S T U D I E S

IN

H A E M A T O L O G Y

with special reference to the

H A E M A T O L O G Y O F B U R N S

BY

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M. B., Ch. B., F. R. F. P. S. G., M. R. C. P. Ed.

- 1945 -

P R E F A C E.

From February 1942 until October 1943, an investigation was conducted into the various aspects of burns, at the Burns Unit of the Glasgow Royal Infirmary, under the auspices of the Medical Research Council. During this period a part-time grant was made to the Author who was responsible for the haematological investigations and for the general medical supervision of the patients.

A report on the results of this study of burns has been published by the Medical Research Council (Study of Burns and Scalds : M.R.C. Spec. Rep. Series No. 249. H.M. Stationery Office, Lond., 1944), and a preliminary account of the haematological investigations is contained therein.

The present work comprises chiefly a more detailed report on the changes occurring in the blood of burned patients, with special reference to haemoconcentration and burns shock, and to the anaemia of burns. These studies involved the use of technical procedures which themselves required investigation. The procedures concerned were the estimation of the haemoglobin concentration in the blood, and the quantitative estimation of the osmotic fragility of the red cells. Accordingly, the work to be described has been divided into Parts I and II. Part I is devoted

to a study of the technical procedures which were of particular importance in the assessment of changes which occur in the burned patient. Part II is devoted to certain aspects of the haematology of burns. A detailed table of contents precedes each Part.

Throughout this work the Author owed a great deal to his environment and associations, and it is with great pleasure that he records his gratitude to Professor J.W.S. Blacklock and the Staff of the Department of Pathology at the Royal Infirmary, Glasgow, where the laboratory work was carried out. Their enthusiasm and co-operation lightened the Author's routine duties and made possible the continuation of this work at a time when all had already accepted extra duties on behalf of those absent on Military Service.

Acknowledgement of thanks is due also to Professor J.A.G. Burton and to Mr. A.M. Clark in whose wards the patients were studied, to Dr. Leonard Colebrook for his encouragement and co-operation, to Dr. A.B. Anderson for information on the biochemical investigation of certain patients described in Part II, and to the Resident Surgeons, Sisters and Nurses whose help at all times was invaluable.

Alexander Brown. 1945.

S T U D I E S I N H A E M A T O L O G Y

P A R T I.

THE ESTIMATION OF HAEMOGLOBIN

AND

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CHAPTER I.

THE ESTIMATION OF HAEMOGLOBIN.

I. METHODS OF ESTIMATION OF HAEMOGLOBIN.

Haemoglobin was first estimated colorimetrically by Gowers in 1878. He diluted blood with water and compared it with a glycerine-gelatine standard coloured with picrocarmine to match normal blood diluted 1 in 100. The result was given as a percentage of this arbitrary normal.

Gowers's picrocarmine standard was unstable, and the colour was considered unsuited to visual comparison. Accordingly, Hoppe-Seyler (1892) who had been working with carboxyhaemoglobin, introduced the pigment for the estimation in a special double pipette. He found, however, that the usefulness of the method was limited by the poor keeping qualities of the carboxyhaemoglobin.

Haldane (1901), recognising that the instability of this pigment was in great part at least, due to unsuitable methods of preservation, substituted carboxy-haemoglobin for Gowers's picrocarmine in a sealed tube containing carbon monoxide. By this method Haldane provided a relatively stable standard, and at the same time the simple instrument for clinical haemoglobinometry

to which his name has since been attached. The value of this instrument was further enhanced by its standardization so that a reading of 100 per cent. was equivalent to a blood with an oxygen capacity of 18.5 volumes per cent., a value which was regarded as the average normal for healthy adult males (Haldane & Smith, 1900: Haldane, 1901).

In 1895 Sahli introduced acid haematin as the haemoglobin derivative for comparison, and as the standard, in an instrument of the Gowers type. It was a simple pigment to prepare and a satisfactory one for visual comparison.

Haessler and Newcomer (1916) employed eleven standards in dilutions from 10 to 110 per cent. with the Sahli instrument, but this added little or nothing to the accuracy of the result. Accurate colour comparison was greatly facilitated by use of the Duboscq colorimeter. Palmer (1918) employed this instrument with carboxy-haemoglobin as the pigment, but Cohen and Smith (1919) could not corroborate his views on the stability of his standard solution. They preferred acid haematin.

Acid haematin in concentrated form keeps relatively well, but the pigment is unsuited for colorimetric estimation of haemoglobin. The shade and intensity of the colour are not dependent entirely on the amount of haemoglobin present (Berczeller, 1918:

Wu, 1922). Full development of the colour may take an hour or more, and the time required may differ with different samples of blood (Meyer & Butterfield, 1914: Brown, 1942: Ponder, 1942: Ashford, 1943). Wu (1922) suggested that the variations in shade and intensity of the colour were due to factors influencing the state of dispersion of the acid haematin, which is in colloidal suspension rather than in true solution. He found that the difficulties could be overcome by alkalinization of the solutions prior to comparison. By Wu's modification of the method of Cohen and Smith, therefore, the advantages of acid haematin as a stable standard, and of alkaline haematin as a solution suitable for comparison, were combined. The alkaline haematin method has the additional advantage that it allows accurate estimation of total haemoglobin even in the presence of methaemoglobin (Wu, 1922: Clegg & King, 1942: King, Gilchrist & Delory, 1944).

Cyanmethaemoglobin, initially described by Kobert in 1891, was first employed in haemoglobinometry by Stadie (1920) in the study of methaemoglobinaemia. The use of this pigment has been further investigated and found to be particularly suited for research purposes (Drabkin & Austin, 1935: Austin & Drabkin, 1935: Evelyn & Malloy, 1938: Reeve, 1944: King, Gilchrist & Delory, 1944).

The use of photoelectric methods in colorimetry has obviated the need for permanent standards so difficult to obtain with haemoglobin derivatives, and accordingly many of the pigments previously discarded on this account have been reinvestigated. Reeve (1944) found that use of oxyhaemoglobin in weak alkali (0.1 per cent. ammonia) provided a convenient and satisfactory method which gave results in close approximation with oxygen capacity. The use of carboxyhaemoglobin has been shown to be satisfactory in the absence of abnormal pigments derived from haemoglobin (King, Gilchrist & Delory, 1944), but acid haematin was found to be unsuited for accurate work (Clegg & King, 1942). Pyridine haemochromogen (Rimington, 1942) was rejected on account of the objectionable nature of the reagents (Clegg & King, 1942), and on account of differences in the rate of colour development between blood and the haemin standard (King, Gilchrist & Delory, 1944). Cyanmethaemoglobin was first adopted for photoelectric use by Evelyn and Malloy (1938), and further experience showed that it was a particularly suitable pigment for the accurate estimation of total haemoglobin (Reeve, 1944: King, Gilchrist & Delory, 1944). The alkaline haematin methods (Wu, 1922: Clegg & King, 1942) were regarded as slightly less accurate, but preferable to carboxyhaemoglobin when abnormal pigments may be present (King, Gilchrist & Delory, 1944).

II. STANDARDS OF REFERENCE IN HAEMOGLOBINOMETRY.

The depth of colour of Gowers's picrocarmine standard was based on the clinical impression of normal haemoglobin. The results were expressed as a percentage of this arbitrary normal.

Haldane appreciated the wide range of haemoglobin concentrations occurring in normal individuals, and the variations occurring in the same individual at different times. He recommended (Haldane & Smith, 1900) that "haemoglobinometer standards be referred to ox blood of known oxygen capacity" since there is complete parallelism between the colouring power of the blood and oxygen capacity (Haldane & Smith, 1900: Haldane, 1901). On the basis of results obtained from twelve healthy men, his haemoglobin standard corresponded to a blood with an oxygen capacity of 18.5 volumes per cent.

Sahli (1907) regarded the use of low standards as undesirable, on the grounds that mild degrees of anaemia were frequently overlooked when values for haemoglobin from such cases were found to be little less than 100 per cent. Accordingly he replaced his original by a more concentrated standard equivalent to blood with an oxygen capacity of 23.2 volumes per cent.

The use of the Haldane and Sahli instruments

has continued to the present time, but in many instances the modern product has deviated from the original specifications. Many instruments of the Sahli type have been supplied with glass standards. Many have adopted lower values as "normal," and with increasing diversity of standards, and of types of comparison tubes, clinical haemoglobinometry has fallen into disrepute which is scarcely merited by the original methods. Numerous criticisms of the available apparatus led to an investigation on the calibration of the haemoglobinometers in use in the clinical laboratories and teaching departments at the Glasgow Royal Infirmary during 1942-1943.

CALIBRATION OF TWENTY HAEMOGLOBINOMETERS OF THE SAHLI TYPE.

In this investigation the object was to obtain the equivalent in grams per cent. of the 100 per cent. reading in each instrument.

Method of Calibration.

The haemoglobin percentage of a blood of known oxygen capacity was estimated in each instrument by the method recommended by the Standing Committee on Laboratory Methods (1944). Full development of the colour was ensured by allowing the undiluted haematin mixture to stand for forty minutes at 20°C. The pipettes used were accurate to within 0.5 per cent. (Certified by the National Physical Laboratory). Colour matching was performed in artificial light. The haemoglobin content of the blood

was calculated from its oxygen capacity by use of Hüfner's factor, and oxygen capacity itself was obtained photo-electrically in the manner described later (p. 26). From the values obtained by the haemoglobinometer and by the photoelectric colorimeter the haemoglobin equivalent of 100 per cent. was calculated for each Sahli instrument.

Results.

Each instrument was tested three times in the manner described, and the average gram-equivalent of 100 per cent. for each is shown in Table I.

TABLE I.

Calibration of Sahli Haemoglobinometers.

Instrument No.	Gm. equiv. of 100%.	Instrument No.	Gm. equiv. of 100%.
1	10.4	11	12.5
2	10.4	12	15.2
3	10.7	13	14.9
4	10.5	14	12.7
5	14.6	15	13.0
6	14.8	16	13.8
7	14.1	17	15.6
8	13.5	18	17.0
9	12.4	19	15.6
10	12.1	20	16.4

The reasons for the very low values obtained for the first four instruments are, that originally they were designed for a gram-equivalent of 12.4, and that in replacement of broken comparison tubes care was not taken to obtain tubes standard for the instrument. Substandard comparison tubes and faded standards were not the only

faults. Three colour standards supplied for use with Sahli instruments were found to be an unsatisfactory match for any commonly employed pigment in any dilution, in daylight or in artificial light. New instruments were found to have an error in the stated gram-equivalent amounting to more than 10 per cent.

DISCUSSION.

These widely divergent results in the use of the Sahli instrument are of great significance in practice. A normal blood containing 15 grams of haemoglobin per 100 ml. may give a reading from 88 to 144 per cent. depending on the instrument used; and an anaemic blood with a haemoglobin concentration of 11 grams per cent. may read 106 per cent. In the indiscriminate use of this series of instruments, the normal range of colour index must be regarded as from 0.88 to 1.44 at least.

Greater control has been exercised in the production of the Haldane apparatus, but comparison tubes of different calibre and faulty standards have from time to time complicated the use of this simple instrument. Even with apparently close supervision, errors have been introduced (Macfarlane, O'Brien, Douglas et al., 1944: King, Gilchrist & Mathieson, 1944).

Whatever the method of haemoglobinometry adopted, it is desirable that the result of the estimation be expressed in grams of haemoglobin per 100 ml. of blood.

But it is impossible to do this directly. Ever since the work of Haldane and Smith (1900), standards for haemoglobinometry have been based on determination of oxygen capacity. There are, however, no reliable data concerning the maximum volume of oxygen or carbon monoxide with which one gram of pure human haemoglobin will combine. Hüfner in 1894 estimated the carbon monoxide combining power of ox haemoglobin to be 1.34 ml. per gram., and it is customary to employ this factor to calculate the gram-equivalent of human blood of stated oxygen capacity. The exact oxygen combining power of pure human haemoglobin is still to be determined; technical difficulties have made it almost impossible to prepare the pure pigment.

Values for total haemoglobin in blood have been calculated from the iron content of washed red cells (Ponder, 1942: King, Gilchrist & Mathieson, 1944: Macfarlane, O'Brien, Douglas et al., 1944) on the assumption that the iron content of pure haemoglobin is a fixed and accurately known quantity. Again, however, precise knowledge is lacking; the exact iron content of pure human haemoglobin is still to be determined.

At the present time, therefore, there is no strictly accurate standard to which estimation of haemoglobin may be referred, but in practice, there is no reasonable objection to the use of Hüfner's factor or to the calculation of haemoglobin from the iron in washed

red cells, provided the standard of reference and the factor used are always stated.

III. THE PHOTOELECTRIC ESTIMATION OF HAEMOGLOBIN.

INTRODUCTION.

The estimation of the concentration of haemoglobin in the circulating blood is of the greatest importance, and it is an estimation in which there is a universal need for greater accuracy.

Simple haemoglobinometers of the Haldane-Gowers and Sahli types even if carefully constructed, are subject to considerable error due to personal difficulties in colour matching. In addition, there is seldom any attempt to record results obtained from such instruments in absolute values, so that no standard of reference is available to permit comparison of values obtained from two different instruments which record haemoglobin levels as percentages of an arbitrary normal. A further source of confusion is provided by the ever increasing diversity of colour standards for use with instruments of the Sahli type. It may be fairly said that clinical haemoglobinometry has deteriorated rather than progressed since its inception by Gowers and Haldane, and the lowest levels of accuracy are reached when not even an approximate idea of the haemoglobin concentration is obtained (p. 7).

Improved methods of colour comparison have led to a considerable increase in the accuracy of the results (Palmer, 1918: Cohen & Smith, 1919), but the personal factor is not eliminated in the use of the Duboscq instrument.

The introduction of photoelectric methods has provided a simple and rapid means for accurate estimation of haemoglobin. Suitable instruments are sensitive and stable, and capable of giving results which are highly reproducible under optimum conditions.

Two types of photoelectric colorimeter have been designed by the Author and they have proved well suited for accurate work over long periods of time. The first instrument, a simple single-cell absorptiometer, has given results with an error not exceeding 0.5 per cent., while the second, a more elaborate mains-operated instrument is subject to even less variation.

THE CONSTRUCTION OF A SIMPLE PHOTOELECTRIC ABSORPTIOMETER.

The first instrument designed and constructed by the Author is a single photocell colorimeter. It has produced over a period of three years, simply and rapidly, results readily reproducible and showing very close correlation with repeated estimations of oxygen capacity (p. 26).

The Components.

(a) One photocell of the selenium barrier layer type.

Fig. 1a.

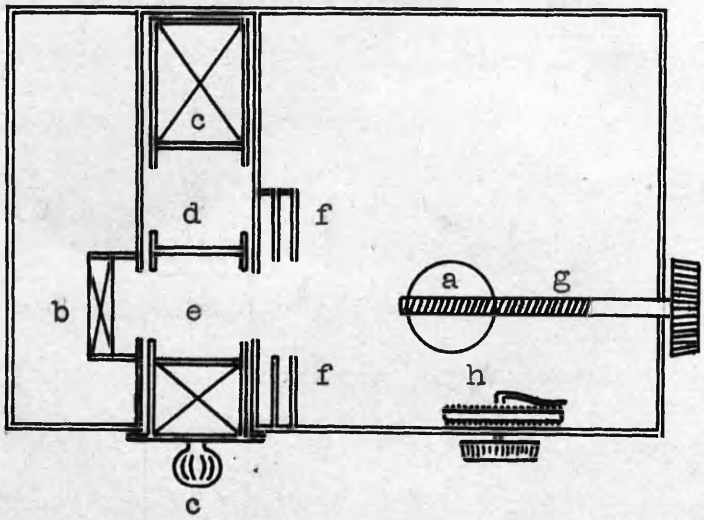
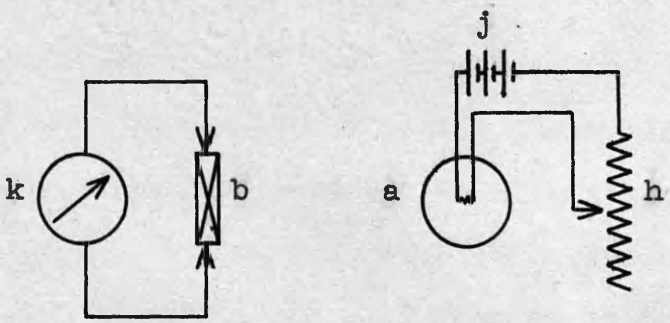


Fig. 1b.



a. lamp.
b. photocell.
c. carrier for optically faced cups.
d. position of cup holding water.
e. position of cup holding solution of pigment.
f. filter-holders.
g. screw adjustment controlling portion of the lamp.
h. variable resistance in lamp circuit.
j. 6 v. accumulator.
k. mirror galvanometer.

(b) One mirror galvanometer with lamp and scale. The instrument used has a coil resistance of 960 ohms, and gives a full-scale deflection with 2 micro-amps.

(c) One 5-ohm, 1-amp. variable resistance.

(d) Sufficient sheet and angle brass (16 gauge) and a short length of screwed brass rod (gauge, 2 B.A.).

(e) Two identical parallel-faced optical glass cells, 5 mm. optical depth and 6 cm. high.

(f) Colour filters (Chance-Watson green and blue-green for haemoglobin).

(g) Two 6-volt accumulators (to be used alternately).

The design of the instrument is shown schematically in Figure 1a and the circuit diagram in Figure 1b.

The Method of Use.

When the galvanometer zero has been adjusted, the light is switched on with the glass cell containing water in position between lamp and photocell. With the lamp in a standard position in the centre of its short travel, the intensity of the illuminant is adjusted so that the galvanometer reading is exactly 200 mm. (a full-scale deflection). This is done as accurately as possible, first by means of the series resistance in the light circuit, and then by means of the fine adjustment controlling the position of the lamp. The cup containing the unknown solution is then inserted between the lamp and the photocell,

replacing that containing water, and the new galvanometer reading (X) noted. The concentration of the pigment represented by X is obtained by interpolation in the graph prepared for the appropriate solution as described below.

Although the instrument is very simple both mechanically and electrically, only careful use will produce accurate results. It is essential to fix the position of the lamp within narrow limits and to use the fine adjustment only between these narrow limits, otherwise the current flowing through the lamp will have to be altered considerably to provide a full-scale deflection of 200 mm. Such an alteration would change the emission spectrum of the lamp to such an extent that the quality of the light reaching the photocell would be altered sufficiently to impair the accuracy of the results.

Recent work on the use of a constant voltage transformer has suggested that under favourable conditions, mains supply may be sufficiently stable for an instrument of this type (Morris, 1944). When, however, the electrical supply shows a voltage fluctuation amounting at times to 10 per cent., the only sufficiently stable source of current is the storage battery. If it is kept in good condition, an accumulator will maintain a relatively stable output during fourteen days' constant use. The freshly charged accumulator should be allowed to discharge slightly and to attain its stable output before a series of estimations is attempted.

THE CONSTRUCTION OF A MAINS-OPERATED PHOTOELECTRIC COLORIMETER.

In addition to the inconvenience associated with the use of accumulators, a single photocell instrument is extremely sensitive to the least imperfection in electrical contacts in the lamp circuit. Even a screw (S.E.S.) lamp-holder may at times be unsatisfactory, and a bayonet fitting is useless.

The instrument to be described was designed by the Author to overcome these minor objections. It incorporates many improvements, allowing greater flexibility in use, and provides a means for obtaining even greater accuracy.

The Components.

(a) Two photocells of the selenium barrier-layer type.

(b) One 8-ohm mirror galvanometer, with lamp and scale. The instrument used has a coil resistance of 8 ohms, and gives 120 mm. deflection with 1 micro-amp.

(c) One 12-volt, 1-amp. bulb, automobile head-lamp type, with S.B.C. or S.E.S. holder.

(d) One 12-volt, 50 watt transformer.

(e) Sufficient sheet and angle brass (16 gauge).

(f) Two identical optical glass cells each 5 mm. optical depth; two similar but of 10 mm. optical depth; and two 'micro' cells of 5 mm. optical depth but with outside width reduced - capacity 1.5 ml. All cups 6 cms. high.

Fig. 2a.

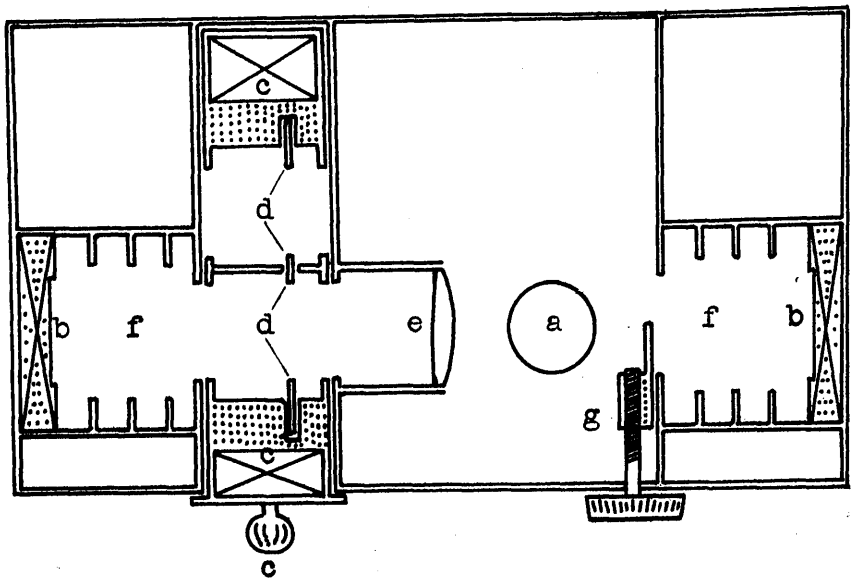
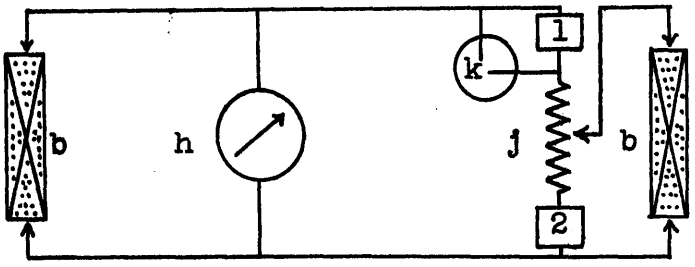


Fig. 2b.



a. lamp. 12 v. 12 w., supplied from A.C. mains through a transformer.
b. photocells.
c. carrier for optically faced cups with removable partition in position (d).
d. partition in position, removed to accommodate double width cup. (10 mm.).
e. condenser lens focussing lamp filament on ground glass in its holder (f) on carrier side of lamp.
f. holders for filters and ground glass.
g. screw-controlled sliding panel between lamp and photocell.
h. mirror galvanometer.
j. shunt resistance, variable portion with block 1 and 2 at each end.
k. mercury switch across block resistance j 1.

(g) Suitable colour filters in pairs: Chance-Watson green and blue-green, or the blue-green with Wratten No. 58.

(h) One potentiometer rheostat of about 8 ohms resistance, with one block resistance of about 10 ohms at each end, connected in series. The rheostat has a scale 4 inches in diameter, with 360 equal divisions. It is made from 1/4 inch ebonite rod screwed with B.A. die and bent when hot after winding with 26 gauge constantan wire.

(i) One mercury switch.

The design of the instrument is shown schematically in Figure 2a. and the circuit is that described by Brice (1937), modified to suit the purpose to which it has been adapted (Fig. 2b.).

The Method of Use.

The galvanometer is used as a null-point instrument. The scale reading is noted with the light switched off and no current flowing through the galvanometer. The light is then switched on, and with the appropriate filters in position, water in the optical cup, and the rheostat pointer at zero on the scale, the sliding shutter is so adjusted that no current flows through the galvanometer. The cup containing water is replaced by that containing the solution of the pigment to be estimated, and the rheostat is adjusted

until the circuit is again balanced and no current flows through the galvanometer. The concentration of the pigment represented by the reading on the rheostat scale is obtained by interpolation in the graph prepared for the appropriate pigment.

CALIBRATION OF THE COLORIMETERS FOR ESTIMATION OF HAEMOGLOBIN.

Before the instrument can be used to estimate the haemoglobin concentration in any sample of blood, a series of haemoglobin solutions of known concentration must be inserted and a graph prepared by plotting the values of X against the values of the various concentrations. The details of the methods employing oxyhaemoglobin, carboxy-haemoglobin, alkaline haematin, and cyanmethaemoglobin will now be described.

Oxyhaemoglobin.

About 10 ml. of blood is taken from an arm vein of a healthy adult male into a test-tube containing 1 mg. dry heparin as an anticoagulant. Gentle repeated inversion ensures that the mixture is homogeneous without the introduction of the minute bubbles of air which render accurate use of a pipette impossible. The specimen is then divided into two portions. The first is used at once for the estimation, in duplicate, of oxygen capacity (Peters & Van Slyke, 1932). The second portion is used to prepare, in 0.4 per cent. ammonia, suitable dilutions of blood equivalent to the range of haemoglobin it is

desired to estimate. With the filters in use (p.15), a dilution suitable for routine use in a cup of 5 mm. optical depth, is 1 in 101. Accordingly, the dilutions are prepared on the basis that the colour produced by the addition of 0.1 ml. of blood to 10 ml. weak ammonia is equivalent to the oxygen capacity (O) obtained from the first portion, or to O/1.34 grams per 100 ml. of blood (using Hüfner's factor). The solutions of oxyhaemoglobin so prepared are inserted into the colorimeter and the corresponding values of X obtained in the manner previously described for each instrument.

Carboxyhaemoglobin.

Calibration of the instruments for use with this pigment is carried out exactly as described for oxyhaemoglobin, with the exception that the oxyhaemoglobin is converted to carboxyhaemoglobin before the solutions are inserted into the colorimeter. This conversion is effected by passing coal-gas through the solutions by means of a fine silver catheter adapted for the purpose. The values of X corresponding to the various dilutions are then obtained.

Alkaline Haematin.

Dilutions of blood of known oxygen capacity are, in this case, made in decinormal hydrochloric acid. These dilutions are allowed to stand at room temperature for about 10 minutes, and then to 10 ml. of each is added 1.0 ml. ten per cent. sodium hydroxide. The solutions of

alkaline haematin are then ready for insertion into the instrument, and the values of X are obtained as described.

Cyanmethaemoglobin.

Into about 10 ml. 0.4 per cent. ammonia in a 25 ml. volumetric flask 0.1 ml. of blood is pipetted. To this 0.1 ml. 10 per cent. potassium ferricyanide is added. The mixture is allowed to stand at room temperature for 15-20 minutes and then 0.05 ml. 10 per cent. potassium cyanide is added. The mixture is made up to the 25 ml. with 0.4 per cent. ammonia and is ready for insertion into the colorimeter in a further 20 minutes. The blood chosen should have a haemoglobin concentration of about 17 gms. per cent. Aliquot portions of the 25 ml. solution already prepared made up in various dilutions with 0.4 per cent. ammonia will then give solutions of corresponding concentrations for purposes of calibration.

Calibration of the Instruments.

At least four different solutions distributed more or less evenly over the required range, should be prepared for each calibration, and a graph should be drawn from the results of at least two separate samples of blood, the oxygen capacity for each having been obtained in duplicate.

Colorimeter No. 1 :- With the instrument described, the values of X obtained by insertion of the various dilutions of the chosen blood pigment should form a straight line or

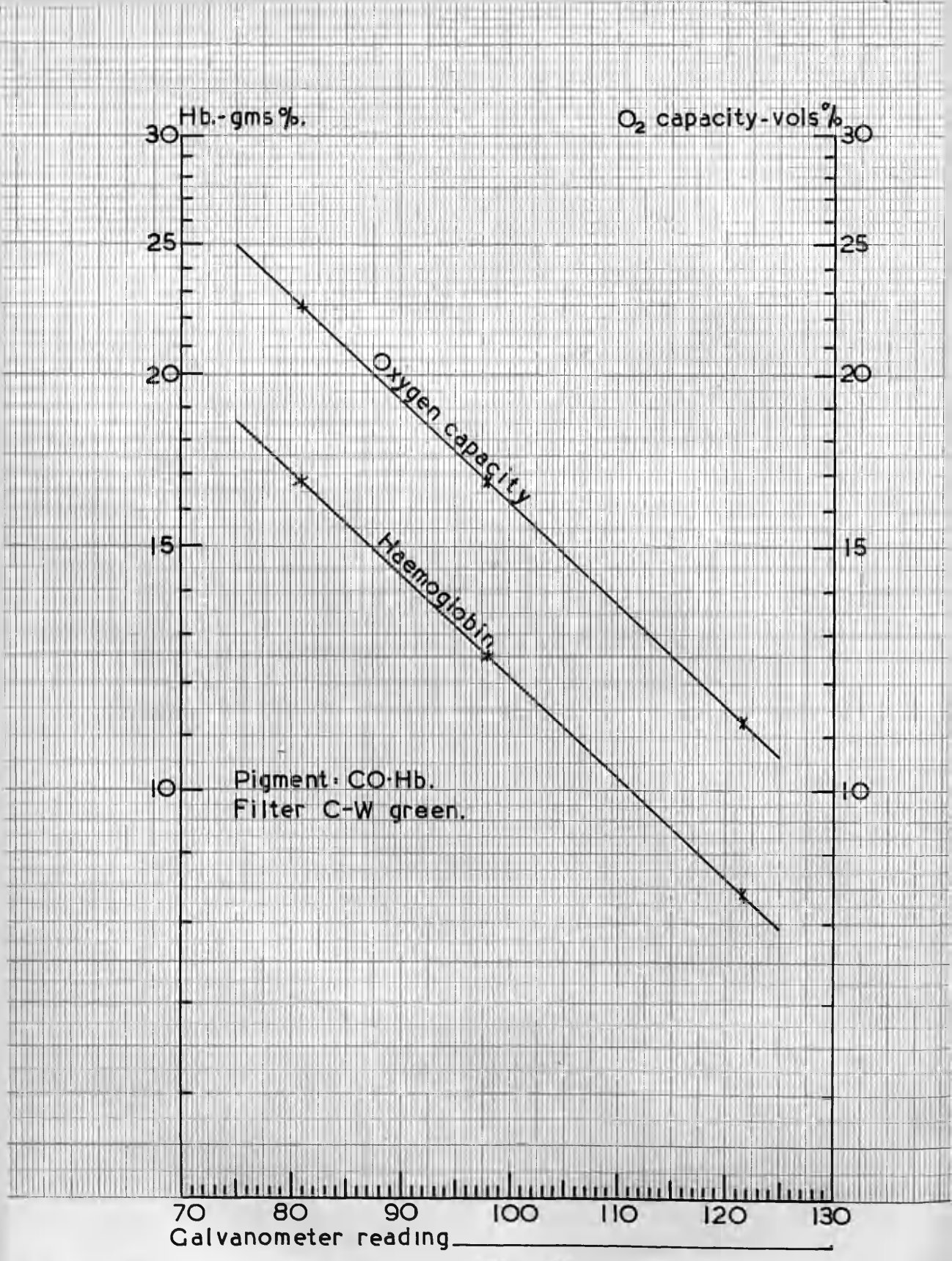

Hb.- gms %.
O_2 capacity - vols %
30
25
20
15
10
Oxygen capacity
Haemoglobin
Pigment - CO·Hb.
Filter C-W green.
70
80
90
100
110
120
130
Galvanometer reading

Fig. 3

or a very shallow curve when plotted against the logarithm of the concentrations. That this requirement is accurately met is seen from Figure 3. which, for convenience, has been drawn on semilogarithmic paper. Equally satisfactory graphs are obtained with all the pigments. The colour filter used must always be stated with each graph.

Colorimeter No. 2 :- With the circuit used in this instrument (Brice, 1937), the rheostat readings bear a direct relationship to the intensity of the colour and therefore to the strength of the solutions. When the scale readings for the various concentrations are plotted against the values for these concentrations, a straight line results (Fig. 4.). The values of the variable and of the two fixed resistances are so computed that a convenient range of values of light transmission is covered by the variable portion of the total resistance. Maximum use is thereby made of the 360 scale divisions which represent a range of about 12 grams haemoglobin per 100 ml. of blood.

Occasionally it is necessary to use solutions the light absorption of which is more than balanced by the first fixed resistance. Under such circumstances, the first block resistance is short-circuited by the mercury switch, and a new total resistance of about 18 ohms is now in use with the rheostat forming its first portion. When a graph is drawn for any pigment, it is therefore necessary to note the resistance used in its construction.

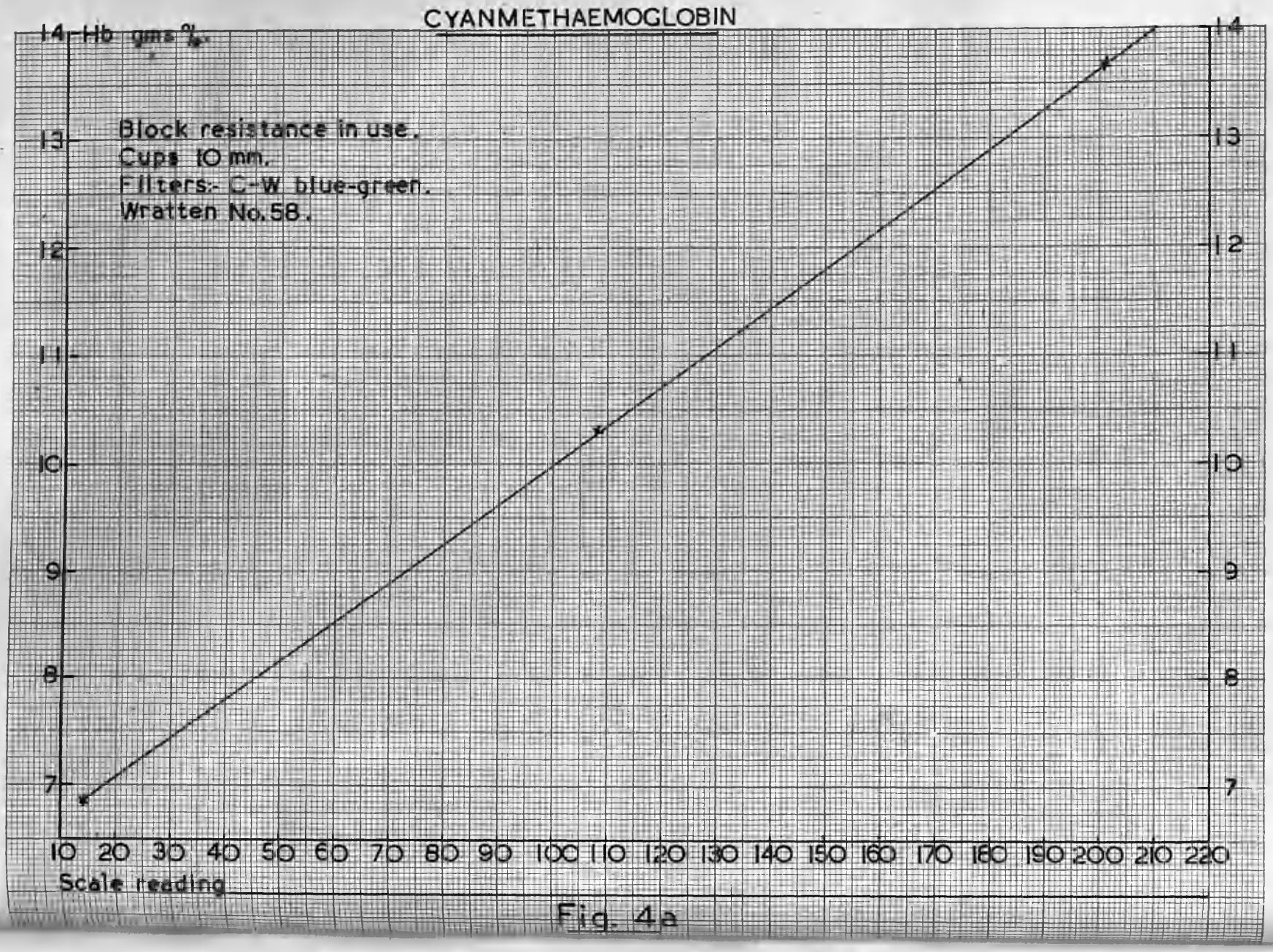

CYANMETHAEMOGLOBIN
Hb gms %.
Block resistance in use.
Cups 10 mm.
Filters:- C-W blue-green.
Wratten No. 58.
14
13
12
11
10
9
8
7
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220
Scale reading

Fig. 4a

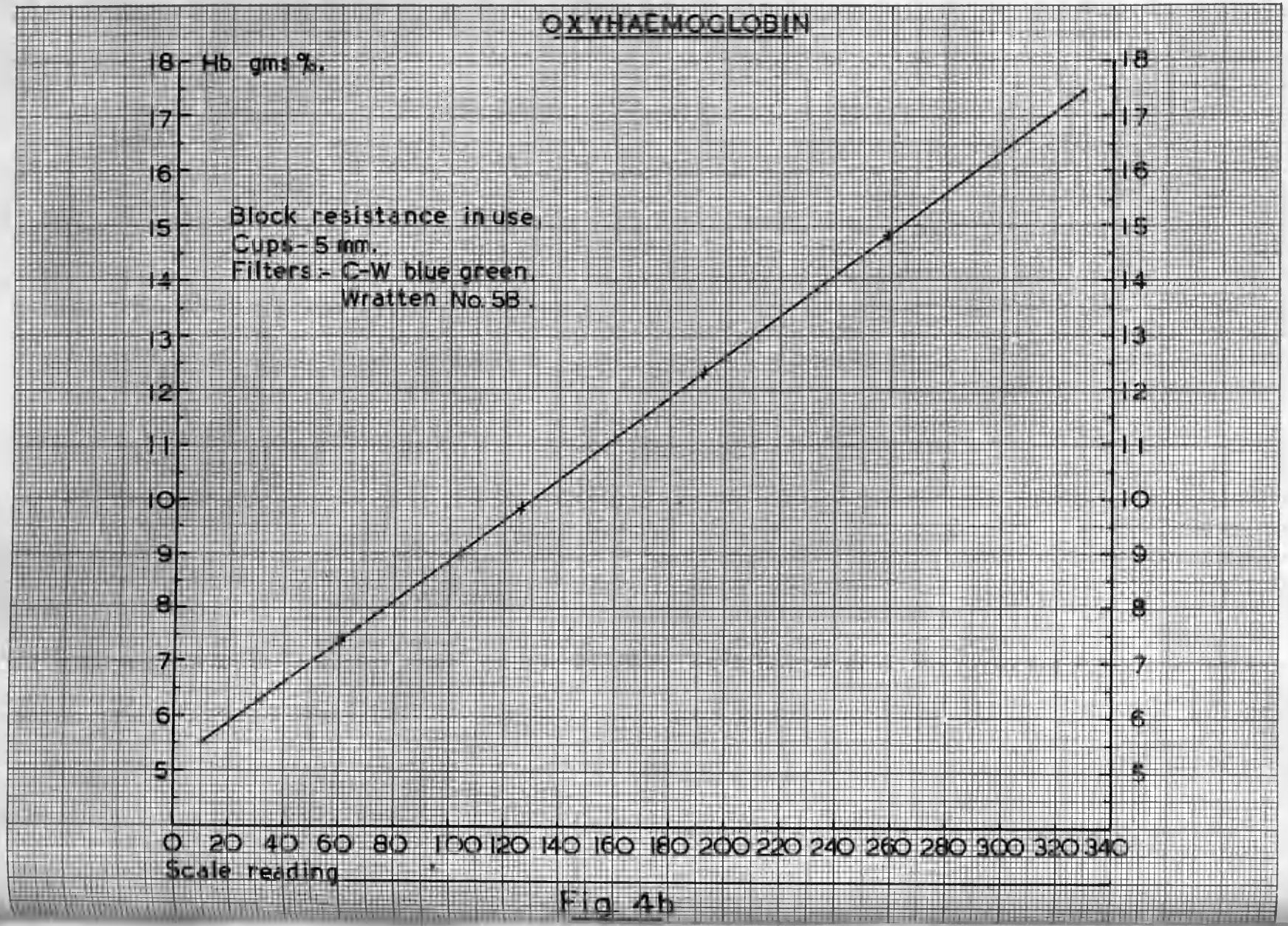

OXYHAEMOGLOBIN
Hb gms %.
Block resistance in use,
Cups - 5 mm.
Filters - C-W blue green
Wratten No. 58.
18
17
16
15
14
13
12
11
10
9
8
7
6
5
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340
Scale reading

Fig. 4b

For many purposes the standard optical cups with a depth of 5 mm. are most convenient, but in some estimations increased depth of solution is required and the 10 mm. cups must be used. In such circumstances the partition in the cell carrier is removed, and both 5 mm. cells are replaced by 10 mm. cells. In the construction of the graph it is therefore necessary to indicate the optical depth of the cells used.

Even the 10 mm. cells require only 4 ml. of solution, but when less is available, the 'micro' cells must be used. These cells have an optical depth of 5 mm., and the instrument is so designed that no adjustment is necessary when the standard 5 mm. cells are replaced by the 'micro' cells for any estimation, and no alteration in accuracy results.

Finally in the calibration of the instrument for any estimation, it is essential to record on the graph the filters used in its construction.

The graphs obtained in the calibration of Colorimeter No. 2 for the estimation of haemoglobin using oxyhaemoglobin and cyanmethaemoglobin are shown in Figure 4.

IV. THE PRACTICE OF HAEMOGLOBIN ESTIMATION. ON VENOUS BLOOD.

In obtaining a sample of venous blood, it is the custom to use, where possible, only a dry sterile

needle. The chance of producing haemolysis is thereby minimised. The blood is collected in a clean dry test-tube containing sufficient dry heparin to prevent coagulation (1 mg. for 10 ml. of blood). Rarely is a syringe necessary, and when one is used it is of the all-glass type, dry and paraffined. Venous stasis assists in the withdrawal of the blood, and under ordinary circumstances, has no effect on the haemoglobin level (p.34).

The Oxyhaemoglobin Method.

In a clean dry test-tube, 0.1 ml. of blood is added to 10 ml. 0.4 per cent. ammonia. The mixture is shaken, and the solution of oxyhaemoglobin is ready for estimation.

The Carboxyhaemoglobin Method.

The solution of oxyhaemoglobin prepared as described above, is gassed in the manner already noted (p.17). Frothing can be prevented by touching the surface of the liquid with a fine wire moistened with caprylic alcohol.

The Alkaline Haematin Method.

To 10 ml. decinormal hydrochloric acid in a dry clean test-tube, is added 0.1 ml. of blood. When the acid haematin suspension has been allowed to stand at room temperature for about 10 minutes, 1 ml. 10 per cent. sodium hydroxide is added. After thorough mixing, the solution of alkaline haematin is ready for estimation.

The Cyanmethaemoglobin Method.

The method is exactly that described in the preparation of the initial 25 ml. solution for purposes of calibration (p.18). In this estimation the 10 mm. cells are used because the dilutions are less than those employed in the other methods (1:250).

ON CAPILLARY BLOOD.

Capillary samples have seldom been used, except in children. In such cases, 0.05 ml. blood is added to the appropriate solvent. In the case of the alkaline haematin method, 0.5 ml. 10 per cent. sodium hydroxide is added before the estimation is completed. The cyanmet-haemoglobin method has not been used for capillary samples.

THE PIPETTES.

Accurately graduated pipettes have been used throughout the work. These used for blood and graduated to contain 0.1 and 0.05 ml. are 'Grade A' tested by the National Physical Laboratory. In use, they are carefully washed clean by the solvent into the solution after their contents have been discharged.

Only one 10 ml. pipette has been used; it delivers 9.998 ml. at 20°C. One 'Grade A' (N.P.L.) 5 ml. pipette has been used for estimations on capillary blood.

V. THE ACCURACY OF PHOTOELECTRIC METHODS.

THE STABILITY OF THE INSTRUMENTS.

Reproducibility of results of estimations performed in duplicate, and the comparison of colorimetric estimations of haemoglobin with estimations of oxygen capacity, are to some extent an indication of the accuracy of photoelectric methods. The former test, however, reveals only the accuracy of technique during the period over which each duplicate estimation is performed: the latter involves comparison with a method which has its own errors.

