ERYTHROCYTE SURVIVAL IN RHEUMATOID ARTHRITIS*

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The cause of anaemia in rheumatoid arthritis has not yet been fully elucidated. Several workers have suggested that haemolysis is at least a contributory factor.

Using the Ashby method for erythrocyte survival study, Alexander, Richmond, Roy, and Duthie (1956) found that normal erythrocytes were eliminated more rapidly than usual from the circulation of patients with rheumatoid arthritis, whereas cells from rheumatoid donors survived normally. Similarly, Freireich, Ross, Bayles, Emerson, and Finch (1957) found a normal survival of patients' erythrocytes in normal recipients but in thirteen rheumatoid recipients normal donor cells were eliminated more rapidly than normal, at a rate which ranged from 1.09 to 2.39 per cent. of the cells per day. This destruction was interpreted as being random, and was thought to suggest the presence of an extrinsic defect.

Radioactive chromium is an isotope which is especially suitable for the study of the life span of erythrocytes, as a patient's cells can be labelled, and after re-injection, studied in their natural environment. The ^{51}Cr now available has a high specific activity (10 mC. per mg.) so that a survival study can be carried out using a low and apparently non-toxic concentration of metallic chromium.

There have been very few previous reports of survival studies in rheumatoid arthritis using this method, and results have been equivocal (Ebaugh, Peterson, Rodnan, and Bunim, 1955; Weinstein, 1959).

In the present paper we describe the results of cell survival studies using ^{51}Cr in fifty patients suffering from rheumatoid arthritis.

Methods

Erythrocyte Survival.—The patients' erythrocytes were labelled with ^{51}Cr by a standard method (Mollison and

Veall, 1955) and then re-injected. Venous blood samples were collected 10 to 15 minutes after the injection and subsequently at regular intervals.

Radioactivity of 3-ml. volumes was measured in a well-type scintillation counter with a thallium-activated sodium iodide crystal of 5 cm. diameter. The measurements of the initial blood samples were taken to represent 100 per cent. radioactivity content of the blood, and by comparison with the radioactivity of subsequent samples of blood the rate of loss of radioactivity from the circulation was calculated. Results were corrected for isotope decay and plotted on arithmetic graph paper.

Occult Blood.—Gastro-intestinal blood loss was estimated by measuring the radioactivity of the stools following the labelling process described above. The stools were collected in waxed cartons of 16 oz. capacity, each carton containing a 24-hour collection. The radioactivity was counted by positioning the carton between two scintillation crystals. It was separated from the lower counter by a plastic ring so that the specimen contained in the carton was approximately equidistant from both crystals. The counting system was surrounded by lead shielding and the responses of both crystals counted together. A standard was prepared from 3 ml. venous blood placed in a similar carton and diluted with approximately 100 ml. water. It was assumed that the radiation-absorbing properties of faeces and water are the same, so that the blood content of the faeces could be calculated directly from the ratio of the two counts. This system of counting was found to have a satisfactory geometry and sensitivity.

Routine Haematological Investigations.—Blood counts and other haematological investigations were carried out using standard methods. The erythrocyte sedimentation rate (E.S.R.) was determined by the Westergren method.

Patients.—Investigations were carried out on sixteen men and 34 women who had definite rheumatoid arthritis according to the revised classification of the American Rheumatism Association (Ropes, 1959). In all the patients the disease was active. In 44 the E.S.R. was 15 mm./hr or more, and in the remainder the disease

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was judged to be active on clinical findings. Ages ranged from 12 to 79 years. Patients with evidence of complicating factors, such as Felty's syndrome or lupus erythematosus, were excluded.

Many of the patients had received various forms of therapy in the past, and salicylates were administered to all during the course of the present investigation. Nine patients were receiving steroids at the time of the commencement of the erythrocyte survival study.

Results

Results of the erythrocyte survival studies were plotted on arithmetic graph paper and compared with the results obtained in a similar study of twenty normal healthy men and women subjects (Fig. 1). They have been corrected for decay but not for elution of chromium from intact erythrocytes. Correction formulae are partly empirical, and it appears to be more satisfactory to interpret results by comparing findings in patients with those in normal subjects without applying any correction factors. The normal range shown consists of the mean measurements in the twenty normal subjects, with two standard deviations. All the measurements in the normal subjects fell within this range. In 46 of the patients with rheumatoid arthritis the measurements of ^{51}Cr activity were within the normal range.

