

DISCOVERY AND DISEASE CONTROL*

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Basic research often does not lead to an immediate solution to problems. Furthermore, applications of major discoveries may take years and may develop in unpredictable ways. Why then do funding agencies and foundations expect a product in the short term? In response to this expectation, scientists and science administrators promise results in the short term and are later criticized for not producing the goods. Even in so-called applied areas of disease control, we usually cannot predict when the next major advance will occur. To some in the endemic areas, it is difficult to justify basic research in the face of major health problems, as basic research cannot give a timetable for when it will make things better. However, where there are no known solutions or only partial or expensive control measures, research, slow and unpredictable as it may be, is the only hope. This is surely the case in malaria.

Malaria and its problems vary for each area of the world. Insecticides sprayed on the walls of houses are of little value in much of Africa because the vector feeds outside the house. As chloroquine resistance increases, so will mortality. In Myanmar and other similar regions of Asia, the forest fringe is the area where control has been extremely difficult. *Plasmodium falciparum* has returned as a major threat in the Indian subcontinent and Sri Lanka. Multi-insecticide resistance is a problem in the cotton growing areas of Central America. Deforestation and movement of populations into the Amazon exposes the immigrants to a high risk of malaria, and then throughout the country as a whole, as these people return to other parts of the country. We have little to offer in these difficult and varied situations that is economically feasible. How are we to proceed? One way to gain some perspective is to review the successes of the past and to trace the critical events that led to these successes.

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ANTIMALARIAL DRUGS

Chloroquine is on a short list of drugs that have decreased suffering and saved huge numbers of lives. The development of synthetic antimalarial drugs began at the turn of the century and brought to the peoples of the world a safe, effective, and cheap antimalarial drug, chloroquine. The discovery of this drug goes to prehistory and the use of cinchona bark by Peruvian Indians and its introduction into Europe by Jesuit missionaries. The history of this early discovery is described by Haggis¹ and by Grammicia.² The amazing observations by the early physicians in realizing that only malaria is affected by quinine is a testament to the acumen of these early physicians³ and a testament to the efficacy of quinine in malaria. During the early part of the 19th century, Caventou and Pelletier purified quinine. This opened the way for the analysis of the chemical structure by the German organic chemists of the latter half of the 19th century. These organic chemists were undoubtedly heavily influenced by the aniline dye industry. Of what use was this chemical research to the peoples of that time? The answer is that the purification of quinine and its chemical structure was not applied for almost 100 years.

The next important event in the discovery of chloroquine was also influenced by the German dye industry. Ehrlich observed that methylene blue stained malaria parasites and speculated that the dye that stains might also kill the malaria parasite. He always thought in terms of specificity of binding of chemicals for targets, a point of view that was to influence many fields of medicine, perhaps his greatest legacy.

Indeed methylene blue did kill the parasite, but it had a low therapeutic index. Subsequently, Schulemann added side chains to methylene blue and obtained increased activity.⁴ Nevertheless, the activity was not adequate for the needs of antimalarial drugs. Now the information from the previous century became of use to the development of synthetic antimalarials. Using the structure of quinine, Schulemann attempted to

put the side chain from the most active methylene blue derivative onto the 4 position of the quinoline ring. Without knowledge of the structure of quinine, he would not have known to place the side chain on a quinoline ring or where to place the side chain. If he had not started with methylene blue, he would not have a nitrogen at the 1 position of the side chain. A carbon at that position, as in quinine, would have led to an inactive compound. Nevertheless, the attempted synthesis was still unsuccessful. Schuermann, in his attempt to make a 4-aminoquinoline, ended up with an 8-aminoquinoline. He knew the side chain was in the wrong position and felt confident that if he could attach the side chain to the 4 position, he would have an active antimalarial drug. Ironically, this first synthetic antimalarial, the 8-aminoquinoline developed in 1925, is still the only class of drugs available against the liver stages. Later, the German organic chemists successfully made the first effective 9-acridine, Atebrine, which includes another side chain from quinine. Interestingly, it is the other half of the molecule that contains chloroquine. The subsequent story describing the pitfalls in the discovery of chloroquine has been beautifully told by Bob Coatney in his presidential address to the Society in 1962.⁵

It is clear that the discovery of chloroquine would not have occurred without the structural work of the organic chemists in the 19th century who purified quinine and defined its structure. Why did the 19th century administrators of science justify the support for work that had no application until the 20th century? The imagination of Ehrlich would have gone unrewarded without this important information. The other part of the story is the serendipity of the discovery of 8-aminoquinolines and the luck in starting the synthesis with methylene blue.

With the problems of drug resistance, the amazing thing to me is how little chemotherapy research today is oriented to the study of parasite molecules that may be vulnerable to attack; that is, molecular mechanisms that are unique to the parasite or are markedly different than those of the host. Our options need to be broadened by a sound biochemical research program, and then perhaps we will discover new classes of antimalarial drugs that we so badly need. Too few scientists are willing to follow this less trendy direction of research.

