Con. A sepharose column binding 4/10/82.
of glycosylated DNA.

(A) Effect of Mannose +
(B) Effect of non-glyosylated DNA.

^3H T_H DNA 2.5×10^5 cpm per microgram
^{14}C E. coli DNA 1.1×10^4 cpm per microgram.

d-Mannose 50 micrograms per ml.

Con-A sepharose 18 mg con A bound per ml of settled gel

Columns.

Con-A sepharose columns 10 mg conA.
0.6 ml settled gel
washed extensively with 1% Triton-X-100
~ 10 column volumes to remove stabilizer
Equilibrated with 20 column volumes
of PBS 0.01 M K-PO_4 pH 7.2
0.15 M NaCl
containing 10 mM $MgCl_2$

Aliquots of
4 micrograms ^3H T4 DNA
~ 1×10^6 ^3H cpm.
mixed $\bar{c}$
(A) PBS + Mg^{++} 0.2 ml
(B) d-Mannose 0.2 ml 4 micrograms.
(C) ^{14}C E. coli DNA 0.2 ml 4 micrograms
~ 4.4×10^4 ^{14}C cpm

Each aliquot fractionated on con-A sepharose columns as in earlier expts.

0.2 ml fractions collected
100 fractions.
Radioactivity checked.

plotted ~~as~~ column elution volume vs % of Input

^3H T_4 DNA on con A sepharose — Total volume (0.5 ml)
(c) Effect of ^{14}C E. coli DNA

	FR #	^{14}C cpm per Aliquot	Total	%	^3H cpm per aliquot	Total	%
	Sample (5λ)	400	40000	100	9560	956000	100
	Sample (5λ) Eluate	130	13000	32.5	10		> 0.01
	PBS + Mg Cl₂						
200 λ	1	11600	11600	29	10		> 0.01
	2	7600	7600	19	20		
	3	4000	4000	10	10		
	4	2400	2400	6	10		
	5	1200	1200	3	10		
	6	400	400	1	10		
	7	180		0.45	10		
	8	60		0.15	15		
	9	30		0.075	10		
	10	40		0.1	8		
	1	60		0.15	10		
	2	30		0.075	6		
	3	20		0.05	10		
	4	10		0.025	10		
	5	15		0.03	6		
	6	20		0.05	5		
	7	15		0.03	8		
	8	28		0.04	10		
	9	10		0.025	8		
	20	8			10		
	1	18			10		
	2	10			10		
	3	20			20		
	4	30			10		
	5	60			10		
	6	30			10		
	7	30			8		
	8	60			6		
	9	16			8		
	30	5			2		
	1	28			10		
	2	28			10		
	3	80			10		
	4	16			10		
	5	30			10		
	6	20			10		
	7	10			10		

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^3H T_4 DNA on con A sepharose (0.5 ml Total Volume)

(B) Effect of α Mannose.

FR #	^3H cpm per aliquot	Total	% Input
	(5λ)		
Sample	9880	988000	100
	5λ		
Sample wash	4610	461000	46.7
PBS+Mg^{++}	5λ		
1	2470	247000	25.00
2	1285	128440	13.00
(100λ) 3	34086	68172	6.9
(100λ) 4	9880	19760	2.00
(100λ) 5	24700	49400	5.00
(200λ) 6	1620	1620	0.164
7	860	860	0.087
8	220	220	0.022
9	110	110	0.011
10	36		> 0.01
11	40		
12	33		
13	20		
14	21		
15	30		
16	56		
17	30		
18	11		
19	60		
20	16		
1	5		
2	10		
3	18		
4	13		
5	12		
6	13		
7	14		
8	13		
9	13		
30	13		
1	12		
2	10		
3	20		
4	25		
5	30		
6	36		
7	38		
8	30		
9	38		
40	40		

FR #	^3H cpm	Total % Input
41	23	> 0.001 %
45	20	
50	10	
55	5	
60	8	
65	2	
70	3	
75	5	
80	8	
85	10	
90	3	
100	8	
Tris pH 8.2		
101	6	> 0.001
2	8	
3	10	
4	3	
5	15	
6	8	
7	10	
8	3	
9	3	
110	3	
1	8	
2	10	
3	20	
4	30	
5	5	
6	6	
7	3	
8	2	
9	3	
120	1	

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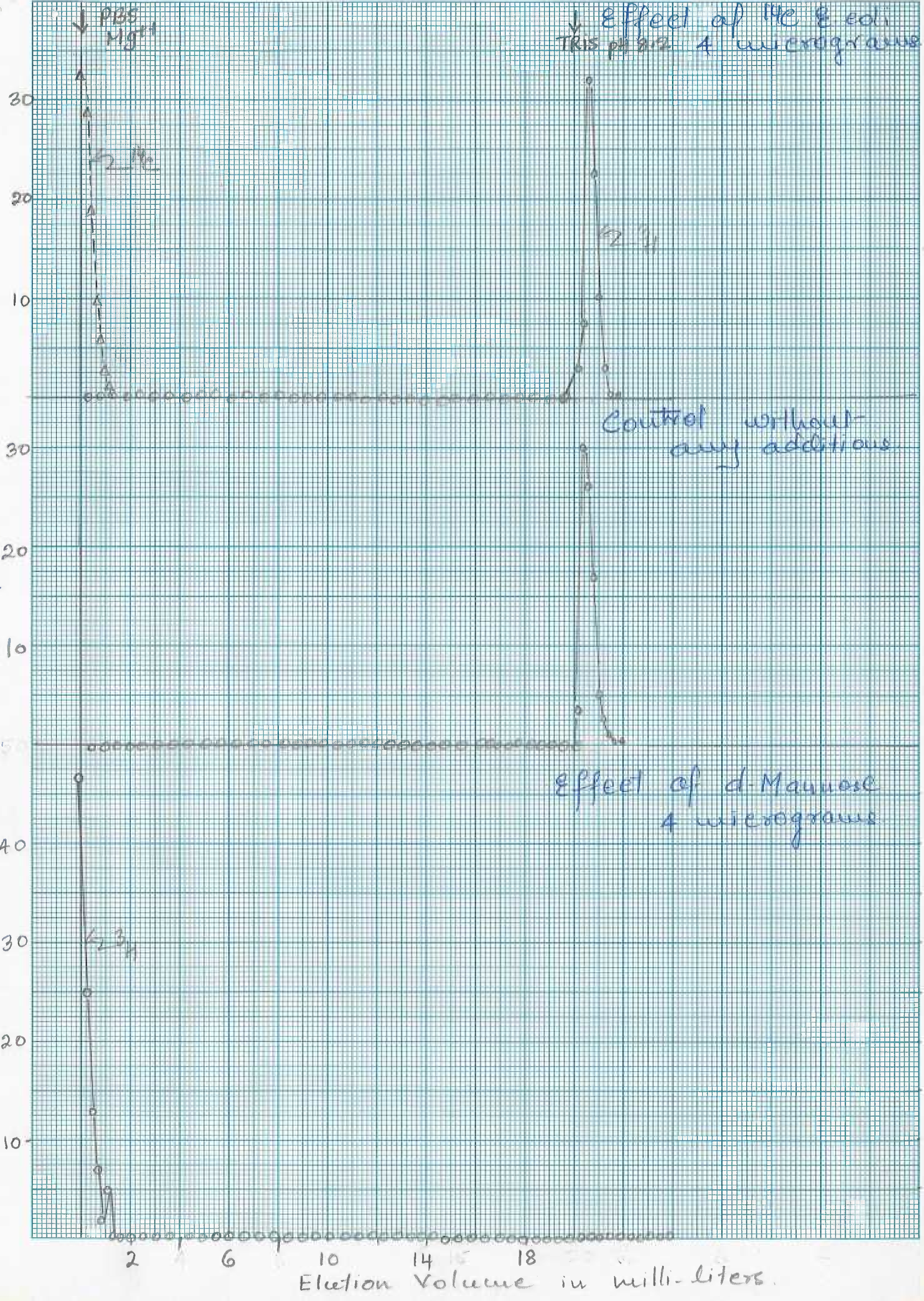
(C)

FR #	^{14}C cpm Aliquot	Total	% Input	^{3}H cpm Aliquot	Total	% Input
40	20		> 0.001	10		> 0.001
45	10			18		
50	20			16		
55	15			15		
60	30			19		
65	10			17		
70	30			16		
75	8			15		
80	6			12		
85	3			11		
90	2			10		
100	1					
Tris pH 8.2						
101	3		> 0.001		28680	3
2	2				71700	7.5
3	0				308788	32.3
4	3				215100	22.5
5	0				95600	10.0
6	5				27724	2.9
7	2				4780	0.5
8	0					
9	0					
110	2					
1	1					
2	0					
3	20					
4	0					
5	0					
6	30					
7	0					
8	3					
9	0					
120	0					

^3H T4 DNA on con A sepharose (0.6 ml columns)



K+E 10 X 10 TO THE CENTIMETER 18 X 25 CM. KEUFFEL & ESSER CO. MADE IN U.S.A. 46 1521

5/25/82.

Denaturation of DNA   0.3 M NaOH 10' RT.
chill

T4 DNA 200 γ/ul in 10 mM NaCl
10 mM Mg^{++}

1. 25 λ DNA
112.5 λ NaCl Mg^{++}
18.75 λ 2.0 M NaOH

2. 125 λ DNA
18.75 λ 2.0 M NaOH.

3. 500 λ DNA 50 γ/ul
18.75 λ 2.0 M NaOH

λ p lac DNA 515 γ/ul.

4. 4 λ DNA
121 λ NaCl Mg^{++}
18.75 λ 2.0M NaOH

5. 20 λ DNA
105 λ NaCl Mg^{++}
18.75 λ 2.0 M NaOH

(6). 500 γ DNA 50 γ/ul
18.75 λ 2.0M NaOH

10' at RT.
chill dilute c̄
equal vol. 10X SSC.

Dot blot experiments. 5/25/82.

Nitrocellulose filters S & S
47 mm size. 0.45 microns.

① wash the filters in H_2O
② " " " in 5 x SSC
③ Dry air.
④ T4 and λplac DNA A_{260} 1.0 ~ 50 micrograms per ml in 10 mM NaCl
10 mM Mg^{++}

denatured by incubating in boiling HOH bath for 5'

chilled immediately to keep them denatured.

⑤ ~~Na~~ 20 x SSC added to DNA solutions to bring SSC concentrations to 5 x SSC
⑥ applied to washed filters.

T4	NaCl Mg^{++}	λplac
1λ	1λ	1λ
1.5 λ	1.5 λ	1.5 λ
2.0 λ	2.0 λ	2.0 λ
2.5 λ	2.5 λ	2.5 λ

⑦ Filter was then air-dried

(8) Filter washed with 5 x SSC

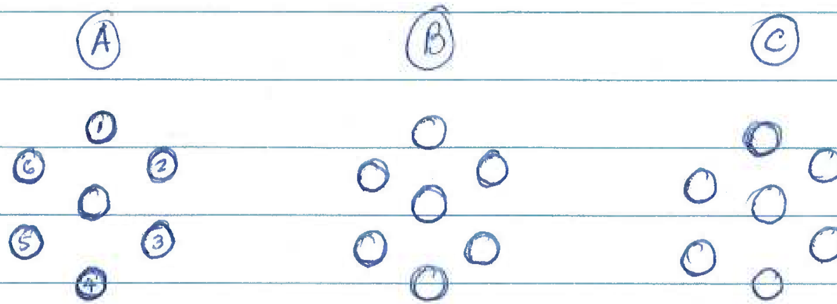
(9) " baked at 80° for 2 hours.

(10) " blocked c̄ 3% BSA in 2x SSC
at 37° for 2 hours in sealed ~~tube~~ bag.

(11) Washed c̄ 0.2 mg con A in 5.0 ml 5x SSC
containing 20 mM Mg^{++}
incubated at R.T. for 30 - 60 minutes.

(12)

Detection by Ochterlony techniques. 5/18/82.



Center Well

Con A	1 mg/ml.	A
	2.5 mg/ml	B
	7.5 mg/ml.	c

(2.30 pm.)

1 PBS

2 Mannose

3 Maltose

4 λ plac DNA

5 T_4 DNA

6 ATP

left at 4° in humid atmosphere for 42 hrs.

4.8.82.

DNA binding to con-A sepharose columns.

^{3}HT4 DNA   2.5 x 10^5 cpm per microgram.
^{14}C E. coli DNA   1.1 x 10^4 cpm per microgram.

Con-A sepharose   18 mg con A bound per ml of settled gel.

Columns.

Con-A separose columns
0.6 ml settled gel ~ 10 mg con A
washed with 1% Triton-X-100
10 column volumes to remove the stabilizer.
Equilibrated with 10-20 column volumes of PBS   0.01 M KPO_4 pH 7.2
0.15 M NaCl
containing 10 mM. $MgCl_2$

2-5 micrograms DNA in 100 microliters of 10 mM TRIS. NaCl pH 7.2
applied to the column.
Column washed extensively with equilibrating

buffer ~ 30 column volumes
~ 20 ml.

