Human fetal liver (HFL) cell culture was initiated from a pool of six normal human liver tissues. The proliferation and viability of HFL cells were evaluated using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay, and the cells increased by more than 100-fold by culture for 15 d. The levels of expression of albumin (ALB), hepatocyte nuclear factor 4α, hepatocyte growth factor, CYP3A4, CYP3A5, and CYP3A7 mRNA in HFL cells increased with culture period, while that of α-fetoprotein (AFP) mRNA decreased gradually. In HepG2 cells, however, the expression levels of ALB and AFP mRNAs were not changed, and the levels of expression of CYP3A4, CYP3A5, and CYP3A7 mRNAs decreased gradually. The mRNA expression of major CYP isoforms including CYP3A4, CYP3A6, CYP2B6, CYP2C (2C9 and 2C19), CYP2D6, and CYP2E1, could be detected in HepG2 cells. With the exception of CYP1A2, all of the CYP mRNAs expressed in HepG2 cells were detected in HFL cells. In HFL cells, CYP3A4 and CYP3A7 mRNA expression levels were markedly up-regulated by dexamethasone (DEX), but not by rifampicin (RIF). CYP3A5 mRNA expression was increased to a level 3-fold greater than control by DEX. On the other hand, CYP3A4, CYP3A5, and CYP3A7 mRNA expression levels in HepG2 cells were increased from 2- to 3-fold by treatment with DEX and RIF. Pregnane X receptor mRNA was expressed in HepG2 cells, but not HFL cells. These results indicate that the character of HFL cells with regard to CYP expression was different from that of HepG2 cells.

Key words human fetal liver cell; HepG2 cell; CYP3A4; CYP3A7; induction; glucocorticoid receptor
the culture reached 90–100% confluence, and suspended in Cell Banker after proliferation. Aliquots of the suspensions were cryopreserved at −150 °C until use. HepG2 cells were obtained from Cell Resource Center for Biomedical Research, Tohoku University, Japan.

**Evaluation of Cell Proliferation by MTT Assay** The HFL cells at passage numbers 2, 6, and 10 cryopreserved at −150 °C were used for evaluation of cell proliferation. Proliferation of the cells was evaluated using the MTT assay as described previously. The MTT-derived blue formazan product formed in viable cells was dissolved with DMSO and quantified by determining its absorbance at 570 nm.

**RESULTS**

**Influence of Passage on Cell Proliferation** First, the influence of passage on cell proliferation was examined in HFL cells. Proliferation and viability of the cells were evaluated using MTT assay as described in Materials and Methods. Cells at passage numbers 2, 6, and 10 were seeded onto collagen-coated culture dishes. The MTT-derived blue formazan product formed in viable cells increased by more than 100-fold for the first 15 d in culture, and then reached a plateau (Fig. 1). There was no marked difference in the rate of proliferation among the cells, although cells at passage number 6 showed somewhat slower proliferation than those at passage 2 and 10.

**Morphology of HepG2 and HFL Cells** To highlight the morphological differences between HepG2 and HFL cells, micrographs of both cell lines are shown in Fig. 2. At low plating density, many of the individual HFL and HepG2 cells exhibited epithelial-like morphological features (Fig. 2). HFL cells uniformly proliferated on plates until reaching 100% confluence and formed a flattened monolayer. In early culture of HFL cells, the cells were predominantly small hepatocytic cells with a granular cytoplasm and a centrally placed nucleus. The main morphological features of these cells were homogeneous, and the cells were closely attached to each other forming a continuous monolayer. On the other hand, HepG2 cells overlapped each even at low cell density and formed colonies, because the cells did not exhibit uniform proliferation. Many of the HepG2 cells maintained a polygonal shape culture conditions even after overlapping with each other in the colonies. HFL cells did not exhibit contact inhibition of growth despite being normal cells (Fig. 2). HFL cells achieved a higher saturation density at confluent cell proliferation, and displayed reduced adhesion and enhanced emigra-

