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Book Reviews

The electric microbe acid test

IMPEDANCE MICROBIOLOGY

by Ruth Firstenberg-Eden and Gideon Eden, *Research Studies Press, Letchworth/John Wiley, Chichester & New York, 1984. £24.50 (xiii + 170 pages) ISBN 0 86380 020 3*

'These germs – these bacilli – are translucent bodies, like glass, like water. To make them visible you must stain them. Well, my dear Paddy, do what you will, some of them won't stain. They won't take cochineal: they won't take any methylene blue: they won't take gentian violet: they won't take any colouring matter. Consequently, though we know, as scientific men, that they exist, we cannot see them.'

Sir Ralph Bloomfield Bonington; G. B. Shaw, *The Doctor's Dilemma.*

In contrast to Sir Ralph, most microbiologists require quantitative data concerning the microbial load in a sample, and the standard, traditional method to determine the number and nature of microorganisms present in a food, clinical or environmental sample is, of course, the plate count method.

The major disadvantages associated with this method are: (1) that a long time must elapse before a sample may be declared 'positive' (contaminated) or 'negative' (safe); and (2) that the method is extremely monotonous in use. Such considerations have led to the development of a number of approaches to the rapid, automated estimation and identification of microorganisms, a field that has come to be known as 'Automated Microbiology'.

Apart from direct epifluorescent microscopic techniques, it is the purely physical techniques of 'Automated Microbiology' which hold the greatest promise for constituting a rapid and automated means of estimating the microbial content of a sample and, amongst these, the impedance method referred to in the title of this book is perhaps most likely to become the method of choice.

The measurement of biological impedances has a long and distinguished history; it was impedance measurements on erythrocytes by Fricke in 1925 that first showed that the cell membrane was of molecular thickness (3–10 nm): similarly, the observation by Cole and Baker in 1941 that the squid axon possesses inductive properties led to the analysis of non-linear, voltage-dependent ionic conductances embodied in the Hodgkin–Huxley (1952) equations. What, then, is the impedance method, and how do we apply it in microbiology where the organisms are too small for intracellular measurements?

It has been known, at least since the work of G. N. Stewart in 1897, that the presence or activity of microorganisms can influence the electrical impedance of the medium in which they are growing, most usually by decreasing the conductance resulting from the production of small, charged metabolites from macromolecules or from uncharged species such as sugars. In principle, then, changes in the impedance of a microbial growth medium might be used to gain information about the number and/or nature of microorganisms in the culture. However, it was not until the 1970s when Cady, amongst others, demonstrated a monotonic relationship between the microbial content of a sample and the time needed to change the culture impedance by a certain amount that the technique began to find its feet. The present monograph is an attempt to review, mainly for the benefit of microbiologists lacking a background in the physical sciences, the fairly substantial literature describing the principles and practice of the impedance technique. There is special reference to the optimization of the protocols to be used, for a given type of measurement, with the objective of minimizing the extensive scatter in the data.

Whilst the Coulter Counter works on the principle that microorganisms passing a narrow orifice take up a significant volume fraction and strongly affect the (DC or low frequency) impedance of the system, the volume fraction taken up by the microorganisms in the type of arrangement with which we are here concerned is negligible, so that it is their influence on the conductance of the *medium*, and on the impedance of the *electrode/electrolyte interface*, that we wish to measure. Rather little is known about the exact factors determining the electrode impedance, but the authors note that isoconductive solutions containing H^+ rather than other cations have a much greater electrode capacitance (measured with stainless steel electrodes), so that we may determine the presence of yeasts by measuring the capacitance under conditions in which the conductance changes but little.

This type of finding, which greatly enhances the utility of the method, came out of the work of the authors at the laboratories of the Bactomatic firm, and nicely illustrates my own prejudice

that whilst the impedance technique is very convenient and powerful, and will become more so as the cost of computer hardware and software drops yet further, the greatest improvements in the use of this technique will come from: (1) an improved understanding of the metabolic and electrochemical bases for the observed changes in the admittance, the conductance and the capacitance; and (2) the optimization and redesign of selective bacteriological media specific for use with the impedance technique. On this basis, one would argue that there remains a great deal of interesting and fundamental scientific work to be done on this topic.

