714816

THE PATHOGENESIS OF THE ANEMIA OF CHRONIC LEUKEMIA: MEASUREMENT OF THE LIFE SPAN OF THE RED BLOOD CELL WITH GLYCINE-2-C^{14}*

Nathaniel I. Berlin, John H. Lawrence, and Helen C. Lee

Section on Research Medicine, Donner Laboratory and Donner Pavilion

University of California, Berkeley

*These studies were supported by U. S. Public Health Service Grant C-1440 (C2), and the labeled glycine and the equipment used were provided by the Atomic Energy Commission.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title Scientists Papers - Dr. John Lawrence
Accession No. 434-90A-0168
File Code No. 19-14-6
Carton No. 4
Folder No. O-R 1954
Notes
Found By Karen Holmes
Dates
COPY

3004024

Anemia not resulting from extravascular blood loss can arise either from a decrease in the rate of production of red blood cells or a decrease in the life span of the red blood cells or from a combination of these two factors. For many years it has been held that the anemia of leukemia results from a crowding out of the erythropoietic fraction of the bone marrow by leukemia tissue, resulting in a decreased rate of formation of red cells. Often the histological appearance of the bone marrow would tend to confirm this opinion. Certainly in some cases the marrow appears to be deficient in erythropoietic elements. However, in 1949, investigations in this laboratory showed surprisingly but conclusively that in most cases of chronic leukemia, the rate of production of red blood cells is normal or even greater than normal (1,2). Since these patients are in many instances anemic by blood volume standards, it was postulated that the red cell life span was decreased in this disease. Direct measurement of the life span of the red cell in several patients with chronic leukemia (3,4) indeed did show a shortened life span. Similar findings based on the Ashby differential agglutination technique were reported from several other laboratories (5-7). However, the Ashby method measures only the survival of normal donor cells in the patient.

With the demonstration that glycine is a specific precursor for the porphyrin of hemoglobin, it became possible to measure the life span of the red blood cell in its natural environment (8). The present paper is a more detailed and extended report on the life span of the red blood cell in eight patients with chronic leukemia as measured by the carbon-14 labeled glycine method. Three of these patients have been briefly reported in our original paper (4).

Methods

Each patient was given approximately 100 microcuries of carbon-14 as glycine-2-C^{14} (8.2 mgm. of glycine) intravenously. Blood samples were

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office

Records Series Title

Accession No. 434-90A-0168
File Code No. 19-14-6
Carton No. 6
Folder No. 0-R 1954
Notes
Found By
Dates

COPY

3004025

obtained at frequent intervals. The blood was centrifuged, and the plasma removed. The hemoglobin was extracted by a modification of the method of Drabkin (9), in which the cells were washed three times with physiological saline. When a definite layer of white blood cells was present, this too was removed. After the third washing, the cells were laked in distilled water. Toluene was added, the mixture shaken and then allowed to stand overnight. The stroma and the lipid-containing toluene layers were separated by centrifugation. From the intervening clear layer, an aliquot of the hemoglobin solution was aspirated. This was then dried in vacuo and combusted to carbon dioxide with a chromic-sulfuric acid mixture (10). The specific activity of the carbon dioxide was measured in a 100 cc. ionization chamber, at atmospheric pressure, using a vibrating reed electrometer and a recording potentiometer (11). Analysis of the dry hemoglobin powder for iron content showed that this material was approximately 95% hemoglobin.

Results and Discussion

Lymphatic leukemia: Three patients with chronic lymphatic leukemia were studied. The hematological course and therapy, together with graphs for the specific activity of the hemoglobin are presented in Figure 1-3.

Patient 1, F.E., a 40 year-old white male. This patient was studied during a period when the leukemia process was relatively well stabilized, testosterone being the only therapy given at that time. The red cell life span under these conditions was 113 days, which is normal. Throughout the course of the study, the spleen was palpable at the costal margin, but the patient was not anemic.

Patient 2, E. H., a 63-year-old white male. This patient was studied when the white blood count was showing a slow, progressive rise, while at the same time the red blood count and hemoglobin showed a progressive decline and the frequency of radioactive phosphorus therapy was increasing. The red cell life span was 102 days, only slightly, if at all, shorter than normal. The

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title
Accession No. 434-90A-0168
File Code No. 19-14-6
Carton No. 6
Folder No. O-R 1954
Notes
Found By
Dates
COPY
3004026

slow, continual rise in specific activity during the period of the 30th to 60th day probably results from some depression of the rate of red cell production due to the P^{32} administered just before these studies were begun. This results in a rate of production of red blood cells less than the rate of destruction, with the net effect being an increase in the relative concentration of cells containing carbon-14. Throughout the period of the study the spleen was palpable two to three fingerbreadths below the costal margin.

Patient 3, J.R., a 68-year- old white male. This patient was studied during the terminal phase of his disease. Under radioactive phosphorus therapy, the white cell count declined, and the red cell count also continued to decrease. The spleen was very large in this patient, extending to the right lower quadrant. The red cell life span in this patient does not fit the pattern observed in the first two patients, nor that observed in normals with nitrogen-15, but the data can be interpreted as indicating random destruction of red cells. The decreasing portion of the curve of C^{14}-labeled red cells can be fitted to a straight line when plotted on semilogarithmic paper, which indicates a fractional destruction rate of the circulating labeled cells at any time irrespective of the length of time that the cell has already lived. This decreasing rate is similar to that which has been observed in sickle cell anemia (12) and in experimental animals after hemorrhage (13,14). This interpretation would indicate that this patient's cells have a mean life span of 18 days. (This is only approximate, since four transfusions were given during the period of study.) This type of result would indicate a hemolytic process. It should also be noted that this patient achieved a peak hemoglobin specific activity approximately three times as high as any other individual, indicating a greater utilization of the radioactive glycine for hemoglobin formation than in other patients. In this case there was classical evidence of a hemolytic process

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title
Accession No. 434-90A-0168
File Code No. 19-14-6
Carton No. 6
Folder No. a-R 1954
Notes
Found
Dates
3004027

as demonstrated by a marked reticulocytosis and increased fecal urobilinogen excretion, although the increase in urobilinogen excretion was much smaller than would be expected as calculated from the life span of the red cell and the total red cell volume.

