The oxygen-transferring ferment of respiration

Nobel Lecture, December 10, 1931

References to the transfer of oxygen by iron in the older literature generally apply to the iron in the blood pigment which carries molecular oxygen from the lungs to other parts of the body. The erroneous view that the iron of blood pigment causes combustion of foodstuffs, and thus transfers the oxygen not only spatially but also by chemical catalysis, is the meaning of Liebig's theory of respiration. "The blood corpuscles", said Liebig, "contain an iron compound, no other constituent of living material contains iron."

It has only recently been recognized that iron is present in all cells, that it is vitally important, and that it is the oxidation catalyst of cellular respiration. Catalytic oxidation in living substances rests upon change of valency in an iron compound which is the respiratory oxygen-transferring ferment.

The concentration of the ferment iron in living substance is very small, being in the region of 1 g to 10 million g of cellular substance. The effects of iron are very great, and it follows that oxidation and reduction of the ferment iron must occur extremely rapidly. In fact, almost every molecule of oxygen that comes into contact with an atom of ferment iron reacts with it. In this way, ferment iron fulfils its function in almost perfect manner. The space required for a given amount of reaction is reduced to a physically possible minimum, and the only limit set to the separation of reaction from non-reaction in the microstructure of living material is the spatial arrangement of the molecules. This is the physiological meaning of the great reactivity of the cell ferments or of the fact that the concentration of ferments in the cellular substance is very small.

Mechanism of cell respiration

Of the two processes concerned in the oxidation and the reduction of ferment iron, the former is not in any way problematical. Complex-bound bivalent iron in compounds reacts, in vitro as well as in the cell, with molecular oxygen. The primary respiratory reaction can be imitated in the test-tube
with pure substances of known composition; but it is not yet possible to reduce in vitro trivalent iron with the cell fuel: it is always necessary to add a substance of unknown composition, a ferment, that activates the combustible material for the attack of the iron. It must, therefore, be concluded that activation of the combustible substance in the breathing cell precedes the attack of the ferment iron; this corresponds with "hydrogen activation" as postulated in the theory of Wieland and Thunberg. According to the results of a joint research with W. Christian, this is a cleavage comparable with those known as fermentation.

It is possible that the interplay of splitting ferment and oxygen-transferring ferment does not fully explain the mechanism of cellular respiration; that the iron that reacts with the molecular oxygen does not directly oxidize the activated combustible substances, but that it exerts its effects indirectly through still other iron compounds - the three non-autoxidizable cell haemins of MacMunn, which occur in living cells according to the spectroscopic observations of MacMunn and Keilin, and which are reduced in the cell under exclusion of oxygen. It is still not possible to answer the question whether the MacMunn haemins form part of the normal respiratory cycle, i.e., whether respiration is not a simple iron catalysis but a four-fold one. The available spectroscopic observations are also consistent with the view that the MacMunn haemins in the cell are only reduced when the concentration of activated combustible substance is physiologically above normal.

This will suffice to indicate that oxygen transfer by the iron of the oxygen-transferring ferment is not the whole story of respiration. Respiration requires not only oxygen-transferring ferment and combustible substance, but oxygen-transferring ferment and the living cell.

Inhibition technique

The usual methods of analytical chemistry have not been employed for investigation of the chemical constitution of the oxygen-transferring ferment, because it was felt that the infinitesimally small concentration of the ferment and its sensitiveness made such procedures unprofitable. Instead, the "inhibition technique" was used, that is, substances were looked for which have a specific and reversible inhibitory effect on the oxygen-transferring ferment, i.e., which inhibit oxidation of the living substance. It is obvious that any substance that inactivates the ferment must react chemically with the
ferment, so that inferences as to the chemical nature of the ferment can be drawn from knowledge of the character of the inhibitory substances and of the conditions under which it reacts. That is to say, the chemical reactions of the ferment are investigated, using the inhibition of some catalytic action as the indicator, instead of the usual colour reactions or production of precipitates. Obviously, in such conditions the ferment concentration may have any value as long as the intensity of its action is sufficiently great. And it is an advantage that the ferment can then be investigated under the most natural conditions in the intact and breathing cell.

