THE EFFECTS OF TRYPSIN ON FUSION AND CONTRACTILITY IN CHICK SKELETAL MYOBLASTS

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Trypsin is the substance most commonly used to disaggregate tissues to form primary cell cultures. Digestion of the intercellular material however is necessarily accompanied by damage to the cell surface. The present work reports the effects of trypsin on subsequent retention of function in cultured myoblasts. Previously Dawson & Abdul-Jabar (1980) had shown that trypsin concentration, and time and temperature of contact were critical in preparing cultures of beating heart cells, and also that ATP had a protective effect against trypsin damage.

In the present work chick embryo thigh muscle was disaggregated by trypsin solutions, and their effects on subsequent fusion of myoblasts and contractility of myotubes observed.

Fusion was studied, by phase-contrast microscopy, by counting the numbers of myotubes formed and measuring their length and width. Contractility was more difficult to assess, as the myotubes, though contractile in nature, do not normally contract spontaneously and certainly do notcontract rhythmically like heart cells. However it was found that they did contract when co-cultured with beating heart cells. When a heart cell and a myotube were in contact, the heart cell depolarised the skeletal muscle membrane, resulting in rhythmic contraction of the myotube. It appeared from phase-contrast microscopy that there is a definite cell-cell attachment between the two cell types, possibly to the extent of membrane fusion where attached, but not total engulfment of one cell within the other. Large numbers of these heterogeneous cell complexes could be seen in the cultures, the myotube contracting at the same rate as the heart cell. In a smaller number of the cell complexes, the myotube was moving only passively, being pulled at its point of attachment by the heart cell but not itself contracting. By the fifth or sixth day of culture large tracts of adjacent myotubes were rhythmically contracting. It is of interest that beating mouse cells could not initiate contractions in the chick myotubes.

The skeletal muscle tissue could withstand trypsin up to 0.2% during disaggregation and still retain some function, although less than with 0.025%, whereas with cardiac muscle the best concentration was only 0.0125%. Presumably, in view of the protective role found for ATP, loss of contractility was brought about by trypsin damage to membrane ATPase.

Dawson, M., Abdul-Jabar, Z., in Richards, R.J., Rajan, K.T., (Editors) (1980) Tissue Culture in Medical Research (II), 71-77. Oxford: Pergamon.

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