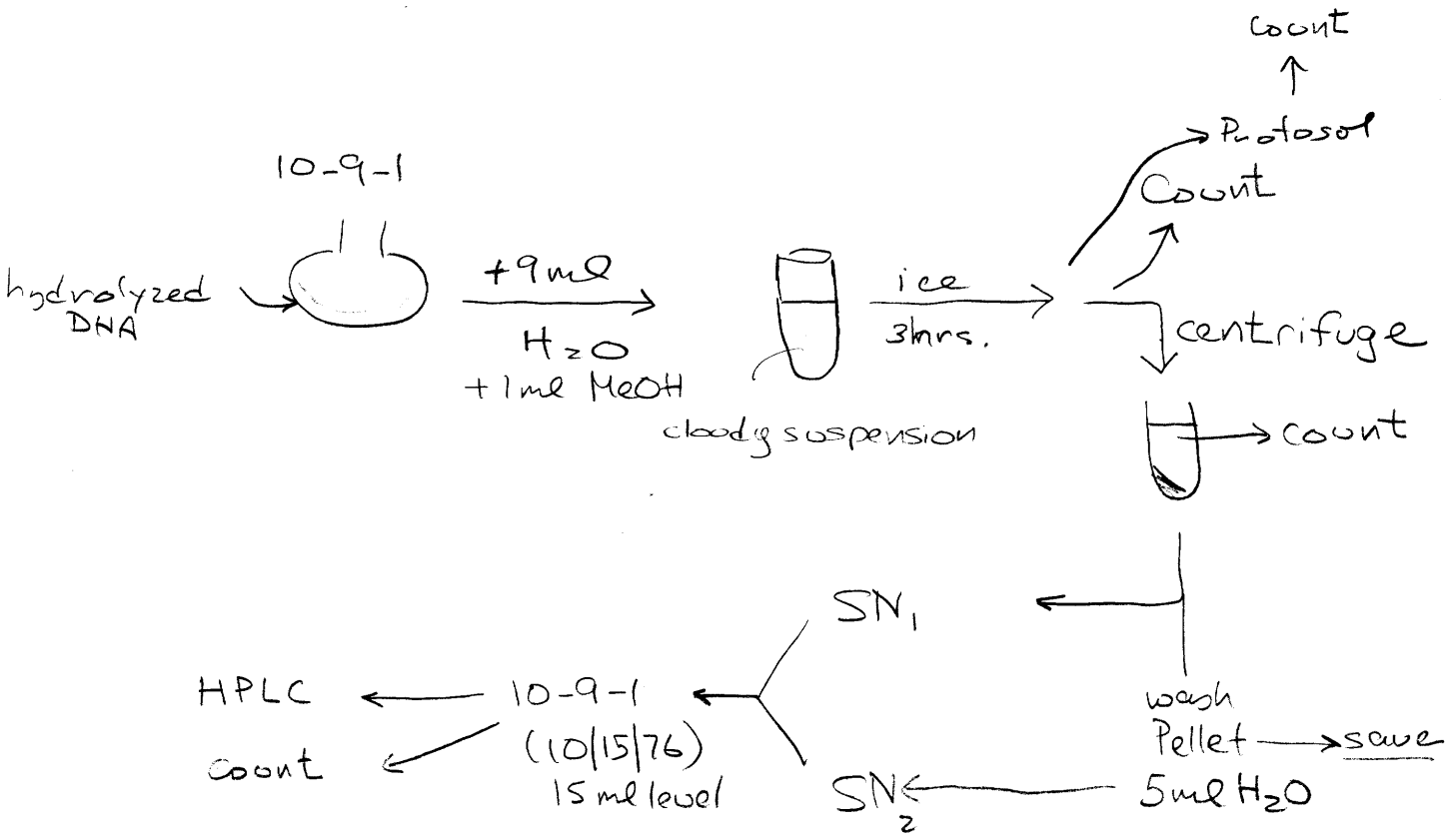
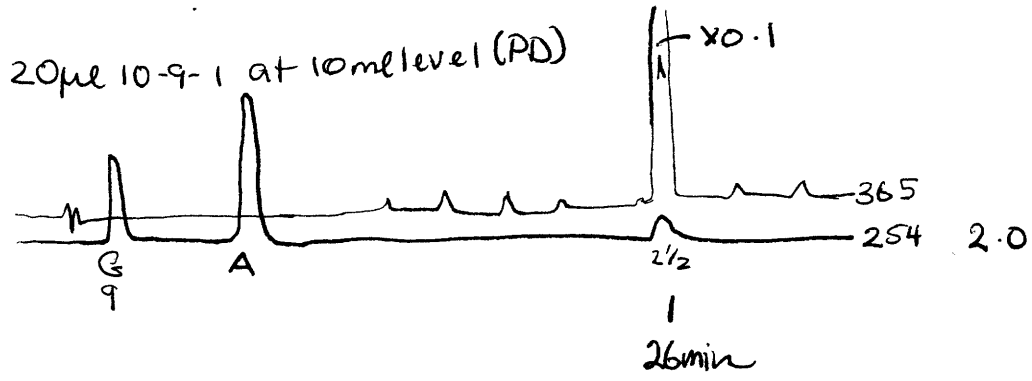

