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Splenic Origin of Early-Labeled Bilirubin in Rats Exposed to High Altitude[1] (41060)

LO-CHANG OU AND ROGER P. SMITH

Department of Physiology and Department of Pharmacology and Toxicology. Dartmouth Medical School, Hanover, New Hampshire 03755

Abstract. The formation of early-labeled [^{14}C]bilirubin (ELB) from the pulse-injected heme precursor, [2-^{14}C]glycine was measured in rats. Chronic exposure to a simulated altitude of 18,000 ft increased the rate of ELB formation two- to three-fold over that in sea level controls for periods of up to 40 hr following precursor injection. Splenectomy significantly reduced the rate of ELB formation in altitude-exposed rats in the 0- to 5-hr interval after precursor injection, but splenectomy had no effect in sea level controls. Conversely, hypoxia increased the rate of ELB excretion in splenectomized rats in the 10- to 20-hr interval after precursor injection, but not at earlier time intervals. These results are consistent with our previous hypothesis that an exaggerated splenic erythropoiesis with both effective and ineffective components develops in the rat as a result of chronic exposure to extreme altitude.

We have recently documented the occurrence of hemoglobinemia in rats (1–3) and mice (4) chronically exposed to a simulated altitude of 18,000 ft (5500 m). Independently, Altland and Parker (5) also observed this phenomenon in rats under similar conditions of altitude exposure. In the past, hemoglobinemia in man invariably has been associated with intravascular hemolysis and a shortened erythrocytic life span (6). In contrast, hypoxia-induced hemoglobinemia in rats is characterized by a normal erythrocytic life span, and it occurs in the face of a greatly elevated hematocrit (1). The excretion rate for bilirubin exceeded predictions made from the estimated rate of destruction of circulating, senescent red cells by more than two-fold (3). It appeared unlikely that heme proteins other than hemoglobin could account for changes of that magnitude. Indeed, the degree of hemoglobinemia in altitude exposed animals could account for the excretion of the "extra" bilirubin, and acute splenectomy greatly reduced the magnitude of both (3).

These findings suggested that the "extra" bilirubin originated from the free hemoglobin in plasma which in turn must have originated from a source other than senescent circulating erythrocytes (3). We, therefore, suggest that the hypoxia-induced hemoglobinemia resulted from splenic ineffective erythropoiesis (3). This hypothesis is substantiated by our findings that splenic erythropoiesis plays the key role in the high-altitude polycythemic response in intact rats (7). Hypoxia may stimulate effective as well as ineffective components of erythropoiesis. As a result of the exaggerated ineffective component, however, large numbers of imperfect erythrocytes are formed. The breakdown of these imperfectly formed erythrocytes *in situ* or soon after their release into the peripheral circulation could result in hemoglobinemia and account for the observed excretion of "extra" bilirubin. It is unlikely that these imperfect erythrocytes could survive the ^{51}Cr-labeling procedure for erythrocyte survival studies. Thus, the loss of these imperfect cells during the labeling procedure would result in an apparently normal survival time for the remaining erythrocytes.

It has been well documented in man (8) and in animals (9–11) that ineffective erythropoiesis of pathophysiological origin is associated with an increased formation of so-called early-labeled bilirubin (ELB) from heme precursors such as [2-^{14}C]- or [^{15}N]glycine. The early-labeled fraction appears within a few hours of precursor injection; it may last for as long as 60 hr, but it is

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many days in advance of that fraction derived from hemoglobin in circulating red cells. The formation of the early-labeled [^{14}C]bilirubin from [2-^{14}C]glycine in intact and acutely splenectomized rats exposed to a simulated altitude of 18,000 ft was therefore investigated.

