

Sulphonamides: glory and misfortunes

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Abstract

The author tells how the discovery of sulphonamides happened and refers to some of the misfortunes that, in some way, reduced

the glory of this important scientific event.

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In February 1935, the magazine *Deutsch Medizinische Wochenschrift* published an article called “Ein Beitrag zur Chemotherapie der bakteriellen Infektionen” (A contribution for the chemotherapy of bacteria and infections). The author, Gerhard Domagk, who had been working for a number of years at Bayer I. G. Farbenindustrie, in Elberfeld, was showing this way the antibacterial action of the chemical substance, Prontosil, in infected mice with lethal doses of pathogenic streptococcus.

The results were surprising, revealing that after all it was actually possible to find what many scientists were looking for several decades: an antibacterial agent capable of acting in living organisms, without producing prohibitive side-effects.

Such discovery, as it is obvious, had behind it a long history which had started taking shape from the second half of the 19th century onwards. In those days, England, France and Germany, already in full industrialization process, were disputing the world hegemony and were throwing themselves into a voracious conquest of raw materials and new markets. Among these three European powers there was an atmosphere of rivalry not always peaceful that would spread towards Medicine, mainly in the area causing more concern at the time: infectious and parasitic diseases. (To be remembered that European explorers, leaving their sanctuary, would add to the already known diseases, others with non-less lethal effects that were abundant in the tropical regions, as Malaria and the sleeping disease).

In France of Napoleon III and the Third Republic, it would then emerge Louis Pasteur; in Kayser and Bismarck's Germany, Robert Koch; in Victorian England, Joseph Lister. Well it was thanks to these three men that in a short while the definite relationship between infectious-contagious disease and micro-organisms became fully known; the basic rules of asepsis and antisepsis were defined; and modern numerology was founded (based on the triad bacteria – antigen – antibody) that would remain operational until half of the 20th century.

Only after such knowledge was acquired, it was possible to move to the next stage: the search of medicines capable of restraining infections in humans. It is true that at the time the so-called antiseptic substances were starting to be developed which would destroy *in vivo* bacteria. But their toxic effects were so severe that excluded any chance of therapeutic application.

When passing from the 19th to the 20th century another remarkable personality of Medicine emerged: the German Paul Ehrlich. With sound knowledge and a strong personality he would influence some of the key ideas, and a great part of the investigation of the following decades.

At the time, the scientific world was deeply divided about the roads to follow regarding the therapy of infectious diseases. On one hand, those following Pasteur, were absolutely convinced that the solution would be reinforcing the organic defenses. (Ironically among those was Almroth Wright, a laboratory director where at a later stage, Fleming would find penicillin). On the other side, there were those believing to be possible to find one or more substances capable of acting selectively against bacteria.

Ehrlich, belonging to this second group, used for the first time the expression “chemotherapy” persuaded as he was that sooner or later a chemical substance with a specific action against bacteria, but harmless to the hosting organism, would appear. Such

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substance would be, in his own words, similar to a “magic bullet” capable of destroying, without damaging, a clearly identified target. In this line of thought, he adopted the method of trying blindly several compounds, numbered by order of entry in the trials persuaded that some of them would end up revealing the aimed properties. To achieve his targets, he developed an idea which would mark, in the future, the medical investigation: “animal models”. Thanks to this it was possible to test the pharmacological and therapeutic effects of the several chemical products in laboratory animals and extrapolating afterwards the results for the human body. On the other hand, Ehrlich impressed with the affinity of certain stains for the bacterial walls, has admitted that would be probably among this group of chemical substances that the so sought “bullet magic” would be found.

Inspired in Schaudinn's works, that in Berlin Serologic Institute, had discovered the *Treponema pallidum* (erroneously thought to be a relative of *Trypanosoma*), Ehrlich started to blindly trying a long list of organic arsenical compounds. When in 1910 he reached the number 606 of such series, he had finally found a substance with an inhibitory action against the *Treponema*. It was born, Salvasan, that although with a modest effect and some side effects was the first sign of therapy against bacterial infection and could be within reach of Medicine.

This was the situation when in 1921, Gerhard Domagk, then a young physician, had started working at the City Hospital in Kiel. There, besides his clinical activity, he was doing research with a dedication, since a very early stage, to the study of the reticulo-endothelial system (RES) which had been described by Aschoff shortly before. In 1923, his growing interest on Pathological Anatomy, made him acquainted with Prof. Walter Gross, director of the Institute of Pathology of Greifswald University, who invited him as his assistant. Domagk accepted and in spite of the difficult financial conditions, resulting on the rampant inflation, in Germany at the time that would restrain the resources for research, he has resumed with enthusiasm the task of clarifying the problem which was still seducing him: the importance of the RES. It was when making experiments in splenectomised rats that he observed, for the first time, what he thought to be a new phenomenon, but that he himself verified afterwards that have been already described: red blood cells phagocytosis by the cells of the liver endothelium

and by Kupffer cells.

