Title

Splenic Destruction of Newly-Formed Red Blood Cells and Shortened Erythrocyte Survival in Mice with Congenital Microcytosis

Authors

Landaw, Stephen A
Russell, Elizabeth S
Bernstein, Seldon E

Publication Date

2023-09-06

SPLENIC DESTRUCTION OF NEWLY-FORMED RED BLOOD CELLS AND SHORTENED ERYTHROCYTE SURVIVAL IN MICE WITH CONGENITAL MICROCYTOSIS

Submitted to Blood:
June 15, 1970

Stephen A. Landaw
Elizabeth S. Russell, and
Seldon E. Bernstein

From the Donner Laboratory, University of California, Berkeley 94720, and the Jackson Laboratory, Bar Harbor, Maine 04609

Portions of this work were performed during a Visiting Investigatorship at the Jackson Laboratory, Summer, 1968, and supported in part by a Special Fellowship and a Career Development Award (1-K04-HE17693-01A1) from the National Heart Institute (S.A.L.)

This work was supported in part by the U.S. Atomic Energy Commission (Contract AT(30-1)-1800), the National Aeronautics and Space Administration (Contract W-7405-eng-48), and by National Institutes of Health grants from the National Cancer Institute (CA-01074) and the National Institute of Child Health and Human Development (HD-00254).

Address reprint requests to Stephen A. Landaw, M.D., Donner Laboratory, University of California, Berkeley, Calif. 94720

DISCLAIMER

ABSTRACT

Red blood cell survival and patterns of erythropoiesis-associated heme destruction were studied in mice with microcytosis (mk/mk) of the MK/Re and SEC/1Re strains, and their normal controls (+/+ and mk/+) using the endogenous production of ^{14}CO following glycine-2-^{14}C injection. In affected homozygotes of both strains, there was evidence for splenic destruction of reticulocytes and increased random hemolysis, with higher rates of random hemolysis in affected mice of the SEC/1Re strain. In addition, alterations in the "early labeled peak" of the mk/mk mice of the SEC/1Re strain suggested the presence of a small component of "ineffective erythropoiesis", which was not seen in affected mice of the MK/Re strain. The "ineffective erythropoiesis" and splenic reticulocyte destruction were abolished by prior splenectomy, but the rate of random hemolysis was only minimally lessened and splenectomized mice remained anemic. These results offer direct insight into RBC survival patterns in genetically determined hemolytic anemias, and document results of the interaction of a single gene defect (mk) with the rest of the genome.

INTRODUCTION

Microcytosis, an autosomal recessive trait in the mouse, has recently been described by investigators at the Jackson Laboratory[1-5] and elsewhere[6]. The anemia is characterized by microcytosis and anisocytosis, reduced mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), and by reticulocytosis and a typical "Christmas wreath" appearance of reticulocytes in smears on glass slides[2,5]. Although absolute red blood cell counts are often elevated in affected (mk/mk) mice, due to the extreme microcytosis and hypochromia the animals may be severely anemic, are paler than their unaffected littermates at birth[2], and in many stocks have reduced viability[5].

In this paper, red blood cell survival in affected and control mice was studied via the endogenous production of carbon-14 labeled carbon monoxide (^{14}CO) in the expired air following injection of glycine-2-^{14}C. Previous studies have shown that endogenously-produced CO arises solely from heme catabolism[7-9], and that the fate of a cohort of labeled circulating red blood cells can be followed via ^{14}CO production following injection of labeled glycine[10]. This technique is particularly applicable to the study of congenital murine anemias for the following reasons:

1. It allows heme catabolism to be studied without venesection or collection of body fluids, avoiding possible unphysiologic effects of such sampling, or the requirement for large numbers of animals, and

2. It is capable of giving information concerning destruction of labeled cells during maturation in the bone marrow ("ineffective erythropoiesis")[11].

The usefulness of this technique in assessing the destruction of newly-formed red blood cells by the spleen (splenic sequestration and destruction of reticulocytes) will form a portion of this communication.

MATERIALS AND METHODS

Animals studied were adult male or female mice of the MK/Re or SEC/1ReJ strain homozygous (mk/mk) for the microcytosis defect and their normal littermates (mk/+ and +/+). Mice were studied for ^{14}CO production approximately 2 to 4 months after birth, and routine blood studies were performed using standard techniques on blood obtained from the orbital sinus at the completion of each experiment, when the mice were 5 to 9 months old.

Endogenous ^{14}CO production was studied in groups of two to five mice, each animal being injected intravenously with 10 μCi of glycine-2-^{14}C, using techniques previously described[9,10]. Immediately after glycine injection the mice were placed as a group into metabolism chambers, and breath collected continuously for the first 48 hours, and at frequent intervals thereafter up to 100 days after isotope injection. Results of each experiment were reported in terms of disintegrations per minute of ^{14}CO collected per hour per individual animal.

