Yonago Acta medica 1999;42:103–112

Biochemical Functions and mRNA Expression of Rat Hepatocytes in a Bioartificial Liver Module

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Evaluation of hepatic functions of the bioartificial liver (BAL) module is essential for developing an optimal BAL system which can be applied to a patient with fulminant hepatic failure. Although hepatic function in BALs has been evaluated only in biochemical activities in previous reports, we assessed a newly designed BAL from a hollow fiber type not only in biochemical activities such as gluconeogenesis, ureogenesis and albumin synthesis but also in the expressions of albumin messenger RNA (mRNA) and cytochrome P450 2B1 mRNA by Northern blot analysis. One to 3×10^8 hepatocytes isolated from adult rats were suspended in hydrated collagen, loaded into a hollow fiber module and perfused with L-15 medium supplemented with various hormones for 2 to 13 days. Both production rates of glucose and urea nitrogen were highest on day 1 (610 μg glucose/10^7 cells/h and 76.1 μg urea nitrogen/10^7 cells/h, respectively). Ureogenesis was maintained for 8 days in BAL, but gluconeogenesis decreased after 7 days. Albumin production rate increased to day 3, reached its maximum (11.0 μg/10^7 cells/h) and then gradually decreased. The expression of albumin mRNA was highest on day 2 and detectable until day 11. Cytochrome P450 2B1 mRNA was detected only on day 2. Northern blot analysis showed that the maintenance period in each hepatic function was different. These results suggested that assessment of the functions at a molecular level was invaluable in the development of a potential BAL for clinical use. It might be necessary to reconstruct the lobular system as well as a 3-dimensional environment for hepatocytes in the BAL to maintain the ability of detoxification such as cytochrome P450s.

Key words: albumin mRNA; bioartificial liver; cytochrome P450 2B1 mRNA; hepatic function

Despite recent advances in intensive medical treatment, a patient with fulminant hepatic failure has a poor prognosis (Pappas, 1988). Liver transplantation is an effective therapy, but is not always available. As another strategy for the treatment of severe hepatic failure, several cases using a bioartificial liver (BAL) system mainly for bridging to a transplant liver have been reported (Chen et al., 1997). There are, however, only a few cases that have recovered using only a BAL (Matsumura et al., 1987; Sussman and Kelly, 1993; Chen et al., 1997). It is mainly due to the reason why the ability of BAL as a "biochemical factory" as the liver in vivo is not completely maintained for a prolonged period. Therefore, accurate and specific assessment of hepatic functions of bioartificial modules, such as gluconeogenesis, ureogenesis, albumin synthesis and also ability of detoxification, is essential for developing an optimal BAL system.

In previous reports, the hepatic functions of BALs were evaluated only in the biochemical activities of glucose, urea nitrogen or albumin production (Shatford et al., 1992; Takeshita et al., 1995; Naruse et al., 1996a, 1996b; Sielaff et

Abbreviations: BAL, bioartificial liver; CYP 2B1, cytochrome P450 2B1; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; hEGF, human epidermal growth factor; L-15, Leibovitz's L-15 medium; PBS, phosphate buffered saline; PVLA, poly-N-p-vinylbenzyl-D-lactonamide; WE, Williams' E medium

al., 1997) but not on the gene expression level, although hepatocytes of conventional cultures are investigated extensively using molecular methods (Kocarek et al., 1993; Kimball et al., 1995; Nagaki et al., 1995; Tamura et al., 1995). In this study, we designed a potential BAL of a hollow fiber type loading rat hepatocytes at a high density which maintained hepatic functions. The medium for perfusion and basement matrix of a BAL were selected based on results from hepatocytes cultured on dishes. Evaluation of maintenance of the liver functions of BALs over 13 days was performed not only by biochemical assays but also by Northern blot analysis of albumin messenger RNA (mRNA) and cytochrome P450 2B1 (CYP 2B1) mRNA.

