

5/16/69

Ideas for NB exp.

1. Split tumor growing. Each as separate

1. Effect neurohormones (diff. conc.) on <sup>cell growth</sup> levels

- 2) cell growth
- 3) cell type
- 4) act. potential

a) Compds for Tyrosine Hydroxylase (NB-Dopa oxidase) COMT, MAO, etc.

- 1) Norep.
- 2) Ep.
- 3) Tyrosine
- 4) DiOH (DOPA)
- 5) DiOH-ethylester (DOPA ester)

- 6) ~~MAO~~ MAO inhibitors - effect on COMT
- 7) 3'-5' - cyclic AMP
- 8) " " butyl AMP

10) All known metalloids of E + more

11) Good inhibitors of specific enzymes  
 Effect of blocked path on enzyme in pathway + effect of metal

- effect of hormones
- 12) Ac Chl
  - 13) Glu
  - 14) GABA
  - 15) Histamine
  - 16) Serotonin 5-OH-trypt

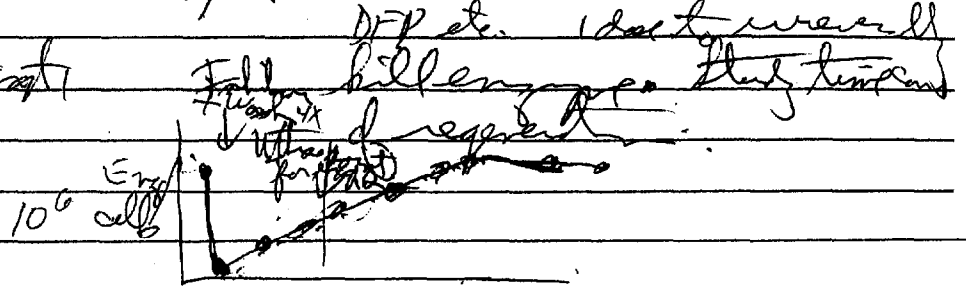
butyl feed

h) Cholin acetylase

- 3) True + False Cholinesterase
4. Cholinesterase Vesicles
5. " " " protein (soluble)

1. Acetyl Cholin
2. Cholin
3. acetyl
4. Butyl Cholin
5. Specific inhibitor of cholinesterase

\* 2) Expt 1



Myers

Disturbance of cells with constant rate of activity or ~~proportion~~ fall.

1) Expt 2

Effect of ~~enzyme~~ on rate of enzyme ~~activity~~ at 50% reagent ~~activity~~ (below).

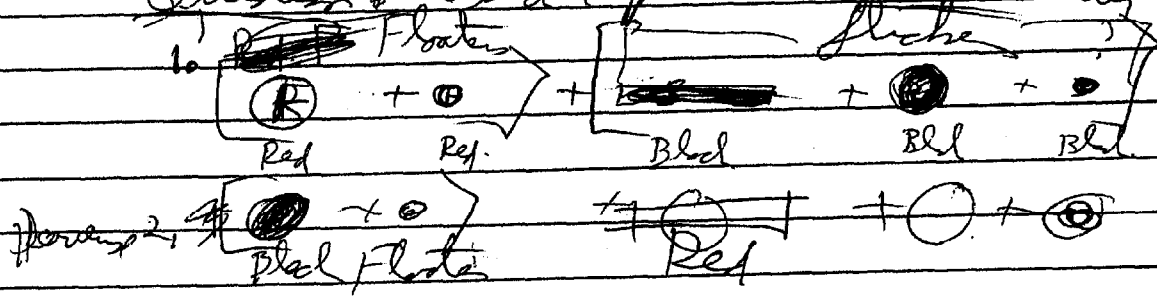
1.

~~Mark float~~

**\*\* Idea** • Do different types of cells that stick & look like they have synapses/divide?   
 • Deleted from plates become floaters?

