GLYCINE-2-C-14 ERYTHROCYTE LIFE SPAN IN NORMAL AND HEMORRHAGED RATS

by

Deane E. Smith

for the degree of

Master of Science

in

Biology Department

Program

Dr. James R. Jasper 3/9/77

Advisor Date

Dean Rand 3/9/77

Dean of Graduate School Date

YOUNGSTOWN STATE UNIVERSITY

March, 1977

ABSTRACT

GLYCINE-2-C-14 ERYTHROCYTE LIFE SPAN
IN NORMAL AND HEMORRHAGED RATS

Deane E. Smith
Master of Science
Youngstown State University, 1977

The life spans of red blood cells produced in normal and hemorrhaged adult male rats were measured by administering 10 μCi of glycine-2-C-14 to each rat. Blood samples were periodically collected over a 100 day period and assayed for radioactivity by liquid scintillation counting. Hemorrhage reduced the life span of the red blood cells from 67 days to 27 days. These findings support the results of others who have measured the life span of red blood cells in rats using other techniques (Berlin 1951, Dornhorst 1951, Amatuzio 1953, Eadie 1953).

ACKNOWLEDGMENTS

I wish to acknowledge the assistance of Professor James R. Toepfer, Biology Department, Youngstown State University, who advised and directed my research and gave editorial criticism of the thesis.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE PAGE

Glycine-2-C-14 Erythrocyte Life Span in Normal and Hemorrhaged Rats

CHAPTER 1

INTRODUCTION

Under normal conditions the rate of production and liberation of erythrocytes by the marrow evenly matches the rate of their destruction by the reticuloendothelial system, so the normal level of red cells in the circulation is maintained (Ham 1965). If the number of red cells in the blood is reduced, as a result of hemorrhage the marrow can increase its production up to 10 times in order to maintain the balance (Stohlman 1961). The method by which regulation is achieved is only partly understood.

It is believed that hypoxia, the fundamental erythropoietic stimulus, triggers the release of erythrogenin from the kidney, which reacts with a serum protein, probably of hepatic origin to produce the circulating erythropoietin. (Stohlman 1959). This interaction, occuring in either the kidney or in the plasma or both, may involve the splitting off of a portion of the serum substrate by the enzymatic action of erythrogenin.

It is conceivable that the interaction of erythrogenin and serum yields a labile form of erythropoietin (possibly a "pro-ESF") which requires a protective glycoprotein attachment for safe passage in the circulation to the blood forming tissues. The ESF then stimulates

ESF-committed cells to begin proerythroblast production.

The stages of erythrocyte development, in order of differentiation from the hemocytoblast, are proerythroblast, basophilic erythroblast, polychromatophilic erythroblast, normoblast, and erythrocyte. It should be borne in mind that the principal processes involved in the differentiation of erythrocytes are reduction in size, loss of nucleus and cellular organelles, and acquisition of hemoglobin.

The proerythroblast is the youngest precursor of the erythrocyte, possessing a relatively large nucleus with a thin rim of basophilic cytoplasm. This cell is intermediate both in size and degree of basophilia of its cytoplasm between the hemocytoblast and the basophilic erythroblast.

The basophilic erythroblast is a smaller cell than the proerythroblast and contains a nucleus with a coarser network of chromatin material. The cytoplasm shows intense basophilia, more so than that of the hemocytoblast, owing to an increased content of ribonucleic acid. The basophilic erythroblasts undergo numerous mitotic divisions and produce cells called polychromatophilic erythroblasts which acquire a small amount of hemoglobin. After staining with Wright or Giemsa stain, the cytoplasm varies in color from a purplish-blue to a lilac or gray owing to the presence of varying amounts of pink-staining hemoglobin within the basophilic cytoplasm of the erythroblasts. Thus they are termed polychromatophilic. The nucleus of the polychromatophilic erythroblast has a denser chromatin network than that of the

basophilic erythroblast and the cell is smaller.

The polychromatophilic erythroblasts undergo numerous mitotic divisions. Some remain in a resting condition as a reservoir of cells. In others the basophilia of the cytoplasm decreases and the amount of hemoglobin increases to such an extent that the cytoplasm stains approximately as acidophilic as that of the mature erythrocyte. Cells which exhibit this degree of acidophilia within their cytoplasm are referred to as normoblasts. The normoblast is smaller than the polychromatophilic erythroblast and contains a smaller nucleus which stains densely basophilic. Gradually the nucleus becomes pyknotic. There is no further mitotic activity. Finally the nucleus is lost by a process thought to be simple extrusion from the cell, although a few investigators believe the process to be one of karyolysis. The young erythrocytes, called reticulocytes, contain a delicate network which can be demonstrated by supravital staining with dyes such as new methylene blue or phase microscopy. The majority of the reticulocytes lose their reticular structure before leaving the bone marrow, the reticulocyte count of peripheral blood normally being less than 1 per cent of the erythrocytes(Leeson and Leeson 1970).

The spleen removes most of the worn-out red blood cells of the body from the circulatory system. From the hemoglobin of these worn-out cells it manufactures bilirubin which it liberates into the blood, from which the liver

collects it. The spleen also extracts iron from the hemoglobin of the worn-out cells and liberates this into the bloodstream in a form that permits it to be used over again in the manufacture of new red blood cells in the red bone marrow.

Protoporphyrin IX, which serves as nucleus for the heme in hemoglobin and cytochrome enzymes, is synthesized from glycine and acetate(White, Handler, and Smith 1974). Isotopic labeling studies have indicated that the acetate has been metabolized through the tricarboxylic acid cycle and actually participates as a succinyl-CoA (Grinstein 1949).

