'To search and studdy out the secrett of tropical diseases by way of experiment'

David A Warrell

ABSTRACT – William Harvey wrote about malaria, snake bite and rabies, three diseases now having their greatest impact in tropical developing countries. Global malarial mortality has not declined for 50 years. The most effective control measure would be a vaccine. Temporary immunity in humans, through hundreds of bites by irradiated infected mosquitoes, was achieved in the 1970s. A promising current strategy is effector T-cell vaccination directed at infected hepatocytes. RTS,S/(SB)AS02, an adjuvanted fusion protein, produced transient protection in 70% of vaccines. Prime (DNA vaccine) boost (poxvirus recombinant) is particularly immunogenic. Pyrethroid-treated bed nets reduce childhood mortality and deplete the mosquito population, interrupting transmission. Chlorproguanil-dapsone is more effective than pyrimethamine-sulfadoxine in treating uncomplicated chloroquine-resistant malaria. Artemisinin derivatives are as effective as quinine in severe disease. Snake bite is an underestimated and neglected cause of morbidity and mortality in rural communities in tropical countries. Sutherland's pressure-immobilisation technique is recommended first-aid for victims of neurotoxic elapid snakes. Rabies post-exposure prophylaxis, using new generation cell culture vaccines, is now feasible in developing countries, employing an economical 8-site intradermal regimen. This Harveian Oration, the first in 350 years to be devoted to tropical medicine, emphasises the importance of this speciality in the twenty-first century.

Above all, William Harvey (1578–1657) believed in the experimental method and wanted to encourage the Fellows of this College to do research. He exhorted 'the ffellowes and members of the Colledge ... to search and Studdy out the secrett of Nature by way of Experiment' (Harvey Trust Deed 21 June 1656). My title reflects a personal enthusiasm for experiment in the field of tropical medicine. I cannot pretend that Harvey shared this interest, but he certainly mixed with people for whom diseases of warm climates were of real practical importance. His brothers traded with the Levant and the Far East and a colleague at St Bartholomew's Hospital, John Woodall (1556?–1643), was surgeon-general to the East India Company from 1613 to 1635[1]. As an example of the application of the experimental method to tropical medicine, I will discuss three diseases which I have found particularly interesting and challenging. I was delighted to find evidence in Harvey's writings that he had come across these conditions even though he never travelled outside Europe.

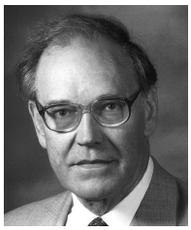
Malaria

Harvey experienced malaria both as patient and pathologist. Discussing the anatomical position of the liver and spleen in his *Prelectiones anatomiae universalis*, he mentioned that his spleen was enlarged during a quartan ague[2] and in *De motu cordis et sanguinis*, he described the effects of tertian fever on the heart and lungs, adding: 'I speak with experience on this point through my dissections of subjects who have died at the beginning of attacks'[3].

In those days, *Plasmodium vivax* (and possibly *P. malariae*) infections, described as tertian or quartan agues, were common in estuarine and marshland areas of England. The character of vivax malaria, now known as benign tertian malaria, was much more severe in the sixteenth and seventeenth centuries, when it is thought to have increased average crude death and infant mortality rates by one-third to one-half and to have reduced average life expectancy by 25 years in residents of malarious areas, compared to those living in upland areas of England[4].

The global malaria crisis

The WHO estimated that, in 1997, among 300–500 million cases of malaria world-wide, there were 1.5–2.7 million fatalities, 75% of them young children in sub-Saharan Africa[5]. Malaria also creates morbidity through chronic anaemia and neurological impairment after cerebral malaria and by contributing to the low birth weight of perhaps one million babies born annually to mothers who contract malaria during pregnancy[6]. Inhabitants of malaria endemic areas are further impoverished through the devastating effects of this disease on



The Harveian Oration is given annually at the Royal College of Physicians of London under an indenture of William Harvey in 1656. This article is based on the 2001 Oration given on 18 October 2001 by

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social, economic and even perhaps intellectual development[6]. Paradoxically, poverty may be associated with high fertility, thus increasing the demands on scarce resources: '*A mesa do pobre é escassa, mas o leito da miséria é fecundo*' (The table of the poor is meagre, but fertile is the bed of misery)[7].

Three of the most useful responses to the global malaria challenge are the development of a malaria vaccine, the use of insecticide-treated bed nets and the improvement of anti-malarial treatment.

Malaria vaccines

Natural immunity

By the age of 7–10 years, children who have survived growing up in malarious areas will have acquired immunity naturally, through repeated infections. A vaccine that could produce this level of immunity would be invaluable for infants and pregnant women in sub-Saharan Africa. A second type of vaccine would be aimed at protecting non-immune travellers to malaria-endemic areas against all symptoms and effects of malaria. A third type, by targeting sexual stages of the parasite in human and mosquito, could help to reduce malaria transmission.

