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ON THE FORMATION OF ORGANIC COMPOUNDS FROM INORGANIC BY THE INFLUENCE OF LIGHT

The incentive for my first photochemical research, which was begun in 1911, was received through reading Schimper's work on the relation between nitrate reduction in green leaves and exposure to light. According to Schimper, leaves in the shade are always much richer in nitrates than leaves which have been exposed to direct sunlight. Little or nothing was known at that time in regard to the chemical aspect of nitrate reduction under the influence of light. It was not until later that I became acquainted with the important work of E. Laurent in which the formation of oxygen gas and potassium nitrite by the action of sunlight on sterilized potassium nitrate was described.

My photochemical experiments, carried out in Zürich, between 1911 and 1914, were concerned with the reduction of nitrates by light energy, either alone or in the presence of organic substances. At that time I did not employ iron in my experiments. My attention was first called to the importance of iron in nitrate reduction by some bacteriological experiments which I carried out during the war. In my work with cholera bacilli I found that their iron absorption and assimilation must be in direct relation with their power of reducing nitrates to nitrites and ammonia. On searching the literature for similar observations, I found that Schimper describes an experiment in which iron free (chlorotic) leaves were unable to reduce any nitrate at all, even on exposure to intense light.

It is very striking to observe the great rapidity with which cholera bacilli, taken from the intestine of a cholera victim immediately after death, reduce nitrates to nitrites in a peptone culture. In the case of cholera bacteria the reduction proceeds just as rapidly in the dark as it does in the light. The reduction of nitrates, however, as I have shown, stands here in direct relation to the oxygen respiration of the bacteria and also to their iron content. These observations led me to use iron in my purely chemical investigations on nitrate reduction.

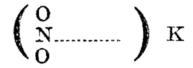
As regards the chemistry of the process, the only relation known at that time was that ferrous hydroxide reduced potassium nitrate and in the process became oxidized to the ferric compound at the expense of the nitrate oxygen. From the physiological standpoint this stoichiometric process could have no importance, since only traces of iron are present in cells and these traces must become inactive at once because of their oxidation to the ferric compound.

I therefore had to search for a process in vitro which would show that the smallest traces of iron were capable of reducing large quantities of nitrate or nitrite and that this reduction failed entirely in the absence of iron.

In other words, I tried to find a chemical reduction process in which the iron acted as a catalyst. I succeeded in doing this when investigations with nitrites and sugar in carbonate solution were carried out. Grape sugar solutions do not reduce nitrates or nitrites, even when they are heated under pressure, but the smallest trace of an iron salt is sufficient to reduce a large amount of nitrite on warming with glucose in weakly alkaline solution. Under these conditions nitrates remain entirely unattacked.

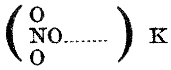
The rôle which traces of iron play in this reduction process was entirely incomprehensible. Further experiments have thrown light on this chemical process, but a detailed description would be out of place here. The main points, however, should be discussed, *i. e.*, why nitrates remain unattacked and why nitrites are reduced extremely easily to ammonia, with the successive formation of the intermediate products KNO_2 and KNO.

The character of the alkali nitrites is determined by the auxiliary valencies of the nitrogen central atom:



$$\left(\begin{matrix}\mathrm{O}\\\mathrm{N}\cdots\cdots\cdots\\\mathrm{O}\end{matrix}\right)\mathrm{K}$$

while that of the alkali nitrates is determined by one oxygen atom, which I call the nitrate oxygen atom.



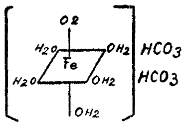
$$\left(\begin{matrix}\mathrm{O}\\\mathrm{NO}\cdots\cdots\\\mathrm{O}\end{matrix}\right)\mathrm{K}$$

This atom possesses residual affinity, but the nitrogen atom is completely masked. Nitrogen has a great affinity for iron and the reduction of alkali nitrites is induced by a primary linkage of the nitrite nitrogen to the iron nucleus. On the other hand, the affinity of iron for nitrate oxygen is not sufficiently great to bring about a primary addition and subsequent splitting off of an oxygen atom.

