Magic bullets and moving targets: antibiotic resistance and experimental chemotherapy, 1900-1940

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SUMMARY: 1.—Histories of antibiotic resistance. 2.—A therapy with side effects. 3.—The impact of resistance. 4.—A «wholly mysterious phenomenon». 5.—A revival. 6.—Conclusions.

ABSTRACT: It was in the 1940s that antibiotic resistance arose as an object of study for clinical medicine. Somewhat earlier it had become an important analytical tool for bacterial geneticists. However, the concept of antibiotic resistance as an induced and inheritable trait of microbial species was introduced a generation earlier in the years preceding the First World War. The paper reconstructs the concept that was put forward by the German immunologist Paul Ehrlich in 1907. He came across the phenomenon when trying to develop chemotherapies for trypanosomiasis, the best known of which is African sleeping sickness. However, resistance was studied by him for other than therapy-related purposes. It provided a productive laboratory model for the study of cell functions. Induced resistance to chemicals facilitated the development of ideas on the relation of a parasite's cellular metabolism and of drug action, i.e. by providing a negative proof for the existence of chemoreceptors on the surfaces of parasite cells. This approach does also serve to explain why British and German researchers continued to study the phenomenon of induced resistance in microbes for decades —despite it being absent from clinical medicine. After all, there existed very few chemotherapies of infectious diseases prior to the arrival of the sulfa drugs. Moreover, resistance to such medicines was rarely observed. However, being part and parcel of Ehrlich's theories, his views on resistance were also criticised together with these. It was in particular Henry Dale who would challenge Ehrlich's views of resistance being an inheritable and stable trait of microbes. Instead he insisted that understanding this «wholly mysterious phenomenon» required taking into account some host interaction. Induced resistance, which had come into being as a chance discovery on the chemotherapy of sleeping sickness, thus became one of the more important laboratory models of twentieth-century immunological research. Its early history is largely discontinuous with later work, and antimicrobial resistance as it evolved from 1900 to 1940 followed other trajectories than those which became relevant after 1940.

KEY WORDS: Antibiotic resistance, trypanosomiasis, atoxyl, Paul Ehrlich, drug receptors.

PALABRAS CLAVE: Resistencia a antibióticos, tripanosomiasis, atoxyl, Paul Ehrlich, receptores.

1. Histories of antibiotic resistance

Few historical phenomena seem so closely linked as the histories of twentieth-century anti-infective chemotherapy and the history of antibiotic resistance. Recent histories of sulfa drugs and antibiotics have left no doubt that the arrival of such medicines from the mid 1930s on was followed quickly by that of resistant strains of the microbes targeted by such medicines[1]. Yet, the history of this phenomenon had other dimensions and we should be cautious of associating it only with histories of pharmacology, clinical medicine and public health. An important but neglected example is the place of antibiotic resistance in the history of bacterial genetics from the 1930s, which pre-dates its occurrence in clinical medicine[2]. In this paper I use the term «antibiotic resistance» in its widest sense to include synthetic chemicals, like arsenical and the sulfa drugs, as well as naturally occurring substances, such as penicillin and streptomycin. I argue that before the 1940s, resistance for most physicians meant bodily resistance, e.g. the organism's capacity to withstand an infection. Germany's leading handbook of infectious diseases would, in 1930, define the term in that way. In relation to drug-induced, inheritable traits of microbes, the author, himself being an adherent of Paul Ehrlich's immunology, would speak of secondary resistance[3]. Others would use Ehrlich's original term «Festigkeit»[4] or its

1. On antibiotics: Bud, Robert. Penicillin: triumph and tragedy. Oxford: Oxford University Press; 2007; Lesch, John E. The first miracle drugs: how the sulfa drugs transformed medicine. Oxford: Oxford University Press; 2007. On resistance: Summers, William C. Microbial drug resistance: a historical perspective. In: Wax, Richard G.; Lewis, Kim; Salyers, Abigail A.; Taber, Harry, eds. Bacterial resistance to antimicrobials. Boca Raton: CRC Press; 2008, p. 1-9; Straand, Jørund; Gradmann, Christoph; Lindbæk, Morton; Simonsen, Gunnar Skov. Antibiotic development and resistance. In: Heggenhougen, Kris; Quah, Stella, eds. International encyclopaedia of public health. San Diego: Academic Press; 2008, p. 200-211.
2. Brock, Thomas D. The emergence of bacterial genetics. Cold Spring Harbour: Cold Spring Harbour Laboratory Press; 1990; Creager, Angela N. H. Adaption or selection? Old issues and new stakes in the postwar debates over bacterial drug resistance. Studies in History and Philosophy of Biological and Biomedical Sciences. 2007; 38: 159-190.
3. Schlossberger, Hans. Chemotherapie der Infektionskrankheiten. In: Kolle, Wilhelm; Kraus, Rudolf; Uhlenhuth, Paul, eds. Handbuch der pathogenen Mikroorganismen. Jena-Berlin-Wien: Gustav Fischer-Urban und Schwarzenberg; 1930, p. 551-730.
4. Fischl, Viktor; Fischl, Lili. Arzneifestigkeit, Avidität, Interferenz. Zeitschrift für Immunitätsforschung und experimentelle Therapie. 1934; 83: 324-335.

