In 1988 the Nobel Prize in Physiology or Medicine was awarded jointly to Sir James W. Black, Gertrude B. Elion, and George H. Hitchings “for their discoveries of important principles for drug treatment.” The abstraction of the award citation subsumed two distinct lines of research. Black’s work introduced the first members of what became new classes of drugs: beta-blockers used in treatment of cardiovascular and other conditions, and H2-blockers, used to treat acid-peptic disorders. Hitchings’ and Elion’s collaboration had yielded effective drugs for use in a remarkable variety of conditions, including cancer, gout, organ transplantation, malaria, and bacterial and viral infections.

This chapter is a first approach to a description and analysis of what may be called the Hitchings-Elion research program, which spanned more than four decades by the time that the investigators received their Nobel award. Three aspects of the Hitchings-Elion program deserve particular emphasis: its coherence and unity across almost half a century of work that engaged a variety of collaborators; its embodiment of both rational and empirical elements; and its character as industrialized research. The close interdependence of these characteristics of the program may best be appreciated by tracing its course from its beginnings in the early 1940s through its various embodiments up to the eve of its recognition by the Caroline Institute in 1988.


J.E. Lesch
University of California, Berkeley, CA, USA
email: jlesch@calmail.berkeley.edu

16.1 Formation of a Research Program

When George Hitchings joined the Wellcome Research Laboratories in Tuckahoe, New York in 1942 as “head and sole member of the Biochemistry Department,” he had already been working in the field of biochemistry for over a decade. Following bachelor’s and master’s degrees in chemistry at the University of Washington, he began graduate school at Harvard in 1928. Working in Cyrus Fiske’s laboratory in the Medical School’s Department of Biological Chemistry, Hitchings was assigned to develop analytical methods for the purine bases, a project that became his dissertation and yielded several early publications. Taking his doctorate in 1932 in the midst of the Depression, he was able to continue working for several years with temporary appointments at Harvard in cancer and nutritional research, and at Western Reserve University in electrolyte research.²

At Wellcome Hitchings was given modest resources, but also a free hand to develop his own program. He later recalled that by that time he had been interested in chemotherapy for several years, but that

academia stood, rather disdainfully, apart from all this activity, and stated that it was premature to attempt chemotherapy because there was not sufficient knowledge of biochemistry, physiology, and pharmacology to sustain any kind of meaningful operation. . . . But when we came on the scene in 1942, there was a bright, shining star on the horizon, which had arisen from the work on Prontosil and its active principle sulfanilamide, and from the recognition by Woods and Fildes that this was a case of metabolite antagonism. Thus, the antimetabolite theory was born.³

Hitchings referred here to the work of British medical bacteriologist Paul Fildes and biochemist Donald Woods. In publications that appeared in 1940 Woods and Fildes asserted that sulfanilamide, and by extension, other sulfa drugs, acted on bacteria by interfering with an enzyme that helped to synthesize a nutrient the bacteria needed for growth and reproduction. Sulfanilamide closely resembled a compound (substrate) acted upon by the enzyme to produce the needed nutrient, so sulfanilamide was able to compete with the substrate and displace it. In this way sulfanilamide prevented the formation of the nutrient and thereby blocked the growth and reproduction of the bacteria. Unable to increase in numbers, the invading bacteria were then destroyed by the defenses of the human or animal host. Woods and Fildes identified the substrate as p-aminobenzoic acid.⁴

Fildes went on to generalize these findings into a program for the discovery of new antibacterial drugs. In 1940 he published “A rational approach to research in chemotherapy” in *The Lancet*. His argument was that antibacterial substances as a group function by interfering with an essential metabolite in the bacterial cell. The kind of inhibitions produced by sulfanilamide required “an inhibitor so closely related in formula to the essential metabolite that it can fit the same enzyme, and sufficiently unrelated to be devoid of essential metabolic activity.” For Fildes, this involved the further conclusion that “chemotherapeutic research might reasonably be directed to modification of the structure of known essential metabolites to form products which can block the enzyme without exhibiting the specific action of the metabolite.” With this statement, Fildes had converted a particular, if spectacular, result with a known antibacterial agent into a proposal for a research program that might identify many others yet unknown.5

In the 1940s and 1950s other researchers put Fildes’ program into practice in the search for new antibacterial drugs. More important, other researchers were inspired to take a second step of generalization that opened up a still wider research horizon. This involved the definition of the concept of antimetabolite as a substance that interfered with the action of an essential metabolite in a living cell. This could mean bacteria (as it did for Fildes), but it could also mean other kinds of infectious microorganisms, eventually including viruses, or neoplastic (cancerous) cells that appeared within an organism.

The beginnings of a transition to the broader concept can be seen as early as 1941 in a paper by John Lockwood, and American surgeon and bacteriologist at the University of Pennsylvania. Lockwood saw reason for optimism in the Woods-Fildes theory, and said that

it is perhaps pardonable to suggest that we may be provided with a new method of approach to the treatment of cancer, a disease in which unrestrained proliferation of tissue cells is similar in some respects to the proliferation of bacteria in invasive infections. If the difference between malignant cells and normal cells should be found to be due to the local activity of some chemical growth factor, a compound of similar chemical configuration might be administered to cancer patients which would block the activity of the proliferative factor without exhibiting its physiological effects.6

After he joined Wellcome Hitchings saw an opportunity to use the expanded concept of antimetabolite to bring together in a novel way his interest in the biosynthesis of nucleic acids and a search for new chemotherapeutic agents.