The question as to the manner in which nitrates were reduced to nitrites in biological processes was still unsolved. From my experiments with cholera bacteria and from Schimper's observation, it could be seen that oxygen appeared to be directly involved in the reduction of nitrates. In following up this idea experimentally I observed the surprising fact that oxygen in the presence of ferrous salts reduces nitrates instantaneously, even in the cold, with the formation of nitrites. This observation was fundamentally new. It was known that ferrous hydroxide or ferrous bicarbonate reduces alkali nitrates, but it was believed up to this time that the iron was oxidized at the expense of the nitrate oxygen. I have found that ferrous bicarbonate or hydroxide in the absence of oxygen even at the temperature of boiling water does not attack alkali nitrates to the slightest extent; the presence of oxygen, however, brings about immediate reduction to nitrite and there is a direct relation between the amount of oxygen dissolved in the water and the amount of nitrite formed.

This chemical process I should like to designate as the origin of fertilization. Two inorganic compounds of great energy content, such as ferrous bicarbonate and potassium nitrate, remain unchanged in an oxygen free aqueous solution. In contact with air, moist ferrous bicarbonate rapidly absorbs oxygen, either from the air or from water, and links a molecule in the inner sphere of the iron nucleus. This oxygen absorption I should like to compare with the fertilization of an ovum, since from the almost non-magnetic ferrous hydroxide or ferrous bicarbonate there is formed a peroxide compound which closely approaches metallic iron in its magnetic properties. This extremely energetic peroxide compound draws a molecule of potassium nitrate into the inner sphere of the iron nucleus and splits an oxygen atom from it with the formation of nitrite. There follows further reduction of the nitrite to the extremely reactive compound potassium nitrosyl, K(NO), which, at the same time, reacts with organic compounds present, especially aldehydes, with the formation of carbon and nitrogen containing compounds. Ferrous bicarbonate and oxygen in this case assume the rôle of light and reduce alkali nitrates to nitrosyl, which is a very reactive amphoteric intermediate product either of nitrate reduction or of ammonia oxidation. Ferrous bicarbonate peroxide, however, can give up the temporarily loosely linked oxygen molecule, which is thus somewhat activated, to oxidizable compounds which are present in the same solution, just as hæmoglobin absorbs oxygen and gives it back again for oxidation or dehydrogenation purposes.

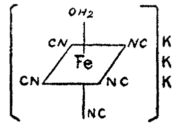
In this reaction, however, it is found that there is a selective action which depends upon the affinity of the compound to be oxidized to the central iron atom of the peroxide.



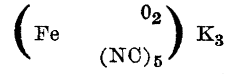
Compounds which are not primarily linked in the inner sphere by the iron atom are not oxidized. There is an analogy here to enzymatic reactions, which are characterized by their selective action brought about by residual affinity. In the reactions which we have described, residual affinity or auxiliary valence powers in the sense of Werner's theory play a decisive rôle. The selective character of this chemical reaction is probably based on them alone. Also, I believe that the reduction of nitrate to nitrite by light is introduced by the taking up of energy and the consequent activation of the auxiliary valence powers of the nitrate oxygen atom and of the oxygen of the water; the subsequent processes are reactions which can take place in the dark. In the case of the reduction of alkali nitrates with ferrous hydroxide peroxide, the energy is transmitted to the nitrate molecule by the strongly magnetic peroxide compound, with the splitting off of an oxygen molecule. The further reduction of alkali nitrite or nitrosyl to ammonia is doubtless brought about by nascent hydrogen formed by the decomposition of water molecules in the inner sphere through the action of the central iron atom in the peroxide compound.

From the preceding discussion it is apparent that many reactions are brought about by the fertilization of the iron molecule by molecular oxygen; there is, I believe, considerable evidence that the magnetic properties of the peroxide are in direct relation to its great chemical reactivity. It is worthy of note that complex ferric compounds are easily transformed into ferrous compounds by the influence of light or by hydrogen peroxide and thus are continually brought back into a reactive form which has the power of taking up oxygen. The well-known example is the reduction of potassium ferricyanide to ferrous compounds.

