Bio Factsheet

Number 100

Antibiotics and Antibiotic resistance

This Factsheet describes;

- **the nature of antibiotics, their mode of action and their uses, including the treatment of infectious diseases;**
- **the development of antibiotic resistance and its impact on the treatment of disease;**
- **the process of penicillin production.**

Definitions

Antibiotic: A chemical produced by a microorganism that is able to inhibit the growth of or kill other microorganisms. They are effective in low concentrations and act on specific species of microorganism (usually bacteria but some also act on fungi).

Bacteriocidal: An antibiotic, such as penicillin, that will kill bacteria.

Bacteriostatic: An antibiotic, such as streptomycin or tetracycline, that will prevent bacterial growth.

Gram positive: Refers to bacteria, for example, *Clostridium sps*. and *Bacillus sps.* which when stained with Gram stain retain crystal violet within their cells and thus appear blue or purple under the microscope. Gram negative bacteria, for example, *Pseudomonas sps.* do not retain the crystal violet stain. The difference between the two groups is due to their different cell wall compositions.

Broad and narrow spectrum antibiotics: Broad spectrum antibiotics, for example, tetracyclines, chloramphenicol and streptomycin, act effectively against many different bacterial species. Narrow spectrum antibiotics, for example, penicillins, are only effective against a few different bacterial species.

The nature of antibiotics

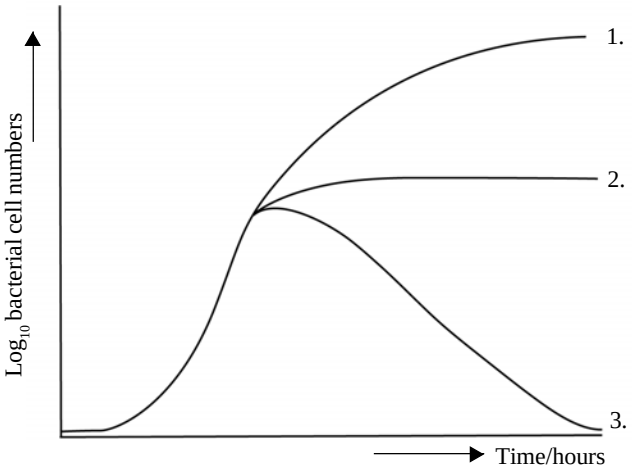
Antibiotic activity was first observed in 1896 by Ernest Duchesne and 'rediscovered' in 1929 by Alexander Fleming. Both scientists noted the inhibition of bacterial growth on an agar plate due to contamination with the fungus *Penicillium notatum.* However, it was several years before the compound causing the inhibition was isolated and identified. Reasonable amounts of the penicillin could not be produced until the early 1940s and it only came into widespread use after 1945. Since then many hundreds of antibiotics have been discovered but relatively few are used as medicines.

Remember *- many of the antibiotics known only affect organisms that are not pathogenic to humans or animals and so there is no need to develop them. Some are also too toxic to human or animal cells for use as drugs. Others are difficult to manufacture in large enough quantities for medical use.*

Ideally an antibiotic which can be used as a medicine should have the following properties:

- it should effectively target pathogenic microorganisms (usually prokaryotic), either by inhibiting their growth (bacteriostatic) or by killing them (bacteriocidal). See Fig 1.

Fig. 1. The effect of anitbiotics on bacterial growth



Key

1. = **control** (no antibiotic - normal growth)
2. = **bacteriostatic** (antibiotic stops growth, body's immune response eventually kills bacteria)
3. = **bacteriocidal** (antibiotic kills bacteria)

- it should have minimal or no adverse toxic effects on human or animal cells (eukaryotic).
- it should be cheap to manufacture in bulk.
- it should be easy to administer by mouth or injection.
- it should persist in the human or animal body, at concentrations that are effective, long enough to remove infection.
- it should be effective against a wide range of pathological microorganisms, including gram positive bacteria, gram negative bacteria and fungi.

Most antibiotics work by inhibiting a specific biochemical pathway that is present in the target microorganism but not present in the human or animal cells. For example, penicillin and its derivatives inhibit the synthesis of peptidoglycans in developing bacterial cell walls. The weakened cell walls disintegrate due to osmotic lysis. Since gram positive bacteria have much more peptidoglycan in their walls (up to 95%) than gram negative bacteria (as little as 5%), it follows that penicillins are most effective against gram positive species.

