7-26-76

1. Count 100μl of 7-21-1 (this is the HCOOH hydrolysate – brought up in 10% MeOH).

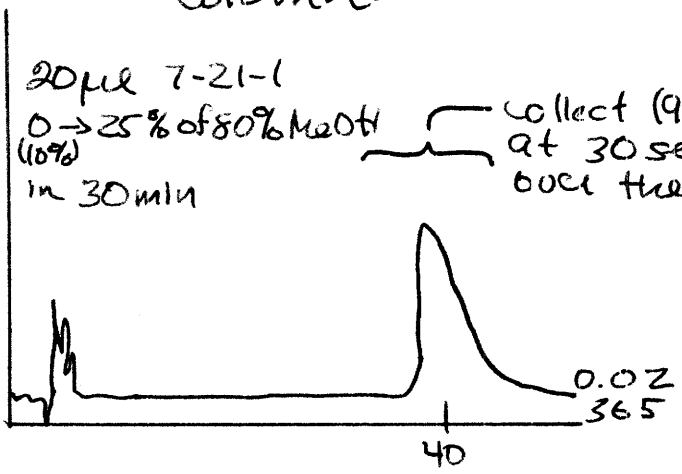
7/23 measurement 70,445 cpm / 50μl = 1408.9 cpm/μl

7/26 measurement 169,403 cpm / 100μl = 1694.0 cpm/μl

12.5ml in entire sample = 2.12×10^7

there is a 17% difference

2. Chromatogram of 7-21-1 on Reeve Angell Column-



fraction	cpm/μl
1	39.5
2	244.8
3	355.9
4	366.8
5	162.0
6	66.4
7	32.2
8	16.5
9	13.0
TOTAL	1299.12

(x20 = 25,982)

Compared with 1694 cpm/μl,

$\frac{1299}{1694} = 0.77 \Rightarrow$ 77% of counts on column were eluted in peak I

Observation: The resolution and efficiency of this column have severely degraded.

7-26-76 3. Sample 7-21-1 was then loaded onto the precolumn in (2) parts: 4.5 ml and 7.5 ml. Peak I was isolated in each case.

note peak I went on full scale on 0.50 in each case

- peak I vol. was ~ 25 ml, and most of the liquid was removed by rotary evaporation at 60°C (took ~ 45 min)
- sample was rinsed into grad test tube with 4.0 ml 10% MeOH

50 µl were counted = 37,103 cpm
=> 742.06 cpm/µl
=> 4000 x 742.06 = 2.968×10^6 cpm total in the sample from the 100 mg of DNA.

NOTE: a small (~ 10 units x.01 axis) peak was observed at 25 min - it was collected from one of the samples - but it had very little radioactivity.

4. Precolumn Effluent Processing

- 60 ml of effluent were collected from the effluent of the precolumn after the samples were loaded and washed on the PC.
- add 180 ml of EtOH (-20°C) to the effluent mixture Obs - turned only slightly cloudy - store in freezer.
- total vol = 240 ml; take 100 µl for counting