Thus, in the first two cases of lymphatic leukemia, the red cell life was normal, but in the third patient, in the anemic, terminal phase, the red cell life span was considerably shortened and corresponded to a pattern of random destruction. The anemia, then in this case, was probably due in a large part to this hemolysis.

Utilizing the Ashby differential agglutination technique in three cases of chronic lymphatic leukemia, Brown and co-workers (6) found two with finite, normal red cell life spans and one with shortened random red cell destruction; while in nine cases studied by R. Berlin (5), five may be interpreted as indicating a finite, an almost normal red cell life; two cases showed random destruction with markedly shortened red cell life; and in two cases the follow-up was not sufficient. In agreement with Brown, Elliot, and Young (4) and in partial agreement with Berlin (3), the red cell life span may be either normal or very short in chronic lymphatic leukemia, and the destruction process may be random, suggesting splenic destruction, as in case 3, or, as in cases 1 and 2, the cells may have a normal life span, or a slightly shortened but finite life span (case 2), suggesting a defective cell. In patient 3, with a large spleen, the destruction of red cells was random, but in cases 1 and 2, although the spleen was enlarged, the red cell life span was finite. Presumably case 3 presents the type of situation in which splenectomy might temporarily benefit the patient.

Myelogenous leukemia: Five patients with myelogenous leukemia were studied. The hematological course and red cell life data are presented in Figures 4-8.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title
Accession No. 434-90A-0168
File Code No. 19-14-6
Carton No. 2
Folder No. 0-R 1954
Notes
Found By
Dates
COPY
3004028

Patient 4, P. N. This patient was a 56-year-old white male in the chronic myeloid leukemic phase of polycythemia vera.

Patient 5, W.F. This patient was a 24-year-old white male with chronic myelogenous leukemia of two years' duration at the time of this study.

Patient 6, E.P. This patient is a 32-year-old negro female with chronic myelogenous leukemia of three months' duration at the time of this study.

Patient 7, G.J. This patient is a 52-year-old white female with chronic myelogenous leukemia of six months' duration at the time of this study.

Patients 5, 6, and 7 had classical chronic myelogenous leukemia. These individuals had finite red cell life spans of 70 to 83 days. Patient 8 had a finite red cell life span of 100 days. Although this is not as short as the others, it is shorter than normal. Thus, in these patients with chronic myelogenous leukemia, the red cell life span is shortened, but finite. In patients 2 and 5 the variation in the specific activity of the hemoglobin observed during the period of the 20 th to 60 th day probably represents changes in the relative concentration of labeled cells resulting from changes in the rate of production of red cells induced by radiation therapy. The patient was anemic throughout the course of the study.

With the exception of patient 8, these individuals with myelogenous leukemia had large, palpable spleens, generally extending below the umbilicus at the time of these studies. R. Berlin (5), using the Ashby differential agglutination technique in 15 cases of chronic myelogenous leukemia, found red cell 50% survival times varying from 5 to 63 days, or red cell life spans of 10 to 126 days; however, in only two cases was the 50% survival greater than 44 days, and in the majority of cases it was less than 30 days. In these 15 cases the graphs indicate a finite and shortened red cell life in three, and in one case it is difficult to decide. Thus, in general, his survival times are shorter than would be predicted from the present study.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title
Accession No. 434-90A-0168
File Code No. 19-14-6
Carton No. 6
Folder No. D-R 1954
Notes
Found By
Dates
COPY

3004029

This apparent difference may reflect either different types of patients or the result of different methods.

In an attempt to correlate the red cell life span with other clinical data, Table I shows the reticulocyte count, serum bilirubin, fecal urobilinogen, and rates of red cell production, as measured with Fe^{59}. This table shows that of our three patients with lymphatic leukemia, only one (case 3) with a short red cell life span had reticulocytosis. All five patients with myelogenous leukemia had a reticulocytosis. In the three patients with lymphatic leukemia the serum bilirubin was normal, but it was not measured in the myeloid group. The fecal urobilinogen excretion was variable; in one patient with myelogenous leukemia, it appears to be elevated, in two patients with myelogenous leukemia, it is low. In two patients with lymphatic leukemia the fecal urobilinogen appears to be within normal limits, while in patient 3, there is some increase, although not marked. This patient (case 3), with a very short red cell life span, had the highest urobilinogen excretion in this group. Of these laboratory determinations, the one test that correlated best qualitatively with the measurement of red cell life span was the reticulocyte count, but the reticulocyte count alone could not be used to predict red cell life span.

