Harveian oration

"To search and Studdy out the secrett of Tropical Diseases by way of Experiment"

David A Warrell

Above all, William Harvey (1578–1657) believed in the experimental method and wanted to encourage the fellows of the Royal College of Physicians, London, to do research. In an indenture establishing this oration, he exhorted "the ffellowes and members of this Colledge . . . to search and Studdy out the secrett of Nature by way of Experiment" (Harvey Trust Deed, June 21, 1656). My title reflects a personal enthusiasm for research in the field of tropical medicine. 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 and thought about their pathophysiology even though he had never travelled outside Europe.

Malaria

In 16th and 17th century England, *Plasmodium vivax* and possibly *P malariae* infections ("agues") were common in estuarine and marshy areas. 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 own spleen had been enlarged during a quartan ague[1] and in *De motu cordis et sanguinis*, he described the effects of tertian fever on the heart and lungs: "I speak with experience on this point through my dissections of subjects who have died at the beginning of attacks".[2]

The global malaria crisis

The WHO estimated that, in 1997, among 300 million to 500 million cases of malaria worldwide, there were 1·5 million to 2·7 million fatalities, 75% of them young children in sub-Saharan Africa.[3] Malaria also creates morbidity through chronic anaemia and neurological impairment after cerebral malaria and by contributing to the low birthweight of perhaps 1 million babies born annually to mothers who contracted malaria during pregnancy.[4] Inhabitants of malaria endemic areas are impoverished through the devastating effects of this disease on social, economic, and even perhaps intellectual development.[4]

Among the most useful responses to the global malaria challenge are the development of a malaria vaccine, the use of insecticide-treated bednets, and the improvement of antimalarial treatment.

Malaria vaccines

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 beings and mosquitoes, could help to reduce malaria transmission.

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-specific and stage-specific. The genome of *P falciparum* (25–30 megabases with 5000–6000 genes, many of them polymorphic, on 14 chromosomes) exhibits great diversity and each infection involves a number of different *P falciparum* strains. Antigenic variation of some parasite proteins 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 human beings to pathological effects of malaria infection.[5] Immune response to vaccines can also be determined genetically.[6]

The first successful attempt to immunise a human being against malaria, by David Clyde and colleagues,[7] was based on studies in mice infected with *P berghei*,[8,9] in which protection was induced by bites of irradiated infected mosquitoes. This technique was re-examined by Steve Hoffman and colleagues.[10,11] 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 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 impractical for immunising small groups of non-immune travellers, let alone endemic populations.

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.[12] The design of effector T-cell

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vaccines, targeting pre-erythrocytic stages of the life cycle in infected hepatocytes, is based on these findings. The two most productive strategies have been use of protein-adjuvants (eg, in the RTS,S/AS02 vaccine) and heterologous prime-boost immunisation.

RTS,S—a fusion protein combining most of the circumsporozoite protein of *P falciparum* and HBsAg with 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 6 months, only one in five was protected.[13,14] Field trials in The Gambia showed an efficacy against infection of 71% (95% CI 46–85) during the first 9 weeks, but no protection after that. A single booster vaccination achieved protection of 47% (4–71, p=0·037) during the next malaria season.[15] Protection correlated with a short-lived vaccine peptide-specific CD4 T-cell response. It is hoped that this vaccine could be improved 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.

In Oxford, UK, 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 cytotoxic T-lymphocyte epitopes and the entire thrombospondin-related adhesion protein (TRAP). The poxvirus recombinant is a highly attenuated vaccinia virus strain (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.

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 bednets

Untreated bednets (mosquito nets) can protect their occupants against the nuisance of mosquito bites, but have not proved consistently effective in preventing malaria.[16] Insecticide-treated nets (ITNs), first used in the 1940s, are now impregnated with quick-acting synthetic pyrethroids. Randomised controlled trials have shown that ITNs can achieve an overall reduction in all-cause childhood mortality of 17%.[17] 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.[18] In a larger study, there was a 25% reduction in all-cause mortality in children aged 1–9 years.[19] However, in the following year, when villagers were asked to pay US$0·50 for retreatment of their nets with insecticide, use of ITNs declined and child mortality rates returned to preintervention 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 the incidence 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 (ter Kuile F, Phillips-Howard P, personal communication).