English translation as fastness or drug resistance[5]. Experimental medicine would not use the term to describe a trait of a microbe before the 1930s[6]. It was only in specialised immunology, pharmacology and genetics that the term appeared in this meaning with some regularity.

Thus, antibiotic resistance, though only known to a few, was not a new concept in the 1940s. A literature review published in 1944 holds a few surprises[7]. While the authors did consider questions that we would expect to be discussed, for example, whether resistance to chemicals as a trait of microbial species results from mutation or selection, they did not do so with respect to bacterial species we would expect to be addressed by clinicians or geneticists like *Staphylococcus aureus* or *Escherichia coli*[8]. Instead the review deals almost exclusively with a certain genus of unicellular parasites, trypanosomes, some of which are known as the pathogen of human trypanosomiasis. In relation to the various species of trypanosomes —and this is the real surprise— the authors point to some 40 years of research and a long list of resistances to various chemicals that could be artificially induced in these organisms. On top of this, the authors do not mention that this work had any clinical relevance in relation to the many trypanosomiases of men and animals, such as sleeping sickness, Chagas disease or Ngana.

So why was antibiotic resistance studied? To answer this question, I will reconstruct the historical situation in which drug resistance in trypanosomes was first observed. I will then follow the development of what seems to have been a sustained interest for decades and finally ask how this research contributed to the more familiar debates about resistance as they

5. Browning, C. H.; Gulbransen, R. The treatment of relapses in experimental trypanosome infections: cures after repeated relapses without increasing the dose of the chemotherapeutic agent. Journal of Pathology and Bacteriology. 1928; 31 (1): 134-136.
6. If we take the *Journal of Bacteriology* as an example, the term resistance is fully absent in the 1920s, in the 1930s it would appear in two different meanings related to either bodily or bacterial resistance. Only from the 1940s would it be exclusively employed to describe a trait of a microbe. Moberg, Carol L. Launching the antibiotic era. Personal accounts of the discovery and use of the first antibiotics. New York: The Rockefeller University Press; 1990, p. 563.
7. Eagle, Harry; Magnuson, Harold J. The spontaneous development of arsenic-resistance in Trypanosoma equiperdum, and its mechanism. Pharmacology and Experimental Therapeutics. 1944; 82 (2): 137-151.
8. An introduction in: Summers, n. 1. Clinical researchers in those days would e.g. focus on gonococci where resistance had been resulting from therapy with sulphonamides (Lesch, n. 1, p. 277). Fleming and Florey would observe it for example in staphylococci when working with penicillin (Bud, n. 1). Bacterial geneticists would try to build laboratory models of mutation with bacteria such as Escherichia coli (Creager, n. 2).

evolved from the 1940s. All this will be based on the guiding hypothesis that antimicrobial resistance as it evolved from 1900 to 1940 followed other trajectories than those which became relevant later on.

2. A therapy with side effects

There are good reasons to place the beginning of our story in the year 1905, when at the Liverpool School of Tropical Hygiene two researchers, Anton Breinel and Harold Wolferstan Thomas, observed that the arsenical compound Atoxyl was capable of controlling the growth of trypanosomes[9]. Atoxyl, an ostensibly non-toxic arsenical, had been synthesised in the 1880s and had so far enjoyed a somewhat inconspicuous career in the treatment of, for example, syphilitic disorders of the skin[10]. Trypanosomes, which had been known for a few years had only two years earlier been identified as the pathogens of sleeping sickness[11]. Following that there had been a rush to explore possibilities for their control and the two Liverpool researchers had observed that the parasites were vulnerable to the substance[12]. From then on the drug followed two interconnected trajectories. The first of these is not so interesting for us. It was a short lived euphoria connected to Atoxyl as a remedy for sleeping sickness. From 1906 on it was tested on several expeditions in Africa. Yet Atoxyl did not fully live up to the therapeutic expectations: What it brought about was a slowing down of the progression

9. Thomas, H. Wolferstan. Some experiments in the treatment of trypanosomiasis. British Medical Journal. 1905; 2317: 1140-1143. Dale, Henry H. Chemotherapy. Physiological Reviews. 1925; 3 (3): 359-393, here p. 373
10. On Atoxyl: Riethmiller, Steven. Ehrlich, Bertheim, and Atoxyl. Bulletin of the History of Chemistry. 1999; 23: 28-33.
11. Lyons, Maryinez. African Trypanosomiasis. In: Kiple, Kenneth F., ed. The Cambridge world history of human disease. Cambridge: Cambridge University Press; 1993, p. 552-561. David Bruce has been credited with that discovery, but not without dispute: Boyd, John. Sleeping sickness: the Castellani-Bruce controversy. Notes and Records of the Royal Society of London. 1973; 28: 93-110
12. There had been predecessors starting with arsenical acid in 1899, but they were either less effective or too toxic (or both) (Dale, n. 9, p. 373). Pearce, Louise; Brown, Wade H. Experimental trypanosomiasis: its application in chemotherapeutic investigations. The Journal of Experimental Medicine. 1918; 28: 109-147 for an overview of substances used in experimental research on trypanosomes.

of an otherwise still 100% lethal disease[13]. On top came violent side-effects. For lack of alternatives and a few other reasons it enjoyed a certain popularity, but around the First World War it was pushed aside by more effective medicines, most notably Bayer 205, which became known as Germanin[14].