Materials and Methods

Reagents

Leibovitz's L-15 medium (L-15), Williams' E medium (WE) and fetal calf serum (FCS) were obtained from Gibco BRL (Rockville, MD). Collagenase was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Prolin, insulin, dexamethasone and glucagon were from Sigma (St. Louis, MO). Human epidermal growth factor (hEGF) was from Wakunaga Pharmaceutical Co. (Hiroshima, Japan). [α-^{32}P]dCTP was from Amersham (Amersham, United Kingdom). Other reagents except described above were of analytical grade.

Isolation and dish culture of hepatocytes

Hepatocytes were isolated from adult male Wistar rats (140–170 g) by in situ collagenase perfusion method modified in our laboratory as described previously (Kohno et al., 1991). In preliminary experiments, to assess maintenance of the liver-specific functions in a long-term culture, 2 different types of media and 3 types of extracellular matrices were examined with hepatocytes cultured on dishes. The media were based on WE or L-15 supplemented with 5% FCS, 5000 KIU/L aprotinin, 10^{-7} mol/L insulin, 10^{-7} mol/L dexamethasone, 10^{-8} mol/L glucagon and 10 ng/mL hEGF. Proline (30 mg/L) was added only to L-15. Three types of matrices tested were non-coated plastic (non-coated), hydrated collagen gel (collagen gel) (Enami et al., 1987) and poly-N-p-vinylbenzyl-D-lactonamide (PVLA) (Akaike et al., 1993). The culture media were changed every 24 h and the hepatocytes were cultured for 24 h to 96 h in a humidified incubator at 37˚C under 5% CO_2 in air, and were evaluated by biochemical assays.

Bioartificial liver module

Hepatocytes were loaded into a hollow fiber cartridge manufactured for clinical hemodialysis (SULFLUX FS-03, from Kaneka Co., Osaka) as a bioartificial liver module. The cartridge had 2 separate chambers: the space around the hollow fibers (extracapillary) and the space within the hollow fibers (lumen). The fiber-surface area was 0.3 m^2 and volumes of the lumen and extracapillary space were 35 mL and 55 mL, respectively. The device consisted of polysulfone fibers of a 340 μm inside diameter, a 50 μm membrane thickness and a 0.2 μm porous size. The extracapillary surfaces of hollow fibers were precoated with 0.015% type I collagen solution (cellmatrix, from Nitta Geratin Co., Osaka) before the inoculation of hepatocytes. Isolated hepatocytes with a viability more than 80% by trypan blue exclusion test were suspended in 0.1% type I collagen solution with concerted L-15 medium (Enami et al., 1987). In each experiment, 1.0 to 3.0 $\times$ 10^8 hepatocytes in 40 mL of the collagen gel solution were loaded into the extracapillary space of the cartridge. After 4 h incubation to accelerate gel formation in a humidified atmosphere of 95% air and 5% CO_2 at 37˚C, perfusion of the luminal space was started with approximately 200 mL of perfusion medium consisting of L-15 supplemented with 5% FCS, 5000 KIU/L aprotinin, 30 mg/L prolin, 10^{-7} mol/L insulin, 10^{-7} mol/L dexamethasone, 10^{-8} mol/L glucagon and 10 ng/mL hEGF. The perfusion medium was preincubated at 37˚C, given oxygen at 500 mL/min and circulated at approximately 20 mL/min. The perfusion medium was

changed every 24 h and continued to perfuse for 2 to 13 days under a sterilized hood.

Biochemical assay of metabolic activities

Gluconeogenesis

After removal of the perfusion medium, modules were perfused in the luminal space for 2 h with glucose-free Hanks' solution (pH 7.4), supplemented with 10 mmol/L HEPES, 10^{-5} mol/L glucagon, 2 mmol/L L-alanine, 2 mmol/L DL-lactic acid. Glucose in the perfusate was measured by a Glucose C II-test Wako kit (Wako) using the mutarotase-glucose oxidase method (Miwa et al., 1972).

