Gachelin G, Garner P, Ferroni E, Tröhler U, Chalmers I (2016). Evaluating Cinchona bark and quinine for treating and preventing malaria.

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Part 1: Evaluation of Cinchona bark in the 17th and 18th centuries

We know a lot about malaria: the epidemiology of the disease, and the genome of the parasites that infect us. We have made great advances, with new and effective drugs, methods to control the vectors, rapid diagnostic tests, and the potential for effective vaccines. We have seen malaria gradually disappear from temperate climates by the 1950s. Despite all these advances, the disease still remains a cause of widespread poor health in many tropical areas.

Although we know malaria causes fever, to this day defining when a fever is caused by malaria remains a challenge. The clinical signs of fevers, including those caused by malaria, have been known for centuries, if not millennia (see Aulus Cornelius Celsus as an example, translation 1876). From the beginning of the 18th century, however, malaria gradually emerged – from at least 128 different fevers recorded between 1774 and 1794 by Vicq d'Azyr (Peter 1972) – as a distinct clinical entity within the still complex group of intermittent (periodic) fevers (Alibert 1804).

During the 18th century, albeit with some continuing ambiguity (Coster 1829), malaria gradually became accepted as a defined set of intermittent fevers responding to 'therapeutic tests' using *Cinchona* bark, or, from, from the 1820s, by using quinine. But difficulties in the diagnosis of genuine malaria persisted until the causal parasite and vectors had been recognized at the end of the 19th century.

After examining clinical and statistical data on malaria gathered during the 19th century, the Malaria Commission of the League of Nations concluded that few of the data were sufficiently reliable to be used in comparative studies (Malaria Commission 1925). The principal writer of the report, Nicolaas Swellengrebel (Verhave 2011), pointed out that a shift in the meaning of the word malaria had occurred: from 'mal-aria' (bad air) as the cause of many fevers, to the name of a disease ('plasmodiosis') reflecting the causative parasites – *Plasmodia*.

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Comparison with past clinical experience and the rapidity of the response to both *Cinchona* bark and quinine in many reported cases and case series left comparatively little room for doubt that the drug had beneficial effects on the disease. As noted at the end of the 19th century by a German writer promoting careful statistical evaluation of treatments in general, "to substantiate the efficacy of…quinine in malaria, one may not need statistics" (Ephraim 1890-94).

By contrast, it was not possible to make similarly confident causal inferences about the effects of most other interventions, whether purported advances in treating malaria, or measures to control and prevent malaria at the population level. Multiple interventions were often poorly characterized, and delivered without any formal comparison groups. Uncertainties and disputes resulted from this lack of formal comparative studies, with inevitable confusion about which anti-malarial policies to apply, particularly at the population level (Malaria Commission 1925; Gachelin 2013).

From the end of the 19th century, when the life cycle and vector of malarial parasites were identified, other antimalarial interventions were deployed beyond drugs, including, for example, approaches to limit breeding places for mosquitoes; provide physical protection of buildings and people against the insects; and to deploy anti-larval and anti-mosquito procedures, such as petrol, larvivorous fish, and Paris Green as a pesticide.

In this paper we consider methods used from the 17th to the 21st centuries to assess the effects of *Cinchona* bark and of quinine and its derivatives for (i) treating and (ii) preventing malaria in individuals, and (iii) in attempts to control malaria in populations.

The introduction of Cinchona bark to Europe during the 17th century

From its first documented use by the Spanish in Peru around 1630, the history of *Cinchona* bark is a mixture of facts and legends, first compiled by Baldi as early as 1663 (Baldi 1663), and subsequently researched by numerous authors (for recent examples, see Jarcho 1993; Maehle 2011; Rocco 2004; Honigsbaum and Wilcox 2004; Boumediene 2013). Although the bark was not included in the Inca pharmacopeia, it appears to have been used by Andean populations to combat shivering. The medicine became known, among other names, as *Cortex peruanus*, or Jesuit's powder, since it had been imported into Europe from Latin America by the Loyola Order. During the second half of the 17th century, the bark was used increasingly to treat fevers – and intermittent fevers in particular, which were shown 200 years later to be caused by malarial parasites.

The *Schedula Romana*, published in 1649, is an early example of an efficient anti-malaria recipe, generally assumed to have been designed by the Spanish cardinal Juan de Lugo and to have summarized trials that he probably carried out (Jarcho 1993). The doses recommended are likely to have been established by trial and error, and it can reasonably be assumed that de Lugo relied on results obtained using the various recipes proposed by Roman apothecaries. In brief, two drachms (7.5 to 9 grams) of selected bark (no details specified) were to be ground to a very fine powder (a special mill was established in Rome) and infused in hot, strong wine. This mixture was to be administered every day (sometimes several times a day). A rough computation based on the probable content in quinine of red bark leads to the conclusion that doses used in 1650 were in the range of those used after the isolation of quinine, that is, between 0.75 and 1.5 grams of quinine a day.

The market became flooded with *Cinchona* barks of various efficacy, as well as with ineffective and even poisonous, barks from other trees, such as holly. In 1640, the efficacy of different parts of the tree, as well as the role of growth conditions in their content of the unknown 'active principle', was unknown – or only suspected. *Cinchona* as a tree was not known to Europeans, at least until its

description by genuine botanists around 1730, so that fakes could easily be sold instead of the genuine product. Also, search for less expensive alternatives to *Cinchona* contributed to market confusion. From the very beginning, both Jesuits and the Spanish crown attempted to control the quality of the material sent to, and sold in, Europe. It is worth noting that the genuine 'good' starting material (red and bitter bark) and a reproducible method for extracting its active principle was not established and publicized until the late 1730s. This goes some way to explaining large disparities in the reported efficacy of bark in malaria. Knowledge about that was empirically acquired largely by merchants and apothecaries. The scientific reasons for differences among barks and different parts of the tree were not identified until 1842, after a systematic survey of their quinine content by Bouchardat (1842).

By the middle of the 17th century bark as a treatment was becoming widely discussed, especially in the Protestant world, which was suspicious of any remedy promoted by the Jesuits: religious bigotry played a large part in the arguments that raged about the bark's efficacy (Rocco 2004). This may have been the reason that Robert Talbor's 'discovery' of an effective treatment for ague (malaria), published in 1672 as *Pyretologia, or a rational account of the cause and cure of agues*, revealed no details of the treatment other than it had four ingredients, two of which were native to England (Honigsbaum and Wilcox 2004). Talbor may have been among the first practitioners to carry out clinical trials, and one of the reasons for his success may have been his access to the best species of bark through his smuggling contacts in Essex (Honigsbaum and Wilcox 2004).

Talbor's claims may have been one of the reasons that Thomas Sydenham (dubbed 'the English Hippocrates') may have changed his mind about the bark. In his first book on methods for curing fevers published in 1666 Sydenham was somewhat lukewarm about the role of bark. Ten years later he endorsed it heartily while, by implication, condemning all other touted 'specifics': 'I am sure of this, that the only specific is the Peruvian bark', describing it as his 'sheet anchor' (cited in Cook 2011). Sydenham's change of heart may have resulted from using an infusion of bark supplemented with tincture of bark (prepared according to Talbor's recipe) instead of crude bark powder (Boumediene 2013).

Formal evaluations of the effects of *Cinchona* bark during the 18th century

By the end of the 17th century, the notion that *Cinchona* bark was specific for fevers had become progressively firmly established, and several early therapeutic 'trials' have been documented, for example, at the Chinese court (de Fontaney 1703; Cook 2010) and at the court of Louis XIV of France (Vallot 1711; Ferez 2003).

