$.

Undried NA's weighed into bomb tubes, add 0.2 ml 72% $HClO_4$, cork, cook 60' 100°. Cool. Add 0.4 ml H_2O, mix, spin. Pipette 18 μl spots onto paper & for P.

P:		Repeat. Graph of 3-VII.		
30 γ	.255	.268		
40 γ	.346	.352		
X - BSNA	.345 } .338	.353 }	.345 = 39 γ P	= $\frac{.039}{21.0} \times \frac{0.6}{.018}$ = 6.2% of wt.
Z "	.331	.337 }		
P_6 - YNA	(.461)	.344 }	.337 = 38 γ P	= $\frac{.038}{15.2} \times \frac{0.6}{.018}$ = 8.3%.
W "	.325	.330 }		

~~Repeat~~:

26-VII.		A	G	C	U	
YNA	1	.737	.747	.472	.401	
	2	.737	.734	.478	.409	
	$\bar{x}$	.737	.740	.476	.405	

BSNA					T	MC 27~~5~~ 283 290.		
	1	.906	.623	.571	.582	.025	.027	.023
	2	.894	.610	.558	.610	.020	.022	.07
	$\bar{x}$	.900	.617	.565	.571 ~~.621~~		.024	

Bottle + vaccinia after removal

	7.3012
	.2798
	21.4 mg.

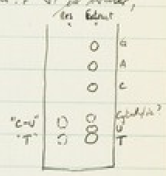
	Yield/ml	For incomplete extraction Add 10% to pyrimidine	Ratios
A	2.53 (~~1.96~~)	2.53	1.03
G	2.19	2.19	0.89
C	1.80+0.19=1.99	2.19	0.89
T	2.05	2.25	0.91
U	0.65	0.71	0.29
		9.87	4.01

Total μ moles nucleotide/mg virus = $\frac{9.9}{100} \times 5 \times \frac{49}{18} \times \frac{1}{21} = 0.064$

% calc. NA in virus = $0.064 \times \frac{310}{10} = 2.0\%$.

30-VII-50 Vaccinia NA

Analyzed, freeze-dried Vaccinia virus from McFarlane (V94/95) weighed into centrifuge tube. Add 1 ml 5% TCA, heat 90° 15 min., spin, decant & evap. supernat. to dryness. Re-extract residue c̄ 1 ml TCA 15 min at 90°, add sol'n to first & evap. to dryness; then finally wash residue c̄ 0.5 ml TCA, add to above & dry. Also dry residue in oven. Hydr. in 18 μl 72% $HClO_4$, dil. c̄ 31 μl water, spin, put 18 μl spots – 2 of extract + 1 of residue.
Extract shows G, A, C, U, T, & spot above U (cytidylic?)
Residue shows ~~water~~ non-spec. absorption, & spots of T & diffuse spot in cytidylic–uracil portion ("C-U")



NA Extract	G 250	A 260	C 275	U 250	U 255	U 260	U 265	T 260	T 265	T 270	"Cytidylic" 280	275	270	265	260	255
1	.242	.352	.185	.081	.084	.082	.075	.162	.164	.13	.025	.026	.027	.029	.029	.027
2	.239	.326	.194	.071	.072	.069	.062	.156	.161	.149	.023	.024	.026	.029	.028	.026
x̄	.241	.329	.190			.076			.163		.024					

(under U: ↓ assume 2/3 uracil; .051)

Residue.

"C-U" (against cytidylic blank)

255	.086
260	.094
265	.106
270	.123
275	.134
280	.118

"T"

270	.094
265	.101 (assume ¼ T = 0.025)
260	.102
255	.105
250	.109
245	.116
240	.144

Uracil & Cytidylic from NA extract concentrated & re-run in BuOH-formic → small uracil spot, no evident spot of cytidylic.

31-VII-50. <u>Bm DNA</u> (made by Sylvia)

Full-grown silkworms killed c̄ ether, weighed (= 234 g.) & immediately minced c̄ Waring blender in 500 ml 1 M-NaCl 0.02 M-Na citrate. Contains much mulberry leaf! Stand at room temp. 2 hrs. Strain & spin clear (MSE 30 mins), add to 1 vol EtOH → big ppt. Susp. in 50 ml 1 N-NaOH, leave overnight at 37°.

Spin off small residue. Ppt c̄ HCl + EtOH. Susp. in M-NaCl + NaOH to weak alkaline, big insol. residue spun off & discarded. Sol'n Sevag ca. 5x (removes yellow), ppt → big ppt. Dissolve in M-NaCl, dilute sample for Beckman → no peak at 260. Add spit, leave overnight, ppt. still → big ppt. Dissolve in 10 ml M-NaCl, Sevag 4 more x → more gel.

Dil 1:25 for Beckman

265	.297
260	.314
250	.287
240	.224
235	.201
230	.193
225	.214

Total NA ≏ 0.3 × 0.04 × 25 × 8 = 2.4 mg.

Ppt. c̄ HCl + EtOH → still pretty big ppt. Spin down, wash c̄ EtOH & ether. Add 0.04 ml 72% $HClO_4$, cook 1 hr at 100°, add 0.06 ml water, spin. Hydrolysate is practically solid.

Bottle 7.2084
+ NA (undried) .2864
NA 78.0 mg. ↑ 5.0 ml N/25 NaOH

Vol. flask (20 ml) MT 13.4167

+ thymine	.4407	T = 24.4 mg.	↑ 20 ml 2 N-HCl.
+ guanine	.4652	G = 24.5	
+ adenine	.4918	A = 26.6	

12-ix-50. Hydrolysis of pure bases along c̄ NA, by HCOOH & $HClO_4$.

T, G, & A dried 1½ hrs at 110°, weighed into vol. flask, dissolved in 2 N-HCl.

BSNA. not dried, dissolved in N/25-NaOH.

Pipette 0.5 ml portions BSNA sol'n into 4 tubes, dry down. To 2, add 0.3 ml T, G, A sol'n, dry down.

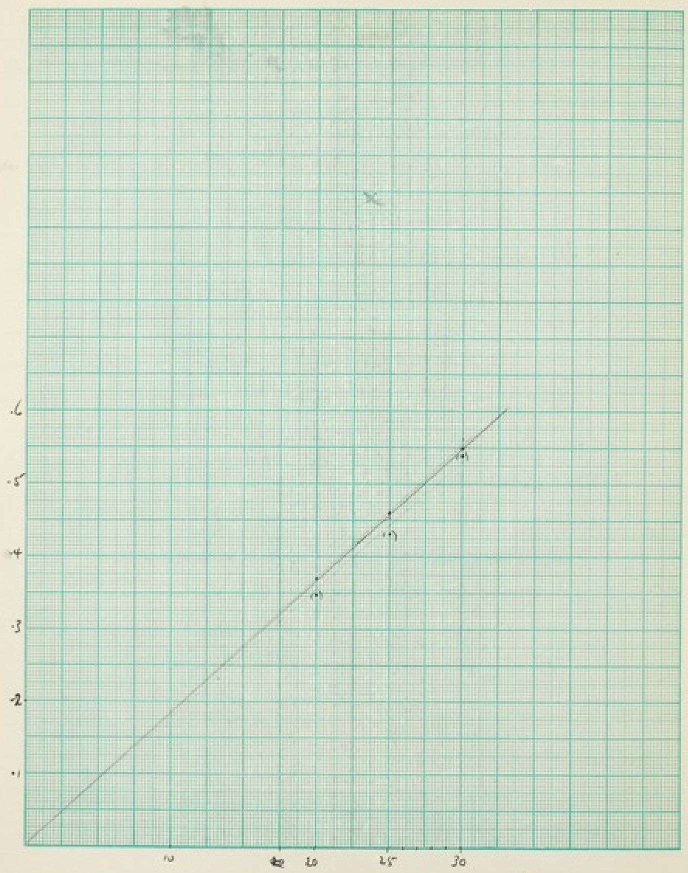
HCOOH – to 1 tube BSNA & 1 c̄ added bases add 0.5 ml 98% HCOOH, hydr. 175° 30 min., dry down, ↑ 0.25 ml 1N-HCl.

$HClO_4$ – to identical pair of tubes add 0.1 ml 72% $HClO_4$, cook on B.W.B. 1 hr., add 0.15 ml H_2O, spin.

