CHAPTER I

INTRODUCTORY

SIR MICHAEL FOSTER(1) likened the growth of knowledge to the ascent of a spiral stair from which the observer periodically surveys the same landscape, but each time from a higher level than the last. In the decade before the war great advances were made in an understanding of hæmoglobin, and even then sufficient was known about it to elicit, if not to justify, the statement(2) that hæmoglobin "was perhaps the second most interesting substance in the world"—the first presumably being chlorophyll. The landscape spread out before the observer then was that of hæmoglobin behaving differently under all sorts of circumstances. The point of view on the spiral which was attained was the discovery that circumstances themselves had a quite unexpectedly great effect in regulating the action of hæmoglobin, so that the question was seriously asked whether all hæmoglobin was not really the same. The answer at one time appeared almost to be "yes." Those whose philosophy ran in the direction of a single hæmoglobin, had still to face the fact that the pigment from the corpuscles of different animals crystallised differently(3). That, in 1910, seemed a small matter as compared with the divergences which had been shown merely to be a matter of circumstance—solvent, temperature, hydrogen-ion concentration and the like. With an understanding of the necessity for the study of hæmoglobin under uniform conditions the whole question of the uniformity of hæmoglobin was ripe for reinvestigation. A few experiments made on the subject by Douglas, Haldane and Haldane(4) before the war pointed to the existence of essential differences in the hæmoglobin of different species.

Since 1921 the whole matter has been gone into in great detail, and to-day we are confronted with the same landscape, which ten years previously appeared to consist merely of a few massive features: from our present height it consists of endless detail.

Both abroad (Vlès(5)) and at home(6) it was shown that the hæmoglobin of *Arenicola* differed essentially from that of man, both spectroscopically and in its gas-binding properties. The discovery of this difference led to a systematic comparison of the hæmoglobins

of many forms of life(7). Of these one of the most fruitful was the comparison between the hæmoglobin in the larva of the fly *Gastrophilus* and that of the horse(7), (8), the point of the comparison being that the *Gastrophilus* larva grows in the equine stomach. But though the larval hæmoglobin is manufactured in the horse it is not the same spectroscopically as the horse's hæmoglobin. Moreover it is not evident why the *Gastrophilus* larva should contain hæmoglobin at all. The primary use of hæmoglobin in the Mammalia is for oxygen transport. In *Gastrophilus* larva the hæmoglobin is fixed and the same is true of the hæmoglobin in mammalian muscles and many other places. Keilin therefore was led into an investigation of the structure and function of hæmoglobin in muscle—this research led to his discovery of cytochrome(8), a body closely related to hæmoglobin, which is very widely distributed throughout the animal and vegetable kingdoms—so much so as to challenge the question whether it or its constituents are not really more primitive than chlorophyll.

What is the nature of the differences between one hæmoglobin and another?

Clearly there is the possibility that as between two different samples of hæmoglobin, the hæmatin may be uniform and the globin may differ. Or the globin may be the same and the hæmatin may differ. Or both the globins and the hæmatins may differ.

One method of attacking the question was to treat the hæmoglobin with alkali in the presence of a reducing agent. The idea—erroneous as it turned out—was that the globin would be split from the hæmatin and the resulting spectrum would be that of reduced hæmatin in alkaline solution. In this task Anson and Mirsky(9) repeated (though quite independently) an observation which had appeared sporadically in the previous literature but the importance of which had never been appreciated (Bertin-Sans and Moitessier(10), and Dilling(11)), namely, that hæmochromogen is not reduced hæmatin in alkaline solution, but is a compound of that body with globin. Anson and Mirsky(9), working over a number of mammalian bloods, found that the spectra of the hæmochromogens which they yielded were identical. From this it may be gathered that in this range of hæmoglobins the hæmatin portion is identical in all, and that the globins are different.

The question arises then: If the globins are different in the hæmoglobins of the horse and the mouse, why have the hæmochromogens identical spectra? That question leads to another: What is the

essential difference between hæmoglobin and hæmochromogen? Anson and Mirsky (9) answer this question as follows: Hæmoglobin is hæmatin united with undenatured globin—hæmochromogen is united with denatured protein.