Ureogenesis

After the gluconeogenesis assay, Hanks' solution (pH 7.4) with 10 mmol/L HEPES, 5 mmol/L NH_4Cl was perfused in the luminal space for 2 or 3 h. Urea nitrogen in the perfusate was measured using a Urea-Nitrogen test kit (Wako) by the diacetylmonoxime method (Coulombe and Favreau, 1963).

Albumin assay

After the Hanks' solution was perfused for assaying gluconeogenesis and ureogenesis, the luminal space was perfused with a fresh L-15 medium supplemented with 5% FCS, 5000 KIU/L aprotinin, 30 mg/L prolin, 10^{-7} mol/L insulin, 10^{-7} mol/L dexamethasone, 10^{-8} mol/L glucagon and 10 ng/mL hEGF for 12 to 25 h. The albumin concentration in the perfusate was measured by enzyme-linked immunosorbent assay (ELISA) method (Dunn et al., 1992). Purified rat albumin and peroxide-conjugated rabbit anti-rat albumin antibody was obtained from ICN Pharmaceuticals (Costa Mesa, CA). The antibody was preincubated with an excess of bovine albumin (30 mg/mL in 0.5% Tween-20 in PBS) before the sample addition in order to block any cross reaction (Shatford et al., 1992).

Northern blot analysis

Induction of CYP 2B1

To induce CYP 2B1, 1 mmol/L phenobarbital (Marco Seiyaku Co., Ltd., Nagoya, Japan) was added to the perfusate for 24 h before the day of harvesting hepatocytes.

Harvest of hepatocytes

After removing the perfusion medium, the module was perfused in the extracapillary space with RNase free 0.05% collagenase solution for 1 h followed by PBS for 1 h to harvest hepatocytes. The collected cell pellets by centrifugation ($50 \times g$, 2 min, 4˚C) were stored at –80˚C until RNA extraction.

RNA extraction and cDNA probes

Total RNA of hepatocytes was extracted using an acid guanidinium thiocyanate phenol chloroform method (Chomczynski and Sacchi, 1987) (ISOGEN, from Nippon Gene Co., Ltd., Toyama, Japan). The liver RNA was extracted by the modified guanidine ultracentrifugation method using Beckman SW41Ti roter (25,000 rpm, 20 h, 20˚C) (Ullrich et al., 1977) from normal liver specimens and liver specimens of adult Wistar rats, which were induced CYP 2B1 by intraperitoneal injections of phenobarbital at a dose of 150 mg/kg weight at 48 h and 24 h before sacrifice. The rat albumin complementary DNA (cDNA) probe (HG293: pAct-Alb) was obtained from the Health Science Research Resources Bank (Osaka), the rat CYP2B1 cDNA probe (rat pcP-450pb1) (Fujii-Kuriyama et al., 1982) was from the Riken Gene Bank (Ibaragi, Japan) and the rat β-actin cDNA probe was a kind gift from Dr. Ueta (The Gene Research Center, Tottori University).

Northern blot analysis

Total RNA (20 μg) of each sample was hybridized with [^{32}P]-labeled cDNA probes of rat albumin and CYP 2B1. The same blots were rehybridized with rat β-actin cDNA probe to assess equal loading of the blots. Autoradiograms were quantified by densitometric scanning using a Phosphor Image Scanner (Molecular Imager system GS-525, BioRad, Hercules, CA).

Statistical analysis

Experimental dates were expressed as mean ± SD (Tables 1 and 2) and mean ± SE (Figs. 1–3).

Table 1. Metabolic activities of hepatocytes cultured on non-coated dishes with WE or L-15 medium for 24 h or 72 h

Medium†	Production rate‡ (μg/10^7 cells/h)					
	Glucose		Urea nitrogen		Albumin	
	24 h	72 h	24 h	72 h	24 h	72 h
WE	466 ± 30.4	156 ± 9.60	8.40 ± 1.60	6.20 ± 1.60	11.2 ± 5.00	5.30 ± 1.40
L-15	275 ± 22.8	200 ± 8.10	27.8 ± 2.20	15.9 ± 2.60	22.0 ± 11.2	11.0 ± 3.00
P value	0.0103	0.0038	0.0101	0.0039	0.0768	0.0004

FCS, fetal calf serum; hEGF, human epidermal growth factor; L-15, Leibovitz's L-15 medium; WE, Williams' E medium.

