Gastroenterologia Japonica
Copyright © 1990 by The Japanese Society of Gastroenterology

Vol. 25, No. 6
Printed in Japan

Ethanol and acetaldehyde metabolism in cultured hepatocytes from chronic alcoholic rats

Yasuhiro UESHIMA, Shujiro TAKASE, and Akira TAKADA
Division of Gastroenterology, Department of Internal Medicine, Kanazawa Medical University, Ishikawa 920-02, Japan

Summary: In this study the authors analyzed ethanol (Et-OH) and acetaldehyde (Ac-CHO) metabolism in cultured hepatocytes isolated from chronic alcoholic rats. Hepatocytes were isolated and cultured from two groups of rats, one was fed with a liquid diet containing Et-OH and another was pair-fed with a control diet for 4 weeks. After 48 hours of the primary culture, Et-OH was added to the culture medium at a final concentration of 5 mM with or without 2 mM 4-methyl pyrazole (Py). Serial changes of Et-OH and Ac-CHO levels in the medium for 48 hours were determined in 4 groups of the alcohol alone, alcohol-Py, control and control-Py groups. In the alcohol alone and control groups, Et-OH disappearance rates (EDR), which are roughly equivalent to Et-OH oxidation rates in the hepatocytes, were significantly higher than those in the corresponding Py treated groups. In the two alcoholic groups, the EDR was significantly higher than those in the corresponding control groups. In cultured hepatocytes, 75-80% of Ac-CHO produced from Et-OH was oxidized. The increasing rates of Ac-CHO (AcICR), a function of Ac-CHO production and oxidation rates in the hepatocytes, increased in parallel with the increase in the EDR. However, the AcICR/EDR rate, which is a parameter of the Ac-CHO oxidation rate in the hepatocytes, was not different among the 4 groups. These results indicate that the cultured cells maintain the characteristics of Et-OH metabolism in chronic alcoholic rats and may also be used for the study of Et-OH and Ac-CHO metabolism as an *in vitro* model. *Gastroenterol Jpn 1990;25:708–714*

Key words: *acetaldehyde metabolism; chronic alcoholic rats; cultured hepatocytes; ethanol metabolism*

Introduction

Almost all ingested ethanol (Et-OH) is metabolized by the liver; only a trace of Et-OH is metabolized by other organs[1,2]. Acetaldehyde (Ac-CHO) produced in the liver is also oxidized mainly in the liver. It was supposed that the hepatic share of Ac-CHO metabolisms may be smaller than that of Et-OH[3,4]. The metabolism of Ac-CHO in the liver, however, is not as well understood as that of Et-OH.

Culture of hepatocytes has recently become common. When cultured hepatocytes are used for a study, Et-OH and Ac-CHO metabolism in the liver can be analyzed, excluding the effects of the extrahepatic organs. Such studies, however, are relatively few[5-9], especially on Et-OH metablism in chronic alcoholic animals and Ac-CHO metabolism. In the present study, Et-OH and Ac-CHO metablihsm in cultured hepatocytes obtained from chronic alcoholic rats were analyzed to determine the usefulness of cultured cells for the study of Et-OH and Ac-CHO metabolism.

Materials and Methods

One group of male Wistar strain rats, weighing 150–200g, was fed with a liquid diet containing

Received July 3, 1989. Accepted April 20, 1990.
Address for correspondence: Akira Takada, M.D., Division of Gastroenterology, Department of Internal Medicine, Kanazawa Medical University, Uchinada, Ishikawa 920-02, Japan.

Et-OH (36% of total clories, alcohol group) and another group of rats was pair-fed with a control diet (Et-OH replaced by isocaloric sucrose; control group) for 4 weeks. In each diet 35% of the total calories were fat calories. The regimens and caloric composition of the diets were previously reported[10].

