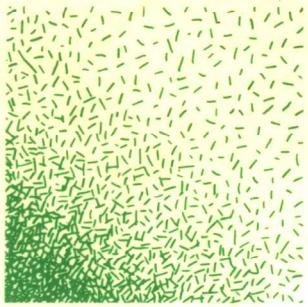
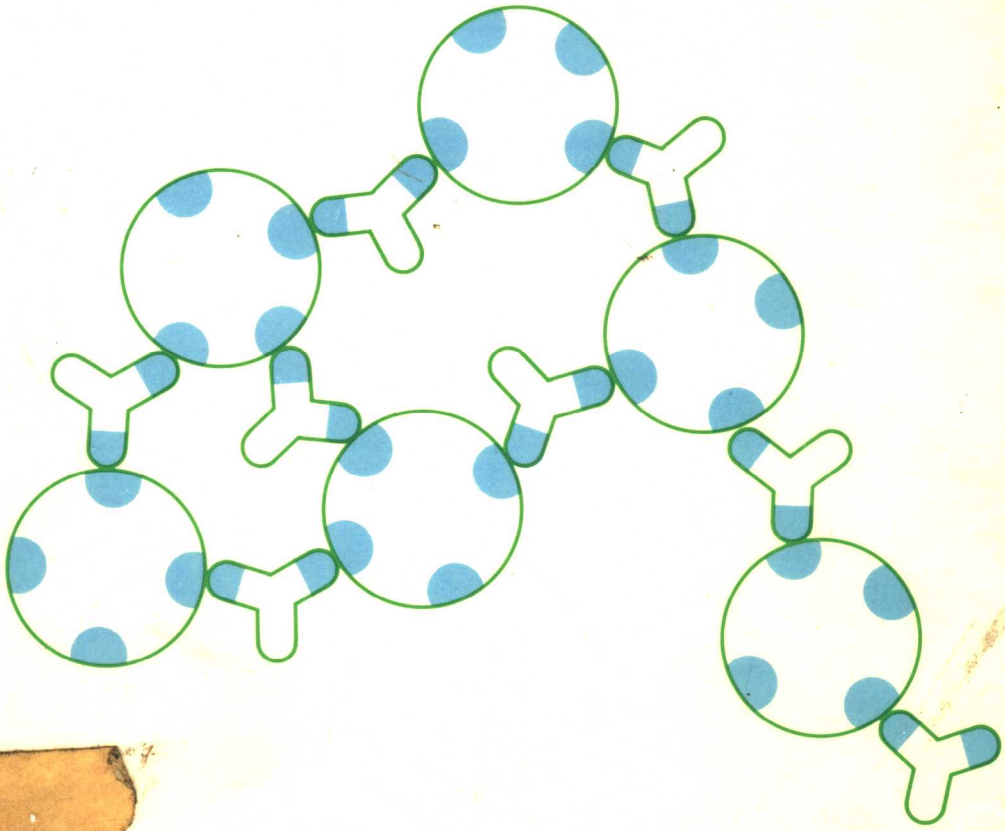


Introduction to Microbiology

THIRD EDITION

BASIC MICROBIOLOGY SERIES VOL 1

J F Wilkinson



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Introduction to Microbiology

BASED ON THE ORIGINAL TEXT BY

J.F. WILKINSON MA, PhD
Professor of Microbiology
University of Edinburgh

THIRD EDITION REVISED AND EDITED BY

I.R. BOOTH BSc, PhD, G.W. GOODAY BSc, PhD

N.A.R. GOW BSc, PhD, W.A. HAMILTON PhD, FRSEd

& J.I. PROSSER BSc, PhD

All of the Department of Microbiology
University of Aberdeen

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Editorial offices:
Osney Mead, Oxford, OX2 0EL
8 John Street, London, WC1N 2ES
23 Ainslie Place, Edinburgh, EH3 6AJ
52 Beacon Street, Boston
Massachusetts 02108, USA
667 Lytton Avenue, Palo Alto
California 94301, USA
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Preface

When this book first appeared it was designed as an introductory book for those with little or no knowledge of microbiology. In the intervening fifteen years not only has the understanding of micro-organisms advanced considerably but we have witnessed many changes in secondary education such that microbiology is now a component of most school curricula in biology. This revision has attempted to be true to the aim of the original author whilst strengthening the breadth of coverage through revision of the component chapters. In many cases this has involved a substantial rewriting of the material with the introduction of more recently developed concepts to give the reader a more balanced impression of microbiology in the 1980s. We have also recognized the growing importance of the biotechnological applications of micro-organisms by devoting a single chapter to this important aspect of microbiology. Thus, by reading this text it is hoped that the reader will gain an overview of the current status of the many diverse and interesting aspects of modern microbiology.

