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微生物学——1975年

这是一本根据美国微生物学会1974—1975年召开的学术会议中的一些论文编辑的。内容较新，反映了微生物学，尤其是医学微生物学的某些新进展，可供微生物、医学微生物、抗菌素、生物制品、真菌毒素等有关的科研人员参考。全书分五部分：(一)临床微生物学的快速诊断法(14篇)；介绍电流阻抗法、微量热量法、生物发光法、逆向免疫电泳法、气相色谱法和电子计算机法等新技术用于临床微生物诊断。(二)细菌疾病的致病机制(43篇)；论述微生物在体表的相互作用，渗透、发炎及细胞的去向，外毒素和内毒素等。(三)真菌毒素(5篇)。(四)新疫苗(4篇)；论及流感嗜血菌、脑膜炎奈瑟氏球菌，淋病奈瑟氏球菌，铜绿假单孢菌和呼吸道病毒的疫苗。(五)细胞的分化和联系(11篇)，论述原核细胞和真核细胞的细胞分化的基础研究等。

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Introductory Note

Readers will easily note that this volume has a "perspectives" quality, particularly in relation to clinical microbiology. With the very great range of topics in clinical microbiology, we expect that the surveys from the American Society for Microbiology (ASM) Conference on Pathogenic Mechanisms in Bacterial Diseases will provide many members, including teachers, with useful summaries and keys to the very latest literature. Neither diagnosis nor treatment has been totally left out, and parts of the successful ASM Conference on Rapid Diagnostic Techniques, as well as some discussions of prospects for certain New Vaccines, are included.

The interactions of "basic" and "applied" microbiology are so strong that they can be outlined for nearly every one of the contributions to this volume. An example of the fact that microbiology never strays too far from sweaty relevance is the Symposium on Mycotoxins from the 1975 Annual Meeting of the ASM. The mycotoxins surely are members of only one of the vast numbers of new classes of microbial products yet to be studied. They show the characteristic usefulness in research and the potential importance in human disease that all scientists have grown to expect of microbial products.

Comparable examples could be multiplied from a section on a new trend in "basic research": the material from the ASM Conference on Cell Differentiation and Communication. The eukaryotic microbes provide a seemingly unlimited array of models—but the fascination and liveliness of this new field can be seen from the conference, without further comment from me. Especially at a time when pressures on graduate students and microbiologists are increasingly intense, students can surely take heart from the number of exciting topics just coming under investigation!

David Schlessinger

I. RAPID DIAGNOSTIC TECHNIQUES IN CLINICAL MICROBIOLOGY

Introduction

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The ASM Conference on Rapid Diagnostic Techniques in Clinical Microbiology was held in Tucson, Arizona, 4–6 December 1974. Just over 300 microbiologists attended, with roughly equal distribution between representatives of universities, private hospital laboratories, and industry. The purpose of the conference was to define the extent to which new microbiological techniques which give rapid and/or automated results can be applied to problems in clinical microbiology. The emphasis in program planning was on chemical, electrical, radiometric, and immunological methods fundamentally different from those in current use in all but a few clinical microbiology laboratories. The conference was organized into formal presentations, formal and informal discussion, and demonstrations of equipment relevant to the subject matter of the conference.

The papers which follow represent the majority of the formal presentations at the conference. These papers and other activities clearly demonstrated that clinical microbiology is entering a period of considerable change involving a move away from the classical, cultural, and biochemical techniques in use over the past 50 years. Some methods, such as the radiometric detection system discussed by Eileen Randall and the rapid, automated susceptibility testing system described by Thomas Gavan (Antimicrob. Agents Chemother. 7: 466–480), have been extensively evaluated in the clinical laboratory setting and are being introduced into routine use in laboratories which find them appropriate to their needs. Others, such as the electrical detection systems and the direct detection of microbial metabolic products by gas chromatography, look very promising but further evaluation is necessary before introduction into the clinical laboratory can be considered. In addition to the more technical topics discussed at the conference, one morning was devoted to the rapid handling of microbiological data by computers. In that session complete laboratory reporting systems were discussed as well as the selective use of computers for problems in bacterial identification, susceptibility testing, and hospital epidemiology.

