FOUR CASES OF FAMILIAL ACHOLURIC JAUNDICE :

The effect of splenectomy on red cell morphology

BY

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The lowered resistance to hypotonic saline of the red cells in acholuric jaundice has for long been known, but it is only since the advent of the Price-Jones method of accurately measuring red cell diameters, and the introduction of the cell volume index that the unique shape of the red cells has been recognized. Of late the work of Vaughan¹ has added to the knowledge of the concentration of haemoglobin within the corpuscle and more recently, while this work was in progress, Hawksley and Bailey² have published a paper showing the effect of splenectomy on the red cell diameter. The following series of cases largely confirms the results of these observers.

Case records.

The four cases comprise a family of two boys and two girls. The disease was transmitted to them through their father who has been proved to be a typical example of the malady. Some of his brothers and sisters and their children are also affected. Probably the paternal grandfather of the cases under discussion also suffered from this disease as he was noted for his sallow complexion. Details of the family history, and a report of a haemolytic crisis occurring in each of the cases described in this article have been published by Dr. Angus Scott³.

All four cases were admitted to the Royal Hospital for Sick Children, Glasgow, where splenectomy was performed by Mr. Alex. MacLennan. Below is a summary of each case history. The details of differential leucocyte counts are omitted to conserve space.

Case 1. A girl aged twelve years was admitted 13.9.34; she was slightly undersized and thin. Considerable pallor and slight jaundice of skin were present and sclerotic icterus was marked. The spleen was enlarged, the lower pole being palpable two-and-a-half finger-breadths below the costal margin. The liver was not palpable. The urine contained urobilin in large amounts.

BLOOD EXAMINATION (13.9.34):—Haemoglobin (Haldane) 75 per cent.; red cells, 3,190,000 per c.mm.; colour index, 1.19; leucocytes, 6,600 per c.mm.; reticulocytes, 13.2 per cent.;

platelets, 127,600 per c.mm. Van den Bergh reaction, indirect positive ($9\frac{1}{2}$ units). The fragility of red cells was increased (haemolysis present in 0.62 per cent. saline which was the highest percentage used). A Price-Jones curve showed a marked shift to the left, M.C.D., 6.57μ , microcytosis, 16.8 per cent. The differential leucocyte count was normal. A very occasional normoblast was seen.

Splenectomy performed 17.9.34. The spleen weighed 340 gm. Microscopically the picture was typical of acholuric jaundice. There was increase of the fibrous tissue of the capsule and trabeculae. The pulp was hyperplastic, the pulp spaces being engorged with blood and the sinusoids empty and flattened. The Malpighian bodies were scattered and compressed, and in most there was central and peripheral fibrosis. In this case there was almost complete absence of free iron in the spleen.

The jaundice disappeared rapidly. A blood count one week after operation (25.9.34) showed:—Haemoglobin, 85 per cent.; red cells, 3,920,000 per c.mm.; colour index, 1.08; leucocytes, 4,800 per c.mm.; reticulocytes, 2.2 per cent.; platelets, 768,000 per c.mm. M.C.D. of red corpuscles, 6.95μ , microcytosis, 7 per cent.

The patient made steady progress, and on 22.10.34, five weeks after splenectomy, the blood count was:—Haemoglobin, 96 per cent.; red cells, 4,920,000 per c.mm.; colour index, 0.98; reticulocytes, less than 1 per cent.; platelets, 270,000 per c.mm. The fragility of red cells after operation was slightly decreased, beginning at 0.58 per cent. NaCl; M.C.D. of corpuscles, 6.71μ ; microcytosis, 6 per cent. The van den Bergh reaction 0.5 units, indirect positive.

Case 2. A girl aged ten years was admitted 13.9.34. She was a pale and thin child, with a very sallow complexion and sclerotic icterus. The spleen was palpable, two-and-a-half finger breadths below the costal margin. The liver was not palpable. The urine contained urobilin in large quantities.

A blood examination on 13.9.34 showed:—Haemoglobin, 64 per cent.; red cells, 2,980,000 per c.mm.; colour index, 1.08; leucocytes, 11,000 per c.mm.; reticulocytes, 12.8 per cent.; platelets, 151,980 per c.mm. The van den Bergh reaction: indirect positive ($9\frac{1}{2}$ units). The fragility was increased as in case 1. A Price-Jones showed a shift to the left, M.C.D., 6.417μ , microcytosis 24.8 per cent. The differential leucocyte count showed no special features. No nucleated red cells were present.

Splenectomy was performed on 17.9.34. The spleen weighed 300 gm. The microscopical appearance was the same as in case 1. A slight deposit of free iron was detected in the pulp cells, at the periphery of some of the Malpighian corpuscles.

