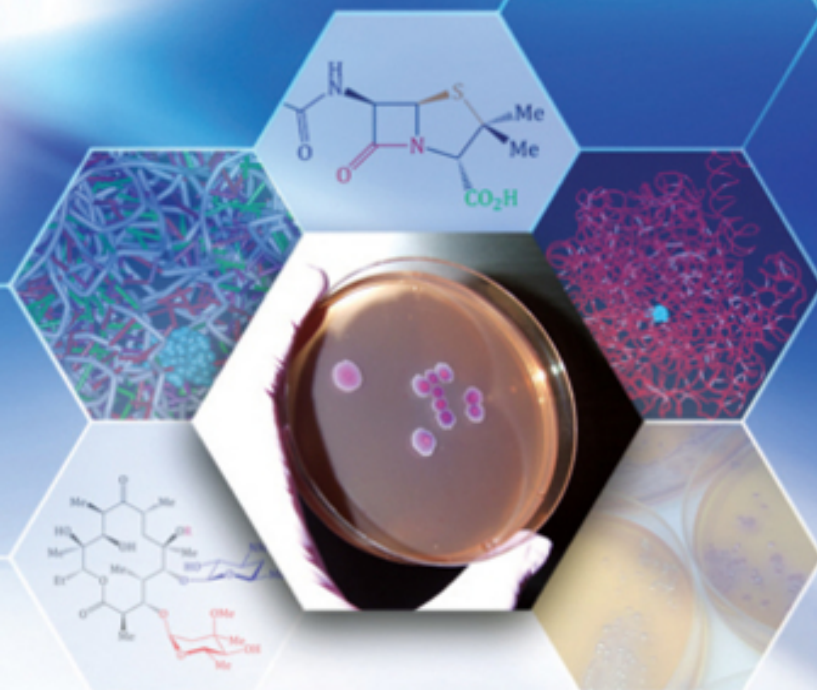# ANTIBACTERIAL AGENTS

CHEMISTRY, MODE OF ACTION, MECHANISMS OF RESISTANCE AND CLINICAL APPLICATIONS

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Contents

Section 2: Agents Targeting DNA

Chapter 2.1: Quinolone Antibacterial Agents

Chapter 2.2: Rifamycin Antibacterial Agents

Section 3: Agents Targeting Metabolic Processes

Antibacterial Agents

Chemistry, Mode of Action, Mechanisms of Resistance and Clinical Applications

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FOR OUR FAMILIES

Preface

Since the introduction of benzylpenicillin (penicillin G) in the 1940s, it is estimated that over 150 antibacterials have been developed for use in humans, and many more for veterinary use. It is the use of antibacterials in the treatment of infections caused by pathogenic bacteria that led to them being labelled as 'miracle drugs', and, considering their often simple pharmacology, the effect that they have had upon infectious diseases and population health is remarkable. We are lucky enough to have been one of the generations for whom antibiotics have been commonly available to treat a wide variety of infections. In comparison, our grandparents were from an era where bacterial infection was often fatal and where chemotherapeutic agents were limited to the sulfonamides and antiseptic agents. This golden age of antibacterial agents may, however, soon come to an end as more and more bacteria develop resistance to the classes of antibacterial agents available to the clinician.

  The timescale of antibacterial development occupies the latter half of the 20th century, with the introduction of the sulfonamides into clinical use in the 1930s, shortly followed by the more successful penicillin group of antibiotics. The discovery of penicillin by Sir Alexander Fleming in 1928, for which he received the Nobel Prize jointly with Howard Florey and Ernst Boris Chain, represents one of the major events in drug discovery and medicine. The subsequent development and wartime production of penicillin was a feat of monumental proportions and established antibiotic production as a viable process. This discovery prompted research which was aimed at discovering other antibiotic agents, and streptomycin (the first aminoglycoside

identified) was the next to be isolated, by Albert Schatz and Selman Waksman in 1943, and produced on a large scale. Streptomycin became the first antibiotic to be used to successfully treat tuberculosis, for which every city in the developed world had had to have its own specialised sanatorium for the isolation and rudimentary treatment of the 'consumptive' infected patients. It was estimated at the time that over 50% of the patients with tuberculosis entering a sanatorium would be dead within 5 years, so the introduction of streptomycin again proved a significant step in the treatment of infectious disease.