The success of the conference was due in large part to the advice and effort of a great many microbiologists, particularly Paul Ellner, Alex Sonnenwirth, John Sherris, and Lawrence Kunz, who as session conveners acted as an unofficial organizing committee. It is my hope and expectation that this field will continue to advance at a rate which will make another conference appropriate soon.

Determination of Bacterial Cell Concentrations in Urine Specimens by Electrical Measurements

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INTRODUCTION

Urinary tract infections are by far the most common of the urological diseases (4, 7, 9). Bacteriuria usually follows the use of indwelling catheters and various surgical procedures in most hospitals (1). The unsuspected and elusive asymptomatic urinary tract infections among children, pregnant women, diabetics, and geriatric populations also pose serious problems (9). Thus, the need exists for rapid, inexpensive screening tests for the presence of bacteriuria. These should be sensitive and yet simple to perform.

In the microbiological methods presently used by clinical microbiologists, progeny are detected after a varied period of growth by counting colonies, by bioluminescence, or by observing various biochemical reactions such as formazan formation, etc. (4, 6, 10). With this in mind, we examined the possibility of using electronic impedance or electrical characteristics of bacterial cells as a rapid screening method which bypasses colony formation and growth. Kass (7) defined a bacteriuria as a urinary tract infection containing 10^5 (or greater) organisms per ml of urine. A rapid detection system should be capable of measuring above and below this level both for detection and for continued monitoring purposes.

IMPEDANCE MEASUREMENTS

Principles of Impedance

Impedance is a measure of the resistance ("opposition") of a circuit element to the flow of a sinusoidal alternating current. When the sinusoidal current in an electrical circuit containing only resistance (i.e., a "resistor") is in phase with the alternating current and there is no variation in time, the resistance value is the voltage to current ratio (Fig. 1a). When the current is in a circuit containing only an inductance (i.e., an inductor coil), it lags behind the voltage by 90°, and $2\pi fc$, the inductive value, is the voltage to current ratio (Fig. 1b). In a circuit containing only capacitance, the current leads the voltage by 90°, and $\frac{1}{2\pi fc}$ is the value of the voltage to current ratio (Fig. 1c). When any two or all three are present in the circuit, the result is an adjustment to some value and phase angle with respect to the voltage which will be intermediate between ±90° depending on the relative amounts of resistance, inductance, and capacitance in the circuit.

The concept of impedance, actually denoted by the symbol Z and readily manipulable by the rules of vector algebra, represents the total "opposition" to the flow of the sinusoidal al-

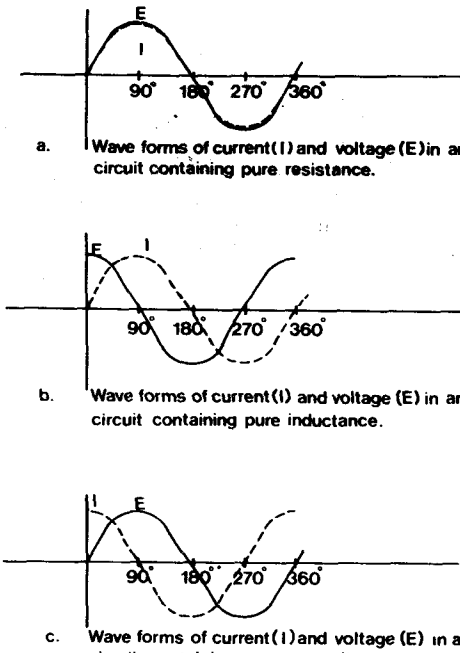

FIG. 1. *Various wave forms and relationship of current (I) and voltage (E) in an alternating current (A-C) circuit containing (a) pure resistance, (b) pure inductance, and (c) pure capacitance*

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ternating current in a circuit containing resistance, inductance, and capacitance (the latter two together are termed the "reactive" part of the circuit).