Tetracyclines prevent tRNA coupling to bacterial ribosomes and so prevent protein synthesis, growth and cell division in many bacterial species. Streptomycin interferes with amino acid assembly into bacterial protein molecules and so is also effective against a wide range of bacteria. Chloramphenicol blocks peptide bond synthesis during protein synthesis in a wide range of bacteria.

Many known antibiotics affect processes within human and animal cells and so are normally too toxic for use as medicines. However, some may have to be used, with great care, in special cases where other less aggressive treatments are not available. Even penicillin and its derivatives must be used with care because a significant proportion of the population are allergic to penicillin, suffering skin rashes and fever or even death from anaphylactic shock, (due to an extreme immune reaction), if exposed to it. Prolonged use of streptomycin may cause damage to the vestibular (balancing) apparatus of the ear, hearing loss and kidney damage. Overuse of chloramphenical can produce severe blood diseases due to its action in preventing peptide bond formation which thus may inhibit blood cell formation. Tetracyclines can seriously disrupt the normal intestinal bacterial population allowing its replacement with 'superinfecting' fungi and other bacteria. See Table 1.

Other uses of antibiotics

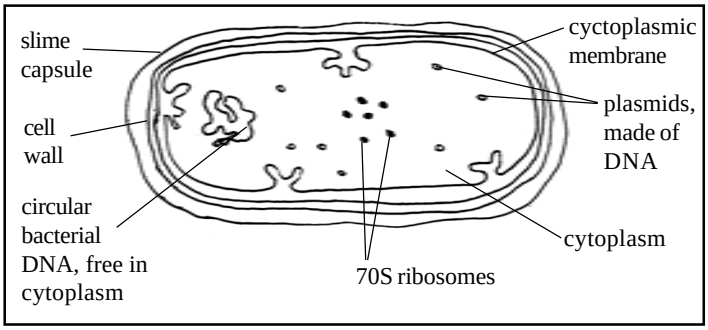
- small concentrations of antibiotics may be incorporated into cattle, sheep, and pig food – this stimulates growth, speeds up meat production and so increases profits.
- small concentrations of antibiotics may be incorporated into poultry and other animal feeds as a prophylactic – intended to prevent infection before it occurs.
- antibiotics are used to treat bacterial plant diseases in potatoes, tomatoes and fruit trees.
- antibiotics are included in tissue culture media to prevent unwanted microbial contamination.
- antibiotics are included in some soaps as a prophylactic – to reduce the risks of skin infection.

***Remember** – the indiscriminate use of antibiotics in feed stuffs means that humans may receive unwanted doses of antibiotics in meats, eggs and milk. This exposure may cause the development of an immune or inflammatory response to the antibiotic so that the human cannot be treated with the drug at a later date. The practice has also resulted in the development of antibiotic-resistant strains of bacteria.*

The development of antibiotic resistance

Antibiotic resistance is shown by certain bacteria which can survive and flourish in the presence of certain antibiotics. These antibiotics have no bacteriostatic or bacteriocidal actions on the bacteria. If the resistant bacteria are pathogenic than they can cause antibiotic resistant diseases (Fig 2).

Fig 2. Electron micrograph appearance of a rod-shaped bacterium, showing bacterial DNA and plasmids.



Antibiotic resistance arises by spontaneous gene mutation, either in genes of the main bacterial DNA or in 'R-factor genes' located in bacterial plasmid DNA. (R-factors cause resistance to antibiotics, virus infections and UV radiation). If a population of bacteria is exposed to an antibiotic then any non-resistant bacteria will be killed or inhibited but any mutant resistant bacteria will survive and multiply, passing on their resistant genes from generation to generation. In effect, natural selection is occurring. For example, a mutation in the main DNA of *Mycobacterium tuberculosis* produced modified ribosomes, to which the antibiotic streptomycin could not attach, in order to inhibit protein synthesis and so inhibit bacterial growth. Thus streptomycin lost its effectiveness in treatment of tuberculosis.

Antibiotic resistance has a chemical basis, the mechanisms depending on the bacterial species and antibiotic involved. For example, penicillins contain a ring structure, known as a β–lactam, which normally inhibits the synthesis of peptidoglycans in bacterial cell walls, resulting in a loss of strength and death due to osmotic lysis. Penicillin-resistant bacteria, for example, *Staphylococci*, produce an enzyme called 'penicillinase' or 'β–lactamase' which destroys the β–lactam structure of penicillin, rendering it ineffective.

