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Strategies and Technologies for Controlling Tropical Diseases

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INTRODUCTION

Throughout history, diseases have been brought under control by a range of direct and indirect measures. In most cases, disease control has been an unplanned bonus of economic and social development. The opportunities for controlling a disease either directly or indirectly depend on the relationships among the disease-producting organism, the environment, and humans. The more complex the relations, the more points for intervention, but not necessarily for success in controlling the disease. Experience shows that the most complex infectious diseases are, in general, the most poorly controlled.

In the developed world, the control of infectious diseases has come about largely as a result of improvements in living conditions. Because of better sanitation, uncontaminated potable water, uncrowded housing, and an adequate diet, exposures to pathogens are less frequent and less intense, and people are better able to fight off severe infection.

Many recent declines in U.S. mortality rates are due to specific interventions, such as new vaccines and drugs, that are targeted at specific diseases (e.g., vaccines and antiviral drugs for hepatitis B). Three types of biomedical tools are used to control many of the infectious diseases of the developed world: 1) immunization techniques to prevent disease; 2) diagnostic technologies; and 3) therapy, including surgical and medical treatment. A fourth tool important to controlling many tropical diseases is vector control—i.e, control of the insects and other organisms that transmit disease-producing organisms to humans (e.g., mosquito vectors of malaria) or are otherwise necessary for the disease-producing organism to complete its life cyle (e. g., aquatic snails that serve as intermediate hosts for schistosomes).

These four tools are the primary direct measures that can be used to control diseases in individuals. Along with disease surveillance (which may involve screening segments of the population for signs of disease or immunity) and keeping track of vector populations and their infection rates, these tools are also the means for controlling infectious diseases in populations.

The remaining chapters of this report review the status of vector control (ch. 6), immunization (ch. 7), diagnosis (ch. 8), and treatment (ch. 9) for the tropical diseases of interest in this report: malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, leprosy, tuberculosis, diarrheal diseases, acute respiratory infections (ARIs), and arboviral and related viral infections. Current research and promising developments are highlighted for each. This chapter provides background for the chapters that follow by laying out the strategies, using single and multiple interventions, for controlling tropical diseases.

Some gains in understanding tropical diseases have yet to be applied to any specific control measure. The methods of biotechnology (e.g., the use of recombinant DNA technology), for example, have led to new knowledge about the immunology of parasitic disease organisms. Although some of this knowledge may eventually be applied to the development of vector control, vaccines, diagnostic or therapeutic measures, it currently has no practical application. Biotechnology's contributions to advance in research toward disease control are brought to light in several chapters of this report. In the latter part of this chapter, some of the methods basic to biotechnology are described.

STRATEGIES FOR CONTROLLING TROPICAL DISEASES

Most **developing countries of the tropics are still at** the stage where increased investment in sanitation and clean water would have a tremendous benefit in the control of disease. Extending clean water and sanitation facilities to as many of the world's people as possible continues to be a goal of development agencies and international health groups. Although this report does not evaluate the success of such efforts, it is clear that large portions of the world's poor remain unserved. The tropical disease control measures in the biomedical spectrum, which are the focus of this report, will not solve the underlying problem of the lack of sanitation and clean water, which in many cases contributes to disease transmission. In many cases, however, these measures can protect against contracting disease and reduce morbidity and mortality due to disease.

Worldwide eradication is possible for very few diseases. Smallpox was eradicated through a worldwide vaccination program, and the eradication of measles may also be possible. For most of the diseases considered in this report, however, the most realistic goal at present is not eradication but control. Control means lowering morbidity and mortality to tolerable levels and containing the spread of disease.

Certain features of smallpox were essential to its eradication: 1) smallpox is transmitted directly from person to person, with no vector; 2) smallpox is transmitted only through fairly close contact with an infected person; 3) there are no animal reservoirs for smallpox; 4) there are no subclinical cases of smallpox (virtually everyone who is infected breaks out in the smallpox rash); and 5) smallpox develops within a relatively short time after exposure.

Most other major parasitic diseases—certainly the five included in the Special Program for Research and Training in Tropical Diseases (TDR)—depart from these characteristics. The parasites that cause malaria, trypanosomiasis, leishmaniasis, and filariasis are transmitted by vectors, and the parasites that cause schistosomiasis live part of their lives in intermediate snail hosts. The probability of eradicating vector-borne diseases by killing off all vector species or intermediate hosts appears slim. The story of the worldwide "malaria eradication campaign" waged by the World Health Organization (WHO) beginning in the 1950s has taught important lessons in that regard (see ch. 6).

Diseases that affect both humans and other animals pose special problems for control. The existence of animal reservoirs of disease means that even if all human cases are eliminated at any one time, the disease will still exist in animals and will eventually pass into humans once again. One strain of African trypanosomiasis is actually a disease of livestock, called nagana, which can be transmitted to humans. Yellow fever is an arboviral disease that exists in monkeys as well as humans. A highly effective, long-lived vaccine for yellow fever has existed for decades, and in many areas, transmission to humans has been largely curtailed. Because of the monkey reservoir, however, the absence of cases in humans does not allow discontinuing vaccination for yellow fever as was possible in the case of smallpox. Workers in development projects in Brazil, in which jungle is cleared for roads or agriculture, have been the victims of a type of yellow fever ("jungle yellow fever") in which the virus picked up by mosquitoes from monkeys is transmitted to humans. The control of yellow fever is quite good, but realistically, there will be no eradication.

The control of most of the other tropical diseases considered in this report is not as successful as that of yellow fever. The development of successful malaria vaccines, however, could put malaria control on a similar footing.

If eradication is not the goal, the options for disease control are varied, but it has been difficult to discover the most effective and most cost-effective mix of control measures. The "St. Lucia Experiment, " for the control of schistosomiasis on the West Indian Island of St. Lucia, is one example of a successful research effort to compare the effectiveness and costs of several control measures (see box 5-A).

