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Research Article

THE HAEMOLYTIC FACTOR IN THE ANAEMIA OF LYMPHATIC LEUKAEMIA[1]

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Cases of lymphatic leukaemia complicated by a haemolytic anaemia have been reported by Haden (1), Singer and Dameshek (2), Wiseman (3), Feldman and Yarvis (4) and by Stats, Rosenthal and Wasserman (5) among others. It has been the commonly held view that such cases are rare and that the cause of the anaemia usually found in association with lymphatic leukaemia lies in a disturbance of erythropoiesis the result of replacement of erythropoietic tissue by leukaemic tissue. That haemorrhage is also an etiological factor in the anaemia in some cases is clear to everyone, but that excessive haemolysis may also be a factor is by most authors considered an improbability. Because of the haemosiderosis which they found and the hyperplasia of erythropoietic tissue which was demonstrated in some of their cases, earlier workers (6–8) believed that excessive haemolysis probably played an important role. This view was disputed by Forkner (9) who cited the work of Whipple and Robscheit-Robbins (10) which showed that there was only a little more iron in leukaemic than in normal livers. Recently the problem has been reviewed by Collins and Rose (11) who admit that excessive haemolysis may be present but state that in the case of lymphatic leukaemia the anaemia is due in most cases to depression of erythropoiesis.

It has been the purpose of the present investigation to determine by means of the differential transfusions of Ashby whether or not excessive haemolysis is an important factor in the production of the anaemia of lymphatic leukaemia even when the usual signs of excessive haemolysis are absent and whether in any cases there is evidence of the presence of a haemolytic process not seen in normal subjects.

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EXPERIMENTAL METHOD

The cases studied were transfused with 500–1,500 cc. of stored whole blood. The blood had been stored for one to four days in the proportion of 400 cc. of blood to 150 cc. of an anticoagulant mixture containing 2.3 gm. dextrose U.S.P. and 1.7 gm. sodium citrate U.S.P. per 100 cc. The inagglutinable erythrocytes were counted the day after transfusion, then every other day for 10 days and subsequently at intervals of 10 to 14 days. The method of differential agglutination was that described by Dacie and Mollison (12). With this method it has been found that the standard error of the difference between duplicate observations is of the order of 6% of the inagglutinable count (13). In the present study four counts were made on each specimen, and in some cases two counts by two observers. The results have been expressed numerically in the manner described by Brown and associates (13) who worked on the hypothesis that in some cases of anaemia two processes of erythrocyte destruction could be distinguished and who named these the *linear haemolytic mechanism* and the *exponential haemolytic mechanism.* This method of description provides *1)* an estimate of the average life of the transfused erythrocytes ($\bar{t}$), *2)* the fraction of the transfused erythrocytes which has been destroyed by the *exponential haemolytic mechanism* (F_e) and *3)* a fictitious average life which would have obtained if the linear haemolytic mechanism had been acting alone ($\bar{t}_s$). These are derived from the equation.

$$\frac{N}{N_o} = (1 - L_s t)\,\frac{R \exp(-L_e t) + 1}{R + 1}$$ [4]

which has been found to be the simplest equation which reasonably fits the data in those cases where the plotted results show considerable curvature. When the decay curve is linear the equation becomes

$$N = N_o(1 - L_s t)$$

It has been found by Callendar and colleagues (14) that

[4] Notation

- N = Donor cell count in recipient at time t, million per c.mm.
- N_o = Donor cell count in recipient immediately after transfusion, million per c.mm.
- t = Time measured from transfusion (days)
- L_s, L_e, R = Constants.
- $\bar{t}$ = Average life of transfused cells measured from t = 0 (days)
- $\bar{t}_s$ = Average life corresponding to linear component (days)

in a normal recipient the decay curve is linear and that the average life of the transfused erythrocytes is about 60 days. This corresponds well with the estimates of life span of the erythrocyte which have been made by those who fed a normal subject glycine containing N^{15} (15) and by those who have used pigment excretion studies as the basis for estimation (16).

The cases which are reported now were not selected except with respect to suitability of blood group and accessibility during the period of observation.

