Notebook 3

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NUCLEIC ACIDS

Oct. '49 - Feb. '50

3

PP/GRW/A/2

231
181
50

G. R. Wyatt
Molteno Institute
Cambridge. phone 4577.

Deoxyribonuclease prep.

26-x. Ca. 4 lb beef pancreas ground, resp. in 2½ l. cold 0.25 N. H_2SO_4, leave overnight in frig. pH ca. 2.

8-xi. Ppt. been standing ca. 1 week at pH 3, but not Alk'd. Bring to pH 4.5 by portions Na_2HPO_4 + add 1 vol. chloroform.

Specimen tube 7.606

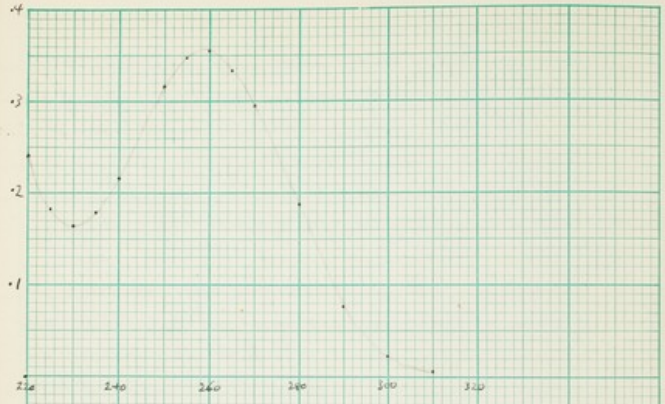
" + semi-dry N.A. 7.901

N.A. = .295 g.

27-X. Beef spleen N.A. prep.

2 beef spleens minced, susp. in 2 litres 0.9 % NaCl, steam (difficult, goody, would not squeeze by), wash 3 times E 0.9 % NaCl, susp. in 2 litres 10 % NaCl, leave at 4° overnight, spin, decant, suck thro gauze, pour into equal vol 90 % EtOH (pH of stuff 6.5), spool off on stirring rod (more non-fibrous ppt. later just taken), resuspend in Waring blendor in ca. 200 ml 10 % NaCl, ~~[illegible]~~ deproteinize 7 times = chloroform-octyl, emulsifying in Waring blendor, pour into equal vol 90 % EtOH, spool on rod, susp. in 100 ml 10 % NaCl, spin 10 min 10,000 (small brown pellet), re-ppt. in EtOH, wash stuff on rod in 50 % EtOH, 90, 100, ether, evt off rod.

Very stringy.



Bon NA

	D		Subtract RNA contaminants	Molecular ratios Total = 4
Adenine	.618	476	.399	1.11
Guanine	.414	391	.314	.87
Cytosine	.376	372	.295	.82
Thymine	.331	432	.432	1.20
Uracil	.101	77		
			1440	4.00

1-xi-48 Ovaert N.A.

Bon: 13 virgin mates (kept 24° ca. 10 days) + 30 effete moths, ground, emuls in 10% HClO4 ca. 80 ml,

Bonart: 4 adults 2 pupation (kept at 4° ca. 14 days) ground in 10% HClO4 ca. 25 ml. Discard on spilling fresh material

Stand overnight in frig. pH ca. 6

2-xi Spin down large ppt. Pour each into equal vol 90% EtOH, leave in frig.

9-xi Bon. Spin down large flocculent ppt. Suspend, shake once [illegible] Little than paper. [illegible] slight turbidity. Spin 11,000 [illegible]

87

Buckland, D.C., Foster, R.E. and Nordin, V.J.
1949. Studies in forest pathology. VII. Decay in western hemlock and fir in the Franklin river area, British Columbia.

Can. J. of Res. 27: 312-331.

$D_{260}/D_{230} = 2.16$

10-xi [illegible] 1.0 cc 4% NH_3, read

1:100 dilution

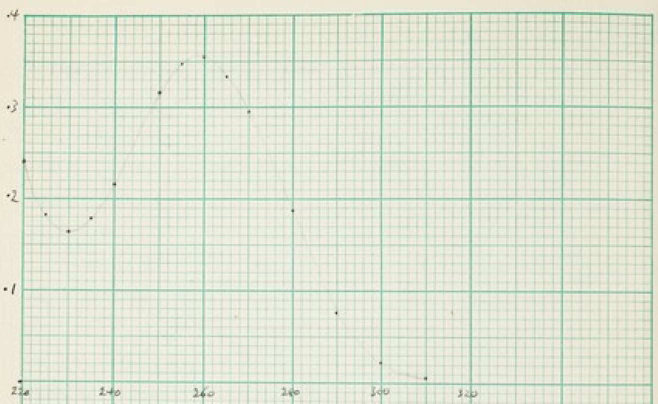
260 .63 $D_{260}/D_{230} = 2.14$

230 .295

Total N.A. $= \frac{.63}{1.25} \times .04 \times 100 = 2$ mg.

Oxydown. Add ½ ml HClO4, cook 17½° 30 min, oxydown ↑ 0.15 ml.

	Guanine	Adenine	Cytosine	Uracil	Thymine
1	.396	.610	.364	.083	.323
2	.415	.618	.382	.105	.334
3	.432	.625	.382	.114	.385
x̄	.414	.618	.376	.101	.331



Bm NA

λ	~~Yield/ml~~ D	Yield/ml	Subtract RNA contaminants	Molar ratios total = 4
Adenine	.618	476	.399	1.11
Guanine	.414	391	.314	.87
Cytosine	.376	372	.295	.82
Thymine	.331	432	.432	1.20
Uracil	.101	77		
			1.440	4.00

1-xi-49 Insect N.A.

Bm: 13 virgin moths (kept at 4° ca. 10 days) + 30 effete moths, ground, susp in 10% NaCl ca. 80 ml,

Locust: 4 adult L. migratoria (kept at 4° ca. 14 days) ground in 10% NaCl. ca. 25 ml. ~~Often~~ Discard on getting fresh material

Stand overnight in frig. pH ca. 6

Numbers [illegible]

85
86
87

... equal vol 90% EtOH, leave in frig.

... large flocculent ppt. Suspend, shake once ...

... spin → only minute gel. Filter thru paper.

... acidified EtOH → only slight turbidity. Spin 11,000

...l ppt, dissolve in 1% NH_4OH

240	280 .188		$\frac{D_{260}}{D_{280}} = 2.16$
.264	290 .077		
.272	300 .021		
317	310 .008		
355			
295			

...ks/ml ... Dissolve in 1.0 ml dil. NH_3, read

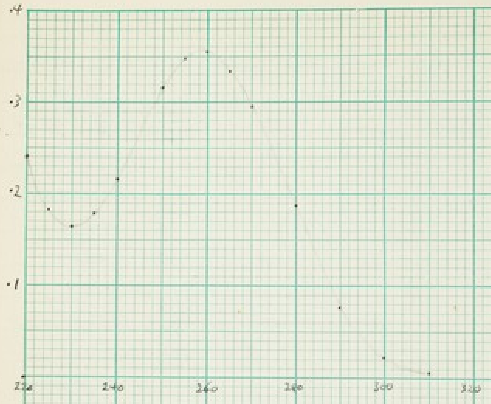
... = [illegible] mg. = 25 γ [illegible]

...3 $\frac{D_{260}}{D_{230}} = 2.14$ 40 μl [illegible] 2 [illegible]

...75 Total N.A. = $\frac{.63}{1.25} \times .04 \times 100$ = 2 mg.

Dry down. Add 5 ml HCOOH, cook 175° 30 min, dry down, ↑ 0.15 ml.

	Guanine	Adenine	Cytosine	Uracil	Thymine
1	.396	.610	.364	.083	.323
2	.415	.618	.382	.105	.334
3	.432	.625	.382	.114	.335
$\bar{x}$	.414	.618	.376	.101	.331



Bm NA

λ	~~Yield/µmol~~ D	Yield/µmol	Subtract RNA contaminants	Molar ratios total = 4
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Cytosine	.376	372	.295	.82
Thymine	.331	432	.432	1.20
Uracil	.101	77		
			1.440	4.00

1-xi-49. Insect N.A.

Bm: 13 virgin moths (kept at 4° ca. 10 days) + 30 effete moths, ground, susp. in 10% NaCl ca. 80 ml,

Locust: 4 adult L. migratoria (kept at 4° ca. 14 days) ground in 10% NaCl. ca. 25 ml. ~~Ofte~~ Discard on getting fresh material

Stand overnight in frig. pH ca. 6

2-xi. Spin down large stuff
Pour each into equal vol 90% EtOH, leave in frig.

9-xi Bm. spin down large flocculant ppt. Suspend, shake once c chloroform-octanol, spin → only minute gel. Filter thru paper. Pour into 1 vol acidified EtOH → only slight turbidity. Spin 11,000 30 min. Small ppt, dissolve in 1% NH₄OH

Dil. 1:10

220	.240	280	.188	$\frac{D_{260}}{D_{230}} = 2.16$
225	.264	290	.077	
235	.272	300	.021	
240	.276	310	.008	
250	.317			
255	.340			
260	.355			
265 / 270	.331 / .295			

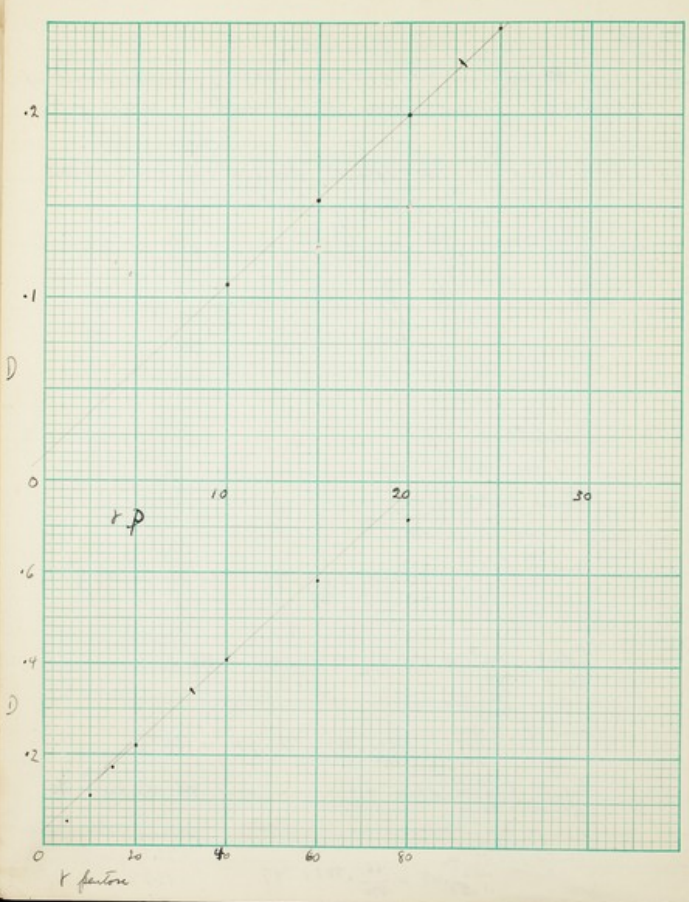
16-xi Dried down: 1 book/mouth. Dissolve in 1.0 ml dil. NH_3, read 1:100 dilution = 1 µmol. = 25 µ of each

Read 260 .63 $\frac{D_{260}}{D_{230}} = 2.14$ 40 µl ... 2 ... µl ...

230 .295 Total N.A. = $\frac{.63}{1.25} \times .04 \times 100 = 2$ mg.

Dry down. Add 5 ml HCOOH, cook 175° 30 min, dry down, ↑ 0.15 ml.

	Guanine	Adenine	Cytosine	Uracil	Thymine
1	.396	.610	.364	.083	.323
2	.415	.618	.382	.105	.334
3	.432	.625	.382	.114	.335
$\bar{x}$	.414	.618	.376	.101	.331



H-xi. Repeat P. Allen's method.
Make up to 25 ml, read in 2 ml cell, against water.

10 γ	.107
15	.153
20	.200
25	.247
.5 ml SNA 1:10	.226
"	.230
"	.227

.228 ≡ 23.0 γ = 46 γ/ml = 8.6 %

5-xi. Spleen N.A. on ml Ilford 608.