The development of antibacterials continued throughout the latter part of the 20th century, with the introduction into the clinic of the cephalosporins, chloramphenicol, tetracyclines, macrolides, rifamycins, quinolones, and others. All of these agents have contributed to the arsenal of antibacterial chemotherapy and all have a specific action on the bacterial cell and thus selective activity against specific bacteria. We hope that this book will serve to highlight the development of the major antibacterial agents and the synthesis (where plausible) of these drugs. In addition, as health care professionals, we hope that students of medicinal chemistry, pharmacy, pharmaceutical sciences, medicine, and other allied sciences will find this textbook invaluable in explaining the known mechanisms of action of these drugs. We believe that knowledge of the mode of action and pharmacology of antibacterial agents is essential to our understanding of the multidrug approach to the treatment of bacterial infections. Several administered antibiotics acting upon different bacterial cell functions, organelles, or structures simultaneously can potentiate the successful eradication of infection. In addition, by understanding the action of the antibacterial agents at a cellular level, we are able to envisage those mechanisms involved in drug toxicity and drug interactions. As is

demonstrated with the majority of the available therapeutic agents, antibacterial toxicities are observed with increased doses, as well as idiosyncratically in some patients and in combination with other therapeutic agents in the form of a drug interaction.

We have endeavoured to provide the major clinical uses of each class of antibiotic at the time of writing. As bacterial resistance may develop towards these therapeutic agents, and as other antibiotics are developed, the prescribed indications of these agents may change. Antibacterial prescribing worldwide is a dynamic process due to the emergence of resistance, and consequently some drugs have remained in clinical use, while others have 'limited' use.

In most developed countries in the world, the use of antibiotics is second to analgesic use, and with such extensive use, antibacterial resistance has inevitably become a major global concern. Rational prescribing of antibiotics is a key target for the World Health Organization, which endeavours to limit the use of antibiotics in an attempt to reduce the incidence of drug resistance. Despite these attempts, it is the nature of bacteria that resistance will inevitably occur to some agents, and this should prompt the further development of new antibiotics by the pharmaceutical industry. If we revisited the topic of antibiotic use, development, and mechanisms of action in 10–20 years (this is not to be taken as a hint as to when we might revise this book), we would hopefully find that several new drugs had been developed, while some of the classes with which we are familiar would have disappeared. Perhaps the clinical picture would appear to be similar, but drug treatments would probably have changed.

Section 1

Introduction to Microorganisms and Antibacterial Chemotherapy

Chapter 1.1

Microorganisms

Key Points

- Bacteria can be classified according to their staining by the Gram stain (Gram positive, Gram negative, and mycobacteria) and their shape.
- Most bacterial (prokaryotic) cells differ from mammalian (eukaryotic) cells in that they have a cell wall *and* cell membrane, have no nucleus or organelles, and have different biochemistry.
- Bacteria can be identified by microscopy, or by using chromogenic (or fluorogenic) media or molecular diagnostic methods (e.g. real-time polymerase chain reaction (PCR)).
- Bacterial resistance to an antibacterial agent can occur as the result of alterations to a target enzyme or protein, alterations to the drug structure, and alterations to an efflux pump or porin.
- Antibiotic stewardship programmes are designed to optimise antimicrobial prescribing in order to improve individual patient care and slow the spread of antimicrobial resistance.

1.1.1 Classification

There are two basic cell types: prokaryotes and eukaryotes, with prokaryotes predating the more complex eukaryotes on earth by billions of years. Bacteria are prokaryotes, while plants, animals, and fungi (including yeasts) are eukaryotes. For our purposes in the remainder of this book, we will further subdivide bacteria into Gram positive, Gram negative, and mycobacteria (we will discuss prokaryotic cell shapes a little later).

