CLINICAL HAEMATOLOGY

BY

R. D. EASTHAM

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Consultant Pathologist to the Frenchay Group of Hospitals, Bristol; Lately Consultant Pathologist to the Newcastle General Hospital, Newcastle upon Tyne

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PREFACE TO THE FOURTH EDITION

DURING the four years since the appearance of the third edition of this book, many significant advances have been reported in haematology. For instance, the clinical significance of the concentration of 2,3-diphosphoglycerate (2,3-DPG) in red cells is better understood. New sections dealing with haemopexin, dyserythropoietic anaemia, and the HL-A antigen system have been included, and the sections on vitamin B_{12}, erythropoietin, sideroblastic anaemia, and haemolytic anaemia have been extensively rewritten, and an attempt has been made to classify many recently described disorders of neutrophil function.

In the section dealing with blood clotting, information on plasma coagulation factors and platelet function is expanded. Fibrin degradation products and their clinical significance are described, and the sections dealing with serum complement and anticoagulant therapy have been rewritten.

A number of diseases covered in the book are rare, and are marked [*R*] to distinguish them clearly from common conditions.

Finally, with the adoption of the Système International d'Unités (S.I. Units) by many countries throughout the world, a table is included to enable simple interconversion with previous routine established units.

Bristol R.D.E.
May, 1974

PREFACE TO THE FIRST EDITION

THIS book is similar in design and intent to *Biochemical Values in Clinical Medicine* in that I have attempted to provide an accurate summary of the ways in which various clinical conditions can be related to haematological results.

Since clinical haematology is a different discipline from clinical biochemistry, it was not considered adequate merely to list the various laboratory tests and the significance of their results. Accordingly, the book falls into the following sections:

1. Haemoglobins and associated pigments.
2. Red blood-cells.
3. Anaemia.
4. Peripheral white blood-cells.
6. Bleeding, clotting, and transfusion.

The relevant laboratory tests are included in each section. Individual coagulation factors (e.g., Factors V, VII etc.) are considered in detail. Where known, their physical characters are listed. In this respect the recently agreed International Nomenclature for the various factors has been used (e.g., Factors I, II, III, IV, V, VII, VIII, IX, X).

The aetiology of certain clinical conditions (e.g., pancytopenia, purpura in children, macrocytic anaemia) have been considered. Because the rate of expansion of knowledge in Haematology, as in Clinical Biochemistry, is very great at the present time, many new tests which are not, as yet, performed in routine laboratories have been described. This extension of recent knowledge has also been used as an excuse for giving details, for example, of such conditions as von Willebrand's disease, which, although rare, has a very interesting relationship with haemophilia and other conditions with reduced plasma antihaemophiliac globulin content. (The name von Willebrand has been used in connexion with four syndromes.)

It is hoped that this book, which is not intended to replace standard haematology text-books, will encourage more discussion of haematological cases and problems between clinicians and clinical haematologists. It is also hoped that it will be of some assistance to junior medical hospital staff in their requests for tests, and interpretation of results of these tests.

I am grateful to *Dr. A. B. Raper*, Consultant Haematologist, Bristol United Hospitals, for reading the manuscript, and to *Mr. K. W. Denson, F.I.M.L.T.*, for helpful advice, criticism, and much valuable information relating to coagulation problems.

Bristol,
January, 1961

R.D.E.

CONTENTS

Section I.—Haemoglobin and Associated Pigments

WHOLE BLOOD HAEMOGLOBIN

S.I. units= g./dl.
Conversion factor from g./100 ml. = 1·0.

Normal Range:

Infant (full term, cord blood):	13·6–19·6 g./100 ml.
Infant (3 months):	9·5–12·5 g./100 ml.
Infant (1 year old):	11·0–13·0 g /100 ml
Children (10 years old):	11·5–14·8 g./100 ml.
Adults (ignoring sex difference, mean):	14·8 g./100 ml.
Adult males:	13·5–18·0 g./100 ml.
Adult females:	11·5–16·4 g./100 ml.
Elderly males (65–85 years + mean):	13·62 g./100 ml.
Elderly females (65–85 years + mean value):	13·11 g./100 ml.

