

Notebook 8

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CHEMISTRY

November 1952 - July, 1955.

8

PF/GRW/A/7

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6/5

Supplied by
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CAMBRIDGE

Summary Index to Blood P Work

<u>Pages</u>	<u>Date</u>	<u>Subject</u>	
	15-X-54	1. Test of Brown - I sol.	- nucleotides
	26-X	Col. 2 - " "	" "
	1-A-i	3 - " "	" - good separation
	8-X-i	Test of Loring P method	
	8-X-i	Col. 4 - test separation of GGP, P _i & FDP	
	11-X-i	5 - " "	GIP, AGP, PGA, P _i - good sep'n
	15-X-i	6 - " "	known amino acids - no sep'n.
	17-X-i	7 - " "	all of above sp'd.
	22-X-i	8 - " "	- Brown acetate
	30-X-i	9 - " "	" "
	23-X-i	Test of anthrone reaction	
	6-Xii	Col. 10 - test separation of mixture : Brown acetate	
	26-i-55	Homoamines	
	28-i	" "	
	2-ii	N.ac - homoamines - BBm	
	31-V	Col. 11 - test separation : Benzacetate w pH 6	
	10-Vi	12 - " "	pH 8
	13-Vi	13 - " "	- acid
	16-Vi	14 - " "	Brown 1 x 8 format
	20-Vi	P _i in Ma r Tp - 10 - Loring & Lopez method.	✓
	22-Vi	P _i & P ₂ in Tp - " "	✓
	21-Vi	Col. 15 - test separation of GGP, AGP, P _i & glutamine	
	24-Vi	Col. 16 - " "	no " - Brown x 8
	30-Vi	Col. 17 - " "	" - Brown x 10
	30-Vi	18 - Tp blood - v/c	
	6-Vii	19 - " "	- good fractionation but R = 200 % ✓
	11-Vii	20 - " "	- no run from col. 19
	14-Vii	21 - " "	- no run - 20 - diff at length pH

		A		C		OC		
P for 2.5 ml	x 200	unhydrolyzed	hydrolyzed P	unhydrolyzed	hydrolyzed P	unhydrolyzed	hydrolyzed P	$\frac{P}{R}$
7.557	7.56	22.6	5.56	0.246		7.45	0.330	
7.587	7.60	22.7	5.79	0.255	4.46	0.1965	7.21	0.318
7.621								96.5

20-XI-52 Loss of OC hydrolyzed $\pm$ DNA ⑤

BSNA 5.0 mg. } $\uparrow$ 3.0 ml 88% HCOOH. 2 0.7-ml
 hydrolyzed OC 0.5 mg }
 portions sealed in commercial 10 mm. paper

tubes. (a) 100° 2 min.

(b) 175° 30 min.

(Translucent surface blue color on sealing tubes!). Dry down, $\uparrow$ 25°.

Take 7.75 μ l spots, 2.10 for P.

5-XII head after drying spots in dry tubes.

		A_{260}	OC 200	C_{220}
a	B 1	123	87	87
	2	126	108	94
	$\bar{x}$	125	98	
	spots 1	843	818	
	2	850	824	
	$\bar{x}$	847	821	
	$\bar{x}-3$	722	723	

		A_{260}	OC 200	C_{220}	
b	B 1	126	89	88	
	2	110	80	74	
	$\bar{x}$	118	85	81	
	spots 1	882	799	548	} note from reading of unhydrolyzed!
	2	878	770	550	
	$\bar{x}$	880	785	549	
	$\bar{x}-3$	762	700	468	

	a		b	
	Wards	Rates	Wards	Rates
A	3.86	32.1	3.87	32.3
T	3.98	33.1	3.97	33.2
G	2.16	18.0	2.14	17.9
OC	2.01	16.7	1.99	16.6
	12.01	99.9	11.97	100.0
P, V	4.287 4.287	4.287 4.287	4.33 4.25	4.29
x 198	12.74		12.98	
R	9.44		9.38	
%P	3.49		3.49	

20-xi-52. Phages

- (a) 1.49 avg T4+7 (31-x) } ↑ 0.5" and 88% HCOOH in over 6 min.
 (b) 1.49 avg T4+7 (31-x) } Repeat to 175" 30 min.
 Breakdown: ↑ 25% T4, 7.75% HCOOH, 2.10 for P.

5-xii Because of Beckman not working, kept spore out and in dry hole until 6-xii.

		Gr	A	OC	T
B	1	102	69	58	100
	2	97	63	55	86
	$\bar{x}$	100	66	57	93
a	1	342	570	257	418
	2	333	566	246	401
	$\bar{x}$	338	568	252	410
	$\bar{x}-3$	238	502	195	317
b	1	333	568	250	418
	2	336	570	249	399
	$\bar{x}$	335	569	250	409
	$\bar{x}-8$	235	503	193	316

(a) T7

(b) T2r.2

(c) T4r.7

	Reindeer	Reindeer	Reindeer	Reindeer
A	0.839	25.8 24.5	2.255	31.5
T	1.031	30.1	2.518	35.1
G	0.755	23.3 22.0	1.163	16.2
OC	0.800	24.7 23.3	1.216	17.1
	3.425	99.9	7.162	99.9
P.Y	2.43 2.41	2.84 2.87	3.36 3.27	
	2.89	2.89	3.38	
R	7.19	8.55	10.04	
90P	4.79	83.8	96.2	
	2.16	2.35	3.85	

6-xii-52.

Phages. incl. T7.

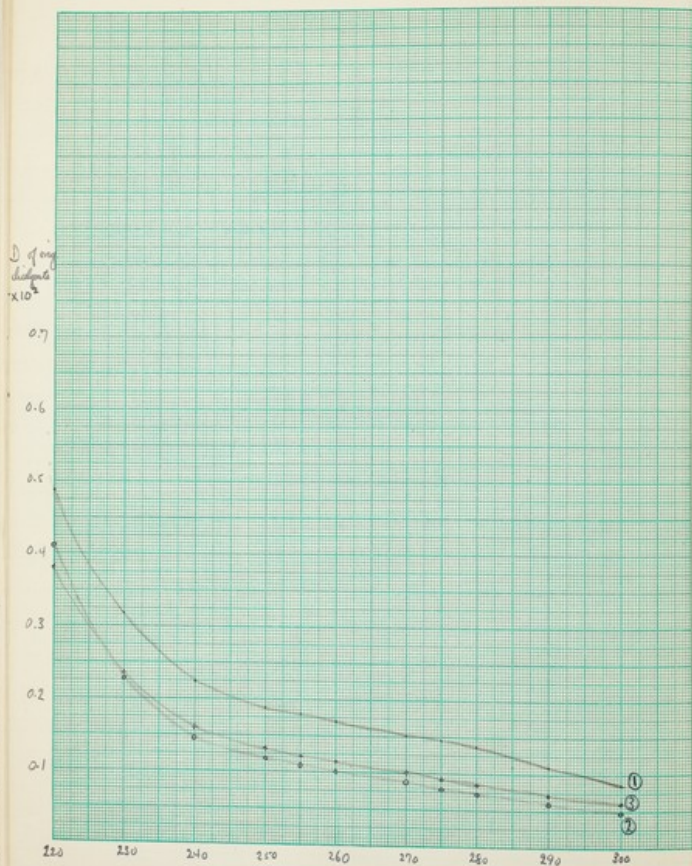
(a) Negliff's T7 1.35 ang.

(b) T2r.2 (22-xi) 1.46 ang.

(c) T4r.7 (3-xi) 1.48 ang.

↑ 0.5 and HERTH 175° 30 min., Aug.
 ↑ 25 µl, took 7.75 µl after, 2.11 µl for P.

	B	A	C ₅₀₀	OC ₅₀₀	T
1	99	65	55	54	81
2	101	67	56	55	84
$\bar{x}$	100	66	56	55	83
a	1	185	175	138	134
2	181	175	142	138	170
$\bar{x}$	183	175	140		165
$\bar{x}-B$	83	109	84		82
b	1	226	386	168	170
2	229	361	176	178	291
$\bar{x}$	228	359		174	283
$\bar{x}-B$	128	293		119	200
c	1	298	469	204	208
2	294	470	204	208	339
$\bar{x}$	296	470		208	337
$\bar{x}-B$	196	404		153	254



8-xii-62 PBm 80/35₂ diapause.
Concentrated by distillation in vac. Final concentrations fairly trusted.

	①	②	③
	Days 1+2	Days 3+4	Days 5+6
	3400 ml $\rightarrow$ 55 ⁺ ml x 55/3400	3600 ml $\rightarrow$ 19.5 ml x 19.5/3600	4600 ml $\rightarrow$ 55 ⁺ ml x 55/4600
300	84 110	90 60	.051 61
290	69 110	110 60	.060 72
280	86 127	134 73	.072 86
275	92 146	145 79	.077 92
270	98 151	162 88	.084 101
260	.107 170	187 101	.096 115
255	.113 179	202 109	.096 122
250	.119 189	218 118	.110 132
240	.141 224	268 145	.134 160
230	.200 318	419 227	.196 234
220	.308 489	756 410	.318 380

	T7		T6 175°		T6 165°		T2 at 165°	
	a		b		c		d	
	Time	Rate						
A	1.461	2.5	3.97	32.1	4.09	32.2	4.78	32.0
T	1.786	30.0	4.02	32.7	4.08	31.9	4.865	32.8
G	1.292	2.7	2.38	18.4	2.35	18.75	2.70	18.2
C+OC	1.419	23.8	2.08	16.8	2.18	17.17	2.815	16.97
[U]	[0.767] [6.2]		[0.06] [0.8]		[0.025]		[0.13]	
	5958		12.37		12.70		14.83	
P.V	4.12 / 4.06 4.00		4.24 / 4.26 4.18		4.32 / 4.38 4.29		5.13 / 5.10 5.07	
1.298	12.08		12.53		12.88		15.20	
	49.4		98.8		99.1		97.7	
90P	2.05		7.24		7.30		4.04	

9-xii-52 Phase. T7. 165° hydrolysis

- (a) Mg/eff. T7 2.405 mg } ↑ 0.5 ml H₂O 175° 30 min. } Dry 2.25 ml
 (b) Run T6 at 2NA 0.69 } } H₂O - 30% CH₂Cl₂
 (c) " 0.70 } } 7.75 ml defec
 (d) T2 at 5 (22.0) 1.53 } } 2.11 gwt.

		G ₂₀₀	A ₂₀₀	C ₂₀₀	O C ₂₀₀	T ₆₅	215	U	265
B	1	96	64	54	53	83	77	63	56
	2	96	67	53	52	86	83	69	62
	$\bar{x}$	96	66	54	53	85	80	66	57
(a) T7	1	237	252	203	196	225	104	91	83
	2	238	259	203	197	229	112	99	90
	$\bar{x}$	238	256	203	197	227	108	95	87
$\bar{x} - B$		142	190	149	144	142	28	29	28
(b) T6 175	1	347	580	249	254	402	83	70	63
	2	347	584	251	256	409	86	72	65
	$\bar{x}$	347	582	250	255	406	85	71	64
$\bar{x} - B$		251	516	196	202	321	5	5	5
(c) T6 165	1	360 (340)	596		265	406	79	66	60
	2	355	600		264	407	82	69	62
	$\bar{x}$	358	598		265	407	81	68	61
$\bar{x} - B$		262	532		212	322	1	2	2
(d) T2 at 165	1	393	682		298	467	86	73	66
	2	393	684		296	476	92	79	72
	$\bar{x}$	393	683		297	472	89	76	69
$\bar{x} - B$		297	617		244	387	9	10	10

16-xii-52. T7 NA (Lysogen) Repeat search for OC.

Integrate each of 2 tubes, 5.0 mg T7 NA $\uparrow$ 2 ml H₂O. 175° 30 min.
Combine 2 wafers to ~0.5 ml, spread on 8" wide what wafers. #3,
run in wafers - 195°.

11-xii-52

T7 phageoff Search for OC.

"C" elutes from 2 hydrolyses combined, wafers to degrees (colony
forming) wafers on paper.

"V" elutes from hydrolysis no. 2 likewise. Run in 195°-197°.

"V" $\rightarrow$ no visible wafers off. Unwafers.

"C" $\rightarrow$ wafers off C, none off OC. Elute both regions.

Read directly against blanks.

	OC	C
270	.007	.230
275	.007	.248
280	.007 .006	.226
285	.0004	

260	.015
265	.012
270	.016
275	.010
280	.022
285	.014
290	.021
295	.024

$$\text{Mean OC} = \frac{.006 \times 105}{.243} \times 100 = 2.7\%$$

	(a) BaV not dial		(b) BaV dial		(c) TNA	
	Time	Ratio	Time	Ratio	Time	Ratio
A	1.38	28.2	1.01	27.5	2.12	28.8
T	1.52	31.0	1.16	31.5	2.20	28.8
G	1.00	20.4	0.786	20.0	1.74	22.2
C	1.00	20.4	0.772	21.0	1.66	21.2
	4.90		3.678		7.72	98.6
					(0.40)	1.2
					7.52	99.9
PY	2.21		1.50		2.53	
	2.15	2.19	1.52	1.51	2.53	2.54
	2.70				2.58	
X						
195	6.46		4.45		7.50	
R	7.6%		83.7%		104%	
		(a)		(b)		
68	5.236 mg in 25 x 97% + 2.4		1.992 in 25 x 97% + 1.5			
	= 26%					
	= 0.121 mg/ml					
P	2.19 x in 2.16 ml = 1.041 / ml		1.51 x in 2.11 ml = 0.72 / ml			
70P	0.86%		0.93%			
70LNA	6.5%		7.7%			

5-1-53. PBM 80/35₂ dial. & not.

(a) PBM 80/35₂ not dial. 3.236 mg (on side of tank)

(b) dialyzed 1.992 mg

(c) 1.992 mg + 1.992 mg = 3.984 mg. Cook 100° (water bath) 2 hrs. Each 10 ml 70% HCl. shaking periodically. Add 15 ml H₂O, mix, take 2.10 ml for P & 7.72 ml for L.

		G	A	C	T
B	1	99	68	61	89
	2	101	72	66	93
	$\bar{x}$	102	70	63	91
a	1	209	281	166	208
	2	214	247	170	216
	$\bar{x}$	212	249	168	212
$\bar{x}-B$		110	179	105	121
b	1	180	201	143	180
	2	183	201	144	186
	$\bar{x}$	183	201	144	183
$\bar{x}-B$		81	131	81	92
c	1	291	344	234	261
	2	295	346	239	270
	$\bar{x}$	293	345	237	266
$\bar{x}-B$		191	278	174	175

T2r-3		T6r-1			
	Yrds	Ratio		Yrds	Ratio
A	A	4.04	32.4	3.90	32.7
T	T	4.11	33.0	3.99	33.4
G	G	2.22	17.8	2.165	18.15
C	HMC	<u>2.09</u>	<u>16.8</u>	<u>1.865</u>	<u>15.65</u>
		2.26	100.0	11.92	99.9

P, V	4.26, 4.27	4.00	4.03
P, 298	12.73	12.00	
R	97.9	99.3	
X 298 P	3.50	3.26	

R

Elementary analyses

	Mg/mol	P/0.2 mol	T ₆ P	N/mol	298	P/H
T2r-3 (1-1-53) 2.46 mg + 3.0 mol % NaOH	0.820 668/669	6.97	4.08	105.17	12.83	0.38
T6r-1 () 2.53	0.843 674/679	6.97	4.14	113.97	13.62	0.36

12-i-53. Phages - New prep.

2.76	T2r-3 (1-1-53)	1.48 mg.	↑ 0.5 and 58% H ₂ O, 17% 30 min. by
2.52	T6r-1 (1-1-53)	1.50 mg.	↑ 25% sol. In 2.15 for P, 7.72 after.

Refers over heated in drying by mistake. Maybe phage too?

	G	A	HMC	T
B 1	176	167	80	198
2	161	181	84	218
Σ	169	174	82	208
T2r 1	424	761	279	531
2	401	697	291	539
Σ	413	699	285	535
Σ-B	244	525	203	327

T6r 1	412	680	287	515
2	402	682	268	534
Σ	407	681	263	525
Σ-B	238	507	181	317

	T ₁₂ -6 a		T ₁₂ -3 b		T ₁₂ -1 c	
	Ymole	Ratio	Ymole	Ratio	Ymole	Ratio
A	29.9	32.7	4.39	32.6	4.23	32.7
T	3.14	34.3	4.54	33.8	4.41	34.1
G	1.50	16.4	2.34	17.4	2.24	17.3
HMC	1.515	16.35	2.175	16.2	2.07	16.0
	9.145	99.95	13.445	100.0	12.95	100.1
P ₁ /	3.54 } 3.49		4.81 } 4.80		4.37 } 4.37	
	2.63 }		4.78 }		4.37 }	
x ₂₅	10.69		14.30		13.03	
R	85.6		94.2		99.4	
P _{OP}	2.89		3.86		3.52	

15-1-53

Phages.

- (a) T₆-6 (v.52) 1.51 mg. } ↑ 0.5 ml 88% HCOOH, 175° 30 min.
 (b) T₂-3 (1-1-53) 1.51 mg. } ~~Indicate by multiple spots~~
 (c) T₆-1 (1-1-53) 1.51 mg. } day 4 ↑ 25% H₂O, Tube 2-11 = 772 pl.
 Chromatograms run to bottom of paper.

		G	A	HMC	T
R	1	113	80	78	117
	2	115	79	70	115
	$\bar{x}$	114	80	74	116
(a)	1	280	469	225	363
	2	278	467	216	369
	$\bar{x}$	279	468	221	366
	$\bar{x}-B$	165	388	147	250
(b)	1	367	649	291	474
	2	374	653	278	479
	$\bar{x}$	371	651	285	477
	$\bar{x}-B$	257	571	211	361
(c)	1	358	626	276	461
	2	361	633	273	473
	$\bar{x}$	360	630	275	467
	$\bar{x}-B$	246	550	201	351

Phosphorus analysis

10.4 mg vaccine + 2.5 ml 1% HCl → 4.16 mg / ml.

$$P: 0.05 \text{ ml} \rightarrow \frac{5.78}{5.92} \times \frac{5.44}{4160 \times 0.05} = \frac{5.88}{4160 \times 0.05} \times 100 = 2.83\%$$

$$DNA: 0.05 \text{ ml} \rightarrow \frac{43.0}{42.7} \times \frac{42.9}{4160 \times 0.05} \times 100 = 2.06\%$$

$$RNA: \text{based on } \frac{P}{N} \text{ ratio} = 2.0 \times \frac{2.0}{5.4} = 0.56\%$$

16.1-53. Vaccinia.

