Biochemical Pharmacology, Vol. 33, No. 1, pp. 71–77, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

HUMAN FETAL LIVER CULTURES: BASAL ACTIVITIES AND INDUCIBILITY OF EPOXIDE HYDROLASES AND ARYL HYDROCARBON HYDROXYLASE

DUN-REN PENG, GIAN MARIA PACIFICI and ANDERS RANE*
Department of Clinical Pharmacology, Huddinge University Hospital, 141 86 Huddinge, Sweden

(*Received* 18 *April* 1983; *accepted* 13 *July* 1983)

Abstract—The environmental influence of various drugs on the epoxide hydrolase with styrene oxide (EH_{so}) or benzo(a)pyrene-4,5-oxide (EH_{BPox}) as substrate and the aryl hydrocarbon hydroxylase (AHH) activity was studied in monolayer cultures of human fetal hepatocytes (HFH) obtained at legal abortions. Hepatocytes were isolated by trypsin treatment of liver fragments and primary HFH cultures were maintained in Eagle's minimum essential medium supplemented with 15% newborn calf serum. The HFH were plated on culture dishes and allowed to 'settle' for one day before adding various drugs (in 1 μl dimethylsulfoxide/ml) or solvent only and assay 1–2 days later.

The basal AHH activity [assayed with ^{3}H-benzo(a)pyrene as substrate] varied between 2 and 8.4 pmoles/min/mg protein and the basal EH_{so} activity was 0.3–4.9 nmoles/min/mg protein ($n = 6$) after one or two days' culture. The corresponding activity of EH_{BPox} was 0.23–1.48 nmoles/min/mg protein ($n = 5$). Exposure of cultures to 2 mM phenobarbital (Pb), 2.5–25.0 μM benzanthracene (BA), 0.1 mM trans-stilbene oxide (TSO), or 5 μM β-naphtoflavone (βNF) resulted in a 1.2–3.7-fold induction of EH_{so}. Induction of EH_{BPox} was also observed with Pb, βNF, BA and TSO as inducers. Pb gave a dose-dependent induction of both EH at 0.1, 1.0 and 2.0 mM.

Our results demonstrate that EH and AHH activities in HFH cultures are inducible by classical *in vivo* inducers. Although difficult to prove, it is plausible that such induction takes place also in intrauterine life.

Drug oxidation is not catalyzed in the fetal liver of any of the non-primate experimental animals investigated so far, at least not until just prior to birth [1]. It develops rapidly in the perinatal period. In contrast, the human fetus is able to catalyze drug oxidation reactions as early as the first trimester [2]. This has been demonstrated for many different drugs and other xenobiotics [1, 3, 4]. The benefit of this capacity is obvious in case the effect is terminated upon metabolism of the drug. However, it seems to be a potential disadvantage for the fetus if the drug is bioactivated, i.e. transformed to active or toxic metabolites. A large number of drugs [5] and other foreign compounds, e.g. polycyclic hydrocarbons [6] are known to mediate pharmacological or toxic effects via their degradation products.

Given this situation the questions about the biological function of the various drug metabolizing enzyme systems and their regulation mechanisms have gained increasing interest. Does the species difference (prenatal drug metabolism in man only) represent a constitutional difference or is it a result of environmental influence, specific for man? In order to elucidate the latter question we wanted to test whether cultured hepatocytes from the human fetus could be used for studies of environmental influence on drug metabolic processes.

* All correspondence and reprint requests should be sent to: Anders Rane, M.D., Dept of Clinical Pharmacology, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

This paper describes a technique for primary culture of fetal hepatocytes, earlier described for rat [7] but now adopted for application on human fetal liver. It also presents data on the activity and inducibility of aryl hydrocarbon hydroxylase (AHH) and epoxide hydrolase (EH). The influence of phenobarbital (Pb), benzanthracene (BA), *trans*-stilbene oxide (TSO) and β-naphtoflavone (βNF, 5,6-benzoflavone) on these enzyme activities was tested in cultures of human fetal livers.

MATERIALS AND METHODS

Chemicals

Eagle's minimum essential medium (MEM) with Hank's salts, newborn calf serum, trypsin and penicillin–streptomycin were all purchased from Gibco Europe (Glasgow, U.K.). NADPH, glucose-6-phosphate dehydrogenase, unlabelled benzo(a)pyrene and 1,2-BA were purchased from Sigma. βNF was purchased from Ega-Chemie (Steinheim, F.R.G.). The thin layer chromatography (TLC) plates (19-channel LK5DF) were purchased from Whatman (Clifton, NY, U.S.A.). Sodium Pb and solvents were purchased from standard commercial sources. TSO was a gift from Dr. J. de Pierre.

