

This Week's Citation Classic

Rosalki S B. An improved procedure for serum creatine phosphokinase determination. *J. Lab. Clin. Med.* **69**:696-705, 1967.
[St. Mary's Hospital, London, England]

The creatine kinase activity of serum is determined by a procedure in which adenosine triphosphate, liberated by the action of the enzyme on creatine phosphate and adenosine diphosphate, is linked to the reduction of nicotinamide-adenine dinucleotide phosphate with glucose, hexokinase, and glucose-6-phosphate dehydrogenase, and the reaction followed spectrophotometrically at 340 nm. All reagents are combined in a single stable lyophilisate requiring only reconstitution with water. [The SC[®] indicates that this paper has been cited in over 825 publications since 1967.]

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"I had previously described a test for myocardial infarction (' α -hydroxybutyrate dehydrogenase').¹ I found that an American company was marketing the procedure with altered determination conditions so that its diagnostic performance would be impaired, and wrote complaining to the company president. On a visit to the United Kingdom in November 1964, he invited my wife and me to join him for dinner. During the course of the meal, he suggested that instead of complaining about an existing product, I might propose something novel. At that time, creatine kinase methodology was abysmal. The only convenient test² frequently gave negative values, and other methods were so prolonged and labour-intensive that few laboratories carried out determinations. On the back of the menu card, I sketched out my idea for modification of the Kornberg adenosine triphosphate (ATP) assay³ to creatine kinase measurement by the addition of creatine phosphate and adenosine diphosphate (ADP), and the combination of all reagents in a single lyophilisate which would require only aqueous reconstitution and sample addition. In correspondence, I outlined more fully the required reagent composition, which was prepared and presented in individual gelatin capsules.

"Method optimisation and evaluation were carried out under very adverse conditions. At the time, I was a consultant in clinical pathology and responsible for all the clinical chemistry, haematology, microbiology, and even histopathology for two acute children's hospitals—all on a parttime basis! I had received negligible support from my hospital for my research work; I had no research assistant and no suitable apparatus.

"I persuaded the American company to donate to me a simple fixed-wavelength (340 nm) single cuvette spectrophotometer, cost \$250. To measure enzyme reaction rates on this instrument, a needle was held at null point by rotating a knob connected to a numbered dial. Each Sunday, my wife would accompany me to the hospital. I would set up the reaction and read out the figures on the dial at minute intervals with a stopwatch. My wife would record the readings. Subsequently, we would calculate and plot the change in absorbance per minute. The reaction had a six-minute lag phase and the linear phase required a further five-minute monitoring period. Each single enzyme determination required 15 minutes instrument time. Each reaction mixture contained ten constituents, each of which had to be individually varied during optimisation studies. The labour involved was considerable, and can scarcely be imagined in these days of multi-sample, microprocessor-controlled automated enzyme analysers.

"Despite all the difficulties, method and clinical studies were completed in 1965. Details were submitted and immediately accepted for publication in 1966, and appeared in 1967.

"This paper has been highly cited because the hitherto complex creatine kinase determination was now so simple, requiring only sample addition to a single pre-prepared substrate mixture, and so sensitive that the procedure was adopted worldwide for creatine kinase determination in clinical biochemistry laboratories. This facilitated wider recognition of the outstanding value of creatine kinase determination in the investigation of heart and muscle disease, and in turn prompted increased use of the method."

1. **Rosalki S B & Wilkinson J H.** Reduction of α -ketobutyrate by human serum. *Nature* **188**:1110-11, 1960.
2. **Tanzer M L & Gilvarg C.** Creatine and creatine kinase measurement. *J. Biol. Chem.* **234**:3201-4, 1959.
3. **Kornberg A.** Reversible enzymatic synthesis of diphosphopyridine nucleotide and inorganic pyrophosphate. *J. Biol. Chem.* **182**:779-93, 1950.