Hepatocytes were isolated from rats of both groups using the collagenase digestion method of Seglen[11]. The isolated cells were suspended in a medium containing a 3:7 mixture of Ham F-10 and L-15 (Flow Laboratories Inc., Md.), 10% fetal calf serum, insulin, dexamethasone and penicillin G, according to the description of Odashima et al[12], and cultured in Corning plastic culture flasks (25 cm^2, 70 ml) precoated with fibronectin, at a seeding density of 1×10^6 cells per flask. Culture was carried out at 37°C in a humidified atmosphere consisting of 5% CO_2 in air. The culture medium was changed every 2 days. Usually, two culture flasks were obtained from one rat.

At 48 hours of the primary culture, the culture flasks were tightly sealed with rubber caps, immediately after inflow of 95% oxygen gas at a speed of 0.5 l/min for 5 minutes into the air space of the flasks. Then Et-OH was added to the cultured medium at a final concentration of 5 mM through the rubber caps. Into half of the culture flasks, 4-methyl pyrazole (4-MP) at a final concentration of 2 mM was also added at the same time. Consequently, the experimental groups counted of 4 groups of alcohol alone, alcohol-pyrazole, control, and control-pyrazole.

An aliquot of the culture medium was obtained at 0, 3, 6, 12 and 24 hours after the addition of Et-OH by a needle through the rubber caps from each culture flask. Et-OH and Ac-CHO levels in each sample were measured by the method of Eriksson et al[13], using gas chromatography. And the partial pressures of oxygen and carbon dioxide (PO_2 and PCO_2) and pH of each sample, except for 3 hours, were measured using a pH/blood gas analyzer (IL system 13036; Instrumentation Laboratory, Boston). Pyruvate and lactate concentrations of the medium at 0 and 24 hours were measured by the method of Olsen[14]. Serial changes of PO_2, PCO_2 and pH of the medium were also determined in non-sealed control cultures without oxygen in-flow and Et-OH addition.

In the isolated and cultured hepatocytes from the control group, alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and p-nitrophenol hydroxylase (PNPH) which is a specific marker for the induction of microsomal P450 II E1[15,16], were determined by the methods of Bonnichen and Brink[17], Sugata et al[18], and Koop[15], respectively. The isolated cells and cultured cells which were scraped off at 24 and 48 hours of the primary culture after washing with ice-cold phosphate buffered saline at pH 7.2 (PBS) were sonicated in PBS at 0°C for 30 seconds using an Ultrasonic Disrupture, Model UR-200 (Tomy Seiko Co. Ltd, Tokyo). After centrifugation at 9,000 xg for 30 sec at 4°C, the supernatants was used for measurement of enzyme activity. In an aliquot of the sonicated cell fraction, DNA and protein contents were measured by the methods of Labarca and Paigen[19] and Lowry et al[20], respectively. Activities of the enzymes per μg DNA and per mg protein were calculated.

All experimental values were expressed by the mean ± standard deviation. Student's t-test was applied for group comparisons.

Results

The enzyme activities in hepatocytes from rats of the control group, are shown in **Table 1**. The ADH and ALDH activities expressed both per μg DNA and per mg protein tended to be lower in the cultured cells than in the isolated cells. However, the changes were not significant statistically. On the other hand, the PNPH activity in cultured cells, except for the activity per mg protein at 24 hours, were significantly lower than that in the isolated cells.

Serial changes of PO_2, PCO_2 and pH of the medium are shown in **Table 2**. The PO_2 in the experimental groups was high compared to the physiological condition (non-sealed, no oxygen and no Et-OH control), because of the in-flow of oxygen gas. The PO_2 tended to decrease in accordance with the time of incubation in all groups.

Table 1 Alcohol dehydrogenase, aldehyde dehydrogenase and p-nitrophenol hydroxylase activities in cultured hepatocytes

	isolated cells	cultured cells	
		24 hr	48 hr
alcohol dehydrogenase			
(p mole NADH/min/μ gDNA)	11.76±2.95	10.10±2.59	9.14±2.09
(p mole NADH/min/mg protein)	0.73±0.35	0.57±0.09	0.56±0.05
aldehyde dehydrogenase			
(p mole NADH/min/μ gDNA)	52.88±9.99	44.17±14.74	36.54±8.00
(p mole NADH/min/mg protein)	3.32±1.15	2.48± 0.76	2.36±0.44
p-nitrophenol hydroxylase			
(n mole PNC/min/μ gDNA)	0.88±0.13	0.47±0.04	0.47±0.13
(n mole PNC/min/mg protein)	0.45±0.06	0.29±0.05	0.28±0.12

