Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 12155–12159, December 1992
Genetics

High expression of human β^S- and α-globins in transgenic mice: Erythrocyte abnormalities, organ damage, and the effect of hypoxia

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Communicated by Helen M. Ranney, September 3, 1992 (received for review May 13, 1992)

ABSTRACT **A line of transgenic mice with two cointegrated transgenes, the human β^S- and α2-globin genes, linked to the β-globin locus control region was produced and bred with mice carrying a deletion of the mouse β^{major}-globin gene. In transgenic mice homozygous for the β^{major} deletion ($\alpha^H\beta^S[\beta^{MDD}]$; where α^H is human α-globin and MD is mouse deletion), 72.5 ± 2.4% (mean ± SD) of the β-chains are β^S and the ratio of α^H- to β^S-globin was 0.73. Introduction of a heterozygous mouse α-globin deletion into mice homozygous for the β^{major} deletion ($\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$) resulted in 65.1 ± 8.5% β^S and a human α/β ratio of 0.89 ± 0.2. Sickling occurs in 95% of erythrocytes from $\alpha^H\beta^S[\beta^{MDD}]$ mice after slow deoxygenation. Transmission electron microscopy revealed polymer fiber formation but not fascicles of fiber. Increased organ weight was noted in lung, spleen, and kidney of transgenic mice vs. controls that may be due to hypertrophy or increased blood volume in the lungs and/or increased tissue water content. The hemoglobin content of lung, spleen, and kidney was also elevated in transgenic animals due to trapped hemoglobin and/or increased blood volume. When transgenic and control mice were examined by magnetic resonance imaging at 9.4 tesla, some transgenic animals had enlarged kidneys with prolonged relaxation time, consistent with increased organ weight and water content. The glomerular filtration rate was elevated in transgenic animals, which is characteristic of young sickle cell patients. Furthermore, exposure to hypoxia resulted in significantly decreased hematocrit, increased erythrocyte density, and induced a urine-concentrating defect. We conclude that the transgenic mouse line reported here has chronic organ damage and further hematological and organ dysfunction can be induced by hypoxia.**

The development of several different transgenic mouse models for sickle cell disease (1–7) has the potential of elucidating the mechanism of vasoocclusion in sickle cell anemia. Sickle cell vasoocclusion is a multifactorial event that involves obstruction of the microcirculation by irreversibly sickled cells (8), nondeformable polymer-filled deoxygenated cells, and adhesion by deformable discocytes capable of contributing to the initiation or aggravation of vasoocclusion (9–11). The time interval or delay time between deoxygenation and the onset of polymer formation may play a role in determining both the frequency and severity of vasoocclusion (12).

Several organs are particularly susceptible to obstruction and ensuing damage in sickle cell disease. In this paper we will focus on the spleen, the kidneys, and the lungs. In the mouse, the spleen is both the site of erythrocyte (RBC) production and destruction. In sickle cell patients, the spleen is the site of infarction (13), potential sequestration (14), and autospenectomy in the second decade of life. The detailed pathophysiology of vasoocclusion need not be the same in each organ. For example, in some organs, such as the lung and kidney, the response to hypoxia is vasoconstriction instead of vasodilation, which will tend to make any occlusive events self-perpetuating.

Because of anatomic and physiological differences between animals and humans, any particular animal model may reproduce only some aspects of human sickle cell disease; however, insights may be gained from these differences since comparison of their impact on pathology may allow us to rank the relative importance of a particular feature.

We report here studies on sickling tendencies and organ damage (under ambient conditions and after exposure to hypoxia) in a line of transgenic mice that was created by the simultaneous microinjection and cointegration of LCR–β^S and LCR–α^H (LCR, locus control region; α^H, human α-globin) constructs on a normal mouse background (15). Higher levels of human β^S were achieved by breeding the transgenic mice with mice bearing a deletion of the mouse β^{major}-globin gene; when the β^{major} deletion was bred to homozygosity ($\alpha^H\beta^S[\beta^{MDD}]$, where MD is mouse deletion), expression of β^S averaged 72.7 ± 2.4% (mean ± SD) and the α^H/β^S ratio averaged 0.73. To reduce the synthesis of mouse α chains, the mice were bred to heterozygosity with mice carrying an α-globin deletion; the resulting transgenic mice ($\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$) displayed an increased α^H/β^S ratio of 0.89 but expressed a somewhat reduced level of β^S chains (65.1 ± 8.5%). A preliminary report of this work has appeared (16).

