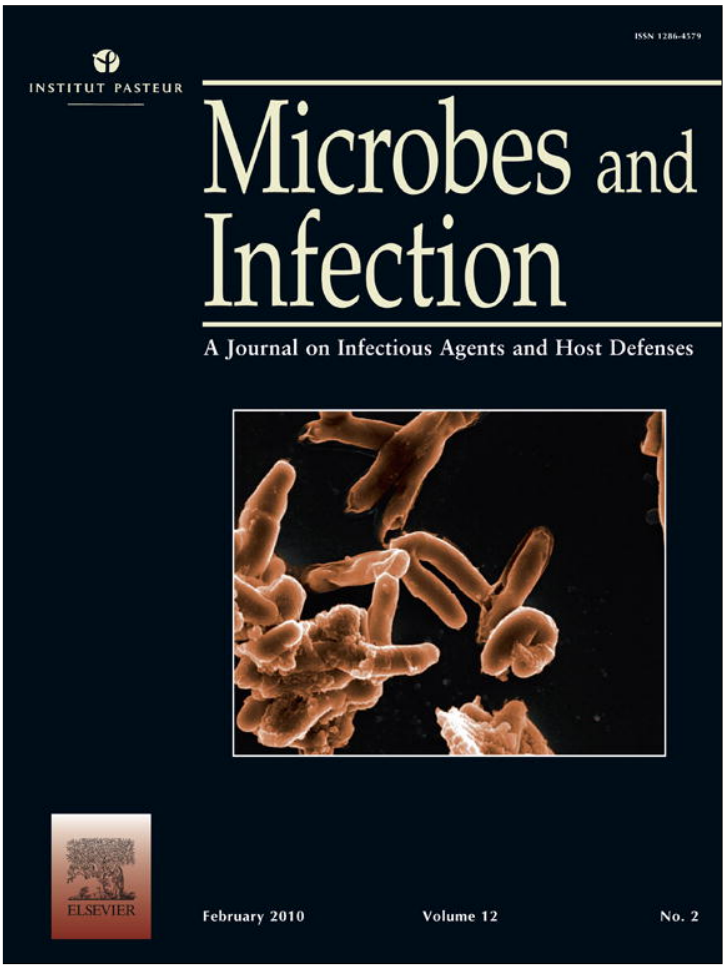

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Review

Tuberculin immunotherapy: its history and lessons to be learned

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Abstract

The use of tuberculin for the therapy of tuberculosis was attempted more than 100 years ago and abandoned because of its adverse reactions. In this historical review we point out that some of the intensive efforts to avoid the reactions were based on the best scientific rationale available at that time. Balancing the dosage and intervals of tuberculin delivery with clinical and laboratory monitoring of patients achieved a limited success, with implications, toward current research in the field. The role of economical and social aspects at that time is also a lesson to be learned toward current approaches to tuberculosis control.

Keywords: Tuberculosis therapy; Therapeutic use of tuberculosis vaccines; Tuberculosis history

1. Discovery and reactions to tuberculin therapy

It is reasonably well known that soon after discovering the tubercle bacillus, Robert Koch studied how inoculation of cultured bacilli affected previously infected or healthy guinea pigs. He observed that the first infection established a degree of protection against re-infection. Inoculation of dead tubercle cultures provoked no reaction in healthy animals, but in infected animals, minute doses tended to heal the tubercular lesions while higher doses could kill the animals. His discovery of a possible remedy against tuberculosis (TB) was announced on the 4th of July 1890 at the International Medical Congress in Berlin. Subsequently, his paper "A further report on a remedy for tuberculosis" was published on the 13 November 1890. Here, Koch elucidated: "in spite of all precautions, too many accounts have reached the public, and that in an exaggerated and distorted form, so that it seems imperative, in order to prevent all false impressions, to give at once a review of the position of the subject" [1]. He described the reactions encountered in the treated individuals and cautioned that the suitability of tuberculin treatment would depend on the form and status of the illness. Koch believed that tuberculin treatment destroyed the necrotic tuberculous tissue, rather than killing the bacilli. Causing this, the propagation of the disease in the organism would have been avoided, as the bacilli would die, being not fed within the nidus or focus of the infection.

