Chapter 10
Drugs and Healthcare

Drug Discovery and Development

SRI has had an extended, almost 50-year, history in the exploration of new drugs and a bit of that history is related here. But before proceeding, it is helpful to understand drug development and testing at SRI and the process shown in Figure 10-1, which shows the phases needed to bring a drug into use. This process is long, expensive, and sometimes tortuous, littered with opportunities to fail.[1] As a research institute, and a player lying outside the normal pharmaceutical industry, SRI has characteristically worked in the earliest phases of drug discovery. Here the research content and the risks are high, but the costs are—relatively speaking—low, and the special facilities and personnel qualifications found in a clinical setting are not required. SRI has also engaged in some preclinical testing, and this somewhat broader charter has prompted some initiatives that may alter its more limited discovery role.

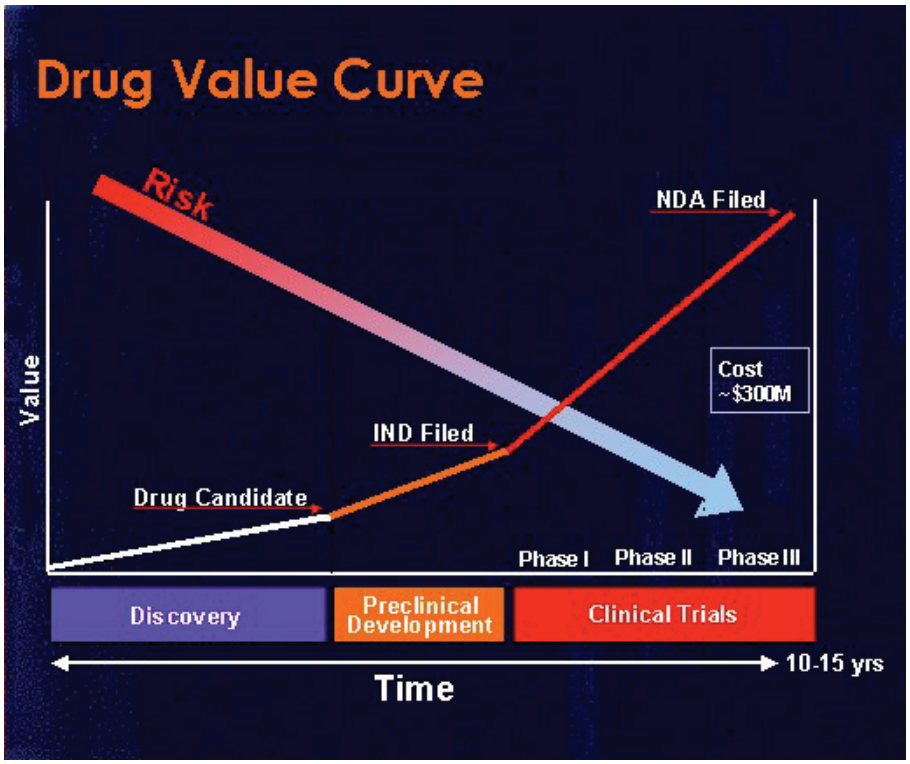

Figure 10-1. Notional model of drug development and testing showing the phases and the exponentially increasing costs.

Although drug discovery is just the first part of drug development, the discovery process itself is complex, time-consuming, and exceedingly dependent upon the competency and imagination of the research staff. Figure 10-2 shows the major steps involved in discovery itself and, like the overall drug development process, each step can be a showstopper.

With over 40 years in the field of drug discovery, SRI is now trying to broaden its role in drug development. Doing so is very consistent with its new orientation toward a greater participation in the commercialization of its intellectual property. The reasoning goes something like this. Over time and in a somewhat piecemeal fashion, SRI has acquired the competency and the facilities for conducting many preclinical tests. Both government regulatory and research agencies and drug companies have sponsored these

[1] According to a recent article in *Science*, the average cost of developing, testing, and gaining approval for a new medicine is a staggering $897 million. This cost includes the 99.9% of compounds that wash out in the development pipeline! But of even those drugs that enter human testing, only 1 in 5 get approved for sale. Obviously, the earlier in the development process an unmarketable drug is eliminated, the better. (Robert F. Service, "Surviving the Blockbuster Syndrome," *Science*, 303, 1796–1799, March 19, 2004)

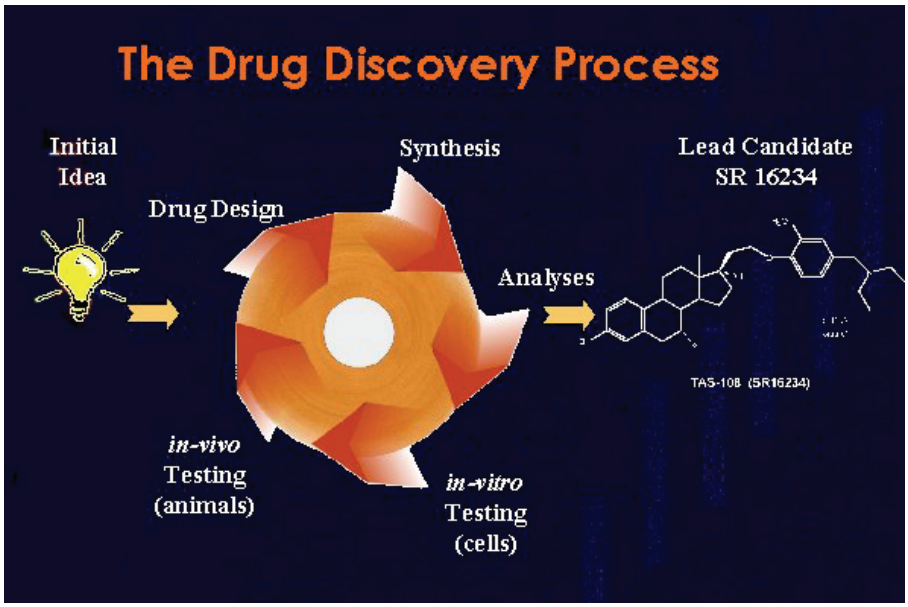

Figure 10-2. Steps in the drug discovery process. The wheel depicts it as an iterative exploration.

activities. There arises the notion, then, of offering a more comprehensive drug development service. SRI already has experience in screening drug candidates and in testing for stability, manufacturability, and toxicity. Because of this established capability, SRI claims that it can now perform preclinical work faster and cheaper than pharmaceutical houses, whether those houses are established biopharmaceutical companies or simply financial frameworks for drug exploration and development. A vice president of a local biotech company told the *San Francisco Business Times*, "We've looked into the matter of doing it ourselves versus getting it outside. There's just no way you can compete with an established firm that already has its facility in place and established procedures and staff."[A] Time will reveal the value of this initiative.

With that background, we now review some of the specifics in SRI's drug discovery and development history. Work in testing the toxicology of drugs and some environmental pollutants will also be covered as well as SRI's work in the healthcare sciences.

Antimalaria Drugs

Although malaria was once considered a controllable disease, its effect on the world is not only staggering but is rapidly rising. About 40% percent of the world's population is at risk in about 90 countries. Estimates from the World Health Organization and other interested groups place the number of new clinical cases each year at several hundred million with the annual death toll over 1 million.[2] As bad as that seems, it is actually a lower rate than existed, say in the 1920s, when most cases were in Asia (see Figure 10-3).

Today, malaria is largely controlled in Asia, relatively speaking, but is actually a growing threat in Africa where over 90% of those one million malarial deaths now occur. That rise in Africa began in the 1950s and 1960s. When the major inroads on malaria were made starting in the 1930s, it was mostly because of better control of the vector, the mosquito, and because of introduction of drugs like quinine (or more important, chloroquine), which were hostile to the responsible blood parasite. The parasite associated with malaria invades the red blood cells and causes them to stick to the linings of capillaries and small blood vessels. This blockage effectively shuts off the supply of glucose and oxygen to the body's cells by decreasing the number of viable red blood cells present.

The *Plasmodium* parasite responsible for malaria has several types. One of the more prevalent is called *Plasmodium vivax*, which results in a recurrent infection that attacks only a certain age of red blood cells (about 5% of the total) and is therefore usually not fatal. Another form, *Plasmodium falciparum*, attacks all red blood cells regardless of their age and is lethal, responsible for about 95% of malarial deaths. This most deadly strain has been reason alone to seek new and more powerful drugs.

[2] The Canada-based International Development Research Center places the annual death rate at between 1.5 to 2.7 million. Other sources, such as the presentation from WHO in Figure 10-3, are closer to 1 million.

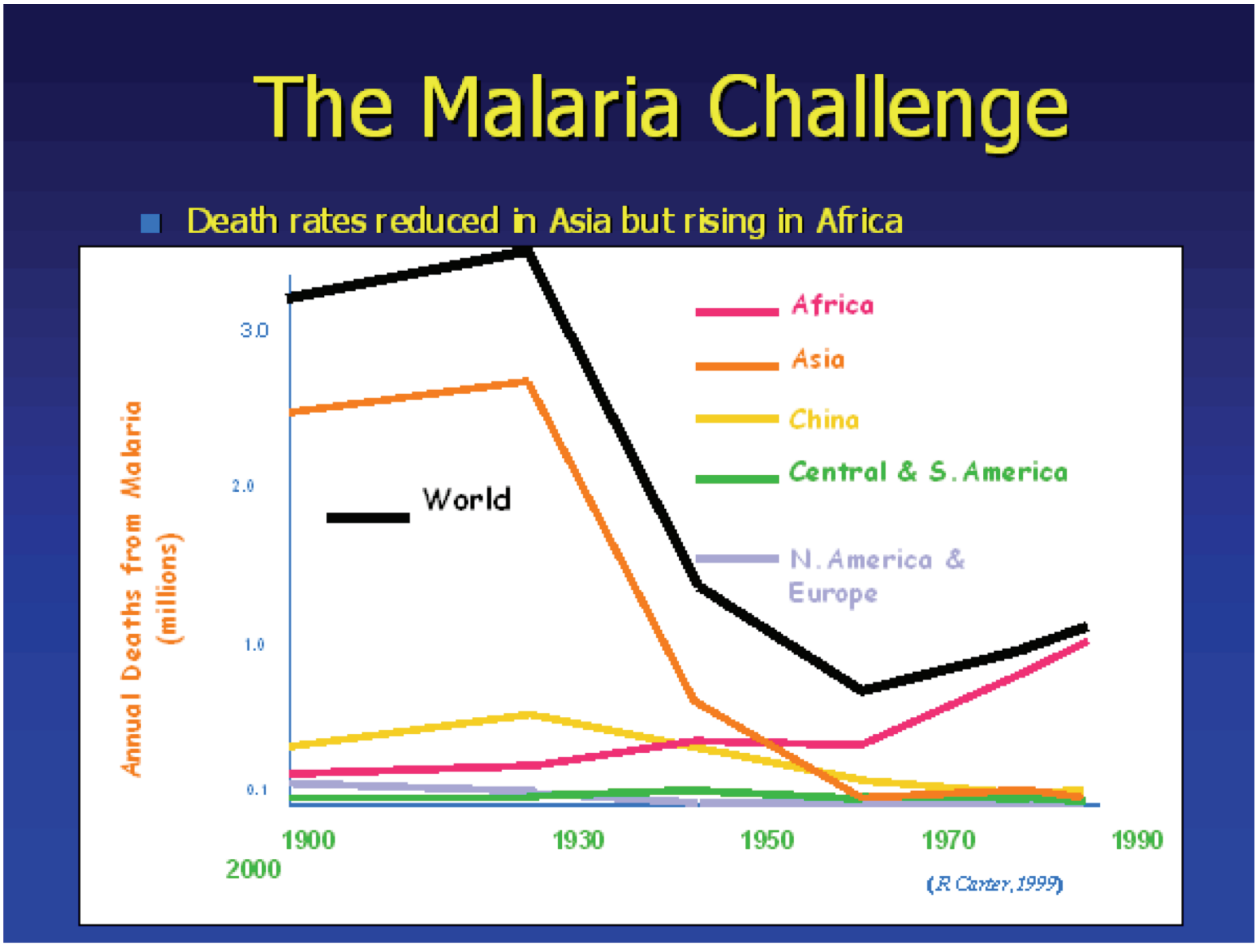

Figure 10-3. The worldwide mortality from malaria (from a WHO presentation found at www.who.int/rbm/presentations/us-canada/uscong/sld006.html).