†Both media were supplemented with 5% FCS, 5000 KIU/L aprotinin, 10^{-7} mol/L insulin, 10^{-7} mol/L dexamethasone, 10^{-8} mol/L glucagon and 10 ng/mL hEGF. the L-15 was added 20 mmol/L $NaHCO_3$ and 30 mg/L proline also.

‡Production rates were calculated as μg products/10^7 cells/h and expressed as mean ± SD of 6 dishes in one experiment.

Mann-Whitney's *U* test was performed between WE and L-15 medium.

The differences between any 2 groups were analyzed by Mann-Whitney's *U* test. Values of $P < 0.05$ were considered significant.

Results

Metabolic activities of hepatocytes cultured on dishes

Glucose, urea nitrogen and albumin production rates in hepatocytes cultured on plastic dishes compared to WE and L-15 medium are summarized in Table 1. After 24 h in culture, urea nitrogen production was higher in hepatocytes cultured with L-15 based medium than the hepatocytes cultured with WE based medium ($P < 0.05$) although gluconeogenesis was higher in hepatocytes cultured with WE medium ($P < 0.05$). All production rates, glucose, urea nitrogen and albumin in hepatocytes cultured with L-15 were significantly higher than with WE after 72 h in culture ($P < 0.01$, $P < 0.01$, $P < 0.001$, respectively). Marked differences in these activities were observed in hepatocytes cultured on different types of extracellular matrices (Table 2). Production rates of glucose and urea nitrogen at 48 h ($P < 0.001$, $P < 0.001$,

Table 2. Metabolic activities of hepatocytes cultured on 3 types of matrix-coated dishes†

Matrix-coated	Production rate‡ (μg/10^7 cells/h)					
	Glucose		Urea nitrogen		Albumin	
	48 h	96 h	48 h	96 h	48 h	96 h
Non-coated	158 ± 17.1	151 ± 27.5	78.8 ± 25.5	66.2 ± 55.4	21.0 ± 5.10	37.7 ± 21.9
Collagen gel	1780 ± 238***	914 ± 239***	329 ± 57.5***	245 ± 153*	19.0 ± 6.00^{NS}	53.9 ± 26.9*
PVLA	159 ± 8.70^{NS}	140 ± 22.8^{NS}	66.9 ± 27.0^{NS}	55.4 ±41.0^{NS}	26.9 ± 8.20*	39.5 ± 19.7^{NS}

FCS, fetal calf serum; hEGF, human epidermal growth factor; L-15, Leibovitz's L-15 medium; PVLA, poly-N-p-vinylbenzyl-D-lactonamide.

†Hepatocytes were cultured with the L-15 medium supplemented with 20 mmol/L $NaHCO_3$, 5% FCS, 5000 KIU/L aprotinin, 30 mg/L proline, 10^{-7} mol/L insulin, 10^{-7} mol/L dexamethasone, 10^{-8} mol/L glucagon and 10 ng/mL hEGF for 48 h or 96 h.

‡Production rates were calculated as μg products/10^7 cells/h and expressed as mean ± SD of 6 dishes in 2 separate experiment.

Mann-Whitney's *U* test was performed between non-coated and collagen gel, between non-coated and PVLA. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$; NS, not significant.