As you are probably already aware, we can use the Gram stain to distinguish between groups of bacteria, with Gram positive being stained dark purple or violet when treated

with Gentian violet then iodine/potassium iodide (Figures 1.1.1 and 1.1.2). Gram negative bacteria do not retain the dark purple stain, but can be visualised by a counterstain (usually eosin or fuschin, both of which are red), which does not affect the Gram positive cells. Mycobacteria do not retain either the Gram stain or the counterstain and so must be visualised using other staining methods. Hans Christian Joachim Gram developed this staining technique in 1884, while trying to develop a new method for the visualisation of bacteria in the sputum of patients with pneumonia, but the mechanism of staining, and how it is related to the nature of the cell envelopes in these different classes of bacteria, is still unclear.

Figure 1.1.1 Dyes used in the Gram stain

Figure 1.1.2 Example of a Gram stain showing **Gram positive** (*Streptococcus pneumoniae*) and **Gram negative** bacteria (Image courtesy of Public Health Image Library, Image ID 2896, Online, [http://phil.cdc.gov/phil/home.asp, last accessed 26th March 2012].)
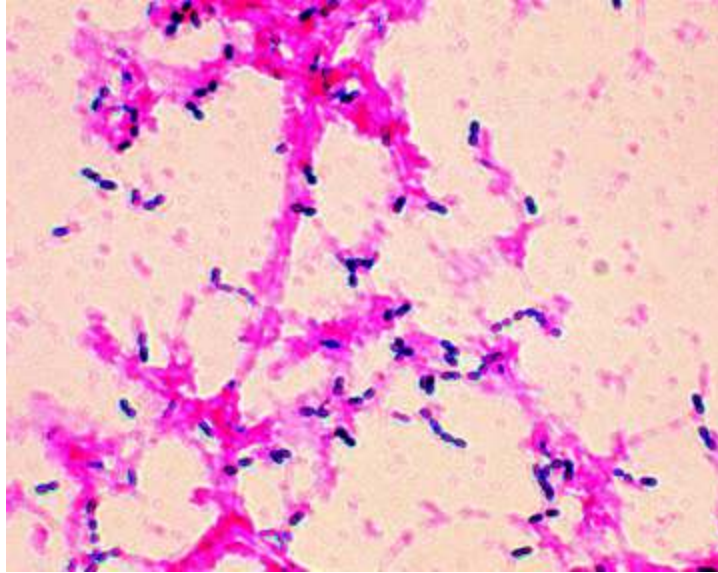
Some of the Gram positive and Gram negative bacteria, as well as some mycobacteria, which we shall encounter throughout this book, are listed in Table 1.1.1.

Table 1.1.1 Examples of Gram positive and Gram negative bacteria, and mycobacteria.

Gram positive	Gram negative	Mycobacteria
Bacillus subtilis	*Burkholderia cenocepacia*	*Mycobacterium africanum*
Enterococcus faecalis	*Citrobacter freundii*	*Mycobacterium avium* complex (MAC)
Enterococcus faecium	*Enterobacter cloacae*	*Mycobacterium bovis*
Staphylococcus epidermis	*Escherichia coli*	*Mycobacterium leprae*
Staphylococcus aureus	*Morganella morganii*	*Mycobacterium tuberculosis*
Meticillin-resistant *Staphylococcus aureus* (MRSA)	*Pseudomonas aeruginosa*	
Streptococcus pyogenes	*Salmonella typhimurium*	
Listeria monocytogenes	*Yersinia enterocolitica*	

1.1.2 Structure

The ultimate aim of all antibacterial drugs is selective toxicity – the killing of pathogenic[1] bacteria (bactericidal agents) or the inhibition of their growth and multiplication (bacteriostatic agents), without affecting the cells of the host. In order to understand how antibacterial agents can achieve this desired selectivity, we must first understand the differences between bacterial (prokaryote) and mammalian (eukaryote) cells.

The name 'prokaryote' means 'pre-nucleus', while eukaryote cells possess a true nucleus, so one of the major differences between bacterial (prokaryotic) and mammalian (eukaryotic) cells is the presence of a defined nucleus (containing the genetic information) in mammalian cells, and the absence of such a nucleus in bacterial cells. Except for ribosomes, prokaryotic cells also lack the other cytoplasmic organelles which are present in eukaryotic cells, with the function of these organelles usually being performed at the bacterial cell membrane.