---


6 John S. Lockwood, “Progress toward an understanding of the mode of chemotherapeutic action of sulfonamide compounds,” in *Chemotherapy*, University of Pennsylvania Bicentennial Conference (Philadelphia: University of Pennsylvania Press, 1941), 9–28 (on 26).
Research following the Woods-Fildes theory had shown that sulfanilamide was antagonized not only by p-aminobenzoic acid but also by the bases of the nucleic acids and by some amino acids, in certain combinations. Growth factors (later called folic acid) involved in the synthesis of purine and pyrimidine bases had also been identified. Hitchings reasoned that preparation of synthetic analogs of the purine and pyrimidine bases might provide antimetabolites that would serve at the same time as tools for the biochemical study of nucleic acid synthesis and as potential chemotherapeutic compounds. “It seemed that this was a fertile field to explore,” he later recalled, “and that one might use the antimetabolite principle to explore folic acid’s enzymes and metabolic pathways. We felt that it was highly probable that, in the course of these explorations, we would discover exploitable information that could be used in chemotherapy.”

To implement this project Hitchings little by little assembled a small group of collaborators. His first recruit was Elvira Falco, then an assistant in Wellcome’s Bacteriology Department. Hitchings and Falco together designed a system to screen purine and pyrimidine compounds for biological activity, using the bacterium *Lactobacillus casei*. Gertrude Elion, a chemist, joined the group in 1944, and concentrated mostly on synthesis of purine analogs. In 1947 Peter B. Russell arrived from Cambridge University, bringing expertise in organic chemistry and some familiarity with medicinal chemistry.

Hitchings later recalled that when this project began, “none of the enzymes and metabolic pathways toward the nucleic acids were known.” Nevertheless the black box screening system devised by himself and Falco using *L. casei* quickly yielded promising results. *L. casei* would grow either on a growth factor (folic acid) or on a mixture of purine and the pyrimidine thymine. The system was set up so that it could show either stimulation effects or antagonistic effects of analogs of bases of the nucleic acids. Early screening revealed that analogs could be found that had a marked inhibitory effect not only on *L. casei*, but also on some pathogenic bacteria. Encouraged by these results, Hitchings and his colleagues expanded the biological screening procedures, and added toxicity testing on growing rats.

A few others joined the Hitchings research group in the mid-1940s, but the number remained small, and all shared a single large laboratory. Fortunately,
collegial relations were friendly. “Under the leadership of Falco,” Hitchings later recalled, “a constant flow of banter developed covering a wide range of subjects and degrees of seriousness. We never had any obstacles to interpersonal communication.”

Encouraged by the results of expanded biological screening using the *L. casei* system, Hitchings in 1947 entered into arrangements with two outside entities for expanded testing of the purine and pyrimidine analogs being prepared in his laboratory. One of these was with the Sloan Kettering Institute in New York, which would test compounds for antitumor activity using the sarcoma 180 model in mice. The other was with laboratories that would conduct expanded antibacterial and antimalarial testing.

In addition to making possible increased numbers of tests for antitumor activity, the connection with Sloan Kettering benefited Hitchings’ research group in other ways. Impressed with the potential of the compounds and associated biological information coming from the Wellcome team’s work, Cornelius P. Rhoads, the Sloan Kettering director, offered the group increased financial support. This assistance, which continued into the early 1950s when it was replaced by internal money from Burroughs Wellcome, allowed for a doubling of the number of members of Hitchings’ group, to a total of around fifteen people. The link with Sloan Kettering also led to contacts with researchers and clinicians that proved valuable as the research proceeded.

One of the first compounds sent by the Hitchings group to Sloan Kettering for testing in 1948 was 2,6-diaminopurine, synthesized by Gertrude Elion. Sloan Kettering researchers found it to be active in sarcoma 180 tests in mice, and clinical trials conducted by Joseph H. Burchenal at Memorial Hospital gave promising results in treatment of patients with leukemia. Hitchings later recalled that these early results were “sufficient to establish cancer chemotherapy as a continuing primary goal of our group.”

The early findings on 2,6-diaminopurine were also one of the first visible results of Gertrude Elion’s concentration on the chemistry and metabolism of purines, an assignment she had taken on not long after joining the Hitchings group. The daughter of immigrant parents, Elion had followed education in New York City public schools with four years at Hunter College, where she graduated in 1937 with a major in chemistry. Unable to afford graduate school, she found jobs scarce, and as she later recalled, “the few positions that existed in laboratories were not available to women.” After working in a temporary

---

10 Hitchings, Autobiography (ref. 2).
11 Hitchings, “Selective inhibitors” (ref. 7), 476–477; and Hitchings, Autobiography (ref. 2).
12 Hitchings, Autobiography (ref. 2).
teaching position and as an unpaid laboratory assistant, she began graduate studies in chemistry at New York University in 1939, supporting herself by teaching in New York City secondary schools. With her masters degree in hand in 1941, she spent a year and a half doing routine quality control work for a food company, then six months in a laboratory at Johnson and Johnson in New Jersey. When the latter position ended, she found herself with multiple job offers from research laboratories. Among these was an invitation to join the Hitchings group, which she accepted in 1944.14