From then onwards, Domagk started taking an interest on the study of degradation and phagocytosis by bacteria. In infected rats with *Staphylococcus*, he has succeeded to demonstrate that the RES cells would phagocyte micro-organisms while nearby and at the same time amyloid substance would be accumulated. However when previously he was carrying on the desensitization of animals or making coccus fragile, phagocytosis phenomena would intensify and involve the activity of other cells, namely mononuclear monocytes. These findings induced in Domagk the idea that there could be substances capable of acting upon bacteria, making them more vulnerable to organic defenses, in the end, it was a mitigated form of “chemotherapy” of bacterial infections.

The results of such research were published in “*Virchows Archiv fur Pathologische Anatomie und Physiologie*” with this title: “The RES importance in destroying the pathogenic agents and the origin of the amyloid substance”.

Meanwhile in 1925, Prof. Walter Gross was invited to take his seat in Münster University and Domagk followed him, certainly without crossing his mind that 60 years later, the Pathology Institute where he was working in, would receive his name. There Domagk was in charge of a laboratory where he had the opportunity of carrying on developing the experimentation in the area of bacterial infections and tumors.

In a country as Germany, which was then at the forefront of the research and technological progress, Domagk's work could not be unnoticed. Therefore it's not surprising that in 1927, Prof. Heinrich Hörlein, who was directing the pharmacological research of I.G. Farbenindustrie in Elberfeld, had invited him to set up and run an Institute of Pathology and Experimental Bacteriology.

In Elberfeld, Domagk has initially focused his attention on the same issues he had been working on in Münster: the fight against infectious disease and malignant tumors. But his first attempts were a total failure. They consisted in studying the influence, on bacteria, of certain proteins extracted from the RES of healthy animals. It was after this failure that he decided to concentrate his attention on the understanding of chemical compounds capable of damaging bacteria and making them more vulnerable to the natural mechanisms of defense.

It started then by imagining an experimental mo-

del appropriate to his research: infected rats with a viral strain of *Streptococcus* obtained from patients with sepsis. Using such method, he could then observe the histological changes caused by the infection and evaluating to which point the tested substance would reveal the capacity to damage or destroy bacteria.

It was at that time that he started this close cooperation with two Bayer chemists: Fritz Mietzsch and Joseph Klarer. They synthesized hundreds of compounds delivering them to Domagk, who would evaluate their antibacterial properties, first *in vitro* and than in infected mice. It was a thorough and methodical work, involving a great number of substances, heavy metal salts, arsenium and antimony compounds, acridine derivatives, etc. But the initial results were disappointing. Sometimes, substances would reveal antibacterial activity but would have severe side-effects; others would act *in vitro* but its efficacy in infected animals was none.

In spite of that, Domagk persevered and in 1931, started trying a group of azoic stains with a chemical structure similar to acridine. Such stains had a special feature that would make them different from others: they had a sulphonamide radical, considered responsible for the strong adherence to wool proteins, reason why they were so appreciated by the technicians of the textile industry.

When the compound of such series was given to him with the code KL 695, Domagk verified that *in vitro*, it had no activity against the *Streptococcus* but even so, he decided to try it in infected rats. Why? It might be a moment of inspiration, or the stubbornness of a researcher who would not give up facing the first failure? The most likely is that Domagk had just complied with his initial idea that antibacterial agents would limit to weakening and making fragile micro-organisms, creating the conditions for the natural mechanisms of defense to act effectively. Therefore it would not be odd to find that its activity would reveal itself exclusively *in vivo*. The truth is that KL 695, apart of being well tolerated, had in mice an antibacterial action much higher than any other substance previously studied.

Facing such results, Domagk kept on researching and decided to experiment KL 730, another azoic stain with a much smaller molecule, initially known as *Streptozone* and later registered as *Prontosil rubrum*. Just five days to Christmas 1932 when he performed a decisive trial in two groups of rats, infected with

lethal doses of streptococcus. At 48 hours, the rats injected with KL 730 30 were still alive and active, while the rats in the control group were dead. Besides, no bacteria or any lesion on the tissues of the treated rats was found. Domagk has repeated several times this experiment and always got the same results. Ehrlich's old dream of one day to find a chemical substance with bacteriostatic activity and neglectable side-effects had come true.

However in spite of being so important such findings were kept in the highest of secrets for two years, for reasons never quite clear. Some think that Domagk did not want to take risks and has attempted to confirm his own results while, at the same time, waiting the clinical trials with Prontosil to be carried out. It was speculated that this pause of two years would not be motivated by delays on registering the patent. But a patent for what? For Prontosil? Or has Domagk suspected that the bacteriostatic action did not reside in the stain, but in one of the radicals and he was trying to gain some time until this question was clarified?