A schematic diagram of a bacterial cell is given in Figure 1.1.3, showing the main features of the cells and the main targets for antibacterial agents. As eukaryotic cells are much more complex, we will not include a schematic diagram for them here, and will simply list the major differences between the two basic cell types:

- Bacteria have a cell wall and plasma membrane (the cell wall protects the bacteria from differences in osmotic pressure and prevents swelling and bursting due to the flow of water into the cell, which would occur as a result of the high intracellular salt concentration). The plasma membrane surrounds the cytoplasm and between it and the cell wall is the periplasmic space. Surrounding the cell wall, there is often a capsule (there is also an outer membrane layer in Gram negative bacteria). Mammalian eukaryotic cells only have a cell membrane, whereas the eukaryotic cells of plants and fungi also have cell walls.

- Bacterial cells do not have defined nuclei (in bacteria the DNA is present as a circular double-stranded coil in a region called the 'nucleoid', as well as in circular DNA plasmids), are relatively simple, and do not contain organelles, whereas eukaryotic cells have nuclei containing the genetic information, are complex, and contain organelles,[2] such as lysosomes.
- The biochemistry of bacterial cells is very different to that of eukaryotic cells. For example, bacteria synthesise their own folic acid (vitamin B9), which is used in the generation of the enzyme co-factors required in the biosynthesis of the DNA bases, while mammalian cells are incapable of folic acid synthesis and mammals must acquire this vitamin from their diet.

Figure 1.1.3 Simplified representation of a prokaryotic cell, showing a cross-section through the layers surrounding the cytoplasm and some of the potential targets for antibacterial agents
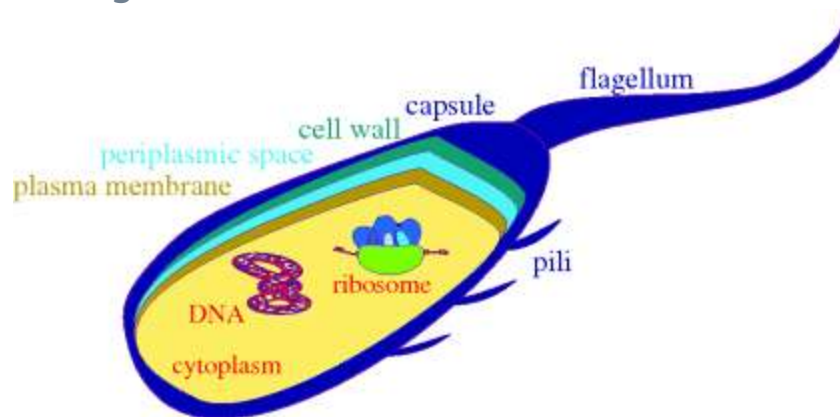
Whenever we discuss the mode of action of a drug, we will be focussing on the basis of any selectivity. As you will see from the section headings, we have classified antibacterial agents into those which target DNA (Section 2), metabolic processes (Section 3), protein synthesis (Section 4), and cell-wall synthesis (Section 5). In some cases, the reasons for antibacterial selectivity are obvious, for example mammalian eukaryotic cells do not have a peptidoglycan-

based cell wall, so the agents we will discuss in Section 5 (which target bacterial cell-wall synthesis) should have no effect on mammalian cells. In other cases, however, the basis for selectivity is not as obvious, for example agents targeting protein synthesis act upon a process which is common to both prokaryotic and eukaryotic cells, so that in these cases selective toxicity towards the bacterial cells must be the result of a more subtle difference between the ribosomal processes in these cells.

   We will now look at these antibacterial targets in detail, in preparation for our in-depth study of the modes of action of antibacterial agents and bacterial resistance in the remaining sections.