The decision to join the Wellcome Research Laboratories proved decisive for Elion’s career. In Hitchings’ group she found a work environment that gave full scope to her drive and intellectual ambition. Encouraged to learn and to take on increasing responsibility, she found her opportunities quickly expanding. “From being solely an organic chemist, I soon became very much involved in microbiology and in the biological activities of the compounds I was synthesizing,” she later recalled. “I never felt constrained to remain strictly in chemistry, but was able to broaden my horizons into biochemistry, pharmacology, immunology, and eventually virology.”15

That this was the case was no doubt due in part to Hitchings’ own qualities as colleague and research manager. The small size of his research group, especially in the early years before it acquired support from Sloan Kettering, was also a factor, since it mitigated against a highly specialized division of work. Equally or more important were the specifically industrial goals of the research, which aimed not simply at new biochemical knowledge, but also at the development of effective chemotherapies. Implementation of such goals called for use or creation of whatever kinds of knowledge, skills, or instruments could be brought to bear on the problems, regardless of their provenance in specialized academic fields.

The modest but unmistakable success of 2, 6-diaminopurine brought Elion and the Hitchings group squarely into the emerging field of cancer chemotherapy. In 1948, the same year that the Wellcome Research Laboratories sent 2, 6-diaminopurine to Sloan Kettering for testing. Sidney Farber and his colleagues at the Children’s Medical Center in Boston published a paper reporting promising results in treatment of acute leukemia in children, using aminopterin, a folic acid antagonist. Farber was careful in his conclusions, stressing the small number of patients in the study, the temporary character of the remissions obtained, and the toxicity of the compound. With these reservations, he nevertheless saw in his results “a promising direction for further research concerning the nature and treatment of acute leukemia in children.”16

15 Elion, “Autobiography” (ref. 14), 967.
In his article Farber credited the contributions of researchers in the Lederle Laboratories and the Calco Chemical Division, both components of the American Cyanamid Company, “who are responsible for the chemical research that made possible these studies on children.” Behind this acknowledgment lay several years of a collaboration between industrial and clinical researchers that was distinct from, but that in some respects paralleled, the collaboration that had begun to develop between the Hitchings group and Sloan Kettering.\textsuperscript{17}

The involvement of Lederle Laboratories in cancer chemotherapy appears to have been prompted in the first instance by a collaboration that it began in 1944 with another medical researcher, Richard Lewisohn. In 1937 Lewisohn, a surgeon at Mount Sinai Hospital in New York City, had begun investigating the antitumor effects of spleen extracts, and in 1939 he had set up a screening program to identify other chemical agents that might cause regression of tumors. By 1941 he was focusing on a search for B group vitamins in yeast, and then barley, extracts. Lewisohn reported promising results in treatment of breast cancer, but an independent investigation conducted in 1943 at Memorial Hospital in New York City at the instigation of Lewisohn’s sponsor, the International Cancer Research Foundation, failed to confirm his findings. When folic acid was isolated by Lederle researchers led by Yellepragada SubbaRow in 1944, Lewisohn surmised that this compound might be the active substance in his yeast and barley extracts.\textsuperscript{18}

In 1944 Lederle researchers supplied Lewisohn with a growth factor isolated from \textit{Lactobacillus casei}, presumed to be pteroylglutamic acid (folic acid). With this substance Lewisohn obtained inhibition of cancers in mice. Further investigation, however, showed that the substance supplied was a related but distinct compound, pteroyltriglutamic acid, and that pteroylglutamic acid itself was ineffective in treatment of mouse cancer.\textsuperscript{19}

Prompted by this finding, Lederle chemists synthesized both pteroyldiglutamic acid and pteroyltriglutamic acid, naming them dipterin and teropterin, respectively. By 1947 the Lederle researchers had begun a collaboration with Farber, and the compounds were passed on to him for clinical testing. In a preliminary clinical report published in late 1947, Farber called for further investigation of teropterin in clinical trials. He also noted in this report and in his 1948 paper that treatment with either dipterin or teropterin accelerated the leukemic process in patients, in comparison to patients not so treated. Based on this finding, Farber suggested two distinct therapeutic approaches. One of these would make use of the acceleration phenomenon by following administration

\textsuperscript{17} Farber, “Temporary remissions in acute leukemia in children” (ref. 16), 787, 793.


\textsuperscript{19} Sneader, \textit{Drug Discovery} (ref. 18), 249.
of dipterin or teropterin with radiation or nitrogen mustard therapy. The other would employ treatment with folic acid antagonists supplied by the chemists. Among these antagonists was aminopterin, the subject of Farber's 1948 paper.²⁰

Behind the synthesis of aminopterin was an effort on the part of chemists at Lederle Laboratories, and also at laboratories in Bound Brook, New Jersey that were part of the Calco Chemical Division of American Cyanamid, to prepare other folic acid analogs as possible antagonists of folic acid. The beginnings of this program remain to be clarified. Part of the background lies in the expansion of pharmaceutical research and production by American Cyanamid beginning in 1936, when Calco set up a new pharmaceutical division to conduct research on sulfonamides and built the first American pilot plant for production of sulfanilamide. American Cyanamid became a leader in the sulfa drugs field, manufacturing not only sulfanilamide, but also sulfapyridine (under license from the British firm May & Baker), sulfathiazole, and sulfaguanidine, and in 1940 introducing sulfadiazine, all of which were heavily used during World War II. In 1937 American Cyanamid set up new general research laboratories in Stamford, Connecticut, and from this time on the company's pharmaceutical research involved collaborations of Bound Brook with either Lederle (at Pearl River, New York) or Stamford.²¹