13-ix 3 14.0 μl spots of each (after hydrolysates stood overnight), & aliquots for P at same time.

~~Theoret. amt. of added bases in hydrolysate if no destruction.~~

Amounts of bases added: A 0.399 mg.
G 0.368 mg.
T 0.366 mg.



13-ix-50. P estimations.

		Incubated. [illegible] 4 cm. cells. Against reagent blank.		Re-done without incubation; clean cylinders	
HCOOH – BSNA	A	·502		·545	
	h	·561	·532	·535	·541
	d	·533		·524	= 29.5 γP
HCOOH – BSNA + T, G, A	X	·518		·533	
	Lx	·539	·533	·546	·532
	D	·543		·516	= 29.1 γ
$HClO_4$ – BSNA	Z	·500		·478	
	a	·500	·493	·490	·484
	P_2	·479		·484	= 26.3 γ
$HClO_4$ – BSNA + T, G, A.	P_3	·531		·507	
	g	·516	·515	·524	·514
	P_4	·499		·510	27.9 γ
Blanks – ~~[illegible]~~ Incubation blank	W	– ·021	– ·022		
	P_5	– ·023			

	beginning	end			
20 γ	·344	– ·350	= ·347	·369	[illegible]
25 γ	·430	– ·432	= ·431	·460	[illegible]
30 γ	·538	– ·541	= ·540	·549	[illegible]

BSNA - HCOOH $HClO_4$

	Knudsford	Ratio	Knudsford	Ratio
A	4.90	1.156	5.12	1.280
G	3.72	0.877	3.75	0.896
C	3.69	0.871	3.58	0.860
T	4.66	1.099	4.23	1.017
	16.97	4.003	16.66	4.003
Corresp. P	19.0		17.0	
% P recovered	89%		98%	

Factor for ~~any~~ total sum = $\frac{5}{4.14} \times \frac{.265}{.261} \times 1.01 = 90.1$

		A	G	C	T
HCOOH	Total mg. base found in mixture	0.386	0.823		0.845
	" in NA	0.579	0.505		0.531
	Extra recovered	0.387	0.318		0.314
	Total extra added	0.399	0.368		0.366
	% recovery	97	86		86
$HClO_4$	Total found in mixture	1.035	0.899		0.870
	" in NA	0.694	0.564		0.536
		0.341	0.335		0.334
	% recovery	85	91		91

14-i-50. Base extinctions of hydrolysis mixt.
All read on Beckman against water.

		A_{260}	G_{250} (260)	C_{275} (260)	T_{265} (260)
Blanks	1	.029	.026 (.024)	.018 (.023)	.060 (.056)
	2	.024	.028 (.025)	.022 (.021)	.053 (.051)
	x̄	.024	.027	.020	.057
HCOOH-BSNA	1	.652	.490	.405	.414
	2	.669	.490	~~.416~~ ~~.407~~ .404	.443
	3	.661	.439	~~.393~~	.426
(Blank off)	x̄	.637 ✓	.409 ✓	.388	.371 ✓
HCOOH-NA+	1	1.05	.690	.431	.632
	2	1.08	.700	.439	.651
	3	1.09	.688	.429	.641
	x̄	1.05 ✓	.666	.413	.591
$HClO_4$-BSNA	1	.660	.459	.384	.381
	2	.698	.468	.416	.396
	3	~~.720~~ .713	.465	.389	.401
	x̄	.665	.410	.376	.336
$HClO_4$-NA+	1	1.05	.719	.394	.621
	2	1.10	.731	.418	.648
	3	1.06	.713	.394	.640
	x̄	1.05	.694	.382	.579

Factor for mononucleotide mixture, based on P: $\frac{29.3}{26.3} = 1.11$

$\frac{29.3}{27.9} = 1.05$

Tube MT 8.9644
+ bugs 9.0324
bugs 68.0 mg

14-ix-50. Extraction of NA bases from TB bacilli by TCA.

Bovine tubercle bacilli weighed into hypo tube (68 mg, wet basis), extracted 3 x each for 15' = 1 ml 5% TCA at 100°, spinning down after each in heated centrifuge bucket (cooled during first spin; otherwise kept at 50-60°). Bugs down efficiently, the last + combined add 0.05 ml 72% $HClO_4$, heat 100° 1 hr. Add 0.1 ml water, spin, filter on Gelman.

16.IX.30. Repeat hydrolysis of bases along ± NH

To remainder of DNA soln from 48 mg material in 3 ml N/25-NaOH, add 7/8 vol DNA + 2 ml water → 119 mg in 5 ml.

Dry down H 1 ml portions (≙ 20 mg NA), 0 to 2 add 1 ml of soln of G, A + T (≙ 1.2 mg each). + dry down.

$HClO_4$: 0.1 ml, hydr. 95°-100°, add 0.2 ml water.

HCOOH: 0.7 ml, dry down (title ± added bases buffer) Atmosphere-bone on P, ↑ 0.3 ml,

Discarded.

NA.	Test tube MT	28.5695			
	+ BSNA	.7143			
	Undried NA =	144.4 mg.	↑ 6.0 ml N/50 NaOH → 24 mg/ml		
Bases	Test tube MT	27.5607			
	+ thymine	.5792	T = 18.5 mg	↑ 5.0 ml 86% HCOOH →	3.70 mg/ml
	+ adenine	.6043	A = 25.1 mg		5.02 "
	+ guanine	.6316	G = 27.3 mg		5.46 "

17-ix-50. __Hydrolysis of pure bases along $\bar{c}$ NA - 3rd try.__

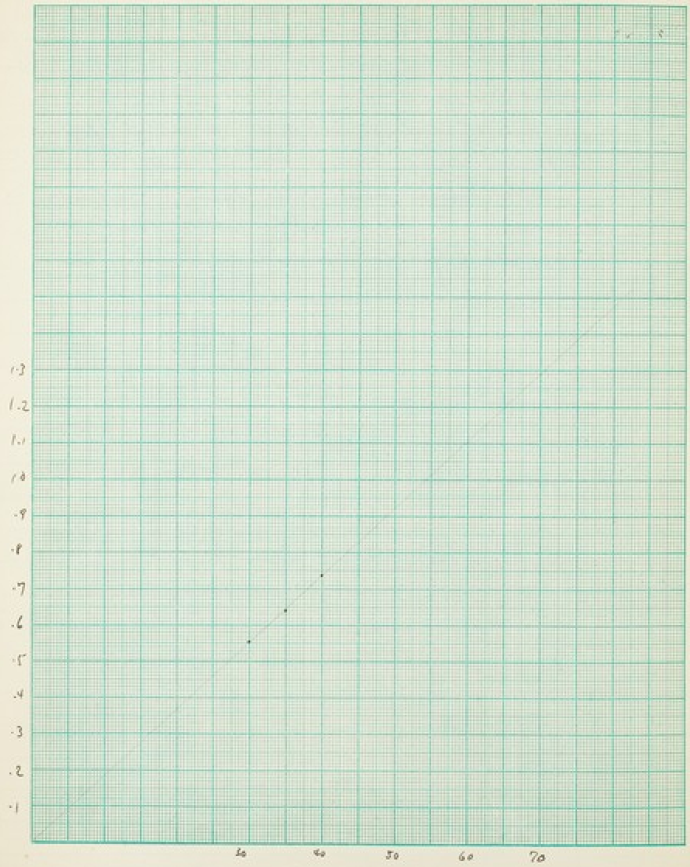
BSNA made up 24 mg/ml (undried) in N/50 NaOH, & 1.0 ml portions dried down in 4 tubes.

A, G, & T made up in 86% HCOOH, & 0.3 ml pipetted into 2 of the tubes; dry down.

HCOOH hydrolysis: 0.5 ml, 175°, 30', dry down, ↑ 0.4 ml N-HCl.

$HClO_4$ " : 0.15 ml 100° 60', add 0.25 ml water.

18 μl aliquots for chromatograms & for P.



17-ix-50. Pectinations. Without incubation. 4 mm cell, 608 filter.

30 γ	.554	
35	.640	
40	.736	
HCOOH	1.23	1.23 ≡ 66.5 γP
"	1.23	
" +	1.22	1.23 ≡ 66.5 γP
" +	1.23	
$HClO_4$	1.16	1.16 ≡ 63 γP
"	1.16	
" +	1.09	1.10 ≡ 59.5 γP.
" +	1.10	

Pcontent of NA = $.0665 \times \frac{0.4}{.018} \times 1.01 \times \frac{100}{24} = 6.2\%$.

