Red cell survival in biliary cirrhosis

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SYNOPSIS Studies were carried out on eight patients with primary biliary cirrhosis. Four patients were found to have a haemolytic anaemia; one had Coombs red cell antibodies. Two patients had evidence of splenic sequestration of red cells using the radio-chromium technique. There was a significant correlation between the red cell survival and the bromsulphthalein retention test and the red cell survival and the serum level of bilirubin. It was concluded that the anaemia of cirrhosis of the liver, including primary biliary cirrhosis, may be due to a number of mechanisms and a unifying hypothesis based on the degree of liver dysfunction is suggested.

A review of the literature (Subhiyah and Al-Hindawi, 1967) has shown that 54% of patients with cirrhosis of the liver have a shortened red cell survival, measured by the radiochromium technique. However, patients with primary biliary cirrhosis, as a group, have not been investigated from this point of view. The need for such a study was prompted by a patient who presented with a Coombs-positive autoimmune haemolytic anaemia and in whom investigations also revealed coincident biliary cirrhosis. As far as we are aware this combination of disease has not been previously reported.

Material and Method

Eight female patients with primary biliary cirrhosis were studied (Table I). The diagnostic criteria (Table II) were raised serum alkaline phosphatase and gamma globulin together with a positive immunofluorescent mitochondrial antibody test and a liver biopsy showing the histological features described by Goudie, MacSween, and Goldberg, (1966), and with or

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without raised serum bilirubin and cholesterol levels. Bromsulphthalein retention studies were carried out on seven patients.

Red cell survival studies (Table III) were carried out in seven patients by the radioactive chromium technique (Mollison and Veall, 1955). (Red cell survival studies were not carried out in case 1 before therapy because of the acuteness of the haemolytic process.) This gave a measure of the red cell half life ($T_{\frac{1}{2}}$ ^{51}Cr) for each patient and thus of the degree of haemolysis. The mean $T_{\frac{1}{2}}$ ^{51}Cr in six haematologically normal patients was 28 ± 5 (SD), range 22-35 days (Hume, Dagg, Fraser, and Goldberg, 1964). In order to determine whether or not the spleen was acting as an organ of erythrocyte sequestration, radioactivity was also measured by surface counting over the heart, liver, and spleen as described by Jandl, Greenberg, Yonemoto, and Castle (1956). A spleen/liver ratio at $T_{\frac{1}{2}}$ of at least 2.3 to 1 was considered to be evidence of splenic sequestration of a degree sufficient to suggest that splenectomy would relieve a haemolytic anaemia (Goldberg, Hutchison, and MacDonald, 1966). None of the patients lost blood from the alimentary tract during the study as determined by tests for faecal occult blood.

No.	Sex	Age (yr)	Jaundice	Pigmentation	Xanthomata	Spleen Size (in.)	Liver Size (in.)
1	F	50	+	+	—	0	0.5
2	F	53	+	—	—	2	1.5
3	F	59	—	—	—	0	0.5
4	F	54	+	—	—	(—)	2
5	F	67	+	+	+	0.5	2½
6	F	61	+	—	—	2.5	2.5
7	F	53	—	+	—	0.5	1.5
8	F	54	+	+	—	0	1.0

Table I Clinical features

No.	Serum Bilirubin (mg%)	Serum Cholesterol (mg%)	Serum Alkaline Phosphatase (K.A. units)	Gamma Globulin (g%)	Mitochondrial Antibody Titre	Liver Biopsy Histology	Percentage BSP Retention at 45 min
1	6.0	600	30	3.2	1/512	+	38
2	2.2	392	70.8	3.8	1/2000	+	33
3	0.5	312	49.5	3.6	1/2048	+	11
4	2.2	276	27.6	3.7	1/128	+	—
5	1.9	460	36.0	3.9	1/128	+	6
6	4.3	312	44.9	3.7	1/512	+	25
7	0.3	247	19.8	3.9	1/1024	+	4
8	4.1	276	31.6	4.6	1/512	+	26

Table II Diagnostic features

No.	Hb (g%)	Reticulocytes (%)	WBC (per cmm)	Platelets (per cmm)	T½ ⁵¹ Cr (days)	Spleen/Liver Ratio	Coombs Test
1	6.8	34	29,000	140,000	—	—	+
2	14.0	<2	7,500	178,000	18	1.1:1	—
3	12.5	<2	4,800	245,000	23	1.1:1	—
4	12.7	<2	11,300	164,000	22	(—)	—
5	12.3	<2	8,700	224,000	24	3:1	—
6	7.7	<2	2,800	39,000	18	2.5:1	—
7	12.5	<2	6,800	233,000	29	1.1:1	—
8	11.7	<2	10,100	121,000	17	2.1:1	—

Table III Haematological data

An immunofluorescent antibody test was performed by the method of Goudie *et al* (1966).