But something else began to raise concern in the post World War II era. New malarial strains were becoming resistant to chloroquine, the 1940s drug that had gained such wide use because of its effectiveness and its reduced side effects relative to quinine. By the 1960s, this resistance was also beginning to surface in *falciparum*.[B] Later, it was observed that a newer antimalarial drug, mefloquine, synthesized to help chloroquine resistance, was itself losing some of its effectiveness. The bugs are relentlessly and frustratingly adaptable.

SRI's Halofantrine

Malaria has long been a particular concern to members of the armed forces, who may be called on to serve in the regions of the world where malaria is still prevalent. So, in about 1964 the Walter Reed Army Institute of Research (WRAIR) entertained a proposal from SRI to look into drugs that could address the more lethal malarial forms. A contract was awarded in 1965 that began a decade-long search at SRI for a new and effective antimalarial drug, particularly for the deadly *falciparum* form of the *Plasmodium* parasite.

The opportunity came to an SRI medicinal chemist, Dr. William Colwell (see Figure 10-4), and to the leader of the Bio-Organic Chemistry Program, Dr. David Henry, who had developed a considerable background in anti-parasitic chemotherapy. Before them lay this always present, fine line between a compound that was powerful enough to kill the parasite and specific enough to avoid damaging side effects. Most of the antimalarial drugs, such as quinine, have terrible side effects and often unreasonably difficult regimens. SRI examined a wide variety of compounds that had displayed antimalarial behavior but suffered from clinical ineffectiveness.

In the fall of 1970, Colwell investigated the "docking" of some compounds to a likely active site on a yard-long ball-and-stick model of a generic segment of DNA. He began to understand how he might improve the efficacy

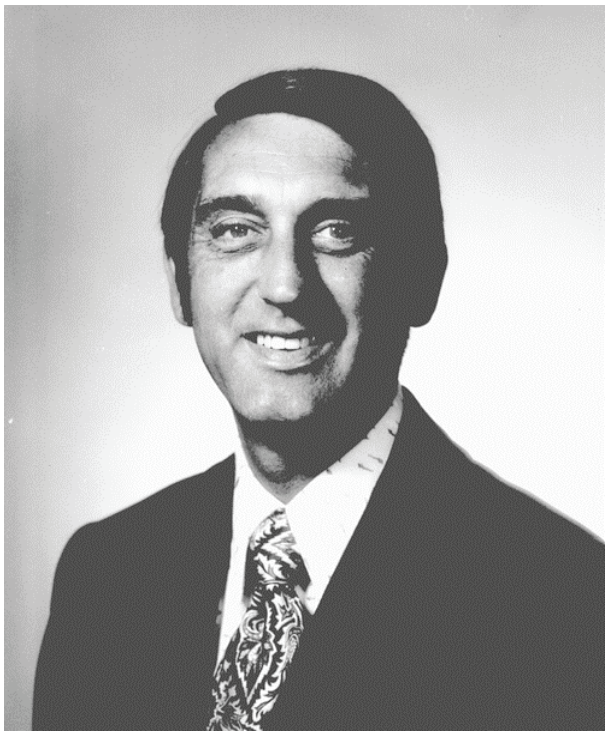

Figure 10-4. SRI organic chemist, Dr. Bill Colwell.

of a class of compounds, called arylamino alcohols. These were already known to have some activity against the *falciparum* parasite. Although the exact mechanism is even today a matter of conjecture, Colwell's radioisotope studies showed a high absorption of the compound into the unicellular parasite and, in particular, a concentration around its nucleus. So, Colwell and his colleagues were convinced that they could find something in that family that was more effective than the existing set of malarial drugs.

Having synthesized a series of new variants from this arylamino family, they submitted them to a clinically predictive mouse assay developed for WRAIR by the University of Miami. The arylamino propanols they had focused on using their model predictions proved four to six-fold more effective than the predecessor (aminoethanols). Preclinical toxicity (side-effects) was at least comparable. The researchers published the drug series and its bioassay results in the technical literature in 1972.[C] The first compound selected was named halofantrine by WRAIR, and it and four promising relatives were assigned to the sponsor for further action.

Halofantrine entered into several trials; perhaps the earliest was done using U.S. volunteers in 1974. Later, trials were conducted in many African, Southeast Asian, and Middle East countries. The license to produce the drug was awarded by the Army to Smith Kline & French in 1984. Halofantrine became authorized for use in France in 1988 and in the United States in 1992. It was not marketed in the United States until 1998, presumably due to the small market. Under the arrangements of the day, the drug became the property of the U.S. Army and SRI has never received any commercialization income from it. Regrettably, except for a few well-known medical indices, SRI has also been left unrecognized as the inventor of halofantrine.

As to its impact, halofantrine has successfully treated the strain of malaria for which it was designed and has been used worldwide for the successful treatment of millions of people. It is an important member of the group of antimalarial drugs recommended by the WHO and is used in all regions where malaria is present. Colwell's original choice, the mono-butyl form of halofantrine, now called desbutylhalofantrine, with perhaps even fewer side effects, was placed on Phase I trials by the Army in 1998. Specifically, this form of halofantrine has a much-reduced tendency to decrease heart rate through a prolongation of the heart's electrical cycle.[D]

As with earlier malaria drugs, there remains the crucial question of whether halofantrine will remain effective in this critical battle with the *falciparum* parasite. Will it develop any cross-resistant characteristics with other antimalarial drugs; will malaria parasites such as *falciparum* that have developed a resistance to, say mefloquine, also lose their susceptibility to halofantrine? We still don't know. In the meantime, halofantrine is likely to become a primary pharmaceutical for preventing malaria for travelers from western countries in addition to its continued role for the treatment of critical infection in the host countries. Reasons also exist to be cautious of halofantrine. One is that, taken in combination with mefloquine, another antimalarial drug, halofantrine can cause serious changes to heart rhythms. Halofantrine is also much more expensive than the first line drugs such as chloroquine and mefloquine.

Two additional compounds from the SRI program were among the total of five, including halofantrine, selected for clinical trial by WRAIR. These two compounds successfully passed Phase II and III investigation and have

been reserved for future use in case of resistance development to the current drugs.

Artemisinin

Although antimalarial drugs have a broad range, they are up against a formidable and highly adaptive enemy, so new counter-measures are being sought. Today, there is a groundswell of interest in developing an understanding of the genetics of how both the *falciparum* parasite and its mosquito vector reproduce and adapt. Such solutions still have their controversy, however, and their promises are a long way off.[E] In the meantime new drugs are needed, and one that has shown promise in Asia is a class called artemisinins.[F] In the mid-1980s SRI chemist Mitchel Avery heard of the possible efficacy of this drug from visitors to a Chinese folklore medicine institute in Shanghai.[G] The drug was made from the sweet wormwood (*Artemisia annua*) or ginghaosu weed and was reported to be effective against *falciparum*.

Over a period of 3 three years, Avery, with colleagues Wesley Chong and Clive Jennings-White, made 55 different analogs of artemisinin. These were sent for testing in whole blood at the Walter Reed Army Hospital in Washington DC. Though there have been considerable reported successes of the drug, the WHO and the U.S. AID have been cautious in its approval partly because of the drug's expense and the dangers of its dosage.[H] To those working in the field, however, artemisinin in combination with more conventional but increasingly ineffective drugs like chloroquine, is the next best hope. SRI is no longer involved in the synthesis of artemisinin.

So, this SRI research into antimalarial drugs is over, yet the death rate from malaria is still enormous, with some 2,000-3,000 African children dying from it each day.[I] Much of the research battlefield has now switched to studies of genomics, including the parasite and the mosquito carrier, and to those humans who have a natural high resistance to the disease.

Anticancer Drugs

Before describing SRI's contributions in cancer drugs, we explain briefly some of the concepts and terminology that will appear.[J] Much of the approach to chemotherapy involves the basic cell processes enabled by the protein-building template that is DNA. While we think of DNA in the production of normal cells, it is necessary for the replication of all living cells, including those of cancer. So, understanding the cell replication process in general may help identify the vulnerabilities of rapid-growing cancer.

DNA, or deoxyribonucleic acid, is the heredity-defining molecule in living cells. It is a polymer consisting of two very long, interconnected helical strands, each of which is made up of structural subunits called nucleotides. These nucleotides are the building blocks or monomers of each strand of the helix. Removal of a monomer-linking phosphate group from a nucleotide leaves a nucleoside of which, with almost no exception, there are but four types in all of DNA.[3] It is the order of these four nucleosides in their extensive repetition along the DNA molecule that gives rise to the genetic code. The two strands are complementary in structure and are reversibly bound in a double helical coil. For DNA to replicate, its components, including the above building blocks and their constituents, must be available. This means individual elements such as phosphorus, carbon, nitrogen, and oxygen must also be present and available in some form or the replication process ceases and cells and their host die.

It was toward inhibiting the formation of the DNA-building components that SRI targeted its early anticancer investigations. This included denying the availability of some of the components of the nucleotides. Since the replication of normal cells must also go on, interrupting that process without discrimination produces undesirable side effects. SRI's early anticancer drug research focused importantly on the synthetic chemistry of nucleosides, with an objective of altering nucleoside structures so that their chemical incorporation into the DNA replication process would effectively terminate it. Because cancer

[3] A nucleoside is a structural subunit of nucleic acids, which control the hereditary properties of all living cells. It consists of a sugar molecule and one of four attached nitrogen bases: two purines (adenine and guanine) and two pyrimidines (cytosine and thymine). With the addition of a phosphate group, nucleosides form nucleotides, which are the repetitive basic building blocks of the DNA molecule. The sugar molecules can be of two types: deoxyribose and ribose. The former is in DNA and the latter in RNA.

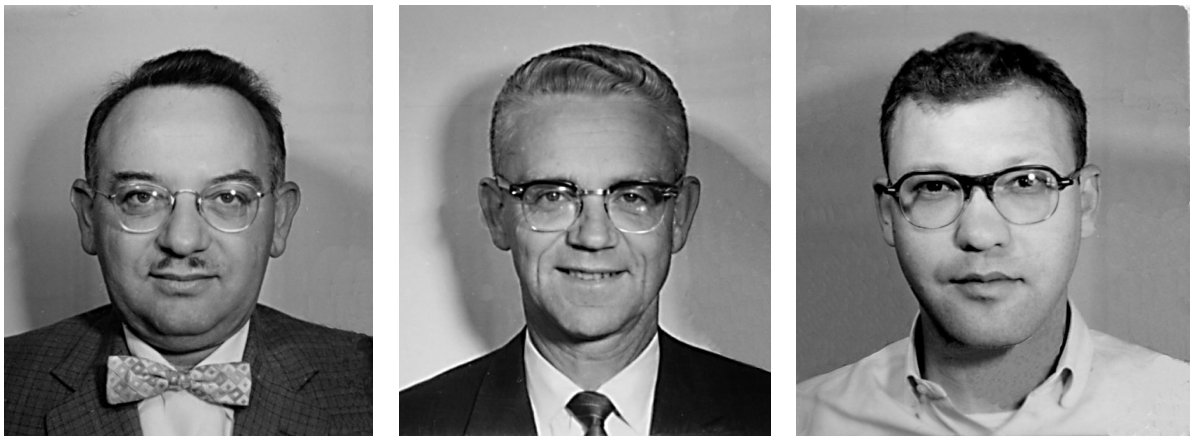

Figure 10-5. Organic chemists, Drs. Bernard R. Baker, Bruce Graham, and Leon Goodman.

cells are characteristically fast growing, a drug lacking tissue-specific attributes would still cause greater cell termination in cancer than in normal cells.

