Notebook 1

Publication/Creation

31 January 1949 - 26 October 1949

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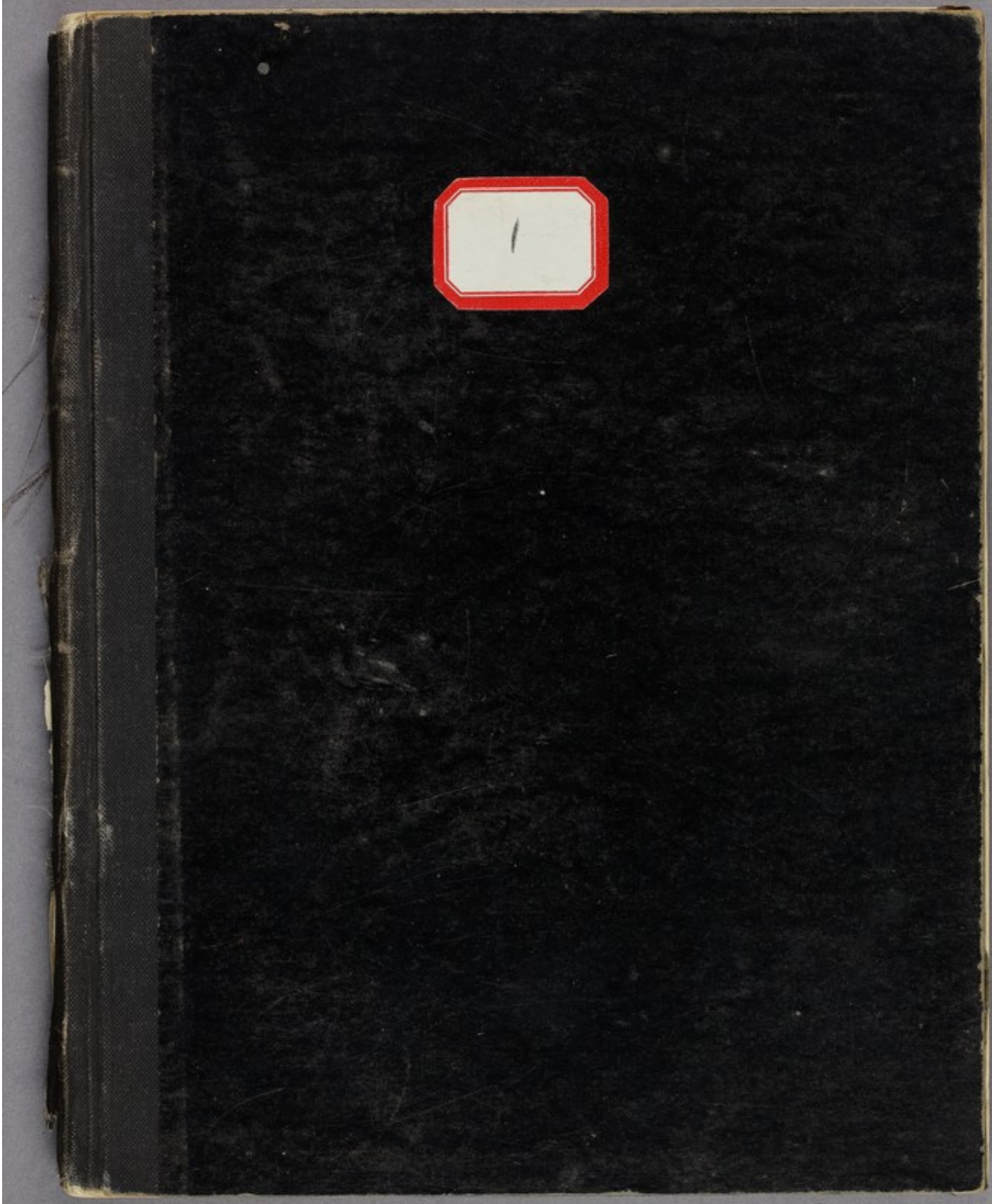
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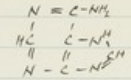
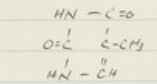
PP/GRW/A/1

G. R. Wyatt
Molteno Institute, Cambridge.
Jan. 1949.

First calculation of DNA molar ratios



Adenine



Thymine

PP/GRW/A/1

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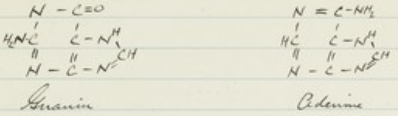
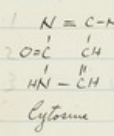
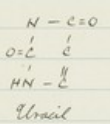


Guanine

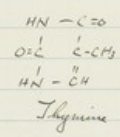
Adenine



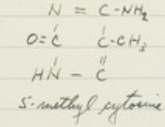
Cytosine



Uracil



Thymine



5-methyl cytosine

31-1-49

<u>Solution of Ld-polyhedra in gut secretion</u>

A ~~[illegible]~~ large I instar larva was exposed to ether, c̄ drop of greenish exudate from mouth caught in capillary. Place on slide, add loopful of polyhedral suspension (3-Ld-36, ex K.S.), c̄ examine in dark ground. Dissolve gradually, being all broken open in ca. 15 min. Repeat c̄ second larva: pa. dissolve very quickly – all gone in 5 min. – much more quickly than in $M/50$ Na_2CO_3. Exudate from 3rd larva: take pH c̄ phenol violet & borate buffer. Approx. 9.5 – 10, but inaccurate due to small amt. & masking by green pigment.

Draw drop of blood by puncturing larva, add pa.: no solution or swelling.

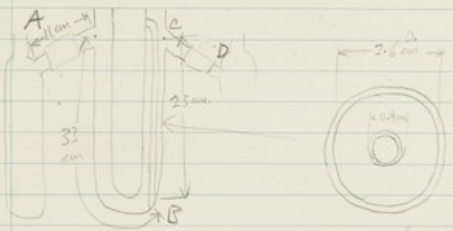
Amp-hours = 2 × 6 = 1.2

At .02 amps, can leave $\frac{1.2}{.02}$ = 60 hrs.

Preparative Electrophoresis

25-11-49 Plating Method

Run at 0.6 amps. in approx ½ sat KCl. 2 hours.



V of open tube = $(1.3)^2\pi$ = 5.30 cm³ / cm length.

V of coiled " = 5.30 − $(.4)^2\pi$ = 5.30 − .64 = 4.66 cm³ per length

Capacity: Open tube: 5.2 × 55 = 290 ml

Coiled " : 4.7 × 23 = 110 ml

Excess between coil = 540 ml measured.

Total 1480 ml

Gradient:

specific resistance = r

Total . $R = \frac{55}{5.3} r + \frac{23}{4.7} r = (10.4 + 4.9) r = 15.3 r$

Cooled portion potential diff $= \frac{4.9}{15.3} \times 200 = 54$ volts.

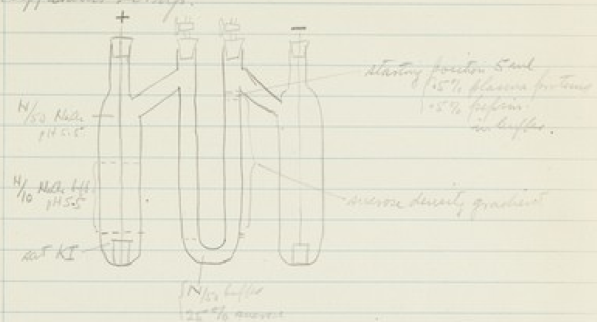
Gradient $= \frac{54}{23} = 2.4$ volts/cm.

Assuming $\mu = 10^{-4}$ cm/sec/volt/cm

migration in 15 hours $= 10^{-4} \times 2.4 \times 3600 \times 15 = 13$ cm.

Electrophoresis.

28-11-49. Apparatus set up:



Switched on current 4.30 pm.

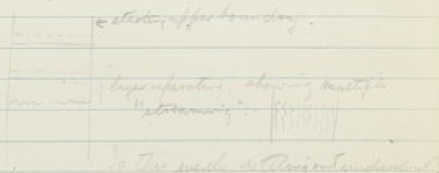
Potential across terminals = 220 v.

Current = 14 milliamps.

Some of protein appears to be in large particles, & is settling out.

5.30 pm. C = ·016 amp.

6.30. Appearance



Is this really settling and [illegible]?

1-11-49. 9.30am. Protein layer very diffuse – greatest conc. around 9 cm, but some (larger particles?) moved up to 18 cm. downwards. No visible separation of 2 proteins!

1-iii-49. Run 2.

Same buffer etc undisturbed; remnants of last test proteins left in. Reverse + & − to electrodes.

Put in 5 ml containing: 1 ml Pottel sol'n (ca 1%?)
1.5 ml Hb soln
2.5 ml M/50 buffer 25% sucrose.

Layer in middle of gradient (sol'ns not adequately mixed, ∴ layer spread over 5 cm.

11 AM. Turn on.

12.30 - Hb moving to −

Was not moving much, but flocculating & beginning to settle. Getting some refraction. Stopped.

Run 3 Hb + Cyt. c. sucrose gradient 0-25%. Refill U-tube & electrode vessels.

Material streams badly downward from lower boundary

1.45 pm start. ·015 amp.

4.0 pm. spreading has continued, little migration of upper boundary. Visible particles (Hb debris or impurities?) are settling out.

1-iii-49 Room 4.

Re-fill U-tube but not electrode vessels. Left arm of U-tube 4/10 acetate pH 5.6; right arm 4/50 acetate, sucrose 0–35%

Layer in at mid-pt of gradient 4 ml containing:

0.7 ml 1% C solⁿ
0.7 ml TVH "
2.0 ml 35% sucrose 4/50 acetate.

6.05 pm – start .012 amps.

7.00 pm Hb moved 1 cm to – (down)
TVH not moved, but aggregating & some settling.
– getting good separation.

9.30 pm .016 amps. TVH has moved 3.5 cm to + (up) & upper boundary is still quite sharp, lower hazy, spread due to aggregation & settling – some aggregates settled 10 cms, then Hb.
Hb has moved 5 cm to – (down), & has not spread hardly. – Layer spread to about 4 cm originally.
Would have complete separation if not for aggregation of virus.

temp in column = 24°
Room temp = 17°

Phosphate, pH 7.

Need: 1000 ml N/10 = 390 ml KH_2PO_4 + 610 ml Na_2HPO_4
100 ml N/50 35% sucrose. 100 ml buffer + 54 g sucrose.
400 ml N/50

Need: TYM 2 ml
TMV 1 ml
N/10 buff 1 ml
water 1 ml
sucrose 2·15 g.
} 4 ml

2-iii-49. Run on TMV + TYM.
– Left electrode vessel + arm of U-tube: M/10 phosphate buffer pH 7.0.
+ Right " " : lower part M/50 buffer
upper part M/50 buffer.
Right arm of U-tube M/50 buffer, pH 7.0, sucrose 0—35%.
Layer in TYM-TMV mixture + ml near bottom of gradient

6.30 pm start. ·024 amps. 220 v.

8.15 pm. ·038 amps. very warm (35°?)
Lower boundary of layer moved 3.5 cm to – (down)
Upper " " " 3.7 cm to + (up)
Suggestion of separation into 2 layers, but no clear division.
Some running by convection?
Reduce voltage to 110, current = 0.016 amps.

3-iii 4.30 p.m. ·012 amps. cool.
Some has found into electrode vessel, by moving to top of column.—
Below this, globulin tails off & no definite boundary. No separation.
– perhaps both move at same rate at this pH.

Mix: 1·5 ml 1·3% cyt c (Jsos)
1 ml M/10 phos buff 7·0
1·5 ml aq. dest.
2·2 g. sucrose.

Mix: 3 ml cyt. c 1·3%
1 ml M/10 buffer
2 ml M/50 buffer containing 35% sucrose
2·1 g. sucrose.

5 cm 17 hrs
15 cm 51 hrs.

3-iii Electrophoresis of cytochrome c. (Theorell. Ark. f. Kem. Min. & Geol. 16B: 1804)

Use same column as last run. = some water still in upper part column. Reverse water: +↓

Into lower part of column pipette cytochrome sol'n.

12 M. start. adjust voltage (= ca. 180) to give .020 amp. .02 amp at 158 volts.

6:00 pm. upper boundary moved 2·8 cm., lower boundary unmoved, a smear of material still in original layer.

4-iii 10.00 am Red pigment upper boundary moved 8·7 cm. 5·9 from this zone decreases downwards to very little in original portion.

Moved 0·5 ml to = is band of equal width to original layer = still sharp, of pinkish slightly turbid material. Looks like impurities.

Some mess of TMV & TYM.

12 M. top boundary now moved 9·5 cm.

Impurity layer = " 0.7

Two factions drawn off.

— 4 —

2nd run on cytochrome.

Empty & refill either apparatus, = phosphate, pH 7.0. left arm = M/10, right arm M/50. Sucrose 0–35%. Layer in at bottom of gradient 6 ml containing 0·6% cyt. c. (ca. 40 mg.)

4.30 pm Start. Keep at .02 amp. by reducing voltage from 200

5-iii 9.45 am Dropped to .010 amps (130 v.), increased to 220 v., .014 amp.

Upper boundary moved 5·1 cm.

6·15 pm .024 amp - slug warm exp. at bottom of U-tube.
Upper boundary marked 7.6 cm. Lower boundary of moving component was moved 2 ¼ cm. & below this faint brownish material, which has true boundary moved down (correction

Expt. c. 2nd run, cont'd.

6-iii. 10.45 a.m. Column has been displaced (by dint of pressure from heat?) downwards, xs liquid escaped thro left electrode, which leaks, & some cyt. flowed over to left arm of U-tube. Circuit broken by drop in liquid level in right tube.

Shift liquid levels & decant as much as possible of purified cyt. c. (80%?)

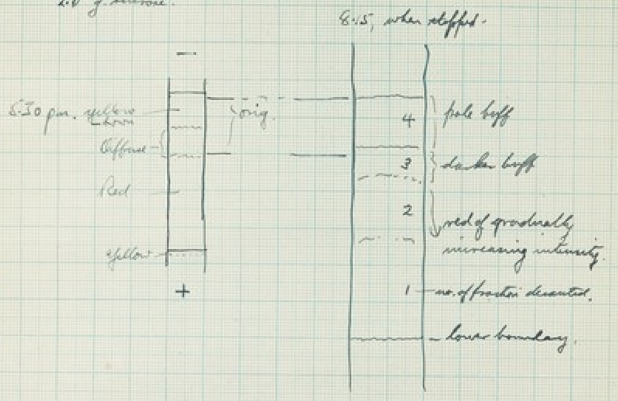
Moral (1) make electrodes watertight

(2) run with stopcocks open & columns balanced.

7-iii. Noted that − electrode (wt.) is black, & has black ppt in bottom of vessel − evidently run too long, gave off H_2 − made pressure.

Capacity: ca. 24 hours at 20 milliamps.

5 ml cyt c (modified)
1.2 ml M/10 phosphate.
2.0 g. sucrose.



8-iii-49. <u>Electrophoresis of Tsou's modified Cyt. c.</u>

Set up c̄ phosphate pH 7.8. M/10 and M/50. Sucrose 0-15%.

Layer 1/3 ~~of way~~ from bottom of gradient 7 ml containing 5 ml of Tsou's M-C-32, 2 mg/ml.

3.10 pm. start. Run at 18-20 milliamps. ca. 120 volts.

5.30 pm Lower boundary moved 2.6 cm.

8.15 pm " " " 5.0 cm.

Decant in 4 fractions.

2nd run on Mod. cyt. c.

Draw off gradient & replace, but use same left arm & electrode vessels without emptying.

Mix: ~~3.5~~ 3.0 ml cyt. c.
0.8 ~~1.0~~ ml M/10 buffer. } Layer near top of gradient.
0.5 35% sucrose.

9.20 pm start.

10.30 am stop. 14 milliamps. cool.

Lower boundary moved 10 cm.

7-10-iii-49. Chromatograms on Bm virus & poly prot. (6-Bm-3A & 3B).

6-Bm-3A - virus - used ½ of ~~ac~~ hydrolysate - should be ca. ·7 mg. -

6-Bm-3B - poly prot - " ·1 ml of BaOH " " " 1 mg.

Run in Bút-formic. phenol (4 drops NH₃).

- absent
+ present (little)
++ " (mod)
+++ " (much)

	Virus.	Poly. prot.
Aspartic	+++	++
Glutamic	++	++
Valine	+	++
Alanine	+++	++
Leucine + iso	+	+++
Lysine	++	++
Hist + arg.	+	++
Methionine	—	+
Proline	—	+
Tryptophan	—	+
Tyrosine	+	++
Phalanine	—	+
Serine	+++	++

11-iii-49. Repeat, ~~for~~ 6-B-3A - virus - remainder of hydrolysate

3B - poly prot. ca. 0·1 ml.

Bút.-acetic (long way of paper) start 7 PM.

14-iii-49. Run 26 hours in Bút. ac.; 40 hrs in phenol

poly prot. - good separation, but cannot identify spots.

virus - no good - Too much salt!

.1 M Na_2CO_3	2.0 ml
.15 M NaCl	8.0 ml
Polyhedra	2.5 ml
aq dest	12.5
	25 ml

Nitrogen on Polyhedra prep 7-iv-49. Total prep. 4.2 ml.
Acid: 1 ml ≡ 490.7 γ N.

A	Blank	0.055	.050
B	"	0.045 ml acid	
D	0.1 ml poly. prep.	0.700	.720 − .050 = .67 ≡ .67 × .491 × 100/10 × 10 = 32 mg/ml
F	0.1 ml " "	0.740	

Nitrogens on finished preps:

A	Blank	0.056	~~.057~~
B	"	.060	.060
C	0.2 ml Poly. prep.	0.355	.350 − .060 = .29 ≡ .29 × .491 × 100/10 × 5 = 4.76 mg/ml
D	0.2 ml "	0.345	
E	0.2 ml virus	0.120	.120 − .060 = .060 × .491 × 100/10 × 5 ≡ 1.098 mg/ml.
F	0.2 ml "	0.110 (some lost in forming)	

Preparation: Polyhedra = 2.5 × 22 = 55 mg.
Poly. prep = 10.0 × 4.75 = 47.5
Virus = 3.1 × .098 = 3.0 mg
50.5

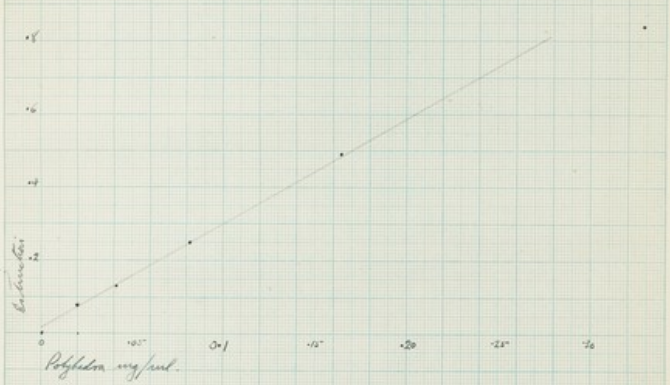
8-iv-49 Ld virus prep.