Difficulties facing the development of a malaria vaccine

Despite 70 years of endeavour, no satisfactory malaria vaccine has been developed. A major problem is the impracticability of producing large quantities of attenuated micro-organisms, the basis for most effective viral and bacterial vaccines. Other difficulties relate to biological attributes of the malaria parasite, selected during evolution to enable it to survive in the human host until it is taken up by a mosquito and propagated. Immunity to malaria is strain- and stage-specific. The genome of *P. falciparum* (25–30 megabases with 5–6,000 genes, many of them polymorphic, on 14 chromosomes) exhibits great diversity and each infection may involve up to eight different *P. falciparum* strains. Antigenic variation of some parasite proteins, notably PfEMP-1 expressed on the surface of infected erythrocytes, enables *P. falciparum* to evade the host's immune response. Another problem facing the widespread use of a malaria vaccine is the variation in innate genetic resistance of humans to pathological effects of malaria infection such as that conferred by polymorphisms of globin and glucose-6-phosphate dehydrogenase genes[8].

Pre-erythrocytic stage vaccines

Irradiation-attenuated sporozoites

The first successful attempt to immunise a human against malaria, by David Clyde and his colleagues[9], was based on studies in mice infected with *P. berghei*[10], in which protection was induced by irradiation-attenuated sporozoites. This technique was re-examined by Hoffman and his colleagues[11,12]. A group of 11 volunteers, immunised by receiving more than a thousand bites from irradiated mosquitoes harbouring infectious *P. falciparum* sporozoites, were protected against 33 out of 35 challenges by non-irradiated infected mosquitoes. Protection lasted for at least 36–42 weeks and extended to a strain of parasite different from those used for immunisation. However, such a laborious process is impracticable for immunising small groups of non-immune travellers, let alone endemic populations.

Pre-erythrocytic stage effector T-cell vaccines

The immunological mechanism of protection conferred by irradiated sporozoite immunisation involves $CD8^+$ and $CD4^+$ T-cell recognition of sporozoite proteins expressed within infected hepatocytes and humoral antibodies to sporozoite surface proteins[13]. The design of effector T-cell vaccines, targeting pre-erythrocytic stages of the life cycle in infected hepatocytes (Fig 1), is based on these findings. The two most productive strategies have been the use of protein-adjuvants (RTS,S/(SB)AS02) and heterologous prime-boost immunisation.

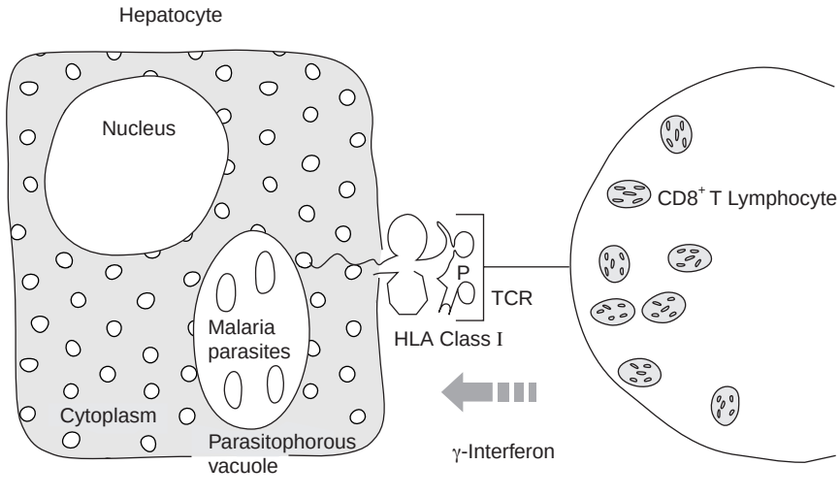

Fig 1. T-cell attack on a malaria-infected hepatocyte, leading to γ-interferon-induced lysis; the mechanism of pre-erythrocytic malaria T-cell vaccines. P=malaria peptide; TCR=T-cell receptor. Redrawn after AVS Hill, with permission.

RTS,S/(SB)AS02 malaria vaccine – RTS,S, a fusion protein combining most of the circumsporozoite protein of *P. falciparum* with HBsAg and a complex adjuvant (AS02), is capable of inducing strong antibody and $CD4^+$ T-cell responses. It protected 50% of volunteers challenged within 2–3 weeks of their last immunisation but, after six months, only one in five was protected[14,15]. Field trials in the Gambia showed an efficacy against infection of 71% (95% CI, 46–85%) during the first nine weeks but no protection after that. A single booster vaccination

achieved protection of 47% (3.8–71%, p=0.037) during the next malaria season[16]. Protection correlated with a short-lived vaccine peptide-specific $CD4^+$ T-cell response. It is hoped to improve this vaccine by modifying the adjuvant, by boosting with a vaccinia recombinant circumsporozoite protein and by addition of a blood stage (MSP-1) antigen. Trials in Gambian children are underway.