Massive deployment of ITNs in China and Vietnam has been associated with an impressive reduction in malaria

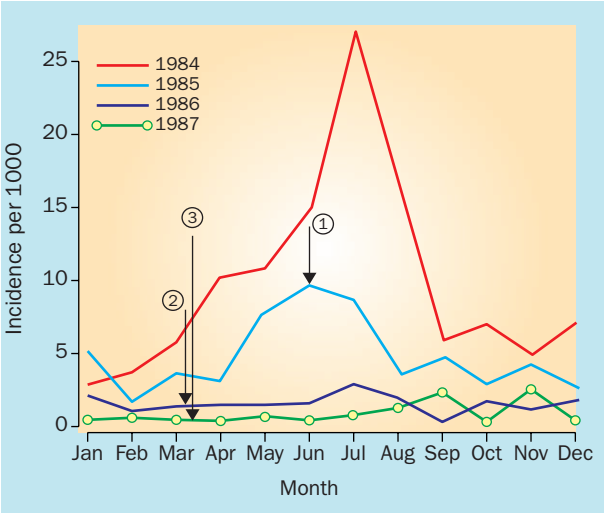

Figure 1: **Monthly incidence of *Plasmodium vivax* malaria in Buji District, Guangdong Province, China, before (1984) and after introduction of deltamethrin-treated bednets**
1, 2, and 3 in circles represent time of impregnation of nets in 1985, 1986, and 1987, respectively. From reference 20, with permission.

(figures 1 and 2; National Malaria Control Programme, Ministry of Health, Vietnam; Allan Schapira, personal communication), although other interventions and changes may have contributed, notably the use of artemisinin drugs in Vietnam.[20]

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.[21] 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 WHO's Expanded Programme on Immunisation vaccines and house spraying with residual insecticides, should surely be provided by international agencies or governments.[22,23]

Chemotherapy of uncomplicated falciparum malaria

Cheap synthetic drugs such as chloroquine, amodiaquine, pyrimethamine, and sulphonamides have been available for half a century. Unfortunately, resistant strains of *P falciparum* emerged less than 2 years after the introduction of pyrimethamine and 15 years after chloroquine.[24] The effect of chloroquine resistance on malarial mortality is now evident. Between 1984 and 1995, in three areas of Senegal, malaria mortality among children younger than 5 years increased 2·3-fold, 2·5-fold, and 11-fold, in association with the emergence of chloroquine-resistant *P falciparum* infections.[25] The drug most commonly used to replace chloroquine (recently in Tanzania, Kenya, and Malawi) has been the antifolate-sulphonamide combination pyrimethamine-sulfadoxine, but in southeast Asia, serious resistance developed in about 5 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 resistance to pyrimethamine-sulfadoxine, are increasingly prevalent in Africa, and a fourth mutation, *dhfr*164, has been detected recently in *P falciparum* isolates from Tanzania (Sibley C, unpublished),[26] suggesting that the efficacy of pyrimethamine-sulfadoxine will be short-lived.

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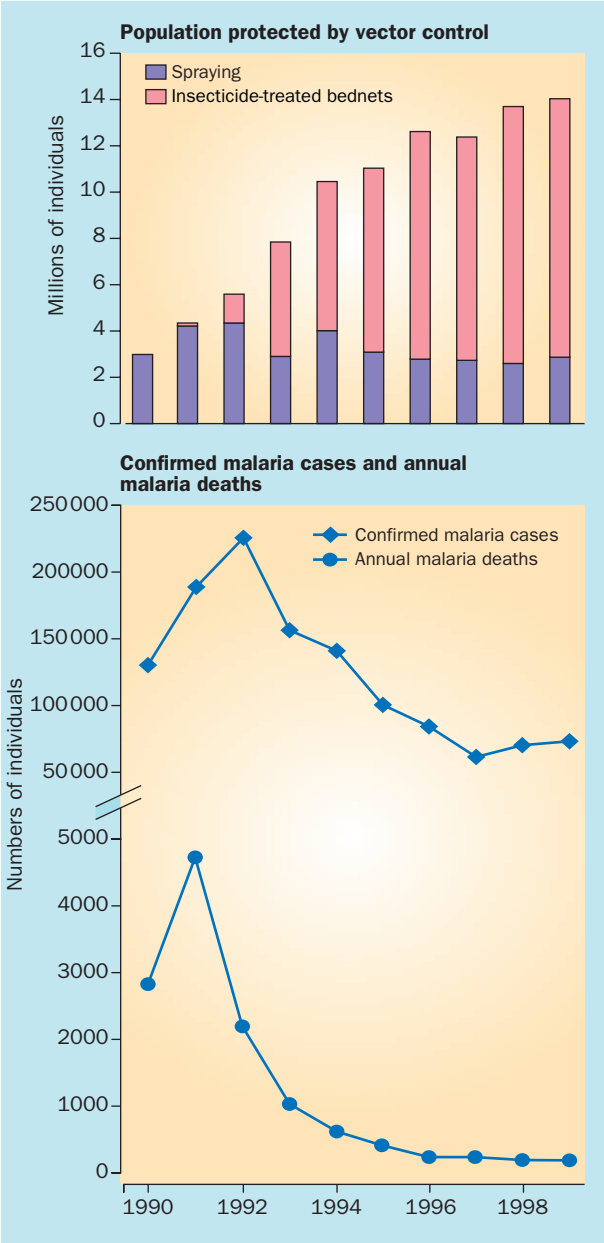