Neither test gives accurate information regarding the stability of the value of X in relation to light absorption, over the whole period of study. Errors in this respect may be introduced by changes in the lamp spectrum, in the filters, and in the photocells. The stability of response to a particular light transmission is most conveniently tested by the insertion, from time to time, of a suitable neutral screen. A Chance-Watson grey filter was used for this purpose. Tested in this way, Colorimeter No. 1 has shown a variation in response of about 0.5 per cent.; Colorimeter No. 2, a variation of about 0.2 per cent. In neither case does the variation affect significantly the results to be described.

REPRODUCIBILITY OF RESULTS.

In the majority of instances, haemoglobin

estimations have been performed in duplicate. The same 10 ml. pipette has been used throughout, but any of six 0.1 ml. pipettes has been used indiscriminately for measurement of the blood.

In 100 consecutive duplicate estimations using Colorimeter No. 1 and carboxyhaemoglobin as the pigment, the average difference was 0.0625 gm., and the maximum difference of 0.3 gm. occurred on only one occasion. A difference of 0.2 gm. occurred on four occasions.

Corresponding values in the use of Colorimeter No. 2 and oxyhaemoglobin as the pigment were 0.0620 gm. and 0.2 gm. The latter difference occurred on two occasions.

Any slight difference in the results with the two instruments can be explained on the basis of the slight differences in stability. They are not significant.

THE CHOICE OF A PIGMENT FOR HAEMOGLOBINOMETRY.

The ability of any one pigment to represent by its light absorption the concentration of haemoglobin present in any sample of blood depends on the degree to which its light absorption is affected under the conditions of the estimation, by the presence of other pigments in the sample. The most common haemoglobin derivative of significance in this respect is methaemoglobin, and when it is likely to occur, cyanmethaemoglobin is the pigment of choice for the estimation. Alkaline haematin, though

not quite as satisfactory, is to be preferred to oxyhaemoglobin or carboxyhaemoglobin (Wu, 1922: King, Gilchrist & Delory, 1944).

When abnormal haemoglobin derivatives are not present in significant amounts, little benefit is to be gained by the slightly more complicated method using cyanmethaemoglobin. For investigations under these circumstances, both oxyhaemoglobin (Reeve, 1944) and carboxyhaemoglobin (King, Gilchrist & Delory, 1944) have been found satisfactory.

Both oxyhaemoglobin and carboxyhaemoglobin are unstable and it is important to know how long solutions of these pigments may be kept before their light absorption undergoes significant change. It was found (Table II) that cyanmethaemoglobin remains unaltered for at least 24 hours at 20°C.; that oxyhaemoglobin is stable for this period if kept at 0°C.; and that carboxyhaemoglobin is unstable even in the cold.

TABLE II.

Spec. No.	Oxyhaemoglobin Change in 24 hrs.		Carboxyhaemoglobin Change in 24 hrs.		Cyanmethaemoglobin Change in 24 hrs.	
	0°C.	20°C.	0°C.	20°C.	0°C.	20°C.
1	0	+0.05	+0.05 +0.10*	+0.15 +0.20*	0	0
2	0	0	0 0*	0 0*	0	0
3	0	0	0 0*	-0.05 0*	0	0
4	0	+0.10	+0.10 +0.15*	+0.20 +0.20*	0	0

*effect of re-gassing.

VI. PHOTOELECTRIC METHODS AND OXYGEN CAPACITY.

INTRODUCTION.

Haldane was the first to suggest that haemoglobinometers should be standardised in terms of oxygen capacity, since he found complete parallelism between the latter and the depth of colour due to haemoglobin (Haldane & Smith, 1900). Although this statement appears to have been correct, it was scarcely justified on the published evidence. A small range of oxygen capacity had been investigated (14.68-22.02 volumes per cent. - Haldane & Smith, 1900: Haldane, 1901), and in the previous paper by these authors (1900), the parallelism alleged, was based on the examination of blood from several animals and one man. In the case of the man, the authors themselves regarded the oxygen capacity estimation as inaccurate.

In 1908 Morawitz and Röhmer compared the depth of colour of the blood with its oxygen capacity in various anaemic states, and in 1910 Douglas pursued a similar investigation during the period of regeneration after haemorrhage. Both confirmed Haldane's suggestion.

Since an accurate method for the estimation of haemoglobin was available, it was decided to investigate how closely colorimetric estimation of haemoglobin compared with the values for oxygen capacity obtained by the method of Van Slyke.

TABLE III.

Comparison of Photoelectric Estimation of Haemoglobin and Oxygen Capacity.

Specimen No.	Oxygen Capacity.	
	Photoelectric	Gasometric.
1	19.50	19.45
2	8.85	8.86
3	12.05	12.01
4	13.05	12.20
5	9.20	10.07
6	13.95	13.59
7	11.90	12.52
8	15.45	15.52
9	18.49	18.06
10	16.48	16.65
11	17.82	17.82
12	17.35	16.43
13	15.28	15.88
14	18.75	18.83
15	19.01	19.00
16	21.80	21.50
17	19.75	19.77
18	13.12	13.13

METHOD OF INVESTIGATION.

No attempt was made to limit the investigation to samples of normal blood, but specimens likely to contain abnormal pigment were excluded.

Venous blood was collected in the manner previously described (p. 20). The specimen was divided into two portions, one for the determination of oxygen capacity, and the other for the estimation of haemoglobin. Oxygen capacity was obtained by the method of Van Slyke (1932). Haemoglobin was estimated using carboxyhaemoglobin as the pigment in Colorimeter No. 1.

At first a pipette delivering 1 ml. was used

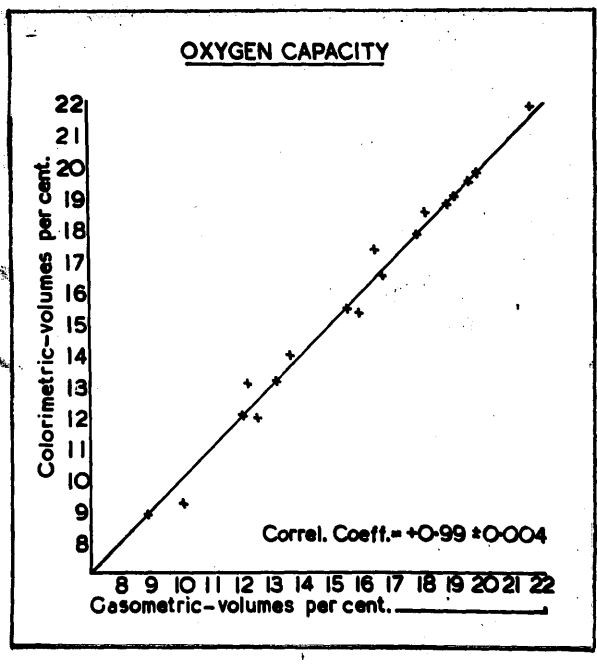

OXYGEN CAPACITY
Colorimetric-volumes per cent.
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
Correl. Coeff.= +0·99 ±0·004
8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Gasometric-volumes per cent.

Fig. 5

for the Van Slyke estimation, and one containing 0.1 ml. for the haemoglobinometry. Each was used by a different individual.* It was thought that greater accuracy and consistency might be obtained if one person pipetted the blood for both estimations, with as little time interval as possible, and using the same pipette. Accordingly, the 1:100 dilution of the blood for colorimetry was prepared by adding 1 ml. of blood (Van Slyke pipette) to 100 ml. 0.4 per cent. ammonia in a volumetric flask. The solution was mixed thoroughly and transferred to a larger vessel for gassing.

RESULTS.

The results, expressed in terms of oxygen capacity from the original graph (p.19), are compared with those obtained by the gasometric method (Table III) and the degree of correlation is shown in the accompanying Figure (Fig. 5).

There is no doubt that the results obtained in the use of the photoelectric instrument and those obtained for oxygen capacity are almost perfectly correlated. The numbers of abnormal bloods examined are too few to indicate the full range over which this degree of correlation exists.

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* I am very greatly indebted to Dr. A.B. Anderson, of the Biochemical Laboratory of the Department of Pathology at Glasgow Royal Infirmary, for all the determinations of oxygen capacity.

C H A P T E R II.

HAEMOGLOBIN LEVELS AND THEIR SIGNIFICANCE.

INTRODUCTION.

In Part II. of the present work, variations in haemoglobin are regarded as giving some indication of changes in the volume of the circulating plasma due to burns. Small changes have been found to occur in patients with minor injuries, and in order to assess these changes it has been found necessary to investigate the physiological variations in haemoglobin, and also some of the factors which may influence the results.

I. THE EFFECTS OF VENOUS STASIS ON BLOOD VALUES.

Criticism of the values obtained from venous blood, withdrawn under conditions of venous stasis, has been made on the grounds that obstruction of venous return alters the fluid balance between the capillaries and the tissue spaces, and causes loss of fluid from the vessels with concentration of the formed elements in the blood-stream.

In 1889, Cohnheim showed experimentally in animals, that venous obstruction in a limb increases the proportion of corpuscles in the blood of the affected part.

Local polycythaemia has resulted from progressive obstruction to the return of blood from the head and neck (Reckzeh, 1905). The local effect of vasomotor influences has been regarded as producing similar changes (Cohnstein & Zuntz, 1888: Chéron, 1895: Grawitz, 1895), but the evidence is not convincing as factors influencing the splenic reservoir were not considered.

The effects of venous congestion in a limb in the human subject have been investigated by Lewis (1927). He showed that if a pressure of 25-30 mm. Hg. be applied to the veins of the upper arm, the forearm swells rapidly. The change is not great, but it reaches a maximum in 15-20 seconds. If higher pressures are used, the swelling is almost complete in 30 seconds and thereafter continues to mount gradually. This second rise is due to loss of tone and distension of the vessels and also in small part to the formation of oedema - a very slow but continuous process (Lewis & Grant, 1926).

With the derangement of fluid balance associated with venous stasis, there is a rise in the protein content of the plasma in the affected area (Rowe, 1916), in the cell:plasma ratio (Peters, Eisenman & Bulger, 1925: Plass & Rourke, 1927) and in the oxygen capacity (Peters, Eisenman & Bulger, 1925). The results obtained by these authors are shown in the accompanying Tables (Tables IV. & V.). The values in Table V. have been calculated from the authors' data.

TABLE IV.

The Effect of Venous Stasis on Haemoglobin and Haematocrit (Peters, Eisenman & Bulger,1925).

Case No.	Condition of test	O_2 Capacity	P. C. V. †
1	Before tourniquet	18.61 vol. %	42.7 %
	After "	22.45 vol. %	51.2 %
2	Before "	18.42 vol. %	41.5 %
	After "	22.72 vol. %	52.1 %

The pressure applied was sufficient to "obstruct the venous return without completely obliterating the arterial pulse. Blood was withdrawn from the arm vein immediately before the tourniquet was applied and again after the tourniquet had been in place for about five minutes."

TABLE V.

The Effect of Venous Stasis on Haematocrit (Plass & Rourke, 1927).

Duration of Stasis.	Effect on P. C. V. †	Duration of Stasis.	Effect on P. C. V. †
5 minutes	+3.5%	20 minutes	+ 8.8%
8 minutes	+2.0%	20 minutes	+16.1%
12 minutes	-0.5%	21 minutes	+ 7.5%
15 minutes	+5.4%	22 minutes	+13.3%
15 minutes	+8.2%	26 minutes	+ 3.0%
16 minutes	+2.9%		

In this case the tourniquet pressure was between 80 and 100 mm. Hg.

If venous stasis is capable of causing haemo-concentration amounting to almost 20 per cent. in five minutes, significant errors may be introduced even if stasis is only of short duration. Such errors would be

† The abbreviation 'P. C. V.' is used in Tables and Figures to indicate the haematocrit reading or packed cell volume.

of great importance in the accurate assessment of minor changes in haemoglobin levels such as may occur in patients with mild burns. Accordingly, the effect of venous stasis of 90 seconds' duration on blood values has been investigated. This period of time was chosen because it represents a maximum allowance for routine withdrawal of blood by the experienced worker using the method previously described (p.20).

METHOD OF INVESTIGATION.

In the removal of samples of venous blood for various purposes, the opportunity of studying the effects of venous stasis was taken. Cases of peripheral vascular disease were excluded, but apart from this no selection was made.

A sphygmomanometer armlet was applied to the upper arm in such a manner as to leave venous return unobstructed. A dry sterile needle (No. II) was inserted into an antecubital vein and about 5 ml. of blood was withdrawn by means of a clean, dry, paraffined all-glass syringe, into a 'Pyrex' test-tube containing 0.5 mg. of dry heparin. The needle was kept in the vein, and bleeding was prevented by application of the thumb to the open end. The pressure within the cuff was raised to 60 mm. Hg., and maintained at this level for 90 seconds. At the end of this period blood was allowed to run from the needle until about 5 ml. had collected in a second prepared tube. The

pressure was maintained until the whole sample had been obtained; the period of time involved was usually about 30 seconds. Determination of haemoglobin, haematocrit, and in some cases, red cell levels, was then made on each sample.

Haemoglobin was estimated in the mains-operated colorimeter using oxyhaemoglobin as the pigment.

Determination of the packed cell volume was made using Wintrobe's haematocrit tubes (Wintrobe, 1933). They were spun at 3,000 revolutions per minute for 30 minutes.

Enumeration of red cells was performed in the same counting-chamber throughout. Suitable dilutions of blood in Hayem's solution were made using 10 ml. of the latter and adding 0.05 ml. of blood. The pipettes used were of guaranteed accuracy (N.P.L. Grade A.). This method ensures standard accuracy in preparing the dilutions, and greatly facilitates the preparation of homogeneous suspensions.

RESULTS.

The results of the investigation in 10 cases are shown in Table VI.

No significant alteration occurred in haemoglobin values, but in 8 of the 10 cases a slight rise in packed cell volume occurred. This rise cannot be regarded as within the error of the method which is accurate to 0.5 per cent. The four observations on the red cell levels reveal no significant change.

TABLE VI.

The Effect of Venous Stasis on Red Cell Values.

Case No.	Hb.		P. C. V.		R. B. C.	
	1	2	1	2	1	2
1	12.70	12.70	43.5	44.0	-	-
2	14.70	14.70	50.0	52.0	-	-
3	12.50	12.45	41.0	43.0	-	-
4	15.60	15.50	54.0	55.5	-	-
5	10.25	10.15	35.0	35.5	-	-
6	13.75	13.65	47.5	48.0	-	-
7	10.10	10.05	36.5	36.5	3.52	3.52
8	15.35	15.35	49.5	51.0	5.07	5.11
9	12.00	11.95	41.5	43.0	4.71	4.67
10	14.45	14.40	46.0	46.0	4.53	4.61

Column 1. = values before application of tourniquet.
Column 2. = values after application of tourniquet.

DISCUSSION.

No references to observations on the effect of venous stasis of short duration have been discovered. In the cases described by Peters, Eisenman and Bulger (1925), marked changes were produced by stasis lasting five minutes. The authors give no indication of the accuracy of their methods. The minimum duration of the congestion in the investigation of Plass and Rourke (1927) was five minutes, and sequelae, both subjective and objective, were noted in the majority of cases. Discomfort, pain, and even tetanic spasm were seen, and cyanosis and occasionally purpura occurred. In such cases the stasis was greatly in excess of that usually employed in withdrawing blood from a vein. The magnitude of the changes might be explained on this basis alone. In addition, the methods used were not

conducive to accurate results, and this may explain also the disproportionate effects recorded.

It is not surprising that stasis lasting only 90 seconds has not been found to cause a significant variation in haemoglobin concentration. Although such stasis induces volume changes in the affected part almost at once (Lewis, 1927), these changes are due chiefly to distension of the vessels; the escape of fluid is a slow process.

Alterations in the packed cell volume are not comparable with changes in haemoglobin. Changes not only in the numbers of the red cells, but also in their size, may alter the cell mass. The haemoglobin results indicate that the number of the cells remains constant. The slight increase in the packed cell volume may be due to an increase in the mean corpuscular volume. Such a change may be due to local acidosis such as may occur with prolonged venous congestion (Whitby & Britton, 1937a).

CONCLUSIONS.

There is no evidence that the application of a tourniquet at a pressure of 60 mm. Hg. for 90 seconds, prior to the withdrawal of blood, significantly affects haemoglobin values. Slight changes in the haematocrit may occur due to alterations in the corpuscular volume induced by local acidosis.

II. NORMAL VARIATIONS IN HAEMOGLOBIN.

INTRODUCTION.

In attempting to assess the significance of changes in haemoglobin concentration following burns, it is important to know how much variation may occur in normal individuals during the day, and from one day to the next. The evidence at present available is more confusing than helpful.

Dreyer, Bazett and Pierce (1920) recorded the haemoglobin levels of normal adults at intervals during the day, and found a maximum variation of 30 per cent. They regarded changes of 10 per cent. as common. Rabinovitch (1923) noted a diurnal variation of 26 per cent. in two cases, 15-20 per cent. in four, and 10-15 per cent. in six. Short (1935) reported a possible variation of 17 per cent.

Considerably less diurnal variation in haemoglobin levels was found by McCarthy and Van Slyke (1939) and McCarthy (1943). Using the method of Van Slyke and Hiller (1928) for the estimation of carbon monoxide combining capacity, they showed in 18 cases that the average range between the highest and the lowest values was 1.3 volumes per cent. The greatest observed range was 2.3 volumes per cent., which was equivalent to 11 per

cent. of the mean haemoglobin for the day. No uniform direction of change was encountered, and dissimilar trends were seen in the same individual on different days.

It is evident from this published work that haemoglobin levels vary significantly during the day, but there is little agreement on the magnitude of the variation which is to be regarded as physiological.

METHODS OF INVESTIGATION.

The subjects chosen for the investigation comprised (a), healthy senior medical students and resident medical officers, who were pursuing their usual routine during the period of observation, and (b), a series of adults confined to bed. In the latter group, the patients were either awaiting operation for an anatomical defect, such as a hernia, or suffering from some minor complaint not associated with any disturbance of fluid balance. Details relevant to these cases are recorded with the results of the investigation.

Investigations on the day-to-day variations in haemoglobin were made on both groups, and samples of venous blood were withdrawn at 11.00-11.15 a.m. daily for 4-5 days.

Investigations on diurnal variations in haemoglobin were made only in the patients confined to bed. Samples of blood were obtained at intervals of 3 hours from 9 a.m. until 6 p.m.

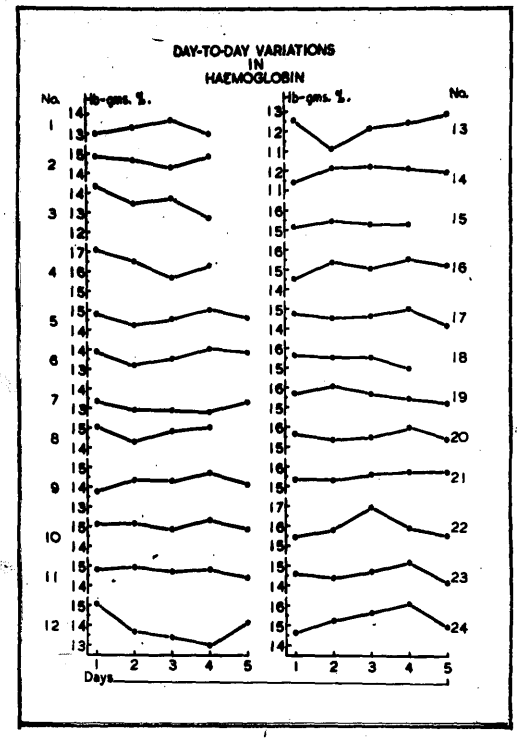

DAY-TO-DAY VARIATIONS
IN
HAEMOGLOBIN
No.
Hb-gms. %.
Days
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

Fig. 6

The blood samples were obtained by the method previously described (p. 20), without the use of a syringe. Undue stasis was avoided. The estimations were performed in duplicate within 2 hours of withdrawal of the blood, using oxyhaemoglobin as the pigment in Colorimeter No. 2.

TABLE VII.

Variations in Haemoglobin.

Case No.	Daily Haemoglobin Values.					Remarks.
	1	2	3	4	5	
1	13.00	13.30	13.65	12.95	-	Healthy adult male.
2	14.85	14.65	14.25	14.80	-	Healthy adult male.
3	14.35	13.45	13.70	12.65	-	Healthy woman: menstruating.
4	17.10	16.50	15.65	16.25	-	Healthy adult male.
5	14.80	14.25	14.55	15.00	14.60	Healthy adult male.
6	13.90	13.20	13.50	14.00	13.80	Healthy adult male.
7	13.35	12.90	12.85	12.80	13.30	Healthy adult male.
8	15.05	14.30	14.80	15.00	-	Healthy adult male.
9	13.75	14.35	14.30	14.70	14.10	Healthy adult male.
10	15.10	15.15	14.85	15.30	14.85	Healthy adult male.
11	14.80	14.95	14.70	14.80	14.40	Healthy adult male.
12	15.05	13.65	13.40	13.00	14.15	Healthy adult male.
13	12.05	11.10	12.10	12.35	12.70	Dyspepsia.
14	11.40	12.10	12.15	12.00	11.80	Chronic arthritis.
15	15.15	15.45	15.25	15.20	-	Hyperacidity.
16	14.50	15.35	15.00	15.45	15.10	Hyperacidity.
17	14.75	14.50	14.60	14.95	14.05	Lipoma.
18	15.65	15.50	15.50	14.90	-	Hernia.
19	15.70	16.05	15.65	15.40	15.15	Dyspepsia.
20	15.65	15.35	15.45	15.95	15.35	Old injury.
21	15.35	15.30	15.60	15.70	15.70	Arthritis.
22	15.45	15.80	16.95	15.90	15.50	Hyperacidity.
23	14.60	14.40	14.70	15.15	14.15	Dyspepsia.
24	14.65	15.25	15.65	16.10	14.95	Hernia.

RESULTS.

The results of the examination of the blood of 24 individuals, at the same time daily for 4-5 days, are shown in Table VII and graphically in Figure 6. An analysis of these results is presented in Table VIII. The maximum

variation recorded in any case over the entire period of the investigation was 2.05 gms. per 100 ml., equivalent to 14.7 per cent. of the mean haemoglobin for the period. The average maximum variation was about half the greatest recorded, and equal to 6.5 per cent. of the average mean haemoglobin.

TABLE VIII.

Variations in Haemoglobin.

Case No.	Variations in 4-5 day period.			Maximum change in 24 hours.		
	Mean Hb.	Max. var.	Max. % of mean	Max. var.	Mean Hb.	Max. % of mean
1	13.23	0.70	5.30	0.70	13.30	5.26
2	14.64	0.60	4.10	0.55	14.53	3.78
3	13.54	1.70	12.55	1.05	13.18	7.96
4	16.38	1.45	8.85	0.85	16.08	5.28
5	14.64	0.75	5.12	0.55	14.53	3.78
6	13.68	0.80	5.85	0.70	13.55	5.16
7	13.04	0.55	4.21	0.50	13.05	3.83
8	14.79	0.75	5.07	0.75	14.68	5.11
9	14.24	0.95	6.67	0.60	14.05	4.27
10	15.05	0.45	2.99	0.45	15.08	2.98
11	14.73	0.55	3.73	0.40	14.60	2.74
12	13.85	2.05	14.80	1.40	14.53	9.75
13	12.06	1.60	13.28	1.00	11.60	8.40
14	11.89	0.75	6.30	0.70	11.75	5.96
15	15.26	0.30	1.97	0.30	15.30	1.96
16	15.08	0.95	6.30	0.85	14.93	5.68
17	14.57	0.90	6.18	0.90	14.50	6.20
18	15.39	0.75	4.87	0.60	15.20	3.95
19	15.59	0.90	5.78	0.40	15.85	2.52
20	15.55	0.60	3.86	0.60	15.65	3.83
21	15.53	0.40	2.57	0.30	15.45	1.94
22	15.92	1.50	9.42	1.15	16.48	6.97
23	14.60	1.00	6.85	1.00	14.65	6.33
24	15.32	1.45	9.45	1.15	15.53	7.40
Average	-	0.93	6.50	0.73	-	5.46

The variation in any period of 24 hours was less marked (Table VIII). This may have been due to a

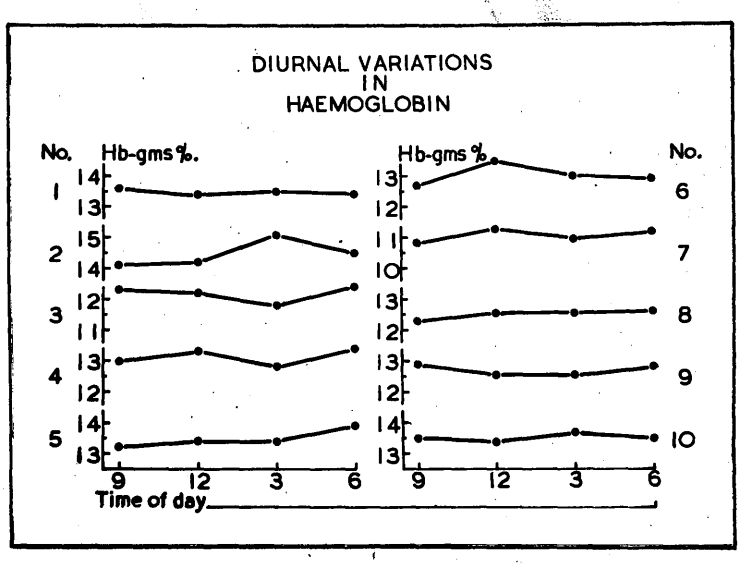

DIURNAL VARIATIONS
IN
HAEMOGLOBIN
No.
Hb-gms%.
1
2
3
4
5
Hb-gms%
No.
6
7
8
9
10
9
12
3
6
Time of day

Fig. 7

progressive change, such as loss of blood, in some of the patients, but there was no evidence of this except in No. 3 in whom menstruation began on the first day of the test.

The 10 individuals in whom diurnal changes were investigated formed a separate series, and the results and relevant details are shown in Table IX and Figure 7. The maximum variation was 1.00 gms. per 100 ml., equivalent to 6.9 per cent. of the mean: the average maximum was 4.12 per cent. of the mean (Table X). There was no uniformity in the variations shown by the different patients, and it was impossible to predict a period during which the greatest changes might be expected to occur.

TABLE IX.

Diurnal Variations in Haemoglobin.

Case No.	3-Hourly Haemoglobin Values				Remarks.
	9 a.m.	12 m.d.	3.p.m.	6 p.m.	
1	13.60	13.40	13.50	13.45	Peptic ulcer.
2	14.10	14.20	15.10	14.50	Peptic ulcer.
3	12.30	12.20	11.80	12.40	Anal stricture.
4	12.95	13.30	12.75	13.40	Hernia.
5	13.20	13.40	13.40	13.90	Cyst of toe.
6	12.70	13.50	13.00	12.90	Hernia.
7	10.83	11.30	10.95	11.20	Peptic ulcer.
8	12.30	12.55	12.55	12.60	Painful scar.
9	12.90	12.55	12.55	12.85	Old injury.
10	13.50	13.40	13.70	13.50	Chronic cholecystitis.

TABLE X.

Diurnal Variations in Haemoglobin.

Case No.	Variations from 9 a.m. to 6 p.m.			Maximum change in 3 hours.		
	Mean Hb.	Max. var.	Max. % of mean	Max. var.	Mean Hb.	Max. % of mean
1	13.49	0.20	1.49	0.20	13.50	1.48
2	14.48	1.00	6.90	0.90	14.65	6.14
3	12.18	0.60	4.92	0.60	12.10	4.95
4	13.10	0.65	4.95	0.65	13.08	4.97
5	13.48	0.70	5.20	0.50	13.65	3.66
6	13.03	0.80	6.13	0.80	13.10	6.11
7	11.07	0.47	4.25	0.47	11.07	4.25
8	12.50	0.30	2.40	0.25	12.43	2.05
9	12.71	0.35	2.75	0.35	12.73	2.75
10	13.53	0.30	2.22	0.30	13.55	2.22
Average	-	0.54	4.12	0.50	-	3.86

From consideration of the results in both series of cases it is evident that the more frequently estimations were made in a given period, the greater was the variation which was revealed. The average maximum change in haemoglobin levels at an interval of 9 hours (calculated from Table IX) was 2.07 per cent. of the mean, while the average change recorded in 3-hourly estimations over the same period was 4.12 per cent. of the mean. When, however, four estimations were made over a period of 72 hours the average maximum change revealed was 5.67 per cent. of the mean (calculated from Table VII). Thus both the number of estimations and also the length of the period over which they are made, influence the magnitude of the variations recorded - at least within a 4-5 day period.

DISCUSSION.

These results, obtained by the careful use of

accurate photoelectric methods, are not in agreement with many of the results obtained in the past by other methods. Only one report on diurnal variations agrees with the findings already presented. In 1939, McCarthy and Van Slyke described the diurnal variations in haemoglobin in 18 healthy adults. The carbon monoxide capacity method of Van Slyke and Hiller (1928) was used, and estimations performed in duplicate were in very close agreement. The results indicated an average maximum diurnal variation of about 6.3 per cent., and a maximum of about 11 per cent., of the mean level. The longer the period of the survey (9 a.m. until 12 midnight) and the more frequent observations probably explain the magnitude of the variations which are slightly greater than those recorded in the present series.

The greater diurnal variations recorded by Dreyer, Bazett and Pierce (1920), have not been confirmed. These authors noted a maximum variation of 30 per cent., and stated that changes of 10 per cent. were more or less common. The reason for these high values is due, in part at least, to the method used in the estimation of haemoglobin. Samples of capillary blood without anti-coagulant were allowed to collect in a paraffined watch-glass from which 0.1 ml. was removed and added to 19.9 ml. of physiological saline. Lysis was produced by addition of a roughly measured amount of saponin which was allowed

to act at 30°C. for a few minutes. Comparison was then carried out in a Duboscq colorimeter.

The method of sampling seems unsatisfactory, though the authors claim close agreement in duplicate estimations. No statement is made regarding the accuracy of the improvised pipettes. The method of producing lysis is unduly complicated and introduces a small but unknown variable - the saponin. Finally, visual colorimetry is less accurate than the method used in the present investigation, and also less accurate than the method of McCarthy and Van Slyke (1939).

Rabinovitch (1923) reported variations as great as 26 per cent. on the basis of determinations of oxygen capacity. The method used has been regarded as unsatisfactory and inaccurate (McCarthy and Van Slyke, 1939), and no duplicate estimations are recorded. The results, therefore, cannot be regarded as an accurate index of normal diurnal variations.

Short (1935) reported a maximum range of 17 per cent. Here also the methods are open to criticism. Duplicate counts of red cells and duplicate estimations of haemoglobin differed by as much as 195,000 - 540,000 per c.mm. and 0.35 - 0.80 gms. per 100 ml. respectively. The method of haemoglobin estimation is not mentioned.

McCarthy and Van Slyke (1939) have criticised colorimetric estimations of haemoglobin on the grounds that

variable factors in the plasma might alter light absorption and so introduce errors. In the absence of marked lipaemia, the variable but weak pigmentation of normal plasma in a dilution of 1:202 (approximately) does not produce a significant error. Even if it were to do so, the apparent diurnal variation would be increased in comparison with the results of these authors, not diminished as in the present series.

SUMMARY AND CONCLUSIONS.

In patients without evidence of active disease and confined to bed, the average maximum diurnal variation in haemoglobin (from values obtained from venous blood at intervals of 3 hours, between 9 a.m. and 6 p.m.) was 0.54 gms. per 100 ml. This is equivalent to 4.12 per cent. of the mean haemoglobin for the period.

In 24 adults of whom 11 were ambulant and 13 confined to bed, the average maximum difference in haemoglobin estimations performed at intervals of 24 hours was 0.73 gms. per 100 ml., equivalent to 5.04 per cent. of the mean. In the same series, daily estimation of haemoglobin for 4-5 days showed an average maximum variation of 0.93 gms. per 100 ml., or 6.33 per cent. of the mean value for the period.

Slightly greater diurnal and day-to-day variations may be recorded if observations are made more frequently, and if the period is extended.

The maximum recorded changes in haemoglobin in the various periods of observation were as follows:-

Maximum diurnal variation ..... 1.00 gm. = 6.90% of mean.

Maximum variation in 24 hours .... 1.40 gm. = 9.75% of mean.

Maximum variation in 72-96 hours . 2.05 gm. =14.70% of mean.

III. THE SPLENIC RESERVOIR AND THE EFFECT OF ADRENALINE ON HAEMOGLOBIN CONCENTRATION.

INTRODUCTION.

Knowledge of the function of the spleen as a blood reservoir has been derived chiefly from the work of Barcroft and his associates. They observed (1922) that changes in haemoglobin concentration occurred in relation to changes in environmental temperature and atmospheric pressure. In some instances, this variation occurred too rapidly to be explained on the basis of production or destruction of red cells, and it was concluded that they were due to release of cells from stores within the body. The spleen was suspected of being the reservoir, and subsequent experiments proved that this was so.

The sequestration of blood within the spleen was demonstrated by exposing rats to an atmosphere containing 0.06-0.1 per cent. of carbon monoxide (Barcroft & Barcroft, 1923). The gas was rapidly absorbed into the circulating blood, but its appearance in the spleen was

much delayed. A similar delay, amounting at times to 90 minutes, was observed in the disappearance of the gas from splenic blood when the animal was restored to a pure atmosphere.

In acting as a blood reservoir, the spleen exercises a real function in adjusting the circulating functional haemoglobin according to the needs of the animal. In 1925 it was shown (Barcroft, Harris, Orahovats & Weiss) that after death, which in most cases was preceded by general anoxaemia, the spleen was much smaller than it had been in the normal living animal. In death due to haemorrhage the spleen was reduced to a fifth of its former normal size. Less severe haemorrhage, (8 per cent. of blood volume in the cat) induced splenic contraction which contributed to the circulation an amount of blood approximately equal to that lost. Exposure to carbon monoxide caused splenic contraction, and if the animal were deprived of this source of additional haemoglobin by splenectomy, its ability to withstand exposure to the gas was significantly reduced (Barcroft, Murray, Orahovats, Sands & Weiss, 1925). Exposure to undue concentrations of carbon dioxide caused splenic contraction (Hargis & Mann, 1925). Exercise (Barcroft, Harris, Orahovats & Weiss, 1925: Barcroft & Stephens, 1927) and excitement (Hargis & Mann, 1925: Izquierdo & Cannon, 1928) cause splenic contraction. Both exercise and excitement have been shown to cause

appreciable haemoconcentration in the peripheral blood (Ferrari, 1897: Barcroft, Harris, Orahovats & Weiss, 1925: Izquierdo & Cannon, 1928: Short, 1935: Kaltreider & Meneely, 1940).

The reservoir function of the spleen has been investigated in relation to the action of drugs of different types. Ether causes contraction of the spleen and haemoconcentration (Hausner, Essex & Mann, 1938: Searles, 1939: Jascho, 1943). Sodium amytal causes splenic enlargement and peripheral haemodilution (Hausner, Essex & Mann, 1938: Searles, 1939). Pentobarbital causes enlargement and haemodilution (Hausner, Essex & Mann, 1938: Green, Nickerson, Lewis & Brofman, 1943: Hann, Bale & Bonner, 1943: Jarcho, 1943). Experiments with the sodium salt (Nembutal) and radioactive iron have shown that up to 30 per cent. of the circulating red cells may become segregated in the enlarged spleen which may weigh, when removed, about four times as much as the organ of the same animal (the dog) removed under ether (Hann, Bale & Bonner, 1943). Pentothal has an effect similar to that of pentobarbital (Hausner, Essex & Mann, 1938).

Sympathomimetic drugs have a common effect in producing contraction of the spleen, and haemoconcentration has also occurred following their administration. Adrenaline itself has an effect on the spleen apparently out of proportion to its influence on blood pressure or renal

volume (Hoskins & Gunning, 1917). Hargis and Mann (1925) also found that the threshold for contraction was lower than that for changes in arterial blood pressure. The reduction of the size of the spleen is not confined to the normal organ. It has been observed to occur following the administration of adrenaline in a patient with hepatic cirrhosis and splenomegaly (Ravenna, 1940). Injection of adrenaline into the splenic artery prior to splenectomy for various conditions, was found to cause contraction of the spleen, and a great increase in the red cell concentration in the blood of the splenic vein (Watson & Paine, 1943). The latter finding suggests the discharge of red cells previously in a more concentrated form in the splenic pulp than obtains in the peripheral circulation. This is supported by the observations of Millgrew (1939) who found the values for packed cell volume in the splenic reservoir blood and in the blood from the systemic veins to be 64 and 41-44 per cent. respectively.

Both ephedrine (Davis, 1937) and benzedrine (Pinkston & Pinkston, 1939) have an action similar to that of adrenaline.

These investigations on splenic function and its relation to alterations in haemoglobin levels due to excitement, exercise, and anaesthetics have shown very clearly that in animals at least, very significant haemoconcentration or haemodilution may be produced by

alterations in the volume of the spleen. This is of great importance in considering experimental work on burns. Though the animal may be kept at rest, the possible effects of excitement or of the anaesthetic used, have frequently been ignored, with the result that, in attributing the observed changes in haemoglobin solely to the burn, considerable confusion has occurred.

The possibility of a similar relationship must be considered also in the clinical study of burns. Investigations on the changes in the blood and in the blood pressure to be described later have shown that, even with minor injuries, the blood pressure may be raised and at the same time slight haemoconcentration may occur. It is thought that these findings may be related, and that both may be due to excitement.

In the course of investigations on the glycogenolytic action of adrenaline as a test of liver function, advantage was taken of the opportunity to observe the effect of the drug on haemoglobin levels. Adrenaline plays a prominent part in the production of the excitement syndrome, and it was thought that some idea would be gained as to the magnitude of the resulting changes in man, and as to the possible relation between the alterations in blood and blood pressure already mentioned.

The nature of the primary investigation explains the relative frequence of liver disease among the patients

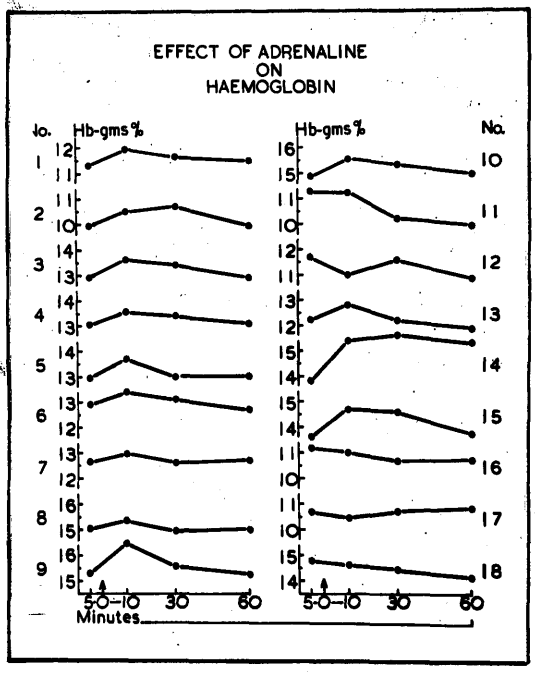

EFFECT OF ADRENALINE
ON
HAEMOGLOBIN
No.
Hb-gms%
Hb-gms%
No.
Minutes
5-0-10
30
60

Fig. 8

selected. Several relatively normal individuals have also been included.

METHOD OF INVESTIGATION.

The patients were confined to bed on the day of the test, which began at about 11.15 a.m. and ended before the mid-day meal. Samples of venous blood were obtained 5 minutes before, and 10, 30, and 60 minutes after, the subcutaneous injection of 1 ml. of 0.1 per cent. adrenaline hydrochloride.

Haemoglobin was estimated photoelectrically using oxyhaemoglobin as the pigment. All estimations were made in duplicate and no two such estimations differed by more than 0.15 gms. per cent. Two patients were given 1 ml. of 0.85 per cent. sodium chloride instead of adrenaline.

RESULTS.

The changes in haemoglobin levels in 16 patients given 1 mg. of adrenaline hydrochloride are shown in Table XI and in Figure 8. With three exceptions, a significant rise in haemoglobin followed the injection. It reached a maximum in 10 minutes, when an average rise of 0.73 gms., equivalent to 5.53 per cent. of the mean original level, was recorded. A fall occurred in 30 minutes, and return to pre-adrenaline levels was noted at the end of one hour. There is no evidence that the results were affected by the nature of the illness present.

TABLE XI.

The Effect of Adrenaline on Haemoglobin Concentration.

Case No.	1st Hb.	Time after adrenaline (minutes) 10	30	60	Max. rise	Max. % of 1st	Remarks.
1	11.30	11.95	11.65	11.50	0.65	5.75	Hepatic cirrhosis.
2	9.95	10.50	10.70	9.95	0.75	7.54	Hepatic cirrhosis.
3	12.90	13.60	13.40	12.90	0.70	5.42	Healed peptic ulcer.
4	13.05	13.55	13.40	13.05	0.50	3.83	Healed peptic ulcer.
5	12.95	13.65	13.00	13.00	0.70	5.38	Healed peptic ulcer.
6	12.90	13.40	13.10	12.70	0.50	3.94	Sciatica.
7	12.65	12.95	12.60	12.70	0.30	2.36	Infective hepatitis.
8	15.05	15.35	14.95	15.00	0.30	2.00	Healed pneumonia.
9	15.30	16.45	15.55	15.25	1.25	8.20	Fibrositis.
10	14.90	15.55	15.30	14.90	0.65	4.36	Intestinal taeniasis.
11	11.30	11.25	10.20	9.90	fall	--	Fibrositis.
12	11.70	11.00	11.55	10.80	fall	--	Healthy male.
13	12.25	12.80	12.20	11.85	0.55	4.64	Infective hepatitis.
14	13.85	15.40	15.60	15.30	1.55	10.01	Infective hepatitis.
15	13.65	14.70	14.60	13.75	1.15	8.48	Infective hepatitis.
16	11.20	11.05	10.70	10.70	fall	--	? Lymphadenoma.
17	10.70	10.45	10.70	10.85	fall	--	No adrenaline given.
18	14.80	14.60	14.45	14.15	fall	--	No adrenaline given.
Average (excluding 11, 12, 16-18)					0.73	5.53	

Unusual results occurred in Cases 11, 12 and 16, in whom the highest haemoglobin levels occurred in the first sample of blood withdrawn, though a fall occurred later.

Blood pressure readings were obtained in the first six patients. Variations amounting in some cases to 5 mm. Hg. were noted but they had no relation to the injection of adrenaline, or to the changes in haemoglobin.

DISCUSSION.

Although it has been suggested that the spleen in man may behave as an elastic, rather than as a muscular, organ (Ravenna, 1940), there is evidence that,

in its function as a blood reservoir, it acts like the animal spleen, and that in man also, exercise and excitement may cause a rise in haemoglobin concentration in the peripheral blood.

Ferrari (1897) was probably the first to study the influence of excitement on the blood count in the human subject. In his observations on students at an examination he recorded an increase in red cells amounting to about 10 per cent. The original observations of Barcroft and his associates (1922) were made on healthy adults, and the haemoglobin changes in relation to exercise recorded by Short (1935) were part of a clinical study.

The effect of adrenaline on the size of the spleen in man was observed by Ravenna (1940), and by Watson and Paine (1943). Contraction of the spleen occurred, and red cell levels increased in the blood of the splenic vein. Again in man, a rise in red cell count of about 20 per cent. occurred in two individuals 10-20 minutes after the administration of adrenaline; but no significant change occurred in two patients whose spleens had previously been removed (Benhamou, Jude & Marchioni, 1929).

The average rise in haemoglobin of 5.53 per cent. observed in the present study falls far short of the average rise of 20 per cent. recorded in animals

(Izquierdo & Cannon, 1928) and also of the rise of 20 per cent. recorded by Benhamou, Jude and Marchioni (1929) in man. Nevertheless, the rise is sufficiently great to indicate that adrenaline in man may cause the addition of a significant number of red cells to the circulating blood. The investigation does not prove conclusively that excitement would have a similar effect, but by analogy from the experimental and clinical work already reviewed, the possibility cannot be doubted. Indeed, the unusual response noted in Cases 11, 12 and 16, and the changes in those given only saline, may be explained on this basis alone.

