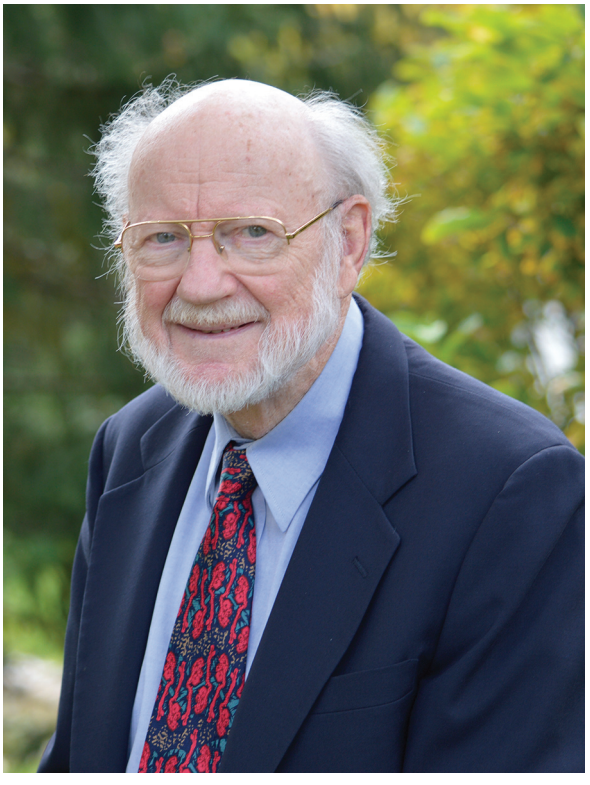

Lessons from the History of Ivermectin and Other Antiparasitic Agents

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Abstract

The twentieth century's arsenal of chemical anthelmintics brought manifold improvement in human health and, more abundantly, in animal health. The benefits were not only in health per se but also in agricultural economics, livestock management, and the overall production of food and fiber to support expanding human populations. Nevertheless, there remains (due in large part to drug resistance and paucity of available vaccines) a great need for new means of controlling disease caused by parasitic worms. Prudence should persuade us to look to our past for lessons that might help in our quest for new drugs. The lessons suggested here derive from the history of ivermectin and other anthelmintics. They deal with the means of finding substances with useful antiparasitic activity and with alternative approaches to drug discovery.

INTRODUCTION

Thousands of years ago, Scribonius Largus taught that "medicine" is derived from the Latin *medicamentum*, referring narrowly to what we call medication. Although the etymology has been disputed, there is no doubt that medicine remains largely a matter of medicines. The question of how we acquire medicines is therefore worth some consideration.

It is a pleasure on this occasion to contemplate the subject in the context of the animal sciences, for my own experience with antiparasitic drugs has kept animal science very much in my mind for many decades. My ruminations about chemotherapy may have some relevance to pathogens other than parasites, but I do not presume to suggest that they will be pertinent beyond the area of infectious diseases. In any case, the study of host-pathogen relationships may eventually be reduced to biophysics, the distinction between drugs and vaccines may be erased, and chemotherapists may die out.

As a chemotherapist of a sort, I would like to think that the chemotherapy community will not face extinction any time soon. I do not actually think of myself as a chemotherapist. Like most practitioners of the art, I am a chemotherapist secondarily to being something else. I am a zoologist and parasitologist. But the bulk of my research over five decades has been devoted to antiparasitic agents. Experimental chemotherapy is my occupational calling and is my subject here. Because biographical narrative has been incorporated in previous prefatory articles in the *Annual Review of Animal Biosciences*, I should perhaps explain how my unplanned career came about.

My interest in parasitic worms, if not actually congenital, was present at an early age. Growing up in the small town of Ramelton in rural County Donegal (Ireland), I would naturally have been exposed to the idea of treating domestic animals to cure their diseases, and I remember being fascinated as a teenager by a leaflet advertising Imperial Chemical Industries' hexachloroethane for the treatment of liver-fluke disease in sheep and cattle. Whatever its origin, my interest in parasitic worms was soon to expand into a lifelong occupational addiction.

For this preoccupation with parasites, the late Professor J.D. Smyth of Trinity College, Dublin University, must in large part be held accountable. Throughout my undergraduate years, Smyth was the sort of inspiring mentor every student should have [as I was privileged to acknowledge just before he died (1)]. Desmond Smyth would go on to become an international luminary in parasitology, but he was then just beginning to gain attention for his discovery of the value of semipermeable tubing in the in vitro culture of tapeworms. His technique is still in use. It was because of Smyth that I was given an opportunity to do postgraduate studies in the United States, and because of him I was emboldened to set off for the University of Wisconsin and the laboratory of the late Professor Arlie Todd.

