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Trails of inquiry and thought leading toward today's bioenergetics

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Significant experimental observations and concepts leading from various sources toward today's bioenergetics are briefly sketched. To limit the essay to manageable proportions main consideration is given to origins in research on metabolism and biological oxidations and attendant energy conversions. Relevant data and dates are summarized in table form.

Introduction

It was somewhat of a surprise to me, when Dr. Wikström asked me to speak here at this occasion, as I am one who is not really in what is called bioenergetics today – but may be was in it before it was called bioenergetics. I, therefore, assumed that he would not expect me to tell you the latest in your field, but rather some of the earliest. With this thought in mind, I accepted the challenge; however, in trying to refresh my memory and reading up on old papers and reviews, I was overwhelmed by the magnitude of the task and I realized how inadequate a substitute I am for those who should speak to you here. Unfortunately, many of them are no longer with us; the last decade has taken a heavy toll of those whom I would consider pioneers in this field. In comparison, I was more an interested bystander. However, during my school and student days I have been close to some of the people who have contributed, I have been at some of the locations where they worked, I heard them lecture and, what also seems important to me in the historical perspective, I have grown up in the intellectual and social atmosphere of those days, which was indeed in many ways different from the present one. The development of a field of science is not independent of these factors. Of course, this takes us back only 60 years or so; however, I have still heard stories from witnesses of the second half of the last century. Thus, for instance, I knew a chemist, who was 'scientific assistant' to Justus Liebig, who made his great contributions by the middle of the 19th century; or my grandfather told me of lectures by Wilhelm Bunsen.

When did bioenergetics begin as a field? I do not remember having heard the word before about 1960. I was working in 'Biological Oxidation' as a postdoctoral fellow in the fifties, and it seems to me that this field was a major forerunner of bioenergetics; and even one or two decades earlier many referred to it as 'Metabolic Biology'. I would like now to trace this path further into the past and try to convey to you some of the spirit, the attitudes, thoughts and endeavors of those bygone days, as I perceive them from the record and my own impressions. We may then see how this path will eventually connect with our endeavors of today. I am always particularly fascinated by the visions, which some of the pioneers had of the future, often centuries ahead of their days; visions, which were later verified, and in some cases I would like to quote directly from them.

Blood circulation and respiration

Alert human beings were at all times curious what brings about and sustains life, with the most obvious features of life being heartbeat, blood flow, breathing, production of heat, digestion, motion and growth. In the tables I have assembled instances of decisive progress, starting from about 1600 A.D. * The individual tables cover parallel developments in different areas and are arranged on an appropriate timescale. As usual, structure is the safest thing to explore and thus anatomy and later microscopy have made their contri-

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* The extensive historical accounts of David Keilin [1] and of Joseph S. Fruton [2] have been very helpful guides to the older literature.

butions. The major and then the capillary circulation were explored, the latter establishing the continuity of blood flow. Red blood cells, arteries and veins were observed and so was the difference in color of arterial and venous blood. It was found that only part of the air is consumable by a flame or a respiring animal. This part was called 'vital air' or 'vital spirit'. At that time no consideration was given yet to the eventual product of respiration. It was only by the middle of the 18th century that the presence of a 'fixed' air was observed, later identified as CO_2. Then in rapid succession nitrogen (azote) was identified as well as hydrogen and oxygen. Priestley even observed the regeneration of the vital air by a green plant, closely missing the phenomenon of photosynthesis. Lavoisier's contributions toward the end of the 18th century stand out in accuracy and significance. His work was instrumental in effecting the demise of the 'Phlogiston' theory, which dominated thinking in this field up until then and inhibited progress. According to this theory oxidation consisted in expelling something – namely phlogiston – from the oxidized substance rather than combining the substance with oxygen. However, even Lavoisier did not proceed beyond the concept that during respiration 'combustion' occurs in the lungs and the blood and that the substrate is the 'chyle', entering the blood from the liver and containing the nutrients. That respiration is actually occurring in tissues, even in the absence of blood, e.g., in some insect tissues, was only proposed at the turn of the century and in fact established as late as the latter part of the 19th century. By the middle of the century, G. Liebig observed that respiration in muscle increased with activity and concluded from his work that blood was only a transport agent, but not the site of respiration. Pflueger, who convincingly demonstrated that the tissues were the site of respiration, still held the view that the tissues themselves were consumed in the process.