The failure to demonstrate any significant blood pressure response is not surprising in view of the small dose of adrenaline used. The threshold for contraction of the spleen is lower than that for changes in arterial pressure (Hoskins & Gunning, 1917: Hargis & Mann, 1925).

SUMMARY AND CONCLUSIONS.

It is evident that the spleen in man and animals has a significant function as a reservoir from which red cells, present in concentrated form, may be discharged into the circulation when the need arises. Among the conditions causing splenic contraction and peripheral haemoconcentration are excitement and the administration of adrenaline and related drugs. From observations recorded in the past on the effects of

these drugs, it appears that haemoconcentration may be produced without significant alterations in arterial blood pressure. The results of the present investigation support this view.

An investigation on the effect of 1 mg. adrenaline hydrochloride in patients suffering from hepatic and splenic disease, and also in relatively healthy individuals, has shown that a rise in haemoglobin of as much as 10 per cent. may result. The average variation is greater than the normal diurnal variation already recorded.

It seems possible that excitement in the anticipation of venepuncture may contribute to the observed normal variations in haemoglobin. It seems highly probable that the excitement following a burn may cause mild degrees of haemoconcentration. In some cases this may be associated with a rise in blood pressure.

Reference has been made to the effects of various anaesthetics on the spleen and on red cell levels in the peripheral blood. These effects are of great importance in the assessment of blood changes following burns in animals under deep anaesthesia with one or more of the anaesthetics already mentioned.

C H A P T E R III.

THE QUANTITATIVE ESTIMATION OF SALINE FRAGILITY.

INTRODUCTION.

The lytic effect of hypotonic salt solutions has been used as a diagnostic procedure in haematology for many years, but it is only within comparatively recent times that the test has been subject to careful investigation. The more popular methods indicate only the concentration in which lysis begins, and that in which it is complete. No account is taken of variations in resistance to lysis exhibited by different portions of the red cell population. The phenomenon of partial haemolysis in the intermediate concentrations of the salt is ignored.

The significance of partial haemolysis has been debated, but the available evidence indicates that it is due to complete destruction of a proportion of the red cells, the rest remaining intact. There is no evidence that it is due to partial liberation of haemoglobin from all the cells (Koopman & Falker, 1936). This concept is of great importance. It means that an accurate quantitative estimation of haemolysis, throughout a suitable range of salt solutions, will detect undue

fragility of a small number of corpuscles in a sample of blood in which most of the cells exhibit normal fragility. Such a test is of great value in the investigation of fragility in relation to the blood changes which follow severe burns. In such cases a proportion of the circulating cells may be rendered unduly fragile, due to exposure to abnormal temperatures.

The method of quantitative estimation of erythrocyte fragility which has formed the basis of all subsequent work was devised by Simmel (1923). He used a mixture of salts similar to those found in blood serum, and with an equal osmotic pressure. From this solution he prepared various dilutions, to each of which was added capillary blood in the proportion of 1:200. Red cell pipettes were used, and by relating the red cell counts obtained with the various hypotonic solutions to the normal red cell count made in the usual manner, he was able to prepare a graph illustrating quantitatively the complete range of haemolysis occurring in the different solutions.

Simmel's method was adopted with little modification by Leake and Pratt (1925), and by Waugh and Chase (1928), but in a detailed study of saline fragility, Whitby and Hynes (1935) used pure sodium chloride instead of the mixture of salts. They regarded the latter as unnecessary on the grounds that the test cannot, under

any circumstances, be compared with the influences likely to affect the red cells in the circulation. Subsequent work has been confined almost entirely to the use of sodium chloride, though sodium sulphate has also been employed (Lepeschkin, 1932).

Various methods have been adopted in estimating the amount of haemolysis in the different solutions. Leake and Pratt (1925), Waugh and Chase (1928), and Whitby and Hynes (1935) performed red cell counts according to the method of Simmel (1923). Satisfactory results have also been obtained by comparing the amount of haemolysis against standards representing 10-90 per cent. haemolysis, prepared from each sample of blood tested (Vaughan, 1937: Creed, 1938: Dacie & Vaughan, 1938: Dacie, 1941 & 1943). Photoelectric methods were used by Hunter (1940). Lepeschkin (1932) employed a tedious and much less accurate method involving the dilution technique popular in haemoglobinometry. The height of the column of packed red cells obtained by centrifuging the blood-saline mixture, was used by Beebe and Hanley (1936) and by Guest and Wing (1939), but the results were influenced by alterations in corpuscular volume. Such a method cannot be considered satisfactory.

The development of accurate methods has stimulated investigation of the factors influencing the results. Whitby and Hynes (1935) found that arterial

blood was less fragile than venous, and that the fragility of capillary samples occupied an intermediate position. They suggested that the differences were related to the degree of oxygenation of the blood, and showed that saturation with carbon dioxide caused a great increase in fragility. Creed (1938) confirmed these findings and observed that aeration of a sample of blood initially with a low oxygen content, reduced its fragility to a constant level. Dacie and Vaughan (1938) in discussing the measurement and significance of erythrocyte fragility, recognise two types of factor influencing the results, intrinsic and extrinsic. In the former group are the shape of the cell and its chemical composition; in the latter, the oxygen and carbon dioxide content of the blood, the temperature and pH of the haemolytic solution, and the concentration of the red cells. The amount of plasma is also an important factor, because in proportion to its effect in raising the osmotic pressure of the saline, it protects the red cells from lysis. For this reason, it has been suggested that washed red cells should always be used unless the plasma is sufficiently diluted to eliminate the protective factor (Whitby & Britton, 1937b). On the other hand, unless the pH of the solutions is accurately controlled, the buffer action of the plasma is an actual advantage, and whole blood is to be preferred (Creed, 1938). Under these circumstances, a correction

may require to be made for the rise in osmotic pressure produced when an unusual amount of plasma is added, as may occur in the use of anaemic blood. The addition to the saline solutions of "double or treble the quantity of blood ... in very severe anaemias" suggested by Waugh and Chase (1928) may so reduce the apparent fragility of the red cells as to render the results almost useless. A much more satisfactory procedure is to adjust the red cell:plasma ratio to about 45:55 by removing an appropriate amount of plasma after sedimentation of the red cells has been hastened by gentle centrifugation.

In view of the many and variable factors which may influence the results obtained from the estimation of saline fragility, it is essential in any investigation to indicate not only the method used but also the results to be expected in normal circumstances. In Part II. of the present work, the estimation of saline fragility provides much information on the changes in the red cells following severe burns. In consequence a preliminary consideration of the methods used, and of the range of normal values obtained, is of great importance.

I. THE ESTIMATION OF SALINE FRAGILITY.

THE METHOD OF ESTIMATION.

Venous blood was used throughout the investigations recorded here, samples being obtained in the manner already described (p. 20). Prior to sampling, thorough mixing was ensured by agitation in an automatic shaker. This served also to re-oxygenate the blood, which was removed under conditions of venous stasis.

The various dilutions of saline used in this test were prepared as stock solutions from sodium chloride of analytical purity, and checked by titration. The range of strengths employed varied from 0.24 per cent. to 0.72 per cent. NaCl, by steps of 0.02 per cent. Five ml. of the respective saline solutions were added to a series of centrifuge tubes, and then, to each of these was added, by means of a microburette with a ground and vaselined tip, an equal volume of blood (0.10 ml.). A similar amount of blood at the beginning, and again at the end, of the manipulation was added to 10 ml. distilled water to provide standards equivalent to 50 per cent. haemolysis. The contents of the tubes were mixed by inversion, and allowed to stand not less than 15 minutes at 20°C., and not more than 2 hours at 20°C., or 24 hours at 0°C. The tubes were then centrifuged, and the supernatant solutions compared with the standards in a Duboscq colorimeter.

Comparison of the two standards revealed that, in the short time taken to add the blood to the saline solutions, no appreciable error occurred due to red cell sedimentation in the burette.

This method of estimation of fragility is the one to which reference is made below in considering normal values for fragility, and the changes which occur in relation to burns. The conditions of the test have been based on investigations on the factors which may influence the results. These factors are discussed in a later section.

THE METHOD OF RECORDING THE RESULTS.

The results are recorded graphically by plotting the degree of haemolysis (as a percentage of complete lysis) against the concentration of the sodium chloride used (Figs. 9a-9d). From the graphs, the concentrations of the salt in which lysis begins, and in which it is complete, can be seen at a glance. The concentration of the salt in which 50 per cent. haemolysis occurs can be readily obtained by interpolation. This value is termed the Median Corpuscular Fragility (M. C. F.).

FACTORS INFLUENCING THE RESULTS.

Apart from the intrinsic factors (Dacie & Vaughan, 1938) there are numerous factors which influence the results of the fragility test. It is evident from the work of Whitby and Hynes (1935), and of Creed (1938), that

the proportion of blood to saline should be kept constant. In the present study this proportion was maintained at 1:50. Under these circumstances the amount of plasma added with the blood is sufficient to have some buffer effect without unduly adding to the osmotic pressure of the saline unless the blood is very anaemic. No attempt was made to adjust the pH of the solutions used.

The Effect of Delay in Setting up the Blood-Saline Mixtures.

It is important to know how long a sample of blood may remain either at room temperature (20°C.), or at 0°C., before a significant alteration in fragility occurs.

In the course of many fragility estimations no significant alteration has been found to occur if the blood is left for three hours at 20°C., but delay of longer duration results in a variable but frequently significant increase in fragility.

In investigating some of these changes a complete fragility curve was unnecessary, and the amount of haemolysis in 0.4 per cent. sodium chloride was determined photoelectrically. In this estimation, 0.100 ml. of whole blood or of a suspension of washed red cells in 0.85 per cent. saline, was added to 5.0 ml. of 0.4 per cent. saline in a centrifuge tube. The mixture was allowed to stand at 20°C. for 15-20 minutes, and then centrifuged. The haemoglobin content of the supernatant fluid was estimated photoelectrically in Colorimeter No. 2 and compared with

the haemoglobin content of the whole blood or red cell suspension estimated in the usual manner.

The increase in fragility which may occur if blood is left at 20°C. for 24 hours is shown in Table XII. The first 8 estimations were made by the photoelectric method just described, and the last 6 were derived from the complete curves obtained by the standard method (p.60). From these curves it is also possible to record the variations in M.C.F. caused by the delay in the estimations.

TABLE XII.

Alterations in the Fragility of Red Cells allowed to remain at 20°C. for 24 hours.

Spec. No.	% Lysis in 0.4% NaCl.			M.C.F. - % NaCl.		
	Immediate	After 24 hours	Increase	Immediate	After 24 hours	Increase
1	56.5	67.8	11.3	-	-	-
2	76.4	77.0	0.6	-	-	-
3	77.5	78.5	1.0	-	-	-
4	51.7	67.0	15.3	-	-	-
5	58.3	68.7	10.4	-	-	-
6	75.5	74.6	0.9	-	-	-
7	53.6	69.3	13.7	-	-	-
8	73.2	78.5	5.3	-	-	-
9	31.0	43.0	12.0	0.372	0.386	0.014
10	31.0	44.0	13.0	0.374	0.390	0.016
11	85.0	85.0	0.0	0.434	0.460	0.026
12	84.0	86.0	2.0	0.434	0.452	0.018
13	52.0	75.0	23.0	0.426	0.430	0.004
14	74.0	76.0	2.0	0.402	0.428	0.026

It is evident that delay of 24 hours at 20°C. may increase the lysis occurring in 0.4 per cent. saline by 15 per cent., and the M.C.F. by 0.026 per cent. It is of interest that the increase in fragility is not equal

throughout the whole curve, and the samples showing the greatest increase in lysis in 0.4 per cent. NaCl do not necessarily show the greatest increase in M.C.F.

These alterations are very significant in view of the small variations in normal fragility during the day, and from one day to the next, and although less change occurs if the blood is kept at 0°C., it is essential to perform the estimation within a few hours, and it is preferable to do so immediately, after withdrawal of the blood.

The Effect of Delay in Reading the Results.

Although the blood-saline mixtures should be prepared with the minimum delay, it is not necessary to complete the test at once. Haemolysis in hypotonic saline occurs within a few minutes, and even at 20°C. the mixtures may be left for 6 hours without risk of further lysis. If kept at 0°C. for 24 hours no alteration in the fragility has been observed even in samples of blood removed soon after injury in patients severely burned.

The Effect of the Anticoagulant.

Throughout the investigations recorded here, heparin was used as the anticoagulant. There is no evidence that it affects red cell fragility, and the results obtained with Wintrobe's oxalate mixture (Whitby & Britton, 1937c), have been found to be identical.

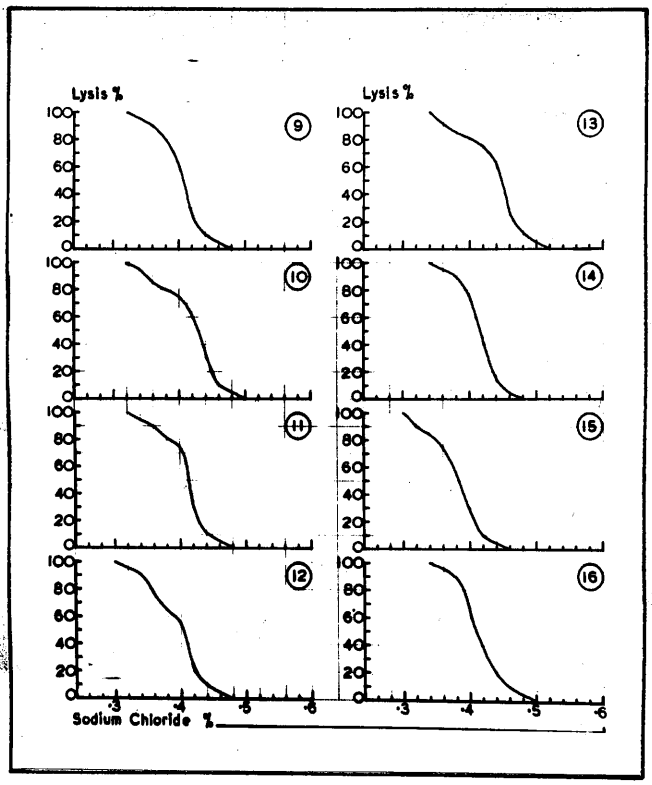

Lysis %
100
80
60
40
20
0
9
10
11
12
13
14
15
16
·3
·4
·5
·6
Sodium Chloride %

Fig. 9b

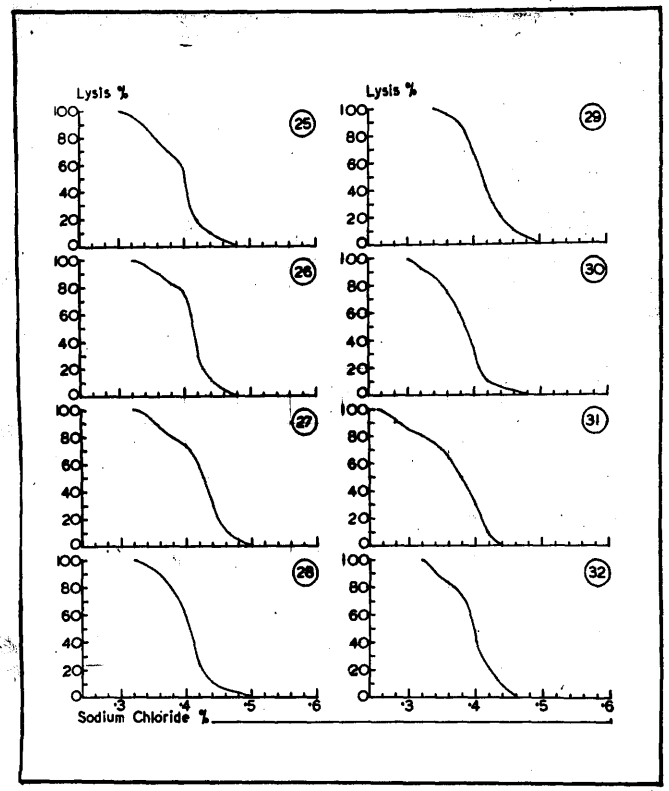

Lysis %
100
80
60
40
20
0
25
26
27
28
29
30
31
32
·3
·4
·5
·6
Sodium Chloride %

Fig. 9d

II. QUANTITATIVE ERYTHROCYTE FRAGILITY IN HEALTHY ADULTS.

METHOD OF INVESTIGATION.

The values obtained in health form a satisfactory basis for the assessment of the changes related to severe burns, if the same methods are used throughout the investigation. Therefore, in the present study, as in that described later in relation to burns, the method used in the estimation of fragility was that already described (p.60).

The normal adults comprising the present series of cases, were healthy blood donors. The samples of blood were obtained at the evening Session of the Blood Transfusion Service, between 5.30 and 7.30 p.m. The first 5 ml. of blood flowing from the vein at the process of bleeding was collected in a heparinized test-tube, and the fragility estimation was performed without delay. Haemoglobin and haematocrit levels were estimated on each sample; all were within normal limits.

RESULTS.

The results in 32 healthy individuals are shown graphically in Figure 9, and the values for the M.C.F. obtained from these graphs are recorded in Table XIII.

The shape of the curve shows slight variation in the different individuals, but the general configuration is very similar throughout. Slight difficulty has frequently been experienced in recording the point at which

complete lysis occurs, but there has never been any doubt as to the toe of the curve in samples from normal persons. This is of importance in that after severe burns, the fragility curve may show persistent haemolysis at a level unchanged throughout the concentrations of saline above a certain strength. The significance of this is discussed later.

TABLE XIII.

Erythrocyte Fragility in 32 Healthy Adults.

Case No.	M. C. F. NaCl%.	Lysis Begins	Lysis Complete	Case No.	M. C. F. NaCl%.	Lysis Begins	Lysis Complete
1	0.374	0.44	0.28	17	0.386	0.44	0.32
2	0.416	0.46	0.34	18	0.362	0.42	0.28
3	0.434	0.48	0.34	19	0.368	0.42	0.28
4	0.432	0.48	0.34	20	0.362	0.42	0.28
5	0.406	0.46	0.30	21	0.400	0.46	0.32
6	0.424	0.46	0.32	22	0.424	0.48	0.32
7	0.408	0.46	0.34	23	0.414	0.48	0.34
8	0.430	0.48	0.34	24	0.434	0.48	0.34
9	0.406	0.46	0.32	25	0.400	0.46	0.30
10	0.430	0.48	0.32	26	0.414	0.46	0.30
11	0.413	0.46	0.32	27	0.427	0.48	0.32
12	0.404	0.46	0.30	28	0.404	0.48	0.32
13	0.448	0.50	0.34	29	0.410	0.48	0.34
14	0.416	0.46	0.34	30	0.386	0.46	0.30
15	0.384	0.44	0.30	31	0.379	0.42	0.26
16	0.410	0.48	0.34	32	0.398	0.44	0.32

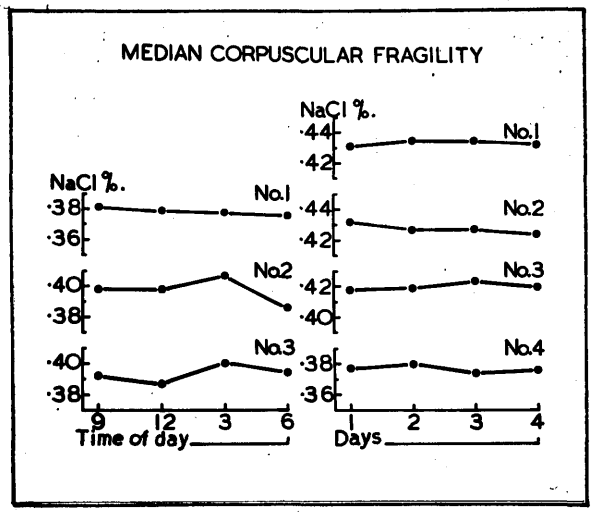
Minimum M. C. F. = 0.362%NaCl. Minimum fragility = 0.42-0.26%NaCl.
Maximum M. C. F. = 0.448%NaCl. Maximum fragility = 0.50-0.34%NaCl.

DISCUSSION.

The average fragility values obtained in the present series differ slightly from the results recorded by other workers in this field. Differences in technique

are probably responsible. In the study of 50 normal adults, Whitby and Hynes (1935) found lysis commencing in 0.45-0.51 per cent. saline, and from their graphs, it appears that the M.C.F. was 0.405-0.445 per cent. NaCl. The normal M.C.F. recorded by Creed (1938) was 0.36-0.40 per cent. NaCl. Slightly lower values have also been recorded by Dacie and Vaughan (1938) and Dacie (1941). These differences are due, in great part, to differences in technique. The lower values were obtained from venous blood carefully aerated before use, and in addition, the ratio of blood to saline was twice that used in the present series. The wide range of normal values in different individuals recorded by Leake and Pratt (1925) and by Waugh and Chase (1928) were probably due to use of capillary blood and to variations in its oxygenation.

As far as can be ascertained, the results presented here are in agreement with those obtained by other workers. More important is it that the normal range of saline fragility has been established for a method which has been used to investigate the changes following severe burns described in Part II.


MEDIAN CORPUSCULAR FRAGILITY
NaCl %.
·38
·36
No.1
·40
·38
No.2
·40
·38
No.3
9
12
3
6
Time of day
NaCl %.
·44
·42
No.1
·44
·42
No.2
·42
·40
No.3
·38
·36
No.4
1
2
3
4
Days

Figs. 10 & 11

III. NORMAL VARIATIONS IN ERYTHROCYTE FRAGILITY.

INTRODUCTION.

Much significance is attached to variations in erythrocyte fragility following severe injuries due to burning. It is therefore important to have some normal values with which these variations may be compared. To some extent, the desired information is supplied by the range of values occurring in healthy persons, but it is also important to know how much variation is likely to occur during the day, and from one day to the next. The following investigation on the diurnal and daily variations is therefore presented.

THE METHOD OF INVESTIGATION.

The test was performed in the manner described (p.60). The curves of quantitative fragility were very similar to those shown in Figure 9, and only the variations in M.C.F. are illustrated graphically here (Figs. 10 & 11).

The individuals selected for the investigation were those in whom normal variations in haemoglobin were being studied. Saline fragility and haemoglobin levels were estimated on the same sample of blood. Diurnal variations in fragility were estimated in Cases 1-3 (Table IX, p.40) and daily variations in Cases 1-4 (Table VII, p.38).

RESULTS.

The curves of the estimations of fragility

are not shown, but the values for the M. C. F. obtained from them by interpolation, are recorded in Tables XIV and XV.

TABLE XIV.

Diurnal Variations in Median Corpuscular Fragility.

Case No.	3-hourly M. C. F. values.				Maximum variation
	9 a.m.	12 m.d.	3 p.m.	6 p.m.	
1	0.381	0.379	0.378	0.376	0.005
2	0.398	0.398	0.407	0.386	0.021
3	0.392	0.387	0.400	0.394	0.013

TABLE XV.

Daily Variations in Median Corpuscular Fragility.

Case No.	Daily M. C. F. values.				Maximum variation
	1	2	3	4	
1	0.431	0.435	0.435	0.432	0.004
2	0.432	0.427	0.428	0.424	0.008
3	0.418	0.419	0.424	0.420	0.006
4	0.377	0.380	0.374	0.376	0.006

The variations in M. C. F. are very small. The greater change noted in the diurnal figures is of doubtful significance in such a small series. It may be a chance occurrence due to slight variations in oxygenation. A slightly greater delay in performing the tests may also have operated in increasing the values.

Reference to these normal variations in fragility will again be made when the changes occurring in relation to burns are discussed.

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S T U D I E S

IN

H A E M A T O L O G Y

with special reference to the

H A E M A T O L O G Y O F B U R N S

BY

ALEXANDER BROWN,

M. B., Ch. B., F. R. F. P. S. G., M. R. C. P. Ed.

- 1945 -

S T U D I E S I N H A E M A T O L O G Y

P A R T II.

THE HAEMATOLOGY OF BURNS.

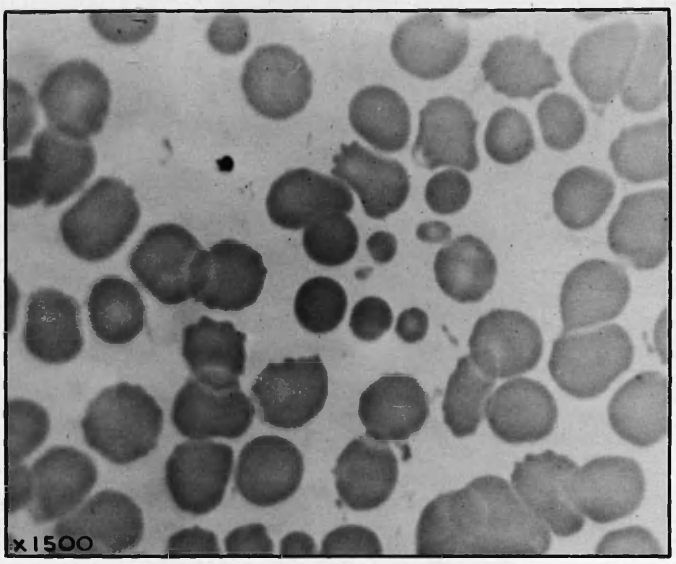

Plate I.

PLATE I.

FRAGMENTATION AND MICROSPHEROCYTOSIS IN THE BLOOD AFTER SEVERE BURNS.

Case 46: Blood taken 1.5 hours after injury.

(p.156)

CONTENTS.

CHAPTER I.

SECONDARY SHOCK IN BURNS.

CHAPTER II.

INTRODUCTION TO THE STUDY OF BLOOD CHANGES AND BLOOD PRESSURE IN RELATION TO SHOCK.

CHAPTER III.

HAEMOCONCENTRATION AND SHOCK.

CHAPTER IV.

HAEMOCONCENTRATION AND SHOCK.

(Continued).

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SECONDARY SHOCK IN BURNS.

I. INTRODUCTION: THE MORTALITY FROM BURNS.

Injuries due to burning have become a very great social problem, the magnitude of which is well illustrated by the mortality figures recorded in Britain and the United States of America (Table I).*

TABLE I.

The mortality from Burns in Britain and America.

	Scotland 1937.	England & Wales, 1937.	United States 1939.
Population.	4,976,610	41,031,000	128,000,000
Deaths from burns.	256	1,318	6,240
Death rate per million.	51	32	49

In Glasgow the problem is no less serious. The progressive increase in the numbers of cases of burns admitted to hospital in Glasgow over a period of 100 years has been shown clearly by Dunbar (1934). It is probable, as Dunbar himself suggests, that part of this increase

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* Figures for Britain obtained from the Report of the Registrar General, 1937.
Figures for United States of America obtained from the United States Bureau Vital Statistics Special Report, 1939, 7:77 (McClure, 1939).

was due to a greater liability or inclination of burned patients to be treated in hospital in the latter part of the period surveyed, but this can hardly be the only factor concerned. Thus at the beginning of the period, in the decade 1833-1842, the maximum number of cases admitted to the Glasgow Royal Infirmary in 12 months was about 80. At that time the Royal Infirmary was the only Glasgow hospital to which patients with burns were admitted. In the last decade considered by Dunbar, 1923-1932, a level of about 320 admissions in 12 months was reached, in spite of the fact that by this time two other large voluntary hospitals, and also the Royal Hospital for Sick Children, were treating these injuries. The figures obtained from the records of the Royal Infirmary for the next decade, 1933-1942, reveal an average annual admission rate of 266 cases, and an average annual death rate of 13.1 per cent. of the admissions. (Table II).

The increasing numbers of burns noted by Dunbar, together with these more recent figures, indicate the importance of the problem. Close inspection of the available evidence suggests that there is every prospect of maintenance of these high figures (Brown, Lewis-Faning & Whittet, 1945). This being so, it is of the utmost importance that an understanding should be sought as to the cause of death in burns. Only with this understanding can there be any prospect of rational and effective treatment.

TABLE II.

The Mortality in Admissions to the Burns Unit, Glasgow Royal Infirmary: 1933-1942.

Year.	Admissions.	Deaths.	Mortality %.
1933	269	37	13.8
1934	261	46	17.7
1935	327	27	8.3
1936	320	43	13.4
1937	227	33	14.5
1938	256	29	11.3
1939	208	24	11.5
1940	263	29	11.0
1941	244	47	19.3
1942	283	37	13.1
Total.	2,658	352	13.1

II. THE CAUSE OF DEATH IN BURNS - BURNS SHOCK.

INTRODUCTION.

The great majority of deaths from burns occur within a short time of injury. In a series of 1,200 cases admitted to the Burns Unit of the Glasgow Royal Infirmary, about half the deaths occurred during the first 24 hours, and almost three-quarters occurred within the first 3 days (Gibson & Brown, 1944). It is a finding upon which there is general agreement, that death occurs after a variable period during which the patient presents a train of signs and symptoms to which the term "burns shock" has been applied.*

* Throughout this volume, the term "burns shock" is used to indicate this clinical syndrome, and the term "shock period" is applied to the period during which this syndrome tends to appear, i.e. - the first 2-3 days after the burn.

The clinical picture of shock is universally so well known that a full description is unnecessary here, but some features of burns shock are of special interest in relation to haematological changes. Even with severe burns, patients may have little pain: they may remain alert and rational with almost no general upset, until within half an hour of death. With the onset of stupor or coma, a rising temperature is a common feature, and in several cases temperatures of 106-108°F. have been recorded. A constant feature of burns involving more than 10 per cent. of the body surface is thirst, which is often very severe. The significance of hyperpyrexia and thirst in relation to circulatory changes and to alterations in fluid balance is considered in later chapters.

BLOOD CHANGES AND SHOCK.

Introduction.

A vast amount of literature has accumulated on the subject of burns shock, but it is only within comparatively recent years that an explanation has been sought on a factual basis. The first post-mortem examinations reported on fatal cases of burns were carried out by Cumin of Glasgow in 1823. In a study of five cases, he noted extreme congestion of the abdominal and thoracic viscera, and small haemorrhagic areas on the pleura, peritoneum and meninges. There was nothing which could be regarded as peculiar to this type of injury, and nothing

to indicate the fundamental cause of death.

Later in the nineteenth century a more definite attempt was made to explain the cause of death following severe burns. Changes in the blood formed the basis of most of the theories at this time, and it seems probable that they owed their origin primarily to the observations of Baraduc (1862). He was the first to report the association of increased blood viscosity, which he attributed to loss of serum found in the blisters.

Ponfick (1867-1883) regarded red cell destruction as of greater etiological significance in burns shock. This view was supported by von Lesser (1880), but opposed by Klebs (1877), and by Tappeiner (1881), both of whom considered the changes in the circulation to be of much greater importance. Hoppe-Seyler (1881) criticised both the work and the conclusions of von Lesser. He failed to find sufficient changes in the red cells to account for death, and favoured the belief that the responsible factor was absorption of toxic products from the burned tissues.

Silberman (1890) amplified the hypothesis of Baraduc by postulating a sequence of events beginning with transudation of fluid from the blood-stream and haemo-concentration, and ending with the death of the patient. He thought that the concentration of the blood resulted in capillary stasis and dilatation, with thrombosis, leading to embarrassment of ventricular action and "arterial anaemia."

Capillary thrombosis was given greater prominence as an etiological factor in burn shock by Markusfeld and Steinhaus (1895). Continuing the observations of Schultze (1865) on the effect of heat (51-52°C.) on red cells in vitro, they found that similar changes occurred in the experimental animal. These morphological changes were followed, they said, by chemical changes leading to thrombosis. That the red cell changes do occur is fully corroborated by the present investigation. But the suggestion that thrombosis occurred in the vessels at a stage sufficiently early in the shock period for it to be regarded as of etiological significance, was so poorly substantiated by Markusfeld and Steinhaus that the conclusions of these authors must be accepted with reserve. Subsequent work on the pathology of shock in burns has not lent support to their statement.

From time to time thereafter, fresh attempts were made to explain burns shock. Vasomotor paralysis and cardiac failure, due to excessive stimulation of peripheral nerves by injury, pain, fear, anxiety or infection, was the mechanism favoured by Crile (1903, 1923), but the investigations of Porter and his colleagues showed very clearly that such a theory was not in accordance with fact. In the first place, the effect of afferent impulses on the vasomotor centre was shown to be as great whether the blood pressure was normal, or as low as 25 mm. Hg., (Porter, 1907-8).

In addition, it was found impossible to produce fatigue of the vasomotor centre and a fall in blood pressure by stimulation of afferent nerves (Porter, Marks & Swift, 1907-8). Finally it was concluded that the centre was neither fatigued nor inhibited in shock (Porter & Quinby, 1907-8). The findings of Henderson (1908-9, described below), and of Mann (1914), supported this conclusion. The latter found that stimulation of a mixed nerve, or asphyxia, caused a rise in blood pressure even in the presence of an extreme degree of shock, and maintained that, far from being fatigued or inhibited, the centre was most resistant and actually in a very active state, since the peripheral arterioles were constricted until the late stages. Erlanger, Gesell and Gasser (1919) supported these views, and concluded that the initial changes could best be explained by a reduction in the volume of the circulating blood, and that terminal failure of vasomotor tone was the result, and not the cause, of the low blood pressure.

The concept that the circulatory failure of shock results from inadequate venous return to the heart and not from vasomotor exhaustion or inhibition, is due in great part to the work of Henderson (1908-9). He showed that during a more or less prolonged period which always preceded the shock stage, the arterial pressure was maintained at a normal level in spite of diminishing venous return to the right auricle and reduced diastolic filling. Administration

of saline intravenously immediately restored normal conditions, but without this infusion, diastolic filling of the ventricles diminished progressively, and the output of the heart suffered in consequence. Finally, when the arterial blood flow had fallen to about 30 per cent. of normal, arterial pressure fell rapidly. No failure of the vasomotor centre could thus be postulated. On the contrary, the fact that the blood pressure was maintained in spite of a diminished cardiac output demonstrated that the centre was in a state of extreme activity.

While subsequent evidence has supported this concept to a degree which may be regarded as conclusive, Henderson's views on the cause of the inadequate venous return met with much opposition. He believed that it was due to failure of the venopressor mechanism: that this failure was due to deficiency of carbon dioxide in the blood and tissues: and that the deficiency was caused by hyperventilation. Subsequent work, and also careful clinical observation have, however, shown that shock frequently occurs in the absence of hyperventilation, and it seems more probable that acapnia is the result and not the cause of the circulatory failure. More recently Henderson (1930) advanced the theory that failure of the venopressor mechanism in shock was due to diminished tone in skeletal and visceral muscles, particularly in the diaphragm. He believed that the intramuscular pressure

was an important factor in promoting return of blood to the heart, and that the tone of the muscles of respiration was particularly important in this respect. His view was that, in shock, depression of general muscle tone resulted in stagnation of the blood in the peripheral vessels, and circulatory failure. He did not explain the loss of muscle tone, nor did he present any evidence in support of his theory. The low intramuscular pressure which he found may well have been a result, and not a cause, of shock.

Haemoconcentration and Capillary Permeability.

A great advance in the investigation on the etiology of secondary shock was provided by Keith's contribution to the Special Investigation on Surgical Shock and Allied Conditions (1919) under the auspices of the Medical Research Council. Using the method of Keith, Rowntree and Geraghty (1915) for the estimation of blood volume, he showed that reduction of blood volume and of plasma volume was an important and striking feature of wound shock; that the degree of reduction was related to the severity of the shock; and that recovery was associated with an increase of blood volume. On this basis he regarded the administration of fluid to maintain the volume as the essential basis of treatment. Keith's findings were confirmed experimentally by Gasser, Meek and Erlanger (1919).

Attention was directed more specifically to the circulatory changes in burns shock by the work of Underhill,

Carrington, Kapsinow and Pack (1923). In the intervening years the observations of Baraduc (1862) and of Silberman (1890) had been ignored or forgotten, and Underhill and his colleagues were the first to stress anew the changes in the red cell:plasma ratio (haemoconcentration) which occur in cases severely burned. This change appeared to vary with the severity of the burn, and haemoglobin values nearly twice normal were recorded in some instances.

Further investigation on the effects of superficial burns (Underhill, Kapsinow & Fisk, 1930a) demonstrated that when a rabbit received a superficial burn, subcutaneous oedema was produced, and that its production was associated with marked haemoconcentration. The development of the oedema was rapid, reaching a maximum after approximately 24 hours. Reabsorption was slow, being completed only at the end of 5-6 days. From a study of the distribution of dyes in experimental burns, the same authors (1930b) showed that substances which normally do not pass the capillary wall, appeared in the oedema fluid. Support was thus lent to their view that haemoconcentration after burning is due to loss of fluid from the blood through capillaries, the permeability of which has been increased. Continuing their investigations they showed (Underhill, Fisk & Kapsinow, 1930) that a superficial burn involving approximately a sixth of the body surface, induced within 6 hours a significant loss of fluid from the blood in the

form of subcutaneous oedema. The fluid reached its maximum accumulation in the first 24-36 hours, after which it was gradually absorbed. It was then shown (Underhill & Fisk, 1930) that the oedema fluid was identical with plasma in composition. This observation was found by McIver (1933) to apply also to man.

The concept was thus definitely established that after superficial burns a great increase in permeability occurs in the capillaries in the region of the injury; that the increased permeability allows the escape from the blood stream into the tissues of fluid indistinguishable from plasma in composition; and that the resulting diminution in blood volume reduces the venous return to the heart, and produces the sequence of effects first postulated by Henderson.

This view has remained unshaken to the present time, and it has been of immense practical value since it has provided a satisfactory basis for rational treatment - treatment directed to the maintenance of the blood volume, a subject which will be discussed in a later chapter. There have, however, been differences of opinion on several points related to this theory. Although there has been general agreement that a great increase in capillary permeability occurs in the region of the burn, it is not generally agreed that the vascular defect is confined to this site. Underhill (1930), and Blalock (1934), for example, believe

that it is so, but Moon (1938, 1942) is of the opinion that there is a more general and widespread increase in capillary permeability. This question is considered more fully in a later chapter.

The Rate and Magnitude of Fluid Loss.

A matter of great importance is the rate at which fluid loss may be expected to occur into the tissues. It was shown experimentally (Underhill, Kapsinow & Fisk, 1930a: Underhill, Fisk & Kapsinow, 1930) that significant loss occurred within 6 hours: that tissue oedema was maximum 24-36 hours after injury: and that absorption was slow, being complete in 5-6 days. Blalock (1931) and Beard and Blalock (1931) observed an accumulation of fluid in the burned area amounting to an average of 57 per cent. of the total plasma volume, and stated that the passage of fluid into the tissues continued to the 40th hour. Elkinton, Wolff and Lee (1940) corroborated this statement by reporting that the capillary walls recovered their tone in about 40 hours after the injury. Harkins (1934) showed, in animals under barbital anaesthesia, that about 50 per cent. of the total plasma shift occurred in the first hour. Keeley, Gibson and Pijoan (1939) also emphasised the rapidity with which reduction of plasma volume occurred, but did not carry their experiments beyond the 14th hour.

These findings were obtained experimentally using animals under the influence of an anaesthetic. In

view of the fact that animals show little tendency to lose fluid from the surface of the body (Blalock & Duncan, 1942), and in view also of the effect of anaesthetics on the blood volume and haemoconcentration, (Part I, p.47), there should be some hesitation in applying such experimental findings to the problem of burns in man. Confirmation should first be sought from clinical studies. Such confirmation has not been forthcoming from reports of observations in the human subject, chiefly because patients are seldom admitted to hospital sufficiently soon after burning to permit the study of early changes. In addition, knowledge of blood values before the injury is denied to the clinical worker, who is thus deprived of very valuable information in the estimation of changes in fluid balance. In the report of Underhill, Carrington, Kapsinow and Pack (1923), although the blood changes were investigated in considerable detail, observations were not begun until 3 p.m. on the day following the accident. This means that at least 15 hours had elapsed since burning. Tenery (1940), however, reports from a clinical study, the details of which have not been published, that half the haemoconcentration to be expected occurred in the first 6 hours. Penberthy (1941) also found that haemoconcentration was of rapid onset. One case showed a haematocrit reading of 70 per cent. at the end of the first hour.

Haemoconcentration and Capillary Permeability in Relation to Treatment.

Although the importance of the blood changes in burns is almost universally appreciated, and the value of therapy directed to the maintenance of the blood volume generally accepted, insufficient stress has been laid on the rapidity with which the changes may take place. The expected duration of the increased permeability of the capillaries has been almost universally ignored. Underhill and his colleagues attributed the disappearance of haemoconcentration, which occurred in their cases at the end of 20-40 hours, to the administration of large amounts of fluid by mouth. On the contrary, the results of this treatment were not spectacular, and, with burns of the severity described, there is no evidence that the development and disappearance of haemoconcentration were affected by the measures adopted.

In the light of evidence on the duration of the increase in capillary permeability, it would be expected that if adequate plasma transfusion were given during the first 48 hours, there should be little or no need for further addition of fluid to the circulation to maintain blood volume. Yet Strumia, Wagner and Monaghan (1940) continued the administration of plasma to the 11th day, and Minot and Blalock (1940) to the 19th day.

It is evident that if the period of treatment

requires to be prolonged to this extent, there is an unexplained discrepancy between the theoretical basis and its practical application. The evidence presented in later chapters will do much to dispel this confusion. There is no clinical evidence that haemoconcentration progresses beyond 72 hours after the injury in any patient, treated or untreated, and there is no justification for continuing plasma infusion beyond that short period, as far as the maintenance of blood volume is concerned. Its value as a source of protein in cases of severe burns cannot be stressed too highly, but this is a matter which is not relevant to the present discussion.

Summary.

It is evident that changes in the circulating blood have been recognised for almost ninety years. From the beginning of the present century there has been a growing realisation that a significant reduction in the circulating volume is of great etiological significance in the production of the syndrome of secondary shock in burns. The concentration of the blood must be regarded as indicating, more or less accurately, loss of plasma into the tissues, from vessels the permeability of which has been increased.

Confusion still exists on many points in relation to the causation of the state of shock. It is uncertain that oligaemia is the sole factor, and several writers have

postulated the action of toxic substances absorbed from the burned area. Although there is undoubted evidence of toxic absorption, it must be considered with full appreciation of the magnitude of the circulatory changes which follow serious burns.

In relation to the occurrence of oligaemia, it is uncertain whether the plasma loss into the tissues is a local or a general phenomenon. Experimental work has shown that under certain conditions, the fluid loss is of very rapid onset, and that a maximum increase in the permeability of the capillaries is rapidly attained. It is not clear whether these findings are applicable to man, but the importance of problem in relation to transfusion therapy demands its further investigation.

BLOOD PRESSURE AND SHOCK.

It is generally accepted that a low blood pressure is one of the characteristic features of shock, and there is a widespread belief that alterations in the level of the blood pressure provide an early indication of the onset of this condition. On the other hand, from time to time, attention has been directed to the occurrence of the syndrome in the absence of a fall in blood pressure.

The belief that shock was due to vasomotor paralysis and cardiac failure served a useful purpose in stimulating investigation on the changes in the peripheral vessels in this condition. The experiments of Porter and

his colleagues showed that the vasomotor centre is neither fatigued nor inhibited. Meltzer (1908) observed that fatal experimental shock may not, at any stage, be associated with a very low blood pressure, and that a low blood pressure may occur without shock. Further corroboration of the activity of the vasomotor centre in shock was presented by Henderson who demonstrated that the blood pressure may be maintained, by a process of peripheral vasoconstriction, until the blood flow falls to about 30 per cent. of normal. Moon (1934) reviewed additional clinical and experimental work indicating that changes in the blood pressure are neither the first nor the most accurate, criteria of shock. In a recent review (Leading Article, Lancet, 1941) the need for frequent estimations of the blood pressure in cases in whom shock may develop is stressed, but it is admitted that the circulation may be poor in spite of a normal blood pressure. Keckwick, Maycock, Marriott and Whitby (1941) reported that the fall in blood pressure in haemorrhagic shock was proportional to the severity of the injury, but observed that in extreme exsanguination, compensatory vasoconstriction might maintain the blood pressure at a deceptively high level, especially in young subjects. More recently, and again in the experimental animal, Olson and Necheles (1943) found that a normal or even a high blood pressure may occur in severe shock.

