

THE NATURE OF ANAEMIA IN RHEUMATOID ARTHRITIS

V. RED CELL SURVIVAL MEASURED BY RADIOACTIVE CHROMIUM

BY

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A number of studies have been undertaken to assess the importance of shortened red cell survival in the causation of anaemia complicating rheumatoid arthritis. Freireich, Ross, Bayles, Emerson, and Finch (1954), using the Ashby technique, found reduction of the life span of transfused erythrocytes from normal individuals in patients suffering from the disease. Alexander, Richmond, Roy, and Duthie (1956), using the same technique, confirmed this observation in all of eighteen patients, as did McCrea (1957) in ten out of fifteen patients and Freireich, Ross, Bayles, Emerson, and Finch (1957) in "more than half" of thirteen patients. However, Freireich and others (1954, 1957) observed that cells from patients with rheumatoid arthritis survived normally in the circulation of normal individuals; Alexander and others (1956) reported that the survival time of cells from patients with rheumatoid arthritis, although shorter than normal, exceeded that of cells from normal subjects when transfused to other patients with rheumatoid arthritis.

The survival of a patient's own cells in his own circulation cannot be measured by the Ashby technique. Hence it is interesting to review the evidence for a haemolytic process in rheumatoid arthritis that has been obtained with the use of chromium-51 as a red cell label. Bunim (1954) reported the first such study and observed reduction of the survival time of the patients' own cells in all the cases investigated. This was confirmed in a report by Ebaugh, Peterson, Rodnan, and Bunim (1955). Weinstein (1959), also measuring the survival of the patient's own cells in his own circulation, stated that "hyperhaemolysis" contributes to the anaemia of rheumatoid arthritis. However, a study of his data shows that nine of the eighteen

patients investigated fell within the normal range of the author's series and that in only four patients (two of whom had splenomegaly) was the rate of disappearance of injected radioactivity clearly increased. Lewis and Porter (1960) used chromium-51 in a study of fifty patients; in only four was survival of the individual's own cells (after correction for elution of chromium) below the normal range, and in these patients the finding appeared to be due to a loss of radioactivity from the circulation, in the first few days of the study, slightly greater than that which is known to occur in normal subjects. These authors, who took care to exclude gastro-intestinal bleeding as a cause of loss of labelled red cells from the circulation, concluded that "haemolysis is not a feature of rheumatoid arthritis and is not a significant factor in the development of the anaemia which is often associated with the disease".

Because of the divergent results of different investigators, it was considered that the following report, which deals with the survival of the subject's own cells and of donor cells from normal individuals in patients with rheumatoid arthritis, would be of interest. The preliminary findings in this investigation have been reported elsewhere (Richmond, 1957).

Methods

21 patients suffering from rheumatoid arthritis were investigated; in each patient the diagnosis of "definite rheumatoid arthritis" had been established by the criteria recommended in the revised classification of the American Rheumatism Association (Ropes, 1959). All were receiving in-patient treatment at the Rheumatic Diseases Unit, Northern General Hospital, Edinburgh.

The patients were unselected; none showed an unusually severe degree of anaemia, clinical evidence of haemolytic disease, or palpable splenic enlargement.

The survival of the patient's own red cells in his own circulation was studied in each case, and in six of the patients the survival of cells freshly withdrawn from compatible healthy donors was also measured.

In five normal individuals the measurement of survival of the subject's cells in his own circulation was used as a standard of normality.

Red cell survival was estimated using the radioactive isotope chromium 51 as a red cell label; the method was that described by Mollison and Veall (1955). Because of the elution of chromium from labelled cells (approximately 1 per cent. per day) it is accepted that the rate of disappearance of injected radioactivity from the patient's circulation can only serve as an index of cell survival. No attempt has been made to correct the results in the present investigation for elution. The first blood sample was withdrawn 1 hour after the injection of chromium-labelled cells and its content of radioactivity constituted the initial level of "100 per cent." Two further samples of blood were withdrawn in the next 3 days; thereafter samples were obtained twice weekly for at least 20 days.

The methods for determining the peripheral blood counts, the erythrocyte sedimentation rate (E.S.R.), and the agglutination titre of sensitized sheep cells (S.S.C.T.) have been described in previous communications (Richmond, Gardner, Roy, and Duthie, 1956; Bremner, Alexander, and Duthie, 1959).

Results

Clinical and Haematological Status of Patients.—Eleven female and ten male patients were investigated. Clinical and other data on these patients are shown in Table I.

There was a wide range for age, clinical duration of disease, haemoglobin level, erythrocyte sedimentation rate, and agglutination titre for sensitized sheep cells.