Fermentation

During these developments attention was also focussed on fermentation, which was the area of most practical importance among those mentioned so far. Black, who had identified the 'fixed' air, also found that the gas bubbles rising in a fermenting liquid were CO_2. Lavoisier recorded a complete balance sheet of all the known substances participating in the alcoholic fermentation of yeast. Sterilization became known and was practically exploited by the turn of the 18th to the 19th century. Gay-Lussac concluded from his study of the sterilization procedure, that it was the absence of oxygen that prevented the onset of fermentation in sterilized samples. Although Antonie van Leeuwenhoek had reported seeing small particles in a fermenting solution late in the 17th century, it remained for Cagniard-Latour to provide proof by direct microscopic observation of samples during the brewing process that, as the process went on, the yeast multiplied (1838). Berzelius and J. Liebig, two of the most renowned chemists of that time, remained unconvinced that the particles seen were living organisms and violently rejected the interpretation that living organisms were causing fermentation. Both of these men are recognized for their original and progressive views and accomplishments at that time. However, not having available in their mind any picture of macromolecules and the intricate chemical makeup of cells which we now enjoy, they were unable to visualize that there were indeed some chemical structures akin to those known to them, that were present in those tiny particles or organisms and brought about fermentation. Although J. Liebig together with Woehler had discovered the enzyme emulsin, which hydrolyzes the glycoside amygdalin, he failed to make the association between the findings on fermentation and enzyme action as he had observed it. It was only very reluctantly that both Berzelius and Liebig, shortly before their death (1839 and 1870, respectively), accepted the view of living yeast being involved in fermentation. Pasteur's brilliant studies in the last decades of the 19th century had brought the inescapable evidence, which Liebig could not reject. However, Pasteur himself, who, in addition, had identified aerobic and anaerobic microorganisms and had done much to lay the foundations for modern microbiology, fell victim to a similar misjudgment, as had Berzelius and Liebig. Convinced from his studies of the enormous versatility and viability of microorganisms, he rejected the idea that was more and more taking hold at the end of the 19th century, namely that reactions akin to those occurring in fermentation, were catalyzed by what was called 'ferments' at that time. He stated clearly that in his opinion living yeast itself is the ferment and that there was no need for additional special substances, called 'unorganized ferments' (corresponding to today's 'enzymes', a word introduced by Kuehne in 1877). It was only two years after his death that the crucial experiment was done by E. Buchner, which showed that fermentation could be obtained in a filtered press-juice of yeast. While there was skepticism about the validity of this experiment, as it probably always is, if someone has done a startling experiment (which it indeed was at that time), the awarding of the Nobel prize in chemistry to Buchner in 1907 proves that Pasteur's view did not prevail.

Catalysis

We referred to Berzelius above and his views of contemporary scientific questions and also to what was known as ferment at that time. It seems, therefore, appropriate to consider developments in the field of catalysis. Around 1800 Vogel has shown that O_2 and H_2 could be brought to react over charcoal to form water. Similarly, Humphry-Davy had been able to oxidize ethanol to acetic acid over platinum black. Berzelius was deeply impressed by these experiments and stated that one was obviously dealing here with a hitherto unknown 'new force' which he called 'catalytic force'. He went on to say: * "We can now assume on very good grounds that in living plants and animals thousands of catalytic processes take place between tissues and fluids, thus giving rise to multitudes of chemical compounds. We have so far been unable to propose acceptable mechanisms for the formation of these compounds from common raw materials, plant juice or blood, but the future may well reveal them in the catalytic power of the tissues from which the or-

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* Presented in the translation by D. Keilin from the original German text with permission from Dr. Joan Keilin Whiteley and Cambridge University Press.