Materials and Methods. Male Sprague–Dawley rats weighing 250 to 300 g were exposed to a simulated altitude of 18,000 ft for 30 days (1). Control rats were kept in the same room at sea level pressure. Two days before the end of the exposure, a biliary fistula (12) with or without splenectomy was performed under ether anesthesia. Beginning 2 hr after the surgery, with the animal fully awake in a restraining metabolic cage, 100 μCi [2-^{14}C]glycine (5–20 mCi/mmole) was injected through a catheter inserted in the external jugular vein. Bile was collected in the dark over ice. The bile collection was conducted under room air for sea level control rats, and under hypoxic conditions for the altitude-exposed groups. The volume and bilirubin concentration (13) of each sample was measured. Bilirubin from each sample was separated, recrystallized and its specific activity measured in a Packard liquid scintillation spectrometer (14). Counting efficiency (%) was determined by a channel ratio method. The rate of labeled bilirubin production was expressed as dpm (disintegration per minute) in [^{14}C]bilirubin per 24 hr 100 g body wt. Values were plotted at the median time for each collection period and smooth curves were drawn by eye through these points.

Results. The increases in hematocrit and plasma hemoglobin, and the loss of body weight in intact altitude-exposed rats were comparable to those changes previously reported (1, 2). The effect of high-altitude exposure on the excretion of early-labeled bilirubin (ELB) in such animals is summarized in Fig. 1. The rate of excretion of ELB after [2-^{14}C]glycine injection in hypoxic rats was greater than that in sea level controls by a factor of 2 to 3 over the entire time course examined.

After acute splenectomy in both groups (Fig. 2) the rate of ELB formation in altitude-exposed rats did not differ from

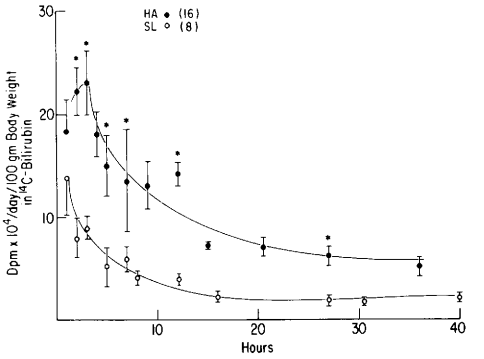

FIG. 1. Formation of early-labeled bilirubin in the bile of sea level (SL) and high altitude (HA) rats following intravenous injection of 100 μCi [2-^{14}C]glycine. Vertical bars = ±1 SD, * denotes $P < 0.05$ for comparison between SL and HA groups at that time. Figures in parentheses are the numbers of animals studied. HA rats were maintained at 18,000 ft for 30 days.

that in sea level controls over the first 10 hr following precursor injection. However, the rate of ELB excretion became significantly greater in hypoxic rats in the 10- to 20 hr interval after precursor injection.

For convenience the data in Figs. 1 and 2 have been summarized for equivalent 5 hr time intervals as Table I. It can be seen that the effect of splenectomy in hypoxic rats is to produce a decrease in the ELB excretion during the first 5 hr after precursor injec-

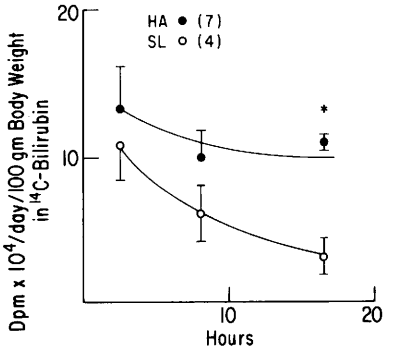

FIG. 2. Formation of early-labeled bilirubin in the bile of sea level (SL) and high-altitude (HA) rats following splenectomy in both groups and the intravenous injection of 100 μCi [2-^{14}C]glycine. Vertical bars ± 1 SD, * denotes $P < 0.05$ for comparison between SL and HA groups at that time. Figures in parentheses are the numbers of animals studied. HA rats were maintained at 18,000 ft for 30 days.