METHODS

Sickling. For kinetic measurements, blood samples were collected into heparinized saline and washed into 10 mM Hepes (pH 7.4) at 37°C containing 10 mM glucose, 10 mM KCl, and enough NaCl to adjust the osmolality to 330 milliosmolal. A plasma osmolarity of 327 milliosmolar was previously reported for mice (17) and was confirmed for both control and transgenic animals by measuring 20 plasma osmolarities (332 ± 16 milliosmolar, mean ± SD). Cells (100 μl) with a hematocrit (Hct) of 10% were placed into a stoppered vial and deoxygenated by an alternating vacuum/N_2 flow system for 5 min at 25°C. A 10 mM dithionite solution (4 μl) was added to the cell suspension (final value, 0.4 mM dithionite, pH 7.32, 336 milliosmolal); cells were removed anaerobically at intervals and added to a vial containing degassed 2% (vol/vol) glutaraldehyde in phosphate-buffered saline (PBS) at pH 7.4.

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Abbreviations: EM, electron microscopy; MRI, magnetic resonance imaging; GFR, glomerular filtration rate; RBC, erythrocyte; Hb, hemoglobin; MCHC, mean corpuscular Hb concentration; Hct, hematocrit; SS, sickle cell disease; SC, double heterozygote expressing both Hb S and Hb C.

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To study the extent of sickling after slow deoxygenation, blood samples were collected from the tail into heparinized capillary tubes, immediately sealed on both ends, and held at 25°C for 24 h. The ends of the tubes were cut off in a nitrogen-filled glove bag and the cells were extruded into vials containing degassed 2% glutaraldehyde in PBS (pH 7.4).

Scanning and Transmission Electron Microscopy (EM) of RBCs. Cells were washed in PBS (pH 7.4) and fixed in buffered 10% (vol/vol) formaldehyde, or for transmission EM in 2.5% glutaraldehyde, and examined as described (18, 19).

Organ Weights, Pathology, and Hemoglobin (Hb) Content. To determine organ weight, both control (C57BL/6J) and transgenic mice of various ages were anesthetized and partially exsanguinated by cardiac puncture; their organs were excised; fat and membranes were removed, blotted dry, weighed, and fixed in buffered 10% formaldehyde. To determine Hb content in tissues, 0.2 ml of blood was obtained from each mouse and labeled with ^{51}Cr by incubating the cells with 4 μCi of $Na_2{}^{51}CrO_4$ (1 Ci = 37 GBq) at room temperature for 1 h; 2 mg of ascorbic acid per ml of incubation mixture was added to the incubation vials at the end of 1 h to reduce any unbound dianionic ^{51}Cr. The cells were washed three times with sterile nonpyrogenic saline. Each mouse received 0.3 ml at Hct 25% of its own Cr-labeled cells by intraperitoneal injection. After 30 days, the specific activity per ml of cells was determined for each mouse, and the animals were sacrificed; their organs were weighed, and radioactivity was measured in a γ counter. Hb per gram of tissue was calculated for both control and transgenic animals.

Magnetic Resonance Imaging (MRI). Five control mice (two FVB/N and three C57BL/6J) and seven transgenic mice were examined by MRI at 9.4 tesla in a GE vertical widebore microimaging magnet. The images (see Fig. 4) were collected in a 25-mm i.d. probe, and the remaining animals were imaged in a 35-mm i.d. probe; both probes had birdcage coils. The repetition rate was 15 sec with an echo time of 20 msec. A slice thickness of 1 mm was used and 256 × 256 data points were collected with two averages. A slice selective 90° was used with a sinc pulse.