Todd had an abiding interest in livestock farming and livestock parasitology, as well as a warmhearted interest in the welfare of his graduate students. His laboratory was in the Department of Veterinary Science, so the ambiance was conducive to research on the treatment of disease. By the time I had finished my graduate program, I was ready (to my surprise) to accept a job in industry—a decision made easier by the name of the particular branch of the company in question, the Merck Institute for Therapeutic Research.

Companies vary in their geographical and organizational maps, in their founding ethos and the constancy of their mission, and in the liberality with which they view "basic" exploratory research. All these things (even constancy) are liable to change over time. For these reasons, I was fortunate in 1957 in joining the Merck organization as a member of the Department of Parasitology, which was then led by Dr. Ashton Cuckler [whose marvelous flair for industrial research I have sketched elsewhere (2)]. The research division of Merck turned out to be a cauldron of intellectual stimulation and excitement that far exceeded my expectations. My knowledge of

chemistry being meager, I was fortunate indeed to find myself surrounded by brilliant chemists and (as projects matured) similarly impressive scientists of every description. I had decided to try industrial research (as I had decided to try the United States) for a year—perhaps two at most. I remained at Merck for 33 years (and made the United States my home). Since retiring from industry, I have been privileged to be a member, for the past 25 years, of the incomparable Research Institute for Scientists Emeriti at Drew University in Madison, New Jersey. Can there be a more perfect postindustrial "retirement" than continuing to pursue one's professional interests with student colleagues? Through it all, chemotherapy has remained my focus—and my delight.

For many years, it has seemed to me that there has been a glaring need for a more candid appraisal of the role of scientific research in the acquisition and deployment of agents for the control of infectious diseases (3). Throughout those years, valuable new drugs have continued to appear, so that perhaps the need I proclaim has more of gossamer than of glare. Yet we must ask whether the therapeutic benefits have been commensurate with the investment in time, treasure, and scientific talent of the period. In a world in which demographic and climatologic change add to the complexity of our struggle to control disease, are we getting enough bang for our buck?

For food and fiber, human beings depend to a large extent on nonhuman animals, which in turn depend on plants; our objective should be to control (but not eliminate) the pathogenic populations of those life-forms. The question is how best to do that. The following paragraphs explore whether recent chemotherapeutic experience offers any lessons that might enable us to use our resources more effectively. And, foolhardy though it may be, a proposal is made for trying out a somewhat different approach to the discovery of drugs for infectious diseases.

A note for the nonbiological reader: Zoologically, the early developmental stages of nematode worms are "juveniles," not "larvae." Popular use has, as it so often has, legitimized misuse, and now "larva" and "larvae" are widely applied to juvenile nematodes even in the (nonzoological) scientific literature. This lax convention is used here in the interest of making the text more readily understood. The term "microorganism" is here used interchangeably with the venerable though unfashionable word "microbe."

LESSONS FROM THE HISTORY OF IVERMECTIN

The biggest single factor leading to the discovery of ivermectin was steadfast reliance on empirical principles of drug discovery. The antiparasitic efficacy of the chemical class was revealed by a process that is part of a long tradition and that has many components. It represents an approach to drug discovery that is mostly, but not exclusively, concerned with the treatment of infectious disease. Its core element is the testing not only of substances that may reasonably be expected, on scientific grounds, to possess therapeutic activity, but also (and especially) of substances for which no such rationale exists.

Ivermectin (**Figure 1**) was introduced commercially in 1981 (4) for the control of parasitic worms (helminths) and ectoparasitic arthropods. Early scientific reports included a description of the microorganism that produces ivermectin and its precursor abamectin (avermectin) as well as evidence of their exceptional potency against intestinal and extraintestinal parasites (5–8).