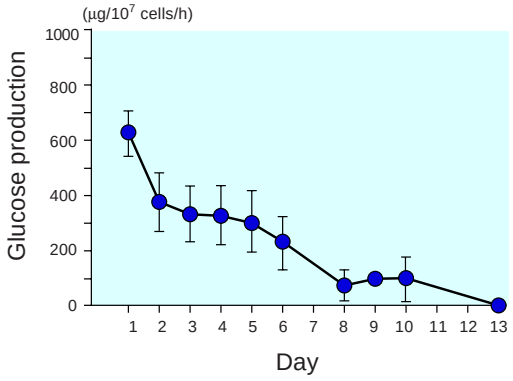

Fig. 1. Gluconeogenesis of hepatocytes in the bioartificial modules. Production rates were calculated as μg glucose/10^7 cells/h and expressed as mean ± SE of 10 samples in 5 separate experiments. The highest glucose production rate was observed on day 1 (610 μg/10^7 cells/h).

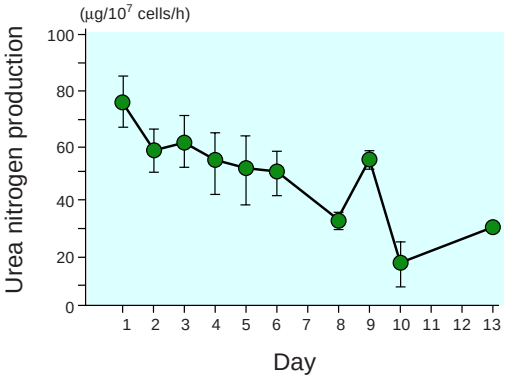

Fig. 2. Ureogenesis of hepatocytes in the bioartificial modules. Production rates were calculated as μg urea nitrogen/10^7 cells/h and expressed as mean ± SE of 10 samples in 5 separate experiments. The production of urea nitrogen was highest on day 1 (76. 1 μg/10^7 cells/h).

respectively) and all the rates of glucose, urea nitrogen and albumin at 96 h ($P < 0.001$, $P < 0.05$, $P < 0.05$, respectively) of hepatocytes entrapped in collagen gel were significantly higher than those of the hepatocytes on non-coated dishes or on PVLA coated dishes. According to these results, L-15 based medium and collagen gel were selected as the perfusion medium and the extracellular matrix for a bioartificial liver module.

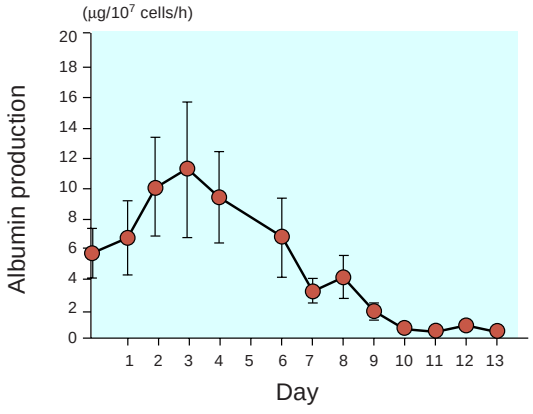

Fig. 3. Albumin production rate of hepatocytes in the bioartificial modules. Production rates were calculated as μg albumin/10^7 cells/h and expressed as mean ± SE of 18 samples in 6 separate experiments. The albumin production rate was at its maximum on day 3 (11.0 μg/10^7 cells/h).

Metabolic activities of hepatocytes of the bioartificial liver module

To evaluate metabolic activities of hepatocytes in the long-term culture of all modules, the production rate was calculated from total cell numbers at the time of inoculation and expressed as μg products/10^7 cells/h. The highest glucose production rate was observed on day 1 (610 μg/10^7 cells/h), gradually decreased to 70.2 μg/10^7 cells/h on day 8 and was maintained until day 10 on the same level (Fig. 1). The production of urea nitrogen was highest on day 1 (76.1 μg/10^7 cells/h) and was maintained to day 8 (32.8 μg/10^7 cells/h) as shown in Fig. 2. Albumin production rate was at its maximum on day 3 (11.0 μg/10^7 cells/h), then gradually decreased and was measurable until day 13 in the module (Fig. 3).

