Notebook 4

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NUCLEIC ACIDS

Feb. '50 — May '50.

4

PP/GRW/A/3

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Feb. 1950.

20 ii-50. Ram sperm nucleic acids

Mann's preps 1, 2 & 3.

① Sperm separated from sem. plasma, washed, extracted 2 TCA, residue 2 EtOH-ether, residue dried.

② Sperm separated from sem. plasma, washed, disintegrated, most tails removed, sperm heads together 2 some tails acetone-dried.

③ Sperm separated from sem. plasma, subjected to fractional centrifugation so as to remove most tails. Sperm-heads washed ultrasonically and acetone-dried.

500 mg. each extracted & ppted by Schmidt & Thannhauser. Material ppted 2 HCl + EtOH has Prosthetic: ① 3.87%
② 3.63%
③ 4.25%.

	①	②	③
Dried N.A. in bottle:	4.4404	2.1259	3.1319
Bottle	4.4046	2.0916	3.0521
	35.8	34.3	79.8
Dissolved in aq. dist. vol	5.0	6.1	5.0
→ mg/ml dry wt.	7.16	5.62	15.9

24-ii Kjeldahls:

Blank v. TKA Bailey

h - 0.25 ml sol. in ① .536 } .538 - .018 = .520 = .258 mg N = 14.3 % N
j - 0.25 ml " .540 }

i 0.5 ml sol. in ② .928 } .931 - .018 = .913 = .448 mg N = 15.9 % N
k 0.5 ml " .934 }

E 0.2 ml sol. in ③ 1.125 - .018 = 1.107 = .544 mg N = 17.1 % N
F 0.2 ml " (some lost) 1.025

1-iii Repeats

8 Blank .02
9 " .03

h 0.15 ml sol. in ① 0.82 = .80 × .491 × [illegible] × [illegible] = 16.4 % } 16.8 %
j " 0.86 (flask dirty?) = 17.3 }
l

20-ii-50 Portions weighed out by Mann: ① 95.2 mg
② 101 mg
③ 174.5 mg.

All treated identically: dissolve in 12 ml N/10 NaOH, leaving 9 × [illegible] → v. little more gel. Gels dissolved in EtOH, add 3 drops glac. HAc, give to Mann.
Supernat. add ·1 ml glac. HAc + 15 ml abs EtOH → big stringy ppt. Spin down, wash ō 90% EtOH, abs. EtOH, ether, Dry overnight over $CaCl_2$ salt, then $2\frac{1}{2}$ hrs over P_2O_5 @ 110° in low vac.

22-ii Dissolve in aq. dest. take 1.0 ml aliquot for bases.

P estimation:

			D	
①	P_2 - 0.2 ml		.408	~~.425 = 100 γP = 7.0~~
	P_4 - 0.2 ml		.442	~~104 = ...~~ Repeat.
②	P_6 - 0.2 ml		.370	} .372 = 87 γP = 7.7 % P.
	a - 0.2 ml		.374	
③	b - 0.1 ml		.537	} .537 = 126 γP = 7.9 % P
	g - 0.1 ml		.537	
Repeat ①	P.		.406	} .404 = 95 γP = 6.6 %
	R		.402	

Ram sperm NA. ①, ② & ③ Yield N

		D	Yields/ml	Mol. ratio	Yields/mg dry wt.	% dry wt.
①	A	·527	4.06	1.13	·570	2.25
	G	·345	3.25	0.91	·456	2.28
	C	·312	3.09	0.86	·434	1.30
	T	·303	3.95 / 14.35	1.10	·555	1.11
	MC	·011	·11	·03	·015 / 2.030	·05 / 7.49
	P				2.13	
2 ②	A	·652	5.02	1.14	·641	3.21
	G	·416	3.92	0.89	·501	2.50
	C	·383	3.80	0.86	·486	1.46
	T	·379	4.94 / 17.68	1.12	·630	1.26
	MC	·017	·17	·04	·022 / 2.283	·07 / 8.50
	P				2.48	
③	A	·621	4.78	1.15	·647	3.23
	G	·382	3.60	0.87	·488	2.44
	C	·364	3.61	0.87	·489	1.47
	T	·358	4.67 / 16.66	1.12	·633	1.27
	MC	·015	·15	·04	·020 / 2.277	·06 / 8.47
					2.55	

Dry wt.

① Bases 26.6
Rest as for NA 36.1
62.7

② Bases 29.7
Rest 40.6
70.3

③ Bases 29.7
40.5
70.2

23-11-50 Ram sperm DNA estimation of bases —

Adenine
Guanine
Cytosine
Thymine
Me Cytosine
P
N
% of P
% of N
% of [illegible]

Ram sperm N.A. ①, ② & ③

		D	μmoles/ml	Molar ratio	μmoles/mg dry wt.	% of dry wt.
①	A	·527	4.06	1.13	·570	2.25
	G	·345	3.25	0.91	·456	2.28
	C	·312	3.09	0.86	·434	1.30
	T	·303	3.95 / 14.35	1.10	·555	1.11
	MC	·011	·11	·03	·015 / 2.08	·25 / 7.49
	P				2.13	
②	A	·652	5.02	1.14	·641	3.21
	G	·416	3.92	0.89	·501	2.50
	C	·383	3.80	0.86	·486	1.46
	T	·379	4.94 / 17.68	1.12	·630	1.24
	MC	·017	·17	·04	·022 / 2.283	·17 / 8.20
	P				2.48	
③	A	·521	4.78	1.15	·647	3.23
	G	·382	3.60	0.87	·488	2.44
	C	·364	3.61	0.87	·489	1.47
	T	·358	4.67 / 16.66	1.12	·623	1.27
	MC	·015	·15	·04	·020 / 2.277 / 2.55	·06 / 7.47

Dry wt.

① Bases 26.6, Rest [illegible] 36.1 = 62.7

② [illegible] 29.7, [illegible] 40.6 = 70.3

③ Bases 29.7, 41.5 = 70.2

23-11-50 Ram sperm DNA estimation of bases —

	① % of dry wt.	① μg moles/mg	① Molar ratio	② % of dry wt.	② μg moles/mg	② Molar ratio	③ % of dry wt.	③ μg moles/mg	③ Molar ratio	Mean ratio
Adenine	7.7	·570	1.13	8.55	·641	1.14	8.7	·647	1.15	1.14
Guanine	6.9	·456	0.91	7.55	·501	0.89	7.35	·488	0.87	0.89
Cytosine	4.8	·434	0.86	5.4	·486	0.86	5.45	·489	0.87	0.86
Thymine	7.0	·555	1.10	7.95	·630	1.12	8.0	·623	1.12	1.11
Me-cytosine	0.2	·015	·03	0.3	·022	·04	0.25	·020	·04	·04
P	6.6	2.13		7.7	2.48		7.9	2.55		
N	14.3	10.2		15.9	11.4		16.8	12.0		

	①	②	③
% of P accounted for	95	92	89
% of N accounted for	73	75	71
% of dry wt. accounted for	63	70	70

Ram sperm N.A. ①, ② & ③

	D	Yield/ml	Wt/dilution	Yield/mg dry wt.	Yield N % dry wt			Dry wt.

Ram sperm MC by elution & re-running.

		Ratio	$x-\bar{x}$	$(x-\bar{x})^2$
1-iii	③ N.A.	0·042	3	9
20-iii	① Orig	·035	4	16
	③ N.A	·043	4	16
28-iii	① Orig	·041	2	4
	① S.prod.	·043	4	16
From general analysis		·031	8	64
		·039	0	0
		·037	2	4
		8) 311		129
		39.		

$V_{\bar{x}} = 1.8$

$\sigma_{\bar{x}} = 1.3$

$\bar{x} = 0.039 \pm .0013$

23-ii-50 Ram sperm DNA estimation of bases —

1 ml each ~~NA~~ sol'n dried down, hyd. HCOOH 175° 30 min,

$x \frac{5}{.0275} \times \frac{.35}{.38} \times \frac{1}{7.16} =$ ~~140~~ 136 — put up: ① 0·35 ml (7.16 mg dry wt.) 1 N. HCl

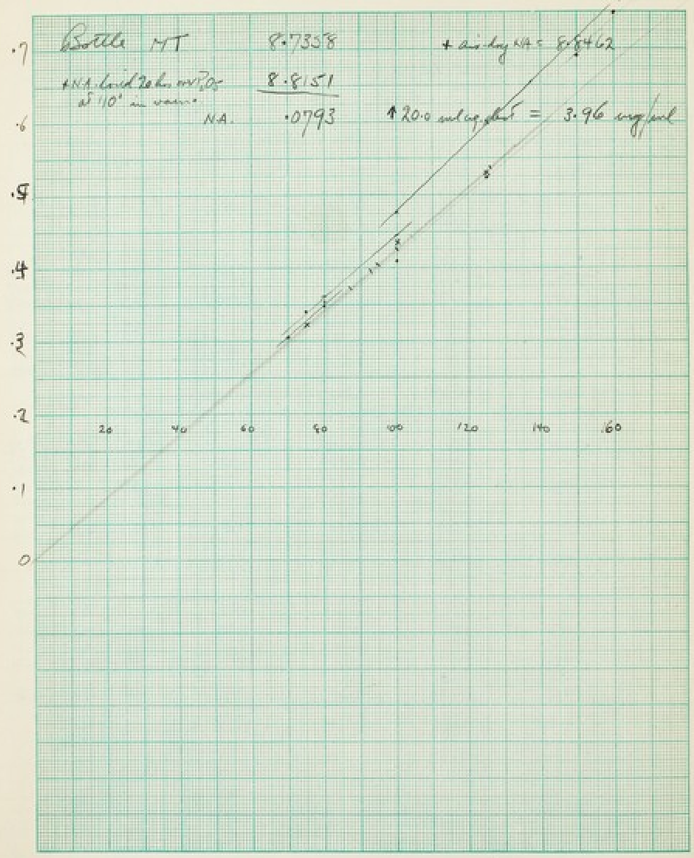
$x \frac{5}{.0274} \times \frac{.25}{5.62} =$ ~~124~~ 128 — ② 0·25 ml (5·62 mg)

$x \frac{5}{.0274} \times \frac{.75}{15.9} =$ ~~126~~ 131 — ③ 0·75 ml (15 mg)

17.4 µl spots, 3 of each, arranged alternately on paper. Single blank.
Re-read after cleaning cells!

		A_{260}	G_{250}	C_{275}	T_{265}	MC 278	MC 282	MC 286
①	1	·525 ~~·550~~	·343 ~~·372~~	·310 ~~·323~~	·302 ~~·323~~	·009 ~~·028~~	·009 ~~·029~~	·009 ~~·026~~
	2	·530 ~~·559~~	·347 ~~·364~~	·314 ~~·336~~	·309 ~~·333~~	·011 ~~·037~~	·011 ~~·037~~	·011 ~~·035~~
	3	·527 ~~·528~~	·344 ~~·387~~	·313 ~~·313~~	·298 ~~·298~~	·014 ~~·010~~	·014 ~~·011~~	·013 ~~·009~~
	$\bar{x}$	·527	·345	·312	·303		·011	
②	1	·648 ~~·669~~	·411 ~~·441~~	·382 ~~·395~~	·362 ~~·392~~	275 ·010 ~~·026~~	282 ·012 ~~·025~~	290 ·009 ~~·023~~
	2	·656 ~~·691~~	·427 ~~·452~~	·386 ~~·442~~	·394 ~~·424~~	·019 ~~·042~~	·020 ~~·043~~	·016 ~~·038~~
	3	·651 ~~·649~~	·409 ~~·409~~	·381 ~~·380~~	·381 ~~·382~~	·017	·018 ~~·017~~	·015 ~~·016~~
	$\bar{x}$	·652	·416	·383	·379		·017	
③	1	·619 ~~·634~~	·376 ~~·407~~	·366 ~~·381~~	·352 ~~·371~~	·009 ~~·024~~	·011 ~~·024~~	·009 ~~·023~~
	2	·628 ~~·656~~	·397 ~~·424~~	·364 ~~·389~~	·370 ~~·399~~	·012 ~~·037~~	·014 ~~·052~~	·011 ~~·034~~
	3	·616 ~~·619~~	·373 ~~·373~~	·362 ~~·360~~	·353 ~~·349~~	·019 ~~·024~~	·020	·016
	$\bar{x}$	·621	·382	·364	·358		·015	

Bottle MT	8.7358	+ air-dry NA = 8.8462
+ N.A. kiln dried 20 hrs over P_2O_5 at 110° in vacuo	8.8151	
N.A.	.0793	↑ 20.0 ml aq. soln = 3.96 mg/ml



21-ii-50. Thymus nucleic acid N. Bailey.

22-ii P estimation – Allen's method – Ilford 608 filter 1 cm cells (↑25 ml)

① – 0.25 ml N.A. sol'n. ·398 } ·396 = 93 γP = 9.4% P

② " " ·394

		Repeat
75 γP	·340	·323
100 γP	·410	·435
125 γP	·523	·530
80 γP (Mann's)	·349	

Kjeldahl: HCl 1 ml = ·491 mg N.

A – blank ·018

D – blank ·018

d – 0.5 ml NA sol'n ·596 – ·018 = ·578 = ·284 mg N = 14.3% N

k – 0.5 ml " ·582 (some lost)

1-iii Repeats – blank 0.02

d – 0.5 ml ·580 } ·59 – ·02 = ·57 = $\frac{.57 \times .491}{.5 \times 3.96}$ = 14.1%

l – " ·60

Repeat – ammonia overnight –

8 – blank ·02

9 " (yearly) ·03

h – 0.5 ml ·575 } ·582 – ·02 = ·562 = ·276 mg N = ~~13.9~~ 14.0% N.

i " ·575

j " ·595

Mean: 14.1%

TNA Barley

	D	µmols/ml	Molar ratios	Orig. sol'n µmols/ml	µmols/mg. dry wt.	µmols N	%
A	·676	5·20	1·10	2·99	0·755	3·78	10·2
G	·428	4·04	0·855	2·32	·585	2·92	8·8
C	·425	4·21	0·89	2·42	·611	1·83	6·8
T	·419	5·46	1·15	3·14	·794	1·59	10·0
		18·91					
MC	·025	0·25	0·053	·144	·036	·11	·45
					2·781	10·23	36·25
						+ 2·78 × 190	52·9
							89·2

P accounted for: 92%.

29.iv.50

Re-calculated c new absorption values & pipette calibration

	D	µmols/ml	Ratios	µmols/mg dry wt.	% of dry wt.	µmols N
A	·676	5·20	1·116	·730	9·86	3·65
G	·428	3·89	0·834	·546	8·25	2·73
C	·425	4·03	0·865	·566	6·28	1·70
T	·419	5·28	1·131	·740	9·33	1·48
MC	·025	0·265	0·056	·0358	0·45	0·11
Total			4·002	2·618	34·17	9·67
					49·8	
					84·0	

25.ii.50

TNA Barley - estimation of bases

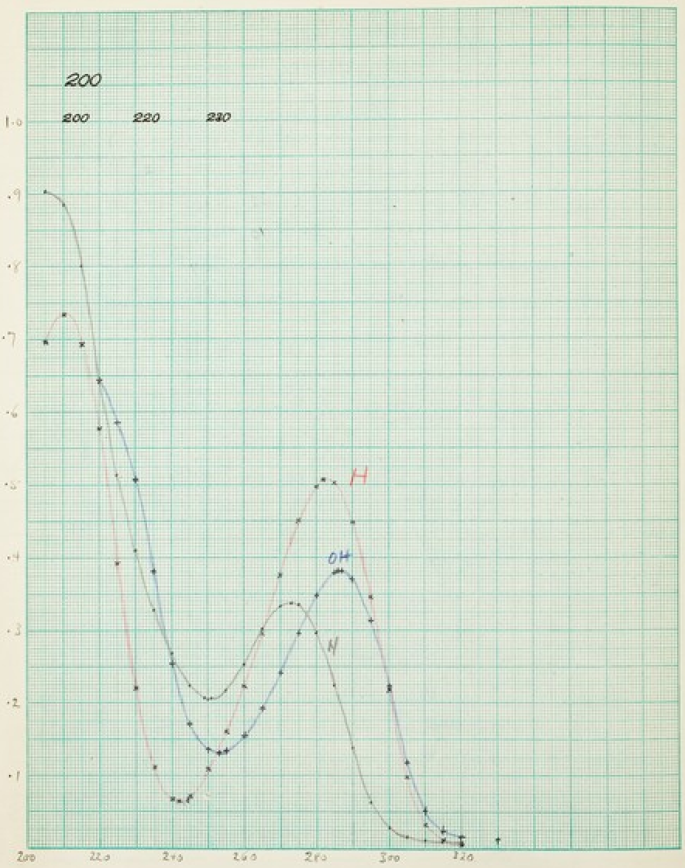
2·0 ml NA sol'n dried down, hydr. HCOOH 175° 30 min, ↑ 0·4 ml, 17·4 µl spots.