review

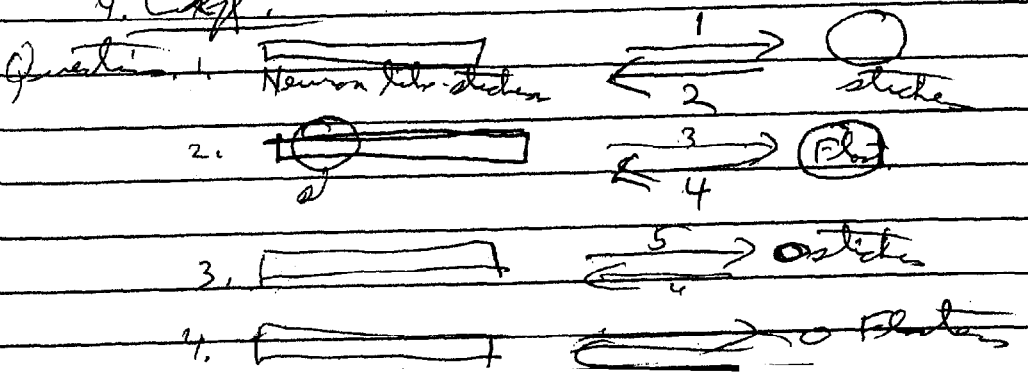
Mark floaters with carrier red   
 Mark ~~different~~ like stick in plate   
 Question: Which types are interconnected?



3. ~~Assay~~

- 1) ~~Test~~ 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
- 2) Number balls, each type float
- 3) ↓ of blood ... " float

4. Expt.



5. Cell Types

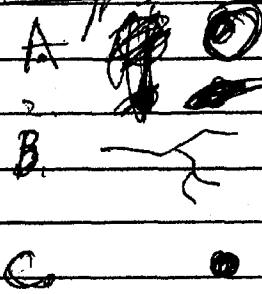
1. ~~Large~~ Cell

- 1. Round attached to flesh
- 2. Round attached to cell
- 3. Round (single floater)
- 4. Branched attached to flesh
- 5. " " " cells

2. Small cell

- 1. Float - angle
- 2. " - all

Cell Types

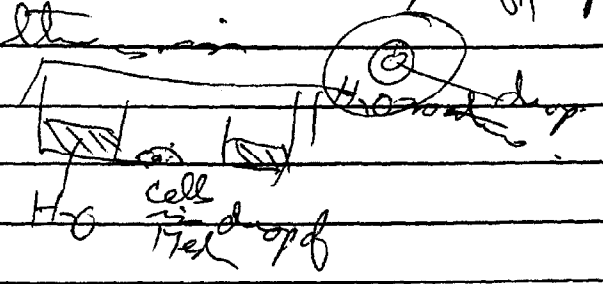


Cell Types	<del>Notes</del>	B	C	Flesh
A	+	[?]		[?]
B	+	[?]	+	+
C		[?]	?	

\*\*\*6. Quantities

- 1) Stand with log phase only. Distinct
- 2) " " " " only
- 3) " " " " only ↓

~~\*\*\*\*~~ <sup>immediately</sup> Do with hand-picked cells. Minimum <sup>cells 5000</sup>  
 Arrows. (-100 cell and type of drop) <sup>cells 5000</sup>  
 Arrow culture in



or if can't see, make a scope, use  
 rose glass or Maxima  
 or cover slip on drop.

50% <sup>cells</sup> conditioned medium + 50% Nutrient

1) ~~Flt~~ Popult

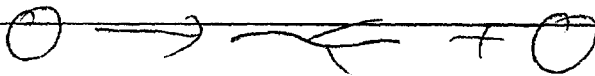
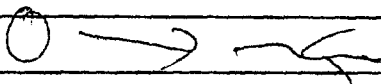
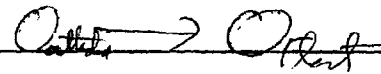
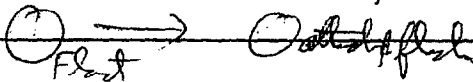
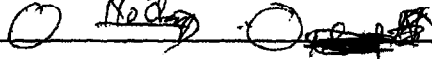
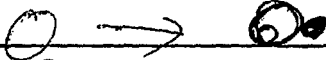
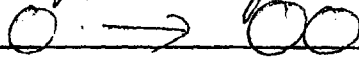
2) drop.

3) attached to gel + small cover slip. Pick off all other cell types.

~~Account~~ <sup>cell types</sup> Time Course 0T, 1, 2, 3, 4, 5, etc.