TABLE I

Early contributions in areas leading toward bioenergetics

	Anatomy/physiology	Microscopy/spectroscopy	Chemistry/gas analysis	Fermentation/putrefaction
1600				
	Blood circulation (Harvey)		Consumable part of air: 'vital spirit'/ no consideration of product of respiration (Boyle, Hooke, Lower)	
		Capillary circulation/continuity of circulation (Malpighi)		
1700		Arterial color in aerated blood (Hooke, Lower, Mayow)		
		Red cells, arteries, veins/yeast cells (Van Leeuwenhoek)		
			'Fixed air' as product: CO_2 (Black) Nitrogen (Rutherford)	Gas bubbles observed in fermentation: 'fixed air' (CO_2) (Black)
			Regeneration of vital air by a plant (Priestley)	
	Tissue respiration (Spallanzani) even with bloodless organisms/tissue is thought to be substrate		Quantitative analyses: O_2, N_2, CO_2, H_2; O_2/CO_2 exchange in blood and lungs; 'chyle' consumed in blood	Quantitative balance sheet for fermentation/internal oxidoreduction postulated (Lavoisier)
1800			'Phlogiston' theory abandoned (Lavoisier)	Sterilization by heating and storage in closed containers (Appert)
		Yeast multiplies as fermentation proceeds (Cagniard-Latour)		O_2 assumed to produced putrefaction (Gay-Lussac)
1850	Respiration of muscle increases with activity/blood is only transport agent (G. Liebig)			Identification of 'sugar fungus' (Schwann)
		Spectrum of HbO_2 (Hoppe-Seyler)		Microscopic observation of yeast multiplication during fermentation (Cagniard-Latour).
		Reversible 'reduction' of HbO_2 (Stokes)		Rejection of the idea that living organism causes fermentation (Berzelius, J. Liebig)
				Detailed study of fermentation (Pasteur)
	Respiratory gas exchanges of blood; foodstuffs, not tissue is consumed. (Bert)			Definition of aerobic and anaerobic microorganisms.
1875	Respiration located in cells/tissues consumed in respiration (Pflueger)	UV-bands in heme-proteins (Soret) Myohematin, histohematin (Mac Munn)		Yeast is 'ferment': rejection of idea of special catalytic substance (Pasteur). Acceptance of Pasteur's view that fermentation requires live organism (Berzelius 1839, Liebig 1870)
1900				Fermentation in cell-free extract of yeast (Buchner)
				Identification of hexosephosphates in cell-free fermentation of yeast (Harden and Young)

gans of the living body are built up." We can only marvel at this visionary statement, which, however, at the time it was made, was not able to elicit any further advances. The mechanism of function of these catalysts remained mysterious. They were also termed 'contact substances' and were thought to act by close contact with their substrates and to impart on them some of their energy. There was no basis at that time to be more specific. Table II shows that in the latter part of the 19th century a number of ferments were found, a development, which was bound to lead to more advanced views in the decades that followed.

Biological oxidations and dehydrogenation

Table II shows progress from 1800 A.D. until the first third of the 20th century in biological oxidations, which we can consider now as an offshoot from what was the subject of Table I. Schoenbein, who discovered ozone in 1840, observed blueing of a resin (guaiac) that was in use for pharmaceutical purposes. He found that this reaction, while also elicited by oxygen and H_2O_2, was particularly strong with ozone. It was reversible with reducing substances. Similar reactions were later observed with slices of mushrooms. These observations were probably the beginning of the use of oxidation-reduction dyes and indicators, which flourished later in the century, when the new synthetic industrial dyes became available. Schoenbein and his contemporaries were impressed by the power of the 'active' oxygen which they saw in ozone and it was suggested that this was somewhat akin to what was observed with ferments and that ferments might simply generate active ozone-like oxygen, which would bring about the degradation of foodstuffs. This view, however, was strongly opposed by Pflueger, who showed in the 70's that cellular combustion was largely independent of the partial pressure of O_2. It was, however, not clear which intracellular substances were metabolized.

