Preparation of TTP^{32} of High Specific Activity 10/4/52

2.5 g E coli cells (Summio 10/3) Au 10/2 S.A. = 4.1×10^7 cpm/μM. Transfered to 500 ml erlenmeyer with about 25 ml water. Then 100 ml Bloor's solution (3 parts 95% EtOH - 1 part anhydrous ether) added. Suspension brought to boil on steam bath, then boiled gently for 10 min. with swirling. Suspension filtered on Buchner (Whatman #1) with suction. Residue washed three times with 20 ml portions of Bloor's solution then 3, 20 ml portions of ether. Powder dried with air suction (5'). Dry powder suspended in 10 ml 1N NaOH 17 hrs 37°.
V.f. = 10.0

10/5/52

Precipitation of DNA:

Some undissolved material remaining in suspension. Centrifuged but centrifugation did not give any packing of the precipitate. 3 drops BTB added. 1.5 ml 35% cold PCA added with stirring. Indicator just ↑ yellow. Then 1.9 ml more PCA added with stirring. Copious precipitate formed. final PCA conc. ca 5%, centrifuged immediately. Supernatant poured off Vol = 13.4 ml. Supernatant saved.
precipitate suspended in 5 ml cold H_2O — 1.0 ml 1N NaOH added. pH ~ 9. most of ppt dissolves approx 0.2 ml cold 7% PCA added to bring pH to approx 8.5. Stand in ice ~20' V.f. = 6.2 ml. Somewhat Alkaline suspension centrifuged 20' at setting of #50 in Servall. Supernatant drawn off. 1 ml H_2O added to ppt, centrifuged ppt did not pack properly. Sup drawn off. This was repeated. The two water extracts combined with supernatant. V.f. = 6.5 ml. To poorly packed precipitate was added 0.1 ml 2N NaOH ppt rubbed up, solumentation not possible 0.1 ml 1N HCl added to pH ~ 6. V.f. ~ 2 ml - saved.

Diphenylamine test

Sample	ml	water	Diphenylamine	1:96		μM	μM/ml	μM total
DBTSP 1 μM/ml	.10	.20	1.0	.267	2.78 D.U./μM			
" " "	.05	.25	↓	.145				
Alkaline susp.	.02	.28		.134		.048	2.40	24.0
Dissolved PCA ppt	.02	.28		.175		.063	3.15	19.5
PCA sup	.10	.20		.032		.015	.15	2.0
Blank		.30						

Counts:

Samples diluted 1/1000

Sample	Vol counted			
PCA sup	.01	7393	$\times 10^5 \times 13.4 =$	9.8×10^9 cpm total
PCA ppt	.05	3196	$\times 10^5 \times 6.5 =$	20.8×10^8 "
undissolved residue	.05	8782	$\times 2 \times 10^4 \times 2 =$	3.6×10^8

1.23×10^{10} cpm total.

expected: 1.33×10^{10} (calculated from S.A. of P_i of growth medium & uptake by the cells).

1st Reprecipitation of DNA

To dissolved PCA ppt - 0.7 ml 35% PCA added, ppt centrifuged immediately. Supernatant saved. ppt washed with 6 ml cold water. Precipitate suspended in 5 ml cold water. 0.1 ml N/1 NaOH added to pH ~8.0 almost all of precipitate dissolved.

Diesche Hots

Sample	vol	water	Δ596	μM	μM/ml	total μM
Blank	–	.30	–			
.1mM ATP	.1 ml (1mM/l)	.20	.262	2.780/μM		
.05μM "	.05	.25	.147			
PCA sup	.20	.10	.009			
PCA wash	.20	.10	.006			
Redissolved ppt	.02	.28	.185	.067	3.35	17.4
Undissolved residue (above)	.05	.25	.187	.067	1.34	2.7

∴ including the 2.7 μM with the 19.5 μM in the redissolved PCA ppt + 2.0 μM in the PCA sup. this gives a recovery of 24.2 μM Deoxyadenosine out of the 24.0 μM present in the whole alkaline digest.

