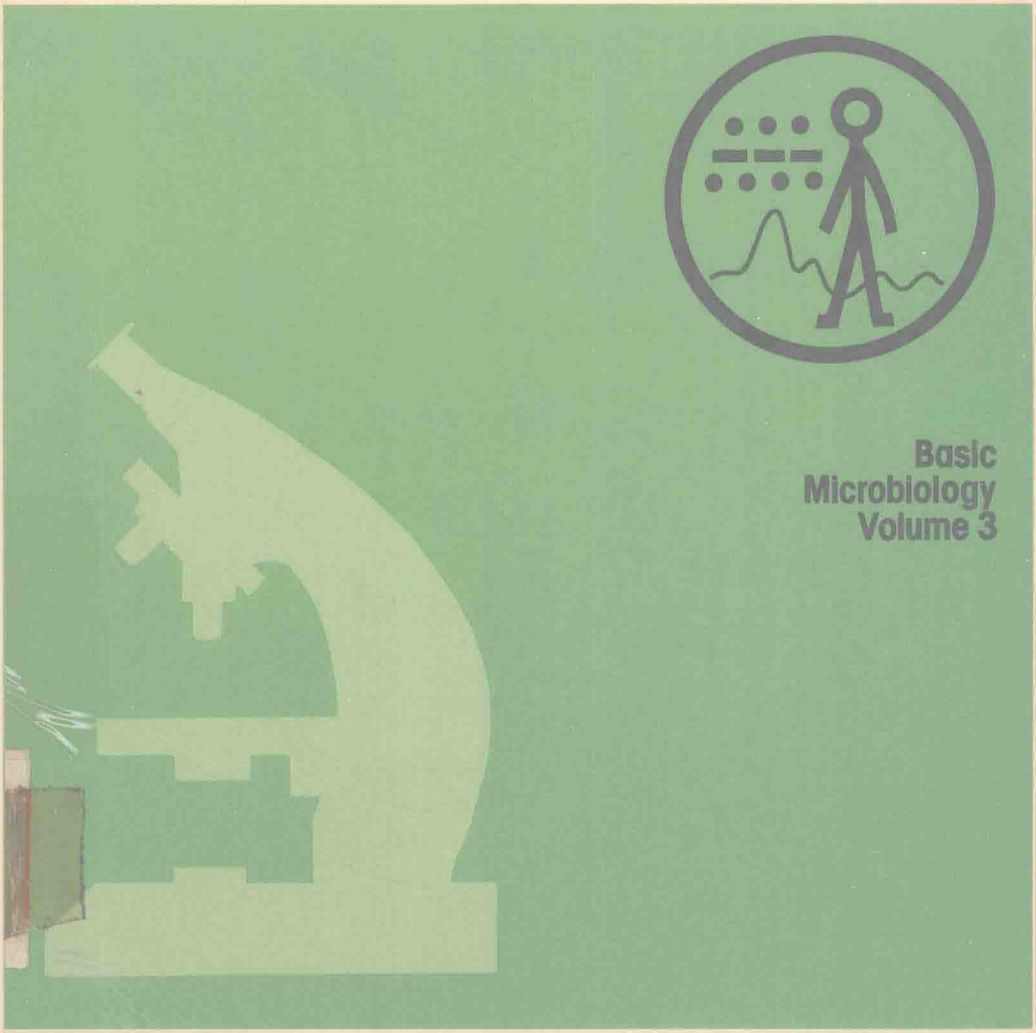
J. G. Collee
Applied Medical Microbiology

Second edition



Basic Microbiology Volume 3

A Halsted Press Book

Basic Microbiology

EDITOR: J. F. WILKINSON

Volume 3

Applied Medical Microbiology

J. G. COLLEE
M.D., F.R.C.Path., F.R.C.P.E.
Professor of Bacteriology
University Medical School
Edinburgh, and
Honorary Consultant Bacteriologist
to the Lothian Health Board
Scotland

