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Monooxygenation, cytochrome P450-mRNA expression and other functions in precision-cut rat liver slices*, [1]

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With 5 figures and 1 table

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Summary

Precision-cut rat liver slices (KRUMDIECK slicer, slice thickness 200–250 µm) were incubated in rollers containing modified William's medium E at 37 °C for 2, 24 and 48 hrs. Protein, DNA, potassium and glutathione concentrations did not decrease during 48 hrs. Lactate dehydrogenase (LDH) leakage into the medium was relatively marked during the first 2 hrs of incubation, from the 2nd to the 48th hr LDH leakage was very low. The same is true of the release of thiobarbituric acid-reactive substances. Albumin synthesis and transport into the medium decreased to about 70 % after 48 hrs. Cytochrome P450 (CYP)-dependent 7-ethoxycoumarin O-deethylation rate was relatively stable up to 48 hrs, whereas testosterone hydroxylation decreased significanly without alterations of the proportions of the 7 quantified hydroxylated metabolites. After exposure of the slices to β-naphthoflavone for 6 hrs CYP1A1-mRNA expression, measured by competitive RT-PCR, was increased by a factor of at least 1000. Precision-cut liver slices are a useful tool for the study of various hepatic functions, drug metabolism and its induction *in vitro*.

Introduction

The use of hand-cut tissue slices was started by WARBURG (1923). The disadvantage of such slices was the poor reproducibility and their thickness, which did not allow sufficient oxygen and substrate supply. After the development of rat hepatocyte isolation (BERRY and FRIEND 1969), isolated hepatocytes became the *in vitro* model of choice for pharmacological, toxicological, metabolism and transport studies (SKETT 1994). Since the development of the Krumdieck slicer (KRUMDIECK et al. 1980), which allows the preparation of thin slices with reproducible thickness and a minimal trauma to the tissue, these precision-cut slices have become a suitable *in vitro* model for the investigation of hepatic xenobiotic metabolism, hepatotoxicity, transport and other functions. In recent reviews (PARRISH et al. 1995; EKINS 1996b; OLINGA et al. 1997b) detailed information on the use of precision-cut liver slices can be found, in part including comparisons of drug metabolism between slices and isolated hepatocytes as well as between the *in vivo* situation and *in vitro* systems. In spite of many open questions (e.g. adequate culture methods, supply of inner cell layers with substrates), which need further investigations, precision-cut slices are even now successfully used for many purposes.

To further characterize the liver slices we investigated, additionally to relatively general viability parameters such as potassium, glutathione, DNA, protein concentrations and lactate dehydrogenase (LDH) leakage, the following important liver functions:

1. Albumin synthesis is a liver-specific function. So far the maintenance of albumin synthesis in liver slices has not been investigated. In the case of dedifferentiation of hepatocytes albumin formation would disappear.

2. Cytochrome P450(CYP)-dependent monooxygenation of xenobiotics is very important for the effects of drugs and toxic substances. So far a lot of biotransformation reactions have been investigated, which have been

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more or less maintained during culturing of the liver slices for some days. For a standardized use of liver slices it is important to show that the metabolic pattern of a substance, i.e. the ratio between the metabolites, is not changed during culturing. Therefore we determined the hydroxylation of testosterone during a culture period of up to 48 hrs, a hormone which is metabolized at different CYPs to form a lot of metabolites hydroxylated at different positions. So far only a few investigations are known. GOKHALE et al. (1997) found very poor activities of testosterone hydroxylation in liver slices; even the usual main metabolite 6β-hydroxytestosterone was not detectable during the first 24 hrs of culture. Other authors found a well measurable formation of hydroxylated testosterone metabolites in rat and dog liver slices (EKINS 1996a; EKINS et al. 1996), the maintenance of these biotransformation reactions during culturing, however, was tested for only 4 hrs (rats) or not at all (dogs). DE KANTER and KOSTER (1995) compared testosterone hydroxylation in fresh and cryopreserved liver slices, but without quantitative information about the formation of the metabolites. OLINGA et al. (1997a) only measured the formation of the main metabolite 6β-hydroxytestosterone.

3. Induction of CYP by xenobiotics is of toxicological and pharmacological relevance. Investigations of the last years demonstrate that liver slices can be used for the detection of induction after *in vitro* exposure of the slices to inducers. By measurement of enzyme activities and/or concentrations of various CYP forms (Western immunoblotting or ELISA) rat liver slices have been used to study CYP induction (LAKE et al. 1993, 1996; GLÖCKNER and MÜLLER 1995; MÜLLER et al. 1996; DRAHUSHUK et al. 1996). Using the same methods, induction was also detected in human liver slices (LAKE et al. 1996, 1997). In some investigations the detection of induction needed incubation of the slices for 48 to 72 hrs. At these times control values of metabolism had markedly decreased. To avoid longer incubation times, which is particularly important if cryopreserved liver slices are used (cf. GLÖCKNER et al. 1998), we determined CYP1A1-mRNA by RT-PCR after incubation of the slices in the presence of β-naphthoflavone (BNF) and found a marked CYP1A1-mRNA formation within 6 hrs incubation (MÜLLER et al. 1996). Later DRAHUSHUK et al. (1996) found an increase in CYP1A1- and 1A2-mRNAs after 24 hrs incubation in the presence of TCDD. In this paper we demonstrate the use of competitive RT-PCR to quantitate the expression of CYP1A1-mRNA after *in vitro* induction.