SRI's Entry into Anticancer Drug Discovery

SRI's work in cancer chemotherapy began in the mid-1950s with the arrival of Dr. Bernard R. Baker from Southern Research Institute (see Figure 10-5). His prior accomplishments had included the early synthesis of a nucleoside, puromycin, which had anticancer properties. His purpose in coming to SRI was to start a group in the search of new anticancer agents. This opportunity came in response to an initiative from the Cancer Chemotherapy National Service Center (CCNSC), a part of the National Cancer Institute (NCI).[4] Baker was a brilliant chemist who had a close relationship with that Center. He actually left a Center-sponsored project at Southern Research to come to SRI to establish yet another. To give the new SRI laboratory a sense of completeness, he organized it into a synthesis group, which could produce testable quantities of both final drugs and their intermediates, and an analytical group with capabilities in infrared and magnetic resonance spectroscopy. To round out this mix, there was an existing SRI group that was already doing drug screening in mice for the same NCI. This ability to perform locally the efficacy testing of new drugs on mice would provide rapid feedback.

The synthesis group consisted of 15 chemists whose objective was to synthesize gram quantities of target drugs as well as larger quantities, sometimes in the hundreds of grams, of chemical intermediates. These efforts were divided between the syntheses of analogs of known anticancer compounds and creating new compounds of interest, based on postulated biochemical mechanisms. The analytical group supplied analytical and spectral data to check the identity and purity of the various chemical agents. The major emphasis of the CCNSC was to increase the general awareness of new drugs and their effects by placing all results in the open literature. This stipulation had long-term consequences for SRI. Although SRI was to gain national prominence from its work in chemotherapy and nucleosides, SRI did not create any patent position on the corresponding work at that time.

An early success of the program was the first synthesis anywhere of a natural purine deoxynucleoside (called 2'-deoxyadenosine), one of the four building blocks of DNA.[5] From this work also came a drug called adenine B-D-arabinofuranoside, a compound intended to be a tumor inhibitor with minimal side effects. Unfortunately, it was too easily deactivated for that role by other body

[4] A leader in this emerging part of SRI was Dr. Bruce Graham (see Figure 10-5). He had received a phone call from the San Francisco airport from a staff member of the NCI, informing him of new programs they were promoting and that he thought SRI might be interested. Graham drove up to meet him and learned about the programs and also that there was someone the NCI thought would be qualified to lead an anticancer effort should SRI be interested; namely, Bernard Baker. Since this was a good opportunity to participate in a large NCI activity, Graham followed up.

[5] See Footnote 3.

enzymes, but the drug turned out to have good anti-herpes attributes.[6]

In 1957 Baker began examining certain biochemical targets in the DNA replication process. The approach was to inhibit the metabolism of folic acid, which had a critical role as a "carrier" for the carbon atoms needed in the DNA replication process. These antifolates effectively shut down the production of the nitrogen bases needed for the biosynthesis of the nucleosides that make up DNA and RNA. The problem was how to make such an inhibitor selective for only cancer cells. The early antifolate inhibitors, unfortunately, were also toxic to normal mammalian cells, so that kind of work slid briefly into the background at SRI.

In about 1959, Baker initiated another program at SRI in specific enzyme inhibitors. This was a search for certain compounds that he liked to call "fraudulent nucleosides" that took advantage of an existing concept of agent intervention called a "lock and key mechanism." Here the target enzyme represents the lock and the synthesized chemical agent the key, which, in this case, had the property to seek out the lock. Once the key enters the lock, the other end of the active molecule would bind to another reactive site on the enzyme in a process called alkylation. If this latter bond were strong enough, the binding would be irreversible and could block the enzyme from further biological functioning.[K] This approach gained broad use in the anticancer community and Baker later wrote a book about it.

With the departure of Baker in 1961 and under the new leadership of Dr. Leon Goodman (see Figure 10-5), SRI's chemotherapy work brought a new emphasis in the exploration of two areas: a family of drugs called nitrogen mustards and a revisit of ways to inhibit the metabolism of folic acid and its analogs. The nitrogen mustards, a type of alkylating agent,[7] were among the first anticancer drugs used anywhere (e.g., chlorambucil and cyclophosphamide for various forms of lymphomas). These substances interfere with the DNA/RNA replication process. In that process, however, they also affect normal cells and thus produce serious side effects. This family also happens to have antibiotic as well as anticancer properties and two members of this family received special emphasis at SRI.

The analytical chemistry group that Baker started also needs to be mentioned. Under the direction of Dr. Peter Lim, it was able to win its own NCI support as early as 1956. Remarkably, that analytical chemistry project has continued for nearly 50 years, making it SRI's longest continuous project.

The Antibiotic Called Adriamycin

By the 1970s Adriamycin (doxorubicin) and Daunomycin (daunorubicin) were two antibiotics that had come to be very popular as chemotherapy drugs. As with many pursuits in drug discovery, the work begins with efficacious drugs and looks for closely related analogs that have higher success rates, lower side effects, or both. That was what Dr. Ed Acton and others at SRI undertook in the 1970s (see Figure 10-6). Both drugs had shown reversible binding to DNA and an interference with DNA/RNA synthesis. Adriamycin was then called the most active compound against cancer because of its broad range of activity—notably against major tumors that did not respond to other drugs. In many cases, however, the efficacy rate was too low—perhaps in only 1 in 3 patients. Further, toxic side effects limited both the dose level and the duration of treatment. Hence improved analogs of these new drugs were widely sought, and soon the drug development community had created some 2,000 active analogs of adriamycin.[L]

This large number of analogs illustrates a recurring problem in drug discovery: how to choose for further study from among analogs that are active in a primary screen and thus displaying some clinical potential. To assist in dealing with this at SRI, Dr. Dave Henry set up one laboratory in conjunction with the synthesis group to do simple *in vitro* screens for inhibition of DNA and RNA synthesis, supplemented by data from the NCI's own mouse screens. From this filtering, the SRI group came up with at least two new analogs that received wide attention in the cancer drug development field. One was 5-iminodaunorubicin, which was significantly less cardiotoxic, and the other was cyanomorpholinodoxorubicin (CMDR), which

[6] SRI's virologist, Dr. Gus Freeman, suggested that adenine B-D-arabinofuranoside's characteristics might make it a good antiviral agent, but NCI nixed that investigation. A few years later, Parke-Davis isolated the same drug from other natural sources and applied it to antiherpes use. Parke-Davis gave it the trade name Virazole and it is still in use today.

[7] An agent that can transfer a chemical alkyl group onto DNA. Widely used in chemotherapy.

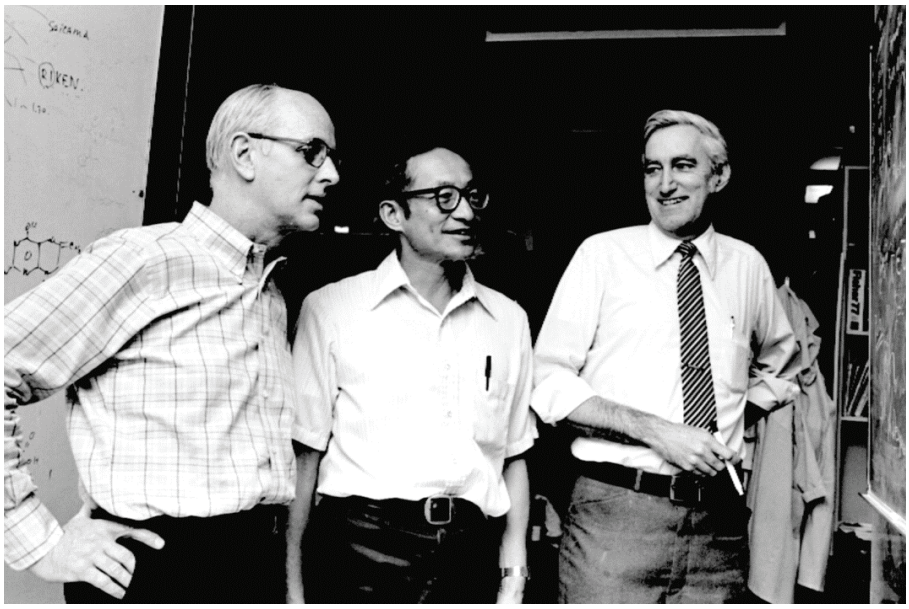

Figure 10-6. Organic chemists, Drs. Ed Acton, Bill Lee, and Joe DeGraw.

was 600 times more potent, in terms of the required dose amount, than adriamycin, and its binding to DNA was irreversible.

A significant academic achievement by SRI in this family was the first synthesis of daunosamine, the sugar component within each of these more effective drugs followed by a formal total synthesis of adriamycin in 1983.[M] Adriamycin is still used worldwide in the treatment of a variety of cancers and is of great clinical importance in chemotherapy.

During the late 1970s and early 1980s, two important policy changes occurred in drug development that affected SRI. First, in 1977 the NCI stopped awarding contracts for drug synthesis. After some 20 years of sponsorship by the CCNSC, SRI's efforts in cancer drugs now rested not on an Institute relationship with a major client but on the much smaller grant petitions from individual researchers. Second, SRI changed its policy on owning and holding patents.[8] As a result, SRI came to have good patent coverage on these and related drug candidates. A license for the CMDR was awarded to a drug company, but despite widespread interest and attention at the preclinical level, neither the 5-imino nor the CMDR was entered into clinical trial. This failure is not uncommon in drug development for a variety of reasons, but it does illustrate the need of an independent research house like SRI to have drug development partners. No matter the merit of the SRI work and the promise of the drug, a hand-off in the development cycle must occur. Because of the high cost of proceeding and the in-house competition from potential drug-development partners, the transition is difficult.

Antifolates

A revived attention given to antifolates also produced important results at SRI. As just mentioned, most drug development efforts require both a chemist and a clinician. In this case, the medical partner was Sloan Kettering in New York City. The principal SRI organic chemist was Dr. Joe DeGraw, working with colleague Dr. William Colwell. DeGraw and Colwell were familiar with the folate process and its inhibitors. In particular, they focused on an enzyme that helped stage the acquisition of carbon by folic acid. That enzyme, called a reductase, was a target for chemists as early as 1950. The existing inhibitor for that enzyme, called methotrexate, was effective only in limited forms of leukemia or lymphomas. It also had serious side effects, particularly on fetuses, and was essentially ineffective against solid tumors. Therefore, SRI undertook a search for related forms that would gain a greater concentration in tumor cells than in normal ones.

The first major discovery in this search was a modification of methotrexate that would still be a powerful enzyme inhibitor but would be more easily absorbed through the walls of tumor cells than through normal ones. Two forms of the drug were synthesized: one called 10-deazaaminopterin (or 10-DA) and one called 10-ethyl-10-deazaaminopterin (10-ET). SRI developed an efficient synthesis method that could produce enough of these two drugs for clinical trials, and by early 1985 Sloan Kettering began Phase I testing.

[8] This change in policy is keyed to the 1984 passing of the Bayh-Dole act that allowed nonprofit organizations to take ownership of intellectual property developed under Government sponsorship. See Appendix B.

