Brit. J. Haemat., 1963, 9, 552.

The Origin of 'Burr' Erythrocytes

R. E. Bell

Clinical Laboratory Services, University of Alberta Hospital, Edmonton, Alberta, Canada

The term 'burr' red cell was introduced by Schwartz and Motto (1949) to describe irregularly shaped erythrocytes 'having one or more large spiny projections' along their periphery. These abnormal erythrocytes are found most frequently in uraemia and gastric carcinoma and less frequently in association with bleeding peptic ulcer (Aherne, 1957). They are also seen in haemolytic anaemia where they are associated with fragmented erythrocytes (schistocytes), triangular forms, or other unusual shapes (Brain, Dacie and Hourihane, 1962). Recently they have also been described in aplastic anaemia (Lewis, 1962). On rare occasions they may be seen in blood smears of normal subjects. Very little is known about how these abnormal erythrocytes are produced. Dacie (1960) suggested that burr cells were artefacts produced on the slide as the cell dries. There is evidence suggesting that mechanical injury to the cell may be a major factor (Brain *et al.*, 1962; Sayed, Dacie, Handley, Lewis and Cleland, 1961) but they may also result from the action of unknown metabolic or 'toxic' factors. It is the purpose of this paper to present some morphological evidence that suggests a mechanism for their development. The sequence of events ending in a burr cell appears to be the formation of a vacuole at the periphery of the cell that subsequently ruptures through the cell membrane leaving a crater and a deformed erythrocyte.

MATERIAL

The observations reported herein were first made on the blood of a patient who had a suspected recent massive gastro-intestinal haemorrhage and in whose blood many burr cells appeared. The patient was a 46-year-old male admitted to this hospital with a history of diarrhoea which had commenced 3 weeks before admission. This had persisted for about 10 days. He was given some pills at the onset of diarrhoea which he stated 'made his stools black' for several days. Following the diarrhoea he became very weak and pale. Three days before admission he began to develop swelling of both legs.

He had been pensioned for rheumatoid arthritis but had minimal clinical evidence of the disease. He gave a history of transient swelling of his legs 20 years ago and 6 years ago he was in another hospital with haematuria, attributed to acute glomerulonephritis.

Physical examination revealed pallor, some puffiness around the eyes and moderate pitting oedema of his legs up to his knees. The liver was just palpable. There were no other significant physical findings aside from his arthritis. The blood pressure was 140 systolic 85 diastolic.

The haemoglobin on admission was 6.7 g. per 100 ml. The next day the haemoglobin was 8.0 g. per 100 ml., WBC 3800 with 62 per cent polymorphonuclear neutrophils and 8.1 per cent reticulocytes. No protein, sugar or red blood cells were found in the urine. Examination of a peripheral blood film showed an increase in regenerative macrocytes and many burr cells. There were also rare hypochromic cells present. Blood platelets were increased in number. A bone marrow biopsy performed 6 days after admission showed moderate normoblastic hyperplasia and decreased iron stores. Other studies showed a blood urea nitrogen of 8 mg. per 100 ml., serum iron 25 µg. with a total iron-binding capacity of 705 µg. per 100 ml. serum.