1.1.3 Antibacterial targets

1.1.3.1 DNA Replication

DNA replication is a complex process, during which the two strands of the double helix separate and each strand acts as a template for the synthesis of complementary DNA strands. This process occurs at multiple, specific locations (origins) along the DNA strand, with each region of new DNA synthesis involving many proteins (shown in italics below), which catalyse the individual steps involved in this process (Figure 1.1.4):

- The separation of the two strands at the origin to give a replication fork (*DNA helicase*).
- The synthesis and binding of a short **primer** DNA strand (*DNA primase*).
- DNA synthesis, in which the base (A, T, C, or G) that is complementary to that in the primer sequence is added to the growing chain, as its triphosphate; this process is continued along the template strand, with the new base

always being added to the 3′-end of the growing chain (*DNA polymerase*) in the leading strand.
- The meeting and termination of replication forks.
- The proofreading and error-checking process to ensure the new DNA strand's fidelity; that is, that this strand (red) is exactly complementary to the template (black) strand (*DNA polymerase and endonucleases*).

Figure 1.1.4 DNA replication fork (adapted from http://commons.wikimedia.org/wiki/File:DNA_replication_en.svg, last accessed 7 March 2012.)
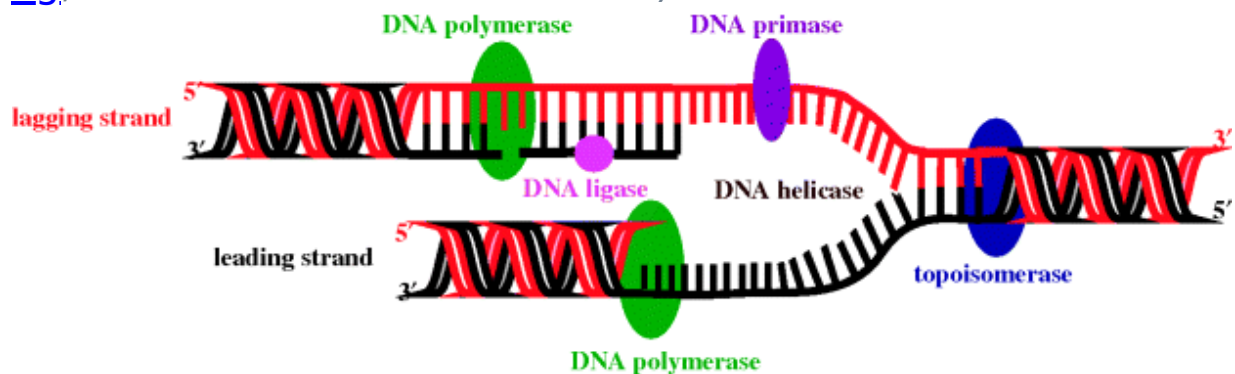
Due to the antiparallel nature of DNA, synthesis of the strand that is complementary (black) to the lagging strand (red) must occur in the opposite direction, and this is more complex than the process which takes place in the leading strand.