Koch was by then a respected researcher, admired by most of the scientific community. As TB was an emotive health issue of the 19th century, his announcement created a general excitement. Due to apparently exaggerated rumours, the treatment attracted many physicians to Berlin to learn how to use it. Despite personal, professional and political temptation [2–4], Koch didn't act hastily, cautiously pointing from the outset at the limitations and weaknesses of the remedy. He earnestly warned people against indiscriminate application of the remedy to all patients and specified that "the most important point to be observed in the new treatment is its early application" [1]. In the same issue of the journal, a letter by Dr. Delépin warned: "I feel sure from what we all know that Dr. Koch wishes us to receive his discoveries, not with scepticism, but with a reasonable and

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scientific spirit. Any other course will certainly lead to disappointment and perhaps to a reaction still more unwise than the present excitement.''

One year later, Koch commented on the reactions observed by other physicians when using tuberculin as a therapeutical agent [5]. Koch's remedy rapidly achieved lots of attention. Some praised the benefits to joints, bones and lupus in combination with surgery, as noted also by Koch himself [6–8]. However, most comments were critical of the severe reactions; soon, the adverse effects overrided the possible moderate benefits. Ultimately, the indiscriminate use of tuberculin resulting in mortality combined with the difficulties of manufacturing and distribution of tuberculin tarnished Koch's reputation and discredited tuberculin therapy [4,8–10]. Following 1891, the treatment had been confined only to a few remaining enthusiasts in Europe and USA, who following Koch's recommendations aimed to avoid the serious adverse reactions [5,10]. Following Lichtheim's endeavours in 1891 toward reactionless tuberculin treatment 1901, Goetsch reported the results of his 10 years experience of tuberculin treatment in patients of a provincial hospital in 1901. He observed severe reactions, but noted that they could be avoided if tuberculin dosage was properly adjusted [5]. Nevertheless, the risk of adverse reactions was discouraging extended use of the treatment. Attempts to explain the reactions considered: (a) synergy between toxins contained in tuberculin and in the infected body; (b) 'disbalancing of host antitoxins' by tuberculin, (c) lysis of tuberculin to smaller toxins (Wolff-Eisner theory); (d) antibody mediated allergy (Von Pirquet) and (e) 'Hypersensitiveness' [9].

Local, as well as general reactions were observed. Local redness, pain and swelling were quite well tolerated. General symptoms were fever, headache, malaise and loss of appetite. Focal reactions associated with the pulmonary disease were haemoptysis, pleuritic pain, greater cough and swelling of lymphatic glands. The latter were the most wanted but also the most feared ones, as the first goal of the treatment was to provoke changes in the focus of the infection. As the outcome of treatment was not predictable, major efforts were made to adjust the tuberculin dosage and schedule to the patient's general clinical condition.

2. Manufacture of new tuberculins

The first attempt to avoid reactions was to generate a new product. ''Old tuberculin'' was based on human tubercle cultures grown on nutrient broth with a 5% of glycerin, sterilised by steam, evaporated, filtered and adding 0.5% of phenol to be further filtered, this first attempt of remedy was known as Old Tuberculin. Koch himself developed new versions of it: the New tuberculin, the steamed cultures ground and mixed with glycerin to obtain only the insoluble parts of bacillary bodies (1897) and the Koch's bacilli emulsion on 1901, the powdered tubercle suspended in a mixture of half part of glycerin and half of distilled water, in order to obtain an emulsion. Other tuberculins were designed: the Albumose-free tuberculin from cultures grown in albumose-free medium and Beraneck tuberculin, a mixture of filtered culture of tubercle bacilli grown in albumose-free medium plus an extract of bacillary bodies in 1% of phosporic acid [11]. One of the main problems of the products was its preparation, as they required dilutions and these were performed by the physicians themselves, a fact that the manufacturer of the Beraneck tuberculin improved (as its dilutions could be already provided) ensuring a better uniformity of concentration [9]. New tuberculins appeared all over the world: Hunter's modification B, von Ruck's Watery Extract, Behring's TC, and many others [12]. Unfortunately, irrespective of which 'new' tuberculin was used, the reactions appeared to be similar, though varying somewhat in intensity [9].

