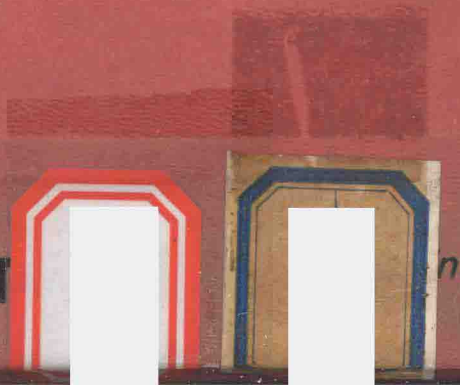
LEHMANN
HUNTSMAN

man's haemoglobin



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MAN'S HAEMOGLOBINS

Including the haemoglobinopathies and their investigation

H. LEHMANN
Cambridge

and

R. G. HUNTSMAN
London

WITH A PREFACE BY

F. G. YOUNG, F.R.S.

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PREFACE

by

F. G. YOUNG, F. R. S.

Two different, and indeed almost opposite, trends in the development of Biochemistry during the present century can be discerned. The first is the revelation in ever-growing detail of a similarity in the metabolic patterns which underlie all forms of life – the existence of a common metabolic ground plan in living cells. The second is a growing recognition of the chemical distinctiveness of the individuals who make up a population.

In his classical work 'Inborn errors of Metabolism' the late Sir Archibald Garrod pointed out fifty years ago that certain recognized metabolic aberrations in man could be ascribed to genetical abnormalities which might or might not result in recognizable disease. At the opening of the Courtauld Institute of Biochemistry in London in 1928 Garrod said that 'evidence is accumulating that no two individuals of a species are any more identical in chemistry than in form. It would seem that there is a chemical basis for those departures from type, which are styled mutations, and I believe that the liabilities of certain individuals to certain maladies have chemical origins'. In no branch of medical science have Garrod's ideas been better illustrated as yet than in the study of the haemoglobins, a subject on which Dr. H. Lehmann and Dr. R. G. Huntsman have written this authoritative work. The volume provides an invaluable conspectus of a subject of which the pabulum ranges from medicine to laboratory chemistry, from physiology to physics.

When in 1949 Linus Pauling and his colleagues showed that the electrophoretic mobility of the circulating haemoglobin of people suffering from sickle-cell anaemia was significantly different from that of what could be termed ordinary human haemoglobin, few realized what a rich field of new discovery had been entered. After all, at that time little certain could be said about the detailed primary structure of proteins in general, and although haemoglobin was of importance both to physiologists and to crystallographers, any final common pathway in their interests was far off. But slowly, owing much to the pioneer researches of A. J. P. Martin and R. L. M. Synge on methods for the separation of amino acids, and to the means

developed by Frederick Sanger for ascertaining the sequence of the amino acids in proteins, the chemistry of the haemoglobins has become well understood. And as a result of this knowledge many individual differences in human haemoglobins which are now recognized can safely be attributed to clearly defined, though small, chemical differences.

The manner in which proteins are built up in living cells is now clear and an example of the value of the recognition of a common ground plan has lain in the fact that much about this process has been learned, and learned more easily, from an examination of the process in lower organisms, but the knowledge thus acquired seems also to apply in general terms to higher animals. In the gametes the information about the nature of the chemical structures to be built up in the offspring is carried in the molecules of deoxyribonucleic acids (DNA) and the order in which the component nucleotides appear in a molecule of DNA determines the order of amino acids in the proteins which are built up with the aid of the genetical information thus provided. The manner in which the sequence of the nucleotides in the DNA determines the sequence of amino acids in a protein – the genetic code – has been ascertained largely by experiments on bacteria and the viruses which infect them – the bacteriophages – and the information derived in this way has been found to agree with that obtained from investigations on the tissues of higher animals. An exciting outcome of the researches of Dr. Lehmann and his colleagues, which is described in chapter 7 of this book, is the realization that the structural differences which naturally occur among the human haemoglobins are consonant in every way with the genetic code deduced from experiments on very different biological materials.