Renal Function. Glomerular filtration rate (GFR) for control (C57BL/6J) and transgenic mice (three $\alpha^H\beta^S[\beta^{MDD}]$ and one $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$) was examined by inulin clearance. The mice were anesthetized by i.p. injection of inaction at 10 mg/100 g (body weight), and PE10 catheters were inserted into a jugular vein for i.v. infusion of [^{14}C]inulin (New England Nuclear) in Ringer's solution and into a carotid artery for continuous monitoring of mean arterial blood pressure. A PE50 catheter was sutured into the urinary bladder for time collections of urine. The i.v. infusion rate was 1 ml per h per 100 g (body weight). Urine was collected into preweighed vials for determination of urine flow rate. Inulin clearance was calculated as described (20).

Induction of Hypoxia. Four transgenic (two $\alpha^H\beta^S[\beta^{MDD}]$ and two $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$) and two control (C57BL/6J) mice were subjected to 3–7 days of 8% O_2/0.5% CO_2/91.5% N_2. A previously established baseline Hct was used for all animals and small daily blood samples were taken from two of the transgenic animals; the other three animals were sampled at the end of 3 and 5 days of hypoxia. After 5 days of hypoxia, a density gradient determination (21) was done on an $\alpha^H\beta^S[\beta^{MDD}]$ mouse and a control mouse. A second group of animals were subjected to 7 days of hypoxia in 8% O_2/0.5% CO_2/91.5% N_2, two of the four transgenic animals, one $\alpha^H\beta^S[\beta^{MDD}]$ and one $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$ mouse, died on days 5 and 7, respectively. Urine osmolality was measured on day 2 and day 7 after overnight deprivation of water.

RESULTS

Sickling and RBC Morphology of Oxygenated and Deoxygenated Cells. Scanning and transmission EM were performed on $\alpha^H\beta^S[\beta^{MDD}]$ mouse cells subjected to both fast (alternate N_2 and vacuum) and slow (overnight in a capillary tube) deoxygenation, where 95% of the cells were found to be sickled. Many cells had the characteristic sickled and holly leaf morphology (Fig. 1*A*). Transmission EM revealed diffuse but clearly identifiable strands of polymer, but the well-organized fascicles characteristic of human sickled cells were not seen (Fig. 1*B*). In another set of experiments, cells were rapidly deoxygenated by alternate N_2 and vacuum and were then further treated with dithionite at low (0.4 mM) concentrations. The cells were examined for sickling at timed intervals from 1 min to 24 h. Under these conditions the percent sickled cells ranged from 22 to 75% at 1 min to 95% at 24 h (Fig. 2). The cells that sickled between 3.5 and 24 h were notable in that most of these cells were either single-domain cells or cells with a few parallel domains—that is, long thin bipointed cells rather than holly leaf forms.

Organ Weights and Hb Content. Thirty-seven transgenic (23 $\alpha^H\beta^S[\beta^{MDD}]$ and 14 $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$) and 16 C57BL/6J control mice of various ages were sacrificed and their lungs, spleens, and kidneys were weighed. Organ weight was normalized to total body weight (Fig. 3). There was no statistically significant difference in total body weight between adult control and transgenic animals; however, the variability of normalized organ weight between transgenic animals was notable. We found that there was a statistically significant increase in the weight of spleen, kidney, and lung in the transgenic animals. Abnormally large organs (defined as exceeding 1% of the animals total body weight) were excluded from the calculation of average weights. Normalized kidney weight was 0.58 ± 0.06 g/kg (mean ± SD; $n = 9$) for control mice and 0.68 ± 0.10 g/kg ($n = 25$) for all transgenic mice, which is significantly different with $P < 0.02$. For $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$ mice, kidney weight increased with age with a slope of 0.24% body weight/day, which was significant at $P < 10^{-4}$ ($r^2 = 77\%$). The same trend was noted for $\alpha^H\beta^S[\beta^{MDD}]$ mice but appeared to occur at an older age. Normalized spleen weights were higher for both $\alpha^H\beta^S[\beta^{MDD}]$ mice [0.40 ± 0.10 g/kg ($n = 11$)] and $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$ mice [0.42 ± 0.11 g/kg ($n = 9$)] than for C57BL/6J control mice [0.27 ± 0.06 g/kg ($n = 12$)], which was statistically significant at $P < 0.0006$ for all transgenic mice vs. control. The size of the spleen did not appear to be age dependent in mice >60 days of age. Normalized lung weight was 0.49 ± 0.3 g/kg ($n = 9$) for C57BL/6J control mice and 0.61 ± 0.08 g/kg ($n = 25$) for all transgenic