count
Protosol
Count
10-9-1
hydrolyzed DNA
+9ml
H2O
+1ml MeOH
ice
3hrs.
centrifuge
cloudy suspension
count
SN1
HPLC
10-9-1
(10|15|76)
15 ml level
count
wash
Pellet
save
SN2
5ml H2O


20µl 10-9-1 at 10ml level (PD)
x0.1
365
254
2.0
G
9
A
2½
1
26min

Subject JE-4 DNA: 10-9-1 Processing Instructor's Name

10-11-76 Lyophilization of 10-9-1

Sample 10-9-1 contained 1ml HCOOH and 1ml H_2O in a 10 ml flask. The contents (frozen originally) were rinsed into a fifty ml RB flask with 3ml H_2O (total vol ~ 5 ml)

-freeze, place in ice bath and lyophilize, as described previously.

-a white powder was obtained

10-15-76 Dissolution of 10-9-1 (done by Paul D.)

- See flow sheet on opposite page. The DNA initially was dissolved in 9 ml H_2O to yield a milky sospension. 1 ml MeOH was added and the mixture was held in an ice bath for 3+ hrs to ppt nucleic acids.
- after standing in ice, a portion was counted:

  100 µl : 44,000 counts/4 min
- after centrifugation:

  100 µl : 43,300 counts/4 min

10,825 cpm / 0.1 ml → X10 ml → 1.08 X10⁶ total cpm

- The phases were separated and the pellet was washed with 5 ml H_2O
- sample stored in refrigerator

HPLC Results:

G and A peaks were seen and a very large adduct peak (~1 ~50%) - there wasn't as much ^3H in the adduct as I'd expected - but haven't done the calculations yet.