0.2 ml fractions collected
aliquots checked for radioactivity.

Elution continued with ~~high pH buff~~
(a) high pH buffer Tris. NaCl pH 8.2
(b) mannose containing PBS-Mg^{++} buffer

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^{3}H T4 DNA on Con A sepharose.

FR #	^{3}H cpm	Total		FR #	^{3}H cpm
	(10λ)	x ~~100~~			
Sample	2136	~~427~~ 4272 00		43	10
				4	3
				5	4
Sample Eluate	194	1940		6	3
				7	2
				8	3
PBS-Mg^{++} wash				9	3
				50	2
				1	3
1	5			2	6
2	6			3	20
3	0			4	3
4	3			5	8
5	2			6	18
6	8			7	10
7	6			8	7
8	5			9	6
9	8			60	3
10	6			1	6
1	5			2	3
2	8			3	3
3	3			4	5
4	4			5	6
5	5			6	2
6	8			7	10
7	9			8	5
8	3			9	30
9	2			70	6
20	1			1	3
1	5			2	7
2	6			3	8
3	8			4	3
4	9			5	9
5	10			6	10
6	3			7	0
7	7			8	3
8	6			9	4
9	5			80	3
30	6			1	8
1	3			2	13
2	4			3	33
3	3			4	3
4	2			5	20
5	0			6	21
6	3			7	3
7	1			8	15
8	2			9	18
9	3			90	3
40	3			95	4
1	5			100	5
2	10				

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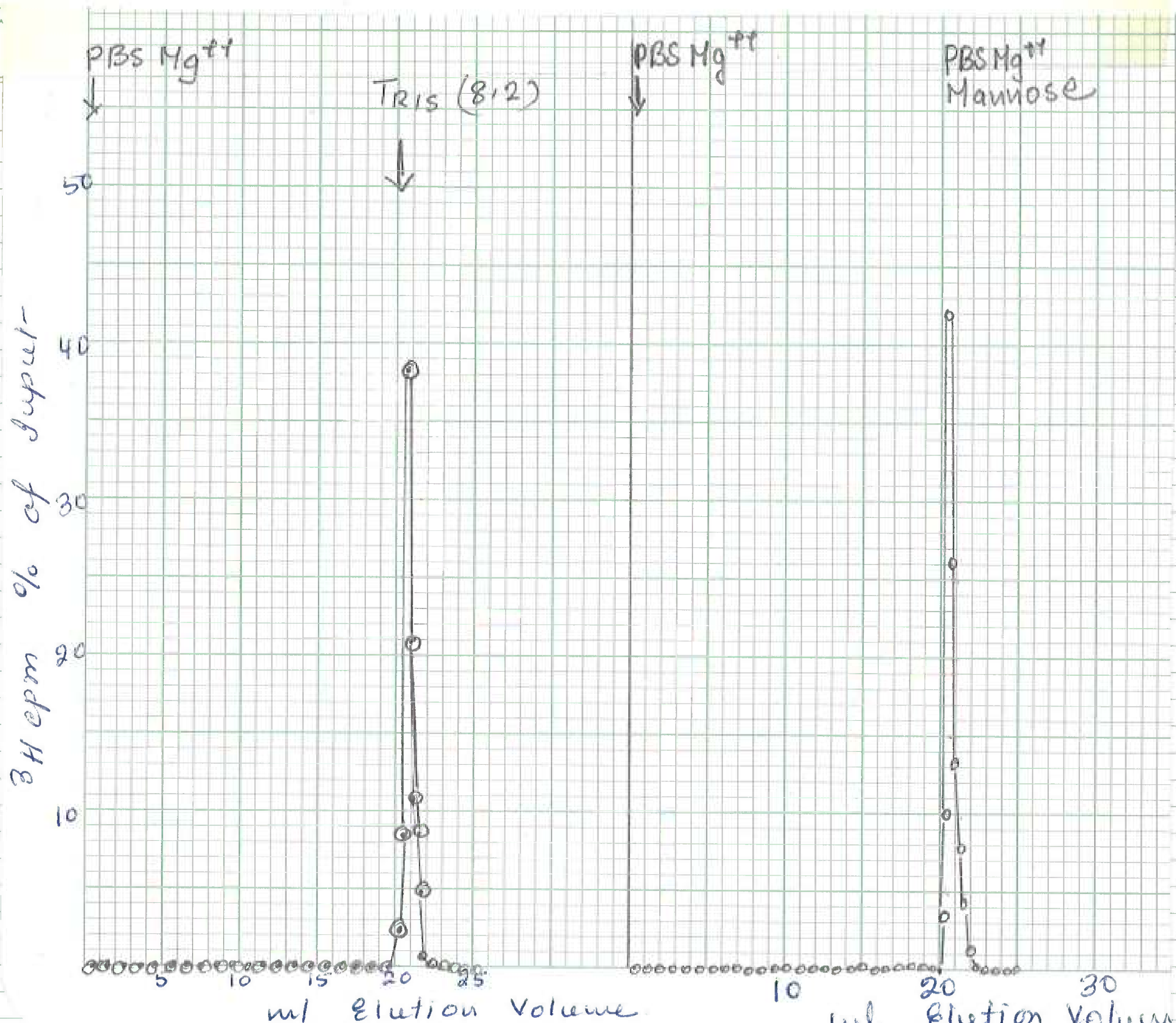


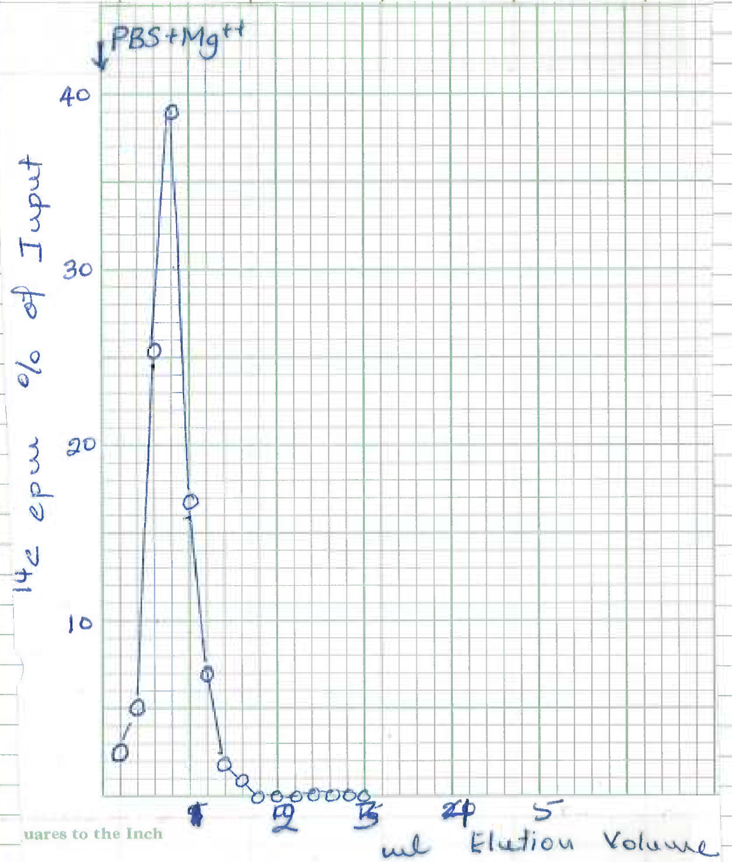
4	5613	112260	26.28
5	2923	58460	13.68
6	1705	34100	7.98
7	983	19660	4.60
8	310	6200	1.45
9	39	780	0.18
10	23	460	0.11
11	11	220	0.052
12	3		
13	3		
14	10		

FR #	^3H cpm 10x	Total	% Input
Tris 8.2 wash			
1	561	11220	2.63
2	1843	36860	8.63
3	8190	163800	38.34
4	4486	89720	21.00
5	2328	46560	10.9
6	1859	37170	8.7
7	1053	21060	4.93
8	200	4000	0.94
9	38	760	0.18
10	4	80	0.019

FR #	^3H cpm	Total	% Input
Mannose 2 micrograms in PBS+Mg^{++} wash.			
1	765	15300	3.58
2	2238	44760	10.48
3	9056	181120	42.397
4	5613	112260	26.28
5	2923	58460	13.68
6	1705	34100	7.98
7	983	19660	4.60
8	310	6200	1.45
9	39	780	0.18
10	23	460	0.11
11	11	220	0.052
12	3		
13	3		
14	10		

^{14}C E. coli DNA on Con A Sepharose

FR #	^{14}C cpm per Aliquot	Total	% Input
	(10λ)		
Sample	229	22900	100 %
	(200λ)		
Sample Eluate	448	448	1.96
	(200λ)		
PBS+Mg⁺⁺ wash			
1	613	613	2.68
2	1212	1212	5.29
3	5865	5865	25.61
4	8954	8954	39.10
5	3870	3870	16.90
6	1603	1603	7.00
7	458	458	2.00
8	241	241	1.05
9	36	36	
10	16	16	
1	10	10	
2	11	11	
3	36	36	
4	25	25	
5	13	13	
6	14	14	
7	30	30	
8	26	26	
9	15	15	
20	18	18	
1	10	10	
2	36	36	
3	30	30	
4	31	31	
5	21	21	
6	3	3	
7	5	5	
8	3	3	
9	2	2	
30	3	3	
1	3	3	
2	0	0	
3	2	2	
4	5	5	
5	10	10	



4.6.82.

Concanavallin A precipitation of radioactivity from ^3H Thy labelled T4 DNA.
Comparison with ^{14}C labelled non-glycosylated DNA

DNA

(a) ^3H(Thy) T4 DNA from ^3H labelled T4 phage T4 am 82 (44⁻62⁻)
260 micrograms per ml in
10 mM Tris pH 7.2
10 mM NaCl
Sp act. 2.5×10^5 cpm per microgram

(b) ^{14}C labelled E. coli DNA
hi mol wt 454 micrograms per ml in 10 mM Tris pH 7.2
10 mM NaCl
Sp. act 1.1×10^4 cpm per microgram.

Concanavallin A 20.0 mg per ml in 2.0 M NaCl solution.

4.6.82

Incubation mixtures of 0.5 ml consisted of

NaCl 1.0 M

PO_4 0.01 M pH 7.2

^3H or ^{14}C DNA 1 – 10 micrograms.

Con A 1 – 6 mgm.

Reactions started by adding con A to assay mixtures containing DNA mixed well by vortexing

Incubated at 25° [R.T.) for 10 minutes

Centrifuged and radioactivity determined on aliquots of supernatee.

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4.6.82

DNA		con A	^3H cpm ~~per aliquot~~ Supernatant per aliquot 10λ	Total	% non ppted
^3H T4 DNA	2	0	1650	82500	100
	4	0	3490	174500	100
	6	0	5000	250000	100
	8	0	7135	356750	100
	10	0	10,030	501500	100
	2	1 mg	48	2400	2.9
	4	↓	156	7800	4.46
	6		235	11750	4.7
	8		410	20500	5.7
	10		636	31800	6.3
	2	2 mg	23	1150	1.39
	4	↓	54	2700	1.5
	6		68	3400	1.36
	8		120	6000	1.68
	10		256	12800	2.55
			(100λ aliquots)		
	2	4 mg	22	110	0.13
	4	↓	38	190	0.11
	6		49	245	0.098
	8		65	325	0.091
	10		75	375	0.074
	2	6 mg	36	180	0.218
	4	↓	65	325	0.186
	6		83	415	0.17
	8		120	600	0.168
	10		195	975	0.194