5 γ xylol	.054
10 γ	.108
15 γ	.173
20 γ	.220
0.2 ml SNA	.339
"	.339
46 γ	.406
60 γ	.582
80 γ	.718

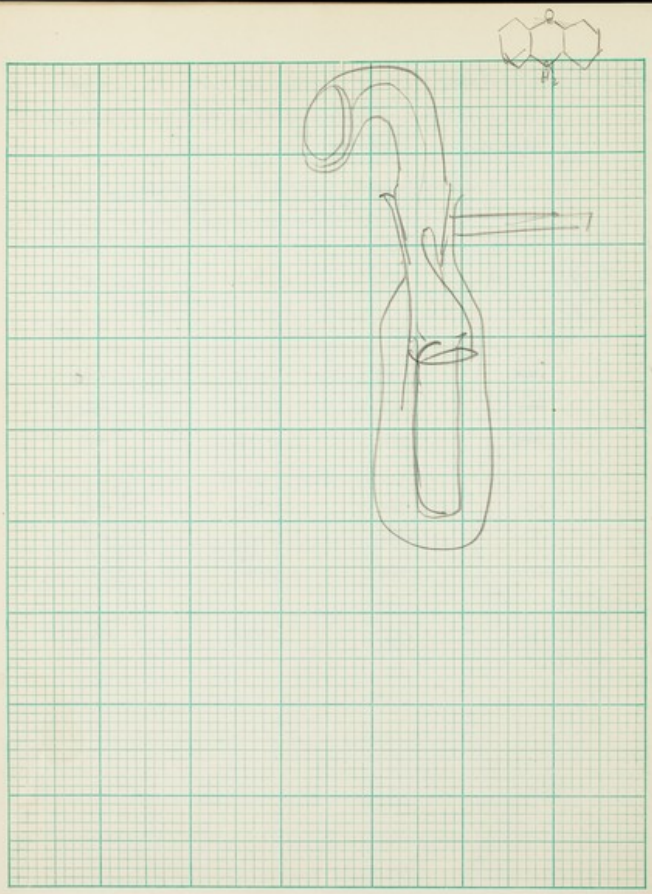
.339 ≡ 32.5 γ = 162 γ/ml

	D	mmoles/ml (?)	mmoles/ml orig. SNA (?)	Molar ratios	μmoles N /ml (?)
A	.690	.0530 ~~[illegible]~~	3.82 ~~[illegible]~~	1.05	19.10
G	.468	.0441	3.17	0.875	15.85
C	.448	.0443	3.19	0.88	9.57
T	.4605	.0601	4.32	1.19	8.65
			14.50	3.99	53.17

3.17	.875
3.19	.88
4.32	1.19
10.68	2.945

4-xi SNA bases. 1.0 ml + 0.25 1N HCl. 17.4 μl spots. First run n.g. – repeat after standing 1 day (evap.?). 70% 90% EtOH 2.5 N HCl. 2 hydrolyses. Factor 719 (?)

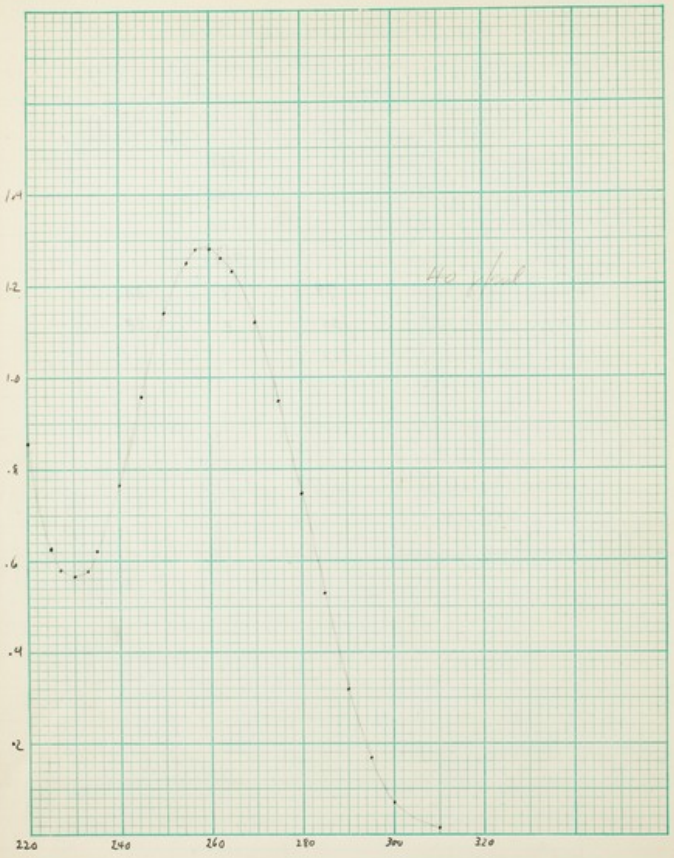
		A_{260}	G_{250}	C_{275}	T_{265}
① Blanks		.035	.031	.038	.065
② Blanks against against ①		.049	.029	.030	.083
① against blanks ①	1	.698 ~~[illegible]~~	.489	.449	.500
	2	.695 ~~[illegible]~~	.453	.446	.463
	3	.692 ~~[illegible]~~	.455	.442	.455
② against blanks ②	1	.676	.460	.465	~~.472~~ .467
	2	.700	.490	.449	.464 .459
	3	.681	.460	.436	.431 .424
$\bar{x}$		.690	.468	.448	.4605



Spleen N.A. proportions. From summary 10-xi

	µg/mg dry wt.		µmols/mg dry wt.		Molar ratios
	Found	Calc. for bases	Found	Calc. for bases	
Adenine	89.8		·666		1·36
Guanine	83.8		·555		0·875
Cytosine	62.1		·560		0·885
Thymine	94.6		·752		1·19
P	86	79	2·78	2·54	
N	137	130	9·78	9·29	
N.A. calc. as Na salt		813			

Deoxyribose, calc. from bases = 340 µg/mg.
Purine-bound ditto 163 "
Pentose found by orcinol 304 " = 18·5 % of calc. from bound deoxyribose
= 16·5 % of pentose if 100% RNA



5-xi

Repeat hydrolysis on SNA. ③ = ④ 1 unit ↑ .25°.
④ dropped, maybe lost some by splash. ③ OK.
Solvent off. A & C not perfectly separate. Cut out 4 corners of ③ anyway, for check on base : N:P ratios.

		A	G	C	T
③	1	.703	.466	411	.426
	2	.676	.438	.411	.419
	3	.687	.430	.408	.420
	4	.693	.460	.402	.418
	Σx	2.759	1.804	1.630	1.683
	x̄	.684	.451	.409	.421

Curve of SNA 1:100 pH 7.3 against sp. leaf.

220	.887	260	1.28	310 .017
225		262.5	1.26	
225	.626	265	1.23	
227.5	.580			
230	.565	270	1.12	
232.5	.578			
235	.621	275	.944	$\frac{D_{260}}{D_{230}} = 2.27$
240	.764	280	.743	
245	.968	285	.527	
250	1.14	290	.319	NA conc. for tissue = 5.34 × 8.13 = 43.34 mg/ml
255	1.25	295	.163	
257.5	1.28	300	.069	Conc. for $D_{260}=1$ = $\frac{43.34}{100} \times \frac{1}{1.28} = 3.39$

	D	Eluate μg/ml	Orig μg/ml	Orig μmols/ml	Molar ratios	μg mols N/ml
G	.438	6.23	448	2.96	.875	14.80
C	.420	4.62	332	2.99	.885	8.97 ~~14.95~~
T	.428	7.04	506	4.01	1.19	8.02
				9.96		
A	.644 by proportion from last run	6.68	480 ~~309~~	3.55 ~~2.24~~	1.05	17.75
			1766	13.51	4.00	49.54
Resid. N.A. Na salt = 13.5 x 190			2570			
			4336			

10-xi 3rd hydrolyses on SNA ⑤ + ⑥ 1 ml N ·25 17.4 ml for...

	A	G	C	T
1	·706	·444	·423	·414
2	·687	·421	·419	·391
3	·696	·426	·430	·412
4	·674	·433	·407	·404
5	·661	·428	·420	·415
6	·670	·434	·421	·433
7	·655	·447	·418	·424
	pyridine!	·438	·420	·428

9-xi Locust DNA.

50 g. fresh wt. locusts, [following ether] killed by CO_2, degutted & de-winged. Macerated in Waring blender c̄ 400 ml 0.9% NaCl, strain thru muslin, spin 2400 20 min. re-susp. in ·9% NaCl & spin down. Susp in 200 ml 10% NaCl → gel, leave in frig.

13-xi Add few drops NH_3 → alkaline, & 150 ml more 10% NaCl, emulsify on W.B. spin 9000 15 min. Filter to remove some scum. Still pink-turbid. Add HAc → pH 5. Pour into equal vol 90% EtOH → large stringy ppt. Centrifuge off, dissolve in 150 ml 10% NaCl + 2 drops NH_3. pH 9.5
Swing c̄ W.B. 3x → v. little more gel. Filter off last gel. add 1 N. HAc → pH 5. pour into equal vol 90% EtOH, spin off stringy ppt., dissolve in 10 ml ·01 M. Na_2CO_3, dialyse overnight against running water, then 24 hrs ag. dist.

16-xi. Spin 12000 30 min → no pellet, some scum (fat?); shake c̄ ether, let stand in sep. funnel.

18-xi. Run off → 20 ml, slightly turbid.

Dil. 1:100

220	·383	255	·568
230	·367	260	·586
235	·356	265	·541
240	·395	270	·476
250	·535	280	·317
		290	·150
		300	·046

ca. 2 mg/ml.

Amt N.A. = $\frac{·586}{1·2}$ × ·04 × 100 × 20 = 39 mg.

Orcinol reaction: 0·2 ml → color hard to estimate because of interfering yellow; guess at 30 γ pentose. To ca. 8 ml of N.A. add 1 drop ribonuclease, incubate 37° overnight. Acidify (HCl) spin, dissolve ppt. in 4 ml dil NH_3
Orcinol: ·2 ml → ca. 50 γ pentose. Add 2 drops ribonuclease, dialyse running water overnight. 19-xi. 0·2 ml → 25 γ pentose, return to dialysis.

9-xi. Herring Sperm N.A. (Sylvia)

Ca. 400 g. frozen herring soft roe thawed in 800 ml water, made 5% in HAc, macerated on Waring blender, spun 2000 15 min., re-susp in 5% HAc, spun again. Susp in 2 liters 10% NaCl. add 10% NaOH to → pH 6.5. Gels firmly on addition of the NaOH. Leave overnight in frig. (large gelled sediment)

10-xi Dilute to 4 liters, centrifuge, decant (turbid), pour into equal vol EtOH, collect stringy ppt. on rod, leave dispersed ppt. dissolve in 10% NaCl + few drops NH_3. Repeat 3x (not v. much gel)

13-xi Ld HA.

5 virgin moths, 3♂, 2♀, kept 2–10 days in frig. De-winged, ground in 30 ml ·9% NaCl, spin 9000 5 min. Resusp. tissue & spin down again. Then susp. in 40 ml 10% HCl + 2 drops 880 NH_3 & leave in frig.

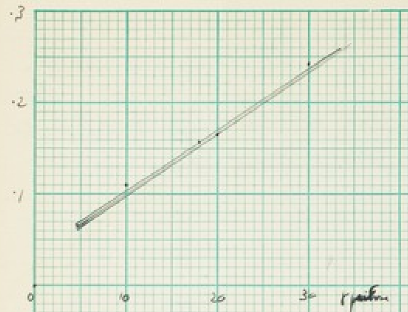
20-xi After week in frig spin clear, pour into equal vol. with EtOH (pH 4.5)

21-xi Spin off small ppt. dissolve in 15 ml 10% HCl

	large	small
Beaker MT	95.4884	36.2962

1-xi-49. Further purification of SNA.

Aq semi-dry fibrous NA, ca 15 mg dissolved in 10% NaCl + few drops NH_3. & remaining ca 100 mg in 1N. NaOH. Latter incubated overnight @ 37°, then both dialyzed 24 hrs in cyl. 48 hrs in aq. bath. Slightly turbid soln 20 min 11,500, decant into beaker, freeze-dry.



Locust	D	γ mol/ml	molar ratio	Re-calc.	
Adenine	·901	692	1.15	·674	1·183
Guanine	·525	495	·82	·478	·816
Cytosine	·521	516	·86	·495	·845
Thymine	·535	698	1.16	·674	1.150
		2401		2341	3.994

Locust NA cont'd.

21-xi. After second overnight dialysis c̄ ribonuclease, spin off small turbidity.

·2 ml → 25 γ protein = 125 γ/ml

1:100 D_{260} = ·734 ≏ 25 γ/ml. = orig. 2·5 mg/ml.
D_{230} = ·365

Theoret. protein. bound sugar ≏ $\frac{20}{100}$ × 2500 = 500

% RNA = $\frac{125}{500}$ × 100 = 25

Add 2 drops ribonuclease, incubate @ 37° 1hr, dialyze overnight.