Second Edition

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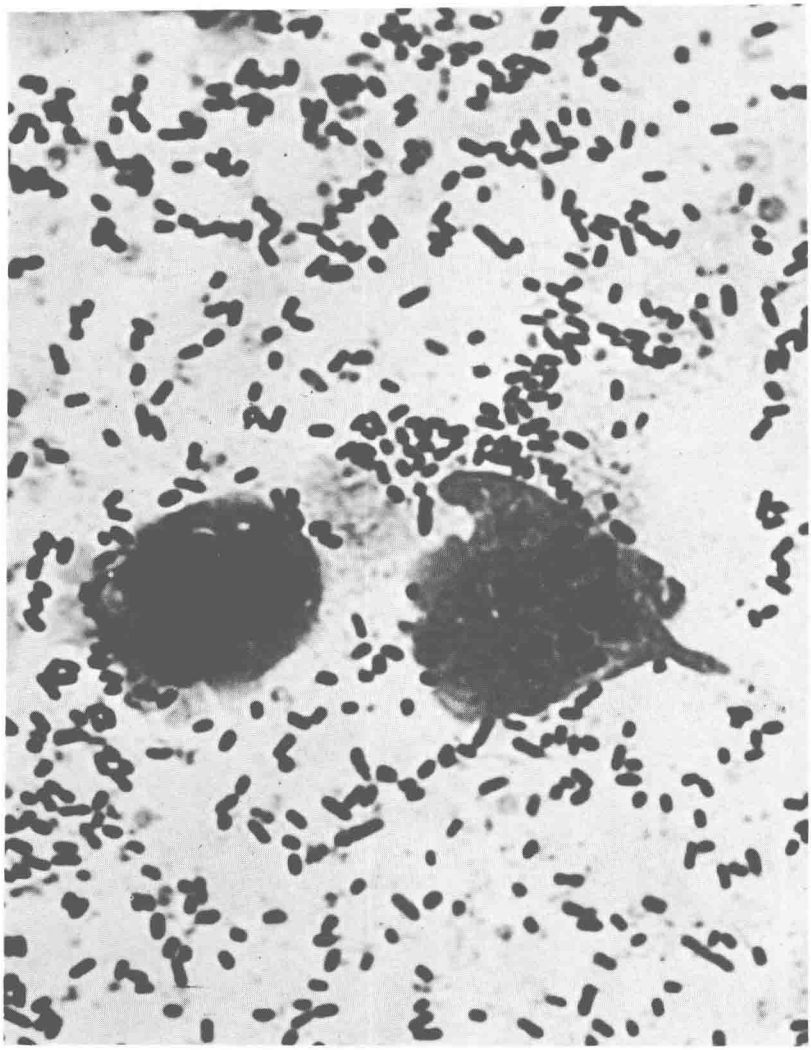

Bacteria and pus cells in a specimen of infected urine from a patient with cystitis.
(× approx. 2000.)

Preface to First Edition

There are several good general textbooks of medical microbiology and many specialist texts that deal with aspects of bacteriology, virology, mycology and protozoology in relation to infectious diseases of man.

This small book cannot bear comparison with any of these volumes. It has been written in the hope that it may set the scene usefully for students of biology, junior medical and dental students, nurses and paraclinical technicians who may seek a gentle introduction to some clinically related areas of microbiology. The aim has been to present concepts and to bring in new facts and terms as they are needed to develop the individual chapters. The use of a limited number of illustrative examples and the repetition of some examples to demonstrate different aspects, or to emphasise important points, is intentional.

It is hoped that, despite the many inadequacies and omissions of this slim volume as a general source of information, it might drive the reader to fuller texts in due course, sufficiently equipped with some of the language and the concepts of medical microbiology to tackle the further reading with a measure of understanding and infectious enthusiasm.

I am indebted to many colleagues, particularly to Dr Andrew Fraser, Dr Brian Duerden, Mr William Marr, Dr John Peutherer and Dr Donald Weir, for their constructive advice. I am also grateful to Mr James Paul who did the photomicrography and to Mr Ian Lennox who helped me with some of the original artwork. Special thanks are due to Mr Robert Campbell and his colleagues at Blackwell for their care.

Preface to Second Edition

It is encouraging to be asked to produce a second edition. Medical microbiology has moved considerably in five years. This fact, and the constructive suggestions of reviewers and colleagues, have obliged me to re-write many sections and to make additions or amendments to the tables and figures.

I am again grateful to my friends for their continuing help. On this occasion, thanks are also due to Dr Leslie Milne who gave me much-needed tutorials in elementary mycology, to Dr Sebastian Amyes who reviewed and advised me on aspects of disinfection and sterilization, and to Dr Brian Watt who kindly guided me in the revision of the chapter on antimicrobial drugs. I am grateful to my publishers, particularly Mr Per Saugman and Mr Nigel Palmer for their forbearance, and my special thanks are due to Mrs Sheila Boyd whose care and ability are evident throughout this edition.