The experiments to be reported consisted of four +/+ control mice of the SEC/1ReJ strain, three mk/+ mice of the SEC/1ReJ strain, two mk/mk mice of the SEC/1ReJ strain, and two mk/mk mice of the MK/Re strain. An additional three mk/mk mice of the SEC/1ReJ strain were studied 11 days following surgical removal of the spleen. Parameters of red blood survival were analyzed for all mouse groups by fitting

^{14}CO data to appropriate mathematical functions[10] which yielded estimates of the rate of random destruction of RBC (random hemolysis), the mean potential lifespan and the spread of lifespans about this mean (phase of senescent death), and the fractional incorporation of glycine into RBC hemoglobin heme. From such parameters, and from graphical analysis of "early peak" data (see Results and Discussion sections), mean overall RBC lifespan (average RBC survival time for all modes of RBC death), and the fractionation of heme destruction among the various phases of RBC death ("ineffective erythropoiesis", splenic destruction of reticulocytes, random hemolysis, and senescence) could be estimated.

Blood from a single +/+ control and an affected mk/mk mouse of the MK/Re strain was prepared for examination by scanning electron microscopy as follows: freshly drawn orbital sinus blood was allowed to clot on a paraffin surface under conditions of high humidity. After clot retraction was complete, the clot was fixed in formalhehyde for 48 hours. The clot was then placed on a glass slide, covered with a thin metal film by evaporation, and examined with a scanning electron microscope (Japanese Electronic Optical Laboratory Corporation, Model JSM-1).

RESULTS

The range of hemoglobin concentration in circulating blood of control (+/+) mice was 16.1 to 17.0 grams per 100 ml, while it was 6.1 to 7.8 in mk/mk mice of the SEC/1ReJ strain and 8.2 to 10.6 in mk/mk mice of the MK/Re strain. In three mk/mk mice of the SEC/1ReJ strain post-splenectomy, the range was 5.0 to 6.7 grams per 100 ml. Reticulocyte concentration was 3 to 4 per cent in +/+ controls, while

it was in the range of 15 to 23 per cent in mk/mk mice of the SEC/1ReJ strain following splenectomy as well as in unsplenectomized mk/mk mice of the MK/Re and SEC/1ReJ strains. These values correspond well with more complete blood studies of mice of the same genotypes raised in the same laboratory[2,5] except that in the previous studies the hemoglobin content in SEC/1Re-mk/mk mice was somewhat higher (10.6 ± 0.2 for mk/mk females, 11.2 ± 0.3 for mk/mk males (mean ± S.E.)).

Production of ^{14}CO during the first 5 days after glycine-2-^{14}C injection ("early labeled peak") in heterozygotes (mk/+) was normal, with peak ^{14}CO production 1 to 2 hours after isotope injection, and a rapid decline thereafter (Figures 1 and 2). In affected mice (mk/mk) of the SEC/1ReJ strain (Figure 1) or the MK/Re strain (Figure 2), following this initial peak and a subsequent decline in ^{14}CO production, there was a second peak noted starting about 24 hours after isotope injection, which was maximal at 35 to 50 hours, and terminated about 72 hours after glycine injection. In splenectomized affected (mk/mk) mice of the SEC/1ReJ strain (Figure 1), the initial peak at 1 to 2 hours was seen, but the second peak at 24 to 72 hours was absent, being replaced by a plateau production more than twice that seen in the controls or heterozygotes.

Although adequate criteria for the precise definition and quantitation of "ineffective erythropoiesis" have yet to be made, certain observations[10-12] suggest that such processes should result in additional ^{14}CO production directly following the initial (1 to 2 hours after isotope injection) peak of ^{14}CO activity. No such increase is seen in normal rats or mice[9,12,13] or in any of the animal groups herein studied, with the exception of the mk/mk mice of the SEC/1ReJ

strain (Figure 1). In these mice, a "shoulder" was seen in the ^{14}CO curve about 10 to 15 hours after isotope injection, with a maximum and statistically significant difference from control animals seen at about 12 hours following glycine-2-^{14}C injection.

Production of labeled CO 3 to 100 days after glycine injection in control, homozygotes, or splenectomized homozygotes of the SEC/1ReJ strain is shown in Figure 3. Data points, representing 5-hour collection of expired ^{14}CO are shown as solid or open circles, while the least-squares best-fit of these data points to mathematical functions used to compute RBC survival[10] is shown as solid, dotted, or dashed lines. In the control animals there is a well-defined "late peak" of ^{14}CO production 30 to 60 days after glycine injection, representing primarily the senescent destruction of RBC labeled in the initial cohort. This "late peak" was not seen in the mk/mk mice, although its presence is suggested by the slight dip in the curve at about 45 to 50 days after glycine injection. In these animals, ^{14}CO production in the time period of 20 to 60 days fell exponentially, reflecting a markedly increased rate of random hemolysis. In the splenectomized microcytic mice the slope of this exponential fall was less than that seen in non-splenectomized mice of the same strain. The parameters of RBC survival derived from such curves, and also from the curves in the mk/mk mice of the MK/Re strain (not shown in Figure 3) are shown in Table I. To such results were added a graphical estimation of the amount of labeled heme accounted for in the abnormal peak seen 24 to 72 hours after glycine injection, and also for the amount of "ineffective erythropoiesis" present. In estimating the magnitude of these latter two processes, it was assumed that

they were absent in normal controls, and that their magnitude in affected mice could be approximated by subtracting data points obtained in the affected mice from corresponding data points in the controls. Results of this analysis are shown in Table II.