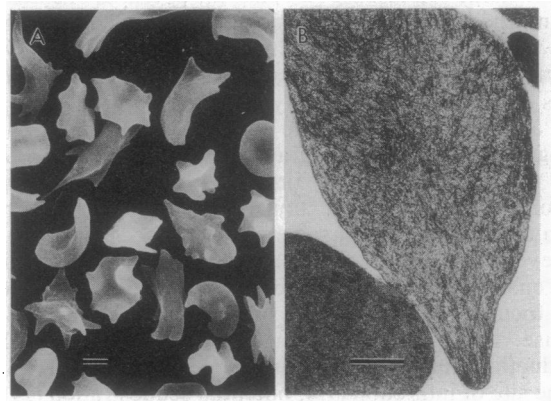

FIG. 1. (*A*) Scanning EM of cells from an $\alpha^H\beta^S[\beta^{MDD}]$ mouse that has been slowly deoxygenated and fixed. Note the small size of the mouse RBCs. (Bar = 2 μm.) (*B*) Transmission EM of sickled mouse RBCs from an $\alpha^H\beta^S[\beta^{MDD}]$ mouse. Note the presence of polymer but the absence of fascicles. (Bar = 1 μm.)

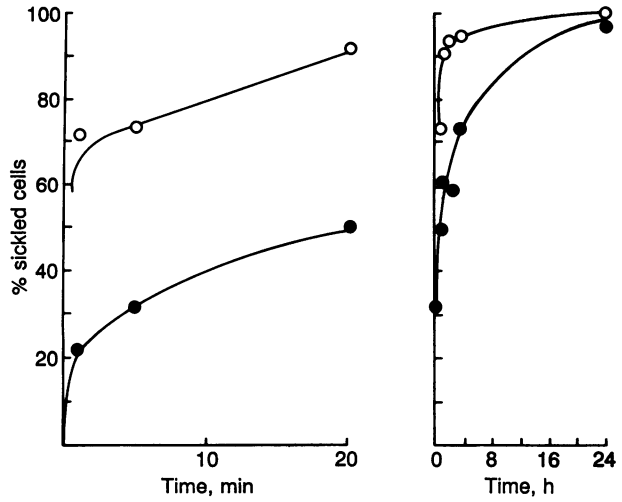

FIG. 2. Rate of sickling for $\alpha^{H}\beta^{S}[\beta^{MDD}]$ (open circles) and $\alpha^{H}\beta^{S}[\alpha^{MD}\beta^{MDD}]$ (solid circles) mice. Note that ≈95% of the cells sickle at 24 h.

mice, which is significantly different with $P < 0.023$. The weight of the lungs did not appear to be age dependent in mice >60 days of age.

During the course of dissection it was noted that the lungs of the transgenic animals were red rather than the normal pink-white of control mice. The Hb content of the lungs per gram of tissue was determined by ^{51}Cr labeling of the mouse RBCs and then sacrificing the animals 30 days after injection of the ^{51}Cr-labeled cells. The Hb content of the lungs was strikingly elevated. Control mice had 179 ± 37 cpm/g of tissue ($n = 2$) and the transgenic animals had 1067 ± 518 cpm/g of tissue ($n = 7$) with $P < 0.05$.

Pathology. $\alpha^{H}\beta^{S}[\beta^{MDD}]$ and $\alpha^{H}\beta^{S}[\alpha^{MD}\beta^{MDD}]$ mice were examined for gross pathological changes using light microscopy (Table 1). Abnormalities observed in mice expressing β^{S} included in some but not all individuals: iron deposits, focal scarring and erythropoiesis in the spleen, a striking expansion of the red pulp in the spleen of $\alpha^{H}\beta^{S}[\alpha^{MD}\beta^{MDD}]$ mice, congestion and septal thickening in the lungs, and congestion of the kidneys.