Ivermectin and its microbial precursor abamectin (avermectin) were the first of the macrocyclic lactone antiparasitics. They, like other anthelmintic classes, proliferated through structural modification, in response to market need and competitive pressure. Eprinomycin, for example, is a chemical derivative selected on the basis of pharmacological distribution in treated cattle. But having originated as products of microbial fermentation, macrocyclic lactones were also amenable to modification through fermentation research. A mutant strain of *Streptomyces avermitilis* was altered by changes in growth medium to produce doramectin; chemical derivitization of

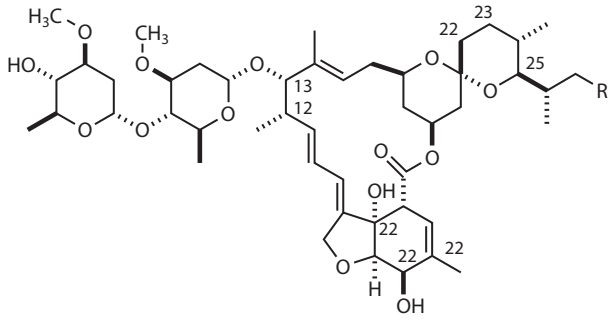

Figure 1

The structure of ivermectin (22,23-dihydroavermectin). R = CH_3, ivermectin B1a; R = H, ivermectin B1b. The drug may contain up to 20% of the B1b homolog.

doramectin produced selamycin. A strain of *Streptomyces cyanogriseus* was found to produce a macrocyclic lactone that was developed as nemadectin; derivitization of nemadectin produced moxidectin. Milbemycin oxime was developed not from an avermectin but from an older macrocyclic lactone structure, milbemycin. These and many other basic and applied scientific aspects of the group have been documented in comprehensive multi-chapter reviews (9–11).

The macrocyclic lactone class soon came to dominate the control of parasitic diseases of livestock and companion animals. Because the drugs are effective against both endo- and ectoparasites, they are known by the infelicitous but convenient term "endecticides."

Ivermectin is the macrocyclic lactone with which I am most familiar, and experience with ivermectin is the main source of the lessons offered here. It was clear almost from the beginning that, in financial terms, the potential value of ivermectin in the animal-health market was very large and in the human-health market was very small. Nevertheless, the drug has been widely distributed not only for livestock and companion animals but also (in a non-market context) for humans.

The Value of Casting a Wide Net

The discovery of ivermectin resulted from screening microbial fermentation products for antiparasitic activity. The microbes themselves were gathered from a wide range of sources to enhance the diversification of assay input. Among those sources was the Kitasato Institute in Tokyo. A group of Kitasato scientists, led by Professor Satoshi Omura, routinely isolated microorganisms from soil by allowing them to grow in laboratory culture, after which they were tested for activity against a variety of other microorganisms. Under an agreement with Merck & Co., Inc., isolated microbes that were considered unusual in appearance or cultural characteristics were dispatched to Merck laboratories in the United States. When a batch was sent in 1974, it was understood by Merck microbiologists that those isolates had shown little antimicrobial activity in the Kitasato tests, and that was in accord with the results of routine antimicrobial tests done at Merck. In 1975, the isolates were transferred from the Merck Microbiology Department to the Parasitology Department, where they could be tested in a new assay that had been devised especially for testing microbiological fermentations for activity against parasites. One of those isolates, when regrown in the Merck laboratories and tested in the new antiparasitic assay, yielded a potent anthelmintic substance. That substance (a mixture of abamectin and several related structures known collectively as avermectins) would be the forerunner of ivermectin and, by extension, the macrocyclic

lactone class of antiparasitic agents. The self-evident lesson is that increasing the magnitude and diversity of screening input will enhance the probability of screening success.

The Value of Managers and Technicians

Those looking for new drugs today are unlikely to be solitary workers; they are likely to depend on the assistance of technicians for the quality of their research and on managers or directors for the continued existence of the research. Directors and managers allocate resources, financial and otherwise. Technicians may be scientifically trained, but in cases involving routine projects, such as screening operations, many of the technicians learn on the job. Managers and technicians should of course be accorded due recognition and authorship for identifiable and specific scientific contributions. As was evident in the case of ivermectin (12), the outcome of research will often depend crucially on the wise judgment of those who determine strategy and tactics, and on the steadfastness of technicians who remain alert throughout repetitive operations. Where authorship is not appropriate, other forms of recognition are warranted. To bend a trope from an illustrious nonscientist, they also serve who only hover above or labor below.

Managers must confront imponderables. In developing a new chemical class intended for general use, how soon should a member of the class be subjected to an Ames (teratogenicity) test? How long (and how much) should resources be invested in supporting an assay that has failed to turn up "actives"? In such circumstances, a judgment must be made on other than scientific grounds. Common sense and experience in experimental chemotherapy will help, but science itself will not.

