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ACTIVITIES OF SEVERAL PHASE I AND PHASE II XENOBIOTIC BIOTRANSFORMATION ENZYMES IN CULTURED HEPATOCYTES FROM MALE AND FEMALE RATS

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Abstract—Hepatocytes were isolated from adult male and female rats and maintained in monolayer culture for up to 24 hr. The degree of preservation of representative phase I and phase II xenobiotic biotransformation enzymes was studied in these cells immediately after isolation, after attachment in culture, and after 24 hr in culture. Regarding phase I pathways, hepatocytes during 24 hr lost 50% of cytochrome P-450, but maintained high mixed function oxidase activities; 75% of aryl hydrocarbon hydroxylase and 65% of benzphetamine demethylase activities were preserved in hepatocytes from males, whereas in hepatocytes from females 70 and 50% of these activities, respectively, were maintained. Of phase II pathways, glutathione transferase activity after 24 hr, tested toward 1,2-dichloro-4-nitrobenzene as substrate, was diminished in male hepatocytes to 20% of the initial liver activity and in female cells, to 35%, whereas the activity tested toward 1-chloro-2,4-dinitrobenzene as substrate was stable. UDP-glucuronosyltransferase activities, tested toward *p*-nitrophenol and phenolphthalein as substrates, were slightly increased during 24 hr of culture of hepatocytes to levels higher than in liver before perfusion. The level of UDP-glucuronic acid, the endogenous substrate for the enzyme, was reduced after isolation to only 6% of the initial liver value, and then increased during culture to a level approximately 60% of normal. Thus, the changes in xenobiotic biotransformation enzymes and associated constituents in cultured hepatocytes were not uniform, although biotransformation capability remained reasonably intact.

Hepatocytes in primary monolayer culture are widely used to study mechanisms of cytotoxicity and genotoxicity of xenobiotics [1–8]. Metabolism and toxicity studies indicate that cultured hepatocytes retain much of the capability of liver to biotransform xenobiotics [9–14], but significant alterations do occur in culture [10, 11, 15].

The major classes of enzymatic processes that are involved in the biotransformation of xenobiotics are categorized as phase I oxidation–reduction reactions and phase II conjugative reactions. In phase I biotransformation, the mixed function oxygenase (MFO) system oxidizes xenobiotics, and then in phase II conjugation enzymes convert xenobiotics to water soluble metabolites. The oxidation of xenobiotics sometimes results in them being activated to reactive toxic species. These are detoxified by conjugation. Thus, the balance between phase I and phase II reactions determines the final toxic effects of a chemical to cells. However, these two pathways are not tightly coupled [16] and, consequently, culture-dependent changes in one or the other could substantially alter the biologic effect of a xenobiotic.

The MFO system is dependent upon the cytochrome P-450 system for oxidation reactions [17–19]. Several studies have shown that the level of cytochrome P-450 and the associated MFO activities of hepatocytes in primary culture can decline rapidly [10, 20–22]. A variety of approaches have been tried to maintain either the level of P-450 or the activity of associated enzymes [23–31]. The studies available thus far, however, have used only male rats, which have higher levels of P-450 and MFO activities than females [32–34]. Moreover, comparison of activities of different enzymes that are dependent upon cytochrome P-450 has not been extensive. Also, although cultured hepatocytes can perform phase II conjugation of xenobiotics [9, 11–13, 30, 35, 36] few comparisons of activity levels to those of MFO activities have been reported [30]. Furthermore, few data [30] are available on an important hepatic detoxification reaction, glutathione transferase activity [37–39] in hepatocytes in primary culture.

The present study profiles representative phase I and II biotransformation pathways in hepatocyte primary cultures isolated from male and female rats during the first 24 hr in culture. Cytochrome P-450 content and MFO activities such as aryl hydrocarbon hydroxylase and benzphetamine demethylase were measured, and glutathione transferase and UDP-glucuronosyltransferase were assayed for conjugative activities. The phase II enzymes were each

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measured toward two substrates. The results show that hepatocytes from both male and female liver undergo only a small to moderate decrease in the activity of some enzymes, while others are constant or increased.

