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OBTAINING AND APPLICATION OF SULFANILAMIDE MEDICINES

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The method of sulfanilamide synthesis described in the article. The historical facts of discovery and application of sulfanilamide drugs are considered. Modern and more perspective derivatives of sulfanilamide are reviewed.

Key words: *sulfanilamide, para-aminobenzoic acid, dihydrofolic acid, microorganisms, bacteriostatic effect, sulfamethoxazole, trimethoprim.*

Introduction. Sulfonamides were the first chemotherapeutic drugs with wide spectrum of activity that have application in practical medicine. Since discovery in 1935 of antimicrobial properties of sulfanilamide 6 000 sulfa substances were synthesized and studied, 40of these compounds are used in medical practice.

Early efforts in the development of anti-infective drugs. For much of history, infectious diseases were the leading cause of death in most of the world. The widespread use of vaccines and implementation of public health measures, such as building reliable sewer systems and chlorinating water to assure safe supplies for drinking, were of great benefit in decreasing the impact of infectious diseases in the industrialized world. However, even with these measures, pharmaceutical treatments for infectious diseases were needed. The first of these was arsphenamine, which was developed in 1910 by the German medical scientist Paul Ehrlich, who is a founder of modern chemotherapy, for the treatment of syphilis. Arsphenamine was the 606th chemical studied by Ehrlich in his quest for an antisyphilitic drug. Its efficacy was first demonstrated in mice with syphilis and then in humans. Arsphenamine was marketed with the trade name of Salvarsan and was used to treat syphilis until the

1940s, when it was replaced by penicillin. Ehrlich referred to his invention aschemotherapy, which is the use of a specific chemical to combat a specific infectious organism. Arsphenamine was important not only because it was the first synthetic compound to kill a specific invading microorganism but also because of the approach Ehrlich used to find it. In essence, he synthesized a large number of compounds and screened each one to find a chemical that would be effective. Screening for efficacy became one of the most important means used by the pharmaceutical industry to develop new drugs.

The discovery of sulfanilamide. The next great advance in the development of drugs for treatment of infections came in the 1930s, when it was shown that certain azo dyes, which contained sulfonamide groups, were effective in treating streptococcal infections in mice. One of the dyes, known as Prontosil, was later found to be metabolized in the patient to sulfanilamide, which was the active antibacterial molecule. In 1933Prontosil was given to the first patient, an infant with a systemic staphylococcal infection. The infant underwent a dramatic cure [1].

Daniel Bovet's team at the Pasteur Institute in Paris discovered that sulfanilamide (4-aminobenzenesulfonamide), was itself active both *in vitro* and *in vivo*. Intestinal enzymes in the human body convert Prontosil into sulfanilamide, the active molecule; there were no enzymes in the bacterial cultures upon which Prontosilhad been tested, which is why Prontosil was inactive *in vitro*. One advantage of sulfanilamide over Prontosil was that the skin of patients was not turned red. Moreover, sulfanilamide was much cheaper than Prontosil. It had first been prepared in Vienna in 1908 by Paul Gelmo, in the course of his PhD researches. It was patented in 1909, so that the patent had expired by the time that Bovet's team made their discovery. Bovet was later to receive the 1957 Nobel Prize for Physiology or Medicine [2, 3].

In subsequent years many derivatives of sulfonamides, or sulfa drugs, were synthesized and tested for antibacterial and other activities.

Interesting historical facts about sulfanilamide application. The 69-year-old British Prime Minister, Winston Churchill, had two bouts of pneumonia in 1943, the

first one in February. At the Teheran conference, concerned with finalising strategy for the war against Nazi Germany held between November 28th and December 1st that year, he met the President of the United States, Franklin Roosevelt, and Joseph Stalin, Leader of the Soviet Union.

