Large Scale Exchange Reaction

8/30/57

8/29

1.6 ml of P^{32} (8/20/57) = 10 mC. Evap to dryness. Add water + repeat. Repeat. Add 0.2 ml Na_2HPO_4, 0.1 M. Add 4-5 drops of 0.1 N NaOH to bring pH to dirty blue (thymol blue) equivalent to that of the Na_2HPO_4. Muffle furnace – 1 hour – 400° C. (Bathing = 4.5).

8/30 : Dissolve PP^{32} product in water. Add 0.2 ml Morita. Centr. Pipet off clear solution from under Morita "fines"; lost about 0.1 ml in leaving "fines" behind.

Incubation Mixture:

PP^{32}, ~ 10 mC, ~ 10 μ mole	1.50
Tris, 1 M, 7.5	.20
$MgCl_2$, 0.1 M	.20
TTP, 0.5 μM/ml	.20
DATP "	.20
DCTP "	.20
DGTP "	.20
DNA, 7 γ/ml	.20
DEAE, Fr. 4 7/19 (undil)	.04
Water	.06
	3.00

60' at 37°. Faintly turbid. Now add: (after chilling)

albumin 10 mg/ml	0.50
PCA, 7.0 %	2.50
Acidified PP	5.00

V_f = ~ 11.0 Centr. Supt = S1

Treat with 0.2 ml of Morita. Supt = S2

Wash Morita 3x with 15 ml - volumes of cold water to which 2-3 drops of 7 % PCA was added each time.

(Complete over)

	vol	dilu	cpm/0.1 ml	Σ
Supr. #1	11	10^6	122	1.34×10^{10}
" #2 (after Monte)	11	10^6	162	1.78×10^{10}
Wash #1	15	10^4	101	1.5×10^8
2	"	10^3	30	4.5×10^6
3	"	10^2	116	1.8×10^6
Monte susp. after Wash #3	"	10^2	(.05 ml) 3127	93×10^6

Electrics : 50% EtOH (NH_3) ; 2 ml each time.

	vol	dilu	cpm/.02 ml		
El. 1+2	4	10^3	265	53×10^6	88×10^6
3	2	"	31	3	
Monte susp	2	"	308	31	
4	2	"	10	1	

Take El. 1, 2 and 3 and blow off EtOH at about 40°.
Vol = 3.0 ml. Store in deep freeze.

9/3/57

Chromatography of Pyro-discharged Triphosphates.

To monte eluate 8/30/57 1.K. (2.9 ml) add following:

✓	DCTP	1.25 μM/ml	0.80 ml
✓	DGTP	2.5 μM/ml	0.40 ml
✓	TTP	3.55 μM/ml	0.28 ml
✓	ATP	3.5 μM/ml	0.29 ml

} = 1.0 μMole of each Triphosphate

Uf = 4.8 μl - pH 7.2 - 7.5 (BTB + Light Blue)

Spectrum - 1:20 + neutral pH

λ 250	λ 260	λ 280	0.D./ml
.409	.472	.268	9.48

Counts - plate .02 of 1:400 Std = 8×10 cpm.

Cp 2' = 1079, 967 } 1023 ÷ 2 = 512 cpm

20 ÷ 6

$5.12\times10^{2} \times 4\times10^{2} \times 5\times10^{1} = 1.02\times10^{7}$ /ml = 4.9×10^{7} cpm total

Column - 8.0 × 1.0 cm Dowex 1 Cl⁻ 2% × 4 (fast running).

Material - 4.4 ml of above mixture put on in 30'. Wash with 8 ml H_2O.

Elute at rate of ~ 0.5 ml/minute

Tube #	Vol.	E_{250} 1:20	E_{260} 1:20	E_{280} 1:20	$\frac{E_{250}}{E_{260}}$	$\frac{E_{280}}{E_{260}}$	ΣE_{260}
0 eqml	4.4	.409	.472	.268	.57	.87	41.5
PH wash	13.0	.048	.025	.013			
1	14.0	.057	.069	.050			
2	13.0	.055	.075	.085			
3	13.5	.038	.050	.025			
4	13.5	.032	.025				
5	13.5	-.010	.000				
6	16.5						
7	16.5						
8	16.5		.020				
9	22.5		.015				
10	21		.020				

Vol plate 1:400	Cpm	Σ cpm
.02	512	4.48×10^{7}
.02 undil	7	
.02	700	$.49\times10^{6}$
.02	712	$.46\times10^{6}$
.02	388	$.26\times10^{6}$
.02	260	$.16\times10^{6}$
.02	165	$.11\times10^{6}$
.02	104	
.02	48	
↓	74	Counted 9/4/57
	130	$.15\times10^{6}$ ↓
	516	$.54\times10^{6}$