MATERIALS AND METHODS

Young adult male or female Fischer F344 rats (13 weeks old) (Charles River Breeding Laboratories, Wilmington, MA) were used. Rats were maintained on NIH-07 laboratory chow (Zeigler Bros., Gardners, PA) and received water and food *ad lib.*

Isolation of hepatocytes

Isolation of hepatocytes utilized the two-step liver perfusion method of Seglen [40] as modified in this laboratory [41]. Animals were anesthetized with sodium pentobarbital, 50 mg/kg body weight. The caudal lobe of the liver was removed just before the perfusion to determine the basal levels of liver constituents and activities of enzymes. In the first step of the perfusion, the portal vein was cannulated and the liver was washed out for 4 min with a buffered, 37° solution of 0.5 mM ethylene glycol-bis-(β-aminoethyl ether)*N*-*N*′-tetraacetic acid in Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution buffered with 0.05 M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid to pH 7.2. In the second step, a 37° buffered solution (pH 7.4) of type I collagenase (Sigma Chemical Co., St. Louis, MO), 100 units/ml in Williams medium E (WME) supplemented with 50 μg/ml gentamicin sulfate (Flow Laboratories, McLean, VA), was circulated through the liver for 9–11 min. The flow rate in step 1 was 35–40 ml/min and in step 2, 18–20 ml/min; the variation of flow was a function of the size of the rats.

Following perfusion, cells were detached by gentle combing into fresh collagenase solution at room temperature. This procedure produces a selective recovery of hepatocytes. After adding WME supplemented with 10% calf serum (CS) (North American Biological, Inc., Miami, FL), the cell suspension was centrifuged at 50 *g* for 4 min, and the pellet was resuspended in WME plus 10% CS. Hepatocytes ($5–8 \times 10^6$) were then inoculated into 100 × 20 mm disposable plastic petri dishes (Falcon Plastics, Oxnard, CA) in a final volume of 15 ml of WME containing 10% CS and 50 μg/ml gentamicin sulfate. Ten to fifteen dishes were seeded for each time point. An attachment interval of 2.5 hr was used to permit attachment of only viable cells.

After the attachment interval the unattached cells were removed, and the viable cells were washed with 5 ml of WME without CS and refed with 10 ml of WME. Incubations were performed in a humidified 37° incubator with 95% air–5% CO_2.

Initial viability of freshly isolated hepatocytes was determined by trypan blue exclusion as previously described [20]. Dissociates from male rats were 90–94% viable cells, while those from female were 87–88% viable.

Preparation of livers and cell fractions

The caudal lobe of the liver was placed in a large volume of 4° sucrose buffer: 0.3 M sucrose, 1 mM EDTA and 5 mM Tris–HCl, buffered to pH 7.4. The tissue was blotted dry, weighed and homogenized in a small volume of cold sucrose buffer with a tight fitting motor-driven Teflon pestle. The homogenate was centrifuged at 9000 *g* for 30 min, yielding a supernatant fraction which was recentrifuged at 100,000 *g* for 60 min. Aliquots of the 100,000 *g* supernatant fraction were stored in liquid nitrogen for no more than 2 weeks. The microsomal pellet was suspended in a final volume of 1.5 ml of sucrose buffer and stored in liquid nitrogen for no more than 2 weeks.

For assays on freshly isolated hepatocytes (1 hr after beginning of perfusion), between 5×10^7 and 1×10^8 cells were added to a 4-fold excess of sucrose buffer and centrifuged at 1000 *g* for 5 min. The cell pellet was homogenized in sucrose buffer, and the homogenates were treated as described above.

For assays on cultured hepatocytes, cells were scraped off each dish in 5 ml of sucrose buffer using a rubber policeman; then the cells were pooled ($5 \times 10^7–1 \times 10^8$ total) and processed as described above. Measurements were made after the 2.5-hr attachment interval and after 24 hr from the end of perfusion.

Enzyme assays

Aryl hydrocarbon hydroxylase (AHH). AHH was measured in microsomal fractions using the methods of Nebert and Gelboin [42] with slight modifications described by Yang *et al.* [43]. The assay was done in duplicate. Under dim light, the reaction was started by addition of 80 nmoles benzo[*a*]pyrene (Sigma) dissolved in 25 μl acetone for each sample (total volume of 1 ml). Standards with known amounts of 3-hydroxybenzo[*a*]pyrene (National Cancer Institute, Chemical Repository, Chicago, IL) in the incubation mixture were run in parallel with the assay for calculating the amount of product formed, which was measured using an Aminco–Bowman spectrofluorimeter of the American Instrument Co. (Silver Spring, MD). The enzyme activity is expressed as nmoles product formed per min per mg of microsomal protein ± the standard error.

Benzphetamine demethylase (BZD). BZD was determined in microsomal fractions according to the procedure of Prough and Ziegler [44]. The assay was done in duplicate. Benzphetamine (provided by Upjohn, Kalamazoo, MI), dissolved in methanol (20 μl each 1 ml of sample), was used to start the reaction; the concentration of the substrate was 1 mM. The formation of CH_2O was measured colorimetrically using the Nash reagent [45]. The enzyme activity is expressed as nmoles CH_2O formed per min per mg of microsomal protein.