The rate of production of red cells was measured with radioactive iron and, as previously reported from this laboratory, was normal or greater than normal in the cases studied (1). It would be anticipated that this would also hold for the other two patients who died before this determination could be done. With the demonstration by radioiron that the bone marrow is producing a normal or greater than normal number of red blood cells and that, in one of three cases of chronic lymphatic leukemia and five cases of chronic myelogenous leukemia, the red cell life span is shortened, the pathogenesis of the anemia in the absence of hemorrhage is clearly then a result of the

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Scientists' papers -
Records Series Title Dr. John Lawrence
Accession No. A34-90A-0186
File Code No. 14-14-6
Carton No. (6)
Folder No. O-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004030

shortening of the life span of the red blood cell. However, the bone marrow in these individuals, although producing a normal or greater than normal amount of red cells per day as measured with radioiron, is still not producing a sufficient number to compensate for the decreased red cell life span, and furthermore is not responding to this stimulus with an increase in production of the magnitude to which it appears to be capable. Patients with polycythemia vera (2) and hereditary hemolytic anemia (16) appear to be able to increase the production rate about six to nine times, while in leukemia, the maximum observed increase is approximately fourfold. In this sense, in leukemia the bone marrow is abnormal with respect to erythropoiesis. This does not, however, deny the conclusion that the fundamental defect leading to anemia is a decrease in the life span of the red blood cell.

In vivo studies with radioactive iron in patient 2, with chronic lymphatic leukemia, who was not anemic, disclosed a normal iron turnover pattern. However, in six other cases with chronic lymphatic leukemia studied in the same manner (18) it was found that there was a definite secondary rise in the counting rate over the spleen site in three patients; in two this was slight; and in one there was no such rise. This secondary rise has been interpreted to indicate premature destruction of erythrocytes in the spleen (17,18). Of these seven patients with in vivo radioiron studies, five had normal total red cell volumes. In three of these five patients there was evidence for splenic erythroclasis. The two definitely anemic patients both showed a secondary spleen rise, indicating premature breakdown of red cells in the spleen.

In patient 5 (W.F.) with chronic myelogenous leukemia, who had a shortened, finite life span of the red cells, the in vivo radioiron study showed a rapid early rise in the counting rate over the spleen site, which was maintained at a high level during the course of observation. The early rise in counting rate here was interpreted as indicating splenic production of red cells, while

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Scientists' papers-
Records Series Title Dr. John Lawrence
Accession No. 254-90A-0186
File Code No. 14-14-6
Carton No. (6)
Folder No. D-R 1954
Notes
Found By Karen Holmes
Dates 1952-1954
COPY

3004031

The failure of the counting rate to fall may be interpreted as being due to presence of radioiron in the spleen resulting from premature breakdown of red blood cells by the spleen. This case is characteristic of all other cases (four) of myelogenous leukemia studied with radioiron (17,18). Thus, in two cases of chronic lymphatic leukemia where anemia was present, there was a premature destruction of red cells in the spleen, while in chronic myelogenous leukemia, there is both production and premature destruction of red cells in the spleen.

It seems that the red cell life may be shortened by at least two mechanisms. One possible mechanism is a shortened cell life due to an intrinsic defect within the cell itself. Such a defect might express its influence in a red cell life that is either finitely or randomly shortened. An example of finite shortening as a type of intrinsic defect is possibly seen in the lessened red cell life span after hemorrhage (13,14) and an example of randomly shortened red cell life span is the varyingly shorter-lived red sickle cell disease (12). The second general type of mechanism responsible for shortening red cell life is seen in the hemolysis resulting from mismatched transfusions. The finding in all five cases of chronic myelogenous leukemia that the red cell life was finite and shortened leads to speculation that the basic defect is inherent in the red cell and not due to some extracorpuscular factor, such as hypersplenism, which should produce a random type of destruction. In one of the three cases of lymphatic leukemia such a random type of destruction was seen, suggesting that in that instance hypersplenism or some other extracorpuscular factor was producing the anemia, although even in this instance it is possible for the defect to be within the red cell.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Scientists' papers-
Records Series Title Dr. John Lawrence
Accession No. 454-90A-0186
File Code No. 14-14-6
Carton No. (6)
Folder No. D-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004032

Conclusions

(1) The red cell life span was approximately 18 days in one case of lymphatic leukemia and 102 and 113 days, respectively, in two additional cases.

(2) The red cell life span in five cases of myelogenous leukemia was 70 to 100 days.

(3) The anemia of leukemia, when not due to hemorrhage, may be the result of shortening of the life span of the red cell and not due to crowding out of the red cell precursors in the bone marrow.

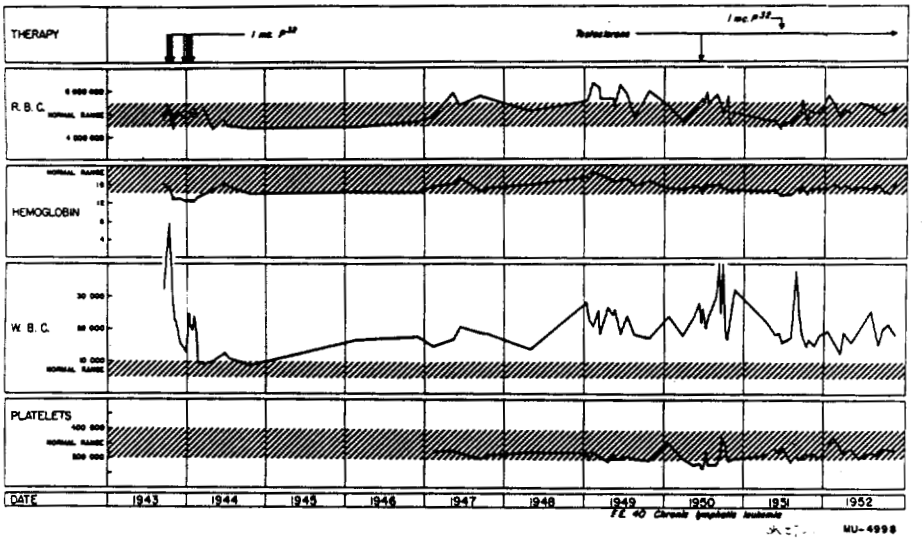
(4) Reticulocyte count correlated negatively better with red cell life span than did serum bilirubin or fecal urobilinogen, but none of these tests can predict with any accuracy the degree of shortening of the life span of the red cell.