Box 5-A.—The St. Lucia Experiment

The St. Lucia experiment began in 1965 with a memorandum of understanding between the Government of St. Lucia and the Rockefeller Foundation to "cooperate in the study of matters affecting the health of the people of St. Lucia, and in seeking and applying measures to control disease, and, more specifically, schistosomiasis." In addition to the Rockefeller Foundation, the British Medical Research Council and Overseas Development Association sponsored the project, and the Edna McConnell Clark Foundation contributed funds.

Schistosomiasis is a debilitating disease caused by trematode worms that live in the human host's blood vessels. Eggs of the New World species (*Schistosoma mansoni*) are excreted in feces. The eggs hatch in water, enter certain types of snails, and eventually emerge from the snail in a form able to penetrate the unbroken skin of a person in the water. The link between feces-contaminated water and this disease is obvious.

Three interventions to control schistosomiasis were compared in different parts of the island of St. Lucia:

- controlling snails with molluscicides (chemicals that kill mollusks, the group of invertebrate animals to which snails belong);
- providing piped water to each house; and
- treating all infected people with drugs.

All three interventions were successful, and as a result, the incidence of schistosomiasis is now at an all-time low in St. Lucia.

The St. Lucia experiment began with a careful study of the social and economic factors affecting schistosomiasis. The St. Lucians' use of water and their beliefs about different sources of water affected, and in some cases, changed plans.

In the area of the island chosen for molluscicide treatment, snails were monitored for infection with schistosomiasis before spraying began. Rather than blanket spraying all waters, the approach was to spray only those areas with infected snails ("focal" spraying). In this area of the island, the schistosomiasis incidence rate (the rate of new cases) in children (up to 10 years old) fell from 22 percent in 1971 to 4 percent in 1974, and the prevalence (the rate of existing cases) gradually dropped over all age groups. In addition, the monitored snail populations showed a drop in infection from 3.9 percent in 1971 to 1.1 percent in 1974. By 1974, transmission was virtually nil. Those few people still excreting schistosome eggs were treated with chemotherapy; and focal spraying was continued at sites routinely used by people. In 1980, only 2 children of 700 were infected.

In the five villages of the island chosen for the second intervention, water was piped to each house, and a laundry and shower unit were built in each village. The rate of new infections fell, but transmission continued until an education campaign was instituted to decrease use of the river for washing. The rate of new infections in children fell from 30.6 to 12 percent between 1970 and 1975. Chemotherapy further reduced the rate to about 6 percent.

In the area of the island chosen for the third intervention, all people who were excreting schistosome eggs were treated with drugs. With fewer people excreting eggs, there was less contamination of water and a lower probability of infection. Three rounds of treatment brought new infection rates among children down to about 3 percent, both in areas where rates were high to begin with (23 percent) and in areas where rates were low to begin with (6 percent). Before the program, about 54 percent of the population was infected. After 3 years of chemotherapy, prevalence was down to 7 percent. The infection rate among snails also fell to almost zero.

Of the three interventions, chemotherapy produced the quickest, greatest decline in new infections, at the lowest cost. Annual per capita costs for the first 2 years were: $1.10 for chemotherapy; $3.70 for snail control; and $4.59 for water supply.

Each intervention had different advantages. Molluscicide does not require the cooperation of all residents and is effective even if infected people move into the area. It is expensive, however, and getting more so as the cost of petroleum (the feedstock for molluscicide production) rises. Molluscicides kill aquatic life other than snails and cannot be used where people fish. Furthermore, in the absence of chemotherapy, this intervention does nothing for people already infected.

Piped water is fairly expensive and the equipment requires continued maintenance, but this intervention has the benefit of controlling more than just schistosomiasis. In the St. Lucia experiment, the villages with piped water had lower

rates of infant diarrhea. Where water supply is part of an overall development effort, it should control schistosomiasis. Education about the benefits of using the clean water supply would probably increase the benefit.

Chemotherapy, the quickest and most effective single intervention, requires continued participation and cooperation from the community. Surveillance, particularly for immigrants from infected areas, must be kept up as well. And even though chemotherapy is relatively cheap, it still will be too expensive for many countries.

The St. Lucia experiment illustrates two important points. First is the encouraging fact that disease can be controlled successfully through several different methods. Second is the focus on control, rather than eradication. Maintenance measures to keep transmission to a minimum at a relatively small cost have been recommended to the Government of St. Lucia by the St. Lucia study team.

SOURCE: J.A. Cook, P. Jordan, and K.S. Warren, "The St. Lucia Experiment and the Evolution of a Contemporary Global Strategy for the Control of Schistosomal Disease," typescript, Rockefeller Foundation, New York, 1981.

TOOLS FOR CONTROLLING TROPICAL DISEASES

General Measures

The infectious diseases that killed most Americans at the turn of the century are now largely under control in the United States. Improved sanitation, the presence of adequate nutrition, and higher levels of education claim much of the credit. Those three conditions are not met in most developing countries.

Providing a secure, clean water supply for drinking and washing, and successfully educating people in how to use it, can reduce the transmission of diseases such as schistosomiasis (which is transmitted to humans through contact with water in which infected snails live) and diarrheal diseases caused by water-borne and food-borne pathogens. Sanitary disposal of human and animal waste further reduces the chance of spreading disease. In southern Africa, south Asia, east Asia, and the Pacific, less than half the population has access to a secured water supply or excreta disposal. In general, access is much greater in urban than in rural areas. In India, for instance, 80 percent of the urban population, but only 18 percent of the rural population, has water supplied, In Ethiopia, the corresponding figures are 58 and 1 percent (412).

Adequate nutrition buoys functioning of the immune system, increasing the individual's resistance to contracting diseases and his or her ability to fight a disease once contracted. Nutrition is particularly critical for pregnant women and infants.