- 1) <sup>100</sup> Cell Number.
- 2) Number Flashes  $\Delta$  O
- 3) " " B  $\bullet$
- 4) " " ~~Flashes~~ - used hand doublets etc.
- 5) " " ~~sticks~~ C  $\bar{r}$
- 6) " " O
- 7) assessments O

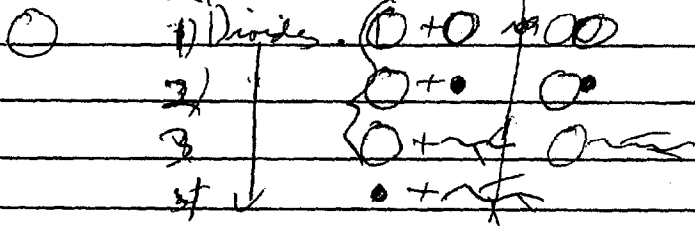
Q. 8) Single cell extra in drop. ~~all~~



Summary

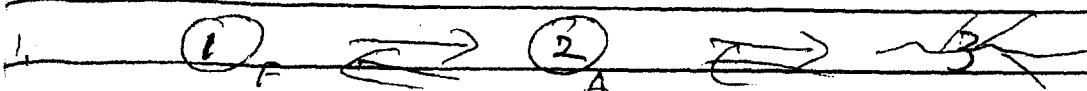
- |                |                   |    |
|----------------|-------------------|----|
| 1) No division | ○ flat with       | 90 |
| 2) No          | ○                 | 70 |
| 3) No division | ○ <del>flat</del> | 90 |

Cell A

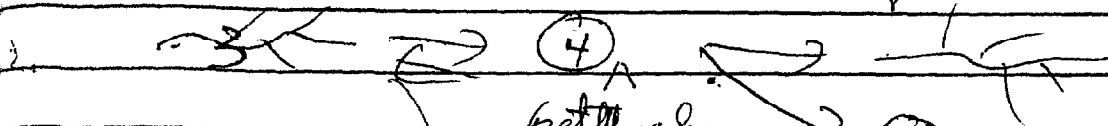


Cell B

- Nodules
1. ● <sub>flat</sub>
  2. ○
  3. ~~flat~~
- Downs

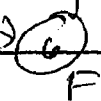


are different frequency

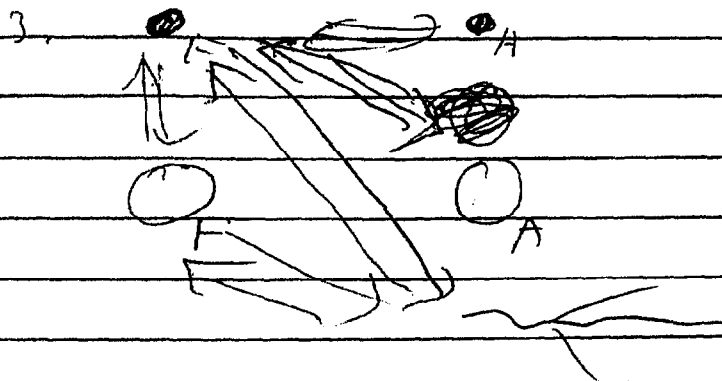


get the by shaking particles

F are the same?