19-i. Into 2 tubes: 26.7 mg + 23.9 mg (total 50.6 mg). In each tube 2.5 ml 88% HCl, seal, cook 175° 30 min. Open. Turbid. Spin 7000 5 min. → clear soln but almost no pellet (some surface remain). Evap. in vac. to ~0.3 ml, spread on 8" strip What #3, oven in moist HCl.

20. Elute bands G, A, C, U, T, in vol. 0.5 ml each, evap. down, + 20 µl, spread on What #3 along 5 markers. ^{in PAB-103} Run, cut out, elute in 4 ml 1% HCl, read directly against blanks:

	G	A	C	$\frac{1}{2} HMC$	U	T
255	970	265 164	278 140		263 .152	270 .534
250	96	262.5 166	275 143	283 0.076	259 .158	265 .589
245	91	260 164	272 139	279 0.087	255 .150	260 .567
				275 0.091		

Yields, 10°

G 8.7

A 12.8

C 13.6

U 2.0

T 7.4

$$44.5 + 10\% \text{ for loss} = 50. \quad \text{Total in 4 ml} = 200 \times 10^{-3} \times 2.00$$

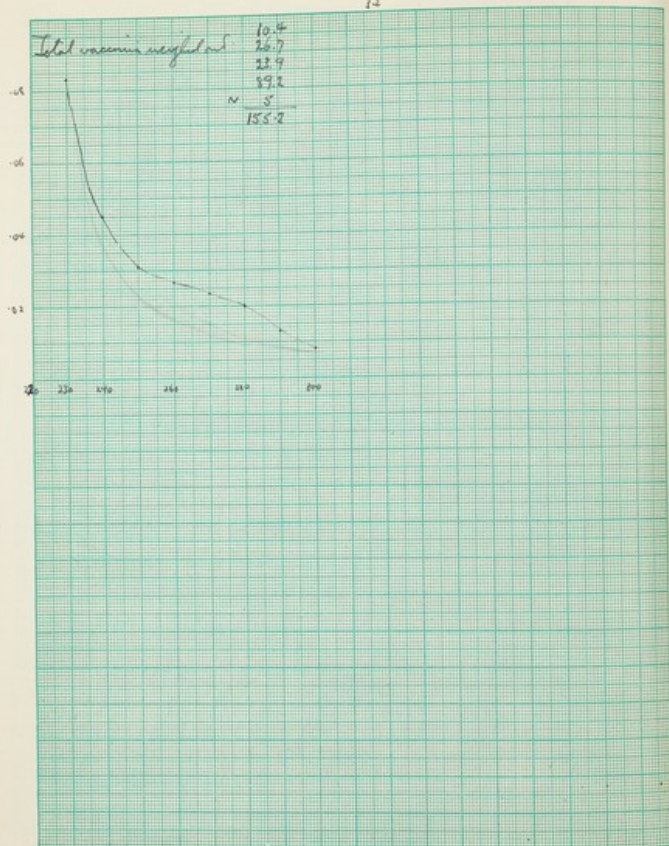
Assume mean molecular wt = 315.

$$\text{NA} = 200 \times 315 = 630 \text{ } \checkmark$$

$$\% \text{ NA} = \frac{0.63 \times 100}{50.6} = 1.2\% \quad \text{Must be loss in handling.}$$

$$\text{Mass HMC} = \frac{0.087 \times 2}{0.97} \times \frac{1.00}{1.43} \times 100 = 13\% \text{ of C}$$

$$2 \times \frac{1}{2} = 0.06$$



23-i-53

Vaccinia NA

89.2 mg vaccinia $\uparrow$ 10 ml ~ 0.15 M-NaCl. Add 3.6 g urea, shake till dissolved. Then after ~ 20 min add ~ 5 ml chloroform-octanol, shake, spin, repeat twice. Third time $\rightarrow$ small gel. Ppt $\uparrow$ EtOH, spin, ppt again $\uparrow$ EtOH, spin, dry = PROT. Ppt add ~ 2 vols EtOH $\uparrow$ 1 drop HCl $\uparrow$ few drops Mg Ac. $\rightarrow$ ppt, gradually flocculating. Spin down, $\uparrow$ 2 M NaAc (doesn't dissolve completely). Ppt $\uparrow$ 2 vols EtOH, spin down, wash = EtOH, dry = NA-A.

NA-A, scraped into second tube, weighs 9.67 mg. (after removal of 0.04)

23.8	0.01
23.6	0.01
23.5	0.01
23.4	0.01
23.3	0.01
23.2	0.01
23.1	0.01
23.0	0.01
22.9	0.01
22.8	0.01
22.7	0.01
22.6	0.01
22.5	0.01
22.4	0.01
22.3	0.01
22.2	0.01
22.1	0.01
22.0	0.01

40 $\uparrow$ 3.2 ml 0.01 M-NaOH. Assume NA contribution is ~ 0.01

Then total NA = $0.01 \times 0.04 \times 32 = 9.7 \times 0.31$

24-i

PROT. from above $\uparrow$ 5 ml N-NaOH, remove room temp. 48 hrs.

26-i

Swing N-NaOH. spin $\rightarrow$ little gel. Ppt $\uparrow$ EtOH $\uparrow$ HCl $\rightarrow$ bigger ppt. $\uparrow$ 3 M-NaAc + N-NaOH $\uparrow$ pH > 10 . Dissolves and v. well. Swing 3x. Ppt $\uparrow$ HCl $\uparrow$ 2 vols EtOH $\rightarrow$ fine ppt. Spin down, dissolve in 0.3 ml 3 M-NaAc-NaOH, ppt in small tube, spin down, wash twice = EtOH, dry $\rightarrow$ NA-B. NA-B, dried overnight, weighs 1.21 mg.

Vaccinia DNA

	Units	Percent
A	3.60	29.5
T	3.65	29.9
G	2.51	20.6
C	2.45	20.0
	12.21	100.0

P.V
 $\frac{4.14}{4.08} \times 100 = 101.2\%$

x 298

R

84P = $\frac{4.10}{12.06} \times \frac{30}{2.10} \times 100 = 48.5\%$

27.1.53 Vaccinia NA analysed.

(A) "NA-A", 9.7 mg containing 0.3 mg NA } Load 0.75 µl 85% (14000) and
 (B) "NA-B" 1.21 mg } 175° 30 min. After centrifuge
 to remove some acid, flaky material, dry down.

(A) 30 µl, spread on band on paper.

(B) 30 µl. Take 3 x 2.1 for P (3rd spot out into 2 for estimation.
 2 x 7.7 for spots, remainder as 3rd spot.

"A" → weak bands of G, A, C, U (?) & T (?). Elute C & U+T, & run in BuOH-NH₃.

"B" → good clean chromatogram: U, T, G, A, C, & T only. Elute "C+U" portion from 3rd lane & run in BuOH-NH₃.

Elutes from "B" (DNA) directly against blanks:

	HMC portion	C	
275	.002	.286	
279	.0015	.272	Max HMC as % of C in vaccinia DNA
283	.001		$= \frac{.0015}{.286} \times \frac{1.05}{.097} \times 100 = 0.6\%$

		G	A	C ₂₀₀	T
8	1	104	69	63	108
	2	100	68	61	101
	$\bar{x}$	102	69	62	105
NA-B	1	376	539	318	374
	2	379	535	317	375
	$\bar{x}$	378	537	319	375
22-3		276	468	257	290

	a		b		c		d	
	Value	Ratio	Value	Ratio	Value	Ratio	Value	Ratio
A	3.96	33.0	4.20	32.6	32.4	32.1	3.78	32.1
T	4.05	33.7	4.225	32.8	4.00	32.6	3.87	32.8
G	2.065	17.2	2.32	18.0	2.227	18.15	2.155	18.30
HMC	1.93	16.1	2.13	16.5	2.112	17.2	1.99	16.9
	12.005	100.0	12.875	99.9	12.279	100.05	11.995	100.1
P, Y	4.35 4.38	4.37	4.43 4.49	4.46	4.06 4.21	4.14	3.89 3.86	3.88
x 278	13.02		13.28		12.33		11.55	
R	92.2		97.1		99.7		102.0	
% P	7.20		7.15		6.93		6.40	

Leak of 98% HCOOH.

- (a) 0.72 mg. TB⁺ DNA + 0.1 ml. Analar 98% HCOOH
 (b) 0.74 " " 0.25 ml. " "
 (c) 0.71 " " 0.5 ml. " "
 (d) 0.72 " " 0.5 ml. Analar 98% HCOOH
- 175° 30 min. day.
 + 25% N-H₂, End
 7.7 g. of solids, 2.1 g. of P.

		G	A	HMC	T
B	1	100	69	62	98
	2	98	67	60	94
	$\bar{x}$	99	68	61	96
a	1	326	583	249	422
	2	326	582	246	413
	$\bar{x}$	326	583	248	418
$\bar{x}-B$		227	515	187	322
b	1	356	616	268	436
	2	351	611	268	428
	$\bar{x}$	354	614	268	432
$\bar{x}-B$		255	546	207	336
c	1	346	575	267	413
	2	342	584	264	415
	$\bar{x}$	344	580	266	414
$\bar{x}-B$		245	512	205	318
d	1	339	589	285	407
	2	332	588	282	400
	$\bar{x}$	336	589	284	404
$\bar{x}-B$		237	491	193	308

Chromatograms

2.23 mg $\uparrow$ 3.0 ml 0.1 N-NaOH, stand overnight.

Q: 0.5 ml $\rightarrow$ 110 DNA $\% \text{ DNA} = 24.6$
 P 0.15 ml $\rightarrow$ 3.00 $\% \text{ P} = 2.59\%$

Yields Ratios

A	3.53	29.2
T	3.92	32.4
G	2.22	18.4
C	2.43	20.1
	12.10	100.1

P, Y 4.78 } 4.77
 4.76 }

x 298 14.21

R 85.23

TS.

Rec'd 2-ii-53, from Lark via Cohen.

3-ii-53 2.11 mg $\uparrow$ 0.5 ml 88% HCOOH cook 17 $\frac{1}{2}$ 30 min.

	G	A	273	275	277	279	T
B	99	63	57	56	56		87
2	95	60	53	52	52		86
$\bar{2}$	97	62	55		54		87

TS	1	341	522	319	311	307	299	388
	2	341	520	317	309	307	298	410
	$\bar{2}$	341	521	310		299	299	399
	$\bar{2}-\bar{3}$	244	459	255		245		312

T6 DNA	1	351	602	261		268		425
- NaOH	2	351	604	260		267		429
(w. only)	$\bar{2}$	351	603	261		268		427
	$\bar{2}-\bar{3}$	254	541	206		214		340

TS "C" washed to dryness, run in BuOH-NH₃ stain 17 MC-C protein.

17 MC		271	316
278	.002	328	
279	.003	316	
283	.003		

$$\text{Max. 17 MC} = \frac{3}{328} \times \frac{97.105}{100.97} = 1.0\%$$

Chen's Analysis

1.80 mg $\uparrow$ 1.0 ml 0.1N-NaOH, stand overnight.

0.5 ml. biuret $\Rightarrow$ 54 $\%$ DNA, but color not right - guess less than half of this
 $\Rightarrow$ DNA - $\approx$ 20 $\%$ - Approx 2 $\%$

P: 0.1 ml $\Rightarrow$ 467 $\times$ P = 2.6 $\%$

Meningopneumonia virus

Killed virus rec'd 2-11-53 from Sigel in Colon.

3-12-53 14.84 mg $\uparrow$ 2.5 ml 88% HCOOH, cook 176° 30 min. Evap. to 40% sol.

spread in band ($\approx$ 4") on What. #1, run in isopropanol-HCl

4-11 $\Rightarrow$ G, A, C, T. Elute 4 bands, G, A, C+MC, U+T, each 0.3-0.5 ml.

Freeze dry.

5-11 Transfer almost quantitatively to paper, run in 80% NH₃ along: markers.
 Elute in 4 ml.

	G	A	HMC	C	U	T
			270 .021			
246	.054	256 .920	278 .022	271 .591	256 .046	261 .499
280	.154	260 .970	279 .022	275 .614	260 .042	265 .575
254	.050	264 .980	283 .021	279 .590	264 .040	269 .494

$V_{\text{index}} \times 10^3$ 0.49 7.5 [0.23] 5.85 6.5

Assume 5.8

Total V_{index} nucleotide/mol = 25.6×10^3

Total = = $0.186 \times 4 = 1.02$

Add 20% for handling loss = 1.23

Weight of nucleotide = 310 = 381

$\% \text{ HPA in virus} = \frac{0.381 \times 100}{14.8} = 2.6 \%$

Misc. HMC = $\frac{0.23}{5.85} \times 100 = 3.9 \%$ of C

T₆ DNA - NaOH

	Units	Ratio
A	4416	32.3
T	478	33.2
G	2.31	17.9
HMC	2.12	16.5
	12.87	99.9
P, Y	44.58 44.64	44.61
x298	13.72	
R	93.8%	

2-ii-53

Effect of N-NaOH on T₆ DNA.

0.81 mg from T₆ DNA + 0.2 ml N-NaOH, stand at room temp, overnight.

3-ii

After 20 hrs., add 0.015 ml glacial HCl & 0.4 ml EtOH, spin down ppt. by

add 0.5 ml 88% HCOOH, wash 175° 3 min.

Dry down, + 2.5 ml N-HCl, take 7.72 spots, 2.10 ml for P.

Readings 2 pp. back under T5.

	T2r3 phage		TS DNA	
	Yields	Ratio	Yields	Ratio
A	3.76	21.9	3.85	30.4
T	3.94	33.4	3.94	31.1
G	2.08	17.6	2.46	19.4
HMC	2.00	17.0		
C			2.43	19.2
	11.78	99.9	12.68	100.1
P, V	4.55 4.29 4.03	4.29	4.47 4.38 4.35	4.47
x298	12.78		13.31	
R	92.4		95.4	96

TS DNA

6-11-53 6.2 mg TS + 1.5 ml N-NaOH overnight at room temp.
 7-11-53 Ppt = 0.1 ml HCl + 3 ml EtOH. Ppt + 3 ml M-NaCl-0.05 N-NaOH.
 leaving twice → little gel second time. Ppt = HCl + EtOH.
 + 0.4 ml NaCl-NaOH solution, divide between 2 hydrolysis tubes, ppt.
 wash once in EtOH, dry, weigh. → 0.99 & 1.01 mg.

1.01 mg TS DNA + 0.7 ml 88% HCOOH 175° 30 min. Byham, + 30 ml
 HCl. Take 2.1 for P + 2.7 for T.

Simultaneously, do 1st mg T2r3 phage + 0.5 ml HCOOH, dry, + 25 ml.

	G	A	C		T
B 1	98	66	58	57	93
2	96	62	56	54	84
3	97	64	57	56	89
T2r3 1	327	554	246	250	410
2	325	550	246	250	394
3	326	552	246	250	402
4-8	229	488		194	313
TS 1	368	567	314	302	409
2	368	560	309	298	395
3	368	564	312	300	402
4-8	271	500	255		313

	TS DNA		T2r-3		T6r*-1	
	Yields	Ratios	Yields	Ratios	Yields	Ratios
A	3.29	30.2	4.81	32.1	4.29	32.1
T	3.31	30.4	5.02	33.4	4.46	33.4
G	2.12	19.5	2.64	17.6	2.325	17.6
H	2.16	19.8	2.64	1		
C			2.535	16.9	2.235	16.8
HRC	10.88	99.9	15.005	100.0	13.340	99.9
P, D, V	4.11 } 4.44 3.97 }		5.16 } 5.22 5.28 }		4.53 } 4.58 4.63 }	
x298	12.02		15.55		13.64	
x: R	9.26		96.5		97.9	
R						

12.ii.53

TS DNA & phages

TS DNA 0.98 mg } ↑ 0.5 ml 88% HCOOH, 175° 30 min.
 T2r-3 phage 1.58 } Dry down. TS ↑ 30 ml, water ↑ 25 ml.
 T6r*-1 1.54 } Take 2.10 l for P, 7.72 afri.

	G	A	273	C	279	T
B	1	104	74	65	64	104
	2	106	70	62	62	106
	̄	105	72	64	63	105
TS	1	338	500	293	283	368
	2	338	498	288	277	367
	̄	338	499	291		368
x-B		233	427	227		263
T2r-3	1	395	698		812	504
	2	395	696		806	504
	̄	395	697		809	504
x-B		290	625		246	297
T6r*-1	1	366	654		281	461
	2	362	624		279	459
	̄	364	629		280	460
x-B		259	557		217	355

18-ii-53

Examination of natural HMC for comparison to HMU.

0.4 mg natural HMC + 40 μ l 2M-HNO₃ + 8 μ l glacial HOAc. 27° overnight.

19-ii.

Add 0.15 ml H₂O $\Rightarrow$ ~ 2 mg/ml.

Standard: Synthetic HMU ~ 2 mg/ml in water.

— H —

16-VI-53

Effect of HClO₄ on synth. HMC.

1.25 mg synth. HMC + 0.125 ml 72% HClO₄. (= 12.5 mg/ml)

Take 2 portions each 0.05 ml. (a) Heat 100° 1 hr

(b) Control, room temp. (2 vials)

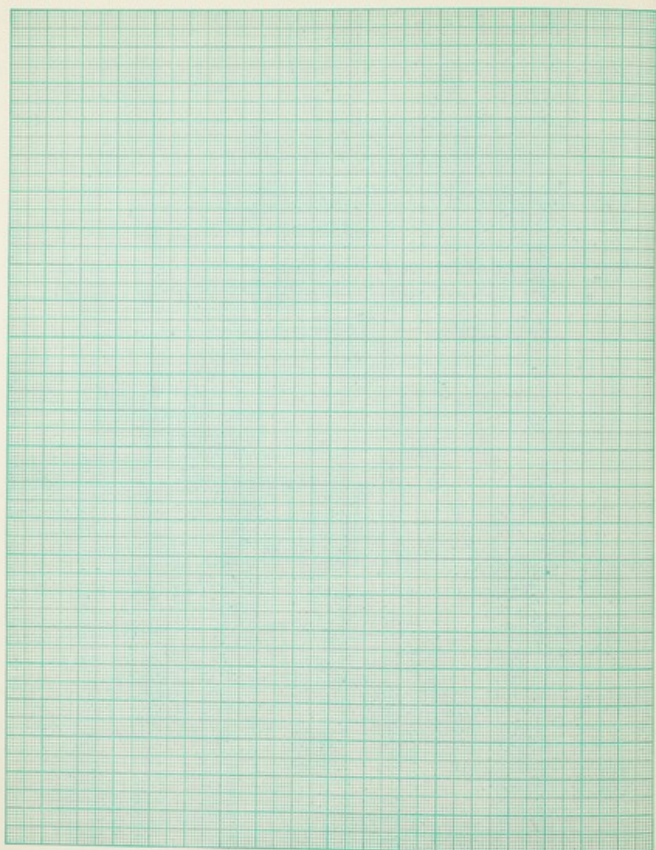
To each, add 0.1 ml of ket. reagent, & take 4 x 7.72 μ l spots.