3. Inoculation schedules

Amelioration of tuberculin therapy was attempted by finding a safe schedule of inoculations. There were two schools of tuberculin therapists: (1) believing in immunization by large tuberculin dose and (2) believing in healing action (recall of host immunity) by small tuberculin doses [5,9]. Koch favoured large doses, since reactions were followed by tolerance, to be overrided by the next higher dose. Small doses being gradually increased at short intervals were introduced by Ehrlich and coworkers on 1891 (Fig. 1). Goetsch on 1901 introduced long treatment and later Petrushky intended interrupted treatment for mild cases. Eventually, Sir Almroth Wright introduced the use of small doses at spaced intervals to overcome the so called 'negative phases' [13].

The approaches to tuberculin therapy were influenced by the existing understanding of host responses to infections. The concept that prophylactic vaccination can lead to resistance toward infections, was established by Jenner (vaccination with cowpox to prevent smallpox, 1796) and by Pasteur (live attenuated bacilli to protect against anthrax, in 1870s). In 1896, G.F.I. Widal demonstrated in the sera of individuals with typhoid fewer agglutinins, considered to be 'protective substances'.

With the idea that vaccination may also generate protective substances, A.E. Wright approached therapeutical vaccination with the assumption that a vaccine could not only prevent, but also heal, by boosting the levels of protective substances. However, the observed aggravation of the patient's condition, called a 'Negative Phase' was attributed to the exhaustion of the protective substances [14]. Once that phase passed, a ''positive phase'' with elevated protective substances followed [15]. Wright thought that the intensity of both phases could be related to tuberculin dosage i.e. if the dose was not sufficient, the Negative Phase decreased, but the Positive Phase could not appear; on the other hand, if the dose was too high, the Negative Phase would be too long, and the Positive Phase would arrive too late or would not arrive [16].

The considerable inflammatory swelling which could be observed in the infection site regardless the infection treated, remembered him the reaction described in tuberculin patients. He thought that tuberculin inoculation disrupted the bacterial nidus and spread by the lymph, thus generating new infection

