

The Liver as a Source of Extrarenal Erythropoietin Production

By Walter Fried

Nephrectomized rats exposed to intense hypoxia produce sufficient erythropoietin (Ep) to detectably increase their plasma Ep titers. Extrarenal Ep production is no longer detectable if nephrectomized rats are also subjected to 80% hepatectomy prior to being made hypoxic at 0.465 atmos and is barely detectable but significantly lower than in nephrectomized rats when exposed to 0.435 atmos. These results suggest that the liver plays an important role in the production of extrarenal erythropoietin.

NEPHRECTOMY causes the plasma erythropoietin (Ep) level of experimental animals and of man to fall to a fraction of the level observed in comparably stimulated intact subjects.¹⁻⁴ Nephrectomized rodents⁵⁻⁷ and humans^{3,4,8} do, however, have detectably elevated plasma Ep titers when intensely stimulated; and the titer of extrarenally produced Ep is related to the intensity of the hypoxic stimulus.² The site of extrarenal Ep production has not been identified. However, the results of several studies suggest that the liver is involved in extrarenal Ep production.^{9,10} The following studies were performed to further elucidate the role of the liver in extrarenal Ep production.

MATERIALS AND METHODS

Sprague-Dawley male rats were used. All surgical procedures were performed under ether anesthesia. Partial hepatectomy (80%) was performed by slight modification of the Higgins and Anderson method.¹¹ This consisted of removal of the middle and left lobe and two-thirds of the right lobe of the liver. The caudate lobe remained intact. Nephrectomy was performed via a ventral incision. Total hepatectomy and evisceration was performed by a two-stage procedure. The first stage consisted of ligation of the inferior vena cava just proximal to the entry of the right renal vein. Six weeks later, the abdomen was reopened along the same ventral incision; the stomach, small and large bowels, pancreas and spleen were removed in block, and then the total liver was removed. Rats subjected to this procedure were injected with either 5 ml of a 20% dextrose solution s.c. postoperatively or 3 ml i.v. postoperatively and again after 4 hr. Adrenalectomy was performed through a ventral incision, and operated rats received 1 mg of hydrocortisone i.p. immediately after the procedure. Orchiectomy was performed via a scrotal incision in rats anesthetized with ether. Hypoxia was induced in a steel chamber attached to a vacuum pump and fitted with a spring valve designed to maintain the desired hypobaric pressure.

Plasma Ep titers were assayed in polycythemic mice according to the method of Weintraub et al.¹² The results are expressed in Std B units calculated from a dose-response curve drawn from assay of a standardized Ep preparation in doses of 0.01-2.0 U. (For the standardized preparations, erythropoietin was collected and concentrated by the Depart-

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Table 1. Experiment 1

Group	Procedure Prior to Hypoxia (0.4 atmos)	Procedure After 4-Hr Hypoxia*	No. of Donor Rats	No. of Assay Mice	Plasma Injected (ml)	RBC ⁵⁹ Fe Uptake of Assay Mice Mean ± SE (%)	Ep (U/ml)
1	Bilateral nephrectomy	Hepatectomy and evisceration	9	16	0.8	0.9 ± 0.08†	Nondetectable
2	Bilateral nephrectomy	Evisceration	6	8	0.8	2.5 ± 0.16‡	0.08
3	Bilateral nephrectomy	—	15	20	0.8	6.5 ± 0.5	0.16
4	—	Hepatectomy and evisceration	6	7	0.4	19.0 ± 1.7	2.03
5	—	—	15	8	0.4	15.9 ± 1.2	1.23
6	—	No Hypoxia	5	7	0.4	0.9 ± 0.06	Nondetectable

* Mice were reexposed to hypoxia for 4 hr after this procedure.

† Differed from group 2 ($p < 0.01$).

‡ Differed from group 3 ($p < 0.01$).

ment of Physiology, University of the Northeast, Argentina; it was further processed and assayed by Hematology Research Laboratories, Childrens Hospital of Los Angeles under Research Grant HE 10880, and was authorized for distribution by the Erythropoietin Committee of the National Heart and Lung Institute.) Minimum significantly detectable dose was 0.03 U, and the linear portion of the curve ranged between 0.05–1.0 U.