Noninvasive Analysis by MRI of β^{S} Transgenic Mice. Transgenic mice with $\alpha^{H}\beta^{S}[\beta^{MD}]$ and $\alpha^{H}\beta^{S}[\beta^{MDD}]$ were examined by proton MRI at 9.4 tesla. Transgenic mice were contrasted to thalassemic trait mice {in which the β^{S} gene was not introduced $((-)[\beta^{MD}])$} and to the C57BL/6J mice. In the control mice the intensity of the kidney in a proton density weighted sequence was less than that of the skeletal muscle of the back, whereas in the β^{S} mice the intensity of the kidney was equal to that of the skeletal muscle. Some β^{S} mice were found to have enlarged kidneys with prolonged transverse relaxation time (T_2) (Fig. 4, skeletal muscle, straight arrow; kidney, bent arrow), which suggests chronic kidney damage and edema; their spleens had short longitudinal relaxation time (T_1) and T_2, suggestive of iron overload. In some mice an intense band was also observed between the cortex and medulla, which is a region in which extravasation of contrast material due to disruption of the microcirculation has been reported in sickle cell disease patients. Our present data suggest that chronic renal damage occurs, since the prolonged T_2 is consistent with elevated tissue water content or edema, which frequently accompanies organ damage (22–24). Three out of seven transgenic mice examined had enlarged kidneys.

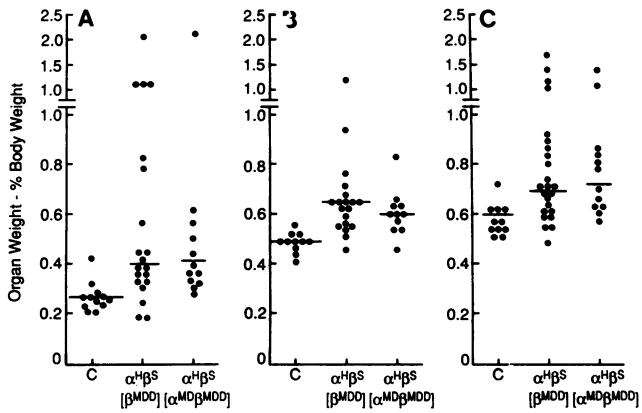

FIG. 3. Normalized organ weight for C57BL/6J (C), $\alpha^{H}\beta^{S}[\beta^{MDD}]$, and $\alpha^{H}\beta^{S}[\alpha^{MD}\beta^{MDD}]$ mice. (*A*) Spleen. (*B*) Lung. (*C*) Kidney. Note the wider variance of the transgenic animals.

Table 1. Organ pathology in the β^{S} transgenic mouse

Organ	$\alpha^{H}\beta^{S}[\beta^{MDD}]$	$\alpha^{H}\beta^{S}[\alpha^{MD}\beta^{MDD}]$
Lung	Congested (8/8) Thickened septa (2/8)	Congested (4/4)
Spleen	Fe^{2+} (7/7) Fibrosis (2/7)	Fe^{2+} (3/3) Expanded red pulp (3/3)
Kidney	Congested (7/7)	Congested (7/7)

Numbers in parentheses are number of animals in which condition was observed over number of animals examined.

Renal Function. Measurements of inulin clearance (GFR) in normal mice and $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mice revealed that GFR is ≈25% higher in transgenic animals (13.52 ± 0.3 ml/min·kg; $n = 4$) vs. control animals (9.74 ± 0.6 ml/min·kg; $n = 6$; $P < 0.01$). Urine osmolality measured after overnight water deprivation was not different than in control mice. This was the case both for mice studied under room air and after 48 h of hypoxia (10% O_2) in an environmental chamber.