Education, particularly of women, has beneficial effects on maternal and child health. The World Bank estimates that for every year of maternal education, infant mortality rates drop by nearly 1 percent (412). Although the exact sequence of events has varied around the world, in general, birth rates drop as the level of health increases. Fewer births lead to healthier children and women, and, in the long run, can help to improve nutrition.

Chagas' disease (American trypanosomiasis) could be controlled through the application of present-day knowledge. In Panama, for example, the Corozal palm *(Schelia* spp.), which provides thatch for rural dwellings, has been found to be a mini-ecosystem, harboring both reduviid bug vectors and mammalian reservoirs of *Trypanosoma cruzi.* If houses were constructed with corrugated metal or adobe tile roofs rather than thatch, then human contact with the reduviid bug vector of the disease could be limited considerably. Any attempts to control the reduviid bugs by insecticide only must be continuous, because the mammalian reservoirs of Chagas' disease provide a steady supply of T. *cruzi* parasites ready to infect the next generation of reduviid bugs that feed on them.

Vector Control Technologies (see ch. 6)

Many important tropical diseases, including malaria, trypanosomiasis, leishmaniasis, filariasis, and arboviral infections, are transmitted to humans by mosquitoes or other arthropods (e.g., ticks, sandflies, midges, and gnats). One, schistosomiasis, requires a second host, a freshwater snail, for the parasite to complete its life cycle. Control of vectors and snails in endemic areas is one way to control the transmission of these diseases.

Before about the middle of this century, most vector control efforts used mechanical approaches such as catching insects, removing breeding sites, or setting up physical barriers to the vectors' migration (e.g., by clearing strips of forest). Most vector control activities today use pesticides. The pesticide era began with the introduction of DDT (dichloro-diphenyl-trichloro-ethane) after World War II and has had some significant accomplishments. It also has taught that insects have a great capacity to evolve pesticide-resistant forms. In many parts of the tropics, stronger and stronger pesticides have been used against ever more resistant insects.

Other vector control strategies include biological control measures (e.g., the introduction of predators or pathogens of vectors) and environmental control measures (i. e., altering the environment to eliminate conditions necessary for the vectors' survival). The use of a combination of measures to control vectors is generally referred to as "integrated pest management" (1PM).

Immunization Technologies (see ch. 7)

The purpose of immunization is to prevent an individual from getting a disease if exposed to the agent that causes the disease. Vaccines are the most important tools of immunization. The functional components of vaccines are "antigens," substances that cause the body to marshal its own

Photo credit: Dr. Robert Edelman, National Institutes of Health

Irrigation ditches are frequently infested with the snail intermediate hosts of schistosomes.

immune system by activating one or more types of immune system cells. Some cells may be programmed to physically attack disease organisms and others are primed to produce "antibodies," substances that act to neutralize or kill the disease organism should the organism invade the body. Less important than the active immunity induced by vaccines is a passive immunity produced in an individual by the transfer of antibodies in blood serum from other, actively immune individuals.

All the human vaccines in use today are vaccines for bacterial and viral diseases. There are currently no vaccines for human parasitic diseases, but there is a great deal of research activity toward their development. The furthest along is a vaccine against one stage of the malaria parasite *Plasmodium (see Case Study B: The Development of a Malaria Vaccine).*

Vaccines benefit both the individuals immunized by them, and the community as a whole. Even if every person in the community is not vaccinated, if a high proportion are vaccinated or have acquired immunity from having had the disease, the nonimmune members will also be protected because there are not enough susceptible people to sustain disease transmission. This phenomenon is called "herd immunity."

In the United States most, but not all, children are immunized against measles, mumps, rubella, poliomyelitis, tetanus, diphtheria, and pertussis. In general, immunization levels are high enough to produce herd immunity. Outbreaks do occur, however, particularly outbreaks of measles. Investigations usually reveal that a critical mass of unvaccinated children existed in the community, and at least one was exposed to a case of measles. Although there are seldom deaths associated with measles in the United States, that is not the case in the developing world.

Immunization programs are potentially the most effective means of disease control for most infectious diseases, but they require a long-term commitment of public health resources to be successful. The initiation of vaccine programs requires careful analysis of the health, social, political, and economic conditions of the population to be immunized. (Table 5-1 lists the biologic considerations for using a vaccine against a tropical disease.) Since 1974, WHO has been the major promoter of childhood immunization, through its

Table 5.1.- Considerations for Use of a Vaccine for a Tropical Disease

Regarding the target organism:
- Complexity of antigenic structure
- Multiple stages of the organism's life cycle
- Accessibility of infection site within the host
- Occurrence of different species, geographic strains, local and temporal variants
- Inherent genetic variability of organisms
- Possibility of genetic selection for vaccine resistance

Regarding the vaccine:
- Immunogenicity of antigen: efficacy and effectiveness
- Use and type of adjuvant or diluent
- Possible undesirable side effects
- Targeted at one or several variants of the disease organism
- Separate administration or combined with other target vaccines
- Storage requirements (e. g., refrigeration and shelf life)

Regarding the vaccinee:
- Age
- Health status, including pregnancy
- Degree of possible resistance
  - —due to genetic or ethnic factors
  - —due to prior exposure or acquired immunity
  - —due to current infection
- Protocol; type, number, and time of other immunizations
- Access to prophylactic and therapeutic drugs
- Route, mode, dosage, and number of inoculations
- Measurement of immune response
  - —comparison with age-matched groups in developed countries
  - —monitoring for side effects or adverse reactions
- Degree and duration of protection
  - —from infection
  - —from pathologic effects of disease

Regarding the vaccine trial:
- Randomized selection of vaccinee and control groups
- Characteristics of placebo used in control group
- Availability of baseline disease incidence and prevalence data
- **Ethical aspects**
- **Training of field teams and standardization of procedures**
- **Site selection: number and distribution of trial populations**
- **Identifying populations at risk but not already infected or immune**
- **Determining exposure to and risk of infection by target organism**
- **Statistical validation of data; required number of participants**
- **Presence of related or competing organisms**
- **Length of followup**
  - **–for safety**
  - **—for efficacy**
- **Coordination with other public health programs—effect of vector control or other simultaneous projects**
- Source and adequacy of funding

SOURCE: P. Basch, "The Role of Biotechnology in Tropical Disease Research," contract report prepared for the Office of Technology Assessment, U.S. Congress, Washington, DC, 1984.