From neutral potassium ferrocyanide an alkaline compound is formed on exposure to sunlight for a period of a few seconds; on further illumination in the presence of air there results, besides free ferrous ions, an extremely reactive complex ferrous compound (potassium pentacyano-aquo-ferroate)



which links oxygen, nitric oxide or carbon monoxide to the central iron atom, just as hæmoglobin does. In air potassium pentacyano-aquo-ferroate absorbs and carries oxygen. Also in this case the oxidation of the oxidizable substances present takes place only when these substances are first linked coordinately in the inner sphere of the iron salt and, therefore, we are again dealing with a case of selective oxidation. Potassium pentacyano-peroxo-ferroate



$$\left(\mathrm{Fe}\;\begin{matrix} & O_2 \\ & (NC)_5 \end{matrix}\right) K_3$$

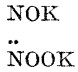
behaves like an iron catalyst and its action as an oxygen carrier and activator and as an activator of inorganic and organic compounds which show affinity to iron I should like to designate as the origin of respiration. The entrance of certain groups, in this case five cyanide groups, into the inner sphere of the iron nucleus, raises the power of the ferrous ion to absorb oxygen from the air. Especially interesting is the fact, observed by Miss Pfaltz and myself, that one reactive auxiliary valency of the iron nucleus can be made inactive by equivalent amounts of potassium cyanide, arsenic trioxide, carbon monoxide, or other blood poisons.

These purely chemical experiments harmonize with experiments of Jacques Loeb and of O. Warburg.

In 1906 Jacques Loeb expressed the view that the essential feature (or possibly one of the essential features) of the process of fertilization was the increase of the rate of oxidations in the egg and that this increase was caused by the membrane formation alone. These conclusions have been confirmed by the measurements of O. Warburg as well as those of Loeb and Wasteneys, both showing that the alteration or cytolysis of the cortical layer of the eggs is in some way connected with the increased rate of oxidations. O. Warburg has further shown that the oxidations in the sea urchin egg are due to a catalytic process in which iron acts as a catalyzer. The active iron ions of egg cells or of algæ can be made inactive by equivalent amounts of hydrocyanic acid, thereby causing respiration and nitrate assimilation to cease.

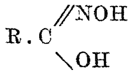
A neutral, unreactive aqueous solution of potassium ferrocyanide, which is quite stable in the dark, is extremely sensitive to light. The most important rays are those of long wave lengths, such as the bluish-yellow and red of daylight. These bring about the formation of an alkaline aquo base, as has been shown by L. W. Bass and myself. The reduction of alkali nitrates, on the other hand, is brought about only by light of short wave lengths, the ultra-violet and violet rays. In sunlight, during the summer, these rays are present in considerable quantities and the reduction of alkalinitrate to nitrite can be brought about in a short time if the oxygen which is split off is taken care of chemically, a process which may be brought about by addition of complex ferrous salts such as potassium ferrocyanide. The photochemical reaction $KNO_3 \rightarrow KNO_2 + O$ is thus accelerated by very small quantities of complex ferrous salts, a fact which was not known up to this time.

Let us now turn our attention to a compound which has not yet been isolated, but which is formed as a labile intermediate product in the reduction of nitrates with light energy or with ferrous hydroxide and oxygen. This compound is potassium nitrosyl K(NO), which was first described by A. Angeli in the so-called Angeli Salt



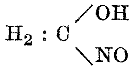
$$\begin{array}{c} NOK \\ \ddot{} \\ NOOK \end{array}$$

Potassium nitrosyl reacts extremely readily with aldehydes with the formation of hydroxamic acids



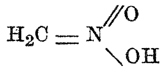
$$R.C\begin{array}{l} /\!\!/NOH \\ \backslash OH \end{array}$$

In this reaction it forms an intermediate product, as I found with Coert, which is an addition product of potassium nitrosyl to the aldehyde group, entirely analogous in composition to the product formed when hydrocyanic acid adds to an aldehyde with the formation of a cyanhydrin. This labile addition product is nitroso-methyl alcohol, if formaldehyde and nitrosyl react together



$$H_2 : C\begin{array}{l} /OH \\ \backslash NO \end{array}$$

The stable product of the reaction, however, is formhydroxamic acid, which by itself is quite stable in the dark, but in light partially rearranges into aci-nitro methane.



