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A REVIEW OF ANTIBIOTIC-INDUCED DRUG ALLERGIES: MECHANISMS, PREVALENCE, AND FUTURE PERSPECTIVES

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ABSTRACT
Drug allergies pose a significant public health challenge, contributing to a substantial proportion of adverse drug reactions, particularly among antibiotics. This review explores the mechanisms underlying drug allergies, emphasizing the role of antibiotics, which frequently induce hypersensitivity reactions. Drug allergies are classified into immediate and delayed hypersensitivity types, with immediate reactions mediated by immunoglobulin E (IgE) and delayed responses involving T lymphocytes. A critical aspect of antibiotic-induced allergies is haptenization, where antibiotics interact with host proteins, forming antigenic determinants that trigger immune responses. Recent studies highlight the prevalence of allergic reactions to specific antibiotics, such as amoxicillin and sulfamethoxazole, raising concerns regarding their clinical use and management. The review examines the emerging pharmacologic interaction (p-i) concept, which suggests that certain antibiotics may activate T lymphocytes directly without requiring haptenization. Additionally, the diagnostic challenges associated with distinguishing true allergic reactions from adverse drug effects are discussed, emphasizing the need for more sensitive and specific testing methods. This review aims to synthesize current knowledge on antibiotic-induced allergies, identify gaps in understanding, and propose future research directions. By enhancing our understanding of the immunological mechanisms involved, this work seeks to improve diagnostic and therapeutic strategies, ultimately contributing to better patient safety and care in clinical settings.

KEYWORDS: Drug allergies; Antibiotics; Hypersensitivity; Haptenization; Immunoglobulin E (IgE); Pharmacologic interaction (p-i); Diagnosis; Amoxicillin.

INTRODUCTION
Drug allergies represent a significant public health concern, contributing to a substantial portion of adverse drug reactions.[1] These immune-mediated reactions occur when typically harmless substances trigger hypersensitivity responses in susceptible individuals.[2] The complexity of drug allergies lies in their unpredictable nature, which can lead to serious and sometimes fatal outcomes, including anaphylaxis.[3] This review aims to explore the mechanisms underlying drug allergies, particularly focusing on the role of antibiotics, which are among the most frequently implicated agents.[4]

The classification of drug allergies into distinct types—ranging from immediate hypersensitivity reactions mediated by immunoglobulin E (IgE) to delayed-type hypersensitivity reactions mediated by T lymphocytes—highlights the intricate interplay between the immune system and pharmacological agents.[5] The immediate hypersensitivity reactions often manifest as skin rashes, urticaria, or respiratory distress, while delayed reactions may present with more complex symptoms, including systemic involvement and organ dysfunction.[6]

A notable aspect of antibiotic-induced hypersensitivity is the phenomenon of haptenization, where the antibiotic interacts with host proteins to form antigenic determinants.[7] This process can lead to the production of specific antibodies and sensitization of T lymphocytes, resulting in a heightened immune response upon subsequent exposures.[8] The understanding of haptenization is crucial, as it elucidates how certain antibiotics, such as penicillins and sulfonamides, can provoke allergic reactions in genetically predisposed individuals.[9]

Recent studies have shown that the prevalence of allergic reactions to antibiotics varies significantly, with certain agents exhibiting a higher incidence of hypersensitivity.[10] For instance, amoxicillin and sulfamethoxazole have been identified as common culprits in allergic reactions, raising concerns regarding their widespread use.[11] The incidence of these reactions not only impacts patient safety but also complicates clinical management, as alternatives may not be as effective or available.[12]

This review will delve into the various mechanisms of antibiotic-induced allergies, examining the role of pharmacological interactions with immune receptors.[13] The emerging pharmacologic interaction (p-i) concept posits that some antibiotics may activate T lymphocytes directly without undergoing haptenization, thereby bypassing traditional metabolic pathways.[14] This concept has significant implications for our understanding of drug allergies and highlights the need for further research to elucidate the underlying mechanisms.[15]

Additionally, we will explore the diagnostic challenges associated with drug allergies, particularly in distinguishing between true allergic reactions and adverse drug effects.[16] The limitations of current diagnostic methods underscore the necessity for the development of more sensitive and specific tests to identify allergic responses to antibiotics.[17]

The field of drug allergy research, particularly concerning antibiotics, is rapidly evolving.[18] A deeper understanding of the immunological mechanisms involved, along with improved diagnostic and therapeutic strategies, is essential for enhancing patient care and safety.[19] This review aims to synthesize current knowledge and identify gaps in understanding antibiotic allergies, ultimately contributing to better management practices in clinical settings.[20]

I. General information on drug allergies

Drug allergies are immune reactions triggered by a typically harmless active ingredient. Allergies are characterized as hypersensitivity reactions initiated by immunological mechanisms. Hypersensitivity itself is defined as causing reproducible clinical symptoms or signs that occur upon exposure to a defined stimulus at a dose tolerated by normal subjects.[21]

Allergies account for approximately one-third of adverse drug reactions.[22] The incidence of allergic reactions ranges from 1.8 to 4.2 per thousand hospitalizations, as reported in a review that consolidated studies on allergy incidence and prevalence from 1983 to 2008. Incidence refers solely to newly identified cases during a specified period, whereas prevalence encompasses all existing cases, including both new and previously identified instances.[23]

Drug allergies cannot be predicted, presenting a significant societal challenge due to the severity of some reactions, which can result in patient mortality.[24] Symptoms of drug allergies may include skin rashes and urticaria, digestive disturbances (such as vomiting and diarrhea), respiratory issues (like cough and sneezing), eye irritations, or edema, with cutaneous reactions being the most common. In cases of exacerbation, characterized by mucosal involvement (e.g., exanthema, urticaria, edema) or cardiac rhythm disturbances, potentially leading to a drop in blood pressure and collapse, the term "anaphylactic shock" is employed.[25] Depending on its severity, this condition can lead to death due to circulatory and/or respiratory failure.[26]

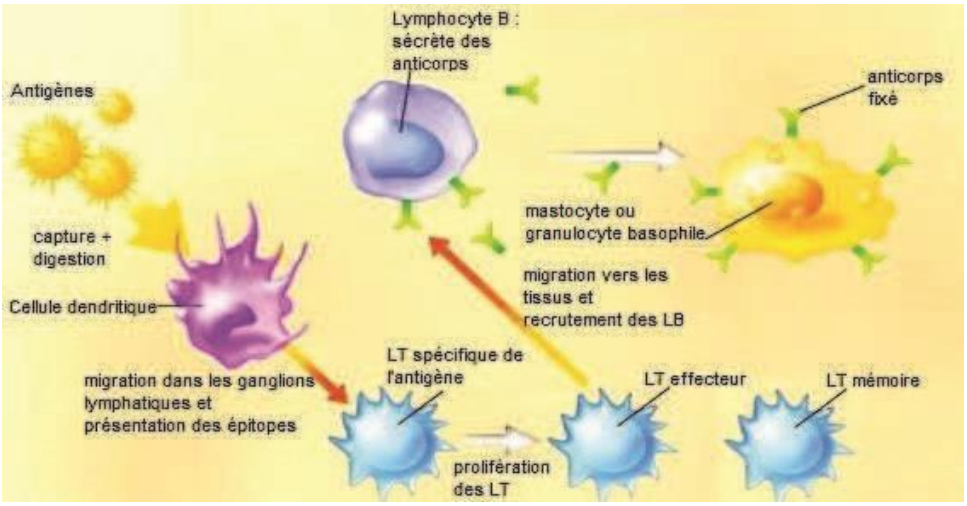
More rarely, certain allergic reactions manifest as potentially fatal syndromes, such as Lyell's syndrome, Stevens-Johnson syndrome, or drug reaction with eosinophilia and systemic symptoms (DRESS).[27] Lyell's and Stevens-Johnson syndromes represent epidermal necrolysis, differentiated by severity: Stevens-Johnson syndrome involves less than 10% detachment of the epidermis, while Lyell's syndrome involves over 30%.[28] DRESS syndrome begins with fever, malaise, edema, and skin eruptions, followed by eosinophilia and various hematological, hepatic, and renal abnormalities.[29] The mortality rate for DRESS is approximately 10%, largely due to the challenges associated with its diagnosis.[30]

In 1963, two English immunologists categorized allergic hypersensitivities into four main groups (I, II, III, and IV) based on the mechanism of action and the time course of the response, ranging from immediate for Type I to 2 to 14 days for Type IV. Types I, II, and III are mediated by humoral mechanisms, involving B lymphocytes and the antibodies they produce.[31] In contrast, Type IV hypersensitivity is mediated by T lymphocytes specific to the drug. Types II and III are clinically rarer.[32] It is common to encounter allergic mechanisms that combine two types of hypersensitivity, such as Types I and IV.[33]

The mechanism of humoral allergy involves antibodies, particularly immunoglobulin E (IgE) for Type I and immunoglobulin G (IgG) for Types II and III. Any allergic response begins with a sensitization phase during the initial contact of antigen-specific lymphocytes (in this case, the drug) with the antigen.[34] This phase is clinically silent, and it is only upon subsequent exposure to the same antigen (the elicitation phase) that allergic symptoms manifest. For hypersensitivities of Types I, II, and III, the initial phase involves the activation of antigen-specific T lymphocytes and the production of specific immunoglobulins E or G by B lymphocytes (which differentiate into plasma cells), along with the proliferation of memory T lymphocytes.[35]

In contrast, Type IV hypersensitivities are mediated by cellular mechanisms, meaning antibodies do not play a role in this process.[36] During the initiation phase, the

immune system first detects a threat and captures the responsible antigens through antigen-presenting cells, such as dendritic cells.[37] Following maturation, these cells migrate to lymph nodes, where they digest the antigen and activate antigen-specific T lymphocytes. These T lymphocytes are referred to as naïve, indicating that they have never encountered the specific antigen. After processing the antigen, the dendritic cell presents a fragment known as an epitope to the T lymphocytes.[38] This presentation occurs via a major histocompatibility complex (MHC) molecule; in humans, this is referred to as human leukocyte antigen (HLA).[39] Antigen-specific T lymphocytes recognize the epitope through their T cell receptor (TCR).[40] This recognition triggers lymphocyte proliferation, leading to the differentiation of two types of lymphocytes: effector cells, which migrate to peripheral tissues, and memory T cells, which are stored in lymph nodes and peripheral sites, becoming active during the elicitation phase **Schema 1**.



Schema 1: General principle of the initiation phase of an allergic reaction.

