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This Week's Citation Classic®

Pradet A. Étude des adénosine-5'-mono, di et tri-phosphates dans les tissus végétaux. I. Dosage enzymatique (Study of adenosine-5'-mono, di and tri-phosphates in plant tissues. I. Enzymatic measurement).
Physiol. Vég. 5:209-21, 1967.
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ATP, ADP, and AMP were estimated in biological extracts using the firefly bioluminescence assay. ATP was estimated directly with luciferin and luciferase. ADP and AMP were estimated after enzymatic conversion into ATP and subtracting the initial value of ATP or ATP + ADP. [The *SCI*® indicates that this paper has been cited in over 140 publications.]

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I started studying *in vivo* aspects of energy metabolism in plants in 1964 with the aim of elucidating certain physiological mechanisms of seed germination such as photosensitivity and dormancy. I hoped that the knowledge of the *in vivo* concentration of molecules with very short turnover times, such as ATP, ADP, and AMP, would give useful information about the physiological status of the cells. Nearly nothing was known because the techniques were not available; the chromatographic studies were too time-consuming and the NAD/NADH enzymatic assays, which allowed some preliminary data to be obtained in mammalian tissues, could not be used with plants.

In 1955 Strehler and Toller[1] had described a method to estimate ATP by using the bioluminescent properties of the firefly extract. Although this method had been rarely used, I was convinced that its very high sensitivity could be exploited for the determination of the mono- and diphosphate adenine nucleotides by transforming them into ATP.

In 1965 I was pleased to find a commercial supply of desiccated firefly tails in the catalog of a firm that has since disappeared. I used the long delay before receiving them to prepare equipment to measure bioluminescence by connecting the high-voltage power of a Geiger-Muller counter, the photomultiplier of an out-of-order spectrophotometer, and the amplifier and the chart recorder of a polarograph.

After having prepared the luciferin luciferase extract from the tails, I tried to produce light by mixing ATP with this extract, but I failed, even using a new batch of fireflies. I suspected that the equipment was not adequate and I tried to see the light after having acclimated my eyes for a long time in a dark room. That was also unsuccessful.

Plant biochemistry was just beginning in France at that time. Plant biochemists had very few contacts with biochemists in other fields and I was unable to get any advice. I was extremely disappointed and put the assay aside. Two years later, during a party, I was introduced to Dr. Nikhorochess, who was an old man working on muscular contractions. I asked him if he knew anything about the firefly assay of ATP. His reply: "*Il y a un seul secret; vous devez acheter les lucioles chez Sigma.*" (There is only one secret; you have to obtain the flies from Sigma.) He was right! Some months later, with the excellent technical assistance of J. Vermeersch, I was able to determine ATP, ADP, and AMP by bioluminescence and wrote the paper that describes the method. (It was my first full paper.) At the same time, my colleague J.L. Bomsel and I published a study of the adenine nucleotides in wheat leaves.[2] The two papers were published in French in a French journal. The method soon proved very useful in plant science as well as in many other fields of biological sciences.

I was extremely surprised and happy to learn that this paper has become a *Citation Classic*, because, in fact, most people who used the method described in the paper referred to the first two authors who used (and quoted) the method and published their data in English in an easier-to-find journal. Now the method is often used without quotation. Its utilization allowed us to demonstrate that adenine nucleotide ratios or adenylate energy charge are high in all normally metabolizing cells and that adenylate kinase maintains the nucleotides under near equilibrium.[3] It is this paper that allowed our method to be known by the international community. Since that time, the adenine nucleotide ratios have been used to approach the energy status of cells or tissues mainly under ATP-regenerating conditions. Today, the results of 31 PNMR call into question the validity of this approach and constitute a fascinating new field of research. Many applications of this method were recently reviewed.[4,5]

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3. ----------. Study of adenosine-5'-mono, di and triphosphates in plant tissues. IV. Regulation of the level of nucleotides *in vivo* by adenylate kinase: theoretical and experimental study. *Biochim. Biophys. Acta* 162:230-42, 1968. (Cited 135 times.)
4. **Pradet A & Raymond P.** Adenine nucleotide ratios and adenylate energy charge in energy metabolism. *Annu. Rev. Plant Physiol.* 34:199-224, 1983.
5. **Raymond P, Gidrol X, Salon C & Pradet A.** Control involving adenine and pyridine nucleotides. (Stumpf P K & Conn E E, eds.) *The biochemistry of plants: a comprehensive treatise.* New York: Academic Press, 1986. Vol. 9. (In press.)

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