RESULTS

Case I, J. H. (48–3506), a man 67 years of age had been a known diabetic for 10 years. For one year before admission he had complained of dyspnoea and substernal distress on exertion and latterly of nocturnal dyspnoea. Examination on admission showed moderate enlargement of the the cervical lymph nodes, enlargement of the liver, a heart which was borderline in size, rales over the lower half of both lung fields, and fluid in the right pleural cavity. Urinalysis—trace of albumen, varying glycosuria. HGb. 5.8 gm.%. R.B.C. 1,740,000. Haematocrit 18%. W.B.C. 650 with 58% lymphocytes, 16% prolymphocytes, 4% lymphoblasts, 20% polymorphonuclear neutrophiles and 2% band cells. Reticulocytes 2.4%. Platelets 31,680. Serum Bilirubin 0.4 mg.%. Fasting Blood Sugar 231 mg.%. Blood Urea 33 mg.%. Serum Chlorides 586 mg.%. Serum Proteins 6.3 gm.%. with 4.12 gm. albumin and 2.18 gm. globulin. Plasma Prothrombin Content 62%. Faecal Urobilinogen 631 mg./day during a three-day collection by the method of Schwartz, Sborov and Watson (17). Bone marrow smear showed 6% small lymphocytes, 25.5% large lymphocytes, 14.5% prolymphocytes, 42% lymphoblasts and an L/E ratio of 9/1. Chest x-ray showed a pneumonic infiltration of both lung roots and increased markings in the upper half of the left lung. C/T ratio of 16.5/30. Electrocardiogram—chronic myocardial pathology. He was treated with rest, digitalis, insulin, a low

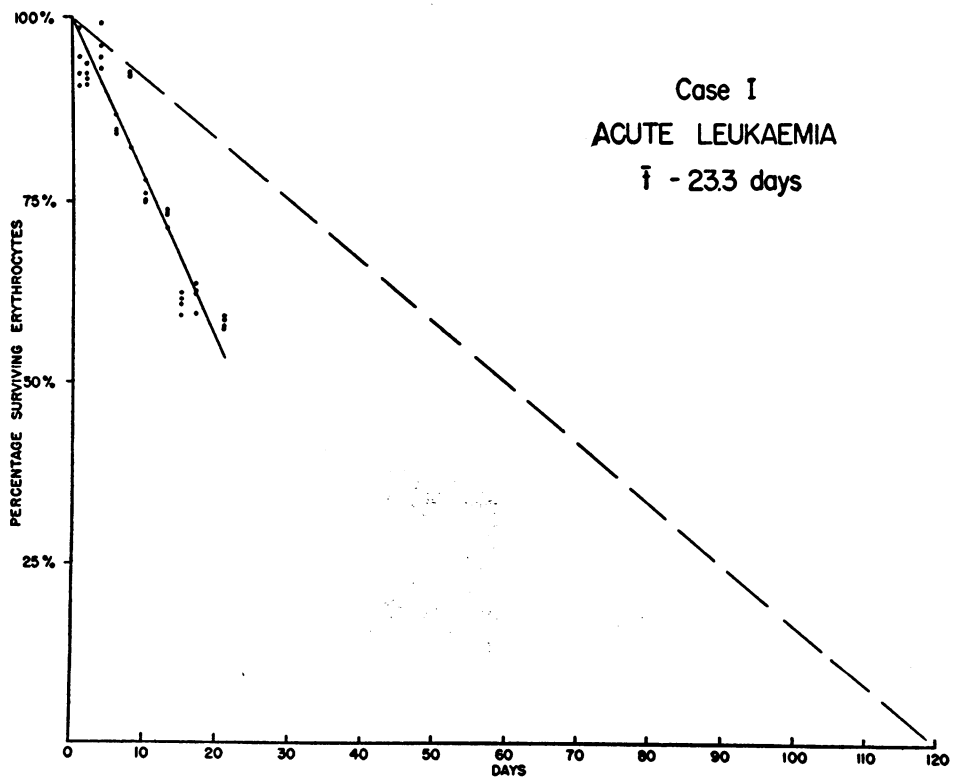

FIG. 1. SURVIVAL OF TRANSFUSED ERYTHROCYTES IN CASE I

The dots represent the actual observations and the solid line has been drawn through points calculated from the fitted quotation. The broken line represents the decay curve in a normal subject.

sodium neutral ash diet, and Fowler's solution. The diabetes mellitus was brought under good control. He died at home eight weeks after his admission to hospital.