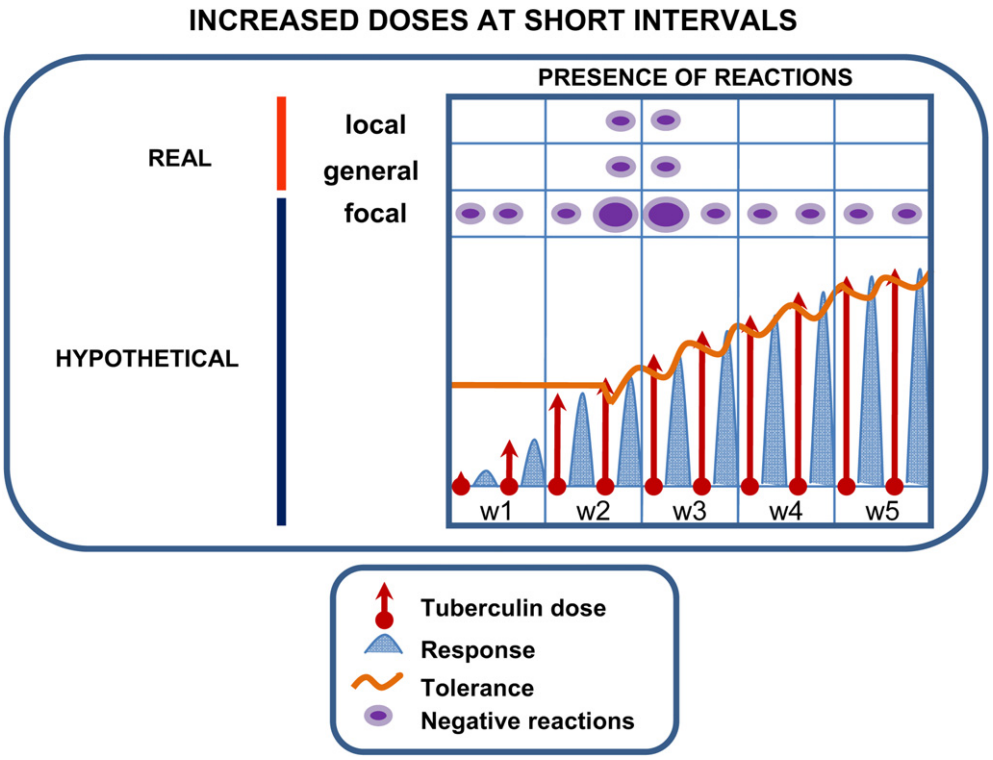

Fig. 1. Inoculation method based on increased doses at short intervals. Tuberculin dose was 10-fold increased until reaching the reaction point, when general and local reactions were detected. From then, little increases of the dose were done in order to obtain a hypothetical focal effect while overcoming the tolerance appeared and avoiding more severe reactions.

foci if the patient was in a negative phase. For avoiding reactions, he suggested to take into account both host resistance and bacterial virulence [14] when optimizing the tuberculin dosage and schedule of inoculations. He believed that reactions were due to the accumulation of Negative Phases and aimed to achieve successive Positive phases by repeated inoculation of the same dose of tuberculin by at long intervals (Fig. 2).

4. The attempt to find a correlate of protection

Wright endeavoured to measure in blood the protective substances which would correlate to the protective effects of tuberculin inoculation, prior to the appearance of adverse symptoms. He would have known that Metchnikoff, following Pfeiffer's studies, described in 1884 the ability of leukocytes to phagocytose and destroy bacteria and Leishman developed the method to measure phagocytosis quantitatively. Wright, considered the existence of an incitor element in the immune serum that generated an opsonic action on the bacteria, preparing them for the phagocytosis. He studied the nature of this incitor element [17] and, by modifying Leishman's method, improved the testing of the opsonic power of the blood, in attempt to correlate it with the vaccine-imparted protection. Counting the phagocyted bacilli by the leukocytes contacting the serum from patients and healthy controls was expressed as the Opsonic Index (OI) [18].

Serum agglutinins, as putative tuberculosis protective substances (i.e. the tuberculo-tropic substances, according to Ehrlich's nomenclature) were first tested by Koch based on previous studies from Arloing.

Wright calculated the OIs in the patients whom he inoculated with the staphylococcus vaccine at St. Mary's Hospital [19] (in order to better predict the negative and positive phases) and later he applied it to tuberculous patients. The OI was supposed to distinguish between tuberculous (decreased OI) and healthy individuals [20], and to be useful to detect the negative and positive phases. Increased OI reflected in good response to the tuberculin treatment [17,21]. This method was widely used by most physicians giving tuberculin therapy, with variable results [12,22–26]. The first criticism was raised in 1910, complaining about its non-specificity and later by statisticians about its subjectivity of the test reading; Clive Riviere implied in 1914, an up to a 20% of error [27]. Wright, admitted in 1923 that retracted the value of these results 20 years later in 1944 [15]. Thus, the mirage of finding a valid correlate of TB protection vanished and it still continues as a challenge to researchers.

5. 'Autoinoculation' and therapy by rest and exercise

When studying the OI in TB patients, Wright observed changes following tuberculin treatment, but also spontaneous fluctuations in feverish patients. He suggested that the variations in the OI values could be related to a disturbance of the