I will mention here two substances that can specifically and reversibly inhibit respiration in living substance: hydrocyanic acid (for historical reasons) and carbon monoxide (for other reasons).

Inhibition of cellular respiration by prussic acid was discovered some 50 years ago by Claude Bernard, and has interested both chemists and biologists ever since. It takes place as the result of a reaction between the prussic acid and the oxygen-transferring ferment iron, that is, with the ferment iron in trivalent form. If trivalent iron is designated by Fe, the reaction underlying the effect of prussic acid can be written as follows:

\[
\text{FeOH} + \text{HCN} \rightleftharpoons \text{FeCN} + \text{H}_2\text{C}
\]  

(1)

The oxidizing OH-group of the trivalent ferment-iron is replaced by the non-oxidizing CN-group, thus bringing transfer of oxygen to a standstill. Prussic acid inhibits reduction of the ferment iron.

Inhibition of respiration by carbon monoxide was discovered only a few years ago. If the initial reaction in respiration is

\[
\text{Fe} + \text{O}_2 \rightarrow \text{FeO}_2
\]  

(2)

then, in the presence of carbon monoxide, the competing reaction

\[
\text{Fe} + \text{CO} \rightleftharpoons \text{FeCO}
\]  

(3)

will also occur and, varying with the pressures of the carbon monoxide and of the oxygen, more or less of the ferment iron will be removed from the catalytic process on account of fixation of carbon monoxide to the ferment iron. Unlike prussic acid, therefore, carbon monoxide affects the bivalent iron of the ferment. Carbon monoxide inhibits oxidation of the ferment iron.
Thus inhibition of respiration by carbon monoxide, unlike that by prussic acid, depends upon the partial pressure of oxygen.

The toxic action of prussic acid in the human subject is based on its inhibitory action on cellular respiration. The toxic effect of carbon monoxide on man has nothing to do with inhibition of cellular respiration by carbon monoxide but is based on the reaction of carbon monoxide with blood iron. For, the effect of carbon monoxide on blood iron occurs at pressures of carbon monoxide far from the level at which cellular respiration would be inhibited.

The effect of light on inhibition of cellular respiration by carbon monoxide.

Not only cellular respiration but also simpler iron catalyses are reversibly inhibited by carbon monoxide and prussic acid. If one compares such iron catalyses and their inhibitions with cellular respiration and its inhibitions, then it appears that the catalyst of cellular respiration behaves like an iron compound in which the iron is bound to nitrogen. But it would never have been possible to reach a definite conclusion if Nature had not endowed the iron compounds of carbon monoxide with the remarkable property of becoming dissociated - with splitting off from the carbon monoxide - under the action of light.

If carbon monoxide is added to the oxygen in which living cells breathe, respiration ceases, as has already been mentioned, but if exposure to ultraviolet or visible light is administered, respiration recurs. By alternate illumination and darkness it is possible to cause respiration and cessation of respiration in living, breathing cells in mixtures of carbon monoxide and oxygen. In the dark, the iron of the oxygen-transferring ferment becomes bound to carbon monoxide, whereas in the light the carbon monoxide is split off from the iron which is, thus, liberated for oxygen transfer. This fact was discovered in 1926 in collaboration with Fritz Kubowitz.