Effect of Hypoxia on Hematology and Renal Function. When the animals were exposed to an atmosphere of 8% O_2/0.5% CO_2/91.5% N_2, the Hct fell to 70% of the control value in transgenic but not control animals (Fig. 5). One animal, an $\alpha^{H}\beta^{S}[\alpha^{MD}\beta^{MDD}]$ mouse, died on the fifth day of hypoxia and a second animal, an $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mouse, was sacrificed after 5 days of hypoxia and was found to have a hemorrhage of the renal papilla. Density gradient centrifugation revealed that the mean corpuscular Hb concentration (MCHC) of transgenic mice subjected to hypoxia increased by 2 g/dl over that of transgenic mice maintained under ambient conditions (Fig. 6); the MCHC of control mice was not affected by hypoxia. The densest cells were isolated and found to consist of ISCs, deformed cells, and cells that had lost membrane area. A second group of animals was subjected to 7 days of hypoxia

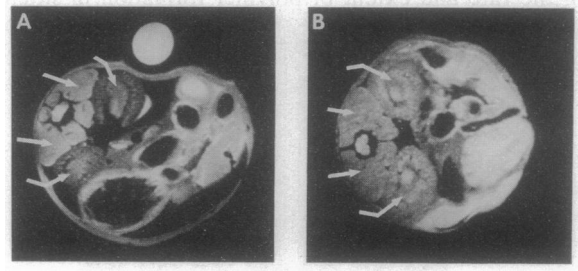

FIG. 4. MRI of the mouse kidney at 9.4 T using a proton density weighted pulse sequence. (*A*) Control mouse C57BL/6J heterozygous for the mouse β^{major} deletion $(-[\beta^{MD}])$. (*B*) Transgenic mouse $\alpha^{H}\beta^{S}[\beta^{MDD}]$. Note the difference in contrast between the kidneys (bent arrows) and paraspinal muscles (straight arrows) in the control and transgenic mouse. The higher intensity of the kidney in the transgenic mouse suggests longer relaxation times and a higher water content.

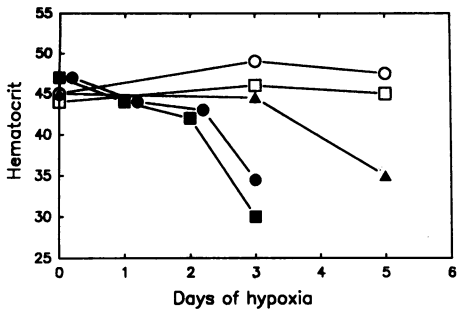

FIG. 5. Effect of hypoxia on the Hct of control (C57BL/6J, open symbols) and three transgenic mice (solid symbols). The mice were exposed to either 3 or 5 days of 8% O_2/0.5% CO_2/91.5% N_2. Control mice, open squares; $\alpha^H\beta^S[\beta^{MDD}]$ mice, solid circles and triangles; $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$ mouse, solid squares.

(8% O_2/0.5% CO_2/91.5% N_2), two of the five transgenic animals, one $\alpha^H\beta^S[\beta^{MDD}]$ and one $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$ mouse, died on days 5 and 7, respectively. Urine osmolality was measured on the seventh day for the surviving three transgenic mice and urine osmolality decreased to 70% of that found in control mice (Fig. 7).

DISCUSSION

In this paper we have examined the sickling tendencies, organ damage in steady state, and the effect of hypoxia in two transgenic lines; one homozygous for the mouse β^{major}-chain deletion ($\alpha^H\beta^S[\beta^{MDD}]$) and the other concomitantly heterozygous for the mouse α-chain deletion and homozygous for mouse β^{major} deletion ($\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$).

Sickling. When RBCs from transgenic mice were deoxygenated by alternating N_2 and vacuum, followed by low levels of dithionite, at 330 milliosmolar (normal mouse plasma osmolarity) between 20 and 75% of the cells sickled in <5 min and sickling continued for >12 h. These results indicate that polymer formation in most mouse RBCs is in the stochastic range in which polymerization mostly occurs from few nucleation sites in the cell and at any time a small but relatively constant percent of cells is at risk of polymer formation and sickling.