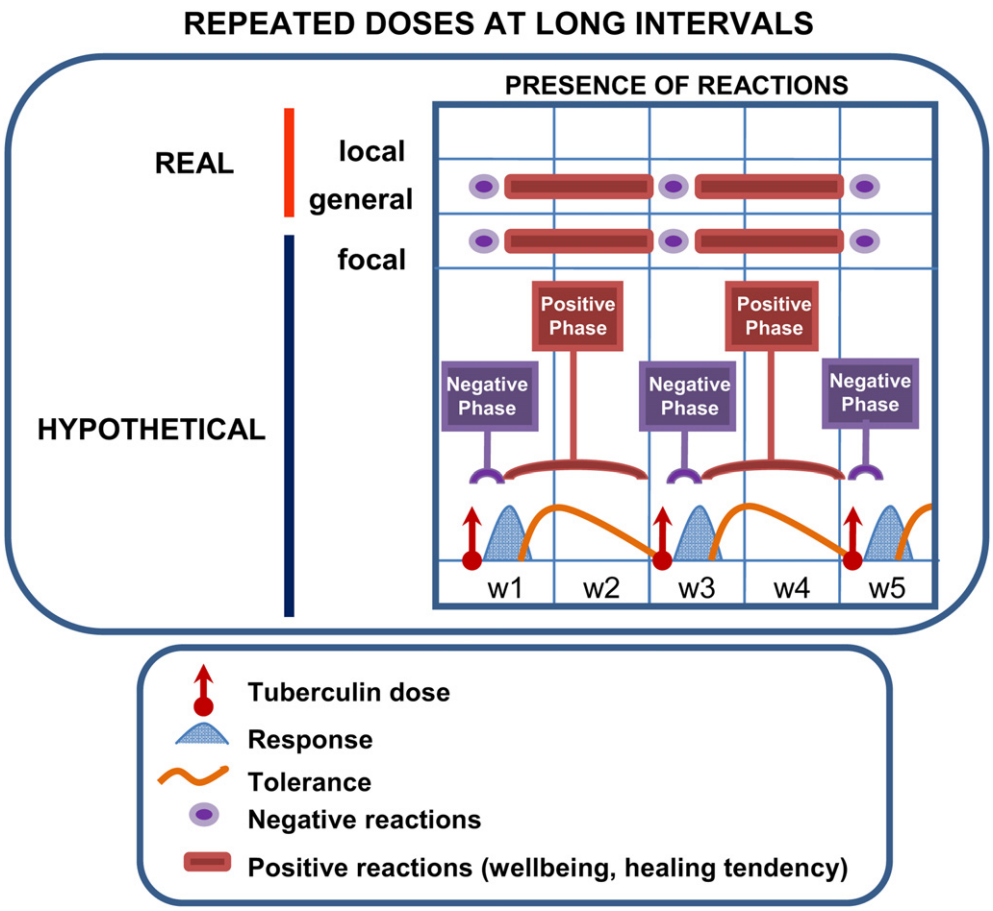

Fig. 2. Inoculation method developed by Wright based on repeated doses administrated at long intervals. By surveilling general reactions, a fixed tuberculin dose considered sufficient to achieve focal reactions was inoculated, the intervals being spaced in order to wait for the tolerance to pass and not to sum negative phases.

site of the disease, which he named 'autoinoculation'. His contention was that continuous periodic escape of bacilli or bacillary toxins to the blood stream caused the episodes of clinical symptoms [20]. This image of autoinoculation as a basis of fluctuations in symptomatology was further developed by Freeman, by massaging of disease joints and others, by exercise, operations and induced hyperaemia. The notion of autoinoculation became the basis of diagnostic and therapeutical strategies. As early as in 1843, Davy reported that strenuous exercise could induce fever in healthy people, and tuberculous patients were believed to be more sensitive on assumption, that toxins from tubercular lesions would be autoinoculaed into the blood stream. Hence, Penzoldt used exercise to diagnose early phtisis without fever, and to establish the gravity of already diagnosed cases.

For the therapy of TB, Patterson and Inman combined exercise with rest and that had been used in most English sanatoria [15,27,28]. Rest was introduced previously by Dettweiler and became a common practice in continental Europe. According to Canetti, diminishing the frequency and extent of respiratory movements by rest reduced the respiratory trauma to lesions. Diminished pulmonary circulation was assumed to deprive the lesions of the factors which initiate their softening and promoted circulatory stasis, which lead to sclerosis and inhibited new bacillary colonization. In line with this concept, lung collapse therapy aimed to increase the effect of rest by allowing the healthy pulmonary regions (instead of the lesions) to distend, thus further reducing the trauma and favouring the healing of the injured parenchyma [29]. Alternatively, Pottenger advocated relaxation or compression of the lung tissue. Relief to the lungs from reduced respiratory movements, autoinoculation was assumed to be diminished. That would have resulted in shrinking and healing the lesions faster, decreasing the blood flow in the infected, inflamed areas and the symptoms reduced [30].