I *Recovery from sol'n of five bases*

	Am't found	Am't taken	% recovery
A	$\frac{.853}{.96}$ ~~.96~~ .15 x .01 = 0.0886 ~~0.0??3~~ mg	0.018 x 5.02 = 0.0903	~~102~~ 98
G	$\frac{.59}{.73}$ x 5 x .01 =	0.0951	.018 x 5.46 ~~3.76~~ = 0.0981	~~108~~ 97
T	$\frac{.797}{.63}$ x 5 x .01 =	0.0633	.018 x 3.70 ~~5.46~~ = 0.0665	~~105~~ 95

II *Molar ratios of BSNA (allow 0.05 for 5-M-C)*

	HCOOH μmol/ml	Ratio	HClO4 μmol/ml	Ratio
A	11.94 ~~?.??~~	1.14	12.00 ~~?.00~~	1.16
G	8.90	0.85	9.64	0.93
C	8.94	0.855	8.74	0.84
T	11.53	1.10	10.61	1.02
	41.31	3.945	40.99	3.95
Amount P	42.9		40.6	
P accounted for	96%		101%.	

18.ix *Base estimations.*

	A_{260} in 10 ml	G_{250}	C_{275}	U .255	U 260	U 265	T_{265}
HCOOH	.781	.980	.934 (36, 24)	.013	.016	.018	.900
	.777	.991	.962 (42, 52)	.016	.015	.013	.924
	.766	.966	.946 (60, 46)	.045	.046	.042	.924
	.775	.979	.941				.916
HCOOH +	1.341	1.84	.925	.052	.055	.052	1.485
	1.362	1.83	.963	.059	.059	.056	1.505
	1.351	1.81	.955	.052	.053	.051	1.490
	1.357	1.83	.948				1.48
HClO4	.776	1.050	.910	.025	.025	.024	.840
	.788	1.06	.934	.032	.030	.029	.848
	.780	1.07	.916	.020	.019	.019	.842
	.780	1.06	.920				.843
Corr. for vol. change by P content: 8 (x 665/63)	.824	1.12	.971				.889
HClO4 +	1.198	1.76	.800	.031	.032	.034	1.293
	1.226	1.80	.837	.039	.040	.041	1.316
	1.246	1.82	.824	.022	.021	.024	1.339
	1.223	1.79	.820				1.316
Corr. for vol. change by P content 8 (x 665/595)	1.37	2.00	.916				1.47
+	.842	1.38					.796
	.860	1.38					.796
	.849	1.41					.798
	.850	1.39					.797

Not corrected for vol. of solutes

Amount in each spot, μg.

	A	G	C	T	Corr. for V of solutes
HCOOH					
Total formed	141.0	125	49.9	117.6 ~~72.6~~	× 1.037
Found in NA	80.8	67	49.5	72.6	1.03
Extra	60.2	58		45.0	
Extra added	66.4	72.1		49.0	
% recovery	91	80.5		92	
$HClO_4$					
Total formed	143.	137	48.2	116.7	X1.037
Found in NA	85.9	76.6	51.1	70.5	1.03
Extra	57.1	60.4		46.2	
Extra added	66.4	72.1		49.0	
% recovery	86	84		94	

Hydrolysis of pure bases, 2 NA - Expt. of 18-IX-50.

μg./spot, corr. to constant hydrolysate vol. = 0.40 ml.

HCOOH	A	G	C	T
Found in mixture	146.0	129.5	51.6	121.8
Found in NA	83.3	69.0	51.0	74.9
Extra	62.7	60.5	-	46.9
Extra used	66.4	72.1		49.0
% recovery	95			96

	Whole vaccinia				+ pepsin residue
Tube MT	18.1436	[illegible].	18.3757		18.3646
+ stuff	.1613	+ remainder of new vaccinia	.3808 ~~[illegible]~~	= 5.1 mg.	.3704
Stuff	17.7 mg	+ remainder of old vaccinia	~~.3822~~ .3847	3.9 mg	5.8 mg.
				9.0 mg.	

18-IX-50. <u>Vaccinia</u>

(1) <u>Total NA of vaccinia.</u> Whole NaCl-treated vaccinia dried 1½ h. @ 110°, weigh (17.7 mg.) extract c 5% TCA @ 100° 4 x: 30', 15', 15', 15' spinning in hot centrifuge bucket. Extracts dried down together, add 33 ul 72% HClO$_4$, heat 100° 60', add 50 ul H$_2$O, spin, put 2 x 33 ul spots on paper. TCA residue: dry & hydr. identically & 1 33 ul spot.

(2) <u>DNA of whole vaccinia.</u> Dry & weigh remnants of old (V 94/95) & new vaccinia (total 9 mg), add 1.5 ml 1N-NaOH, leave @ 37° overnight. Ppt c HCl + EtOH, extract c TCA, dry down, hydr in 18 ul HClO$_4$, add 18 ul H$_2$O, put on 33 ul spot.

(3) <u>NA of pepsin residue.</u> Dry, weigh (5.8 mg.), hydr. whole: add 33 ul 72% HClO$_4$, heat 100° 60', add 50 ul H$_2$O, spin, put 2 x 33 ul spots on paper.

18-ix-50. Vaccinia

(4). NA in NaCl extraction sft. To sol^n (ca. 40 ml) add HAc → pH < 4; a little $Ca(NO_3)_2$, (ca 0.5 g.), + 1 vol abs. EtOH. Stand in frig 4 hrs. Spin 3500 30 min.

Sft. undiluted:

D_{250}	0.656	280	·333
260	·418	290	·296
270	·374		

Max. possible total NA $= \frac{1}{2} \times \cdot 42 \times \cdot 04 \times 75 = \cdot 6$ mg

(does not dissolve)
Ppt: susp. in ca. 1 ml M NaCl – N/20 NaOH, transfer to pyrex tube, re-ppt: HAc + EtOH, extract ppt c̄ (1 ml portion) TCA at 100°: once for 30' + twice for 15".
Dry down, hydr c̄ 18 µl $HClO_4$, add 18 µl H_2O, put on 33 µl spot.

(5) NA in pepsin digestion sft. (ca. 25 mg) ppt. as above + spin off

Sft. undiluted:

D 240	∞	260	·41	280	·532
250	·716	270	·464	290	·461

Max. possible total NA $= \frac{1}{2} \times \cdot 41 \times \cdot 04 \times 30 = 0.24$ mg.

Ppt (v. small) - treat as above, but using only 0.5 ml portions TCA.

Vaccinia.

γ mols base ml eluate x 10²:-

	Whole virus		Pepsin residue		"DNA"		Pepsin spt.		NaCl spt.	
A	1.19	1.09	1.42	1.19	1.62	0.88	0.15	0.54	9.48	0.86
G	0.95	0.87	1.04	0.87	1.08	0.59	0.26	0.93	12.29	1.11
C	[0.79]	0.72	[1.02]	0.85	[1.39]	0.76	0.27	0.96	11.2	1.01
U	0.67	0.61	0.47	0.39	0.85	0.46	0.44	1.57	3.78	0.34
T	0.78	0.71	0.84	0.70	2.40	1.30	0.0	0	7.45	0.67
	4.38	4.00	4.79	4.00	7.34	3.99	1.12	4.00	44.20	3.99

Total γ mols base/aspt	0.22	0.24	0.37	0.056	2.21
≡ γ NA (x315)	69	74	116	18	696
γ NA in whole hydrolysate	174	186	121	20	760
NA as % of virus	0.98 %	3.2 %	1.34 %	0.08 % (of 25 mg virus)	1.52 % (of 50 mg virus)

Conc. in soln, mg per l.

	Untreated	% of amt. applied	After heating in HCOOH	% recovered
A	5.14	102	5.06	98.5
G	5.58	102	5.44	97.5
T	3.76	102	3.71	98.8

10-xi-50. <u>Hydrolysis of pure ~~guanine~~ bases: c HCOOH & $HClO_4$.</u>

0.5 ml mixture of G, A, & T, from 18-ix, pipetted into 2 tubes, dry down.

To one, add 0.5 ml 72% $HClO_4$, stopper, heat 100° 60'

other, add 0.5 ml 98% HCOOH, seal off, heat 175° 30', evap to dryness.