$\times \frac{5}{.0178} \times \frac{4}{2}$ = 7·5 (struck out)

	A_{260}	G_{250}	C_{275}	MC 275	MC 282	MC 285	T_{265}
1	·695	·457	·432	·028	·029	·028	·420
2	·669	·390	·410	·014	·017	·016	·404
3	·674	·432	·434	·027	·029	·027	·438
4	·667	·432	·425	·021	·024	·022	·413
$\bar{x}$	·676	·428	·425		·025		·419

$\times \frac{5}{.018} \times \frac{4}{2} \times \frac{1}{3.96} = 140$

	% of dry weight Found	% of dry weight Calc. for bases	µg mols/mg. dry wt. Found	µg mols/mg. dry wt. Calc. for bases	Molar ratios
Adenine	10·2		·755		1·10
Guanine	8·8		·585		·86
Cytosine	6·8		·611		·89
Thymine	10·0		·794		1·15
Me Cytosine	0·45		·036		·053
P	9·4		3·03	2·78	
N	14·0		10·0	10·2	
N.A. Na salt		89·2			



23-ii-50

MC UV spectra

MC ex HNA, purified on paper chromatograms, eluted from isoprop.-HCl partially pure, finally 3 spots each 2 x 17.4 µl run in BuOH-formic, cut out, eluted in 5 ml H_2O, N/10 HCl, N/10 NaOH. H_2O extract neutralised to pH 7 c̄ 1 drop N/10 NaOH. Read against H_2O, HCl, etc., no paper blanks.

λ	HCl		Neutral		NaOH	
205	·696		·903	203 ·96		
210	·734		·885			
215	·693		·800			
220	·577		·641		·644	
225	·392		·518		·585	
230	·220		·410		·506	
235	·111		·328		·381	
240	·068	242 ·065	·268		·254	
245	·072		·224	249 ·206	·170	
250	·109		·201	251 ·206	·136	
255	·161		·217		·134	253 ·131
260	·223		·254		·154	
265	·296		·301		·192	
270	·375		·333		·241	
275	·450		·331	273 ·337	·296	
280	·497	282 ·506	·297		·348	
285	·502		·224		·379	286 ·381
290	·448		·138		·370	287 ·381
295	·346		·063		·313	
300	·218		·028		·223	
305	·098		·016		·117	
310	·032		·012		·051	
315	·012		·010		·024	
320	·006		·009		·017	
						320 ·013

		Acid	Neutral	Alkali
	Maximum	282	273	287
	Minimum	242	250	253
Cytosine (Hotch.)	Max	275 ·7	267 ·6	279 ·8
	Min	238 ·4	247 ·3	250 ·3

15-iii-50.

MC in Locust N.A

2 old hydrolysates run together as band in isoprop-HCl. → no visible U, scarcely visible MC. Elute, run in BuOH-NH3 → faint spot in MC position, adjoining faint A spot. Cut out MC, elute 2 spots together in 5ml, cut out C, elute each spot in 10ml.

	MC						C
270	275	280	283	285	290		275
						(1)	1·27
·081	·086	·087 ↑	·084	·082	·071	(2)	1·26
						x̄	1·26

Neutral: pH 7.0			Alkaline N/10		
	285	·021		270	·044
	280	·030		275	·047
	275	·038		280	·051
	274	·039		285	·052 ←
	270	·044 ←		287	·052
	265	·042		290	·050

Eluate must contain some C &/or A. Assume 70% MC

$$\text{MC then} = \frac{·7 \times ·084 \times 100}{1·26 \times 4} = 1·2\ \%\ \text{of C!}$$

"Molar ratio" = ·010

25-iii-50. **MC estimations**

Commercial Ca-thymus nucleate, run in isoprop-HCl, elute C+MC, dry down, ↑ 0·05 ml aq. dist., run 40 hrs in BuOH-NH3. Elute MC in 5ml, C in 10ml HCl.

	MC				C		
	278	282	285	290	275		
(1)	·203	·225	·225	·202	1·21		
(2)	·183	·207	·207	·187	1·17		
x̄		·216			1·19 = 2·38		"Molar ratio"
	mols:	·022			2·36	Moles MC = 9.3	·097

Human tubercle bacillus NA treated identically. Only very faint spot in MC position.

	MC				C
	278	282	285	290	275
(1)	−·004	+·009	+·013	·012	1·71
(2)	−·011	+·003	+·006	·006	1·70
x̄		·006			1·70 = 3·40

MC < 0·18%

28-iii Bailey's TNA treated identically

	MC			C
	275	282	290	C_{275}
(1)	·110	·123	·107	1·02
(2)	·091	·109	·096	0·99
x̄		·116		1·01 = 2·02

MC = 5·8 % of C.

"Molar ratio" = ·052

28-ii

Synthetic 5-M.C from Pierce.

Sol'n of ~~hydrochloride~~ "5-methyl-cytosine·HCl", not specially dried, made up 3.96 mg/ml in aq. soln. 17.4 μl spots run in BuOH-HCOOH. MC has tailed a bit, & some T separated. Elute MC spots in 8.0 ml H_2O (distilled dry), N/10 HCl, N/10 NaOH, T spots in 5 ml N/10 HCl & 5 ml N/10 NaOH.

T/

	In H. against paper blank	against H, no paper		In OH. against OH (no paper)
230	·014		250	·168
235	·009		260	·145
240	·014		270	·147
250	·035		280	·150
260	·039		285	·151
264	·061		290	·149
265	·061	·103	295	·141
270	·056			

MC/

	HCl	N	NaOH (no paper blank)
205	206 ·721	·895	
210	·727	·871	
215	·666		
220		·614	221 ·521
225	·366		·517
230		·261	·484
235	·126		·395
240	·097	·112	
245	·100	·105	·216
250	·123	·125	254 ·176 ·161
255	·138	·161	256 ·160 ·163
260			·172
265	·263	·258	·202
270			·240
275	·394	·356	·286
280	282 ·431 ·440	·373	·327
285	288 ·435 ·440	·357	286 ·321 ·312
			287 ·308
290	·389	·306	287 ·339 ·310
295	·301		·287
300	·188	·139	·207
305			
310	·030	·026	·065
315			
320	·009	·010	·035

against paper blank

280	·409	Curiously acid
282	·418	
283	·419	
285	·417	

1-iii

M-C estimations

Bundsome in isoprop-HCl, eluted: N/10 HCl, dried, R .05; 2 ml 17.4 spot run in BuOH-1·NH3 40 hrs.
MC ↑ 5 ml, C ↑ 10 ml.

		MC			C	
HNA		275	282	290	275	
	(1)	·122	·136	·118	·758	
	(2)	·129	·141	·122	·756	
	$\bar{x}$		·138		·757 = 1.514	MC = 9.3% of C
						"Molar ratio" = ·080
Ram spam ③	(1)	·075	·081	·069	·860	
	(2)	·077	·084	·070	·840	
	$\bar{x}$		·082		·850 = 1.70	MC = 4.9% of C
						"Molar ratio" = ·042

Revised Molar ratio = $\frac{.082 \times .096 \; (.102) \times .84}{1.70 \times .095} = 0.043$

2-iii-50

Amino-acids in Ham spleen proteins

In bomb tubes (*) / ml N.A. sol'n ① (7.16 mg = 2 mg protein) ↑ 0.15 ml 1 N. HCl
(b) / ml N.A. sol'n ③ (15.9 mg = 4 mg protein) ↑ 0.3 ml 1 N. HCl.
(c) 5.2 mg "Sheep protein" ① ↑ 0.5 ml 1 N. HCl
(d) 5.2 mg " " ③ ↑ 0.5 ml 1 N. HCl.

Hyds 175° 30 min.

Re-calc.

	Yields/μmol	Ratios				Y/expt
A	5.64	1.17		5.64	1.200	38
G	3.73	0.78		3.60	0.766	27
C	4.18	0.87		4.02	0.856	22
T	5.68	1.18		5.49	1.169	34.5
	19.23	4.00	assume MC	·04	0.009	0.25
				18.79	4.000	

Locust DNA repeat.

3-iii Remainder of Locust NA prep put in 1N NaOH 37° overnight, ppt c̄ HAc + EtOH, hyd. HCOOH 175° 30 min. Run 4 spots in isoprop. HCl, remainder as band for MC.

6-iii

	A	G	C	T	MC 275	282	290
omit	[.562	.538	432	.481]	·010	·010	·008
	·744	·432	·422	·452	·011	·011	·008
	·716	·366	·419	429	·010	·010	·008
	·730	·390	·429	·424	·014	·014	·012
$\bar{x}$	·733	·396	·423	·435			

C + MC ↑ ·05. 2 18 μl spots run in BuOH - NH_3

No MC visible ! Elute C + MC both in 5·0 ml N/10 HCl

MC 275	280	283	285	290	C 275
·038	·028	·018	·06	·012	1.02
·003	·009	·012	·03	·012	1.02

Assume ·01

MC = approx 1% of C. Molar ratio = ·01

Adenine recovery by HCOOH & short HCl hydrolysis.

4-iii-50 2 aliquots 1·0 ml sol'n of Bailey's THA dried down, hydrolysed (1) HCOOH 175° 30 min, (2) 1 N. HCl 100° 15 min, Then dry down, + 0·2 ml N/10 HCl, run 17.4 µl spots in BuOH-NH_3.

	HCOOH	HCl.
(1)	·703 ~~[illegible]~~	·683
(2)	·634 ~~[illegible]~~	·671
x̄	·669	·677.

Not well resolved, & A + MC together.

$\frac{HCOOH}{HCl}$ = ·988

6-iii-50. Repeat. 3 spots each on big paper. MC not clear of A, so cut out together. Other substances well resolved.

	HCOOH	HCl.
(1)	·706	·681
(2)	·707	·691
(3)	·715	·682
x̄	·709	·685

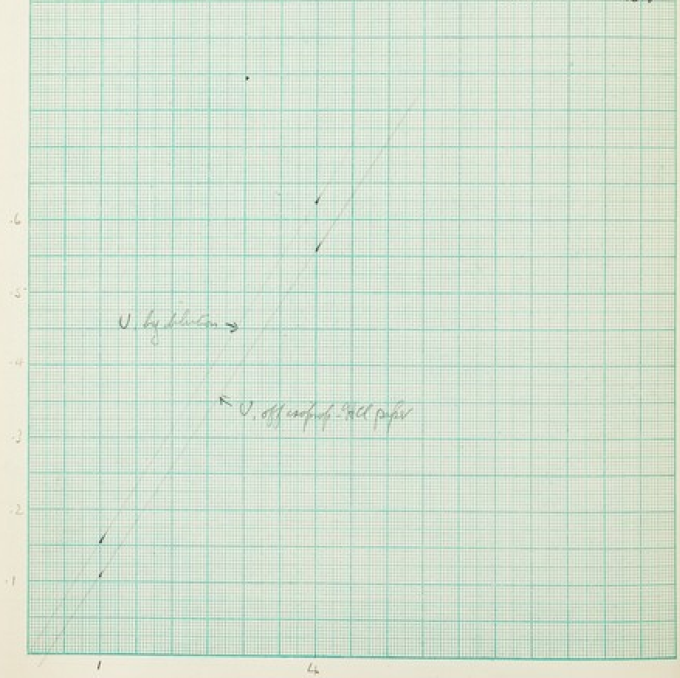
D of MC in HCOOH hyd. = ·025 × $\frac{·25}{·65}$ = ·010

$\frac{HCOOH}{HCl}$ = 1.02

Corrected A D = .699

∴ none destroyed in HCOOH hydrolysis.

	G	U	G	U
Bottle MT	41.0804	41.5935	41.2820	41.2339
+ unknown base	41.1053	41.6169	41.3048	41.2577
dried 2 hrs 105°, cool over P_2O_5	41.1056	41.6168		
			22.8	23.8



7-IV-50. <u>Recovery off paper – uracil & guanine</u>

Standard sol'ns G & U made up: dry 2 bottles, weigh in ca. 25 mgs G & U, dry 2 hrs 105°, cool over P_2O_5, weigh again (no loss of moisture). Add to each 10.0 ml N/10 HCl. Failed to dissolve. Discard. Weigh out fresh U & G, without drying. Add 10.0 ml N/10 NaOH, dissolve. Run 3 18 µl spots of each in ~~Bu~~ isoprop-HCl.

	U_{260}	G_{280}
(1)	·578	·571
(2)	·580	·576
(3)	·569	·568
	·576	·572
By direct dilution:	·623	·620
% recovery:	92	92

Repeat c U sol'n & same dil. 1:4, in BuOH-NH_3 & Isoprop-HCl.

	BuOH-NH_3 0.59	BuOH-NH_3 2.38	Isoprop-HCl 0.59	Isoprop-HCl 2.38	By dilution 0.59
(1)	·128	·621	·107	·550	·157
(2)	·089	·594	·107	·560	·154
(3)	·103	·595	·103	·567	·155
$\bar{x}$	·107	·603	·106	·559	·155

7.iii

Synth. MC.

17.4 µl spots of sol'n 3.48 mg/ml run in [illegible] → MC + T, both cut out. Elute 3 spots each in 5 ml N/10 HCl. 1 spot MC in N/10 NaOH, 1 in H_2O, neutralize c 1 drop N/10 NaOH → pH 7. Paper blanks eluted in HCl, NaOH, H_2O. Read against blanks.

	MC in HCl 282		T in HCl 265	
(1)	·664	·666	·066	·060
(2)	·668		·058	
(3)	·667		·056	
$\bar{x}$				

	HCl (1)	NaOH	pH 7
205			
210	209 ·766 ·712		
215	211 ·748 ·770; 212 ·750; 213 ·769		·96
220	·622	221 ·654	·754
225	·423	·611	·597
230	·228	·516	
235	·096	·362	·380
240	242 ·046 ·042	·218	
245	243 ·049 ·043	·137	·257
250	·088	·104	251 ·229 ·228
255		252 ·106 ·102; 253 ·103	252 ·241 ·229
260	·240	·137	
265			·255
270	·470	·262	273 ·407 ·419
275	·579		274 ·420 ·421
280	282 ·643 ·668	·405	·378
285	283 ·659 ·664	286 ·446 ·450; 287 ·450; 288 ·449	·291 (4% too low)
290	·591	·436	·180
295	·460	·364	
300	·277	·249 (10% too low)	·032
305	·114		
310	·030	·036	·007
315			
320	0	0	·004

8-III-60. Sarkey's THA

Soln 3.96 mg/ml, standing in frig 16 days. dil 1:100 pH 7.0.

210	1.13			
220	·629			
225	·452			
230	·386	232	·384	
235	·415			
240	·521			$\frac{D_{258}}{D_{232}} = 2.47$
250	·816			
255	·92	257	·945	
260	·945	258	·95	
265	·90	259	·95	
270	·820			
280	·527			
290	·248			
300	·059			
310	·018			
320	·011			

39.6 γ/ml → ·95

Soln as diluted contains P: $\frac{9.4}{100} \times 3.96 \times \frac{1}{100} = 3.72$ γ/ml

$= \frac{3.72}{31} = .120$ mM

$\varepsilon_p = .95 \times \frac{1000}{.12} = 7900$

	Hoffman Rale	Roy's recrystallized
Dried Bottle MT	42.3564	40.6142
+ Gramine	42.4144	40.6500
After drying	42.4148	40.6508
Gramine (take lower weight)	58.0	35.8 mg
Both ↑ 20 ml N/10 NaOH: Conc =	2.90 mg/ml	1.79 mg/ml

Kjeldahl

Blank .01

b 0.3 ml H-R	.76	$.745 \times \frac{.59}{2.90} \times \frac{1}{.3} = 42.8\%$	92%
d "	.75		
i 0.5 ml Roy's	.76	$.75 \times \frac{.59}{1.79} \times \frac{1}{.5} = 41.0\%$	88 / 90%
P5 "	lost by bump		

Theoret. N. content $= \frac{5 \times 14.01}{151.1} = 46.4\%$

Standard UV absorption.

Gramine weighed, dissolved in N/10 NaOH, diluted c N/10 HCl: ~~Roy's~~ both, 0.5 ml ↑ 100 ml.

Cell correction (250 mμ): subtract .007 from observed value.

	Roy's	H-R
247	.658	
248	.661	1.08
249	.660	
250	.656	1.07
251	.651	
226	.204	
225	.197	.334
224	.195	.330
223	.198	

D of 10 γ/ml: Roy's: $\frac{.649}{} \times \frac{.01}{1.79} \times \frac{100}{.5} = .725$ (.649 replacing ~~.656~~)

H-R: $1.06 \times \frac{.01}{2.90} \times \frac{100}{.5} = .731$ (1.06 replacing ~~1.07~~)

} .728

Bomb tube MT 10.3387

" + N.A. dried 3 hrs/110° (turned slightly brown) 10.3695

30.8 mg.

Dissolve in 1.0 ml HCOOH. Pipette out 0.25 (0.2) ml & dilute to 2.5 (2.0) ml for P & N.
Remainder (0.8 = 24.6 mg) seal off & hydrolyse, ↑ 0.75 ml.

Kjeldahl: a blank .017
d " .019
g 0.2 ml 1/10 soln .205
l " .195 } .20 − .018 = .182 = 89.1 γN = 14.5%.