(5) The spleen appears to play a significant role in premature breakdown of red cells in the five cases of chronic myelogenous leukemia studied and in five of the seven cases of chronic lymphatic leukemia studied with radioactive iron.

(6) In the five cases of myeloid leukemia and in two of three cases of lymphatic leukemia the life span was finite, but shortened, while in one case of lymphatic leukemia there was random destruction of the labeled red cells.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Scientists' papers-
Dr. John Lawrence
Records Series Title
Accession No. 434-90A-0186
File Code No. 19-19-6
Carton No. (6)
Folder No. D-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004033



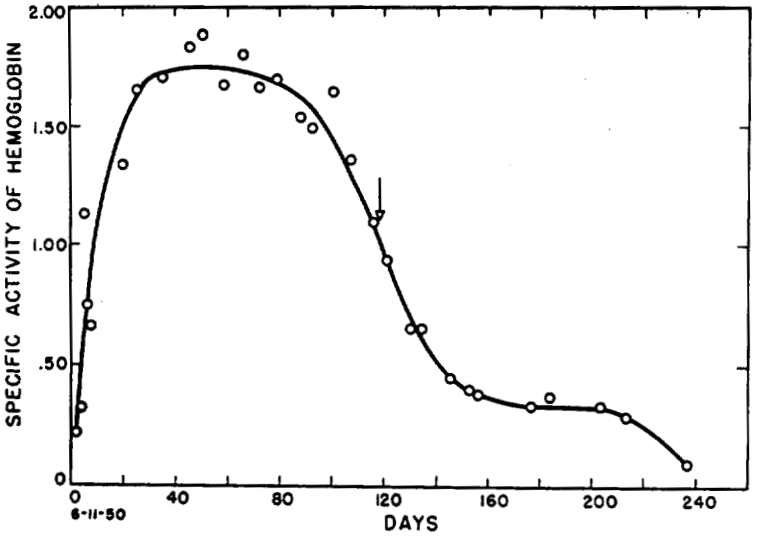
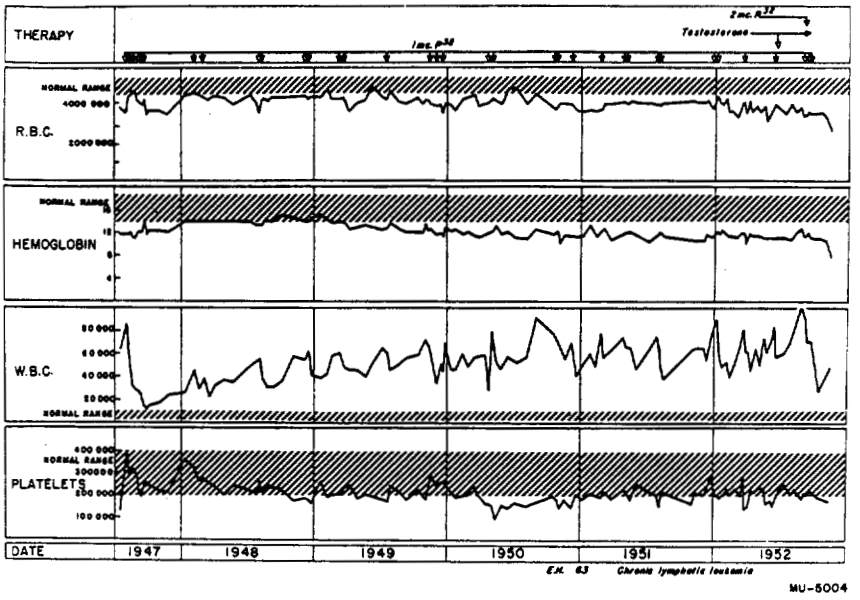


Fig. 1. Hematological course and red cell life span in F. E., a 40-year-old white male with chronic lymphatic leukemia.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title Scientists' papers - Dr. John Lawrence
Accession No. 434-90A-0186
File Code No. 19-19-6
Carton No. (6)
Folder No. D-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004034



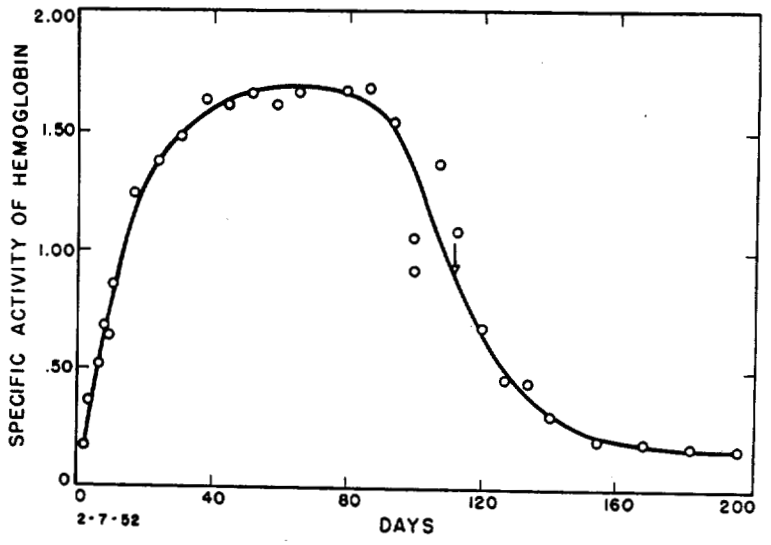


Fig. 2. Hematological course and red cell life span in E. H., a 63-year-old white male with chronic lymphatic leukemia.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Scientists' papers-
Dr. John Lawrence
Records Series Title
Accession No. 434-90A-0186
File Code No. 19-14-6
Carton No. (6)
Folder No. O-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004035