Upon re-exposure to the antigen, a similar capture mechanism occurs, but this time the dendritic cell presents the epitope to a memory T lymphocyte rather than a naïve one. These memory T cells differentiate into effector T lymphocytes and migrate to peripheral tissues to initiate an immune response against the antigen.[41] This response is clinically observable due to the increased number of memory T lymphocytes compared to the naïve T lymphocytes present during the initiation phase, as well as a deficiency in regulatory T lymphocytes.[42] To trigger the immune response, the effector T lymphocyte secretes cytokines that recruit various effector cells depending on the type of hypersensitivity. Our study focuses specifically on immediate hypersensitivities (Type I).[43] The IgE antibodies specific to the antibiotic, synthesized during the initial encounter, bind to the surface of mast cells and basophils.[44] These two types of white blood cells are found in tissues and blood, respectively.[45] While they are nonspecific, they are considered sensitized when specific IgE antibodies are bound to their surface.[46] Upon encountering the antigen, these cells have the capacity to degranulate, releasing chemical mediators contained in granules within the cytoplasm of mast cells and basophils.[47] The released mediators include vasoactive amines such as histamine, enzymes like tryptase, lipid mediators, and various cytokines **Figure1**.

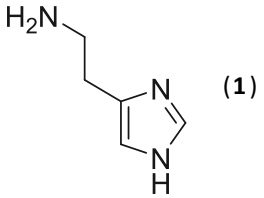


Figure 1: Chemical structure of histamine (1).

Histamine is largely responsible for allergic symptoms such as urticaria, conjunctivitis, and allergic rhinitis.[48] It binds to histamine receptors, particularly those present on the endothelial cells of blood vessels and smooth muscle cells, leading to vasodilation and bronchoconstriction, respectively.[49] This effect is similarly observed with lipid mediators released, such as prostaglandins and leukotrienes. When released in excessive amounts, histamine can cause hypotension and anaphylactic shock.[50] Cytokines play a crucial role in stimulating the recruitment of leukocytes responsible for the later phase of the allergic reaction, which occurs 6 to 24 hours after the second exposure to the antigen. This phase can lead to tissue damage and significant mucus secretion in the respiratory tract.[51]

The hapten theory

The concept of hapten was introduced in 1935. A hapten is an active principle (antigenic molecule) with a molecular weight of less than 1,000 Da. While it can be recognized by the immune system, it cannot independently provoke a response. To be detected by

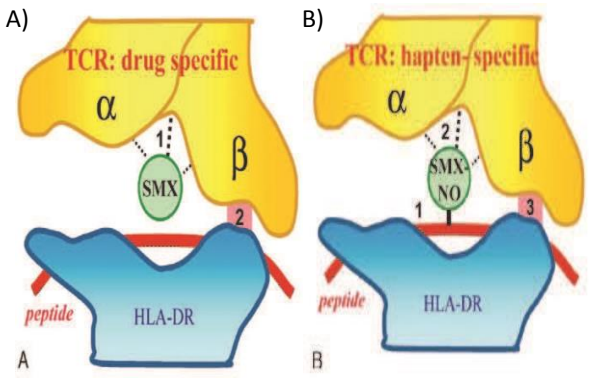
dendritic cells, a hapten must be conjugated to a carrier protein, either cellular or serum-based, such as human serum albumin (HSA). The carrier protein is said to be haptenized by the drug.[52] A pro-hapten refers to an active principle that undergoes enzymatic modification (in situ metabolism of the antigenic molecule) before covalently binding to the carrier protein.[53] In contrast, a pre-hapten undergoes a non-enzymatic (chemical) modification. Immunogenic antigens detected by the immune system are typically proteins that have been covalently modified by an active principle or one of its metabolites.[54] A modified protein can be captured by a dendritic cell, which then digests it into several peptide fragments. Some of these peptides will present the drug or an antigenic metabolite, forming hypothetical epitopes.[55] The dendritic cell will present these peptides, including the epitopes, via major histocompatibility complex (MHC) molecules to a T lymphocyte specific to the antibiotic, which recognizes the epitopes through its T cell receptor (TCR).[56]

There are two classes of MHC (I and II) based on the type of T lymphocyte involved:

- When an epitope is presented to CD8+ T lymphocytes, MHC class I is utilized.
- For presentation to CD4+ T lymphocytes, MHC class II is employed.

Regarding penicillins and sulfonamides, the two antibiotic families most commonly associated with allergic reactions, specific CD4+ T lymphocytes have been found in the blood of allergic patients. Previous studies conducted by our collaborators have highlighted the role of CD4+ T lymphocytes in the mechanism of drug allergies. Consequently, the MHC class II molecules on dendritic cells (specifically HLA-DR, DP, or DQ in humans) are particularly important during the presentation to T lymphocytes, and this will be the focus of our investigation.[57]

However, the research team led by Pichler proposed that certain antibiotics might be presented directly to T lymphocyte receptors, inducing an immune response through a mechanism known as the pharmacologic interaction with immune receptors (p-i concept). This concept involves the direct presentation of the drug to T lymphocytes without the need for the haptenization phase followed by digestion by dendritic cells.[57] The molecule binds, through non-covalent interactions, between the TCR and an HLA on a dendritic cell presenting a non-haptenized peptide to a T lymphocyte, leading to its capture and activation. This mechanism has been particularly illustrated with the sulfonamide antibiotic sulfamethoxazole (SMX), which will be defined in the subsequent section **schema2**.



shema 2: Representation of the presentation of the SMX according to the p-i concept (A) compared to the presentation according to the hapten-theoretic theory (B).

Diagnosing an allergic reaction

Aside from severe symptoms, many other signs of drug allergic reactions are often mistaken for potential side effects of the medications involved, complicating the diagnostic process. The most noticeable symptoms are typically cutaneous, which can be confirmed by a dermatologist.[58] If an allergic reaction is suspected, the patient will first undergo a medical interview to assess the types of symptoms experienced. If a general practitioner validates the suspicion of an allergic reaction, the patient is referred to an allergist for skin and/or laboratory testing.[59] Skin tests include prick tests and intradermal tests, which are implemented in cases of immediate hypersensitivity (mediated by IgE). Prick tests involve placing a drop of diluted allergen on the skin of the forearm or back and penetrating the epidermis with a needle.[60] The appearance of a rash validates the allergy to the tested medication, but a negative result does not exclude the possibility of an allergy. Intradermal tests involve injecting the allergen beneath the skin; if the diameter of the resulting papule increases based on certain criteria, the test is considered positive.[60] Again, a negative result does not rule out the possibility of an allergy. These tests should be conducted in specialized facilities due to the risk of anaphylactic shock in some patients. For delayed hypersensitivities, the skin tests used include delayed reading intradermal tests and patch tests. Patch tests allow for prolonged application of the drug (2 to 3 days) on the patient's back. If reactions are observed upon removal by the allergist, the patient is considered allergic. A lack of reaction, however, does not definitively indicate non-allergy.[61]

As a last resort, if all skin tests return negative despite preliminary indications of a potential allergy, or when skin tests are not feasible, a provocation test may be conducted. This test involves administering the suspected allergen via the same route that led to the hypothetical allergic reaction and is only performed for commonly used, irreplaceable medications. This test is significantly more sensitive than skin tests and is regarded as the "gold standard," with a sensitivity of 100%. However, due to its risks, it must be performed in a facility

equipped with resuscitation services. If the suspected allergic symptoms are associated with Stevens-Johnson syndrome, Lyell's syndrome, or DRESS, skin tests cannot be performed. In addition to skin tests, laboratory tests should also be conducted, which involve detecting specific drug antibodies (IgE for Type I). However, these results alone cannot confirm an allergy. Such tests often exhibit low sensitivity, particularly in the case of penicillins. Tests for basophil degranulation are not reliable due to a sensitivity of only 50%, given the low number of circulating basophils. Tests for the release of sulfidoleukotrienes still require validation due to their lack of sensitivity and specificity.[62-65]

In acute clinical reactions, blood levels of histamine, tryptase, and urinary levels of methylhistamine can help clarify the roles of basophils and mast cells, regardless of the cause of degranulation. In vitro tests on T lymphocytes are also performed, as there are numerous specific memory T lymphocytes present in the blood of allergic patients who have previously encountered the allergen. The lymphocyte transformation test (LTT) quantifies the proliferation of specific T lymphocytes, particularly for delayed hypersensitivities to β-lactams, but it is also utilized for sulfonamide allergies. This test is widely used because it is harmless, making it preferable for patients who have experienced severe hypersensitivity reactions. Another test for delayed hypersensitivities to β-lactams involves detecting interferon-γ using the ELISpot (enzyme-linked immunosorbent spot) technique, though this method is not yet widely adopted and remains in the research domain. While many diagnostic tests exist, none are currently ideal. There is a pressing need to develop new tests that meet criteria for selectivity, sensitivity, and ease of use for medical personnel.[64-68]

Treatment of allergies

Currently, there are very few effective treatments for managing allergies. The most commonly employed approach is allergen avoidance, which is not a true treatment but rather a strategy to prevent any contact with the allergen. In cases involving penicillins, avoidance is relatively straightforward, as alternative antibiotics—such as macrolides, tetracyclines, or quinolones—can effectively substitute for penicillin. However, the use of cephalosporins is generally discouraged due to the risk of cross-reactivity. It is well established that other antibiotics tend to be less effective than penicillins and β-lactams overall, and some bacteria can develop resistance to these alternatives. Therefore, it is strongly recommended to conduct diagnostic tests rather than self-diagnosing an allergy based on misinterpretation of a dermatological reaction.[69-73]

When a medication is essential and irreplaceable, symptomatic treatment may be administered prior to taking the drug. However, this treatment addresses only the symptoms of the allergy rather than its underlying cause. There are two primary types of symptomatic treatments: antihistamines and corticosteroids. Antihistamines work by mitigating the effects of histamine; they act as competitive antagonists of H1 histamine receptors. Additionally, there is a specific antihistamine called tritoqualine, which inhibits the enzyme L-histidine decarboxylase, thereby preventing the synthesis of histamine from L-histidine **Figure2**.

