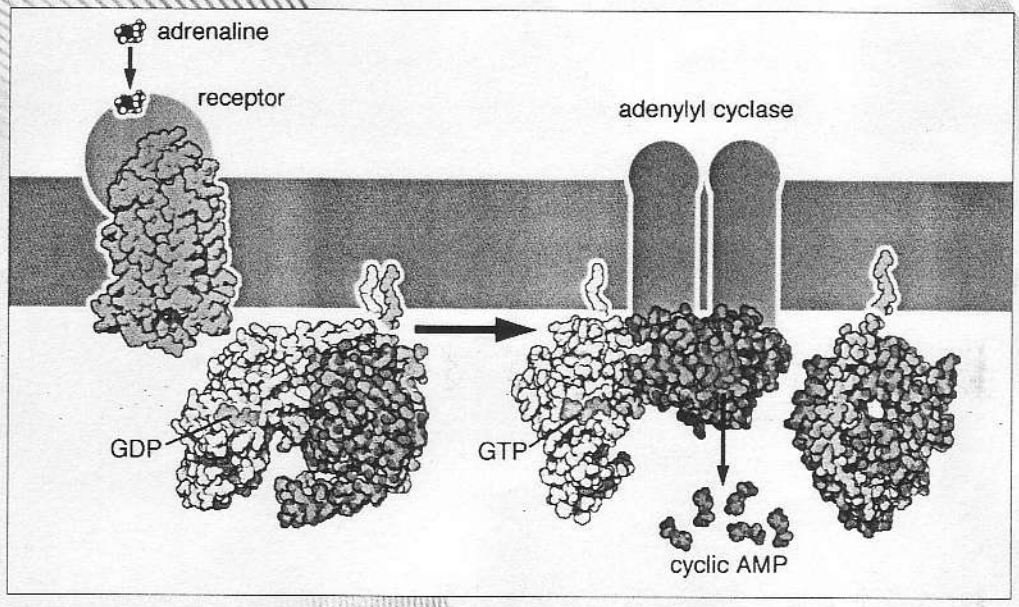
53rd National Meeting of the Italian Society of Biochemistry and Molecular Biology (SIB)

and

National Meeting of Chemistry of Biological Systems Italian Chemical Society (SCI - Section CSB)

Palazzo dei Congressi di Riccione

23rd - 26th September 2008



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8.1

ANTIOXIDANT ACTIVITY OF *OLEA EUROPAEA* DERIVATIVES

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At the present time it is easy to observe an increasing interest for the utilization of natural products as opposed to synthetic ones. The drug, food and cosmetic industry mostly use synthetic phenolic compounds with the aim of scavenging free radicals. In the cosmetic industry, in particular, these compounds are used to delay the skin ageing process.

Grape, olive and orange are fruits rich in antioxidants which are poorly exploited by the industry[1-4].

Persuing our interest in the field of natural antioxidant discovery, we have evaluated "in vitro" in different experimental models (Erythrocytes, Phosphatidylcholine liposomes, DPPH) the radical scavenging activity of raw extracts coming from manufacturing process of olive oil[5].

The products exhibit a marked antioxidant activity with an IC_{50} ranging from 1 to 5 mg/ml which is largely over to that of vitamin E.

In addition the compounds show a sinergistic interaction with this important antioxidant contained in olive oil..

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8.2

NEW APPROACHES TO THE EXPANDED NEWBORN SCREENING PROGRAMS BY TANDEM MASS SPECTROMETRY: REDUCTION OF FALSE-POSITIVES FOR C5, C5OH, C6DC.

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The expansion of newborn screening programs has increased the number of newborns diagnosed with inborn errors of metabolism in the presymptomatic phase but it has also increased false-positive results. False positive results are costly for Public Health Resources and causes unnecessary parental stress. We report an update on the latest developments in the expanded newborn screening programs. Acyl-carnitines C5, C5OH, C6DC are one of the analytes most frequently responsible for false-positive results. We developed a rapid liquid chromatography-tandem mass spectrometry (LC-MS/MS) method that identifies free acids and glicine derivatives: 3-idroxisovaleryl-acids (3-OH-IVA), 2-idroxisovaleryl-acids (2-OH-IVA), 3- MethylcrotonylGlycine (3MCCGly), IsovalerylglycIne (IVAGly), PropionylGlycine (C3Gly), 3-idroxMethyl-Glutaric acid (3-OH-3-MetGlut), 3-Methyl-Glutaconic acid (3-MetGlutac), Lactic acid (LA), Metylmalonic acid (MMA), Succinyl acid (Suc) and TiglylGlycine (TiglilGly) in blood spots thus reducing false-positive rates due to C5, C5OH, C6DC during expanded newborn screening programs. We studied newborn screening spots from 92 healthy controls; 28 from false positives for abnormal C5, C5OH, C6DC and 23 from inborn truly affected. Analytical method consists of chromatographic separation on a C6-Phenyl column of an extracted 3.2 mm dried blood spot and injection into triple quadrupole mass spectrometer equipped with a Turbo Ion Spray Ionization Source. Specific Multiple Reaction Monitoring were carried out and labelled isotopic reagent were used as an internal standard. No derivatization is required and total analysis time is 5 minutes per sample. Intra- and interassay imprecision data were 3.5%-8% and 3.2%-6% for MMA. Limit of detection and limit of quantitation were 0.01 and 0.05 micromol/L, respectively, for C3Gly and IVAGLy. The recoveries were 92.9%-106.1%. No deterioration was noted on the columns after 500 chromatographic runs. The application of this method as second tier test allows to reduce false-positive results, the retesting and the consequent recalls inborn. In addition, the test allows us to diagnose with greater certainty diseases like Isovaleric acidemia (IVA), 3-Methylcrotonyl-CoA carboxylase deficiency or MethylcrotonylGlicinurie (MCC), Biotinidase (BIO) because on the other hand, false-negative cases have been reported by several newborn screening laboratories. We found that in experimental conditions developed specifically is that the following picture of metabolic alteration:

- IVA increase 3-OH-IVA, IVAGly
- MCG increase 3-OH-IVA, 3MCCGly
- BIO increase 3-OH-IVA, IVAGly, C3Gly

CONCLUSIONS: This method has the potential to markedly reduce false-positive results and the associated costs and anxiety. It may also be suitable for diagnosing and routinely monitoring blood spots IVA, BIO, MCC.

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