The jaundice disappeared rapidly. A blood count one week after operation showed:—Haemoglobin, 80 per cent.; red cells, 4,470,000 per c.mm.; colour index, 0.90; leucocytes, 9,000 per c.mm.; reticulocytes, 3.2 per cent.; platelets, 759,000 per c.mm.; M.C.D. of red cells, 6.84μ , microcytosis, 9.4 per cent. No urobilinuria. A blood count (22.10.34), five weeks after operation showed:—Haemoglobin, 82 per cent.; red cells, 4,850,000 per c.mm.; colour index, 0.82; leucocytes, 10,800 per c.mm.; reticulocytes less than 1 per cent.; platelets, 213,000 per c.mm. The fragility of red cells was slightly decreased, lysis beginning at 0.58 per cent. NaCl. M.C.D. of

red corpuscles, 6.33μ , microcytosis, 27.2 per cent. The van den Bergh reaction was negative.

Case 3. A boy aged eight years was admitted 21.9.34. He was moderately well nourished, but pale and with definite icterus. The spleen was palpable, its lower pole two finger-breadths below costal margin. The liver was not palpable. Urobilinuria was present.

Blood count (22.9.34) showed:—Haemoglobin, 66 per cent.; red cells, 3,090,000 per c.mm.; colour index, 1.08; leucocytes, 8,200 per c.mm.; reticulocytes, 16.2 per cent.; platelets, 131,000 per c.mm. The fragility was increased to the same degree as in case 1. The van den Bergh reaction, indirect positive ($4\frac{1}{2}$ units). A Price-Jones curve showed shift to the left, M.C.D. 6.34μ , microcytosis 27.2 per cent.

A transfusion was given (22.9.34) of 350 c.c. of whole blood and splenectomy was performed on 25.9.34. The spleen weighed 280 gm. In this case splenic fibrosis was more marked than in either of the previous cases. There was a diffuse deposit of free iron in the pulp cells, especially marked in those cells surrounding the Malpighian bodies. There was a rapid clearance of jaundice. A blood count one week after operation showed:—Haemoglobin, 74 per cent.; red cells, 4,100,000 per c.mm.; colour index, 0.88; leucocytes, 8,000 per c.mm.; reticulocytes, 1.5 per cent.; platelets, 598,000 per c.mm.; M.C.D. of red cells, 6.41μ , microcytosis, 24.4 per cent. No urobilinuria was present. The child made an uninterrupted recovery. A blood count on 30.10.34, five weeks after operation showed:—Haemoglobin, 86 per cent.; red cells, 4,890,000 per c.mm.; colour index, 0.88; leucocytes, 8,200 per c.mm.; reticulocytes, less than 1 per cent.; platelets, 502,000 per c.mm. The fragility was slightly decreased, lysis beginning at 0.60 per cent. NaCl. M.C.D. of red cells, 6.44μ , microcytosis, 16.8 per cent. The van den Bergh reaction was negative.

Case 4. A boy aged six years was admitted 21.9.34. He was thin and weakly in appearance. Considerable pallor was present with a definite icteric tinge of skin and sclerotics. The spleen was palpable, its lower pole two finger-breadths below costal margin. The liver was not palpable. Urobilinuria was present.

A blood count (22.9.34) showed:—Haemoglobin 48 per cent.; red cells, 2,810,000 per c.mm.; colour index, 0.86; leucocytes, 7,700 per c.mm.; reticulocytes, 16.4 per cent.; platelets, 155,000 per c.mm. The fragility was increased as in case 1. The van den Bergh reaction was indirectly positive ($4\frac{1}{2}$ units). A shift to the left was present in the Price-Jones curve, M.C.D., 6.39μ , and microcytosis, 22 per cent. A differential leucocyte count showed increase of neutrophils (77.5 per cent.) and absence of eosinophiles. Normoblasts amounted to 77 per c.mm. Clinically there was no evidence of acute infection. The patient was transfused 23.9.34, receiving 450 c.c. of whole blood. Splenectomy was performed on 25.9.34. The spleen weighed 240 gm. Fibrosis and destruction of the Malpighian bodies were most obvious in this case, as also was the deposit of free iron, which as well as being present in the pulp cells was also present in the reticulum. The distribution of the iron was chiefly round the Malpighian bodies.

