2

C00-2005-8

FINAL REPORT
to the
U.S. ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
BIOCHEMICAL ASPECTS OF ERYTHROPOIESIS IN
CHRONIC IRRADIATION
UNDER THE DIRECTION OF
CHARLES E. BRAMBEL
PROFESSOR OF CHEMISTRY
(EMERITUS)
IN THE
DEPARTMENT OF CHEMISTRY
OF THE
UNIVERSITY OF NOTRE DAME

MASTER

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

leg

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

INTRODUCTION

Spontaneous recovery of splenic erythropoiesis during chronic irradiation of rats results in the elaboration of an altered hemoglobin molecule. Several basic physical criteria characterize this altered hemoglobin. A number of questions arise regarding this phenomenon:

- (1) is it a response to the stimulus of anemia?
- (2) does the source of hemoglobin reside in the normoblasts arising in the spleen?
- (3) have the normoblasts migrated from the bone marrow compartment to the spleen and are now synthesizing an altered hemoglobin? Is this an effect of the new environment?
- (4) Or, have normoblasts arisen from latent precursors in the spleen and develop in an environment favorable for hemoglobin synthesis?

Experimental studies revealed that splenic erythropoiesis was a response to the anemia which developed as a result of the chronic suppression of erythropoiesis. This was shown by producing marked splenic erythropoiesis at earlier times during exposure to irradiation in response to bleeding, hemolysis, or anoxia.

EXPERIMENTAL PROCEDURES AND MATERIALS

Peripheral blood and organs were obtained from the following groups of experimental rats. The biochemical procedures used are indicated.

I. HEMOGLOBIN

A. polyacrylamide disc gel electrophoresis

1. analytical

- a) non-irradiated
- b) non-irradiated splenectomized
- c) chronically irradiated, hypoxic environment
- d) chronically irradiated, splenectomized, hypoxic environment
- e) erythroid spleen (from chronically irradiated rats in hypoxic environment) (normoblasts)
- f) fetal
- g) components from ion exchange chromatography
 - (1) non-irradiated
 - (2) non-irradiated splenectomized
 - (3) chronically irradiated, hypoxic environment
 - (4) chronically irradiated, splenectomized, hypoxic environment

2. sodium dodecyl sulfate gels: components from ion exchange chromatography
 - a) non-irradiated
 - b) non-irradiated, splenectomized
 - c) chronically irradiated, hypoxic environment
 - d) chronically irradiated, splenectomized, hypoxic environment
- B. ion exchange chromatography; - analytical and preparative CMC cellulose and IRC-50
 - a) non-irradiated
 - b) non-irradiated splenectomized
 - c) chronically irradiated, hypoxic environment
 - d) chronically irradiated, splenectomized, hypoxic environment
- C. alkali resistance
 - a) non-irradiated
 - b) non-irradiated splenectomized
 - c) chronically irradiated, hypoxic environment
 - d) chronically irradiated, splenectomized, hypoxic environment
 - e) chromatographic components (CMC)
 - (1) non-irradiated
 - (2) chronically irradiated, hypoxic environment

II. GLOBIN

A. polyacrylamide disc gel electrophoresis

1. analytical

- a) non-irradiated
- b) non-irradiated splenectomized
- c) chronically irradiated, hypoxic environment
- d) chronically irradiated, splenectomized,
hypoxic environment
- e) chromatographic components
 - (1) non-irradiated
 - (2) chronically irradiated, hypoxic environment
 - (3) chronically irradiated, splenectomized,
hypoxic environment

2. sodium dodecyl sulfate gels: components from ion exchange chromatography IRC-50

- a) non-irradiated
- b) non-irradiated splenectomized
- c) chronically irradiated, hypoxic environment
- d) chronically irradiated, splenectomized,
hypoxic environment

3. urea gels: components from ion exchange chromatography IRC-50

- a) non-irradiated
- b) non-irradiated splenectomized
- c) chronically irradiated, hypoxic environment
- d) chronically irradiated, splenectomized, hypoxic
environment

- B. ion exchange chromatography: - CMC cellulose and IRC-50
 - 1. non-irradiated
 - 2. non-irradiated, splenectomized
 - 3. chronically irradiated, hypoxic environment
 - 4. chronically irradiated, splenectomized, hypoxic environment
- C. sedimentation patterns
 - 1. non-irradiated
 - 2. chronically irradiated, hypoxic environment
- D. amino acid composition of chromatographic components (IRC-50)
 - 1. non-irradiated
 - 2. chronically irradiated, hypoxic environment
- E. end group analysis of chromatographic components (IRC-50)
 - 1. non-irradiated: - N-terminal
 - 2. chronically irradiated, hypoxic environment: - N-terminal

RESULTS

Several modifications of disc gel electrophoresis were used to ascertain the pattern of separation of the different hemoglobins prepared from erythrocytes: - analytical and sodium dodecyl sulfate gels.