Whatever the explanation, the truth is that Domagk's discovery was only published in 1935 together with two clinical trials, one from Klee and Römer and another by Schreus, demonstrating unequivocally Prontosil efficacy.

The impact caused in the world of medicine was enormous and in France, physicians of the Pasteur Institute, headed by Tréfouël, immediately started studying the new substance. The first misfortune of this history of sulphonamide happened then: different from what Ehrlich envisaged and what apparently Domagk thought, the French researchers have demonstrated that in fact the bacteriostatic action was not due to the stain but to the sulphonamide radical, which while separating of the organism, in Prontosil molecule, would be free to act against bacteria. Sulphonamide had already been synthesized by the Austrian chemist, Paul Gelmo, in 1908 and therefore could not be patented.

Of course none of this would withdraw merit to Domagk's extraordinary discovery, which was only possible thanks to the persistence, the professionalism and the rare intuition of a great scientist. Therefore it was not a surprise that in 1939, Stockholm Academy would decide to award him with the Nobel Prize of Medicine.

But another misfortune happened: Hitler, annoyed

with the fact that in 1936 the Nobel Peace Prize had been awarded to Carl von Ossietzky, the editor of the liberal and pacifist newspaper, *Weltbühne*, has decreed that in the future no German would be authorized to accept the prize. Domagk had therefore to wait until the end of World War II and in 1947 to be awarded the Diploma and the Medal but not the money prize: this according to the norms had already been incorporated in the Academy funds.

Meanwhile, Prontosil would trigger the first big revolution fighting infectious diseases. Its efficacy was revealed not only against the streptococcus, but equally against the pneumococcus, gonococcus, meningococcus and many other bacteria. For history purposes, two cases treated at the time with total success were recorded: Domagk's own daughter, carrying a sepsis contracted in the sequence of a needle puncture and at risk of having an upper limb amputated; and the son of President Franklin Roosevelt, suffering of a severe purulent lymphangiitis.

In the following years, Domagk and Prontosil received 60 international prizes (among which the Grand Prix of Paris World Exhibition, in 1937) and were object of several tributes. It was the acknowledgement of the importance of such discovery, capable on its own of modifying the course of Medicine and History.

However another misfortune, the third, would cast a shadow on the sulphamide's history relegating them to a secondary place that actually they did not deserve. In 1941, Florey and Chain, after researching for two years the phenomenon of *Penicillium notatum* antibiosis on the staphylococcus described by Fleming, were making their first therapeutical trials with penicillin. The results were nothing but amazing: a yellow liquid, produced by a fungus, would heal in a few hours severe bacterial infections and besides would reveal important advantages. On one hand it had a bactericidal effect much more powerful and included among its victims the terrible staphylococcus which had escaped sulphamide action; and on the other hand, it seemed not to have any side effects (sulphamide, in spite of being relatively harmless, had revealed in some cases undesirable effects of certain severity). Medicine would enter this way in the antibiotic era with the vast panoply of pharmacological groups that would come to exist: aminoglycosides, tetracycline, chloramphenicol, macrolides, semisynthetic penicillin, cephalosporins, monobactam and thienamycin.

However in the decades following Domagk's discovery, sulphamide would come to know a golden period. Several groups of researchers threw themselves immediately in the search for sulphonamide derivatives: in England sulphapyridine emerged (1938) and in the USA, the sulphathiazole (1939). In 1940, sulphadiazine was introduced, replacing the previous ones and in 60s was still used with great efficacy in the treatment of bacterial pneumonia and meningococcal meningitis. Several changes on the molecule and the addition of new radicals have succeeded to improve solubility, absorption and time of action. Some commercial preparations even acquired enormous popularity, as it was the case of Madribon® (sulphadimethoxine) that for many years has deserved the special preference of our pediatricians.

After a period of highlight, the decline of sulphamide was inevitable. In the 70s, the knowledge of the mechanism of action has led to the association of an anti-malaria agent, trimethoprim that would potentially increase its antibacterial effect. This is how a sulphamide, sulphamethoxazole is still appearing in the pharmaceutical forms, now hidden under the registered name of co-trimoxazole. Apart of this, just another one is mentioned, sulphametizol recommended for the treatment of urinary infections by the coli bacilli. And nothing else.

Obviously this does not withdraw any part of the merit to Domagk's outstanding discovery. By demonstrating the bacteriostatic activity of Prontosil, after years of patient and obstinate work in his Elberfeld laboratory, he made real Ehrlich's old dream turning one of the most brilliant pages in the History of Medicine. ■

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