

HEMOGLOBIN SYNTHESIS IN MICE DOUBLY HETEROZYGOUS  
FOR ALPHA AND BETA THALASSEMIA

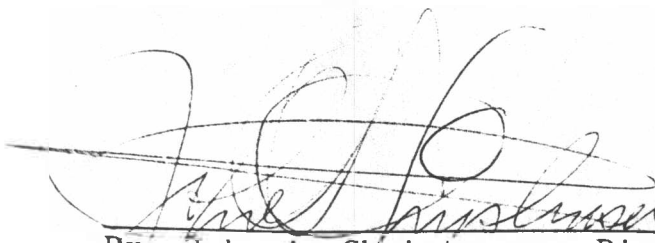
Submitted in Partial Fulfillment of the Requirements  
for Graduation with Honors to the Department of  
Biology at Carroll College, Helena, Montana.

Eric Jay Smith  
April 2, 1984

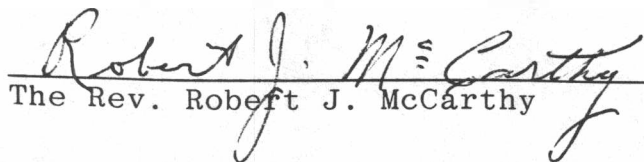
CORETTE LIBRARY  
CARROLL COLLEGE

CORETTE LIBRARY CARROLL COLLEGE  
  
3 5962 00084 660

This thesis for honors recognition has been approved  
for the Department of Biology.

  
Dr. John A. Christenson, Director

  
Dr. James J. Manion

  
The Rev. Robert J. McCarthy

April 2, 1984  
Date

## ACKNOWLEDGEMENTS

My sincere thanks go to Dr. Raymond A. Popp at Oak Ridge National Laboratory, for without his patience, understanding and guidance, I would not have been able to complete my research. I would also like to express my gratitude to Dr. Diana Popp for **instructing me in** hematology, and to thank Richard Winegar and Cindy Wawryzniak for letting me look over their shoulders while they were working.

My research was supported by a grant from the U.S. Department of Energy under contract with Oak Ridge National Laboratory and the Union Carbide Corporation.

I am greatly indebted to my thesis advisor, Dr. John A. Christenson, and my readers, Dr. James J. Manion and The Rev. Robert J. McCarthy, for taking the time and effort to review my thesis. I would also like to thank Cheryl McNurlin for taking on the formidable task of typing my thesis.

Lastly, I would like to express my gratitude to my parents, Lester and Janet Smith, whose never-ending support and encouragement has given me the strength to obtain my educational and career goals. Mom and Dad, I love you both very much.

## ABSTRACT

The extent of imbalance between the synthesis rate of  $\alpha$ -globin polypeptide chains relative to  $\beta$ -globin chains in thalassemic mice is one of the major factors in determining the pathophysiology of thalassemia. Mice doubly heterozygous for both a mutant  $\alpha$ -thalassemic chromosome and a  $\beta$ -thalassemic chromosome exhibited a hypochromic, microcytic anemia with mild reticulocytosis and erythrocytosis. However, the erythrocytes of these mice displayed few signs of hemolysis, or poikilocytosis, and did not contain visible inclusion bodies. Analysis of the relative rates of globin chain synthesis in vitro by  $^3\text{H}$ -leucine incorporation revealed the  $\alpha/\beta$  globin synthesis ratio was slightly greater than one and essentially no different from normal, non-thalassemic mice. Additional data suggests the erythroid cells of doubly heterozygous mice can partially compensate for the loss of almost half of their globin chain genes.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
ABSTRACT.....	iii
LIST OF TABLES.....	v
LIST OF ILLUSTRATIONS.....	vi
INTRODUCTION AND LITERATURE REVIEW.....	1
MATERIALS AND METHODS.....	3
RESULTS.....	11
DISCUSSION AND CONCLUSION.....	15
LITERATURE CITED.....	18

LIST OF TABLES

Table	Page
1. Amino acid concentrations.....	9
2. Hematologic parameters.....	12
3. The relative rates of $\alpha$ - and $\beta$ -globin synthesis by <u>in-vitro</u> $^3\text{H}$ -Leu incorporation into reticulo- cytes.....	14

## LIST OF ILLUSTRATIONS

Figure	Page
1. Cellulose acetate electrophoretic patterns of hemoglobin.....	5
2. A typical globin separation of normal SEC-mouse hemoglobin on an Amberlite IRC-50 chromatography column and corresponding radioactivity count.....	10