Expanded Program on Immunization (see box 5-B).

Diagnostic Technologies (see ch. 8)

Diagnostic technologies serve several purposes:

- to determine pertinent characteristics of an individual's disease, so that appropriate treatment can be given;
- for public health reasons, to determine the prevalence of pathogenic agents in populations; if control measures (e.g., a vaccine) are begun, determining the prevalence of such agents is important to allow evaluation of the success of the measures; and
- for research purposes, to find out about the ranges of diseases affecting a population and the immune status of populations, for instance.

A **wide range of biochemical tests supplements the observational abilities of medical personnel in diagnosis. Many of these diagnostic tests rely on reactions between antigens and antibodies and have radioactive or color markers that signify the test result. Recombinant DNA technologies are contributing to diagnostics in several ways,** including the production of pure reagents for tests and the production of "DNA probes, " which recognize the genetic material of disease-producing organisms.

Therapeutic Technologies (see ch. 9)

The object of therapy is to improve the lot of a person with disease. In the best case, therapy involves ridding the person of disease, but for many conditions, symptomatic relief is equally important. This report concentrates on drug treat-

Box 5-B.—WHO's Expanded Program on Immunization (EPI)

WHO's Expanded Program on Immunization (EPI) began in 1974 with a goal of immunizing all children against diphtheria, pertussis, tuberculosis, measles, tetanus, and poliomyelitis (272).

EPI is an operational attempt to apply safe and efficacious vaccines that already exist. Diphtheria, pertussis, polio, and tetanus vaccines have been available for many years and are recommended for widespread use. The efficacy of tuberculosis vaccine (Bacillus Calmette-Guerin vaccine) is more controversial (see ch. 7). Measles vaccination has also been controversial because of the variable seroconversion rate (percentage of vaccinated individuals who later have measurable antibodies) and protection rates (percentage of vaccinated individuals who do not get disease) obtained in developing countries. Measles vaccine is heat labile and not as effective as desirable in infants under 1 year of age.

Even with efficacious and safe vaccines, however, EPI has encountered problems of cost, logistics, and acceptance by the public, as well as strictly biomedical problems of immunizing malnourished populations already infected with other pathogens. A critical problem, and one that impedes EPI in many countries, is the need for a reliable "cold chain," the means to refrigerate vaccines from manufacture to use. In remote rural areas, the opportunities for the cold chain to break down increase, and with the breakdown, a damaged vaccine becomes more likely.

In countries that, for whatever reasons, cannot maintain an immunization campaign over the long run, vaccination programs may actually exacerbate disease rather than avert it. For diphtheria and polio, in particular, followup and regular boosters are necessary. Individuals who are vaccinated as children but not followed up as adults may still be susceptible to these diseases, potentially more severe than if they had occurred in childhood.

A technical problem is that the effectiveness of vaccines used in EPI may be impaired by the use of other health care measures. Chloroquine given for malaria prophylaxis, taken for long periods of time, can reduce the response to vaccines.

Even if new vaccines are developed, their impact on major public health problems in the tropics will still be problematic. Major questions that remain are whether the vaccines will work in large heterogeneous populations who are malnourished and often carrying other infections, whether the vaccine will be cheap enough and feasible to use (stable enough, easily administered), and what side effects will be encountered.

ment; it does not consider surgical treatment, because surgery plays a relatively minor role in the treatment of most tropical diseases.

Drugs to treat most bacterial infections exist, though bacteria in many cases are resistant to one or many drugs. Drugs to treat helminthic (worm) infections also exist, but these are generally less effective and less safe and require longer periods of treatment than do drugs against bacterial infections. The development of drugs to treat viral infections is just beginning. The antiviral drugs currently available are not commonly used against tropical diseases.

Genetic Tools

The science and industry of present-day biotechnology—the use of recombinant DNA techniques and other sophisticated genetic tools—are outgrowths of the ideas and experiments of a 19th century Austrian monk. In 1865, Gregor Mendel first described the concept that traits of higher organisms are transmitted from one generation to the next as discrete units. Although Mendel's work was unrecognized and unappreciated for half a century, the science of genetics had been born. For decades, the units of heredity, genes, were considered to be vaguely defined units or particles riding in some sort of linear order upon the chromosomes of animals and plants. Beads on a string were used as a popular analogy.

In 1953, Watson and Crick suggested a structure for the precise chemical makeup of Mendel's hypothetical hereditary units. The now familiar double-helix structure of deoxyribonucleic acid, or DNA, is at the core of modern molecular genetics, for it provides the framework of our understanding of the functioning of the genetic material and the basis for manipulating it.

The DNA molecule consists in part of an invariant "backbone" made up of two coiled strands of repeating sugar (deoxyribose)-phosphate components. Each sugar has attached to it one of four chemical bases. These four bases—adenine (A), cytosine (C), guanine (G), and thymine (T)—in certain combinations form bridges, something like the rungs of a ladder, across the paired helically coiled deoxyribose-phosphate strands. An A must pair with a T, in either order (A-T or T-A); and a C must pair with a G. Therefore, given a sequence of bases on one strand of a DNA molecule, say A-T-A-C-G-T, one can deduce the complementary sequence, in this case T-A-T-G-C-A, which must be attached to the other strand.

In the synthesis of new DNA from existing DNA, a process known as "replication," the two strands of the DNA molecule separate, each serving as a template. In the process of "transcription," the DNA strand serves as a template for a strand of a slightly different nucleic acid known as RNA, which in turn codes for the synthesis of proteins (see 357 and 361).