***Exam Hint**:- You do not have to remember the detailed chemical structure of any antibiotics but you must know the basic mechanism of penicillin-resistance in bacteria.*

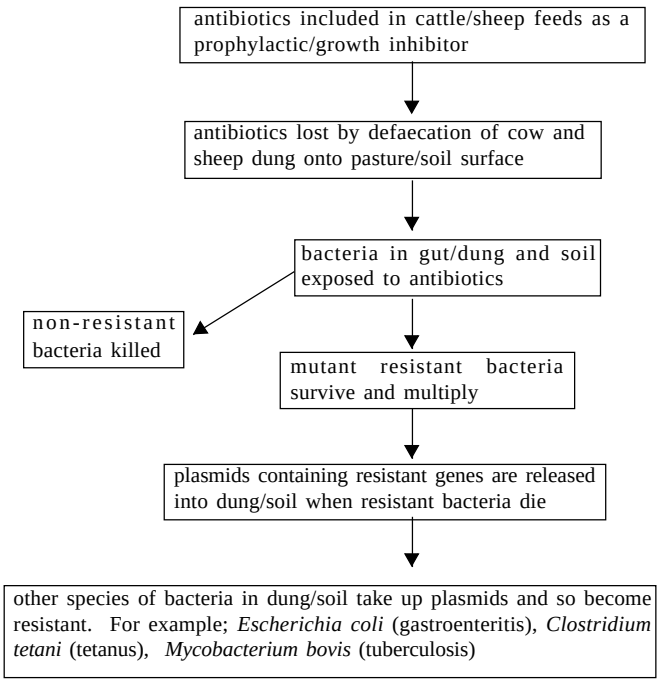
The problem of antibiotic resistance is made worse by the transfer of resistance-conferring genes between bacteria by two methods:

- in the process of transformation, which is a form of bacterial sexual activity, one bacterium can take up DNA from another bacterium. Populations of penicillin-resistant *Neisseria gonorrhoeae*, which causes gonorrhoea, have arisen in this way.

Table 1. Common antibiotics and the diseases they control

Antibiotic	Producer	Disease	Pathogenic organism
Penicillin and its derivatives (narrow spectrum – but some have their spectrum extended by chemical modification)	*Penicillium chrysogenum* (mould)	Diphtheria Syphilis Tetanus Scarlet fever Meningitis Anthrax	*Corynebacterium* *Treponema* *Clostridium* *Streptococcus* *Meningococcus* *Bacillus*
Chloramphenicol (broad spectrum)	Actinomycetes (soil bacteria, for example, *Streptomyces*). Can also be synthesized chemically.	Typhoid fever Meningitis Typhus Skin infections	*Salmonella* *Haemophilus* *Rickettsia* *Staphylococcus*
Streptomycin (broad spectrum)	Actinomycete – *Streptomyces griseus*	Tuberculosis	*Mycobacterium*
Griseofulvin (narrow spectrum)	*Penicillium notatum*	Ringworm	*Trichophyton* (a fungus)

- plasmids, often carrying resistance-conferring genes to several antibiotics, can be exchanged between bacteria, even of different species. The following flow chart indicates one possible way in which several species of bacteria may have become resistant:



Overexposure of the human and animal population to antibiotics due to overuse by doctors and vets has also contributed to the appearance of so many antibiotic-resistant species and strains of bacteria. As a result bacterial diseases such as tuberculosis are becoming very difficult to treat.

The manufacture of penicillin

Penicillin was originally isolated from the fungus *Penicillium notatum.* However, the yields were not high and the fungus preferred to grow in surface culture which limited production. Various strains of *Penicillium chrysogenum* are now used. They have high yields of penicillin and will grow in submerged culture, thus allowing bulk manufacture (Fig 3).