The first step in the sequence of reactions leading to the synthesis of protoporphyrin, the immediate precursor of heme, is catalyzed by delta-aminolevulinate synthetase. The purified enzyme has an absolute requirement for the substrates, glycine and succinyl CoA, and for the cofactor pyridoxal 5' - phosphate and Mg^{2+}. The enzyme contains at its active site an -SH group that reacts initially with pyridoxal phosphate to form a thiohemiacetal, with subsequent formation of a Schiff base with glycine, followed by a condensation reaction with succinyl CoA and the formation of delta-aminolevulinic acid.

Delta-aminolevulinate synthetase is the rate-controlling enzyme in the pathway of heme biosynthesis and is itself rapidly metabolized, having an apparent half-life of about 1 hour in mammalian liver. Two moles of delta-aminolevulinate condense to form porphobilinogen in a reaction catalyzed by delta-aminolevulinate dehydrase. One

molecule of delta-aminolevulinate forms a Schiff base with the enzyme; this is followed by a nucleophilic attack, by this intermediate anion, on the carbonyl carbon of a second molecule of delta-aminolevulinate. The resulting aldol loses the elements of water, and the free amino group of the second molecule of the substrate displaces the amino group of the enzyme by a "trans-Schiff" reaction, forming porphobilinogen.

Since four molecules of porphobilinogen are utilized in porphyrin synthesis, all the porphyrin nitrogens are contributed by the nitrogen of glycine. Isotopic studies have established that eight of the methylene carbon atoms of glycine are used in the synthesis of each porphyrin nucleus (Bale, Yuile, Delavergne, and Miller 1949). Of these eight carbon atoms, four become atom 2 in each pyrrole ring, i.e., are in the ring adjacent to the nitrogen atom of the pyrrole nucleus, and an additional four are utilized for the methene bridges of carbon atoms between the rings. The remaining 26 carbon atoms, not derived from glycine, have their origin in succinyl CoA.

Protoporphyrin synthesis begins with condensation of four molecules of porphobilinogen, under the influence of a porphobilinogen deaminase to a postulated intermediate, polypyrryl methane. The polypyrryl methane, in the presence of an isomerase, may form uroporphyrinogen III, which can in a similar manner yield coproporphyrinogen III. Coproporphyrinogen III may be oxidized and decarboxylated to yield

protoporphyrin IX.

Several hormones and drugs that stimulate erythropoiesis appear to increase the amount of delta-aminolevulinate synthetase, the committed, rate limiting enzyme in the biosynthetic pathway of heme. In the synthesis of heme, iron is incorporated after synthesis of protoporphyrin IX is complete (Sshalnick, Hammmaker, and Maver 1972).

Various methods have been used to estimate the life span of red cells in different species. For erythrocytes of normal man four independent methods have given results which agree closely. Data obtained by estimating with the agglutination method of Ashby (1919) the survival of cells derived from a blood group O donor and injected into a blood group A recipient were subjected to a mathematical analysis by Callender, Powell & Witts (1945) who found an average life span of 120 days. A similar result using an identical mathematical treatment was obtained by Jope (1946) who estimated the disappearance of sulphaemoglobin from the blood of subjects who had been in contact with trinitrotoluene immediately before the beginning of the experiment. The assumption implied in both methods is that the labelled cells have the same life span as the normal cells of the experimental subject.

Shemin & Rittenberg (1946) measured the rate of disappearance of ^{15}N in the haemin isolated from human subjects given isotopic glycine and showed that the average 'life span' of the hemoglobin molecule is about 120-125 days.

A similar figure was obtained by Gray, Neuberger & Sneath (1950) who followed the ^{15}N content of the stercobilin excreted by a normal subject fed isotopic glycine. The good agreement found between methods employing isotopic and non-isotopic labelling indicates that the hemoglobin in the circulating red cell of man is metabolically inert until the cell disintegrates. Isotope methods are more accurate than the other labelling techniques and give more detailed information about the distribution of life spans in a red cell population. The assumption which is made with all four methods is that the rates of daily production and destruction are constant and equal, appears to be justified, at least in man, by the remarkable success which has been achieved in the quantitative interpretation of the data.

Reliable data for few species other than man are available. Hawkins & Whipple (1938), working with bile fistula dogs, measured the interval between maximum regeneration of blood after bleeding or phenylhydrazine administration and the peak of increased excretion of bile pigments and deduced from their results that the average life span of the dog red cell is about 125 days. Grinstein, Kamen & Moore (1949) used carboxy labeled glycine-C-14 and determined the rate of decrease of activity in the globin. For two dogs the values 75 and 95 days respectively were obtained. Bale,Yuile, de la Vergue, Miller & Whipple (1949) fed DL-lysine labeled with ^{14}C to a dog and determined the radioactivity of the globin over a long period. Their figures indicate that the

average life span of the red cell of the dog is about 110-115 days. Ponticorvo, Rittenberg & Block (1949), using the rate of incorporation of deuterium-labeled acetate into the heme of the hemoglobin estimated the average life span of the red cell of the rat to be about 100 days.

The determination of the average survival time or life span of erythrocytes enhances markedly the appraisal of the etiology of anemias and other pathologic states (Callender, Powell and Witts 1945). The most direct approach is to measure the survival of the erythrocytes within their natural environment. Heretofore, technics permitting this approach have involved the use of isotopes of iron (Fe^{59}), nitrogen (N^{15}) and carbon (C^{14}) and have been cumbersome due to the complexities of the analysis of these isotopes and the factor of reutilization of the isotope by erythrocytes. The differential agglutination technique of Ashby, although very useful, permits only the appraisal of donor cells within a second individual.