Belief in the balance between host resistance and the virulence of infection was reflected by the notion, that a certain amount of antigen was constantly needed to immunize, whereas its excess could overpower the host response with a fatal outcome. Rest was considered to be necessary to bring autoinoculation under control and recover the equilibrium, but with some degree of autoinoculation still needed to make the patients to tolerate the effects of 'autotuberculin' and to maintain their immune response. Antigens could be provided by exercise from the infection site, or they could be externally provided by inoculation of tuberculin [31].

6. Transition from therapeutic to prophylactic vaccination with *Mycobacterium chelonae*

Friedrich Franz Friedmann isolated from the lungs of turtles from the Berlin zoo bacilli, indistinguishable from human tubercle bacilli even by Koch. As the reactions to tuberculin were widely acknowledged at that time (early 1900s) Friedmann knew of the attempts to reduce reactions by modifying *Mycobacterium tuberculosis* cultures by mechanical, chemical or by temperature methods and considered that the non-human *M. tuberculosis* bacilli might lead to lesser reactions. The turtle's bacillus (*M. chelonae* or Friedmann's bacillus) was considered avirulent for warm-blooded animal species, and as a live vaccine it was considered to be used for both therapeutic and prophylactic vaccination as an alternative to tuberculin. At that time, Calmette was already working on his live attenuated vaccine for tuberculosis [32]. On the 6th November1912, Friedmann announced to the Medical Society in Berlin his results on the vaccination of more than 1000 individuals. Its subcutaneous, intramuscular, intravenous, oral, via conjunctiva and even intralesion administration was considered to be safe. Intramuscular 1–3 inoculations were separated by long intervals [33].

Pedro Guillermo Belmes in his review [34] remarked the most important factor to avoid reactions was the tuberculin dose used. Local reactions could be massive, e.g. painful inflamed swelling of an orange's size. Inoculations were repeated only once the reaction subsided (3–12 months). Friedmann tested his vaccine first on himself, afterwards in tuberculous adults and later on tuberculous children. The results on a total of 1182 patients in 1912 showed the cure or improvement of lesions in most cases, though Friedmann noted the existence of a high individual variability, which he associated with different phases of the disease. Better response being obtained in those with mild forms of TB (patients carried on working), while the 6 deaths (out of 250 treated) were those suffering of severe TB. But Friedmann went a little bit further. At that time, tuberculin was used (in addition to its main therapeutic use) also to treat prophylactically contacts of tuberculosis patients (mostly children) but this wasn't its primary use [11]. Therapeutic benefits stimulated Friedmann to use also live *M. chelonae* as a prophylactical vaccine. He reported in 1912 on the vaccination of 335 children (1–3 years old) 305 of them being suckling infants; in the case of twins, he vaccinated one only. All children were tested before vaccination using the von Pirquet method for TB diagnosis. The preventive vaccination of suckling infants only generated an infiltration of a size between a pea and a cherry that disappeared within a few weeks without leaving any trace.

Vaccination with *M. chelonae* was tested also by intramuscular inoculation of guinea pigs. The unvaccinated animals survived less than 110 days after challenge, while the vaccinated ones lived approximately a year (363 days). Notably, vaccinated animals given a therapeutic dose following virulent challenge survived about 4 times longer. This confirmed the same vaccine being useful both in a preventive and therapeutical way, and was interpreted in favor of, even if the infection itself could maintain the immunity generated by the vaccine, the need of repeated doses to maintain the response [35]. Friedman's success with both prophylactic and therapeutic vaccination was rewarded by the Prussian Government by endowment of a chair in Research on Tuberculosis at Berlin's University, and an offer of 1,000,000 US$ for the exclusive rights of his remedy from an American investor [35].

