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THE ABRAHAM FLEXNER LECTURES
SERIES NUMBER SIX

ON OXIDATION, FERMENTATION, VITAMINS, HEALTH AND DISEASE

by

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THE ABRAHAM FLEXNER LECTURESHIP

THE Abraham Flexner Lectureship was established November 22, 1927, in the School of Medicine of Vanderbilt University through the generosity of Mr. Bernard Flexner of New York City. An adequate endowment was provided, the income from which is used to secure as lecturer at intervals of two years some eminent physician or scientist who has made a definite contribution in the field of medical science or some science allied with medicine.

This Lectureship was created in recognition of the great service which Doctor Abraham Flexner, as Secretary of the General Education Board, rendered to medical education through the reorganization and development of the new School of Medicine of Vanderbilt University. Mr. Bernard Flexner wished in this way to permanently associate his brother's name with the institution which he had so generously helped to establish and improve.

The significant objective of the Flexner Lectureship was to contribute to the maintenance of those ideals and standards of scholarship in medical education and research which will bring increasing honor to Vanderbilt University and to the memory of Abraham Flexner. The Flexner Lectureship will also be of value in the development of cultural interests in medical education and high ethical standards in medical practice.

It was thought that these purposes could be best

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accomplished by having some eminent physician or scientist remain in residence, biennially, for a period of two months and become associated with the teaching personnel and students of the respective departments. The first series of lectures was given during the session of 1928–29.

The formal lectures will be published in serial volumes by the University authorities and this should afford a means of extending the influence and scientific value of the Lectureship.

JAMES H. KIRKLAND.

FOREWORD

THIS volume contains the five lectures comprising the Sixth Series of Abraham Flexner Lectures delivered by Dr. Albert v. Szent-Györgyi at the School of Medicine of Vanderbilt University during the months of March and April, 1939. They represent a summary of Dr. Szent-Györgyi's experiments in the field of biological oxidation conducted during the past fifteen years.

The present series of lectures is unique in that it has dealt with a field of knowledge of which only a small group of the auditors had ever heard and of these only a few individuals could claim familiarity. To present the subject of biological oxidation in an intelligent, not to say interesting, manner to an audience whose attention is assured by the threat of a subsequent examination is a difficult task, as any professor of biochemistry will admit. Yet in these lectures we listened to a discussion of the subject with no avoidance of technical terms, with no "talking down" to the listeners, but given in such a clear and fascinating manner that no signs of flagging interest were ever apparent. The same individuals returned time after time and by their enthusiastic praise induced others to come with them. The constantly increasing attendance was a splendid demonstration of his listeners' appreciation of the magnificent performance of the speaker.

Nor had we, whose attendance was prompted by

professional interest, any feeling of disappointment. The unique ability which enabled Professor Szent-Györgyi to make his work attractive to and understood by a non-chemical audience enabled him to give it an equal interest and value to his professional confreres. To us it was not only a beautiful exhibition of the art of exposition, but it opened a clearer vision of the subject of biological oxidation.

C. S. Robinson.

INTRODUCTION

Chancellor Carmichael, Dean Leathers, Members of the Faculty, Ladies and Gentlemen:

I have covered many a mile and crossed many a frontier to reach you following the generous invitation of this University. I have come to speak to you in honor of a great man, Abraham Flexner.

As a research man I am seeking truth and you invited me to tell about the truth I think to have found or at least was looking for. I will try to merit your confidence by speaking freely about my problems; I will not try to give you an impersonal review that could better be written and read at home at the fire-side.

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Lecture I

PRINCIPLES OF BIOLOGICAL OXIDATION

WHATEVER a cell does has to be paid for—and the currency of living systems, with which the cell has to pay, is energy. If there is no free energy there is no life. The sole and ultimate source of this energy is the radiation of the sun. This, however, cannot be utilized as such to maintain life, for life would fail at night if this were the case. Therefore, the radiant energy is packed into small parcels by the chloroplasts of the chlorophyll-containing plants. If the cell needs energy, it does not use radiation but unpacks these parcels of energy, called "foodstuff molecules." The two fundamental reactions of life are: (1) The making of these packages, and (2) their unpacking.

$$\text{Energy} + nCO_2 + nH_2O = nO_2 + C_nH_{2n}O_n \quad (1)$$

$$CnH_{2n}O_n + nO_2 = nH_2O + nCO_2 + \text{Energy} \quad (2)$$

Reaction (2) is the opposite of reaction (1). The first of the two reactions is performed only by chlorophyll-containing plant cells, while reaction (2) is performed by all cells, whether of the plant itself, or of the animal which eats the plant (herbivora) or of the animal which eats the animal which ate the plant (carnivora).

Even the mere maintenance of the cellular structure costs energy and if there is no free energy available life stops immediately and the magnificent structure

of life, the cell, collapses. This is the reason why there is "one way traffic only" at the gates of death and we can't come back if we have been dead for some time.

I will talk to you about the second reaction. If you look at the left side of the second equation, you observe that it represents a system in which C, H and O atoms are linked in a certain fashion. In our cells this system is rearranged in a way that C atoms and H atoms are now bound to O in the form of CO_2 and H_2O, as expressed in the right side of the equation. This rearrangement is called oxidation or combustion. In this rearrangement the total energy of the system is liberated.

Wherever there is a chance to liberate energy life appears and pushing itself, so to speak, between the two sides of the equation, makes the reaction take place and uses the energy for its purpose.

At an elevated temperature of a few hundred degrees, this rearrangement, the oxidation of the foodstuff, will take place spontaneously, liberating all the energy in the form of heat. But the cell, unlike the steam engine, is unable to use heat for its functions and the energy turned into heat is practically lost as a source of work. The cell needs thus a special chemical apparatus to effect this rearrangement in such a way that the energy is not liberated in the form of heat but in some other form in which it can leave the scene of oxidation, reach the points of the cells needing energy, and support their cellular functions. How this is done is my problem, the "problem of biological oxidation."

Since all cellular functions need energy, biological oxidation is connected with all chapters of biology. It stands right in the center of life. The problem is as yet solved only to a small extent and so I will take you neither into an isolated field, nor into the domain of petrified knowledge, but into a central problem of biology which is still in rapid transformation and represents one of the most active, most fruitful and fascinating fields of research.

As I just mentioned, if the food oxidizes spontaneously outside the body at an elevated temperature its energy will be liberated in the form of heat. The same is the case with any other spontaneous chemical reaction. It will liberate its energy in the form of heat. It is natural that the same process, if taking place within the cell, will likewise liberate its energy in the form of heat and it will not matter to the reaction whether the cell is present or not. This leads us to the first principle of biological oxidation: The cell cannot use for its maintenance any spontaneous reaction that will run by itself. The cell can use as sources of its energy only reactions that do not run by themselves, only those that are *made* to take place by the cell within the mechanism of the special chemical apparatus that makes the reaction take place in such a way that its energy is not liberated as heat but in some other more useful form. Accordingly we find that our foodstuffs do not oxidize spontaneously outside the cell at body temperature. Definite evidence of this was given a few years ago by Lord Carnarvon and Tut-enkhamon. You remember the exciting story of the grave of the Egyptian emperor.

At its opening the breakfast of the emperor was found unburned though it had been exposed to the action of oxygen during several thousand years at a temperature that was not very different from 37°C. Had the king risen and consumed his breakfast, as he had anticipated doing, the food would have been oxidized in no time, that is to say the cells of the emperor would have made reactions take place that would not run spontaneously.

To sum up: The foodstuff and O_2 represent a system which can be rearranged by the cell into another system with a lower energy level, composed of CO_2 and H_2O, the difference in energy being liberated and used by the cell for its maintenance. We find in Nature that wherever there is such a system with two possible energy-levels life appears, pushes itself between the two levels, makes the energy drop and thrives by means of the energy liberated.

Outside the cell the foodstuff does not oxidize, for both the sugar-molecule as well as the molecule of O_2 are very stable, inert, lazy structures. To make the reaction take place, at least one of the two, the foodstuff or the O_2 molecule, has to be activated. Chemistry knows several active forms of oxygen but knows no active bread-and-butter and so it was natural that science looked first for an activation of the oxygen molecule. There have been presented several very ingenious theories of oxygen activation, but it was reserved to the great German biochemist, O. Warburg, to lay the foundation of our knowledge in this field. Warburg was led into this by his study of the action of cyanide. You all know about this favorite poison

of lovers, one of the most rapid chemical means of transport from this world to the other. If you inject it into your blood, the very first thing you notice is that you are dead. What cyanide actually does is to stop biological oxidation and thus doing it cuts out the source of energy, which means immediate death. Even small quantities of this substance will do the trick. But cyanide is, speaking chemically, a very tame substance and does not give any violent reaction. It has only one very peculiar property; namely that it combines most easily with heavier metal atoms into complex molecules. So Warburg was led to conclude that also in the cell cyanide combines with certain metal atoms which are present in small quantities and which are responsible for the "activation of oxygen." By their combination with cyanide these metals are inactivated, and in this way, no active oxygen being available, biological oxidation stops.

I am sorry that my time is too short to allow me to enter into details of this splendid research work, which started with the study of the catalytic function of charcoal and ended with the discovery of the "respiratory enzyme." Warburg found that the catalytic metal atoms were not present in the free condition but linked to protein and had the properties of an enzyme, which he called in a somewhat dictatorial way the "enzyme of respiration," thinking at that time that by its discovery the whole problem of biological oxidation was resolved.

Another supporting pillar of our knowledge of biological oxidation was erected by H. Wieland who discovered the activation of the foodstuff molecule.

Wieland started his work by the study of palladium catalysis. If we treat an unsaturated substance with H_2 nothing happens. But if we introduce colloidal palladium into the system, H atoms will be added to the unsaturated substance; evidently the palladium combined with the H_2 and "activated" it. As an example, if we treat fumaric acid with H_2 in the presence of palladium, two H atoms will be added and we obtain succinic acid:

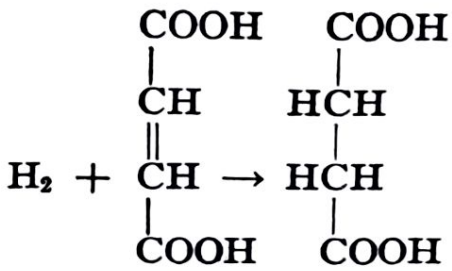


FIG. 1

Such reactions are reversible. Palladium not only can add H to an organic substance, it also can take it off, if, instead of adding H_2, we introduce a substance that can bind H. When an organic substance loses H we say it is oxidized: For the chemist, losing H is just as much an oxidation as is the taking up of oxygen.

That biological oxidations might be such "dehydrogenating oxidations" was shown by Wieland first for the oxidation of ethyl alcohol to acetic acid by the acetic acid bacteria. You know well that if you are careless enough not to empty entirely your bottle of wine and leave the bottle open, that is to say admit oxygen, the wine will turn acid. This is due to the action of acetic acid bacteria which oxidize ethyl alcohol into acetic acid. In a well-closed and full bottle, containing little or no oxygen, naturally there will be no such oxidation.

But Wieland showed that this oxidation might take place even in complete absence of oxygen, provided we add, instead of the O_2, some substance capable of binding hydrogen, e.g., quinone. This shows that in essence this oxidation is but a "dehydrogenation" and that, to use Th. Thunberg's nomenclature, the O_2 acts, analogous to the quinone, simply as an "H acceptor."

Naturally you will raise one objection: if you put the formulas of ethyl alcohol, C_2H_5OH, and acetic acid, $C_2H_4O_2$, side by side, you will find that acetic acid contains not only less H but contains also more oxygen and you will ask, how can a molecule acquire oxygen in the absence of oxygen by mere dehydrogenation. But the solution is quite a simple one. If we split off H and replace it by water, H—OH, the molecule becomes richer in oxygen. So the whole transformation of alcohol into acid can be explained by splitting off H and adding water:

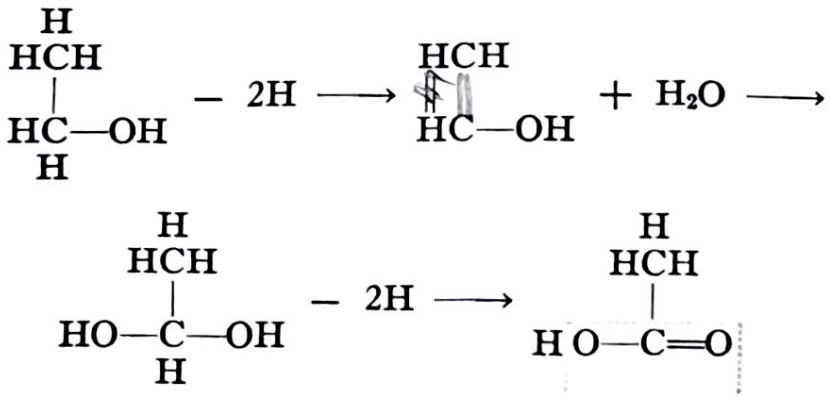


FIG. 2

The natural objection to this is that it is all right for acetic acid but how should this apply to our own metabolism. You know that practically all the carbon of our food is found after its oxidation in the

expired air as CO_2, in accordance with our equation (2) on p. 1. This CO_2 seems to be definite evidence that the molecule and its carbon chain has been directly attacked by O_2! But if you look carefully at the formula of acetic acid on the right side of the series, you will discover in it a CO_2, indicated by the broken line. If conditions are favorable the cell can split off from the carboxylic group a CO_2, a process called "decarboxylation." There are strong reasons for believing that practically all our expired CO_2 is formed by such a decarboxylation, which is effected, as you see, more or less independently of all oxidation, simply following, as an independent process, dehydrogenation. On a later occasion I will show you, how our main foodstuff, sugar, is dehydrogenated and how this dehydrogenation is followed by decarboxylation. I will even show you that one of the most mysterious diseases, beriberi, is in essence but a failure of this decarboxylating mechanism.

But let us come back, after all these formulas, to the question of energy, the liberation of which is the main function of oxidation. How does all this work from the point of view of energy? At which reaction is the energy liberated? Dehydrogenation, the splitting off of H from an organic molecule does not liberate energy, but, on the contrary, by itself, uses up a small amount of it. The addition of water is practically thermoneutral, it neither yields, nor absorbs significant amounts of energy. The same is true for decarboxylation. It is the coupling of H to O, the oxidation, i.e. the combustion of H that yields the energy. In fact in this reaction, the oxidation of H,

the yield of energy, is one of the richest known to the chemist.

Wieland enunciated the theory that most biological oxidations proceed by dehydrogenation and his theory has since been corroborated by the great bulk of modern research.

This theory completely revolutionized most of our conceptions of biological oxidation. It tells us that life knows but one fuel, H, which is the great fuel of life. However your luncheon may taste, in essence it is but H, and its molecules but small parcels of H. The carbon skeleton of the molecule serves to fix these volatile H atoms, it serves as a hat rack on which the H atoms, like hats, are hung up in a row. What the cell does is to take off these H atoms, burn them to water, that is to say link them to oxygen, using the liberated energy for its maintenance.

So Wieland's research has brought hydrogen into the center of biological oxidation and with this into the center of life. It is the apparently simple reaction $2H + \frac{1}{2}O_2 = H_2O$ which is the source of our vital energy. It is to the elucidation of this apparently simple reaction that all of my work has been devoted during the last fifteen years and all my results, however heterogeneous they may seem, have grown out of the study of this reaction.

Thus in the cell not only the oxygen but at the same time the foodstuff molecule is activated for oxidation. This activation is the function of thermolabile catalysts, enzymes, called "dehydrogenases," thoroughly studied by Th. Thunberg, which adsorb the foodstuff molecule and change its structure in such a

way that it will give off H atoms, provided there is present a suitable H acceptor. This process we call "H activation."

When I started working in biochemistry some fifteen years ago a most violent discussion was going on between Warburg and Wieland. Warburg maintained that without active oxygen there was no oxidation, oxygen activation being thus the only essential process. Wieland replied, on the other side, that for biological oxidation no oxygen at all was needed provided we add to our system some suitable H acceptor. My first modest contribution to oxidation was to show, simultaneously with A. Fleisch, that the two theories were not contradictory, both processes were essential and the whole situation could be summed up by saying that "activated oxygen is the H acceptor." And with this we thought to understand biological oxidation!

Since that time research has brought to light an unexpected richness of new data and our knowledge of those days is but the mere outline of our present conception. As I will show you in my second lecture, we know most about the respiration of muscle. In this tissue the activated H is not immediately handed over to the oxygen, but first to another organic substance, an "intermediary H acceptor" or "H carrier." From this substance the H passes to a second, from this to a third, etc., and has to pass thus a series of substances before it approaches its beloved oxygen. Each of these intermediary H acceptors is activated for this function by a specific protein.

But why does Nature effect this simple reaction,

H + O, in such a complicated way? What does this transport of H from substance to substance really mean? This transport of H from substance to substance is, in fact, only its piece-meal oxidation. At every step, at every new reaction a part of the energy of the H is liberated. In this way the oxidation of the H and with it its energy is cut into pieces, the latter being liberated in distinct and small quantities. Apparently, the quantity of energy liberated in the oxidation of an H atom is too big to be dealt with. It represents, so to speak, a big bank-note and the cell wants change for its every-day life; it can't pay with bank-notes for all its petty functions. As I will show you presently, this "change," this currency unit of the cell seems to be about 11 Calories.

We will understand this better if we give a moment's consideration to the oxidation of H in the reaction $2H + O = H_2O$. From a more general point of view this union of H with O is nothing special and H can unite equally well with a great number of other atoms or molecules. The union with O is unique only from the fact that there is no other substance the union of which with H would yield as much energy as is yielded by its union with O. Once the H unites with O to form H_2O all its energy is released and there is no method by which we can release energy from H_2O. That is why we say that the H is burned, oxidized, once it has united with O. If, for instance, we burn H_2 we obtain 68 Calories, the maximum amount we can derive from H_2. But let us imagine that we unite H_2 not with O but with another substance X, that yields on this union instead of 68 only 34 Calories.

