Second Edition

anaerobic bacteriology in clinical medicine

A. Trevor Willis

ANAEROBIC BACTERIOLOGY IN CLINICAL MEDICINE

SECOND EDITION

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FOREWORD

ALL TYPES of bacteria are, I suppose, exciting to work with, and to judge from the spate of work on bacteria by chemists and biochemists, their interest extends far beyond the field of the most general bacteriologist. But it is particularly difficult to resist the fascination of the obligate anaerobes—organisms which have set themselves the problem of a full bacterial life in an oecological niche from which atmospheric oxygen must be wholly or largely excluded.

Their problem has obviously been triumphantly solved; many obligate anaerobes are widespread and common, and notably quick to take advantage of opportunities to exploit their powers. As a consequence they have become extremely important to man, not only in disease, but in many other ways, as, for instance, in the disposal of sewage, the retting of flax and the breaking down of other vegetable and animal matter.

Though the interest and practical importance of the obligate anaerobes have long been obvious, the supposed technical difficulties of isolating and growing them in pure culture have deterred many bacteriologists from studying them seriously. There *are* difficulties; but they are easily overcome, and once this has been done, isolation and biochemical characterization of anaerobes is very little more difficult than comparable work on aerobes.

This book is an admirable guide to the technical procedures necessary for the identification of the pathogenic anaerobes and their close anaerobic associates in pathological lesions. In this the author has used his extensive experience in this field to decide which of the multitude of tests suggested in the literature are really useful for discrimination; this should save the less experienced a great deal of time and exasperation. Beside this, the book provides a succinct account of the diseases produced by anaerobic organisms, and a rather simplified, but practically useful, account of their toxicology; in these, as in the technical part, the author surveys a very extensive and scattered literature.

It is natural that the author's experience should, in the main, incline him to deal with the pathogenic anaerobes. May I make here my perennial plea for similarly thorough work on the non-pathogenic members of the group? They are of extraordinary variety, many of them are of practical importance, and far too little is known about them; surely they must excite somebody!

C. L. OAKLEY

PREFACE TO THE SECOND EDITION

THE preparation of a new edition has enabled me to incorporate suggestions kindly offered by my colleagues. The section on anaerobic cocci has been enlarged, and more attention paid to the practical aspects of immunization against tetanus. Much other material has been included, and appropriate additions made to the bibliography.

I am greatly indebted to Dr. K. I. Johnstone for many helpful suggestions and for reading the revised manuscript, and to Miss B. Stokoe for her help in recasting the bibliography. The work of revision has been lightened by the constant help of my wife, and by the guidance and courtesy of my Publishers.

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PREFACE TO THE FIRST EDITION

MY FIRST thoughts about writing this book were stimulated by the dread often expressed by students and colleagues about the difficulties of growing anaerobes. In looking for a suitable research project, one of my colleagues once remarked, "No anaerobes for me! I want to work on something like staphs. that you can grow without messing about".

Whilst it is true that a certain amount of "messing about" is a necessary part of culturing anaerobes, these preliminaries are not nearly so onerous as many people seem to think. Consequently I resolved to produce a book on anaerobic technique. As the manuscript grew, a little was added here and a little there, until, in its final state, it had developed into something more than its initial title implied. Though the basic techniques for growing anaerobes are the same in all fields of bacteriology, the present title is due to the emphasis which, as a clinical bacteriologist, I have almost unconsciously placed on the medical aspects of the subject. The aim of the book is to provide the reader with concise information on anaerobic technique, and on the determination and evaluation of the properties of the clinically important anaerobic bacteria. A great deal of information has been omitted and the bibliography has been limited, but it is hoped that the references given will lead those interested into the appropriate literature. In order to keep the book to a reasonable size, a basic knowledge of bacteriology has been assumed. I hope that the following pages will be of some help to pathologists who are confronted with the problem of isolating and identifying anaerobes from clinical material.

