DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

www.nih.gov

May 7, 2001

Bob Young
Research Director, Public Citizen's Congress Watch
Public Citizen
215 Pennsylvania Avenue S.E.
Washington, D.C. 20003

RE: FOI Case No. 26177

Dear Mr. Young:

This is a final response to your Freedom of Information Act (FOIA) request dated January 9, 2001. You requested a copy of "NIH Contributions to Pharmaceutical Development," an administrative document dated February 2000, which was described in the "References" section of a May 2000, report by the Joint Economic Committee of the United States Congress.

A search of the Office of Science Policy, NIH, produced 38 pages of records responsive to your request. That document is enclosed.

Provisions of the Act allow us to recover part of the cost of responding to your request. Because the cost fell below the $25 minimum fee, there is no charge for the enclosed material.

Sincerely,

Susan R. Cornell

Susan R. Cornell, Esquire
Freedom of Information Officer
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Enclosure:

38 pages

Administrative document
prepared by NIH staff.

NIH CONTRIBUTIONS TO PHARMACEUTICAL DEVELOPMENT

CASE STUDY ANALYSIS OF THE TOP-SELLING DRUGS

INTRODUCTION

The United States is the acknowledged world leader in innovative biomedical science and technology. U.S. pharmaceutical and biotechnology companies are among the nation's most internationally competitive industries. This economically advantageous position owes chiefly to the continuing stream of advances in the basic biomedical sciences over the last five decades. Efforts to harness the potential of the revolutionary advances of the last several decades in genetics, molecular biology, and related disciplines are now extensive — advanced pharmaceuticals, genetic and other new human health therapies, and a host of commercial applications outside of medicine. The unique U.S. system of public support for science, mainly through the National Institutes of Health, is the foundation of that success.

An ongoing series of studies in the Office of Science Policy at NIH has been designed to analyze the effectiveness of public funding of biomedical research. Because product development is a good measure of the practical usefulness of research advances, case studies of products now on the market have been used to illustrate the health and economic benefits of NIH-funded research. The intellectual history underlying development of eight commercially successful products has been documented, and the results are presented here. These products include the five top-selling pharmaceuticals and three non-medical biotechnology-based products.

Background

The health and economic benefits created by the U.S. pharmaceutical industry result from an informal public-private partnership. Federal support for basic research has been acknowledged by the pharmaceutical industry to be the foundation for its success. According to the largest pharmaceutical industry trade association[1], "the National Institutes of Health plays a vital role in drug discovery by funding basic research into the fundamental mechanisms of disease. This allows industry to focus on finding ways to intercept disease mechanisms."

Some less obvious ways that public funding creates an infrastructure on which privately-funded research builds have been documented. For example, a 1993 report to the U.S. Congress[2] details the role of NIH in training biomedical scientists, many of whom subsequently work in industry, and in supporting the construction of buildings and laboratories at universities across the country in the 1950s and 1960s. A 1994 survey of life science firms also showed that industry funding of academic biomedical research is growing, and that scientists are increasingly combining public and private funds in the same research projects.[3] The authors of the 1994 survey point out that life

sciences companies draw on the scientific knowledge generated by a publicly-funded research infrastructure in academia.

Perhaps most striking are recent studies that suggest that continued Federal support for biomedical research over the past half-century has encouraged pharmaceutical companies to act in ways that enhance their productivity. Based on their detailed analyses of the organization of pharmaceutical research and development, including interviews with senior industry scientists and managers, Cockburn and Henderson suggest that those pharmaceutical companies that organize in ways that most effectively tap the results of publicly-funded science are those that are most successful.[4] For example, they found that those firms whose scientists publish a higher fraction of papers coauthored with university-based biomedical researchers obtained more patents per research dollar, on average, than firms whose scientists work less closely with the public sector.

Two earlier case study analyses have assessed the input of various sectors of the biomedical science community. Maxwell & Eckhardt looked at 32 drugs introduced prior to 1990 and concluded that non-industrial contributions (universities, government labs, non-profit research institutes, hospitals) play an important role in drug discovery. Without these nonindustrial contributions, approximately 60 percent of the drugs would not have been discovered or would have had their discoveries markedly delayed.[5] More recently, Cockburn and Henderson[6] constructed case histories of medications identified by industry experts as having had the most impact upon therapeutic practice between 1965 and 1992. Among these 21 drugs, publicly-funded research was instrumental to the development of 16, or 76%. Comparing their results from a more recent group of drugs to Maxwell and Eckhardt's earlier study, Cockburn and Henderson suggested that "public sector research appears to have become more important over time, as one might expect given the increasing role of modern molecular biology in drug discovery."

Although the intent of these studies is to demonstrate the utility of public funding to industry productivity, they still may under represent the effects of basic research. Other reports have appeared which question or underplay the role of basic research in medical and technological advances. For example, in response to a survey conducted in the late 1980s research and development managers at major U.S. pharmaceutical firms reported that only one-quarter of the products and processes they had commercialized in the previous decade would not have been developed without delay in the absence of recent academic research.[7] A recent survey reported that private industry was the first to synthesize over 92 percent of drugs approved between 1981 and 1990.[8]

Maxwell & Eckhardt state that "the availability of a new and independently discovered drug [often] provided an essential tool that permitted a much needed verification of some at-risk concept." The book concludes that 38 percent of the drugs resulted entirely from industry input and that industry was the largest contributor to drug and medicine production. A similar stance is taken further in an editorial and follow-up letter in recent issues of *Science*,[9] which state that all research that is "unconnected to useful products" should be privatized, although in another paragraph it is noted that companies are phasing out their non-targeted research because it is not cost effective. The letter comments that the flow of usefulness is usually from technology to

science rather than vice versa.

Private industry does play a large and growing role in medical research. By 1994, industry accounted for over half of the total national investment in medical research. However, most of this private investment was for applied research and product development. In 1992 (the most recent year for which figures are available), 38% of the pharmaceutical industry's total R&D investment of $8.8 billion was used for applied research, 48% was spent on product development, and only 14% was applied to basic research.[10] To the extent that basic research into the underlying mechanisms of disease drive new medical advances, the R&D in industry is not performing the role played by public research funding.

Whether the conclusions are reached by survey or case study, what these analyses have in common is that by carefully defining what constitutes a necessary contribution, much of the enabling intellectual background that led to the new product is removed from consideration. Maxwell & Eckhardt define a contribution as separate from its framework of science; work that is "permissive" as distinct from that which is "contributory"; and a "necessary forerunner" as not directly involved in the innovation. The present study was undertaken to determine whether and to what extent public funding of research enabled the development of certain medically or commercially successful products. Additionally, this study begins to lay a basis for discussing the specific ways by which those who expand fundamental understanding of the workings of the natural world are as important to technological advance as those who implement that knowledge.

METHODOLOGY

Case studies were used to illustrate the public/private partnership in drug development. An intellectual history was drawn of the top five drugs from a list of the 13 drugs which sold $1 billion or more in 1994 and 1995. These five top-sellers are the antidepressant Prozac, the two antihypertensives Vasotec and Capoten, the antiherpes drug Zovirax, and the ulcer and gastritis drug Zantac. We choose not to highlight known NIH success stories. Instead, these drugs were selected based on their market success as the objective indication of their benefits to health. The analysis began with a simple Medline search using the chemical name of the drug to find several review articles. These reviews and the original research articles they cited provided a view of the understanding of the disease and the technical capabilities at the time the product was developed. As discussed below, patent citations were not useful in developing these cases.

The scientific discoveries that led to the necessary concepts and techniques were identified, along with the names and affiliations of the scientists performing the work. Rather than attempt to identify a small number of "key papers," which does not accurately represent the way scientific ideas develop in the research community, the approach taken was to identify major areas of research which led to drug discovery and the individuals or laboratories who were significantly involved. Each case is presented in two parts: a story describing how the ideas and events came together, and a table which identifies scientists and their affiliations, contributions, references, and support acknowledgments when available.

Example products for case studies were selected based on their commercial success, where the role of NIH in their development initially was unknown. Example medical products were selected from the broad category of therapeutic drugs. This category of medical products was selected because drugs provide important health benefits, and because they are often more cost-effective than other medical interventions.[11] We obtained a list of the 13 pharmaceuticals with sales over $1 billion in 1994, provided by the Pharmaceutical Research and Manufacturers Association of America (PHRMA)[12], and selected the five drugs with the highest worldwide sales. These are: Vasotec and Capoten (antihypertensives), Zovirax (an antiviral agent), Prozac (an antidepressant), and Zantac (an antiulcer agent).

CASE STUDIES OF TOP SELLING DRUGS

Pharmaceutical discovery and development is an excellent illustration of the benefits to health of publicly funded research. Drugs and medicines are a major tool of health care and the most cost-effective medical intervention. Additionally, the pharmaceutical industry is one of the United States' most competitive international enterprises. A close look at the process of drug discovery reveals the interactive partnership between academic and industry scientists and shows how NIH funded research underlies the development of treatments for disease. The intellectual histories discussed here emphasize the need to keep both industry and medicine supplied with this resource of new ideas and techniques.

Case studies were used to trace the development of the five top-selling pharmaceuticals . The case studies (Appendix A) are presented in two parts, first a story of the ideas and technical advances which led up to the discovery, followed by a table which lists the scientists who were involved in each area of science, and documents their affiliations (NIH-supported academic, foreign academic, or industrial) and their contributions with references to the scientific literature. The science and areas of research underlying the case study drugs are described briefly below, followed by an interpretation of the findings.

Research Summary

Prozac (Fluoxetine) is used for the treatment of depression and several other psychological disorders. It acts by increasing the concentration of the signaling substance serotonin in the connections (synapses) between nerves. Three areas of research underlie its development: 1) research on blood pressure and antihistamine drugs, 2) the neurochemical basis of depression, and 3) the molecular basis of neuronal signal transmission.

Antihistamines, like many other drugs, act on the substances which nerve cells (neurons) use to transmit signals. The first antidepressant was developed following the observation of mood changes after taking certain antihistamines. Basic research on the transmission between neurons had found several of the message molecules, and an early antidepressant drug became an important tool in discovering how the signal is sent and then terminated. Psychiatric conditions could now be studied at the molecular level by showing how these signal molecules and neuron communication mechanisms underlie mental states. Through the interaction of these two fields of study and the use of the first generation drugs as research tools, the correct neurotransmitter, serotonin, was targeted. Industry scientists chose another antihistamine drug as the chemical basis for this search.

Capoten (Captopril) and Vasotec (Enalapril) are used to treat hypertension, and they act by inhibiting a crucial enzyme, ACE, in a cascade of molecular signals which regulate blood pressure. Two areas of research underlie these drugs: 1) research on the renin/angiotensin/ aldosterone (R/A/A) system of blood pressure regulation, and 2) enzyme kinetics studies of the bovine enzyme carboxypeptidase, which is very closely related to ACE.

Research on the involvement of the kidney in initiating hypertension found the substance renin,

which causes vessel walls to constrict, thereby increasing blood pressure. Renin was subsequently shown to act by activating another molecule, angiotensin. Later, angiotensin was found to occur in two forms; AI is converted by angiotensin converting enzyme (ACE) to the active form AII. AII has two hypertensive effects, first by directly causing blood vessels to constrict, and second by inducing aldosterone production by the kidney. Aldosterone causes salt retention, which increases blood volume and thereby raises blood pressure as well. An early ACE-inhibiting drug derived from snake venom became a tool for continued study of the R/A/A system. Using chemical information about ACE, captopril was discovered through the adaptation of a molecular model of bovine carboxypeptidase plus its inhibitor. Captopril had serious side effects, however, and another company was able to alter the chemical structure to produce a drug with longer act[illegible]y while lacking the side effects, enalapril.

