*Biochemical Pharmacology*, Vol. 38, No. 4, pp. 677–684, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00


# GLUCOCORTICOID HORMONES PREVENT THE INDUCTION OF γ-GLUTAMYL TRANSPEPTIDASE BY ETHANOL IN A RAT HEPATOMA CELL LINE

R. BAROUKI,*† N. PERROT,‡ J. BOUGUET,* M. N. CHOBERT,* V. TOFFIS,§ M. PAVÉ-PREUX,* C. S. YANG,‖ P. BEAUNE‡ and J. HANOUNE*

* INSERM U-99, hôpital Henri Mondor, 94010 Créteil; ‡ INSERM U-75, hôpital Necker Enfants Malades, 156, rue de Vaugirard, 75015 Paris; § Laboratoire de Toxicologie, hôpital Henri Mondor, 94010 Créteil, France; and ‖ New Jersey Medical School, Department of Biochemistry, 185 South Orange Av., Newark, NJ 07103, U.S.A.

(*Received* 3 *May* 1988; *accepted* 31 *August* 1988)

**Abstract**—The increase in serum γ-glutamyl transpeptidase (GGT) is a well known marker of chronic alcoholism in man. We have previously shown that ethanol (180 mM) induces GGT activity 2–3-fold in the $C_2$ rat hepatoma cell line. In this study, we have analyzed the interaction of ethanol with steroid hormones and drugs in this well defined cell culture system. Dexamethasone (100 nM), a synthetic glucocorticoid agonist, completely prevented the induction of GGT by ethanol, but had no effect when added alone. This inhibitory effect was also observed with other corticosteroids, but not with sex steroids; it was prevented by RU 486, a glucocorticoid antagonist. These observations suggest that dexamethasone acts through a high affinity glucocorticoid receptor. Conversely, ethanol did not interfere with the glucocorticoid induction of alanine aminotransferase in the same cell. We have analyzed the metabolism of ethanol in the $C_2$ cells. These cells lack significant alcohol dehydrogenase activity as well as any cytochrome P-450 Alc immunoreactivity. Dexamethasone did not modify the disappearance of ethanol in the culture medium of those cells. We conclude that glucocorticoid hormones interact with ethanol at the cellular level, and that this interaction does not involve a modification of alcohol metabolism.

Alcohol alters the functions of fetal as well as adult hepatocytes through a variety of ways. Most of the metabolic consequences of alcohol consumption can be attributed to the oxidation of ethanol which perturbs the NADH/$NAD^+$ ratio in the cell [1, 2]. The toxicity of alcohol is primarily due to its first metabolite, acetaldehyde, which causes lipid peroxidation and cell death [1]. Ethanol also perturbs the fluidity of the plasma membrane and thus modulates the activity of some membrane-bound enzymes [3, 4]. The increase in other enzyme activities has been attributed to protein induction. The actual mechanism by which ethanol increases the number of protein molecules of such enzymes as cytochrome P-450 [5], glucose-6-phosphate dehydrogenase [6] and γ-glutamyl transpeptidase (GGT)¶ [7] remains poorly understood. Finally, ethanol alters cell functions by interacting with drugs, hormones or neurotransmitters [2, 8]. Two different types of interactions can be distinguished. Ethanol can act directly at the receptor site of the drug or at an enzyme mediating the drug's effects as described for the Gaba receptor [9] and the adenylate cyclase system [10] in the nervous system. Alternatively ethanol can modify the metabolism of the drug in the liver which could possibly enhance its toxicity [2].

In order to investigate the mechanism by which ethanol modifies cell functions, animal models [1, 11] as well as cell culture systems [5, 12] have been developed. We have focused on the study of protein induction by ethanol in a number of cell lines derived from the Reuber H35 rat hepatoma [7]. The induction of GGT was used as a test for sensitivity to ethanol. The $C_2$ cell line was thus selected as its GGT activity is increased 2–3-fold following ethanol exposure [7]. In another study using 2 D gel analysis, we have shown that the number of proteins, the synthesis of which is modified by ethanol, is very limited [13]. This reflects the specificity of ethanol effects in this particular cell line.

We have used this *in vitro* system to investigate the interaction of ethanol with drugs and hormones at the cellular level. In the present study we report the interaction of alcohol with glucocorticoid hormones. These hormones specifically inhibit the induction of GGT by ethanol and this effect is probably not mediated by a modification of the metabolism of ethanol in the $C_2$ cell line.

## MATERIALS AND METHODS

### *Chemicals*

[$^{14}CH_3$]NDMA (8 mCi/mmole) was obtained from Amersham (U.K.). NDMA and NADPH were

† To whom correspondence should be addressed.

¶ Abbreviations used: GGT, γ-glutamyl transpeptidase; ALAT, alanine aminotransferase; PBS, phosphate buffered saline; NMDA, *N*-nitrosodimethylamine; Na Dod $SO_4$, sodium dodecyl sulfate; NADPH, nicotinamide adenine dinucleotide phosphate; P-450 Alc, P-450 j = P-450 IIE1.

purchased from Boehringer (Mannheim, F.R.G.). Electrophoresis products were from Serva (Heidelberg, F.R.G.) and peroxydase conjugated immunoglobulins from Dako (Copenhagen, Denmark). L-γ-Glutamyl-paranitroanilide, glycylglycine, dexamethasone, and dimedon were from Sigma Chemical Co. Dimilume was from Packard (Caversham, U.K.). Ethanol was from Merck (réf. 983).