To those who have assisted me in various ways I would express my thanks. I am particularly grateful to Professor R. A. Willis and Dr. W. Goldie for their literary criticism and advice, to the Honourable Zaidée Milner for her help in typing the manuscript, to Miss E. M. Read for her assistance with the bibliography, to Mr. C. N. England and Mr. F. Dexter for production of the

figures, to Dr. K. S. Zinnemann who translated many German papers for me, to Mr. M. Ellis for his helpful criticism of Chapter 5, and to Dr. G. M. Williamson for many helpful and stimulating discussions.

For permission to reproduce some of the tables and figures, I am indebted to Messrs. Baird and Tatlock (London) Ltd. (Fig. 3), and to the Editors of the following journals: *The American Journal of Surgery* (Table XV), *The British Journal of Experimental Pathology* (Figs. 1 and 2), *The Journal of Pathology and Bacteriology* (Tables III, IV, XVI and XVII; Fig. 16), and *The Lancet* (Tables I and X; Fig. 14).

I am deeply grateful to my wife, but for whose constant encouragement and help this book would never have been completed.

The dedication to Professor C. L. Oakley is particularly appropriate, for he is not only my teacher, but also it was he who first introduced me to the exciting study of anaerobic bacteria, and who has given unstintingly of his time, encouragement and advice in all my anaerobic problems and in the preparation of this book.

Finally, my thanks are due to my Publishers, and especially to Mr. J. K. Burgess and Mr. L. E. Rayner, of their Medical Publishing Department, for their patient and helpful guidance.

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CONTENTS

INTRODUCTORY CHAPTER

THERE is a tendency in hospital laboratories to neglect anaerobic culture, chiefly because of the difficulty in isolating many of the anaerobic bacteria. It is true that anaerobes that are pathogenic for man are less frequently encountered in clinical bacteriology than are other pathogenic organisms, but routine cultures made both aerobically and anaerobically show that infection with anaerobes is more common than is generally supposed. This is well illustrated in Table I, which is reproduced here by kind permission of Dr. Joan Stokes.

TABLE I

ANAEROBIC STRAINS ISOLATED FROM VARIOUS SOURCES
(After Stokes, 1958)

Specimen	*Positive cultures*	*Total number yielding anaerobes*	*Anaerobes in pure culture*	*Percentage yielding anaerobes*
Pleural fluid	144	18	10	12·5
Cerebrospinal fluid	33	3	2	9·0
Blood	85	9	8	10·5
Abdominal pus	528	173	30	32·7
Genital pus	220	69	24	31·3
Neck lymph-gland	101	8	3	8·0
Infected sebaceous cyst	66	21	13	31·0
Boils, and so on	1,339	18	4	1·3
Others	2,221	177	45	7·9

During World Wars I and II a great impetus was given to the study of anaerobic bacteria, especially the clostridia, owing to the frequency of serious wound infections due to them. Before World War I, the techniques for anaerobic culture were very imperfect and information about anaerobes was very confused. This confusion was the result of a number of factors other than mere technical faults. The peculiar difficulties inherent in the study of anaerobes were not appreciated, and foremost amongst

these was the unrecognized problem of obtaining the organisms in pure culture. Mixed cultures of anaerobes were frequently examined, described and accepted as new species, and many of the early discrepancies are attributable to this cause. A classic example is the *Bacillus enteritidis sporogenes* of Klein (1897–98), which was almost certainly a mixed culture of *Clostridium welchii* and *Cl. sporogenes*.

Incomplete examination of cultures added to the confusion (and sometimes still does), and many new species were unjustifiably created. A further cause of the multiplication of species was the fact that different strains of a single species were frequently given specific names and recognized as separate species. *Cl. welchii*, for example, has been specified by different workers as *Bacillus aerogenes capsulatus* (Welch and Nuttall, 1892), *B. phlegmones emphysematosae* (Fraenkel, 1893), *B. emphysematosus* (Kruse, 1896), and *B. perfringens* (Veillon and Zuber, 1898).