Zovirax (Acyclovir) treats herpes simplex virus (HSV) infection by inhibiting the ability of the virus to replicate its DNA, thereby blocking its growth. Three research areas contributed to its development: 1) the virology of HSV, 2) studies of the enzymes of DNA replication, and 3) research on nucleotide analogs and their potential as antimetabolite cancer drugs.

The study of the replication of DNA in cells lead to the characterization of the enzymes which are involved. When HSV was isolated from sores on the skin, it was shown to have a large DNA genome which replicates and expresses genes in the same manner as the cells. Closely following the discovery of the cellular enzymes, the DNA replication enzymes of HSV were also found and characterized. During this time, the idea was developing that, since DNA consists of a chain of nucleotides, chemically altered forms, or analogs, of nucleotides could be incorporated into the growing DNA chain or could bind to the replication enzymes, thereby halting cell growth. This would mainly affect rapidly growing cells, such as cancer. Although nucleotide analogs had proven somewhat useful against HSV previously, they acted equally on the body's growing cells and so were very toxic. Once HSV was found to make its own enzymes with slightly different properties from those of the cell, analog drugs for cancer were also screened for one which would preferentially inhibit HSV rather than cellular replication. Acyclovir had this property, and was found to inhibit two of the viral DNA replication enzymes, while not killing the cells.

Zantac (Ranitidine) treats ulcer and gastritis by blocking the signaling molecule histamine from causing cells in the stomach and duodenum to produce acid. An earlier drug from which ranitidine was derived, cimetidine (Tagamet), was the first drug which could distinguish between the two types of molecules on cells, called receptors, which bind to histamine and determine what the cell's response will be. These two drugs specifically block the H2 but not the H1 type receptors, permitting acid secretion to be blocked without inhibiting other necessary functions of histamine. Three lines of research underlie these developments: 1) the discovery and characterization of histamine, 2) the concept of receptors on cells for various signaling molecules, and 3) the discovery of two types of receptors for adrenaline and a drug specific for the second type.

The signaling molecule histamine was discovered early in this century, and its chemical structure and many affects on different tissues became well known. Meanwhile, the mechanisms by which

cells receive and respond to such signals were under intense study, and the idea that cells had specific receptor molecules for these signals was slowly developing. After it was proposed that two different types of receptors for adrenaline would explain its different effects, a chemical was found which blocked only the second type of receptor. Based on this, a drug was developed to protect a weakened heart from overstimulation by adrenaline, by blocking this second receptor type. After this, histamine was also proposed to have two types of receptors, H1 and H2, the second of which stimulated acid secretion. By patterning a search after the discovery of the adrenaline blocker, and by making use of the well-known chemistry of histamine, the H2 receptor and a drug which would specifically block it were found. The resulting drug, Tagamet, was effective, but due to serious side effects it was soon replaced by Zantac, which was the product of research starting with a different chemical backbone.

Analysis of Drug Case Studies

Scientific progress over several decades underlies the development of each of the five top-selling drugs. They were conceived through research conducted in the 1950s, '60s and early '70s, developed and patented in the 1970s, and FDA approval was based on clinical results from the 1970s and '80s. NIH-funded research played a critical role in drug discovery in each of these cases. Researchers at U.S. universities and at NIH contributed by discovering basic phenomena and concepts, developing new techniques and assays, and participated in clinical applications of the drugs. However, these cases also demonstrated that public and private sector biomedical research are interwoven, complementary parts of the highly successful U.S. biomedical sciences endeavor.

Basic research lays the groundwork for drug discovery. The field of research that underlies most pharmaceutical drug development is organic chemistry and synthesis. The first techniques for isolating the active chemical in a natural substance, and then modifying its molecular structure at will, were developed during the previous century. The contemporary organic chemistry that is cited in industry research papers is frequently supported by NIH grants; however, the large majority of chemical methods are unattributed because they are old or widespread enough to be considered part of the "general knowledge." Laboratory models—the cells, tissues, or animals in which the drugs are to be tested—are of equally critical importance. The basic methods of cell culture and animal surgery were developed in the early part of this century. These older methods are unattributed, although they originate in academic science, but most specific models, or assays for specific enzymes, usually have come from the individual academic labs as part of their research results, and are cited by industry researchers in the scientific literature. Most lines of research have strong roots in European universities, but with each decade since World War II, the US contribution rose sharply as a result of NIH funding.

For a pharmaceutical company to target a disease for drug development, there must be an acceptable market potential. If the condition is widespread, causes serious disease and currently no adequate therapy exists, there is likely to be a sufficient demand for the drug to support the cost of development. Also, some insight into the cause of the disease, or a means to approach it, is usually needed. Industry scientists draw upon an existing body of scientific knowledge

when they consider these factors and begin a research and development effort. In some cases, that scientific knowledge is well-developed, but in other cases, less is known when drug development begins.

The research which ultimately led to Prozac began when the mood-altering effect produced by an antihistamine was noticed by a surgeon, at which point industry scientists began supplying him with experimental drugs. At the time, little was known about the physical basis of depression. In the cases of the other drugs, industry entered the field only after academic scientists had clarified the disease to the point of finding the enzyme or hormone that the drug acted upon. Before any work began on the development of Zovirax, publicly-funded basic researchers had discovered the cell's mechanism of DNA replication, followed by discovery of the replication enzymes made by herpes simplex virus (HSV) which were similar but not identical to the cellular enzymes. The use of nucleotide analogues (altered versions of the nucleotide building blocks of DNA) as inhibitors of tumor cell growth was pioneered in academic laboratories also. Based on these advances, industry scientists applied their work on nucleotide analog inhibitors to finding an antiviral drug. An industry scientist developed the precursors to both Zantac and Capoten after publicly-funded scientists identified the signaling substances involved in gastritis and high blood pressure, respectively, and developed the concept of how these substances acted.

Public and private research play complementary roles. The biology of a disease was usually worked out by academic scientists, while the search and testing of drugs was performed by industry, although there is often overlap in these roles. Using existing scientific knowledge, industrial scientists search for a substance with the desired activity. Once a potential drug is discovered, industry scientists conduct extensive in vitro and animal tests until they are ready to patent the invention and publish the results. Then, further studies by the company and by academic researchers on the drug's mechanism of action and its effects on animals and, eventually, on human patients, fit into a framework of continuing basic and applied advances. In many instances, a new drug becomes an experimental tool of academic researchers to understand the physiological system and the disease pathology. These continued studies often further clarify the disease mechanism and provide leads for drug improvement, as well as aid the company in getting FDA approval. Technological innovation did not follow a one-directional "pipeline," in which basic research leads applied research, and applied research leads to product development. The process involved feedback in both directions between publicly funded labs and industrial researchers.

None of the top sellers in these case studies are "first generation" drugs; they are the result of a great deal of basic research on the disease mechanism which allowed more specific targeting of the underlying problem. Sometimes this extension of knowledge included the use of the first generation drug itself. In the development of Prozac, the discovery of an earlier drug by industrial researchers preceded and enabled the discovery by academic scientists of the particular signal transmitter in the brain which the drug was acting upon to alleviate depression. This knowledge permitted the company to hunt for a more active and specific next generation drug. The result was Prozac, which itself then became an important tool in greater understanding of the neurological basis of depression. In the other cases, the particular enzyme or hormone

central to the disease mechanism was already discovered before the first drug was produced. Capoten and its antecedent drug were tools for increasing level of sophistication at which renal hypertension was understood, thereby permitting better management of the disease and pointing the way to the next step in drug design. Zantac and Zovirax were not as important in elucidating the disease process, but nonetheless were very important in ongoing progress in treatment.

The route to drug discovery is unpredictable. When scientists select chemicals to be tested for drug action, they may use either an empirical or rational design approach. In the empirical approach, collections of compounds are screened to find an active drug, where the initial lead is the activity of a natural substance or a chance observation. Knowledge of the biological system or the mechanism of drug action is not needed, although the screening process makes use of models and assays developed through basic research. Rational design of compounds for a particular activity requires knowledge of the biological system. When the target is defined —e.g., the enzyme or gene responsible for a crucial cellular activity—then an inhibitor or modifier of its function can be sought. This rational design step provides a chemical series of potential drugs, and therefore is followed by another round of screening to find the most active form. In practice, most pharmaceutical development has been a combination of these two approaches.

The five cases differ in the degree to which purely empirical discovery versus a rational targeting step were important. The development of Zovirax and the drug that preceded it was based on rational design of specific molecules to inhibit known enzymatic activities, with no purely empirical discovery phases. Capoten, Vasotec, and Zantac were also produced by the use of rational design steps based on the prior discovery of the molecular targets, although the initial drug with the activity of Capoten was derived from the activity of a natural substance. Among the five cases, Prozac was the least dependent on a rational approach, as the first drug with antidepressive properties was an entirely empirical discovery. However, the development of Prozac itself resulted from rational design aimed at one neurotransmitter out of several.

Research may be targeted to the cure of a particular disease, or aimed at understanding basic mechanisms and gaining knowledge for which no immediate application is apparent. Disease-targeted research can be effective in fueling progress in a given area. However, just as often, results from other fields of research led to breakthroughs in disease concepts or in drug discovery. These five drugs all arose from both disease-specific and unrelated fields of research. The discovery of Zantac depended on advances in three fields which were unrelated to gastritis and ulcer disease: the chemical structure and biological activity of histamine, the broad concept of cellular receptors, and the work which culminated in the development of a cardiac drug. Two out of three areas supporting the discovery both of Zovirax and of Prozac were unrelated to the disease that these drugs treat. The discovery of Capoten and Vasotec arose mainly from a broad research effort targeted to the study of hypertension, heart and kidney disease, but knowledge from research in protein chemistry and enzyme kinetics provided the critical lead needed to produce Capoten. It is not always possible to predict the source of new inspiration. Basic research aimed at understanding biological mechanisms and gaining knowledge for which no immediate application is apparent has been a vital supply of new ideas, and can only be sustained

through public support.

In the last few years, the process of drug discovery has been revolutionized by combinatorial chemistry, which is the general name for a collection of new methods to produce enormous numbers of related molecules in an orderly, tagged series. These technologies speed up empirical drug search by generating a diversity of compounds to screen for a lead. Although these methods were to a great extent designed by the industry scientists who need them, they have their roots in publicly-funded basic research. Molecular structure modeling is currently used for rational drug design and lead production. Once again, the initial concepts and techniques needed for structural modeling come from NIH funded labs, while it is now industry scientists who continue to develop the techniques. Because even the most perfect model can only produce a set of potential drugs, structure modeling and combinatorial chemistry are used in concert to maximize the efficiency of drug design, once a target is selected. Knowledge of the underlying disease mechanism which reveals the enzyme, cell, or symptom that should be targeted continues to be generated by NIH-funded research.

Advances in molecular and cellular biology have created the new biotechnology industry, which is based on an entirely new concept of drugs and medicines. Biotech drug and medicine development is, if anything, even more based in and interrelated with public sector research than drug development in the big pharmaceutical firms.

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2. U.S. Congress, Office of Technology Assessment (1993) *Pharmaceutical Industry R&D: Costs, Risks, and Rewards*, OTA- .

3.Blumenthal, David, et. al. (1996) *Relationships between Academic Institutions and Industry in the Life Sciences--An Industry Survey*. The New England Journal of Medicine, 334: 368-373 (Feb. 8).

4.Cockburn and Henderson (1996) op. cit.

5. Maxwell, Robert A. And Shohreh B. Eckhardt (1990) *Drug Discovery: A Case Book and Analysis*. Clifton, NJ: Humana Press).

6.Cockburn, Iain and Henderson, Rebecca, "Public-Private Interaction and the Productivity of Pharmaceutical Research" (June 1996), Prepared for the 1996 Micro-BPEA Conference.