TABLE I. EFFECT OF SPLENECTOMY ON THE FORMATION OF EARLY-LABELED BILIRUBIN

	Sea Level			High Altitude		
	Hours after injection [2-^{14}C]glycine					
	0–5	5–10	10–20	0–5	5–10	10–20
Treatment	(dpm × 10^4 in [^{14}C]Bilirubin/100 g body wt)					
Intact rats	9.6 ± 5.9	5.2 ± 2.0	3.1 ± 1.2	20.0 ± 6.4[a]	13.1 ± 8.4[a]	10.9 ± 4.5[a]
Splenectomized rats	10.7 ± 4.7	6.8 ± 2.0	4.6 ± 2.8	12.7 ± 4.6	9.8 ± 3.8	11.0 ± 0.5[a]
P value	N.S.	N.S.	N.S.	0.01	N.S.	N.S.

[a] Significantly different from comparable values at sea level as indicated in Figs. 1 and 2.

tion, but not in the time intervals thereafter. In contrast splenectomy had no effect on ELB excretion in rats maintained at sea level.

Discussion. All of the previous work on the origins and kinetics of ELB excretion (8–12) was performed on normal animals or in animals where ineffective erythropoiesis was induced in bone marrow as by iron-deficiency anemia (10). In the latter condition the ELB fraction could be further separated into primary and secondary phases. The primary phase was somewhat arbitrarily said to last up to 3.5 hr, was associated with nonerythroid sources, and was tentatively identified as being hepatic in origin. The secondary phase occurred between 3.5 and 40 hr, was associated with erythroid components, and was markedly increased in the ineffective erythropoiesis of iron-deficiency anemia (10). The latter pattern is similar to that which we observed in splenectomized rats exposed to altitude (Fig. 2) where the "primary" phase of ELB excretion was normal but the "secondary" phase (10- to 20-hr period) was significantly increased.

In contrast, when intact and splenectomized hypoxic rats are compared (Table I) the ELB excretion is increased in the primary phase, but not at later time intervals. This pattern is similar to that ascribed to nonerythroid sources (10), but it is obvious that in this case it had a splenic rather than hepatic origin.

When iron deficient rats were treated with iron for 2 days prior to [^{14}C]glycine injection, the primary phase of ELB excretion was sharply elevated whereas the secondary phase seemed to be unaffected (10). This pattern was believed to represent increased ELB production from hepatic rather than erythroid sources since it was independent of either the production or the destruction of red cells. In our hypoxic rats, however, the increased primary phase of ELB excretion must have been of splenic origin since splenectomy decreased the magnitude of that phase in hypoxic rats down to that in rats maintained at sea level (Table I).

Our results in intact rats exposed to altitude (Fig. 1) differ from those in iron-deficient rats given supplemental iron (above) in that our animals seem to show increases in both the primary and secondary phases of ELB excretion. In medullary ineffective erythropoiesis such a pattern would be interpreted as reflecting increases in both nonerythroid and erythroid components of the ELB (10). These distinctions, however, made in iron-deficient rats may not be applicable to hypoxic rats where the ineffective erythropoiesis is of splenic origin. Our apparent ability to influence the ELB excretion pattern with hypoxia and with splenectomy suggests the existence of a two phase process, but we have not yet performed the experiments which would allow us to distinguish between erythroid and nonerythroid components. The differences between the medullary (10) and our splenic models may have some other basis such as anatomy or perfusion by the blood.

The preceding discussion thus makes it clear that the hepatic component does not account for the greatly enhanced primary phase of the excretion of the ELB of the hypoxic rats. It is possible that the enhanced secondary phase of ELB excretion

of the hypoxic animals is the result of a prolonged hepatic component. However, this explanation is unlikely, since it appears that degradation of hepatic cytochrome *P*-450, which is the major source of bilirubin originating from the liver, is suppressed in hypoxic rats (15).

Although the spleen appears to be responsible for the major erythropoietic effort in hypoxic rats, splenectomized animals exposed to similar conditions of hypoxia also become polycythemic and hemoglobinemic (2). Thus, in the absence of the spleen, the bone marrow appears to be activated also with both effective and ineffective components to the erythropoietic effort. The results in Fig. 2 could represent the activation of the ineffective erythropoietic component in bone marrow. An alternative explanation, however, is that the late increase is due to a subpopulation of imperfect cells, possibly reticulocytes, which originated from the spleen, but survived longer in the systemic circulation than the rest.

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