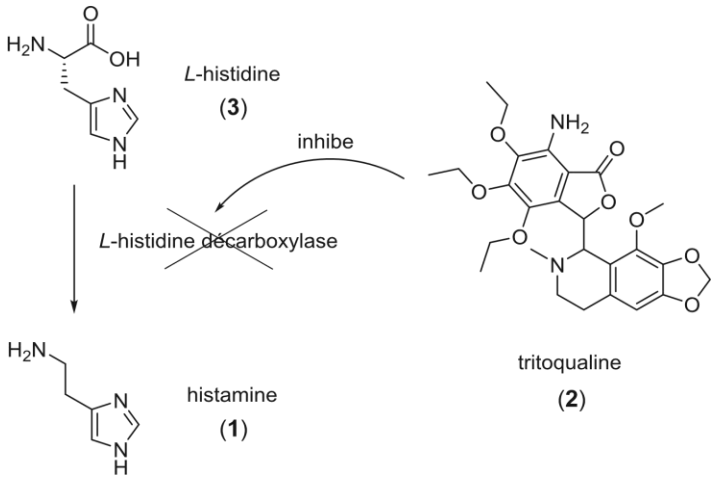


Figure 2: Synthesis of histamine (1) from L-histidine (3) and its inhibition by tritoqualine (2).

Corticosteroids are anti-inflammatory medications administered during severe allergic reactions. It is recommended to use corticosteroids alongside antihistamines, as the latter do not prevent anaphylactic shocks and may even obscure their initial symptoms. However, the combination of corticosteroids and antihistamines has been shown to reduce the incidence of certain allergic reactions.[73]

Desensitization, or specific immunotherapy, targets the immune system by inducing tolerance to the allergen responsible for the symptoms. This treatment involves subcutaneous injections of gradually increasing doses of the allergen, administered at regular intervals over several years. Ongoing advancements in treatment have made options simpler for patients, including sublingual therapies. In the case of penicillin desensitization, oral

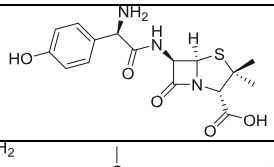
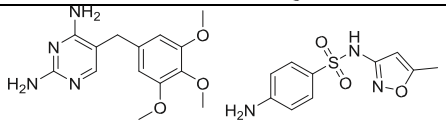
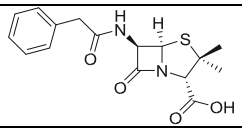
administration is utilized as it significantly reduces the risk of severe reactions. This approach is the only treatment recognized by the World Health Organization as effective and sustainable, offering excellent long-term results by desensitizing the body and preventing allergic responses. Nonetheless, the risk of serious reactions remains, as even a minimal dose of the allergen can pose a threat to the patient's life. Therefore, new treatments must be developed to induce tolerance safely.[74]

In this context, our research aims to identify new molecular tools for developing diagnostic tests and therapeutic desensitization treatments. Our objective is to determine the epitopes that, when presented to specific T lymphocytes, trigger their proliferation and the corresponding immune response. To achieve this, we will first examine the nature of the antigen under study.[75]

II. Antibiotics studied responsible for allergies

The antibiotics examined in this study were selected based on their high prevalence of allergic reactions, as classified by a comprehensive study conducted in 1986, which involved 15,438 hospitalized patients from 1975 to 1982. This classification remains relevant, as evidenced by a review published in 2011 that compiled multiple studies addressing the prevalence and incidence of drug hypersensitivity. Additionally, a publication from 2012 detailed findings from an investigation conducted between 2005 and 2010. In the original study, each allergic skin reaction was validated by a dermatologist and recorded to calculate the prevalence associated with each medication. The overall observed allergic prevalence for antibiotics was found to be 2.2%. This figure was subsequently calculated for each specific drug, revealing that amoxicillin and sulfamethoxazole exhibited the highest rates of allergic reactions table 1.

Table 1: Allergic prevalence of the three drugs studied and their ranking.

Medicine	Structure	Prevalence (%)	Classification
Amoxicilline (**4**)		5,1	1
Trimethoprim (**6**) -sulfaméthoxazole (**5**)		3,4	2
Pénicilline G (**7**)		1,9	10

According to the data, the antibiotic with the highest incidence of allergic skin reactions is amoxicillin, which has a prevalence of 5.1%, corresponding to 63 allergic patients out of 1,225. As the first-line antibiotic, amoxicillin is also the most widely consumed antibiotic in France, with a daily defined dose (DDD) of 10.7 per 1,000 inhabitants. The DDD, as defined by the World Health Organization's Collaborating Centre for Drug Statistics Methodology, allows for the calculation of daily consumption based on the number of units sold relative to the population size.[76]

The combination of trimethoprim and sulfamethoxazole ranks second in causing allergic reactions, with a prevalence of 3.4%, or 36 cases out of 1,066. This combination has a consumption rate of 0.3 DDD per 1,000 inhabitants per day in France, making it one of the less frequently used antibiotics, primarily prescribed for the prevention and treatment of opportunistic infections in HIV-positive patients. Penicillin G is ranked tenth, with a prevalence of 1.9%, accounting for 17 cases out of 918 patients. In France, its consumption is 0.4 DDD per 1,000 inhabitants per day, making it one of the least prescribed penicillins today due to its reduced efficacy compared to amoxicillin against many bacterial strains.[78]

The focus of this study is on penicillins and sulfonamides, given their significant allergic prevalence and clinical importance. To generalize our investigation on penicillins, we have chosen to first study a model β-lactam: penicillin G, which has a more lipophilic structure than amoxicillin. Although it is used less frequently and has a narrower spectrum of action, it still ranks tenth in allergic prevalence (1.9%) according to previous studies. Following the examination of penicillin G, we will proceed with the study of amoxicillin, and finally, we will explore another major group of allergens, the sulfonamides, specifically sulfamethoxazole.[79]

1) Penicillin G

Penicillin G (PG) (**7**), or benzylpenicillin, of natural origin is produced by fungi of the genus *Penicillium* (Figure 3).

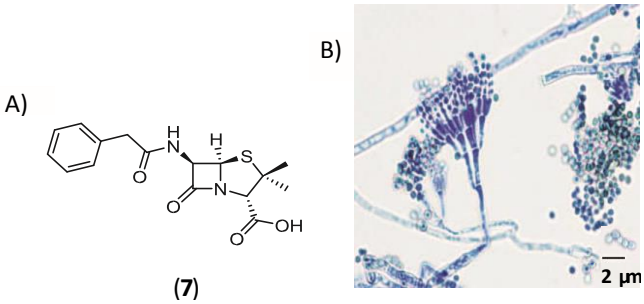


Figure 3: Structure chimique de la pénicilline G (7) (A) photographie d'un champignon *Penicillium chrysogenum* au microscope (www.inspq.qc.ca/compendium-moisissures/penicillium-spp) (B)

a) The history of penicillin

Since ancient times, certain populations have utilized fungi and molds to treat wounds and infections. Beginning in the 1870s, scientists, including Louis Pasteur, began investigating the potential for bacterial growth in the presence of molds.[80]

In 1897, Dr. Ernest Duchesne defended his thesis titled "Contribution to the Study of Vital Competition Among Microorganisms: Antagonism Between Microbes and Molds." He examined the interaction between a fungus he referred to as *Penicillium glaucum* (though the nomenclature of the time suggests it may have involved a different species of *Penicillium*) and *Escherichia coli* bacteria. Although his findings were inconclusive, he acknowledged that his preliminary study required further exploration and could lead to discoveries with practical applications in preventive hygiene and therapeutic contexts.[81]

Penicillin G was accidentally discovered in 1928 by Dr. Alexander Fleming. Upon returning from vacation to his laboratory at St. Mary's Hospital in London, he observed that a mold, *Penicillium notatum*, used by his lab neighbor, had contaminated his Petri dishes containing staphylococcal cultures. Notably, he found that no staphylococci grew where the mold was present. Fleming hypothesized that the mold produced a substance with antimicrobial properties, which he later purified with the help of a chemist and named "penicillin." After a year of studying the antimicrobial effects, stability, and toxicity of penicillin, he published his findings, but it would not be utilized in medicine until the 1940s.

Howard Florey, a pathology professor at Oxford, along with biochemist Ernst Chain, bacteriologist Norman Heatley, and chemist Edward Abraham, successfully produced and purified penicillin for the first time. They documented its bacteriostatic effects and used it to treat infections in mice, rats, and cats.

The progress of their work was interrupted by World War II, but Florey, Chain, and their team persevered, developing therapeutic applications for humans and studying the pharmacokinetics of penicillin. They successfully treated a 15-year-old boy with a suppurating femoral neck, as well as other patients, both adults and children, with eye infections unresponsive to previous treatments, publishing their advancements in 1941. However, widespread production of penicillin was delayed until 1942 in the United States, where another mold, *Penicillium chrysogenum*, was found to produce 200 times more penicillin than *Penicillium notatum*. American laboratories such as Merck, Pfizer, and Squibb then began industrial-scale production and marketing of penicillin as part of a new class of medications known as antibiotics.

Ultimately, in 1945, Fleming, Florey, and Chain were awarded the Nobel Prize in Physiology or Medicine for their discovery of penicillin and its curative effects on numerous infectious diseases.

b) Penicillin production

The syntheses of penicillin G and other natural penicillins were initiated in the 1950s. However, a critical challenge lies in the closure of the β-lactam ring, a tense four-membered structure that preferentially leads to the formation of a more stable five-membered oxazolone ring. This phenomenon was first observed in 1953 by researchers who conducted numerous attempts to close the ring using methyl ester derivatives of penicillin G, employing reagents such as thionyl chloride, phosphorus trichloride, acetyl chloride, and acetic anhydride **Figure4**.

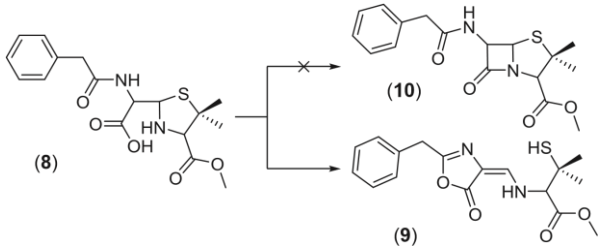


Figure 4: Attempts to close the β-lactam cycle by Sheehan and his collaborators.

They found a method to achieve cyclization, although not directly from the acid derivative of β-methyl benzylpenicilloate. The incorporation of a phthalimide derivative into the side chain of penicillin prevents the formation of the oxazolone ring. However, the yield of cyclization remains low, at only 13%, following the oxidation of sulfur to sulfone by KMnO4 in acetic acid on the corresponding derivative **Figure 5**.

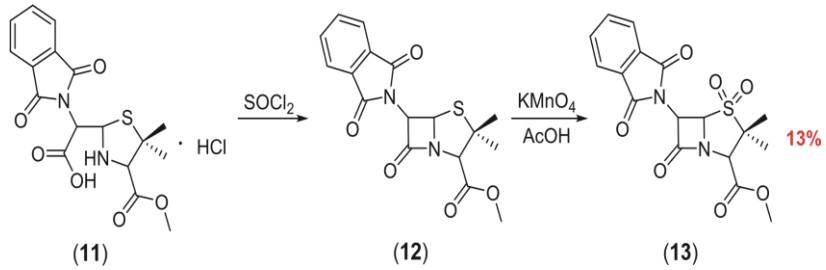


Figure 5: Closure of β-lactam on the phthalimid derivative (11) followed by oxidation of sulphur to sulfone.