*Antibodies*

Anti-rat cytochrome P-450 Alc immunoglobulins were raised in rabbit as described by Patten *et al.* [14]. Anti rat cytochrome P-450 UTA, PB-B, ISF-G, BNF-B, PB-C, PCN-E antisera were obtained in rabbit as described by Guengerich *et al.* [15]. They were used after a 1/200 dilution.

*Cell culture and ethanol treatment*

The $C_2$ cells derived from line $H_4IIEC_3$ of the Reuber H35 hepatoma were grown in a modified Ham's $F_{12}$ medium (GIBCO, Uxbridge, U.K.) supplemented with 5% fetal calf serum (Flow Lab., Irvine, Scotland), 200 units/ml of penicillin, 50 μg/ml of streptomycin (Diamant, Puteaux, France), 0.5 μg/ml of fungizone (Squibb, Princeton, U.S.A.). Cells were routinely cultured as previously described, in a 7% $CO_2$ humidified atmosphere at 37°, on Falcon plastic petri dishes; $2.5 \times 10^4$ to $10^5$ cells and $10^5$ to $10^6$ cells were seeded per 6 cm and 10 cm diameter petri dishes respectively. Experiments were performed during exponential phase of growth. For ethanol and steroid treatment, culture medium was removed and fresh medium containing any of the various steroids with or without ethanol was added for 48 hr. We previously mentioned that GGT induction was not modified whether the plates were wrapped or not in parafilm to avoid ethanol evaporation [7].

*Microsome preparation*

The $C_2$ cells (100 millions) were washed twice with PBS, then scraped and centrifuged at 300 *g*. The pellet was resuspended in 5 ml of buffer (0.25 M sucrose, 10 mM Tris–HCl (pH 7.5), 1 mM EDTA) and homogenized in a tight Dounce homogenizer on ice (40 strokes). The homogenate was centrifuged for 20 min at 13,500 *g* at 4° (SS 34 rotor, Sorvall). The supernatant was collected and centrifuged for 60 min at 105,000 *g* at 4° (Ti 50 rotor, Beckman). The final pellet was then resuspended in 0.3 ml of sodium phosphate buffer 100 mM, pH 7.5 containing 20% glycerol, 10 mM $MgCl_2$, and stored in aliquots at −80°.

*Enzyme assays*

*Gamma-glutamyl transpeptidase.* At each time point, control and ethanol treated cells were washed with phosphate buffered saline (PBS) at 4°, scraped, centrifuged at 300 *g* and resuspended in PBS. GGT activity was determined according to the method of Orlowski *et al.* as previously described [16] using L-γ-glutamyl-paranitroanilide and glycylglycine as substrates. Proteins were estimated by the method of Lowry using bovine serum albumin as standard [17]. Activity was expressed as mU/mg protein. One mU corresponds to 1 nmol paranitroanilide formed per min.

*Alanine aminotransferase.* Cell homogenates were prepared by incubating the cells in PBS containing 0.5% Triton X 100 for 15 min at 37°. Alanine aminotransferase was assayed using a kit distributed by Roche.

*Alcohol dehydrogenase.* The enzyme was assayed on a cell homogenate as described by Arslanian *et al.* [18].

*N-nitrosodimethylamine (NDMA) demethylase activity.* NDMA demethylase activity was determined by the radioactive method of Hutton *et al.* [19] modified by Hawke *et al.* [20]. The assay mixture contained 0.1–0.2 mg of microsomal protein, 100 mM potassium phosphate buffer, 6 mM $MgCl_2$, 1 mM NADPH and 1 mM [$^{14}$C]NDMA (8 mCi/mmol) in a final volume of 0.5 ml. Controls contained no NADPH. The mixture was incubated for 10 min at 37° in a shaking water bath in capped tubes and the reaction was stopped by the addition of 0.3 ml of 1 M sodium acetate, pH 4.5, on ice. Twenty microliters of 0.1 M HCHO and 1 ml of 10 mM dimedon were added. The tubes were then placed in a boiling water bath for 5 min. After cooling the samples, 0.8 ml water and 4 ml hexane were added. The tubes were mixed and centrifugated for 10 min. Three milliliters of the hexane layer were removed, placed in scintillation counting glass vials and evaporated under a flow of nitrogen. The samples were counted in dimilume to avoid chemioluminescence. Activity was expressed as mU/mg protein. One mU corresponds to 1 nmol HCHO formed/min.

*"Western blots" analysis*

Microsomes from isoniazid treated rats, or from $C_2$ cells treated as indicated, were electrophoresed on Na Dod $SO_4$/polyacrylamide gels (9%) according to Laemmli [21]. The separated proteins were electrotransferred to nitrocellulose sheets as described by Guengerich *et al.* [22]. Nitrocellulose sheets were blocked for 60 min at 37° by 3% serum albumin and 10% fetal calf serum in a phosphate buffer (135 mM NaCl, 15 mM $KH_2PO_4$, 81 mM $Na_2HPO_4$, 27 mM KCL) and then incubated with antirat cytochrome P-450 antibodies overnight at 4° (anti-P-450 Alc immunoglobulins were diluted to about 5 μg IgG/ml). The nitrocellulose sheets were washed six times with phosphate buffer containing 0.2% Tween 20 and then incubated at room temperature for 30 min in phosphate buffer containing a 1/250 dilution of peroxidase conjugated swine immunoglobulins raised against rabbit immunoglobulins. Finally, the sheets were washed six times with phosphate buffer and the peroxidase activity was detected with 4-chloro-1-naphtol and $H_2O_2$ as previously described [23].