22-xi. 1:100 : D_{260} = ·621 ≏ 20 γ/ml = orig 20 mg ml
D_{230} = ·378

Orcinol: 10 γ ·110
20 γ ·165
30 γ ·241
.2 ml NA ·157 = 18 γ : 90 γ/ml

Theoret. protein. bound sugar: $\frac{20}{100}$ × 2000 = 400

% RNA = $\frac{90}{400}$ × 100 = 22.5 %.

Ca. 4 ml dried down, hydrolyzed, up in 0·4 ml (?) 17.4 μl spots.

	Guanine	A	C	T
1	·526	·910	·521	·537
2	·543	·882	·517	·532
3	·500	·904	·511	·519
4	·533	·910	·534	·552
x̄	·5255	·9015	·521	·535

	D	Units/unit	Molar ratio	Re-calc.	
A	·158	121	·90	0·93	
G	·101	95	·71	·71	
C	·149	147	1·09	1·08	
T	·134	175	1·30	1·29	
		538	4·00	4·01	

		Units/unit Found	Units/unit Corrected for RNA	Molar ratio	Re-calc.
A	·706	5·43	4·63	·92	·95
G	·519	4·89	4·06	·81	0·80
C	·621	6·15	5·32	1·06	1·05
U	·067	0·83			
T	·461	6·02	6·02	1·20	1·19
			20·03		3·99

27-xi B. coli N.A. ① B.

Extracted by 1 N. NaOH overnight 37°. Not digested → brown streak on paper. Nucleotides unusual.

	G	A	C	T
① 1.	·107	·164	·149	·137
2.	·125	·137	·160	·144
② 1.	·057	·187	·138	·122
2	·113	·146	·149	·133
Σ	·101	·158	·149	·134

15·xii Prep 3/4 ⑧. Contains brown stuff, which mostly runs to front, + small spot in normal position.

G	A	C	U	T
·524	·721	·611	252 ·074 241 ·083 262 ·078	·461
·533	·696	·621	·098	·461
·476	·689	·585	·068	·437
·511	·703	·649	·098	·476
·513	·719	·638	·044	·469
·519	·706	·621	·067	·461

	D	μg mols/ml × 10⁴	Molar ratio
A	·571	4.38	1.11
G	·409	3.85	·97
C	·334	3.31	·84
T	·329	4.30	1.09
		15.84	4.01

Contains RNA

25-xi Locust N.A. (2).

Locusts, mixed ages, de-winged, de-gutted → 30 g., ground in ·9% NaCl (Waring), strained thro muslin, tissue spun down from 100ml " 4 times. Susp. in 100ml 1 M NaCl overnight frig.
spin clear, ~~Sevag (Waring) 4 times, ppt c̄ equal vol EtOH~~
ppt c̄ 6 vols water, dissolve ppt in 1 M NaCl, Sevag 4 times,
ppt c̄ equal vol EtOH, (small brownish ppt), dissolve in 10 ml
1:50 NH_3, dialyze.

29-xi Spin clear.

1:100

λ	D	λ	D
265	·366	270	·324
260	·392	280	·216
255	·385	290	·105
250	·353	300	·036
240	·221	310	·022
235	·165		
230	·137		
225	·141		
220	·175		

$\frac{D_{260}}{D_{230}} = 2.85$

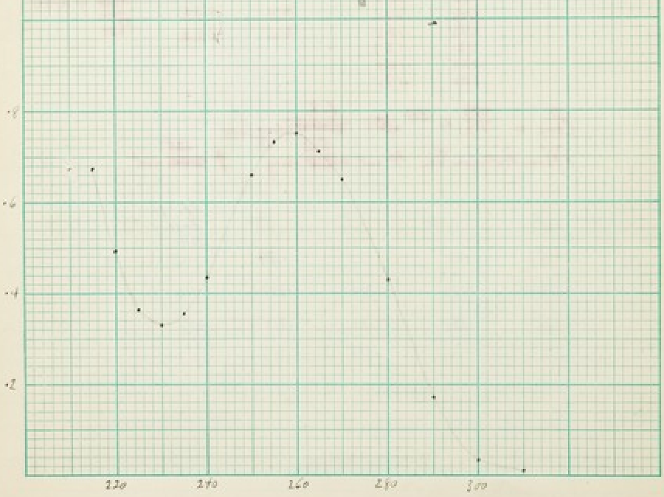
Conc = $\frac{·39}{1·2}$ × ·04 × 100 ~~[illegible]~~ 1.3 mg/ml.
Ppt in hydrolysis tube 4 ml = ~~[illegible]~~ 5·2 mg ↑ ~~[illegible]~~ .25 ml 17.4 μl spots.

	A	G	C	T	Spot just beyond T D240
1	·569	·411	·332	·334	·123 →
2	·580	·423	·339	·329	·110
3	·565	·393	·330	·315	·023
x̄	·571	·409	·334	·329	

265	·075
260	·090
255	·108
250	·114
245	·122
240	·123
235	·135
230	·144
225	·153
220	·162

Protein #II H.T. + N.A.
Dried 18 hrs @ 110° over P_2O_5

7.3630
7.4304
.0674 in 10 ml = 6.74 mg/ml



29-XI Beef spleen NA (2)

3 lbs spleen minced into 2 litres cold .15 M. NaCl, spin down (10 min.), repeat twice. Susp. in 3 l. 1 M. NaCl, macerate 2 min in Waring blender, spin 60 min 3700. Pour supernat. into 20 l. tap water (+ HAc to pH 6.5) → large stringy ppt - centrifuge off, dissolve in ca. 750 ml 1 M. NaCl (+ NaOH → pH 9-10) (doesn't all dissolve) Lsevag many times → much gel. After ca. 8 times, ppt c̄ equal vol EtOH + HAc → pH 6. Stringy-flaky ppt - spin down, dissolve in 200 ml 1 M. NaCl + Na_2CO_3 → pH 8.5; Lsevag again twice → almost no gel. Dialyze. Spin 10,000 30 min., pour into equal vol EtOH. + few drops HAc. Spin down, wash in 90% EtOH, abs, ether, scrape out & let dry.

Yield: 2.1 g, dried.

1:200 dil in cylind. of 6.74 mg/ml soln in M/20 Na_2CO_3

215	.674		
220	.493	260	.752
225	.363	265	.711
230	.330	270	.649
235	.356	280	.414
240	.457	290	.167
250	.660	300	.023
255	.735	310	.009

$\frac{D_{260}}{D_{235}} = 2.28$

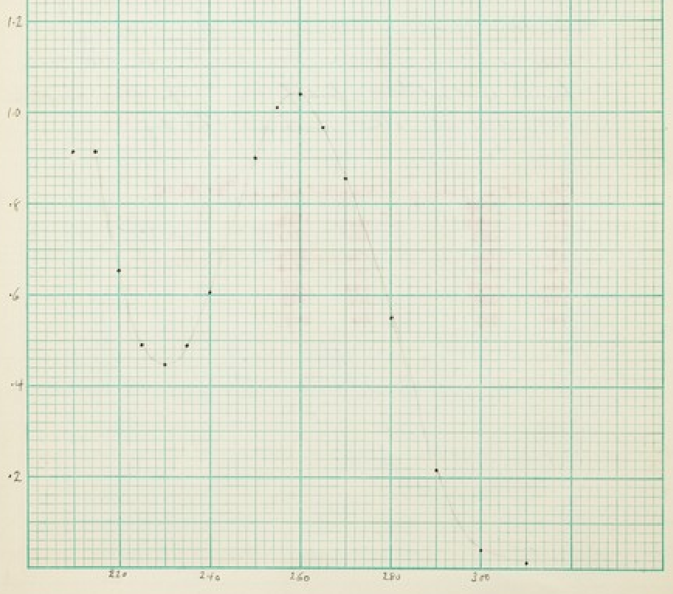
N.A. calc. fr. bases = 6.02 mg/ml.

Conc. for $D_{260} = 1$ $= \frac{6.02}{200} \times \frac{1}{.752} = 40$ γ/ml.

$\varepsilon_p = .752 \times 200 \times \frac{31 \times 100}{6.74 \times 9.35} = 7400$

Batch III HT. 7.3170
+ N.A. 7.5494

N.A. 0.2324 in 25 ml = 9.30 mg/ml in ·02 M. Na_2CO_3



1-xii-49 Herring sperm N.A. (Sylvia's prep.)

240 g. fresh testes suspended in 1 l. 0.9% NaCl, spin down, repeat twice. Susp. in 6 l 1 M. NaCl, spin on big machine 1 hr, then on Sharples (still turbid). Emulsify in portions in Waring mixer → small gel. & some clarification of sol'n. Mix with 24 l water, wind fibres on rod, lift out. Dissolve in 2 l 1 M. NaCl + NaOH → pH 9. Add ca 20 mg papain + pinch KCN, incubate 20 hrs 37°. Add HCl → pH 6, pour into equal vol EtOH, wind on rod, squeeze out, pull off rod, wash overnight with 4 changes 60% EtOH, then with 90, abs, ether & leave in desiccator to dry.

1:200 dil in aq. dil. of 9.3 mg/ml sol'n in M/50 Na_2CO_3

215	·915	·800			
220	·655	·566	260	1.04	·880
225	·490	·424	265	·965	·825
230	·446	·382	270	·858	·738
235	·486	·398	280	·551	·546
240	·604	·476	290	·218	·267
250	·900	·763	300	·040	·070
255	1.01	·875	310	·012	·017

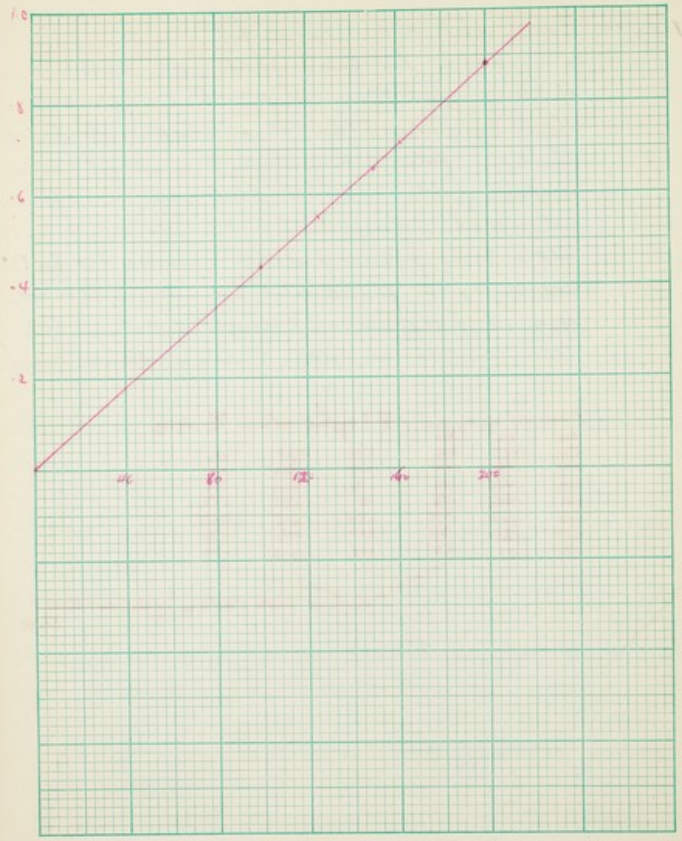
$\frac{D_{260}}{D_{230}} = 2.34$

Ensured dilution of sol'n in aq. dil, not treated with Na_2CO_3

N.A. calc. fr. bases = 7.75 mg/ml

Conc. for $D_{260} = 1$ $= \frac{7.75}{200} \times \frac{1}{1.04} = 37.2$

$\epsilon_p = \frac{1.04 \times 200 \times 31 \times 100}{9.30 \times 8.7} = 7{,}970$



3-XII-49 Potentiometric

Reagent blank, 60% ethanol

1. 0.2 ml SVA(2)	.548	.548	= 126 γ = $\frac{126 \times 5}{6.720}$ = 9.35 %
2 "	.551		
3. 0.2 ml HNA	.706	.710	= 162 γ = $\frac{162 \times 5}{9.30}$ = 8.71 %
4 "	.714		
100 γ ?	.442		
150 γ	.655		
200 γ	.880		

3 xii N. estimations

1 ml HCl : 1.025, 1.025

a 0.15 ml SNA(I) - 2 ml HCl - .45 } .42 = 1.01 mg/ml N = 15.0%
b " " .39
d 0.10 ml HNA " .50 } .535 = 1.40 mg/ml N = 15.1%
g " .57
h Blank .5 ml H_2SO_4 .98
j " .98
l " .98

5 x Sifters 1 ml HCl: 1.030, 1.034, 1.034. After: 1.065, 1.065

a 0.15 ml SNA (I) .505 } .43 = 1.00 mg/ml N = 14.9%
b " .36
c " .43
g 0.10 ml HNA 2 ml HCl 0.48 } .54 = 1.40 mg/ml = 15.1%
h " 0.59
j " .55
k Blank 2 ml HCl 2.07
l " 1 ml .99 + 1 ml HCl: 2.065
n " 1.00 + 1 ml HCl: 2.075

HNA	D	Eluate γ/ml	Orig γ/ml	Orig μmols/ml	Molar ratios
A	·752	7.81	898	6.64	1.10
G	·509	7.25	834	5.52	0.91
C	·481	5.29	608	5.48	0.91
T	·436	7.18	825	6.55	1.08
				24.19	4.00

BSNA (2)	D	Eluate γ/ml	Orig γ/ml	μg mols/ml	Molar ratios	
A	·768	7.96	687	5.09	1.10	1.098
G	·494	7.04	607	4.02	0.87	.869
C	·479	5.27	455	4.09	0.885	.884
T	·472	7.78	670	5.32	1.15	1.148
				18.52	4.00	

4-xii-49 NA bases (tubes left 3½ hrs)

HNA — spot beyond cytosine, thought to be cytidine instead of C — wrongly.