References.—Dacie, J. V., and Lewis, S. M. (1968), *Practical Haematology*, 4th ed. London: Churchill; Myers, Mary, Saunders, Cynthia, and Chalmers, D. C. (1968), *Lancet*, 2, 261.

PLASMA HAEMOGLOBIN

S.I. units = mg./dl.

Normal Range: Less than 1 mg./100 ml. of plasma.

Pathological:

1. Slight Increase (5–10 mg./100 ml.):
   a. Sickle-cell thalassaemia.
   b. Haemoglobin C disease.
2. Moderate Increase (10–25 mg./100 ml.):
   a. Acquired haemolytic anaemia (auto-immune).
   b. Sickle-cell anaemia.
   c. Thalassaemia major.
   d. Sickle-cell haemoglobin C disease.
   e. Prosthetic heart valve replacement. Haem is released, with lactate dehydrogenase (LDH), from cells which are lysed, and from red cells which are damaged but not lysed.

Reference.—Dale, J., and Myhre, E. (1972), *Acta med. scand.*, 191, 133.

3. Marked Increase: Any condition associated with rapid intravascular haemolysis, and haemoglobinuria, i.e., mechanisms for the carriage of free haemoglobin in plasma are swamped.

See also Haptoglobins, Methaemalbumin, Haemoglobinuria, and Haemolytic Anaemia.

The renal threshold for plasma haemoglobin is approximately 150 mg./100 ml. Plasma haemoglobin must exceed 25 mg./100 ml. before it gives the plasma a faint pink hue.

No increase in plasma haemoglobin occurs in hereditary spherocytosis, since red-cell breakdown occurs extravascularly in the spleen.

N.B.—It is important to avoid haemolysis either during or after the collection of the blood sample, otherwise the result is invalid.

REFERENCES.—Pearson, C. M., Beck, W., and Blahd, W. H. (1957) *Archs intern. Med.*, 99, 376; Martinek, R. G. (1965), *Proc. Ass. clin. Biochem.*, 3, 228.

MEAN CORPUSCULAR HAEMOGLOBIN (M.C.H.)

S.I. units—no change.

Normal Range:

Infants (3 months):	24–34 pg.
Infants (1 year):	23–31 pg.
Children (10–12 years):	24–30 pg.
Adults:	27–32 pg.

Pathological:

INCREASE:

Macrocytosis (up to 50 pg.).

DECREASE:

Microcytosis, associated with iron deficiency (down to 15 pg.).

N.B.—When the packed cell volume (P.C.V.) is estimated by centrifugation of whole blood, trapping of plasma between red cells inevitably occurs, with consequent overestimation of red-cell size, especially if there is anisocytosis of the red cells. In these circumstances, the mean corpuscular haemoglobin concentration (M.C.H.C.) is a better measure of hypochromia.

When the P.C.V. is automatically calculated from measurements of the mean red-cell volume (M.C.V.) and the red-cell count, trapping errors are eliminated (e.g., Coulter 'S' counter), and an M.C.H. of less than 27 pg. indicates hypochromia, the M.C.H.C. only falling with very gross hypochromia.

COLOUR INDEX

$$\frac{\text{Haemoglobin (expressed as percentage of normal)}}{\text{Red-cell count (expressed as percentage of normal)}} = \text{Colour Index (C.I.)}$$

The Colour Index is not a useful estimation since it is a relative ratio only.

Mean Corpuscular Haemoglobin = Colour Index × 29·2 when normal red-cell count assumed to be 5 000 000 per c.mm. and normal haemoglobin assumed to be 14·6 g./100 ml.

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (M.C.H.C.)

S.I. units—no change.

The Mean Corpuscular Haemoglobin Concentration M.C.H.C.) is the amount of haemoglobin carried by 100 ml. of

red blood-cells, i.e., it represents the actual haemoglobin concentration in the blood.

