PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 147 430-433 (1974)

Effect of Microsomal Enzyme Inducers on the Liver Changes Produced by Common Bile-Duct Ligature (38356)

B. SOLYMOSS AND G. ZSIGMOND

Département de Pathologie and the Institut de Médecine ét de Chirurgie Expérimentales, Université de Montréal, Montréal H3T 1J4, Québec, Canada

Hypertrophy of the smooth endoplasmic reticulum (SER) is usually associated with increased hepatic microsomal enzyme activity; therefore, SER proliferation is generally regarded as a structural sign of hepatic microsomal enzyme induction. However, it has recently been reported that, in rat hepatocytes, large doses of dieldrin render the SER hypertrophic, yet hypoactive; hence it has been suggested that this alteration may also represent a transition from adaptation to injury (1). Hypertrophic SER has also been found in the presence of obvious hepatic damage such as is produced by biliary obstruction (2), administration of carcinogens (3), or alcoholic liver disease (4).

On the basis of these findings, it seemed of value to know whether the hypertrophic SER elicited by biliary obstruction is hyper- or hypoactive, and, if it is the latter, does it represent an irreversible functional alteration or can it be reactivated by phenobarbital, spironolactone or pregnenolone-16α-carbonitrile, PCN (3β-hydroxy- 20- oxo- 5- pregnene- 16α- carbonitrile). These steroids, like phenobarbital, induce hepatic mixed-function oxygenases, bilirubin–glucuronyl- transferases and glutathion- *S*- aryltransferases (5–8). Furthermore, spironolactone and PCN enhance bile flow (7, 8) by accelerating the secretion of the bile-salt independent fraction (9). These effects are associated with proliferation of the SER in rat hepatocytes (10, 11) and largely explain the beneficial action of the steroids on various drug intoxications (12).

Materials and Methods. Female ARS/Sprague–Dawley rats (Madison, WI), with an initial body weight of 95–105 g, were maintained *ad libitum* on Purina Laboratory Chow (Ralston Purina Co. of Canada) and tap water.

Forty animals were divided into five equal groups, one of which (absolute controls) was not pretreated or operated on. Group 2 received no steroids, whereas the remaining rats were given the following substances by stomach tube for 3 days: Group 3, 84 mg/kg body wt of spironolactone (Searle, Chicago, IL); Group 4, 68.7 mg/kg of PCN (Upjohn, Kalamazoo, MI); Group 5, 50.8 mg/kg of phenobarbital sodium (British Drug Houses, Dorval, Québec, Canada). The doses were equivalent to 20 μmoles and the steroids were administered as aqueous microsuspensions homogenized with two drops of Tween-80/100 ml.

The common bile duct of all the animals of Groups 2–5 was ligated under ether anesthesia on the fourth day. The treatments described above (Groups 3–5) were then continued for 3 days per week — between the fifth and 39th day of the experiment. During this period, two to three animals in each group (except the absolute controls) succumbed to bile-duct infection. The survivors were exsanguinated by decapitation on the 39th day. Small pieces of liver tissue were immediately removed from 3 rats per group and processed for electron microscopy, as outlined elsewhere (10). The livers were then excised, weighed, and — after small pieces were taken for histologic examination — homogenized, as previously described (8, 13). Individual samples were used for the following determinations: microsomal protein content (14); NADPH cytochrome C reductase activity (15); cytochrome P-450 content (16); ethylmorphine *N*- demethylation (17); and bilirubin-UDP–glucuronyltransferase activity (18). The blood collected by exsanguination was utilized for quantitative determination of free and conjugated bilirubin

(19).

The differences between the parameters were statistically evaluated (for all determinations) by Student's t test. $P < 0.05$ values were considered as "significantly different" (20).