While hydrogen of substrates, not only carbon, had been considered earlier as a possible fuel in respiration, the properties of 'nascent' hydrogen discovered in

TABLE II

Progress in biological oxidation and catalysis in the 19th and early 20th century

	Catalysis	Biological oxidation
1800		
	$H_2 + O_2 \rightarrow H_2O$ over charcoal (Vogel)	H_2O_2 discovered (Thenard)
	Ethanol → acetic acid over Pt (Humphry-Davy)	
	'Diastase' (Dubrunfaut)	
	Pepsin (Schwann)	
	Catalytic Power, a new force (Berzelius)	Ozone discovered (Schoenbein)
	'Contact Substances' (Mitscherlich)	
		Blueing of guaiacum (a resin) by ozone and H_2O_2 (Schoenbein)
	Emulsin (Woehler, J. Liebig)	
	Lipase (Bernard)	
1850		
	Definition of 'ferments' as catalysts (Traube, Schoenbein)	
	Invertase (Berthelot)	
	Trypsin (Kuehne)	'Nascent hydrogen' proposed as substrate (Hoppe-Seyler)
1875	Urease (Musculus)	
		Laccase, the term oxidase coined (Bertrand)
		Oxidases as catalytic metal proteins
		Oxidizing ferment, O_2-carrying ferment proposed/muscular activity as respiratory act (Traube)
	Peroxidase (Linoissier)	'Indophenol oxidase'/O_2 transport by organically bound Fe (Spitzer)
1900		
	Catalase (Loew)	
		'Respiratory enzyme', an Fe compound (Warburg)
		CN as reversible inhibitor of respiration (Batelli, Stern)
	Competitive inhibitors (Quastel)	
		Theory of hydrogen activation vs O_2 activation/quinones, methylene blue as oxidants (Wieland)
		Metal containing oxidases; laccase, tyrosinase (Warburg, Keilin and collaborators)
		Dehydrogenations in washed tissue minces/specificity for substrates (Thunberg)
1940		

chemistry induced Hoppe-Seyler to suggest this species as the reactant with respiratory oxygen. While this did not carry much conviction, we can see in it the forerunner of ideas coming up some 40 years later on the activation of hydrogen by dehydrogenases (Wieland, Thunberg). From the present standpoint, the most decisive conceptual and experimental contributions were made in the last quarter of the 19th century by Traube, who expressed advanced views on the mechanisms of oxidation and fermentation. He considered muscular activity as intimately connected with respiration and suggested that specific oxidizing or oxygen carrying ferments are present in tissues. His contemporary Bertrand purified laccase and coined the term 'oxidase'. The oxidation-reduction of indophenol became an indicator of oxidase activity in tissues and Spitzer concluded from his experiments that organically bound iron was involved in the respiration of tissues.

The beginning of the 20th century then brought

TABLE III

Important experimental contributions to our knowledge of oxidative phosphorylation