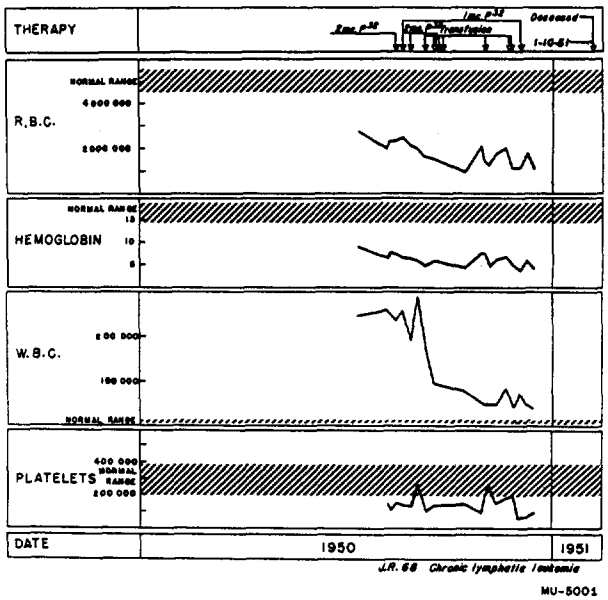


Fig. 3a. Hematological course in J. R., a 68-year-old white male with chronic lymphatic leukemia.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title Scientists' papers - Dr. John Lawrence
Accession No. 434-90A-0186
File Code No. 14-14-6
Carton No. (6)
Folder No. D-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004036

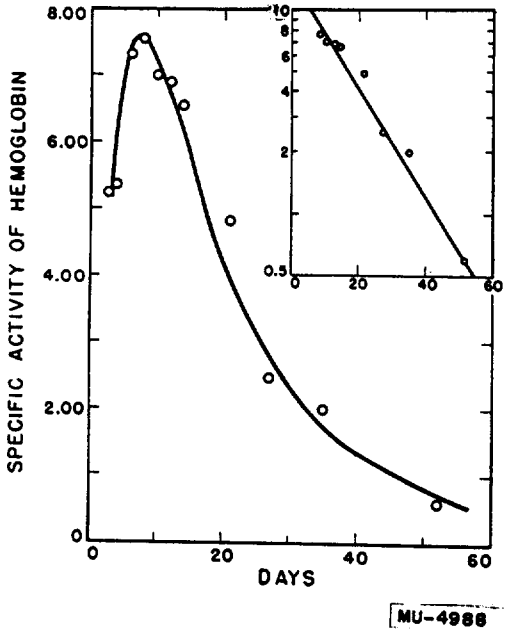
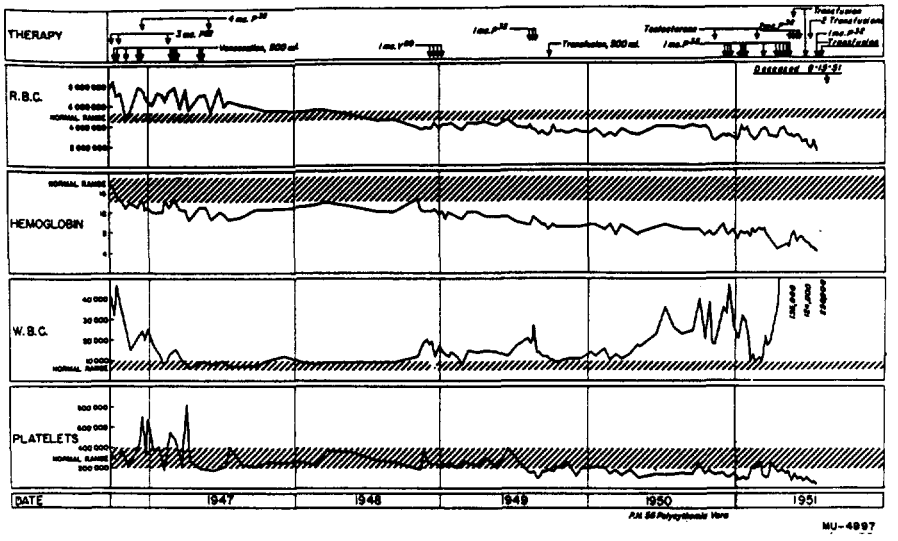


Fig. 3b. Red cell life span in J. R., a 68-year-old white male with chronic lymphatic leukemia.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Scientists' Papers-
Dr. John Lawrence
Records Series Title
Accession No. 88-404-0186
File Code No. 19-19-6
Carton No. (6)
Folder No. D-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004037



