Hydrolysis of 100 mg DNA for For Rak I Isolation

- The same 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 - with sticks in it and intermittent stirring - for one hour.
- Evaporate off HCOOH under N₂

Observation - after n 4 hours there was still about 3-4 ml left - the solution was quite viscous. The sample spent n 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% Me0H to sample (which had a 2.5 ml volume)
- Cool in ice - lot of DNA started to ppt
- Spin at 12 k for 15 min in 40 rotor
- Decant clean 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)

\[
\text{Solute factor} \quad \frac{12.5 \text{ ml}}{0.05 \text{ ml}} = 250
\]

Total counts in 50 µl = 70,445 cpm
Total counts in 12.5 ml = 1.76 \times 10^7

From P24, there are 223,250 cpm/mg DNA
\[ \Rightarrow 2.233 \times 10^7 \text{ cpm in original sample} \]

\[ \frac{1.76 \times 10^7}{2.233 \times 10^7} \times 100 = 79\% \]

% Solubilization could be adduct(s)
degradation products
non-specifically or loosely-bound AF, and metabolites.

PERCENT LIBERATION OF ADDUCT FROM DNA

Amount submitted to HPLC: 50 µl (reps. 1/60 of sample)
Total cpm in two adduct peaks: 49,239

\[ \frac{(49,239)(250)}{} = 1.231 \times 10^7 \text{ cpm in these peaks in the entire sample} \]

\[ \frac{55\%}{\text{of original DNA radioactivity that is liberated as adducts}} \]

\[ \frac{70\%}{\text{of soluble hydrolysate that is adducts}} \]

The rest of the solid material may be AFB, degr. prod., or adduct still bound to soluble DNA.
Sample 7-21-1 was thawed and prepared for analysis. It looked quite clean - cleaner than I remember it looking last night.

- Count 50 µl

50 µl 7-21-1 at the 12.25 ml level:

70,446 cpn
281,778 counts/4 min = 70,446 cpn

- Inject 50 µl into HPLC (Waters Column)

40 min gradient from 10% to 80% Me.OH
T=51, F=1130, V=1.0

Results: 79% of the radioactivity started with was in the liquid that was counted – the DNA (deproteinated) largely was soluble in the 10% MeOH; however.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>141.9</td>
</tr>
<tr>
<td>2</td>
<td>113.5</td>
</tr>
<tr>
<td>3</td>
<td>641.1</td>
</tr>
<tr>
<td>4</td>
<td>41,624.2</td>
</tr>
<tr>
<td>5</td>
<td>786.1</td>
</tr>
<tr>
<td>6</td>
<td>162.2</td>
</tr>
</tbody>
</table>

NOTE: 30 sec fraction intervals

60 µl 7-21-1

SIX FRACTIONS COLLECTED IN THIS AREA
7-23-76 Counting DNA Pellet

A "DNA" pellet formed after centrifuging the cloudy material formed 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 solution was counted.

\[
\begin{align*}
100\mu l & \text{ solubilized pellet: } 14,3,644 \text{ cpm/10 min} \\
& \Rightarrow 1,436,644 \text{ cpm/0.1ml} \\
\text{total volume: } & \approx 10\mu l \text{ (exact vol not meas.)} \\
& \Rightarrow 1,436 \times 10^6 \text{ cpm in entire sample}
\end{align*}
\]

--- This is \( \approx 6\% \) 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).