Routine haematological measurements were carried out by the method of Dacie and Lewis (1963).

Results

The results of the red cell survival studies (Table III) showed that three patients (cases 2, 6, and 8) had a haemolytic anaemia, the red cell survival being 18 days, 18 days, and 17 days respectively, as compared with the normal red cell survival (22-35 days). Four patients had a red cell survival time within the normal range. One patient (case 1) had an acute haemolytic anaemia (Coombs positive). The gamma-globulin neutralization test showed a warm

Fig. 1 Regression line of bromsulphthalein retention on red cell survival expressed as a log-log line.

(IgG type) antibody which was found to have a specificity of anti-Dc. Two patients (cases 5 and 6) had evidence of splenic sequestration of red cells from surface counting, the spleen:liver ratio being 3:1 and 2.5:1 respectively, and one patient (case 6) also had an associated leucopenia and thrombocytopenia with generalized hyperplasia of the bone marrow. There were no correlations found between the red cell survival and the size of the liver or spleen, and no correlation between the red cell survival and serum cholesterol, serum alkaline phosphatase, serum gamma globulin level, and immunofluorescent mitochondrial antibody titre. However, in the six patients (cases 2, 3, 5, 6, 7, and 8) in whom red cell survival (RCS) could be related to the bromsulphthalein retention (BSP) a significant relationship was found between these two measurements (Fig. 1).

The regression of log y (BSP) on log x (RCS) is given by the equation $y = 6.4349 - 4.0095 x$ giving the correlation coefficient (r) of 0.9712 ($P < 0.01$); the reduction in the red cell survival is related to the degree of liver dysfunction when expressed as a log-log line.

In the seven patients (cases 2, 3, 4, 5, 6, 7, and 8) in whom red cell survival could be related to the serum bilirubin level (SB) a significant relationship was found between these two measurements (Fig. 2).

The regression of y (SB) on log x (RCS) is given by the equation: $y = 23.1631 - 15.789 x$ giving a correlation coefficient (r) of 0.8496 ($P < 0.05$); the reduction in the red cell survival is related to the increased level of serum bilirubin when expressed as a log-linear relationship.

Fig. 2 Regression line of serum bilirubin in red cell survival expressed as a log-linear relationship.

Discussion

Reduced red cell survival measured by the radioactive chromium technique has been reported in approximately 54% of patients with liver cirrhosis (Subhiyah and Al-Hindawi, 1967) although the mechanism of this haemolytic anaemia remains in doubt. Subhiyah and Al-Hindawi (1967) assert from their own studies that 'splenic destruction of red cells is an important, if not the main, factor in the haemolytic process'. However, the application of statistical methods to their results shows that there is no significant correlation between radioactive excess spleen counts expressed as a percentage and the red cell survival measured at the half-time disappearance of radiochromium (T_{51Cr}) in their 22 patients ($r = 0.2904$, $p > 0.05$). Furthermore, although they found, by surface counting, an increased splenic accumulation of radioactivity in four of seven patients with cirrhosis whose T_{51Cr} was under 20 days, and an increased splenic uptake in only one of seven patients with cirrhosis whose T_{51Cr} was normal, this difference is not significant using the chi square test ($\chi^2 = 2.8$; $p > 0.05$). However, when Student's t test was applied to the mean spleen counts of these two groups a significant relationship was found ($t = 1.8666$; $p < 0.05$). It can be seen, therefore, that while there is a relationship between red cell survival and the splenic accumulation of radioactive chromium, this is not close. Most observers are convinced that the spleen plays the main part in the haemolytic process of at least some cases of cirrhosis of the liver (Jandl, 1955; Jones, Weinstein, Effinger, and Capps, 1955; Holzbach, Shipley, Clark, and Chudzik, 1964; Silva, Abu Jamra, Maspes, Pontes, Pieroni, and Cintra,

1965; Kimber, Deller, Ibbotson, and Lander, 1965), but the evidence that this is the principal cause in general is lacking. Kimber *et al* (1965) found an abnormal uptake of red cells by the spleen in only three of 18 patients with cirrhosis who had reduced red cell survival. Discrepancies have also been described following splenectomy in such patients. Hyman and Southworth (1951) found splenectomy to be unsuccessful in six patients with cirrhosis with a haemolytic anaemia, while Silva *et al* (1963) found this operation to be successful in three of four patients, although portacaval anastomosis preceded the splenectomy. Chaplin and Mollison (1953) have reported reduced red cell survival in a patient with cirrhosis who had had a splenectomy. It may be relevant to note that splenectomy is successful in only 50% of unselected cases of acquired haemolytic anaemia (Welch and Damashek, 1950).