The second trajectory started in one of the institutions where atoxyl was studied, Paul Ehrlich's Institute for Experimental Therapy in Frankfurt. Yet, here the perspective was different from what researchers in Africa focussed on. Whereas, for example, Robert Koch more or less restricted himself to therapeutic testing of atoxyl on the shores of Lake Victoria[15], Ehrlich pursued a research project in which fundamental immunology and therapy were intimately connected. To understand how research on resistance became part and parcel of such a research program it is useful to remind ourselves of the essential steps of its development. Ehrlich had focussed his early work on histological staining. For that purpose he had employed certain synthetic dyestuffs, known as aniline dyes. Such dyes had two peculiar properties that influenced their further career. First, they were synthetic chemicals and well defined standardised industrial products, implying that anything that was done by their application seemed easily reproducible. Secondly, they had specific affinities for (prokaryotic) bacterial cells, while leaving (eukaryotic) bodily cells unstained. In his dissertation of 1885 Ehrlich had put forward an explanation of such staining that took it to be a physiological rather than a physical process. The event of staining was thus assumed to be bound up with a metabolic intake of colours by a given cell[16]. It was but a small step to research the pharmacological qualities of such phenomena: in 1891 Ehrlich described the application of methylene blue for the staining of the pathogen of malaria, *Plasmodium falciparum* and subsequently tried it as a possible therapy on malarial patients.

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13. Scheube, B. Die Krankheiten der warmen Länder. Ein Handbuch für Ärzte. 3 ed. Jena: Gustav Fischer; 1910 (1896). A curative effect was possible for very early stages that were hard to diagnose, however.
14. Eckart, Wolfgang U. Medizin und Kolonialimperialismus in Deutschland 1884-1945. Paderborn: Schöningh; 1997, p. 505-13. Tropenabteilung, Bayer Leverkusen. Die Chemotherapie der Schlafkrankheit. Leverkusen: Bayer; 1938. Lyons, Maryinez. The colonial disease: a social history of sleeping sickness in northern Zaire, 1900-1940. Cambridge: Cambridge University Press; 1992.
15. Gradmann, Christoph. Laboratory disease: Robert Koch's medical bacteriology. Baltimore: Johns Hopkins Universiy Press; 2009: 213-225.
16. Ehrlich, Paul. Das Sauerstoffbedürfnis des Organismus. Eine farbenanalytische Studie. Diss. Med. Medical Faculty, Friederich-Wilhelms-Universität zu Berlin; 1885.

It was about ten years later, while developing his side-chain theory of immunity, when Ehrlich ventured into making more systematic statements on antibiotic chemotherapy[17]. He proposed that relations between therapeutic molecules and microbes actually resembled those between toxins and antitoxins respectively: So-called chemoreceptors on the surface of microbial cells would facilitate the intake of therapeutic molecules, which would then eventually have an effect on these cells[18]. The main advantage of such a therapy lay, so he stated, in its specificity. Ideally, just as infectious diseases were caused by single bacterial species, these would in turn be susceptible to medicines that would selectively target those cells. In 1906, Ehrlich famously made his claim about the possibility of «magic bullets» that killed germs but not host cells.

> «If we picture an organism as infected by a certain species of bacterium, it will obviously be easy to effect a cure if substances have been discovered which have an exclusive affinity for these bacteria and act deleteriously or lethally on these alone, while at the same time they possess no affinity for the normal constituents of the body and can therefore have the least harmful, or other, effect on that body. Such substances would then be able to exert their full action exclusively on the parasite harboured within the organism and would represent, so to speak, magic bullets, which seek their target of their own accord»[19].

His thinking here can usefully be understood as a «theory of drug action», analogous to his side chain theory of immunity.

It was from 1904 that Ehrlich directed his work towards the development of such therapies. His choice of experimental object may seem strange, since he chose the parasitic pathogens of vector-borne tropical infections, trypanosomiases in particular. Yet these unicellular parasites suited his interests rather well. First of all, two French researchers, Laveran and Mesnil, had

17. For introductions see Prüll, Cay-Rüdiger; Maehle, Andreas-Holger; Halliwell, Robert Francis. A short history of the drug receptor concept. Houndsmills, Basingstoke: Palgrave; 2009, p. 16-40; Silverstein, Arthur. Paul Ehrlich's receptor immunology: the magnificent obsession. San Diego: Academic Press; 2003.
18. Ehrlich's relevant papers are in volume 3 of his collected papers, Ehrlich, Paul. Gesammelte Arbeiten. Zusammengestellt und herausgegeben von F. Himmelweit. 3 vols. Berlin-Göttingen-Heidelberg: Springer; 1956-1957.
19. Ehrlich, Paul. Address delivered at the Dedication of the Georg-Speyer-Haus. In: Himmelweit, Fred, ed. The collected papers of Paul Ehrlich. London: Pergamon Press; 1960 (1906), p. 53-63 (59).