In this case we could say that the H is "half-burned," and transfering it from X to O we could extract another 34 Calories. Let us say we couple our H_2 first with a substance A and derive from this reaction 17 Calories, then transfering it from A to X we can obtain another 17 Calories, and if transfering it from X to B, we release 17 Calories, then there will be still another 17 Calories left that we can release by transfering the H from B to O. In this way, choosing well our "intermediary H acceptors," we can release the energy bit by bit, burning our H piece-meal. This is what is actually happening in the muscle cell.

So the H of the foodstuff and the O are separated by a chain of "H carriers" which must be passed before the H can approach the O.

At the end of the reaction we find both H and O united in the form of H_2O, as evidence that after the H has passed this chain of carriers it does reach oxygen. But this is only apparent and I hope not to shock you unduly by telling you that the fact is that this H never reaches its beloved O_2 at all.

I have mentioned "O_2-activation" before and told you that this was the work of metallic atoms and that O_2 reacts in the cell primarily with them. O_2 in fact, does not oxidize H directly, but a metal atom which is oxidized by O into its higher valency-form. The H also reacts directly with this metal, reducing it again to its lower valency. The metal acts thus as catalyst in this reaction by its valency change.

This catalytic activity of metals, catalysing the oxidation of labile H, is a process well known to the chemist. Most substances, containing a reactive H,

oxidize in the air more or less rapidly, the H being oxidized by the O_2 to water. The chemist calls this process autoxidation. Analysis has shown that the greatest part of these dehydrogenating autoxidations is catalysed by metals—mostly Cu or Fe—present in traces as impurities, and if we eliminate the last traces of metals by special methods autoxidation stops. Only very active H, a very negative and electro-active H, seems to oxidize without metal at a high rate in the dark and at low temperatures.

Thus life, when utilizing metals in the oxidation mechanism of the cell, did not invent a new principle. Life never invents new principles, but only applies principles of inanimate Nature in a very clever way. By fixing the metal to a specific protein the cell not only prevents its loss but also gives to this metal the possibility of working at its best.

I have said that the metal exerts its catalytic function by its valency change, by being oxidized and reduced alternately. But now what is the difference between an oxidized and reduced metal, let's say bivalent copper, Cu^{II}, and monovalent copper, Cu^{I}. The difference, as you know, is that the latter contains one more electron. Thus it follows that H, when reducing Cu^{II} does nothing more than to give an electron to it, and O_2, in like manner, when it oxidizes the metal simply takes this electron over. But what happens now to the H and O_2. If the H gives up an electron, a negative charge, it becomes itself positively charged, becomes what we call an H ion. H ions, H^+, are elements of water for water dissociates into H and OH ions. Thus once the H has given up its

electron it becomes water and its rôle in oxidation is finished. On the other side, O_2, when taking up a negative charge, can unite with the H ions of the water. If the metal is Cu it will let the O_2 go, after it has taken up two electrons. In this case the O_2 will unite with two H ions and form H—O—O—H, hydrogen peroxide; but if the metal is Fe, which has a greater affinity for HOOH, it will let the oxygen go only after it has taken up four electrons and united with four H ions to $2H_2O$. We could sum up the function of the metal, Me, thus:

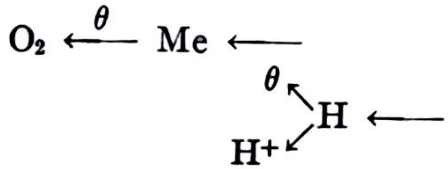


FIG. 3

This function of the metal is not the "whole truth," but truth it is and it will suffice for the moment. The "whole truth" I will tell you later. What interests us at the moment is the rôle of O_2. As you see the whole O_2, which previously has been thought to be the omnipotent master of oxidation, has only a very limited rôle in respiration; it is not even an H- acceptor. Its rôle is that of an "electron acceptor," and once the O_2 has taken up the electron its rôle is finished and it disappears from the scene of oxidation, being lost in the water, the great solvent of the cell. The H of the foodstuff acts, after having lost part of its energy, as "electron donator," while the metal serves as "electron transmitter."

In our cells we do not find a single metal atom, but a series of metal atoms, acting as electron transmit-

ters. As the H itself has been transmitted from substance to substance in a series of H transmitters, releasing its energy piece-meal, so the electron is now transmitted in a series of metals from metal to metal, releasing its energy step by step, being eventually transferred onto O_2.

The chain of the oxidation is thus a linking together of two main parts, viz: a chain of "H carriers" and a chain of "electron carriers." At the one end of the chain stands O_2 as an "electron acceptor," at the other the foodstuff as a "H donator," the H, after its transport, serving in its turn as the "electron donator" for the chain of metals.

In this way we have arrived at a formal description of the mechanism of oxidation but have not yet touched the most important problem: How the energy of these reactions is transmitted for other functions of the cell which need energy—muscular contraction for example. It is evident that all this is done by the cell to release energy in definite quantities, to bind this released energy in such a form that it can be transferred from the seat of oxidation to those points of the cell that need energy.

It was not long ago that we obtained the first information about this fundamental problem. Of central interest in these researches are those amazing substances, the codehydrogenases, which, in the hands of Warburg, have shed a new light on the mechanism of enzymatic activity.

It has been known for some time that the function of certain dehydrogenases depends on the presence of thermostable substances, called coferments. I

showed this some fifteen years ago for the lactic-acid-dehydrogenase. Banga and I have also shown that this coenzyme belongs to the group of nucleotides, substances chemically related to nucleic acid. V. Euler and Nilsson have shown that v. Euler's cozymase, likewise a nucleotide, is also able to act as a coenzyme. Researches took a dramatic turn when Warburg showed that this substance contained a pyridine ring and its function was to take over, by the hydrogenation of one of its double bonds, two atoms of H from the H-donator, and to act, in this way, as an intermediary H-acceptor or H-transmitter in the chain of H transport. This coenzyme is in itself quite inactive and it is activated by the same enzyme as is the donator. The dehydrogenase represents thus a protein which adsorbs and activates the H-donator and the coenzyme in such a way that two H atoms will pass over from the former to the latter. The dihydro-coenzyme can now transfer the H to the next H-transmitter, let's say to oxaloacetate. In this case oxaloacetate and coenzyme will be adsorbed by another specific protein and the H will pass from the coenzyme to oxaloacetate. The dehydrogenase, the specific catalytic protein, is thus a substance which adsorbs and activates two substances and makes them react, makes H pass from one to the other. To put it another way we can say that the coenzyme is the "prosthetic" group, that it is the active group of the enzyme, but we must not give too much importance to words, for it just depends on the quantitative relations which of the substances mentioned will be "substrate" and which a prosthetic group.

If we take a relatively great quantity of reduced coenzyme, and an equivalent quantity of flavo-protein and add these to malico-dehydrogenase containing a trace of oxaloacetate, H will be transferred via oxaloacetate to the flavo-protein. In this case oxaloacetate acts as prosthetic group, dihydro-coenzyme as substrate.

We do best just to register facts, the fact that there are in the cell specific proteins which are capable of adsorbing and activating the coenzyme or dihydro-coenzyme and some other substance and of passing H from one to the other.

The analysis of muscular contraction has shown that this process takes its energy from esters of phosphoric acid, like creatine-phosphate. As shown by Meyerhof this ester-linkage contains 11 Calories which are set free on its scission. If we wish to support muscular contraction by oxidation we must, somehow, transfer the energy of dehydrogenation to the formation of phosphoric acid esters. It is becoming increasingly probable that it is the coenzyme which is instrumental in this energy transfer. This coenzyme is probably so constructed that, like adenylic acid, it can bind phosphoric acid by an ester-linkage. What actually happens is thus, in all probability, somewhat as follows: At the moment that the H is transferred from the donator to cozymase it loses some of its energy. The systems H_2 donator + coenzyme + phosphate is transformed on the protein into the other system viz: oxidized donator + dihydro-coenzyme-phosphate. This energy is used at once to establish a new ester-linkage and to bind a molecule

of phosphoric acid to cozymase. In other words, the energy lost by the H atoms is invested at once in an ester-linkage. Now phosphoric acid can be transferred from one substance to the other without loss of energy, thus it is transferred from adenyl-pyrophosphate to creatine (Parnas, Meyerhof, Needham).

Probably the same happens with the phosphate of the coenzyme and thus we arrive at creatine-phosphate, which represents in essence a small parcel of energy which can be transferred from the seats of oxidation, reach contractile elements and be unpacked to support contraction by its splitting.

Judging from redox-potentials, about one-third of the total energy of the oxidation of the H is liberated during the H transfer. The greater part of this energy could be conserved and transferred by means of the coenzyme into the form of the phosphoric acid-ester-linkage. Two-thirds of the total energy of oxidation is liberated in the electron transfer and it is difficult to believe that this energy is allowed to turn into heat and thus be wasted. We have, however, not the least notion of the mechanism of this part of the energy-transfer; it is at the moment one of the most important problems of biology that awaits solution.

But whatever the mechanism of the energy-transfer of the electron transport may be, the importance of phosphoric acid esters, in the problem of biological energetics, admits of little doubt. The discovery of this energetic significance of phosphate is one of the most important and striking discoveries of modern biological research which, I expect, will, in the future,

open still unforeseen vistas, and will bring us closer to the understanding of life.

Ladies and gentlemen, I think I have summed up the great underlying principles of biological oxidation so far as they are known today. You have seen a dramatic development of our ideas. The apparently simple reaction, the oxidation of the foodstuff molecule, has been decomposed into a long series of most involved and fascinating reactions. Accordingly, we know that the mechanism of biological oxidation represents a very involved mechanism, and also a mechanism of high precision. In order to react, two molecules have to be brought into close proximity. The greatest distance at which one molecule can act on the other is 4.5Å, which is not much of a distance. To interact, the active parts of the molecules must approach to within this minute distance from each other, and we have reasons to suppose that the individual catalysts are so built into the semi-solid protoplasm, as to assume certain steric relations which allow the reactions to take place at the desired speed. All this together gives a very complicated apparatus in which distances are measured by Ångströms, which, by its precision, far surpasses the limits of precision of the finest mechanism that human hand has ever made.

You have also seen that the great driving force of life is the affinity between H and O and all our vital energies are derived from the gradual approach of the H to O. Whatever the mechanism of the oxido-reductions may be, in the end we find the O linked to H and the H linked to O in the form of water, and the energy

liberated in this union is the great spring that is driving life.

To finish our discussion we might ask now: What is it that separates H from O again, what is winding up the great spring of life over and over again? I can be brief about it for you all know that it is the sun, that great benevolent power which is the ultimate source of all vital energy. It is the green pigment, the chlorophyll of the plants that captures the rays of the sun and uses their energy to separate H and O again, fixing H to the carbon of CO_2 and sending the oxygen back into the atmosphere again, according to our equation (1) on p. 1. This H, fixed to C, in the form of carbohydrate is actually the food—the immediate source of energy of plant and animal. The cell then takes the H from the C and lets it unite with oxygen to form water again, using the energy of the reaction for its own purpose, thus liberating and using the energy of the sun which has been packed into the molecule by the vegetable cell.

In this way, all life from the energy point of view is but the play of water, the separation of the water into its elements and its reformation from them. But water is more than the spring, the driving force of life. Zoologists tell us that all life originated in the ocean and water is the mother and cradle of life. Our own blood is, from a certain point of view, but a sample of primordial sea water. Chemical analysis shows that we, ourselves, are composed of 80 per cent of water, and are thus but a fairly dilute, 20 per cent aqueous solution. It is as impossible to separate a girl from her smile and have the girl and the smile apart, as it

is impossible to separate life from matter and have them apart. Life is to a certain extent matter itself, and so water is not only the great spring, but to a great extent it is life itself and we can look upon ourselves as a few gallons of sea water with some solid stuff in it that, some million years ago, grew a skin in order to be able to crawl on land.

Lecture II

THE RESPIRATION OF MUSCLE

WHEN I was a student I often wondered why physiologists devoted so much work to muscle which seemed to be their favorite subject, while to me muscle seemed the least interesting of all organs. Now, after having worked for ten years on muscle, I understand this interest better. By virtue of its mechanical function, the most violent energy changes are found in muscle. While the liver and kidney can work through their daily task in ease, muscle is frequently called upon to do a great amount of work within a fraction of a second. Corresponding to these energy changes we find violent chemical changes which lend themselves much better to measurement than do those in the protracted functions of other organs. Moreover, the less interesting an organ from the human point of view, that is to say, the less involved, the more interesting it becomes for the researcher who must simplify conditions as much as possible.

Correlated with the violent chemical and energy changes we find in muscle a well developed oxidation system, and this is why, after having devoted ten years to the study of muscle, I am now devoting my second lecture to this organ to show you by this example how the principles, treated in my first lecture, apply to the cell.

According to my first lecture we can expect to find a very complicated chemical system, a long chain of reactions, at the one end of which stands the foodstuff molecule, the "H donator," giving off two atoms of H which shoot through the chain of H carriers, then give off their electrons and become transformed into H ions. The electrons continue the journey through the series of metals, the "electron transmitters," and are picked up at the other end of the chain by the oxygen. There is no accumulation of intermediary products anywhere and you might wonder, how on earth did biochemistry find out about all the members of this chain and the sequence of reactions. In fact, I am often asked this question. To understand this we will do best to follow the historical development of the problem and start at the oxygen-end.

As I mentioned, oxygen reacts in the cell with a metal atom, linked to protein—a "metal-proteid." I also mentioned that it was the action of cyanide which led Warburg to the discovery of this "respiratory enzyme." If the metal, by entering into a complex-molecule with cyanide, loses its catalytic activity the whole respiration stops, for it does not matter where a chain breaks, it will become useless.

The first component of the respiratory mechanism was thus discovered by means of a specific poison, which left all other links of the chain intact, but brought to light, by its inhibitory action, the individual member on which it acted.

It hardly needs to be said that for such studies we do not need the whole animal; it suffices to take a bit of its tissue, cut it into slices or small pieces to give it

a great enough surface to allow oxygen to diffuse freely into the cells. Now if we suspend this tissue in a suitable liquid, put it into a vessel and shake it, we can follow the oxygen consumption by measuring the pressure changes in the gaseous phase, the air in contact with our tissue suspension. Such an apparatus, consisting of a vessel and a manometer which indicates the pressure changes, we call a "respirometer."

The second link of the chain was discovered a few decades ago by an English physiologist, MacMunn, who observed that muscle contained a colored substance, related to, but not identical with haemoglobin, which he called "Myo-Haematin." But MacMunn was a fantastic sort of a fellow and nobody believed him and people rebaptised his child from "Myo-Haematin" to "My-Own-Haematin," expressing herewith their belief that nobody on earth except MacMunn himself had such a thing in his cells; so the whole story was forgotten.

It was some fifteen years ago that D. Keilin, a small man with a big head and a very good heart, working at Cambridge rediscovered this substance which gives a beautiful and characteristic absorption spectrum. He called it "Cytochrome." The substance can be observed in cells by means of a spectroscope and is found in the various organs of widely different species, like yeast or insects and mammals, and it is thus one of the basic constituents of the cell.

Cytochrome has the remarkable quality that its color and spectrum fade out when it is oxidized and reappear again when it is reduced. In this way, by observing it directly, we can, under the microscope

armed with a spectroscope, follow its alternate oxidation and reduction without damaging the living cell or the tissue at all. If we center our microscope as described in the classical experiments of Keilin on the pectoral muscle of a bee, we will see the cytochrome appear as soon as the bee begins to move its wings. When the movements stop the cyrochrome disappears again. In this way we can convince ourselves that the cytochrome is actually involved in oxidation and reduction without, in any way, damaging the cell.

Cytochrome is not a single substance, it is composed of three different entities which Keilin called Cytochrome A, B, and C, all of which take part in respiration. Haas, working in Warburg's laboratory, showed that in yeast the oxidation and reduction of the C component was proceeding at a rate which showed that the whole respiration was actually accomplished through cytochrome. The three substances, cytochrome A, B and C do not represent alternate ways of oxidation, they function in series, that is to say that in respiration first one of the cytochromes is oxidized, this cytochrome in turn oxidizes another, and this the third. According to E. Ball, whose results are in agreement with the results of my collaborator, K. Laki, cytochrome A is oxidized first, it then oxidizes C and C oxidizes B.

Keilin has given a clear demonstration that it is the respiratory enzyme of Warburg which oxidizes cytochrome. Since the function of this enzyme is to oxidize cytochrome, it can better be called "Cytochrome-Oxidase." The oxygen reacts thus in the tissue with this metal-proteid, "cytochrome-oxidase,"

oxidizes its metal and this metal of cytochrome-oxidase oxidizes cytochrome A. A oxidizes C and C oxidizes B. B in its turn is reduced by the hydrogen coming from the H donator. There is some uncertainty about the problem whether the cytochrome-oxidase is or is not identical with cytochrome A. Until this problem is definitely settled I will retain the original concept and will talk about cytochrome-oxidase and cytochrome A as different substances.

As shown by Keilin the cytochromes are related to haemin. Of the three cytochromes, C can be extracted and prepared in pure condition and has lately been analyzed by H. Theorell. The most essential part of this molecule is an iron atom held in a tetrapyrrol linkage. The oxidation and reduction of the cytochrome consists of the alternate oxidation and reduction of the iron atom thus: $Fe^{II} \rightleftarrows Fe^{III}$. In essence the cytochromes represent thus a series of metal atoms which transmit electrons, donated by the H and accepted eventually, via cytochrome-oxidase, by the O_2.