[7-^{3}H]Styrene-7,8-oxide (99.6 mCi/mmole) and [G-^{3}H]-benzo(a)pyrene (spec. act. 20 Ci/mmole) were purchased from the Radiochemical Centre (Amersham, U.K.). Unlabelled styrene oxide and styrene glycol were obtained from Aldrich (Beerse,

Belgium). The radiochemical purity of styrene oxide was >99% after purification, as estimated by the TLC system described below. Unlabelled and radioactive styrene oxide were purified before use [8].

Benzo(a)pyrene-4,5-oxide and 4,5-dihydrobenzo(a)pyrene-4,5-epoxide-[G-^{3}H] (spec. act. 382 mCi/mmole) were received from the NCI Chemical Carcinogen Reference Standard Repository, a function of the Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, MA.

Biological material. Livers from human fetuses were obtained at legal abortions performed by vaginal application of prostaglandins or extraamniotic instillation of ethacridine (Rivanol®). Fetal age was determined from the crown-rump length and varied between 10 and 23 weeks. The liver was excised from the fetus (which was kept in ice-cold saline) as soon as possible, usually within 3 hr. It was transferred into ice-cold Dulbeccos Ca–Mg free solution. The preparation of fetal liver cell cultures was performed essentially as described by Goujon *et al.* [7].

Culture medium. Culture medium consisted of Eagle's MEM with Hank's salts supplemented with newborn calf serum (15%), sodium bicarbonate (12 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml).

Isolation of human fetal liver cells. The liver was rinsed and minced with a pair of scissors in a Ca–Mg free Dulbecco's solution. The solution was changed several times until the red colour disappeared indicating that the solution was relatively free from hemoglobin. Thereafter, (1–6 hr after abortion) the liver fragments were suspended in the same solution containing 0.1% trypsin and kept at 0–4° for 1–1.5 hr. The solution was then incubated for 15 min at 37°. After decantation, the large residual fragments were poured directly on a 1 cm layer of glass microspheres (1 mm dia) in a 100 ml Erlenmeyer flask. After addition of culture medium the flask was shaken by hand and the combined cell suspensions were centrifuged gently twice in a conical sterile tube at 4° at 50 *g* for 5 min. The supernatants were then removed and the pellet was resuspended in 50 ml of the culture medium (MEM). The viability of the HFH was estimated by the Trypan blue exclusion test and the cells were counted in a Bürker chamber. All steps of the procedure were performed using mouth shelters, sterile gloves and autoclaved pipettes, glass beads and glass wares.

Culture and treatment of cells. The HFH were plated on tissue culture dishes (Nuncleon, Denmark). The normal cell number per well (33 mm dia) was 5×10^6. The cells were incubated in a CO_2 incubator box (ASSAB, Sundbyberg, Sweden) at 37° in a humidified atmosphere of 5% CO_2 in air. After about 24 hr settlement* of the culture the medium was replaced by fresh MEM containing dexamethasone (1.5 μM). At this time the various drugs or other foreign compounds were added in dimethylsulfoxide (DMSO) 0.1%. Control cultures contained only DMSO, 1 μl/ml medium. Thereafter the medium (with or without inducers) was changed every 2nd day but often the cultures were harvested within 2 days after 'settlement'. The final concentrations of the various drugs or other compounds in the culture medium were as follows: Pb 0.1, 1.0, 2.0 mM; BA 2.5, 25 μM; TSO 0.02, 0.1 mM; and β-NF 0.005, 0.05, 0.1 mM. Harvesting the cell cultures was carried out 1, 2 or 3 days after settlement. Rubber 'policemen' were used to collect the material. All steps of the procedure were made at 4°. Since normally relatively few cells were floating in the medium, these were combined with the main cell population adhering to the bottom of the wells and the material was then centifuged at 4° at 1000 *g*. After decanting the supernatant medium and rinsing with Dulbecco's solution, the pellet was frozen at −20° until the enzyme assay.

Enzyme assays. For the enzyme assays, the frozen cell pellets were thawed in about 0.3–0.6 ml. Tris–sucrose–EDTA buffer (0.1, 0.25, 0.001 M) pH 7.6 and sonicated for 20 sec (divided into four periods of 5 sec) in an ultrasonic disintegrator (MSE Scientific Instruments, U.K.). Epoxide hydrolase activity was determined with styrene oxide (EH_{so}) or benzo(a)pyrene,4-5-oxide (EH_{BPox}) as substrate.