The test transfusion was followed for 21 days. When the number of surviving erythrocytes was plotted against time the decay curve was found to be linear for that period (Figure 1). It showed an increased slope as compared with that found in normal subjects (14) and the calculated average life of the transfused cells ($\bar{t}$) was 23.3 days.

Case II, W. H. (27913), a man aged 54 had been suffering from disseminated sclerosis for 13 years. For six years he had been aware of swellings in his axillae and groins and for one year there had been a mass in his abdomen. He also complained of pallor, increasing dyspnoea on exertion and of oedema of the ankles. Examination showed a pale wasted man with generalized lymphadenopathy, a palpable liver and a greatly enlarged spleen. There was the characteristic evidence of moderately advanced disseminated sclerosis but for two years there had been no increase in the disability from this cause. Urinalysis was negative. HGb. 4.4 gm.%. R.B.C. 1,850,000. Haematocrit 17%. W.B.C. 1,050,000 with 94% lymphocytes. Reticulocytes 1.0%. Platelets 60,000. Serum Bilirubin less than 0.2 mg.%. He lived for about 18 months following this admission to hospital.

The test transfusion was followed for 38 days and the plotted results showed considerable curvature (Figure 2). The calculated average life of the transfused cells ($\bar{t}$) was 18 days and the proportion destroyed by the *exponential haemolytic mechanism* (F_e) was 0.34.

Case III, C. W. (49–4189), a man aged 68 had noted loss of strength and lumps in his neck for one year. There were enlarged lymph nodes to be palpated in the cervical, occipital, axillary, epitrochlear, inguinal and popliteal regions. The liver edge was just palpable and the spleen was enlarged to below the level of the umbilicus. Urinalysis negative. HGb. 9.1 gm.%. R.B.C. 3,200,000