We would like to thank our secretaries, Sylvia McKenzie and Gill Taylor, for their perseverence in the eternal process of redrafting the component parts of the book. Our thanks are also extended to those who have given original illustrations for reproduction in this book. Finally, I would like to thank my co-revisionists for expediting the modernization of this text.

Ian R. Booth

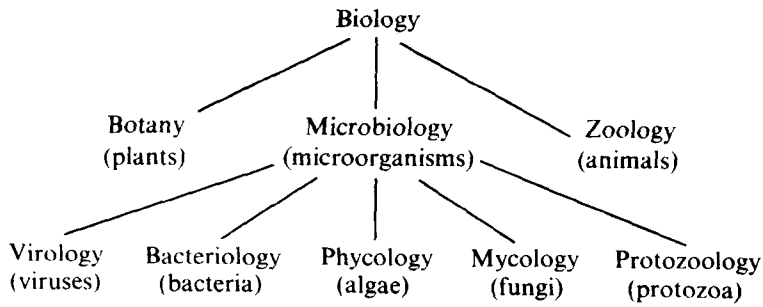
Contents

1 : Introduction

What is microbiology?

Microbiology is the study of micro-organisms, that is organisms which are of microscopic dimensions. Because the human eye cannot resolve any object smaller than 1/10 mm in diameter and most micro-organisms are only a few thousandths of a millimeter in size, they can only be seen with the aid of a microscope. As a direct consequence of the 'invisibility' of microbes to the naked eye and the need for specialized techniques to study them, microbiology was the last of the three major divisions in biology to develop. However, if it was late to arrive, it is a discipline which has been quick to develop.

It is now usual to include five major groups as micro-organisms; the subdivisions of virology, bacteriology, mycology, phycology and protozoology (the studies of viruses, bacteria, fungi, algae and protozoa respectively).



There have been continued attempts to include all organisms under the disciplines of either botany or zoology; bacteria, algae and fungi have in the past been considered to be part of the Plant Kingdom while protozoa have been

included in the Animal Kingdom. This type of classification cannot be supported from a taxonomic point of view.

If we group all living things according to the similarities of certain nucleic acids which are common to all organisms (the ribosomal RNAs), it is found that the most distantly related organisms (ones which diverged earliest during evolution) fall naturally into three groups. Accordingly, all animals, plants, algae, fungi and protozoa belong to just one group, the 'eukaryotes', which are distinguishable from the 'eubacteria' or true bacteria and the archaebacteria or primitive bacteria. Since microbiology encompasses the study of groups of organisms in all three of these kingdoms it may be argued that it covers a greater biological diversity than its sister divisions rather than vice versa.

The diversity of life within the microbiological world may appear less obvious to many than that of types of mammals or flowering plants. However, the striking diversity of microbial organisms lies not in their range of morphologies but rather in their ecological and physiological specializations. Bacteria have existed for at least three 3×10^9 of the earth's 4.5×10^9 year life span and in this time have evolved to cope with and flourish in almost every niche, no matter how inhospitable.

There are microbes which are adapted for life in the coldest oceans and in hot water springs where temperatures approach boiling point. Others are capable of growing in saturated salts, at high pressures, in acid at pH 0.2 or alkali at pH 12.5. The most radiation resistant organism known is a bacterium—*Deinococcus radiodurans*. The versatility of microbes to cope with extremes of environment extends also to the range of substances from which various micro-organisms can obtain energy and cell nutrients. Micro-organisms are the only group of organisms capable of fixing atmospheric nitrogen into utilizable organic compounds, or of growth in the total absence of oxygen. The terrestrial ecosystem depends on the activities of bacteria and fungi to dispose of the organic detritus that would otherwise accumulate and consume the land. To this end there are microbes that are capable of biodegrading every natural organic compound and all but a few of the most recalcitrant man-made organic materials such as certain plastics, dioxin, polyfluorocarbons etc.