Later, in 1955, researchers reported the use of N,N'-dicyclohexylcarbodiimide (DCC) to facilitate the formation of amide bonds between carboxylic acids and amines. They decided to test this amidation method for cyclizing the β-lactam structure in a natural penicillin. In 1957 and again in 1959, they published the total synthesis of a natural penicillin: penicillin V. The tert-butyl phthalimidomalonate reacts with D-penicillamine in the presence of aqueous sodium acetate and ethanol, resulting in the formation of two asymmetric centers and potentially generating four diastereomers. Two diastereomers were observed: one with a yield of 24% and another with a yield of 30%. Although the absolute stereochemistry of these compounds could not be determined, after extensive comparison with literature data, the authors concluded that the product with a yield of 24% exhibited the same stereochemistry as natural penicillin V (2S, 5R, 6R). The diastereomer with a yield of 30% was converted to the desired compound through recrystallization in pyridine, achieving a recrystallization yield of 46%, which provided an additional 14% yield for the desired compound, resulting in a total yield of 38%.

Subsequent deprotection of the phthalimide was carried out using hydrazine followed by treatment with aqueous hydrochloric acid, yielding the primary amine derivative with a good yield of 82%. The researchers noted that from this compound, various penicillins could be synthesized by reacting the primary amine with different acyl chlorides. For the synthesis of penicillin V, phenoxyacetyl chloride was employed in a stoichiometric ratio of one equivalent with two equivalents of triethylamine in dichloromethane. The amidated compound was obtained with a yield of 70%. This compound then underwent deprotection of its tert-butyl ester in an acidic medium, resulting in penicillanic acid quantitatively. This acid was subsequently treated in a basic environment with four equivalents of DCC, yielding penicillin V with a final yield of only 5% **Figure 6**.

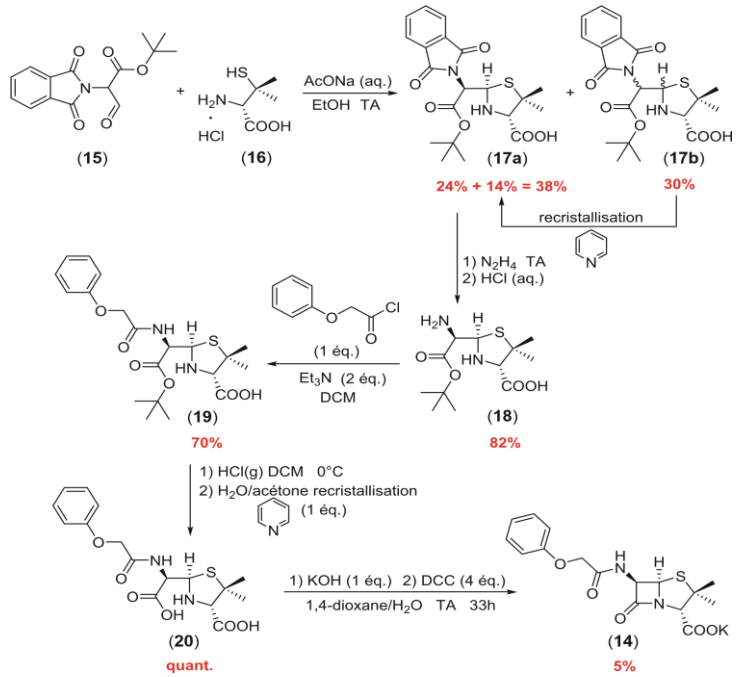


Figure 6: Total synthesis of penicillin V (14).

This total synthesis, comprising five steps, yields the desired product with an overall efficiency of only 1%. The authors indicate that the total synthesis has also been applied to penicillin G; however, it results in a lower cyclization yield compared to the 5% achieved for penicillin V. Research into the synthesis of penicillins has largely ceased, particularly since advancements in mutagenesis have led to the development of fungal strains that produce over 60,000 units/mL in culture within 200 hours, which is more than fifteen thousand times the yield of the original *Penicillium notatum* strain. Numerous patents have been filed regarding the production of penicillin as improvements were made. Today, penicillin G continues to be produced through biotechnological methods.

Bioproduction involving fungi entails the biosynthesis of a tripeptide (ACV) composed of L-aminoadipic acid, L-cysteine, and L-valine, which is epimerized to D-valine during its incorporation into the enzyme L-aminoadipyl-L-cysteinyl-D-valine synthase, known as ACV synthase. This tripeptide subsequently cyclizes to form isopenicillin N. Finally, the acyltransferase catalyzes the substitution of the side chain with another radical, such as phenylacetic acid for penicillin G. This biocatalyzed acylation allows for the use of different precursors in the culture medium to enhance the yield of the desired type of penicillin **Figure 7**.

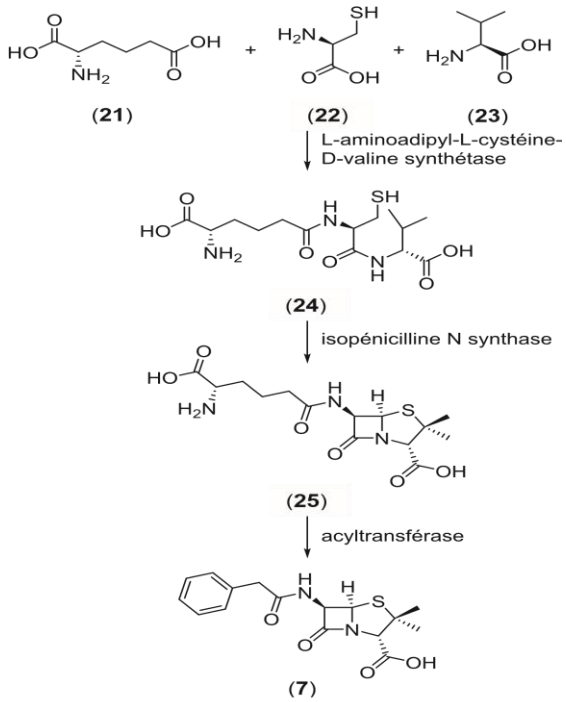


Figure 7: Biosynthesis of penicillin.

Penicillin emerged as a medication produced on an industrial scale even before its chemical structure was fully elucidated and its mechanism of action was entirely understood.

c) Le mode d'action des pénicillines

Penicillins, like all antibiotics, target bacteria. The bacterial cell wall is primarily composed of peptidoglycan, which provides structural integrity and mechanical protection against osmotic pressure and external threats. Peptidoglycan consists of oligosaccharides, specifically a repeating chain of N-acetyl-D-glucosamine (NAG) and N-acetylmuramic acid (NAM), linked by β(1→4) glycosidic bonds **Figure 8**.

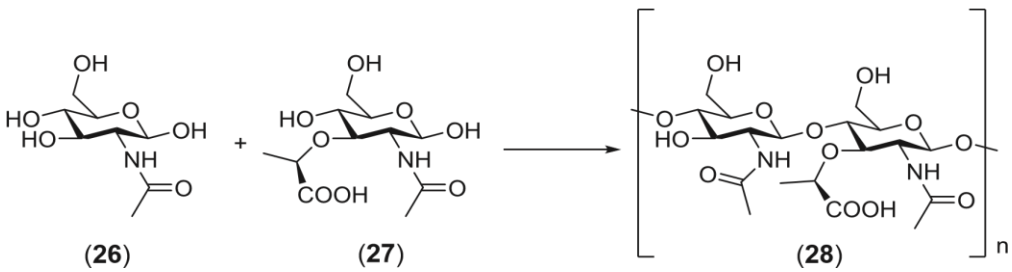


Figure 8: Chemical structure of N-acetyl-D-glucosamine (26) and N-acetylmuramic acid (27) and their sequence (28).

On the carboxylic acid end of certain N-acetylmuramic acid (NAM) molecules, small peptides composed of L-alanine, D-glutamic acid, L-lysine, glycine, or diaminopimelic acid (an analogue of lysine) are attached, ending with two D-alanine residues. The three-dimensional assembly of peptidoglycan to form the bacterial cell wall occurs through the action of an enzyme known as transpeptidase. This enzyme catalyzes the cleavage of the amide bond with the terminal D-alanine residue and facilitates the coupling with a glycine residue from another polypeptide chain. This process is crucial for cross-linking peptidoglycan and providing rigidity to the cell wall **Figure9**.