DNA helicase is the enzyme which separates the DNA strands and in so doing, as a result of the right-handed helical nature of DNA, produces positive supercoils (knots) ahead of the replication site. In order for DNA replication to proceed, these supercoils must be removed by enzymes (known as topoisomerases) relaxing the chain. By catalysing the formation of negative supercoils, through the cutting of the DNA chain(s) and the passing of one strand through the other, these enzymes remove the positive supercoils and give a tension-free DNA double helix so that the replication process can continue. Type I topoisomerases relax DNA by cutting one of the DNA strands, while, you've guessed it,
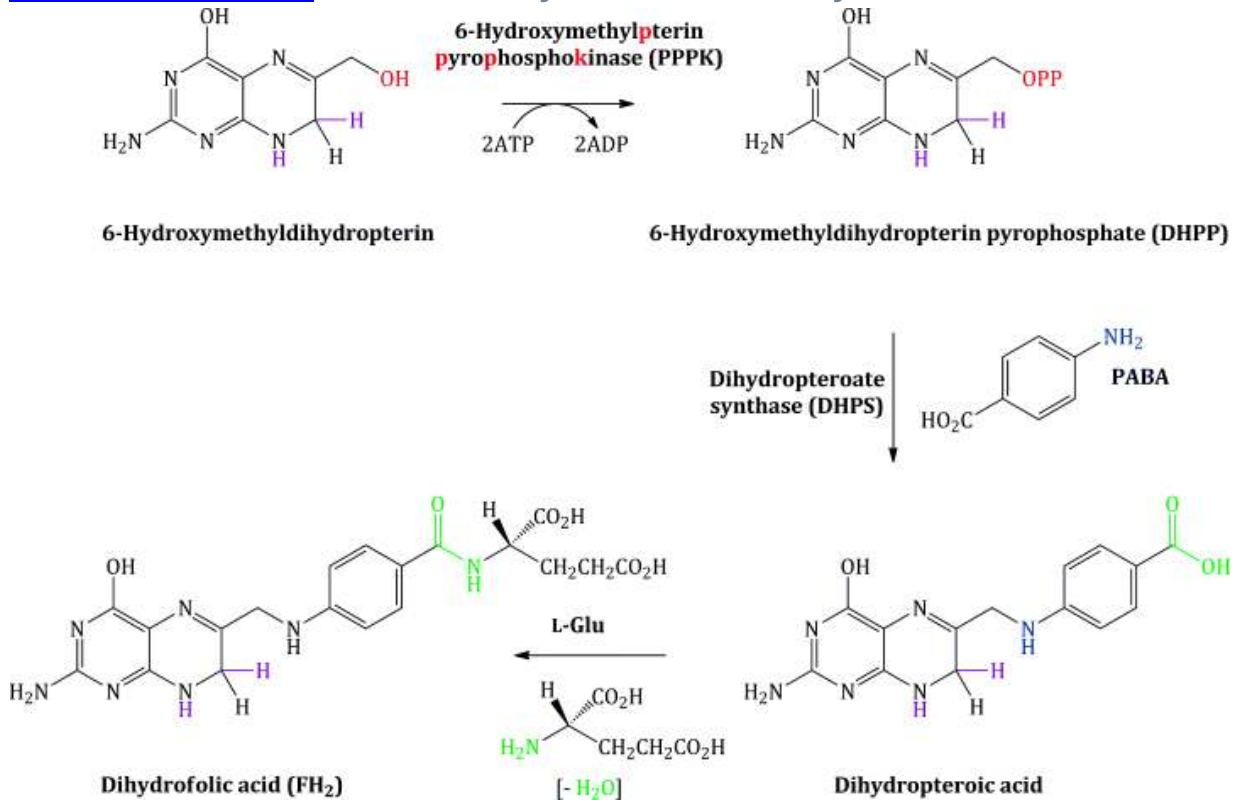
type II cut both strands (Champoux, 2001). In Section 2.1 we will look at a class of drugs which target the topoisomerases: the quinolone antibacterials, which, as DNA replication is obviously common to both prokaryotes and eukaryotes, must act on some difference in the DNA relaxation process between these cells.

1.1.3.2 Metabolic Processes (Folic Acid Synthesis)

As mentioned above, metabolic processes represent a key difference between prokaryotic and eukaryotic cells and an example of this is illustrated by the fact that bacteria require *para*-aminobenzoic acid (PABA), an essential metabolite, for the synthesis of folic acid. Bacteria lack the protein required for folate uptake from their environment, whereas folic acid is an essential metabolite for mammals (as it cannot be synthesised by mammalian cells and must therefore be obtained from the mammalian diet). Folic acid is indirectly involved in DNA synthesis, as the enzyme co-factors which are required for the synthesis of the purine and pyrimidine bases of DNA are derivatives of folic acid. If the synthesis of folic acid is inhibited, the cellular supply of these co-factors will be diminished and DNA synthesis will be prevented.

  Bacterial synthesis of folic acid (actually dihydrofolic acid[3]) involves a number of steps, with the key steps shown in Schemes 1.1.1 and 1.1.2. A nucleophilic substitution is initially involved, in which the free amino group of PABA substitutes for the pyrophosphate group (OPP) introduced on to 6-hydroxymethylpterin by the enzyme 6-hydroxymethylpterinpyrophosphokinase (PPPK). In the next step, amide formation takes place between the free amino group of L-glutamic acid and the carboxylic acid group derived from PABA (Achari *et al.*, 1997).