While a variety of methods have been used for determining erythrocyte life span, the method of isotope tagging of the porphyrin ring is probably the least objectionable and was the one chosen for this study. Glycine is a nitrogenous precussor of the protoporphyrin of the hemoglobin of rats. A prime prerequisite in the use of C-14 is the assumption that hemoglobin in the intact red cell is metabolically inert. In fact the data obtained with this and other labels have been used to support this concept (Berlin, Lawrence and Elmlinger

1957).

The problems involved in tagging the red cell by use of the isotope and determining its longevity are: First, to introduce the isotope into the corpuscle in such a chemical combination that it remains in the corpuscle during its life; second, to choose an isotope of some element that will not be reutilized after the corpuscle has disintegrated; and last to choose an isotope of sufficiently long half life so that the study can be carried out. The purpose of this study is to investigate the effect of hemorrhage on the red blood cell life span in rats using glycine-2-C-14.

CHAPTER II

MATERIALS AND METHODS

Animals

Adult male rats of the Wisconsin Holtzman strain weighing 180 gm were used throughout this study. The animals were fed Purina Rat Chow. Food and water were supplied "ad libitum". The rats were maintained in plastic cages, 5 per cage and acclimatized to laboratory conditions for a period of 2 weeks prior to use.

RBC Life Span Determination

The RBC life span of normal and hemorrhaged rats was determined as follows: fifty normal rats were used as controls. The μCi of glycine 2-C-14 (Amersham/Searle) contained in 1 ml.9% saline was injected intra-peritoneally into each of these animals. They were then arranged into 10 groups of 5 animals each. Blood samples were collected over a period of 100 days by alternating groups so that rats in each group were bled only twice. The first blood samples were taken on day 0 before injection of the labeled glycine and, therefore, served to determine the amount of background activity. This activity was subtracted from samples taken at days 3,5,10 and at 5 day intervals thereafter over the entire period of 100 days. In this manner excessive blood loss to each animal was avoided. Preliminary

studies had indicated that frequent repeated sampling of the same rat over a long period of time resulted in erratic and shortened life-span curves.

A second group of 50 rats (experimental) were bled 5 mls of blood each on 2 successive days by cardiac puncture and glycine-2-C-14 was then given 6 hours after the second hemorrhage. These animals were then arranged into groups and their blood sampled in the same manner as were the animals of the control group.

Collection and Digestion of Blood

Blood was removed from the dorsolateral caudal vein using a 23 guage needle and placed into the small plastic vials containing ammonium oxalate. The vials were prepared by evaporating in them .1 ml of a 1.6 gm % ammonium oxalate solution in an oven. Twenty microliters of blood in each vial was then placed into glass scintillation vials containing .25 ml of NCS. NCS^{TM} solubilizer is a solution of a quaternary ammonium base in toluene which was developed by Amersham/ Searle to solubilize biological samples for liquid scintillation counting.

The vials were placed in an oven and digested at 50^{o}C for 20 minutes. Eight-tenths ml of freshly prepared benzoyl peroxide (1g/5ml scintillation grade toluene heated to 60^{o}C for 20 minutes, then cooled and filtered) was then added as a decolorizing agent. The vials were placed back in the 50^{o}C oven for an additional 30 minutes. Finally, 18 ml of scintillation solution (6g/L PPO and 75 mg/L POPOP)

was mixed with digested decolorized blood after it reached room temperature. The samples were placed in a cool dark room for 24 hours prior to counting to reduce high background activity due to chemolumenescence.

Assay of Radioactivity

Radioactivity measurements on all samples were carried out using a Beckman liquid scintillation counter. The activity obtained was in counts per minute per 20 µl blood. This was then converted to net disintegrations per minute per 20 µl of blood (DPM/20 µl blood)by correcting for quench and then subtracting the background activity. Preliminary experiments in which plasma was separated from centrifuged RBC's indicated that all of the radioactivity resided in RBC's and not in plasma. Therefore, it was deemed unnecessary to separate the cells from plasma for the radioactivity determinations.

Reticulocyte Determination

A total of one thousand RBC's Reticulocytes were counted and the number of reticulocytes counted was expressed as a percentage of total cells counted. Reticulocyte counts were then carried out on a separate group of 4 rats which were first bled 4 ml on 2 successive days and counts were then made starting immediately after hemorrhage and daily for eight days thereafter.

The reticulocyte staining solution consisted of 0.5 gm new methylene blue and 1.6 gm potassim oxalate dissolved in

100.0 ml of distilled water. Approximately equal amounts of staining solution (2 drops) and fresh blood (2 drops) were mixed on a slide. The red cell suspension was placed into a moist chamber, and allowed to stand for 10 minutes in order to stain the reticulocytes and RBC's. The suspension then was drawn into a capillary pipet and expelled on a slide. Thin smears were then made and air-dried. Reticulocytes were counted under oil immersion without fixation or counterstaining. The red blood cells were light greenish blue in these preparations. The reticulum appeared as sharply outlined, deep blue granules or short rods within the stroma of the reticulocytes.

The staining of the reticulum was not affected by relatively wide variations of the proportion of blood and staining solution. However, the proportion of blood and staining solution determined the color of the red cells. A marked excess of staining solution produced a deep blue color in the red blood cells, obscuring the reticulum. A large excess of blood diminished the greenish blue coloration of the red blood cells. Generally a slight excess of blood gave optimal color contrast.