As compared to the controls, rate of random hemolysis in the investigated sample of microcytic anemics congenic with SEC/1Re was increased approximately 16-fold (Table I). Together with the early RBC destruction at 1 to 3 days following labeled glycine injection this resulted in a mean overall RBC lifespan of 15 days, as compared to 45 days in the controls (Table II). Splenectomy resulted in a slight decrease in the rate of random hemolysis to 11 times normal, and increased the mean overall RBC lifespan to 23 days. Anemic mice of the MK/Re strain showed a rate of random hemolysis 6 times that of the normal SEC/1Re-+/+ mice tested, and a fractional glycine incorporation approximately equal to that seen in homozygotes of the SEC/1Re strain, results which are compatible with the higher hemoglobin concentrations seen in the former mice. In all studies, the time of maximum death by senescence (mean potential RBC lifespan) and the spread of lifespans about this mean were essentially unaffected (Table II) although the fraction of all labeled RBC dying of senescence was markedly reduced in all mk/mk mice.

DISCUSSION

In the affected homozygotes with microcytosis (mk/mk), abnormally small and hypochromic RBC are produced in great numbers, along with larger hypochromic RBC, many of which have the appearance of target cells on glass slides. The result of these abnormalities is

a normal or increased number of RBC per unit volume associated with a low mean corpuscular hemoglobin concentration (MCHC), and a decreased hemoglobin content of whole blood. Examples of the morphologic picture seen in the peripheral blood of these animals are shown in Figures 4 and 5. The microphotographs show the above mentioned size and shape alterations, as well as the "Christmas wreath" reticulum pattern of the reticulocytes. At the present time the nature of the gene action leading to the defective erythrocytes of mk/mk mice is not known, but the possibility of abnormality in the hemoglobin structure has been eliminated[5].

Red blood cells in mice with microcytosis have shortened survival, characterized by early destruction of newly-formed RBC, and by increased rates of random hemolysis (Tables I and II). That a portion of reticulocytes die by the process of splenic trapping and destruction was concluded from the following observations:

1. Labeled CO production increased in a well-defined "peak" 24 to 72 hours after glycine injection. The upswing of this extra peak began at 24 hours, which is approximately equal to the mean time of entry of labeled normal mouse RBC into the circulation (26 hours[10]). The end of this peak at 72 hours corresponds to the time by which most or all of the cells labeled in the initial cohort have entered the peripheral circulation[10,14].

2. This increased "peak" of CO production was completely abolished by prior splenectomy (Figure 1).

Unlike the case in congenital spherocytosis in either the deermouse[15] or man[16,17], splenectomy did not improve the anemia of microcytic mice. Presumably, in man and deermouse, spherocytic RBC are

recognized as abnormal and trapped and destroyed only in splenic sinuses and cords[17], while non-splenic sites for RBC destruction (such as the liver) allow such affected cells to survive normally. In mice with microcytosis, on the other hand, the cells which either escape splenic destruction as reticulocytes, or those produced in splenectomized hosts are still subjected to an increased rate of random destruction (Table II). Table II indicates that mk/mk mice of the SEC strain lost about 93 per cent of the initial cohort of labeled RBC from "ineffective erythropoiesis", splenic destruction of newly emerging cells, and random hemolysis. While splenectomy seemed to abolish the first two processes, it only slightly decreased the rate of random hemolysis, so that 76 per cent of the labeled cohort was lost via random hemolysis, resulting in a RBC survival that was still markedly shortened.

Since the spleen is markedly erythropoietic in mk/mk mice[5], removal of this organ might well decrease erythropoiesis after splenectomy. Thus, although the mean RBC lifespan following splenectomy was increased slightly from 15 to 23 days, the decrease in glycine incorporation into RBC heme noted in these mice (last column, Table I) was probably sufficient to counteract this beneficial effect, resulting in no significant change in circulating hemoglobin concentration. Thus, the presence of splenic sequestration of reticulocytes in these animals was no guarantee that splenectomy would be beneficial. Similar circumstances probably explain the failure of splenectomy in patients with documented splenic destruction[18-20] of young RBC.

The production of ^{14}CO was abnormal in the time period of about

10 to 15 hours after glycine injection in mk/mk mice of the SEC/1ReJ strain (Figure 1). Kinetic arguments[10-13,21-27] would suggest that the process(es) of intramedullary destruction of RBC and/or their precursors would be maximal during this interval of time. In any case, "ineffective erythropoiesis" does not seem to be an integral part of the effects of the mk/mk genic substitution, since this alteration was not observed in the experiments with MK/Re-mk/mk mice. Further studies to delineate these processes with more precision are now in progress.

The value of the ^{14}CO method employed in this and similar studies lies in its ability to sequentially study, in the intact experimental animal (or human subject), the various modes of death to which the red cells are susceptible ("ineffective erythropoiesis", splenic destruction of reticulocytes, random hemolysis, and senescence[10,11,27]). With the aid of rigidly controlled genetic backgrounds in anemic mice, the relationship between these phases of RBC destruction, the underlying single gene defect, and other "modifying" genes present in the same animal can be studied in great detail. Such interaction between the underlying single gene defect (mk) and other modifying genes is suggested by the observed difference in rate of random hemolysis (and the presence or absence of "ineffective erythropoiesis") between our samples of mk/mk mice from the SEC/1ReJ and MK/Re strains. The previously noted inability to maintain viable stocks of mk/mk individuals congenic with particular inbred strains[5], strain difference in incidence of skin lesion in mk/mk mice[28], plus differences in deleterious influence of the gene substitution on breeding performance, all demonstrate the importance of interaction between the

single gene, mk, responsible for the microcytosis, and the remainder of the genome. A further study of these interrelationships should give increased insight into the basic hematologic mechanisms influencing health and disease.