Scheme 1.1.1 Bacterial synthesis of dihydrofolic acid

6-Hydroxymethyldihydropterin → 6-Hydroxymethylpterin pyrophosphokinase (PPPK), 2ATP 2ADP → 6-Hydroxymethyldihydropterin pyrophosphate (DHPP)

Dihydropteroate synthase (DHPS), PABA

Dihydrofolic acid (FH₂) ← L-Glu, [- H₂O] ← Dihydropteroic acid

Scheme 1.1.2 Formation of the tetrahydrofolate enzyme co-factors

Dihydrofolic acid (FH₂) → NADPH NADP⁺, DHFR → Tetrahydrofolic acid (FH₄)

serine → glycine

Formate (HCO₂⁻) / Serine, Glycine

N^{10}-Formyltetrahydrofolic acid (N^{10}-CHO-FH₄)

N^5,N^{10}-Methylenetetrahydrofolic acid (N^5,N^{10}-CH₂-FH₄)
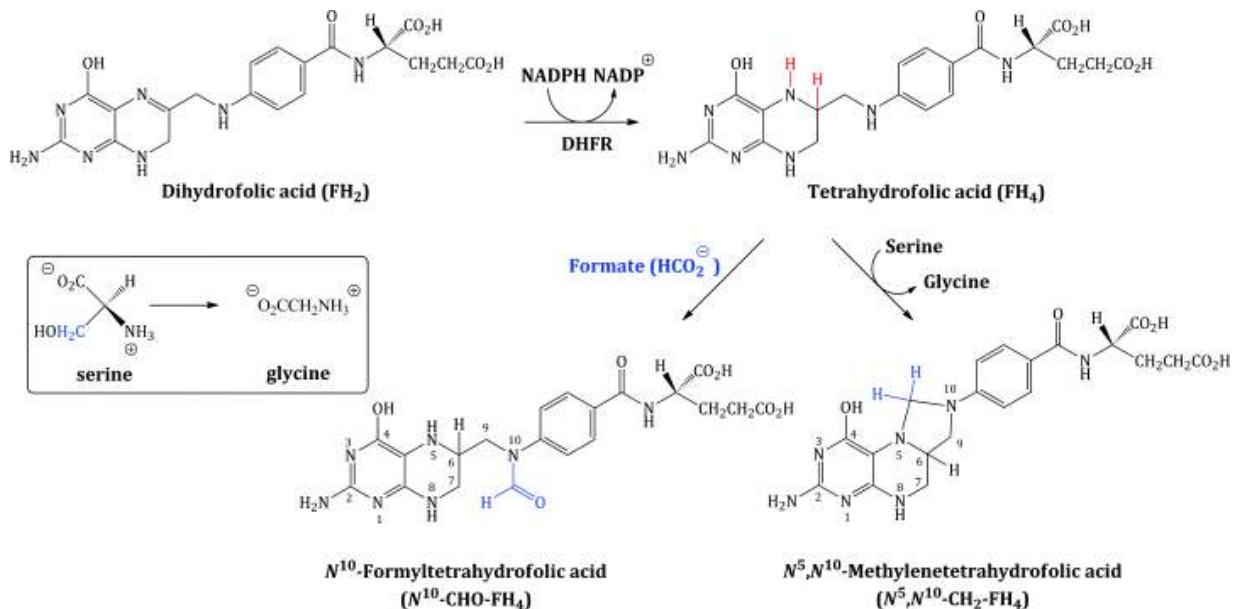
Dihydrofolic acid (FH₂) is further reduced to tetrahydrofolic acid (FH₄), a step which is catalysed by the enzyme

dihydrofolate reductase (DHFR), and FH_4 is then converted into the enzyme co-factors N^5,N^{10}-methylenetetrahydrofolic acid (N^5,N^{10}-CH_2-FH_4) and N^{10}-formyltetrahydrofolic acid (N^{10}-CHO-FH_4) (Scheme 1.1.2).

The tetrahydrofolate enzyme co-factors are the donors of one-carbon fragments in the biosynthesis of the DNA bases. Crucially, each time these co-factors donate a C-1 fragment, they are converted back to dihydrofolic acid, which, in an efficient cell cycle, is reduced to FH_4, from which the co-factors are regenerated. For example, in the biosynthesis of deoxythymidine monophosphate (from deoxyuridine monophosphate), the enzyme thymidylate synthetase utilises N^5,N^{10}-CH_2-FH_4 as the source of the methyl group introduced on to the pyrimidine ring (Scheme 1.1.3).

