MiSACmatters 1

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ABOUT MiSAC

MiSAC*matters*: this publication from MiSAC is aimed at promoting and supporting the post-16 teaching of microbiology in schools and colleges. It will be made available free-of-charge on paper and on the MISAC website.

There are four main sections: a **main article** features current issues in microbiology of relevance to the syllabus; **A Happy Medium** provides guidance on the principles and use of microbiological culture media; **The Culture Column** gives information about micro-organisms suitable for use in schools and colleges; **Investigating Microbiology** offers background information on topics that may form a basis for developing practical investigations.

Suggestions of topics for future issues of MiSAC*matters* and other comments will be most welcome.

Also available: MiSAC*briefings* provide resource material to further the understanding of the principles of microbiology and its applications. MiSAC*activities* provide protocols, technical details and background theory for practical investigations in microbiology. MiSAC*helps* give examples of the responses to enquiries from teachers and technicians on various aspects of microbiology, particularly practical work.

Annual MISAC Competition. The MiSAC Annual Competition for Key Stages 3 and 4 (or equivalent) now attracts up to 2,000 student entries each year. Each year's topic is linked to the National Curriculum. Special sponsorship provides money prizes totalling £1,000 for students and their schools.

Antibiotics *versus* bacteria

Antibiotics have kept bacterial infections at bay for more than 50 years. ***Dr John Grainger*** *looks at their use today and their role in nature.*

In the service of mankind

Antibiotics are produced by some microbes and affect others by either killing them or preventing them from growing. Fortunately, susceptible microbes include those bacteria that cause many common diseases (Table 1, page 2) and some fungi; therefore, antibiotics, are a vital part of the arsenal of chemotherapy. But it must be noted that antibiotics are not effective against all pathogenic microbes. For example, viruses are not susceptible and, therefore, such diseases as influenza and herpes cannot be treated with antibiotics.

Originally, 'antibiotic' was used for wholly natural products, e.g. the penicillins produced by certain fungi, and 'drug' for synthetic substances, e.g. the sulphonamides. However, as now there are semi-synthetic antibiotics, e.g. methicillin, and also wholly synthetic ones, e.g. chloramphenicol, both words tend to be used for any antimicrobial compound that is effective at small concentrations.

Modes of action

A successful chemotherapeutic agent has to be effective against the pathogen but cause minimal damage to the patient. This selective toxicity is expressed as the therapeutic index, i.e. the ratio of the therapeutic dose (the level for clinical treatment) to the toxic dose (the level that causes damage to the patient); the larger this ratio the better.

Explanations for the suitability of various agents are based on an understanding of their various mechanisms of action (Table 2, page 2). For example, the penicillins interfere with the synthesis of peptidoglycan (murein), a characteristic component of the cell walls of bacteria (prokaryotes). This substance provides the strength needed to prevent cells from bursting under natural, large internal pressures - up to those of a motorcar tyre! However, these antibiotics do not affect us in this way because, being eukaryotes, we do not have peptidoglycan.

A novel, speculative train of thought is that antibiotics are relics from a bygone age of RNA

Sulphonamides are antimetabolites, a category of substance which blocks the formation of vital cell components through being a structural analogue of a key intermediate in their production. For example, sulphanilamide (a sulphonamide) is a structural analogue of p-aminobenzoic acid which is needed by bacteria for the synthesis of folic acid, a co-factor of great importance in purine biosynthesis. We are not affected by this drug because of an inability to synthesise folic acid which, therefore, must be supplied in our diet.

Another important feature of a chemotherapeutic agent is the range of pathogens against which it is effective, i.e. a *narrow* or *broad spectrum* of activity (Table 2, page 2). The penicillins, for instance, have a narrow spectrum of activity in being much more effective against Gram-positive bacteria (e.g. *Staphylococcus aureus*) than Gram-negative bacteria (e.g. *Escherichia*

continued on page 2

Antibiotics *versus* bacteria

continued from page 1

coli). This difference is because in Gram-positive bacteria, peptidoglycan is distributed throughout the cell wall and, therefore, is readily accessible to the antibiotic. Peptidoglycan is less accessible in Gram-negative bacteria because it is in an inner layer of the cell wall and protected by an outer membrane which has an outer layer of lipopolysaccharide (see Fig 1).