ACKNOWLEDGEMENT

The authors are indebted to Dr. Thomas C. Hayes for performing the scanning electron microscopic studies on control and affected mice.

REFERENCES

1. Nash, D.J., Kent, E., Dickie, M.M., and Russell, E.S. The inheritance of "mick", a new anemia in the house mouse. Amer.Zool. 14:404, 1964.

2. Russell, E.S., and Bernstein, S.E. Blood and blood formation, In: Green, E.L. (Ed.) Biology of the Laboratory Mouse, 2nd ed. New York, McGraw-Hill, 1966, p. 351.

3. Bernstein, S.E. Hereditary disorders of the rodent erythron, In: ILAR Symposium on Genetics in Laboratory Animal Medicine. Nat. Acad. of Sci. Publ. 1724, 1969, p. 9.

4. Russell, E.S. Abnormalities of erythropoiesis associated with mutant genes in mice, Chapter 31. In: Gordon, A.S. (Ed.) New York, Appleton-Century-Crofts (In press).

5. Russell, E.S., Nash, D.J., Bernstein, S.E., Kent, E.L., McFarland, E.C., Mathews, S.M., and Norwood, M.S. Characterization and genetic studies of microcytic anemia in the house mouse. Blood, (In press).

6. Pinkerton, P.H., and Bannerman, R.M. The hereditary anemias of mice. Hematol. Rev. 1:162, 1968.

7. Sjöstrand, T. The formation of carbon monoxide by the decomposition of haemoglobin *in vivo*. Acta Physiol. Scand. 26:338, 1952.

8. Coburn, R.F., Blakemore, W.S., and Forster, R.E. Endogenous carbon monoxide production in man. J.Clin.Invest. 42:1172, 1963.

9. Landaw, S.A., and Winchell, H.S. Endogenous production of carbon-^{14}labeled carbon monoxide: an *in vivo* technique for the study of heme catabolism. J. Nucl. Med. 7:696, 1966.

10. Landaw, S.A., and Winchell, H.S. Endogenous production of ^{14}CO: A method for calculation of RBC lifespan *in vivo*, Blood, (In press).

11. White, P., Coburn, R.F., Williams, W.J., Goldwein, M.I., Rother, M.L., and Shafer, B.C. Carbon monoxide production associated with ineffective erythropoiesis. J. Clin. Invest. 46:1986, 1967.

12. Robinson, S.H. Increased formation of early-labeled bilirubin in rats with iron deficiency anemia: evidence for ineffective erythropoiesis. Blood 33:909, 1969.

13. Robinson, S.H., Tsong, M., Brown, B.W., and Schmid, R. The sources of bile pigment in the rat: studies of the "early labeled" fraction. J. Clin. Invest. 45:1569, 1966.

14. Altman, K.I., and Russell, E.S. Heme synthesis in normal and genetically anemic mice. J. Cell. Comp. Physiol. 64:293, 1964.

15. Motulsky, A.G., Huestis, R.R., and Anderson, R. Hereditary spherocytosis in mouse and man. Acta Genet. (Basel) 6:240, 1956.

16. Schloesser, L.L., Korst, D.R., Clatanoff, D.V., and Schilling, R.F. Radioactivity over the spleen and liver following transfusion of chromium ^{51}-labeled erythrocytes in hemolytic anemia. J. Clin. Invest. 36:1470, 1957.

17. Jandl, J.H. Hereditary spherocytosis. In: Stanbury, J.B., Wyngaarden, J.B., and Fredrickson, D.S. (Ed.) The Metabolic Basis of Inherited Disease. New York, McGraw-Hill, 1966, p. 1035.

18. Ben-Bassat, I., Seligsohn, U., Leiba, M., Leef, F., Chaitchik, S., and Ramot, B. Sequestration studies with chromium ^{51}-labeled red cells as criteria for splenectomy. Israel J. Med. Sci. 3:832, 1967.

19. Motulsky, A.G., Casserd, F., Giblett, E.R., Broun, G.O., and Finch, C.A. Anemia and the Spleen. New Eng. J. Med. 259:1215, 1958.

20. Veeger, W., Woldring, M.G., Van Rood, J.J., Eernisse, J.G., Leeksha, C.H.W., Verloop, M.C., and Nieweg, H.O. The value of the

determination of the site of red cell sequestration in hemolytic anemia as a prediction test for splenectomy. Acta Med. Scand. 171: 507, 1962.

21. Garcia, J. Radioiron time-distribution studies at various ages in the normal male rat. Am. J. Physiol. 190:31, 1957.

22. Levitt, M., Schacter, B.A., Zipursky, A., and Israels, L.G. The non-erythropoietic component of early bilirubin. J. Clin. Invest. 47:1281, 1968.

23. Craddock, C.G., Anemia and the spleen. Weinstein, I.M. and Beutler, E. (Ed.) In: Mechanisms of Anemia. New York, McGraw-Hill, 1962, p. 237.