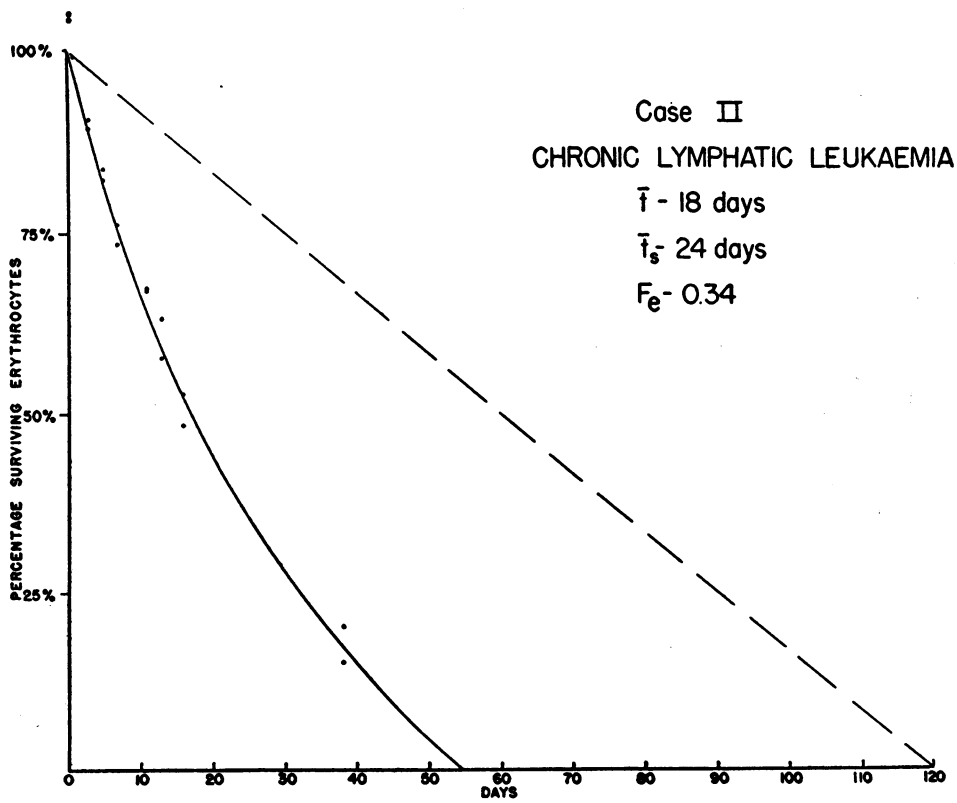

FIG. 2. SURVIVAL OF TRANSFUSED ERYTHROCYTES IN CASE II

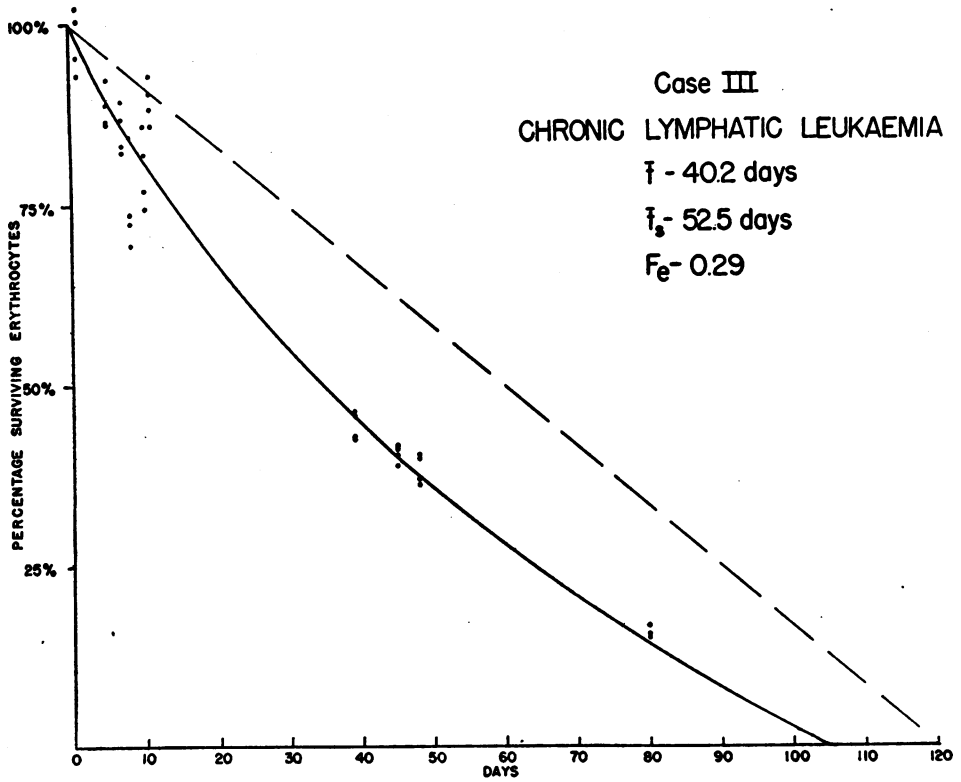

FIG. 3. SURVIVAL OF TRANSFUSED ERYTHROCYTES IN CASE III

Haematocrit 29%. W.B.C. 29,800 with 96% lymphocytes, 2% polymorphonuclear neutrophiles, 1% band cells, 1% myelocytes. Reticulocytes 1.1%. Platelets 169,000. Serum Bilirubin 0.4 mg.%. Faecal urobilinogen 31.9 mg./day. Lymph node biopsy showed the characteristic picture of lymphatic leukaemia. He was given deep x-ray therapy amounting to 300r over both supraclavicular and both axillary regions during a period four to eight days following his test transfusion. Radiotherapy was then discontinued because his white blood count had fallen to 10,000. He received further radiotherapy six weeks later. He is still alive, a year after the test transfusion.