Experiment No. 1

Six groups of five 4-wk-old Sprague-Dawley rats were compared. All animals had their inferior vena cavi ligated as per stage 1 in the two-stage hepatectomy procedure. Six weeks later, rats of groups 1, 2, and 3 had bilateral nephrectomies after which they were made hypoxic for 4 hr. The rats were then removed from hypoxia, those in group 1 were subjected to hepatectomy and evisceration, those in group 2 to evisceration without hepatectomy, and those in group 3 to no operation. Within 45 min, all were again placed into the hypoxic chamber at 0.465 atmos for 4 hr more, after which they were exsanguinated and their plasma was collected and pooled for Ep assay. Rats in groups 4 and 5 were placed into the same hypoxic atmosphere for 4 hr, after which they were removed; those in group 4 had a hepatectomy and evisceration performed, while those in group 5 remained unoperated. They were then replaced into the hypoxic atmosphere for 4 hr more. Their plasma was then collected, pooled, and assayed for Ep. Group 6 rats were unoperated after the initial vena caval ligation 6 wk previously and were not exposed to hypoxia. Their plasma was collected simultaneously with that of rats in groups 1–5 and was pooled for Ep assay. There was a 40%–60% mortality among hepatectomized, nephrectomized, eviscerated (group 1), nephrectomized, eviscerated (group 2), and hepatectomized, eviscerated (group 4) rats. Accordingly, the experiment was replicated three times, and the plasma from these replications was pooled prior to assay.

Experiment No. 2

Groups of five 10-wk-old male rats were subjected to either bilateral nephrectomy (group 1), bilateral nephrectomy and adrenalectomy (group 2), bilateral nephrectomy and gonadectomy (group 3), or bilateral nephrectomy and 80% hepatectomy (group 4). (In group 4, 80% hepatectomy was performed 20 hr prior to nephrectomy and exposure to hypoxia.) As soon as they awakened from anesthesia, all rats were exposed to 0.465 atmos for 8 hr. Immediately afterward they were exsanguinated, and their plasma was collected and assayed for Ep.

Experiment No. 3

Groups of five 10-wk-old rats were subjected to bilateral nephrectomy (group 1), bilateral nephrectomy and 80% hepatectomy (same procedure as in experiment 2) (group 2), or no operation (group 3). Groups 1 and 2 were then exposed to 0.435 atmos for 8 hr, after which their plasma was collected and assayed for Ep. The rats in group 3 were sacrificed at the same time, and their plasma was collected for assay. Because of a 40% mortality in the rats of group 2, this experiment was replicated two times, and the plasmas of the respective groups were pooled prior to assay.

Experiment No. 4

Groups of five rats were subjected to 80% hepatectomy (group 1), or no operation (groups 2 and 3). Rats in groups 1 and 2 were then exposed to 0.465 atmos for 8 hr, after which they were sacrificed, and their plasma was collected for Ep assay. Plasma from rats in group 3 was collected for assay at this time also.

RESULTS

Hepatectomy did not significantly decrease the plasma Ep level of hypoxic rats with intact kidneys but did completely abolish the detectable plasma Ep

Table 2. Experiment 2

Group	Procedure	8-Hr Hypoxia (atmos)	No. of Donor Rats	No. of Assay Mice*	⁵⁹ Fe Uptake of Assay Mice Mean ± SE (%)	Ep (U/ml)
1	Bilateral nephrectomy	0.465	5	11	4.1 ± 0.3	0.10
2	Bilateral nephrectomy and adrenalectomy	0.465	5	5	4.0 ± 0.2	0.10
3	Bilateral nephrectomy and gonadectomy	0.465	5	6	4.4 ± 0.3	0.11
4	Bilateral nephrectomy and 80% hepatectomy	0.465	5	12	0.7 ± 0.05†	Nondetectable
5‡	—	None	5	7	0.9 ± 0.06†	Nondetectable

* Plasma injected: 0.8 ml/mouse.

† Differed from group 1 ($p < 0.01$).

‡ Same as group 6 in experiment 1.

level of nephrectomized hypoxic rats (Table 1). Nephrectomized and eviscerated rats had lower plasma Ep titers than comparably hypoxic nephrectomized rats but had Ep titers that were significantly greater than those of nephrectomized and hepatectomized rats and of controls (Table 1).

The plasma Ep titer of rats subjected to nephrectomy and 80% hepatectomy prior to exposure to hypoxia for 8 hr was undetectable, whereas neither adrenalectomy nor gonadectomy significantly diminished the plasma Ep level of nephrectomized hypoxic rats (Table 2).

After exposure to 0.435 atmos, the plasma of nephrectomized-hepatectomized rats contained a small but significantly detectable Ep titer. This was, however, less than half as high as that in the plasma of nephrectomized rats made comparably hypoxic (Table 3). The plasma Ep titer of hepatectomized hypoxic rats was not significantly different from that of comparably hypoxic unoperated rats (Table 4).