Without going into vector sums and the various mathematical formulas, impedance (Z) can more simply be described as the ratio of the sinusoidal voltage to the sinusoidal current when a sinusoidal alternating current is applied to a circuit and there is a linear time response to the event. Or: impedance = voltage/current ($Z = E/I$). Any change in the components of the circuit, such as the presence of microorganisms or their end products, will thus change the impedance and alter the voltage-current relationship.

Instrumentation Requirements

In measuring impedance or determining other electrical characteristics, the physiological event is placed between the measuring electrodes in such a way that any changes which alter the current density distribution between the electrodes are manifested as a change in impedance. Both bipolar and tetrapolar electrode systems are currently employed to measure impedance.

All impedance circuits have several requirements: (i) a source of sinusoidal alternating current; (ii) an amplifier with an input impedance that is high with respect to the impedance between the electrodes; and (iii) a detector to recover or record the impedance signal. This can be a voltmeter, null indicator, oscilloscope or a phase-sensitive circuit.

Phase-sensitive circuits are necessary if one wishes to dissect the impedance into its "reactive" and "resistive" components. However, if the change is relatively small, the voltage which reflects the changes is usually merely amplified and not broken down into its components. In most cases, only the total impedance change is used.

Geddes and Baker (5) and Schwan (12, 13) have excellent discussions on two-electrode and tetra-electrode impedance instrumentation. In one of the two-electrode arrangements, the impedance "bridge" is arranged so that resistance and capacitance units balance the bridge. When a physiological event is placed in the circuit, this balance is disturbed and the output voltage changes. In another type, current is fed symmetrically to the two electrodes through two resistances (which are high compared to the total impedance), and the detector is connected directly across the electrodes. Here, the voltage change is due both to the nominal impedance and to changes induced by the biological system which is being measured.

In the tetrapolar electrode system, the current enters the two electrodes which are the farthest apart. The two inner or potential measuring electrodes therefore receive a voltage which is determined by the effect of the biological system on the current density distribution in the circuit.

APPLICATIONS TO MICROBIOLOGY

Impedance Measurements of Metabolic Activity

Schwan (13) found that biological materials in suspension exhibit a 1 to 2 log capacitance change as a function of applied frequency. Cole (3) has indicated that bacterial cell suspensions behave similarly. Therefore, the impedance or electrical characteristics of bacterial cells in an appropriate circuit, or impedance changes caused by their metabolism, should be measurable. It is only recently that this type of instrumentation has been used in clinical microbiology to detect bacteria, their growth rate, or the production of metabolites. Three instruments which measure impedance or electrical changes associated with the presence of microorganisms have been reported (14, 15; A. Ur and D. Brown, Symposium on Rapid Methods and Automation in Microbiology, Stockholm, 1973; P. Cady and S. W. Dufour, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, p. 8). Two of these are presently produced commercially. The Strattometer is manufactured by Stratton and Co. (Medical) Ltd., Hartfield, England, and the Bactometer is manufactured by Bactomatic, Inc., Palo Alto, Calif. These two instruments both have added various refinements to the impedance bridge technique by using a circuit containing two measuring cells. Both cells contain the same basic fluid (growth media, special substances, antibiotics, etc.). However, one remains uninoculated as a "reference" well and the other, the sample or "experimental" well, is inoculated with microorganisms. In both of these instruments, a comparison between the impedance of the two wells is recorded. If the initial inocula are low, enough time must elapse to allow measurable impedance changes to occur. This could vary from several hours to days.

Electrical Determinations of Cell Concentration

The third instrument, reported in detail here, uses a tetrapolar steel or platinum electrode system. It differs from the other two

instruments in that it measures the electrical effects of washed bacterial cells suspended in distilled water and not their metabolites. Thus, correlations with concentration can be quickly obtained which bypass growth, colony formation, or production of metabolites. Based on Cole's observations (3), we applied an alternating current square-wave voltage pulse train to bacterial suspensions in distilled water. A sine-wave would have been equally effective, but a square-wave was easier to read on an oscilloscope. In theory, differences in bacterial concentrations should be detectable by measuring the variations in the output peak-to-peak voltage. Since *Escherichia coli* has been implicated in the majority of urinary tract infections, it was chosen as the target organism (2, 8, 11), but other bacteria were also tested.