The plotted results in this case showed an increased slope and also some curvature (Figure 3). The fraction of the transfused cells which

TABLE I

Blood counts following the subcutaneous injection of 1 cc. 1/1,000 adrenalin in Case IV

	Before	5 minutes	10 minutes	20 minutes	35 minutes
HGb., gm. %	10.4	10.4	10.6	10.7	10.6
R.B.C.	3,320,000	3,230,000	3,420,000	3,480,000	3,250,000
W.B.C.	187,000	355,000	299,000	298,000	342,000
Lymphocytes, %	97		81	75	77
Smudge cells, %			18	21	15
Lymphoblasts, %				1	1
Monocytes, %	1				
Polymorphs. %	2		3	3	7
Platelets	172,600	329,400	1,585,000	174,000	435,500

had been destroyed by the *exponential haemolytic mechanism* (F_e) was estimated to be 0.29 and the average life of the transfused cells ($\bar{t}$) was 40.2 days.

Case IV, F. E. B. (49–9730), a man aged 68 gave a history of fatigue and weakness for three years and increasing dyspnoea on exertion for one year. For a month he had had a productive cough. He was pale and emaciated and showed a generalized lymphadenopathy and moderate enlargement of the spleen. There was marked arteriosclerosis of the Monckeberg type and his heart was grossly enlarged. There were numerous coarse rales to be heard over the lower lobes of both lungs. Urinalysis negative. HGb. 6.95 gm.%. R.B.C. 2,340,000. Haematocrit 22%. W.B.C. 330,000 with 97% lymphocytes, 2% polymorphonuclear neutrophiles, 1% eosinophiles. Reticulocytes 1.4%. Platelets 730,080. Blood counts following the injection of 1 cc. 1/1000 adrenalin are shown in Table I. Serum Bilirubin 0.5 mg.%. Faecal Urobilinogen 56.8 mg./day. Serum N.P.N. 48 mg.%. Plasma Protein 6.49 gm.% with 3.96 gm. albumin and 2.53 gm. globulin. Sputum contained large numbers of lymphocytes, some of them lying in sheets. Chest x-rays showed persistent diffuse linear streaking and some mottling in the lower halves of both lung fields. Two sternal punctures yielded no marrow. Treatment with urethane was begun nine days after his test transfusion and in the next three weeks there was considerable improvement with a gain in weight, an increase in haemoglobin from the post-transfusion level of 11 gm.% to 12.1 gm.% and a fall in white blood count to 182,000. The improvement continued with a daily dose of 4.5 gm. urethane so that after three months his white blood count had fallen to 23,000. His haemoglobin at that time was 11.1 gm.%. His lung fields were clear on x-ray.

The result of the transfusion in this case was entirely within normal limits. The decay curve was strictly linear and interrupted the base line at 114 days.

DISCUSSION

To say that evidence of an increased rate of destruction of transfused erythrocytes is evidence of an increased destruction of the patient's own cells is to assume that the two are treated by the body in the same way. That this is not always the case has been shown in congenital haemolytic icterus (18), nocturnal haemoglobinuria (12) and sickle-cell anaemia (19). In these conditions transfused cells from a normal donor may survive for a normal length of time, though it is obvious that the patient's own cells are being destroyed abnormally rapidly. In these three conditions, the reverse also holds, and the patient's cells when transfused into a normal recipient are destroyed more rapidly than the cells of a normal donor. Save in these conditions there is no evidence that cells from a normal donor are destroyed in the recipient faster than the recipient's own cells provided there are not present in the recipient antibodies against the donor cells (mis-matched transfusion) and also, possibly, provided the total erythrocyte count is not raised by transfusion above the recipient's normal count. Such evidence would be admittedly difficult to obtain, and the point of identical treatment of the donor's and the recipient's cells, with the exceptions already mentioned, cannot be proven. It can be said, however, that in those cases in this and in previous work (13, 20) where there was the conventional evidence of excessive haemolysis, *viz.* reticulocytosis, and increase in serum bilirubin and faecal urobilinogen, the average life of the transfused cells has always been markedly diminished. It can also be pointed out that the inferences to be drawn from the present work involving the transfusion of cells from normal donors into abnormal recipients, followed on a comparison of the results of work involving the transfusion of cells from normal donors into normal recipients (14).