Scheme 1.1.3 Biosynthesis of deoxythymidine monophosphate (dTMP)

Deoxyuridine monophosphate (dUMP)

Deoxythymidine monophosphate (dTMP)
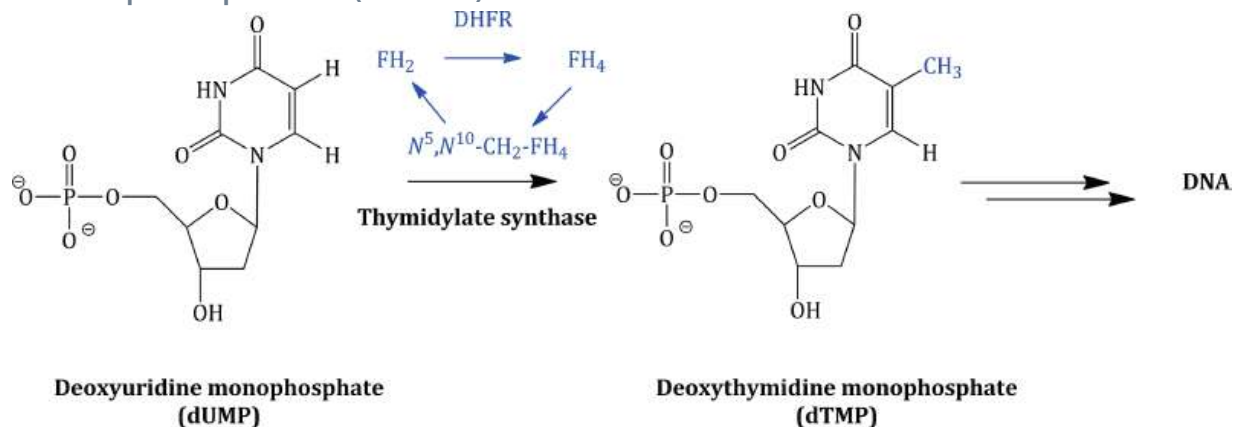
Similarly, N^{10}-CHO-FH_4 serves as the source of a formyl group in the biosynthesis of the purines and, onceagain, is converted to dihydrofolic acid (which must be converted to tetrahydrofolic acid and then N^{10}-CHO-FH_4, again in a cyclic process).

Cells which are proliferating thus need to continually regenerate these enzyme co-factors due to their increased requirement for the DNA bases. If a drug interferes with any step in the formation of these co-factors then their cellular levels will be depleted and DNA replication, and so cell proliferation, will be halted. In Section 3 we will look more closely at drugs which target these processes: the sulfonamides (which interfere with dihydrofolic acid synthesis) and trimethoprim (a DHFR inhibitor).

1.1.3.3 Protein Synthesis

Protein synthesis, like DNA replication, is a truly awe-inspiring process, involving:

- Transcription – the transfer of the genetic information from DNA to messenger RNA (mRNA).
- Translation – mRNA carries the genetic code to the cytoplasm, where it acts as the template for protein synthesis on a **ribosome**, with the bases complementary to those on the mRNA being carried by transfer RNA (tRNA).
- Post-translational modification – chemical modification of amino acid residues.
- Protein folding – formation of the functional 3D structure.

Throughout this process, any error in transcription or translation may result in the inclusion of an incorrect amino acid in the protein (and thus a possible loss of activity), so it is essential that all of the enzymes involved in this process carry out their roles accurately. (For further information on protein synthesis, see Laursen *et al.*, 2005; Steitz, 2008.)

During **transcription**, DNA acts as a template for the synthesis of mRNA ([Figure 1.1.5](#)), a process which is catalysed by DNA-dependent RNA polymerase (RNAP), a nucleotidyl transferase enzyme (Floss and Yu, 2005; Mariani and Maffioli, 2009). In bacteria, the transcription process