Dr. Lehmann and Dr. Huntsman are in some way the biochemical counterparts of the classical naturalists, with rare haemoglobins as their quarry. Dr. Lehmann has travelled to remote parts of the earth in search of his finds, and has discovered some important new variations on the haemoglobin molecule as a result. Unlike the classical naturalist Dr. Lehmann then finds out exactly in which chemical aspect his new find differs from other known species, and his systematic way of ascertaining whether such differences exist, and exactly what they consist of, has been widely adopted. Having got the chemical information Dr. Lehmann and his colleagues then become geneticists and, perhaps by judicious examination of the relatives of the donor of the interesting blood, map out the pathway of the gene which has carried the information leading to the abnormality. In this way most valuable information has been obtained about the genetics of the human race.

This book contains a survey of information which is scattered throughout the literature pertaining to various branches of science but which has been brought together under the authoritative hands of writers who themselves have contributed much to the advance of the subject which they so ably survey. This book can be commended as one which will naturally take its place among those accounts of an important subject which are at once most readable and unquestionably authoritative.

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CHAPTER 1

IRON

Haemoglobin was once described by the American physiologist L. J. Henderson, as the second most interesting substance in the world. It is probable that he thought of chlorophyll as occupying the premier position. However, haemoglobin is not a single substance but a group of related globins to which the same prosthetic group, haem, is attached. The haem is a combination of one iron atom with a molecule of porphyrin, which itself is composed of a ring of four pyrrole units (fig. 1.1).

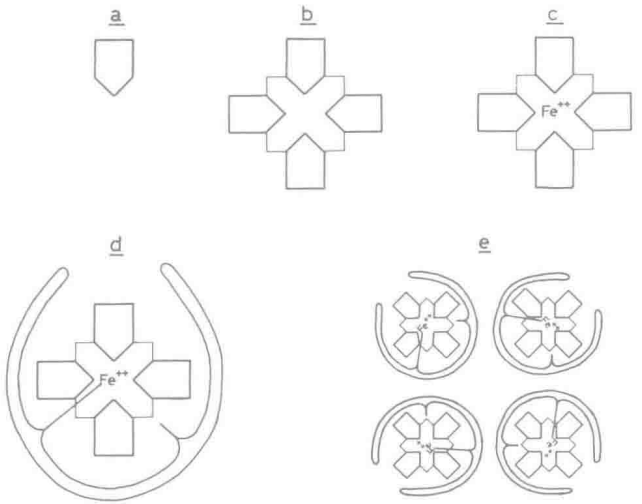

Fig. 1.1. Steps in the development of haemoglobin.
a: pyrrole unit, b: porphyrin ring, c: haem, d: haem–globin complex (e.g. myoglobin), e: four haem–globin units forming one complex molecule (e.g. haemoglobin).

Those haemoglobins that exist in nature today must represent the end-result of a very long period of evolution and are superbly adapted to the specialised and varied functions that they undertake in the cells of even the lowest animal. Haem itself is ubiquitous in the animal kingdom and it has been suggested that its creation, by the substitution of iron for magnesium

in the porphyrin ring of chlorophyll, was the key that opened the door to aerobic life (fig. 1.2).

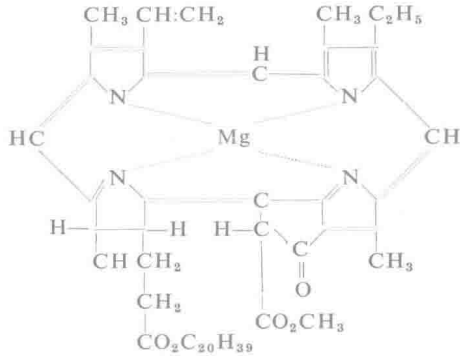

Fig. 1.2. Prosthetic group of chlorophyll, which contains magnesium as a metal and not iron as found in haem.

A normal man contains about 4 g of iron, which are distributed as follows:

Haemoglobin (circulating in the blood)	70%
Iron stores (mainly in the liver, spleen and bone marrow)	20%
Myoglobin (in the muscle)	5%
Respiratory enzymes (in the tissues)	5%

Haemoglobin, myoglobin and the respiratory enzymes, all haem complexes, thus account for four-fifths of the body's iron.