PNC: p-nitrocatechol. (n=4)
*: $P<0.05$

Table 2 Serial changes of partial pressures of oxygen and carbon dioxide and pH in the medium of cultured hepatocytes

groups		0 hr	6 hrs	12 hrs	24 hrs
Alcohol alone (n=10)	PO_2	272±30	267±54	254±38	239±45
	PCO_2	19± 4	20± 4	24± 3	29± 3**
	pH	6.81±0.06	6.81±0.07	6.76±0.05	6.81±0.05
Al-pyrazole (n=10)	PO_2	299±25	309±17	291±23	289±45
	PCO_2	22± 2	24± 2	24± 2	24± 2
	pH	6.79±0.01	6.76±0.03	6.76±0.03	6.81±0.05
Control (n=10)	PO_2	286±25	271±30	269±31	255±43
	PCO_2	20± 1	21± 2	21± 2	24± 3
	pH	6.80±0.03	6.79±0.08	6.78±0.09	6.76±0.21
Cont-pyrazole (n=10)	PO_2	383±23	281±29	286±31	276±33
	PCO_2	21± 1	21± 2	21± 2	22± 3
	pH	6.79±0.03	6.80±0.06	6.79±0.08	6.77±0.12
Non-sealed control# (n=5)	PO_2	157± 4	140± 6	142± 6	144± 1
	PCO_2	16± 4	33± 4	32± 2	34± 5
	pH	6.82±0.07	6.64±0.03	6.68±0.02	6.69±0.02
Sealed control# (n=5)	PO_2	151± 5	149± 1	144± 3	144± 5
	PCO_2	21± 1	19± 2	19± 1	23± 2
	pH	6.74±0.03	6.80±0.03	6.81±0.01	6.78±0.02

PO_2 (mmHg): partial pressure of oxygen.
PCO_2 (mmHg): partial pressure of carbon dioxide.
#: no oxygen, no ethanol.
**: $P<0.01$ vs 0 hour

However, the decrease was very slight and the concentration was still high even at the 24 hour period. PCO_2 and pH did not change significantly during the experimental period in all groups, except for PCO_2 of the alcohol alone group. In that group, PCO_2 significantly, but slightly, increased at the 24 hour period. However, the value was not higher than that in the non-sealed control medium at the 24 hour period. Lactate and pyruvate levels in the medium at the 0-hour and 24-hour periods are shown in **Table 3**. Lactate levels increased and pyruvate levels decreased

Table 3 Lactate and pyruvate levels in the medium of cultured hapatocyte

groups	0 hr			24 hr		
	lactate (mM)	pyruvate (mM)	L/P	lactate (mM)	pyruvate (mM)	L/P
Alcohol alone (n=10)	2.82±0.13	0.45±0.01	6.27±0.24	3.82±0.13**,++	0.13±0.03**,++	30.0±5.33**,++
Al-pyrazole (n=10)	2.71±0.20	0.44±0.01	6.18±0.52	3.11±0.90*	0.37±0.04**	8.40±4.02**
Control (n=10)	2.83±0.26	0.42±0.01	6.76±0.48	4.73±0.63**,++	0.29±0.04**,++	16.4±3.72**,++
Cont-pyrazole (n=10)	2.81±0.71	0.44±0.01	6.42±1.74	3.17±1.03	0.39±0.07	8.70±5.11**

L/P: lacatate/pyruvate ratios.
**: P<0.01, *: P<0.05 vs 0 hour.
++: P<0.01 vs corresponding pyrazole treated group.