The Value of Assay Innovation

The discovery of ivermectin resulted from assay innovation. In the decades following the discovery of penicillin, many new antibiotics were found by screening fermentation products against microorganisms in vitro. Yet it would appear that not a single anthelmintic was found by the primary screening of fermentation products against parasitic worms. That was presumably because no suitable assay then existed. The breakthrough that yielded ivermectin was an in vivo assay. Details of the assay have been published (4, 7, 11). In essence, fermentation products were fed to worm-infected mice, which were later examined for evidence that the infection still existed. The empirical nature of the system is abundantly evident: The mice were fed an arbitrary, though standard, amount of food containing an unknown amount of an unknown substance that might not be there (13). The lesson is that the want of a known assay technique (even a want of long standing) should not dissuade anyone from trying to create an assay to fit the need.

Liabilities, Real and Virtual

In the development of a broad-spectrum anthelmintic, lack of efficacy against a major pathogenic species is generally seen as a liability; it is certainly not likely to be a reason for celebration. Yet such was the case in the development of ivermectin for the "worming" of dogs. The recalcitrant parasite in question is the adult heartworm, *Dirofilaria immitis*. It is not killed by ivermectin even though the drug reaches it through the host bloodstream. The adult stage is the primary pathogen in dirofilariasis, but treatment of dogs to get rid of the adult is hazardous to the infected dog and to the reputation of the veterinarian. It must be done, and can be done safely, but it should not be the incidental result of routine worming. Ivermectin and other macrocyclic lactones are crucially important in the control of heartworm disease; yet, in an apparently made-to-order anomaly, their

success is due to their potency against the essentially nonpathogenic larvae (microfilariae). Lack of efficacy against the adult (pathogenic) stage, an apparent liability, is a real asset.

For the routine anthelmintic treatment of dairy cattle, lipophilicity in a drug is almost sure to be a liability because lipophilic drugs and their metabolites are likely to be eliminated at least partly through the milk of treated animals. The result is that the milk must be withheld from the market until it is essentially free of drug residues (meeting the requirements of relevant regulatory agencies). Ivermectin is distributed generously in the lipid component of treated cattle, resulting in an undesirably long "withdrawal period" (14). Escape from this liability was achieved by testing ivermectin derivatives in lactating cattle to determine their distribution in milk, and then testing the more promising candidates for anthelmintic efficacy. In this way eprinomectin was successfully developed for use in dairy cattle as well as other livestock (15) and was later developed as an exceptional long-lasting injectable product for cattle (16).

It is clear that drug characteristics that are usually liabilities are not always liabilities. And things that really are liabilities may sometimes be converted into assets. There is, however, an important warning to be made in respect to chemotherapeutic agents in general, a lesson that became apparent as a result of the unexpectedly wide spectrum of ivermectin: The wider the spectrum of efficacy, the greater the need for environmental vigilance in the practical application of the agent.

Challenging Conventional Wisdom

When the antiparasitic efficacy of ivermectin was initially announced, there were experts who dismissed it as a curiosity on the grounds that it could never be produced economically in the amounts needed for practical use. It was indeed true that large-scale synthetic production of ivermectin was not feasible, but the filamentous bacterium *Streptomyces avermitilis*, the natural source of the chemical class, could be cajoled into producing unnatural amounts of abamectin (from which ivermectin is made by chemical modification), so production by fermentation became standard.

Another lesson of which we are reminded by the ivermectin saga is that experts may be betrayed by their expertise into viewing new developments in a less-than-expert way. At a time when a macrofilaricide (roughly "adulticide") was a recognized need (as it still is) for the control of river blindness, the value of a microfilaricide (roughly "larvicide") was doubted even in the face of evidence of its potential value. The danger, of course, is that misplaced skepticism might discourage pursuit of a promising development.

The Value of Flexible Clinical Objectives

In disease control, causal prophylaxis would seem a priori to be the ideal. Yet clinical prophylaxis (prevention of disease without eradication of infection) is seen over and over again to be of great value. Indeed, clinical prophylaxis is closer to the biological ideal. Undoubtedly, human squeamishness (together, in some cases, with a modest health penalty) would be a deterrent to clinical prophylaxis in the human context. Even in the case of immunological clinical prophylaxis of hookworm disease in dogs, it was sociological and economic rather than scientific factors that blocked the acceptance of a vaccine (17). The acceptance of ivermectin in the control of human river blindness attests to the value of clinical prophylaxis.

