Warburg optical test

When **Otto Heinrich Warburg (1883–1970)**, a German biochemist and later **Nobel Prize winner in Physiology or Medicine (1931)**, first used the absorption properties of pyridine coenzymes to observe enzyme-catalyzed reactions, he apparently had no idea how significantly this discovery would be applied to clinical chemistry. The vast majority of methods routinely used today to determine the activity of enzymes are based directly or indirectly on photometry of the absorbance of reaction mixtures containing pyridine coenzyme. This principle has also given the method its name "optical test".

Reversible hydrogenation of nicotinamide adenine dinucleotideu (NAD^+), which occurs on the pyridine ring of nicotinamide, leads to the reduced form ($NADH + H^+$) and is accompanied by a significant change in the absorption spectrum. While the oxidized form (NAD^+) has an absorption maximum at a wavelength of 260 nm, the cancellation of the aromatic character of the pyridine nucleus and its transition to the quinoid form ($NADH + H^+$) is accompanied by the formation of another absorption maximum at 340 nm.

The magnitude or change in absorbance at 340 nm is directly proportional to the number of reduced coenzyme molecules. The conversion of one mole of coenzyme corresponds to the conversion of one mole of substrate and, in addition, this conversion can be monitored over time, i.e. the reaction rate can be measured continuously using a spectrophotometeru.

Thus, it is possible to prepare a reaction mixture of the enzyme of interest (or the biological material in which it is being determined), the coenzyme and the appropriate substrate at the optimum concentration, and at the optimum pH and temperature to determine the reaction rate by measuring changes in absorbance on a suitable photometer at a wavelength of 340 nm.

Coupled reactions

In view of the fact that NAD^+ is a coenzyme of only some dehydrogenases and that there are a number of clinically important enzymes from classes other than just oxidoreductases, the use of "compound optical assays" is even more common in addition to the "single optical assay", whereby the above principle is used only as an indicator reaction, while the actual reaction measuring the activity of the enzyme in question is preceded or even coupled in a series with other auxiliary reactions.

enzyme	reaction	auxiliary reaction	indication reactions
alaninaminotransferase EC 2.6.1.2 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC2/6/1/2.html)	alanin + 2-oxoglutarate ⇌ glutamate + pyruvate		***LD:*** pyruvate + NADH + H^+ ⇌ lactate + NAD^+
creatin-kinaze EC 2.7.3.2 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC2/7/3/2.html)	creatine phospate + ADP → creatine + ATP	***HK*** glucose + ATP → Glc-6-P + ADP	***Glc-6-PD*** Glc-6-P + $NADP^+$ ⇌ 6-P-glukonate + NADPH + H^+

Explanatory notes:

LD – lactate dehydrogenase (EC 1.1.1.27 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC1/1/1/27.html))
HK – hexokinaze (EC 2.7.1.1 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC2/7/1/1.html))
Glc-6-PD – glucose-6-phosphate dehydrogenase (EC 1.1.1.49 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC1/1/1/49.html))
Glc-6-P – glucose-6-phosphate

The peroxide reaction is also often used as an indication, e.g. in the determination of glucose or cholesterol.

analyte	reaction	indication reactions
glucose	***GOD*** glucose + O_2 → gluconic acid + H_2O_2	***POD*** H_2O_2 + fenol + 4-aminoantipyrine → chinonimine (pink) + 4 H_2O
cholesterole	***CHE*** cholesterole + O_2 → 4-cholesten-3-on + H_2O_2	***POD*** H_2O_2 + fenol + 4-aminoantipyrine → chinonimine (pink) + 4 H_2O

Explanatory notes:

GOD – glucose oxidase (EC 1.1.3.4 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC1/1/3/4.html))
CHE – Cholesterol oxidase (EC 1.1.3.6 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC1/1/3/6.html))
POD – Perixodation (EC 1.11.1.7 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC1/11/1/7.html))

Note: The work of Otto H. Warburg extends significantly beyond laboratory practice and medicine. For example, Warburg's description of the metabolism of transformed cells has become an important pillar for further cancer research.