The differences in the globin moiety only carry us a certain way. There are certainly hæmoglobins which differ in the hæmatin moiety —hæmatin is essentially a compound of iron with porphyrin, and porphyrin is essentially a compound containing four pyrrol groupings. As there can be many porphyrins, according as the side chains differ slightly, so conceivably there can be as many hæmoglobins. Three such have been prepared by Hill and Holden (12), they are the hæmoglobins which correspond to proto-, meso- and hæmatoporphyrins. There is also one such body which has not been prepared synthetically but which was found in the blood of certain polychæte worms by Ray Lankester (13) and the true nature of which has recently been recognised by Fox (14).

Porphyrins may unite with metals other than iron, as has been shown by numerous observers (Laidlaw (15), Schulz (16), Milroy (17)). Knowledge of the metalloporphyrins has been much extended by R. Hill (18), but though he has made many attempts to prepare hæmoglobins from metalloporphyrins containing metals other than iron, these have been unsuccessful. Globin will not unite with them: and from the other side, though hæmochromogens may be prepared by the addition of many substances (hydrazine, nicotine, pyridine) to hæmatin, globin is the only such material which by its association with oxygen yields a body from which the oxygen is detachable by mere exposure to a vacuum.

Hæmochromogen forms no reversible oxide. Attempts to oxidise it in alkaline solution appear to break it down. An oxide in neutral solution is stable, but it cannot be reduced by a vacuum. It has been studied by Keilin (19) and is called by him "parahæmatin" (formerly it was known as kathæmoglobin). While oxygen does not unite reversibly with hæmochromogen, carbon monoxide does. It is possible to determine the curve, as was done by Anson and Mirsky (9), which expresses the equilibrium between CO and hæmochromogen. Reverting from hæmochromogen to hæmoglobin, the affinity of CO for the pigment is about 400 times that of oxygen in man, and perhaps 100 times that of oxygen in the rabbit—the relative affinities of the two gases differing for the hæmoglobins of different animals.

To the great affinity of CO for hæmoglobin, has been attributed

its danger as a poison, the current view being that CO itself is innocuous, but that uniting with the hæmoglobin of the body it prevents the carriage of the oxygen, necessary for life, to the brain and other organs. It might be inferred that to forms of life which did not depend on hæmoglobin for their oxygen supply, carbon monoxide would be harmless. Yet this is not the case. Warburg (20) has shown that CO inhibits the oxygen uptake of yeast and J. B. S. Haldane (21) that both to growing seedlings and to moths carbon monoxide is poisonous even though these forms of life are devoid of hæmoglobin. Moreover, the poisonous dose does not depend upon the absolute amount of carbon monoxide present but on its concentration relative to that of oxygen.

The union of carbon monoxide in such small concentrations with hæmoglobin has been analysed by Hartridge and Roughton (22). The equilibrium constant of each of the reactions

$$CO + Hb \rightleftarrows COHb$$

and

$$O_2 + Hb \rightleftarrows O_2Hb$$

is of course the quotient of the velocity constants of the two phases of the reaction. Hartridge and Roughton, by extraordinarily ingenious methods, have measured the four velocity constants involved, and found that the great affinity of CO for hæmoglobin is due not to the great magnitude of the velocity constant for the phase

$$CO + Hb \longrightarrow COHb,$$

but to the small value of the constant for

$$CO + Hb \longleftarrow COHb.$$

The equation

$$CO + Hb = COHb,$$

written in that way, expresses a heritage of convention, for the molecular weight of hæmoglobin has at last been determined in two quite different ways independently by two observers. By a curious irony one of these methods depends upon its extreme slowness, the other on its extreme speed. Adair (23), by measurements of the osmotic pressure, each measurement extending over months, has found the osmotic pressure over a great range of conditions to correspond to about 70,000. This figure is the same as that more recently obtained by Svedberg (24), who, spinning solutions of hæmoglobin at about 10,000 revolutions per minute, actually concentrated the

hæmoglobin molecules in the peripheral portion, with sufficient precision to calculate the molecular weight. The molecular weight of 70,000 or thereabouts introduces fresh possibilities as to the differences between one form of hæmoglobin and another. The molecular weight which would correspond to an atom of iron is about 17,000, or approximately one-quarter of the true molecular weight. We do not know how the hæmatin is attached to the globin, but it would appear either that four hæmatins are attached to one globin, or four molecules, each of 17,000 molecular weight, are condensed. In either case the process might admit of great variations in detail. The establishment of the fourfold molecular weight leaves us with some difficulties:

(1) In its relation to oxygen hæmoglobin reacts very much as though one molecule of oxygen united with one of hæmoglobin. Hartridge and Roughton (25) suggest that possibly in this very large molecule the four hæmatins may be so far apart as each to unite with oxygen as though the others were non-existent.