Contents

1 Introduction

The micro-organisms that abound in nature are classified on the basis of their size, structure and other criteria, into the groups indicated in Table 1.1 and Fig. 1.1.

Table 1.1 Major groups of eukaryotic and prokaryotic organisms and the viruses (a simplified classification).

EUKARYOTIC CELLS	Protozoa Algae Fungi (including yeasts)
PROKARYOTIC CELLS	Bacteria (including filamentous bacteria) Spirochaetes Rickettsiae Chlamydiae Mycoplasmas
. . .	Viruses

This book deals mainly with the commensal and pathogenic bacteria of man. It includes a brief consideration of some fungi and viruses of medical importance, but it does not deal with pathogenic protozoa.

Micro-organisms that cause disease in plants, animals or man are called *pathogens* and may be distinguished from the very large group of free-living and generally harmless *saprophytes* that include many organisms involved in natural processes of decomposition and putrefaction. With few exceptions, the pathogens are parasites and cause disease by living on or in a host and interfering in some way with the host metabolism. However, parasitism is not necessarily harmful; there are many micro-organisms that are not free-living but lead a parasitic existence without apparently causing disease in the host. The parasites concerned in this form of peaceful co-existence are known as *commensal organisms*. In some cases, the commensal state is more or less enduring and there are clear examples of symbiosis in which the host-parasite association is mutually beneficial. On the other hand, many commensal organisms may attack the host when special circumstances allow and these organisms are regarded as *potential pathogens*.

Together with other micro-organisms that are not commonly associated with direct aggression, such potential pathogens may share a role as opportunist invaders. It can be argued that any pathogen that exploits a

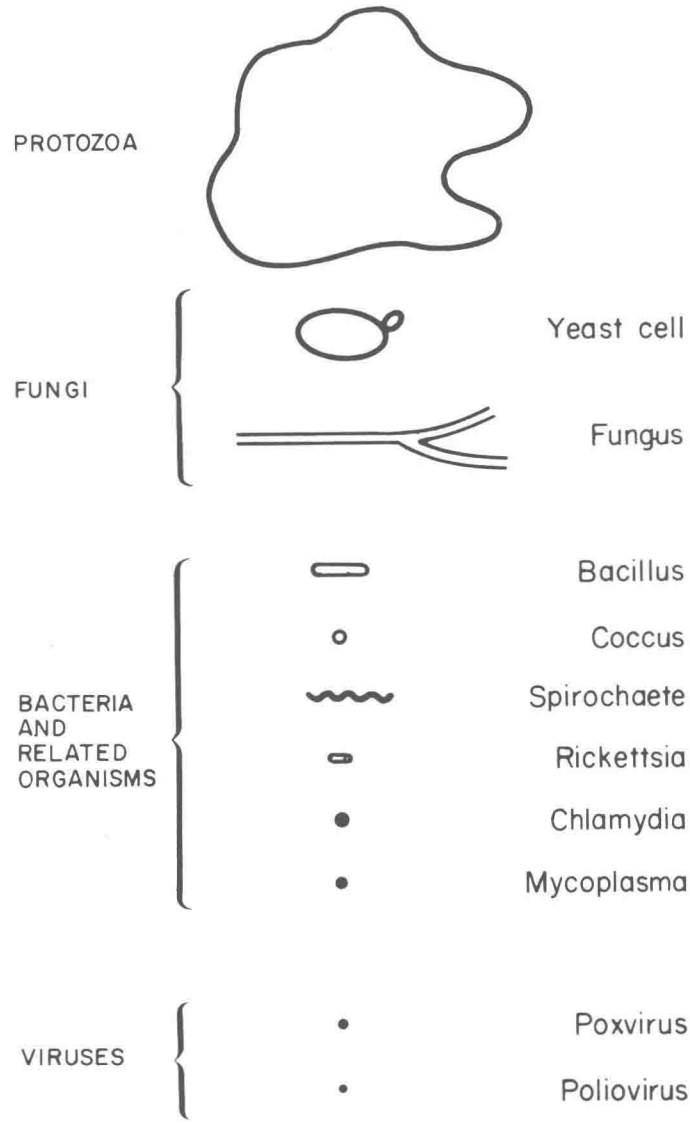

Figure 1.1 An outline of the relative sizes and approximate shapes of some micro-organisms.

weakness in a vulnerable host is opportunist, but the term *opportunistic* is currently applied to a relatively non-aggressive organism when it takes advantage of a weakened or debilitated patient. For example, man's normal defence mechanisms may be upset in drug addicts and in patients who are treated with cytotoxic drugs. It is well recognized that patients in these categories may succumb to infections with organisms that are not generally able to cause disease without the assistance of a debilitating factor to promote their attack (see p. 49).