Glutathione transferase (GT). GT was measured in the cytosol fraction according to the procedure of Habig and Jakoby [46] using either 1 mM 1-chloro-2,4-dinitrobenzene or 1 mM 1,2-dichloro-4-nitrobenzene (Eastman Kodak, Rochester, NY) as substrates. The assays were done in duplicate at room temperature in curvettes in a Beckman model 25 spectrophotometer (Fullerton, CA). The reactions were started with an aliquot of cytosol fraction, and the changes in absorbance reflecting disappearance of substrate were recorded. The enzyme activity is expressed either as nmoles 1,2-dichloro-4-nitro-

benzene conjugated formed per min per mg of supernatant protein ± the standard error or as nmoles 1-chloro-2,4-dinitrobenzene conjugate formed per min per mg of supernatant protein ± the standard error.

UDP-glucuronosyltransferase (UDP-GT). UDP-GT activity in microsomal suspension was measured by a method derived from Duvaldestin *et al.* [47] and Halac and Reff [48] using phenolphthalein and *p*-nitrophenol (Aldrich Chemical Co., Milwaukee, WI) as substrates. The assays were performed in triplicate.

The reaction was started with 0.025 to 0.035 mg of microsomes treated with Triton X-100 (Sigma) for 30 min (final detergent concentration in the incubation mixture 0.01%), pH 8, at 0° to activate the enzyme.

The volume of the incubation mixture for phenolphthalein was 60 μl, containing 6 μmoles Tris–HCl buffer (pH 8 at 37°) 0.5 mg bovine serum albumin, 0.7 nmole $MgCl_2$, 18 nmoles phenolphthalein, 16 nmoles methanol and 170 nmoles uridine-5′-diphosphoglucuronic acid (UDP-GlcUA). The volume of the incubation mixture for *p*-nitrophenol was 45 μl, containing 3 μmoles Tris–HCl buffer (pH 8 at 37°) 0.19 mg bovine serum albumin, 0.19 nmoles $MgCl_2$, 30 nmoles *p*-nitrophenol, 3 nmoles methanol and 170 nmoles UDP-GlcUA.

All the reaction mixtures were incubated for 20 min at 37° and the reactions were stopped with 20–25 μl of a 10% solution of trichloroacetic acid. The reaction mixtures were then incubated for 2 min in boiling water, and afterwards 1 ml of glycine buffer, 1.6 M (pH 10.7), was added to each tube to develop the color. The tubes were spun for 5 min, and the supernatant fraction was read at 555 nm for phenolphthalein and 400 nm for *p*-nitrophenol. The activity is reflected as loss of color due to disappearance of substrates. The enzyme activity is expressed either as nmoles phenolphthalein-glucuronide formed per min per mg of microsomal protein or as nmoles *p*-nitrophenol-glucuronide formed per min per mg of microsomal protein. The rates of glucuronidation varied as a function of the methanol concentration in the incubation mixture (data not shown), but at the concentration used in this study methanol did not affect the UDP-GT activities.

Chemical assays

Cytochrome P-450 was measured in microsomal suspensions according to the method described by McLean and Day [49] and Schoene *et al.* [50]. Absorbance was measured on a DW-2 u.v./vis spectrophotometer (American Instrument Co.). Values are given as nmoles per mg microsomal protein ± the standard error.

UDP-GlcUA was determined according to the method of Singh *et al.* [51] with slight modifications and expressed as nmoles/mg protein ± the standard error. The cells were scraped in cold sucrose buffer (0°) containing 5 mM EDTA and then centrifuged at 1000 *g* for 3 min. The pellet was quickly homogenized in a cold solution of 5 mM EDTA (pH 7.3) and then cooked for 3 min to denature proteins. After cooling on ice, it was centrifuged at 3000 *g*. The supernatant fraction was stored in liquid nitrogen until assay. Liver caudal lobe was washed in cold sucrose buffer containing 5 mM EDTA, then homogenized in a cold solution of 5 mM EDTA and treated as described above for the cells.

Protein was determined by the method of Lowry *et al.* [52]. DNA was determined according to the methods of Burton [53] as modified by Richards [54]. The 9000 *g* pellet was cleaned with cold 10% TCA and with 95% ethanol according to Schneider [55], before the DNA assay.

RESULTS

Cytochrome P-450 content and microsomal protein. Cytochrome P-450 content was measured in male and female liver just before perfusion and in hepatocytes after 24 hr in culture. Slight differences were found between the levels of cytochrome P-450 in male and female livers, and high variability was displayed after culture of female hepatocytes for 24 hr. Male liver contained a 15% higher concentration of P-450 (average 0.786 nmole/mg protein ± 0.033 from three rats) than female (average 0.667 nmole/mg protein ± 0.019 from three rats). After 24 hr in culture, approximately a 50% loss occurred in P-450 content in both male (average 0.410 nmole/mg protein ± 0.012 from three rats) and female (average 0.377 nmole/mg protein ± 0.030 from three rats) hepatocytes.

