Hepatic Erythropoietin Production in the Lead-poisoned Rat

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Extrarenal erythropoietin production is increased above normal in lead-injected rats exposed to hypoxia either immediately after or 1 day after nephrectomy. Extrarenal erythropoietin production in lead-poisoned rats is actinomycin D sensitive, and the major extrarenal site of production or activation of the hormone in normal or lead-poisoned anephric rats is the liver.

Anemia of various degrees of severity occurs following lead poisoning. This anemia is usually hypochromic with an increased reticulocyte count and increased red cell hemolysis. These changes are attributed to an inhibition of several enzymes involved in heme synthesis, an interference with the transport of iron, as well as changes in the erythrocyte membrane. A defect in erythroid differentiation and maturation has also been noted. Lead poisoning also results in renal tubular dysfunction, and characteristic intranuclear inclusion bodies are found in the tubular lining cells of rats.

The importance of functioning renal tissue for the regulation of normal erythropoiesis by the production or activation of the hormone erythropoietin is well established, as is the finding that extrarenal erythropoietin production occurs. Evidence will be presented that in the lead-poisoned rat (1) extrarenal erythropoietin production is increased, and (2) the liver is necessary for extrarenal erythropoietin production to occur.

MATERIALS AND METHODS

All experiments were performed using male Sprague-Dawley rats weighing 320–360 g. Bilateral nephrectomy was performed as described previously. Partial hepatectomy (80%) was performed using methoxyflurane (Metofane, Pitman-Moore) anesthesia. Rats were exposed to a simulated altitude of 22,000 ft (P02 67 torr) for 5 hr. In one experiment, a group of anephric hepatectomized rats were exposed to 28,000 ft (P02 52 torr) for 5 hr, and another group of similarly operated rats were bled (1.5 ml/100 g body wt) immediately before a 5-hr exposure to 22,000 ft. Blood was collected from the abdominal aorta under ether anesthesia, was allowed to clot, and the serum was removed. The serum from two three rats was pooled.

Lead acetate dissolved in 5% dextrose (25 mg/ml) was injected intravenously at a dose of 40 mg/kg body wt (0.105 mM/kg body wt). Actinomycin D was injected intravenously at a dose of 0.5 µg/g body wt. All serum samples were dialyzed against several changes of distilled water, lyophilized, and restored to volume with saline before bioassay.

Erythropoietin was assayed using the 7-day post CO female LAF1/JAX mouse. One milliliter
of test serum was injected subcutaneously. Each sample was assayed in six eight mice weighing 22-26 g. The results are expressed as the mean per cent ± 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 from the 72-hr $^{59}$Fe uptakes by reference to a standard curve prepared using the International Reference Preparation (IRP). It is assumed that the increase in radioiron incorporation in the plethoric assay mice was a direct result of erythropoietin in the injected sera.

RESULTS

The effect of lead, injected 1 day before nephrectomy or hepatectomy or both operations, on the production of erythropoietin as a result of a 5-hr hypoxic exposure, is compared to controls in Fig. 1. It is clear that the total production of hypoxic exposure, is not altered when lead is injected 1 day before the 5-hr hypoxic exposure (group 1 compared to 2). The production of the hormone by rats exposed to hypoxia immediately after nephrectomy is 4.5 times greater than in rats injected with lead 1 day before nephrectomy (group 3 compared to 4), whereas, injection of lead without a hypoxic exposure does not increase the serum erythropoietin level (group 5). Hepatectomy in the normal rat does not alter the amount of hormone produced as a result of the hypoxic exposure (group 6 compared to 1), but removal of the liver of rats injected with lead 1 day before the hypoxic exposure sig-

![Fig. 1. A comparison of the production of serum erythropoietin in rats receiving lead 1 day before a hypoxic exposure. Six-eight mice per group, no detectable (ND) difference from saline-injected controls.](image)
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Fig. 2. A comparison of the production of serum erythropoietin in rats receiving lead 2 days before a hypoxic exposure. Six-eight mice per group, no detectable (ND) difference from saline-injected controls.

A combination of hepatectomy and nephrectomy completely prevents the production of erythropoietin in normal and lead-injected rats. Serum erythropoietin levels are not elevated in anephric hepatectomized rats when the hypoxic exposure is increased to 28,000 ft or when the rats are bled before exposure to an altitude of 22,000 ft. The 72-hr \(^{59}\text{Fe}\) uptakes are, respectively, 0.86 ± 0.09 and 0.98 ± 0.1 compared to an uptake of 0.8% ± 0.1 in uninjected plethoric mice.

The effect of lead injection 1 day prior to nephrectomy on the production of serum erythropoietin, as a result of a 5-hr hypoxic exposure 1 day after nephrectomy, is compared to controls, as shown in Fig. 2. One day after nephrectomy, measurable levels of erythropoietin are not found in rats exposed to this hypoxic exposure for 5 hr (group A); however, if lead is injected 1 day prior to nephrectomy, significant production of the hormone occurs (group B). The production of erythropoietin in the lead-injected anephric rats is almost completely prevented by partial hepatectomy or by the injection of actinomycin D immediately before the hypoxic exposure (groups C and D). The injection of lead alone without a hypoxic exposure does not increase the serum level of the hormone in a similar time schedule (group E).

DISCUSSION

The importance of the kidney in the production or activation of erythropoietin in the rodent has been demonstrated by a number of investigators,\(^4\) since the initial observations of Jacobson and co-workers in 1957.\(^7\) Erythropoietin is produced in anephric rats exposed to hypoxia immediately after operation, but 1 day later the same hypoxic exposure does not result in erythropoietin production.\(^3\,\^8\,\^9\) The erythropoietin produced in anephric rats is indistinguishable immunologically from the hormone produced in normal rats.\(^3\,\^10\)
The production of extrarenal erythropoietin appears to require a more sustained hypoxic stimulus than renal erythropoietin production. The production of renal erythropoietin via a hypoxic stimulus is the result of de novo protein synthesis, and is decreased by lead injection.

The present experiments clearly indicate that, although renal production of erythropoietin in response to hypoxia is depressed by prior injection of lead acetate on this time schedule, extrarenal production of the hormone is much greater in injected animals. The processes involved in extrarenal erythropoietin production in lead-poisoned rats are inhibited by actinomycin D. The data also indicate that the liver is the major site of production or activation of extrarenal erythropoietin in the normal rat, corroborating the recent findings of Fried.

It might be objected that the failure of nephrectomized, partially hepatectomized rats to respond to hypoxia is related to a decreased oxygen demand in animals deprived of so much metabolically active tissue. The fact that these animals do not respond with production of erythropoietin, even when additionally stressed by bleeding prior to the 22,000-ft exposure, or by increasing the simulated altitude to 28,000 ft, strongly suggests that this failure is related to the absence of the liver itself, rather than to a decreased oxygen demand.

The increased extrarenal erythropoietin production in lead-poisoned animals suggests that processes involved in biogenesis of the hormone are increased above normal in the liver to compensate for the kidney dysfunction. Similar changes in the site of production or activation of the hormone may occur in renal disease.

REFERENCES
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