Material and methods

Chemicals: All substances were commercially available and of analytical grade.

Krebs-Henseleit buffer, pH 7.4, was supplemented with HEPES (12.6 mM) and gentamicin (50 mg/l) and gassed with 95 % O_2/5 % CO_2.William's medium E (WME, Biochrom KG) was supplemented with L-glutamine (292 mg/l), insulin (1 μM), gentamicin (50 mg/l) and tylosin (100 mg/l), gassed with 95 % O_2/5 % CO_2.

Preparation of slices: Liver slices were obtained from male Han:Wist rats aged 33–40 days. The rats were housed in plastic cages under standardized conditions (tap water and standard diet (Altromin 1316) ad libitum, light-dark cycle 12/12 h, temperature 22 ± 2 °C, humidity 50 ± 10 %). The rats were sacrificed by decapitation in ether anaesthesia. The liver was immediately perfused with and placed into cold Krebs-Henseleit buffer, and then tissue cores (diameter 8 mm) were sliced with a Krumdieck slicer (Alabama R & D Corp., Munford, AL, USA), filled with Krebs-Henseleit buffer. The slice thickness was 200–250 μm.

Incubation of slices: For incubation the roller system was used (PARRISH et al. 1995), a modification of the dynamic organ culture, where the liver slices are intermittently exposed to the medium and the gas. Two liver slices were put on a stainless steel insert, placed in an incubation vial containing 1.8 ml of WME, gassed with 95 % O_2/5 % CO_2, and incubated in a roller incubator (Vitron Inc., Tucson, AZ, USA) at 37 °C for 2–48 hours. After 2 and 24 hrs the medium was changed. In some experiments (induction of CYP), BNF (final concentration 25 μM) was added to the medium. After incubation the slices were immediately placed into liquid nitrogen for later RNA isolation or were homogenized for further determinations. For monooxygenation and albumin synthesis the continuous submerging of liver slices in the medium seemed to be advantageous. For such experiments, after the above-mentioned incubation times in rollers, slices were placed into Erlenmeyer flasks containing WME and gassed with 95 % O_2/5 % CO_2 and were incubated for a further time (see below).

Determination of protein and DNA contents: Protein was quantified by means of a modified biuret method (KLINGER and MÜLLER 1974). DNA was determined according to DOWNS and WILFINGER (1983), modified by MILLER et al. (1993).

Determination of glutathione and potassium contents: Total glutathione was determined according to KRETZSCHMAR et al. (1989). In brief: three parts of each homogenate were denatured and diluted with 4 parts of metaphosphoric acid (25 % w/v) and 9 parts of 0.2 M sodium phosphate/0.005 M EDTA buffer, pH 8.0, and stored at –80 °C until determination. After thawing and centrifugation at 20,000 g at 4 °C for 30 min, glutathione was measured in the supernatant with Ellman's reagent.

Potassium was measured by flame photometry in a supernatant which was obtained as follows: 1 liver slice was placed into 1.1 ml distilled water and homogenized by sonication. After addition of 20 μl of 70 % perchloric acid and centrifugation (20,000 g for 30 min) 1 ml of the supernatant was further diluted with distilled water and used for K^+ determination.

Determination of LDH leakage and TBARS (thiobarbituric acid-reactive substances) release: After incubation LDH was determined in the slice homogenate and the incubation medium. Slices were homogenized 1:6 in 0.1 M sodium phosphate buffer, pH 7.4, containing 0.1 % triton X. The homogenate was gradually diluted with this buffer to 1:400. This homogenate and the corresponding incubation medium were stored at –80 °C until the LDH determination, which was carried out by means of a test kit (LDH opt. lactatdehydrogenase EC 1.1.1.27 UV-test, Sigma diagnostics). The reaction was started by 10 μl of diluted slice homogenate or 20–100 μl of medium, and the decline of absorbance at 340 nm was measured at 25 °C for 3 min. LDH leakage

is given in % of total LDH (i.e. inside the slices and in the medium) at the end of the incubation period.

TBARS were determined according to YAGI (1987) in 0.1–0.2 ml of incubation medium.

Determination of albumin synthesis: After incubation in rollers for varying times (s. above), 2 liver slices were placed into 4 ml of WME in 25-ml-Erlenmeyer flasks and were incubated at 37 °C for 3 hrs. Thereafter aliquots of the incubation medium were stored at –20 °C for at least 24 hrs. Reference samples containing rat albumin were stored in the same manner. Rat albumin concentration was determined by means of competitive ELISA (according to KEMENY 1994). As antibody a peroxidase-conjugated IgG fraction from rabbits against rat albumin was used (Organon). The peroxidase reaction was performed with o-phenylendiamine as substrate.