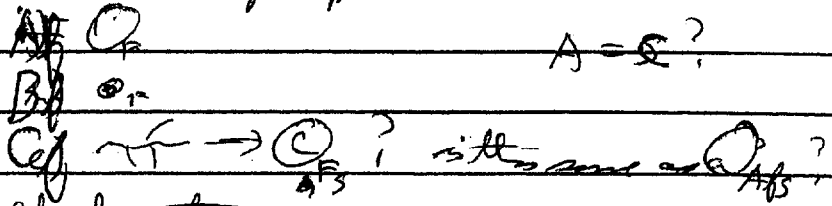


Flat      at the

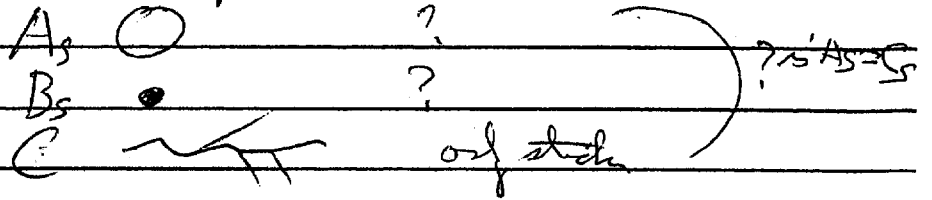


Summary Questions

1. How many <sup>gross</sup> cell types? 3 or 4  
 1. 2-3 Floating types.



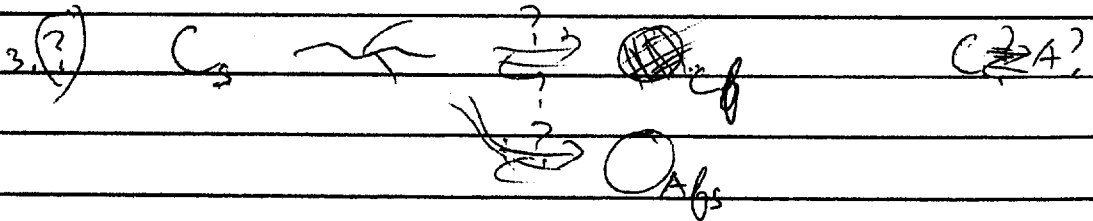
2. 33 stake types



Minim's Cell types = 2-3

1. A<sub>fs</sub>  $\circ$

2. B<sub>fs</sub>  $\odot$

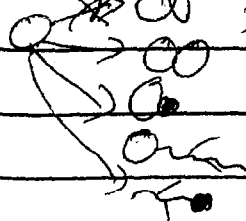


2. How many (%) of each cell type? Don't!  
 ) ~~of~~ local cells.  
 ) ~~of~~ stake cells.

3. Which <sup>cell</sup> types divide? % dividing / 2x hrs. Identify all products.  
 A  
 B  
 C

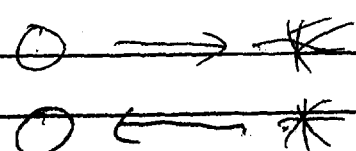
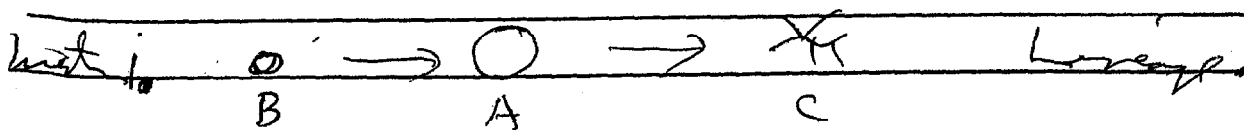


4. Lineage of cell types



Division

	-	NC	?	+?%
	-	<u>7, 2, 2, 2</u>	NC	?
	-	+	?	NC



$$\begin{aligned}
 A &\rightarrow A+B+C \\
 &\rightarrow A+B \\
 &\quad B+C
 \end{aligned}$$

~~Do not~~ ~~XXXX~~ ~~XXXX~~ ~~XXXX~~ ~~XXXX~~ ~~XXXX~~

**Cell Differentiation**

Possible Combinations of Cell Types

3 samples	A	1 of N	±
2	B	1 of N	±
3	C	1 of N	±
<del>6 samples</del>	AB	2 of N	+
5	AC	2 of N	+
6	AA	2 of N	+
7	<del>BB</del>	3 of N	+
8	BC		
9	CC		

Cell Differentiation

2) Reversal of cell type
3) Cell Division
4) Cell Death
5) Cell Migration
6) Cell Fusion
7) Cell Proliferation
8) Cell Differentiation
9) Cell Apoptosis
10) Cell Transcription

10 samples

Combinatorial from

1) 10 samples

10 types

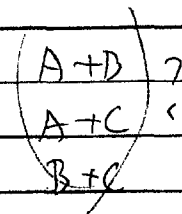
AAA	BBB	CCC
AAB	BBC	CCA
ABB	BCC	CAA

A →

B →

C →

- Manufacturing requirements
- 1) 50 cells / day
  - 2) 50 B "
  - 3) 50 C " on day
  - 4) 50 Comp. → chat



Start with 1 cell / ~~misconception~~ ~~the~~ ~~condition~~ ~~medium~~

- 1-100 Add/day
- 100 B / " "
- 100 C / " " recall that on day covering

Examination . 01 1, 2, 3, 5, 7 10 15

Name of cell type

Type of cell type

Maxim Number of divisions and all types  
\* ~~Expt 2~~ → Co ~~Expt 2~~ → Co ~~Expt 2~~ →  
Efficiency for full cell type

\*\*\* Expt 3  
6 different types of A  
B  
C } are cells selected!

\* Expt 2 + Cloning for Neutrophils

- 1. A<sub>111</sub>
  - 2. B<sub>111</sub>
  - 3. C<sub>111</sub>
- Start with A+B+C cells

\*\*\* Any Clones?

- No. Cells per plate
- 1. % of cells / hrs
  - 2. % of cell types
  - 3. % " " " " " " " "

\*\*\* Do all cells frequent mitose?