24. Gevirtz, N.R., Wasserman, L.R., Sharney, L., and Tendler, D. Studies of plasma Fe^{59} disappearance-- a manifestation of ineffective erythropoiesis and of hemolysis. Blood 25:976, 1965.

25. Pollycove, M. Iron metabolism and kinetics. Seminars in Hematol. 3:235, 1966.

26. Barrett, P.V.D., Cline, M.J., and Berlin, N.I. The association of the urobilin "early peak" and erythropoiesis in man. J. Clin. Invest. 45:1657, 1966.

27. Landaw, S.A. Kinetic aspects of endogenous carbon monoxide production in experimental animals. New York Acad. Sci. (Annals) (In press).

28. Russell, E.S., McFarland, E.C., and Kent, E.L. Low viability, skin lesions, and reduced fertility associated with microcytic anemia in the mouse. Transpl. Proc. (In press).

PARAMETERS OF RBC SURVIVAL IN MICROCYTIC MICE

TABLE I

STRAIN	GENOTYPE	(K) RATE OF RANDOM HEMOLYSIS (PERCENT/DAY)	(T) MEAN POTENTIAL RBC LIFESPAN (DAYS)	(SIGMA) SPREAD OF RBC LIFESPANS ABOUT T (DAYS)	(C) FRACTIONAL INCORPORATION OF GLYCINE INTO RBC HEME (PERCENT)*
SEC/1ReJ	+/+	0.32 ± 0.04**	48.2 ± 0.1	8.8 ± 0.1	0.30 ± 0.01
SEC/1ReJ	mk/mk	5.2 ± 0.10	49.3 ± 1.1	8.0 ± 1.0	0.52 ± 0.01
SEC/1ReJ	mk/mk splenectomized	3.5 ± 0.28	45.4 ± 1.4	15 ± 1.4	0.45 ± 0.02
MK/Re	mk/mk	2.0 ± 0.12	46.3 ± 0.3	10 ± 0.4	0.54 ± 0.01

* INCLUDES "Ineffective erythropoiesis" plus all labeling of RBC hemoglobin heme

** Computer estimate of uncertainty in parameter expressed as MEAN ± 1 S.D.

TABLE II

FRACTIONATION OF RED BLOOD CELL DEATH IN MICE WITH MICROCYTOSIS

STRAIN	GENOTYPE	(PERCENT OF TOTAL ERYTHROPOIETIC HEME TURNOVER)				MEAN OVERALL RBC LIFESPAN (DAYS)
		INEFFECTIVE ERYTHROPOIESIS	SPLENIC SEQUESTRATION	RANDOM HEMOLYSIS	SENESCENCE	
SEC/1ReJ	+/+	0*	0*	14	86	45
SEC/1ReJ	mk/mk	3	21	69	7	15
SEC/1ReJ	mk/mk splenectomized	0	0	76	24	23
MK/Re	mk/mk	0	20	47	33	24

*Assumed to be zero in normal controls

FIGURE LEGENDS

Figure 1. Comparison of "early labeled peak" for ^{14}CO in SEC/1Re-mk/mk mice, in congenic SEC/1Re-+/+ mice, and in splenectomized SEC/1Re-mk/mk mice. Ordinate represents ^{14}CO production rate (dpm/hour/mouse), while abscissa represents time after glycine-2-^{14}C injection (hours).

In the intact SEC/1Re-mk/mk mice, the presence of a "shoulder" at 10 to 18 hours should be noted, along with a secondary rise at 24 to 72 hours. Both of these findings were abolished by prior splenectomy.

Figure 2. Further data on "early labeled peak" of microcytic and normal mice. (Ordinate and abscissa same as for Figure 1) Microcytic mice (o) were congenic with the MK/Re rather than the SEC/1Re strain; they showed the same 24 to 72 hour splenic destruction peak, but gave no evidence of ineffective erythropoiesis.