Remember: *– a* ***primary metabolite*** *is a chemical produced by the chemical processes of the cell involved in growth or energy release. A* ***secondary metabolite*** *is a chemical produced at a later stage in the life cycle, during ageing or spore formation.* ***Batch culture*** *is when all the ingredients are added at the beginning. It is then left until the reaction forming the secondary metabolite has taken place, after which the products can be harvested. In* ***fed-batch culture****, additional nutrients are added when the secondary metabolite is being synthesized – this enhances the yield of secondary metabolite.*

Penicillin is a secondary metabolite and so is produced when the fungal culture is in the mature stage and the nitrogen content of the growth medium is falling to low levels. It is manufactured by batch culture or fed-batch culture.

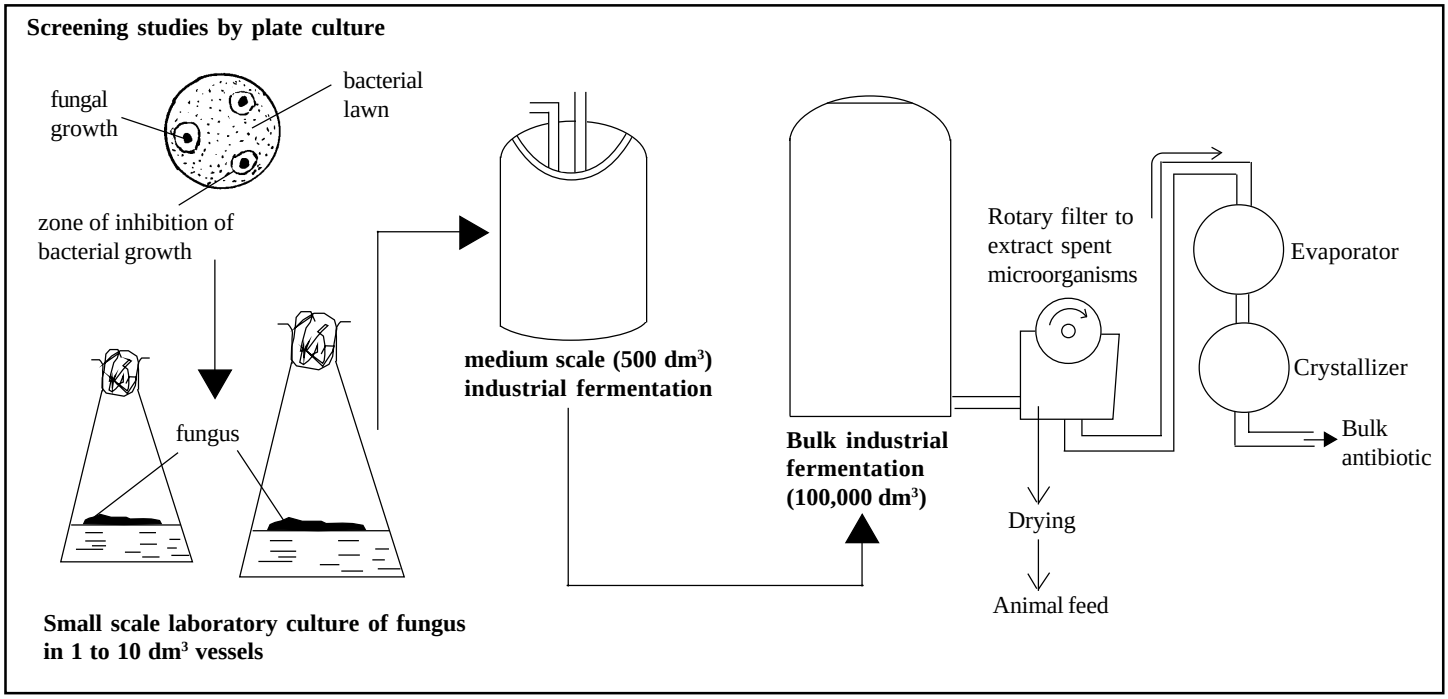
The screening studies tell us whether the particular fungus under consideration produces antibiotics in reasonable quantities and which bacterial species the antibiotic will be active against. Suitable fungi are then grown in laboratory conditions in volumes of growth medium up to 10 dm^3. This enables optimum growth conditions of temperature, pH and nutrient content of the growth medium to be found and also the optimum time for harvesting the antibiotic. With *Penicillium chrysogenum* the growth medium often used is corn steep liquor which contains a high concentration of sugars and also a precursor, ie. a chemical which makes penicillin-G (Penicillin-G is benzylpenicillin). The medium is inoculated with a suspension of conodiospores of *P. chrysogenum*. The yield of penicillin is highest when oxygen availability is kept high, temperature is maintained at 24°C and the pH is slightly alkaline. The mould starts releasing penicillin from about 40 hours onwards which, is after the exponential growth phase has finished.