*Ethanol assay*

Ethanol was assayed in aliquots of the culture medium by gas chromatography [24]. The aliquots were previously centrifuged 10 min at 10,000 *g* to eliminate lysed cells.

## RESULTS

### *Dexamethasone abolishes ethanol induction of GGT*

Gamma-glutamyl transpeptidase activity in the $C_2$ cell line increased 2–3-fold following a 48 hr exposure to ethanol [7]. As shown in Fig. 1, dexamethasone prevented the induction of GGT by ethanol in a dose-dependent manner, when both drugs were added together. Half maximal inhibition was observed at 2 nM and essentially complete inhibition was achieved at 100 nM. Dexamethasone alone had no significant and reproducible effect on basal GGT activity, although a very slight increase was sometimes observed at the highest concentrations.

### *Dexamethasone acts through a specific glucocorticoid receptor*

Various steroids (0.5 $\mu$M except for dexamethasone, 0.1 $\mu$M) were added alone or with 180 mM ethanol to the $C_2$ cells. Gamma-glutamyl transpeptidase activity was measured after a 48 hr incubation. The results shown in Fig. 2 indicate that only dexamethasone and corticosterone had an inhibitory effect. Progesterone inhibited very slightly the induction of GGT by alcohol whereas testosterone and estradiol had no effect. The drug RU 486, a well known glucocorticoid antagonist [25], had no effect by itself, but prevented the inhibitory action of dexamethasone. The relative potency of these various steroids as well as the antagonistic action of RU 486 point to a glucocorticoid receptor mediated effect.

A glucocorticoid receptor was shown to be present in the $C_2$ cells [26]. We have checked that this receptor was functional under our experimental conditions by looking for the induction of alanine aminotransferase (ALAT) by glucocorticoids [27]. Figure 3 shows a dose–response curve of the transaminase activity as a function of dexamethasone concentrations. Dexamethasone induced the transaminase activity 5–6-fold with a half-maximal increase at around 2 nM. In fact, the extent of ALAT induction parallels the extent of inhibition of the alcohol effect on GGT (compare Figs 1 and 3). Interestingly, the addition of 1% ethanol to the various concentrations of dexamethasone did not modify the action of the glucocorticoid on ALAT activity. All the above results indicate that glucocorticoids specifically inhibit the induction of GGT by alcohol whereas alcohol does not modify other glucocorticoid effects.

In Fig. 4, we show that a 48 hr preincubation of the cells with 10 nM dexamethasone does not prevent the induction of GGT by 1% ethanol. Only when dexamethasone was added together with ethanol did it prevent the alcohol effect.

### *Does dexamethasone act by modifying the metabolism of ethanol?*

We have looked for the presence of the two most important *in vivo* metabolic pathways of alcohol, namely alcohol dehydrogenase and cytochrome P-450 dependent oxidase [28]. Confirming previous characterization of liver specific enzymes in the $C_2$

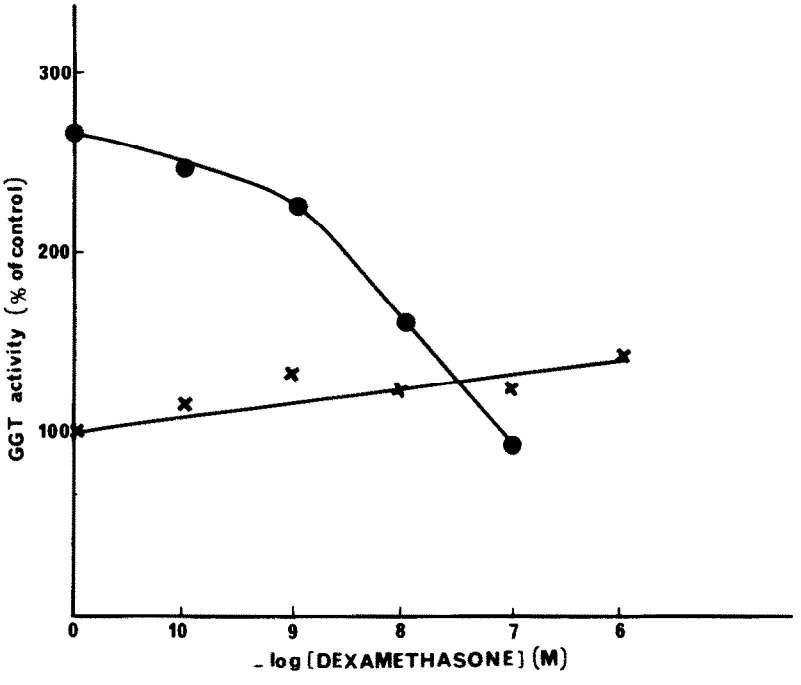


Fig. 1. Dexamethasone dose–response curve on control and ethanol treated cells. Dexamethasone was dissolved in ethanol at a concentration of 2 mM and was further diluted in culture medium. Cell culture was carried out as described in Materials and Methods. Various concentrations of dexamethasone were added to the cells with (●) or without (×) 180 mM ethanol. Ethanol and dexamethasone were added concomitantly. After a 48 hr incubation, the cells were washed, scraped and assayed for GGT activity. The assay was run in duplicate. The experiment was repeated three times with similar results. One hundred per cent activity corresponded to 1.6 mU/mg protein.