- 1. B " " " " " "
- 2. C " " " " " "
- 3. A<sub>111</sub> / A<sub>222</sub> / A<sub>333</sub> Number of Mitoses / hrs
- 4. B " " " " " "
- 5. C " " " " " "
- 6. Total Mitoses

Quest 1. Treatment types

- 1. % A
- 2. % B
- 3. No treated
- 4. % A, B, C
- 5. No

Expt 4. Cell affinities. Log phase  
Don't try mixing  
Get results for expt 1

affinity

Use that also + all

	A	B	C
A	+	+	?
B	+	?	?
C	+	?	+

light, double, triple from size, color, identity, all types  
 50 " A  
 50 " B  
 50 " C

1. Control & number cell.
2. Number aggregates
3. ~~Double cell / aggregate~~
4. For each aggregate  
 1. Number or partial or complete double  
 2. Number A cell  
 3. " B "

\* 5. Doublets are excellent to each kind of doublet

- 1 AA +
- 2 AB + } Floaters, Repeat statistics
- 3 BB 0
- 4-6 Repeat 1-3 } tubes
- 7 CC +
- 8 CA ? } tubes
- 9 CB ?
- 10 C<sub>2</sub>C<sub>1</sub> → C<sub>1</sub>C<sub>2</sub> } Reverse

4. Bright cells

Quest  
 4) Rat of A to C formula, the number of  
 C cells. <sup>2nd object</sup> ~~Indicates for 3~~ <sup>on the</sup> ~~not~~  
~~not~~ ~~by~~ ~~sup~~ ~~that~~ ~~is~~ ~~not~~ ~~the~~ ~~same~~  
 Just attend cells carefully.

data in?

\*\*\* Quest <sup>100%</sup> for working cell

1) Brown + light

1) Total	100%	Control (No Brown)
2) A cells / Total	70	
3) B " / " "	30	
C C " / - "	0	

90 A cells  
 B "  
 C "

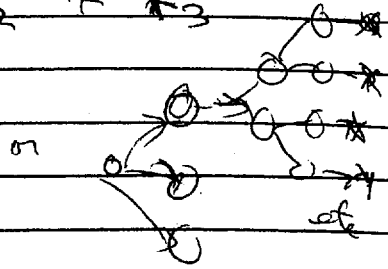
Repeat + Brown  
 Determine cell set  
 Determine Number of cells

C may be end of the cell.

Can 1 cell give rise to diff cell types.  
If so, which cell?

Model 1  $0 \rightarrow \bigcirc \rightarrow *_1 + *_2 + *_3$

Model 2  $0 \rightarrow \begin{matrix} \bigcirc \rightarrow *_1 \\ + \\ \bigcirc \rightarrow *_2 \\ + \\ \bigcirc \rightarrow *_3 \end{matrix}$



Model 3  $\begin{matrix} \bigcirc \rightarrow *_1 \\ \downarrow \\ \bigcirc \rightarrow *_2 \\ \downarrow \\ \bigcirc \rightarrow *_3 \end{matrix}$

Nb of adjacent

Model 4  $0 \rightarrow \textcircled{0} \rightarrow *_{\text{in one cell}}$

Steps in different sequence steps  
 7. ~~Steps~~ different. All modified steps  
 Is cell division required?  
 Steps do differ in sequence, time  
 Reversibility and diff steps

5. Effect all core % A

B

C

OT 1, 2, 3, 4, 5 Days etc.

4. Am plates vs sticks  
Separate all with core. Can trace  
originals.

Put edge ~~to~~ ~~left~~ ~~detached~~ ~~\*~~ from log  
again, ~~to~~ ~~+~~ ~~to~~ ~~keep~~ ~~log~~  
to insert  
Core A  
B  
C  
Tilt

2) ~~to~~ ~~3~~ ~~to~~ A  
B  
C

3) ~~to~~ ~~to~~ ~~to~~ H  
D  
C

10. 17 pages - general and cell type. ~~done~~

11. ~~done~~ Number along former

- 1. Aggr. a) The circle all aggr.
- b) triple triple triple (1/2 grain x)
- Multibeam multi
- + all fragments of grain
- Circle all triple aggr.

12. ~~done~~ c) Pascal thin diff sig mat.