Northern blot analysis of hepatocytes of the bioartificial liver module

The albumin mRNA was expressed in the hepatocytes cultured in the bioartificial liver modules from day 2 to day 11 (Fig. 4A). To assess quantitative expression of mRNAs, the ratio of albumin mRNA/β-actin mRNA and CYP 2B1 mRNA/β-actin mRNA were calculated (Fig. 5). It was most strongly expressed

on day 2 in the ratio of albumin mRNA/β-actin mRNA and then gradually decreased. The expression of CYP 2B1 mRNA by induction with phenobarbital was detected at day 2 but on very low level (Fig. 4B). No inducibility of mRNA of CYP 2B1 was found on day 5 or day 11. The expression of rat β-actin mRNA, used as an internal control, was the same level during culturing in the device (Fig. 4C).

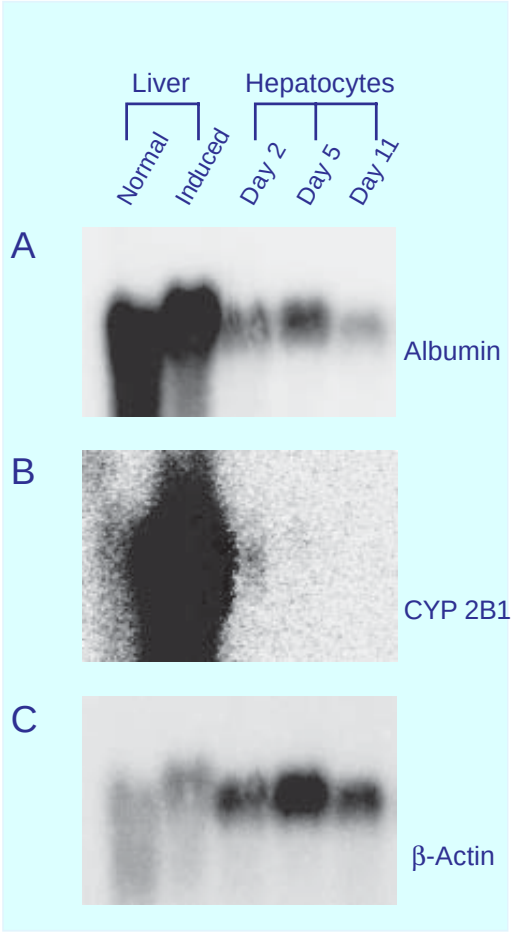

Fig. 4. Northern blot analysis of hepatocytes in the bioartificial modules. **A**) Albumin mRNA. **B**) Cytochrome P450 2B1 (CYP 2B1) mRNA. **C**) β-Actin mRNA. Each lane shows control RNA from a normal adult rat liver (Normal), RNA from an adult rat liver induced with phenobarbital (Induced), RNAs on days 2, 5 and 11 of hepatocytes induced with phenobarbital from bioartificial liver modules.

Discussion

The development of hybrid artificial liver devices has been exhaustively investigated for decades (Matsumura et al., 1987; Takahashi et al., 1992; Fremond et al., 1993; Li et al., 1993; Naruse et al., 1996a; Chen et al., 1997), and yet none of the studies has been established as a standard clinical treatment for liver failure. The complexity of functional activities of the liver makes it difficult to design such systems. In order to develop modules that can support and improve patients with liver failure, it is necessary to evaluate the functional capacity of hepatocytes in the systems over periods. Suitable markers for following quantitative specific functions of the device are required (Lazar et al., 1995). We designed a hollow fiber type BAL inoculating primary hepatocytes from rats cultured with L-15 medium entrapped in collagen gel which was expected to maintain hepatic function for a prolonged period. In addition to assays of biochemical activities, such as gluconeogenesis, ureogenesis, and albumin synthesis, the expression of albumin mRNA