The first to recognize these difficulties was Robertson (1915–16), who pointed out that "workers are inclined to allow an extraordinary latitude in the matter of motility, spore formation, character of colony and cultural reactions to organisms of the anaerobic group . . . and that either we must consider the sporulating anaerobes as extraordinarily polymorphic, or we must conclude that there are some special difficulties in obtaining pure cultures". From her own investigations, Robertson concluded that the study of mixed cultures accounted for most of the anomalous findings of earlier workers, and she drew attention to the extreme difficulty of obtaining anaerobes in pure culture. It was not until the invention by McIntosh and Fildes of the modern anaerobic jar that plate cultures of anaerobes could be easily and consistently obtained, thus enabling these organisms to be isolated and examined with a precision hitherto unknown.

The use of this new technique resolved much of the earlier chaos and provided the first distinctive account of the main species of clostridia. Since then, the accumulation of accurate observations and the development of better techniques have greatly increased our knowledge and understanding of these important organisms.

Today, though the technical approach to the study of anaerobes

is rather more complex than with aerobic organisms, the difficulties are not nearly so great as is generally supposed, and any properly equipped clinical laboratory should be able to isolate and identify any of the well-recognized anaerobic species. Some of the easiest organisms to recognize belong to the genus *Clostridium*, and of these, *Cl. welchii*, the most important causative organism of gas gangrene in man, presents the least difficulty. Unfortunately, many anaerobes are not so accommodating as this, and despite the most scrupulous technique the greatest difficulty may be found in growing some of the species, let alone in isolating or identifying them. This is particularly the case with some fusiform bacilli and some of the more exacting clostridia.

This book describes some of the methods which are used for the culture and isolation of anaerobes of medical importance, and the techniques and tests that are useful in their identification. In these respects I have drawn on my own experience and have indicated where possible the techniques which I have found most useful.

The material on cultural and biochemical characters has been limited to those features of anaerobes that I have found to be of greatest value in their identification, so that the reader is not overwhelmed with a multiplicity of investigations. More detailed attention is paid to toxins, for not only are these enzymes largely responsible for the characteristic diseases produced by the different anaerobes, but also toxins are playing an increasingly important part in the recognition and classification of anaerobes, particularly the clostridia. Little reference has been made to anaerobes other than those of medical importance, and a chapter on anaerobic infections in man has been included so that the relationship between the clinical and laboratory aspects of pathogenic anaerobes may be better appreciated.

It is wise for bacteriologists who have had little experience with anaerobic organisms to study some earlier papers that deal with them, and with the principles of anaerobic culture. From the extensive literature on these subjects (*see* McCoy and McClung, 1939; McClung and McCoy, 1941), the following is a useful selection: Robertson (1915–16); Henry (1916–17); McIntosh (1917); Committee upon Anaerobic Bacteria and Infections

(1919); Douglas, Flemming and Colebrook (1920); Heller (1921); Hall (1922, 1929); Slanetz and Rettger (1933); Spray (1936); Dack (1940); Reed and Orr (1941, 1943); Rosebury (1944); Tulloch (1945); Hayward (1947); McVay and Sprunt (1952); Hare and his colleagues (1952); McClung (1956); and Stokes (1958).

The following are authors of larger works of reference which I have found of particular value and interest: Weinberg, Nativelle and Prévot (1937); van Heyningen (1950); Prévot (1955); Smith (1955); Wilson and Miles (1955); Cruickshank (1960); Stokes (1960); Parish and Cannon (1961); MacLennan (1962); Barber and Garrod (1963); Tolhurst, Buckle and Williams (1963).

There are, unfortunately, no short cuts in anaerobic bacteriology; success is favoured by a good technique, a rigid adherence to the rules, and by a rather greater degree of patience than is required for the successful culture of most aerobic organisms.

CHAPTER 1

METHODS OF GROWING ANAEROBES

WHEN an oxygen-free or anaerobic atmosphere is required for obtaining surface growths of anaerobes, anaerobic jars or tins provide the method of choice. Some of the methods for obtaining anaerobiosis in a jar are also applicable to single tube or plate cultures, but for laboratories that intend to undertake anaerobic bacteriology seriously, anaerobic jars or tins are essential.