Although these findings have been derived chiefly from experimental work, they indicate very clearly the need for an accurate assessment of the changes in blood pressure in relation to shock. It is of the utmost importance to discover whether a fall is an early indication of incipient shock, or whether it is a manifestation only of the established condition, occurring when contraction of the peripheral vessels can no longer compensate for the diminished blood volume. Evidence obtained from the study of burns, and described in later chapters, indicates clearly that in burns shock blood pressure readings may be very misleading, and that normal or high levels may be recorded irrespective of the nature of the burn or of the ultimate issue.

C H A P T E R II.

INTRODUCTION TO THE STUDY OF BLOOD CHANGES AND BLOOD PRESSURE IN RELATION TO BURNS SHOCK.

I. OBJECTS OF THE INVESTIGATION.

A systematic enquiry has been made into the various blood changes in a large series of cases of burns. In particular, it seemed desirable both to ascertain how closely haemoconcentration was dependent on the extent and severity of the burn, and to investigate the rate of occurrence and the duration of this process. The information so gained would be of great value in the correction, by transfusion, of the changes in blood volume which occur soon after burning. From the results of such a study a full appreciation of the magnitude of the alterations in fluid balance occurring in severe burns would permit more accurate assessment of other factors which have, from time to time, been suggested as causes of shock in burns. Observations on the results of maintenance of normal blood volume by transfusion of plasma should serve further to throw light on this problem.

The need for accurate reassessment of the changes in blood pressure in relation to shock has already been

discussed (p.85). It is important to discover the relation between blood pressure and the development of haemoconcentration, and to ascertain how far the changes in pressure can be relied upon as a guide to prognosis and treatment.

II. THE CLINICAL MATERIAL.

Only patients admitted to hospital have been studied. They comprise a series of cases of both sexes, with burns verying in extent from 1 per cent. to 90 per cent. of body surface (Berkow, 1924), and for descriptive purposes they have been grouped as follows:-

Group I ... Burns involving 1-5 per cent. of the body surface.*
Group II ... Burns involving 6-15 per cent. of the body surface.
Group III ... Burns involving 16-30 per cent. of the body surface.
Group IV ... Burns involving over 30 per cent. of the body surface.

III. METHODS.

GENERAL CONSIDERATIONS.

Samples of blood were obtained by venepuncture in almost all cases. Where results recorded were obtained, instead, from capillary samples, attention is drawn to this fact. For the withdrawal of blood from a vein, a

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* For details of individual cases cited in the text - i.e. age, sex, site and degree of burning, ultimate fate, etc. see Appendix (p. x.).

dry sterile needle was used. The blood was collected in a heparinized tube without the use of a syringe, temporary stasis being induced. Examinations were made with the least possible delay, almost always within an hour of bleeding. An automatic shaker was used prior to sampling.

In the great majority of cases haemoglobin estimations, red cell counts and haematocrit determinations were made on the same sample of blood; in the remaining patients, apart from those from whom only capillary samples were obtained, the red cell counts were omitted. In regard to the assessment of haemoconcentration or anaemia, there was no significant difference between the values obtained for red cell counts and haematocrit or haemoglobin determinations.

Except when special reasons to the contrary exist, throughout this study haemoglobin concentrations have been charted. Preference has been given to haemoglobin values, because they are the most accurate of the three estimations.

In cases described here in which only capillary blood was obtained, estimation of haemoglobin was carried out, or a capillary haematocrit tube was used.

HAEMOGLOBIN.

At the beginning, the estimations in a few cases were performed by the alkaline haematin method (Cohen and Taylor, modified by Wu; Peters and van Slyke, 1932),

comparison being carried out in a Duboscq colorimeter. Almost all the results recorded here were obtained by the use of the single cell photoelectric colorimeter (p.11), using carboxyhaemoglobin as the pigment, and a Chance-Watson green filter. Throughout the work, the same 10 ml. pipette, delivering 9.998 ml. at 20°C., was used for 0.4 per cent. ammonia, to which was added 0.100 ml. of blood. The same apparatus was used in calibrating the instrument, a normal blood of known oxygen capacity (van Slyke) being obtained for this purpose.

In the case of capillary samples, 0.05 ml. of blood was delivered directly from the skin puncture into 5 ml. of ammonia.

Throughout the investigation, the instrument was checked against oxygen capacity, and by the insertion of a neutral screen, as described in Part I of this work. No significant variation in sensitivity was found to occur. In recording serial estimations on samples of venous blood, differences of 0.2 gm. haemoglobin per cent. or more are regarded as significant. The almost perfect correlation with oxygen capacity has already been described (p.26).

HAEMATOCRIT.

The packed cell volume was estimated from a sample of venous blood by use of the Wintrobe haematocrit tube. When only capillary blood was available, a heparinized teated capillary pipette was em ployed to transfer

the blood from the needle puncture to the capillary haematocrit tube. An angle centrifuge was used, the specimen being spun at about 3,000 revolutions per minute for 30 minutes.

BLOOD PRESSURE.

Blood pressure readings were obtained by use of a mercurial sphygmomanometer in adults. An instrument of the dial type and with special 2-in. cuff was used for infants and children (Messrs. Down Bros., Ltd.). The readings in this instrument were checked from time to time against the mercury manometer.

C H A P T E R III.

HAEMOCONCENTRATION AND SHOCK.

I. INTRODUCTION.

In attempting to assess the significance of haemoglobin levels in relation to burns, isolated estimations are valueless, both because of lack of knowledge of the pre-burn level and on account of the wide range of values obtained in apparently healthy individuals. The only possible exception to this is the occurrence of a value much above normal. Serial blood examinations are necessary, and the times at which these should be made are conditioned by the rate at which changes may be expected to occur.

In the first part of this report, changes in haemoglobin concentration are regarded as indicating changes in plasma volume, a rising haemoglobin as indicating haemoconcentration due to loss of plasma. The questions of inequality of distribution of red cells, and of the addition of red cells to the circulation from reservoirs such as the spleen, are discussed later. The occurrence of haemolysis, and the excretion of haemoglobin by the kidneys, are also factors which interfere with the

assessment of changes in blood volume on the basis of haemoglobin estimations; they are factors encountered only in very severe and extensive burns.

In the shock period - i.e. in the first 72 hours after burning - haemoconcentration may occur with considerable rapidity and it may continue for a few hours or for almost three days. At the end of this variable period, the red cell values begin to fall. In the severe cases reported here, frequent blood samples were obtained during the first 48 hours, because they were necessary to control the administration of serum or plasma. In the majority of the milder cases, less frequent observations were made, and in some it is impossible to demonstrate a rise in haemoglobin and haemoconcentration. Often, however, a definite fall on the second or third day justified the assumption that some degree of haemoconcentration had probably occurred.

The blood values obtained after the shock period cannot be used to indicate the probable pre-burn levels because, as is shown later, these values may be subnormal.

II. HAEMOCONCENTRATION - GROUP I.

(Burns 0-5 per cent. of body surface).

MAGNITUDE OF HAEMOCONCENTRATION.

This group is represented by males of ages from 16 to 60 years. None was given transfusion and none

Fig. 1

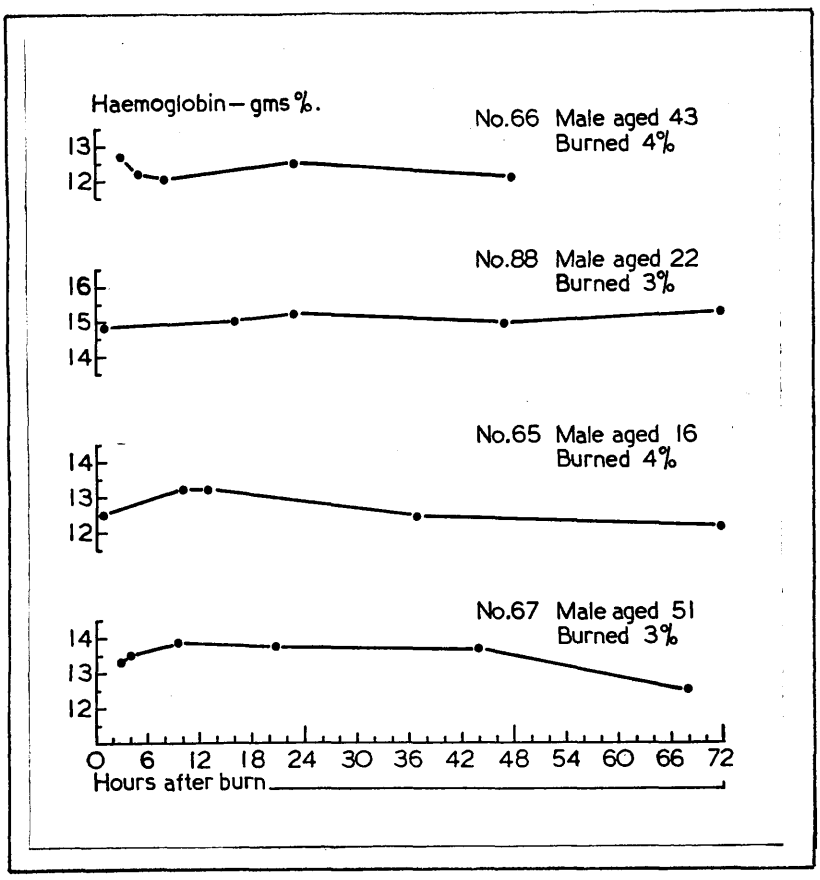
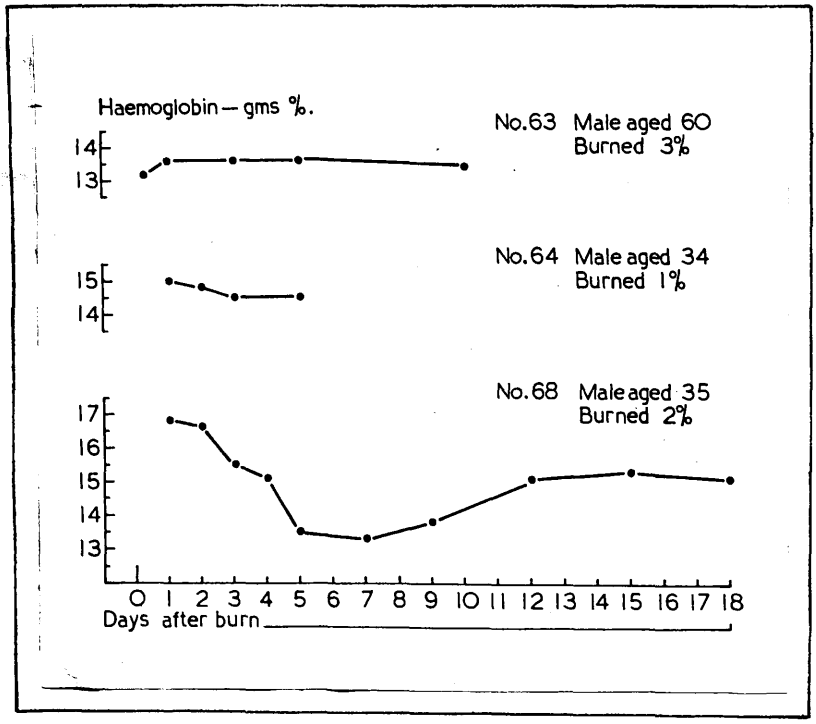

Haemoglobin – gms %.
No.66 Male aged 43
Burned 4%
13
12
No.88 Male aged 22
Burned 3%
16
15
14
No.65 Male aged 16
Burned 4%
14
13
12
No.67 Male aged 51
Burned 3%
14
13
12
0 6 12 18 24 30 36 42 48 54 60 66 72
Hours after burn

Fig. 2


Haemoglobin – gms %.
No.63 Male aged 60
Burned 3%
14
13
No.64 Male aged 34
Burned 1%
15
14
No.68 Male aged 35
Burned 2%
17
16
15
14
13
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Days after burn

developed symptoms of shock. In the majority, a distinct cycle of changes in haemoglobin level occurred during the first week, but little alteration is recorded during the first 72 hours (Fig. 1). These changes in some cases are regarded as beyond experimental error, but, since they do not exceed the daily variations found in normal subjects (p.36), they cannot be regarded as necessarily related to the burn.

This absence of change in the first 72 hours, or in the first week, is not representative of the Group, for in some cases, in which paucity of readings during the first 24 hours precluded their being considered at this stage, much more obvious changes occurred during the first week. Three examples of this are shown in Fig. 2. Case No. 68 is of special interest, in that changes of the magnitude seen here surpass those found in many patients much more extensively burned.

DURATION OF HAEMOCONCENTRATION.

In all the cases in this Group, any tendency to haemoconcentration was seen during the first 12 hours and a fall was apparent by the second day.

BLOOD PRESSURE.

Of the patients in this Group, Case 67 (Fig. 1) showed a blood pressure of 155/80 on admission at 3 hours after burning. The systolic pressure rose to 160 mm. Hg. at the fourth hour, and by the ninth hour a slight fall

had occurred (140/80). A final systolic level of 120-130 mm. Hg. was reached by the 21st hour, and was maintained until the patient's discharge on the 9th day. In this case, the rising blood pressure occurred during the phase of haemoconcentration. In Case 65, though the initial reading of 108/70 was well within normal limits, a final systolic level of 95-102 mm. Hg. was reached at the 9th hour and maintained till dismissal. Such a finding was encountered in all groups. In Case 88 no significant change in haemoglobin levels was recorded (Fig. 1); a blood pressure of 118/80 at 1 hour after burning was unchanged fifteen hours later.

SUMMARY.

In Group I, changes in blood levels probably indicating slight haemoconcentration occurred during the first 72 hours after burning. In the cases recorded, these changes were closely similar, but the total magnitude of the changes occurring in the first week bore little relation to the extent of the burn; and it may be that the changes recorded here in the short period of the study are not entirely representative of this Group.

Blood pressure readings above normal, both absolutely and, in many cases, relatively, have been encountered in the first few hours. The high blood pressure may occur at the same time as haemoconcentration.

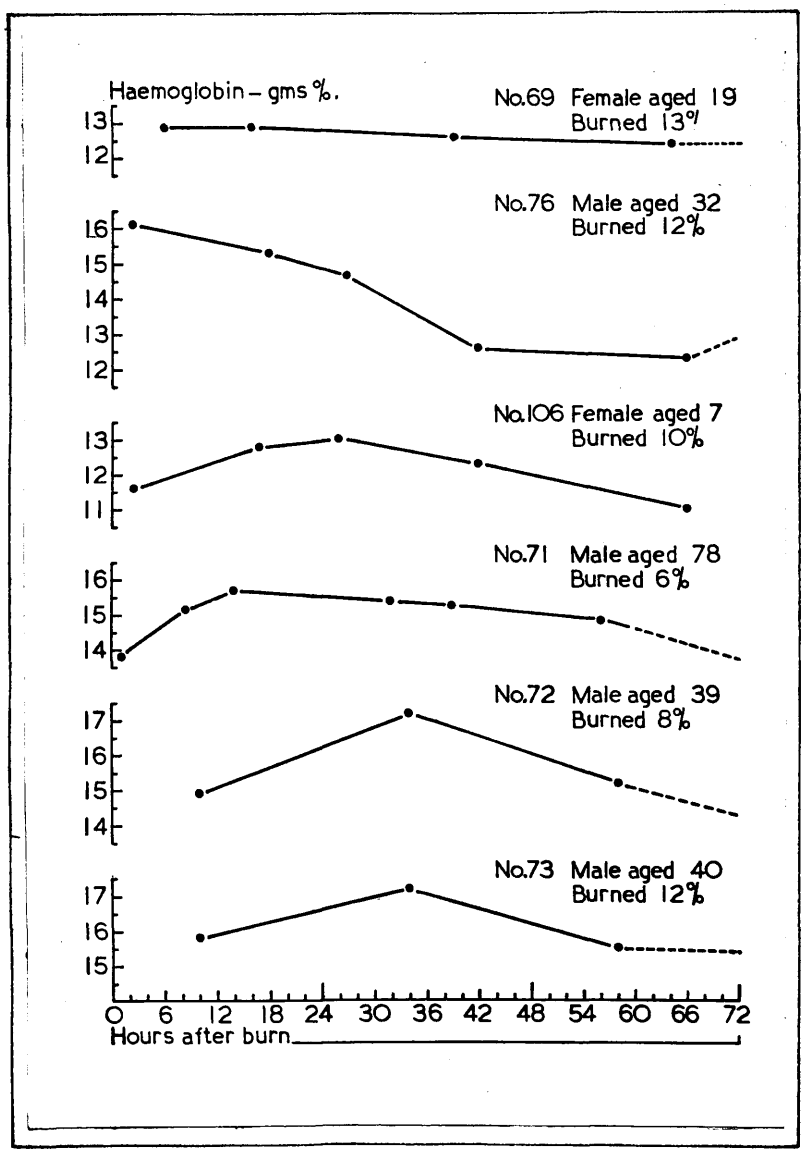

Haemoglobin – gms %.
No.69 Female aged 19
Burned 13%
No.76 Male aged 32
Burned 12%
No.106 Female aged 7
Burned 10%
No.71 Male aged 78
Burned 6%
No.72 Male aged 39
Burned 8%
No.73 Male aged 40
Burned 12%
0 6 12 18 24 30 36 42 48 54 60 66 72
Hours after burn

Fig. 3

III. HAEMOCONCENTRATION - GROUP II.

(Burns 6-15 per cent. of body surface).

The majority of the cases to be described in this Group are included to illustrate the changes in haemoglobin level immediately following injury. In some, the blood pressure is correlated with these changes, and in a few only blood pressure readings are reported. No case developed symptoms of shock.

MAGNITUDE OF HAEMOCONCENTRATION.

In the study of haemoconcentration, the cases have been divided into two groups - 5 patients who received serum transfusion, and 6 who did not. The latter will be considered first.

Reference to Fig. 3 reveals that the blood changes in Group II are more definite than those in Group I. But the magnitude of the changes varies considerably, and the variation is not related only to the extent of the burn. In comparison with those in other cases, the changes in Case 69 are conspicuous by their absence, in spite of the extent of the burn, while relatively minor burns (Cases 71 and 72) are associated with very significant haemoconcentration. Attention is again drawn to Case 68 (Fig. 2) and to Case 69 (Fig. 3). In the latter, no change occurred in haemoglobin levels during the 15 days following the burn.

The changes in haemoglobin levels in the shock

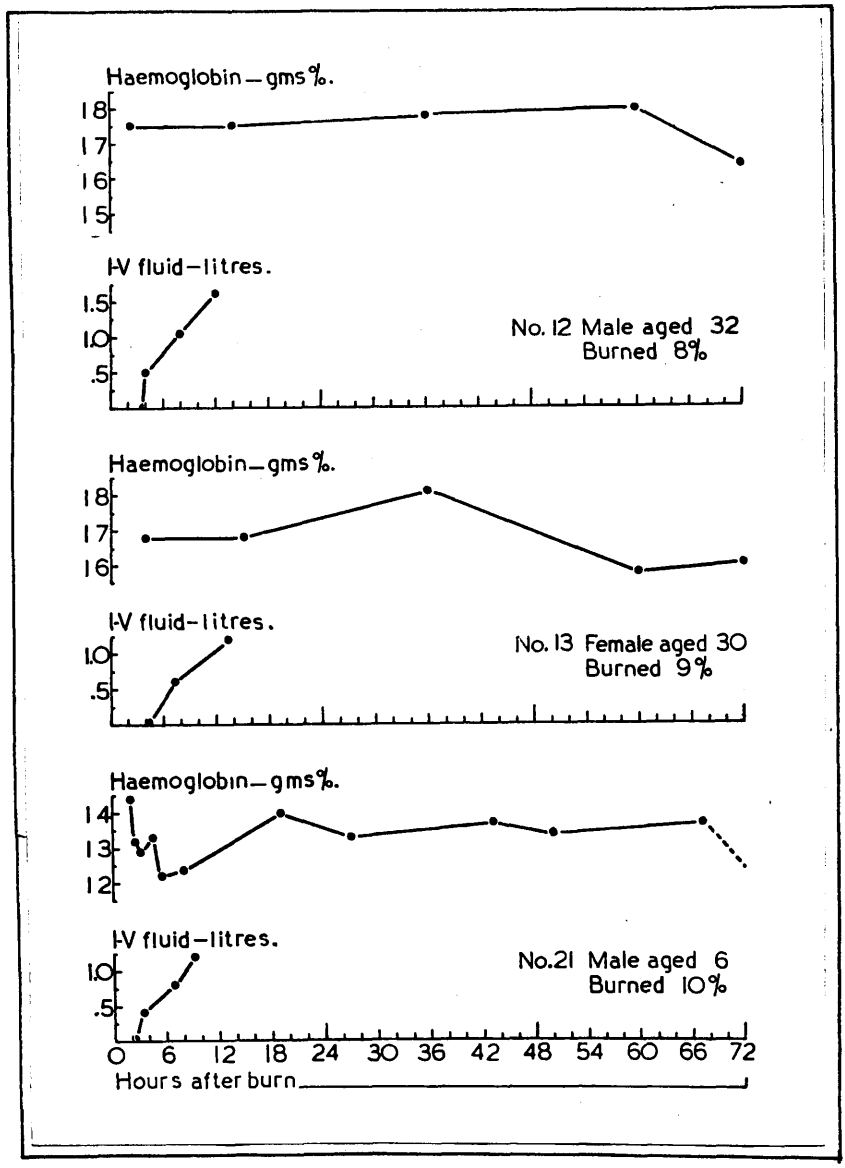

Haemoglobin – gms %.
18
17
16
15
I-V fluid – litres.
1.5
1.0
.5
No. 12 Male aged 32
Burned 8%
Haemoglobin – gms %.
18
17
16
I-V fluid – litres.
1.0
.5
No. 13 Female aged 30
Burned 9%
Haemoglobin – gms %.
14
13
12
I-V fluid – litres.
1.0
.5
No. 21 Male aged 6
Burned 10%
0 6 12 18 24 30 36 42 48 54 60 66 72
Hours after burn

Fig. 4

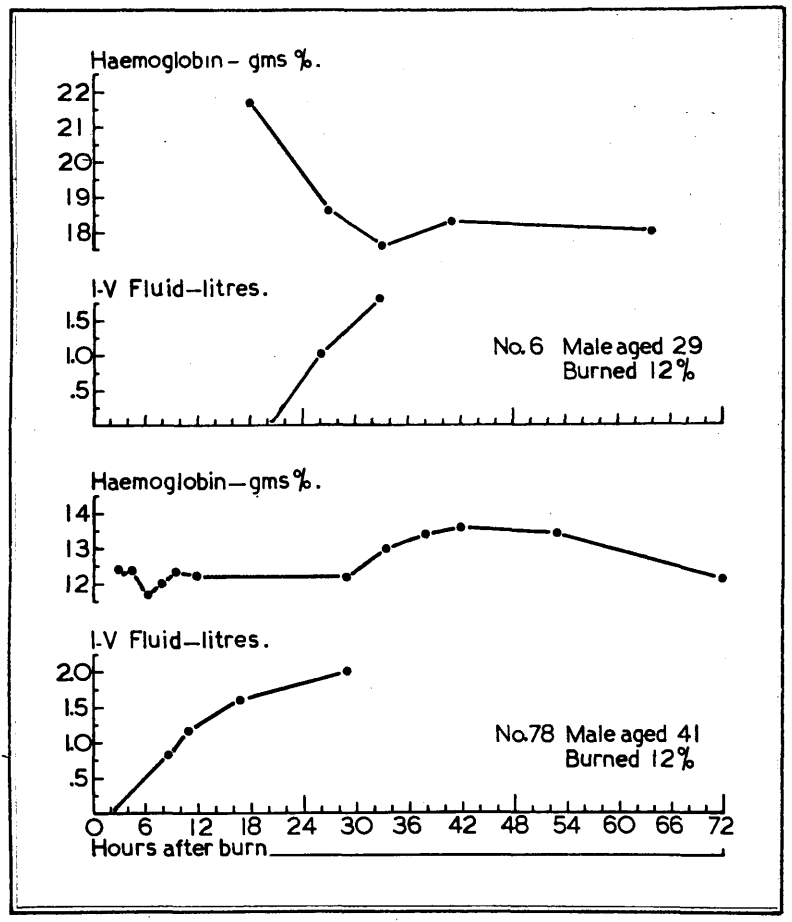

Haemoglobin - gms %.
22
21
20
19
18
I-V Fluid—litres.
1.5
1.0
.5
No. 6 Male aged 29
Burned 12%
Haemoglobin—gms %.
14
13
12
I-V Fluid—litres.
2.0
1.5
1.0
.5
No. 78 Male aged 41
Burned 12%
0 6 12 18 24 30 36 42 48 54 60 66 72
Hours after burn

Fig. 5

period are viewed more closely in patients transfused. Cases 12 and 13 (Fig. 4) are similar in many respects. The subjects were of the same age, though of different sex, and the burns differed little in extent. Given like amounts of fluid in the same period after the burn, the two patients showed very similar haemoglobin curves. In these two cases, administration of serum at a rate of 3.0 and 3.2 ml. per minute for 9 and 8.5 hours, respectively, in the first 12 hours, was accompanied by absolute maintenance of haemoglobin levels, and presumably also of plasma volume. In both, however, as soon as administration of serum ceased, there was a slight tendency, more marked in Case 13, for the haemoglobin to rise.

In Case 21 (Fig. 4), a child of 6 years, administration of serum at about the same rate (2.9 ml. per minute for 7.5 hours in the first 12 hours) resulted in haemodilution, previous levels being rapidly regained after cessation of transfusion.

In Case 6 (Fig. 5) marked haemoconcentration was present at 18 hours. Administration of serum at a rate of 2.3 ml. per minute from 20 to 33 hours sufficed to produce marked haemodilution, and to restore a haemoglobin level on the high side of normal.

In Case 78 (Fig. 5), during the first 12 hours, transfusion of 2.2 ml. of serum per minute maintained steady haemoglobin values. In the following 17 hours,

with administration of 0.75 ml. per minute, these levels remained unchanged. When transfusion was discontinued, the haemoglobin level rose to a maximum at 42 hours and then slowly declined.

These findings are summarised in Table III. It is evident that fluid requirements tend to be greater in the first 12 hours after injury.

TABLE III.

Patient.		Fluid Transfused.			
		First 12 hours.		Second 12 hours, etc.	
Case No.	Extent of Burn (per cent. of body surface)	Rate ml./min.	Effect on Hb.	Rate ml./min.	Effect on Hb.
12	8	3.0	Level maintained	None	Slight rise
13	10	3.2	Level maintained	None	Slight rise
6	12	None	Marked elevation	2.3	Marked fall
78	12	2.2	Level maintained	0.75	Level maintained

DURATION OF HAEMOCONCENTRATION.

Reference to Fig. 3 reveals that in the cases not transfused, maximum haemoglobin levels were recorded for 2.25 to 36 hours after burning. The chart shows the limits within which this is an accurate estimate. Case 76 shows an unusual reaction, with a rapid fall from a maximum level of 16.05 g. haemoglobin per cent. at 2.5 hours to 12.4 g. per cent. at 66 hours. Subsequently, a

steady rise to a level of 15.1 g. per cent. occurred, with stabilisation at this level from the 8th day. If this last level indicates even very approximately the patient's normal level, haemoconcentration must have been of rapid onset and short duration.

It is evident also, from Figs. 4 and 5, that there are great differences in the duration of progress of haemoconcentration - 60 hours in Case 12; 42 hours in Case 78; and about 36 hours in Case 13.

Administration of serum to a normal individual may produce haemodilution which, when transfusion is stopped, will be followed by a rise in haemoglobin - "haemoconcentration"; but in the three cases just cited (12, 13 and 78), from the commencement of the transfusion, no haemodilution was produced. It seems highly probable, therefore, that the subsequent rise in haemoglobin in these patients indicated continuation of plasma loss from the circulation.

BLOOD PRESSURE.

Blood pressure records in this Group (Table IV) show a number of cases in which a high initial level and a subsequent fall occurred in the first few days, or in which a normal or slightly low blood pressure was recorded on admission. Several patients showed a rise in blood pressure in the first 48 hours.

TABLE IV.

GROUP II. - BLOOD PRESSURE READINGS.

(a) No transfusions given.

Case No.	Days after Burning. 0	1	2	3	4	5	6	7	Remarks.
69	108/68	108/58	104/65	106/65	114/60	108/60	108/60		Minimal change in Hb. (Fig. 3)
70		120/74	128/80	124/78	128/74	124/70	120/70	122/80	Moderate Hb. change.
71	130/90 ... 150/100	160/100							Definite Hc. with rise in B. P. (Fig. 3)
75	112/72 ... 110/72	96/66	98/64	98/60	700/60		102/62	100/62	Marked Hb. change.
76	150/90								Marked Hb. change. (Fig. 3)
106	112/64	110/64	110/70	100/72	106/80	95/72	108/76	106/78	Slight blood change. (Fig. 3)
117	104/68	120/72 ... 128/85	116/72	114/72	105/72	94/70	102/68	94/68	Practically no Hb. change.
118		116/70	100/68	98/65		100/68			No bloods examined.
119	125/70	110/70	110/70		108/70				No bloods examined.

TABLE IV. (Contd.)

(b) Transfused.

Case No.	Hours after burning. 3	6	9	12	15	18	21	24	Remarks.
6	108/72	106/72			96/72	100/72	110/74		Transfusion begun at 20 hrs. P.C.V. 58.5 per cent., Hb. 21.8 g. per cent. at 18 hrs.
12	130/30			125/76					Very little change. (Fig.4).
13	120/48								(Fig.4).
21	120/75	118/78	115/78			104/68	106/70		B.P. established ultimately at 100/72-106/75. (Fig.4).
26	108/78			90/68	112/70	120/78	120/80	116/76	
78	170/90	145/100	150/90	148/98	138/85				No excitement at all. Ultimately established at 145/100-140/85.

In Case 69, a steady level was maintained during the first 3 days. No explanation is available for the higher reading on the 4th day. Case 71 shows a rise from 130/90 at 1 hour to 150/100 at 13.75 hours, and to 160/100 at 32 hours. The rise accompanied the process of haemoconcentration in a phlegmatic elderly male. In Case 75, the highest blood pressures recorded occurred at 1 and 5 hours (blood values were not determined until the 17th

hour). Case 76 showed a pressure of 150/90 at 2.5 hours, when the highest recorded haemoglobin occurred (Fig. 3). In Case 106, little definite change in blood pressure or haemoglobin is recorded. Case 117 shows a rise in blood pressure on the 2nd day, associated with little alteration in haemoglobin. A relatively high blood pressure was recorded 24 hours after burning in Case 118, and 1 hour after burning in Case 119.

Among those transfused, Cases 12 and 13 showed no upset in blood pressure. In Case 21 relatively high readings were obtained during the first 12 hours. These high levels were never again recorded during the period of hospitalisation. In Case 26, the initial blood pressure readings were low and administration of fluid was accompanied by a rise. The initial high reading in Case 78 occurred on admission, when the patient was drowsy and under the influence of morphine, and no excitement was apparent. It is probable that in this case the initial haemoglobin level of 12.4 g. per cent. represented haemoconcentration, for the patient was found to be iron-deficient, due to chronic loss of blood from haemorrhoids, and when, after 2 months' intensive iron therapy, his red cells numbered more than 5 million per c.mm., the haemoglobin was still only 12.2 g. per cent.

A rise in blood pressure was thus recorded in several patients at a time when maximum haemoconcentration

was occurring. On the other hand, it occurred in Case 117, who showed no significant alteration in haemoglobin levels; and in Case 106 the maximum reading was recorded when the haemoglobin was at a level again reached and finally maintained at the end of a week. Case 106 was one in whom excitement was obvious on admission, and throughout her stay in hospital she proved to be of very nervous disposition.

SUMMARY.

The great majority of patients investigated in this Group showed blood changes probably indicative of alteration in plasma volume. These alterations were not directly related only to the extent of the burn: degree (depth) and site may have played a part in determining their magnitude. Haemoconcentration ceased within the first 24 hours, or continued well into the third day. The evidence obtained from administration of fluid suggests that the rate at which fluid is lost rapidly reaches a maximum in the first 12 hours; that during the ensuing period - the duration of which is variable - loss occurs less rapidly; and that, finally, at a variable time, alteration in the balance between loss and return allows haemodilution to occur.

In this Group, low blood pressure may be recorded on admission within a few hours of burning, but attention is drawn to the frequency with which high or

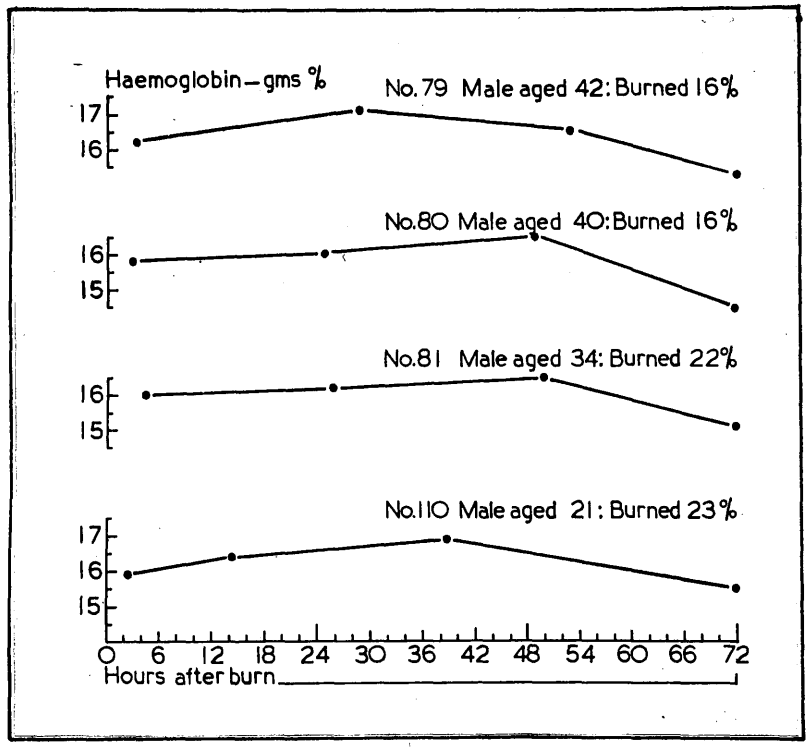

Haemoglobin – gms %
No. 79 Male aged 42: Burned 16%
17
16
No. 80 Male aged 40: Burned 16%
16
15
No. 81 Male aged 34: Burned 22%
16
15
No. 110 Male aged 21: Burned 23%
17
16
15
0 6 12 18 24 30 36 42 48 54 60 66 72
Hours after burn

Fig. 6

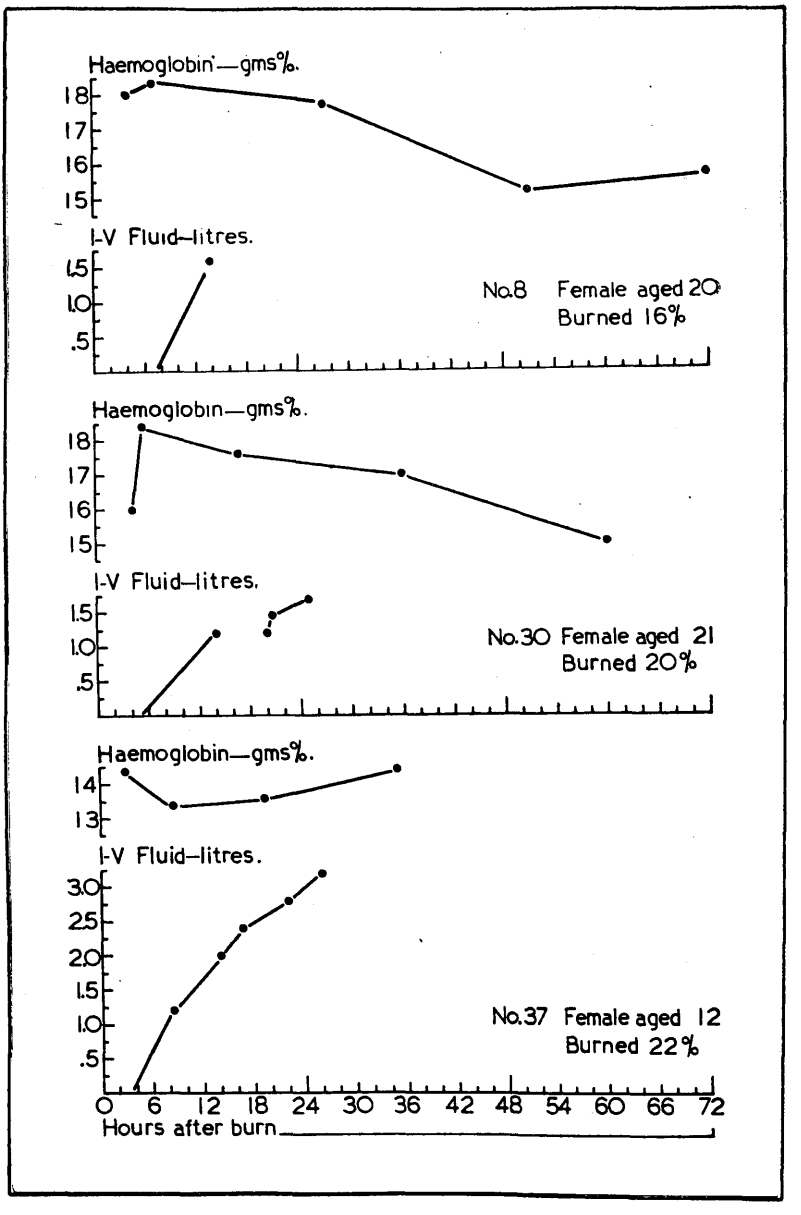

Haemoglobin—gms%.
18
17
16
15
I-V Fluid—litres.
1.5
1.0
.5
No.8 Female aged 20
Burned 16%
Haemoglobin—gms%.
18
17
16
15
I-V Fluid—litres.
1.5
1.0
.5
No.30 Female aged 21
Burned 20%
Haemoglobin—gms%.
14
13
I-V Fluid—litres.
3.0
2.5
2.0
1.5
1.0
.5
No.37 Female aged 12
Burned 22%
0 6 12 18 24 30 36 42 48 54 60 66 72
Hours after burn

Fig. 7

relatively high values occur, and to the occurrence of these at a time when haemoconcentration is at, or is approaching, its maximum. The blood pressure may, however, be high without any apparent change in haemoglobin levels.

IV. HAEMOCONCENTRATION - GROUP III.

(Burns 16-30 per cent. of body surface).

MAGNITUDE OF HAEMOCONCENTRATION.

Few patients in this Group did not receive transfusion, and only four such cases are described (Fig. 6). The changes in the first 72 hours were remarkably similar in all, and differed little from the more obvious alterations encountered in Group II. These patients not transfused were those who, in the early stages of the investigation, were left to be transfused should need become apparent clinically. The changes therefore are probably the least which may occur in burns in This Group. The changes, in addition, may represent roughly the maximum which may be unassociated with symptoms.

The majority of the patients received transfusion of serum (Figs. 7-9). In the two young adults least burned (Fig. 7: Cases 8 and 30) abnormally high haemoglobin levels occurred during the first 6 hours. With commencement of transfusion, no fresh haemoconcentration was recorded, and in the second 12 hours little or no serum was required. A fall of haemoconcentration to relatively normal levels

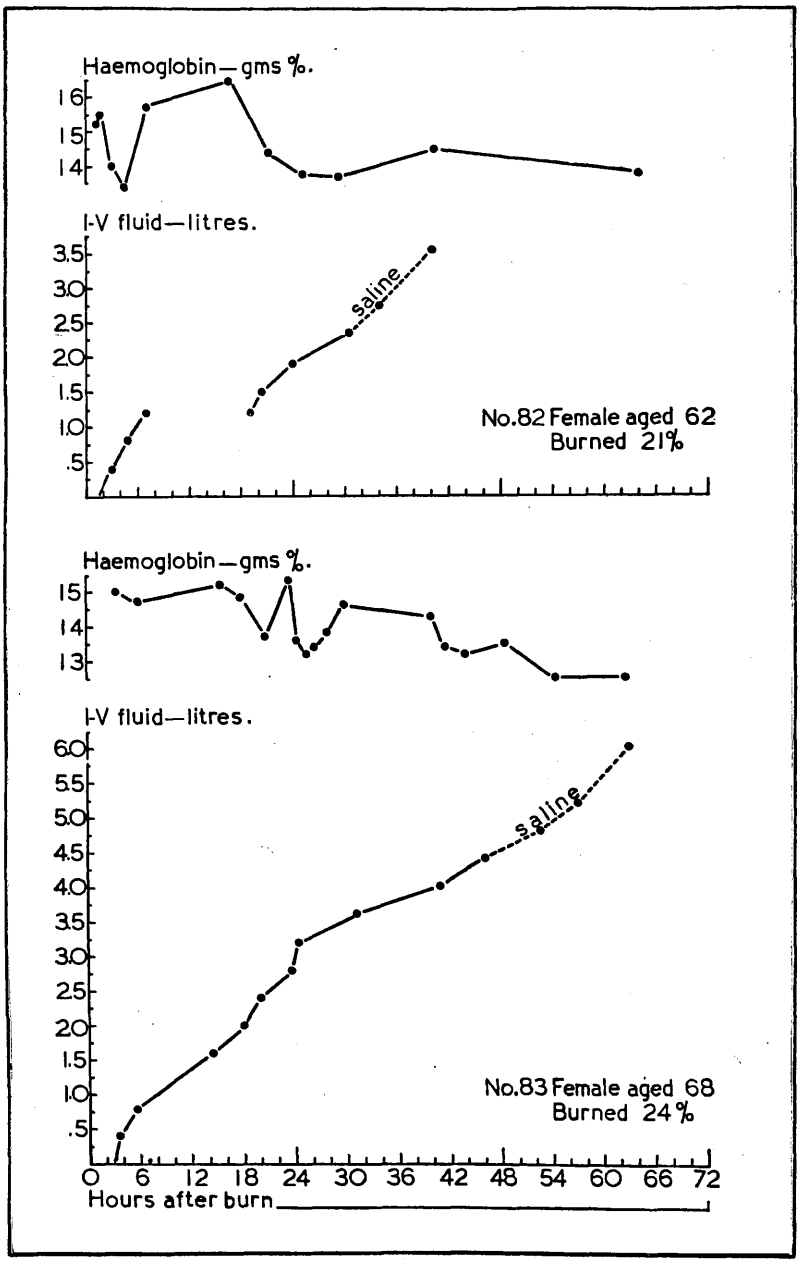

Haemoglobin — gms %.
16
15
14
I-V fluid — litres.
3.5
3.0
2.5
2.0
1.5
1.0
.5
saline
No.82 Female aged 62
Burned 21%
Haemoglobin — gms %.
15
14
13
I-V fluid — litres.
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
saline
No.83 Female aged 68
Burned 24%
0 6 12 18 24 30 36 42 48 54 60 66 72
Hours after burn

Fig. 8

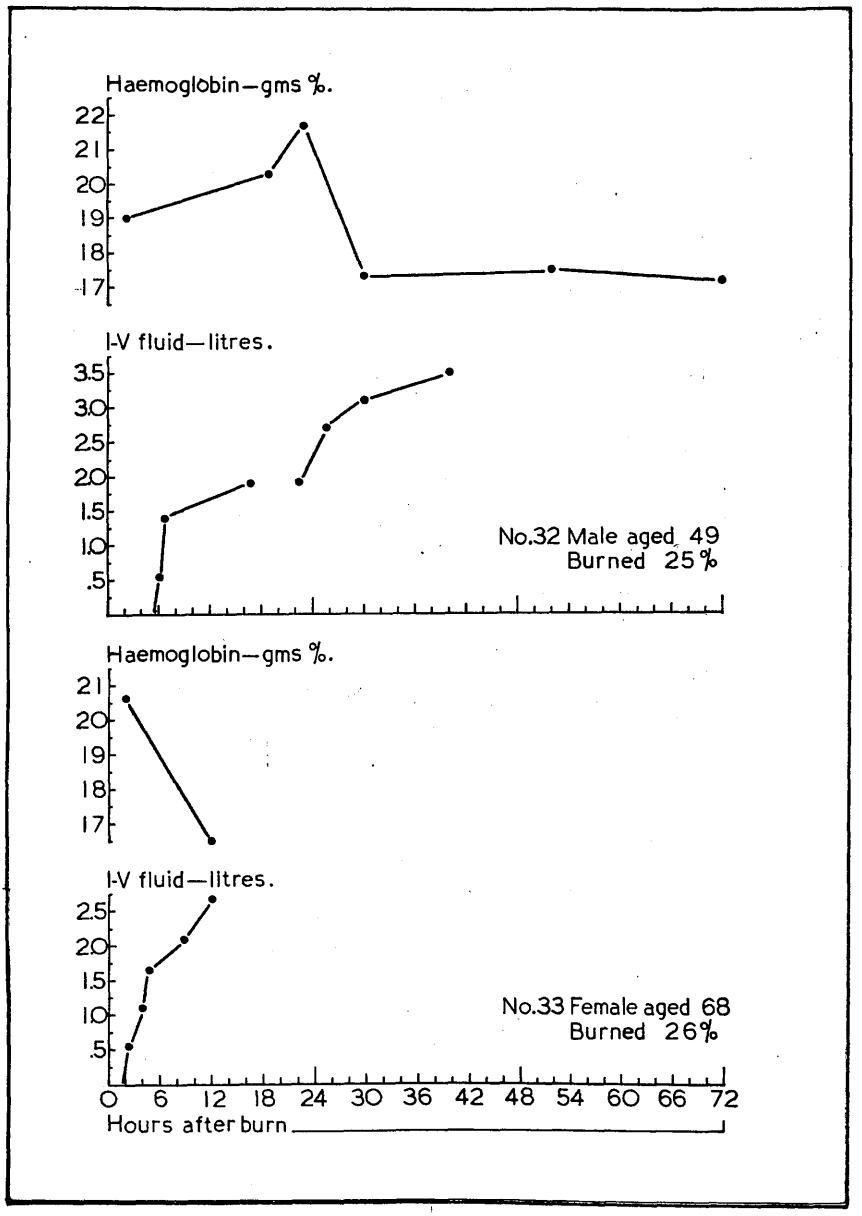

Haemoglobin—gms %.
22
21
20
19
18
17
I-V fluid—litres.
3.5
3.0
2.5
2.0
1.5
1.0
.5
No.32 Male aged 49
Burned 25%
Haemoglobin—gms %.
21
20
19
18
17
I-V fluid—litres.
2.5
2.0
1.5
1.0
.5
No.33 Female aged 68
Burned 26%
0 6 12 18 24 30 36 42 48 54 60 66 72
Hours after burn

Fig. 9

occurred by the end of the third day.