MOSQUITO CONTROL

The discoveries in mosquito control are perhaps more dramatic and more is known of the attitudes of the scientific and public health community at the time. Again, the dissociation between discovery and application is clear.

The exploits of Ronald Ross in India, the influence of his mentor, Patrick Manson, and Ross' conflict with the Italian scientists who independently discovered the role of the mosquito in the transmission of malaria are described in detail elsewhere⁶ and are not the subject of the present talk. The implications for the control of malaria were immediately evident to Ross and he proceeded to sweep the puddles in Freetown, Sierra Leone, to eliminate the areas of mosquito breeding.

Unfortunately, the great scientists are no better at forecasting problems in implementation than are average mortals. In fact, medical entomologists of the day were quite aware of the problems facing control. The discoveries by Ross and by Walter Reed in Cuba on yellow fever gave Gorgas the information that he needed to control the dual plagues for the building of the Panama Canal. The costs, however, were and still are outside the budgets of most developing countries, using the techniques of Gorgas (put oil on standing water, drain ditches, and screen houses). At the beginning of this century, the mosquito control during construction of the Panama Canal cost \$3.50/person/year, or \$3 million for 10 years to suppress malaria in 100 mi.² Many approaches to control of larvae since that day have had small successes in limited areas, but have not found worldwide application. For example, larvivorous fish have had only limited value.

One of the people who had a major impact on our thinking about *Anopheline* vectors was Louis Hackett, who with outstanding scientists in Italy, worked on the control of malaria with the limited tools available.⁷ Hackett was one of a group of malariologists who worked for the International Health Division of the Rockefeller Foundation in the 1920s and 1930s. When Paris green became available, he introduced it into control research in the Italian countryside. As a commentary on the malaria experts of the day, Hackett tells of the lack of interest in a demonstration of the use of Paris green to control mosquito larvae.⁸

The most exciting research by this group was the solution to a problem that faced malariologists of the day. Why was *Anopheles maculipennis* in 1 country or at 1 season associated with transmission and its presence not associated with transmission in another setting? It was known that both mosquitoes, if fed on infected volunteers, became infected with malaria. The solution to this mystery is beautifully told in Hackett's book *Malaria in Europe*.⁷ Accepting the possible prejudice of one of the workers and the limits of my background as an historian, the story is as follows. It was felt by some workers that there were different strains, but the proof was lacking. A public health worker on retirement, Falleroni, had the time to pursue his real hobby of collecting mosquito eggs. He noticed that the eggs of *A. maculipennis* from different parts of Italy had different designs in the eggs and could be separated. What he had discovered was species complexes in vector mosquitoes. However, the implications of this discovery were not appreciated by Falleroni or other scientists of the day. At the same time, Hackett and Missiroli had performed a beautiful study that showed the mosquitoes from a nonmalarious area fed only on animals, and apparently the same mosquitoes from a malarious area preferred to feed on humans. Although they suspected that species differences explained the discrepancy, they had no morphological markers with which to demonstrate a difference. It was at this point that they combined Falleroni's egg observations with their own findings. It was then possible to associate these different egg phenotypes with behavioral differences. The mosquitoes that were efficient vectors preferentially fed on humans; the non-vectors preferentially fed on animals. This concept is extremely important even today in understanding the vector capacity in a seemingly homogeneous mosquito population. In the laboratory, we have extended these markers to biochemical, cytogenetic, molecular, and biological characteristics (the survival of the offspring of a mating). It has been further extended to include incipient speciation where mosquitoes with chromosomal inversions or other genetic changes no longer mate with wild type mosquitoes.

From our perspective, the Italian research program that Hackett developed was magnificent and is still impacting on our thinking and control strategies to this day. Despite this and the other

discoveries that followed Ross, the Expert Committee at the League of Nations said in 1925 that entomology research was doing a disservice to the field and distracting doctors from the important work at hand—the treating of the sick and dying.⁹ Why spend time dipping for larvae in the local bodies of water when quinine was available for treatment? It was true that in most tropical countries it was impossible to apply the revolutionary finding of Ross to actual disease control. Is this the time to quit trying to find a way to control malaria by attacking mosquitoes? This theme is not unfamiliar to us today where many attempts to open new approaches in public health are met with skepticism, coming even from our colleagues. In 1941, just 15 years after the League of Nations report and ~50 years after the seminal work of Ross, a discovery was made that changed the history of malaria control. Never in the history of man had it been possible to approach disease control on such a scale. This discovery was, of course, that of dichlorodiphenyltrichloroethane (DDT) by Müller. He thought that DDT was just the first generation of insecticides and that future modifications would improve on its activity. Little did he know that he had already discovered the best that chemists were to offer us to this day. DDT was cheap, safe, and long-acting. Malathion, which is replacing DDT today in areas of DDT resistance, is a poor second with a short span of activity as a residual insecticide.