(2) Hæmoglobin in its relations with oxygen behaves also as though in solution. This is true whether in ordinary aqueous solution or in the red blood corpuscles. Yet the red blood corpuscles of many animals, if hæmolysed even under the most rigorous conditions and without either concentration or dilution of the hæmoglobin, shed it as crystalline material. Why should the mere breaking of the structure of a cell throw the material within it out of solution? Presumably in the cell the molecules are not free to orientate themselves at pleasure. The direction of their orientation is in some way decided by the structure of the cell and the whole of the biological aspect is regulated by that orientation. If that be true of the hæmoglobin in the red corpuscle, of how many other substances and in how many other cells may it not also be true?

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—— Abstract of Communication to XIIth Internat. Physiol. Congress at Stockholm. *Skand. Archiv.* 1926.
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CHAPTER II

PORPHYRINS

THERE are stories in Cambridge which go back to the days in which Michael Foster sowed such seed as grew up to form the English school of modern physiology. One of these stories has to do with his pupil, Francis Maitland Balfour, the embryologist. It is related (with what truth I cannot say) that Balfour as a young man asked Foster to suggest to him a subject for research. Foster was taking lunch at the time and before him was a boiled egg. He pointed to the egg and said, "What better subject can you have than that?" And there the study of hæmoglobin may commence also; for the particular material which at the moment is regarded as the material starting-point in hæmoglobinology is porphyrin, the brown colouring matter of many eggs. I say "at the moment" because twenty years ago, or even less, the study of hæmoglobin was supposed to have its source in chlorophyll; but the most competent judges, for instance Willstätter (1), regard it as difficult to attach evolutional significance to the chemical similarity between chlorophyll and porphyrin. Even though the view that hæmoglobin in the animal kingdom is the direct result of chlorophyll which is eaten in vegetable food has found recent supporters in Verne (2) and Marchelewski (3), it seems not proven. Let us commence then with porphyrin as being the simplest pigment which has evident chemical relationships with hæmoglobin.

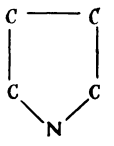
Porphyrin, apart from its existence in the egg-shell, does not bulk very large in warm-blooded animals, though in small quantities it is widely distributed. In the earthworm it constitutes the line of pigment down the back and is found in many other places in invertebrates (MacMunn, Dhéré, Derrien). To distinguish it from the other members of its class (which comprises other natural porphyrins such as uro- and coproporphyrin) it is known as Kämmerer's porphyrin. More recently it has been called by Hans Fischer (4) (who has crowned a compendious series of researches by synthesising some members of the porphyrin group) protoporphyrin. The names ooporphyrin and Kämmerer's porphyrin are significant, for they serve to remind the reader that the material found in the egg-shell is the same as that obtained from blood by Kämmerer. He produced this substance as a degradation product of hæmoglobin by the action on it of

certain bacteria, and so, naturally, the question arises: What is the relation between ooporphyrin and the simplest crystalline product of the hæmatin family—hæmin for instance? So far as their empirical formulæ are concerned the relationship would be as follows:

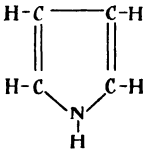
Ooporphyrin	$C_{34}H_{30}N_4O_4 . H_2$
Hæmin	$C_{34}H_{30}N_4O_4FeCl$
Hæmatin	$C_{34}H_{30}N_4O_4FeOH.$

Here let me make a short digression to point out the essential nature of these formulæ. Written as graphic formulæ they, at first sight, look almost as alarming as the modern "cross-word puzzle." Yet a moment's consideration will show that in their main features they are easily grasped.