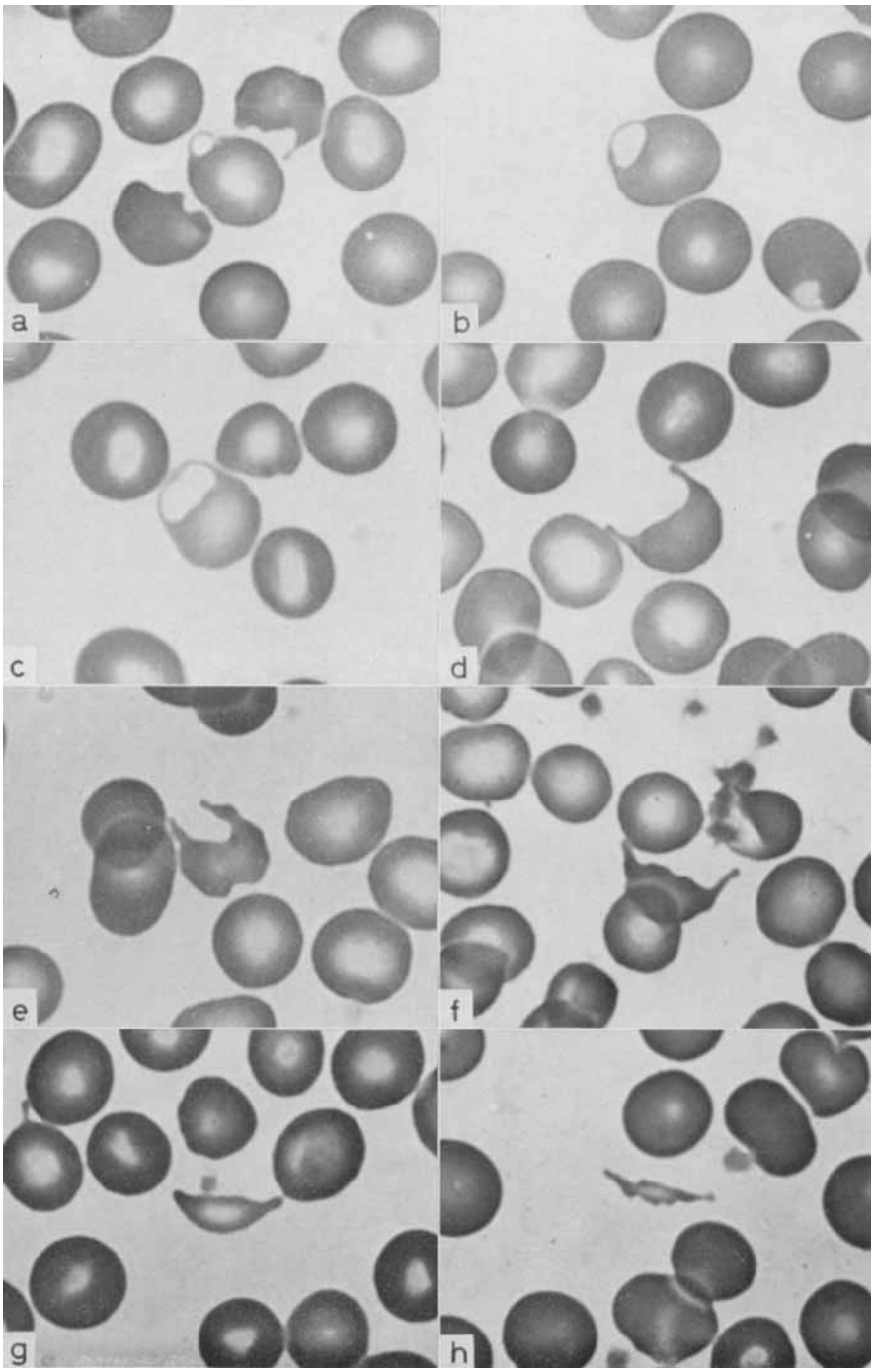

FIG. 1. Photomicrographs taken of a single blood film of the patient. The sequence of development of burr cells which is proposed can be clearly seen. In (b) there is a platelet overlapping the border of an erythrocyte. Slides stained with basic fuchsin and photographed using a green filter Wratten B (×1575). The sequence illustrated in (g) and (h) is regarded as only suggestive.

Occult blood was not found in the stool on five examinations. There was no evidence of haemolysis. Erythrocyte osmotic fragility was normal after 24 hours' incubation. Total serum bilirubin was 0.5 mg. per 100 ml. and only a trace of urobilinogen was found in the urine. Radiological studies of the gastro-intestinal tract and an intravenous pyelogram revealed no abnormalities. Two weeks later examination showed a rise of his haemoglobin to 10.7 g. per 100 ml. with 3.0 per cent reticulocytes, WBC of 10,500 per cu.mm. with a normal differential count. Repeated urinalyses have shown 30–50 RBC per high power field but only a trace of protein. A glucose tolerance curve was of diabetic type.