Erythrocyte Survival.—As a measure of "cell survival" the linear regression coefficient (b) of radioactivity (log. percentage of initial radioactivity) on time (in days) was calculated for the data obtained in each patient. In each case there was a high linear correlation between remaining radioactivity and time (correlation coefficient, $r = -0.9361$ to -0.9985) within the period after injection during which the rate of disappearance was calculated. A plot of the data showed that the relationship was not strictly linear in some cases, but within the chosen limits of 0 to 20 days the linear coefficients were adequate for the purpose of comparing "cell survival" in different individuals and groups.

The following results are summarized in Table II and Fig. 1 (opposite) and Fig. 2 (overleaf).

(a) *Own Cells in Normal Subjects.*—The average linear regression coefficient (b) for the five normal subjects studied was -0.0107 ($r = -0.9937$). These results are similar to those of Mollison and Veall (1955), for whose data the following figures have been calculated: $b = -0.01160$; $r = -0.9950$.

TABLE I
CLINICAL AND HAEMATOLOGICAL STATUS OF 21 PATIENTS WITH RHEUMATOID ARTHRITIS, SHOWING RESULTS OF RED CELL SURVIVAL

Case No.	Age (yrs)	Sex	Clinical Duration of Disease (yrs)	Haemoglobin Level per cent. (100 per cent. = 14.8 g./100 ml.)	Erythrocyte Sedimentation Rate (mm./hr)	Sensitized Sheep Cell Test	Linear Regression Coefficient (b)*	
							Own Cells	Normal Donor Cells
1	65	F	9	58	82	1:1,024	-0.0131	-0.0148
2		F				1:16	-0.0109	
3	52	M	5	89	48	1:512	-0.0121	
4	46	M	3	63	23	<1:8	-0.0117	-0.0103
5	48	F	9	93	29	1:8	-0.0114	
6	55	M	4	92	80	1:128	-0.0123	
7	56	F	11	63	111	1:16	-0.0092	
8	45	F	3	75	82	1:1,024	-0.0121	
9	33	M	12	82	54	1:1,024	-0.0094	
10	58	F	8	86	55	1:1,024	-0.0126	
11	61	F	1	76	68	1:32	-0.0103	
12	63	F	30	77	80	1:64	-0.0090	
13	50	M	7	57	101	1:32	-0.0101	
14	64	M	2	89	43	1:256	-0.0126	
15	38	M	2	87	55	1:1,024	-0.0104	
16	50	F	8	74	70	1:256	-0.0120	-0.0127
17	51	F	4	96	23	1:16	-0.0113	-0.0122
18	46	M	3	94	38	1:1,024	-0.0115	
19	42	F	9	78	26	1:32	-0.0141	
20	38	M	14	90	34	1:256	-0.0128	-0.0123
21	56	M	1	106	46	1:1,024	-0.0133	-0.0146
Mean	48.4		6.9	77.4	55			

* See text.

TABLE II
ERYTHROCYTE SURVIVAL IN DIFFERENT GROUPS OF SUBJECTS

Group of Subjects	Cells Labelled	Linear* Regression Coefficient (b)	Linear* Correlation Coefficient (r)
5 Normal Subjects	Own	-0.01070	-0.9937
21 Patients with Rheumatoid Arthritis	Own	-0.01100	-0.9422
6 Patients with Rheumatoid Arthritis in whom Survival of Own Cells and Donor Cells was Studied	Own	-0.01215	-0.9934
	Donor	-0.01302	-0.9578

* See text.

(b) *Own Cells in Patients with Rheumatoid Arthritis.*—The average linear regression coefficient (b) for the patients with rheumatoid arthritis was -0.01100 (r = -0.9422) and comparison of this result with that obtained in normal subjects did not show a significant difference. However, in fifteen of the 21 patients studied, “cell survival” was

shorter than the mean for the normal controls.

(c) *Normal Cells in Patients with Rheumatoid Arthritis.*—For the six patients (Cases 1, 4, 16, 17, 20, 21) in whom survival of their own cells and those of normal donor cells was measured consecutively, the following results were obtained:

Own cells $b = -0.01215; r = -0.9934;$

Normal cells $b = -0.01302; r = -0.9578.$

Comparison of the curves for “cell survival” between normal cells in patients with rheumatoid arthritis and own cells in normal subjects showed a difference which was highly significant ($p < 0.001$). Comparison between the curves for normal cells and own cells in patients with rheumatoid arthritis showed no significant difference ($0.1 > p > 0.05$).

There was no apparent relationship between “cell survival”, and the age, sex, clinical duration of disease, haemoglobin level, erythrocyte sedimentation rate, and titre in the sensitized sheep cell test.