In 1712, Franceso Torti, physician of the Duke of Modena, appears to have been the first to conduct systematic studies of the effects of *Cinchona* on different types of fever, and the first modern description of intermittent fevers (Torti 1712). He introduced the sensitivity and resistance of fevers to *Cinchona* bark in the nosography of fevers, anticipating the nosological usage of response to quinine used after 1820. For resistant fevers, he recommended giving up to a total of 20 drachms (2.5 ounces) spread over three weeks (Honigsbaum and Wilcox 2004). He summarized his representation of fevers by drawing a symbolic 'life tree' inspired by the *Cinchona* tree: on the left, the list of fevers resistant to bark extracts is associated with flowers and leaves; on the right, intermittent fevers sensitive to quinine are listed and associated with bare branches. See: http://www.biusante.parisdescartes.fr/histoire/images/index.php?refphot=01803

By the middle of the 18th century, it appears to have become widely accepted that *Cinchona* bark was useful in treating at least some fevers. For example, in a book on fevers published in 1763 (Lind 1763; Tröhler 2003), James Lind criticized the "false manner of reasoning" that sometimes led a treatment to be rejected if it had not helped in a particular case:

> *...when, from a few exceptions, an attempt is made to overthrow the established maxims of the science. As, for example; if the bark should fail of curing an ague, or mercury of removing a venereal taint, are we thence rashly to conclude, that either of those medicines will prove, in all other instances and cases, ineffectual?*
> (Lind 1763, p 56)

In the 1768 edition of An essay on diseases incidental to Europeans in hot climates, Lind leaves no doubt about his respect for bark, advising that :

> *All naval and other sea surgeons, whose ships are bound to the East Indies, should take with them ten times the usual quantity of bark, and upon this account be excused from taking other drugs not wanted in that climate, as bark is procured there with great expense and difficulty.* (Lind 1768, p 81)

One of the main difficulties in using *Cinchona* bark was the large variability of anti-malarial effects of the preparations. This variation reflected the type of *Cinchona* tree, the place it was grown, and several other variables that influenced the content of the active principle of the bark. McCausland (1783), a surgeon in the British Army stationed in Canada, reported having cured only 60 (64%) of 94 patients with Peruvian bark compared with 84 (82%) of 103 treated with tartar emetic (antimony potassium tartrate). Aware of the methodological shortcomings of his retrospective analysis, he mentioned that he thought that his comparison may not have been reliable: "Solid and invariable conclusions", he suggested, "can only be drawn from a very large number of experiments and observations (McCausland 1783, pp 281-2; Maehle 2011).

Maehle (2011) has commented on the way that McCausland and other late 18th century medical practitioners attempted to justify and refine therapeutic methods by providing comparative, quantitative information on their successes and failures with different forms of treatment. The quantitative, retrospective analysis of their therapeutic experiences, sometimes with a few, sometimes with hundreds of cases arranged in tables, helped practitioners to shape their preferred methods of treatment. For example, Home (1780) compared giving bark before an anticipated fit with giving it after the fit had occurred, both in group comparisons and in within-patient 'crossover' trials (giving it after the fit seemed more effective); and Collingwood (1785) compared different kinds of bark. A few years later Robert Robertson (1789) tabulated comparisons showing that only 1 of 216 (0.5%) Royal Navy sailors with 'ship fever' died after receiving bark compared with 19 among 296 (6.4%) receiving antimony and other treatments (Maehle 2011). As the fevers were much more likely to be due to typhus than malaria, the difference is more likely to have reflected the toxicity of antimonials than the efficacy of bark.

Part 2: Synthesis and evaluation of quinine during the 19th and 20th centuries

In the light of the increasing number of positive medical reports which had accumulated by the beginning of the 19th century, few doubted that *Cinchona* bark was a 'good' medicine for intermittent fevers. After 1800, improvement in procedures for acid-base extraction of the active principles of medicinal plants yielded a number of purified molecules of medical interest. The treatment of several diseases, formerly based on the use of crude plant extracts, could now be examined on a more quantitative basis. As a consequence, there was an explosion of clinical experiments with novel molecules (Magendie 1829). After quinine and other *Cinchona* alkaloids had been purified in 1820 (Pelletier and Caventou 1821) the molecule was promptly tested in patients. Numerous medical observations and case reports from all over the world soon indicated that quinine was specific for 'malarial' (intermittent) fevers. Treatment of fevers with quinine thus

contributed markedly to the nosography of malaria by distinguishing between fevers responding to the extracts, and those that did not.

Magendie (1829) first used dogs to check whether there was any significant toxicity associated with quinine and its salts. John Elliotson (1823), a physician at St. Thomas' Hospital in London, provided an account in English of Magendie's and other French experience:

> *As soon as the two alkalies of Cinchona were discovered, M. Pelletier sent a quantity to Dr. Magendie, who administered them to dogs in large doses without nausea, vomiting, or other apparent result. The indefatigable and acute physiologist then injected into the veins of these animals from two to ten grains of the sulphate and of the acetate of Quinine and Cinchonina in solution, but with no more effect. Satisfied of the innocence of the substances, he ordered the sulphate of quinine to several scrofulous children affected with ulcers [*possibly tuberculosis of the lymph glands*], and in a fortnight the most decided benefit was obtained.*

After Magendie had satisfied himself that quinine was not toxic in humans, it was widely tested in France in hospital patients suffering from intermittent fevers (Elliotson 1823). Quinine – mostly used as a sulphate, tannate or acetate salt – met with the same success as bark extracts. However, questions were asked about whether it was worth using a costly purified material instead of widely used crude bark extracts.

A fairly definitive answer in favour of quinine came from John Elliotson's review of the experience of French physicians – particularly Double (1820) and Chomel and Villermé (reviewed in Rouzet 1821) – and his own assessment of the effects of free base or sulphate salt of quinine in 16 adult patients in Britain who had intermittent (tertian and quartan) fevers. Elliotson (1823) provided a medical and social history of each of them, including information about unsuccessful previous treatments. He gave each of them 5 grains of quinine or quinine sulphate every 6 hours; noted the rapid decrease in the intensity of rigors; and continued until there had been no rigor for a fortnight. No difference was perceived between those who had received quinine and those who had received its salt. Some side effects were noted, including vomiting, but none was such that the treatment had to be discontinued.

Elliotson's conclusion was straightforward: quinine or its salts should be used instead of bark extracts to treat intermittent fevers. He stated the advantages of quinine over bark as follows:

> *"Quinine is nothing but the new form of an old medicine, but presented in such a way that no intermittent fever can resist it… It is very true that Quinine and Cinchonina cannot strictly be called new medicines, because they exist, one or both, in the Cinchona which we have all been prescribing. We are in the situation of M. Jourdain, in Molière's Bourgeois Gentilhomme, who had been speaking prose all his life without knowing it…But although we have not gained a new medicine, the acquisition of so compendious a form of bark, if one may so speak, is highly important… The patient has only to take a pill, and is spared the annoyance of swallowing any of the mass of inert powder which remains after the extraction of Quinine, and which frequently, whatever may be the disease, so disgusts him, or so oppresses his stomach, and deranges his system at large, that bark cannot be borne in efficient quantity, or at all: and, what is particularly interesting, we find that they succeed when bark has failed – that they cure cases of intermittent fever which have resisted bark, although perfectly well borne, and freely administered.*

Elliotson tended to prefer the free base of quinine because it involved fewer preparation steps and so was less expensive. Some of his comments on cured

cases also implied that treated patients were protected against further fever attacks.

A few years later, Magendie (1829) summarized and discussed a larger number of case reports, alongside similar pharmacological studies of other alkaloïds. He mentions the difficulties in defining intermittent fever as a nosographical entity common to all studies, but, on the basis of many publications from various countries (Great Britain, Ireland, Italy, and France, for example), he concludes that quinine was indeed highly effective against intermittent fevers. Some of the Italian case series he quoted included up to 64 patients with different forms of severe intermittent fevers. The outcome of the treatment was compared to previous experience of treatment with bark extracts, or to what was known to occur in the absence of treatment. Recovery was defined as the disappearance of clinical signs and the absence of relapse in the short term. In that respect, the list of cases of intermittent fevers treated with quinine, as collected by Magendie (1829), appears as a kind of success story: so far, no failure had been noted.

The notion of intermittent fevers 'being cured by quinine' has to be tempered by claims made for the use of quinine in other disorders. Magendie notes that several authors reported using quinine to treat or alleviate a variety of non-malarial diseases – ulcers, haemorrhoids, gastric inflammation, intermittent neuralgia, and haemoptysis, for example. All of these diseases are characterized by some periodicity, as if 'intermittence' itself was more of a cause than a consequence of the disease (Sigaud 1844). In any case, Magendie's thinking probably reflected the still prevalent view that 'intermittent disorders' in general were caused by inflammation of internal organs, particularly the stomach and the bowel, which itself was due to some unspecified intermittent miasmatic influence.

