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Review

Curriculum Vitae

Professor Emeritus at the Department of Plant Sciences, Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University of Göttingen, was born 3 January 1934 in Berlin

1954–1955	Study of chemistry at the University Innsbruck (Austria),
1955–1960	Continuation of study at the University of Marburg (Germany)
1957–1958	Stipend from the Adolf Todt Foundation for half a year study at the Institute of Chemistry, University of Edinburgh, Scotland
1961	Graduation with the degree of a Diplom Chemiker
1960	Married to Fiona Stewart, M.A., three sons
1962	Promotion Dr. Phil. at the University of Marburg

Thesis: Phosphate containing metabolites in tissues and in isolated mitochondria from rat and pigeon. (Institute of Physiological Chemistry) Prof. Theodor Bücher, Prof. Martin Klingenberg

1962–1968	Research Assistant at the Institute of Physiological Chemistry, University Marburg
1968–1976	Senior Researcher at the Institute for Physiological Chemistry and Physical Biochemistry, University of Munich
1976–1978	Professor at the Institute for Physiological Chemistry and Physical Biochemistry, University of Munich
1978	Professor of Biochemistry and Director of the Division of Plant Biochemistry, University of Göttingen
2002	Professor Emeritus of Biochemistry, University of Göttingen

Honors, Awards

1980	Miller Professor University Urbana Ill.
1982	Research Fellow of the Royal Society, University of Sheffield, ARC
1990	Elected Member of the Akademie der Wissenschaften zu Goettingen
1993	Max-Planck-Research Prize of the Alexander-von Humboldt Stiftung and the Max-Planck-Gesellschaft
1996	Corresponding Membership Award of the American Society of Plant Physiologists also: Corresponding Membership Award of the Australian Society of Plant Physiologists
2002	Invited Guest Professor Universities of Delhi and Hyderabad (India)
2002	Highly Cited Researcher, Institute for Scientific Information (ISI)

Textbook

Pflanzenbiochemie (Spektrum-Verlag 1. Auflage 1996, 4. Auflage, 2008) Plant Biochemistry (Elsevier Academic Press, USA 2004), also Japanese, Chinese and Indian editions, Russian edition in preparation.

Activities

2000–2006	Representative of the Union of German Academies of Science in the InterAcademic Panel (IAP), Coordinator of the IAP Initiative on Genetically Modified Plants.

From Liver to Leaves: Memories of a Plant Biochemist

H.-W. Heldt

Contents

Abstract This is a report of more than 40 years of my own work and that with my group, beginning in Marburg in the group of Martin Klingenberg in the institute of Theodor Bücher, continuing in Munich in the institute of Martin Klingenberg and finally in Goettingen.

A wide spectrum of sophisticated methods developed in our group was used to study the metabolism of liver as well as leaves, ranging from the discovery of the mitochondrial ATP/ADP translocator to the finding of a number of chloroplast translocators. It was followed by studies of the regulation of the Calvin Cycle, of starch and sucrose synthesis, the role of mitochondrial oxidative phosphorylation in photosynthesis, the redox transfer within a leaf cell, assay of metabolite gradients in C_4 plants and the relationship between subcellular metabolite concentrations in intact leaves and in the phloem sap. These results were made possible by a very fruitful collaboration of our team and many colleagues from Germany and abroad.

1 Liver

At school, chemistry was my favourite subject, and it had always been my intention to study chemistry in order to become a chemist working in industry. I studied pure chemistry at the universities of Innsbruck (Austria), Edinburgh (Scotland) and Marburg. During my preparation for the diploma exam I came across a section on biochemistry in Klages's textbook on organic chemistry. At the time, biochemistry

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was not taught to chemistry students. What I read about ATP, the glycolytic and citric acid cycle fascinated me and I made up my mind to write my diploma thesis on a biochemical topic which in Marburg was then only possible in the Institute of Physiological Chemistry at the Medical Faculty. I needed the consent of Prof. Karl Dimroth, the head of the department of organic chemistry, which he granted, but asking me: "What do you want to do with this later?"