The effect of the chemotherapeutic agent may be either *static* (*bacteriostatic* if the target organism is a bacterium) when the pathogen regains activity after removal of the agent or *cidal* (*bactericidal*) when the lethal effect on the pathogen is irreversible (Table 2). A cidal effect may become a static one on dilution and the same agent may have a cidal effect against one species but a static one for another.

An understanding of the various modes of action of antimicrobial agents and their other properties (Table 2) provides the basis for the rational choice of treatment. However, the regimes of antibiotics and drugs used are continually changing, due largely to the increasing menace of the spread of resistant strains. This challenge is being met in a variety of ways - by using mixtures of agents, re-designing existing ones and searching for new approaches, not least a renewed effort to observe basic principles of hospital hygiene.

But what is their role in nature?

It may seem obvious that the role of antibiotics in nature is for one microbe to defend itself against competitors. Many antibiotic-producing microbes such as *Penicillium* and *Streptomyces* are common in soil yet the majority of soil microbes do not produce antibiotics and most soil bacteria are not sensitive to those that are produced. Even sensitive types may soon develop resistance to the small doses of antibiotics present and pass the resistance on to the next generation. Also, in the soil, antibiotic-producers are very unlikely to meet the bacteria against which they are most active.

Table 1: Some bacterial infections, their causes and the antibiotics and other chemotherapeutic agents commonly used to treat them

Infection	Typical causative organism	Treatment†
	Gram-positive bacteria	
diphtheria	*Corynebacterium diphtheriae*	clarithromycin, erythromycin
tuberculosis	*Mycobacterium tuberculosis*	isoniazid + rifampicin + ethambutol + pyrazinamide
boils, wounds	*Staphylococcus aureus*	cephalosporins, methicillin
sore throats, sepsis	*Streptococcus pyogenes*	amoxycillin, erythromycin
pneumonia	*Streptococcus pneumoniae*	cephalosporins, erythromycin
meningitis (bacterial)	*Streptococus pneumoniae*	amoxycillin, cephalosporins
	Gram-negative bacteria	
urinary tract, pneumonia	*Escherichia coli*	cephalosporins, sulphonamides, trimethoprin
Legionnaire's disease	*Legionella pneumophila*	clarithomycin, erythromycin
cholera	*Vibrio cholerae*	tetracyclines
whooping cough	*Bordetella pertussis*	clarithomycin, erythromycin
meningitis (bacterial)	*Neisseria meningitidis*	amoxycillin, cephalosporins

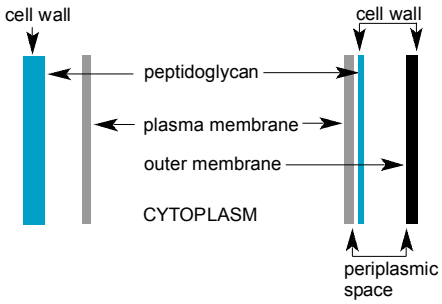
†*The author is grateful to Ramesh Chauhan MRPharmS for advice on current practice*

Table 2: Some antibiotics, their sources and properties

Antibiotic	Source	Spectrum of activity (Gram-reaction of sensitive bacteria)	Type of effect	Process inhibited
penicillin G	*Penicillium**	narrow (+)	cidal	cell wall synthesis
ampicillin		broad (+, some -)		
carbenicillin		broad (+, many -)		
methicillin		narrow (+)		
cephalosporins	*Cephalosporium**	broad (+, some	cidal	cell wall synthesis
streptomycin	*Streptomyces***	broad (+, -)	static	protein synthesis
chloramphenicol		broad (+, -)		
erythromycin		broad (+, some -)		
tetracyclines		broad (+, -)		
rifampicin	*Streptomyces***	broad (+, some -)	static	nucleic acid synthesis
vancomycin	*Streptomyces***	narrow (+)	cidal	cell wall synthesis
polymyxins	*Bacillus***	narrow (-)	cidal	membrane function