1.50×10^{6}

9/4/57

Tube #	Vol	E_{300}	E_{260}	E_{200}	260/200	300/260	ΣE_{200}	Vol plated	cpm	Σ cpm.
11	18.5		.043	.075				.02 ↓	1042	$.90 \times 10^6$
12	17.5		.055	.105					1630	1.43×10^6
13	17.5		.065	.125					2086	1.82×10^6
14	18.5		.062	.118					1706	1.38×10^6
15	18.5		.042	.070					1060	$.98 \times 10^6$
16	18		.027	.035					520	$.47 \times 10^6$
17			.025						300	$.27 \times 10^6$
18			.025						282	$.26 \times 10^6$
19			.027						448	$.40 \times 10^6$
20	18	.050	.040	.027					610	$.55 \times 10^6$
21		.055	.055	.023					878	$.79 \times 10^6$
22		.060	.065	.023					1143	1.03×10^6
23		.080	.080	.031					1410	1.27×10^6
24		.080	.085	.028					1543	1.39×10^6
25	17.5	.082	.087	.028					1655	1.48×10^6
26		.050	.052 .050	.030					1739	1.57×10^6
27		.075	.095 .075	.025					1426	1.28×10^6
28		.062	.062	.020					1154	1.01×10^6
29			.050	.023					760	$.67 \times 10^6$
30	17		.040	.019					531	$.45 \times 10^6$
31	1		.027	.017					300	$.25 \times 10^6$
32			.017						165	$.14 \times 10^6$
33									86	
34			.010						35	
35	17								22	
36			.010						15	
37									15	
38									16	
39									15	
40									3	
41			.015						19	
42	18.5								32	
43									26	
44									21	
45									11	

8.20 x 10^6

12.45 x 10^6

counted 9/5/57 ↓

9/4/57

Cool tubes 10 thru 16. Volume = 130 ml. Wash tubes ☆ H_2O. Volume = 138 ml.
Add 1.4 ml M/1 glycine 9.2 and 0.34 ml 4M LiOH. pH ~ 9.2 (Thymol Blue)
Freeze up.

Pool tubes 20 thru 30. Volume = 180. Wash tubes ☆ H_2O. Volume = 200 ml.
Add 2.0 ml glycine M/1, 9.2 and .48 ml 4N LiOH. pH ~ 9.2

Pool tubes 54 thru 73. Volume = 205 ml. Wash out tubes. Volume = 275. Neutralize (pH 7.5)
with 4N LiOH. Freeze.

Cool tubes 86 thru 98 Vol = 235 - Wash out tubes + Vol = 255.
Since 0.2 ml of ~3N NH_4OH was added to each tube during collection, no
neutralization was required. pH = 8 (BTB and Thymol Blue)

Plate .02 ml of last pool (TTP?). 839 cpm
839 x 50 x 255 = 10.70 x 10^6 cpm total (on 9/6/57)

Add 2.5 ml M/1 glycine 9.2 and 4 drops 4N LiOH. pH ~ 9.2

9/5/57

Time	Vol	E_{250}	E_{240}	E_{230}	$\frac{250}{240}$	$\frac{250}{236}$	Σ_{E240}	Vol Ratio	cpm Corr	Σ cpm	
46								.02	15		
47	15.0							↓	21		
48									12		
49									22		
50									27		
51									48		
52	15.0								63		
53									55		
54									68		
55									90		
56									111	$.08 \times 10^6$	
57	14.8								165	.12 "	
58									156	.12	
59									156	.14	
60									263	.19	
61									321	.24	
62	14.0								308	.22	
63									379	.27	
64									377	.26	3.46×10^6
65									354	.28	
66									403	.28	
67	14								352	.27	
68									336	.24	
69									320	.22	
70									252	.19	
71									228	.16	
72	13.5								157	.11	
73									126	.08	
74									92		
75									94		
76									81		
77									98		
78									66		
79									47		
80									45		