Photochemical dissociation of iron carbonyl compounds was discovered in 1891 by Mond and Langer, by exposing iron pentacarbonyl. This reaction is specific for carbonyl compounds of iron, most of which appear to dissociate in the presence of light, e.g., carbon-monoxide haemoglobin (John Haldane, 1897) carbon-monoxide haemochromogen (Anson and Mirsky, 1925), carbon-monoxide pyridine haemochromogen (H. A. Krebs, 1928), and carbon-monoxide ferrocystein (W. Cremer, 1929). When the
photochemical dissociation of iron carbonyl compounds is measured quantitatively (we followed hereby Emil Warburg's photochemical experiments), by using monochromatic light and comparing the amount of light energy absorbed with the amount of carbon monoxide set free, it is found that Einstein's law of photochemical equivalence is very exactly fulfilled. The number of FeCO-groups set free is equal to the number of light quanta absorbed, and this is independent of the wavelength employed. For example, the equation for the light reaction of carbon-monoxide pyridine haemochromogen is

$$\text{FeCO} + \text{hv} \rightarrow \text{Fe} + \text{CO}$$

Photochemical dissociation of iron carbonyl compounds can be used to determine the absorption spectrum of a catalytic oxygen-transferring iron compound. One combines the catalyst in the dark with carbon monoxide, and so abolishes the oxygen-transferring power of the iron. If then this is exposed to monochromatic light of various wavelengths and of measured quantum intensity, and the effect of light measured the increase in the rate of catalysis - it is found that the effects of the light are proportional to the quanta absorbed.

The arrangement becomes very simple if the catalyst is present, as is usually the case, in infinitesimally low concentration in the exposed system. Then the thickness of the layers related to the amount of absorption of light can be considered to be infinitely thin, the number of quanta absorbed is proportional to the number of quanta supplied by irradiation, and the ratio of the absorption coefficients ($\beta$) of light is:

$$\frac{\beta_1}{\beta_2} = \frac{W_1}{W_2} \cdot \frac{i_2}{i_1}$$

Here, the effects of light $W$, i.e., the rate of increase of catalysis, and the incident quantum intensities $i$ (both easily determinable figures) are on the right, while $\beta$, on the left, is the ratio (that is to be determined) of the coefficient of light $\beta$, so that the relative absorption spectrum of the catalyst, the position of the absorption bands and the intensity ratio of the bands can be estimated.

In collaboration with Erwin Negelein, this principle was employed to measure the relative absorption spectrum of the oxygen-transferring res-
piratory ferment. The respiration of living cells was inhibited by carbon monoxide which was mixed with the oxygen. We then irradiated with monochromatic light of various wavelengths and of measured quantum intensity, and the increase of respiration measured together with the relative absorption spectrum - according to Eq.(4). For only practically colourless cells are suitable for this type of experiment. A prerequisite for Eq.(4) is a layer infinitely thin with regard to light absorption. Thus, for instance, red blood corpuscles and green vegetable cells are not suitable.

Method for determination of the absolute absorption spectrum

The usefulness of the method is not yet attained with the determination of the relative absorption spectrum, rather can it be so elaborated as to supply the absolute absorption coefficient of the ferment.

Imagine living cells whose respiration is inhibited by carbon monoxide. If these are irradiated, respiration does not increase suddenly from the darkto the light-value, but there is a definite, although very short, interval until the combination of carbon monoxide with the ferment is broken down

![Graph](image)

**Fig. 1.** Carbon-monoxide compound of the oxygen-transferring respiratory ferment.
by the light. Even without calculation, it is obvious that the rate of increase in the effect of light must be related to the depth of colour of the ferment. If the ferment absorbs strongly, the carbon-monoxide compound will be rapidly broken down, and vice versa.

The time of increase of the action of light can be measured. The time taken for a given intensity of light to cause dissociation of approximately half the carbon-monoxide compound of the ferment can be measured and, from this time, and from the effective intensity of light, the absolute absorption coefficient of the ferment for every wavelength can be calculated.

The absorption capacity of the ferment, measured in accordance with this principle, was found to be of the same order as the power of light absorption of our strongest pigments. If one imagines a ferment solution of molar concentration, a layer of $2 \times 10^{-6}$ cm thickness would weaken the blue mercury line 436 µµ by half. The fact that the ferment in spite of this cannot be seen in the cells is due to its low concentration.

Absorption spectrum of the ferment

We have determined the absorption coefficients of the ferment for the region between the ultraviolet line 254 µµ and the red line 660 µµ. Monochromatic light of relatively great intensity - 1/100 to 1/10 gramcalories per minute -
was required for this purpose. In the first experiments, in collaboration with Negelein, 16 wavelengths were available. F. Kubowitz and E. Haas have isolated a further 15 wavelengths of sufficient intensity and purity, so that it is now possible to determine 31 points of the ferment spectrum. Our light source was a mercury-vapour lamp, and a specially designed discharger with specially high output (Dr. Hans Boas), flame carbons (from the Siemens-Plania Works*), and finally the new Pirani lamps of the Osram Study Group*. Monochromators and colour filters were used to isolate the lines from these sources of light. Table 1 shows the wavelengths isolated up to the present time, together with the absolute absorption coefficients of the carbon-monoxide compound of the ferment.

If the absorption coefficient is entered as a function of the wavelength, the absorption spectrum of the carbon-monoxide compound of the ferment is obtained, as shown in Fig. 1. The principal absorption-band or y-band lies in the blue, while to the right of this, lie the long-wave subsidiary bands \( \alpha \) and \( \beta \) in the green and yellow, and, to the left of the principal band, lie the ultraviolet subsidiary bands \( \delta \) and \( \epsilon \). This is the spectrum of a haemin compound, according to the position of the bands, the intensity state of the bands, and the absolute magnitude of the absorption coefficients. The ab-

* We thank Dr. Patzelt (of the Siemens-Plania Works) and Dr. Krefft (of the Osram Study Group) for their valuable advice.
sorption spectra of other carbon monoxide haemin compounds are shown in Figs. 2, 3, and 4.

Model of haemin catalysis

It appeared essential to have a control to ascertain whether haemin as an oxidation catalyst of carbon monoxide and prussic acid really behaves like the ferment. If cystein is dissolved in water containing pyridine, and a trace of haemin is added, and this is shaken with air, the cystein is catalytically oxidized by the oxygen-transferring power of the haemin. According to Krebs, the catalysis is inhibited by carbon monoxide in the dark, but the inhibition ceases when the mixture is illuminated. Prussic acid too acts on this model as

Fig. 4. Carbon-monoxide haemoglobin.
on cellular respiration, inasmuch as it combines with the trivalent haemin and inhibits its reduction. Just as in life, inhibition by carbon monoxide is dependent on the oxygen pressure, while inhibition by prussic acid is independent of the oxygen pressure.

Table 1.

<table>
<thead>
<tr>
<th>Wavelength (μμ)</th>
<th>Light source</th>
<th>Absolute absorption coefficient of the carbon-monoxide compound of the ferment (cm²/gram atom iron)</th>
</tr>
</thead>
<tbody>
<tr>
<td>253</td>
<td>Zinc spark</td>
<td>0.70 × 10⁸</td>
</tr>
<tr>
<td>283</td>
<td>Magnesium spark</td>
<td>2.00 × 10⁸</td>
</tr>
<tr>
<td>309</td>
<td>Flame carbon (aluminium salt)</td>
<td>0.55 × 10⁸</td>
</tr>
<tr>
<td>313</td>
<td>Mercury-vapour lamp</td>
<td>0.51 × 10⁸</td>
</tr>
<tr>
<td>326</td>
<td>Flame carbon (copper salt)</td>
<td>0.50 × 10⁸</td>
</tr>
<tr>
<td>333</td>
<td>Zinc spark</td>
<td>0.50 × 10⁸</td>
</tr>
<tr>
<td>344</td>
<td>Cadmium spark</td>
<td>0.50 × 10⁸</td>
</tr>
<tr>
<td>356</td>
<td>Flame carbon (thallium salt)</td>
<td>0.99 × 10⁸</td>
</tr>
<tr>
<td>366</td>
<td>Mercury-vapour lamp</td>
<td>0.51 × 10⁸</td>
</tr>
<tr>
<td>383</td>
<td>Flame carbon (magnesium salt)</td>
<td>0.51 × 10⁸</td>
</tr>
<tr>
<td>405</td>
<td>Mercury-vapour lamp</td>
<td>0.90 × 10⁸</td>
</tr>
<tr>
<td>422</td>
<td>Flame carbon (strontium salt)</td>
<td>3.05 × 10⁸</td>
</tr>
<tr>
<td>436</td>
<td>Flame carbon (calcium salt)</td>
<td>3.70 × 10⁸</td>
</tr>
<tr>
<td>436</td>
<td>Mercury-vapour lamp</td>
<td>3.60 × 10⁸</td>
</tr>
<tr>
<td>448</td>
<td>Magnesium spark</td>
<td>1.30 × 10⁸</td>
</tr>
<tr>
<td>460</td>
<td>Flame carbon (lithium salt)</td>
<td>0.40 × 10⁸</td>
</tr>
<tr>
<td>494</td>
<td>Flame carbon (magnesium salt)</td>
<td>0.15 × 10⁸</td>
</tr>
<tr>
<td>517</td>
<td>Flame carbon (magnesium salt)</td>
<td>0.19 × 10⁸</td>
</tr>
<tr>
<td>524</td>
<td>Flame carbon (strontium salt)</td>
<td>0.18 × 10⁸</td>
</tr>
<tr>
<td>535</td>
<td>Thallium-vapour lamp</td>
<td>0.30 × 10⁸</td>
</tr>
<tr>
<td>546</td>
<td>Mercury-vapour lamp</td>
<td>0.30 × 10⁸</td>
</tr>
<tr>
<td>553</td>
<td>Flame carbon (magnesium salt)</td>
<td>0.26 × 10⁸</td>
</tr>
<tr>
<td>560</td>
<td>Flame carbon (calcium salt)</td>
<td>0.33 × 10⁸</td>
</tr>
<tr>
<td>578</td>
<td>Mercury-vapour lamp</td>
<td>0.30 × 10⁸</td>
</tr>
<tr>
<td>589</td>
<td>Sodium-vapour lamp</td>
<td>0.54 × 10⁸</td>
</tr>
<tr>
<td>596</td>
<td>Flame carbon (strontium salt)</td>
<td>0.38 × 10⁸</td>
</tr>
<tr>
<td>603</td>
<td>Flame carbon (calcium salt)</td>
<td>0.20 × 10⁸</td>
</tr>
<tr>
<td>610</td>
<td>Flame carbon (lithium salt)</td>
<td>0.12 × 10⁸</td>
</tr>
<tr>
<td>652</td>
<td>Flame carbon (strontium salt)</td>
<td>0.02 × 10⁸</td>
</tr>
<tr>
<td>670</td>
<td>Flame carbon (lithium salt)</td>
<td>0.005 × 10⁸</td>
</tr>
</tbody>
</table>

(Measurements according to F. Kubowitz and E. Haas.)
In conjunction with Negelein, this model was also used to test the ferment experiments quantitatively. Haemin catalysis in the model was inhibited by carbon monoxide in the dark. Then monochromatic light of known quantum intensity was used to irradiate it, and the absorption spectrum of the catalyst calculated from the effect of the light which was known from direct measurements on the pure substance. The calculation gave the absorption spectrum of the haemin that had been added as a catalyst, and so the method was verified as a technique for the determination of the ferment spectrum, both the calculation and the measurement method.

Ferment bands

It is our intention to make use of the ferment bands in order to determine the chemical constitution of ferment-haemin. A few remarks on haemin and its bands must serve as an introduction.

The absolute height of the bands varies within definite limits - even for one and the same haemin. Variations depend upon the salt concentration, the solvent, etc. If the height of one band decreases, its breadth generally increases, whereby the area within the limits of the band appears to remain constant. The absolute height of the bands is only significant inasmuch as the dimension must harmonize.

The ultraviolet bands of haemin are indicated in free haemin, but are only fully shown when the haemins are bound to protein. The ultraviolet bands shown to the left of the main bands in Figs. 1 to 4 are related to the protein components of the haemin compound and are not of interest here since it is the constitution of the haemin components that is significant.

The principal absorption band in the blue and the subsidiary band of the longest wavelength (the \( \alpha \)-band) are suitable for the chemical classification of the compounds of haemin. Later it will be necessary to use the second long-wave band (the \( \beta \)-band) also, but its position in the ferment has not yet been determined with sufficient exactness.

The positions of the principal band and \( \alpha \)-band of the ferment are:

<table>
<thead>
<tr>
<th>Carbon-monoxide compound of ferment</th>
<th>Principal band</th>
<th>( \alpha )-band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>433 ( \mu )</td>
<td>590 ( \mu )</td>
</tr>
</tbody>
</table>

These will be referred to as the "ferment bands" because the ferment was the first for which they were determined.
Haemins are the complex iron compounds of the porphyrins, in which two valencies of the iron are bound to nitrogen. The porphyrins, of which Hans Fischer determined the chemical structure, are tetrapyrrol compounds in which the four pyrrol nuclei are held together by four interposed methane groups in the \( \alpha \)-position.

Green, red, and mixed shades of haemins are known. If magnesium is replaced by iron in chlorophyll, green haemins are obtained. Their colour is due to a strong band in the red which is already recognized in chlorophyll. The ferment does not absorb in the red and cannot, therefore, be a green haemin.

Red haemins are the usual haemins in blood pigment and in its related substances, such as mesohaemin and deuterohaemin. Coprohaemin is also a red haemin which is an iron compound of the coproporphyrin that H. Fischer recognized in the body. Other red haemins are pyrrohaemin, phyllohaemin and rhodohaemin, whose porphyrins Willstätter has prepared by complete reductive breakdown of chlorophyll. The positions of the principal absorption band and of the \( \alpha \)-band of the carbon-monoxide compound of red haemins are:

| Carbon-monoxide compound of red haemins | Principal band \( 420 \ \mu \mu \) and shorter wavelengths | \( \alpha \)-band \( 570 \ \mu \mu \) and shorter wavelengths |

The ferment bands are at least 13 to 20 \( \mu \mu \) nearer the red than the haemin bands. It follows that the ferment is not a red haemin.

Between the green and red haemins are those of mixed colour which are so called because, in solution, very slight changes in the thickness of a layer make them appear green or red. The corresponding porphyrins - which their discoverer Hans Fischer called pheoporphyrins - are formed when chlorophyll is carefully reduced with hydriodic acid. Phylloerythrin also, is a pheoporphyrin that is formed by reduction of chlorophyll in the intestinal canal of ruminants and which Lobisch and Fischler have isolated from ox bile. The pheoporphyrins are closely related to blood pigment but, as H. Fischer showed, pheoporphyrin a is simply mesoporphyrin in which the one propionic acid has been oxidized so that ring closure with the porphyrin nucleus is made possible. Pheoporphyrin a is a reduction product of chlorophyll a or an
oxidation product of blood pigment, and connects together, in an ama-
 zigingly simple manner, the principal pigments of the organic world the blood
 pigment and the leaf pigment.

The bands of Fischer’s pheoporphyrins are shifted towards the bands of the
 blood pigment to the red, i.e., in the direction of the ferment bands but not to
 such an extent as to make them identical with them. Chlorophyll b has, in
 general, bands of longer wavelength than chlorophyll a, and for this reason,
 W. Christian and I applied Fischer’s reduction method to it. In this way we
 obtained pheohemoglobin b, which, when linked with protein, corresponds
 with the ferment in respect to the position of the principal band. The bands of
 the carbon-monoxyde compound of pheohemoglobin b are:

<table>
<thead>
<tr>
<th>Carbon-monoxyde compound of pheohemoglobin b</th>
<th>Principal band</th>
<th>α-band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>435 μμ</td>
<td>598 μμ</td>
</tr>
</tbody>
</table>

While the principal band of pheohemoglobin corresponds with the fer-
 ment bands within the permitted limits, the a-band shifts so far beyond
 them because it lies too near the red. It is, nevertheless, interesting that when
 chlorophyll b is reduced, one obtains a pheoporphyrin of which the haemin
 of all the pheohaemins that have been demonstrated up to the present time
 is the most like the ferment.

