Br. J. exp. Path. (1982) **63**, 1

HAEM AND DRUG-METABOLIZING ENZYMES IN REGENERATING RAT LIVER

R. C. SRIVASTAVA*, R. S. DWIVEDI, G. KAUR AND R. SRIVASTAVA

From the Industrial Toxicology Research Centre, P.O. Box No. 80, Mahatma Gandhi Marg, Lucknow-226001, India

Received for publication August 10, 1981

Summary.—Various parameters of haem and drug metabolism were measured during the course of liver regeneration after two-thirds hepatectomy. Partial hepatectomy produced a significant depression in delta-ALA synthetase and delta-ALA dehydratase, and induction in haem oxygenase at an early stage of regeneration. The values returned to normal within 7–14 days. These changes were also accompanied by a marked decline in benzo(a)pyrene hydroxylase and aminopyrene demethylase. The level of glutathione and the activity of glutathione reductase also increased during the early stage of proliferation. The increased level of glutathione with concomitant decrease in drug-metabolizing enzymes and induction in haem oxygenase could be considered as a protective mechanism for the detoxication process, although a contribution from other biotransforming mechanisms cannot be excluded.

LIVER CELLS display several forms of growth in response to altered physiological demands (Bucher and Malt, 1971; Thomson, 1975; Weber, 1975). Rat-liver regeneration after partial hepatectomy provides a model for the study of intracellular metabolic processes involved in rapid cellular proliferation and growth. The biochemical profile of regenerating liver has been studied extensively (Robes *et al.*, 1975; Weber, 1975; Schlaeger and von Seydlitz, 1975). During the early stages of proliferation following partial hepatectomy, several hepatic microsomal components including cytochrome P-450-mediated enzymes decrease, but all the parameters return to normal values within 7–14 days. The depression in catalytic activity has been ascribed to loss of the related enzyme proteins (Henderson and Kerston, 1970; Gram *et al.*, 1968; Woods and Murthy, 1975). Even though changes in the activity of drug-metabolizing enzymes have been investigated in regenerating rat liver, data on the possible alterations in haem-metabolizing enzymes under identical conditions are very scanty indeed. In the present communication, attempts have been made to correlate the alterations in haem-metabolizing enzymes with drug-biotransforming enzymes during the process of liver regeneration.

MATERIALS AND METHODS

Animal and general procedures.—Male albino rats of the ITRC colony weighing 150–200 g were housed in an air-conditioned room and allowed free access to food and water. Animals were subjected either to sham-operation or to two-thirds hepatectomy under mild ether anaesthesia (Higgins and Anderson, 1931).

After fasting overnight, 6 rats from each group—*i.e.*, control (non-operated), sham- and partially hepatectomized—were killed at Days 1, 2, 3, 7 and 14 by cervical dislocation. Livers were excised, weighed and homogenized in 3 vols of ice-cold 0·25M sucrose using a teflon-glass homogenizer.

Enzyme assays.—Hepatic delta-ALA synthetase and delta-ALA dehydratase were assayed

Correspondence to: Dr R. C. Srivastava, Scientist, Industrial Toxicology Research Centre, P.O. Box No. 80, Mahatma Gandhi Marg, Lucknow-226001, India.

* To whom reprint requests should be addressed.

by the methods of Woods and Murthy (1975) and Baron and Tephly (1969) respectively, using whole homogenate as enzyme source. Glutathione content and the activity of glutathione reductase were determined by the methods of Ellman (1959) and Bergmeyer (1965) respectively.

Haem-oxygenase activity was determined in the microsomal fraction according to the procedure of Maines Ibrahim and Kappas (1977).

Benzo(a)pyrene hydroxylase was assayed according to the modified procedure of Dehnen, Tomings and Ross, (1973), the details of which have been described by Husain *et al.* (1978). Aminopyrene demethylase was assayed by the method of Mazel (1971).

Protein was estimated by the method of Lowry *et al.* (1951), bovine serum albumin being used as standard.

The statistical treatment of the data was by Student's t test. The level of significance was chosen as $P < 0.05$.

