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Hemolytic Anemia Induced by Light Therapy in Jaundiced Rats[1] (40144)

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In the presence of increased concentrations of bilirubin, red cells exposed to fluorescent light undergo membrane damage and hemolysis *in vitro* (1–3). Overt hemolytic anemia has been described only rarely in human infants undergoing phototherapy for hyperbilirubinemia (4, 5). However, studies in jaundiced Gunn rats subjected to light therapy (6) have revealed evidence of increased bilirubin production, which was tentatively attributed to an increase in early-labeled bilirubin formation. The present study was performed to determine whether this increase in bilirubin production is in fact due to red cell hemolysis *in vivo,* in analogy with the findings *in vitro.*

Materials and methods. Experiments were performed with male homozygous Gunn rats weighing 300–350g. Since nonjaundiced heterozygotes were not available at the time of study, control experiments were performed with normal male Sprague-Dawley rats of similar age and size. Animals were maintained under normal laboratory conditions for a control period of 2 weeks. Body hair was then shaved off and for the next two weeks the rats were exposed to light under a fan-cooled reflecting canopy (kindly loaned to us by Dr. J. D. Ostrow) containing six 15-W Sylvania F15T8-D daylight fluorescent bulbs and providing approximately 1700 foot candles of light energy; the characteristics of the incident light are specified in a previous publication (7). After 2 weeks of light therapy the animals were returned to ambient laboratory conditions with the cages shielded from incident light because of the animals' exposed skin. After another 2-week control period several rats were re-exposed to light while receiving sodium salicylate in order to displace bilirubin from albumin in plasma (8, 9). These animals received intraperitoneal injections of 26–52 mg sodium salicylate 30 minutes before light therapy was begun and then 5 hr and 1, 2 and 3 days thereafter.

During the total of 6 or more weeks of study, heparinized blood samples were removed from the tail every 2–3 days for measurement of hematocrit, reticulocyte percentage (10), and plasma bilirubin concentration (11). Plasma haptoglobin concentration was measured electrophoretically as hemoglobin-binding capacity (12) at the end of the first control and light periods; electrophoresis of plasma to which no hemoglobin was added served as a screening test for plasma hemoglobin and methemalbumin. Blood was examined for Heinz bodies by incubation with methyl violet several times during the first day of light therapy; this test was also performed in four Gunn rats splenectomized 1 day before exposure to light. Since the animals lost approximately 1 ml blood per week through blood sampling, they were treated with 5 mg iron dextran (Imferon, Lakeside Laboratories) intramuscularly every 3–5 days to prevent iron deficiency. Stools were tested with guaiac during light therapy and uniformly gave negative results for occult blood.

In a separate experiment five Gunn rats received intravenous injections equivalent to 0.2 ml packed ^{51}Cr-labeled (13) red cells. Blood samples were removed at 24 hr and then every 2–3 days throughout 2 weeks under normal laboratory conditions. In three animals this was followed by 18 days of light exposure, while two animals were continued under control conditions. The animals were then sacrificed and the spleens and measured portions of the livers were counted in a γ scintillation spectrometer.

Results. The findings in a single Gunn rat are shown in Fig. 1 and the combined data for all four animals are summarized in Table I. With the onset of light therapy plasma bilirubin concentration fell rapidly to a new plateau approximately 40% below the control level. This change was promptly reversed on

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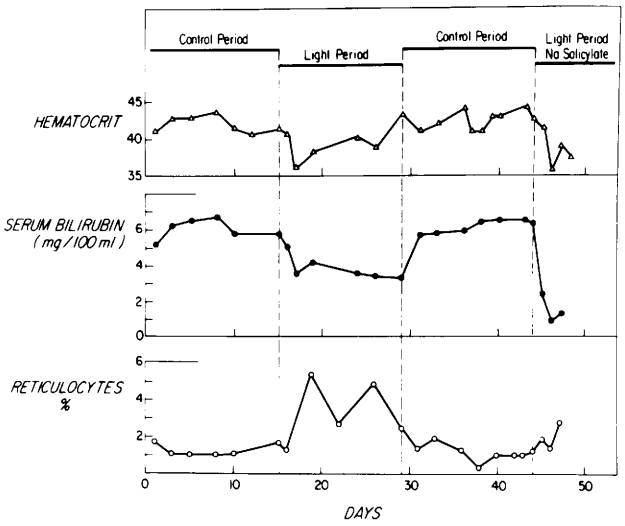

FIG. 1. Changes in hematocrit, reticulocyte percentage and serum bilirubin concentration in a Gunn rat during control periods and periods of fluorescent light therapy. Similar changes occurred in each of the 4 Gunn rats that were studied. During the second light period this Gunn rat also received injections of sodium salicylate in order to displace bilirubin from albumin in plasma.