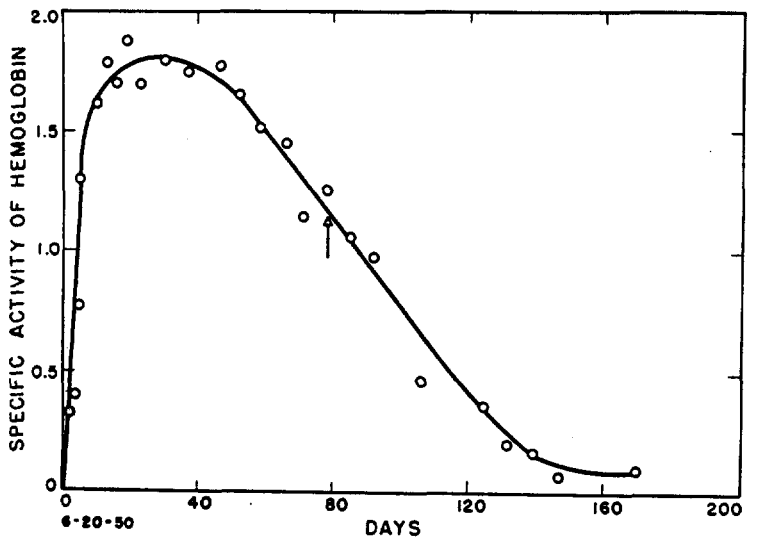
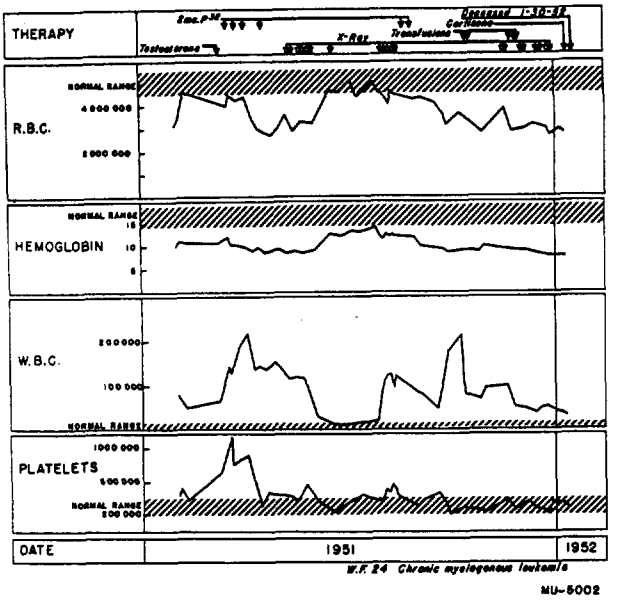


Fig. 4. Hematological course and red cell life span in P. N., a 56-year-old white male in the chronic myeloid leukemic phase of polycythemia vera.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title Dr. John Lawrence
Accession No. 434-90A-0186
File Code No. 14-14-6
Carton No. (6)
Folder No. O-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004038



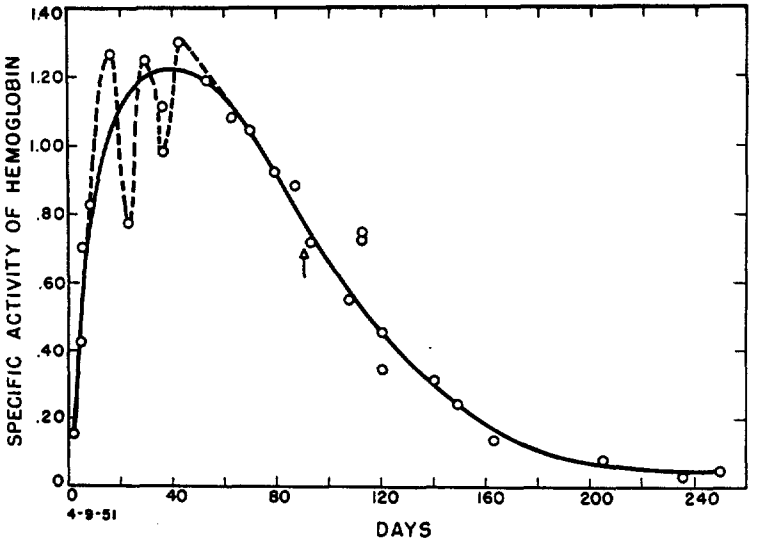
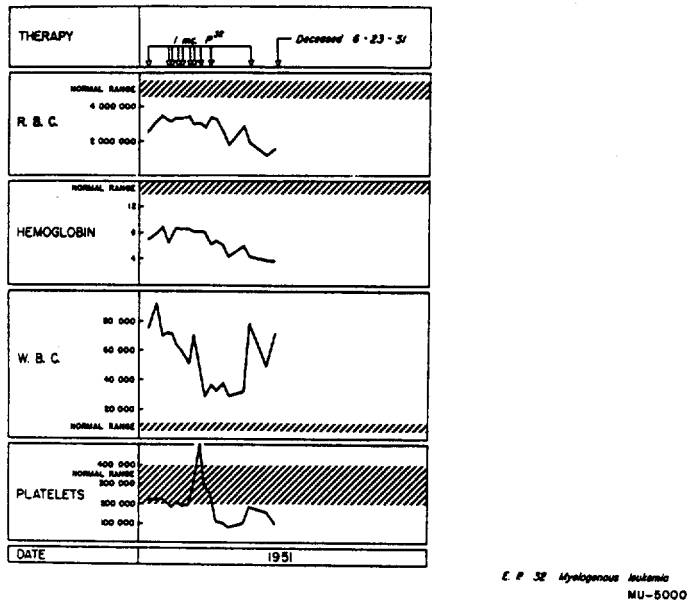


Fig. 5. Hematological course and red cell life span in W. F., a 24-year-old white male with chronic myelogenous leukemia.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Scientists' papers-
Records Series Title Dr. John Lawrence
Accession No. 434-90A-0186
File Code No. 19-19-6
Carton No. (6)
Folder No. O-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004039