The cytochromes are not present in the cell in a free state;—they are linked to the protein. As shown by Laki, by this linking to protein the cytochrome changes its properties, is "activated," if we want to put it that way. Cytochrome C changes its redox-potential about 200 millivolts by its linkage with protein. Since the cytochromes are fixed in the semi-solid protoplasmic structure and have no considerable thermal agitation, we must suppose them to be built into the protein in very definite steric relations which allow their Fe atoms to interact. The Fe atoms of the

three different proteins cannot be farther apart than 4.5 Å, a fact which excludes any haphazard distribution.

The three cytochromes are thus a part of a precision-mechanism with very accurate spacial relations. As you have seen we also have a great deal of very accurate information about their function and structure; all the same, if we are to be quite honest, we must confess that we do not understand them. Why are they there, why are there three? We can only guess and I have given some of my guesses in my first lecture. Judging by redox-potentials the energy liberated in this cytochrome-electron-transfer is very considerable, in reality about two-thirds of the total energy of oxidation. The potentials of the three cytochromes are such as to indicate that this energy is liberated in the transfer of the electron from one to the other in three fairly equal portions. How this energy is conserved and transferred we do not know.

The question now arises: What is the substance which gives up its H and thus directly reduces cytochrome? As we have seen, the H itself travels from the donator to cytochrome. Now what is the last substance that takes up this H and allows it to be oxidized, its electron being taken over in the reaction by cytochrome? This was the question which I, with my faithful collaborators, tried to answer a few years ago after we had found that the activated foodstuff, the final H donator itself, did not react immediately with cytochrome.

There were a few remarkable facts to guide us. It was known from the experiments of Thunberg

that muscle (and most other organs) contained a most active dehydrogenase which acted specifically and oxidized succinic acid at an immense rate to fumaric acid (see page 39). Contrary to other dehydrogenases this succino-dehydrogenase needs no co-dehydrogenase. Why on earth should there be such an extraordinary ferment for a substance that is not one of the most important metabolites? Moreover there was "fumarase" discovered by H. Einbeck. This enzyme is one of the most active ferments known which converts 1.malic acid or fumaric acid into an equilibrium mixture of these two substances. The existence of this fumarase also showed that these dicarboxylic acids with 4 C atoms play a quite extraordinary rôle in cell life.

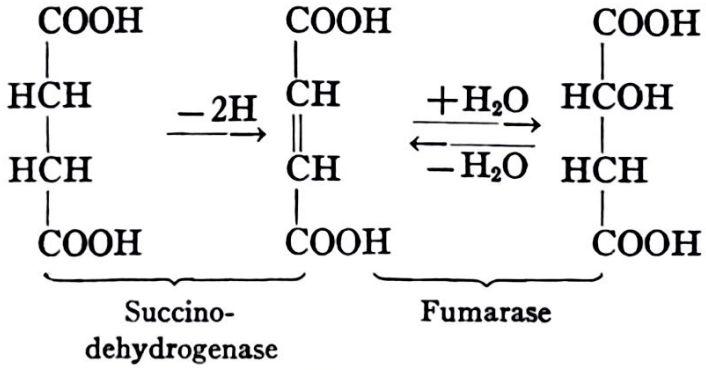


FIG. 4

It might seem strange at first sight that such simple and plain substances should play such important rôles. But looking closer at it we will discover that the C_4 dicarboxylic acids are quite unique substances. Chemists tell us that the H of α and β C atoms, that is to say, the first and second neighbors of a COOH group are quite specially reactive. Now there is only one series of substances that contains two neighboring

secondary C atoms (which can thus form a double bond) which are both at the same time α and β C atoms. A. T. Virtanen found that these acids play also a fundamental rôle in N fixation by plants, one of the most important processes of living nature.

To cut a long story short we arrived at the assumption that the main function of succinate in tissue is not to serve as fuel, but to serve as a catalyst, as a catalytic H-carrier between the foodstuff and cytochrome. The succinate in the tissue would, according to this theory, be activated by the succino-dehydrogenase and then two of its H-atoms would be oxidized by the cytochrome. The dehydrogenated succinate then turns into fumarate which, by its double bond, can take up two new H-atoms coming from the donator.

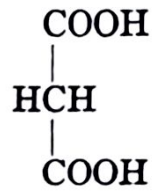
Before more seriously considering such a theory one must first consider quantitative relations. Muscle contains very little succinate, about 10 mg. per cent, and respiration is a very intense process. Should muscle burn its H by way of succinate, then it should be able to oxidize and reduce the small quantity of succinate present at a rate which corresponds to the total respiration. The analysis shows that not only can the succinodehydrogenase oxidize succinate at a rate that exceeds the rate of respiration, but that the kinetics of this oxidation are such (zero type reaction) that they allow the muscle to activate and oxidize this small quantity of succinate present at a maximum rate.

The kinetic relations thus favored our theory so we set out to find more direct evidence for it. To be

accepted, the theory needed strong proof for at that time it was thought that dehydrogenases served only to oxidize foodstuffs. The supposition that a dehydrogenase, together with its substrate, acted as a catalytic H transmitter was a somewhat revolutionary idea. The problem could be approached from different sides.

First we undertook to determine what substances could reduce the bound cytochrome of muscle. We "washed" muscle, that is to say extracted all H donators present. Then we added different possible substances one by one. We found that there was only one that could react immediately with cytochrome and this was succinate. This showed that if succinate and its dehydrogenases were members of the H-transport system their place was next to the cytochrome. The experiment, however, did not prove that succinate actually was a member of the oxidation-chain.

More direct and conclusive evidence for this was found by the method of "specific poisoning." As mentioned, it was this method which led Warburg to the discovery of his enzyme. In our case the specific poison was not cyanide but malonate. This dicarboxylic acid has the same structure as succinate itself, only it has but three C atoms. It is, so to speak, succinate minus one C atom:



Malonic acid

Owing to the similarity of its structure malonate is adsorbed by the succinodehydrogenase, and thus displaced succinate from the enzyme. However, it is not able to take over its function, for it has only one CH_2 group and is thus unable to form a double bond and give off its H-atoms. J. H. Quastel, who discovered this inhibition of the succinodehydrogenase by malonate, also found that this inhibition was quite specific and that no other dehydrogenase was inhibited by malonate.

If succinate and the succinodehydrogenase are involved in respiration we have to expect that malonate would inhibit respiration. We found that malonate, added in 0.001 molar concentration, inhibits respiration to about the same extent as does cyanide. That this inhibition is actually due to the displacement of succinic acid molecules on the enzyme could be demonstrated in a very striking way, simply by the addition of a few milligrams of succinate or fumarate. The succinate, with its concentration elevated, could now compete successfully with the malonate for the enzyme and the respiration thus went on again as if nothing had happened.

This malonate inhibition and its release is a very dramatic and convincing experiment which proves that the succinodehydrogenase is actually involved in the respiratory mechanism. Another very nice piece of evidence for the specificity of the action of malonate is given by the following experiment: If we add methylene blue to the unwashed muscle, this dye will be reduced. The reduction, however,

will be inhibited by malonate. If, however, instead of methylene blue we add gallophenine, malonate not only will not inhibit the reduction, it will enhance it. The explanation is the following: gallophenine is too electro-negative to be reduced by the succinodehydrogenase. The reduced gallophenine, however, can be oxidized by fumarate, but if we add malonate we inhibit this re-oxidizing action.

Finally, there is one other amusing experiment which also proves the catalytic importance of succinate. If we suspend minced muscle in phosphate it shows a vigorous respiration which, however, soon begins to fall. This decrease of respiration is due to the fact that the muscle is losing some of its succinate by diffusion, for if we add a small quantity of succinate all is well again. As shown by F. Stare a few hundredths of a milligram of succinate suffice to stabilize respiration and to make up for the loss by diffusion. It is remarkable that muscle oxidizes large quantities of added succinate irreversibly at a high rate but retains the last traces necessary for the catalysis with great tenacity, conserving this small quantity, probably by adsorbing it on to the enzyme.

As mentioned in my first lecture, other dehydrogenases act on substances in pairs. They do not really split off H from the substrate but adsorb this substrate together with the coenzyme and make the two substances to pass H from one to the other. Succinodehydrogenase needs no coenzyme and we might ask whether this "action in pairs" also holds for succinodehydrogenase and, if so, what is the

partner of succinate. In all probability we may regard cytochrome B as the reaction-partner of succinate on the succinodehydrogenase and regard this pigment as the prosthetic group of the dehydrogenase.

This relation of the succinodehydrogenase to the cytochrome suggests a very interesting problem. In chemistry when we reduce or oxidize a substance the H-atoms are taken off or put on in pairs. So if succinate is oxidized into fumarate it means that it loses two H-atoms. Cytochrome, however, as an oxidizing agent is monovalent, it can oxidize only one H at a time. This makes it probable that dehydrogenases are capable of giving off H-atoms one by one and of working with what the chemist calls "free radicals."

But let us go on now and see whether we can find the next member of the H-transport system. We have seen that there is always a small quantity of succinate in muscle which, in the presence of O_2, is oxidized to fumarate, but there is also "fumarase" which will convert three-fourths of the fumarate into malate. (See p. 37) Malate, in its turn, will be acted on by the "malico-dehydrogenase" which will activate this substance, transmit its two H-atoms of fumarate and through this to cytochrome. The malate, after losing two H-atoms, becomes oxaloacetate. The primary oxidation product is analogous to fumarate, probably oxyfumarate. At the neutral reaction of tissue this substance changes instantaneously into oxaloacetate. The kinetics of this oxida-

tion are in many ways analogous to the kinetics of the succinodehydrogenase. What happens now to oxaloacetate?

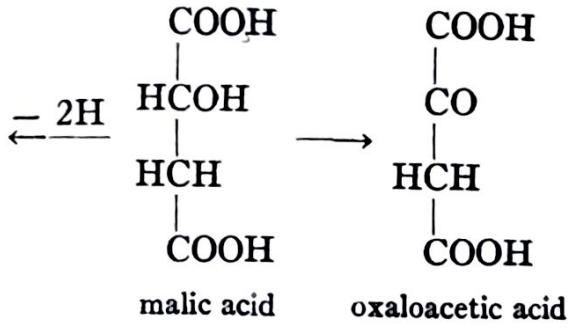


FIG. 5

It is easiest to approach this question by adding some oxaloacetate to muscle and finding out what happens to it. If we do this we will find that the tissue reduces the oxaloacetate at an amazing rate. Within 10 minutes the breast muscle of the pigeon will reduce about 1 per cent of its own wet weight of oxaloacetate. In fact, the oxaloacetate—being activated by the malico-dehydrogenase—will lick up all H coming from the donator and will thus be used by the cell as an intermediary H acceptor.

There is even a very ingenious little mechanism in the cell which prevents any H from being transferred from the donator immediately to fumarate so long as there is any oxaloacetate present. Oxaloacetate very strongly inhibits the succinodehydrogenase; mere traces of it are sufficient to stop the function of the enzyme and to block herewith any H-transport towards cytochrome and O_2. While there is oxaloacetate present no H will be thus transported to O_2 and the only substance capable of taking up H will thus be oxaloacetate itself which will lick up all the avail-

ably hydrogen until it is completely reduced. If we calculate the quantity of oxaloacetate reduced by the tissue in a certain period we will find that it is equivalent to the oxygen that the tissue could take up in the same time.

Malate and oxaloacetate, activated on their dehydrogenase, thus act as intermediary H transmitters. They pass on their H to the succinate-fumarate-succinodehydrogenase system. Now comes the question: What is the next member in the series, from what does oxaloacetate receive its H?

The main H donator of the muscle is triose-phosphate, activated by the triose-phosphate-dehydrogenase, as has been shown by Banga and myself. Now if we put triose-phosphate-dehydrogenase, triose-phosphate, malico-dehydrogenase and oxaloacetate together, the triose will be oxidized, it will lose two H-atoms, and the oxaloacetate will be reduced by accepting these H-atoms. The next neighbor of the malate-oxaloacetate-malico-dehydrogenase system is thus the final donator, viz., triose with its dehydrogenase. Herewith we arrived at the end of the chain. The malico-dehydrogenase and its substrates, malate and oxaloacetate, thus lie in sequence between the final donator and the fumarate. The H given up by the triose is accepted by oxaloacetate which hereby becomes malate, malate passes it on to fumarate which thus becomes succinate, succinate has its H oxidized off by cytochrome B, B is oxidized by C, C by A, A by the cytochrome-oxidase.

This chain appears quite long enough but it we look at it more closely we see that it still stretches

out a bit longer. I just said that the donator, triose-phosphate, was dehydrogenated by a "dehydrogenase." By "dehydrogenase," in this case, I meant the specific protein plus the coenzyme and as we have seen the coenzyme itself is already an H transporter. To be more exact we must thus begin describing the H transport series by saying that the donator, triose-phosphate, is adsorbed together with the coenzyme on the specific protein, the triose-phosphate-dehydrogenase. There the two H-atoms pass from the donator to the coenzyme. Then the dihydro-coenzyme is adsorbed together with oxaloacetate by the "malicodehydrogenase." Now the H passes from the coenzyme to oxaloacetate which, by the acceptance of the two H-atoms, becomes malate. Malate, activated by the malico-dehydrogenase, also gives up its two H-atoms only to the coenzyme adsorbed and activated by the same enzyme, and it is the coenzyme which passes these H atoms to fumarate. Both reductions of the coenzyme, its reduction by the triose and its reduction by malate, are probably linked with a phosphorylation and this gives us the probable reason why Nature shunts the malic-oxalacetic step into the series, viz.: in order to have the phosphorylation repeated.

There is some difficulty in supposing that the same coenzyme would act twice in the same series. How can the coenzyme know when it has to give its H to oxaloacetate and when to fumarate? Probably it is not the coenzyme but the dehydrogenase that decides the question. Possibly the malico-dehydrogenase is built in such a way that the coenzyme adsorbed on

it can react only with fumarte or diaphorase (see later) This naturally is only one of the possibilities and further experiments are needed to answer the question.

According to the conception given, the H of the malate should be transmitted by the coenzyme to the fumarate. At this point of the chain, however, something still seems to be missing. That the coenzyme can transmit H from the triose to oxaloacetate, we can readily understand since both dehydrogenases, the one that activates triose and the other that

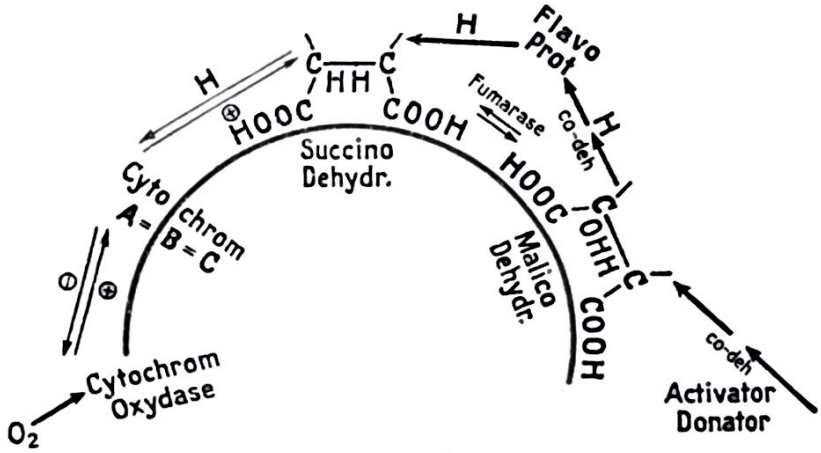


FIG. 6

activates oxaloacetate, are known as enzymes working with coenzyme, adsorbing and activating this latter. The enzyme, however, that activates fumarate, the succino-dehydrogenase, does not activate coenzyme. We need thus a link between the malico-and succino-dehydrogenase, a substance that can transmit the H of the coenzyme to the fumarate which has been activated by the succino-dehydrogenase.

In my little book[1] published two years ago in which

[1] *Studies on Biological Oxidation and Some of Its Catalysts*, J. A. Barth, Leipzig, 1937.

I summed up my own work, I expressed my belief that this link between malate or coenzyme and fumarate was a flavo-protein. I summed up my whole conception in the accompanying figure in which the semicircle represents the protoplasmic surface.

These flavo-proteins are most amazing substances. They consist of a protein and an alloxazine dyestuff, flave or flavine. This dyestuff was first observed by myself and Banga. We were struck by the reversible oxidizability of this substance and postulated that it must be, in some way, connected with H transport, oxidation and reduction. We, however, could not fit it into the oxidation cycle.

We gave the child a name, "flave" or "cytoflave," and left it at that. R. Kuhn, to whom we owe the analysis of this substance, called it "flavine," while Freunberg and Györgyi showed it to be identical with Vitamin B_2.

Our inability to fit this dye into the cycle was explained by Warburg, who showed that it acts in conjunction with a specific protein. This flavoprotein acts as an enzyme, the flavine being the prosthetic group. Free flavine, however, which we had at hand was found to be inactive. These flavines, as has been shown lately by Warburg and his collaborators, are also the prosthetic groups of certain enzymes like the Schardinger enzyme which oxidizes aldehyde to acid, and the d. amino-oxidase which oxidatively deaminates amino acids. Regarding this latter enzyme F. B. Straub, independently, shortly after Warburg, came to identical conclusions. These enzymes activate their substrate and the dye, trans-

fering the H from the former to the latter. Then the reduced dye oxidizes spontaneously in air. This seems to be in contradiction to the statement of my first lecture according to which a spontaneous oxidation cannot be used by the cell as a source of energy. Probably the energy of these reactions is actually wasted by the cell, being allowed to turn into heat. The oxidative deamination as a source of energy is an unimportant reaction. The Schardinger enzyme is found in milk. Since milk does not work, this enzyme probably has some importance quite unrelated to energetics. (See lecture IV.)

