SPECIAL INTEREST BOX 9.2 Hans Krebs and the Citric Acid Cycle

Hans Krebs (1900–1981), a German-born British biochemist, is best known for his discovery of the citric acid cycle, arguably one of the most important contributions to biochemistry in the twentieth century. Krebs's efforts to elucidate the details of oxidative metabolism (the means by which nutrients are converted to energy) were made possible by many factors. As in most scientific research, Krebs was the beneficiary of discoveries by many other scientists that provided information about substrates (e.g., succinate, fumarate, and malate) and reactions (e.g., dehydrogenations, hydrations, and dehydrations) that were known to be involved in cellular respiration. The most prominent of these other scientists were Otto Warburg (1883–1970) and Albert Szent-Györgyi (1893–1986). Krebs was also fortunate to have spent the first few years of his career working in Otto Warburg's laboratory in the Kaiser Wilhelm Institut in Berlin. There Krebs learned techniques, developed by Warburg, that later proved to be crucial in his own research projects. Among these were *manometry* (a method for determining the concentration of a specific substance by using an instrument that measures the uptake of O_2 or the release of CO_2), *spectrophotometry* (a technique that measures the concentration of a substance by determining: the proportion of incident light that is absorbed by the substance at a specific wavelength, see p. 424 for a discussion of light), and the preparation of experimental tissue slices. Over several years, beginning in 1933, Krebs, aided by the prodigious efforts of his research student William A. Johnson, slowly pieced together the elements of the oxidative pathway that he eventually realized was a cycle. It was only some years after Krebs and Johnson reported their work in 1937 that the citric acid cycle was recognized as the principal means by which carbohydrates are oxidized in living cells. In addition to the controversial nature of their work (e.g., Szent-Györgyi believed that molecules such as succinate and fumarate were acting as shuttles for hydrogen atoms to O_2), one of the principal problems that delayed recognition was the identity of "active acetate," the intermediate in the conversion of pyruvate to citrate. Krebs observed that when pyruvate was added to tissue slices, large amounts of citrate were produced. However, the addition of acetate, the expected product of the reaction, had no effect. It was not until much later that Fritz Lipmann and Nathan Kaplan discovered acetyl-CoA (1945) and Severo Ochoa and Feodor Lynen established that acetyl-CoA reacts with oxaloacetate to form citrate (1951). For his efforts Krebs received a knighthood and the Nobel prize in physiology and medicine in 1953 (shared with Fritz Lipmann). Other important contributions of Hans Krebs include the discovery of the urea cycle and the glyoxylate cycle. The *urea cycle* (Section 15.1), the mechanism by which some animals convert toxic waste nitrogen to urea (a water-soluble product that can then be excreted), was discovered in 1932 by Krebs and Kurt Heinsleit, a medical student. In 1957 in a joint publication Hans Kornberg and Hans Krebs reported the discovery of the *glyoxylate cycle* (see p. 293).

Many years after his discovery of the citric acid cycle, when asked to reflect upon why he had succeeded when so many other brilliant scientists had failed to elucidate this mechanism, Krebs replied in part,

> "My outlook was that of a biologist trying to elucidate chemical events in living cells. I was thus accustomed to correlating chemical reactions in living matter with the activities of the cell as a whole. By putting together pieces of information in jigsaw-puzzle manner, and by attempting to find missing links, I tried to arrive at a coherent picture of metabolic processes. So my mind was prepared to make use of any piece of information which might have a bearing on the intermediary stages of the combustion of foodstuffs. This difference in outlook was, I believe, an important factor in determining who first stumbled on the concept of the tricarboxylic cycle."*

*Krebs, H. A., The History of the Tricarboxylic Acid Cycle, *Perspect. Biol. Med.*, 14:166–167, 1970.