		A	G	C	T
HNA 1 ml HClO4 17.4 μl spots. [illegible] bowl 1	1	·752	·524	·480	·440
	2	·752	·502	·475	·433
	3	·754	·515	·478	·448
bowl 2	4	·751	·505	·484	·442
	5	·755	·508	·479	·436
	6	·750	·503	·493	·420
	x̄	·752	·509	·481	·436

		A	G	C	T
BSNA (2) 1 ml [illegible] 17.4 μl — 1	1	·761	·496	·477	·454
	2	·762	·484	·466	·456
	3	·776	·506	·494	·499
[illegible] = 863 — 2	4	·764	·494	·483	·468
	5	·757	·488	·467	·471
	6	·788	·497	·488	·485
	x̄	·768	·494	·479	·472

After NaOH treatment

BSNA	D	weight $\times 10^2$	Molarity	
A	.662	5.09	1.11	1.110
G	.419	3.95	.86	.861
C	.397	3.92	.85	.885
T	.412	5.39	1.17	high 1.73
		18.35		Loss on treatment = 14.5 %
HNA				
A	.659	5.06	1.07	1.067
G	.445	4.19	.88	.884
C	.412	4.07	.86	.859
T	.434	5.66	1.19	1.192
		18.98		Loss on treatment = 9.5 %

8-xii-49. Effect of 1 N. NaOH on DNA.

2 bomb tubes: 1 ml BSNA sol'n, 1 ml HNA sol'n. To each add .11 ml 40% NaOH → 1 N. Leave at 37° overnight.

9-xii Neutralize ~ HAc glac., (1 drop methyl red in tube), add total 2 ml EtOH → ppt, spin down, decant, dry off, hydr. in .5 ml HCOOH 175° 30 min.

HNA ↑ 4 ml, BSNA ↑ 3 ml [illegible]

		A	G	C	T
BSNA	1	.649	.421	.395	.425
	2	.655	.403	.374	.403
	3	.681	.432	.402	.407
	$\bar{x}$	.662	.419	.397	.412
HNA	1	.659	.450	.421	.439
	2	.659	.435	.408	.434
	3	.660	.451	.407	.430
	$\bar{x}$	.659	.445	.412	.434

HNA. Correction for wrong setting of C & T.
~~Estimate~~ Calculate C + T on basis of unit A + G, using ratios from hydrolysis expt.

	µg mol/mg	Molar ratios
A	·714	1·10
G	·594	·93
	1·308	2·03

C = $\frac{\cdot 85}{2\cdot 03}$ × 1·31 = ~~·~~ ·548

T = $\frac{1\cdot 13}{2\cdot 03}$ × 1·31 = ·729

Re-calc. using new absorption values and pipette calibration & adding MC
MC = HNA [illegible] [illegible]

		D	µmoles/ml	µmol/mg	Ratios	% dry wt.	µmols N
BSNA	A	·768	5·91	·730	1·116	9·86	3·65
	G	·444	4·50	·556	0·850	8·40	2·78
	C	·479	4·55	·562	0·859	6·24	1·69
	T	·472	5·95	·735	1·123	9·27	1·47
	MC	·028	0·286	·0354	0·054	0·44	0·11
	Total			2·618	4·002	34·21	9·70
					2·62×19	49·8	
						84·0	
HNA	A	·752	5·79	·691	1·104	9·34	3·46
	G	·509	4·63	·554	0·886	8·36	2·77
	C	·456	4·34	·519	0·831	5·76	1·56
	T	·460	5·80	·693	1·108	8·74	1·39
	MC	·036	0·367	·0438	0·070	0·55	0·13
	Total			2·501	3·999	32·75	9·31
					19×2·50	47·5	
						80·3	

Beef Spleen N.A., second prep.

Date of 4-xii

$\times \frac{5}{\cdot 08} \times \frac{9}{674}$ = ·1256

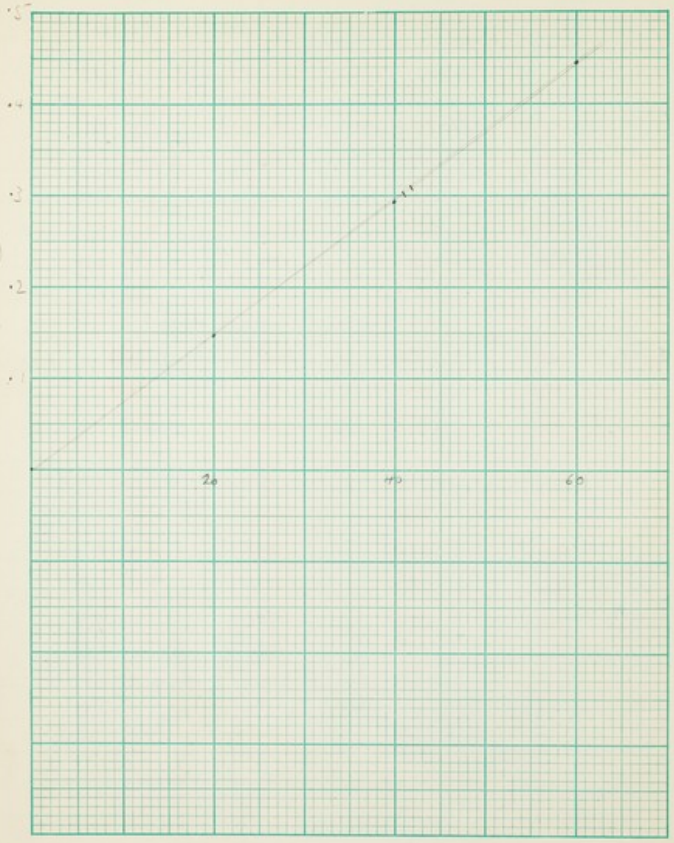
	% of dry wt. Found	% of dry wt. Calc from bases	µg mols/mg dry wt. Found	µg mols/mg dry wt. Calc fr. bases	Molar ratios
Adenine	10.2		·755		1.10 1.10
Guanine	9.0		·596		0.87 .92
Cytosine	6.75		·606		0.89 .85
Thymine	9.95		·790		1.15 1.13
P	9.35	8.50	3.02	2.74	
N	14.9	14.2	10.6	10.16	
N.A. Na salt		88.0			

Herring Sperm N.A.

~~56~~ [illegible] ~~of 18/xi/49~~
Date of 4-xii corrected for ratios.

$\times \frac{5}{\cdot 08} \times \frac{4}{930}$ = ·194

Adenine	9.65		·714		1·10
Guanine	9.0		·594		0·92
Cytosine	6.5		·548		0·85
Thymine	8.9		·729		1·13
P	8.7	8.1	2.71	2·59	
N	15.1	13.6	10.8	9.70	
NA. (Na salt)		83.4			



8-xii Controls

1 cm cell, 608 plate, against water.

20 ✕ myelose	.148
40	.293
60	.445
.2 ml BSNA	.309 ≡ 41.8 γ
.2 ml HNA	.302 ≡ 41.0 γ

BSNA: Purine bound deoxyribose per ml = 7.11 × 134 = 1220 γ

Extinction purine bound portion = 41.8 × 5 = 209 γ/ml

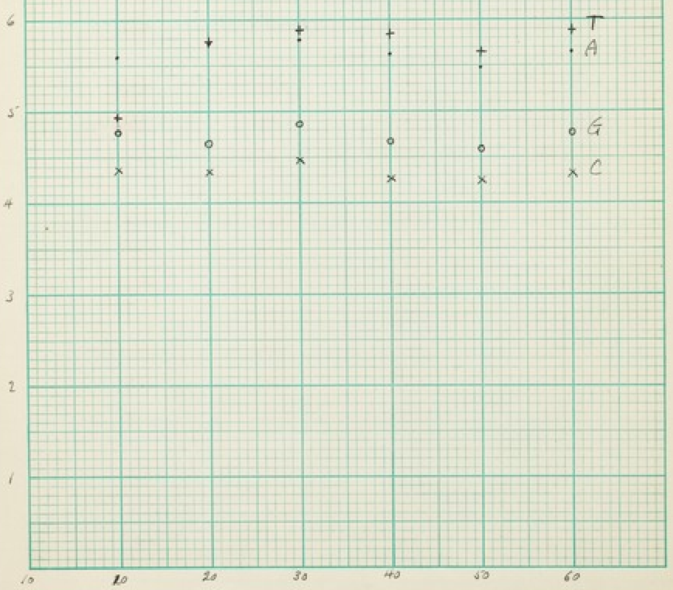
% $\frac{209}{1220}$ = 17.2 %

= 15.4 % of portion of all RNA

HNA. $\frac{205}{7.16 \times 134}$ = 12.6 % = 11.3 % of portion of all RNA.

Molar ratios

	10′	20′	30′	40′	50′	60′
A	1.14	1.12	1.10	1.10	1.10	1.10
G	0.97	·91	·93	·92	·92	·92
C	·89	·85	·85	·84	·85	·84
T	1.00	1.12	1.12	1.14	1.13	1.14
	4.00	2.00				



8-xii-49. Lengths of hydrolysis repeated. 1 ml portions HNA sol'n (9.30 mg/ml dry wt.) dried down, hydrolysed in 0.5 ml HCOOH at 175 ± 2°. Dry down, + 0.4 ml.

		A	[illegible] ×10²	mg/ml	G	[illegible] ×10²	mg/ml	C	[illegible] ×10²	mg/ml	T	[illegible] ×10²		Total [illegible] ×10²
10 min	1	·704			·511			·443			·394			
	2	·732		7.59	·496		6.95	·441		4.64	·376		6.00	
	3	·748			·513			·438			·363			
	x̄	·728	5.60		·507	4.78		·441	4.36		·378	4.94		19.68
20	1	·754			·494			·436			·411			
	2	·718		7.77	·494		6.76	·424		4.60	·434		6.84	
	3	·765			·493			·451			·448			
	x̄	·746	5.74		·494	4.65		·437	4.33		·431	5.76		20.48
30	1	·747			·515			·455			·447			
	2	·758		7.84	·528		7.09	·449		4.77	·469		7.16	
	3	·754			·507			·455			·436			
	x̄	·753	5.79	1.100	·517	4.87	0.927	·453	4.48	0.851	·451	5.89	1.120	21.03
40	1	·709			·480			·432			·440			
	2	·756		7.61	·517		6.79	·439		4.52	·449		7.10	
	3	·724			·490			·428			·451			
	x̄	·730	5.61	1.099	·496	4.67	0.916	·433	4.28	0.839	·447	5.84	1.143	20.40
50	1	·721			·461			·434			·442			
	2	·708		7.42	·497		6.52	·432		4.53	·439		6.87	
	3	·710			·469			·425			·417			
	x̄	·713	5.48	1.097	·476	4.58 (4.48)	0.918	·430	4.25	0.852	·433	5.65	1.131	19.96
60	1	·729			·498			·432		0.5	·443			
	2	·752		7.66	·519		6.91	·437		4.59	·458		7.16	
	3	·725			·497			·438			·453			
	x̄	·735	5.65		·505	4.76		·436	4.31		·451	5.89		20.61

BSNA RNase treated

	Unhydrolysed	Molecular ratio	
A	5.37	1.10	1.104
G	4.16	.855	855
C	4.22	.87	.868
T	5.72	1.18	1.175 % loss = 9.2
	19.47		

HNA RNase treated

A	5.27	1.07	1.068
G	4.33	.88	.877
C	4.35	.88	.882
T	5.78	1.17	1.171 % loss = 6.2 %
	19.73		

9-xii Effect of RNase on HNA, BSNA②, & HNA+YNA.