E. coli K-12 was used for the majority of these experiments. The other organisms tested were *Pseudomonas aeruginosa* ATCC 9721, *Staphylococcus aureus* ATCC 12600, and *Bacillus cereus*. The organisms present in clinical urine specimens were not identified by this laboratory. Bacterial cultures were grown on a rotary shaker at 37 C for 24 h in antibiotic medium #3 (Difco). Amounts of 5 to 10 ml of cells were washed two times with distilled water on 0.45-μm membrane filters. The cells were then thoroughly agitated to break up clumps, resuspended, and diluted to desired concentrations.

Urine specimens were obtained from the clinical microbiology laboratory of the University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston. Specimens were prefiltered through 8-μm filters to remove leukocytes, erythrocytes, and other debris. The bacteria were then washed as usual on the membrane filters and resuspended in distilled water. Debris or inorganic salts would interfere with the test. Spread plate counts on plate count agar (Difco) were made with all bacterial suspensions.

An Exact model 120 waveform generator supplied a square-wave input alternating current at 20 V peak to peak (P-P). There was no visible distortion over a frequency from 1 to 10 Hz as the wave had a constant shape and amplitude within this range. The voltage meter, a Textronix (type 502-A) oscilloscope, has a high impedance input (100 kohm). It also has a band pass which will permit voltage readings to be taken within the input frequency range without distortion (a low pass filter with a cut-off frequency greater than 10 kHz). The (−) terminal of the signal generator and the bottom terminal of the oscilloscope were grounded. The wires from the signal generator were attached to the two outer electrodes, and the wires from the inner ones were attached to the oscilloscope. The probe configuration and this instrumentation are shown in Fig. 2. A four-pronged probe was used (Fig. 3). Either steel or platinum wires served as electrodes. These were fastened to the cover of a 50-mm plastic petri dish and extended down into the bacterial suspension. Measurements were made on the peak-to-peak output voltage. No doubt several other types of voltage meters or signal

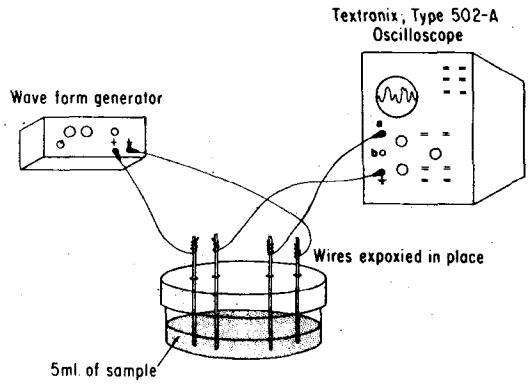

FIG. 2. *Instrumentation employing the input square-wave form generator, tetra-polar electrode probe, and the output voltage meter (oscilloscope). The generator was grounded at the (—) terminal and the oscilloscope at the lower site (⏚). The current enters the two farthest electrodes. The two internal electrodes are connected to the oscilloscope.*

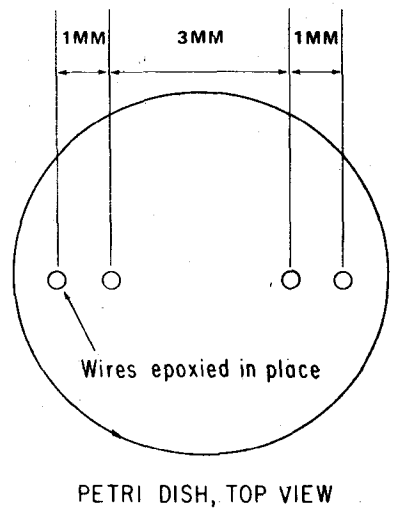

FIG. 3. *Configuration of the four steel probes which were epoxied in place on the cover of a 60 by 15 mm plastic petri dish.*

generators could be used here. The arrangement of the electrical connections proved to be of prime importance.

Several different electrical connections were tried with the tetrapolar probe. The input voltage from the generator is represented by (+) and (−). In the arrangement which gave the highest resolution, there was a single-ended input in which the probe next to the positive input probe was grounded. The signal generator wires were connected to the outer electrodes, and the wires from the internal electrodes were attached to the oscilloscope: (+)(+) (−)(−). The data presented in Fig. 4 and 5 were obtained with this arrangement.