Heterologous prime-boost immunisation – In Oxford, my colleague Adrian Hill and his team have pioneered the strategy of priming with a DNA-based vaccine and boosting with a recombinant poxvirus, a particularly effective way of inducing $CD8^+$ cytotoxic T lymphocytes and enhancing TH1-type $CD4^+$ T-cell responses, both of which correlate with protection. The DNA vaccine encodes a string of sporozoite CTL epitopes and the entire thrombospondin-related adhesion protein (TRAP). The poxvirus recombinant is the highly attenuated vaccinia virus strain (Modified Vaccinia Virus Ankara (MVA)), which does not replicate in mammalian cells, containing the same malaria insert. Phase I and II studies in Oxford and the Gambia have confirmed the safety and immunogenicity of the regimen and challenge studies are underway. An even more promising regimen, based on mouse studies, consists of priming with a Fowlpox (Avipox FP9) recombinant and boosting with the MVA recombinant (AVS Hill, personal communication).

Until an effective malaria vaccine can be deployed throughout the endemic areas, more conventional methods must be used to prevent and treat malaria.

Prevention of malaria by insecticide-treated bed nets (mosquito nets) (ITNs)

History

Mechanical barriers against the nuisance of night-biting arthropods were described centuries before Ross and Grassi proved that malaria was transmitted by mosquitoes[17]. Herodotus (484–425?BC) in the The Histories (completed in about 425BC) described how the Egyptians who lived in low-lying areas which became marshy with standing water after heavy rain (τελματα)[18], coped with the huge numbers of mosquitoes:

> *Every man there has a net which he uses in the daytime for fishing, but at night he finds another use for it: he drapes it over the bed where he spends the night and then crawls in under it and goes to sleep. Mosquitoes can bite through any cover or linen blanket that a person might wrap himself up in when he is in bed, but they do not even try to bite through the net at all[19].*

Commentators disagree about whether this is possible or whether Herodotus was confusing fishing nets with finer-meshed mosquito curtains. If Egyptian seine nets had been folded several times, the resulting mesh might have been fine enough to exclude mosquitoes. However, the word Herodotus used for fishing net is ἀμφίβληστρον which implies a coarse-meshed throwing net rather than a seine net. It has been claimed that fish oil residues on the nets might have had a repellent effect[20]. This seems the most likely explanation if we take Herodotus' observation literally, that the mosquitoes 'did not even try to bite through the net' (Oliver P Taplin, personal communication).

Insecticide-treated nets (ITNs)

Untreated bed nets (mosquito nets) can protect their occupants against mosquito bites. How effective they are at preventing malaria is more controversial, but there is no doubt that treatment of the net with an insecticide or repellent greatly improves the protection it offers. ITNs, first used in the 1940s, are now impregnated with quick-acting synthetic pyrethroids. In a meta-analysis of 18 randomised controlled trials, ITNs achieved an overall reduction in all-cause childhood mortality of 17%[21]. The most dramatic successes were in The Gambia, an area of holoendemic seasonal malaria. Introduction of ITNs was associated, in the first year, with reductions of 70% and 63% in malaria-related and overall mortality in children aged 1–4 years[22]. In a larger study, there was a 25% reduction in all-cause mortality in children aged 1–9 years[23]. However, in the following year, when villagers were asked to pay US $0.5 for re-treatment of their nets with insecticide, use of ITNs declined and child mortality rates returned to pre-intervention levels. In the Siaya/Bondo districts of western Kenya, ITNs reduced the incidence of severe malarial anaemia in pregnancy by up to 50% (p<0.05), the incidence of low birthweight babies by 28% (risk ratio 0.72, p<0.05) and of low birthweight and stillborn babies, abortions and intrauterine growth retardation by 25% (risk ratio 0.75, p<0.05) in women of parities 1–4 (F ter Kuile, P Phillips-Howard, personal communication).

Massive deployment of ITNs in China (2.7 millions protected by 1988) and Vietnam (11.5 millions protected by 1999) has been associated with impressive reductions in malarial incidence and mortality (Fig 2)[24] (National Malaria Control Programme, Ministry of Health, Vietnam; Allan Schapira, personal communication). However, attribution of the decline in malaria in these countries is difficult, as there were other changes and interventions (notably the use of artemisinin drugs in Vietnam) which may well have contributed.

ITNs not only protect their occupants but, acting like traps baited with human odour, kill or repel mosquitoes, so reducing malarial inoculation rates in the community[25]. Use of ITNs in Africa has been frustrated by lack of enthusiasm in some countries and by the cost of providing nets and ensuring their annual retreatment with insecticide. ITNs, like EPI vaccines and house spraying with residual insecticides, should surely be provided free by international agencies or governments[26,27].