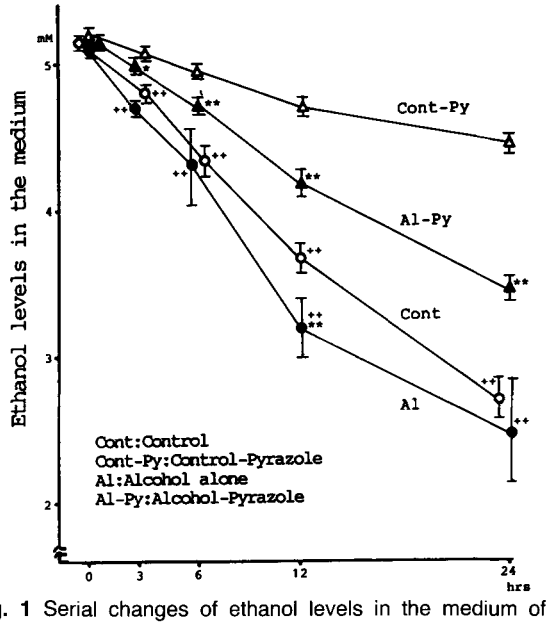

Fig. 1 Serial changes of ethanol levels in the medium of cultured hepatocytes.
++: P<0.01 vs corresponding pyrazole treated group.
*: P<0.05, **: P<0.01 vs corresponding control group.

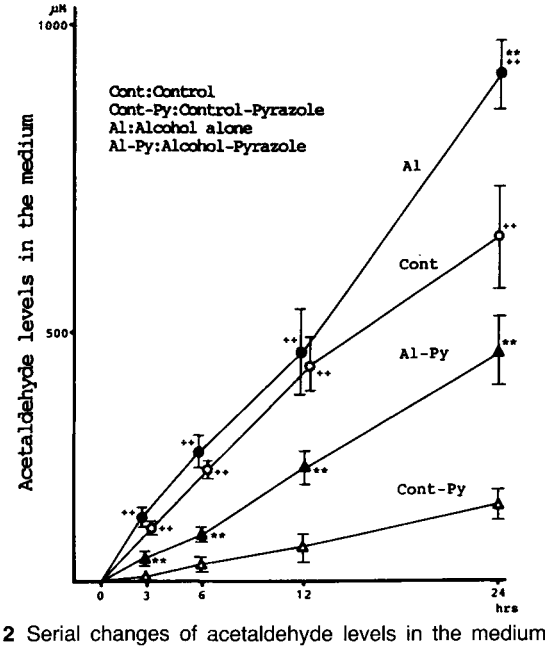

Fig. 2 Serial changes of acetaldehyde levels in the medium of cultured hepatocytes.
++: P<0.01 vs corresponding pyrazole treated group.
**: P<0.05 vs corresponding control group.

significantly at the 24 hour period in all groups, except for the control-pyrazole group. Consequently, lactate/pyruvate ratios significantly increased in all groups. This increase was prominent in the alcohol alone and control groups and the ratios at the 24 hours in these groups were significantly higher than those in the corresponding 4-MP treated groups.

Serial changes of Et-OH levels in the medium are shown in **Figure 1**. The levels decreased gradually with a nearly linear curve in all groups. The decreases were faster in the order of the alcohol-alone, control, alcohol-pyrazole and control-pyrazole groups. The Et-OH levels in the alcohol alone and control groups were significantly lower from the 3-hour period to the 24-hour period than those in the corresponding pyrazole treated groups. In the pyrazole treated groups, the alcohol-pyrazole group showed significantly lower Et-OH levels than the control-pyrazole group throughout the experimental period. The difference in Et-OH level between the alcohol-alone and control groups was significant only at the 12 hour period.

Table 4 Ethanol disappearance rate and acetaldehyde increase rate

	Alcohol alone	Al-pyrazole	Control	Cont-pyrazole
EDR (μM/hr)	161±20.4$^{++,**}$	81.3±4.6**	124±5.9^{++}	30.5±2.4
AclCR (μM/hr)	37.6± 3.2$^{++,**}$	19.7±2.9**	27.6±3.7^{++}	6.1±0.9

EDR: ethanol disappearance rate, AclCR: acetaldehyde increase rate.
++: P<0.01 vs corresponding pyrazole treated group.
**: P<0.01 vs corresponding control group.