To understand further developments we will have to skip for a moment to another problem and go back to Thunberg's experiments. The method that had perhaps the greatest influence on the development of our knowledge of dehydrogenases is the classical one of Thunberg. Thunberg proceeded in the following manner: He cut the muscle to pieces and extracted it with water. Many dehydrogenases are retained in the washed particles while the H donators are washed out. If, however, along with the dye methylene blue we also add an H-donator, a substance for which there is a dehydrogenase present, the H is transferred from the donator to the dye. We can observe this transfer of the activated H directly for the dye loses its color when it accepts H and is thus reduced to a "leuco-dye." It was thought at that time that it was a fundamental property of dehydrogenases to be able to transfer H from the donator on to dyes. The discovery of coenzymes did not change this conception fundamentally; now we simply said

that the H was transferred from the donator to the coenzyme and from the coenzyme to the dye. In fact, we had no other method of finding out whether a dehydrogenase was present at all or not. The detailed knowledge of the coenzyme, however, supplied us recently with a newer method. The coenzyme, when taking up H, shows a characteristic absorption spectrum in the ultraviolet (Warburg). We thus can take the muscle, add coenzyme to it and observe under the spectroscope whether there is H transfer from donator to coenzyme. So we have an entirely new method in hand with which to determine whether or not there is a dehydrogenase present, without using methylene blue, simply by observing the reduction of the coenzyme under the spectroscope.

This new method has brought us some surprises. V. Euler and Adler showed that when treating the muscle with water, the malico-dehydrogenase goes into solution, reduces coenzyme in the presence of malate, but does not reduce methylene blue. Banga and I also had shown that the triose-phosphate-dehydrogenase, when in solution, does not reduce the methylene blue. It thus was not a quality of the dehydrogenase and its coenzyme to reduce methylene blue. If, however, the malico-dehydrogenase is not extracted but is left in the muscle particles, it will reduce methylene blue. The same results were obtained by V. Euler and Adler if they added the extracted muscle to the dehydrogenase solution. It thus became evident that the dehydrogenase with its coenzyme does not reduce methylene blue at all and if it does so in the muscle, this is because there

is some additonal catalyst present which transfers H from the coenzyme to methylene blue. This new factor was called by V. Euler "Diaphorase." At the same time Green and Dewan also found evidence of such a new factor which they called "coenzyme factor."

There is only one dehydrogenase that can directly reduce methylene blue without the intervention of diaphorase and this is the succino-dehydrogenase. Thus if we add methylene blue to the minced muscle and observe its reduction, we know that the H could be transferred on to the dye only by two members of the oxidation chain, either by the diaphorase or by the succino-dehydrogenase.

A few months ago F. B. Straub succeeded in extracting this diaphorase from muscle and preparing it in pure condition. He showed that it is a flavo-protein consisting of an alloxazine-nucleotide combined with a specific protein.

The exact position of this diaphorase in the oxidation chain has not yet been ascertained. The observations, in my opinion, fit best the assumption that its place is between malate and fumarate or coenzyme, and that this diaphorase is identical with the flavo-protein, postulated according to Fig. 6 two years ago. Thus assigning this position, temporarily, to diaphorase, we can try once more to sum up the succession in the stages of transport of the H as it progresses on its way. The H of the donator, activated by its dehydrogenase, is transferred on to the coenzyme. From the coenzyme it is transferred on to oxaloacetate which is activated by the malico-

dehydrogenase. From the malate the H is transferred to coenzyme, from the coenzyme to diaphorase, from diaphorase to fumarate which in turn is activated by the succino-dehydrogenase. Then the two H-atoms, now present in the form of succinate, are oxidized off by cytochrome B which takes over their electron. The H-atoms thus serve as electron-donators and turn into H ions. The electron is transferred from cytochrome B to C, from C to A, from A to the cytochrome-oxidase and from this enzyme to O_2, which taking up the electrons combines with the H ions to form water.

I do not insist here too much on the individual links of this chain. What I want really to show you is that this long chain of reactions represents, in fact, nothing else than piece-meal oxidation of H which is performed by the cell in this complicated way, by means of this very involved mechanism of oxidation which allows the energy of the H to be liberated in small and distinct portions. As I have mentioned before, the cytochromes and their oxidase, the succino-dehydrogenase and the diaphorase are built into the semi-solid protoplasm, and must thus be situated in very distinct steric relations.[2]

[2] I am often asked in discussion: What is the limiting factor of respiration, i.e., which member of the oxidation chain determines the velocity of O_2 uptake? This is, indeed, one of the most interesting and fundamental problems of physiology. We are unable to answer this question yet. The chain is built up in such a way that it can give the most vigorous respiration. This suggests that the limiting factor is the quantity of H donator present. This would mean that simultaneously with a muscular contraction an appropriate quantity of H donator is formed from some reserve-substance like glycogen. The nervous system, when giving an impulse for contrac-

Certain objections have been raised against this conception of respiration from different quarters. I pictured this mechanism of H-oxidation as a strictly prescribed route along which H has to pass once it left the specific donator. One of the greatest experts of intermediary metabolism, F. Knoop, very justly contends that he knows a great number of substances that, if put into the cell, will be oxidized and reduced, give up or take up H; the mechanism thus cannot be very specific. But Knoop's observations and my conception are not contradictory. Naturally, if I put a substance into the cell it might take up H from coenzyme or diaphorase, or give up H to it. There is no reason why it should not do so. This, however, does not say that the cell does not dispose of specific H donators like triose-phosphate, produced at a very high rate from glycogen, the main source of muscular energy, nor that the H of these donators does not cover the greatest part of the energy-need by following the route described in this lecture.

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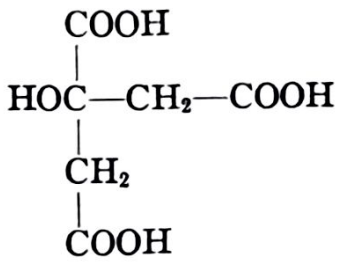
tion, would thus have to give an impulse for the mobilization of an adequate amount of triose-phosphate. This seems to be unlikely. If I would have a chance to work on this problem, and would have to make myself a working hypothesis, I would suppose that one or more of the coenzymes of respiration and fermentation, which are phosphorylated while performing their function, are inactive in the phosphorylated condition. (Adenylic acid, codehydrogenase and thiamine as H transmitters?) When the cell does not work and thus does not split phosphoric acid esters, these catalysts would be present in a phosphorylated, inactive condition. As soon as the cell works and splits its phosphoric acid esters, the catalysts lose phosphate and begin to work, and work until all phosphate acceptors are phosphorylated again. This would mean that it is the need of energy (i.e., need of phosphoric acid esters) that governs and limits the rate of respiration.

Before finishing this lecture I must mention certain experiments of H. A. Krebs and Johnson which, according to these authors, give a different explanation of the function of the four-C-atoms-containing dicarboxylic acids like succinate, fumarate, malate and oxaloacetate. According to Krebs the real function of these acids is not to transfer H but to serve as members of a more complex cycle. To understand all this we have to go back to experiments of Knoop and Martius, who have shown that oxaloacetate can react with pyruvate, in vitro, forming citrate. They have further demonstrated a series of transformations in which citrate can be oxidized to succinate. According to Krebs all these reactions are actually effected in the cell and represent the "mechanism of carbohydrate oxidation." According to him an oxide of triose combines with oxaloacetate with which it forms citrate and becomes in this way, so to speak, a side-chain of oxaloacetate. This side-chain is then oxidized off, the citrate being oxidized to succinate. Succinate is oxidized then through fumarate and malate to oxaloacetate again, and this oxaloacetate can bind new triose. Unfortunately Krebs has failed to give convincing data on the quantitative relation of this cycle to the total oxygen uptake. Breusch and Thomas have failed to convince themselves of the correctness of this theory. As I will show later, it is possible that the greater part of triose is not oxidized completely at all. But even if triose should be burned in muscle to CO_2 and water in this cycle, this function is not *the* function but only an additional function of these C_4 dicarboxylic acids. In order to

combine with oxaloacetate, in forming citrate, triose must first give up 4 H-atoms. It is the function of the C_4 series to transmit these H-atoms to O_2. If citrate is oxidized to succinate it must give up 6 H-atoms. Again it is the catalytic function of these C_4 acids to perform the oxidation of these H-atoms. Banga has shown that the H of the citrate *is* transported by the C_4 acids. Furthermore, Thomas showed recently that the quantity of citrate formed by oxaloacetate is small compared with the quantity of H transported by the C_4 series.



Citric acid

I do not wish, in any way, to be-little the importance of Krebs' experiments. Citric acid has always been a puzzle to biochemists and possibly the cycle of Knoop, Martius and Krebs actually represents an important way of oxidative carbohydrate breakdown. What I want to bring out is only that the experiments of Krebs in no way touch the conception of H transport by C_4 acids and they represent only a possible additional function of these simple and yet so remarkable substances.

Ladies and gentlemen, I have more chance to exhaust you than to exhaust my subject. You are certainly amazed by the richness of data and observations, and by the great complexity of these mecha-

nisms. But if you want really to picture this mechanism to yourself, you must not visualize it in the static way in which I described it. You must try to visualize it in full action. If you can imagine an apparatus producing the donator at a high rate, taking off its H, dealing with its residue, if you can follow this H in your imagination, shooting through the long chain of reactions, giving up it electron in the end, then follow the electron as it continues its journey until it reaches O_2 streaming constantly into the cell, if you can add to this picture the phosphoric esters formed all along the way, and still leave room for other forms of bound energy yet unknown, bound energy reaching those points of the cell which use this energy and perform with it equally complicated functions; if you can imagine all this in full, simultaneous and harmonious action, then you have the first inkling of that most wonderful organization and activity the sum total of which we call by that one simple little name—Life.

Lecture III

ON FERMENTATION AND ITS RELATION TO OXIDATION

THE development of more complicated and pretentious forms of life has become possible through oxidation. We have seen that oxidation is in essence a rearrangement of a system composed of foodstuff and O_2, into another system with a lower energy level, into a system finally composed of CO_2 and H_2O. The existence of these two widely separated energy levels makes the existence of higher forms of life possible. Wherever there are two such possible energy levels with the possibility of liberating energy, life appears, pushes itself between the two levels, makes the system drop to its lower energy level and utilizes the energy liberated for its own purposes. We have also seen that in oxidation the real source of energy is the piece-meal oxidation of hydrogen, the gradual drop of the energy level of the system $2H + \frac{1}{2}O_2$ to the zero level of $H_2O + CO_2$. The source of the H is the foodstuff where it is in a fixed form.

In Nature, however, we frequently find only one-half of the system, the foodstuff alone without oxygen. The foodstuff molecule itself, let us say sugar, represents a system of C, H and O atoms linked in a certain fashion. This form of C, H and O atoms which we call carbohydrate does not represent that form of C, H and O in which this system contains the least

energy. It is possible to rearrange these atoms in such a way that, in their new relationships, they will contain somewhat less energy. The system of atoms called carbohydrate can thus be rearranged even without oxygen into a new system with a somewhat lower energy level. The energy difference, liberated by this change, is not very considerable; in fact it is small compared to the quantity of energy which can be liberated in the presence of oxygen. Nevertheless, energy it is, and so we can expect that wherever there is foodstuff even without oxygen, life will appear, rearrange the atoms of the foodstuff molecules into the new system with a lower energy level, and will thrive by means of the energy thus liberated. This anaerobic, oxygen-free, energy-liberating, rearrangement of foodstuff molecules we call fermentation. There are many forms of fermentation according to the chemical nature of the system into which the foodstuff is rearranged. Let us consider at the outset two classical examples, the alcoholic and the lactic fermentation. In alcoholic fermentation the carbohydrate molecule, $C_3H_6O_3$, is rearranged into a system composed of ethyl-alcohol, C_2H_6O, and CO_2. In a lactic fermentation the sugar is rearranged into lactic acid. The energy difference between sugar on one side, alcohol, CO_2 and lactic acid, on the other side, is not very great, since the greatest part of the energy of the carbohydrate is still contained in the alcohol or the lactic acid. But an energy difference does exist and this will suffice to make life thrive. The energy that can be obtained by the oxidation of a triose molecule is about 340 Calories. By this an-

aerobic rearrangement into lactic acid or alcohol we liberate only about $\frac{1}{30}$ part of the energy. Fermentation, compared with oxidation, is thus a very wasteful process that can cover the energy need only of very simple, modest forms of life such as yeast or bacteria. If we, ourselves, had to live on fermentation we would have to eat about 60 kg. of bread a day which would make life rather difficult.

Accordingly, fermentation is also the much simpler process which probably preceded oxidation in the history of life and it took probably millions of years until Nature found its way to use oxygen, built up its complicated oxidative apparatus and opened in this way the possibility of the development of more complicated and pretentious forms of life. If we let our fantasy go we can picture life originating in some puddle in which, under the catalytic action of a metal, carbohydrate was formed from CO_2 under the influence of sunlight, on which carbohydrate the first simple forms of life thrived. Then, maybe, this simple life learned to bind this catalytic metal within itself better and better and thus began to build its own carbohydrate. Today we find this carbohydrate-producing apparatus in the chloroplasts, the green chlorophyll-containing granules of plants. Then, probably, maybe millions of years later, life learned to use oxygen and to develop a more economical utilization of its carbohydrate. Naturally, I must emphasize that I was not there so I cannot guarantee this sequence of events. All the same we find this sequence reflected even in our own body. The embryo, in its most primitive forms, works with fermentation

rather than with oxidation and even in the developed organism we find fermentative processes yielding directly the energy for certain cellular activities, the process being followed by oxidation. For example, Hopkins, Meyerhof and Hill have shown that the most important source of the energy of muscular contraction is lactic fermentation. Then, in a secondary way, oxidation comes in, oxidizes some of the lactic acid and puts the wasteful energy balance right again.

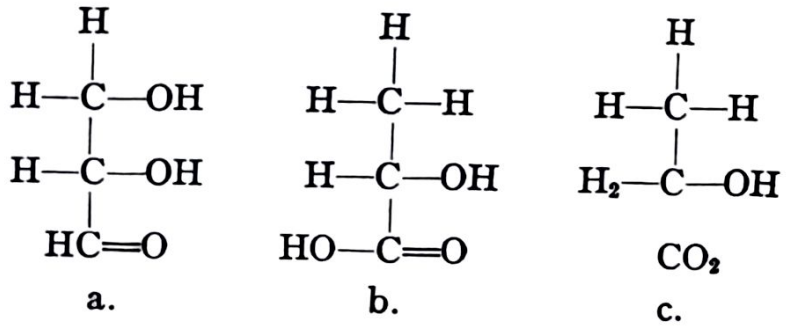


FIG. 7

I will speak later of this relation of oxidation and fermentation. Here, I want to direct your attention to the molecular mechanisms by which the fermentation works. Let us take as an example the lactic acid fermentation.

If we put the two molecules, the sugar triose (a) and lactic acid (b) (Fig. 7), side by side we observe that the difference between the two is that oxygen has been transferred from the top of the molecule to the bottom. One end of the molecule has been reduced from H_2COH to CH_3, while the other end has been oxidized from HCO to COOH, thus at this end of the molecule the C is oxidized, so to speak, to CO_2 which, however, is still bound in the molecule in the

form of COOH. If we split this CO_2 off, we obtain ethyl alcohol (c). So lactic acid fermentation and alcoholic fermentation are nearly identical processes with the one difference that in alcoholic fermentation the CO_2 is eventually detached.

This fermentation is thus a transfer of O from one end of the molecule on to the other, but this is only a final result which does not tell us anything about the mechanism by which it has been reached. The cell possesses no means for such a direct oxygen transfer, which is chemically impossible. The only means the cell possesses are those which we already have seen at work in oxidation, i.e., taking off and putting on H, taking off water and putting it on again. We have to proceed thus from triose to lactic acid by these means.

But here again we must bear in mind that the whole process is effected by the cell, not just in order to transform carbohydrate, but in order to liberate energy and to liberate it in a form other than heat, that is, in a form which can be used for work.

The first step in lactic acid fermentation is to take off two H atoms and replace them by water. (Fig. 8.) The substance that takes over these two H atoms is the cozymase which is also the coferment of dehydrogenases.

You will be struck thus far by the complete identity of the two processes of oxidation and fermentation. Oxidation of triose consisted, as you remember, of the dehydrogenation of triose, the H being taken over by the coenzyme. This was the first step of piece-meal oxidation of H. The energy was used

for phosphorylation, was invested in a phosphoric-ester-linkage. So far, then, oxidation and fermentation are identical. We can also expect that the enzymes, the dehydrogenases, activating the triose, are the same in both processes. The difference between the two processes comes in only at the next step. In oxidation there is oxygen, the final H acceptor of electrons. The H was thus sent on towards oxygen via the C_4 dicarboxylic acids. In fermentation there is no oxygen, no such external H acceptor. But the cozymase must, in some way, get rid of its two H atoms before it can accept new ones. But since there is no external H acceptor present, it has no other possibility except to put it back onto the same molecule (the triose) from which it has taken it.

