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CONJUGATION REACTIONS IN PRIMARY CULTURES OF RAT HEPATOCYTES

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The appeal of using isolated liver cells in the study of drug metabolism is the possibility to study sequences of reactions as well as metabolic events taking place in different parts of the cell, while at the same time having well-defined conditions. With cultures there are also possibilities of long-term experiments, such as studies on induction, carcinogenicity etc. If liver cells in culture are to be used as experimental models, then the effect of culture conditions on the metabolism, and especially on the relationship between phase I and phase II reactions, should be known. It is known that there is a rapid decline of cytochrome P-450 and associated mono-oxygenase activities during culture, but phase II reactions have not been investigated much. In this study a mono-oxygenase activity and two conjugation reactions were measured as a function of culture time in order to investigate their stability and interrelationship during culture.

MATERIALS AND METHODS

Hepatocytes were isolated from Sprague-Dawley rats by the collagenase perfusion method (1), and suspended at 0.75×10^6 cells/ml in growth medium (a 1:1 mixture of Ham F-12 and Waymouth MB 752/1 (Gibco,Paisley, Scotland), supplemented with 5 mmol/l $NaHCO_3$, 20 mmol/l Hepes, 10 mmol/l Tricine, 10 IU/l insulin, 50 μg/l glucagon, 10^{-6} mol/l dexamethasone and 5% fetal and 5% newborn calf serum (Gibco)). 2.5 ml of this suspension was seeded onto collagen-coated 60-mm plastic culture dishes. The medium was changed 3 h after seeding, and then every 48 h. Metyrapone (2-methyl-1,2-(3-pyridol)-1-propanone), which has been shown to reduce the loss of cytochrome P-450, was added to some cultures at 0.5 mmol/l.

7-Ethoxycoumarin-O-deethylase was determined from detached cells after homogenization in 0.15 mol/l KCl (2). To measure conjugation the attached cells were incubated with 100 μmol/l methylumbelliferone; the glucuronide (MUG) and sulfate (MUS) were determined after specific hydrolysis as described before (3), and DNA content was quantitated with diphenylamine (4).

RESULTS AND DISCUSSION

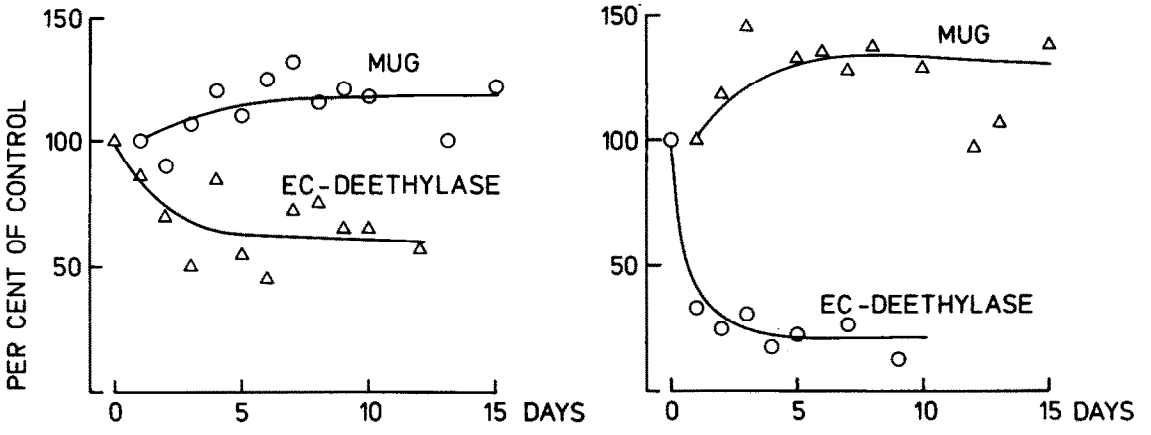

Fig. 1. Ethoxycoumarin-deethylation and methylumbelliferone glucuronidation in primary cultures of rat hepatocytes. EC-deethylase activity in a homogenate of freshly isolated cells is taken as 100% (activity:0.90 nmol/h/ug DNA). The activity of UDP-GT (MU) was calculated per dish, and day 1 in culture taken as 100% (activity:61.2 nmol/h/dish; 2.2 nmol/h/ug DNA). The values are means of 2-3 determinations. Right panel:no metyrapone; left panel:with metyrapone.

Hepatocytes were grown up to 3 weeks in culture. The DNA content stayed constant, or declined slowly for about 10 days, but after that there was enhanced loss. Metyrapone had no effect on the DNA content.

A sharp drop in 7-ethoxycoumarin-O-deethylase activity was seen during the first day, and after 4 days the activity was down to 20%, at which level it remained for several days (Fig.1). The addition of metyrapone reduced this loss (Fig.1), so that after 24 h the activity was about 80%, and then remained at 50% for several days. UDP glucuronyltransferase (MUG) activity stayed at the level of day 1 for over 2 weeks (Fig.1), and considerable activity could be seen even at 3 weeks. The sulfotransferase (MUS) behaved similarly to UDP-GT, with no loss of activity during 2-3 weeks (results not shown). Thus the MUG/MUS ratio was stable during culture, but the EC-deethylation/conjugation ratio fell even in the presence of metyrapone. The fact that the ratio between UDP-GT, a membrane-bound enzyme, and ST, a soluble enzyme, is maintained, suggests that no significant membrane damage is occurring. An unchanged ratio after 24 h has been reported by Fry and Bridges (5).

Conditions to prevent the loss of cytochrome P-450 are actively searched for (e.g.6), and are essential if liver cell cultures are to be used to their potential in drug metabolism studies. However, these results suggest that conjugation activities are maintained. Further studies are needed to confirm that the behaviour of the conjugation reactions in culture is physiological, but it seems that liver cell cultures can be used as a model system for them, e.g. with respect to induction. However, the changed phase I / phase II relationship should be kept in mind.

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