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Oxidation, energy transfer, and vitamins

Nobel Lecture, December 11, 1937

A living cell requires energy not only for all its functions, but also for the maintenance of its structure. Without energy life would be extinguished instantaneously, and the cellular fabric would collapse. The source of this energy is the sun's radiation. Energy from the sun's rays is trapped by green plants, and converted into a bound form, invested in a chemical reaction. It can easily be observed that, when sunlight falls on green-plants, they liberate oxygen from carbon dioxide, and store up carbon, bound to the elements of water, as carbohydrate:

$$\text{Energy} + n\,CO_2\,(H_2O) = n\,O_2 + C_nH_{2n}O_n \qquad (1)$$

The radiant energy is now locked up in this carbohydrate molecule. This molecule is our food and the plant's foodstuff. When energy is required, the above reaction takes place in the reverse direction, i.e. the carbohydrate is again combined with oxygen to form carbon dioxide, oxidized, and energy released thereby:

$$n\,O_2 + C_nH_{2n}O_n = n\,CO_2\,(H_2O) + \text{Energy} \qquad (2)$$

According to our earlier views, carbon and carbon dioxide played the central role in this process. Supposedly, radiant energy was used to break down *carbon* dioxide. On oxidation carbohydrate was again combined with oxygen to form *carbon* dioxide.

Investigations during the last few decades have brought hydrogen instead of carbon, and instead of CO_2 water, the mother of all life, into the foreground. It is becoming increasingly probable that radiant energy is used primarily to break water down into its elements, while CO_2, serves only to fix the elusive hydrogen thus released:

$$\text{Energy} + 2n\,H_2O = 4n\,H + n\,O_2 \qquad (3)$$

$$4n\,H + n\,CO_2 = C_nH_{2n}O_n + n\,H_2O \qquad (4)$$

While this concept of energy fixation was still being developed, the importance of hydrogen in the reversal of this process, whereby energy is liberated by oxidation, had already been confirmed by H. Wieland's experiments. This could be represented as follows:

$$C_nH_{2n}O_n + n\,H_2O = 4n\,H + n\,CO_2 \quad (5)$$

$$4n\,H + n\,O_2 = 2n\,H_2O + \text{Energy} \quad (6)$$

This way of representing it is meant to bring out the fact that our body really only knowns one fuel, hydrogen. The foodstuff, carbohydrate, is essentially a packet of hydrogen, a hydrogen supplier, a hydrogen donor, and the main event during its combustion is the splitting off of hydrogen. So the combustion of hydrogen is the real energy-supplyingreaction; To the elucidation of reaction (6), which seems so simple, I have devoted all my energy for the last fifteen years.

When I first ventured into this territory, the foundations had already been laid by the two pioneers H. Wieland and O. Warburg, and Wieland's teaching had been applied by Th. Thunberg to the realm of animal physiology. Wieland and Thunberg showed, with regard to foodstuffs, how the first step in oxidation is the "activation" of hydrogen, whereby the bonds linking it to the food molecule are loosened, and hydrogen prepared for splitting off. But at the same time oxygen is also, as Warburg showed, activated for the reaction by an enzyme. The hydrogen-activating enzymes are called dehydrases or dehydrogenases. Warburg called his oxygen-activating catalyst, "respiratory enzyme".

These concepts of Wieland and Warburg were apparently contradictory, and my first task was to show that the two processes are complementary to one another, and that in muscle cells *activated oxygen* oxidizes *activated hydrogen.*

This picture was enriched by the English worker D. Keilin. He showed that activated oxygen does not oxidize activated hydrogen directly, but that a dye, cytochrome, is interposed between them. In keeping with this function, the "respiratory enzyme" is now also called "cytochrome oxidase".

About ten years ago, when I tried to construct this system of respiration artificially and added together the respiratory enzyme with cytochrome and some foodstuff together with its dehydrogenase, I could justifiably expect that this system would use up oxygen and oxidize the food. But the system remained inactive. So there had to be other links missing, and I set off in

search of them. To start with, I found that the dehydrogenation of certain donors is linked to the presence of a co-enzyme. Analysis of this co-enzyme showed it to be a nucleotide, identical with v. Euler's co-zymase, which H. v. Euler and R. Nilsson had already shown to accelerate the process of dehydration.

As a result of Warburg's investigations, this co-dehydrogenase has recently come very much into the foreground. Warburg showed that it contains a pyridine base, and that it accepts hydrogen directly from food when the latter is dehydrogenated. It is therefore, the primary H-acceptor.