2.5 ml polyhedral prep of 7-iv added to alkali. 9:50 a.m.
11 a.m. – microscopic exam: v. few pp. still intact.
Spin 10 min 6000. decant from pellet consisting of brown smudge + blue-white polyhedral remnants + virus.
Spin 30 min 12000. Decant, susp. in 25 ml aq dest.
Spin both suspended virus + decanted poly prep. 30 min 12000.
Virus: susp. in aq. dest.
Poly prep.: ppt. @ 5.8 by adding HAc. Spin down. Dissolve in .001 M Na_2CO_3 + make up to 10 ml.

Suspend in aq. dest. to recover more virus. Spin out smudge 5 min 3500, decant, spin 15 min 12000. Susp. small pellet in aq dest, add to main virus, make up to 3.1 ml.

Kjeldahls on Ld polyhedra 22-iv-49.

Blank .062 } .064
" .067 }
0.1 ml polyhedra .560 } .566 − .064 = .502 ≡ .502 x .491 x $\frac{10}{15}$ x 10 = 164 mg/ml
" .553 }



26-iv-49. Turbidity estimation of Ld polyhedra.
Pref. of 22-iv diluted in saline.
Filter 08-2 (blue) 1 cm micro-cells.

1:50	= 0.328 mg/ml.	.84
1:100	.164	.49
1:200	.082	.25
1:400	.041	.15
1:800	.020	.08

10-v. Suspension for agglutination tests.
1 cm cell. 08-2 filter. DE 1.0
0.5 cm cell " 0.55
1 cm micro-cell 0.84 ≡ 0.33 mg/ml.

5·0 ml poly prep. of 22-iv = 82 mg.
2·0 ml M/10 Na_2CO_3
8·0 ml ·9% NaCl
10·0 ml aq. dest.

25·0 ml.

27-iv-49. Rooth Ld virus prep.

Put up mixture 9.40 a.m.

12.15. Dark ground: very largely dissolved.

Spin 5 min @ 3500, suspend pellet, & spin again 5 min @ 3500 to remove more virus.

Spin supernat. 35 min @ 11.000, decant, suspend in aq. dest, & spin down again. Pellet much whiter, less translucent this time. Susp. in aq. dest & examine in dark field: much aggregated.

Spin down again. Attempt to suspend pellet in v. small volume aq. dest – impossible! Portions of coarse suspension put into 5 ml of each of ·15 M, ·05 M, ·01 M NaCl, ·05 M, ·01 M borate pH 8.4, ·01 M Na_2CO_3 – ·05 M NaCl, sol'n containing some poly. protein.

Won't suspend easily in any.

2-V-49. NaCl: all 3 dilutions, virus almost entirely settled out.

Borate: largely still well suspended. ·05 & ·01 same.

Na_2CO_3-NaCl: " " "

Aq. dest. " " " – maybe not so good as borate. but cannot tell, since mostly used for precipitin test.

Sol'n c̄ polyprot: turbid – ppt. – probably aggregated.

$S_{20} = \frac{1}{x\omega^2} \cdot \frac{dx}{dt}$

$S_{20} = 1800 \times 10^{-13}$
$x = 8$ cm
$dx = 6$ cm
$\omega = 1000$

$dt = \frac{dx}{S_{20}\times\omega^2} = \frac{6}{1800\times10^{-13}\times8\times10^6} = \frac{1}{24\times10^{-5}}$

dt in min $= \frac{10^5}{24\times60} \simeq$ 70 min.

$g = \frac{\omega^2 x}{980}$

$8000 = \frac{\omega^2 8}{980}$ $\quad \omega^2 = \frac{8000\times980}{8}$

$\omega = 1080$ $\quad$ r.p.m. $= \frac{1000\times60}{2\pi} = 9500$

Old Sorvall: 140 volts = 6000 r.p.m.
220 " ≡ 9500

Hyddalls:

Blank	.5 ml H_2SO_4 new bottle	.020
"		.020
"		.020
.1 ml urine		.053
"	(some action in tube)	.057
"	(")	.059

.056 − .020 = .036

≡ .036 × .491 × $\frac{100}{15}$ × 10 ≡ 1.18 mg/ml

Total prep: 5 × 1.18 = 5.9 mg.

New Sorvall:

Full speed: 12,000

$g = \frac{\omega^2 x}{980} = \left(\frac{12000\times2\pi}{60}\right)^2 \times \frac{7.5}{980}$

$= (1.25\times10^3)^2 \times \frac{7.5}{980} = 12{,}100$

4-V-49. Ld virus prep.

11.45 a.m. Fresh polyhedra, ground @ 150 mg. put in 37.5 ml .025 M Na_2CO_3 .05 M NaCl.

2.15. Dark ground: almost all dissolved.

Spin 5 min @ 6000, decant, resusp. pellet in aq. dest, spin " " , decant.

Spin 2 supernates 60 min @ 9500 (≡8000 g.)

Suspend 2 pellets in 30 ml aq. dest. & spin 60 min @ 9500.

Suspend pellets in aq. dest (suspends OK). & make up to 6 ml.

10-V. Much aggregated. Slow spin = much goes down. Dark ground: both pellet & supernat contain large aggregates & bugs.

6-V-49. Hydrolyses

A. 5 mg Ld poly prod. of 2-V. 7 N HCl 120° 6 hrs.
B 2.4 mg Ld virus of 4-V. 7 N HCl 120° 6 hrs.
C 1.2 mg Ld virus of 4-V. 100% HCOOH 175° 2 hrs.

Chromatograms

C taken to dryness, resp. in 40 µl aq. dest., put 16 µl on paper. put into butanol 6 p.m.

7-V-49 5 p.m. 23 hrs. taken out & pumt. Shows guanine, cytosine, adenine, thymine, no uracil.

16-V. 16 µl on paper. Butanol-formic 4.30 p.m. (large tank).

17-V. 2.30 p.m. Take off. 22 hrs.

Chromatograms of 16-V.

	Virus	Poly prot.	
Aspartic	+++	+++	
Glutamic	++	+++	
Glycine	++	++	
Alanine	+++	+++	
Valine	++	++	
Serine	++	++	
Threonine	+	+	
Leucines	+++	++	
Tyrosine	+	++	
Phenylalanine	++	+	
Proline	+	+	
Histidine	+	++	←
Lysine	+	++	
Arginine	+++	++	

10-V-49. Ld virus & poly prot. Amino-acid chromatograms.
Hydrolysates taken to dryness & dissolved:
Poly prot in 0.2 ml aq. dest.
Virus .1 ml
Chromatograms: Poly prot: .032 ml ≡ 0.8 mg protein
Virus: .032 ml ≡ 0.7 mg " (1.5% N.A.)
Put in butanol-acetic @ 6.30 p.m.

11-V-49 4 p.m. 21½ hrs. Just reached bottom (long way). Take out.

12-V-49. 12.30 p.m. Into phenol-NH_3.
Virus & poly prot. were mixed on paper: 2 chromatograms the same.
Otherwise OK, but a bit streaked in phenol.

16-V. Repeat. 32 µl of poly prot, & dilute of virus hydrolysates.
4 p.m. into phenol.

17-V 5.30 out of a - 3° from bottom.

18-V 9.30 am. out of butt-acetic.

Spots streaked a bit in phenol — too much salt.

Kjeldahls:

		HCl 1 ml = 491 μg N.	
A	Blank	0.020	.019
B	"	.018	
C	0.2 ml clean prep	.343	.343 - .019 = .324 × .491 × $\frac{100}{15}$ = [illegible] mg/ml × 5.2 ml = 28 mg total
D	" " (splashed!)	.341	
E	0.2 ml dirty "	.354	.344 - .02 = .324 = 5.3 mg/ml × 11.5 ml = 61 mg total
F	0.2 ml "	.334 (T5on!)	

Say 80% polyhedra 20% muck
= 50 mg polyhedra

1.2 ml M/10 Na_2CO_3
7.0 ml 1% NaCl
11 ml polyhedra
.8 ml aq dist
20
} 10.45 am.

17-V. Lm polyhedra & virus preps.

Collection of dead larvae from three strains. Purified by centrifuging in saline (washed 10x), & finally into 2 fractions:
5.2 ml "clean" for injecting bm
11.5 ml "dirty" for virus prep.

18-V. Dissolve all "dirty" polyhedra in .006 M Na_2CO_3 .05 M NaCl.
3½ hrs. Darkground: many few still not burst, tho swollen; virus in brownian movement. Spin 5 min 6000. Large brownish & whitish pellet - susp. in .006 M Na_2CO_3. Darkground: many undissolved polyhedra. Spin again 5 min 6000. Same. Spin combined supernats. 55 min 9550. Susp. pellet in .01 M Na_2CO_3 3 p.m.

4 pm - some more polyhedra dissolved, but some still whole. Spin 5 min 6000.

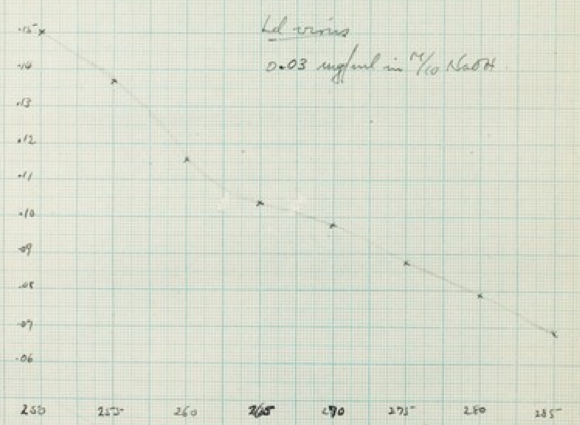
pellet contains brown muck along with virus. Suspend in .006 M Na_2CO_3, Spin 5 min 6000.

10 ml ^saline^ containing ca. 120 mg polytube.
2.0 ml M/10 Na_2CO_3
~~8.0 ml M/10 NaOH~~
13.0 ml aq. dest.

25

Final virus prep. in 5.2 ml.

For beckman, dilute 20 × into M/10 ~~[illegible]~~ NaOH.
0.15 ml into 2.85 of NaOH.



30-V. Ld virus prep.

10.10 a.m. mix.

1 pm. Examine darkground: pa. mostly dissolved; many bugs. Spin 6 min 6000, decant from large pellet (mostly bugs) which was susp. in ·006 M Na_2CO_3 & again spin 5 min 6000. Spin both supernats. 1 hr @ 9500, suspend in ·006 M Na_2CO_3, spin again 1 hr 9500, susp in 5 ml aq. dest, spin 5 min 4000 to remove some more bugs. Dark ground: still a lot of bugs.

Beckman

	255	·073
1/20	260	·067
	265	·062

Add 0.15 ml virus. Now dil. 1/10 in M/10 NaOH.

		Brought to boil & cooled	
255	·177	·137	250 - ·15
260	·175	·116	
265	·159	·104	
270		·098	
275		·088	
280		·079	
285		·069	

Acid 1 ml = 491 μN.

Kjeldahls:	b	Blank	·018	} ·018
	d	"	·018	
11.30 am	J	·5 ml poly prot	·528	} ·541 − ·018 = ·523 × ·491 × 100/15 ×2 = 34 mg/ml × 26 ml = 88 mg total
	k	"	·554	
	J	·25 ml virus	·040	} ·041 − ·018 = ·023 × ·491 × 100/15 ×4 = 0.30 mg/ml × 5.2 ml = 1.55 mg.
	k	"	·042	

8-VII-49.

Gach polyhedra prep.

Final pellet, spun from saline, susp. in aq. dest.

Into 8 bottles, 20 ml each, frozen. = 160 ml.

Kjeldahls:

HCl 1 ml = .491 mg N.

1) Blank - 1 ml H_2SO_4 (decanted acid) }
2 " 0.0 } obviously something wrong. Not enough H₂SO₄??
3 " 0.01 } Assume 0.04

4 0.1 ml prep 2.80 }
5 " 2.73 } $2.75 - 0.04 = 2.71 \times .491 \times \frac{100}{15} \times 10 = 89$ mg/ml
6 " 2.73 }

Total yield $= 164 \times \frac{89}{1000} = 14.5$ g.

In 8 bottles, each containing 20 ml = 1.78 g.

—— || ——

18-VII.

Virus prep.

1.8 g @ 5 mg/ml = 360 ml. $360 \times \frac{.008}{.1}$ $\frac{36}{8}$ 288

Mix: 20.0 ml cont. 1.8 g polyhedra.
30. ml M/10 Na_2CO_3
120 ml .15 M NaCl
190 ml aq. dest.
360

10.40 a.m.

2 p.m. - $3\frac{1}{2}$ hrs. Dark ground: mostly dissolved, many ghosts, some still containing virus. Some big spores.
Spin 5 min 6000.

8-VI-49. Ld virus vaccine

2 ml Virus prep of 5-V containing ca. 0.2 - .3 mg/ml in aq. dest. was stored in frig until 30-V. Then added formalin to make 0.2% in formaldehyde, & kept in frig.

8-VI. Spin down to remove from formalin, susp. in 1.5 ml aq. dest.

Test of acquired immunity.

Inject 40 larvae c .01 ml (3 γ) of vaccine + place in individual tubes. Same time, place 40 untreated larvae in individual tubes.

After 2 days divide each series into 4 series of 10:-

(a) untreated control
(b) fed 5×10^{-8} g polyhedra
(c) 5×10^{-9}
(d) 5×10^{-10}

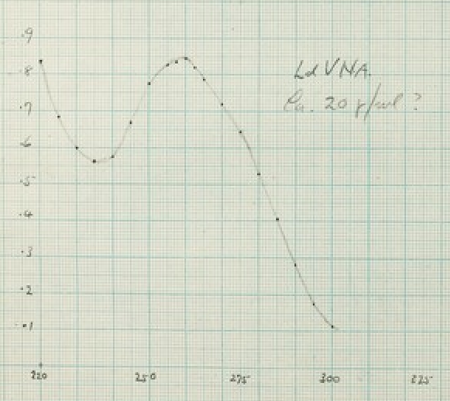
20-VI. Ld V. Kjeldahls

10.10 am.
a Blank 0.033 } 0.028
d " 0.023 }
f 0.2 ml virus 0.189 - 0.028 = .161 = 2.64 mg/ml.
l " " 0.256 - 0.028 = 0.228 ≡ $0.228 \times .491 \times \frac{100}{15} \times 5 = 3.73$ mg/ml

Total vol - 20 ml. ~~yield = 74 mg~~

% virus ex pe. = $\frac{.050}{1.78} \times 100$ = ~~4.2%~~. 2.8%.

21-VI. Repeat.
a Blank 0.015
d " 0.015
f 0.3 ml virus 0.248 }
l 0.3 ml " 0.240 } $.244 - .015 = .229 \times .491 \times \frac{100}{15} \times \frac{10}{3} = 2.50$ mg/ml. Total yield 50 mg.



21-VII-49. <u>Splitting N.A. from Ld V.</u> (Sevag method).

10 ml ~~prep~~ of 16-VII, containing 25.0 mg virus added to 10 ml M/10 Na_2CO_3, heat on W.B. $1\frac{1}{4}$ hours, 55-65°.

Add 5 ml chloroform + 2 ml amyl alcohol, shake 10 min., spin, decant. Supernat. turbid, add 2nd 5 ml chlor. + 2 ml amyl. & shake again. Repeat c̄ reduced amt chloroform, to total of 4 extractions - still v. small gel, & supernat still slightly turbid.

<u>Supernat (N.A. fraction)</u>.

Add 3 ml N. HCl (≃ N/10) - fine ppt. Add ca. ½ vol EtOH, to dissolve chloroform drops. Spin 11,000 20 min. → fine sticky ppt. Dissolve in M/50 NaOH. - viscous, turbid. Add equal vol. aq. dest, spin 15 min 10,000. (Boundary).

<u>Gel (prot. fraction)</u>. Dissolve gel by addition of EtOH, spin down. Tough fibrous pellet. Add aq. dest, then NaOH (to make ca. N/25)

N.A. absorption; dilute 10x. 260 1.72
250 1.49

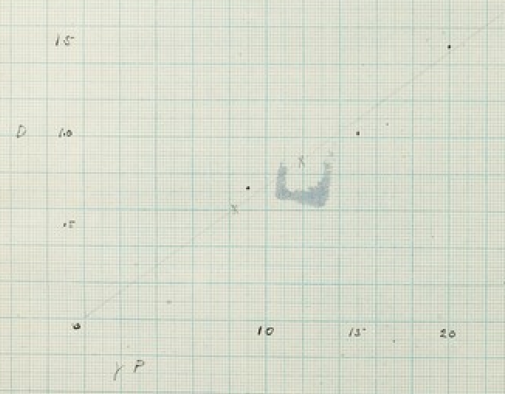
Diluted 20x: in .0025 M NaOH.

257½	.835	262.5	.820
260	.845	265	.785
255	.826	270	.717
250	.776	275	.644
245	.670	280	.528
40	.578	285	.404
235	.561	290	.282
230	.600	295	.178
225	.684	300	.113
220	.835		

Total NA = .845 x .04 x 20 x 3.35 = 2.2 mg.
% recovery, if 16% in virus = $\frac{2.2}{16 \times 25}$ = 57%

3·5 ml contains 25 mg NA. ≡ 400 γ N   250 γ P

Use for P 2 samples 0·25 ml ≡ ea. 20 γ
" N " 0·25   40 γ N.



22-VII-49 Analysis of hd VNA

N. Kjeldahls by Ingle. Incinerate 2 hrs, add 2 drops 10% H_2O_2, + 1½ hrs.

Titrating 1 ml acid ≡ ·705
c NaOH   ·705
                  ·703

Blank   ·658
" lost   —
·25 ml N.A.   ·423 } ·430   ·658 − ·430 = ·228
"   ·436 }

N per ml = $\frac{·228}{·704}$ × ·0983 × 4 = ·127 mg N/ml.

P if pure TNA' = ·127 × $\frac{9.6}{16.4}$ = ·0742 mg/ml
In one sample ·0185

P. Incinerate 2 samples 0·25 ml. Ppt, spin down, wash 3 times c aq. dist. Dissolve in M/10 NaOH up to 10 ml for Beckman test – then add $K_4Fe(CN)_6$ + HCl. 11·30 a.m. (NB: 10 γ is 9 ml out of 10).