The amount of microsomal protein normalized for the amount of DNA averaged 12.6 mg protein/mg DNA in male hepatocytes and 11.3 mg protein/mg DNA in female hepatocytes. The protein content of cells was diminished markedly during cell isolation to 3.8 mg protein/mg DNA in hepatocytes from males and 2.9 in hepatocytes from females. Subsequently, during attachment and culture, the levels remained constant.

Mixed function oxidase activities

AHH and BZD were measured in hepatocyte primary cultures to assess phase I oxidative enzyme activities. The basal liver activities of both enzymes

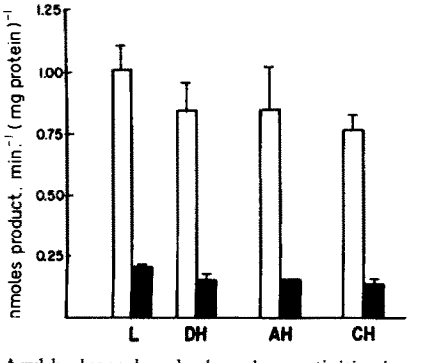

Fig. 1. Aryl hydrocarbon hydroxylase activities in rat liver just before perfusion (L), in dissociated hepatocytes (DH), in attached hepatocytes after 2 hr in culture (AH). and in cultured hepatocytes at 24 hr in culture (CH). AHH activity is expressed as nmoles 3(OH)-benzo[*a*]pyrene formed per min per mg of microsomal protein. Each bar represents the mean ± S.E. of three independent experiments. Where no standard error is indicated, the values represent two independent experiments. Key: (□) male, and (■) female.

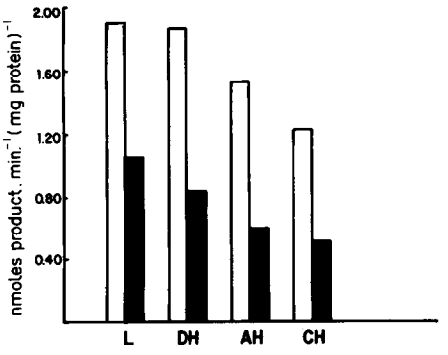

Fig. 2. Benzphetamine demethylase activities in rat liver just before perfusion (L), in dissociated hepatocytes (DH), in attached hepatocytes after 2 hr in culture (AH), and in cultured hepatocytes at 24 hr in culture (CH). BZD activity is expressed as nmoles CH_2O formed per min per mg of microsomal protein. Each bar represents the mean of two independent experiments which did not differ by more than 15%.

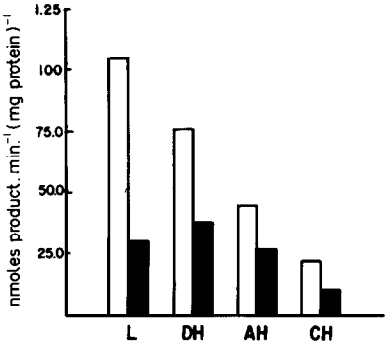

Fig. 4. Glutathione transferase activities toward 1,2-dichloro-4-nitrobenzene in rat liver just before perfusion (L), in dissociated hepatocytes (DH), in attached hepatocytes after 2 hr in culture (AH), and in cultured hepatocytes at 24 hr in culture (CH). GT activity is expressed as nmoles 1,2-dichloro-4-nitrobenzene conjugate formed per min per mg of supernatant protein. Each bar represents the mean of two independent experiments which did not differ by more than 15%.

were different in males and females, and these differences were mainly preserved during isolation, attachment and culture; both activities were greater in male than in female liver, 5-fold for AHH activity (Fig. 1) and 2-fold for BZD activity (Fig. 2). AHH activity was stable during isolation and attachment, and displayed substantial preservation in culture, 75% in male and 70% female hepatocytes (Fig. 1). BZD activities declined slightly more than AHH activities; in culture, the degree of maintenance was 65% in male and 50% in female hepatocytes (Fig. 2).

Glutathione conjugation

As an index of phase II conjugation reactions, GT activities toward two substrates were measured. 1-Chloro-2,4-dinitrobenzene is a substrate for GTs A, AA, B (ligandin) and C, while 1,2-dichloro-4-nitrobenzene is a substrate for GTs A and C as shown by Habig and Jakoby [46]. The conjugation activity tested toward 1-chloro-2,4-dinitrobenzene was the same in male and female liver (Fig. 3). No significant loss of activity occurred during isolation and culture of hepatocytes from either male or female livers.

Using 1,2-dichloro-4-nitrobenzene as the substrate for GT, a markedly greater activity was present in male liver compared to female (Fig. 4). Hepatocytes from male rats displayed a progressive decline in activity during isolation, attachment and culture. Overall, 79% of the initial liver enzymatic activity was lost. Hepatocytes from female rats maintained their activity during isolation and attachment, but during the 24-hr culture period a 66% decline in activity occurred.

Glucuronidation

Another index of conjugation, UDP-GT activity, was measured toward two substrates and the UDP-GlcUA levels were determined. Glucuronidation

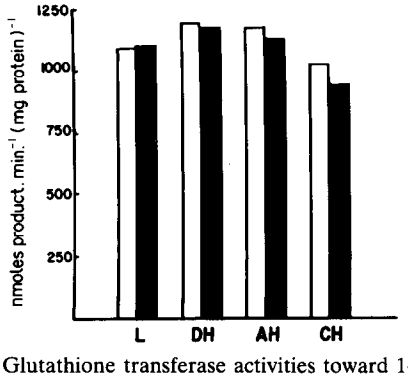

Fig. 3. Glutathione transferase activities toward 1-chloro-2,4-dinitrobenzene in rat liver just before perfusion (L), in dissociated hepatocytes (DH), in attached hepatocytes after 2 hr in culture (AH), and in cultured hepatocytes at 24 hr in culture (CH). GT activity is expressed as nmoles 1-chloro-2,4-dinitrobenzene conjugate formed per min per mg of supernatant protein. Each bar represents the mean of two independent experiments which did not differ by more than 15%.

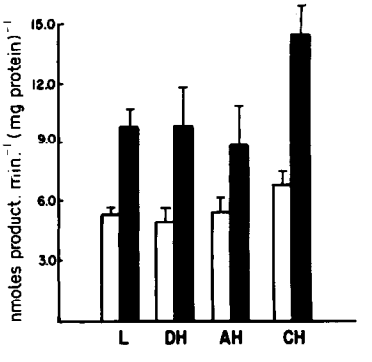

Fig. 5. UDP glucuronyltransferase activities toward phenolphthalein in rat liver just before perfusion (L), in dissociated hepatocytes (DH), in attached hepatocytes after 2 hr in culture (AH), and in cultured hepatocytes at 24 hr in culture (CH). UDP-GT activity is expressed as nmoles phenolphthalein-glucuronide formed per min per mg of microsomal protein. Each bar represents the mean ± S.E. of three independent experiments.

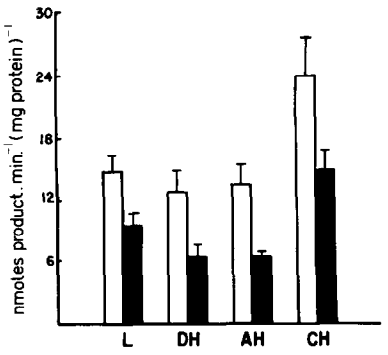

Fig. 6. UDP glucuronosyltransferase activities toward *p*-nitrophenol in rat liver just before perfusion (L), in dissociated hepatocytes (DH), in attached hepatocytes after 2 hr in culture (AH), and in cultured hepatocytes at 24 hr in culture (CH). UDP-GT activity is expressed as nmoles *p*-nitrophenol-glucuronide formed per min per mg of microsomal protein. Each bar represents the mean ± S.E. of four independent experiments.

tested toward phenolphthalein was greater in female hepatocytes than male (Fig. 5), whereas with *p*-nitrophenol UDP-GT was greater in male hepatocytes (Fig. 6).

Phenolphthalein conjugation activity (Fig. 5) was stable during isolation and attachment and, at 24 hr, was even slightly increased in both male and female hepatocytes, 24 and 32%, respectively, of the initial liver values. *p*-Nitrophenol conjugation activity (Fig. 6) likewise did not change much after isolation or attachment, and then increased at 24 hr of culture in both male and female hepatocytes, 40 and 35%, respectively, of the initial liver values.

Phenol-red is commonly used as a pH indicator in commercial culture media and is a component in WME. It is known to be conjugated by UDP-GT [37]. In two experiments, medium without phenol-red was used, but no differences were found in the activities of UDP-GTase assayed in female and male hepatocytes during 24 hr of culture (data not shown).

UDP-GlcUA levels were assayed only in male hepatocytes. The initial level in liver averaged 2.54 ± 0.08 nmoles/mg protein in three rat livers. This was reduced dramatically by isolation to 0.14 ± 0.005 nmole/mg protein (average in three hepatocyte preparations) and then increased during culture to a level approximately 60% of normal, 1.48 nmoles/mg protein ± 0.12 (average from three rats).