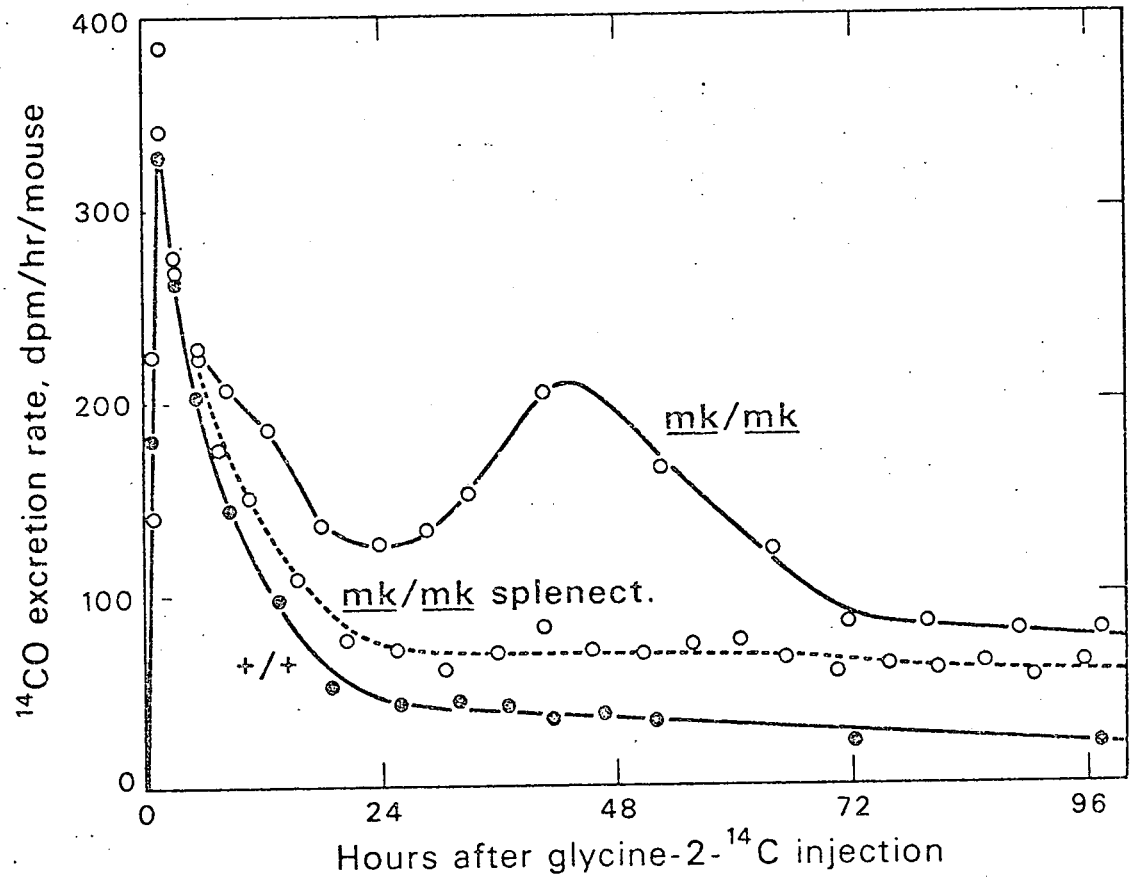
CO excretion curves for SEC/1Re-+/+ mice (repeated from Figure 1) and for SEC/1Re-mk/+ mice (⊙) are almost identical, indicating that the mk gene is completely recessive.

Figure 3. "Late peak" for ^{14}CO in mice of the SEC strain. Ordinate represents ^{14}CO production rate (dpm/hour/mouse) while abscissa represents time after glycine-2-^{14}C injection (days). Data points are shown as solid or open symbols, while the least-squares best fit of these data points to appropriate mathematical functions describing total ^{14}CO production[10] is shown as solid, dashed, or dotted lines. The phase of senescence centered about 50 days in the normal mice (+/+) is markedly diminished in mk/mk mice in the presence or absence

of the spleen.

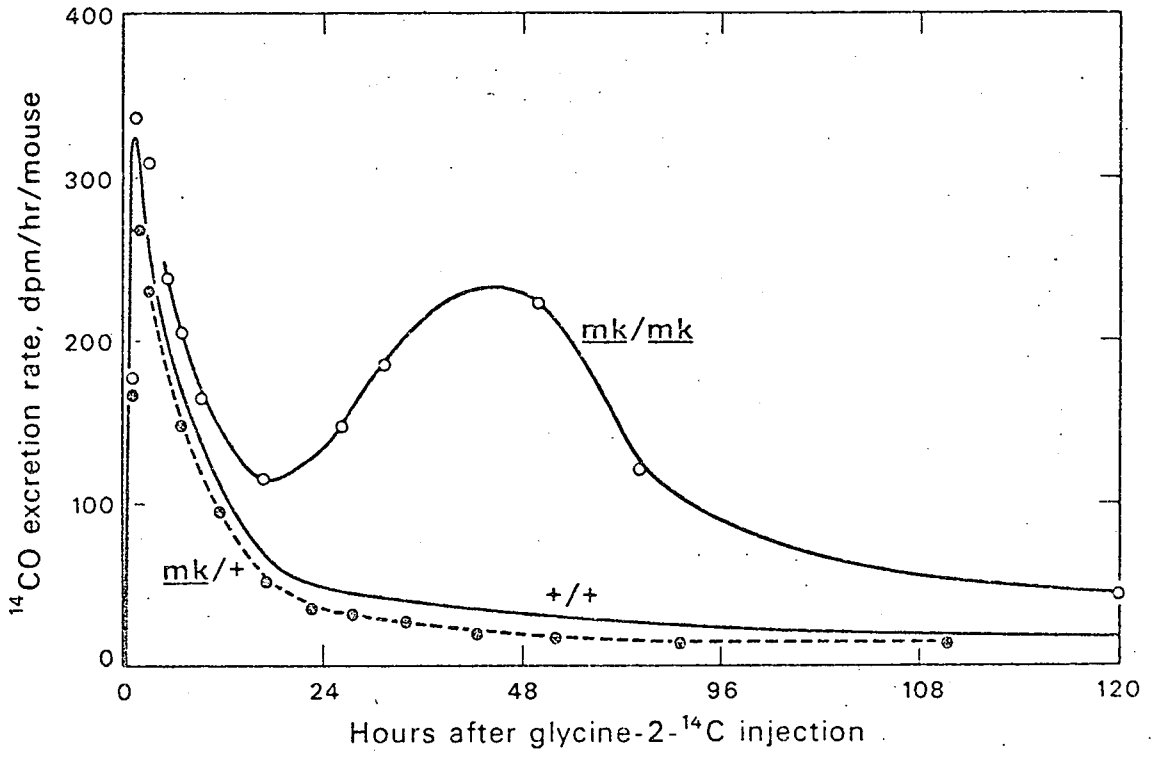
Figure 4. Reticulocyte preparation from a mk/mk mouse of the SEC/1ReJ strain on a glass slide. Note the "Christmas wreath" reticulocytes, the marked hypochromia of the RBC, and the presence of numerous small and misshapen RBC. (New Methylene Blue counterstained with Giemsa. 1440X)