River blindness (onchocerciasis) is one of two major tropical diseases caused by roundworms known as filariae. In river blindness, the first-stage larva (microfilaria) is the primary pathogen. In the other disease, lymphatic filariasis, the adult worm is the primary pathogen. In both,

ivermectin is lethal to the microfilariae but does not kill the adult worms (though it may suppress their reproduction), and transmission to humans can be blocked by ivermectin treatment because microfilariae will no longer be present (in skin or blood, respectively) to be picked up by the insect vector (blackfly or mosquito, respectively). In the case of lymphatic filariasis, diethylcarbamazine can similarly be used to kill microfilariae, and (whether diethylcarbamazine or ivermection is used as microfilaricide) albendazole is administered concurrently to kill adult worms and thereby minimize morbidity.

In several regions of Africa, Central America, and South America, river blindness has been brought under control by repeated administration of ivermectin to kill the larval stage of the parasite. Ecuador and Mexico are examples of countries in which the disease has actually been eliminated (18, 19). For lymphatic filariasis, control programs have reached the point at which a plan has been developed for elimination of the disease from the vast tropical regions where the disease is endemic. The donation of ivermectin and the practical ramifications of its use in international control programs have been discussed by many (see, for example, 20).

LESSONS FROM THE GENERAL HISTORY OF ANTIPARASITIC DRUGS

Patterns and approaches in the field of new drug discovery have changed in recent years (21–23), yet lessons may be derived from the general history of anthelmintic therapy in the second half of the twentieth century. I had the good fortune to be involved, in one way or another, in the development not only of ivermectin but also of thiabendazole, cambendazole, rafoxanide, and clorsulon. The lessons offered here must owe something to that experience and the experience of working on potential drugs that failed to fulfill their potential. It is not always possible to link a particular lesson to a particular drug.

Thiabendazole was the first of the benzimidazoles, which were to become the blockbuster broad-spectrum anthelmintics of their day (24). Its anthelmintic efficacy was discovered empirically in assays conducted by my colleague Dr. John R. Egerton, using nematode larvae in vitro and nematode infections in mice. Dr. Cuckler invited me to share the excitement of exploring its effects on various stages of various roundworm species, especially *Trichinella spiralis*, the agent of trichinosis. (This marked the beginning of a new era for me at the merely personal level. I had been looking forward to devoting my career to the fluke disease schistosomiasis, but I soon discovered that biodiversity can be appreciated at the laboratory bench as well as in the great outdoors.)

Some Challenges of Assay Selection

In the field of infectious diseases, infected laboratory animals have long been used for the detection of efficacious substances. Such screening is profligate of test material and laboratory resources, yet in vivo screening has the advantage of giving hints as to the bioavailability and safety of any new active compound, as well as the advantage of testing compounds against all accessible biological systems of the target parasite at once (unknown systems as well as those known).

As Ehrlich found more than a century ago, a direct correlation between efficacy in vivo and efficacy in vitro cannot be assumed. Comprehensive comparison of in vivo and in vitro assays cannot be undertaken here, nor is this a place for discussion of the ethical aspects of in vivo experimentation. One particular aspect, however, has practical implications for the experimental chemotherapist. As ethical concern about the use of animals in research expands, the use of in vivo assays contracts. This may therefore be a suitable time to raise the question of the appropriate size of groups of experimental animals. Much attention has been devoted to the desirability of reducing group size. The ultimate reduction (a single animal per test substance) was pioneered by Ostlind in

small-animal screening in the parasitology laboratories at Merck & Co. and was shown to be perfectly satisfactory in several assays (25). The key to success is near-perfect control of the experimental infection, and the lesson is this: In a well-regulated assay, one is sufficient and two a surfeit.

A much-neglected weakness of the in vitro assay is the inherent unpredictability of effects associated with the ambient medium. The salinity of the medium, for example, may affect the sensitivity of the assay, and the effect is not necessarily consistent between different drug classes (26).

Lessons from New Actives

When an active compound is turned up by a screen, its biochemical reactivity should be considered. In the normal course of events, the extent to which the mode of action should be explored will be determined by several factors, including the perceived potential of the compound for clinical or commercial utility, the novelty (patentability and prospect for basic research) of the compound, and the reputation of that class of compound for nastiness (is it known, for example, for teratogenicity or stench?).

If the active compound achieves the status of clinical or commercial utility, or even comes close, then the mode of action will naturally suggest itself as a useful target for further drug discovery. The temptation to follow this line of thought into active research should be approached with caution. There is a high probability that chemical structures sharing a given biochemical mechanism will share both the assets and the liabilities of the original, so that drugs so developed will lack true novelty and will likely be subject to resistance already acquired by target pathogens. New actives, as well as established drugs and abandoned leads, should be regarded as potential tools for basic research as well as candidates for therapeutic application (13).