## INTRODUCTION AND LITERATURE REVIEW

Thalassemia is an hereditary disorder in man in which there is a deficient or absent synthesis of one or more of the globin polypeptide chains that comprise normal human hemoglobin (23). In the majority of cases, either the  $\alpha$  or  $\beta$ -polypeptide chain synthesis is defective (5), and has two immediate consequences: 1) the total amount of hemoglobin produced is decreased, and 2) the imbalance between the  $\alpha$  and  $\beta$ -chain synthesis rates results in an accumulation of the excess chains within the erythroid cells (9). In the more severe forms of  $\alpha$  and  $\beta$ -thalassemia, the free globin chains aggregate to form insoluble inclusion bodies which impinge upon the red cell membrane, increase cation permeability, and enhance the probability erythrocytes will be trapped and damaged in the reticulo-endothelial system (10). This leads to a characteristic hemolytic anemia with severe anisocytosis, poikilocytosis, and reticulocytosis (1).

Within the past several years, mouse models of human thalassemia have been discovered. Two heritable, radiation induced mutations and one chemically induced mutation have resulted in a deletion of the two  $\alpha$ -globin gene loci from chromosome eleven (7, 21). These mutations have

generated heterozygous mice with hematological abnormalities and a decreased  $\alpha$ -globin synthesis consistent with  $\alpha$ -thalassemia-1 (minor) in humans (13, 14). Since the homozygous  $\alpha$ -thalassemic condition is lethal in mice (12), as in humans, all further references to  $\alpha$ -thalassemic mice will represent the heterozygous state, unless otherwise noted.

Analogous to the  $\alpha$ -thalassemic mouse, a spontaneous mutation has resulted in the deletion of the  $\beta$ -major globin gene locus from chromosome seven and has produced mice possessing a deficiency of  $\beta$ -major globin polypeptides (18). When mice are homozygous for this aberrant chromosome they exhibit the hematologic anomalies observed in humans with  $\beta$ -thalassemia major or Cooley's anemia (18).

Past studies conducted on humans have shown that clinically severe forms of  $\alpha$  and  $\beta$ -thalassemia are partially ameliorated when there is a single  $\beta$ -thalassemia or  $\alpha$ -thalassemia gene present, respectively, within the same individual (3, 5, 6, 8, 19). Conjunctly, the  $\alpha/\beta$  globin synthesis ratio is more balanced in those individuals who possess both thalassemia genes.

The purpose of this study was to examine the hematologic parameters of mice afflicted with the various types of thalassemias, and in particular, the doubly heterozygous  $\alpha$ -thalassemic  $\beta$ -thalassemic mouse. And then attempt to correlate the observed pathophysiology or clinical severity with the degree of globin chain imbalance.

## MATERIALS AND METHODS

Mice: Mus musculus. The normal, control animals were strain SEC/1Re from inbred stock at Oak Ridge National Laboratory. The  $\alpha$ -thalassemic mice were designated 352HB and were the seventeenth generation progeny of nonirradiated strain 101/R1 females mated to the original  $\alpha$ -thalassemic mutant, a 600R x-irradiated SEC/R1 male (17). This line was inbred by repeated backcross with SEC/R1 mice. The  $\beta$ -thalassemic mice were descendants of B6D2 hybrid mice which were offspring of a  $\beta$ -thalassemic mutant, a DBA/2J male, who was mated to a C57B1/6J female. F<sub>1</sub> hybrids were backcrossed to normal B6 mice to maintain the mutation and were also mated inter se to produce homozygous mutants (18). Mice of inbred strains C57B1/6J and DBA/2J were purchased from Jackson Laboratory, Bar Harbor, Maine. Appropriate matings between  $\alpha$ -thalassemic and  $\beta$ -thalassemic mice were used to produce the desired double heterozygous  $\alpha$ -thalassemic  $\beta$ -thalassemic animals.

Identification of Hemoglobin Types. Alleles at the hemoglobin  $\alpha$ -chain loci are designated Hba<sup>1</sup> for 101/R1 and C57B1/6J mice, and Hba<sup>2,3</sup> for SEC/R1 (the deleted  $\alpha$ -chain locus in thallemic mice is indicated by a bar, -) (4, 11). The hemoglobin of 101/R1 and SEC/R1 mice

possessing the  $\alpha$ -chain deficiency and the hemoglobin of SEC/R1 controls have different solubilities and form unique types of crystals in potassium phosphate buffer (11, 17). Thus these two criteria can be used to classify normal and  $\alpha$ -thalassemic mice. The procedure involved collecting 0.1 ml of whole blood by suborbital sinus puncture (15) and lysing it with 0.6 ml of distilled water. The released hemoglobin was converted to carbon monoxyhemoglobin by bubbling CO through the lysate at a rate of 10 ml/min for 20 sec. Then 4 ml of 3.5 M  $K_2HPO_4$ - $KH_2PO_4$  buffer (pH 6.7) were added to the HbCO, mixed thoroughly, and the preparations placed in a water bath at 30° C for 21 hr (11). The precipitated hemoglobin in the samples was removed by filtration through Whatman No. 1 filter paper and the solubility of the filtrate determined by reading the O.D. at 575 nm. The crystals of the precipitated hemoglobin were examined under a light microscope at 150X. The observed crystalline structures included fine needles, hexagonal plates, large and small crystalline aggregates, and granular precipitates (11).