Normal Range:

Infants (3 months):	27–34 g./100 ml. of red cells.
Infants (1 year):	28–33 g./100 ml. of red cells.
Children (10–12 years):	30–33 g./100 ml. of red cells.
Adults:	30–35 g./100 ml. of red cells.

Macrocytic cells may be normochromic, but are never hyperchromic, since there appears to be a physiological upper limit to haemoglobin concentration per unit of red-cell substance. It is of interest to note that while the red-cell count and red-cell size vary from animal species to species, the M.C.H.C. is remarkably constant throughout the mammals.

Pathological:

INCREASE IN M.C.H.C. (i.e., above 36 g. per cent): Levels above 36 g. per cent may be found in very severe prolonged dehydration.

DECREASE IN M.C.H.C. (i.e., less than 30 g. per cent):

a. *Iron-deficiency anaemia:* The M.C.H.C. may be very low in prolonged iron deficiency. While the M.C.H.C. is usually normal in the megaloblastic anaemias before treatment, the M.C.H.C. frequently falls below normal following successful specific therapy, as the iron stores are used up. Similarly after a severe single haemorrhage, the red blood-cells have the same M.C.H.C. as before the haemorrhage. After repeated bleeds, the cells become hypochromic, as the body iron stores are used up, following increased red cell regeneration.

b. *Overhydration:* The M.C.H.C. can be used as a guide to the success of treatment in cases of overhydration, e.g., water intoxication. During overhydration the M.C.H.C. may fall over 24–48 hours by 2 g. per cent.

c. *Thalassaemia.*

d. *Sideroblastic anaemia.*

N.B.—*See* notes under Mean Corpuscular Haemoglobin for interpretation of results of M.C.H.C., which depend entirely on the method used for its estimation.

PACKED CELL VOLUME OR HAEMATOCRIT

This value can be defined as the volume of red blood-cell per 100 ml. of whole blood. S.I. units conversion factor = 0·01, i.e., 44–62 per cent becomes 0·44–0·62.

Normal Range:

Infant (full term, cord blood):	44–62 per cent.
Infants (3 months):	32–44 per cent.
Children (1 year old, mean):	36–44 per cent.
Children (10 years old, mean):	37–44 per cent.
Adult males:	40–54 per cent.
Adult females:	36–47 per cent.

N.B.—The P.C.V. may be measured by methods involving centrifugation of whole blood with consequent plasma trapping and over-estimation of the P.C.V. or by calculation from M.C.V. and red-cell count measurements, and hence elimination of trapping error (Coulter 'S' counter). *See* notes under M.C.H. section.

The venous blood packed cell volume × 0·91 = the whole body packed cell volume. This ratio changes:

Increase:

Pregnancy.
Splenomegaly.
High altitude.

Decrease:

Extravascular fluid retention.
Congestive cardiac failure.

REFERENCE.—Dacie, J. V., and Lewis, S. M. (1968), *Practical Haematology*, 4th ed. London: Churchill.

The uppermost cell layer in a centrifuged haematocrit tube consists of platelets and normally comprises 0·26–0·44 per cent of the total blood-volume.

Immediately below this cream-coloured layer lies a grey layer (0·4–1·0 per cent) in which lie the leucocytes. Nucleated red cells, when they occur in the peripheral blood, are found in this layer. In all cases of anaemia with peculiar differential white cell counts, unidentified cells, or in cases of suspected megaloblastic anaemia, stained preparations should be made of this 'buffy layer'.

The red blood-cells in the upper layer have a higher concentration of reticulocytes than the lower layers. These upper cells also contain more water, sodium, potassium, chloride, and phosphate than the red cells in the lower layers.

REFERENCE.—Keitel, H. G., Berman, H., Jones, B. H., and MacLachlan, E. (1955), *Blood*, **10**, 370.

Decreased Packed Cell Volume: Any case of anaemia, from whatever cause.