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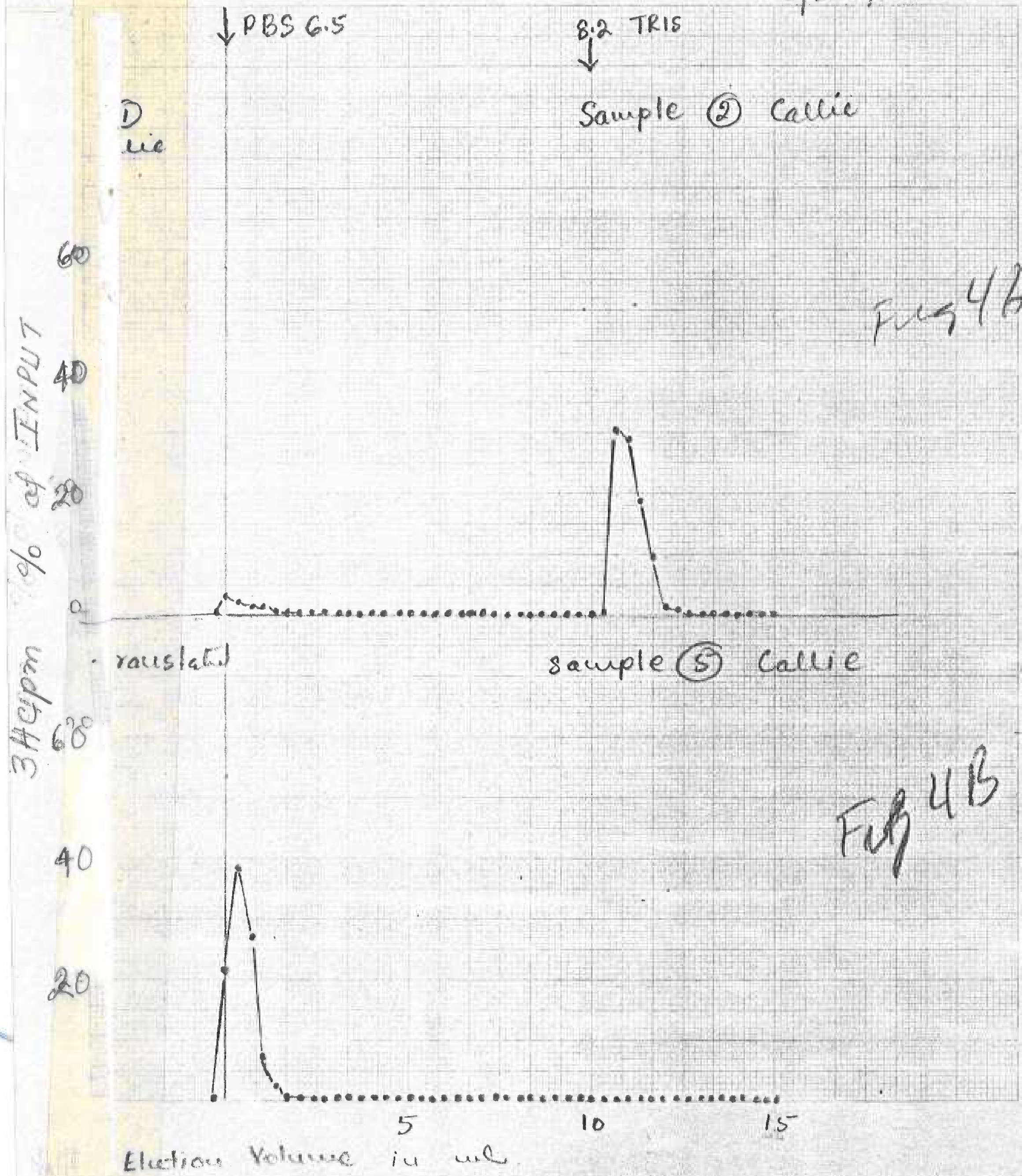
DNA		Con A	Radioactivity in Supernatant per aliquot (10λ)	Total
^{14}C E. coli DNA	2 Microgram.	0	250	12500
	4	↓	386	19300
	6		800	40000
	8		966	48300
	10		1050	52500
	2	1 mgm	248	12400
	4	↓	350	17500
	6		900	45000
	8		958	47900
	10		1090	54500
	2	2 mgm.	256	12800
	4	↓	386	19300
	6		795	39700
	8		980	49000
	10		1100	55000
	2	4 mgm	245	12250
	4	↓	354	17700
	6		810	40500
	8		975	48750
	10		1076	53800
	2	6 mgm	256	12800
	4	↓	400	20000
	6		754	37700
	8		1011	50550
	10		1210	60500


Con-A Sepharose Binding of glucosylated DNAs
3/8-9/82
PBS 6.5
8.2 TRIS
Sample ② Callie
Fig 4A
3H cpm % of INPUT
60
40
20
0
sample ⑤ Callie
Fig 4B
60
40
20
5
10
15
Elution Volume in ml

Con. A sepharose column 3/8/82.
Binding of glycosylated DNAs.

DNAs.

① ^{3}HT λ DNA nick translated 2/26/82.

② Callie's sample I 10λ

③

④

1 41
001.00
003.00
005594.00

1 65
001.00
003.00
006848.00

DNA sample preparations

I
- 100 λ DNA nick translated λ DNA.
- 2.5 λ $MgSO_4 \cdot 7H_2O$
- 5 λ $CaCl_2$.
- 392.5 λ PBS (6.5)

0.5 ml
10λ for counting.

II
- 100 λ DNA Callie sample I
- 2.5 λ $MgSO_4 \cdot 7H_2O$
- 5 λ $CaCl_2$.
- 392.5 λ PBS (6.5)

0.5 ml
10λ for counting.

III
- 100 λ DNA Callie Sample II
- 2.5 λ $MgSO_4 \cdot 7H_2O$
- 5 λ $CaCl_2$
- 392.5 λ PBS (6.5)

0.5 ml
10λ for counting.

IV
- 100 λ DNA Callie sample ⑤
- 2.5 λ $MgSO_4$
- 5 λ $CaCl_2$
- 392.5 λ PBS (6.5)

0.5 ml
10λ for counting.

3/8-9/82.

Con A- sepharose columns.

1.0 ml column equilibrated c̄ PBS (6.5)
50 - 100 ml
DNA sample applied Eluate collected
Aliquot of 0.5 ml PBS 6.5 used to drive
the sample into the gel. Eluate collected.
Column washed with 10-15 ml PBS (6.5)
0.33 ml fractions collected - 30 fractions
Column was then eluted with 10 mM
Tris (8.2) 10 mM NaCl 5-7 ml.
Fractions collected.

All fractions counted using 3.5 ml
Reafluor.

Results expressed as counts percent
of input vs elution volume.

1 01
001.00
005.00
002226.00
1 02
001.00
015.00
000346.00
1 03
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001.00
050569.00
1 04
001.00
001.50
025009.00
1 05
001.00
005.00
002363.00
1 06
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010.00
000512.00
1 07
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1 08
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020.00
000105.00
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080.00
000088.00
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000094.00
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020.00
000105.00
1 84
001.00
080.00

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000969.00
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000354.00
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000784.00
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000349.00
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015.00
000235.00
1 13
001.00
020.00
000189.00
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000134.00
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000122.00
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020.00
000116.00
1 17
001.00
020.00
000131.00
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001.00
020.00
000109.00
1 19
001.00
080.00
000098.00
1 20
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000084.00
1 21
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080.00
000075.00
1 22
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1 03
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003.00
006874.00
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000632.00
1 06
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000157.00
1 07
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003.00
006864.00
1 09
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002128.00
1 10
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000587.00
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015.00
000285.00
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020.00
000140.00
1 16
001.00
080.00
000098.00
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4,023.5
Total = 563,290

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x 140
1609400
40235
563,290.0

DNA sample I – Known negative control
3HTTP - nick-translated λ DNA
2/26/82.

	FR #	3H cpm	% Input		FR #	3H cpm	% Input
original	10λ	2226			28	57	0.051
	x 50	111300	100 %		29	44	0.04
	S. Eluate	346	0.31		30	52	0.047
	W. Eluate	50569	45.44		TRIS (8.2)		
	PBS # 1	25009	22.47		31	32	0.029
	2	4556	4.09		32	38	0.034
	3	2733	2.46		33	78	0.07
	4	2363	2.12		34	71	0.064
	5	713	0.64		35	45	0.04
	6	512	0.46		36	44	0.04
	7	372	0.33		37	19	0.017
	8	312	0.28		38	16	0.014
	9	252	0.23		39	28	0.025
	10	210	0.19		40	16	0.014
	11	155	0.14		41	24	0.022
	12	134	0.12		42	16	0.014
	13	110	0.099				
	14	109	0.098				
	15	98	0.088				
	16	96	0.086				
	17	65	0.058				
	18	70	0.063				
	19	62	0.056				
	20	58	0.052				
	21	66	0.059				
	22	62	0.056				
	23	53	0.048				
	24	53	0.048				
	25	77	0.069				
	26	53	0.048				
	27	49	0.044				

DNA Sample II [# 1 of Callie]

	FR #	^{3}Hepm	% Input
Original 10x		4490	
Column Samp 10x		727	
	x50	36350	100
	S.E	201	0.55
	W.E	3753	10.32
	PBS #1	4048	11.14
	2	1629	4.48
	3	1018	2.8
	4	620	1.71
	5	468	1.29
	6	224	0.62
	7	165	0.45
	8	157	0.43
	9	135	0.37
	10	135	0.37
	11	104	0.29
	12	82	0.23
	13	105	0.29
	14	88	0.24
	15	94	0.26
	16	105	0.29
	17	85	0.23
	18	75	0.21
	19	75	0.21
	20	90	0.25
	21	69	0.19
	22	77	0.21
	23	69	0.19
	24	74	0.20
	25	63	0.17

FR #	^{3}Hepm	% Input
# 26	63	0.17
27	54	0.15
28	67	0.18
29	49	0.13
30	56	0.15
TRIS (8.2)		
31	33	0.091
32	1820	5.01
33	11058	30.42
34	2364	6.5
35	715	1.97
36	373	1.03
37	244	0.67
38	181	0.50
39	124	0.34
40	104	0.29
41	75	0.21
42	55	0.15

DNA Sample III [#2 of Callie]

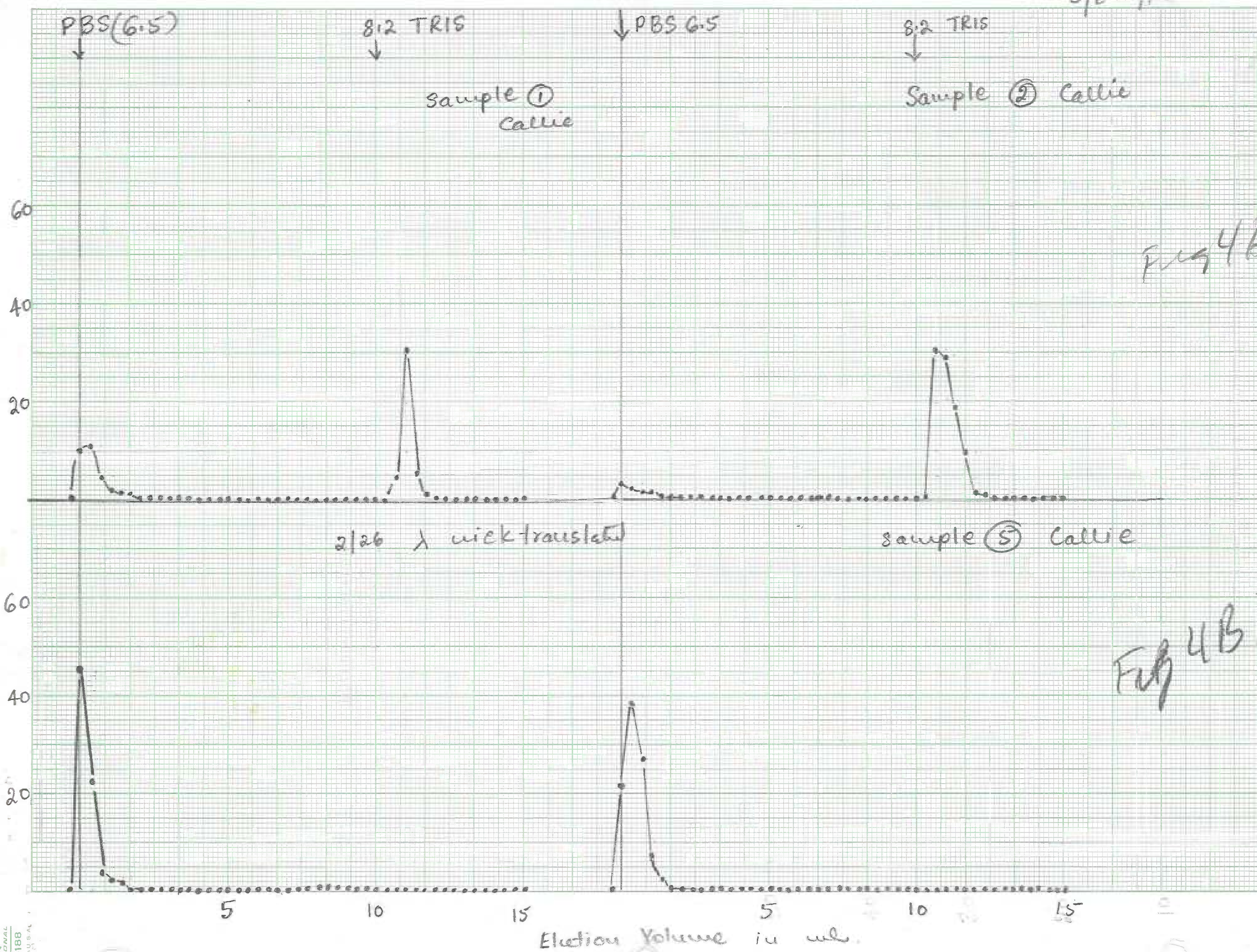
3/9/82

FR #	^{3}Hepm	% Input	FR #	^{3}Hepm	% Input
Original 10λ	5125		27	47	0.098
Sample 10λ	969		28	66	0.14
XS0	48450	100	29	55	0.12
SE	354	0.73	30	46	0.095
W.E	1643	3.39			
PBS 1	1257	2.60	Tris (8.2)		
2	845	1.75	31	282	0.58
3	784	1.62	32	14913	30.78
4	349	0.72	33	14021	28.94
5	235	0.49	34	9168	18.93
6	189	0.39	35	4588	9.47
7	134	0.28	36	820	1.69
8	122	0.25	37	479	0.99
9	116	0.24	38	262	0.54
10	131	0.27	39	138	0.29
11	109	0.23	40	90	0.19
12	98	0.20	41	68	0.14
13	84	0.17	42	50	0.10
14	75	0.15	43	49	0.10
15	71	0.15	44	36	0.074
16	77	0.16	45	27	0.056
17	70	0.15	46	32	0.066
18	55	0.11	47	35	0.072
19	59	0.12	48	31	0.064
20	63	0.13			
21	68	0.14			
22	60	0.12			
23	65	0.13			
24	62	0.13			
25	59	0.12			
26	49	0.10			