tot. vol only 60 ml ← ? 180 ml

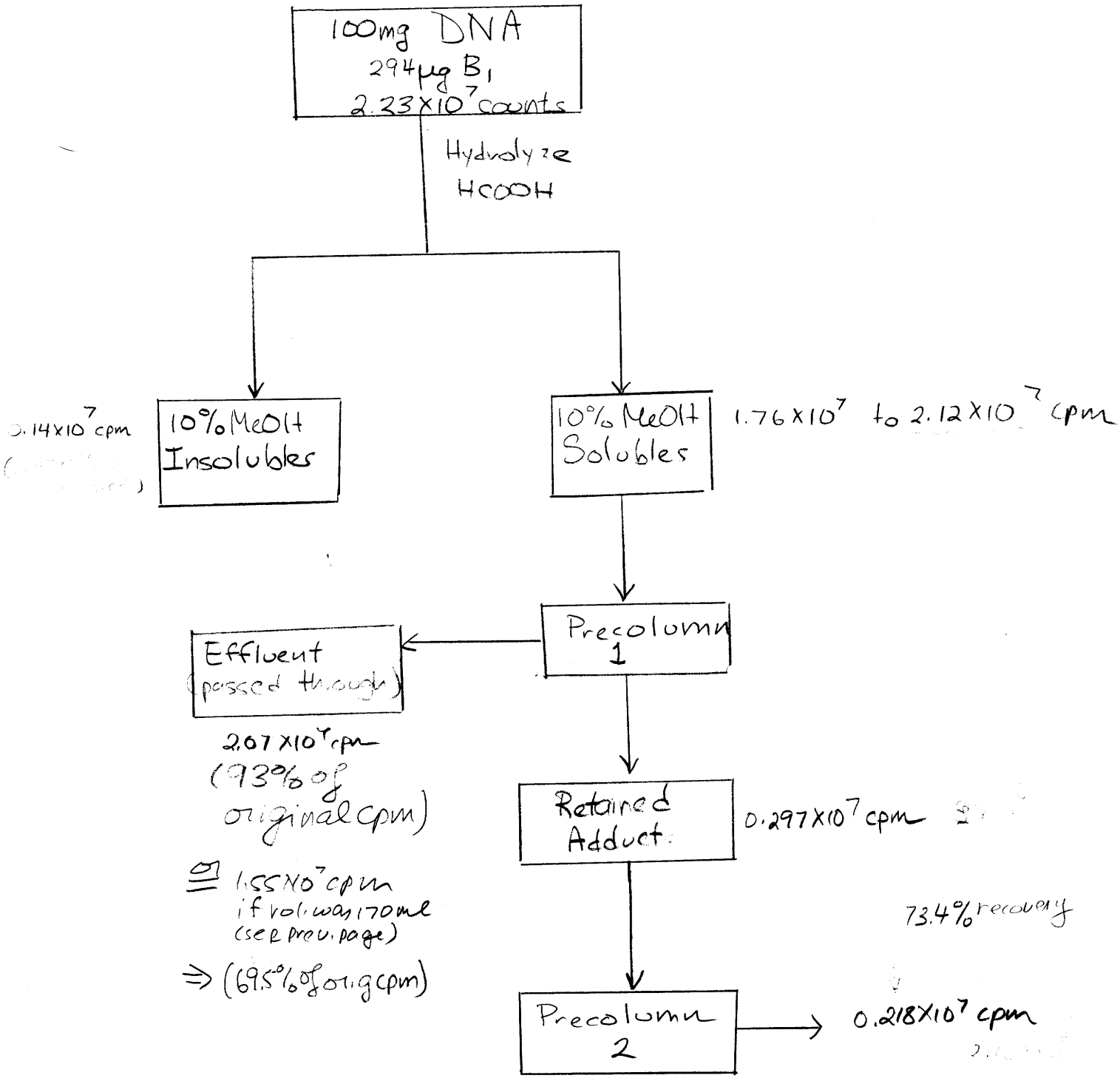
100 µl → 9142 cpm => 91.42 cpm/µl

=> 2.194×10^7 counts went through precol. note that this is large relative to the cpm collected.

recount - 50 µl → 4226.5 cpm => 2.029×10^7 !

↳ or 1.55×10^7 if vol = 170 ml

Flow Sheet on Peak 1 Isolation:



2-26-76 5. ~~Precolum~~
Purge of Analytical Column after Elution of Addact

- Column was purged with 80% MeOH - several strong peaks came out x0.10

total Vol = 13.0 ml
100 µl → 1122 cpm

⇒ 1.459×10^5 counts collected

Conclusions on Peak 1 isolation:

1. only 12% of the hydrolysate was recovered as semipurified peak 1 — this is much lower than expected — and I know that more than 70% of the hydrolysate radioactiv. was present as peak 1
2. Peak 1 probably was there, but it didn't stick to precolumn.

8-2-76 Peak 1 Isolation - 2nd Stage of Purification

- The sample is at 4 ml level in 10% MeOH
- add 4 ml H_2O ∴ conc. MeOH now 5%
- load on precolumn
- Put precolumn in Micromeritics and purge with 22% strong eluent (80% MeOH) = 17.6% MeOH
- Peak 1 eluted at 26 min - most of it was collected in a pear-shaped flask.

Volume: 8.7 ml
25 µl (µpipette): 6,152 cpm
50 µl ("): 12,621
25 µl (Hamilton Syr: 6213
} 250 cpm/µl