~~ProstH-H₂~~

~~ProstH-H₂~~

	Raw	Cooked	Raw	Cooked
B	59		64	
Sample 1	446	447	477	462
2	451	450	475	464
$\bar{x}$	449	449	476	463
$\bar{x} - B$	390	390	412	399

$$\text{Conc. of HMC recovered} = \frac{0.390}{9700} \times 150 \times \frac{4}{0.00772} \times \frac{0.15}{1000 \times 0.05} = 9.4 \text{ mg/ml}$$



Solubility of Water in butanol.

t°	g. H_2O /100g. sol. sol.	Density of sol. d. sol.	(A)	(B)
			g. H_2O / 100 ml	g. H_2O / 100 ml
15	80.14	0.8533	68.3	84.3
20	79.93	0.8484	67.8	83.6
25	79.73	0.8450	67.4	83.0

- data of Phil = Mathias, J. R. C. L., 1926.

According to curve plotted from Jones, 1929, g. butanol/100 g. water
@ 20° = 80.2

11-17-52

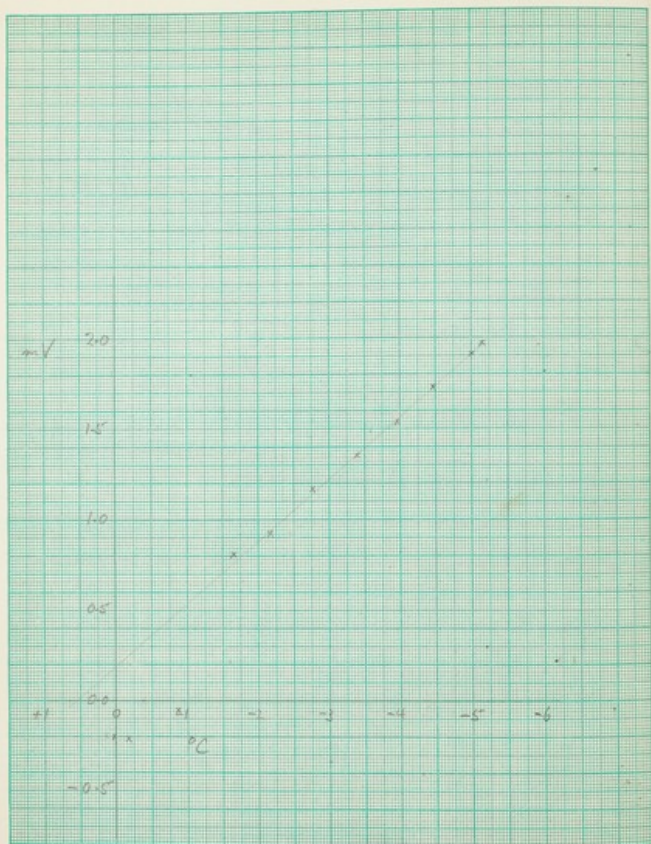
Freezing point depression — thermometer calibration

German Thermometer #110. Room temp. 26°. Distilled water ice crushed & suspended in distilled water in 1000 ml beaker inside larger beaker wrapped in towel. Stirred both mechanically & by hand. Read & line mounted in line — calibration point being read.

Stem immersion depth	Reading
14.5 cm	-0.09°
10.5 cm	-0.01

Whole apparatus moved to cold room (2°) and left 1½ hr to equilibrate

14.5 cm	-0.18° - .19
10.5	-0.16 - .17
6.5	-0.15 - .16



11-ix-53

Thermocouple calibration

Thermocouple from Bill Wellington. E.S. galvanometer.

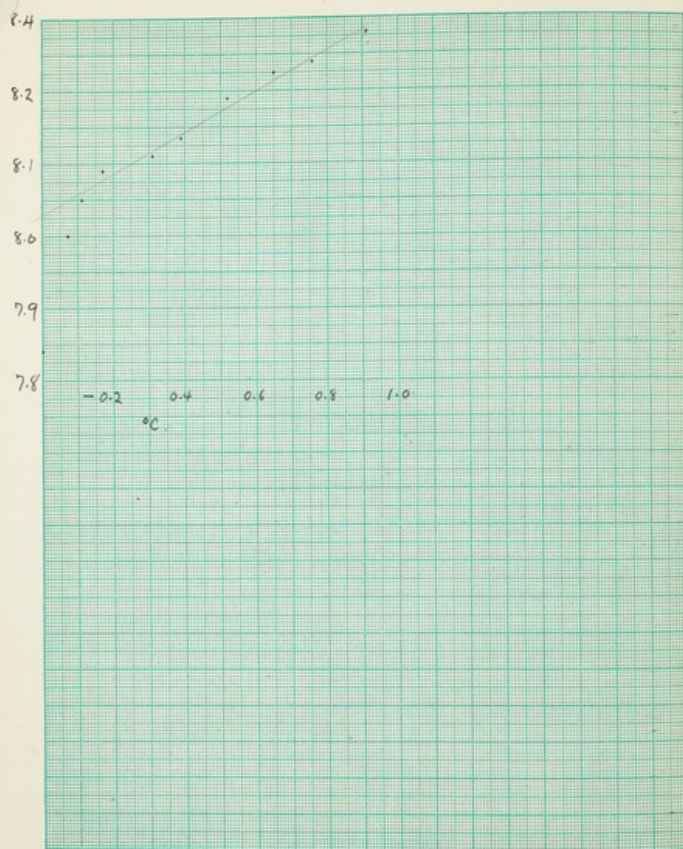
Couples set in glass tubes in paraffin wax.

Ref. couple in hot water-ice stirred bath, assumed to be 0°.

Calibration couple in alcohol tube in ice-water bath, to which salt is gradually added to depress temp.

Thermom. reading	True temp.	mV X 10
+0.02		-0.18
-0.17		-0.21
-0.87		-0.55
-1.65		+0.81
-2.18		+0.92
2.78		1.17
3.40		1.35
3.98		1.54
4.47		1.73
5.00		1.91
5.14		1.97

mV x 10



14-ix-53.

Thermocouple calibration

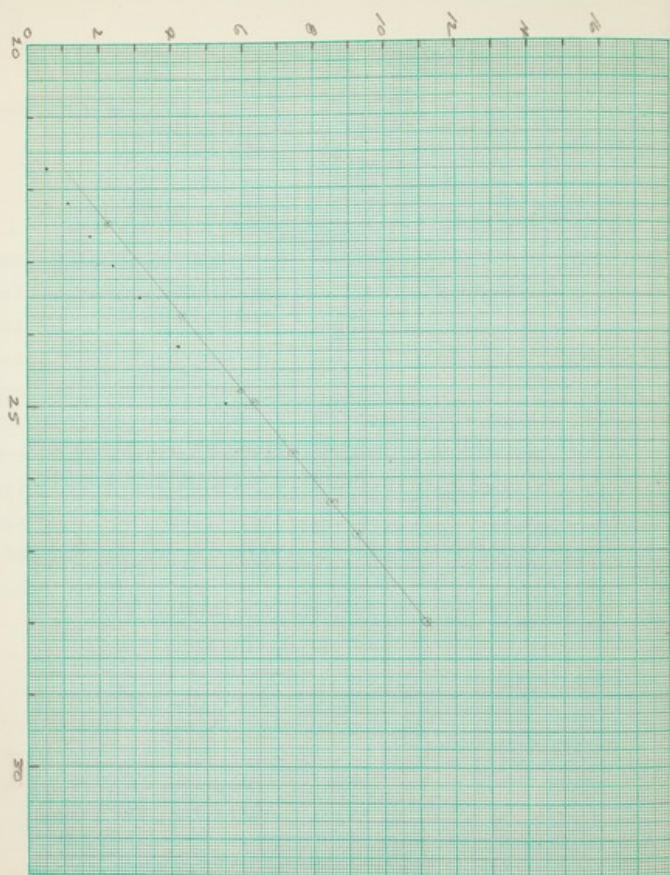
Ref. couple in water - bath at room temp = 21°

immersed 10.5 cm.

Calibration couple in ag. sol. - ice bath, along: Thermocouple no. 110_h

Temp. depressed by small addition of salt, allowing time to reach constancy.

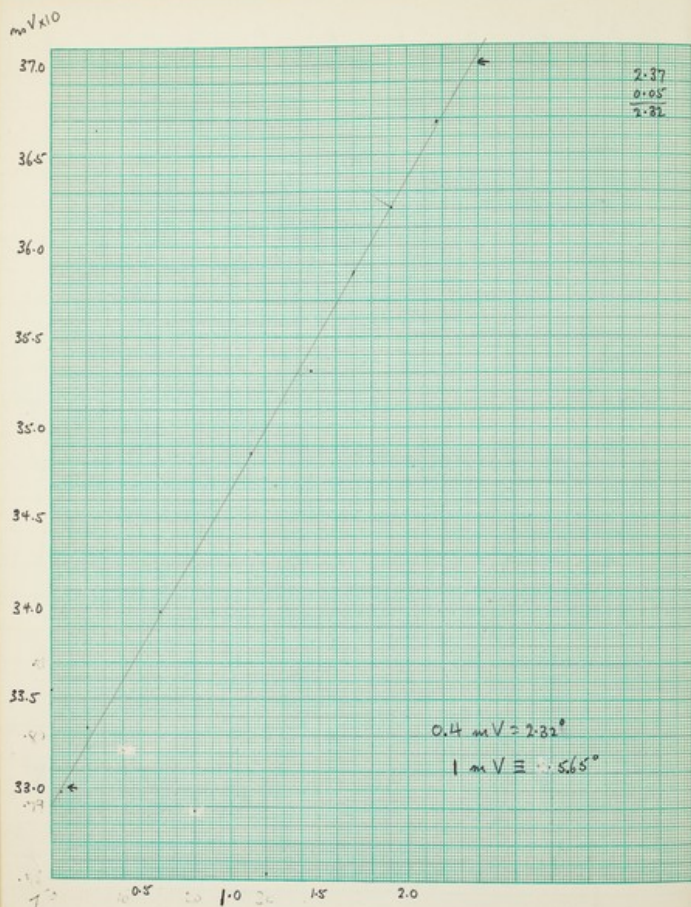
Thermom. reading	True temp.	mV x 10	$\bar{x}$
-0.04	0.00	7.81, 7.71, 7.61, 7.84, 7.84, 7.84	7.84
-0.11	-0.07	7.985, 7.99, 8.01	8.00
-0.15	.11	8.09, 8.08, 7.99	8.05
-0.21	.17	7.99, 8.14, 8.11	8.08
-0.35	.31	8.11, 8.135, 8.09	8.11
-0.43	.39	8.14, 8.13, 8.135	8.135
-0.56	.52	8.215, 8.18, 8.17	8.19
-0.69	.65	8.23, 8.24, 8.28	8.25
-0.80	.76	8.34, 8.26, 8.24	8.28
-0.95	.91	8.34, 8.36, 8.39	8.36



15-ix. 4 couples in this, both ref. c in unknown.

15-ix Thermometer	mV	16-ix	mV
18.94	0.416	22.48	2.26
21.72	0.26	23.75	4.35
22.20	1.08	24.95	6.85
22.65	1.78	24.79	5.99
23.06	2.435	25.65	7.44
23.50	3.16	26.33	8.52
24.19	4.25	26.77	9.25
24.98	5.56	27.99	11.21
25.66	5.86		

25.22 1.49 - 3 functions in parallel
26.11 1.84



Thermocouple calibration

18-in.

4 junctions fully embedded in wax.

Ref. junction in Dewar @ 21° . Room temp.

x junction in ice-water bath (not distilled). Offset by adding salt.

Thermometer #110, $mV \times 10$
immersed 10.5 cm.

0.05	32.98
0.20	33.34
0.61	33.98
1.12	34.86
1.46	35.31
1.70	35.86
1.91	36.21
2.47	36.68

Bov. blood 178 C from storage. F.p. of water held as 3480 mV
In bath: $+8^\circ$

Time	mV	Time	mV	Water f.p. or done
0	0.2346	23 min.	3.634	5 min. 2.521
2	0.2849	24	3.635	10 2.523
3	0.3122	25	3.636	12 2.526
5	0.3538	26	3.6375	14 2.529
7	0.3838	27	3.639	15 2.534
7 1/2	0.418	27 1/2	3.6395	16 2.532
8 1/2	0.4701	28 1/2	3.639	17 2.538
9	0.4758	29	3.6445	19 2.560
10 1/2	0.4851	29 1/2	3.644	20 2.527
11 1/2	0.4912	30	3.644	21 2.527
13	0.4982	31	3.644	22 2.562
14	0.5021	33	3.648	23 2.527
15	0.5066	34	3.650	25 2.528
16	0.5100	35	3.654	27 2.528
17	0.5118	37	3.658	
18 1/2	0.519			
20	0.525			
21	0.5201			
22	0.5207			
22 1/2	0.5234			

Zero before blood: 3.480 mV

" after 2.93 3.558

$\bar{x} = 3.519$

Blood Δ f.p. = $0.125 \pm 0.71^\circ$

30-18

 $\Delta f.p. \text{ det'n's}$

Standard:	Time	Ref. Therapy	Con V, ref. bath to air, water
	3:02 pm	22.6	3.576, 3.579, 3.580, 3.580, 3.580
	3:35	22.7	3.581, 3.583, 3.584, 3.584
	4:35	22.8	3.594, 3.596, 3.598, 3.598, 3.598
	5:35	22.86	3.611, 3.614, 3.614, 3.614
	6:15	22.9	3.617, 3.619, 3.619, 3.619

Blood sample #3 ~ 1.1 ml (arbit sample)

Time	mV	Time	mV
Bleeding #3 3:44	3.474	Bleeding 4:17	4.123
45	3.540	18	196
46.4	3.611	3 would. 19	274
47	3.659	19.6	3.700
48	3.712	20	3.698
49	3.749	21	698
50	3.784	21.6	699
51	3.814	22	701
52	3.844	23	701
53	3.875	23.6	705
54	3.895	24	706
55	3.921		
56	3.936	Bleeding 5:47	3.820
57	3.952	48	720
58	3.968	#4 50	4.039
59	3.972	0.5 ml. 51	4.055
4:00	3.991	53	4.140
4:03	3.986	54	4.161
4:03.6	3.974	55	4.181
4:04	3.967	56	4.186
05	3.965	60.1	4.285
6	3.952	21	4.307
6.6	3.950	41	4.491
7	3.956	42	3.723
7.6	3.959	52	3.718
8	3.962	6	718
8.6	3.964	7	723
		8.2	722
			740

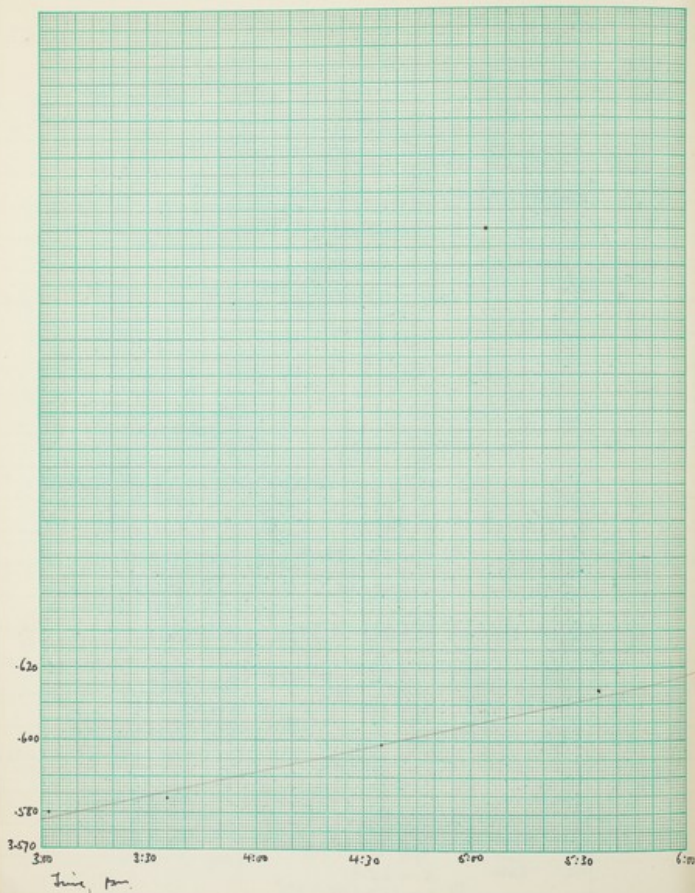
Leveat: 4.272 - 3.596 = 0.678

 $\Delta f.p. = 3.698 - 3.596 = 0.102$ $= 0.58^\circ$

Corr = 0.58

 $\Delta f.p. = 3.717 - 3.618 = 0.099$ $= 0.56^\circ$

Corr = 0.53



1-x-53

$\Delta f.p.$ detms.