$$H_2C = N\begin{array}{l} /\!\!/O \\ \backslash OH \end{array}$$

By longer exposure to light various products result, among them methylamine. By color reactions it can be shown that the first product of the reduction of formhydroxamic acid by light energy is formaldoxime, which on further illumination suffers a partial Beckman rearrangement with the formation of formamide. Another part decomposes into hydrocyanic acid and water. Formaldoxime, which I found as one of the volatile constituents of cholera peptone culture, is doubtless a substance of physiological importance. With trivalent iron it forms very characteristic deep-red complex salts, which in light are transformed rapidly into colorless salts with bivalent iron.

Anhydrous formaldoxime polymerizes readily to a trimolecular form. In the dark in aqueous solution it is comparatively stable. In weakly alkaline solution or on exposure to light, however, there results a rearrangement of the atoms with the formation of an extremely labile intermediate product which links chemically to other formaldehyde molecules; compounds are thus formed which contain several carbon and nitrogen atoms linked together. In sunlight from formaldoxime and formaldehyde are formed cyclic nitrogen compounds which contain pyridine and pyrrol rings and which give the typical alkaloid reactions. Acetaldoxime behaves in an entirely analogous manner. In the spring of 1914 I exposed to sunlight on the roof of the Schatzalpsanatorium in Davos, in sealed glass flasks, a large quantity of acetaldoxime solution containing aqueous formaldehyde; the mixture formed a clear mobile liquid. One year later the flask contained, in place of the mobile liquid, a thick dark brown syrup which I did not have the opportunity to investigate further.

Nitromethane also behaves in a manner entirely similar to formaldoxime, since in weakly alkaline solution or under the influence of light it possesses the property of linking formaldehyde molecules chemically. In the case of nitromethane an intermediate product is known, namely, isonitrobutylglycerine, which Henry obtained from nitromethane and formaldehyde on the addition of traces of an alkali salt. I observed that the same compound results when nitromethane and formaldehyde in aqueous solution are exposed to the light from a mercury lamp. The formation of iso-nitrobutylglycerine proves again that compounds of the type of aci-nitromethane, formhydroxamic acid or aliphatic aldoximes react with formaldehyde readily to form compounds with a larger number of carbon atoms, a fact which is of particular importance in the formation of organic compounds from inorganic. The connection between the formation of iso-nitrobutyl glycerine and dihydroxyacetone oxime with compounds such as alanine, serine, etc., will only be indicated. The formation of compounds of this type from inorganic substances and light has not yet been realized experimentally. The fact should be mentioned that a short exposure of formhydroxamic acid in the presence of traces of iron to mercury light, or a long exposure to mercury light of a mixture of potassium nitrite, carbon monoxide gas and traces of ferric chloride gives a water solution containing amino nitrogen which forms dyestuffs with triketo-hydrindin hydrate, just as α-amino acids do. The completion of this work was interrupted by the war, and because of other circumstances can not be taken up at the present time. Recently E. C. C. Baly has repeated and extended my investigations and has confirmed all the results with regard to the formation of formhydroxamic acid, methylamine, alkaloids and α-amino acids.