Recombinant DNA Technology

In 1973, Cohen, Chang, Boyer, and Helling reported the first in vitro production of DNA molecules that were "replicated" when transferred into living organisms. The details of recombinant DNA work are extremely complex and constitute the subject of many thousands of technical publications (e.g., 187, 132, 224, and 397).

Recombinant DNA technology is the term used for methods to transfer segments of genetic material from one organism to another, to replicate it, and to use it to make chemicals (see box s-C). Recombinant DNA technology represents an extremely powerful and precise set of tools with unknown, but certainly great, implications not only for tropical medicine but for many aspects of life in the future. A widely acclaimed use of recombinant DNA technology is for the production, in commercial quantities, of materials such as human insulin, interferon, or growth hormone. An application of cloned complementary DNA or RNA (see box s-C) is in locating a viral, bacterial, or parasitic DNA sequence within a host organism. These nucleic acid hybridization probes promise to be useful in molecular diagnosis of disease.

Monoclinal Antibodies (MAbs)

MAbs are homogeneous antibodies derived from clones of a single cell. To understand MAbs and the hybridomas that produce them, it is necessary to go back to the work of early immunologists.

Box K.—Recombinant DNA Technology

Recombinant DNA technology is a process whereby pieces of DNA from different sources, as different as humans and bacteria, are joined together. The basic method for recombining DNA follows.

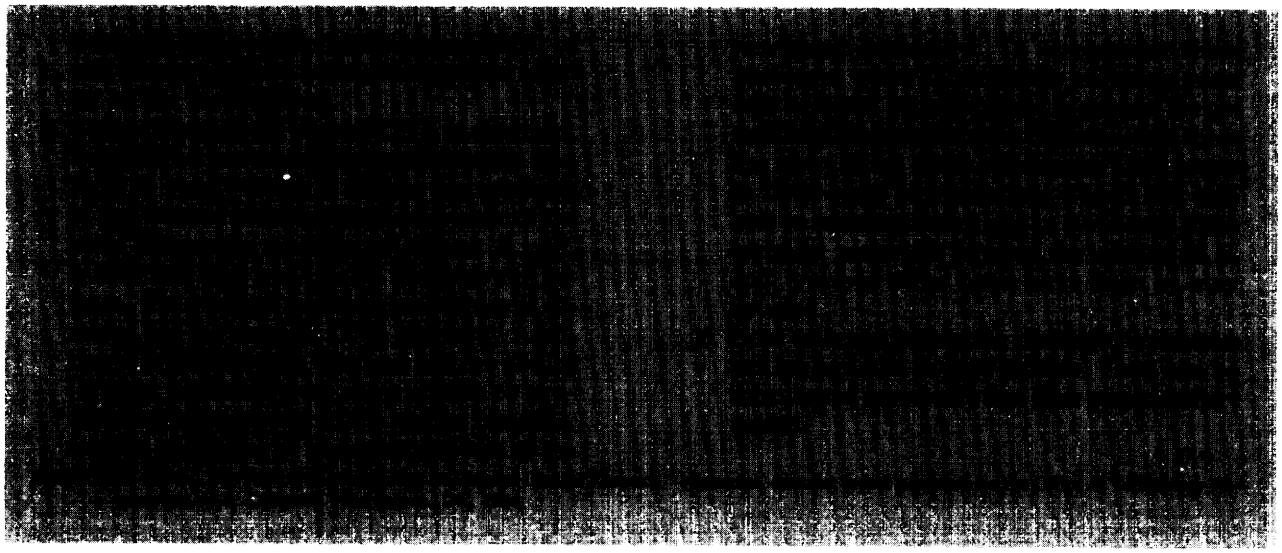
1. Start with a, b, or c:
   a. DNA is extracted from an organism. Within the total DNA (the genome) is the genetic information for making ill of the components and characteristics of that organism. The tick is to pull out from all that DNA the gene of interest, which probably constitutes only l/1,000th or l/10,000th of the total.
   b. A reversal of the transcription process (in which DNA is a template for RNA) is used to make a particular piece of DNA. RNA is usually extracted from cells that are actively producing the desired material. Through the use of a special enzyme called reverse transcriptase, the strand of RNA is used as a template on which a segment of single-stranded DNA is synthesized. Because the structure of this new DNA strand is complementary (like the relationship between a photographic negative and a print made from it) to the structure of the RNA template, the newly formed DNA is called "complementary" DNA. The DNA and RNA strands of the "hybrid" molecule are separated and the DNA strand used as a template with the enzyme "DNA polymerase I" to synthesize a double-stranded DNA configuration.
   c. If the sequence of bases in the desired DNA is known, chemical methods are used for the direct synthesis of DNA. These methods are limited to "shorter" pieces.

z. The extracted DNA *(from* la) orcomplementary DNA (from lb) is cut into pieces by use of restriction enzymes (restriction endonucleases) that cleave the DNA or complementary DNA molecule at certain specific sites. Restriction enzymes have been extractedfrom more than 200 different strains of bacteria and cleave the DNA molecule at any of more than 90 known sites, unique for any particular erkzyme. The cleavage site is determined by the sequence of constituent base pairs. One of the most commonly used restriction enzymes, called "Eco RI" and derived from *Escherichia coli*, cleaves the double-stranded DNA molecule in such a way as to leave overlapping cohesive or "sticky" ends. The resulting segments may contain part of a gene, a whole gene, or several genes depending on the configuration of the particular DNA molecule being cleaved. If complementary DNA was the starting material, it is already enriched for the genes encoding for production of the particular material made by the cells from which the RNA was extracted.

3. The pieces of DNA are inserted into a "vector." [This borrowed term is perhaps an unfortunate selection—the DNA vector has nothing to do with an insect vector of disease. The idea is that the vector transmits something to a new site.] Insertion is accomplished by cutting the vector's own DNA with restriction enzymes and then splicing the fragments of foreign or "passenger" DNA between the cut ends. Vectors are introduced into bacterial cells in which they replicate hundreds or thousands of times. Each copy of the vector contains one copy of the "passenger" DNA. In this way, "clones" of DNA are produced.