Results. There was no significant difference between the growth of the absolute controls (Group 1) and of rats subjected to bile-duct obstruction (Table I). At the end of the experiment, the livers of ligated animals weighed about 2–3 times more than those of the absolute controls (Group 1). If expressed as the ratio per 100 g of body wt, the livers of PCN-treated rats were even larger than those of Group 2 animals. In Groups 2–5, the livers were greenish-brown, and the surfaces moderately granular. There was histologic evidence in these rats of bile-duct proliferation accompanied by slight periportal fibrosis; spotty necrosis was also observed in this area, leading to a focal disappearance of hepatocytes. Bile-plug formation was not as marked as in clinical cases of long-standing total biliary obstruction. The electron microscopic findings confirmed that ligation of the common bile duct induced SER proliferation.

The microsomal protein level in ligated animals per g of liver was considerably lower than in the absolute controls (Group 1). PCN-or phenobarbital-treated rats showed significantly higher values than Group 2 animals (Table I).

The values obtained for the absolute controls (Group 1) and for rats subjected to extrahepatic bile flow-obstruction (Group 2) suggested that this intervention greatly decreased cytochrome P-450 content, NADPH cytochrome C reductase activity, and ethylmorphine N-demethylation (Table II). PCN treatment (Group 4) produced a significant increase in cytochrome P-450 content (as compared with Group 2); it restored NADPH cytochrome C reductase activity to normal, and enhanced ethylmorphine N-demethylation (Table II). The effects of spironolactone and phenobarbital were less pronounced in these respects.

Ligation of the common bile duct decreased bilirubin-UDP- glucuronyl-transferase activity, as compared with the absolute controls, that is, Group 1 (Table III). PCN administration resulted in significantly higher values, not only in comparison with Group 2 but also Group 1. Under identical conditions, phenobarbital failed to elicit higher parameters than those observed in Group 2. The effect of spironolactone in this respect was not as pronounced as that of PCN, but it was more significant than that of phenobarbital. Compared with Group 2, the increased bilirubin-UDP–glucuronyl-transferase activity elicited by PCN (Group 4) and spironolactone (Group 3) did not result in significantly lower serum bilirubin values (Table IV).

Discussion. These data indicate that long-term ligature of the common bile-duct results in functional and structural alterations of the hepatic endoplasmic reticulum. In confirmation of the observations of Steiner *et al.* (2), SER-

TABLE I. Effect of Spironolactone, PCN, and Phenobarbital on Liver Weight and Microsomal Protein Content in Rats following Ligature of the Common Bile-Duct (Means ± SE).

Group	Number of rats	Treatment	Body weight (g)	Liver weight (g)	Liver weight (g/100 g body wt.)	Microsomal protein recovered (mg/g)
1	8	None	242.5 ± 5.1	7.60 ± 0.38	3.13 ± 0.11	30.36 ± 0.87
2	5	Common bile-duct ligation[a]	246.0 ± 7.7	20.29 ± 1.23[b]	8.23 ± 0.31[b]	20.84 ± 0.95[b]
3	6	Common bile-duct ligation + spironolactone	247.0 ± 6.1	18.79 ± 2.35[b]	7.57 ± 0.85[b]	21.83 ± 0.59[b]
4	6	Common bile-duct ligation + PCN	246.8 ± 6.9	23.90 ± 0.93[b]	9.70 ± 0.36[bc]	23.29 ± 0.59[bc]
5	5	Common bile-duct ligation + phenobarbital	238.8 ± 7.6	18.53 ± 0.99[b]	7.74 ± 0.27[b]	24.40 ± 0.83[bc]

[a] Ligation of the common bile-duct was performed 5 weeks prior to the biochemical determinations.

[b] Significantly different ($P < 0.05$) from Group 1.

[c] Significantly different ($P < 0.05$) from Group 2.

TABLE II. Effect of Spironolactone, PCN, and Phenobarbital on Hepatic Microsomal Parameters in Rats Subjected to Common Bile-Duct Ligature (Means ± SE).