These results are in apparent disagreement with measurements of C_{sat} (concentration of deoxyHb in equilibrium with the polymer) that were reported (15) as similar to that of sickle trait hemolysates. The more rapid onset of sickling observed under physiological conditions points out two pitfalls in extrapolating from C_{sat} measurements to *in vivo* severity. In the mouse, the high plasma osmolarity and presence of Hb S result in a high MCHC, which increases both the rate and extent of polymer formation [a situation

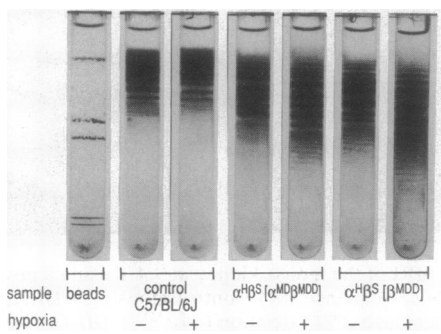

FIG. 6. Effect of hypoxia on the density distribution of control and transgenic RBCs. Note the increase in RBC density for transgenic mice subjected to hypoxia and the slight decrease in RBC density that occurs in the control mice.

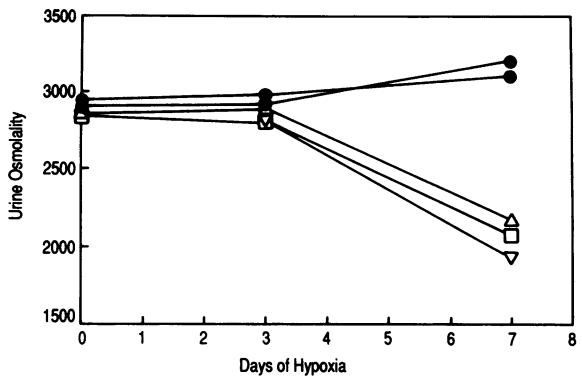

FIG. 7. Effect of hypoxia on the urine-concentrating ability of transgenic (open symbols) and control mice (solid symbols). Note that little change in urine osmolarity occurs in the first 3 days.

analogous to that seen in SC disease (25) where rapid sickling is observed despite a C_{sat} similar to that for sickle trait cells]; furthermore, heterogeneity of Hb distribution may result in some cells sickling much more rapidly than their cohort. Finally, although Rhoda *et al.* (26) demonstrated that a pure solution of one component of the mouse RBC, the tetramer $(\alpha^M\beta^S)_2$, has an extremely long delay time, the complex mixture of 10 tetramers formed from the four human and mouse dimers and heterodimers present in these RBCs may preclude simple extrapolation of delay time from relative oxygen saturation or from results with isolated tetramers.

Organ Damage at Steady State. Transgenic mice expressing high level of human β^S and α^H chains exhibit some chronic organ damage in addition to the mild hematological abnormalities as reported (15). A statistically significant increase in organ weight was noted in spleen, lung, and kidney of transgenic vs. control mice. This effect may be due to edema secondary to vasoocclusion in the kidney and lung, vascular engorgement, or to actual hypertrophy.

Iron was observed in the spleen of all transgenic animals compatible with increased RBC destruction. Three of 10 animals examined had fibrosis of the spleen, which is suggestive of past infarcts; again these infarcts are presumably due to sickling. Neither iron nor fibrosis was noted in control animals. Significantly expanded red pulp was noted in all $\alpha^H\beta^S[\alpha^{MD}\beta^{MDD}]$ mice examined, which is suggestive of both increased RBC destruction and possibly increased RBC production since erythropoiesis occurs in the adult mouse spleen. This result is compatible with the average reticulocyte count of 10% found in these animals that is higher than that found in the $\alpha^H\beta^S[\beta^{MDD}]$ animals, which exhibited only a modest expansion of red pulp.

In the lung, increased Hb content was noted both by gross observation of excised lungs and by measurement of tissue Hb content 30 days after injection of ^{51}Cr-labeled RBCs. A striking 10-fold increase in Hb per gram of tissue was noted in the transgenic animals. This may be due to increased blood volume in the lungs, some contribution from denatured Hb, and/or retention of sickled RBCs post-mortem.

The kidney weight relative to body weight of $\alpha^H\beta^S$-$[\alpha^{MD}\beta^{MDD}]$ mice increases with age, suggesting cumulative damage, vascular engorgement, or hypertrophy with age. MRI indicates prolonged relaxation time consistent with an elevated water content. Kidney weight also increases with age in $\alpha^H\beta^S[\beta^{MDD}]$ mice, but more heterogeneously and at a later average age. Fibrosis was observed in the kidneys of 2 of 10 animals examined, which suggests that infarcts may occur in some animals.