Figure 5. Scanning electron microscopic films of clotted blood from an affected (mk/mk) mouse and a control (+/+) of the MK/Re strain. Note the presence of numerous flattened, distorted RBC (target and crenated cells) in the affected mouse (A) and the regular, rounded appearance of RBC in the controls (B). (Original magnification 3,000.)



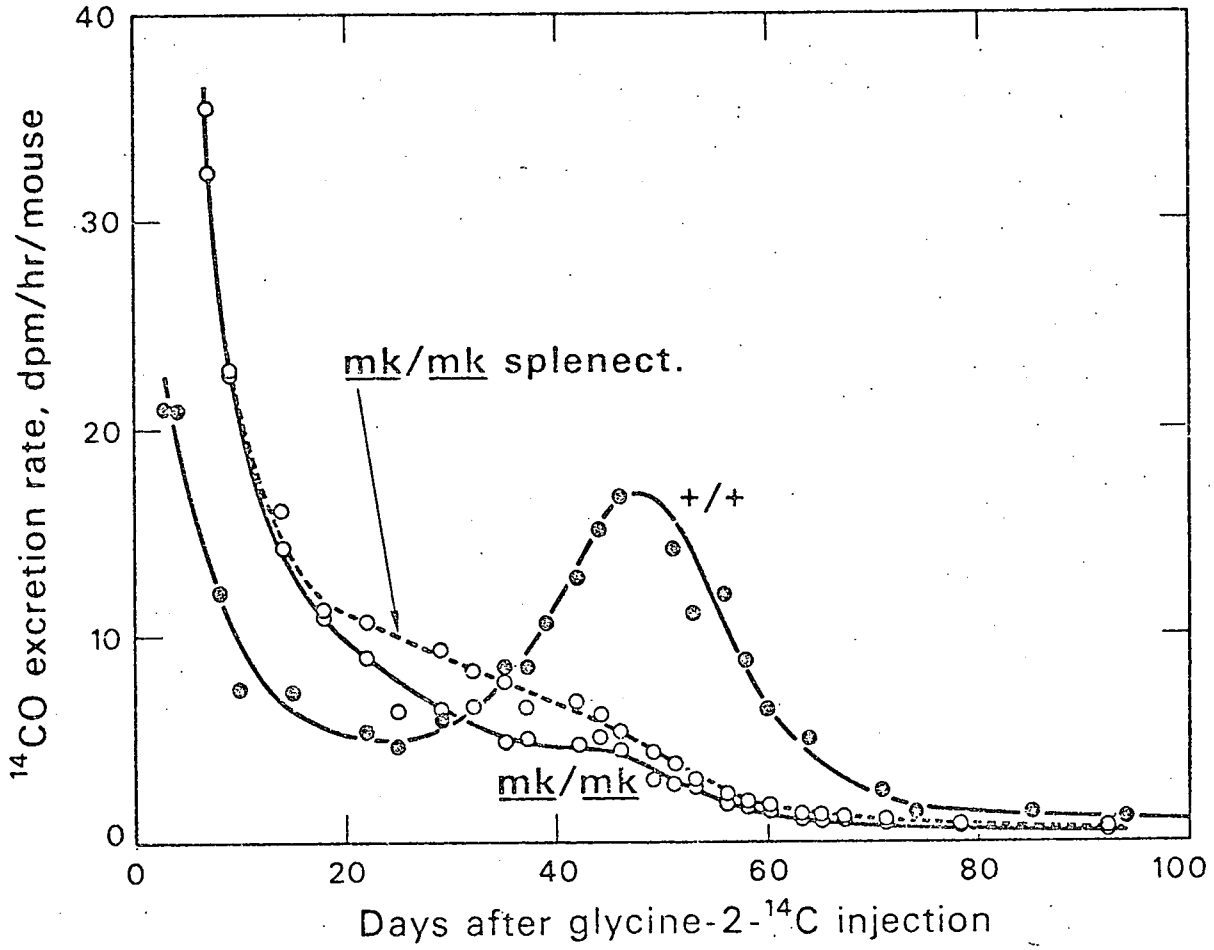
DBL 699-5063

(1)