While working on the isolation of the co-enzyme with Banga, I found a remarkable dye, which showed clearly by its reversible oxidation that it, too, played a part in the respiration.

We called this new dye *cytoflav.* Later Warburg showed that this substance exercised its function in combination with a protein. He called this protein complex of the dye, "yellow enzyme". R. Kuhn, to whom we owe the structural analysis of the dye, called the dye *lactoflavin* and, with Györgyi and Wagner-Jauregg, showed it to be identical with vitamin B,.

But the respiratory system stayed inactive even after the addition of both these new components, codehydrogenase and yellow enzyme.

With the help of my loyal collaborators, especially Annau, Banga, Gözsy, Laki, and Straub, I succeeded over the last few years in showing that the C_4-dicarboxylic acids together with their activators, were involved as links in this chain of oxidation, and that with their addition the system was now complete, showing an oxygen uptake corresponding to normal respiration.

My time is too short to permit me to go into the details of the demonstration and the countless measurements which led to this conclusion. I will only describe the end result of this work in a few words. This is as follows: the C_4-dicarboxylic acids and their activators which Thunberg discovered are interposed between cytochrome and the activation of hydrogen as intermediate hydrogen-carriers. In the case of carbohydrate, hydrogen from the food is first taken up by oxaloacetic acid, which is absorbed onto the protoplasmic protein, the so-called malic dehydrogenase, and thereby activated. By taking up two hydrogen atoms, oxaloacetic acid is changed into malic acid. This malic acid now passes on the H-atoms, and thus reverts to oxaloacetic acid, which can again take up new H-atoms.

The H-atoms released by malic acid are taken up by fumaric acid, which is similarly activated by the plasma protein, the so-called succinic dehydrogenase. The uptake of two H-atoms converts the fumarate to succinate, to

succinic acid. The two H-atoms of succinic acid are then oxidized away by the cytochrome. Finally the cytochrome is oxidized by the respiratory enzyme, and the respiratory enzyme by oxygen.

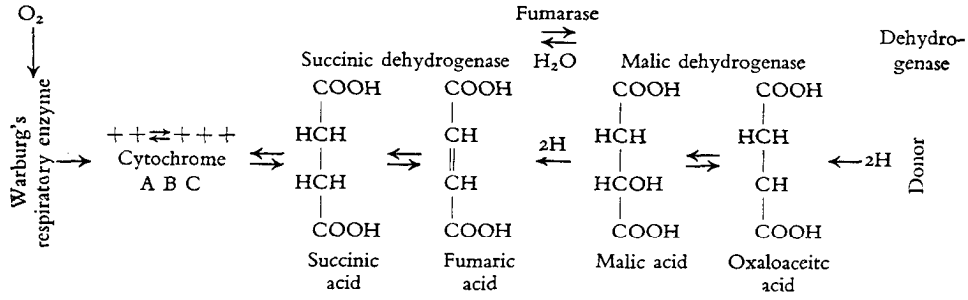

Fig. 1

The function of the C_4-dicarboxylic acids is not to be pictured as consisting of a certain amount of C_4-dicarboxylic acid in the cell which is alternately oxidized and reduced. Fig. 2 corresponds more to the real situation. The protoplasmic surface, which is represented by the semi-circle, has single molecules of oxaloacetate and fumarate attached to it as prosthetic groups. These fured, activated dicarboxylic molecules then temporarily bind the hydrogen from the food.

The co-dehydrogenases and the yellow enzymes also take part in this system. I have attempted in Fig. 2 to add them in at the right place.

