Hepatic Regeneration and Erythropoietin Production in the Rat

Abstract. *The regenerating liver produces erythropoietin in response to hypoxia. The amounts of erythropoietin produced in animals subjected to hepatectomy are significantly higher than those observed in sham-operated animals. Hepatic erythropoietin production appears to be dependent upon the stage of regeneration with the highest levels being produced during the period of greatest proliferation and increase in liver mass.*

Erythropoietin (Ep), a glycoprotein hormone, is generally acknowledged to be the prime regulator of erythropoiesis in higher organisms (*1*). While it is well established that the kidney is the main site of Ep elaboration in the intact adult animal (*2*), it is clear that extrarenal sources of Ep also exist (*3*). Recent evidence has implicated the liver as the primary organ of extrarenal Ep production (*4, 5*). It appears that the liver is capable of elevating its normal baseline production of Ep following removal of the kidneys and exposure to a hypoxic stimulus (*6*). Our experiments demonstrate that the regenerating liver produces Ep in response to hypoxia, with the degree of response being dependent on the time allowed for regeneration after hepatectomy.

The animals we used were male Long-Evans rats, each weighing 120 to 130 g. All experimental and control groups of animals included 15 rats. The experimental animals were subjected to partial hepatectomy under ether anesthesia. Approximately 80 percent of the liver was extirpated and, with the exception of the blood removed with the liver, the operation was relatively bloodless. Hematocrit values were determined in each test group at 0 to 144 hours after hepatectomy. Bilateral nephrectomy was performed at intervals of 0, 3, 6, 12, 24, 48, 72, 96, 120, and 144 hours after hepatectomy. The animals were allowed to recover from the anesthesia for 1 hour after nephrectomy and were then subjected to hypoxia (0.40 atm of air) in a decompression chamber for 6 hours. Immediately after they were subjected to hypoxia the animals were exsanguinated, and the serum from each group was pooled and assayed for the presence of Ep by using exhypoxic polycythemic mice (*7*). Each assay group consisted of five mice, with each mouse receiving 1 ml of test serum. Values for the incorporation of radioactive iron by red blood cells were converted into equivalent IRP units by reference to the standard curve for the international reference preparation of Ep.

Three groups of control animals were examined following the same time intervals as for the experimental animals. Group 1 included animals that were sham-hepatectomized, bilaterally nephrectomized, and rendered hypoxic for 6 hours. Sham operations followed the same procedures as the complete operations with the exception that the organs were not ligated or removed. Group 2 included rats that were hepatectomized, bilaterally nephrectomized, and allowed to remain for 7 hours at ambient pressure (approximately 1 atm of air). Group 3 included animals that were sham-hepatectomized, sham-nephrectomized, and subjected to 6 hours of hypoxia (0.40 atm of air). In addition, two other test groups were examined. In the first group, only the right lateral lobe of the liver was removed (approximately 30 to 40 percent hepatectomy) according to the same procedure as was used for the groups subjected to 80 percent hepatectomy. The second group consisted of rats subjected to 80 percent hepatectomy followed by combined bilateral nephrectomy and splenectomy at the same time intervals as previously described.

When compared to the sham-hepatectomized groups (Fig. 1), the rats subjected to hepatectomy had decreased serum concentrations of Ep for 22 to 26 hours after liver removal; Ep production then markedly increased and attained peak values at 72 hours. The Ep level dropped to that of the sham-operated groups by 96 hours and remained there. The increase in Ep production corresponded to the period of active liver regeneration which, in the rat, nears completion at 72 to 80 hours after hepatectomy (*8*). Animals in which only the right lobe of the liver was removed (Table 1) also exhibited a peak of Ep production at 72 hours, but the increase was only 56 percent of that in animals subjected to 80 percent hepatectomy.