Alleles at the hemoglobin  $\beta$ -chain loci are designated Hbb<sup>d</sup> for 101/R1 mice, Hbb<sup>S</sup> for SEC/R1 and C57Bl/6J mice, and Hbb<sup>th-1</sup> for thalassemic mice (11, 17, 18). Mice possessing the  $\beta$ -thalassemia gene can readily be identified by the banding patterns (Fig. 1) that are produced by cellulose acetate electrophoresis of cystamine-modified hemoglobins (20). Briefly, the procedure was to obtain

a drop of tail vein blood, lyse it in 20 ul of distilled water, add 5 ul cystamine and mix thoroughly. Approximately 1 ul of this preparation was applied to mylar-backed cellulose acetate strips (Titan III, Helena Lab.) that were previously wetted with a buffer solution of 0.18 M TRIS, 0.10 M boric acid, 0.002 M EDTA, pH 8.6. The strips were electrophoresed at 300V/7.6 cm for 15-20 min.

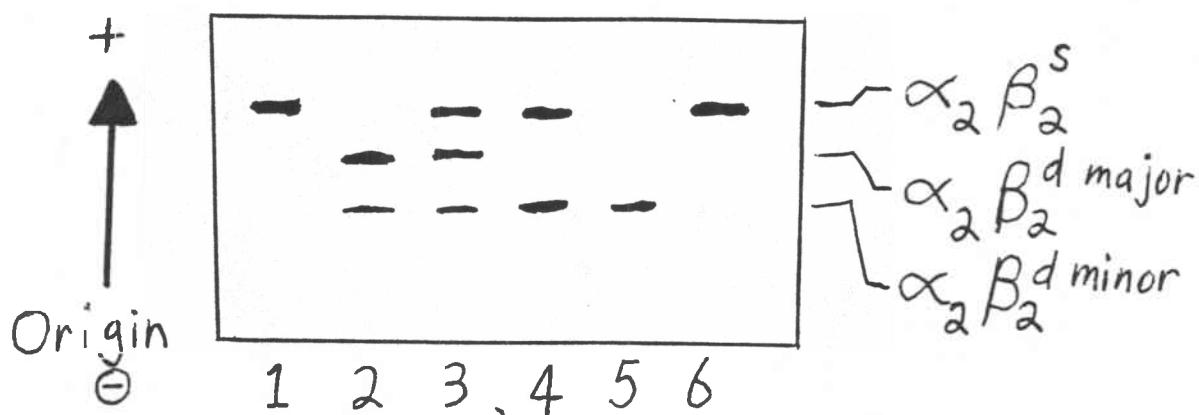


Fig. 1. Cellulose acetate electrophoretic patterns of hemoglobin.

The Hbb<sup>S</sup> haplotype produces only one type of  $\beta$ -globin,  $\beta$ -single. The Hbb<sup>d</sup> haplotype produces two distinct  $\beta$ -globins,  $\beta$ -major and  $\beta$ -minor. The Hbb<sup>th-1</sup> haplotype is a mutant of Hbb<sup>d</sup> in which the  $\beta$ -major gene locus has been deleted and thus only  $\beta$ -minor globin is present in the hemoglobin.

The hemoglobins were from (1) normal C57B1/6J (Hba<sup>1</sup>/Hba<sup>1</sup>; Hbb<sup>S</sup>/Hbb<sup>S</sup>), (2) normal 101/R1 (Hba<sup>1</sup>/Hba<sup>1</sup>; Hbb<sup>d</sup>/Hbb<sup>d</sup>),

(3) normal heterozygote ( $Hba^1/Hba^1$ ;  $Hbb^S/Hbb^d$ ), (4)  $\beta$ -thalassemia heterozygote ( $Hba^1/Hba^{2,3}$ ;  $Hbb^S/Hbb^{th-1}$ ), (5)  $\beta$ -thalassemia homozygote ( $Hba^1/Hba^1$ ;  $Hbb^{th-1}/Hbb^{th-1}$ ), (6) normal C57Bl/6J ( $Hba^1/Hba^1$ ;  $Hbb^S/Hbb^S$ ).