Chemotherapy of uncomplicated falciparum malaria

Extracts from the bark of the cinchona tree, containing quinine and related alkaloids, were the first effective and specific antimalarial treatments used by Europeans, in their Latin American

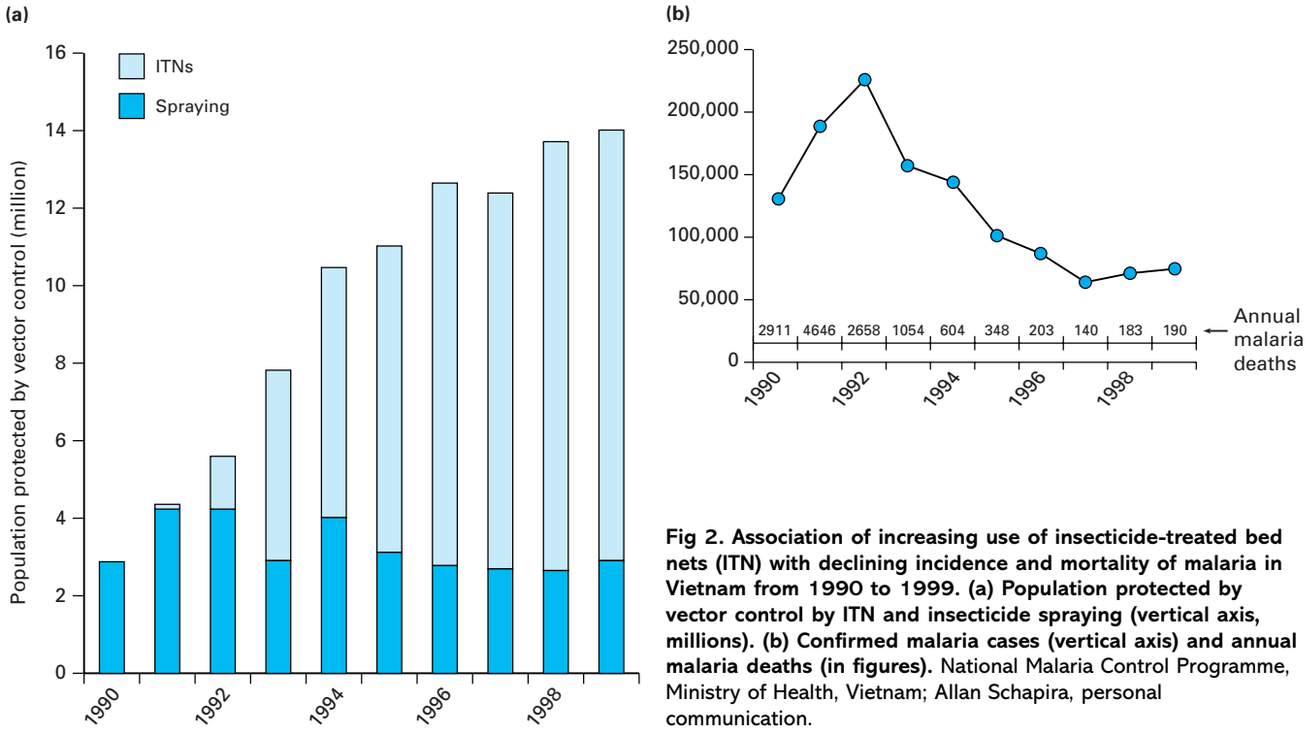

Fig 2. Association of increasing use of insecticide-treated bed nets (ITN) with declining incidence and mortality of malaria in Vietnam from 1990 to 1999. (a) Population protected by vector control by ITN and insecticide spraying (vertical axis, millions). (b) Confirmed malaria cases (vertical axis) and annual malaria deaths (in figures). National Malaria Control Programme, Ministry of Health, Vietnam; Allan Schapira, personal communication.

colonies and at home. Harvey could not have benefited from this discovery made by Jesuit missionaries in Peru in about 1600, as 'Jesuit's bark' was not available in Europe until 1639 and in England until the 1650s[28]. His contemporary, Robert Burton (1577–1640), listed quartan agues among many diseases that physicians could not cure at all[29].

Cheap synthetic drugs, such as chloroquine, amodiaquine, pyrimethamine and sulphonamides, replaced quinine as treatment for uncomplicated falciparum malaria, from the 1940s. Unfortunately, resistant strains of *P. falciparum* emerged less than two years after the introduction of pyrimethamine and 15 years after chloroquine[30]. The impact of chloroquine resistance on malarial mortality is now evident. Between 1984 and 1995, in three areas of Senegal, malarial mortality among children under five years old increased 2.3, 2.5 and 11 fold, associated with the emergence of chloroquine resistant *P. falciparum* infections[31]. The drug most commonly employed to replace chloroquine (recently in Tanzania, Kenya and Malawi) has been the antifolate-sulphonamide combination pyrimethamine sulfadoxine (PSD) ('Fansidar'), but in South East Asia, serious resistance developed in about five years. Resistance to antifolates results from mutations at residues 108, 51, 59, 16 and 164 in the *P. falciparum* dihydrofolate reductase (DHFR) gene and sulphonamide-resistance from mutations at positions 436, 437, 581 and 613 in its dihydropteroate synthase (DHPS) gene. DHFR 108, 51 and 59 mutations, which are associated with PSD-resistance, are increasingly prevalent in Africa, and a fourth mutation, DHFR164, has recently been detected by complementation into yeast in *P. falciparum* isolates from Tanzania[32] suggesting that the efficacy of PSD will be short-lived. An alternative strategy is to combine chlorproguanil ('Lapudrine') with dapsone as 'lapdap'. This has proved more effective than PSD in treating *P. falciparum* with 108, 51 and 59 mutations in Tanzania[33], but there is already evidence that these mutants are being selected in areas where lapdap is being used[34]. It has been suggested that lapdap should be combined with an artemisinin derivative to extend its useful therapeutic life[35], but in mouse malaria, there is some evidence of antagonism between antifolates and artemisinins[36].