One glimpse of the evolving interest in the antimetabolite concept within American Cyanamid by the mid-1940s may be found in a paper published by Richard O. Roblin, Jr. in 1946. A chemist in the Chemotherapy Division of the Stamford Research Laboratories, Roblin set out to survey current literature on what he called metabolite antagonists, remarking that “the concept that substances chemically related to a metabolite may interfere with the normal function of that metabolite in living cells is attracting widespread interest among chemists and biologists.” In an article of 122 pages that included 471 references, Roblin summarized work to date (the paper was received for publication in December, 1945) on antagonists of vitamins, hormones, and cell metabolites, crediting the Woods-Fildes theory as the stimulus to many of these

---


investigations. He concluded with remarks that closely paralleled the views that the Hitchings group was putting into practice:

Since in many respects it is a relatively new and rapidly developing field, it is not possible to assess all the implications inherent in the broad concept of metabolite antagonists. However, as an approach to the mechanism of action of a number of drugs, as a guide in the synthesis of new therapeutic agents, and as a means of evaluating the normal mode of synthesis and function of metabolites in living cells, the concept appears to offer many possibilities as yet unexplored.  

With established expertise in the biochemistry of folic acid and in pharmaceutical and organic chemistry, the researchers at Lederle and Bound Brook were well positioned to supply folic acid antagonists to clinical researchers. The first such compound supplied to Farber in 1947 was pteroylaspartic acid, which he found to have some effect in reducing numbers of leukemic cells. Exploring the effects of molecular modifications, the chemists found that replacement of the hydroxyl substituent on the 4-position of the pteridine ring by an amino group increased the potency of folic acid antagonists. One of the compounds that emerged from this discovery was aminopterin, which reached Farber in November 1947. Another was the compound synthesized at Bound Brook in the summer of 1947 and at first called amethopterin. Found to be effective but less toxic, amethopterin replaced aminopterin in leukemia chemotherapy by the early 1950s, under the new name methotrexate.

Galvanized by the promise of 2,6-diaminopurine and other findings, and with an expanded research staff made possible by support from Sloan Kettering, the Hitchings group in 1948 began to divide responsibilities for the different components of the laboratory’s research. Henceforth Falco, Russell, and Hitchings himself concentrated on pyrimidine analogs. Elion, who had already developed special expertise on purines and purine metabolism, would continue to focus her attention on purine analogs.

In each case the research would continue with two aims. First, it would try to elucidate the roles of purine and pyrimidine bases in nucleic acid synthesis and thus in growth, and the part played by folic acid in the synthesis of these bases. Second, it would try to identify among the analogs new chemotherapeutic agents. The theory was that tissues that depended for survival on rapid

---

24 Hitchings, Autobiography (ref. 2).
growth—parasitic microbial or cancer cells, for example—should be especially sensitive to compounds antagonistic to substances needed for growth. The researchers expected that specific differences in the biochemistry of different kinds of cells would allow for identification of compounds with selective action, although the identity of these compounds could not be predicted in advance of biological and clinical screening. As Hitchings later put it in a revealing passage of his Nobel lecture, “by 1947, six or seven of us were pursuing this work, and the feeling in the group was, ‘Now we have the chemotherapeutic agents; we need only to find the diseases in which they will be active.’”

Although the purine and pyrimidine lines of research undertaken by the Hitchings group were closely related both temporally and conceptually, the sequence of developments within each line may best be understood by considering them in turn. In what follows we will look first at the work related to purines and purine analogs, then at research on pyrimidines and their analogs, in each case focusing on the development of new chemotherapeutic agents. Finally we will consider what may be seen as a related but distinct line of research undertaken primarily by Elion beginning in 1968, namely the search for antiviral drugs and study of their mechanisms of action.

16.2 Purines and Purine Analogs

By 1951 the group led by Elion had synthesized over one hundred purines, all of which were then screened for activity in *L. casei*. In the course of this work they had found that the substitution of oxygen by sulfur at the 6 position of the molecule in the natural purines guanine and hypoxanthine produced purine analogs that were inhibitors of purine metabolism. Two of these compounds were 6-mercaptopurine and 6-thioguanine. The *L. casei* screen showed that the inhibitory effect of 6-mercaptopurine could be reversed by hypoxanthine, a compound described by Hitchings as “more or less the core of purine metabolism.” Animal tests at Sloan Kettering showed that 6-mercaptopurine was active against a number of rodent tumors and leukemias, and in 1952 Cornelius Rhoads organized a cooperative clinical trial with around a dozen investigators, including notably Joseph Burchenal at Memorial Hospital.

Clinical results soon revealed the activity of 6-mercaptopurine in acute leukemia in children. Excited by the preliminary findings, Rhoads passed the news on to journalist Walter Winchell, and soon there were press reports that a

---

25 Hitchings, “Selective inhibitors” (ref. 7), 476.

new leukemia treatment had been found, and that Hitchings had supplied it to Sloan Kettering. Hitchings later recalled what followed:

As you may imagine, the roof fell in on me. Within two days I had 600 letters on my desk and phone calls from all over the world; we were in one terrible bind. We had limited supplies of the drug, no idea what it would cost, and no mechanism for distribution or for dealing with the many pathetic appeals that we received.