In 3 tubes: 1.0 ml HNA sol'n (9.3 mg) p.4

1.0 ml BSNA ② sol'n (6.7 mg) p.3

0.5 ml HNA sol'n (4.6 mg) + 0.25 ml conc. YNA (5 mg) p.4

To each add 0.5 ml M/10 Ⓟ pH 6.8, and 1 drop of RNase, leave 40 hrs at room temp. (14-16°).

Add 4 drops **glac.** HCl, and **3** ml abs. EtOH, mix → large ppt. spin down, decant (forgot to re-ppt & wash), hydr., 17.4 µl spots.

HNA-YNA mixture → small unusual spot ~ ca. 1/10 other spots. Not eluted.

		A	G	C	T
BSNA	1	.691	.425	.424	.428
	2	.689	.440	.415	~~.44~~ .432
	3	.718	.461	.440	~~.459~~ .453
	x̄	.699	.442	.426	.438
HNA	1	.690	.461	.439	.432
	2	.702	.468	.445	~~.444~~ .443
	3	.667	.450	.432	~~.455~~ .453
	x̄	.686	.460	.439	.443

(A)		D	μmol/ml $\times 10^2$	Molar ratio	μmol/ml	
	A	.334	2.57	1.26	2.59	1.292
	G	.147	1.48	.73	1.43	0.719
	C	.120	1.24	.61	1.19	0.599
	T	.219	2.86	1.40	2.76	1.388
			8.15	4.00	7.95	3.998
B	A	.340	2.61	1.33	2.62	1.373
	G	.169	1.60	.82	1.54	0.808
	C	.081	0.80	.41	0.76	0.399
	T	.215	2.82	1.44	2.71	1.421
			7.83		7.63	4.001

12-xii T. NA. (A).

	A	G	C	T
1.	.330	.151	.124	.214
2.	.337	.163	.125	.224
$\bar{x}$	.334	.157	.125	.219

14-xii (B) Extract $\frac{1}{2}$ conc. HCl @ pH 10. A·1, 17.1 µl

	A	G	C	U	T
1	.328	.162	.078	255·112 / 265 ·019	.209
2	.340	.185	.082	260 ·027	.217
3	.351	.160	.084	260 ·038	.222
$\bar{x}$	.340	.169	.081		.215

255	·112	
260	·019	
265	·024	
270	·025	
275	·022	
280	·018	
260	·038	·012
265	·032	·017
270	·038	·013
275	·031	·017
280	·027	·016

Avg. total μ mols of bases = $\cdot0783 \times 5 \times \frac{1}{\cdot0174} = 2.25$

μg. of NA = $\frac{2.25}{1.000} \times 320 = \underline{0.72}$ mg.

13.xii

Dendrolimus pini DH.17 (Sylvia)

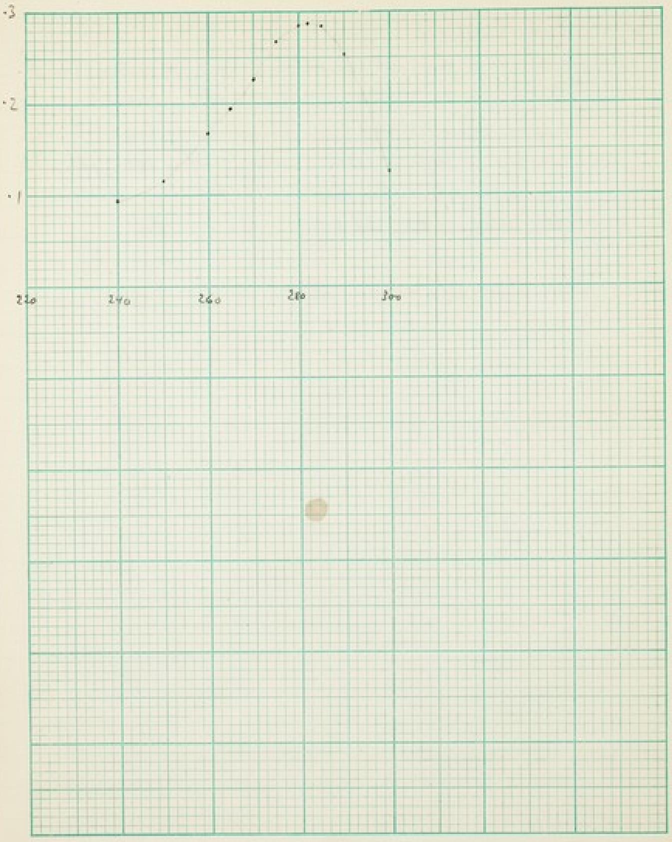
27 g. fresh wt. 2/3 grown caterpillars killed ē ether frozen overnight, susp. (Waring bl.) in 150 ml .9% NaCl, spin 3500 10 min, re-susp. & wash in .9% NaCl 4 times → clear supernat. Susp (W.bl.) in 150 ml 1M NaCl, spin 20 min 8000 rpm. Pour into 900 ml water → nothing. Leave overnight 4°; susp. residue in 100 ml 1M NaCl & leave 3 d. 4°. Spin clear, pour into 600 ml water → minute ppt. Discard

— " —

13-xii-49 <u>Locust DNA ⑤</u>

21 g. fresh-m. adults de-winged, de-gutted, susp. (W.B.) in ca. 150 ml .9% NaCl, spin. Repeat 3x. Susp. in 200 ml 1M NaCl → v. viscous, leave overnight 4°, spin 8000 8 min., pour into 1200 ml water. Spin down ppt. ↑ 1 M NaCl pH 9, spin clear. Re-ppt (?). Sevag. Ppt $\bar{c}$ EtOH. ↑ in dil NH_3. Leave in frig.

10-1-50 Ca. 15 ml soln. Spin clear.



First mention of extra spot (= 5MC)

TNA . position of cytidine - just below cytosine, in p.p.-HCl.
...natogram, re-hydrolyse ~~one~~ half 175°. HCOOH 1½ hrs →
2 17.4 μl spot in N/10 HCl :-

C blank

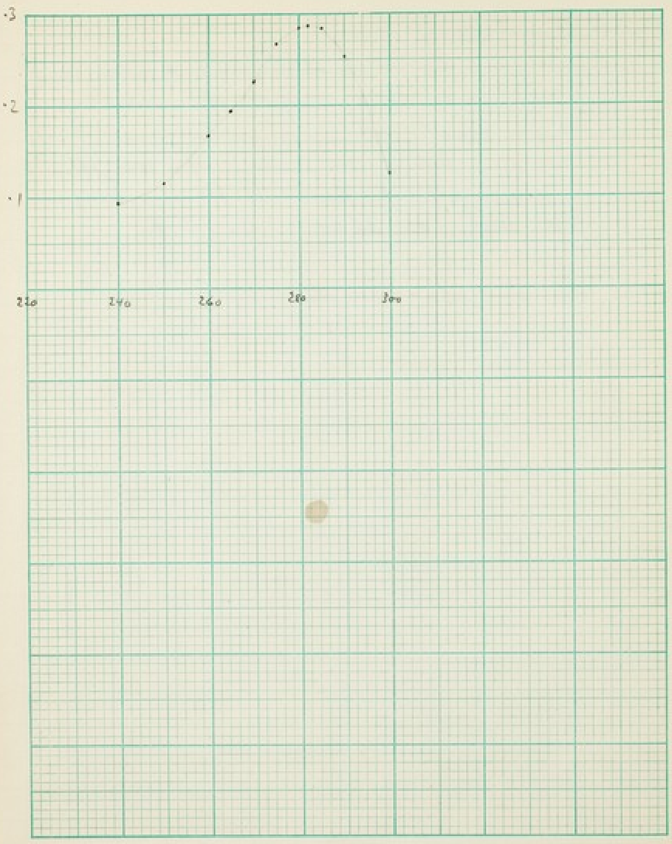
+ 2 days 40% HCOOH

·241
·250
·263
·267
·252

...no ninhydrin color.

... re-hydrolysed extract dried down. + 3.0 ml aq. dist.
..., then add ·03 ml conc HCl, mixed, then ·06 ml 40% NaOH.

		N	H	
		~~·900~~	·950	
		·792	·737	
		·669	·560	
		·588	·448	
		·525	·398	243 ·388
		·488	·390	
		·467 252 ·465	·408	
		·469	·434	
	·526	·498	·457	
		·504 260 ·509	·482	
		·508 272 ·509	·505	
		·488	·522	278 ·526
	·52	·447	·524	
		·376	·496	
		·306	·436	
		·240	·354	
300	[illegible]	·203	·260	
310	·253	·168	·130	
320	·198	·146	·107	



14-xii

Extra spot re (TNA) - position of cytidine - just below cytosine, in prop-HCl.
Elute from strip chromatogram, re-hydrolyse one half 175°, HCOOH 1½ hrs → no change. Elute 2 17.4 μl spots in N/10 HCl:-

	Against C blank N/10 HCl	+ 2 days 40% HCOOH
240	·053	
250	·116	
260	·168	
265	·194	
270	·227	
275	·268	·241
280	·254	·255
282	·257	·263
285	·257	·267
290	·252	·252
300	·126	

1 17.4 μl spot → no ninhydrin color.
Remaining extract & re-hydrolysed extract dried down. In 3·0 ml aq. dist., neutralize. Read N, then add ·03 ml conc. HCl, read, then ·06 ml 40% NaOH.

	OH	N	H
220		~~·900~~	·950
225		·792	·737
230	·89	·669	·560
235	·749	·588	·448
240	·646	·525	·398
245	·579	·488	·390 243 ·388
250	·544	·467 252 ·465	·408
255	·529	·469	·434
260	·523 262 ·526	·488	·457
265	·527	·504 268 ·508	·482
270	·537	·508 272 ·504	·505
275	·541	·488	·522 278 ·526
280	·551 282 ·552	·447	·524
285	·548	·376	·496
290	·520	·306	·436
295	·464	·240	·354
300	·387	·203	·260
310	·258	·168	·130
320	·198	·146	·107

Locust NA, spotted from N NaOH.

	D	(mol/ml)	Molar ratios	Recalc.	
A	·812	6.24	1.12	6.25	1.147
G	·509	4.80	·86	4.63	0.850
C	·463	4.58	·82	4.39	0.806
T	·512	6.69	1.20	6.46	1.185
		22.31			
MC	·015	0.15	·03	assume 0.05	0.009
				21.78	3.997

21-xii ~~Preparation of "MC"~~
~~824 mg. semi-dry HNA divided into 3 bombs. Each + 2 ml HCOOH. 40 min 175°~~

19-i-50 Locust NA. Prep. in frig. of earlier date than 14-xii marked "ca. 2 mg/ml". 3 ml taken, ppt. c̄ NaAc + EtOH, ↑in N NaOH, overnight 37°. ppt = NaAc + EtOH in bomb tube. Hydrolyse 30 min HCOOH (→ 185°-190° for short time)

	A_{260}	G_{260}	C_{275}	MC	T_{265}	Cell blanks water
1	·806	·507	·464	·013 (275 ·018)	·536	260 ·002; 280 ·003
2	·802	·486	·455	·005 (275 ·004)	·488	260 −·001; 280 ·002
3	·827	·534	·471	·027 (275 ·024)	·512	260 ·002; 280 −·003
$\bar{x}$	·812	·509	·463	·015	·512	

(·027 →) contains C.

	"NaOH"		"Control"	
	Yield/μmol	Molar ratios	Yield/μmol	Molar ratios
A	5.69	1.07	5.30	1.06
G	5.24	.99	4.57	.90
C	4.13	.78	4.31	.86
T	6.20	1.16	5.75	1.15
Total	21.26		19.93	
MC	.32	.06	.30	.06
U	.63	.12	.01	0.00

16-xii-49. Repeat effect of 1N. NaOH on MNA. To 0.5 ml MNA soln added .85 ml 40% NaOH (→1.2N), mix, leave overnight ca. 34°C., acidify c HCl, add 1.5 ml EtOH, spin, dry, hydrolyse, ↑ 0.2 ml. Control, same minus NaOH. Try to make out MC & U also, but not well resolved from C & T.