Treatment of severe malaria

Artemisinins

In 1971–72, Chinese scientists first isolated the active principle, *qing hao su* (artemisinin), from *Artemisia annua*[37]. The efficacy and safety of its derivative artemether for treatment of severe *P. falciparum* malaria has been compared with that of quinine in a series of large, randomised trials. A meta-analysis of seven trials involving 1,919 patients found no statistically significant difference in case fatality, duration of coma, persistence of fever or the incidence of neurological sequelae in patients treated with artemether or quinine. However, the combined 'adverse outcome' of either death or neurological sequelae was significantly less common in the artemether group, and artemether cleared peripheral parasitaemia more rapidly than quinine. Subset analyses suggested an advantage of artemether treatment in adult patients and in those with renal failure, hypoglycaemia

and jaundice[38]. In thousands of carefully observed patients, there has been no evidence of the severe neurotoxicity induced by artemisinins in animals. The use of anti-malarial agents in combination, to prevent or delay emergence of drug resistant genotypes of *P. falciparum*, has long been argued by Wallace Peters[30] and the special advantages of drug combinations including a rapidly-acting artemisinin derivative, by Nick White and his colleagues[39].

For early anti-malarial treatment, at village level, before profound and irreversible pathophysiological disturbances have evolved, artemisinins can be administered as suppositories. This is particularly valuable in the treatment of infants and young children who may not tolerate tablets.

Anti-cytokine treatment for severe malaria

Experience with ancillary treatments such as dexamethasone[40], anti-pyretics[41], prophylactic anticonvulsants[42], iron chelation[43], malarial hyperimmune globulin[44]; heparin, dextrans, osmotic agents, adrenaline and cyclosporin A[45] has been uniformly disappointing.

In 1978, Ian Clark suggested that a malarial toxin or pyrogen released at merogony (schizont rupture) might stimulate macrophages to release mediators that were ultimately responsible for lethal pathophysiology[46]. This hypothesis gained ground when tumour necrosis factor (TNF) and other cytokines were detected in a mouse malaria model[47], high circulating levels of TNFα correlating with severity were found in African children with cerebral malaria[48] and genetic variation within the TNF promotor region was found to be associated with susceptibility to cerebral malaria[49]. Anti-cytokine immunotherapy seemed logical, but in a large randomised, placebo-controlled trial in children with cerebral malaria in the Gambia, an anti-TNFα monoclonal antibody failed to improve case fatality and was associated with a significant increase in neurological sequelae[50].

The Jarisch-Herxheimer reaction of louse-borne relapsing fever

Malaria is one of a number of diseases in which, despite compelling pathophysiological evidence, cytokine antagonists have failed to produce a demonstrable lowering in mortality[51]. The exception is louse-borne relapsing fever (LBRF) (*Borrelia recurrentis* infection) which is now virtually confined to the Horn of Africa[52]. It was once a common epidemic disease in Britain (up to 1870) and Europe, accompanying famine, poverty and crowding – conditions encouraging lousiness in the population. In Harvey's time, it was probably responsible, with louse-borne typhus, for cases of 'famine fever', and earlier – in the sixteenth century – may have contributed to the epidemics of 'sweating sickness' described by John Caius (1510–1573)[53] and others[54]. In the first half of the twentieth century, LBRF is thought to have caused 50 million cases with a 10% case fatality in Eastern Europe, North Africa and the Middle East[52].

Treatment with antibiotics such as penicillin and tetracyclines is essential, as the untreated case fatality has exceeded 70% in some epidemics. But this treatment provokes a life-threatening Jarisch-Herxheimer reaction (J-HR) in the majority of patients[55]. Clark's prediction that such reactions might be mediated by macrophage-derived products[47] was confirmed in LBRF[56]. The borrelial pyrogen is its variable major lipoprotein[57]. In a randomised, placebo-controlled trial, an ovine polyclonal anti-TNFα Fab antibody ('CytoTAb'), infused just before the administration of intravenous tetracycline, reduced the incidence and severity of the reaction without impairing the clearance of spirochaetaemia[58]. The J-HR of LBRF – a predictable, intense inflammatory response to the necessary treatment of a dangerous infection – is a good example of Harvey's dictum that rare conditions can be valuable in the elucidation of medical problems[1].

