Resume of Polymerization Experiments
Winter 1963 (Maleady)

In this book

Ja-9163 C^{14} PolyA: made with Brenneman pH 6.3.
96.6 μmoles ADP, 11.3 mg plus 3.9 μmoles.

Enzyme Ja 101 G-200 1-10-63
polym c ADP at least 10× stim by $(pA)_3$

1-14, Ja101 cont. Primer stim c UDP and ADP
apparent absolute primer dependence at
50 μmole/ml diphosphate, 0.01 M Mg^{++}
Duplicated 1-30-63

1-17 Kornberg type exp't on primer formation.
Failed.

1-21-63 UDP + poly U primer. Made poly U c Brenneman pH 6.3

1-29-63 UDP + poly U. Check stimulation Brenneman pH 6.3 enzyme.
About 2× stimulation of rate

2-1,4-63 CDP best at lower concentrations. (about 10 μmoles/ml)
better if Mg^{++} at least equal to CDP

1-7-63

ADP polymerization ± C^{14} ADP (exp 1)

Combine 0.25 ml ADP Schwarz lot 6101, 250 mg/ml
and 0.1 ml C^{14} ADP 6204 lot C8AD Schwarz
Take .01 ml and dil to 100 ml ± 1 ml 1N HCl & H_2O.
Abs_{257} vs .01N HCl = .427 conc = 284 µmm/ml total units 1500

		CPM	−13	CPM/ml	Av	
Plate	0.25	71.1 +2.6	58.1	2320000	2254000	22.54×10^5 cpm/ml
diluted	0.50	122.4 −1.3	109.4 −3	2188000 +660		

$\frac{22.54 \times 10^5}{284} = .793 \times 10^4$ cpm/µmole = 7930 cpm/µmole

Set up the following mixture and incubate at 37 °C after a zero time sample of .020 ml is taken & added to .23 ml H_2O. Take samples at 1, 2 & 3 hrs. also.

ADP above 96.6 µmm	.3 ml	Add .25 ml Ur Ac $HClO_4$ reagent to sample. Stand 10 min. Spin 10 min & do a 7.S. Phosphate on .3 ml of the sup.
.5 M Tris pH 9.0	.150	
.2 M $MgCl_2$	.100	
.004 M EDTA	.15	
Enz pH 6.3 III 3	.15	Start 1:16 PM (4 hr total)
(PA) 4.2 2.9 µmm/ml	.20	Freeze at 5:16 – do assays in morning
H_2O	.50	(ADP = 96.6 µm / 2 µ = 48 µm/µ

		1N H_2SO_4	2.5% Mol	Reduce	1:16 Ac	H_2O	Abs 820
1	Bl —	.8	.16	.08	.3	.26	
2	Std 1 µmm/ml .080	.8	.16	.08	.3	.18	
3	Zero Sple .3	.8	.16	.08	—	.26	

	Abs	µmm/sple	µmm/ml	Δ
Zero	.033	.0131	1.094	
Std	.201			
1 hr	.446	.1722	14.35	13.26
Std	.207			

	Abs	µmm/sple	µmm/ml	Δ
2 hr	.603	.228	18.95	17.86
Std	.212			
3 hr	.644	.237	19.76	18.67
Std	.217			

4 hour total incubation (~40 µmoles/µgm total)

For 20 μmoles Pi/ml = 40 μmoles total or approx 20 mg. polymer

1-8-63

ADP polym. $\bar{c}$ C^{14} cont'd. (page 2)

Thaw mixture and do sevags. 1/4 vol $CHCl_3$ 1/10 vol isoamyl alcohol. Shake 5 min. Cent. 5 min. Take off water layer saving the $CHCl_3$ layer. Repeat $\bar{c}$ water layer until no interface appears.

	1)	2)	3)	4)	5)	6)
vol	= 2 ml	1.6	1.4	1.5	1.2	1.2
$CHCl_3$	.5 ml	.4	.35	.375	.30	.30
alc.	.2 ml	~~.32~~ .16	.14	.15	.12	.12

Combine the $CHCl_3$ layers and wash twice adding the wash to the H_2O layer. Vol = 7.1 ml

Dilute .005 to 1 ml $\bar{c}$ H_2O and read OD vs H_2O

Abs_{257} = .546 undil = 109.2/ml

total = 77.50 (or about 77.5 μmoles on 6s1)

Wash $CHCl_3$ layer again $\bar{c}$ 3 ml H_2O keeping it separate and reading the OD. Abs_{257} = .293 discard this 3rd washing

Save above H_2O layer + 2 washings, tot vol 7.1 ml — Dialyze vs 1 l .025 M KCl, start 12:00 PM 1/8 change at 5:00 ℄ in morning 24 hrs later take out. Check OD then ale. ppte. 1-9-63

Vol = 8.2 ml Dil. .005 to 1 ml $\bar{c}$ H_2O Abs_{257} vs H_2O = .150

add 3 vols ale., 24.6 ml, .6 ml .2M $MgCl_2$ undil = 30 total units = 246 or about 25 μmoles polymer

Wash ppt $\bar{c}$ ~~95%~~ 100% ~~Ether~~ Store in freezer over night Label Ja 9 63 expect 12 mg.