Table 5 The ratios of AclCR/EDR, differences between EDR and AclCR, degradation percentage of Ac-CHO

	Alcohol alone	Al-pyrazole	Control	Cont-pyrazole
AclCR/EDR	0.24±0.04	0.24±0.06	0.22±0.04	0.20±0.04
EDR − AclCR (μM/hr)	123.4±21.1$^{++,**}$	61.6±6.5**	96.8±9.6^{++}	24.5±2.0
degradation % of Ac-CHO	76.2± 4.1	75.6±4.4	77.6±3.9	80.2±2.6

EDR: ethanol disappearance rate, AclCR: acetaldehyde increase rate.
++: P<0.01 vs corresponding pyrazole treated group.
**: P<0.01 vs corresponding control group.

Serial changes of Ac-CHO levels in the medium are shown in **Figure 2**. The levels increased linearly in all groups. The increases were greater in the order of the alcohol-alone, control, alcohol-pyrazole and control-pyrazole groups. The Ac-CHO levels in the alcohol-alone and control groups were significantly higher from the 3-hour period to the 24-hour period than those in the corresponding pyrazole treated groups. In the pyrazole treated groups the alcohol-pyrazole group showed significantly higher Ac-CHO levels than in the control-pyrazole group throughout the experimental period. The difference in Ac-CHO level between the alcohol-alone and control groups was significant only at 24 hours.

Disappearance rates of Et-OH (EDR) from the medium, which is roughly equivalent to the ethanol oxidation rate (EOR) in the hepatocytes and increasing rates of Ac-CHO (AcICR) in the medium for 24 hours, which is a function of the balance between Ac-CHO production and oxidation rates in the hepatocyte and may be equivalent to the accumulation rate of Ac-CHO outside hepatocytes, were calculated from the linear sections of the decreasing and increasing curves, respectively. The EDR in the alcohol-alone and control groups was significantly higher than those in the corresponding pyrazole treated rats. In the two alcohol groups, the EDR was significantly higher than those in the corresponding control groups. The AcICR in the groups increased in parallel with the increase in EDR. The rates in the two pyrazole non-treated groups and those in the two alcoholic groups were significantly higher than those in the corresponding pyrazole treated groups and than in the corresponding control groups, respectively (**Table 4**). The ratio of AcICR/EDR, which may be a parameter of Ac-CHO degradation in the hepatocytes, did not differ among the 4 groups. The amounts of Ac-CHO that were oxidized in the hepatocytes may be calculated by subtracting AcICR from EDR. The differences between EDR an AcICR were greater in the alcohol and pyrazole non-treated groups than in the corresponding control groups or corresponding pyrazole treated groups (**Table 5**). The percent degradation of Ac-CHO to its production in the hepatocytes were calculated by the following formula: AcICR − EDR/EDR ×100. The percentages were not different among the 4 groups.

Discussion

Et-OH and Ac-CHO easily evaporate at temperatures for culturing. Therefore, in the present

study, cultured flasks were tightly sealed after saturation of oxygen in the culture medium. Despite the fact that the flasks were sealed, PO_2 did not decrease and PCO_2 did not increase significantly, except for the alcohol-alone group, throughout the experiment. PO_2 was higher and PCO_2 was lower than in the non-sealed control culture at 24 hours in all experimental groups. These results indicate that hypoxia for hepatocytes may not develop under the present experimental conditions.

In the hepatocytes isolated from chronic alcoholic rats, Et-OH metabolic rates increased. The increase in Et-OH metabolic rates was also clearly observed in the alcohol-pyrazole group, suggesting that the 4-MP insensitive system, namely microsomal ethanol oxidizing system (MEOS), activity increases even in the cultured cells from chronic alcoholic rats. It is well known that Et-OH metabolism increases in chronic alcoholic rats, mainly due to the increase in MEOS activity[10,21]. The present results in the cultured cells were similar to those of an *in vivo* study in chronic alcoholic rats[10,21]. Matsuzaki et al[22] reported that the rates of Et-OH oxidation increased in the isolated hepatocytes from chronic alcoholic rats, compared to those from normal rats. These results suggest that cultured cells maintain the characteristics of hepatocytes in chronic alcoholics for the Et-OH metabolism and that cultured hepatocytes may be able to be used for the study of Et-OH metabolism in chronic alcoholic rats as an *in vitro* model.