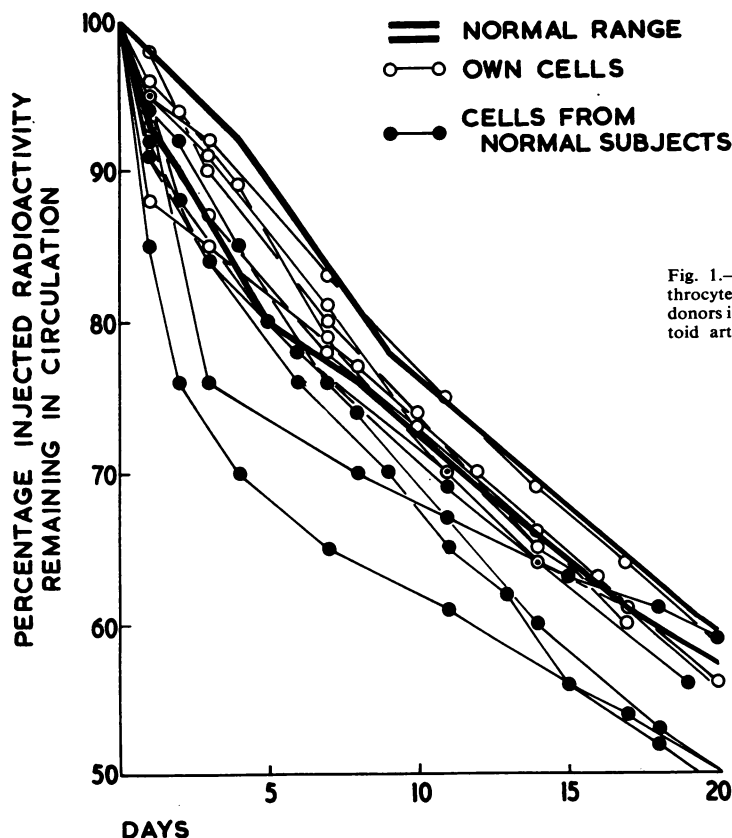


Fig. 1.—Survival of the subject's own erythrocytes and of erythrocytes from healthy donors in six patients suffering from rheumatoid arthritis measured by the radioactive chromium technique.

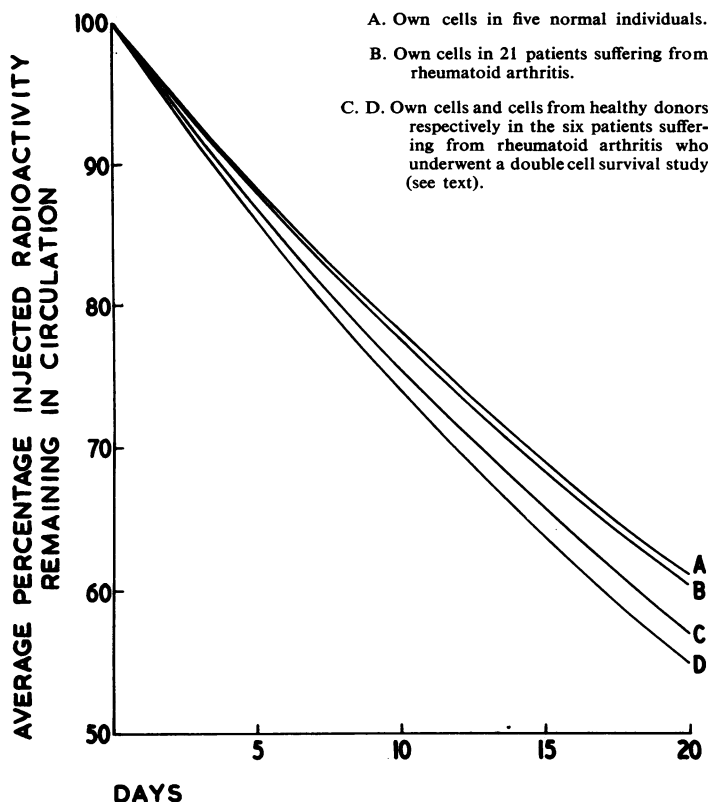


Fig. 2.—Survival of erythrocytes in normal subjects and in patients with rheumatoid arthritis measured by the radioactive chromium technique. The curves have been constructed from the average linear regression coefficient for each group (see text).