recently demonstrated that the blood of mice infected with animal trypanosomiasis (Ngana) could be cleared of these by the injection of an arsenical that was known as Fosters solution[20]. Thomas' and Breinel's discovery of the effect of Atoxyl on these parasites followed suit and thus there were good reasons to see trypanosomes as promising candidates to build a laboratory model of the chemotherapy of infectious disease. They responded well to toxic chemicals[21], they were easy to cultivate, and as unicellular organisms they offered bigger, more distinct cell-functions than bacteria.

Ehrlich tested a large number of chemicals and the first effective preparation he came up with was a red synthetic dye, which subsequently became known as trypan red[22]. Even though this constituted a working animal model of therapy through its success with mice, it was only of theoretical value as it turned out that it was not effective against varieties of trypanosoma pathogenic to humans[23]. Trypan red was suited to demonstrate the principle of specific chemotherapy, but was unlikely to have any practical relevance.

The second preparation that Ehrlich came up with was of a more traditional design. The arsenical atoxyl had been synthesised in the 1880s. As I have mentioned, Ehrlich had not even discovered its suitability to treat trypanosomiasis himself, yet he delivered a new chemical formula of the substance and working on this chemical took his research a large step forward[24]. It was on 4 March 1907 that he delivered a paper in Berlin about his experimental studies on trypanosomes that had by then occupied him for almost three years. Together with Kiyoshi Shiga, he had succeeded in identifying a whole series of chemicals that were effective in animal experiments. By doing so, they had eventually come across a bewildering phenomenon: susceptibility to such preparations could eventually drop and even disappear altogether after their prolonged application. This kind of reduced susceptibility was obviously a property of the pathogen, since it

20. Pearce; Brown, n. 12, p. 110.
21. Better than most bacteria: Ehrlich, Paul; Shiga, Kiyoshi. Farbentherapeutische Versuche bei Trypanosomenerkrankung. In: Himmelweit, Fred, ed. The collected papers of Paul Ehrlich. London: Pergamon Press; 1960 (1904), p. 24-37 (24).
22. Ehrlich; Shiga, n. 21.
23. On the testing of various anti-trypansomal preparations by Robert Koch in East Africa: Koch, Robert. Schlußbericht über die Tätigkeit der deutschen Expedition zur Erforschung der Schlafkrankheit. In: Schwalbe, Julius, ed. Gesammelte Werke von Robert Koch. Leipzig: Verlag von Georg Thieme; 1912 (1907), p. 534-546 (542). Gradmann, n. 15, p. 221.
24. Riethmiller, n. 10.

could be inherited over generations of the parasites and even transferred together with the manipulated microbes between different laboratory animals at will. The first observation of that sort had been made in relation to fuchsin, a red dye:

> «There must have been produced a fuchsin-fast or «fuchsin-resistant» strain. I have chosen this expression because I found that such a resistance, once acquired, appears to remain unaltered [...]»[25].

Upon further investigation, it turned out that the phenomenon could be induced by a host of other chemicals. Ehrlich suspected that it would eventually occur with any chemical effective against trypanosomes:

> «Judging from our experience this should be a general phenomenon. In the likely event that more trypan-hostile chemicals will be revealed, it is also very likely that it will be possible to produce strains that are resistant to these»[26].

3. The impact of resistance

By the time induced drug resistance was first observed, it was a laboratory event in the sense that it was not observed outside of this environment. The phenomenon as Ehrlich could produce it was, for example, not noted by Robert Koch in East Africa, when he treated a large number of patients on Lake Victoria[27]. What appear to be irreconcilable observations can be explained by the rather different setups of experimental therapy in Frankfurt and East Africa. Resistance in Ehrlich's laboratory had been the result of a carefully monitored therapy that evolved over months. Koch, however, had usually confined treatment to massive shots of the medicine administered over just a few days. His patients would usually run from his camp anyway as soon as they felt better; without follow ups there was little chance of observing the phenomenon. Curiously enough, however, Ehrlich's and Koch's ideas on the subject even clashed in a single event. In Koch's institute in

25. Ehrlich, Paul. Chemotherapeutische Trypanosomenstudien. Berliner Klinische Wochenschrift. 1907; 44: 233-236; 280-283; 310-314; 341-344; 349-50. Ehrlich's collaborator Röhl had taken the resistant strain through 36 generations and observed no alteration in its qualities (p. 341).
26. Ehrlich, n. 25, p. 314.
27. Gradmann, n. 15, p. 213-225

Berlin a laboratory servant had incidentally been infected with sleeping sickness in August 1906. With Koch away in East-Africa, his treatment was largely guided by Ehrlich's proposals. An atoxyl treatment was tried and half a year later, parasites turned out to have been made resistant. After this unwelcome discovery, which actually occurred almost simultaneously with Ehrlich's paper on chemotherapy being presented in Berlin in February 1907, the patient's body became a laboratory tool for Ehrlich's pharmacopeia of synthetic dyestuffs of which a number were tested on the patient subsequently. Koch however, after his return to Germany, when presented with the phenomenon, insisted that he had never encountered it. For him, doctors in Germany had simply been using an inappropriate scheme of therapy instead of following the one he had recommended in his letters from Lake Victoria. He concluded that there was no such thing as drug resistant trypanosomes and consistently resumed atoxyl therapy which, he claimed, cured the patient[28].