Hematologic Analysis. Hematology was performed on freshly drawn blood collected in heparinized microtubes from the retroorbital sinus. Hematocrit (microhematocrit method), red blood cell and nucleated cell (hemocytometer), hemoglobin (cyanmethemoglobin colorimetric method), and reticulocyte (new methylene blue supra vital staining) values were determined by standard procedures as described by Wintrobe *et al.* (23). Values for the mean corpuscular hemoglobin and mean corpuscular cell volume were subsequently determined. The nucleated cell counts represent the white blood cells plus the nucleated erythrocytes (normoblasts) that may be found in the blood of thalassemic mice.

Globin Chain Synthesis: Preparation of Reticulocytes. To increase the number of reticulocytes in the peripheral blood, the mice were given three subdermal injections of 0.5 cc phenylhydrazine (2 mg/ml 0.85% saline) administered at 48-hr intervals. Two days after the final injection, 1.5 ml of blood was drawn by suborbital sinus puncture from three mice of the same genotype. The cells were washed once in five volumes of 2% sodium citrate - 0.5% saline, centrifuged at 1500 rpm for 15 min, the supernatant drawn off, and the cells washed in five volumes of 0.85%

saline, centrifuged and washed in saline two additional times.

In Vitro  $^3\text{H}$ -Leucine Incorporation. The freshly prepared reticulocytes were incubated in 10 ml of a medium containing: 1 ml 5x Delbecco's minimal essential medium, 1 ml 2.2%  $\text{NaHCO}_3$ , 0.2 ml human transferrin (2.5 mg/ml), 1 ml  $^3\text{H}$ -Leucine (0.5 mCi/ml made 50  $\mu\text{l}$ /1.95 ml  $\text{H}_2\text{O}$  Schwartz/Mann Co), 1 ml amino acid (A.A.) pool minus Phe, Met, Leu, Gly<sup>a</sup>, 1 ml Phe and Met, 1 ml Gly, 3.55 ml distilled  $\text{H}_2\text{O}$ , and 0.25 ml ferrous ammonium sulfate (100 mg/10 ml). The pH of the medium was adjusted to 7.1 with 0.1 N HCl and the prepared reticulocytes added to the medium and incubated 90 min at 37° C in a 5%  $\text{CO}_2$  atmosphere. The mixture was then centrifuged at 2000 rpm for 10 min and the supernatant drawn off. The packed cells were washed in 0.85% saline, recentrifuged and washed two additional times, and lysed in four volumes distilled  $\text{H}_2\text{O}$ . The globin chains were precipitated out of solution, drop by drop, in 30-40 volumes of cold acidified acetone (-20° C) (16) and the mixture centrifuged at 2500 rpm for 5 min. The globin pellet was reprecipitated in cold acidified acetone, centrifuged, and the pellet dissolved to 5 ml with distilled  $\text{H}_2\text{O}$ .

Column Chromatography. The globin chains were separated from each other by chromatography through a column, size 1.9 x 90 cm, prepared with Amberlite IRC-50 (CG-50, Type 2) resin equilibrated in 11.7% formic acid (22).

<sup>a</sup>See Table 1, pg. 9, for A.A. concentrations.

The globin sample was applied to the column and washed in with 100 ml 11.7% formic acid, followed by 200 ml of 3 M urea (pH 1.9). Finally, 1400 ml of a 3M to 8M log gradient of urea (pH 1.9) were run over the column. All solutions were pumped through the column at a rate of 3.5-4 ml/min and fractions collected every five min. Absorbance at 280 nm of the protein in the eluate was recorded and 1 ml aliquots of each fraction were analyzed by  $^3\text{H}$  counts per minute in a liquid scintillation counter (Packard).