Lessons from Weak or Abandoned Actives

In empirical random screening, failure to find an active substance is the default day-to-day outcome. Confirmable actives are rare. Among them are substances that, despite their activity, are of little interest to the chemotherapist. Historically, neither authors nor editors have been prepared to clutter up the pages of journals with lists of compounds that were inactive or marginally active in empirical screens, and the proper disposition of abandoned actives remains difficult to resolve. The enhancement of leads through molecular alteration or developmental research is an established feature of experimental chemotherapy. But in random screening, a weak active is often dismissed without being accorded the status of lead. Some of them are abandoned because efficacy is weak at maximal tolerated dosage (though often it has not been feasible to test this point adequately). Others are abandoned because they are found to be members of a chemical class tainted by toxicity. Still others are dropped from consideration for reasons peculiar to the particular active substance. Questionable leads cannot all be pursued indiscriminately, but they should not be discarded hastily.

In the 1950s, a particular compound was weakly but reproducibly active against *Schistosoma mansoni* when given to mice according to a specific protocol (27). I believe it will not be a betrayal of a significant secret to identify the substance, some half-century after the fact, as O-methyl threonine. The discovery was greeted by my more experienced colleagues with a stunning lack of interest. It did not seem worthwhile to publish a finding so destitute of promise, but to this day I wonder if that bit of information might sometime be useful to someone somewhere.

If there is a lesson here, it may be that actives of little apparent value should be published. Indeed the suggestion has been made that inactives should be made public to reduce pointless

screening by others. In this era of informatics and the posting of massive amounts of information in supplemental depositories, this question might warrant renewed attention.

The Lesson of "Inactives" That Are Active

A parasitologist once told me with pride that he had tested thiabendazole against *T. spiralis* in rodents before I had done it. He added that he had not taken the matter further because the drug had been inactive. He probably thought it unnecessary to mention that what he meant to say was that the drug had been inactive when given at the dosage and in the regimen he had chosen for his experiment. It is seldom unnecessary to mention such information. (The efficacy of thiabendazole in rodent trichinellosis is widely recognized.) In random-screening assays the vast majority of tested substances will be "inactives" even though some of them might have been recorded as "active" if they had been tested at dosages higher than that selected arbitrarily for that particular assay. Screening at multiple dosages would do much to offset the risk but is almost always rejected as profligate of assay resources. The risk of "false negatives" is, for the most part, accepted as a built-in defect of random screening.

The Lesson of Multiple Biodynamic Actions

Discovery that an anthelmintic causes a particular biochemical action on a parasite does not mean that the mechanism of anthelmintic action has been found. Thiabendazole's efficacy appears to reside in its disruption of microtubule formation rather than in its inhibition of fumarate reductase. The paralyzing effect of ivermectin on nematodes appears to result from binding to a neuronal glutamate receptor rather than from potentiation of the release and binding of the neurotransmitter GABA (*gamma*-aminobutyric acid). Other compounds have cholinergic effects that may or may not be primary (28). Drugs may also have multiple clinical effects. Thiabendazole not only has a lethal effect on parasitic nematodes but also has anti-inflammatory activity in mammals, and it has been widely used as an agricultural fungicide.

TRADITIONAL DISCOVERY SYSTEM

Good but Not Great

Throughout the centuries, drugs were discovered empirically—that is, by chance clinical observation or trial-and-error testing. It is impossible to say when deliberate attempts were first made to test a variety of substances in the hope that a particular thing might have a particular therapeutic effect, but Redi's seventeenth-century experiments, in which he dunked parasitic (and nonparasitic) worms into various liquids, may well be the earliest on record (29). A prehistoric parent might have been the first to plunge a worm into a jar of alcohol to reinforce the lesson that drinking hard liquor can be lethal to the drinker. Only a bold and astute child would have countered that evidently people with worms would be well advised to drink alcohol. [Under very limited circumstances there may be a trace of truth in that (30).]