5/5/57

Tube #	Vol	ϵ_{250}	ϵ_{260}	ϵ_{270}	250/260	250/270	$\Sigma_{\epsilon 260}$	√plated	cpm corr	Σ cpm
81	3							.02	36	
82								↓	31	
83									44	
84									36	
85									20	
86									136	
87									600	.39
88	18.0								2384	Counted 5/6/57 2.14×10^6
89									3003	↓ 2.70×10^6
90									2108	1.90 "
91									1101	.99 "
92									632	.57 "
93	18.0								568	.51
94									536	10 .71 .48
95									463	.42
96									304	.27
97									255	.23
98	18.0								124	.11
99									35	
100									9	
101									2	
102									17	
103	17.5								8	
104									-1	
105									•	
106									9	
107									.	
108	17.0								-1	
109										
110									6	
111										
112	16.5								0	
113										
114										
115									2	

9/6/57

Tube #	Vol	Vol plated	eq. conc	Σ eq.
116	15			
117				
118			0	
119				
120	15		12	
121	↓		7	
122			7	
123			< 5	
124			< 5	
125			< 5	
126			< 5	

0.5 M HCl
0.5 M Li Cl

9/7/57

Harvest of DATP and TTP (pyro labeled)

DATP

Count 0.02 ml. of pooled DATP tubes (see 9/4/57).
979 cpm. $979 \times 50 \times 200 = 9.8 \times 10^6$ cpm total
Add 0.92 ml 3M $BaBr_2$ (This adjusts Ba^{++} conc. to .02 M)
Add 140 ml Abs. ethanol at -12°. Let stand at 2° for 1 hr.
Spin at #60 4'. Plate 0.2 ml of ppte cpm corr = 0
Harvest remainder of ppte. Store in vacuum over KOH @ 2°.
Decompose ppte in usual manner.

TTP

Take pooled TTP tubes (see 9/4/57) which have volume of 265 ml and have pH adjusted to 9.2. Total cts in solution = 10.7×10^6 on 9/6/57
Add 1.77 ml 3M $BaBr_2$. (this adjusts Ba^{++} Conc. to 0.02 M).
Add 265 ml Abs. ethanol at -12°. Let stand at 2° for 1 hr.
Spin at #60 4'. Count 0.2 ml. of ppte = 37 cpm corr.
$37 \times 5 \times 265 \times 2 = 9.9 \times 10^4$ ct non-precipitated = << 1% total
Harvest remainder of ppte. Store in vacuum over KOH @ 2°.
Decompose ppte in usual manner.

DGTP } work up as above.
DCTP }

9/12/57

Is Pyrophosphate Released in Absence of DNA?

	1	2	3	4	5	6	7	8	9	10	11	12
√ TTP .107 μM/ml	.05	.05				.05				.05	.05	*
√ DATP .117 (.093) μM/ml (hyperlabeled)	.04		.04				.04			.04	.04	*
√ DGTP .119 μM/ml	.05			.05				.05		.05	.05	*
√ DETP .245 (.093) μM/ml	.03				.03				.03	.03	.03	*
√ $MgCl_2$ 0.1 M	.02 →											
√ KPO_4 M/15 7.4	.02 →											
√ Thymus DNA .5 mg/ml (reheat. 9/9/57)	.02 mm	—	—	—	—	.02	.02	.02	.02	—	.02	02
√ DEAE fr. 4	—	.01 →										
√ H_2O	.09	.20	.21	.22	.20	.18	.15	.20	.18	.08	.06	19
	.30 →											

30' 37°

Add .20 ml carrier (DNA, 2.5 mg/ml) + 0.5 ml 7% PCA. 3'0°. Add 0.1 ml Norit (25% P.V.). Add .01 ml BSA (50 mg/ml). Mix. Sp. 5'0°. Spin 5'. Plate 0.60 ml of supe. Add 0.16 ml 2N KOH & neutralize PCA.

Std = 756

Bkg = 13 cpm

	1	2	3	4	5	6	7	8	9	10	11	12
c/2 m.												
c/m. corr	5046	1876	2102	585	786	1606	3156	1470	1892	4350	5912	

9/13/57

Nonit Treatment of Pyro Labeled DCTP

To 2.0 ml of DCRPPP* Add .02 ml of 1N HCl. pH ~ 2. Add 0.015 ml Nonit 20% P.V. Mix intermittently 5' @ 0°. Spin. Do not decant.

Counts

Original 1:10, plate .02; cpm = 1300 ($1.3\times10^3 \times 10^1 \times 5\times10^1 \times 2 = 1.3\times10^6$ cpm total)

Nonit supe undiluted, plate .02; cpm = 4350 (= 33% of counts remain in supe)

Add .015 ml more Nonit. Stir up 5'. Spin. Do not decant.

Plate .02 undiluted; cpm = 1350

$\frac{1350}{13000} \times 100 = 10.4\%$

From data of 9/12/57 one can calculate that 7% of DCTP counts remain most non-absorbable. ∴ about 93% of most absorbable counts have been absorbed.

Spin resuspension once more 3'. Decant supe. Wipe out tube. Wash precipitate 2 × @ 2.0 ml ice-cold $\frac{M}{100}$ HCl: Discard washes.

Elute most with 1.5 ml volumes of EtOH : H_2O : Conc. NH_3 mixture (50 : 50 : 0.3 by vol.) 10' 0°. Collect supe after centrifugation:

Elute I Plate .01 = 4400 cpm.

$4.4\times10^3 \times 10^2 \times 1.5 = 6.6\times10^5$ cpm total (56% of that absorbed)

Elute II plate .01 cpm = 650 $6.5\times10^2 \times 10^2 \times 1.5 = .97\times10^5 = 8\%$

	250	260	280	OD 260/ml	OD 280/ml	mM/ml 250	mM/ml 260	mM/ml Average
1:20 pH 2	.057	.098	.143	1.96	2.86	.22	.32	.27

plate 0.01 ml of 1:20 = 516 cpm.

$5.16\times10^2 \times 10^2 \times 2\times10^1 = 1.03\times10^6$ cpm/ml

$\frac{1.03\times10^6}{0.27} = 3.8\times10^6$ cpm/μM

9/13/57

Does Reactor Require DNA When measured by Pyro-release from DCRPP^{32}?

	1	2	3	4	5	6
DCTP 0.27 mM/ml 3.8×10^6	.02 →					
DATP	.01	—	—	.01	.01	—
DGTP 0.5 mM/ml Cold	.02	—	—	.02	.02	—
DTTP	.01	—	—	.01	.01	—
$MgCl_2$ 0.1M	.02 →					
KPO_4 1M 7.4	.02 →					
Thymus DNA .5 mg/ml refrozen	.02	—	.02	—	.02	—
DEAE fr 4 9/9	—	.01	.01	.01	.01	—
H_2O	.18	.23	.21	.19	.17	.24
	Vf .30 →					

37° 30'

Add 0.20 ml. Carrier DNA (2.5 mg/ml.) 0.05 ml 1/N HCl + 0.35 ml H_2O + 0.01 ml B.S.A. + 0.10 ml norit, (20% P.V.) Mix, 5' 0° Spin.

Norit packs well. Plate 0.5 ml

Std = 815
Reg = 15

C/2 ml	128	119	1027	168	2814	109
C/ml Corr	49	45	498	70	1392	40
Δ = 49						
~~mμmoles phos~~	0	-4	449	21	1343	-9
mμmoles/gm	0	0	.236	.011	.710	0

VI

9/16/57

De Nonte Treatment of TTP, DATP, DGTP to reduce blanks (Pyro release)

To 3.0 ml of each of TTP, DCTP DGTP add 0.03 ml M/7 HCl.
Add 0.045 ml Nonte (70% P.V.). Stir intermittently 5'. Spin.
Do not decant. Plate 0.02 ml of supno.

	Cpm 1241	Cpm/ml
TTP	~~2201~~	6.2×10^4
DATP	2201	1.1×10^5
DGTP	333	1.6×10^4

Plate 02 of a 1:10 dilution of orginal soln.

	Cpm	Cpm/ml
TTP	926	4.6×10^5
DATP	1576	7.9×10^5
DGTP	311	1.5×10^5

Add 0.02 ml more of Nonte to each tube. Stir 5'00 Spin. Plate 02 of supt.

TTP		
DATP	1515	7.6×10^4
DGTP	264	1.3×10^4

Elute ~~=~~ 2.0 ml of 50% etoH containg 0.3% Conc. NH_3 30'00 Spin. Plate .01 of supt

TTP	3154	3.19×10^5/ml
DATP	6075	6.08×10^5/ml
DGTP	1087	1.09×10^5/ml

UV on solutions diluted 1→1.1

	350	260	280	$E_{260/ml}$	$\mu M/ml$	Sp. Activ
TTP	.065	.070	.054	.77	.092	3.4×10^6
DATP	.131	.138	.057	1.52	.107	5.078×10^6
DGTP	.107	.088	.084	.97	.083	1.31×10^6

TTP and DATP Concentrated 2.5-fold and DGTP concentrated 3 fold by blowing air three at rm temp.
Final Concentrations: TTP .23 $\mu M/ml$
DATP .27 $\mu M/ml$
DGTP .25 $\mu M/ml$

9/16/57

Check Whether Single Deoxynucleoside Triphosphates Release Pyro in Presence of DNA + Absence of DNA

	1	2	3	4	5	6	7	8	9	10	11
√ TTP	.02			02			02			.01	.01
√ DATP } to 0.25 mM/ml		.02			02			02		.02	.02
√ DGTP			.02			02			02	.02	.02
√ DCTP 0.25 mM/ml (cold)										.01	.01
√ M/15 KPO4 pH 7.4	.02 →										
√ $MgCl_2$ 0.1 M	.02 →										
√ Thymus DNA .5 mg/ml (refrigerator)	.02	.02	.02	—	—	—	.02	02	02	.02	—
√ DEAE fr. 4 9/9	—	—	—	.01 →							
√ H_2O	.22	.22	.22	.23	.23	.23	.21	.21	.21	.17	.15
	.30 →										

37° 30'

Add 0.20 ml Carrier + 0.40 ml 5% HCl (cold). Add 0.01 ml Albumin (50 mg/ml) followed by 0.10 ml. Vortex. Mix, 5°0. Spin. Plate 0.50 ml/Aliquot.

Old = POC

Reg = 2

	1	2	3	4	5	6	7	8	9	10	11
c/2'	151	158	92	140	267	335	425	1176	1668	6454	1319
c/m corr.	63	67	34	58	122	156	200	576	822	3200	647
Δ - Enzyme	0	0	0	-5	55	122	137	509	758	3136	483
mμmoles split	—	—	—	0	.02	.186	.08	.180	.202		

Sp. Activities:

TTP	3.96×10^6
DATP	5.07×10^6
DCTP	1.31×10^6

9/17/57

Is it P^{32} that is released from DATP when incubated $\bar{c}$ Enzyme + DNA?

DATP 5.4×10^6/μM ~.25 μM/ml		.40
$\frac{M}{15}$ KPO_4	7.4	.40
$MgCl_2$	0.1 M	.40
Thymus DNA	.5 mg/ml	.40
DEAE purified	9/8	.20
H_2O		4.20
30' 37°		6.00

Add 4.0 ml "Carrier" and 8.0 ml $\frac{N}{10}$ HCl (cold). Add 0.20 ml Albumin (50 mg/ml) and 2.0 ml Norite (20% P.V.). Mix. Sit 10' 0° with intermittent mixing. Spin 5'. Decant supe.

Volume of Norite supe = 15.5 ml Neutralize $\bar{c}$ 0.3 ml 2N KOH. Vol = 15.8 ml
Count 0.5 ml = 564 cpm. Set aside 1.0 ml. Put remainder (13 ml) on column.
(Add 0.05 ml $\frac{N}{10}$ PP pH 7 A.K.)

Column - Dow 1 Cl^- 2% xc 10 cm x 1 cm.

	Tube #	Volume	Vol plated	cpm corr 545	Σ cpm 15,600		Vol for Paneyan	μM P	Σ μM P
	original	18	0.5						
	PH work	27	1.0	0					
HCl →	1	9	↓	85	800		.10	~~.087~~ .00	
	2	14		3			↓	~~.131~~ 0	
	3	15		-3				0	
	4	15		3	~~45~~ ~~50~~ 140			0	
	5	23		6	~~250~~			~~.087~~ 0	20
	6	15		2	~~30~~ 60			.131 ~~.087~~	15.6
	7	15		5½	~~170~~			.160 ~~.131~~	24
	8	15		10	~~250~~			.188	47
	9	15		9	~~835~~	counted 9/18/57		.188	28
	10	15		7	~~805~~				
	11	8		4	~~59~~				
HCl →	12	18		34	648				
	13			8	150				
	14			2	35				
					1663				

VI

9/18/57

Hydrolysis in 1N H_2SO_4 15' 100°

Tube #	Volume	Vol plated	Cpm corr	Σ cpm		inorganic /ml	P cpm/ml	Σ P cpm	Sp. Activity
15	15	1.0	-3						
16		↓	4						
17			-7						
18	19		4						
19			-1						
20			7						
21	15		1	19					
22			34	645					
23			100	1900					
24	19		218	4160		000	.155	2.85	2.7×10^3
25			230	4370	15,310	000	.146	2.78	3.1×10^3
26			130	2470		000	.091	1.73	2.5×10^3
27	15		67	1270					
28			21	400					
29			5	95					
30	19.5								
31									
32									
33									
34									
35									

Summary - 84% of Counts eluted are in Pyro region. Looks definite that Pyro is split off DATP in presence of DNA in limit reaction.