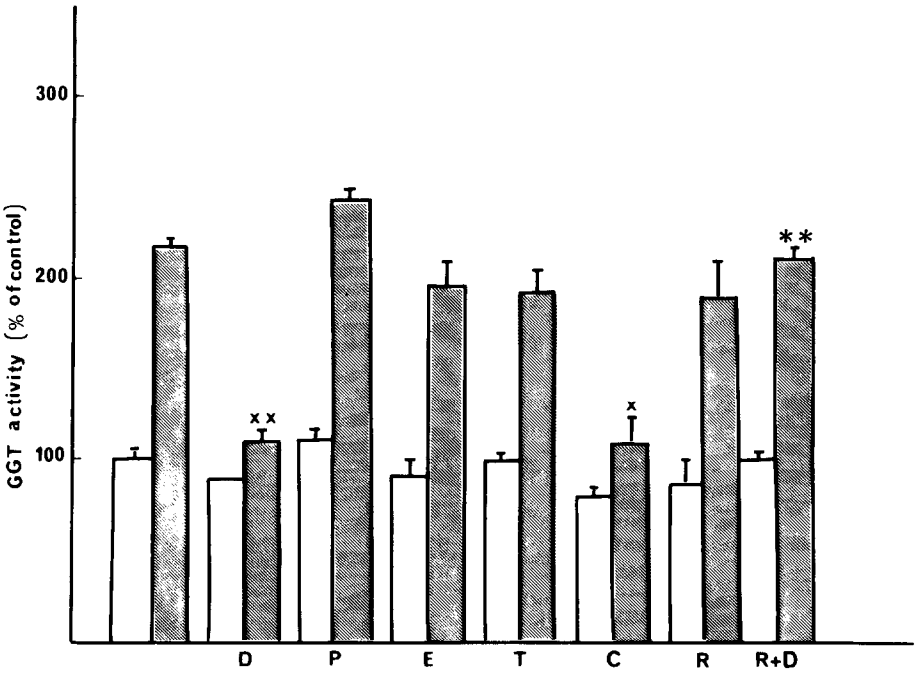


Fig. 2. Effect of various steroids on induction of GGT activity by ethanol. All steroids were used at a concentration of 0.5 $\mu$M except dexamethasone which was at a concentration of 0.1 $\mu$M. Ethanol (180 mM) and the various steroids were added at the same time and the GGT activity was measured after an incubation of 48 hr. Each point is the average ± SEM of four independent determinations. For statistical significance the Mann–Whitney U test was used: $^{xx}$ $P < 0.01$ when compared to ethanol treated cells; $^{x}$ $P < 0.05$ when compared to ethanol treated cells; ** $P < 0.01$ when compared to ethanol + dexamethasone treated cells. Dark bars represent activities in cells treated with ethanol. D, dexamethasone; P, progesterone; E, estradiol; T, testosterone; C, corticosterone; R, RU 486; 100% activity corresponded to 3.17 mU/mg protein.

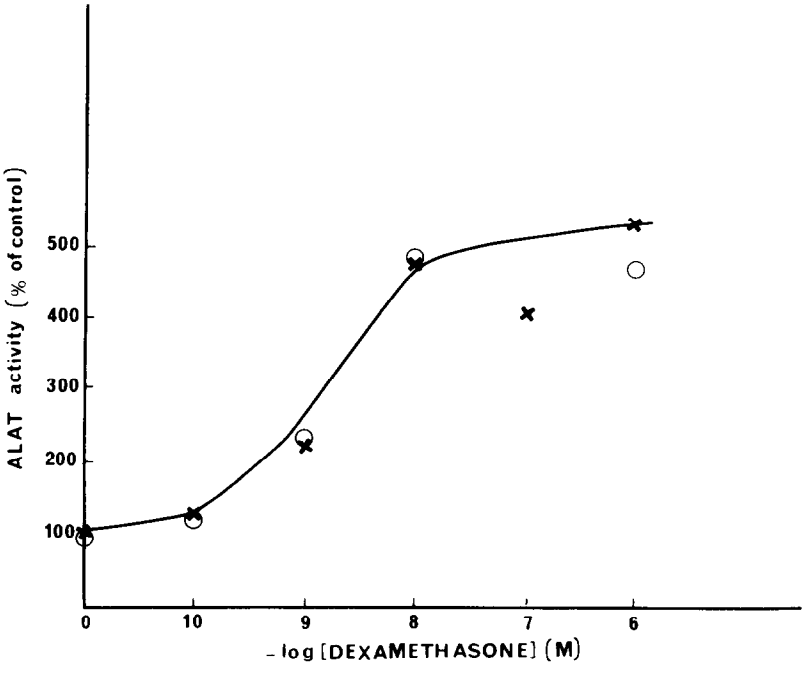


Fig. 3. Effect of dexamethasone on alanine aminotransferase in $C_2$ cells. Various concentrations of dexamethasone were added in the absence (○) or the presence (×) of 180 mM ethanol to the $C_2$ cells under the same conditions as those described in the legend of Fig. 1. One hundred per cent activity corresponded to 0.13 U/mg protein.