TABLE I. EFFECT OF LIGHT THERAPY ON GUNN RATS.[a]

	Control period	Light period		Control period
	2 weeks	1st week	2nd week	2 weeks
Hematocrit, %	42.0 ± 0.6	38.4 ± 0.5	40.6 ± 0.6	42.2 ± 0.4
Reticulocytes, %	1.7 ± 0.1	2.8 ± 0.3	2.4 ± 0.3	1.3 ± 0.1
Bilirubin, mg %	5.9 ± 0.2	3.6 ± 0.2	3.6 ± 0.1	6.0 ± 0.1
Haptoglobin[b], mg %	147.6 ± 8.3	66.7 ± 13.3		—

[a] Values are means ± SE for all measurements in four Gunn rats during each period of study. Hematocrit, reticulocyte % and bilirubin concentration all differed significantly during the light as compared to both control periods, with $P < 0.01$ or 0.05 by 3-way analysis of variance and Duncan's multiple range comparison test.

[b] Haptoglobin was measured as hemoglobin-binding capacity at the end of the 2-week control and light periods. Values for control and light periods differed significantly, with $P < 0.04$ by paired-t test.

return to normal light conditions. Hematocrits were relatively stable during the initial control period and then decreased during the first few days of light therapy in all four animals. The nadir in hematocrit ranged from 35 to 38 and was reached at 2 days in two animals and at 4 days in the other two animals. Thereafter hematocrits increased slowly, returning to, or close to, baseline values by the end of the 2-week period of light therapy. Reticulocyte percentages increased during the light period in all four rats. Peak reticulocyte counts ranged from 3.4 to 5.4% and were usually observed on the fourth day of therapy. Reticulocyte percentages fluctuated during the light period, so that the mean values shown in Table I are lower than these peaks. However, they remained elevated throughout the entire light period and then returned to control levels within a few days after the animals were placed back under normal light conditions. Four nonicteric rats studied during similar periods of normal and light conditions showed no significant change in hematocrit or reticulocyte percentage.

In all four Gunn rats plasma haptoglobin concentration at the end of the light period was lower than control values (Table I). Trace amounts of hemoglobin were detected on electrophoresis of several plasma samples but these were from both the control and light periods and doubtless reflected some hemolysis during sampling of tail blood. Methemalbumin was not observed in any of these samples. No abnormalities in red cell morphology were discerned in peripheral blood smears during the light period. No Heinz

bodies were observed either in these animals or in four splenectomized Gunn rats on exposure to light.

After the second control period three Gunn rats were re-exposed to light while receiving injections of sodium salicylate. As anticipated (8, 9), serum bilirubin concentrations fell more dramatically than with light alone, reaching levels of 0.7–1.2 mg/100 ml within 2 days (Fig. 1). Hematocrits also decreased, but only to the extent observed in the earlier light period when no salicylate was given.

Computer analysis (PROPHET) of the ^{51}Cr red cell survival curves demonstrated that the rate of decline of ^{51}Cr cpm/ml RBCs or whole blood fell more rapidly in three Gunn rats after they were transferred from control to light conditions, in contrast to the findings in two Gunn rats that were continued under control conditions. However, the curves did not conform to the usual exponential kinetics, perhaps because of the absence of a steady-state of red cell turnover during light treatment. It was therefore not possible to establish accurate $t_{1/2}$ values for these curves. Additional ^{51}Cr studies were not performed for this reason and because these preliminary data served to corroborate the results based on hematocrit, reticulocyte % and plasma haptoglobin concentration. Measurements of organ sequestration (Table II) indicated similar values for ^{51}Cr in liver in light-treated and control animals. However, splenic ^{51}Cr levels were lower in the three light-treated rats than in the two controls.