In the years that followed, quinine was very widely used as a specific treatment for intermittent fevers, albeit, not universally. For example, based on his investigations in Algeria, Boudin (1842) judged arsenic oxide to be a preferable anti-malarial to quinine. Numerous preparations contained either quinine alone or combined with alkaloïds like morphine, or with arsenic or tartar emetic (antimony potassium tartrate). Jourdan's *Pharmacopée universelle* (1840) describes more than a hundred official preparations based on quinine, bark powder or bark extracts, all believed to be endowed with distinct properties on a huge variety of diseases, for example, typhus (Thomson 1846), or used as a tonic (Dorvault 1858, first ed. 1843). Like arsenic (Whorton 2010), quinine became a kind of panacea.

Choice of quinine dose for treating malaria

How was an appropriate dose of quinine first defined? The dose of quinine used in 1820 and in later studies appears to have been adapted from the quantity of bark powder previously used for intermittent fevers. Depending on the origin of the bark, the content as quinine lies in the range of 1-4% of dry material (Magendie 1829; Delondre and Bouchardat 1854; Dorvault 1858). For example, at the court of Louis XIV (de Blegny 1680; Vallot et al. 1711; Ferez 2003), 'pre-quinine' physicians had routinely used one and a half to two ounces of bark every day for a minimum of 6 days, as powder, extract, or pills. This is in the range of 1-2 grams of quinine a day. A rough computation based on more accurate prescriptions for the preparation of bark powder potions for fevers (Jourdan 1840) suggests daily doses equivalent to about 1 gram of quinine, distributed in several doses. Such a computation is obviously subject to substantial uncertainty, particularly concerning the concentration of quinine in the original material. It suggests, however, that the daily doses used after 1820 were merely adaptations from a century and a half of use of bark powder.

Not only did isolation of quinine offer patients a more convenient way of being treated, it offered physicians the possibility of treating malaria using a quantified

protocol for drug administration. The dose of pills of quinine to be administered was established after the first wave of trials on humans between 1820 and 1822. Most authors mentioned by Magendie (1829) used an initial 3-5 grains of quinine powder in pills. The administration was repeated, usually no more frequently than twice a day, until complete disappearance of rigors. Elliotson kept to 5 grains every 6 hours. Italian doctors reported the daily use of up to 25 grains to treat severe and relapsing intermittent fevers. Despite the variations and uncertainties in the reports, Magendie (1829) gathered evidence that looks like a crude dose-response study: less than 2 grains a day was ineffective, and more than 15 grains a day was found to be either actually toxic (leading to tinnitus and vomiting, for example) or simply badly tolerated by patients. Assuming that quinine preparations were pure and identically prepared – which is far from certain – consensus on the treatment of intermittent fevers rested on the administration of 5 to 15 grains of quinine per day, usually in several doses, although with noticeable local variations.

The range of prescribed doses endured. Eighty years later, medical text books, including Manson's *Tropical Medicine* (1898), recommended doses across a similar range. Depending on the authors and the forms of malaria, 1g of quinine sulphate, chlorhydrate or tannate was administered before the acute phase, followed by smaller daily doses, and 0.25 to 0.4 g per day in the longer term. Later on, there was some variation in treatment protocols depending on the *Plasmodium* species targeted, and discussion about dosage continued (Marchoux 1933); but there was actually little change in protocols over time. The effects of different doses of quinine in neuro-syphilis patients infected with malaria was studied alongside mean plasma quinine concentration. The "class of therapeutic effect"- a measure of efficacy in terms of parasite presence and concentration in the blood – provided estimates of dosage, mean quinine concentration and therapeutic effect in a case series of patients (Earle 1948). By contrast with dosage, duration of treatment continued to vary greatly, ranging from a few days to months.

Side effects of bark, particularly nausea and vomiting, were recognized in the 17th century. Cinchonism – a combination of tinnitus, high-tone hearing impairment and nausea – was clearly defined by Magendie (1822) after he had administered pure quinine to dogs and later to patients. Case studies published after 1830 report hearing loss, headaches, vertigo, disturbances of vision, bradycardia, and digestive problems. Different alkaloids were compared to identify which had fewest of these problems while retaining anti-malarial effectiveness (Honigsbaum and Wilcox 2004). Very high doses of bark and later quinine were used in fevers that were unresponsive to lower doses. One medical dictionary considered (unspecified) large doses of quinine as about as poisonous as morphine (Adelon 1842) and Dechambre's dictionary (1877) contains a lengthy discussion of the side effects of large doses (up to 4 grams) of quinine. Honigsbaum and Wilcox (2004) conclude that a combination of the alkaloids at the same total dose (but a lesser dose of each individual component) reduces the frequency of at least some of the adverse effects.

Controlled trials to compare anti-malarial drugs in the early 20th century

In 1866, the Madras Government appointed a Commission to examine the respective efficacy of different alkaloids in treating malaria. In an article published some time later, Dymock, Warden and Hooper (1891) wrote:

> *From the Report it appears that the number of cases of paroxysmal malarious fevers treated was 2,472, – namely, 846 with quinine, 66 with quinidine, 559 with cinchonine and 403 with cinchonidine. Of these 2472 cases, 2,445 were cured and 27 failed. The difference in remedial value of the four alkaloids as deduced from these experiments may be thus stated:*

Quinidine	*ratio of failure per 1000*			*..... 6*
Quinine	*“*	*“*	*“*	*..... 7*
Cinchonidine	*“*	*“*	*“*	*..... 10*
Cinchonine	*“*	*“*	*“*	*..... 23*

Dymock and his colleagues do not report whether the Commission’s ‘experiments’ were ‘natural’ or ‘planned’.

Quinine alkaloids were not the only drugs tested as anti-malarials, however. After Paul Ehrlich had discovered that some dyes stained microorganisms, methylene blue became used as an anti-malarial (Guttman and Ehrlich 1891). A brief contemporary report of a controlled comparison of methylene blue with quinine concluded that the former was superior (Maitland 1900), and interest in methylene blue continues today (Meissner et al. 2006). The introduction of methylene blue led to chemical substitutions of the original aromatic nucleus and identification of a number of other molecules active against malaria (most of which were not tested until the 1930s – see below). In addition, as arsenic and arsenic derivatives had long been used as anti-malarials, organic derivatives of arsenic (Atoxyl and Salvarsan) developed to treat syphilis (Williams 2009) were also tested in malaria patients (Marchoux 1933). However, the studies were poorly controlled and small scale, and none of the molecules proved sufficiently active to dislodge quinine and its alkaloids from their dominant position in the treatment of malaria.

Planned, alternate allocation to treatment comparison groups began to be used in India at the end of the 19th century in controlled evaluations of interventions to prevent and treat cholera and plague (Choksy 1900; Haffkine 1900; Chalmers et al. 2011; Ramanna 2014). Over the first two decades of the 20th century, criteria for treatment tests in other diseases, including malaria, began to be developed. In 1920, Acton reported his research on the treatment of malaria in India using different quinine alkaloids. He mentions that he had consulted the statistician Karl Pearson and the physiologist Henry Dale and made the following recommendations:

1. *The population under investigation should be sufficiently large and homogeneous. At least 100 men should be treated, and the parasites must be of the same species and found microscopically in every case.*
2. *Reinfections must be excluded during treatment and observation.*
3. *Eight weeks should be the minimum period of observation required after treatment.*
4. *The experiment should be repeated if necessary to eliminate the errors due to chance distribution.*

An important omission from Acton’s list of conditions was the need to generate groups of patients who were otherwise comparable by using alternate (or random) allocation to the treatments being compared. Uncertainty about the validity of Acton’s conclusions appears to have been one of the reasons that the British Medical Research Council decided that it was important to check Acton’s conclusion that quinidine and cinchonidine were outstandingly effective in achieving a permanent cure of benign tertian malaria (*Acton 1920*). A *Cinchona* Derivatives and Malaria Committee was formed and one of its eight requirements for comparisons was that:

> *Alternate patients will be treated with quinine and quinidine respectively, until 50 cases have been treated with each* (Medical Research Council 1925).