OBSERVATIONS

Smears were made on 3×1 in. microscope slides by standard technique from blood taken from the tip of the needle used to collect specimens from the antecubital vein for haematological or other procedures. They were stained with Wright's stain. Examination of these smears showed numerous burr cells. In addition, there were present about equal numbers of erythrocytes with a clear vacuole or blister located at the periphery of the cell (Fig. 1). These were usually round but sometimes oval or irregular in outline and were about 1–3 μ in greatest diameter. The erythrocytes maintained their normal shape when the vacuoles were small, but the border of the cells over the larger vacuoles was irregular. Rarely more than one vacuole was present in a single cell. In some erythrocytes the thin cell membrane enclosing the vacuole had broken, leaving an open crater at the border of the erythrocyte with two remnants of cell membrane extending from the edge of the defect left by the vacuole. Cells at this stage had a crescent or quarter-moon shape and bore a close resemblance to burr cells. On examining these smears, one could without difficulty place individual cells in a sequence starting with those with a small intracellular vacuole and ending with an 'early' burr form (Fig. 1). These changes are illustrated diagrammatically in Fig. 2.

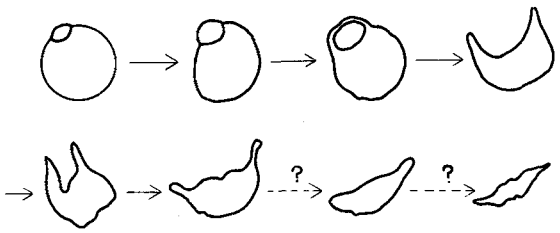

FIG. 2. A diagram illustrating the proposed sequence of development in the formation of burr red cells.

Since making these observations on the patient whose history has been outlined, we have looked for similar cells in other smears, particularly those in whom we have previously reported burr cells. In smears of ten such patients we have without exception found vacuoles in a small but significant proportion of erythrocytes. Included among these were smears from both males and females with hypochromic anaemia and patients with azotaemia. With one exception, the number of burr cells and what we now regard as precursor forms of these cells were not as numerous in blood smears of these patients as in the patient herein reported in detail and therefore the developmental sequence outlined was more difficult to discern. The exception, a male with a hypochromic anaemia resulting from chronic intestinal haemorrhage of unknown cause had numerous burr cells and other representative morphologic

forms of the sequence described. Rare burr cells and rare erythrocytes with vacuoles can be found after a meticulous examination of some normal smears. We have not been able, however, to trace the full development in these instances.

Other studies that have been done include staining of the smears for fat, glucogen, glycoprotein and iron. The material in the vacuole did not stain with any of these cytochemical procedures. Reticulocyte stains showed that the cells did not stain with the vital stain (new methylene blue) and, therefore, did not represent cells recently released from the bone marrow. Heinz bodies could not be demonstrated in the cells. We have also observed these vacuoles in erythrocytes suspended in normal saline or solutions of vital dyes and examined with the ordinary light microscope. In such wet preparations the vacuoles are not refractile, and retain a relatively constant size and shape during a 20–30 minute period of observation.

DISCUSSION

These observations have been interpreted as showing the following sequence of developments in the erythrocyte preliminary to the formation of a burr cell. A small vacuole appears at the periphery of an otherwise normal erythrocyte. This enlarges and may distort the cell to some degree, particularly where the vacuole is only covered by a thin cell membrane. The vacuole ruptures the cell membrane, leaving a defect with sharp peaked edges from which thin remnants of the cell membrane extend. Flattening of the crater with further distortion of the cell produces a typical burr cell.

While the occurrence of such abnormalities in individual cells does not necessarily prove that they represent a sequence of steps in the formation of burr cells, we feel that the evidence that this is so is highly suggestive. The possibility that other abnormal shapes arise from further distortion of cells of this type or eruption of additional vacuoles may offer an explanation for many of the other bizarre shapes of erythrocytes found in peripheral blood under varying circumstances. Very rarely we have found a peripheral vacuole in erythrocytes already distorted.

Other mechanisms have been proposed for the origin or erythrocytes of unusual shape. Dacie (1960) suggested that burr cells are the result of irregular crenation *in vitro* but concluded that the exact pathogenesis is unknown. Aherne (1957) favoured a metabolic origin possibly as a result of inactivation or abnormality of enzyme systems essential for the maintenance of the structure of the cell. No 'toxic' substance or metabolic abnormality common to all clinical circumstances where these cells are seen has been demonstrated. It is also possible they represent a normal mode of cell destruction. This is supported by the observation that some of the stages in the development described in this paper are seen in blood smears from healthy individuals. The abnormalities that these cells manifest may be of degree rather than kind.

