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Scientific Correspondence

Dear Editor,

Biochemists, cytologists, pharmacologists, pathologists and biophysicists, all over the world use subcellular fractionation. This consists of a number of biochemical procedures, intended to find out the distribution and activities of enzymes, receptors, binding sites and other tissue components, as well as lesions, in the cell organelles, such as the membranes, the nuclei, the mitochondria, etc. Obviously, these research workers believe that such experiments inform them what happens in the same organelles in the living intact animals or plants. Such studies are carried out with the inescapable assumption (not stated) that the procedures for isolating the fractions do not themselves alter significantly the distribution, activities, reactions, or rate of reactions of the substances being studied[1]. If this assumption were untrue, the validity of all the experiments in which it was implied would have to be re-examined and, perhaps they would have to be re-interpreted.

I have examined the literature[2] and questioned my colleagues for about 35 years (cf. ref. 3, pp. 22–38), although the procedures have been used for about 50 years. The only control experiments which I can find are measurements of the 'recovery' of activities. If the quantities or activities of all the fractions added together at the end of the procedures represent, say, 70% to 130% of the activity of the crude homogenate, this is considered satisfactory. If they represent a smaller proportion, the total of the activities of the final fractions are added up, and the results are expressed as percentage of these total activities.

The use of percentage of recovery as a proportion of that of the original crude homogenate does not take into account changes occurring during the dying of the animal, the addition of the homogenising medium, or the homogenisation itself. Furthermore, neither of these two recovery measurements takes into account the likelihood that both the substances in the organelles and any co-factors, activators or inhibitors (both known and unknown) will diffuse from their native locations during dilution, homogenisation, centrifugation and extraction, which would change the apparent locations of the chemical, reaction, activity, binding site or metabolism, being studied. It should be born in mind that the aim of subcellular procedures is to find out what happens in different parts of the living cell, and their usefulness depends upon how accurately measurements made in the tests at the end of the procedures reflect what occurs in the intact living organism.

There seem to be two ways of addressing these problems. The first one is to do the long overdue control experiments, if one believes that an experiment is only as good as its controls. I can not find in the literature systematic experiments examining the effects of homogenisation, centrifugation and addition of powerful unnatural reagents, or natural reagents in unphysiological concentrations, on the activity or distribution of substances or activities, during the procedures of subcellular fractionation. The other approach is to use non-disruptive techniques, of which there are many; these include: examining blood cells, sperm, protista; analysing extracellular body fluids; microdissection of cells; microdialysis in vivo; positron emission tomography; nuclear magnetic resonance imaging (cf. ref. 3, pp. 256–58).

Can those who derive conclusions from subcellular fractionation indicate references to literature reporting the former control experiments, or do they believe that it is not necessary to carry out control experiments for these procedures? I believe that our students and those who finance our research are entitled to know the answers to these proper questions. History may take a harsh view of the failure to address them.

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1 Hillman, H., Certainty and Uncertainty in Biochemical Techniques, pp. 1–40. Surrey University Press, Henley on Thames 1972.
2 Hillman, H., Biochem. Soc. Bull. *1* (1979) 11.
3 Hillman, H., The Case for New Paradigms in Cell Biology and in Neurobiology. Mellen Press, Lampeter 1991.