The Committee made arrangements for ‘confirmative trials’ in Khartoum, El-Obeid, Lagos, Port of Spain, Georgetown, Dar-es-Salaam, Nairobi, Entebbe and

Kuala Lumpur, using the disappearance of parasites from the peripheral blood as the principal measure of effect.

The hoped-for data was not forthcoming from most of the centres. By far the most complete information (from 72 patients alternately allocated to quinine or quinidine) was supplied by William Fletcher from the Institute for Medical Research at Kuala Lumpur, in the Malay States (Fletcher 1925). Seventeen years earlier, Fletcher had used alternate allocation to create comparable groups of asylum inmates in a comparison of the effects of unpolished and polished rice on the incidence of beri-beri (Fletcher 1907; Vandenbroucke 2003). In his report of a controlled comparison of quinine and quinidine for the British Medical Research Council Fletcher described his use of a similar approach (Fletcher 1925):

> *The patients were not selected in any way; all who came into the hospital with malaria were treated with purified drugs until the supply was exhausted. Seventy- two patients were numbered consecutively on their admission to hospital. The first patient and all those bearing odd numbers were given quinine; those with even numbers (i.e. the second and alternate patients) were given quinidine.*

Fletcher concluded that the immediate effect of quinidine bisulphate "is as good as, or slightly better that quinine bisulphate". However, as illustrated by a controlled comparison of the two drugs reported in 1932 (Sanders and Dawson 1932), uncertainties about their relative merits persisted; and even today, quinidine remains a parenteral drug used for severe malaria in the USA, where quinine is often not available (White et al. 1981).

Although Fletcher had used alternation twice in treatment evaluations early in the history of controlled trials, he does not appear to have adopted this as a routine design feature in his research on treatments for malaria at the Institute for Medical Research (Fletcher 1928). However, others at the Institute used alternate allocation to compare quinine with different quinine alkaloids and mixtures of alkaloids (Institute for Medical Research 1933; 1934) and with the new synthetic anti-malarial drugs, such as atebrin (Field 1934; Field et al. 1937).

In 1926, the Indian Journal of Medical Research published the first of a series of eleven reports of research on the treatment of malaria by Major John Alexander Sinton. In the first article (Sinton 1926a), Sinton itemizes the 'steps necessary to test the efficacy of any treatment:

> *It is very necessary in conducting experimental investigations into the effects of any drug in the treatment of malarial fevers that strict precautions should be taken to ensure:*

1. *that the disease being treated is malaria, diagnosed not merely by clinical signs and symptoms, but by the finding of the parasites immediately before the commencement of treatment;*
2. *that the patient has no other disease, the signs and symptoms of which might obscure the effects of the drug being tested;*
3. *that the drug being tested is actually being taken and retained in the amounts prescribed;*
4. *that no other drug is being taken at the same time, which might vitiate the results of the experiments;*
5. *that, in comparing different treatments, infections due to the different species of malaria parasite are considered separately;*
6. *that in comparing the effects of one treatment with another, the populations treated by the different methods should be as far as possible homogenous;*

7. *that a sufficient number of patients are treated, in order that the results may not be vitiated by errors of chance distribution;*
8. *that controls are used to eliminate, as far as possible, any possible variations in the results, due to season, virulence of the parasites, immunity, etc.;*
9. *that a strict standard as to what is to be considered as a 'cure' of the infection is laid down; and*
10. *that if this standard depends on a period of observation, chances of re-infection are excluded during this time.*

In subsequent reports, Sinton reports that "The alternative case control method was used, and the cases as diagnosed were placed alternately in a control and an experimental group so that there should be no bias in the choice of patients on account of any apparent severity or otherwise in the infection" (Sinton 1926b); "patients were allotted to the different series in strict rotation to avoid any personal bias in the selection of the cases" (Sinton 1927). In the last report in the series (Sinton and Bird 1929) Sinton reiterates an essential feature of his studies:

> *In all tests at least two treatments, of which one was a quinine treatment, were carried on at the same time, so that the result of one treatment might act as a control on the other. In several instances, three or even four forms of treatment were conducted during the same period, the alternative case method being used.*

As in other spheres at the time (Chalmers et al. 2011), alternation was increasingly adopted during the 1930s as a feature of controlled comparisons of alternative anti-malarial treatments. A large multicenter comparison of quinine and Totaquina (a mixture of *cinchona* bark alkaloids) was conducted under the auspices of the League of Nations involving participants in Algeria, Bulgaria, China, France, Italy, the Federated Malay States, Morocco, Roumania and Spain (Pampana 1933). Although the report refers to the need to "to standardize so far as possible the technique of the experiments and to prevent any selection of cases likely to give misleading results", there is no mention of the method used to allocate patients to the comparison groups, and no suggestion from the Tables that alternation was used. By contrast, Hicks and Diwan Chand (1935) reported that in their study "quinine sulphate and Totaquina, types I and II, were given to alternate cases of benign tertian, and similarly to alternate cases of malignant tertian."

Summarizing their assessment of these and similar studies done during the 1920s and early 1930s, Honigsbaum and Wilcox (2004) concluded that crude extracts of *cinchona* bark were as effective as quinine in the treatment of both vivax and falciparum malaria, and might be even more effective in dealing with quinine resistant *Plasmodium falciparum*. A limitation of these and later controlled studies was that treatment benefit was assessed only by parasite clearance in the short term.

Alternation was also used in some of the comparisons of quinine with new synthetic antimalarial drugs in studies organised under the aegis of the Malaria Commission of the League of Nations (1937). As noted earlier, controlled comparisons of quinine with atebrine were done in the Malay States (Field 1934; Field et al. 1937). In Bolshevo in the Soviet Union, all patients suffering from acute attacks were given either quinine, acriquine, quinine plus plasmocide or acriquine plus plasmocide "in the order of their arrival at the dispensary" (p 1066). Alternation was also used in comparisons of atebrine alone with atebrine and plasmoquine/chloroquine (Bastianelli et al. 1937) and alternation may have been used by Mezincesco and Cornelson in Roumania (p 979). Swellengrebel's and de Buck's studies in the early 1930s assessing the prophylactic efficacy of plasmoquine in previously healthy volunteers in the Netherlands (Swellengrebel and de Buck 1931;1932), and Soesilo's and Gilbert's (1934) similar assessment of

atebrin given prophylactically among volunteers in the Dutch East Indies, were small scale and contributed little to an understanding of the potential use of these agents in field conditions. Alternate allocation trials involving quinine continued to be reported several years after the end of the 2nd World War (see, for example, Johnstone 1947) until concealed random allocation became widely adopted as the most secure way of preventing allocation bias (Chalmers et al. 2011).

Part 3: Using bark and quinine to prevent malaria in individuals

Whereas the efficacy of *Cinchona* bark and quinine for treating intermittent fevers had become widely accepted by the turn of the 18th and 19th centuries, their role in preventing fevers had not been convincingly established. However, as early as 1711, Vallot had reported in the *Journal de la santé du roi Louis XIV* that he had learned from experience that long term administration of *Cinchona* bark prevented relapse of fevers (Vallot 1711). Long after the King had been declared cured after treatment for intermittent fevers, he was prescribed bark to prevent further attacks. At that time, people all over Europe lived with mild malaria, and travelers, sailors and soldiers experienced severe fevers in Africa, Central America, and India, where fevers were often named after the place where they had been observed (Coromandel, Guzzarat, Bengali, for example).

The role of armed forces in the development of prophylactic use of *Cinchona* bark was important. The Count of Bonneval claimed (albeit without any quantitative evidence) that bark had been used with success in 1717 during the siege of Belgrade (quoted in Rey 1871). However, most historical sources suggest that it was not until the end of the 18th century that European armed forces became aware of the importance of acute malaria for naval and ground operations (Osborne 2000). Both of the British naval surgeons sharing the name 'James Lind' wrote about bark for prophylaxis as well as for treatment of intermittent fevers (agues) (Lind 1771; Lind 1776).

Alan Magill states in an article on the Centers for Disease Control website that, in 1768, Lind recommended that "every man receives a daily ration of cinchona powder". We have been unable to locate these words in the 1768 edition of 'scurvy' Lind's '*An essay on diseases incidental to Europeans in hot climates*' (Lind 1768). Lind does note in the book that:

> *…[U]pon an eclipse of the moon, the English merchants and others who had left off taking the bark, suffered a relapse…and recommends that bark should be taken at the full and change of the moon, as being the seasons most dangerous for an attack or relapse into those intermitting fevers* (Lind 1768, pp 81-82).