1.1. Storage and transport iron

The storage iron is not an iron porphyrin complex but a core of an inorganic ferric salt, probably the hydroxide, surrounded by a shell of protein. This is a soluble complex, called ferritin. When the ferritin aggregates to form an insoluble complex it is called haemosiderin. When the tissue iron stores are at a normal level there is a preponderance of ferritin but when iron overloading occurs the proportion of haemosiderin greatly rises.

Some thirty years ago it was still widely believed that there was no iron in the plasma. It is a measure of the recent development of microchemical techniques that the man who has done most to establish the concept of serum iron, Professor Heilmeyer, is an active professor of medicine in Freiburg today.

Only 3 to 4 mg of iron are in the plasma at any one time but the importance of this fraction is out of proportion to its minuteness, because this iron in the plasma is the sole link between the sites of iron metabolism in the body.

The main traffic is made up of the return of iron, derived from red cells destroyed in the reticulo-endothelial system, back to the marrow for re-incorporation into new red cells. The iron is transported in the ferric form and is attached to a series of related globulins which are collectively called transferrin. The type of transferrin a man carries is genetically determined and the distribution of these related proteins in different populations can be used as anthropological markers, because different races of mankind carry these sub-groups in different proportions. There is also one type of transferrin, discovered by Kirk in Asiatics, which up to the present has never been shown in any other racial group.

It is normal for the plasma transferrin to be about one-third saturated with iron. The extent to which it could combine further with iron is called the unsaturated iron-binding capacity. The total iron-binding capacity therefore equals the plasma iron plus the unsaturated iron-binding capacity. The level of plasma iron tends to be somewhat lower in women than in men and also varies considerably with the time of day. In the evening the plasma iron may well be 25% lower than in the morning.

The plasma iron is reduced when the body stores of iron have become exhausted. It is also low in many chronic illnesses unassociated with iron deficiency, but these can be distinguished from straightforward iron deficiency because the total iron-binding capacity is low in toxic states and tends to be high when iron is deficient.

Plasma iron may be increased if the delicate balance between the rate at which iron enters and leaves the plasma becomes disturbed. One obtains a rise, therefore, both when there is increased haemolysis and also when there is a marrow disorder resulting in impairment of haemoglobin synthesis. Conditions where a haemolytic state is coupled with impaired formation of haemoglobin are therefore doubly likely to give rise to considerable increases in the level of plasma iron. Thalassaemia, untreated pernicious anaemia and lead poisoning would be examples.

The degree to which the transferrin is saturated with iron influences its function. In order for the developing red cells to wrest the iron from the transferrin effectively its saturation must be above 30%. Below this figure the uptake of iron from the transferrin by the marrow becomes reduced. If, however, the percentage saturation is above 60%, the iron begins to be deposited in quantity in tissue store sites such as the liver. Thus the percentage saturation of transferrin gives a conception of the likely fate of circulating iron.

1.2. The respiratory enzymes

1.2.1. The cytochrome system

The molecular oxygen of oxyhaemoglobin is not utilised directly for the oxidation of metabolic substrates in the body cells. Between oxygen and the substrate is inserted a series of linking reactions, 'the respiratory chain'. Iron appears in this chain in the form of haem in the important compounds collectively called cytochrome, and also as non-haem iron in one of the enzymes involved. The different cytochromes, which are a group of intracellular proteins with iron porphyrin prosthetic groups, have alterations in the side chains of the porphyrin ring, which cause differences in their absorption spectra. The cytochromes fluctuate in the tissues between the oxidised form, with the iron atom in the ferric state and the reduced form, with the iron atom in the ferrous state.