In Case 37, that of a child, a relatively large transfusion was required, but the evidence available does not suggest any diminution in the rate of haemoconcentration during the second 12 hours (Fig. 7).

During the first 7 hours in Case 82 (Fig. 8), the result of serum transfusion at a rate of 4 ml. per minute was to leave the haemoglobin value little removed from its original level. But between the 18th and 29th hours, transfusion at the rate of 1.6 ml. per minute was associated with haemodilution, in spite of the fact that a rise in the haemoglobin value followed substitution of saline for serum at the end of this period.

In Case 83 (Fig. 8), in the first 12 hours, transfusion of serum at a rate of 2.4 ml. per minute maintained haemoglobin levels practically unaltered, while in the second 12 hours 2.2 ml. per minute caused slight haemodilution. Reduction in the rate of administration to 1 ml. per minute during the following 7 hours was followed by a rise in blood levels for a short period.

In Case 32 (Fig. 9), serum transfusion at a rate of 3.2 ml. per minute between 5.5 and 17 hours was inadequate, while between 22 and 31 hours, 2.5 ml. per minute caused marked haemodilution.

In Case 33 (Fig. 9), 4.5 ml. per minute from the 2nd to the 12th hour corrected a marked degree of haemoconcentration.

These findings are summarised in Table V.

TABLE V.

Patient.		Transfusion.			
		First 12 hours.		Second 12 hours.	
Case No.	Extent of Burn (per cent. of body surface)	Rate ml./min.	Effect on Hb.	Rate ml./min.	Effect on Hb.
82	21	4	Level maintained	1.6	Marked fall.
83	24	2.4	Level maintained	4.2	Marked fall.
32	26	2.9	Moderate rise.	2.5	Marked fall.
33	26	4.5	Marked fall.	-	-

DURATION OF HAEMOCONCENTRATION.

Though greater haemoconcentration and fluid requirements were noted in this Group, no case showed prolongation of this phase beyond that found in Group II, in which the burns were less extensive.

In Cases 79, 80, 81 and 110 (Fig. 6), none of whom received transfusion, the duration of progressive haemoconcentration varied from about 36 to 60 hours.

In Cases 8 and 30 (Fig. 7), haemoconcentration proceeded for not more than 24-36 hours. In Case 82 the duration of haemoconcentration was about 40 hours; in Case 83, it was not more than 48 hours, and in Case 32 about 52 hours.

BLOOD PRESSURE.

Blood pressure records in 10 patients in this

Group are summarised in Table VI. An initial high reading was recorded in six.

TABLE VI.

Case No.	Hours after Burning. 1-2	3	6	9	12	15	18	21	24	Remarks.
8		125/80	90/54	Treat-ment.			118/?			Allowed to develop Hc.* (Fig.7)
19	90/60	120/70	120/70	120/70		125/70	150/80	130/80		B.P. rose with treatment.
30	120/?	116/86	98/56		100/56			108/56	114/60	Allowed to develop Hc. (Fig.7)
32			144/98				102/82		96/80	B.P. fell with progress. (Fig.9)
33	200/100	125/30	165/100	150/100						B.P. fell with haemodilution. (Fig.9)
37		110/80	90/60	110/80	104/60	108/70	95/60		100/70	Treated at once. (Fig.7)
38	165/92 148/86	128/92				110/90		126/90		Same as Case 32.
82	179/94	122/80	120/70			110/70	95/68	115/78	128/76	Treated at once. (Fig.8)
83		192/90	188/90	120/75		169/90	160/88	160/84	166/90	Treated at once (Fig.8)
110		150/90								(Fig.6)

*Hc. = haemoconcentration.

In Cases 8 and 30, the blood pressure, normal at first, fell below normal and rose again with

transfusion. The normal initial level was associated with haemoconcentration in both.

In Case 19, it is of interest that, with haemoconcentration on admission, a low blood pressure was recorded. It rose to normal level with slight haemodilution, and to 150/80 when haemoconcentration again developed due to inadequate treatment.

In Case 32, transfusion was inadequate in the first 24 hours, and the blood pressure fell below normal. The slightly high pressure recorded at 6 hours was associated with haemoconcentration.

In Case 38, the high level at 0.75 hour fell to normal at 3 hours when the haemoglobin was recorded at 18.6 g. per cent.

In Cases 82 and 83, both elderly women, initial hypertension was associated with haemoglobin levels within normal limits. It is impossible to say whether the levels actually represented haemoconcentration in these patients.

In Case 110, a reading of 150/90 was associated with a haemoglobin of 15.9 g. per cent.

In these 6 cases, the high blood pressure levels were recorded with the highest haemoglobin levels in 3 where definite haemoconcentration was present in 2 and at a time when haemoconcentration might be expected, in all. A normal blood pressure in association with haemoconcentration is shown in 2 cases.

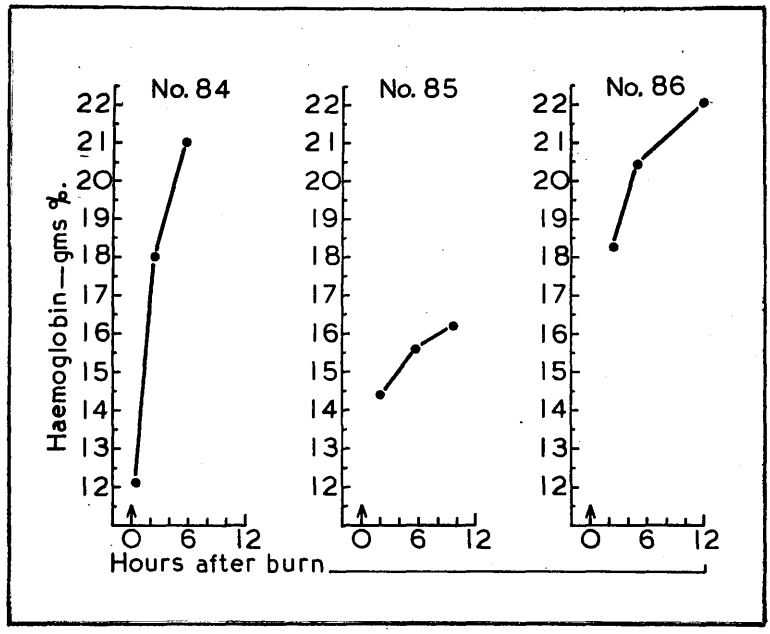

No. 84
No. 85
No. 86
Haemoglobin—gms %.
22
21
20
19
18
17
16
15
14
13
12
0
6
12
Hours after burn

Fig. 10

SUMMARY.

All patients in Group III showed marked blood changes, but no extension of the progress of haemoconcentration beyond 72 hours is seen. Low blood pressure was recorded 1.25 hours after burning in Case 19, but attention is drawn to the occurrence of high and normal blood pressures associated with haemoconcentration in the untreated cases. In some cases, these high or normal levels preceded a fall to subnormal levels, accompanied by a state of shock, for which transfusion had to be given.

V. HAEMOCONCENTRATION - GROUP IV.

(Burns over 30 per cent. of body surface).

Only 3 patients not transfused are described in this Group. All suffered extremely extensive burns. They received only morphine, and all died between 10 and 12 hours after the injury.

MAGNITUDE OF HAEMOCONCENTRATION.

Case 84 (Fig. 10) indicates how rapidly haemoconcentration may supervene. Assuming a previously normal haemoglobin level of 12 g. per cent., the findings suggest that approximately 50 per cent. of the plasma volume was lost from the active circulation in the first 2.5 hours and 70 per cent. 5.5 hours after injury. Assuming a previous normal plasma volume of 3 litres, the calculated rate of plasma loss is 12.5 ml. per minute in

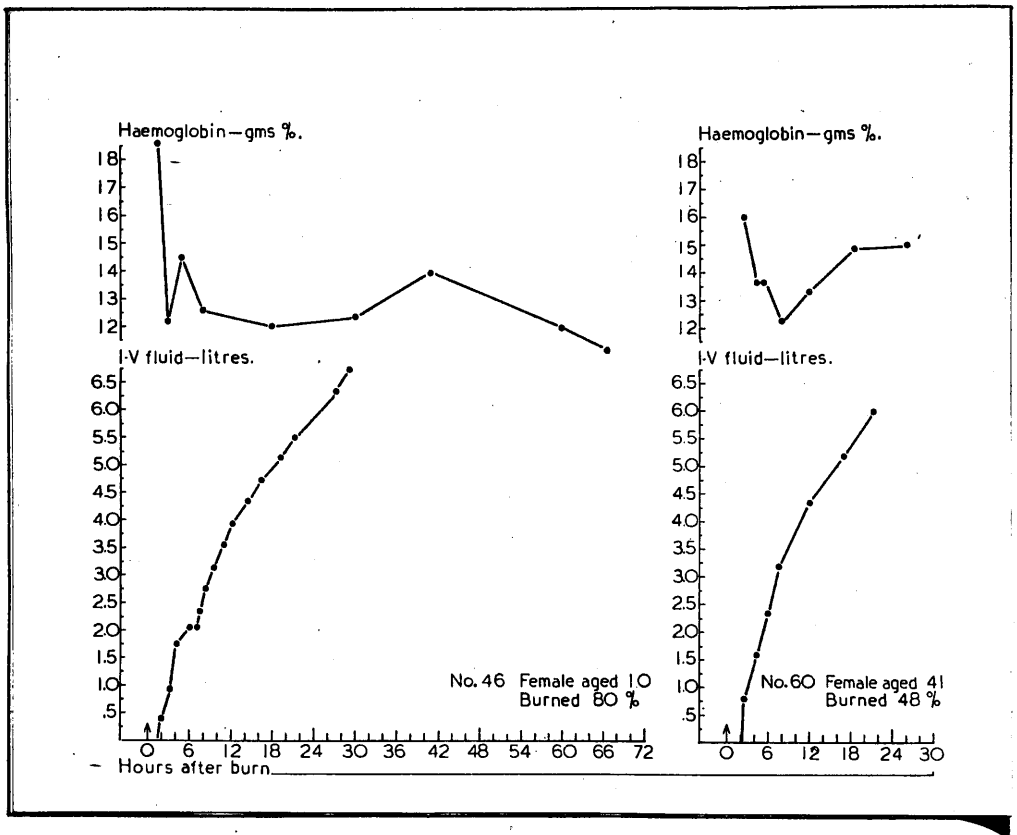

Haemoglobin—gms %.
18
17
16
15
14
13
12
I·V fluid—litres.
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
No. 46 Female aged 10
Burned 80 %
0 6 12 18 24 30 36 42 48 54 60 66 72
Hours after burn
Haemoglobin—gms %.
18
17
16
15
14
13
12
I·V fluid—litres.
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.5
No. 60 Female aged 41
Burned 48 %
0 6 12 18 24 30

Fig. 11

the first 2.5 hours, and 3.3 ml. per minute in the next 3 hours, with an average of 7 ml. per minute during the first 5.5 hours. From the other two Cases (85 and 86), no blood records were obtained in the first hour, and, in view of the findings in Case 84, assessment of the degree of haemoconcentration present is not possible. The rate of development of haemoconcentration in the period 2.5-5.5 hours after injury in Case 85 is less rapid than in Case 84. In Case 86, the curve resembles closely that of Case 84.

Among the cases transfused (Fig. 11) are several which compare very closely with No. 84 in many respects. In Case 46, a child of 10 years, administration of serum at a rate of 6.7 ml. per minute produced rapid haemodilution. In Case 60, not quite so extensively burned as No. 84, administration of serum at a rate of 7.3 ml. per minute in the first 12 hours produced only slight haemodilution and, during 12-21 hours, 3.1 ml. per minute was associated with haemoconcentration.

Any attempt at calculation from haemoglobin levels of the amount of haemoconcentration, and of the amount of plasma lost during a period of hours, assumes that the plasma:cell ratio is the same throughout the whole circulatory system. This is not so. There is evidence (McIver, 1933) that the cell and haemoglobin content of capillary blood may be higher than that of

venous blood. The tendency, therefore, in any assessment of plasma loss based on values obtained from venous blood- as in the Case 84 - is towards an understatement rather than an exaggeration of the loss. At least, however, some idea is obtained from these figures of the rapidity with which the circulating fluid volume may become diminished, and of the extent of reduction which may take place before death in cases of major burns.

DURATION OF HAEMOCONCENTRATION.

Several patients with extensive burns transfused from an early hour, but not recorded graphically here, serve to confirm the rapid and extreme haemoconcentration which may follow extensive burns. The numbers studied are insufficient to correlate extent of injury with magnitude of change, but in those examined there is no evidence of progress of haemoconcentration beyond 60 hours. In some cases in this group, there is met with for the first time a complication which to a certain extent invalidates any attempt to assess the significance of the haemoglobin values, namely, the occurrence of haemoglobinaemia and haemoglobinuria. This complication will be discussed when the phase of anaemia is considered.

BLOOD PRESSURE.

Accurate readings are often difficult or impossible to obtain in cases of widespread and deep burns. In Case 52, however, a youth of 14 burned over 31 per cent.

of his body surface, the blood pressure at 1 hour after injury was 165/114. A haemoglobin level, high but still within normal limits, was recorded at this time (16.3 g. per cent.), but, on the evidence already reviewed, this may have indicated haemoconcentration. With rising blood values, the blood pressure fell to normal. A second case, a child of 9 (No. 49), showed a blood pressure of 130/110 9.75 hours after the burn.

SUMMARY.

In Group IV the development of haemoconcentration was extremely rapid, and the reduction in blood volume - indicated by the magnitude of the change in haemoglobin level - was extreme in many extensive cases, considerably more than half the original plasma volume being lost to the circulation within a few hours.

In spite of the extent of the burns - and the rapidity and degree of haemoconcentration liable to occur - hypertension in the early hours after injury is again recorded. Nevertheless, at least in the patients transfused (they were the only ones who survived the shock period) there was no evidence of the progress of haemoconcentration beyond the first 60 hours after injury.

C H A P T E R IV.

HAEMOCONCENTRATION AND SHOCK.

(Continued).

I. DISCUSSION.

It has long been known that haemoconcentration occurs in many forms of shock, both clinical and experimental. In this work, it has been shown that in patients with burns the degree of haemoconcentration is roughly proportional to the extent of the burn. Attention has been drawn to cases showing disproportionate changes, and it is evident that factors other than extent of the injury may sometimes operate. Some indication of these factors may be obtained by consideration of the pathogenesis of the reduction in circulatory blood volume.

OLIGAEMIA AND CAPILLARY PERMEABILITY.

It is generally agreed that diminution of blood volume in burns shock is due to loss from the circulation of almost unaltered plasma (Underhill & Fisk, 1930: Beard & Blalock, 1931: McIver, 1933). Some of this fluid may be lost from the body as exudate and blister fluid; it varies very much from patient to patient. A considerable quantity escapes into the tissue spaces. This loss may occur

locally at the site of the injury (Underhill, 1930: Underhill, Fisk & Kapsinow, 1930: Blalock, 1931: Minot & Blalock, 1940); by others (e.g., Moon, 1942), it is believed to occur both locally and generally throughout the capillary bed. It is probable that the extent of the burn will influence greatly the local fluid loss, and that the degree (depth) of burning will have much less effect. Only capillaries damaged to an extent causing increased permeability, but at the same time compatible with active, though perhaps sluggish, circulation through them, can contribute to progressive reduction in blood volume. The zone of capillary damage satisfying these criteria around a deep burn may be a little larger than a similar zone related to a more superficial injury similar in extent.

Extent and depth of the injury are not the sole governing factors in the production of haemoconcentration (Fig. 3, p. 97). Since blood volume is largely dependent on the establishment of a balance between the circulating plasma and the extracellular tissue fluid (Best & Taylor, 1939a), it is evident that normally the passage of fluid from the capillaries into the tissue spaces is balanced by the return of fluid from the tissues to the blood stream. Reduction in blood volume will be directly related to the amount by which loss exceeds return. If loss is excessive, haemoconcentration may be prevented by

a corresponding increase in lymph flow. It is probable, therefore, that factors favouring stagnation of fluid in the tissue spaces will favour the occurrence of haemoconcentration and a reduction of blood volume. Absence of muscle movement, laxity of tissues, and obstruction to the flow of lymph may operate in this way.

Muscular movement has been shown to be a powerful factor in promoting flow of lymph (White, Field & Drinker, 1933), and it is of interest to note that sedation by morphine in cases of extensive burns in this investigation seemed to hasten death. There is, however, no proof that the sedative increased the rate of haemoconcentration, and other factors may be involved.

Laxity of tissues allows the accumulation of greater amounts of fluid before a given tissue pressure is reached, and, therefore, before a balance is established between extra- and intravascular fluid. No conclusive clinical evidence relating these features to the degree of haemoconcentration is at present available, but the use of pressure dressings (Siler and Reid, 1942: Swingle, Remington, Kleinberg, Drill and Eversole, 1942) and the closed plaster technique (Katz, Shleser, Asher & Perlow, 1942: Glenn, Gilbert & Drinker, 1943: Katz, Asher & Perlow, 1943: Sellars & Willard, 1943) in the treatment of burns is based on these considerations.

Obstruction to the flow of lymph may occur

directly or indirectly due to burning. Coagulation occurs sooner or later in the plasma distributed through injured tissues (Glenn, Gilbert & Drinker, 1943), and the fibrin slowly produced hinders lymph flow. Experimentally, progressive oedema may be in great part prevented by heparinising the animal prior to burning. The burn may itself interfere directly with lymph flow, particularly if it is situated on the proximal part of a limb, and especially if the proximal part of the burn is deep. In such a case, the flow of lymph may be much affected and maximum accumulation of fluid and reduction of blood volume may be reached at a relatively early stage. This may be the explanation of unusually great reaction seen during the first week in Case 68 (Fig. 2, p. 95). In this patient, an area of complete skin loss extended across the entire width of the arm, just above and below the antecubital fossa. Marked oedema of the forearm and hand occurred within 12 hours, at the end of which time the maximum haemoglobin level was recorded. The oedema had disappeared by the 5th day, and by this time a marked fall in haemoglobin level had occurred.

These considerations are based on the assumption that at least the greater part of the fluid from the circulation escapes through the damaged capillaries at the site of the burn. Underhill, Fisk and Kapsinow (1930) and Blalock (1931) have shown that much loss of fluid

occurs locally into the tissues after burns and other injuries. Moon (1942), on the other hand, supports the contention that there is a generalised increase in capillary permeability, though this view has been disputed by many other authors (e.g. Dunphy, Gibson & Keeley, 1941: Glenn, Muus & Drinker, 1943). Moon cites as evidence the escape of dyes circulating in the plasma at sites remote from the injury during the development of shock; he regards, also, as significant the post-mortem evidence of increase in capillary permeability - capillary engorgement, oedema and petechiae in the lungs and other organs. There is no doubt that extremely congested and oedematous lungs are usually seen in patients dying of shock after burns, and there is no doubt that petechial haemorrhages are frequently seen in the viscera, especially under the endocardium. But these findings indicate only the ultimate result of the burn. They are not necessarily evidence of the primary derangement. The great haemoconcentration occurring in the skin capillaries remote from the injury indicates loss of fluid from these vessels, but this may be the result of capillary anoxaemia, which itself is only secondary to oligaemia produced by loss of fluid into and around the injured area. In other words, the general may follow the local increase in capillary permeability. A vicious circle is thereby established.

This conception is important from another point

of view. Fishberg (1940) points out that if fluid exchange between blood and tissues functioned as usual, reduction in blood volume would be followed by resorption of fluid from, and dehydration of, the tissues. He regards the fact that a fall in plasma protein may not occur in such conditions as indicating general abnormality in fluid exchange. Clinically, however, all patients with any but minor burns complain of thirst, which is frequently very severe in those extensively burned. This suggests tissue dehydration. In addition, an initial fall in plasma protein may be recorded simultaneously with rising haemoglobin values. The evidence is therefore incomplete, and the general increase in capillary permeability may well be a secondary though perhaps an important occurrence, as already suggested.

OLIGAEMIA AND SHOCK.

Primary shock was seen rarely during the period of this study, and in those patients in whom it was thought to be present on admission, it was impossible to exclude the presence of some reduction in blood volume. Whether or not some reduction is present, the disturbance in peripheral circulation associated with the fall in blood pressure may predispose to general capillary upset and the development of secondary shock.

In all patients with severe burns in whom serial blood readings were recorded, haemoconcentration was found

to occur. This change is of significance only in that it reflects the basic upset - a reduction in blood volume. Haemoconcentration per se cannot be regarded as the significant feature in production of shock (Wood & Blalock, 1941). Serial blood readings are the most satisfactory practical method of assessing changes in blood volume, in spite of the various sources of inaccuracy (Underhill, 1927). This author cites inequality of distribution of red cells as one of these. From the evidence discussed above, it is apparent that venous blood shows less haemoconcentration than capillary blood. In practice, it has been found that blood from either source is satisfactory in the control of administration of fluid.

Rising haemoglobin levels may be due to the addition of concentrated red cells to the circulation from a reservoir such as the spleen (Part I, p.45). The occasional occurrence of a slight increase in haemoglobin concentration with a rise in blood pressure may be explained on the basis of this mechanism alone. According to the evidence at present available, however, the possible "haemoconcentration" so produced in man is of minor degree; it may explain small changes, but it does not affect the broad principles already discussed.

Symptoms are probably only roughly proportional to the degree of haemoconcentration present. Their appearance bears a more constant relation to the severity

of the burn. Few patients with burns involving more than 15 per cent. of the body surface fail to develop the features of shock if untreated. It is difficult to correlate symptoms with blood changes, on account of the absence of precise knowledge of the magnitude of these changes; but it is probable that only rough correlation exists. This does not imply that oligaemia is not wholly responsible for the symptoms, because similar reduction in blood volume need not affect different vascular systems to the same degree.

It is difficult to assess the role of oligaemia in the production of shock in burns. A toxic factor has been alleged to play an important part, but the evidence of this is not convincing when the possibility of absorption of toxic substances from dressings has been excluded (Wells, Humphrey & Coll, 1942: Erb, Morgan & Farmer, 1943: Barnes & Rossiter, 1943: Cameron, Milton & Allen, 1943: Gibson, 1944). Nevertheless, that a toxic factor does circulate cannot be doubted. The initial rise in temperature which occurs in all cases of severe burns is not due to skin infection (Colebrook, Clark, Gibson & Todd, 1944). Mobilisation of leucocyte reserves (p.190) almost certainly occurs as a response to products of tissue breakdown. Although it is true that circulatory changes are sufficient to account for most of the unfavourable sequelae, the significant fact is that

oligaemia does not account for all the complications which may occur within the shock period. Even with apparently adequate fluid replacement and maintenance of an efficient circulation, vomiting may occur, oliguria may persist, and death may result. The question of intravascular haemolysis and haemoglobinuria must not be forgotten in such cases, but renal damage from this cause does not appear to be the only factor concerned. Death may occur without evidence of gross haemolysis.

It must be admitted that although oligaemia is a factor of prime importance, a factor which of itself may cause death, and a factor which can be eliminated by prompt and adequate plasma transfusion, some additional factor contributes to the early mortality of burns. It must also be admitted that there is good evidence that absorption of products of tissue destruction occurs in sufficient degree to produce a general reaction. Whether this reaction explains the mortality in cases severely burned when the circulatory changes have been controlled by transfusion, is a matter which has yet to be settled.

In reviewing the magnitude of the blood changes during the first 72 hours, it has been shown that the rate of fluid loss increases rapidly to a maximum in the first 12-18 hours. In no case was it found to progress beyond 72 hours, and in many it was of much shorter duration. If general increase in capillary permeability

be regarded as a secondary phenomenon, early transfusion must be regarded as prophylactic; but, by maintaining the blood volume and so preserving an efficient peripheral circulation, it favours continued loss of fluid from the damaged capillaries at the site of the burn. The amounts of plasma or serum found necessary to maintain blood volume are thus frequently much in excess of the calculated amount lost from the circulation in patients with burns of similar extent who are not transfused. The difference is obvious in the more serious cases, in which amounts of fluid considerably in excess of the normal total plasma volume may be required.

BLOOD PRESSURE, VASOCONSTRICTION, AND SHOCK.

A low blood pressure is almost invariable in that fully-developed clinical state which is called "shock." In the early stages, however, when other significant changes are occurring, blood pressure levels actually higher than normal may be found.

Attention has already been directed to the early occurrence of normal or elevated blood pressures in the various Groups of Cases reported above, and to the occurrence of these normal or high levels with demonstrable haemoconcentration. From the practical point of view, it is important to assess the value of blood pressure readings obtained in the first few hours after injury, in relation to the ultimate fluid requirements of the Case.

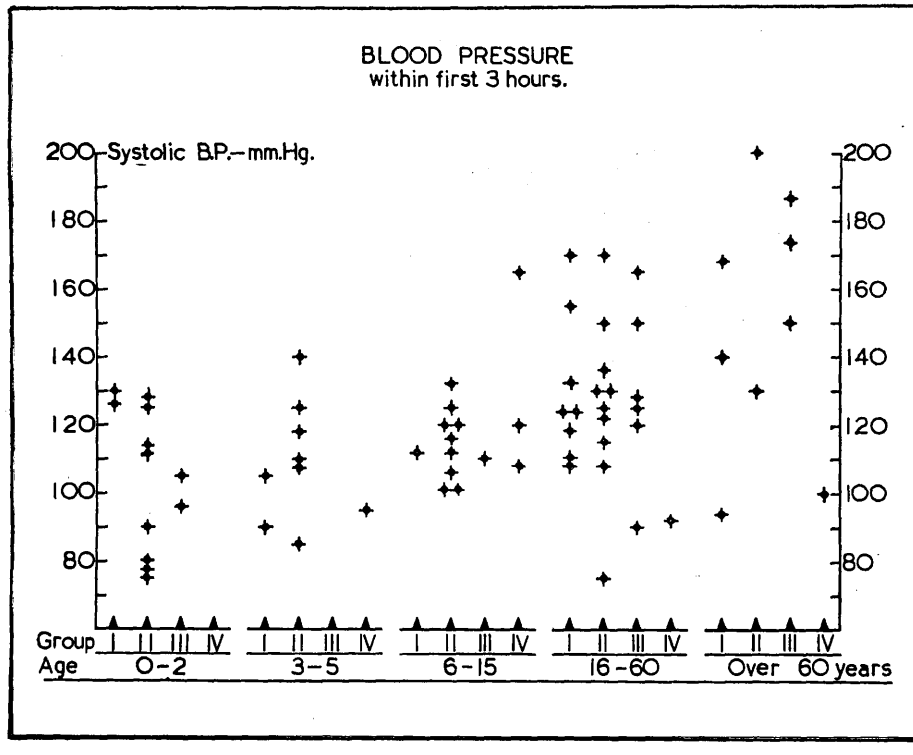

BLOOD PRESSURE
within first 3 hours.
Systolic B.P.—mm.Hg.
200
180
160
140
120
100
80
Group I II III IV
Age 0-2
3-5
6-15
16-60
Over 60 years

Fig. 12

Of a series of 68 patients in whom an initial blood pressure reading was recorded within 3 hours of burning, the great majority showed a blood pressure within normal limits. In 20-25 per cent. of the cases, the values were higher than those usually accepted as normal (Fig. 12).

In some of these patients, the unexpectedly high blood pressure levels may not have been related to the injury, and, certainly, among the elderly adults, several cases of essential hypertension were encountered. But in many, even those with a diseased vascular system, subsequent stabilisation of blood pressure at significantly lower levels has been recorded.

In some, the initial high blood pressure was probably due to excitement, a factor difficult to assess. The possible effect of the associated vasoconstrictor activity on the splenic reservoir has already been discussed. In such cases, the rise in haemoglobin and blood pressure may be results of the same process.

The phenomenon of vasoconstriction and a normal blood pressure in the presence of a reduced blood volume and symptoms of shock has been noted for many years. Meltzer (1908) recorded the occurrence of all the clinical features of shock in the experimental animal, yet there was little if any reduction in blood pressure. Mann (1914) pointed out that there is no vasomotor depression or

fatigue in shock, and that the peripheral vessels are constricted. This vasoconstriction favours the maintenance of normal blood pressure in the presence of a reduced blood volume.

Since haemoconcentration, and presumably therefore a reduced blood volume, may be associated with a normal or high blood pressure, and since (Fig. 12) patients severely burned (Groups III and IV) may share in the high blood pressure levels recorded above, it would seem that demonstration of haemoconcentration is a more reliable guide than the blood pressure to treatment, because transfusion, to be of maximum benefit, must be given early.

The misleading information which may be given by blood pressure is illustrated by Case 52, a youth of 14, burned over 31 per cent. of his body surface. The blood pressure one hour after injury was 165/114. It fell to 76/50 at 14 hours, and the patient died with clinical features of severe shock, the need for early transfusion not having been fully appreciated at that stage of the investigation.

In conclusion, it must be emphasised that a fall in blood pressure is a secondary phenomenon and indicates a relatively late stage in the circulatory failure which follows injury.

VASOCONSTRICTION AND TEMPERATURE REGULATION IN SHOCK.

Peripheral vasoconstriction favours selective distribution of the available blood when the total circulating volume is reduced. This is beneficial in so far as it improves the circulation in the viscera, but ill effects may follow interference with temperature regulation. About 85 per cent. of the total heat loss from the body occurs via the skin (Best and Taylor, 1939b). This loss is reduced by vasoconstriction, reduction in blood volume, and reduced circulatory rate - all conditions obtaining in the severely burned patient. In burns shock, therefore, heat production may exceed heat loss on this basis alone. An additional factor operating in the same direction is the extensive leathery coagulum found in the severely burned patient, and it would seem that in such cases conditions are very favourable for the development of hyperpyrexia.

In this study, a (mouth) temperature of over 105°F. has been recorded in seven cases, and in two it exceeded 107°F. All but two of the patients had burns involving more than 40 per cent. of the body surface; these two were children with burns, chiefly whole skin loss, involving 10 and 20 per cent. body surface, respectively. In all cases, the hyperpyrexia was recorded between the 10th and the 31st hour after burning. In three cases, shock was inadequately treated, and a

cold skin was present for a variable period. In three, haemoconcentration was apparently adequately dealt with from the beginning; the skin appeared flushed and hot. It therefore appears that, though vasoconstriction and skin loss may favour the development of hyperpyrexia, some other factor may also be involved, e.g. reaction to plasma or serum.

It is difficult to justify any special application of warmth to the shocked patient. The skin is cold, but application of warmth to it, if effective in producing active skin circulation, will do so at the expense of tissues more sensitive to ischaemia. In addition, though the vasodilatation so produced restores the function of the skin in temperature regulation, the environment renders the restoration ineffective. If the heat is ineffective in improving skin circulation, it increases the metabolism without increasing the transport of metabolites. Neither result is desirable.

The only treatment which will maintain adequate circulation both in the skin and internal organs, and so prevent the ill effects of ischaemia, is transfusion. The special application of warmth to the skin seems inadvisable, at least until the additional circulatory requirements have been anticipated by this means.

II. SUMMARY AND CONCLUSIONS.

I. HAEMOCONCENTRATION.

(a) A rise in the red cell count, haematocrit and haemoglobin levels, has been recorded after burns involving over 5 per cent. of the body surface. Significant changes may occur in burns even less extensive.

(b) Haemoconcentration is roughly proportional to the extent of the burn; and symptoms of shock develop, in association with haemoconcentration, with increasing frequency as the extent of the burn increases. Few patients with burns involving more than 15 per cent. of the body surface fail to develop these symptoms if untreated.

(c) Haemoconcentration is regarded as reflecting the significant factor in the production of secondary shock in burns - reduction in blood volume. It can be controlled by transfusion of serum or plasma.

(d) Reduction in blood volume probably begins immediately after burning, and as much as 50 per cent. of the total circulating plasma may be lost from the circulation within 2 hours.

(e) Loss of fluid from the circulation occurs most rapidly during the first 12 hours; it may continue until the 3rd day; but no patient has been observed, treated or untreated, in whom haemoconcentration progressed beyond the first 72 hours.

(f) The possibility of a toxic element in burns shock has been discussed. The evidence is inconclusive.

II. BLOOD PRESSURE.

(a) Observations made in 68 cases within 3 hours of burning have shown that at this stage in the majority the blood pressure is within normal limits. It may be above normal.

(b) The normal or high blood pressure levels found at this time are unrelated to the nature of the burn, or to the ultimate issue.

(c) Normal and high blood pressures have been recorded in association with definite haemoconcentration.

III. INDICATIONS FOR TRANSFUSION, AND ITS CONTROL IN THE SHOCK PERIOD.

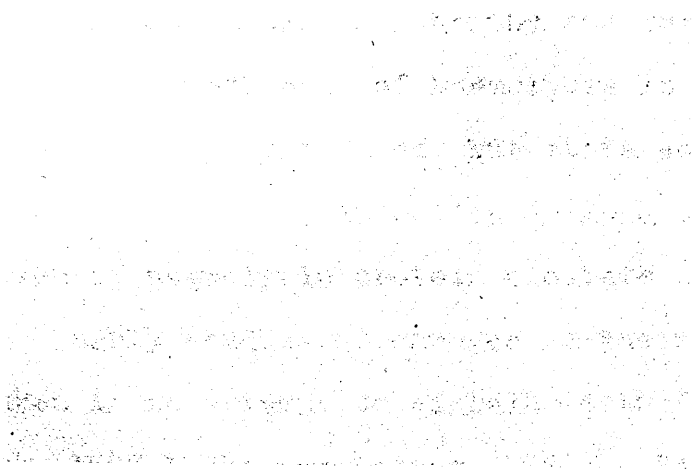
(a) If the effects of oligaemia, haemoconcentration and circulatory stasis are to be avoided, it follows from the above considerations (Id) that early transfusion is essential in extensive burns. The greater part of the transfusion will probably be required in the first 12 hours (Ie), and, as far as blood volume changes are concerned, transfusion will not be necessary beyond the third day.

(b) Blood pressure readings obtained within 3 hours of burning are of no value in indicating the need for transfusion; and an isolated haemoglobin or red cell estimation will give no more indication of the

requirements than knowledge of the extent of the burn, since pre-burn levels are not known.

(c) Ideal treatment by transfusion comprises the commencement of fluid therapy at an early stage, when little change has occurred, and the maintenance of a circulating blood volume as nearly unchanged as possible.

The initial assessment of the needs of any case must be based on the area involved (Ib), and the rate and magnitude of transfusion are best controlled by repeated estimations of red cell, haemoglobin or haematocrit levels.



C H A P T E R V.

ANAEMIA IN BURNS.

I. INTRODUCTION.

It has long been known that morphological changes occur in the blood of the burned patient, but the nature of these changes and their etiology have been little investigated in man. The only information to be gleaned from Wintrobe (1942) is that "severe hemolytic anaemia with hemoglobinuria is said to occur ... sometimes following extensive burns." Whitby and Britton (1942) state that "the mechanism of haemolysis in severe burns has not been fully elucidated, but it is supposed to be due either to secondary infection or more probably to absorption of haemolytic protein cleavage products."

Early studies of changes in the red cells were undertaken in an attempt to explain death from burns shock (Ponfick, 1867-1883: von Lesser, 1880). Schultze (1865) observed that crenation and fragmentation of red cells followed exposure of the blood to temperatures of 51-52°C. Similar findings were recorded by von Lesser (1880), Silberman (1890), Burkhardt (1905), Helsted (1906) and Shen, Ham and Fleming (1943). Experimental burns in

animals were found to be followed by similar changes in the blood within a short time of the injury (von Lesser, 1880: Markusfeld & Steinhaus, 1895: Pfeiffer, 1905). Von Lesser (1880) observed haemoglobinaemia and haemoglobinuria in animals burned by scalding, and in animals transfused with blood from a burned animal. Isaacs, Brock and Minot (1925) confirmed the morphological changes occurring in heated blood and observed that red cells heated above 50°C. showed an increase in osmotic fragility.

In man, Locke (1902) noted fragmentation of erythrocytes in blood smears prepared from two fatal cases half an hour and one and a quarter hours after burning, respectively. Lucido (1940) described the occurrence of anaemia in an adult male burned over 40 per cent. of the body surface. The red cell count fell from 7 million on the third, to 3.5 million on the fourteenth day. Haemoglobinuria was not observed.

In the Cocoanut Grove fire disaster of 28th November, 1942, 27 of a total of 81 cases, showed anaemia. Twenty presented a picture of progressive anaemia reaching a height between the eighth and sixteenth day after admission. There appeared to be a definite relation between the severity of the burn and the degree of the anaemia (Lund, Taylor, Finland et. al., 1943). Haemoglobinuria was noted in about 75 per cent. (9 cases) of those who had second and third degree burns involving

45-75 per cent. of the body surface. In investigations on these, and some additional, cases, Shen, Ham and Fleming (1943) found that the urine contained haemoglobin, methaemalbumin and haemosiderin. It was devoid of red cells. The plasma or serum showed haemoglobinaemia. No significant anaemia was observed in the first 48 hours. In some cases the red cells showed an increased osmotic fragility which was associated with spherocytosis. No close correlation between spherocytosis and fragility was recorded.

It has thus been demonstrated that when blood is exposed to temperatures of about 50°C., changes occur in the red cells which render them more sensitive to osmotic influences. These changes have been shown to occur in the experimental animal, and both experimentally and clinically intravascular haemolysis has been observed to occur following severe burns. It would therefore appear that anaemia of a haemolytic type may develop after severe burns, but the evidence is incomplete on many points. No comprehensive study of the blood changes has hitherto been attempted in burns and the evidence upon which the rather vague statements on the anaemia are based comprises a small number of disconnected investigations. The occurrence of anaemia has never been adequately investigated in burns of all degrees of severity and the relation of its development to the nature of the injury still remains in

doubt. The time of onset of anaemia is obscure, though it may reach its height on the eighth to the sixteenth day. No statement of its further progress is available, and it is not evident if the anaemia is ever severe enough in those who survive the injury to prove a major problem in treatment.

There is evidence that haemolysis may occur after very severe burns, but there is nothing to indicate whether other factors may, by themselves, or in association with lysis, cause a fall in blood levels. Although increased osmotic fragility of the red blood cells has been demonstrated in connection with severe burns its correlation with morphological changes in the red cells, and with the severity of the injury has not been fully described.

In the following pages, the results of an investigation on the anaemia of burns are reported. The study includes data on the anaemia (or anaemic trend) after burns of different degrees of severity (Groups I-IV), together with observations on changes in red cell size, alterations in saline fragility, the reticulocyte count, plasma bilirubin, and also haemoglobinaemia and haemoglobinuria.

II. METHODS OF INVESTIGATION.

GENERAL CONSIDERATIONS.

Samples of blood were obtained by venepuncture in almost all cases. Where results recorded were obtained, instead, from capillary samples, attention is drawn to this fact. For the withdrawal of blood from a vein, a dry sterile needle was used. The blood was collected in a heparinized tube without the use of a syringe, temporary stasis being induced. Examinations were made with the least possible delay, almost always within an hour of bleeding. An automatic shaker was used prior to sampling.

In describing the time of onset and the magnitude of the anaemia haemoglobin levels have been charted. The scale employed in recording these levels has been reduced by half, compared with that used in the previous section.

Since the patients' red cell or haemoglobin levels before burning were not known, a condition of "anaemia" has been held to exist wherever serial blood estimations show a significant depression in these values; or when the post-burn level continues strikingly below the normal average.

HAEMOGLOBIN AND HAEMATOCRIT.

The method of estimating haemoglobin concentration and the packed cell volume has already been described (p. 90).

PLATELETS, RETICULOCYTES, AND WHITE CELLS.

The method of Salter (1941) was used for these estimations. It was found to be satisfactory, but satisfaction appears to depend on the suitability of the cresyl blue employed. (Some samples of the stain produce turbidity when mixed with the cyanide and render the method quite useless.) Platelets were found to persist numerically unchanged for several hours at room temperature, and no difficulty was experienced in counting them unless there had been difficulty in obtaining blood from the vein.

QUANTITATIVE ERYTHROCYTE FRAGILITY (SALINE).

The various dilutions of saline used in this test were prepared as stock solutions from sodium chloride of analytical purity, and checked by titration. The range of strengths employed varied from 0.24 per cent. to 0.72 per cent. NaCl, by steps of 0.02 per cent. Five ml. of the respective saline solutions were added to a series of centrifuge tubes, and then to each of these was added, by means of a microburette with a ground and vaselined tip, an equal volume of blood (0.10 ml.). A similar amount of blood at the beginning, and again at the end, of the manipulation, was added to 10 ml. of distilled water to provide standards equivalent to 50 per cent. haemolysis. The contents of the tubes were mixed by inversion, and allowed to stand not less than 15 minutes at 20°C. and not more than two hours at 20°C., or 24 hours at 0°C.

The tubes were then centrifuged, and the supernatant solutions compared with the standards, in a Duboscq colorimeter.

Comparison of the two standards revealed that, in the short time taken to add the blood to the saline solutions, no appreciable error occurred due to red cell sedimentation in the burette.