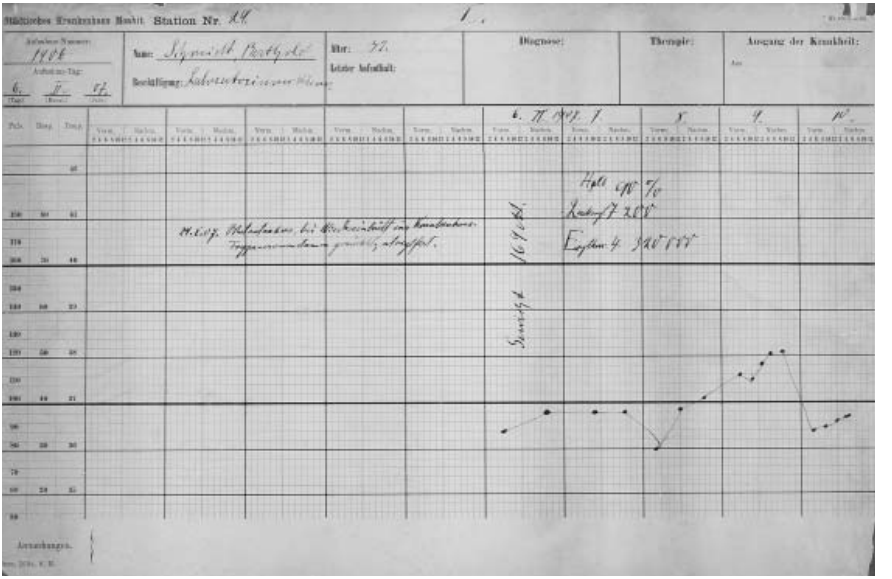

Figure 1. Medical records of B Schmidt with (translated) comment: «24.1.07. Blood smear taken upon re-entry to hospital. Trypanosome strain cultivated; resistant to atoxyl». Source: Robert Koch Institute, Berlin.

28. Gradmann, n. 15, p. 155-166.

Of course, resistance indicated the limitations of atoxyl as a therapy for sleeping sickness. Ehrlich was, however, also deeply intrigued by the phenomenon in its own right. Resistance had some peculiar qualities that made it possible to investigate what he called the «therapeutic biology of the parasite»[29]. Once acquired, it was an inheritable trait. It was also exclusively a trait of the parasite, since it could be transferred together with the latter at will between various individual animals and even species, including humans. It was also specific in the sense that it would be confined to one chemical substance or sometimes closely related groups of these.

But what did this peculiar discovery actually mean for contemporary medicine? If we consider antibiotic resistance as a clinical phenomenon, which has to be taken into account in the treatment of individual patients, the relevance was close to nothing. Ehrlich's interest in atoxyl as a medicine for trypanosomiasis ceased in 1907 and this can be attributed to the above mentioned limitations of this kind of medicine, but also to the instance that severe side effects of this medicine, such as loss of eyesight, had become public[30]. In this situation, Ehrlich himself took his interest in antibiotic chemotherapy from sleeping sickness to syphilis and from trypanosomes to spirochetes. As is well known, this resulted in the development of Salvarsan, which was marketed from 1910 on[31]. However, no such thing as a resistant strain of the *Spirochaeta pallida* to Salvarsan ever turned up[32]. Moreover, in quite a contrast to what everybody expected, Salvarsan was not first of a series of effective chemotherapies that would target common infectious diseases other than syphilis. Instead this medicine and its modifications remained the only effective antibacterial chemotherapy for almost 30 years, that is, until the arrival of the first sulfa drugs in the mid 1930s[33]. Once it had been observed, resistance did not gain importance as a clinical phenomenon. Not even in relation to the parasitic vector-borne infection of sleeping sickness, which was after all one of the major public health issues in colonial Africa, did resistant trypanosomes continue to be of some relevance. The reason is that in the treatment of sleeping sickness

29. Ehrlich, n. 25, p. 313.
30. Gradmann, n. 15, p. 219; Tropenabteilung, n. 14, p. 31-34.
31. On the introduction of Salvarsan: Sauerteig, Lutz. Ethische Richtlinien, Patientenrechte und ärztliches Verhalten bei der Arzneimittelerprobung 1892-1931. Medizinhistorisches Journal. 2000; 35: 303-334.
32. Tropenabteilung, n. 14, p. 63-71.
33. Lesch, n. 1.

atoxyl was soon pushed aside by Tryparsamid and right after the First World War by Bayer 205, better known as Germanin[34]. Both of these proved to be potent medicines which also had the advantages of fewer side effects and not inducing resistance in the trypanosomes. Atoxyl, even though it continued to be used, was now an outdated, yet cheap therapy with known side-effects.