7. Edwin Mansfield, "Academic research and industrial innovation," Research Policy 20 (1991): 1-12.

8. Tufts University, 1991 (need complete cite on this).

9. Rustum Roy, "Roads Not Taken, Yet," editorial, Science, v. 273, July 19, 1996.

10. National Science Foundation, National Patterns of R&D Resources: 1994, NSF 95-304 (Arlington, VA, 1995).

11. Frank R. Lichtenberg, "The Effect of Pharmaceutical Utilization and Innovation on Hospitalization and Mortality," National Bureau of Economic Research Working Paper No. 5418, January 1996.

12. Judy Moore, Consultant, National Health Policy Forum, "The Pharmaceutical Industry," background paper distributed by the Pharmaceutical Research and Manufacturers Association, January 1996.

Ranitidine (Zantac)

Ranitidine is used to treat gastric ulcers by blocking acid secretion in the stomach, allowing them to heal. It is more active than the first acid inhibitor, and it is better tolerated and lacks the serious side effects of the previous drug. It was the number one selling drug in 1994 and 1995, which indicates the demand for a treatment of gastritis and ulcers. The development of the first "histamine H2-receptor antagonist" drug, which preceded ranitidine, represented a new concept in ulcer treatment. This class of drug acts by controlling acid secretion by blocking the substance that signals the stomach to produce acid. A very large body of research on histamine, its physiological effects, and its mechanism of action are behind the targeted research which resulted in these drugs. Two other areas that figured significantly were the developing concept of receptors on cells for biological signaling molecules, and the research leading to the cardiac drugs known as the β-blockers which grew from it.

The substance histamine was discovered near the turn of the century by European scientists, although at the time it was thought to be the result of bacterial growth. Another European academic scientist conducted an extensive set of experiments demonstrating histamine's complex physiological actions, which included the ability to alter blood pressure through effects on blood vessels, and to cause constriction of the bronchiolar and other smooth muscle (ie, the muscle of organs and vessels.) Ten years later, a U.S. researcher demonstrated that histamine was a normal component of the tissues, and by 1926 the European group had confirmed this. It was also shown, in two European academic labs, that histamine induces secretion of acid in the stomach. During this time, the chemical properties and methods of purification of histamine were also being defined. In 1937, an academic scientist in France developed the first inhibitor of histamine, but it was not until 1942 that industry scientists developed an antihistamine which could be used as a drug. Over the next 8 years, a large number of antihistamines were developed by foreign and US companies, although notably one US academic researcher developed the highly successful drug diphenhydramine (Benadryl) which also became the chemical basis of Prozac. These drugs blocked some but not all of the actions of histamine.

The ideas leading to the production of ranitidine had their beginning in the study of neurotransmission in the sympathetic nervous system and the development of the cardiac drugs known as β-blockers. The sympathetic nerves are part of the autonomic nervous system which regulates the functions of organs. The neurotransmitter adrenaline was first characterized and purified at the turn of the century by the U.S. scientist who later identified histamine as a normal substance in tissues. Understanding of the function of adrenaline and noradrenaline had progressed to the point in the 1930s where it was known that they could cause either excitatory or inhibitory responses in tissues, but there was a great deal of confusion as to how this happened. Several groups developed drugs which blocked the excitatory response to adrenaline in all tissues except the heart. The most successful of these adrenaline antagonist drugs was one produced by a US academic scientist, which became a widely used research tool, as well as a chemical lead for industry pharmaceutical development.

During this time, the notion of specific "receptors" on cells, which mediate the cell's uptake and response to a variety of substances both natural and medical, was slowly emerging. The receptor concept was highly controversial, mainly due to the variety of phenomena it had to account for. In 1948, a U.S. academic scientist performed experiments with several of the known adrenaline blockers and showed that two separate types of receptors, α and β, must exist, which could not be classified simply as excitatory and inhibitory. All the drugs available at the time blocked the α type of receptor. However, this work ran counter to the prevalent theory of the day, and was not accepted. Ten years later, an industry team began

testing analogs of adrenaline to find one with improved bronchodilator activity. An analog is a molecule similar enough to adrenaline, for example, that it will bind to the adrenaline receptor, but has a modification that prevents the physiological activity from occurring while also preventing the binding of adrenaline itself. These scientists reported the first compound, DCI, which blocked the inhibitory actions of adrenaline. A U.S. academic scientist realized the potential of DCI to act on the heart, and performed the experiments which put the new inhibitor together with the α and β receptor concept. He pointed out that the receptors which DCI blocks in the heart had to be the β type, which stimulate the heart but cause inhibition in most other tissues. Upon hearing these results, a scientist at ICI in Europe, who was seeking drugs to protect the heart from excitation by adrenaline, realized that DCI had the activity he was looking for. His team eventually produced the first of the β-blocker drugs, propranolol, in 1962, which was a breakthrough in treatment of heart disease. This scientist, James Black, emphasized in his papers that his work was initiated based on the concept of α and β adrenergic receptors published in 1948. He is also the connection between the cardiac drugs and the antihistamine drugs for treatment of ulcers.

As the various effects of histamine were studied through the use of antihistamines, it became clear that several effects were not inhibited by these drugs, including the secretion of gastric acid. In keeping with the developing concept of specific receptors, a European lab defined the receptors which were blocked by these antihistamines as H1 receptors, and postulated that one or more additional types must exist. Based on this and his previous experience with β-blockers for the heart, it was obvious to Black to look for the other form of histamine receptor which mediated the effects on acid secretion by the stomach, and a chemical to specifically block them. Now at Smith-Kline French (SKF), Black and his team made analogs of histamine to test for inhibition, using the knowledge of the isolation and chemistry of histamine. But even with this auspicious start, it was 8 years later, after testing 700 compounds, before one having the required activity without the negative effects was found. The article describing the discovery of H2 receptors and the production of a chemical which inhibited them was published by the SKF research team in 1972. In it, they remark that the work was based on analogy with the β-receptors in the heart and on the structure of histamine. This first inhibitor was not useful as a drug, but after several years of additional research, the SKF scientists published the description of cimetidine (Tagamet) in 1975, the first H2-receptor antagonist drug. Cimetidine was widely tested in clinical trials, and proved useful, but had a number of serious side effects stemming from the fact that it also inhibited another physiological system.

Over the next 3 or 4 years, scientists at several companies tested different types of organic molecules for H2 antagonist activity. Certain molecular features of cimetidine, which probably were responsible for its side effects, were also thought to be a necessary part of its activity. The Glaxo team began experiments with a different molecular basis, and in 1979 they published the discovery and initial testing of ranitidine. This drug is 5 to 10 times as potent per chemical weight as cimetidine and is longer lasting. Also, since ranitidine is more specific for the H2 histamine receptors, it lacks the side effects that were problematic with cimetidine. Over the next 10 years a very large number of trials of ranitidine were conducted in US and foreign academic clinical centers, testing its usefulness for a variety of conditions and its effects on many functions of the body such as the immune system, the liver, blood pressure, respiration, and many aspects of the gastrointestinal system.

The story of ranitidine also offers an example of how continuing research and more understanding of the underlying disease process leads to improvements in treatment. It had long been thought that the excess production of stomach acid seen in ulcer and gastritis patients was itself the cause of the conditions. This idea was bolstered by the observation that when acid secretion was suppressed with H2-antagonist drugs the ulcers would heal, although recurrence was the rule. In 1983, just as ranitidine was brought to market,

the bacterium Helicobacter pylori was isolated from the stomachs of gastritis and ulcer patients by a scientist at an Australian university medical center. He proposed that the bacteria were the cause of the patients' conditions, and described methods for culturing the organism. Within the year, 4 additional reports of the same finding were published from other European medical centers, and many more followed once researchers knew to look for it. Over the next 5 years, many medical centers in the U.S. and Europe began clinical trials to test the association of H. pylori with disease, and to try various antimicrobial treatments to eliminate it.

Bacteria had been found in ulcer patients before the 1983 discovery, but the difficulty of culturing the organism was not realized, and most researchers found no bacteria. One laboratory which had isolated bacteria in the 1970s concluded they were not causative, since healing with antacid did not affect the infection. After the reports linking H. pylori to ulcer and gastritis appeared in 1983 and 1984, academic researchers continued to improve culture methods. As they discovered features of the organism's biology, new detection assays rapidly appeared. One significant feature is its production of the enzyme urease. Urease had been discovered well before H. pylori, but it was thought to be produced by the stomach. Forty years later, in 1968, it was proven to be bacterial in origin, although its importance still was not recognized. Following the discovery of H. pylori, a European group showed that these bacteria produce the urease, and soon thereafter another foreign research team developed a diagnostic assay based on urease. Subsequently, easier and more accurate assays were developed in two U.S. laboratories. One of these was the scientist who discovered H. pylori, who returned to the U.S. and established a laboratory at the University of Virginia.

The idea that H. pylori was the cause of most chronic gastritis and ulcers was not readily accepted by the medical community. Increased understanding of the organism not only improved treatment, it explained features of the pathology so that physicians would accept the new concept and adopt the treatment regimen for their patients. The association of chronic hyperacidity with ulcer was an argument against the significance of H. pylori, especially when it turned out that in culture the bacteria are susceptible to acid. Soon, academic researchers in Europe and the U.S. demonstrated that the bacteria alter the secretion of acid from the stomach while having a mechanism of creating a "microenvironment" that is protected from acid. Clinical trials have shown that acid secretion returns to normal after the bacteria is eliminated. Also, H. pylori is killed by a wide spectrum of antibiotics in culture, yet treatment with many of these antibiotics resulted in only temporary relief of the condition, followed by recurrence. The discoverer of H. pylori, now in the U.S. and using NIH funding, found that in the stomach the bacteria are much more resistant, and treatment with multiple antibiotics is required. Another objection was based on consistent recovery of H. pylori from stomachs of people without gastritis or ulcer. A U.S. research team with NIH funding studied the means by which the bacteria colonize the epithelium of the stomach and upper small intestine. He saw that inherited differences in proteins on the surface of the cells determined the ability of H. pylori to grow and therefore determined the individual's susceptibility. By the late 1980s, several US and foreign academic groups had realized that H. pylori is linked to gastric cancer. The ability of the organism to increase acid production by the stomach is thought to be linked to its defenses, its resistance to antibiotic treatment, and to its involvement in cancer, although the mechanism is not yet known.

A one time antibiotic treatment regimen to eliminate H. pylori, as opposed to long term maintenance with H2-antagonist drugs, recurrence, and sometimes surgery as a last resort, is an obvious benefit both to the patient and to the health care insurer. However, this story highlights the risky nature of pharmaceutical development, given the possible decline in sales of ranitidine, which itself was the product of some relatively low investment chemical manipulation by Glaxo once Smith-Kline French had invested in the

effort and expense of proving the concept and developing the prototype. Clinical studies have shown that ranitidine it is effective in relieving pain and speeding the healing during treatment regimen to eliminate H. pylori. Also, several conditions that are not caused by H. pylori, such as ulcer caused by aspirin, gastric-esophageal reflux and a hereditary hyperacidic condition, respond well to ranitidine.