The results obtained by this method in normal individuals have been described (p. 65).

RED CELL MEASUREMENTS.

The mean corpuscular volume (M.C.V.) and other absolute values were calculated from the red cell, haemoglobin and haematocrit figures. The mean corpuscular average thickness (M.C.A.T.) and construction of Price-Jones Curves required measurement of red cell diameter. This was carried out by a process of microprojection with a ribbon filament lamp as a source of light, and with the microscope optical axis in the horizontal position. The image, at a magnification of 1,000 diameters, was projected on to millimetre-ruled graph paper in a horizontal plane, by means of a prism attached to the eyepiece. Each red cell diameter recorded is the average of two measurements of diameter at right angles to one another.

Evenly spread coverslip preparations stained by Leishman's stain were examined and 400 red cells were measured in each case.

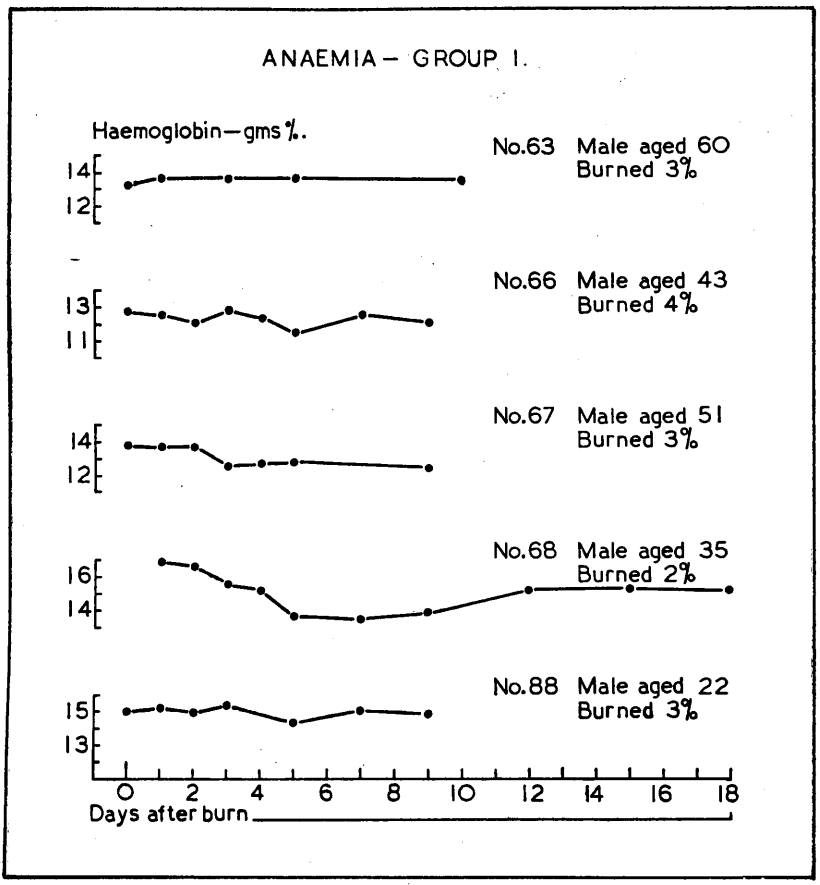

ANAEMIA – GROUP I.
Haemoglobin—gms%.
No.63 Male aged 60
Burned 3%
No.66 Male aged 43
Burned 4%
No.67 Male aged 51
Burned 3%
No.68 Male aged 35
Burned 2%
No.88 Male aged 22
Burned 3%
14
12
13
11
14
12
16
14
15
13
0 2 4 6 8 10 12 14 16 18
Days after burn

Fig. 13

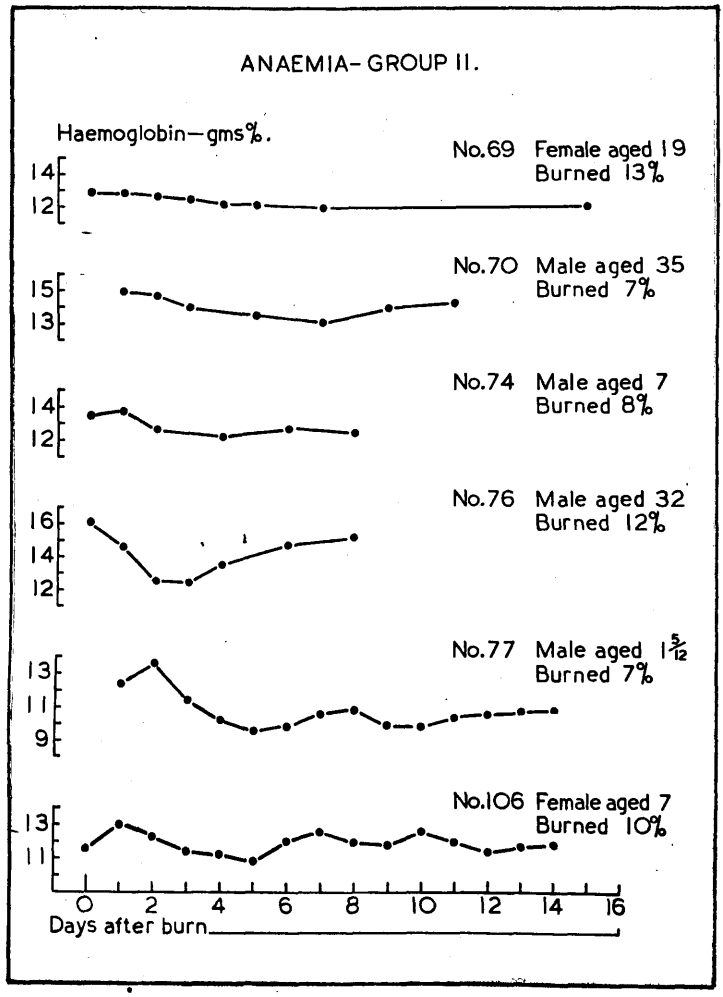

ANAEMIA- GROUP II.
Haemoglobin—gms%.
No.69 Female aged 19
Burned 13%
No.70 Male aged 35
Burned 7%
No.74 Male aged 7
Burned 8%
No.76 Male aged 32
Burned 12%
No.77 Male aged 1 5/12
Burned 7%
No.106 Female aged 7
Burned 10%
Days after burn

Fig. 14

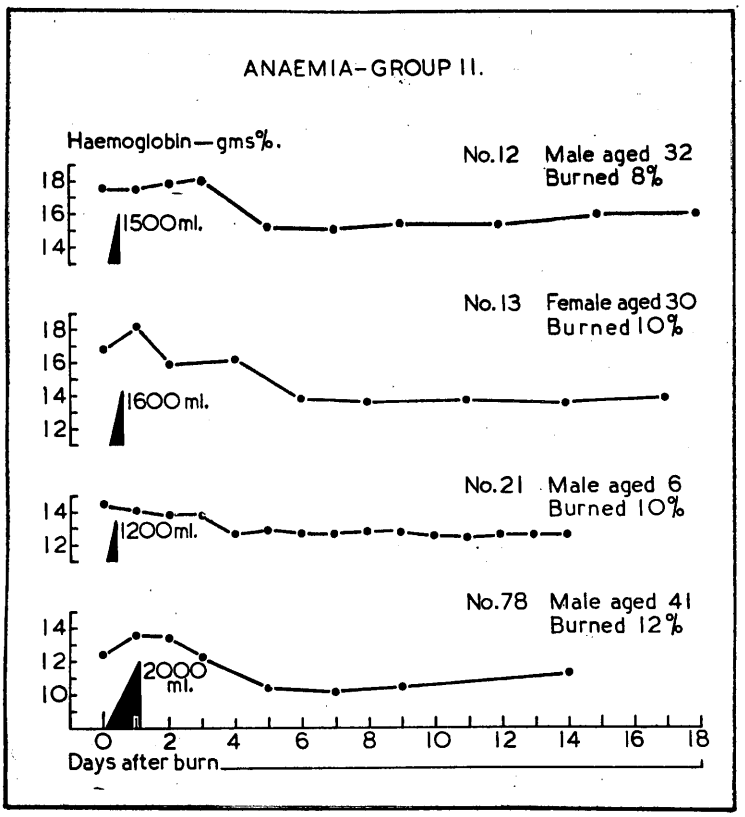

ANAEMIA-GROUP II.
Haemoglobin—gms%.
No.12 Male aged 32
Burned 8%
1500ml.
No.13 Female aged 30
Burned 10%
1600 ml.
No.21 Male aged 6
Burned 10%
1200ml.
No.78 Male aged 41
Burned 12%
2000 ml.
Days after burn

Fig. 15

III. ANAEMIA - GROUP I.*

(Burns 0-5 per cent. of body surface).

Changes in the haemoglobin level in 5 patients during the first 9-18 days after burning are recorded in Fig. 13. Only two show a significant variation (Nos. 67 and 68), and in Case 68 the levels recorded between the 5th and the 9th day indicate a temporary phase of anaemia. The others show either no change at all (Case 63), or variations from day to day such as may occur in the normal subject.

IV. ANAEMIA - GROUP II.

(Burns 6-15 per cent. of body surface).

Ten cases are described in this group; of these, 4 received transfusion of serum or plasma. Those not transfused are considered first (Fig.14).

Cases 69, 74 and 106 show no significant change, in spite of the extent of burning, while Cases 70, 76 and 77 show a definite fall in haemoglobin, with a subsequent rise. (All blood samples from Case 77 were capillary).

Four cases transfused during the shock period are illustrated in Fig. 15. The shaded area in each indicates the amount of fluid transfused, and the duration of the transfusion. Case 11, in this group, is shown in

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* For details of individual cases cited in the text - i.e. age, sex, site and degree of burning, ultimate fate, etc. see Appendix (p. x.).

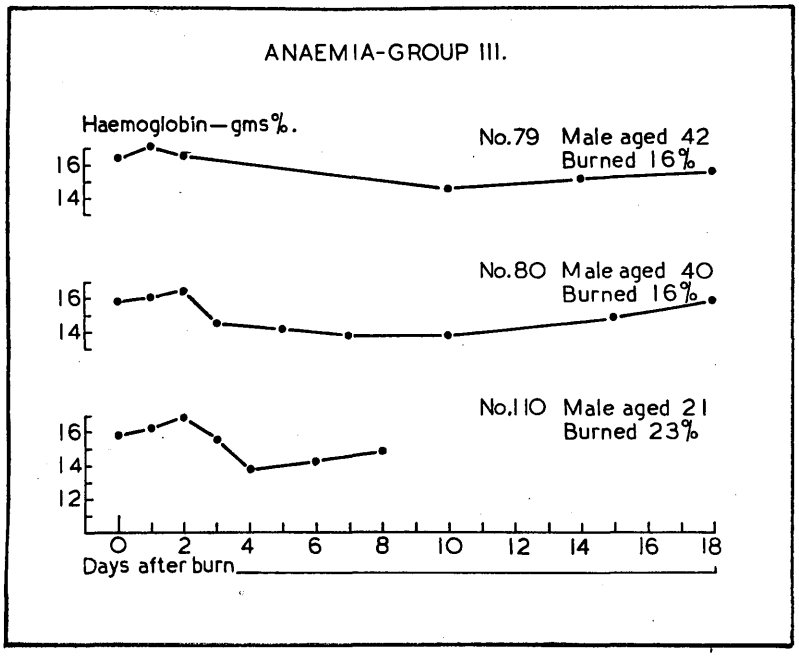
ANAEMIA-GROUP III.
Haemoglobin—gms%.
No.79 Male aged 42
Burned 16%
No.80 Male aged 40
Burned 16%
No.110 Male aged 21
Burned 23%
16
14
12
0 2 4 6 8 10 12 14 16 18
Days after burn

Fig. 16

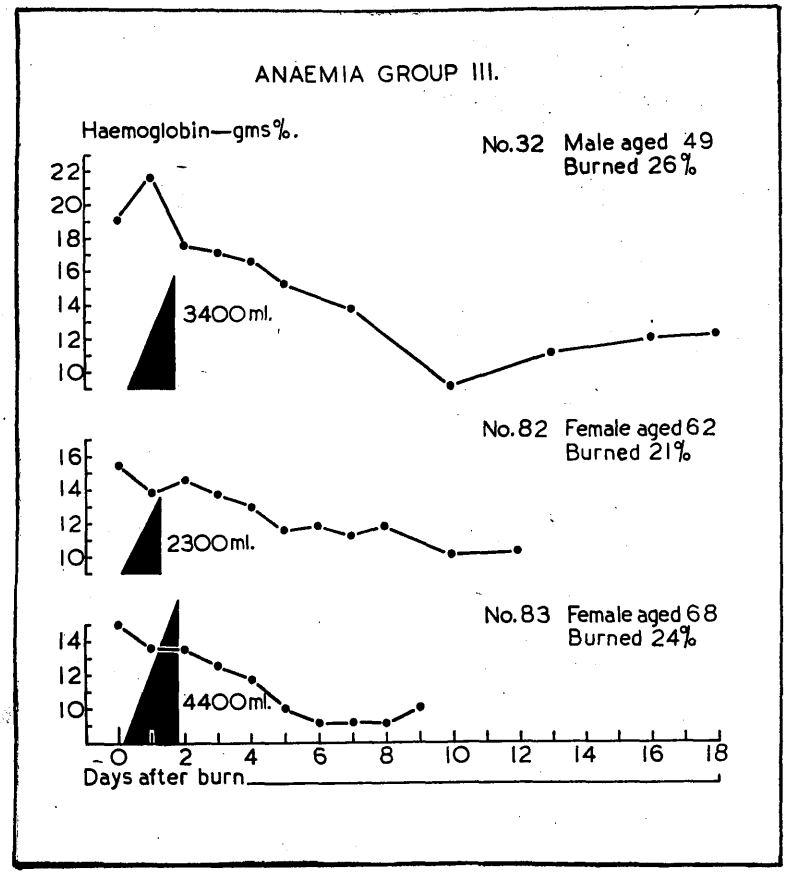
ANAEMIA GROUP III.
Haemoglobin—gms%.
No.32 Male aged 49
Burned 26%
22
20
18
16
14
12
10
3400ml.
No.82 Female aged 62
Burned 21%
16
14
12
10
2300ml.
No.83 Female aged 68
Burned 24%
14
12
10
4400ml.
0 2 4 6 8 10 12 14 16 18
Days after burn.

Fig. 18

Fig. 28 (p. 176). Only two of the four patients recorded in Fig. 15 show an anaemic phase. It is transient.

In all the cases of Group II, the anaemia, if any, has been temporary and of short duration. The general tendency follows that shown by Case 68 (Group I). The fall in haemoglobin is maximal by the 3rd to the 7th day (usually the 7th), and levels at which stabilisation subsequently occurs are reached by the 7th-15th day.

V. ANAEMIA - GROUP III.

(Burns 16-30 per cent. of body surface).

Few patients in this group were not transfused in the shock period, and only three cases are described here (Fig. 16). All show a phase of temporary anaemia. In Case 110 the burns were extensive but superficial. His discharge on the 9th day precluded further observations.

Six cases given serum or plasma during the shock period are described here (Figs. 17 and 18).

Case 8 shows an initial fall, maximal by the 7th day and followed by a rise to the 12th day (thus conforming to the type of temporary anaemia previously seen). After the 12th day, a second fall occurred, resulting in anaemia from which recovery was not recorded during the following six weeks. During the first 5 weeks, mild and irregular pyrexia was noted daily. At the end of this period a haemoglobin level of 10.5 g. per cent. was reached - the lowest recorded. By the end of the 6th week,

pyrexia had disappeared, and from then onwards a spontaneous rise in blood values occurred. A haemoglobin level of 12.9 g. per cent. was reached on the 9th week after burning.

In none of the other Cases (Figs. 17 and 18) was a phase of temporary anaemia recorded. A gradual fall, however, occurred from the 2nd or 3rd day, and by the 10th day abnormally low levels were present.

In Case 30 (Fig.17), a maximum level is recorded on the 1st day - due to haemoconcentration. During the following 11 days, the haemoglobin concentration dropped from 18.4 to 8.2 g. per cent. At this point, transfusion of whole blood was given. The patients subsequent course was one of prolonged anaemia, without complete return to normal, during the following four months. During the first month, her burned surfaces were unhealed and she showed daily pyrexia, varying in degree. Three blood transfusions were given during this period, and no evidence of spontaneous rise in blood values was seen. During the second month, the mild febrile attacks disappeared, though many areas were still unhealed; and from the beginning of the second month, slight but sustained spontaneous rise in blood values was recorded. A haemoglobin level of 11.2 g. per cent. was present eight weeks after burning.

In Case 31 (Fig.17), a steady fall in haemoglobin values is recorded during the first 14 days. Little

alteration occurred during the third week, but by the end of the fourth week the haemoglobin level reached 7.7 g. per cent., and a transfusion of whole blood was given. During the second month, further transfusions were necessary to maintain a haemoglobin level above 10 g. per cent. During the third month, haemoglobin levels were maintained at about 12 g. per cent. without transfusion, and in the fourth month quite normal levels were reached spontaneously (13.6-14 g. per cent.). During the first two months, the general course was pyrexial, but towards the beginning of the third month the temperature gradually settled. A temporary exacerbation of fever occurred between the 9th and 10th weeks, due to a respiratory complication, but thereafter the course was afebrile.

Case 32 (Fig.18) shows a rapid drop in blood levels, the lowest haemoglobin value being recorded on the 10th day. From the 13th day until the 50th day, no significant alteration in haemoglobin level was recorded (10-11 g. per cent.). By the beginning of the third month, a slight spontaneous rise in haemoglobin occurred (12 g. per cent.) and this rise was maintained until the beginning of the fourth month, when normal blood values were recorded (haemoglobin 16 g. per cent.).

During the first seven weeks, the general course of this case was pyrexial, a temperature of 100-101°F. being noted daily. Practically no rise in temperature

above normal was recorded thereafter.

Cases 82 and 83 (Fig.18) are similar in many respects. Both elderly women they sustained burns differing little in extent or severity. Both survived the shock period, to die during the second week; and both developed a significant degree of anaemia. In neither case was any sustained rise of haemoglobin apparent before death.

Summary.

A temporary reduction in haemoglobin values is recorded in three cases of relatively mild, though fairly extensive, burns. These patients did not receive transfusion during the shock period.

Of the patients more severely burned - and transfused - a phase of temporary anaemia occurred in one; it was followed by a more chronic phase. In all the other cases no temporary phase was seen. Instead, a moderate degree of anaemia developed progressively in the first 14 days, and the low levels were maintained for a month or more. Recovery did not occur spontaneously during the febrile period.

VI. ANAEMIA - GROUP IV.

(Burns over 30 per cent. of body surface).

Patients with burns involving more than 30 per cent. of their body surface who were not given plasma or serum transfusions during the shock period did not survive

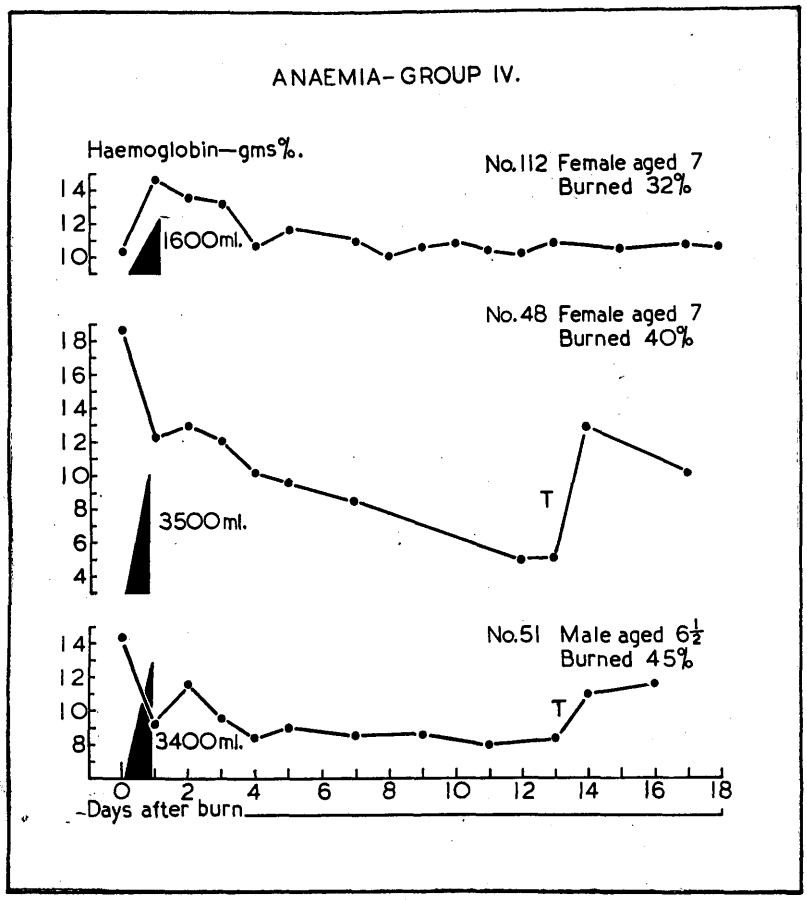
ANAEMIA- GROUP IV.
Haemoglobin—gms%.
No. 112 Female aged 7
Burned 32%
1600ml.
No. 48 Female aged 7
Burned 40%
3500ml.
T
No. 51 Male aged 6½
Burned 45%
3400ml.
T
Days after burn

Fig. 19

beyond the second day, and the development of anaemia has not been recorded.

Six cases are described to illustrate the blood changes which may follow very severe and extensive burns.

Case 112 (Fig. 19) though burned extensively and moderately severely (32 per cent. whole skin loss), showed remarkably little systemic upset clinically, and remarkably little derangement in blood values. Stabilisation of haemoglobin at a level of 10-11 g. per cent. occurred from the 4th day.

Case 48, a girl of 7 years, burned 40 per cent. showed the most severe degree of anaemia recorded in any of a series of 33 cases burned over more than 30 per cent. of the body surface. On the 12th day, the haemoglobin reached a level of 4.8 g. per cent. (R.B.C. 1.57 million per c.mm.). The case is exceptional also, in that death occurred at the end of the third week, due to agranulocytosis. A more detailed statement of the blood findings in this case is made later (p. 200).

Case 51 (Fig. 19) shows the development of anaemia by the 4th day and the maintenance of a haemoglobin level of about 8.5-8.0 g. per cent. until blood transfusion on the 13th day. This transfusion was followed by little alteration in blood values until about the end of the third week. The gradual development of anaemia necessitated transfusion again at the end of the first

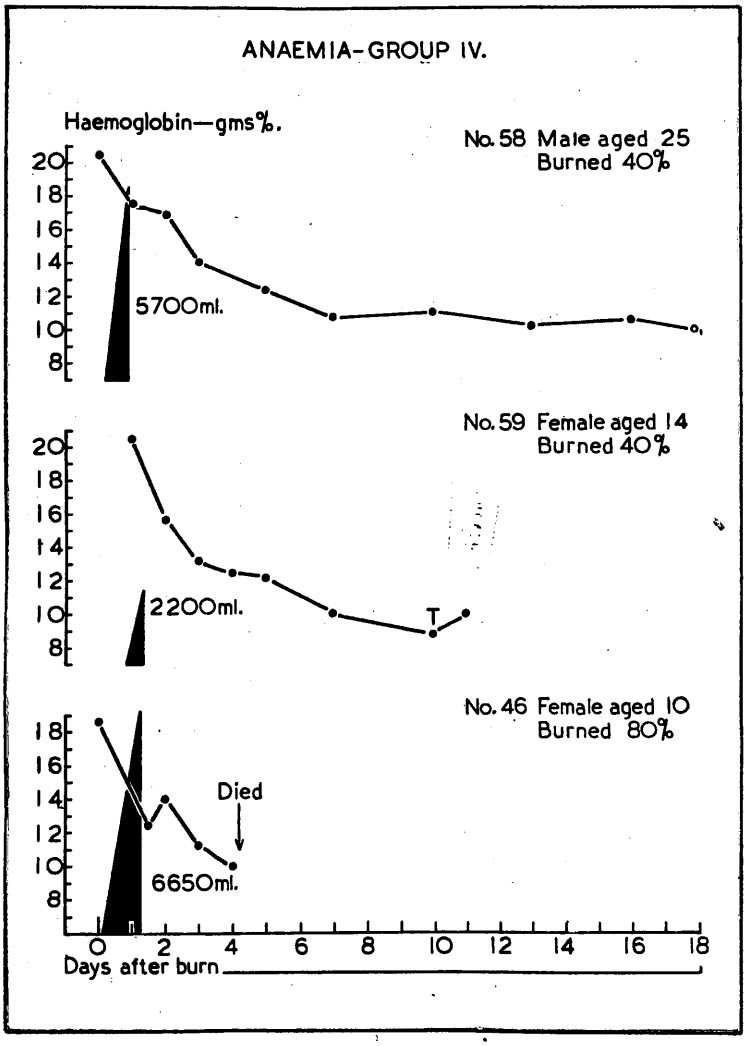
ANAEMIA-GROUP IV.
Haemoglobin—gms%.
No. 58 Male aged 25
Burned 40%
5700ml.
No. 59 Female aged 14
Burned 40%
2200ml.
T
No. 46 Female aged 10
Burned 80%
Died
6650ml.
Days after burn

Fig. 20

month. At the end of the sixth week, the haemoglobin level was 9.4 g. per cent., and the child died with hypoproteinaemia and with extensive unhealed burned areas.

Case 58 (Fig. 20) showed a moderate degree of anaemia by the 12th day. A gradual fall in blood values occurred into the fourth week, when blood transfusion was given. The lowest haemoglobin value reached was 9.55 g. per cent. Transfusion raised this level to 12.4 g. per cent., and at the end of the second month no significant alteration had occurred. By the end of the third month, normal levels were reached spontaneously. In spite of the extensive nature of the burn in this case, a temperature reading above 100°F. was recorded on only three occasions after the 14th day.

Case 59 (Fig. 20) was given blood transfusion on the 10th day. In spite of two more transfusions, the haemoglobin level two months after burning was 6.9 g. per cent. Seen a year later, with a portion of her burn not yet healed, this patient had a haemoglobin concentration of 9.3 g. per cent. During her six months stay, in the Burns Unit, this patient was never completely afebrile for more than 24 hours. She was subsequently transferred to an Emergency Hospital.

Case 46 (Fig. 20) shows a fall in haemoglobin levels more or less parallel with that recorded in the previous case. Death occurred on the 4th day. This case is discussed in more detail in connection with studies of

red cell fragility.

Summary.

In this Group, though transfusion was sometimes given, haemoglobin values below 8 g. per cent. were rarely recorded in the first three weeks. Most of the patients showed a tendency to persistent anaemia lasting a month or more. In Case 58, the anaemia was relatively mild and spontaneous recovery occurred by the end of the second month. Little febrile upset was recorded in this patient. In Case 112 no anaemia is demonstrable. This patient was undoubtedly the least severely burned of all those recorded in this Group.

An extreme degree of anaemia is recorded in one Case (Case 48). It is possible that some factor not operative in the other patients was in part responsible for the apparently disproportionate anaemia, as evidence of other haematological upset also exists. The patient died of agranulocytosis.

VII. GENERAL SUMMARY AND CONCLUSIONS.

In all patients with minor burns (Group I), only one of five showed a definite fall in blood values during the first week. In Group II, at least five of ten cases showed the phase of temporary anaemia. In Group III, all three of the patients with milder burns showed a temporary drop in haemoglobin. These patients were burned superficially though fairly extensively;

they were not given saline or plasma in the shock period, and they showed no general clinical upset. In these cases, a closely similar pattern of change is followed by all: the phase of haemoconcentration disappears, and the haemoglobin level continues to fall usually until the 5th-7th day, after which there is a gradual rise. The level at which stabilisation subsequently is shown to occur is reached about the 12th-14th day.

In patients burned over 20 per cent. of the body surface, the temporary phase of anaemia followed by quick recovery has rarely been noted. In Group III there is seen for the first time a progressive fall in red cell values from the shock period until about the 10th-14th day. Thereafter there is a marked tendency for a state of anaemia to persist for a month or more. Spontaneous recovery from this state is usually associated with disappearance of fever, and progressive healing of the raw areas.

Patients in Group IV, with one exception, showed the most severe reaction. Red blood cell levels about 50 per cent. of normal were reached on the 10th-14th day. Thereafter, almost all cases remained more or less anaemic until the burns were well on the way to being healed.

In the more serious cases in Group III, and the great majority of those in Group IV, blood transfusion was required, either at about the 12th day after burning or

during the phase of chronic anaemia which followed.

From consideration of these findings, there appear to be three types, or phases, of anaemia which may follow burns:-

(i) A phase of temporary anaemia, which is maximal about the 5th-7th day and which has disappeared by the 10th-14th day.

(ii) A phase of rapidly developing and moderately severe anaemia, less readily reversible and reaching a maximum in 10-14 days.

(iii) A phase of chronic anaemia, the duration corresponding roughly with that of the febrile period, after which gradual and spontaneous recovery occurs.

CHAPTER VI.

ANAEMIA IN BURNS.

MORPHOLOGICAL CHANGES IN THE RED CELLS.

I. INTRODUCTION.

Variations in the mean corpuscular volume (M.C.V.), in the mean corpuscular diameter (M.C.D.) and in the mean corpuscular average thickness (M.C.A.T.), are indications of the effect of the burn on the circulating red cells. The early changes are probably the direct result of heat on the cells: they are similar to the changes which have been produced by heat in vitro. The changes occurring at the end of a week, or even longer after injury, are probably the result of various factors, such as the reticulocyte response following the development of anaemia, and the depression of haemoglobin synthesis and of haemopoiesis due to chronic infection of the raw surfaces.

Values obtained for M.C.V. are the most sensitive index of changes in red cell size, since this measurement is a function of both diameter and thickness. This, however, applies only when one measurement increases and the other remains unaltered, or when both increase.

But if thickness is increased at the expense of diameter, microspherocytosis may occur without any alteration in the M.C.V. Such a change may occur after severe burns.

II. ALTERATIONS IN MEAN CORPUSCULAR VOLUME.

A summary of the changes in M.C.V. is presented in Table VII. The cases described here have already been discussed in the previous chapter.

It is difficult to interpret the changes in M.C.V. shown by these cases. In general it may be said that there is a tendency for the cell volume to increase during the first few days after injury, but this increase shows little or no relation to the severity of the burn. Its significance is further in doubt in view of the fluctuations which tend to occur, and assessment of the changes is best left until alterations in diameter and thickness are discussed.

After the first week, changes in cell volume are seen only in cases severely burned. These occur gradually and the tendency is towards an increase in M.C.V. at a time when the patient is anaemic and a degree of reticulocytosis is present.

There is no evidence that the changes described are related to the administration of serum or plasma, or to any other therapeutic measure. This aspect is considered more fully later.

TABLE VII.

	Case No.	Fig.	Serum or Plasma (ml.)	Variations in M.C.V. - (c.microns).
Group I	63	13	-	Initial M.C.V. 99; rise to 107-109 on first to fifth day. Subsequent stabilisation 96-100 from seventh to twenty-eighth day.
	66	13	-	Initial M.C.V. 98; rise to 107 by sixth day; fell to 87 on ninth day.
	68	13	-	M.C.V. 95, 99, 105, during first 4 days; stabilisation at 95 from twelfth to eighteenth day.
Group II	12	15	1,500	M.C.V. 101, 101, 105 during first day; stabilisation at 87-93 from fifth day.
	13	15	1,600	Fall from 108 to 90 during first 3 days; stabilised at 92-98 for month.
Group III	80	16	-	Stable at 96-100 during first 2 days. Rise to 110 on third day followed by gradual fall and stabilisation at 93 on tenth and fifteenth days.
	8	17	1,600	No significant variation at any time; no reticulocytosis.
	30	17	1,700	Rise from 100 to 110 in thirty-six hours; thereafter stable at 100 till twelfth day, when blood transfusion was given.
	31	17	1,600	No significant upset till fourth week - reticulocytosis.
	32	18	3,400	M.C.V. maximum at 99 in shock period; fell to 90 on seventh and tenth days; rose to 100 later when anaemia developed.
Group IV	48	19	3,500	Initial level 75; rose to 98 in twenty-four hours.
	58	20	5,700	For details see Fig. 29.
	59	20	2,200	Gradual rise from 88 at twenty-four hours to 99 on eighth day.
	46	20	6,650	M.C.V. 104-94 in first eighteen hours; rise to 108 at fifty-four hours and maintained till fourth day (death).

III. RED CELL MEASUREMENT IN THREE CASES OF SEVERE BURNS.

CASE 46 (Female aged 10, burned 80 per cent. of body surface).

This child sustained very severe burns when her clothing caught fire at 3.50 p.m. on the 24th February, 1943. She was admitted to hospital within an hour, and the first blood examination was performed almost at once. The findings in the 4-day survival period in this Case are recorded in Table VIII.

TABLE VIII.

Blood Findings.	Time after burning (hours).							
	1.5	3.5	5	8	18	54	72	96
R.B.C. ($\times 10^6$)	4.39	3.43	4.06	3.36	3.59	3.48	2.99	2.88
Hb. (gms.%)	18.6	12.1	14.5	-	12.4	14.0	11.2	10.5
P.C.V. (%)	46.0	32.0	37.0	35.0	30.0	37.5	32.5	30.5
M.C.V. (c.μ)	104	94.0	94.0	104	83.0	108	109	105
M.C.D. (μ)	6.23	6.56	6.52	6.78	7.15	7.75	7.87	7.75
M.C.A.T. (μ)	3.40	2.82	2.80	2.88	2.06	2.30	2.25	2.23

The initial blood findings reveal a normal red cell count with a slightly high haemoglobin concentration, or M.C.V. slightly increased, and a M.C.D. slightly below normal. The most striking feature is the degree of spherocytosis indicated by a great increase in the M.C.A.T. It is seen to fall within the next two hours, and remain at a level on the upper limit of normal thereafter until death on the fourth day by which time a very significant fall in

the red cell count had occurred. A general increase in M.C.D. occurred to the fourth day.

CASE 48 (Female aged 7, burned 40 per cent. of body surface).

This child's clothing caught fire at 4 p.m. on 23rd October, 1942. She was admitted to hospital about two and a half hours after burning and the first sample of blood was obtained almost immediately (Table IX).

TABLE IX.

Blood Findings.	Time after burning (hours).								
	3	6	16	26	40	65	96	120	168
R.B.C. ($\times 10^6$)*	6.67 6.70	4.10 4.10	3.56 3.42	3.42 3.41	4.18 3.86	3.61 3.66	2.95 3.05	2.83 2.81	2.71 2.67
Hb. (gms.%)	18.7	12.6	10.7	12.2	12.0	12.0	10.1	9.5	8.4
P.C.V. (%)*	50.0 50.0	35.0 35.0	28.7 28.5	33.0 33.0	38.0 37.0	35.0 35.0	29.5 29.5	24.5 24.5	21.0 21.0
M.C.V. (c.μ)	75.0	86.0	80.0	98.0	93.5	96.5	98.5	87.0	78.0
M.C.D. (μ)	6.75	6.27	6.89	7.50	7.63	7.91	8.08	7.75	7.98
M.C.A.T. (μ)	2.10	2.80	2.14	2.22	2.04	1.97	1.95	1.85	1.56

*Red cell counts and P.C.V. are given in duplicate.

The initial findings indicate marked haemoconcentration with microcytosis and normal values for diameter and thickness. At the sixth hour, spherocytosis was evident but it was only a transient phenomenon as far as it was revealed by the M.C.A.T. In the stained films examined, however, microspherocytosis was evident as far as the ninety-sixth hour. This change was less marked than in Case 46. An increase in M.C.D. amounting to megalocytosis was observed by the seventh day.

CASE 58 (Male aged 25, burned 40 per cent. of body surface).

This man's clothing caught fire at 9.30 p.m. on the 19th November, 1942 and he sustained extensive burns almost wholly in third degree. He was admitted to hospital about three hours later. The relevant findings from blood examination during the first five days are shown in Table X.

TABLE X.

Blood Findings.	Time after burning (hours).							
	3	5	12	16	36	60	72	120
R.B.C. ($\times 10^6$)	5.09	5.18	5.60	3.47	4.50	5.20	4.68	3.34
Hb. (gms.%)	19.0	19.1	20.4	13.2	17.5	16.9	14.0	12.4
P.C.V. (%)	50.0	50.5	52.5	31.5	46.0	41.5	40.0	34.5
M.C.V. (c.μ)	98.0	98.0	94.5	91.0	102	81.0	85.5	103
M.C.D. (μ)	6.77	6.67	7.15	6.92	7.06	7.24	7.49	7.66
M.C.A.T. (μ)	2.73	2.82	2.35	2.41	2.60	1.97	1.95	2.12

The initial findings indicate haemoconcentration, an M.C.V. on the upper limit of normal and considerable spherocytosis. The increase in M.C.A.T. was maintained at a slightly lower level throughout the first 36 hours. Thereafter normal values were obtained. A gradual increase in red cell diameter occurred towards the fifth day.

DISCUSSION.

The three cases had much in common. All were severely burned and all developed a significant degree of anaemia. Initial values for M.C.D. in all cases were

slightly below the average normal, and in each case the average diameter gradually increased until values in excess of normal were reached by the second or third day. In association with the early microcytosis, the M.C.A.T. was found to be increased, in one case (No.46) the most severely burned, by almost 50 per cent. With the development of anaemia and an increase in the M.C.D., the M.C.A.T. fell to normal (Cases 46 and 58) or subnormal (Case 48).

Microspherocytosis has been observed in the past as a result of heating a sample of blood to 51-52°C. It has also been observed in the circulatory blood following experimental burns in animals. More recently reference has been made to such changes in the blood of patients with extensive deep burns. (See p.131). The cases just described afford ample evidence of the degree of spherocytosis which may be encountered, and it is of interest to note that the most marked example occurred in the patient most severely burned (Case 46). Since only the mean alteration is given by the values quoted in Table VIII, the figures do not indicate whether the change in the affected cells is greater, or whether more cells are affected. A study of the distribution of the changes in the red cell population will be more informative.

IV. THE DISTRIBUTION OF THE CHANGES IN CELL DIAMETER IN SEVERE BURNS.

INTRODUCTION.

In this study blood films were prepared from all samples obtained for the various purposes already described. No significant change in the shape or size of the red cells was obvious in cases of mild burns. These cases showed either a temporary phase of anaemia, or no significant alteration in red cell levels.

In cases of severe burns in whom anaemia developed, it was evident, even from a cursory inspection of stained films, that a distinct change had overtaken many of the red cells. Spherocytosis and microspherocytosis were marked features in samples obtained within a few hours of burning and they persisted in gradually diminishing degree until 2-4 days after the injury. * Fragmentation of red cells was a feature observed only in films made within a few hours of burning. In such cases, small irregular portions of red cells, apparently fully haemoglobinised, and measuring as little as 0.5-2 μ in diameter, were numerous. In some instances a few "ghost" cells were also seen.

In preparing distribution curves illustrating changes in red cell diameter, fragmented cells have, as far as possible, been omitted.

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* See Plate I.

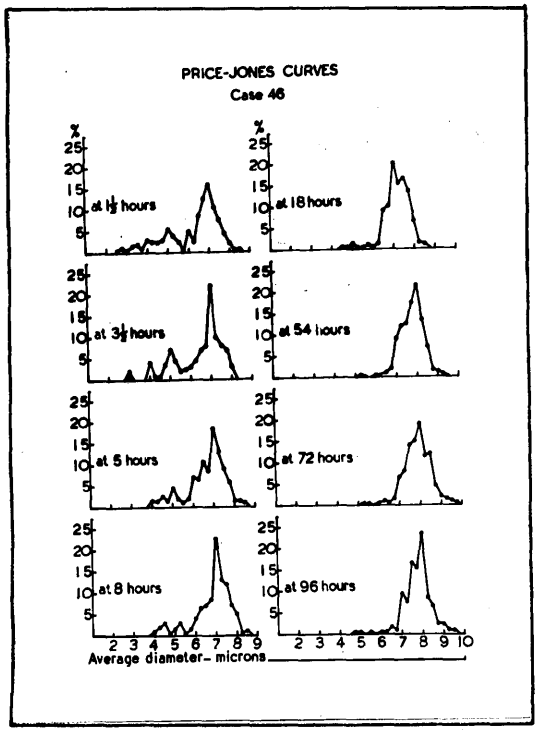

Fig. 21

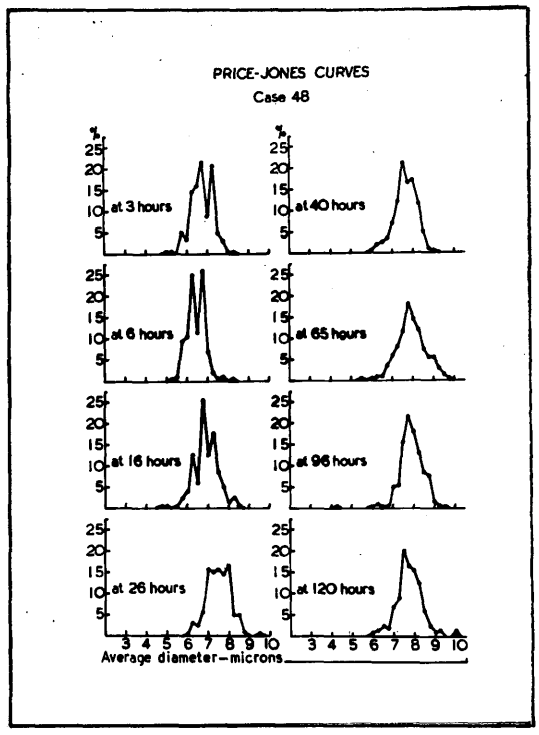

Fig. 22

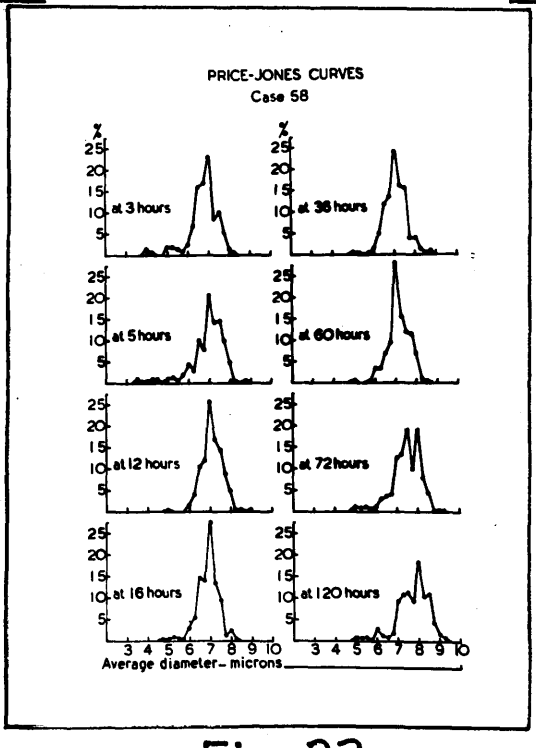

Fig. 23

PRICE-JONES CURVES IN CASES SEVERELY BURNED.

In Case 46, the degree of microcytosis was very great (Fig. 21), and as time elapsed and anaemia developed, the small cells, which were for the most part spherocytes, disappeared, and a degree of megalocytosis became apparent. A few spherocytes still remained in the last sample of blood examined, 96 hours after burning. Fragmented cells were numerous in the first two films examined. Few were found thereafter.

In Case 48 (Fig. 22) the degree of microspherocytosis was less pronounced, but megalocytosis was apparent 26 hours after the injury.

In Case 58 (Fig. 23) the findings were very similar in type to those obtained in Case 46. The degree of microspherocytosis in the first two samples was less marked, and the change was no longer evident after 36 hours.

DISCUSSION.

In all cases the curves show a shift to the left immediately after injury, thus indicating a corresponding degree of microspherocytosis produced by the burn. As is shown later, this change is associated with a proportionate increase is osmotic fragility, with intravascular haemolysis and occasionally haemoglobinuria.

