Available online at www.sciencedirect.com
SCIENCE DIRECT®

Medical Hypotheses (2003) **61**(2), 190–193

doi:10.1016/S0306-9877(03)00005-7

Crucial questions in cell biology and in neurobiology

H. Hillman

Unity Laboratory of Applied Neurobiology, Guildford, Surrey, UK

Summary Forty-two proper questions about the research methods, the structure, physiology, biochemistry, pathology and theory of cell biology and 27 more about neurobiology are listed. These have not hitherto been addressed in the literature, other than in my previous publications. The answers to some of them show anomalies in current views, or draw attention to control experiments, which have never been done. Progress will be limited until these important questions are addressed.

Questions are the engines of progress. One may regard it as axiomatic that one has an inescapable duty to answer all proper questions asked as a response to one's publication of a book or article in a learned journal, or if one has spoken as an authority on television or radio. That is, the duty of a scientist is to engage in dialogue.

Over the past 30 years, I have asked and answered a large number of crucial questions, which have otherwise never been posed. Therefore, I like to engage the interest of young students and research workers, before they have published sufficient research to give them an interest in *not* asking the following questions. (My publications in which some of these are addressed are indicated.)

CELLS

Methods

1. Can one calibrate substances *extracted* from tissues, using pure solutions of those substances?

Received 30 July 2002
Accepted 19 December 2002

Correspondence to: **Harold Hillman**, Unity Laboratory of Applied Neurobiology, 76 Epsom Road, Guildford, Surrey GU1 2BX, UK.
Phone: +44-1483-568332;
E-mail: hillmanh@breathemail.com

2. To which parameters of the tissue should concentrations be referred (1)?

3. Can one use a single pure protein to calibrate different subcellular fractions, each containing different mixtures of proteins?

4. Does the 'enrichment' of a subcellular fraction or cell type, change its chemical properties (2) (pp. 1–40)?

5. Does one assume that the preparation of tissue for histology, histochemistry, immunocytochemistry or electron microscopy does not change its chemical or biological properties (3)?

6. What control experiments have ever been carried out to warrant the assumptions of 4 and 5, or is it not necessary to do them (2, pp. 39–40, 52, 64–65)?

7. Does the finding of a chemical activity or a structure in a subcellular fraction, or in a subcellular location, prove that it has not moved during the preparation?

8. What is the evidence that solutes, liquids and particles do not relocate during fixation and freezing?

9. Does the shrinkage of tissues and their sectioning in any histological technique permit one to measure the dimensions of cells and subcellular organelles?

10. Can an electron microscopist looking at a heavy metal deposit on a dehydrated tissue derive any information about its biochemistry in life?

11. How can one assess the dimensions and structure of cell membranes by electron microscopy, when the

preparation involves the use of ethanol, acetone and propylene oxide, which extract lipids, which are believed to be major components of the cell membranes (4)?

12. Can one know the thickness in life of cell membranes?

13. How valid is the use of agonists, antagonists, inhibitors and ligands, to detect receptors, in preference to the transmitters, hormones, drugs and toxins, themselves?

14. Why is it assumed that homogenisation, washes and centrifugation, do not affect the affinity of receptors for transmitters, hormones, drugs, toxins and ligands?

15. Why should it be necessary to tilt the stages of electron microscopes to see randomly orientated membranes and other structures in all orientations, when this is not necessary with the light microscope?

16. Why have so few experiments been done to compare the chemistry and staining properties of the *same* cells in the parent tissue and in tissue cultures subsequently?

17. Is it reasonable to believe that incubation of tissues does not result in an exchange of substances between them and the incubating media?

18. Does growth of tissues in culture change their structures, biochemistry, staining or immuno-reactivity?

19. Can intracellular pipettes measure activities of a single intracellular ion, or membrane potential accurately?

Structure

20. Why do the cell, the nuclear and the mitochondrial membranes, as well as the mitochondrial cristae appear in electron micrographs nearly always to be cut in near perfect transverse sections (5)?

21. If the cell membrane is fluid mechanicallly, how can cells maintain their integrity?

22. How is intracellular movement of light microscopically visible particles in the cytoplasm, possible in life, when it is believed to contain the cytoskeleton, lysosomes and mitochondria, and it has a low viscosity (5)?

23. Why has only *one* of the many *thousands* of receptors, cell surface antigens and channels, which have been isolated, sequenced and characterised, their structures have been elucidated and their dimensions calculated, been seen by electron microscopy on the cell membranes, when, they are believed to be located there, and it is claimed that the thickness of the membrane is within the resolution of the electron microscope.

24. Why do the lamellae of the myelin sheath always appear to be uniformly distant apart on longitudinal section?

25. What is the evidence that the microsomal fraction consists of cell membranes and endoplasmic reticulum?

26. Where are the liposomes in life?

27. Can a lysosome be a particle or a vacuole?

28. Why is it assumed that each cell of a particular organism contains the same quantity of DNA, despite the huge variation in dimensions of their nuclei?

29. Where do protein synthesis and acid hydrolysis occur in cells, in which ribosomes and lysosomes cannot be seen?

Physiology, biochemistry and pathology

30. How can carriers assist the passage of ions and amino acids across membranes, when the complex of the carrier and the substance carried *must* be larger than the substance carried itself?

31. How can the nuclear pores prevent the ions and other smaller molecules crossing the nuclear membrane, when the messenger RNA moves from the nucleus to the cytoplasm?

32. How do the ions cross the membranes of the non-excitable cells, if a signal does not cause the ionic channels to open and close?