Research Leading to the Development and Use of Ranitidine

USA = affiliated with academic institution or NIH in the U.S.
Frn = foreign academic institution
Ind = industry labs (other than Glx)
Glx = Glaxo researchers

✍ = referenced in SQ or MK papers
★ = key contribution
✏ = review article

Adrenergic receptors and cardiac β-blocker drugs

Scientist	Affiliation	Contribution [references] support acknowledgements
Goodman	USA	Made the adreneline antagonist most widely used in studies of its action and as the basi... for further drug design [Nickerson & Goodman 1947 J. Pharmacol. Exp.T... 99:167]
Ahlquist	USA	★Early studies of substances with adrenergic effects; proposed separate α and β type receptors for adrenaline, which may be either excitatory or inhibitory in different tissues [Ahlquist 1948 Am.J.Physiol. 153:586]
Slater, Powell	Ind	Synthesized the first antagonist of adrenaline's inhibitory effects, DCI, based on an adrenaline analog asthma drug [Powell & Slater 1958 J.Pharmac.Exp.Ther. 122:480]
Moran	USA	First showed that DCI would relax the response of the heart to adrenaline; identified it as a blocker of Ahlquist's β type adrenergic receptors [Moran & Perkins 1958 J.Pharmac.Exp.Ther. 124:223] NIH H-2953; PHS Sr. Research Fellowship
Black	Ind	Developed first β-blocker drug for use in cardiac patients [Black & Stevenson 1962 Lancet ii:311; Black et al. 1964 Lancet i:1080] ICI

Histimine and its two types of receptors

Scientist	Affiliation	Contribution
Windaus, Vogt	Frn	First synthasized histamine [Windaus & Vogt 1907 Ber. 40:3691]
Dale	Frn	★Demonstrated the physiological effects of histamine, and that its effect is on smooth muscle; proved that it is a natural constituent of tissues [Barger & Dale 1910 J.Physiol London 40:38; ibid. 41:318; Best et al. 1927 ibid. 62:397; Dale 1929 Lancet i:1233]
Ackermann	Frn	Early work on the isolation and chemical properties of histamine [Ackermann 1910 Ztschr.f.physiol. Chem. 65:504; Ackermann & Fuchs 1938 ibid. 257:153; Ackermann & Mohr 1938 ibid. 255:75]
Abel	USA	First purified and characterized adrenaline. Isolated histamine from tissues, and showed it was had physiological activity [Abel 1898 Proc.Am.Physiol.Soc. P.3-5; Abel & Kubota 1919 J.Pharmac.Exp.Ther. 13:243; Abel & Nagayama 1920 ibid.15:347]
Popielski	Frn	First showed that histamine induces gastric acid secretion [Popielski 1920 Pfluger's Arch. 178:214]
Bovet	Frn	Developed the first antagonist of excitatory adrenaline response, and based on that, later synthesized the first antagonist of histamine [Fourneau & Bovet 1933 Arch.Int.

		Pharmacodyn. 46:178; Bovet & Staub 1937 C.R. Seances Soc.Biol.Paris 124:547]
Code	Frn	Important method of recovering histamine from tissues and blood; relationship of histamine to gastric ulcer and anaphylactic shock [Code 1937 J.Physiol. 89:257; Code & Ing 1937 J.Physiol. 90:501; Code 1939 Am.J.Physiol. 127:78; Code & Varco 1942 ibid. 137:225]
Loew, Chickering	Frn	Histamine stimulates acid secretion from the stomach [Loew & Chickering 1941 Proc.Soc.Exp.BiolMed. 48:65]
Schild	Frn	Developed a widely used assay for determining the potency of a chemical relative to histamine. Showed that certain histamine actions were inhibitory rather than excitatory, proposed multiple receptors, naming the exitatory ones H1-receptors. [Schild 1942 J.Physiol.101:115; Ash & Schild 1966 Br.J.Pharmac.Chemother. 27:427]
Black	Ind	Described the H2 histamine receptors responsible for acid secretion, and made the first inhibitor of them [Black et al. 1972 Nature 236:385] Smith-Kline French

Drug development and testing

Scientist	Affiliation	Contribution
Ganellin, Parsons Brimblecomb	Ind	Testing and chemical analysis of cimetidine and its precursor H2 antagonists [Black et al. 1973 Agents & Actions 3:133; Brimblecomb et al. 1975 J.Int.Med.Res. 3:86; Durant et al. 1975 J.Med.Chem. 18:905; Ganellin et al. 1976 Fed.Proc. 35:1924; Brimblecomb et al. 1978 Gastroent. 74:339] Smith-Kline French
Brittain	Glx	Designed, synthesized, and tested ranitidine. [Bradshaw et al. 1979 Br.J.Pharmacol. 66:464P; Brittain & Daly 1981 Scand.J.Gastroent. Suppl. 69:1]
Richards	Glx	Testing of many effects of ranitidine in animals and humans, comparison to cimetidine [Richards 1983 J.Clin.Gastroent. 5 Suppl. 1:81]

Clinical trials

Scientist	Affiliation	Contribution
Hirschowitz	USA	Compared efficacy and dose of cimetidine with the earlier drugs in animals; reviewed a large body of clinical trials of cimetidine [Gibson et al. 1974 Gastroent. 67: 93; ✏Hirschowitz 1979 Ann.Rev.Pharm.Tox. 19:203; Danilewitz et al. 1982 NEJM 306:20] NIH AM-09260; VA support
Walt	Frn	Efficacy and side effects of ranitidine studied, in comparison to cimetidine [Walt et al. 1981 Scand.J.Gastroent. 16(Supp 69):81; Walt et al. 1981 Gut 22:49 & 319; Walt et al. 1981 Gastroent. 80:1311]
Boyd, Peden	Frn	Efficacy and side effects in comparison to cimetidine [Peden et al. 1979 Lancet i:690; Boyd et al. 1980 Gut 21:A922; Boyd et al. 1981 Scand.J.Gastroent. 16(Supp 69):81; Peden et al. 1981 Scand.J.Gastroent. 16:325]
Langman	Frn	Comparison of ranitidine and cimetidine for several activities [Langman et al. 1980 Br.Med.J. 281:473; Henry et sl. 1980 ibid 281:775; Langman et al. 1981 Scand.J. Gastroent. 16(Supp 69):115]

Gibinski	Frn	Large scale trial for efficacy of ranitidine for ulcer; reviewed multicenter trials [Gibindki et al. 1981 Hepatogastroent. 28:216; ✏Gibinski 1981 Curr.Med.Res.Opin. 7:516]
Conner,Sawyer	USA	Thorough review of early clinical trials for ranitidine [✏Berner et al. 1982 Clin. Pharm. 1:499]
ADIS	Frn	ADIS Drug Information Services, Auchland, New Zealand - Thorough review of large scale ranitidine trials up to 1989 [✏Grant et al. 1989 Drugs 37:801]

Helicobacter pylori

Scientist	Affiliation	Contribution
Davies		Urease is of bacterial origin [Delluva et al. 1968 Biochim. Biophys. Acta [illegible]:646]
Colin-Jones, Steer	Frn	Isolated bacteria from ulcer patients, but did not conclude they were causative [Steer & Colin-Jones 1975 Gut 16:590]
Marshall	Frn/USA	★Isolated and cultured H. pylori from stomach of ulcer patients; continued experiments proving it is the cause of ulcer and gastritis. At UVA: diagnostic assay; virulence determinants and growth requirements discovered; clinical trials of several antibiotic treatments [Marshall & Warren 1983 Lancet i:1273; Marshall et al. 1985 Med.J.Austr. 142:439; ✏at UVA: Marshall 1989 Gastroent.Clin.Biol. 13:50B; Marshall et al. 1990 Gastroent. 99:697; Marshall 1991 J.Gastroent. Hepat. 6:121; Marshall et al. 1993 Dig.Dis.Sci. 38:1674; Peura et al. 1996 Am.J. Gastroent. 91:233] NIH S07RR-05431
Tytgat	Frn	H. pylori makes the urease; early clininal trials of antibiotic therapy [Langenberg et al. 1986 Lancet 1:1348;✏Tytgat & Rauws 1987 Aliment. Pharmacol.Ther.1:527S]
Wise, McNulty	Frn	Devised a simple assay of urease to diagnose H. pylori [McNulty & Wise 1985 Lancet 1:1443]
Graham	USA	Many clinical studies on epidemiology and antibiotic efficacy; devised simplified assay for H. pylori infection; studied its genetics, and identified cellular receptors and bacterial proteins involved in infection [Graham et al. 1987 Lancet 1:1174; Evans et al. 1988 Infec. Immun. 56:2896; Graham et al. 1989 Gastroent. Clin.Biol. 13:84B; Evans et al. 1993 J.Bacteriol. 175:674; al-Assi et al. 1995 Am.J.Gastroent. 90:1411; Yousfi et al. 1995 Alim.Pharmacol. Ther. 9:209] NIH M01RR-00350; R01DK-39919; Veterans Administration

Acyclovir (Zovirax)

Acyclovir is used for the treatment of herpes simplex virus (HSV) infection. Acyclovir is not only a much more effective inhibitor of HSV replication than previous antiviral drugs, it was the first to specifically inhibit replication of the virus without interfering in the cell's replication. Because of this specificity, and unlike the earlier nonspecific antivirals, its toxicity to the patient is very low. The main areas of research used by Burroughs Wellcome (BW) scientists to produce acyclovir were the virology of HSV, the characterization of the enzymes of DNA replication, and the synthesis and use of nucleoside analogs as antimetabolite drugs. The development of several specific cell culture techniques for growing and testing the virus were necessary as well. The discovery of acyclovir was based on acquired scientific knowledge rather than on the observation of an unexpected action by another drug or the action of a naturally occurring substance. Only when the understanding of HSV and of DNA replication and cell division had reached the point where a degree of rational design could be used was the first generation drug, and later acyclovir itself, produced.

HSV was isolated from oral and genital lesions in the 1920s. Once identified, it was shown to be widespread and responsible for several different diseases. HSV causes cold sores and a common venereal disease in a large percent of the normally healthy population. If it infects the cornea and conjunctiva of the eye, there will be recurrent outbreaks which eventually can cause loss of vision. Various neurological syndromes can occur, chiefly encephalitis. All of these symptoms are especially severe and persistent in newborns and in immunosuppressed patients, where HSV can cause massive outbreaks of sores at the local point of infection or disseminated infection throughout the body. Additionally, it may cause abortions and birth defects, and was thought at the time acyclovir was developed to be oncogenic. The clinical and basic research describing these diseases demonstrated that HSV was an appropriate target for industry antiviral drug development.

Around 1930, a European scientist showed that cold sores were the result of a virus, and people with recurrent outbreaks had antibodies against this virus in their blood. In the 1960s, the structure of the HSV particle and its mechanism of budding from cells was described. Researchers in NIH-supported laboratories found that HSV enters neurons through their endings in the skin, remains permanently latent in the central nervous system, and reactivates from these neurons to produce the skin lesions and other complications. European scientists first reported that HSV's genome is a large double strand of DNA. Subsequently the viral DNA was sequenced and mapped, and the mechanism of its replication was described. A large number of genes were discovered, and their expression was studied and related to phases of the viral life cycle. HSV was found to encode its own enzymes for DNA replication, rather than using cellular enzymes as some viruses do. The two viral enzymes which are specifically inhibited by acyclovir were among those that were detected, purified and characterized. This understanding of HSV's life cycle was gained through research in publicly funded academic laboratories mainly but not entirely in the US. The knowledge and methods developed in these labs permitted testing of acyclovir for efficacy and mechanism of action, diagnosis in patients, and appropriate application of the drug to the particular manifestations of HSV infection.

During this time, the details of the synthesis of DNA by dividing cells were being discovered. In the early 1960s, the enzyme activities involved DNA replication were detected. Over the following ten years, the details of DNA replication were worked out, and the cellular enzymes DNA polymerase (pol) and thymidine kinase (TK) were extensively characterized. DNA pol adds one nucleotide at a time to the growing DNA chain during replication, while TK is one of a number of enzymes which prepare the nucleotides in the form which DNA pol can use. Assays for measuring the activity of these enzymes were developed as this research proceeded. Closely following the progress with the cellular enzymes, HSV researchers detected and purified the TK and DNA pol made by the virus, which are the enzymes that are inhibited by acyclovir. This work

was mainly performed in NIH-funded academic research labs, several of which were referenced in BW literature for techniques of enzyme assay and purification.