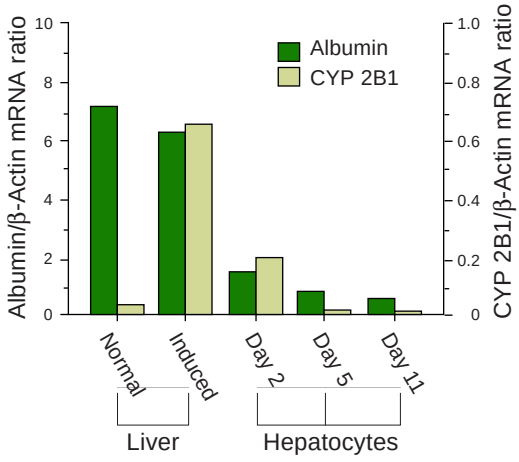

Fig. 5. The ratio of albumin mRNA/β-actin mRNA expression and cytochrome P450 2B1 (CYP 2B1) mRNA/β-actin mRNA expression. Each lane shows control RNA from a normal adult rat liver (Normal), RNA from an adult rat liver induced with phenobarbital (Induced), RNAs on days 2, 5 and 11 of hepatocytes induced with phenobarbital from bioartificial liver modules.

and CYP 2B1 mRNA by Northern blot analysis was achieved to clarify whether or not these functions were present in sufficient amounts.

We selected the L-15 medium as the perfusion medium for the module, but the WE medium had been used in many previous reports (Shatford et al., 1992; Takeshita et al., 1995; Naruse et al., 1996a). The differences between the L-15 medium and the WE are as follows: the concentration of amino acids in L-15 is higher than in WE, galactose is used instead of glucose in L-15, and the pH of L-15 medium is stable in an atmosphere without 5% of CO_2. Tanaka and others (1979) reported that hepatocytes cultured at a high amino acid concentration were consuming amino acids and forming a large amount of urea under an anabolic state. Although both media contain high concentrations of amino acids, there was a significant difference in ureogenesis in the experiments of hepatocytes cultured in dishes. The stability of the pH of the medium was also an important factor in selecting the type of medium, because the L-15 medium can be recirculated for a long time without CO_2 supplementation. As well as the type of medium, the hormonal addition of 10^{-7} mol/L insulin, 10^{-7} mol/L dexamethasone, 10^{-8} mol/L glucagon and 10 ng/mL hEGF was crucial for the maintenance of hepatic functions (data not shown). The levels of biochemical activities in BAL were found to be lower than those in the dish-cultures. It could be because the amounts of the perfusion medium were not sufficient and hepatocytes in BAL could not exert full hepatic functions. Culturing hepatocytes in an atomosphere without CO_2 might be another cause of low hepatic functions of BALs. Further studies are needed on these aspects.

It was also important to choose an appropriate basement matrix for hepatocytes in the hollow fiber module. The data of hepatocytes cultured on dishes showed marked differences in the production rates of glucose, urea nitrogen and albumin between the collagen gel and non-coated dishes. Many investigations on the effects of the extracellular matrix revealed that the constituents of the extracellular matrix for hepatocytes in vitro strongly influence expression and maintenance of liver specific genes (Kocarek et al., 1993; Nagaki et al., 1995; Tamura et al., 1995). Two different types of matrices, collagen gel and PVLA, both providing a 3-dimensional configuration for hepatocytes, were studied (Akaike et al., 1993; Lazar et al., 1995). Hepatocytes entrapped in collagen gel were found maintaining much higher levels of biochemical activities consistant with several reports of BAL systems using collagen gel as a cellular matrix in the hollow fiber cartridge (Shatford et al., 1992; Sielaff et al., 1995a; Takeshita et al., 1995). To obtain a firm attachment of hepatocytes in the extracapillary membrane of fibers, we modified the method of hepatocyte inoculation; firstly, we coated the extracapillary membrane with a low concentration (0.015%) of collagen solution, then loaded the hepatocytes in 0.1% hydrated collagen and then incubated them at 37˚C allowing them to gel rapidly to avoid a disproportional distribution of collagen gel in the extracapillary space.