No immediate response to this demand was possible. The company did promptly file a New Drug Application with the Food and Drug Administration. Following personal visits to the sites of the clinical trials by the FDA official in charge of reviewing new applications, 6-mercaptopurine was approved for commercial release in September, 1953. Even then, production problems remained, and the clinical studies were not made public until the end of April, 1954, when the New York Academy of Sciences held a symposium on the new drug.27

When 6-mercaptopurine entered medical practice in 1953, standard drug treatment for acute childhood leukemia consisted of methotrexate and steroids. Median life expectancy for children so afflicted was three to four months, and only about thirty percent of patients lived for a year. In some individuals the disease was entirely resistant to chemotherapy. Treatment with 6-mercaptopurine raised median survival time to twelve months, and some patients treated with 6-mercaptopurine and steroids were able to remain in remission for years.28

6-mercaptopurine did not solve the problem of childhood leukemia, but did indicate a way forward. Elion, Hitchings, and their co-workers were encouraged to continue, and other cancer researchers joined the search for antimetabolites of nucleic acid bases. With the development of other drugs and of combination chemotherapy, physicians were eventually able to cure around eighty percent of patients with acute childhood leukemia.29

As the position of 6-mercaptopurine in the clinic was consolidated, Elion and her co-workers carried on with metabolic studies of the compound, hoping to find ways to improve its therapeutic properties in cancer treatment. Meanwhile, from the mid-1950s other researchers were elucidating pathways of

---

28 Elion, “The purine path to chemotherapy” (ref. 26), 449; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 844.
purine biosynthesis. Elion and Hitchings realized early on that much of the 6-mercaptopurine administered to a patient was metabolized in vivo, so that very little of the compound was excreted unchanged. Especially important was the breakdown of the medicine by the enzyme, xanthine oxidase, yielding 6-thiouric acid. Other reactions affected the sulfur on the molecule. In an effort to modify the metabolism of 6-mercaptopurine so that it would not be readily converted into other compounds in vivo, Elion’s group first introduced various substituents on the purine ring. With the exception of thioguanine, a derivative they already knew, the resulting compounds lacked antitumor activity. So they tried a different approach, adding removable “blocking groups” to the molecule’s sulfur atom, in the hope that these groups might protect the sulfur from oxidation and hydrolysis. The idea was that once inside cells the blocking group might be removed, releasing 6-mercaptopurine, and that ideally this would be effected by an enzyme specific to tumor cells.30

The most promising compound to come out of this approach was azathioprine, synthesized in 1957. Able to act as a pro-drug for 6-mercaptopurine, azathioprine also proved to have a better chemotherapeutic index than its parent compound in a mouse cancer, adenocarcinoma 755. Unfortunately its chemotherapeutic index for human leukemia was not significantly better than that of 6-mercaptopurine, ending its prospects as an improved replacement for the latter in cancer chemotherapy.31

Azathioprine might have been shelved, had it not been for the intervention of clinicians interested in a different kind of chemotherapy. At Tufts University in Boston, William Dameshek and Robert Schwartz were seeking drugs that might enable human bone marrow transplantation as a means of treating aplastic anemia, leukemia, or radiation damage, but none of the compounds they tried had succeeded in suppressing the immune response. It occurred to Schwartz that the immunoblastic lymphocytes formed in an immune response were very similar to leukemic lymphocytes. If this was so, he reasoned, might not proliferation of the cells formed in the immune response be suppressed by the same agent that suppressed proliferation of leukemic cells, that is, by an antimetabolite? With this idea in mind, he wrote to Hitchings to obtain 6-mercaptopurine, and to Lederle Laboratories to obtain methotrexate. Hitchings replied immediately with a supply of 6-mercaptopurine, but Schwartz’s letter to Lederle did not reach its destination. Hitchings later reflected that if the circumstances had been reversed, Schwartz’s experiment might have ended, since methotrexate was not active in the system he was using. Instead, a new opening appeared for Dameshek’s and Schwartz’s investigations.32

30 Elion, “The purine path to chemotherapy” (ref. 26), 449–451; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 844.
31 Elion, “The purine path to chemotherapy” (ref. 26), 451–452; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 844; Sneader, Drug Discovery (ref. 18), 253.
32 Elion, “The purine path to chemotherapy” (ref. 26), 452; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 844–845; Sneader, Drug Discovery (ref. 18), 253.
In a series of experiments Schwartz showed that in rabbits injected with a foreign antigen, such as bovine serum albumin, immune response was suppressed by 6-mercaptopurine, and that the suppression effect was strongest when 6-mercaptopurine was given at the same time as the antigen. In the latter case the lack of response could persist for weeks. He also showed that with the right combination of drug and procedures the response could be made specific, with rabbits becoming tolerant to one antigen while mounting an immune response to others. Prompted by Schwartz, the Hitchings-Elion group set up an immunological screening test in which they measured the immune response of mice to sheep red cells, a test that enabled them to identify new drugs and drug combinations, and to extend the investigation in other ways. As Hitchings later pointed out, Schwartz's demonstration of a chemically induced immune tolerance using the antimetabolite, 6-mercaptopurine, represented "an extremely important breakthrough in the field of immunology."33