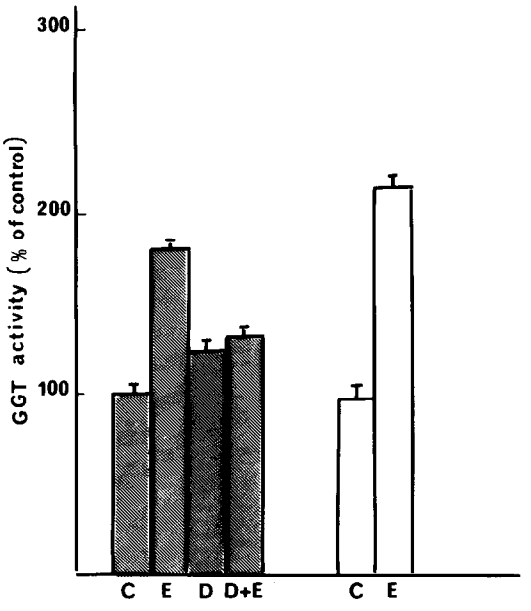


Fig. 4. Effect of preincubation with dexamethasone. Cells were either preincubated (dark bars) or not (empty bars) with 10 nM dexamethasone for 48 hr. They were then washed and fresh medium was added alone (control: C), with 180 mM ethanol (E), 10 nM dexamethasone (D), or both 180 mM ethanol and 10 nM dexamethasone (DE). After an incubation of 48 hr, cellular GGT activity was assayed. Each point is the average ± SEM of four independent determinations. One hundred per cent activity correspond to 1.4 mU/mg proteins.

Table 1. Disappearance of ethanol from the cell medium of $C_2$ cells

| | Time (hr) 0 | 6 | 24 | 48 |
|---|---|---|---|---|
| Ethanol 8.8 g/l (191 mM) | | | | |
| Medium alone | 8.8 | 6.8 | 3.4 | 2.3 |
| Medium + cells | | 7.2 | 3.7 | 1.9 |
| Medium + cells + dexamethasone | | 6.3 | 3.9 | 2 |
| Ethanol 1.4 g/l (30 mM) | | | | |
| Medium alone | 1.4 | 1.5 | 0.8 | 0.6 |
| Medium + cells | | 1.0 | 0.7 | 0.7 |
| Medium + cells + dexamethasone | | 1.3 | 0.9 | 0.6 |

Ethanol concentrations are expressed in g/l; dexamethasone was used at a concentration of 0.1 μM. Each value is the average of two independent determinations.

cells [27], we have not been able to detect alcohol dehydrogenase activity in this cell line under conditions where such an activity was found in isolated hepatocytes (8.2 mU/mg protein in rat hepatocytes and less than 0.4 mU/mg protein in $C_2$ cells). Alcohol dehydrogenase activity was still undetectable when cells were incubated with 1 μM dexamethasone or 180 mM of ethanol.

A possible cytochrome P-450 mediated oxidation of alcohol was assessed using two independent techniques. Microsomes were prepared from control, glucocorticoid and alcohol treated cells. Samples containing total microsomes were run on polyacrylamide gels, blotted on nitrocellulose filters and incubated with anticytochrome P-450 Alc antibody. Cytochrome P-450 Alc is responsible for the microsomal metabolism of ethanol. The results of such Western blots are shown in Fig. 5. While a cytochrome P-450 Alc band was clearly detected in rat liver, it was not found in microsomes from treated or control $C_2$. Moreover, P-450 Alc was not induced by other drugs such as phenobarbital and 3-methylcholanthrene (Fig. 5). In addition, control and treated $C_2$ cell microsomes were also tested using other antibodies with different specificities (UT-A, PB-B, PB-C, BNF-B, PCN-E) and no band corresponding to any of these cytochromes P-450 was found (data not shown). In the same experiments, a specific band was observed in microsomes isolated from isoniazid treated rats (isoniazid is a selective inducer of P-450 Alc [29]). Finally, a band corresponding to epoxide hydrolase, a marker of microsomes was detected in $C_2$ cell microsomes (data not shown). A functional assay for cytochrome P-450 Alc based on the measurement of NDMA oxidation by microsomes confirmed the previous result. Indeed, NDMA metabolism was recently shown to be mediated by cytochrome P-450 Alc [30]. An activity of 0.6 mU/mg of proteins was detected in normal liver and essentially no activity (less than 0.05 mU/mg of protein) was found in the $C_2$ cells under any of the conditions tested.

Finally we have measured the disappearance of ethanol (191 mM or 30 mM initially) in the culture medium of cells treated or not with dexamethasone. The rate of decrease in ethanol concentration in the medium was essentially similar with or without cells (Table 1) which indicates that the metabolism of ethanol by the cells, if present, is extremely poor. In the presence of dexamethasone the rate of decrease in ethanol concentration was not significantly modified (Table 1).