+ 0.5 ml 1N-HCl (must warm to dissolve).

12-xi. 14.0 μl strips of each & of orig. soln on paper.

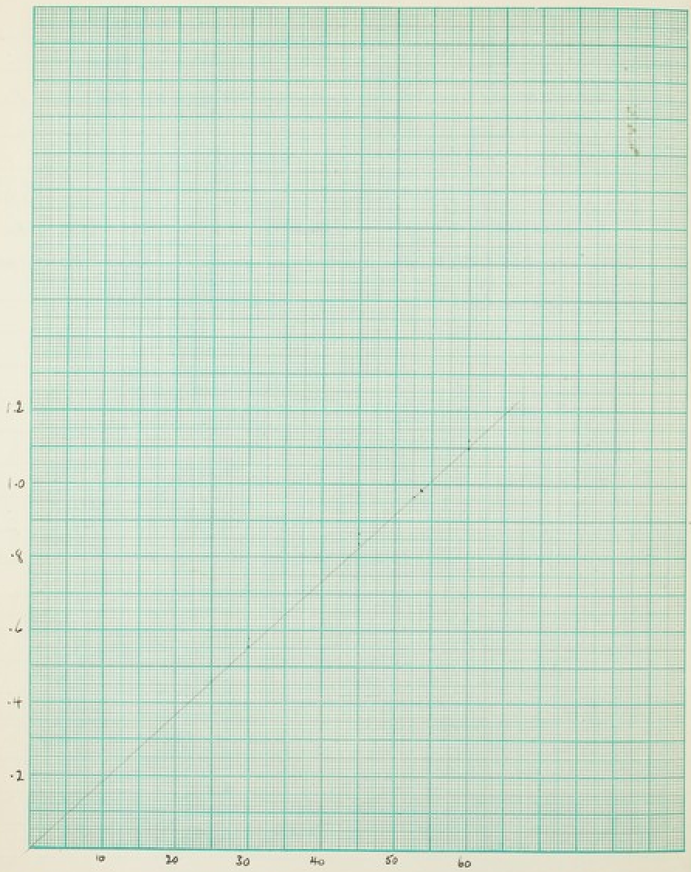
$HClO_4$ streaked badly – evidently cannot apply undiluted.

Cut out orig soln spots & HCOOH, elute in 5 ml N/10 HCl, read all & blanks, in same cell against water.

		A_{260}	G_{250}	T_{265}
Blank		.029	.034	.089
orig	1	1.415	1.17	0.746
	2	1.415	1.19	.743
	3	1.402	1.15	.768
		1.41	1.17	.752
		.03	.03	.089
		1.38	1.14	.663
HCOOH	1	1.364	1.13	.721
	2	1.390	1.12	.707
	3	1.384	1.13	.784
		1.38	1.13	.737
		.03	.03	.089
		1.35	1.10	.648
+1% corr. for vol. of solutes		1.363	1.111	.655

Tube MT 23·6+2
+NA 7884

NA ·114·2 mg undried BSNA ↑4 ml 98% HCOOH, with warming



20-x-50. Pertumations

P_2 = blank

		Read against inein blank		
P_6	HCOOH	·975	·984	= 535 γP
GWA	"	·992		
LX	HCOOH +I,G,A	·511	·516	=
P_1	"	·521		
A	$HClO_4$	·957	·966	= 52.5 γP
Z	"	·975		
k	$HClO_4$ + T, G, A	·510	·506	
j	"	·501		

HCOOH not [illegible]	1.02	− ·024 = 1.00	0.95
"	0.92	·90	
30 γP	·579	·555	
45	·863	·839	
60	1.12	1.10	

[illegible] blank read against reagent blank = 0.024 (i.e. [illegible] blank absorbs less)

Pin [illegible] NA = $·0535 \times \frac{·5}{·014} \times \frac{100}{28.5}$ = 6.7 %

HCOOH.	HCOOH µmol/ml	Ratio	HClO₄ µmol/ml	Ratio
A	8.85	1.127	8.77	1.121
G	6.58	0.837	7.01	0.898
C	6.82	0.869	6.60	0.845
T	8.78	1.117	8.46	1.083
	31.03	3.950	30.84	3.947
Conc of P	34.5		33.9	
Accounted for	90%		91%	

Added bases; conc. in orig. soln. µg/ml

A $\frac{1.445}{0.96} \times \cdot 31 \times \frac{5}{\cdot 004}$ = 5.23

G $\frac{1.146}{0.73}$ × " = 5.61

T $\frac{663}{\cdot 663}$ × " = 3.75

21-x-50. Base estimations.

All read in same cell against N/10 HCl.

		A	G	C	T	Actual vol. of hydrolysate
Blank		·035	·049	·036	[·125] Apparently in error – assumed 0.080	
HCOOH	1	1.18	·771	·739	·767	0.5 × 102.5 = ·513
	2	1.18	·774	·767	·802	
	3	1.19	·770	·751	·766	
	$\bar{x}$	1.183	·772	·752	·778	
		·035	·049	·036	·080	
		1.15	·723	·716	·698	
Actual wt./ml. eluate		11.98	9.91	7.54	11.09	
HCOOH +	1	1.27	·971	·403	·767	0.5 × 102 = ·51
	2	1.28	·955	·409	·774	
	3	1.28	·958	·408	·753	
	$\bar{x}$	1.273	·961	·407	·759	
		0.035	·049	·036	·080	
		1.238	·912	·361	·679	
Actual wt./ml eluate		12.89	12.50	3.80	10.59	
HClO₄	1	1.17	·826	·724	·744	·5 × 102.5 × 102 = ·523
	2	1.18	·819	·739	·746	
	3	1.18	·816	·724	·768	
	$\bar{x}$	1.177	·820	·729	·753	
		·035	·049	·036	·080	
		1.14	·771	·693	·673	
Actual wt./ml eluate		11.87	10.56	7.30	[illegible]	
HClO₄ +	1	1.27	·995	·392	·707	·5 × 102 × 102 = ·52
	2	1.27	·990	·388	·731	
	3	1.28	·990	·394	·729	
	$\bar{x}$	1.273	·992	·391	·722	
		·035	·049	·036	·080	
		1.24	·943	·355	·642	
Actual wt./ml eluate		12.91	12.91	3.74	[illegible]	
+ (added base)	1	1.44	1.19		·739	
	2	1.44	1.20		·746	
	$\bar{x}$	1.440	1.195		·742	
		·035	·049		·080	
Actual wt./ml eluate		1.405 14.62	1.146 15.69		·662 10.51	

Recovery of bases after hydrolysis & BSNA.

μg. / chromatogram spot, corrected to whole hydrolysate vol. = 0.50

		A	G	C	T
HCOOH	Found in mixture	65·8	63·8	19·4	55·0
	Found in NA	30·7	25·4	19·3	28·4
	Extra formed	35·1	38·4	-	26·6
	Amt added	36·6	39·2	-	26·3
	% recovery	96	98	-	101
HClO4	Found in mixture	67·2	67·2	19·4	53·1
	Found in NA	31·1	27·6	19·1	27·9
	Extra formed	36·1	39·6		25·2
	Amt added	36·6	39·2		26·3
	% recovery	99	101		96

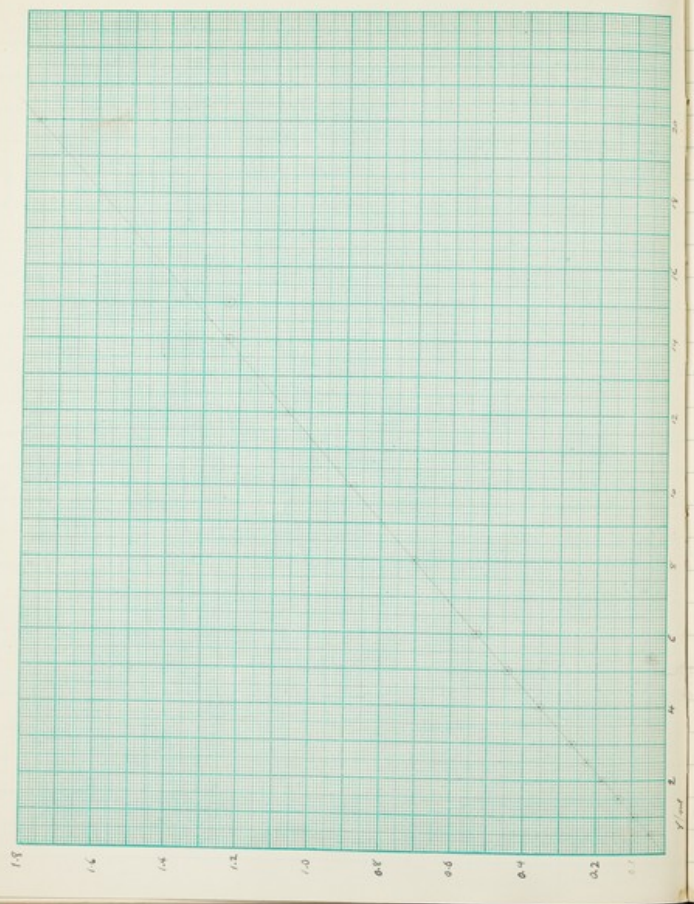
Recovery of bases after hydrolysis of BSNA.