Monolayers of cells that were shown to be susceptible to HSV, as well as embryonated chicken eggs, were used to detect HSV growth and isolate its enzymes. These methods were also used by the industry scientists to test the inhibition by antivirals, and cell culture and plaque assay were used to test numerous compounds at different concentrations and conditions. Then, appropriate animal disease models that mimic the natural disease were used in the next phase of testing. All these assays and models were developed in academic research labs. The BW scientists conducted extensive tests of the activity, efficacy, and concentration of acyclovir in established cell lines, using their own modifications of previously developed assays.

A great deal of research during the 1940s and 1950s was being directed towards developing antimetabolite drugs - chemicals that poison growing cells - because of their potential usefulness against cancer. The nucleotide bases were known by this time to be the constituents of DNA, and in 1954 a team of scientists at a university in the U.S. showed that tumor cells incorporate nucleotides more rapidly than do normal cells. Interest began to grow in nucleotide analogs - nucleotides with a chemical modification - as antimetabolite drugs which could inhibit DNA synthesis. The idea was that an analog which could be incorporated into the growing chain of DNA but would then block any further elongation of the chain would mainly kill rapidly growing tumor cells. Two different U.S. academic scientists developed the firsttwo nucleotide analog drugs for treating cancer. Soon after, in 1959, another publicly funded U.S. researcher synthesized the nucleotide analog idoxuridine, which eventually was found to have antiviral activity when applied to skin infected with HSV. This was the first clinically effective antiviral drug. However, its usefulness was limited by its high toxicity, since it acted by inhibiting DNA synthesis and therefore affected the cells of the body as well as the virus.

Development of these drugs by the academic scientists interested BW in nucleotide analogs as replication enzyme inhibitors. Along with the growing understanding of enzymology and the enzymes of DNA synthesis, scientists at BW were extensively researching the enzyme adenosine deaminase, and designing and testing inhibitors of it. They found that a part of the chemical structure of nucleotides which is required for normal DNA synthesis is not required for a nucleotide analog to enter the first step of the reaction, binding to the enzymes. Therefore, these analogs offered a means of inhibiting the enzymes. One of the potential inhibitors designed and tested by this team, the nucleotide analog acycloguanosine (acyclovir), was found in the UK labs of BW to have excellent and highly specific activity against HSV.
In 1977, BW scientists published the article detailing their tests of the selective action of acyclovir on HSV growing in cell cultures. To define and test the mechanism of action of acyclovir, understanding of the existence and mechanism of DNA pol and TK, and the discovery that HSV makes its own enzymes with distinct properties, was needed. The BW team demonstrated that acyclovir acts preferentially to inhibit viral but not cellular enzymes in two ways: first, only the viral TK activates the drug to a usable form; second, the viral DNA pol is inhibited approximately 3000-fold over the cellular DNA pol. This paper referenced 33 articles, 20 articles by researchers at United States universities receiving NIH and NSF support; 5 articles by researchers at European academic institutions; and 8 articles by industry scientists. In 1978, a second article reported the actual synthesis of acyclovir, and it cited 13 articles, 3 from NIH supported labs, 7 from industry, and 3 by foreign researchers. In this article, 5 papers from publicly funded research were cited for nucleotide organic chemistry, techniques for which companies usually cite only their own chemical methods, not academic research.

After the production and in vitro and animal testing of acyclovir by BW scientists, clinical trials were

conducted by academic clinical institutions. Most of these were supported by a combination of NIH grants and funds from BW. The drug's efficacy was tested for different manifestations of HSV infection, in different modes of application, and for other herpes viruses. Improvements in acyclovir's effectiveness through combination with other drugs were reported. The mechanisms of acyclovir resistance in HSV strains, which arise frequently in immunosuppressed patients, were studied. BW has continued to synthesize new forms of the drug and to test them for efficacy and pharmacokinetic properties, and these also were incorporated into the research of academic virologists and clinicians. The delivery vehicle of the topical form of acyclovir changed from DMSO to polyethylene glycol to propylene glycol; the use of PEG was described by US academic scientists and the use of propylene glycol was described by European academics.

Research Areas Leading to Production and Testing of Acyclovir

USA = affiliated with academic institution or NIH in the US
Ind = industry or private foundation
Frn = foreign academic institution

BW = Burroughs Wellcome
✍ = referenced in BW's papers
★ = key article

1. Culture techniques for growing virus in cells

Scientist	Affiliation	Contribution [references] support acknowledgements
Dulbecco	USA	Invented the technique of producing viral plaques in monolayers of cells, widely used to test the antiviral activity of large numbers of compounds. [1952 PNAS 38:747-52]
Niven	Frn	Cell differentiation state and susceptibility to viral infection; first cultivation of HSV in human cells [Bang & Niven 1958 Br.J.Exp.Path. 39:317]
Scherer	USA	Detailed the use of a pure strain of mouse cells for the cultivation of virus. [1953 Amer. J. Pathol. 29:113] National Foundation for Infantile Paralysis, Inc.
Tyrell	Frn	Different cell types or stages of cell development affect susceptability to virus; defined cellular conditions required for viral growth. [Tyrell et al., 1958, Brit.J.Exp.Path.39:178; Hoorn&Tyrell, 1965, ibid 46:109]

2. Isolation and description of herpes simplex virus

Scientist	Affiliation	Contribution
Andrews	Frn	★First showed presence of antibodies in serum to herpes, and showed relationship between these and recurrent cold sores. Also, one of the first reports of growth of virus in cell cultures. [Andrews, 1929 Brit.J.Exp.Path. 10:188; Andrews & Carmichael 1930 Lancet 1:857.]
Kucera	USA	Described growth of HSV in cell monolayers. [1966, Proc. Soc. Exp. Biol. Med 122:258.] NIH grants # CA-12197 and CA-12382.
Sarov	Frn	Showed that HSV has a large, double-stranded DNA genome. [1968, Becker et al. Virology 36:184.]
Keir , Gold	Frn	First demonstrated induction of a DNA pol by HSV.Showed that the pol was immunologically distinct from the host cell pol. [Keir & Gold,1963 Biochem Biophys Acta 72:263-76; Keir et al., 1966 Virology 30:154-7.]
Roizman	USA	Defined the features of the HSV genome, and details of its gene expression. Purified HSV specific proteins, permitting immunological studies of HSV. [Kieff et al. 1971, J. Virol. 8:125; Frenkel & Roizman 1971, J. Virol. 8:591; Spear & Roizman 1972, J. Virol. 9:143] NIH grants # CA-08494 and CA-19264; NSF grants # BMS73-06940 and GB38799; U. Chicago Cancer Research Ctr. CA-14599; Amer. Cancer Soc. VC103I
Nahmias	USA	Antibodies to HSV-1 and -2 permit identification of the virus in clinical specimens. [Nahmias et al., 1970 Am. J. Epidem. 91:539.] NIH CA-11433, CC-00555, NS-22301.

Kit, Dubbs	USA	★Cited for describing the HSV thymidine kinase activity and an assay for it. [✍Kit & Dubbs '63 Biochem. Biophys. Res. Comm. 11:55; ✍Dubbs & Kit '64 Virology 22:493.] NIH grant CA-06829-02 and 06656-01; NSF GB620; Amer. Medical Assoc. ERF 71; grant from the Leukemia Society
Bastian, Tralka	USA	Demonstrated that HSV is latent in ganglia. [Bastian et al., 1972 Science 178:306.] NCI intramural
Barringer	USA	Demonstrated that HSV is latent in neurons of the sacral ganglions. [Barringer, 1974 NEJM 291:828.] VA hospital; National Multiple Sclerosis Society.
Overall	USA	In an animal model, recurrant skin lesions result from reactivation of latent virus in the nervous system. [Stanbury et al., 1982 J. Infec. Dis. 146:397.] NIH AI-42524, AI-10217.
Honess, Watson	Frn	Isolated HSV TK. [1974, J.Gen.Virol. 22:171; 1977, J.Virol. 21:584.]
Purifoy	USA	★First purification of HSV-encoded DNA pol. [Purifoy & Schaffer, 1975 J.Virol.16:498; Powell & Purifoy, 1977 J.VirolL24:618] NIH PO1CA-10893
Huang	USA	Cited for method of purifying DNA pol. [✍ 1975. J. Virol. 16:298.] NIH grants NHLI-72-2911, AI-12717, and fellowship F22 CA-04032

3. Enzymes involved in DNA replication

Scientist	Affiliation	Contribution
Monod, Jacob	Frn	Early description of mechanisms and principles of enzyme action. [Monod & Jacob1961 Cold Spr. Harbor Symp Quant. Bio 26:389.]
Maley	USA	Described cellular thymidine kinase (TK) activity [Maley & Maley 1962 Biochemistry 1:847] NIH # CA-5119; Am. Heart Assn.
Kornberg	USA	★Described several distinct DNA polymerase (pol) activities in bacterial cells, distinguished the main pol of replication, its physical properties and mechanisms, and the mechanism of its use of dNTPs [Bruttag et al. '71 PNAS 68:2826; Englund et al. '69 J. Biol. Chem. 244:3045& 3048; Deutscher & Kornberg '69 J. Biol. Chem. 244:3019.Gefter et al. '71 PNAS 68:3150.] NIH R01GM-07581
Kessel	USA	Cited for assay of levels of nucleoside phosphorylating activity. [✍Kessel, 1968 J.Biol.Chem. 243:4739] NIH grants # PH 43-66-541, C6516 (NCI)
Lerman	USA	Cited for assay of pol activity. [✍Altman & Lerman '70 J. Mol. biol. 50:235] NIH GM-13767; NSF GB-4119; Altman was supported as a University Fellow at U. Colorado.
Livingston	USA	★Purified the main polymerase of cellular replication and described features of its mechanisms. [Livingston et al. 1975 J.Biol.Chem.250:] NIH grant # AI-06045.

Cheng, Ostrander	USA	Cited for method of preparation of cytosol fractions to assay phosphorylation by TK, and for technique to purify HSV TK from cells. [Lee & Cheng, 1976 J.Biol.Chem. 251:2600; Cheng & Ostrander, 1976 ibid 251:2605] NIH grant # CA-05298, CA-13038; Amer.Cancer Soc. # CH-29

4. Nucleoside analog antimetabolite drugs and organic chemical synthesis

Scientist	Affiliation	Contribution
Cantarow, Paschkis	USA	Tumor cells incorporate nucleosides more rapidly than normal cells [Cantarow & Paschkis, 1954]
Heidelberger	USA	Designed and tested one of the first nucleoside analog antimetabolites, fluorouracil, in collaboration with Hoffman-La Roche chemists [Heidelberger et al. 1957 Nature 179:663]
Dekker	USA	Designed and tested the nucleoside analog cytarabine [Walwick et al. 1959]
Prusoff	USA	Designed and synthesized the first nucleoside analog antiviral agent, idoxuridine. [Prusoff, 1959, Biochem.Biophys.Acta 32:295.] CY-3076
Preiss, Handler	USA	Cited in early BW articles for organic synthetic methods. [Preiss & Handler 1957 JBC 225:759.] NIH grant # RG-91; contract with Atomic Energy Commission and Duke U. # AT-(40-1)-289.
Flaks	USA	Cited for synthetic methods. [Flaks et al. '57 JBC 228:201.] NIH, NCI, PHS and NSF support acknowledged but no grant #s given.
Seegmiller	USA	Cited for organic synthesis methods. [Seegmiller et al., 1967 Science 155:1682.]
Rozenberg	Fra	Cited for organic synthesis methods. [Holmsen & Rozenberg, 1968, Biochem.Biophys.Acta 157:266.]