Ref. both standardized } Time Therman mV
to dist. water - ice : 2.50 pm. 23.1 3.652, 3.654, 3.654, 3.654
3.50 23.1 3.649, 3.650, 3.651, 3.650

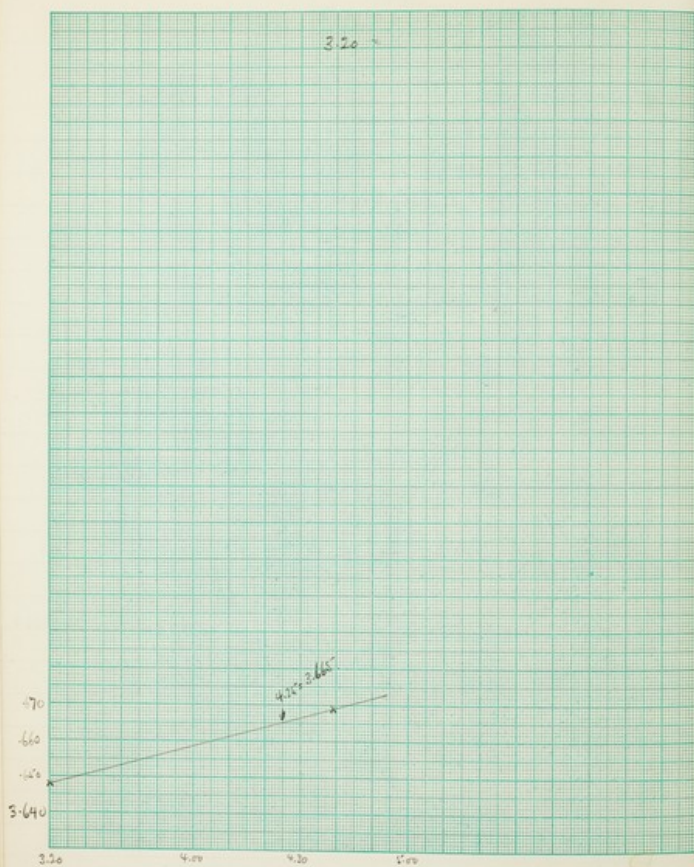
Physd sample 5, 0.5ml.

Time	mV
3.18	3.760
3.19	3.764
20	2.764
2	.782
21	.804
22	.836

$$\Delta f.p. = 3.760 - 3.651 = 0.109 \text{ mV}$$

$$\approx 0.62^\circ$$

$$\text{Corr} = 0.59^\circ$$



20 x 53

ASP Data

Bld sample #14

0.5cc

Ref bath standardized

Time

Thermom

mV

to Dist H₂O + ice

4.39

23.25

34.85

36.01 36.48 36.70 36.68

Bld sample #14

0.5cc

Time

mV

3.33

24.18

4.03

37.43

3.35

31.28

4.14

43.95

3.37

34.45

4.18 1/2

49.62

3.39

37.07

4.21

43.77

3.41

38.95

4.21 1/2

37.99

3.42

40.31

4

37.55

3.44

41.34

4.25

37.85

3.45

41.68

26 1/2

37.90

3.46

42.07

27 1/4

37.93

3.47

42.40

28 1/2

37.97

3.48

42.72

29

37.99

3.49

42.97

29 1/2

37.51

3.51

42.97

31 1/2

38.10

3.54

42.84

3.55

43.03

Taken out

to an.

3.58

31.14

3.59

32.78

3.60

34.52

4.01

36.22

4.02

36.57

2 1/2

37.38

$$\Delta f_p = 3.785 - 3.665 = 0.120$$

$$\text{Corr} = 0.115 \text{ mV}$$

$$= 0.65^\circ \text{C.}$$

20-x

Reference dist H₂O + ice mixture

Time	mV				
2.16	30.77	Thermom	2.20 gm	23.39	4.41) 36.76 37.00
2.17	36.93		2.26	25.37	37.02 37.04 37.07
2.19	36.97	Thermom	4.05	23.50	37.05 37.06 37.05
2.21	36.98	Thermom	4.35	23.53	4.43) 37.05
2.22	36.99				
2.23	36.99				
2.24	36.99				

Standardize Δ Sp. of 0.345 H. ammonia

Time	mV	Sp.			
2.41	27.83	3.45	26.83		
2.43	32.70	3.30	32.17		
2.47	34.96	3.05	33.52		
2.49	36.71	3.1	35.23		
2.53	38.95	3.2	36.42		
2.55	38.650	3.25	38.38		
2.60	39.55	3.4	40.41		
3.02	39.68	3.55	41.33		
3.04	39.73	3.6	38.51		
3.08	39.95	3.65	38.47		
3.12	39.91	3.65	38.51		
3.13	39.95	3.7	38.54		
3.17	39.98	3.75	38.60		
3.19	39.96	3.8	38.55		
3.21	39.76	3.9	38.71		
3.17	39.56	4.1	38.75		
3.2	39.41				
3.21	39.55				
3.22	39.68				
3.24	39.70				
3.25					

ice - 0.5°

0.12°

Standardize Δ Sp. of 0.1944 M NaCl

Time	mV				
4.15	34.54				
4.16	36.73				
4.17	37.76				
4.18	38.75				
4.18 1/2	39.80				
2.0	41.38				
2.1	42.11				
2.2	42.50				
2.3	38.76				
2.4	38.38				
2.4 1/2	38.36				
2.4 3/4	38.36				
2.5 1/4	38.39				
2.5 3/4	38.38				
2.6 1/2	38.42				
2.7 1/2	38.44				
2.8	38.50				
2.9	38.50				
3.0	38.53				
3.1					

ice - 12.0°

Corr = 0.71°

7-iv-54.

F.p.c (In chromatography room. Ref. couple in lab. burner flask, = small burner mounted on top.

Ref. to 0°: Time Room temp. mV

10.50		3.111, 3.115, 3.115, 3.115
11.15	20.4	3.112, 3.115, 3.113, 3.113
11.45	20.6	3.114, 3.115, 3.117, 3.117
12.00	20.0	3.115, 3.116, 3.116, 3.116
12.10		3.115, 3.115, 3.115, 3.115
12.58		3.114, 3.115, 3.115, 3.115

Std. soln.	11.27	3.176	11.58	3.170
NaCl	28	3.193	59	3.190
0.1744 M.	29	3.193	12.00	3.190
	30	3.193	01	3.190
	1	3.193	02	3.190
	2	3.193	03	3.190
	3	3.193	04	3.190
	4	3.193	05	3.190
	5	3.193	06	3.190
	6	3.193	07	3.190
	7	3.193	08	3.190

1 mV = 5.65°C.

0° = 3.114

$\Delta f.p. = 243 - 114 = 129$

$= \frac{0.72^\circ C}{0.70}$

0° = 3.117

$\Delta f.p. = 244 - 117 = 127$

$= \frac{0.72^\circ C}{0.69}$

Antone	4.03	3.540
0.378 M.	4	3.505
	1	3.536
Thermt. $\Delta f.p.$	5	3.526
= 0.704°C.	6	3.527
	7	3.527
	8	3.527
	9	3.527

$\Delta f.p. = 257 - 119 = 138$ mV

$= 0.78^\circ C$

Corr = 0.75°

Medium ①	4.31	3.290
	32	3.290
	33	3.290
	34	3.290
	35	3.290
	36	3.290
	37	3.290
	38	3.290
	39	3.290
	40	3.290
	41	3.290
	42	3.290
	43	3.290
	44	3.290
	45	3.290
	46	3.290
	47	3.290
	48	3.290
	49	3.290
	50	3.290

41.3 2.210

42 2.210

1 2.210

2 2.210

3 2.210

4 2.210

5 2.210

6 2.210

7 2.210

8 2.210

9 2.210

10 2.210

11 2.210

12 2.210

13 2.210

14 2.210

15 2.210

16 2.210

17 2.210

18 2.210

19 2.210

20 2.210

21 2.210

22 2.210

23 2.210

24 2.210

25 2.210

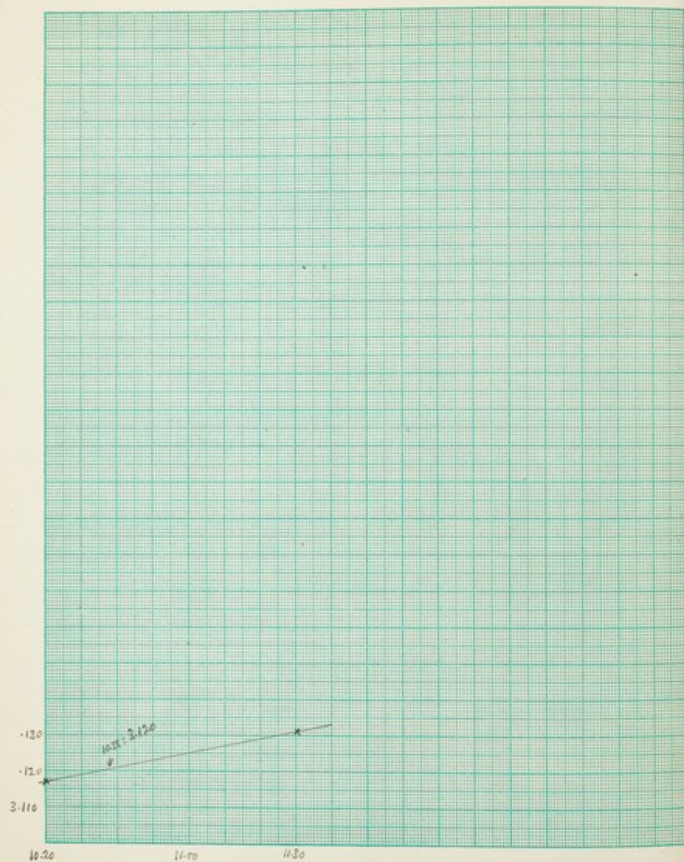
26 2.210

27 2.210

28 2.210

29 2.210

30 2.210



8-14. Time Room t. Amv
 zero: 10.20 am. 20.6 2.117, .117, .117
 11.30 21.0. Strid: .123, .130, .130, .131, .131, .131
 wistrid: .127, .130, .129, .128, .126, .126, .128

land dist. 10.33 3.270 Repeat: eq. dist.: 11.18 3.170
 water, to 34 $\frac{1}{2}$.140 19 .520
 w. dist. 35 $\frac{1}{2}$.130 20 .430
 zero! 36 $\frac{1}{2}$.128 21 .139
 37 $\frac{1}{2}$.126 22 .121
 38 $\frac{1}{2}$.128 23 .121 3.131 = True gas
 39 $\frac{1}{2}$.128 24 .122 3.130 = True gas
 40 $\frac{1}{2}$.126 25 .132 .001 correction
 41 $\frac{1}{2}$.128
 42 $\frac{1}{2}$.133
 True gas = 3.127 = True gas
 3.120 = True gas
 + .007 correction

8. m. Dry in both, stored well: zero = 3.136 (Room t. = 21.1)
 Thermocouple in big test tube, & crushed dist. water in
 & dist. water, stored; took in ice-water bath. zero = 3.144
 Correction = +0.008

f.p. of eq. dist.: 5.12 3.420
 .13 .530
 $\frac{1}{2}$.145
 14 .145
 15 .145
 16 .147

Zero included, & big test tube of ice within big beaker of ice: 3.144.

* MEAN CORRECTION, SUBTRACT FROM PREVIOUS VALUES OF ΔP = -0.005 mV.
 = -0.028° C.

3-xi-53

N & P

BBm 1

7.32 mg $\uparrow$ 5 ml H_2O 0.5 ml $\rightarrow$ 9.60 γ total P = 1.31% of blood solids.1.0 ml $\rightarrow$ 71.0 γ N = 4.85% "

BBm 15

11.09 mg $\uparrow$ 5 ml H_2O 0.5 ml $\rightarrow$ > 10 γ P1.0 ml $\rightarrow$ 107.3 γ N = 4.84% "
$$\frac{107.3 \times 5}{11.09} = 48.4$$

11-xi

Prevention of blackening by hydroxyamine

Into each of 7 small tubes weigh 1 mg BBm 12

0.1 ml

Add: ~~10%~~ hydroxyamine HCl solution made from 10%:

Time (approx)	10%	3%	1%	0.3%	0.1%	Water	90%
5 min.	clear	clear	clear	clear	slightly turbid	slightly turbid	clear
1 1/2 hrs.	turbid	clear	clear	sl. turbid	turbid	as turbid darkening	slightly clear
20 hrs.	{ turbid but not dark	slightly turbid	clear	slight ppt.	slight ppt.	heavy dark ppt.	clear, clouded
16-xi				still clear.			

5-1-54

Nucleic acid deriv. in BBm.

19 x BBm-1 extract analyzed } run in isopropanol-91%
 9.5 x BBm-14 " ex. - high. (a.e. apt. 31) } = DNA markers.
 → nothing, as viewed by fluor. penicling. Marker OK.

WT. of blood solidified ≈ 2 mg. resp.

Assuming sensitivity of 1%, mass. of any NA deriv. = 0.1% of solids.
 = 0.01% of blood
 = 10 mg %.

 WT. of ash 1.77 1.69 2.25 4.64
 % ash (by wt.) 9.60 9.54 10.23 14.05

Ultracentrifugation

Cellophane bag made from 11 cm dia. disk, in 10 x 75 mm Pyrex tube. Add 0.7 ml rabbit serum.

Spin on Beavell SS1 at 30,000 rpm 1 hr: OK

35,000 rpm 1/2 hr: OK

40,000 rpm 20 min: 1 bag

hook, other bag found some protein.

Dry wt. & ashing of BBm samples.

7-1-54 BBm samples from desiccator weighed into crucibles weighed in weighing bottles, then dried in oven at 225° F overnight & re-weighed.

Bottle no.	33	97	86	43
BBm no.	1	1	14a	12
Fresh sample	18.46 mg.	17.73	21.99	33.00
Dried sample	17.28	16.72	20.64	29.95
Water loss	1.18	1.01	1.35	3.05
% water	6.4	5.7	6.2	9.2

H₂O + any
Volatiles

Ashing. Crucibles heated to 550° C. for 3 hrs. Still gray, but cool & weigh.

Wt. of ash	1.80	1.76	2.38	5.06
% ash (dry wt.)	9.8	9.9	10.8	15.3

Re-ash at 650° for 7 hrs (by mistake - intended 2 hrs.). (Note: some white except for a few black specks - but?)

Wt. of ash	1.77	1.69	2.25	4.64
% ash (dry wt.)	9.60	9.54	10.23	14.05

Corrected ΔT data compiled Aug. 23/54.

Date	Log	Material	ΔT p. $^{\circ}C$
8-1X	GW	BB-3	0.55
		BB-4	0.53
1-X	"	BB-5	0.59
13-X	"	0.345 M. NaCl	0.72
		BB-12	0.67
20-X	SW	BB-14	0.65
		NaCl	0.77
7-11-54	GW	NaCl	0.70
		NaCl 0.378 M	0.75
		Mulsion 3	0.50
7-11	GW	BB-22	0.50
		Mulsion 6	0.36

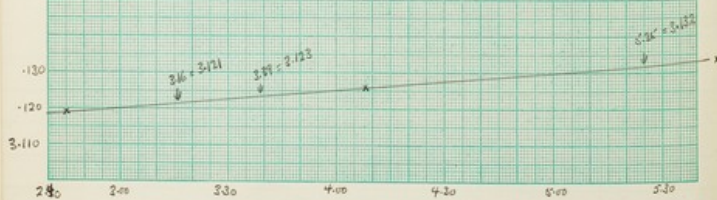
Throat = 0.64

Throat = 0.72 ± 0.66

Throat = 0.72 ± 0.66

Throat = 0.70

Corr. = 0.53



7-VI-54.

Freezing points getting good from ΔT of ag. dist. treated same way as sample.

Overall average of 2.33 = 3.114

Ag. dist.; 1 ml. RT = 20°C

BB-22. 1 ml.

2.44 3.020

43 1.130

45 1.119

46 1.119 = 3.119

solid.

17 2.10

18 2.10

19 2.11

20 2.14

21 2.19

22 2.19

23 2.19

24 2.19

25 2.19

26 2.19

27 2.19

28 2.19

29 2.19

30 2.19

31 2.19

32 2.19

33 2.19

34 2.19

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54 2.19

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56 2.19

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59 2.19

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61 2.19

62 2.19

63 2.19

64 2.19

65 2.19

66 2.19

67 2.19

68 2.19

69 2.19

70 2.19

71 2.19

72 2.19

73 2.19

74 2.19

75 2.19

BB-22. 1 ml.

3.13 3.170

14 3.140

15 3.15

16 3.16

17 3.17

18 3.18

19 3.19

20 3.20

21 3.21

22 3.22

23 3.23

24 3.24

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69 3.69

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71 3.71

72 3.72

73 3.73

74 3.74

75 3.75

76 3.76

77 3.77

78 3.78

79 3.79

80 3.80

81 3.81

82 3.82

83 3.83

84 3.84

85 3.85

86 3.86

87 3.87

88 3.88

89 3.89

90 3.90

91 3.91

92 3.92

93 3.93

94 3.94

95 3.95

96 3.96

97 3.97

98 3.98

99 3.99

100 4.00

Nat Haven. Oct. 1954.

241 -

$$r = 0.4 \text{ cm} \quad A = \pi r^2 = 0.503 \text{ cm}^2$$

$$r = 0.15 \text{ cm} \quad A = 0.0785 \text{ cm}^2$$

Test soln: AA 38.9 mg }
 GA 37.2 } $\uparrow$ 25 ml water + enough N-H₂SO₄ (v. conc)
 CA 25.8 }
 VA 36.3 }
 for detection & make good basis

Fraction No.	275	280	275	260	275	260	275
1	0.04	11	.32	.25	21	.12	.12
2	0.04	12	.18	.135	22	.19	.09
3	0.04	13	.18	.165	23	.19	.120
4	0.04	14	.126	.095	24	.324	.220
5	0.08	15	.137	.100	25	.68	.91
6	0.08	16	.161	.118	26	.115	.67
7	0.08	17	.178	.122	27	.161	.94
8	0.08	18	.187	.110	28	.152	.107
9	0.08	19	.187	.102	29	.166	.97
10	0.08	20	.187	.093	30	.121	.72
11	0.08	21	.187	.093	31	.76	.44
12	0.08	22	.187	.093	32	.44	.27
13	0.08	23	.187	.093	33	.26	.47
14	0.08	24	.187	.093	34	.08	.08
15	0.08	25	.187	.093	35	.08	.08
16	0.08	26	.187	.093	36	.08	.08
17	0.08	27	.187	.093	37	.08	.08
18	0.08	28	.187	.093	38	.08	.08
19	0.08	29	.187	.093	39	.08	.08
20	0.08	30	.187	.093	40	.08	.08
21	0.08	31	.187	.093	41	.08	.08
22	0.08	32	.187	.093	42	.08	.08
23	0.08	33	.187	.093	43	.08	.08
24	0.08	34	.187	.093	44	.08	.08
25	0.08	35	.187	.093	45	.08	.08
26	0.08	36	.187	.093	46	.08	.08
27	0.08	37	.187	.093	47	.08	.08
28	0.08	38	.187	.093	48	.08	.08
29	0.08	39	.187	.093	49	.08	.08
30	0.08	40	.187	.093	50	.08	.08

Column chromatography for P-esters

Run 1
 15-x

Column made up then:
 1. Dimer - 10 x 200-400 μ m, packed by L. E. Carter.
 Packed in 1/4" 2.5 cm layers & pressure of 6 ft. water.
 Leave 3 days & water flowing from safety funnel, about
 6" pressure. Bed vol = 5 ml.