μg./chromatogram eqvt, corrected to mole hydrolysate wt. = 0.60

		A	G	C	T
HCO$_2$H	Found in mixture	65.8	63.8	19.4	55.0
	Found in NA	30.7	25.4	19.3	28.4
	Extra formed	35.1	38.4	-	26.6
	Amt added	36.6	39.2	-	26.3
	% recovery	96	98	-	101
HClO$_4$	Found in mixture	67.2	67.2	19.4	53.1
	Found in NA	31.1	27.6	19.1	27.9
	Extra formed	36.1	39.6		25.2
	Amt added	36.6	39.2		26.3
	% recovery	99	101		96

SAULT STE. MARIE.

December, 1950.



9-xii

Linearity of Beckman.

Ardamin sulfate (not dried) made up 0.132 mg/ml.

Diluted as follows:

Vol. Taken	Diluted to	$D_{262.5}$		nominal γ/ml
0.25 ml	50 ml	0.049		0.5
.5	"	.094		1.0
.75	"	.138		1.5
1.0	"	.185		2.0
1.25	"	.228		2.5
1.5	"	.267		3.0
2.0	"	.357		4.0
2.5	"	.443		5.0
3.0	"	.531		6
3.0	25	.705		8
2.5	"	.884		10
3.0	"	1.06	1.064	12
5.5	"	1.22	1.233	15
5.0	"	1.70	1.762	20

HT cell after: 0.002

Check cells. Cleaned by Smith-Dorsten. 13209 as blank, read on 13217. M/10 1961.

λ	D		
300	0.000	230	.000
280	.000	225	−.003
260	.001	220	−.006
250	.001	215	−.012
245	.002	210	−.016
240	.002	207.5	−.019
235	.002		

13-xii Calibration of micro pipettes – gravimetric

						D263 for pipetteful in 5 ml	Repeat 18-xii
①	Full MT	9.7812	.7812	.7811	.7811	.677 } .675	678 } .677
	water	.7777	.7776	.7776	.7776	.674 }	676 }
		3.5	3.6	3.5	3.5		709
							$V = 100 \times \frac{.677}{.997} \times \frac{5}{100} = 3.40$
②	Full	7.3104	.3105	.3104		1.640 }	
	MT	.3019	.3020	.3019		1.655 }	
		8.5	8.5	8.5			
③	Full	6.7800	.7800	.7800			
	MT	.7693	.7693	.7693			
		10.7	10.7	10.7			
④		4.5868	.5867	.5867		2.32	
		.5748	.5748	.5748		2.43	
		12.0	11.9	11.9			
⑤		8.8750	.8750			.844 }	
		.8692	.8692	(size reduced)		.851 }	
		5.8	5.8				
⑥		6.5				1.291 }	
						1.291 }	

Blank from MT test tube: 0.003, 0.003

Standard: 0.10 ml → 50 ml 1.977 } 1.974   0.1 → 100 0.995 } 0.997
　　　　　　" 　　　　　 1.972 }　　　　　　　　　　　 1.000 }
　　　　0.20 – → 100 1.920

BSNA-$HClO_4$.

	Yield/mol	Ratio
A	1.135⁻	1.14
G	0.985⁻	0.99
C	0.835⁻	0.825⁻
T	1.01	1.00
	3.985⁻	3.935⁻

14-xii-50. BSNA.

20·0 mg undried weighed into tube, add 0·3 ml 70% $HClO_4$, real, heat on BSWB 60'. Add Ort and H_2O.
Put aliquots on paper 3 × 3·5 µl + 3 × 11·9.
P. 3·5 µl aliquots in flasks 4, 5, 16.

NA-$HClO_4$ 6·7%

3·5 µl aliquots + blanks alongside put out, elute in 5 ml 4% $HClO_4$, stand overnight, shake, but do not centrifuge. Pour into cuvs of well once before reading.
3 clean tubes + $HClO_4$ D_{260} = 0·002, 0·000, 0·001

		A_{260}	G_{250}	C_{275}	T_{265}
Blanks	(1)	0·010	0·009	0·012	·026
	2	·011	·012	·011	·024
	3	·016	·013	·012	·027
	$\bar{x}$	·012	·011	·011	·026
Spots	1	·156	·121	·100	·110
	2	·164	·117	·101	·106
	3	·167	·120	·095⁻	·102
	$\bar{x}$	·162	·119	·099	·106
Spots − blank		·150	·108	·088	·080

Successive readings on G_1 after pouring in c̄ out of solution tube:

0.113, ·119, ·121, ·122, ·123, ·123 After standing 5 min. ·122

BSNA – $HClO_4$

	Nucleotide	Ratios
A	1.17	1.12
G	1.01	0.97
C	0.885	0.85
T	1.045	1.00
	4.110	3.94

20-xii. BSNA hydrolysate of 14-xii re-run on paper pre-treated by running isoprop.-HCl down it. 3.5 µl spots.

		A	G	C	T
Blanks	1	0.012	0.009	·010	·014
	2	·019	0.011	·012	·016
	3	—	[0.016 pencil mark]	[·015 pencil mark]	—
	$\bar{x}$	·016	·010	·011	·015
Spots shaken well c̄ centrifuged	1	·163	[·113 before spinning] ·120	·10[illegible]	·095
	2	·162	(·119) ·119	·100	·094
	$\bar{x}$	·163	·120	·101	·095
Blanks re-read shaken c̄ centrifuged	1	·009	·009	·008	·011
	2	·014	·009	·007	·012
	3	—	(·012 (pencil))	[·011 pencil]	—
	$\bar{x}$	·011	·009	·008	·012
Spot – blank		·152	·111	·093	·083

Conclude: (1) Pre-running of paper reduces T blank by 40%, does not reduce others.
(2) Centrifuging reduces all blanks by ca. 25%, c̄ improves reproducibility.

8-ii-51 Prep after Schmidt & Thannhauser treatment.

	A	G	C	MC	U	T	Total	
Blank	.017	.015	.015	.010	.018	.032		
1	.406	.293	.213	.068	.048	.296		
2	.409	.294	.213	.068	.044	.296		
$\bar{x}$	.408	.294	.213	.068	.046	.296		
$\bar{x}$ - B	.391	.279	.198	.058	.028	.264		Compt. P
Y moles/ml	3.00	2.54	1.88	0.59	0.355	3.32	11.68	11.9 98%
Ratios (=4.00 A+)	1.06	0.90	0.66	0.21	0.13	1.17		

14-ii. Re-treated c NaOH. ↑ 3.98 ml Chromatogram lying in drawer several days.

	A	G	C	MC	U	T	Total
B	.015	.021	.014	.009	.024	.038	
1	} .638	.488	} .314	.097	.069	.454	
2		.489		.091	.075	.446	
$\bar{x}$	.638	.489	.314	.094	.072	.450	
$\bar{x}$ - B	.623	.468	.300	.085	.048	.412	
Y moles/ml	4.79	4.26	3.96 (~~3.00~~)	0.87	0.61	5.19	18.68 ~~17.52~~ 14.9
Ratios	1.06	0.94	0.66	0.19	.135	1.15	

20-1-51. German NA prep.

1 pkt. "TONIK" processed wheat germ = 395 g. susp. in 2 l 1 M NaCl – 0.01 M Na citrate, left 2 hrs. @ 4°

22-i Add 1 l M-NaCl, mix on Waring blender, spin. Pour off (1.8 l.) into 6 vols. (11.7 l) tap water, let stand overnight.