Counts Samples diluted 1/1000

Bkg: 26/2

Sample	Vol. counted	
redissolved ppt	.01	$3355 \times 5 \times 5.2 = 1.75 \times 10^9$
PCA sup	.05	$2052 \times 20 \times 10^3 \times 7.2 = 2.9 \times 10^8$
PCA wash	.05	$393 \times 20 \times 10^3 \times 6.0 = 4.7 \times 10^7$

St: 1067

2nd reprecipitation of DNA

0.7 ml 35% PCA added to redissolved ppt. 5' 0° ppt centrifuged. supe. poured off. ppt washed with 6 ml cold water. ppt suspended in 5 ml cold water 0.16 ml 1.0 N NaOH added to pH ~7.5. precipitate dissolves completely.

Counts: – sample dil 1/100

Blg: 35/2
St: 1036

Sample	vol		
Redissolved ppt	.01	2684 / 2801	$2743 \times 10^5 \times 5.2 = 1.4 \times 10^9$ cpm
PCA supe.	.05	72 / 60	$70 \times 10^3 \times 20 \times 6.0 = 8.4 \times 10^6$
~~PCA~~ wash	.05	48	$48 \times 10^3 \times 20 \times 6.0 = 5.8 \times 10^6$

Dilute:

Sample	Vol	water	λ96		μM	μM/sol	μM total
Blank.		.30					
0.1 μM ASP	μMolut .1 ml	.20	.280	2.78 D.U./μM			
0.05 μM ASP	.05	.25	.137				
redissolved ppt	.02	.28	.162		.060	3.00	15.6

Specific activity $\frac{1.4 \times 10^9}{31.2 \mu M} = 4.5 \times 10^7$ cpm/μM.

DNA solution frozen up overnight

10/6/56

DNAase Digestion of P^{32} Labeled Ecoli DNA.

Col DNA	5.20 ml
DNAase Worthington 1x crystl. 5mg/ml 7/9/56	.15
Tris M/1, 7.5	.20
$MgCl_2$ - 0.1M	.25
V.f.	5.80

2hr 37°. Some precipitate formed during incubation

Test for completeness of digestion:
samples removed at 0' and 2hr, treated as follows:

Sample	0'	2hr	thymus DNA control
thymus DNA - 5 mg/ml	.04	.04	.04
H_2O	.13	.13	.13
7% PCA	.20	.20	.20
Digest	.03	.03	—

5', 0° centrifuged, sup. poured off and tested for Dische and counts.

Dische:-

Sample		vol	water	λ596		μM
1	Blank	—	.30	—		
2	.1 μM ASP	.10 μm ASP/ml	.20	.270	2.73 D.U./μM	
3	.05 " "	.05	.25	.138		
4	0'	.20	.10	.007		
5	2hr	.20	.10	.110		$.040 \times 5 \times \frac{.4}{.03} \times 5.8 =$ 15.6 μM total
6	thymus DNA control	.20	.10	.003		

counts:

2hr dil 1/100 - 0.01 plated - $\frac{1135}{1181}$ $\times 10^4 \times \frac{.4}{.03} \times 5.8 = 9.0 \times 10^8$ cpm (this is low).

0' undil - 0.01 plated $1104 \times 10^2 \times \frac{.4}{.03} \times 5.8 = 8.5 \times 10^6$ cpm

Digest frozen until next week

Assay of Monoamine (Heppel) 10/6/56

Human semen monoamine from Heppel 5-29 $A_{\frac{1}{5}H}$ ~ 8000 U/ml diluted .02/2.0 *

	1	2	3	4	5	6
K Thymidylate 14 µM/ml	.10 →					
$MgCl_2$ - 0.3 M	.03 →					
NaAc pH 5.0 M/1	.10 →					
monoamine .05/1.0 *	.01	.02	.05			—
Diaminase (Kornberg) Acetone-3 *				.02		—
Diaminase (Kaplan II) see Kornberg note					.10	—
water	.26	.25	.22	.23	.17	.27
V.f. =	.50 →		20' 37°			

then following additions made:-

$5N H_2SO_4$	.2 →					
water	1.0 →					
2.5% Molybdate	.2 →					
Reducer	.1 →					
V.f.	2.0	15'	R.T.			
λ 700	.288	.556	1.105	.043 slightly turbid	.034	.021
corr for P_i	.267	.535	1.084	.022	.015	
MMP: split 20'	.127	.255	.516	.011	.007	
" " final assay	2540	2550	7064	.275	.07	
U/ml	7658	7655	6700	.83	.21	

1 µMP - St - .208 } mean = .210 per 0.1 µM
.05 µMP St - .106 }

* Dilutions in 1/10 Tris pH 7.5.