It was an extremely fortunate choice and when I started my work in 1959 at the Institute of Physiological Chemistry in Marburg, it was probably one of the most modern in the world. Its director, Prof. Theodor Bücher, had been working in Otto Warburg's laboratory of the Kaiser-Wilhelm-Institut in Berlin when he discovered the glycolytic enzyme phosphoglycerate kinase. This was only published after the war (Bücher 1947). He then had a laboratory at the Eppendorf University clinic in Hamburg, where, together with Dr. Netheler, he designed the legendary Eppendorf photometer to be used as a tool to carry out enzymatic assays according to Otto Warburg, where reactions are coupled to pyridine nucleotide dehydrogenases. He established an enzymic assay of ethanol based on alcohol dehydrogenase, which became very important for identifying drunk drivers. He also worked out a protocol to isolate five glycolytic enzymes in one process from rabbit muscle. This procedure was applied by the Boehringer Company, marking its start as a supplier of biochemicals, and opened up the prospect of using enzymatic assays as a routine method.

In 1953, Theodor Bücher was appointed professor in Marburg and transformed the erstwhile small division into the large Institute of Physiological Chemistry with completely modernised laboratories. He designed the institute to facilitate the study of metabolism from many different angles by a number of research groups led by senior researchers. The group of Rudolf Czok isolated enzymes, that of Dirk Pette (1965) assayed the proportions of the activities of a large number of metabolic enzymes in different tissues, that of Hans Jürgen Hohorst assayed metabolite levels in freeze-stopped tissues (Hohorst et al. 1959), Hans Schimassek (1963) performed metabolic studies with perfused rat liver, Roland Kirsten and his wife performed amino acid analyses in tissues with a novel automated microscale amino acid analyser designed in the institute (Kirsten and Kirsten 1962), Klaus Papenberg assayed the nucleotide content of human liver biopsy samples by ion exchange chromatography (Schnitger et al. 1959) and last but not least Dr. Klingenberg studied the respiratory metabolism of isolated mitochondria (Klingenberg and Slenczka 1959). Later, many of these persons became full professors at various universities. One very important person was Hans Schnitger, an ingenious inventor of scientific instruments. As the ingredients of enzymatic analyses were valuable, the reaction volumes had to be reduced, which led to the invention in the institute of the so-called microliter system. Hans Schnitger designed the Eppendorf pipette, which was built in our workshop and which we used long before it became commercially available. The plastic Eppendorf vessel, now used in almost every laboratory in the world, was developed. In a way, Marburg was then a birthplace of modern biochemistry.

It was the aim of Theodor Bücher to utilise the knowledge of biochemical studies in medicine. Many prominent medical professors worked in his institute to learn

biochemical methods or cooperated with him. He cooperated closely with the married couple Dr. Schmidt and Dr. Schmidt in Kassel, pioneers in the introduction of the measurement of enzyme activities in blood samples, nowadays a medical routine. I explain all this in so much detail, since for me as a chemistry student with no knowledge whatsoever of biochemistry or physiology, the scientific environment of the Marburg institute was of crucial importance for my later work.

Prof. Bücher assigned me to Klaus Papenberg, who used a microscale ion exchange chromatography apparatus, constructed by Hans Schnitger and built in the workshop of the institute, to analyse nucleotide patterns in human liver biopsy samples of about 40 mg (Schnitger et al. 1959). The ion exchange column was a 200-cm long plastic tube with an inner diameter of 0.5 mm and filled with Dowex anion exchanger. It was a forerunner of high pressure liquid chromatography. The fluid was initially pumped by a peristaltic pump and later by a high pressure piston pump designed once more by Hans Schnitger. A chromatogram took about 7 days, during which about 500 samples of 0.12 ml each were collected in Teflon racks. Each sample had to be transferred to a microcuvette for the measurement of UV-absorption by a Beckmann DU photometer, and transferred back into the rack, where the samples were dried and digested by acid for phosphate determination by the molybdate method, where each sample had to be assayed again photometrically. For this reason, the whole method was very time consuming and required the assistance of a technician. Later, the ultraviolet measurements were carried out with a micro-flow cuvette. It was my job to run the chromatographies and to identify the peaks of the ultraviolet and phosphorous measurements. The whole method was designed to analyse the nucleotide pattern of extracts from liver biopsies as a potential diagnostic tool for liver diseases. I sometimes accompanied Klaus Papenberg to a hospital in Kassel to hold the vessel with liquid nitrogen for fixing a liver biopsy from patients in an operation theatre. These biopsies were taken in addition to those taken for normal diagnostic purposes. Later, however, it was realised that the analytical approach was not viable, because the 5-s between the withdrawal of the biopsy and the fixing were enough to distort the nucleotide pattern. Unfortunately, one day on his way from Kassel to Marburg Klaus, Papenberg had a serious car accident and never returned to the laboratory. I, an inexperienced diploma student, was left with a laboratory and a technician. As nobody told me what to do, I decided, using the available technique, to analyse the content of nucleotides and other metabolites containing phosphate in rat liver and presented these data in my diploma thesis (Heldt 1963). I presume Prof. Bücher was satisfied with my work, since I had described the entire nucleotide outfit of liver tissue.