The most useful index of erythrocyte elimination is the time taken for half the originally administered chromium to disappear from the circulation ($T_{1/2}^{51}\text{Cr}$). The normal range has been reported variously to be between 23 and 40 days (Berlin, Waldmann, and Weissman, 1959). In our normal subjects it was found to be 25 to 35 days.

In 46 of the patients with rheumatoid arthritis,

$T_{1/2}^{51}\text{Cr}$ was between 25 and 35 days. In the remaining four cases $T_{1/2}^{51}\text{Cr}$ was 24, 23, 23, and 19 days, respectively.

As stated above, correction formulae are generally unsatisfactory, but by applying an elution correction it is possible to determine, at least approximately, the pattern of a survival curve and the mean cell life.

In Cases 47 to 50, graphs of erythrocyte survival have been replotted, after correction for an elution half-time of 64 days, as suggested by Hughes Jones and Mollison (1956). The results have been compared with the normal range, similarly corrected (Fig. 2, overleaf).

In these four cases there is an early loss of approximately 15 per cent. of the erythrocytes and thereafter the remaining cells appear to be eliminated at a normal rate, so that the slope of elimination parallels that of the normal subjects. In Cases 48 and 49, survival studies were repeated approximately 6 months later. On this occasion there was no demonstrable early loss of ^{51}Cr , and $T_{1/2}^{51}\text{Cr}$ was 35 days and 29 days respectively, with a normal slope of elimination.

During the erythrocyte survival studies all stools were collected and the blood content estimated. The figures in the Table (overleaf) represent the average daily blood loss over a 30-day period. In Case 48, this was 20 ml. per day, and at least theoretically this could account for the increased rate of elimination of ^{51}Cr from the circulation. On the second labelling there was a loss of only 1 ml. per day when the survival study showed a normal rate of elimination. However, in Case 49, there was no such correlation, and 5 ml. per day was the average loss throughout both survival studies. Moreover, the relatively large average amounts of blood lost in

Fig. 1.—Erythrocyte survival studies in fifty patients with rheumatoid arthritis. Results have been corrected for isotope decay but not for chromium elution. The normal range (twenty subjects) is shown by the interrupted lines.

Fig. 2.—Erythrocyte survival studies in four patients in whom the results fell outside the normal range. Corrections have been applied for isotope decay and for elution of chromium.

Cases 17 and 23 were not associated with measurable increased elimination from the circulation. In some cases the daily amount of blood loss varied from nil to 50 ml., but the "early loss" of radioactivity from the circulation in Cases 47, 48, 49, and 50 was not associated with increased gastrointestinal bleeding during that period.

A number of the patients were anaemic but there was no obvious close relationship between the degree of anaemia and the activity of the disease (Table, opposite).

Discussion

There have been few reports of erythrocyte survival studies in rheumatoid arthritis and the principal previous observations have been made on the results of studies using the Ashby method. However, this technique has limitations; it is now recognized that cross transfusions not infrequently result in diminished survival even when there are no demonstrable antibodies (Mollison, 1959). It is conceivable that the incompatibility may be even greater in patients with rheumatoid arthritis. By the use of radioactive chromium it has been possible to study the survival of the erythrocytes of such patients in their natural environment.

Erythrocyte survival studies have been carried out on fifty patients with active rheumatoid arthritis in a number of whom there have been varying degrees of anaemia.

In 46 of the fifty patients, erythrocyte survival was normal and in only four was there evidence of an increased rate of erythrocyte elimination. This was due to an "early loss" of approximately 15 per cent. of the labelled erythrocytes, with a subsequent normal slope of the curve. The reason for the early loss of radioactivity from the circulation is unexplained, though an early loss of approximately 10 per cent. is often encountered in normal subjects (Mollison, 1959). But even if the "early loss" in the patients with rheumatoid arthritis had been of greater significance than that which occurs in normal subjects, the increased rate of cell elimination is only slightly greater than normal. The early loss was not associated with any concomitant fluctuation in packed cell volume or with excessive blood loss. It was not seen on a subsequent occasion in two of the patients in whom survival studies were repeated under conditions similar to those of the first study.