The GFR was found to be increased by ≈25% in transgenic animals maintained under ambient conditions when compared to control animals. Increased GFR is a characteristic of

young sickle cell patients and is usually attributed to anemia (27); since these mice are not anemic, this explanation for hyperfiltration is insufficient. No urinary concentrating defect was detected in animals maintained in room air.

Effect of Hypoxia. After 7 days of hypoxia, urine osmolality decreased to 70% of that found in control mice, which suggests that a urine-concentrating defect similar to that observed in sickle cell anemia (SS) patients after the first decade of life is induced by the higher levels of *in vivo* sickling that occur during hypoxia. The absence of the urine-concentrating defect under ambient conditions might be related to the unique vascular structure of the mouse medulla: The vascular bundles of the mouse renal medulla fuse into giant vascular bundles (28) that may affect the probability of obstruction due to sickled cells.

When control and transgenic animals were subjected to 3–5 days of hypoxia, the Hct decreased in transgenic but not in control animals. The MCHC of the transgenic animals also increased, which suggests that hypoxia may have a "snowballing" effect in which hypoxia results in sickling and altered intracellular cation content, which then leads to dehydration and more rapid polymer formation, sickling, and more severe vasoocclusion. Three of the eight transgenic animals subjected to hypoxia died within 5–7 days possibly of vasoocclusion; all of the four control mice survived.

Because hypoxia induces RBC destruction, generation of dense cells (cells with high intracellular Hb content), irreversibly sickled cells, and a urine-concentrating defect in transgenic but not in control mice, these effects are probably the result of increased *in vivo* sickling of transgenic mouse RBCs. These effects may be either due exclusively to an increase in the percent of nondeformable cells and/or the increased number of young cells that are potentially capable of adhesion and may play a role analogous to that postulated in human sickle cell vasoocclusion (11, 29). Further studies of this model may help distinguish between these alternatives.

In studies by Lutty *et al.* (30), retinopathy was detected in $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mice. The onset of retinopathy was age-dependent with no changes at 4 months, no change to severe changes by 12 months, and moderate to severe changes in a higher proportion of animals by 18 months. In animals >1 year of age, occlusion of arterioles, resulting in nonperfused areas of retina, and arteriovenous anastomoses were observed. Retinal and choroidal neovascularization and fibrous and fibrovascular preretinal membranes were found. Large numbers of retinal pigment epithelial cells migrated into the sensory retina and were observed in preretinal membranes and ensheathing major veins and venules. These findings are similar to those found in SS and SC disease in humans.

A major advantage of the transgenic mouse model is the possibility of studying chronic processes and the possibility of inducing with hypoxia abnormalities that then become amenable to pathogenic studies. Nevertheless, the transgenic mouse (as any other animal model) will necessarily differ substantially from the sickle cell disease patient because mouse anatomy and physiology differ from human (31). These differences can be turned into advantages because they might allow the assessment of the relative contribution of each of these factors to the pathogenesis of the disease. In addition, by reducing the severity of the phenotype in the steady state, it allows us to challenge the animal with hypoxia, low pH, or hyperosomolar solutions and follow the development of the abnormality. In a sicker animal model, in which spontaneous vasoocclusion is common and the phenotype is very severe, this opportunity would not be available.

In conclusion, the β^{S} transgenic mouse lines described here exhibit chronic organ alterations (spleen, lungs, kidney, and retina) under ambient conditions. In addition, the exposure to hypoxic conditions induces anemia, an increase of dense cells, and a renal concentration defect. These findings establish this animal model as a useful tool to understand vasoocclusion in organs and the processes leading to organ damage.

The assistance of Dominique Freeman of General Electric Nuclear Magnetic Resonance Instruments (Fremont, CA), in obtaining some of the magnetic resonance images is gratefully acknowledged. We are indebted to the Ultrastructural Center of Albert Einstein College of Medicine (Jane Fant and Frank Macaluso). The technical assistance of Fanya Schonbuch is gratefully acknowledged. This work was partially funded by National Institutes of Health Grants HL 37212, HL 21016, and HL 28381 and the American Heart Association, New York Affiliate.

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