1906	Harden and Young	Phosphorylation of hexose in cell-free fermentation of yeast: 'coferment' of fermentation	[6]
1914	Embden	Phosphorylation of hexose in muscle glycolysis	[7]
1917	Meyerhof	Coferment of fermentation in animal tissues	[8]
1925	Keilin	Cytochromes	[9]
1927	Eggleton and Eggleton	Labile phosphate in muscle contraction	[10]
1928	Warburg and Negelein	Photodissociation spectrum of the 'Atmungsferment'	[3]
1929	Fiske and SubbaRow	Labile phosphate in muscle contraction is creatine phosphate	[11]
1929	Lohmann	Adenine nucleotides; ATP as possible energy source in muscle contraction	[12]
1930	Lundsgaard	Muscle contraction in absence of lactic acd	[13]
1932	Engelhardt	Oxidative phosphorylation in pigeon red cells	[14]
1936	Schlenk	Structure of 'Cozymase' (DPN)	[15]
1936	Warburg and Christian	TPN and DPN as cofactors	[16]
1938/39	Kalckar	Oxidative phosphorylation in cell-free extracts of kidney	[17]
1939	Warburg and Christian	Substrate level phosphorylation	[18]
1939	Negelein and Brömel	Substrate level phosphorylation	[19]
1939	Lipmann	Phosphorylation on oxidation of pyruvate in *Lactcbacillus delbrueckii*	[20]
1939	Belitser and Tsibakowa	Oxidative phosphorylation of creatine in minced heart muscle; $P/O > 1$	[21]
1940	Lipmann	Pyruvate → acetylphosphate in *Lactobacillus delbrueckii*	[22]
1940	Krebs and Eggleston	Tricarboxylic acid cycle	[23]
1941	Claude	Mitochondria	[24]
1941	Ochoa	Ox. phos.[a] in cell-free brain preparation; $P/O > 1$	[25]
1941	Engelhardt	Myosin has ATPase activity	[26]
1945	Lipmann	Coenzyme A	[27]
1949	Kennedy and Lehninger	Ox. phos. associated with particles	[28]
1949	Friedkin and Lehninger	Ox. phos. with NADH as substrate	[29]
1951	Kielley and Keilley	Ox. phos. is associated with mitochondria	[30]
1951	Lynen et al.	Acetyl-CoA as 'active acetate'	[31]
1952	Lardy and Wellman	Respiratory control in mitochondria	[32]
1953	Sjöstrand, Palade	High resolution EM pictures of mitochondria	[33,34]
1953	Cohn	Oxygen exchange between water and ATP	[35]
1954	Arnon et al., Frenkel	Photophosphorylation	[36,37]
1956	Boyer et al.	^{32}P-ATP exchange	[38]
1956	Chance and Wiliams	Spectroscopic definition of components of respiratory chain	[39]
1957	Crane et al.	Ubiquinone as mitochondrial constituent	[40]
1960	Beinert and Sands	Additional components of the respiratory chain	[41,42]
1962	Hatefi et al.	Separation and reconstitution of respiratory chain components	[43,44]
1966	Jagendorf and Uribe	'Acid bath' phosphorylation of chloroplasts	[45]
1971	Kagawa and Racker	Liposomes; reconstitution of ox. phos. in liposomes	[46]
1973	Boyer et al.	Not synthesis but release of ATP from synthetase requires energy	[47]
1976	Drachev et al.	Direct measurement of membrane potential with liposomes inserted into membrane	[48]
1977	Wikström	Cytochrome *c* oxidase as proton pump	[49]

[a] Ox. phos. stands for oxidative phosphorylation.

increased activity in the field, when Warburg started his classical experiments on the 'Atmungsferment', the respiratory enzyme. However, under the influence of contemporary thinking about the nature of proteins and enzymes, the earlier work of Warburg on biological oxidation by undefined iron compounds did not proceed significantly beyond the colloid-chemical model (see below: Development of concepts). It was only in his studies on the inhibition of the respiratory enzyme by CO that Warburg was able to make substantial progress. From this epoch then stemmed his brilliant work on the photochemical action spectrum of the respiratory enzyme [3] which was recognized by the Nobel Prize in Physiology and Medicine in 1931. While this was a great step forward, the question as to the specificity of the oxidative process was not satisfactorily answered.

In the second decade of our century, which includes the period of World War I, Wieland studied the anaerobic reduction of artificial oxidants such as methylene-blue or quinones in model systems. He also found that bacteria were able to oxidize ethanol to acetic acid and that xanthine oxidase ('Schardinger's enzyme') could oxidize its substrates under these conditions in the absence of oxygen [4]. He proposed that it is not oxygen that has to be 'activated' for interaction with substrates, but that there are dehydrogenases in tissues which 'activate' the bound hydrogen. This idea was supported by Thunberg [5] who introduced the method of measuring the bleaching time of methylene-blue in an evacuated tube with a sidearm, from which a specific substrate can be added to a tissue mince washed free of endogenous substrates. Through differential sensitivity of the individual enzymes present to heat and freezing, Thunberg could show that there must be a variety of dehydrogenases and that there was indeed a remarkable specificity for substrates. It was then Keilin's (re)discovery of the cytochromes in 1925 that provided the link between Warburg's oxidizing Atmungsferment and the Wieland-Thunberg dehydrogenase systems in tissues. There followed a new period in studies on biological oxidations, which one may call the period of phosphate and phosphorylation.