### Table 1. PCR Primers and Conditions

<table>
<thead>
<tr>
<th>Primer</th>
<th>Length/annealing temperature</th>
<th>Sense primer 5′→3′</th>
<th>Antisense primer 5′→3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALB</td>
<td>396 bp/57 °C</td>
<td>TCTATCCGTGGTCTGAAACC</td>
<td>CTTAAGGCAAGCTGTACCTG</td>
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<tr>
<td>AEP</td>
<td>320 bp/60 °C</td>
<td>TAACACCCCTGTTGAGCAAG</td>
<td>ATTAAACCTCCAAAAGACGAC</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>415 bp/58 °C</td>
<td>AGTGGCTACTGCTGAGTA</td>
<td>TCTGGATGAGTGTGCTTC</td>
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<tr>
<td>CYP2A6</td>
<td>708 bp/58 °C</td>
<td>GAACACAGACAGATGTTCA</td>
<td>TCCGTTGTTGGGTTCTC</td>
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<td>CYP2B6</td>
<td>753 bp/58 °C</td>
<td>ATGACATTACCGGCAACATC</td>
<td>TCTTTTTCAGTTCCCAATTG</td>
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<tr>
<td>CYP2C20</td>
<td>540 bp/60 °C</td>
<td>ACACAAAGATCAATGGGACA</td>
<td>GCTGAGAAAGGCTAGAAGTA</td>
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<td>CYP2D6</td>
<td>299 bp/56 °C</td>
<td>GTGCGTACCTGTTCTC</td>
<td>TTTGGTATGATGTTGCTC</td>
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<tr>
<td>CYP2E1</td>
<td>353 bp/60 °C</td>
<td>AACTTGGGATGTTGGAACAG</td>
<td>CTCTTTCACCTTTGACAGA</td>
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<td>CYP3A4</td>
<td>626 bp/60 °C</td>
<td>CTTGTGTTCCTCTTGGAAAGTACCT</td>
<td>ACCTATGCAATCGATGCCTT</td>
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<tr>
<td>CYP3A5</td>
<td>230 bp/62 °C</td>
<td>TGACCCAAAGTGACCGACAG</td>
<td>TGAAGAAATCCTTGCGGTTC</td>
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<tr>
<td>CYP3A7</td>
<td>475 bp/54 °C</td>
<td>CTATGTATCTGTCAGTA</td>
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<td>GAPDH</td>
<td>307 bp/54 °C</td>
<td>CATCACCACCTTTAGGAGG</td>
<td>CATGAGAAAGGCTAGAAGTA</td>
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<tr>
<td>GRα</td>
<td>557 bp/54 °C</td>
<td>ACACAGGCTTCAGGTATCTT</td>
<td>ACCTGTTCGTTGCACAG</td>
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<td>HGF</td>
<td>303 bp/62 °C</td>
<td>TCCAGGACATGACATGCTCC</td>
<td>AGCTTACCTTGCAGGTTTCC</td>
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<td>HNF4α</td>
<td>275 bp/60 °C</td>
<td>GCCATCTTCAAGGCATCAT</td>
<td>GACCCTTCAACGACATCTC</td>
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<tr>
<td>PXR</td>
<td>442 bp/68 °C</td>
<td>CAAACGCGGAGAAAGTGAAGCAG</td>
<td>CTGGTCCTGATGAGGCAAGT</td>
</tr>
</tbody>
</table>

a) CYP2C9 and CYP2C19.
tion from confluent cell layers. Finally, not only HepG2 cells but also HFL cells overlapped each other and formed clusters on prolonged culture.

DISCUSSION

It has been difficult to estimate alterations in drug metabo-
lism induced by candidate drugs and chemicals in humans due to species-related differences, although this has been researched extensively in experimental animals. Primary cultures of human hepatocytes have been used extensively for evaluation of induction of drug-metabolizing enzymes in humans. Thus, primary cultures of hepatocytes are among the most useful cell lines as human liver models. Our previous study, however, suggested that the induction mechanisms of CYP3As in HFL may be different from those in the adult liver. Therefore, experiments using HFL cells need to estimate the effects of drugs on human fetal drug-metabolizing enzymes, as research in pregnant human subjects should be restricted due to ethical considerations. On the other hand, HepG2 cells that express fetal-specific proteins, such as CYP3A7 and AFP, have been used in a number of studies of drug metabolism. However, there have been no comparative studies of gene expression between HepG2 cells and cultured HFL cells, and only limited data are available regarding the comparative cellular responses of these two systems to inducers. The present study was performed to further characterize the utility of HFL cells as an in vitro system in which to assess the effects of drugs and chemicals on CYP expression in the fetal liver.