Monooxygenation in intact liver slices: *7-ethoxycoumarin O-deethylation (ECOD):* 2 liver slices were placed into 4 ml of WME in 25-ml-Erlenmeyer flasks. The reaction was started by the addition of 20 µl of 160 mM 7-ethoxycoumarin dissolved in methanol (final concentration 0.8 mM). No cofactors were added. After 10 min incubation at 37 °C the reaction was stopped by the addition of 4 ml of ice-cold 0.31 M trichloroacetic acid (TCA). The medium/TCA mixture (without slices) was centrifuged, and to 1 ml of the supernatant 4 ml of 0.24 M borate buffer, pH 10.0, were added. In this alkaline solution the metabolite 7-hydroxycoumarin was determined fluorometrically (375/454 nm). *Testosterone hydroxylation:* This reaction ran under the same conditions as ethoxycoumarin O-deethylation including the addition of TCA, with the exception that the reaction was started with testosterone dissolved in 10 µl of methanol (final concentration 0.25 mM). From 2 ml of medium/TCA mixture testosterone and its hydroxylated metabolites (OH-TE) were isolated by solid phase extraction on LC8 columns. After the elution of the substances from the column with 2 ml methanol, methanol was evaporated. For derivatization of the steroids 0.5 ml of N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) was added to the dry residue (30 min, 80 °C), then the surplus MSTFA was evaporated, and the trimethylsilyl derivatives were dissolved in 0.5 ml of hexane. Testosterone and its metabolites were determined by GC-MS (Finnigan MAT GCQ). The carrier gas was helium (linear velocity 40 cm/s). The splitless injection of 1 µl of the steroid-containing hexane solution was carried out using a CTC Analytics liquid sampler A200SE in a split/splitless injector (injector temperature 275 °C). A fused-silica capillary column (25 m x 0.22 mm ID) bonded with 8 % phenyl(equiv.) polycarborane-siloxane (SGE) was used. The column oven temperature was set at 60 °C for the initial 30 s and was raised to 200 °C in steps of 40 grd/min, and then to 300 °C in steps of 2 grd/min. The transfer line and ion source temperatures were 275 and 150 °C, respectively. The ionization energy was 70 eV for the EI mode.

Determination of CYP1A1-mRNA: Determination of CYP1A1-mRNA was performed by means of competitive RT-PCR (SIEBERT and LARRICK 1992; FOLEY et al. 1993). The RNA used as internal standard was prepared according to VAN DEN HEUVEL et al. (1993). The required composite primers were purchased from Pharmacia, Freiburg, Germany. The obtained RNA was quantitated fluorometrically (SCHMIDT and ERNST 1995) with Sybr Green II (FMC Bioproducts, Rockland, ME, USA).

Total RNA was prepared by means of an RNA Kit II (InViTek, Berlin, Germany). After storage in liquid nitrogen liver slices were lysed with a guanidinium-containing lysis solution. The lysates were treated with a DNA adsorber, followed by phenol extraction and precipitation of the remaining RNA. Precipitates were washed twice with 75 % ethanol and dissolved in nuclease-free water after drying. For RNA quantitation the absorbance at 260 nm was determined.

Reverse transcription was performed in a final volume of 20 µl containing 0.2 µg of total liver RNA, varying amounts of internal standard RNA from a dilution series, RT-buffer, 5 mM $MgCl_2$, 1 mM each dNTP, 1 unit/µl ribonuclease inhibitor (RNasin), 0,25 µg oligo dT_{15} primer, 7 units AMV reverse transcriptase (reagents obtained from Promega, Madison, WI, USA). The samples were incubated at 42 °C for 30 min.

Usually 2 µl of reverse transcription products served as templates for PCR performed in a final volume of 25 µl containing PCR buffer, 1,5 mM $MgCl_2$, 0,2 mM each dNTP, 2 units Taq polymerase (reagents obtained from Gibco Life Technologies, Eggenstein, Germany) and 2 µM each of CYP1A1-cDNA forward and reverse primers (primers obtained from Pharmacia, Freiburg, Germany). The samples were incubated at 94 °C for 1 min, followed by 35 cycles each consisting of a denaturing step at 94 °C, an annealing step at 62 °C and an elongation step at 72 °C. Incubation of the samples were performed in a personal cycler (Biometra, Göttingen, Germany).

The amplification products were resolved by agarose gel electrophoresis (1,5 % in TBE) and visualized by ethidium bromide fluorescence. Quantitation was performed by comparing the RT-PCR signals generated from CYP1A1-mRNA (341 bp) with the RT-PCR signals generated from a series of known internal standard RNA concentrations (240 bp).

Without preceding reverse transcription of the RNA, no PCR signals were observed, i.e. the isolated liver RNA was not contaminated by genomic DNA.

Statistics: The results are given as arithmetic means ± SEM of up to 7 independent slice preparations (7 livers). The paired t-test was used to estimate group differences (pairs from each liver, $p \leq 0.05$).