P 0.5 ml 1/10 soln (micro cell, 608 filter, against water) .69
" .72 } .705 = 148 γP
100 γP .476 = .148×2 / 3.08 = 9.6%
125 γP .600

Repeat: 0.25 ml against reagent blank .341
0.25 ml .330 } .335 = 76 γP = 9.9%
70 γP .305
80 γP .354

Mean = 9.7% P

	μmols/ml	μmols/mg dry wt.	Ratios	% of dry wt.	μmols N
A	8.16	.690	1.060	9.33	3.45
G	7.19	.608	0.934	9.19	3.04
C	5.37	.455	0.699	5.05	1.36
T	8.27	.700	1.076	8.82	1.40
MC	1.76	.149	0.229	1.86	0.45
	32.07	2.602	3.998	34.25	9.70

μmols/mg dry wt. = 84.6

.26 × 190 = 49.4
83.7

% of P accounted for 87%

11-iii-50. Bemax N.A. (Sylvia)

232 g. Bemax in 1M. NaCl to 1500 ml, leave in frig. 2 days. Dilute to 2½ litres.

13-iii Spin, pour slightly viscous supernat into 12 l. tap water, collect stringy ppt. by straining through muslin. Susp. in 200 ml 1 M. NaCl, made pH 9, spin 15 min on Sorvall, (→ biggish pellets), to soln add 20 ml 40% NaOH, leave overnight at 37°.

14-iii Ppt c̄ HAc + EtOH, susp. in 200 ml M NaCl pH 10 (turbid), Sevag till v. little gel (only 3×), dialyse overnight (Permutit water). Spin on Sorvall 30 min → clear soln. Ppt c̄ HAc + EtOH → only faint turbidity. Add HCl to pH 3, then some M NaCl → more ppt. Salt evidently necessary. Spin off.

Factor: ×·7½ · 5 / ·018 · 24.6 = 146

24-iii

		A	G	C	T	275	MC 283	290
Blank		.047	.052	.030	.084	.029	.025	.022
	(1)	1.11	.845	.595	.744	.187	.199	
	(2)	1.11	.846	.597	.744	.184	.199	
	(3)	1.11	.842	.596	.733	.179	.194	
	(4)	1.11	.835	.594	.739		.195	
	x̄	1.11	.842	.595	.740		.197	
Subtract blank		.05	.052	.030	.084		.025	
		1.06	.790	.565	.656		.172	
μmols/ml		8.15	7.46	5.50	9.14		1.72	
Ratios		1.08	0.98	0.74	1.27		0.23	

Bottle	11.6685
+ Beef spleen N.A., not specially dried	11.7562
N.A.	87.7 mg.

P estimation: (1 cm cells, 608 filter, against incin. blank)

a – 0.05 ml soln. .318
d " .330 } .324 = 72 γ P $= \frac{72}{.05} \times \frac{4}{877} = 6.6\%$.

80 γ P .361
100 γ P .445

% of total released

Hydrolysis time	A	C	T	P (estimated by Mann)	
0				0.12% of prep. =	1.8 % of total P
7'	100			.85	13
15'	97	3		1.49	23
30'	95	7	11.5	2.34	35
60'	94	12	20	2.65	40
120'	90	21	35	3.12	47
Total P (Mann, 5 min incineration)				6.15	
(self, 1 hr ")				6.6	100

14.iii Rate of release of bases by hydrolysis in N. HCl at 100° (with Mann).

87.7 mg (air-dry) beef spleen N.A. dissolved in 4.0 ml N/20 NaOH. 0.5 ml portions pipetted into bomb tubes; to each add 0.5 ml conc. HCl → 1 N., stopper, place on B.W.B. 7—120 min. Inorganic phosphate estimated by Mann. 2 18 μl spots from each run in BuOH-NH_3 44 hrs. (T very close to bottom).

		A	C	T
7'	(1)	.581		
	(2)	.529		
	$\bar{x}$	.555		
15'	(1)	.547	.008	
	(2)	.527	.012	
	($\bar{x}$)	.537	.010	
30'	(1)	.579	.024	.041
	(2)	.576	.024	.039
	($\bar{x}$)	.578	.024	.040
60'	(1)	.508	.036	.068
	(2)	.540	.042	.068
	($\bar{x}$)	.524	.039	.068
120'	(1)	.500	.069	.127
	(2)	.500	.074	.115
	($\bar{x}$)	.500	.072	.121
Total, by ratio (19.iii.49) to 7' A		.555	.338	.349

11-iii-50 5-M-C, synth & ex. HNA, UV curves.

18 μl synth "5-M-C", 3.48 mg/ml, & 18 μl conc. fresh MC ex HNA, run 3 spots of each, in BuOH-NH$_3$.

Extn from N/10 HCl, N/10 NaOH, & aq. dest., neutralized c̄ ca. 0.1 ml N/10 NaOH to → pH 7.0. Read against corresp. blanks.

	N Synth	N Nat	H Synth	H Nat	pH 14 Synth	pH 14 Nat
205			208 ·778	·778		
210	·835	·861	211 ·791 ·790	·786 ·781		
215	·774	·788	·765	·746		
220	·621	·674	·632	·622	·22 ·569	·598
225	·514	·529	·431	·428	·553	·576
230	·433	·416	·240	·245	·479	·498
235	·360	·360	·115	·128	·328	·363
240	·307	·326	242 ·069 ·065	·087 ·088	·205	·236
245	·255	·273	247 ·070 ·065	·087 ·083	·133	·161
250	251 252 ·224 ·223 ·221	·238 ·236 ·234	·104	·115	252 253 ·104 ·102 ·106 ·102	·127 ·123 ·124 ·122
255	·230	·239	·168	·167	·136	·148
260	·276	·279	·246	·242	·136	·148
265	·334	·333	·351	·328	·188	·195
270	273 ·382 ·391	·376 ·384	·458	·449	·256	·258
275	274 ·391 ·392	·383 ·385	·574	·568	·332	·327
280	·348	·337	·650	·616	·396	·387
285	·260	·251	283 ·661 ·666	·627 ·632	287 ·435 ·427	·423 ·416
290	·151	·144	·595	·564	·424	·412
295	·069	·055	·452	·431	·510	·342
300	·016	·016	·282	·271	·238	·235
305	·001	·004	·116	·115	·112	·115
310	0	·002	·033	·021	·028	·037
315	0	·002	·005	·009	0	·027
320	0	0			0	0

Thymine D_{265}: ·051

BEMAX N.A.

	Fractional	Ratios	Re-calc. from new D values. Combined	Ratios
A	4.89	1.10	4.89	1.066
G	4.44	1.00	4.29	0.936
C	3.27	0.74	3.13	0.683
T	5.16 / 19.76	1.16	4.99	1.087
NC	~~4.83~~ 1.12	0.25	1.04	0.227
			18.34	3.999

14-iii-50. Bemax N.A.

First prep, before ca. 8-iii, made by method similar to that of 11-iii. (viz. 1 N. NaOH).
Spots extracted only 2 hrs.

	A	G	C	T	NC 275	NC 283	NC 290
1	.633	.468	.330	.388	.089	.099	.088
2	.630	.466	.330	.408	.095	.105	.091
3	.651	.478	.331	.398	.096	.106	.094
4	.632	.474	.328	.391	.092	.103	.090
&	.636	.471	.330	.396		.103	

	280	285	287	290	295
NC, after adding 1/2ml H_2SO_4:	.062	.086	.087	.086	.058

Pipette taken as 18.1 µl.

Ram sperm fractions weighed (air-dry) directly into [illegible]

	%P (Mann)	Wt. hydrolysed	Vol. HNO₃ [illegible]	Hydrolysate mg P/ml
(1) Orig. prep.	2.13	42.6 mg	0.5 ml	1.81
Levag prot.	1.33	14.1	0.15	1.25
(2) Orig. prep.				
Levag prot.	1.17	—		
(3) Orig. prep.	3.14	39.6	0.5	2.48
Levag prot.	2.22	31.0	0.4	1.72

	(1) Orig. prep.		(1) Levag prot.		(3) Orig. prep.		(3) Levag prot.	
	γ/ml	Ratio	γ/ml	Ratio	γ/ml	Ratio	γ/ml	Ratio
A	4.70	1.08	4.24	1.02	7.40	1.06	4.92	.93
G	3.74	.86	3.90	.94	6.37	.92	5.16	.98
C	3.76	.87	3.64	.87	5.91	.85	4.44	.84
T	5.14	1.19	4.86	1.16	8.17	1.17	6.58	1.25
MC	< .32	< .07	< .32	< .08	< .32	< .05	< .28	< .05
Bases Total	17.66		16.96		28.17		21.38	
Correct. P found	21.0		14.5		28.8		19.9	

15-iii-50. **Ram sperm fractions**

Mann's original prepns (1) & (3) (see 20-ii-50), & "Levag proteins" (1) & (3) hydrolysed HCOOH 175° 30 min. Run 3 18 µl spots of each run in isoprop.-HCl. → brown streak.

		A_{260}	G_{250}	C_{275}	T_{265}	MC 275	MC 283	MC 290
(1) Orig	(1)	602	394	379	408	038	037	031
	(2)	611	401	376	386	029	028	023
	(3)	630	397	385	389	031	031	026
	$\bar{x}$	.611	.397	.380	.394		.032	
S. prot	(1)	550	407	381	365	034	032	023
	(2)	550	418	361	378	034	029	022
	(3)	556	416	361	373	041	026	028
	$\bar{x}$	.552	.414	.368	.372		.032	
(3) Orig	(1)	.970	673	602	635	036	036	030
	(2)	.970	678	598	630	029	029	025
	(3)	.950	678	595	614	030	031	026
	$\bar{x}$	.963	.676	.598	.626		.032	
S. prot	(1)	.643	549	452	482	026	025	019
	(2)	.636	542	444	504	027	027	021
	(3)	641	554	450	526	035	032	025
	$\bar{x}$	.640	.548	.449	.504		.029	

P/ette = 18.1

RAT NUCLEIC ACIDS

	Bone Marrow		Spleen	
	Yield/ml	Ratios	Yield/ml	Ratios
A	6.22	1.14	5.75	1.09
G	4.75	.87	4.61	.87
C	4.74	.87	4.45	.84
T	6.20	1.13	6.29	1.21
MC	< .22	< .04	< .26	< .05
Total bases	22.13		21.36	
Corresp. P found	21.0		23.5	

Re-calc: B.M.

	Yielded	Ratios
A	6.24	1.160
G	4.59	0.854
C	4.55	0.846
T	5.99	1.114
MC	0.12	0.022 (guessed)
	21.49	3.996

15 · iv · 50. Rat nucleic acids (Mrs. Mann's preparations)

Bone marrow (clear sol'n): 27 mg, 5.9% P, in 5 ml water. P = .27 mg/ml
Spleen (brown sol'n): 100 mg, 3.02% P, in 10 ml water. P = .302 mg/ml
Of each, 2.0 ml dried down, hydr HCOOH, ↑ 0.3 ml.

Orig: eluate factor $= \frac{2}{.3} \times \frac{.0181}{5} = 241$

		A	G	C	T	MC 275	MC 283	MC 290
Marrow	1	836	523	490	475	041	033	025
	2	802	502	471	488	022	019	015
	3	802	499	479	466	021	019	016
	4	802	494	476	472	021	018	014
	$\bar{x}$	.810	.504	.479	.475		.022	
							Say .012	
Spleen	1	745	473	449	469	024	021	016
	2	750	478	451	478	036	033	026
	3	753	484	454	497	025	024	018
	4	743	482	445	481	029	025	018
	$\bar{x}$	.748	.479	.450	.481		.026	

		Molar ratio Marrow	Spleen
MC estimation:	23-iii	.043	.053
	14-v	.046	.056

20-iii-50. MC estimations - Ram sperm fractions & Rad.

Run in usual way, elute band ft. isoprop. HCl, 1·05; 2 18μl spots in BuOH·NH3. MC ↑5ml, C ↑10ml.

		MC 275	MC 283	MC 290	C 275	Molar Ratio
Ram sperm ③ orig	(1)	·109	·120	·105	1·53	
	(2)	·109	~~·122~~ 118	·106	1·55	
	$\bar{x}$		·119		1·54 = 3·08	·119/3·08 × ·102/·095 × ·54 = 0·035
③ NA	(1)	~~·094~~ ·097	·108	·088	1·10	
	(2)	·100	·106	·091	1·10	
	$\bar{x}$		·105		1·10 = 2·20	·105/2·20 × ·102/·095 × ·54 ... = ·043
③ S. prot	(1)	·189	·203	·179	1·39	
(burnt in	(2)	·180	·194	·170	1·33	
drying down?)	($\bar{x}$)		·198		1·36 = 2·72	·198/2·72 × [illegible]

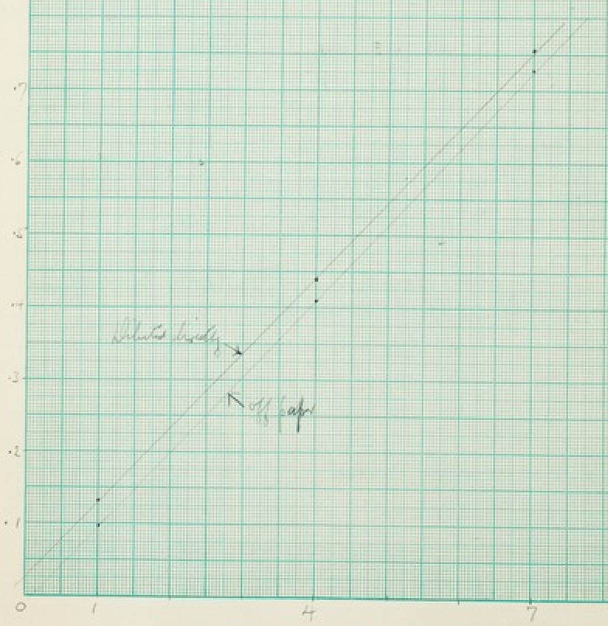
28·ix Repeat fractions of prep ①, but n. little S. prot., so only one spot. Read against blanks, in usual way.

		MC ↑5ml 275	283	290	C ↑10ml 275		Ratio
① Orig	(1)	·061	·057	·049	·625		
	(2)	·053	·056	·049	·625		
	$\bar{x}$		·057		·625 = 1·25	[illegible]	·041
① S. prot.		·074	·077	·066	·804 = 1·61	[illegible]	·043

Bottle MT 11.6680

" + dried re-crystallized thymine 11.7858

Thymine 117.8 mg. in 25 ml 1 N. HCl = 4.71 mg/ml.



16-iii **Thymine - standard absorption & recovery from paper.**

Commercial thymine, once recrystallized from water, dried 3 hrs at 110°, weighed & dissolved in 1 N. HCl.

Make dilutions: 7 ml sol'n + 3 ml N/10 HCl

4 + 6

1 + 9

Run 2 18·1 μl spots from each in [illegible]-HCl & [illegible]-NH_3, but not orig. sol'n, which has crystallized out!

18-iii 1/10 4/10 7/10

·18 ml 750 (1) ·749, (2) ·752 } ·750

D for 10 γ/ml = ·750 × ·01/4.71 × 10/7 × 50/·18 = ·632

Pipette [illegible] (1) ·139, (2) ·126 } ·132 ; (1) 440, (2) ·440 } ·440 ; (1) ·746, (2) ·766 } ·756

Re-read after adding bits of clean filter paper - standing overnight.

1/10	4/10	7/10
·140	·445	·746
·131	·446	·776

no adsorption to paper.

off NH_3 (1) ·097, (2) ·099 } ·098 ; ·408, ·414 } ·411 ; ·729, ·738, ·726 } ·731

·096 ·410 ·728 — discrepancies ·036, ·030, ·028 $\bar{x}$ = ·031

off HCl (1) ·094, (2) ·094 } ·094 ; ·404, ·413 } ·409 ; ·733, ·718 } ·726

Theory, of D = ·632 and pipette [illegible] } ·107 ·429 ·750

Theor. D in 7/10, from Hotchkiss absorption value

$= 4.71 \times \frac{7}{10} \times \frac{\cdot 0181}{5} \times \frac{1000}{10} \times \cdot 608 = \cdot 726$

18.iii. Thymine recovery after various lengths of run.
4 strips paper, each c̄ 2 18 µl spots of 4/10 thymine, run in isoprop. HCl, & taken off after: $2\frac{1}{2}$, $7\frac{1}{2}$, $19\frac{1}{2}$, 25 hrs

Hours run:		0	$2\frac{1}{2}$	$7\frac{1}{2}$	~~20~~ $19\frac{1}{2}$	25
Distance run:		0	4 cm	11 cm	20 cm	23 cm.
T_{265}	(1)	.449	.417	.410	.412	.414
	(2)	.433	.424	.427	.406	.416
	$\bar{x}$	.441	.420	.418	.409	.415

Bomb tube		10·8398
" + N.A. dried 3 hrs @ 105°		10·8458
	N.A.	6·0 mgs. Hydrolyse ↑ 0·25 ml

16·iii·52 **Locust N.A. (4)**

Locusts killed = ether, de-winged, de-gutted, then weight 34 g. Waring blend in ·9% NaCl, strain, spin 8 min MSE. give supernat to f O.8 for RNA, susp in ·9% NaCl + spin again. Susp in 125 ml water, add 125 ml 2 M. NaCl, leave in frig overnight.