"Put it back," this is easily said. It could be taken off with liberation of energy, but how to put it back! The molecule of glyceric acid, into which triose has been transformed after its dehydrogenation, has no grouping in its molecule that could accept H, that could act as an oxidizing agent toward dihydro-coenzyme; neither the COOH nor the COH are suitable for this purpose, none of these can be reduced under these conditions. We actually need a carbonyl, a CO group to accept the H. The cell must thus develop a CO group out of the two COH groups, that is to say, one at the expense of the other. This is done by splitting off water, one H and one OH (see Fig. 8 encircled), establishing temporarily a double bond. This substance is called enol-pyruvic acid that can easily rearrange itself by the transfer

of one H and the elimination of the double bond. In this way we obtain pyruvic acid. Now this pyruvate can accept by its CO group the two H atoms from cozymase, relieving this substance of its burden and itself being reduced to lactic acid. (In alcoholic fermentation pyruvate is decarboxylated, that is to say, its bottom CO_2 is split off, leaving acetaldehyde behind which accepts the 2 H atoms and is reduced in this way to ethyl alcohol.) We thus finish with lactic acid (or alcohol) while the energy difference between these two substances and triose (12 Calories) has been invested in the phosphoric ester linkage which represents about 11 Calories. The energy in this form can be transferred to other parts of the cell which need it, and which are able to split the ester into coenzyme and free phosphate again thus liberating the 11 Calories which had thus been impounded.

You have seen that oxidation is, in its first step, identical with fermentation. The dehydrogenated triose is, in oxidation, transformed into pyruvate in both cases. In fermentation the H is put back onto this molecule but in oxidation there follows a long chain of reactions in which the H of the cozymase is eventually oxidized to water.

Oxidation is thus, in essence, fermentation plus a long chain of reactions, a mechanism in which the H is burned, yielding up its total energy. (Fig. 8 downward arrow.)

But if you give your attention to this diagram of formulae for one more minute you might make another most striking discovery. In fermentation it is

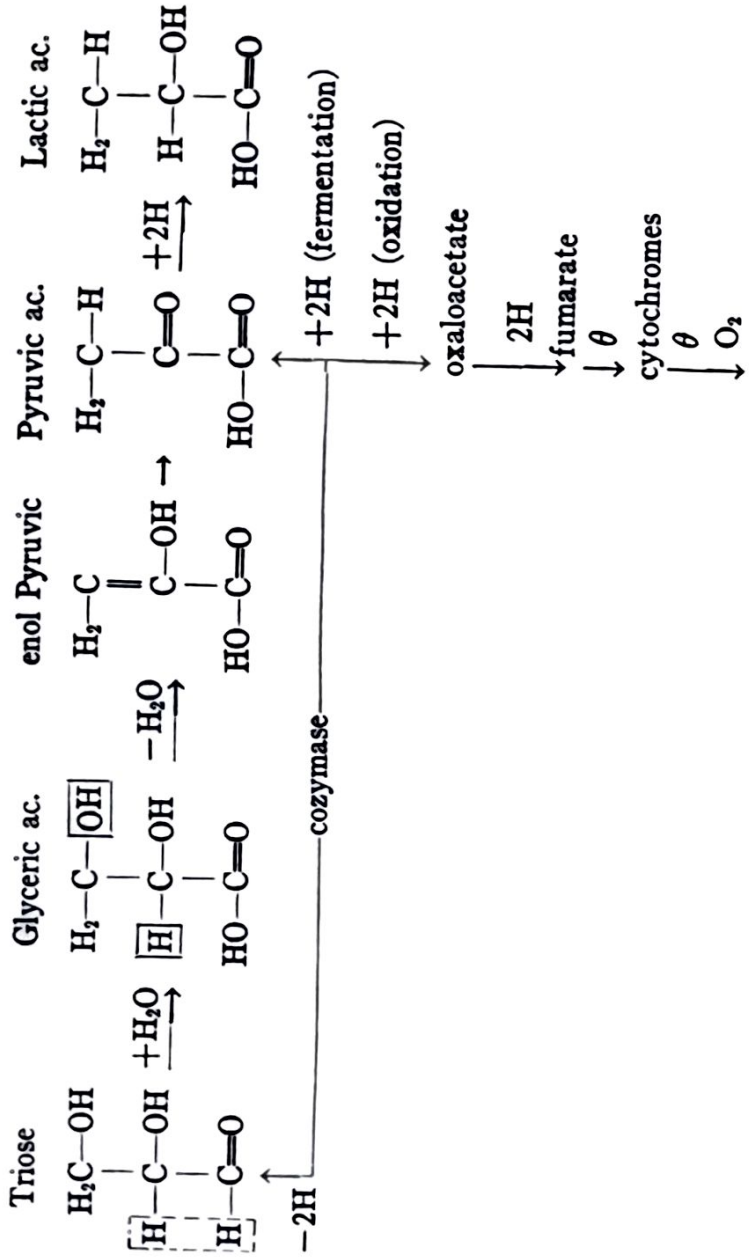

Triose
Glyceric ac.
enol Pyruvic
Pyruvic ac.
Lactic ac.
$H_2C—OH$
$H_2—C—OH$
$H_2—C$
$H_2—C—H$
$H_2—C—H$
$+H_2O$
$-H_2O$
$+2H$
$-2H$
cozymase
$+2H$ (fermentation)
$+2H$ (oxidation)
oxaloacetate
2H
fumarate
θ
cytochromes
θ
O_2

FIG. 8

pyruvic acid, in oxidation it is oxaloacetic acid that accepts the 2 H atoms from the coenzyme. If you now put the formulae of these two substances, pyruvic and oxaloacetic acid, side by side you will discover that oxaloacetate, the H acceptor of oxidation, is in essence only carboxy-pyruvate. When Nature thus built its way toward oxygen, by its first step, it did nothing more than carboxylate a pyruvic acid, thus taking it out of the carbohydrate cycle, and labeling it as "catalyst." This relation between oxidation and fermentation is perhaps the one result of my experiments that has hitherto given me the most mental satisfaction.

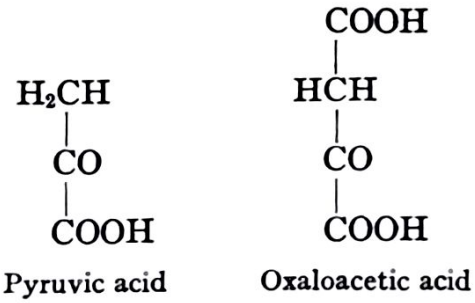


FIG. 9

The pyruvate molecule is held by its enzyme, as seems probable from the results of K. Laki, by means of the carboxyl group. By the introduction of a second COOH the molecule thus obtains a second handle by which the enzyme can hold it. This explains why oxaloacetate has a greater affinity for the activating enzyme than pyruvate has, hence if both pyruvate and oxaloacetate are present, it will be the latter that will take over the H.

These relations are quite important, for they, at least partly, explain to us a reaction that has been

for a long time a puzzle for biochemists. Pasteur observed that if he admitted O_2 to fermenting yeast, oxidation started and at the same time fermentation (production of alcohol and CO_2) decreased. If he stopped oxidation by taking oxygen away, fermentation started up again. This phenomenon we call the "*Pasteur reaction.*" The relation of pyruvic and oxaloacetic acid considered above gives us a simple explanation of it. If there is oxygen there is oxaloacetate, succinate and malate being oxidized into this substance. If there is oxaloacetate it will take over the H of the coenzyme and this is oxidation. If there is no oxygen present the only H acceptor present will be pyruvate which will take up the H, and this is fermentation.

As I mentioned at the outset, fermentation is the simpler and older process; nevertheless you must not look upon it as an antiquated procedure, limited to micro-organisms, for even at the present day the greatest part of the energy-need of contracting muscle is supplied primarily by lactic fermentation. This is easy to understand because oxygen cannot be accumulated very well in the muscle and a rapid contraction would not find enough of it; furthermore oxidation is a complicated business. On the other hand, carbohydrate in the form of glycogen can well be accumulated and can readily be fermented. Thus the muscle covers, as shown by Hopkins, Hill and Meyerhof, its sudden need for energy by fermentation. This by itself would be a wasteful process because the greatest part of the energy of the sugar is left in the lactic acid. The cell solves the problem in the

following way: It does not throw the lactate away but oxidizes part of it at its "early convenience." The energy hereby obtained is used to resynthesize the rest of the lactate to glycogen (Meyerhof). Thus only part of the fermented glycogen is lost and the whole process is paid for in the end by oxidation, which puts the wasteful energy-balance right again. Thus the phylogenetic sequence is repeated: first fermentation and then oxidation.

This procedure reminds me of certain financial methods which developed after the war in my country. We had no money but had some property left from better days. If one suddenly needed money, as the muscle needs energy, there were two possible ways of getting it, either to take your belongings to your "uncle," or to sell them. "Uncle" gave little for them but you got the money readily. This corresponds to fermentation. The other possibility was to sell your goods, this way you got more for them but this took time. This corresponds to oxidation. The method followed in case of sudden need was thus the following: you took your things, let's say a dozen silver spoons, to "uncle" and got the money, then you had two of the spoons sold, and with the money thus obtained you retrieved the other ten. The muscle ferments sugar into lactate and then oxidizes part of it, and by the energy produced it apparently resynthesizes the rest into sugar.

I want to devote the rest of my time to this reaction, in which part of the lactate is burned and the rest resynthesized to sugar. Meyerhof sums things up by saying that part of the lactate is burned and the

energy produced is used to resynthesize the rest into carbohydrate. To be more accurate one-third to one-sixth of the lactate is burned, two-thirds to five-sixths resynthesized. It would be every third or every sixth molecule that thus is burned.

This sounds simple enough but if we try to picture the mechanism of this process we run into insurmountable difficulties. If lactic acid is oxidized, it is activated by the lactico-dehydrogenase, loses two H atoms and turns into pyruvate. There is no difficulty up to this point for tissues contain a very active lactico-dehydrogenase. There is no difficulty either with the oxidation of pyruvate, for tissues contain an active oxidase for this substance also. But if we would want to go on oxidizing pyruvate right down to CO_2 and water, we would need, after the loss of every two H atoms, a new dehydrogenase. A great number of additional dehydrogenases would thus be needed for these substances between pyruvate and CO_2 and no such dehydrogenases are known. Their existence can even be excluded. Not only would we need a great number of enzymes for the breakdown of lactate, but we also would need a number of catalysts for the resynthesis of sugar and a complicated apparatus for the transfer of energy from oxidation to resynthesis. This is almost unthinkable. Moreover, the resynthesis of lactate involves the reduction of the carboxylic group and as yet we have no evidence that any such reduction is even possible in the cell.

Some ten years ago J. B. Conant proposed a much simpler explanation for Meyerhof's observations, at least for the quotient one-third. He thinks that it

is not every third molecule of lactate but one-third of every molecule that is oxidized. Let us see how this works. Lactate is oxidized to pyruvate. There is no difficulty about this, nor is there any difficulty about the oxidation of pyruvate, two H atoms being split off from this substance. If, at the same time, we split off one CO_2 we have a substance with the formula, $C_2H_4O_2$, which corresponds to the general formula, $C_nH_{2n}O_n$, of a carbohydrate. It corresponds to a biose which could simply be polymerised into hexose. This process does not involve any transfer of energy for it runs all the time with liberation of energy or is thermoneutral. The liberated energy is not lost for lactic acid, and probably pyruvic acid, are dehydrogenated by means of the coenzyme which conserves the liberated energy. This process is so much simpler that we have to accept it until evidence to the contrary is produced. I, myself, have had this theory in mind for ten years. I have not been able to prove it yet but all I have seen is more in favor of than against it. It represents a most ingenious mechanism. The cell splits the sugar into lactate, produces the energy it needs, then oxidizes four H atoms, producing still more energy, after which it has two-thirds of its sugar back and everything is ready again.

This cycle of carbohydrate breakdown and resynthesis is one of the most essential processes of cell life, whatever its mechanism may be. This cycle is also occasionally involved in some most important pathological derangements, so let us consider it more in detail.

We have started with triose and taken two H atoms off of it by means of the coenzyme. The dehydrogenated triose was transformed into pyruvate. In oxidation these H atoms are burned and pyruvate is left behind. In fermentation pyruvate takes the two H atoms back and becomes lactate. But now, if this lactate is oxidized, at the first step it again loses these H atoms and again we have pyruvate. So whatever path we follow we encounter pyruvate. As in the ancient world all roads led to Rome, so in biochemistry all roads lead to pyruvic acid. This substance, pyruvic acid, is one of the queerest molecules; it can do almost anything. It can oxidize, can reduce, can decarboxylate, can polymerise. These many sided possiblities make pyruvic acid the horror of the chemist and at the same time they give a fascination to the molecule in the eyes of the biochemist. These many sided possibilities make it probable, as emphasized by F. Knoop, that pyruvic acid represents, in fact, the great cross-road of the main metabolic processes, not only between oxidation and fermentation, but also between fat and carbohydrate metabolism.

As Embden has shown one of the many reactions pyruvate can give is to be transformed in the tissue to acetone. This is a most important finding, for the formation of acetone gives a basic chemical symptom of that disturbance of carbohydrate and fat metabolism which is known to you as diabetes. Acetone was thought of as a substance that can only be formed from fat. Embden's observation opens new possibilities and pyruvate with its cycle might lead us to a

deeper understanding of this mysterious pathological disturbance, and of the interrelation of fat and carbohydrate metabolism.

But what about CO_2? I have been talking about oxidation and fermentation and all the time I did not mention CO_2 which is, after all, together with H_2O, the main final product of our metabolism. We have followed the dehydrogenative oxidation of sugar into pyruvate. There was no CO_2 formation. But pyruvate is a very badly balanced molecule. Its one end is reduced to CH_3, its other end is maximally oxidized to COOH. Next to the COOH we find a CO group. Thus one-half of the molecule is very heavily loaded with O. If COOH has as neighbor a CO group, the CO_2 of the carboxylic group comes off rather easily. In alcoholic fermentation of yeast it is at this stage that actually CO_2 is split off. This splitting off of CO_2 is, however, not an oxidation. It is simply due to the action of a special enzyme, the decarboxylase, which is capable of effecting this scission.

We are not yet very well informed about this decarboxylation in animal tissues. The experiments of my collaborator, E. Annau, rather suggest that in the liver, and this is probably true also for muscle, the CO_2 can be split off from pyruvate only after the further loss of two H atoms. Be this as it may, the surprising fact remains that the production of CO_2 and oxidation are more or less independent processes—and this is one of the most unexpected results of modern biochemistry.

What lends additional interest to this decarboxy-

lation is the discovery of K. Lohmann that the coenzyme of the decarboxylase is nothing else than phosphorylated vitamin B_1. The work of R. A. Peters and of his collaborators has shown that in beriberi, the disease caused by the lack of vitamin B_1, there is a disturbance of pyruvic acid metabolism. Now, by the discovery of Lohmann all this becomes clear; in absence of codecarboxylase, B_1, the decarboxylation of pyruvic acid is disturbed. In this way this mysterious disease, beriberi, is reduced to an equation, and can be described in the plain language of chemistry. The observations of Peters and F. Lipmann make it probable that the vitamin is involved not only in the decarboxylation, but also in the oxidation of pyruvic acid.

It seems not unlikely that decarboxylation might also explain the reduction of carbohydrate to fat. It has always been a puzzle how this reduction is effected. The COOH represents the highest oxidation level of a C atom, so if CO_2 is split off, the average oxidation level of the molecule is diminished, the molecule is reduced. If, for instance, lactic acid, the isomer of carbohydrate, would be decarboxylated, we would get alcohol which can, on paper, be polymerised into fat. Decarboxylation without oxidation is, in fact, a reduction.

Before closing this lecture I must correct an error made deliberately. I have talked about "triose" into which hexose is split and which is oxidized and fermented. In fact it is not hexose but hexose-phosphate that is fermented, the reserve carbohydrate, glycogen being phosphorylated prior to its hydrolysis.

Hexosephosphate is split into triose-phosphate and it is this substance that undergoes the further changes and not simply triose. According to Parnas and his collaborators, the phosphate molecule describes in these changes a closed cycle. According to the school of this eminent investigator, it is the adenyl-pyrophosphate that gives its phosphate to the sugar when it is phosphorylated, and it is adenylic acid that again takes over the phosphate from the triose after its oxidation. To transfer phosphate to adenylic acid requires energy. This energy is donated by the triose molecule itself. As demonstrated by Meyerhof, the triose molecule, after its dehydrogenation, does not lose energy in its transformation to glyceric acid and from glyceric acid to enol-pyruvic acid phosphate. All the energy drop is concentrated at one point, at the transformation from enol-pyruvic into pyruvic acid. There is a sudden drop of energy at this point which energy is used to couple the phosphate moiety on to adenylic acid. The system enol-pyruvic acid-phosphate plus adenylic acid is thus transformed into pyruvic acid and phosphorylated adenylic acid which latter, in its turn, can again give its phosphate to a new sugar molecule which is being formed from glycogen. We do not understand yet why this cycle is made by Nature at all, nor why sugar is phosphorylated if it really is such a closed cycle, and the cell does not gain by it a new molecule of phosphoric ester. Probably, we do not yet know all about it.

On the harmonious interaction of all these processes —oxidation, and fermentation, carbohydrate breakdown and resynthesis, transition into fat etc.,—our

health depends, and the disturbance of this delicate regulation may play an important primary or secondary rôle in many pathological conditions. It would not be permissible to close this discussion without mentioning Warburg's work on cancer. As is widely known, Warburg has found in rapidly growing malignant tissue a disturbance in the interrelation of fermentation and oxidation. Oxidation is unable to keep up with fermentation and clear away all the lactic acid formed. We find the same relations also in embryonal tissues. The more primitive embryonal tissues seem to depend for their energy upon the more primitive process of fermentation rather than upon oxidation. As was nicely shown by my collaborator, A. Blazso, the oxidation apparatus is but very weakly developed in embryos. The cancer cell, reverting to its more primitive embryonal type, reverts to this primitive type of metabolism. Fermentation is in itself a wasteful process, but is in agreement with the parasitic nature of cancer and embryos. Only a very few normal tissues can live by fermentation only, as for instance the retina, the optical requirements of which could not function in the presence of a cytochrome system.