Industrially the fermentation is initially carried out as a medium scale 'pilot' operation. If this produces the antibiotic efficiently it is scaled up to bulk manufacture.

The penicillin dissolved in the nutrient broth is obtained by 'down-stream' processing. This involves removing the spent fungus by filtration, evaporating off the water from the broth and crystallizing the penicillin. The process mainly yields penicillin-G which can be treated chemically to make other penicillins, for example, ampicillin and cephalosporins.

The spent fungus can be dried and sold for use as farm animal feed.

Fig. 3. Flow chart illustrating the industrial production of an antibiotic



Practice Questions

1. a) Antibiotics are 'secondary metabolites'. What does this mean? 2

   b) Name the organisms which would be used in the industrial manufacture of

   (i) penicillins

   (ii) streptomycin

   (iii) griseofulvin 3

   c) Suggest two advantages and two disadvantages of using the spent microorganisms as animal food. 4

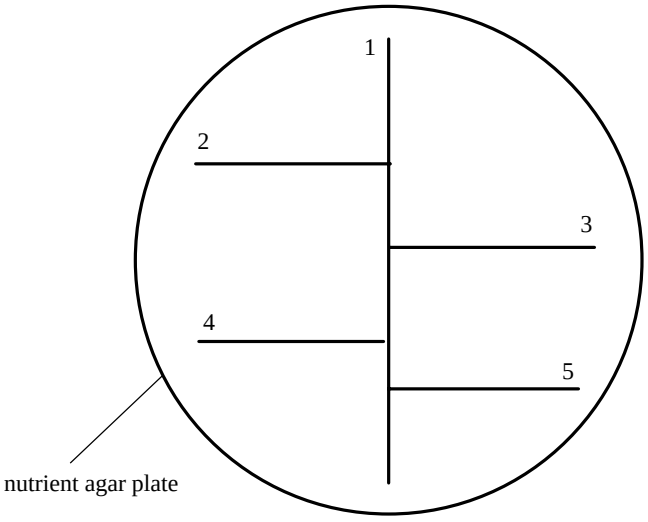
2. a) `Distinguish between each of the following pairs, naming an example in each case:

   (i) gram positive and gram negative bacteria. 4

   (ii) broad spectrum and narrow spectrum antibiotics. 4

   (iii) bacteriocidal and bacteriostatic antibiotics. 4

   b) The diagram below shows a screening test to find out if a newly discovered microorganism secretes an antibiotic. The organism was first inoculated onto a nutrient agar plate along line 1. Four different known species of bacteria were then inoculated along lines 2, 3, 4 and 5.



   What results would you expect to see if the microorganism,

   (i) had no antibiotic activity? 1

   (ii) secreted a broad spectrum antibiotic? 1

   (iii) secreted a narrow spectrum antibiotic? 1

3. a) Describe how penicillin affects bacterial growth. 3

   b) Why is penicillin more active against gram positive bacteria than against gram negative bacteria? 2

   c) Produce a flow chart illustrating the bulk industrial manufacture of penicillin. 12

4. a) Outline the process by which many species of bacteria can become resistant to antibiotics. 7

   b) Suggest three ways in which the chances of developing antibiotic resistant strains of bacteria may be reduced. 3

   c) Why is it becoming increasingly important to immunise the human population against diseases such as tuberculosis? 2

Answers

1. a) a secondary metabolite is produced during the ageing stage of microbial growth/when the nutrient medium is becoming exhausted;
it is not a product of cellular respiration/may be an excretory product; 2

b) (i) *Penicillium chrysogenum/P. notatum*;
(ii) *Streptomyces griseus*/Actinomycetes;
(iii) *Penicillium notatum;* 3

c) advantages:
provides income for the manufacturer;
low concentration of antibiotic in the animal feeds may act prophylactically to prevent infection/may stimulate growth; 2

disadvantages:
antibiotics in the animal feeds may result in the emergence of antibiotic-resistant strains of bacteria;
antibiotics in the animal feeds may sensitize the animals against the antibiotics so that they cannot subsequently be given the antibiotics to treat disease; 2