Increased Packed Cell Volume:

1. *a.* PRIMARY POLYCYTHAEMIA VERA: This condition is one of the myeloproliferative diseases. Whole blood haemoglobin concentrations of 18–24 g./100 ml. are usual. The red blood-cell count ranges from 7 000 000 to 10 000 000 per c.mm. or more, with frequently a reduced mean red-cell volume. The whole blood-volume may be increased to 83–133 ml./kg. body-weight. The bone-marrow shows proliferation of all the cellular elements.

b. BENIGN FAMILIAL ERYTHROCYTOSIS: A rare benign familial condition with abnormally increased red-cell mass, without leucocytosis or thrombocytosis. There exists a rare benign familial polycythaemia occurring in childhood. There is no accompanying leucocytosis or thrombocytosis, such as occurs frequently in polycythaemia vera. Possibly some cases are due to electrophoretically undetected haemoglobinopathies with impaired haemoglobin-oxygen dissociation curves.

REFERENCES.—Cassileth, P. A., and Hyman, G. A. (1966), *Am. J. med. Sci.*, 251, 692; Fairbanks, V. F., Maldonado, J. E., Charache, S., and Boyer, S. H. (1971), *Proc. Staff Meet. Mayo Clin.*, 46, 721.

2. SECONDARY POLYCYTHAEMIA:

a. Relative polycythaemia: Following reduced fluid intake and/or excess loss of body fluids, although the total red-cell mass is not increased, the fall in plasma volume leads to an increased P.C.V.

b. Absolute polycythaemia:

i. Newborn (transient).
ii. Congenital heart disease.
iii. Acquired heart disease.
iv. Lung disease.
v. High altitudes.
vi. Cushing's syndrome (many cases show increases 5 per cent above normal).
vii. Occasionally following chronic poisoning with aniline dyes, etc.
viii. Renal disease. Polycythaemia may be associated with (*a*) renal carcinoma, especially clear cell type, (*b*) renal sarcoma, (*c*) nephroblastoma, (*d*) benign renal tumour, (*e*) hydronephrosis, and (*f*) polycystic kidneys.
ix. Cerebellar haemangioblastoma—in 9–20 per cent of cases.
x. Stress erythrocytosis due to chronic alcoholism.

REFERENCES.—Jones, N. F., Payne, R. W., Hyde, R. D., and Price, T. M. L. (1960), *Lancet*, I, 299; Smith, J. F. B., and Lucie, N. P. (1973), *Ibid.*, 1, 673.

N.B. In pregnancy the plasma volume increases to a maximum of +55 per cent above the previous normal value by approximately 60 days before delivery. The whole blood-volume increases at the same time by approximately +45 per cent (i.e., approximately 1800 ml.) and the red-cell mass increases by about +30 per cent. Thus, although the total body haemoglobin increases, the whole blood-haemoglobin level falls.

BLOOD-VOLUME

Normal Range:

1. TOTAL BLOOD-VOLUME:
   Full term infant ≏ 85 ml./kg. body-weight.
   Premature infant ≏ 108 ml./kg. body-weight.
   (At birth, delayed clamping of the cord is equivalent to a transfusion of about 150 ml. of whole blood in a 3·5-kg. infant.)
   a. 72–100 ml./kg. body-weight.
   b. 2500–4000 ml./sq.m. body surface area.
2. TOTAL PLASMA VOLUME:
   a. 49–59 ml./kg. body-weight.
   Infants ≏ 41·3 ml./kg. body-weight.
   Adult males ≏ 46 ml./kg. body-weight.
   Adult females ≏ 45 ml./kg. body-weight.

b. 1400–2500 ml./sq.m. body surface area.

(The plasma volume is very unstable, and difficult to measure. It cannot be used to deduce the red-cell volume.)

3. TOTAL RED-CELL VOLUME:

Newborn infant $\simeq$ 29 ml./kg. body-weight.

Adult males $\simeq$ 30 ml./kg. body-weight.
(25–35 ml./kg. $\pm$ 2 S.D.)

Adult females $\simeq$ 25 ml./kg. body-weight.
(20–30 ml./kg. $\pm$ 2 S.D.)

Red-cell volume increased:

a. Primary polycythaemia vera.

b. Secondary erythraemia:

i. Anoxia.

ii. Renal.