		A	G	C	T	MC	U
"NaOH"	1	.732	.561	.421	.473	270 .030 275 .037 280 .038 285 .037	250 .036 255 .042 260 .039 265 .031
	2	.733	.560	.416	.468	.280 .028	260 .024
	3	.754	.546	.417	.485	" .029	260 .089 265 .060
	x̄	.740	.556	.418	.475	.032	.051
"Control"	4	.704	.547	.448	.458	275 .031 280 .033 285 .032	260 .015
	5	.696	.484	.433	.454	280 .031	" → .010
	6	.669	.424	.426	.408	" .026	" → .002
	x̄	.690	.485	.436	.440	.030	.001

Probably got labels crossed. Results a bit queer anyway, but shows elimination of U, initially 10% of other bases, c presence of MC = 5% of T.

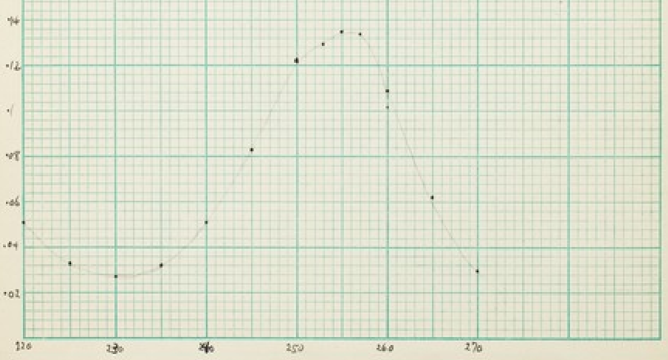
	Orig HNA		NaOH-treated		RNase-treated	
	Yield/µl	Ratios	Yield/µl	Ratios	Yield/µl	Ratios
A	5.89	1.14 35	5.46	1.11 07	5.70	1.13 29
G	4.77	.920	4.51	.915	4.68	.93 28
C	4.48	.864	4.16	.844	4.31	.854
T	5.61	1.081	5.58	1.131	5.50	1.09 89
Total	20.75		19.71		20.19	
"MC"	.36	.069	.34	.069	.37	.073
"U"	.14	.03	.17	.03	.15	.03

19-xi.

HNA re-runs. Orig. hydrolysates – untreated (1), NaOH-treated (1), & RNase-treated. 3 spots 17.4 µl each, on longest possible paper. Good for ratios only. All on same paper, same tank.

		A	G	C	T	MC	U
Orig. prep.	1	.755	.536	.58	.433	275 .032 280 .034 285 .033	255 .004 260 .007 265 [illegible]
	2	.779	.521	.464	.428	280 .039	255 .010 260 .012 265 [illegible]
	3.	.764	.493	.447	.433	.035	255 .006 260 .013 265 [illegible]
	x̄	.766	.506	.453	.431	.036	.011
NaOH	4	.713	.483	.48	.445	.035	255 .022 260 .025 265 .024
	5	.707	.481	.425	.424	.038	255 .015 260 .016 265 [illegible]
	6	.711	.469	.416	.415	.030	255 −.006 260 .000 265 .003
	x̄	.710	.478	.420	.428	.034	.014
RNase	7	.745	.47	.434	.417	275 .034 .036 285 .035	60 .008
	8	.736	.528	.438	.432	.040	260 .016
	9	.743	.494	.433	.416	.035	260 .012
		.741	.496	.435	.422	.037	.012

	Orig BSNA		NaOH-treated		RNase-treated	
	µmols/ml	Ratios	µmols/ml	Ratios	µmols/ml	Ratios
A	5·94	1·13 (24)	5·24	1·13 (26)	5·45	1·13 (26)
G	~~5·24~~ 4·56	·864	3·89	·84 (36)	4·10	·85 (48)
C	4·71	·893	3·96	·851	4·31	·890
T	5·90	1·12 (17)	5·51	1·184	5·49	1·14 (35)
	21·11		18·60		19·35	
"MC"	·29	·055	·24	·052	·23	·048
as % of C		6·2		6·1		5·4



20-xi

BSNA re-runs.

	A	G	C	T	MC	U	
1	·772	·497	·467	·456	255 ·030 ·029	255 ·129 260 ·110 265 ·062	
2	·782	·482	·478	·455	255 ·028 ·031	255 ·102 260 ·086 265 ·057	
3	·765	·472	·483	·446	·026	255 ·079 260 ·067 265 ·041	
$\bar{x}$	·773	·484	·476	·452	·029		
						D_{255}	D_{260}
4	·689	·427	·400	·424	255 ·020 ·023	·136	·105
5	·686	·404	·398	·420	255 ·029 ·031	·032	·029
6	·668	·404	·401	·423	255 ·015 ·018	·027	·025
$\bar{x}$	·681	·412	·400	·422	·024		
						255	260
7	·708	·437	·439	·425	·024	·045	·042
8	·704	·437	·432	·436	·028	·039	·038
9	·711	·430	·435	·403	·018	·109	·088
$\bar{x}$	·708	·435	·435	·421	·023		

"U₁":

	M/10 HCl	+ 3 drops 40% NaOH	2 drops 40% NaOH in 3 ml alcohol
270	·030	·096	·036
265	·062	·120	·039
260	·109	·121	·043
257	·134		
255	·135	·128	·047
252 ·129 250	·122	·134	·059
245	·083	·132	·054
240	·051	·134	·064
235	·082	·142	·079
230	·027	·152	·107
225	·033	·188	
220	·051	·358	

NaOH-treated BSNA

	mol/gm	Ratios
A	5.54	1.14
G	4.33	.89
C	3.96	.811
T	5.70	1.17
MC	.24	.049

% recovery = $\frac{19.53}{21.05}$ = 91%

Molar MC = 6.1% of C

to check % recovery.

21-xii Repeat effect of 1N. NaOH on BSNA, 1.0 ml soln dried down in bomb tube, add 0.5 ml 1N. NaOH, leave overnight 37°, acidify c. glac. HAc., add 1.5 ml 90% EtOH, ether ppt., dry, add NaOH, hydrolyse 175° 35 min., PO3 ml. 17 ppt after

	A	G	C	T	"MC"
1	.724	.478	.404	.440	.027
2	.734	.458	.401	.439	.021
3	.703	.442	.394	.432	.028
x̄	.720	.459	.400	.437	.024

21-xii-49. <u>Preparation of "MC"</u>

800 mgs semi-dry HNA dissolved in 6 ml HCOOH in 3 bombs, hydrolyze 175° 40 min. Open, dry down.

9-i-50 + several ml 1N. HCl, filter off bigger bits carbon, dry down, + ca. 2 ml N. HCl, spread all along 22" of Whatman #3, run to bottom in isoprop.-HCl. Brown streaks. Run 30 min. Not resolved - cut bands & run one containing MC again - still not resolved from C - cut out & run in BuOH-NH_3 - Almost resolved. Extract "MC", "MC+C" fore-running, & "C". "MC" - ca. 5 ml. -

Dil. 1:50 in N/10 HCl.

275	.708
280	.757
282	[illegible] .756
283	.750
285	.756

Extract conc.: $\frac{.756}{.8} \times 50 \times 10 = 470\gamma/ml$.

10-i-50 Deaminations

Cytosine.

Mixt in centrifuge tube: 0.25 ml cytosine (1.2 mg)
2.5 ml 50% (2 M.) $Ba(NO_2)_2$
1.0 ml 10 N. H_2SO_4.

→ thick ppt $BaSO_4$, ± bubbling ± frothing. Some brown fumes. 11 a.m. Leave 4½ hrs. Add solid $Ba(OH)_2$ → pH > 1.5°, spin off $BaSO_4$, evap → dryness. Large xtals. ↑ water, add N. H_2SO_4 → ppt. Trans and ppt., spin, filter, dry down. ↑ perhaps N/10 HCl. Run spot in isopr.-HCl. Mostly still C; not > 20% converted to U.

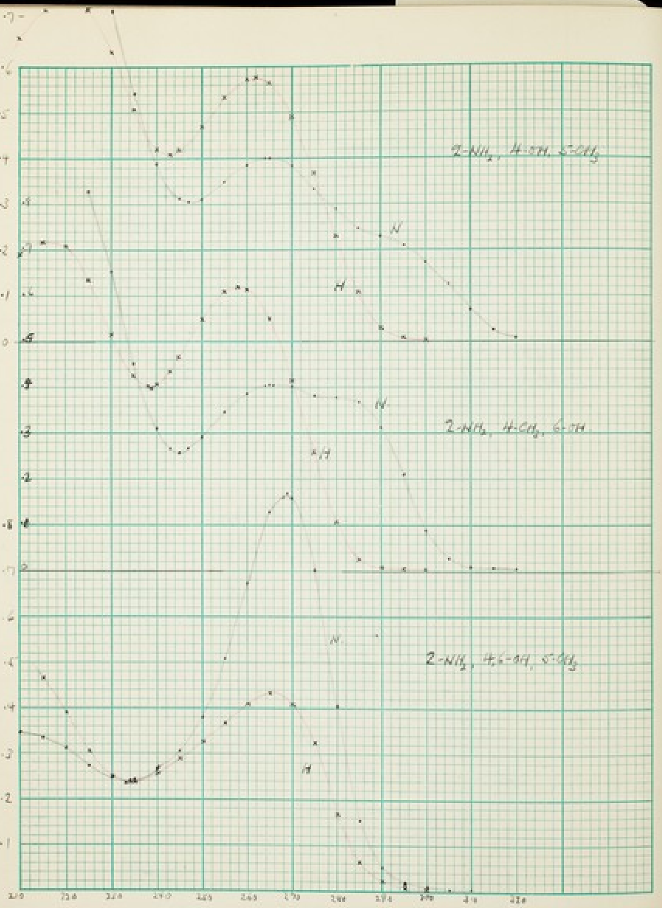
11-i-50 Repeat ± XS $Ba(NO_2)_2$

Mixt in flask: {.2 ml Cyt.
{2 ml 2 M. $Ba(NO_2)_2$
{0.5 ml 10 N. H_2SO_4

Incubate ± HAc - {.2 ml Cyt
{2 ml 2 M. $Ba(NO_2)_2$
{0.4 ml glac. HAc.

} Room temp. overnight

Spots: ± HAc, C → U almost completely
H_2SO_4, " only ca 25%.



Methyl-pyrimidine UV curves. Stock sol^ns 1:400 = 10γ/ml. Titrated to pH 7.

Neutral pH 7

	Mol. 100 C 2-NH$_2$, 4-OH, 5-CH$_3$	2-NH$_2$, 4-CH$_3$, 6-OH	2-NH$_2$, 4,6-OH, 5-CH$_3$
220	·97	·97	·313
210	1·10	1·24	·345
215	1·08	1·10	·337
225	·86	·83	·274
230	·721	·618	·244 237 ·241
235	·542	·454	·242
240	·390	243 ·310 ·267	·264
245	247 ·315 ·306	247 ·258 ·266	·306
250	·312	·292	·379
255	·349	·344	·507
260	264 ·384 ·400	264 ·386 ·402	·674
265	·400	266 ·404 ·403	268 ·830 ·860
270	·382	·401	269 ·860 ·870
275	·337	·381	·701
280	·288	·379	·408
285	·248	·367	·166
290	·229	·314	·053
295	·210	·210	·020
300	·176	·090	·008
305	·126	·027	
310	·069	·009	·003
315	·027	·007	
320	·009	·006	·003

	acid	alk	acid	alk	acid	alk
210	·654		·694			
215	·721		·718		·965	
220	·766		·709		·593	
225	·742		·639		·304	
230	·631		·518		·248 233 ·257	
235	·507		·434 238 ·463 ·468		·237	
240	·421 242 ·410		243 ·484		·258	
245	·420		·464		·291	
250	·471		·548		·326	
255	·537		·607 258 ·616 ·620		·366	
260	·575 262 ·578				·409	
265	·563		·532		·436	
270	·490		·416		·409	
275	·367		·261		·324	
280	·231		·106		·169	
285	·107		·026		·063	
290	·032		·007		·022	
295	·008		·003		·009	
300	·003		·003		·005	
305						
310						

14-1-50 HNA hydrol 6 N. HCl 175° 60 min.
Pro G, bottle A; big glycine methyl ester ppt.

Estimate C, MC, U, T (U-T not perfectly resolved)

	C_{275}	MC_{250}	U_{260}	T_{265}	
		275-271			
(1)	·586	·678	·267	·978	
		285-276			
		275-258			
(2)	·614	·067	·252	·944	
		275-264			
$\bar{x}$	·600	·071	·260	·961	
μ mols/ml	5·94	0·71	3·23	12·60	C+U
Ratios (T=1·10)	·52	·06	·28	1·10	0·80

17-1-50. Urea spot examination

Urin: 1 ml conc. 0.48 mg nitrogen / Control: 0.1 ml conc. 0.5 mg 2·4 N, 4·04, slurry opposite
2 ml 1 M $Ba(NO_3)_2$ 1 ml water.
0.5 ml glac. HAc. Same $Ba(NO_3)_2$ + HAc.