Figure 2: **Effect of increasing use of insecticide-treated bednets on incidence of and mortality from malaria in Vietnam, 1990–99**

Data provided by Allan Schapira (National Malaria Control Programme, Ministry of Health, Vietnam).

An alternative strategy is the combination of chlorproguanil with dapsone. This combination has proved more effective than pyrimethamine-sulfadoxine in treating *P falciparum* with 108, 51, and 59 mutations in Tanzania,[27] but there is already evidence that these mutants are being selected in areas where chlorproguanil-dapsone is being used.[28] It has been suggested that chlorproguanil-dapsone should be combined with an artemisinin derivative to extend its useful therapeutic life,[29] but in mouse malaria, there is some evidence of antagonism between antifolates and artemisinins.[30]

Treatment of severe malaria

In 1971–72, Chinese scientists first isolated qing hao su (artemisinin), from *Artemisia annua*.[31] The efficacy and safety of the artemisinin derivative artemether and quinine for treatment of severe *P falciparum* malaria has been compared in a series of large, randomised trials. A meta-analysis of seven trials involving 1919 patients found no 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.[32] In thousands of carefully observed patients, there has been no evidence of the severe neurotoxicity induced by artemisinins in animals. The use of antimalarial agents in combination, to prevent or delay emergence of drug-resistant genotypes of *P falciparum*, has long been argued by Peters,[24] and the special advantages of drug combinations including a rapidly-acting artemisinin derivative have been maintained by White and colleagues.[33]

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

Results of randomised controlled trials of ancillary treatments such as dexamethasone,[34] antipyretics,[35] prophylactic anticonvulsants,[36] iron chelation,[37] malarial hyperimmune globulin,[38] heparin, dextrans, osmotic agents, epinephrine, and ciclosporin[39] have been uniformly disappointing.

In 1978, Ian Clark suggested that a malarial toxin or pyrogen released at merogony might stimulate macrophages to release mediators that were ultimately responsible for lethal pathophysiology.[40] This hypothesis gained ground when tumour necrosis factor (TNF) and other cytokines were detected in a mouse malaria model,[41] high circulating concentrations of TNFα, correlating with severity, were found in African children with cerebral malaria,[42] and genetic variation within the TNFα promotor region was found to be associated with susceptibility to cerebral malaria.[43] Anticytokine 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.[44]

Malaria is one of a number of diseases in which, despite compelling pathophysiological evidence, cytokine antagonists have failed to produce demonstrable benefit.[45] The exception is louse-borne relapsing fever (*Borrelia recurrentis* infection) which is now virtually confined to the horn of Africa. 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 in most patients.[46] Clark's prediction that such reactions might be mediated by macrophage products[41] was confirmed in louse-borne relapsing fever.[47] The borrelial pyrogen is its variable major lipoprotein.[48] In a randomised, placebo-controlled trial, an ovine polyclonal anti-TNFα Fab antibody, infused just before the administration of intravenous tetracycline, reduced the incidence and severity of the reaction without impairing the clearance of spirochaetaemia.[49] The Jarisch-Herxheimer reaction of louse-borne relapsing fever—a predictable, intense inflammatory response to the necessary treatment of a dangerous infection—is a good

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example of Harvey's dictum that rare conditions could be valuable in the elucidation of medical problems.[50]

First-aid treatment for snake bite

In Britain, the only 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.[51] The problem of snake bite is worst in rural parts of the tropics where patients might 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 toxinology when he self-experimented with spider's venom.[50] He was also interested in first aid for snakebite.

