would be in agreement with the decrease of dermal and aortic GAG observed in diabetic rats[16,17]. The absence of this effect after treatment of the diabetic rats with E is of interest in view of observations suggesting that the hormone does not only inhibit the synthesis[18,19], but also decreases the degradation of GAG[20].

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3 C.A. Hernberg, Acta physiol. scand. *79*, 351 (1952).
4 S. Forgacz, Radiologe *13*, 167 (1973).
5 R. Silberberg, The Pituitary in Relation to Skeletal Aging and Disease, in: Hypothalamus, Pituitary and Aging. Ed. A.V. Everitt and J.A. Burgess. C.C. Thomas, Springfield, Ill., 1975.
6 R. Silberberg and G. Gerritsen, Diabetes *25*, 477 (1976).
7 J. Menczel, M. Makin and G. Robin, Isr. J. med. Sci. *8*, 918 (1972).
8 J.V. Santiago, W.H. McAlister and S.K. Ratzan, Clin. Endocr. *45*, 845 (1977).
9 P. McNair, S. Madsbad and C. Christiansen, Diabetologia *17*, 283 (1979).
10 R. Silberberg, M. Aufdermaur and J.H. Adler, A.M.A. Archs Path. Lab. Med. *103*, 231 (1979).
11 A.M. Cohen, A. Teitelbaum and R. Saliternik, Metabolism *21*, 235 (1972).
12 A.M. Cohen, Hormone metab. Res. *10*, 85 (1978).
13 M. Silberberg and R. Silberberg, Steroid Hormones and Bone, in: Biochemistry and Physiology of Bone, vol. III, 2nd edn. Ed. G.H. Bourne. Academic Press, New York and London 1972.
14 J.E. Scott and J. Dorling, Histochemie *5*, 221 (1965).
15 I. Pousty and W.F. Butler, Res. Vet. Sci. *25*, 182 (1978).
16 S. Schiller and A. Dorfman, Biochim. biophys. Acta *78*, 373 (1963).
17 M.P. Cohen and V.G. Foglia, Diabetes *19*, 639 (1970).
18 R.E. Priest and R.M. Koplitz, J. exp. Med. *116*, 565 (1962).
19 G. Herbai, Acta physiol. scand. *79*, 351 (1970).
20 J.A. Rosner, V.M. Goldberg and L. Getzy, Arthritis Rheum. *22*, 52 (1979).

Effect of acute and chronic ethanol ingestion on the content of reduced glutathione of various tissues of the rat[1]

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Summary. The effect of acute ethanol ingestion (5 g/kg) by fasted rats, or chronic treatment in fed animals, revealed a significant decrease in the content of reduced glutathione of the liver and kidney. No changes were observed in reduced glutathione levels of the pancreas, intestines, stomach or spleen in the acute model. In this condition, the time course study of the decrease in reduced glutathione levels showed a progressive effect in the liver and a rapid and constant effect in the kidney.

Hepatic reduced glutathione (GSH) seems to play a significant role in the protective mechanisms against the toxic effects of some xenobiotics and/or their metabolites[2]. It has been suggested that GSH depletion could lead to lipoperoxidation and, consequently, to cell damage and lysis[3]. Previous studies by our group have shown that the administration of a single dose of 5 g of ethanol/kg to rats fasted overnight induced a drastic decrease in the content of liver GSH after 6 h of intoxication[4], concomitant with an enhancement of lipoperoxidative processes[4,5]. Since the metabolic and toxic effects of ethanol consumption are not limited to the liver, but occur in several other organs as well[6], it was of interest to study the effect of acute ethanol administration to rats on the content of GSH of tissues such as kidney, pancreas, intestines (jejunum), stomach and spleen, including the liver for comparison. GSH levels in liver and kidney were studied in animals following chronic ethanol ingestion, a situation in which hepatic lipoperoxidation has been found to increase markedly[7–9].

Materials and methods. Studies of the effect of acute ethanol ingestion were carried out in male Wistar rats (Facultad de Medicina Occidente, Universidad de Chile) weighing 150–200 g and fasted overnight (16 h), intubated with 5 g of ethanol/kg as a 40% w/v solution in saline. Control animals received isovolumetric amounts of saline or isocaloric amounts of glucose[4]. Determinations were done after 6 h of treatment in animals kept in a warm environment (25 °C). The time course study of changes in liver and kidney GSH levels was performed in fasted rats given 5 g of ethanol/kg sacrificed after 1, 2, 3, 4, 5 and 6 h of intoxication.