EH_{so} activity was measured according to Jerina *et al.* [9] with some modifications. The incubation mixture consisted of 0.2 M Tris–HCl buffer, pH 8.7, 0.5 μmole [^{3}H]-styrene oxide (150,000 dpm) and an aliquot of the sonicated homogenate corresponding to 0.15 mg of protein in a final volume of 0.5 ml. The reaction was started by addition of substrate in 5 μl of acetonitrile. After 30 min of incubation at 37° the reaction was terminated by adding 100 μl of tetrahydrofuran solution containing styrene oxide and styrene glycol. After mixing 40 μl were applied on the preadsorbent phase of the TLC plates and chromatographed in a system of chloroform; ethylacetate (8:2, v/v). After drying, the styrene glycol was visualized under u.v.-light. The area of fluorescence was scraped off and mixed with 1 ml of methanol and 10 ml of Luma Gel® for liquid scintillation. The procedures for determining EH_{BPox} was the same as that for EH_{SO} with the following modifications: the incubation mixture, in a final volume of 0.1 ml, contained 0.01 μmole benzo(a)pyrene-4,5-epoxide containing [^{3}H]-benzo(a)pyrene4,5oxide (600,000 dpm). The reaction was carried out in a shaking water bath at 37° for 4 min and stopped by adding 20 μl of tetrahydrofuran solution. Chromatography was performed in a system of benzene:methanol (93:7, v/v) and the further procedure was the same as described above for EH_{so}.

AHH activity was measured as described by van Cantfort *et al.* [10].

Protein was determined according to Peterson [11].

RESULTS

Preparation and maintenance of cultures. All steps of the cell preparation were performed under semi-sterile conditions. Using this approach the rate of bacterial overgrowth was less than 1 in 10 cultures, which was considered acceptable.

* Hereafter the word 'settlement' is used to denote the first 24 hr after the plating before change of medium took place.

Various concentrations of trypsin were tested. The large variation in quality between the cultures precluded any systematic comparison of yields between cell preparations with different trypsin concentrations. However, the lower (0.1–0.15%) concentrations were seemingly best when consideration was taken both to yield and the subsequent growing capacity in the culture dishes. Too high concentrations probably affect components of the cell membrane which are of importance for adherence of cells to the bottom of the well.

Refrigeration (at 4°) of the hepatic tissue fragments in trypsin enriched Dulbecco's solution longer than 1–1·5 hr did not improve the yield of cells. It is our experience that tissues from older fetuses (18–24 weeks of gestation) gave rise to better cultures than tissues from young fetuses. Similarly, the shorter interval between the abortion and collection of the tissue the better. No given limit may be recommended since the survival (lifetime) of the cultured hepatocytes varied tremendously depending on the gestational age, the abortion course etc. Generally, attempts to culture liver cells from prostaglandin aborted fetuses were more successful than if the tissue originated from ethacridine aborted fetuses.

Epoxide hydrolase with styrene oxide as substrate (EH_{so}). There was no significant difference in EH_{so} activity between cultures with and without addition

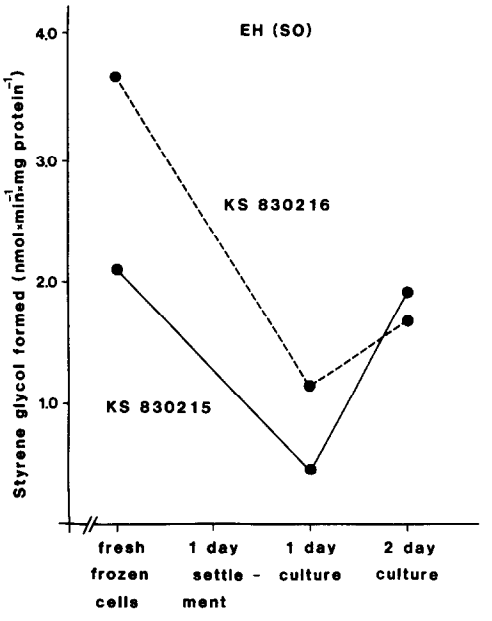

Fig. 2. Epoxide hydrolase basal activity (without induction) in fresh hepatocytes that were frozen and then sonicated as compared to sonicates from hepatocytes cultured for different times without inducing agents in the culture medium. Upper and lower curves represent data from a 20- and 22-week-old fetus, respectively.

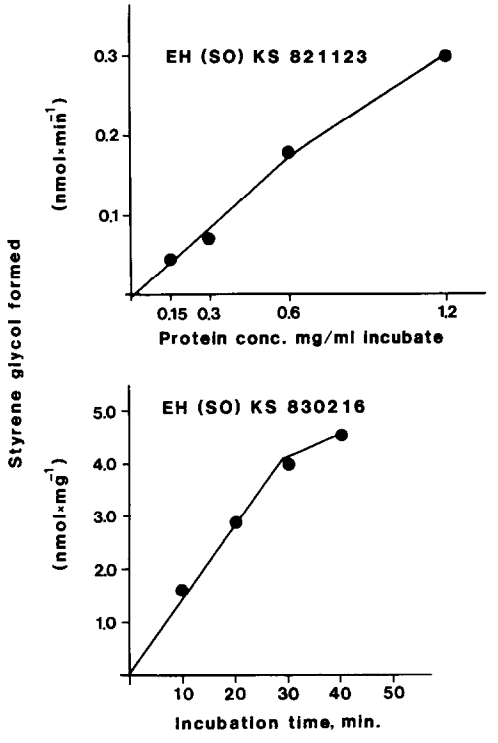

Fig. 1. Epoxide hydrolase activity with styrene oxide as substrate; styrene glycol formed as a function of protein concentration (upper panel) or incubation time (lower panel) in sonicated homogenates of cultured hepatocytes from a 17- (upper panel) and a 20- (lower panel) week-old human fetus. The figure shows two representative experiments.

of the solvent, DMSO. Cultures (without DMSO) maintained at room temperature for 1 or 2 days had no or considerably lower EH_{so} activity than controls (without DMSO).