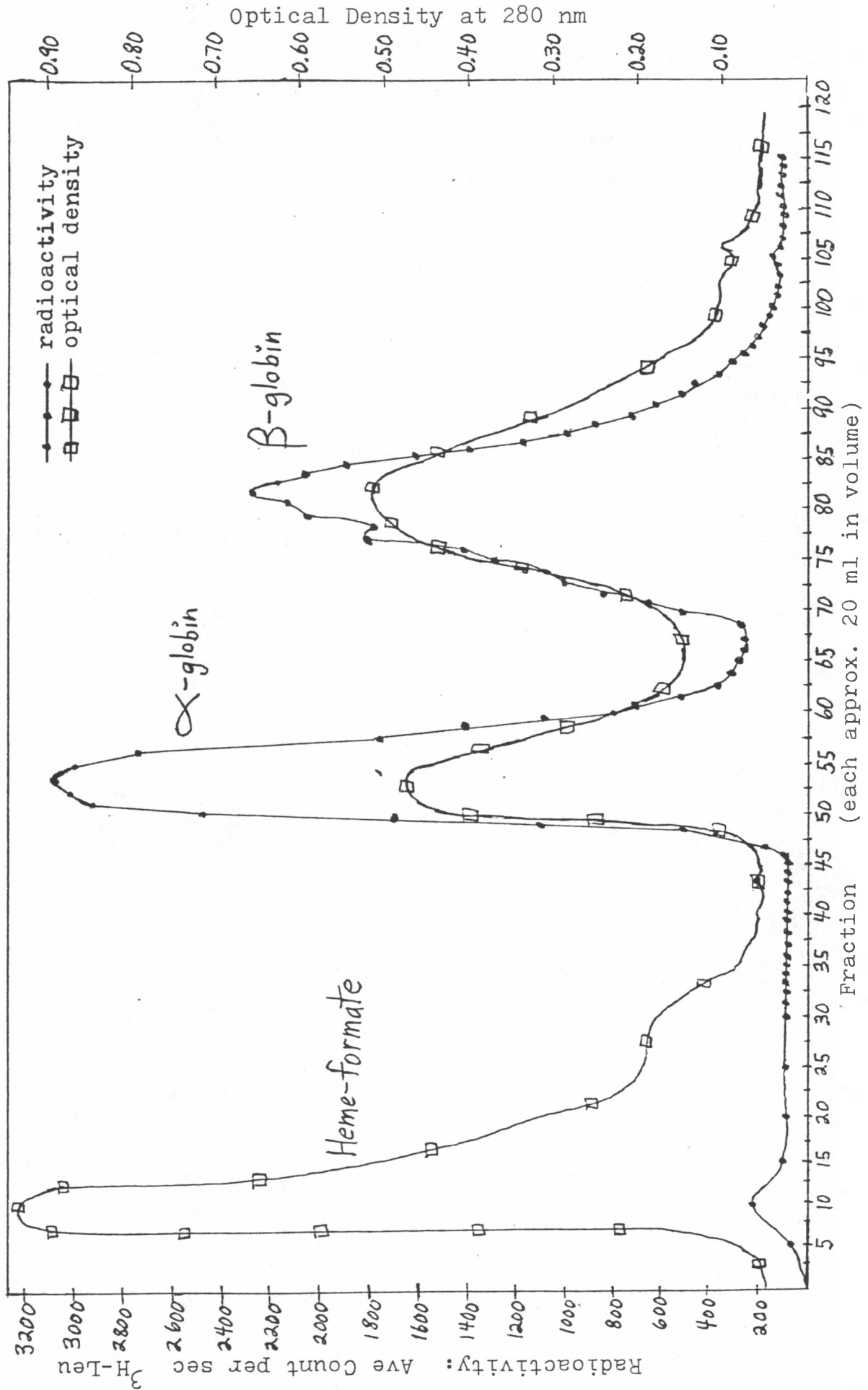
The  $\alpha/\beta$  ratios were calculated by finding the total counts per min for each globin chain using the corresponding curve of radioactivity, minus the background radiation (Fig. 2).

Statistical Methods. The mean and standard error of the mean were determined for each hematologic parameter and for the  $\alpha/\beta$  ratios. A t-test for two means was utilized to determine if the hematologic values of the double heterozygotes differed from normal mice (2).

Table 1. Amino acid concentrations (mg/100 ml)

Ala	66.8	Lys	73.0
Arg	21.0	Met	2.5
Asp	66.5	Phe	6.5
Asn	66.0	Pro	23.0
Cys	12.1	Ser	104.0
Gln	73.5	Thr	104.0
Glu	36.5	Trp	204.0
His	46.5	Tyr	27.7
Ile	39.3	Val	70.2
Leu	19.7	Gly	18.75

Fig 2. A typical globin separation of normal SEC-mouse hemoglobin on an Amberlite IRC-50 chromatography column and corresponding radioactivity count.



## RESULTS

Hematology. Wright's stained blood films from doubly heterozygous  $\alpha$ -thalassemic  $\beta$ -thalassemic mice revealed hypochromic erythrocytes with a fair number of microcytic cells. However, only a small number of poikilocytes were observed and inclusion bodies could not be detected within the red blood cells. Table 2 contains a summary of the hematologic parameters evaluated for not only the doubly heterozygous and normal mice, but also for the other various types of thalassemic mice, so that a quick visual comparison could be made.

The double heterozygotes exhibited a reduced hematocrit, Hct. ( $48.2 \pm 2.4\%$ ), and a statistically significant increase in both the red blood cell counts, RBC ( $13.90 \pm 0.8 \times 10^6/\text{mm}^3$ ), and the reticulocyte counts ( $5.2 \pm 0.8\%$ ), when compared to the normal control mice.<sup>a</sup> They also displayed a significant reduction in hemoglobin, Hb ( $14.92 \pm 0.60$  g/dl), mean corpuscular hemoglobin, MCH ( $10.8 \pm 0.7$  pg/rbc), and mean corpuscular volume, MCV ( $34.8 \pm 2.4$   $\mu\text{m}^3$ ).<sup>b</sup> The nucleated cell counts were not substantially different from the normal.