In studies involving chronic ethanol administration, male Wistar rats (Canadian Breeding Laboratories, Quebec) weighing 168.2 ± 1.0 g ($n = 12$) were matched by weight. Half of the animals were given an ethanol-containing liquid diet (composition as percent of total calories: 35% ethanol, 19% protein, 41% fat and 5% carbohydrate) and the other half received a liquid diet in which ethanol was replaced isocalorically by sucrose[10], for 4 weeks. The caloric intake was 310 ± 4 kcal/kg/day and the ethanol consumption was 15.5 ± 0.2 g/kg/day ($n = 21$ determinations in 6 pairs of rats). In order to minimize possible differences in the time of food intake preceding the experiments, which could alter GSH levels[4], 1/3 of the diet consumed by the ethanol-treated rat the day before was given at 14.00 h to the corresponding pair of animals and the remaining 2/3 at 21.00 h. On the following day, 09.00 h, the animals were given 4 ml/100 g b.wt of the respective liquid diets by gastric tube[11], 2 h before sacrifice.

For measurements of the GSH content, the tissues were washed in cold 0.15 M KCl, homogenized in 0.5 N $HClO_4$ and centrifuged at 4500 rpm for 10 min. GSH was immediately determined in aliquots of the supernatants obtained with 5,5′-dithiobis-(2-nitrobenzoate) at 412 nm according to Ball[12]. All reagents were obtained from Sigma (St. Louis). Results are expressed as means ± SEM and the statistical analysis was performed by Student's t-test.

Results. The administration of a single dose of 5 g of ethanol/kg elicited a 50% decrease in the concentration of GSH in the liver of fasted animals as compared to the corresponding control rats (table, A). Apart from the liver, the kidney also showed a significant decrease in GSH levels

but of a smaller magnitude (23%), no changes being observed in pancreas, intestines, stomach or spleen under these conditions (table, A). The time-course study of the decrease in GSH levels due to acute ethanol ingestion revealed a slow but progressive effect in the liver (figure, A) and a rapid effect in the kidney, already seen after 1 h of treatment and which is approximately constant throughout the intoxication period studied (figure, B).

The study of the effect of chronic ethanol ingestion on the hepatic and renal GSH contents showed a 26% and 21% decrease respectively, when compared with sucrose-control animals (table, B).

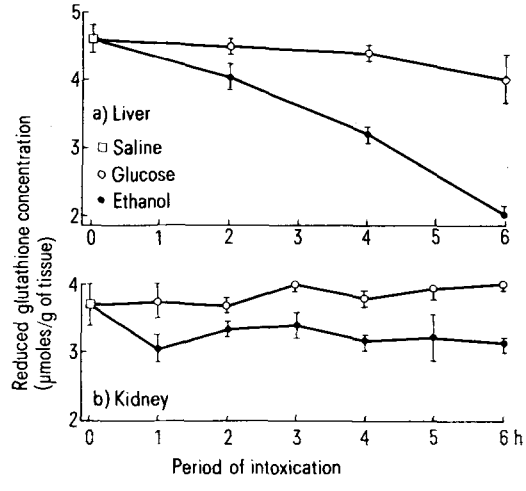
Discussion. Data presentd here indicate that both acute and chronic ethanol ingestion induce a significant decrease in the levels of GSH of the liver and kidney, organs that manifest metabolic and functional abnormalities in these conditions[6,13].

In the acute model, the differences in the magnitude and time course of the effect of ethanol treatment on GSH levels of the liver and kidney could conceivably be due to differences in the metabolic characteristics of these organs related to ethanol and glutathione metabolism. In fact, ethanol oxidation occurs primarily in the liver[6] and the kidney is considered as the major site for glutathione degradation inthe body[14]. No significant changes in GSH levels were observed in pancreas, intestines, stomach or spleen in these experimental conditions.

In the chronic model, a decrease in hepatic and renal GSH levels was observed following 2 h of an oral administration of 4 ml/100 g b.wt of the respective liquid diet which represents a dose of 2 g of ethanol/kg in the treated animals. This probably represents an effect of the treatment per se rather than an acute action of the ethanol present in the body, since no major changes in liver GSH content are detected when naive rats are given 2 g of ethanol/kg and are studied at 6 h, or when they received 5 g of ethanol/kg and are studied 2 h later[4]. Theses findings are at variance with recent studies by Hassing et al.[15] showing a significant increase in the concentration of GSH in the liver. In this study, however, chronic treatment with ethanol was carried out for only 2 weeks, and the nutritional status of the animals as well as the presence or absence of ethanol in the body at the time of sampling are not stated[15], factors that could possibly explain the discrepancy observed.

Tissue GSH depletion seems to be of importance in the induction of cell damage by a) diminishing its capacity to eliminate some reactive compounds or their metabolites by conjugation, thus allowing the alkylation of biomolecules to take place[2] and/or b) decreasing the catabolism of peroxides produced in the cell by the stimulation of lipoperoxidative processes[3].

It has been proposed that mechanism (a) plays a role in the hepatotoxicity induced by drugs such as acetamoniphen and isoniazid[16], as well as renal injury after phenacetin administration[16]. Acetaldehyde, the first metabolite produced during ethanol oxidation, is able to react with several sulfhydryl compounds to produced excretable thiazolidinecarboxylic acid derivatives[17,18]. Recent studies in isolated hepatocytes have suggested that acetaldehyde is responsible for the ethanol-induced GSH depletion as this effect can be prevented by inhibition of alcohol dehydrogenase by pyrazole and it can be potentiated when acetaldehyde oxidation is blocked by disulfiram[19]. Furthermore, mitochondrial alterations[20], lipoperoxidation[7] and GSH depletion[19] induced by acetaldehyde can be prevented by sulfhydryl agents such as cysteine, penicillamine, GSH or methionine, under different experimental conditions[7,19,20]. In relation to mechanism (b), it has been proposed that acute ethanol



Time course of ethanol-induced depletion of reduced glutathione in rat liver (A) and kidney (B). Groups of 4–8 rats fasted overnight (16 h) were given either 5 g of ethanol/kg, isovolumetric amounts of saline or isocaloric amounts of glucose, and were sacrificed at the selected times shown.

Changes in the content of reduced glutathione of several tissues of the rat induced by acute and chronic ethanol administration

Tissue		GSH concentration (μmoles/g of wet weight of tissue)			Effect (%)	P
		Saline	Glucose	Ethanol		
A Acute ethanol treatment[a]	Liver	4.10±0.22 (8)[c]	3.85±0.35 (8)	2.00±0.15 (8)	−48-51	0.001
	Kidney	3.45±0.11 (8)	3.61±0.12 (8)	2.73±0.07 (8)	−21-24	0.001
	Pancreas	1.00±0.28 (3)	1.04±0.21 (4)	0.99±0.13 (4)	− 1-5	NS[d]
	Intestines	2.55±0.37 (3)	2.59±0.30 (4)	2.73±0.29 (4)	+ 5-7	NS
	Stomach	1.53±0.23 (3)	1.66±0.18 (4)	1.65±0.21 (4)	+ 0-8	NS
	Spleen	2.49±0.21 (4)	2.46±0.09 (4)	2.68±0.22 (4)	+ 8-9	NS
		Sucrose-control	Ethanol-treated			
B Chronic ethanol treatment[b]	Liver (6)	7.32±0.69	5.42±0.40		−26	0.05
	Kidney (6)	2.24±0.21	1.77±0.29		−21	0.01

[a] Tissue GSH levels were measured in fasted animals (16 h) given a single oral dose of 5 g of ethanol/kg, isovolumetric saline or isocaloric glucose and were studied 6 h later.

[b] Tissue GSH levels were measured in fed rats given sucrose or ethanol containing liquid diets for four weeks, 2 h after the administration of 4 ml/100 g b.w. of the respective liquid diets by gastric tube.

[c] Number of animals used.

[d] NS = not significant.

intoxication imposes an oxidative pressure on the liver by increasing the metabolism of superoxide radicals, thus stimulating lipoperoxidation and decreasing the levels of GSH by peroxide catabolism through the glutathione peroxidase reaction[4,5].

The influence of the hepatic GSH depletion and the increased lipoperoxidation induced by ethanol ingestion on liver pathology are currently under study in our laboratory. Lipoperoxidation, apart from being deleterious to the cell by itself[3], is an oxygen-dependent process whose enhancement could possibly contribute to the increased oxygen consumption of the liver observed after acute[21] and chronic[22] ethanol intake. This, in turn, has been suggested to be of importance in the production of liver necrosis in the periacinar zone as the result of a reduced oxygen supply to this area[23].