Gastro-intestinal bleeding occurred to a varying extent in all but one of the patients, but except possibly in one instance (Case 48) the amount was not sufficient to have had a significant influence on the rate of elimination of the labelled erythrocytes from the circulation.

The occurrence of haemolysis in patients with lupus erythematosus and Felty's syndrome is well recognized, and has been demonstrated in several

TABLE
CLINICAL AND HAEMATOLOGICAL DATA AND RESULTS OF ^{51}Cr STUDIES
IN FIFTY PATIENTS WITH RHEUMATOID ARTHRITIS

Case No.	Age (yrs)	Sex	Duration of Disease (yrs)	Hb (g./100 ml.)	E.S.R. (mm./hr)	$\text{T}_{1/2}^{51}\text{Cr}$ (days)	Daily Mean Gastro-Intestinal Blood Loss (ml.)
1	49	F	1	11.9	125	35	2
2	54	F	5	11.8	50	35	3
3	55	M	3	12.8	117	34	1
4	61	F	4	11.1	78	34	3
5	25	M	12	15.8	30	33	3
6	63	F	19	12.9	80	33	4
7	55	F	5	11.9	85	33	3
8	51	F	1	14.2	18	31	2
9	63	F	19	11.6	94	31	2
10	48	F	10	11.0	55	31	1
11	34	F	1	10.4	108	31	1
12	60	F	16	11.5	80	30	2
13	65	M	16	11.4	80	30	2
14	78	F	20	11.2	66	30	2
15*	29	F	10	10.9	10	30	1
16†	66	F	14	10.8	69	30	2
17†	50	F	1	8.9	70	29	9
18†	58	F	4	8.1	112	29	3
19†	79	F	6	12.7	63	28	2
20†	39	F	8	12.5	82	28	1
21*†	54	F	2	12.4	28	28	1
22*	51	F	7	11.5	73	28	2
23	65	F	16	10.5	88	28	7
24*	48	M	20	16.0	37	27	3
25	64	F	5	15.2	60	27	1
26*	73	M	16	14.9	15	27	0
27	56	M	15	14.5	10	27	3
28	46	M	2	12.9	39	27	2
29*	49	F	4	12.8	25	27	1
30	40	F	1	10.3	83	27	2
31†	25	M	3	10.2	70	27	1
32†	50	M	1	15.2	5	26	1
33	57	F	5	12.5	60	26	3
34†	61	F	17	12.3	14	26	3
35	52	F	17	11.1	80	26	1
36*	69	M	20	10.8	76	26	1
37	42	F	20	10.6	45	26	1
38	74	F	15	9.1	24	26	1
39†	63	M	5	14.8	30	25	2
40	50	F	25	14.1	31	25	1
41*	69	F	23	13.6	10	25	2
42	67	M	13	13.4	30	25	1
43†	51	F	12	13.2	10	25	2
44	12	M	1	11.8	63	25	5
45†	54	M	4	10.8	90	25	4
46	18	M	10	9.7	56	25	6
47	47	F	13	12.4	68	24	1
48†	21	F	4	12.4	40	23	20
49†	53	F	20	13.6	100	23	5
50†	40	M	23	14.3	26	19	3
Normal Range		M F		13.5-18.0 11.5-16.4	3-5 4-7	25-35 25-35	0 0

* Patient on steroid therapy before and during survival study.

† D.A.T. less than 16, but diagnosis established by presence of nodules and/or x-ray changes.

‡ Survival studies were repeated approximately 6 months later with the following results:

Case No.	Hb (g./100 ml.)	$\text{T}_{1/2}^{51}\text{Cr}$ (days)	Daily Mean Gastro-Intestinal Blood Loss (ml.)
48	11.9	35	1
49	12.6	29	5

patients investigated by us, but not reported here. The present series did not include any patients with such complicating features.

Several of the patients with normal erythrocyte survival had received steroid therapy before and at

the time of the investigation. It is, of course, possible that cell survival was influenced by this treatment, but by far the greatest number of patients with normal survival had not received any treatment other than salicylates.

Summary

Using the isotope ^{51}Cr as a label, erythrocyte survival studies were carried out in fifty patients with active rheumatoid arthritis. In all cases the patient's own cells were labelled.

No cases of Felty's syndrome or lupus erythematosus were included in the series.