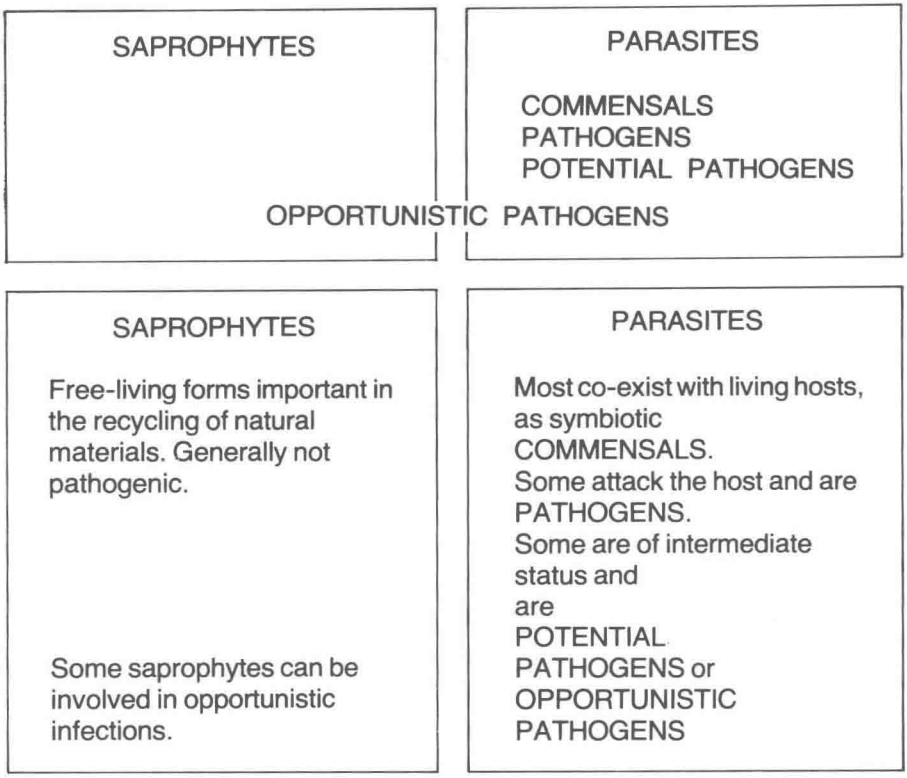

Figure 1.2 A simplified outline: the interrelationship of saprophytes, parasites, commensals, opportunist invaders, potential pathogens and pathogens.

SPECIES-SPECIFICITY IN HOST-PARASITE ASSOCIATIONS

The pathogens that attack plants are different from those that attack animals. In turn, those that attack certain animals demonstrate affinities for specific hosts that may or may not include man. Many pathogens of man are strictly host-specific and do not naturally infect other animals; examples are the typhoid bacillus, the organisms of bacillary dysentery, the diphtheria bacillus, the cholera vibrio, and the viruses of smallpox and measles. However, some organisms are pathogenic for both man and certain animals, and these are discussed in Chapter 11.

DISEASE SYNDROMES

Patterns of signs and symptoms that characterize diseases are referred to as *syndromes*. A disease syndrome may be clearly specific for a certain disease; for example, the clinical signs and symptoms of fully developed cases of leprosy, mumps or tetanus are not likely to be confused. These particular

Table 1.2 A simple classification of common bacteria of medical interest.

FILAMENTOUS BACTERIA (Higher bacteria). *Streptomyces* species produce various antibiotics; *Actinomyces israeli* causes actinomycosis.

