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(viii)
SYMPOSIA SERIES No. 42

BIOCHEMISTRY OF
THE CELL NUCLEUS

Edited by P. B. Garland and A. P. Mathias
(ISBN 0 904498 03 4) £15.00 U.S. $30.00

The articles of this Symposium deal with aspects of the structure and function of the cell nucleus, at several levels of molecular and biological organization. Although much is already known of the way in which the genetic information of eukaryotic cells is stored, replicated, transcribed and processed, the scale and intricacy of the operation is immense in comparison with the simpler and more amenable bacterial systems. Accordingly our knowledge of the more highly evolved eukaryotic systems is far from complete, both in concept and detail. The articles not only review present knowledge; no less importantly they identify areas where mystery is more obvious than mechanism, and they pose some of the central questions that future research will have to answer.

Biochemistry of the Cell Nucleus will be timely reading for those in life or medical sciences who, either by their teaching or research, or just general intellectual curiosity, desire to deepen their understanding of how the nucleus masterminds the incredibly complex but beautifully co-ordinated activities of the cell.

List of contents and authors:

Multiplicity of Animal Cell Deoxyribonucleic Acid Polymerases by G. Brun & F. Chapeville.
The Deoxyribonucleic Acid Polymerases of Non-Vertebrate Eukaryotes by A. G. McLennan & H. M. Keir.
An Approach to the Understanding of Messenger Ribonucleic Acid Synthesis, Processing and Regulation in Eukaryotes by R. Williamson.
Chromatin Structure by J. O. Thomas.
Structure and Function of Nuclear Membranes by W. W. Franke.
Mitosis and Microtubule Assembly by M. Jacobs & T. Cavalier-Smith.

THE BIOCHEMICAL SOCIETY BOOK DEPOT
P.O. Box 32, Commerce Way, Colchester CO2 8HP, Essex.
7-Ethoxycoumarin: substrate for a rapid, sensitive, fluorometric assay of microsomal monooxygenase activity

The mixed-function oxidase system in liver microsomes plays an important part in the metabolism of drugs. For this reason, sensitive and reliable tests are necessary for determining enzyme activity and consequently the drug-metabolizing capacity of the system.

7-Ethoxycoumarin has been found1 to be an excellent substrate for the direct fluorometric determination of microsomal monooxygenase activity. The assay is based on the O-dealkylation of 7-ethoxycoumarin to produce the highly fluorescent 7-hydroxycoumarin (umbelliferone, catalog number H2400-3).

This system involves cytochrome P-450 containing monoxygenases and is dependent on NADPH and molecular oxygen.

\[
\text{CH}_2\text{CH}_2\text{O} \longrightarrow \begin{array}{c}
\text{NADPH, O}_2 \\
\text{cytochrome-P-450} \\
7\text{-ethoxycoumarin}
\end{array} \rightarrow \begin{array}{c}
\text{O-deethylase} \\
7\text{-hydroxycoumarin}
\end{array}
\]

Since it is well known that microsomal monoxygenases are induced typically by phenobarbital and polycyclic aromatic hydrocarbons, e.g., 3-methylcholanthrene (MC), the effect of these on O-deethylation of 7-ethoxycoumarin was studied. Phenobarbital and MC induced O-deethylation of 7-ethoxycoumarin in hepatic tissues2 and in isolated rat liver cells,3 whereas only phenobarbital induced the O-deethylation in extrahepatic tissues.4

It has also been demonstrated that MC inducibility of 7-ethoxycoumarin O-deethylase and aryl hydrocarbon hydroxylase is determined genetically in the Ah locus.5 Poland et al.6 have shown that O-deethylation is also inducible by 2,3,7,8-tetrachlorodibenz-p-dioxin (TCDD).

An improved fluorometric assay for microsomal monoxygenase determination via 7-ethoxycoumarin O-deethylation has been developed recently.7 This in vitro fluorometric assay is at least ten times as sensitive as present methods, and has facilitated the kinetic studies of O-deethylation activity as well as a reevaluation of the use of 7-ethoxycoumarin O-deethylation as an indicator of phenobarbital-induced monooxygenase activity in mice.8

This assay enables nearly quantitative recovery of the major product, 7-hydroxycoumarin, by extraction and the product is essentially free of fluorescent contaminants. Maximal fluorescence of 7-hydroxycoumarin in aqueous solution is obtained at pH 9.5 or higher. O-Deethylase activity induced by phenobarbital, MC and TCDD was studied by this method.9

A modified, simple and sensitive assay of 7-ethoxycoumarin O-deethylation was reported recently, by which a level of 170 pmol of 7-hydroxycoumarin could be measured.8

The advantages of using 7-ethoxycoumarin as a substrate are that the compound is not known to be carcinogenic, it is not particularly light-sensitive, and it is dealkylated to a single, highly fluorescent product.7 7-Ethoxycoumarin has been rigorously purified to eliminate as much background fluorescence as possible.

7-Ethoxycoumarin should prove to be a useful indicator of a wide range of monoxygenase inducers, particularly in cancer research.

References:

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