17·iii Spin off residue, ppt from supernat by pouring into 6 vols tap water. Spin down ppt, susp. in 1 M. NaCl + NaOH → pH 9, spin off insol. material, add 1/10 vol 40% NaOH, leave overnight @ 37°.

18·iii Filter off some scum, ppt = HAc + EtOH, dissolve in 1M NaCl

Sevag 4 x. ppt. ↑ H_2O

1:100					
220	·546	257	·712		
230	·317	260	·721	300	·077
232	·312	262	·717	310	·021
235	·324	270	·654		
240	·398	280	·474		
250	·619	290	·254		

$\frac{D_{260}}{D_{232}}$ =

Sevag again several times, ppt, wash = EtOH + ether, dry.

Against N/10 HCl Blank: A , G , C , T , MC 275 283 290

(1) Not read, because blank too big.

(2)

(3) MC estimation. C + MC band eluted all put in one spot.

(4)

	275		270 275	283	290	265
Blank	·034	Blank	·046	·037	·031	
C in Total	2·00	MC in Total	·121 ·120	·108	·089	·118
C − B	1·97	MC − B	·074	·071	·058	

Assume 40% MC = ·028

MC Ratio = $\frac{·028}{1.97 \times 2} \times \frac{102}{95} \times 84$ = ·006

Bomb tube 10.2021

" " + uridroid DNA 10.21.70

Uridroid DNA 1H.9 mg 1·5 ml

IV·iii Schwartz DNA.

Stiff viscid opals. Read U + T only, against proper blanks.

	U_{260}	T_{260}
(1)	·166	·586
(2)	·168	·566
(3)	·168	·568
$\bar{x}$	·167	·573
Rmols/ml	2·07	7·48

Uppon % RNA = $\frac{2·07}{9·65}$ = 21·8 %

	Vendo	Retro
A	4.14	1.51 ~~0.60~~
G	2.13	0.60
C	3.37	0.95
T	4.59	1.03
	14.23	4.09

11.12 Earthworm DNA.

	220	·758	
1:100	230	·542	235 · 534
	240	·594	
	250	·761	
	260	·83	
	270	·744	

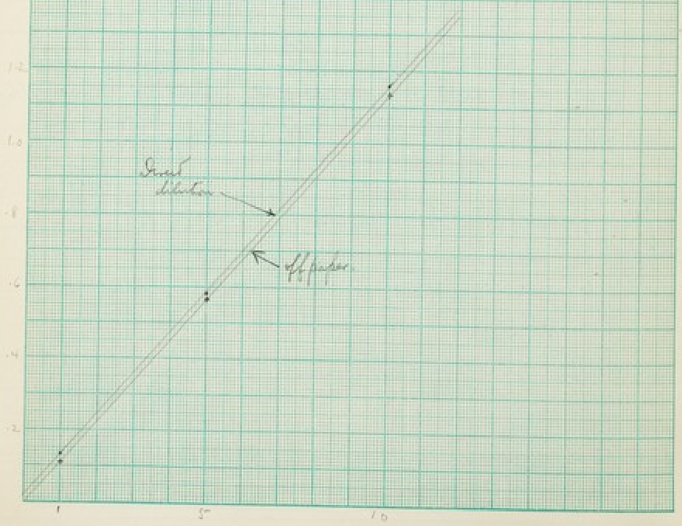
N.A conc. = ·83 × ·04 × 100 = 3.3 mg/ml. Total N.A. = 3.3 × ·5 = 1.6 mg.
All hydrolysed, + ·08, final in 3 ml to 18 µl. Some brown streak.
Read in usual way against proper blanks.

	A_{260}	G_{250}	C_{278}	T_{265}	MIC: 3 black : one 270 · 283 · 290
(1)	·542	·234	·347	·369	
(2)	·530	·224	·358	·357	·076 ·066 ·057
(3)	·542	·244	·357	·369	
$\bar{x}$	·538	·234	·354	·364	

Bottle MT

+ dry thymine

13·5329	6·3125
·6393	6·4406
·1064 mg.	128·1 mg. ·925 ml = 5·14 mg/ml



21-ii Thymine, re-crystallized & dried, made up 5·14 mg/ml in N/10 NaOH, & diluted 1/2 & 1/10 in N/10 NaOH.
From each dil. 1 pipette-ful in 5 ml N/10 HCl, & same time 2 spots on paper. Also 0·18 ml → 50 ml N/10 HCl

D_{265}:

	1/10		1/2		1/1		1/1 ·18 → 50
(1)	·138	·132	·580	·583	1·16	1·16	1·16
(2)	·127		·587		1·16		1·16

D_{265} of soln containing 10γ/ml = $1·16 \times \frac{·01}{5·14} \times \frac{50}{·18} = ·629$

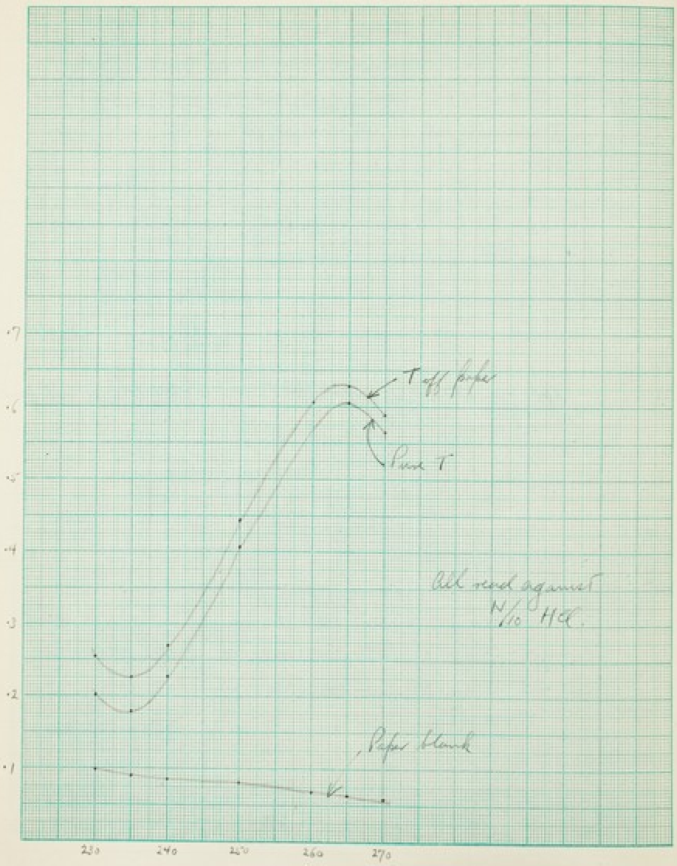
22-iii Spots run overnight in isoprop. HCl. Cut out blank beside each spot, & read against blanks:

	1/10		1/2		1/1	
	·112	·110	·561	·566	1·14	1·135
	·108		·571		1·13	
[illegible]	·116		·582		1·16	
Recovery	95		97		97	

23-iii Re-read to test formula, against N/10 HCl:

	1/10 (1)	(2)	$\bar{x}$	1/2 (1)	(2)	$\bar{x}$	1/1 (1)	(2)	$\bar{x}$
235	·109	·114	·111	·220	·229	·224	·372	·372	·372
265	·172	·172	·172	·621	·636	·629	1·19	1·19	1·19

	1/10	1/2	1/1
by formula:	T = ·118	T = ·595	T = 1·17
	B = ·061	B = ·040	B = ·13
	·179	·635	



22-iii. **Estimation of blank behind a spot**

All read ~~Blanks~~ against N/10 HCl :

	Blanks of paper				Thymine on paper			Pure Thymine		
	(1/10)	(2)	(3)	$\bar{x}$	(1)	(2)	$\bar{x}$	(1)	(2)	$\bar{x}$
230	·108	·103	·087	·099	·254	·256	·255	·206	·196	·201
235	·096	·092	·081	·090	·225	·228	·226	·182	·176	·179
240	·090	·087	·079	·085	·267	·274	·270	·229	·225	·227
250	·083	·080	·076	·080	·438	·448	·443	·411	·404	·407
260	·072	·066	·066	·068	·601	·611	·606			
265	·066	·061	·061	·063	·628	·633	·628	·611	·599	·605
270	·062	·058	·058	·059	·584	·594	·589	·572	·558	·565

To find true blank (in presence of T spot) at 265; $D_{B_{265}}$:–

$D_{235} = D_{B_{235}} + D_{T_{235}}$

$= 1.43\, D_{B_{265}} + .296\, D_{T_{265}}$

$3.38\, D_{235} = 4.83\, D_{B_{265}} + D_{T_{265}} = 3.38 \times .226 = .764$

And $D_{265} = D_{B_{265}} + D_{T_{265}} = .628$

Subtract. $3.83\, D_{B_{265}} = .136$

$\therefore D_{B_{265}}$ (true blank) = $\underline{.036}$

cf observed blank = ·063

Difference = $\underline{.027}$

General formulae: $D_{T_{265}} = 1.26\, D_{265} - 0.88\, D_{235}$ (→ ·592)

$D_{B_{265}} = 0.416\, D_{265} - 0.99\, D_{235}$ (→ ·027)

23-iii.

Thymine recovery + blanks.

To 3 blank strata of yesterday added 1 pipette full "3·4 μg" thymine, mixed, read against N/10 HCl.

	1/10	1/2	1/1	2
230				
235	·260	·259	·234	·251
240				
250	·471	·476	·468	·472
260				
265	·666	·671	·670	·669
270				

$D_{T_{265}} = 1{\cdot}26\, D_{265} - 0{\cdot}48\, D_{235} = {\cdot}842 - {\cdot}221 = {\cdot}621$ (should be ·605)

$D_{B_{265}} = 0{\cdot}416\, D_{265} - 0{\cdot}99\, D_{235} = {\cdot}278 - {\cdot}248 = {\cdot}030$ (should be ·063)

23-iii

MC ex Rat NA's

Read allagainst N/10 HCl.

	MC Blank	Marrow MC₁	MC₂	MC₂ − B	Spleen MC₁	MC₂	MC₂ − B
235	·107	·104	·116				
242	·093	·097	·101		·121	·112	
250	·088	·100	·099				
260	·088						
270	·088						
275	·081	·144	·131	·056	·157	·141	·068
283	·058	·124	·116	·062	·140	·129	·077
290	·048	·106	·101	·055			

	C blank	Marrow C₁	C₂	C₂−B	Spleen C₁	C₂	
230	·120	·331	·343				
235	·098	·226	·240		·231	·232	
240	·089	·206	·221		·212	·214	
245		·252	·266				
270	·072						
275	·067	·101 (1.01)	·182 (1.82)	0·95	·101 (1.01)	·101 (1.01)	0·94
280	·061						

Subtracting blanks: "Molar ratio"

Marrow: C = 0·94 = 1·89 = 1·86 µmols [illegible] $\frac{\cdot 062}{1\cdot 89} \times \frac{1.02}{0.95} \times \frac{1}{0.82} = 0.043$

MC = 0·062 = ·062 µmols [illegible]

Spleen. C = 0·94 = 1·88 [illegible] µmols $\frac{\cdot 076}{1\cdot 88} \times \frac{1.02}{0.95} \times \frac{1}{0.82} = 0.053$

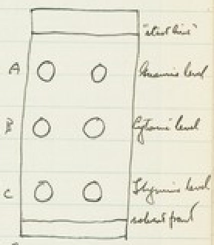
MC = 0·076 = ·076 µmols [illegible]

30.iii

Variation of Paper blanks.

2 sheets of Whatmans #1 (cut from one sheet) run in isoprop-HCl 24 hrs. Dry one over heat (1 Kw. radiator), let other dry at room temp overnight.

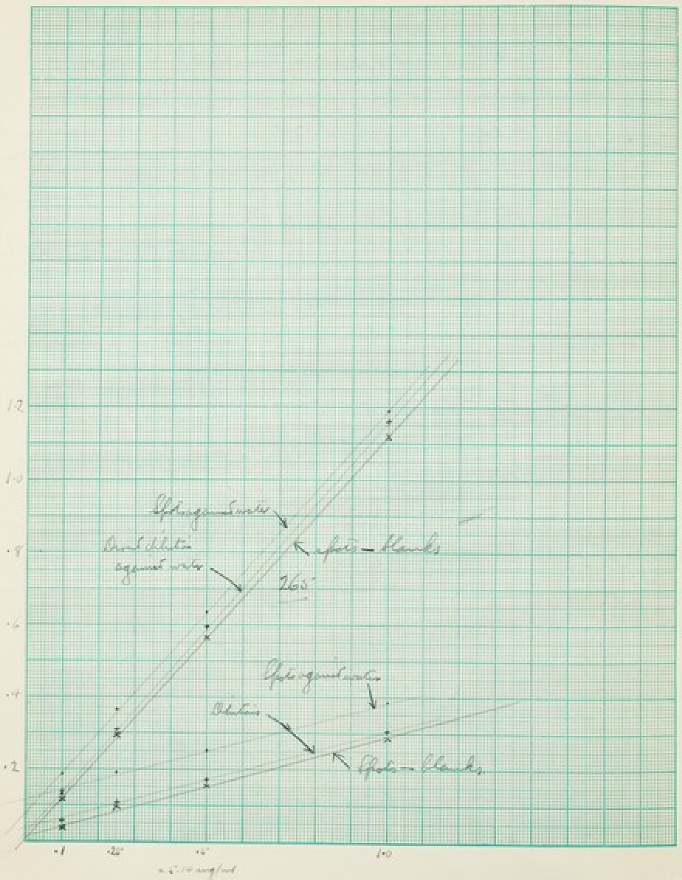
Cut discs 4.5 cm dia., thus from each.



Also from untreated What. #1 paper:

	265		235	
(1)	·044	·051	·051	·055
(2)	·057		·058	

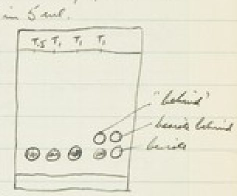
		A 265	A 235	B 265	B 235	C 265	C 235
No heat,	(1)	·038	·059	·035	·046	·069	·092
	(2)	·034	·050	·035	·052	·068	·099
	$\bar{x}$	·036	·055	·035	·049	·069	·096
Heat	(1)	·055	·057	·058	·059	·090	·102
	(2)	·044	·051	·053	·058	·083	·101
	$\bar{x}$	·050	·054	·056	·059	·087	·102



30·10

<u>Recovery of thymine repeated.</u>

Soln 5·14 mg/ml in N/10 HCl diluted ·1, ·25, ·5, 1. From each, put 3 18.1 µl spots on paper, run 24 hrs in isoprop.-HCl. Cut out all as 4.5 cm dia circles, elute in 5 ml.
Cut also blanks thus:
Elute in 5 ml N/10 HCl, & read against water.



<u>Blanks:</u>

	"Beside" 265	235	Behind 265	235	Beside behind 265	235
·5 × 5·14	·069	·091	·054	·062	·050	·064
1 × 5·14	·068	·097	·051	·062	·045	·064
$\bar{x}$	·069	·094	·053	·062	·048	·064

<u>Thymine spots</u>

	·1 265	235	·25 265	235	·5 265	235	1 265	235
Spots off paper (1)	·188	·128	·364	·183	·620	·238	1·19	·379
(2)	·185	·137	·368	·194	·646	·265	1·17	·377
(3)	·186	·132	·364	·193	·638	·249	1·20	·394
$\bar{x}$	·185	·131	·364	·190	·634	·251	1·19	·383
subtract blank	·116	·036	·295	·095	·565	·156	1·12	·288
theory	·116		·290		·582		1·16	
% recovery	100		102		97		97	
Same solution diluted & pipetted to 5 ml, not run on paper (1)	·144	·058	·311	·104	·597	·174	1·16	·304
(2)	·134	·052	·309	·108	·588	·167	1·16	·306
$\bar{x}$	·139	·055	·310	·106	·593	·171	1·16	·305
Blank by difference	·046	·076	·054	·084	·041	·080	·03	·078

Bottle 7.7724

" + S-M-C. 7.8024

30.0 mgs. ↑ 10.0 ml aq. sol. (duplicate?)

Kjeldahls:

Blank	·027	·025	
"	·022		
0.5 ml sol'n	·915		
"	·905	·905 − ·025 = ·88	$= \frac{·88 \times ·491 \times 2}{3.00} = 28.8\%$
"	·895		

Theoret. N content $= \frac{42.03}{125.1} = 33.6\%$ If 1 H_2O, $\frac{42}{143} = 29.4\%$.

Repeat
P, Blank ·025
P₂ ·025
k ½ ml sol'n ·91
k " ·(?)

Re-crystallized from water. bottle 7.4553
+ M.C 7.4986
M.C 43.3 mgs. ↑ 10.0 ml water → 4.33 mg/ml

Kjeldahls: P, blank ·04 (acid from still?)
P_3 – ·01
k 0.3 ml M.C sol'n ·74
j " ·80
l " (washed) ·79

$\frac{·79 \times ·491}{4.33} \times \frac{1}{·3} = 29.9\%$ N.

30.iii.50. <u>5-methyl cytosine standard absorption data</u>

Pirie's 5-M-C-HCl purified by twice pptng as picrate, & finally crystallizing at neutrality from methanol.