7. Tuberculin treatment in sanatoria and dispensaries

Despite the rapid discredit of tuberculin therapy, its use continued and even expanded in the light of Wright's contribution. Many physicians reported benefits with acceptable degree of risk. When tolerance occurred, the option was between increasing the dose at short intervals (Fig. 1) or waiting for it to pass. Wright aimed to avoid tolerance using small constant doses at long intervals which seemed to work better for localised infections (Fig. 2) [11].

Tables for dosage helped physicians to choose the correct dosage and developed a skill to treat avoiding fatal results, generating many successes which were reported [5,9,11,12,23,24,31,36]. Besides, treatment was combined with rest, open air; better nutrition and hygiene. Tuberculin use was widespread: it was performed in more than 200 institutions in United Kingdom (UK) in 1912. Use by sanatoria in Germany increased from 29% to a more than 70% between 1910 and 1912 [11].

However, TB mainly affected the poor, who were unable to attend sanatoria because of their high price. Nevertheless, the impact of TB on the economy in the UK and the aim to isolate the infectious sources led to the construction of sanatoria charities offering help. But most of poor patients couldn't permit themselves to give up their jobs. The first TB dispensary (The Victoria Dispensary for Consumption and diseases of the chest) was opened in 1887 in Edinbourgh by Sir Robert Philip, with the aim to avoid this problem. Soon dispensaries flourished all over the world: from 1914 to 1917, their number rose from 4 to 371 in UK; up to 600 dispensaries existed in Germany in 1912 and about 450 in America [28]. Dispensaries became important as they could ensure the therapeutical administration of tuberculin remedy at that time (at least 10 times cheaper than in sanatoria) permitting the individuals to retain their jobs. This has emphatically been described by Wilkinson, from his experience in his ''Dispensary for the poors'' at Kennington Road in London [23], which performed also the screening and surveillance of contacts and follow-up the patients, retaining invaluable epidemiological records.

In 1912, tuberculin was a cheap remedy: it cost between 6.5 pences and 8 shillings depending the tuberculin used (before 1971, one pound was divided in 20 shillings, and each shilling into 12 pence) [11]. But dispensaries had a further advantage: the tuberculin treatment cost 2£/case, while in sanatoria costed up to 32£ only for the constantly medical supervision [23]. The request of the Tuberculin Dispensary League (founded by Wilkinson) to the Medical Research Council (MRC) for funding tuberculin therapy was declined perhaps because was involved in the development of an improved tuberculin,

though it admitted in 1924 that it failed to achieve better results [28]. Tuberculin therapy continued to be widely used until the appearance of chemotherapy, when it was slowly abandoned for its efficacy being variable depending on its complicated application and its failure feared for the consequently reactions which it could bring.

8. Lessons to be learned from tuberculin therapy

A number of the quoted brave endeavours from history deserve admiration for the dedication to their cause. Which of the ideas from that debate are pertinent to present research for improved control of TB? The reported therapeutic achievements deserve to be acknowledged as 'proof of principle' for therapeutic vaccination, despite the associated risk of adverse side effects. The need to find a correlate of protection to monitor any form of vaccination is as open and pressing as ever before and remains a major goal for TB research. The autoinoculation theory of Wright brought up the concept that tubercle bacilli are not held in a closed nidus but able to pass into the blood stream from time to time; this premise is mandatory for the current theories of the pathogenesis of TB [37]. Wright's principles on the need of boosting the host immunity to prevent the dissemination of infectious bacilli and to inhibit their growth in the local nidus of infection [38] also retained their validity. Immunotherapy of any kind remains to be of interest as long as chemotherapy regimens take too long to complete, and these last years, many research groups on TB have been focused on new vaccines' development. Perhaps there could still be merit in following partly Wright's principles by aiming to combine a short regimen of chemotherapy to remove the bulk of dividing bacilli followed by vaccination targeting the remaining persister organisms thus preventing the dissemination of the infection.

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