Apart from the role of the spleen other mechanisms have also been implicated in the haemolytic process of cirrhosis of the liver. The Coombs antiglobulin test has revealed the presence of red cell antibodies on occasion (Hyman and Southworth, 1951; Jones *et al*, 1955) although such a finding seems not to be very frequent. Other studies have suggested that in some cases, at least, there is probably both an intracorporeal and an extracorporeal factor involved in the anaemia (Fauvert, Loverdo, Nicollo, and Boivin, 1958; Hall, 1960; Combrison, 1960; Pitcher and Williams, 1963; Katz, Velasio, Guzman, and Alessandri, 1964; Smith, Lonergan, and Sterling, 1964).

The present series illustrates many of the factors so far described. Of the eight patients with biliary cirrhosis studied, four (cases 1, 2, 6, 8), ie, 50%, had a shortened red cell survival which is similar to that in patients with cirrhosis in general. One patient (case 1) had an acute Coombs-positive autoimmune haemolytic anaemia. The significance of the red cell antibodies in the production of the anaemia is suggested by the remission in the anaemia when steroid therapy was introduced. The haemoglobin rose from 6.8 g to 12.5 g% and the PCV rose from 20% to 36%, and the Coombs test became negative. Red cell survival is not usually affected by steroid therapy in cirrhosis (Williams and Billing, 1961; Katz *et al*, 1964). Two patients (cases 5 and 6) had evidence, from surface counting, of splenic sequestration of red cells of a degree which suggested that splenectomy would influence the haemolytic process. Case 6 also had leucopenia and thrombocytopenia with generalized hyperplasia of the bone marrow which was in keeping with the diagnosis of hypersplenism. Radiation to the spleen of this patient caused a reduction in splenic size from 3 in. to 1½ in. below the costal margin and a remission in the anaemia. The haemoglobin rose from 7.7 g to 12.7 g% and the PCV

Fig. 3 Hypothetical relationship between cirrhosis of the liver and anaemia.

from 28% to 36%. There was a moderate rise in the white cell count from approximately 2,500/cmm to 3,600/cmm. There was no obvious change in the platelet count which continued around 40,000/cmm. The remaining two patients (cases 2 and 8) had reduced red cell survival times which did not appear to relate to any of the factors so far discussed. Further analysis of the group as a whole failed to show any relationship between the red cell survival and the size of the liver or spleen or between the red cell survival and the serum cholesterol, alkaline phosphatase, serum gamma globulin, or the immunofluorescent mitochondrial antibody titre. However, there was a significant correlation between the serum bilirubin level and the red cell survival ($P < 0.05$). This unexpected finding may have relevance to the production of the haemolytic process when considered in the light of the experimental findings of Powell, Duncicliiff, and Billing (1968). These workers, following experiments in rats whose bile ducts had been ligated, suggested that 'the haemolytic process accompanying cholestatic jaundice may be related to the increased levels of conjugated bilirubin in the plasma, but not to the accumulation of conjugated bile acids, phospholipids, and cholesterol'. In the present series, it was not determined what percentage of the total serum bilirubin was made up of conjugated bilirubin, but it is tempting to suggest that conjugated bilirubin is the extracorporeal factor in the haemolytic anaemia of biliary

cirrhosis. Furthermore, in the six patients in whom there was both a red cell survival and a BSP retention test available for analysis, a significant relationship was found between these two measurements ($P < 0.01$). This is in keeping with the observations of Kimber *et al* (1965) made on a group of 24 patients with chronic liver disease resulting from alcoholism, post-necrotic scarring, or haemochromatosis. Statistical analysis applied to their results shows a significant correlation between red cell survival and the BSP test ($r = 0.422$; $P < 0.05$). It can be concluded from this that the degree of liver dysfunction is also an important factor in the production of the haemolytic anaemia and the one which initiates all the other mechanisms.

From the available evidence, therefore, it would appear, after excluding obvious causes such as iron deficiency due to haemorrhage, vitamin B₁₂ and folic acid deficiency, which are probably of dietary origin, and relative anaemia due to increased plasma volume, that the anaemia of cirrhosis of the liver, including primary biliary cirrhosis, results from an interaction of different mechanism. We would suggest the following unifying hypothesis for the mechanisms (Fig. 3): cirrhosis of the liver, however produced, is associated with reticuloendothelial hyperplasia and increased production of gamma globulin (Glynn and Holborow, 1965). This increase in the reticuloendothelial cell system causes, on the one hand, splenic enlargement with, on occasion, 'hypersplenism' and on the other hand, on occasion, Coombs red cell antibodies with resulting haemolytic anaemia. The damaged liver also produces a 'metabolic' disturbance, including a raised serum conjugated bilirubin level which affects the red cells either intracellularly or extracellularly or both, rendering the cells more susceptible to destruction by the reticuloendothelial system generally, resulting in shortened red cell survival times. Finally, for some reason unexplained, although probably also related to the 'metabolic' changes produced by the diseased liver, the bone marrow fails to utilize iron for haem synthesis (Kimber *et al*, 1965) resulting in an inadequate marrow response to the haemolytic process. This model may provide a useful guide to further research into this very complex subject.