Oxidation may be considered as the removal of negative charges (electrons) from the substance being oxidised, which thereby gains a positive charge. A chemical undergoing reduction acquires an electron and gains a negative charge. However, oxidation–reduction processes appear superficially to manifest themselves in somewhat different ways, for example:

1. Oxidation of ferrous to ferric salts
   $Fe^{++} \rightarrow Fe^{+++}$ + electron with negative charge (e^-).
2. Oxidation of succinic acid to fumaric acid (dehydrogenation)

$$\begin{array}{l} COOH \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array} \quad \rightarrow \quad \begin{array}{l} COOH \\ | \\ CH + 2H \\ | \\ CH \\ | \\ COOH\ . \end{array}$$

The hydrogen is not given off as a gas (H_2) but is passed to a coenzyme acting as a hydrogen acceptor. If the H is in fact considered as a hydrogen ion (H^+) with simultaneous formation of a negatively charged electron (e^-)

$$2H \rightarrow 2H^+ + 2e^-$$

succinic acid has been oxidised to fumaric acid with the removal of two electrons and the formation of two hydrogen ions (H^+).

As free electrons are unstable their removal from the metabolites implies acceptance of the electrons by a receptor. The electron donor undergoes oxidation and at the same time the electron receptor undergoes reduction. This process is described as a 'redox' process. Redox processes supply

energy to all forms of life of varying complexity from a man enjoying champagne and oysters to the amoeba sharing the man's pleasure in his colon.

The link between the hydrogen donated by a cellular metabolite and molecular oxygen is provided by a series of redox reactions, forming a respiratory chain (fig. 1.3).

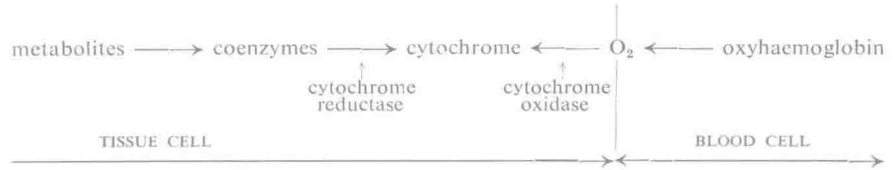

Fig. 1.3. The respiratory chain. The link between the coenzyme and the cytochrome is provided by the cytochrome reductase, and the link between cytochrome and oxygen by cytochrome oxidase. All these reactants fluctuate between reduced and oxidised form. *Metals*: cytochrome: haem iron; cytochrome reductase: non-haem iron; cytochrome oxidase: copper.

The last link is cytochrome oxidase which joins a reduced cytochrome with molecular oxygen provided by oxyhaemoglobin, the reduced cytochrome thereby becoming re-oxidised. During the final reaction in the chain the oxygen can be considered to have become reduced ($O \rightarrow O^{--}$). The place of the cytochrome system in the electron transfer chain can be therefore summarised as in fig. 1.4.

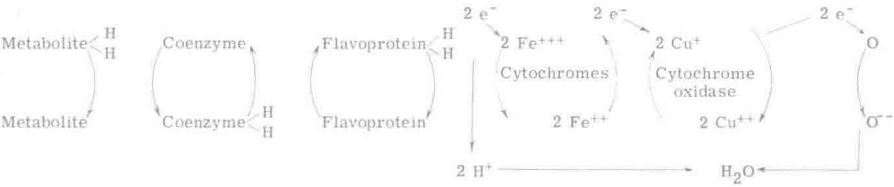

Fig 1.4

The carriage of hydrogen, donated by the metabolite undergoing oxidation to the cytochrome system is achieved by intermediate links provided by coenzymes; firstly NAD (nicotinamide adenine dinucleotide or coenzyme I or diphosphopyridine nucleotide) or NADP (nicotinamide adenine dinucleotide phosphate or coenzyme II or triphosphopyridine nucleotide) and subsequently a flavoprotein. The reader will note that these complexes are formed from vitamins of the B_2 group, nicotinamide and riboflavin.

In the series of reactions outlined above the hydrogen atom becomes a hydrogen ion and electron, $H \rightarrow H^+ + e^-$, and the electrons travel along the chain of carriers called the cytochrome system. As the electrons are passed along the chain the coupling reactions that occur produce energy in the form of energy-rich phosphate bonds. Instead of the hydrogen combining with oxygen in an explosive way, the energy of the reaction is released in a gradual, controlled manner. By this means the energy requirements of the cell are met by building up energy-rich phosphate bonds.