DISCUSSION

This study characterized several aspects of the xenobiotic biotransformation capability of cultured hepatocytes isolated from adult male and female rat liver. A significant finding in this study was that six enzyme activities were almost the same in isolated cells as in intact liver. Superficially, this might appear appropriate, but, in reality, the two populations are quite different; isolates prepared by the techniques used here are at least 98% hepatocytes, whereas liver is only 60% hepatocytes, although in volume hepatocytes constitute more than 90% of the liver. The reason for this correspondence is obscure, but it can be concluded that isolated hepatocytes represent well hepatic xenobiotic biotransformation systems.

In previous studies on cultured hepatocytes, many investigators have observed a severe loss in cytochrome P-450 and the related enzyme activities from male rat hepatocytes [10, 20–22, 30]. To compensate for this, attempts have been made to maintain P-450 and MFO activities in rat hepatocytes [23–31]. In the present study, the decline of MFO activities was less than that observed under other conditions, although no supplementation of the medium was employed except for calf serum during the period of attachment. A combination of improvements in the perfusion techniques [21, 41], the use of high quality collagenase, and culture in an enriched medium probably contributed to these good results.

An interesting finding was that although hepatocytes lost 50% of their cytochrome P-450 during 24 hr in culture, they maintained relatively high MFO activities; 75% of AHH and 65% of BZD activities were preserved. This correlates with our earlier observation that in hepatocyte cultures the generation of benzo[*a*]pyrene metabolites is linear or even increased over 24 hr in culture [14]. Current studies show the same for *N*-2-fluorenylacetamide metabolism (C. A. McQueen and G. M. Williams, manuscript in preparation).

Both AHH and BZD are sex-dependent in the rat, with males having higher activities than females [56]. During 24 hr in culture, hepatocytes maintained the sex differences in activities of both enzymes. To some extent, the persistence of this sex difference in culture in the absence of sex-dependent hormones may be due to neonatal hormonal imprinting [57, 58]. In support of this interpretation, it has been reported that castration performed in adult rats does not reduce the male MFO activities to the same levels observed in females [33, 59].

Regarding phase II pathways, GT activity assayed using 1,2-dichloro-4-nitrobenzene as substrate was diminished from the initial activity in liver by 80% in male or 70% in female hepatocytes. In contrast, the GT activity using 1-chloro-2,4-dinitrobenzene as substrate was stable. These activities probably represent different forms of GT, which have been purified and characterized [38, 46, 60, 61]. 1,2-Dichloro-4-nitrobenzene is a specific substrate for GT forms A and C, while 1-chloro-2,4-dinitrobenzene is also a substrate for these forms and, in addition, AA and B (ligandin) [61, 62]. The B form is the major form of GT present in rat liver with a level at least three times higher than all other forms of GT combined [61]. The differences in activities in cultured hepatocytes could be accounted for if GT forms A and C were reduced in culture while forms B and AA activities were increased, as the sum of A, AA, B, and C activities remained constant as shown by 1-chloro-2,4-dinitrobenzene conjugation.

Another indicator of phase II reactions, UDP-GT activity, was assayed with *p*-nitrophenol and phenolphthalein as specific substrates for two forms of the enzyme. *p*-Nitrophenol is considered to be more specific for the late fetal form and phenolphthalein for the neonatal form of UDP-GT [63, 64]. UDP-GT activities were slightly increased in hepa-

tocytes between attachment and 24 hr in culture to levels higher than in liver before perfusion. The increase was greater for glucuronidation of *p*-nitrophenol than for phenolphthalein. Thus, the conditions used in the present studies better preserve this activity than those used by Schwarz *et al.* [39] that led to a marked decrease of the UDP-GT tested toward morphine and naphthol during 10 hr of culture of hepatocytes.

The level of the endogenous substrate for UDP-GT, UDP-GlcUA, dropped dramatically during cell isolation and then increased in culture to values close to those of intact liver. The initial loss of UDP-GlcUA may be caused by pentobarbital anesthesia; recently Watkins and Klaassen [65] have reported that hepatic UDP-GlcUA content was diminished after treatment of rats with low doses of sodium pentobarbital. Alternatively, the UDP-GlcUA may have been leached out of the cells during perfusion. Obviously, the hepatocytes recovered from the initial perturbation of dissociation and restored their UDP-GlcUA. The fact that the UDP-GT activities were only slightly affected in spite of these fluctuations is consistent with other evidence that UDP-GlcUA does not regulate the synthesis of the enzyme [66, 67].