However, Ehrlich, after losing interest in the therapy for sleeping sickness, continued his interest in the resistant trypanosomes. He returned to the issue in several papers around 1910 which discussed the questions of chemotherapy and immunity[35]. At the same time the phenomenon became generally known and it was for instance reproduced in bacteria such as pneumococci[36] and two researchers even wrote on laws of resistance[37]. To understand the fascination in the phenomenon we need to examine more closely why Ehrlich was so intrigued by it. A paper delivered in 1909 provides us with some insight: «The discovery of resistant strains and their inspection in detail turned out to be the most valuable means to study the truly intimate chemical structure of the parasite»[38]. Ehrlich's theory of drug action linked chemical compounds to those parasite cells with suitable chemoreceptors on their surface, in much the same way by which toxins linked to antitoxins. Yet, it was not an easy task to find any evidence for the existence of such sites, let alone to describe single receptors in more detail[39]. Antibiotic resistance, however, provided a means of doing precisely this. Antibiotic chemotherapies had been envisioned as an

34. Tropenabteilung, n. 14.
35. Ehrlich, Paul. Über Partialfunktionen der Zelle. In: Himmelweit, Fred, ed. The collected papers of Paul Ehrlich. London: Pergamon Press; 1960 (1909), p. 171-182; Ehrlich, Paul; Gonder, Richard. Experimentelle Chemotherapie. In: Himmelweit, Fred, ed. The collected papers of Paul Ehrlich. London: Pergamon Press; 1960 (1914), p. 559-582.
36. Marks, Lewis H. Über einen arsenfesten Bakterienstamm. Zeitschrift für Immunitätsfoschung. 1910; 6: 293-298. In the *Zeitschrift für Immunitätsforschung* around 1910 several such papers by Constantin Levaditi from the Institut Pasteur are reviewed.
37. Morgenroth, J.; Rosenthal, F. Experimentell-therapeutische Studien bei Trypanosomeninfektionen. III. Mitteilung. Arzneifestigkeit der Trypanosomen gegenüber Verbindungen der Hydrocupreinreihe. Zeitschrift für Hygiene und Infektionskrankheiten. 1912; 71: 501-535.
38. Ehrlich, Paul. Über die neuesten Ergebnisse auf dem Gebiet der Trypansosomenforschung. In: Himmelweit, Fred, ed. The collected papers of Paul Ehrlich. London: Pergamon Press; 1960 (1909), p. 195-212 (195).
39. On Ehrlich's side chain theory: Prüll, Cay-Rüdiger. Part of a scientific master plan? - Paul Ehrlich (1854-1915) and the origins of his receptor concept. Medical History. 2003; 47: 331-354, Prüll; Maehle; Halliwell, n. 17, p. 16-40; Silverstein, n. 17.

application of immunology in the first place. Yet, for the time being the traffic was in the opposite direction, as resistance became instead a tool to study mechanisms of immune response and address questions about the structure of cells. From 1910 to 1914, after he had suspended his work on the chemotherapy of sleeping sickness, Ehrlich published a whole series of papers on principal questions of chemotherapy, in all of which inducing resistances is introduced as a handy analytical tool. In his Nobel Prize lecture «On Partial Functions of the Cell», he came back to the issue and described induced resistance as the best means of substantiating his claim that specific receptors existed on the surface of cells[40]. The host of resistances that could be produced in trypanosomes made them the ideal model organism for studying the therapeutic biology of the cell.

The evidence that could be produced this way was manifold: on a very basic level, resistance supplied evidence to Ehrlich's theory of drug action that required a physiological uptake of therapeutic molecules by cells. Such observations were also confirmed for bacteria[41]. For example, given a certain quantum of medicine that was injected into a laboratory animal's bloodstream, this would disappear from the bloodstream if a susceptible pathogen was present. If resistance of this pathogen did arise, this uptake would gradually be suspended in a plausible relation to the strength of resistance. Secondly, the phenomenon of so-called cross-resistances could be exploited. If, for instance, induced resistance to arsenicals as a side-effect produced resistance to other, chemically unrelated, dyes that had not been applied, this led Ehrlich to the conclusion that a single receptor on the surface of the cell was susceptible to both of these substances. The arsenic-resistant strain had also consistently lost its ability to be stained by these dyes, an ability which, of course, it had possessed in its non-resistant form[42]. Resistance offered positive evidence for the existence of a device, namely the chemo-receptor, whose existence had so far been more or less theoretical.

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40. Ehrlich, Paul. Über Partialfunktionen der Zelle. In: Himmelweit, Fred, ed. The collected papers of Paul Ehrlich. London: Pergamon Press; 1960 (1909), p. 171-182 (178-179).
41. Levaditi, Constantin; Twort, C. Su la trypanotoxine du bacillus subtilis. La toxo-résistance. Comptes rendus Soc. Biol. 1911; 70 (18): 799 did so in relation to bacterial toxins using Bacillus subtilis as an experimental model.
42. Ehrlich, Paul. Über die neuesten Ergebnisse auf dem Gebiet der Trypansosomenforschung. In: Himmelweit, n. 35, 195-212 (209).