The work of Schwartz and Dameshek drew the attention of Roy Calne, a young British surgeon investigating kidney transplantation in dogs. Collaborating with Hitchings' and Elion's Burroughs Wellcome colleagues in Beckenham, England, Calne used 6-mercaptopurine to suppress immune response, and succeeded in extending the life of a transplanted kidney from the usual 8–10 to 44 days, a new record.34

Calne subsequently came to the United States on a Commonwealth Fund Fellowship, with the plan of continuing his work at Peter Bent Brigham Hospital in Boston. Peter Bent Brigham was then a major center for transplantation research, but with the exception of a donation between identical twins, all transplanted kidneys had been rejected. On the advice of the Burroughs Wellcome group in Beckenham, Calne made a stop in Tuckahoe on his way to Boston, and came away with several compounds, including what the Hitchings-Elion group then called 57-322, or azathioprine. Soon Calne reported to Hitchings that azathioprine was superior to 6-mercaptopurine in suppressing immune response, and that one dog had already carried a transplanted kidney for several months. Similar successes led to the first human kidney transplantation with azathioprine as the only immunosuppressive agent. The recipient had been near death, but recovered and lived more than two years after the surgery.35

Under the trade name Imuran, azathioprine was joined with prednisone in a standard immunosuppression regimen in the early 1960s. Between 1965 and 1972 some 25,000 kidney transplantations were done in the United States, and

33 Elion, “The purine path to chemotherapy” (ref. 26), 452; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 845.
34 Elion, “The purine path to chemotherapy” (ref. 26), 452; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 845.
35 Elion, “The purine path to chemotherapy” (ref. 26), 452; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 845.
numbers increased thereafter. Improvements came from new drugs, and from antigen typing and matching, and transplantation of other kinds of organs became possible. Subsequent investigations showed that azathioprine, 6-mercaptopurine, and thioguanine were also useful in treatment of autoimmune disease, including systemic lupus and rheumatoid arthritis. The antimetabolite concept, embedded in the Hitchings-Elion program, had helped to open another field of medicine.36

Elion and her co-workers were not done with 6-mercaptopurine. They knew from metabolic studies that 6-mercaptopurine was broken down in the organism, and that the enzyme responsible for its oxidation was xanthine oxidase. They reasoned that they should be able to potentiate 6-mercaptopurine in treatment of leukemia by inhibiting xanthine oxidase with an antimetabolite. Since xanthine oxidase had been a test enzyme in the Hitchings group’s early search for substrates and inhibitors of the natural purines, there were a number of inhibitors at hand. The one they chose was allopurinol, an analog of the natural purine hypoxanthine that early screening had shown to have no inhibitory effect on bacteria or tumors, and to be non-toxic. Mouse studies showed that allopurinol did potentiate the antitumor and immunosuppressive effects of 6-mercaptopurine. Similar results emerged from studies of use of the compound in treatment of human granulocytic leukemia, undertaken in collaboration with a physician, Wayne Rundles, at the Duke University School of Medicine. Later clinical studies showed, however, that the potentiation was accompanied by a proportional increase in toxicity, so that the chemotherapeutic index of 6-mercaptopurine remained unchanged.37

With their attention focused on xanthine oxidase, Elion and Hitchings realized that the enzyme was responsible not only for the oxidation of 6-mercaptopurine, but also for formation of uric acid from the natural purines hypoxanthine and xanthine. Since the painful condition of gout is due to deposits of uric acid crystals in joints or kidneys as a result of excess uric acid in the blood or urine, treatment with allopurinol to inhibit the formation of uric acid opened the way to a new and effective treatment for this disease. Several problems had to be confronted in animal and human studies, including the potential long-term effects of a drug that would need to be taken for the patient’s lifetime. One especially significant finding was that in the organism allopurinol not only acted as an inhibitor of xanthine oxidase, but also as a substrate of the same enzyme, which converted allopurinol by oxidation into


37 Elion, “The purine path to chemotherapy” (ref. 26), 453; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 845–846; Sneader, Drug Discovery (ref. 18), 254.
the xanthine analog, oxypurinol. This result was clinically significant, since oxypurinol was found to bind to and inactivate the enzyme. It was also found to have a longer half-life in the organism, enabling steady-state levels of the drug to be more readily achieved in patients. Since allopurinol was completely absorbed in oral administration while oxypurinol was not, Elion and her co-workers concluded that allopurinol was the ideal pro-drug for oxypurinol. Allopurinol went on the market in 1966, and by the 1970s was among the standard drugs used in treatment of gout.\textsuperscript{38}

### 16.3 Pyrimidines and Pyrimidine Analogs

In 1948, the same year that Elion launched the development of purine analogs as drugs with synthesis of 2, 6-diaminopurine, her colleague Elvira Falco initiated a second line of research with synthesis of a pyrimidine analog, p-chlorophenoxy-2,4-diaminopyrimidine. The Hitchings group had begun work with pyrimidines and their analogs as early as 1945. Now the compound prepared by Falco indicated that pyrimidines with the 2, 4-diamino structure could be of special interest for the antimetabolite research program. The lead was intensively pursued by Falco and Peter Russell. They found that not only did compounds of this group strongly inhibit \textit{L. casei}, but that molecular modification also yielded compounds that were markedly selective in their inhibitory action on different species of organism. The practical implications were clear. As Hitchings later recalled, “it appeared probable that we would be able to tailor such compounds for specific actions against pathogenic species of many kinds.”\textsuperscript{39}