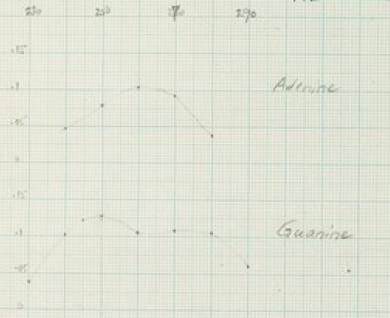
Hilger: 9 γ   ·720
15   1·02
20   1·50
unknown   ·605   8·5 γ } 10·1   N.A = 55% pure.
" "   ·873   11·9 }   11·9 → 65% N.A.
                     20·2

Calculating back from NA analysis, should be at least 18 γ

% P ~~from~~ original virus recovered in N.A. = $\frac{10.1 \times 4 \times 3.35}{25000}$ × 100 = 5·4
(Total vol. N.A. = 2·3 + 1·0 + 0·05 = 3·35)

Amt NA in ppt = ~~[illegible]~~ 18 × 10 × 4 × 3·35 = ~~1·35~~ 2·4 mgs., from 25 mg virus = 5·4%
NA N calc. from P = $\frac{15}{10}$ × 18 = 27 γ   ⅓ of NA lost.
NA found = 32 γ   Purity of NA = 84%.

	Peak in N/10 HCl.	(Hotchkiss) N_{260}	M	10γ/ml. D
Guanine	250	40	151	0.703
Adenine	260	105	135	0.964
Cytosine	275	53	111	0.910
Thymine	265	59	126	0.608
Uracil	260		112	0.720



LdVNA

26-VII **Bases**. 2 samples each 1.15 ml (≡ 50.5 γ D), into bomb tubes.
(1) add HCl to make 1N, heat on B.W.B. 1 hr. – purines
(2) evap. to dryness, add ·5 ml HCOOH, give 2 hrs 175°. – pyrimidines.
Both opened, evap. to dryness, dissolve (1) in 0.1 ml N/10 HCl.
(2) in 0.1 ml aq. dest.

27. ~~Run~~ Spot on paper – 5 0.016 ml spot of each. forgot to neutralyse, + run: (1) purines Butanol-water 24 hrs
(2) pyrimidines Butanol NH_3 9 hrs.

28 Dry, print, cut out spots ē blanks, + elute 4 hrs in 5 ml N/10 HCl, read on Beckman.

28-VII **Adenine**

(1) 260 ·103
250 ·079
240 ·047
270 ·092
280 ·032

Mean H_{260} = ·100 N_{260} = ·100/·96 = ·104

Adenine conc. = ·104/105 × 1000 = 0.99 γ/ml. — Corrected 1.04

(2) 260 ·102
[(3) 260 ·078]
(4) 260 ·099
(5) 260 ·098

μMolar conc. in extract = ·99/135 × 1000 = 7.33 — 7.70

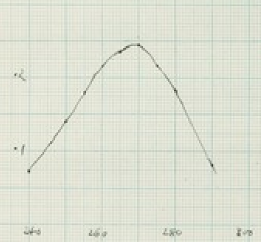
Guanine
(1) 250 ·087
240 ·102
245 ·125
250 ·128
260 ·104
270 ·108
280 ·104
290 ·060

Mean H_{245} = ·122 N_{260} = ·122 × 100/99 × 1/1.18 × 71/100 = ·074

Guanine conc. = ·074/40 × 1000 = 1.85 γ/ml. — Corrected 1.73

= 1850/151 = 12.25 μM. — 11.45

(2) 245 ·109
(3) 245 ·127
(4) 245 ·118
(5) 245 ·122



Base	Beckman γ/ml	Extract μM.	Molar ratio
Guanine	1.85	12.45	1.18
Adenine	0.99	7.70	0.74
Cytosine	1.52	13.7	1.33
Thymine	1.07	8.5	0.82
		41.35	4.00

In original prep., molar conc. of total base $= 41780 \times \frac{5}{.016} \times \frac{1}{1.15} = 2.28$ mM. (×2 written above the 5; the original figure before 2.28 is struck out)

" " of $P = \frac{10.1 \times 4}{31} = 1.30$ mM.

" "

28-VII Thymine (1) 245 ·176
255 ·131
265 ·135
275 ·056
285 ·042

(2) 245 ·026
255 ·049
265 ·064
275 ~~·045~~ ·048
285 ·015

Using this value only:

$H_{260} = .064 \times \frac{.955}{.985} = .063$ (one figure struck out)

(3) 245 ·242
255 ·148
265 ·126
275 ·102
285

Thymine conc. $= \frac{.063}{59} \times 1000 = 1.07$ γ/ml.

$= \frac{1.07}{126} = 8.50$ μM.

(4) 245 ·248
265 ·126

245 ·258
(5) 265 ·182

29-VII Cytosine (1) 290 ·080
(+adenine?) 280 ·181
275 ·216
270 ·238
265 ·231
260 ·216
255 ·180 (·160)
240 ·072

Means: $H_{280} = .182$

$H_{270} = .238.4$

$H_{255} = .179$

Simultaneous equations:

(2) 280 ·177
270 ·232
255 ·174

$H_{270C} = 1.74H_{280} - 0.76H_{270} = .315 - .182 = .133$

$H_{270C} = 1.75H_{270} - 1.6H_{255} = .416 - .286 = .130$

(3) 280 ·183
270 ·242
255 ·184

$N_{260C} = \frac{.132}{1.60} \times \frac{1.00}{.95} \times \frac{.93}{1.00} = .081$

(4) 280 ·183
270 ·240
255 ·178

Cytosine conc. $= \frac{.081}{53} \times 1000 = 1.52$ γ/ml.

(5) 280 ·187
270 ·240
255 ·179

$= \frac{1.52}{111} = 13.7$ μM.

MT Weighing: tube dried @ 110°5 over low P_2O_5 — 9.6005 g.
" + vac.-dried virus 9.6309 g.
Virus .0309 — made up to 10 ml.

N- aliquots of 10 ml: 10.54 a.m.

				Bangha
a	Blank 0.5 ml H_2SO_4	.796	1 ml acid =	.863
b	"	.796		
c	"	overrun		
d	0.16 ml virus	.124		
e	"	.133	.129	
f	"	.131		

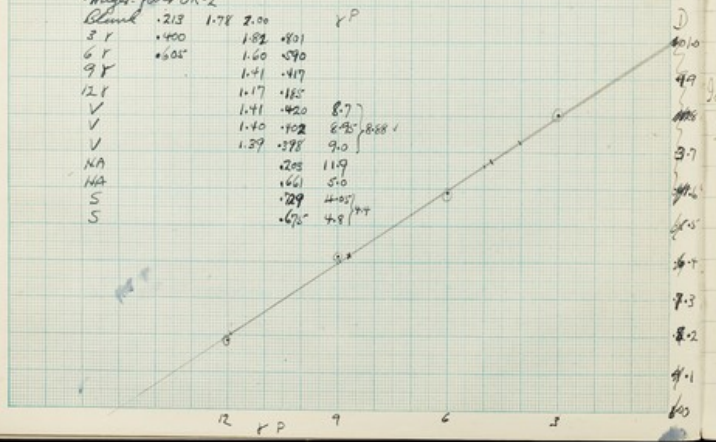
$.796 - .129 = \frac{667}{.863} \times .0983 \times \frac{1}{.16} = .475$ γ N/ml

$\%N$ in dry virus $= \frac{.475}{3.09} \times 100 = 15.4\ \%$

P- 3 aliquots each 0.16 ml — $\%P_2O_5$ in virus: $.00858 \times \frac{10}{.16} \times \frac{100}{43.69} = 1.80\ \%$

Wilgs. filt. OR-2

				γ P	
Blank	.213	1.78	2.00		
3 γ	.400		1.82	.801	
6 γ	.605		1.60	.590	
9 γ			1.41	.417	
12 γ			1.17	.185	
V			1.41	.420	8.7
V			1.40	.402	8.95 / 8.88
V			1.39	.378	9.0
NA				.209	11.9
NA				.661	5.0
S				.229	4.05 / 4.4
S				.675	4.8



1/11

2-VIII-49. Analysis of Ld V - second prep

1.75 g. washed Geck prep in dissolved 3 hrs in .008 M NH_4Cl, .05 M NaCl. Spin slow spin, ± sediment resusp. ± spun down again. Recover more virus. Virus spun down, brown ? ghosts washed off surface - pipette. Spun down again, given slow spin, spun down third time, remaining dark material washed from pellet to redeem virus fraction. Clear pellets, entirely white including about 2/3 of prep, susp. in 1 ml aq. dest. & washed into weighing bottle & further 1 ml. Freeze @ -10°.

3-VIII. Lo desiccator over P_2O_5, vac. down, leave overnight.

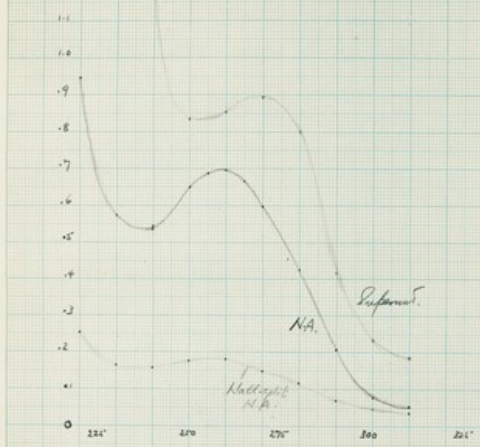
4-VIII. Weigh. Add to dried residue ca. 5 ml 1 N. NaOH, & place @ 37°. After 8 hrs, still large lumps. Break up & transfer quantitatively to 10 ml vol. flask, making vol. to ca. 9 ml & N. NaOH.

5-VIII. Isolation of N.A.: After 24 hrs @ 37° mostly dissolved, still turbid, & some small lumps. Made up to 10 ml, shaken, ± aliquots taken for N & P. Remaining 9.04 ml diluted to 20 ml ± aq. dest., shaken, ± 5 ml chloroform + 2 ml amylalcohol, spun. Only small protein gel - small amount have hydrolysed. Repeat extraction.

Chloroform phase: dissolve in CCl4, spin down protein. Not much. Add 2.0 ml 10 N. H_2SO_4 to dissolve for N & P.

Aqueous (NA) phase: spin 30 min @ 12000. V. little sediment, ± supernat. pipette clear. Add 1.5 ml 6 N HCl (made ca. N/10) - milky ppt. Spin down. Yellowish pellet. Dissolve (gumming) in N/20 NaOH.

Ld VNA of 5.VIII. From vol. of 3.0 ml in .02 M NaOH, diluted 50 x in aq. dil.



Hydrolysis:

1 ml acid = .905, .878, .870, .864, .844

Blank .780
Blank .786
average .790

0.1 ml N.A. .495, .497 } .496. .790 − .496 = .294/.864 × .0895 = 30.4 γ N = .304 mg/ml N.

0.2 ml supernat. .604
0.2 ml " .613 } .609. .790 − .609 = .181/.864 × .0895 = 18.7 γ N = .093 mg/ml N.

		mg N	mg P	N/P	% DNA
Summations:	30.9 mg. Ld V.	4.76	.586	6.52	19
	3.0 ml N.A.	0.91	.36	2.52	67 %
	30 ml supernat	2.79	.066		
	Recent T.N.A	16	9.5	1.68	

6-VIII. Ld VNA dil. 50x

260	.695	255	689
265	.666	250	625
270	.559	240	.387
280	.423	230	.272
290	.204	220	.945
300	.077		
310	.052		

Total NA = .695 × .04 × 50 × 30 = 41.2 mg.

% moving, of 18 % in NA = 4.2 / 18 × .90 × 21 = 64

Supernat. from which N.A. ppted. (contains hydrolyzed protein + N.A.?) from total vol. 27.5 ml.

Undiluted

260	862	255	.825
270	895	240	1.17
280	800	230	2.90
290	416		
300	.224		
310	188		

8-VIII. P. NA 2 samples 0.1 ml out of total 3.0 ml NA prep. 5.0 = .150 mg Total / 11.9 = .357 mg/ml

Supernat. 2 samples 2.0 ml out of total 27 ml (35 before Precipitation)

4.4 γ / 2 ml = .006 mg total

1.30 N. N.A. 2 samples 0.1 ml

Supernat. 2 samples 0.2

N.A. hydrolysis. To each of two tubes 1.25 ml N.A. prep. Dry down.

Purines: add 1 ml 6N HCl. Heat on B.W.B. 1 hr. } open - evap. to dryness.

Pyrimidines: 0.5 ml 100% H_2SO_4, 175° 2 hrs. }

9-VIII. Dissolve each in 0.1 ml N/10 HCl, put 5 · .01875 ml aliquots on paper, & start chromatograms at 5:45 p.m. Purines: but-formic. - 17 hrs.

Pyrimidines: but-water; 9:30 p.m. add 4 drops NH_3. - 16 hrs.

Data from J. D. S. – Purines & Pyrimidines in N/10 HCl.

		λ peak	D for 10 γ/ml	M	atoms N	1/DM
John	Adenine	260	0·964	135	5	~~[illegible]~~ 769
	Guanine	250	·703	151	5	~~[illegible]~~ 943
Hotchkiss	Cytosine	275	·910	111	3	~~1009~~ 990
	Uracil	260	·720	112	2	~~[illegible]~~ 765
	Thymine	265	·608	126	2	~~760~~ 1305

Variation

Adenine:

	$x-\bar{x}$	$(x-\bar{x})^2$
362	+7	49
368	+13	169
352	−3	9
346	−9	81
347	−8	64
$\bar{x}$ = 355		5)372
		74·5

σ = 8·63

S.E. $\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$ = 3·9

S.E. of molar ratio = $\frac{\cdot 0039}{\cdot 964 \times 1\cdot 35} \times \frac{4}{10\cdot 32}$ = ·0115

Guanine:

	$x-\bar{x}$	$(x-\bar{x})^2$
268	+20	400
280	+32	1024
221	−27	729
222	−26	676
251	+3	9
248		5)2838
		567·6

σ = 23·8

S.E. = 10·6

S.E. of molar ratio = ·0106 × $\frac{\cdot 83}{\cdot 2484}$ = ·0356

Thymine:

198	−8	64
209	+3	9
201	−5	25
202	−4	16
220	+14	196
206		5)310
		62

σ = 5·9

S.E. = 3·2

S.E. of ratio =

Cytosine:

358	−3	9
352	−9	81
366	+5	25
356	−5	25
371	+10	100
360·6		5)240
		48

σ = 6·9

S.E. = 3·1

S.E. of ratio = ·0031 × $\frac{1\cdot 26}{\cdot 361}$ = ·011

10·VIII. Neither chromatogram had run far enough. Both sprayed & butanol & replaced further 5 hours. Still only just separated. Cut out spots & elute in 5 ml N/10 HCl.

11-VIII. Beckman:

	λ	(1)	(2)	(3)	(4)	(5)	Mean	Factor	γ/ml	Molar ratio
Adenine	240	·156								
	245	·213								
	250	·278	297	270						
	260	·362	368	352	346	347	·355 ±·0039	1/·964	3·68	2·72 / 0·96
	275	·317								
	280	·145								
Guanine	240	206								
	245	251								
	250	268	280	221	222	251	·2484 ±·0106	·71, 1/·703	3·54	2·34 / 0·83
	260	202								
	275	182								
	290	104								
Thymine	255	166								
	265	198	209	201	202	220	·206 ±·0049	1/·608	3·38	2·68 / 0·95
	275	152								
	285	057								
Cytosine	260	227								
	270	338								
	275	358	352	366	356	371	·3606 ±·0031	·93, 1/·910	3·96	3·56 / 1·26
	285	269								
										11·38

Molar ratios:	Aug. 11 (wrong)	July 29	Aug. 19
Adenine	0·96 ±·01	0·74	0·80
Guanine	0·83 ±·04	1·11	1·03
Thymine	0·95	0·82	0·89
Cytosine	1·26 ±·01	1·33	1·28
Total bases per mol P	0·74	0·57	0·58

In orig. N.A. prep. molar conc. of total bases = 11·30 × $\frac{5}{\cdot 0158}$ × $\frac{\cdot 1}{1\cdot 25}$ = 2·86 mM.

" " " of P = $\frac{11\cdot 9}{31}$ = 384 mM.

Pigeon tube: MT dried: Ld V 13.9468 Ld PP 13.0496
+ 4 proteins 13.9535 13.0672
.0067 .0176

Correction for 2 ml $\frac{M}{100}$ NH_4CO_3 = $\frac{2}{1000} \times \frac{1}{100} \times 106$ = .0021 .0155

	15-VIII. Blend front		18-VIII. Butanol front	
15-VIII.	V ions	Poly pept.	V ions	P.P.
Aspartic	++	++	++	++
Glutamic	++	+++	++	++
Glycine	++	++	++	+
Serine	+++	++	++	+
Threonine	+	++	✓	+
Alanine	+++	++	++	+
Tyrosine	+	++	/	+
Valine	+	++	++	+++
Cystamine	+	++	+	++
Leucines	++	+++	+++	+++
Proline	+	++	+	++
Arginine			++	++
Histidine	++	++	−(trace?)	+
Lysine			+	++
Diaminopimelic				+ (?)

Hydrolyses for amino acids.

To 2 bomb tubes previously dried & weighed:

(1) ca. 10 mg. fmce 3 times washed Czech Ld V in aq. dest.

(2) ca. 40 mg twice ppted Czech Ld PP in 2 ml $\frac{M}{100}$ NH_4CO_3.

Evap. to dryness, place 30 min @ 110°, cool over P_2O_5.

5-VIII. Weigh.

8 Add 1—1.5 ml 6N HCl, seal off, to 120° oven 12.30 pm.–6.30 pm.

8-VIII. Open, evap to dryness.

9-VIII. Dissolve in aq. dest: virus 0.2 ml (:)
poly pept 0.4 ml

Chromatograms run 30 hrs in phenol–H_2O
16 µl. 17 hrs in butanol – acetic (but. 40 H_2O 10 aq. dest 50)

11-VIII. Results: spots spread (tailed) in phenol, run v. slowly in butanol (leucines R_F ca. .3 !)

12- Remainder of hydrolysate – evap to dryness twice, then dissolve in same vols. again aq. dest. – V 0.2 ml, PP 0.4 ml.
Chromatograms – 18 µl on each. To phenol 11.40 am.

13-VIII. Off 5.40 pm. – 30 hrs. Dry off phenol: heat & Long overnight.

14-VIII 11 am. to but-acetic.

15-VIII. 9 am. Out – 22 hrs.
Better than last time, but still some tailing in phenol & reduced R_F's in butanol.

12.15 pm. Set up 2 more: Ld V 0.018 ml, Ld PP 0.016. 2 to butanol-acetic first.

18-VIII. Good chromatograms. Photographed – blackwhite & Kodachrome.

Kjeldahls

1 ml amide : ·896, ·890, ·904, ·896, ·892, ·894 = ·894

10.15 Blank 0.5 ml H_2SO_4 ·846, ·840, ·842 } ·843

" ·842

0·062 ml virus ·420, ·420, ·417 } ·419. ·843 − ·419 = ·424 × $\frac{·0698}{·894}$ × $\frac{1}{·062}$ = ·684 mg N/ml

≅ 4·44 mg virus/ml.

Total vol. = 6·5 + 0·12 + 0·2 + 0·4 = 7·2 ml = 32 mg virus.

Aggregated virus: ca. 6 ml, 1·6 mg/ml = $\frac{10}{42}$ mg total.