Results

During the incubation of precision-cut liver slices for 48 hrs protein and potassium concentrations did not change (fig. 1). This constant potassium level was reached as early as 20 min after the beginning of slice incubation; before potassium content was much lower (not shown). Glutathione concentration even increased from the 2nd to the 24th hr, after that time no changes were observed (fig. 1). DNA content was stable between the 24th and 48th hr, after 2hrs, however, it was somewhat lower (fig. 1).

LDH leakage was, as expected, relatively high (about 9 %) during the first 2 hrs of incubation. Between the 2nd and 24th hr, however, only 2.5 % were released into the medium. Between the 24th and 48th hrs LDH leakage increased to about 5 % (fig. 2). The formation and release of TBARS was considerable during the first 2 hrs, from the 2nd to the 24th and from the 24th to 48th hr the release per hr was much less (fig. 2).

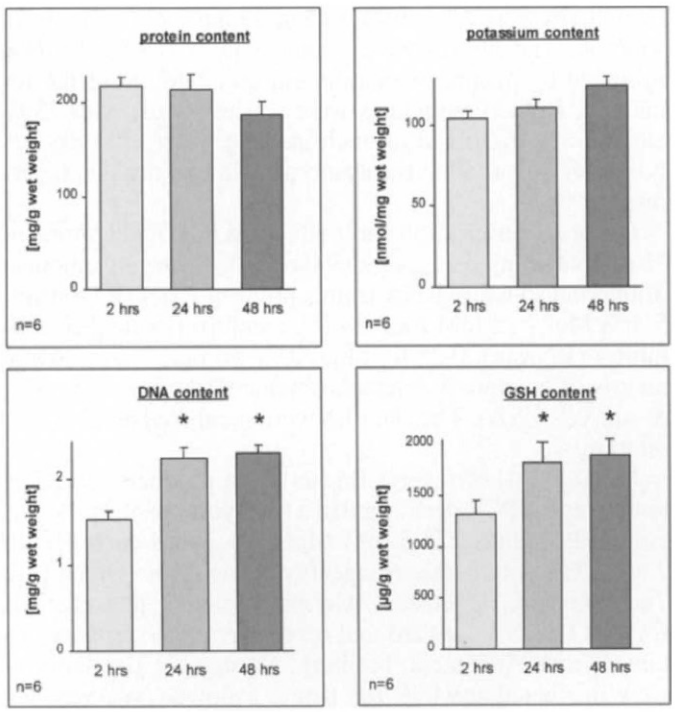

Fig. 1. Protein, potassium, DNA and glutathione (GSH) contents in rat liver slices after incubation for 2–48 hrs.

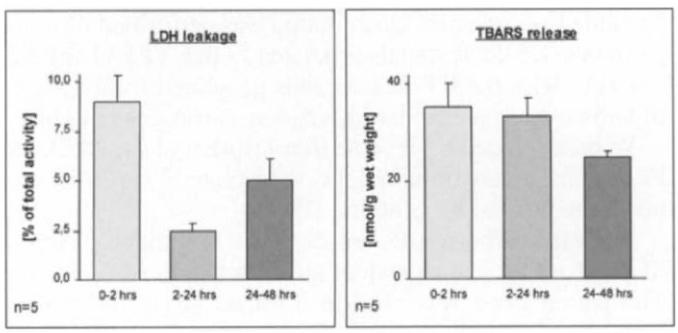

Fig. 2. LDH leakage and TBARS (thiobarbituric acid-reactive substances) release from rat liver slices into the medium during incubation for 2–48 hrs

Liver slices were able to produce and secrete considerable amounts of albumin. There was a decrease to about 70 % from the 2nd to the 48th hr of incubation (fig. 3). CYP-dependent ECOD in intact slices remained relatively constant up to 48 hrs of incubation (fig. 3).

Intact liver slices could hydroxylate testosterone at different positions. 7 out of a higher number of metabolites were quantified in this experiment. The main metabolite was 6β-OH-TE, followed by 16α-OH-TE and 2α-OH-TE. The amounts of 2β-OH-TE and 7α-OH-TE were even lower, and only traces of 6α-OH-TE and 15α-OH-TE were found. There was a significant decrease in the hydroxylation rates at all positions after 24 and 48 hrs incubation. The relation between the metabolites did not change during this long-term incubation (fig. 4).

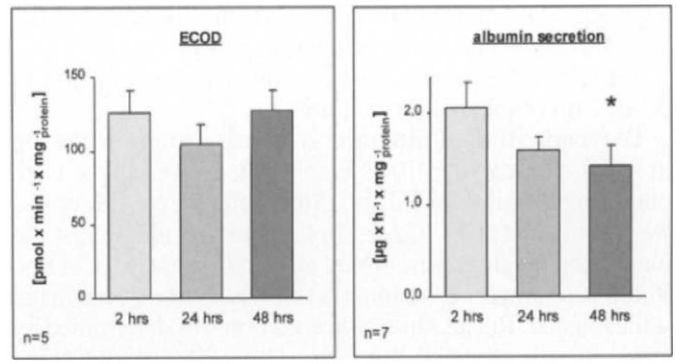

Fig. 3. Albumin formation and secretion and 7-ethoxycoumarin O-deethylation (ECOD) in rat liver slices after incubation for 2–48 hrs

Table 1. CYP1A1-mRNA content in rat liver slices incubated for 6 hrs in the absence (control) or presence of 25 µM β-naphthoflavone (BNF).