DISCUSSION

Erythropoietin titers become elevated in bilaterally nephrectomized, hypoxic experimental animals⁵⁻⁷ and humans,^{3,4,8} and this is due to production of Ep in extrarenal sites rather than release of preformed Ep from storage sites.¹³ This extrarenal Ep has antigenic components that cross-react with an anti-

Table 3. Experiment 3

Group	Procedure	8-Hr Hypoxia (atmos)	No. of Donor Rats	No. of Assay Mice*	⁵⁹ Fe Uptake of Assay Mice Mean ± SE (%)	Ep (U/ml)
1	Bilateral nephrectomy	0.435	10	15	8.0 ± 0.6	0.15
2	Bilateral nephrectomy and 80% hepatectomy	0.435	6	10	3.6 ± 0.3†	0.07
3	No operation	None	10	8	1.4 ± 0.1	Nondetectable

† Differs from group 1, $p < 0.01$; differs from group 3, $p < 0.01$.

* Plasma injected: 0.8 ml/mouse.

Table 4. Experiment 4

Group	Procedure	8-Hr Hypoxia (atmos)	No. of Donor Rats	No. of Assay Mice*	⁵⁹ Fe Uptake of Assay Mice Mean ± SE (%)	Ep (U/ml)
1	80% Hepatectomy	0.465	5	10	26.1 ± 1.3†	>2.0
2	No operation	0.465	5	10	23.0 ± 1.4†	>2.0
3	No operation	None	5	10	1.1 ± 0.1	Nondetectable

† Differs from group 3, $p < 0.01$.

* Plasma injected: 0.5 ml/mouse.

serum produced against Ep from patients with intact kidneys² and that is equally effective in vitro and in vivo.² This latter observation indicates that extrarenal Ep is a complete and biologically active molecule that is not dependent on the intact kidney of the assay animal for its activation. The site of extrarenal Ep production has not yet been identified. However, both Reissmann and Nomura⁹ and Burke and Morse¹⁰ have shown that perfusion of the liver results in release of a substance into the perfusate that is capable of stimulating erythropoiesis of assay mice. Others have not observed this to be the case.¹⁴

The results described above indicate that removal of the liver from nephrectomized rats abolishes their ability to detectably increase their plasma Ep titer in response to exposure to 0.465 atmos. Total hepatectomy and evisceration is a very debilitating procedure, as evidenced by significant mortality in rats subjected to this procedure. Accordingly, one might interpret the inability of hepatectomized and nephrectomized rats to produce Ep in detectable titer on exposure to hypoxia as resulting from the animals being so sick that the sites of extrarenal Ep production are unable to function. Although this possibility cannot be totally excluded, it is unlikely to be the case, since Ep production of hepatectomized but nonnephrectomized rats is not detectably altered and since nephrectomized rats subjected to 80% hepatectomy, which appear to tolerate the procedure quite well, are also unable to elevate detectably their plasma Ep titer in response to exposure to 0.465 atmos. In addition, earlier experiments, which have been confirmed here, have shown that neither total evisceration nor 80% hepatectomy significantly alters the plasma Ep level of hypoxic rats with intact kidneys.¹⁵ This is in contrast to the results of Katz et al.¹⁶ who observed that hepatectomized rats, exposed to 5 hr of hypoxia, had lower plasma Ep titers than did unoperated ones. This difference may result from the shorter period of hypoxic exposure of rats in Katz's experiments, an experimental design that could conceivably be more sensitive to small differences in capacity for Ep production. Evisceration of nephrectomized rats does significantly decrease the plasma Ep titers of these rats as compared to nephrectomized rats made comparably hypoxic. This may result, as previously discussed, from the rigor of the procedure. It may also result from the resultant decrease in the amount of hepatic blood flow. The reason for this phenomenon is currently under investigation.

Katz et al.¹⁶ have suggested that the substrate for erythropoietin is produced by the liver. This raises the interesting speculation that this substrate can undergo spontaneous conversion to active Ep but at a rate that is only a fraction of that which occurs in the presence of erythropoietin. This is unlikely to be the explanation for the results reported in this paper, since one would then expect a significant decrease in the plasma Ep level of hepatectomized rats with intact kidneys, which was not observed either here or previously.¹⁸ It is, however, possible that the liver produces either erythropoietin in smaller amounts than does the kidney, or an enzyme with similar but less effective ability to convert the plasma substrate to active Ep. This possibility remains to be tested.

In conclusion, the results of experiments described above are, in the opinion of the author, most consistent with the concept that the liver is a source of extrarenal erythropoietin and/or erythropoietin, although not necessarily the sole one. The observation of a small but significant rise in plasma Ep titer on exposure of nephrectomized and partially hepatectomized (80%) rats to 0.435 atmos may result either from Ep produced in the remaining 20% of liver or from Ep produced outside the liver and kidneys on intense hypoxic exposure. It is interesting to note that, excluding hypernephroma, the tumors most commonly associated with polycythemia and elevated plasma Ep levels are hepatomas.¹⁷ Gordon et al. have shown in one patient with hepatoma that the tumor was producing excess substrate for erythropoietin rather than erythropoietin or Ep per se.¹⁸ Several investigators, on the other hand, have extracted Ep from hepatocellular carcinomas,¹⁹⁻²⁰ although others were unable to demonstrate erythropoietic-stimulating factor in extracts from hepatocellular carcinomas.²¹ This suggests that the liver may produce both substrate for erythropoietin and erythropoietin or Ep.