After this meeting, he went to General Eisenhower's villa near Carthage but *en route* spent an hour sitting on an airfield in a cold wind. He slept for the rest of the day, December 11th, then complained of a sore throat. Next day, his temperature rose to 101°F. A portable X-ray machine revealed a shadow on the lung; it was pneumonia again. Immediately Churchill was given the new antibiotic made by the British firm of May and Baker; the tablets were simply known as 'M&B'. Churchill had a mild heart attack on December 15th, but his condition improved and he convalesced by having *Pride and Prejudice* read to him. He compromised on his habits by agreeing to drink only weak whisky and soda and not to smoke at all. No cigars. He is quoted as saying: "*Dear Nurse, pray remember that man cannot live by M & B alone*." His doctors, Lord Moran and Dr Bedford, were also M & B.

Subsequently Churchill issued a saying "*Excellent nurses and the highest medical authorities in the Mediterranean arrived from all quarters as if by magic. This admirable M & B, from which I did not suffer any inconvenience, was used at the earliest moment and after a week's fever the intruders were repulsed*."

At this distance of time, it is not clear whether the drug used was M&B 693, sulfapyridine, or else M&B 760, sulfathiazole, though it is more likely to have been the former. And if it had not been for a German scientist, it is quite possible that this drug might not have been available [4].

The discovery of Sulfanilamide greatly affected the mortality rate during World War II. American soldiers were taught to immediately sprinkle sulfa powder on any open wound to prevent infection. Every soldier was issued a first aid pouch that was designed to be attached to the soldier’s waist belt. The first aid pouch contained a package of sulfa powder and a bandage to dress the wound. One of the main components carried by a combat medic during World War II was sulfa powder and sulfa tablets.

Synthesis of sulfanilamide. Sulfanilamide can readily be made from aniline in four steps (Fig.1). Aniline is first acetylated to protect the easily oxidised amine group; sulfonated using chlorosulfonic acid; ammonia is added to generate the sulfonamide group; and finally boiling with dilute HCl removes the protecting acetyl group, which is more easily hydrolysed than the sulfonamide group [5–7].

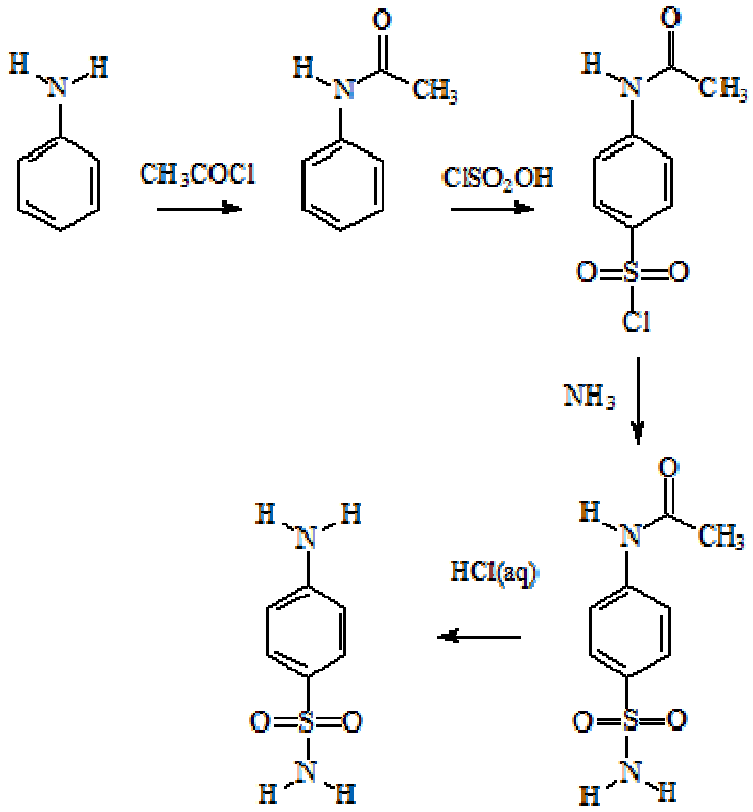
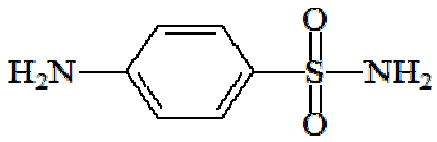


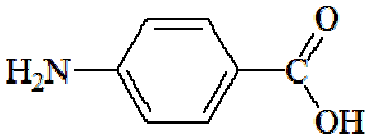
Fig. 1. Sulfanilamide synthesis

Mechanism of sulfanilamide action. The mechanism of sulfanilamide's action was discovered in 1940 by Donald Woods, researching in Oxford. He found that *para*-aminobenzoic acid – PABA (Fig. 2) stopped sulfanilamide's antibacterial effects, and noted that these two molecules had very similar structures.

