CC/NUMBER 22
MAY 30, 1983

This Week's Citation Classic

Williamson D H, Mellanby J & Krebs H A. Enzymic determination of D(—)-β-hydroxybutyric acid and acetoacetic acid in blood. *Biochemical J.* **82**:90-6, 1962. [Medical Research Council Unit for Research in Cell Metabolism, Dept. Biochemistry, Univ. Oxford, England]

A rapid and specific enzymic method for measuring ketone bodies (acetoacetate and 3-hydroxybutyrate) is described using a purified D-3-hydroxybutyrate dehydrogenase from *Rhodopseudomonas spheroides.* [The *SCI®* indicates that this paper has been cited in over 755 publications since 1962.]

Dermot H. Williamson
Metabolic Research Laboratory
Nuffield Department
of Clinical Medicine
Radcliffe Infirmary
Oxford OX2 6HE
England

March 22, 1983

"In 1960, I was invited by Sir Hans Krebs to return to the MRC Unit for Research in Cell Metabolism in Oxford, a group I had left some six years previously when it was still located in Sheffield. On discovering that in the interval I had acquired some expertise in the use of enzymes as analytical tools, it was suggested that I should develop an enzymic method for the determination of ketone bodies (acetoacetate and D-3-hydroxybutyrate) using the D-3-hydroxybuty-rate dehydrogenase known to be active in rat liver mitochondria. The existing chemical methods for the determination of ketone bodies were time-consuming and unspecific.

"After three months of abortive attempts to solubilize this enzyme which is tightly bound to the inner mitochondrial membrane, the project seemed doomed to failure. A chance conversation between Sir Hans and an Australian microbiologist, June Lascelles, who was a lecturer in the department, provided the vital clue to further progress. Certain bacteria, including *Rhodopseudomonas spheroides*, are known to accumulate a polymer, poly-hydroxybutyrate, as a reserve fuel and they also contain soluble 3-hydroxybutyrate dehydrogenase to oxidize the monomer, D-3-hydroxybutyrate.[1] Within a few weeks I had grown sufficient amounts of *Rhodopseudomonas spheroides* to obtain a partially purified preparation of the enzyme and to show that it could indeed be used for the enzymic determination of acetoacetate and hydroxybutyrate. Perhaps more importantly, the enzyme appeared stable. It was at this stage that Jane Mellanby, a PhD student working on the metabolism of ketone bodies, joined me in the large-scale production of the enzyme. Soon we were growing 30 litre aspirators of the red-coloured bacteria and occasionally covering the floor of the 37°C room because of a too effective aeration system. At a later stage we encouraged H.U. Bergmeyer of Boehringer Mannheim to take up production of the enzyme and this resulted in a joint publication on the crystalline protein.[2] "We realized the potential clinical value of the method and described its application to blood samples in the original paper. The method has been widely used for research on the regulation of ketone body production[3] and of ketone body utilization.[4] In particular, the method was used by Cahill and his colleagues[5] to demonstrate that ketone bodies are an important substrate for brain in starvation. Despite numerous minor modifications by other workers, the original method appears to be firmly established."[6]

1. **Carr N G & Lascelles J.** Some enzymic reactions concerned in the metabolism of acetoacetyl-coenzyme A in Athiorhodaceae. *Biochemical J.* **80**:70-7, 1961.
2. **Bergmeyer H U, Gawetui K, Klotzsch H, Krebs H A & Williamson D H.** Purification and properties of crystalline 3-hydroxybutyrate dehydrogenase from Rhodopseudomonas spheroides. *Biochemical J.* **102**:423-31, 1967.
3. **McGarry J D & Foster D W.** Regulation of hepatic fatty acid oxidation and ketone body production. *Annu. Rev. Biochem.* **49**:395-420, 1980.
4. **Robinson A M & Williamson D H.** Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol. Rev.* **60**:143-87, 1980.
5. **Owen O E, Morgan A P, Kemp H G, Sullivan J M, Herrera M G & Cahill G F.** Brain metabolism during fasting. *J Clin. Invest.* **50**:1589-95, 1967.
6. **Bach A, Métals P & Jaeger M A.** Etude critique de la détermination enzymatique de l'acétoacétate et du D-(—)- bêta-hydroxybutyrate par la méthode de Williamson: application au plasma et au tissu hépatique. *Ann. Biol. Clin. Paris* **29**:39-50, 1971.