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2 J.R. Mitchell, V.R. Snodgrass and J.R. Gillette, Envir. Health Persp. *15*, 27 (1976).
3 J. Högberg and A. Kristoferson, Eur. J. Biochem. *74*, 77 (1977).
4 L.A. Videla, V. Fernández, G. Ugarte, A. Valenzuela and A. Villanueva, FEBS Lett. *111*, 6 (1980).
5 A. Valenzuela, N. Fernández, V. Fernández, G. Ugarte, L.A. Videla, R. Guerra and A. Villanueva, FEBS Lett. *111*, 11 (1980).
6 R.D. Hawkins and H. Kalant, Pharmac. Rev. *24*, 67 (1972).
7 N.R. Di Luzio and T.E. Stege, in: Alcohol and the Liver, p.45. Ed. M.M. Fisher and J.G. Rankin. Plenum Press, New York 1977.
8 H. Remmer, D. Albrecht and H. Kappus, Naunyn-Schmiedebergs Arch. Pharmak. *298*, 107 (1977).
9 H. Sies, O.R. Koch, E. Martino and A. Boveris, FEBS Lett. *103*, 287 (1979).
10 J.M. Khanna, H. Kalant and G. Bustos, Can. J. Physiol. Pharmac. *45*, 777 (1967).
11 E. Baraona, M.A. Leo, S.A. Borowsky and C.S. Lieber, J. clin. Invest. *60*, 546 (1977).
12 C.R. Ball, Biochem. Pharmac. *15*, 809 (1966).
13 D.H. Van Thiel, W.D. Williams, J.S. Gavaler, J.M. Little, L.W. Este and B.S. Rabin, Am. J. Path. *89*, 67 (1977).
14 A. Wendel, R. Hahn and W.G. Guder, in: Renal Metabolism in Relation to Renal Function, p.426. Ed. U. Schmidt and U.C. Dubach, H. Huber, Vienna 1975.
15 J.M. Hassing, A.L. Hupka, S.J. Stohs and P.C. Yoon, Res. Commun. chem. Path. Pharmac. *25*, 389 (1979).
16 S.D. Nelson, M.R. Boyd and J.R. Mitchell, in: Drug Metabolism Concepts, p.155. Ed. D.M. Jerina. A.C.S. Symposium Series 44. Am. Chem. Soc., Washington, DC 1977.
17 H. Sprince, C.M. Parker, G.G. Smith and L.J. Gonzalez, Agents Actions *4*, 125 (1974).
18 H.T. Nagasawa and D.J.W. Goon, Life Sci. *17*, 707 (1975).
19 J. Viña, J.M. Estrela, C. Guerri and F.J. Romero, Biochem. J. *188*, 549 (1980).
20 A.I. Cederbaum and E. Rubin, Biochem. Pharmac. *25*, 2179 (1976).
21 L.A. Videla, Clin. Sci. molec. Med. *55*, 341 (1978).
22 L. Videla, J. Bernstein and Y. Israel, Biochem. J. *134*, 507 (1973).
23 Y. Israel, L. Videla and J. Bernstein, Fedn Proc. *34*, 2052 (1975).

Microinterferometric characterization of isolated human hepatocytes[1]

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Summary. Hepatocytes isolated from liver tissue taken by biopsy from 18 patients with hepatic or extrahepatic diseases displayed a weight class-organization similar to that of other animal species. In most cases the cell classes had a period of 108 pg varying in number from 4 to 14; cell weight range was 96–432 pg as a minimum (4 classes) and 108–1536 pg as a maximum (14 classes). In 5 cases cell classes showed a period of 120 pg resulting 7–9 in number; cell weight range was 216–960 pg (7 classes) or 216–1200 pg (9 classes). No correlation was found between sex, age, liver histopathology, disease of the patients and the various parameters measured.

Isolated hepatocytes of several mammalian species[2,3] can be arranged in an orderly series of discrete classes on the basis of the total solid content (dry weight or dry mass) of the single cells. In adult animals each class has a normal distribution and a modal weight which differs from that of the preceding or following one by a constant amount (class period) so that all the classes are in an arithmetical progression. Here we report data referring to the human species, which up to now has hardly been studied.

Liver tissue from patients with hepatic or extrahepatic diseases (18 cases in all) was taken by biopsy in the course of abdominal surgery. For this reason, no healthy subject could be examined. Part of the tissue was used for histology (H & E) in order to assess liver pathology, if any. The rest was immediately processed to disperse the cells according to Rappaport and Howze[4]: after a 2-h incubation at the temperature of cracked ice in an aqueous medium containing Na tetraphenylboron (a K-complexing agent) 0.001 M, sucrose 0.05 M, NaCl 0.14 M, Na phosphate buffer 0.005 M, ph 8.5, the cells were dispersed by pipetting the tissue fragments up and down in a series of pipettes with decreasing bore size. Then, the isolated hepatocytes were washed with the same medium by centrifuging at low speed, and resuspended in anhydrous glycerol. Their dry weight (100 cells in each case) was determined with an integrating microinterferometer[5] assuming for α (Beneke[6]) a value of 0.0009[7]. Errors in these measurements have been discussed[5,8].

The results show that human hepatocytes display a class-organization substantially similar to that of other animal species[2,3] (figure).

In the majority of the cases (table; cases 1–13) the number of cell classes varied between 4 and 14, with a corresponding variation of the average dry weight of the cells between 204 and 680 pg (pg = 10^{-12} g). The modal values of the classes were 108, 216, 324, ... pg, i.e. an arithmetical progression, with a period of 108 pg. Cell weight range was 96–432 pg as a minimum (4 classes) and 108–1536 pg as a