5. Drug development and testing

Scientist	Affiliation	Contribution
Schaeffer	BW	Described the organic synthesis of acyclovir, and reported toxicity testing in animals. [Schaeffer et al., 1978, Nature 272:583-5]
Elion	BW	Described the mechanisms of action of acyclovir on viral polymerase and thymidine kinase, and demonstrated preferential inhibition of viral rather than cellular replication through specific interaction with both enzymes. [Elion et al., 1977, PNAS 74:5716-20.]
DeClercq	Fra	Extensive testing of acyclovir sensativity of different strains of HSV. [DeClercq et al., 1980, J.Inf.Dis.141:563.]

6. Clinical trials

Scientist	Affiliation	Contribution
Benjamin	USA	Cited in clinical trials for viral assay and typing in clinical specimens. [1977 J. Clinical Micro. 6:571]
Crumpacker	USA	Clinical trial of effectiveness on primary lesions. [Crumpacker et al., 1979 Antimicrob.Ag. and Chemo.15:642.] NIH grant # CA13431; grant from BW.
Corey, Nahmias	USA	Clinical trials of acyclovir. [Corey et al., 1982 NEJM 306:1313; Corey et al., 1983, Ann.Int. Med. 98:914.] NIH grants # AI-14495 and AI-20381; grant from BW.
Meyers, Wade	USA	Efficacy trials of oral and intravenous acyclovir for HSV and CMV infections, especially in immune suppressed patient; frequency of resistant HSV strains with multiple treatment cycles. [Wade et al., 1982 Ann. Intern.Med. 96:265; Meyers et al.,1982 Am.J.Med.73:229; Wade et al., 1983 J.Inf.Dis.148:1077; Wade et al., 1984 Ann.Intern.Med.100:823.] NIH CA-18029 and CA-26966.
Whitley	USA	Efficacy trial for severe and neonatal HSV infection. [Whitley et al., 1982 Amer. J. Med 73:165.] NIH grant # AI-12667, CA-13148 and RR-032; grant from BW.
Fiddian, Mindel	Frn	Efficacy in primary herpes infection. [Mindel et al. 1982 Lancet 1:697; Fiddian et al., 1983 J.Antimicrob.Chemo.12:67; Mindel et al., 1984 Lancet 2:57.].
Bryson	USA	Efficacy of oral acyclovir in genital infection. [Bryson et al., 1983 NEJM 308:916; Reichman et al., 1984 JAMA 251:2103.]
Pagano	USA	Efficacy trial for EBV [Pagano et al., 1983 J.Antimicrob.Chemo.12, SupplB:113; Pagano & Datta 1982 Am.J.Med.73:18 . NIH grant # AI-17205; grant from BW. J. S. Pagano referenced as the source of HSV for testing in Elion et al 1977.
Mertz	USA	Efficacy trial of oral acyclovir in genital herpes [Mertz et al. '84 JAMA 252:1147]

Captopril (Capoten) and Enalapril (Vasotec)

Captopril and enalapril are drugs that control hypertension by inhibiting an enzyme (ACE) that is critical in blood pressure regulation. Hypertension is a complex disorder which can be based on the malfunction of several normal mechanisms, and the primary initiating causes of it are still not entirely understood. Left untreated, hypertension causes progressive damage to kidneys, heart, and systemic blood vessels. Captopril and enalapril are highly effective in breaking the chain in the system that is involved in up to 70% of hypertension. Captopril, developed by Squibb, is a novel drug resulting from an extended research effort. Enalapril, made by Merck, is an improved ACE inhibitor based on an alteration of the chemical structure of captopril, so the development of these two drugs is combined in a single story. The discovery of ACE inhibitors began with a natural substance, but the production of captopril was one of the earliest successful examples of rational drug design using molecular modeling to provide the lead.

In addition to organic chemistry, there is one broad field of research which underlies captopril and enalapril discovery: the study of the hormones renin, angiotensin and aldosterone (the R/A/A system) and their involvement in hypertension and congestive heart failure. Also, at one small but crucial point, very basic research into enzyme biochemistry and kinetics provided the necessary insight that directly lead to the development of captopril. As early as 1898, the kidney was suspected of involvement in hypertension, and a substance extracted from kidneys which raised blood pressure in rabbits was termed renin. In the 1930s, the idea of a renal source of hypertension was revived by a US research group, who isolated renin and showed that it strongly elevated blood pressure. About 5 years later, a foreign and a US group both found that renin is an enzyme that activates another protein, which eventually was named angiotensin. Angiotensin increases blood pressure by its very powerful constricting effect on arteries and capillaries. In the mid-1950s, NIH-funded researchers were surprised to observe that angiotensin is actually two separate proteins, and an enzyme converts angiotensin I (AI) to the active form, angiotensin II (AII). They had discovered angiotensin-converting enzyme (ACE), the enzyme which is inhibited by captopril and enalapril. A number of laboratory models were developed along the way by this group, which later were used by Squibb and Merck in their drug tests. These included the use of strips of guinea pig ileum for initial test of inhibitory effect, and several rat and dog models of hypertension, for which US and foreign academic researchers are referenced in the companies' publications.

In 1962, researchers in Europe found that a snake venom had the effect of relaxing blood vessels, thereby rapidly lowering blood pressure. These scientists subsequently localized ACE to the lungs, and showed that the substance in snake venom blocked the conversion of AI to AII by inhibiting ACE. Scientists at Squibb isolated the active molecule in the snake venom and called it teprotide. Although it did not prove feasable as a human drug, it was used in many studies of the involvement of renin and AI/AII in hypertension, many of which were conducted in US academic labs. During this time, an NIH-funded scientist in the US showed that another hormone made in the kidney, aldosterone, is involved in blood pressure regulation by causing the kidney to retain sodium and increase the blood volume. In addition to its vasoconstrictive effect, this researcher and his group found that angiotensin also induces aldosterone secretion by the kidney, thereby increasing blood pressure by a second mechanism. When Squibb developed teprotide, the company scientists provided it to this academic group in the clinical trials which were the first to show that ACE inhibitors could decrease blood pressure in humans. This academic researcher and the many scientists who worked in his laboratory played a central role in working out the R/A/A mechanism of hypertension and convincing other medical researchers of its importance. They performed many animal and human studies both before and after the discovery of teprotide which were critical to the understanding of blood pressure regulation, and performed many clinical studies of captopril as well.

Another system of heart and blood pressure regulation had been discovered a few years earlier, the adrenergic system, and drugs known as "β-blockers" had been developed to control it. The concept that the R/A/A system could be the cause of hypertension at first was discounted by most researchers because the adrenergic system alone was thought to explain the condition. A large number of animal and clinical studies ensued using the β-blockers, teprotide, and several drugs which act at other steps in the R/A/A system, and the importance of renin, angiotensin, and aldosterone in maintaining normal and hypertensive blood pressure was slowly accepted by the medical community. These studies, in the large laboratory mentioned above and in several others, also revealed that the two systems are linked through the involvement of renin, and began to show the significance of hypertension in congestive heart failure. Several important assays, for renin, aldosterone, and the relative concentration of AI and AII, were developed along the way. The majority of these studies came from US academic labs receiving public funding, with several foreign academic ar Squibb group contributions as well. Since market potential is needed for a company to begin an R&D effort, the increasing evidence of the importance of the R/A/A system in hypertension, kidney and heart disease was a factor in the decisions of Squibb and later of Merck to push their efforts to develop ACE inhibitor drugs.

The Squibb group worked for the next six years to improve on teprotide. Their breakthrough idea came when a US academic lab with NIH funding discovered an inhibitor of a bovine enzyme that is related to ACE. They published structural data and a model proposing that the inhibitor fit into the enzyme's active site. The scientists at Squibb made a chemical series based on the inhibitor, and found one that weakly inhibited ACE. Finally, they designed a model of ACE and inhibitor interaction based on the structural model of the bovine enzyme. The necessary data for the Squibb group to make this model came from the studies of another NIH-funded US academic lab on the structural and catalytic properties of ACE. The Squibb group, with data from their own inhibitor studies, used computer graphics to predict inhibitor structures that would bind better to the ACE active site. From this model, they synthesized a series of potential inhibitor molecules to test, and the result was captopril.

In 1977, Squibb scientists published the description of the modeling, synthesis and initial in vitro and animal testing of captopril. They demonstrated that captopril inhibits the action of AII and lowers blood pressure in hypertensive rats. This article references 15 papers, 7 from US universities receiving NIH funding, 7 from their own or other industrial labs, and one article by researchers at a European university. The US articles were cited for background knowledge, clinical trials with teprotide, and mechanisms of ACE action and testing. In 1978, they published two detailed reviews which describe extensive enzyme activity studies and animal testing, in which they acknowledge the many academic research groups whose work they drew upon. The majority of these were publicly funded US researchers.

The first clinical trial of captopril was performed by a Swiss research group in collaboration with Squibb researchers. Subsequently, captopril was used in a large number of studies revealing the fundamental relationship of AII to several aspects of hypertension and congestive heart failure. Clinical studies also showed the importance of determining whether the mechanism driving the hypertension is the R/A/A system or the adrenergic system. NIH funded researchers developed a very effective diagnostic test, using a single dose of captopril to measure the degree to which the R/A/A system is at fault, which was widely used in choosing between the ACE inhibitors, the beta-blockers, and several other types of drugs that are available. Several groups proved the importance of ACE inhibition in treating congestive heart failure. It is important to the company to know when and how the drug should be given so that its effectiveness and therefore the demand for it is maximized. The clinical trials revealed several side effects of captopril as well.

Scientists at Merck sought to modify captopril to remove some of the side effects. They made the

observation that the captopril molecule had a certain side chain shared by another drug that caused the same side effects. They synthesized a series of substitutions of this side chain, and tested them in vitro for enzyme inhibition, then in animals for effect on blood pressure. This series produced enalapril, which has slightly higher activity than captopril, with much longer duration of action. The article describing the design, synthesis and initial testing of enalapril appeared in 1980. It contained 20 references, 9 to NIH funded labs, 9 to their own and Squibb's publications, and two to foreign researchers. US research papers were cited for background knowledge of R/A/A, clinical results, and chemical and enzymatic methods.

The effectiveness of ACE blockade for lowering blood pressure was already proved as a concept with teprotide and captopril. Clinical trials of enalapril were performed in both US publicly funded labs and in foreign medical institutions. It was shown to be effective in lowering blood pressure, while lacking the side effects of captopril. A US team saw that with long term use of enalapril, a persistent blockade of the R/A/A system and therefore improvement in the hypertensive cycle occurs. The US researchers performing clinical trials were supported by a combination of NIH grants and money from Merck.of obesity, alcoholism, premenstrual syndrome, and various phobias and mental disorders.