** fungus; ** bacterium*

Fig 1: Diagram of an idealised bacterial cell showing the cell wall structure in Gram-positive (left) and Gram-negative (right) cells



Any feasible explanation for their role must also be examined in the light of the properties of the antibiotics. For example, antibiotics (and some other substances that have no antibiotic activity) are *secondary metabolites*, i.e. their production does not begin until towards or just after the end of the period of active growth. Yet microbial growth in soil is usually either absent or very slow because of a lack of the nutritional and other conditions necessary for good growth. This also questions the effectiveness in soil of the many antibiotics, e.g. the penicillins, which affect only cells that are actively multiplying. Also, the unusual structure and small size of the molecule of most antibiotics appear to have no obvious affinity with the more complex molecules of the main processes of microbial cell metabolism.

The search for a more satisfactory explanation for the role of antibiotics in nature has yielded an interesting idea that antibiotics are relics of a bygone age of RNA-life. This derives in part from the contemporary role of RNA in cell multiplication. The idea is based on speculation that there was an era of RNA pre-dating the emergence of DNA when RNA had a function that was different from that in the DNA world. The suggestion is that in this RNA era, 'pre-antibiotics' may have been formed along with nucleotides (which evolved into self-replicating RNA) and functioned as regulators of or templates for biochemical activities and helped in the transmission of genetic information to the next generation.

But would not a mechanism that now seems useless have been eliminated in the course of evolution? Not if it has a yet unidentified purpose, for concentrations of antibiotics too small to interfere with bacterial growth have been shown to stimulate the normal genetic machinery and cell growth.

For a popular account of this speculative idea, see 'Chemical warfare' by John Postgate (TES, 19 February 1993, page 17).

Next issue: *Bacteria versus antibiotics*

A HAPPY MEDIUM: TYPES OF CULTURE MEDIA

COMPLEX MEDIA

Complex media (sing. -ium) commonly contain ingredients extracted from meat, yeast and malt (0.5-1 %, w/v). They are convenient for the routine cultivation of a wide range of micro-organisms. However, the identity and concentrations of the large number of specific nutrients present, i.e. sources of carbon, nitrogen and other elements, and various growth factors (amino acids, vitamins, purines, pyrimidines, etc.), are not precisely defined.

Nutrient agar and **nutrient broth** are common complex media used for growing bacteria. Both forms contain the same nutrients but the former is solidified by the inclusion of 1.2-1.5% (w/v) agar; 'broth' is literally a thin (meat) soup. They have a pH value of c. 7.0-7.4. Agar is a complex mixture of polysaccharides extracted from red seaweeds and it has little nutritional value. A sugar, commonly glucose (1%, w/v), is added for organisms that require a fermentable substrate, such as a yeast growing anaerobically or lactic acid bacteria, e.g. *Streptococcus*. Malt extract agar or broth (pH value c. 5.4) is used for growing moulds and yeasts.

CHEMICALLY DEFINED MEDIA

In a chemically defined medium (also known as *synthetic*, *minimal* or *mineral*), each ingredient is added separately and in known amount. They are usually used in liquid form because even the traces of nutrients present in agar might be sufficient to compromise an investigation. The formulation is chosen according to the nutritional requirements of the micro-organisms involved. The basic component is a mixture of mineral salts. Some mixtures are available from suppliers already prepared.

Other nutrients including sources of carbon and energy (C&E) and nitrogen (N) are added to the mineral base. The most usual source of C&E is glucose but no added C source is required for microbes that can utilise CO_2 from the air. N is supplied in either inorganic or organic form depending on the requirements of organisms. There may also be a need for growth factors. The simplest form of this type of medium may contain only four ingredients.