In an Appendix in the 1771 edition of the same book (Lind 1771), Lind's views on bark for prevention is clearer:

> *[S]trangers in aguish places, and persons subject to agues should take, every other night, two or three teaspoonfuls of tinctura sacra, or a few grains of pilula Rufi, so as to prove gently purgative. For farther prevention, they may take every morning before breakfast, a wine glass of an infusion of bark and orange peel in water; or, what will prove more effectual, a tablespoonful of a strong tincture of the bark in spirits, diluted occasionally with water.*

Honigsbaum (2001) has reported that, in 1771, Lind persuaded the Admiralty to introduce *Cinchona* bark in wine to crews on 'ships of war on the Guinea station', but the author gives no reference to this and we have been unable to find the quoted passage. Honigsbaum also reports that, in December 1803, Admiral Nelson had directed that 'a dose of Peruvian bark, in a preparation of good wine or spirits' be given to sailors in the morning before going ashore in marshy areas,

'and the same in the evening on his return on board' (again, no reference is given).

Cinchona bark, and quinine soon after, was gradually defined as a prophylactic by the Royal Navy to address medical problems that emerged within a very particular political and military context. The Slave Trade Act (1807) had forbidden the importation of African slaves to British colonies. A similar law was enacted by the USA in 1808, followed by another in France in 1815. As a result, the British and then the Americans (in 1840) established permanent anti-slavery squadrons to patrol the African coast. They used rivers to enter deep into the interior, thus operating in highly malarious areas. One historian has estimated that half of all Europeans who arrived in West Africa were dead within a year (Curtain 1998, cited in Mitcham 2010).

Cinchona bark had been included in the Navy medicine chest in 1814; the Admiralty began issuing regulations on fevers in 1816; quinine replaced bark in the medicine chest in 1830; and a Royal Navy department of statistics was created in 1831 to gather observations contained in ships' log-books (Mitcham 2010). There were increasing numbers of case reports of apparently successful prophylactic use of quinine. For example, Thomas Thomson (1846) recorded a personal experience on the West Coast of Africa in 1842:

> *I determined to commence the experiment in my own person, taking daily one or two full doses of quinine; and although I may with truth say that I was more exposed than any other person to the exciting and predisposing causes of remittent fever... I quite escaped both forms of fever. On being ordered to England...I considered it necessary to reduce gradually the quantum of quinine; and just before arriving home, had left it off entirely: when, strange to say, I was for the first time attacked with tertian ague in England, under which I suffered for some time; and it returned again at the same season, September, the following year.*

No comparative clinical trials with and without quinine prophylaxis appear to have been reported until 1847 (Carter 1914), which saw publication of the first of two important reports by Alexander Bryson, a British naval surgeon (Bryson 1847; 1854). The first of these contains the following passages:

> *Cinchona bark and the sulphate of quinine are both extremely useful agents for the prevention of fever; and although it would appear their powers have been considerably underrated...still the numerous instances on record in which they have been successfully employed leave no room to doubt that their more general use upon the station is most urgently required. In the North Star, for example, twenty men and one officer were employed on boat duties at Sierra Leone; they all took wine and bark with the exception of the officer; he was the only person who suffered an attack of fever.*
>
> *Two boats were detached from the* Hydra *in the year 1844 to examine the Sherbro river; the whole of the men were supplied with bark and wine, and not one of them was taken ill, while the whole of the gig's crew, with the exception of the captain, who were similarly exposed for two days only, without being supplied with either, contracted fever of a dangerous character. Facts like these are not to be mistaken; the previous pages of this report contain many others of nearly equal value.* (Bryson 1847, p 218)

Bryson concluded that quinine should be employed as a prophylactic in the Navy instead of bark,

> *'and that its use should be continued, not only while the men were exposed in unhealthy localities, but for at least fourteen days after they*

returned on board, in order that the antagonistic influence of the medicine might be kept up until the incubation period of the disease had expired. The suggestion was adopted, and the results, upon the whole, are most satisfactory' (Bryson 1847).

The 9th article of the Royal Navy's instructions to physicians specified quinine as the medicine to use to prevent malaria (Bryson 1847).

[As an aside, Bryson goes on to remark that, given claims that tobacco possessed prophylactic properties, there were "not any just grounds for believing it to be of the slightest value in this respect" (Bryson 1847, p 219).]

Bryson's observations also prompted him to guess how quinine might be working.

> *Although neither bark nor quinine has the power of preventing the germs of fever from lodging in the system, there they may lie dormant for a period of from fourteen to twenty days or even longer, nevertheless, from their peculiar antagonistic properties, they most decidedly have the power in many instances of preventing their development in pyrexial action. Hence the frequently supposed failure of the medicine is undoubtedly to be attributed to its use not having been persisted in for a sufficiently long time after exposure to the exciting causes; namely, throughout the entire probable period of incubation.*
>
> *It is therefore suggested that it would be advisable not only to administer, daily, one of these febrifuges to men so long as they are exposed to the influence of the land, and the vicissitudes of the weather in open boats, but to continue its use for at least fourteen days after their return on board. As the sulphate of quinine is more certain in its action, infinitely less nauseous than bark, and therefore less objectionable to fastidious people, it should invariably be preferred for exhibition: whether it be given in wine, water, or rum is of no great consequence: the latter will generally be the most acceptable to seamen, although they will seldom object to it in wine.* (Bryson 1847, p 219).

Based on notes taken out of ships' log books, similar conclusions were reached a few years later by a US Sanitary Commission (1861) based on reports such as the following:

> *"During our stay in the river Lagos, quinine wine was regularly offered to the men, morning and evening — all took it, I believe, except two mid-shipmen and two seamen belonging to the galley. These four persons subsequently each suffered a severe attack of fever. While in the whole force, consisting of upwards of 220 men, there occurred only a few other cases of trifling importance.* (Report of Mr. Heath, Surgeon of the *Teazer*)
>
> *"Thirty-six men belonging to the* Water-Witch *were employed in the attack on Lagos; they were in the river four or five days, and, with the exception of three, all took quinine wine while there, and for fourteen days after they left it. Of the whole number, five only were attacked with fever, namely, the three men who did not take the wine, and other two, who most imprudently exposed themselves to the sun, and bathed while much heated by violent exercise.* (J. Henderson, Esq, M.D.)
>
> *"On the morning of the 25th of November, seventy-seven men of the ship went up the river Lagos to attack the town. Before starting, every officer and man was ordered to take a glass of quinine wine, and a sufficient quantity was put into the boats to repeat the same at night. All, to the best of my knowledge, took it, with the exception of Mr. D., master's assistant, who rather plumed himself on having escaped taking a dose of physic.*

> *This young gentleman, on the 10th of December, just a fortnight after, was seized with a violent attack of remittent fever; and, of the whole number who entered the river, he is the only one who, up to this date (the 7th of January), has been attacked."* — (F. Stupart, Esq., Surgeon.)

Collation of these reports of dramatically successful protection may have been a mere summing up of positive and negative instances, but this was fairly typical of the approach used routinely throughout the 19th century (Jorland 2005). The results of the observations listed on data sheets assembled at the Royal Navy Bureau of Statistics suggested strongly that prophylactic use of quinine was beneficial, provided it was taken before, during and after leaving a malarious area (Bryson 1847; McShane 1856).

The observations made were not formal comparisons of sailors who did or did not receive prophylactic quinine. Other practices had been adopted which afforded protection from malaria, even though the role of mosquitoes in causing the disease was unknown. Several military and civilian sources had already shown that the risk of developing malaria could be reduced or avoided by observing some simple rules (Montfalcon 1826). At least as far as the armed forces were concerned, these included not staying on shore at night, staying in closed quarters after sunset, anchoring ships at a distance from the coast (usually one nautical mile), and not swimming in rivers.