Assay of Deoxyribonucleases

10/6/52

	1	2	3	4	5	6	7
Glycine M/1 8.5	.03 →						
$MgCl_2$ - 0.3 M	.03 →						
DNAase Digest 10/4	.10 →						
Acetone 3 (10/2) (1:10) Kornberg	.02	.04	.08				
Kaplan II (10/4)	—	—	—	.01	.02	.04	—
water	.32	.30	.24	.33	.32	.30	.34

V.f. = .50 → 20' 37°

following additions made:

NaAc, pH 5.0 M/1	.10 →
1N HCl	.01 →
Monoxidase ~40 U/ml	.10 →
water	.04 →
V.f.	.75 → 20' 37°

following additions made:

5N H_2SO_4	.20 →
water	.75 →
2.5% Molyden.	.20 →
Reducer	.10 →
V.f.	2.0 15' R.T.

λ 700 mμ	.820	.840	.860	.307	.450	.600	.097

0.1 μM St: .207

.05 μM St: .107

mean = .210 per 0.1 μM

too much enzyme used - Acetone-3 at (1:10) in volumes added gives complete splitting.

10/6/58

Repeat Diesterase Assays (lower enzyme levels)

	1	2	3	4	5	6
Glycine pH 8.5	.03 →					
$MgCl_2$ – 0.3 M	.03 →					
DNAase Digest	.10 →					
Acetone 3 (1:40)	.01	.02	.04	–	–	–
Kaplan II (1:2)				.01	.02	–
water	.33	.32	.30	.33	.32	.34
V.f. = .50 →		25', 37°				

following additions made:-

IN HCl	.01 →	
1M NaAc pH 5.0	.10 →	
manganese 100 µg/ml	.10 →	
water	.04 →	
V.f. = .75 →		20', 37°

following additions made:-

$1N H_2SO_4$	.20 →	
water	.75 →	
25% Polydek	.20 →	
Reducer	.10 →	
V.f. 2.0	15', R.T.	

λ_{700}	.324	.435	.615	.193	.300	.088	0.1µM St: .210
corr	.236	.347	.527	.105	.212	–	0.02µM St: .106
0.1µM µM P_i split 20'	.112	.166	.250	.050	.100		
" " 60'	.336	.500	.750	.150	.300		
U/ml	1344	–	–	30			

Assay of Monoesterase (Heppel) for Diesterase Activity

10/9/56

	1	2	3	4
NaAc M/1, pH 5.0	.10 →			
DNAase Digest	.10 →			
Monoesterase 40 U/ml (10/6)	.02	.02	.10	.20
$MgCl_2$ - 0.3 M	.03 →			
water	.25	.22	.17	.07
V.f. =	.50 →			

20' 37°

following additions made :-

5 N H_2SO_4	.20 →			
2.5% Molybdate	.20 →			
Reducer	.10 →			
water	1.00 →			
V.f.	2.00	20' R.T.		
λ 700	.060	.072	.087	.103
μM P_i split	.028	.034	.041	.048

0.1 μM P_i St: .214

Diesterase Digestion of DNAase Digest

10/8/12

DNAase digest 10/4 transfered to graduated tube, tube washed out with cold water. pH adjusted to ~8.5 with 0.1N NaOH (0.3 ml). final volume 10.0 ml.

The following reaction mixture set up:

DNAase Digest:		10.00 ~30 μM nucleotide
$MgCl_2$ - 0.3M		1.80
Glycerol M/1, 8.5		1.80
Diesterase - Acetone-3 (10/2)		.06
Water		16.34
	V.f.	30.00

2 hr 37° then placed in ice bath

Assay for completeness of diesterase action:-

	1	2
Digest	.10	.10
Monoesterase 500γ/ml	.01	—
Na Ac M/1, pH 5.0	.10	.10
$MgCl_2$ - 0.3M	.01	.01
V.f	.22	.21

20' 37°

Then 0.2 ml 20% TCA added 5', 0° centrifuged.

Ortho P test

TCA supp.	.25	.25
5N H_2SO_4	.20	.20
2.5% Molybdate	.20	.20
water	1.25	1.25
reducer	.10	.10
X 700	.117	.006
μM P_i split	.056	.003

0.1 μM P_i SV: 1/10

Δ = .053 ; .053 x .43/.25 x 30 x 10 = 26.6 μM total of nucleotide