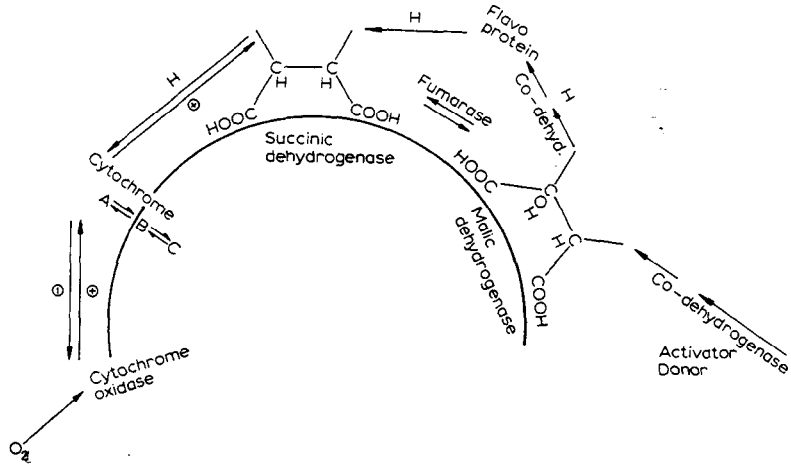

Fig. 2

This diagram, which will probably still undergo many more modifications, states that the "foodstuff" - H-donor - starts by passing its hydrogen, which has been activated by dehydrase, to the co-dehydrogenase. The co-enzyme passes it to the oxaloacetic acid*. The malic acid then passes it on again to a co-enzyme, which passes the hydrogen to the yellow enzyme. The yellow enzyme passes the hydrogen to the fumarate. The succinate so produced is then oxidized by cytochrome, the cytochrome by respiratory enzyme, the respiratory enzyme by oxygen.

So the reaction $2H + O \rightarrow H_2O$, which seems such a simple one, breaks down into a long series of separate reactions. With each new step, with each transfer between substances, the hydrogen loses some of its energy, finally combining with oxygen in its lowest-energy compound. So each hydrogen atom is gradually oxidized in a long series of reactions, and its energy released in stages.

This oxidation of hydrogen in stages seems to be one of the basic principles of biological oxidation. The reason for it is probably mainly that the cell would not be able to harness and transfer to other processes the large amount of energy which would be released by direct oxidation. The cell needs small change if it is to be able to pay for its functions without losing too much in the process. So it oxidizes the H-atom by stages, converting the large banknote into small change.

I myself was led into the territory of oxidation some 15 years ago by a false supposition. I was interested in the function of the adrenal cortex. When this organ ceases to function, life itself ceases (Addison's disease). But before life ceases, a brown pigmentation makes its appearance in the individual, as happens with certain fruits: apples, pears, bananas, etc., which, as they decay, also turn brown. As a result of investigations by Palladin, the great Russian botanist, it was known that this brown discolouration was related to the damaged oxidation mechanism. I myself was (and still am) convinced, that, with regard to basic functions, as oxidation may be regarded representative of them, there are in principle no fundamental differences between animals and plants. So I set out to study the oxidation system in the potato, which, if damaged, causes the plant to turn brown. I did this in the hope of discovering, through these studies, the key to the understanding of adrenal function.

It was already known that the plants which turn brown when damaged -

* One cannot exclude the possibility that the yellow enzyme may also mediate the transfer of hydrogen between carbohydrate and oxaloacetic acid.

about half of all plants - contain a polyphenol, generally a pyrocatechol derivative, together with an enzyme, polyphenoloxidase, which oxidizes polyphenol with the help of oxygen. The current interpretation of the mode of action of this oxidase was a confused one. I succeeded in showing that the situation was simply this, that the oxidase oxidizes the polyphenol to quinone with oxygen. In the intact plant the quinone is reduced back again with hydrogen made available from the foodstuff. Phenol therefore acts as a hydrogen-carrier between oxygen and the H-donor, and we are here again faced with a probably still imperfectly understood system for the stepwise combustion of hydrogen. In the damaged plant, reduction of quinone cannot keep pace with the mounting oxidation of the phenol, and quinones remain unreduced and form pigments.

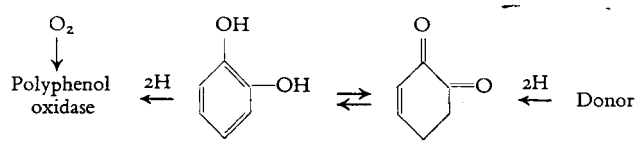

Fig. 3

However, this system gave me no information about adrenal function. So I turned to the plants which do not turn brown when they die, and therefore had to contain an oxidation system with a different structure. All that was known of these plants was that they contained a very active peroxidase. This peroxidase is able to activate peroxide. In the presence of this enzyme, peroxide can oxidize various aromatic substances to coloured pigments. This reaction does not occur without peroxidase. For example, if benzidine is added to a peroxide in the presence of peroxidase, a deep-blue colour appears immediately, which is caused by the oxidation of the benzidine. This reaction, which also serves to indicate the enzyme's presence, does not occur without peroxidase.

But if, for this reaction, I simply used some juice which had been squeezed from these plants instead of a purified peroxidase, and added benzidine and peroxide, the blue pigment appeared, but only after a small delay of about a second. Analysis of this delay showed that it was due to the presence of a powerful reducing substance, which reduced the oxidized benzidine again, until it had itself been used up.