Micro-organisms, as we shall see, also form a range of associations with other microbes and with higher plants and animals. They can be pathogens, parasites, symbionts, commensals and saprophytes, and thus their ecological influences infiltrate into all the trophic levels of life and the gamut of possible ecosystems. The microbiological world

may be hidden from sight but it is a microcosm whose activities are of central importance to the structure of the biosphere.

Microbes and man

Self-centredly we tend to evaluate the importance of things by their impact on ourselves. Despite their small size, microbes are certainly of immense importance to man; they cause disease, provide us with various foods and medicines, and dispose of our wastes. In a real sense they are responsible for the very air we breathe since free molecular oxygen was completely absent from the pre-biotic atmosphere and has accumulated only as a product of the metabolism of primeval photosynthetic bacteria.

Mankind has made use of micro-organisms, or their biochemical activities, since long before he even knew of their existence. We know that in 6 000 BC the ancient Babylonians and Sumerians were brewing beer much as we do today and that the Egyptians were baking leaven bread 2 000 years later. Despite the antiquity of these microbiological practices, the first documentations of the structure of micro-organisms did not occur until the advent of the first microscopes in the seventeenth century. Although the spontaneous generation of mice from old rags and of maggots from meat had been disproved by this time, it was not until last century that Louis Pasteur showed that microbes were not produced *de novo* from muds or decaying organic matter.

If Pasteur was the founder of industrial microbiology, Robert Koch was the forefather of medical microbiology. Koch, a German doctor, showed in 1876 that the causative agent for bovine anthrax was a bacterium, *Bacillus anthracis*. He isolated the bacillus from diseased cattle, cultured it on nutrient jelly and showed that the cultured micro-organisms would cause the symptoms of anthrax when inoculated into healthy cattle. This remarkable work remains a milestone in microbiology and revolutionized mankind's perception of the nature of disease.

We live in a time when microbiology has come of age. Industrial microbiologists produce microbial products on a huge scale—14 000 tons of penicillin, 300 million tons of the flavour enhancer MSG (monosodium glutamate), and 1 430 million tons of vinegar are manufactured per annum. We use microbes to make beer, wine, cheese, yogurt, sauerkraut, soya sauce, antibiotics, pesticides, gels and many other products. Microbiological reactions are used to process sewage, transform the chemical structures of drugs, clean

clothes (bacterial enzymes are used in biological detergents) and even to extract precious metals such as copper and uranium from their mineral ores. Within the last decade new technologies have been developed, such as gene cloning, which will use microbes as factory cells for the synthesis of valuable pharmaceutical products such as human insulin, hormones, antiviral drugs and vaccines. When somatostatin, a growth hormone secreted by the brain, was first purified it took 500 000 sheep brains to produce a five milligram quantity. In 1977, genetic engineers produced an equal quantity of somatostatin from two gallons of bacterial culture at a cost of less than £5.

Despite the dramatic advances in medical microbiology since the time of Robert Koch, micro-organisms will continue to be a major problem in medicine and in diseases of plants. The outbreak of influenza in 1918–1919 claimed more than 20 million lives—far more than were killed during the World War which was then coming to an end. Each year fungal, bacterial and viral diseases of plants cause some 3.3 billion dollars loss in the world's food crops. There is still no cure for the common cold or any of the more serious viral diseases of man and even eminently curable bacterial diseases such as tuberculosis and leprosy still affect millions of people in the underdeveloped nations of the world. We continue the work towards producing an effective vaccine against the protozoan malarial parasite which kills over a million people each year. Also, we will have to continue to meet the challenge of diagnosis and treatment of 'new' diseases such as Legionnaires' disease and AIDS. It has also become evident that viruses are involved in the aetiology of some forms of cancer. It would, however, be grossly misleading to create the impression that micro-organisms such as bacteria are by their very nature pathogenic. A normal healthy human body harbours on its surface and within its alimentary canal ten times as many microbial cells than it has cells of its own kind. Many of these are of positive benefit to the digestive process and the rest are mostly harmless passengers which we never notice.

The fascination of microbes to microbiologists lies not only in their utility and pathogenic activities but also on the powerful insights we glean from studies of microbes about the ways in which living things of all types grow and multiply. It may seem strange to some that we know more about the detailed biology of a bacterium (*Escherichia coli*) which lives in the human intestine than any other organism. Indeed, this bacterium has been used as the organism of choice during the past few decades in research concerned with the formulation

of general biological principles and particularly of those at a molecular level. As Francis Bacon noted, the nature of things is commonly better perceived in small than in great. Our relationships with the microbes is and will continue to be dichotomous—they are our deadliest adversaries but also our closest allies.