4. A «wholly mysterious phenomenon»

Resistance had by the First World War become firmly connected to Ehrlich's side chain theory of immunity and to his related views on chemotherapy. These, however, were not undisputed[43] and thus Ehrlich's views on resistance were an attractive point of attack for those who wished to challenge his theories at large. The quality of evidence that resistance offered depended to a large extent on the stability of the phenomenon as being inheritable, transferable, quantifiable and specific. This, however, was doubted almost immediately. In 1908 Mesnil and Brimont from the Institut Pasteur showed that the stability and degree of resistances did vary when the parasites were transferred between species. Resistance produced in mice could disappear in rats, but «re-emerged» if the parasites were re-transferred to mice[44]. While the stability of the phenomenon was reasserted in Ehrlich's group, it was through such work that the specificity now seemed less impressive. Of course, cross-resistances had been noted from the outset, but were considered to be rare. Yet, as Gonder and Kudicke noted in 1912, there were now many cases where resistance to one chemical led to the same phenomenon arising in connection with other unrelated chemicals[45]. Finally, the question of whether resistance arose from mutation and selection or adaption was open. While Ehrlich had opted for the first, he showed little interest in the issue as such; others, such as Constantin Levaditi, insisted that the issue was open and relevant[46].

Also it was unfortunate, and frequently pointed to, that there was in fact no action at all of arsenicals such as atoxyl on trypanosomes *in vitro:* an effect, however, which only took place *in vivo* was in any likelihood dependant on some host interaction. The nature of this was unknown and also poorly accounted for in Ehrlich's theory of chemotherapy, which centred on the interaction of parasite and drug[47]. It was from such observations that Henry Dale launched his fundamental critique of Ehrlich's chemotherapeutic

43. From the recent work by Prüll; Maehle; Halliwell, n. 17, p. 64-92 the impression can be gained that Ehrlich's view on immunity was a minority opinion in the interwar years.
44. This was demonstrated by Mesnil and Brimont in 1908, Dale, n. 9, p. 380.
45. Reported in Dale, n. 9, p. 381.
46. Levaditi; Twort, n. 41, reported in Zeitschrift für Immunitätsforschung. 1911: 419, 471. Pringsheim, E. Zeitschrift für Immunitätsforschung. 1912, 287-288.
47. The argument was put forward by Voegtlin and Smith. Dale, n. 9, p. 376.

theories in 1923[48]. Deconstructing resistance was an important part of it. The phenomenon, he conceded, did indeed stand out as evidence for Ehrlich's statements such as on specific affinities of chemicals to parasite cells and of the existence of chemoreceptors. However, substantial evidence suggested that things were ultimately more complicated. Transferability of resistance was not a general rule and cross resistances to chemically non-aligned substances were so widespread that Ehrlich's explanation was insufficient. Dale also stressed that the whole model of chemotherapy demanded an immediate interaction of drugs and parasite cells, which was far from proven in a model that worked only *in vivo* in important examples, such as atoxyl. All in all, while not doubting the existence of drug resistance as such, Dale turned it from a cornerstone of Ehrlich's theory into a «wholly mysterious phenomenon»[49].

5. A revival

One might expect that in the mid-1920s, ten years after Ehrlich's death and after having been subject to major challenges, resistant trypanosomes would cease to be important as laboratory models and would be relegated to the status of a curious phenomenon arising in strange parasites treated in strange ways. Yet, apart from Dale's critique, there were other factors lending stability. Beyond the standing of its followers, in German science[50] it came to be of importance that the program had been developed in co-operation with the pharmaceutical industry. The best documented case is the cooperation of the Bayer Company and Ehrlich's institute, where one of his assistants, Wilhelm Roehl, became head of the research department in 1911. In the following decades Bayer had a long-term involvement in synthetic remedies for tropical diseases, such as malaria and sleeping sickness. None of these produced resistance, but at Bayer no one doubted the phenomena itself: Roehl in the early 1920s stated his allegiance to Ehrlich's theory and reaffirmed that the phenomenon of resistance offered an outs-

48. Dale, n. 9.
49. Dale, n. 9, p. 381.
50. Fischl; Fischl, n. 4; Schlossberger, n. 3.

tanding way to understand drug action – even if it did not have immediate practical relevance[51].