Again there was rapid disappearance of jaundice. A blood count one week after operation showed:—Haemoglobin, 68 per cent.; red cells, 4,450,000 per c.mm.; colour index, 0·8; leucocytes, 8,700 per c.mm.; reticulocytes, less than 1 per cent.; platelets, 842,000 per c.mm. M.C.D. of red cells, 6·65 μ ; microcytosis, 10·2 per cent. There was no urobilinuria. A blood count, four weeks after operation showed:—Haemoglobin, 80 per cent.; red cells, 4,780,000 per c.mm.; colour index, 0·84; leucocytes, 6,000 per c.mm.; reticulocytes, less than 1 per cent.; platelets, 809,000 per c.mm. (One week later the platelets were reduced to 576,000 per c.mm.)

The fragility of red cells was slightly reduced beginning at 0·60 per cent. NaCl. M.C.D. of red cells, 6·38 μ ; microcytosis, 18·0 per cent. The van den Bergh reaction was negative.

Discussion.

It will be seen from the above summaries that each of the children was suffering from a considerable anaemia showing all the typical features of acholuric jaundice, namely, normal or slightly raised colour index, increased fragility of the red cells, reticulocytosis, excessive bilirubinaemia and urobilinuria, and in addition the history of a familial and hereditary transmission. In each case the expected and now well-recognized group of changes resulting from splenectomy in acholuric jaundice was found. There was immediate cessation of excessive blood destruction, and consequent diminution of bone marrow activity, resulting in a rise of the red cell count and percentage of haemoglobin to normal or almost normal levels, and a fall of the number of reticulocytes. At the same time bilirubinaemia and urobilinuria were reduced to within healthy limits with subsequent disappearance of jaundice. As in all cases in which the spleen is removed a remarkable increase in the platelet count to 700,000 or 800,000 per c.mm., occurred and was thereafter followed by a gradual return to normal numbers. Little alteration in fragility was noted, although cases have been described where the fragility became normal or was even increased after splenectomy. In the present cases there was slight lessening of fragility.

In recent years interesting observations have been made on the peculiarity of the red corpuscles in acholuric jaundice. Although the diameter of the corpuscle is reduced, there is little, if any, change in volume, the inference being that the cell has assumed a globular shape.

Recently Vaughan and Goddard¹ have shown that the concentration of haemoglobin in the red corpuscle in acholuric jaundice is higher than in health, and in various diseases including different types of anaemia. In our cases similar findings were obtained. The technique used was similar to that described by Vaughan and Goddard, except that blood was spun in graduated centrifugal tubes instead of in the haematocrit. The calculations of the various factors were done by the formulae given by these workers.

Briefly, mean corpuscular volume (M.C.V.) is the average volume of the red corpuscle and is expressed in cubic μ . Mean corpuscular haemoglobin (M.C.H.) is the average amount of haemoglobin by weight in the corpuscle

and is given in micro-micrograms ($\gamma\gamma$). Mean corpuscular haemoglobin concentration (M.C.H.C.) gives as a percentage the amount of haemoglobin per unit volume of red cell.

The data from the present cases are shown in table I where for comparison the average figures for a group of normal children are given.

TABLE 1.

COMPARISON OF BLOOD FINDINGS IN NORMAL CHILDREN AND IN MICROCYTIC HYPOCHROMIC ANAEMIA WITH THE FINDINGS IN ACHOLURIC JAUNDICE.

	RED CELLS : MILLIONS PER C.MM.	HAEMO- GLOBIN : GM. PER CENT.	VOL. OF PACKED RED CELLS PER CENT.	M.C.V. c. μ .	M.C.H. $\gamma\gamma$.	M.C.H.C. PER CENT.	M.C.D. μ .
AVERAGE OF 5 NORMAL CHILDREN	5.17	13.02	40.60	78.54	25.17	32.06	
AVERAGE OF 4 CASES OF ACHOLURIC JAUNDICE	3.02	8.52	23.95	79.08	28.2	35.46	6.41
ONE CASE OF MICRO- CYTIC HYPOCHROMIC ANAEMIA	3.76	4.83	21.67	57.63	12.84	22.24	

M.C.V. = Mean corpuscular volume.
M.C.H. = Mean corpuscular haemoglobin.
M.C.H.C. = Mean corpuscular haemoglobin concentration.
M.C.D. = Mean corpuscular diameter.