In both veterinary and human medicine, anthelmintics are used as chemoprophylactic agents—providing clinical prophylaxis by means of routine herd treatment. Some political and economic aspects of agricultural use have been reviewed (22). The essential goal of the chemotherapist remains the discovery of substances that adversely affect parasitic worms (or render them harmless, in which case they are no longer parasitic worms). The two traditional approaches to this objective are empirical testing, as mentioned, and the identification and exploitation of biochemical targets. For many decades it was inculcated in the minds of many that the empirical approach was intrinsically

less scientific than the more rational mechanism-based approach. Empirical screening, however, is not an anti-intellectual endeavor, and empiricism is in no way inimical to the biochemical and molecular pursuit of knowledge, or to the mechanism-based pursuit of new leads.

The empirical (screening) approach has been undertaken primarily by industrial laboratories. Its successes have been important (they are the only practical successes so far), but they have been more sporadic than would be wished. The mechanism-based approach has traditionally been the focus of academic laboratories. Its failure to deliver useful new anthelmintics has been attributed mostly to insufficient information, so the phenomenal recent growth in biochemical knowledge offers hope for a new era of drug discovery. At some point probability may come to favor the mechanism-based approach over the empirical. Unfortunately, we will not know when the tipping point has been reached until some considerable time afterward. In the meantime, we should deploy our resources according to what we perceive to be the probability of success.

Exciting scientific progress is being made on both fronts, and the distinction between corporate and university projects is being increasingly blurred. Within the mechanism-based approach to drug discovery, new avenues are being explored in parallel with advances in underlying biochemical and molecular knowledge (31–35). Modern instrumentation technology, including automation and digitized readout, has yielded increases in the efficiency of in vitro screening and in the sophistication and objectivity of phenotypic data collection [as seen in a recent example (36)]. The benefits and limitations of in vivo assays for primary and secondary screening are widely appreciated, and the evolution of improved animal models has been slow but steady (37). In vivo screening can no longer be equated with whole-animal screening. Cell-culture technology has enabled the testing of substances against live parasites within live cells. Understandably, this has been used mostly in the context of protozoan parasites, but there is no reason it could not be used for tests against intracellular stages of helminths (e.g., juveniles of *Trichinella* sp. and *Trichuris* sp.). Surely great promise resides in the recent creation of the microengineered cell culture systems known as organs-on-chips (38).

WHAT LIES AHEAD?

Strategy

Almost 40 years ago I suggested that "the probability of finding the drugs needed by the developing nations could be increased by bringing the industrial type of screening into the overall picture" (3, p. 24). Since then, as noted above, there have been new developments in both the empirical and the mechanism-based approaches to drug discovery. They are extensions, offshoots, and spin-offs, and they are important. Yet the overall success rate of the traditional methods has been uneven, and it may be declining. What is needed is a radical departure from current approaches. Not having one to suggest, I propose merely a radical reinvigoration of a traditional method.

We do not know how to discover new drugs predictably, but we know how to discover them unpredictably. The empirical random-screening approach can be expected to yield truly novel drugs, and to yield them at a rate that will increase according to increases in the number and in the diversity of things tested. The industrial type of screening has become less common at a time when it should have become more common. It is time to seriously consider a massive mechanism-blind screening of microbes gathered from all corners of the Earth, with the objective of finding novel antiparasitic agents (and, ideally, other biodynamic substances).

The screening of synthetic chemicals may sometimes be limited by a limited supply of diverse chemicals to be tested. But that becomes a problem only where there is a finite stockpile of chemicals to begin with. It may hold true, for example, for an institution's or company's collection

of synthesized chemicals, or for a library of molecules generated by some specific research project. The production of novel synthetic chemicals is limited more significantly by the boundaries of human knowledge, expertise, and creativity. The synthetic resources of the natural world are vastly greater. An awesome chemist could have synthesized (or at least imagined) the avermectin molecule before its natural biosynthesis was known, but even my most awesome chemistry friends say that they cannot imagine having done so. That is a lesson we should not ignore. We should screen naturally occurring substances, whether animal, vegetable, or mineral. In the context of drug discovery, the term "natural products" generally refers to the products of microbial metabolism. Although the screening of substances made by higher organisms should not be ruled out, we are concerned here with what can loosely be called "fermentations." A renewed focus on the screening of microbial metabolites would seem to be worthwhile.

Tactics

Past proposals have included the creation of discovery centers in the tropics (under the sponsorship of an international health agency) coupled with development operations in existing industrial laboratories, as well as a reverse variant of that concept, with the discovery phase in pharmaceutical companies and development in tropical laboratories (4). (Those proposals were made in the context of human health, but new drugs are needed for infections regardless of who is infected.) The present proposal does not envision (or reject) any realignment of regional or institutional responsibilities. Screening centers could be large or small, centralized or scattered, but the focus should always be on the output, not the throughput.