Phosphorylations during fermentation and oxidations

The period of phosphate and phosphorylation – not to be confounded with the much more recent period of protein phosphorylation – was ushered in by the finding that sugar phosphates are formed in fermentation and also in glycolysis (Table III). As this period is much closer to our days, Table III may serve as a guide through the decisive events without much additional comment. The discovery of a 'coferment of fermentation', of phosphocreatine, of the adenine nucleotides, the finding that phosphocreatine is a more immediate source of energy for muscle contraction than lactic acid, the observation of oxidative phosphorylation in minced tissue and then in cell-free extracts, the first documented case of substrate-level phosphorylation, namely on oxidation of phosphoglyceraldehyde, the development of the concept of 'high-energy' phosphate compounds and the recognition of the central position of ATP in energy conversions and then the observation of oxidative phosphorylation during electron transport followed each other rapidly within about 15 years. The basis for this last discovery, namely phosphorylation during e^- transport, was Keilin's discovery of the cytochrome system, which linked the site of substrate dehydrogenation to that of oxygen uptake, and the establishment of the tricarboxylic cycle as the principal device for the oxidative degradation of C_2 fragments to CO_2 and water. While in the earlier work in this period most interest focussed on obtaining cell-free preparations or active extracts, it became evident in the late forties that for oxidative phosphorylation during e^- transport some elements of structure are required. This led to an interest in subcellular structures – with the electron microscope making decisive contributions – and mitochondria were eventually identified as the sites of ATP production. This was a critical finding, inasmuch as it introduced the transition to the period we might call that of the membrane and vectorial transport.

Development of concepts

We must, of course, view all of these developments on the background of contemporary progress in adjacent fields such as inorganic and physical chemistry, which took large strides forward in the latter part of the 19th and early 20th century. I would, therefore, like to draw attention to the subtle interplay of experimental and conceptual contributions before I illustrate what has been said with quotes from some of the principal contributors.

It is beyond the scope of our theme to try going into any detail, however, as we are aiming toward bioenergetics, we should briefly mention the definition and elaboration of some basic concepts in physical chemistry, foremost among them the laws of thermodynamics and the principles of electrochemistry, from which follows the concept of 'free energy'. We find in the literature, well into the 20th century, neglect of the distinction between free energy and the 'heat' of a reaction. It is only through clarification of these terms and underlying principles that progress in the understanding of chemical reactions and their relationship to physiological phenomena could eventually be reached. The names of Robert Mayer, Hermann Helmholtz, J. Willard Gibbs, James Joule, Wilhelm Ostwald and Walther Nernst come to mind here.