As a result of the trials, 10-ET was shown to be more effective that either 10-DA or methotrexate and its side effects were less pronounced. 10-ET was then selected for Phase II trials both alone and in conjunction with existing drugs. The results were excellent in breast and lung cancer. Early reports from Sloan-Kettering stated that its use in a new chemotherapy regimen yielded 59% tumor regression and major relief of symptoms for lung cancer. It was also "far more effective" in the most common form of unresponsive lung cancer, non-small-cell carcinoma.[N]

With that kind of encouragement, the drug was licensed to Ciba-Geigy for Phase III clinical trials. Here the testing leaves the high scrutiny and familiarity with which the creators administer the drug and enters the real world where new risks are faced. Is the drug reliably taken and in the correct amounts? Do the side effects alter the physician's approach to the protocol? Although the drug, now called edatrexate, passed Phase III trials, there arose enough toxicity issues (primarily mucositis[9]) that physician compliance with dosage stipulations could not be guaranteed.[10] Such real-world conditions are important and legitimate experiences for the Phase III trials, but they represent one of the many potholes along the road to commercial use. There are others.

In the mid-1980s, when Ciba-Geigy took edatrexate into the regulatory process, getting a new drug approved by the FDA was very difficult. Clinical trials had to prove not just that the drug was effective but that it was clearly more effective than, in this case, methotrexate, already in use. That was harder to prove significantly. Because of this hurdle, the Phase III results described above, and some internal difficulties at Ciba-Geigy, such as being bought out by Roche, Ciba-Geigy gave the rights to edatrexate back to SRI and its co-owner, Sloan Kettering. Edatrexate was then licensed to a small start-up company in California, SciClone Pharmaceuticals, which, after three years, also failed to commercialize the drug.

So, in spite of its efficacy in early testing at Sloan Kettering and some successes in its Phase III trials,[11] edatrexate is no longer on the path for production. Interestingly, Ciba-Geigy produced so much of the drug it is still being studied and applied within the medical research community. Although its use is still being explored, its patent has only a year or so to run and edatrexate's future, at least for SRI, is about over.

But the last word on SRI's work on antifolates has not been written. SRI is still pursuing an analog of edatrexate for lung cancer that SRI was the first to synthesize. This analog is called 10-propargyl-10-deazaaminopterin, or PDX, and it is working its way, with partnerships, through clinical trials.[12]

Radiosensitizers

Most cancer patients who have localized tumors receive radiation therapy either by itself or in combination with other treatments. The ability of such radiation to kill cancer cells is in part determined by the amount of oxygen the cells contain. Cancer cells need some amount of oxygen to live, but there is a class of such cells that are oxygen-poor, or hypoxic. Simply put, hypoxia develops when the oxygen demand of a growing tumor exceeds its oxygen supply (see Figure 10-7). Hypoxic cells are less sensitive to radiation and can enter a resting state from which they can later multiply. There is evidence that significant portions of tumor cells are hypoxic, and the types of drugs that can help attack them are called radiosensitizers. Other types of drugs, which attack hypoxic cells directly, have the logical and rhythmic name of hypoxic cytotoxins.

To be successful in killing a cancer cell, radiation must ultimately modify, either directly or indirectly, the DNA of the cell,

[9] Inflammation of the lining of the gastrointestinal tract.

[10] This kind of outcome was at odds with earlier experience according to the inventor Joe DeGraw. When combined with mitomycin and vinblastine (together called EMV), the side effects were so small that it could be administered on an outpatient basis and quadrupled life expectancy. (Ref. *SRI Journal*, 10(1), 1990)

[11] According to medical science bulletins (http://pharminfo.com/pubs/msb/edatrexate.html and htm), edatrexate has been successful in treating non-small-cell lung cancer, mesothelioma (a cancer of the lining of the lung and abdomen generally from asbestos exposure), metastatic breast cancer, colon cancer, non-Hodgkin's lymphoma, and other forms of cancer.

[12] It is called PDX and it is a propargyl derivative of edatrexate, targeted specifically for smoking-induced lung cancer. SRI, with collaborators Sloan-Kettering and Southern Research Institute, has licensed PDX to Allos Therapeutics and it is now in Phase II trials by Sloan-Kettering (personal communication from Mas Tanabe and from the Sloan-Kettering web site, January 17, 2003).

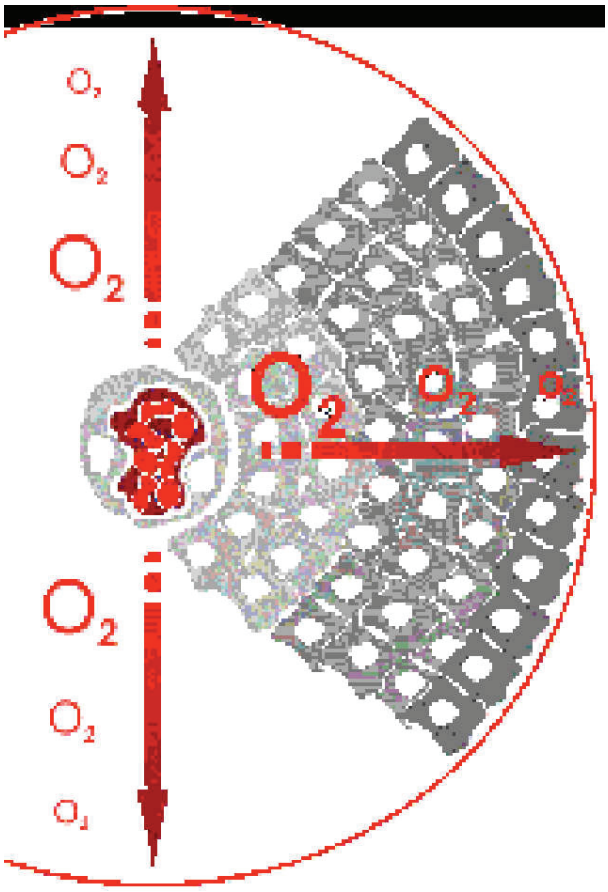

Figure 10-7. Diagram showing how oxygen consumption near a feeding blood vessel (center blotch) creates oxygen gradients within a few cell diameters. (Thus, unlike normal tissues, tumors contain zones of oxygenated, hypoxic, and dying/necrotic cells.)

making it nonreplicating. This means adding lesions to or, preferably, breaking either one or both DNA strands. The process is generally one of oxidizing DNA. Since normal cells are rich in oxygen, the question becomes one of how to raise the oxidizing vulnerability of those cells with little oxygen while avoiding a compounding effect on normal cells.[13]

In the late 1970s, SRI began exploring the field of radiosensitizing agents in collaboration with the Stanford Medical Center and under the sponsorship of the National Cancer Institute. The analytical and synthesizing work for the search was done under the leadership of SRI biochemist Dr. William W. Lee.[14] He knew that, as with most all anticancer drugs, a successful radiosensitizer must

- Selectively sensitize hypoxic cells and have insignificant effects on normal cells
- Be nontoxic at clinically acceptable dose levels
- Diffuse to and through the tumor tissues efficiently
- Be metabolized slowly enough that radiosensitizing concentrations can be achieved
- Be effective against hypoxic cells at all stages of the cell cycle.

So the search began for a compound that would mimic the effects of oxygen. The two major ways oxygen interacts chemically are through free radicals and oxidation. Compounds having these properties had been under investigation for some 20 years. Free radicals had the problem of instability and being so reactive that they might not have time to migrate to the target site. So the SRI search first concentrated on agents that had oxidation-like potential, that is, those that had an affinity for electrons.

One problem presented by the radiosensitizing agents already in existence was that they dangerously attacked the nervous system. That toxicity posed another design constraint to SRI chemist Lee. Here Lee and Stanford colleague Martin Brown reasoned that a nitro group in the structure of these drugs might be the cause. Eliminating that group, together with eliminating the entire drug as rapidly as possible following radiation, should reduce the toxicity. Following this reasoning, Lee synthesized one of SRI's early offerings showing reduced neurotoxicity. It was called etanidazole. In mice, it showed three times the radiosensitizing effect of existing drugs at doses inducing no observable nerve damage.

[13] While one might suggest simply adding oxygen to the blood before irradiation, the vulnerability of hypoxic cells is more complex than such a simple notion implies. There are both chronic (long lasting) and acute (temporary) cellular hypoxia. Blood oxygenation addresses only the chronic type. (See Footnote 14.)

[14] The principal investigator at Stanford was biologist Dr. J. Martin Brown. Brown won the 1999 Cain Award of the American Association for Cancer Research for his work on hypotoxicity, including radiosensitizers. In his acceptance speech, he showed a picture of SRI's Bill Lee and acknowledged that he owed his award to Lee's drug synthesis work. (J. Martin Brown, The Hypoxic Cell: A Target for Selective Cancer Therapy, Eighteenth Bruce F. Cain Memorial Award Lecture, *Cancer Research*, 59, 5863-5870, December 1, 1999.)

But as etanidazole was introduced into some Phase III trials, it surprisingly failed to show a significant overall benefit. The answer lay, apparently, in the increasingly complex picture that hypoxia was presenting. Etanidazole, as it turned out, was effective mainly against the most oxygen-starved cells and was less effective against those with oxygen in intermediate amounts. Moreover, the dosage of this radiosensitizer that would make the cell vulnerable increased exponentially with the amount of cellular oxygen present. On the surface this sounds good because a dose adequate to kill hypoxic cells might leave the more normal, oxygen-rich cells unaffected. Alas, tolerable dosages left too many intermediate level cells untouched.[15] Could the team find a drug that could kill hypoxic cells directly so that, when used in combination with radiation therapy, the overall result would have greater efficacy than either used alone?

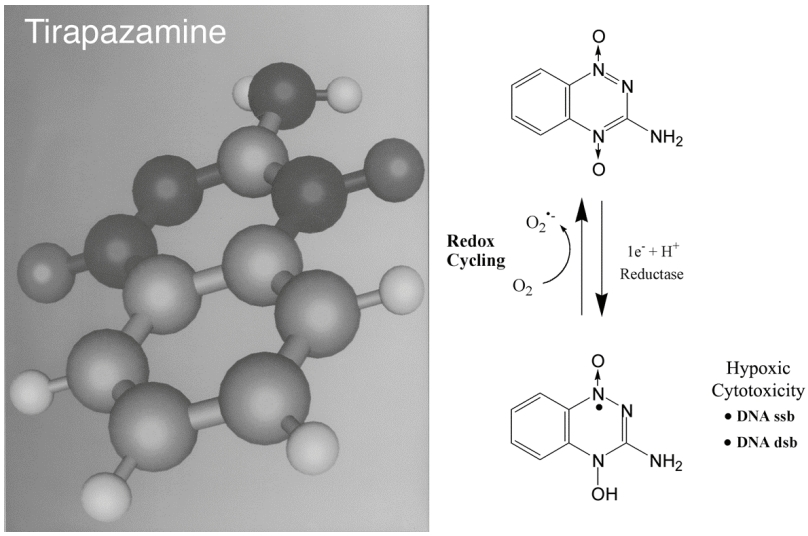

Figure 10-8. The molecular model of tirapazamine together with the hypoxic versus aerobic reaction it produces.

In the mid-1980s Lee and Brown were still searching for better radiosensitizers that didn't cause nerve damage. With some serendipity, when testing one class of their trial compounds, they noticed that the compound's lethality to the hypoxic cells was about the same whether they were irradiated or not![16] This class not only didn't damage nerve cells but, using much lower concentrations, it killed as many hypoxic cells as the best radiosensitizers. More good news: their differential toxicity to hypoxic versus non-hypoxic cells was greater than any other known drug.

They called the lead compound TPZ or tirapazamine, and in rough terms its mechanism is as follows. In the intercellular structure, the drug undergoes a reaction that transforms it into a highly reactive radical. In the absence of oxygen, this negatively charged radical is capable of drawing a hydrogen atom from a nucleotide segment of one or both strands of a DNA molecule, thus killing it. However, when oxygen is present, that radical either never forms or is quickly neutralized, effectively returning the drug to its parent form. That exchange is shown in Figure 10-8. Tirapazamine was thus attractive enough to enter into clinical trials.