There was great excitement in my little basement room in Groningen, when I found that the adrenal cortex contained a similar reducing substance in relatively large quantities.

Both my means and my knowledge of chemistry were inadequate for investigating the substance more closely. But thanks to the invitation from F. G. Hopkins and the help of the *Rockefeller Foundation,* I was able ten years ago to transfer my workshop to Cambridge, where for the first time I was able to pay more serious attention to chemistry. Soon I succeeded in isolating the substance in question from adrenals and various plants, and in showing that it corresponded to the formula $C_6H_8O_6$ and was related to the carbohydrates. This last circumstance induced me to apply to Prof. W. N. Haworth, who immediately recognized the chemical interest of the substance and asked me for a larger quantity to permit analysis of its structure. Unfortunately it appeared that the only material suitable for preparation on a large scale was adrenal gland. All my efforts to find a suitable plant raw material remained unsuccessful, and adrenals-were not available in large quantities in England.

Prof. Krogh tried to help me, generously sending me adrenals from Copenhagen by plane. But unfortunately the substance perished in transit. Then the Mayo Foundation and Prof. Kendall came to my help on a large scale, and made it possible for me to work, regardless of expense, on the material from large American slaughter-houses. The result of a year's work-was 25 g of a crystalline substance, which was given the name "hexuronic acid". I shared this amount of the substance with Prof. Haworth. He undertook to investigate the exact structural formula of the substance. I used the other half of my preparation to gain a deeper understanding of the substance's function. The substance could not replace the adrenals, but caused the disappearance of pigmentation in patients with Addison's disease.

Unfortunately it turned out that the amount of substance was inadequate for finding out its chemical constitution. Through lack of means the preparation could not be repeated, and no cheaper material was found from which the acid could have been obtained in larger quantities.

From the beginning I had suspected that the substance was identical with vitamin C. But my unsettled way of life was not suited for vitamin experiments, concerning which I had not had any experience either. In 1930 I gave up this way of life, and settled down in my own country at the University of Szeged. Fate, too, soon sent me a first-rate young American collaborator, J. L. Svirbely, who had experience in vitamin research, but besides this experience brought only the conviction that my hexuronic acid was not identical with vitamin C. In the autumn of 1931 our first experiments were completed, and showed unmistakably that hexuronic acid was power-

fully anti-scorbutic, and that the anti-scorbutic acitvity of plant juices corresponded to their hexuronic acid content. We did not publish our results till the following year after repeating our experiments. At this time Tillmans was already directing attention to the connection between the reducing strength and the vitamin activity of plant juices. At the same time King and Waugh also reported crystals obtained from lemon juice, which were active anti-scorbutically and resembled our hexuronic acid.

Suddenly the long-ignored hexuronic acid moved into the limelight, and there was an urgent need for larger amounts of the substance, so that on the one hand its structural analysis could be continued and on the other its vitamin nature confirmed. However, in the course of our vitamin experiments we had used up the last remnants of our substance, and we had no chance of preparing the substance from adrenals, and, as already mentioned, every other material was unsuitable for large-scale work.

My town, Szeged, is the centre of the Hungarian paprika industry. Since this fruit travels badly, I had not had the chance of trying it earlier. The sight of this healthy fruit inspired me one evening with a last hope, and that same night investigation revealed that this fruit represented an unbelievably rich source of hexuronic acid, which, with Haworth, I re-baptized ascorbic acid. Supported on a large scale by the American *Josiah Macy Jr. Foundation,* it was still possible by making use of the paprika season, which was then drawing to a close, to produce more than half a kilogram, and the following year more than three kilogram of crystalline ascorbic acid. I shared out this substance among all the investigators who wanted to work on it. I also had the privilege of providing my two prize-winning colleagues P. Karrer and W. N. Haworth with abundant material, and making its structural analysis possible for them. I myself produced with Varga the mono-acetone derivative of ascorbic acid, which forms magnificent crystals; from which, after repeated dissolving and recrystallization, ascorbic acid can be separated again with undiminished activity. This was the first proof that ascorbic acid was identical with vitamin C, and that the substance's activity

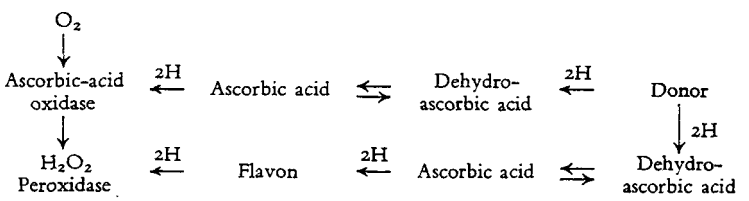

Fig. 4

was not due to an impurity. I do not wish to linger any more over this well-known story, which developed in such a dramatic fashion. Thanks to international collaboration, in the unbelievably short space of two years the mysterious vitamin C had become a cheap, synthetic product.