Group	Number of rats	Treatment	NADPH cytochrome C-reductase (n*M*/reduced per min/microsomes of 1 g liver)	Cytochrome P-450 (n*M*/microsomes of 1 g liver)	Ethylmorphine *N*-demethylation (μM formaldehyde formed per hr/microsomes of 1 g liver)
1	8	None	1820 ± 46	16.63 ± 0.27	2.14 ± 0.16
2	5	Common bile-duct ligation	891 ± 62[a]	2.27 ± 0.95[a]	0.58 ± 0.28[a]
3	6	Common bile-duct ligation + spironolactone	1318 ± 159[ab]	3.44 ± 0.44[a]	1.82 ± 0.76
4	6	Common bile-duct ligation + PCN	1749 ± 55[b]	10.38 ± 0.20[ab]	6.74 ± 0.56[ab]
5	5	Common bile-duct ligation + phenobarbital	1332 ± 63[ab]	5.73 ± 0.24[ab]	1.34 ± 0.39[b]

[a] Significantly different ($P < 0.05$) from Group 1.
[b] Significantly different ($P < 0.05$) from Group 2.

proliferation occurred in animals subjected to this intervention. However, instead of being associated with hyperactivity, this change was accompanied by decreases in microsomal protein and cytochrome P-450 content, NADPH-cytochrome C reductase activity, ethylmorphine *N*-demethylation, and bilirubin-UDP-glucuronyl transferase activity. Furthermore, the hypoactivity produced in the endoplasmic reticulum by extrahepatic bile flow-obstruction was reversible to a certain degree. Apparently, administration of microsomal enzyme inducers can improve some functions of the endoplasmic reticulum, restore others to normal, and increase certain activities above the norm. Among the inducers tested, PCN was the most effective in reversing the detrimental alterations of the endoplasmic reticulum.

These observations also suggest that, in certain diseases of man leading to long-standing bile flow-obstruction, a decrease can be expected in the activity of drug-metabolizing enzymes, which may result in adverse pharmacologic reactions. Administration of some microsomal enzyme inducers could possibly restore microsomal enzyme activity in such cases, influencing first of all the excretion of those polar products that are eliminated via the urine.

TABLE III. Effect of Spironolactone, PCN, and Phenobarbital on the *in Vitro* Glucuronidation of Bilirubin in the Hepatic Microsomes of Rats Subjected to Common Bile-Duct Ligature (Means ± SE).

Group	Number of rats	Treatment	Bilirubin-UDP-glucuronyl transferase activity (n*M* of bilirubin–glucuronide formed in 10 min) (mg of microsomal protein)	(microsomes of 1 g liver)	(microsomes of total liver)
1	8	None	2.28 ± 0.20	70.32 ± 7.98	536 ± 68
2	5	Common bile-duct ligation	1.60 ± 0.16[a]	33.54 ± 3.62[a]	684 ± 90
3	6	Common bile-duct ligation + spironolactone	2.86 ± 0.26[b]	62.30 ± 6.50[b]	1114 ± 84[ab]
4	6	Common bile-duct ligation + PCN	4.62 ± 0.32[ab]	107.08 ± 6.04[ab]	2538 ± 102[ab]
5	5	Common bile-duct ligation + phenobarbital	1.86 ± 0.20	45.44 ± 4.82[a]	818 ± 56[a]

[a] Significantly different ($P < 0.05$) from Group 1.
[b] Significantly different ($P < 0.05$) from Group 2.

TABLE IV. Effect of Spironolactone, PCN, and Phenobarbital on the Plasma Concentrations of Bilirubin in Rats with Biliary Cirrhosis (Means ± SE).