Other significant, and more or less novel, points made by the authors are that: (1) the optimal temperature and/or medium for growth of a given set of organisms in a culture is not necessarily the optimum temperature/ medium for their estimation; (2) since one is interested in many cases (such as food microbiology) more in the *activity* of the microorganisms than their *number* in a sample, impedimetry may actually be *conceptually superior* to plate counts; and (3) the generation time of the microorganisms *actually in the matrix in which they are living or to be tested* may be obtained from the slope of the curve relating the impedance detection time to the logarithm of the initial cell number. Like Sir Ralph, therefore, we do not need to see the microorganisms to know that they are there!

What, then, are my quibbles about this book? First, the level of explanation of the theory of AC impedance and electrochemistry is, in my view, neither sufficiently elementary nor extensive for the typical microbiological audience at whom the book is primarily aimed. Second, the legends to the Figures and Tables are in most cases appallingly skimpy. Third, nowhere is even the approximate, and not insignificant, cost of the commercially available instrumentation mentioned.

However, this said, I am certain that the impedance method has a splendid future in Automated Microbiology, and I believe that this useful monograph will do much to stimulate interest in the existing implementations of these approaches, and to help the development of related ones. Other books will surely be written about this method of microbial biomass estimation, but this is the first, and *ergo* the best.

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Using serum-free media

MAMMALIAN CELL CULTURE: THE USE OF SERUM-FREE HORMONE-SUPPLEMENTED MEDIA

edited by Jennie P. Mather, *Plenum Press, 1984. $39.50 (xvii + 284 pages) ISBN 0 306 41584 4*

This timely volume consists of articles selected to illustrate the increasing utility and interest in systems for cell culture using serum-free media. An exhaustive survey on this subject would not have been possible in a single volume, at least in the chapter form as presented here. Rather the intent was to include in-depth coverage in a number of different areas to provide readers with data and reviews, giving examples of the various experimental possibilities of this general approach.

Accordingly, the first chapter provides insight into the roles of PDGF, FGF, EGF, somatomedin C and other factors on progression through the G_0/G_1 phase of the cell cycle of quiescent 3T3 cells, and the second compares the reduced requirements for growth factors and hormones of transformed cells from human, hamster, rat and mouse tissues with those of normal cells. It is clear that the use of limiting-serum or serum-free culture media permits interpretations which would be difficult or impossible with the more standard milieu.

This argument is extended in Chapters 3 and 4 for differentiated cells such as neurons, keratinocytes, adipocytes and pigmented epithelia where the effects of insulin, FGF, hGH and other factors on adipogenic cell lines and the requirements for growth and function of human myelomonocytic lines (HL-60, K562, U-937 and THP-1) are discussed. The elements of hybridoma technology are reviewed concisely in Chapter 5, with an interesting summary of reports on defined media which permit both growth of the cell lines and the synthesis and release of immunoglobulins. Of course these media provide a significant advantage for those desiring to concentrate and purify the monoclonal products.

The kidney cell lines MDCK and LLC-PK_1, isolated and serially propagated in media containing serum, have been used for many years for model studies on trans-epithelial water flux and other proximal tubule functions. More recently, supplementation of defined medium with insulin, transferrin, triiodothyronine, hydrocortisone and prostaglandin E_1 has permitted propagation and study in the complete absence of serum (Chapter 6). Not only was contamination of the study system with enzymes, other proteins and serum factors reduced, thereby simplifying interpretation, but also primary kidney epithelia could be maintained without fibroblast overgrowth, a common problem encountered in most attempts to isolate and maintain epithelia from mixed tissues. The media used were designed specifically to stimulate proliferation of the epithelial cell type desired, by the introduction of the appropriate hormone(s) or growth factor(s) while, at the same time, avoiding conditions which favor fibroblast growth (e.g. higher serum concentration).

Studies *in vitro* on interactions between cells and extraneous agents (e.g. carcinogens), among the same or differing cell types within an organ and between cells and their matrices are also described. Interestingly, factor(s) which stimulate DNA synthesis in normal heptocytes were found in sera of rats treated with carcinogens and from tumor-bearing humans (Chapter 7). Autocrine control and paracrine interactions among Sertoli, Leydig, peritubular myoid and germinal cells of the testis are reviewed, and an interesting model for intratesticular cell regulation is described. Our understanding of the regulatory roles played by individual cell types in organs such as the testis, which are composed of a number of differing cell types in close association, can certainly be advanced with definition of conditions appropriate for *in vitro* function of clonally derived lines.

The biochemical and biological properties of attachment factors (collagen, fibronectin, laminin, chondronectin, serum-spreading factor, epibolin and fetuin) are summarized in Chapter 9,