<sup>a</sup>Level of significance, 0.05 and 0.15, respectively.

<sup>b</sup>Level of significance, 0.2, 0.01, and 0.05, respectively.

Table 2. Hematologic Parameters<sup>a, b</sup>

MICE		Hct. (%)	RBC ( $\times 10^6$ mm <sup>3</sup> )	Nucleated Cells (/mm <sup>3</sup> )	Hb (g/dl)	MCH (pg/rbc)	MCV ( $\mu^3$ )	Retics. (%)
# Tested	Phenotype							
9	Normal	49.7 $\pm$ 1.7 (47-53)	12.03 $\pm$ 0.40 (11.63-12.63)	7,033 $\pm$ 1,215 (4,800-8,800)	15.99 $\pm$ 0.47 (15.40-16.63)	13.3 $\pm$ 0.4 (12.9-13.8)	41.3 $\pm$ 1.7 (39.6-43.6)	3.3 $\pm$ 0.8 (2.0-4.8)
9	Double Heterozygote	48.2 $\pm$ 2.4 (45-52)	13.90 $\pm$ 0.80 (13.00-15.63)	6,894 $\pm$ 2,589 (3,600-11,800)	14.92 $\pm$ 0.60 (14.09-15.92)	10.8 $\pm$ 0.7 (9.9-12.0)	34.8 $\pm$ 2.4 (32.0-39.1)	5.2 $\pm$ 0.8 (3.2-5.8)
3	$\alpha$ -Thalassemia	44.7 (44-45)	13.75 (12.50-14.63)	4,067 (3,400-4,400)	13.03 (12.77-13.07)	9.5 (8.7-10.5)	32.6 (30.1-36.0)	6.7 (5.0-7.6)
3	Heterozygous $\beta$ -Thalassemia	49.7 (47-53)	12.57 (11.63-14.25)	8,733 (7,000-10,700)	15.15 (14.80-15.86)	12.1 (11.1-12.7)	39.7 (37.2-42.1)	5.5 (4.8-5.8)
3	Homozygous $\beta$ -Thalassemia	35.7 (34-38)	9.65 (9.07-10.25)	10,967 (9,400-13,200)	11.46 (10.99-11.85)	11.9 (10.7-13.1)	37.0 (36.3-37.5)	19.3 (18.6-20.6)
3	$\beta^s$ -Duplication	48.0 (46-49)	11.67 (11.25-12.50)	4,267 (3,000-5,000)	15.01 (14.17-15.22)	12.9 (12.2-13.9)	41.2 (39.2-43.6)	6.3 (4.4-9.2)

<sup>a</sup> The range of values for each parameter are in parentheses, below the mean value.

<sup>b</sup> The standard error of the mean was computed only for normal and doubly heterozygous thalassemic mice.

Note: Hbb<sup>s</sup>-Hbb<sup>s</sup>/Hbb<sup>s</sup> denotes a duplication of the Hbb<sup>s</sup> locus.

Globin Synthesis. Measurements of the relative rates of synthesis of  $\alpha$ - and  $\beta$ -globin by  $^3\text{H}$ -leucine incorporation into the pooled reticulocytes are displayed on Table 3. The average  $\alpha/\beta$  synthesis ratio for the doubly heterozygous mice was  $1.06 \pm 0.06$  (standardized =  $1.04 \pm 0.02$ ), and could not be considered statistically different from the value obtained for normal mice,  $1.02 \pm 0.04$  (standardized = 1.00).

Table 3. The relative rates of  $\alpha$ - and  $\beta$ -globin synthesis by in-vitro  $^3\text{H}$ -Leu incorporation into reticulocytes.

Mouse Phenotype	Total Counts per min		$\alpha/\beta$	Standardized $\alpha/\beta$
	$\alpha$ -globin	$\beta$ -globin		
Normal: $\alpha^{\text{SEC SEC}} \beta^{\text{S S}}$	25,138	25,572	0.98	1.00
Double Heterozygote: $\alpha^{\text{C57 S}} \beta^{\text{S th}}$	16,018	15,982	1.00	1.02
Normal: $\alpha^{\text{SEC SEC}} \beta^{\text{S S}}$	9,105	9,053	1.01	1.00
Double Heterozygote: $\alpha^{\text{C57 S}} \beta^{\text{S th}}$	12,988	12,301	1.06	1.05
$\alpha$ -Thalassemia: $\alpha^{\text{SEC}} \beta^{\text{S S}}$	9,986	16,170	0.62	0.61
Homozygous $\beta$ -Thalassemia: $\alpha^{\text{C57}} \beta^{\text{th}}$	13,553	8,285	1.64	1.63
Normal: $\alpha^{\text{SEC SEC}} \beta^{\text{S S}}$	3,816	3,552	1.07	1.00
Double Heterozygote: $\alpha^{\text{C57 S}} \beta^{\text{S th}}$	14,088	12,537	1.12	1.05
Heterozygous $\beta$ -Thalassemia: $\alpha^{\text{C57 SEC}} \beta^{\text{S th}}$	13,217	10,669	1.24	1.15
$\beta^{\text{S}}$ -Duplicate: $\alpha^{\text{SEC SEC}} \beta^{\text{S S}}$	16,311	16,399	0.99	0.93