Evidently, the mass of regenerating tissue determines the amount of Ep that can be produced in response to hypoxia. In hepatectomized, nephrectomized animals kept at ambient pressure, the amount of Ep produced was barely detectable. Sham-hepatectomized, sham-nephrectomized rats exposed to hypoxia showed Ep levels that were approximately equal to those of unoperated hypoxic animals. These values were much higher than those noted in the hepatectomized, nephrectomized rats, indicating

Table 1. Serum erythropoietin (Ep) concentrations and hematocrit values in rats during hepatic regeneration. The results are expressed as the mean ± standard error of the mean for each experimental group, with the number (*N*) of experimental trials given in separate columns. The data for Ep represent equivalent units of Ep derived from the standard dose-response curves for the international reference preparation (IRP) of human urinary Ep, assayed in the exhypoxic polycythemic mouse.

Time after hepatectomy (hours)	Hepatectomy (70 to 80 percent), nephrectomy, and hypoxia			Hepatectomy (30 to 40 percent), nephrectomy, and hypoxia		Hepatectomy (70 to 80 percent), nephrectomy with splenectomy, and hypoxia	
	Ep	*N*	Hematocrit value*	Ep	*N*	Ep	*N*
0	0.055 ± 0.0049	4	38.3 ± 1.29	0.056 ± 0.0072	3	0.049 ± 0.0036	4
3	0.059 ± 0.0005	3	39.2 ± 1.60			0.051 ± 0.0025	4
6	0.086 ± 0.0097	3		0.058 ± 0.0029	3	0.054 ± 0.0025	4
12	0.160 ± 0.0260	3	38.0 ± 1.45	0.070 ± 0.0005	3	0.148 ± 0.0099	4
24	0.245 ± 0.0071	3	39.1 ± 1.23	0.186 ± 0.0085	3	0.237 ± 0.0120	3
48	0.442 ± 0.0433	3	38.6 ± 1.09	0.359 ± 0.0100	3	0.451 ± 0.0173	3
72	0.780 ± 0.0100	4	39.4 ± 1.52	0.590 ± 0.0150	3	0.783 ± 0.0093	3
96	0.264 ± 0.0099	3	38.1 ± 1.42	0.325 ± 0.0179	3	0.276 ± 0.0123	3
120	0.251 ± 0.0133	3	37.3 ± 2.10	0.268 ± 0.0013	3	0.262 ± 0.0096	3
144	0.253 ± 0.0192	3	38.4 ± 1.26	0.259 ± 0.0017	3	0.263 ± 0.0133	3

*The hematocrit value in a normal rat is 38.7 ± 1.46.

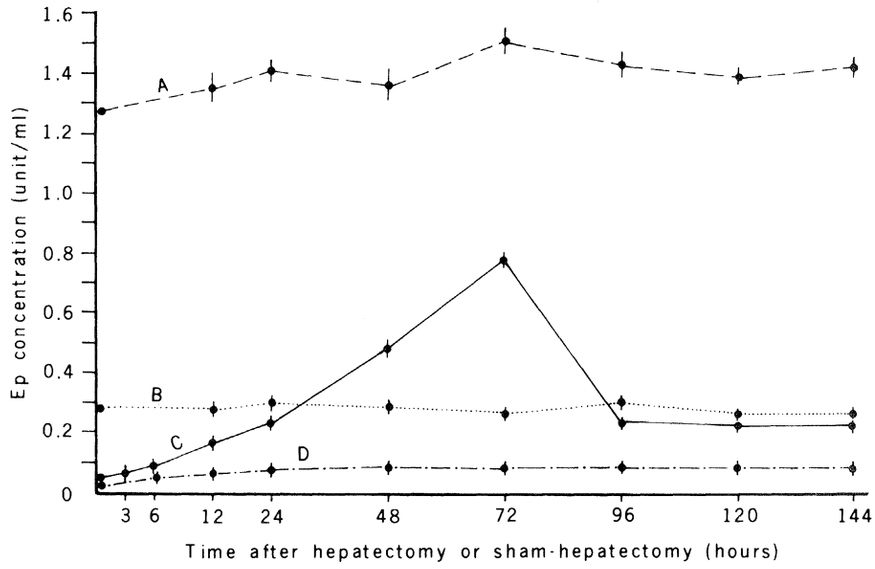

Fig. 1. Mean amounts of Ep in the serum at various time intervals during hepatic regeneration. Vertical bars through the means indicate ± 1 standard error of the mean. The curves are for rats treated as follows: curve A, sham hepatectomy, sham nephrectomy, and hypoxia; curve B, sham hepatectomy, nephrectomy, and hypoxia; curve C, hepatectomy, nephrectomy, and hypoxia; and curve D, hepatectomy, nephrectomy, and room pressure.