The following are some of the methods which have been used for obtaining anaerobic conditions in sealed containers: (1) McIntosh and Fildes anaerobic jar and tin; (2) alkaline pyrogallol anaerobic jar; (3) chromium and sulphuric acid jar; (4) phosphorus jar; and (5) vegetable tissue jar.

McINTOSH AND FILDES ANAEROBIC JAR

In the method employing the McIntosh and Fildes anaerobic jar, hot platinum or palladium is used to catalyse the combination of oxygen with hydrogen to form water—a principle first applied to the culture of anaerobes by Laidlaw (1915). McIntosh and Fildes (1916) adapted Laidlaw's method to a number of devices for anaerobic growth, and modified forms of their anaerobic jar today provide quite the best means of obtaining anaerobic conditions.

There are three important types of McIntosh and Fildes anaerobic jars, all basically the same in construction, but differing in the form of the catalyst capsule.

TYPES OF McINTOSH AND FILDES ANAEROBIC JARS

(1) In the original jar, and in some modified types of it, the capsule—for example, Wright's capsule (Wright, 1943)—is composed of asbestos wool impregnated with finely divided palladium and enclosed in a fine-mesh brass or copper gauze envelope. The

palladium is activated by heating it in a Bunsen burner flame immediately before sealing the jar, after which hydrogen is run in. This method of operation is still widely used, since the capsule is easily made in the laboratory (*see* Appendix) and can be used with any air-tight container of suitable size.

(2) In electrically operated anaerobic jars the palladinized asbestos, similarly enclosed in a wire gauze capsule, is heated by a 12 V element (Fig. 1), which is connected to two terminals

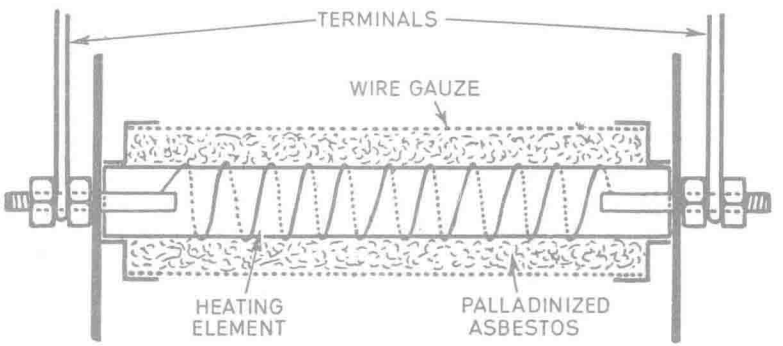

FIG. 1.—Palladinized asbestos heater. (*Reproduced by courtesy of P. Fildes and the Editor of The British Journal of Experimental Pathology.*)

in the lid of the jar. Here, the catalyst is not heated until the jar has been sealed and filled with hydrogen.

Electrical heating of the catalyst was first introduced by Smillie (1917) and was subsequently adopted by Fildes and McIntosh (1921) and Brown (1921). Various minor modifications of this type of anaerobic jar have since been made (*see* Brewer, 1938–39; Evans, Carlquist and Brewer, 1948).

(3) More recently, Messrs. Baird and Tatlock (London) have developed an anaerobic jar which utilizes a room-temperature catalyst (Heller, 1954). The catalyst, which is manufactured under patent as Deoxo pellets by Baker Platinum Ltd., London, consists of pellets of alumina coated with finely divided palladium. The Baird and Tatlock anaerobic jar using this type of capsule is very efficient and can be set up in a few minutes (Test Report, 1958). Figs. 2 and 3 depict the McIntosh and Fildes