To the outside spectator, all this work of the biochemist in which he shifts little H atoms and the phosphate molecules from one substance to the other, must seem a little play for big children. Thank heaven that this is really so and that biochemistry is a lovely game of refined cookery, very fit for the amusement of big children. I often suspect my really good colleagues of being attracted to their working

desk less by the desire to elucidate profound problems or save humanity than by the childish pleasure of playing about to make fluids boil, to pour them from one bottle into another, to see manometers sway right and left and to solve crossword-puzzles of Nature. Any criticism is a confession. It *is* a lovely game. But at the same time this innocent game of H atoms jumping about does give us a deep insight into the ways and means of Nature; it even explains mysterious diseases in simple terms and helps us to build a firmer foundation for medical science. So these little H atoms may eventually help to relieve human suffering and in the fight against disease become weapons not less sharp than the surgeon's best knife.

Lecture IV

ON VEGETABLE OXIDATION SYSTEMS AND THE MECHANISM OF DEHYDROGENATING AUTO-OXIDATION

THE students of medicine present in this audience will, perhaps, be annoyed to hear that I will devote this lecture to vegetable respiration. It is man they want to cure, not cabbages. This is right, but after all it is life they have to understand if they want to cure, and it is the same old life whether it is in us or in our green fellow-creatures. Both are but relatively recent leaves on the same old tree of life. The difference between the two is much smaller than we would be inclined to think. This generalization is one of the most delightful vistas modern biochemistry has given us, this vista of the great unity of life. Biochemistry teaches us that many constituents of our body are found with equal frequency in plants and animals, fulfilling analogous functions in each. These substances of plants, just as they are, or with little alteration, fit into the machinery of our cells. Two machines, the parts of which are interchangeable, cannot be very different and so anything we learn about the plant will lead us closer to the understanding of ourselves. The plant, as an object of the study of life, has, compared to man, some very great advantages. The plant does not have to go to school, attend lectures,

search for food, so it can dispense with many of the unessential complications found in our body which enable us to walk, hear, see, smell and think. Life in the plant will present itself in much simpler forms, and thus allow the great fundamental principles to come to the fore. Moreover according to the widely differing conditions here or there, e.g., roots and leaves, one of the different mechanisms of the cell will be found more developed, lending itself the better for study. All animal cells live in a common constant "internal environment," which is identical in most parts of our body.

I was driven to study plants in a somewhat unusual way some fifteen years ago. My object was the function of the adrenal cortex, the function of the cortical part of that little organ above our kidney. At that time we knew very little about it. The one thing we knew was that human patients suffering from an insufficiency of this organ (Addison's disease) die, but turn brown before doing so. They turn brown just as does the dying banana, apple or potato. Biochemistry, at that time, did not fully realize the great fundamental unity of plants and animals, but I, myself, was convinced that there could be no principal difference between "cabbages and kings." Cabbages, then, being cheaper, I began to study cabbages. At that time it was already known from the work of the Russian, Palladin, that the turning brown of potatoes has something to do with the disorganization of respiratory systems. This led me to study vegetable respiration.

We still know very little about vegetable respira-

tion. The chemical changes are much slower in the plant than in the animal and thus more difficult to measure.

I will start my story with the brown discoloration of potatoes I have just mentioned. This brown discoloration is due to the formation of quinones.

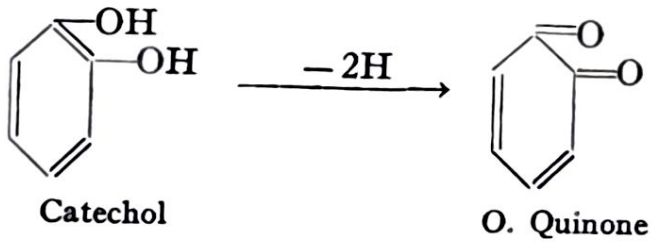


FIG. 10

If we oxidize off two H atoms of an o-diphenol like catechol (Fig. 10), it becomes a quinone which is slightly yellow. If this quinone is not reduced to catechol at once and is allowed to stand in contact with the proteins and amino acids present in the tissue, it will combine with these to turn brown or black, forming so-called melanoid pigments. Such catechol derivatives are found in the potato and many other plants. Associated with this catechol we find an "enzyme" which strongly catalyzes the oxidation of catechol by O_2 which oxidizes off the two phenolic H atoms. If the plant is damaged these enzymes (or the catechol) are released and there ensues a rapid oxidation. At the same time the plant seems to lose its ability to reduce these quinones which are thus free to form pigments. Hence this pigment formation is the expression of the action of "polyphenoloxidase" on catechol derivatives.

Kubowitz and Keilin have shown lately that the catechol-oxidase is a copper-proteid, i.e., a protein

containing copper. Without copper the protein is inactive, without protein the copper is but slightly active.

Thus we can sum up the situation by saying that in this system two H atoms of the catechol molecule are oxidized off, under the catalytic influence of a metal Cu, linked to a specific protein as Cu-proteid. This Cu-proteid is what we call an enzyme, and the Cu atom is its active "prosthetic" group. Cu, in the free condition, will catalyze the oxidation of the catechol even without protein but infinitely more slowly. One milligram of free Cu will oxidize in an hour's time a few milligrams of catechol. In the form of Cu-proteid as "catecholoxidase," it will oxidize as calculated by Keilin, $3\frac{1}{2}$ kg. catechol in the same time. When the cell applied this Cu for the oxidation of catechol it did not invent a new principle, it only availed itself of a basic property of copper. By linking the Cu, however, to a specific protein, the cell not only protected its Cu atoms from being lost but also gave them a chance to act at their very best.

The oxidation of the catechol consists of the loss of two H atoms and it looks as though the metal had done nothing but speed up the union between these H atoms and O_2. But, as stated in my first lecture, we must realize that this reaction is only apparent, that the O_2 really does not unite directly with the H atoms of catechol but only takes over their electrons, the function of the metal being to transmit the electron from H to O. The oxidation of the H consists only of the loss of an electron and the reduction of O_2 consists of taking over this electron. The metal

passes on the electrons from H to O_2 one by one. The O_2 molecule (O=O) accepts the first two electrons by opening up the double bond which connects its two O atoms. Now this doubly charged—O—O—can bind two H^+ (H ions) of the water and form H—O—O—H which is hydrogen-peroxide. Cu has no affinity for peroxide and so it lets it go; the story is finished and we obtain from O_2 + catechol, quinone + peroxide.

Unlike Cu, iron has a great affinity for peroxide. So if it is Fe that catalyzes the oxidation HOOH is not formed, for the iron atom holds the peroxide tightly until it accepts two more electrons and can thus bind two more H^+ (H ions) and be reduced to H_2O, water.

This explains for us the existence of another enzyme, peroxidase, always found in presence of catechol-oxidase. This enzyme, the peroxidase, contains iron which will react with the HOOH formed by the catechol-oxidase and will oxidize, with this peroxide, a second molecule of catechol. In this way the HOOH is reduced to water, all the four valencies of the O_2 having been utilized for the oxidation of catechol.

The catechol-oxidase system was thus cleared up, but it gave no clue to the function of the adrenal cortex. It is true the catechol-derivative of the polyphenoloxidase system of the plants is closely related to adrenalin, the specific hormonal product of the adrenal medulla, but it was the cortical part of the adrenal gland that primarily interested me. So, obtaining no answer to my question from the

polyphenoloxidase system and the plants that discolored on dying, I turned to the plants that did not turn brown. I switched the question around and asked no more why patients with defective adrenals turned brown, but why it was that we, who had good adrenals, remained colorless. This question could be answered only by the other plants which, like ourselves, remained colorless on dying, so I asked these plants whether they could tell me anything about the function of the adrenals.

Very soon I found that many plants that belonged to this group not only failed to turn brown themselves, but their juice, if added to the juice of polyphenoloxidase plants, prevented these from coloring for some time. The delay is mostly but a few seconds; this delay of a few seconds has cost me several years of work for I wanted to know its reason. I found that the reason lay in the presence of a most fascinating substance which can reduce all quinones to phenol and in this way prevent pigment formation. In other words, the substance had two labile H atoms which it very readily gave up to the quinone. The more I learned about this new substance, the more interesting it seemed to be. Eventually I crystallized it, that is to say, peeled it out in a pure condition which made analysis possible. It was an acid and it seemed to be related to an unknown sugar which I called "Ignose," the substance itself being called "Ignosic acid." But the editor of the journal to whom I sent my paper did not like jokes and rejected the name. "Godnose" being no more successful, we agreed that the child's name should be "hexuronic

acid." Later, with advancing knowledge of its structure it had to be rebaptized in haste and it is now called ascorbic acid (sometimes, cevitamic acid) because it is identical with Vitamin C and prevents scurvy. In this way I became a father without wishing it, the father of a vitamin. Such accidents seem to happen even in science.

You will understand my excitement upon finding that the adrenal gland contained relatively great quantities of ascorbic acid. In fact, before the advent of paprika, the adrenal gland was the only source that admitted its large-scale preparation. You also will understand my enthusiasm when I found that ascorbic acid bleached out patients with Addison's disease. Hopes of solving the color problem by ascorbic acid were disappointed, for ascorbic acid reduces only certain pathological melanoid pigments. I thought I had solved the problem of the function of the adrenal gland, but today we know that ascorbic acid is not the specific product of the adrenal gland and all my work is just a new example of the old experience that even a wrong theory may lead to good results. All the same, there still seems to be some relation, not understood, between the adrenal gland and ascorbic acid.

I also found in cabbages an enzyme which, by means of O_2, could oxidize off reversibly the two labile H atoms of ascorbic acid. The ascorbic acid, after this oxidation, again could take up two new atoms of H. This enzyme, the ascorbic-acid-oxidase, analogous to the polyphenoloxidase, is apparently a specific metal-proteid, probably a cupro-proteid. The two

H atoms with the O_2 will also give HOOH, and in the presence of the oxidase we find also a peroxidase which, by means of the HOOH, oxidizes a second molecule of ascorbic acid. The difference, as compared with the polyphenoloxidase system, is only that the peroxidase needs for this oxidation an intermediary phenolic substance. Peroxidase does not oxidize substances of the type of ascorbic acid, it oxidizes only phenols. The peroxidase, in this case, oxidizes a phenol, the phenol oxidizes a second molecule of ascorbic acid.

The specific phenol acting in this system was a benzo-pyrane, a member of the great group of substances studied by Kostanecky and Perkins and called, according to details of their structure, flavons, flavanons, or flavonols, which substances, according to Robezinicks, have a specific affinity for peroxidase. In citrus fruits we found an especially active member of this group present as a glucoside which up to that time had been unknown in this form. We called it, with V. Bruckner, "Eriodictin." The plant seems to produce in its early days the flavon for all its life. But in the unripe plant we find this substance in a methylated, inactive and stable form, which was known as "Hesperidin" for a long time. In this substance, one of the two o-phenolic OH groups, necessary for activity, is inactivated by methylation, but in the ripe plant the substance is demethylated to the active eriodictin.

I am talking in such detail about this substance because of a small accident that happened to us at that time. I had a letter from an Austrian

colleague who was suffering from a severe hemorrhagic diathesis (vascular type). He wanted to try ascorbic acid in his condition. Possessing at that time no sufficient quantities of crystalline ascorbic acid, I sent him a preparation of paprika that contained much ascorbic acid and the man was cured by it. Later, with my friend, St. Rusznyak, we tried to produce the same therapeutic effect in similar conditions with pure ascorbic acid but we obtained no response. It was evident that the action of paprika was due to some other substance present in this plant. It would have been a hopeless job to try to find and isolate this substance had we not had our experience with the flavons. So we set out to prepare flavons, in the first place eriodictin, that can easily be injected and we found that similar pathological conditions, not previously amenable to therapy, could be cured by it with regularity. The effect had several characteristics of vitamin-action, so, tentatively, I called it Vitamin P in honor of Paprika and Permeability, on which later it was found to have an influence. As yet, I have failed to demonstrate its vitamin nature by animal experiments and until such proof is given the vitamin nature of this substance is not beyond doubt.

But let us come back to respiration. We found an oxidation system composed of ascorbic acid, ascorbic acid oxidase, and flavone. This system was analogous in many ways to the polyphenol-oxidase system. There were reasons to believe that these two systems represent the O_2-end of the oxidation system of plants, a system, analogous to the animal oxidation, in which the H, mobilized from the donator, is handed

over from substance to substance in a chain of intermediary H-transmitters until it reaches the polyphenol or the ascorbic acid, or their oxidase. Here the H gives up its electron to the metal, and this electron reduces the O_2. In this conception the polyphenol and the ascorbic acid are analogous to the succinic acid in the muscle.

This is a most fundamental problem and I must stop to discuss it. Does the polyphenol and ascorbic oxidase system really represent the O_2-end of the normal respiratory mechanism of plants? Is this the door through which the O_2 enters into the life of the plant cell? To be brief about it, I do not think so.

First, consider the ascorbic-oxidase system; it is present only in about every tenth plant, and oxidation is such a universal phenomenon! All plants respire and it is difficult to believe that there should be among them such great differences in the mechanism of such a fundamental phenomenon as respiration!

But it is different with the polyphenoloxidase system; this is present in about half of the plants. This is a better proportion though not quite satisfactory. But here we have another trouble. The situation, somehow, is analogous to the situation in detective stories in which the suspicion of murder is raised by the fact that the evidence of suicide is too plain. Polyphenoloxidase is a very active enzyme which oxidizes the polyphenol to quinone at an immense rate. But in the intact plant we find no quinone, thus, if the quinone is formed it is reduced at the same rate. But this oxidation and reduction together must give a most violent oxygen uptake,

almost an explosion, and potatoes, as you know, do not explode; they hardly respire. Thus we must suppose that the polyphenoloxidase, in the intact plant, either does not act on polyphenol at all or at least that only a minute fraction of its potential activity is available. But if it is only a minute fraction of its possible activity that can be exercised, then, maybe, it is not active at all and has some quite different significance. My collaborator, Vietorisz, could show that the polyphenoloxidase begins to exert its violent action on the polyphenol only at the moment when the cell is damaged and one may ask whether this oxidase is not connected with the "damage," rather than with respiration.

There is also another circumstance that makes one reflect. The quinone is such an active oxidizing agent that it destroys any living structure at once. Is it possible that the cell, that labile structure, could work with such a dangerous substance?

We can fit all these data into a very neat little theory by supposing that the polyphenoloxidase is not connected with respiration but with the natural immunity of the plant. This theory is supported by the distribution of the polyphenoloxidase. We do not find it, for example, in the parts of the plants that respire strongly and are exposed to air. We do find it in cells, as in the interior of potato tubers where there is practically no oxygen. Its distribution rather follows the exposure to damage. If the plant cell is damaged by insects or bacteria, the enzyme begins to produce quinone; this quinone kills

the invader and by combining with the protein shuts the door to the extension of the damage.

Possibly oxidation enzymes are more frequently involved in immunity than we think. So for instance, the immunity of milk against B. coli might be explained by the Schardinger enzyme. B. coli produces aldehydes, aldehydes are oxidized by the Schardinger enzyme and produce peroxide (HOOH) which kills B. coli. The same enzyme will not inhibit the B. lacticus, which produces no aldehyde.

All this might have given much pleasure to an immunologist but was very disquieting for a student of oxidation. The fact is that after all we do not know how vegetable cells respire and how O_2 enters into the respiration mechanism of the plant cell.

There was also another very disquieting circumstance. Whatever might be the function of catechol and ascorbic-acid-oxidase, there they were. Together with the succinic-acid oxidation system, these were the three known metallic oxidases. Succinic acid-catechol-ascorbic acid! It would be difficult to find three more heterogeneous substances and yet one would expect in such a fundamental process as oxidation, some basic plan, some semblance of unity. We were unable to give any reason why Nature should have availed herself of three such different substances as the last connecting links of H with O_2. Moreover there were some plants which were neither catechol-oxidase-plants, nor did they show any signs of an ascorbic system. Something very important was apparently missing.

The best thing one can do in such a plight is to forget for a while all about these complex systems and to go back to something simpler, still more fundamental from which these systems might have developed.

So I set out with my faithful collaborators, Banga, Gerendas, Laki and Straub, to study simply the metal-catalysed oxidations in which, as in the case of succinate, ascorbic acid or catechol, two H atoms were oxidized off from an organic molecule under the catalytic influence of a metal atom (dehydrogenating auto-oxidation). We did not use Cu, but Fe as the catalyst, and first tried to understand the Fe-catalysed oxidation of catechol. We trusted that the experience gained with this system could be applied to other metals and also to the metal-proteids.

I will not try to lead you along the arduous road we have to follow ourselves, but will simply give you the results of our experiments.

Our experiments showed that the mechanism of this apparently simple reaction is quite a complicated one. The whole reaction starts with the dissociation of the catechol whereby it loses its two phenolic H atoms, and the rest of the molecule assumes a negative charge as a catechol anion. This loss of H is not oxidation, it is merely a dissociation, the place of the H atoms being taken by electrical charges. The real oxidation of the catechol consists in giving up these negative charges. They are given to the metal but not, as one would expect, by a simple collision. The doubly charged catechol anion unites first to a single molecule with the Fe atom. To unite with the

catechol anion the Fe does not use its primary electrovalencies. As you know, Fe has two or three primary so-called electrovalencies by which it can combine, for instance with Cl as $FeCl_2$ or as $FeCl_3$. But quite apart from these primary valencies the Fe atom is surrounded by a strong field of force by which it can bind six more radicals. By means of these six "secondary or coördinate valencies" the Fe can give, with different molecules or atoms, very stable so-called coördinate compounds if certain conditions are fulfilled. One condition, in the case of organic molecules, is that the Fe should find on that organic molecule two atom groups capable of reacting with two of its coördinate valencies.