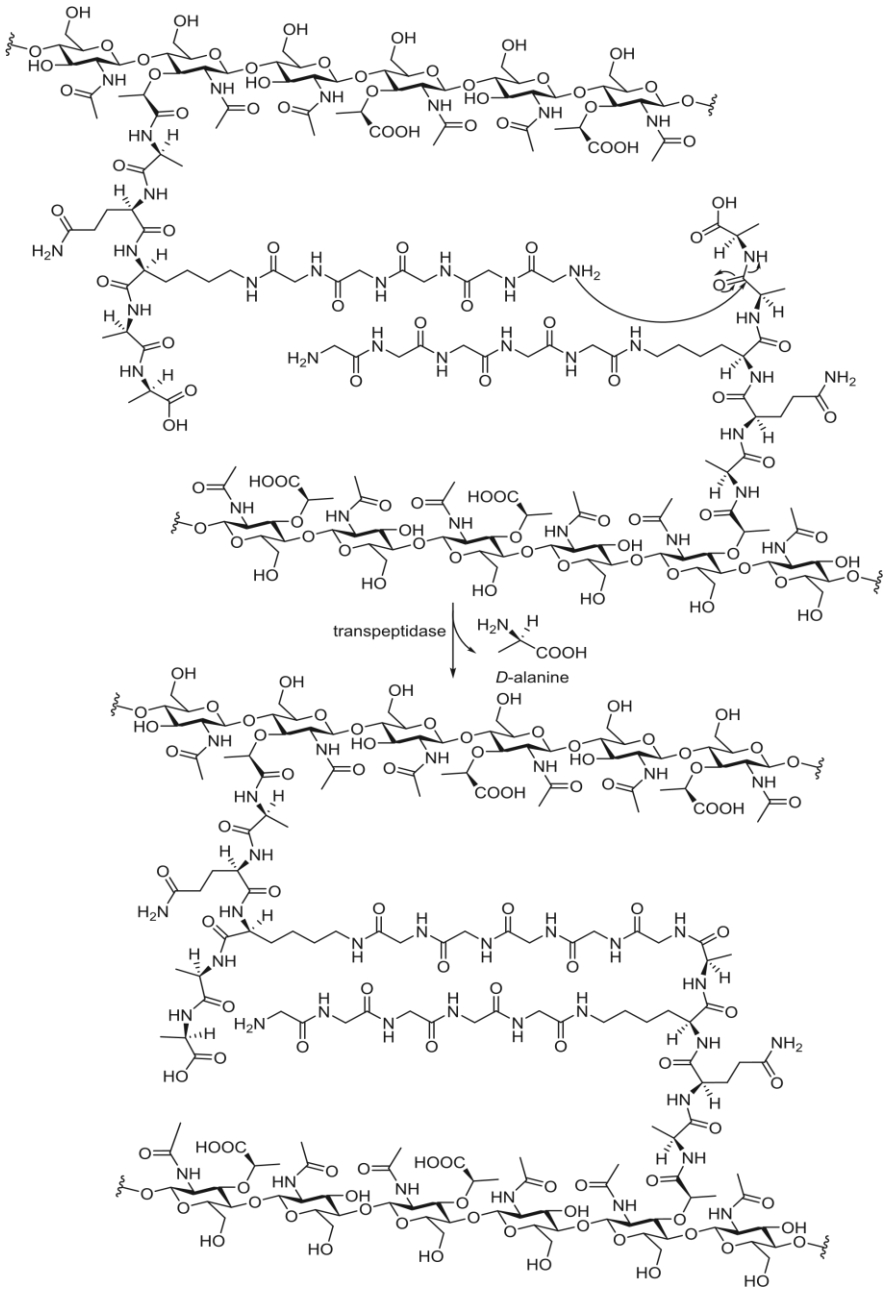


Figure 9: Binding between two peptidoglycan chains by the action of transpeptidase.

The isosteric relationship between the terminal dipeptide D-alanine-D-alanine and penicillin G allows the latter to access the active site of transpeptidase, effectively blocking the enzyme by binding to its serine residues through the opening of its β-lactam core. This interaction inhibits the formation of the bacterial cell wall, preventing cell division and the generation of new bacteria. Consequently, the bacteriostatic effect of penicillins is only active against bacteria that are in the growth phase, rather than those in the stationary phase that have already synthesized their peptidoglycan. The bactericidal effect, which leads to bacterial destruction, arises from the bacteria's own mechanisms; the alteration of their peptidoglycan activates their peptidoglycan hydrolases, also known as autolysins, which induce bacterial lysis **Figure10**.

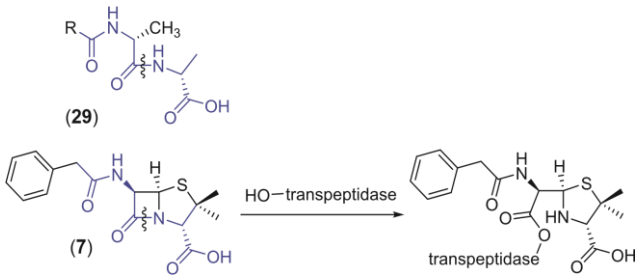


Figure 10: Isosteria between D-alanine-D-alanine (29) and penicillin G (7), followed by its opening by serine residues of the transpeptidase leading to inhibition of the enzyme.

The thickness of the cell wall is significantly greater in Gram-positive bacteria, which have direct contact with the extracellular environment. In contrast, Gram-negative bacteria possess a thinner peptidoglycan layer embedded between two plasma membranes; the inner membrane is primarily phospholipidic, while the outer membrane is predominantly composed of lipopolysaccharides. This structural arrangement reduces the effectiveness of natural β-lactam antibiotics. However, Gram-negative bacteria feature porins in their outer membrane. These porins are channel-forming proteins that facilitate the passage of small hydrophilic molecules across the outer membrane, allowing penicillins to reach the peptidoglycan and transpeptidase. The limited efficacy of penicillin G against Gram-negative bacteria can be attributed to its relatively low hydrophilicity, which hinders its passage through the outer membrane via porins. Nevertheless, it can still diffuse into certain Gram-negative bacteria through the outer membrane, although this process is slow. Following the action of penicillin, Gram-positive bacteria lose their cell membrane and are referred to as protoplasts. In contrast, Gram-negative bacteria cannot completely lose their cell membrane and are therefore termed spheroplasts **Figure11**.

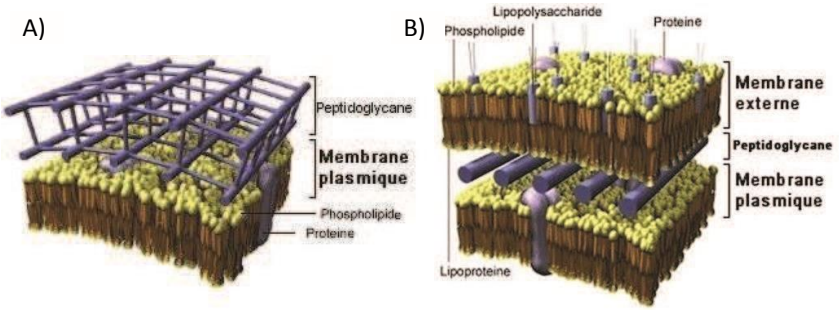


Figure 11: Representation of the wall of a Gram+ (A) bacterium compared to that of a Gram- (B) bacterium.

d) The haptenization mode

Penicillin G, with a molecular weight of 334.39 g/mol, must bind to a protein to be recognized by the immune system and trigger an allergic reaction. As a hapten, the nucleophilic groups of proteins, particularly lysine residues, attach to penicillin through the opening of its β-lactam core.

Compared to a free β-lactam, the bicyclic β-lactam structure of penicillins exhibits enhanced reactivity. This increased reactivity arises from the cyclic strain at the junction of the rings, which prevents the nitrogen atom from adopting a planar geometry, thereby disrupting the electronic delocalization of the amide. In the absence of this delocalization, the carbonyl double bond (C=O) of the lactam function in penicillin is significantly more electrophilic than that of a free β-lactam, facilitating nucleophilic attacks by amino acid residues such as the amine function of lysine. The interaction of a nucleophilic residue with the β-lactam motif results in the formation of a bioconjugate **Figure12**.

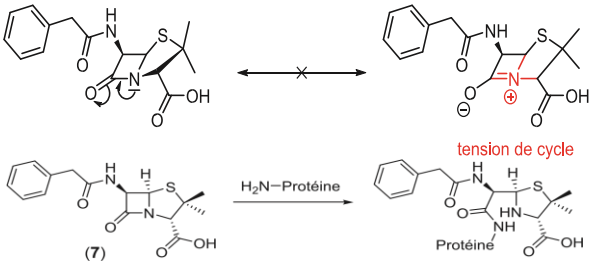


Figure 12: Hapentenization of penicillin G (7) by opening of the β-lactam by the aminoterminal amino acids of a protein.

The open penicilloyl moiety that substitutes the protein serves as the primary antigenic determinant of penicillin G. Additionally, other minor antigenic determinants have been described in the literature, such as benzylpenicilloyl-protein and penicillamine-protein, which result from the reaction of cysteine residues in proteins (including those involved in disulfide bonds) with penicillin G. However, these minor determinants have not been observed in vitro using human proteins or patient sera **Figure13**.

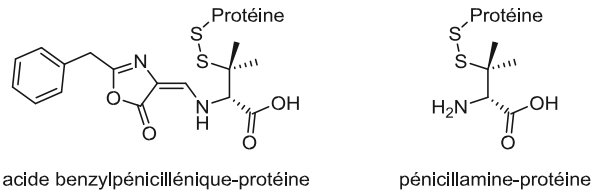


Figure 13: Minor antigenic determinants of penicillin G (7).

These adducts arise from the metabolism of penicillin G. In acidic aqueous environments, such as the stomach, penicillin G is metabolized into penicilloic acid, as well as penicillanic acid **Figure14**. Penicilloic acid rapidly rearranges into penicillinic acid. Additionally, penicillanic acid decomposes into penicillamine **Figure15**.

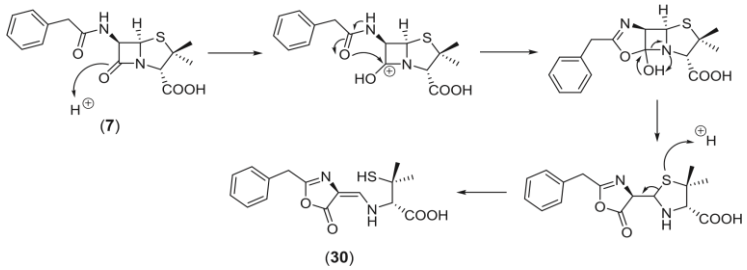


Figure 14: Mechanism of formation of penicillen acid (30) from penicillin G (7) in an acidic medium.

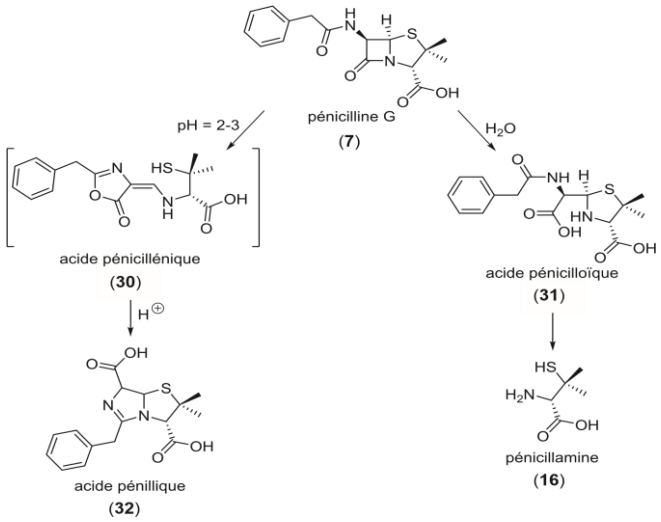


Figure 15: Metabolization of penicillin G (7) in acidic aqueous medium.

Penicilloic acid can also form open adducts with lysine residues in a protein, leading to epimerized compounds at position 6, while position 5 predominantly maintains an R configuration due to steric constraints within the thiazolidine ring. The bioconjugate of the protein with the 5R,6S diastereomer is reported in the literature to be potentially more immunogenic than that derived from its 5R,6R isomer **Figure16**.