6 hrs room temp. Add H_2SO_4 to XS, spin, add $Ba(OH)_2$ to XS, filt., dry down → some salt ($Ba(NO_3)_2$?). Add few drops H_2SO_4, drying, till some moisture (H_2SO_4) does not evaporate. Add few ml H_2O, trans into tube, dry down, + 0·1 ml H_2O. Spots ca 10–15 µl.

Both → thymine green intensity.

24-1 To check identity, run conc spot of T ex dr urn MC, along: Tex HNA

	pH 7		+ 1 drop 40% NaOH		+ 2 drops conc HCl	
	Tex MC	Tex HNA	ex MC	ex HNA	ex MC	ex HNA
230	·158	·225			202	·282
235	·126	·191	·257	·325	·172	·244
240	·131	·216	·207	·267	·180	·269
245			·177	·237		
250			·173	·246		
255			·184	·279		
260	·298	·381			·323	·346
265	·290	·531	·214	·341	·327	·338
270	·276	·500			·309	·323
280			·229	·374		
285			·232	·377		
290			·233	·378		
295			·219	·359		

Preparation of 5MC

dissolved in 20 ml HCOOH. spread
0 min @ 175°. Over bust. Leaves 3.3 g.
, boil c charcoal, filter. Still brown.
from water.

HNO_3, add 10 ml sat. ca. 2.0 g $AgNO_3$
ff c wash c 2 10 ml portions warm ·2 N HNO_3
6 N. HCl, filter off AgCl, wash c N HCl.
3 x. Yellow, c some syrupy liquid
d few ml water, then few ml sat $Ba(OH)_2$
— ppt forms, but re-dissolves on mixing:
Vac down, ↑ ca. 2 ml water, frig overnight

rcoal contains much of all bases
nic ppt: G & A only
purine ppt: T + C about equal. 1/10 MC
MC sol'n: also contains T + unknown c trace A.
portions hot ·2 N. HNO_3 c repeat Ag. purine
ation.
ned on Holberts #3 paper c run in $PrOH/NH_3$

18·i·50. <u>Mess-up second prep.</u>

40g. air-dry HNA dissolved in 20 ml HCOOH. Spread over 6 bomb tubes, 30 min @ 175°. One burst. Leaves 3·3g. Washout c̄ some N HCl, boil c̄ charcoal, filter. Still brown. Vac. to dryness, twice repeat from water.

Dissolve in 20 ml ·2 N. HNO_3, add 10 ml sat. aq. 2·0 g $AgNO_3$ → large ppt. Filter off & wash c̄ 2 10ml portions warm ·2 N HNO_3.

Filtrate: add few drops 6 N. HCl, filter off AgCl, wash c̄ N HCl.

Filtrate: vac to dryness 3×. Yellow, c̄ some syrupy liquid does not evaporate. Add few ml water, then few ml sat $Ba(OH)_2$ in case $Ba_3(PO_4)_2$ forms — ppt forms, but re-dissolves on mixing. Still strongly acid. Vac down, + ca. 2 ml water, frig overnight.

19-i Filter off ppt.

Chromatogram shows: Charcoal contains much of all bases
Purine ppt: G & A only
Pyrimidine ppt: T + C about equal. No MC
C+MC soln: also contains T & unknown & trace A.

Re-extract charcoal c̄ 4 portions hot ·2 N. HNO_3 & repeat Ag-purine pptn & T+C crystallization.

20-i All C+MC fractions spread on 4 sheets #3 paper & run in $BuOH\,NH_3$.

14

	Top		Bottom	
	μmols/ml	Ratios	μmols/ml	Ratios
A	5·41	1·14	4·78	1·12
G	4·46	·93	4·35	1·02
C	4·04	·85	3·56	0·83
T	5·21	1·09	4·41	1·03
	19·12		17·10	
MC	0·28	·06	0·36	·08

Re-calc, using new D, & disregarding bad G value.

	Top		Bottom	
	μmols/ml	Ratios	μmols/ml	Ratios
A	5·43	1·13	4·79	1·14
G	4·30	0·90	3·79	0·90
C	3·87	0·81	3·42	0·81
T	5·29	1·10	4·47	1·06
MC	·29 ~~0·49~~	0·06	·37 ~~0·389~~	0·09
	19·18	4·00	16·84	4·00

25-1-50. Centrifugal fractions of HNA

0·5% about 1 hr @ 30,000. pH 8·5 borate 1M NaCl.

		A_{260}	G_{250}	C_{275}	MC_{280} (283 2% higher.)	T_{265}
Top	1	·702	·460	·416	275 ·021 ·025	·418
	2	·711	·485	·399	275 ·029 ·032	·429
	$\bar{x}$	·706	·472	·407	·028	·420
Bottom	3	·621	·508 (omit)	·360	275 ·038 ·040	·356
	4	·628	·414	·360	275 ·032 ·033	·355
	$\bar{x}$	·622	·461	·360	·036	·355

50 ml: 200 mg T
500 mg C
150 mg MC
850 mg × 4 = 3.4 g $AgNO_3$

N.A. in aq. dil. arbitrary conc.

λ		λ	
220	·514	265	·709
225	·565	275	·566
230	·303	285	·347
235	·314	295	·131
245	·538	305	·024
255	·710		
260	·740		

Filter off ppt, decompose in 6 N HCl, filter off AgCl. Filtrate to dryness. Material soluble in 5 ml 85% isopropanol 1 N HCl, run on column in this solvent. Thymine separated, but C + MC together.

2-11. Fractions containing C + MC dried down several times, then add BuOH–water–NH_3 → separate aqueous layer (too much NH_4Cl?). Dry again, extract material soluble in ca. 10 ml dry BuOH, dry down, + 8 ml BuOH H_2O NH_3, put on column & run in this solvent.

24.i.50. Herring NA prep for MC.

4 tins (= 2 lb) soft roe suspend in 2 l. 0.5 N NaOH 1 M NaCl. Leave at room temp 4 hrs. Spin on big machine 1 hour → small sediment & turbid supernat. Suspend sediment in 1 M NaCl. Neutralise both, add papain, HCN, leave overnight 37°.

25.i. Sediment fraction: spin again. Both supernats, ~~[illegible]~~ made alkaline, shake c chloroform, spin off gel, clarify on Sorvall. Acidify c HAc. ppt c equal vol. EtOH. Spin down big ppt, wash c 60% EtOH, then 90. Leave overnight to dry.

26. Semi dry: 50 g.

43 g (30 g dry NA?) put in flask c 300 ml methanol, bubble HCl gas thru for several hours. Stand overnight 37°.

27. NA still not all dissolved – maybe 3–4 g. left.

Filter off purine HCl ppt. Filtrate vac down almost to dryness → brown tar. Dissolve in ca. 50 ml 6 N HCl, cook in bombs 125° 2 hours. Open, heat c charcoal, filter, wash charcoal c 1 N HCl hot. Filtrate: vac down. Add water & vac down twice, but does not go dry, → goo. Put up in ca. 100 ml hot H_2O, neutralise c $Ba(OH)_2$ in hot H_2O (ca. 20 g) → big ppt. Filter, wash c hot H_2O.

Filtrate vac. down to ca 50 ml, leave in frig overnight.

28 Filter off large silvery crystalline ppt. (Thymine)

Filtrate: vac down to dryness. + 10 ml hot water, let cool 3 hrs → thick ppt. (ppt + some thymine)

Filter, wash ppt c water. Filtrate is v. acid – make alkaline c NaOH.

Add 2 g $AgNO_3$ in water → big ppt, then more $AgNO_3$ till XS (ca. 4 g in all)

		A	G	C	MC 275	MC 280	MC 285	T
Hydrolysate of ppt	(1)	·545	·623	·507	·012	·009		·355
	(2)	·545	·648	·516	·000	·001		·338
	(3)	·516	·619	·495	-·002	-·001		(·289) omit
	(4)	·564	·596	·493	·001	·000		·336
	$\bar{x}$	·542	·621	·503				·343
N.A. sol'n	(1)	·477	·500	(·513)	·029	·027		·296

LdVNA, fraction spun down from NH_3:

	μmols/ml	Ratio	Recalc	
A	4.17	·86	4.17	0.883
G	5.85	1.20	5.65	1.196
C	4.98	1.02	4.77	1.010
T	~~4.48~~	·92	4.32	0.914
	19.48		18.91	4.003

28-i-50 LdVNA Sylvia's prep

Polyhedra from dirty residue of Geoh prep. stood 6 mos. in water @ room temp. cleaned up. Guessed 600-800 mg. Ovoidin spin off much, then virus. Much "virus" aggregated lost on slow spin. Virus + N-NaOH (1 M NH_4) overnight @ 37°, then

2 spin clear (v. small residue), lovig, ppt c HAc + $EtOH$, + dil NH_3. Some residue is insoluble - spun off.

Clear sol'n vol. ca 6 ml

1:25 in aq. dist.

220	·525	275	·345
225	·403	285	·146
230	·319	295	·067
235	·271	305	·032
240	·257		
245	·272		
255	·315		
260	·320		
265	·304		

Total amt = $\frac{·32}{1} \times 25 \times ·04 \times 6$ = 1.9 mg. NA.

Start: Pa. 600 mg. Virus 24 mg. NA = 15% = 3.5 mg

Ppt from clear sol'n + NH_3 insol. residue both hydrolysed, each + 0.08 ml NA sol'n. 3 x 17.4 μl spots - spoilt by running solvent over paper.

Hydrolysate of ppt: 4 x 17.4 μl spots → good chromatogram. Also one spot from sol'n.

Bovine Tubercle bacillus NA

	Moles/unit	Ratios	Re-calc	
A	2.45	·69	2.46	0.713
G	4.19	1.18	4.05	1.173
C	4.87	1.37	4.67	1.354
T	2.70	·76	2.61	0.757
	14.21	4.00	13.79	3.997

T.B. NA ex bugs.

30-i-50 ca. 2 g. dried bovine T.B. bugs ↑ 10 ml N NaOH @ 37°.

31-i. Spin off bugs - still v. large volume. Supernat. ppt. c HAc + EtOH → fair-sized ppt.

De-fat bugs residue c EtOH + ether. ↑ NaOH, leave overnight at room temp. (by mistake), then overnight at 37°.

2-ii Spin off bugs, filter off some scum. Ppt supernat c HAc + EtOH → small ppt. Combine ppts ↑ water, neutralize, add spit.

3-ii Swing ca. 5 times, ppt c HAc + EtOH, ↑ 3 ml 1 N NH_4OH.

4-ii 1:50

260	·795	270	·695
250	·788	280	·516
240	·729	290	·276
230	1.01		
220			

Total NA = ·8 × ·04 × 50 × 3 = 4.8 mg.

Ppt: HAc + EtOH → big ppt - must contain carbohydrate. Dry down, hydrolyse, ↑ 0.2, [illegible] H. 17.4 μl [illegible].

A	G	C	T	MC 275	MC 280	MC 285
·315	·449	·483	·199	·003	·007	·010
·327	·480	·502	·206	·005	·009	·011
·309	·424	·484	·179	·000	·004	·006
·325	·426	·499	(·246) av	·006	·007	·008
·319	·445	·492	·207			

Tuberculin NA (1)

	Readings	Ratios	Means
A	1.44	.80	.82
G	1.76	.98	.97
C	2.53	1.41	1.40
T	1.47	.81	.81
	7.22		

T.B. NA ex tuberculin

30-i-50 2·5 ml Old Tuberculin T precipitated = ca. 6 ml 90 EtOH + few drops HAc glacial. Spun down ppt., ↑ 5 ml M NaCl ⅟10 NaOH. Swung. Ppt: HAc + EtOH. ↑ ca 5 ml dilute NH_3, spun clear.

1:50 : 230 ·115 240 ·104 250 ·125 260 ·128 270 ·106

Conc. = ·127 × ·104 × 50 = 0·25 mg/ml

Total only 1·2 mg!

Ppt very large ppt – must be carbohydrate.

Cold fresh tuberculin, then ppt., leave overnight

31-i Ppt: HAc + EtOH. → v. small ppt. Spin off. ↑ HCl/EtOH, hydrolyse.

2-ii ↑ 0·5 ml HCl, 17·4 ml spots:

	A	G	C	M/C 275	M/C 280	T
(1)	·197	·188	·247	·018	·012	·118
(2)	·177	·186	·270	·018	·017	·109
$\bar{x}$	·187	·187	·258			·113

3-11-50.