Much of the original work on cytochrome was based on the difference in the spectrum of the oxidised and reduced form (fig. 1.5). It is remarkable

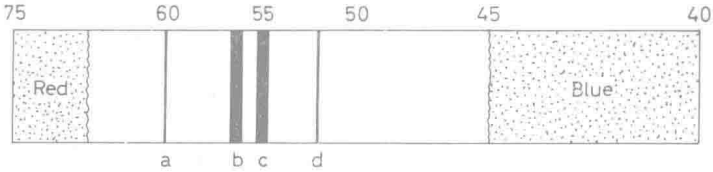

Fig. 1.5. Spectrum of reduced cytochrome. It was the change in the absorption spectrum of cytochrome which gave early workers an insight into its function in cellular oxidation processes. (After Keilin.)

how the investigations on the absorption spectrum, which are only just about a hundred years old, have led to an increase of knowledge in the fields of chemistry, biochemistry and physics.

We often do not appreciate enough how much we are standing on the shoulders of great men of the nineteenth century and the giant strides made by these often isolated research workers are difficult for us to appreciate. We are bemused and excited by the splitting of the atom and by the visualisation of protein molecules, just as our forefathers must have been equally excited when it was possible to look through a piece of glass at the stars and to pronounce on their composition.

Perhaps the introduction of spectral analysis is one of the best examples of how a new technique, apparently of theoretical interest only, can subsequently open up whole continents of knowledge. We knew a man who in his youth was a student at Heidelberg, and who found much amusement in listening to Kirchhoff describing in his lectures on physics the spectral lines discovered by his friend Bunsen. Later in their lectures on chemistry, they were told by the very same Bunsen of this fascinating phenomenon first observed by his colleague Kirchhoff!

1.2.2. Peroxidase and catalase

Of these two haem-containing enzymes, catalase exists mostly in animals and peroxidase mostly in plants. Both can dispose of hydrogen peroxide (H_2O_2) when it is formed during oxidation processes in the cells. In contrast to haemoglobin with its ferrous haem, these enzymes have a ferric haematin prosthetic group. The prosthetic group itself, and to a lesser extent even inorganic iron, may act as comparatively sluggish catalysts in the breakdown of hydrogen peroxide. Catalase and peroxidase are essentially similar enzymes. Catalase, however, acts on H_2O_2 directly by using one molecule of hydrogen peroxide as a hydrogen donor for another molecule of hydrogen peroxide, whereas peroxidase uses a separate substrate (H_2R) for that purpose.

$$\underset{\text{(white benzidine)}}{H_2O_2 + H_2R} \xrightarrow{\text{Peroxidase}} \underset{\text{(blue benzidine)}}{2H_2O + R}$$

$$H_2O_2 + H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Many medical laboratories use the peroxidase activity of haem derived from digested haemoglobin to demonstrate bleeding in the intestinal canal. The faeces are mixed with H_2O_2 and with colourless hydrogen donors such as guaiac or benzidine, which turn blue when they become oxidised. By acting as a catalyst very small amounts of haem can thus be discovered. When this reaction was studied more than one hundred years ago, the ability of the peroxidase to detect such minute amounts of the chemicals involved caused its discoverer to marvel, and he referred to the peroxidase reaction as 'a chemical microscope'.

1.3. Haemoglobin and myoglobin

Myoglobin resembles both the haem compounds we have mentioned up to now, and haemoglobin. Like the first it is situated inside metabolically active tissues and like haemoglobin it acts not by oxidation or reduction of its iron but by becoming oxygenated and deoxygenated. If the oxygen pressure is high, an oxygen molecule becomes attached to the ferrous atom in the centre of the haem group. The oxygen molecule is subsequently released when the pressure of oxygen falls. In haemoglobin and in myoglobin this oxygen is attached to the molecule but on release of the oxygen the iron atom will again be in the ferrous state: