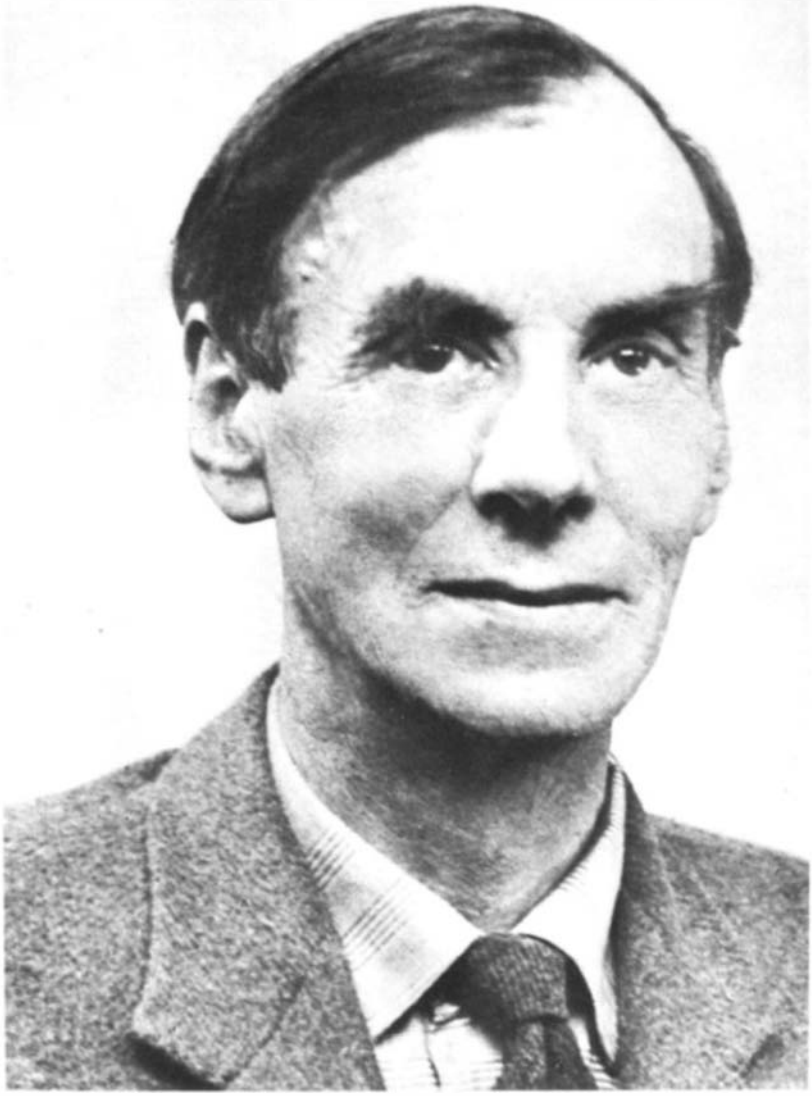

Robert Hill

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DAYS OF VISUAL SPECTROSCOPY

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To have been asked by the editor of the *Annual Review of Plant Physiology* to prepare an introductory chapter is indeed an honor. I accepted because it might be taken as an acknowledgment of help and encouragement given to me by so many different people and also from many different plants. I choose a title which refers to the time up to the year 1945. That is, the time before the advent of photoelectric spectrum photometry which has been responsible for many major scientific advances since that date.

At Stresa in 1971 the organization by Giorgio Forti of the Second International Congress on Photosynthesis enabled many of us to celebrate the bicentenary of Joseph Priestley's discovery of reversal of animal respiration by vegetation. At that time there was some thought of having a later continuation of the history of photosynthesis after 1800. But plowing through the nineteenth century in detail would be heavy going. Even Eugene Rabinowitch hinted as much at the end of his superb historical review where he had shown the effect of the four most remarkable investigators following Priestley: Ingen-Housz, Senebier, de Saussure, and Robert Mayer. Their work had led to the overall representation of photosynthesis as we see it today in the vegetable kingdom. As given by Rabinowitch in 1945, referring to the year 1845, the equation reads:

$$\text{carbon dioxide} + \text{water} + \text{light} \xrightarrow{\text{plant}} \text{organic matter} + \text{oxygen} + \text{chemical energy}$$

It happened that Dr. A. San Pietro had asked me to contribute a lecture to the Gordon Research Conference on Regulatory Mechanisms in Photosynthesis, held in August 1973. There was discussion about later publication of my talk. So, with permission, this present contribution is based on that lecture, entitled An Appreciation of Some of the Former Searches Leading to a Knowledge of Photosynthesis. The first part is concerned with influences through the years since I started as a

research student in 1922; the second half indicates how, from 1935 onwards, green plants attracted much of my scientific inquisitiveness.

Chlorophyll and hemoglobin are perhaps the two most interesting coloring matters on this earth. Not so very long ago, while he was still with us, the Sage had asked me, "Who said why is grass green and blood red?" I do not know; perhaps my readers will; but this question summed up in a few words the interests I hope now to share with them.

To begin with, let us go back to 1837, when H. von Mohl described chloroplasts as discrete green bodies seen in plant cells under the microscope. He also observed the presence of starch in several kinds of plants. Later, Julius von Sachs (1832–1897) proved that the starch in chloroplasts was only formed in the light and could disappear from the chloroplasts in darkness. The starch, detected by the color with iodine, was formed very rapidly in some plants. This made it appear that starch might be the first detectable product of photosynthesis and that the whole process actually took place in a chloroplast. The classical demonstration with a stencil cut to form the word STÄRKE placed on a leaf showed how sharply defined the effect of light could be. In 1921 Schroeder partially covered a single algal cell and only the illuminated part showed starch formation. Autonomy of chloroplasts has been questioned many times, for example in 1883, when Josef Böhm showed that starch could be formed in chloroplasts if leaves were floated on sugar solutions in darkness. This led to the view that the first product of photosynthesis could be a simple sugar; besides, it can be observed that many plants do not form starch in their leaves. D. Tollenaar in 1925 made a detailed study of conversion of carbohydrates in leaves of *Nicotiana tobacum* L., which like many other solanaceous plants is a notable starch former. He found that feeding leaves with a solution of sucrose of only 0.025% gave the formation of starch at 28°. Czapek in 1911 found that for many plants about 1.0% was required. The time required for the first appearance of starch formed in darkness is measured in days, while in the light the time may be measured in minutes. The difference in time scales could be due to the slow diffusion of the carbohydrate through the cut portion of the leaf to the chloroplasts. Yet the concentration of sugar solution needed to enable the leaf of a particular plant to form starch in the dark is of the same order as that estimated for starch formation in the light. For example, leaves of the wheat *Triticum sativum* Lam. were found by Tollinaar to require feeding with a 10% solution of sucrose, and when growing in the field the leaves contain starch only under conditions allowing a rapid rate of photosynthesis. The snowdrop (*Galanthus nivalis* L.) does not form starch in the leaves and could not be induced to do so with any concentration of sucrose. The regulatory mechanisms which determine the appearance and disappearance of starch in a chloroplast would still seem to offer some challenging problems.

The study of plant physiology as a part of botany seemed to have been started by Sachs and also by Bussingault, who had a practical interest in agriculture. The influence of Sachs on the teaching of plant physiology continued long after the beginning of the present century. His *Handbuch der Experimental Physiologie,* published in Leipzig in 1865, is still regarded as a classic. The simple representation of photosynthesis as the formation of carbohydrate from CO_2 and water with

elimination of oxygen stimulated theories by many of the organic chemists in the nineteenth century. In particular, J. von Liebig (1803–1873) proposed the gradual reduction of CO_2 through a series of organic acids finally to give carbohydrate. The formaldehyde hypothesis of A. von Baeyer (1835–1917), published in 1870, involved the production of formaldehyde from CO_2 as an intermediate stage in the formation of carbohydrate. This derived strong support from the discovery by A. M. Butlerow (1828–1886) that formaldehyde was condensed to a hexose sugar by means of aqueous alkalies. While Baeyer's formaldehyde hypothesis exercised a strong influence on the study of photosynthesis for over 50 years, no direct support could be found for it from experiments with living plants, except for the one fact that in a continuous run the photosynthetic quotient CO_2/O_2 is unity or very near to it.

Although little was added in chemical terms to the formulation of the production of carbohydrates from CO_2, there were many important developments in relation to biological studies throughout the last century. In 1894 T. W. Engelmann used motile bacteria under the microscope and showed that oxygen is produced by illuminating a part of the chloroplast in a living cell of *Spirogyra.* By the same method, G. Haberlandt in 1888 observed oxygen produced from an isolated chloroplast of a moss (*Funaria hygrometrica* Hedw.). This was obtained by cutting the leaf under sucrose solution so that a chloroplast could leave the cell without either osmotic or mechanical damage. In 1896 A. J. Ewart extended this method with several plants, notably with *Selaginella helvetica* (L.) Link, but *Elodea canadensis* Michx. and other phanerogams that were tried did not yield active chloroplasts. These experiments with chloroplasts probably represent the first isolation of an intracellular organelle showing some recognizable activity both inside and outside the cell. It was only later that the mitochondrion was recognized to be the structure concerned in active uptake of oxygen in intracellular respiration. We can appreciate this early work in two ways: either by putting ourselves in the position of the knowledge at the time by a great effort, or by enjoying the numerous detailed descriptions in the old literature from the advantage of present knowledge. In 1888 F. Noll described the "cavern moss" (*Schistostega osmundacea* Mohr., *S. pennata* Hook.) and showed how the lens-shaped cells of the protonema can focus the image of the limited area of light from outside onto the chloroplasts contained in them. The diffused light in the habitat of this moss may be too feeble to support the growth of any other plant. Noll's drawing of the cells of the protonema is reproduced (p. 15) in the book on photosynthesis by Walter Stiles. This book, published in 1925, was one of the first books in English devoted to this subject. It was followed in 1926 by H. A. Spoehr's monograph, published under the auspices of the American Chemical Society. The two books together give a fine critical review of the early experimental work.

From the beginning of the twentieth century the available scientific methods for the study of photosynthesis began to show continuous and rapid improvement. Experiments requiring a strong source of illumination were no longer dependent on sunlight. The methods of analysis of gases had been greatly improved, together with the methods of controlling temperature. These technical improvements were used successfully by F. F. Blackman, who carried out (in my opinion) the first *real* kinetic

analysis concerning rates of photosynthesis. This was to determine the effect of varying three external factors—light intensity, carbon dioxide concentration, and temperature—one at a time, keeping the other two at constant level. He assumed that the rate was completely determined by the slowest or limiting factor (meaning a limiting *process* depending on the factor). The rate showed a linear relation at first, as the magnitude of the particular factor was increased, and then tended to become constant when one or other of the factors held at constant level became limiting. This simple conception of a limiting factor gave a plot of the rate as a uniform slope with a sharp bend to the horizontal—a situation rarely approached in practice. Usually the curve is convex to the coordinate, which refers to the variable factor, showing a uniform slope at first and then gradually reaching the horizontal region which has been referred to as "the ceiling." However, this simplified hypothesis did not obscure the importance of the experimental results. They showed that at a constant low light intensity a moderate increase in temperature did not affect the rate, while at a high light intensity the rate increased with temperature. Blackman concluded that the process of photosynthesis consisted of two parts: the light reaction, a process limited by low light intensity with the Q_{10} of unity, and the dark reaction, with a higher Q_{10} between 2 and 3 at high light intensity. However, if the temperature was raised above 40°C, the Q_{10} diminished and at about 45° was reduced to zero. His analysis showed that the dark process had the properties of enzyme reactions as it had a high Q_{10} and was destroyed above a certain range of temperature. The work of Blackman provided a basis for developments in the study of photosynthesis which have continued to the present time.

It should be emphasized that in those days the chlorophylls were the only chemical substances known to be specific for the process of photosynthesis. This made the chlorophyll molecule the key for the source of energy derived from light. In 1913 Willstätter and Stoll published their researches on the chemistry of hemin and chlorophyll from the Kaiser Wilhelm Institut für Chemie in Berlin. Willstätter had succeeded Baeyer at the Institute. A photograph of Willstätter in his laboratory has been published showing von Baeyer's wash bottle on the bench, the traditional type bottle, exactly like those we used in the 1920s. Baeyer was the famous figurehead in the rapid development in the nineteenth century of synthetic organic chemistry, culminating in his synthesis of the natural blue dye, indigo. It is said that Baeyer's success stimulated Willstätter to study the chlorophyll structure as a green pigment.

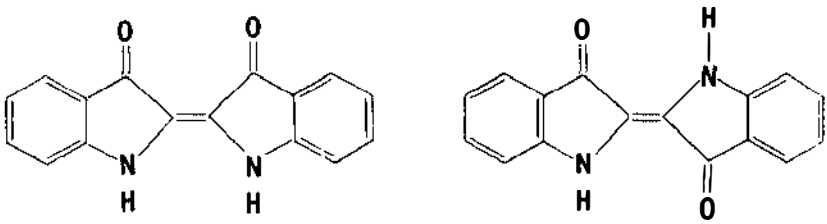

Figure 1 *(left)* Baeyer formula for indigo. *(right)* Formula for indigo established by X-ray crystal structure in 1928.

Willstätter and Stoll developed Blackman's work and also worked with leaves of land plants. The apparatus used for the measurement of photosynthesis at that time now looks formidable. The air with added CO_2 was passed over the leaf and analyzed before and after going through the leaf chamber. This meant that conditions could be maintained constant for the concentration of CO_2, but the analysis had to be very accurate as differences were small. Leaves of land plants were chosen with the object of getting variations in the internal factors, because this would entail variations in the content of chlorophyll and the enzymic factor as conceived by Blackman. Willstätter was notable for his representation of experimental results in terms of a number. His Purpurogallinzahl or PZ, which refers to the specific activity of a peroxidase, is widely known. The "Assimilationszahl" was defined as the ratio: hourly assimilated CO_2(g)/chlorophyll(g), while its reciprocal multiplied by 176 gave the value of the "Assimilationszeit": the time in seconds for one molecule of CO_2 to be converted by one molecule of chlorophyll in the leaf. These numbers refer to measurements of photosynthesis in approximately saturating light, with the activity expressed in terms of chlorophyll content. The values of the Assimilationszahl was found to vary greatly with the type of leaf. Some varieties of plants with yellow-green leaves gave very high values which were less affected by change in temperature than those found with leaves of the normally green variety. This showed that there could not be a constant ratio between the amount of chlorophyll and the activity of the enzymic dark process.

This division of photosynthesis into just two processes seems strange to us now, but we have to carry this idea through several more stages in following the development of the subject. Willstätter and Stoll found that yellow-green leaves which were chlorotic as a result of growth under iron deficiency gave a low photosynthetic number; this suggested that iron nutrition was important, not only for the formation of chlorophyll but also for activity of the enzymic process. The work on photosynthesis was partly carried out in Munich, and in 1917 Willstätter and Stoll collected their papers into a volume entitled: *Untersuchungen über die Assimilation der Kohlensaure,* which appeared in 1918. This book has always been a pleasure to take in hand with its fine printing, wide margins, and fascinating discussions of theory and experiment.

In 1908 Joseph Barcroft described his differential manometer which he used for the estimation of respiratory gas exchanges. This was partly derived from the constant volume apparatus for blood gas analysis originally developed by J. S. Haldane in 1902. His discovery that ferrocyanide liberated the whole of the oxygen combined with hemoglobin gave a new method for study of its physiological function. I think that the first description of the differential manometer came from botanical studies; it was given in 1902 by H. H. Dixon, who showed how it could be used to measure the respiration and photosynthesis of a seedling and moreover to prove that the plant had no use for carbon monoxide. Was this the first application of manometry to measure the rate of photosynthesis?

However, in 1908 Otto Warburg realized that a manometric method could be used for continuous observation of gas exchange on a suspension of respiring cells such as sea urchin eggs, which he had been studying at Naples. Warburg then developed his constant volume manometer and showed the advantages of using cell

suspensions of a microorganism. The unicellular alga *Chlorella* was introduced by Warburg for the study of a green plant. His technique created a new epoch for experimentation with respiration and photosynthesis. With the leaf the diffusion of gas via the stomata through intercellular spaces represented an important internal factor which was operative in the analysis of Blackman. With the cell suspension the diffusion path is shorter and more easily determined.

In 1919 Warburg, following Blackman's analysis, showed that the "dark enzymic process," which he called the "Blackman reaction," was inhibited by cyanide. The "light reaction" he found to be extremely sensitive to narcotics, more sensitive than the respiration. By the use of intermittent illumination he was able to show a time separation of the light and dark processes. The brilliant development of this method in 1931–1932 by Robert Emerson and William Arnold enabled them to measure the time for completion of a dark process. This gave a most surprising result. The number of chlorophyll molecules concerned in reduction of one molecule of CO_2 was found to be between 1000 and 2000, and the time of this dark process was about 1/100th of a second. The values of the "assimilation time" given by Willstätter and Stoll for normal green leaves were usually between 10 and 30 seconds; these referred to all the chlorophyll, and division by a factor based on the result of Emerson and Arnold would make the values obtained from the leaves and from the *Chlorella* appear in reasonable agreement. But the values of the assimilation time for leaves of a golden variety were between 1.5 and 2 seconds because of the lower content of chlorophyll. So would a smaller number of chlorophyll molecules be concerned in the assimilation of one molecule of CO_2? While Emerson preferred to interpret his results in terms of an essential catalyst present in very small quantity in relation to the chlorophyll content of the algae, the idea of a photosynthetic unit was developed. This came to be thought of as representing a structure containing a definite number of chlorophyll molecules.

The widely adopted assumption that the chlorophyll molecule had a direct chemical relationship with the reduction of CO_2, leading to a subsequent production of oxygen, seems to have been a dominating idea up to the 1930s. No experiments with isolated chlorophyll and CO_2 seemed to give any positive clue to finding the mechanism in a living cell. Willstätter and Stoll in their book gave reference to 30 previous and current theories. But in 1926 a far-reaching change in outlook was on the way. By that time A. J. Kluyver and H. J. L. Donker had developed the conception of many biological processes, including photosynthesis, in terms of hydrogen transport. C. B. van Niel was developing the study of photosynthetic bacteria which he obtained in pure culture. Among the bacteria, "chemosynthetic" forms were known which could reduce CO_2 in the dark by utilization of energy derived from oxidation of components in their environment. So chlorophyll, CO_2, and light together were not fundamental for assimilation of carbon! Yet it was felt that there was nothing in this that would apply to the green plant, and besides, the properties of the chlorophyll molecule provided such an esthetic attraction. So even when van Niel announced his generalized scheme for photosynthesis,

$$CO_2 + 2H_2A \xrightleftharpoons{\text{Light}} (CH_2O) + 2A + H_2O$$

which was based on his accurate quantitative experiments with bacteria, there was no immediate stir in the domain of plant physiology. Thus I published my contribution on the green plant in 1937 and 1939 with no reference to van Niel's work; I think he forgave me. I had found that chloroplasts from higher plants would produce oxygen in light by the reduction of substances other than CO_2. This property of isolated chloroplasts eventually emerged in the literature under the name of "the Hill reaction," indicating perhaps that none of us knew exactly how it worked. Since I have been asked occasionally how this reaction was discovered, what follows now is an attempt to record in a subjective sense some of the influences and experiments leading up to it.

In my early days I had acquired a keen interest in botany from my father and in artists' colors from my maternal grandfather, who taught art during the second half of the nineteenth century. During the first World War there was a serious shortage of dyestuffs because they were produced mainly in Germany. So during my school days the idea of reviving the traditional dyes from plants became a powerful influence. Indigo from the woad plant (*Isatis tinctoria* L.) exercised a special fascination both chemically and botanically. There was a recorded locality on a bank above the river Severn where I got some seed in 1920 and have had it growing ever since. The lower epidermis of a leaf of the woad in its first year is easy to skin off. It used to be fun to put the skinned fragments in dilute $NaHCO_3$ and to demonstrate photosynthesis by counting bubbles from the broken end of a vascular bundle. Then the gas could be collected and shown to rekindle a glowing splint.

In 1922 I was fortunate to become a research student with the active group headed by F. G. Hopkins in the biochemical laboratory in Cambridge. In those days Hopkins did not consider that plants were interesting for biochemical research. On one occasion he indicated that he thought they were rather disgusting because they had no mechanism for excretion; characteristically this was a really stimulating idea! He directed me into the study of the blood pigment, hemoglobin and its derivatives. In this field I had two notable rivals, M. L. Anson and A. E. Mirsky, in the department of physiology under the direction of Joseph Barcroft. They usually won out on most points, but I probably supplied them with their first lot of pure hemin! I think the work with blood pigments in the department of Hopkins, and later in the Molteno Institute with David Keilin, was the most important and exciting time in my scientific life. Hopkins was keen to have an explanation for the easy removal of the iron from hemoglobin (yielding the porphyrin) by dilute acid in absence of oxygen, while in oxyhemoglobin the metal had become so firmly fixed. To begin with, one had to learn to follow changes in absorption spectrum with the Zeiss direct vision spectroscope. All physiological and medical students then were taught to use this instrument for observing the reactions of blood pigments. I was led to examine the combining properties of the porphyrin with different metals. The ferrous iron porphyrin when separate from the protein (globin) would form a characteristic compound with CO both in water and when dissolved in an organic solvent, but all attempts to show the formation of a reversible compound with O_2 were fruitless. In a collaboration with H. F. Holden, we were able to split off the protein from hemoglobin reversibly. It was a great moment when we observed the two absorption

bands of oxyhemoglobin with our resynthesized product, showing that the protein had retained its specific activity in directing the properties of the ferrous iron porphyrin towards molecular oxygen. Hopkins at once asked us what happened if we put the porphyrin back instead of the hematin. This produced a dramatic change in the absorption spectrum of the aqueous solution of the porphyrin. The absorption bands became very narrow and sharp, even more so than if the porphyrin was dissolved in an organic solvent.

The molecule of hemoglobin seems to have been responsible for initiating an amazing number of scientific developments. The study of the function of the hemoglobin in larvae of *Gastrophilus* had led Keilin to discover in cytochrome the key to the understanding of intracellular respiration. When J. D. Bernal (known privately as Sage) first showed us the X-ray diffraction pattern of spots from a crystal of hemoglobin, he excited all of us with wonder and anticipation. The S-shaped O_2 dissociation curve of the blood had led to the famous equation of A. V. Hill and to Gilbert Adair's determining the molecular weight of hemoglobin by his superlative skill in the construction of osmometers and in the interpretation of the measurements of osmotic pressure. The different hemoglobins provided material for molecular weight determinations with the ultracentrifuge when it was being developed by Svedberg in Sweden. Then one might have been hit by a high pressure jet of a dilute hemoglobin solution, as happened once to Hopkins when Hamilton Hartridge and F. J. W. Roughton were developing techniques for the measurement of high velocity reaction rates. R. A. Peters had succeeded in putting the chemical studies with hemoglobin on a sound basis, for in 1912 he had shown in Barcroft's laboratory that when hemoglobin was saturated with oxygen the ratio of O_2 to Fe was unity.

The biochemical department at Cambridge moved into its new building in 1923. When this was formally opened in 1924 we all had to produce some kind of display. The different metalloporphyrins made a fine lot of colors to cheer up the bench, and I had filled a large glass jar with a bright purple solution of hematoporphyrin in acid. One of the eminent guests indicated that it just looked like methylated spirit; my resulting deflation was compensated because the show of pigments led to contact with David Keilin. This soon became intimate, as indeed all Keilin's contacts through the Molteno institute were to become. I helped him in the preparation of cytochrome *c* from yeast. It was a baker's yeast of a Delft strain, very pleasantly aromatic, very pink, and we used to eat bits of it. It was in this yeast (which was lost during the last war) that Keilin observed for the first time the cytochrome spectrum in a microorganism. We had difficulties in preparation of cytochrome *c* from muscle until A. Szent-Gyorgyi showed us how to use trichloracetic acid as a start. For his work on cytochrome Keilin used the Zeiss microspectroscope. This had a right angle prism which could cover half the slit, giving a comparison spectrum so that the instrument could be used as a spectrum colorimeter. Keilin described this in his book, *The History of Cell Respiration and Cytochrome.* He emphasized the advantage of using a small dispersion spectroscope for direct observation of pigments; the absorption bands are less spread out, showing more contrast than they would with greater dispersion. Under Keilin's kind guidance I was able

to use this apparatus to follow up the very important work of M. L. Anson and A. E. Mirsky on the nature of the pigment known as hemochromogen from a quantitative standpoint. The results showed that the ferrous porphyrin could form dissociable compounds with two nitrogen-containing molecules. This result, taken together with the structure Willstätter had proposed for the porphyrin, would have indicated that the iron was 6 covalent in Werner's sense and was surrounded by the nitrogen atoms forming an octohedron. But unfortunately there was a bond between the two methene carbons which joined the four pyrrol rings together. Kuster had proposed a formula in 1912 that would have been perfect for representing the hemochromogen type of structure. Willstätter and Stoll pointed out that the disadvantage of Kuster's formula was that it had the 4 nitrogens and 12 carbons forming a 16-membered ring. Adolph Baeyer's powerful influence from his "strain theory" was still operative. Yet again in the 1920s, after Hans Fischer had actually synthesized porphyrins, the results were used as evidence to support a structure he called the "indigoid," which nevertheless allowed the presence of two 8-membered rings. When Joseph Barcroft was revising his book, *The Respiratory Function of the Blood,* first published in 1913, with a second edition in 1928, he asked me for information about porphyrins. I gave him the "indigoid" structure. I have felt excessively stupid on many occasions, but when the correct formula appeared, actually before the publication of Barcroft's book, this seemed the worst. I had such a great admiration for the way Barcroft had dealt with the oxygen dissociation curve of hemoglobin; if only pages 9–11 could have been replaced! Barcroft was able to get a footnote added on page 9 indicating that Kuster's formula had been accepted.

Then I became anxious to measure oxygen dissociation curves of hemoglobin. I wanted a compact and rapid method which could be applied to quite small quantities of the blood pigment, so a spectroscopic method seemed most suitable. Malcolm Dixon very kindly loaned me the Zeiss microspectroscope he had obtained through the Royal Society. The double wedge trough for the comparison spectrum was discarded in favor of a pair of cups and plungers from an old Dubosq colorimeter. The diluted sample of hemoglobin was put in a Keilin-Thunberg vacuum tube to which a graduated pipette could be attached (see Figure 2). The method was made easy because Nature gives us a readymade standard solution of oxygen, about millimolar in terms of H equivalents. That was the only standard needed for measuring the equilibrium in dilute solutions of hemoglobin. So after removing all the oxygen from the sample in the vacuum tube, known amounts of oxygen could be added with the pipette. After the equilibrium had been established in the vacuum tube, the ratio HbO_2/Hb could be determined. The comparisons between this method and the standard procedure then in use were better than one had dared to hope for. In 1925 I had a most fascinating collaborative effort using the spectroscopic method with H. P. Wolvekamp from The Netherlands, who was working with Barcroft.

It was simple to change over and use a hemoglobin with a known affinity to determine the amount of oxygen in solution over the dissociation range of that particular hemoglobin. So it would seem interesting to put some chloroplasts in with some myoglobin, but nothing happened in the light when I had hoped to see some

oxyhemoglobin appear. The only working hypothesis was a light and a dark reaction. So I decided to add the dark reaction, based on the current studies with muscle, in the form of an aqueous extract of acetone leaves, very strong and soupy. It was a very thrilling moment when I saw the spectrum of oxymyoglobin. Then later on a sad disappointment: the presence or absence of CO_2 made no difference. This was really lucky, however, because if CO_2 had "worked," I might well have got no further. It was shown that the oxygen produced in light corresponded with the reduction of a hydrogen or electron acceptor. One of the reactions catalyzed in light was the reduction of ferric potassium oxalate to the ferrous state. It seemed that the oxygen must have come from the water, thus confirming van Niel, provided that this property shown by chloroplasts was part of the process of photosynthesis.

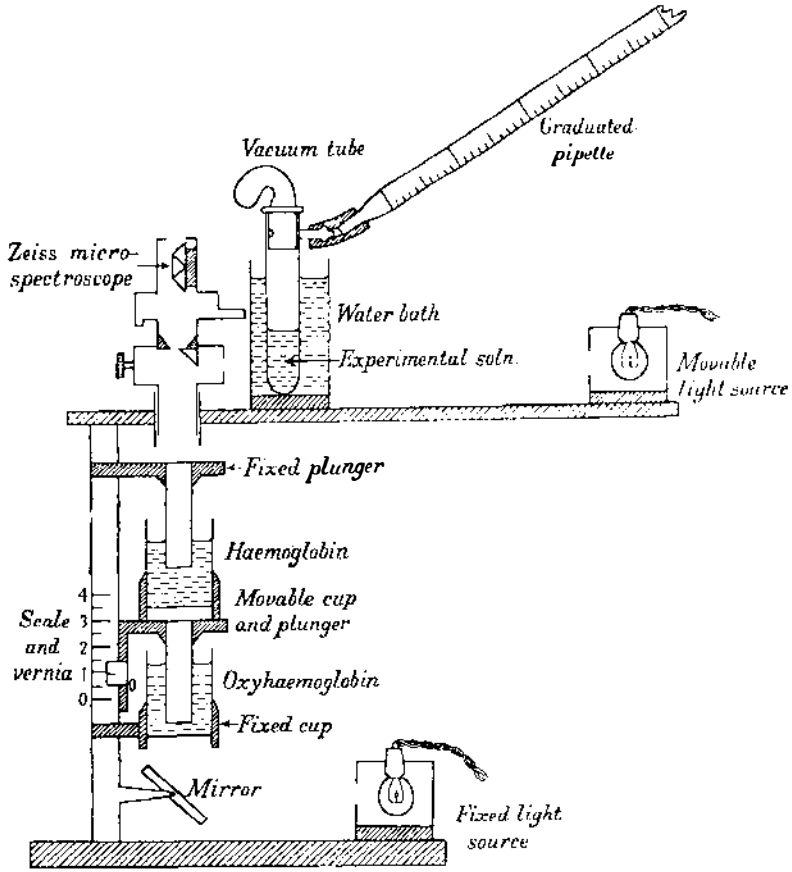

Figure 2 The Zeiss microspectroscope used to measure oxygen dissociation curves of hemoglobin.

The effort at this critical stage was enormously helped by Otto Warburg's work with *Chlorella,* which he published in 1919. Ronald Scarisbrick and I found that the production of oxygen by chloroplasts was very sensitive to urethanes. The concentrations for inhibition agreed with those Warburg had found for whole cells. Also, we found that the production of oxygen was not sensitive to cyanide. This gave evidence for assuming that the chloroplasts plus hydrogen acceptor were doing the light reaction in the sense that F. F. Blackman used. But the light curve with our isolated chloroplasts tended to a dark reaction ceiling and was sensitive to temperature at the higher light intensities. No longer then did we have to be content in photosynthesis with just a light and a dark process.

The chloroplast activity seemed to resemble the cytochrome system working the wrong way round. Keilin, by his use of the two types of inhibitor, cyanide and urethane, was led to interpret the respiratory function of cytochrome components in relation to the transfer of H or of electrons. Scarisbrick and I found that the chloroplasts had a cytochrome component very much like cytochrome *c* which was associated with their structure. Warburg had introduced the idea of structure bound constituents, and inhibition by urethane was used as an indication that a process was structure bound. It has always seemed remarkable to us that while in respiration the reaction with O_2 is sensitive to cyanide, in photosynthesis the process of producing O_2 does not show the same type of sensitivity. Scarisbrick had suggested a model for the photochemical production of oxygen. This was in terms of a heme protein compound like ferrihemoglobin. By the loss of three negative charges it might be brought to the state of oxyhemoglobin; the loss of a further negative charge would act like adding ferricyanide and liberate the oxygen, restoring the ferrihemoglobin. But the insensitivity of the chloroplast activity to cyanide militated against this very suggestive picture. It was an effort to be convinced that we were really on the right lines with photosynthesis. The idea of photosensitization of CO_2 by chlorophyll seemed to be so fixed in people's minds. Through Margory Stephenson we heard about the chemosynthetic organisms and about the varied activities shown by bacteria. This comparative view gave us the necessary confidence. So in 1939 Scarisbrick and I had distant visions of something like a photosynthetic chain. Then came the war and we both started different work. The description of cytochrome *f* was not published until 1950. In the meantime the first volume of *Photosynthesis and Related Processes* by Rabinowitch had appeared, in which he discussed the work by Scarisbrick and myself. Meanwhile, *Photosynthesis in Plants,* edited by James Franck and W. Loomis, had appeared in 1949, with a contribution on the photochemical liberation of oxygen from water by A. S. Holt and C. S. French. By this time the search for the path of CO_2 was well on the way. It was started in 1939 by Ruben, Kamen, and Hassid, with the short-lived tracer carbon ^{11}C, and continued from 1945 when supplies of ^{14}C became available. Accounts of this early work are given in the book by Franck and Loomis.

In conclusion, I must record my appreciation of having been able to witness brilliant scientific developments over the years, seemingly emanating from the colors of blood and grass, and express my gratitude for the friendship of many of the participants in this search for knowledge.