RESULTS

Alterations in haem- and drug-metabolizing enzymes during the course of liver regeneration are shown in Tables I and II. Partial hepatectomy produced a significant lowering in the activity of delta-ALA synthetase and delta-ALA dehydratase at Day 1 and 2. No significant change in the activities of these enzymes was observed in sham-operated rats. Table I also shows that the level of haem oxygenase was significantly raised up to Day 3 after hepatectomy, with a pronounced rise at Day 1 and 2, and returned to normal within 7 to 14 days. A significant increase in haem oxygenase was also observed in sham-operated rats only at Day 1.

The data as shown in Table II indicate that benzo(a)pyrene hydroxylase and

TABLE II.—*Changes in the activity of drug-metabolizing enzymes during liver regeneration*

Time after operation (in days)	Benzo(a)pyrene hydroxylase (fluorescence/ min/mg protein)	Aminopyrene demethylase (nmoles formaldehyde/ min/mg protein)
Control	1·76 ± 0·16	1·12 ± 0·10
Sham operation		
1	1·59 ± 0·13	0·98 ± 0·14
2	1·81 ± 0·20	1·10 ± 0·8
3	1·69 ± 0·17	1·20 ± 0·12
Partial hepatectomy		
1	0·82 ± 0·12[a,b]	0·22 ± 0·02[a,b]
2	0·97 ± 0·07[a,b]	0·42 ± 0·06[a,b]
3	1·24 ± 0·10[a,b]	0·78 ± 0·08[a,b]
7*	1·55 ± 0·20	1·22 ± 0·12
14*	1·81 ± 0·15	1·18 ± 0·10

Each value represents mean ± s.e. of 6 animals.
[a] $P < 0.05$; when compared with control.
[b] $P < 0.05$; when compared with their respective sham-operated controls.
* 7-day and 14-day data from partially hepatectomized animals were compared with control as there was no change in the sham-operated and control groups.

TABLE I.—*Changes in the activity of haem-metabolizing enzyme during liver regeneration*

Time after operation (in days)	δ-ALA-synthetase (nmoles ALA/h/mg protein)	δ-ALA-dehydratase (nmoles PBG/h/mg protein)	Haem-oxygenase (nmoles bilirubin/h/ mg protein)
Control	0·33 ± 0·02	5·27 ± 0·18	2·19 ± 0·09
Sham operation			
1	0·30 ± 0·03	4·98 ± 0·30	2·70 ± 0·18[a],
2	0·31 ± 0·03	5·02 ± 0·20	2·37 ± 0·21
3	0·33 ± 0·02	5·30 ± 0·31	1·96 ± 0·31
Partial hepatectomy			
1	0·17 ± 0·02[a,b]	3·90 ± 0·18[a,b]	5·72 ± 0·14[a,b]
2	0·24 ± 0·02[a]	3·96 ± 0·20[a,b]	4·88 ± 0·12[a,b]
3	0·27 ± 0·04	4·89 ± 0·11	3·73 ± 0·10[b]
7*	0·33 ± 0·04	4·96 ± 0·26	2·73 ± 0·29
14*	0·30 ± 0·03	5·04 ± 0·18	2·81 ± 0·36

Each value represents mean ± s.e. of 6 animals.
[a] $P < 0.05$; when compared with control.
[b] $P < 0.05$; when compared with their respective sham operated controls.
* 7-day and 14-day data from partially hepatectomized animals were compared with control as there was no change in the sham-operated and control groups.

aminopyrene demethylase decreased significantly up to Day 3 with a maximum reduction at Days 1 and 2. No significant change in the activity of these 2 enzymes was noticed in sham-operated rats.

The data given in Table III show that, unlike the enzymes of haem biosynthesis and drug metabolism, glutathione content and activity of glutathione reductase increased significantly at Day 1 and 2. In sham-operated rats, glutathione reductase increased only at Day 1, while the glutathione reductase increased only at Day 1, while the glutathione level remained unaltered.