In initial experiments the standard induction time of 2 days (after 1 day's settlement of the culture) was employed for this assay. After 2 days' induction the inducibility of EH_{so} was variable although the cultures were still metabolically active on day 4 and 6. The reaction was found to be linear with respect to protein and time at least up to 0.6 mg/ml incubate and 30 min, respectively (Fig. 1). This incubation time was usually used. In order to save the material for as many incubations as possible 0.30 mg protein/ml was used routinely. The basal activity of the enzyme was measured in freshly isolated cells that were frozen immediately and in control cultured cells at different times. The activity profile with time is shown in Fig 2. After 1 or 2 days' culture (after settlement) the basal activity was 0.3–4.9 nmole/min/mg protein.

Phenobarbital (2 mM) exerted a variable degree of induction, from 1.2- to 3.1-fold in 6 of 7 (6/7) investigated livers. To evaluate the possibility of concentration dependency of the induction three consecutive liver cultures were induced with Pb at various concentrations (0.1, 1.0 and 2.0 mM). All liver cultures displayed a dose-dependent inducibility of the EH_{so}. The two highest concentrations exerted significant induction already after 1 day (2/3), 2 days (3/3) and 3 days (1/2). The response to the lowest Pb concentration was variable and usually not significant, irrespective of the duration of induction. In some cultures the lowest concentration gave an

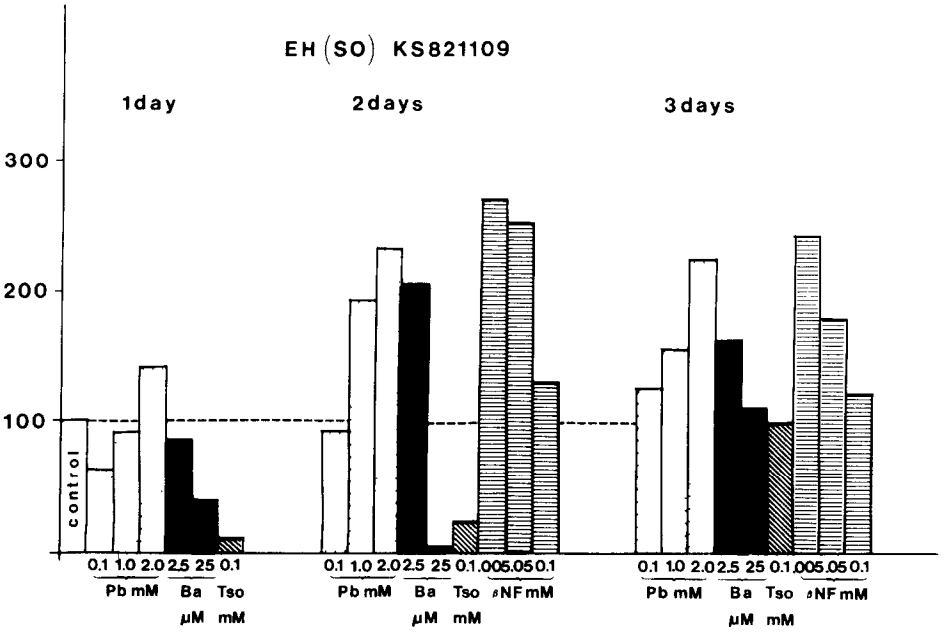

Fig. 3. Inducibility of epoxide hydrolase with styrene oxide as substrate in human fetal hepatocytes (from a 20-week-old fetus) exposed to different agents for 1, 2 or 3 days after 24 hr settlement. Activities are given in relation to control (= 100). Inducing agents and their concentrations in culture medium are given below the bars. Pb, phenobarbital; Ba, benzanthracene; TSO, trans-stilbene oxide, βNF, β-naphtoflavone.

apparent depression of the activity as compared to the control cultures. A representative experiment is shown in Fig 3. The specific EH_{so} activity increased with the Pb concentration in the medium as shown in Fig. 4(a) both 1 and 2 days after addition of the drug.

Benzanthracene was initially only tested in the 2 day cultures at 25 μM concentration. In all of 8 liver cultures, this agent induced the EH_{so} 1.3- to 2.9-fold after 2 days' exposure. In subsequent experiments (N = 4) it was found that lower concentration of this agent (2.5 μM) in half of the cultures had a stronger inducing effect.