TRUE BACTERIA Morphology and Gram reaction	Nature	Genus	Important infections caused by individual species	Group reference
Gram-positive bacilli	Aerobic	*Mycobacterium*	Tuberculosis; leprosy	Acid-fast bacilli
		Corynebacterium	Diphtheria	Corynebacteria
		Bacillus	Anthrax	Aerobic sporeformers
	Anaerobic	*Clostridium*	Tetanus; gas gangrene	Anaerobic sporeformers
		Bifidobacterium	?	Bifidobacteria
Gram-positive cocci	Aerobic	*Streptococcus*	Tonsillitis; various infections	Pyogenic cocci
		Staphylococcus	Boils; various infections	
Gram-negative cocci	Aerobic	*Neisseria*	Meningitis; gonorrhoea	
	Anaerobic	*Veillonella**	Various infections	
Gram-negative bacilli	Aerobic	*Escherichia*, *Klebsiella*, *Proteus*	Various infections including wound infections and urinary tract infections	Enterobacteria
		Salmonella	Typhoid fever; food poisoning	
		Shigella	Bacillary dysentery	
		Pseudomonas	Wound infections; urinary tract infection etc.	
		Vibrio	Cholera	
		Haemophilus	Meningitis; respiratory tract infections	Parvobacteria
		Bordetella	Whooping cough	
		Brucella	Undulant fever	
		Pasteurella	Various infections	
		Yersinia	Plague	
		Francisella	Tularaemia	
	Anaerobic	*Bacteroides*, *Fusobacterium*	Various infections	*Bacteroides-Fusobacterium* group

*These are Gram-negative, but some anaerobic cocci are Gram-positive.

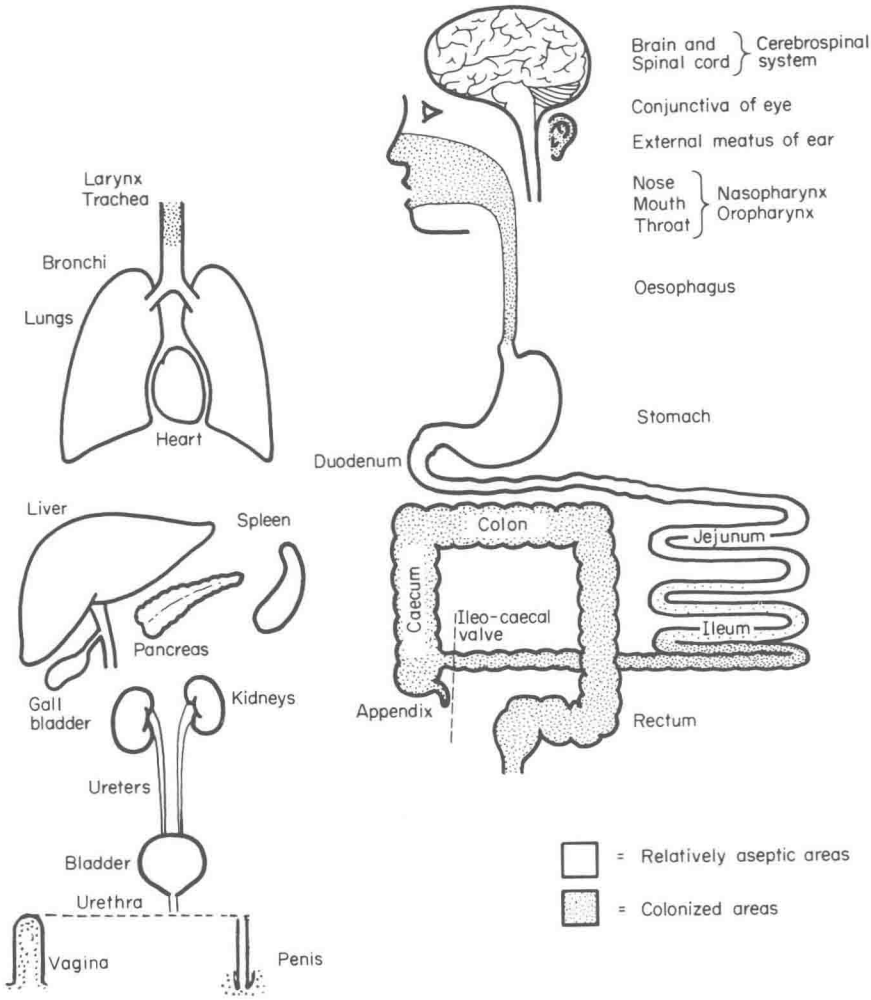

Figure 1.3 A simplified diagram of some relatively aseptic areas and colonized areas of the body. Various other aseptic areas including muscles, joints and blood vessels are omitted.

disease syndromes are only produced as a result of infection with the leprosy bacillus, the mumps virus or the tetanus bacillus respectively, and the 'differential diagnosis' in typical cases is limited and relatively straightforward. However, the clinical syndrome associated with a simple wound infection can be produced by any one of a variety of organisms acting alone or in combination. Similarly, the clinical picture of meningitis—an infection of the central nervous system in which an ill patient has headache with neck stiffness and dislikes light shining on his eyes (photophobia)—can be produced by a meningeal infection caused by any one of several bacteria or viruses (p. 66).