Recently, it has been reported that ADH activity in cultured hepatocytes decreased markedly during the early days of the primary cultrue[5,6,9]. Grunnet et al[9] reported that the decrease in ADH activity was related to the concentrations of glucose, amino acids and hormones in the cultured medium and that a decrease in ADH activity was not observed in the medium containing 25 mM glucose. In the present study, ADH and ALDH activities decreased slightly, but not significantly, at 24 and 48 hours of primary culture. The culture medium used in the present study contained 35 mM galactose, instead of glucose. This may be the reason for the slight decrease in enzyme activities in the present experimental system. On the other hand, PNPH activity in the cultured cells decreased significantly and the activity was about 60% of that in isolated cells, suggesting that the mechanism to keep the MEOS activity in cultured cells may be somewhat different from that in ADH and ALDH. However, metabolic analysis of Et-OH in the present study suggested that the difference of the MEOS activity between chronic alcoholic rats and normal rats was still kept in the cultured cells.

It has been proposed that Ac-CHO produced from Et-OH may be mainly metabolized in the liver, because very low blood Ac-CHO levels were observed compared to its Et-OH levels[3,4]. However, the hepatic share of Ac-CHO oxidation is less well known. There is no report on direct evaluation of the rate of Ac-CHO oxidation in the liver. Lindros[4] tentatively calculated that over 90% of Ac-CHO from Et-OH might be metabolized in the liver, based on the results of Forsander et al[3], which determined the difference of Ac-CHO and Et-OH levels between the portal and hepatic veins, and the results of Ross[23], which determined hepatic blood flow. In the present study, the degradation percentages of Ac-CHO were 75 to 80%. These values were compatible with the values calculated indirectly in *in vivo* studies[3,4,23], suggesting that cultured hepatocytes may also be able to be used for studies on Ac-CHO metabolism.

Ac-CHO in the medium accumulatd in parallel with the amounts of Et-OH oxidation. Therefore, accumulation of Ac-CHO was greater in the alcohol groups and the pyrazole non-treated groups than in the corresponding control and pyrazole groups. However, AcICR/EDR ratios, which are a parameter of Ac-CHO oxidation, were not different among the 4 groups. Moreover, the calculated degradation percentages of Ac-CHO in the hepatocytes were also not different among the 4 groups. These results indicate that the oxidation rates of Ac-CHO in the hepatocytes were the same in all groups under the present experimental conditions.

We reported that the degradation of Ac-CHO produced by the non-alcohol dehydrogenase

(non-ADH) pathway, probably by MEOS, may be slower than that produced by the ADH pathway in the *in vivo* study[24]. However, these results were not supported by the present *in vitro* study, because the degradation percentages of Ac-CHO in the hepatocytes were not different between the 4-MP treated and non-treated groups. We have reported that Ac-CHO added to the culture medium of hepatocytes metabolized quickly[25]. Therefore, the possibility that in the present experimental system Ac-CHO flowed out into the medium, and was perhaps re-utililized for oxidation, should be considered. Another possibility that the decrease in PNPH activity in the cultured cells may make unclear the difference between Et-OH metabolism through the ADH and non-ADH pathways, should be also considered. In the present study, no difference of oxidation of Ac-CHO produced by the two different pathways was detected, indicating that some device to prevent re-utilization of Ac-CHO for its oxidation in the hepatocytes, such as trapping of Ac-CHO in the medium, and prevention of the decrease in PNPH activity in cultured hepatocytes may be needed to detect such a delicate difference.