Trans-stilbene oxide at 0.1 mM induced the EH_{so} activity 1.4- to 3.7-fold after 2 days' exposure. Induction was observed in 7/9 livers. Subsequent

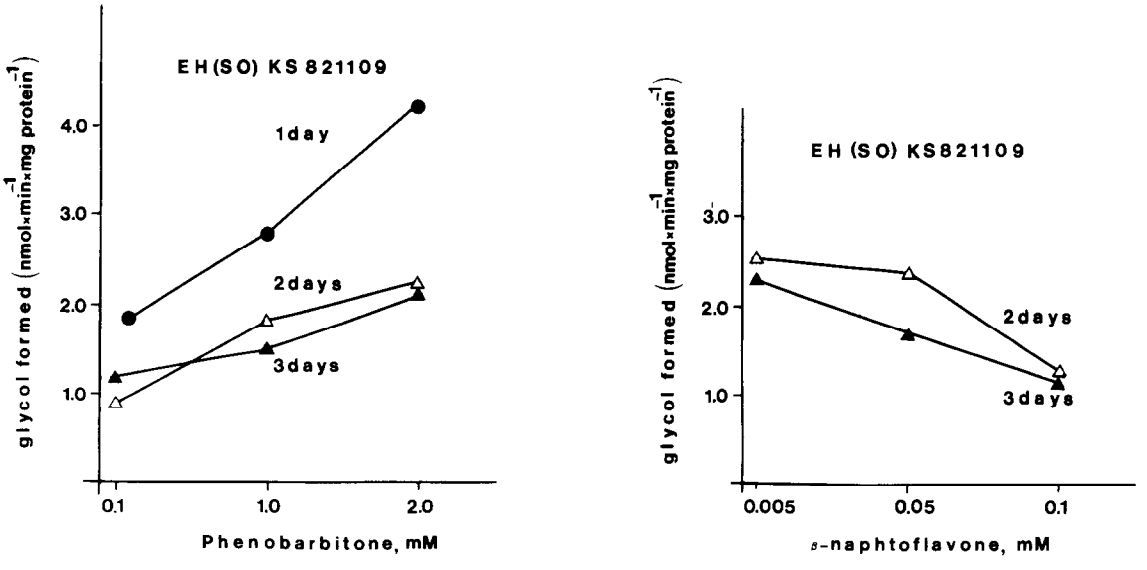

Fig. 4. Epoxide hydrolase activities (with styrene oxide as substrate) in human fetal hepatocyte cultures, exposed to different concentrations of phenobarbital (a) or β-naphtoflavone (b) for 1, 2 or 3 days (see Materials and Methods). Hepatocytes were obtained from a 20-week-old human fetus.

experiments revealed that lower concentrations had more pronounced effects and concentrations of 0.002, 0.02 and 0.1 mM were therefore tested. Only a few cultures showed induced EH_{so} activity after only 1 day's exposure to TSO. Significant induction was observed after 2 days in 7/9 different cultures without signs of concentration dependency.

β-Naphtoflavone was tested as an inducer at several concentrations, 0.005, 0.05 and 0.1 mM. The highest concentration sometimes gave rise to crystals in the medium and therefore, was not considered to give reliable results. The inducibility (Fig. 3) as well as the specific activity (Fig. 4b) increased with decreasing concentrations of βNF. At 0.005 mM there was a 1.7–2.7-fold induction of the EH_{so} activity on day 2 in 3/5 liver cultures investigated. Two liver cultures were induced only by 0.05 and 0.1 mM βNF on day 2.

Epoxide hydrolase with benzo(a)pyrene-4,5-oxide as substrate (EH_{BPox}). Because of limited supply of human fetal tissues EH_{BPox} was tested only in 5 liver cultures with a limited number of inducing agents at different concentrations. The basal activity varied between 0.23 and 1.48 nmoles/min/mg protein after 1 or 2 days' culture (after settlement). As was the case with EH_{so} this enzyme was consistently induced by phenobarbital in 4/5 cultures, the fifth of which did not respond to any inducing agent at all. There was 1.3- to 2.8-fold induction by 2.0 mM Pb on day 2. Dose dependency was tested in only one of the cultures and from the results (Fig. 5) There seems to exist such a concentration dependency, in particular on day 2.

β-Naphtoflavone (0.1 mM) also induced EH_{BPox} by a factor of 1.3–2.0 in 3/3 cultures on day 2. Significant inducing effect by this agent was observed also on day 3–4 in 2/3 liver cultures.

Benzanthracene (25 μM) exerted a strong inducing effect (1.9–2.5 times) in 3/4 investigated cell cultures. Trans-stilbene oxide had a variable effect on EH_{BPox}. Usually it induced the EH_{BPox}, as shown in Fig. 5. The induction varied between 1.2- and 2.2-fold on day 2.