Many of the areas of the world endemic for malaria are not amenable to insect control. This is most dramatically true in Africa. One approach that is being tested in Africa is the use of bednets impregnated with long-acting pyrethroids.¹⁰ It is too early to evaluate its impact on malaria in this area, and the research on this method must have the highest priority. The studies must not measure just mosquito numbers and attack rate, but also the effect on disease and mortality over an extended period of time.

The interval between the discovery of the mosquito as the vector of malaria and the broad application of this to disease control was almost 60 years as a result of the serendipity of the discovery of DDT. Huge problems in vector control continue to exist and the full application of this discovery remains unfulfilled almost 100 years later.

If there is one area that needs a technological

fix equivalent to the quantum leap offered by DDT, that area is mosquito-oriented methods in malaria control. There is every reason to believe that problems with malaria will be getting worse in the years ahead. Why then is there no major program in basic research on mosquito biology? Although I cannot define the way it will be applied, are not the problems and the potential of research in this area enough of a justification? Genetic manipulations of mosquitoes that lead to refractoriness or recombinant microorganisms that kill mosquitoes raise the specters of either the impossibility of the task or the potential risks. This type of attitude interferes with the scientific exploration of the area. As I have noted above, it is the sequence of discoveries that presents new possibilities. Some might ask, can we afford to open a new area of research when we can not support the research that is now under way? I would answer, can we afford not to undertake this new direction?

VACCINE RESEARCH

Vaccine research in malaria is the most misunderstood and misrepresented. Why do we have to sell malaria vaccine research on the basis of a product in the immediate future? Can not science administrators and the financial organizations be educated to the reason for the research and its justification? Clearly a vaccine that works would have a major impact on disease. The undertaking is of unbelievable dimensions if we remember that malaria has the ability to persist in immune humans. What viral vaccine has been produced that can induce immunity to a persistent infection? Furthermore, it is required that the vaccine be produced by subunits of the whole organism. Malaria vaccines will have to improve on nature, if our goal is solid immunity, or reduce mortality, a result which we would all accept. The extent and pace of malaria vaccine research worldwide outstrips any other research in tropical medicine. Why then are funding agencies rethinking their involvement? For example, the information on immunity to pre-erythrocytic parasites has blossomed since the seminal work of Ruth Nussenzweig and her colleagues. Despite the multiple problems that face the implementation of a vaccine against this stage,¹¹ I believe that we will find a way around the problems, assuming that we keep at it. The asexual blood stages are even more of a challenge because there

are billions of parasites from which to choose an escape mutant within one individual. The immune pressure over time has already selected for multiple variants. That is, polymorphism is a well described characteristic among malaria clones. Does that mean that we give up and fund other areas of research? We cannot afford not to continue because only with such an effort will we eventually see ways around this problem.

Mosquito research is now almost 100 years old and is still in need of major support. Chemotherapy is too narrowly based despite the huge effort over centuries. Vaccine research with its potential for great gifts to the curious needs the same patience and long view.

CONCLUSIONS

Malaria was used as an illustrative example of many problems that face the tropical world today. Problems exist that are insoluble because we lack affordable tools and methods to apply the tools that exist. As shown in malaria, the problems will increase when chloroquine is no longer an effective anti-malarial drug. Is it reasonable to expect that basic research can solve these problems within our lifetime? The honest answer to this is that we cannot predict when the control measures will be available. These are not engineering problems, where it is possible to know the tools at hand and from these develop a reasonable time table. In complex biological questions, there is an indeterminacy about the correct approach and the expected outcome of any research program. We can say with certainty that the solution will only be found by the seemingly chaotic efforts of many scientists. Julius Comroe, in his wonderful book *Retrospectroscope, Insights into Medical Discovery*, cites predictions by scientists and other respected authorities that were wrong.¹² One example describes the predictions for a successful polio vaccine. Simon Flexner, Director of the Rockefeller Institute for Medical Research, in 1911 predicted that a vaccine would soon be available; Macfarlane Burnett in 1949 could see no hope for a vaccine in the near future. Enders, Weller, and Robbins in 1949 solved the problem of growing large quantities of the virus in nonneural tissue.

Although the problems in tropical medicine are worldwide, we in the United States must work out our own way of adequately financing research in this area. The altruistic feelings of North

Americans, our need for tourism and travelers, our need for the military, and our needs within the United States, require a more coordinated effort among the various responsible agencies. Why have the people responsible for this area from HHS, AID, and DOD never met to discuss the common needs of the United States in this area? Is it possible that there is too much self interest and parochialism to permit these organizations to work together towards the common good? Foundations have also played an important role, but their interests seem to fluctuate as the directors change. The work, although itself chaotic, needs a steady hand in positions of leadership in government and in foundations. The agency with the greatest consistency in recent years has been the Tropical Disease Research and Training Programme of the World Health Organization. Because of their responsibility for the health of the developing world, they recognize the need for a balanced program of basic research, applied research, and training. They are willing to go the course in difficult areas such as malaria vaccines despite the perceived problems in the biology of the malaria parasite. They realize the risks but are willing to balance these against the benefits of a successful vaccine. Funding groups in the United States need to take a similar long-range view.

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