33. Do different cells in the same body contain different antigens?

34. What evidence is there that the body produces proteins, which are toxic to itself?

35. In diseases believed to be auto-immune, either *organ*-specific or *tissue*-specific, why does the body not reject the whole organ or tissue, as it rejects incompatible transplanted organs or blood of the wrong group, often making patients ill, or even killing them?

36. Is it possible to study the chemistry of necrosis by disruptive biochemical procedures, or procedures in which tissue is killed?

Theoretical

37. Should one entertain a hypothesis, some of whose elements are unproved or unprovable?

38. Are *living systems* open, partially open or closed, thermodynamically (6)?

39. Are tissues studied by *biochemical and biophysical procedures* open, partially open or closed, thermodynamically?

40. How can one distinguish between the causes and effects of a disease process, that is, identify its genesis?

41. Should one ever accept the results, interpretations or hypotheses arising from experiments in which no, or inadequate, controls have been carried out?

42. Is it a reasonable assertion that scientists have a duty to enter into dialogue with all interested parties?

NEUROBIOLOGY

Methods

1. Why are what are believed to be different kinds of neuroglial cells so rarely seen by light microscopy of healthy central nervous systems?

2. Why is there no common agreement about the staining procedures, which are believed to identify neurons, astrocytes, oligodendrocytes and microglia (7, pp. 64–71)?

3. Does the term neuroglia or glia implies that neurobiologists and pathologists cannot distinguish between the alleged sub-types, or that there are no differences?

4. Since the three alleged types of neuroglial cells were *originally* classified using histological techniques, does not this imply that anyone using antibodies to identify each type, should correlate these two criteria for the identification of what they believe to be the different cell types?

5. Is it surprising that allegedly different neuroglial cells grown in completely different cell culture conditions show different morphological, biochemical and immunological properties?

6. Is it reasonable to believe that neuronal and astrocytic cell bodies contain different antigens than their processes?

7. How specific are antibodies and other markers to neurons and different neuroglial cells?

8. Why are synapses not seen in unfixed and unstained preparations of the nervous system by light microscopy (8)?

Structure

9. Why are the dimensions and numbers of synapses so different by light and electron microscopy (8)?

10. Why is it so difficult to find the non-myelinated segment of the neuron continuous with the cell body and the myelinated sheath or an axon?

11. Why does one not see the bifurcation of myelinated fibres, except in diagrams?

12. Why do light micrographs in the literature never show the connection between the dendrite of one neuron to the presynaptic fibre and synapse on the next neuron (8)?

13. Why are synaptic vesicles so uniform in diameter in sections of the nervous system?

14. Why do there not appear to be cell membranes around the cytoplasm of what are believed to be neuroglial cells?

15. Why do axons from ganglion cells of the autonomic nervous system appear to have only one axon, rather than an afferent and efferent fibre?

16. Why do synaptic clefts, tight junctions and desmosomes nearly always appear in electron micrographs to be equally spaced apart, or why does one rarely, if ever, see them obliquely or face on?

17. Can one measure dimensions of cell bodies and axons in sections?

Physiology, biochemistry and pathology

18. How is axonal transport of particles possible when the axon contains networks of endoplasmic reticulum, microtubules and microtrabeculae?

19. Why is it assumed that evidence derived from experiments on neuromuscular junctions is relevant to understanding of transmission in the central nervous system?

20. How can reactive astrocytes move within hours to the sites of injury of the brain and spinal cord, if these structures are packed with neurons and neuroglial cells?

21. What evidence is there that high concentrations of 'excitotoxic' transmitters damage adjacent cells?

22. If high concentrations of K^+, low concentrations of Ca^{2+} and mechanical damage can induce neurons to fire, are they not transmitters (9)?

Theoretical

23. How can synapses store information, when they are also believed to transmit it?

24. What evidence – as opposed to hypotheses – is there that learning, memory, self-consciousness or mind affect synapses?

25. Can one devise experiments on living animals that test *only* learning or memory, without inducing other behavioural and physiological changes?

26. Does the chemical theory of transmission contain unproved and unprovable sub-hypotheses (9)?

27. Why are synapses regarded as necessary for the transmission of information, when electric conduction conveys information without crossing synapses (10)?

These questions have been addressed in my previous publications, and I invite any interested parties to provide different answers to them. Any of these questions may be quoted, and, or used in examinations, preferably with acknowledgement of source.

REFERENCES

1. Hillman H. Some fundamental theoretical and practical problems associated with neurochemical techniques in mammalian studies. *Neurochem Int* 1983; **5**: 5–20.
2. Hillman H. *Certainty and Uncertainty in Biochemical Techniques*. Henley on Thames: Surrey University Press, 1972.

3. Hillman H. Limitations of clinical and biological histology. *Med Hypotheses* 2000; **54**: 553–564.
4. Chughtai I., Hillman H., Jarman D. The effects of daematoxylin and eosin, Palmgren's and osmic acid procedures on the dimensions and appearance of isolated rabbit medullary neurons. *Microscopy* 1986; **35**: 625–629.
5. Hillman H., Sartory P. *The Living Cell.* Chichester: Packard Publishing, 1980.
6. Hillman H. Thermodynamics biochemistry and microscopy in the 21st Century. *Physiol Chem Phys Med NMR* 2002, in press.
7. Hillman H. *The Cellular Structure of the Mammalian Nervous System.* Lancaster: MTP Press, 1986.
8. Hillman H. The anatomical synapse by light and electron microscopy. *Med Hypotheses* 1985; **17**: 1–32.
9. Hillman H. A re-examination of the vesicle hypothesis of transmission in relation to its applicability to the mammalian central nervous system. *Physiol Chem Med NMR* 1991; **23**: 177–198.
10. Hillman H. A new hypothesis for electrical stimulation in the mammalian nervous system. *Med Hypotheses* 1991; **34**: 220–224.