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References

- Chaplin, H., Jr., and Mollison, P. L. (1953). Red cell life-span in nephritis and hepatic cirrhosis. *Clin. Sci.*, 12, 351-360.
- Combrisson, A. (1960). L'équilibre erythrocytaire des cirrhoses du foie. *Sang*, 31, 885-904.
- Dacie, J. V., and Lewis, S. M. (1963). *Practical Haematology*, 3rd ed. Churchill, London.

- da Silva, L. C., Jamra, M. A., Maspes, V., Pontes, J. F., Pieroni, R. R., and Cintra, A. B. DeU. (1963). Pathogenesis of indirect reacting hyperbilirubinemia after portacaval anastomosis. *Gastroenterology*, **44**, 117-124.
- Fauvert, R., Loverdo, A., Nicollo, S., and Boivin, P. (1958). L'hémolyse dans les cirrhoses. Etude de 40 cas par la méthode des hématies marquées du chrome 51. *Sem. Hôp. Paris*, **34**, 1801.
- Glynn, L. E., and Holborow, E. J. (1965). *Autoimmunity and Disease*. Blackwell, Oxford.
- Goldberg, A., Hutchison, H. E., and MacDonald, E. (1966). Radiochromium in the selection of patients with haemolytic anaemia for splenectomy. *Lancet*, **1**, 109-114.
- Goudie, R. B., MacSween, R. N. M., and Goldberg, D. M. (1966). Serological and histological diagnosis of primary biliary cirrhosis. *J. clin. Path.*, **19**, 527-538.
- Hall, C. A. (1960). Erythrocyte dynamics in liver disease. *Amer. J. Med.*, **28**, 541-549.
- Haozbach, R. T., Shipley, R. A., Clark, R. E., and Chudzik, E. B. (1964). Influence of the spleen size and portal pressure on erythrocyte sequestration. *J. clin. Invest.*, **43**, 1125-1135.
- Hume, R., Dagg, J. H., Fraser, T. N., and Goldberg, A. (1964). Anaemia of Felty's syndrome. *Ann. rheum. Dis.*, **23**, 267-271.
- Hyman, G. A., and Southworth, H. (1951). Hemolytic anaemia associated with liver disease. *Amer. J. med. Sci.*, **221**, 448-456.
- Jandl, J. H. (1955). The anemia of liver disease: observations on its mechanism. *J. clin. Invest.*, **34**, 390-404.
- Jandl, J. H., Greenberg, M. S., Yonemoto, R. H., and Castle, W. B. (1956). Clinical determination of the sites of red cell sequestration in hemolytic anemias. *J. clin. Invest.*, **35**, 842-867.
- Jones, P. N., Weinstein, I. M., Ettinger, R. H., and Capps, R. B. (1955). Decreased red cell survival associated with liver disease. *Arch. intern. Med.*, **95**, 93-102.
- Katz, R., Velasco, M., Guzman, C., and Alessandri, H. (1964). Red cell survival estimated by radioactive chromium in hepatobiliary disease. *Gastroenterology*, **46**, 399-404.
- Kimber, C., Deller, D. J., Ibbotson, R. N., and Lander, H. (1965). The mechanism of anaemia in chronic liver disease. *Quart. J. Med.*, **34**, 33-64.
- Mollison, P. L., and Veall, H. (1955). Use of the isotope ⁵¹Cr as label for red cells. *Brit. J. Haemat.*, **1**, 62-74.
- Pitcher, C. S., and Williams, R. (1963). Reduced red cell survival in jaundice and its relation to abnormal glutathione metabolism. *Clin. Sci.*, **24**, 239-252.
- Powell, L. W., Dunncliff, M. A., and Billing, B. H. (1968). Red cell survival in experimental cholestatic jaundice. *Brit. J. Haemat.*, **15**, 429-435.
- Smith, J. A., Lonergan, E. T., and Sterling, K. (1964). Spur-cell anemia. Hemolytic anemia with red cells resembling acanthocytes in alcoholic cirrhosis. *New Engl. J. Med.*, **271**, 396-398.
- Subhiyah, B. W., and Al-Hindawi, A. Y. (1967). Red cell survival and splenic accumulation of radiochromium in liver cirrhosis with splenomegaly. *Brit. J. Haemat.*, **13**, 773-778.
- Welch, C. S., and Dameshek, W. (1950). Splenectomy in blood dyscrasias. *New Engl. J. Med.*, **242**, 601-606.
- Williams, R., and Billing, B. H. (1961). Action of steroid therapy in jaundice. *Lancet*, **2**, 392-396.