~~done~~ 12. Roger - Freeze several hundred inches NB  
of 1st test inches. Freeze → thin  
EOI

~~done~~ 13. little to my EOI format

14. Est curve fall and before

15. Cont

16. Flake on own abs

17. Small bricks



Hil

12. Identify C<sup>14</sup> adding 1) interval. }  
 How much? } 2) extend } - extend  
 3/4 } } - extend  
 } } - extend

13. Base Histone  
 " histone  
 " Citab

13. ~~Identify~~ of structural forms that is sedimentally? (K.A. ad.) ant. 7/9

14. Identify differential at top? fill  
 Conditions for differentiation

1. 17ed
2. Log growth no sth
3. Don't
4. thick all different
5. Cells
6. Light touch of top
7. Put all on plate with little medium  
 no serum for 24 hrs with  
 2% stud then dilute
8. Agon
9. Airy cultures
10. Cells
11. Suspension cultures
15. Rb

- 16. Neurospora also glucose st:
- 17. Hormones also top of aggregates with
- ~~18. Flucl Horde st:~~ aggregates with aggregates with
- 19. Diff cell st: like like
- 20. Diff cell

Beautiful test in for control inhibit  
st: with no glucose  
also for high cell density without acid  
front

15) Gen all A Nematosporia  
B  
C

Supplies  
No Nuclei  
simple alms just A, B, detached round C

16.) Radiation H3T to  
identify during my cell tryp

$\sim 2.5 \times 10^{-6}$   $\mu\text{g}$  Thymine / lymphoid cell.

$\sim 10^{-5}$   $\mu\text{g}$  DNA / lymphoid cell.

Assume 20 C/molecule  $\text{H}^3\text{T}$

20,000  $\mu\text{C}$  /  $\mu\text{mole}$

1  $\mu\text{mole} \sim 350 \mu\text{g}$

20  $\mu\text{C}$  /  $\mu\text{mole}$

1  $\mu\text{C} \sim 0.35 \mu\text{g}$

0.002  $\mu\text{C}$  /  $\mu\text{mole}$

1  $\mu\text{C} \sim 0.00035$

needs  $\sim 150$  cells.

$\sim 35 \times 10^{-5}$

$\sim 0.0001$   $\mu\text{C}$  / cell

$10^{-4}$   $\mu\text{C}$  / cell

$2 \times 10^6$   $\mu\text{C}$  /  $\mu\text{C}$

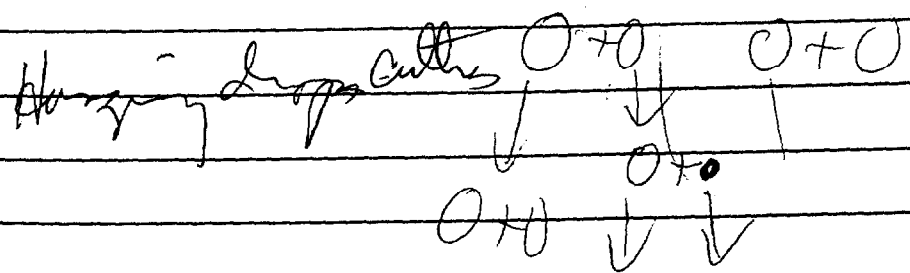
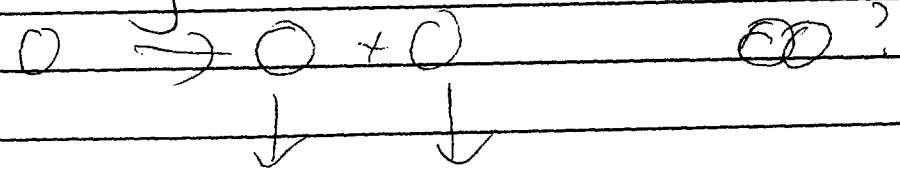
~~20 cells  $\sim 0.5 \times 10^6$   $\mu\text{C}$~~

$\sim 50$   $\mu\text{C}$  T / cell at 25% cell

20  $\mu\text{C}$  /  $\mu\text{mole}$

or 100  $\mu\text{C}$  T / " " " " if 40%  $\mu\text{C}$  /  $\mu\text{mole}$

17) For  $\text{H}^3$  incorporation of DNA in cells,  
Must separate  $\text{H}^3$  cells & incubate  
separately.



Memorized all.

16) final cell est. test  
 2000 cells/ml. 1) ~~1) 1000~~  
 2) ~~1) 1000~~  
 3) ~~1) 1000~~  
 4) ~~1) 1000~~  
 etc.

then identify the <sup>drop count</sup> fish No. with  
 1 A cell  
 1 B cell  
~~1 C cell~~

Forget about the drops  
 or, Put drops on ~~piece of cover slip~~  
 & transfer app. to drops tank  
 for est.

19. To collect ~~portion~~ of ~~cells~~  
 in ~~cover slip~~ ~~slide~~  
 I collect cells that ~~dry~~