The shift to the right corresponding to an increase in the M.C.D. is present in the curves from all three cases in the late stages. The reason for this is

is not obvious. It cannot be due only to removal of the spherocytes from the circulation, because the curves show very clearly that larger cells have appeared in the films. The possibility that these larger cells are reticulocytes, or at least young cells recently added to the circulation, must be considered.

The significance of the spherocytosis, and the relation of the changes to factors other than the effect of heat are discussed in a later chapter.

V. OBSERVATIONS ON THE RETICULOCYTES IN THE PERIPHERAL BLOOD.*

All degrees of reticulocytosis up to about 11 per cent. have been recorded in the cases described in previous chapters. The more marked reticulocyte responses were noted in patients in whom anaemia developed early and rapidly. (Table XI).

TABLE XI.

The Reticulocyte Percentage after Burns.†

Case No.	Days after Burn.								Further Response.
	0	1	2	3	4	5	6	7	
11	0.1	0.2	0.1	0.3	0.1	-	0.1	0.0	None.
12	0.0	0.1	0.1	0.0	-	0.1	-	0.1	None.
13	0.1	0.0	0.1	0.3	0.3	-	0.3	-	None
14	0.0	0.1	0.1	0.1	-	0.2	-	0.1	None
32	0.1	0.1	0.1	0.2	0.4	-	0.4	0.4	1.1% on 16th day.
48	0.1	0.3	0.3	0.7	1.6	1.6	-	2.1	10.9% on 13th day.
58	0.1	0.2	0.5	0.8	-	1.2	1.0	3.0	6.4% on 21st day.

†In the cases recorded here, reticulocyte counts were performed on all specimens of blood from which haemoglobin values were determined (see figures above), and every third or fourth day subsequently for at least five weeks.

In Group II, (Cases 11-14) no anaemia was recorded, and no significant rise in reticulocytes occurred.

In Group III (Case 32) a slight degree of anaemia developed (Fig.18). The maximum reticulocyte level recorded was 1.1 per cent.

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*Salter's method of counting reticulocytes gives results per c.mm., but in this section the numbers are expressed as a percentage of the red cells. Fallacies introduced by the presence of haemoconcentration or haemodilution are thus obviated.

In Group IV (Cases 48 and 58) the anaemia was still more severe (Figs. 19 and 20) and a more pronounced reticulocyte response occurred.

The evidence suggests that the reticulocyte response is roughly proportional to the degree of anaemia, and the occurrence of such a response indicates at least in these early stages, a normal degree of marrow activity. In the later stages, when anaemia was associated with extensive unhealed areas, a reticulocytosis was unusual. The highest recorded was 5 per cent. It occurred on the twenty-eighth day in Case 31 (Fig.17). It was ineffective in restoring the blood levels to normal.

In relation to the megalocytosis which has been found to develop 26-60 hours after burning (Tables VIII - X) it would seem at first sight that the appearance of reticulocytes alone could not be responsible. But it depends on how many new cells have been added to the circulation and not on how many young cells can be demonstrated on a sample of blood by the staining of a short-lived reticulum. It seems much more probable that the degree of megalocytosis indicates more accurately than does the reticulocyte count, the new cells released by the bone marrow.

VI. GENERAL SUMMARY AND CONCLUSIONS.

In this chapter, observations on the alterations in red cell size have been recorded. Throughout the

various Groups, comprising burns involving 2-80 per cent. of the body surface, a tendency has been observed for the M.C.V. to increase early after the injury. This increase has not been shown to be significantly related to the severity of the burn and other factors may be responsible. It may result from mere alteration in shape with little alteration in volume.

A much more significant change in relation to the injury has been recorded in the study of changes in cell diameter and thickness. Immediately after the burn, and in proportion to its severity, a degree of microspherocytosis has been shown to occur. This change is associated with, but outlasts, the occurrence of red cell fragmentation. The latter has been seen only with a few hours of burning, while a few spherocytes may still be present on the fourth day. These findings are in agreement with those of previous workers, and are regarded as reflecting the effect of heat on a proportion of the red cell population exposed to its influence during the process of burning.

With the disappearance of the microspherocytosis, a degree of megalocytosis becomes apparent. This may be due to the commencing addition of young cells to the circulation, a process which is inadequately measured by the reticulocyte count. The disappearance of the smaller cells from the blood will emphasize the change.

CHAPTER VII.

ANAEMIA IN BURNS.

VARIATIONS IN OSMOTIC FRAGILITY: HAEMOGLOBINAEMIA AND HAEMOGLOBINURIA.

I. INTRODUCTION.

This section comprises a report on the results of serial quantitative estimations of saline fragility in cases of both mild and severe burns. These results are summarised in the accompanying figures (Figs. 24 and 25) as variations in the concentration of sodium chloride in which 50 per cent. haemolysis occurs, that is, as variations in the Median Corpuscular Fragility (M.C.F.). The curves from which the M.C.F. values were obtained by interpolation are, with one exception (Case 46), not shown here.

II. VARIATIONS IN MEDIAN CORPUSCULAR FRAGILITY (M.C.F.).

In seven patients, fragility tests were made on frequent samples of blood during the shock period, and thereafter at intervals of about three days until the end of the fourth week. The occurrence of death on the fourth day in Case 46, and on the twenty-second day in Case 48,

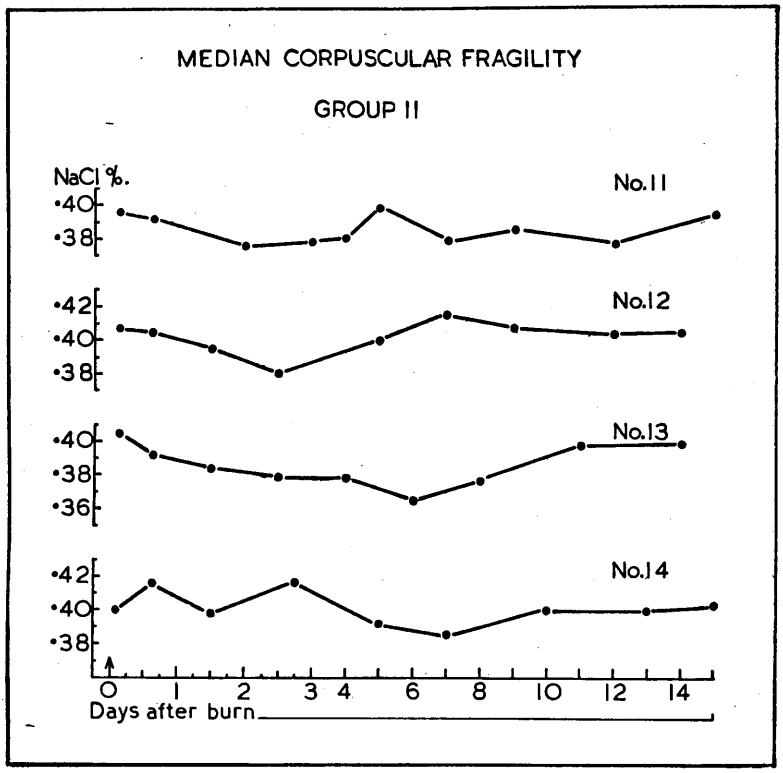
MEDIAN CORPUSCULAR FRAGILITY
GROUP II
NaCl %.
No.11
·40
·38
No.12
·42
·40
·38
No.13
·40
·38
·36
No.14
·42
·40
·38
0 1 2 3 4 6 8 10 12 14
Days after burn

Fig. 24

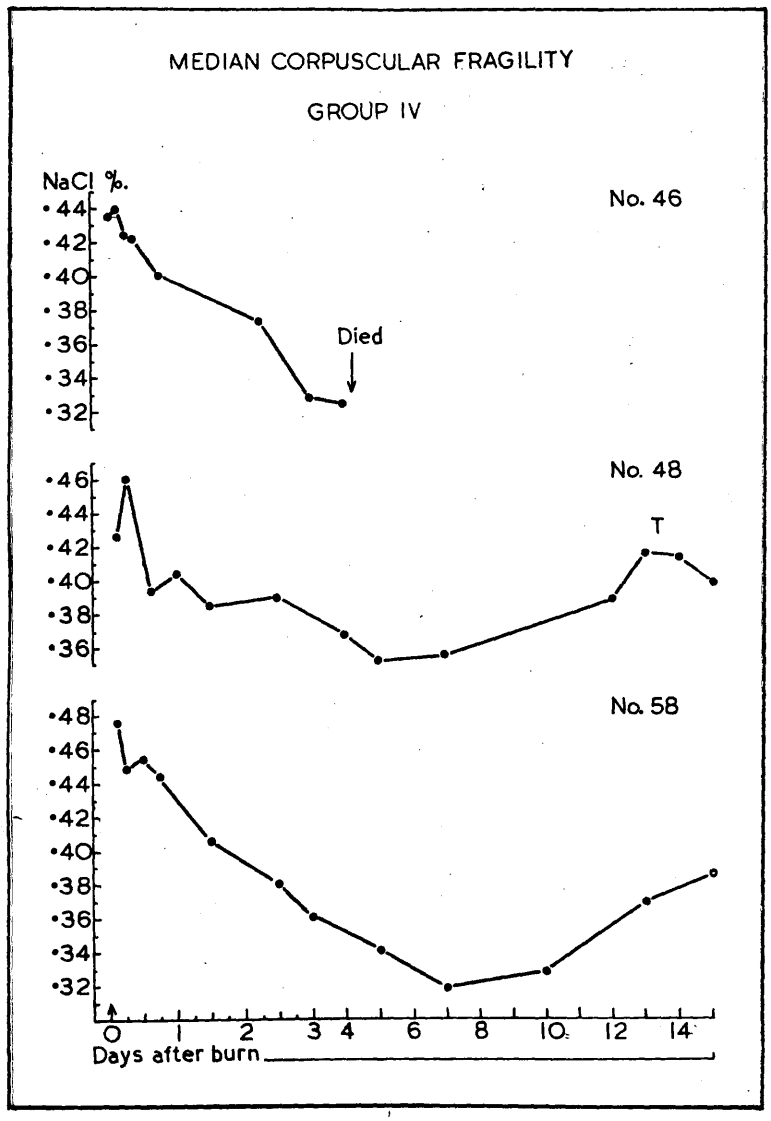
MEDIAN CORPUSCULAR FRAGILITY
GROUP IV
NaCl %.
No. 46
·44
·42
·40
·38
·36
·34
·32
Died
No. 48
·46
·44
·42
·40
·38
·36
T
No. 58
·48
·46
·44
·42
·40
·38
·36
·34
·32
0
1
2
3
4
6
8
10
12
14
Days after burn

Fig. 25

limited the period of investigation in these cases. Only the changes occurring during the first 15 days are illustrated here (Figs. 24 and 25).

M. C. F. IN GROUP II (BURNS INVOLVING 6-15 PER CENT. OF THE BODY SURFACE).

The changes in fragility found in four patients with burns involving 6-10 per cent. of the body surface are shown in Fig. 24. As isolated values, the figures obtained for M. C. F. at any stage in the shock period are within normal limits, but the serial records of every case show a range of variation greater than has yet been found by the same method. It would appear, therefore, that even in cases of minor burns a significant alteration in saline fragility may occur.

If a general tendency to change in the M. C. F. is exhibited, it is towards a reduction during the first 3-4 days after burning. The fall is not sustained, and the subsequent rise may equal or exceed the initial fall.

M. C. F. IN GROUP IV (BURNS INVOLVING OVER 30 PER CENT. OF THE BODY SURFACE).

In Fig. 25 are shown, drawn to the same scale, the variations in M. C. F. encountered in three patients very severely burned.

In each the initial values are much above normal. A rapid fall occurs almost at once, and within 36 hours figures within normal limits are recorded in all cases.

The reduction in M.C.F. continues more or less regularly until the fifth day (Case 48) and seventh day (Case 58), when the levels are markedly subnormal. The M.C.F. in Case 46 a few hours before death on the fourth day is as low as any recorded in the patients who survived the period reviewed. In these patients, the fall in M.C.F. ceased spontaneously on the fifth and seventh days, respectively, and thereafter a rapid rise towards normal is recorded.

It is of interest at this stage to compare the changes found in Group II (Fig.24) and in Group IV (Fig.25). In the former, there is a tendency for a fall to occur during the first 3-7 days and for a rise to follow, previous levels being reached or exceeded by the tenth to fifteenth day. In Group IV, high initial levels are replaced by the lowest recorded levels on the fifth to seventh day, after which a rise occurs. In Cases 48 and 58, during the second and third weeks, levels in the upper limits of normal were reached (not shown on graph).

Thus, burns mild and severe may show the same type of variation in M.C.F., the magnitude of the changes being greater the more severe the burn. In the severe cases, the initial levels are much above normal. In the milder cases, though the initial levels are within normal limits, it is impossible to say whether the values recorded are normal for each patient. As in the case of haemoglobin

levels, it has not been possible to relate the initial levels to the intermediate or final values - even at the end of four weeks, because there is evidence that abnormal variations in fragility may occur for several weeks after the injury.

III. FRAGILITY IN RELATION TO HAEMOGLOBINAEMIA AND HAEMOGLOBINURIA.

In estimating saline fragility, it was found impossible in some cases to obtain a haemoglobin-free saline, even in concentrations up to 0.9 per cent. Above a certain salt concentration, the amount of haemolysis persisted unchanged. (The degree of this "residual haemolysis" is expressed below as a percentage of the value for complete haemolysis.) The saline solutions were checked by titration, and fresh stock solutions were prepared; but the residual haemolysis occurred as before. The bloods in question could not be added even to 0.9 per cent. saline without the occurrence of haemolysis.

Allied to this finding was the observation that, among the routine samples of blood collected through a dry sterile needle into a dry heparinized tube, were a few which showed gross haemolysis. All these specimens were obtained from patients extensively burned. In addition, when specimens of urine could be obtained from these patients during the shock period, they contained blood pigment.

A similar haemoglobinaemia and haemoglobinuria has been reported recently after severe burning by Cope and Rhinelander (1943), and a recent account of alteration in erythrocyte fragility associated with this condition has been given by Shen, Ham and Fleming (1943).

Details of the cases showing haemoglobinaemia are given in Table XII.

TABLE XII.

Case	Extent of Burn (per cent. of body surface)	Hb. g. per cent. Plasma	Time (hours)	Haemo-globinuria	Fragility	Notes.
45	75-80	Slight.	1.75	1 spec. 8 hours.	Fig. 26 No. 7	-
46	80	1.54 0.50 0.58 0.38 trace	1.5 3.5 5 8 18	Specimen obtained at 16 hrs.	Figs. 25 and 27	Blister fluid at 3.5 hrs. contd. 0.45 g. % Hb.
84	90	Not obvious 1.2 1.1	.5 2.5 5.5	Marked in only sample obtained.	Fig. 26 Nos. 5 and 6.	Capillary sample. Venous sample Venous sample
85	70	Not obvious. Rapid lysis in vitro. 0.225	2 5.5 9	Not seen.	Fig. 26 Nos. 1, 2 and 3.	—

All the patients showing haemoglobin in the plasma in large amounts were very extensively and severely burned, and all except Case 46 died within 12 hours of

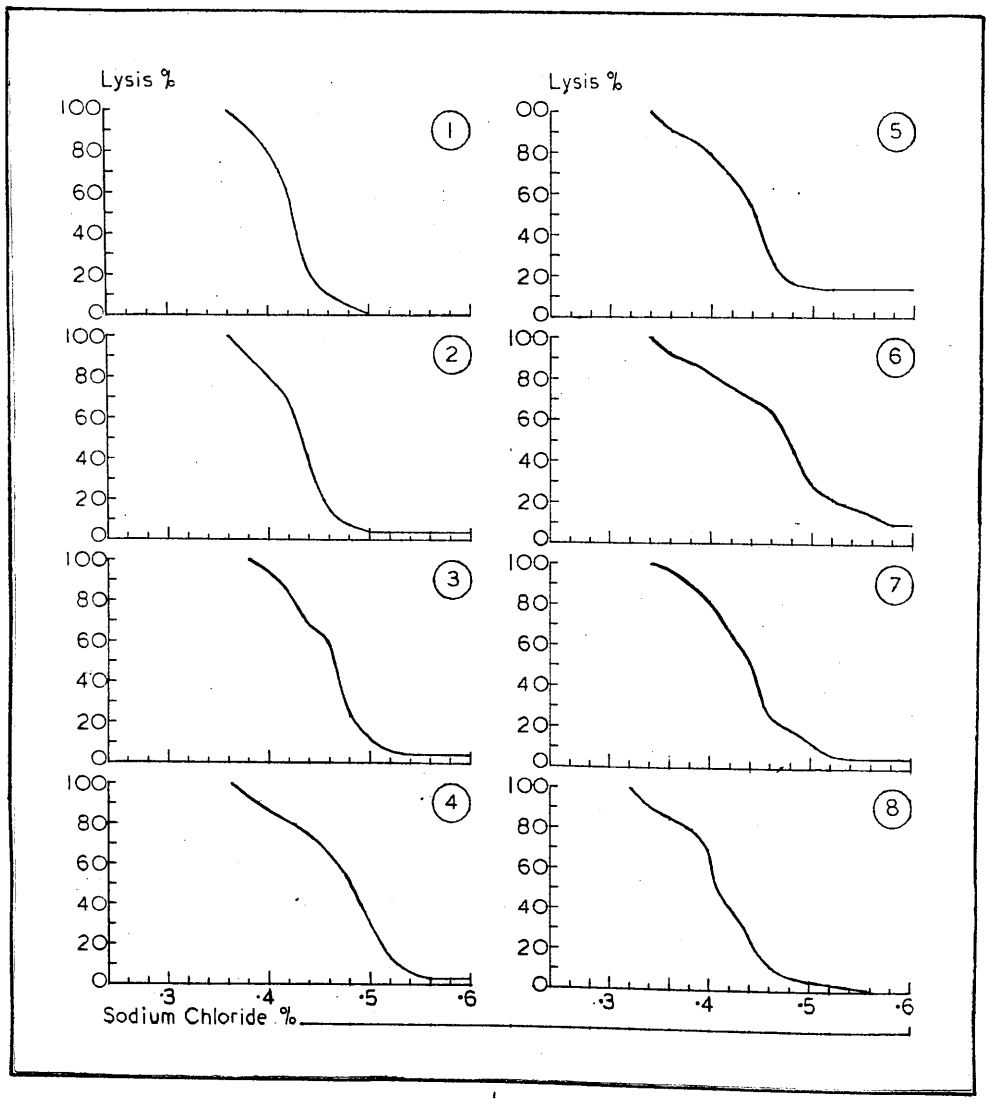

Fig. 26

the injury. It is possible that other cases had traces of haemoglobin (less than 0.1 g. per cent.) but no investigation was made into this matter.

Case 85 is of special interest, because it was noted that the second sample of blood showed no initial haemolysis but that haemolysis developed rapidly in vitro. The haematocrit tube, filled and centrifuged within 5 minutes of withdrawal of blood, showed no obvious haemoglobin in the plasma, but when sedimentation occurred in the specimen, which had been subject to no undue manipulation, marked haemoglobin staining of the plasma was found to be present 1.5 hours later.

IV. FURTHER OBSERVATIONS ON RED CELL FRAGILITY AND RESIDUAL HAEMOLYSIS.

Detailed results of the fragility tests performed on samples of blood from patients very extensively burned, and showing residual haemolysis or haemoglobinaemia, are recorded in Fig. 26.

In case 85 (Fig. 26, Nos. 1-3), at 2, 5.5 and 9 hours an increase in M.C.F. from 0.424 per cent. NaCl to 0.466 per cent. NaCl is seen. In specimens 2 and 3, residual haemolysis of about 5 per cent. occurs in concentrations above 0.50 per cent. and 0.54 per cent. NaCl, respectively. In the sample No. 3, slight haemoglobinaemia was observed (Table XII).

In Case 115 (Fig. 26, No. 4) the only sample, obtained at 5.5 hours, shows a M.C.F. much increased (0.480 per cent. NaCl) and a residual haemolysis of about 5 per cent. The size of the blood sample precluded more than a capillary haematocrit estimation in addition to the fragility test. No gross haemolysis was evident in the fresh specimen, and a single sample of urine showed no haemoglobinuria.

In Case 84 (Fig. 26, Nos. 5-6), the M.C.F. is already above normal at 2.5 hours (0.442 per cent. NaCl) and it rises at 5.5 hours to a very high level (0.476 per cent. NaCl). On both occasions 10-15 per cent. haemolysis persists in the higher saline concentrations. On both occasions haemoglobinaemia was marked (Table XII).

In Case 45 (Fig. 26, No. 7) only one sample of blood was obtained. It shows a M.C.F. on the high side of normal (0.440 per cent. NaCl) and 5 per cent. haemolysis in all saline above 0.54 per cent. Slight haemoglobinaemia was recorded in this sample, and haemoglobinuria was marked.

In Case 56, burned 75 per cent., a sample of blood obtained at 1 hour shows a M.C.F. within normal limits (0.408 per cent. NaCl) and no residual haemolysis.

In attempting to explain the residual haemolysis, two possibilities have to be considered:-

(a) that it was brought about by manipulation

of the blood in the microburette, or by this factor in association with (b);

(b) the presence of some degree of haemolysis in the fresh blood.

It seems very unlikely that injury of the cells by manipulation played a part, for the following reasons: The method used in these cases was the same as that employed throughout the entire series, and no residual haemolysis was encountered in any patient with mild burns. No haemolysis was noted after manipulation in the microburette if it were not previously present. As will be seen later, the most careful addition of the blood to the saline from a chemically clean pipette did not alter the results in any way.

It seems equally unlikely that the residual haemolysis noted in the tests was due to the haemoglobin present in the fresh plasma. In Case 115 (Fig. 26, No. 4), 5 per cent. residual haemolysis occurred, but the fresh specimen showed no gross haemolysis. The maximum plasma haemoglobin recorded in the whole series was 1.54 g. per cent. (Case 46, Table XII). Since the whole blood (with a haematocrit of 46 per cent.) was used for the fragility test, the plasma contained only 0.83 g. haemoglobin per 100 ml. blood, or 4.5 per cent. of the total haemoglobin present at that time (18.6 g. per cent.). Yet the same sample of blood showed a residual haemolysis amounting to

more than 15 per cent. in all strengths of NaCl above 0.52 per cent. The initial haemolysis in this case was not responsible for the residual haemolysis found in the test.

This conclusion is also borne out by the fuller analysis of the readings obtained with the bloods from Case 84 (see Table XII and Fig. 26, Nos. 5 and 6). The 1st sample (capillary blood) showed no haemolysis; the 2nd and 3rd (venous blood; Fig. 26, Nos. 5 and 6) showed an equal amount of haemolysis in their plasma, though they differed in their red cell content. It is seen from consideration of the facts related to these two samples of blood (Table XIII) that the plasma haemoglobin cannot account for more than 2.5 per cent. lysis in the fragility tests.

TABLE XIII.

Case 84.

Blood Spec.	Blood Hb. gms. per cent.	Haemato-crit.	Plasma Hb. per 100 ml. plasma.	Plasma Hb. % of total Hb.	Residual lysis in Fragility Test.
2 (Fig. 26, No. 5)	19	60 per cent.	1.2 g.	2.5	15 per cent.
3 (Fig. 26, No. 6)	21.5	70 per cent.	1.1 g.	1.5	10 per cent.

To confirm this conclusion, a known amount of plasma was added to 0.85 per cent. saline as in the

fragility test. Figures corresponding exactly with those obtained by calculation were obtained from the blood sample.

In order to make still more certain that manipulation was not responsible for the residual haemolysis, the following experiment was carried out with Specimen No. 3 (Fig. 26, No. 6):-

1.0 ml. blood No. 3 was added to 1.0 ml. 0.9 per cent. saline, using a chemically clean pipette, and the mixture was gently inverted several times.

A control sample undiluted was pipetted in the same way, and inverted for the same period at the same time.

The red cells were then counted:-

Undiluted sample .. .. R.B.C. 5.69×10^6/c.mm.

Diluted sample (×2) .. R.B.C. 5.22×10^6/c.mm.

Loss red cells .. - 0.47×10^6.

= 8.2 per cent. original number.

About 8 per cent. of the cells had been lysed by the addition of the blood to 0.9 per cent. NaCl. But the estimation of the amount of haemoglobin in the fresh plasma had shown it to be 1.5 per cent. of the total present in the whole blood (Table XIII). It was to be expected, therefore, that when added to saline the total

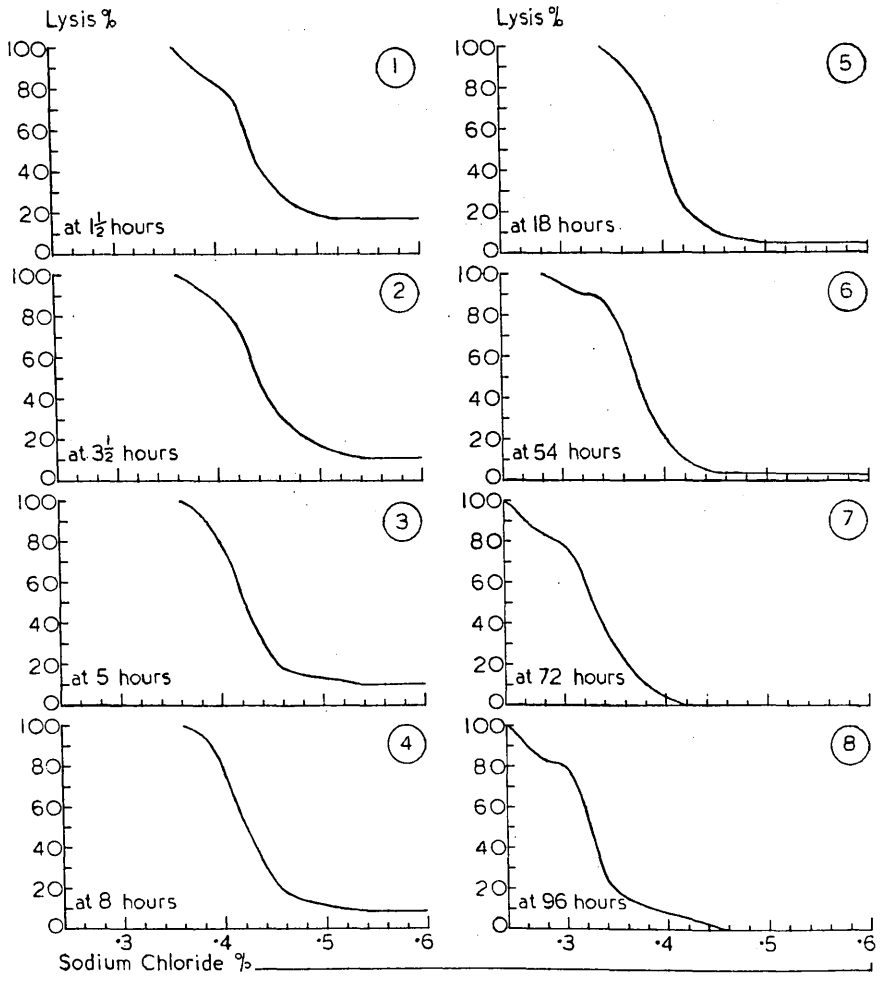
Lysis %
100
80
60
40
20
0
1
at 1½ hours
2
at 3½ hours
3
at 5 hours
4
at 8 hours
Lysis %
5
at 18 hours
6
at 54 hours
7
at 72 hours
8
at 96 hours
·3
·4
·5
·6
Sodium Chloride %

Fig. 27

lysis would be about 9.5 per cent. The figure actually found for residual haemolysis in this sample of blood (Fig. 26, No. 6) was almost exactly the same - viz. 10 per cent.

The fact that the residual haemolysis maintained the same level in all concentrations of saline beyond 0.58 per cent. strongly suggests that the 8 per cent. of cells so represented form a group much more readily destroyed than the remaining 92 per cent. the fragility of which is only slightly increased.

A similar state of affairs may be said to have occurred in the bloods of all the other patients who showed a fragility curve of the type just considered.

RESIDUAL HAEMOLYSIS, HAEMOGLOBINAEMIA, AND ANAEMIA IN A PATIENT WITH BURNS INVOLVING 80 PER CENT. OF THE BODY SURFACE.

It has just been shown that residual haemolysis in the fragility test is due to changes in the red cells which render them unable to withstand dilution of the plasma by 0.9 per cent. NaCl, even in the proportions 1:2.

The changes in the fragility curves in Case 46 during the four days of survival after burning are shown in Fig. 27.

At 1.5 hours (Fig.27, No. 1), immediately before transfusion was begun, a normal M.C.F. was recorded (0.436), and a residual haemolysis of 15 per cent. was found in all

concentrations of NaCl above 0.50 per cent.

A slight rise in M.C.F. occurred at 3.5 hours; and, thereafter, as the M.C.F. progressively declined, the amount of residual haemolysis fell until, at 72 hours, when the M.C.F. was 0.328, no residual lysis was evident (Fig. 27, No. 7). The final result on the 4th day shows no residual lysis and a M.C.F. of 0.324.

Reference to Table XII reveals that haemoglobinaemia was most marked when the residual lysis was at a maximum - early after burning - and that when the residual haemolysis was not found in the fragility test, gross haemoglobinaemia had ceased and haemoglobin had disappeared from the urine.

During the first 4 days, the haemoglobin level fell from 18.6 g. per cent. to 10 g. per cent. (Fig. 20), but, owing to major changes in plasma volume which may have occurred during this period, it is impossible to estimate the loss of red cells indicated by this fall. That considerable loss was occurring is certain. The development of haemoglobinaemia amounting to as much as 8 per cent. of the total blood haemoglobin indicates, to some extent, the degree of haemolysis which had already occurred in the first 1.5 hours. It is difficult to say just how much haemolysis is indicated by gradual fall of plasma haemoglobin to the 18th hour; and by the still more gradual disappearance of residual haemolysis in the

fragility tests. Even in normal subjects, given not more than 10 g. of stroma-free haemoglobin intravenously, it takes at least 10 hours for the plasma to be cleared (Ottenberg & Fox, 1938). The failure to clear the plasma of haemoglobin until the end of 24 hours in Case 46, severely burned, with oliguria, and haemolysis amounting to about 8 per cent. of the circulating red cells at 1.5 hours, cannot therefore be taken to indicate continued marked haemolysis throughout the first day. In this case marked haemolysis occurring during the process of, or very soon after, burning, by itself appears sufficient to explain the persistence of obvious haemoglobinaemia until the 18th hour, though the evidence obtained from study of erythrocyte fragility suggests that slight continued haemolysis may have contributed. The gradual disappearance of the residual haemolysis may indicate either that the red cells represented by this part of the curve were recovering their former resistance, or that they were being destroyed. The latter seems the more probable, in view of the degree of increased fragility shown by these cells.

The evidence reviewed suggests, therefore, that marked haemolysis may occur during, or shortly after, the process of burning in cases extensively injured, and that a less rapid process of haemolysis may continue during the following 24 hours at least.

On this basis, it seems possible that, at the time of burning, a certain proportion of red cells exposed to heat are lysed in situ; that the residual haemolysis in the fragility tests indicates the proportion of cells which are severely damaged, but survive immediate destruction, to be destroyed more gradually in the next day or two; and that a slight increase in the average fragility of the remaining cells indicates a variable and slight amount of damage sustained by some or all of these cells.

CHAPTER VIII.

THE ETIOLOGY OF ANAEMIA IN BURNS.

I. INTRODUCTION.

It has been shown that the burned patient, roughly in proportion to the severity of his burn, is liable to become anaemic within the first week of injury. A reduction of red cell levels to about 50 per cent. of normal has been the usual finding after severe burns, and patients severely burned have required transfusion on the 10-12th day and later.

Certain changes in the diameter and thickness of many of the red cells have been demonstrated at an early stage after the injury. These changes consist of the development of a degree of microspherocytosis. Fragmentation of the red cells has also been observed.

Observations on variations in the osmotic fragility of the red cells have revealed that immediately after a severe burn there is a great increase in saline fragility, and that a proportion of the red cell population may be so severely affected that lysis occurs even in 0.9 per cent. saline. Intravascular haemolysis and haemoglobinuria may occur.

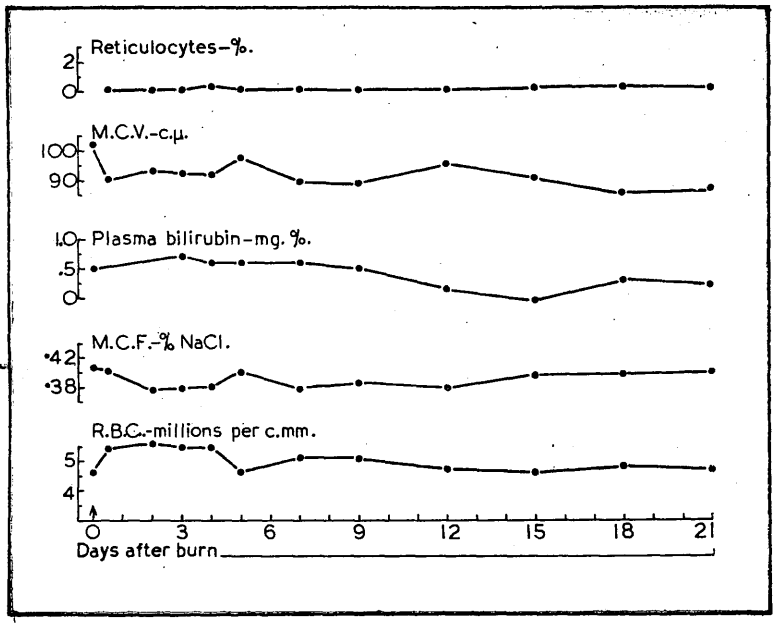
Reticulocytes–%.
2
0
M.C.V.–c.μ.
100
90
1.0
Plasma bilirubin–mg. %.
.5
0
M.C.F.–% NaCl.
·42
·38
R.B.C.–millions per c.mm.
5
4
0
3
6
9
12
15
18
21
Days after burn

Fig. 28

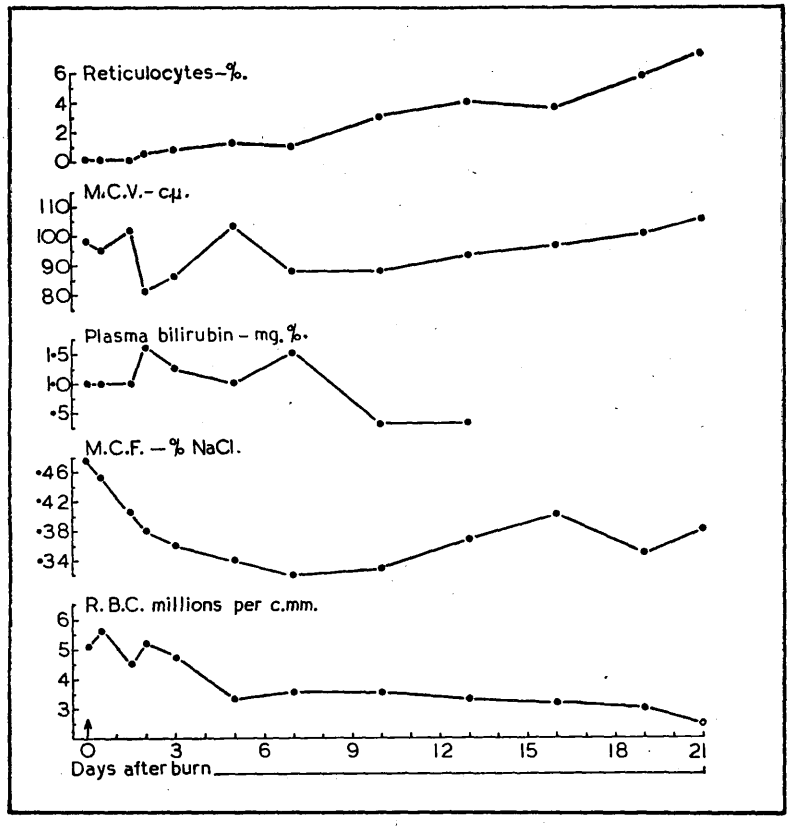
Reticulocytes–%.
6
4
2
0
M.C.V.– cμ.
110
100
90
80
Plasma bilirubin – mg. %.
1·5
1·0
·5
M.C.F. – % NaCl.
·46
·42
·38
·34
R.B.C. millions per c.mm.
6
5
4
3
0
3
6
9
12
15
18
21
Days after burn

Fig. 29

These findings have been described individually. In order to correlate them two typical cases, one mildly the other severely burned, are illustrated (Figs. 28 and 29). Both received serum transfusion in the shock period.

Case 11 (Fig. 28) suffered a relatively mild burn (6 per cent.) and received 1,000 ml. of serum in the first 24 hours. In this patient, there is no definite evidence of the development of even a mild degree of anaemia, as shown by red cell, haemoglobin and haematocrit estimations. The mean corpuscular volume (M.C.V.) is highest at 2 hours; it fluctuates during the first 12 days, and thereafter slightly lower levels are maintained. The M.C.F. also shows its highest recorded value in the first specimen. A distinct fall occurs till the 4th day, when a rise in M.C.F. takes place. Subsequently, stabilisation is evident from about the 15th day. The plasma bilirubin* is slightly increased during the first 7 days; thereafter, normal values are recorded. No significant alteration in reticulocytes is observed in the first 21 days after injury.

Case 58 (Fig. 29) suffered a severe burn (40 per cent.) and received 5,700 ml. of serum during the shock period. Marked anaemia is seen to have developed by the end of the first week. The anaemia persists, and by the

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* I am very greatly indebted to Dr. A.B. Anderson for the estimations of plasma bilirubin recorded here. A full account of his work on the biochemical changes in burns is given elsewhere (Anderson & Semeonoff, 1944).

end of the second week, a reticulocyte response is evident. The M.C.V. varies significantly during the first week, in the form of a slow steady rise in M.C.V. accompanying the reticulocyte response. The M.C.F. is markedly increased on admission, but by the 7th day a steady and rapid fall has produced values abnormally low. Thereafter, a gradual rise to normal is seen. The plasma bilirubin, already above normal at 3 hours, rises on the 2nd day to 1.7 mg. per cent., a level from which there is little deviation until after the 7th day. A return to normal then occurs.

It is evident that in these two cases the general trend of the variations is very much the same. They differ only in degree. In both cases, the changes are confined almost entirely to the first 7 days following injury. By this time, anaemia is maximal and spontaneous arrest in its progress occurs. Irregularly high values for M.C.V. occur especially in the first few days, and in the second week stabilisation tends to occur. Significant changes in fragility occur in the first week. The highest values for M.C.F. are recorded in the first few hours, and the lowest at about the 7th day, after which a rise occurs spontaneously. These high values were obtained (Case 58) from samples showing a considerable degree of microspherocytosis (Table X: Fig.23). The low values were obtained when an increase in M.C.D. and a normal or slightly low M.C.A.T. were present. Comparison

of the data from this case illustrates this correlation very clearly (Figs. 23 and 29: Table X).

II. DISCUSSION.

The anaemia which may follow burns has been shown to vary from a transient change of mild degree to a rapidly progressive and ultimately severe anaemia, requiring several blood transfusions during the first month. In patients extensively and severely burned, a variable degree of anaemia tends to persist for several months.

In relation to the cause of these anaemias, the following possibilities have to be considered:-

1. That the changes are due to haemodilution following the period of haemoconcentration and occurring with the return of fluid from the tissue spaces.
2. That red cells may be irregularly distributed, an excess being stored in the spleen.
3. That actual destruction of red cells occurs.
4. That there is marrow dysfunction, resulting in failure to maintain or produce normal blood levels.

HAEMODILUTION.

Since plasma may escape from the circulation into the tissue spaces in sufficient amount to cause demonstrable haemoconcentration, it would not be surprising

if the return of this fluid into the blood stream were demonstrable as a reduction in haemoglobin levels. But it is unlikely that return of this fluid would cause apparent anaemia if fluid had not been added to the circulating blood in the meantime, during the period of haemoconcentration. The addition of fluid to the blood-stream from tissues remote from the burn has already been discussed, and it is possible that this actually does take place, at least to a minor extent. It seems possible that the return of the fluid from the tissues around the burn, if in sufficient amount, might then cause excessive haemodilution and apparent anaemia. Attention has been drawn to the factors favouring the accumulation of fluid in the tissues. These factors would be expected to increase the tendency to a phase of haemodilution. Loss of fluid from the surface, and from the body, would have the reverse effect. The occurrence of oedema during the period of haemoconcentration, and its disappearance during the development of apparent and temporary anaemia, has been noted.

A final statement on the role of changes in plasma volume in the production of the mild and temporary "anaemia" encountered in cases of Groups I and II might best be made on the basis of direct studies of the volume changes, but these studies could not be attempted.

DISTRIBUTION CHANGES.

Variations in haemoglobin level may be produced by the action of the spleen (Part I, p.45). It seems probable that such variations would be slight, and it is unlikely that they could explain the anaemia encountered in patients severely burned. Such a mechanism might contribute to the minor change in haemoglobin already discussed. Splenic enlargement has not been observed clinically, and at autopsy no significant increase in the size of the spleen has been noted.

HAEMOLYSIS.

The various changes occurring in the blood, especially after burns involving more than 10-15 per cent. of the body surface, are compatible with the occurrence of haemolysis, of a degree varying roughly with the severity of the burn.

The early phase of temporary "anaemia" is rarely seen in patients with burns involving more than 15 per cent. of the body surface. Instead, there is a phase of rapidly developing and severe anaemia, reaching a maximum in 10-14 days. In the early stages, spherocytosis develops and erythrocyte fragility (M.C.F.), increases. Later, with the development of the anaemia, the M.C.F. falls rapidly. The occurrence of haemoglobinaemia and haemoglobinuria has been recorded after very severe burns, and these changes are closely related to the more marked

alterations in saline fragility. It would seem that, in all groups, changes of the same type are occurring, and that these changes differ only in degree.

The occurrence of hyperbilirubinaemia and urobilinuria (Anderson & Semeonoff, 1944) is compatible with, though not necessarily indicative of, a process of haemolysis; and the same may be said of the almost invariable finding of splenic siderosis in cases coming to autopsy after the first week.

The fundamental cause of the changes in the red cells leading to haemolysis is probably the direct action of heat. When blood is heated even rapidly to temperatures of 51-65°C., and then immediately cooled to 37°C. the abnormal changes which occur (Shen, Ham & Fleming, 1943) are similar to those found in the patients described in this study. The first detectable alteration is the formation of bud like projections connected at first, and finally disconnected from the red cell. Progressive fragmentation is associated with spherocytosis. Coincident with the appearance of spherocytes and microspherocytes in the blood film, the osmotic fragility of the red cells becomes increased. When maximum fragility is reached, a proportion of the cells are lysed in 0.85 per cent. sodium chloride solution.

These experimental findings are exactly in agreement with the observations in man already described

in detail, and when it is considered that blood which has undergone such changes is abnormally susceptible to mechanical trauma artificially produced (Shen, Ham & Fleming, 1943) the probable mechanism of the haemolysis in burns becomes increasingly evident.

All the clinical and experimental evidence strongly supports the view that a haemolytic type of anaemia occurs in relation to severe burns, that the haemolysis is due to intravascular destruction of red cells rendered unduly susceptible to the physiological trauma of the circulation, and that this susceptibility is the direct result of heat on the cells whereby fragmentation and spherocytosis occur. The evidence is that the change induced by heat is inherent in the red cells: it is independent of the fluid in which they are suspended (Shen, Ham & Fleming, 1943).

On this basis, the occurrence of severe haemolysis will depend on several factors. The most important are (1) the temperature to which the blood has been subjected, (2) the duration of heating, and (3) the volume of blood subjected to these conditions. On the same basis, strict correlation between severity of burn and degree of red cell involvement is improbable. It is to be expected that, although marked red cell changes are likely to be associated with severe burns, all cases of severe burns may not show obvious haemolysis. These

expectations have been fulfilled in the present investigation. Several cases of very extensive burns showed no evidence of intravascular haemolysis within a few hours of injury.