Precipitate almost invisible. ~~Qu~~ Quite gelatinous on centrifuging

1-10/63

ADP polym. c C^{14} cont'd (page 3)

Centrifuge Ja 9163 in Internat at 35 for 30 min. Gelatinous precipitate
Wash c 95% EtOH – 10 ml
100% EtOH – 10 ml
Ether – 5 ml. Solid didn't look too good. Very hard, small pieces. Therefore, plan to lyophilize. 1-11-63

Dissolve Poly A in H_2O. Needed .12 ml .2M versene, Adj. pH to neutral with .030 ml 1N NH_4OH.
pH was ~5, final pH ~ 6.5.

Lyophilize. Weight = 11.27 mg 1-15-63
Rinse lyophilizer tube c 1/2 cc. H_2O dissolving the poly A. Store in conv. cent. tube. Dilute 0.02 ml + 0.98 ml 0.01 M KPO_4 pH 7.0. Abs_{257} = 1.537 unde = 76.85. About 7.7 μmole/ml = 7930 × 7.7 = about 61,100 cpm/ml
Must remember that this polymer has versene in it.

Plate 0.01 ml of solution. count in gas flow counter.
Bkgd = 11.3
Duplicates cpm #1 558, 598 } av 593 corr for Bkgd = 582
cpm #2 598, 616 }
58200
~~5820~~ cpm/ml of solution

for 7.3 μmole/l (ϵ_p = 10.5)
$\frac{58,200}{7.3}$ = 7970 cpm/μmole

1-10-63

G-200 Sephadex

Pool fractions 7, 8, 9 & 10 (stored in deep freeze, assayed on 1/7)

λ	Abs
280	091
260	091

Label Ja 10163

mg Pro/ml = .0792

Repeat 280/260 ☆ BSH in Reference cell

280 = .099
260 = .057

280/260 = 1.74

mg Pro/ml = .109

Assay 2 mg/ml

Sample	vol	BSA	H_2O	CPM	−Bkg 13.4	−Bl	Pi/pple	Pi/ml	s.a.	s.a./Pro .109/ml
1 Bl	—	.005	.045	28.1	14.7	—				
2 Ja10163	.010	.005	.035	421.0	407.6	392.9	.00509	.509	6.43	4.68
3 "	.020	.005	.025	816.0	802.6	787.9	.01023	.511	6.45	4.68
4 "	.030	.005	.015	1220.0	1206.6	1191.9	.01547	.515	6.50	4.83

in each: .010 PolyA 5 mg/ml
.030 Rx Mix 1/10/63
.010 .1M K_2HPO_4

Reassay 2-7-63

Freeze Ja10163, fract 6, 11 + 12. Store in deep freeze in hall

New Rx Mix

.3 ml	P^{32} 12/5/62
4.0 ml	.5M Tris pH 8.2
.4 ml	.05M EDTA
.5 ml	.2 M $MgCl_2$
.8 ml	H_2O
6.0	

Dilute .05 → 5 Plate .05, .10

	CPM	−Bkg 13.4	CPM/ml	
.05	1400	1386.6	27732×10^5	27.45×10^5
.10	2730	2716.6	27166×10^5	

$$\text{s.a. ltd assay} = \frac{27.45 \times 10^5 \times .03}{1 + \left(\frac{45.1 \times .03 \times .3}{6}\right)}$$

$$1.068$$

S.a. = 77100

1-10-63

To test polymerization activity of enzyme Ja 9T63 purified from the G-200 Sephadex.

Incubate + take samples etc. as on 1-4-63
20µl samples.
Start 3:13
take sample at 10 to 5

	I	II	III	IV	V
ADP 1-4-63	.015	.015	.015	.015	.015
.5M Tris @ H 9.0	.025	.025	.025	.025	.025
.2M $MgCl_2$	.005	.005	.005	.005	.005
.004M EDTA	.005	.005	.005	.005	.005
Ja 9T63	.010	.010	.060	.040	—
(pA)$_{4.2}$ 2.9 µmm/ml	—	.010	—	.010	—
H_2O	.040	.020	—	—	.050

Phosphate det.

Zero Time	vol	$1N_2SO_4$	Amm Mol	Reducer	1:1 HCl	H_2O	Abs 820	−Bl	µm/Δ hr	µm/ml
1 Bl		.8	.16	.08	.3	.26	.034	—		
2 Std	.080				.3	.18	.243	.209		
3 10	.3				—	.26	.087	.053	.0203	1.69
4 20					—	.24	.069	.035	.0134	1.12
5 30					—	.26	.063	.029	.0111	.925
6 40					—	.26	.072	.038	.0145	1.21
7 50					—	.26	.075	.041	.0157	1.31

Hour	Abs	−Bl	µm/Δ hr	µm/ml	Δ
1	042	—			—
2	234	.192			—
3	088	.046	.0191	1.59 / 1.69 I	0
4	250	.218	.0907	7.56 II	6.44 III
5	093	.051	.0212	1.76	.83
6	.402	.360	.150	12.5 III	11.29 IV
7	063	.021	.0087	.726	0

Plan regular time curve to determine actual extent of stimulation. Then Kornberg type investigation of lag period.