that the kidney is a much more potent producer of Ep in adolescent animals than in adults (Fig. 1). When splenectomy was added to the procedure, Ep levels decreased in the group nephrectomized at 6 hours ($P < .05$), but did not change at later times (Table 1). Therefore, the spleen, a second site of extrarenal Ep production (*5*), was of minimal importance in this test system.

A necessary consequence of partial hepatectomy is a decrease in the total blood and plasma volume due to loss of blood within the liver at the time of surgery, although systemic anemia does not develop. This is evident from the fact that, in all the experimental groups, there were no detectable hematocrit changes in animals examined 0 to 144 hours after hepatectomy (Table 1). It has also been demonstrated (*9*) that hepatic blood flow is increased, in the regenerating liver, by dilation of sinusoidal beds, as seen by an increase in perfusion rate per gram of liver tissue. Majumdar *et al.* (*10*) have shown, in the rat, that there is an increase in albumin and fibrinogen synthesis beginning 4 hours after hepatectomy, although, in the dog, the total serum protein and albumin remain relatively stable throughout the course of regeneration (*11*). The amount of free amino acids in the liver, an indicator of protein synthesis, is increased, but practically no change occurs in the total free amino nitrogen in either systemic blood or plasma after partial hepatectomy (*12*). Increases in free amino nitrogen and protein synthesis may be due to increased extracellular space in the remaining stump of hepatic tissue (*10, 11*). The increased Ep production observed in regenerating livers was not the result of any metabolic or circulatory alterations induced by the regenerative process, because the nonhypoxic control animals showed no significant increase in serum Ep throughout the experimental period.

The data presented here demonstrate the capacity of the regenerating liver to respond to a hypoxic stimulus with elevated concentrations of serum Ep. Of importance is the observation that these Ep levels are significantly higher ($P < .005$) than those noted for the hypoxic, sham-hepatectomized animals. It is probably the regenerating portion of the liver that is responsible for the observed higher levels of the hormone. It is also interesting that the degree of hepatic Ep production appears to be dependent on the stage of regeneration. Thus, the greatest concentrations of serum Ep occur at 24 to 72 hours after hepatectomy. This corresponds to the period of greatest cell proliferation (*8, 13*) and increase in mass of liver tissue (*14*) in the rat.

While the kidney is the major site of Ep production in the adult, the liver is the primary locus of Ep production during fetal (*15*) and neonatal (*16*) development. Preliminary observations (*17*) have implicated the Kupffer cell as the site of Ep or erythrogenin production in the intact liver. Widmann and Fahimi (*18*) have observed that Kupffer cell activity is markedly increased during hepatic regeneration. The above evidence suggests that the Kupffer cell is involved in extrarenal Ep production in the regenerating liver. The similarity between the regenerating liver and the embryonic liver has previously been reported (*19*), and it is tempting to speculate that regeneration confers upon hepatic tissue the ability to revert to the fetal state in its capacity to produce Ep.