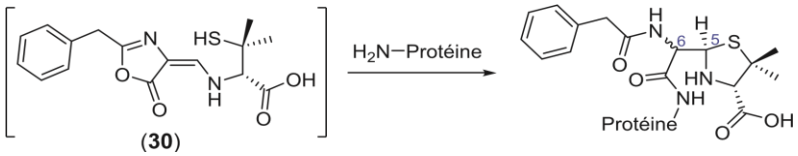


Figure 16: Formation of the major epimerized antigenic determinant by way of penicillen acid (30).

The metabolite penicilloic acid is detected in vivo at very low levels, prompting us to focus this thesis on studying the major antigenic determinant resulting from the opening of the β-lactam ring of penicillin G by lysine residues in proteins.

In summary, the use of penicillin G significantly advanced medicine from its discovery until the 1960s, but its limitations soon became evident. Its antibacterial spectrum is restricted, particularly against Gram-negative bacteria due to its low bioavailability. Additionally, penicillin G is rapidly eliminated via the renal route, with 50% to 80% of the administered antibiotic dose excreted within two to three hours. This necessitates frequent administration, which is burdensome since penicillin G can only be given parenterally due to its instability in acidic environments (such as gastric juice). This rapid renal elimination results in over 80% recovery of the active compound, while the remainder is excreted in urine as an inactive metabolite: penicilloic acid.

Consequently, efforts have been made to synthesize penicillin derivatives to address the issues associated with penicillin G.

2) Amoxicilline

Amoxicillin is a synthetic aminopenicillin with the broadest spectrum of activity among penicillin derivatives. Since its introduction to the market in 1972, it has largely replaced penicillin G and has become the antibiotic of first choice. However, amoxicillin is also associated with the highest prevalence of allergic reactions, occurring in approximately 5.1% of the population.

a) The amoxicilline history

In 1959, Batchelor, Doyle, Nayler, and Rolinson developed an industrial method for isolating 6-aminopenicillanic acid (6-APA), the core structure of penicillins, from the fermentation of Penicillium chrysogenum, without the introduction of any acylating reagent into the culture broth. This bicyclic structure allows for the synthesis of various side chains, leading to the production of so-called semisynthetic penicillins **Figure17**.

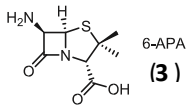


Figure 17: Chemical Structure of 6-Aminopenicilllan Acid (33).

Researchers aimed to enhance the properties of penicillin G due to its hydrophobic nature, which limits its oral administration and results in a narrow spectrum of activity. To increase its effectiveness against Gram-negative bacteria, the hydrophilicity of penicillin needed to be improved. Consequently, aminopenicillins, which feature a free amino group, are more water-soluble. Additionally, it was observed that the spectrum of activity of these aminopenicillins is significantly broader than that of penicillin G. The first of these, ampicillin, was discovered in 1961; however, it still exhibited poor oral bioavailability. Amoxicillin, discovered in 1964, has enhanced hydrophilicity due to its phenolic group, allowing for oral administration. Its broad spectrum of activity and improved oral bioavailability have made amoxicillin the antibiotic of first choice **Figure18**.

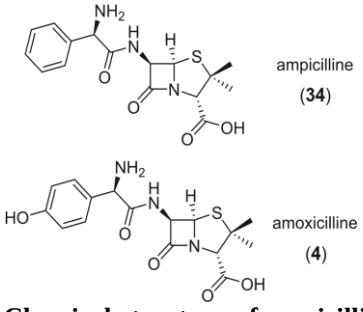


Figure 18: Chemical structure of ampicillin (34) and amoxicillin (4).

b) Industrial synthesis

Currently, amoxicillin is the most widely consumed antibiotic globally, with a consumption rate of 10.7 daily defined doses per 1,000 inhabitants per day in France. The challenge of synthesizing penicillin-type β-lactams with high yields and enantioselectivity has made a fully synthetic industrial production of these aminopenicillins difficult. Consequently, when Batchelor and colleagues described a method for extracting 6-aminopenicillanic acid in 1959, pharmaceutical industries opted for a semisynthetic approach to produce synthetic aminopenicillins like amoxicillin.

There are various pathways to access these semisynthetic penicillins. First, the intermediate 6-aminopenicillanic acid can be obtained either through the method outlined by Batchelor and his team in 1959, which involves culturing Penicillium chrysogenum in a broth without an acylating agent, or from a natural penicillin (such as penicillin G) obtained through the culture of Penicillium chrysogenum, followed by selective hydrolysis of the amide side chain via enzymatic or chemical means. From this 6-APA intermediate, acylation with the desired side chain can be accomplished through either enzymatic or chemical methods **Figure19**.

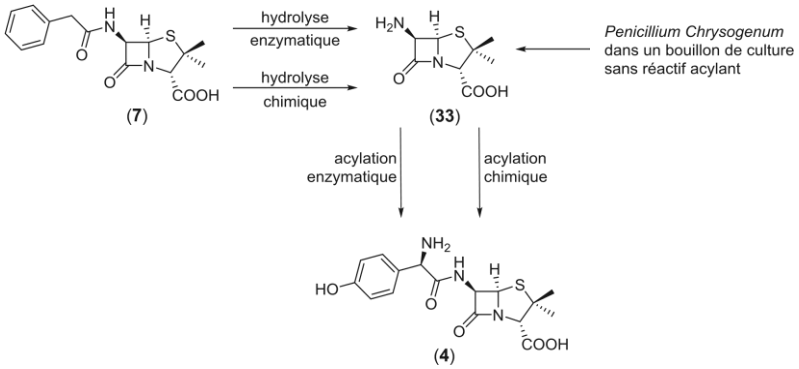


Figure 19: Different routes of access to hemisynthetic penicillins.

Numerous publications on the enzymatic hydrolysis of penicillin G into 6-aminopenicillanic acid emerged in 1960. Notably, an enzyme derived from Escherichia coli cells achieved a 95% conversion rate in four hours.

Additionally, a penicillin amidase from Alcaligenes faecalis resulted in conversions of approximately 6 mg/mL of culture, while a penicillin acylase from Nocardia produced 2.4 g/L of 6-APA from 5 g/L of penicillin G in 16 hours, yielding an 80% efficiency. However, by the 1970s, the enzymatic approach was largely abandoned in favor of chemical methods that avoided issues associated with enzymes, such as low product concentrations, contamination risks from bacterial strains, and high isolation costs.

Published by Weissenburger and Van der Hoeven in 1970, this synthetic method allows for the efficient production of 6-APA with an excellent yield of 91% over four steps. Given that penicillin G contains both an amide and a highly reactive lactam function under basic and nucleophilic conditions, it could not be hydrolyzed using conventional methods. Starting from the potassium salt of penicillin G, the acid function is protected by a silyl group through treatment with phosphorus pentachloride at low temperatures, resulting in the iminochloride derivative. Subsequently, at low temperatures, an excess of n-butanol facilitates the formation of the imino-ether via nucleophilic substitution. Ammoniacal hydrolysis followed by pH adjustment to 4.1 enables the isolation of 6-aminopenicillanic acid, which crystallizes with a high yield **Figure20**.

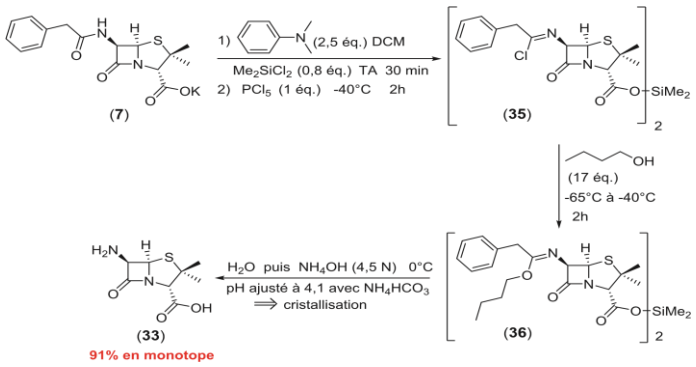


Figure 20: Chemical synthesis of 6-APA (33).

The chemical synthesis process in question presents significant environmental challenges and incurs excessive solvent and energy costs at an industrial scale, making it unsustainable. As a result, enzymatic pathways are being revisited to address these issues, particularly through the development of immobilized enzymes that facilitate recycling. Initial attempts to utilize enzymes from native bacterial strains yielded unsatisfactory conversion rates, prompting researchers in the 1980s to enhance bacterial strains. The advent of recombinant DNA technology enabled the creation of a strain with ten times the activity of the native variant. The most effective penicillin amidases currently in use originate from these improved strains of *Escherichia coli*.

The second step in the synthesis of semisynthetic penicillins involves the acylation of 6-aminopenicillanic acid with derivatives of D-phenylglycine, which has also been developed through both chemical and enzymatic methods. The patent documenting the discovery of amoxicillin was published in 1965, at which point the synthesis was primarily chemical. The patent describes a racemic mixture of N,O-diprotected 4-hydroxyphenylglycine; however, the inventors also note that synthesis was accomplished using both D and L series. This compound reacts with ethyl chloroformate to form a mixed anhydride, which subsequently reacts with 6-aminopenicillanic acid in a basic medium to yield the sodium salt of N,O-diprotected amoxicillin, achieving an overall yield of 61% across both steps. The deprotection of amoxicillin is performed via double hydrogenation, resulting quantitatively in the desired product. However, the purity of the final product is not addressed in the patent **Figure21**.