In summary, during culture of hepatocytes, significant changes in the activities of some xenobiotic biotransformation enzymes occurred. Generally, the changes were similar for male and female hepatocytes, but they were not uniform for different enzymes. Indeed, some activities increased while others decreased. The overall impact of these changes on the disposition of substrates cannot be projected but, based on the fact that MFO activities diminished while several conjugative activities were stable or increased, a shift to more effective detoxification might occur. Clearly, therefore, the changes documented here need to be considered in studies of the metabolism by cultured hepatocytes of both endogenous and exogenous substrates.

REFERENCES

1. C. O. Abernathy, L. Lukacs and H. J. Zimmerman, *Biochem. Pharmac.* **24**, 347 (1975).
2. G. M. Williams, *Cancer Lett.* **1**, 231 (1976).
3. J. R. Fry and J. W. Bridges, *Biochem. Pharmac.* **26**, 969 (1977).
4. D. C. Anuforo, D. Acosta and R. V. Smith, *In Vitro* **14**, 981 (1978).
5. J. D. Yager and J. A. Miller, *Cancer Res.* **38**, 4385 (1978).
6. G. M. Williams, in *Organ Directed Toxicity, and Chemical Indices and Mechanisms IUPAC* (Eds. S. S. Brown, D. S. Davies), p. 131. Pergamon Press, New York (1981).
7. J. Inman, A. Stead, M. D. Waters and J. Lewtas, *In Vitro* **71**, 1004 (1981).
8. C. A. McQueen and G. M. Williams, *Fund. appl. Toxic.* **2**, 139 (1982).
9. J. R. Fry and J. W. Bridges, in *Progress in Drug Metabolism* (Eds. J. W. Bridges and L. F. Chasseand), Vol. 2, p. 71. John Wiley, Chichester (1977).
10. P. S. Guzelian, D. M. Bissel and U. A. Meyer, *Gastroenterology* **72**, 1232 (1977).
11. A. F. Sirica and H. C. Pitot, *Pharmac. Rev.* **31**, 205 (1980).
12. M. Koike, K. Sugeno and M. Hirata, *J. pharmac. Sci.* **70**, 308 (1981).
13. D. P. Jones, H. Thor, B. Andersson and S. Orrenius, *J. biol. Chem.* **253**, 6031 (1978).
14. I. Schmeltz, J. Tosk and G. M. Williams, *Cancer Lett.* **5**, 81 (1978).
15. R. E. Billings, R. E. McMahon, J. Ashmore and S. R. Wagle, *Drug Metab. Dispos.* **5**, 518 (1977).
16. J. W. Bridges, P. Wiebkin and J. R. Fry, in *Microsomes, Drug Oxidations and Chemical Carcinogenesis* (Eds. M. J. Coon, A. H. Conney, R. W. Estabrook, H. J. Gelbein, J. R. Gillette and P. J. O'Brien), Vol. 2, p. 619. Academic Press, New York (1980).
17. T. Omura, R. Sato, D. Y. Cooper, O. Rosenthal and R. W. Estabrook, *Fedn Proc.* **24**, 1181 (1965).
18. S. J. Orrenius, *Cell Biol.* **26**, 713 (1965).
19. R. Kato, *J. Biochem., Tokyo* **59**, 574 (1966).
20. A. J. Paine and R. F. Legg, *Biochem. biophys. Res. Commun.* **81**, 672 (1978).
21. N. Kaplowitz, *Am. J. Physiol.* **239**, 439 (1980).
22. C. J. Maslansky and G. M. Williams, *In Vitro* **18**, 683 (1982).
23. G. Michalopoulos, G. Sattler, C. Sattler and H. C. Pitot, *Am. J. Path.* **85**, 755 (1976).
24. M. Dickins and R. E. Peterson, *Biochem. Pharmac.* **29**, 1231 (1980).
25. D. M. Bissel and P. S. Guzelian, *Archs Biochem. Biophys.* **192**, 569 (1979).
26. S. Newman and P. S. Guzelian, *Proc. natn. Acad. Sci. U.S.A.* **79**, 2922 (1982).
27. A. J. Paine, L. J. Hockin and R. F. Legg, *Biochem. J.* **184**, 461 (1979).
28. K. F. Nelson and D. Acosta, *Biochem. Pharmac.* **31**, 2111 (1982).
29. B. G. Lake and A. J. Paine, *Biochem. Pharmac.* **31**, 2141 (1982).
30. J. A. Holme, E. Soderlund and E. Dybing, *Acta pharmac. toxic.* **52**, 348 (1983).
31. R. P. Evarts, E. Marsden and S. S. Thorgiersson, *Biochem. Pharmac.* **33**, 565 (1984).
32. R. Kato, K. Onoda and Takayanagi, *Jap. J. Pharmac.* **20**, 157 (1970).
33. R. Kato and R. J. Gillette, *J. Pharmac. exp. Ther.* **150**, 279 (1965).
34. R. Kato and T. Kamataki, *Xenobiotica* **12**, 787 (1982).
35. R. G. Thurman and F. C. Kauffman, *Pharmac. Rev.* **31**, 229 (1980).
36. B. Andersson, M. Berggren and P. Moldeus, *Drug. Metab. Dispos.* **6**, 611 (1978).
37. J. L. Driscoll, N. T. Hayner, R. W. Holland, G. S. Karotkin, P. M. Galletti and H. O. Jaureagui, *In Vitro* **18**, 835 (1982).
38. M. J. Pabst, W. H. Habig and W. B. Jakoby, *J. biol. Chem.* **249**, 7140 (1974).
39. L. R. Schwarz, R. Götz, T. Wolff and F. J. Weibel, *Fedn Eur. Biochem. Soc. Lett.* **98**, 203 (1979).
40. P. O. Seglen, *Meth. Cell Biol.* **13**, 29 (1976).
41. G. M. Williams, E. Bermudez and D. Scaramuzzino, *In Vitro* **13**, 809 (1977).
42. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **243**, 6242 (1968).
43. C. S. Yang, F. S. Strickhart and L. P. Kicha, *Biochem. Pharmac.* **27**, 2321 (1978).
44. H. R. A. Prough and D. M. Ziegler, *Archs Biochem. Biophys.* **180**, 363 (1977).
45. T. Nash, *Biochem. J.* **55**, 416 (1953).
46. W. H. Habig and W. B. Jakoby, *Meth. Enzym.* **77**, 398 (1981).
47. P. Duvaldestin, J. L. Mahu and P. Berthelot, *Biochim. biophys. Acta* **384**, 81 (1975).
48. E. J. Halac and A. Reff, *Biochim. biophys. Acta* **139**, 328 (1967).