If two of the six coördinate valencies of Fe are bound this way by two catechol radicals we can write the formula as follows:

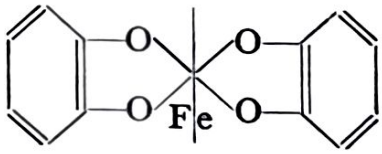


FIG. 11

If we observe the molecule more accurately we see that the Fe has actually become a member of two heterocyclic rings. Such rings can be formed only by five or six atoms, hence it is necessary that the two complex-forming groups of the organic molecule should be on two C atoms which are neighbors or are separated only by one C atom.

Now the Fe has two more coördinate valencies left and by these it can bind an O_2 molecule. Thus we attain the formation of an involved coördinate mole-

cule in which the metal acts as the central atom. This central atom, in case of Fe or Cu, can change its valency number by one from ferric to ferrous or from cupric to cuprous. The only difference between these two forms is that the one contains one more electron than the other. By this valency-change the metal can take up or give up one electron. In this way the metal will be able to take over, one by one, the two electrons of the catechol radicals and pass them over to the O_2 which, after having accepted four electrons, can unite with four H-ions to form water. The catechol itself, having lost its charge, is let loose by the metal and thus we finish with free metal, quinone and water, and the metal can start up a new reaction again.

In this complex the constituents mutually activate each other. By its binding to catechol the metal is enabled to react with O_2; by its combination with the metal the O_2 is enabled to accept electrons.

A great number of organic anions can combine with Fe, but only a few can give over electrons to the metal. Catechol is capable of doing this because it can rearrange its double bonds in such a way that no free valency will be left after the loss of electrons:

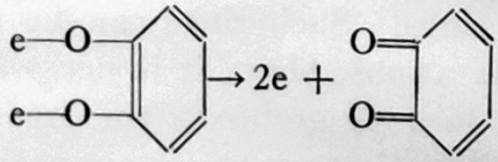


FIG. 12

(e means in this formula the electron which has taken the place of the H when this was lost by dissociation.)

We see thus, once again, how appearances are often misleading. From the formula,

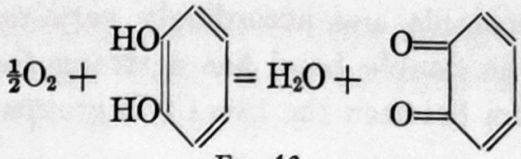


FIG. 13

it looks as though the oxygen would simply oxidize off two H atoms from catechol. Now you have seen that in reality this apparently simple reaction represents a long series of events in which complex-formations, the possible importance of which has been emphasized by H. Wieland and his collaborators, play an important rôle. The H of the catechol plays no rôle in the cycle since it is lost by dissociation at the first step.

To be able to give all this long series of reactions, catechol or any other substance must answer to a great number of specifications. It must contain, first of all, two atomic configurations which are capable of dissociation, and complex-formation and which lie at the right distance from one another, and so are capable of giving up their charges by a simple rearrangement of double bonds.

To my knowledge there is only one atomic group which has all these qualities, viz. the dienol-group, $\begin{array}{l} HO—C— \\ \quad\;\; \| \\ HO—C— \end{array}$, which can rearrange to the diketone arrangement $\begin{array}{l} O{=}C— \\ \quad\;\; | \\ O{=}C— \end{array}$.[1] The dienol group, however,

[1] Theoretically there is one other group which might play a similar rôle; this is the SH group which, after giving up its charge, can become stable through the disulphide linkage. This group is actually found in glutathione which seems to play an important but hitherto unknown rôle in cell life.