In addition, the physical condition of sailors and marines may also have improved as a result of better sanitary conditions aboard, particularly the quality of drinking water, an improvement largely reflecting the opinion that malaria was caused by a water-borne or an air-borne 'poison' (Watt 2002). Anyway, after 1850, quinine associated with a combination of other anti-malarial procedures was adopted as standard procedure in the British and American navies. The mortality in American and British African stations and squadrons, despite a lack of precision in the records, declined abruptly after 1845 (Mitcham 2010).

After progress had been made in protecting the health of Royal Navy personnel, Bryson expressed obvious frustration that a proven measure was not yet being used to preserve the crews of some merchant vessels on the coast of Africa. He pointed out that "As these vessels generally carry (for the prevention of scorbutic disease) a supply of lemon juice, which, in consequence of the great abundance of yams and fruit, is nearly if not entirely useless, they ought to carry instead of the lemon juice a sufficiency of quinine wine for the crew, which should be administered in the same manner as in the men-of-war on the station" (Bryson 1854).

The crews of some non-military vessels were protected using prophylactic quinine. For example, in his account of his exploration of the Niger and Tsadda rivers in 1854, William Balfour Baikie reported:

> *Being now fairly in the river, we commenced giving, morning and evening, to all Europeans on board, two thirds of a glass of quinine wine, which contained about five grains of quinine, believing that this would act as a prophylactic or preventive, while exposed – as everyone must be while in the Delta – to the influence of malaria* (Baikie 1856, p 34; McConnaha 2007).

By 1861, Report 31 of the US Sanitary Committee (1861) contained material derived from interviews with civilian and military physicians about their practice in malarial areas. The accumulation of personal experiences, whether in America, Africa or Asia, led to the conclusion that quinine sulphate – as pills or as quinine wine, in doses of between 3 and 5 grains a day – was effective in preventing malaria. Some of the reports deal with personnel working in plantations in the USA and in Asia, thus confirming the extension of quinine use to malaria-

infected agricultural locations. Van Buren, in the name of the Sanitary Committee, wrote:

> *In conclusion, it may be fairly assumed, even from the evidence thus imperfectly and hastily collated, that the power of Quinine as a preventive of miasmatic disease, is fully established as a medical fact; and that it can be employed, not only with entire safety, but with the greatest advantage, even to the saving of life, by healthy persons exposed to malarial influences. Viewed in the light of humanity, as well as of economy — both of men and money — the prevention of disease is of far greater importance than its cure, and your Committee venture to express the opinion that intelligent and judicious action on this important subject at the hands of the proper authorities would save much sickness and many valuable lives during the present campaign* (US Sanitary Commission 1861)

France and French armed forces are surprisingly absent from the debate on quinine as a prophylactic agent. The probable reason was suggested by Laveran (1896) in a critical review of the French attempts to use quinine in this way written after the disastrous Tonkin expedition, during which malaria affected 50% of French troops. For unknown reasons, the data reported in Laveran's review suggest that quinine used by French physicians appears significantly less effective than that used in the Royal Navy. Laveran (1896) points to inappropriate doses and regimens and to indiscipline. In concluding his paper, he proposes the following controlled trial:

> *Il y a lieu d'instituer des expériences dans les conditions suivantes. Soit un corps de troupe qui occupe une position insalubre, on le divisera en trois groupes aussi homogènes que possible dont on excluera les hommes ayant déjà eu une fièvre palustre : au premier groupe on donnera une dose quotidienne de quinine (0gr, 20 à 0gr, 30), au deuxième on prescrira la quinine tous les deux jours (0gr, 40 à 0gr, 60), le troisième groupe ne prendra pas de quinine et ne prendra rien.*
>
> *It sounds sensible to institute experiments under the following conditions. Troops in insalubrious circumstances will be divided into three groups as homogeneous as possible after exclusion of men who have already suffered palustral (marsh) fever: the first group will be given a daily dose of quinine (0.2 to 0.3 g), the second group will be given quinine (0.4 to 0.6 g) every other day, the third group will not receive quinine and will not take anything.*

The controlled trial proposed by Laveran does not appear to have been carried out either by him or by any other French physicians, although it was done in Italy a few years later (see below).

By the second half of the 19th century quinine had become an obligatory accompaniment for most Europeans traveling or working in malarial areas. Recognition that a combination of procedures was needed to achieve some control of malaria in certain environments predated the complex array of strategies developed after the origin and transmission of the disease had become understood. It also became clear that, other than in 'disciplined local environments', these measures were unlikely to achieve eradication of malaria. But even in the context of a prison, the effects of prophylactic use of quinine could be disappointing: in a study using alternate allocation of 120 prisoners to 20 grains of quinine on two successive days, or to nothing, no differences between the two groups in subsequent hospital admissions with malaria or length of stay were detected (Waters 1903).

Part 4: Efforts to control malaria in populations using mass medication with quinine in the 20th century

The very end of the 19th century was a turning point in parasitology in general and malariology in particular. Physicians had accepted that a parasite caused malaria and that the parasite could be killed or weakened by quinine. These discoveries provided a rationale for improving the use of quinine for prophylaxis. In addition, new medical measures had been introduced to quantify and monitor malaria by identifying the vectors and searching for parasites in them, and by screening human populations for evidence of infection, including parasites in the blood, and spleen enlargement.

Targeting the parasite: Robert Koch's use of microscopy and quinine to control malaria

By the 1890s, having discovered and isolated the causative agents of cholera, tuberculosis and anthrax, Robert Koch was prestigious and influential. Since 1891 he had directed the huge Institut für Infektionskrankheiten created for him in Berlin. In the early 1890s Koch had developed and successfully applied a strategy against cholera focusing on the isolation and elimination of the causative agent, the parasite vibrio cholerae. By the mid-1890s Koch assumed that the two preconditions for applying this approach in malaria – tracing and destroying the parasite (*a Plasmodium*) – could equally easily be achieved by microscopic examination of the blood and administering quinine. By analogy, Koch decided that "[t]he experiment (Versuch) to fight malaria according to the same principles […] absolutely had to be done" (GW 2; 1, p 456).

Clinical experimentation, with a protocol and precise numerical statements, was not Koch's forte, and it has been something of a struggle to piece together, using the online edition of Koch's Collected Works (Gesammelte Werke, GW), relevant information from over a hundred pages of his letters, reports and other documents about malaria written between 1898 and 1908 :*Koch R. Gesammelte Werke, Berlin 1912. Page numbers that follow in this section refer to volumes 2.1 or 2.2, respectively of this edition, see http://edoc.rki.de/browsing/rki_rk (last checked 18 January 2017).*

Koch's intensive involvement in attempts to control malaria appear to have begun in 1898, when he asked his administrative superior, the Prussian minister of religious, education and medical affairs, for money to fund an expedition to Italy and the Dutch Indies (GW 2: 2, p 883-887). He started off with a campaign in the Tuscan *maremma* around Grosseto, together with an Italian colleague, Professor Gosio. Gosio had hitherto fostered physical antimalarial measures directed against the vector – petroleum on marshes, draining stagnant water, and mosquito nets – which had been promoted by the Italians and the British. Koch's work in Italy appears to have continued until at least 1903, and although some letters about it were published, these do not provide any basis for strong inferences about the success of Koch's malaria control methods there.

Soon after starting his work in Italy, "an extraordinarily lucky opportunity" presented itself to Koch to perform a further 'experiment' in New Guinea. He found in New Guinea an almost untouched, beautiful and luxuriant country, yet infested by malaria (p 440). This meant that he could start his experiment from scratch. Koch's stay in New Guinea from 29 December 1899 till 8 August 1900 proved decisive for his future statements about the control of malaria. His experimental community was the German plantation site of Stephansort (Koch 1900), which had 734 inhabitants and two "hospital houses" (one for the roughly five dozen Europeans [GW 2, 1, p …], the other for the natives).

First, the blood of all 734 inhabitants was examined for parasites. The population was submissive, and small gifts were given to the children to secure their compliance (p 414). Parasites were found in the blood of 157 of the 734 inhabitants (GW 2: 1, pp 404-411, 443) and they were treated immediately with quinine. Koch stipulated that patients were to be given 1 gram of quinine daily until the malaria parasite had disappeared from their blood, then, after an

interval of seven days, a gram of quinine was to be given on each of two days, followed by another seven day interval, and so on, for at least two months (p 411). Upon re-examination two months later, "only a very small number" remained carriers of parasites, and "fresh cases were hardly observed thereafter"(p 895).