Group	Number of rats	Treatment	Conjugated bilirubin (mg/100 ml)	Unconjugated bilirubin (mg/100 ml)	Total bilirubin (mg/100 ml)
1	8	None	0.21 ± 0.03	0.85 ± 0.05	1.05 ± 0.02
2	5	Common bile-duct ligation	4.79 ± 0.40	5.71 ± 0.92	10.50 ± 1.14
3	6	Common bile-duct ligation + spironolactone	5.48 ± 0.33	4.43 ± 0.37	10.24 ± 0.33
4	6	Common bile-duct ligation + PCN	4.01 ± 0.32	3.86 ± 0.59	7.87 ± 0.77
5	5	Common bile-duct ligation + phenobarbital	5.71 ± 0.51	5.37 ± 0.53	11.08 ± 0.90

Summary. In female rats, ligature of the common bile duct for 5 weeks caused hepatic enlargement, bile duct-proliferation and hyperplasia of the smooth endoplasmic reticulum (SER). Biochemically, however, there was a decrease of microsomal protein and cytochrome P-450 content, of NADPH cytochrome C reductase and bilirubin-UDP-glucuronyl-transferase activities, as well as of ethylmorphine *N*-demethylation indicating that bile duct-obstruction induced hypertrophic but hypoactive SER. Prior and continued administration of pregnenolone-16α-carbonitrile (PCN) to ligated animals corrected all these biochemical changes showing that the hypoactivity of the SER is reversible.

This work was supported by the Medical Research Council of Canada. (MA-4669).

The authors thank the companies listed in the Materials and Methods section for the compounds used in this study.

The competent technical assistance of Mrs. C. Jubert and Miss L. Robitaille is also gratefully acknowledged.

1. Hutterer, F., Schaffner, F., Klion, F. M., and Popper, H., Science **161,** 1017 (1968).

2. Steiner, J. W., Carruthers, J. S., and Kalifat, S. R., Ztschr. Zellforsch. **58,** 141 (1962).

3. Bruni, C., Lab. Invest. **9,** 209 (1960).

4. Klion, F. M., and Schaffner, F., Digestion **1,** 2 (1968).

5. Solymoss, B., Classen, H. G., and Varga, S., Proc. Soc. Exp. Biol. Med. **132,** 940 (1969).

6. Solymoss, B., Werringloer, J., and Toth, S., Steroids **17,** 427 (1971).

7. Zsigmond, G., and Solymoss, B., J. Pharmacol. Exp. Ther. **183,** 499 (1972).

8. Solymoss, B., and Zsigmond, G., Can. J. Physiol. Pharmacol. **51,** 319 (1973).

9. Zsigmond, G., and Solymoss, B., Proc. Soc. Exp. Biol. Med. **145,** 631 (1974).

10. Garg, B. D., Kovacs, K., Blascheck, J. A., and Selye, H., J. Pharm. Pharmacol. **22,** 872 (1970).

11. Horvath, E., Kovacs, K., Blascheck, J. A., and Somogyi, A., Virchows Arch., Sect. B., Cell Pathol. **7,** 348 (1971).

12. Selye, H., "Hormones and Resistance." Springer-Verlag, Heidelberg (1971).

13. Solymoss, B., Toth, S., Varga, S., Werringloer, J., and Zsigmond, G., Can. J. Physiol. Pharmacol. **49,** 841 (1971).

14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).

15. Ernster, L., Acta Chem. Scand. **12,** 6 (1958).

16. Sladek, N. E., and Mannering, G. J., Biochem. Biophys. Res. Commun. **24,** 668 (1966).

17. Mannering, G. J., *in* "Selected Pharmacological Testing Methods" (A. Burger, ed.), p. 51. Marcell Dehker, New York (1968).

18. Van Roy, F. P., and Heirwegh, K. P. M., Biochem. J. **107,** 507 (1968).

19. Weber, A. P., and Schalm, L., Clin. Chim. Acta **7,** 805 (1962).

20. Snedecor, G. W., and Cochran, W. G., "Statistical Methods," 6th ed. The Iowa State University Press, Ames, IO (1967).

Received December 18, 1973. P.S.E.B.M., 1974, Vol. 147.