Methods in microbiology

A subject can only develop according to the techniques available. This may sound platitudinous but a study of the history of microbiology demonstrates the prime importance of suitable methodology. Three techniques in particular had to be perfected before the science of microbiology could evolve beyond a primitive visionary state.

1 *Microscopy*. Since microbiology is mainly concerned with the study of living organisms of microscopic dimensions, its development depended for its initiation entirely upon the refinement of the microscope.

2 *Sterilization methods*. Media to be used for growth of a particular micro-organism had to be freed from all other living organisms; in other words, sterilization methods had to be developed.

3 *Pure culture methods*. Once it was possible to obtain sterile growth media, it became practicable to introduce methods to separate different micro-organisms from each other and to maintain them in pure culture. Their individual characteristics could then be studied. Let us consider each of these critical developments in turn.

The microscope

Prior to the seventeenth century there had been various reports of the existence of invisible living creatures but, before the development of suitable means of magnifying them, no proof was obtainable. To Anthonie van Leeuwenhoek, a Dutch merchant and amateur scientist living in Delft, belongs the honour of providing the first accurate report of the occurrence of bacteria. Leeuwenhoek employed his spare time in pursuing his hobby of making lenses which he used to build magnifying glasses of high resolving power. These single-lens microscopes were of the simplest possible design (see Fig. 1.1) but were still capable of magnifying an object by about 200-fold. As a result of his exceptionally painstaking care in the building and use of his microscopes, Leeuwenhoek was able to make descriptions of many micro-organisms including some which were almost certainly bacteria.

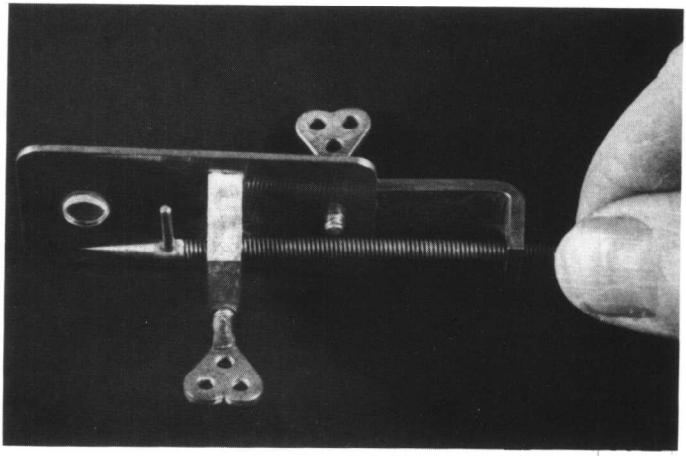


Fig. 1.1. The type of microscope used by Leeuwenhoek. The object is placed at the end of a spike attached to a screw and is viewed through a small lens.

Anybody who has tried to use a reproduction of one of his instruments will soon realize that his particular genius fulfils the criterion of an infinite capacity to take pains. Using a racey style that would nowadays be the subject of multitudinous editorial transformation, he communicated the results of his work in the form of letters to the recently founded Royal Society of London. An example is given in this excerpt of a letter of 1684 which gives the first description of bacteria:

> 'Though my teeth are kept usually very clean, nevertheless when I view them in a magnifying glass, I find growing between them a little white matter . . . I took some of this flower and mixed it with pure rain water wherein were no animals . . . and to my great surprise perceived that the aforesaid matter contained many small living animals, which moved themselves very extravagantly. The biggest sort had the shape of A, their

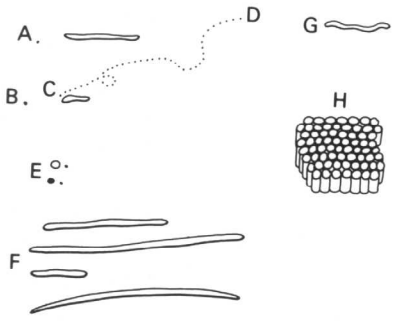


Fig. 1.2. Leeuwenhoek's famous drawing of micro-organisms published in 1604. Different shaped organisms can be seen, some of which were most certainly bacteria.

motion was strong and nimble, and they darted themselves through the water as a Jack or Pike does through water.... The second sort had the shape of B. These spun about like a Top.... In the third sort I could not well distinguish the figure, for sometimes it seemed to be an oval and other times a circle. These were so small that they seemed no bigger than E'.