Returning to the processes of oxidation, I now tried to analyse further the system of respiration in plants, in which ascorbic acid and peroxidase played an important part. I had already found in Rochester that the peroxidase plants contain an enzyme which reversibly oxidizes ascorbic acid with two valencies in the presence of oxygen. Further analysis showed that here again a system of respiration was in question, in which hydrogen was oxidized by stages. I would like, in the interests of brevity, to summarize the end result of these experiments, which I carried out with St. Huszák.

Ascorbic acid oxidase oxidizes the acid with oxygen to reversible dehydro-ascorbic acid, whereby the oxygen unites with the two labile H-atoms from the acid to form hydrogen peroxide. This peroxide reacts with peroxidase and oxidizes a second molecule of ascorbic acid. Both these molecules of dehydro-ascorbic acid again take up hydrogen from the foodstuff, possibly by means of SH-groups.

But peroxidase does not oxidize ascorbic acid directly. I succeeded in showing that another substance is interposed between the two, which belongs to the large group of yellow, water-soluble phenol-benzol-γ-pyran plant dyes (flavone, flavonol, flavanone). Here the peroxidase oxidizes the phenol group to the quinone, which then oxidizes the ascorbic acid directly, taking up both its H-atoms.

At the time that I had just detected the rich vitamin content of the paprika, I was asked by a colleague of mine for pure vitamin C. This colleague himself suffered from a serious haemorrhagic diathesis. Since I still did not have enough of this crystalline substance at my disposal then, I sent him paprikas. My colleague was cured. But later we tried in vain to obtain the same therapeutic effect with pure vitamin C. Guided by my earlier studies into the peroxidase system, I investigated with my friend St. Rusznyák and his collaborators Armentano and Bentsáth the effect of the other link in the chain, the flavones. Certain members of this group of substances, the flavanone hesperidin (Fig. 5) and the formerly unknown eriodictyolglycoside, a mixture of which we had isolated from lemons and named citrin, now had the same therapeutic effect as paprika itself. It is still too early on in our experience for us to make any definitive statements. But it does seem that these substances possess great biological activity. They influence most ob-

viously the capillary blood vessels, whose permeability and resistance suffer gravely in many disease states. These dyes are able to restore the state of affairs to normal, and to judge by the first experiences, it seems that these substances will enrich the doctor's inventory with a really useful new weapon for him to fight illnesses with. Our experiments made it probable that certain members of this group possess vitamin-like properties. For this reason I called the substance vitamin P. Unfortunately these vitamin-like properties have not yet been successfully demonstrated in a completely irreproachable and reproducible fashion.

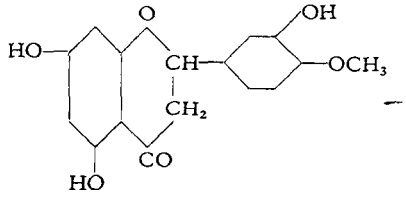

Fig. 5

Ladies and Gentlemen, I have tried to sketch out for you a rapid picture of my work. When I myself look back, I am always only aware of the distressing puniness of my efforts, as compared with the magnitude of Nature and of my problems. One circumstance, however, fills me always with the greatest happiness and gratitude, when I look back on my own struggles. From the moment I seized my staff, a novice in search of knowledge, and left my devastated fatherland to tread the wanderer's path - which has not been without its privations - as an unknown and penniless novice, from that moment to the present one, I always felt myself to belong to a great, international, spiritual family. Always and everywhere I found helping hands, friendship, cooperation and international solidarity. I owe it solely to this spirit of our science that I did not succumb, and that my endeavours are now crowned with the highest human recognition, the award of the Nobel Prize. This Nobel Prize, too, is but a fruit of this spirit, of this pan-human solidarity. I can but hope, my heart filled with gratitude, that this spirit may be preserved and that it may spread its bounteous rays beyond the limits of our knowledge, over the whole of humanity.