Known as SR 4233, tirapazamine's record in clinical trials has been good. Phase I studies at Stanford, Harvard, and Glasgow Universities set the acceptable dosage level on the basis of side-effects. Phase II trials showed the drug to be effective against a wide range of cancers, and it is now in Phase III trials, particularly exploring its use in combination with other drugs for non-small-cell lung cancer (NSCLC). This is an important area because lung cancer is by far the leading cause of cancer mortality in the United States and 75% - 80% of lung cancer is NSCLC. During these trials, it was noted that tirapazamine was useful not only in conjunction with radiation therapy but also as an adjunct in chemotherapy. Tirapazamine has also entered Phase I trials at Stanford using oral delivery.

But, what of the drug's commercial side? In spite of its apparent potential, the licensing of tirapazamine has been checkered at best. In the beginning, SRI and Stanford became interested in a newly developing arm of Eastman Kodak as it decided to enter the pharmaceutical business. However, Kodak later abandoned that initiative and sold the new division to a somewhat old and staid drug company, Sterling Winthrop, which would later be bought out by the French pharmaceutical company, Sanofi (now Sanofi Winthrop Pharmaceuticals in the United States). Each of these corporate shuffles required a reexamination of the kind of drug business SRI was in and which of those companies were

[15] Ibid.
[16] These compounds were benzotriazine di-N-oxides.

to be SRI's heroes. The fate of tirapazamine is still before us.

An Hypoxia Probe

One of the limitations on the use of radiosensitizers, of course, is knowing whether hypoxic regions of a solid tumor exist and, if so, exactly where they are located. The nature of those regions and their vascular characteristics are described in the next section, but it is enough to say that hypoxia arises because of the disorganized nature of blood delivery systems in rapidly growing tumors. The need to identify such locations and the need to monitor the properties of a growing tumor motivated development of a drug now known as SR 4554, which is now in the first phase of clinical trials. Its role is that of a diagnostic probe rather than a therapeutic agent.

The roots of SR 4554 are a bit unusual.[O] The work on radiosensitizers described above was, as Lee approached retirement, being continued by Dr. Mike Tracy. As a consequence, Tracy was continuing to ponder that area of drug development as he attended a meeting at the University of Oxford in December of 1987. On that occasion he renewed an acquaintance with a long-time colleague, Dr. Paul Workman of the University of Glasgow. The venue was a local pub and the conversation centered on the nature of a molecule that would help identify the presence of hypoxia in solid tumors. The molecule should be as benign as possible and cause high illumination under magnetic resonance examinations. As Workman rattled off the molecule's needed characteristics, Tracy was jotting on a napkin how the molecule could be realized. Tracy returned to SRI, and within about a month, including the holidays, he had made the first synthesis of the molecule.[P]

The resulting drug is known as a fluorinated 2-nitroimidazole and it began with three design goals. It was to have

- A nitro group that would have an oxygen reduction potential to act selectively on just hypoxic tumor cells
- An affinity for water and hydrogen bonding that would limit its penetration to nerve tissue, thus giving it low neurotoxicity
- Fluorine components that would make whatever they were bound to more visible under magnetic resonance imaging and spectroscopy.

These design goals were met *in vitro* with hypoxic tumor cells showing a greater affinity for the drug than, say, normal brain or other cells. Its working levels were low enough that it was also nontoxic. Preclinical trials were slowed because of its nontherapeutic nature, but over almost a decade it proceeded toward clinical trials.

All syntheses of the drug have taken place at SRI, including those necessary for clinical trials. Phase I trials began in late 2000 and are still under way in England. These are sponsored by what was called the Cancer Research Campaign, now known simply as Cancer Research U.K. Again, because the probe is diagnostic and does not offer immediate relief to a tumor patient, its somewhat limited volunteers are acting solely for the advancement of science. The Phase I tests are continuing.

Fighting Blood Vessel Growth in Tumors

There is one more vulnerability in a tumor's blood supply that SRI is addressing. As mentioned, for a tumor to keep growing, it must develop and maintain an adequate blood supply. Interestingly, tumors begin this process while still 1-2 mm in size and do so by inducing new and sometimes rapid blood vessel growth; a process called angiogenesis. Biochemically, tumors may induce angiogenesis by tripping the "angiogenic switch," which is a dynamic balance of angiogenic inducers and angiogenic inhibitors, to favor the effect of the inducers.

These growing tumor vessels are an attractive target for anticancer therapy not only because they are accessible but because they are composed of normal cells, called endothelial cells, which make up the blood vessels in all tissues. Unlike cancer cells, endothelial cells are genetically stable and therefore unlikely to develop resistance to anticancer drugs, currently a major problem in the treatment of tumors by chemotherapy. The tumor blood vessels are also abnormal in ways beyond the oxygen starvation or hypoxia mentioned earlier. Because of the excessive demand for blood, tumor-feeding blood vessels also have important structural and functional differences, the former of which is illustrated in Figure 10-9. Functional differences offer another avenue of attack.

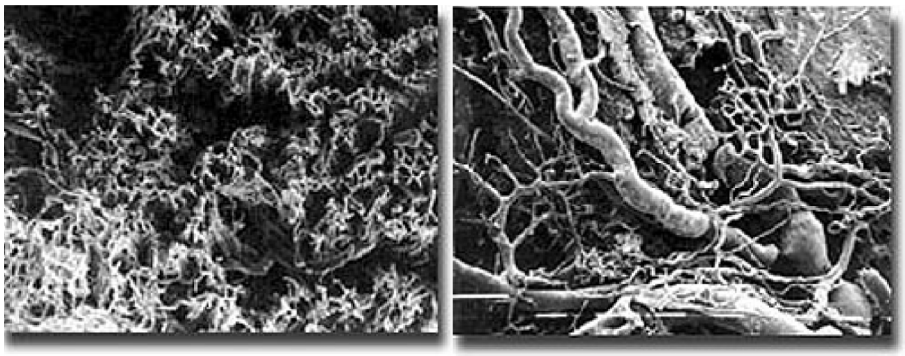

Figure 10-9. Normal and cancer-induced blood vessels. An image of normal blood vessels in mouse muscle is shown on the left while the one on the right shows the aberrant blood vessels in a mouse tumor. Notice the tumor vessels have chaotic structures in both size and placement and there are areas with no blood supply at all.

Note that this tendency for blood vessel growth is not common in adult humans, occurring only in situations like menstruation, pregnancy, and wound healing. So, assuming some stability in the life of normal human blood vessels, drugs selective to angiogenesis will target mainly those vessels contributing to cancer growth. Scientists at SRI investigating this new area in cancer therapy have come up with several trial drugs, but none more important that those steroidal ones dealing with breast cancer, as discussed below.

Steroid Hormones and Breast Cancer

In the world's war on cancer there are many fronts, but few get more attention than the hormone-sensitive cancers of the breast and prostate. Important in combating these cancers are steroid hormones, compounds discovered in the 1920s that help define us as male or female. These steroids have been the center of work for SRI's Dr. Mas Tanabe and his colleagues for over 40 years (see Figure 10-10).[17] They consist of such well-known drugs as cortisone, estrogen, and testosterone plus those from the oral contraceptive field. While not extensive, cortisone work at SRI has explored its impact on such diverse topics as mitigating stress in Air Force pilots and the treatment of rheumatoid arthritis.

In the mid-1960s, however, funding for such work on steroid hormones began to dry up, at least for the treatment of cancer. This was largely because their broad activity produced too many side effects. In contrast, however, and as an indication of the exceptional competence at SRI, the NIH and drug companies like Schering Plough and Taiho have sponsored work on steroid hormones here continuously for over 30 years. The contract with Schering Plough began in July 1957 and was still going strong in 1983! By that point, SRI had granted Schering eight patents and more than 30 journal articles in the area of new anti-inflammatory steroids.[Q] Now a resurgence of interest has occurred because of their ability to be selective and to activate or repress genes.

Although the field of steroid hormones has again become "hot," there is a shortage of the chemists needed to build the compounds for the biologists to test. The big goal at the moment and a focus of the SRI work is to try to build modifications of the natural hormones that are very tissue-selective. To illustrate one

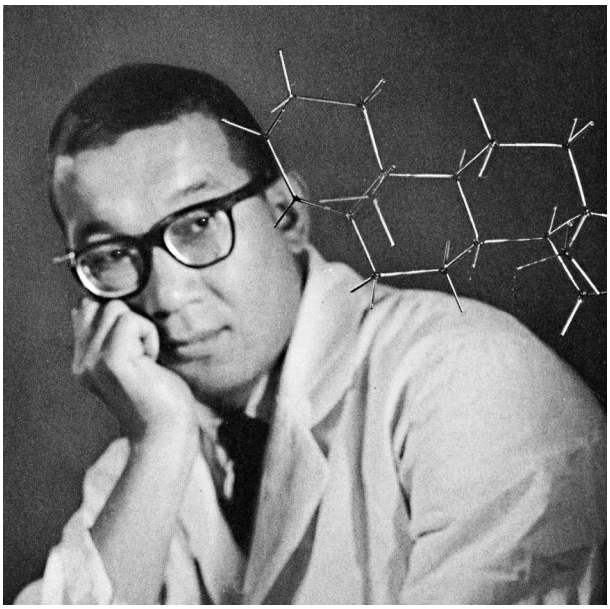

Figure 10-10. Dr. Masato Tanabe about 1960.

[17] Dr. Tanabe is, in the opinion of many of his SRI associates, one of the most dedicated and brilliant chemists to have worked at SRI. Stories are told about his not only dragging technical papers on what few vacations he took, but actually studying them. His probing mind never seems at rest. In the days when traveling to conferences meant sharing hotel rooms with colleagues, one roommate remembered being awakened in the night when Tanabe rose up in bed and shouted, "But we should try the acetate!"

CHRONOLOGY OF TWO SRI ANTI-ESTROGEN DRUGS	
July 1996	Synthesis search began
December 1998	Discovery phase ended for SR 16234 and 16287
April 1999	Preclinical testing of absorption, toxicity, etc.
December 1999	Synthesized 1.4 kg in tablet form Began investigational new drug (IND) testing
August 2000	IND application submitted to Food and Drug Administration
September 2000	Phase I trials began
March 2002	Phase I trials ended

avenue, consider the currently popular drug for breast cancer treatment—tamoxifen.

The natural hormone estrogen is required for the growth of cancer cells in about two-thirds of breast cancer cases. Tamoxifen is a purely synthetic compound that acts like natural estrogen in that it is accepted by the estrogen receptors of breast cancer cells but, once attached, normally does not permit the cell to replicate. Hence, tamoxifen is called an anti-estrogen. At the moment, tamoxifen is the most widely used drug for breast cancer therapy, but it is not perfect because it does have some estrogen activity. That is, it appears to some tumor cells as natural estrogen and thus enables their growth. Approximately half of the patients using tamoxifen will not be helped. So tamoxifen resistance has become an unmet medical need. In addition, tamoxifen increases the risk for endometrial (uterus-lining) cancer.

The goal for SRI was to create a very tissue-selective estrogen that would act like an anti-estrogen in the breast and uterus but would appear as normal estrogen to bones and other responsive tissue such as cardiovascular tissue. The possibility of this duality first arose from a lead compound found under an SRI contract from NIH.[R] That idea, then, was proposed to Japan's largest anticancer pharmaceutical house, Taiho Pharmaceuticals, in July 1996. Taiho's acceptance of the proposal began a six-year attempt to find, develop, and test such a compound. The above table shows the incredibly short time frame over which the development took place.