Red cell hemolysates from non-irradiated intact and splenectomized rats produced 6 hemoglobins compared to five for the chronically irradiated animal in an hypoxic environment with component VI missing. Hemoglobin from chronically irradiated splenectomized animals in an hypoxic environment yielded a pattern identical to that obtained from non-irradiated rats, i.e. 6 hemoglobins in the same general distribution. For comparison, fetal hemoglobin was found to contain 6 hemoglobins (day 14 and day 16 after onset of pregnancy) which suggests that fetal hemoglobin was not produced by the intact chronically irradiated animal.

Hemolysates from washed normoblast suspensions isolated from erythropoietic spleens (from chronically irradiated rats) exhibited an electrophoretic pattern identical with the one obtained from peripheral blood of the chronically irradiated rat. This observation suggests the possible origin of the modified hemoglobin pattern described above, particularly in view of the normal profile observed in the irradiated splenectomized animal.

Analytical disc gel electrophoresis was used to ascertain the homogeneity of the hemoglobin components obtained by ion exchange

chromatography of erythrocyte hemolysates. All components migrated as single bands. They moved with the same rate as the discs seen when the corresponding multiple hemoglobin was electrophoresed.

The molecular weights of each of the hemoglobins comprising the multiple hemoglobins prepared from the following groups of experimental animals: - a) non-irradiated (6 hemoglobins); b) non-irradiated, splenectomized (6 hemoglobins); c) chronically irradiated, hypoxic environment (5 hemoglobins) and d) chronically irradiated, splenectomized, hypoxic environment (6 hemoglobins) were determined by the use of sodium dodecyl sulfate gels. Each hemoglobin migrated as a single disc. An approximate weight of 15,000 was obtained from a standard calibration curve for all species tested.

Red cell hemolysates were prepared from the following groups of experimental animals: (a) non-irradiated, (b) non-irradiated, splenectomized, (c) chronically irradiated, hypoxic environment, and, (d) chronically irradiated, splenectomized, hypoxic environment were examined using ion exchange chromatography. The elution profiles from CMC with a pH gradient manifested satisfactory resolution of the different hemoglobins: 6 hemoglobins for group (a); 6 hemoglobins for group (b); 5 hemoglobins for group (c); and, 6 hemoglobins for group (d). The number of hemoglobins for each group are in complete agreement with the results obtained with analytical polyacrylamide disc gel electrophoresis.

Alkali resistance tests were run on hemolysates from the four groups of experimental animals indicated above. The multiple hemoglobin from non-irradiated intact rats and non-irradiated splenectomized animals yielded similar rates of denaturation to alkali. The chronically irradiated hypoxic environment rats revealed a slower rate to alkali denaturation compared to the non-irradiated animals, i.e., increased resistance. A marked increase in the rate of denaturation was noted in the chronically irradiated splenectomized hypoxic environment rats.

Individual hemoglobins separated on CMC from non-irradiated rats and chronically irradiated animals reveal additional differences in behavior with respect to denaturation. Each hemoglobin reveals a characteristic reaction rate. All the hemoglobins from the irradiated animals manifested greater resistance to alkali denaturation compared to the non-irradiated rat.

Six globins were demonstrated by analytical polyacrylamide disc gel electrophoresis for intact and splenectomized non-irradiated rats; and, from the chronically irradiated splenectomized hypoxic environment rats. Five globins were found in animals maintained in chronically irradiated hypoxic environment rats. The banding patterns were identical to the ones obtained from multiple hemoglobin.

The chromatographic profiles from CMC yielded the following results, i.e., 6 globins for group (a); 6 globins for group (b); 5 globins for group (c); and 6 globins for group (d). These profiles were similar to those obtained when multiple hemoglobins were chromatographed.

The molecular weights of each of the globins were determined by the use of sodium dodecyl sulfate gels. Each globin migrated as a single band. An approximate weight of 14,500 for all species tested.

Urea-polyacrylamide gel electrophoresis was carried out on five components obtained from chromatography of globins on IRC-50 with a urea gradient. Five components varying in distribution were found in all four groups of experimental animals. Each component revealed a characteristic heterogenous electrophoretic pattern. There was a marked difference in the banding distribution in the different groups. This procedure yielded a multiplicity of discs that cannot be readily interpreted.

A homogenous peak was obtained from the globin from the non-irradiated rat compared to a major and minor peak for the chronically irradiated animals. Although these data support the difference in globins, further studies in this area are indicated.

The amino acid composition of the chromatographic components from IRC-50 with urea gradient elution for the different groups of experimental rats was determined. There was a decrease in the number of acidic residues of the fastest moving component in the chronically irradiated rat.

The N-terminal amino acid for all five components of the non-irradiated and chronically irradiated rats was valine.

COMMENTS

1. The foregoing biochemical data support the suggestion that a modified polymorphic hemoglobin is produced when rats are maintained in a chronically irradiated hypoxic environment.
2. The evidence suggests that the spleen is the site of production of the modified polymorphic hemoglobin.
3. Biosynthesis of the modified hemoglobin may be response to stress associated with chronic irradiation.
4. The significance and biochemical role of polymorphic hemoglobins merits further investigation.
5. The mechanism of biosynthesis of a complex macromolecule during continuous radiation exposure is a challenging problem.

Charles E. Brambel

Charles E. Brambel
Professor of Chemistry (Emeritus)