Red-cell volume normal, but plasma volume decreased in 'stress' erythraemia. This condition should not be treated as though it were primary polycythaemia vera. P.C.V. may exceed 55 per cent with haemoglobin exceeding 17 g./100 ml. but red-cell volume within normal range.

The *Interstitial Fluid Volume* is three times as great as the plasma volume, and protects the plasma volume from marked change following loss.

In anaemia (other than immediately after haemorrhage) the normal blood-volume is maintained by a compensatory increase in the plasma volume.

Increase:

1. Normal pregnancy. Both the red cell and plasma compartments increase, the greater increase being in plasma volume. This results in the apparent anaemia in normal pregnancy (i.e., haemoglobin remains *above* 10 g./100 ml.). The total blood-volume increases by up to 45 per cent maximal at the thirty-second week, while the total plasma volume increases by 25–55 per cent and the red-cell mass increases by 20–40 per cent.
2. Polycythaemia vera (red-cell mass increased).
3. Occasional cases of congestive cardiac failure.
4. Administration of excess saline solution, glucose solution, or water after pitressin, results in increased volume until diuresis occurs.
5. Administration of glucose solution during hypothermia. The body enzymes act much more slowly at lower body temperatures. Glucose acts as a relatively inert expander of the extracellular space, until the body temperature rises again towards normal.
6. Over-transfusion with blood, plasma, serum, or dextran.

Decrease:

1. Haemorrhage. The plasma volume is rapidly restored from the fluid reserve in the interstitial fluid space. Rapid increase in the plasma volume compensates for the loss of red-cell mass.
2. Water and/or electrolyte deficiency:
   a. Starvation and water deficiency.

b. Persistent vomiting.
c. Prolonged diarrhoea.
d. Addison's disease.

The plasma volume is increased by an average of +16 per cent, with gross reduction in red-cell volume.

3. The blood-volume is said to be reduced in myxoedema.
4. Chronic nutritional anaemia. The plasma volume remains constant, but the red-cell volume falls with the falling haemoglobin concentration. Thus, whole blood haemoglobin estimations read too high as compared with the total circulating haemoglobin. (This error may be as high as +50 per cent.)

REFERENCE.—Tasker, P. W. G. (1959), *Lancet*, **1**, 807.

BLOOD VISCOSITY

Whole Blood Viscosity: This value is of clinical importance, since it is directly related to the packed cell volume. When the P.C.V. exceeds 50 per cent the work required of the heart to pump the blood round the body is twice normal. In polycythaemia vera the whole blood viscosity may be six times normal. Using a cone-in-cone viscometer the whole blood viscosity in normal males = 4·59 centipoises, and in normal females = 3·95 centipoises, at 37° C. Normal red cells are readily deformable and in plasma probably are equivalent to a deformable fluid drop suspended in a second immiscible liquid.

It is possible that with increased blood viscosity, reversible red-cell aggregates form, resulting in hypoxia of cells and consequent changes in the cell surface charges, leading to irreversible agglutinates. These could, in turn, result in platelet aggregates and eventually thrombosis.

In sickle-cell disease the red-cell viscosity, and hence the whole blood viscosity, increases markedly with deoxygenation within the normal physiological range, resulting in impaired blood-flow through the smaller blood-vessels, causing tissue anoxia and damage. This is due to the increased rigidity and inability to deform of red cells containing HbS with a low Po_2.

Whole blood hyperviscosity occurs through:

a. Excessive aggregation of erythrocytes
b. Abnormal rigidity of red cells
c. Abnormal increase in haematocrit
d. Abnormal increase in plasma proteins, especially fibrinogen and/or globulins

} singly or in combination.

e.g., 1. Congestive cardiac failure.
2. Cyanotic heart disease.
3. Excessive placental transfusion in newborn.
4. Polycythaemia.
5. Macroglobulinaemia.
6. Hyperlipaemia.
7. Collagen disorder.