DNA sample #4 [Sample (5) of Callie]

	FR #	^3H cpm	% Input	FR #	^3H cpm	% Input
Original 10x		6874		# 27	58	0.18
DNA Sample 10x		632		28	57	0.18
	x 50	31600	100	29	53	0.17
S.E.		157	0.5	30	35	0.11
W.E.		6804	21.53			
PBS # 1		12128	38.38	TRIS (8.2)		
	2	8587	27.17	31	63	0.20
	3	2285	7.23	32	48	0.15
	4	899	2.85	33	37	0.12
	5	285	0.90	34	30	0.095
	6	179	0.57	35	47	0.15
	7	141	0.45	36	28	0.09
	8	132	0.42	37	38	0.12
	9	140	0.44	38	19	0.06
	10	98	0.31	39	59	0.19
	11	79	0.25	40	43	0.14
	12	78	0.25	41	51	0.16
	13	89	0.28	42	42	0.14
	14	81	0.26	43	20	0.06
	15	75	0.24	44	19	0.06
	16	80	0.26	45	18	0.06
	17	52	0.17	46	16	0.05
	18	66	0.21	47	18	0.06
	19	49	0.16	48	16	0.05
	20	61	0.19			
	21	52	0.17			
	22	50	0.16			
	23	41	0.13			
	24	59	0.19			
	25	54	0.17			
	26	47	0.15			

AMPAD · EFFICIENCY LINE® 22-206


Con-A Sepharose Binding of glucosylated DNAs
3/8-9/82
PBS(6.5)
8.2 TRIS
PBS 6.5
8.2 TRIS
Sample ① Callie
Sample ② Callie
Fig 4A
3H cpm % of INPUT
60
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2/26 λ nick-translated
sample ⑤ Callie
60
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Fig 4B
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Elution Volume in ml.
NATIONAL
12-188
10 Millimeters to the Centimeter

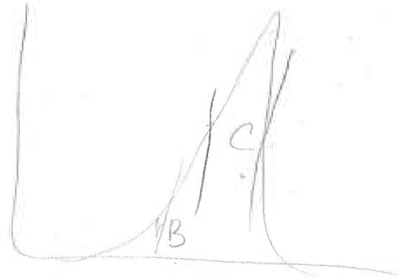
① fraction B 35.8% 0.7×10^6 g/μg

② fraction C 39.3% $.8 \times 10^6$ g/μ

~~③~~ λ 0.6×10^6 cpm/μg

% of

Approx. 6 K bases.



Con-A sepharose binding 3/6/82

~~FR01~~

Samples from Stan

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Dollie.

Samples are numbered (#1), ~~(#2)~~, (#3) and (#4).

They are in 4.0 ml test tubes in a beaker on top of the rack that we indicated in the freezer. In 50 mm KPO_4 pH 7.0

Sample	Vol.	cpm/10λ	cpm Total
# 1	2.2	5236, 4671 > 4953.5	1,089,770
# 3	1.9	1480, 1448 > 1464	278,160
# 4	2.0	1547, 1482 > 1514.5	302,900

→

Samples for Column. 0.5 ml. in PBS 6.5, $MgSO_4$ 5 mM, $CaCl_2$ 1 mM.

	I	II	III
DNA sample (1)	① 45λ	③ 180λ	④ 165λ
$MgSO_4 \cdot 7H_2O$	2.5λ	2.5λ	2.5λ
$CaCl_2$	5λ	5λ	5λ
PBS	447.5λ	312.5λ	327.5λ

3/6/82.

① One ml con-A sepharose columns equilibrated
c̄ 50 – 100 ml 10 mM Na-K-PO_4 pH 6.5 } PBS
150 mM NaCl }
buffer.

② DNA samples under Test were applied to
the column — 0.5 ml volume.
Eluate collected

③ Samples were driven into the gel c̄ two
aliquots of 0.25 ml each PBS (6.5) buffer.
Eluate collected

④ Columns were eluted with 10 ml PBS (6.5)
buffer
0.33 ml — 10 drop — fractions collected.

~~⑤~~ 30 fractions total.

⑤ Column was then washed with
6.0 ml 10 mM TRIS } pH 8.2
10 mM NaCl }
10 drop ~ 0.33 ml fractions collected

To each fraction added 3.5 ml Reafluor
and all fractions counted.

⑥ Results are plotted as
3H cpm percent ~~of~~ total Input
vs elution volume.

3/6/82

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3/6/82

Sample # 1. from Callie.

DNA sample made up as follows.

	DNA	45 λ
1 M	$MgSO_4$	2.5 λ
0.1 M	$CaCl_2$	5 λ
	PBS (6.5)	447.5 λ

} Total 0.5 ml.

FR #	^{3}H cpm	% Input	~~FR#~~	FR #	^{3}H cpm	% Input	FR #	^{3}H cpm	% Input
10λ DNA	3628			# 18	24	0.15	39	185	1.1
x 4.5	16326	100		19	24	0.15	40	110	0.67
S. Eluate	16	0.098		20	16	0.098	41	107	0.66
W Eluate	556	3.41		21	25	0.15			
PBS # 1	671	4.11		22	10	0.06			
6.5 (10 ml) 2	259	1.59		23	18	0.11			
3	51	0.31		24	21	0.13			
4	23	0.14		25	19	0.12			
5	24	0.14		26	21	0.13			
6	19	0.12		27	28	0.17			
7	19	0.12		28	31	0.19			
8	26	0.16		29	27	0.17			
9	23	0.14		30	19	0.12			
10	28	0.17		31	20	0.12			
11	20	0.12		32	12	0.074			
12	12	0.074		33	18	0.11			
13	17	0.10	TRIS	34	51	0.31			
14	26	0.16	(8.2)	35	5067	31.04			
15	20	0.12		36	8356	51.18			
16	19	0.12		37	1213	7.43			
17	21	0.12		38	511	3.13			

3/6/82

Sample # 3 from Callie

	DNA	180 λ
1 M	$MgSO_4$	2.5 λ
0.1 M	$CaCl_2$	.5 λ
0.5	PBS	312.5 λ

} Total 0.5 ml.

FR #	^3H cpm	% Input	FR #	^3H cpm	% Input	FR #	^3H cpm	% Input
Sample (10λ)	1500							
X 18	27000	100%	# 19	44	0.16	40	468	1.73
S. Eluat.	15	0.06	20	39	0.15	41	239	0.89
W. Eluat.	1209	4.48	21	36	0.13	42	161	0.6
PBS 6.5			22	29	0.11	43	108	0.4
1	1353	5.01	23	34	0.13	44	89	0.33
2	1105	4.09	24	27	0.1	45	45	0.17
3	522	1.9	25	35	0.13	46	56	0.21
4	327	1.2	26	31	0.11	47	45	0.17
5	138	0.51	27	34	0.13	48	40	0.15
6	140	0.51	28	34	0.13	49	41	0.15
7	96	0.36	29	39	0.15	50	55	0.21
8	61	0.23	30	32	0.11			
9	68	0.25	31	26	0.096			
10	67	0.25	32	23	0.085			
11	60	0.23	TRIS 8.2					
12	49	0.18	33	24	0.09			
13	52	0.19	34	21	0.08			
14	57	0.21	35	34	0.13			
15	40	0.15	36	485	1.8			
16	46	0.17	37	6932	25.67			
17	37	0.14	38	5625	20.83			
18	28	0.1	39	1776	6.6			

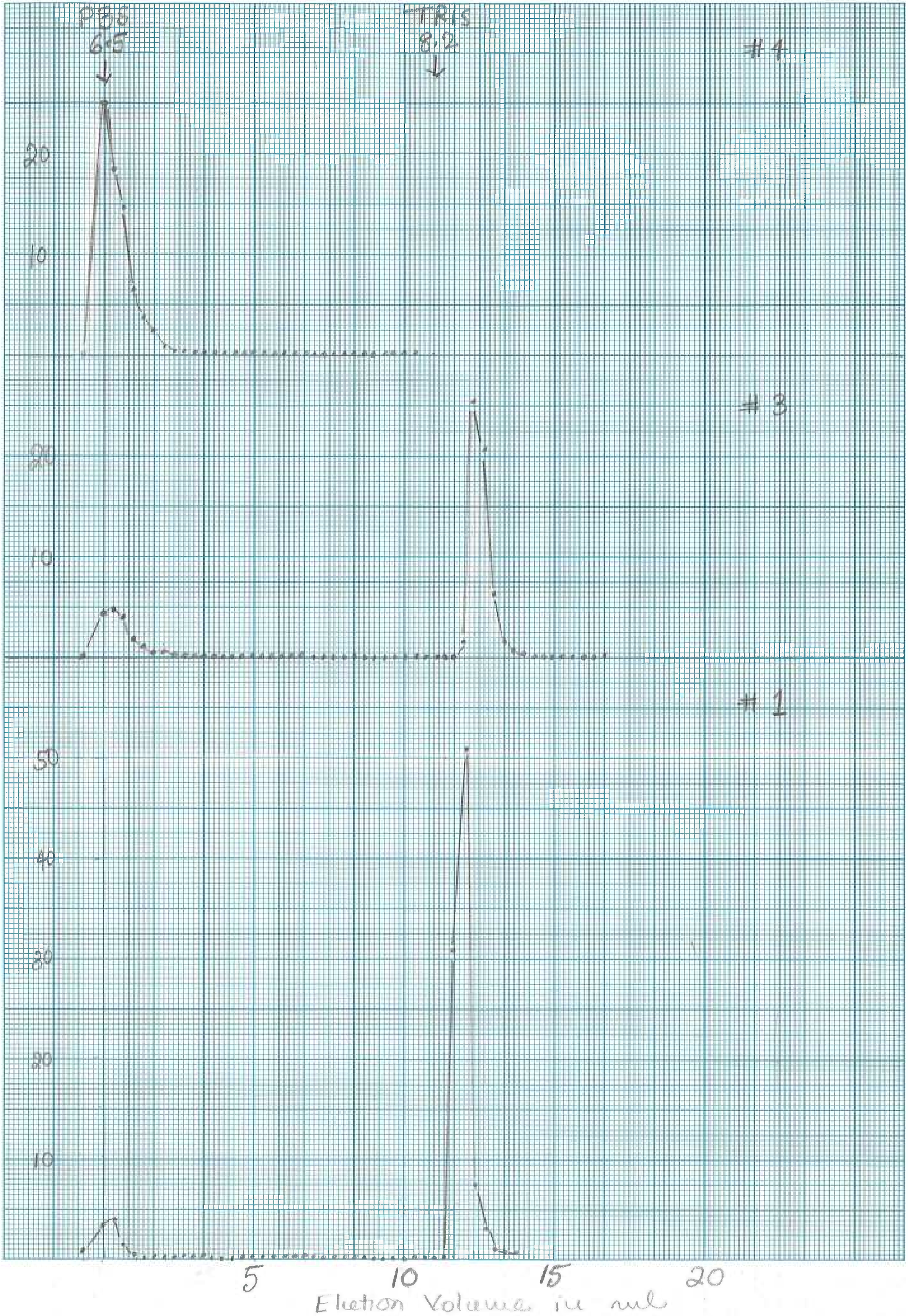
3/6/82.