Moisture content of B.D.H. Potato starch

Bottle ~~+ fresh~~ MT ~~starch~~	11.6687
" + fresh starch	13.0903
Fresh starch	1.4216
After 4 hrs @ 110°	12.8121
Water loss	0.2782

Water content = $\frac{.278}{1.422}$ = 19.5% of orig. wt.

Column: 100 g starch (= 80 starch + 20 water) 150 ml BuOH + 4 ml
~~75~~ 60 15 add 100 ml BuOH + 7.5 ml H_2O.

NA ex human T.B. bacilli:

	Vmols/ml	Ratios	New	
A	3.27	0.70	3.28	0.724
G	5.37	1.15	5.18	1.143
C	6.30	1.35	6.05	1.336
T	3.75	0.80	3.60	0.796
	18.69		18.11	3.999

NA ex Human TB bacilli:

ca. 3.5 g. dried heat-killed bacilli ~~for~~ extracted 3 days c̄ EtOH-ether. Then dry c̄ ether, grind c̄ 5 g. alumina powder c̄ a little water, susp. in ca. 20 ml 1 N. NaOH, c̄ overnight 37°.

15-11-50. Spin off big remnants c̄ alumina, re-extract c̄ few ml 1 N. NaOH, liquid: spin clear, ppt c̄ HCl c̄ EtOH, dissolve ppt in N/10 NaOH, several many x.

16-11. Ppt: HCl c̄ EtOH → big ppt. Dissolve in ~~[illegible]~~ 3.8 ml 1 N. NH_4OH.

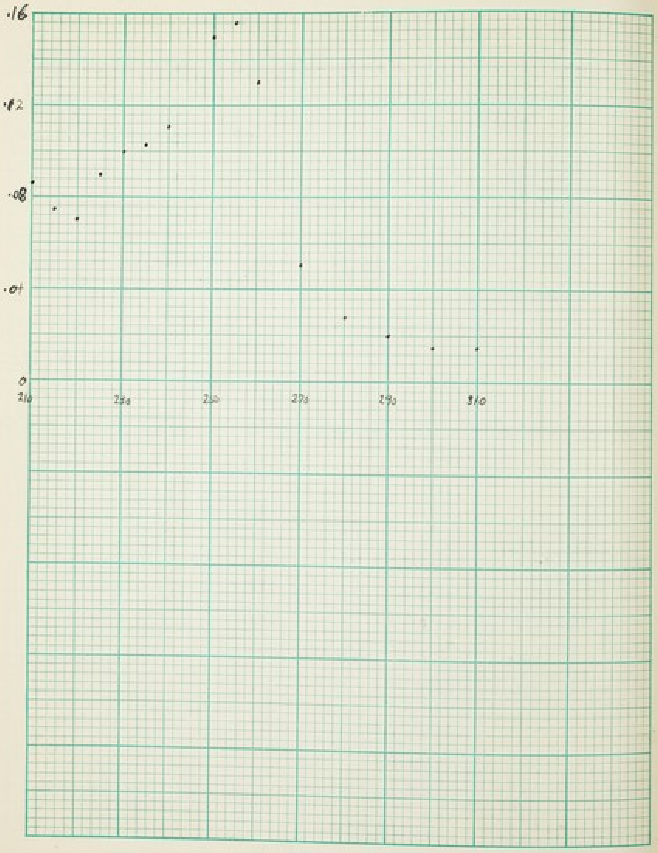
1:200.

230	.590	265	.568
235	.467	275	.454
240	.460	285	.278
245	.509	295	.094
255	.600	305	.026
260	.600		

Total amt NA = .6 × .04 × 200 × 3.8 = 18.2 mg.

2.0 ml pptd in bomb tube, hydr. HCOOH 175° 30 min, ↑ 0.4 ml. Hydrolysate → some brown streak

	A_{260}	G_{250}	C_{275}	T_{265}	$_{270}MC_{280}$	
1	.426	.583	.634	.296	.018	.013
2	.427	.567	.636	.282	.032	.026
3	.431	.553	.644	.273	.017	.017
4	.420	.573	.633	.295	.012	.012
5	.426	.569	.637	.286		



10-11-56 "Pre-T" eluted ex strip chromatogram of HNA. Rerun in isoprop. HCl.

Spot eluted: in N/10 HCl

250	.150	240	.111
255	.156	235	.103
260	.130	230	.100
270	.051	225	.090
280	.028	220	.071
290	.020	215	.075
300	.015	210	.086
310	.015		

Estimation of MC in nucleic acids.

15·ii·50. **BSA** Band containing C + MC eluted quantitatively from isoprop. ·9HCl chromatogram, dried down, ↑ ·05 ml, 2 17·4 µl spots run in BuOH-H_2O. Cut out C & MC spots., MC 5 ml, C 10 ml.

	MC_{280}	C_{275}
(1)	·079	·631
(2)	·069	·621
$\bar{x}$	·074	·626 ≡ 1·252
(µmols/ml:	0·74	11·2

Moles MC = 6·6 % of C

"Molar ratio" = ·057

20·ii. Same c̄ HNA - elute from isoprop. 9HCl, run 40 hrs in BuOH-NH_3.

	MC_{280}	C_{275}
(1)	·163	·975
(2)	·176	·970
$\bar{x}$	·169	·972 = 1·944
mols/ml:	1·69	19·38

Moles MC = 8·7 % of C)

"Mola ratio" = ·075

23·ii. Same c̄ bound NA ③ (contains much aut. RNA)

	MC in 5 ml				C_{275} in 10 ml
	270	275	280	285	
(1)	·036	·035	[·034]	·030	~~[illegible]~~ 1·09
(2)	·016	·018	·019	·018	~~[illegible]~~ 1·06
$\bar{x}$			·020		1·08 = 2·16

- close to solubility limit!

Moles MC = 0·9 % of C

Molar ratio < ·008

Commercial Ca thymonucleate

	Found/mol	Ratios
A	5·05	1·04
G	4·58	0·94
C	4·00	0·83
T	5·76 / 19·39	1·19
MC	0·31	0·064

20·ii·50. Commercial "Ca nucleate ex thymus gland".
10·8 mg., air-dry, hyde. H'oon 176° 30 min, ↑
(Some solvent spilt over chromatogram).

	A	G	C	T	MC 275	MC 280
1	·646	·503	·407	~~·456~~ ·460	·027	·029
2	·648	·478	·407	~~·472~~ ·410	·028	·031
3	·666	·477	·403	~~·498~~ ·430	·029	·033
4	·696	·486	·401	·460	·029	·032
$\bar{x}$	·664	·486	·404	·440		·031

23-ii "MC" in YKA.

(incl. both commercial & re-purified)

YKA hyd. HCOOH 175° 2 hrs sealed in bomb, run in isoprop-HCl. Elute C + MC position, dry down, in 0.05 ml aq. dil., run 2 17.4 μl spots in PhOH-NH3, → big C spot, faint spot in MC position.

	MC in 5 ml								C in 10 ml
	250	255	260	265	270	275	280	285	275
	·078	·074	·074	·074	·066				
(1)						·056	·048	·040	·796
	·026	·029	·036	·044	·045				
(2)						·038	·032	·024	·797

Van Slyke, D. D.
1911. A method for the quantitative determination of aliphatic amino groups. J. B. C. 9: 185–204.

Using $NaNO_2$ (30 g in 100 ml water) 5 equiv. glac. HAc. at 20°. time for quantitative liberation of N_2 was:

α-amino acids	5 min.
lysine amino	½ hr.
NH_3 & CH_3NH_2	1.5–2 hrs.
Urea	8 hrs. (5% in 1 c.c.)
Purines & pyrimidines	2–5 hrs.

Peters & Van Slyke, 1932. use $NaNO_2$ 800 g in 1 liter water. For 2 ml mixture use 1 ml glac. HAc. & 2 ml $NaNO_2$. Amino acids need 3–4 min.

Schneider, W. C.
1945. Phosphorus compounds in animal tissues.
I. Extraction & estimation of desoxypentose nucleic acid and of pentose nucleic acid.

<u>Diphenylamine reaction</u> (Dische). PNA does not interfere.
1 mole DNA gives color = 2 moles desoxycytidine, desoxyguanosine, or desoxyadenosine. Pyrimidine desoxyribosides react only slightly.

<u>Orcinol reaction</u> (Mejbaum, Z. physiol. Chem. 258, 117). PNA + DNA
.0166/.135 = 12.3% give E values for γP 0.135 and 0.0166 respectively. All samples DNA gave same color for γP, as did desoxycytidine & desoxyribosides.
∴ Must apply correction for DNA in estimating PNA.

<u>Splitting nucleoproteins.</u> 5% TCA @ 90° for 15 min. extracts quantitatively, as measured by color reactions. Carry out by adding to tissue suspension equal vol. 10% TCA.
If necessary, first extract acid-soluble P with cold 10% TCA quickly & phospholipid with boiling 3:1 alcohol-ether (remove TCA with 90% EtOH).

Allen, R. J. L.

1940. The Estimation of Phosphorus. B.J. 34: 858–865.

Ammonium molybdate, 8·3% in aq dest. (can add a little NH_4OH to help solution)

Perchloric acid – 60%.

Amidol reagent – 2 g. amidol + 40 g. Na bisulfite in 2 g. dest to 200 ml.
Keep in stoppered bottle in the dark – best to discard after ca. 10 days.

Estimation of orthophosphate.

Soln. + 20 ml. + 400 γP, is put in 25 ml flask.
Add 2 ml perchloric acid, 2 ml amidol reagent, 1 ml ammonium molybdate in that order, then water to 25 ml. Read a deckford filter after 5'–30 min.
Nearly linear 10 – 400 γP.

Total P.

Digest c. 2·2 ml perchloric acid until colorless (for drops 30% H_2O_2 help if much organic material). Cool, rinse into 25 ml flask, add reagents as above. Can develop color in incineration flask by adding 20 ml water, then reagents.

Color is constant between 5'–30 min., but increases after several hours. Temp. variation 8–26° does not affect; for 1·0–2+ ml perchloric, 1·7–2·2 ml amidol, 0·9–1·5 ml molybdate on 5P.

Interference – permissible = 10 γP: $FeCl_3$ ·0013 M – more decreases color
Na_2SiO_3 ·00011 M. (Si : P = 7·5).

In case of turbidity, run extract color & absorbtyf abs. after addition of 1 ml 10% oxalic acid (contains = XS molybdate).

McCarty, M. 1946.

Purification & properties of desoxyribonuclease isolated from beef pancreas.
J. Gen. Physiol. 29 : 123 – 139.

Activity measured by viscosity change of DNA in thymus.
Activation: Mg^{++} ·003 M. is optimal, or can use Mn.
Enzyme is v. active down to 0·006 mg/ml, & to 10x greater dilution if some gelatin is added as stabiliser.
Expts. were run at 30°C.

Enzyme ~~dissolved~~ dissolved in water has pH 4-5, which is max. stability range. Above pH 7, loss of activity is more rapid. Heating 55° 15 min inactivates 90%.
Optimal pH for action 6.8 – 8.2
Inhibitors: fluoride, citrate

Levene, P.A., & E. Lorpes.

1930. A method of separation of ribopolynucleotides from thymonucleic acid and on the conditions for a quantitative separation of the purine bases from the ribopolynucleotides.

J.B.C. 86: 389–401.

To obtain 10% soln of Na salt of pancreas N.A.'s is added large XS of glacial acetic acid. Filter. Ppt contains RNA, & from soln may be ppted DNA by addition of 1.5 vols EtOH.

Extract 67.5% Isopropyl 2.5 N. HCl: 95% Isopropyl: 71 ml
10.3 N. HCl: 24.2 ml
water 4.8
100.0

Ll

Lymantria

Lymantria
dispar
ADENEIC

Data on Purines & Pyrimidines

	Peak	N/10 HCl D for 10 γ/ml in 1 cm layer		M	DM	μ moles/ml for D = 1.0 10	Atoms N.
Adenine	260	101	0.964	135	.0769		5
Guanine	250	66	.703	728	151	.0943	5
Cytosine	270		.910	930	111	.0990	3
Uracil	260		.720	709	112	.1241	2
Thymine	265		.608	639	126	.1305	2
Σ M-C	283		~~.74~~ .68	784	125	~~.107~~ .114	3

Short composition of DNA

Deoxyribose M = 134
H_3PO_4 = 98
232
Subtract $3H_2O$ = 54
∴ Attach to every base 178
Or for H_2 not add 22 = 190

Mean M of deoxynucleotide — H_2O = 309
∴ ribonucleotide — H_2O = 321

P in hypothetical deoxytetranucleotide = $\frac{31}{309}$ = 10.0%