REFERENCES.—Dintenfass, L. (1962), *Circ. Res.*, 11, 233; Rosenblatt, G., Stokes, J., and Bassett, D. K. (1965), *J. Lab. clin. Med.*, 65, 202; Dintenfass, L. (1971), *Blood Microrheology. Viscosity Factors in Blood Flow, Ischaemia and Thrombosis*. London: Butterworth; Goldsmith, H. L. (1972), *Progress in Hemostasis and Thrombosis* (ed. T. H. Spaet), p. 97. London and New York: Grune & Stratton.

Plasma Viscosity: At 25° C. the normal plasma viscosity ranges from 1·50 to 1·72 centipoises. During normal pregnancy the range is increased from 1·50 to 1·80 centipoises at 25° C.

In solution, weight for weight, fibrinogen has a greater viscosity than globulin, which in turn has a greater viscosity than albumin. The E.S.R. depends on rouleaux formation, which in turn depends on the concentrations of fibrinogen and the globulins. Thus it has been found that the E.S.R. and the plasma viscosity increase in parallel until the plasma has become so viscous that it slows the fall of the red-cell rouleaux.

Measurement of the plasma viscosity is a very sensitive index of change in the plasma proteins in response to inflammation and tissue damage. Unpredictable variations in the E.S.R. due to the effects of variation in the haematocrit and in the red-cell surface are avoided, and plasma may be kept for over 24 hours without alteration in the result. Using a simple viscometer results with a coefficient of variation of less than 1 per cent are obtained, with each set taking less than 2 minutes to perform.

In the author's laboratory this test has replaced the E.S.R.

REFERENCES.—Eastham, R. D. (1954), *J. clin. Path.*, 7, 66, 164; Eastham, R. D. (1965), *J. Obstet. Gynaec. Brit. Commonw.*, 70, 763; Eastham, R. D., Jancar, J., and Duncan, Ethel H. L. (1965), *Br. J. Psychiat.*, 111, 999.

HAEMOGLOBIN OXYGEN DISSOCIATION CURVE

S.I. Unit conversion factor = 0·133, i.e., Po_2 90–100 mm.Hg becomes 12–15 kPa.

The speed of reaction of uptake of oxygen or its release by haemoglobin is 0·07 sec. Since blood is in the alveolar capillary for 0·5 sec., there is adequate time for oxygen uptake in the lungs, and similarly there is adequate time for release of oxygen to the tissues.

When the oxygen content of haemoglobin is plotted against the partial pressure of oxygen to which the haemoglobin is exposed, a sigmoid curve is obtained, which demonstrates the ability or inability of the haemoglobin to take up or release oxygen at different oxygen partial pressures. In the lungs the arterial Po_2 is 100 mm. Hg and in the tissues the venous Po_2 is about 40 mm. Hg. In the normal adult at a Po_2 of more than 60 mm. Hg haemoglobin has little oxygen to release to tissues. The steepest part of the sigmoid curve is also the part at which a relatively small change in oxygen tension results in the rapid release or uptake of oxygen by the red cells. Where the curve is less steep, at either end of the sigmoid curve, relatively great changes in oxygen tension are required to cause release or uptake of oxygen by the red cells (i.e., the red cells are inefficient

carriers of oxygen over these parts of the curve). In the normal adult, haemoglobin has little oxygen to release to tissues at a Po_2 of 60 mm. Hg, whereas at a Po_2 of 4–40 mm. Hg haemoglobin releases oxygen rapidly to the tissues.

A 'shift to the right' of the curve implies greater ease of release of oxygen to the tissues, but if this shift is excessive, there is little effective oxygen transport by the blood at moderate Po_2 tensions. Conversely, a 'shift to the left' of the curve implies a greater avidity in the red cells for oxygen, and therefore no release of oxygen to the tissues until very low Po_2 values are reached.

In patients with acid-base imbalance, it is very useful to measure the partial pressure of oxygen corresponding to 50 per cent saturation of haemoglobin with oxygen at pH 7·4 and at 38° C., ($P_{50}O_2$, $Po_{2(sat.50)}$), since this should reveal any tendency to severe tissue anoxia. The normal value for $P_{50}O_2$ = 27·0 ± 1·2 mm. Hg.