The SRI team was able to design and synthesize these drugs so quickly because of their many years of work dedicated to steroids. In a comparatively short time and a discovery phase cost of less than $3 million, SRI invented two new anti-estrogen agents that are much more tissue-specific than tamoxifen. Their code names are SR16234 and SR16287 and they were to be directed at those patients who had experienced tamoxifen failure. In an important step, SRI also designed the compounds so that the drug could be taken orally, thus substantially lowering the cost of administration.

Amazingly, the SRI drugs had other important advantages beyond just estrogen-blocking. The two SRI drugs are members of a general class of compounds capable of tissue differentiation called selective estrogen receptor modulators or SERMs. Tamoxifen is a member of that family but has the above-mentioned limitations. During the discovery phase of SR 16234, chemist Richard Peters and biochemist Wan-Ru Chao discovered that the "234" compound not only inhibited blood vessel growth (angiogenesis), it accelerated the programmed death of cancer cells (apoptosis). As mentioned earlier, cancer tumors need blood vessel growth to deliver nutrients, but because the human body grows blood vessels only during pregnancy, menstruation, and wound healing, inhibiting angiogenesis outside those times might make it an effective anticancer mechanism, as in postmenopausal women. So, SR 16234 has several ways to fight cancer and its trials are encouraging.

As the SRI team continued the refinement of SR 16234, the drug showed six important benefits. It:

- Inhibited tumor cell growth through estrogen-blocking (SERM)
- Inhibited blood vessel growth (anti-angiogenesis)
- Somewhat accelerated cancer cell death (apoptosis)
- Is safe for estrogen-responsive, normal tissue
- Showed no toxicity for other normal cells
- Could be administered orally.

The combination of SERM and anti-angiogenesis means that the drug can potentially be used for breast cancer, even after metastasis. SRI also pursued and secured its intellectual property on SR 16234, obtaining a worldwide patent position including a general formulation process for the drugs.

With this attractive outlook, Taiho Pharmaceuticals bought a worldwide license to the two compounds in the spring of 1999, targeting them as replacements for tamoxifen. Taiho also agreed to conduct preclinical testing at SRI, and with favorable outcome, carry it through Phase I trials. Those trials have now been successfully completed. [18] They will also continue to explore acceptable manufacturing costs as the testing explores its value against tamoxifen-resistant strains of cancer.

SRI researchers believe that their new agent may even have a potential for *preventing* breast cancer and a useful role in hormone-replacement therapy, where estrogen has proved to have significant risk. Many researchers were involved with Tanabe in this work[19] and, as of this writing, SR 16234 is about to enter Phase II clinical trials. Because of excellent Phase I results, Taiho will continue to carry the large financial burden of the Phase II clinical trials.

Other Tanabe contributions also deserve mention. The first is his pioneering use of stable isotopes to do fundamental studies of metabolism. More specifically, he showed how to insert the traceable isotope C_{13} into antibiotics to show how a microbe could actually assemble a particular antibiotic to defend itself. Before this technique was developed, people had to break down the antibiotic and guess how it may have been constructed. Tanabe tagged small, elementary, one- and two-carbon chains and placed them in the presence of the microbe. He then took the resulting antibiotic and analyzed it using magnetic resonance spectroscopy to reveal the presence of the imposed C_{13} and thus the manner by which antibiotic material was formed.

On March 27, 2001, Tanabe was awarded the Japanese Pharmaceutical Society's Distinguished Service Award for a long history of helping Japanese academic scientists and companies in the chemical and pharmaceutical fields. In particular, the award recognized his dedication to the scientific exchange represented by the 45 scientists who have come to SRI to study under him. He is the first person outside of Japan to win this honor.

[18] Richard Peters mentioned that in one situation with 16 nearly terminal patients, 40% were stabilized. Metabolic absorption has been very good, particularly when taken after food, and from CancerEDGE.com comes a report that SR 16234 is doing well against similar anti-estrogen drugs such as tamoxifen, raloxifene, and Faslodex (March 20, 2002).

[19] Besides Richard Peters and Wan-Ru Chao are Andrew Kelson, Ling Jong, Robin Van Lengen, John Johansson, Cris Olsen, Jyanwei Liu, and Barbara Sato. Chao and Sato are biochemists while the rest of the team are organic chemists.

Chemical Hazards in Our Environment

Pollution, Pesticides, and Pests[5]

It was exactly on the third anniversary of SRI's very first project when in December of 1949 SRI agreed to help Alcoa assess the biological effects of an effluent from its fertilizer plants. The effluent, airborne fluorides, was affecting the plants and animals downwind of its reduction factories in several western states. The chemical was part of the phosphate ore rather than one introduced into the reduction cycle. Alcoa went outside its corporation for the study not only because Alcoa didn't have the capability internally, but because doing so no doubt gave the work some useful objectivity. The specific quest was to assess the effects of fluoride on cattle, and to Alcoa's credit, the work was to be done openly and the results published in the open literature.

So, SRI's 259th project started what would become a six-year investigation into the measured effects of fluoride on milk production as well as the teeth and bones of cows. The most obvious effects from the ingestion of plants laden with the chemical were the softening of the cows' teeth and some changes in the structure of their leg bones, making it difficult for the cows to walk. These effects were known in general terms, but the important question was how much fluoride it took to cause noticeable damage.

To help measure and evaluate the effects on individual cows, SRI enlisted a veterinarian from Modesto, CA, and the cooperative use of his dairy farm. By measuring the daily amounts of sodium fluoride fed to the cattle, photographing the incisor teeth of each animal at regular intervals throughout the multi-year experiment, recording daily milk production and documenting the condition of the autopsied cattle that had undergone various levels of exposure, SRI was able to quantify the levels of impact. These were used to resolve several litigations and settlements concerned with safe ingestion levels of fluorides. Later, scientists from SRI's Los Angeles Laboratory also investigated for Alcoa the damage of fluorides on fruits and vegetables. The main effect in this case was a pockmarked appearance on the skin that could ruin the marketability of the product.

In a related vein, SRI undertook work for Shell Development as Shell decided to go into the agricultural product line of insecticides and pesticides. Beginning in 1953 and continuing for about 20 years, this work lasted long enough to look into the safety and toxicity of Shell's agricultural products on certain animals and their offspring.

The same expertise in insecticides enabled SRI to evaluate the efficacy and safety of flea collars. Shell was a pioneer in this area. SRI was able to show that when flea collars were applied too tightly, skin lesions occurred, in either dogs or cats, but with a looser application, the collar was effective in killing fleas and yet not harm the pet.

In another pet-related project for the California Fish Canners Association, SRI identified the reason red meat tuna was causing a disease called steatitis (yellow fat disease) in cats. Red meat tuna had little vitamin E and because of lax processing conditions, the unsaturated fats were allowed to oxidize, thus producing a toxic product. Thus, there was a need to keep the tuna's fatty acids from oxidizing. Vitamin E had been added by the processors to improve the health of the cats and to preserve the tuna. However, the form of vitamin E they had chosen simply wasn't biologically available to the animals. So a change in the chemical structure of the antioxidant, vitamin E, and improvement in the sanitation conditions during processing, cured the problem. Beyond pets, the effect of toxins on fish, upland game birds, and other animals were also examined for several clients. As we will now see, humans can also be victims of airborne toxins.

Animal Models in the Pursuit of Preventing Chronic Lung Disease

Today's attention given to the discharge of chemicals into our atmosphere obviously came from our heightened awareness of their effects on humans and other living things. In the 1940s and 1950s, pollutants had some fairly direct and lethal effects in the "killer fogs" of London and similar but more modest effects in the eastern United States. The effects of less severe concentrations, however, weren't so measurable or clear. Because many of the effects of such exposure in humans do not manifest

themselves until middle age or beyond, our awareness would have come much slower if we had relied only on the measurable air pollution for its ultimate effects on humans.

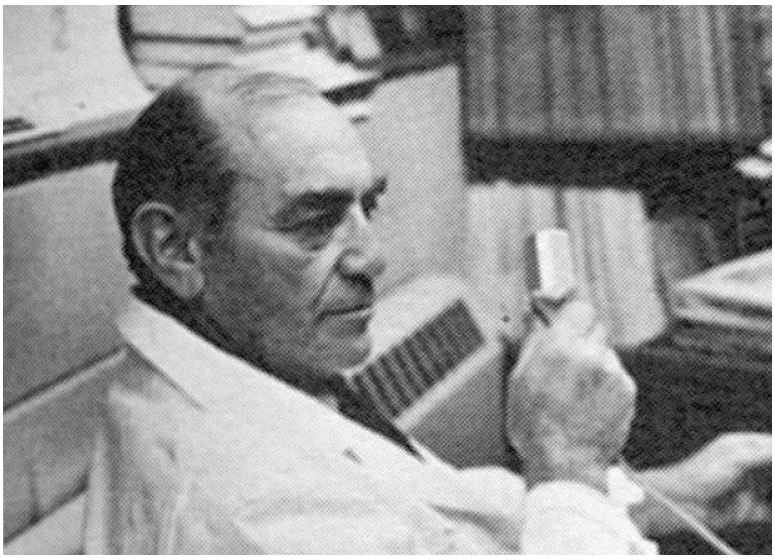

Figure 10-11. Respiratory disease researcher, Gustave Freeman, M.D.

In the early 1960s, SRI, in the person of Dr. Gus Freeman, began an inquiry into the environmental causes of two respiratory diseases. One is termed chronic obstructive pulmonary disease (COPD) and the other is emphysema. COPD is a loss of elasticity in the air sacs (alveoli) of the lungs, whereas emphysema is the loss of their surface area. Both are overwhelmingly associated with smoking but also have ties to the quality of urban air. The number of cases of emphysema is growing; it currently affects about 2 million Americans.

One of the first invitations for SRI to investigate what was becoming an increasing health problem came from the California Department of Health. Recognizing the growing problem in the Los Angeles Basin, they sought some insight into the causative mechanisms. The representative calling on SRI claimed the only person who could effectively examine the toxic effect of the oxides of nitrogen was SRI's Freeman, who had looked into the subject while in the Army and who had just come to SRI from the NIH's National Cancer Institute by way of Cal Tech (see Figure 10-11).[T] Freeman saw the problem more as a regulatory issue, but his interest in the cause and pathogenesis of respiratory diseases persuaded him to undertake the study. Because of its general importance, the work also came to be supported over the 1960s and 1970s by NIH's National Institute of Environmental Health Sciences and the Environmental Protection Agency.

Because of the slow development of emphysema in humans and particularly the difficulty in obtaining human tissue during its early stages, it became necessary to look for an analog in animals. Earlier attempts at this approach had failed, mainly because of a lack of adequate consideration of the importance of the slow onset of the disease. Recognizing this, Freeman's SRI team worked for over 10 years to develop a representative model in rats. The approach came to consist of their exposure to nitrogen dioxide, an important constituent in tobacco smoke and present in automobile exhaust and other urban smog pollutants including power plants. Various levels of nitrogen oxide (NO_2) and ozone (O_3) were introduced.

Some lower levels produced no clinically observed discomfort in rats but, over a period of months, did cause morphological changes to their lungs. Such changes were observed to depend on the concentration and the duration of exposure. Consistent exposure levels of 10-15 parts per million over several months were enough to cause life-threatening COPD. Because it was important to watch the development of COPD over the *normal* life span of the rat (2 to 3 years), exposures were also made intermittently and at varying concentrations. "Smog" level concentrations of 0.8-2.0 ppm, offered consistently, showed cellular changes characteristic of the more heavily exposed rats but still permitting a more normal life span, perhaps suffering a reduced lung capacity.

Using this model in rats, SRI was able to show that there were important thresholds in the levels of NO_2 and O_3 and its consequential effects. Levels in the vicinity of 1-2 ppm caused cellular damage, some of which could be recovered from, and were not life threatening. Levels 10-15 ppm, however, were fatal under long exposure and caused permanent damage at lesser exposure.