When mentioning Warburg's early experiments on model systems of biological oxidation, we touched on an example as to how contemporary thinking in related fields may influence the interpretation of experimental results. At the time when these experiments were done what was called 'Colloid Chemistry' was on the rise and, according to the views then current in this field, the properties of gel-like substances, to which proteins were also counted, were explained in terms of aggregates of smaller molecules, as they were then familiar to chemists, and adsorption to surfaces of colloids was an important topic. As there is a connection here to the model for the action of catalysts (see above) which was thought to occur by surface contact, these concepts were very much on the minds of those dealing with ferments. It may be of interest in the context of colloidal aggregates vs. individual macromolecules that one of the greatest chemists of that time, Emil Fischer, stated in 1913 that a synthetic carbohydrate derivative of M_r 4021, obtained in his laboratory, was larger or as large as most proteins [50]. The idea of macromolecules with a linear primary structure was developed only a decade or more later. It is well to remember here that Richard Willstaetter, one of the most esteemed bioorganic chemists of that time maintained that enzymes are not proteins as late as 1927 [51]. Concerning more recent progress, I have listed in a separate table (Table IV), what one might see as the main conceptual contributions. There is, of course, some overlap, when experimentalists have also been the ones to formulate the concepts. It will be evident that, without some of the concepts that were elaborated, progress would most likely have been much slower. The concept of the high-energy compounds, of the 'squiggle', was so successful and dominated thinking in the 40's and 50's that the primary high energy intermediate was generally visualized as being of an analogous nature, as it was expressed in the 'chemical hypothesis' of oxidative phosphorylation. Thus, it took someone raised outside the mainstream of biochemistry, to point to the – literally – missing dimension, namely to the likelihood that reactions in structured and compartmented organelles will have a directional component, which is not considered in homogeneous solution chemistry. In the statements (Table IV, 1977) by the principal participants in the debate about the mechanism of oxidative phosphorylation it is made clear that, while the general principle of vectorial transport is recognized as one of the, or even *the* governing principle in the process, there should still be room for other types of high-energy intermediates. Depending on the life-time one has in mind for such intermediates, there may, of course, be an almost infinite number of them. Just as the properties of subcellular structures make it possible to conceive of vectorial reactions, so it seems equally possible to conceive of the structures of protein molecules as temporarily assuming 'energy-rich' conformations and thus representing transitional intermediates, as has been suggested in the 'conformational' hypothesis.

Quotes from contributors

One of the great events – if we may consider them as a single one – were the two consecutive Symposia on Phosphorus Metabolism at the McCollum-Pratt Institute at Johns Hopkins in 1951/52. They were an effective forum for ideas and for the latest experimental information. Just a few months before his untimely death, Otto Meyerhof gave the introductory lecture and we can sense his personal involvement and his appreciation for the significance of the event in his

TABLE IV

Concepts in the development of the field of oxidative phosphorylation

1925	Keilin	The respiratory chain	[9]
1937	Krebs and Johnson	Citric acid cycle	[52]
1939	Belitser and Tsibakowa	P/O > 1, phosphorylation during e^- transport	[21]
1941	Ochoa	P/O > 1, phosphorylation during e^- transport	[25]
1941	Kalckar	General significance of transphos-	[53]
1941	Lipmann	phosphorylations; 'high energy phosphate'	[54]
1941	Johnson	Idea of respiratory control	[55]
1943	Lardy and Phillips	Uncoupling of oxidation and phosphorylation	[56]
1945	Lardy and Elvehjem	ATPases as transporylases	[57]
1951/52	McElroy and Glass	McCollum-Pratt symposia on phosphorous metabolism	[58]
1953	Slater	'Chemical' hypothesis of oxidative phosphorylation	[59]
1961	Mitchell	'Chemiosmotic' hypothesis of oxidative phosphorylation	[60]
1961	Williams	Idea of localized protons in ATP synthesis	[61]
1971	Singer	Fluid mosaic model of membrane structure	[62]
1973	Boyer et al.	'Conformational' hypothesis	[38]
1975	Mitchell	Q cycle	[63]
1977	Boyer, Chance, Slater Racker, Ernster	Acceptance of general principle of chemiosmotic hypothesis	[64]

words. "We can proudly state that this whole subject did not exist 25 years ago, but since then has developed rapidly...the most interesting phases of phosphate metabolism...had their very first start with the discoveries of Arthur Harden...". He then portrayed the events up to 1918 (Table III) and continued: "However, there then followed a long lag of about 10 years, until suddenly, in the decade after 1926, several facts emerged which were of fundamental importance to our problems." He then enumerated the following points: (1) the formation of the sugar ester is not a side-reaction; rather a whole series of phosphate compounds arise in subsequent metabolic steps. (2) In these reactions there is an interplay between three coenzyme systems, viz., the adenylates, the pyridinenucleotides and diphosphothiamine. (3) There is transfer of organically bound phosphate without going through the stage of inorganic phosphate, establishing the new principle of group transfer. (4) The most important: the discovery of 'high energy' phosphates. "We know how this notion has modified all our thinking in various fields of physiology..." he went on to say. Meyerhof ended his presentation with these words: "I believe, therefore, that just as the role of iron in biological reactions is now made completely understandable by the work of Otto Warburg...so the role of phosphate compounds in the organisms is made understandable by their importance for energy transfer. For this reason, I think that these two subjects belong to the most advanced chapters of biochemistry."