Still it was from quite another angle that studies in drug resistance and the trypanosome model for their investigation was revitalised. From 1931 onwards, a group of researchers at the Liverpool School of Tropical Hygiene began to investigate the experimental treatment of trypanosomiasis and resistances in great detail. Their strategy was two-fold. Firstly, to reaffirm the central observations that Ehrlich had built upon (quantifiability, transferability, stability and hereditary character) and, secondly, to investigate the «weak spot», that is, to investigate more closely the stages in which the medicines were metabolised by parasite and host[52]. The group's work started with trypanosomes, but was gradually expanded to other parasites such as spirochetes. In the papers of Frank Hawkings, Frederick Murgatroyd and Warrington Yorke the sphere of interest was still very much experimental chemotherapy. It is surprising to see that there are no connections whatsoever of their work to the emerging field of bacterial genetics where antibiotic resistance was also exploited. What is important to see is that both approaches not only differed in focus, but also in the experimental models employed. While geneticists were investigating whether resistance in bacteria, such as *Escherichia coli,* resulted from mutation or selection within a bacterial culture, such questions were absent in the work of the Liverpool researchers who continued to employ the trypanosome model-system[53]. At the same time, the argument was put forward by the director of the trypanosome research centre in Entebbe/Uganda, Herbert Charles

51. In 1926 he delivered an address to the 6th Annual meeting of the Deutsche Pharmakologische Gesellschaft on «Theoretische Grundlagen der Chemotherapie» [theoretical foundations of chemo therapy], where he explicitly referred to Ehrlich's theory as a frame for Bayer's work on drug development. (Bayer archive/Leverkusen).
52. Yorke, Warrington; Murgatroyd, Frederick; Hawking, Frank. Studies in chemotherapy: V. Preliminary contribution on the nature of drug resistance. Annals of Tropical Medicine and Parasitology. 1931; 25: 351-358. This was followed by a whole series of papers in the following years, which were published in the *Annals of Tropical Medicine* down to 1938. Much of it was based on Frank Hawking's work, who in 1933 had delivered a thesis on induced drug resistance in trypanosome. Power, Helen J. Tropical medicine in the twentieth century: a history of the Liverpool School of Tropical Medicine 1898-1990. London and New York: Keagan Paul International; 1999: 90-93.
53. Borman, Earle K. Comparative studies on the natural and acquired resistance of certain strains of Escherichia Coli to the bacteriostatic and germicidal effects of cations. Journal of Bacteriology. 1932; 23: 315-329.

Lyndhurst-Duke, that trypanosome studies of this kind also had little if any relevance for clinical medicine. This was less motivated by the fact that there were enough medicines for sleeping sickness that did not induce resistance. What seems more important is that the Liverpool group had specialised in other varieties of parasite, *T. rhodesiense* and *T. equiperdum* rather than *T. gambiense* which causes sleeping sickness. Since, however, Atoxyl was still employed to some extent for various reasons, this was unfortunate. As Lyndhurst-Duke reminded his readers, it was far from clear whether drug resistance, as it was studied by the Liverpool group, could be equated with the phenomena that occurred in patients with sleeping sickness[54].

This type of criticism pointed to a feature that was characteristic for research into the chemotherapy of infectious disease from 1900 to 1940, namely its increasingly distant relationship to clinical medicine. It is in this sense no coincidence that, as it had been mentioned at the outset, the term resistance, understood as a trait of a parasite, had not found its way into the language of contemporary clinical medicine. Resistance for most meant bodily resistance e.g. the capacity of an organism to withstand an infection. Even in experimental medicine, it was only from the 1930s that the term resistance came to be employed to describe a trait of a microbe. Until those days such a usage would be confined to specialised immunology, pharmacology and genetics.

6. Conclusions

The early history of antimicrobial resistance was that of a chance discovery, resistance in trypanosomes, which was developed into a laboratory model delivering evidence to support Ehrlich's theory of chemotherapy. Trypanosomes had been popular in chemotherapeutic research before, yet resistance made them even more attractive, since there was no other known microbe in which such a multitude of resistances could be induced. Thus, an irritating phenomenon from the early days of chemotherapy became a laboratory model to study (parasite) cell functions in relation to immunity. That this exclusive status was diminished by the rise of bacterial genetics

54. Lyndhurst Duke, Herbert. The trypanosomes of man: their resistance to arsenical drugs. The Lancet. 1933: 553-557, Lyndhurst Duke, Herbert. Arsenic resistance in trypanosmomes, Letter to the editor. The Lancet. 1935: 903-904.

and by the arrival of sulfas and fungal antibiotics points to another reason for it being popular for 30 years: the fact that Ehrlich's chemotherapy program had not resulted in a long line of such medicines and that even in the case of those that existed, their effect could not be explained sufficiently. Even his former collaborator Browning commented in 1935 that Ehrlich's grand scheme had resulted in nothing but «four distinct malarials —six groups of trypanocidal compounds— many new antiseptics and an almost miraculous spirochaeticide»[55]. In the mid 1930s Ehrlich's chemotherapy looked like a once promising child that had died in junior boots. In this sense it seemed to share the fate of Ehrlich's side-chain theory of immunity to which it was closely linked. All in all, one should be careful not to interpret the early history of antibacterial chemotherapy from the perspective of the post-1940 period when it became a success story. Perceived from within, it was rather a period of disillusionment. In the absence of working therapeutic molecules, the study of chemotherapy in general developed into an ivory tower inhabited by immunologists and pharmacologists, and resistant trypanosomes were among the most popular toys in that setting.

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55. Quoted in Galdston, Iago. Some notes on the early history of chemotherapy. Bulletin for the History of Medicine. 1940; 8: 806-818 (817).