References

1. Lieber CS: Metabolism of ethanol. Medical Disorders of Alcoholism: Pathogenesis and Treatment. Chap. 1. WB Saunders Company, Philadelphia. 1982;1-42
2. Winkler K, Lindquist F, Tygstrup N: The hepatic metabolism of ethanol in patients with cirrhosis of the liver. Scand J Clin Lab Invest 1969;23:59-69
3. Forsander OA, Hillbom ME, Lindros KO: Influence of thyroid function on the acetaldehyde level of blood and liver of intact rats during ethanol metabolism. Acta Pharmacol 1969;27:410-416
4. Lindros KO: Acetaldehyde oxidation and its role in the overall metabolic effects of ethanol in the liver. Lindquist F, Tygstrup N, ed. Regulation of Hepatic Metabolism: Proceeding of the Alfred Benson Symposium VI, Copenhagen 1973, Munksgarrd, Copenhagen. 1974;417-432
5. Lad PJ, Shier WT, Skelly H, et al: Adult rat hepatocyte in primary culture. VI. Developmental changes in alcohol dehydrogenase activity and ethanol conversion during the growth cycle. Alcoholism: Clin Exp Res 1982;6:64-71
6. Dich J, Vind C, Grunnet N: Long-term culture of hepatocytes: Effect of hormones on enzyme activities and metabolic capacity. Hepatology 1988;8:39-45
7. Crabb DW, Li TK: Expression of alcohol dehydrogenase in primary monolayer cultures of rat hepatocytes. Biochem Biophys Res Commun 1985;128:12-17
8. Mezey E, Potter JJ, Diel AM: Depression of alcohol dehydrogenase activity in rat hepatocyte culture by dihydrotestosterone. Biochem Pharmacol 1986;35:335-379
9. Grunnet N, Vind C, Dich J: Maintenance of alcohol dehydrogenase activity in long-term culture of hepatocytes from female rat. Alcoholism: Clin Exp Res 1989;13:25-28
10. Kanayama R, Takase S, Matsuda Y, et al: Effect of dietary fat upon ethanol metabolism in rats. Biochem Pharmacol 1984;33: 3283-3287
11. Seglen PO: Preparation of isolated rat liver cells. Methods in Cell Biology 1976;13:29-83
12. Odashima S, Sturgess JM, Rothstein A: Studies with mature rat hepatocyte in monlayer culture. Cell Tis Res 1976;169:167-168
13. Eriksson CJP, Mizoi Y, Fukunaga T: The determination of acetaldehyde in human blood by the perchloric acid precipitation method: The characterization and elimination of artefactual acetaldehyde formation. Anal Biochem 1982;125:259-263
14. Olsen C: An enzymatic fluorometric method for determination of acetaldehyde, ß-hydroxybutyrate, pyruvate, and lactate. Clin Chim Acta 1971;33:293-300
15. Koop DR: Hydroxylation of p-nitrophenol by rabbit ethanol inducible cytochrome p450 isozyme 3a. Mol Pharmacol 1986;29: 399-404
16. Renke LA, Moyer MJ: P-nitrophenol hydroxylation. A microsomal oxidation which is highly inducible by ethanol. Drug Metab Dispos 1985;13:548-552
17. Bonnichen RK, Brink NG: Liver slcohol dehydrogenase. Methods enzymol 1 Academic Press, New York. 1955;495-500
18. Sugata K, Takada A, Takase S, et al: Determination of aldehyde dehydrogenase isozyme activity in human liver. Alcohol 1988;5:39-43
19. Labarca C, Paigen K: A simple, rapid and sensitive DNA assay procedure. Anal Biochem 1980;102:344-352
20. Lowry OH, Rosenbrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-275
21. Lieber CS, DeCarli L: Ethanol oxidation by hepatic microsome: Adaptive increase after ethanol feeding. Science 1968;162: 917-918
22. Matsuzaki S, Gordon E, Lieber CS: Increased ADH independent ethanol oxidation at high ethanol concentration in isolated rat hepatocytes: The effect of chronic ethanol feeding. J Pharm Exp Ther 1981;217:133-137
23. Ross BD: Perfusion Techniques in Biochemistry. Claredon Press, Oxford. 1972;145
24. Yasuhara M, Matsuda Y, Takada A: Degradation of acetaldehyde produced by the nonalcohol dehydrogenase pathway. Alcoholism: Clin Exp Res 1986;10:545-549
25. Kawahara H, Matsuda Y, Takada A: Effects of ethanol on the microtubules of cultured rat hepatocytes. Alcohol & Alcoholism 1987; Suppl 1:307-311