BRIAN A. NAUGHTON
STEPHEN M. KAPLAN, MARTIN ROY
ALLEN J. BURDOWSKI
ALBERT S. GORDON
Department of Biology, Graduate School of Arts and Science, New York University, New York 10003
SAM J. PILIERO
Departments of Physiology and *Histology, New York University College of Dentistry, New York 10010*

References and Notes

1. A. S. Gordon, *Vitam. Horm.* **31**, 105 (1973); S. B. Krantz and L. O. Jacobson, *Erythropoietin and the Regulation of Erythropoiesis* (Univ. of Chicago Press, Chicago, 1970).
2. L. O. Jacobson, E. Goldwasser, W. Fried, L. Plzak, *Nature (London)* **179**, 633 (1957); Z. Kuratowska, B. Lewartowski, E. Michalak, *Blood* **18**, 527 (1960).
3. E. A. Mirand, T. C. Prentice, W. R. Slaunwhite, *Ann. N.Y. Acad. Sci.* **77**, 677 (1959); R. D. Lange and N. I. Gallagher, in *Erythropoiesis*, L. O. Jacobson and M. Doyle, Eds. (Grune and Stratton, New York, 1962), p. 361; J. C. Schooley and L. J. Mahlmann, *Blood* **39**, 31 (1972); *ibid.* **40**, 662 (1972); *Proc. Soc. Exp. Biol. Med.* **145**, 1081 (1972).
4. W. Fried, *Blood* **40**, 671 (1972).
5. S. M. Kaplan, S. A. Rothmann, A. S. Gordon, I. A. Rappaport, J. F. Camiscoli, C. Peschle, *Proc. Soc. Exp. Biol. Med.* **143**, 310 (1973).
6. W. Fried, T. Kilbridge, S. B. Krantz, T. P. McDonald, R. D. Lange, *J. Lab. Clin. Med.* **73**, 244 (1969); W. Fried, W. H. Knospe, F. E. Trobaugh, *Proc. Soc. Exp. Biol. Med.* **137**, 255 (1971).
7. J. F. Camiscoli and A. S. Gordon, in *Regulation of Hematopoiesis*, A. S. Gordon, Ed. (Appleton-Century-Crofts, New York, 1970), p. 369.
8. N. L. R. Bucher, *Int. Rev. Cytol.* **15**, 245 (1963).
9. T. M. Saba, *RES J. Reticuloendothel. Soc.* **7**, 406 (1970).
10. C. Majumdar, K. Tsukada, I. Lieberman, *J. Biol. Chem.* **242**, 700 (1967).
11. R. J. MacKenzie, C. M. Furnival, M. A. O'Keane, L. H. Blumgart, *Br. J. Surg.* **62**, 431 (1975).
12. V. Ferrari and R. D. Harkness, *J. Physiol. (London)* **124**, 443 (1954).
13. K. Weinbren and A. Taghizadeh, *Br. J. Exp. Pathol.* **46**, 413 (1965); R. J. Stenger and D. B. Confer, *Exp. Mol. Pathol.* **5**, 455 (1966); F. S. Talarico, D. D. Feller, E. D. Neville, *Proc. Soc. Exp. Biol. Med.* **131**, 430 (1969).
14. J. L. Norris, J. Blanchard, C. Povolny, *Arch. Pathol.* **34**, 208 (1942); E. D. McLaughlin, *Surgery* **47**, 420 (1960).
15. E. D. Zanjani, E. Horger, A. S. Gordon, L. Cantor, D. L. Hutchinson, *J. Lab. Clin. Med.* **74**, 782 (1969).
16. S. M. Kaplan, S. R. Weinberg, A. S. Gordon, *Biol. Neonate* **24**, 145 (1974).
17. C. Peschle, G. Marone, A. Genovese, I. A. Rappaport, M. Condorelli, *Blood* **47**, 325 (1976); D. F. Gruber, J. R. Zucali, Sr., J. Wleklinski, V. La Russa, E. A. Mirand, *Exp. Haematol.* **4**, 117 (Suppl.) (1976); E. J. Carson, M. Roy, B. A. Naughton, A. J. Burdowski, A. S. Gordon, in preparation.
18. J. J. Widmann and D. H. Fahimi, *Am. J. Pathol.* **80**, 349 (1975).
19. H. P. Popper and F. Schaffner, *Liver: Structure and Function*, (McGraw-Hill, New York, 1957), p. 166.
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