TABLE III.—*Changes in glutathione level and glutathione reductase activity during liver regeneration*

Time after operation (in days)	Glutathione (μmole/g wet tissue)	Glutathione reductase (unit†/mg protein)
Control	8·26 ± 0·52	49·67 ± 3·20
Sham operation		
1	8·79 ± 0·56	56·35 ± 1·1[a]
2	8·13 ± 0·49	51·96 ± 3·1
3	7·98 ± 0·53	47·48 ± 2·6
Partial hepatectomy		
1	12·31 ± 0·78[a,b]	65·50 ± 1·4[a,b]
2	10·42 ± 0·67[a,b]	62·04 ± 1·6[a,b]
3	8·55 ± 0·55	54·00 ± 2·9[a]
7*	8·27 ± 0·51	51·00 ± 1·8
14*	8·01 ± 0·48	53·10 ± 2·7

Each value represents mean ± s.e. of 6 animals.
[a] $P < 0·05$; when compared with control.
[b] $P < 0·05$; when compared with their respective sham-operated controls.
* 7-day and 14-day data from partially hepatectomized animals were compared with control as there was no change in sham-operated and control groups.
Unit†—A unit of enzyme activity is the amount of enzyme which at 25° in a 3ml assay mixture changes optical density by 0·001 at 340 nm.

DISCUSSION

A major fraction of the haem synthesized in liver is diverted towards the formation of cytochrome P-450 (Sassa *et al.*, 1979; Estabrook, Shigematsu and Schenkman, 1970). This cytochrome plays an important role in the microsomal electron transport required for the biotransformation of a variety of endogenous and exogenous chemicals (Maines and Kappas, 1977). The process is affected by drugs, hormones, metals and a number of environmental pollutants (Maines and Kappas, 1977).

The results of the present study reveal an impairment of the metabolism of haem and xenobiotics in regenerating liver. The most pronounced effect is on haem oxygenase, which is activated, leading to a relatively fast degradation of haem and a concomitant inhibition of delta-ALA synthetase, the rate-limiting enzyme in this process. The result of this effect is reflected in the marked impairment of mixed function oxidases dependent on cytochrome P-450.

The reduced levels of such enzymes have been ascribed to the reduced concentrations of the microsomal haem proteins (Henderson and Kerston, 1970; Gram *et al.*, 1968). Since total hepatic protein did not change significantly during liver regeneration, it appears that the protein-synthesizing apparatus is mostly serving the cellular proliferation at this stage, as evidenced by enhanced synthesis of nucleic acids (Weber 1975). The effect on the individual enzymes could also be due to other specific mechanisms (Bresnick, Williams and Mosse, 1967; Ohtake, Hasegawa and Koga, 1978; Ohtake and Koga, 1979).

A reciprocal relationship between the increased level of glutathione and diminished level of drug-metabolizing enzymes together with the activation of haem oxygenase, could be considered as a protective mechanism for the detoxication process, although contributions from other biotransforming mechanisms cannot be excluded. Enhancement of glutathione is shown by the increased activity of glutathione reductase, which transforms oxidized glutathione to reduced glutathione. In sham-operated rats, an increase in glutathione reductase at Day 1, further suggests that it is needed for the repair process.

It is, therefore, conceivable that regenerating liver, like neonatal and newborn livers (Maines and Kappas, 1977),

cannot cope with the increased requirement for the biotransforming activity when called upon to deal with xenobiotics.

The authors are grateful to Dr C. R. Krishna Murti, Director, Industrial Toxicology Research Centre, Lucknow, for his encouragement and keen interest in the work.

REFERENCES

BARON, J. & TEPHLY, T. R. (1969) Effect of 3-Amino-1,2,4-triazole on Induction of Rat Hepatic Microsomal Oxidases, Cytochrome P-450 and NADPH-cytochrome-C Reductase by Phenobarbitol. *Molec. Pharmacol.*, **5**, 10.

BERGMEYER, H. U. (1965) *Methods of Enzymatic Analysis*. New York: Academic Press. p. 875.

BRESNICK, E., WILLIAMS, S. S. & MOSSE, H. (1967) Rates of Turnover of Deoxythymidine Kinase and of its Template RNA in Regenerating and Control Liver. *Cancer Res.*, **27**, 469.

BUCHER, N. L. R. & MALT, R. A. (1971) *Regeneration of Liver and Kidney*. Boston: Little Brown Co. p. 17.

DEHNEN, M., TOMINGS, R. & ROSS, J. (1973) A Modified Method for the Assay of Benzo(a)pyrene-hydroxylase. *Anal. Biochem.*, **53**, 373.

ELLMAN, G. (1959) Tissue Sulfhydryl Groups. *Arch. Biochem. Biophys.*, **82**, 70.