These observations of Leeuwenhoek were followed by a period of nearly two centuries during which there was little further descriptive work on the smaller micro-organisms because nobody was capable of designing a microscope which had a sufficient resolving power but which could, at the same time, be used by a worker of average dexterity and patience. Further progress depended upon the development of a compound microscope with an eyepiece and objective lens allowing an increase in the magnification obtainable and greater ease of manipulation. It is true that Robert Hooke had used compound microscopes in the seventeenth century but they were incapable of the performance given by Leeuwenhoek's single-lens microscopes. The reason for this lay in defects such as chromatic and spherical aberration inherent in their basic design. During the eighteenth century these defects were gradually overcome by the following refinements:

1 Corrected complex eyepiece and objective lenses.

2 A condenser to focus light on the object.

3 A thin glass coverslip to place over a liquid drop on a glass slide so that objects within the liquid could be viewed in a flat plane.

4 The oil-immersion lens. The resolving power of a microscope can be increased by using a material lens of higher refractivity than air between the objective. The material most commonly used is a special immersion oil.

In conjunction with these developments in microscopic design, staining methods were perfected so as to allow a simple classification of micro-organisms based on morphological grounds. However, the theoretical limit of resolution of the light microscope is about 0.2 μm and clearly gives little hope of seeing much internal structural detail in a typical bacterium of 0.5–1.0 μm diameter.

Just as there was a quiescent period of microbial cytology between the work of Leeuwenhoek and the sophistication of the compound microscope, so there was little real progress between the latter half of the nineteenth century and the development of the electron microscope in the 1940s. The small wavelength of an electron beam allows a theoretical resolving power down to 0.01 nm and for the first time, viruses could be demonstrated as physical entities. Practical

difficulties in instrument design such as the development of magnetic lenses have prevented resolutions as low as this but it is still possible to see the larger molecules that make up the architecture of a cell. In practice only thin objects can be viewed with any real hope of obtaining good definition of internal structure and so methods had to be developed in which cells are fixed, dehydrated, embedded in plastic and sectioned to give a preparation about 100 nm thick (i.e. about 10 sections to a bacterial cell). Some increase in contrast can be obtained by using electron-dense stains like osmic acid, permanganate, or uranium salts. Another problem in electron microscopy is the possibility of artefacts caused by fixation, drying and embedding; this difficulty can be partly obviated by the use of freeze-etching in which a carbon replica is made of a frozen surface in a cell (Fig. 2.8). In spite of the many difficulties involved in its use and particularly in the interpretation of results, the electron microscope has opened up a new world to microbial cytologists.

Methods of sterilization

Sterilization involves the complete destruction or removal of all living organisms from the object being sterilized. The development of methods for sterilization was very largely a happy consequence of the controversy over spontaneous generation culminating in the work of Pasteur.

Experiments designed to prove or to disprove spontaneous generation depended upon two general principles:

1 The complete sterilization of a suitable growth medium so that no living organisms exist at the start of the experiment.

2 The design of the vessel so that it is impossible for micro-organisms to enter from the outside. This was necessary following the realization of the existence of micro-organisms floating around in the air. For example, even 'fresh' air may contain one particle carrying a micro-organism per cubic foot while the figure may be a hundred to a thousand times greater in a crowded room.

Provided these principles are rigidly adhered to and provided the conditions are otherwise suitable for microbial multiplication, any growth occurring must be the result of spontaneous generation. Clearly the key question was how good the methods were for attaining and maintaining sterility. Such was the emotional fervour aroused by a controversy which involved the very nature of life that many important scientists became involved. The technical 'fall-out' was the development of sterilization methods. Let us consider the two principles in greater detail:

1 The attainment of sterility. The usual method depended upon heat treatment, which was known to be inimical to most forms of life. However, it was soon realized that micro-organisms vary widely in their resistance to heating (Fig. 1.4) and sterilization clearly must be gauged to the most resistant form. In general, bacteria required higher temperatures than larger forms and some micro-organisms can produce specialized heat-stable structures called spores (p. 27). Boiling at normal pressure was insufficient to kill these spores and so autoclaves were designed to increase the pressure, and, thereby, the temperature.