Stock sol'n, 3.00 mg/ml diluted 0.5 ml ↑ 100 N/10 HCl

Against water:

286	1.08	245	·126
284	1.10	243	·116
283	1.10	242	·115
282	1.10	241	·116
280	1.08	239	·130

D of sol'n of 10 γ/ml $= 1.10 \times \frac{·01}{3.00} \times \frac{100}{·5} = ·735$

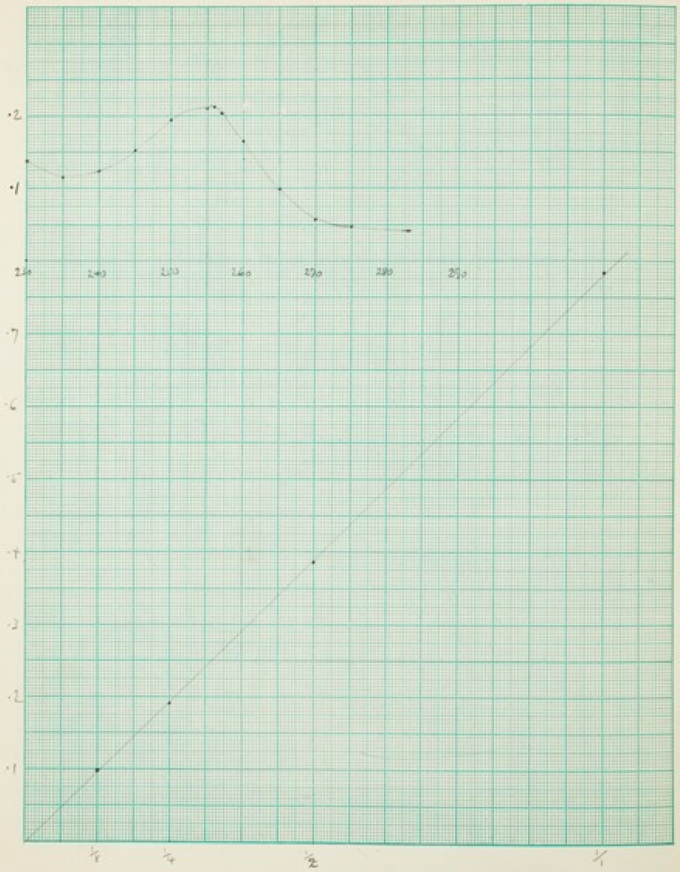
3.iv. From Kjeldahls, obviously not pure. Re-crystallize from water, wash c EtOH & ether, dry 2 hrs 105°

Sol'n 4.33 mg/ml dil. 1:400 in N/10 HCl

286	·798	243	·084
283	·801	242	·083
280	·784	241	·084

no cell correction needed.

D of 10 γ/ml $= ·801 \times \frac{·01}{4.33} \times \frac{400}{1} = ·740$



31-iii-50. <u>5-M.C recovery from paper.</u>

Stock 5-M.C. 3.0 mg/ml in water, diluted 1/2, 1/4, 1/8.

3 18.1 μl spots of each on paper, run in isoprop.-HCl.

All read against N/10 HCl.

<u>Blanks</u>:

	242	283
"1/4"	·121	·036
"1/2"	·121	·034
"1/1"	·145	·041
	·129	·037

230	·128
235	·115
240	·123
245	·152
250	·195
255	·210
256	·212
257	·203
260	·165
265	·099
270	·057
275	·048

Paper has taken up something – phenol? pyridine?

Spots eluted

	1/8 242	1/8 283	1/4 242	1/4 283	1/2 242	1/2 283	1/1 242	1/1 283
(1)	·123	·142	·132	·223	·145	·416	·216	·815
(2)	·132	·131	·147	·227	·179	·429	·240	·824
(3)	·139	·135	·147	·232	·199	·423	·227	·821
$\bar{x}$		·136		·227		·423		·820
Subtract blanks		·099		·190		·386		·783
Direct dilution: (1)	·049	·117	·039	·205	·053	·404	·091	·792
(2)	·057	·125	·051	·205	·073	·408	·107	·798
$\bar{x}$		·121		·205		·406		·795
Blank by difference		·015		·022		·017		·025
Theoret. D, from D_{orig} = ·785		·099		·198		·397		·794
% recovery		100%		96%		97%		98%

3·iv·50. Mushroom DNA.

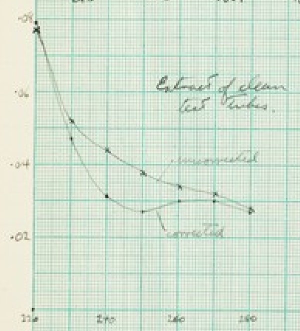
215 g. substituted mushrooms examined for maggots. Mixing blend in 0.9% NaCl, add 0.1 vol. 40% NaOH, leave overnight 37°.

4·iv Spin clear (black), fff = HAc + EtOH, ⇒ big greyish fff.

↑ in 1M NaCl pH 9. spin clear, fff = HAc + EtOH.

3 "clean" test tubes from basket at random, add 5 ml N/10 HCl, shake, read against N/10 HCl.

	1	2	3	Σ	Corrected for cells
220	·061	·115	·055	·077	·079
230	·038	·075	·043	·052	·047
240	·032	·061	·039	·044	·031
250	·030	·052	·032	·038	·027
260	·029	·047	·027	·034	·030
270	·026	·045	·025	·032	·030
280	·023	·038	·024	·028	·027



Blank in presence of low-density spots.

all read out against air? (margin note)

T, 5·14 mg/ml, & 5-M-C, 4·33 mg/ml, each diluted 1:20.
From each, 4 18·1 µl spots run overnight in ascending ·9 HCl, along with sheet of paper for blanks. Also 3 18·1 µl pipettes diluted directly to 5 ml.

		T 235	T 265	MC 242	MC 283
Blank	(1)	·083	·062	·048	·026
	(2)	·094	·064	·056	·033
	(3)	·099	·072	·058	·037
	(4)	·077	·059	·042	·032
	$\bar{x}$	·088	·064	·050	·032
					~~·097~~
Eluted spots	(1)	·095	·115	·051	·099
	(2)	·127	·139	·061	·089
	(3)	·117	·133	·057	·085
	(4)	·094	·115	·048	·089
	$\bar{x}$	·108	·125	·054	·090
Subtract blanks from eluted spots		·020	·061	·004	·058
Diluted directly	(1)	·043	·078	·050	·077
	(2)	·070	·095	·065	·103
	(3)	·058	·085	·056	·092
	$\bar{x}$	·057	·086	·057	·091
Subtracting tube dist. blank shown.		·010	·053	·015	·066
		·015	·058	·008	·061

Good agreement with theoretical. (note pointing to ·058)

Good agreement! (note beside ·066 / ·061)

	μg nucleotide (?)	Ratios
A	2.48	~~1.37~~ 1.44
G	1.01	~~0.66~~ 0.56
C	1.21	~~0.67~~ 0.68
T	2.46	~~1.32~~ 1.43
	7.16	

Correct G for cell absorption from blue on
subtract 0.008 D.

24-iv-50. <u>L. monacha DNA.</u>

Full-grown healthy larvae starved overnight, killed
c ether, weighed (= 117 g.), susp. in 500 ml .9% NaCl
(Waring blendor), strain & squeeze thro muslin, spin
15' min @ 2400. Discard supernat. Susp tissue in ca. 200
ml 1 M. NaCl, leave overnight in frig.

25-iv. Spin down, pour into 6 vols. water → flocculent ppt.
Spin off. ↑ 1M NaCl pH9. All dissolves. Add 1/10 vol 40%
NaOH, leave overnight @ 37°

26-iv. Ppt c HAc & EtOH. → brown ppt. ↑ 1M NaCl pH9. Savag 4x.
Ppt. ↑ s. dil. NaOH. Read on Beckman. total ca 4 mg impure NA.
Savag 2x more. Dialyse overnight → no ppt.

27-iv. Ppt., ↑ 1 ml water, dry down in bomb tube, hydrolyse HCOOH, ↑ 0.15 ml.
1 M. HCl. 18.0 μl spots.
Spots but weak. No MC visible

	A	G	C	T
1	·327	·120	·134	·212 ~~·206~~
2	·319	·128	·127	·186
3	·328	·119	·124	·181
4	·316	·116	·127	·208
5	·322	·119	·128	·195
		·111		

24-iv-50. Yeast DNA.

2 lb bakers yeast susp. in 4 l. ·1 M NaCl ·05 M Na citrate, add 150 g. Kieselguhr (standard Kg), filter, discard filtrate. To yeast add 750 g sand, grind ĉ mechanical mortar 2 hrs, examine microscopically. Cells all still intact! Add some water, then 1/10 vol 40% NaOH, stir, leave overnight at 37°.

25-iv. Spin, decant, ppt from supernat. ĉ HAc + EtOH. → big ppt. Filter off. ↑ 1 M NaCl + NaOH till alkaline; spin off small undissolved material. Supernat. ppt ĉ HAc + EtOH, spin down, ↑ 1M NaCl pH 9, spin off some undissolved material, add 1/10 vol 40% NaOH, leave overnight @ 37°.

No good. Big yield of gum.

Bottle 7.1432
+ undried TNA .1881
TNA 4.49 mg. ↑ 1.5 ml N/100 NaOH

BSNA in perchloric

	Yield	Ratios	
A	6.71	~~1.1~~	1.115
G	5.36	0.901	.892
C	4.86	0.806	.809
T	6.73	~~1.1~~	1.119
MC	0.37	~~0.06~~ .064	.062
	24.03		3.997

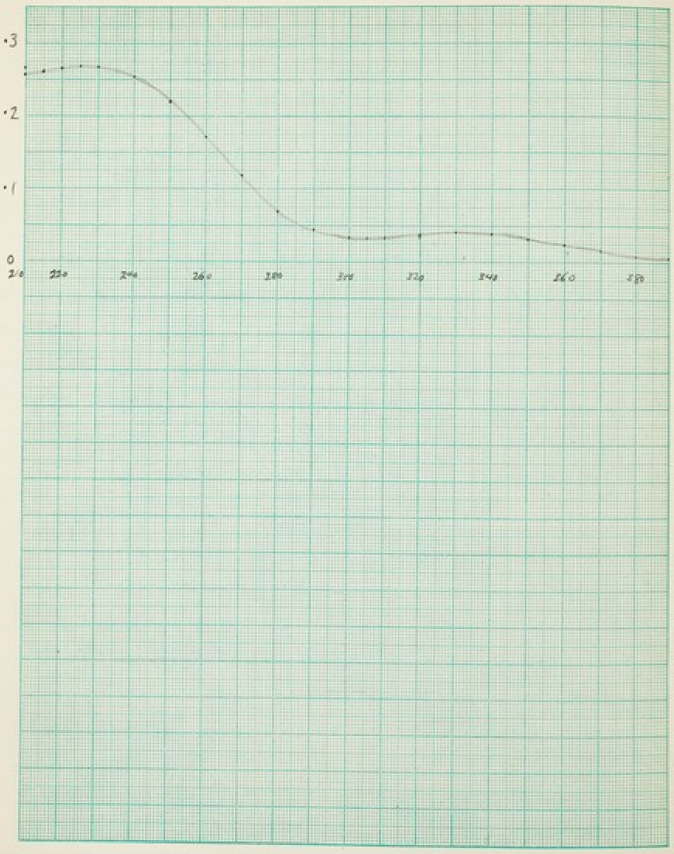
Bailey's TNA

	Perchloric Yield	Perchloric Ratios	Formic Yield	Formic Ratios	Formic / Perchloric
A	5.62	1.129	5.79	1.120	1.03
G	4.49	0.895	4.50	0.871	1.00
C	4.26	0.849	4.37	0.847	1.03
T	5.41	1.078	5.70	1.103	1.05
MC	0.30	0.060	0.29	0.056	0.97
Total	20.08	~~4.000~~ 4.001	20.65	~~4.000~~ 3.997	1.03

Test perchloric hydrolysis

29-iv-50. Bailey's TNA, undried, made up 30.0 mg/ml. 0.5 ml portions dried down in 2 bomb tubes. ① Add 0.5 ml HCOOH, hydrolyse in usual way, ↑ 0.5 ml 1N HCl.
② Add 0.1 ml 60% (9.2 N) $HClO_4$, stopper, cook on B.W.B. 1 hr., cool, add 0.4 ml water, shake well (much coke).
From both, 18.0 µl spots run in isoprop.-HCl.
All read against one set of blanks.

		A	G	C	T	MC 275	MC 283	MC 290
BSNA perchloric	1	.880	.589	.510	.532	.051	.052	.042
	2	.868	.611	.513	.547	.049	.050	.040
	3	.868	.592	.513	.524	.048	.051	.040
	x̄	.872	.597 (8) .589	.512	.534		[.051] by [illegible] = 0.036	
TNA perchloric	1	.729	.500	.450	.426	.024	.027	
	2	.738	.513	.446	.435	.029	.030	
	3	.722	.490	.448	.426	.026	.027	
	x̄	.730	.501 (8) .493	.448	.429		.029	
TNA formic	1	.749	.496	.462	.454	.026	.029	
	2	.757	.520	.463	.455	.022	.026	
	3	.750	.494	.456	.446	.024	.029	
	x̄	.752	.503 (8) .495	.460	.452		.028	



DNA ex Schwarz yeast N.A.

244 mg. Schwarz NA in 4 ml 1 N NaOH 24 hrs. @ 37° by Roy. Ppt = HAc + EtOH → bry ppt. Wash ppt twice = cold N/10 HCl, (redissolved and reprecipitated) dissolve in NH_4OH, dry down in bomb tube, hydrolyse HCOOH 175° 30'. ↑ 0.15 ml.

Isoprop. HCl → 4 DNA spots, + yellow spot between MC & U positions.

Elute yellow spot in N/10 HCl:

260	·171	270	·118	340	·038
250	·220	280	·069	350	·032
240	·254	290	·044	360	·024
230	·267	300	·033	370	·016
225	·268	305	·033		
220	·265	310	·033	380	·009
215	·261				
210	·257	320	·037	390	·006
		330	·040	400	·003
				420	·000

4

Run 4 180 µl spots. Faint U spot, & yellow spot just beyond T.

A	G	C	255	265	MC 275	283	290	U 255	260	265
·286	·741	·273	·120	·238	·014	·018	·008		·030	·031
·299	·769	·277	·106	·243	·021	·018	·014		·022	·028
·289	·748	·272	·106	·246	·020	·017	·012		·015	·021

(see Duchin)

	Unhydrol.	Ratios	
A	5.98	1.152	1.158
G	4.~~50~~ 48	~~0.886~~	0.868
C	4.32	~~0.834~~	0.838
T	5.65	1.089	1.094
MC	0.224	0.043	0.043
	20.~~73~~ 65	3.998	4.001

30-iv-50 <u>Bailey's TKA re-run for standard error.</u>

10.8 mg (not dried) Bailey's TKA in bomb tube. Hydrolysis, ↑ 0.4 ml. 18.0 μl spots. Read against paper blanks.

(Orig. cells)

	A	G	C	T	MC 275	MC 283	MC 290
1	·776	·491	·452	·449	·021	·023	·019
2	·790	·524	·461	·448	·023	·024	·021
3	·761	·487	·451	·447	·017	·020	·017
$\bar{x}$	·776	·501	·455	·448		·022	
		9					
		·492					

Flask 25.2018
+NA 27.5.18
NA 2.310 g. ↑ 10 ml 1 N. NaOH.

	Found/mol	Factor
A	1.37	0.98
G	1.27	0.91
C	1.42	1.02
T	1.52	1.09
	5.58	4.00

2-V-50 DNA ex Schwarz N.A.

2.31 g. NA ↑ 10 ml 1 N. NaOH 37° overnight.
Add 1 ml HCl, then 10 ml 90% EtOH ⇒ ppt. Stand 1 hr @ 4°. Spin off EtOH. To ppt add 25 ml ½ HCl, mix, stand 3 hrs @ 4°. Spin down. Dissolve ppt in 10 ml 1 N. NaOH, dialyse.
⇒ small brown ppt. Spin off. ↑ 10 ml 1 N. NaOH = A
Supernat. Add HCl + EtOH ⇒ big white ppt. (ppt = HCl alone). Spin off. ↑ 10 ml ·05 N. NaOH. (pH of mixture > 10). Mineral does not dissolve. Spin off, ↑ 10 ml ½ NaOH (dennis all dissolve) = B. Supernat = C.

Add dil 1:20 A dil again 1:4 = 1:80

270	·81
260	·945
250	·86
240	·663
230	·58
220	·89

} characteristic ↑.

B 1:160		C 1:160	
270	1.75		2.3
260	2.2		2.7
250	1.95		2.4
240	1.3		1.7
230	0.96		1.3
220	1.5		2.2

Combine B + C, add 0.1 vol 10 N. NaOH, leave overnight @ 37°.
Filter off some material which has not dissolved. To filtrate add HCl + 1 vol. EtOH ⇒ fine white ppt. Spin off.

6-V ↑ 1.0 ml. Dil 1:100 $D_{260} = 0.147$, $D_{230} = 0.073$. Total NA 0.6 mg.
Ppt in bowl tube. Hydr. ⇒ 0.2 ml 1 KOH. ↑ 0.04 ml HCl. 2.18 µl after. MK Monitor 275 283 290.