Haematocrit Determination

Micro-haematocrit values were established on the same group of rats used in the reticulocyte determination. Values were made immediately after hemorrhage and then daily for eight days.

CHAPTER III

RESULTS

Life span of Normal Rats

Figure 1 presents the results of the normal rats. After injection of C-14 labeled glycine the activity of hemoglobin reached a maximum of 525 DPM/20 µl blood on day 5 and thereafter remained constant for the next 55 days. At day 60 the activity began to decrease rapidly and disappeared at day 74. The midpoint of this rapid decrease in activity is 67 days and represents the mean life span of the normal erythrocytes in rats (Shemin and Rittenberg 1946).

Life span of Hemorrhaged Rats

The results of hemorrhage are shown in Figure 2. The C-14 level reaches a maximum of 1034 DPM/20 µl blood on day 2. In contrast to normal animals the activity falls rapidly in two different plases. In the first phase, the activity decreases from 903 DPM/20 µl blood at day 5 to 426 DPM/20 µl blood at day 25. In the second phase the activity decreases quite rapidly from 426 DPM/20 µl blood on day 25 to 59 DPM/20 µl blood on day 30.

Figure 3 indicates that an increase in reticulocytes from 1% at day 0 to 38% at day 3 has occurred. This was then followed by a rapid decrease to approximately normal values

(3.9%) on day 4. This was followed by an increase to 14% on day 5 and a return to normal by day 8.

In contrast, Figure 4 shows a rapid inital decrease in hematocrit to 20% at 15 minutes post-hemorrhage. This is then followed by an increase over a period of six days reaching a maximum of 54% at day 6. By 8 days after hemorrhage, hematocrit values were approximately normal, 49%.

Figure 1

Graph showing mean red blood cell life span of normal adult male rats. Each point shows the mean of observations in 5 rats, bars are ($\pm$) S.E. of means. Broken line represents death of red blood cells.