Flow rate = 2 drops/min.

34 drops = 1 ml. Apply 6 ft. water pressure. Flow = 16 drops/min.

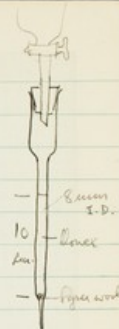
Wash: ~ 40 ml 3 M. Na formate. Effluent $\rightarrow$ ppt & light, even
 to end of this. Flow rate reduced to 9 drops/min.
 Wash: ~ 40 ml 6 N H₂SO₄ - 0.5 M. Na formate. Effluent $\rightarrow$ finally no ppt.
 Reduce pressure to ~ 6 drops/min. & wash overnight & water.

19-x 5:30 pm. Red sample 5 ml uncolored. Wash in 2 2.5 ml portions
 water. Apply 4 N-H₂SO₄ & start collecting 100 drops sample
 7.5 drops/min.

20-x 11 am. 90 fractions collected. Stop machine.

21-x. Make detection 0.5 ml F.S. and record at 260 & 275.

Cytophys & aldehydes came through together because of wrong
 straight 4 N-H₂SO₄ instead of gradient from water.



Columns

Run 2. 26x. Col. from run D washed: 8 H. H form, 88% H form, & copious water. Had some air in for top 2.5 cm., but bubbles seemed to disappear after.

Add 5 ml. methoxide conc. & force in at ~ 6 drops/min. Wash in: 2.5 ml. portion's water.

Apply gradual elution: conc. = 25% and water in 500 ml. flask, conc. = 25% and 4 H. H form. However, due to pressure changes, about 100 ml. must be added into mixture before feeding column.

J. 4. 1.55 pm. Elute at 6 drops/min., collecting 100 drops fractions.

10.30 pm. 37 tubes collected. Reduce pressure: down overnight.

27-x. 10 am. 56 tubes. Elute for reading 0.4 ft 4 ml. & water.

Fr. no.	D ₁₆₀	D ₂₇₀	270 260	270 260	270 260
B ₁	.06	.04		29	.06
B ₂	.05	.04		30	.06
1	.05	.04		31	.06
2	.06	.05		32	.06
3	.07	.05		33	.06
4	.07	.05		34	.06
5	.22	.17		35	.06
6	1.30	1.81	1.39 C	36	.06
7	5.2	>3.5		37	.06
8	2.9	1.48	.51	38	.07
9	3.4	2.00	.59 A	39	.10
10	1.82	1.79	1.02	40	.15
11	.22	.16		41	.22 .17
12	.12	.08		42	.28 .21
13	.10	.08		43	.29 .22
14	.08	.06		44	.30 .23
15	.06	.05		45	.38 .28
16	.06	.05		46	.37 .43
17	.07	.05		47	.89 .66 74
18	.06	.05		48	1.11 .82 74
19	.06			49	1.14 .83 72
20	.06			50	.90 .67 74
21	.06				
22	.06				
23	.06				
24	.06				
25	.06				
26	.06				
27	.06				
28	.06				

Perchlorate 0.1 ml sample, 0.4 ml sodium persulfate, 2 hrs at 95°C, H₂O and reagent, 22 hrs at 37°C, 0.6 N₂O (only 10 min at 16°C), 1 hr, 2 hrs at 37°C.

Tube no. Vol. eld. YP %T. 634/15

1	0	0	88.47
2	0	0	89.6
3	0.02	0.2	73
4	0.02	0.2	36
5	0.05	0.5	67
6	0.05	0.5	92
7	0.1	1.0	85
8	0.1	1.0	84
9	0.2	2.0	59
10	0.2	2.0	70
11	0.5	5.0	46
12	0.5	5.0	47

Run 3
Nov. 1

Fresh column. 8 mm x 10 mm. Washed: 88% HCOOH, water until neutral. Apply 5 ml undistilled water, wash in: 8 ml H₂O.

Apply gradient elution: 150 ml 4 N HCOOH in reservoir, 150 ml water in mixer. adjusted so that HCOOH enters mixer dropwise from beginning. 100 drop samples.

2-ml, 1120 min. 44 tubes collected. Soln. in mixer vessel is tested; but dead is clear.

Tubes 7-8, apolar.

Reacts 0.4 T 4.0 ml for W reading.

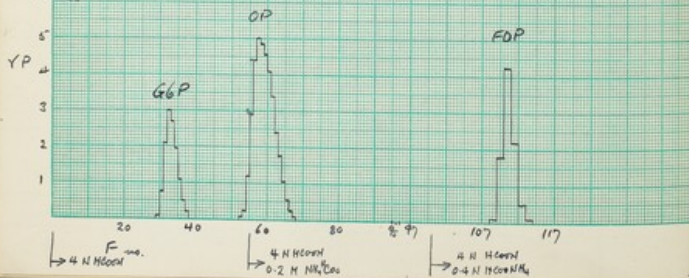
Fraction	Det.	D ₂₇₅	275/260	Parting	Tube	%T	YP	Yod.	D ₂₆₀	D ₂₇₅	275/260
8	.048	.04						31	.06		
9	.048	.04						32	.07		
1	.05	.04		13	100			33	.07		
2	.05	.04		14	94			34	.07		
3	.06	.05			99			35	.06		
4	.07	.05			101			36	.06	.05	
5	.08	.05			100			37	.06		
6	.13	.09			100			38	.09	.07	
7	.22	.22			98			39	.21	.16	
8	1.28	1.80	1.31		64			40	.37	.28	.76
9	2.45	2.80	1.14		22			41	.40	.30	.75
10	.20	.20			89			42	.71	.53	.75
11	.08	.09			99			43	1.43	1.05	.74
12	.15	.09			96			44	1.61	1.19	.74
13	1.55	.66	0.43		58			45	0.99	.72	.73
14	1.70	.72	0.42		54			46	0.37	.28	.76
15	2.85	1.21	0.46		32			47	.14	.11	
16	1.45	0.61	0.42		59			48	.09	.07	
17	.33	.16	0.48		84			49	.08		
18	.12	.08			96			50	.07		
19	.09				99			51	.07		
20	.08				99			52	.06		
21	.06				100			53	.06		
22	.07				100			54	.05		
23	.07				100			55	.24	.15	
24	.46				100			56	.78	.46	.59
25	.46							57	1.57	.91	.58
26	.46							58	1.88	1.09	.58
27	.46							59	1.60	.92	.57
28	.46							60	1.05	.61	.58
29	.46							61	.58	.35	.60
30	.46							62	.30	.19	
								63	.17	.11	
								64	.12	.09	
								65	.10	.07	

8.21

Ortho-P ester. Test of Lowry et al. modified method.
 Reagent: 15.3 ml concd. H_2SO_4 } $\uparrow$ 400 ml : water.
 1.25 g am. molybdate. (MoO_4)
 Before use, add to 8 ml, 1 ml 10% ascorbic acid.

Tube no.	ml. Pstd.	ml. water	Y.P.	630 filter % T
1	0	0.5	0	89.1
2	0	0.5	0	88.4
3	0.02	0.48	0.2	84.2
4	0.02	0.48	0.2	83.9
5	0.05	0.45	0.5	77.7
6	0.05	0.45	0.5	77.5
7	0.1	0.4	1.0	68.3
8	0.1	0.4	1.0	68.0
9	0.2	0.3	2.0	53.0
10	0.2	0.3	2.0	53.0
11	0.5	0	5	24.4
12	0.5	0	5	24.8
13	0	0.5	0	89.2
14	0	0.5	0	89.1

To each, add 2.0 ml freshly prepared reagent, mix, & let each stand in incubator $1\frac{1}{2}$ hrs.



Column over (4)

8021-24 Leave column as ③, re-packed & washed by conditions same. 8 x 115 sec.
Apply 5 ml mix of G6P, FDP 10P, wash in 5 shots =
10 p.m. ~~the~~ water → 4N HCOOH. Start 5 g drops/min., 100 drop sample.
Put some air in upper part of column.

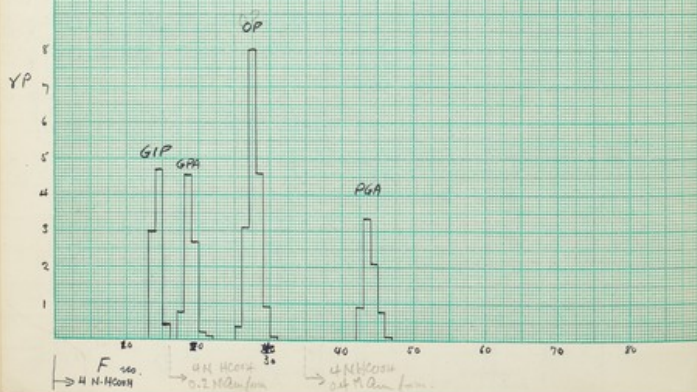
9. xi. 9. 1955. 55' tubes collected. Reservoir empty. Flood in main tunnel. Rats down to 4 days/week. Put 150 ml 0.2M. NH_4 formate in 4N HClO₄ in reservoir.

6. pm Increase loop count to 250. F96 = 250 loops.
8. pm Sample 100! apply 0.4 g NH₄ to male in 4 NH₄ETH

Pectas. 0.1 ml samples + 0.2 ml alkyling reagent, into oven at 100° (11-10). 17M, rain
the methyl to → 16°. 1-15°, t = 46. 2-45° - end of oven. And 2 ml reagent.

[illegible]

Lat	Water	Yp	%T	F	%T	Yp	%Water
A-02	0.0%	0.2	82	14	36	2.9	62
		0.2	82	18	87	0	0
.10	0.0	1.0	63	27	7	~8	100
		1.0	63	27 (0.05)	23	4.4	
B-04	.06	2.0	47	43	86	0.0	0
		2.0	47				
.10	0.0	5.0					
		5.0	19				
-	0.1	0	87				
-	0.1	0	87				



Colerum Run (5)

11-xi-54. Fresh column Doses 1. 8 x 110 mm. Affy P. virus as shown.
12 M. Affy 150 ml water → 150 ml 4N-1500M. Rubber-free system.
200 deep troughs, 10 droops/minute.
8 gms. ~~all~~ Reservoir M.T. Aff 150 ml 0.2 M N16-formate-4N 1500M.
12-xi 9 am " Aff 150 ml 0.4 M " "

M2-xi 9.2m

12-81 Pestus. No eggs or B's. Larvae 1-56. 0-1 and small. Charn 11-30 mm. 1.59 per - trans to 160.
5-30 out of form. 5-30 to incubator. 5-30 - read.

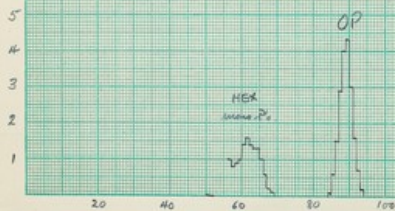
	IP	ST	IP
1	87	33	87
2	87	24	86
3	87	25	87
4	87	26	87
5	86	27	86
6	85	28	87
7	85	29	87
8	87	40	86
9	87	41	86
10	87	42	66
11	87	31.5	0.9
12	86	44	8.35
13	85	45	2.1
14	21.5	46	0.15
15	79	47	0.1
16	86	48	86
17	69	49	86
18	22	50	86
19	28	51	87
20	82	52	86
21	81	53	87
22	86	54	87
23	87	55	86
24	87	56	86
25	78	22	
26	74	15.5	1.5
27	7	28	
28	22	42	
29	26	46	
30	15	0.1	
31	87	2.7	
32	87	17.8	

15-xi Tubes 57-80 $\rightarrow$ negative P test. Mod read on charinets.

Tube 7-8398 = 3.0 mg 7-6186 = 3.0 mg
 + GIP-K 8428 = 3.9 6216 = 3.0 } 1 N 5' ml water + 100 ml
 + GIP-Ba 8467 = 3.9 6216 = 3.0 } 100 ml to be divided
 + FGP-Ba 8412 = 4.8 6286 = 4.0
 OP (KX70) 0.5 ml = 0.5 mg P

16-xi. F 1-20 not done 17-xi. F56 →
 F 21 → 0.1 ml sample, no cells (52.5 x 60)

F	YT	YP	F	YT	YP	F	YT	YP
21	86	63	1.0	76	86	84	86	
22	87	67	0.8	76	86	85	86	
23	88	67	0.9	77	87	86	87	
24	89	63	1.0	78	87	87	87	
25	86	57	1.4	79	87	88	88	
26	81	54	1.6	80	87	89	87	
27	82	57	1.4	81	87	89	86	
28	83	58	1.3	82	87	↓		
29	84	58	1.8	83	86	111	no color	
30	85	63	1.0	84	83			
↓	86	72	0.6	85	73			
31	87	82	0.2	86	84			
	88	82	0.1	87	86			
	89	86		88	26			
	90	86		89	24			
	91	86		90	83			
	92	86		91	83			
	93	87		92	71			
	94	86		93	82			



Column Run 6

15-xi

P. spds. as shown placed on column

6 pm. 150 ml water → 1 N H₂SO₄ 6 drops/min. F = 100 drops.

16-xi. 9 am. F 50 collected + 4 fractions missed because no tubes. Add tubes
 + collect F55 →. Remove MT. Add 150 ml 4N H₂SO₄.

16-x

11.1375

+ GIP-K -1454 = 7.6 mg
 + GEP-Ba -1537 = 8.3
 + FGP-Ba -1613 = 2.6
 + FGP -1665 = 5.2
 + PGP-Ba -1748 = 7.8
 + PGP-Na -1800 = 8.7
 + 1 ml KH_2PO_4 1.0 mg P
 + 10 ml H_2O + 11 ml + Bunkel 1850

16-x. P. ext.

F	%T	VP	F	%T	VP
1	87	16	86		
2	87	17	86		
3	86	18	86		
4	87	19	86		
5	87	20	86	6.1	
6	87	21	82	6.2	
7	87	22	88	8.0	
8	77	23	18	8.2	
9	76	24	16	8.1	
10	5	25	84	6.1	
11	6	26	87		
12	69	27	87		
13	8	28	87		
14	77	29	87		
15	88	30	87		
		31	87		
		32	87		

83
 ↓
 65
 33
 66
 67

Column Run 7

17-xi

Re-use column from ⑥. 11 x 0.9 cm. Apply 5.5 ml (half) used as shown. Test Hatched & L¹⁰⁰ formate system.

9.30 pm. 150 ml water → 150 ml 1 M NH_4 formate. 6.6 drops/min. F:150

17-xi

9.45 am. F 31. Re-run MT. Add 150 ml 2 M NH_4 formate, increase flow to 9.5 drops/min.

Tube Volume Calibration

Approx 13 x 100 mm. Tubes, selected for B.L. column. Pipette in water & measure height of meniscus from outside bottom of tube.

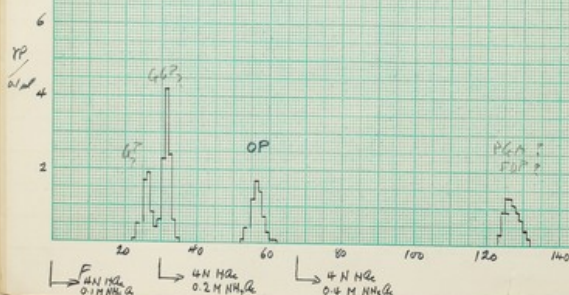
Tube no.	Height for 2 ml. water	+ 5 ml	(a) (b)
1	22 mm.	44	52
2	21	51	50
3	22	53	51
4	22.5	52.5	50
5	22.5	54.5	52
6	22.0	54	52
7	22.0	53.5	51.5
8	22	53	52
9	22.5	54	51.5
10	23	56	53
	22.25	53.75	51.50

Flash 18.0790

+ G.P. Ba 0.990 = 20.0 mg.
 + F.D.P. 1.105 = 11.5
 + G.P.A. Na 1.306 = 20.1
 + P.G.A. Ba 1.476 = 17.0
 + 2 ml. K₂Cr₂O₇ = 2.0 mg P
 + 2 ml. N-NH₄OH
 (+ ppt; charcoal filter?)
 + 17 ml. H₂O (intended 16)
 + 100 ml. Na₂CO₃ until all dissolved

Precipitate (incubated on bath at 34° → 34°)

3-xii	F	%T	YP	F	%T	YP	F	%T	YP	F	%T	YP
1				35	87		61	86		123	72	
↓ Blank				36	87		62			124	61	
20				37			63	Blank		125	54	
21	36			48			64			126	53	
22	35	0.1								127	52	
23	74	0.5		49	86		3-xii			128	62	
24	50	1.7		50	85 (Gold Tarn)		67			129	64	
25	47	1.9		51	86		↓ Blank			130	71	
26	67	0.8		52	84	0.1	115			131	78	
27	77	0.4		53	76	0.4	4-xii			132		
28	72	0.6		54	60	1.2	116	80				
29	42	2.3		55	50	1.7	117	80		2-xii	47	
30	23	4.2		56	53	1.4	118	79	high?	5Y	46	
31	40	2.4		57	69	0.7	119	79	Blank?		18	
32	73	0.6		58	80	0.3	120	80	Blank? 0.3Y			
33	84	0.1		59	84	0.1	121	80	from readings			
34	86			60	85	0.1	122	79				



Col. Run 9.

30-xi

Tube tip enlarged to 5 mm. dia.

Lab. 9: Drop size: water: 100 drops = 7.0 ml.

Tube #2: 100 drops water = 7.4 ml.

4 N-HAc = 4.5 ml

Column 8 x 120 mm. acetate. All 5' ml. Porin.

Eluting; 200 ml. running volume:

1-xii 1.46 pm F 1 200 ml. water → 4 N-HAc - 0.1 M NH₄Cl. F = 100 Lp. 6.55 / min.

6.16 F 16

10.11 pm F 30 Res still contains ~50 ml. Remove this & add 200 ml. 4 N-HAc - 0.2 M NH₄Cl. Success to 6.5 drops/min.

2-xii 10.10 am F 68 " ~40 ml. " 4 N-HAc - 0.4 M NH₄Cl

Rate down to 5.5 / min. Return to 6.1

5.30 pm Reduce flow rate to leave overnight.