When the following probe configurations were used, there was poor resolution between 10^3 and 10^9 cells/ml:

(+)(−) (−)(+) differential input to oscilloscope

(+)(−) · (+) single-ended input next to positive probe

(+) · (−)(+) single-ended input next to negative probe

The mechanical features of the probe itself had little effect on the resolution. Even when distances between the steel probes were as small as 3 mm, it was possible to distinguish a 2 log change in concentration. The only effect noted was a shift in frequency and a small decrease in resolution with the concentrations containing 10^3 and 10^5 cells/ml. The depth that the probes were inserted into the sample had no appreciable effect on the steady-state readings. It was noted, however, that upon complete submersion the time constant of the transient response increased as a function of the distance between the probe and the surface of the sample. It was suggested that this phenomenon might be due either to polarization of the electrodes or to gas concentration gradients formed between the probes and surface of the media as a result of the high input voltage of 20 V (peak-to-peak). To avoid this possibility, 5 ml of sample was used and the input voltage was reduced to 10 V peak-to-peak.

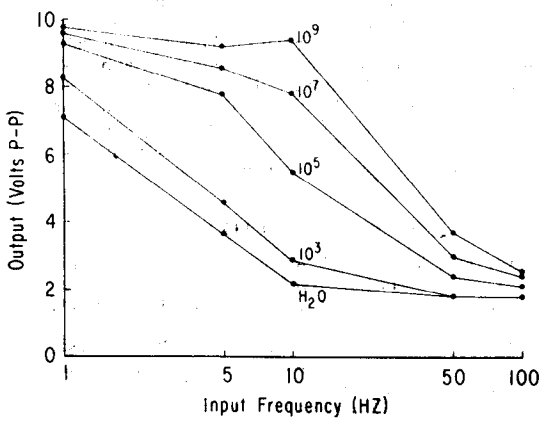

FIG. 4. *Effect of input frequency on resolution of various concentrations of washed cells of Escherichia coli suspended in distilled water. Bacterial concentrations (per ml) are indicated numerically.*

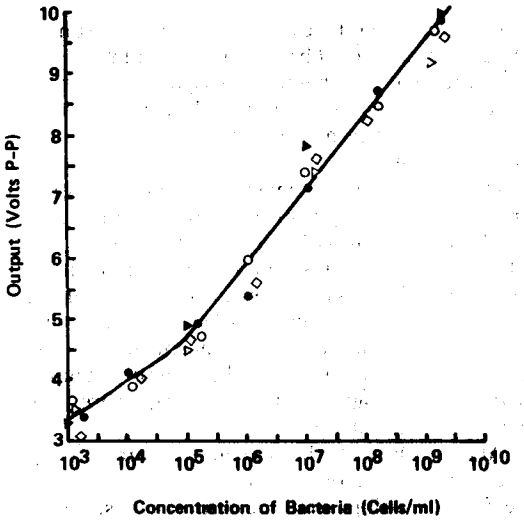

FIG. 5. *Effect of the concentration of cells on the electrical response as reflected in the output voltage. Each symbol represents an experiment done on different days (△ = day 1; ● = day 2; etc.).*

When samples of *E. coli* at different concentrations were tested, the resolution between readings taken with the different cell suspensions was found to be a direct function of the input frequency. As can be seen in Fig. 4, the maximal resolution between concentrations occurred at 10 Hz. The output voltages refer to the wave heights measured on the oscilloscope. Cell suspensions of *S. aureus*, *P. aeruginosa*, and *B. cereus* gave similar curves, indicating that the shape or size of the organisms did not particularly effect their electrical characteristics.

Using the data from Fig. 4 and plotting only the 10 Hz readings of the voltage at various concentrations, we formed a "nomograph." With this, we can correlate cell numbers with the output voltage in known and unknown specimens.

Rapid Detection of Bacteriuria

E. coli and other organisms were suspended in sterile urine, washed, resuspended in dis-