% yield of virus from polyfilm = $\frac{42}{1780}$ × 100 = 2·4 %.

12-VIII. Ld Virus prep.

1 bottle (1·78 g.) Each polyfilm into ·0085 M $NaCO_3$ · ·05 M NaCl

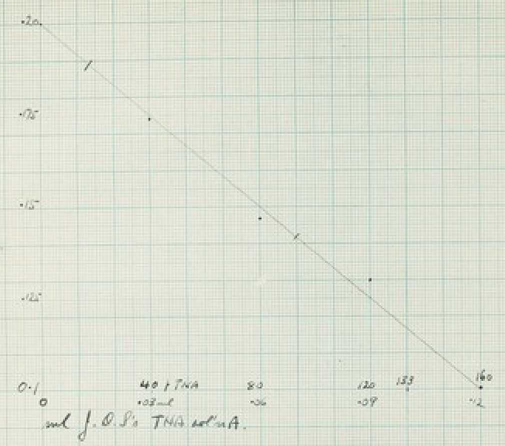
3¾ hrs. Dark-pomed. Less undissolved.

Spin 6800 8 min. Decant from biggish brown pellets + spin 9600 50 min (2x). Susp. pellets in ·008 M $NaCO_3$ + spin 6600 8 min, + spin virus out of this supernat.

All virus pellets susp. in about ag. dest + spin 9600 50 min. (4x), after brownish glass washed from top of pellets.

Poly prot twice ppted + spun 12000 60 min to remove virus. Divide in 100 ml M/50 Na_2CO_3. Half kept, half dialyzed for dry wt.

15·VIII. Susp. in ca. 8 ml aq. dest. Take Kjeldahl aliquots .62 ml + ? aliquots - 0·1 ml.



John's data on Roy's purified TNA (sol'n A)

N 245 μg/ml (theort. from bases: 230)

P 126 μg/ml (" " 128)

					Chargaff's molar ratios J.B.C. 177.43	
Adenine	154	1.10	molar ratios	1.14 mM.	A 1·6	= 1.19
Guanine	123	0.78		·815	G 1·3	0.96
Cytosine	99.6	0.87		·896	C 1·0	0.74
Thymine	162	1.24		1·286	T 1·5	1.11
	538.6			4.137	5·4	

~~N.A. = P/(P/x) × 100/9.5 = ·126 × 100/9.4 = 1.34 mg/ml~~

Calc. from bases, N.A. in 1 ml = ·539 (bases) + ·00414 × 178 (sugar+phos.) = 1.275 mg/ml (1.27)

Theort. % P in TNA = (·00414 × 31)/1·275 × 100 = 10·1 %

13-VIII. Test of Dische reaction

·074	Sol'ns: 0·03 ml J.O.S.'s TNA prep A, up to 4.0 ml ē aq. dest.
·147	·06 " "
·130	·09 " "
·100	·12 " "
·142	·09 " to 4·0 ml ē N/10 HCl, hydrolyse on B.W.B. 15 min
·189	·09 " " ē 1N. HCl " " 30 min.
·192	4·0 ml N.A. supernatant
0·20	4·0 ml aq. dest. blank.

Add Dische reagent, heat 5 min on B.W.B.

Cool in cold water. Read after 20–30 min

Helger readings 606 filter. 1 cm cells.

Destruction of deoxypentose: 15 min boil in N/10 HCl: 22 % lost

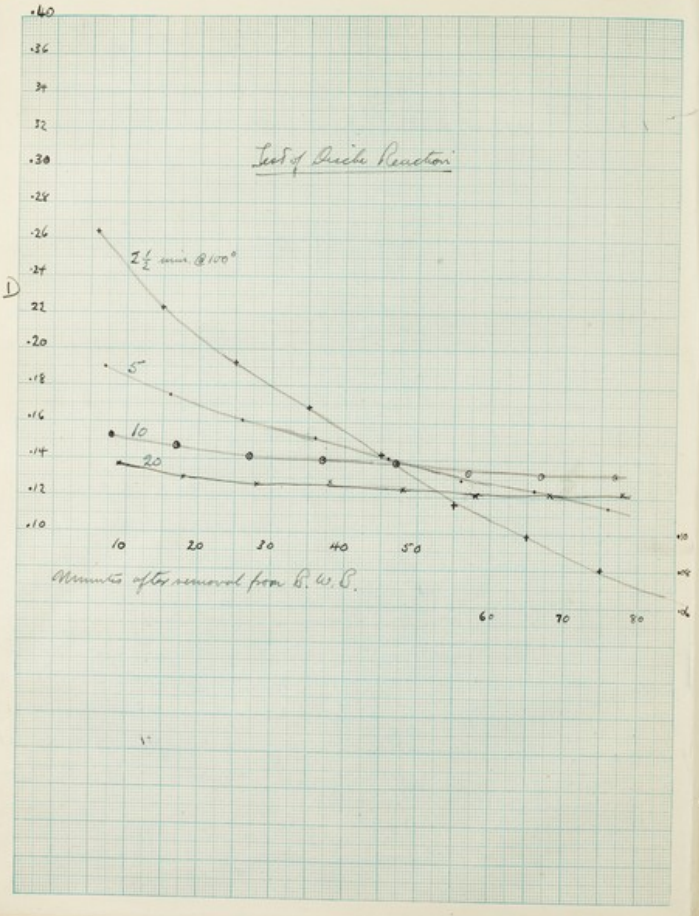
50 min. " " N HCl: 86 % lost.

15-VIII. Into 4 tubes, each ·25 ml sol'n A, 2·75 ml aq. dest, 6 ml Dische reagent.

B.W.B. 20, 10, 5, 2½ min. Out at 3.18.

	min. after removal from BWB	reading	Spec. 606										3 hrs.
20	9	·137			·130 (18)	·127 (28)	·128 (38)	·124 (48)	·121 (58)	·122 (68)	122 (78)	118
10	8	·153	·148 (10)	·147 (12)	·147 (17)	·144 (27)	·140 (37)	·138 (47)	·135	·132	132	122
5	7	·190			·175 (16)	·161 (26)	·152 (36)	·144 (46)	·129	·124	115	066
2½	6	·264			·223 (15)	·192 (25)	·168 (35)	·142 (45)	·116 (55)	·097 (65)	·081 (75)	–·03 ppt.

Water blank 0·4

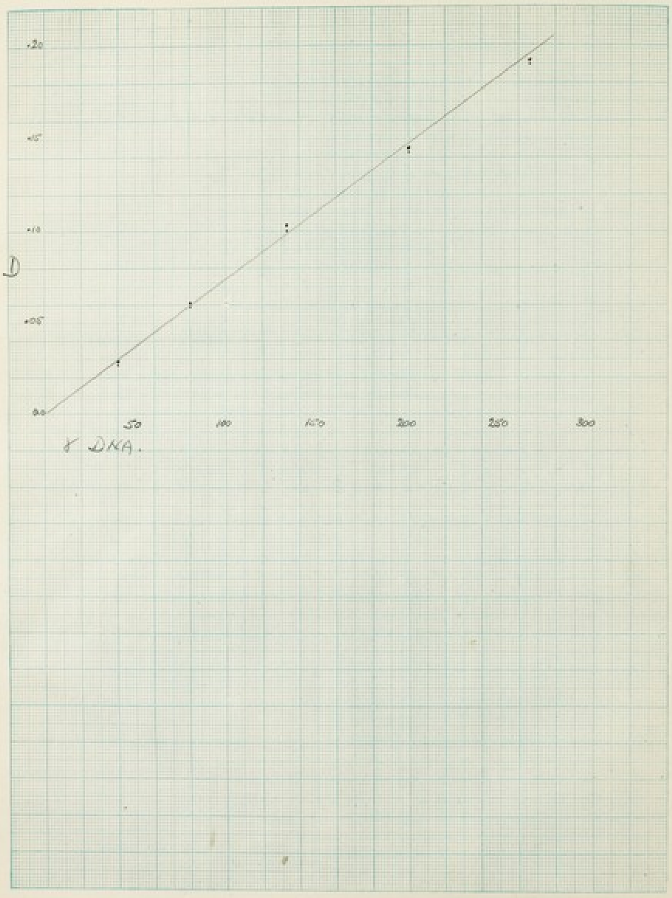


W 1

Effects of NaCl + NaOH on LdV

14-VIII. Remnant of LdV prep of 2-VIII. divided into 2 parts, and 0·65 ml.
Add to (a) 4 ml 1 N. NaOH
to (b) 3 ml 9% NaCl + 1 ml M/10 Na_2CO_3
(a) goes clear in few min cold, is slightly viscous
(b) does not clear, but shows some stringy aggregation, is more viscous.
11·30 am both to 37°.

15-VIII. Spin both 12,000 30 min (10·45) - v. small pellet fr. NaOH. Large from NaCl.
Supernate - make ca. N/10 acid by adding HCl. - NaOH gives fine ppt, NaCl, cundy.
(11·45) Spin 12000 20 min. Make up each supernat to 6·0 ml, + dissolve each N.A. pellet in 3·0 ml N/10 NaOH. Also orig. undissolved pellet of NaCl treatment in 3·0 ml N/10 NaOH.

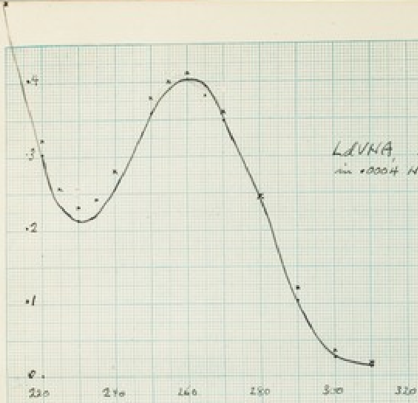


Dische tests

17·VIII.

	Ilford 606 test 30 min	55–60 min		γ DNA
Standards: ·03 ml John's TNA "A"	·027	·029		40
·06 "	·059	·061		80
·10	·100	·103		133
·15	·143	·145		200
·20	·190	·192		266
(a) ·20 ml Ld V of 15·VIII	·100 }			126
(b) " "	·095 }			
NaCl test of 14·VIII: total undivided ppt.	·362		intense green	
" N.A. ppt.	·050		green	
½ N.A. supernat.	·004			
NaOH test of 14·VIII: total undivided ppt.	·034		faint green	
" N.A. ppt.	·023		"	
½ N.A. supernat.	·007			
LdVNA prep ③: 1.0 ml (of 6.0) insoluble "a"	·180		strong green	
3 ml (of 38) supernat "b"	·027		faint green	
1 ml (of 6.0) insol. "c"	·080		slightly turbid	
0·2 ml N.A.	·100 ~~·010~~			127

All made up to 3·0 ml c̄ aq dest., add 6·0 ml Dische reagent (c̄ to water blank) (fresh reagent ~~strong~~ mixed c̄ some 2 days old in frig). B.W.B. 15 min. cool in water 25 ~~min~~, read 30–50 min. after removal from B.W.B.



Kjeldahl — 1 ml acid ≡ ·910, ·910, ·913 — Cond of titration: ·912

Blank ·5 ml H_2SO_4	·855		
"	·858	·858 − ·720 = $\frac{·138}{·911}$ × ·0983 = ·0149 mg N	= ·15 mg N/ml
"	·863		
0·1 ml LdVNA ③	·734	·720	
"	·706		

NA N, calc. from P = $0·11 \times \frac{16}{10}$ = 0·176 mg N/ml (?)

Distribution of P & DNA in prep.

	mg P mg/ml	ml	total	mg DNA (Dische)
Starting virus	(1·83)	6·5	·519	4·09
Undissolved remnant "a"	·0018	6·0	·011	
Supernatant "b"	·0018	38·0	·069	
Undissolved material "c"	·0044	6·0	·026	
N.A.	·111	5·0	·555	3·18
Total				

15-VIII. LdVNA prep. 3

7 p.m. 6·5 ml LdV prep. of 12-VIII. (= 28·8 mg) added to 25 ml 1 N. NaOH (makes 0·8 N.) & to 37°C.

16-VIII. 15 hrs @ 37°. Still turbid, though less so than when first mixed. Spin 12000 30 min (10.10), & decant from smallish pellet ("a" in 6·0 ml NaOH). Ppt ~~[illegible]~~: 4·5 ml 6N HCl → large curdy ppt. Spin. Supernat 38·0 ml = "b".

Pellet: dissolve (turbid) in 20 ml N/10 NaOH, add 5 ml chloroform-amyl alc., shake, spin. Repeat twice with reduced amt chloroform.

Aqueous phase (N.A.): acidify, spin.

Chloroform gel: add 20 ml water (unstable). Add EtOH till homogeneous, acidify, spin. Suspend in 6·0 ml N/10 NaOH. Turbid. = "c"

N.A. pellet: susp. in 5·0 ml N/50 NaOH. Viscous, turbid. Spin 12,000 30 min (add pellet to "c")

17-VIII. Beckman. Prep. dil. 1:50 in aq. dist.

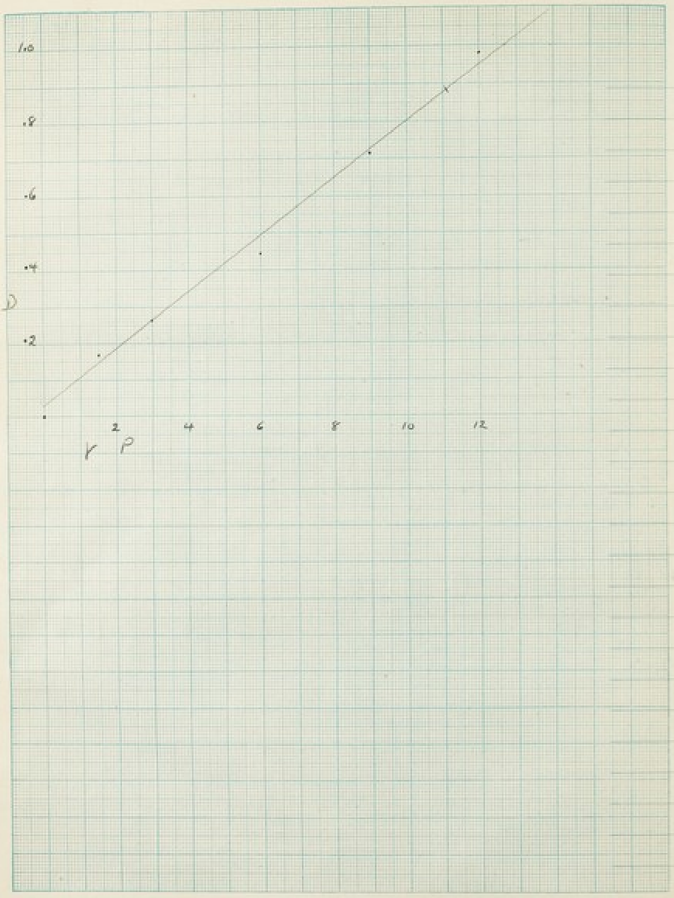
λ	D		λ (18-VIII. Re-check)	D
220	0·303	·606	210	·540
230	·213	·426	220	·325
240	·256	·512	225	·257
250	·358	·716	230	·232
255	·407	·814	235	·242
260	·404	·808	240	·281
265	·395	·790	250	·380
270	·348	·696	255	·400
280	·245	·490	260	·411
290	·103	·206	265	·382
300	·026	·052	270	·360
310	·014	·028	280	·246
			290	·122
			300	·037
			310	·018

$\frac{D_{260}}{D_{235}} = 1·9$

Total NA = ·404 × ·04 × 50 × 5 = 4·0 mg

% yield (of 16% in V) = $\frac{4·0}{28·8 \times ·16}$ = 87%

Dische - DNA in orig. virus = $\frac{·630}{4·44}$ × 100 = 14·2 %.



17-VIII. <u>P estimations</u>

		ORZ filter water blank		
Standards:	1.5 γ P	·169		
	3	·264		
	6	·483		
	9	·715		
	12	·980		
Insufficient incinerated	(1) 0.1 ml hd V of 15-VIII.	·235		all low < 1.7% P in hd V
	(2) "	·145		
	(3) "	·301	3.5	
Assay have contained lumps	(1) 0.031 ml same hd V	·150	1.5 γ P = 48.5 γ P/ml = 1.1% in hd V	
	(2) " "	·480		
	(1) 0.10 ml hd UNA of 16-VIII.	·892	·884 ≡ 11.1 γ P	
	(2) "	·875		
	0.5 ml insol. material "a" of 16-VIII.	·100	0.9	
	1.0 ml supernat "b"	(1) ·178, (2) ·158 — ·168	1.8	
	0.5 ml insol. material "b"	·206	2.2	

17-VIII-49. LAVNA (3) Bases.

Bases: 2.0 ml prep. dried down, add ·5 ml HCOOH, cook 175° 2 hrs. (+ +0)

2.0 ml " made N in HCl, cooked 100° 1 hr. (415)

Dry down, dissolve each in 0·1 ml N/10 HCl, put 5° 15·75 μl spots on paper.

Purines: BuOH-formic 10 pm

Pyrimidines: BuOH-NH₃ 10 pm.

18·VIII. 5 pm. take off (19 hrs.) found, cut out, elute in 5·0 ml N/10 HCl.

19·VIII. Beckman.

		D_{260}	$x-\bar{x}$	$(x-\bar{x})^2$		γ/ml	μmoles	molar ratio
Adenine: (1)	all well resolved	·327	−15	225				
(2)		·338	−4	16				
(3)		·361	+19	361				
(4)		·350	+8	64				
(5)		·332	−10	100				
$\bar{x}$		·3416 ± ·0055		5|766 / 153	σ = 12.4	35·4	2·62	0·80

		D_{250}	$x-\bar{x}$					
Guanine (1)		·364	+2	4				
(2)		·358	−4	16				
(3)	tailed	·294	−68	4624				
(4)		·436	+74	5436				
(5)		·360	−2	4				
		·3624 ± ·0200		5|10084 / 2017	σ = 44.9	5·14	3·40	1·03

Theoret. N content:

	per mol	per mol N
Adenine	262	131
Guanine	340	170
Cytosine	421	126
Thymine	294	59
		486

Molar conc. in orig. prep. = $\cdot 486 \times \frac{5}{\cdot 0158} \times \frac{\cdot 1}{2} = 7.68$ mM

$= 7.68 \times 14 = 107$ γ/ml = 16.7%.