Snake bite

In Britain, our one species of venomous snake, the adder (*Vipera berus*), causes barely 100 hospital admissions annually and has killed only 14 people since 1876, the last in 1976. But snake bites are a common medical emergency in many parts of the tropical world. The annual global mortality from snake bite is said to range from 50,000 to 100,000, but these largely hospital-based estimates are unreliable since most victims seek traditional treatment and may die at home unrecorded. Population-based studies have revealed unexpectedly high rates of bites and deaths[59]. For every person killed, more than ten may be left with some permanent physical handicap. The problem of snake bite is worst in rural parts of the tropics where patients may have to travel for hours or even days before reaching a clinic where they can be treated with antivenom, the only effective antidote. In this situation, safe and reliable first-aid methods are specially important to delay the development of life-threatening paralysis, shock, bleeding diathesis and acute renal failure.

Harvey has been credited with one of the earliest controlled observations in clinical toxicology when he self-experimented (toxinology) with spider's venom[1]. He was also interested in first-aid for snake bite.

First-aid treatment of snake bites: use of ligatures/tourniquets

In *De motu cordis et sanguinis*, Harvey cited the spread of venom from the site of a snake bite as proof of his hypothesis: 'in the bite of serpents ... it comes about that the whole system is vitiated through the relatively harmless touching of but a small part of it.' This suggested a drastic first-aid method: 'a man might be saved ... if a very strong ligature were made above the wound immediately and the mortified part below the ligature were cut off presently[1].'

Ligatures were not mentioned in the earliest known treatise on the treatment of snake bite, the Brooklyn papyrus, dated the fourth century BC, but possibly much older[60,61]. However, this technique was recommended by Nicander of Colophon (180BC?–?)[62], Galen (129?–200?)[63] and, justified by his

Harveian concept of the circulation, by Francesco Redi (1626–1697/8): 'apply a tight ligature at the part not far above (the bite) so that the circulation of the blood does not carry the venom to the heart and the whole mass of blood be infected[63].' It was left to Felice Fontana (1730–1805) to prove ligatures effective by experiment but he recognised the risk of applying them for too long: 'The ligature, by confining the blood to the part, produces a greater local complaint, and a stronger disposition to gangrene: for this reason it should be pretty loose and ought to be removed as early as possible[64].'

However, the danger of premature release was illustrated vividly by Jacob Bontius (1592–1631) in his description of a case of cobra bite in The East Indies: 'a very tight ligature was put round his arm, which occasioned such intolerable pain, as to oblige it to be slackened. The poor man immediately expired[65].'

After more than a century of neglect, Hamilton Fairley revived interest in ligatures in the early twentieth century. Australian snake venoms were found to be absorbed very rapidly, through lymphatics and veins, depending on the molecular size of particular venom components. Ligatures delayed the absorption of neurotoxins only while they were in place and did not prevent fatal paralysis after release[66]. The use of tourniquets in human victims proved extremely painful and carried a formidable array of complications, of which the most debilitating was gangrene. Studies in Oxford by Barnes and Trueta suggested a more acceptable first-aid technique. In rabbits, absorption of black tiger snake (*Notechis ater*) and Russell's viper (*Daboia russelii*) venoms could be prevented by lymphatic obstruction or by complete immobilisation of the envenomed limb. However, this method was not effective for cobra venom, whose lethal toxins had a lower molecular weight and were probably absorbed directly into the bloodstream[67].

Pressure immobilisation

In Melbourne, my colleague Struan Sutherland[68] realised that these observations had important implications for snake bite first-aid. In animals, he found that immobilisation of the envenomed limb combined with compression at about 55 mmHg greatly reduced the systemic spread of tiger snake (*Notechis scutatus*) venom and its principal neurotoxin, notexin (Fig 3). Compression was best achieved by firm binding with a crêpe bandage, over the whole length of the bitten limb and immobilisation by splinting. No formal clinical trials have been carried out, but a number of persuasive anecdotal case reports have shown that the pressure-immobilisation (PI) technique (Fig 4) can delay the appearance of systemic envenoming[69]. However, there are practical difficulties in bandaging at the correct pressure and, even in Australia where PI has been enthusiastically promoted, it is used in only about 18–45% of snakebite cases and, at least in children, is rarely applied properly (JH Pearn, personal communication). Lymphoscintigraphy studies in healthy volunteers injected with a labelled 'mock' venom colloid showed that crêpe bandaging was effective only within a narrow pressure range and with the patient completely immobilised[70].

Pressure immobilisation could exaggerate the tissue necrosis caused by the venoms of many species of viper and some elapids occurring outside Australia. Results of controlled clinical trials are needed but, in the meantime, PI is recommended only for the first-aid treatment of bites by snakes capable of causing early life-threatening paralysis.

Mad dog bite and rabies

Rabies encephalomyelitis is one of the most agonizing and certain deaths imaginable[71] and fear of rabies affects the millions of people bitten by potentially rabid animals each year. Some idea of the burden of human suffering from rabies is provided by WHO's estimates of 60,000 deaths from dog-mediated rabies and the use of 50 million doses of vaccine for post-exposure prophylaxis in 1997[4]. Even these figures are probably underestimates.