Discussion

The majority of investigators who have sought to demonstrate a haemolytic process in patients suffering from rheumatoid arthritis have concluded that reduction of red cell survival has been a factor in the aetiology of anaemia in this condition. The survival curves have indicated random destruction of red cells. The fact that cells from patients with rheumatoid arthritis survive normally when transfused to normal individuals (Freireich and others, 1954, 1957) suggests that the disorder is due to some extracorporeal factor rather than to an intrinsic abnormality in the erythrocyte.

The largest study was that of Lewis and Porter (1960). These authors, after measuring the survival of the individual's own cells, using the radioactive chromium technique, in fifty patients, reported that in only four instances was there evidence of a slight increase in the rate of red cell destruction. The present investigation confirms this finding; in a

proportion of patients with rheumatoid arthritis the injected radioactive chromium disappeared slightly more rapidly than in normal individuals, and while the average curve for "cell survival" was slightly steeper than that for normal subjects, it was not significantly different.

However, when cells from normal donors were transfused into six of the patients with rheumatoid arthritis, the rate of disappearance of injected radioactivity was faster than when the survival of the patients' own cells was measured and significantly faster than in normal subjects.

Mollison (1959) has drawn attention to the misleading results which can arise in cross-transfusion experiments and are presumably due to incompatibility which has not been detected by the usual *in vitro* tests. However, this explanation seems unlikely to obtain in the present investigation. Not only would incompatibility be expected to lead to a much greater increase in the rate of red cell destruction than has been observed, but also the finding of accelerated destruction of normal donor cells compared with the patients' own cells confirms our previous results (Alexander and others, 1956) when, because the Ashby technique was used, *all* the studies involved cross-transfusion.

Mollison (1959) suggests that, in a patient with a random destructive process, a sample of the patient's own cells probably contains more young cells and cells that have resisted haemolysis than does a population of cells from a normal individual. Hence more rapid destruction of donor erythrocytes than of the patient's own erythrocytes is characteristic of an extracorporeal type of haemolytic disorder. This was the finding in the present investigation; in fifteen of the 21 patients with rheumatoid arthritis, "cell survival" was shorter than the average for the normal group, but the transfusion of erythrocytes from healthy donors was necessary before any significant difference could be shown to exist between patients with the disease and normal subjects.

When the present observations are considered, together with the results of previous investigators, it seems likely that there is a mild haemolytic factor

in the causation of the anaemia that is found in patients suffering from rheumatoid arthritis.

Summary

(1) The literature relating to red cell survival in patients suffering from rheumatoid arthritis has been reviewed.

(2) The survival of the subject's own cells has been measured using radioactive chromium in 21 patients suffering from rheumatoid arthritis. In six of these the survival of normal donor erythrocytes was also measured.

(3) The rate of removal of injected radioactivity from the circulation was slightly, but not significantly, more rapid than normal when the patients' cells were labelled. The rate of elimination of labelled donor erythrocytes was significantly faster than normal.

(4) It is concluded that there is a mild haemolytic process of extracorporeal type in rheumatoid disease.

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Nature de l'anémie dans l'arthrite rhumatismale. V. Survie des globules rouges mesurée par le chrome radioactif

RÉSUMÉ

(1) On passe en revue la littérature concernant la survie des globules rouges des malades atteints d'arthrite rhumatismale.

(2) La survie des érythrocytes propres du sujet fut mesurée à l'aide du chrome radioactif chez 21 malades atteints d'arthrite rhumatismale. Chez six d'entre eux on mesura aussi la survie des globules rouges provenant d'un donneur normal.

(3) Le taux d'élimination de la radioactivité injectée de la circulation fut légèrement mais non pas appréciablement au dessus de la normale en ce qui concerne les globules marqués propres du malade. Le vélocité d'élimination des globules marqués du donneur fut appréciablement augmentée.

(4) On conclut que dans la maladie rhumatismale il existe un benin processus hémolytique du type extracorpulaire.

Naturaleza de la anemia en la artritis reumatoide. V. Supervivencia de los hematias medida con cromo radioactivo

SUMARIO

(1) Se pasa en revista la literatura referente a la supervivencia de hematias de enfermos con artritis reumatoide.

(2) La supervivencia de los eritrocitos propios del sujeto se midió por medio de cromo radioactivo en 21 enfermos con artritis reumatoide. En seis de éstos se midió también la supervivencia de hematias procedentes de donante normal.

(3) La velocidad de desaparición de la radioactividad inyectada de la circulación fué ligeramente pero no apreciablemente superior a la normal en el caso de eritrocitos propios del paciente. Los eritrocitos marcados de donante sano fueron eliminados con rapidez apreciablemente superior a la normal.

(4) Se concluye que en la enfermedad reumatoide existe un ligero proceso hemolítico del tipo extracorpular.