Sample # 4 from Callie

DNA volume	165 λ	} 0.5 ml Total
1 M $MgSO_4$	2.5 λ	
0.1 M $CaCl_2$	5 λ	
PBS (6.5)	327.5 λ	

FR #	^3H cpm	% Input	FR #	^3H cpm	% Input
DNA 10λ	1158				
x 16.5	19107	100 %	19	39	0.21
S.E	69	0.36	20	34	0.18
W.E	4804	25.14	21	39	0.21
PBS (6.5)	~~3590~~		22	26	0.14
10 ml 1	3590	18.79	23	19	0.10
2	2817	14.74	24	28	0.15
3	1234	6.46	25	30	0.16
4	735	3.85	26	29	0.16
5	499	2.61	27	26	0.14
6	174	0.91	28	30	0.16
7	76	0.4	29	33	0.17
8	74	0.39			
9	42	0.22			
10	56	0.29			
11	40	0.22			
12	48	0.25			
13	40	0.22			
14	30	0.16			
15	23	0.12			
16	38	0.2			
17	45	0.24			
18	34	0.18			

3/6/82



3/1/82

Objective :

A To determine whether, glycosylated DNA, e.g. T4 phage DNA, binds to concanavalin-A sephanose columns at neutral or slightly acidic pH

B. To check ~~for~~ whether this binding is irreversible, i.e. whether the bound DNA can be replaced from the column, either by,

(I.) increasing the pH of the elution buffer,

or, by

(II) monosaccharides such as mannose

C. To compare binding of non-glycosylated DNA, e.g. phage lambda DNA to concanavalin-A-sepharose columns under similar conditions.

D. To determine whether presence of d-mannose will affect binding of glycosylated DNA to con-A sepharose columns.

Materials & Methods:

DNAs Nick-translated, ^3H-T labelled

(i) λ DNA 3.615 μg/ml
10780 ^3H cpm per 10 μl.

(ii) T4 DNA 3.29 μg/ml
16372 ^3H cpm per 10 μl.

in H_2O

Affinity Matrix

Concanavalin-A sepharose
18 mg concanavalin-A bound per ml of settled gel.

Buffers. Phosphate Buffer saline pH 6.5 (PBS)

10 mM TRis-HCl } Buffers at
10 mM NaCl } pH 7.2
7.4
7.6
7.7
7.8
7.9
8.0
8.1
8.2.

Monosaccharide d-Mannose at 0.056 M in d.H_2O

Experimental protocols:

1. One ml concavallin-A sepharose columns in sterile capillary pipettes were equilibrated ~~with~~ excessively with PBS [50 – 100 ml]
2. DNA sample [0.5 ml in PBS supplemented with 5 mM $MgSO_4 \cdot 7H_2O$ and 1 mM $CaCl_2 \cdot 2H_2O$] was applied to the column.
3. Sample was driven into the ~~column with~~ gel with 2 aliquots ~~[of ½ volume~~ of [½ Sample volume] PBS.
4. Column was successively eluted with

I ⓐ 10 ml portions of PBS

ⓑ 5 ml portions of Tris NaCl (7.2)

ⓒ " " " Tris NaCl (7.4)

ⓓ " " " " (7.6)

ⓔ " " " " (7.7)

ⓕ " " " " (7.8)

ⓖ " " " " (7.9)

ⓗ " " " " (8.0)

ⓙ " " " " (8.1)

ⓚ " " " " (8.2)

II ⓐ 10 ml portions of PBS

ⓑ 10 ml portion of d-Mannose (0.056 M)

5. Ten-drop fractions were collected, when the elution was started (three fractions per ml, of eluting buffer) directly into the scintillation vial.

6. 3.5 ml of scintillation cocktail Reafluor was added, mixed well and counted.

7. Results are plotted as ~~per cent counts recovered~~ counts recovered percent of the input vs elution volume.

Experimental data :

Nick-Translated (^3H) T4 DNA sample (0.5 ml.)
10λ of original 17622
100 λ used in sample

Nick-Translated ^3H λ DNA sample 0.5 ml
10 λ of original 10823 100λ used/sample.

Total counts applied to the columns.

T4 · DNA	column ①	176220
	②	176220
λ DNA	column ①	108230
	②	108230

I pH elution

T4 DNA

		^3H cpm	Percent
Input ~~cpm~~		176220	100 %.
Sample eluate		30	0.017
$Wash_1$ eluate		28	0.016
$Wash_2$ eluate		36	0.02
PBS	FR # 1	31	0.018
(10 ml.)	2	38	0.022
	3	36	0.02
	4	35	0.02
	5	37	0.02
	6	35	0.02
	7	37	0.02
	8	40	0.023
	9	43	0.024
	10	45	0.026
	11	46	0.026
	12	47	0.026
	13	48	0.026
	14	45	0.026
	15	44	0.025
	16	43	0.025
	17	48	0.026
	18	49	0.026
	19	45	0.025

T4-DNA I pH elution

FR #		^3H cpm	% of Input	FR #		^3H cpm	% of Input
PBS	20	40	0.023	Tris NaCl (7.2)	43	38	0.021
10 ml.	21	41	0.023	(5 ml)	44	35	0.02
	22	40	0.023		45	36	0.02
	23	38	0.021	Tris NaCl (7.4)	46	32	0.018
	24	36	0.02	(5 ml)	47	41	0.022
	25	35	0.02		48	43	0.024
	26	37	0.02		49	36	0.02
	27	36	0.02		50	37	0.02
	28	38	0.021		51	38	0.022
	29	35	0.019		52	35	0.02
	30	34	0.019		53	34	0.02
Tris NaCl	31	32.	0.018		54	20	0.011
(7.2) 5 ml.	32	33	0.018		55	21	0.011
	33	38	0.021		56	35	0.02
	34	32	0.018		57	36	0.02
	35	36	0.02		58	37	0.02
	36	31	0.018		59	30	0.011
	37	32	0.018		60	31	0.011
	38	38	0.021	Tris NaCl (7.6)	61	32	0.011
	39	31	0.018	(5 ml)	62	33	0.011
	40	32	0.018		63	35	0.02
	41	33	0.018		64	32	0.011
	42	33	0.018		65	38	0.022
					66	36	0.02

T_4 DNA I - pH elution

FR #	^3H cpm	% Input	FR #	^3H cpm	% Input	FR #	^3H cpm	% Input
TRis NaCl (7.6) 67	86	0.049	(7.8) 91	105	0.06	115	89	0.05
5ul. 68	85	0.049	5ul 92	106	0.06	116	104	0.059
69	100	0.056	93	113	0.064	117	113	0.064
70	112	0.064	94	107	0.062	118	100	0.058
71	157	0.086	95	108	0.062	119	83	0.05
72	162	0.092	96	133	0.075	120	86	0.05
73	185	0.105	97	123	0.07	(8.0) 5ul 121	38	0.02
74	193	0.109	98	151	0.086	122	78	0.044
75	200	0.113	99	121	0.07	123	48	0.027
pH 7.7 76	212	0.12	100	130	0.071	124	35	0.02
(5 ul) 77	215	0.122	101	121	0.07	125	69	0.039
78	1589	0.9	102	122	0.07	126	35	0.02
79	3875	2.198	103	186	0.106	127	90	0.05
80	6219	3.53	104	130	0.074	128	20	0.011
81	43212	24.52	105	115	0.065	129	100	0.058
82	80861	45.89	(7.9) 106	113	0.06	130	35	0.02
83	28183	15.99	5ul 107	119	0.068	131	86	0.05
84	8161	4.63	108	118	0.068	132	100	0.058
85	3809	2.16	109	129	0.073	133	86	0.05
86	1081	0.61	110	153	0.086	134	69	0.039
87	236	0.134	111	196	0.111	135	72	0.041
88	105	0.06	112	113	0.064	(8.1) 136	38	0.02
89	136	0.077	113	151	0.086	5ul 137	45	0.024
90	118	0.069	114	121	0.07	138	46	0.025

T4 DNA pH elution

FR#	^3H cpm	% Input	FR #	^3H cpm	% Input	FR #	^3H cpm	% Input
(8.1) 5 ml 139	25	0.014	(8.1) 5 ml 148	36	0.020	(8.2) 157	41	0.023
140	30	0.017	149	18	0.01	158	38	0.022
141	32	0.018	150	23	0.013	159	43	0.023
142	85	0.048	(8.2) 5 ml 151	25	0.014	160	90	0.051
143	80	0.045	152	41	0.023	161	100	0.057
144	68	0.039	153	94	0.053	162	38	0.022
145	39	0.022	154	48	0.026	163	25	0.014
146	32	0.018	155	95	0.053	164	46	0.026
147	20	0.011	156	100	0.055	165	32	0.018

T4 DNA II mannose - elution.

FR#	^3H cpm	% Input	FR #	^3H cpm	% Input	FR #	^3H cpm	% Input
S eluate	86	0.049	(PBS) 9	35	0.02	(PBS) 20	89	0.051
W_1 eluate	45	0.026	10	48	0.028	21	35	0.02
W_2 eluate	49	0.028	11	45	0.026	22	36	0.02
(PBS) 1	41	0.023	12	61	0.035	23	90	0.051
10 ml 2	46	0.026	13	41	0.023	24	45	0.026
3	38	0.022	14	48	0.028	25	38	0.022
4	35	0.02	15	56	0.032	26	60	0.034
5	38	0.022	16	59	0.033	27	30	0.017
6	39	0.022	17	51	0.029	28	50	0.028
7	41	0.023	18	50	0.029	29	36	0.02
8	46	0.026	19	53	0.03	30	90	0.051

T4 DNA II Mannose Elution.

FR #	3H cpm	% Input	FR #	3H cpm	% Input	FR #	3H cpm	% Input
Mannose 0.056M 31	89	0.051	41	211	0.12	51	95	0.054
10ml 32	90	0.051	42	220	0.13	52	196	0.11
33	84	0.048	43	280	0.12	53	201	0.11
34	7258	4.12	44	83	0.047	54	38	0.022
35	56295	31.95	45	200	0.12	55	49	0.028
36	80118	45.46	46	38	0.022	56	38	0.022
37	13913	7.895	47	69	0.039	57	101	0.057
38	1354	0.77	48	211	0.12	58	36	0.022
39	861	0.49	49	38	0.022	59	25	0.014
40	301	0.17	50	86	0.049	60	100	0.057

λ DNA Total Input 108230
I pH Elution.

FR #	^3H cpm	% Input	FR #	^3H cpm	% Input	FR #	^3H cpm	% Input
S eluate	6227	5.76	21	19	0.011	TRis(7.2) 44	40	0.02
W1 eluate	34230	31.63	22	14	0.011	45	35	
W2 eluate	34983	32.32	23	15	0.011	TRis 7.4 46	85	
PBS 1	16831	15.55	24	23	0.02	47	30	
10 ml. 2	11000	10.16	25	23	0.02	48	92	
3	226	0.21	26	23	0.018	49	60	
4	112	0.10	27	29	0.02	50	35	
5	104	0.096	28	16	0.01	51	32	
6	40	0.02	29	25	0.02	52	38	
7	40	0.02	30	25	0.02	53	39	
8	43	0.02	TRis(7.2) 31	61	0.022	54	30	
9	20	0.01	2	38	0.02	55	32	
10	29	0.015	3	33	0.02	56	28	
11	28	0.014	4	55	0.03	57	38	
12	35	0.022	5	32	0.02	58	88	
13	21	0.01	6	38	0.02	59	35	
14	32	0.018	7	89	0.05	60	69	
15	24	0.011	8	32	0.02	TRis 7.6 61	30	
16	19	0.011	9	30	0.02	62	30	
17	18	0.011	40	49	0.024	63	31	
18	14	0.01	41	35	0.02	64	32	
19	21	0.02	42	38	0.02	65	33	
20	16	0.01	43	39	0.02	66	40	

λ DNA Input 108230
pH - Elution

FR #	^3H cpm	% Input	FR #	^3H cpm	% Input	FR #	^3H cpm	% Input
(Tris 7.6) 67	22		(Tris 7.7) 90	41		(Tris 7.9) 112	35	
68	21		(Tris 7.8) 91	38		113	36	
69	22		92	40		114	30	
70	25		93	29		115	29	
71	38		94	51		116	38	
72	36		95	35		117	30	
73	32		96	43		118	81	
74	38		97	25		119	30	
75	41		98	86		120	41	
(Tris 7.7) 76	38		99	89		(Tris 8.0) 121	36	
77	44		100	38		122	38	
78	45		101	35		123	33	
79	96		102	32		124	25	
80	100		103	41		125	28	
81	38		104	40		126	31	
82	20		105	38		127	32	
83	151		(Tris 7.9) 106	39		128	30	
84	35		107	46		129	86	
85	36		108	52		130	56	
86	38		109	38		131	35	
87	49		110	39		132	45	
88	30		111	69		133	44	
89	31					134	29	
						135	10	

λ - DNA   Mannose ELUTION   3/1/82

INPUT 108230

FR #	^{3}H cpm	% of Input	FR #	^{3}H cpm	% of Input	FR #	^{3}H cpm	% of Input
Sample Eluate	5804	5.36	PBS 21	82	0.05	Mannose 44	30	
Wash$_1$ Eluate	82968	76.50	22	87	0.06	45	25	
Wash$_2$ Eluate	13916	12.86	23	65	0.05	46	20	
PBS #1	2150	1.98	24	40	0.03	47	19	
10ml. 2	914	0.85	25	38	0.022	48	18	
3	820	0.76	26	36	0.022	49	16	
4	633	0.59	27	35	0.022	50	18	
5	375	0.35	28	38	0.025	51	35	
6	239	0.22	29	39	0.025	52	30	
7	200	0.18	30	40	0.02	53	31	
8	234	0.22	Mannose 31	23	0.02	54	32	
9	178	0.16	32	23	0.02	55	33	
10	148	0.14	33	23	0.02	56	34	
11	159	0.14	34	24	0. —	57	35	
12	136	0.13	35	25		58	36	
13	135	0.13	36	26		59	38	
14	173	0.15	37	29		60	30	
15	115	0.11	38	31				
16	131	0.12	39	30				
17	136	0.13	40	31				
18	136	0.13	41	34				
19	116	0.1	42	30				
20	98	0.09	43	30				

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3/2/82

D. Effect of mannose (in reaction mixtures) on DNA binding to con-A sepharose columns.

Experimental protocols

1. One ml con-A sepharose columns were equilibrated with PBS (6.5) 50 - 100 ml

2. DNA samples were made up in 0.5 ml volume containing at F.C.
   - 10 mM Na-K-PO_4 6.5
   - 150 mM NaCl
   - 5 mM $MgSO_4 \cdot 7H_2O$
   - 1 mM $CaCl_2 \cdot 2H_2O$
   - 40 ug DNA [T_4-DNA 21500 cpm (^3H)
     λ-DNA 12000 cpm (^3H)
   - 0.056 M Mannose.