While at these symposia there was optimism that it might be only a few years until the mechanism of oxidative phosphorylation would be understood, there followed another period of at least two decades, until one had come substantially closer to this goal. This is expressed by the authors of the chapter on oxidative phosphorylation in the Annual Reviews of Biochemistry in 1969 in their introductory statement [65]. "Those who have followed the lore and vagaries of oxidative phosphorylation since the late 1930s are beginning to believe that this area of science may be an exception to Einstein's dictum concerning the rationality of nature." This dictum was "Der Herrgott ist raffiniert, aber nicht boshaft." (The Lord is sophisticated, but not malicious.)

Only a year later E. Racker wrote about the same dilemma in the book that he edited in 1970 on 'Membranes of Mitochondria and Chloroplasts' [66]: "Having been raised by the music of substrate-level phosphorylation my own prejudices induce me to lean toward some aspects of the chemical hypothesis, e.g., the phosphorylated high-energy intermediate. In its favor is the tendency of nature to repeat itself with respect to the mechanism...On the other hand, the experimental evidence in favor of an intimate relation between oxidative phosphorylation and the formation of a membrane potential is mounting."

Five years later Racker edited another book: Energy Transducing Mechanisms [67]. The preface starts with the following words: "The historians of energy transducing mechanisms will perhaps refer to the years following 1970 as the period of conversion. Oxidative phosphorylation, ion transport, muscular contraction and membranous excitability have been more or less duplicated in simple model systems and basic mechanisms of operation have been formulated....we begin to recognize some remarkable similarity between them...We see a new unity of biochemistry that extends not only horizontally through the various kingdoms of life, but vertically through the diversity of functions."

Finally, I would like to quote once more from the Annual Reviews, this time from 1977, where my table ends. Peter Mitchell's final statement reads like this: "It is now widely appreciated that the intrinsically vectorial osmotic properties of the reactions involved in oxidative phosphorylation and related energy transductions...are the very properties that, until they were recognized, held back the progress of research in this important field."

Concluding remarks

Do I read a touch of bitterness out of these last words? Or do I read that into them? It might have been the complicated detailed postulates that were attached to the chemosmotic hypothesis and the seeming non-specificity of the bulk proton gradient that made many hesitant to accept it outright. Had we had the picture of the intricate features of membranes with their inserted and penetrating proteins on our mind that we have today, specificity may not have been so hard to imagine. Be that as it may, but we have seen, even from the greatly abbreviated history that I have given, that there has been and still is a continuous struggle for the truth, or the best approximation to it, which, however, in turn, helps to solidify what finally emerges as the better approximation; and this may well be worth some delay. We have also seen that scientists, who have greatly advanced science, such as Liebig, Pasteur and Willstaetter, by their perseverance with certain ideas, even in the face of persuasive evidence to the contrary, may also have delayed progress because of the authority they commanded. And people in general were much more outspoken, assertive and less tolerant, even only as far back as the beginning of the century. In addition, those with authority or in power were able to wield much more influence over the less recognized, e.g., via their prestigious university positions or as editors of journals. Thus, not only science seems to have advanced, but also mutual relationships among those dedicated to it. A new problem has emerged that plagues us more today; it is the unmanageable flood of information. We may sometimes feel overwhelmed and discouraged in the face of all the things we don't know and which we think we should know or learn. Then, however, as we now have a chance, from the most recent advances in our field, to recognize more and more of the unity of biochemistry that Racker alluded to in the quote above, and to take exciting glimpses far back into the history of the evolution of proteins and living organisms, we may again feel it has been worth it after all.

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