23-i Suck off spt. spin down big ppt. ↑ 400 ml M NaCl + NaOH → pH 8. spin off little insol. material, reppt. by pouring into 2.4 l water → big ppt. Dissolve in 400 ml M NaCl, sevag 3 x (still big gel). Ppt c EtOH c HAc. Take small sample & hydrolyse c 70% HClO₄ 110° 30 min → much RNA. Dissolve all in 150 ml 1 N. NaOH, leave overnight at room temp + overnight at 28°C. (no 37°!). Ppt c HAc & EtOH. ↑ all. M NaCl, sevag ca. 4 x → no more gel, ppt c HAc + EtOH → strings. Collect strings only, wash c 70% EtOH, 95%, ether. Leave to air dry.

29-i. Air-dry = 390 mgs.

31-i 7.5 mgs hydr 90% HCOOH 175° 30 min ↑ 0.25 ml N HCl Considerable U spot. Dissolve all prep in ca. 10 ml N-NaOH, leave 18 hrs at 37°. ppt c HAc & EtOH, wash c EtOH, ether.

6-ii-51 Weigh 10.2 mg air dry, hydr 30 min 0.5 ml 90% HCOOH

P: 2λ → 3.7, 3.9, 3.7 = 3.77 γ 175°, dry, ↑ 0.3 ml N-HCl. [illegible] ·35 [illegible] spots. Some U.

9-ii 9.9 mg re-treated c NaOH & hydrolysed to see if U reduced.

P: 2λ → 4.25, H₂O, 4.25 = 4.30 γ

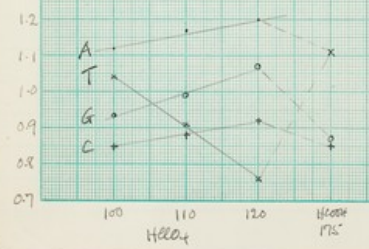
24-1.54.

$HClO_4$ hydrolysis at diff temps. HCOOH?

BSNA undried 63.4 mg ↑ 2.5 ml 86% $HClO_4$. (= 12.6 mg)
0.5 ml aliquots into 4 tubes dried down. To 3 add
NA in $HClO_4$ = 6.3% 0.2 ml 70% $HClO_4$, cook 60' at 100°, 110°, 120°; 60 min.
each, add 0.2 ml aq dest, ofnin. 4th tube ↑ 0.5 ml
96% HCOOH, cook 175° 30 min, dry down, ↑ 0.4 ml N-HS.
From each, aliquots: 2 × 2 µl (remanial pipette) for P 1.94 µl
2 × 16 µl (" ") for N 15.4
2 × 8 µl (" ") for bases 7.7

	N			P		
	Vol. used	N found		Vol. used	P found	
$HClO_4$ - 100	16 µl	46.8 48.9	47.9 γ	2 µl	3.56 3.73	3.65 γ
$HClO_4$ - 110		49.9 47.5	48.7 γ		3.79 [4.13]	3.79 γ
$HClO_4$ - 120		50.6 51.1	50.9 γ		3.88 3.83	3.86 γ
HCOOH		53.4 53.1	53.3 γ		3.77 3.78	3.78
Pipet blank	negligible					

	$HClO_4$-100	$HClO_4$-110	$HClO_4$-120	HCOOH 175°
	Yield and Ratios			
	N	N	N	N
A	2.69 ×4 = 1.12	2.69 ×4 = 1.17	2.58 ×4 = 1.20	2.67 ×4 = 1.11
G	2.24 ×4 = 0.935	2.27 ×4 = 0.99	2.29 ×4 = 1.07	2.09 ×4 = 0.87
C	2.04 ×4 = 0.85	2.03 ×4 = 0.88	1.98 ×4 = 0.92	2.04 ×4 = 0.85
T	2.49 ×4 = 1.04	2.09 ×4 = 0.91	1.64 ×4 = 0.76	2.66 ×4 = 1.11
	9.46 3.945	9.08 3.95	8.49 3.95	9.46
Group P =	9.30	9.65	9.83	9.62
(P/31 × 27/1.95 × 1/5) % P assembled =	102	94	86	98
Yield N found	35.75	35.07	33.67	35.24
Corresp N found (N = 1/5.414 ×)	34.2	34.8	36.3	38.1
% recovered for	104	101	93	93



30-1-51 BSNA bases.

5 ml N/10 HCl pipetted by hand, stood overnight, shaken mechanically 5' min., after 3 min. Read all against aq. blk.

		A	G	C	T	
Blank	1	.016	.016	.016	.036	Pattern of variation suggests pipetting error.
	2	.015	.013	.019	.039	
	3	.015	.015	.017	.040	
	$\bar{x}$	.015	.015	.017	.038	
$HClO_4$ 100°	1	.360 .360*	.259 .260*	.228	.234	
	2	.370 .369	.264 .261	.233	.237	
	3	.366 .365	.260 .256	.231	.238	
	$\bar{x}$	.365 = .350	.261 = .246	.231 = .214	.236 = .198	
$HClO_4$ 110°	1	.364 .365*	.265	~~.234~~ .230	.204	
	2	.365 .363	.265	~~.234~~ .230	.203	
	3	.367 .364	.265	~~.234~~ .230	.204	
	$\bar{x}$	.365 = .350	.265 = .250	.230 = .213	.204 = .166	
$HClO_4$ 120°	1	.354	.271	~~.234~~ .227	.169	
	2	.352	.269	~~.234~~ .224	.168	
	3	.348	.268	.223	.167	
	$\bar{x}$	.351 = .336	.267 = .252	.225 = .208	.168 = .130	
HCOOH 175°	1	.364	.249	.235	.252	
	2	.362	.244	.230	.249	
	3	.362	.243	.227	.246	
	$\bar{x}$	.362 = .347	.245 = .230	.231 = .214	.249 = .211	

* after more shaking + standing ~ no 4th min

	Found	Ratios
A	4.47	1.09
G	3.49	0.85
C	3.23	0.79
T.	5.01	1.22 ← T too high from non-specific absorption
	16.20	3.95

Composition of Sputum V:

			P % of component	% of total
Prot.	86.3 mg	= 85%	0.05	0.04
NA (dock 10% P)	15.5	= 15%	10.0	1.5
	101.8			

31-1-51 "Synthetic Virus" – HCOOH.

86.3 mg air-dry Lab PP ↑ 5 ml M/10 NaOH

17.2 mg " BSHA ↑ 2 ml M/100 NaOH

Dilute ⇒ no ppt. Filter (to remove fluff) into 10 ml 95% EtOH containing 0.03 ml HAc ⇒ flo. ppt. Allow to H6 Spin, wash = 95% EtOH, then = ether, let dry in air.

1-ii-51. 5.1 mg weighed into small tube. Hydr 0.1 ml 90% HCOOH 175° 30 min., dry down, ↑ 20 µl N-HCl, took 2 × 8.5 µl spots. Chromatogram has brown stain + fluorescent spots, but not usual non-spec absorption except below thymine.

		A	G	C	T
Blank		.018	.018	.013	.035
Spot	(1)	.597	.401	.354	.433
	(2)	.601	.403	.355 .276	.435 .433
	x̄	.599	.402	.354	.434
– blank		.581	.384	.339	.399

2-11-51. Calibration of micro-overflow pipets on Beckman.
Empirical soln of A + T diluted.
Standard: 0.1466 mL ↑ 100 mL M/10 HCl (German pipet)
D_{265} = 0.984, 0.982 = 0.983

Calibrations: each pipet measured 3 × onto plate paper, & then eluted, along = blanks in 5.0 mL (overflow onto pipet) M/10 HCl.
Read at 265 mμ. St = D_{265} = 0.985 0.986