is very unstable and accordingly very rare, partly because the double bond has a strong tendency to escape from between the two COH groups and form

```
HOC=
    |   , thus losing its ability to rearrange its double
HOC=
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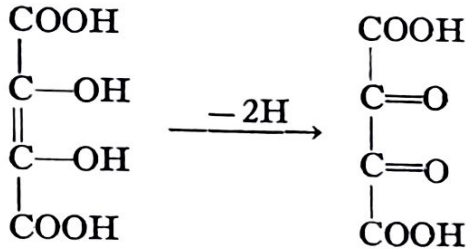
bonds and to give up its electrons. Accordingly we find this dienolic group only in a very small number of naturally occurring substances. One such substance is catechol in which the dienol is stabilized by the aromatic ring. Thus here we have the explanation why Nature utilizes catechol or its derivatives as members of an oxidative mechanism.

One other substance which contains a dienolic group is ascorbic acid, in which the dienol is stabilized by the acid-anhydride ring and thus we have the reason why Nature utilizes ascorbic acid. At the same time we see that in spite of their apparently great difference, both substances, catechol and ascorbic acid, are not so very different after all. They are both in essence dienol groups with a few other C atoms around to carry and stabilize this dienol formation.

These experiments on metal catalysis have thus explained to us why there is a catechol-oxidase and why there is an ascorbic-acid-oxidase; at the same time they have given some unity to the whole problem. But possibly these experiments have explained to us even more how plants really respire, what the door is through which O_2 enters into the vegetable respiration. There is besides catechol and ascorbic acid, a third quite common substance that contains a

stable dienolic formation and this substance is dioximaleinic acid in which the dienol is stabilized by the two neighboring COOH groups.

If all our ideas and deductions were correct, we might expect that dioximaleinic acid would also play a rôle in oxidation systems. To approach this problem Banga and I first asked whether there is an enzyme in plants, analogous to catechol or ascorbic-oxidase, and capable of oxidizing reversibly dioximaleinic acid into the corresponding diketone.



Dioximaleinic acid

FIG. 14

The experiments showed that some plants contained a most active and specific dioximaleinic-acid-oxidase. Banga and Philipot later extended these experiments and examined a great number (25) of plants and found that they all contained such an enzyme. In some plants the enzyme was most incredibly active, the crude juice of the plants oxidizing in two minutes its own dry weight of dioximaleinic acid and it looks as if, in this enzyme, we have really found the enzyme system by which O_2 enters into vegetable cellular respiration.

This enzyme also has the properties of a metal proteid and also produces HOOH. The HOOH

reacts in the plant with the peroxidase, oxidizes a phenol which in its turn oxidizes a second molecule of dioximaleinic acid. The structures of the three systems, catechol-oxidase, ascorbic-acid-oxidase and dioximaleinic-acid-oxidase, are thus analogous. In all three the substrate is oxidized reversibly and the peroxide formed oxidizes, by means of the peroxidase, a second molecule of the substrate.

But what about succinic acid which plays, in muscle, an analogous rôle to catechol, ascorbic or dioximaleinic acid? Succinic acid is not a dienol nor is it autoxidizable! How did Nature come to select it? I cannot answer this question definitely yet, but the dioximaleinic-acid-oxidase gives us, if not an answer or mental rest, at least a hope of it. If the animal developed from more primitive vegetable forms and if dioximaleinic acid is, in the vegetable, the last H transmitter, then one might expect that the succino-oxidase could develop from this dioximaleinic-oxidase. In fact, succinic acid is most closely related chemically to dioximaleinic acid; dioximaleinic acid is just an oxide of succinic acid. This close chemical relationship between these substances actually makes a phylogenetic relation probable, the succino-oxidase having developed from the dioximaleinic oxidase. Dioximaleinic acid is a very active and labile substance. One could think that Nature replaced it in the course of phylogenetic development by the more stable and inactive succinate, plus an activator in the form of succino-dehydrogenase.

All these possible relationships become still more fascinating in the light of H. Theorell's most recent

experiments. Theorell tried to purify the dioxi-maleinic-oxidase and found in the preparation a strong haemochromogen reaction. Peroxidase, which was also present, gives a similar reaction, being, in fact, a haemin-proteid. At the same time Theorell found that by dialysing the enzyme it was inactivated but could be reactivated by a mere trace of manganese. This makes it probable that the enzyme is a haemin-mangano-proteid, possibly a peroxidase combined with Mn. In fact, Theorell found that a haemin cytochrome C + Mn acted together as a dioximal-einic-acid-oxidase. The pages on this question are not yet closed, in fact they are hardly opened. A special interest to all these findings is given by the fact that cytochrome is probably also a constituent of the succino-dehydrogenase, and that the succino-dehydrogenase behaves, as was shown lately by my collaborator, L. Massart, in many respects as a proteid of a lighter metal like Mn, which draws the analogy and the possibility of intimate phylogenetic relations of succino-oxidase and dioximaleinic-oxidase still closer.

I am conscious of having left somewhat the solid ground of experiment, for the less firm footing of speculation when I talk about phylogenetic relations. But having already done so, allow me to finish this lecture by losing ground completely and going still further back with phylogenesis, right to the origin, the first and simple beginnings of life.

You have seen that in oxidation, metals played an important rôle, and this not only by their primary valencies but rather by those diffuse and strong fields

of force that surround the atom, forces which fall into the definition of secondary or coördinate valencies. By these forces the metal atom is capable of binding groups like COH, NH_2, CO etc., which could not be bound by primary valencies. By this binding of a molecule not only the metal atom but also the bound molecule is strongly distorted. Thus far, this is not speculation, it is just a repetition of chemical experience. But let us speculate now on the first form of life arising somewhere and somewhen in the ocean. What tools were there for this first system to use in doing chemical reactions—none other than the cations and anions found in sea water, atoms with strong surrounding electric forces. We can expect thus that these first forms of life availed themselves of these ions or atoms as tools, binding the heavier and rarer ones to the organic structure, using and binding common and light ones just at the moment of the reaction into a dissociable form.

It is not surprising thus to hear day by day about new enzymes that have been revealed to be metal-proteids or at least proteins acting only in the presence of a lighter metal. It is not surprising to read that G. Bertrand finds that a considerable number of the not too rare cations are indispensable for life; it is not surprising to find the rare halogen, iodine, bound and fixed within a hormone, thyroxine, or that muscular contraction can be transmitted from muscle to muscle by the humoral way by potassium. (Szent-Györgyi, Bacq and Goffart.)

I think that the great importance of metal atoms and anions, these primitive tools of life, is recognized

as yet only to a small extent. But thinking of a metal atom and its biological function, we have to think rather in terms of secondary than primary electrovalency which, owing to its great electro-affinity, dissociates and gives no compounds at all. Physiology has hitherto stood too much under the ban of the primary or electro-valency idea and has had to introduce mystic concepts like "ionic equilibration." By considering the fields of forces around atoms, we may not only understand many biological reactions better, but we may some day get an insight into the nature of the first and simplest forms of life.

Lecture V

ON VITAMINS, HEALTH AND DISEASE

THERE is no other domain in biology that, for a while, promised such easily won laurels to the researcher as did the field of vitaminology. That is why vitamins were for a long time my pet aversion. It is the irony of fate to have made me, without my willing it, father of one of the vitamins and to have brought me in close touch with others. Nature, as the old physiologist, Bernstein, used to say, is not a mother, but a mother-in-law of the scientist.

To understand what vitamins mean for medicine, we must transport ourselves into the atmosphere prevailing at the end of the last century. Pasteur had shown that epidemic diseases were caused by micro-organisms, and it seemed that every disease must have its positive causative agent. On the other hand, extensive studies on nutrition, pursued chiefly in Germany, showed that food had to contain, besides, a sufficient number of calories, proteins, fats and carbohydrates in a suitable proportion to be satisfactory. The great problem of feeding had been reduced to a simple mathematical formula. It was in the haze of victory of these two doctrines that Eijkman, a young Dutch doctor, published his observations, according to which beriberi was not caused by a positive infectious agent, but by a lack of something missing from food—a negative factor. F. G. Hopkins at Cam-

bridge showed that this something, necessary for health, acted in minimal, insignificant quantities when one considers their energy content. The name *Vitamin* invented by C. Funk was accepted for this group of substances with the presumption that they were amines, and were connected somehow or other with the mystery of life. They were considered to be something quite special, a peculiar group of mysterious agents. These vitamins prevented certain diseases and were thus the supporters of health. Public interest turned in full measure towards these mysterious agents; special laboratories were founded for their study and a great host of specialists, the vitaminologists, set out to study them, to ascertain their number, their relative quantities in food, the quantity of each necessary for health and to find suitable experimental animals for their study.

I am not a vitaminologist myself and in discussing vitamins I can depend only on the meager experience I have had as a biochemist who has devoted his life to the study of a cellular function, oxidation. What this experience is, I have told you in my previous lectures. I have shown you that biological oxidation is the function of a very involved chemical mechanism in which a great number of different molecules interact harmoniously like wheels in a machine. To me the cell is an immense mechanism with thousands of wheels, the molecules, the harmonious interaction of which is that wonderful thing we call by the one little simple word, "life." The harmony, the smooth running, of this machine is what we call health.

In this mechanism we find wheels, the molecules, of

the most varied kinds. We find huge, labile molecules with a weight of several millions, and small molecules acting by themselves or acting together with their more bulky brothers. There are substances that are present only in small quantities and there are others like water making 80 per cent of the whole. All these substances are equally as important, equally indispensable as are all the wheels of your watch and whenever a wheel is missing there is disturbed function; there is disease.

Talking now about the more simple molecules present in relatively small quantities, we will find that most of them are fabricated by the cell itself from other elements. A few, however, cannot be made by our cells and must be obtained from food ready-made or at least in such a condition that they will fit into our mechanism after a small adjustment.

This theoretically insignificant and uninteresting quality, of not being fabricated by our cells, gives to this small group of substances, called Vitamins, their great medical importance, for if any of the other substances which we make ourselves is missing from the food there will be no serious consequence. If, however, a vitamin is missing, a substance that we can't make ourselves, we will be unable to replace the corresponding molecules of our cells if they are worn out; there will be a wheel missing, there will be a shortage, disturbed function—there will be disease. These vitamins, thus, can make us ill by their mere absence. This paradoxical behavior is their most characteristic feature. While the great host of all other innu-

merable substances can make us ill only if we eat them, vitamins make us ill if we do not eat them.

But vitamins have not only a medical importance, they also have a philosophical importance; for what is the source of these vitamins? It is mostly the plant. Now, if we say that we take these substances from the plant, what we really say is that we can take the wheels of the plant cell and put them into our own mechanism and that they will fit in perfectly. Now, two machines, the wheels of which are interchangeable, cannot be very different. In this way vitamins give us a most entrancing vista of the great chemical unity of all living nature.

Now let us start out from the modest experience of the biochemist and examine one by one the ideas which vitaminology has given us and see whether we can accept them. Let us start with the name and its implications. Let us begin with the tail end of vitamin, with the "amin." As far as we know most of them are not amines—that's that.

As regards the "vita," their mysterious vital function, there is no more mystery about that. To be sure, life is a mystery and all its instruments are mysterious but there is no difference at this point between vitamins and common salt or water.

Their function is not a mystery either. In our study of oxidation we have met several vitamins. There was ascorbic acid which has, in all probability, the simple function of giving off and taking up H atoms as do so many other substances. There were co-dehydrogenases, with their pyridine rings; they

also are H acceptors and members of the vitamins of the B group. There were alloxazines with an apparently analogous function acting as B_2 vitamin. There was co-decarboxylase with the simple function of being instrumental in splitting off CO_2 from COOH, a substance known as vitamin B_1 and causing, by its absence, beriberi. All these are quite plain and simple functions and we have no reason to suppose that other vitamins should act differently, even if the reaction each may carry out is a different one.

The substances themselves, the vitamins, are no more mysterious either. The biochemist has isolated, analyzed and synthesized them. You can find them now in bottles on the bench of the biochemist, standing in one row with other chemicals such as salt, sugar or oil, looking in no way different. The only difference may be that the biochemist shows them with more pride.

The name vitamin conveys another implication which we must consider more seriously. The name suggests that in some way vitamins form by their very nature a special group of substances. Accordingly, we find theories in literature about "equilibrium of vitamins and hormones," and the like. We must thus consider whether vitamins really constitute a special group, or whether they are just a small number of substances linked together in a more or less haphazard way by the accidental circumstance that we are unable to make them? If they do not form a special group based on some essential quality, then, naturally, all speculations about equilibria between vitamins and other groups have to be rejected. Natu-

rally there is a relation between all substances in our body and the change of any one will induce a change in all the rest. What I mean is, do vitamins as such form a closed group which could act as a whole, having special relations with other groups of substances.

In what way could vitamins be different from the rest? What unity could there be between the different vitamins? Their structure? The chemical structures of these substances are the most varied imaginable. There is no unity in them. There are sugar derivatives, acyclic and cyclic molecules, alloxazines, pyridines, phenanthrenes and others. One could invoke as a special quality the minuteness of quantity in which they act. But hormones and metals act in the same minimal quantities and there are vitamins on the other hand, like vitamin C, which we find in tissue and of which we need correspondingly quite considerable quantities.

We are thus unable to define the vitamins by any other quality than by their capacity to make us ill if if we don't eat them, in other words, by the fact that we are unable to synthesize them. This quality is not too characteristic either, for histidine, tryptophane and many other substances share this property. Thus, we must ask, is this quality, failure of synthesis by our body, due to some special quality of the vitamin molecule which makes its synthesis impossible, or is this rather a defect of our own cells which determines their inability to make them?

There can be no real difficulty in making these molecules or else the plants would be unable to make them.

It is difficult to believe that cabbages should be so much cleverer than we. Thus it looks as if it were not a quality of the vitamin molecule itself, but rather our own cellular limitation which determines that we cannot make them.

After all what is behind all this, why can't we make them? Is it some essential difference between plant and animal which makes the synthesis possible in the former and not in the latter? First of all, is it really true that animals can't make vitamins? There have been some early observations which suggested that it is not a general defect, shared by all animals, for it had been stated that rats, for instance, can make vitamin C. One of the early American polar explorers made the observation that a rat soup would prevent scurvy. Later, M. Pearce showed experimentally that rats really can make ascorbic acid.

Most of the animals of the temperate climates do not get scurvy if fed on vitamin C free diet, but this is, as we know today, not because they don't need ascorbic acid but because they make it themselves.

The cow, the cat, the fowl and rats all can make ascorbic acid, and if you tell a rabbit that ascorbic acid is a vitamin he will laugh at you because to him it is not a vitamin—he can make it. Why is it then that it is only man, the monkey and the guinea-pig that can't make ascorbic acid? The explanation is, I think, a quite simple one. It lies in one of the fundamental laws of Nature which is laziness. Nature never makes anything unnecessarily, and any function that is not used will be lost. Naturally it will not be lost from one day to the other, but if you

bring up a race of animals during a hundred thousand years in a tropical jungle where there is green food all year round with plenty of ascorbic acid in it, you may be sure that that race will forget how to make it. This is the reason why our chief experimental animal for vitamin C is the "guinea pig," which comes from the ever-green New Guineas. An equally good object would be the monkey, but not the rabbit, whose race developed in our zone where there is no green food and thus no ascorbic acid during the winter. If the rabbit could not make ascorbic acid, it would die of scurvy during the winter for no animal can live without ascorbic acid. Our inability to make ascorbic acid is evidence of our tropical origin.

You see thus that it is not the quality of the vitamin molecule which prevents its synthesis in the body. It is our defect, that we can't make it and the reason does not lie in the fundamental structure of our cells but in the accidental circumstances of our origin.

The vitamin nature of a substance has thus no theoretical importance. Its practical medical importance is of greater significance so let us devote the rest of our time to this aspect of the problem as I think certain principles of medical application can be derived even from the modest experience of the biochemist.

We have seen that the vitamins we have already met had a certain function, for instance, decarboxylation. A certain number of pyruvic acid molecules have to be decarboxylated every day and for this we need a certain number of vitamin B molecules. If there are enough of these molecules, the decarboxyla-

tion will run smoothly. An excess will do no good, and were there enough, we could not expect a favorable result from their greater administration. While an overdosage will do no good, we can expect a therapeutic action only if there is a shortage. Thus all that the doctor has to do is to see that the human body should have enough of each individual vitamin, and he will ask us two questions: (1) how many vitamins are there, and (2) how much of each must we ingest daily?

The first problem seems to be hopeless but is, in fact, quite simple. The second seems to be simple but can't be answered at present. As to the first, the number of vitamins, we can make no definite statements. New vitamins are discovered almost every year and there is no reason to suppose that our list is complete. But how can one hope to administer them correctly if he doesn't know how many there are? Nevertheless, I can give a rather consoling answer. We mostly discover a vitamin by the corresponding deficiency disease. The easier a vitamin leads to such a deficiency disease, the greater its practical importance, but, at the same time, the more easily this vitamin will be discovered. Thus it follows that the first vitamins discovered, A, B, C and D, are the most important ones; thus the later a vitamin will come to stand in the alphabet, probably the smaller its importance. Thus the doctor need not think about the vitamins still undiscovered; he may limit his attention to the vitamins designated by the first four letters of our alphabet.

Now let us turn to the second problem, how much of

each single vitamin must we eat? To put it more scientifically, what is the minimum daily dose D.M.Q. (dosis minima quotidiana) of every single vitamin? A very great deal of sane and careful work has been done to find this D.M.Q. Let us watch the vitaminologist at his work and see how he arrived at his answer. Let us take, for example, a C-vitaminologist. What he does is this: he takes half a dozen young guinea pigs and puts them on a diet which contains all necessary ingredients except vitamin C. He finds that for a week or so all goes well. The second week the first symptoms of scurvy appear and growth stops. The third week the animals begin to lose weight and at the end of the fourth week they die with typical symptoms of scurvy, loose teeth, swollen joints and multiple hemorrhages.

To a parallel set of control animals he gives increasing quantities of ascorbic acid and finds that 1.5 mg. a day will prevent the appearance of all scorbutic symptoms. Therefore he says this is the D.M.Q., the quantity necessary to maintain health.

This experience was extended to man. There is practically no scurvy or beriberi in our climate, thus, we argued, there can be no lack of the vitamins C or B.

All would have been well had the chemist not made trouble by isolating and synthesizing the single vitamins, and putting them into the hands of the clinicians who started injecting them in unlimited quantities to all sorts of patients. These experiments gave unexpected results. To take one example, the clinician found that alcoholic neuritis could be cured by the application of massive doses of vitamin B. What is

especially striking and agreeable is that we can go on drinking and yet our neuritis will be cured if only we take enough vitamin B. Now what conclusions must we draw from all this? We have thought that there was no lack of vitamin B in our climate, and anyway alcoholic neuritis is not an avitaminosis. Does B act then as a chemotherapeutic agent like aspirin or the like? This is in contradiction to our conceptions. How then, are we to understand these effects. Something seems to be missing or to be wrong in our fundamental conceptions and we must find out what it is, or where we have been led astray.

In my opinion, the solution lies in the unwarranted conclusions we made from animal experiments. Let us repeat once more how these were done. We found that if we gave, for instance, 1.5 mg. ascorbic acid daily to guinea pigs, our animals had no scurvy, were thus healthy. But is this true? Is it right to divide the world into "scurvy" and "no scurvy." How do we know that they were healthy just because they had no scurvy—is "no scurvy" the same as health? Can we talk at all about health if we have nothing more before us than a few guinea pigs sitting in a well protected cage, and without scurvy? Do we call it health if we sit in a corner and have no scurvy? No, certainly not.

What, then, do we call "health," "perfect health"? We must answer this question, for "health" is the ideal, the highest aim of medicine. I think we must call "perfect health" not the absence of scurvy or other disease, but a condition of the body in which it is capable of the highest performances, in which it

shows the greatest resistance against all noxious influences, physical, chemical and biological.

It thus follows that should we wish to find out whether our guinea pigs were healthy, we should not allow them to sit quietly in a corner but should subject them to all sorts of strain, physical, chemical, and biological, should make them work, expose them to cold, toxins, infections and see what is the quantity of vitamin at which they do best. The dose of vitamin, the further increase of which would fail to give increased protection, would be the real optimal daily dose.

We must distinguish between two dosage requirements, the D.M.Q., the quantity of the single vitamins sufficient to prevent scurvy or other manifest deficiency disease, and the D.O.Q., (the dosis optima quotidiana) the quantity necessary to maintain our body in optimal condition—in perfect health. Experimental vitaminology has taught us about the first, but has misled us in regard to the second.

In the literature we might find some indication about this D.O.Q. Thus, S. S. Zilva finds that he needs 1.5 mg. of ascorbic acid to keep his guinea pigs "healthy," but must administer about 15 times this quantity to keep them saturated. He thinks this 20 mg. of ascorbic acid a day for a small guinea pig is a crazy amount and has no biological significance since his animals were healthy anyway. But is 20 mg. such a crazy amount? Such a small guinea pig consumes about 100 g. of green food daily in his original surrounding and this green food contains on the average 20 to 30 mg. of ascorbic acid, just the quantity

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Zilva finds necessary for saturation. This saturation is thus the normal state of the animal. I expect that for any vitamin, the D.O.Q. will be found to be the quantity the animal consumes normally in his natural habitat

Naturally, the D.O.Q., found in the animal experiment can tell us little about man. To establish the necessary data for man we must work on man, and it is most important that this should be done.

The only way to approach this question for man is by statistics and mass experiment. We would have to increase the quantity of vitamins in a big group of the human population and see at which quantity the incidence of disease becomes the least, or at which quantity certain performances become the best. So long as resistance or performance can further be improved, we cannot talk about perfect health, for to increase resistance means to improve health and a health that can be improved is not perfect health.

I find this problem so very important that some years ago I prepared $3\frac{1}{2}$ kg. of pure ascorbic acid in the hope of finding some organization or individual who would care to make such mass experiments. I was disappointed for there is no organization which can undertake such work, and it is difficult to find individuals who care to participate in a work in which personal merit gets lost in a mass of statistics.

If we thus define health not as lack of scurvy, but as something much higher and subtler, all those contradictory clinical experiments become at once intelligible. They simply tell us that there is a great difference between scurvy or beriberi and perfect health.

The two conditions are separated by a wide margin, corresponding to the condition of hypovitaminosis, a condition in which there is not enough vitamin to keep us healthy but too much to allow us to develop scurvy or another deficiency disease, a condition which has no other symptom than that our body is unable to develop its maximum resistance against noxious influences, a condition in which it will stand a greater chance of becoming ill, and once ill will not be able to develop to the maximum its defense. If we administer vitamin in this situation we will have introduced no chemotherapeutical agent but only will have paid our old debt to Nature, thus enabling the body to develop all its natural forces in its fight against disease.

A considerable part of mankind seems to go through life with such a hypovitaminosis. Most of us may perhaps get through unpunished. If, however, this hypovitaminosis associates itself with some lesion like an excess of alcohol, it might lead to a neuritis. If it is associated with an infection, it will lead to some infectious disease. If it is associated with a congenital debility of the capillary vascular system, it will lead to hemorrhagic diathesis. So, if in a thousand people having exactly the same food, only a few become ill; still a lack of vitamin might have a deciding influence in the development of their disease. It might just have been "the straw that broke the camel's back." How far the administration of the vitamin will have a curative action in that disease will depend on the extent to which the vitamin played a rôle in its genesis, and on the question of how far the pathological changes are still reversible.

Whenever I think about these curative effects obtained by the administration of vitamins, there is always one problem that suggests itself to my mind. The problem is this: If we can cure, or at least ameliorate, some disease by the application of a vitamin, what would have happened if our patient had had plenty of vitamin before he contracted his disease? It is an age old experience that it is much easier to prevent than to cure a disease. So the probable answer to my question is that, if our man had had enough vitamin, he would never have become ill.

This leads me to the most important aspect of vitaminology, to the problem how far vitamins can help us to prevent disease. In the end the ideal of modern medicine is not so much to cure as to prevent disease.

This general problem of health and disease which has occupied my mind very much for my whole scientific career, is dominated by two big contradictory impressions. As a medical student, I learned about all those thousand diseases humanity is suffering from. I was 32 years old when I failed in my medical examination because I could not remember the names of all these thousand ailments. Since then, as a biochemist I am living in silent admiration of the wonderful precision, adaptability and perfection of the living organisms. Medicine taught me the shocking imperfection, biochemistry the wonderful perfection of our body and I have wondered where the contradiction lies. Anything Nature produces seems to be perfect. Should, then, man be the only imperfect creature kept alive in face of all his imperfections only by

means created by his own mind? If not, where do all these ailments come from, how must we understand them. This is the great fundamental problem of medicine, the great fundamental problem of health and disease, and we must try to answer these problems and try to advance from the description of the single diseases to some more general conception of health and disease; such a concept might help us in our efforts to lead humanity towards a period of better health and greater happiness.

Allow me to terminate this series of lectures by giving you the general theory of health and disease as I have formulated it for myself. Naturally, you will have to excuse me if I, for the last time, forsake the solid ground of experiment.

To understand ourselves better we must not study the man on the pavement of some big city; for such a study we had better go back to the surroundings of our remote ancestors in the tropical jungle.

The "individual" has little meaning in the jungle; the whole jungle represents one big organism. The nitrogenous matter of the jungle, is in its greatest part, always present in some living form. This nitrogenous organic material is not the private property of the living individual, he got it from the jungle and soon will have to return it to the jungle. Individual life is but a short temporary assemblage of a quantity of matter which, after the individual's end, will soon be dispersed again and will appear in new living form. But even that quantity of organic material which makes an animal, let us say a monkey, is not sharply separated from its surroundings. In the form of food

ingested and air respired, a great number of different molecules pass through our monkey's body. In this way our monkey not only lives in his surrounding, he is, in fact, a part of his surrounding. To be able to exist, he must be perfectly adjusted to his surroundings. As soon as his adaptation becomes incomplete he will be ruthlessly destroyed. So long as he is perfectly adjusted he is safe and healthy. Health means, then, no more than such a perfect adaptation to his environment into which our monkey's body fits as a cogwheel into a machine. This is health-adaptation. There is nothing like "healthy" or "unhealthy" in the world. What is "meat for the one is poison for the other." If you put a rabbit under water he will say water is unhealthy; the fish will feel uneasy on land. There is nothing like healthy or unhealthy and it all depends on what we are made for, and all living creatures have to be perfectly adjusted to their surroundings. And they are adjusted, perfectly adjusted, for this is a law of Nature that any individual mal-adjusted to his surroundings must perish. Anything Nature produces has to be perfect in its essence, and by this rule the monkey is perfect. There is no reason, then, to suppose that our own body is made less perfectly than that of any other individual.

But take now such an individual, perfectly adjusted to the jungle, and put him suddenly on the pavement of New York, give him smoky and dusty air to breathe, put him in this environment which might be too hot or too cold, too dry or too humid; shut him up in a dark room, cover up his body and shut out the sunshine, expose him to noise and to all sorts of undue

excitement, put substances into him, feed him with things to which he is unaccustomed, and the formerly perfect body will fail. He will go to the doctor and the doctor will tell him all sorts of funny names of the diseases he has got.

To cut a long story short, I believe that the human body is just as perfect in itself as the body of any other individual, that disease is not the innate quality of our body but arises from the disharmony of our make-up and our surrounding. There have been one or two big experiments in history which prove this point. One of them is the story of the island of Tristan da Cunha where man of our own race lives a natural life and knows no disease.

Naturally, if you accept this general theory of disease, you will ask what to do about it. Shall we go back to the trees? This would not work; maybe there are not even enough trees for all of us. No, we can't go back. But, there is science to help us to find out which of the factors originally present in our original surroundings are essential and which are not; and then we must see to it that we get these factors into our homes, into our lives.

These factors might be manifold: the purity, the humidity, and the temperature of the air, the quantity of noise and the excitement, physical work, insolation, etc. But certainly one of the most essential factors in our coordination with our surroundings is our food, for food is the form in which our surroundings penetrate into our body in rough, big quantities; and it is equally certain that vitamins are a most important factor in this coordination. Very much has been done

already along this line. Modern transport makes it possible to bring home to us our original primitive surroundings in the form of fruits of the South and it is partly due to this that, as old doctors tell me, running ears, open submaxillary glands, curved and suppurating bones are no more the usual aspect of a child, as they used to be in some European countries a few decades ago. But, there is still a great deal to be done and even expensive food is sometimes shockingly bad from a medical point of view.

I think we all have still a somewhat medieval outlook on disease. We accept disease as one of the innate qualities of life. We think that as death is a quality of life and our machine has to stop some day, so it has to go wrong now and then; this is quite wrong. Death, yes; but disease, no. Disease is not an attribute of life. It is innate in life to be perfect and to know no disease. There are two reservations I must make. The one refers to epidemic infectious disease where our life has to fight another life, that of bacteria which is equally as perfect as ours. Epidemics are known also in the animal world. This danger is not too grave for modern hygiene has dealt with it.

The second reservation is more serious. Any product of Nature is perfect, since any deviation, any imperfection will be ruthlessly culled out—the weaker animal will be eaten by the other. So, Nature gives no chance to the degenerate to reproduce his imperfections. But man, by his charity and other artificial means, keeps the weak alive and gives him a chance to propagate his own weakness; so, unlike other beasts,

there has been ample chance for man to produce imperfect forms.

Do not accuse me of having lost myself in useless speculation. These thoughts are capable of immediate practical application. In fact, I am applying them myself in my own life and small family rather successfully. In the course of the autumn, being closely confined to my laboratory, my resistance decreases and in December I usually get my first cold or some other disease. In the early spring I often had to spend a few weeks in bed. I used to call in the doctor who told me a number of Latin names ending with "itis," and gave me pills which I took in good faith but with little success. Now, I deny the existence of all these "itises" and at the first sign of disease I put my body, with a pair of skiis, on a snowy slope where I can find the greatest number of my ancestral rights fulfilled, such as fresh air, exercise and sunshine, and the "itises" exist no more for me.

These theories have been, in fact, applied unconsciously. Tuberculous patients are sent to the mountains and it is known that no TB exists above 9000 feet. It is not that the mountain air has a curative effect for it is not a therapeutic agent; it is simply the air we are made for, and if we have what we are made for there will be no TB.

At any rate, I think a wider outlook on life, on health and disease, a wider application of scientific methods and their results might still lead to a drastic reduction of disease and human suffering and a corresponding increase in human happiness. Vitamins,

their proper application and knowledge, certainly represent one of the most important factors in this connection. If, on the top of this, humanity would learn to use science really to its advantage and would learn to use its methods and results for edification and not for destruction, and in the end, if we could even learn to apply between social groups and nations the same simple code of morals which we accept for our individual life, humanity would march towards a period of health and happiness unheard of in the annals of our history.

Ladies and gentlemen, I have arrived at the end of my story. Allow me in closing these lectures to sound a personal note. I have given you a summary of all the problems that have occupied me during these last fifteen years, which have been the source of my great pleasures and disappointments. I am unable to review this work without being filled with the deepest gratitude to all those who helped me on my way, which has not been devoid of hardship. My way took me through many countries and wherever I went I found kindness, good-will and international, intellectual solidarity. Towards your country with its well known broad magnanimity, I feel a double obligation. Some ten years ago when difficulties began to weigh too heavily on me and I staggered under the load, it was the Rockefeller Foundation which stretched out its helping hand, and led me out of my small cellar room and opened the opportunities of the wide world of science to me. I owe it to the Josiah Macy, Jr., Foundation, New York, that I could put, during these last years, all my thoughts to a test unhampered by

material difficulties, and could build up a school of young, happy and devoted research men. In fact, the greatest part of the results reported here were reached under the auspices of this foundation. Your kind invitation which brought me from Hungary to Nashville, your kind patience and your splendid hospitality are expressions of the same spirit.

I can only wish that this spirit of human solidarity may spread beyond the limits of science and may conduct us all towards a brighter future.

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