-21-76 Hydrolysis of 100 mg ~~R~~ DNA for For Rat I Isolation

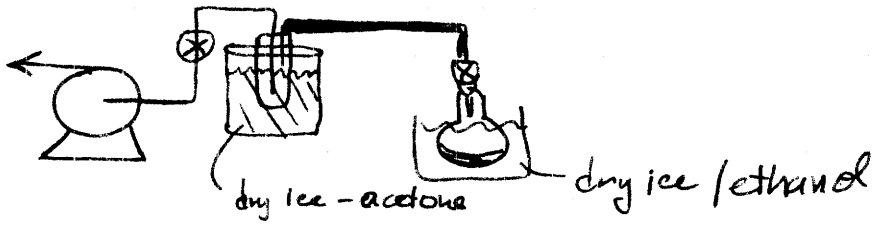
- The ~~conc~~ amount of DNA on one rod from JE-3 is ~10 mg (previous page)
- take 10 rods –
- put 20 ml (HCOOH (88%) into 50 ml beaker
- dissolve DNA from each stick into the HCOOH and let the beaker stand (60 min) – with sticks in it and intermittent stirring – for one hour.
- evaporate off HCOOH under N_2 –

Observation – after ~4 hours there was still about 3-4 ml left – the solution was quite viscous. The sample spent ~5 min at 45°C, while I tried to rotovap it.

→ did not freeze

- sample stored in freezer overnight – at ~4 ml level.
- freeze sample in dry ice-ethanol and try to lyophilize with this system:

The sample froze



- This system was good for keeping the sample in the solid state, but lyophilization proceeded very slowly
- sample was thawed, and kept at reduced P, but volume reduction again was slow.
- add 10 ml 10% MeOH to sample (which had a 2.5 ml volume)
- Cool in ice – lot of DNA started to ppt
- spin at 12K for 15 min in 40 rotor
- decant clear liquid, store in freezer.

SOLUBILIZATION OF RADIOACTIVITY

50 µl sampled out of 12.25 ml → good recovery
(actual original vol = 10 + 2.5 = 12.5 ml)

DILUTION FACTOR $\frac{12.5\ ml}{0.05\ ml} = 250$

total counts in 50 µl = 70,445 cpm
total counts in 12.5 ml = 1.76×10^7

from P 24, there are 223,250 cpm/mg DNA
⇒ 2.233×10^7 cpm in original sample

% Solubilization of Radioactivity $\frac{1.76 \times 10^7}{2.233 \times 10^7} 10^2 =$ **79%**

} could be adduct(s), degradation products, non-specifically or loosely-bound AFB, or metabolites.

PERCENT LIBERATION OF ADDUCT FROM DNA

amount submitted to HPLC: 50 µl (reps. 1/250 of sample)
total cpm in two adduct peaks: 49,239

⇒ (49239)(250) = 1.231×10^7 cpm in these peaks in the entire sample

% of original DNA radioactivity that is liberated as adducts : **55%**

note that hydrolyzed cond. were not opt for adduct lib.

% of soluble hydrolysate that is adducts : **70%**

The rest of the sol material may be AFB, degr. prod. or adduct still bound to soluble DNA.

Subject Peak 1 Isolation (cont) Instructor's Name

7-23-76 Sample 7-21-1 was thawed and prepared for analysis. It looked quite clear - clearer than I remember it looking last night.

- count 50 µl

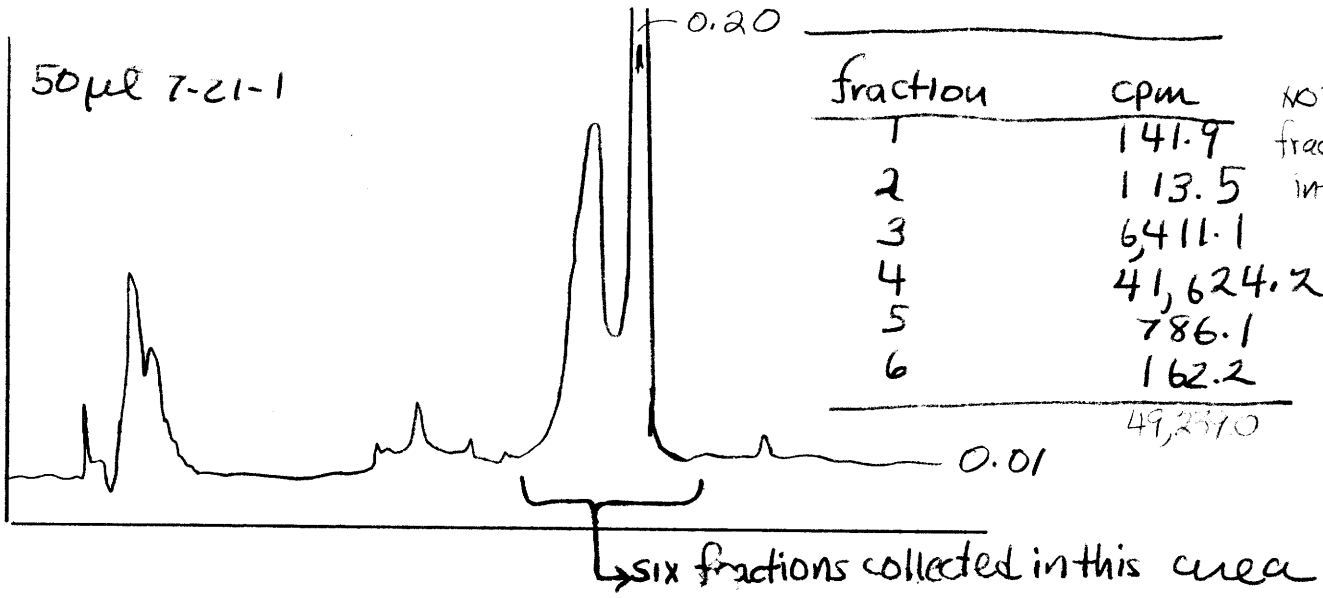
50 µl 7-21-1 at the 12.25 ml level:

281,788 counts/4 min = 70,445 cpm ~~[illegible] cpm~~

- Inject 50 µl into HPLC (waters Column)

40 min gradient from 10% to 80% MeOH
T=51, P=1150, V=1.0

Results: 79% of the radioactivity started with was in the liquid that was counted - the DNA (depurinated) largely was soluble in the 10% meOH, however.



fraction	cpm
1	141.9
2	113.5
3	6411.1
4	41,624.2
5	786.1
6	162.2
	49,237.0

NOTE: 30 sec fraction intervals

Subject Peak 1 Isolation (cont.) Instructor's Name

7-23-76 Counting "DNA" Pellet

A "DNA" pellet formed after centrifuging the cloudy material down that formed after addition of 10% MeOH. This was washed with MeOH, EtOH and then dissolved in H_2O : HCOOH (~ 1:2). 100 µl of the ~~solo~~ solution was counted

100 µl solubilized pellet : 143,644 c / 10 min
⇒ 14,364.4 cpm / 0.1 ml
total volume : ~ 10 ml (exact vol not meas)

⇒ 1.436×10^6 cpm in entire sample

~ THIS IS ~ 6% OF THE TOTAL CPM IN THE DNA ~ which isn't really enough to worry about. (note, a little may have been lost in the isolation, but not enough to make a huge difference).