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

Mean dry weight ± SEM, number of classes and class-period of isolated human hepatocytes

Case (name, sex, age)	Dry weight per average cell (pg)	Number of classes	Period of the classes (pg)	Disease and main liver and hepatocyte changes
1 M.G.L., ♀, 36	204± 8*	4	108	A: acute pericholangitis; perilobular cell swelling
2 A.F., ♀, 15	209± 8	4	108	B: chronic pericholangitis; few fatty, many swollen cells
3 M.G., ♂, 37	344± 12	6	108	E: normal liver picture
4 M.T., ♀, 35	397± 14	6	108	A: chronic pericholangitis; many neoformed bile ductules
5 A.N., ♀, 58	431± 15	6	108	A: light chronic pericholangitis; normal cytology
6 A.R., ♀, 43	326± 14	8	108	G: normal liver picture
7 M.M., ♀, 35	374± 17	8	108	A: chronic pericholangitis; some fatty or swollen cells
8 T.B., ♀, 79	376± 16	8	108	A: chronic pericholangitis; fatty change in many cells
9 A.Z., ♂, 58	494± 25	9	108	C: portal cirrhosis; variable degrees of cell degeneration
10 C.S., ♀, 38	526± 21	9	108	A: light chronic pericholangitis; a few swollen cells
11 L.G., ♀, 44	587± 34	13	108	A: light chronic pericholangitis; normal cytology
12 A.C., ♂, 55	615± 32	13	108	F: light chronic pericholangitis; bile pigments in most cells
13 A.M., ♂, 39	680± 36	14	108	C: portal cirrhosis; marked fatty change
14 P.M., ♂, 25	418± 15	7	120	A: light perilobular fibrosis; marked glycogenosis
15 R.G., ♂, 43	445± 18	7	120	D: chronic pericholangitis; ceroid pigments in some zones
16 L.L., ♂, 56	462± 15	7	120	A: chronic pericholangitis; marked glycogenosis
17 G.P., ♀, 60	486± 22	9	120	A: normal liver picture; some pale and swollen cells
18 I.N., ♀, 52	596± 24	9	120	A: chronic pericholangitis; some fatty or swollen cells

A: Chololithiasis; B: chronic viral hepatitis; C: liver cirrhosis; D: gastric ulcer; E: duodenal ulcer; F: pancreas carcinoma; G: rectum carcinoma. * Significance of the mean of the cell dry weights: $p < 0.001$ in all cases.

maximum (14 classes). Most cells belonged to classes from II to V. 2 cases are reported in the figure (a, b), as an example.

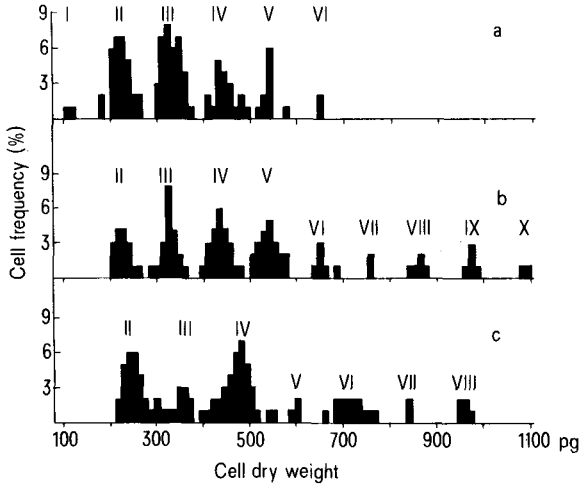
In the other 5 cases (table; cases 14–18) the number of classes varied from 7 to 9, with the average dry weight of the cells varying between 418 and 596 pg. The modal values of the classes were 240, 360, 480, ... pg also in arithmetical progression, with a period of 120 pg. The weight range of the cells was 216–960 pg (7 classes) or 216–1200 pg (9 classes). The cells tended to group in classes II and IV, and to a less extent VI and VIII, leaving a few cells in the intermediate classes. A case is reported in the figure (c), as an example.

No correlation appears between sex, age, liver morphology, disease of the patients and the various parameters measured (table). Liver histology showed no change at all in some patients, whereas in others acute or chronic pericholangitis was found, with or without enlargements of portal triads, neoformation of intrahepatic bile ductules and variable degrees of involvement of the hepatocytes. In 2 patients a liver picture consistent with portal cirrhosis was found, where the regenerating liver plates were formed by several layers of normal and, to a variable extent, fatty or swollen hepatocytes (table).

The class periods of the human hepatocytes are apparently different from those of other mammalian species, in which they were 132 pg (rat, hamster, guinea-pig) or a multiple thereof (528 pg, mouse), 480 pg (rabbit), 216 pg (dog)[2,3]. However comparison is difficult because, by necessity, different procedures for the cell isolation were used.

The procedure employed for the isolation of human hepatocytes, because of the prolonged contact with an aqueous medium, causes a loss of water-soluble constituents, which was found to be about 30% for rat and hamster hepatocytes[9]. On the contrary, the procedure used for the isolation of animal hepatocytes – dog excluded, for which the Rappaport and Howze's method[4] was also employed – prevents that loss[8,10], as the liver tissue is dispersed directly in anhydrous glycerol after a short perfusion in vivo with a Ca^{2+} complexing agent; when, after perfusion, tissue dispersion is made in an aqueous medium, a decrease of about 40% in dry weight was found to occur for white mouse and golden hamster hepatocytes[10,11].

A different extraction of solids could also be one of the explanation for the observed differences in class period in humans (108 vs 120 pg).



Dry weight distribution of human hepatocytes. *a* M.G., ♂, 37: duodenal ulcer. *b* A.Z., ♂, 58: liver cirrhosis. *c* R.G., ♂, 43: gastric ulcer.

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2 R. Tongiani, Boll. Soc. Med. Chir. Pisa *38*, 13 (1970).
3 E. Puccinelli and R. Tongiani, in: New Trends in the Therapy of Liver Diseases, p. 13. Ed. A. Bertelli. Karger Basel 1975.
4 C. Rappaport and G.B. Howze, Proc. Soc. exp. Biol. Med. *121*, 1010 (1966).
5 R. Tongiani, Acta histochem. (Jena) *33*, 13 (1969).
6 G. Beneke, in: Introduction to Quantitative Cytochemistry, p. 63. Ed. G.L. Wied. Academic Press, New York and London 1966.
7 W. Grampp, O. Hallén and B. Rosengren, Exp. Cell Res. *19*, 437 (1960).
8 R. Tongiani and E. Puccinelli, Histochemie *21*, 33 (1970).
9 M. Rossini and R. Tongiani, Experientia *27*, 685 (1971).
10 R. Tongiani, Sperimentale *118*, 263 (1968).
11 R. Tongiani, Z. Zellforsch. *122*, 467 (1971).