There is a low mean corpuscular red cell diameter with a normal cell volume, a slightly increased content of haemoglobin, and increased concentration of haemoglobin. Actually in cases 1 and 2, the M.C.V. (84.6 and 85.2 c. μ respectively) was just above the upper limits of the normal series (82.13 c. μ). In case 3, M.C.V. fell within normal limits but in case 4, M.C.V. (70.21 c. μ) was slightly below the lower limits of normal (74.21 c. μ). The haemoglobin content varied directly with the size of the cell, being rather higher in cases 1 and 2 (32.2 $\gamma\gamma$ and 29.5 $\gamma\gamma$) in which there was definite enlargement of the cells and only very slightly increased in case 3 and slightly below normal in case 4 (23.56 $\gamma\gamma$). This is of course what would be expected, the larger cells being able to hold more haemoglobin. To show the small extent of the variations of cell volume and haemoglobin from the normal, the figures from a case of microcytic hypochromic anaemia in a child of three-and-a-half years are added. In all four examples of acholuric jaundice, however, the mean corpuscular haemoglobin concentration was higher than in the normal series, i.e. the amount of haemoglobin per unit volume of red cell

was high. This is apparently a peculiarity of the corpuscle in this disease, and as suggested by Vaughan and Goddard may be a corollary of the unique shape of the corpuscle.

The effect of splenectomy on the size and shape of the red cell in acholuric jaundice is variously stated by different authors, some asserting that normal diameter was attained, and others that no change occurred. Recently, Hawksley and Bailey² observed the influence of splenectomy on the corpuscle by making a series of Price-Jones curves after operation. In all their cases the mean diameter increased within the first few days (one reaching almost normal figures) but in most cases, this change was transient

FIG. 1.—Price-Jones curves in a case of familial acholuric jaundice. Mean diameter before splenectomy (continuous line) 6.57μ ; mean diameter seven days after splenectomy (broken line) 6.95μ .

and a partial return to the pre-operative condition occurred. Coincident with the increase in mean diameter was a flattening of the Price-Jones curve. In the four cases discussed in this paper, Price-Jones curves were constructed before, one week after, and a month or six weeks after splenectomy. In all cases there was definite lowering of the peak in the first post-operative curves. In cases 1 and 2 a marked shift to the right occurred, the mean diameters being within normal limits although a slight microcytosis persisted in each case. An example is given in fig. 1 where, in order to make the

diagram less complicated, the final Price-Jones curve is omitted. In the remaining two cases the increase in mean diameter was of less extent. Probably the maximum increase occurred earlier than one week after operation. In all four cases, the curves constructed four to six weeks after operation had assumed a high peak, and in three cases the mean diameter had returned to almost the pre-operative figures. In the remaining case (case 1) the mean diameter (6.71μ) remained above Price-Jones' lowest normal limit (6.686μ), microcytosis being only 6 per cent. It will be interesting to note later whether the last case finally reverts or permanently maintains practically normal figures. A Price-Jones curve made by the writer on one case of acholuric jaundice, two years after splenectomy, had a mean diameter of 6.3μ , and is quite indistinguishable from the curves of the mother and other children, all of whom are affected by the disease, and who have not, so far, undergone splenectomy.

It would appear, as Hawksley and Bailey² suggest, that the spleen causes a further distortion of the already abnormal cell. They also suggest that possibly the remainder of the reticulo-endothelial system assumes some of the functions of the removed spleen, although if this were the case it is difficult to understand why excessive destruction of red cells should cease. It is an old argument, whether a faulty bone marrow, producing abnormally fragile red cells which are easy prey for a normally functioning spleen, or an anomalous action of the spleen on the erythrocytes is the primary factor in acholuric jaundice. Certainly the persistence of undue fragility to hypotonic saline after splenectomy in the great majority of cases seems to point conclusively to the primary implication of the bone marrow, but the effect (above described) of removal of the spleen on the size of the red cells would suggest that there are two factors concerned. Even in the early days of life increased fragility and diminished diameter of the red cells are present, so that no evidence of priority of one over the other is available.

The suggestion that the spleen exerts an influence on the red cell diameter is strengthened by the fact that in icterus gravis neonatorum, a haemolytic anaemia in which the spleen is enlarged, there is a definite diminution in the red cell diameter⁴. Further evidence should be obtained by measuring the red cell diameters in cases where the spleen has to be removed and the cell diameter is known to be normal (cases such as thrombocytopenic purpura and splenic rupture) before discussing the phenomenon with particular relation to the haemolytic anaemias with reduced cell diameter.

Summary and conclusions.

The clinical investigation of four cases of familial acholuric jaundice before and after splenectomy is reported. By measurement of the red cell diameter and volume, the globular shape and the unique increase of the concentration of haemoglobin of the corpuscle in this disease is demonstrated.

The suggestion that the spleen has a part in causing the reduced red cell diameter is supported, as after splenectomy an increase of diameter was found.

I am indebted to Prof. G. B. Fleming and Dr. N. Morris for helpful criticism of this paper. The work was done during the tenure of a grant from the Rankin Medical Research Fund.

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