Theoret. DNA:

To every base is attached: desoxyribose $-H_2O_3 = C_5H_8O = 83$

phosphate $HPO_4 = \frac{96}{179} - 1H$ from base $= 178$

DNA in 1 ml orig. prep. = $\frac{5}{\cdot 0158} \times \frac{\cdot 1}{2} \left(17.05 + \cdot 1317 \times 178 \right) = 640$ γ

% P in Ld VNA, calc. from bases = $\cdot 1317 \times \frac{5}{\cdot 0158} \times \frac{\cdot 1}{2} \times \frac{31}{640} \times 100 = 10.1\%$

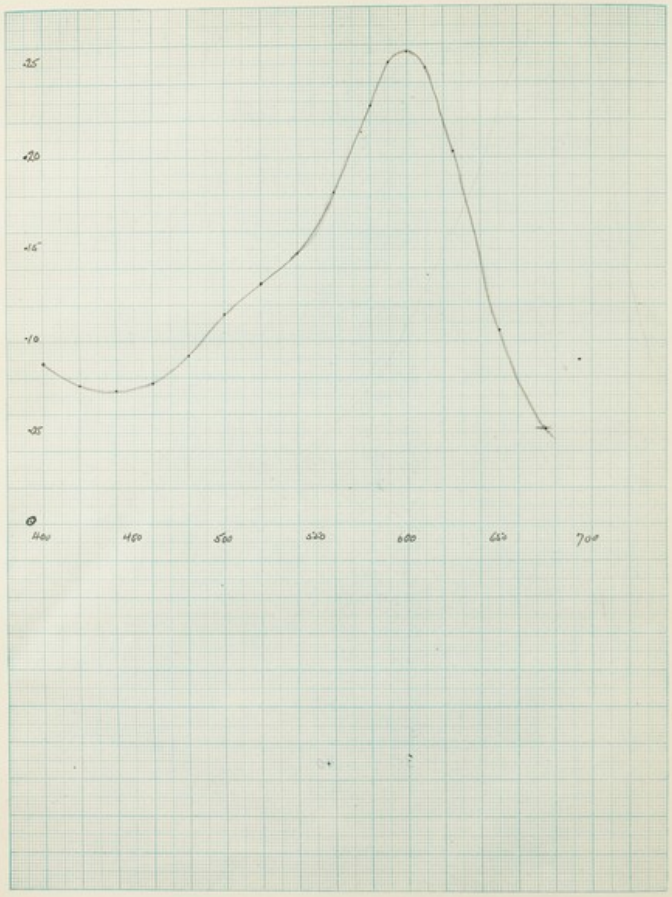
19-VIII.

		D_{274}	x − x̄		γ/ml	μ moles/ml	moles/mole
Cytosine	(1)	·442	+ 18	324			
	(2)	·429	+ 4	16			
all well washed	(3)	·410	− 15	225			
	(4)	·427	+ 2	4			
	(5)	·416	− 9	81			
		·425 ± ·0051		5)1650 / 185 γ = 11.4	4.67	4.21	1.28
Thymine	(1)	·248	+ 23	529			
	(2)	·207	− 18	324			
	(3)	·218	− 7	49			
	(4)	·229	+ 4	16			
	(5)	·222	− 3	9			
		·2248 ± ·0061		5)1927 / 185 γ = 13.6	3.70	2.94	0.89
					17.05	13.17	4.00

In orig. N.A. prep. molar conc. of total bases: $\cdot 1317 \times \frac{5}{\cdot 0158} \times \frac{\cdot 1}{2.0} = 2.03$ mM.

" " P $= \frac{111}{31} = 3.58$ mM. $\frac{\text{Bases}}{P} = 0.581$

Orig. prep. γ/ml	Found	Calc. from bases
P	111	62
N	150	107
DNA (Ouchi)	635	640



18-VIII. Blanks both of 17-VIII read on Beckman.

.20 ml standard = reagent blank

λ mμ	D
420	.076
440	.073
460	.077
480	.092
500	.114
520	.132
540	.148
560	.181
580	.228
600	.258
625	.202
650	.106
675	.052
680	

Sample	D_{600}
.10 ml TNA	.137
.15	.196
.20	.258
.2 Ld V	.122
.2 Ld V	.140
③ a	.258
d	.046
c	.967
N/A	.132

24-VIII. <u>Check purity of guanine & adenine from chromatograms of 19-VIII.</u>

Eluted sol'ns guanine ② & adenine ② evap'd to dryness, susp. each in 31 μl N/10 HCl, put on 2 spots 11.5 μl, run in BuOH-H_2O & BuOH-formic. 6 pm.

25-VIII. 2 pm. Run 20 hrs.
Both show only one spot in each run - adenine & guanine look pure, except for small starting spot (= paper extract?).

—— II ——

<u>Repeat P estimation on Ld VNA ③.</u>

5 ml 1:50 NA from Beckman run 17-VIII, now buggy, & absorption D_{260} = ·275.

3.10. Incinerate 2 aliquots 2.0 ml (ca 5 γ P?). Add 1 drop HNO_3 3x.

6.15. Ppt. ē newly filtered molybdate. Reg. blank.

26-VIII. Left in sunshine after addition of K_4FeCN_6. → fluorescent blue! Discard.

38
16
550
228
608

Flask MT dried 74.4115 g.

Kjeldahl on virus used, aggregated with V of 12.VIII.
1 ml HCl ≡ .988, .990, .996, .998 = .996
Blank 5.4.10 .920 .920 } .920 − .841 = .079 × .0983/.996 × 100/15.4 × 1/.331 = 1.63 mg/ml virus
31/ul V .852 .830 } .841

29.VIII. Micro-balance:
Zero: 20 right
MT filter: wide 0.4, 76 right = .476 − 20 = .456
.500 4 R. = .504 − 20 = .484
.400 1/2 5 1/2 − 20 .492
.400 1/9 −20 .499 } .495 + .499
.500 10 R −20 .490
Zero after: +16 ∴ wt. mean of last 2 weighings = .499 mg over counterpoise.

30.VIII. Zero: − 20
Filter + dried virus: 3.300 − .019 = 3.301 mg.
% virus = 2.802 mg.

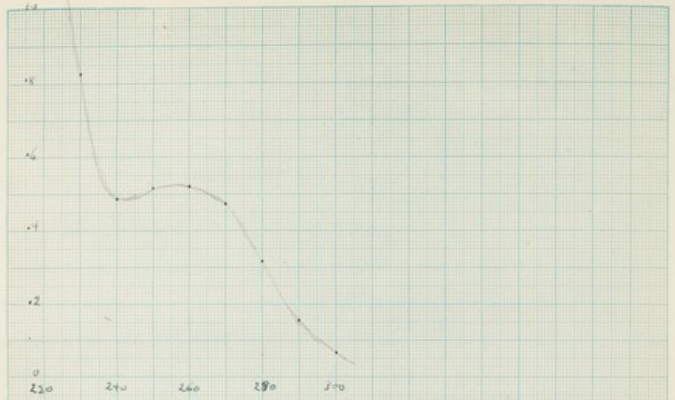
31.VIII. Zero: −16
Filter + extracted virus 3.200 + .045 = 3.261 − % virus = 2.762
After fat-mix: 3.200 + .082 = 3.298
− continuous increase as if % taken up.
Fat-mix was 3.200 + .110 = 3.310
Loss .040

% loss = .040/2.802 × 100 = 1.4 %

26.VIII. Extraction of fat from virus.
Flask bonded + knife 3 hrs 110°, cool over P_2O_5.

29.VIII. 3.8 ml virus = 6.08 mg. Not enough for Soxhlet.
Small sintered glass filter dried 3 hrs 110° cool over P_2O_5, weigh on micro-balance.
Virus − ppt ≤ Am_2SO_4, try to filter. Clogs filter. Pour out, bring to boil to coagulate, clean filter, try again. Still clogs. Spin down virus susp in ca. 2 ml 50% EtOH, filter out, wash ag. dist. Put in oven 110° overnight.

30.VIII. Cool, weigh.
Extract ca. 15 ml petroleum ether 40°-60° B.pt., collect.
Place in oven 110°.



27-VIII. Effect of sat. NaCl on L of V.

1.0 ml aggregated L of V of 12.VIII. (≡ 1.6 mg) treated ≡ 5 ml sat NaCl 2 days room temp.

Add 2 ml chloroform, shake, spin. Repeat.

To aqueous phase added 2 vols 90% EtOH. Only slight ppt.

Spun 12000 20 min. Dissolve insoluble pellet in 5 ml N/50 NaOH.

Spin " 15 min.

Protein gel: dissolve in 3 ml N NaOH. dilute up to 10 ml.

Wavelength	"NA"
220	1.50
230	.823
240	.487
250	.515
260	.520
270	.473
280	.317
290	.134
300	.068

Total N.A. in < .52 × .104 × 5 = 0.14 mg

To yield, if 16% of V = $\frac{.14}{16 \times 10^{-2}}$ =

Yield: ca 55% of N.A., very impure.

N.A. not split from protein by sat. NaCl.

1-ix Extraction thimble MT not specially lined — 4.~~[illegible]~~ 4958
" " + polyhedra, air-dried room temp. — 5.5680

1.0722

Weighing bottle + dry polyhedra. 9.6080
" MT (from day 2) 9.6005
.0075

Wt in 25.0 ml = $\frac{25}{.2}$ × .0075 = 0.94 g.

31.VIII.49. Extraction of fat from polyhedra.

1 bottle (1.78 g) Geoh Lil pe. washed practically free of bugs & much by spinning down 3 times & slow spin, ∴ loss of maybe 50 mg. Susp. in 25.0 ml aq. dist.; 0.2 ml to weighing bottle, dry overnight 110°.

ix. ~~Brooke~~ Soxhlet.

Theor. uracil in extract = $800 \times \frac{1}{\cdot 7} \times \frac{\cdot 0174}{5} = 3.98 \times \frac{94}{100} = 3.74$

Theor. thymine in extract = $1620 \times \frac{\cdot 5}{\cdot 7} \times \frac{\cdot 0174}{5}$ 4.04

Hydrolysis of uracil & TNA.

1-ix-49.

0·5 ml Roy's purified TNA (conc. 2? mg/ml)
+ 0·2 ml containing 0·80 mg uracil } =0·7 ml.

0·1 ml of mixture into each of 4 bomb tubes, dry down, add 0·5 ml HCOOH, hydrolyse 175° 1, 2, 3, 4 hrs.
2 + 4 hr bombs went dry (hole?).
Chromatograms: 2 spots 17·4 ml each 0, 1, 3 hrs hydrolyzed.
Into 5%BuOH+HCOOH 5.15 pm.

2-ix Out 11.45. 18½ hrs. Thymine & uracil well resolved. adenine = cytosine = guanine not quite. Cut out & elute thymine & uracil.

Beckman

				Found	Theor.
Uracil unhydrolyzed	D_{265}	·217 ·248	·232 × $\frac{1}{\cdot 720}$ =	3.22	3.74
1 hr.		·237 ·268	·250	3.47	
3 hrs		·234 ·267	·250	3.47	
Thymine 1 hr	D_{265}	·232 ·216	·224		
3 hrs		·240 ·212	·226	× $\frac{1}{\cdot 608}$ = 3.70	4.04

2-dimensional on ½ hr HCl hydrolysis.

See Sys BuOH-2% HCOOH then 2e. BuOH-N/10 HCl.
Large starting spot remains. Only very small portion of mobile(s) has moved in BuOH-N/10 HCl. Cauliflower, thymine, adenine, cytosine well separated. Guanine not visible. Nothing else out.

3-ix-49. Hydrolysis of TNA.

Roy's purified TNA diluted 1:10.

1 ml portions hydrolyzed: 1 N. HCl 100° ¼, ½, 1, 2 hrs.
100% HCOOH 175° ¼, ½, 1, 2 hrs.

Dry down, susp in 0.15 ml N/10 HCl.

17.4 µl spots: { 1 of each to run 1 dimension in 80% sec BuOH-2% HCOOH.
6 p.m. 1 of HCl ½ hr for 2-dimension – " " ,
then 80% sec BuOH-N/10 HCl.

H-ix. Taken off ca. 3 p.m. (?) – 21 hrs.

5-ix. HCl hydrolysates all show residual mobile(s) and H bases free – even ½ hr shows small thymine. } cytosine-guanine not well resolved.
HCOOH – no mobile(s) in any. Guanine faded.
Run out everything. (see over)

Milder HCOOH hydrolysis

3 portions 0.5 ml 1:10 TNA dry down.
Add 0.5 ml pure HCOOH. ~~Bomb 125°~~ ½, 1, 2 hrs.
[½ hr tube-some spilled-relative values only]
Dry down, dissolve each in 85.75 µl, put on 17.4 µl spots.
80% sec BuOH 86% HCOOH 6 p.m. Run very slowly (big load)

6-ix Duplicate same put on 6 pm

7-ix 9.30 am take off both. See over.

μg/ml from Roy's TNA 1:10.

	N. HCl 100°	HCOOH 175°	John's analysis	HCOOH 125°	9-ix ① HCOOH 175°
Guanine	[20]	[80]	123	130	153
Adenine	163	167	154	147	170
Cytosine	[71]	[142]	99.6	59.6	122
Thymine	67	154	162	76.4	150

"Ad 1 ½"
250 .178
260 .225
270 .196

Ad 2. ½
250 .177
260 .232
270 .200

Thymine ½
255 .028
265 .080
275 .023

[illegible]
265 .043
275 .042
285 .037

Gua. ½
240 .403
250 .132
260 .097

5-ix Beckman on TNA hydrolyses.

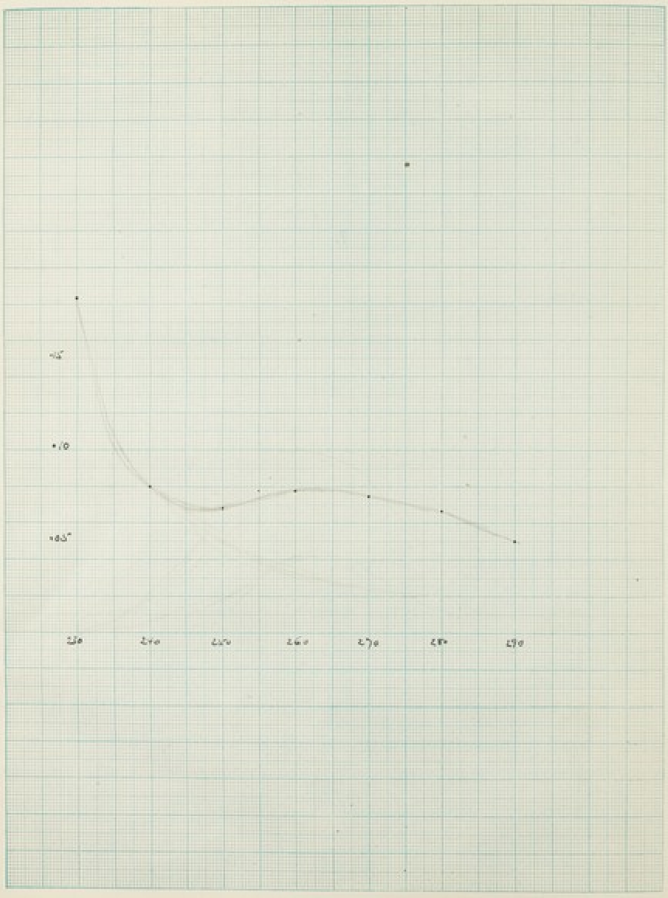
(from ① spot:)
240 +.012
250 .062
260 .033
270 .057
280 .024
290 .038

	Thymine D_{265}	γ/ml	Cytosine D_{275}	γ/ml	Adenine D_{260}	γ/ml	Guanine D_{250}	γ/ml	[illegible] D_{270}
HCl ¼	.088		.147		.376		.015		.452
½	.102	.095 1.56	.128	.150 1.65	.340	.366 3.79	.062	.032 0.46	.390
1	.096		.149		.392		.038		.337
2	.094		.178		.356		.012		.327
HCOOH ¼	.223		.349		.374		.101		−.016
½	.208	.217 3.57	.280	.300 3.30	.386	.374 3.88	.157	.130 1.85	−.023
1	.209		.298		.370		.107		−.014
2	.228		.273		.366		.160		−.029

Blank read against
N/10 HCl:
220 .498
230 .368
240 .238
250 .125
260 .088
270 .068
280 .045

7-ix.

HCOOH 125°		Thymine		Cytosine		Adenine		Guanine	
Chromatogram ①	[illegible]	.030		.052		.226			
	1	.094	1.55	.168		.277	2.88		
	2	.077		.082		.267			
Chromatogram ②	½			.053		.230		.182	
	1			.110	1.21	.297	3.08	.185	2.64
	2			.083		.279		.181	



6-ix-49. <u>Locusta migratoria DNA</u>

1 locust ground in 1 N. NaOH (ca. 25 ml), stand overnight 37°.
Strain, spin. Acidify c̄ HCl → much ppt. Spin out.
Dissolve in ca. M/20 H_2SO_4, spin (only small residue).
Shake c̄ chloroform-amylate, spin, repeat 4 x, till no gel.
... → v. fine ppt. Spin 12000 10 min.
... ppt. ... Spin again. Dissolve pellet in
1% NH_3 ... . Vol ca. 5 ml.

	1:10	1:100
230	∞	1.05
240	1.6	·487
250	1.55	·398
260		·404
270		·395
280		·354

Add equal vol sat. NaCl, c̄ solid NaCl till sat.
7-ix. Spin off small brown ppt. Dialyse.
8-ix. Spin off " " " , pptd by dialysis.
Make ca. 0·2 N c̄ HCl – v. fine ppt. spin 15 min 12000.
Dissolve v. small ppt in 2·0 ml 1% NH_3.

1:50	230	·153
	240	·080
	250	·068
	260	·078
	270	·075
	280	·069
	290	·020

Suppose absorption at 260 peak is ½ due to NA.
– ½ D_{260} = ·04 i.e. ca. 1·5 γ/ml
Then total = 150 γ.

Dry down. 0·5 ml HCOOH ½ hr 150–175°.
Dry down, take up in 0·05 ml N/10 HCl, put 23 μl on paper.

$\frac{.484}{.720} \times 5$

<u>Distribution of stuff in spots on paper.</u>

5.ix. 6 spots uracil 9.15 μl (= 36.6 γ). Run in [illegible]
ca 8 hrs.

6.ix. Print. 3 are fairly round. Cut out disks 3.0 cm dia., which just includes all visible spot, and 0.5 cm ring around and corresp. blank. above uracil.

7.ix. D_{260}

	1	2	3.	$\bar{x}$	γ uracil
Disk	.484	.483	.486	.484	33.6
Ring	−.017	−.018	−.015	−.017	
Total					

Areas: Disk: $(1.5)^2\pi = 7.06$ cm².
Ring $(2)^2\pi - 7.06 = 12.57 - 7.06 = 5.51$

Using rings as blank: must add to reading $.017 \times \frac{7.06}{5.51} = .022$

$\bar{x}$ Reading then .506, ∴ uracil = 36.1.

0.00915 ml uracil soln in 5 ml $^{N}/_{10}$ HCl, read against $^{N}/_{10}$ HCl.

: (1) .493
(2) .494
(3) .496
} .494 = 34.6 γ.

8 ix-49. Hydrolysis of TNA cont'd.

2 samples 1.0 ml Prep. TNA 1:10 hydrolyzed hydrolyze HCOOH 150° ½, 1, 2½ hrs.

Take up in 0.15 ml, put on 17.4 ml yeast.

9 ix. Only 2½ hr hydrolysis is complete.