In general, the maintenance of highly differentiated primary hepatocyte cultures, such as adult liver cells, has many limitations and difficulties. Typically, these cells rapidly lose differentiated phenotypic traits, and have a low replication capacity and features that prevent long-term maintenance and subculture. It was demonstrated that HFL cells have extremely high proliferative ability (Fig. 1). Recently, Lazaro et al. reported that HFL cells can be kept in primary culture for 2 to 4 months without apparent loss of hepatocytic traits in serum-free medium in the presence of epidermal growth factor and can be subcultured at least twice. We found that HFL cells can be subcultured at least 10 times without apparent loss of proliferative activity despite cryopreservation once at −150 °C. The plating efficiency was high through experiments when HFL cells were cryopreserved as suspen-
sions in Cell Banker. These results suggested that the HFL cells could be serially passaged and stored at −150 °C until use.

The morphological characteristics and epithelial cell shape of HepG2 cells are compatible with those of liver parenchymal cells. However, there were significant morphological differences between HepG2 and HFL cells. HepG2 cells formed clusters before reaching 100% confluence, while HFL cells proliferated uniformly on the plates and formed flattened monolayers.

Leeder et al. reported that CYP3A7 is the most abundant CYP3A mRNA species present in 54 liver samples 76 d to 32 weeks estimated gestational age, and that the expression levels of CYP3A5 and CYP3A4 mRNAs are 100- and 1000-fold lower than CYP3A7 expression, respectively. We found that HFL cells constitutively express not only fetal CYP3A isoforms, CYP3A7 and CYP3A5, but also the adult isoform, CYP3A4. In general, the function of hepatocytes in primary culture decreased in a manner dependent on number of days in culture. Especially, CYP3A4 level in primary hepatocytes isolated from adult humans was decreased markedly by incubation for several days. In the present study, the expression levels of CYP3As in HFL cells increased gradually along with ALB by cultivation for 20 d, although CYP3A7 mRNA expression showed an initial increase, followed by a decrease, and a later increase (Fig. 3). On the other hand, the expression level of AFP decreased

Fig. 4. Basal Expression and Induction by DEX of CYPs in HepG2 and HFL Cells

HepG2 and HFL cells were seeded 1×10⁴ cells/well onto 3.5 cm culture plate coated with type I collagen. HepG2 and HFL cells were treated with vehicle (DMSO) or 100 μM DEX for 72 h. Total RNA was extracted from HepG2 and HFL cells cultured for 20 d. Expression of mRNA was analyzed by semi-quantitative RT-PCR as described in Materials and Methods. An image of ethidium bromide-staining agarose gel is shown.

Fig. 5. Effects of Inducers on CYP3As mRNA Expression in HepG2 and HFL Cells

HepG2 and HFL cells were treated with vehicle (DMSO), 100 μM DEX, or 40 μM RIF for 72 h. Total RNA was extracted from HepG2 and HFL cells cultured for 10 d. After treatment, mRNA was analyzed by semi-quantitative RT-PCR as described in Materials and Methods. Left panel: An image of ethidium bromide-staining agarose gel is shown. Right panel: Data presented are the ratio of CYP3A4, CYP3A5, or CYP3A7 to GAPDH and normalized at 1.0 for DMSO treatment alone. Values are expressed as the mean ± standard deviation of three experiments. Significantly different from DMSO group (*p<0.05; **p<0.01).