The observations we have made do not provide an explanation as to why these intracellular vacuoles form. They could be the result of mechanical, 'toxic', or metabolic injury to the cell or be a manifestation of senescence. Recently Brain and associates (1962) have suggested these cells may form as a result of some injury resulting from their contact with diseased blood vessels. They have referred to this as a micro-angiopathic haemolytic anaemia. There has also been a report of a patient in whom irregularly shaped erythrocytes and haemolytic anaemia were probably produced by a jet of blood that was continuously impinging on a bare teflon prosthesis in the atrial septum (Sayed *et al.*, 1961). The haematological abnormalities in this patient disappeared on correction of this mechanical defect.

Other vacuoles have been described in the erythrocytes of patients after splenectomy (Kayama, Kihira, Aoki and Ohnishi, 1962). These are small, usually multiple and are located anywhere in the cell. They are seen best with dark, low contrast phase microscopy, but can be seen on ordinary microscopy as well. Their size, number per cell and location suggest that they are different from the vacuoles described here as the precursor of the burr cell.

The possibility that the vacuoles associated with burr cells are artifacts has been considered. They have been found consistently in many smears of several patients as well as in vital preparations. It is interesting to note that many erythrocytes like those we have illustrated are present in other published photographs (e.g. Allison, 1957, Fig. 1; Aherne, 1957, Figs. 1 and 4). What may be an erythrocyte with a vacuole has also been shown by Brain and associates (1962, Fig. 7). There appears, therefore, to be little doubt regarding the authenticity of these vacuoles within erythrocytes, when large numbers of burr cells are being produced.

We believe that the observations reported here demonstrate the probable stages of development ending in burr red cells. These changes may also be important in the destruction of both normal and abnormal erythrocytes.

SUMMARY

Morphological evidence has been presented suggesting the mechanism of development of burr red cells and possibly other abnormally shaped forms.

Burr cells are believed to develop from a vacuole which appears at the periphery of the cell, enlarges and finally ruptures the cell membrane, leaving an abnormally shaped erythrocyte with fine projections extending from the shoulders of the defect so formed.

ACKNOWLEDGMENTS

I am indebted to Dr. T. Shnitka for his help and advice, to Dr. J. A. L. Gilbert for permission to study his patient and to Mr. A. Eaton and Mrs. B. Motyl for technical assistance.

REFERENCES

AHERNE, W. A. (1957). 'The "burr" red cell and azotaemia.' *J. clin. Path.*, **10**, 252.

ALLISON, A. C. (1957). 'Acute haemolytic anaemia with distortion and fragmentation of erythrocytes in children.' *Brit. J. Haemat.*, **3**, 1.

BRAIN, M. C., DACIE, J. V. and HOURIHANE, D. O'B. (1962). 'Microangiopathic haemolytic anaemia: the possible role of vascular lesions in pathogenesis.' *Brit. J. Haemat.*, **8**, 358.

DACIE, J. V. (1960). *The Haemolytic Anaemias. Part I. Congenital Anaemias*, 2nd edn, p. 23. Churchill, London.

KAYAMA, S., KIHARA, H., AOKI, S. and OHNISHI, H. (1962). 'Post-splenectomy vacuole. A new erythrocyte inclusion body.' *Mie med. J.*, **11**, 425.

LEWIS, S. M. (1962). 'Red-cell abnormalities and haemolysis in aplastic anaemia.' *Brit. J. Haemat.*, **8**, 322.

SAYED, H. M., DACIE, J. V., HANDLEY, D. A., LEWIS, S. M. and CLELAND, W. P. (1961). 'Haemolytic anaemia of mechanical origin after open heart surgery.' *Thorax*, **16**, 356.

SCHWARTZ, S. O. and MOTTO, S. A. (1949). 'The diagnostic significance of "burr" red blood cells.' *Amer. J. med. Sci.*, **218**, 563.