Estimated delivery volume					
	Blank	= .010	.009	.010	= 0.010
		(.004	.003	.008)	
1.97 μl	Commercial 2 λ	= .270	.274	.281	= 0.275 − 8 = 0.265
		(.267	.269	.274)	after reducing = 0.672 − 462
4.94	" 5 λ	= .675	.668	.674	
		(.654	.611	.624 ;	.670 .651 .665)
7.55	" 8 λ	1.050,	1.044,	.985	= 1.026 = 1.016
3.53	Own #1 (3.5)	.484	.484	.486	= 0.485 = 0.475
8.37	#2 (8.5)	1.135	1.135	1.137	= 1.136 = 1.126
11.67	#4 (11.9)	1.583	1.545	1.606	= 1.578 = 1.568
6.53	#6 (6.5)	.891	.886	.884	= 0.887 = 0.877
21.2	#7	1.432	1.432	1.432	= 1.432 = 1.422 × 2 = 2.84

$$V = \frac{D}{0.986} \times \frac{14.66}{2}$$

New gravimetric calibration

4.8

7.7

3.5

8.4

11.9

21.1

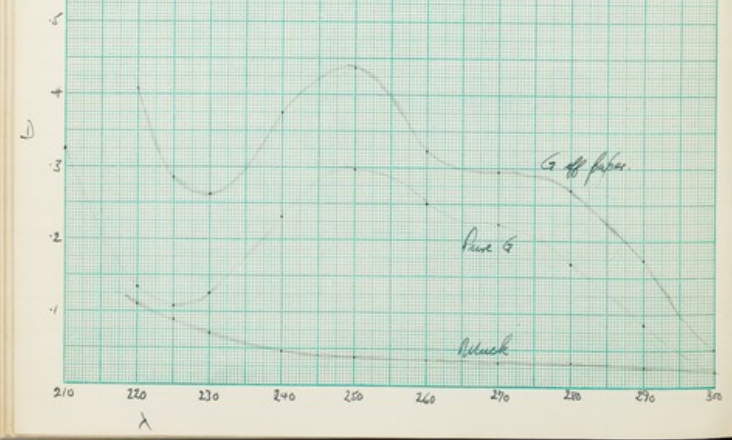
6-ii-51. Repeat pipette calibration.

Same stock soln, same procedure, but tubes left overnight to elute then shaken 10 min by machine, then stood 30 min. 5 ml N/10 HCl : accurate bulb pipette Dies.

Standard: 0.1466 ml ↑ 100 ml

	Paper blank	.012, .011, .011	= .011			
1.92	Commercial 2 λ	.278, .266, .273	= .272 - B	= .261		
4.82	" 5 λ	.660, .660, .677	= .666 -	= .655		
7.68	" 8 λ	1.044, 1.059 1.059	= 1.054	= 1.043		
3.47	Coon # 1 (3.8)	.486 .476 .487	= .483 - B	= .472		
8.32	" 2 (8.5)	1.140 1.143 ~~1.124~~ 1.142	= 1.142	= 1.131		
11.9	" 4 (11.9)	1.626 1.625 1.625	= 1.625	= 1.614		
6.51	" 6 (6.5)	.898 .892 .897	= .896	= .885		
21.0	" 7 (21) ↑ 10 ml	1.460 1.480 1.440	= 1.460	= 1.429		

Unconcentrated	1 hr.		2 hr.	
	Umol/ml	Ratio	Umol/ml	Ratio
A	4.87	1.12	4.36	1.09
G	4.05	0.93	3.74	0.94
C	3.63	0.83	3.40	0.85
T	4.66	1.07	4.31	1.08
	17.21		15.81	



6-ii-51. Synthetic Virus - $HClO_4$ hydrolysis.

2 small tubes: 5.0 mg synth V + 8.35 µl 70% $HClO_4$ 100° 60 min

HA in $HClO_4$ = 9%

5.1 mg " " " 100° 120 min.

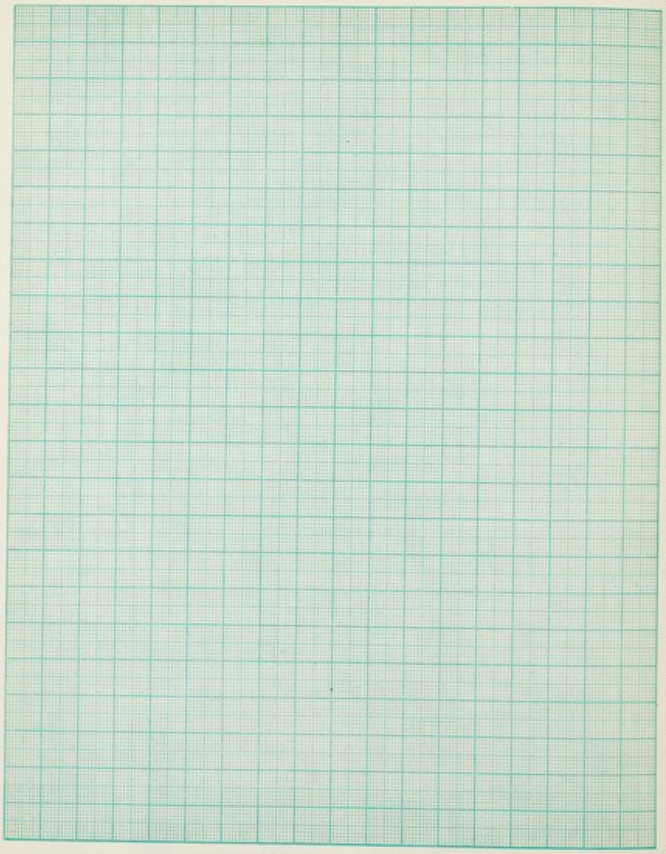
To each add 11.9 µl H_2O, mix, take 2×8.35 µl aliquots.

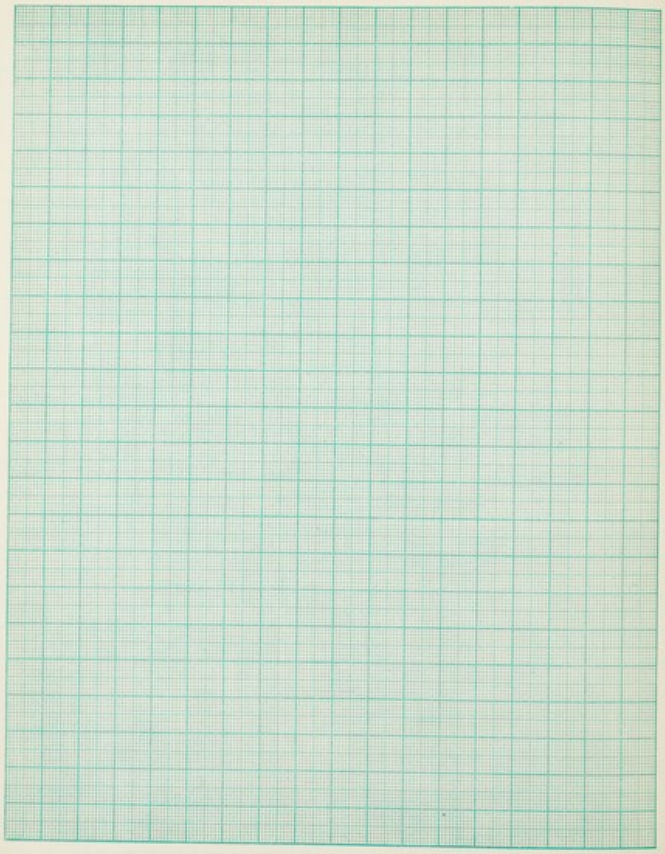
Some brown streak from starting point onto G, but not beyond.

Cut out spot above G to get spectrum of mixed.

		①	②	G_1	Pure G-HClO 225 - 210
	220	.180		.408	.183
9-ii-51 "Blank": read against its blank (cell correction added in)	225	.089	.078	.284	.107
	230	.070	.060	.262	.125
	240	.046	.041	.278	.231
G_1 read vs in G	250	.038	.031	.187	.297
	260	.034	.031	.128	.248
	270	.032	.030	.094	.222
	280	.033	.029	.070	.158
	290	.027		.174	.082
	300	.019	.08	.050	.019

		G 225	G 250	G 300	A	C	T
Blank	1	.016	.011	.005	.011	.009	.033
	2	.014	.009	.004	.010	.009	.033
		.015	.010	.004	.011	.009	.033
Synth V. HClO 100° 60 min	1	.305	.446	.053	.631	.381	.395
	2	.318	.464	.055	.658	.399	.413
	$\bar{x}$	.312	.455	.054	.645	.390	.404
	$\bar{x} - \bar{B}$	.297	.445	.050	.634	.381	.371
Boiled 120 min	1	.284	.420	.051	.578	.366	.378
	2	.284	.421	.051	.580	.366	.377
	$\bar{x}$	.284	.421	.051	.579	.366	.376
	$\bar{x} - \bar{B}$	.269	.411	.047	.568	.357	.343





NH_3 from ammonium alum. (Aluminum ammonium sulfate)

1.323 g ↑ 200 ml

2 ml distilled. $N = \frac{1401}{453.3} \times 1.323 \times \frac{2}{200} = 409\ \gamma$

HCl: 0.81M, .780, .826 = .807 − .009 = 0.798

$N = .798 \times 0.479 = 382\ \gamma$

$\frac{382}{409} = 93.5\%$

Distilling into 1 ml 2% boric acid.