Determination of albumin production in perfusates has been supposed to be an optimal index of the metabolic activities of hepatocytes because albumin is stable in media and only hepatocytes synthesize it (Rothschild et al., 1972). But the biochemical assay of albumin in perfusates does not completely reflect the newly synthesized albumin. Even if the perfusion medium was changed every day and there were be no stored albumin in the medium being used in our experiments, the albumin stored in the extracapillary space of the hollow-fiber module could not be neglected. Thus, we performed an albumin mRNA analysis in addition to a biochemical assay by ELISA. Northern blot analysis reflects the transcriptional activity of the albumin gene at the time of cell harvest so that the existence of stored albumin in the extracapillary space or cross-reaction to FCS in the medium can be neglected. There are no previous reports evaluating the functional activity of BALs in mRNA levels, mainly because hepatocytes must be collected from devices, which means the end of perfusion experiments. The albumin mRNA in our module was maintained for 11 days. The change of expression

levels of mRNA (Fig. 4) was parallel to the change of albumin production rates measured by ELISA in the media (Fig. 3). This result suggests that the measurement of albumin production in perfusates correctly reflects the newly synthesized albumin in hepatocytes of a BAL.

We also performed Northern blot analysis of CYP 2B1 mRNA as an index of the ability of detoxification of the module. In previous reports, the drug metabolizing activity of a BAL was assessed by measuring concentrations of certain drugs, for example lidocaine, and their metabolites by high performance liquid chromatography (Shatford et al., 1992; Nyberg et al., 1993; Rozga et al., 1993). The increased concentration of metabolites shows the activity of cytochrome P450 related drug enzymes and a part of the drug metabolic pathway, but does not show general levels or the inducibility of cytochrome P450s. It was, therefore, thought to be valuable to perform Northern blot analysis.

In the present study, the inducibility and expression of CYP 2B1 mRNA was maintained for a shorter term than the expression of albumin mRNA. Although albumin synthesis might be a useful marker of hepatocyte viability (Sielaff et al., 1995b), in the sense of potential therapeutic value for patients with liver failure, cytochrome P450 activity is suggested to be the critical function that must be provided by successful liver assistance devices (Nyberg et al., 1993). A number of researchers (Kocarek et al., 1993; Sidhu et al., 1993; Donato et al., 1994) have demonstrated that hepatocytes in culture expressed constitutive cytochrome P450 activity only in a differentiated environment. It is known that their distribution of the expression of cytochrome P450s in the hepatocytes in vivo depends on the location of the hepatocytes in the lobules, for example Zones 1, 2 and 3 (Wojcik et al., 1988). In order to maintain high levels of the expression of cytochrome P450s, the reconstruction of the lobular system in the BAL seemed necessary. Hepatocytes in this study were entrapped in collagen gel and loaded into the device evenly so that hepatocytes should have been under a 3-dimensional configuration (Lazar et al., 1995). Even under these conditions, hepatocytes maintained CYP 2B1 mRNA only for 2 days in the BAL, which is not a completely suitable environment for hepatocytes. It is necessary for hepatocytes to have proper polarity and microcirculation likely in vivo, including the presence of vessels, nonparenchymal cells and bile duct systems.

In summary, we evaluated the maintenance of hepatic functions of a potential hollow fiber type BAL by biochemical activities and mRNA levels. The albumin mRNA was detected for 11 days parallel to albumin production measured by ELISA. The expression of CYP 2B1 mRNA, however, was maintained only for a short term even though it was expected to maintain itself for as long a period as albumin. It should be necessary to reconstitute a microenvironment for hepatocytes in a BAL as in vivo, probably retaining zone lobulation, to achieve the total functions of the liver.

Acknowledgments: The authors thank Prof. Hironaka Kawasaki (Second Dept. of Internal Medicine, Faculty of Medicine, Tottori University) and Prof. Kazuo Yamada (Dept. of Biochemistry, Faculty of Medicine, Tottori University) for their valuable suggestions and critical readings of the manuscript. We also thank Dr. Eiji Nanba and Dr. Etsuko Ueta (The Gene Research Center, Tottori University) for assistance with the Northern blot analysis.

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(Received December 21, 1998, Accepted January 11, 1999)