PABA is used by bacteria to make dihydrofolic acid, which is then used to make other molecules including the purine bases necessary to construct DNA, so it is absolutely vital to the bacteria. One stage of the synthesis of dihydrofolic acid is catalysed by the enzyme dihydropteroatesynthetase; sulfanilamide fits the active site of this enzyme, and blocks the site to PABA, so that the bacterium cannot multiply and grow. Thus the immune system has the opportunity to clear up the infection [8,9].



Sulfanilamide



p-aminobenzoic acid

Fig. 2. Structure of sulfanilamide and *p*-aminobenzoic acid

Unlike bacteria, humans do not make dihydrofolic acid. They take in folic acid from their diet as a vitamin (from green vegetables for example), so we don't have any dihydropteroatesynthetase, and that's why sulfanilamide is safe for humans.

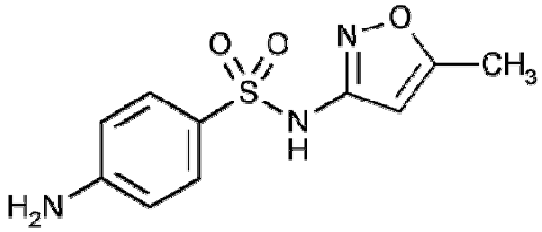
Some inhibitors prevent, or block, enzymatic action by reacting with groups at the active site. The nerve gas diisopropylfluorophosphate, for example, reacts with the serine at the active site of acetylcholinesterase to form a covalent bond. The nerve-gas molecule involved in bond formation prevents the active site from binding the substrate, acetylcholine, thereby blocking catalysis and nerve action. Iodoacetic acid similarly blocks a key enzyme in muscle action by forming a bulky group on the amino acid cysteine, which is found at the enzyme’s active site. This process is called irreversible inhibition.

Some inhibitors modify amino acids other than those at the active site, resulting in loss of enzymatic activity. The inhibitor causes changes in the shape of the active site. Some amino acids other than those at the active site, however, can be modified without affecting the structure of the active site; in these cases, enzymatic action is not affected. Such chemical changes parallel natural mutations. Inherited diseases frequently result from a change in an amino acid at the active site of an enzyme, thus making the enzyme defective. In some cases, an amino acid change alters the shape

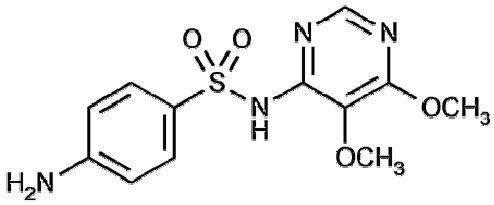
of the active site to the extent that it can no longer react; such diseases are usually fatal. In others, however, a partially defective enzyme is formed, and an individual may be very sick but able to live [10].

Modern derivatives of sulfanilamide. Sulfa drugs are not used as much now as 50 years ago, since some bacteria have acquired immunity to them; there is also the possibility of liver damage and of allergic reactions, such as Stevens-Johnson syndrome. However, other molecules which retain their activity for longer have been synthesized; they tend to be used to treat infections of the urinary tract and in certain sexually transmitted infections like *Chlamydia*.

A mixture of sulfamethoxazole and trimethoprim, known as Co-trimoxazole (Biseptol), has been used to treat a variety of infections, though this is now more restricted. It has been recommended for HIV-exposed infants and children in certain situations. It can be an effective "broad spectrum" antibiotic against a variety of illnesses because sulfamethoxazole (Fig. 3) and trimethoprim (Fig. 4) target successive steps in the biosynthesis of tetrahydrofolic acid (Fig. 5), so lower doses of each molecule can be used in the drug. It's a kind of double-whammy treatment, but concerns about side-effects mean that it sees limited use [11].