2. a) (i) gram positive bacteria retain crystal violet in their cells during a gram stain but gram negative bacteria do not;
for example, *Bacillus sps/Clostridium sps* are gram positive;
Pseudomonas sps/Escherichia sps are gram negative;
ref to higher content of peptidoglycan in gram positive bacterial cell walls is thought to retain the crystal violet/small content of peptidoglycan in gram negative bacterial cell walls allows release of crystal violet; 4

(ii) a broad spectrum antibiotic will be effective against a wide range of bacterial species;
for example, tetracyclines/chloramphenicol/streptomycin;
a narrow spectrum antibiotic only acts against one specific/ a few species of bacteria;
for example, penicillins/griseofulvin; 4

(iii) a bacteriocidal antibiotic kills the (target) bacteria;
for example, penicillin acts by weakening the cell wall so that the bacteria lyse due to osmotic uptake of water;
a bacteriostatic antibiotic prevents the (target) bacteria from growing and dividing;
for example, streptomycin/chloramphenicol/tetracyclines which act by inhibiting protein synthesis in bacteria; 4

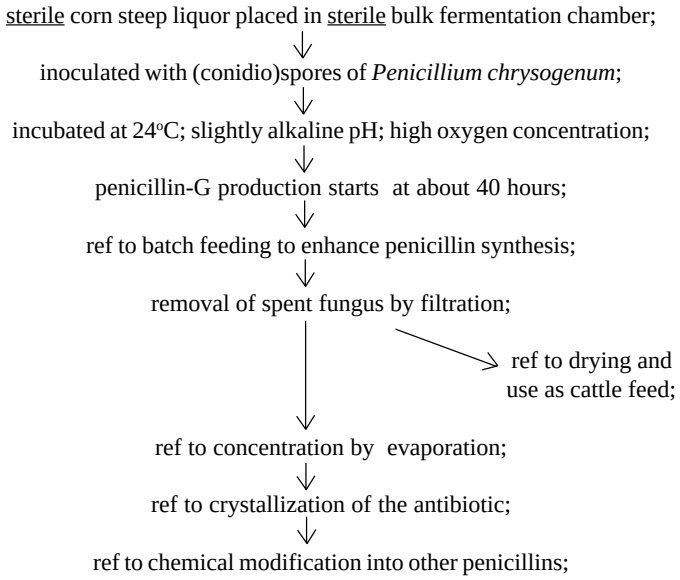
(b) (i) all four species of bacteria would grow up to line 1/bacterial growth on lines 2, 3, 4 and 5 would appear up to line 1; 1

(ii) no/little growth of all four species of the bacterium would occur/growth would be inhibited in three or four of the four species; 1

(iii) (probably) only one of the four bacterial species would be inhibited; 1

3. (a) bacteriocidal effect;
penicillin inhibits the synthesis of peptidoglycans in bacterial cell walls;
weakened walls burst due to osmotic uptake of water/ref to osmotic lysis; 3

(b) peptidoglycan is the main component of gram positive bacterial cell walls/only a small part of gram negative bacterial cell walls;
thus penicillin causes most damage to gram positive bacterial cell walls; 2

c)


12

4. a) ref to overexposure of bacteria to antibiotics;
by inclusion in animal feeds/overprescribing by doctors/vets/ incorrect use by patients;
gene mutation in bacteria resulting in genes conferring antibiotic resistance;
especially in R-genes of bacterial plasmids;
ref to selection of resistant bacteria/non-resistant bacteria do not survive;
ref to transfer of resistance genes to bacteria of the same species by transformation;
ref to plasmid transfer passing resistance genes to other species of bacteria; 7

b) stop exposing animals to antibiotics in feeds;
stop overprescribing antibiotics for disease/stop prescribing antibiotics in cases of virus infection (unless there is a medical need);
use narrow spectrum antibiotics to target only the offending bacteria rather than broad spectrum antibiotics which affect many species; 3

c) tuberculosis bacteria/*Mycobacterium* are resistant to most antibiotics making TB very difficult to treat;
thus vaccination is very important to prevent people catching the disease/to stop the spread of the disease; 2

Acknowledgements:
This Factsheet was researched and written by Martin Griffin
Curriculum Press, Unit 305B, The Big Peg, 120 Vyse Street, Birmingham. B18 6NF

ISSN 1351-5136