Aryl hydrocarbon hydroxylase (AHH). AHH activity was tested in sonicated homogenates of HFH cultured for 1 to 4 days (after 1 day's settlement period). It became apparent early that the activity and the degree of inducibility was lower the longer the culture had been maintained. Moreover, the results from different cultures were variable at longer culture periods. The best results were obtained in 1 or 2 days' cultures.

The AHH activity was negligible in 4 cultures, one of which originated from a liver that was obtained 4 hr after abortion (fetus stored in refrigerator). The activity in the other cultures varied between 2 and 8.4 pmoles/min/mg.

Phenobarbital exerted a concentration dependent induction when tested at concentrations of 0.1, 1.0 and 2.0 mM. For unknown reasons, the lowest concentration sometimes apparently suppressed the activity as compared to the control culture whereas 2 mM was most effective and induced 2.3–5.5 times. Induction was observed in 4/5 different cultures.

β-Naphtoflavone was the only other agent that consistently induced AHH in the HFH cultures. The inducibility varied from 3.3- to 8.2-fold in 2 different 1 day-cultures. After 2 days' exposure there was an equally great induction in one of the cultures.

Trans-stilbene oxide and benzanthracene had no

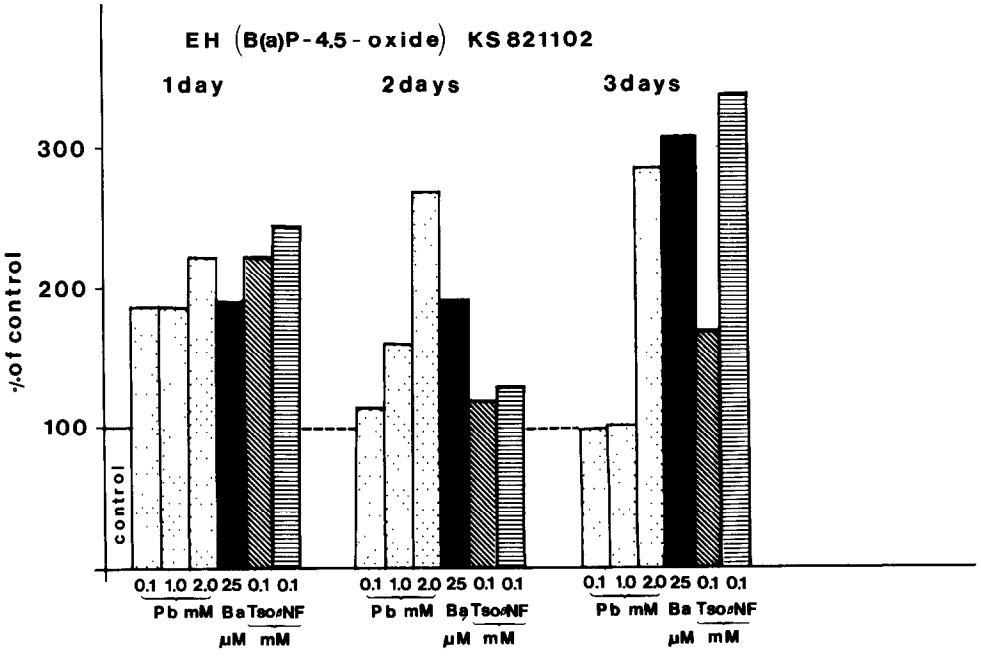

Fig. 5. Inducibility of epoxide hydrolase with benzo(a)pyrene-4,5-oxide as substrate in human fetal hepatocytes (from a 20 week old fetus) exposed to different agents for 1, 2 or 3 days after 24 hours' settlement. Activities are given in relation to control (= 100). Symbols and abbreviations as in Fig. 3.

inducing effect on AHH. Addition of BA inhibited the activity in most cases.

DISCUSSION

Primary cultures of HFH offer a unique possibility to study drug metabolic reactions and the regulatory mechanisms under physiological conditions [7, 12, 13]. They are particularly useful for studies of the influence of exogenous inducers or inhibitors on various reactions, since these drugs cannot be administered to pregnant women on other than therapeutic indications. Moreover, attempts to correlate maternal drug exposure to enzyme activities in the human fetal abortion material have been only partly successful [14].

In our hands the purity of the cell cultures was high and seemingly comparable to that described by Bisell and Tilles [15], who also used 0.1% trypsin for the cell isolation. It was also comparable to the results of Guguen-Guillouzo *et al.* [16] who used 0.025% collagenase for cell isolation. None of these investigators gave however any quantitative information about the purity of the preparations.

Our data show that EH_{so} as well as EH_{BPox} are present at measurable activities in monolayer cultures of HFH. We have also measured AHH in these cultures. All enzymes were found to be influenced by various agents.

Different degrees of induction were attained in different cultures. This may have various explanations, e.g. constitutional-genetic differences between individual fetuses or differences in the technical quality of the culture. In order to minimize technical differences between adjacent cultures from the same tissue as a cause of the variation the enzyme preparations were always generated as a mixture from three or more different wells. Although confluency of the monolayer cultures varied from preparation to preparation we believe that this factor was not of major importance for the results. Enzyme activities were measurable even in non-confluent or floating cultures. Pelkonen *et al.* [17] also reported the same degree of induction of AHH in nonconfluent as in confluent cultures of HFH. In their study the nonconfluent cultures had higher basal AHH activity than the confluent cultures.

The activity of EH_{so} was maintained for several days in culture. This enzyme was less susceptible than AHH to bad conditions of the collected liver specimens. If the liver tissue was dispersed in the fetal abdomen due to, for example, compression the cell culture was generally of bad quality. Nevertheless, the EH_{so} activity was maintained for several days in culture and inducible even though AHH activity had disappeared. Greater persistency of EH_{BPox} than AHH has also been noted by Holme *et al.* [18] in rat hepatocyte cultures. The activity of EH_{so} was measured in fresh isolated HFH that were centrifuged and frozen as a pellet. This activity was somewhat higher than the activity in the cultured cells as shown in Fig. 2.

The activity in the liver cultures was inducible for at least 3 days although maximum inducibility was achieved after 2 days' exposure to the inducer.

Only Pb and βNF induced the EH_{so} consistently and equally in all investigated liver cultures. Little is known from the literature about the effect of these agents on EH_{so}. However, their effects on EH_{BPox} have been compared in fetal rat hepatocytes [7]. Pb was a stronger inducer than βNF.

There was a conspicuous dose dependency of the induction of EH_{so} by Pb and βNF (Fig. 3). When tested at 0.1, 1.0 and 2.0 mM, Pb gave a gradually increased activity as compared to the control cultures containing only solvent. This concentration dependency is in agreement with the observation made by Goujon *et al.* [7] and Gielen *et al.* [12] for EH_{BPox} in cultures of fetal rat hepatocytes, in which 2 mM Pb gave maximal induction whereas concentrations above that were associated with signs of toxicity. Occasionally, the 0.1 mM concentration gave an apparent depression of the activity of EH_{so}. The reason for this is unclear.

The aromatic inducer βNF may be metabolized to toxic epoxides in similarity with BA and other polycyclic hydrocarbons. This may be the reason for the decrease in EH_{so} activity observed at increasing concentrations of these agents. Pelkonen *et al.* [17] tested the influence of various concentrations of BA on AHH in human fetal liver monolayer cultures. In some of their cultures, the AHH activity increased with increasing concentrations of this agent (22–220 μM) whereas in other cultures, a decrease was observed. In our experiments 2.5 μM BA and 5 μM βNF was optimal for induction of EH_{so}. This concentration of BA is far below that observed to give maximal induction of EH_{BPox} in fetal rat liver cultures [7, 19] and from this point of view the human fetal EH_{so} seems to be more sensitive to this polycyclic aromatic compound.

In contrast to the findings by Goujon *et al.* [7] we found only a slight inducing effect on EH_{so} by TSO, added at a concentration of 0.1 mM. This concentration was sometimes more effective than 0.5 mM, which was reported to give the strongest inducing effect on EH_{BPox} in fetal rat hepatocyte cultures [7]. However, in some culture preparations 0.1–0.5 mM TSO depressed the EH_{so} activity considerably, perhaps as a result of direct toxicity of this agent or its metabolites.

The relative activity of enzymes that catalyze the formation [cf. 6] and degradation [20] of reactive intermediates such as epoxides is believed to be of great importance for the ultimate toxic effects of epoxides [20, 21]. *In vivo* treatment of animals lead to changes in EH and AHH activities and hepatic bioactivation of carcinogens. However, relatively few studies have attempted to link together such enzyme perturbations with the biological effects on bacteria, cell cultures or intact animals. Oesch *et al.* [21] demonstrated a relation between the *in vitro* activities of EH and AHH in rat or mouse liver microsomal enzymes and their mutagenic effect on bacteria, when incubated with benzo(a)pyrene. Nebert [22] showed that mouse fetal cell culture toxicity from polycyclic hydrocarbons reflects the toxicity in the intact animal and suggested that the teratogenicity of drugs may be better predicted if the cell culture metabolism of the agent is characterized.

In another study [23] in pregnant rats it was shown that Pb induces both fetal hepatic EH and AHH

whereas only the latter enzyme was induced by 3-methylcholanthrene. The biological significance was shown using the Ames test. This shows the biological significance of differential induction of AHH and EH and highlights the importance of direct studies on human cultured tissues.