Blank 0.008

HCl: .843, .849, .849 = .847 − .008 = .839

$N = .839 \times .479 = 402$ $\quad \frac{402}{409} = 98.5\%$.

21-VII. Kjeldahl burette – gravimetric check.

Horizontal burette:

Stoppered bottle MT	9.5994		
+ 1 ml water from burette	10.6322		
	1.0328		
	10.6325		
+ 1.0 ml water	11.6622	=	1.0297
+ 0.5 ml (0 – .5)	12.1720	=	0.5098 } 1.0285
" (.5 – 1)	12.6907	=	0.5187 }
+ 1.0 ml	13.7201	=	1.0294

Vertical burette:

Bottle MT	9.5993
+ 1.0 ml water	10.5958
	0.9965

Horizontal

Bottle MT + .5 g.	10.1029	
+ 0.2 (0 – 0.2)	.3059	.2030
.2 – .4	.5112	.2053
.4 – .6	.7160	.2048
.6 – .8	.9229	.2069
.8 – 1.0	11.1326	.2097
		1.0297

Check on Kjeldahls ± ferrous ammonium sulfate.

Watch Beaker + salt 36.0288
− salt 33.2605
Salt 2.768 g. + 2 ml conc. H_2SO_4 ↑ 500 ml.

2 ml in Kjeldahl still
HCl: 1.495, 1.458, 1.500, 1.465 = 1.480 − .009 = 1.471
Blank: .009, .009
Normality = $\frac{1000}{1.480 - 1.471} \times \frac{2.768 \times 2}{392.1} \times \frac{2}{1000} = .0382$ N.

Repeat. Beaker + salt 35.0132
33.3556
Salt 1.6576

Distill 2 ml.
HCl: 0.916, .931, .908, .928 = .921 − .009 = .912
Normality = $\frac{.6658 \times 2 \times 2}{3922} \times \frac{2}{.912} = .0369$ N.
Mean of 2 trials: 0.0375

Normality 0.0342
Factor to correct results = $\frac{375}{342} = 1.10$

	Influenza A	Influenza B
Whole virus:		
N	10.0	9.7
P	0.97	0.94
Carbohydrate (as [illegible])	12.5	13.1
DNA	2.1	3.7
Lipid	24	23

<u>Taylor, 1944: Chemical Analysis of Influenza Virus</u>

<u>Preparation of virus:</u>

After 42 hrs. incubation at 37° allantoic fluid was harvested in groups of 800–1000 embryos, which → 100–200 mg virus. Chilled. Spin 2000 g 10 min. Add fowl RBC 3–4 ml packed cells / 100 ml fluid, chill, spin down. Susp. in Ringer & spin down. Elute in Ringer ~~37°~~ 25° 3 hrs. Spin at 2000 g to remove cells. Spin 1 hr at 20,000 g in 15 ml tubes. Susp. in Ringer $CaCl_2$ pH 7.2, about 1/100 vol of orig. fluid. Clarify 5 min at 5000 g. 2–5 mg virus/ml.

50% infectious unit: IA: $10^{-13.4}$ g. virus IB: $10^{-11.6}$; swine: $10^{-12.7}$

HA activity (2+ end pt.): $10^{-6.5}$ $10^{-6.4}$ $10^{-6.3}$

Can store at 2–5° months without change in sedimentation, Tiselius or electron micro. appearance. On heating, coagulates & becomes insoluble at 56–60°.

Dialyze, freeze-dry. Extract lipid by reflux c̄ 3:1 alcohol-ether 2 hrs., dry down extract, & from this extract remove lipid c̄ petrol ether.

Carbohydrate: Bial's test is +, but after hydrolysis c̄ 1.0% H_2SO_4 → –, indicating that the pentose is desoxypentose. No evidence of ribose. DNA was estimated by Dische, against TNA (9.4% P)

Johnson & Coghill. 1925.

Tuberculinic acid was prepared by extraction of dried T.B. bugs with 3 & 5% NaOH at room temp., pptd with HCl & EtOH, & hydrolysed in 25% H_2SO_4 for 35 hrs @ 125°

Purines were pptd as Ag salt in acid, then ammoniacal in alkali. Cytosine was pptd as the phosphotungstate, this decomposed, & cytosine [illegible] by concentration. [illegible] was treated with picric acid, & 2 kinds of picrate [illegible] were formed. After re-crystallization, the [illegible] were examined by Prof. Wm. E. Ford, head of dept of Mineralogy, Yale.

1-VI-50. Absorption of old (Beckman) silica cells.

Cleaned by [illegible] & filled c aq. dest. Read against #6970 (cracked) as blank. re-read after re-cleaning

	6957		6573		6594		$\bar{x}$
220	+.029	+.024	-.005	-.006	+.001	+.005	+.008
230	+.012	+.009	-.011	-.012	-.009	-.007	-.005
240	+.006	+.002	-.019	-.021	-.018	-.015	-.012
242	+.006	+.002	-.019	-.020	-.018	-.017	
245	+.006		-.018		-.018		
250	+.009	+.005	-.012	-.014	-.012	-.012	-.007
260	+.007	+.005	-.002	-.004	-.003	-.002	.000
270	+.005	+.004	+.001	-.001	.000	.000	+.001
280	+.004		+.001		.000		+.001
290	+.002	+.001	.000	-.001	-.001	.000	.000

12-vi Against new cell, 6214 as blank

250	+.019	-.002	+.002	+.006
260	+.007	-.003	.000	+.001

Molecular Weights

Free base	Deoxyribonucleic (add 116)	Deoxy-nucleotide (add 80 more)	Mononucleotide in nucleic acid (minus 18)	
Adenine 135	251	331	313	
Guanine 151	267	347	329	
Cytosine 111	227	307	289	5MC 303
Thymine 126	242	322	304	

Theoret P content of DNA having bases in ratios of calf thymus
DNA = 10.0%.

TABLE of Gms. solid $(NH_4)_2SO_4$ to be added to 1000 cc. Solution of concentration S_1 to bring to the final desired concentration S_2

General formula: $X = \frac{533(S_2 - S_1)}{1 - 0.3S_2}$; S = fract. sat.; X = gms. solid $(NH_4)_2SO_4$ to be added to bring from $S_1 \rightarrow S_2$

S_1 FROM \ S_2 TO	0.1	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7	0.75	0.8	0.85	0.9	0.95	1.00
0	55	113	144	175	209	242	278	312	350	390	430	474	519	560	608	657	708	760
0.1		57	87	118	149	182	215	250	287	325	365	405	448	494	530	585	634	685
0.2			29	59	90	121	154	188	225	260	298	337	379	420	465	512	559	610
0.25				29	60	91	123	157	192	228	265	304	345	386	430	475	521	571
0.3					30	61	93	125	160	195	232	270	310	351	394	439	485	533
0.35						30	62	94	128	163	199	235	275	315	358	403	449	495
0.4							31	63	96	131	166	205	240	280	322	365	410	458
0.45								31	64	98	133	169	206	245	286	330	373	420
0.5									32	65	100	135	172	211	250	292	335	380
0.55										33	68	101	138	176	214	255	298	344
0.6											33	67	103	140	179	219	261	305
0.65												34	69	105	143	182	224	267
0.7													34	70	108	146	187	228
0.75														35	72	110	149	190
0.8															36	73	112	152
0.85																37	75	114
0.9																	37	76
0.95																		38

1240

230	.348
240	.317
250	.300
260	.281
270	.251

$\frac{1}{100}$

260	.361
250	.342
240	.256
235	.253

Total $600 \times \frac{.36}{1.2} \times .04 \times 100 = 72$ mg.

1.5 mg/ml.

$\frac{5 \times 140.1}{151.1}$ wants 0.4.

0.8

1.04

.86

.87

1.21