'Shift to the Right' of Haemoglobin Oxygen Dissociation Curve:

1. Increasing accumulation of carbon dioxide.
2. Falling blood pH.
3. During the first 24 hours at high altitudes.
4. Sickle-cell anaemia. Even though HbS has a greater affinity than normal Hb for oxygen, the increased red-cell 2,3-DPG content reduces the haemoglobin's affinity for oxygen.

   REFERENCE.—Charache, S., Grisolia, S., Fiedler, A. J., and Hellegers, A. E. (1970), *J. clin. Invest.*, 49, 806.
5. Pyruvate kinase deficiency. The red-cell 2,3-DPG content is high and the $P_{50}O_2$ = 38 mm. Hg.
6. Hyperthyroidism and patients treated with tri-iodothyronine. The red-cell 2,3-DPG content is high.

   REFERENCE.—Schussler, G. C., and Ranney, Helen M. (1971), *Ann. intern. Med.*, 74, 632.
7. Anaemia of chronic renal failure.

'Shift to the Left' of Haemoglobin Oxygen Dissociation Curve:

1. Fetal haemoglobin F when compared with adult haemoglobin A, in solution *in vitro*.

In infants older than 1 month, when the Po_2 falls below 60 mm. Hg there is an increase in red-cell 2,3-DPG with decreased red-cell affinity for oxygen and increased oxygen availability to the tissues. In infants under 1 month old, there is no proportional increase in red-cell 2,3-DPG related to falling Po_2 tension, since fetal HbF does not bind 2,3-DPG.

REFERENCE.—Oski, F. A., Gottlieb, A. J., Miller, W. W., and Delavoria-Papadopoulos, Maria (1970), *J. clin. Invest.*, 49, 400.

2. Increased external oxygen tension, e.g., hyperbaric chamber.
3. During hypothermia.
4. Haemoglobin H and haemoglobin Barts. These tetramers have an increased affinity for oxygen.
5. Megaloblastic anaemia.
6. Hypochromic anaemia. The red-cell 2,3-DPG content is increased.

REFERENCE.—Slawsky, P., and Desforges, Jane F. (1972), *Archs intern. Med.*, **129**, 914.

7. Normochromic anaemia.
8. Carbon dioxide poisoning
9. Methaemoglobinaemia
10. Sulphaemoglobinaemia

} The shape of the dissociation curve is altered.

11. Red-cell hexokinase deficiency. $P_{50}O_2$ = 19 mm. Hg with a low red-cell 2,3-PDG concentration.
12. Blood stored with acid citrate glucose solution. In stored blood containing acid citrate dextrose, 2,3-DPG concentrations fall progressively. When such stored blood is transfused into a severely anaemic patient, it is not effective for oxygen transfer to anoxic tissues for some hours after transfusion. Blood stored with citrate phosphate dextrose does not suffer from this defect.
13. Hypophosphataemia. Red-cell 2,3-DPG concentrations are low, with marked haemoglobin affinity for oxygen. Similar low red-cell 2,3-DPG concentrations are found in severe diabetic ketosis, and bicarbonate infusions make this situation worse, increasing potential tissue anoxia. It is possible that phosphate should be added to infusions. There is a direct correlation between arterial blood pH and red-cell 2,3-DPG concentration.

REFERENCES.—Lichtman, M. A., Miller, D. R., Cohen, J., and Waterhouse, Christine (1971), *Ann. intern. Med.*, **74**, 562; Alberti, K. G. M. M., Darley, J. H., Emerson, Pauline M., and Hockaday, T. D. R. (1972), *Lancet*, **2**, 391.

Red-cell 2,3-Diphosphoglycerate (2,3-DPG):

A moderate 'shift to the right' with a higher $P_{50}O_2$ is associated with reduced erythropoiesis; a moderate 'shift to the left' with a lower $P_{50}O_2$ than normal is associated with increased erythropoiesis. Some abnormal haemoglobins are associated with polycythaemia.