To better approximate the condition in humans, Freeman began what turned out to be an 11-year study of the effects on primates.

Exposure during these tests was a nearly constant levels of 9 ppm of NO_2 and 4 ppm of ozone. The results were evaluated with the help of the University of California, Davis, School of Veterinary Medicine. Ozone proved to be over ten times as toxic to the rat population as nitrogen oxide. Emphysema was present in 1 in 6 of the primates. While admittedly a simplistic model, these thresholds of NO_2 became an important guide to regulatory groups interested in the health aspects of air pollution. The results of the NO_2 work were presented at conferences of the World Health Organization in 1977-1978. The EPA limit on the amount of allowed NO_2 is in continuing flux but is now less that 0.1 ppm.

So, for over 15 years Freeman and his colleagues such as Glen Haydon, Bob Stephens, and Mike Evans revealed the toxic effects of airborne oxidants on the bronchial lining and the alveolary surfaces of the lungs. They also examined the ability of the body to heal itself from such damage. Freeman's passion was understanding enough of the pathogenesis of the respiratory system so that meaningful preventive measures could be taken. All this helped set the limits on the environmental concentrations of the atmospheric pollutants, the nitrogen oxides, and ozone.[U]

Comparative Metabolism and the Role of Human Tissue

Over the last 40 or 50 years, the number of chemicals in the world has increased dramatically. Some find use in the industrial world, some in farming and other food production, and many in new forms of drugs for curing what ails us. Surprisingly, the consequences on humankind for many of these chemicals are still largely unknown. Whether a new chemical or drug is hostile to our health should be a question answered *before* it goes to production, both to protect us and to avoid the high capital investments needed for its production or remediation.

Although there is little such screening of industrial chemicals until after there is a perceived problem, drugs are directed, under government regulation, through well-defined procedures from which both their danger and their efficacy are measured. However, these trials are expensive and in some cases risky to the human subjects participating. Needed is a way to determine, in a very complete sense, just how dangerous a specific drug is. Can its side effects be gauged well enough to decide whether its harmful effects outweigh its potential benefit *before* it is even taken to Phase I trials where its incidental risks are evaluated? Is there a way to predict the effects of potentially toxic substances on humans before some subpopulation actually has to experience their risk?

In 1977 Dr. Charlie Tyson, a chemist working at the University of Wisconsin, came to SRI and began looking into the promotion of some projects that interested him (see Figure 10-12). A try at one with a colleague at UCLA didn't pan out. Then Bill Skinner, Tyson's boss, approached him with some overhead funds and, in one of those seemingly prescient moments, asked him to look into new assay methods in toxicology. Tyson, who had some background in toxicology, soon recognized that, since the point of such evaluative experiments was to determine the effect on humans, why not expose human tissue rather than animal tissue to a toxin of interest. In particular, since many foreign things we ingest encounter first the liver, its tissue would be a good starting point for the detection of toxicity. This concept led to SRI's liver cell toxicology assay system.

Figure 10-12. Sr. scientist Dr. Charles Tyson and toxicologist Dr. Carol Green.

Because the concept rested on the presence of living human liver cells *in vitro*, building the liver cell assay system first meant finding enough post-mortem human donors and then determining how to separate their liver tissue into enough functional cells, called hepatocytes,[20] to reliably measure effects. This separation process is called isolation. Tyson examined several isolation methods around the world and concluded that one developed at the University of California, Davis, was the best. It had high tissue yield; that is, a small number of cells would retain all the properties of the liver for that cell type. Gaining access to that process was very important to the SRI work, but one of its best consequences was that UC Davis graduate student, Dr. Carol Green, would come to SRI as a postdoctoral associate (see Figure 10-12).

Figure 10-13. SRI's liver cell toxicology facility. Though not easily recognized, they are, from left to right, Kathy Allen, Toyomitsu Sato, Jack Dabbs, and Carol Green.

The liver cell facility was completed in 1983, making it the first such facility in the world operated as a normal service (see Figure 10-13). Now the real purpose of Tyson's idea could be tested: Would the exposure of human hepatocytes *in vitro* to a specific exogenous agent adequately predict the effect of that same agent to human exposure; that is, *in vivo*?

This approach would require analysis of the *in vitro* data and construction of models to predict *in vivo* response. This had not been done before. The model building would also benefit from those special cases where considerable human testing had already been done, as in drug trials already completed. The team included Tyson, Green, Sue LeValley, Jack Dabbs, Shirley Gee, and Katherine Hawk-Prather from the Toxicology Laboratory and other SRI people including Ron Spanggord, Bob Stephens, and Dave Thomas. The SRI team began a variety of experiments and together wrote a series of papers that put all these new ideas on firmer ground. This new approach brought SRI a reputation that has continued to the present as one of the foremost laboratories in the development of *in-vitro* human and animal tissue model systems. From its beginnings in the early 1980s, to the construction of the facility, to a number of supporting papers in the mid-1980s, SRI's work became widely known.

Beginning in 1983, certain progress was evident. The isolation techniques had been perfected from both animal and human sources. The resulting hepatocytes were characterized in terms of their metabolic capability and their response to both toxic and nontoxic agents. These results were then compared with known *in vivo* responses to the same agents. Agents included standard liver toxins such as carbon tetrachloride and some traditional antidotes for cyanide poisoning. But it was a 1986 paper in the *Journal of Pharmacology and Experimental Therapeutics* that gave the team and their approach wide validation and recognition.[V] The authors were able to show that the *in vitro* responses to the chemical amphetamine by tissue from the livers of rats, dogs, squirrel monkeys, and humans retained the same metabolic distinctions or differences that they displayed *in vivo*. This similarity extended to the production of certain

[20] Literally, liver cells.

metabolic products unique to the different species examined. Comparative metabolism across these species was possible; that is, the *in vitro* process was indeed a legitimate surrogate for human and animal responses.

These results, probably more than anything else, led to Green getting a contract from the National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK) for establishing a liver tissue bank. This was a service type of contract, but it led to the formation of the SRI liver tissue facility, which is still used in metabolism studies. In a related vein, Tyson has now been placed on the Board of Directors of the National Disease Research Interchange, a leading group that acquires human tissues from tissue banks and distributes them to where they are needed.

Another fairly obvious benefit of the use of human cells in testing is the decreased emphasis on animal testing. Almost from his original concept, Tyson recognized this advantage. Other work outside SRI had shown that, for many chemicals, you didn't need to use animals to gauge the potency needed to kill humans. Because the SRI work has been primarily in human tissues, Tyson was asked to co-chair a workshop in October 2000 on how to do away with animal tests for acute lethality. Other leadership roles in the alternatives to animal testing have followed.

Up until now, the *in vitro* systems and their models have been simple ones—simple in the method of isolation or the fact that only one cell type was involved. But the real world is much more complex and so, as most people would anticipate retirement, Tyson is beginning, as he says, the most exciting part of his career. To continue and improve on SRI's liver work, it is necessary to look at more than one of the 3 to 5 liver cell types. With some well-qualified colleagues in the field of human tissue toxicology, Tyson will be trying to move the field forward so that we can make reliable predictions about the effect of a drug on humans when no human has ever been in contact with it. Over the next few years, they will be looking beyond the liver to other organs, such as the heart, lungs, kidneys, and even the brain. They are already looking at bone marrow. There would be a different assay for each of these. Tyson says, "We may have to look in detail at how proteins, or parts of proteins, behave to understand and predict an outcome." (Tragically, Charlie Tyson died unexpectedly as this book was going to press.)

SRI's contribution to the ability to anticipate in the laboratory human responses to toxic agents has now taken the form of supplying the method as opposed to the testing itself. Through the literature, consultation, and the liver tissue bank, SRI has had global influence. Today, the FDA and the drug companies themselves are increasingly requiring this kind of testing against human tissue before drugs proceed very far in the development cycle. It is cost effective and safe. As a validation of its approach and reputation, SRI was recently awarded a $2 million NIH grant to further its work. The goal of this project will be to look for more quantitative, predictive models of the toxicologic impacts on human tissue.

Health Sciences

In addition to its contributions in medical technology and drug research, for many years SRI has also performed important work in selected areas of health science. The precursor of this work began in the early 1970s with a focus on the monitoring of the alcoholism programs of the NIH.[21] This work continued for years and involved the difficult subject of surveying that segment of the population affected by alcoholism in order to provide needed feedback on the efficacy of the sponsored activities. Thus, the SRI emphasis at that time was on health *services* and would remain so over the course of a decade.

Also during that time, separate areas arose within the group that began to examine the interplay of health and the environment. With that additional perspective, the group became known as the Behavioral Medicine Program, and it proceeded to gain a good reputation in the study of cardiovascular disease, including associated risk factors such as stress. But it was the extended experience with the monitoring of alcoholism that would eventually lead, around the early 1980s, toward trying to understand the effects and reasons for addiction to alcohol and tobacco. In each of these, the emphasis

[21] Specifically, the National Institute on Alcohol Abuse and Alcoholism.

Figure 10-14. Health researchers Drs. Dorit Carmelli and Gary Swan.

became more one of *science*, as opposed to service. In about 1986, under the leadership of Dr. Gary Swan (Figure 10-14), the program began a decade-long growth that resulted in its becoming laboratory size. With this new prominence, it became known as the SRI Center for Health Sciences, but two of its research emphases would remain the causes and treatments of our common addictions, such as alcohol and tobacco.

Another aspect of the Center's growth came in 1995 with the arrival of Dr. Adolf Pfefferbaum and his field of brain imaging. That work has led to examining the impacts of aging and other factors, such as alcoholism, on the brain, its composition, and its performance. By 2000, expertise in behavioral pharmacology was introduced; in 2001, the study of human sleep; and by 2002, molecular genetics. Throughout this steady expansion, the Center has maintained its high quality of work, as evidenced by its prolific publishing record. Over the past 3 years, working on individual grants and through collaboration with medical schools such as Stanford, UCLA, UCSF, UC Davis, Indiana University, Emory University, the University of Texas, and others, more than 200 published papers or symposium presentations have been produced by the researchers in the Center.

As another recognition and to aid in its important work on medical imaging, SRI has partnered with GE Medical Systems, which has elected to base the western part of its Advanced Systems Laboratory on the SRI campus. This facility comprises two research magnet systems and houses several of GE's prominent developers and researchers in the field who actively collaborate with SRI Drs. Pfefferbaum and Ian Colrain. The center has also established a state-of-the-art, four-bed human sleep facility. This is being used to support several NIH-funded projects on human sleep and daytime electrophysiology. A few examples will relate a sense of the work done in the Center and its value.[W]

Human Addiction—The Role of Nicotine

Though smoking has developed enough of a stigma in our society that laws restricting it in many public places have been passed, there are still approximately 50 million Americans, or one in five, who smoke. While a few special interests continue to minimize the overall impact of smoking, the social and healthcare costs of this addiction are, by all accounts, staggering. Smoking leads to approximately 415,000 premature deaths each year, and the consequences for U.S. society in medical costs and lost productivity total about $157 billion during 2004.[X] Worldwide, the problem is enormously greater, with about a billion smokers and approximately 3 million people per year dying of tobacco-related illness. It is the leading cause of preventable death in the United States, and in spite of the omnipresent warnings, the success rate for smoking cessation is a dismal 20%. I remember my own father often jokingly repeating that old adage, "Quitting is easy. I've done it a hundred times!" The addictive nature of nicotine is clearly a problem worthy of study, and the Center continues to seek the answer to a number of questions: What causes nicotine to become habit-forming? Why is it much more difficult for some to quit than for others? What are effective treatments for those who want to quit?

