

This Week's Citation Classic

Wollenberger A, Ristau O & Schoffa G. Eine einfache technik der extrem schnellen Abkühlung grösserer Gewebestücke. (A simple technique for the extremely rapid cooling of larger tissue samples.) *Pflugers Arch. Physiol.* **270**:399-412, 1960.

Tissue and organs can be frozen in situ in a fraction of a second by being compressed to a thin layer between two aluminum blocks that are precooled in liquid nitrogen and for convenient handling form part of a clamp. [The *SCI*[®] indicates that this paper has been cited over 490 times since 1961.]

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"This paper was previously classified in *Current Contents*[®] as belonging to the category of *Uncitedness III*,¹ thanks to a practice among authors of referring in their publications to its content without taking pains to cite it as a reference.

"The technique of rapid tissue fixation with the type of freezing clamp described in this 1960 paper was for the first time made public by myself and Bozkourt Wahler at the 20th International Physiology Congress in Brussels in August 1956. When I later asked Dr. Wahler to continue working with me in an attempt to verify our claims by actual measurements of tissue cooling rates, he declined because, as he said, he did not wish to waste his time on technical trivialities. I thereupon engaged the collaboration of two colleagues from a neighboring department—Georg Schoffa, who today is professor of biophysics at the Technical University of Karlsruhe, and his technician, Otto Ristau, now a research chemist at the Central Institute of Molecular Biology of our Academy here in Berlin-Buch. The thermoelectric measurements were done by Ristau and myself, both Schoffa and Ristau

were helpful in the theoretical analysis of the tissue cooling process, and I wrote the main part of the paper, which was submitted to *Pflugers Archiv*.

"Unfortunately, the paper appeared in print in a somewhat mutilated form, because a figure documenting the adequacy of our instrumental set-up was deleted at the insistence of one of the editors. This figure, which never was published, showed that as soon as the bare thermocouple used for monitoring tissue temperature was clamped between aluminum blocks precooled in liquid nitrogen to -196°C , its temperature fell without delay and at an initial rate of approximately $20,000^{\circ}\text{C}/\text{sec}$. There was thus no need to correct the thermo-oscillograms presented in our paper for instrumental inertia.

"The freezing clamp as a tool for the instant cryofixation of tissues owes its popularity partly to the simplicity of its design. Various modifications and more complicated freezing devices based on the clamping principle were introduced in the course of the years, the most sophisticated version being an apparatus that automatically freezes the heart of small open-chest animals at any predetermined point of the electrocardiogram. It was constructed in Moscow by Dr. A. N. Medelyanovski. Making use of this apparatus through collaboration with Medelyanovski's wife, Dr. Yenia Bogdanova, who was not exactly a newcomer to cryobiology after having been national women's champion of the USSR in figure ice-skating, my Berlin coworkers and I were privileged to have a part in the demonstration of systematic oscillations of myocardial cyclic nucleotide levels during the cardiac contraction cycle of the frog.² For less extravagant purposes, however, and in many laboratories throughout the world the primitive clamp described in 1960 continues to serve as a satisfactory tool."

1. **Garfield E.** Uncitedness III—the importance of *not* being cited. *Current Contents* (8):5-6, 21 February 1973.
2. **Wollenberger A, Babskii E B, Krause E G, Genz S, Blohm D & Bogdanova E V.** Cyclic changes in levels of cyclic AMP and cyclic GMP in frog myocardium during the cardiac cycle. *Biochem. Biophys. Res. Commun.* **55**:446-52, 1973.