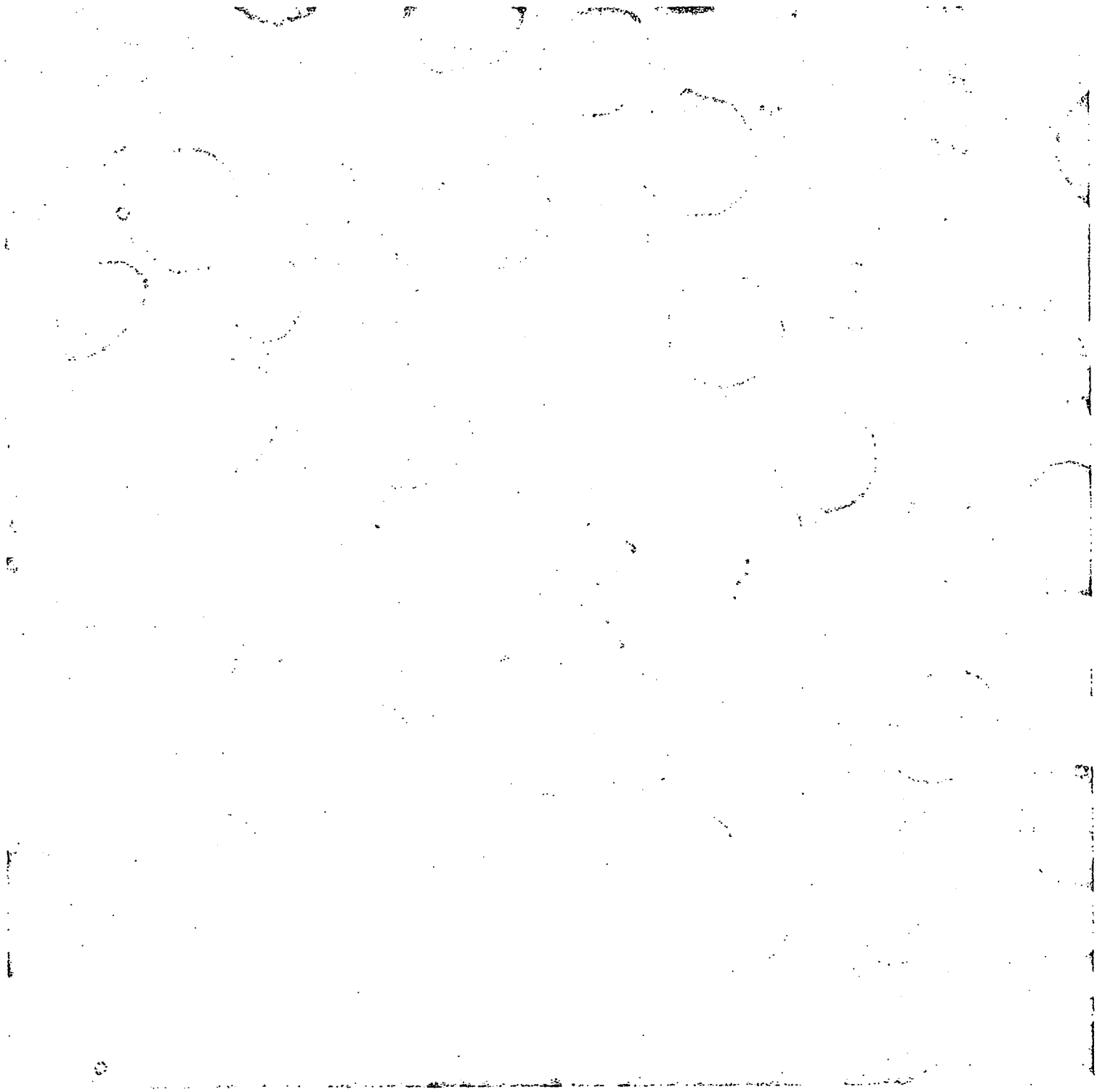
DBL 699-5068

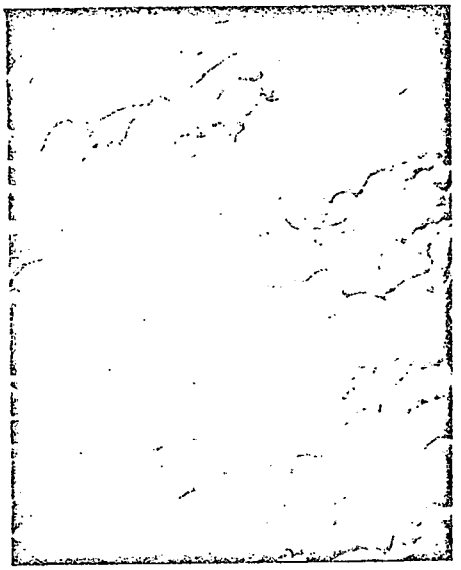
(2)



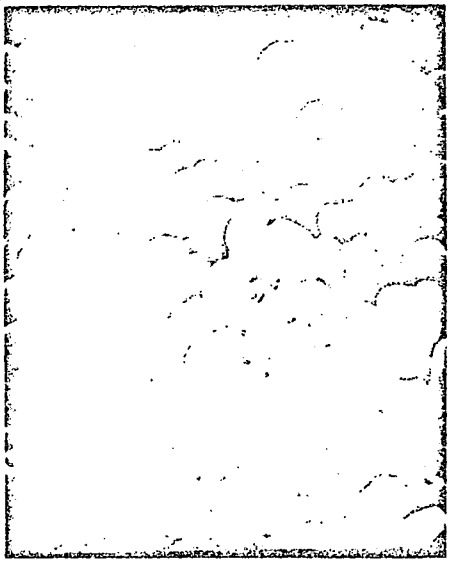
DBL 699-5067

(3)





A



B