By illumination of alkali nitrates with daylight in the presence of formaldehyde gases are formed which in their qualitative composition agree with the gases formed by soil bacteria. They are chiefly nitrous oxide, hydrogen, nitrogen, oxygen, carbon dioxide, carbon monoxide, traces of nitric oxide and hydrocyanic acid. The formation of formhydroxamic acid and of formaldoxime by the reduction of nitrates with oxygen in the presence of iron salts [$Fe(HCO_3)_2$] has been carried out by the writer experimentally. There is direct connection between the formation of these compounds and the formation of the aforementioned gases. The fact that soil bacteria (cholera bacilli also belong to this class) form the same gases which result from the reduction of nitrates in the presence of formaldehyde in sunlight, and the indication that formaldoxime is a product of bacterial activity and the photochemical reduction of nitrates with formaldehyde or with oxygen and iron lead to the conclusion that the chemical reduction processes in both cases are the same or are very similar. The bacteria, which do not need light, most probably use the energy of the iron peroxide in order to make nitrates available for building up proteins. It would be of the greatest interest to investigate whether nitrous oxide or nitrogen, with the help of complex iron salts and oxygen, can be linked in the inner sphere and thereby activated. Such experiments would lead to the still unsolved problems of the assimilation of free nitrogen by bacteria. I consider it to be possible that many soil bacteria and also green leaves utilize nitrous oxide for nitrogen nutrition and, just as they absorb carbon dioxide, they absorb this gas and assimilate it from the air, where, as I found, it is formed continuously by the action of sunlight on nitrogen and oxygen. Nitrous oxide resembles carbon dioxide in its physical properties; according to Langmuir they are isosteric and isoelectric molecules. Experiments in the dark and in light with green plants and with soil bacteria exposed to an atmosphere of nitrous oxide or nitrogen, in combination with purely chemical experiments on the reaction of nitrous oxide with iron salts, should make it possible to obtain a better knowledge of the problem of nitrogen assimilation.

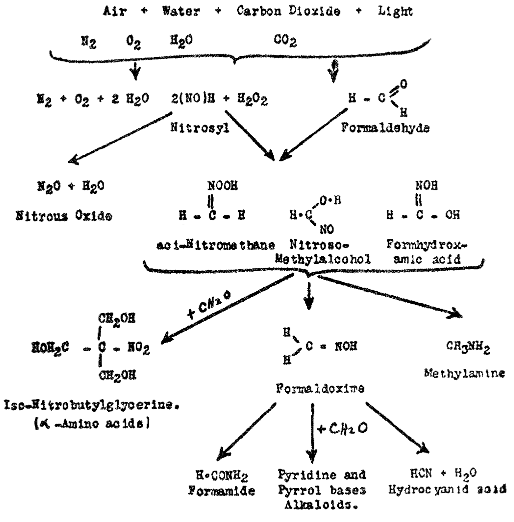
I shall now say just a few words on the reduction of carbon dioxide by light.

I believe that we are still far from the reduction, *in vitro,* of carbon dioxide to formaldehyde in large quantities by sunlight. The reduction of bicarbonates with mercury light gives only very small quantities of formaldehyde or sugar and it is questionable whether this process can be placed in parallel with the photochemical reduction of carbon dioxide in green plants.

It is quite different if the origin of the formation of organic compounds from inorganic is considered. Through exposure to intense ultra violet rays of very short wave length $(\alpha = 200\mu\mu)$, such as one finds, for example, even at the altitude of the Monte Rosa peak, or by the influence of silent electric discharges, it has been shown that carbon dioxide is reduced to formaldehyde, and it is possible that this substance reacts with nitrous oxide formed from atmospheric nitrogen and oxygen to yield formhydroxamic acid. The synthesis of formhydroxamic acid from nitrous oxide and formaldehyde by exposure to sunlight has been carried out by the writer at the Mosso Institute on the Capanna Margheritta (4,600 m). There is as yet no basis for assuming that the formation of formaldehyde from carbon dioxide of the air and sunlight can be carried out at sea level without the aid of chlorophyll.

The formation of organic nitrogen containing substances from inorganic with the aid of light energy is, as you see, solved experimentally, and the methods which have been used lead to the assumption that organic substances found in nature may possibly be formed from air and light in the same or a similar manner. However, I am far from expressing the opinion that, in bacteria or in green plants, the synthesis of simple carbon and nitrogen containing compounds which I have outlined above is the correct one. E. C. C. Baly, who has repeated and extended my experiments, goes very far in his conclusions. For example, he writes the following sentence: "There is no doubt that formhydroxamic acid marks the first step in the phyto synthesis of the nitrogen compounds found in the plant." He proposes a table in which he begins with carbon dioxide and potassium nitrate and ends with proteins.

I believe that the further investigation of the synthesis of organic from inorganic compounds by light will lead to many interesting results, but the exact experimentation, with the isolation and analysis of the intermediate products, will require very great care on the part of the investigator. In sunlight at sea level most light reactions proceed extremely slowly and the secret of how green plants build up their protein from inorganic substances with the help of daylight will probably be unsolved for a long time.



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