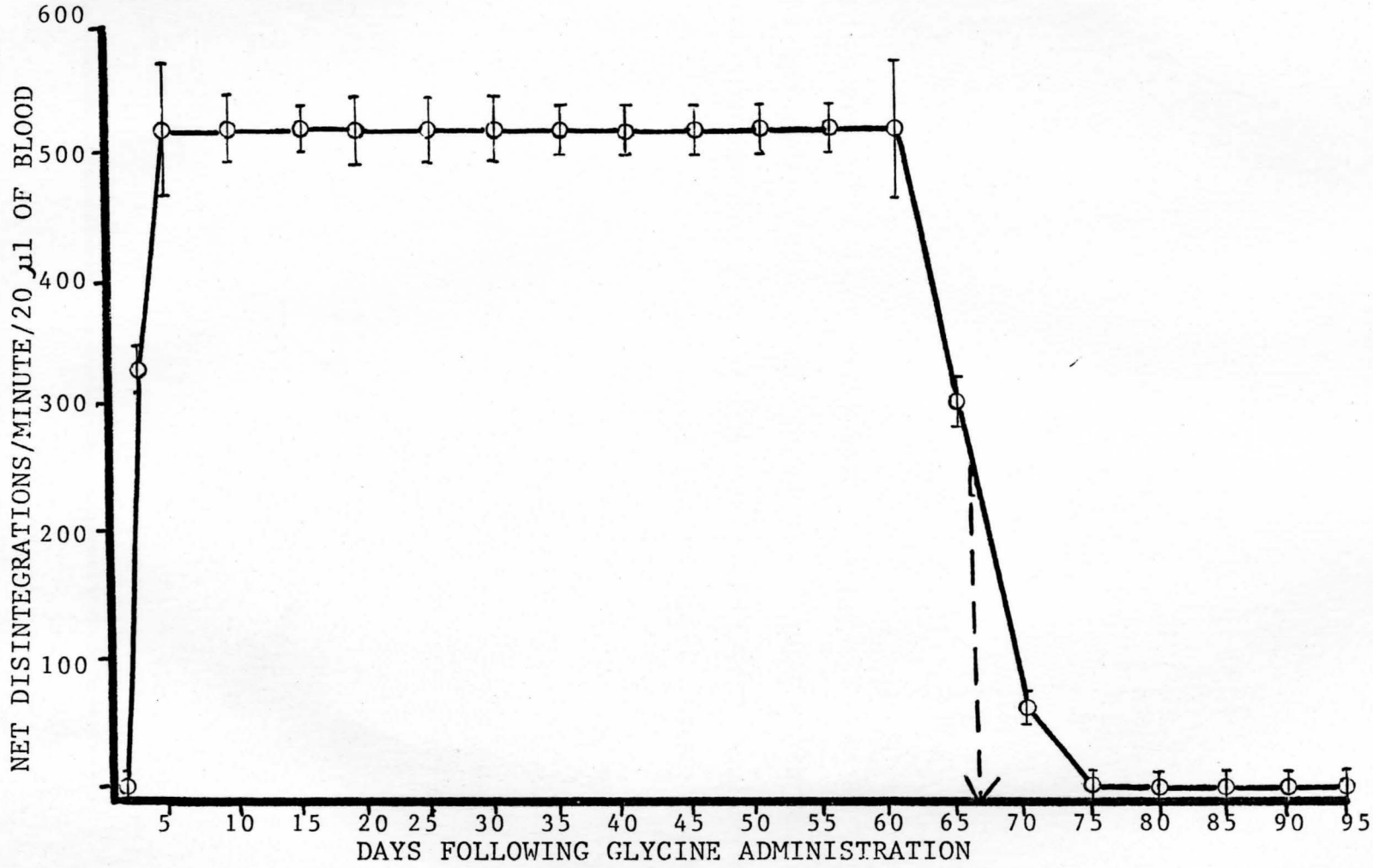
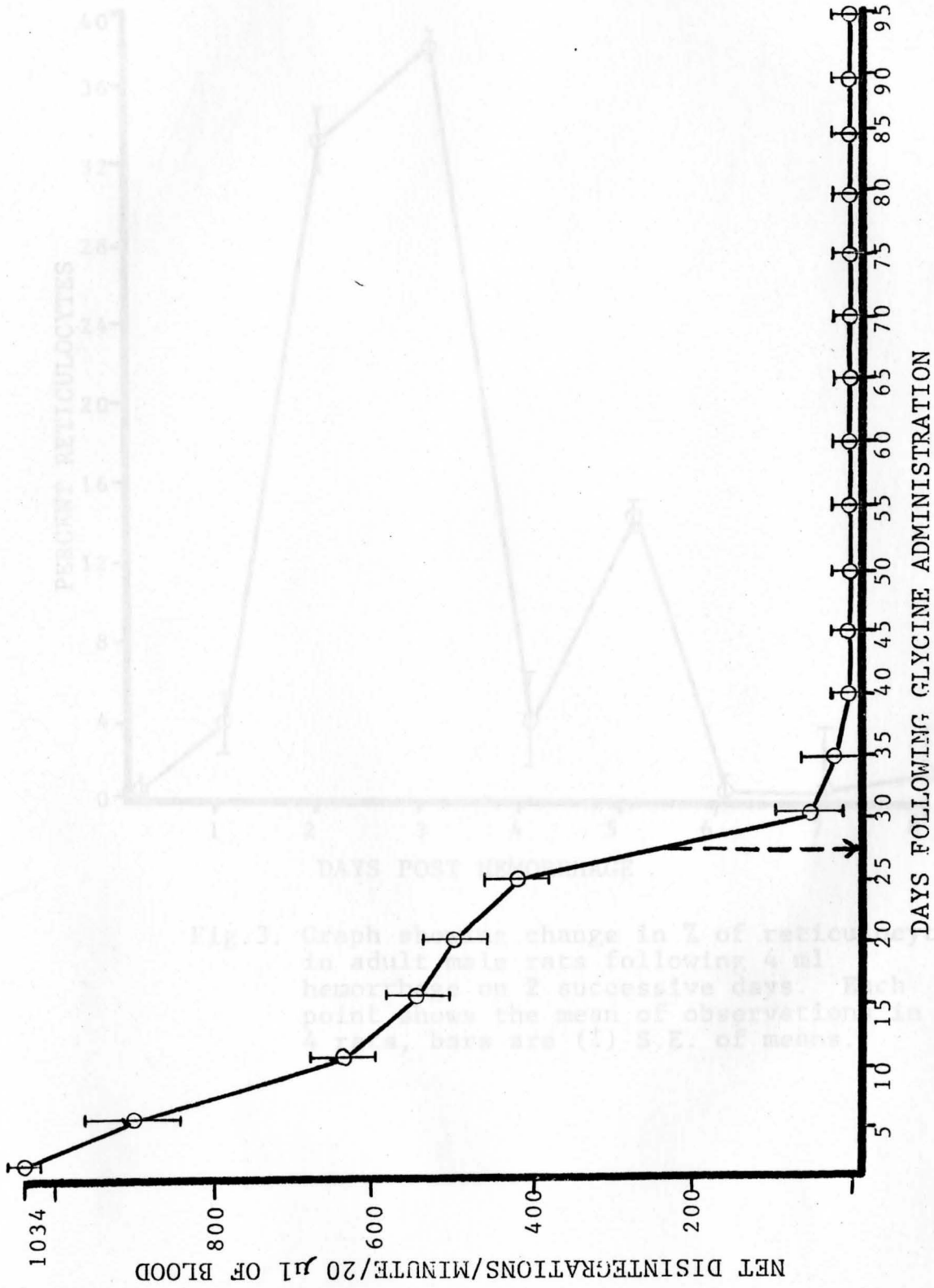

NET DISINTEGRATIONS/MINUTE/20 µl OF BLOOD
600
500
400
300
200
100
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
DAYS FOLLOWING GLYCINE ADMINISTRATION

Figure 1

Graph showing mean red blood cell life span of normal adult male rats. Each point shows the mean of observations in 5 rats, bars are (±) S.E. of mean. Broken line represents death of red blood cells.

Figure 2

Graph showing mean red blood cell life span of adult male rats following hemorrhage. Each point shows the mean of observations in 5 rats, bars are ($\pm$) S.E. of means. Broken line represents death of red blood cells.