## DISCUSSION

The present study has allowed us to demonstrate a direct interaction between ethanol and glucocorticoids at the cellular level. In rat liver, independent studies have shown that both alcohol and glucocorticoids induce GGT activity [31, 32]. In the Fao cell line, GGT activity is induced only by glucocorticoids [16], whereas, in the $C_2$ cells, ethanol but not glucocorticoids induce GGT activity [7]. In fact, glucocorticoids prevent the induction of GGT by ethanol in the $C_2$ cells. Such an interaction has not been studied *in vivo*. Such *in vivo* studies would be difficult to interpret since both drugs modify basal GGT activity. In contrast, the absence of glucocorticoid hormones effect on basal GGT activity in the $C_2$ cells has probably allowed us to uncover their action on the induction of the enzyme by ethanol. The inhibitory effect of glucocorticoids is specific and is mediated by a classical glucocorticoid receptor. This is in contrast to the induction by dexamethasone of other drug metabolizing enzymes such as cytochrome P-450 PCN [33, 34] and digi-

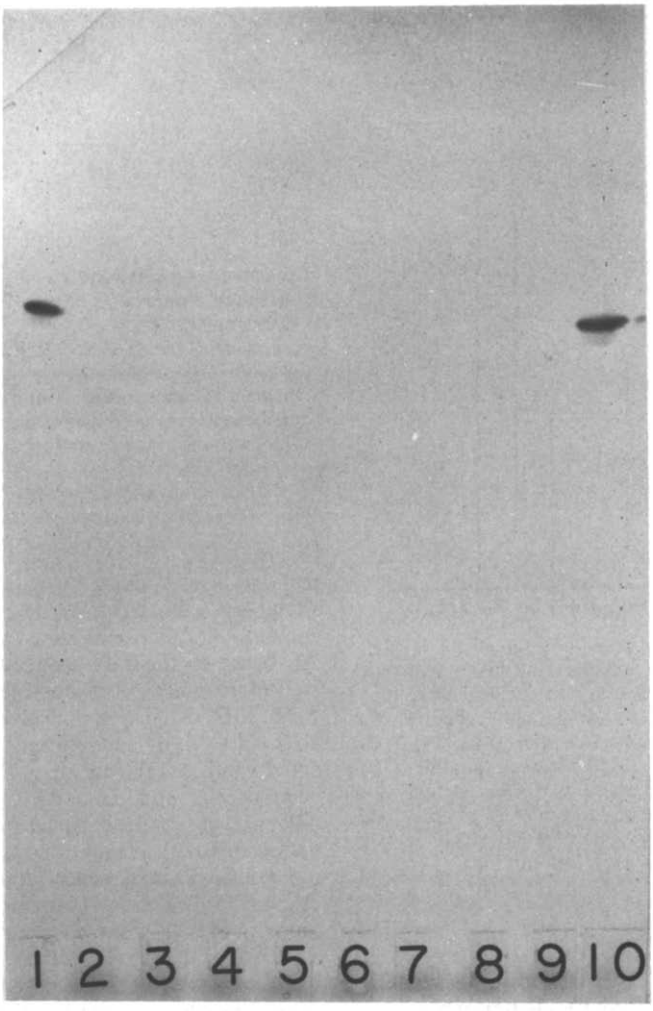


Fig. 5. "Western blots" of microsomes from $C_2$ cells or rat liver. After electrophoresis and transfer the nitrocellulose was incubated with anticytochrome P-450 Alc immunoglobulins which were stained as described in Materials and Methods. 1, 10, Microsomes from isoniazid treated rats (1 $\mu$g, 5 $\mu$g protein); 2, microsomes from control $C_2$ cells (20 $\mu$g protein); 3, microsomes from ethanol treated cells (20 $\mu$g protein); 4, microsomes from dexamethasone treated cells (20 $\mu$g protein); 5, microsomes ethanol + dexamethasone treated cells (20 $\mu$g protein); 6, microsomes from control $C_2$ cells (20 $\mu$g protein); 7, microsomes from phenobarbital treated cells (20 $\mu$g protein); 8, microsomes from phenobarbital + ethanol cells (20 $\mu$g protein); 9, microsomes from 3-methylcholanthrene cells (20 $\mu$g protein); 2–5 and 6–9 were from two different preparations. Similar results were obtained with 50 $\mu$g protein. The sensitivity of the technique allows us to conclude that $C_2$ cells microsomes contained less than 10 pmoles of each P-450 tested per mg protein.

toxigenin monodigitoxoside UDP-glucuronosyltransferase [35], which is observed at high concentrations of the steroid and is mimicked by steroids with antiglucocorticoids activity such as pregnenolone 16 $\alpha$-carbonitrile. Moreover, glucocorticoids do not act by modifying the metabolism of ethanol in the $C_2$ cell and, particularly, they do not induce alcohol dehydrogenase or cytochrome P-450 mediated alcohol oxidation. This metabolism-independent interaction appears to be quite different from other drug interactions particularly those involving ethanol [2]. Indeed, acute ethanol ingestion results in competition with other drugs for microsomal metabolism. In addition, chronic exposure to a variety of drugs including ethanol induce the microsomal metabolism responsible for the oxidation of alcohols and other xenobiotics [36].

Two possibilities for glucocorticoid action can be distinguished. First, glucocorticoids could act by modifying the availability of ethanol to the cell. We have already mentioned that glucocorticoids do not modify the metabolism of ethanol. We cannot eliminate the possibility that these hormones could modify the entry of ethanol in the cells. However, it should be noted that the induction of GGT in the $C_2$ cell by another drug, phenobarbital, is also inhibited by glucocorticoids (unpublished data). Thus, for this hypothesis to be true, glucocorticoids should be able to alter the availability of both alcohol and barbiturates.