Research Leading to the Development and Use of Captopril and Enalapril

USA = affiliated with academic institution or NIH in the U.S.
Frn = foreign academic institution
SQ = Squibb researchers
MK = Merck researchers

✍ = referenced in SQ or MK papers
★ = key contribution
✏ = review article

Renin, Angiotensin, Aldosterone, and Hypertension

Scientist	Affiliation	Contribution [references] support acknowledgements
Tigerstedt, Bergmann	Frn	Described a substance from the kidney, renin, which elevates blood pressure. [Tigerstedt & Bergman 1898 Skand.Arch.Physiol. 8:223]
Goldblatt	USA	Isolated renin and proposed a renal origin of hypertension. [Goldblatt et al., 1934 J.Exp.Med. 59:347] American Medical Assn. grant
Page	USA	Renin acts as an enzyme to increase blood pressure by converting a substance in blood, later seen to be angiotensin; AII stimulates the heart and effects other tissues [Kohlstaedt et al. 1938 Proc.Soc.Exp.Biol.Med. 39:214; Page & Olmstead 1961 Am.J.Physiol. 201:92] Cleveland Clinic Foundation
Munoz	Frn	Discovered angiotensin and showed that renin is an enzyme that activates it in the blood. [Munoz et al. 1939 Nature 144:980; Braun-Menendez et al. 1940 J.Physiol. 98:283]
Skeggs	USA	★Discovered and characterized ACE, as cited in SQ and MK papers; cited by MK for method of purifying ACE [✍Skeggs et al. 1954 J.Exp.Med. 99:275; Skeggs et al. 1958 J.Exp.Med. 108:283; ✍Dorer et al. 1974 Circ.Res. 34:824; Skeggs et al. 1976 Am.J.Med. 60:737] NIH R01HL-17243
Ferreira, Vane	Frn	★Substance in snake venom relaxes blood vessels, from which teprotide was derived. Localized ACE and showed that the snake venom substance inhibited it. [Ferreira 1965 Br.J.Pharma.Chemother. 24:163; Ng & Vane 1967 Nature 216:762; Bakhle 1968 Nature 220:919; Collier et al. 1973 Lancet 1:72]
Laragh	USA	★Role of renin, angiotensin and aldosterone in hypertension, and many details of the system. Importance of aldosterone induction by angiotensin. Rat model of renal hypertension. Use of captopril to test the underlying cause of hypertension [Laragh et al. 1960 J.Clin.Invest. 39:1091; ✍Gavras et al. 1975 Science 188:1316; Laragh 1978 Prog. Cardiovasc.Dis. 21:159; Niarchos et al. 1979 Circ.Res. 45:829; Cody & Laragh 1982 Am. Heart J. 104:1184] NIH # HL-18323; P17HL-14148; M01RR-00645
Case	USA	Role of renin and aldosterone in hypertension; comparison of different types of drug treatment for hypertension; worked closely with Laragh [Case et al. 1976 Am.J.Med. 61:790; Case 1977 NYState J.Med. 77:2100; Atlas & Case 1981 Clin.Endocrin.Metab. 10:537; Atlas et al. 1983 Am.J.Nephrol. 3:118] NIH P50HL-18323
Tree, Robertson	Frn	Relationship of renin, angiotensin, and aldosterone in hypertension; significance of salt regulation; developed several widely used assays for renin and angiotensin in blood serum [Brown et al. 1964 Biochem.J. 93:594; Davies et al. 1973 Lancet 1:683; Waite et al. 1973 J.Endocrin. 57:329; Lebel et al. 1974 Lancet 2:308]

Davis	USA	Aldosterone is involved in hypertension and congestive heart failure; it is induced by renin and angiotensin. Angiotensin and aldosterone maintain blood pressure. [Yankipoulos et al. 1959 J.Clin.Invest. 38:1278; Carpenter et al. 1961 ibid. 40:2026; Davis et al. 1962 ibid. 41:378; Johnson & Davis 1973 Science 179:906] NIH # R01HL-10612
Erdos	USA	Exact enzymatic action and substrate specificity of ACE determined [Igic et al. 1972 Circ.Res. SuppII II-51; Oshima et al. 1974 Biochim.Biophys.Acta 350:26] NIH HE-08764; 5T01-HE-05859; Office of Naval Research contracts N00014-68-A-0496 and N00014-69-A-0385.
Soffer	USA	★Structural, catalytic and physiologic properties of ACE described; data used in Squibb's model of ACE. [✍Das & Soffer 1975 J.Biol.Chem. 250:6762; ✍✍Soffer 1976 Ann.Rev.Biochem. 45:73; Soffer & Sonnenblick 1978 Prog.Cardiovasc.Dis. 21:167] NIH # R01AM-12395; P01GM-11301; P. ...-15088; HL-21394; HL-07071
Johnson, Needleman	USA	Cited for rat model of renal hypertension. Role of autonomic nerves versus renin & angiotensin in hypertension [✍Douglas et al. 1976 J.Pharm.Exp.Ther. 196:35] HE-14397; He-14509; RR-5418-12; HL-19586; training grant GM-02016

Drug concept, synthesis and testing

Scientist	Affiliation	Contribution [references] support acknowledgements
Wolfenden	USA	★Cited by SQ for the model of an enzyme and its inhibitor which was used to model and design captopril [✍Byers & Wolfenden 1973 Biochemistry 12:2070] NIH #R01-GM-18325
Rubin	SQ	ACE inhibitors can lower blood pressure in rats. Efficacy and dose range of captopril in animals [Engel et al. 1973 Proc. Soc.Exp.Biol.Med. 143:483; Rubin et al. 1978 Prog.Cardiovasc.Dis. 21:183]
Ondetti, Cushman	SQ	Modelling, synthesis, and testing of captopril [Ondetti et al. 1977 Science 196:441; Prog. Cardiovasc. Dis. 21:176,183]
Patchett	Mk	Synthesized and described the action of enalapril. [Patchett et al., 1980 Nature 288:280]

Clinical trials

A large number of clinical trials appeared rapidly after the introduction of captopril and of enalapril. Far from all groups involved can be cited; instead, trials by groups which were heavily involved and ones by the Squibb or Merck groups are cited, as well as review articles summarizing the outcomes of many trials.

Scientist	Affiliation	Contribution, [references] support acknowledgements
Laragh	USA	First test of ACE inhibitor, teprotide, in humans. Efficacy trial comparing captopril with another type of drug for hypertension [Gavras et al. 1974 NEJM 291:817; Case et al. 1977 NEJM 296:641; Gavras et al. 1978 NEJM 298:991; Case et al. 1978 Prog.Cardiovas.Dis. 21:195; Niarchos & Laragh 1984 Am.J.Med. 77:407] NIH # HL-18318; grant from Squibb

Cohn, Levine	USA	Clinical application of assay for renin in blood; use and efficacy of teprotide, captopril, and enalapril for congestive heart failure [Cohn & Notargiacomo 1969 Am.J.Med Sci. 257:344; Levine et al. 1979 Trans.Assoc.Am.Physicians 92:203; Levine & Cohn 1982 Am.Heart J. 104:1159; Levine et al. 1980 Circulation 62:35] NIH R01HL-09785; R01HL-11533
Atkinson Robertson	Frn	Efficacy of captopril and its effect on levels of renin, angiotensin, and aldosterone; reviewed clinical studies in 1979 [Atkinson et al. 1979 Clin. Sci. 57: ✏Atkinson & Robertson 1979 Lancet 2:836]
Tarazi	USA	Clarified relationship of R/A/A and adrenergic systems; role of R/A/A in heart failure; early clinical testing of enalapril [Wollam et al. 1977 Drugs 14:420; Tarazi 1980 J.Lab.Clin.Med. 95:155; Fouad et al. 1983 J.Hyperten. 1(Supp):135; Fouad et al. 1984 Hyperten. 6:167; Bravo & Tarazi 1979 Hypertension 1:39] NIH R01HL-15837
Cody	USA	Efficacy and long term effects of enalapril tested; reviewed clinical trials comparing captopril to enalapril, and their usefulness in heart disease; longtime associate of Laragh [Cody et al. 1984 Am.Heart J. 108:81; Kubo et al. 1984 Clin.Res. 32:182A; ✏Cody 1984 Am.J.Med. 77:71] NIH M01RR-47033, 47034, and 47035

Fluoxetine (Prozac)

Fluoxetine is the most widely used drug for the treatment of depression, and is also effective for several other psychological disorders. Fluoxetine was the first "selective serotonin reuptake inhibitor" (SSRI), meaning that, unlike the previous drugs, it does not affect other signaling molecules. For this reason, it lacks the serious side effects which caused a majority of patients to stop taking the earlier antidepressant drugs. The discovery of fluoxetine by Eli Lilly is an example in which an element of rational design was made possible by research into the underlying pathology of mental illness, combined with some very astute observation which twice prompted the selection of a chemical for screening. Fluoxetine is also a good example of academics and industry working closely as partners, because drugs provided by industry scientists permitted advances in understanding of neurotransmission and depression by academic researchers, which in turn suggested the next step in drug design. As for most drugs, the methods of organic synthesis were a necessary background for the development of fluoxetine, and numerous cell culture and animal models were required for its testing. Three other research areas were involved also: the molecular basis of neuronal signal transmission, the neurochemical basis of depression, and surprisingly, research on blood pressure and antihistamine drugs.

The initial observations of mood altering activity came as side effects in the search for drugs with antihistamine effects. These antihistamines provided the chemical basis for both the earliest antidepressive drugs and for fluoxetine itself. Early in this century, a French surgeon, along with scientists at Rhone-Poulenc, observed that antihistamine drugs which stabilized blood pressure also elevated the mood of surgical patients. Based on these results, a Swiss doctor tested a series of antihistamine drugs provided by the Geigy Company for a number of psychiatric conditions, and in 1958 found one which had pronounced antidepressive activity. This was imipramine, the first of a series of antidepressive drugs which were widely used in key studies that determined some of the physiological basis of depression. Those studies were closely tied to the developing knowledge of neuronal signaling mechanisms, and together they permitted a focused search by Eli Lilly scientists. When the Eli Lilly team began their search for a drug with a more specific action against depression, they selected another antihistamine drug as the chemical basis, diphenhydramine (Benadryl), which was developed in an NIH-funded university in the U.S. Eli Lilly synthesized a series of molecules from this basic structure and tested them with assays developed by researchers studying neurochemistry and psychiatry.

The discovery of many cardiovascular, antihistamine, tranquillizing and antidepressive drugs is interrelated because they all act on the substances which nerve cells (neurons) use to transmit stimulatory or relaxing signals. The neurons in the autonomic nerves, which regulate organ function, use some of the same signal molecules used by neurons in the brain. These substances were first discovered around the turn of the century, and later came to be known as neurotransmitters. In the mid 1950s, serotonin was simultaneously found by U.S. and foreign academic researchers. In the early 1960s, norepinephrine (NE), dopamine, and later serotonin, were identified as neurotransmitters in the brain. It was discovered that when a neuron sends a signal, it releases a neurotransmitter into the synapse, where the neuron receiving the signal picks it up. Then, the signal is terminated by "reuptake" of the excess neurotransmitter at specific sites on the first neuron. If this reuptake is blocked, the availability of the neurotransmitter is increased. It is at the step of blocking the reuptake site that fluoxetine has its effect. When it was discovered that different neurotransmitters are localized to different, specific neuronal systems in the brain, scientists realized that these molecules played distinct roles in mental function. These are the basic features of brain physiology and the basis of depression that were understood at the time and were required to determine the mechanism of imipramine action. All of this research was performed in academic institutions in the US and in Europe. The majority of important findings on neuronal signaling were performed in US laboratories funded by NIH grants. The key process of reuptake upon which fluoxetine is based was discovered by a Nobel-winning scientist at the NIH.

As the neurotransmitters were discovered, scientists also began to recognize their relationship to various psychiatric conditions. In early studies, before their function was understood, NE and dopamine were found in the urine in different amounts in normal, depressed or manic patients, which first suggested their involvement in mental states. Later, clinical improvement in depressed patients treated with imipramine correlated with an increase in NE excretion. During the 1960s, U.S. and foreign researchers who were studying the mechanism of imipramine action saw that this drug increased the concentration of NE and serotonin in the neuronal synapses of the brain by inhibiting their reuptake. Eli Lilly scientists began to look for an antidepressive drug without imipramine's side effects on the heart and other functions. Two sets of observations led them to seek a drug that would preferentially inhibit serotonin but not NE reuptake. First, serotonin was being increasingly recognized as an important brain neurotransmitter, and its levels were low in the cerebrospinal fluid of depressed patients. Second, in testing the mechanism of action of a series of drugs based on imipramine, increasing inhibition of serotonin reuptake, not NE reuptake, correlated with antidepressive effect. Both sets of observations were made by US publicly funded and foreign academic scientists in about equal proportion.