NET DISINTEGRATIONS/MINUTE/20 μl OF BLOOD
1034
800
600
400
200
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
DAYS FOLLOWING GLYCINE ADMINISTRATION

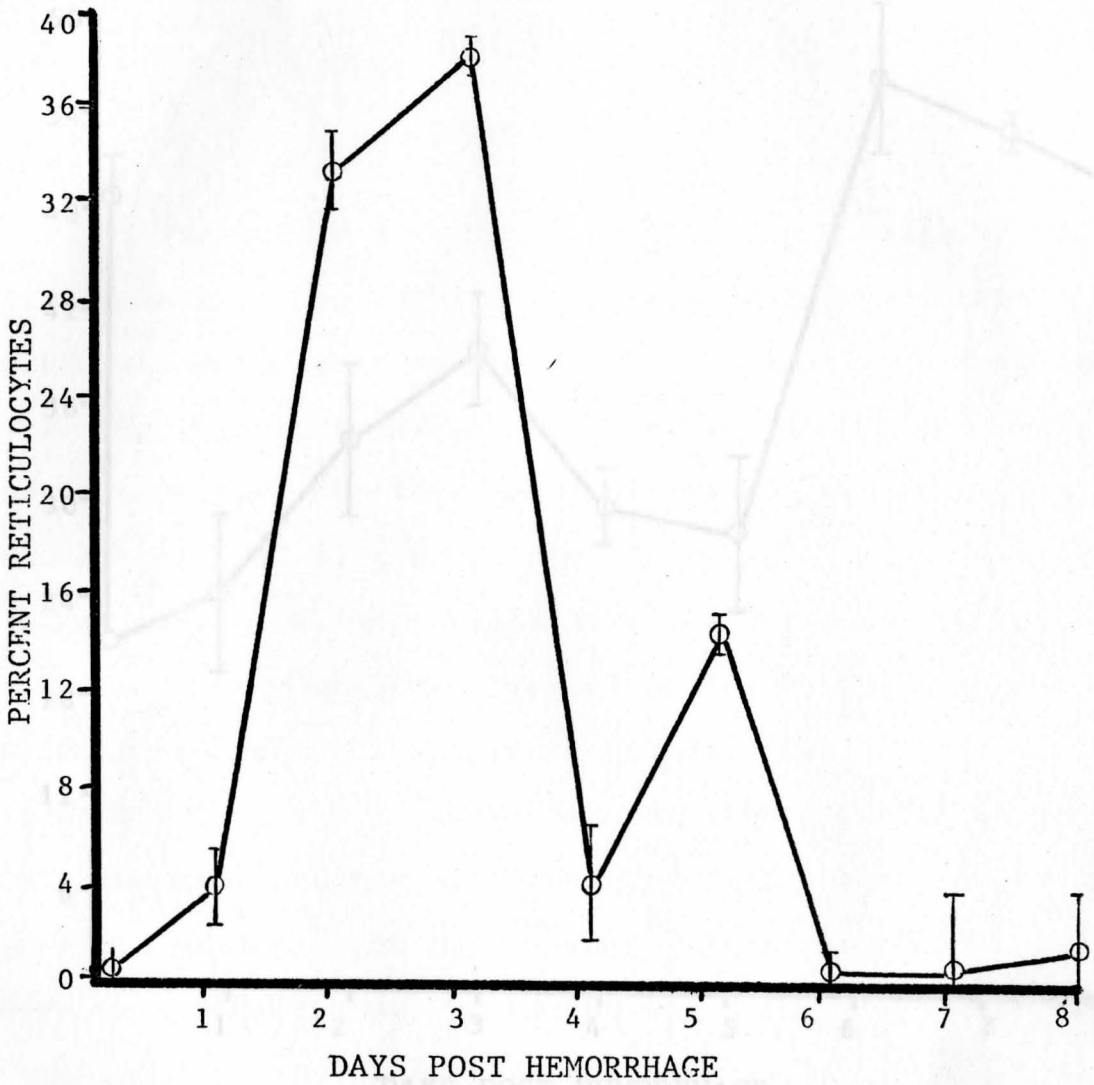


Fig.3. Graph showing change in % of reticulocytes in adult male rats following 4 ml hemorrhage on 2 successive days. Each point shows the mean of observations in 4 rats, bars are (±) S.E. of means.