Discussion. These studies demonstrate that light therapy in jaundiced Gunn rats leads to the rapid development of mild-moderate anemia associated with reticulocytosis. In the absence of blood loss, this constellation of findings reflects red cell hemolysis, as confirmed by depression of serum haptoglobin concentration and preliminary studies of ^{51}Cr-labeled red cell disappearance. Hematocrits gradually returned toward normal after the first few days of exposure to light, but haptoglobin levels remained depressed and reticulocytosis persisted, reflecting continuing hemolysis with improving marrow compensation throughout the duration of light therapy.

TABLE II. ORGAN SEQUESTRATION OF ^{51}Cr.[a]

	Spleen	Liver
	% of injected dose	
Controls (2)[b]	19.1, 22.5	2.9, 2.1
Light-treated (3)[b]	10.2, 8.5, 8.3	2.1, 2.1, 2.4

[a] Organ counts from ^{51}Cr-labeled red cells were measured after 2 weeks of control conditions followed by 18 days of light therapy. Controls were maintained under normal conditions during this entire period. Spleen and liver weights were comparable in treated and control rats.

[b] Number of Gunn rats studied.

Hemolysis was not observed in nonicteric animals subjected to light treatment. Hence, both hyperbilirubinemia and exposure to light must be present for hemolysis to occur. Studies in vitro have suggested that bilirubin acts as a photosensitizer, leading to photooxidative damage to the red cell membrane followed by cell lysis (1–3, 14). This might be expected to give rise to intravascular hemolysis *in vivo;* the decreased splenic uptake of ^{51}Cr in rats with light-induced hemolysis (Table II) appears consistent with this conclusion, although this might also reflect altered tissue affinity for ^{51}Cr as the result of light treatment. In the *in vitro* studies albumin had a protective effect on red cells (1–3), presumably by reducing the concentration of free bilirubin in plasma. In the present study attempts to exacerbate hemolysis by displacing bilirubin from albumin with sodium salicylate were unsuccessful, possibly because this may have caused only transient elevation of free bilirubin.

The clinical aim of phototherapy is to reduce plasma bilirubin concentration, and this was accomplished in all of the animals studied despite the increased pigment load imposed by the moderate hemolysis. Thus, acceleration of bilirubin excretion outweighed the increase in bilirubin metabolism. It should be noted that the amount and duration of light exposure in this study in rats exceeded that employed in most instances for human infants. Nevertheless, it seems possible that an increase in bilirubin formation as the result of hemolysis could sometimes offset the beneficial effect of phototherapy on bilirubin excretion. Brisk hemolysis has been described in one infant who also exhibited bronze skin pigmentation in the setting of light therapy (4). A controlled study of 22 infants (5) failed to demonstrate any decrease in hematocrit with phototherapy; however, other more sensitive indices were not mea-

sured to rule out a compensated hemolytic state. Serum bilirubin levels are sometimes not altered by phototherapy in infants (15, 16) or rats (6) even though bilirubin excretion is enhanced (6). Failures of phototherapy have been reported particularly in infants with jaundice due to Rh or ABO incompatibility (15, 16). Although this is probably due to the initial high rate of bilirubin production, it could be related in part to a direct hemolytic effect of light and high serum bilirubin concentration on red cells with preexisting membrane changes.

Summary. In jaundiced Gunn rats, but not in nonicteric animals, light therapy induced a moderate fall in hematocrit accompanied by reticulocytosis and diminished plasma haptoglobin concentration. There was decreased ^{51}Cr sequestration in the spleens of these animals, suggesting that the hemolysis was intravascular. Hematocrits rose again progressively after the first few days of light therapy, but the other parameters reflected continuing hemolysis with improved marrow compensation. Plasma bilirubin concentration fell in all animals studied. Hence, the increase in bilirubin production caused by this moderate hemolytic anemia was more than offset by light-induced enhancement of bilirubin excretion. However, it is possible that light-induced hemolysis could sometimes interfere with the beneficial effect of phototherapy on hyperbilirubinemia.

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