In 46 of the patients (92 per cent.), erythrocyte survival was normal. In the remaining four patients, there was only a slight increase in the rate of cell elimination, caused by the early loss of approximately 15 per cent. of the labelled cells. The reason for this "early loss" is unknown. Gastro-intestinal bleeding occurred to a varying extent in almost all of the patients, but in only one case was the amount sufficient to have had a detectable influence on the rate of elimination of the labelled erythrocytes from the circulation.

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.

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Discussion.—DR. W. R. M. ALEXANDER (*Edinburgh*) said that unpublished work by Richmond had confirmed these findings. The thought had occurred to him that, in rheumatoid arthritis, the erythrocytes reaching the peripheral circulation were relatively resistant to destruction, the more susceptible cells being destroyed in the marrow. This might explain the normal elimination of rheumatoid erythrocytes and slightly more rapid elimination of healthy erythrocytes in rheumatoid arthritis.

DR. J. J. R. DUTHIE (*Edinburgh*) said that they should remind themselves that normal cells were undoubtedly destroyed more rapidly when introduced into the rheumatoid circulation. A rheumatoid red cell might develop a resistance to this process.

PROF. J. H. KELLGREN (*Manchester*) said that he understood from the authors' definition that any patient showing presumptive evidence of haemolysis, e.g. large spleen, leukopenia, etc., was eliminated from the study, and asked whether that would be a correct estimation?

DR. LEWIS replied that in the few cases in which there was an early loss of a percentage of the radio-activity,

this occurred rapidly and appeared to conform to the pattern which is frequently seen in normal subjects, in whom the $T_{\frac{1}{2}}$ of this early loss has been estimated to be $1\frac{1}{2}$ days. In normal subjects it has been thought unlikely that this represents a population of cells especially susceptible to destruction, but rather to some process such as elution of the ^{51}Cr . In any event, this early loss occurred in only four of the patients studied.

With regard to selection of material, the investigation was undertaken in order to assess whether haemolysis occurred in uncomplicated rheumatoid arthritis and whether it could be blamed for the anaemia which may be found even in the absence of complications such as hypersplenism. Several of the patients included in the series were anaemic with haemoglobin values as low as 8 g. per cent. In cases with hypersplenism, haemolysis is well recognized, and had been demonstrated in several patients studied during the course of the present investigation.

Survie des érythrocytes dans l'arthrite rhumatismale

RÉSUMÉ

On étudia la survie des érythrocytes chez cinquante malades atteints d'arthrite rhumatismale évolutive; l'isotope ^{51}Cr fut utilisé pour marquer des globules rouges de chaque malade.

La vie moyenne des érythrocytes fut normale chez 46 malades (92%). Chez les quatre malades restants, le taux d'élimination des globules rouges n'augmenta que très peu; cette augmentation fut causée par une perte initiale de 15 pour cent environ des érythrocytes marqués. On ne connaît pas la raison de cette "perte initiale". Chez presque tous les malades on nota des hémorragies gastrointestinales d'intensité variable, mais dans un cas seulement la perte sanguine fut suffisante pour se refléter dans le taux d'élimination des érythrocytes marqués.

L'hémolyse n'est pas un caractère de l'arthrite rhumatismale et ne constitue pas un facteur appréciable dans l'anémie qui souvent accompagne cette maladie.

Vida media de los eritrocitos en la artritis reumatoide

SUMARIO

Se estudió la vida media de los eritrocitos en cincuenta enfermos con artritis reumatoide evolutiva, sirviéndose del isótopo radioactivo ^{51}Cr para marcar los hematias de cada enfermo.

La vida media de los hematias fué normal en 46 de los enfermos (92%). En los otros cuatro enfermos hubo solamente un ligero aumento en la tasa de eliminación de los eritrocitos, ocasionado por la temprana destrucción de un 15 por ciento aproximadamente de los hematias marcados. La causa de esta "temprana destrucción" es desconocida. En casi todos los enfermos se detectó hemorragia gastrointestinal de grado variable, pero solamente en un caso fué lo suficiente copiosa para reflejarse en la cifra de eliminación de los eritrocitos marcados de la circulación.

La hemolisis no es una característica de la artritis reumatoide, y por tanto no puede ser considerada como un factor significativo en la anemia que con frecuencia aparece en dicha enfermedad.