Still nearer the ferment in its spectrum, is a haemin occurring in Nature.
 This is spirographis haemin, which has been isolated from chlorocruorin,
 the blood pigment of the bristle-worm Spirographis, in collaboration with
 Negelein and Haas. The bands of spirographis haemin, coupled to globin,
 are:

<table>
<thead>
<tr>
<th>Carbon-monoxyde compound of spirographis haemin</th>
<th>Principal band</th>
<th>α-band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>434 μμ</td>
<td>594 μμ</td>
</tr>
</tbody>
</table>

Constitution of spirographis haemin

It follows from what has already been said that the chemical structure of
 spirographis haemin is important, but because of the difficulty of obtaining
 sufficient quantities of crystallized, analytically pure haemin, experiments on
 it are still incomplete. Working with Negelein, it has so far been found that
 spirographis haemin and also crystallized spirographis porphyrin (that has
also been analysed) contain two carboxyl groups and five oxygen atoms, and therefore a surplus oxygen atom. With hydroxyl amine, this gives an oxime and is then a typical ketone oxygen. Spirographis haemin differs from the red haemius by the surplus or ketone oxygen-atom, and is classified as pheohaemin. Like Fischer’s pheohaemins, spirographis haemin is intermediate between chlorophyll and blood pigment in respect of the degree of oxidation of the side-chains.

Formation and disappearance of the ferment bands

The two haemins with a spectrum most like that of the ferment - pheohaemin b and spirographis haemin - possess a remarkable property. If they are dissolved in dilute sodium-hydroxide solution, in the form of ferrous compounds, the absorption bands slowly wander towards the blue, near the bands of blood haemin. In this way, mixed-colour haemins have been converted into red haemins. On acidification, the change reverts: the <<blood bands>> disappear and the ferment bands appear. This experiment shows that oxidation of the side-chains does not suffice to give rise to the ferment bands, but some process of the type of anhydride formation must also occur.

This reaction, which is the chemical basis of the development of the ferment bands, will not be further discussed here: only the principle on which our work is based will be indicated. Physics brings the ferment bands into existence but organic chemistry is necessary for the identification or creation of these bands. As Anson and Mirsky have said, the procedure is similar to the spectroscopic analysis of the stars. Indeed, the ferment substance - though being so near to us - is, like the substance of the stars, inaccessible for us.

The common origin of haemoglobin and chlorophyll

If oxygen is passed through an aqueous solution of spirographis haemin, at ordinary temperature and under certain conditions, the haemin is oxidized. The previously mixed colour of the solution becomes green, and a band resembling that of chlorophyll appears in the red at 650 \( \mu \mu \). On the other hand, if hydrogen is passed through a solution of spirographis haemin at 37º, in the presence of palladium, the spirographis haemin undergoes reduction
in the side-chain and a haemin resembling that of blood is formed. This is a genuine red haemin* which does not become mixed-coloured when acidified.

The unique intermediate status of the ferment-like haemins demonstrated by these simple experiments suggests the suspicion that blood pigment and leaf pigments have both arisen from the ferment - blood pigments by reduction, and leaf pigment by oxidation. For evidently, the ferment existed earlier than haemoglobin and chlorophyll.

The investigations on the oxygen-transferring ferment have been supported from the start by the Notgemeinschaft der deutschen Wissenschaft and the Rockefeller Foundation, without whose help they could not have been carried out. I have to thank both organizations here.

* According to its spectrum and the hydrochloric-acid number of its porphyrin, this haemin closely resembles mesohaemin, but has a free methine group in the B-position. For this reason spirographis haemin (C₃₂) contains two C atoms less than blood haemin (C₃₄). (Experiments in collaboration with E. Negelein.)