49. A. E. M. McLean and P. A. Day, *Biochem. Pharmac.* **23**, 1173 (1974).
50. B. Shoene, R. A. Fleishchmann, H. V. Remmer and H. F. Oldershausen, *Eur. J. clin. Pharmac.* **4**, 65 (1972).
51. J. Singh, L. R. Schwarz and F. J. Wiebel, *Biochem. J.* **189**, 369 (1980).
52. O. H. Lowry, N. J. Rosebrough, A. L. Farr and J. R. Randall, *J. biol. Chem.* **193**, 265 (1951).
53. K. Burton, *Biochem. J.* **62**, 315 (1956).
54. G. M. Richards, *Analyt. Biochem.* **57**, 369 (1974).
55. W. C. Schneider, *J. biol. Chem.* **161**, 293 (1945).
56. D. E. Ryan, P. E. Thomas, L. M. Reik and W. Lerin, *Xenobiotica* **12**, 727 (1982).
57. J. A. Gustavsson, A. Mode, G. Norstedt, T. Hokfelt, C. Sonnenschein, P. Eneroth and P. Skett, in *Biochemical Actions of Hormones* (Ed. G. Litwack), Vol. 7, p. 47. Academic Press, New York (1980).
58. G. W. Lucier, in *Developmental Toxicology* (Eds. C. A. Kimmel and J. Buelki-Sam), p. 101. Raven Press, New York (1981).
59. S. Katayama, T. Ohmori, Y. Maeura, T. Croci and G. M. Williams, *J. natn. Cancer Inst.* **73**, 141 (1984).
60. A. M. Sarrif, P. V. Danenberg, C. Heidelberger and B. Ketterer, *Biochem. biophys. Res. Commun.* **70**, 869 (1976).
61. W. Habig, M. Pabst, G. Fleischner, Z. Gatmaitan, I. Arias and W. B. Jakoby, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3879 (1974).
62. G. J. Smith, V. S. Ohl and G. Litwack, *Cancer Res.* **37**, 8 (1977).
63. Z. Yin, K. Sato, H. Tsuda and N. Ito, *Gann* **73**, 239 (1982).
64. G. J. Wishart, *Biochem. J.* **174**, 671 (1978).
65. J. B. Watkins and C. D. Klaassen, *Drug Metab. Dispos.* **11**, 37 (1983).
66. B. Haeger, R. DeBrito and T. Hallinan, *Biochem. J.* **192**, 971 (1980).
67. D. Zakim and D. A. Vessey, in *Enzymes of Biological Membranes* (Ed. A. I. Martonosi), Vol. 25, p. 443. Plenum Press, New York (1980).