CHARACTERIZATION OF PROTEIN AND FUNCTION

RESIDENTIAL SCHOOL—WYE COLLEGE, KENT 5–9 SEPTEMBER 1977

This School follows naturally the two previous ones which dealt specifically with the binding of small molecules and ions by biological macromolecules. It is once again aimed at Scientists with some experience in protein research, or division and section heads wanting to acquaint themselves with the current state of knowledge.

In particular, attempts will be made to emphasize the chemical, structural and conformational aspects of protein function.

The School will feature review lectures, and a selection of small group tutorial/discussions dealing with the detailed aspects of experimental techniques and the behaviour of proteins *in vivo* and *in vitro.* One session will be set aside for the presentation and discussion of original contributions by participants. A trip to Canterbury will be arranged on Wednesday afternoon.

Lectures will provide reviews of the main protein functions and the factors which influence protein conformation and aggregation behaviour.

LECTURE PROGRAMME:

FUNCTIONAL CLASSIFICATION OF PROTEINS
STRUCTURAL FUNCTIONS
TRANSPORT PROTEINS
SOLVENT MEDIATED INFLUENCES ON CONFORMATION AND ACTIVITY
CHEMICAL/MECHANICAL ENERGY TRANSDUCTION
CATALYTIC FUNCTIONS
DEFENCE PROTEINS
THE CONFORMATION AND STABILITY OF FOLDED GLOBULAR PROTEINS
SELF-ASSEMBLY AND FUNCTIONS OF MULTI-SUB-UNIT STRUCTURES
BIOLOGICALLY MODIFIED PROTEINS—GLYCOPROTEINS AND LIPOPROTEINS

There will be many lecturers and speakers at the course from Universities, Institutes and large industrial concerns in Europe, United States of America and the United Kingdom.

SEMINAR PROGRAMME:

1. ANALYTICAL TECHNIQUES APPLIED TO THE STUDY OF PROTEIN FUNCTION:

Fluorescence Spectroscopy
NMR Spectroscopy
Immunochemical Techniques
Thermodynamic Methods
Chemical Modification
Theoretical Techniques
Affinity Labelling

2. CONFORMATIONAL PROPERTIES AND INTERACTIONS OF PROTEINS IN VIVO AND IN VITRO:

How do Globular Proteins Fold Up?
Chromosomal Proteins
Hormone-receptor Interactions
Protein Hydration, Conformation and Binding of Small Molecule Species
Fibrillar Proteins
Structural Proteins
Membrane Proteins
Contractile Systems
Active Site Chemistry
Glycoproteins

There will be a total of up to 50 sessions (10 per day for 5 days, i.e. most topics will be dealt with on three sessions). Each participant will be able to attend five of the seminars offered.

A fully descriptive leaflet and application form are obtainable from:

Miss Sara Urwin, The Chemical Society, Burlington House, London WIV 0BN. Tel: 01 734–9971 Ext. 60.

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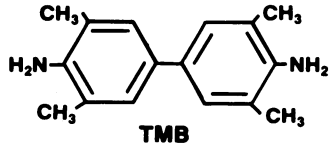
Tetramethylbenzidine

A reported noncarcinogenic analog of benzidine



TMB

For many years benzidine has been used as a sensitive and specific reagent for the detection of blood.[1] However, its extreme carcinogenicity has curtailed its use in recent years. In fact, in 1974, the Occupational Safety and Health Administration banned its manufacture and use in the United States.[2]

Several chemical transformations are thought to be responsible for the carcinogenicity of aromatic amines. An early hypothesis involved possible *ortho*-hydroxylation.[3] Later views suggested that N-oxidation is important. Since *o*-tolidine (3,3′-dimethylbenzidine) retains the sensitivity in the detection of blood, it seemed likely that **3,3′,5,5′-tetramethylbenzidine (TMB)** (in which *ortho*-hydroxylation is impossible) might also be an effective but *safe* substitute for benzidine.[4] Indeed, it was found that subcutaneous injection of **TMB** into rats "*produced no tumors specifically attributable to it, in doses greater than those in which benzidine or o-tolidine cause a high yield of neoplasms.*"[4]

The simple *Salmonella*/microsome microbial test (Ames test)[5] for the detection of mutagenic activity showed **TMB** to be nonmutagenic.[6,7] Results of these studies suggest that **TMB** is also noncarcinogenic.

Garner *et al.*[8] have recently evaluated the use of **TMB** as a presumptive test for blood in forensic work. As might be expected from its structural similarity to benzidine, **TMB**, in various concentrations of glacial acetic acid, reacted with blood in the presence of hydrogen peroxide to form a colored product. Comparative studies with benzidine showed **TMB** to be equally sensitive in the detection of blood.

The specificity of **TMB** and its tendency to give a false positive reaction with substances known to interfere with the benzidine test for blood were examined using various vegetables and applying several methods of testing.[8] The results indicate that **TMB** is as specific as benzidine. Also, **TMB** and benzidine reacted similarly to chemical oxidants and catalysts before addition of hydrogen peroxide.[8]

Since benzidine in solution is well known to lose its sensitivity with storage, the effects of time, light and heat on **TMB** solutions were studied.[8] Results indicate that the stability of **TMB** is the same as that of benzidine.

The only significant difference observed between **TMB** and benzidine was in their solubilities in glacial acetic acid. The concentration of a saturated solution of **TMB** approaches 0.2*M*, while that for benzidine ranges between 0.7 and 1.0*M*.

Thus, the sensitivity, specificity, stability and apparent noncarcinogenicity of **TMB** are very important criteria in determining the usefulness of **TMB** as a substitute for benzidine in the detection of blood.

References:

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8) D.D. Garner, K.M. Cano, R.S. Peimer, and T.E. Yeshion, *J. Forensic Sci.*, **21**, 816 (1976).

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