## DISCUSSION AND CONCLUSION

From a molecular standpoint, one might predict that the doubly heterozygous  $\alpha$ -thalassemic  $\beta$ -thalassemic mouse would have a drastically reduced hemoglobin synthesis, perhaps as much as 50%. These mice contain only two out of a normal complement of four  $\alpha$ -globin genes (7, 21), and are missing a  $\beta$ -major globin gene which codes for approximately 40% of the  $\beta$ -globin polypeptides found in the hemoglobin of normal  $\underline{Hba}^1/\underline{Hba}^1$ ;  $\underline{Hbb}^S/\underline{Hbb}^d$  mice ( $\beta$ -minor globin comprises 10% and  $\beta$ -single globin is found in 50% of the total hemoglobin) (14, 18). However, the hematologic data does not indicate such a severe depression in the amount of hemoglobin produced. The fact that the mean corpuscular hemoglobin is 81% (10.8 pg/rbc / 13.3 pg/rbc) of the normal suggests there is an increased expression of both the remaining  $\alpha$  and  $\beta$ -globin genes. A similar modulation of gene expression has been documented in both  $\alpha$ -thalassemic and in  $\beta$ -thalassemic mice (7, 18).

The clinical hematology of the double heterozygotes reveals a hypochromic anemia. Although the amount of hemoglobin within the erythroid cells is reduced, as is the size of the red blood cells, a compensatory erythrocytosis has increased the number of erythrocytes and brought

the total amount of hemoglobin in the blood up to 93% of the normal. The nucleated cell counts of the double heterozygotes are normal and show there is no increase in the amount of circulating nucleated erythrocytes. This evidence, along with the observed mild reticulocytosis, slight poikilocytosis, and the absence of inclusion bodies and cellular debris, suggests little hemolysis is occurring (5). This seems to be correlated with a balanced  $\alpha/\beta$  globin synthesis rate, where there are no excess globin chains accumulating within the cell.

Further support that hemolysis in thalassemia is associated with unpaired, excess globin chains in the erythrocytes can be seen by examining the hematologic data reported for the other types of thalassemic mice. The hematologic values presented on Table 2 and previously described by Popp and Enlow in 1977 and Skow et al. in 1983, show that  $\alpha$ -thalassemic and homozygous  $\beta$ -thalassemic mice exhibit a hypochromic, hemolytic anemia, whereas heterozygous  $\beta$ -thalassemic mice show no anemia. Correspondingly, the reticulocytes of the  $\alpha$ -thalassemic and homozygous  $\beta$ -thalassemic mice possess unbalanced  $\alpha/\beta$  globin synthesis rates, as shown in Table 3 and as previously reported by Martinell et al. in 1981 (0.75 to 0.80) (7) and Skow et al. in 1983 (1.28) (18), respectively. In comparison, the heterozygous  $\beta$ -thalassemic mice have a much more balanced globin synthesis rate of 1.24 (Table 3), 1.09 and 1.02 as reported by Skow et al. in 1983 (18).

It should be noted that while a balanced  $\alpha/\beta$  synthesis rate precludes a more normal hematological profile, it is only one of the factors which determine the pathophysiology of thalassemia. The other major determinant is the total amount of hemoglobin produced, and when this is decreased, an anemic condition will result (23).

The concurrent existence of  $\alpha$ -thalassemia and  $\beta$ -thalassemia genes in doubly heterozygous mice has lessened the clinical severity that might be expected when almost half of the globin genes are deleted. A similar mild thalassemia occurs in humans who possess both  $\alpha$  and  $\beta$ -thalassemia genes (5).

The development of a doubly heterozygous thalassemic mouse will be extremely useful in investigating the modulation of gene expression that occurs due to the interaction of  $\alpha$  and  $\beta$ -thalassemia genes. Hopefully, the information gained by studying these animals will help provide some insight into the gene regulation and expression that occurs in humans afflicted with thalassemia.