2 The maintenance of sterility. In experiments claiming to show spontaneous generation, a cork was often used to prevent the entry of contaminants from outside. Unfortunately, this method was ineffective in practice since micro-organisms could enter round the side of the cork as the vessels cooled after sterilization. Although a flask could be hermetically sealed, this led to the objection that oxygen, a substance known to be essential for many forms of life, could no longer enter the vessel. It was necessary, therefore, to include some sort of filter to prevent the entry of micro-organisms but not of air. This led to the development of the cotton wool plug which was soon adopted universally by microbiologists. However, one of the simplest and most elegant means of preventing the entry of micro-organisms can be seen in Pasteur's swan-necked flask (see Fig. 1.3). This worked on the basis that organisms in the atmosphere entering the open end of the tube would, in their slow passage through the convolutions, be deposited by the pull of gravity. Pasteur showed that such flasks, although left open, remained sterile indefinitely. By such simple means, he finally disproved the idea of spontaneous generation, a result aided by his skill as an expositor of his own work and by an almost evangelical zeal.

However, although the brilliance and the force of Pasteur's personality were able to catalyse the early adolescence of microbiology, he later acted as an inhibitor of progress since it was often felt that 'surely Pasteur can't be wrong'. For example, he firmly believed that metabolic

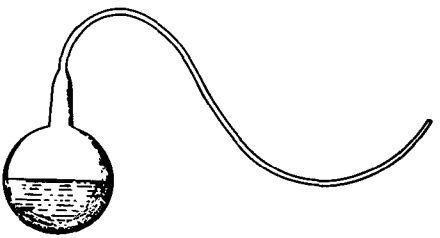


Fig. 1.3. The Swan-necked flask used by Pasteur to demonstrate the absence of spontaneous generation.

processes such as fermentation were an essential property of the 'living force' and could not occur in the absence of living cells, and for a long time this view was generally supported. In science, as elsewhere, an inhibition of progress is the price we may have to pay for an innovative genius.

By the end of the nineteenth century most of the methods currently used for sterilization had been developed. These are briefly summarized below.

Heat

If the percentage of survivors of a micro-organism is plotted against time a logarithmic relationship is found. The slope of the line varies from organism to organism but in the choice of a general sterilization method we must use a time and temperature that will kill all organisms including heat-resistant spores.

This is illustrated in Fig. 1.4 where it can be seen that if a time of 30 minutes is used for sterilization, micro-organism A will require a temperature of only 50° while B needs 60–70° and the bacterial spore suspension C, 120°. The methods generally adopted are as follows:

1 *Wet heat in an autoclave.* The usual method is a time of 30 minutes at a pressure of 1.05 kg/cm^2 which will give a temperature of 121°. This is the best method if it is practicable.

2 *Tyndallization.* A course of three periods of boiling at 100° for 30 minutes at daily intervals. The spores remaining

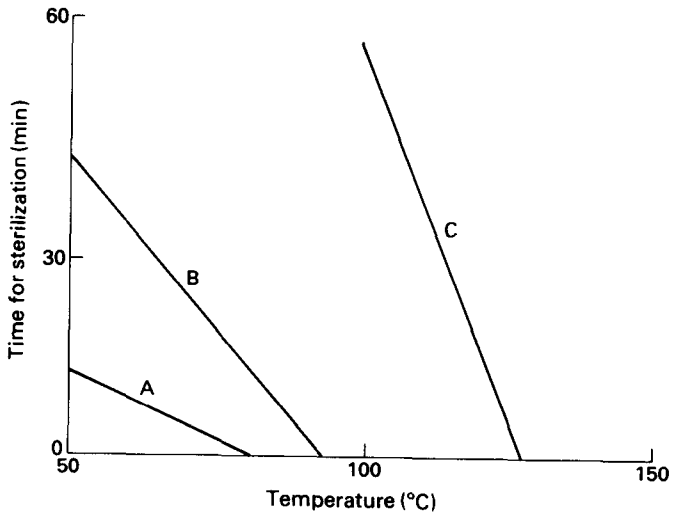


Fig. 1.4. The death curves resulting from heat treatment of two suspensions of vegetative cells of micro-organisms A and B and a spore suspension from micro-organism C.