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Heller, Adam; Ulstrup, Jens

Published in:
Analytical Chemistry

Link to article, DOI:
10.1021/acs.analchem.1c01191

Publication date:
2021

Document Version
Publisher's PDF, also known as Version of record

analytical chemistry

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Adam Heller* and Jens Ulstrup

Cite This: *Anal. Chem.* 2021, 93, 7148−7149

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The historical misattribution of the discovery of flavoenzymes is corrected. It was 26 years old Danish botanist Detlev Müller who discovered glucose oxidase in 1925. In 1925, during his studies of fungi, 26-year old Detlev Müller (see Figure 1), a Danish botanist at the then Royal Danish Veterinary and Agricultural University (KVL), published a brief note in a somewhat inaccessible journal reporting his discovery of a new enzyme, glucose oxidase.[1] He precipitated the glucose oxidase-enriched mixture from the pressed aqueous sap of *Aspergillus niger* by adding 2:1 (v/v) ethanol/ether. After drying the precipitate, he kept it in a desiccator over $CaCl_2$, finding that the enzyme activity of the dried powder was retained for months. He monitored the activity of the powder after dissolving it in water by transferring the solution absorbed by filter paper to a mercury manometer-connected, stoppered, water-containing jar. When he added glucose, O_2 was consumed and the pressure dropped. Simultaneously, the pH of the solution in the jar dropped from about pH 5.5−6.6 to about pH 4.0. From the consumed glucose to O_2 ratio and other tests, Müller concluded, in a paper published in 1928 in a widely read journal,[2] that the acid produced was gluconic acid, i.e., that the enzyme catalyzed the reaction of eq 1.

$$\text{glucose} + O_2 + H_2O \rightarrow \text{gluconic acid} + H_2O_2 \quad (1)$$

In a third paper, published in 1929 also in a widely read journal, Müller[3] showed that the enzyme was selective: When he added mannose, galactose, fructose, arabinose or xylose to the jar, the rate of pressure drop was lower than it was for glucose and was nil for fructose, xylose, and arabinose. The optimal pH, i.e., the pH where the O_2 consumption was most rapid, was pH 5.5−6.5. The rate of O_2 consumption increased with increasing temperature between 0 °C and 30 °C, and at 73 °C O_2 was no longer consumed. Other than from the pressed sap of *Aspergillus niger* Müller prepared a glucose oxidase-rich powder also from the sap of *Penicillium glaucum*.

Müller must have seen that his powders and solutions were yellow. However, being a botanist, he apparently did not consider the yellow color to be of sufficient significance to report it. In contrast, Nobel Prize winner Otto Warburg and Walter Christian, who in 1932−1933 discovered a different enzyme, glucose 6-phosphate dehydrogenase, in baker's yeast *Saccharomyces cerevisiae*,[4−7] did recognize the significance of their enzyme's yellow cofactor and characterized its chromophore. Being unaware that the glucose oxidase of Müller was also a yellow enzyme, Warburg and Christian did not cite his 1925−1929 studies in their 1932−1933 publications on 6-phosphate dehydrogenase. Consequently, they were mistakenly credited with the discovery of the first flavoenzyme, even by Vincent Massey, the late Master of the yellow enzymes.[8] In our 2008 review[9] (Heller and Feldman), we were also mistaken when we attributed the isolation and characterization of glucose oxidase to Warburg and Christian.[4]

Müller continued to work at KVL serving as Full Professor in plant physiology at KVL (1935−1949) and then at University of Copenhagen (1949−1969). He became an Honorary Doctor in Medicine at the University of Copenhagen in 1991.[10] He passed away in 1993 at the age of 94.

As of March 8, 2021 SciFinder Scholar listed 2 758 patents and 11 306 letters or journal articles on sensors or biosensors made with glucose oxidase. The world's most widely used subcutaneously implanted continuously glucose monitoring system for diabetes management, the FreeStyle Libre system of Abbott Diabetes Care, comprises a miniature amperometric sensor made with the enzyme that is electrically wired with an $Os^{2+/3+}$complex comprising redox polymer.[11] Few know that it was Detlev Müller who discovered the enzyme when he was 26 years old and had only the simplest tools.

AUTHOR INFORMATION

Corresponding Author

Adam Heller − *University of Texas, Austin, Texas 78712, United States;* orcid.org/0000-0003-0181-1246; Email: heller@che.utexas.edu

Author

Jens Ulstrup − *Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark;* orcid.org/0000-0002-2601-7906

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.1c01191

Notes

The authors declare no competing financial interest.