3-xii

6-xii Other and Labile P. 0.1 ml. transfer + 2 ml. reagent:

B	87
B	87
1-xii	84
2-xii	64
5-Y	19
5-Y	19
F 24	86
25	86
29	87
30	86
53	50 = 1.8 / P
56	53 1.6
126	85
126	86

29711

Autosome Reaction (Mushinski)

Pipette 2 ml diluted
 reagent into 13x100 mm. tubes. ^{Chill.} Add 0.1 ml water (blank) or
 Glucose (5%) or fructose (5%). Chill. Mix (not thoroughly enough
 and to again later). Chill. To w.b. at 80° (actually 78°) for
 various times. Remove tubes & chill. Read when all complete, at
 620 on Beckman, using blocks under cell, against water blank.

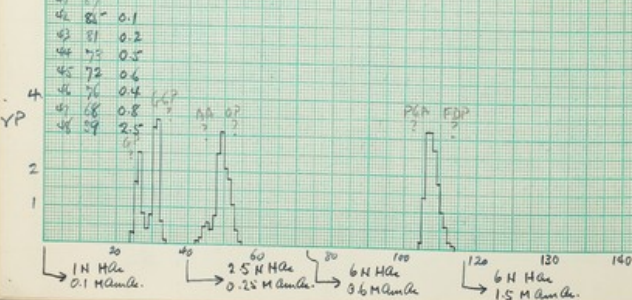
Time	B	G	G-B	F	F-B
2 min		.021		.193	
3		.028		.194	
5		.038		.202	
10	.028 .047	.066		.195	
15		.090		.189	
20		.097		.174	
25		[.140]		.175	
30		.117		.166	

Blank 997.7

+ Blank 990.4 = 47.7 mg

+ 5 ml from 9.000

8-XII	F	%T	YP	F	%T	9-XII	F	%T	12-XII	F
1				49	32	31	97	86	137	
2	Blank			50	44	21	98	87	138	
15				51	49	18	99	87	139	
16	86			52	62	11	100	87	140	
17	87			53	77	0.4	101	87	141	
18	87			54	84	0.1	102	87	142	
19	86			55	86		103	86	143	
20	87			56	87		104	81	0.2	
21	87			57	87		105	87	1.4	
22	86			58	89		106	32	3.2	
23	86			59	87		107	32	3.2	
24	80	0.3		60	87		108	38	2.7	
25	50	1.7		61			109	36	1.5	
26	40	2.8		62			110	62	1.0	
27	68	0.8		96			111	72	0.6	
28	76	0.4					112	81	0.2	
29	67	0.8					113	83	0.1	
30	32	3.2					114	86		
31	30	3.4					115	87		
32	71	0.6					116	87		
33	83	0.1					117	87		
34	86						118	87		
35	86						119			
36	86						120			
37	87						121			
38	87						122			
39	87						123			
40	87						124			
41	87						125			
42	87						126			
43	87						127			
44	87						128			
45	87						129			
46	87						130			
47	87						131			
48	87						132			
49	87						133			
50	87						134			
51	87						135			
52	87						136			
53	87						137			
54	87						138			
55	87						139			
56	87						140			
57	87						141			
58	87						142			
59	87						143			
60	87						144			
61	87						145			
62	87						146			
63	87						147			
64	87						148			
65	87						149			
66	87						150			
67	87						151			
68	87						152			
69	87						153			
70	87						154			
71	87						155			
72	87						156			
73	87						157			
74	87						158			
75	87						159			
76	87						160			
77	87						161			
78	87						162			
79	87						163			
80	87						164			



Col. Run 10 pH 3.7 system

6-XII-54 Re-use col. from 9, washed = Ac^- water.

Apply 5 ml Pamin + AA, wash in = 10 ml water.

5:30 pm 200 ml water $\rightarrow$ 200 ml 1 N HAc - 0.1 M NH_4Ac . 4.5 drops/min F100.

7-XII 9:30 am F40. Re MT, 5 min down to 150 ml. Add 200 ml 2.5 N HAc - 0.25 M NH_4Ac .

Rate = 4 drops/min.

10:45 pm F73 Re MT. Add 200 ml 6 N HAc - 0.6 M NH_4Ac .

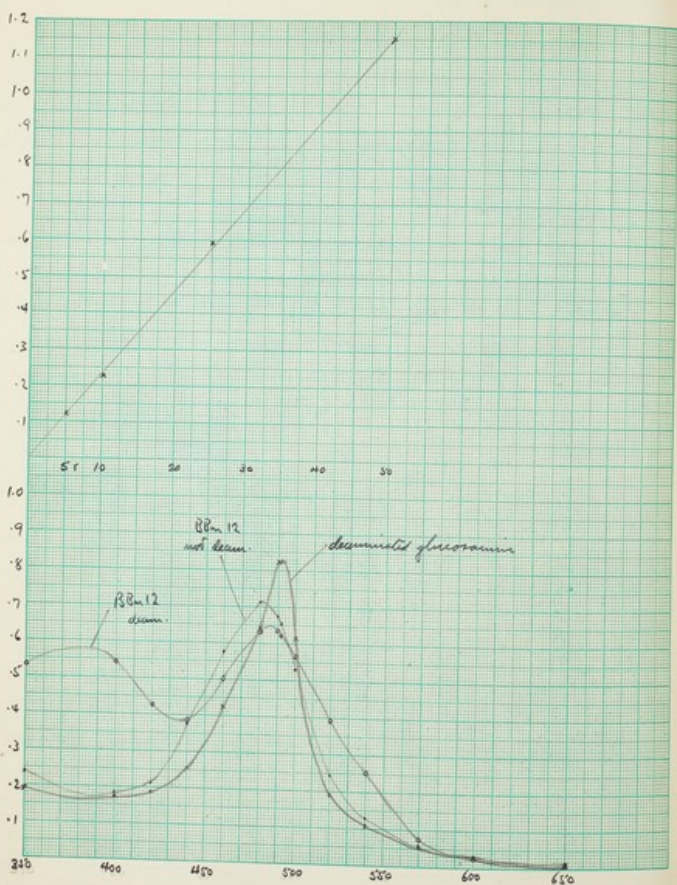
8-XII 9:15 am F97 Re $> \frac{1}{2}$ full - flow rate greatly reduced by stronger acid = 3.8 drops/min.

Raise to 5 drops/min.

4 pm F117 Re MT. Add 200 ml 6 N HAc - 1.5 M NH_4Ac . Change F to 200 drops.

9-XII 12 N F142 Re just MT.

10-XI 9 am F164 Reuse from MT. Shut down.



26.11.55

Haeussler's. Decker - Rosenmund indole method.

10.0 mg BBu-12 + 0.5 ml 14.0 for each of control + deam. 52. Carry through reaction as described, + protein present; then finally centrifuge to clarify.

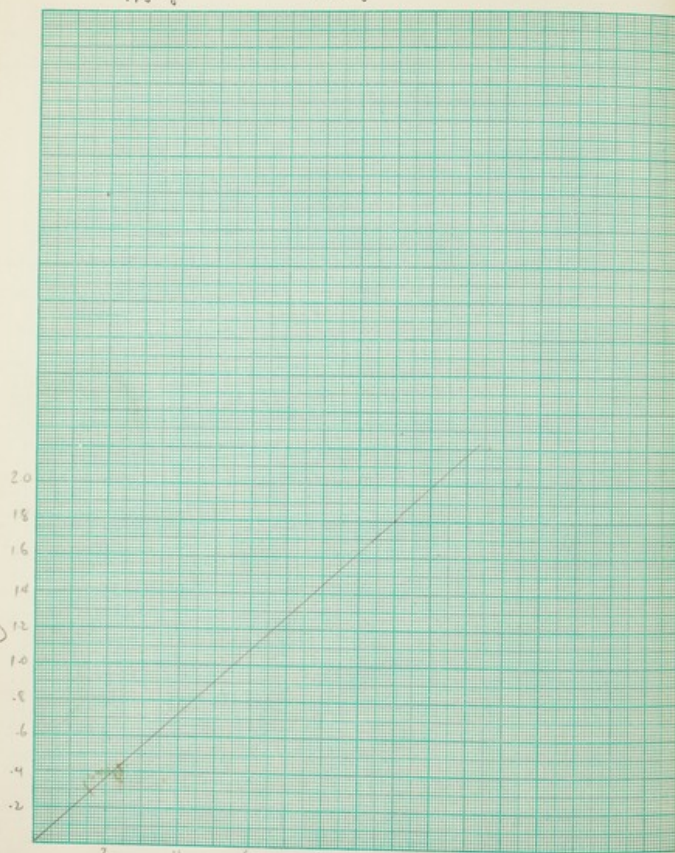
		Not deam.		Deaminated		Δ	$\Delta_0 - \Delta$
	492	520	27	492	520		
Blank	1	27	27	76	26		
		27	27	74	22	50	
BBu-12	2	565	204	563	367		
		492	361	74	21	196	
5.1 ml	3	86	25	237	63		
		87	25	76	21	174	123
10.1	4	74	22	383	78	278	226
25.1	5	74	22	822	179	643	591
50.1	6	76	21	1440	234	1206	1151

Absorption curves: (control blank)

λ	BBu-12 not deam.	BBu-12 deam.	25.1 deam.
350	240	335	196
400	186	344	179
420	214	427	187
440	377	389	256
460	576	503	425
480	711	633	638
490	675	634	825
492	652	623	826
500	530	565	614
520	245	294	191
540	120	250	111
570	52	74	57
600	30	24	22
650	17	9	12

July 3, 9114
432.12 - 9307
19.3

July 3, 9284
+ 88.15 - 9782
19.8



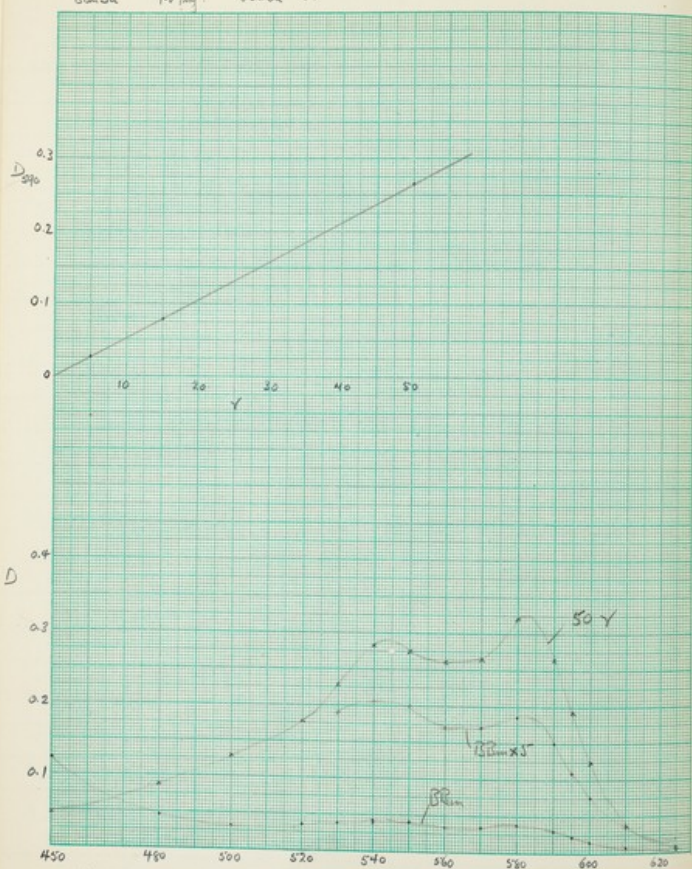
28.1

Hinosaminis

BB-12 r 15" and 1.0 ml N-HClO₄. Stand 1 hr in cold, stir
spin 10,000 10 min. Pipette off clear spf. → turbid on standing.
Mentholyl = 0.18 ml 5% N-KOH (XS). Chill. Spin off big KClO₄ ppt.
Carry thru procedure; necessary to spin BB samples ahead to remove
some ppt.

	Not deamm			deamm.			
	492	520	O _N	492	520	Δ ₀	Δ ₀ -O _N
B	56	14	42	56	16	40	
10 Y	63	18	45	354	71	283	238
10 Y	62	17	45	367	89	278	233
20 Y	58	16	42	641	129	512	470
BB-12	700	315	385	922	669	253	-132
BB-15	450	162	288	534	360	194	-94

Jule 3.9585 4.0163
 663 249
 BB-5a 7.8 mg BB-6a 8.6



2.6.55 N-acetylglucosamine (Aminoff et al method)

BBm samples each + 1.0 ml 0.5 N-H₂CO₃

Stand 3 hrs. Spin + decant off. Add to each 0.1 ml 5% KOH → put off. Spin off KOH.

		D ₅₉₀	D ₅₈₀	% acetyl amino N	%
0.43, 7.8 = 2.8 12	1) BBm-5a 0.43 ml (total), water 0.57	26	32	6.2	0.22
1.2, 8.6 = 7.16 12	2) BBm-6a 1.0 ml	30	38	7.4	0.10
	3) Water 1.0 ml (blank)	0	0		
	4) N-acetylglucosamine 5%, 0.05 ml, water 0.95	26	27		
	5) " 15%, 0.15 ml, " 0.85	79	79		
	6) " 50% 0.50 ml, " 0.50	264	264		

18.6 To each add 0.1 ml 0.25 M. Na₂CO₃, heat 4 min. Add 2 ml glacial HAc. then 1 ml DPAAB reagent.

	BBm-5a	BBm-6a	X.S.
550	275	40	200
560	236	34	170
570	265	34	170
580	321	37	184
590	265	30	150
595	193	22	110
600	124	16	75
610	38	7	35
625	8	5	20
540	284	41	205
530	228	38	190
520	177	35	
500	129	32	
480	75	45	
450	49	124	

N-acetylbenzamide in meat blood

Blood solids weighed into tubes. Each $\uparrow$ 1.1 ml N-144₂ (available for 0.5 N).

Stand 1 hr in cold, spin, decant off

Neutronenanalyse: Tube 7 - 0.20 und SH KTH + 0.04 und M-Holzer $\rightarrow$ 14.7. Totalwert 1.34
(broad thermal line) 8 - 0.194 " " " 1.29
10 - 0.195 " " " 1.29
11 - 0.195 " " " 1.29

Spin, decant off, 1.0 ml + deliver into pyrex tubes

Make blank & stds. also to vol. 1.0 ml. To each add 0.1 ml 0.25

M Na_2CO_3 , heat on BWR 4 min, let cool, add 2.0 ml H₂O
+ 1.0 ml DMAB reagent. Place at 25° in incubator 1 hr.

	Blank	5Y	15Y	40Y	BB6a	11	12	15	BB61	BB61
540	1	33	91	95	239	37	27	49	92	38
545	3	35	94	98	242	35	26	49	89	37
550	2	33	90	93	230	33	24	47	84	35
560	3	30	78	81	200	33	23	42	73	32
580	2	38	103	106	266	32	22	43	79	27
585	2	39	104	104	263	31	21	43	77	25
590	1	38	91	90	228	29	19	38	67	21
610	2	6	15	17	34	16	9	22	27	10
D ₅₈₅ - D ₆₁₀	0	33	89		225	15	12	21	50	15
Apparent % ^{apparent} transmittance						27	22	38	87	27
% of dry wt.						0.054	0.035	0.037	0.130	0.041
Mg/100 ml						5.3	3.3	3.0	13.1	6.5

BBm	8	9	12	13	14a	14b
Tube no	1	2	3	4	5	6
Tube Hg	2.1868	2.9836	2.3238	2.1524	2.7603	2.5992
+ H ₂ O	1996	9966	8382	1621	2702	2122
Blood solids	12.8	11.0	14.4	9.7	9.9	14.0
Blank tube no.	7	8	9	10	11	-

$D_{525} - D_{610}$
 $\times 10^3$

200
100
80
60
40
20

2 4 6 8 10 20 30

Balance: 2.4
1.6
3.5

21-11

N-acetylphenanthrene estimation

Blood solids $\uparrow$ 0.6 ml 0.5 N-HClO₄. Stir. Stand at room temp. 1 1/2 hrs. Spin 10,000 5 min. & draw off into numbered tubes (Re ppt. in tube #6, leave in tube). Neutralize (pH 6.5-7.5) ~ 0.050 ml 0.5 N KOH each. Spin. Draw off 0.50 ml to test tube, add 0.05 ml 0.25 M Na₂CO₃. Test w/ thymol blue (8.0-9.6); blank & H₂O are above range, some unknowns are out quite - add, to unknowns only, additional 0.05 ml 0.25 M Na₂CO₃. Heat 4 min. 100°. Cool. $\rightarrow$ ppt in unknowns, incl. 14b. Add 2 ml H₂O $\rightarrow$ clear soln. Add 0.5 ml DMAP reagent. Leave at 25°

Blank 5Y 20Y 40Y BB8 9 12 13 14a 14b

540				196	70	64			
546				200	68	62	46	78	41
550				190	63	57			72
560				167	57	50	38	59	35
560				220	54	48			64
585	0	30	110	216	52	46	35	56	33
590				187	48	42			55
610	0	4	15	34	30	22	25	20	22
$D_{525} - D_{610}$		26	95	182	22	24	10	36	11
Offset Y acetylphenanthrene					4.5	5.0	2.1	7.5	2.3
% drug ads.					.035	.055	.018	.094	.028
mg/100g					3.3	4.3	1.4	9.4	2.9

$$\left(\frac{Y}{100} \times \frac{0.605}{0.500} \right) \times 1.21$$

G-6-P Ba 20 mg.)