A review article [Schildkraut, 1973 Ann.Rev.Pharmacol. 13:427] written one year before the appearance of fluoxetine by a US academic scientist who was a major contributor to the field gave a thorough view of biochemical psychiatry at the time Eli Lilly scientists began their work. By 1970, it was known that drugs that had an effect on mania or depression increase the concentration of one or another of the neurotransmitters at certain sites in the brain. The article documents the large amount of interest in the various neurotransmitters and their involvement in these mental conditions, and the large number of studies involved in revealing the details. Also shown is the great number of studies and clinical trials attempting to alter these states by adding the deficient neurotransmitters, and to understand how the body processes the added neurotransmitters and the drugs. Once again, US and foreign labs were cited in approximately equal numbers. Eli Lilly researchers also were cited for experiments with several antidepressant drugs.

In addition to the foundational scientific knowledge that underlies fluoxetine development, many critical laboratory systems and assays were developed in the academic research labs. Several behavioral tests for depression or stimulation in rats and mice permitted the assay of potential drug series. The method of preparing "synaptosomes," a specific fraction of nerve tissue, permitted all of the work on reuptake inhibition, as well as all of Eli Lilly's drug testing. This method was initially developed in a US lab receiving NIH grants. Several assays and imaging methods were developed by both US and foreign academic scientists to measure the amount of neurotransmitter at specific sites in the brain. Many of these assays were used in testing fluoxetine prior to its FDA approval.

Experiments on the physiological responses to the antihistamine diphenhydramine were conducted by Eli Lilly and foreign researchers. These studies suggested that this drug might have the desired effects on NE and serotonin, and led the Eli Lilly team to use it as the chemical basis for synthesis and screening. Eli Lilly had also been studying serotonin action in rat brain and the effects of several earlier drugs on this system, drawing upon the methods and findings of US and European researchers. They established very detailed quantitative comparisons of the effects, which became standards for future comparisons of fluoxetine activity. Eli Lilly's documentation of the synthesis and preliminary testing of fluoxetine was first published in 1974. This paper cited 17 references, 7 from publicly funded US labs, 8 from foreign academic labs, and 2 of their own papers. One of the latter two references was for the major testing method using synaptosomes, mentioned above, and cited their own work for modifications to the procedure. The US labs were referenced for background knowledge in both neuronal transmission and biochemical psychiatry, for methods, and for means of analyzing and comparing drug activity.

Clinical trials were published by US and European academics and by Eli Lilly scientists. The drug's efficacy was tested and compared with other antidepressants. A standard scale to score symptoms of depression for severity and improvement was referred to in many of these trials, which was developed by NIH scientists. Trials included determination of dose range, tissue distribution, effects in different patient populations, side effects, effects of long term treatment, and other features which relate to the clinical use of the drug. Fluoxetine received FDA approval for use to treat depression in 1988. Many clinical studies were conducted after that for treatment

Research Leading to the Development and Use of Fluoxetine

USA = affiliated with academic institution or NIH in the U.S.
Frn = foreign academic institution
EL = Eli Lilly researchers
Ind = other industry researchers

✍ = referenced in EL papers
★ = key contribution
✏ = review article

Antihistimine Drugs as Antidepressants

Scientist	Affiliation	Contribution [references] support acknowledgements
Laborit, Charpentier, Courvoisier	Frn/ Ind	First observed mood-a!' :ing effect of an antihistimine, and developed the first psychoactive drug [Charpentier, 1947 Comptes Rendus 225:306]
Kuhn	Frn	★Tested drug series based on antihistimine, and developed imiprimine. [Kuhn, 1958, Am.J.Psychiat.115:459]
Rieveschl	USA	Developed the antihistimine diphenhydramine, used as the starting point for fluoxetine. [

Neurotransmission

Scientist	Affiliation	Contribution
Axelrod, Kopin	USA	★Mechanism of neurotransmitter signal termination by reuptake; action of imipramine and other early antidepressants in blocking reuptake. Cited for assay of drug effect on reuptake of NE [Axelrod & Tomchick 1959 Nature 184:2027;✍Hertting et al., 1961 J.Pharmacol.Exp.Ther. 134:146; Herttig et al., 1961 Nature 189:66 Glowinsky et al., 1965 J.Neurochem. 12:25; Axelrod, 1965 Rec.Progr. Hormone Res. 21:597] NIH intramural Z01MH-00401-4; Z01MH-00421-3
Page	USA	Discovered serotonin, simultaneously with Gaddum [Twarog & Page 1954 Am.J.Physiol. 175:157] Cleveland Clinic Foundation
Gaddum	Frn	Discovered serotonin, simultaneously with Page [Amin et al. 1954 J.Physiol. 126:596]
Vogt	Frn	Neurotransmitters function in brain activity, and are differently distributeded in the regions of the brain. [Vogt1954 J.Physiol. 123:451]
Iverson	USA	Function of neurotransmitters in synapses of the brain. Developed widely used method of brain dissection, brain injection, and extraction and measurement of substances. [Glowinsky & Iversen, 1966 J.Neurochem. 13:655; Iverson & Dravitz 1966 Mol.Pharmacol, 2:360] NIMH intramural
Schanberg	USA	Measurement of serotonin accumulation and uptake in synaptosomes. [Schanberg 1963 J.Pharmacol.Exp.Ther. 139:191] NIH B-940; USPHS predoctoral fellowship
Green	USA	Serotonin accumulates in brain slices and subcellular fractions, methods of measuring it. [Robinson et al. 1965 J.Pharmacol.Exp.Ther. 147:236] GM-10313; 2K3-GM-2459;5T1-GM-5903

Snyder	USA	Serotonin reuptake in different regions of rat brain, kinetic measurement, and the effects of imiprimine on the system. [Snyder & Coyle 1968 J.Pharm.Expr.Ther 165:78; Shaskan & Snyder, 1970, J.Pharm.Expr.Ther. 175:404] NIH RO1-NS-07275, PO1-GM-16492, K3-MH-33128, postdoctoral support on GM-01183.
Merrills	Ind	Imipramine blocks reuptake of serotonin, as well as NE; reuptake occurs in synaptosomes [Blackburn et al., 1967 Life Sci. 6:1653] Phizer
Renyi,Ross	Frn	Relative effects of imipramine and other drugs on serotonin reuptake [Ross & Renyi 1967 Life Sci. 6:1407]

Biochemical psychiatry

Scientist	Affiliation	Contribution
Schildkraut	USA	Role of serotinin in depression, and the effects of imipramine on neurotransmitter metabolism. [Schildkraut et al., 1966 Am.J.Psy. 123:690; Schildkraut & Kety, 1967 Science 156:21; ⇨Schildkraut 1973 Ann.Rev.Pharmac. 13:427] MH-15413
Carlsson, Fuxe	Frn	★Block of serotonin reuptake is basis of antidepressive action of imipramine. Distribution of neurotransmitters in brain, the idea that they are the molecular signals of neurons, and effects of drugs on the reuptake of the neurotransmitters. [Carlsson et al., 1958 Science 127:471; Dahlstrom & Fuxe, 1964 Acta Physiol.Scand. 62:232; Fuxe 1965 ibid 64:247; Carlsson et al., 1968 J.Pharm.Pharmacol. 20:150; Carlsson & Lindqvist, 1969 ibid 21:460; Carlsson 1970 ibid 22:729; Carlsson et al., 1969 Eur.J.Pharmacol. 5:357]
Brodie	USA	A drug causing depression decreases serotonin in brain, the first link of drug action and mood to brain biochemistry. Developed widely used animal model of depression. Compared actions and effects of imipramine and its metabolites [Shore et al. 1955 Science 122:284; Pletscher et al. 1955 Science 122:374; Sulser et al. 1962 Ann.NY Acad.Sci. 96:279; Sulser et al. 1964 J.Pharmac.Exp. Ther. 144:321] NIH NIMH intramural
Goth	USA	Diphenhydramine effects on reuptake of serotonin and NE detailed, which caused the EL scientists to select it as the basis of drug series testing for SSRI action. [Isaac & Goth, 1965 Life Sci. 4:1899; Isaac & Goth, 1967 J.Pharmacol.Exp.Ther. 156:463] 5T1-GM-74203
Roth	Ind	Developed a mouse standard antidepressant assay [Barnett et al. 1969 Int.J.Neuro Pharmac. 8:73] Shering
Weil-Malherbe	Frn/USA	First observed that NE and dopamine are excreted in urine in different amounts in manic or depressed patients. Importance of serotonin in depression. Several collaborative papers with Axelrod in US. [Strom-Olsen & Weil-Malherbe, 1958 J.Mental Sci. 104:696; Weil-Malherbe et al. 1959 Science 129:1226; Whitby et al., 1961 J.Pharmacol. Exp.Ther. 132:193]
Sharman	Frn	Neurotransmitter concentrations are decreased in the cerebrospinal fluid of depressed patients. [Ashcroft & Sharman, 1960 Nature 186:1050]

Design and testing of Fluoxetine

Scientist	Affiliation	Contribution
Lineweaver, Burke	USA	Cited for method of measuring affinity constants, to test drug effects on serotonin reuptake. [✍Lineweaver & Burke, 1934 J.Amer.Chem.Soc. 56:685] USDA
Dixon	Fra	Cited for a method to determine enzyme inhibitor constants. [✍Dixon 1953 Biochem.J. 55:170]
Wong, Molloy	EL	★Selected diphenhydramine for synthesis of drug series; made and tested fluoxetine. Extensive assay of its actions and selectivity [Wong et al. 1974 Life Sci. 15:471; Wong et al. 1975 J.Pharmac.Exp.Ther. 193:804; Wong et al. 1983 Biochem.Pharmacol. 32:1287; 33,69; Wong et al. 1991 Neuropsychopharm. 5:43]
Fuller	EL	Extensive testing of fluoxetine in animals for efficacy, toxicity, and metabolism [Fuller et al. 1974 Life Sci. 15:1161; Fuller et al. 1975 J.Pharmacol. Exp.Ther. 193:796; Fuller & Wong 1977 Fed.Proc. 36:2154]
Mandell	USA	Developed assay to measure serotonin in brain, and showed increase caused by fluoxetine [Geyer et al. 1978 J.Pharmacol.Exp.Ther. 207:650] DA-00265

Clinical Trials

A large number of clinical trials appeared after the introduction of fluoxetine, as well as additional SSRI drugs and trials comparing them with fluoxetine. It would not be possible to cite all groups involved; instead, trials by groups which were heavily involved and ones from the EL team are cited, as well as review articles summarizing the outcomes of many trials.

Scientist	Affiliation	Contribution
Lemberger	EL	Specific reuptake inhibition in patients shown, and method of measuring fluoxetine potency on serotonin uptake in patients. Tissue distribution, absorption, and excretion in normal, elderly, and kidney patients; tested drug interactions [Lemberger et al. 1978 Clin.Pharmacol.Ther. 23:241; Lemberger et al. 1978 Science 199:436; Lemberger et al. 1985 J.Clin.Psych. 46:14]
Bremner	USA	Initial dose range trial for fluoxetine [Bremner 1984 J.Clin.Psych. 45:414]
Stark, Hardison	EL	Multicenter trials showed fluoxetine as effective as imipramine without side effects [Stark & Hardison 1985 J.Clin.Psych. 46:53]
Guy	USA	Manual for scoring symptoms on a depression scale measuring severity and improvement, cited in several clinical trials [ECDEU Assessment Manual for Psychopharmacology, US HEW, Bethesda, MD]
Cohn, Wilcox	USA	Fluoxetine more effective in major depression than imipramine, w/o side effects. [Cohn & Wilcox 1985 J.Clin.Psych. 46:26]
Masco, Sheetz		Fluoxetine compared with other drugs is as effective w/o side effects [Masco & Sheetz 1985 Adv. In Ther. 2:275]