   Samples were applied to the columns.
   Eluate was collected.
   Columns were washed with 2 aliquots of 0.25 ml of PBS (6.5)
   Both washes were collected separately

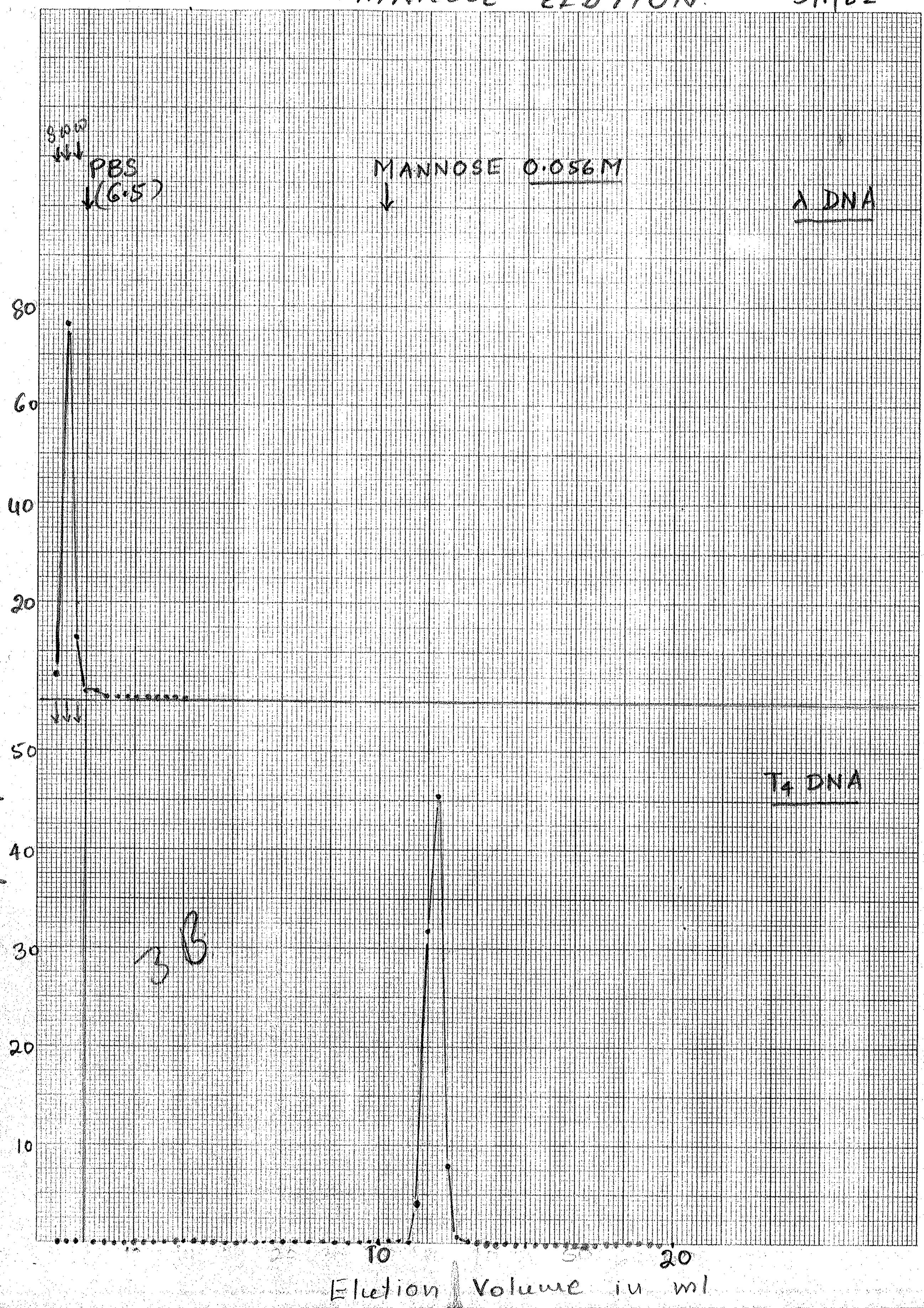
3. Columns were eluted with 10.0 ml PBS (6.5).

4. Ten drop fractions [0.33 ml] were collected directly in scintillation ~~cocktails~~ vials. Added 3.5 ml of cocktail and counted.

5. Results are plotted as ~~counts per~~ recovered percent of Input vs elution volume

FR #	^3H cpm	% Input	FR #	^3H cpm	% Input	FR #	^3H cpm	% Input
T4 DNA	21500	100	13	14	0.065	30	16	8
E. Sample	1505	7	14	10	0.05	λ DNA	12000	100
Wash 1	10110	47.02	15	19	0.09	E Sample	2800	23.3
Wash 2	~~8~~243	38.34	16	16	0.075	E Wash 1	4872	40.6
PBS 1	1005	4.67	17	30	0.14	E 2	3480	29.00
2	236	1.097	18	18	0.084	PBS 1	220	1.83
3	89	0.414	19	22	0.102	2	85	0.71
4	35	0.16	20	16	0.075	3	31	0.26
5	37	0.17	21	18	0.084	4	25	0.21
6	12	0.06	22	20	0.10	5	17	0.14
7	19	0.09	23	23	0.107	6	18	0.15
8	18	0.08	24	15	0.07	7	20	0.17
9	16	0.07	25	10	0.05	8	16	0.14
10	20	0.09	26	7		9	15	0.13
11	25	0.10	27	19	0.09	10	18	0.15
12	11	0.04	28	22	0.11	11	19	0.15
			29	17	0.075	12	20	0.17

DNA - BINDING TO CON - A SEPHAROSE
MANNOSE ELUTION 3/1/82



2/23/82

Check of DNA binding to Con-A Sepharose

Con-A Sepharose 18 mg/ml gel

DNA (A) ^3H E Coli DNA

~ 140,000 cpm/λ

(B) T4 DNA 0.28 mg/ml

Eluting Buffer PBS (6.5)

(A) ^3H E coli DNA

10λ in 1.0 ml PBS containing

5 mM $MgSO_4 \cdot 7H_2O$

1 mM $CaCl_2 \cdot 2H_2O$

0.10 ml used ~ cpm

1.0 ml con A sepharose column equilibrated
c̄ 50 ml PBS 6.5

DNA sample applied to the column.
Sample driven into the column c̄ 2 aliquots
of ~~100~~ 50 λ each PBS.
Column eluted c̄ PBS (6.5) ~~25 ml~~. 4.0 ml.
2 drop fractions collected
checked for radioactivity.
Column eluted successively c̄ Tris. NaCl pH 7.2,
pH 7.4, pH 7.6, pH 7.8, pH 8.0 buffers. 4 ml each

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FR #	^3H cpm.	FR #	^3H cpm.		FR #	^3H cpm.
10x Sample	14154					
Sample Eluate	383	20	1523		43	521
		21	1464		44	497
Sample wash 1	16	22	1560		45	573
2	20	23	1459		46	536
PBS FR. # 1	23	24	661		47	225
2	23	25	1136		48	202
3	25	26	582		49	371
4	24	27	792		50	191
5	119	28	302		51	358
6	542	29	475		52	186
7	1767	30	417		53	166
8	3814	31	253		54	130
9	5681	32	383		55	284
10	9008	33	164		56	224
11	10421	34	413		57	126
12	11356	35	243		58	274
13	10341	36	313		59	168
14	8809	37	378		60	273
15	7328	38	364		61	229
16	4374	39	361		62	246
17	4069	40	115		63	233
18	3380	41	159	TRIS 7.12	64	255
19	2436	42	144	[illegible]	65	171

FR #	^3H cpm
66	154
67	159
68	119
69	121
70	224
71	120
72	284
73	154
74	268
75	283
76	274
77	225
78	263
79	197
80	190
81	173

Last elution $\bar{c}$ Tris NaCl pH 8.2.

All samples diluted $\bar{c}$ (2.0 ml) respective buffers and A_{260} checked.

Buffer	FR #	A_{260}	Buffer	Fr #	A_{260}
Sample		011	Tris 7.9	1	135
Wash	I	006		2	071
	II	003		3	041
PBS	1	004		4	011
	2	005	Tris 8.2	1	006
	3	006		2	004
	4	008		3	003
Tris 7.2	1	004		4	002.
	2	003			
	3	004			
	4	006			
Tris 7.4	1	001			
	2	002			
	3	003			
	4	004			
Tris 7.6	1	006			
	2	010			
	3	023			
	4	043			

Sample diluted to 3.0 ml.	A_{260}	A_{280}
	0.326	046

Sample	A_{260}	~~µg DNA~~ Corrected	µg DNA
Diluted 3 fold	0.326	0.978	50
S.E. x 3	0.011	0.033	1.65
W_I x 3	0.006	0.018	0.9
W_{II} x 3	0.003	0.009	0.45
PBS 1	0.004	0.012	0.6
2	0.005	0.015	0.75
3	0.006	0.018	0.9
4	0.008	0.024	1.2
Tris (7.2) 1	0.004	0.012	0.6
2	0.003	0.009	0.45
3	0.004	0.012	0.6
4	0.006	0.018	0.9
Tris (7.4) 1	0.001	0.003	0.15
2	0.002	0.006	0.3
3	0.003	0.009	0.45
4	0.004	0.012	0.6
Tris (7.6) 1	0.006	0.018	0.9
2	0.01	0.03	1.5
3	0.023	0.069	3.45
4	0.043	0.129	6.45
Tris (7.9) 1	0.135	0.405	20.25
2	0.071	0.213	10.65

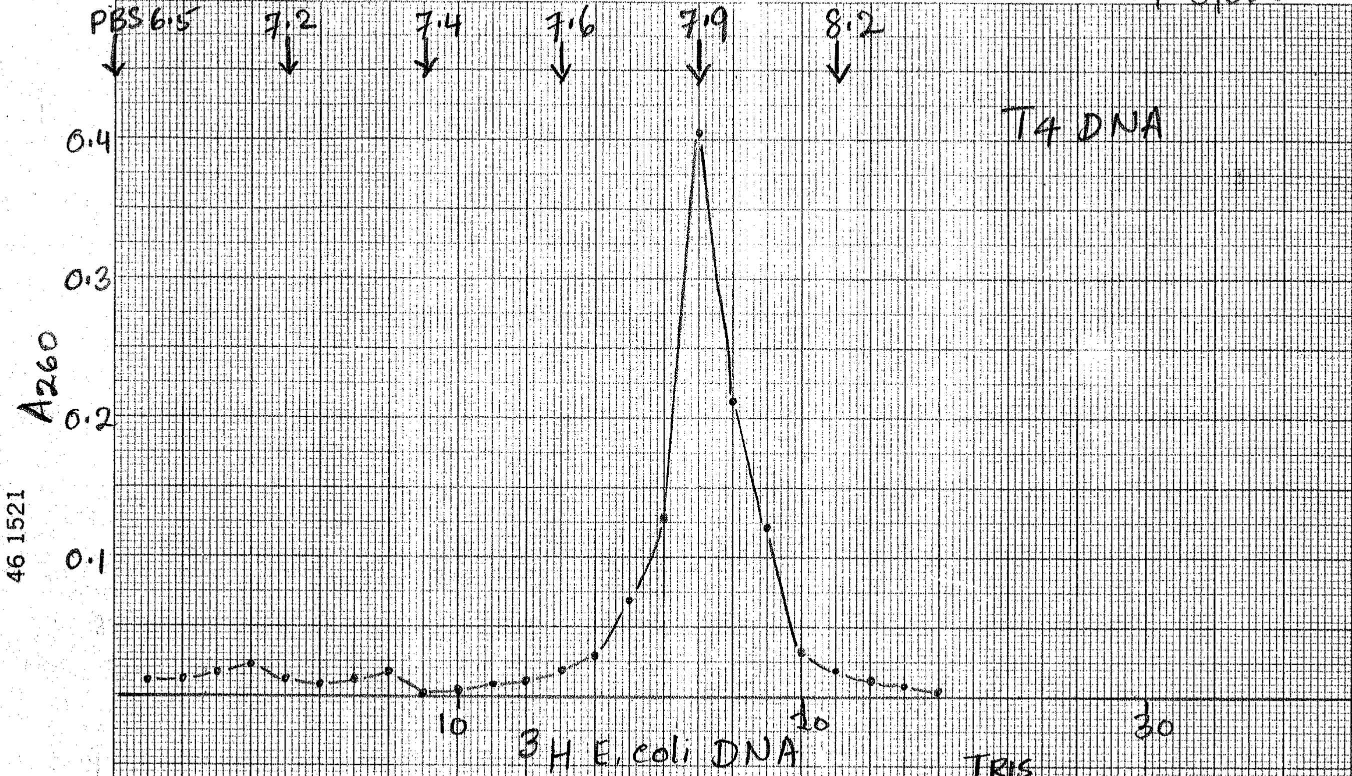
Sample		A260	Corrected	μg DNA
Tris (7.9)	3	0.041	0.123	6.15
	4	0.011	0.033	1.65
Tris (8.2)	1	0.006	0.018	0.9
	2	0.004	0.012	0.6
	3	0.003	0.009	0.45
	4	0.002	0.006	0.3

B) T4 DNA 0.28 mg/ml in 10 mM TRIS (7.2)
10 mM NaCl.

