



## **A segment of purine metabolism: an experimental tool for important biochemical concepts.**

Isabella Marini (1,2,3)

(1) Liceo Scientifico "U. Dini" Pisa Italy (imarini@biologia.unipi.it), (2) Dipartimento di Biologia- Sezione di Biochimica- Università di Pisa, (3) ANISN- Sede di Pisa

A major challenge to biology educators consists in teaching the molecular and cellular basis of life so that students can understand the complexity of the processes involved. One way to achieve this understanding is to link theoretical with experimental knowledge. Students can achieve understanding by participating in an activity that mirrors biochemical practice and thought. This approach provides opportunities to learn about individual or related concepts and about the nature of scientific knowledge. Purines are an attractive choice for an integrated theory-laboratory course because: 1) they are precursors and constituents of nucleic acids and coenzymes metabolic regulators and protagonists of bioenergetics; 2) important purines occur in plants, and some have important pharmacological actions; 3) some purines are chemical signals and neurotransmitters; 4) important metabolic diseases such as gout, severe combined immunodeficiency (SCID) and Lesch-Nyhan disease are due to an alteration of purine metabolism. Study of purine catabolism allows discussion of a variety of metabolic concepts, it can be based on experiments that require only common prior chemistry and biology laboratory training, it can be successfully completed within reasonable time, use the versatile and simple technique of thin layer chromatography (TLC) and easily available and inexpensive materials.

The experiments of this proposal are:

1. Separation and identification of purine mixtures.
2. Detection of adenosine deaminase activity by TLC.
3. Purine nucleoside phosphorylase catalyzes a reversible reaction.
4. Preparation of crude bovine liver or muscle extract.
5. Adenosine deaminase and purine nucleoside phosphorylase in bovine muscle extract.
6. Adenosine deaminase and purine nucleoside phosphorylase in bovine liver extract.

The first three experiments are essentially a preparation for the last three, that are the most meaningful for students. In experiment 2 and 3 students were required to use hypothetic-predictive arguments about reaction reversibility/irreversibility. When they formulated their hypothesis and verified it by starting from the products of the ADA and PNP reactions, they reasoned from effects to causes. This type of thinking is expected to increase their understanding of science as an intellectual activity. The transition from crude tissue extract to the detection of ADA and PNP activities involves the conceptual passage from macroscopic to microscopic to molecular and is easily grasped and really appreciated. The semi-quantitative determination of ADA activity in muscle and liver allows students to better understand the idea of specific tissue patterns of enzyme activity. But the most important insight of these experiments is that consecutive reactions can constitute a pathway. This, connected with the study of the common parameters of a single enzyme activity, allows students to think in terms of metabolic flexibility and complexity. We have found that in performing these experiments students become personally and intensely involved in learning and develop an increased interest in life sciences. A hands-on approach, appropriately interwoven with relevant theoretical themes, helps to develop critical thinking and creativity and to increase appreciation for scientific work.

Living in Pisa, Galileo's city, as we do, we are reminded of the synergic link between the Galilean "sensate esperienze" (experiences carried out with our senses) and "necessarie dimostrazioni" (subsequent rational demonstrations).

### Reference

Marini I., Ipatà P.L. (2007) From purines to basic biochemical concepts. Experiments for high school students. *Biochemistry and Molecular Biology Education* 35: 293-297.