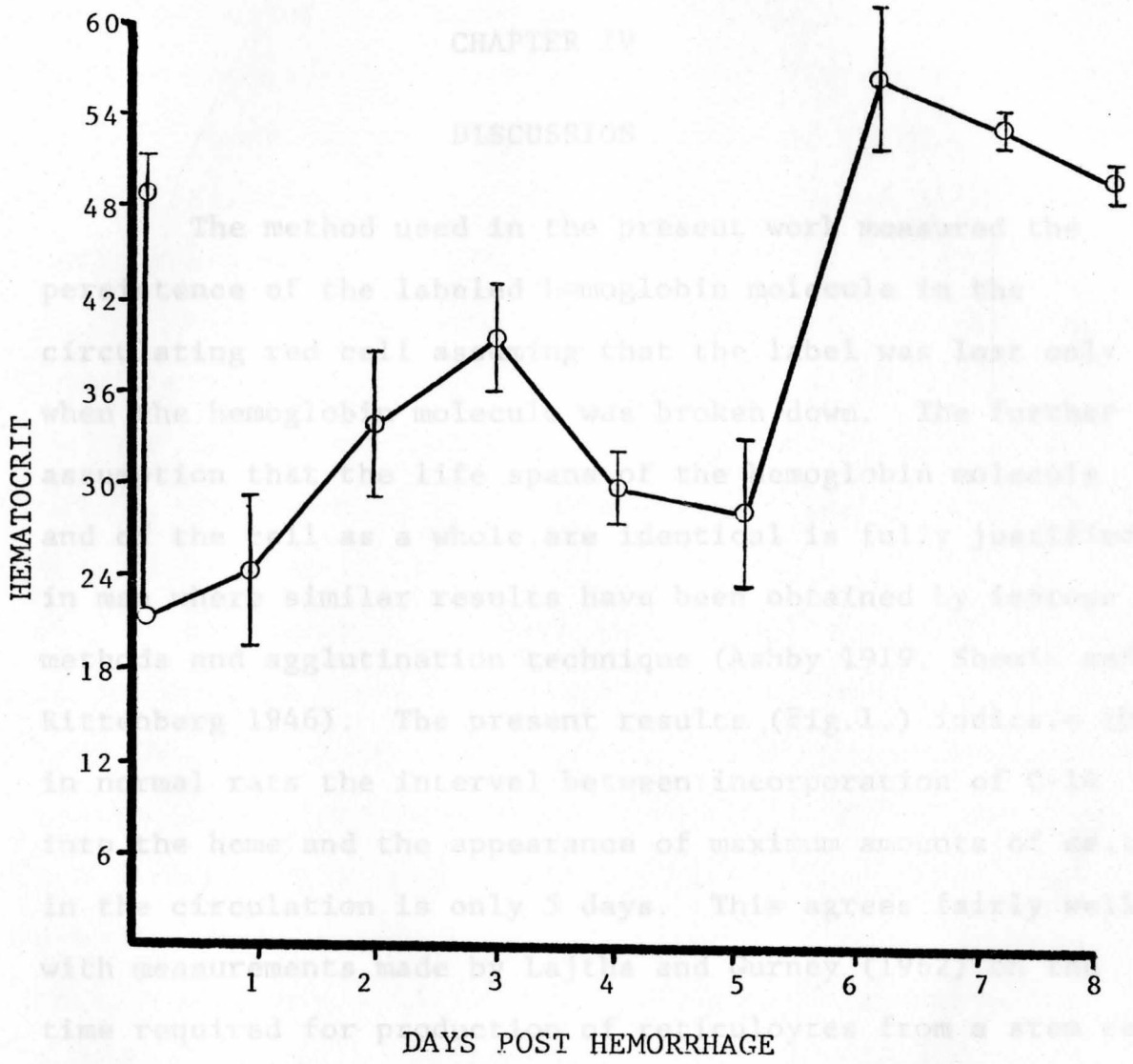


Fig.4. Graph showing change in hematocrit in adult male rats following 4 in 1 hemorrhage on 2 successive days. Each point shows the mean of observations in 4 rats, bars are (±) S.E. of means.

CHAPTER IV

DISCUSSION

The method used in the present work measured the persistence of the labeled hemoglobin molecule in the circulating red cell assuming that the label was lost only when the hemoglobin molecule was broken down. The further assumption that the life spans of the hemoglobin molecule and of the cell as a whole are identical is fully justified in man where similar results have been obtained by isotope methods and agglutination technique (Ashby 1919, Shemin and Rittenberg 1946). The present results (Fig.1.) indicate that in normal rats the interval between incorporation of C-14 into the heme and the appearance of maximum amounts of cells in the circulation is only 5 days. This agrees fairly well with measurements made by Lajtha and Gurney (1962) on the time required for production of reticuloytes from a stem cell in bone marrow.

The rapid drop in activity beginning at day 60 can be interpeted as meaning that the protoporphyrin moiety of heme is not reutilized in erythropoiesis (Grinstein, Kamen and Moore 1949, Berlin, Lawrence and Elmlinger 1957).

Also this drop indicates that the labeled red blood cells are all disappearing at about the same time. Therefore the midpoint of this line can be taken as the mean life span of normal erythrocytes (Shemin and Rittenberg, 1946). In this study the mean life-span was found to be about 67 days. This agrees well with values determined by (Berlin, Lazarus and Meyer 1950).

With the exceptions of the 100 days span obtained by Ponticorvo et.al (1949) using deuterum labeled acetate and as calculated from iron kinetic studies by Burwell et.al. (1953), the reported values are close to the range of 50-60 days (Berlin et.al, 1952).

In contrast, the life span of red blood cells produced in response to acute blood loss is shorter than in normal rats (Fig.2). Shortened life spans from hemorrhage have also been obtained from rats (Berlin and Lotz 1951), rabbits (Card and Valberg 1967), and cats (Valentine et.al. 1951). Certain aspects of these life span curves are similar with the one obtained in this study. There is a greater amount of activity initially present in the blood compared to controls. This corresponds with the time when large numbers of reticulocytes are present in the blood. (Fig.3). This also correlates well with the fact that the number of circulating red blood cells are starting to increase after hemorrhage (Fig.4). This is probably a result of the increase in rate of red blood cell production. The measurement of the actual

life span of red blood cells in bled rats in this study is made difficult by the presence of two phases, (Fig.2), in the disappearance of labeled RBC's from the blood. The first phase is characterized by a rapid decrease in activity. The curve of the first phase is in the shape of a hyperbola. The second phase of the label disappearance curve is very steep. Its slope is similar to the slope obtained from normal rats (Fig.1).

If one takes the midpoint of this slope, the mean life span is about 27 days. This is much shorter than the normal life span. This value is in agreement with the value of 27 days obtained by Berlin (1951).

The two phases of the labeled RBC's disappearance curve seems to indicate two different processes taking place. The first phase seems to indicate a random loss of cells over a longer period of time, whereas, the second phase seems to indicate the disappearance of a population of cells at about the same time. Perhaps this first phase decline in activity is caused from dilution of the total red cell volume with production of unlabeled cells 2 days after the initial reticulocytosis (Fig. 3) as seen from the large increase in hemotocrit from day 5 to day 6 (Fig.4). The possibility also exists that two different populations of cells are produced in response to hemorrhage. This latter assumption may be more correct due to the fact that the decrease in activity continues even after the hemotocrit has returned to normal at 8 days.