   A vector may be:
   a. A "phage" (=bacteriophage), which is a virus that parasitizes certain bacteria. Phages normally grow by injecting their DNA into the bacterium. The phage DNA takes over the replication machinery of the bacterium and makes many copies of itself and ultimately new phage particles which can then infect additional bacteria; or
   b. A plasmid, which is a circular DNA molecule that normally occurs in many bacteria. Many carefully engineered plasmids that contain information necessary for replication, as well as sites for the insertion of segments of foreign DNA and genes useful in later selection processes are available. Because plasmids are smaller than the bacterial chromosome and replicated independently of it, a single cell can contain many plasmids.

4. The passage of the vector through bacterial hosts produces millions of new vectors. A number of clever methods can be used to sort through the new vectors to select the vector that contains the desired sequence of DNA. There may be very few, if any, of them: most



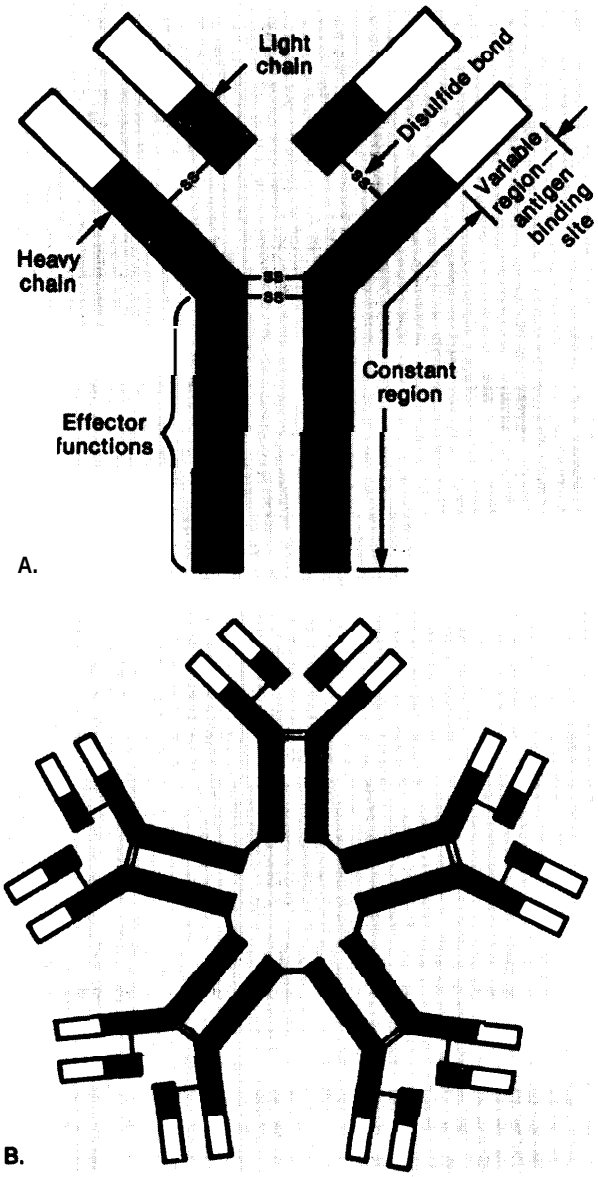
Investigators had observed around the turn of the century that in response to the natural or intentional introduction of foreign materials ("antigens," animals produce specific substances which circulate in the blood. These responsive substances became known as "antibodies," and early researchers such as Paul Ehrlich believed that the antibody and its inducing antigen fit together physically like a lock and a key.

Various theories of antibody formation and action were proposed by many workers, but it was not until 1959 that the Australian immunologist Sir Macfarlane Burnet conceived the theory of clonal selection. Burnet's idea, which led to a Nobel Prize, was that a great variety of quiescent but potentially antibody-producing cells exist in the normal human or animal, each with the inherent capability of secreting one specific kind of antibody. These quiescent but potentially antibody-producing cells are now known to be a certain type of white blood cells, which are called B-lymphocytes. According to Burnet's clonal selection theory, the introduction of any particular antigen into the body causes only those cells preadapted to it to become stimulated and to proliferate and differentiate into plasma cells that release specific antibody in measurable amounts. The many cells created by the repeated division of the originally stimulated cell are "clones," whose secreted antibody mixes in the bloodstream with the products of all other clones of antibody-producing cells.

The structure of antibodies is now known with great precision. Although there are several subtypes of antibodies, their basic composition and behavior is quite similar. As shown in figure 5-1, an antibody molecule has two symmetrical halves, each containing one "heavy" and one "light" protein chain. Each of the four chains contains a constant region (for certain generalized functions), and a variable region (on which are located the antigen-binding sites that determine the antibody's specificity).

For decades, immunologists had obtained antibodies for their research by the inoculation of animals, usually rabbits, with antigen. The antigenic dose could be as simple as a single purified protein, or complex as a whole-organ extract. After some time, the rabbit was bled, its blood allowed to clot, and the clear serum, containing the antibodies, was collected. At that point, the problem was always how to separate the particular antibody of interest from the mixture of very similar materials in the rabbit's blood serum. Scientists devised various chemical precipitations and immunologic binding methods to try to fish the desired antibody out of the complex "polyclonal" pool. Many problems were encountered in these procedures.

Figure 5.1.—Structure of Antibody Molecules



A. The basic unit of all antibodies is a protein molecule made up of two "light" and two "heavy" chains joined by disulfide bonds. Foreign antigens are recognized by regions at one end of the molecule (variable regions). Other parts of the molecule are specialized for "effecter functions" that aid in deactivating and removing the foreign antigen. Antibodies in the immunoglobulin classes lgG, lgD, and lgE exist as single monomers.