		CYP1A1-mRNA/µg total RNA
control	rat 1	$< 1.36 \times 10^{-19}$ mol
	rat 2	$< 1.36 \times 10^{-19}$ mol
	rat 3	$< 1.36 \times 10^{-19}$ mol
	rat 4	$< 1.36 \times 10^{-19}$ mol
BNF	rat 1	1.15×10^{-16} mol
	rat 2	0.90×10^{-16} mol
	rat 3	1.40×10^{-16} mol
	rat 4	1.65×10^{-16} mol

Figure 5 and Table 1 indicate that rat liver slices can be used for the detection of *in vitro* CYP induction by means of RT-PCR. Fresh rat liver as well as control liver slices after 2 or 6 hrs incubation did not contain CYP1A1-mRNA in measurable amounts, whereas after 6 hrs exposure to BNF a considerable amount of this mRNA was found (fig. 5, upper part). The quantification was carried out by means of competitive RT-PCR (fig. 5, lower part). Table 1 shows that the *in vitro* exposure of the slices to BNF enhanced CYP1A1-mRNA by a factor of about 1000, if we assume that the CYP1A1-mRNA level in control slices reached 1.36×10^{-19} mol/µg total RNA. This is the lowest mRNA content we looked for. Since this mRNA content was never achieved in control slices, even higher induction factors are probable.

Discussion

During culturing of precision-cut liver slices in a dynamic roller culture for up to 48 hrs some general parameters, which are a sign of viability, were stable. Potassium

