first visit to — Tübingen

Change of interest: apologies

An Approach to Developmental Biology 23 Nov. '70

apologies for only visiting developmental biologists

molecular biology — what lessons can we carry over to cell biology?

dangers — Hinshelwood.

the "phase" of development ie one (family of) organism(s), especially convenient experimentally, — then apply results to other cases.

our approach — divide into different problems, choose one organism for each one.

what problems?

① classical molecular biology of eucaryotes. repetitive DNA nuclear RNA puffing, ~~etc~~ excretion of proteins etc.

② membranes : ~~[illegible]~~

③ mitosis. ~~[illegible]~~

④ cell movement — how it moves at all. — why it moves in a particular direction.

⑤ cell shape

⑥ ~~co~~ cell contact phenomenon

⑦ ~~[illegible]~~ hormones ie chemical signals between cells.

⑧ "positional information"

Role of genetics was very useful in molecular biology

may hint at "logic" of situation.

~~[illegible]~~ almost certainly useful as a tool when the right conceptual framework has been found.

but not necessarily always useful.

Kempstone . Ampthill Road

Bed. General Hospital

South Lane.

Outpatient

Clinic 4

South of River

Then left when right in

PP|CRI|E|1|18|14

Level of explanation for cell biology

not always necessary to go right down to the molecular level.

eg. existence of enzymes – may not need to know exact molecular mechanism of enzyme action.

but usually useful to go down one level in order to prove a ~~mech~~ postulated mechanism.

moreover experimental techniques at the molecular level ~~more~~ very powerful

eg. finger-printing to show if two proteins are related.

Examples of our work

Sydney Brenner (outline only)

small soil-nematode nematodes (caenorhabditis)

~ 1 mm long – 4 day life cycle – 100 to 200 pg/day
(egg, moults.

Grow on – E. coli – defined medium:

– 3 months cool – indefinitely in liquid N_2

diploid $n=6$

Chose a self-fertilising hermaphrodite. – occasional males to transfer gene

Summary

genetics – excellent – so behavioural genes.

neuroanatomy – very hard work.

behaviour – ~~not yet~~ only described not measured

embryology – not yet started; looks difficult (impermeable egg, etc)

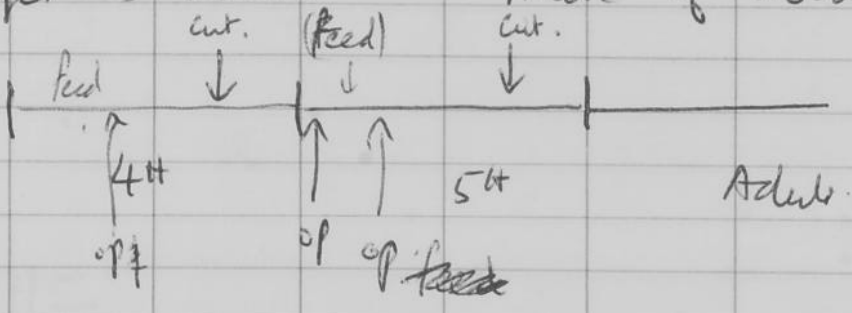
Insect cuticle mainly our own work. (3)

Peter Lawrence
(Mary Munro) Wigglesworth.

Michael Locke Rhodnius 3 stages — S.

5 larval stages → adult meal of blood

moult



ripples only on adult body — S

bristles rather sparse.

cut and splice : only get a snapshot.

move sideways, — S

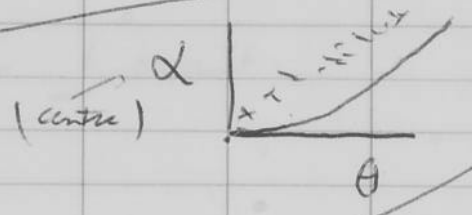
move ↕ — 2S

90° — 2S

ie some "averaging"

Simple model : source, sink, gradient (segmentally repeated)
concentration — gradient = greatest slope — wrinkles — pseudocontours
90° + diffusion : pattern depends on time.

measuring angles of pattern — S



SONK model : allow infinite time.
"pumping" constant. one parameter.
better fit.

what does α depend on : time ? double adult
moulting
? cell "division" ? — doubling.
computer simulation.

SLIDES

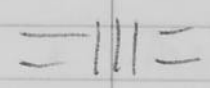
(1) Rhodonius - 3 stages

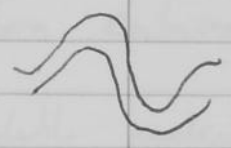
(2) " - body

(3) ⟷

(4) ↕ equiv.

(5) ↕ diff.

(6) 90 

(7) 90° 

(8) ~~Exp~~ 90° Sink, expr. diff.

(9) 90° Sink - relaxed.

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cont ~~but~~ ~~th~~ Thus 90° ok.

~~Computer sim~~ but ~~q~~ 180° result asymmetrical.

but not too asymmetrically after 1 result.

trying simulation.

Source model — but source-sink appears stronger.

Cell motility — mainly work of others
but rapidly developing.

old speculation that due to an actin-myosin system.

Hugh Huxley — ~~colloid~~ acellular slime mould.
extracted actin
decorated actin with rabbit heavy meromyosin.
angle, spacing, ATP diss.