The fundamental grouping of atoms on which the whole pivots is the pyrrol ring, consisting of four carbon atoms and one nitrogen atom joined together in the form of a five-pointed figure

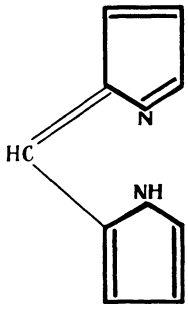


pyrrol itself being

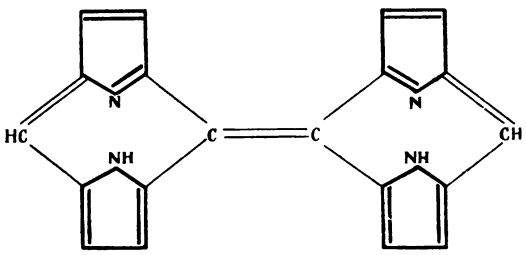


The above is written in an abbreviated form

It is when two such rings are joined together in the following way that the molecule takes on the first vestige of porphyrin character:



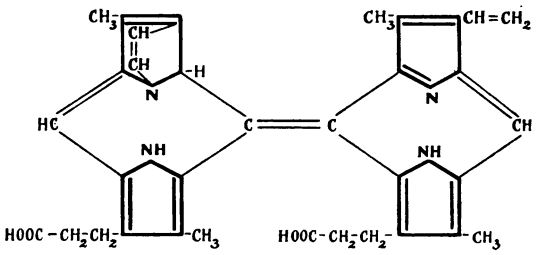
Let us double once more and we obtain[1]



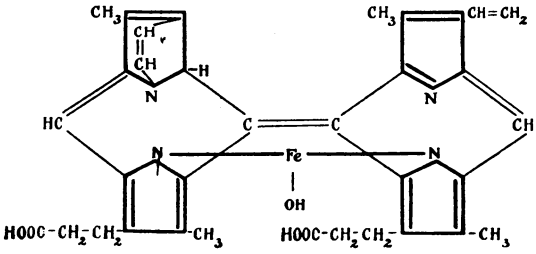
(Hans Fischer.)

This is clearly the skeleton, so to speak, to which the soft parts in the way of side chains are attached. On this skeleton are built up not only all the porphyrins but so far as is known all vertebrate blood pigments.

Let us fill in the "soft parts."



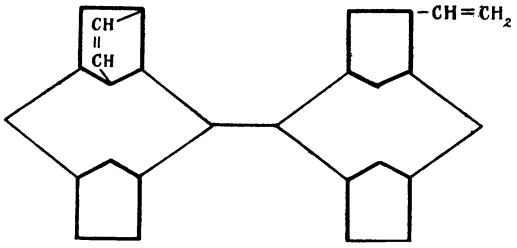
This probably represents the brown pigment of the egg-shell: its relation to the blood pigments is a matter of exchanging for two hydrogens the group FeOH thus:



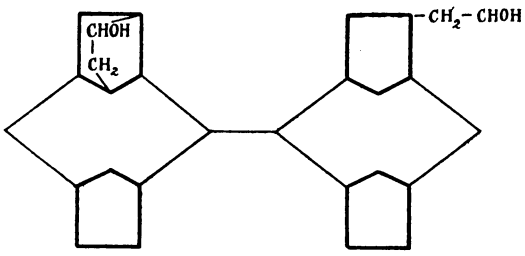
which is hæmatin, or by FeCl which gives hæmin.

[1] Kuster had published an alternative linkage of the four pyrrols which Fischer, since the above went to press, has adopted. [*Berichte Jahrg.* LX. Seite 2611, 1927.]

To return from the digression on the pyrrol structure of porphyrin, I had pointed out that the essential difference between hæmatin and ooporphyrin was the replacement of the FeOH group of the hæmatin by two hydrogen atoms, and further I had said that the replacement could be effected by the action of certain bacteria, at all events when blood was used as the starting-point. The usual method, however, of removing the iron from hæmin is by the action of strong sulphuric acid, or better by hydrobromic acid; in that case the FeCl is replaced not merely by two hydrogen atoms, but two molecules of water are added at the same time. The substance is not ooporphyrin but hæmatoporphyrin, $C_{34}H_{38}N_4O_6$. The hydration takes place in the two groupings indicated below:



Put in two molecules of water H.OH and you might get:



but equally if the water went in to the same groupings in another way, the following might be obtained: