Purification of P Enzyme

7/18/56

	1	2	3	4	5	6	7	8
✓ TTP (C^{14}) .55 μM/ml, 1.7×10^6 cpm/μM	.01 →							
✓ DATP^{32} - 17 μM/ml (~300,000 cpm/μM)	.03 →							
✓ DCTP - 0.50 μM/ml	.01 →							
✓ DGTP - 0.59 μM/ml	.01 →							
✓ Glycine M/1, 9.2	.02 →							
✓ $MgCl_2$ - 0.1 M	.01 →							
✓ Thymus DNA - 0.5 mg/ml	.02 →							
✓ Sonorate (1:4)		.02						
✓ S ppt (1:4)			.02					
✓ Spermo sup (1:4)				.02				
✓ Nuclease treated Spermosup (1:4)					.02			
✓ Lyophilized Enz (1:24)						.02		
✓ pH Sr 1 (1:24)							.02	
✓ pH Sr 2 (1:24)								.02
✓ water	.19	.17	.17	.17	.17	.17	.17	.17

V.f. = .30 ml

30' 37°

Bkg: 20/2, 24/2 mean = 11 cpm

St: 1139 - 11 = 1128

	1	2	3	4	5	6	7	8
cpm/min:	127	537	650	567	489	493	296	233
cpm corr:	52	258	314	273	234	236	137	106
- 52	-	206	262	221	182	184	85	54
mμM Incorp:		.16	.20	.17	.14	.14	.066	.042

Preparation of Fractions:-

1 - E coli Sonorate 5/29 ~20 mg/ml 225 ml + 75 ml M/20 G.G. pH 7.0 to bring protein concentration to 15 mg/ml Sonorate. This is based on 100 γ protein = 0.580 at A 660

2 - 150 ml sonorate + 150 ml M/20 Tris pH 7.5 (0°) 22.5 ml 5% Streptomycin Sulfate added as flask was stirred manually. 10' 0° Centrifuged in 8 plastic tubes. Supernatant poured off and saved (Strep sup 7/13). precipitate rubbed up with 10 ml M/20 KPO_4, 7.4

then 8.7 ml more buffer added to each and precipitates rubbed up again. 10' 0°. Centrifuged - Supernatant - S ppt. Residue discarded.

3- S ppt centrifuged 40,000 RPM. Spinco 2 hours. Residue discarded. Sup = Spinco Sup. (two tubes broken in centrifuge - volume 110 ml)

4- Spinco sup - 110 ml + 1.8 ml .3 M $MgCl_2$
+ .11 ml of 100 γ DNAase/ml (final conc. of DNAase = 0.1 γ/ml)
+ .011 ml of 1 mg RNAase/ml (" " RNAase = 0.1 γ/ml)

5 hours, 37°

0.5 ml + 0.5 ml 7% PCA, 5' 0° centrifuged.

E_{260} of sup dil .05/1.0 = .572 or 22.9 U/ml

original Spinco sup - dil .02/1.0 = .340 = 17.0 U/ml - hypochromic effect = 35%

Incubation flask chilled at 5 hours and dialyzed 13 hours against 8 liters M/100 Tris pH 7.5. Some ppt formed centrifuged out and discarded. Sup - Nuclease treated + Dialyzed Spinco sup. (vol = 120 ml)

5- Nuclease treated + dialyzed spinco sup lyophilized to small volume and taken up to 20 ml Lyophilized Renzyme.

6- 1.0 ml Lyophilized Renzyme + 0.18 ml M/5 NaAc pH 4.0 - 5' 0° centrifuged. ppt dissolved in 1 ml M/20 Tris pH 7.5. pH fr 1. To sup was added 0.5 ml M/5 NaAc pH 4.0 5', 0° centrifuged. Supernatant discarded. ppt dissolved in 1 ml M/20 Tris, 7.5 pH fr-2.

Protein Determinations
(all samples ppted with 5% TCA)

Sample	vol ppted	ppt dissolved in	vol for test	E_{660}	γ Protein	mg Protein/ml	Q
Sonicate	0.05	2.0	.10	.223	54	21.6	0.15
S ppt	0.10	1.0	.20	.082	20	1.00	4.0
Spinco sup	0.50	1.0	.20	.168	41	.41	8.3
Dialyzed Renz	—	—	.10 (direct)	.140	34	.34	8.3
Lyophilized Renz	.10	1.0	.20	.120	29	1.45	11.6
pH fr 1	.20	1.0	.50	.340	83	.83	9.6
pH fr 2	.40	1.0	.50	.182	44	.22	23.0
BSA std (.20γ)	—		.25	.205	50		

samples dialyzed and assayed directly

Sample	vol for test	E_{660}	γ Protein	mg Protein/ml
Lyophilized Renz	.02	.130		1.57
pH fr 2	.20	.205		.80
BSA std (.20γ)	.25	.200	50	

7/19/56

Relative "Rates" of Incorporation of Deoxytriphosphates. Thymus DNA as Primer

RMS

	1	2	3	4	5
M/1 Glycine, 9.2	.06	→			
$MgCl_2$, 1M	.03	→			
TTP^{32} ~40 μM/ml 210,000 cpm/μM	.08	.08	—	—	—
TTP, .50 mM/ml	—	—	.06	.06	.06
Deoxy ATP^{32} ~15 μM/ml ~270,000/μM	—	—	.20	—	—
Deoxy ATP, .50 μM/ml	.06	.06	—	.06	.06
Deoxy GTP^{32} ~17 μM/ml 140,000 cpm/μM	—	—	—	.18	—
Deoxy GTP .59 μM/ml	.05	.05	.05	—	.05
Deoxy CTP^{32} ~.25 μM/ml 158,000 cpm/μM	—	—	—	—	.12
Deoxy CTP .50 μM/ml	—	.06	.06	.06	—
PCenzyme ~45 μ/ml	.10	→			
Thymus DNA .5 mg/ml	.09	→			
W	.46	.40	.28	.29	.36

V_R = 0.90 ml 5 mc 60' at 37° - Add .10 ml of 10' col. sonicate, then .90 ml of cold 7% PCA, rock up ppt., add 2.0 ml of ice water, disperse ppt. Centrifuge, collect ppt. wash 2x by rubbing up in 0.5 ml of 3.5% PCA + 2.0 ml of ice water, Dissolve ppt. in .04 of 0.5 N NaOH add .16 ml of water, mix, plate, dry & count on Gas-Flo.

c per 2 min.	208	2840	5148	2229	2264
St = 1100 corr.		2632	4940	2021	2056
cpm		1316	2470	1010	1028
mμM incorporated		6.2	8.5	7.2	6.5
		TTP	DATP	DGTP	DCTP

Relative "Rates" of Incorporation of Deoxy Triphosphates

Phage DNA as Primer.

RM's

	1	2	3	4	5	6
M/4 Glycine, 9.2	.06					
$MgCl_2$, .1M	.03					
TTP^{32} See 7/18	.08	–	–	–	–	
TTP, .50 uM/ml	–	.06	.06	.06	.06	
Deoxy ATP^{32} .085 uM/ml ~ 200,000/um	–	.35	.35	–	–	
Deoxy ATP, .50 uM/ml	.06	–	–	.06	.06	
Deoxy GTP^{32} See 7/18	–	–	–	.18	–	
Deoxy GTP, .50 uM/ml	.05	.05	.05	–	.05	
Deoxy CTP^{32} See 7/18	–	–	–	–	.12	
Deoxy CTP, .50 uM/ml	.06	.06	–	.06	–	
Phage DNA	.12 →					
P Enzyme	.09 →					
W	.36	.09	.15	.25	.32	

V_F = 0.90 - Inc 60' at 37°

cpx'	2584	909	295	1390	1162
corr.	2289	714	–	1095	867
cpm	1144	357		547	433
mu M/incorp.	5.45	1.25		3.9	2.85
	TTP	DATP		DGTP	DCTP

SA = 115 a

Purification of P Enzyme. 7/19/58

	1	2	3	4	5	6	7	8
✓ TTP (C^{14}) .55 μM/ml. (1.29×10^6 cpm/μM)	.01 →							
✓ DATP - .5 μM/ml	.01 →							
✓ DCTP - .5 μM/ml	.01 →							
✓ DGTP - .59 μM/ml	.01 →							
✓ Glycine M/1, 9.2	.02 →							
✓ $MgCl_2$ - 0.1M	.01 →							
✓ Thymus DNA 0.5 mg/ml	.02 →							
✓ Lyophilized Enz (1:24) 7/17	—	.02						—
✓ AS-1 (1:48)			.02					.02
✓ AS-2 (1:48)				.02				.02
✓ AS-3 (1:48)					.02			.02
✓ AS-4 (1:48)						.02		.02
✓ AS-5 (1:48)							.02	.02
✓ water	.21	.19	.19	.19	.19	.19	.19	.11

V.f. = .30 ml →

30' 37°

BKg: 22/2, 22/2, mean = 11 cpm

St: 1156 − 11 = 1145

	1	2	3	4	5	6	7	8
cpm min:	111	413	127	178	210	193	109	440
cpm corr:	44	196	52	78	94	86	43	209
−44	—	152	8	34	50	42	0	165
mμM		.12	—	.026	.039	.083	—	

Preparation of Fractions:-

Ammonium Sulfate Fractionation.

2.0 ml Lyophilized Penzyme + 0.49 g AS. 5' 0° centrifuged. ppt dissolved in 1 ml M/20 Tris, 7.5 AS-1

To sup. was added 0.18 g AS. 5' 0°, centrifuged ppt dissolved as above AS-2

To sup. was added 0.20 g AS. 5' 0°, centrifuged ppt dissolved as above AS-3.

To sup. was added 0.37 g AS 5' 0° centrifuged ppt dissolved as above AS-4

To sup was added 0.3 ml M/1 NaAc pH 4.0 1 hr 0° centrifuged. ppt dissolved as above AS-5

Supernatant discarded

Protein Determinations

All samples dialyzed against 1% KCl, pH 7.4 and determined directly.

Sample	Vol	E_{660}	mg Protein	mg Protein/ml	Q
AS-1	.1	.170	38.7	.387	—
AS-2	.1	.315	71.6	.716	8.7
AS-3	.2	.255	58.0	.290	32.2
AS-4	.2	.530	120.0	.600	13.2
AS-5	.2	.120	27.3	.136	—
BSA std	.05	.220	50	—	
					10.0

Lyophilized Prep

total = 2.129

= 2.13 mg.

Purification of P Enzyme

7/20/52

	1	2	3	4	5	6
✓TTP (C^{14}) 1.29×10^6 cpm/µM, .55 µM/ml	.01 →					
✓DATP - 0.54 µM/ml	.01 →					
✓DCTP - 0.50 µM/ml	.01 →					
✓DGTP - 0.59 µM/ml	.01 →					
✓Glycine, M/1, 9.2	.02 →					
✓$MgCl_2$ - 0.1M	.01 →					
✓Thymus DNA - 0.5 mg/ml	.02 →					
✓Lyophilized P enz 7/17 (1:24)		.02				
✓AS-1 (1:48)			.02			.02
✓AS-2 (1:48)				.02		.02
✓AS-3 (1:48)					.02	.02
✓water	.21	.19	.19	.19	.19	.15

V.f. = .30 ml

30' 37°

Blq: 22/2, 19/2 means 11 cpm

St: 1161 - 11 = 1150

	1	2	3	4	5	6
cpm min:	122	348	158	221 / 202	119	336
cpm cor:	50	163	68	101 / 95	49	157
-50		113	18	51 / 45 } 48	0	107
mµM incorp		.09	.014	.037		

Preparation of Fractions: -

Ammonium Sulfate Fractionation

2 ml Lyophilized P enzyme + .63 g solid A.S. 5' 0° centrifuged. ppt dissolved in 1 ml M/20 Tris pH 7.5 AS-1.

To sup. was added .35 g solid AS. 5' 0° centrifuged. ppt dissolved in 1 ml M/20 Tris pH 7.5 AS-2

To sup was added .28 g solid AS. 5' 0°. centrifuged. ppt dissolved in 1 ml M/20 Tris pH 7.5 AS-3.

Supernatant discarded

Protein Determinations

Direct - on dialyzed solutions

Sample	Vol	E_{660}	% Protein	mg Protein/ml	Q
AS-1	.05	.265	63	1.26	2.1
AS-2	.10	.240	57	.57	15.6
AS-3	.10	.202	48	.48	—
BSA St	.05	.210	50		
				Total: 2.31	
Lyophilized Prep.					7.4