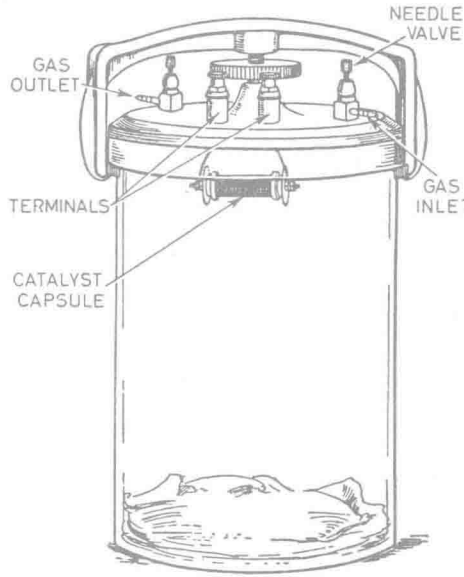

FIG. 2.—McIntosh and Fildes anaerobic jar. (*Reproduced by courtesy of P. Fildes and the Editor of The British Journal of Experimental Pathology.*)

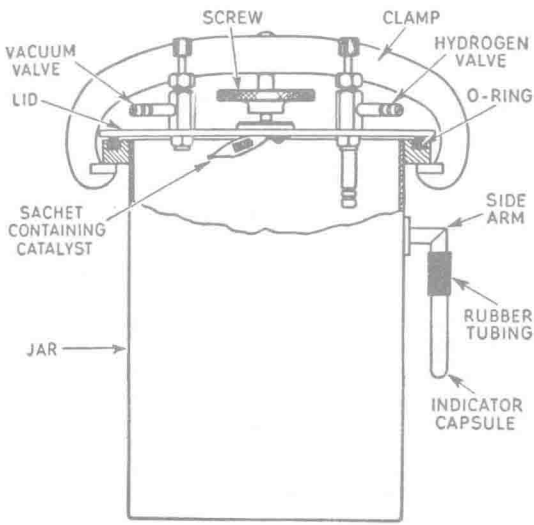

FIG. 3.—B. T. L. anaerobic jar. (*Reproduced by courtesy of Messrs. Baird and Tatlock (London) Ltd.*)

electrically heated jar and the Baird and Tatlock jar respectively. They are cylindrical vessels made of metal (bronze or aluminium) or glass, flanged at the top to carry an air-tight lid which is held firmly in place by a clamp. The lid is provided with two taps, and carries on its undersurface the catalyst capsule. Glass jars are less satisfactory than the metal type owing to their fragility and the greater danger they present should an explosion occur—their use is not recommended.

THE HYDROGEN SOURCE

Hydrogen is obtained from a Kipp's or similar apparatus in which hydrochloric acid is allowed to act on zinc, or more conveniently from a cylinder of the compressed gas. The use of coal gas as a source of hydrogen should be avoided since it contains carbon monoxide which is inhibitory to some organisms. The use of "illuminating gas" is quite inappropriate, since it consists mainly of methane ("marsh-gas" or "fire damp"), and is usually deficient in hydrogen. Since it is important that the hydrogen should be supplied to the jar at low pressure, the hydrogen cylinder should be fitted with a reducing valve and the gas delivered at a pressure of not more than 0·5 lb./in.^{2}. If a reducing valve is not available, a convenient low-pressure source is either a football bladder (Fig. 4) or an aspirator bottle system (Fig. 5) filled from the cylinder.

The pressure-reducing mechanism is attached to a gas-washing bottle, which acts as a flow meter, and this in turn is connected to the anaerobic jar. If the hydrogen has been prepared from the action of hydrochloric acid on zinc, it must be passed through a 10 per cent solution of lead acetate to remove hydrogen sulphide and then through a 10 per cent solution of silver nitrate to remove arsine, since the palladium catalyst is inactivated by both arsine and hydrogen sulphide.

Brewer, Heer and McLaughlin (1955) criticized the use of hydrogen compressed in cylinders. They described a technique for producing hydrogen inside the closed jar, using moist sodium borohydride which slowly liberates hydrogen in the presence of a cobalt chloride catalyst. The advantages claimed for this method of hydrogen production, namely that it eliminates the