	A	G	C	T			
1	·179	·136	·147	·126	·016	·013	·012
2	·177	·148	·132	·116	·009	·007	·006
ā	·178	·140	·150	·121			

Flask 25.2018
+NA 27.51 18

NA 2.310 g. ↑ 10 ml 1 N. NaOH.

	Yield/ml	Ratios
A	1.37	0.98
G	1.27	0.91
C	1.42	1.02
T	1.52	1.09
	5.58	4.00

2-V-50 **DNA ex Schwarz N.A.**

2.31 g. NA ↑ 10 ml 1 N. NaOH 37° overnight

3-V. Add 1 ml HAc, then 10 ml 90% EtOH → big ppt. [illegible] EtOH. To ppt add 25 ml N/10 HCl, mix, stand 3 hrs @ 4°C, spin clear. Dissolve ppt in 10 ml 1 N. NH₄OH, dialyse.

4-V. → small brown ppt. Spin off. ↑ 10 ml ·1 N. NaOH = A

Supernat. Add HAc + EtOH → big white ppt (ppt c HAc alone). Spin off. ↑ 10 ml ~·05 N. NaOH. (pH of mixture > 10). Material does not dissolve. Spin off; ↑ 10 ml N/10 NaOH (almost all dissolves) = B. Supernat = C

All dil 1:20 A dil again 1:4 = 1:80

270	·81	characterist A.
260	·945	
250	·86	
240	·663	
230	·58	
220	·89	

	B 1:160	C 1:160
270	1.75	2.3
260	2.2	2.7
250	1.95	2.4
240	1.3	1.7
230	0.96	1.3
220	1.5	2.2

Combine B + C, add 0.1 vol 10 N. NaOH, leave overnight @ 37°.

5-V. Filter off some material which has not dissolved. To filtrate add HAc + 1 vol. EtOH → fine white ppt. Spin off.

6-V. ↑ 1.0 ml. Dil 1:100 $D_{260} = 0.147$, $D_{230} = 0.073$. Total NA 0.6 mg.

Ppt in bomb tube. Hydr. c 0.2 ml HCOOH. ↑ 0.04 ml HCl. 2.18 µl [illegible].

	A	G	C	T	PC position 275	283	290
1	·179	·136	·147	·126	·016	·013	·012
2	·177	·143	·152	·116	·009	·007	·006
x̄	·178	·140	·150	·121			

T .4 ml ↑100 D_{265} = 1.27 = 5.0 mg/ml

6-V. Ad hoc mixture itself, without hydrolysis, run in 18 µl spots.

	A	G	C	T	MC 260	275
1	.195	.144	.227	.187	.027	.024
2	.195	.157	.227	.189	.026	.024
3	.199	.151	.235	.196	.027	.025
x̄	.196	.151	.230	.191	.027	

	Amt. present	Recovery from formic	%	Recovery from perchloric		Recovery from mix	
A	0.594	0.506	85	0.532	90	0.567	96
G	0.580	0.256	44	0.442	76	0.574	99
C	0.708	0.663	94	0.670	95	0.674	95
T	1.010	0.974	96	0.941	93	0.842	84
MC	0.087	0.119	137	0.099	114	0.096	110

1-V-50. Ad hoc mixture of N.A. constituents.

			Mg/ml
Mix:	1 ml containing	2.97 mg A	0.594
	1 " "	2.90 mg G	.580
	1 " "	3.54 mg C	.708
	0.1 " "	0.434 mg MC	.087
	1 " "	5.05 mg T	1.010
	0.5 " "	15 mgs xylose + 12 mgs H_3PO_4	
	0.4 ml ~~water~~ 1N-NaOH, to dissolve G.		
	5.0	≡ ca. 36 mgs DNA ≡ 7.2 mg/ml.	

F 1.0 ml dried down in bomb tube, ↑ 0.5 ml HCOOH, [illegible] 30' 175°; dry down, ↑ 0.4 ml 1N-HCl

P. 1.0 ml dried down, ↑ 0.2 ml 60% perchloric, heat on BWB 55', cool, add 0.2 ml water. Both: 3 18.0 µl spots. F over-run.

		A	G	C	T	MC 275	MC 283
F	1	.434	.176	.563	.545	.073	.078
	2	.447	.173	.568	.542	.078	.083
	3	.431	.176	.571	.573	.086	.090
	x̄	.437	.175	.567	.553		.084
Eluate µg/ml		4.56	2.40	5.97	8.78		1.07
P	1	.456	.281	.580	.532	.065	.071
	2	.461	.298	.569	.541	.064	.070
	3	.463	.294	.573	.529	.066	.070
	x̄	.460	.291	.574	.534		.070
Eluate µg/ml		4.79	3.98	6.04	8.48		0.89

Bottle MT 7.9592

+ G	7.9685	G =	9.3 mgs.	↑ 10.1 ml 0.1 N NaOH.
+ T	7.9790	T =	10.5 mgs.	
+ BSNA	8.1118	NA =	132.8 mgs.	

Control BSNA: 23.6 mgs ↑ 0.5 ml perchlor. hydr., add 0.5 ml aq. [illegible].

		Recorded/ml	Ratios	✓
BSNA perchlor	A	4.64	1.090	1.096
	G	4.08	~~0.987~~	0.924
	C	3.39	~~0.764~~	0.768
	T	5.07	~~1.142~~	1.149
	MC	0.29	~~0.065~~	0.066
	Total	17.63 / .67	3.998	4.003

BSNA + G + T

	Perchloric: Found/ml	Found NA alone ×.525	Extra	mg/ml	Σ mg	Added mg	%	Formic: Found/ml	Found NA from A ratio	Extra
A	2.58							2.54		
G	4.47	2.20	2.27	3.43	9.44	9.3	101	4.16	1.89	2.27
C	1.78							1.62		
T	5.45	2.68	2.77	3.2	9.96	10.5	95	5.42	2.54	2.90

5.V.

Recovery of bases added to DNA.

← Mixture in N/10 NaOH. 2×1.0 ml dried down in tubes.

F. add 0.5 ml formic, hydr 175° 30', dry down, ↑ 1 ml 1N HCl.

P. add 0.5 ml 60% perchlor., heat on BWB 60', add 0.5 ml aq. [illegible].

×5 [illegible] .244 / .48 1.01 — 3 18 µl spots of each of BSNA in perchlor. on What. #1.

		A	G	C	T	MC 275	283	290
BSNA perchlor	1	.634	.462	.361	.398	.022	.024	.019
	2	.623	.454	.358	.400	.032	.034	.028
	3	.629	.457	.352	.408	.025	.026	.022
	$\bar{x}$	.629	.458 / 4 / .449	.357	.402		.028	
+G+T perchlor	1	.337	.488	.189	.433			
	2	.333	.488	.182	.433			
	3	.339	.500	.194	.429			
	$\bar{x}$	.336	.492 / 9 / .483	.188	.432			
+G+T formic	1	.323	.457	.177	.444			
	2	.329	.456	.163	.419			
	3	.338	.458	.172	.426			
	$\bar{x}$	.330	.457 / 9 / .448	.171	.430			

Weighed into bomb tube: 10·6 mgs. ↑ 0·6 ml HCOOH

~~0·4~~ 0·0857 ml pipetted off for P estimation

Remainder, (0·517 ml ≡ 9.14 mg) hydrolysed 35' 175°; dry down, ↑ 0·4 ml N HCl

(against water, ·700)

P: D = ·685 = ·151 mg. = $\cdot 157 \times \frac{\cdot 6}{\cdot 0859} \times \frac{100}{10\cdot 6}$ = 9.95 %!

150 + P ·69 (against water)

160 + P ·75

– presumably some concentration by evaporation

	① Isolated	Ratios	✓	② Isolated	Ratios	✓
A	5·47	~~1·274~~	1.279	5·29	1·210	1.213
G	~~3·43~~ 3·35	0·799	·784	~~3·54~~ 3·45	0·802	0.787
C	3·08	0·718	·721	3·35	0·766	0.769
T	5·04	~~1·174~~ 1·178	1.179	5·19	1·187	1.190
MC	0·16	0·037	·037	0·17	0·039	0·039
	17·10	4·006	4·000	~~17·54~~ 17·43	4·004	3.998

Echinoderm sperm N.A.

5·v. Ca. 5 ml semen of *Echinus esculentus* coll. yesterday & dried & stored in frig. dil. to ca. 25 ml ē ·9% NaCl & spun 10 min. 9600 g. Susp. in 25 ml ·9% NaCl & spin again. Susp. in ca. 25 ml saline, add equal vol. 2 M. NaCl → gel. Mix on Waring B., leave overnight in frig.

6·v. Gelled. Add N/10 NaOH → pH 9.0, spin in Waring b., spin 11,000 1 hr. Supernat still turbid, pour into 500 ml water → stringy ppt. Strain off, ↑ 1 M NaCl, leave in frig.

9·v. Sylvac Sevag 3x. 1st time → considerable gel. 3rd time → almost none.

Sol'n dil. 1:50.

270	·131
260	·152
250	·131
240	·090
230	·084
220	·142

232 ·080

Total NA ·15 × ·04 × 50 · 55 = ~~225~~ 165 mgs.

Ppt ē HAc & EtOH, wash ē 60, 90, abs EtOH, ether, dry briefly in oven. Total wt 19.4 mgs.

180 µg/plate on What #2.

	A	G	C	T	275	MC 283	280
1	0·710	0·369	0·323	0·388	0·031	0·032	0·028
2	0·720	0·368	0·326	0·409	0·031	0·031	0·029
3	0·704	0·~~396~~ ·374	0·322	0·403	0·017	0·017	0·015
$\bar{x}$	0·711	0·377 9 ·368	0·324	0·400		·027 by 60% MC ·016	

11·v. Rerun on #1

	A	G	C	T			
1	·686	·384	·353	·416	·013	·014	·013
2	·678	·392	·343	·419	·016	·018	·015
3	·700	·383	·364	·403	·017	·018	·016
$\bar{x}$	·688	·386 9 ·377	·353	·412		·017	

	HNA	BSNA
Boat	7.4994	7.3463
+ NA	~~.5179~~ .5282	7.3708
NA	~~.05~~ 28.8 mgs.	24.5 mgs. each ↑ 20 ml aq. dist.

	1 ml all against blank		
Total P in 1.0 ml HNA soln	.445	[illegible]	= 2.3%
do 1.0 ml BSNA	.439	[illegible]	= 2.4%

"Inorg."

HNA	.020	= 4 γP	= 0.2% of total
BSNA	.030	= 6 γP	= 0.3% of total

8. V. "Inorganic" P in TNA & HNA.

Portions of BSNA & HNA, (not dried), weighed out & dissolved in water up to 20 ml. Pipette from each 1 ml for digestion for total P.

To remaining 19 ml of each, add $HClO_4$ & P reagents (Allen) → N.A. ppt. Spin off & read supernat, but ppt. is faintly blue!

---

16. V. Echinus C + MC

	MC in 5 ml			C in 10
	275	283	290	
1	.083	.094	.082	.905
2	.084	.093	.081	.930
$\bar{x}$		.0935		.918

Molar ratio $= \frac{.0935}{2 \times .918} \times \frac{102}{95} \times \frac{1}{.745}$

$= 0.073$

	Mols/mol	Ratios	
A	4.52	1.130	1.137
G	3.~~40~~ 36	~~0.862~~	~~0.845~~
C	3.19	0.800	0.802
T	4.69	1.172	1.179
MC	·18	·037 (ground)	0.045
	~~16.00~~ 15.94	~~4.001~~	4.001

Rat C+MC.

Rat N.A.'s re-run.

9-V-50. Remainder of Mrs Mann's Rat Bone Marrow N.A., dried, dissolved in 1 ml. Dil 1:100. D_{260} = ·158 D_{270} = 0.78. Total NA 6 mg. Pptd in bomb tube, hyd. ·5 ml HCOOH. ↑

3 18 μl spots.

	A	G	C	T	MC 275	MC 283	MC 290
	·596	·384	·344	·382	·020	·020	·18
	·575	·379	·336	·378	·024	·024	·023
	·594	·375	·329	·361	·018	·017	·016
$\bar{x}$	·588	·379	·336	·372	·021	·020	·019
		9 / ·370				MC as mol ~~·018~~ ·018	

14-V. Bone marrow C+MC:

	MC in 5 ml 275	283	290	C in 10
1	·038	·044	·038	·656
2	·044	·049	·043	·661
$\bar{x}$		·046		·658

Ratio $= \frac{·046}{2 \times ·658} \times \frac{102}{95} \times \frac{1}{·82} = ·046$

16-V. C+MC ex spleen (still a bit of streak on chromatogram).

	MC in 5 ml 275	283	290	C in 10
1	·104	·113	·101	1·31
2	·112	·120	·107	1·31
$\bar{x}$	·108	·116	·104	

·116 − ·004 = ·112

Molar ratio = $\frac{·112}{1·31 \times 2} \times \frac{102}{95} \times \frac{1}{·82} = ·056$

Bailey's TNA low conc hydrolysis.

	μmol/ml	Ratios
A	5.51	1.166
G	4.21 ~~5.12~~	0.890
C	3.96	0.838
T	4.95	1.048
MC	0.28	0.058
	18.91	4.000

BSNA perchloric ③

	μmol/ml	Ratios	√
A	4.39	1.100	1.105
G	3.59 ~~3.64~~	0.921	0.904
C	3.32	0.831	0.836
T	4.37	1.094 ~~1.050~~	1.100
MC	[0.22]	0.055	0.055
	15.98 89	4.001	4.000

Test of hydrolysing little N.A. in small formic

8.V.50. 0.4 ml containing 1.2 mg Bailey's TNA pptd in bomb tube, hydr. c 0.5 ml HCOOH 175° 30'. Dryblown, ↑ 0.04 ml, 2 × 15 μl spots on What. #2. A+C not perfectly resolved.

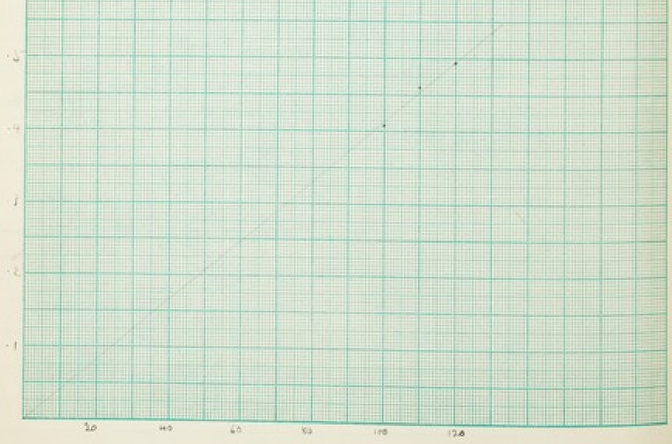
	A	G	C	T	MC 275	283	290
1	0.706	0.458	0.417	0.391	0.017	0.019	0.016
2	0.728	0.467	0.418	0.394	0.036	0.035	0.033
$\bar{x}$	0.717	.463	.418	.393		.027	

10.V.50. BSNA perchloric - 3rd run.

New hydrolysis. 3.180 μl spots on What. #1.

	A	G	C	T	MC 275	283	290
1	.583	.393	.351	.346	.040	.037	.030
2	.580	.410	.430 Tube dirty	.346	.042	.042	.036
3	.580	.410	.346	.348	.021	.022	.019
$\bar{x}$	.571	.404 9 .395	.349	.347		.022	(assume)

	Benzer, dried	BSNA, not dried
Bottle	8.3618	7.1887
+ NA	8.3730	7.2077
NA	11.2 mgs.	19.0 mgs.
	↑ 10.1 ml N/10 NaOH	↑ 10.0 ml H_2O



BSNA }
Benzer N.A. } P & UV curve...

P/.

		60' fells, 1 cm cells, against incubation blank	
P_1	1.0 ml Benzer sol'n	.456	.455 = 110 γ = $\frac{.110}{4.1}$ × 100 = 9.9 %
P_2	1.0 ml "	.454	
P_3	0.75 ml BSNA sol'n	.411	.422 = 102 γ = $\frac{.102}{1.9}$ × $\frac{1}{.75}$ × 100 = 7.15 %
P_5	0.75 ml " "	.433	
	100 γ P	.409	
	110 γ P	.460	
	120 γ P	.494	

UV

	Benzer sol'n 1:25 taken to pH 7.0	BSNA sol'n 0.75:25
310	.051	.021
300	.111	
290	.353	
280	.757	
270	1.12	.774
265	1.19	.855
262	1.22	
261	1.22	
260	1.22	.900
255	1.17	.880
250	1.07	
240	.781	
235	.697	
233	.688	
230	.709	.394
220	1.12	

Benzer $\epsilon_p = 1.22 \times \frac{31}{.110} \times 25$
$= 8600$

BS $\epsilon_p = 0.90 \times \frac{31}{.102} \times 25 = 6740$

Gordon's TNA

	[illegible]	Ratios	[illegible]
A	5.65	1.11	38.2
G	4.34	0.86	32.7
C	4.44	0.87	24.6
T	5.51	1.09	34.8
MC	0.35	0.07	2.2
	20.29		

18-V-50. Gordon's TNA (from Hammarsten's lab).