#### LITERATURE CITED

1. Briton, C.J., and L.E. Howard. 1963. Disorders of the Blood. J. & A. Churchill Ltd., London. pp. 370-2, 480-4.
2. Fisher, R.A., and F. Yates. 1963. Statistical Tables for Biological, Agricultural and Medical Research. Oliver and Boyd, Ltd., Edinburgh.
3. Furbetta, M., R. Galanello, A. Ximenes, A. Angius, M.A. Melis, P. Serra, and A. Cao. 1979. Interaction of alpha and beta thalassaemia genes in two Sardinian families. J. Haemat. 41: 203-210.
4. Hilse, K., and R.A. Popp. 1968. Gene duplication as the basis for amino acid ambiguity in the alpha-chain polypeptides of mouse hemoglobins. Proc. Natl. Acad. Sci. USA 61: 930-936.
5. Kan, Y.W., D.G. Nathan. 1970. Mild thalassemia: the result of interactions of alpha and beta thalassemia genes. J. Clin. Invest. 49: 635-642.
6. Loukopoulos, D., A. Loutradi, and P. Fessas. 1978. A unique thalassaemic syndrome: homozygous alpha-thalassaemia and homozygous beta-thalassaemia. Br. J. Haemat. 39: 377-389.
7. Martinell, J., J.B. Whitney, R.A. Popp, L.B. Russell, and W.F. Anderson. 1981. Three mouse models of human thalassemia. Proc. Natl. Acad. Sci. USA 78: 5056-5060.
8. Melis, M.A., R. Galanello, and A. Cao. 1982. Alpha globin gene analysis in a Sardinian family with interacting alpha and beta thalassaemia genes. Br. J. Haemat. 53: 667-671.
9. Nathan, D.G., R.B. Gunn. 1966. Thalassemia: the consequences of unbalanced hemoglobin synthesis. Am. J. Med. 41: 815-830.

10. Nathan, D.G., T.B. Stossel, R.B. Gunn, H.S. Zarkowsky, and M.T. Laforet. 1969. Influence of hemoglobin precipitation on erythrocyte metabolism in alpha and beta thalassemia. J. Clin. Invest. 48: 33.
11. Popp, R.A. 1969. Studies on mouse hemoglobin loci. VIII. A fourth alpha-chain phenotype. J. Heredity 60: 126-133.
12. Popp, R.A., B.S. Bradshaw, and L.C. Skow. 1980. Effects of alpha thalassemia on mouse development. Differentiation 17: 205-210.
13. Popp, R.A. and M.K. Enlow. 1977. Radiation-induced alpha-thalassemia in mice. Am. J. Vet. Res. 38: 569-572.
14. Popp, R.A., L.P. Stratton, D.K. Hawley and K. Effron. 1979. Hemoglobin of mice with radiation-induced mutations at the hemoglobin loci. J. Mol. Biol. 127: 141-148.
15. Riley, V. 1960. Proc. Soc. Exp. Biol. Med. 104: 751-754.
16. Rossi-Fanelli, A., E. Antonini, and A. Caputo. 1958. Biochem. Biophys. Acta. 30: 608.
17. Russell, L.B., W.L. Russell, R.A. Popp, C. Vaughan, and K.B. Jacobson. 1976. Radiation-induced mutations at mouse hemoglobin loci. Proc. Natl. Acad. Sci. USA 73: 2843-2846.
18. Skow, L.C., B.A. Burkhardt, F.M. Johnson, R.A. Popp, D.M. Popp, S.Z. Goldberg, W.F. Anderson, L.B. Barnett, and S.E. Lewis. 1983. A mouse model for beta-thalassemia. Cell.
19. Weatherall, D.J., L. Pressley, W. Wood, D.R. Higgs, and J.B. Clegg. 1981. The molecular basis for mild forms of homozygous beta-thalassaemia. Lancet 1: 527-529.
20. Whitney, J.B., III. 1978. Simplified typing of mouse hemoglobin (Hbb) phenotypes using cystamine. Biochem. Genet. 16: 667-672.
21. Whitney, J.B., III, J. Martinell, R.A. Popp, L.B. Russell, and W.F. Anderson. 1981. Deletions in the alpha-globin gene complex in alpha-thalassemic mice. Proc. Natl. Acad. Sci. USA 78: 7644-7647.

22. Wilson, S. and D.B. Smith. 1959. Separation of the valyl-leucyl and valyl-glutamyl-polypeptide chains of horse globin by fractional precipitation and column chromatography. Canad. Jour. Biochem. Physiol. 37: 405-416.
23. Wintrobe, M.M., G.R. Lee, D.R. Boggs, T.C. Bithell, J.W. Athens, and J. Foerster. 1981. Clinical Hematology. Eighth Ed., Lea and Febiger, Philadelphia. pp. 7-32, 869-903.