FDP 15

Na₂B₄O₇ P 20

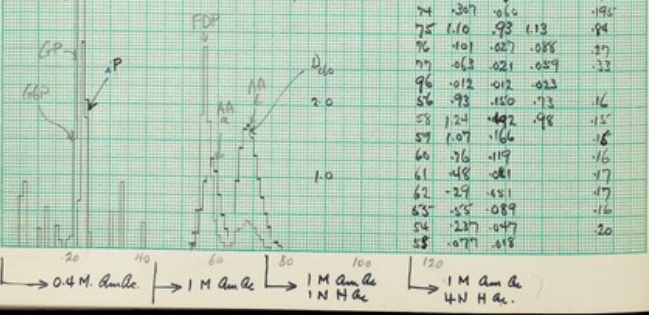
Plat Ba 20

Adonitol 20

Ad. B. P. 2 mg

Pentamethine (100% dist. dist. reagents)

2-v	B	SS	21	12	8-v	AM-20	Blank	74	84
0.4 Y	77	23	23	3.6	52	24		76	74
1.0 Y	66.5	24	78	0.4	53	31	0.1	77-119	Blank
4.0 Y	20	25	84		54	62	1.1		
F1	86	26	84		55	30	2.7	B	85
2	85	27	85		56	17.5	5.6	0.83	72
3	85	28	85		57	34	3.5	2.08	46
4	85	29	85		58	49	2.0	5.01	22
5	89	1.4	30	66	59	67	0.8		
6	82	1.8	31	83	60	76	0.4	113-191	Blank
7	85	32	83		61	79	0.2	192-218	"
8	85	33	83	1.6	62	82	0.1		
9	86	34	84		63	82	0.1		
10	75	0.5	35	85	64	80	0.2	16-v	UV absorption
11	86	36	82		65	73	0.5	D ₆₀	0.30
12	84	1.1	27	84	66	72	0.6	F 65	0.48
13	85	37	85		67	82	0.1	0.98	0.75
14	89	38	85		68	82	0.1	1.37	0.21
15	84	0.6	40	85	69	68	0.8	1.66	0.27
20	56	1.6	52	0.2	70	70	0.6	1.71	0.28
21	81	0.4	54	0.2	71	75	0.4	1.55	0.24
22	25	0.4	51	0.2	72	77	0.3	1.29	0.20
23	12	1.9	50	0.3	73	77	0.3	0.97	0.16
(X 2)	20	29	3.0	85	74	82	0.1	0.49	0.06
					75	82	0.1	1.30	0.10
					76	1.10	0.93	1.13	0.94
					77	1.01	0.87	0.88	0.77
					78	0.63	0.81	0.89	0.33
					79	0.12	0.12	0.23	
					80	0.93	0.50	0.73	0.16
					81	1.24	0.92	0.98	0.15
					82	1.07	0.66		0.18
					83	0.76	0.19		0.16
					84	0.48	0.01		0.17
					85	0.29	0.01		0.17
					86	0.55	0.89		0.16
					87	0.23	0.47		0.20
					88	0.07	0.18		



* Adonitol and many have been added?

Add 3 ml N-HAc. Small bubbles. Add 10 ml H₂O.

50 ml from still all distill. then 10 ml H₂O.

Slowly off. Bubbles + bubbles. 5 ml H₂O.

= total vol. 20 ml.

Add 0.5 ml 40% H-NH₄ → solution.

8-v. Crystals have formed. Add (to remain, 4 ml) 1 ml water.

31-V-55

Column Run 11. Test of acetate pH 6 system.

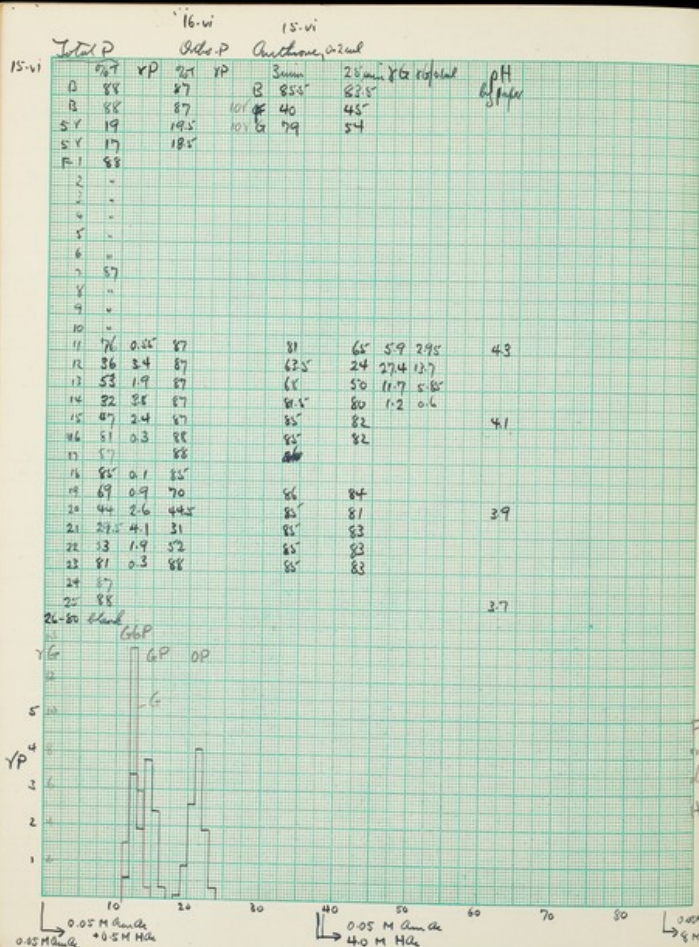
Box 1 converted to acetate form. Column 12.5 cm. washed overnight: 2 MHA - 0.5 M NaAc. then 4 hrs: water → pH 7. Apply 5 ml. next run, wash in 5 ml water.

NH₄Ac buffer: made up 4 M. NH₄Ac, adjust: HAc (~30 ml 5N/l.) to pH 6.2. Diluted 1:5, pH = 5.8.

Electron

0.4 M.
11-V 5:30 pm FD 280 ml water → 250 ml NH₄Ac buffer. 6.5 drops/min. F=100
2-V 9:30 am F43 Res. MT, must down to ~200 ml. Add 250 ml 1 M. NH₄Ac buffer, force them enough to reduce pressure to 250 ml. Adjust to 6.5 drops/min.
F50 load, F57 partly load, because of tubes moving.
5:30 pm F74 Res. MT. Add 250 ml 1 M. NH₄Ac - 1 N. HAc.
3-V 9:00 F114 " Add 100 ml 1 M. NH₄Ac - 4 N. HAc. } F → 150 drops.
3:30 pm F135 " Add 150 ml " " } F → 200
F156 Res. MT. Paris. must to down F → 200
5:45 pm F182 Add ~100 ml N-HAc.
6:45 pm Max. H₂O
7:45 pm F196 Add 200 ml N-H₂SO₄

15-vi	Max. anthracene	γ (25 mm)	γ (5 mm)
B	0.7	0.7	0.7
10Y F	0.7	0.7	0.7
10Y G	0.7	0.7	0.7
F20	0.7	0.7	0.7
21	0.7	0.7	0.7
22	0.7	0.7	0.7
23	0.7	0.7	0.7
24	0.7	0.7	0.7
25	0.7	0.7	0.7
26	0.7	0.7	0.7
27	0.7	0.7	0.7
28	0.7	0.7	0.7
29	0.7	0.7	0.7
30	0.7	0.7	0.7
31	0.7	0.7	0.7
32	0.7	0.7	0.7
33	0.7	0.7	0.7
34	0.7	0.7	0.7
35	0.7	0.7	0.7
36	0.7	0.7	0.7
37	0.7	0.7	0.7
38	0.7	0.7	0.7
39	0.7	0.7	0.7
40	0.7	0.7	0.7
41	0.7	0.7	0.7
42	0.7	0.7	0.7
43	0.7	0.7	0.7
44	0.7	0.7	0.7
45	0.7	0.7	0.7
46	0.7	0.7	0.7
47	0.7	0.7	0.7
48	0.7	0.7	0.7
49	0.7	0.7	0.7
50	0.7	0.7	0.7
51	0.7	0.7	0.7
52	0.7	0.7	0.7
53	0.7	0.7	0.7
54	0.7	0.7	0.7
55	0.7	0.7	0.7
56	0.7	0.7	0.7
57	0.7	0.7	0.7
58	0.7	0.7	0.7
59	0.7	0.7	0.7
60	0.7	0.7	0.7
61	0.7	0.7	0.7
62	0.7	0.7	0.7
63	0.7	0.7	0.7
64	0.7	0.7	0.7
65	0.7	0.7	0.7
66	0.7	0.7	0.7
67	0.7	0.7	0.7
68	0.7	0.7	0.7
69	0.7	0.7	0.7
70	0.7	0.7	0.7
71	0.7	0.7	0.7
72	0.7	0.7	0.7
73	0.7	0.7	0.7
74	0.7	0.7	0.7
75	0.7	0.7	0.7
76	0.7	0.7	0.7
77	0.7	0.7	0.7
78	0.7	0.7	0.7
79	0.7	0.7	0.7
80	0.7	0.7	0.7
81	0.7	0.7	0.7
82	0.7	0.7	0.7
83	0.7	0.7	0.7
84	0.7	0.7	0.7
85	0.7	0.7	0.7
86	0.7	0.7	0.7
87	0.7	0.7	0.7
88	0.7	0.7	0.7
89	0.7	0.7	0.7
90	0.7	0.7	0.7
91	0.7	0.7	0.7
92	0.7	0.7	0.7
93	0.7	0.7	0.7
94	0.7	0.7	0.7
95	0.7	0.7	0.7
96	0.7	0.7	0.7
97	0.7	0.7	0.7
98	0.7	0.7	0.7
99	0.7	0.7	0.7
100	0.7	0.7	0.7



13-vi Run 13. Acetate - H_2O system.
Column Bore 1 Acetate 12 cm. Wash with, then a drop
 NH_4OH → effluent pH. Apply 7.5 ml each (1 mg/ml)
G6P, FDP, β -GP, PGA, +1.0 mg OP.

Electron

13-vi 1800 F1 500 ml 0.05 M NH_4Cl → 500 ml 0.05 M H_2O . F = 200 Lp.

14-vi 1700 F38 → 500 ml 0.05 M NH_4Cl → 500 ml 4.0 M H_2O .

15-vi 1000 F66 → 250 ml 0.05 M NH_4Cl → 250 ml 8.0 M H_2O . F → 400

14-vi 930 F101 100 ml N-HCl directly to column (no mixer)

16-vi Total P

F 81-100 blank

101 88

102 15 (3%) FDP

103 79

104 87

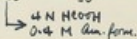
8 87

57 17

57 18

$$\begin{aligned} 10.95 &< \\ r_p & \\ \times 12.8 & \\ = 1.40 & \end{aligned}$$

N-Hept



Elston:

F-62

$$F \rightarrow 300$$

1

2

54

20-vi

P of insect blood. Trial of Lowry & Lopez method.

Blood samples: (1) Ma 28-iv-a. (not fully thawed)

(2) Tp-10 13-vi 07

0.1 ml each into 6x50 tube. Add 0.1 ml 0.6 N HClO₄. Stir. Heavy ppt. Stand 30 min. in frig. Spin 12,000 5 min., decant off supernat. Wash ppt twice to 0.2 ml portions 0.3 N HClO₄, spinning & adding washings to extract. To combined extract add 2.4 ml 0.1 M NaClO₄ → pH ~ 4.5.

Reaction tubes

Extract or std	pH 4 buffer	19xaniline 0.2 ml ↓ Nucleofast 0.2 ml ↓ Dye	7 min	17-20 min YP
B	2.0 ml	"	.001	.002
B	2.0	"	.001	.001
Ma - 0.5 ml	1.5	"	.090	.092
Ma "	"	"	.092	.092
TP 10	1.0	"	.329	.330
TP 0.5	1.5	"	.164	.166
1Y std 0.1 ml	1.9	"	.055	.057
1Y "	"	"	.055	.056
2Y 0.2 ml	1.8	"	.109	.112
2Y "	"	"	.111	.112
5Y 0.5 ml	1.5	"	.276	.277
5Y "	1.5	"	.278	.280

Ma: $1.60 \times 3.0 = 9.6$ avg % 3.1
 TP: $5.96 \times 3.0 = 17.9$ avg % 5.8
 $2.95 \times 3.0 = 8.85$ 17.7

mM

24-vi. Total P & extracts extd. by L & B. method.

0.05 and 0.1 ml of each inoculated, mixed 2 and 3, developed in water bath, 5 read in 0-4 colonies with 0.05

	P _{AT}	rP/water	Avg % avg blood atm
Ma 0.08"	38.6	5:26	153 50.0
a.i.	18.0	5:07	
To 0.05"	34.1	5:08	178 57.4
a.i.	14.2	5:52	
a 0.04"	38.8	5:24	131 42.3
a.i.	12.1	5:24	
L 0.05"	33.6	6:12	153 49.4
a.i.	13.1	6:10	
c 0.05"	40.0	5:02	122 39.4
a.i.	19.9	4:28	
d 0.05"	21.0	6:46	167 53.9
a.i.	11.3 (2nd)	6:46	
e 0.05"	28.6	7:18	179 52.8
a.i.	7.7 (2nd)	7:16	
f 0.05"	28.0	5:34	131 42.3
a.i.	17.9	5:10	
B -	88.0		
	88.2		
5Y	74.3		
	74.1		
2Y	46.3		
	47.1		
5Y	19.0		
	18.2		

Angle: 150 from P to test P
distance between 0-5 = 1 cm
= 5 mm P
 $\frac{1}{2}$ in. = 3.2 mm

27-vi	Repeat iP	3	0	20	248	YP	only 2.
-------	-----------	---	---	----	-----	----	---------

	nut	nut buffer	2T	YP	mg%
B	0	20	788		
B	0	20	848		
2Y	0.5	1.8	720		
"	"	1.8	720		
5Y	0.5	1.5	530		
"	"	1.5	528		
Ma	0.5	1.5	740	175	10.5
TP			624	326	19.6
a			596	544	27.2
L			324	920	47.5
c			648	660	33.0
d			533	495	24.8
e			486	580	29.0
f			512	530	26.5

22-VI

$P_{\text{of } T_p \text{ blood}}$

Top of 1 p. Road.

6 Tubes:

a	Tp. 10	8. vi.	not incubated yet?
b	Tp-1		
c	Tp-10,	13. vi	♂ inc. 9 days
d	"	"	♀ "
e	"	18. vi	♂ " 14 "
f	"	"	♀ "

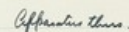
Each sample = 0.1 ml. Add 0.1 ml 0.6 N HClO₄, stir, let stand in frig 1½ hrs., spin 12,000 5 min. Break off ppt., wash ppt. twice ± 0.2 ml portions 0.3 N HClO₄. To combined ~~total~~ supernatants add 2.0 ml 0.1 M Na₂O → pH 4.0 (vol. of protein + contained liquid estimated at 0.1 ml ∴ add 4 × 0.5 = 2.0)

	Buffer	B.L. % ⁵ T	10'	Y.P./paul	mg % ⁵ blood	in M.
a - 0.5' and	1.5	54.7	54.5	9.00	9.03	22.6
a 1.0	1.0	33.6	33.2	9.06		
L 0.5'	1.5	34.9	34.2	17.60	17.6	44.0
L 1.0	1.0	14.6	13.0	-		14.2
C 0.5'	1.5	44.1	44.1	12.9	13.2	33.0
C 1.0	1.0	22.3	21.9	13.14		
d 0.5'	1.5	56.7	56.3	8.4	8.48	21.2
d 1.0	1.0	35.5	35.1	8.55		
e 0.5'	1.5	52.0	52.6	9.68	9.79	24.5
e 1.0	1.0	21.3	20.3	9.90		
f 0.5'	1.5	59.0	58.7	7.60	7.63	19.1
f 1.0	1.0	29.2	28.6	7.66		
B -	2.0	88.2	88.6			
B -	2.0	88.5	88.0			
2 YP 0.2	1.8	70.7	71.0			
" 0.2	1.8	71.1	71.0			
5 YP 0.5'	1.5	52.4	51.9			
" 0.5'	1.5	51.3	51.0			

$$FD = 22(1) \rightarrow 5.66 \times 2 / 0.1 = 5.66 \times 20 = 113.2 \text{ PPLK?}$$

$$\text{Apply: } 4.2 \times 80 = 336 \quad \{ R = 200\%$$


Run 15. Formic acid ^{with linear} gradient, for G6P, GP, + OP.



Column 8 x 120 mm, format X8.

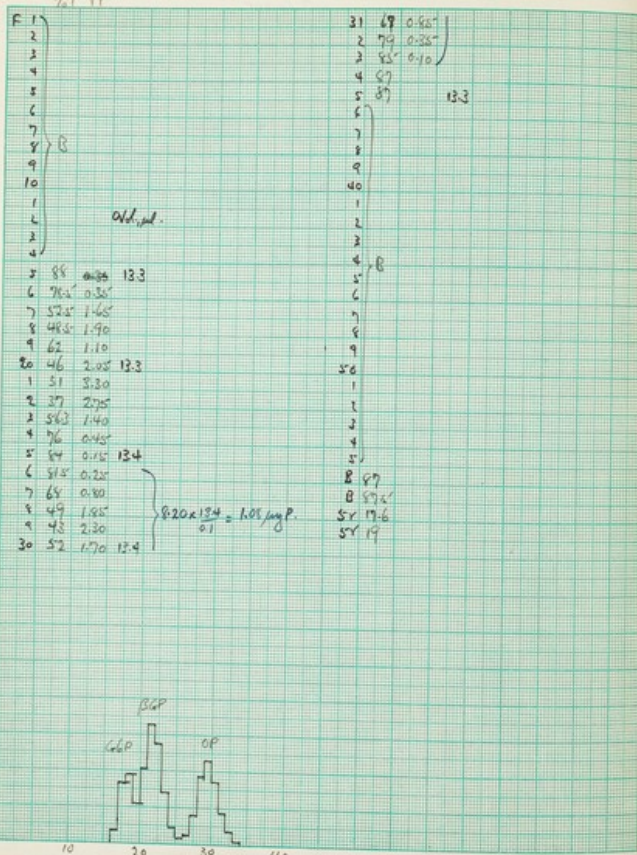
Par on 4.3 ml each GbP soln + glycerol-P soln + 1.0 mg OP.

Election:

21-Vi 14:30 FI 250 ml 2 N-HCOOH into 250 ml water. $\frac{\text{Solids}}{\text{Reduction to FI}}$ F = 200.

22-41 8.45 F36 500 mil goes through

27-VI-50
Total P 30
9.7 YP



24-VI.

Column 16. HCOOH without gradient.
Re-use column 15, washed & water neutralized.
Apply: 13.4 mg BGP } weighed, susp. in water & dissolved
11.5 mg GGP } in acid of Row 50 H₂O.
1.0 mg OP.
Elution: 500 ml 0.5 N-HCOOH.
Add further 250 ml "

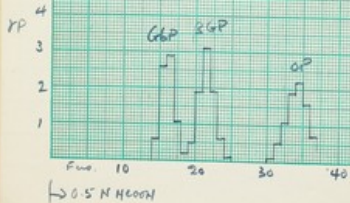
25-VI

Na₂B₄O₇ 10.4 mg
 BaGP 12.9 mg
 GP 1.0 mg

Total P (in 38' cell only 1 hr.)