The suggestion has been made that in patients with enlarged spleens, some of the transfused erythrocytes may be "lost" in the spleen giving a falsely low survival time, but there seems no reason to suppose that the transfused erythrocytes are more likely to be contained in the spleen than are the patient's own cells. That both may disappear from the peripheral circulation into the spleen is obvious. If the transfused erythrocytes were indiscriminately "lost" in the splenic pulp, an increase in the exponential component of the decay curve would follow and there would

be no increase in the activity of the *linear haemolytic mechanism* if indiscriminate "loss" in the spleen were the only abnormal process at work. Data which would obtain in this situation have not been found in this or earlier series (13, 20). Abnormally rapid removal of the transfused erythrocytes by the spleen from the main mass of circulating erythrocytes according to some characteristic of the erythrocytes themselves, such as age, would result in a linear decay curve with increased slope. Any significant return of these cells to the systemic circulation would cause upward deviations of the decay curve which have not yet been observed. In summary rebuttal of the suggestion that an enlarged spleen may vitiate the results in the present experiments, it may be said that there is no reason to suppose the transfused erythrocytes are removed from the circulation more rapidly by the spleen than are the patients' own cells and that if both are removed abnormally rapidly there is no evidence to date that they are returned to the circulation in significant numbers.

In three of the four cases of lymphatic leukaemia which have been studied, the transfused erythrocytes were destroyed at a rate faster than normal, which is interpreted as evidence of excessive haemolysis in these cases. What part, if any, the urethane therapy had in the results of Case IV, it is impossible to say. During the period of observation his haemoglobin was maintained at the immediate post-transfusion level and there was a marked improvement in his general condition, with diminution in size of his spleen and accessible lymph nodes. He was, in fact, in a remission. In Cases II and III the reticulocyte count and the serum bilirubin, and in Case III the faecal urobilinogen, were within normal limits, despite the definite increase in rate of destruction of transfused erythrocytes. This suggestion that the present experimental method is a more sensitive indicator of excessive haemolysis than those more commonly employed has been presented before (20). In Cases II and III there was also evidence of activity of the *exponential haemolytic mechanism.* The *exponential haemolytic mechanism* has not been seen in normal recipients except when the post-transfusion erythrocyte count has been above normal and it has not been evident in this or previous series except when there has also been increased activity of the *linear haemolytic mechanism.* In Case I, the acute case, where the observed portion of the decay curve was sensibly linear, it should be remembered that the period of observation was only 21 days and that further counts might have provided evidence of the *exponential haemolytic mechanism.*

TABLE II

Survival of transfused erythrocytes in four cases of lymphatic leukaemia

Case No.	Average life ($\bar{t}$ days)	Average life corresponding to linear mechanism ($\bar{t}_s$ days)	Fraction destroyed by exponential mechanism (F_e)
I	23.3	23.3	0
II	18	24	0.34
III	40.2	52.5	0.29
IV	57	57	0

These results would seem to show that in at least some cases of lymphatic leukaemia the anaemia is contributed to by increased haemolysis and that in some cases there is evidence not only of increased haemolysis but also of haemolysis of an abnormal type. It is not necessary in three of the present four cases to explain the anaemia entirely on the basis of diminished erythropoietic activity, and indeed in Case II where the rate of haemolysis was more than three times that of normal, the haemolytic activity was of an order which might entirely explain the anaemia found. The fact that excessive haemolysis has been demonstrated in cases where the more usual indices of haemolysis have been lacking suggests that it may be an important factor more often than is now believed. In what proportion of cases this holds, remains to be determined by further work. The physiological mechanism and the sites of the different types of haemolysis which have been observed also remain speculative. The question of a relation between the two types of haemolytic activity which have been demonstrated here and the activity of immune bodies which have been demonstrated in some cases of haemolytic anaemia in leukaemia (21) will have to be answered by further work.

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