	250	·282		270	·244		245	·234
Adenine ①	260	·390	G.T. ①	275	·256	Gu ①	250	·244
	270	·350		280	·241		255	·223
							270	·166

× 5 × ·15
·0174

Solvents: 10% HAc. = 1.7 M. [H] = ~~[illegible]~~ 5.5×10^{-3} pH = ~~[illegible]~~ 2.3

10% H formic = 2.2 M. [H] = ~~[illegible]~~ $[illegible] \times 10^{-2}$ pH = ~~[illegible]~~ 1.7

5% formic = 1.1 M [H] = 1.4×10^{-2} pH = 1.9

Try:	(a)	(b)
Sec. But.	80	80
H_2O	20	20
H formic	5	—
H Ac		10
H Cl		·01 N.

10% acetic + ·01 N HCl. [H] = $.55 \times 10^{-2} + 10^{-2} = 1.55 \times 10^{-2}$ pH = 1.8

Locust: Thymine

9-ix.

	Thymine D_{265}		γ/ml	Cytosine D_{275}			Adenine D_{260}			Guanine D_{250}		
① TNA HCOOH 175°	·208			·256			·390			·244		
¼ hr. 17.4 µl.	·213	·212	3.49	·254	·258	2.84	·385	·380	3.94	·251	·249	3.54
80% Sec. B.S. 10% H formic	·215			·264			·364			·247		
							·388					
④ TNA HCOOH 175°	·201						~~[illegible]~~					
¼ hr. 17.4 µl.	·204	·205	3.37				·382	·382	3.96			
75% sec. B.S. 5% HCOOH	·209						·376					
③ TNA HCOOH 175°	·232			·294			·385			·167		
½ hr. 17.4 µl.	·182	·213	3.50	·283	·290	2.8	·377	·382	3.96	·176	·170	2.42
75% sec. B.S. 5% HCOOH.	·224			·294			·386			·168		

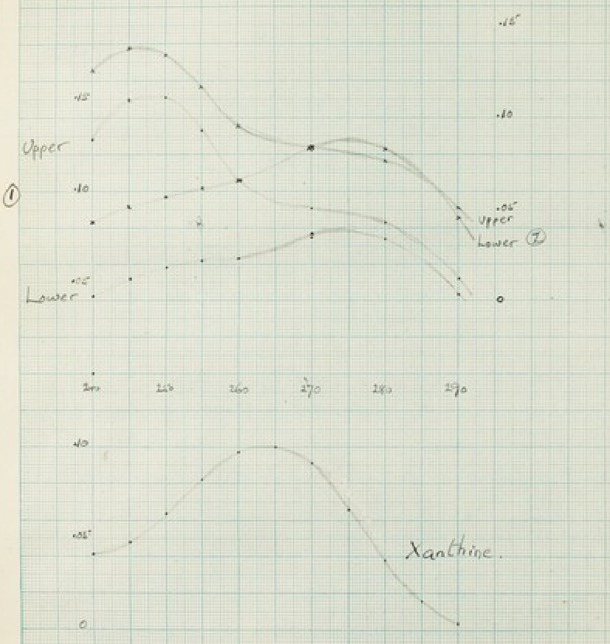
10-ix.

	Thymine	Cytosine	Adenine	Guanine
③ TNA HCOOH 130° 2½ hrs. Sec. But. 75% HAc 10	·198	·276	·376	·137
(4) Ditto Sec. But. 75% H formic 10	·201	·218 ~~[illegible]~~	·360	·212
Locust — Sec. But. 75% H formic 10	260 ·055 265 ·058 270 ·064	270 ·072 275 ·076 280 ·071	255 ·056 260 ·067 265 ·072 270 ·067	250 —·015

Locust brown after new front:

240	·054
250	·038
260	·042
270	·043
280	·044
290	·032

Factor: × 5 × $\frac{·15}{·016}$ = 47



TNA.

12-ix. TNA HCOOH 1 hr 175°. 16·0 μl.

① on Whatman 85 HAc 10 HCl ·01 N. Brown line at acid front across paper at level of cytosine.

		Blank above line	Blank including line	Blank below line
Read against N/10 HCl.	240	·006	·024	·046
	250	·009	·019	·042
	260	·017	·027	·041
	270	·016	·028	·037
	280	·013	·025	·033

Use blanks from appropriate levels.

Guanine spot is bilobate.

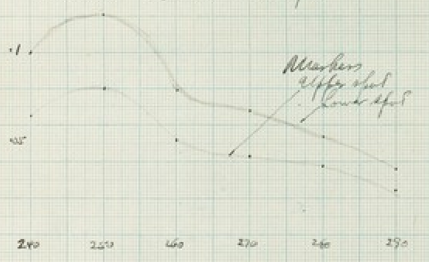
	① upper guanine	lower guanine	② upper G	lower G
240	·128	·042	·126	·043
245	·130	·052	·138	·052
250	·151	·058	·134	·057
255	·133	·062	·117	·062
260	·106	·068	·096	·066
270	·091	·077	·084	·084
280	·083	·074	·077	·083
290	·052	·048	·051	·046

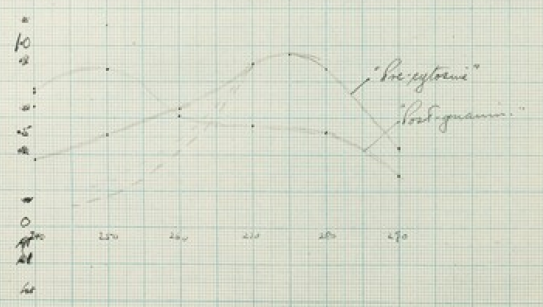
		①	②	mean γ/ml	Orig. γ/ml
Cytosine	D_{275}	·285	·286	3·13	147
Adenine	D_{260}	·413	·413	4·28	201
Thymine	D_{265}	·233	·245	3·94	185

13-ix. Third "epiguanine" spot run down with eluted -3 combined, dried down, hydrolyse 2 hrs HCOOH 175°. Run in Whatman-formic → 2 spots, corresp. guanine & slightly lower. Not cytosine.
Guanine run down in Whatman-HAc-HCl → 2 spots. see over.

Try: ① n But 85 .01 N HCl.
n But 2% HCOOH .01 N HCl
n But 10% HCOOH 5% HAc. – not bad.
n But 85 1% NH$_4$ oxalic. – N.G.!

n But 85 8% HCOOH – 8% HAc.
tert But 70 .01 N HCl. Conc HCl vapor.
n But 85 8% HAc HCl vapor.





14-ix. Several guanine markers run in n But-HAc-HCl → 2 spots. Elute (quickly, warm).

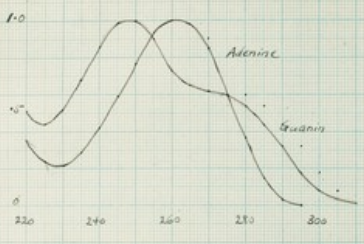
	Guanine	"Epiguanine"
240	.316	.329
250	.388	.388
260	.284	.277
270	.239	.237
275	.237	.256
280	.219	.219

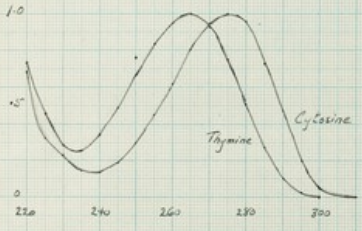
15-ix. TNA (175° 2 hr & 165° ½ hr) run in n But formic 10 acetic 7. Guanine marker in 2 spots. TNA: some tailing between guanine & cytosine. Cut out 2 lanes together: main guanine, "post-guanine", pre-cytosine & cytosine.

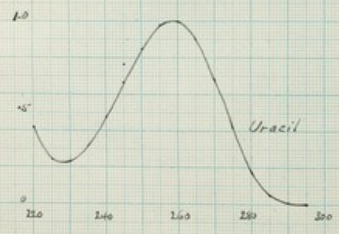
λ	Guanine marker Upper	Guanine marker Lower	TNA Guanine	TNA Post-guanine	TNA Pre-cytosine	TNA Cytosine
240	.065	.100	.282	.074	.037	.066
250	.080	.121	.241	.087	.051	.111
260	.052	.079	.248	.061	.065	.196
270	.043	.068	.216	.056	.090	.265
280	.038	.057	.199	.053	(275) .095 / .087	.299 / .277
290	.025	.036	.125	.029	.074	.149

Try: tert But-HAc-H form (esterification less in tert alc.)
n-But-HCl (" – in 1° alc.)

STANDARD ABSORPTION CURVES IN 0.1 N HCl (HUTCHISON).







Bottle MT	7.7035 g.
" + fresh gonads	7.7959
Gonads (8 pairs ♂ + 2 pr ♀)	.0924 g.

15-ix-49. <u>Bm larval N.A.</u>

Gonads dissected from 10 supposedly healthy full-grown Bm larvae – 8 ♂♂ + 2 ♀♀. Weigh. Ground in sat. NaCl suspend in ca. 10 ml sat NaCl + 5 drops chloroform, stand overnight @ 37°.

16-ix. Spin down undissolved tissue. Acidify supernat. Only v. small ppt. Spin this down.
Suspend N.A. ppt. & NaCl residue each in 1 N. NaOH, & leave @ 37°.

17-ix. Both: spin clear, acidify. Larger ppt in NaCl residue fraction. Combine & spin down, dissolve in 1% NH_4OH. Leave in frig.
(ppt suspended first by mistake in N HCl ! – damage?)

19-ix. Deproteinize by Sevag.

γ/ml in orig. prep. TNA.

	HCOOH 175° ½ hr. n-but-formic	Tert-but-HCl
Guanine	168	156
Adenine	175	176
Cytosine	112	133
Thymine.	162	166

25-IX. TNA. Tert-but HCl & n-but-formic chromatograms on HCl & formic hydrolyses. 1 ml ? 0.15"; 17.4 μl spots.

	Thymine	Cytosine	Adenine	Guanine.
23-IX				
(1) TNA N/10 HCl ½ hr (1)			260 ·354	250 ·214
n-but-formic (2)			260 ·350	·214
(2) TNA HCOOH 175° ½ hr (1)	·227 } ·2285	275 ·233 } ·2365	·390 } ·3915	253 ·264 } ·2725
n-but-formic (2)	·230 } 3.76 γ/ml	·240 } 2.60 γ/ml	·393 } 4.06 γ/ml	250 ·279 } 3.89 γ/ml
24-IX				
(1) TNA N/10 HCl ½ hr (1)			260 ·369	250 ·294 255 ·293
tert-but 75 HCl ·0.15 (2)			260 ·365	250 ·303 255 ·295
			Hydro- adenine: 255 ·356 260 ·367 265 ·368 270 ·364	
(2) TNA N/10 HCl 1 hr (1)			260 ·374	
tert-but HCl ·15 (2)			260 ·383	
(3) TNA HCOOH 175° ½ hr (1)	·233 } ·234	·276 } ·282	·393 } ·394	·260 } ·255
t. b. 75 HCl ·015 (2)	·235 } 3.85 γ/ml	·288 } 3.10 γ/ml	·395 } 4.09 γ/ml	·247 } 3.61 γ/ml
(4) HCOOH 175° 1½ hr (1)	·207	·279	·384	·218
t. b. 75 HCl ·015 (2)	·224	·324	·405 brown line	·218

Roy's TNA 1:10

Kjeldahl:

1 ml HCl: ·925, ·893, ·873, ·870, ·877, ·880

0.3 ml (add 1 ml acid) ·096

" ·053

"

Stuff was dissolved in NH_4OH!

Blank ·771

" ·814

" ·810

28-x Roy's TNA Kjeldahl. Boil = MgO first.

1 ml HCl: ·884, ·885, ·887

·3 ml 1:10 TNA ·114 }

" ·115 } ·115

" ·116 }

Blank ·794 }

" ·784 } $·792 - ·115 = \frac{·677}{·886} \times ·0983 \times \frac{1}{·3} = 250$ γ/ml

" ·798 }

3-x. Repeat, as values for bases were low in above dilution of TNA. 1 ml Roy's TNA made to 10 ml in vol. flask. Boil = MgO treatment overnight.

1 ml HCl: ·860, ·866, ·856, ·858 = ·857

Blank ·5 ml new H_2SO_4 ·697

" ·777 }

" ·765 } $·771 - ·081 = \frac{·690}{·857} \times ·0983 \times \frac{1}{·3} = 264$ γ/ml.

·3 ml 1:10 TNA ·09 }

" ·114 } ·081

" ·040 }

·856

·857

·110

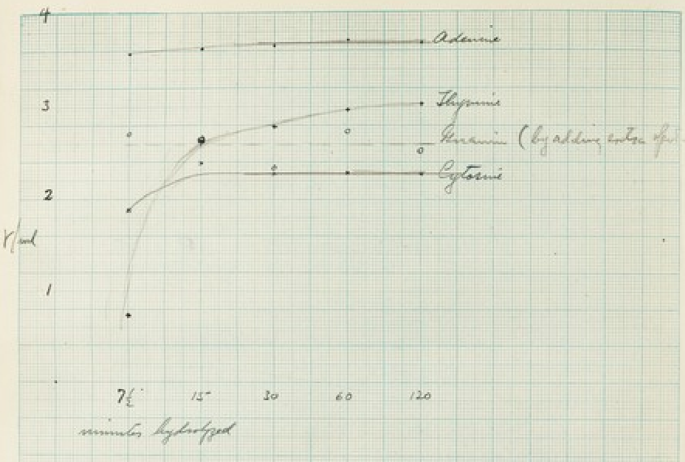
·09

·114

·040

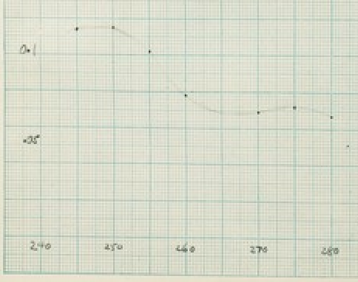
2 ml HCl ·466

·360



Mean maximum values

	γ/ml extract	γ/ml orig TNA
Guanine	2.59	111
Adenine	3.67	158
Cytosine	2.28	98.3
Thymine	3.03	130



30-ix Roy's TNA 1:10 1 ml samples dried down hydrolysed in HCOOH 165° (fairly accurate ± 2°). 1 hydrolysed in HCl. 0.15 ml, 17.4 μl spots. Chromatograms run 24 hrs

	Guanine + E		Adenine		Cytosine		Thymine	
1 7½ min	250 ·093 (+·115 = ·208) / ·079 (+·092 = ·171)	·190 270	260 ·347 / ·342	·344 3.57	275 ·178 / ·166	·172 1.89	265 ·045 / ·044	·045 0.74
2 15 min	·103 (+·067 = ·170) / ·109 (+·091 = ·200)	·185 264	·343 / ·356	·349 3.62	·214 / ·222	·218 2.39	·158 / ·159	·159 2.62
3 30 min	·088 (+·073 = ·161) / ·102 (+·064 = ·166)	·165 235	·348 / ·357	·352 3.65	·213 / ·200	·207 2.28	·168 / ·169	·169 2.78
4 60 min	·148 (+·057 = ·205) / ·083 (+·076 = ·159)	[illegible] 273	·358 / ·358	·358 3.71	·212 / ·205	·209 2.29	·183 / ·176	·180 2.97
5 120 min	·145 (+·024 = ·169) / ·162 (+·020 = ·182)	·176 257	·348 / ·361	·354 3.67	·198 / ·214	·206 2.27	·184 / ·183	·184 3.03
6 N.HCl 100° 30 min	·202 / ·196	·199	·336 / ·333	·335 3.47				

E		1E₁	1E₂	2E₁	2E₂	3E₁	3E₂	4E₁	4E₂	5E₁	5E₂
	245	·114									
	250	·115	·093	·067	·091	·073	·064	·077	·076	·024	·020
	258	·102									
	260	·078	·069	·051	·060	·052	·048	·053	·062	·012	·008
	270	·069									
	275	·072	·068	·051	·054	·049	·048	·052	·047	·010	·007
	280	·067									

To samples E in ¼ HCl add 1.0 ml 1 N. NaOH.

	1E₁	2E₂
245	0	
250	·019	0·0
260	·044	
270	·067	·043
275	·068	·048
280	·028	·059

— slight bypass of gramin.

Combine 10 E₁ tubes } dry down.
10 E₂ tubes }

29·ix. Crushed bd pa.

Unmeasured am't (200 mg?) bd pa (hone power) in saline in shaker tube ⅔ full of balantidi. Shake by machine 1½ hrs. Wash from beads, diluting to ca 25 ml.

Examine: all sized fragments; many elongate or crescent shaped. Practically no whole pa.

Mix 1 ml portions c Na_2CO_3 solns to give conc ·01, ·008, ·007, ·006, ·005, ·004 M.

Leave in frig overnight

30·ix. Darkground:

·01 M.	Appears clear.	Bugs c small particles.
·008	" "	" " c some larger fragments
·007	slightly turbid	" " c more " "
·006	" "	" " " "
·005	fruits	" " c particles of all sizes.
·004	milky	" " " some whole pa.

Virus cannot be freed from crushed pa. by weaker alkali than from whole ones.

Remainder of crushed pa. make ·008 M in Na_2CO_3, ca ·05 M NaCl. leave 4 hours, spin 6800 10 min. Suspend pellets in Na_2CO_3-NaCl c

635 spin same again. Combine supernates, spin ca 10,500 45 min.

Pellet brown, not white. Supernat still slightly opalescent.

Hydrolate: 1 ml HCl: .795, .815, .810 = .810

Blank .750 / .770 / .756 } .76

.20 ml virus 2 ml HCl .560
.2 (") " " .995 } .675
20 " " .695

$.76 - .67 = .09 \qquad .09 + .81 = \frac{.90}{.81} \times .0983 \times \frac{10}{2} \times \frac{100}{15.4} = 5.7$ ~~[illegible]~~ mg virus/ml.

10-X. Repeat

1 ml HCl = .835, .835 = .835

Blank .790 / .770 / .790 } .783

0.005 ml Geol. pe standard .16 / .165 / .145 } .157 $= \frac{.626}{.835} \times .0983 \times \frac{1}{.005} \times \frac{100}{15.4} = 96$ mg/ml polyhedra

85.75 μl LdV 5-X .295 / .310 } .302 $= \frac{.481}{.835} \times .0983 \times \frac{1}{.08575} \times \frac{100}{15.4} = 4.28$ mg/ml virus

17.4 μl PP from virus prep. .540 / .530 } .535 $= \frac{.248}{.835} \times .0983 \times \frac{1}{.0174} \times \frac{100}{15.4} = 10.9$ mg/ml p.p.

Total quantities:		mg.	%
Polyhedra	96 × 6.5 =	624	100
Virus	4.28 × 5	21.4	3.4

5-X-49 LdV prep.

~~[illegible]~~ 6.5 ml Geol. pe (≏ ~~[illegible]~~ 580 mg) in NaCl
10 ml $\frac{M}{10}$ Na_2CO_3
35 ml .9% NaCl
70 ml aq. dest.
121.5
} mix 9.45.

