THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 50, Issue of December 10, p. e10, 2004

Printed in U.S.A.

Classics

A PAPER IN A SERIES REPRINTED TO CELEBRATE THE CENTENARY OF THE JBC IN 2005

JBC Centennial
1905–2005
100 Years of Biochemistry and Molecular Biology

Britton Chance: Olympian and Developer of Stop-Flow Methods

The Kinetics of the Enzyme-Substrate Compound of Peroxidase (Chance, B. (1943) *J. Biol. Chem.* 151, 553–577)

Britton Chance was born in Wilkes-Barre, Pennsylvania in 1913. He spent many summers during his youth sailing, and his love of the sea was the catalyst for his first significant contribution to science and technology. When he was just a teenager, Chance invented an autosteering device that detected deviations in a ship's course and generated a feedback signal to redirect the ship's steering mechanisms. Later in life, his love of sailing and intense competitive spirit landed him a spot on the U.S. yacht Olympic team where he won a gold medal in the 1952 Olympics.

Chance received his Bachelor's of Science degree from the University of Pennsylvania in 1935 and remained there for graduate school. Around this time, Glenn Millikan, at Cambridge University, had developed a novel stop-flow apparatus to measure the formation of oxymyoglobin resulting from the combination of oxygen with myoglobin (1). During his thesis research, Chance observed that "adding H_2O_2 to a crude preparation of peroxidase gave a colored compound that could go on to oxidize a variety of phenols" (2). He believed it might be possible to study the intermediates in the peroxide interaction if he could make a "micro" version of Millikan's apparatus. So, in 1937, under the supervision of Martin Kilpatrick, he started construction of a rapid-flow apparatus.

In 1938, while still a graduate student at the University of Pennsylvania, Chance was offered a contract by the British General Electric Company to test his autosteering device on a ship going from London to New Zealand and Australia. Shortly after his arrival in London, Chance visited with Millikan hoping to study under him. Millikan accepted him as his research student, and after Chance returned from his voyage he started constructing a second microflow apparatus. He completed the apparatus by 1939 and did some initial studies on luciferase O_2 reactions.

In 1940, Chance returned to Pennsylvania to visit his parents but was unable to return to Cambridge due to the onset of World War II. Fortunately, he was accepted back at the University of Pennsylvania and began construction of a third version of his rapid-flow instrument. Once it was completed, Chance used this new instrument to elucidate the peroxidase enzyme-substrate reactions, which is the subject of the *Journal of Biological Chemistry* (JBC) Classic reprinted here.

At this time, there were many theories about how enzymes worked. Leonor Michaelis, the subject of a previous JBC Classic (3), and Maud Menten had proposed their theory which stated that the relationship between enzymatic activity and enzyme and substrate concentration could be explained by the existence of an intermediate enzyme-substrate compound (4). Their theory was modified by Thaddeus Briggs and J. B. S. Haldane who pointed out that the rate of formation of the intermediate could be limited by the number of collisions between the enzyme and substrate (5). However, at the time, there were no experimental observations of the intermediates, and the reaction velocity constants were lumped into one term, the Michaelis-Menten constant, rather than determined separately.

In his paper, Chance used the reaction between horseradish peroxidase and hydrogen peroxide to prove the existence of the intermediate compound and to determine the rates of

Britton Chance. Photo courtesy of the National Library of Medicine.

$$\text{Peroxidase} + H_2O_2 \rightarrow \text{peroxidase-}H_2O_2 \rightarrow \text{peroxidase} + H_2O + O$$

REACTION 1

formation and breakdown of the enzyme-substrate compound. Chance detected the products of the reaction with ascorbic acid and leucomalachite green, which formed the colored malachite green when oxidized. In his experiments, he filled one syringe of his microflow apparatus with hydrogen peroxide, or a combination of hydrogen peroxide and leucomalachite green, and the other syringe with peroxidase. Pushing the syringe plungers simultaneously caused the reactants to be mixed and to flow down the observation tube. The solution eventually stopped at a photocell and light beam where the progress of the reaction was followed by measuring the concentrations of malachite green and enzyme-substrate compound. By varying substrate or acceptor concentration and measuring the resulting changes in the rate of formation and equilibrium concentration of the peroxidase-H_2O_2 compound, Chance was able to study the kinetics of the reaction intermediate.

Using his data, Chance solved the equations representing the Briggs and Haldane modifications to the Michaelis-Menten theory. He found that the activity of peroxidase could be described by a second-order reaction of the enzyme with H_2O_2, followed by a second-order irreversible reaction of the peroxidase-H_2O_2 intermediate with the hydrogen donor. By making a point by point comparison between experiment and theory, Chance was able to confirm the validity of the Michaelis-Menten theory and the Briggs and Haldane modifications. He later won the Paul Lewis award in enzyme chemistry for this work.

Chance ended up getting two Ph.D. degrees, one in physical chemistry from the University of Pennsylvania and one in biology and physiology from Cambridge University. After the war, he went to Stockholm on a 2-year Guggenheim Fellowship to work with Hugo Theorell. He and Theorell used another version of the stop-flow apparatus to study the kinetics of NAD in alcohol-aldehyde interconversion and found that product release was rate-determining (6). This is now called the Theorell-Chance (T-C) mechanism.

Chance returned to the University of Pennsylvania after his fellowship was over and became Professor of Biophysics and Physical Biochemistry and Director of the Johnson Foundation in

1949. In the early 1950s, he shifted his focus more toward biological phenomenon and studied the control of oxidative phosphorylation in mitochondria. Chance, along with Henry Lardy and later Ron Williams, worked out methods to separate mitochondria from cells and preserve their metabolic activity *in vitro* and invented the dual wavelength spectrophotometer to watch ATP synthesis in mitochondria. Spurred by this success, Chance later developed methods for using optical spectroscopy to study living tissues. In the late 1970s, he was the first to use magnetic resonance spectroscopy on a whole organ, the excised brain of a hedgehog.

Chance has received many honors in recognition of his contributions to science, including election to the National Academy of Science in 1954. He was presented the National Medal of Science in 1974 by President Ford, "For his contributions to our knowledge of cellular and subcellular physiology made through work on enzyme-substrate complexes, on the kinetics of enzyme action, and on the mechanism and control of membrane-bound electron transfer during cellular respiration." Today he remains at the University of Pennsylvania where he is still actively involved in research.[1]

Nicole Kresge, Robert D. Simoni, and Robert L. Hill

REFERENCES

1. Millikan, G. A. (1936) Muscle hemoglobin. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **120**, 366–388
2. Chance, B. (2004) The stopped-flow method and chemical intermediates in enzyme reactions—a personal essay. *Photosynth. Res.* **80**, 387–400
3. JBC Classics: Granick, S., and Michaelis, L. (1943) *J. Biol. Chem.* **147**, 91–97 (http://www.jbc.org/cgi/content/full/279/49/e9)
4. Michaelis, L, and Menten, M. L. (1913) Die Kinetik der Invertinwerkung. *Biochem. Z.* **49**, 333–369
5. Briggs, G. E., and Haldane, J. B. S. (1925) A note on the kinetics of enzyme action. *Biochem. J.* **19**, 339–339
6. Theorell, H., and Chance, B. (1951) Studies of liver alcohol dehydrogenase. II. The kinetics of the compound of horse liver alcohol dehydrogenase and reduced diphosphopyridine nucleotide. *Acta Chem. Scand.* **5**, 1127–1144
7. Yodh, A. G., and Tromberg, B. J. (2000) Celebrating Britton Chance. *J. Biomed. Optics* **5**, 115–118

[1] All biographical information on Britton Chance was taken from Refs. 2 and 7.