0.180 ml sample in PBS (6.5) } 1 ml.
MgSO4. 5 mM } sample.
CaCl2 1 mM }

1.0 ml con-A column equilibrated c̄
35.0 ml PBS.
DNA sample applied. eluate collected
Sample driven c̄ 0.5 ml aliquots of PBS
two aliquots collected.
Column eluted c̄ 4.0 ml PBS (6.5)
1.0 ml aliquot collected

Column eluted c̄ 10 mM TRIS 7.2
10 mM NaCl
4 aliquots collected of 1 ml each
Column eluted c̄ 10 mM TRIS 7.4
10 mM NaCl
4 aliquots collected. 1 ml each.
Column eluted c̄ 10 mM TRIS 7.6
10 mM NaCl
4 aliquots
Column eluted c̄ 10 mM Tris 7.9
10 mM NaCl



^{3}H E. coli DNA

TRIS

PBS

7.2 7.4 7.6 7.9

^{3}H cpm x 10^{-3}

12
10
8
6
4
2

10 20 30 40 50 60 70 80

1 ml 2 ml FR # 3 ml 4 ml

46 1521

10 X 10 TO THE CENTIMETER 18 X 25 CM.
KEUFFEL & ESSER CO. MADE IN U.S.A.

II Concavallin-A — Concavallin-A - Sepharose affinity matrix used. One milliliter of settled gel has 18 mg con-A bound to it.

III Buffers.

Phosphate Buffered Saline pH 6.5

Tris.HCl	10 mM	pH 7.2
NaCl	10 mM	
Tris.HCl	10 mM	pH 7.4
NaCl	10 mM	
Tris.HCl	10 mM	pH 7.6
NaCl	10 mM	
Tris.HCl	10 mM	pH 7.9
NaCl	10 mM	
Tris.HCl	10 mM	pH 8.2
NaCl	10 mM	

Temp. — Room temp 25° ~ 28° C

Experimental Protocol

1. 1.0 ml concavallin-A sepharose columns in capillary pipettes were equilibrated with PBS (6.5) excessively (50 ~ 100 ml).
2. DNA sample was applied to the column.
3. Sample was driven into the gel with 2 aliquots of (½ ~~volume~~ sample volume) PBS (6.5)
4. Column was successively eluted with ~~4 ml~~ portions of
   a. PBS (6.5) 4.0 ml
   b. Tris.NaCl (7.2) 4.0 ml
   c. Tris.NaCl (7.4) "
   d. Tris.NaCl (7.6) "
   e. Tris-NaCl (7.9) "
   f. Tris. NaCl (8.2) "
5. Appropriate fractions were collected and checked for DNA either, by
   a. counting radioactivity, or by
   b. checking UV absorbance at A_{260} against appropriate buffer.

~~Results~~ ~~Experimental data~~

A. ^{3}H E.coli DNA

100 microliters DNA used. Aliquot of sample was checked for radioactivity to determine the radioactivity applied to the column.

DNA sample was driven into the column with 2 aliquots of 50 ul each of PBS (6.5)

Sample eluate as well as sample wash - eluates were collected separately to determine the radioactivity

~~when the~~

Column elution with different buffers was collected directly in scintillation vials 2 drop per fraction

16 fractions per ml.

Results were plotted as.

~~percent radioactivity~~

percent radioactivity of the input vs elution volume.

B. T4 DNA

0.18 ml [50 μg] DNA in
1.0 ml PBS (6.5)
$MgSO_4 \cdot 7H_2O$ 5 mM
$CaCl_2 \cdot 2H_2O$ 1 mM
applied to the column.
DNA sample driven into the column with 2 aliquots of 0.5 ml each of PBS (6.5)
Sample eluate as well as wash-eluate collected separately in sterile tubes [nuclease-free].
Column elution with respective buffers was collected as fractions of one ml each.
4 fractions per buffer.
All the fractions were diluted with 2.0 ml of respective buffers and A_{260} vs that buffer was recorded.

Results were plotted as percent A_{260} units of the total ~~uni~~ (input applied) vs column elution volume.

^3H(T) E. coli DNA   Total Input 141540 ^3H cpm.   2/23/82.

pH Elution.

FR #	^3H cpm	% of Input	FR #	^3H cpm	% of Input	FR #	^3H cpm	% of Input
Sample E.	383	0.27	(PBS) 21	1464	1.03	(PBS) 44	497	0.35
W_1 E	16	0.01	22	1560	1.10	45	573	0.40
W_2 E	20	0.01	23	1459	1.03	46	536	0.38
PBS # 1	23	0.01	24	661	0.47	3.681 47	225	0.16
(4 ml) 2	23	0.01	25	1136	0.80	48	202	0.14
3	25	0.01	26	582	0.41	49	371	0.26
4	24	0.01	27	792	0.56	50	191	0.13
5	119	0.084	28	302	0.21	14.75 51	358	0.25
6	542	0.38	29	475	0.34	52	186	0.13
7	1767	1.25	30	417	0.29	53	166	0.12
8	3814	2.69	31	253	0.18	54	130	0.09
9	5681	4.01	32	383	0.27	55	284	0.20
10	9008	6.36	51.99 33	164	0.12	56	224	0.16
11	10424	7.36	34	413	0.29	57	126	0.089
12	11356	8.023	35	243	0.17	58	274	0.19
13	10341	7.31	36	313	0.22	59	168	0.12
14	8809	6.22	37	378	0.27	60	273	0.19
15	7328	5.18	38	364	0.26	61	229	0.16
16	4374	3.09	39	361	0.26	62	246	0.17
17	4069	2.87	40	115	0.081	63	233	0.16
18	3380	2.39	41	159	0.11	64	255	0.18
19	2436	1.72	42	144	0.10	TRIS 7.2 65	171	0.12
20	1523	1.08	43	521	0.37	66	154	0.11

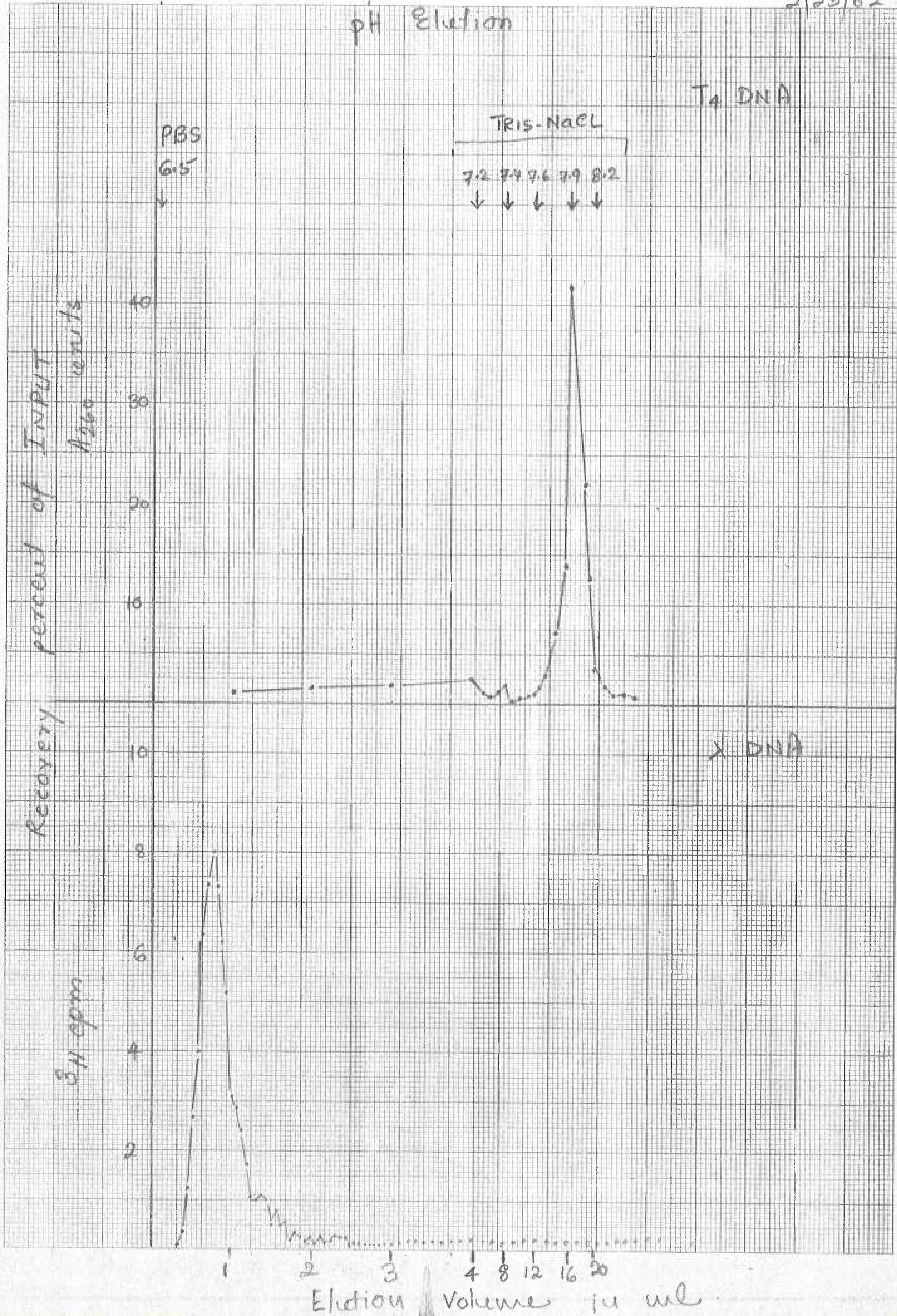
2/23/82

^3H [T] E. coli DNA Total Input 141540 cpm.
pH Elution

FR #	^3H cpm	% of Input	FR #	^3H cpm	% of Input
67	159	0.11	(8.2) 89	89	0.06
68	119	0.085	90	113	0.07
69	121	0.085	91	158	0.11
70	224	0.16	92	133	0.093
(7.4) 71	125	0.088	93	211	0.15
72	284	0.2	94	230	0.16
73	154	0.11			
74	268	0.18			
75	283	0.2			
76	274	0.19			
(7.6) 77	225	0.16			
78	263	0.18			
79	197	0.14			
80	190	0.14			
81	173	0.12			
82	186	0.13			
(7.9) 83	153	0.11			
84	148	0.11			
85	113	0.08			
86	112	0.08			
87	118	0.08			
88	100	0.06			

T4 · DNA Total A260 units applied 0.978

FR #	A260	% of Input	FR #	A260	% of Input
Sample E	0.033	3.37	TRis NaCl 7.9 17	0.405	41.41
Wash1 E	0.018	1.84	18	0.213	21.78
Wash2 E	0.009	0.92	19	0.123	12.58
			20	0.033	3.37
PBS 6.5 1	0.012	1.22	TRis NaCl 8.2 21	0.018	1.84
2	0.015	1.54	22	0.012	1.22
3	0.018	1.84	23	0.009	0.92
4	0.024	2.45	24	0.006	0.61
TRis NaCl 7.- 5	0.012	1.22			
6	0.009	0.92			
7	0.012	1.22			
8	0.018	1.84			
Tris NaCl 7.4 9	0.003	0.31			
10	0.006	0.61			
11	0.009	0.92			
12	0.012	1.22			
Tris NaCl 7.6 13	0.018	1.84			
14	0.030	3.07			
15	0.069	7.1			
16	0.129	13.19			



46 1521
K+E 10 X 10 TO THE CENTIMETER 18 X 25 CM.
KEUFFEL & ESSER CO. MADE IN U.S.A.