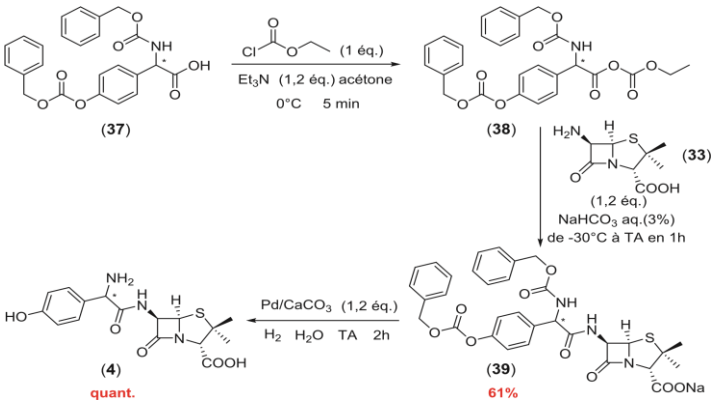
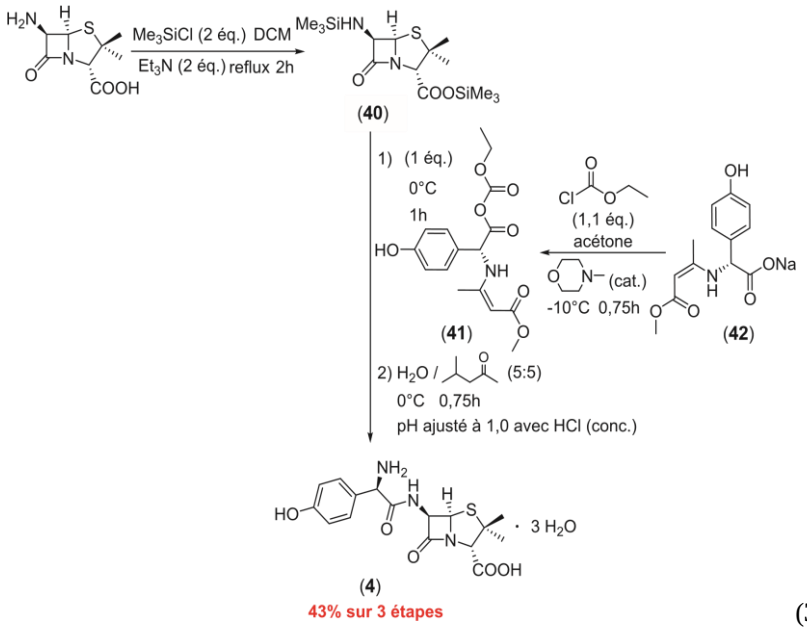


Figure 21: Chemical synthesis of amoxicillin (4).

In 1971, a new patent detailed the synthesis of amoxicillin from a disilylated derivative of 6-aminopenicillanic acid and N-protected D-4-hydroxyphenylglycine. Among the various protective groups mentioned in the patent, only those outlined in the accompanying diagram yield the highest efficiency of 43% with a final purity of 80%. By altering the solvent and treatment conditions, the inventor was able to increase the purity to 93%, although this resulted in a reduced yield of 37%. The 6-aminopenicillanic acid is silylated at its acid and amine functions to form a compound that is subsequently coupled with a mixed anhydride of N-protected D-4-hydroxyphenylglycine, which has been modified with a 1-methoxycarbonyl-2-propenyl group. This group is obtained through the condensation of ethyl chloroformate with the sodium salt of the corresponding acid. Following the coupling reaction, an acid hydrolysis is performed to yield trihydrate amoxicillin, which crystallizes with a yield of only 43% **Figure22**.



(33)

Figure 22: Synthesis of amoxicillin (4).

In 1978, researchers from the Netherlands patented an optimized synthesis of amoxicillin utilizing the economical Dane salt. By adjusting reaction temperatures, times, and maintaining precise pH levels, they achieved a purity of 97.7% and a yield of 83.5%. During the exploration of penicillin acylases, an investigation into chemical acylation using acid chlorides yielded satisfactory results, although the details were not extensively elaborated. This synthetic route was not pursued for amoxicillin but was applied to other semisynthetic penicillins, such as ampicillin.

Subsequent studies revealed the potential for enzyme reversion, enabling the enzymatic synthesis of novel penicillins. For instance, in 1973, researchers developed a method to hydrolyze penicillin G into 6-aminopenicillanic acid using an immobilized penicillin acylase derived from *Escherichia coli*. Two years later, they utilized this same enzyme for the enzymatic synthesis of amoxicillin and other new penicillins. Under slightly basic conditions (pH 8), the enzyme favored hydrolysis of penicillin, while slightly acidic to neutral conditions (pH 6) favored synthesis. Consequently, the synthesis was conducted at neutral pH using 6-aminopenicillanic acid and D-4-hydroxyphenylglycine, achieving a maximum conversion of 30% before hydrolysis became predominant, resulting in low concentrations of amoxicillin.

Over time, numerous improvements were made, and by 1996, a continuous preparation process for amoxicillin was patented, yielding up to 90% from 6-aminopenicillanic acid and activated D-4-hydroxyphenylglycine, with a productivity of 156 mmol/h/L. Approximately 900 g of amoxicillin trihydrate could be produced in 14 hours. The equilibrium between acylation and hydrolysis was shifted in favor of acylation due to the low solubility of amoxicillin, which precipitated in highly concentrated solutions, thereby reducing its availability for hydrolysis.

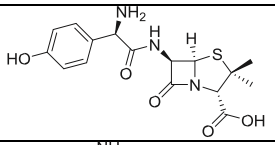
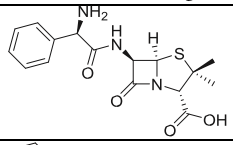
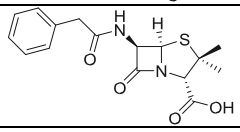
Despite these advancements towards enzymatic industrial synthesis, the chemical method using Dane salt remains the predominant approach for amoxicillin production at an industrial scale today.

c) The mode of action of amoxicillin

The mechanism of action of amoxicillin is similar to that of penicillin G. Amoxicillin demonstrates a higher degree of hydrophilicity compared to penicillin G, which enables it to penetrate Gram-negative bacteria more effectively. As previously discussed, Gram-negative bacteria possess porins in their outer membrane that

facilitate the passage of small, preferentially hydrophilic molecules. This increased hydrophilicity of amoxicillin allows it to traverse the outer membrane more easily than penicillin G. Consequently, amoxicillin exhibits a broader spectrum of activity, targeting both Gram-positive and a wide range of Gram-negative bacteria table 2.

Table 2: logP of amoxicillin (4), ampicillin (34) and penicillin G (7).

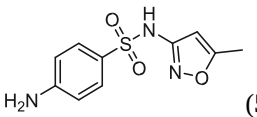
Medicine	Structure	logP
Amoxicilline (**4**)		0,87
Ampicilline (**34**)		1,35
Penicillin G (**7**)		1,83

d) The mode of haptenization of amoxicillin

Amoxicillin has a molecular weight of 365.40 g/mol. Like penicillin G, it acts as a hapten. The primary mechanism of haptenization for amoxicillin is similar to that of penicillin G and other penicillins, involving the opening of the β-lactam ring by the nucleophilic groups of proteins, particularly lysine residues. Amoxicillin is metabolized more slowly than penicillin G due to its greater chemical stability; notably, the formation of minor antigenic determinants, as seen with penicillin G, has not been observed to date.[82]

3) Sulfaméthoxazole

Sulfamethoxazole (SMX) has a prevalence of 3.4% when used in combination with trimethoprim. This combination is commonly employed to prevent resistance and facilitate a more rapid action against bacterial infections **Figure23**.

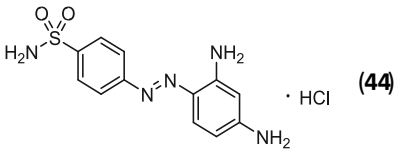


(**5**)

Figure 23: Chemical structure of sulfamethoxazole (5).

a) The history of sulfamethoxazole

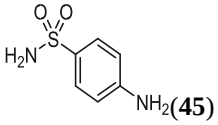
During World War I, many wounded soldiers succumbed to infections, prompting a vigorous search for anti-infective agents. In Germany, two chemists at Bayer, Josef Klarer and Fritz Mietzsch, synthesized sulfamidochrysoidine, which was tested on rat infections and shown to be effective in December 1931. Between 1932 and 1935, German physician Gerhard Domagk, also from Bayer, discovered the antibacterial properties of Prontosil (the hydrochloride of sulfamidochrysoidine) in patients. For his work on the therapeutic activity of Prontosil, Dr. Domagk was awarded the Nobel Prize in 1939 **Figure24**.



(**44**)

Figure 24: Chemical structure of Prontosil (sulfamidochrysoidin hydrochloride) (44).

The efficacy of this antibiotic has garnered significant interest from researchers across Europe, particularly in France, where Ernest Fourneau's team at the Pasteur Institute demonstrated that Prontosil is metabolized into sulfanilamide within the body, serving as the active metabolite against bacteria. It was marketed in 1937 in France, Great Britain, and the United States **Figure25**.



(**45**)

Figure 25: Chemical structure of sulfanilamide (45), a metabolite of Prontosil, an active ingredient against bacteria.

The sulfonamide family subsequently expanded, leading to the synthesis and testing of numerous other compounds, including sulfamethoxazole in 1956. Sulfonamides thus became the first synthetic antibiotics; however, their spectrum of activity is relatively narrow, and their metabolism in the body frequently produces toxic byproducts. This situation further fueled the development of penicillin.[83]

b) Industrial synthesis

The industrial synthesis of sulfamethoxazole involves a classic method that includes the condensation of 3-amino-5-methylisoxazole with an activated p-aminobenzenesulfonic acid (such as a sulfonyl chloride or ester) or with a protected N-activated p-aminobenzenesulfonic acid (for example, protected by an acetyl group). This process results in the condensation product with excellent yields. Following this, a deprotection step of the primary aromatic amine leads to the desired product in a few steps and with nearly quantitative yields **Figure26**.

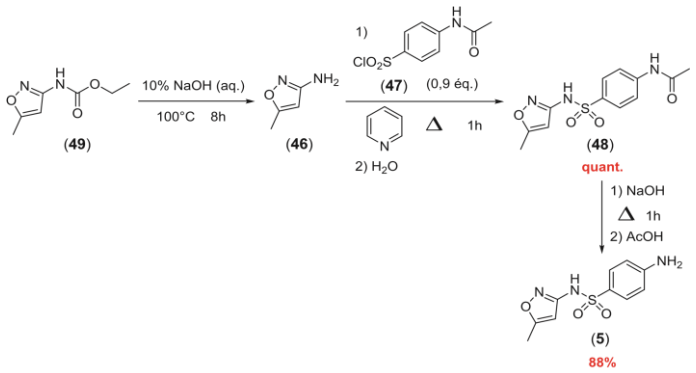


Figure 26: Industriell synthesis of SMX(5).

c) The mode of action of sulfamethoxazole

The mechanism of action of sulfamethoxazole involves the dihydrofolate anion and its reduced analogue, tetrahydrofolate. In the body, tetrahydrofolate is synthesized from dihydrofolate through the action of an enzyme known as dihydrofolate reductase. Tetrahydrofolate is essential for the organism, playing a critical role in the biosynthesis of certain amino acids and nucleic acids **Figure 27**.