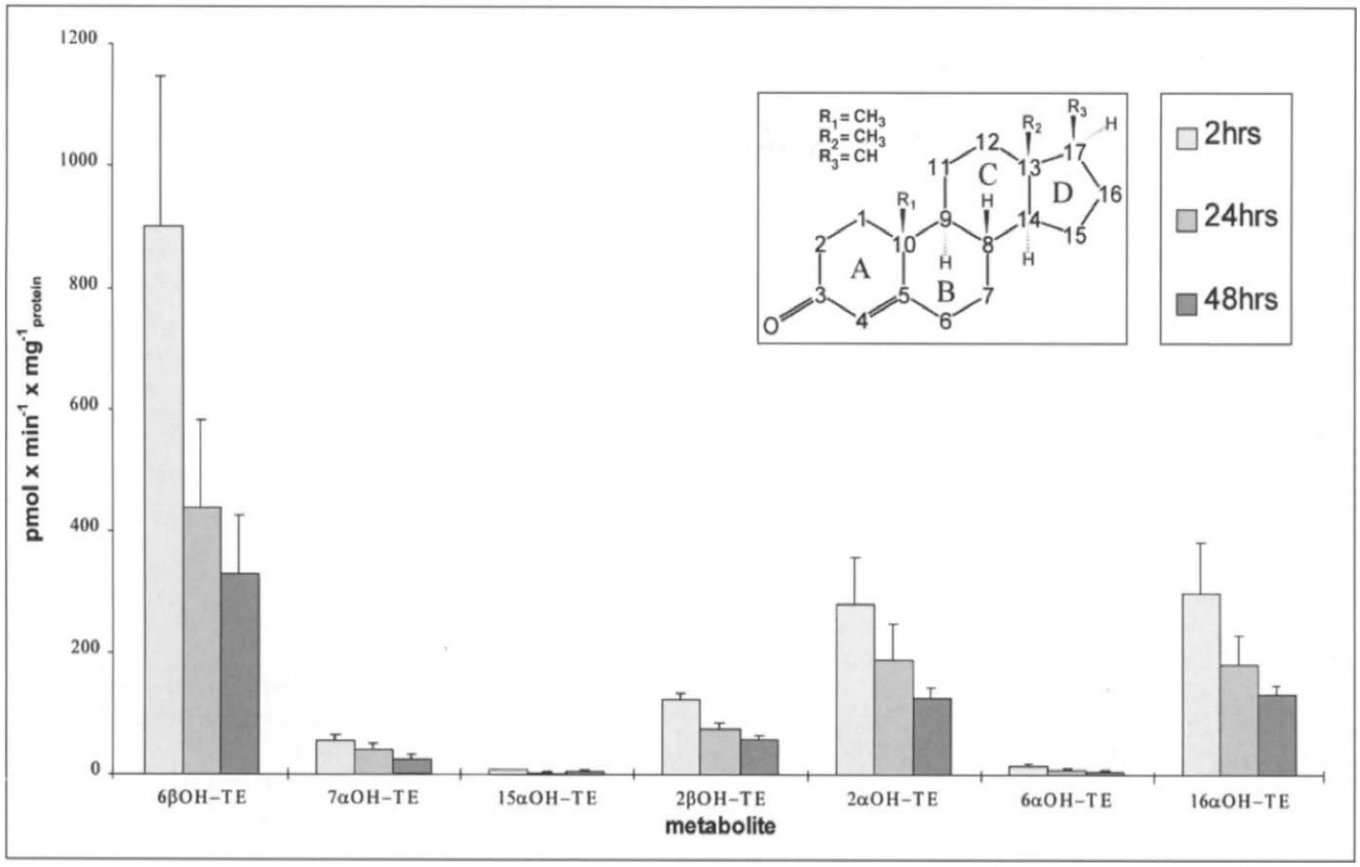

Fig. 4. Formation of hydroxylated metabolites from testosterone in rat liver slices after incubation for 2–48 hrs.

concentration did not change from 2 to 48 hrs culturing. This corresponds in principle to investigations of other authors, who found a stable potassium content for 72 hrs (FISHER et al. 1995a, b) or for 24 hrs (OLINGA et al. 1997a), if the roller culture was used. However, some other culture systems (FISHER et al. 1995b; OLINGA et al. 1997a) or inappropriate culture media (FISHER et al. 1995a) caused a distinct decrease in potassium concentration during culturing. In previous own investigations (GLÖCKNER and MÜLLER 1995; MÜLLER et al. 1996) with thicker slices (0.5 µm) having been shaken in flasks, potassium concentration was also maintained at a constant level for up to 24 or 48 hrs. Glutathione concentration even increased from the 2nd to the 24th hr and was then constant until the 48th hr. Obviously this parameter had not recovered within 2 hrs incubation after slicing. Similar results were obtained with 500 µm slices cultured in flasks (GLÖCKNER and MÜLLER 1995; MÜLLER et al. 1996). DNA content did not change between the 24th and 48th hr and was similar to that in fresh liver tissue. After 2 hrs the concentration per wet weight was a little lower, apparently due to cell residues without nuclei on the surface of the slices, which become detached after longer times of culturing. As expected, LDH leakage into the medium was relatively high within the first 2 hours due to the detached cell debris. From the 2nd to 24th hr, however, only 2.5 % and until the 48th hr additional 5 % of total LDH were released. This indicates a good viability. FISHER et al. (1995a, b) found an LDH leakage of about 20 % during 48 hrs under

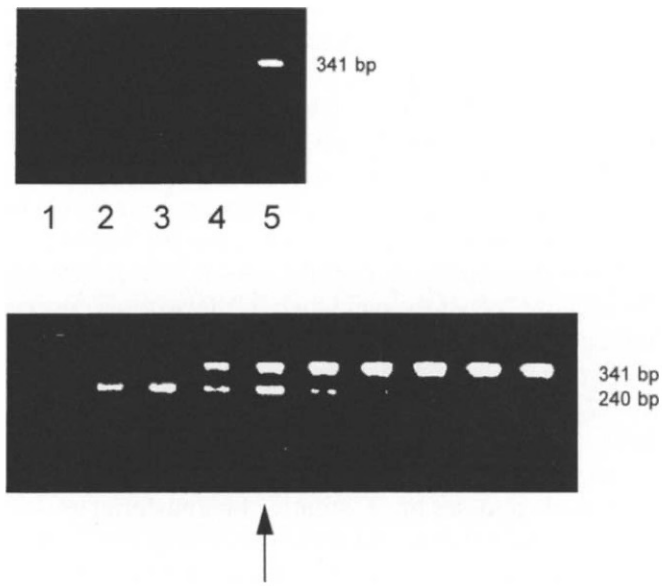

Fig. 5. Expression of CYP1A1-mRNA in liver slices exposed *in vitro* to β-naphthoflavone (BNF). **Upper part:** Electrophoresis of RT-PCR products (341 bp) corresponding to CYP1A1-mRNA. Liver before slice preparation (lane 1), control liver slices before incubation (lane 2), after incubation for 2 hrs (lane 3) or 6 hrs (lane 4); liver slices after incubation for 6 hrs in the presence of BNF (lane 5). **Lower part:** Competitive RT-PCR using liver slices exposed to BNF for 6 hrs . Electrophoresis of RT-PCR products (341 bp) corresponding to CYP1A1-mRNA and of RT-PCR products (240 bp) corresponding to internal standard RNA. The arrow indicates approximately equal concentrations of both RNAs.

optimal conditions; under less suitable conditions LDH leakage was even much higher.

The results show that liver slices can synthesize and release albumin into the medium. The activity was about 4 times as high as or comparable to that found in hepatocyte cultures by ROTEM et al. (1995) or PANG et al. (1997), respectively. In spite of the stability of the above-mentioned general viability parameters, albumin synthesis decreased to about 70 % after 48hrs culturing. It is not clear, if it is a sign of dedifferentiation or the absence of extrahepatic factors which could play a role *in vivo*.