8.0 mgs (not dried) hyd. in 0.5 ml HCOOH, ↑ 0.3 ml HCl.
18.0 μl spots. No U visible. G + A not v. well resolved.

	A	G	C	T	MC 275	MC 283	MC 290
1	725	474	.459	.442	.032	.034	.031
2	.736	.484	.469	.431	.034	.037	.034
3	.741	.496	.473	.437	.030	.032	.029
av	.734	.485 -.008 .477	.467	.437	.034		

	Cytidine (C)	Deoxy A	Deoxy B
Bottle	7.5065	7.2178	7.7046
+ nucleoside	.5243	7.2304	.7144
Nucleoside	17.8 mgs.	12.6 mgs.	9.8 mgs.
↑ 5 ml water → conc.	3.56 mg/ml	2.52 mg/ml	1.96 mg/ml

19-V-50. UV absorption of Cytosine riboside and deoxyriboside.

, 2 samples cytosine deoxyriboside from Brown & Dekker, dried 2 hrs @ 105°, weighed out, ↑ 5 ml H_2O, dil each 1:100 in aq. dest.

	A (deoxy ~~cytidine~~)	B (deoxy)	C (cytidine ~~deoxy~~)
300	.053	.053	.065
290	.342	.275	.444
280	.880	.681	1.14
275	1.04	.798	1.33
273 272, 271	1.05 – 1.06, 1.05	.814 – .816, .816	1.34 1.36, 1.36
270	1.05	.818	1.36
260	.825	.643	1.11
252			.939
250	.645	.509	.924
245	.657	.516	.935
242	.668	.514	.961
240	.721	.557	1.03
230	.868	.673	1.17
220	1.03	.810	1.37
210	1.34	1.08	1.88

Molecular extinction coefficients.

Deoxycytidine (M = 227)
A $1.06 \times \frac{227}{2.52} \times 100 = 9560$
B $.816 \times \frac{227}{1.96} \times 100 = 9450$
} 9500

Cytidine (M = 243)
C $1.36 \times \frac{243}{3.56} \times 100 = 9300$

Hotchkiss cytidine $.30 \times \frac{1}{.765} \times 243 \times 100 = 9500$

20-V.

<u>Cytosine riboside & deoxyriboside in acid and alkali.</u>

Soln. B dil 1:100, C dil. 1:200, in N/10 HCl + NaOH, & read against HCl + NaOH.

	HCl B (deoxy)	HCl C (ribo)	NaOH B (deoxy)	NaOH C (ribo)
310	.014	.004		
300	.228	.196	.016	.017
290	.830	.726	.202	.211
280	1.14	.970	.624	.546
279	1.13	.965		
278	1.13	.964		
275	1.09	.920	.756	.638
272			.785	.655
270	.940	~~.794~~ .785	.780	.646
260	.546	.430	.631	.519
255			.558	.467
251			[illegible]	[illegible]
250	.247	.198	.530	.415
245			.554	.485
241	.149	.114		
240	.147	.115	.607	.530
235	.195	.156		
230	.320	.263	.691	.581
220	.764	.624	.760	.634
215	.890	.708		
211, 212, 213	.854, .890	.705, .722		
210	.876	.700		
209	.865	.648		
206	.810	.643		

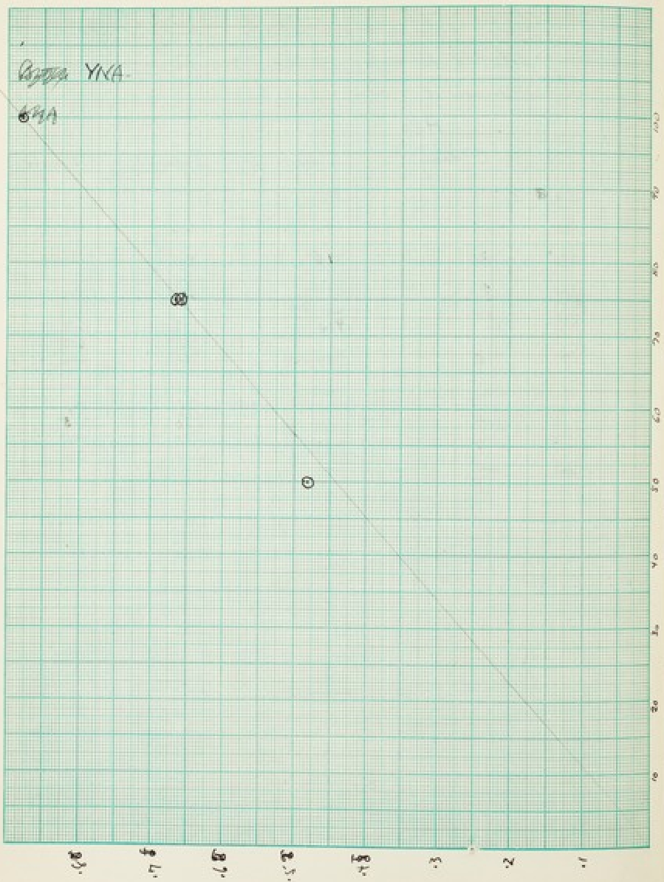
<u>Millimolar extinction coefficients:</u>

Acid: Deoxy $\epsilon = 1.14 \times \frac{.227}{1.96} \times 100 = 13.2$

Ribo $\epsilon = .97 \times \frac{.243}{3.56} \times 200 = 13.2$

Alkaline: Deoxy $\epsilon = .785 \times \frac{.227}{1.96} \times 100 = 9.1$

Ribo $\epsilon = .655 \times \frac{.243}{3.56} \times 200 = 8.95$



21-V-53. Ep of DNA & RNA before & after {NaOH / nuclease} treatment.

Weighed out, without drying,

Purified YNA. 18.4 mgs ↑ 5.05 ml ·01 N NH₄OH → 3.65 mg/ml

BSNA 21.0 mgs ↑ 5.0 ml H_2O → 4.2

B6 TNA 4.1 mgs ↑ 2 ml H_2O → 2.0

HNA 11.7 mgs ↑ 5 ml H_2O → 2.34

From YNA & BSNA solns, take 1 ml, add 0.1 ml 40% NaOH, leave ~~overnight~~ 22 hrs 37°

From YNA, take 1 ml, add 1 drop RNase + 1 drop chloroform, leave overnight 37°

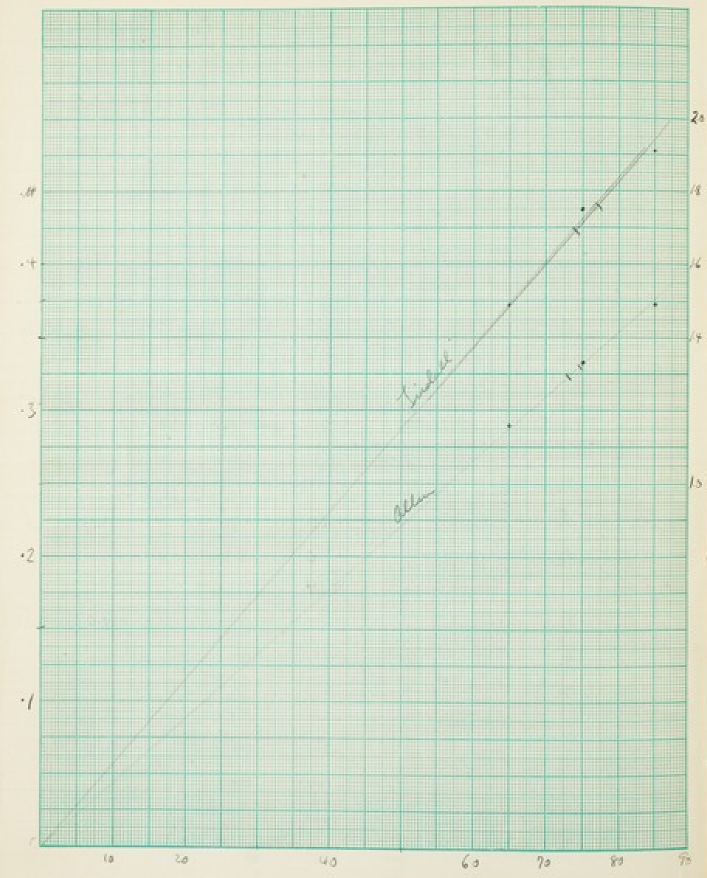
From BSNA, take 1 ml, add 1 drop DNase + 1 drop chloroform + [illegible] $MgSO_4$

+ NaOH → pH 7, leave overnight 37°.

22-V. Neutralize NaOH bottles c 0.057 ml glac HAc. Take from each 0.25 ml for P & 0.25 ml for [illegible].

P estimations - from each, 0.25 ml. (Zamecnik, 60% filter, reagent blank.)

P_2	BSNA	·660	74 γP	7.05% of wt.
P_3	BSNA - NaOH	·580	65·5	
P_4	BSNA - DNase	~~·624~~ ·60	68	
P_5	YNA	·725	82	= 9.0% of wt.
6	YNA - NaOH	·660	74	
g	YNA - RNase	·720	81·5	
j	B6 TNA	·448	51	
i	HNA	·438	49·5	8·5% of wt.
	50 γP	·480	~~54·5~~	
	75 γP	·665		
	100 γP	·880		



22-V. ϵ_p's. 0·25 ml sol'n (same as for P) dil. to 25.

	260 mμ. D	260 mμ. ϵ_p	230 mμ. D	Corr.	$\frac{D_{260}}{D_{230}}$
BSNA	·610	~~5800~~ 6400	·248		2·46
" NaOH	·642	7600	·292	·283	2·27
" DNase	·804	9200	·329		2·44
YNA	~~·850~~ ·900	~~8500~~ ~~9200~~ 9050	·348		2·58
" NaOH	·940	9800	·366	·357	2·63
" RNase	·970	9200	·378		2·57
B'a TNA	·350	5300	·137		2·56
HNA	·360	5600	·154		2·34

23-V. Repeat P estimations — reagent blank 1 cm cells

__Allen__:

P_2 BSNA 0·25 ml	·318	·323 = ~~74.5~~ 73 γ
P_3 "	·328	
P_4 YNA 0·25 ml	·330	·330 = 74·5 γ
P_5 "	·330	
65 γP	·290	
75	·333	
85	·373	

__Tisdall__: Incin. c̄ 0·5 ml HClO4 — ·5 mm cells 608 filter.

b BSNA 0·25 ml	1·69	1·69 = 74 γP
d "	1·70	
j YNA 0·25 ml	1·74	1·76 = 77 γP
l "	1·78	
65 γP	1·49	
75	1·75	
85	1·91	

Ep

BSNA untreated. $Ep = .756 \times \frac{31}{.082} \times \frac{50}{2} = 7150$

after DNase 2hrs 37° $.894 \times \frac{31}{.082} \times \frac{50}{2} \times \frac{5.5}{5} = 9300$

+ 48 hrs 20° $.934 \times \frac{775}{.082} \times \frac{5.5}{5} = 9700$

YNA, in 0.15 N. NH_4OH $.996 \times \frac{775}{.087} = 8900$

after RNase 2hrs 37° $.969 \times \frac{775}{.087} \times \frac{5.54}{5} = 9600$

+ 48 hrs 20° $.989 \times \frac{775}{.087} \times \frac{5.54}{5} = 9800$

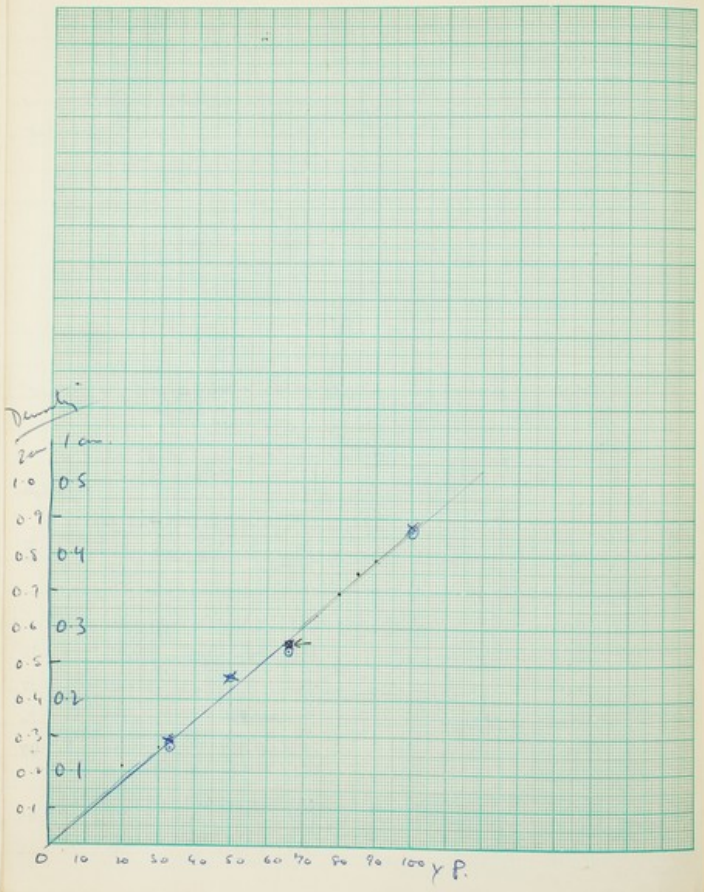
24-V-50. Repeat Ep before & after Nase treatment.

YNA weighed 20.3 mgs. ↑ 10.1 ml 0.15 N NH_4OH → 2.01 mg/ml
BSNA " 24.9 mgs ↑ 10.0 ml water → 2.49 mg/ml.

P (Allen) a 0.5 ml YNA } 87 γP (accurate) = 8.65% of wt.
d "
j 0.5 ml BSNA } 82 γP = 6.6% of wt.
l "

YNA: Pipette out 5.0 ml, add 0.04 ml HAc (→ pH 6), + 0.5 ml containing 1 drop of RNase → 5.54 ml
BSNA: Pipette out 5.0 ml, add 0.5 ml containing 1 drop DNase + 1 stab $MgSO_4$ → 5.50 ml + 1 drop of chloroform.
Put both @ 37° for 2 hrs. 1 drop into M/10 HCl or 50% EtOH - no ppt.

		D_{260}	Ep	D_{230}	$\frac{D_{260}}{D_{230}}$
Beckman. 0.5 ml → 4.25	DNase, 10x conc. in BSNA sol'n	.057		.54	
	RNase, 10x " YNA sol'n	.058		.268	
	BSNA untreated	.756		.317	2.38
	YNA "	258 1.01 .996		.425	2.35
	BSNA + DNase	.900 - .006 = .894		.379	2.36
	YNA + RNase	258 .98 .975 - .006 = .969		.391	2.47
27-V. Re-read Nase sol'ns, after 48 hrs at room temp. (final dilution 5→25)	BSNA + DNase	.940 = .934		.386	2.42
	YNA + RNase	.995 = .989		.434	2.28



	D 1 cm cell	Allen P 1 cm cell
33y SCA New	0·264	0·143
50y SCA Old	0·460	0·231
66y SCA New	0·535	0·278
100y SCA Old	0·863	0·440

TYM ① 0·785 } 0·78
② 0·780 }
③ dil 5 50 by mistake 0·361

BS ① 0·723 } 0·71 = 82 YP
BS ② 0·696 }

Y ① 0·615 } Lifted → 0·72 | Expect 0·74
Y ② 0·720 }

Y 1 1 cm cell 0·364 } ·38 = ·76
Y 2 1 cm cell 0·417 }

Recalc YHA 2 cm cells
66 Y new std ·585
85 Y old std ·752
Y .5 cm ·765 } ·750 = 87 YP
Y .5 cm ·735 }

Prot. 7.0836
+NA .0936
10.0 mgs.

25-V-50. YNA E_p Thiol estimation

10.0 mgs YNA ↑ 5.0 ml 0.05 M. NH_4SH.

0.5 ml ↑ 25 for Beckman. Read against aq. dest.

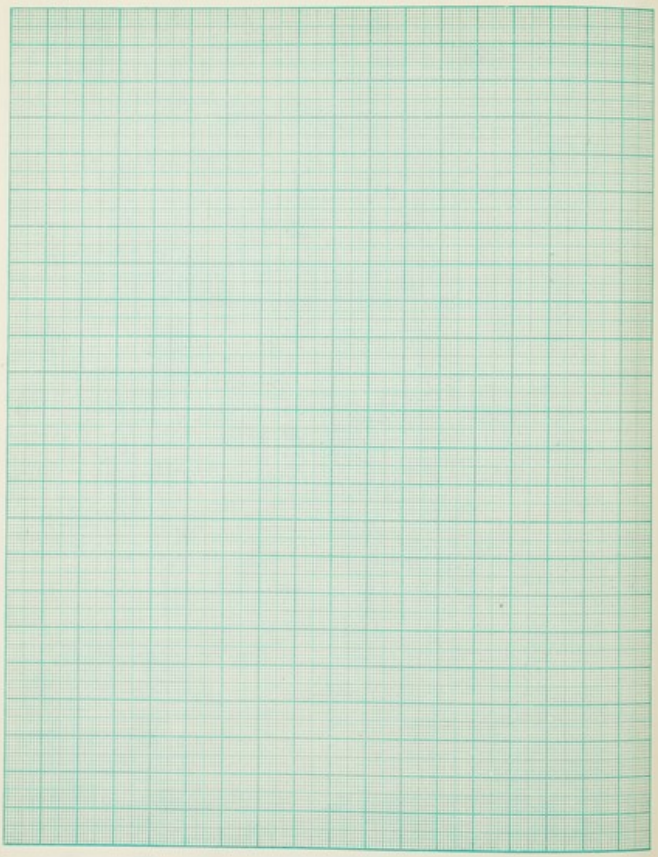
pH 6.9

270	·756		
265	·868		
260	·940		
255	·958		
254	·980		
250	·936	252.5 ·894	$\frac{D_{260}}{D_{230}}$ =
245	·700		
240	·554		
235	·431		
230	·366		
225	·349		

P Alka. 2 ml cells

0.5 ml YNA ·716 } ·708 = 81 γP
0.5 " ·700 }
80 γP ·695
90 γP ·785

$E_{p\,260} = ·940 \times \frac{78.5}{81} = 9000$



YNA - Volkin's analysis.

