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Extrarenal Erythropoietin Production by the Liver in the Weanling Rat[1] (37957)

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Erythropoietin production is considerably reduced but not abolished in adult rats exposed to hypoxia immediately after nephrectomy (1–4), whereas in young weanling rats exposed to hypoxia, removal of the kidneys has little effect on the production of the hormone (4–6). Thus, the hormone is produced or activated by extrarenal as well as renal tissues. Fried (7) has recently presented evidence implicating the liver as a site of extrarenal erythropoietin production in the adult anephric rat.

In the present paper, evidence will be presented that the liver is normally an important site of erythropoietin production or activation in the weanling rat exposed to hypoxia.

Materials and Methods. Groups of male or female Sprague–Dawley rats weighing about 70 g were used at 21–22 days of age. Bilateral nephrectomy was performed as previously described (4). Partial hepatectomy was performed using the procedure of Higgins and Anderson (8) with methoxyflurane anesthesia (Metaphane, Pitman–Moore). About 70–80% of the liver was removed and, except for the blood removed in the liver, the operation was essentially bloodless. Rats were exposed to a simulated altitude of 22,000 ft (P_{O_2} 67 torr) in a decompression chamber for 5 hr immediately upon recovery from the anesthesia. Immediately after the hypoxic exposure, blood was collected from the abdominal aorta under ether anesthesia. Hematocrits were taken and the remainder of the blood was allowed to clot and the serum removed. The serum from 6–8 rats was pooled for erythropoietin assay.

Erythropoietin was assayed following the sc injection of 1 ml of serum in the 7-day post-CO female LAF_1/Jax plethoric mouse (4). The results are expressed as the mean % ± standard error of the injected radioiron incorporated in 72 hr into the calculated blood volume, which was assumed to be 7% of the body weight. Estimations of units of erythropoietin were made by reference to a standard curve prepared from the International Reference Preparation (IRP).

In a separate experiment, the blood volume of control and hypoxic hepatectomized rats was determined using ^{59}Fe-labeled red blood cells. Plasma volumes were estimated from the hematocrit. Blood pH was measured in these rats and in a group of nonhypoxic hepatectomized rats using the Radiometer BMS-3 pH electrode.

Results. The effect of partial hepatectomy and/or nephrectomy on the ability of weanling rats to respond to a brief hypoxic exposure with increases in the serum levels of erythropoietin is shown in Table I. The level of serum erythropoietin elicited by this hypoxic exposure is depressed from normal about 40–50% in male and female rats following partial hepatectomy. Removal of the kidneys, in addition to the partial hepatectomy, almost completely prevents erythropoietin production in weanling male rats.

The hematocrits of the normal and hepatectomized rats exposed to altitude were not significantly different; the values were

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TABLE I. Effect of Partial Hepatectomy and/or Nephrectomy on the Serum Erythropoietin Levels of Weanling Rats Exposed to Hypoxia for 5 hr.

Treatment	Serum erythropoietin	
	72-hr ^{59}Fe uptake	IRP units/ml serum
Male		
Normal + hypoxia	23 ± 0.91[a]	1.5
Hepatectomy + hypoxia	13 ± 1.4	0.9
Hepatectomy and nephrectomy + hypoxia	1.7 ± 0.38	0.05
Female		
Normal + hypoxia	29 ± 0.79	2.2
Hepatectomy + hypoxia	19 ± 2.1	1.1
Saline	0.72 ± 0.09	

[a] Standard error of the mean; 6–8 assay mice/group.

30.5 ± 0.9% and 30.8 ± 1.0%, respectively. The total blood volume of normal rats was 6.65 ml/100 g body wt, whereas the blood volume of the hepatectomized rats was 6.19 ml/100 g body wt. Thus, about 0.46 ml of blood or 6.9% of the total blood volume of the weanling rat was removed by hepatectomy. Estimation of the plasma volume from the measured hematocrits indicates that the plasma volume of the hepatectomized rat was 7.3% less than that of the control rat. About 0.32 ml of plasma/100 g body wt was removed by hepatectomy.

The blood pH of the control and hepatectomized rats after the hypoxic exposure were not significantly different; the values were 7.352 ± 0.021 and 7.348 ± 0.009, respectively. The blood pH of nonhypoxic hepatectomized rats 5 hr after recovery from anesthesia was 7.347 ± 0.008.

Discussion. It is clear that removal of a substantial portion of the liver in weanling rats significantly reduces the production of erythropoietin induced by a brief hypoxic exposure, which is in contrast to the adult rat in which partial hepatectomy does not interfere with erythropoietin production following the same hypoxic stress (9). Partial hepatectomy, combined with bilateral nephrectomy, almost completely prevents erythropoietin production in the weanling rat exposed to hypoxia immediately after operation, whereas bilateral nephrectomy alone has been previously demonstrated to have little effect on erythropoietin production in rats of this age (4, 5). In contrast to the weanling rat, bilateral nephrectomy in adult rats markedly reduces, but does not abolish, the production of erythropoietin following an hypoxic exposure. Removal of the kidneys and most of the liver also prevents erythropoietin production in adult rats exposed to hypoxia immediately after operation (7, 9). Erythropoietin produced in the anephric rat is indistinguishable immunologically from the hormone produced in the intact rat (2, 4).

The fact that the hematocrits of hepatectomized and control rats exposed to hypoxia are not different suggests that the observed reduction in serum erythropoietin titer is not because of expansion of the plasma volume. Indeed, the hormone content of the total plasma volume of the hepatectomized rat, compared with that of the control rat, is even less than the erythropoietin titer per ml of serum indicates, because the plasma volume of the hepatectomized rat is about 9% less than the plasma volume of the control rat.

It appears unlikely that the decreased production of erythropoietin in the hepatectomized rat is related to a shift in the oxygen dissociation curve via the Bohr effect with more efficient presentation of oxygen to the erythropoietin-generating tissues prior to the hypoxic exposure, because the blood

pH of the nonhypoxic hepatectomized rat is indistinguishable from that of the control rat.

Lucarelli *et al.* (10) previously concluded that erythropoiesis in the newborn rat was independent of renally produced erythropoietin. We previously concluded (11) that neonatal erythropoiesis was, however, erythropoietin dependent, as it was abolished by antierythropoietin antibody. The present experiments indicate that the liver and kidneys are major sites of erythropoietin production or activation in the weanling rat. We suggest that the extrarenal source of erythropoietin production, demonstrated by Carmena *et al.* (6) in neonatal rats, is probably the liver.

Summary. The ability of the weanling male and female rats to elevate their serum erythropoietin levels after a brief hypoxic exposure is significantly depressed following partial hepatectomy, and almost completely abolished following partial hepatectomy and nephrectomy.

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