Examples of the use of chemically defined media include demonstrating the diversity of nutritional requirements of micro-organisms and studying specific nutritional types, e.g. photosynthetic forms, nitrifying bacteria.

***Next issue**: Making and using culture media*

THE CULTURE COLUMN

Bacterial genus *Pseudomonas*

Gr. *pseudes* false
Gr. *monas* a unit, monad

CHARACTERISTICS

- **Habitat**: widely distributed in soil, water and on plants, also found on animals.
- **Cultivation**: grows well on nutrient agar (NA) and in nutrient broth (NB) incubated in air (most are strict aerobes) at room temperature for 1-2 days; optimum temperature is 25°C but also able to grow slowly at c. 5°C.
- **Cell morphology** (see Fig 2): young (1-2 days) cultures are Gram-negative, straight rods, 0.3-0.5 by 1.0-1.8 µm, occurring singly and in pairs; rapidly motile by one or more polar flagella (sing. -um).
- **Colony form**: off-white, shiny, 2-4 mm diameter.
- **Physiology**: mostly strictly aerobic; oxidative metabolism of glucose; many produce water-soluble, fluorescent pigments that diffuse through the medium; produce extracellular hydrolytic enzymes; nutritionally non-exacting; utilize a very large number of organic sources of carbon; grow on inorganic sources of nitrogen; do not require growth factors; some species capable of denitrification, e.g. *Ps. denitrificans*.

IMPORTANCE

- Many are **plant pathogens** causing diseases of commercial significance, e.g. wilt, canker, blight, leaf spot.
- Some serious **animal pathogens** infect burns and surgical wounds and cause pneumonia, especially in immunosuppressed patients; also found in biofilms lining the lungs of cystic fibrosis patients.
- May cause **food spoilage**, especially of chilled foods.
- **Bioremediation** of oil spills, using the ability of many species to break down complex carbon compounds.

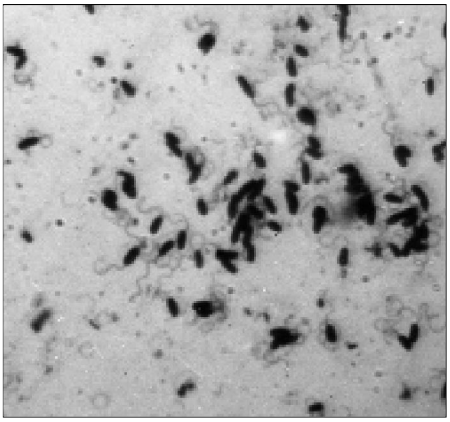
PRACTICAL INVESTIGATIONS

- *Ps. fluorescens* is suitable for use in schools.
- **Morphology:** shape, arrangement and Gram-reaction from young agar cultures; compare with e.g. *Micrococcus luteus* (Gram-positive cocci).
- **Motility** by hanging drop method from moist NA slope or young NB cultures; use a high power dry objective lens (x40); compare with e.g. *Micrococcus luteus* (non-motile).
- **Growth temperature range** at intervals up to 30°C; allow at least 1 week for growth at refrigerator temperatures (4-5°C); compare with e.g. *Escherichia coli* (no growth at 4-5°C).
- Green fluorescent water-soluble **pigment production**; made visible by illumination with ultraviolet radiation (\ 365 nm); incorporation of glycerol (1%, v/v) in the medium (NA or NB) enhances pigment production; incubate for at least 1 week.
- **Extracellular protease activity** shown by zones of clearing around spot inocula on plates of NA + 10% (v/v) skimmed milk after incubation for up to 1 week; compare with e.g. *Bacillus subtilis* (also proteolytic) and *Escherichia coli* (non-proteolytic).
- **Nutritional versatility**: utilisation of various carbon and energy sources (e.g. glucose, citrate) in a medium[1] containing also an inorganic source of nitrogen and mineral salts; compare with e.g. *Escherichia coli* (cannot utilize citrate).