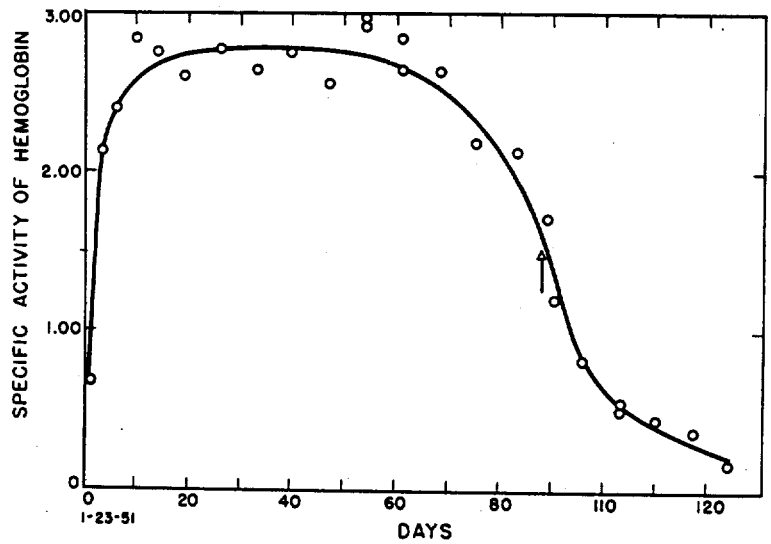
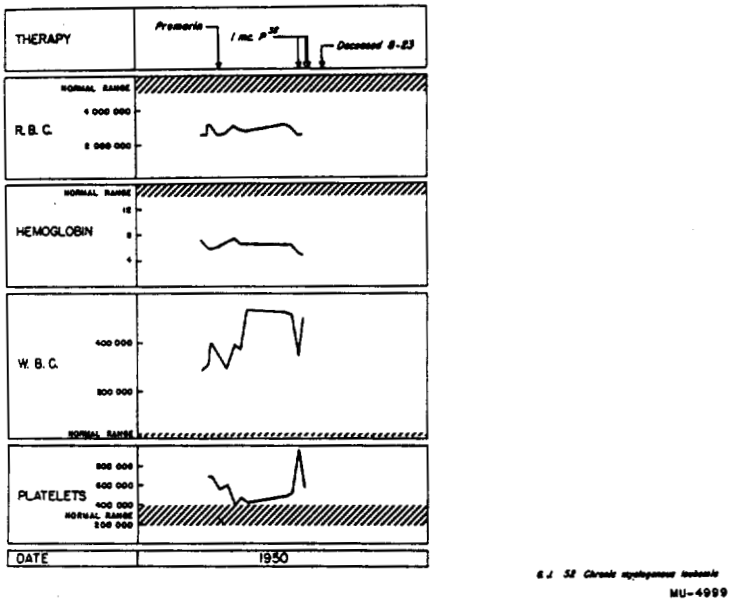


Fig. 6. Hematological course and red cell life span in E. P., a 32-year-old negro female with chronic myelogenous leukemia.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title Scientists' papers - Dr. John Lawrence
Accession No. 434-90A-0186
File Code No. 19-19-6
Carton No. (6)
Folder No. O-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004040



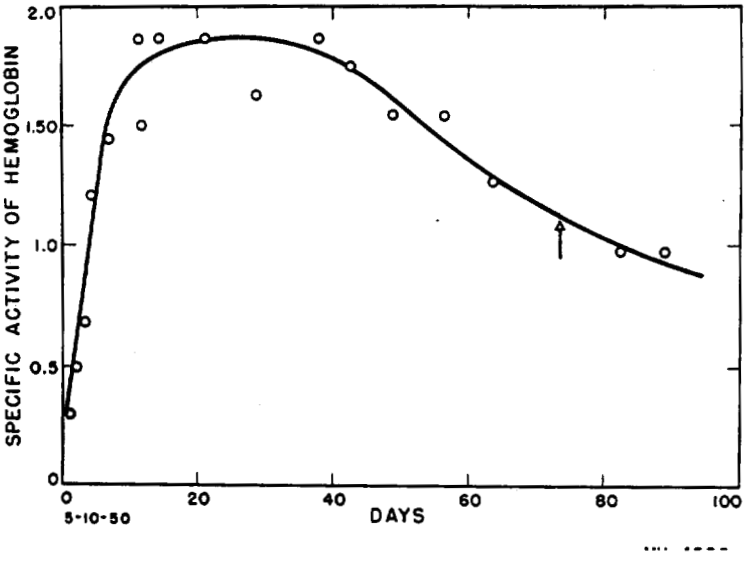
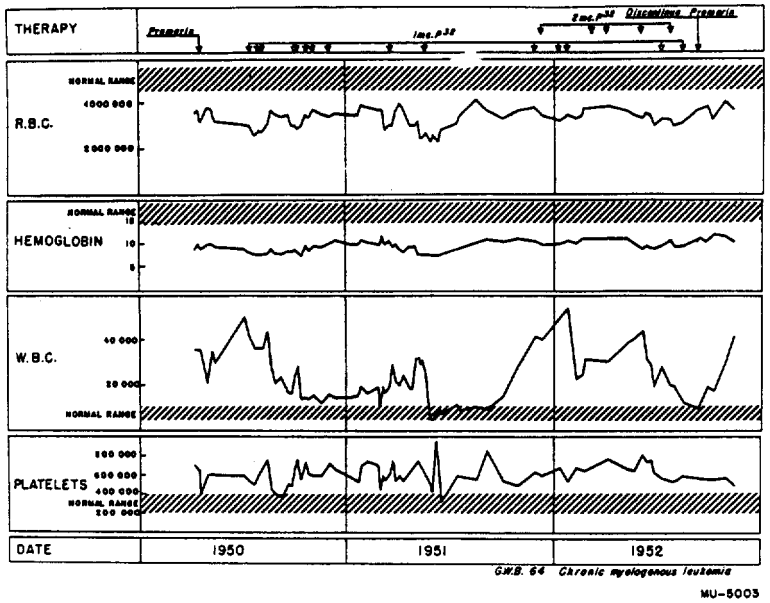