Holtzer did it in glycerated cells — chick
+ then decorated. [eg fibroblasts, neuroblasts etc.]

Denis Bray + Dick Fine

from embryonic brain of chick — decorated with Hugh Huxley

two soluble proteins there in large amounts
— one (55,000) binds colchicine \ different
— one (48,000) may be actin — finger-prints.

Cytochalasin B Carter (Nature, Jan 1967)

mould: stops movement of fibroblasts — quick
— reversible.
tends to flatten cells.
— produces bi (multi)nucleate cells.

Wessells + co-worker (recent PNAS)

stops movement of growth-cone of nerve cells
— confirmed by us
etc.

not yet known for — acellular slime moulds
— plants
etc.

claim disorganization.

Thus two aims (1) find (molecular) sight of action of cytochalasin in the test-tube
(2) natural history of fibrillar molecules in cells.

Reason for meeting: to initiate a general discussion, based on the private discussions held earlier.

our interests

mainly development (incl. nervous system)

also, but less so at the moment, action of ~~[illegible]~~ nervous system.

approach

① avoid classical problems.

② break problem up. eg. classical mol. biol. (for enc.)
positional information.
~~[illegible]~~ smelling "gradients"
~~[illegible]~~ cell movement
cell shape
cell division
~~[illegible]~~ components: cell surface,
membrane component eg. special lipids permeation
fibrillar elements.

choose "best" system for each sub-problem
(but, are expected dividends).

The perfect problem
- significant
- no long investment
= techniques available, or easily devised
- "interesting" results "certain"
- no one else working.

Our work: ours immune system

no-one else working ① nematode, heavy investment.
~~[illegible]~~ results - behaviour, pol.
- development ?

② insect cuticle + arabeanine } predi emphasis ok
the hill - difficult ??

others working
- fibrillar elements - importance of ~~[illegible]~~ antibiotics
- cell fusion
- ~~[illegible]~~ cell surface
- BVdR

level at which answer required

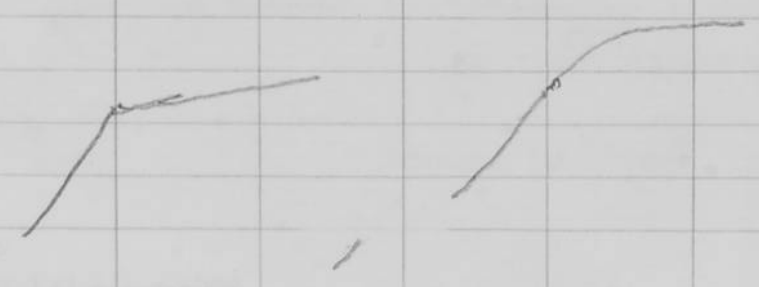
no point in molecular information unless it helps at the cellular level

at this stage

not always easy to see how deep to probe

in ~~any case~~

(ultimately all molecular processes are of interest)



(1)

embryology of multicellular organisms

many problems – eg. gene control – nucleic acid?
hormones.
cell migration eg. pigment cells.
mitosis; control of.

but also how to determine shape
and specific connections eg. ~~nerve~~ nerves.

neuronal specificity

Sperry's early experiments on adult newts: rotated eyes.
Studied ① behaviour
② histology

Gaze & Jacobson, on developing Xenopus.
① early
② later.
Studied by electrodes in tectum
+ visual stimulation.

found diff. times for the two axes.

ie. not due to function.
postulated two systems of coordinates in eye

[recent results on ipsilateral connections]

How does one set up "coordinates"?

① ~~most~~ phase model of Morrell Cohen + Brian Goodwin.
ie send a constant velocity signal.

② "gradient" models. [historical – got a bad name]
~~ie~~ for example, due to diffusion.

Source + sink – reading concentrations.
never mind how.

(2)

is diffusion fast enough? random walk ~ $\frac{L^2}{T}$

values of D. water 20°C. small molecule. 5×10^{-6}

small protein 1×10^{-6}

viscosity in cell? between cells?

times involved – prob. a few hours.

suggest distances about 1 mm.

ie basic theorem. (Spetsai)

Wolpert statements:

typically 50 cells. – linearly ~ 500μ = ½ mm

(up to 100)

Saze quoted 20 cells.

Note however that it is the process

many have both ie signals for big distances.

– eg. ~~stone~~ colonial acrobe? (– mushroom growth)

ie. diffusion does mean ~~mean~~ an actual diffusion of a marked molecule.

really bilateral interaction ~ random walk

v. one directional interaction – wave

~~other explication~~

diffusion models (not ie. waves)

~ pumping mechanisms.

– all cells ~~have~~ become sources. sourcing

strategy

very unlikely to be able to prove

a model by results from simple manipulation (cut + splice)

because can easily make more complicated models.

theories valuable because they suggest new types of

experiment.

eg. ① see what goes from cell to cell

eg. tight junction

② are cells polar inside.

microtubules?

③ can added chemicals interfere ie an array system.

model systems

3D looks too complicated

2D – sheets of cells. visual cuticle. one layer of cells.

Wigglesworth – Peter Lawrence

1D system. Sydney. Richard Wilcox.

algae – will it be relevant

too early to say.

Finally, for nerves, other problems

– the reading of the conductor

– transmission to growing axon. (the idea of the addition)

– expression, to make right contact.

Harris, Bristles etc.