Fig. 6. Expression of PXR and GRα in HepG2 and HFL Cells

HepG2 and HFL cells were seeded 1×10⁴ cells/well onto 6 well-plate coated with type I collagen. Total RNA was extracted from the cells cultured for 20 d. Expression of mRNA was analyzed by semi-quantitative RT-PCR as described in Materials and Methods. An image of ethidium bromide-staining agarose gel is shown.
gradually during culture. AFP and ALB have frequently served as model genes for developmentally regulated gene expression of the hepatic cell lineage.\textsuperscript{30,31} Among the secretory liver proteins, AFP is expressed already in early hepatoblasts and its expression levels increase throughout fetal development. After birth, the AFP gene is selectively silenced.\textsuperscript{19,20} In contrast, expression of ALB, the most abundant serum protein synthesized by hepatocytes, begins in early fetal hepatocytes and reaches the maximal level in adult hepatocytes.\textsuperscript{21} These results suggest that HFL cells containing immature hepatocytes differentiate into more mature cells during culture. On the other hand, the expression of these mRNAs in HepG2 cells did not change appreciably during culture. These results indicate that the character of HepG2 cells is different from that of HFL cells.

HepG2 cells are deficient in the expression of many CYPs\textsuperscript{32} that would provide a basis for differential responses to chemical exposure. Schuetz et al.\textsuperscript{17} reported that HepG2 cells contained only CYP3A7 protein even after treatment with inducers, such as DEX and RIF. Ogino et al.\textsuperscript{33} also reported that CYP3A4 is scarcely expressed and not enhanced by treatment with RIF despite the presence of PXR in this cell line. However, CYP1A2, CYP2A6, CYP2B6, CYP2C (2C9 and 2C19), CYP2D6, CYP2E1, CYP3A4, CYP3A5, and CYP3A7 mRNAs were detected in HepG2 cells by semi-quantitative RT-PCR (Fig. 4). Furthermore, we found that CYP3A mRNA expression was also enhanced by RIF together with DEX (Fig. 5). These results were consistent with those reported previously by Sumida et al.\textsuperscript{25} and Usui et al.\textsuperscript{34} Hewitt and Hewitt\textsuperscript{6} reported that HepG2 cells have phase I enzymes, i.e., CYP1A, CYP2C9, CYP2D6, CYP2B6, CYP2E1, and CYP3A, but their activities and levels are dependent on the source and culture conditions. This discrepancy may have been due to differences in source and/or culture conditions.

HNF4\(\alpha\) is an important transcription factor regulating basal and PXR-mediated CYP3A4 promoter activation.\textsuperscript{35} PXR mRNA was not detectable in HFL cells, although it was expressed in HepG2 cells. On the other hand, HNF4\(\alpha\) mRNA was detected in HepG2 and HFL cells. PXR has been identified as the major receptor responsible for transcriptional activation of CYP3A gene expression.\textsuperscript{36} Another cellular factor, GR\(\alpha\),\textsuperscript{37,38} is known to influence xenobiotic-mediated transcription induction, through cooperative activation of PXR expression. Expression level of GR\(\alpha\) mRNA was almost identical in HepG2 and HFL cells. One reason why CYP3A4 and CYP3A7 mRNA expression were not induced by RIF in HFL cells may be that PXR expression level is very low in these cells as compared with HepG2 cells. On the other hand, these CYP enzymes in HFL cells were induced significantly by DEX. These results suggest that PXR is not an essential factor for basal expression and induction of CYP3A enzymes by DEX in HFL cells.

There are some reports about the expression of CYP enzymes and activity of drug-metabolizing enzymes in HFL.\textsuperscript{27,40—42} However, little is known concerning the induction of drug-metabolizing enzymes in HFL, because research with pregnant or fetus should be never attempted due to ethical considerations. The induction of CYP in HepG2 cells has been studied as a model of human liver.\textsuperscript{15,25} In the present study, however, it was demonstrated that character of HepG2 cells with regard to CYP expression was different from that of HFL cells, although HepG2 cell lives has features of hepatoblast-like cells.\textsuperscript{6,15} The maintenance of HFL cells is easier than that of the adult liver cells as described above. These results indicated that HFL cells might be most suitable as an \textit{in vitro} system in which to assess the effects of drugs and chemicals on CYP expression in the fetal liver.

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