B. Antibodies belonging to immunoglobulin classes lgM and lgA exist as molecules with multiples of the basic unit. lgM (in diagram) is a pentamer (5 units). Different types of lgA exist in monomers, dimers (2 units), and trimers (3 units). The units are joined by disulfide bonds.

SOURCE Office of Technology Assessment, 1985.

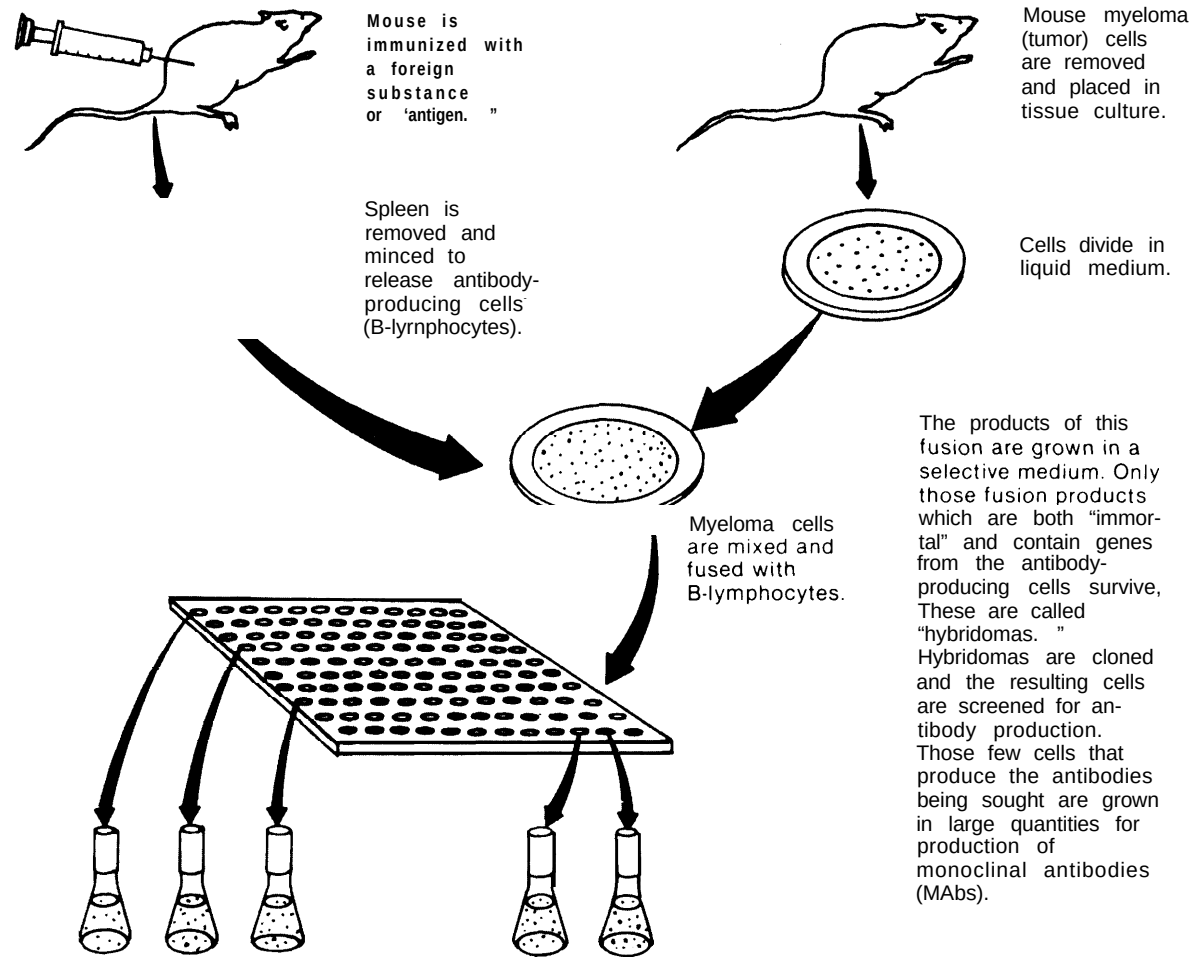
One problem was that complex antigens, as large molecules, usually bear several different structural antigenic determinants called "epitomes," each of which is recognized by distinct antibody-producing B-lymphocytes. The inoculation of a complex antigen results in the production by the immune system of several different antibodies, each of which is specific to a portion of the same antigen. Conversely, several distinct antibodies may combine with the same portion of the antigen. An additional complication is that rabbits, even from closely inbred lines, vary in their individual ability to recognize and respond to particular antigens: the antibodies produced are sometimes heterogeneous and vary from rabbit to rabbit or even within the same animal over time.

Scientists' inability to obtain a uniform, reproducible, and above all, specific product hindered biomedical research until the invention of hybridoma technology in the mid-1970s. This invention, by Georges Kohler and Cesar Milstein at Cambridge University in England, was a landmark in the history of immunology.

For some time, immunologists had attempted' to isolate and clone antibody-forming B-lymphocytes in artificial culture media, but these cells normally have a limited life span and clones cannot be established. Malignant tumors of the immune system called "myelomas" are capable of continuous proliferation in cultures, but cultured myeloma cells produce little or no antibody. Kohler and Milstein devised a method for forcing the recalcitrant myeloma cells to produce specific antibodies in quantity. Their work, published in 1975, has led to an explosion of subsequent studies. What Kohler and Milstein did was to combine an immortal but nonsecreting myeloma cell line with a secreting but nonculturable B-lymphocyte in order to produce "chimeras" having the desirable properties of both parental types. This is the "hybridoma" (=hybrid myeloma) so commonly used today. Hybridomas and MAbs have been reviewed several times (e.g., 6), and step-by-step instruction manuals for their preparation are available (206,437).

The method for preparing MAbs is shown in figure 5-2. First, a mouse is immunized with an antigen and permitted some time to respond.