Use of ligatures and 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,[2] and elsewhere he proposed the application of a ligature and amputation of "the mortified part below" as treatment.[50] Ligatures had been recommended by Galen (129–200)[52] and justified by a 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."[52] It was left to Felice Fontana (1730–1805) to prove ligatures effective by experiment, although he recognised the risk of their causing gangrene if applied for too long.[53]

In Australia, Hamilton Fairley revived interest in ligatures in the early 20th 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.[54] 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, UK, 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.[55]

Pressure-immobilisation

In Melbourne, Australia, Struan Sutherland and colleagues[56] realised that these observations had important implications for snake-bite first aid. In animals, they found that immobilisation of the envenomed limb combined with compression at 55 mm Hg greatly reduced the systemic spread of tiger snake (*Notechis scutatus*) venom and its principal neurotoxin, notexin (figure 3). Compression was best achieved by firm binding with a crepe 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 technique can delay the appearance of systemic envenoming.[57] However, there are practical difficulties in bandaging at the correct pressure and, even in Australia where pressure-immobilisation has been enthusiastically promoted, it is used in only about 18–45% of snake-bite cases and, at least in children, is rarely applied properly (Pearn JH, personal communication). Lymphoscintigraphy studies in healthy volunteers injected with a mock venom, colloid, showed that crepe bandaging was effective only within a narrow pressure range and with the patient completely immobilised.[58]

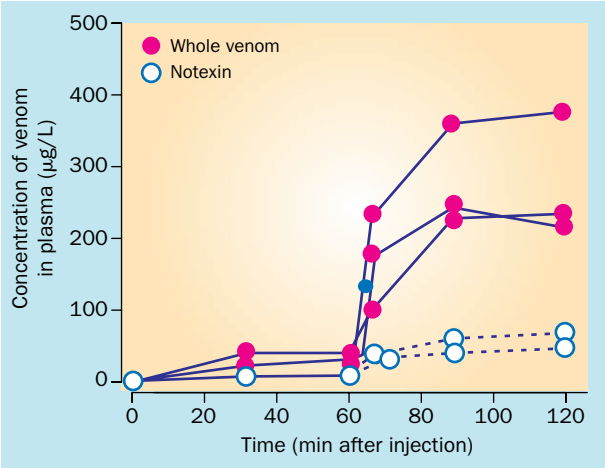

Figure 3: **Concentrations of Australian tiger snake (*Notechis scutatus*) venom in plasma of restrained animals whose envenomed limbs were immobilised and bound firmly with crepe bandages for 60 min**
From reference 56.

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, pressure-immobilisation is recommended 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 agonising and certain deaths imaginable,[59] 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 probable underestimates of 60 000 deaths from dog-mediated rabies and the use of 50 million doses of vaccine for post-exposure prophylaxis in 1997.[3]

In support of his concept of the circulation of the blood, 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."[2]

This notion of rabies spreading through the bloodstream 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.[60] Virus inoculated into the bite wound can replicate locally in

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muscle cells or attach directly to nerves. At the motor endplate, it competes with acetylcholine to bind with the α unit of the acetylcholine receptor before entering the presynaptic nerve ending by endocytosis.[61] Inside peripheral nerves, virus is carried towards the central nervous system by fast retrograde axonal transport[62] probably through interaction of viral phosphoprotein P with LC8 protein, part of the dynein motor in microtubules.[63,64]

Post-exposure vaccination, first used by Pasteur in 1885, was used increasingly during the first half of the 20th century, but its efficacy remained uncertain. The bite of a mad dog, even in the prevaccine era, caused rabies in only about 30% of untreated patients,[65] 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. 13 were bitten on the head (including a 6-year-old boy in whom the wolf's teeth penetrated the dura mater) and 11 on the limbs and trunk. Results of treatment suggested that the combination of equine antirabies serum with vaccine was necessary to prevent rabies after head bites.[66] Rabies immune globulin 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.[67]

Modern post-exposure prophylaxis—comprising wound cleaning, rabies immune globulin, and the safe and potent cell-culture rabies vaccines[68]—has proved highly effective. However, the cost of cell-culture vaccines made them impractical for tropical developing countries. In 1985, a randomised controlled trial demonstrated the efficacy of an economical eight-site intradermal regimen of human diploid cell strain vaccine,[69] leading 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 rabies immune globulin is a 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 passive immunisation.[70]

Conclusion

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. All three conditions attracted William Harvey's attention and interest. I believe that he would have welcomed the application of the experimental method to these diseases and would have sought the relevance of the results to his practice of science and medicine in 17th century England.

In the 350 years of its history, this is the first Harveian Oration to be devoted to tropical medicine. Does this signify that my specialty is at last accepted into the mainstream of medicine in England? The continuing challenge posed by tropical diseases re-emphasises the importance of the discipline of tropical medicine in the 21st century.

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