I was able to retain my laboratory, consisting of two rooms, a technician and with a great view of the Elisabeth Church. As I received no further instruction, but had access to all the resources, it was more or less up to me how to continue these studies for my Ph.D. thesis. The work often kept me occupied late into the night. That was when I became acquainted with Martin Klingenberg, who had his laboratory at the other end of the building. Martin Klingenberg had been a post-doctoral student with Britton Chance at the Johnson Foundation in Philadelphia, USA, where he used a dual-wavelength spectrophotometer to study cytochromes and by which he

discovered cytochrome P450. When coming to Marburg, Martin Klingenberg built a dual-wavelength spectrophotometer for himself and used it for his fundamental studies of the function of the mitochondrial respiratory chain. One of his achievements was the unveiling of the relationship between the redox state of the mitochondrial respiratory carriers and the phosphorylation potential of the generated ATP as assayed with isolated mitochondria (Klingenberg and Schollmeyer 1960). Theodor Bücher and he published the legendary report "Wege des Wasserstoffs in der lebendigen Organisation" in which the different redox potentials of NADH and NADPH in the mitochondria and the cytosolic compartment were characterised and their function defined (Bücher and Klingenberg 1958).

During these long nocturnal discussions, Martin Klingenberg became my mentor and directed my interest towards mitochondria. With my chromatography, I assayed nucleotides and other phosphorylated metabolites contained in whole tissues, isolated mitochondria from rat liver, rat heart and pigeon breast and determined how much of the total metabolites in these tissues were located in the mitochondria. In order to learn about the turnover of the mitochondrial metabolites, I briefly incubated isolated mitochondria with ^{32}P-phosphate and assayed the incorporation by attaching to the chromatography apparatus a flow detector for ^{32}P- radioactivity which I had built for myself (Heldt and Klingenberg 1965). I incorporated all these results in my Ph.D. thesis. Officially, Theodor Bücher was my "Doctor Father", but in actual fact it was Martin Klingenberg.

Upon finishing my Ph.D. it would have been normal for me to go into industry, where the prospects for a career were then very good. To my great surprise, Martin Klingenberg asked me if I would not like to stay and I very gladly agreed. So I became a member of the Klingenberg group, but with my laboratory at the opposite side of the building, and with my own technician. Hans Jacobs, a medic seeking experience in biochemistry, joined me for a longer period. Chromatographic results had shown that creatine phosphate was radioactively labelled in isolated mitochondria. This was surprising since at the time creatine kinase was known as a cytosolic enzyme. From these studies, we discovered a mitochondrial creatine kinase isoenzyme different from the cytosolic one (Jacobs et al. 1964).

My investigations had shown that mitochondria contain endogenous nucleotides, which cannot be washed out. The question arose: how are these mitochondrial adenine nucleotides able to communicate with those outside? To study the kinetics of the incorporation of ^{32}P into the endogenous and external ATP of isolated mitochondria, I constructed a reaction vessel with an outflow at the bottom, which was closed by a programmable electric valve, and attached to a manually driven sample collector containing reaction vessels with perchloric acid for a metabolic quench. The mitochondria were kept in a deenergised state by incubating them in an anaerobic medium, and the reaction was started by blowing oxygen together with ^{32}P-phosphate into the stirred mitochondrial suspension, of which samples were taken at intervals of seconds. These studies showed that the internal ADP was more rapidly phosphorylated than the external ADP, especially at low temperatures (Heldt et al. 1965; Heldt and Klingenberg 1968). The inhibitory effect of atractyloside was particularly interesting in these experiments. It has been