2/23/82

Objective To check whether,

ⓐ DNA binds to concavallin A ;

ⓑ glycosyl residues on DNA have anything to do with binding ;

ⓒ the binding of DNA is irreversible ;

ⓓ bound DNA can be eluted off by pH or any other compound.

Materials & Methods:

~~DNA~~

I DNA - A ^3H E coli DNA - 140,000 cpm/λ
in 0.1 M NaCl pH 7.0
10 λ diluted in
1.0 ml Phosphate buffer Saline
pH 6.5 containing
5 mM $MgSO_4 \cdot 7H_2O$
1 mM $CaCl_2 \cdot 2H_2O$

B T_4 DNA 0.28 mg/ml
in 10 mM TRIS (7.4)
10 mM NaCl
50 μg DNA diluted to 1.0
~~[illegible]~~ ml in PBS (6.5)
containing 5 mM $MgSO_4 \cdot 7H_2O$
1 mM $CaCl_2 \cdot 2H_2O$

2/22/82.

Objective:

To check whether,
glucosylated DNA, e.g. T4 phage DNA
is precipitated by concanavalin A.

Materials & Methods.

T4 DNA — T4 am 82 [44 62] DNA
0.28 mg/ml in
10 mM Tris } pH 7.2
10 mM NaCl }

Calf thymus DNA [worthington] — 0.5 mg/ml in
10 mM Tris } pH 7.2
10 mM NaCl }

Concanavalin A (pharmacia) — 10 mg per ml in PBS pH 6.5

Experimental protocol

1. Fixed amount of DNA [either T4 or calf-thymus] in a series of sterile 1.5 ml eppendorf tubes.
2. Added increasing amounts of con-A [50 to 2500 μg]
3. Volume made up to 1.0 ml by addition of PBS, $MgSO_4$ and $CaCl_2$ so that final concentration will be

   10 mM Na-K-PO_4 } pH 6.5
   150 mM NaCl }
   5 mM $MgSO_4 \cdot 7H_2O$
   1 mM $CaCl_2 \cdot 2H_2O$

4. Mixed well and left at room-temp. for 60 minutes.
5. All tubes were spun at 12,000 x g for 15-20 minutes.
6. Supernates were carefully removed without disturbing the pellets and diluted 3 fold with PBS and A_{260}, A_{280} determined.
7. Controls without DNA were run under similar conditions.

DNA μg / Rx	Concanavalin A μg Rx	Visible ppte		Supernate A260	Supernate A280
0	0	–		0	0
0	50	–		0.06	0.045
0	100	–		0.078	0.096
0	250	–		0.192	0.237
0	500	–		0.288	0.453
0	1000	–		0.522	0.864
0	2500	–		1.35	2.25
T4 50 μg	0	–		0.51	0.276
"	50	–	0.36	0.42	0.246
"	100	–	0.27	0.348	0.372
"	250	±	0.06	0.252	0.513
"	500	+	0.129	0.129	0.75
"	1000	+	-0.204	0.318	1.143
"	2500	+	-0.717	0.633	2.553
CT 50.4 μg	0	–		0.51	0.279
"	50	–	0.51	0.57	0.324
"	100	–	0.522	0.6	0.36
"	250	–	0.511	0.702	0.519
"	500	–	0.522	0.81	0.756
"	1000	–	0.564	1.086	1.146
"	2500	–	0.525	1.875	2.55

2/22/82.

To check whether T_4 DNA is precipitated by con A.
compare with non-glycosylated DNA.

Rx mixtures of 1.0 ml.

PBS (6.5 pH)
$MgSO_4 \cdot 7H_2O$ 5 mM
$CaCl_2 \cdot 2H_2O$ 1 mM
T_4 ~~or CT~~ DNA 50.4 μg.
(or CT DNA 50.00 μg)
Con A 50 — 2500 μg.

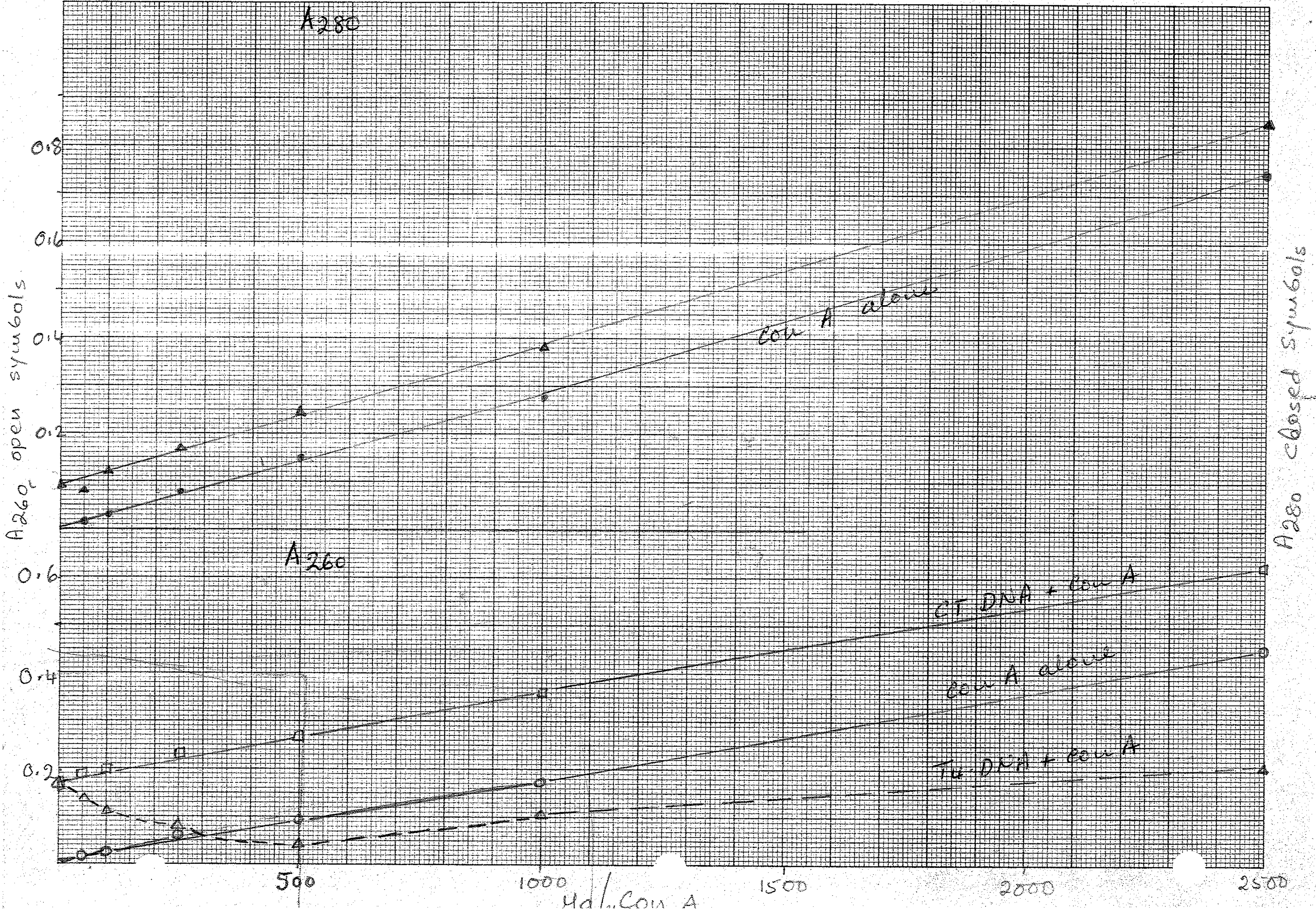
mixed well and incubated at RT for 60'
Tubes spun in microfuge for 15-20 minutes
Supernates diluted 3 fold with PBS (6.5)
and A_{260}, A_{280} checked

Reviewed on 2/22/82 - 7:04 PM
Norman Keller

2/22/82.

T4 DNA 0.28 mg/ml Buffer PBS 6.5
CT DNA 0.5 mg/ml RX at RT.
Con A 10 mg/ml.

	Con A	T4 DNA	CT DNA	PBS	Mg	Ca	Visible ppte	A_{260} (x3.)	A_{280}
0	0	0	0	0.98	10λ	10λ	0	0	0
1	5λ	0	0	975	10		0	02	015
2	5λ	180λ	0	795			0	140	082
3	5λ	0	100λ	875			0	190	108
4	10λ	0	0	970			0	026	032
5	10λ	180λ	0	790			0	116	124
6	10λ	0	100λ	870			0	200	120
7	25λ	0	0	955			0	064	079
8	25λ	180λ	0	775			±	084	171
9	25λ	0	100λ	855			0	234	173
10	50λ	0	0	930			0	096	151
11	50λ	180λ	0	750			+	643	250
12	50λ	0	100λ	830			0	270	252
13	100λ	0	0	880			0	174	288
14	100λ	180λ	0	700			+	106	381
15	100λ	0	100λ	780			0	362	382
16	250λ	0	0	730			0	450	758
17	250λ	180λ	0	550			+	211	851
18	250λ	0	100λ	630			0	625	850
00	0	180λ	0	800	↓	↓	0	170	092
000	0	0	100λ	880	↓	↓	0	170	093



K+E 10 X 10 TO THE CENTIMETER 18 X 25 CM.
KEUFFEL & ESSER CO. MADE IN U.S.A.
46 1521
A280
A260
A260 open symbols
A280 closed symbols
0.2
0.4
0.6
0.8
500
1000
1500
2000
2500
Con A alone
CT DNA + Con A
Con A alone

2/20/82.

	T4 DNA 0.28 mg/ml	Con A 5 mg/ml	Mg^{++} 1 M	Ca^{++} 10 mg/ml	PBS. (6.5)	R.T. incubation. 15' A260 of Sup.
①	0	0.5	10λ	10λ	0.48	
②	0.36	0	10λ	10λ	0.62	
③	↓	0.02	10λ	10λ	0.60	
④		0.05	10λ	10λ	0.57	
⑤		0.10	10λ	10λ	0.52	
⑥		0.15	10λ	10λ	0.47	
⑦		0.2	10λ	10λ	0.42	
⑧		0.5	10λ	10λ	0	
⑨	0	0	10λ	10λ	0.98	

Visible pptе pelletable

	15'	30'	60'	o/n at 4°
①	±	–	–	
②	–	–	–	
③	–	–	+	
④	–	–	+	
⑤	–	±	+	
⑥	–	±	+	
⑦	–	+	+	
⑧	+	+	+	
⑨	–	–	–	

DNA binding to concavallin A

Apriori assumptions.

1. Only glycosylated DNA will bind to con A.
2. Binding of glycosylated DNA is reversible bound DNA can be released by increase in pH
3. Binding of DNA will be inhibited by carbohydrates [which have stronger affinity for con A].

Experimental set-ups.

check for binding of glycosylated DNA to con A

a. precipitation.
b. affinity chromatography.

a. Precipitation.

checked by observing

① A_{260} post Rx of con A and DNA

~~chec~~

② radioactivity.

To check whether glycosylated DNA will bind to con A.

T4 DNA — T4 am 82 [44⁻62⁻] DNA
in 10 mM Tris pH 7.4
10 mM NaCl
A_{260} vs buffer

Con A — 5 mg/ml in PBS (pH 6.5)
[~99% pure protein
less than 0.1% CHO]
metal content at least
4 g ions [Mn^{++} + Ca^{++}] per g mole dimer.
In solution less dimeric form at ~~this~~ pH range used.

Exptl. protocol

To Fixed amt of T4 DNA is added ↑ amt of con A in reaction mixtures containing Ca^{++} and Mg^{++} and PBS (6.5).
The samples are spun ~~after 2 hrs~~ ~~at~~ after ~~ofn at 4°~~ 1 hour at room temp. and supernates measured for A_{260}
checked also for visible precipitate.

~~Use~~

1. Lectins bind sugars
   Lectin-binding to CHO ↑ c̄ sugar residues
   monosaccharides > disaccharides > oligo
2. DNA from phages T_4 ; T_6 is glycosylated

3. A priori assumptions.
   (a) T_4 DNA will bind to ~~Con A~~ s~~epharose~~ concanavallin A
   (b) Binding not inhibited by non-glycosylated DNA
   (c) Bound DNA quantitatively replaced by monosaccharides such as mannose

5×10^5

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×

3615 | 1078000000