In our experiments CYP-dependent ECOD rate in intact liver slices was considerably higher than in other investigations due to different substrate concentrations or incubation conditions (EKINS 1996a) or than in previous own experiments with liver slices of 500 µm thickness (GLÖCKNER and MÜLLER 1995). The latter results indicate that not all layers of cells in the slices take part in drug metabolism, if a certain thickness is exceeded. Under our incubation conditions this biotransformation activity was relatively stable until 48 hrs.

As a measure of testosterone hydroxylation, 7 metabolites, which are formed at different CYP forms, were quantified. EKINS (1996a) found somewhat lower activities in rat liver slices, the quantitative order of the main metabolites 6β-, 16α- and 2α-hydroxytestosterone was the same. OLINGA et al. (1997a) only determined 6β-hydroxytestosterone formation while investigating the influence of various culture systems. After 1.5 hrs culturing the activities were generally lower than in our experiments after 2 hrs. They found a stability of this reaction in the 6-well-shaker for 24 hrs, whereas in the roller culture a marked decrease was observed. Under our conditions we also found a decrease after 24 and 48 hrs. However, all metabolites were concerned, i.e. the metabolic pattern was not significantly changed, which is very important for the successful use of liver slices for drug metabolism studies.

Liver slices are useful for the detection of *in vitro* induction of CYP. If the increase in CYP isozyme concentrations or monooxygenation rates were measured as a proof of induction, slices were incubated for 24 or mostly 48–72 in the presence of the inducer (LAKE et al. 1993; 1996, 1997; GLÖCKNER and MÜLLER 1995; MÜLLER et al. 1996). This long incubation times were partly associated with marked losses of constitutive biotransformation activities and CYP concentrations, so sometimes it is difficult to distinguish between a real induction or a stabilization, i.e. a slower loss, of CYP. In this investigation we could demonstrate an induction by BNF after a slice incubation for only 6 hrs by the measurement of CYP1A1-mRNA formation by means of competitive RT-PCR. This method allows a quantification of specific mRNA. BNF was able to enhance 1A1-mRNA by a factor of at least 1000. With a simpler RT-PCR we had already proved an induction by BNF within 6 hrs incubation of slices (MÜLLER et al. 1996), but a quantification was not possible. Such a quantitative method is preferentially important for the study of *in vitro* induction of other CYP forms and their mRNAs, which are, in contrast to CYP1A1 and CYP1A1-mRNA, present in considerable amounts in control livers. After 6 hrs incubation without BNF no increase in CYP1A1-mRNA was detectable. In cultured hepatocytes without the addition of an inducer, however, PADGHAM and PAINE (1993) found an considerable increase in CYP1A1-mRNA within 1 hr, a sign of rapid dedifferentiation of cultured hepatocytes. Such alterations did not take place in precision-cut slices.

Altogether, our results demonstrate that rat liver slices under our conditions are relatively stable for 48 hrs and can be used for various investigations, e.g. drug metabolism studies, induction etc.

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References

BERRY MN, FRIEND DS: High-yield preparation of isolated rat liver parenchymal cells: A biochemical and fine structural study. J Cell Biol 1969; **43:** 506-520.

DE KANTER R, KOSTER HJ: Cryopreservation of rat and monkey liver slices. ATLA 1995; **23:** 653-665.

DOWNS TR, WILFINGER WW: Fluorometric quantification of DNA in cells and tissue. Analyt Biochem 1983; **131:** 538-547.

DRAHUSHUK AT, MCGARRIGLE BP, TAI H-L, et al.: Validation of precision-cut liver slices in dynamic organ culture as an *in vitro* model for studying CYP1A1 and CYP1A2 induction. Toxicol Appl Pharmacol 1996; **140:** 393-403.

EKINS S: Short-term maintenance of phase I and II metabolism in precision-cut liver slices in dynamic organ culture. Drug Metab Dispos 1996a; **24:** 364-366.

EKINS S: Past, present, and future applications of precision-cut liver slices for in vitro xenobiotic metabolism. Drug Metab Rev 1996b; **28:** 591-623.

EKINS S, WILLIAMS JA, MURRAY GI, et al.: Xenobiotic metabolism in rat, dog, and human precision-cut liver slices, freshly isolated hepatocytes, and vitrified precision-cut liver slices. Drug Metab Dispos 1996; **24:** 990-995.

FISHER RL, HASAL SJ, SANUIK JT, et al.: Determination of optimal incubation media and suitable slice diameters in precision-cut liver slices: Optimization of tissue slice culture, part 2. Toxic Methods 1995a; **5:** 115-130.

FISHER RL, SHAUGHNESSY RP, JENKINS PM, et al.: Dynamic organ culture is superior to multiwell plate culture for maintaining precision-cut tissue slices: Optimization of tissue slice culture, part 1. Toxic Methods 1995b; **5:** 99-113.