The second possibility is that glucocorticoids could specifically interfere with the process of GGT induction by ethanol. In fact, as mentioned above, glucocorticoids induce GGT activity in rat liver and in a highly differentiated hepatoma cell line, Fao [16]. Their inability to induce GGT in $C_2$ cells is due neither to the absence of a functional glucocorticoid receptor, nor to the presence of a high constitutive expression of GGT in this cell line. Indeed, GGT activity in $C_2$ cells is similar to that of Fao cells and of adult rat liver [7], but much smaller than that found in undifferentiated liver cells. Despite their inability to modify basal GGT activity in $C_2$ cells, glucocorticoids still influence GGT expression since they prevent its induction by ethanol and phenobarbital. A hypothetical model accounting for these observations is that the glucocorticoid receptors would be able to bind to the GGT gene in $C_2$ cells without inducing the transcription of the gene but preventing its induction by other factors. In Fao cells, the binding of the receptor to the GGT gene would be efficient and would increase transcription as has been described for other glucocorticoid regulated genes [37].

In the course of the study of the interaction between ethanol and glucocorticoids, we have been able to shed some light on the mechanism of GGT induction by ethanol. Indeed, not only have we shown that dexamethasone does not modify ethanol metabolism, but it also appears that $C_2$ cells do not metabolize ethanol to a significant degree. Therefore the induction of GGT by ethanol is not dependent on the metabolism (or on the metabolites) of this drug in the cell.

*Acknowledgements*—We wish to thank Pr. F. P. Guengerich for kindly providing some of the cytochrome P-450 antigens and antibodies and Drs. P. Berthelot, B. Nalpas, G. Guellaën, Y. Laperche, D. Stengel, N. Ferry, and S. Pol for critical reading of this manuscript, and Ms L. Rosario for excellent secretarial assistance.

This work was supported by the Institut National de la Santé et de la Recherche Médicale, the Délégation Générale à la Recherche Scientifique et Technique, the Université Paris-Val-de-Marne and the Haut Comité de l'Etude et d'Information sur l'alcoolisme.

## REFERENCES

1. Lieber CS, Alcohol and the liver, 1984 update. *Hepatology* **4**: 1243–1260, 1984.
2. Koop DR and Coon MJ, Ethanol oxidation and toxicity: role of alcohol P-450 oxygenase. *Alcoholism Clin Exp Res* **10**: 445–495, 1986.
3. Dawidowicz EA, The effect of ethanol on membranes. *Hepatology* **5**: 697–699, 1985.
4. Gonzalez-Calvin JL, Saunders JB, Crossley IR, Dickenson CJ, Smith HM, Tredger JM and Williams R, Effects of ethanol administration on rat liver plasma membrane-bound enzymes. *Biochem Pharmacol* **34**: 2685–2689, 1985.
5. Sinclair JF, Sinclair PR, Smith EL, Bement WJ, Pomeroy J and Bonkowsky H, Ethanol mediated increase in cytochrome P-450 in cultured hepatocytes. *Biochem Pharmacol* **30**: 2805–2809, 1981.
6. Kelley DS and Kletzien RF, Ethanol modulation of the hormonal and nutritional regulation of glucose-6-phosphate dehydrogenase activity in primary cultures of rat hepatocytes. *Biochem J* **217**: 543–549, 1984.
7. Barouki R, Chobert MN, Finidori J, Aggerbeck M, Nalpas B and Hanoune J, Ethanol effects in a rat hepatoma cell line: induction of γ-glutamyl transferase. *Hepatology* **3**: 323–329, 1983.
8. Teschke R and Gellert J, Hepatic microsomal ethanol-oxidizing system (MEOS): metabolic aspects and clinical implications. *Alcoholism: Clin Exp Res* **10**: 205–325, 1986.
9. Suzdak PD, Schwartz RD, Skolnick P and Paul SM, Ethanol stimulates γ-aminobutyric acid receptor mediated chloride transport in rat brain synaptoneurosomes. *Proc Natl Acad Sci USA* **83**: 4071–4075, 1986.
10. Gordon AS, Collier K and Diamond I, Ethanol regulation of adenosine receptor-stimulated cAMP levels in a clonal neural cell line: an *in vitro* model of cellular tolerance to ethanol. *Proc Natl Acad Sci USA* **83**: 2105–2108, 1986.
11. Alderman J, Takagi T and Lieber CS, Ethanol-metabolizing pathways in Deermice. Estimation of flux calculated from isotope effects. *J Biol Chem* **262**: 7497–7503, 1987.
12. Schanne FAX, Zucker H, Farber JL and Rubin E, Alcohol-dependent liver cell necrosis *in vitro*: a new model. *Science* **212**: 338–340, 1981.
13. Chobert MN, Vincens P, Guellaën G, Barouki R, Laperche Y, Aggerbeck M, Aissani T, Pawlak A, Tarroux P and Hanoune J, Specific modulation by ethanol of the protein synthesis pattern in the $C_2$ rat hepatoma cell line. *J Hepatol* **6**: 85–93, 1988.
14. Patten CJ, Ning SM, Lu AYH and Yang CS, Acetone-inducible cytochrome P-450. Purification catalytic activity and interaction with cytochrome bs. *Arch Biochem Biophys* **251**: 629–638, 1986.
15. Guengerich FP, Danan GA, Wright ST, Martin MV and Kaminsky LS, Purification and characterization of liver microsomal cytochromes P-450: electrophoretic, spectral, catalytic and immunochemical properties and inducibility of eight isozymes isolated rats treated with phenobarbital or beta-naphtoflavone. *Biochemistry (USA)* **21**: 6019–6030, 1982.
16. Barouki R, Chobert MN, Billon MC, Finidori J, Tsapis R and Hanoune J, Glucocorticoid hormones increase the activity of γ-glutamyl transferase in a highly differentiated hepatoma cell line. *Biochim Biophys Acta* **721**: 11–21, 1982.
17. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
18. Arslanian MJ, Pascoe E and Reinhold JG, Rat liver alcohol dehydrogenase: purification and properties. *Biochem J* **125**: 1039–1047, 1971.
19. Hutton JJ, Meier J and Hackney C, Comparison of the *in vitro* mutagenicity and metabolism of dimethylnitrosamine and benzopyrene in tissues from inbred mice treated with phenobarbital, 3-methylcholanthrene or polychlorinated biphenyls. *Mutat Res* **66**: 75–94, 1979.
20. Hawke RL and Welch RM, Major differences in the specificity and regulation of mouse renal cytochrome P-450 dependent monooxygenases. *Mol Pharmacol* **27**: 283–289, 1985.
21. Laemmli UK, Cleavage of structural properties during the assembly of the head of bacteriophage $T_4$. *Nature (Lond)* **227**: 680–685, 1970.
22. Guengerich FP, Wang P and Davidson NK, Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits and humans using immunochemical staining coupled with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Biochemistry (USA)* **21**: 1698–1706, 1982a.
23. Beaune P, Flinois JP, Kiffel L, Kremers P and Leroux JP, Purification of a new cytochrome P-450 from human liver microsomes. *Biochim Biophys Acta* **840**: 364–370, 1985.