Fig. 7. Hematological course and red cell life span in G. J., a 52-year-old white female with chronic myelogenous leukemia.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title Scientists' papers - Dr. John Lawrence
Accession No. 234-90A-0186
File Code No. 19-17-6
Carton No. (6)
Folder No. D-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004041



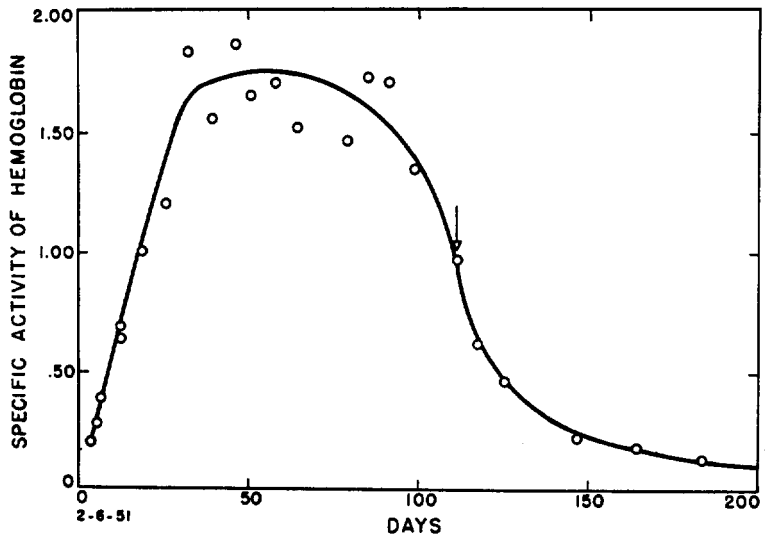


Fig. 8. Hematological course and red cell life span in G. B., a 64-year-old white male with chronic myelogenous leukemia.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Scientists' papers-
Records Series Title Dr. John Lawrence
Accession No. 434-90A-0186
File Code No. 14-14-6
Carton No. (6)
Folder No. O-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

TABLE 1

	Reticulocytes (%)	Fe Turnover* (mgm./kg. of body wt./day)	Fecal Urobilinogen (mgm./day)	Serum Bilirubin (mgm.%)
Lymphatic				
1. F.E.	0.2-1.2	0.33	110	0.083
2. E.H.	0.1-1.2	0.26	182	1.31
3. J.R.	19-26	--	252	0.2
Myelogenous				
4. P.N.	0.2-18.5	0.55	487	
5. W.F.	1.3-8.5	1.32	203 (2/21/51) 47 (3/26/51)	
6. E.P.	0.6-10.0	--	--	
7. G.J.	1.6-5.2	--	--	
8. G.B.	1.6 -3.2	0.26	24	

*Fe turnover = The iron turnover as measured with Fe^{59}, expressed as mgm./kg. of body weight/day for hemoglobin formation (normal, 0.26 mgm./kg./day).

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Records Series Title Scientists' papers - Dr. John Lawrence
Accession No. 434-90A-0186
File Code No. 14-14-6
Carton No. 6
Folder No. 0-R 1954
Notes
Found By Karen Holmes
Dates 1952-1954
COPY

3004043

Bibliography

1. Huff, R. L., Hennessy, T. G., and Lawrence, J. H. J. Clin. Invest., 28:790, 1949.

2. Huff, R. L., Hennessy, T. G., Austin, R. E., Garcia, J. F., Roberts, B. M., and Lawrence, J. H. J. Clin. Invest., 29:1041, 1950.

3. Monthly Progress Report No. 93, January 30, 1951, University of Calif. Radiation Laboratory No. 1110.

4. Berlin, N. I., Lawrence, J. H., and Lee, H. C. Science, 114:385, 1951.

5. Berlin, R., Suppl. 252, Acta Med. Scand., 1951.

6. Brown, G. M., Elliot, S. W., and Young, W. M., J. Clin. Invest., 30:130, 1951.

7. Ross, J. F., Crockett, C. L.,Jr.,and Emerson, C. P. J. Clin. Invest., 30:668, 1951.

8. Shemin, D., and Rittenberg, D. J. Biol. Chem., 166:627, 1946.

9. Drabkin, D. L. J. Biol. Chem., 164:103, 1946.

10. Van Slyke, D., and Folch, J. J. Biol. Chem., 136:509, 1946.

11. Tolbert, B. M. To be published.

12. London, I. M., Shemin, D., West, R., and Rittenberg, D. J. Biol. Chem., 179:463, 1949.

13. Neuberger, A., and Niven, T. J. Physiol., 112:292, 1951.

14. Berlin, N. I., and Lotz, C. Proc. Soc. Exp. Biol. Med., 78:788, 1951.

15. Sheets, R. F., Janney, C. D., Hamilton, H. E., and de Garvin, E. L. J. Clin. Invest., 30:1272, 1951.

16. Crosby, W. H., and Akeroyd, J. H. Amer. J. Med., 13:273, 1952.

17. Elmlinger, P. J., Huff, R. L., Tobias, C. A., and Lawrence, J. H. Acta Haematologica, 9:73, 1953.

18. Elmlinger, P. J. Personal Communication.

DOCUMENT SOURCE
Lawrence Berkeley Laboratory
Archives and Records Office
Scientists' papers-
Dr. John Lawrence
Records Series Title
Accession No. 424-90A-0186
File Code No. 19-19-6
Carton No. 6
Folder No. D-R 1954
Notes
Found By Karen Holmes
Dates 1953-1954
COPY

3004044