12.30. $2\frac{3}{4}$ hrs. Spin 6800 10 min., susp. pellet in Na_2CO_3-NaCl, re-spin. Combine supernats, spin 9600 60 min. Blue-white pellet, small

3.45 amt brown stuff on top (< 5% ?). Susp. in aq. dest, spin 9600 60 min. Only small brown layer. Susp in 5 ml aq. dest.

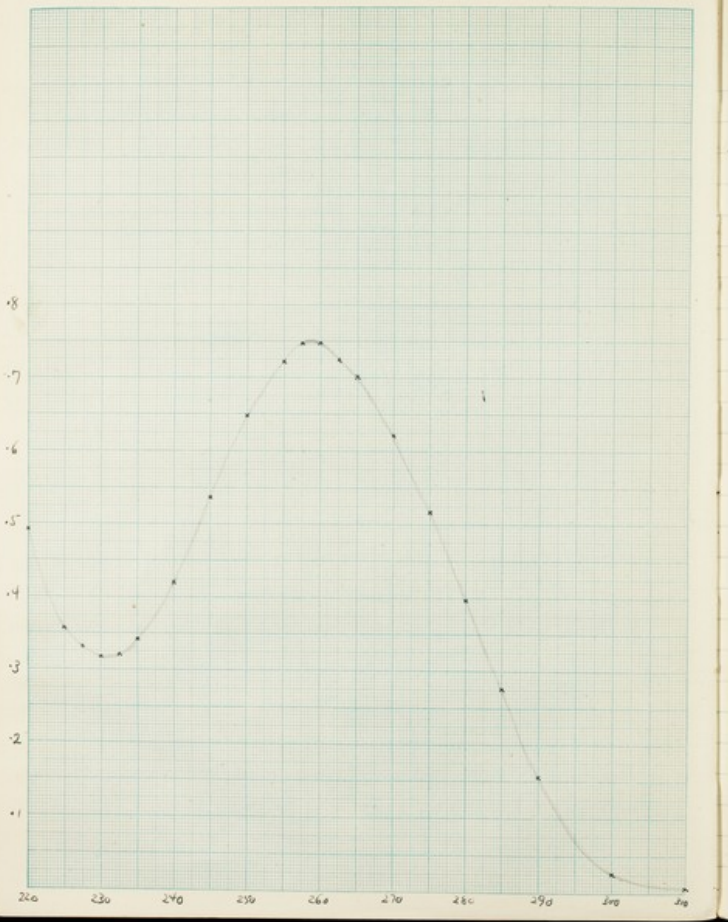
Polyhed. once pptd, susp in 30 ml M/50 Na_2CO_3, spin 2000 1 hour. Skim partially purified by spinning 6800 10 min.

11-X. ~~Hydr~~ Hydrolysis for carbohydrate. 1 ml (4.28 mg LdV) made

9.40–11.40 1 N. ĉ HCl, heat on BWB 2 hrs. (Cf. few mg sucrose in 1 ml N HCl)

5-X-49 TNA hydrolysate same as run on 30-ix. HCOOH 165° 120 min.
Run in ② on Dowex-8% formic. Guanine → double spot.
③ on Dowex-NH_3. Guanine tails to starting spot: cut out whole.

		Starting spot	Guanine	Adenine	Cytosine	Thymine
②	17.4 μM (1)	270 1003 260 822 270 ·853	250 ·387 225 ·208	26 ·427	225 ·267	265 ·236
	(2)	250 ·626 260 ·614 270 ·612	200 ·290 225 ·192	·414	·264	·239
③	17.4 μM		210 ·275	·371	·237	·205
	9.15 μM.		·169 ÷ ·110	·189 ÷ ·360	·103 ÷ ·210	·103 ÷ ·192



5-X-49 TXA standard curve.

Recrystd TXA 1:5000 (1:10 x 1:50) in very weak NH_3. pH ca. 8.7

220	·492
222.5	·356
227.5	·331
230	·317
232.5	·321
235	·342
240	·420
245	·537
250	648
255	·722
257.5	·748
260	·748
262.5	·725
265	·702
270	621
275	·517
280	·398
285	·276
290	·153
300	·026
310	·007

$\frac{D_{260}}{D_{250}} = 2.36$

3-x.49 Effect of things on bd gr.
1 drop (ca 4 mg) Gesh bd gr. ~~[illegible]~~ dil to 1.2 ml, & add trypsin, papain, Na thioglycolate (neutral) ca 5%, & tryps & pap. to gr. already overnight in thioglycolate.

5-x. Examine dark ground
Trypsin – look normal
Papain – polyhedra intact, but many of bd ragged at surface & granular within
Thioglycolate – look normal
Thioglycolate – papain. aggregated, otherwise normal.
" – trypsin. look normal.

14-x $N/10$ HCl, cold 10 min. Do lose refractility, are granular, denatured. but without brownian movement within
$N/10$ HCl 100° 10 min. Still intact, slightly granular within show slight movement within.

18-x Polyhedra dried at 110 for weighing – less refringent than normal, & do not dissolve in 0.01 M Na_2CO_3 ·05 M NaCl, nor in ·25 N. NaOH, tho swell a bit & → granular in latter (no movement).

10-X-49. Effect of Teepol on Ld V.

Add 0·2 ml (mg) Ld Vin aq. dest to 1 ml teepol dilutions in aq. dest 1:100, 200, 400, 800, 1600.

100, 200, 400 gelled, 400 watery less.

800 v. slightly gelled. partially clarified

1600 not at all "

add 0·5 ml sat. NaCl.

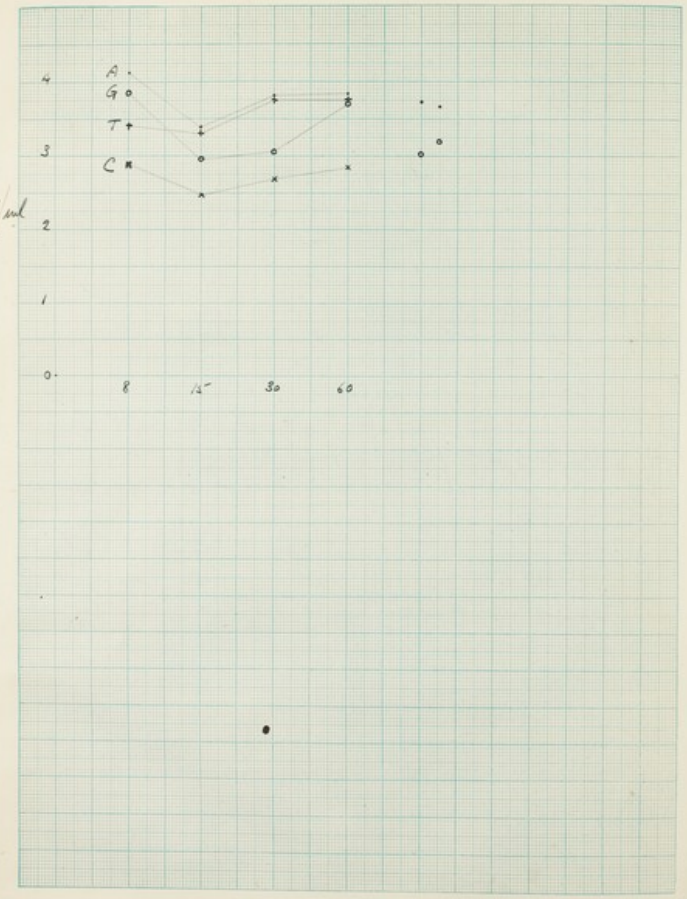
All gel more, incl. 1600, + become turbid. 1:100 much more turbid than others. pH ca. 7. Add ·5 ml pH 5 ·2 M citrate buffer → v. slight increase in turbidity.

To 1:1600 add 1 ml 1:100 Teepol. → as turbid as 1:100 tube.

Combine all 5 tubes, add 2 more ml 1:100 Teepol, leave overnight.

11-X. Spin down goopy pellet. Supernat still slightly turbid. Acidify → no more ppt — H.A. must have come down already.

Repeat same extract. Mix ca 0·8 ml Ld V, 5 ml 1:50 Teepol, 5 ml aq. NaCl, leave, then ppt. pH ca. 8 After few min. spin - good mix to top. Filter off. Acidify → only minute ppt (<100 γ ?) - obviously not all H.A. ppt'd off.

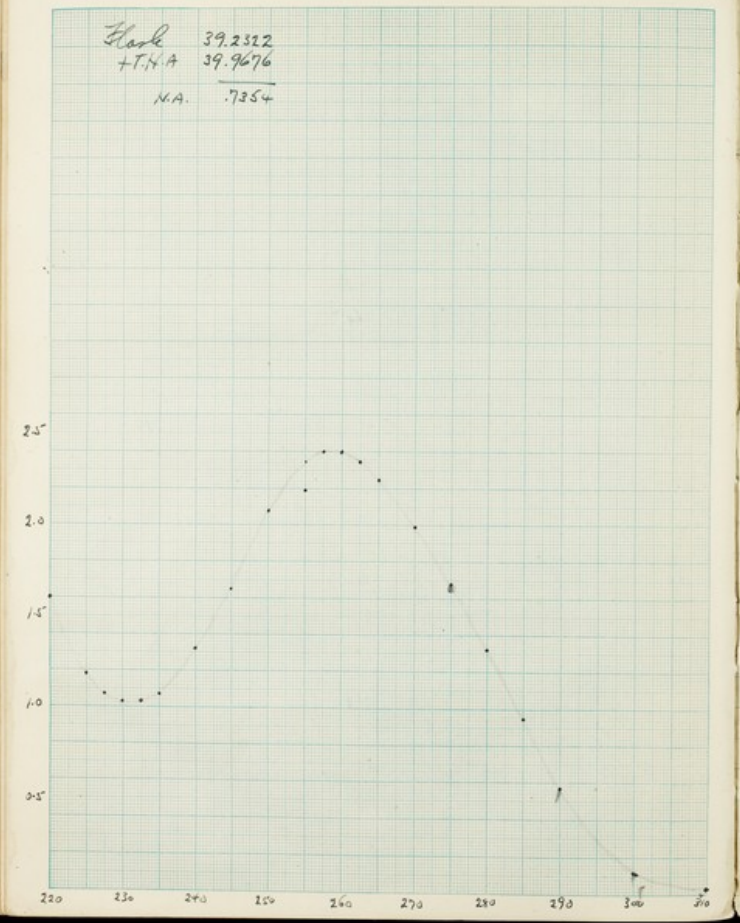


13 x 49. TNA hydrolysates run in 66% isopropanol - 2.0 N HCl
1 ml 1:10 TNA + 15 ml, HCOOH 175° 8–60 min
Blanks read against ½ N HCl.

	E_{S_3}	ET_3	E_{S_4}	ET_4
250	.043	.050	.033	.063
260	.037	.054	.037	.071
270	.016	.028	.014	.059

	Adenine 260		Guanine 250		Cytosine 275		Thymine 265	
① 8 min (1)	.390	.398	.272	.271	.250	.262	.207	.207
(2)	.390	4.12	.269	3.85	.273	2.88	.207	3.41
② 15 min (1)	.320	.326	.203	.208	.221	.224	.209	.200
(2)	.331	3.38	.213	2.96	.227	2.47	.190	3.30
③ 30 min (1)	.363	.368	.233	.231	.258	.245	.226	.228
(2)	.373	3.81	.228	3.05	.232	2.69	.229	3.75
④ 60 min (1)	.378	.370	.266	.261	.259	.259	.221	.227
(2)	.362	3.83	.256	3.71	.259	2.85	.233	3.74
⑤ N. HCl 30 min (1)	.359	.359	.205	.218				
(2)	.358	3.73	.231	3.02				
NHCl 30 min (1)	.347	.353	.208	.224				
run in 1 hour (2)	.358	3.66	.240	3.19				
10% HCOOH								
8-x								

Flask 39.2322
+T.N.A 39.9676
N.A. .7354



13-x-49. Purification of TNA.

E.D.H. TNA (P = 6.65%), not specially dried, weighed into flask, added ca. 20 ml 1 N. NaOH, leave @ 37° overnight.

14-x. Filter off undissolved residue, wash then ÷ 20 ml aq. dest. Acidify ÷ HCl, spin down big very goopy ppt, dissolve in NH_3, spin off small insol material. Shake ÷ chloroform-octyl 8:1. 4 times – still some gel. Ppt ÷ HCl, spin. Dissolve in NH_3, dialyze overnight against running water, then 48 hrs against demineralized water.

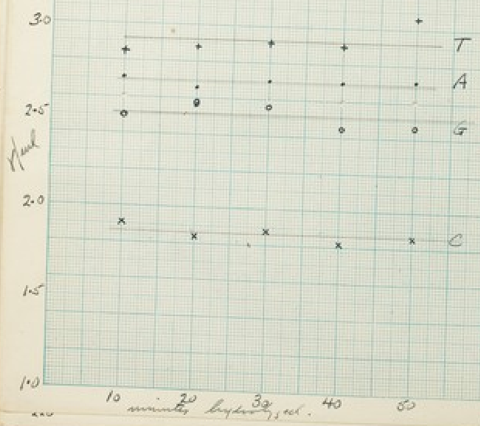
17-x. Spin 10,000 (0 min. Put in 2 bottles (total vol. ca. 35 ml) pH 5.0. Reserved ē ca .5 ml TNA negative. Dilute 1:100 in aq. dest for absorption curve. pH 7.0

220	1.575	255	2.19	290	.57
~~25~~		257½	2.40		
25	1.18	260	2.30	300	.116
270	1.07	262½	2.35		
80	1.03	65	2.25	310	.032
325	1.03				
35	1.07	70	1.99		
40	1.32	75	1.68		
~~45~~	1.65	80	1.32		
50	2.08	85	0.95		

$\frac{D_{260}}{D_{220}} = 2.27$

N.A. per ml org sol n = $\frac{2.40}{.748} \times \frac{9}{5} \times 1.275 = 8.2$ mg/ml.
Dry Wt: 10.8 mg/ml.

		D	Eluate µl/ml	mean	Orig soln µl/ml	µmoles/ml	Molar ratios
Adenine	10 mins	·261	2·71				
	20	·256	2·66				
	30	·260	2·70	2·696	134	0·994	1·04
	40	·260	2·70				
	50	·261	2·71				
Guanine	10	·176	2·50				
	20	·181	2·58				
	30	·180	2·56	2·610	125	0·828	0·87
	40	·172	2·45				
	50	·173	2·46				
Cytosine	10	·174	1·91				
	20	·167	1·84				
	30	·171	1·88	1·862	93	0·838	0·88
	40	·165	1·82				
	50	·169	1·86				
Thymine	10	·173	2·85				
	20	·175	2·88				
	30	·177	2·81	2·920	145·5	1·153	1·21
	40	·176	2·90				
	50	·186	3·06				
						3·813	4·00



Repts

16-X-49 TNA hydrolyses in HCOOH 175° 0·5 ml 1:10 TNA + 86·75 ul, 17·4 µl spots, run 22 hrs in 66% isopropanol 2N HCl.

Blanks against N/10 HCl

		G	A	C	T
		250	260	275	265
(1)		·058 ~~·023~~	·027	·018 ~~·026~~	·037
(2)		·021 ~~·054~~	·020 ~~·040~~	·023 ~~·037~~	·045 ~~·050~~

All read against blanks No 1.

Cells cleaned

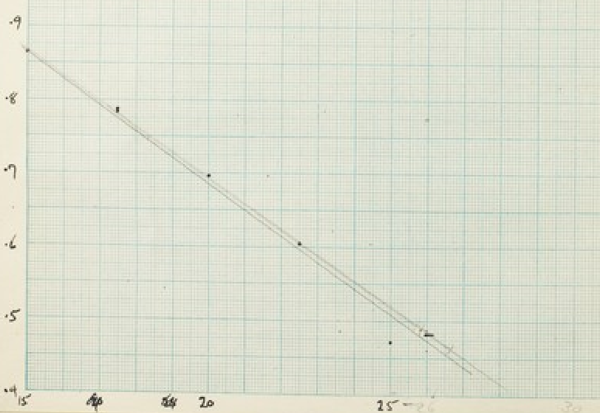
		Adenine 260	x-x̄	(x-x̄)²	Guanine 250		Cytosine 275	Thymine 265
10 min	1	·256	4	16	·191 ~~·185~~	·167	·173	·177
	2	·208	2	4	·212 ~~·209~~	·175	·174	·162
	3	·268	8	64	·183 ~~·185~~	·188	·175	·179
		·261				·176	·174	·173
20 min	1	·245	15	225	·199	·178	·166	·190
	2	·263	3	9	·210	·176	·168	·178
	3	·260	0	0	·183	·190	·167	·178
		·256				·181	·167	·175
30 min	1	·254	6	36	·195	·176	·169	·178
	2	·260	0	0	·208	·173	·172	·178
	3	·267	7	49	·180	·190	·173	·179
		·260				·180	·171	·177
40 min	1	·248	12	144		·168	·158	·166 10 100
	2	·261	1	1		·168	·172	·185 9 81
	3	·271	11	121		·181	·164	·178 2 4
		·260				·172	·165	·176
50 min	1	·248	12	144		·164	·169	·182 14 16
	2	·264	4	16		·171	·169	·189 3 9
	3	·272	12	144		·183	·168	·188 2 4
		·261	Σ	973		·173	·169	·186

S = ·0048
SM = ·0022

Bottle MT 9.6002
+2 ml TNA B dried 110° 9.6218
4 hrs. .0216 g TNA = 10.8 mg/ml.

Kjeldahls:
1 ml HCl : .842, .842, .844 = .843

Blank incl H_2SO_4	.766	.773	
"	.788		
"	.765		
.05 ml TNA B	.069	.064	$= \frac{.709}{.843} \times .0983 \times \frac{1}{.05} = 1.65$ mg/ml
"	.057		
"	.065		
" boiled Aug	.078	.082	$= \frac{.691}{.843} \times .0983 \times \frac{1}{.05} = 1.61$ mg/ml
"	.086		
"	.079		



18.x.49 Analysis of TNA "B"

Aliquots from stock sol'n for dry wt., from 1:10 dilution for N, P.