Our data show that the AHH and the EH in cultured HFH have the potential of being induced by various xenobiotics, e.g. polycyclic hydrocarbons. The inducibility varies between individual cultures depending on constitutional or technical factors. Whether these enzymes are induced in the intact human fetus *in utero* after exposure of the mother to various environmental agents remains to be corroborated. Inducers of drug metabolism *in vivo* may have the potential both to activate and induce drug metabolizing enzymes in cultures. The mechanisms of the enhancement of the enzyme activity are unclear but currently under investigation.

It is plausible that some of the drug induced fetotoxic effects, transplacental carcinogenesis or teratogenesis are regulated by the activity of AHH and EH [23–25]. We therefore propose that the approach to study metabolic reactions in human fetal tissue cultures might be valuable for risk assessment of various chemically mediated toxic effects in man and also for studies of the physiologic regulation of drug metabolic reactions.

Acknowledgements—We thank Mrs. Jolanta Widén for excellent technical assistance. This work was supported by grants from the Swedish Medical Research Council (14x-04496) and the Expressen Prenatal Research Foundation.

REFERENCES

1. O Pelkonen, *Pharmac. Ther.* **10**, 261 (1980).
2. S. J. Yaffe, A. Rane, F. Sjöqvist, L. O. Boréus and S. Orrenius *Life Sci.* **9**, 1189 (1970).
3. D. Rollins, C. von Bahr, H. Glaumann, P. Moldéus and A. Rane, *Science* **205**, 1414 (1979).
4. E. Steiner, C. von Bahr and A. Rane, *Devl Pharmac Ther.* **5**, 14 (1982).
5. S. Garattini, F. Marcucci and E. Mussini, In *Concepts in Biochemical Pharmacology*, Part 3 (Eds. J. R. Gillette and J. R. Mitchell), pp. 113–129. Springer Verlag, New York (1975).
6. D. M. Jerina and J. W. Daly, Arene oxide; a new aspect of drug metabolism. *Science* **185**, 573 (1974).
7. F. M. Goujon, J. van Cantfort and J. E. Gielen, *Chem.-Biol. Interactions* **32**, 361 (1980).
8. G. M. Pacifici and A. Rane, *Drug Metab. Dispos.* **10**, 302 (1982).
9. D. M. Jerina, P. M. Dansette, A. Y. H. Lu and W. Levin, *Molec. Pharmac.* **13**, 342 (1977).
10. J. van Cantfort, J. de Graeve and J. E. Gielen, *Biochem. biophys. Res. Commun.* **79**, 505, (1977).
11. G. L. Peterson, *Analyt. Biochem.* **83**, 346 (1977).
12. J. E. Gielen, J. de Graeve, Fr. Goujon, P. Kremers and J. van Cantfort, In *Biological Reactive Intermediates* II, Part A (Eds. Snyder, Parke, Kocsis, Jollow, Gibson, Witmer), pp. 87–98. Plenum Press, New York (1981).
13. A. Guillouzo, J. F. LeBigot, C. Guguen-Guillouzo and J. R. Kiechel, *Biochem Pharmac.* **31**, 2427 (1982).
14. O. Pelkonen, J. Jouppila and N. T. Kärki, *Archs int. pharmacodyn. ther.* **202**, 288 (1973).
15. D. M. Bissell and J. G. Tilles, *J. Cell Biol.* **50**, 222 (1971).
16. C. Guguen-Guillouzo, J. Marie, D. Cottreau, N. Pasdeloup and A. Kahn, *Biochem. biophys. Res. Commun.* **93**, 528 (1980).
17. O. Pelkonen, P. Korhonen, P. Jouppila and N. Kärki, *Life Sci.* **16**, 1403 (1975).
18. J. A. Holme, E. Söderlund and E. Dybing, *Acta Pharmacol. Toxicol.* **52**, 348 (1983).
19. J. E. Gielen, R. Gol-Winkler, F. Goujon and J. van Cantfort. In *Neoplastic Transformation in Differential Epithelial Cell Systems* in Vitro (Eds. L. M. Franks and C. B. Wigley), pp. 205–234. Academic Press, London (1979).
20. F. Oesch, Epoxide hydratase. In *Progress in Drug Metabolism* (Eds. J. W. Bridges and L. F. Chasseaud), Vol 3, pp. 253–301. John Wiley, Chichester (1979).
21. F. Oesch, D. Raphael, H. Schwind and H. R. Glatt, *Archs Toxicol.* **39**, 97 (1977).
22. D. W. Nebert, *Clin. Pharmac. ther.* **14**, 693 (1973).
23. F. Oesch, *J. biol. Chem.* **251**, 79 (1976).
24. F. Oesch, In *Basic and Therapeutic Aspects of Perinatal Pharmacology* (Eds. P. L. Morselli, S. Garattini and F. Sereni), pp. 53–64. Raven Press, New York (1975).
25. A. Rane and G. M. Pacifici, In *Drug Metabolism in the Immature Human* (Ed. L. F. Soyka and G. P. Redmond), pp, 29–35. Raven Press, New York (1981).