The usefulness of counting 'heat-affected' red cells as a guide to the risk of the later disappearance of red cells after burns

ELIZABETH TOPLEY¹

From the M. R. C. Industrial Injuries and Burns Research Unit, Birmingham Accident Hospital

SYNOPSIS The technique for counting heat-affected red cells ('microcytes') in the head of the blood film taken from patients on admission is described. Although crude, it does appear to increase the precision with which it is possible to predict the disappearance of red cells in the first 24 hours after a burn, and therefore to decide those cases in which ⁵¹Cr red cell volume estimates may be clinically useful.

When a blood film is taken from a patient with a severe burn half an hour or more after injury, a small proportion of the red cells are very small. Occasionally 'budding' red cells are seen from which these small red cells are presumably derived. In the present communication these very small red cells have, when they are less than 3μ in diameter, been called 'microcytes'. These microcytes, which retain the smooth contour of a red cell, are often associated with red cell fragments and spherocytes. If an anaesthetized rat is given a full-thickness skin loss burn involving more than 10% of the body surface, similar microcytes, red cell fragments, and occasional spherocytes appear in the tail blood within five minutes. Similar findings can be produced by heating whole blood under suitable combinations of time and temperature in a test-tube. The presence of these abnormal cells, produced almost certainly by the direct effect of heat on the red cells in the area of the burn, has been described by Shen and Ham (1943) and Ham, Shen, Fleming, and Castle (1948).

The microcyte count on the blood film on admission on 134 patients has proved of clinical value as one of the signs used to predict the risk of red cells later disappearing (Jackson, 1959; Topley, Cason, Jackson, and Davies, 1961). For this reason the technique of counting microcytes is described and evidence presented on the association between the blood film on admission and the later disappearance of red cells measured by red cell volume estimates.

TECHNIQUES

BLOOD FILM The aim is to count the proportion of red cells less than 3μ in diameter. Such cells can be more precisely counted than fragments, which merge imperceptibly into 'red cell dust', and than spherocytes, which merge imperceptibly into normal red cells. Microcytes appear in largest numbers in the head of the blood film immediately in front of the starting drop and decrease in numbers towards the tail. Most consistent counts of these cells have been obtained by confining the counting to fields close to the head and where red cells do not clump. The blood film was stained by a Romanowsky stain, usually Leishmann-Giemsa. At least 500 cells were counted, using a micrometer eye-piece and oil immersion objective of such a magnification that one division on the micrometer scale was equivalent to 1μ .

The depth of staining of the haemoglobin is consistent with these cells being very small red cells rounded off from larger red cells. The small cells are therefore very pale and can be missed if the staining is not efficient and the focus poor. With Romanowsky staining it was usually easy to differentiate 'microcytes' (less than 3μ) from platelets even though they can be of the same size. Small red cells and a platelet are shown in Fig. 1 and in the figures of Ham and his colleagues (1948).

Observer bias influences the decision to make new films or further films. It also influences the fields selected, and the red cells not counted because they were 'fragments' and not 'cells'. All the observations were made by one observer. The films from the first 68 cases were recounted using a numbering system by which the observer did not know which film was from which patient. Almost all the films from more recent cases have been counted within a few hours of admission before the later disappearance of red cells had been measured. Recounts on many of these cases have shown that the report 'less

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¹Now at the National Blood Transfusion Service, Birmingham

FIG.1. A typical microcyte in the blood film taken within four hours of burning injury.

than 2% of microcytes' was almost invariably consistently obtained on recounts. When there were more than 2% of microcytes there was more inconsistency but the results almost invariably agreed to within $\pm 5\%$.

This technique carries all the disadvantages of differential counting of cells on spread blood films but no more satisfactory method has been found. The heat-affected red cells have not been separated satisfactorily by differential centrifugation or differential sedimentation in a variety of solutions.

RED CELL DISAPPEARANCE This has been measured by red cell volume estimates using ^{51}Cr - or ^{32}P -labelled red cells, combined with a knowledge of the patient's average normal red cell volume calculated from height and the volume of blood transfused. The method and calculation of the 'red cell volume estimate of red cell disappearance' has been described elsewhere (Davies and Topley, 1959). The results have been expressed as a percentage of the total circulating red cell volume. A 20% disappearance

would therefore be equivalent to 0.3 litres of whole blood in a child of 6 years (115 cm. tall) and 1.0 litres of whole blood in an adult man (175 cm. tall).

AREA OF FULL-THICKNESS SKIN LOSS This was assessed by the clinical staff on admission, and checked in the light of subsequent grafting operations.