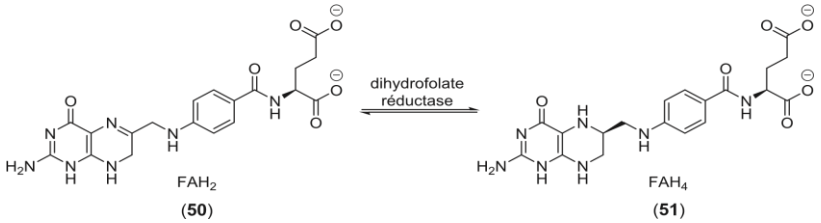


Figure 27: Synthesis of FAH4 (51) from FAH2 (50) *via* dihydrofolate reductase.

The selectivity of sulfamethoxazole stems from a key difference between bacteria and mammals: mammals obtain dihydrofolate through their diet, while bacteria must synthesize it. The same applies to tetrahydrofolate, which bacteria access only via dihydrofolate reductase. Sulfamethoxazole, particularly the derived sulfanilamide, is an isostere of para-aminobenzoic acid, which is involved in the de novo synthesis of dihydrofolate in bacteria. Bacteria incorporate sulfanilamide into their metabolism, where it acts as a competitive inhibitor of dihydropteroate synthase, an enzyme essential for dihydrofolate synthesis. By inhibiting dihydrofolate production, bacteria cannot access tetrahydrofolate, hindering their ability to synthesize certain DNA bases. Consequently, they are unable to multiply, rendering sulfamethoxazole a bacteriostatic antibiotic **Figure 28**.

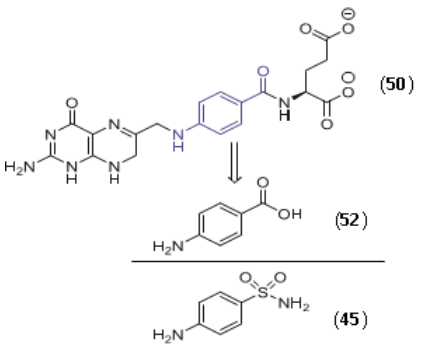


Figure 28: Isosteric structures between sulfanilamide (45) and p-aminobenzoic acid (52), a precursor of FAH2 (50)

To enhance the bacteriostatic effect, sulfamethoxazole is commonly combined with trimethoprim. This combination also helps mitigate the development of resistance, as the two antibiotics operate through different mechanisms. Trimethoprim acts as a competitive inhibitor of the enzyme dihydrofolate reductase, preventing the synthesis of tetrahydrofolate from dihydrofolate that may have been produced prior to the action of sulfamethoxazole. Its specificity arises from the subtle structural differences between the enzymes in bacteria and those in mammals. This dual mechanism of action, through the combination of trimethoprim and sulfamethoxazole, facilitates a more rapid depletion of tetrahydrofolate, quickly halting bacterial growth.[84]

d) Modes of presentation of sulfamethoxazole to T lymphocytes

As previously discussed, there are two modes of presentation for sulfamethoxazole to T lymphocytes. The pharmacological interaction concept does not require the formation of an amino acid-antibiotic adduct and will therefore not be included in this study. The second mode is based on the hapten theory, as previously described for penicillins.

Sulfamethoxazole has a molecular weight of 253.28 g/mol. It must bind to a protein to become immunogenic, but it requires enzymatic metabolism before this binding can occur, making it a pro-hapten. Initially, sulfamethoxazole undergoes oxidation by cytochrome P450 enzymes (CYP2C9 or CYP2C8) or by

myeloperoxidase, which is present in dendritic cells and keratinocytes, transforming it from its amine form to a hydroxylamine derivative. This hydroxylamine then spontaneously oxidizes to a highly reactive nitroso derivative, which readily binds to proteins, particularly to cysteine residues that are not involved in disulfide bonds.

Research suggests that the cysteine derivatives involved in the haptenization of the nitroso derivative may be oxidized to sulfenic acid derivatives, leading to the formation of N-hydroxysulfinamides. Without this oxidation, the resulting derivatives would resemble those observed when glutathione in its thiol form directly binds to the nitroso derivative of sulfamethoxazole. This coupling produces a semimercaptal derivative (N-hydroxysulfenamide), which rapidly rearranges to a more stable sulfinamide derivative. However, this sulfinamide is not stable under heat or acidic conditions, such as those encountered during protein digestion in dendritic cells. This instability could explain why, after treating their haptenized human serum albumin samples at 55°C for 15 minutes, only the N-hydroxysulfinamide derivative was observed **Figure 29**.

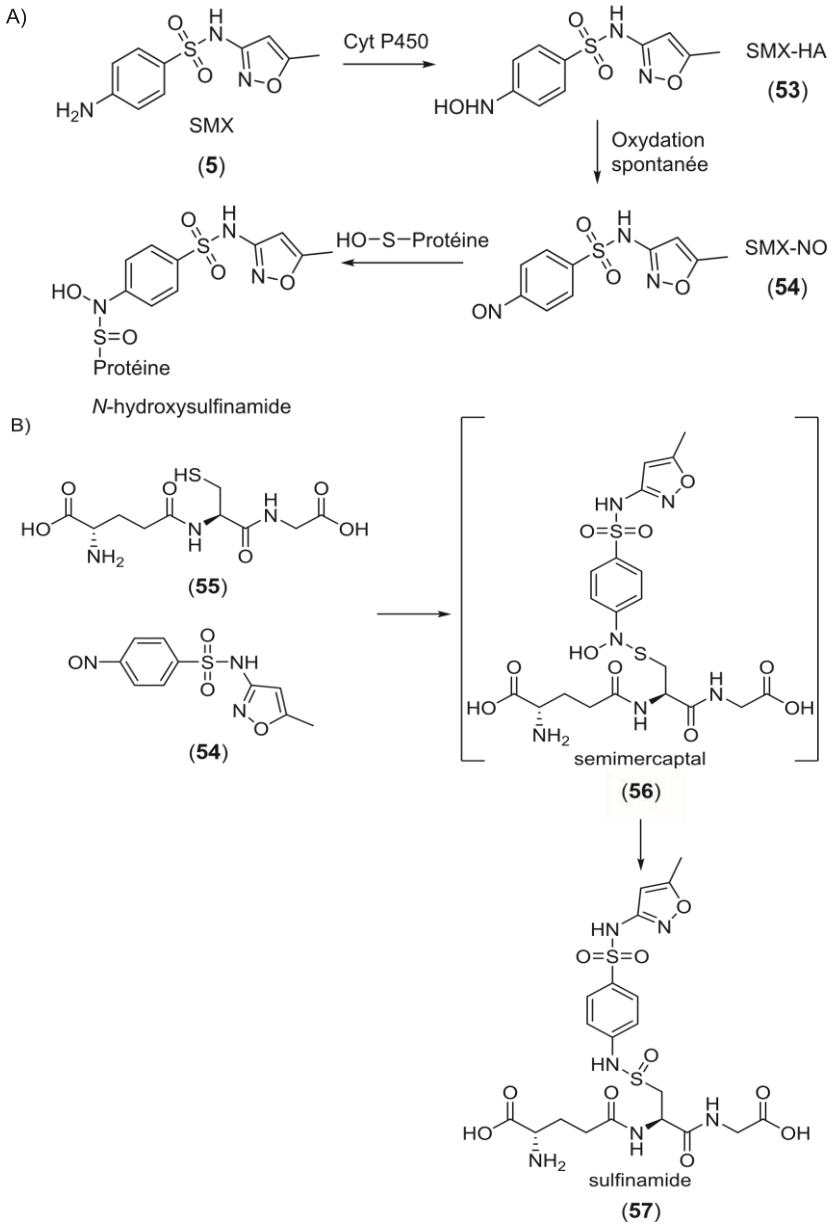


Figure 29: Haptenization of the nitroso metabolite of sulfamethoxazole (54) by oxidized cysteine derivatives of a protein (A) or by glutathione (55) (B).

Generally, the degradation pathway of sulfamethoxazole involves N-acetylation in the liver, with oxidation by cytochrome enzymes occurring only in a small proportion of the antibiotic. However, certain patients classified as "slow acetylators," as well as those with conditions such as HIV that disrupt the acetylation pathway, exhibit a higher prevalence of hypersensitivity to sulfamethoxazole.

With the forms of haptens for the three antibiotics of interest established, the second step of the immune process remains to be investigated. This step involves the digestion of the haptenized protein by dendritic cells and the identification of the resulting peptides that will trigger the T lymphocyte response.

CONCLUSION

Drug allergies, particularly those related to antibiotics, represent a complex and significant challenge in modern medicine. The unpredictable nature of these hypersensitivity reactions complicates clinical management and poses serious risks to patient safety. This review has highlighted the intricate mechanisms underlying drug allergies, with a specific focus on the roles of immediate and delayed hypersensitivity reactions. Understanding these mechanisms is essential for healthcare professionals to effectively diagnose and manage allergic responses. The phenomenon of haptenization has emerged as a critical factor in antibiotic-induced allergies, illustrating how antibiotics can modify host proteins to elicit immune responses. This process is particularly relevant for commonly used antibiotics, such as penicillins and sulfonamides, which can provoke reactions in genetically predisposed individuals. The exploration of the pharmacologic interaction (p-i) concept further enriches our understanding by proposing that certain antibiotics may activate T lymphocytes directly, bypassing traditional haptenization pathways. This insight underscores the need for continued research into the immunological interactions between drugs and the immune system.

The prevalence of allergic reactions to antibiotics, especially amoxicillin and sulfamethoxazole, raises concerns about their widespread use in clinical settings. The high incidence of hypersensitivity reactions necessitates vigilance from healthcare providers, as alternative therapeutic options may not always be readily available or as effective. Therefore, a thorough understanding of the risks associated with these antibiotics is imperative for informed prescribing practices. Diagnostic challenges remain a significant barrier in the identification of drug allergies. Current testing methods often lack sensitivity and specificity, leading to potential misdiagnoses and inappropriate management strategies. The development of more advanced diagnostic tools and methods is crucial for accurately identifying allergic responses and distinguishing them from other adverse drug reactions. Improved diagnostic capabilities will enhance patient safety and facilitate better therapeutic outcomes.

Future research should focus on elucidating the specific epitopes involved in antibiotic allergies and the mechanisms by which they trigger immune responses. Furthermore, investigations into the genetic and environmental factors that contribute to the development of drug allergies will aid in identifying at-risk populations and optimizing treatment strategies.

Addressing the public health challenge posed by drug allergies requires a multidisciplinary approach that combines immunological research, clinical practice, and pharmacological innovation. By enhancing our understanding of the mechanisms involved and improving diagnostic and therapeutic strategies, we can significantly advance patient care and safety in the context of antibiotic use. Continued collaboration among researchers, clinicians, and regulatory bodies will be essential in developing effective interventions to mitigate the impact of drug allergies in the population.

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