10-16-76

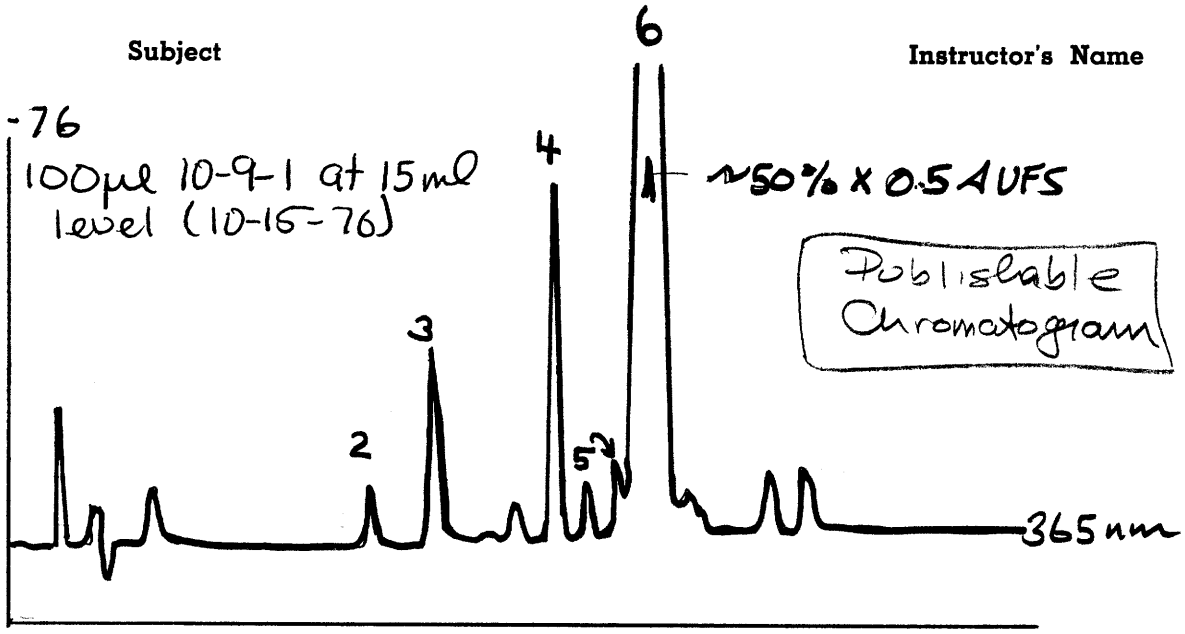
2 : 50 cpm
3 : ?
4 : 108 cpm
5 : 58 cpm
6a : 203 cpm
6b : 227 cpm

215 cpm $\times \frac{2\text{ml}}{0.1\text{ml}}$ (X20 dilution) = 4300 cpm collected from column.

note:

10-16-76

100μl 10-9-1 at 15ml level (10-16-76)



- collect 2-6; count all of 2-5 and ~~collect~~ count 100 μl (2ml total volume) of peak 6

10-16-76 Calculations:

Sample 10-9-1 (at 10 ml level): 1.083×10^5 cpm/ml

$\Rightarrow 1.083 \times 10^6$ total cpm

Sample 10-9-1 (at 15 ml level)

should be: $\frac{1.083 \times 10^6}{15} = 7.22 \times 10^4$ cpm/ml

I foond: 1553.5 cpm/20μl → 77.68 $\frac{\text{cpm}}{\mu\text{l}}$ = 7.77×10^4 cpm/ml

→ 7768 cpm

Inject 100μl 10-9-1 into LC (at 15 ml level)

peak 1 had 215 cpm/100μl of 2ml ⇒ 4300 cpm collected

note that $\frac{4300}{7768} \overset{\text{only}}{=} 55\%$ of injected ^3H was recovered in this peak.

10-16-70 Reversed-phase separation of tRNAs

samples 60% pure Phe tRNA (Avi)
unfractionated tRNA (thesis)

10-16-76

Rough estimation of Amount of Boond B, in Sample 10-9-1

100 µl of 10-9-1 (15 ml level) gave 55% on 0.5 A_{365} UFS.

∴ since this is 1/150th of sample

⟶ 37.5 A_{365} units

20 rods of DNA total ⟶ **750 A_{365} units**

now, Sample 8-3-1 (100µl) gave 9.7 µg addact with 0.2 AUFS, 70% full scale ⇒ 14% on 1 AUFS

$\frac{1.94\ \mu g}{14\%} \Rightarrow 13.9\ \mu g$ / full scale × 1.0

so. $\left(13.9\ \frac{\mu g}{AU}\right)(750\ AU) =$ **10.4 mg addact in sample**