One notable compound to emerge from this line of research in the 1950s was pyrimethamine, an antimalarial. Peter Russell had pointed out earlier the resemblance of a particular compound of the 2, 4-diaminopyrimidine group to a hypothetical structure of a known antimalarial, proguanil, and it was on the basis of this insight that the Hitchings group made an arrangement in 1947 for an outside laboratory to conduct the testing of compounds as antimalarials. The first commercial product to come out of this testing, marketed with the trade name Daraprim, pyrimethamine was a potent and highly selective anti-malarial. Another important compound that came out of the Hitchings group’s 2, 4-diaminopyrimidine program was trimethoprim, an equally potent and highly selective antibacterial.\textsuperscript{40}

\textsuperscript{38} Elion, “The purine path to chemotherapy” (ref. 26), 453–456; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 845–846; Sneader, \textit{Drug Discovery} (ref. 18), 254.

\textsuperscript{39} Falco, Hitchings, and Sherwood, “The effects of pyrimidines on the growth of \textit{Lactobacillus casei}” (ref. 9); Hitchings, Autobiography (ref. 2); Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 846.

\textsuperscript{40} Hitchings, “Selective inhibitors” (ref. 7), 476–477; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 846.
How to account for the remarkable specificity of these compounds? While the Hitchings group pursued its investigations in the 1940s and early 1950s, other researchers were elucidating the biochemistry of folic acid and its metabolism. To Hitchings and his colleagues the action of pyrimidine analogs on $L.\ casei$ suggested that they were in some way antagonistic to folic acid, probably by inhibiting an enzyme that reduced folic acid to folinic acid. By 1950 they had concluded that their compounds were indeed acting as selective inhibitors of this enzyme. Continuing biochemical investigation led to the enzyme’s isolation and the specification of its action as the reduction of dihydrofolate to the biologically active tetrahydrofolate, and thus its name, dihydrofolate reductase.\(^{41}\)

Hitchings and his colleagues conjectured early on that the fine structure of dihydrofolate reductase varied from species to species. They reasoned that an analog that closely resembled the substrate, dihydrofolate, in structure, such as methotrexate, would fit most of the binding sites of the enzyme, regardless of its variations, and thus would not be selective in its activity. Smaller molecules, in contrast, would bind to only some of the sites of the enzyme, and might at the same time bind to sites that were distinct in each species. If so, this would account for the high specificity of action of compounds such as pyrimethamine and trimethoprim. This view of a structural basis for selectivity of action was later confirmed by further investigations, including amino acid sequencing and x-ray crystallographic studies of purified enzymes from various species.\(^{42}\)

For bacteria, at least, another form of selectivity was available in addition to the inhibition of dihydrofolate reductase by trimethoprim. Pathogenic bacteria, unlike humans, are able to synthesize their own dihydrofolate. Research following the Woods-Fildes theory had shown that it is this synthesis that sulfonamides inhibit by competing with an essential substrate, para-aminobenzoic acid. This opened the possibility of what Hitchings called a “sequential blockade,” in which the combination of a sulfonamide and trimethoprim would inhibit the same metabolic pathway at two distinct stages, producing a stronger effect on the bacterium than either drug alone. From this reasoning came the major antibacterial co-trimoxazole, a combination of trimethoprim and sulfamethoxazole, approved by the FDA in 1973 and marketed under different trade names including Septra and Bactrim.\(^{43}\)

\(^{41}\) Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 846; Hitchings, “Selective inhibitors” (ref. 7), 477–478.


\(^{43}\) Hitchings, “Selective inhibitors” (ref. 7), 482; Hitchings, “A biochemical approach to chemotherapy” (ref. 3), 847. On trimethoprim, including doubts about the utility of the trimethoprim-sulfamethoxazole combination, and the eventual marketing of trimethoprim...
16.4 Antivirals

In 1967 Burroughs Wellcome appointed Hitchings Vice President in Charge of Research. At the same time Elion became head of the company’s Department of Experimental Therapy, a position she was to hold until her retirement in 1983. Elion later remarked that colleagues sometimes described her department as a “mini institute,” since it included sections of chemistry, enzymology, pharmacology, immunology and, eventually, virology. By whatever name, she found that the interdisciplinary arrangement “made it possible to coordinate our work and cooperate in a manner that was extremely useful for development of new drugs.”

Within a year Elion’s department began to turn its attention to antivirals. Looking back from a later vantage point, Elion advanced three reasons for this change of direction. Twenty years of work on purine analogs and the new drugs it had yielded, including 6-mercaptopurine, thioguanine, azathioprine, and allopurinol, had accomplished much, and opened the way for a fresh start. The compound that had initiated all of this, 2,6-diaminopurine, had already shown intriguing antiviral activity in 1948, although Elion and her co-workers had not followed up on that lead. Finally, a recent publication by Frank Schabel, Jr., of Parke, Davis & Company and the Southern Research Institute in Birmingham, Alabama, had reported that a purine nucleoside, adenine arabinoside (ara-A) inhibited growth of both DNA and RNA viruses.