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REFERENCES

1. Jacobson, L. O., Marks, E. K., and Gaston, E. O.: Studies on erythropoiesis. XI. Reticulocyte response of transfusion-induced polycythemic mice to anemic plasma from nephrectomized mice and to plasma from nephrectomized mice exposed to low oxygen. *Blood* 14:635, 1959.
2. Fried, W., Kilbridge, T., Krantz, S., McDonald, T. P., and Lange, R. D.: Studies on extrarenal erythropoietin. *J. Lab. Clin. Med.* 73:244, 1969.
3. Mirand, E. A., Murphy, G. P., Steeves, R. A., Weber, H. W., and Retief, F. P.: Extra-renal production of erythropoietin in man. *Acta Haemat. (Basel)* 39:359, 1968.
4. Naets, J. P., and Wittek, M.: Presence of erythropoietin in the plasma of one anephric patient. *Blood* 31:249, 1968.
5. Mirand, E. A., and Prentice, T. C.: Presence of plasma erythropoietin in hypoxic rats with or without kidneys and or spleen. *Proc. Soc. Exp. Biol. Med.* 96:49, 1957.
6. Gallagher, N. I., McCarthy, J. M., and Lange, R. D.: Erythropoietin production in uremic rabbits. *J. Lab. Clin. Med.* 57:281, 1961.
7. Erslev, A. J.: Erythropoietin function in uremic rabbits. *Arch. Intern. Med. (Chicago)* 101:407, 1958.
8. DeGowin, R. L., Lavender, A. R., Forland, M., Charleston, D., and Gottschalk, A.: Erythropoiesis and erythropoietin in

patients with chronic renal failure treated with hemodialysis and testosterone. *Ann. Intern. Med.* 72:913, 1970.

9. Reissmann, K. R., and Nomura, T.: Erythropoietin formation in isolated kidneys and liver. *In* Jacobson, L. O., and Doyle, M. (Eds.): *Erythropoiesis*. New York, Grune & Stratton, 1962, p. 71.

10. Burke, W. T., and Morse, B. S.: Studies on the production and metabolism of erythropoietin in rat liver and kidney. *In* Jacobson, L. O., and Doyle, M. (Eds.): *Erythropoiesis*. New York, Grune & Stratton, 1962, p. 111.

11. Higgins, G. M., and Anderson, R. M.: Experimental pathology of the liver. *Arch. Path. (Chicago)* 12:186, 1931.

12. Weintraub, A. H., Gordon, A. S., and Camiscoli, J. F.: Use of the hypoxia-induced polycythemic mouse in the assay and standardization of erythropoietin. *J. Lab. Clin. Med.* 62:743, 1963.

13. Schooley, J. C., and Mahlmann, L. J.: Erythropoietin production in the anephric rat. I. Relationship between nephrectomy, time of hypoxic exposure, and erythropoietin production. *Blood* 39:31, 1972.

14. Kuratowska, Z., Lewartowski, B., and Michalak, E.: Studies on the production of erythropoietin by isolated perfused organs.

Blood 18:527, 1961.

15. Jacobson, L. O., Goldwasser, E., Fried, W., and Plzak, L.: Role of the kidney in erythropoiesis. *Nature (London)* 179:633, 1957.

16. Katz, R., Cooper, G. W., Gordon, A. S., and Zanjani, E. D.: Studies on the site of production of erythropoietin. *Ann. N.Y. Acad. Sci.* 149:120, 1968.

17. McFadzean, A. J. S., Todd, D., and Tso, S.: Erythrocytosis associated with hepatocellular carcinoma. *Blood* 29:808, 1967.

18. Gordon, A. S., Zanjani, E. D., and Zalusky, R.: A possible mechanism for the erythrocytosis associated with hepatocellular carcinoma in man. *Blood* 35:151, 1970.

19. Santer, M. A., Waldmann, T. A., and Fallon, H. J.: Erythrocytosis and hyperlipemia as manifestations of hepatic carcinoma. *Arch. Intern. Med. (Chicago)* 120:735, 1967.

20. Nakao, K. K., Kimura, K., Miura, Y., and Takaku, F.: Erythrocytosis associated with carcinoma of the liver. *Amer. J. Med. Sci.* 251:161, 1966.

21. Lehman, C. V., Erslev, A. V., and Myerson, R. M.: Hepatocellular carcinoma with erythrocytosis. *Amer. J. Med.* 35:439, 1963.



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