USE OF BLOOD FILM ON ADMISSION AS A GUIDE TO RED CELL DISAPPEARANCE DURING THE ENSUING DAY

The result of the blood film taken on admission has been compared with the red cell volume estimate of red cell disappearance 'about 24 hours' (12-36 hours) after admission. Because the area of full-thickness skin loss is itself a useful guide to disappearance of red cells the patients have been divided into four groups according to this area.

From 1954 to 1959 blood films on admission were taken from many patients with burns involving more than 20% of the body surface. The radioactive red cell volume estimate of red cell disappearance was used only when red cell disappearance might be clinically important. Table I summarizes the results.

TABLE I

RELATION OF BLOOD FILM FINDING TO AREA OF FULL-THICKNESS SKIN LOSS IN 134 PATIENTS

Area of Full-thickness Skin Loss	Microcytes (%) in Blood Film on Admission			Total Patients
	Less than 2	2 to 9	10 or more	
Less than 15%	24	11	0	35
15-29%	20	11	9	40
30-40%	5	20	6	31
More than 40%	3	16	9	28
Total patients	52	58	24	134

of all blood films on admission, probably a fair sample of more than 15% full-thickness skin loss burns, but including a selection of the clinically more severe smaller burns. This table can be used for relating the blood film to the area of full-thickness skin loss. Table II summarizes the results on all patients who had a blood film on admission and a

TABLE II

RELATION OF BLOOD FILM FINDING AND AREA OF FULL-THICKNESS SKIN LOSS TO RED CELL DISAPPEARANCE MEASURED BY RED CELL VOLUME ESTIMATE ABOUT 24 HOURS AFTER BURNING IN 55 PATIENTS

Microcytes in admission blood film	Less than 2%			2-9%			10 or more %			Total Patients
	<20	20 +	Total	<20	20 +	Total	<20	20 +	Total	
Red cell disappearance (%)										
<15% full-thickness skin loss	6	0	6	4	1	5	0	0	0	11
15-29% full-thickness skin loss	6	0	6	2	2	4	0	4	4	14
30-40% full-thickness skin loss	1	0	1	5	6	11	1	4	5	17
>40% full-thickness skin loss	0	0	0	3	4	7	2	4	6	13
Total patients	13	0	13	14	13	27	3	12	15	55

red cell volume estimate after injury. This is not a fair sample of all shock cases because increasingly the presence of less than 2% of microcytes on the blood film was taken as one part of the evidence for predicting that red cells were unlikely to disappear and a radioactive chromium red cell volume estimate was therefore contraindicated. Table II can be used to relate blood film findings to red cell disappearance but must not be used to relate blood film findings to the area of the burn.

LESS THAN 2% OF MICROCYTES have never been associated with a more than 20% disappearance of red cells (0/13 patients, Table II). In less than 15% full-thickness skin loss burns less than a third of patients (Table I) had 2% or more of microcytes in their blood film; but such a finding when present was on one in five occasions associated with a more than 20% disappearance of red cells (Table II). Less than 2% of microcytes were found in half (20/40) the 15-29% full-thickness skin loss burns (Table I). In larger areas of full-thickness skin loss there were almost invariably larger numbers of microcytes. The finding of less than 2% of microcytes may therefore be added evidence that not more than 20% of red cells are going to disappear, rendering the prediction more precise than if the burn area alone were used. This added precision is of particular value in less than 30% full-thickness skin loss burns.

MORE THAN 10% OF MICROCYTES were not found in less than 15% full-thickness skin loss burns (Table I). When present in larger burns they were in 12 out of 15 patients associated with a more than 20% disappearance of red cells (Table II). The amount of measured disappearance ranged from 21 to 46% of the total circulating red cell volume.

DISCUSSION

As reported elsewhere (Jackson, 1959; Topley *et al.*, 1961), the area of full-thickness skin loss, the presence of haemoglobinuria 12-48 hours after injury, the

amount of colloid transfused, and the microcytes in a blood film on admission are useful guides to predicting to the nearest 20% the likely disappearance of red cells during the first 24 hours after injury in more than 15% patients with full-thickness skin loss burns. All the results together are more precise than one result alone, and help to decide whether a ^{51}Cr red cell volume estimate is required to assess red cell loss more precisely. It is just in these cases, with more than 15% full-thickness skin loss, that mortality might be reduced during the shock stage by a nice appreciation of red cell and plasma losses, although in practice the mortality was not reduced over the period of this investigation (Bull, 1958). Such burns are a minority of the cases requiring colloid transfusion. The man-hours in the laboratory to count such films would be minimal. The main criticism of the method is the inherent inaccuracy of a technique based on differential counting in a selected area of a blood film.

The reason for finding an association between the blood film on admission and later disappearance of red cells is not clear. Although the possibility exists that a later haemolytic anaemia or haemorrhagic diathesis is triggered off by the initial heat effect on red cells and capillaries in the burn area, there are many other equally plausible explanations for this association.

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