The subject here is anthelmintic discovery, but a focus on particular types of biological activity (directed against nematodes, trematodes, cestodes, or even other infectious and noninfectious agents of disease) would be governed by therapeutic needs, assay capabilities, and other such factors.

Larval parasites, as well as small invertebrates of various phyla, would be suitable for in vitro assays requiring minimal technical resources. Some form of in vivo screening would be preferable, though the disadvantages of such tests are widely acknowledged. The assay system used in the discovery of the avermectins offers one model for testing fermentation products against worm parasites. Other models are presumably being evaluated. As mentioned, isolated living cells or tissues may provide alternative models. Pressure to reduce whole-animal testing is, and should be, strong, but in vivo testing is still widely accepted in principle. Perhaps we can find (or breed into existence) a test animal more suitable even than a mouse—preferably a tiny and largely insensate creature of repellent visage and of a disposition that is vicious in public and angelic in the laboratory.

Variety in the kind of activity being sought in primary screening could be achieved by having more than one type of parasite in each test animal (care being taken to avoid levels of infection that might reasonably be expected to cause pain, and to use established standards of animal care). In addition to the well-known assay systems, consideration should be given to systems that have been generally overlooked. For example, an assay could make use of murine *Trichinella* infections in which enteral and parenteral stages are briefly concurrent, and in which the readout would need only gross inspection of lesions on the thoracic diaphragm (a suggestion briefly pursued by a World Health Organization committee on which I served many years ago). Other unconventional systems would include the filarial larva-in-mouse-ear system, and assays based on transient survival of helminth larvae in isolated host cells or tissues.

Phenotypical in vivo endpoints would not necessarily be focused on parasite death or removal. In some trematode diseases, prophylaxis could be achieved by a drug that blocked metacercarial

excystation, and such bioactivity could be sought by host treatment or in vitro assay (39, 40). Similarly, consideration could be given to blockade of cestode evagination, nematode molting, insect ecdysis, and other development processes, as well as reproductive processes, such as egg-shell formation.

Consideration must be given to the relative long-term cost of any shift to a different mode of drug discovery. The question is not whether the proposed investment would be sound, but whether it would be more sound, or less sound, than other investments in drug-discovery programs. In that context it should be kept in mind that the cost of developing a new drug in the conventional mode has recently been estimated at $2.6 billion (41).

CLOSING REMARKS

Those engaged in studies on experimental chemotherapy of parasitic disease will undoubtedly have some lessons of their own and may wish to compare them with the lessons offered here. Those just entering the field may find help in avoiding some of the pitfalls of the undertaking. Drawing on the lessons and experience of many years, it is here concluded that it would be prudent to take advantage of recent technology to focus attention on the screening of natural products for anthelmintic activity. Whether undertaken by the private sector or the public sector, screening should be based primarily on the detection of activity in microbial metabolites. Ideally, the test microorganisms would be collected from every ecological niche on Earth. I have no doubt that such a program would yield new biological actives, and that some of them would prove to be of great value. If the political and managerial challenges could be met, the combination of chance and human ingenuity would likely triumph.

Over the past 100 years, the use of chemical anthelmintics has brought manifold improvement in human health and, more abundantly, in animal health. The benefits were not only in health per se but also in agricultural economics, livestock management, and the overall production of food and fiber to support expanding human populations. Nevertheless, there remains (due in large part to drug resistance and paucity of available vaccines) a great need for new means of controlling parasitic disease. As long as we live in a world in which supplies of human food vary regionally and irregularly from scarcity to abundance, a world in which meat is increasingly in demand as a foodstuff, and in which many are clothed in fabrics of hide and hair, the need will persist. Parasitic worms of plants, though not considered here, present a comparable set of threats to Earth's inhabitants. With challenges of such magnitude, we must reach expansively for solutions. Theoretically, at least, we have known that (in Donne's sense) "no man is an island entire of itself." We are coming now to understand that no living thing exists in isolation, and so we must keep in mind that the goal here is the absence of parasitic disease, not the absence of parasites.

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In the course of talks on the ivermectin saga, I sometimes point out pitfalls that might be encountered by others engaged in similar work. The idea of using those lessons as scaffolding for an article was suggested in correspondence with Professor Richard J. Martin and with Professor R. Michael Roberts. I am grateful to both. My thanks to Dr. Ronald J. Doll for providing **Figure 1**.

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