Published: April 27, 2021

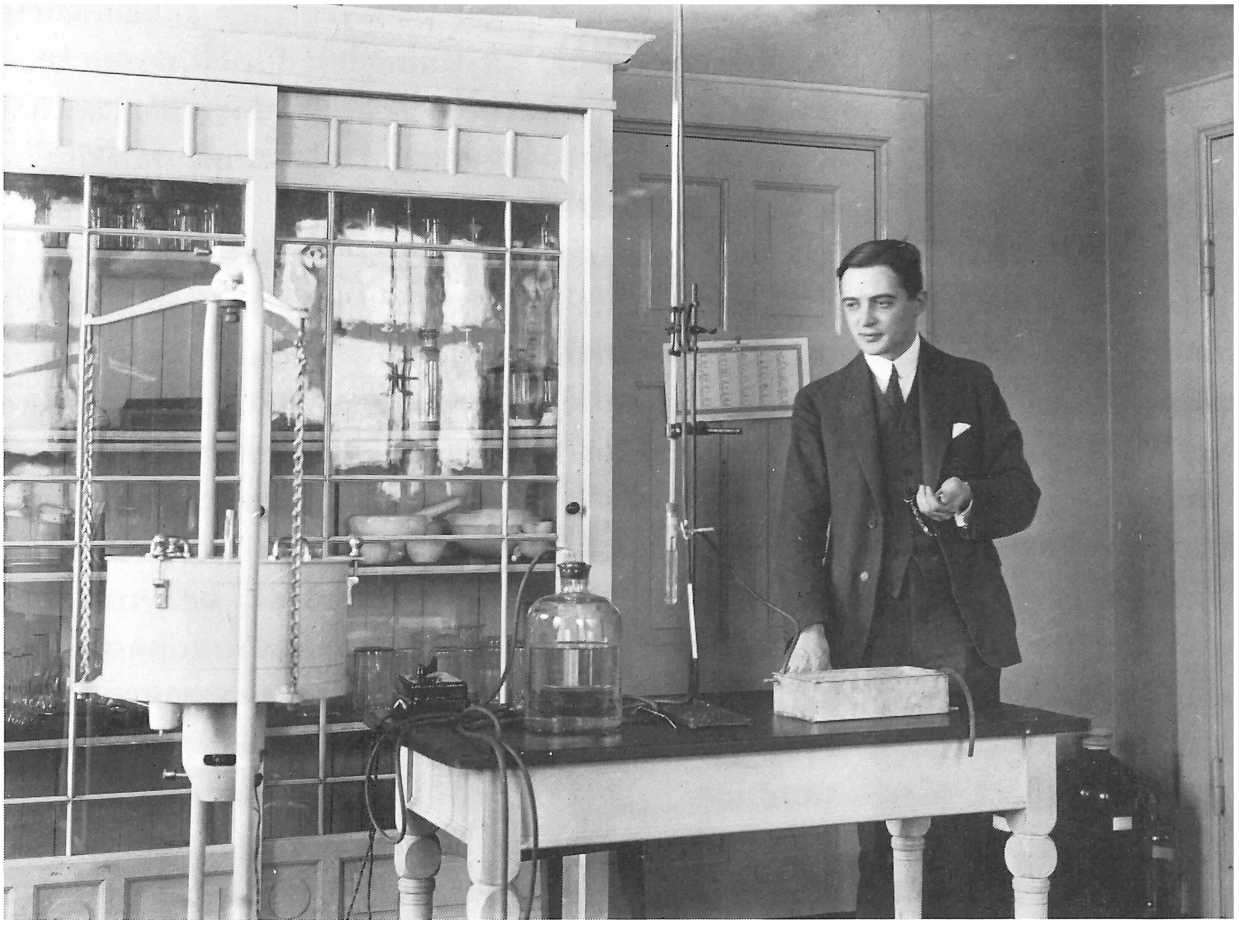

Figure 1. The photograph of Müller, taken in 1931, shows in its center the jar with the attached mercury manometer that he used to follow the kinetics of O_2 consumption. On the left, the press that he used to press the glucose oxidase containing sap from the ground molds. Behind these, the mortar and pestle that he used for grinding the molds with sand and kieselguhr. The photograph, of 31-year old Detlev Müller in his KVL lab, was provided by Morten Fink-Jensen, Science Historian and Christian Knudsen, Natural Science Librarian, both of the University of Copenhagen. It was published in 1979 on the occasion of the 500th anniversary of The University of Copenhagen (*Københavns Universitet 1479–1979*, vol. 13 (2), pp 509–510, *The Mathematics and Natural Science Faculties*). With permission of Copenhagen University, through Morten Fink-Jensen.

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https://doi.org/10.1021/acs.analchem.1c01191
Anal. Chem. 2021, 93, 7148–7149