The importance of intravascular haemolysis in relation to renal function is well known in connection with incompatible transfusion. That the haemolysis of severe burns may be productive of similar effects has been known for many years, and it is of interest to note that pathological changes have been found in the kidneys of several of the cases described in this study. Case 46, in whom marked intravascular haemolysis and haemoglobinuria occurred, and in whom plasma transfusion was apparently adequate, died on the 4th day with a blood urea level of 232 mg. per cent. At autopsy the kidneys were found to show extensive damage of a type similar to that occurring after incompatible transfusion or crush injury (Gibson, 1944). It is thus apparent that in severe cases of burns, death may occur in the shock period from a cause other than oliguria.

MARROW DYSFUNCTION.

Failure of red cell production cannot explain the rapid development of anaemia and the early development of a reticulocyte response, and it seems probable that at this stage marrow dysfunction is not a significant factor. But in the later stages, with failure to recover after a

month or more and without evidence of progressive blood destruction, defective marrow function may well be responsible for the chronic anaemia which persists until the body temperature has settled and the burned areas are healing satisfactorily. Such an anaemia may well correspond with that encountered in many chronic infections (Vaughan & Saifi, 1939).

There is no evidence that this type of anaemia has been related to the level of plasma proteins, and while depletion of body protein may be a contributory factor, it is not necessarily so (Rytand, 1942).

THE INFLUENCE OF TREATMENT.

Haemolytic anaemia, agranulocytosis and thrombocytopenia have been reported following the use of the sulphonamide drugs, to the actions of which all the patients in this series were exposed. Slight depression of platelet counts on beginning treatment with sulphathiazole, and increase immediately on its withdrawal, have been noted (Kracke & Townsend, 1943). Changes in erythrocyte fragility have followed the use of sulphanilamide (Antopol, Goldman & Sampson, 1941). Slight increase in reticulocytes has been recorded after moderate doses of sulphanilamide for various diseases (Campbell, 1938); and there is evidence that drugs of this group may cause liver damage (Schattenberg & Harris, 1943).

The demonstration of appreciable absorption of the drug when sulphonamides are applied to burned surfaces (Anderson & Semeonoff, 1944) suggests at once the possibility that the various haematological abnormalities recorded may have been due, at least in part, to treatment.

In severe burns, haemolytic anaemia is a fairly constant sequel. In fact, it occurs too frequently to be due to sulphonamides alone. No control series untreated with these drugs was, however, available, as patients treated with penicillin had sulphonamide applied to some area in the first few days at least. It is impossible to assess the part played by these drugs in the production of minor alterations in red cell levels, but it should be noted that many patients showing appreciable blood concentrations of the drugs developed no anaemia and no increase in reticulocytes.

The burn must be regarded as responsible for the immediate and great increase in red cell fragility recorded in all the cases of severe injuries. This abnormality was recorded before any treatment, local or general, had been instituted. In addition, a return to normal levels occurred before application of any dressing. By the time, however, that a great decrease in fragility was observed, dressings had been applied in all the cases studied. Since the changes in erythrocyte fragility

recorded by Antopol, Goldman and Sampson (1941) were of the nature of increased resistance to hypotonic saline, it is impossible at present to assess the role of chemotherapy in the development of the low levels of M.C.F. recorded here (Fig.25). It can be said, however, that there was no obvious relationship between the changes in fragility and the blood concentration of the drug.

The role of the sulphonamides in production of changes in the leucocytes is discussed later (p.205).

TABLE XIV.

PLATELETS IN BURNED PATIENTS.

Platelet Counts.

	1st Week.		Following 3 - 4 Weeks.		
Case No.	Average Count.	Number Performed.	Highest Count.	Time of Occurrence.	Number Performed.
11	330,000	2	480,000	24th day	8
12	317,000	4	450,000	12th day	7
13	263,000	3	490,000	18th day	8
14	224,000	1	400,000	22nd day	7
8	-	-	375,000	12th day	5
31	173,000	1	500,000	22nd day	10
32	136,000	3	504,000	26th day	3
38	69,000	1	424,000	8th day	-*
45	440,000	1	-	-	-*
48	320,000	8	536,000	12th day	-†
54	135,000	2	-	-	-*
58	332,500	4	520,000	18th day	8

*Case 38 died on the 8th day: Case 45 at the 10th hour: Case 48 on the 2nd day.
†Daily from 12th - 14th day.

The observations of Kracke and Townsend (1943), on the reduction of platelets in relation to treatment

with sulphathiazole, are to some extent reflected in the figures obtained during the first 4 weeks in twelve cases of burns. No gross abnormality in platelets was found, even in patients burned very extensively, but a tendency was observed for the platelet counts to be rather low during the first week, and for slightly high counts to appear temporarily at the end of this somewhat variable period (Table XIV). These alterations bore no relation to the severity of the injury. While marked thrombocytopenia was not seen, Case 45, burned 75-80 per cent. of the body surface, showed a purpuric eruption on the remaining intact skin, within 2 hours of injury. The platelets numbered 440,000 per cubic millimetre at this time.

Changes in M. C. V. bore no evident relation to the blood concentrations of sulphanilamide.

Plasma and serum therapy influenced red cell levels during the period of administration, but there is no evidence of any other effect. Temporary haemodilution occurred whether or not these fluids had been given in the shock period. Abnormalities in M. C. V. and M. C. F. were found before the administration of serum or plasma, and in patients not transfused at any stage. Marked haemolysis was observed in cases not transfused.

There is thus no evidence that serum or plasma therapy played any significant part in the production

of blood changes, apart from the mechanical effect of haemodilution during the period of their administration.

III. SUMMARY AND CONCLUSIONS.

ANAEMIA.

A reduction in red cell levels is a common feature of burns of all degrees. The magnitude of the change is roughly proportional to the burn.

Patients with burns involving up to 15 per cent. of the body surface frequently show a mild temporary fall in red cell levels, maximal by the 5th-7th day, with recovery by the 10th-12th day. This change may be due to haemodilution.

Burns involving over 15 per cent. of the body surface are frequently followed by a rapidly occurring, moderately severe anaemia, the development of which is associated with a slight and variable increase in red cell size (M.C.V.), spherocytosis, increased erythrocyte fragility, and an increase in plasma bilirubin and urobilin excretion. A reticulocyte response follows. This anaemia is haemolytic in origin, damage to a proportion of the red cells occurring at the time of burning.

After extensive severe burns with considerable skin loss, a state of anaemia tends to persist until the patient is apyrexial and healing is well advanced. This anaemia is regarded as belonging to the type found in association with chronic infection.

HAEMOGLOBINAEMIA AND HAEMOGLOBINURIA.

Four cases are described, all with very extensive burns. The condition was associated with a great increase in saline fragility of the red cells, and marked spherocytosis.

The condition is regarded as differing only in degree from the less obvious blood destruction occurring in patients less seriously burned.

C H A P T E R IX.

CHANGES IN THE LEUCOCYTES IN BURNS.

I. INTRODUCTION.

The changes in the white cells following burns have been recorded on numerous occasions in the past, and little important could be contributed to present-day knowledge were it not that certain changes found in this study have not been recorded previously. These changes are almost certainly related to the treatment of the injury by the local application of drugs of the sulphonamide series.

II. LEUCOCYTOSIS IN BURNS.

INTRODUCTION.

Leucocytosis is a common and early finding after burns. Locke (1902) recorded its occurrence within an hour of injury. In four non-fatal cases white cell counts up to 28,000 per c.mm. were observed. No striking increase in neutrophil polymorphs was seen, but degenerative changes were common. Myelocytes appeared in the blood in two fatal cases. A feature of importance emerging from his work is the serious prognostic significance of very high

leucocyte counts. In this respect his findings support the view of Dorrance (1932) that a leucocytosis of 50,000 per c.mm. or over, in the shock period is a very unfavourable sign.

In a study of extensive cutaneous burns, McIver (1933) describes the white cell response in sixteen cases, four of whom died within four days of the injury. In these fatal cases, the lowest white cell count recorded in the first two days was 23,000 per c.mm. Two showed a leucocytosis of over 50,000 per c.mm. in this period. In eleven non-fatal cases, the highest white cell count recorded in the first two days was 22,000 per c.mm.

THE TOTAL WHITE CELL COUNT.

In this investigation, the variations in the white cells have been studied in 29 cases (Table XV). For descriptive purposes the cases are again divided into four Groups.

A rise in the total white cell count after burning was almost a constant finding. In degree, it was roughly proportional to the area burned, and in the more severe cases very high values were found.

A striking feature of the leucocytosis was the rapidity with which it might appear. It was usually established within the first 3 hours. At this time, there might be a great increase in neutrophil polymorphs (Table XX).

In all groups, two phases of leucocyte response

TABLE XV.

Total White Cell Count (in thousands per c.mm.)

Group and Case No.	Hours after Burn.*					Days after Burn.						
	1-3	6	9	12	18	1	2	3	4	5	6	7
63						2.6	5.2				4.4	
64						9.2	11.2	10.4		7.4		
I. 65	4.8		6.1	9.7			6.8	5.0		3.6		
66	9.9	8.4	7.0			12.0	11.2	7.1	4.6	8.6		11.2
67	6.7		3.1			3.1	3.5	6.3	5.9	4.4		
68							13.0	17.6	14.6	10.0		8.8
11	11.3			10.0			15.6	8.8	10.0	8.2		9.0
12	8.4			11.0		8.0		13.6		4.4		11.8
13	12.6			14.6		19.2		20.8	16.0		18.2	
14	4.6				12.0		14.6	9.4		12.2		12.4
II. 16	8.6				9.5	7.0	4.5	4.3	3.1	6.2	11.2	
21	13.2	13.2	7.9		8.8	18.4	13.0	11.2	4.2	4.0	8.0	10.8
69		9.2				18.1	11.7	6.0	6.1	4.3	5.4	
76	9.6				13.0	6.0	12.5		7.0		11.0	
78	12.3	9.1	6.5	14.6		8.9	6.7	8.6		5.6		
8	5.0	15.2				14.8	14.0	14.2				10.0
30	14.4	16.4			19.6		4.5	11.0				11.0
31	15.4	15.8	21.2			20.8	22.0	17.2		10.6		9.6
III. 80	19.2					15.4	11.6	9.8		11.6		8.8
82	14.0				13.7	18.0	12.3	9.7	12.4	3.1	3.5	6.5
83					10.4	10.8	25.2	6.7	4.15	3.2	5.6	6.1
110	13.0			21.6			11.0		10.3		10.2	
45	80,000 at 1.75 hours.											
46	25.4	19.5	23.8		20.6	12.2	17.6	9.6	8.6			
48	18.8	14.3			12.8	29.2	21.0	20.0	15.5	12.0		13.0
IV. 54	19.8		28.0			13.0	15.8	16.8				
58	20.8	32.6		43.6	35.5	29.2	44.4	16.8		12.2		19.8
59						15.5	6.4	6.4	4.2	7.2	12.2	
85	6.6	15.1										

* Hours only approximately.

tended to occur. Typified by Case 21, there was an initial high white cell count within the first two days. A fall then occurred and normal or low levels might be present on the 4th or 5th day, after which a second leucocytosis frequently occurred-in this case (No. 21) the secondary

response reached 18,000 per c.mm. on the 8th day.

In Group IV, one patient showed a very high white count. It preceded an early fatal issue.

THE POLYMORPHONUCLEAR LEUCOCYTES.

In most cases the neutrophil count followed the total white cell count very closely. From Table XV it is observed that many cases during the first 7 days showed a fall in the total count amounting to leucopaenia. It will be shown later that actual neutropaenia occurred in the majority, and complete agranulocytosis in one, of these.

Neutrophil Polymorphs in Case 11 (Burned 6 per cent. of body surface).

A neutrophil leucocytosis was evident in this case within 3 hours of the injury. The maximum response in the period reviewed occurred on the 2nd day (Table XVI).

TABLE XVI.

Neutrophil Polymorphs in Case 11.

Time after burn.	Total W. B. C.*	Neutrophil Polymorphs.*	Vacuolated forms.
3 hours	11,300	9,300	6 per cent.
12 "	10,000	8,600	3 " "
2 days	15,600	14,300	few
3 "	8,800	7,500	-
4 "	10,000	8,700	-
5 "	8,200	6,800	-
7 "	9,000	6,500	-

* per c.mm.

Toxic granulations and cytoplasmic vacuolation was observed in a small proportion of the cells during the first two days.

Neutrophil Polymorphs in Case 12 (Burned 8 per cent. of body surface).

No obvious leucocyte response was apparent at the 3rd hour. A moderate response was seen at 12 hours and the maximum white cell count was recorded on the 3rd day (Table XVII).

TABLE XVII.

Neutrophil Polymorphs in Case 12.

Time after burn.	Total W.B.C.*	Neutrophil Polymorphs.*	Vacuolated forms.
3 hours	8,400	7,100	12 per cent.
12 "	11,000	8,900	5 " "
1 day(s)	8,000	6,500	4 " "
3 "	13,600	11,600	9 " "
5 "	4,400	3,500	10 " "
7 "	11,800	9,400	2 " "

* per c.mm.

Cytoplasmic vacuolation was more obvious in this case and it continued throughout the period reviewed. Its temporary increase at a time when slight neutropaenia occurred is a fact to which attention will again be directed later.

Neutrophil Polymorphs in Case 13 (Burned 10 per cent. of body surface).

A well-marked neutrophil leucocytosis was observed 3 hours after burning. It reached a maximum on

the 3rd day (Table XVIII).

TABLE XVIII.

Neutrophil Polymorphs in Case 13.

Time after burn.	Total W. B. C. *	Neutrophil Polymorphs. *	Vacuolated forms.
3 hours	12,600	11,700	15 per cent.
12 "	14,600	13,900	14 " "
1 day(s)	19,200	18,500	5 " "
3 "	20,800	20,200	6 " "
4 "	16,00	11,900	5 " "
6 "	18,200	14,200	4 " "

* per c.mm.

Cytoplasmic vacuolation was more marked here than in the previous cases. In addition, nuclear degenerative change was present in almost all the neutrophils seen in the first specimen. Staining was diffuse and no structural detail was apparent. In the last two preparations, about 50 per cent. of the neutrophils showed toxic granulation, but this degenerative change was no longer evident.

Neutrophil Polymorphs in Case 14 (Burned 10 per cent. of body surface).

This is one of the few cases in whom a low white cell count was found 3 hours after the injury. A leucocytosis was not recorded until the 18th hour. It reached a maximum on the 2nd day (Table XIX).

TABLE XIX.

Neutrophil Polymorphs in Case 14.

Time after burn.	Total W. B. C. *	Neutrophil Polymorphs. *	Vacuolated forms.
3 hours	4,600	2,800	+++
18 "	12,000	11,200	+++
2 days	14,600	12,700	++
3 "	9,400	7,100	+
5 "	12,200	7,700	rare
7 "	12,400	8,700	none seen

*per c. mm.

Vacuolated forms and toxic granulations were common in smears made during the first two days. In this period also many of the neutrophils had pyknotic and structureless nuclei.

Neutrophil Polymorphs in Case 58 (Burned 40 per cent. of body surface).

In this patient a marked leucocytosis was evident at the 3rd hour, and it continued to increase rapidly, a level of 43,600 per c. mm. being reached at the 18th hour. A second peak occurred on the 2nd day (Table XX).

TABLE XX.

Neutrophil Polymorphs in Case 58.

Time after burn.	Total W. B. C. *	Neutrophil Polymorphs. *	Cook Index - lobes%.				
			1	2	3	4	5
3 hours	20,800	17,700	16	46	30	8	0
6 "	32,600	32,300	43	47	10	0	0
12 "	43,600	43,400	48	39	12	1	0
18 "	36,500	33,200	55	38	5	2	0
1 day(s)	29,200	27,400	36	48	15	1	0
2 "	44,400	40,300	48	37	12	3	0
3 "	16,800	15,600	57	33	9	1	0
5 "	12,200	11,000	70	29	1	0	0
7 "	19,800	15,000	66	29	4	1	0

*per c. mm.

In this case degenerative changes of the type described were numerous during the first 3 days. It is a striking feature that although the neutrophils numbered 17.700 per c.mm. at the 3rd hour, there was little or no alteration in age distribution of the cells as shown by the Arneth-Cook Index. By the 6th hour, however, the percentage of single-lobed neutrophils had increased by 27.

ASSOCIATED CHANGES.

Eosinophil Polymorphs.

Changes in the eosinophil polymorphs followed the general tendency exhibited in most acute infections with neutrophil leucocytosis. They disappeared soon after the injury and reappeared after a variable period.

In Case 11, none was seen until the 7th day by which time all degenerate forms had disappeared. The eosinophils then amounted to 2 per cent. of the leucocytes present.

In Case 12, eosinophils were first seen (1 per cent.) on the 5th day. They did not reappear during the following week.

In Case 13, no eosinophils were seen during the first 6 days.

In Case 14, none was seen during the first 4 days, but on the 5th, eosinophils amounted to 4 per cent. of all the white cells present. At this stage, degenerate polymorphs had just disappeared from the blood.

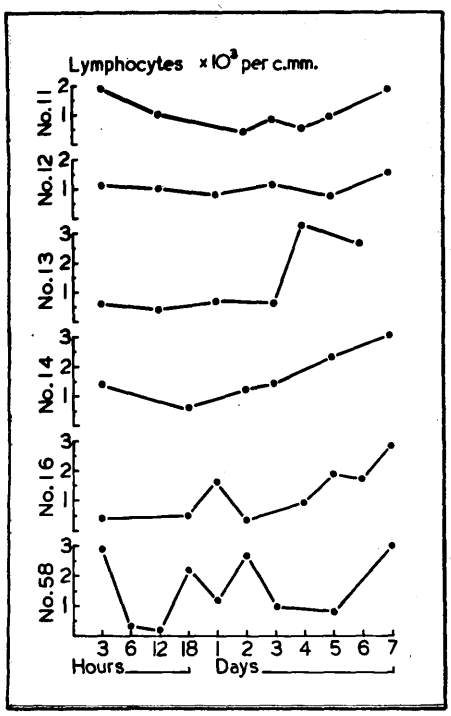

Lymphocytes x 10³ per c.mm.
No. 11
No. 12
No. 13
No. 14
No. 16
No. 58
3 6 12 18 1 2 3 4 5 6 7
Hours
Days

Fig. 30

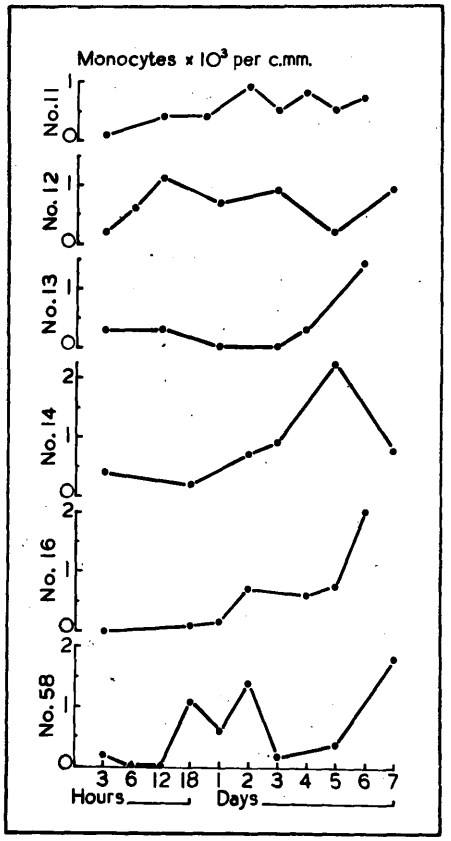

Monocytes x 10³ per c.mm.
No. 11
No. 12
No. 13
No. 14
No. 16
No. 58
3 6 12 18 1 2 3 4 5 6 7
Hours
Days

Fig. 31

In Case 58 the first sample showed 1 per cent. eosinophils. These cells were not seen again in films until the 7th day.

Lymphocytes.

The normal limits in health of the absolute lymphocyte count are 1,500 - 3,000 per c.mm. (Whitby & Britton, 1937: Wintrobe, 1942). In burned patients, significant variations in these cells have been observed during the first week (Fig.30). Lymphopaenia has been observed at least during the first two days in all the cases investigated. In Case 11, the lowest level reached was 400 per c.mm. In the others, in the order as shown in Fig. 30, it was 800, 400, 600, 380 and 200 per c.mm. respectively. The reduction is thus roughly in proportion to the severity of the burn. In Case 48 (Fig.32) the lowest count obtained was 300 per c.mm. at the 16th hour.

A similar trend in the lymphocyte response is shown by all cases during the first week, at the end of which time levels the upper limit of normal are uniformly present.

Monocytes.

Wintrobe (1942) regards the limits of the monocyte count in health to be 275-500 per c.mm. Whitby and Britton (1937) also consider 500 per c.mm. as the upper limit.

In the investigation on the changes in the

monocytes illustrated by Fig. 31, only one case showed a significant reduction in these cells soon after burning (Case 16). Without exception, however, they all showed a monocytosis towards the end of the first week. In Cases 11 and 12, with very mild burns, the highest counts were 900 and 1,100 per c.mm. respectively. In Case 13 a level of 1,400 was reached by the 6th day. Counts in the region of 2,000 per c.mm. were obtained in Cases 14, 16 and 58.

III. LEUCOPAENIA AND AGRANULOCYTOSIS.

LEUCOPAENIA.

Although leucocytosis occurred in the majority of patients, ten (34.5 per cent.) showed total white cell counts under 4,500 per c.mm. during the first seven days. In six of these cases, the leucopaenia was present on the fifth day, and more detailed study of one of these (Case 16) shows that actual granulocytopaenia occurred (Table XXI).

TABLE XXI.

The White Cells in Case 16 - Leucopaenia.

Time	Total W.B.C.	Total*			Arneth-Cook Index - Lobes					Notes.
		P	L	M	1	2	3	4	5	
3 hrs.	8,600	8,250	350	0	40	42	12	4	2	8% vacuolated.
17.5 hrs.	9,500	8,900	500	100	46	40	12	2	0	0% "
21.5 "	7,000	7,740	1,120	140	48	40	8	4	0	0% "
2nd day	4,500	3,440	340	720	56	38	5	1	0	0% "
3rd "	4,300	-	-	-	-	-	-	-	-	
4th "	3,100	1,620	880	600	56	42	2	0	0	6% "
5th "	6,200	3,600	1,860	740	60	34	6	0	0	8% "
6th "	11,200	7,500	1,680	2,020	55	35	10	0	0	0% "
8th "	18,200	14,850	2,800	550	44	43	11	2	0	0% "
10th "	7,500	6,300	750	450	28	52	17	3	0	0% "
12th "	4,800	3,900	650	250	33	50	16	1	0	0% "

* P = polymorphs. L = lymphocytes. M = monocytes. per c.mm.

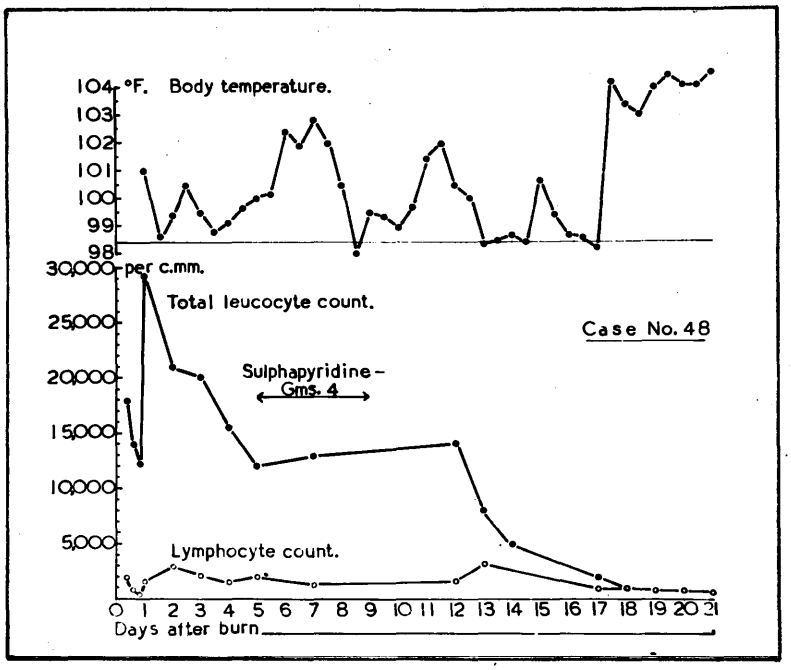

104 °F. Body temperature.
103
102
101
100
99
98
30,000 per c.mm.
Total leucocyte count.
25,000
Case No. 48
Sulphapyridine –
Gms. 4
20,000
15,000
10,000
5,000
Lymphocyte count.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days after burn

Fig. 32

It will be seen, also (Table XXI), that a marked shift to the left in the Arneth-Cook index accompanied the development of the neutropaenia, and that vacuolation of the polymorphs, first noted at the third hour, returned with the fall in the polymorphs, and disappeared with the return to normal levels. These changes are of special interest when compared with those shown by Case 48 in the development of agranulocytosis (Table XXII).

AGRANULOCYTOSIS.

In the cases of leucopaenia just described, the low white cell levels lasted 1 - 4 days and were not associated with any clinical upset. In one patient, however, complete agranulocytosis developed, and she died with staphylococcal septicaemia. The changes in the white cells in this case are summarised in Table XXII and illustrated in Fig. 32.

This patient, a girl aged 7 years, with burns involving 40 per cent. of her body surface, had the burned areas dressed with 10 per cent. sulphanilamide cream 4.5 hours after injury. The dressing was renewed on the 8th, 11th and 14th days. From the 5th to the 9th day, a total of 4 g. of sulphapyridine was given by mouth, on account of a respiratory complication. Some improvement followed, though the temperature did not settle completely. A sudden change for the worse occurred on the 18th day, when the

TABLE XXII.

The White Cells in Case 48 - Agranulocytosis.

Time	Total W. B. C.	Total* P	Total* L	Total* M	Arneth-Cook Index - Lobes. 1	2	3	4	5	Notes.
3 hours	18,800	15,700	1,900	1,200	46	42	8	4	0	36% vacuolated.
6 "	14,300	13,500	800	0	63	31	5	1	0	26% "
16 "	12,800	12,500	300	0	51	38	8	3	0	41% "
26 "	29,200	27,200	1,700	300	64	30	6	0	0	30% "
40 "	21,000	16,200	3,000	1,800	52	36	10	2	0	17.5% "
65 "	20,000	16,600	2,200	1,200	54	36	8	2	0	12% "
4 days	15,500	12,600	1,500	1,400	56	33	8	2	1	15% "
5 "	12,000	9,200	1,900	900	59	30	16	4	0.5	10% "
7 "	13,000	11,100	1,200	700	47	39	12	2	0	5% "
12 "	14,100	11,600	1,800	700	54	33	10	3	0	2.5% "
13 "	8,300	4,500	3,300	500	75	23	2	0	0	30% "
14 "	5,200	-	-	-	-	-	-	-	-	
17 "	2,000	800	1,160	40	80	19	1	0	0	30% "
18 "	1,060	40	1,000	20	All one lobe.					
19 "	0,760	Only one polymorph (basophil) seen.								
20 "	0,756	No polymorphs seen.			—	—	—	-	-	
21 "	0,611	No polymorphs seen.			—	—	—	-	-	

* P = polymorphs. L = lymphocytes. M = monocytes. per c.mm.

temperature rose suddenly, with a rigor, to 104.2°F. This level of fever was maintained with little fluctuation until death on the 21st day.

A blood transfusion had been given for severe anaemia (Fig. 19) on the 13th day. Repeated transfusions, pentnucleotide and liver extract were given from the 18th day, without benefit.

Post-mortem examination revealed a slightly enlarged, soft spleen, from which Staphylococcus aureus was cultured. Both this organ and the liver showed marked siderosis. The bone marrow was hyperplastic, and the

granular series showed a preponderance of myelocytes and premyelocytes with an almost complete absence of more mature forms.

In comparing the changes in the polymorphs seen here with those occurring in Case 16, which showed only leucopaenia, it is of interest that again there was a sudden increase in the vacuolated cells with the falling white cell count; and again a marked (and in this case sudden) increase in the shift to the left was noted at that stage in the Arneth-Cook index.

IV. DISCUSSION.

LEUCOCYTOSIS.

The findings in this study with regard to the leucocytosis following burns are in agreement with those already recorded by other workers in this field.

The rise in the white cell count is roughly proportional to the severity of the burn and an undue response appears to reflect a very severe reaction to the injury. One case in the present series showed a leucocytosis of 80,000 per c.mm. within two hours of the injury. Death occurred eight and a half hours later.

The rapidity with which a marked leucocytosis may occur in the burned patient is a noteworthy feature. The change is frequently well established within the first hour or two. At this stage degenerative changes may be much in evidence, in the form of toxic granulations,

cytoplasmic vacuolation, and nuclear abnormalities. Even with a marked leucocytosis, however, there may be little evidence of the addition of young cells to the circulation (see 1st specimen in Case 58 - Table XX). This finding supports the view that the leucocytosis is not primarily due to increased production by the bone marrow. A process of redistribution and mobilisation may be in great part responsible for this early increase. In this process the spleen probably plays an important, but not the whole, part (Garrey & Bryan, 1935). Circulatory changes such as may occur in burned patients may be contributory factors. The administration of adrenaline causes a temporary leucocytosis. In the study of the effect of this drug on haemoglobin levels (p.45), variations in the leucocytes were observed in six patients. The average rise in the white cell count following 1.0 mg. of adrenaline was 3,250 per c.mm. The maximum rise recorded was 6,000 per c.mm. Such a reaction can occur after splenectomy (Garrey & Bryan, 1935).

Similar changes have been reported in relation to fear and apprehension, and during attacks of paroxysmal tachycardia, white cell counts of over 20,000 per c.mm. have been observed (Levine & Golden, 1922).

The occasional occurrence of mild lymphocytosis with this polymorph reaction is of interest with regard to the lymphocyte count at the 3rd hour in Case 58 (Fig.30)

(Ernst & Herxheimer, 1924: Martin, 1932: Garrey & Bryan, 1935).

Reference to the alterations in the Arneth-Cook Index reveals that within a day or two young cells predominate in the peripheral blood. The period of mobilisation is over, and production has taken its place. This second phase continues, unless interrupted by some other factor, until the infection of the raw surfaces is overcome and healing is progressing.

The disappearance of the eosinophil polymorphs with the development of the leucocytosis, and their return with local and general improvement in the patient, are in keeping with the behaviour of these cells in acute inflammatory processes in general.

The first definite change in the lymphocytes common to all the cases investigated, was lymphopaenia. The cause is obscure. In view of the very short life of the lymphocyte, it is possible that a temporary failure of production might produce temporary lymphopaenia soon after burning. Redistribution of the cells within or without the vascular system might equally well be responsible. Present knowledge does not supply an answer to this problem. It is of interest that similar changes in the lymphocytes have been observed after severe operations. Lymphopaenia occurred in animals within a few hours, and 22 of 27 patients showed a post-operative fall in lymphocytes (Cruickshank, 1942).

The lymphocytosis which tended to occur during the first week may have been due to increased activity of the lymphoid tissue associated with increased lymph flow.

The occurrence of a monocytosis towards the end of the first week after burning may be analogous to the change which accompanies recovery from severe infections. It may be regarded as an accompaniment of the increased activity of the myeloid system. In either case the mechanism is obscure.

LEUCOPAENIA AND AGRANULOCYTOSIS.

The development of leucopaenia in 34.5 per cent. of the cases examined is of interest, for such an occurrence has not been previously reported. The similarity between Case 16 with leucopaenia, and Case 48, with agranulocytosis, has been noted, and it is possible that the changes are related etiologically.

Drugs of the sulphonamide series must be regarded as a possible cause of this unusual white cell response. All the patients had a sulphonamide cream applied as a dressing to the burned surfaces, and it has been shown that marked absorption of the drug may occur within a short time of each application (Hooker & Lam, 1941: Gordon & Bowers, 1942). Studies of the blood levels of the drug exhibited by some of the patients described above were made by Anderson and Semeonoff (1944) and recorded by these authors and by Colebrook, Clark, Gibson and Todd (1944).

In Case 19, with a burn involving 19 per cent. of the body surface, blood levels of 3-4 mg. per cent. followed each application of 3 per cent. sulphanilamide cream. In Case 58 with a burn involving 40 per cent. of the body surface, the application of a cream containing 10 per cent. sulphanilamide resulted in a blood level of the free drug amounting to 30 mg. per cent. By contrast, patients dressed with sulphathiazole showed only a small absorption, as was expected.

Agranulocytosis is regarded as being due to sensitisation to the drug concerned (Reznikoff, 1939: Rosenthal & Vogel, 1939: Park, 1944), irregular and prolonged exposure to the drug are important predisposing factors (Goldman, Applebaum & Antopol, 1941). The total dosage is less important (Reznikoff, 1939: Semon, 1940: Spain, 1940). These are conditions produced by the repeated application of a dressing from which considerable absorption of sulphanilamide or related compound may occur into the blood stream. It is not surprising, therefore that a case of agranulocytosis was observed among about 2,000 cases exposed to drugs of the sulphonamide series. In considering the changes of such local treatment of burns, the rare occurrence of agranulocytosis must be balanced against the efficiency of the dressings. The use in lower concentrations of drugs less liable to cause agranulocytosis - sulphapyridine, sulphathiazole and sulphadiazine (Dowling

& Lepper, 1943: Dowling, Dumoff-Stanley, Lepper & Sweet, 1944: Plummer & Wheeler, 1944) - will tend to minimise still further the danger of this serious but rare complication.

The allergic nature of the leucopaenia which may occur due to drugs of the sulphonamide series is less evident. As a complication of drug therapy it is much more common than agranulocytosis, and it is much less serious. The occurrence of leucopaenia in 34.5 per cent. of the patients investigated in this series is in keeping with the studies of Britton and Howkins (1938) on ambulant patients, and of Bigler, Clifton and Werner (1938) on cases with and without infection. The former authors record leucopaenia in 46 per cent. of 50 individuals given 21 gms. of sulphonamide in 14 days, while the latter believe that small doses over a shorter period cause significant depression of the white cells.

V. SUMMARY AND CONCLUSIONS.

Leucocytosis is almost invariable after burns. It occurs roughly in proportion to the severity of the injury, and it may be well marked within 3 hours of burning. An unduly great response reflects a severe reaction to the injury, and it is of serious significance.

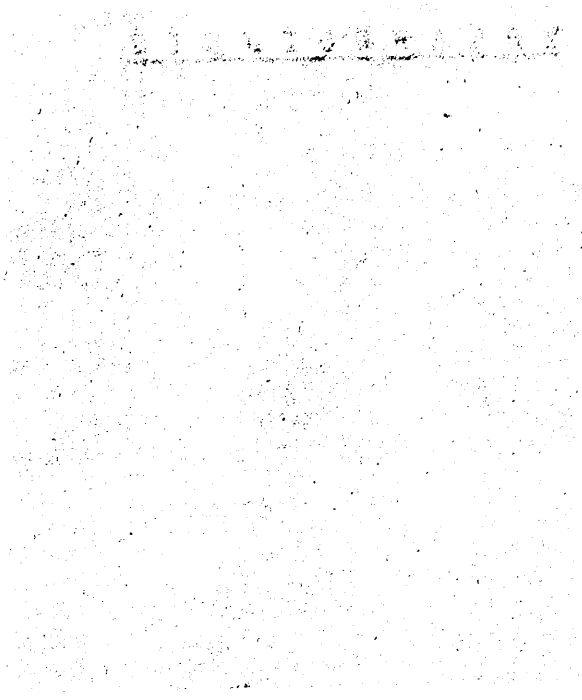
The immediate leucocyte response to the injury is, in great part, of the nature of a mobilisation of the white cells. Evidence of immaturity may be lacking in the

cells added to the general circulation, though toxic and degenerative changes may be present in both nucleus and cytoplasm. From the second or third day onwards, increased production of white cells becomes increasingly evident in cases severely burned, and a well marked leucocytosis may be established by the seventh day. The immediate response may be partly due to circulatory change associated with the shock period, but the possibility of its being due to the circulation of toxic products absorbed from the burned area cannot be excluded. That substances can be absorbed from such a region is beyond question, and the leucocytes themselves show changes very suggestive of such an occurrence. The later response on the part of the white cells is due to infection of the raw surfaces, and it continues in greater or less degree according to the state of healing in these parts.

The variations in the eosinophil polymorphs are similar to those occurring in acute inflammatory processes in general. The same may be said of the increase in monocytes observed to occur towards the end of the first week. An early lymphopaenia occurred in all the patients examined for this change. The same change has been reported to occur after serious operations. The cause is obscure. The lymphocytosis which may occur at the end of the first week may be associated with increased lymph flow.

Agranulocytosis has been observed in one patient,

and leucopaenia was found to occur during the first seven days in 10 of 29 patients in whom serial white cell counts were performed. Both changes are regarded as due to the absorption of sulphonamide compounds from the dressings applied to the burned surfaces.



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A P P E N D I X.

APPENDIX I.

SUMMARY OF PATIENTS DISCUSSED IN PART II.

(SBB = Superficial blister burn; PSL = Partial skin loss; WSL = Whole skin loss).

Case No.	Age.	Sex.	Extent of burn and sites.	Depth of burning.	Total fluid transfused (ml.)	Lived or died? Day of death.
6	29	M.	12%; buttocks and thighs.	6% SBB 6% PSL	1,000	L.
8	20	F.	16%; breast, arm, thigh, abdomen.	9% PSL 7% WSL	1,600	L.
11	32	F.	6%; hands, face.	5% SBB 1% WSL	1,000	L.
12	32	M.	8%; face, palms.	5% SBB 3% PSL	1,500	L.
13	30	F.	10%; face, hands, ankles.	3% SBB 7% WSL	1,600	L.
14	27	F.	10%; back and shoulder.	7% SBB 3% WSL	2,500	L.
16	31	F.	15%; back and arm.	8% (SBB (PSL 7% WSL	1,200	L.
19	51	M.	19%; R. leg.	16% WSL	3,600	L.
21	6	M.	10%; shoulder, chest.	6% PSL 4% WSL	1,200	L.
26	4 1/2	M.	12%; thigh, buttock, legs.	6% PSL 6% WSL	1,600	L.
30	21	F.	20%; buttocks, thighs, arms and back.	Mostly WSL	2,200	L.

Case No.	Age.	Sex.	Extent of burn and sites.	Depth of burning.	Total fluid transfused (ml.)	Lived or died? Day of death.
31	22	F.	22%; R. thorax, axilla, arm	Mostly WSL	1,600	L.
32	49	M.	26%; face, arms and legs.	Mostly PSL	3,400	L.
33	68	F.	26%; neck, trunk, thighs hands.	10% PSL 16% WSL	2,700	D. 3
37	12	F.	22%; abdo., thighs, arms.	6% WSL 16% SBB and PSL	3,200	L.
38	19	F.	22%; buttocks, thighs	5% SBB 17% WSL	4,420	D. 8
45	6	M.	75-80%; trunk, legs, arms.	Mostly WSL	1,500	D. 1
46	10	F.	80%; trunk, arms, legs.	Mostly WSL	6,600	D. 4
48	7	F.	40%; trunk, thighs.	5% PSL 35% WSL	3,600	D. 22
49	9	F.	40%; arm, abdo., legs.	20% PSL 20% WSL	2,200	D. 31
51	6 1/2	M.	45%; trunk, arms, legs.	20% PSL 25% WSL	3,400	D. 43
52	14	M.	31%; neck, trunk, arms, thighs.	19% PSL 12% WSL	2,300	D. 2
54	40	M.	40%; trunk, face, arms.	Mostly WSL	2,900	D. 3
56	51	M.	75%; trunk, arms, legs.	35% PSL 40% WSL	7,600	D. 1

Case No.	Age.	Sex.	Extent of burn and sites.	Depth of burning.	Total fluid transfused (ml.)	Lived or died? Day of death.
58	25	M.	40%; neck, trunk, arms, hands, thighs.	Mostly WSL	5,600	L.
59	14	F.	40%; trunk, buttocks, thighs.	5% SBB 5% PSL 30% WSL	2,200	L.
60	41	F.	45-50%; trunk, arms, thighs.	Mostly WSL	7,200	D. 64
63	60	M.	3%; R. foot.	WSL	-	L.
64	34	M.	1%; R. wrist.	PSL	-	L.
65	16	M.	4%; head, arms.	SBB, PSL	-	L.
66	43	M.	4%; face, hand.	2.5% SBB 1.5% WSL	-	L.
67	51	M.	3%; face, neck.	SBB	-	L.
68	35	M.	2%; R. arm.	1% PSL 1% WSL	-	L.
69	19	F.	13%; buttock, legs, arm.	6% SBB 7% PSL	-	L.
70	35	M.	7%; head, forearms.	3% SBB 4% PSL	-	L.
71	78	M.	6%; face, hands.	3% SBB 3% PSL	-	L.
72	39	M.	8%; face, arms.	4% PSL 4% SBB	-	L.
73	40	M.	12%; face, neck, forearms.	6% SBB 6% PSL	-	L.

Case No.	Age.	Sex.	Extent of burn and sites.	Depth of burning.	Total fluid transfused (ml.)	Lived or died? Day of death.
74	7	M.	8%; face, neck, forearms.	4% SBB 4% PSL	-	L.
75	1 5/12	F.	14%; back, buttock.	6% SBB 6% PSL 2% WSL	-	L.
76	32	M.	12%; face, arms, back.	Mostly SBB	-	L.
77	1 5/12	M.	7%; neck, elbow.	PSL	-	L.
78	41	M.	12%; hands, forearm, leg.	4% SBB 4% PSL 4% WSL	2,000	L.
79	42	M.	16%; face, neck, arms.	10% SBB 6% PSL	-	L.
80	40	M.	16%; face, arms.	8% SBB 8% PSL	-	L.
81	34	M.	22%; face, trunk, arms.	10% SBB 12% PSL	-	L.
82	62	F.	21%; neck, chest, fore-arms, thighs, legs.	7% SBB 7% PSL 7% WSL	2,300	D. 41
83	68	F.	24%; trunk, shoulder, thigh.	4% SBB 10% PSL 10% WSL	4,400	D. 11
84	39	F.	90%; all but left flank.	Mostly WSL	-	D. 1
85	78	F.	70%; trunk, arms, thighs.	20% PSL 50% WSL	-	D. 1
86	35	M.	67%; head, trunk, arms, legs.	Mostly WSL	-	D. 1

Case No.	Age.	Sex.	Extent of burn and sites.	Depth of burning.	Total fluid transfused (ml.)	Lived or died? Day of death.
88	22	M.	3%; foot	WSL	-	L.
106	36	F.	6%; shoulder, arm.	4% SBB 2% WSL	-	L.
110	21	M.	23%; arms, neck, chest.	11% SBB 12% PSL	-	L.
112	7	F.	32%; face, trunk, arms, thighs.	12% WSL 20% (SBB (PSL	1,600	L.
115	17	F.	66%; head, trunk, arms, legs.	Mostly WSL	-	D. 2
117	2	F.	12%; back, shoulders.	Mostly SBB	-	L.
118	1 8/12	F.	8%; chest, abdo., arm.	4% SBB 4% PSL	-	L.
119	8/12	M.	8%; leg.	6% SBB 2% PSL	-	L.

A P P E N D I X II.

ESTIMATION OF AREA BURNED.

Throughout this report, calculation of the areas burned has been based on Berkow's values (Arch. Surg., 1924, 8, 138), which are shown in the table below.

	Berkow.
Head	6 per cent.
Arms	13.5 per cent.
Hands	4.5 per cent.
Trunk	38 per cent.*
Thighs	19 per cent.
Legs	12.7 per cent.
Feet	6.3 per cent.

* (anterior 20, posterior 18 per cent.)

The trunk includes the neck; the lower extremities the buttocks.

Following Berkow, allowance for the different values applying in childhood was made as follows:-

The trunk was reckoned at 40 per cent., the upper extremities at 16 per cent.; for the head, substract the child's age from 12 and add remainder to the adult value (6 per cent.); for the lower extremities (including the buttocks), subtract the child's age from 12 and subtract the remainder from the adult value (38 per cent.).