Thus, disease patterns are recognized in which experience teaches us to associate the probability of infection with certain ranges of organisms, some of

which are more likely to be involved than others. In relatively few situations, such as in leprosy or mumps or tetanus, the probabilities are essentially restricted, but the more common situation is that one of a variety of recognized causative organisms may be involved in producing an infective illness. For example, in the case of an infection of the urinary tract, the commonest pathogen is *Escherichia coli*, but urinary tract infections with *Klebsiella* species, *Streptococcus faecalis, Proteus* and *Pseudomonas* species, alone or in combination, are everyday occurrences (p. 62). Moreover, a urinary tract infection caused by the tubercle bacillus must not be missed, and fungal and viral infections are sometimes associated with urinary tract symptoms. These special problems are dealt with elsewhere in this book. The important point is that the clinical microbiologist must restrict his search within limits demanded by practicability and experience when he attempts to isolate a causative organism and hold it responsible for a particular illness. He is often obliged to produce a reasonably accurate report as quickly as possible. It follows that he must have a considerable knowledge of the likely pathogens in a given situation. To some extent, the medical microbiologists have accordingly developed their own practical classification of bacteria and other micro-organisms associated with disease in man and animals.

A simple classification of some micro-organisms of medical importance is given on pages 3 and 4.

COMMENSALISM

Although various antimicrobial mechanisms operate in the tissues and at epithelial surfaces to control bacterial colonization and microbial attack, there is an abundant and varied commensal flora that flourishes on the skin and on some of the mucous surfaces of the human respiratory, gastro-intestinal and genito-urinary tracts. The 'closed' systems such as the internal surfaces of joints, the cerebrospinal system, the cardiovascular system, muscles and solid organs such as the liver, spleen and brain are relatively free from bacteria and have active defence mechanisms to protect them. However, the upper respiratory tract, the terminal part of the urethra, the vagina, the mouth and throat and the terminal ileum and large bowel—and, of course, the skin—are essentially open to colonization and each has a recognized resident flora. In addition to the resident flora, other organisms may be present, usually in relatively smaller numbers, as transient flora.

The commensals are most numerous in the large bowel where anaerobes of the *Bacteroides-Fusobacterium* group number tens of thousands of millions (10^{10}) per gram of intestinal content. Here they slightly outnumber the bifidobacteria and together these groups greatly outnumber the commensal aerobic coliform organisms (10^{6-8} per gram). A simplified summary of the commensal flora of man is given in Figure 1.3 and Table 1.3.

Anatomical sites such as the oropharynx, the gut and the female genital tract are not single microbiological environments. For example, the flora of the lower vagina differs from that of the cervix of the uterus. The commensal

Table 1.3 A list of aseptic and colonized areas of the body.

ESSENTIALLY STERILE AREAS
Brain and spinal cord (central nervous system)
The nervous system
Bones, joints and muscles
ASEPTIC AREAS WITH EFFECTIVE CLEARING MECHANISMS
Heart and blood vessels (cardiovascular system)
The lymphatic system
Lungs and terminal bronchi (lower respiratory tract)
Liver
Spleen
Kidneys, ureters and bladder
TRANSIENTLY CONTAMINATED AREAS
Skin (see below)
Conjunctiva of eye
Upper respiratory tract (see below)
Stomach and proximal small intestine (duodenum, jejunum, upper ileum)
COLONIZED AREAS
Skin
External meatus of ear
Nasopharynx / Oropharynx — Upper respiratory tract
Lower small bowel (terminal ileum)
Large bowel (caecum, colon, rectum)
Vagina
Terminal urethra

bacterial populations of the surfaces of the tongue, and those of the other mucosal surfaces of the mouth and the surfaces of the teeth all differ significantly. Dental caries and periodontal disease provide good models to illustrate how the commensal flora may participate in disease processes when circumstances allow (p. 51).