1. Medium (%, w/v): mineral salts, e.g. potassium dihydrogen phosphate (0.1) and magnesium sulphate, hydrated ($7H_2O$) (0.02); N source, e.g. ammonium hydrogen phosphate (0.15); C&E source, e.g. glucose, citrate, etc. (0.2); pH value 7.0. Inoculum: small amount of growth from an agar culture as there may be carry-over of nutrients from a broth culture. Note: no growth factors added.

Next issue: Mucor *and related fungal genera*

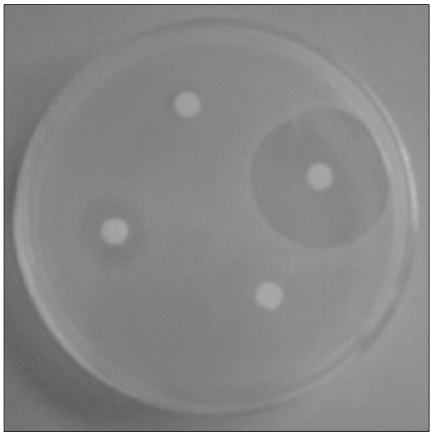
Fig 2: **Stained preparation of *Ps. fluorescens* showing polar flagella**



Testing susceptibility to antimicrobial agents

The disc diffusion method for testing the susceptibility of microbes to inhibitory substances was introduced more than 50 years ago and is still the standard procedure. It is used in hospitals as an aid to the choice of antibiotic for treating a bacterial infection. In the pharmaceutical industry it features in research and development of new antimicrobial agents including the exploitation of traditional plant remedies, e.g. tea tree oil and garlic. The

Fig 3: A disc diffusion test



procedure can also be used with antiseptics and disinfectants, and with Fleming's other famous discovery lysozyme, present in e.g. egg white.

The procedure[1] involves first preparing a uniformly inoculated pour plate or spread plate in a Petri dish. A disc moistened with an inhibitory agent is then placed as soon as possible on the surface of the agar medium. After incubation, the occurrence of an inhibitory effect is seen by the naked eye as a clear zone of no growth around the disc, contrasting with a surrounding haze of growth (see Fig 3).

The edge of a zone of inhibition represents the minimum inhibitory concentration (MIC) at which the agent is effective against the developing microbial population. The diameter of a zone of inhibition including the disc is taken as a measure of the effectiveness of the agent.

The procedure lends itself to project work as it is technically straightforward and provides the basis for investigations that incorporate a quantitative element. However, a reliable outcome depends on the use of a precise methodology because of the involvement of several interacting factors.

The underlying principle is that during incubation, two processes are taking place simultaneously, i.e. growth of the microbe and diffusion of the inhibitory agent away from the disc causing a concentration gradient through the medium. Therefore, changes in any factor that affects the balance between these two processes will influence the outcome.

1. See *Basic Practical Microbiology* (SGM, 2003). Pour plate and spread plate, pp 12-15; Testing sensitivity to antimicrobial substances, p 21.

Some factors to explore

- Type, sensitivity and growth rate of culture.
- Preparation of inoculum (e.g. concentration, age).
- Plating method.
- Nature, potency and diffusion rate of agents.
- Type of culture medium (e.g. composition).
- Volume of medium in the Petri dish.
- Incubation conditions of the test (e.g. temperature).

Acknowledgements: Fig 2: Dr Muriel Rhodes-Roberts; Fig 3: Society for General Microbiology (SGM)

Next issue: *Measuring mould growth*

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Microbiology in Schools Advisory Committee, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AG.
E: *microbe@misac.org.uk*. W: *www.misac.org.uk*.

The **Microbiology in Schools Advisory Committee** (MiSAC), was founded in 1969 as an independent voluntary body to promote the teaching of microbiology in schools and colleges. MISAC is recognised by Government as an authority on the safe use of micro-organisms, provides advice, develops practical protocols, produces factsheets and MiSAC*matters*, works with examination bodies, advises publishers, liaises with suppliers of biological materials, and runs an annual competition for schools. MISAC has charitable status.

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