Sulfamethoxazole



Sulfadoxine

Fig. 3. Structure of the sulfadrug derivatives

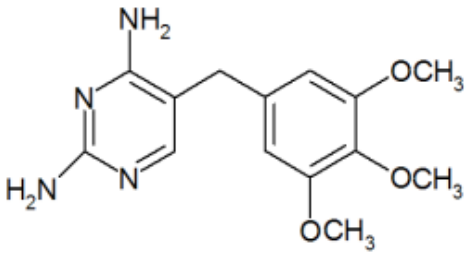


Fig. 4. Structure of trimethoprim

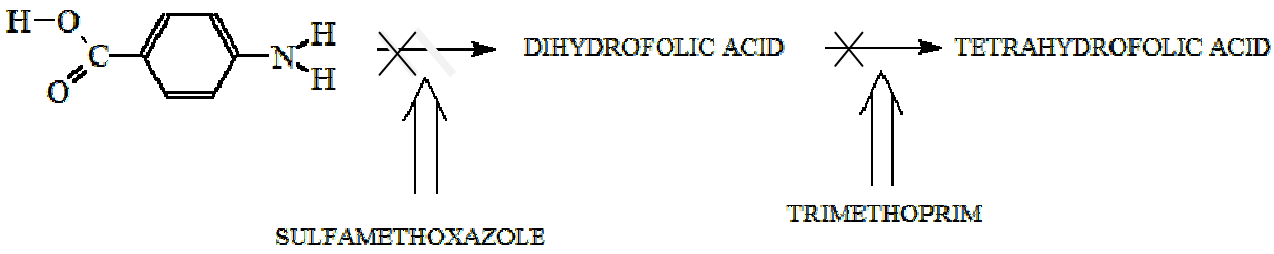


Fig. 5. Blocking of synthesis of tetrahydrofolic acid

Sulfa drugs were first used in malaria treatment in the 1930s, but were superseded by other agents like chloroquine, which in its turn has become less effective. A combination of sulfadoxine (Fig. 3) and pyrimethamine (Fansidar) has been used to treat chloroquine-resistant *Plasmodium falciparium malariae*. This similarly targets the two enzymes in the synthesis of tetrahydrofolic acid. Side effects means that it is only employed for severe cases, and its use is now discontinued in the UK [12].

Despite this, sulfonamides were the first widely used and successful examples of chemotherapy, using synthetic chemicals to fight diseases. They were also the first example of the use of a competitive enzyme inhibitor as a drug.

CONCLUSIONS

Sulfanilamide is one of the first representatives of the antimicrobial drugs. Despite great advances in the production of antibiotics, today sulfonamides remain among the most commonly used antibacterial agents.

The mechanism of antibacterial action of sulfonamides is inhibition the formation of folic and dihydrofolic acids that need to the microorganisms for the synthesis of purines and pyrimidines. Blocking of this metabolic chain leads to stopping of growth and reproduction, i.e. a bacteriostatic effect.

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ПОЛУЧЕНИЕ СУЛЬФАНИЛАМИДНЫХ ПРЕПАРАТОВ И ИХ ПРИМЕНЕНИЕ

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В статье описан метод синтеза сульфаниламида (стрептоцида), приведены исторические факты, связанные с открытием и применением сульфаниламидных препаратов. Рассматриваются современные и перспективные производные сульфаниламидов.

Ключевые слова: *сульфаниламид, пара-аминобензойная кислота, дигидрофолиевая кислота, микроорганизмы, бактериостатический эффект, сульфаметоксазол, триметоприм.*

ОДЕРЖАННЯ СУЛЬФАНІЛАМІДНИХ ПРЕПАРАТІВ ТА ЇХ ЗАСТОСУВАННЯ

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У статті описано метод синтезу сульфаніламіду (стрептоциду), наведено історичні факти, пов'язані з відкриттям й застосуванням сульфаніламідних препаратів. Розглянуто сучасні та перспективні похідні сульфаніламідів.

Ключові слова: *сульфаніламід, пара-амінобензойна кислота, дигідрофолієва кислота, мікроорганізми, бактеріостатичний ефект, сульфаметоксазол, триметоприм.*