ESTABROOK, R. W., SHIGEMATSU, A. & SCHENKMAN, J. B. (1970) The Contribution of the Microsomal Electron Transport Pathway to the Oxidative Metabolism of Liver. *Adv. Enzyme Regul.*, **8**, 121.

GRAM, T. E., GUARINO, A. M., GREEN, F. E., GIGON, P. L. & GILLETTE, J. R. (1968) Effect of Partial Hepatectomy on the Responsiveness of Microsomal Enzymes and Cyto. P-450 to Phenobarbital or 3 Methylcholanthrene. *Biochem. Pharmacol.*, **17**, 1769.

HENDERSON, P. T. & KERSTON, K. S. (1970) Metabolism of Drug During Rat Liver Regeneration. *Biochem. Pharmacol.*, **19**, 2343.

HIGGINS, G. M. & ANDERSON, R. M. (1931) Experimental Pathology of the Liver. I. Restoration of the Liver of White Rat following Partial Surgical Removal. *Arch. Path.*, **12**, 186.

HUSAIN, M. M., DEHNEN, W. & MONCH, W. (1978) Comparative Metabolism of Benzo(a)pyrene in some Rodents. *Toxicol. Europ. Res.*, **2**, 75.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951) Protein Measurement with Folin Phenol Reagent. *J. biol. Chem.*, **193**, 265.

MAINES, M. D. & KAPPAS, A. (1977) Metals as Regulators of Haem Metabolism. *Science*, **198**, 1215.

MAINES, M. D., IBRAHIM, I. & KAPPAS, A. (1977*b*) Solubilization and Partial Purification of Haem-oxygenase from Rat Liver. *J. biol. Chem.*, **252**, 5900.

MAZEL, P. (1971) Experiments Illustrating Drug Metabolism *in vitro*. In *Fundamentals of Drug Metabolism and Drug Disposition*, Eds B. N. Ladu, H. G. Mandel and E. L. Way. Baltimore: Williams & Wilkins. p. 546.

OHTAKE, H., HASEGAWA, K. & KOGA, M. (1978) Zinc-binding Protein in the Livers of Neonatal, Normal and Partially Hepatectomized Rats. *Biochem. J.*, **174**, 999.

OHTAKE, H. & KOGA, H. (1979) Purification and Characterization of Zinc-binding Protein from the Liver of the Partially Hepatectomized Rat. *Biochem. J.*, **183**, 683.

ROBES, H. M., TUOZEK, H. V. & WIRSCHING, R. (1975) Kinetics of Hepatocellular Proliferation after Partial Hepatectomy as a Function of Structural and Biochemical Heterogeneity of the Rat Liver. In *Liver Regeneration after Experimental Injury*, Eds R. Lesch and W. Reutter. New York: Stratton Intercontinental. p. 35.

SASSA, S., KAPPAS, A., BERNSTEIN, S. E. & ALVARES, A. P. (1979) Heme Biosynthesis and Drug Metabolism in Mice with Hereditary Hemolytic Anemia. *J. biol. Chem.*, **254**, 729.

SCHLAEGER, R. & VON SEYDLITZ, H. (1975) Alkaline Phosphatase in Regenerating and Cholestatic Rat Liver: Correlation with Protein, RNA and DNA Synthesis. In *Liver Regeneration after Experimental Injury*, Eds R. Lesch & W. Reutter. New York: Stratton Intercontinental. p. 189.

THOMSON, R. Y. (1975) A Biochemical Comparison between Liver Regeneration and Other Types of Compensatory and Induced Growth. In *Liver Regeneration after Experimental Injury*, Eds R. Leach & W. Reutter. Stratton Intercontinental. p. 119.

WEBER, G. (1975) Biochemical Aspects of Hepatic Regeneration in Reaction to Experimental Injury or Liver Loss. In *Liver Regeneration after Experimental Injury*, Eds R. Lesch & W. Reutter. New York: Stratton Intercontinental. p. 103.

WOOD, J. S. & MURTHY, V. V. (1975) δ-Aminolevulinic Acid Synthetase from Fetal Rat Liver: Studies on Partially Purified Enzyme. *Mol. Pharmacol.*, **11**, 70.