In this work, and at the base of much of the SRI work in addiction, is the question of what drives addiction: genetics or the environment—i.e., nature or nurture? To address this question, the staff has made extensive use of twin registries, including those of the National Academy of Sciences, the NIH's National Heart, Lung, and Blood Institute, and SRI's own Northern California Twin Registry. Exploring

the susceptibility and impact imposed by one's genes, they use the similarities and differences between identical and nonidentical (fraternal) twins. These databases are now large enough to give meaningful statistical bounds when the hypotheses are well drawn.

One of the Center's most important contributions has been to affirm the role of genetics in nicotine dependence. The first paper from SRI to receive widespread recognition from the field appeared in 1992. Before that time, the scientific community was uncertain, perhaps even skeptical, about a genetic contribution to smoking or, if so, its real significance. To examine that point, the SRI staff determined, using more than 20,000 returned questionnaires from the National Academy of Sciences/National Research Council World War II Twin Registry, that an important part, perhaps 50%, of the susceptibility to nicotine dependence is genetic.[Y] Further work showed that the weight gain incurred by many as they try to stop smoking also has a large genetic component. Even in the strong, cross-population correlation between cigarettes, alcohol, and coffee consumption, genetics plays a role. The attribution of the controlling factors is too complex to relate here, but SRI has published articles detailing them, and the results have been corroborated by others, some using molecular genetic strategies.

Another facet of the nicotine addiction question is the problem of quitting, including the efficacy of different approaches. For example, the transdermal approach, the so-called nicotine patch, was examined as a function of cigarette consumption, motivation to quit, sex, body mass index, and age. Expectedly, motivation was an important factor; but unexpectedly, women relapsed sooner than men, and those with high body mass indices sooner than those with low indices.[Z] The effectiveness of certain cessation drugs, such as bupropion, was also examined.

The SRI work in nicotine and alcohol addiction is of high quality and widely respected. Perhaps the best evidence of that reputation is NIH's creation in 2000 of a multidisciplinary team of SRI experts to constitute the Nicotine Addiction Research Center. In this context, nicotine addiction research at SRI continues to be addressed in terms of genetics, environmental factors, and their interactions. Further, the work explores the determinants of smoking onset in youth, the factors that help in smoking cessation, and the basis for addiction through genotyping, gene expression, and proteomics. In addition to SRI staff, this Center has a worldwide core of collaborators representing a broad range of research practices and disciplines.

Cardiovascular Risk Factors and the Brain

Because of the importance of cardiovascular disease in the U.S. population, there have been continuing and obvious reasons to explore its root causes. One of those pursuits has to do with stress and behavior patterns. This area of research came to SRI through the extended tenure of Dr. Ray H. Rosenman, the co-author of the concept of Type A behavior. Although he created that notion before coming to SRI, he continued to examine and refine the relationships between behavior patterns, stress, and cardiovascular risk over the dozen or so years he researched here. With Drs. Margaret Chesney, Marcia Ward, Michael Hecker, and others, he looked into the medical consequences of Type A behavior in the work setting and also into new interview methods to determine the presence of Type A behavior. Furthermore, he refined the most important components that comprise it. The findings included the now-confirmed cardiovascular consequence of chronic hostility.

Other factors in cardiovascular risk continued after Dr. Rosenman's departure in 1990; in particular, the hereditary question arose in the mind of Dr. Dorit Carmelli (Figure 10-14).[AA] How much of cardiovascular disease is genetic, and are there important environmental factors? Carmelli and Swan again explored this question by including an examination of twin registries. In a 1999 paper they showed that, independent of shared genetic or familial influences, midlife cardiovascular risk factors such as high blood pressure, lower levels of high-density lipoprotein cholesterol, and high 1-hour blood glucose levels were predictive of structural differences in the brain at old age. High alcohol consumption and low physical activity also contribute to poor brain morphology. Structurally, lower volumes of gray matter and degrees of brain interconnectivity (white matter) were evident in such cases, and both have influences on cognitive and physical functioning. In other words, the same activities that lead to increased cardiovascular risk are also inimical to brain

function in old age. Other work on the detailed connectivity, gray-matter volume, and other physical composition of the aging brain continues.

Magnetic Resonance Imaging (MRI)

One of the most important and creative aspects of the Center's work addresses the ability to actually look at the structure of the brain by using high-resolution imaging and doing so at a time of one's choosing. Using such methods means being able to watch the progression of changes in response to a variation in pathologies, medicines, or aspects of lifestyles such as alcohol abuse. This capability is far better than examining the cumulative effects one sees at autopsy. Using high-resolution MRI, SRI's Dolf Pfefferbaum has become widely known for his insight and innovative computational techniques to image brain features over time, such as the changes in brain connectivity or the shrinking of gray matter.[22] One remarkable contribution has been to document the ability of the brain to restore itself following the removal of a particular brain-impinging factor. This reversibility can be studied in animals as well as humans. The two GE MRI units now at SRI are part of a collaborative effort between the two organizations to further both the science underlying brain morphology and the instrumentation with which to better understand it. A major ambition of the Center is to use such imaging as a basis for identifying interventions that can limit damage and improve function. Can brain changes, identified early, help prevent downstream problems or, perhaps, illuminate possible windows in brain development when certain brain-enhancing opportunities exist? At the moment, such imaging is directed mostly at the recognition of certain brain pathologies and whether they respond to specific medicines or lifestyles.

Representative New Areas of Study

Lately, the Center has been investigating the reasons why various identified risk factors contribute to cardiovascular, brain, and other pathologies. As just one example, the Center has done a longitudinal study on the genetic causes of obesity, finding that, with aging, certain genes begin to contribute to both the distribution and amount of body fat.

We end this section with a word on the increasing insight we are gathering on the role of genetics in important diseases. The Center is assessing how that knowledge can be used in ways that are beneficial to early intervention and how its use can be made consistent with our social and ethical practices. It is a new frontier with some risk for such problems as unwarranted discrimination in employment or the availability of health and life insurance, yet with vast potential for improving the health and well-being of us all.

[22] Using high-resolution MRI, new cutting-edge imaging techniques have been created by SRI's Pfefferbaum in collaboration with colleagues from Stanford University, including Dr. Edie Sullivan and Dr. Elfar Adalsteinsson. One MRI technique shows the sophistication of these new analytic tools. The team uses something called "echo planar diffusion tensor imaging" to define the orientation and coherence of the microstructure of white matter. The health of this tissue is related to how the cerebral fluid diffuses either along or across the aligned microstructure, with cross-structure or isotropic flows indicating an aging or deteriorating condition in that part of the brain.

Endnotes

[A] *San Francisco Business Times*, January 23, 2003.

[B] R. J. Horton, Introduction of Halofantrine for Malaria Treatment, *Parasitology Today*, 4(9), 1988.

[C] W. T. Colwell, V. Brown, P. Christie, J. Lange, C. Reece, K. Yamamoto, and D. W. Henry, Antimalarial Arylaminopropanlols, *J. Medicinal Chemistry*, 15(7), 771–775, 1972.

[D] The cardiotoxicity of normal halofantrine is being monitored. One paper describes patients tolerance even with some heart interval prolongations (F.W.H. Ombhanje et.al.,"Halofantrine in the treatment of uncomplicated falciparum malaria with a three-dose regimen in Paupa New Guinea: a preliminary report," *Papua New Guinea Med. J.*, 41(1), 23–20, March 1998). Also, the desbutyl form of halofantrine is being verified as safer (D.L. Wesche et.al., "Mechanism of cardiotoxicity of halofantrine," *Clinical Pharmacology & Therapeutics*, 67(5), 521–529, May 2000).

[E] C.F. Curtis, "The Case for Deemphasizing Genomics in Malaria Control," *Science*, 290, 1508–1509, November 24, 2000.

[F] Special Section on Malaria, *Science*, 290, 437–441, October 20, 2000.

[G] *SRI Journal*, 10(1), 3, Spring-Summer 1990.

[H] *New York Times*, Late Edition-Final, Tuesday, 28 May 2002

[I] *Science*, 294, 1439, November 16, 2001.

[J] Some of this account was drawn from draft papers by Drs. Leon Goodman and Elmer Reist, entitled "The History of Cancer Chemotherapy at SRI International, written in February 2000, and a "History of the Life Sciences Division at SRI/SRI International" by Dr. Laszlo Juhos, dated January 24, 1999. Advice also came from Dr. Ed Acton.

[K] Interview with Dr. Elmer Reist on February 3, 2003.

[L] This account of SRI work was aided by input from Ed Acton on February 3, 2003.

[M] J. P. Marsh Jr., C. W. Mosher, E. M. Acton, and L. Goodman, *Chemical Communications*, 973, 1967; and E. M. Acton, A. N. Fujimora, and D. W. Henry, *J. Medicinal Chemistry*, 17, 659, 1974.

[N] "New drugs flow from SRI development pipeline," *SRI Journal*, 10(1), 3–4, Spring-Summer 1990.

[O] Phone conversation with Michael Tracy on March 13, 2003.

[P] Eric O. Aboagye, Andrew B. Kelson, Michael Tracy, and Paul Workman, "Preclinical Development and Current Status of the Fluorinated 2-Nitroimidazole Hypoxia Probe N-(Hydroxy-3,3,3-trifluoropropyl)-2-(2-nitro-1-imidazolyl) Acetamide (SR 4554, CRC 94/17): A Non-invasive Diagnostic Probe for the Measurement of Tumor Hypoxia by Magnetic Resonance Spectroscopy and Imaging, and by Positron Emission Tomography," *Anti-Cancer Drug Design*, 13, 703–730, 1998. Aboagye was a brilliant student of Workman's and Kelson was a chemist associate of Tracy at SRI.

[Q] *The SRI Journal*, 4(5), August 1984.

[R] A study in contraception for the National Institute of Child Health and Development.

[S] From conversations with early SRI staff member and toxicologist Dr. Gordon Newell on February 11, 2003 and May 22, 2004 and an informal account of SRI's work in pollution from metal and fibreboard factories by Dr. Konrad Semrau.

[T] Much of the story surrounding Freeman comes from an account of his professional life compiled by SRI pulminary physiologist and toxicologist and co-worker, Dr. Laszlo Juhos.

[U] Presented to the World Health Organization for inclusion in finding regarding Oxides of Nitrogen and Photochemical Oxidants in 1977 and 1978, respectively. The results are also given in *Biochemical Studies of Environmental Pollutants: Health Effects of Nitrogen Oxides*, S.D. Lee (Ed.), Ann Arbor Science Publishers, Inc., 1980, pp. 243–265. Also, Freeman's group published over 60 other papers about the biochemistry of these diseases of the lungs.

[V] Carol E. Green, Susanna E. LeValley, and Charles A. Tyson, Comparison of Amphetamine Metabolism Using Isolated Hepatocytes from Five Species Including Human, *J. Pharmacology and Experimental Therapeutics*, 237(3), 931–936, 1986.

[W] Some of this account came from discussions with the present head of the Center for Health Sciences, Dr. Gary Swan, on March 17, 2003.

[X] U.S. Surgeons General's Report on Tobacco for 2004 (www.cdc.gov/tobacco/sgr/sgr_2004.index.htm).

[Y] Ibid. and Carmelli, Dorit, Gary E. Swan, Dennis Robinette, and Richard Fabsitz, "Genetic Influence on Smoking–A Study of Male Twins, *New England Journal of Medicine*, Vol. 327, pp 829–833, September 17, 1992.

[Z] Swan, Gary E., Lisa M. Jack, and Marcia M. Ward, Subgroups of smokers with different success rates after use of transdermal nicotine, *Addiction*, Vol. 92, No. 2, pp 207–218, Carfax Publishing Co., 1997.

[AA] Carmelli, D., et al., Midlife cardiovascular risk factors and brain morphology in identical older male twins, *Neurology 52*, April (1 of 2), 1999.