Pathogenesis of rabies

In support of his concept of the circulation of the blood, William Harvey wrote:

> *I have known fever or the other dread symptoms come on after the wound made by the bite of a mad dog has been cured ... the contamination is first imprinted into the part, then reaches the heart in the returning blood, and thereafter from the heart pollutes the whole body[3].*

This notion of rabies spreading through the blood stream has, however, been proved incorrect by experiments carried out over the past 120 years. The virus travels from the site of the infective bite through nerves to reach the central nervous system[72]. Virus inoculated into the bite wound may replicate locally in muscle cells or attach directly to nerves. At the motor end plate, it competes with acetylcholine (and with Chinese krait *Bungarus multicinctus* α-bungarotoxin and HIV external envelope glycoprotein – gp 120 – with both of which rabies glycoprotein G has sequence homology) to bind with the α unit of the acetylcholine receptor before entering the pre-synaptic nerve ending, probably by endocytosis[73]. Inside peripheral nerves, virus is carried towards the central nervous system by fast retrograde axonal transport[74] probably through interaction of viral phosphoprotein P with LC8 protein, part of the dynein motor in cytoplasmic and axonal microtubules[75,76].

Rabies post-exposure prophylaxis

Post-exposure vaccination, first tried by Pasteur in 1885, was employed increasingly during the first half of the twentieth century, but its efficacy remained uncertain. The bite of a mad dog, even in the pre-vaccine era, caused rabies in only about 30% of patients[77], although with wolf bites, the risk was higher. In 1954 a rabid wolf bit 29 people in a village in rural Iran during the course of one night. Eighteen had been bitten on the head (including a six-year-old boy in whom the wolf's teeth had penetrated the dura mater): 13 of these were given vaccine plus equine antirabies serum (mortality 1/13) while five were given vaccine alone (mortality 3/5). The six-year-old boy survived.

Eleven were bitten on the limbs and trunk: four were given vaccine plus antiserum and seven vaccine alone (mortality 0/11). Results of this natural experiment suggested that the combination of equine anti-rabies serum with vaccine was necessary to prevent rabies after head bites[78]. Rabies immune globulin (RIG) is presumed to neutralise virus locally in the bite wound and to enhance cell mediated immunity during the first few days after the bite, before it has gained entry to the nervous system[79].

Modern post-exposure prophylaxis; comprising wound cleaning, RIG and the safe and potent cell culture rabies vaccines[80]; has proved highly effective. However, the cost of cell culture vaccines made them impracticable for tropical

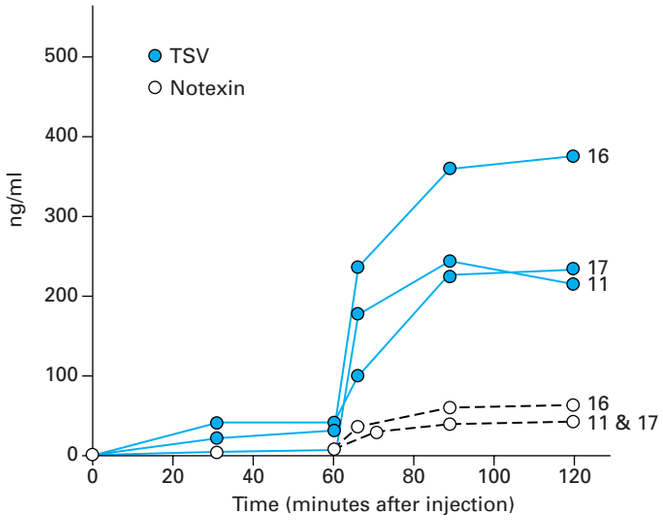

Fig 3 Plasma concentrations of Australian tiger snake (*Notechis scutatus*) venom (whole venom=TSV) and notexin in restrained animals whose envenomed limbs were immobilised and bound firmly with crêpe bandages for 60 minutes. Reprinted with permission from Elsevier Science (Sutherland *et al*., *The Lancet*, 1979, **i**:183–6). Reproduced by permission of Oxford University Press Australia from *Australian Animal Toxins* 2e by SK Sutherland and J Tibbals © Oxford University Press, www.oup.com.au

