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UPTAKE OF LATEX MICROPARTICLES BY RAT HEPATOCYTES IN TISSUE CULTURE

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Tissue culture is a convenient system in which to investigate the factors controlling the release and metabolism of a drug by hepatocytes from an intracellular particulate store. Hepatocytes contain the spectrum of drug metabolising enzymes (Fry 1982) and in vivo have been shown to take up latex particles (Illum et al 1986). As a first step we have used stereological methods (Williams 1977) to quantify particle uptake by cultured hepatocytes, in order to establish data for the presentation of an optimum dose in relation to particle size.

Primary cultures of hepatocytes (5×10^5 cells/culture well: 6 wells/treatment) were prepared from rats (Warren et al 1985). After 24 hours, 200µl (2.5% solid dispersion) of either 500nm or 200nm Polybead carboxylated microspheres (Polysciences Ltd, Northampton) was added to each experimental well. After 1hr the cultures were rinsed and the tissue fixed and processed for transmission electron microscopy. Dried mounts of microparticles on piloform coated grids were photographed in a calibrated Phillips 410 electron microscope. 400 particles were measured in order to confirm the manufacturer's specifications. Similarly calibrated electron micrographs of control (80 micrographs) and experimental (30 micrographs) cultures were subjected to morphometric measurements using the GIS Imagan System on a Hewlett Packard 86B.

The ultrastructure of the cultured hepatocytes was well preserved and there was no difference, except for the presence of microparticles, between control and experimental cultures. The ratio of hepatocyte nuclear volume to cell volume was 5:26. The mean nuclear volume was 356µm³. The mean cell volume was 2227µm³. Using a plot of the progressive mean, counts were made of the number of microspheres/unit of cytoplasm.

Table 1: Values for microparticle diameter; particle number uptake into unit volume of cytoplasm and unit cell; particle volume uptake into unit cell.

Manufacturer's Size Mean ± SD	Measured Size Mean ± SD	No particles/ µm³ Cytoplasm	No particles/ Cell	Vol particles (µm³)/ Cell
500 ± 6nm	449 ± 19nm	0.14	312	14.8
190 ± 9nm	198 ± 7nm	0.42	931	5.2

The difference between the manufacturer's data and measured data clearly indicated the importance of particle measurements before use in such experiments. Although an equal dose by volume and solid was delivered the ratio of the numbers of small to large particles in the dose was 15.1. This ratio was not sustained in terms of particle uptake where the ratio of the number of small to large particles/unit cytoplasm or /unit cell was 3:1. However, if uptake was measured as volume of particle per cell, then a greater intracellular reservoir was obtained using the larger particles. The results show that size is an important variable in determining an appropriate dose to maximise the volume of the carrier within the cell.

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