24. Dubowski KM, *Methodology for Analytical Toxicology*. Sunshine I, CRC Press, Cleveland, 1975.
25. Chobert MN, Barouki R, Finidori J, Aggerbeck M, Hanoune J, Philibert D and Deraedt K, Antiglucocorticoid properties of RU38486 in a differentiated hepatoma cell line. *Biochem Pharmacol* **32**: 3481–3483, 1983.
26. Venetianer A, Pinter Z and Gal A, Examination of glucocorticoid sensitivity and receptor content of hepatoma cell lines. *Cytogenet Cell Genet* **28**: 280–283, 1980.
27. Deschatrette J and Weiss MC, Characterization of differentiated and dedifferentiated clones from a rat hepatoma. *Biochimie* **56**: 1603–1611, 1974.
28. Nalpas B and Berthelot P, Foie et Alcool: aspects métaboliques. *Gastroenterol Clin Biol* **6**: 85–92, 1982.
29. Ryan DE, Ramanatahn L, Iida S, Thomas PE, Hanin M, Shively JE, Lieber CS and Levin W, Characterization of a major form of rat hepatic microsomal cytochrome P-450 induced by isoniazid. *J Biol Chem* **260**: 6385–6393, 1985.
30. Wrighton SA, Thomas PE, Molowa DT, Hanin M, Strively JE, Maines SL, Watkins PB, Parker G, Mendez-Picon G, Levin W and Guzelian PS, Characterization of ethanol inducible human liver N-nitrosodimethylamine demethylase. *Biochemistry* **25**: 6731–6735, 1986.
31. Nishimura M, Stein H, Berges W and Teschke R, $\gamma$-glutamyl transferase activity in the liver plasma membrane: induction following chronic alcohol consumption. *Biochem Biophys Res Commun* **99**: 142–148, 1981.
32. Billon MC, Dupré G, and Hanoune J, *In vivo* modulation of rat hepatic gamma-glutamyl transferase activity by glucocorticoids. *Mol Cell Endocrinol* **18**: 99–108, 1980.
33. Schuetz EG, Wrighton SA, Barwick JL and Guzelian PS, Induction of cytochrome P-450 by glucocorticoids in rat liver. I evidence that glucocorticoids and pregnenolone 16 $\alpha$-carbonitrile regulate *de novo* synthesis of a common form of cytochrome P-450 in cultures of adult rat hepatocytes and in the liver *in vivo*. *J Biol Chem* **259**: 1999–2006, 1984.
34. Schuetz EG and Guzelian PS, Induction of cytochrome P-450 by glucocorticoids in rat liver: II evidence that glucocorticoid regulate induction of cytochrome P-450 by a non classical receptor mechanism. *J Biol Chem* **259**: 2007–2012, 1984.
35. Schuetz EG, Hazelton GA, Hall J, Watkins PB, Klaassen CD and Guzelian PS, Induction of digitoxigen in monodigitoxoside UDP-glucuronosyltransferase activity by glucocorticoids and other inducers of cytochrome P-450p in primary monolayer culture of adult rat hepatocytes and in human liver. *J Biol Chem* **261**: 8270–8275, 1986.
36. Whitlock JP, The regulation of cytochrome P-450 gene expression. *Ann Rev Pharmacol Toxicol* **26**: 333–369, 1986.
37. Danesch U, Hashimoto S, Renkawitz R and Schutz G, Transcriptional regulation of the tryptophan oxygenase gene in rat liver by glucocorticoids. *J Biol Chem* **258**: 4750–4753, 1983.