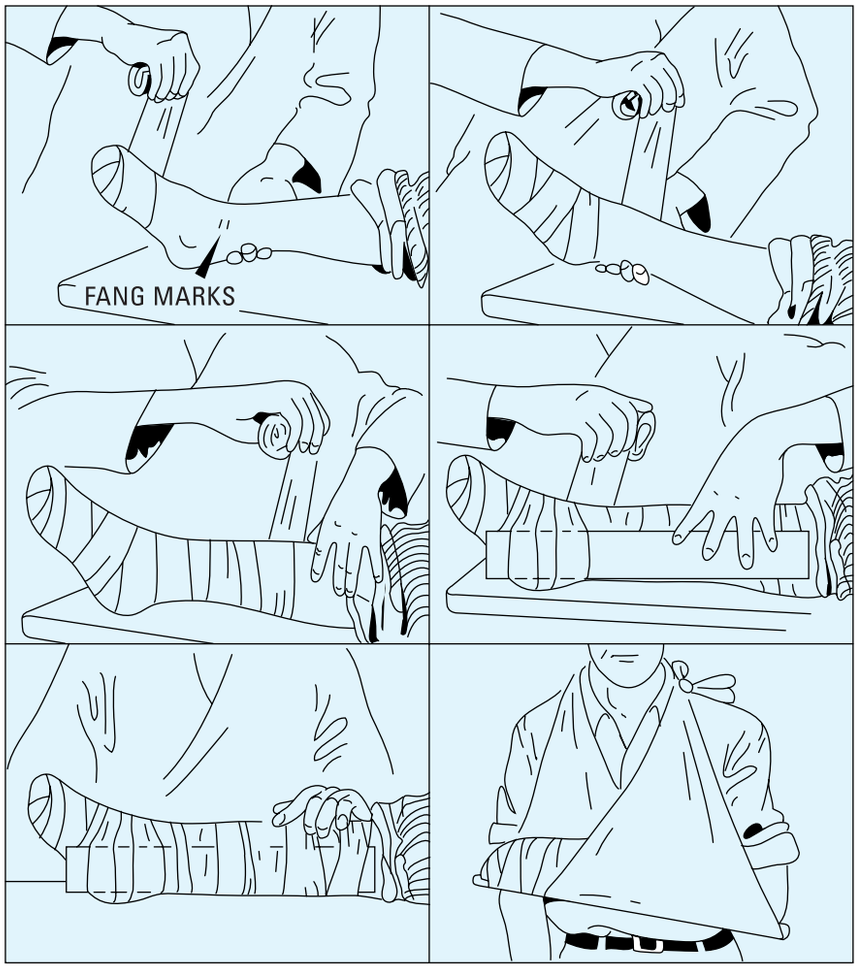

Fig 4 Struan Sutherland's pressure-immobilisation method for first-aid of snake bite From Australian Venom Research Unit, Department of Pharmacology, University of Melbourne, with permission.

Key Points

Malaria causes two million deaths each year mainly in Africa

Effector T-cell malarial vaccines may offer a promising method of prevention

Insecticide-treated bed nets reduce malarial morbidity and mortality and must be deployed more widely

Proguanil-dapsone is preferred to pyrimethamine-sulfadoxine for treatment of uncomplicated chloroquine-resistant falciparum malaria.

Pressure-immobilisation is recommended for bites by neurotoxic elapid snakes

For rabies post-exposure prophylaxis, an economical 8-site intradermal regimen allows use of cell culture vaccines in developing countries

developing countries. In 1985, a randomised controlled trial demonstrated the efficacy of an economical 8-site intradermal regimen of human diploid cell strain vaccine[81], that required 60% less vaccine than conventional regimens. This led the way to deployment of new generation vaccines in the tropical countries where 99.9% of all human deaths from rabies are thought to occur. The provision of RIG is an even greater challenge. In many tropical countries, it is simply not available and, outside wealthy countries, fewer than 2% of rabies post-exposure prophylaxis courses include this component[82].

Conclusion

IN THE MODERN EPIC OF HEALTH, a hero's part has often been assigned to tropical medicine ...: intrepid doctors going off to the steaming jungles and overcoming some of the most lethal diseases besetting mankind[83].

Roy Porter's clever caricature would compel anyone to re-examine their motives for embarking on a career in tropical medicine. Although I cannot completely dissociate myself from this image, I can reassure you that tropical medicine has buried its post-colonial prototype. Many of the pioneers of tropical medicine have been indigenous medical scientists. In Brazil these have included Adolfo Lutz (1855–1940), Adolfo Lindenberg (1872–1944), Carlos Chagas (1879–1934) and Gaspar Vianna (1885–1941). Nowadays, most clinical research on tropical diseases is carried out by local clinicians and scientists, but some institutions in the West such as the Centre for Tropical Medicine at the University of Oxford, and the Liverpool and London Schools of Tropical Medicine, are privileged to maintain collaborations with colleagues and institutions in tropical countries.

This review has focused on research into selected aspects of three apparently very different diseases of tropical countries. However, the diseases that follow bites by mosquitoes, snakes and mad dogs have a crucial zoological component that determines the pathogenesis and epidemiology of infection or envenoming. Understanding these aspects is essential for the development and testing of methods for prevention and treatment. Each of these three diseases attracted William Harvey's attention and interest and I imagine that he would have welcomed achievements of the experimental method applied to tropical diseases and would have sought the relevance of the results to his practice of science and medicine in seventeenth century England.

In the 350 years of its history, this is the first Harveian Oration to be devoted to tropical medicine. I hope that this signifies that my speciality is at last accepted into the mainstream of medicine in this country. The continuing challenge posed by tropical diseases re-emphasises the importance of the discipline of tropical medicine in the twenty-first century.

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