FOLEY KP, LEONARD MW, ENGEL JD: Quantitation of RNA using the polymerase chain reaction. Trends Genet 1993; **9:** 380-385.

GLÖCKNER R, MÜLLER D: Ethoxycoumarin O-deethylation (ECOD) activity in rat liver slices exposed to beta-naphthoflavone (BNF) *in vitro*. Exp Toxic Pathol 1995; **47:** 319-324.

GLÖCKNER R, STEINMETZER P, DROBNER C, et al.: Application of cryopreserved precision-cut liver slices in pharmacotoxicology – principles, literature data and own investigations with special reference to CYP1A1-mRNA induction. Exp Toxic Pathol 1998; **50:** 440-449.

GOKHALE MS, BUNTON TE, ZURLO J, et al.: Cytochrome P450 isoenzyme activities in cultured rat and mouse liver slices. Xenobiotica 1997; **27:** 341-355.

KEMENY DM: ELISA – Anwendung des Enzyme Linked Immunosorbent Assay im biologisch/medizinischen Labor. Gustav Fischer, Stuttgart 1994

KLINGER W, MÜLLER D: The influence of age on the protein concentration in serum, liver and kidney of rats determined by various methods. Z Versuchstierkd 1974; **16:** 149-153.

KRETZSCHMAR M, FRANKE H, ZIMMERMANN T, et al.: Glutathione synthesis and export in experimental liver cirrhosis induced by thioacetamide: Relations to ultrastructural changes. Exp Pathol 1989; **36:** 113-122.

KRUMDIECK CL, DOS SANTOS JE, HO KJ: A new instrument for the rapid preparation of tissue slices. Anal Biochem 1980; **104:** 118-123.

LAKE BG, BEAMAND A, JAPENGA AC, et al.: Induction of cytochrome P-450-dependent enzyme activities in cultured rat liver slices. Fd Chem Toxic 1993; **31:** 377-386.

LAKE BG, CHARZAT C, TREDGER JM, et al.: Induction of cytochrome P450 isoenzymes in cultured precision-cut rat and human liver slices. Xenobiotica 1996; **26:** 297-306.

LAKE BG, BALL SE, RENWICK AB, et al.: Induction of CYP3A isoforms in cultured precision-cut human liver slices. Xenobiotica 1997; **27:** 1165-1173.

MILLER MG, BEYER J, HALL GL, et al.: Predictive value of liver slices for metabolism and toxicity *in vivo* – use of acetaminophen as a model hepatotoxicant. Toxicol Appl Pharmacol 1993; **122:** 108-116.

MÜLLER D, GLÖCKNER R, ROST M: Monooxygenation, cytochrome P4501A1 and P4501A1-mRNA in rat liver slices exposed to beta-naphthoflavone and dexamethasone *in vitro*. Exp Toxic Pathol 1996; **48:** 433-438.

OLINGA P, GROEN K, HOF IH, et al.: Comparison of five incubation systems for rat liver slices using functional and viability parameters. J Pharmac Toxic Methods 1997a; **38:** 59-69.

OLINGA P, MEIJER DKF, SLOOFF MJH, et al.: Liver slices in *in vitro* pharmacotoxicology with special reference to the use of human liver tissue. Toxic in vitro 1997b; **12:** 77-100.

PADGHAM CRW, PAINE AJ: Altered expression of cytochrome-P-450 messenger RNAs, and potentially of other transcripts encoding key hepatic functions, are triggered during the isolation of rat hepatocytes. Biochem J 1993; **289:** 621-624.

PANG J-M, ZALESKI J, KAUFFMAN FC: Toxicity of allyl alcohol in primary cultures of freshly isolated and cryopreserved hepatocytes maintained on hydrated collagen gels. Toxicol Appl Pharmacol 1997; **142:** 87-94.

PARRISH AR, GANDOLFI AJ, BRENDEL K: Precision cut liver slices: application in pharmacology and toxicology. Life Sci 1995; **57:** 1887-1901.

ROTEM A, MATTHEW HWT, HSIAO PH, et al.: The activity of cytochrome P450IA1 in stable cultured rat hepatocytes. Toxic in vitro 1995; **9:** 139-149.

SCHMIDT DM, ERNST JD: A fluorimetric assay for the quantification of RNA in solution with nanogram sensitivity. Anal Biochem 1995; **232:** 144-146.

SIEBERT PD, LARRICK JW: Competitive PCR. Nature 1992; **359:** 557-558.

SKETT P: Problems in using isolated and cultured hepatocytes for xenobiotic metabolism/metabolism-based toxicity testing-solutions? Toxic in vitro 1994; **3:** 491-504.

VAN DEN HEUVEL JP, TYSON FL, BELL DA: Construction of recombinant RNA templates for use as internal standards in competitive RT-PCR. BioTechniques 1993; **14:** 395-398.

WARBURG O: Versuche an überlebendem Carcinomgewebe. Biochem Z 1923; **142:** 317-333.

YAGI K: Lipid peroxides and human disease. Chem Phys Lipids 1987; **45:** 337-351.