2 Laboratory investigation of bacterial infection

When a bacterium produces an infective disease, it multiplies in the host and can often be isolated from the patient by various microbiological procedures that include the culture of material submitted to the laboratory. The material may be a specimen of a discharge such as sputum, urine or faeces, or it may be frank pus. If pus is present in considerable amount, it is sent in a small sterile bottle; a swab is generally used when smaller quantities are involved. In some cases, a fragment of infected tissue removed by a surgeon may be submitted for investigation. Attention to detail in the prompt and proper submission of all such material to the laboratory is of great importance; some organisms are very susceptible to deleterious influences such as desiccation or overgrowth by

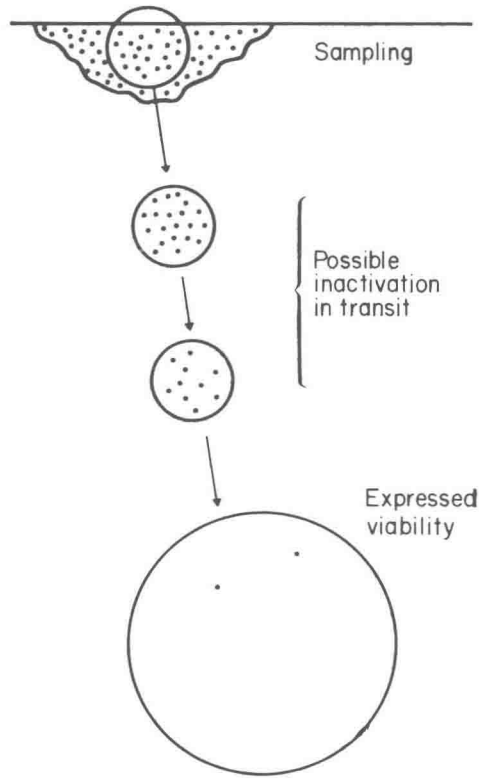

Figure 2.1 Although there may be large numbers of organisms available for sampling, losses in transit may result in a very poor yield on the culture plate.

more hardy commensals or contaminants; many specimens are wasted by carelessness (Fig. 2.1). Special *transport media* may be used to preserve delicate organisms in transit. In some cases, special procedures are required to obtain certain samples for microbiological investigation. If it is suspected that the organism is present in the blood, samples of blood are obtained from the patient under strictly aseptic conditions and submitted in special bottles for *blood culture*. If meningitis is suspected, a sample of cerebrospinal fluid (CSF) is obtained by a procedure known as lumbar puncture and the CSF is then sent for prompt examination.

MICROSCOPY

Some of the material submitted is usually examined promptly by microscopy, either as an unstained 'wet' preparation or, more commonly, as a stained smear. Special microscopy is sometimes required. This examination may provide direct evidence of the nature of the infecting agent; bacteria may be seen, or structures in host cells may suggest a viral infection. In addition microscopy may provide evidence of a host reaction; for example, the presence of pus cells confirms that there is an inflammatory reaction in progress (p. 33).

Despite the successful development of many special culture media and procedures, it is still not possible to grow some recognized pathogens as a routine. For example, the causative organism of syphilis may be recognized in material viewed by dark-ground microscopy, and the causative organisms of Vincent's infection of the gums or those of leprosy are not routinely cultured but may be identified on the evidence of a stained smear or tissue section. In other cases, the urgency of the situation demands a prompt presumptive microbiological opinion before the result of culture is available. Under these circumstances, presumptive identification may rest upon microscopic observations with the light microscope or the electron microscope. The prompt diagnosis or exclusion of smallpox may be facilitated by direct electron microscopy.

CULTURE PROCEDURES

Different organisms with different growth requirements are cultured on different ranges of media under various conditions. Most of the bacterial pathogens of man are grown at 37°C under aerobic conditions on nutritive media and they generally produce recognizable colonies within 24–48 hours. Some require to be grown anaerobically. Some bacteria require carbon dioxide for optimal growth. Some take several days to produce colonies and some, such as the tubercle bacillus, require several weeks.

It is sometimes relatively easy to isolate a pathogen from an ill patient by culture methods and to claim with confidence based on experience that the pathogen is the cause of the presenting illness. Most often, however, the exercise is complicated by several factors: (i) The specimen submitted may be