Cytological evidence exists from other studies that a different sized RBC is produced in response to acute severe anemia in rats (Stohlman 1968). In these rats, the release of "younger" reticulocytes which are much larger than normal reticulocytes takes place. It may be the normal macrocytes have a shortened cell survival due to their size and shape, much as do spherocytes in hereditary spherocytosis. The rapid rate of formation of cells may lead to other defects which affect life span.

REFERENCES CITED

Amatuzio,D.S. and R.L.Evans. Isotope curves in red blood cell studies. Nature 171:797-798,1953.

Ashby,W. Determination of length of life of transfusion blood corpuscles in man. J.Exper.Med. 29:267-281, 1919.

Bale, W.F., C.L.Yuile, L.Delavergne, L.L.Miller and G.H.Whipple. Hemoglobin labeled by radioactive lysine: erythrocyte life cycle. J.Exper.Med. 90: 315-320, 1949.

Berlin, N.I., J.H.Lawrence and P.J.Elmlinger. Recent advances in knowledge of total red cell volume, production and destruction. Blood 12: 147-164, 1957.

Berlin, N.I. and C.Lotz. Life span of red blood cell of rat following acute hemorrhage. Proc.Soc.Exper. Biol. & Med. 78: 788-790, 1951.

Berlin, N.I., L.M.Meyer and M.Lazarus. Life span of rat red blood cell as determined by glycine-2-C^{14}. Am.J. Physiol. 165: 565-567, 1952.

Burwell, E.L., B.A.Brickley and C.A.Finsh. Erythrocyte life span in small animals: Comparison of two methods employing radioiron. Am.J.Physiol. 172: 718-724, 1953.

Callender, S.T.E., E.O.Powell and L.J.Witts. Life span of red cell in man. J.Path & Bact. 57: 129-139, 1945.

Card,R.T. and Valberg,L.S.Characteristics of shortened survival of stress erythrocytes in the rabbit. Amer. J. Physiol. 213, 566-672,1967.

Courtice, F.C. and R.W.Gunton. J.Physiol. 108, 405 1949.

Davis, W.M., E.L.Alpen and A.K.Davis. Radio-iron utilization and erythrocyte life span in rats following thermal injury. J.Clin. Invest. 34: 67-74, 1955.

Dornhorst, A.C. Interpretation of red cell survival curves. Blood 6: 1284-1292, 1951.

Eadie, G.S. and I.W.Brown,Jr. Red blood cell survival studies. Blood 8: 1110-1136, 1953.

Gray, C.H., A.Neuberger and P.H.A.Sneath. Incorporation of N^{15} in stercobilin in normal and in porphyria. Biochem. J. 47: 87-92, 1950.

Grinstein, M., M.D.Kamen and C.V.Moore. Utilization of glycine in biosynthesis of hemoglobin. J.Biol. Chem. 179: 359-364, 1949.

Ham, Arthur W. Histology. Philadelphia: Lippincott, 1965.

Harris, E.B., Belcher, E.H. Proc. 9th Int. Cong. of Radiology, Georg Thieme Verlay, Stuttgart, 1960, p.905.

Hawkins, W.B. and G.H.Whipple. Life cycle of red blood cell in dog. Am.J.Physiol. 122: 418-427, 1938.

Jope, E.M. Disappearance of sulphemoglobin from blood of TNT workers in relation to dynamics of red cell destruction. Brit.J.Indust.Med. 3:136-142, 1946.

Lajtha, L.G., and C.W.Gurney. Kinetic model of a bone marrow stem cell population. Brit.J.Haemat. Oct. 1962.

Leeson, Thomas S. and Leeson, C.Roland. Histology. Philadelphia: Saunders, 1970.

Neuberger, A. and J.S.F.Niven. Haemoglobin formation in rabbits. J.Physiol. 112: 292-310, 1951.

Ponticorvo, I., D.Rittenberg and K.Bloch. Utilization of acetate for synthesis of fatty acids, cholesterol and protoporphyrin. J.Biol.Chem. 179: 839-842, 1949.

Schalnick, L.E.Hammaker, and H.S.Maver. J.Biol.Chem. 242: 4132, 1972.

Shemin, D. and D.Rittenberg. Life span of human red blood cell. J.Biol.Chem. 166: 627-636, 1946.

Stohlman, Frederick,Jr. Observations on the Physiology of Erythropocetin and its role in the regulation of red cell production. Annals of the New York Academy of Sciences, 77: 710-727, 1959.

Stohlman, Frederick,Jr. Humoral regulation of Erythropoiesis VII, Shortened survival of Erythrocytes produced by Erythropoietive or severe anemia. Pro.Soc. Exp.Biol. and Med. 107: 884-887, 1961.

Stohlman, Frederick,Jr. Regulation of Erythropoiesis XX. Kimetics of red cell production. Annals of the New York Academy of Sciences, 149: 156-172, 1968.

Strumia, M.M. Life span of erythrocytes and their post-transfusion survival. Progr.Hematol. 1: 74-90, 1956.

Valentine, W.W., M.L.Pearce, R.F.Riley, E.Richter and J.S.Lawrence. Heme Synthesis and Erythrocyte Life Span in the cat Proceedings. Society for Experimental Biol. & Med. 5, 244-245, 1951.

Van Putten, L.M. Life span of red cells in rat and mouse as determined by labeling with DFP in vivo. Blood 13: 789-794, 1958.

White,Abraham, Philip Handler and Emil L.Smith. Principles of Biochemistry. New York: McGraw-Hill, 1973.