Figure 5-2.–Preparation of Monoclinal Antibodies (MAbs)



SOURCE: Office of Technology Assessment, *Commercial Biotechnology: An International Analysis*, 1984. Adapted from Y. Baskin, "In Search of the Magic Bullet," *Technology Review*, October 1982.

Lymph nodes or spleens of immunized animals are then removed, and their cells are dispersed and added to cultures of certain types of mouse myeloma cells. The mixed cells are incubated so that their cell membranes are allowed to fuse together, producing hybrid cells with two nuclei. After a period, the hybrid cell nuclei stabilize, and the cultures, consisting of thousands of cells, are placed into a medium in which the growth of the original myeloma cell line is prevented. Only the hybrid cells are now capable of proliferation in the new culture conditions, and the task at this point is to identify and separate those cells that are active secretors of a desired antibody. This is accomplished through any of several types of screening assay, in which the overlying culture medium is tested for the presence of antibody by incubation with a particular antigen labeled (with a radioactive or fluorescent marker) to permit the ready identification of resultant antigen-antibody complexes. Those cell populations demonstrating antibody production are then diluted, and each surviving hybridoma cell is isolated to start a clone of genetically identical cells. The result of this process is the development of large colonies, each derived from a single hybrid cell, and each secreting one specific—hence "monoclonal"-antibody.

Milstein has written (223):

> A monoclinal antibody is a well-defined chemical reagent that can be reproduced at will, in contrast to a conventional antiserum, which is a variable mixture of reagents and can never be reproduced once the original supply is exhausted.

All progeny cells from a single hybridoma clone secrete antibody molecules whose variable regions are identical—the variation is only between, not within, clones.

A single experiment may yield a large number of hybridoma clones, about 10 percent of which secrete a monomolecular antibody specific to that particular cell line. Some of these maybe of little or no interest to the investigator, who now faces the lengthy and arduous task of determining the specificity of each secreting hybridoma clone and deciding on its relevance. In some experiments, it may be the goal to generate a variety of different hybridomas. More commonly, an investigator wants to obtain a highly specific antibody. Therefore, he or she attempts to inoculate a correspondingly purified antigen into the B-lymphocyte-contributing mouse in order to reduce the "noise" of unwanted hybridomas.

Hybridoma-derived clones of secreting cells may be propagated in culture media, from which the specific antibody may be separated. Since these cells are derived in part from mouse myelomas, they will induce tumors in mice, with the production of relatively large volumes of antibody-containing ascites fluid, This fluid can be harvested for use, and the hybridoma line can be perpetuated by transfer from mouse to mouse.

A particularly intriguing application of MAbs is in the production of what are referred to as "anti-idiotypic" antibodies. In this technique, animals are immunized with a previously prepared MAb made against a disease-causing organism. The idea is to stimulate the production of a complementary "anti-antibody" which may substitute for the antigen against which the original monoclinal was raised. In an early application of this method to a tropical disease pathogen, Sacks and Sher (303) found that several specific anti-idiotypic antibodies were able to protect mice against challenge with *Trypanosoma rhodesiense,* while others were ineffective. Other investigators have recently reported successful immunization of mice against the bacterium *Streptococcus pneumonia* with an anti-idiotype vaccine (22). The technique is certain to have wider application, although its eventual usefulness is unknown.

Applications of Genetic Tools to Tropical Diseases

Applications of genetic tools to tropical medicine have been discussed extensively by Falkow (112) and Cross (81), who indicated some uses and advantages of cloned genes in medical and veterinary science:

- elucidation of genetic basis of normal and abnormal cell function;
- diagnosis of disease and disorders;
- synthesis of biologically active proteins; and
- production of subunit vaccines, which may provide:
  —improvements in existing vaccines,
  —production of new vaccines,
  —reduction in adverse side effects as a result of increased purity,
  —safety in manufacture and use, and
  —consistency in manufacture and use.

The many applications of MAbs to parasitic tropical diseases have been discussed by Mitchell and Cruise **(237),** Mitchell (235,236), McBride (218), and Rowe (295). In brief, the uses of MAbs in tropical diseases caused by parasites are:

- for diagnosis of infection and identification of the particular type or strain of parasite,
- for information about the genetics and relationships among the organisms,
- as "probes" for defining the antigenic composition of the parasites and of their products,
- for identification of the parasite components likely to stimulate host protective responses, with possible vaccine production,
- for standardization of diagnostic reagents and vaccines,
- possibly for drug targeting or other means of immune-based treatment of infected hosts, and
- to effect host protection in vivo.

MAbs can also stimulate production of anti-idiotypic antibodies and are widely used as probes and reagents in molecular biology laboratories to detect expression of cloned parasite **DNA by host cells.**

In the area of tropical medicine, the genetic tools find their greatest potential application in the search for protective vaccines. If a parasitic organism contains a particular antigenic portion that can stimulate protective immunity in humans, it would be desirable to obtain large amounts of that specific antigen (isolated from the remainder of the organism) to use as a vaccine. One way to obtain such material might be to grow the parasite either in animal hosts or in laboratory cultures, and then extract the desired antigen by chemical means. Because of the time and expense involved, this approach is feasible only for experimental work in the laboratory. However, identification, isolation, replication, and production of the antigen by the expression of the DNA sequence responsible for antigen production could provide access to an essentially unlimited and relatively inexpensive source of the immunizing material.

Biotechnology research with agents and vectors of tropical diseases requires a laboratory equipped with a wide range of modern apparatus, including optical and photographic equipment, gel electrophoresis devices and power supplies, centrifuges, refrigerators and freezers, sterilization facilities for culture media, biological safety cabinets, and myriad smaller devices. A great number of chemicals and reagents are also necessary. Facilities for growing the disease organism must be maintained or the organism must be obtained from elsewhere. Trained technical personnel are indispensable. The work is demanding of effort, skill, and imagination, and requires a certain level of financial support. Most developing countries do not have the capacity to do this type of research, so nearly all of it is done in the United States and other developed countries.