In terms of weight (dried = 89.5 %).

A	0.754 μmols/mg.	1.03
G	.915	1.25
C	.580	0.79
U	.681	0.93
	2.93	
P	2.76 μmols/mg = 8.56 %	
N	15.5 %	

ε_p = 11,000

ε_p calc. from molecules 11,500.

Ehrlich's acid on E H 9.89
N 9.3

Botanical Labs, U. of Penn.
Philadelphia

Ogur, M. & G. Rosen.

1950. The nucleic acids of plant tissues. I. The extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid.

Archives of Biochem. 25: 262-276.

PNA & DNA are extracted ē 1 N. perchloric acid, & estimated by P &/or by UV absorption.

Mean of several ~~tests~~ samples, after perchloric treatment: PNA (yeast) ϵP = 10,816

Before treatment, PNA 9,800 DNA (thymus) ϵP = 8,780

Extraction:

Tissue is homogenized in cold 70% EtOH, spun @ 4°C, & washed once ē 75% EtOH containing 0.1% $HClO_4$.

Residue is boiled 3 min. in 3:1 EtOH-ether, & repeat.

Residue is extracted twice quickly ē cold 0.2 N $HClO_4$.

PNA: Residue ~ ē 1 N. $HClO_4$ @ 4°C. overnight, ē twice washed ē 1 N. $HClO_4$. Combine supernatants for PNA

DNA: Residue is susp. in 0.5 N. $HClO_4$, ē heated on W.B. 20 min. @ 70°. Repeat & combine extracts for DNA

Results are reproducible ē std. dev. of 2%, & agree well ē Schneider's method.

Corn root tip meristem: PNA 4.6 % of dry wt.
DNA 0.74 % ”

Bottle MT

+ Adenine (not dried) A: 8.2231 − .2528 = 29.7 mg ↑ 10.0 ml N/10 HCl = 2.97 mg/ml

Kjeldahls: (adding 2 drops 10% CuSO₄ at beginning; hot plate, minimum 7 hrs)

HCl: 1 ml = .476 mg N

					Theory %N	
P_1	Blank	.005				
P_2	"	.018				
a	0.25 ml A	.750	$\frac{.727 \times .476}{.25 \times 2.97}$	= 46.6%	51.8	10% low
b	"	.735				
P_6	"	.740				
h	0.4 ml U	.880				
i	"	.870	$\frac{.857 \times .476}{.4 \times 4.90}$	= 21.7%	24.6	12% low
j	"	.865				

<u>Adenine standard absorption</u>

Soln 2.97 mg/ml dil 0.5 ↑ 200 ml N/10 HCl.

260	.710
262.5	.719

D_{260} 10 γ/ml = $.710 \times \frac{.01}{2.97} \times \frac{200}{.5}$ = .956

Kjeldalls: HCl 1 ml = .476 mg N.

P_4	Blank		.015				
P_6	"		.015				
a	0.3 ml	G	.765	$\frac{.750 \times .476}{.3 \times 2.90}$	= 41.0 % N.	46.4	12%
b	"	" (dumped)	.735				
k	0.4 ml	V	.885	$\frac{.870 \times .476}{.4 \times 4.70}$	= 22.0 % N	24.6	11%
l	"	lost					
g	0.3 ml	C	.795	$\frac{.782 \times .476}{.3 \times 3.54}$	= 35.0 % N.	37.9	8%
h	"		.800 .770				
c	0.3 ml	MC	.835	$\frac{.828 \times .476}{.3 \times 4.34}$	= 30.3 % N.	33.6	10%
i	"		.850				
?	(						
P_2	0.4 ml	T	.875	$\frac{.882 \times .476}{.4 \times 5.10}$	= 20.6 % N	22.2	7%
P_3	"		.900				

Beckman: D

G	0.25 ↑ 50 ml	250	1.07
		248.5	1.08
U	0.3 ↑ 100	260	.985
		259	.986
C	.3 ↑ 100	275	1.01
MC	.3 ↑ 100	285	1.00
		283	1.02
T	~~0.2~~ 0.2 ↑ ~~100~~ 50	265	1.28
		264	1.29

26-IV-50. Re-check standard absorptions & N contents.

	Conc. taken made up	Beer's conc by Beckman	Percent conc by N content
G	2.90 mg/ml	2.90	
U	4.70	4.70	
C	3.54	3.54	
MC	4.34	4.34	
T	5.14	5.10	

6-vi-50. Re-read same stock sol'n 0.06 ↑ 20 ml. Check Beckman cells c water first – good match.

	·06 ↑ 20 HCl		·06 ↑ 25 N			·06 ↑ 25 OH	
283	1·02				287	·568	·710
		272	·504				
		274	·506	← ·632			
		276	·496				
250	·157						
245	·104						
242	·095	252	·299	·374	253	·126	·157

·591

26-iv-50 5-M.C absorption curves.

Purified synth. M.C. made up as overleaf. Stock dil ·3 ↑ 100

	HCl	28-V-50 H	N	OH
320	·006		·009	·018
310	·054		·014	·075
300	·424		·054	·419
290	·895		·284	·704
				287 ·721
285	1·01	1·02		·718
283	1·02	1·03		
280	1·00	1·00	·590	·613
			275 ·656	
			273 ·658 274 ·658	
270	·731	·723	270 ·638	·425
260	·401	·385	·463	·242
			253 ·377 252 ·376	255 ·199 253 ·194
250	·183	·162 245 ·106	·379	·201
242	·125	·098		
240	·128	·104	·513	·391
230	·407	·378	·751	·746
220	1·07	1·02	1.12	1.12
215	1·27 211 1·36	1·21		
		1·28 212 1·26		
210	1·38	1·26	1·36	
209	~~1·32~~ 1·35			
205 ~~203~~ 2	~~1·62~~ 1·32	206 1·18		

$\frac{4.38 \times .3}{100}$ = ·0013 mg/ml

$\times \frac{10}{438} \times \frac{100}{.3}$

$\times \frac{100}{.3} \times \frac{125}{438}$ ·954

	Peak	D 1 cm	mE
H	283	·785	9·81
N	274	·501	6·26
OH	287	·550	6·87

Minimum

H	242	~~·242~~ (·100)	3·95
N	252	(·376)	3·68
OH	253	(·125)	1·67

	Uracil	Cytosine	5-m-Cytosine
Bottle wt	7.4575	7.8633	7.7725
+ stuff (not dried)	7.4810	7.8812	7.7942
stuff	23.5 mg	17.9 mg	21.7 mg
Dissolve in: (added from pipette)	5.0 ml N/10 HCl	5.05 ml N/10 HCl	5.0 ml H_2O
Conc.	4.70 mg/ml	3.54 mg/ml	4.34 mg/ml

Kjeldahls:

							Ideal N content
P_1	Blank		·018	·018			
P_3	"	(½ hr)	·015				
b	0.4 ml	U	·870	·852 × $\frac{.500\ (std)}{.4}$ × $\frac{100}{470}$ = 22.3 % N.	= 90		24.6 %
d	"	(½ hr)					
h	0.3 ml	C	·796	= ·772 × $\frac{.500}{.3}$ × $\frac{100}{3.54}$ = 35.6	94		37.9
i	"		·784				
j	0.3 ml	MC (transferred)	·78				
l	"	(transferred)	·834	·835 × $\frac{.500}{.3}$ × $\frac{100}{4.34}$ = 31.3	= 93% of		33.6
P_5	"		·853				

7.iv.53 { Uracil standard absorption repeat.
Cytosine – ex HNA, re-crystallised twice from alcohol, once from water, dried at 110-115° 5 hours, and over P_2O_5.
5-M-cyt. as of 3-iv. but further dried as above

For N readings, 3 sol'ns each dil. 0.3 ml ↑ 100 ml N/10 HCl

				cell correction			D for 10γ/ml
Uracil:	260	(1.02)	·995	–·003	=	·992	·704
	259	(1.03)	1·00			·997	
	258	1.02					
	229	(·244)	·201				
	228	(·243)	·198	+ ·002	=	·200	
	227	·248					
	274	1.00					
Cyt.	275	1.01		–·002		1·008	·950
	276	1.00					
	239	·14					
	238	·152		–·007		·145	
	237	·154					
	236	·157					
	235	·164		–·004			
	234	·172					
	284	1.015					
M-C	283	1.02		–·001		1.02	·784
	282	1.01					
	243	·132					
	242	·132		–·009		·123	
	241	·133					

Chargaff, E. & S. Zamenhof
1948. The isolation of highly polymerized desoxypentose nucleic acid from yeast. J.B.C. 173: 327-335

1 Kg. baker's yeast washed; 1 l. 0·1 M. Na acetate, susp. in Na citrate, crush in [illegible] bacterial mill, spin.
Take upper layer of sediment (lower layer is unbroken cells), susp in 850 ml cold 1M NaCl, leave in frig 72 hrs.
Spin clear, ppt c 2 vols. EtOH, wind threads on rod.
Dissolve in 1M NaCl, Sevag, ppt. Crude prep →
19 % DNA, 64% RNA (→20% RNA)
To purify, ppt as Ca salt, then use RNase (→3% RNA)

MC standard absorption. MC-119

18.1 ml of soln containing 3.96 mg final product in 5 ml.

		T_{255}	MC_{255}
28-ii	Pen01-HCO$_2$H	·361	·670
7-iii	Dry sup. HCl	·066	·664
		·058	·668
		·056	·667
11-iii	Pen01-NH$_3$	·051	·666
		·058	·667

Total weight in soln = 3·96 × ·0181 × 1000 = 14·3 γ/ml

Weight of T = $\frac{\cdot058}{\cdot608} \times \frac{5}{10}$ = 1·2 (T=7% of weight)

∴ Wt. of MC hydrochloride = 13·3

Depth of free MC ·570 = 12·3 × $\frac{13.3}{12.3}$ = 10·3

D for 10 γ/ml = $\frac{\cdot667}{10.3}$ = ·64

Absorption of Beckman cells (Spectral)

Cleaned ⊂ HNO_3 ⊃ aq. dest., filled ⊂ aq. dest, ⊃ read 1B, 2A, ⊂2B, ⊃ 1A as blank.

λ	1B	2A	2B		$\bar{x}$
205	·005	·011	·007		
220	−·002	−·002	−·003		−·002
230	+·003	+·007	+·006		+·005
235	·007	·012	·009		
240	+·009	+·017	+·014	243: ·011 ·018 ·014	+·013
245	·011	·017	·013		
→ 250	+·008	·013	·011		+·011
260	·004	·004	·003		+·004
270	·003	·002	·001		+·002
280	·002	·001	·001		+·001
290	·002	·001	·001		+·001
300	·002	·001	·001		+·001

Uracil in N/10 HCl

D for 10 γ/ml = $\cdot 669 \times \cdot 01 \times \frac{250}{2\cdot 38} = \cdot 703$

Hotchkiss: $\cdot 72 \times \frac{1\cdot 00}{1\cdot 00} = \cdot 72$

Guanine

D = $\cdot 675 \times \cdot 01 \times \frac{250}{2\cdot 28} = \cdot 740$

Hotchkiss: ~~[illegible]~~ $0\cdot 40 \times \frac{1\cdot 00}{\cdot 71} \times \frac{1\cdot 18}{1\cdot 00} = \cdot 665$

8-iii-50. Uracil 2·38 mg/ml in N/10 NaOH diluted:

	1 pipetteful up to 50 ml in N/10 HCl (D_{260})	0·20 ml up to 50 ml in N/10 HCl	
(1)	·621	·665	
(2)	·620	·668	
(3)	·628	·674	
$\bar{x}$	·623	·669	Vol = $\frac{\cdot 623}{\cdot 669} \times \cdot 02 = \cdot 0186$

Correct for dirty tubes

Guanine 2·28 mg/ml diluted same way.

	Pipette ↑ 5·0 (D_{250})		·2 ↑ 50	
(1)	·608	·612	·670	
(2)	·624	·630	·675	
(3)	·615	·618	·679	
$\bar{x}$	·616	·620	·675	Vol = $\frac{\cdot 620}{\cdot 675} \times \cdot 02 = \cdot 0184$

8-iii-50

8-iv-50.

~~titrate~~ Titrate 1.00 N. NaOH with .0357 N. HCl

1 pipette full: .504
.504

Vol. = .504 × $\frac{.0357}{1}$ = .0180 mol

25-v.

~~0.1~~ 1.8 ml 1:100 NaOH .515
.520
.510
.510

Pipette filled 1:1 ~~.515~~
.510
.510

Calibration of micro-pipette

By weighing:—(Cartomis)

	Full						
Full	16.7658	658	657	657	657	656	658
MT	474	471	477	475	475	478	478
	180	184	180	182	182	178	180

$\bar{x}$ = 18.0 µl.

By dilution & Beckman reading.

1 pipette-full "acid thymin" made up to 10.0 ml H2O

D_{265}.

0.20 ml dilute made up to 100 ml

(1)	.399	(1)	.439
(2)	.402	(2)	.437
(3)	.404	$\bar{x}$	.438
$\bar{x}$	.402		
− blank	.033		
	.369		

Vol = $\frac{.402}{.438} \times 20$ = 18.4

16.9

8-iii Repeat by weighing (on scale) (Stanton)

Full	25.9444	443	443	443	446
MT	.9262	262	262	264	264
	182	181	181	179	181
	blown out on filter paper			blown out on glass	

$\bar{x}$ = 18.1

Read against distilled water.

	N/10 HCl	N/10 NaOH (Volumetric solution)
290	·001	·018
280	0	·022
270	0	·027
260	−·001	·035
250	−·006	·044
240	−·012	·058
230	−·010	·093
225	−·011	·172
220	−·013	·500 !

200 220 240

27 Apr. 1950.

Standard absorption data, revised.

in N/10 HCl

	M	Atoms N	UV peak	D for 10 γ/ml	10/DM	ε
Adenine	135	5	260 (262.5)	.96	.077	13,000
Guanine	151	5	250 (249)	.73	.091	11,000
Uracil	112	2	260 (259)	.705	.127	7,900
Thymine	126	2	265 (264)	.63	.126	7,960
Cytosine	111	3	270	.95	.095	10,500
MeCytosine	125	3	283	.785	.102	9,800
Cytosine Glycoribonde						13,200
Thymidine Thiothio						9,500

PLANT VIRUSES.

The Bial Reaction for Pentoses (Hilitzer's modification).

Pentoses react with orcinol in hot HCl in the presence of ferric ions to give a green solution. When diluted with butyl or iso-butyl alcohol the solution turns blue and the intensity of the colour is proportional to the amount of pentose.

Solutions.

Orcinol. 1 gm. in 100 ml. water.
For use, dilute 10 ml. with 40 ml. conc. HCl and add 1 ml. of 10% $FeCl_3$.

Butyl alcohol.

Xylose solution. 50 mgs./100 ml. (100γ in 0.2 ml.).

Turnip yellow mosaic virus. 20 mgs./ml.

Method.

0.2 ml. of solution containing approximately 50γ of pentose is mixed with 2 ml. of the orcinol reagent in a test tube and heated in a boiling water bath for 8 mins. It is cooled and the volume is made up to 10 ml. with butyl alcohol.

Micro-biuret reaction for proteins.

The biuret reaction may be made very sensitive.

Reagents: 0.5% $CuSO_4$ solution.
90% Alcohol.
KOH pellets.

Procedure

Take the solution of protein provided and add to 1 ml., containing 0.1 mg.-5 mgs. of protein, 1 drop of the $CuSO_4$ solution.

Add 1 ml. of alcohol and an excess of KOH pellets and mix. The KOH salts out the alcohol which carries the biuret colour with it.

Molisch Reaction for carbohydrates.

The Molisch reaction is a test for all carbohydrates bound or unbound (including filter paper) and is a very useful and sensitive test.

Procedure.

Dissolve a few crystals of α-naphthol in 90% alcohol or chloroform. Add one drop to the solution to be tested and mix. Pour conc. H_2SO_4 the tube and allow it to layer below the solution. Then mix carefully by gentle shaking. The liquid at the interface becomes warm and a brilliant cherry-coloured ring forms. Any green, black or brown ring is unspecific.

Some other substances such as acetone give a slight reaction.