The concentration of 2,3-DPG plays a very important part in compensating to reduce gross shifts in the haemoglobin oxygen dissociation curve. 2,3-DPG is an intermediate metabolite of glucose catabolism in the red cell, and is normally present in the same molar concentration as haemoglobin. It binds preferentially to deoxygenated haemoglobin rather than to oxygenated haemoglobin. The concentration of 2,3-DPG in the free form is maintained by enzymatic action. In anaemia, the tissues remove relatively more oxygen from the circulating haemoglobin, and the increased concentration of deoxygenated haemoglobin binds available 2,3-DPG. This reduces the amount of free 2,3-DPG, resulting in increased 2,3-DPG synthesis, and hence increased total 2,3-DPG in the red cells in anaemia. This increased 2,3-DPG concentration is accompanied by a tendency to 'right shift' of the haemoglobin oxygen dissociation curve, compensating for the tendency to 'left shift' in anaemia.

Patients with a 'left shift' of the dissociation curve (e.g., hexokinase deficiency) respond to exercise with increased cardiac output and a fall in the central venous Po_2, disadvantageous as compared with normal subjects. On the other hand, patients with a 'right shift' of the dissociation curve (e.g.,

pyruvate kinase deficiency), on exercise show only a gradual fall in the central venous Po_2, a greater ability of tissues to abstract oxygen from the circulating blood, and a much smaller increase in cardiac output, demonstrating what an important part 2,3-DPG plays in oxygen transport and release.

2,3-DPG is thought to bind to haemoglobin in the region of the NH_2 terminal of the beta-chains, and is present in high concentration in the red cells of man, horse, dog, rabbit, rat, and guinea-pig. Only low levels of 2,3-DPG are present in the red cells of sheep, goat, cow, and cat, revealing significant species differences.

REFERENCES.—Beutler, E. (1969), *Blood*, **33**, 496; Torrance, J., Jacobs, P., Restrapo, A., Eschbach, J., Lenfant, C., and Finch, C. A. (1970), *New Engl. J. Med.*, **283**, 165; Oski, F. A., Marshall, B. E., Cohen, P. J., Sugarman, H. J., Miller, L. D. (1971), *Ann. intern. Med.*, **74**, 44; Bunn, H. F. (1971), *Science, N.Y.*, **172**, 1049.

BLOOD-OXYGEN

One gramme of haemoglobin when fully converted to oxyhaemoglobin will combine with 1·36 ml. of oxygen at N.T.P.

Thus the oxygen capacity is a measure of the total effective haemoglobin (oxyhaemoglobin and reduced haemoglobin). Carboxyhaemoglobin, methaemoglobin, and sulphaemoglobin are not included.

Oxygen Saturation:

$$\frac{\text{(Oxygen content of sample} - \text{oxygen in physical solution)}}{\text{(Oxygen capacity of sample} - \text{oxygen in physical solution)}} \times 100 \text{ per cent.}$$

NORMAL ARTERIAL BLOOD: Not less than 94 per cent saturated.

NORMAL VENOUS BLOOD: Approximately 70–90 per cent saturated (average value).

In Rh incompatibility due to 'blocking' antibodies, the oxygen-carrying capacity of the infant's red blood-cells is reduced by 15–31 per cent, presumably as a result of red cell membrane damage. This loss of efficiency in the red cells will exaggerate the effects of any associated anaemia.

Estimation of oxygen saturation of samples obtained at various sites during cardiac catheterization is useful when used in conjunction with intracardiac pressure measurements in the detection of intracardiac abnormalities.

Flick Principle: If the arteriovenous oxygen difference and total oxygen consumption over a timed period is known, then

Cardiac output =

$$\frac{\text{Total oxygen consumption per minute (ml./min.)}}{\text{Arteriovenous oxygen difference (ml.)}}.$$

N.B.—During cardiac catheterization, the blood in the right auricle will give a good average venous sample: the blood from the lung periphery will give an oxygenated arterial sample.

REFERENCE.—Hawk, P. B., Oser, B. L., and Summerson, W. H. (1949), *Practical Physiological Chemistry*, 12th ed., 638. London: Churchill.