This 'experiment' was deemed by Koch to have been "perfectly successful" (p 443), and he suggested that that the treatment principles were also applicable for prophylaxis (p 413).

Newcomers to New Guinea were usually very susceptible to malaria: 47.4% of recruited workers from the Gardner Islands fell ill soon after their arrival at Stephansort. They were treated immediately and cured. Those who had remained healthy received quinine prophylactically, and Koch reported that "not one of them got malaria" (p 413). In February 1900 a ship carrying workers recruited from the island of Ambon arrived.

> "*About half of them were given quinine prophylactically, the other half was not; the first group remained healthy, whilst all of the second [group] fell ill with malaria, except a woman. They were all treated at Stephansort, and soon recovered*" (GW p 413; Koch 1900).

The three members of Koch's expedition used quinine prophylaxis regularly. None had contracted malaria after 4 months (p 413), although Koch noted that prophylaxis with quinine was "somewhat tiresome" and "disagreeable" (p 414).

In June 1900 Koch insisted that this success was not accidental since there had been very few new cases in Stephansort, even during the rainy season – six in May, one in June (Koch 1900)). Since the experiment had been conducted over a period of six months he deemed the result to be "unequivocal" (p 416). "Our experiment at Stephansort proves that our procedure also works in tropical climates, and it works quickly" (p 428).

What was the evidence for Koch's strong claim? From Stephansort he reported one prospective comparative trial and a retrospective comparison, yet both were presented in very vague numerical terms: the number of persons observed was not stated, and the results were given using terms such as "all" and "none" or as "decrease by over 50%". This imprecision may be well due to the fact that people were hard to register and follow up in these colonies, with rapidly fluctuating migrant populations.

Koch's assertions about the success of his approach (Koch 1901) depended rather on the logic of focusing on the parasite and not the vector.

> *"My procedure is something completely different. I have to stress this explicitly. The "quinine-prophylaxis" wants to prevent the infection of men as such. My procedure is directed to towards the parasites in the infected men. It aims at healing all patients (p 428) [...]. Physical measures and desiccation of marshes may be done, "but by themselves they will achieve nothing against malaria"(p 446),*

As the mosquitos would persist, Koch's aim was to eliminate the parasite, and this had become more possible on a mass scale because quinine had by then become less expensive (p 427-428).

Koch listened to criticism. Although he debated questions of dosage and presentation of quinine (p 429-430), however, he stuck to what he referred to as "my rather conspicuous experience" (p 432). He was aware that his results at Stephansort might be coincidental and/or might be true only because this was a small local community. He replied to these criticisms by observing what happened over time, and by having his method applied in Italy and East Africa where thousands of people were examined and treated (Ollwig 1903). He also

suggested, however, that such experiments be conducted in Germany, where longer term follow up would be easier (p 416). Later that year he was able to further document the success of his approach by publishing tables of incidence of malaria among the military in Northern Germany. It had decreased from 54.9 per 1000 in 1896 to 0.45 per 1000 in 1897 (p 444).

In October and November 1902, in two letters to his administrative superior, the Prussian minister, Koch reported again on his Stephansort experiment and the further successful applications of his procedure, at his instigation, in North-West Germany; on the Croatian coast; and in German East Africa (Tanganyika). At the first site there had been no outbreak of malaria during dyke building works at Wilhelmshaven; at Brioni (Croatia) malaria had been eradicated; and in Daressalam, "the number of those ill with malaria had decreased by more than 50%" (p 895).

Koch emphasized in his letters that his approach had been intentionally restricted to microscopy and quinine to prove that this approach was capable of eliminating malaria in a variety of climatic and social circumstances (p 896). In his view, physical anti-malarial measures were at best only "a support for my procedure" (p 896).

In 1903, Koch concluded robustly: "...By this experiment it is proved that malaria can be fought by the same principles as cholera. Of course one may also use other measures to restrict malaria as, for instance, the elimination of the mosquitos recommended by Ross or the protection against mosquito bites with bed nets as tried out in Italy" (GW 2: 1, p 457). In 1908 he added that nets might be tried in the colonies "in order to prevent the possible reproach of an omission" (p 897).

Ronald Ross's book "The Prevention of Malaria" (New York, Dutton 1910) was published in the year Koch died. It contained contributions by authors from all parts of the world. Koch's method was initiated by Ollwig in Daressalam in 1901-1903 (Ollwig 1903) and appears to have continued at least until 1914 (Orenstein 1914), was reported as having been "successful in so far as it has at least considerably reduced the incidence of malaria." Yet, in the longer term, Koch's approach did not seem to have worked consistently. In a 1913 paper, Manteufel, a German working in Daressalam showed "from carefully kept records,[...] not subject to the usual errors of such compilations" (Orenstein 1914) that, despite continued administration of quinine between 1903/04 and 1912/13, both the incidence and the mortality of malaria had increased noticeably among Europeans and the native population (Manteufel 1913).

References to the Daressalam experience in Ross's book and elsewhere, and Manteufel's statistics, prompted AJ Orenstein – whose principal duty in Daressalam had been to institute a campaign against malaria – to report his test of Koch's theory that malaria could be eradicated by attacking the parasite within the human host (Orenstein 1914). This was a replication of Koch's Stephansort experiment which involved treating all infected students in the Daressalam Trade School according to Koch's regimen and then comparing the infection rates up to 9 months after quinine treatment. More than a third of the students became reinfected during the Christmas holidays (Orenstein 1914).

In parallel, Orenstein did the same test with 150 natives (adults and children), chosen at random, living near a mosquito-breeding pond, before and after a 5-month period during which the pond had been cleaned and treated with phenol at 10-day intervals. The climatic conditions had remained practically constant, and the blood tests were performed in both studies by the same person "who had no idea whatsoever of the purpose or nature of the experiment." The decrease in infection rate was only 13.9% in the schoolboys and about 20% in the native population (Orenstein 1914).

Orenstein concluded that Koch's prophylaxis should not be "condemned as useless. It may have a certain degree of usefulness, but it is, much to my regret, a very insignificant degree when applied to a permanent community of considerable size in a country where anophelines [...] abound", and where, in addition, immigration continuously introduces carriers (Orenstein 1914;Ewers 1972).

Angelo Celli's use of mass medication with quinine to control malaria

Italy provides the earliest and best example of formal, state-organized quinine prophylaxis, based on the then recent scientific achievements. The Italian government passed a law in 1902 establishing a state monopoly for quinine trade and distribution so the drug became widely and regularly distributed among industrial workers and children (Corbellini 2003). The Torino quinine factory, which was placed under the control of the Ministry of Finance, produced 60 tons of quinine a year (a further 27 tons were imported). The drug was sold at fixed prices and distributed either through municipal dispensaries or through charities. Children were particularly targeted and special preparations were used for them (quinine in sweets or in syrups). About 10,000 specialized physicians (*medici condotti*) were responsible for malaria surveys and treatment, and the Directorate of Public Health was in charge of malaria sanatoria and mobile and static dispensaries (Corbellini 2003).

The earliest comparative trial of quinine used prophylactically in populations appears to have been that organized by Angelo Celli. Celli had previously reported on his controlled evaluation of physical measures to protect Italian railway workers and their homes from mosquitos (Celli 1900a; 1900b; Ferroni et al. 2011). In 1903 he reported an evaluation of the effects of prophylactic use of quinine in a number of Italian cities (Celli 1903):

> *"the first year we treated part of the population with quinine prophylaxis, leaving the rest as control. The year after, thanks to the results on the effectiveness of the treatment, no prejudice or diffidence from the population will emerge and this prophylaxis will reach popular consent".*

Celli's 1903 report is not clear about how the allocation to prophylaxis or control was made, and no comparative statistics were presented in his report.