F 1-10 B YP	F 21 76 0.5
11 83	2 62 1.1
12 88	3 48 1.9
3 88	4 44 2.2
4 72 0.6	5 54 1.6
5 89 2.6	6 70 0.7
6 35 2.9	7 100 0.7
7 63 1.08	8 100 0.7
8 82 0.2	9 100 0.7
9 75 0.5	10 100 0.7
20 48 1.9	11 100 0.7
1 323 3.1	12 100 0.7
2 49 1.9	13 100 0.7
3 72 0.6	14 100 0.7
4 85 0.1	15 100 0.7
5 87	16 100 0.7
6 88	17 100 0.7
7 88	18 100 0.7
8 89	19 100 0.7
9 88	20 100 0.7
30 84 0.1	21 100 0.7

0.02 mol T₂ per mol 4.8 (19.8)
 (mol. wt.) 4.7 (19.0/2)



30-vi Column 17. x10 resin = HCOOH without gradient (y. 116).
 29-vi Column of resin orig. from Carter, poured 12.5 cm. Wash: water
 = 8 N HCOOH → swelling to 13 cm. Wash: water.
 Apply P. eff. resin.

30-vi 17.30 Apply 500 ml 0.5 N HCOOH 6 drops/min F 3200.
 1-vii 10.00 F 32 Running slowly!

8-01 11-01

Vol.		Total P.		iP		Antenna		per cent		G/P		Vol.		Total iP		Antenna	
mil	Y	Y	3 min	25'	Y	mil	Y	Y	2'	25'	mil	Y	Y	2'	25'		
F0	12.8	10.9	0.7	16.8x3	264	4.2	5	12.3	-	-	5	12.3	-	-	-		
1	12.0	9.2	0.7	16.8x3	250	4.7	6	-	-	-	6	-	-	-	-		
2	14.1	0.7	0	78	58	4.5	7	-	-	-	7	-	-	-	-		
3	12.7	1.0x	0.4	79	68	2.8	8	-	-	-	8	-	-	-	-		
4	13.9	0.8	0.4	81	72	1.5	9	-	-	-	9	-	-	-	-		
5	12.9	0.1	-	-	-	-	10	12.1	1.0	0.1	83	78	-	-	-		
6	-	-	-	-	-	-	1	-	0.6	0	83	76	-	-	-		
7	-	-	-	-	-	-	2	-	0.4	0	82	75	-	-	-		
8	-	0.1	-	-	-	-	3	-	0.2	0	-	-	-	-	-		
9	-	-	-	-	-	-	4	-	0.1	-	-	-	-	-	-		
10	12.7	-	-	-	-	-	5	11.9	-	-	-	-	-	-	-		
1	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-		
2	-	0.1	-	-	-	-	7	-	-	-	-	-	-	-	-		
3	-	0.1	-	-	-	-	8	-	-	-	-	-	-	-	-		
4	-	0.1	-	-	-	-	9	-	0.1	-	-	-	-	-	-		
5	12.6	0.1	-	-	-	-	6	11.7	0.1	-	-	-	-	-	-		
6	-	0.2	-	-	-	-	1	-	-	-	-	-	-	-	-		
7	-	0.1	-	-	-	-	2	-	0.1	-	-	-	-	-	-		
8	-	0.1	-	-	-	-	3	-	0.1	-	-	-	-	-	-		
9	-	0.1	-	-	-	-	4	-	0.1	-	-	-	-	-	-		
10	13.3	0.3	-	-	-	-	5	11.5	0.3	-	-	-	-	-	-		
1	-	2.6	0	82	79	-	6	-	0.2	-	-	-	-	-	-		
2	-	3.5	0	83	76	-	7	-	0.1	-	-	-	-	-	-		
3	-	2.2	0	84	80	-	8	-	-	-	-	-	-	-	-		
4	-	0.3	-	-	-	-	9	-	-	-	-	-	-	-	-		
5	12.3	0.1	-	-	-	-	10	11.2	-	-	-	-	-	-	-		
6	-	0.1	-	-	-	-	1	-	-	-	-	-	-	-	-		
7	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-		
8	-	0.1	-	-	-	-	3	-	-	-	-	-	-	-	-		
9	-	0.4	0.4	82	78	-	4	-	-	-	-	-	-	-	-		
10	12.1	1.1	1.1	83	79	-	5	11.2	-	-	-	-	-	-	-		
1	-	1.5	1.4	84	77	-	6	-	-	-	-	-	-	-	-		
2	-	0.6	0.6	80	71	-	7	-	-	-	-	-	-	-	-		
3	-	0.1	-	-	-	-	8	-	-	-	-	-	-	-	-		
4	-	-	-	-	-	-	9	-	-	-	-	-	-	-	-		
5	12.1	-	-	-	-	-	10	-	-	-	-	-	-	-	-		
6	-	-	-	-	-	-	1	-	0.2	-	-	-	-	-	-		
7	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-		
8	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-		
9	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-		
10	12.8	0.6	-	-	-	-	5	10.6	-	-	76.5	56	-	-	-		
1	-	0.0	-	-	-	-	6	10.1	-	-	42	45	-	-	-		
2	-	-	-	-	-	-	7	-	-	-	5.1	80	-	-	-		
3	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-		
4	-	-	-	-	-	-	9	-	-	-	-	-	-	-	-		

Column 19. Tp blood. Doser 1 x 10 formate.

6 puffs Tr-1, expir weight 26.90 g.

Bled, onto ~~water~~ ^{new distill} of thoria in ice. Blood = 6.08 g.

Spices (They have got some good spices - greenish color). Spices are added to remove salts, pour off very completely. Did not re-weigh.

Calc. 6.0 ml 0.6 N-HCl, let stand in frig. 1 hr. Spin.

Wash off once: 10 ml 0.3 N-HCl. Spin. Combine with next washing.

Mentholamine = 1.42 ml 5 N KOH. $\xrightarrow{(OH^-)}$ immediate alkaline ppt. Lat stand

in bio 2 hrs. Lp₁ 41 ml. Lp₂ → 19.6 ml. Take 0.5 for P₂⁺.

but 19.1 on column. Heavy brown sand sticks to top page of column.

Column: Jones 1 x 10 female from run 17 after washing.

P/L 5. Effect has not additive unknown.

Collect effluents from first morning

Track table numbered 0 Election:

F37 250 ml $\frac{4}{0.4} \text{ N HCOOH}$ form } into 250 ml 1 N-HCOOH.

8.vii F80 100 ml 4 N HCOOH - 1 M Am form. directly.

0.011	1.0	1.000000	1.000000	1.000000

P. strus - F0-1 $\rightarrow$ brown after over incubation. Heart over brown $\rightarrow$ clear. Some lost by spatter from F1.

11-vii Repeat FO + 1 : 0.0% and sample:

* Percent of solid part on column

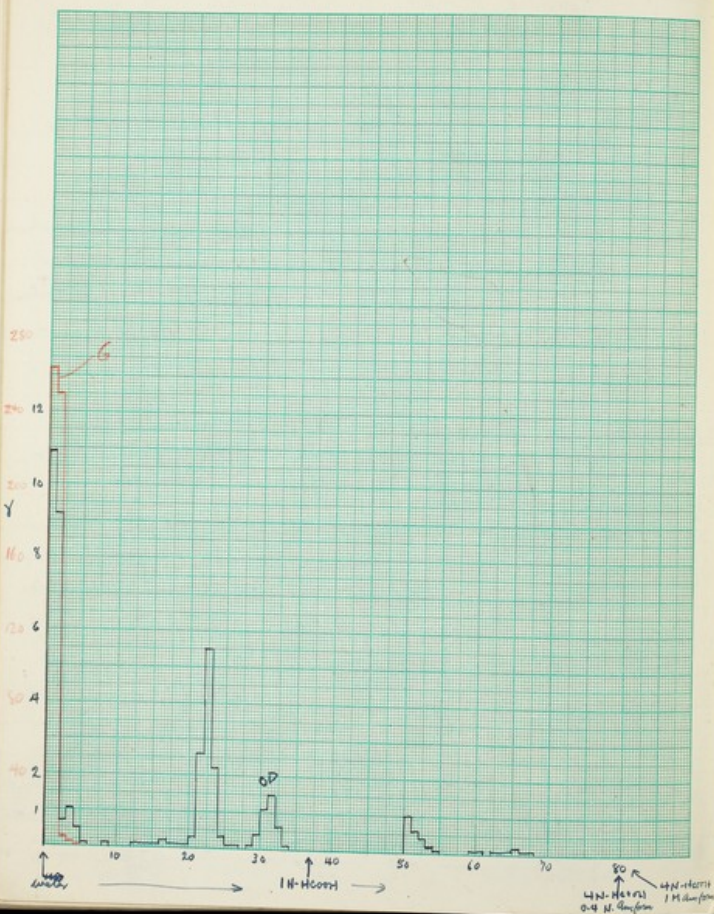
iP + lafite (L+B): 0.1 ml $\rightarrow$ 3.6 YP, but mixture is turbid.

Total P: 0.01 mol $\rightarrow$ 2.6 Y, 2.6 Y
0.02 mol $\rightarrow$ 5.2 Y, 5.2 Y

Total applied to column: $iP: \frac{19.1}{0.1} \times 2.6 = 0.69 \text{ mg}$ $\frac{19.1}{0.1} \times \frac{2.6}{51} \times \frac{1000}{6.15} = 0.69 \text{ mg}$

Total P: $\frac{19.1}{31} \times 26 = 4.96 \text{ mg}$ $\frac{26}{31} \times \frac{19.1 \times 2600}{0.01 \times 6.6} \times 10^6 = 28.0$

0.01



Col. 19 contd.

Sugar content in blood (by anthrone or fraction).

$$F.O.: \frac{264}{0.1} \times 12.8 = 338 \text{ mg.}$$

$$1: \frac{250}{0.1} \times 12.0 = 325$$

$$2: \frac{4.5}{0.1} \times 14.1 = 0.6$$

$$294: \frac{4.0}{1000} \times 12.8 = 0.6$$

67.5 mg. apparent glucose, from 6.0 g. blood.

$$\text{Concn in bld} = 67.5 \times \frac{100}{6} \times 603.5 = 1160 \text{ mg/100ml.}$$

Recovery of P applied to column

$$iP: \frac{3.5}{0.1} \times 13.1 = 0.46 \text{ mg. (} \approx 0.49 \text{ applied)}$$

$$\text{Total P: } 10.9 \times 12.8/6 = 1.40$$

$$9.2 \times 13.0/6 = 1.20$$

$$0.7 \times 14.1/6 = 0.10$$

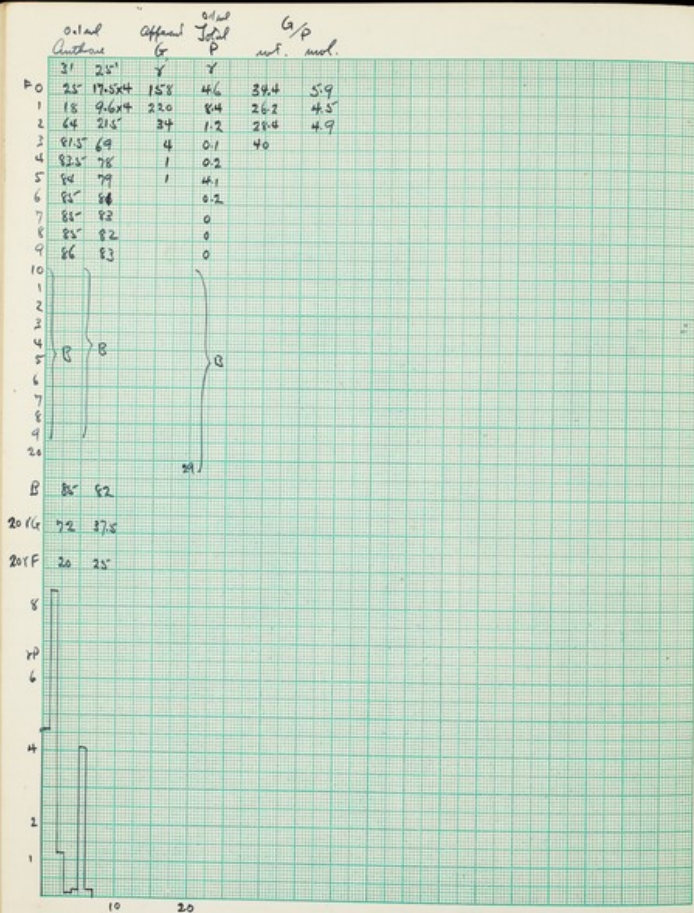
$$1.75 \times 13.8/6 = 2.41$$

$$11.0 \times 13.3/6 = 1.46$$

$$2.7 \times 13.1/6 = 0.49$$

$$2.3 \times 12.0/6 = 2.76$$

$$9.82 \quad (\approx 4.96 \text{ applied!!})$$



11-11

Col. 20. Re-run F0-F1 of col. 19.

Boxes 1 x 8 12.5 x 8 cm. Ventral

Mix F0-F1 of col. 19, add 1 ml 1 N-NH₄OH → pH ~ 10 - off,
to column. (~ 25 ml)

Electron:

F 2 250 ml N-HCOOH with 250 ml water

8 drops/min F = 200.

13-16

Paper chromatograms

~ 40 µl F1 along = markers to 2 solvents:

(a) EtAc 3 HAc 3 H₂O 1

(b) MeOH 12 NH₄OH 2 H₂O 3

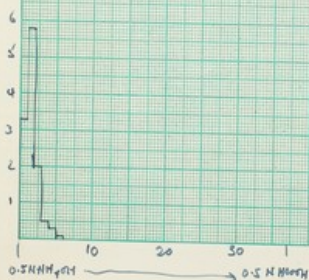
HAc. washed What⁴¹.
Run overnight at
r.t. = ~ 28°C.

Rf's

	(a)	(b)
FDP	.29	.36
G6P	.31	.42
βGP	.62	.59
PGA	.54	.47
Col 20/F1	.78	.54

Total
P. r

F 6 3.3
1 5.8
2 2.0
3 0.5
4 0.2
5 0.1
6 0
7 0
8 0
9 0
10 0
11 0
12 0
13 0
14 0
15 0
16 0
17 0
18 0
19 0
20 0
21 0
22 0
23 0
24 0
25 0
26 0
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96 0
97 0
98 0
99 0
100 0



14-vii

Col. 21. Re-run F0 + F1 of Col. 20.

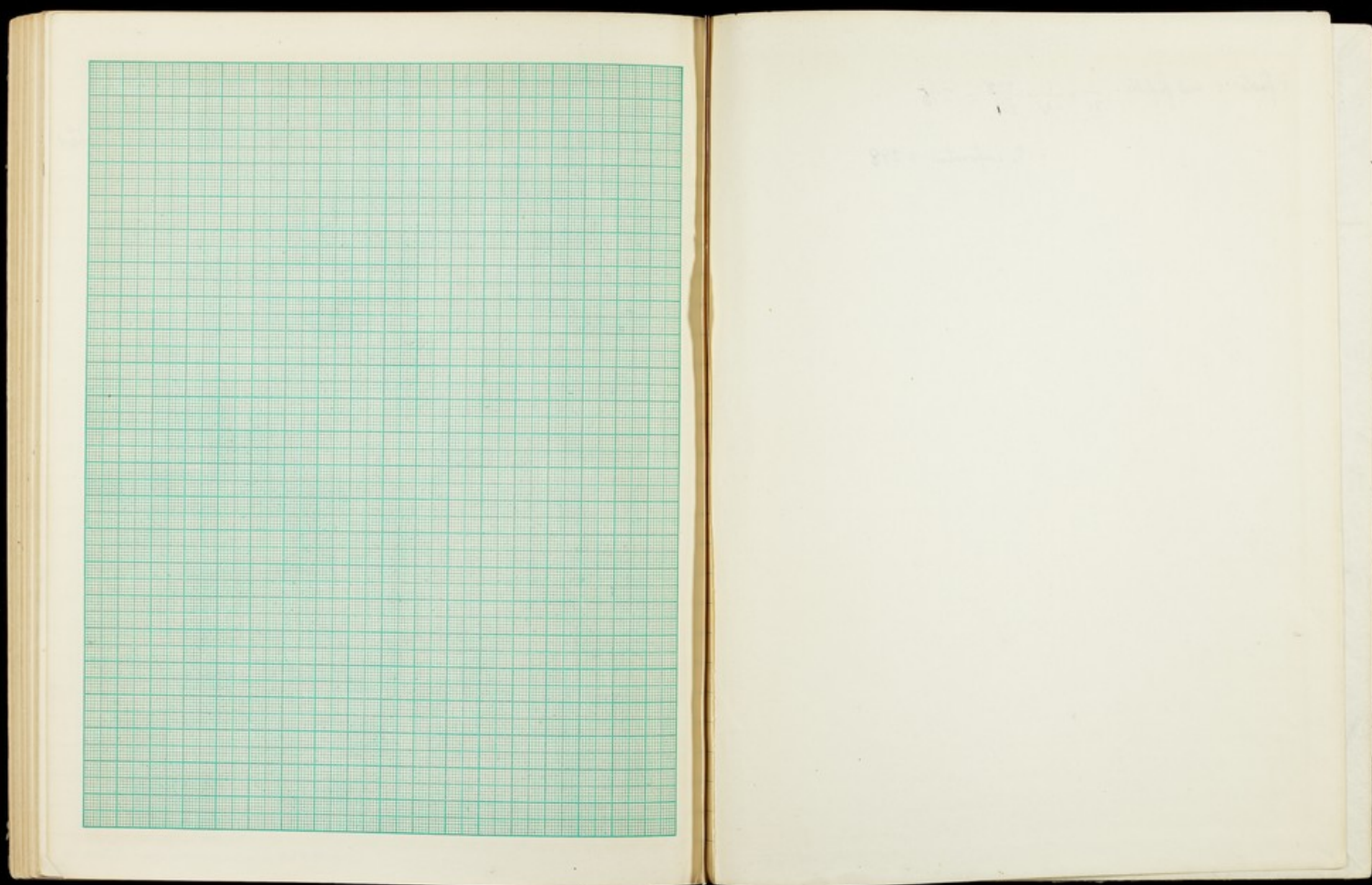
Dowex 1 x 8 col. 20 re-used.

9 min F0 + F1 of col. 20. Add 2 ml conc. NH_4OH $\rightarrow$ 1.0
After addition of ammonia, found cloudy ppt. forms.

Elution:

F2 250 ml 0.5 N HCOOH into 250 ml 0.5 N NH_4OH .

15-vii F37 All through.



P factor = aus pipette: $\frac{1}{31} \times \frac{1}{4.00} \times \frac{7.78}{2.16} = 295$

+ 1% evaporation = 298

