A METHOD OF PREPARING BEATING HEART CELLS FOR TISSUE CULTURE

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A technique which would result in cultured heart cells which would continue to beat has been a long-felt need by tissue culturists. Attempts to achieve this date back more than 50 years. The reasons for seeking such cultures are various. Some workers wish to study the anatomy, physiology and biochemistry of such cells and some, including ourselves, wish to examine and quantify the effects of medicinal substances. Several workers have described methods of preparing beating heart cells, but the cells have mostly been of limited life-span in culture or have contained an undesirably high proportion of non-beating cells. The only commercially available heart cells are non-beating, and in fact totally de-differentiated epithelioids. We would prefer to use cells of human origin for studying drugs for use in humans, but human heart, including aborted foetal, is not easily obtained and indeed illegal to use in some countries. We have used as our source chick embryos, easily obtained, relatively cheap and usually bacteriologically sterile. Ultimately we would wish to establish a line of cells which could be frozen down and remain viable, so that the same material could be used for successive experiments or for concurrent experiments in different laboratories. Meantime we report a method resulting in cultures of useful life-span and containing a high proportion of beating cells.

Method Six to thirteen day embryos are removed aseptically from the egg, the heart put into Ca^{++}- and Mg^{++}- free balanced salt solution at 20°, and cut into fragments. These are placed in 10ml of 0.0125% Difco 1 : 250 trypsin solution in phosphate-buffered saline (pH 7.3) at 37° and stirred gently for 5 minutes. The liquid, containing mostly blood cells, is discarded, a further 4 ml added and this stirred for 3 minutes. The supernatant liquid now contains heart cells and is transferred to 20 ml of trypsin inhibitor solution at 37°. Further portions of 4 ml are added and stirred for 3 minutes until the tissue is completely dissociated. The cell suspensions in inhibitor must be filtered immediately through sterile gauze to remove clumps, centrifuged at 128g for 3 minutes, the supernatant liquid discarded and the cells resuspended in culture medium to a plating density of about 1×10^6/ml. The medium may be any of the standard media, containing 5% of foetal calf serum and gassed with 5% CO_2 in air.

Discussion Success depends on strict aseptic technique, on the type of trypsin, its concentration and time of contact, and on immediate centrifugation and resuspending. Contrary to the findings of previous workers, we find that, provided the cells are undamaged by trypsin and mechanical procedures, then embryo age, batch of serum, presence or absence of chick embryo extract and nature of the media are of less importance. The object of our procedure is to minimise chemical and mechanical cell injury. The less injury there is, the more quickly the cells adhere to their culture vessel. Such adhesion is necessary for the growth of these and of most non-malignant cells. The more rapid the adhesion the better the beating and the sooner its onset. Disturbance while adhering is also inimical to beating.

Results The cultures contain about 90% beating cells and these continue to beat for up to 30 days provided the medium is changed every 5 days. If a beating cell comes into contact with a non-beating cell it will usually also start to beat.

Conclusion Heart cells prepared thus are of value for pharmacological studies.