11/6/67

*** Idea *

1) Get dividing neurons in vitro by fusing (cell hybrids) neurons + growing cell line via UV inactivated Sendai or Parainfluenza type 3 virus.

Need purified fusing factor from virus

a) Cloning in vivo
1) Ascites tumor + Neuron + Virus in peritoneal cavity
2) Same solid
3) In vitro

b) Assay or select for line until can
1) Still synthesize / secrete neurohormones. Synthetic function still intact.
2) Still react to neurohormones. Receptor function intact
Ultimate 3) Still propagate 1 cell type or 2 cell types [illegible]

+ 2) Use UV inactivated Sendai to fuse presynaptic ~~isolated pinched off~~ terminals. Get functional circuits.
Build diff kinds of circuits ~~[illegible]~~ mixing diff purified preps.

3) ~~Isolate~~ propagate 1 kind of neuron. Fuse cells (very conc.).
1) Look for very large cells (syncytium). [illegible] or
2) What is maximum size of syncytium possible.

3) ~~Selection technique~~: Neural Function
~~No selection, Many clones~~
1) ~~Produce neurohormone~~. Excrete
1). AC Choline, ~~Have Chol. Esterase in agar. (Make black ppt of choline Acetate)~~. Dye, Turn color ~~due~~ to acid production when Ach hydrolyzed

2) Form synapses. — Morphology

3) Uptake Neurohormone
a) ~~Hormone impregnated~~

4) ~~Replica plate~~ (or use 2 thin agar plts) ~~Sensitivity to~~ inhibitors of neurons. 1) Tetrodoxin
2) ~~Eserine~~

Use filter (paper) under agar. Remove paper after clones grown. Paper will pick up diffused hormones.

5). ~~Stain part of culture~~ — Neuron stains

3) Make colored Neurons for use in tracing connections. Fuse with pigmented cell line. Retinal pigment cells in culture.
Melanoma.

a) Use original technique, put cells very dilute, fed, certain flourophores which will get inside

4) Red Blood Cells (- nucleus but large) + Neurons.

~~5)~~

4) Have 1) micro electrodes, at fixed sites, in large wounds + Neurons + Virus.
2) Dye in small wounds. Uptake of dye.
) Method for fixing cells to stated sites which are monitored.

5) Try Embryonic Neuroblasts + Invitro Cell line + Virus