P. OR2 filter
Subtract from 1.5, against aq. dist.

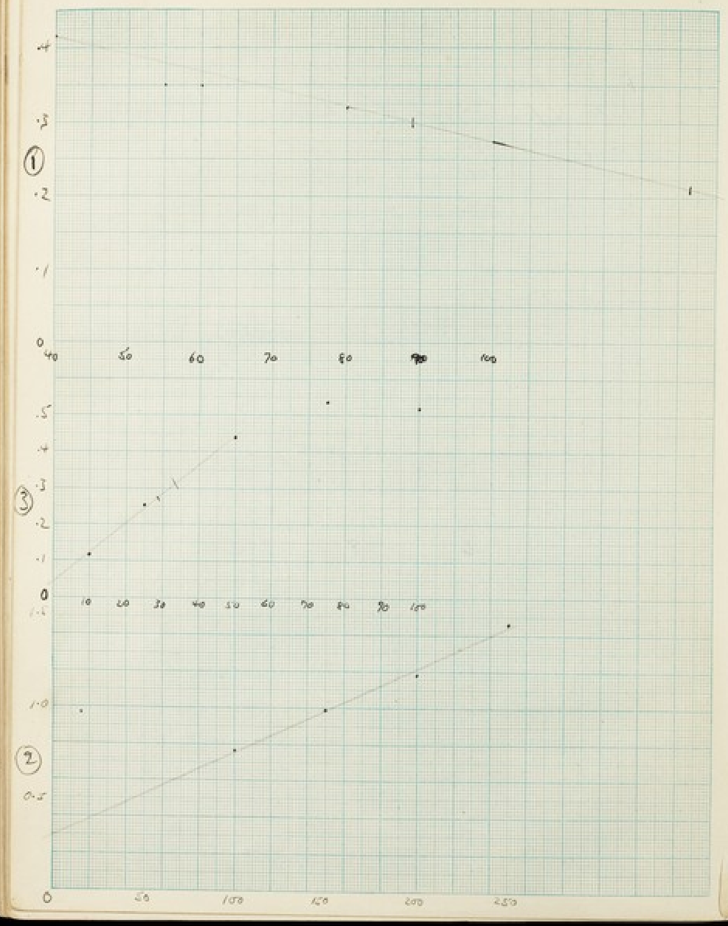
15 γ	.865
17.5	.783
20	.696
22.5	.605
25	.471
P_1 .025 ml NA "B"	.463
P_2 "	.494
P_3 "	.490

.482 26.0 γ = 1.04 mg/ml = 9.6%

Analysis

	mg/ml	% of dry wt.	μmols/ml	μmols/ml calc for bases	
TNA	10.8	100			
N	1.61	14.9	115	112	
P	1.06	9.8	34.2	30.5	12%
A	1.07		8.0		
G	0.98		6.5		
C	0.73		6.55		
T	1.19		9.45		
Total purines			14.5		
Total pyrimidines			16.0		

TNA calc. from bases as straight polynucleotide = 9.41 mg/ml.
= 87% of dry wt.



19-x **TNA orcinol.**

OR·2 filter. water on 0·5

40 γ xylose	·415	
60	·350	
80	·320	
① 100	·275	
0·1 ml TNA "B"	·300	≡ 89 γ
0·1 ml Roy's TNA (= 1·0 ml TNA A)	·210	≡ 131 γ

Sample all made to 1·0 ml, then add 2·0 ml reagent, BWB 8 min, cool, add 8 ml n BuOH.

old xylose

% purine-bound pentose in TNA = $\frac{·089}{10·8}$ × 100 = 0·825 %

≏ 5·0 % RNA !

Repeat c̄ fresh standard 1mg/ml. Read forward against reagent blank.

100	·22
150	·995
② 200	1·18
250	1·46
·2 ml TNA "B"	·324 } ·322
"	·320
·1 ml TNA Roy's	·285

20-x. Repeat. Read forward against aq. dist. Same standard soln. Stood 3 hrs before reading.

10 γ xylose	·115
25	·255
③ 50	·439
75	·552
100	(·619)
·2 ml TNA "B"	·312
"	·309

} ·311 ≡ 33 γ pentose = 165 γ/ml

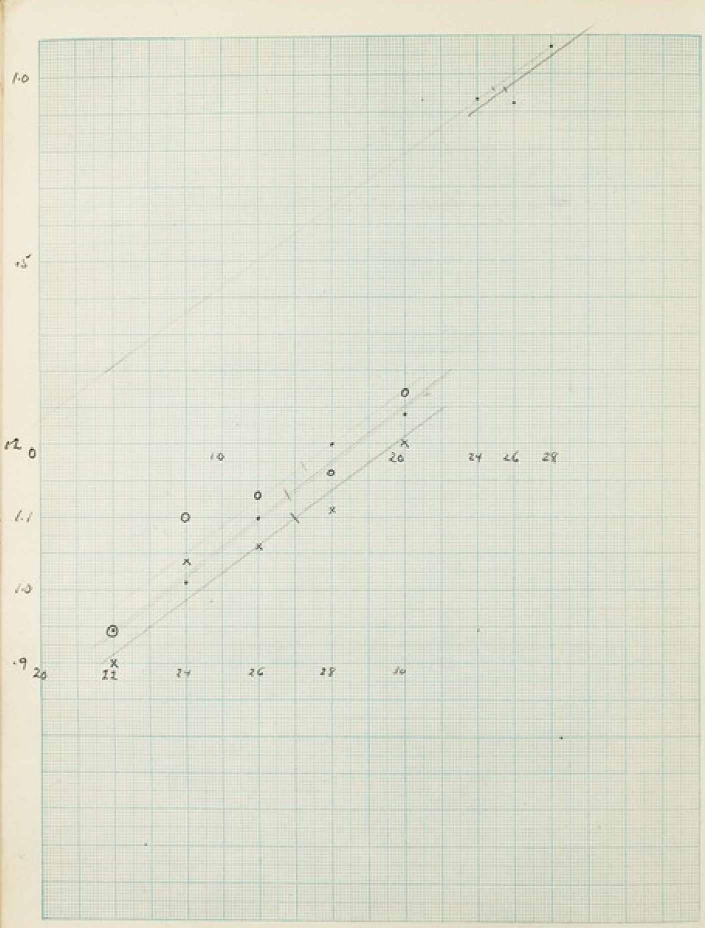
→ ·286 × $\frac{·311}{·322}$ = ·276 = 28 γ = 280 γ/ml

Pentose in 1 ml of pure RNA = ·196 x 150 = 2930. D/D of 100 % RNA = 9·6 %
mM ATP

20-x. TNA 8 bases. 3 bomb, each 1.0 ml TNA 8 dried down up in .5 ml HCOOH, cook 30 min. 175° (165–178). dry down. Much coke. Dissolve in 0.3 ml M/10 HCl. 17.4 µl spots, 3 of each bomb. (#3 ~~up~~ came unstoppered overnight). Run in 65% EtOH 2.5 N. HCl.

		Adenine 260	Guanine 250	Cytosine 275	Thymine 265
(1)	1.	1.12	.358	.753	.766
	2	1.10	.310	.762	.739
		1.11	.334	.758	.753
(2)	1	240 .440 250 .862 260 1.13 270 .975 280 .422	245 .304 250 .327 255 .296 265 .225 275 .217 285 .166	255 .320 265 .587 275 .738 285 .586 295 .160	245 .352 255 .619 265 .757 275 .577 285 .221
	2	1.17	.404	.759	.790
	3	1.07	.391	.665	.778
		1.19	.374	.721	.775
(3)	1	1.13	.342	.781	.786
	2	1.12	.340	.766	.769
	3	1.15	.329	.768	.804
		1.13	.337	.772	.786
Mean of 3 means:		1.14	.348	.750	.771
$\frac{D\ 20\text{-}x}{D_{mean}\ 16\text{-}x}$		4.38	1.97	4.44	4.32

(1)-3 sprayed with ninhydrin – no color at all – hydrolysate of 0.6 mg N.A.



Repeat P on TNA B

In 50 ml, against agdest at 0 OR-2 filter.

24 γ	.955	
26	.925	
28	1.07	
.025 ml TNA	.940	
"	.970	.963 = ~~24.9~~ 25.6 γ = ~~.996~~ 1.02 mg/ml
"	.980	= 9.5 %

22-x. <u>Third Pasteurisation</u> - read overnight.

22	22 γ	.945
24		1.01
26		1.10
28		1.20
30		1.24
.025 ml TNA		1.15
"		1.09 — 1.13 = 26.6 γ = 1.06 mg/ml = 9.8 %
"		1.14

24-x. 4th Read after 2 hrs Overnight

22	.900	.945
24	1.04	1.10
		1.13
26	1.06	1.16
28	1.11	
30	1.20	1.27
.025 ml TNA	1.14	1.20
"	1.10 — 1.10 = 27.0 = 1.08 mg/ml = 10.0 %	1.14 — 1.17 = 27.2
"	1.06	1.18

$\bar{D}$		Eluate γ/ml	Orig. sol'n γ/ml	μmoles/ml	Molar ratios	
A	.720	7.47	1073	7.96	1.04	±.005 S.E.M.
G	.480	6.83	981	6.50	.85	±.007
C	.460	5.06	727	6.55	.86	±.006
T	.503	8.28	1189	9.45	1.24	±.011
			3970	30.46	3.99	
Wt of sugar + P_4 (1780 μmoles)			5440			
Total N.A. per ml			9410			

20-x. TNA B. bases. To hydrolyzate #3 added 31.0 μl conc HCl to dissolve guanine 17.4 μl spots. Run in EtOH-HCl. Guanine spots much bigger. Not saturated.

21-x New hydrolyses: ~~1.0~~ 0.5 ml TNA B in ~~1.0~~ 0.5 ml HCOOH 175° 30'. ~~1.0~~ 0.25 ml. 1 N. HCl. [illegible]

		A_{260}	G_{250}	C_{275}	T_{265}
Blanks read against eyepiece	4	.028	.024 (260 .022)	.025 (260 .029)	.071 (260 .074)
	5	.024	.011 (260 .018)	.030 (260 .034)	.080 (260 .092)
	6	.022	.018 (260 .025)	.028 (260 .030)	.073 (260 .073)
(4)	1	.723 +3 9	.600 +20 400	.497 +17 289	.488 -15 225
	2	.718 -2 4	.476 -4 16	.453 -7 49	.496 -17 289
	3	.734 +14 196	.488 +8 64	.465 +5 25	.495 -18 324
		.725	.488	.465	.496
(5)	1	.710 -10 100	.485 +5 25	.458 -2 4	.518 +15 225
	2	.727 +7 49	.483 +3 9	.459 -1 1	.513 +10 100
	3	.720 0 0	.459 -21 441	.445 -15 225	.506 +3 9
		.719	.476	.454	.512
(6)	1	.711 -9 81	.479 -1 1	.453 -7 49	.504 -1 1
	2	.708 -12 144	.468 -12 144	.460 0 0	.508 +5 25
	3	.733 +13 169	.483 +3 9	.468 +8 64	.515 +5 225
		.717	.477	.460	.510
Mean of 3 means		.720 ±.0032	.480 ±.0039	.460 ±.0031	.503 ±.0045
$\Sigma(x-\bar{x})^2$		752	1109	706	1423
V		940	138.6	88.3	178
σ		9.70	11.77	9.40	13.3
S.E.M.		3.2	3.9	3.1	4.45

24-X.

Repeat dry wt on TNA B.

Wt bottle dried.	19.7297		·0627		
5.0 ml TNA B frozen, dried,	19.7924	N.A. =	~~[illegible]~~	= ·0125	g/ml.
then dried at 115°	19.7857		·0560	= ·0112	
Re-dried	19.7855		·0558	= ·01116	

Dry wt. = 11.16 mg/ml

25-X-49. TNA "B" proportions

·	µg/mg dry wt. Found	Calc. for bases	µg mols/mg dry wt. Found	Calc. for bases	Molar ratios
Adenine	96		·71		1.04 ± ·005
Guanine	88		·58		·85 ± ·007
Cytosine	65		·59		·86 ± ·006
Thymine	116		·85		1.24 ± ·011
P	95	85	3.07	2.73	
N	144	141	10.3	10.0	
N.A.		844			

Desoxypentose calc. from bases = 352 µg/mg.
Purine-bound ditto = 168 "
Pentose found by orcinol = 14.8 " = 8.8 % of calc. purine-bound sugar.
= 7.9% of pentose of all R.N.A.

26-x. TNA "B" parcel. 3 chromatograms 17.4 μl in hydrolysate run in Butanol/ammonia.
Cut out U & T only.

	Uracil $_{260}$	Thymine $_{265}$
1	·044	·666
2	·052	·645
3	·067	·668
$\bar{x}$	·054	

Berenblum, S. & E. Chain
1938. An improved method for the colorimetric determination of phosphate.
B. J. 32: 295.

Chromatogram sugar reagent

(Partridge, 1949, Nature 164: 443).

Aniline 0.93 g. } in 100 ml H_2O sat. butanol
Phthalic acid 1.66 g. }
Spray on, then heat 5 min 105°.

Aldo-pentoses – bright red
Aldo-hexoses, deoxy-sugars, uronic acids – various shades green & brown.
Ketoses – dependent solvent. Fructose → little or no color
+ phenol – Partridge system

Detects 1 γ pentose + aldo-hexoses.

Benzidine for reducing sugars

Horrocks, 1949, Nature 164: 444.

Benzidine AR 0.5 g. }
Glacial HAc 20 ml } Spray. Heat 105° 15 min, observing
Abs EtOH 80 ml } at intervals.

Pentoses → chocolate brown within 5 min.
Hexoses → dark brown spot 10 min.
Ascorbic acid – faint brown after 15 min

Detects 5 μg of all reducing sugars.

Bial Reaction for Pentoses (Mejbaum's modification)

Mix freshly: 1% orcinol 10 ml
Conc HCl 40 ml
10% $FeCl_3$ 1 ml

0.2 ml (~50γ pentose) unknown + 2 ml reagent in test tube. B.W.B. 8', cool, add butanol to vol 10 ml. Absorption peak 680 mμ.

Molisch Reaction for Carbohydrates

Few KClO3 & naphthol dissolve in 70% EtOH.
Add 1 drop to unknown + min. Pour conc H_2SO_4 to layer beneath, + shake gently. Cherry-colored ring.

Strychnine phospho-molybdate method for P.

Incubate 2 hrs ± 0.5 ml 10N. H_2SO_4 + 1 drop HNO_3, then dilute up to 4 ml.

Add 1.5 ml strychnine molybdate soln. (1 vol S.M. soln to 3 vols. unknown, which should not contain mineral acid > 0.5 N, nor alk > 2 N.) Max ppt'd by 1 ml S.M. is 0.2 mg)

Nitro molybdate. 50 g. NH_4 molybdate dissolved & made up to 150 ml. Add to 450 ml HNO_3 (2 vols conc. to 1 vol water).

Strychnine nitrate. 1.5 % in water.

S.M. reagent. 1 vol S. nitrate stirred into 3 vols nitromolybdate, prepared fresh.

1 mg P ≡ 89.3 mg ppt.

Tisdall colorimetric method

Spin out ppt & wash twice. Add 0.5 ml 1% NaOH & make up to 2.5 ± water. Transfer to 25 ml flask, washing in ± twice 2.5 ml.

Add 5 ml fresh 20 % $K_4Fe(CN)_6$ + 2.5 ml conc HCl. Make up to 25 ml ± water.

$Na_2CO_3 = 46+12+48 = 106$

106 g/l = 2 N. = 1 M

∴ g/l = N/10. = M/20

Swaag et al.

1938 The isolation of the components of streptococcal nucleoproteins in serologically active form.

J. B. C. 124. 725.

Nucleoprotein components are freed by sonic vibrations, then melted and split from protein by 1–2 hrs in M/20 Na_2CO_3 50–55° C.

To separate protein from nucleic acid. Add 0·25 vol. chloroform + 0·1 vol. amyl alcohol (foam preventor). shake 15–60 min., centrifuge, decant aqueous sol'n of nucleic acid from chloroform-protein gel.

Chloroform shaking detects ovalbumin 1 in 25,000 by thin-like gelad interface — can use as test of contaminating protein in N.A. preps.

Liberate prot. from gel + alcohol. So isolated, it is serologically active.

Dische Diphenylamine Test

Sevag M.G., J. Smolens & D.B. Lackman
1940. J.B.C. 134 523.

1 g. diphenylamine dissolved in:
2 ml H_2SO_4, then add:
98 ml glac. HAc.

Hilger cells hold 8 ml.

6 ~~8~~ ml added to 3 ~~4~~ ml filtered extract (4 ml N/10 HCl ~~for~~ 25-35 mg bugs hydrolyzed 15 min on B.W.B.)
Put abs. 12 ml on B.W.B. 3 min & cool. Read after 5 min.

If no prelim. hydrolysis, cook 15 min, read after 30.

Hydrolyzed mixture was centrifuged and the clear supernatant used for the analysis. Use of more conc. acid or longer time gave same results.

Bezold & Pister. 1948. Z. f. Naturforsch. 3b. 406-410.
Max. color is developed 25-30' after addition of the reagents. Procedure: to (A) .5 ml unknown containing at least 20 γ DNA add 1 ml Dische reagent & to (B) another same add 1 ml blank reagent of HAc + H_2SO_4 without diphenylamine. Into B.W.B. 10 min. Then cool in running water 10 min. then to A add 4.5 ml blank reagent, & to B add 3.5 ml blank + 1 ml Dische. Read c̄ filt. OG-2 – VG5 at intervals until color begins to drop (ca. 40 min.) & use highest value.

Hauriatur

14-ix-49.

Bomb tube 9.2531

" + cytidine 9.3026

∴ cytidine .0495

Theor. cytosine = 49.5 × 111/227 = 24.2

Actual recovery = 23.6 = 23.6/24.2 × 100 = 97.5%

111
116
227

Kjeldahl micro-analytic mixture

K_2SO_4 80 g

$CuSO_4 \cdot 5H_2O$ 40 g

SeO_2 0.5 g.

1·781 8·54 8·301

25·52 9·641 5·301

Hunterian

14-ix-49.

Bomb tube 9.2531

" + cytidine 9.3026

∴ cytidine .0495

Theor. cytosine = 49.5 × 111/227 = 24.2

111
116
227

Actual recovery = 23.6 = 23.6/24.2 × 100 = 97.5%

6-Bu-1
14-xii-48.

Silkworm hepes, broken up in 2 M H2SO4 + dialysed back to neutrality.

Approx 0.23 mg/ml.

2.0
1.5
1.0
0.5

230 240 250 260 270 280 290 300

Hunterian

14-ix-49.

Bomb tube 9.2531

" + cytidine 9.3026

∴ cytidine .0495

Theor. cytosine = 49.5 × 111/227 = 24.2

Actual recovery = 23.6 = 23.6/24.2 × 100 = 97.5%

111
116
227

6 + Expt 1
3-xii-48.

Silkworm urine (3-8m-8)
0.35 mg/ml in 0.2 M. NaOH.

6
2·5
4
3
2
1
2·0
9
8
7
6
1·5
4
3
2
1
1·0
9
8
7
6
0·5
4

230 240 250 260 270 280 290 300

Hunterian

14-ix-49.

Bomb tube 9.2531

" + cytidine 9.3026

∴ cytidine .0495

Theor. cytosine = 49.5 × 111/227 = 24.2

Actual recovery = 23.6 = 23.6/24.2 × 100 = 97.5%

111
116
227

Silkworm worms in 0.2 N. HCl
Appx. 0.2 mg/ml.

6-Dec-1
13-xii-48.

20
15
10
05

+ HCl.

After dialyzing back to neutrality

220 240 250 260 270 280 290 300