In the second part of a paper devoted to malaria in Italy in 1902, Celli describes a large scale experiment on malaria prophylaxis using quinine (Celli 1903). Following an initial experiment conducted in 1901 which had given promising results, the intervention was extended in 1902 to 16 rural (for example, Agro romani) and urban (for example, Milano and Mantova) malarial areas, mostly in northern and central Italy, including the Pontine Marshes and Ostia. Prophylactic quinine was withheld from part of the population at each location as controls. The intervention was used in two different ways: either continuous (daily) administration of quinine (in most of the areas), or discontinuous (weekly) administration of quinine. Free quinine pills were distributed to the rural populations by physicians. The trials were conducted from May to December, depending on the locations. However, physicians did not use a standard protocol, which makes it difficult to compare different local trials.

The Table summarizing the data in Celli's 1903 report can be analyzed in two ways. By summing up the results of all the studies, it appears that, in the context of daily administration of quinine, 923 people received quinine every day (25-30 centigram) and 44 (4.4%) of them developed malaria. In the control groups, the proportion of people who became ill varied between 12 and 82%. Of 2133 people who received from 1-2 gr per week to 3 gr every 9 days, 191 (about 10%) became ill compared with between 40 to 80% in the control groups. Out of a total of 3055 people treated daily or weekly, only 235 (7.7%) became ill or experienced a relapse compared with 12 to 82% among controls. The results suggest a

protective effect of quinine, particularly daily administration. However, the wide range of malaria incidence among untreated patients precludes firm conclusions.

The second way of reading the data involves comparing the values obtained within each location. This reveals marked variations in the apparent efficacy of quinine prophylaxis, and suggests that intermittent administration is less effective than daily administration. Celli does not discuss the variations, nor does he describe the characteristics of the treated and untreated populations, or how they were chosen. In the same article, he reports prophylaxis using a combination of salts of iron, arsenic, and quinine, promoted by industry and administered in 1901 to railway workers in Foggia, south Italy. Daily administration of quinine (0.15 gr), arsenic and iron was used in 54 individuals, 8 (14.8%) of whom became ill. Weekly administration of quinine (1 gr), arsenic and iron (one spoon daily, 0.01 gr) was used in 52 individuals, 10 (19.2%) of whom became ill. Celli concluded that combined treatment was no more effective or better tolerated (but was more expensive) than quinine alone.

Celli's study is one of the first quantitative attempts to examine, at a population level, the prophylactic use of quinine. Despite the obvious limitations of the approach, the message was clear: quinine has a significant prophylactic activity when administered daily to populations exposed to the parasite. The earlier conclusion reached by Navy physicians was not only confirmed but extended. Thus it seemed that an anti-malaria public health policy should, at least in part, include systematic administration of anti-malarial drugs.

However, it rapidly became apparent to Italian malariologists that quinine-based prophylaxis of malaria was not easy to scale up, and in the end, this strategy failed to control the disease. It certainly helped, but not to the extent that had been hoped. It had met with a number of unexpected difficulties and limitations. Snowden (2006) describes the ambiguities of the quinine-based campaign against malaria in Italy and the multiple economic, societal and political reasons for its failure in many areas. People did not take the drug as frequently as they needed to, and some refused to take it. Reinfection was the rule. The side-effects of quinine (nausea and tinnitus) are not mentioned in the trials, but they may have contributed to the rejection of quinine prophylaxis by some within the population. The diffuse resistance of the general population to quinine prophylaxis is not documented in the Italian medical writings, but it is in a later report by the League of Nations (Malaria Commission 1925).

This factor had been emphasized in 1914 in a review of quinine prophylaxis by a senior surgeon in the United States Public Health Service (Carter 1914). He contrasted Celli's experience in towns and villages with that in the penal colony in Castiadas, Sardinia, where quinine had been given under orders. During 1904, 1905 and 1906, no prophylactic quinine had been given, and 76 per cent of the prison population developed malaria. After four years of prophylaxis the percentage affected in 1911 was just 5 per cent.

The important additional factor that needed to be taken into account was thus the influence of social factors on the effectiveness of quinine prophylaxis (Malaria Commission 1925). Some kind of social organization strongly supporting anti-malaria campaigns was needed for quinine prophylaxis to become effective: that was the case among armed forces and railway workers, then characterized as 'disciplined populations', as were mine workers in Spain (Rodríguez-Ocaña 2005) and rice-workers in Italy (Snowden 2006).

In the latter two cases, efficient quinine prophylaxis relied on the existence, or on the construction, of a strong social organization of workers, which in turn resulted in trade unions. 'Civilian societies' had to be completely convinced that it was sufficiently in their interests to take quinine regularly. The affected populations had thus to be educated: the place of individual and collective

responsibility was the lesson drawn from attempts at malaria prophylaxis. In summary, efficient campaigns of quinine prophylaxis at the population level were those which had been 'unionized', 'militarized' and 'politicized' (Snowden 2006; Gachelin 2013).

League of Nations assessment of mass medication with synthetic anti-malarial drugs

Mass medication with quinine and with the new synthetic anti-malarial drugs was attempted in the 1930s in French Indochina (Robin and Truong-Van-Huan 1935); the Malay States (Field et al. 1937); Algeria (Parrot et al. 1937); and Italy (Bastianelli et al. 1937), among other places (Malaria Commission 1937). Reviewing the experience, the Malaria Commission of the League of Nations observed (p 995):

> *In only a few of the experiments were there control groups or villages kept wholly without treatment [prophylaxis]. As previously, lack of adherence to the regimens prescribed meant that early promise was not sustained. Owing to the natural variations occurring from season to season in the density of the anopheline fauna, as well as the disease itself, the results of experiments without controls can only be accepted with caution.*

The Commission concluded its report with some practical suggestions for treatment and prophylaxis (pp 1012-1016). With regard to the treatment of individuals, the Commission referred to the desirability of microscopic examination of the blood, and that mass treatment with quinine or atebrine should be accompanied or followed by plasmoquine to reduce the risk of relapses. The report notes that there were large malarial areas where mass treatment was impossible for financial or other reasons. The conclusions with respect to mass drug prophylaxis were even more sobering. Without 'disciplined communities under strict supervision' mass drug prophylaxis was highly unlikely to be useful. Indeed, experience had shown that the eradication of malaria by treatment and prophylaxis with the drugs then available was 'practically impossible'.

With regard to the choice among the several anti-malarial drugs then available for curative or prophylactic mass treatment, the Commission ranked quinine first because of its clinical effectiveness, almost complete absence of serious toxicity, and the widespread knowledge of its use and dosage. Indeed, quinine remained unchallenged for a further half century, when controlled trials showed that artemether and artenusate – which had also been derived from plants and used to treat fever (Tu 2011) – were shown to be superior to quinine in treating severe malaria (Sinclair et al. 2012; Esu et al. 2014).

In Summary

The sometimes dramatic results of treatment of intermittent fevers with *Cinchona* bark dates back to the 17th century, and bark was used effectively throughout the 18th century and beginning of the 19th century. Quinine – the active principle in bark – was isolated at the end of the second decade of the 19th century, and because it was more palatable and could be more confidently dosed than bark, it was widely adopted during the second half of the 19th century and the 20th century.

In addition to the clear effectiveness of bark and quinine in treating intermittent fevers, their prophylactic administration also resulted in dramatically effective protection of individuals against these fevers. The therapeutic and preventive effectiveness of bark and quinine in individuals was obvious without carefully controlled trials, and was identified and adopted a century before the cause of malaria and the cycle of *Plasmodium* had been elucidated.

Although the important role of quinine in treating and preventing malaria in individuals became clear during the 19th century, differences between the effects

of different quinine alkaloids, and between quinine and bark and other anti-malarial drugs were not dramatic. This led to a recognition that formally planned experiments were required to obtain trustworthy results. Methodological ground rules began to emerge during the 1920s and 1930s, particularly in India and the Malay states, that comparison groups should be generated by alternation and that groups of sufficient size were required to obtain reliable evidence of treatment differences on substantive treatment outcomes.

Although the use of quinine to treat and prevent malaria in individuals was dramatically successful, research in Italy in particular showed that scaling this up to prevent malaria in populations was unsuccessful, both because of poor compliance with self-medication, and because of the frequency of reinfection. This general conclusion applied except in circumstances – prisons and navies, for example – in which community self-discipline could be assured.

It was not until the 21st century that any alternative anti-malarial drugs were shown in well designed, large controlled trials to be superior to quinine, and then only in severe malaria. The enduring beneficial effects of Cinchona bark and quinine over three and a half centuries is remarkable.

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