As she reflected on Schabel’s findings, it occurred to Elion that the arabinoside of 2,6-diaminopurine might be as active against viral DNA and RNA as adenine arabinoside, given known biochemical similarities of diaminopurine and adenine. An organic chemist colleague, Janet Rideout, synthesized diaminopurine arabinoside, and since at the time Elion’s department lacked a virus laboratory, Elion sent the compound on to John Bauer at Wellcome Research Laboratories in Britain for antiviral screening. Soon Bauer reported that diaminopurine arabinoside was very active against both herpes simplex virus and vaccinia virus, with less toxicity to mammalian cells than adenine arabinoside. Elion later recalled that this promising result “began our antiviral odyssey,” and initiated several years of work in her department on purine arabinosides.

In 1970 Elion’s department moved with the rest of Wellcome Research Laboratories from Tuckahoe, New York to North Carolina. At the same time as a stand-alone drug, see also David Greenwood, Antimicrobial Drugs: Chronicle of a Twentieth Century Medical Triumph (Oxford and New York: Oxford University Press, 2008), 254–256.

44 Hitchings, Autobiography (ref. 2); Elion, “Autobiography” (ref. 14), 967.


46 Elion, “The purine path to chemotherapy” (ref. 26), 457–458.
time Howard Schaeffer joined the group as head of the Organic Chemistry Department, bringing with him a lead into a new approach to the antiviral research. Schaeffer’s work had shown that acyclic nucleosides, and not only nucleosides in which the sugar ring was intact, could be acted on by enzymes. This finding opened the possibility of use of acyclic nucleoside analogs as antimetabolites.47

Promising early results of antiviral screening led the Wellcome researchers to focus on acyclic nucleoside analogs, with the labor divided among three groups. The chemists, Schaeffer and Lilia Beauchamp, synthesized the compounds, the U. K. Wellcome unit that included Bauer and P. Collins conducted the antiviral screening in animals, and Elion’s department studied mechanisms of action, enzymology, and \textit{in vivo} metabolism. The researchers were not surprised to find that, in a parallel with earlier work on purine arabinosides, the 2, 6-diaminopurine analog proved highly active against herpes simplex virus. They were surprised to find that the guanine analog, acycloguanosine or acyclovir, was more than one hundred times as active as the 2, 6-diaminopurine analog. Elion and her colleagues published the early results with acyclovir in 1977 and 1978.48

Acyclovir was impressive in its selectivity as well as its potency. Highly active against herpes simplex viruses and the varicella zoster virus that causes chicken pox, and with some activity against other herpes viruses, it lacked activity against other kinds of virus, and was not toxic to the mammalian cells in which the herpes viruses grew. Convinced that understanding the biochemical basis of this selectivity would yield valuable insights into the herpes viruses, Elion and her colleagues dedicated resources to this project, including the establishment of an in-house virus laboratory that expanded the capabilities of Elion’s department. They found the basis of acyclovir’s selectivity in an enzyme specific to herpes viruses, viral thymidine kinase, which in the infected cell begins a process that leads to the incorporation of acyclovir triphosphate into the viral DNA and termination of the DNA chain. These studies opened the way for further research on enzyme differences in normal and virus-infected cells, and on other enzymes specific to viruses, investigations that would contribute to the search for other antiviral drugs.49

\begin{flushleft}
47 Elion, “The purine path to chemotherapy” (ref. 26), 458.
\end{flushleft}
Entering medical practice in the 1980s, acyclovir had a major impact on treatment of herpes virus infections. In genital herpes it alleviated symptoms and reduced time to healing in first infections and, used prophylactically, reduced the frequency of recurrences. It reduced the period of acute pain in shingles (herpes zoster). In immunosuppressed individuals, such as those undergoing bone marrow transplantation, acyclovir could prevent activation of herpes simplex infections during the period of greatest vulnerability to infection. It could save the lives of people with herpes encephalitis, if given in time. Acyclovir could also be an effective treatment for cold sores, caused by herpes simplex infection.\textsuperscript{50}

16.5 Conclusion

By the time that Hitchings and Elion delivered their Nobel lectures in December, 1988, the AIDS epidemic had emerged as a major health crisis. Scarcely mentioned in their talks is that Burroughs Wellcome researchers were largely responsible for the first antiretroviral drug used to treat HIV, zidovudine (azidothymidine, AZT), which had received a product license from the FDA just one year earlier and which the company marketed under the trade name Retrovir.\textsuperscript{51}

That the Burroughs Wellcome researchers were able to respond so quickly in the wake of identification of the retrovirus (LAV, later HIV) in 1983 was due to the prior existence and the characteristics of the research program examined here.

In June 1984 the Burroughs Wellcome researchers set up a program to identify compounds that might act against HIV. A nucleoside chemist, Janet Rideout, was put in charge of selecting compounds for testing. One of those she selected was AZT, a compound originally synthesized twenty years earlier by Jerome Horwitz at the Michigan Cancer Foundation as a possible chemotherapeutic agent in leukemia. It was probably chosen in part because of its known activity against animal retroviruses. But it helped that the Burroughs Wellcome researchers had already tested it for antibacterial action, and had it at hand. By December 1984 they had positive results for AZT against two types of animal retroviruses, Friend leukemia virus and Harvey sarcoma virus. They then sent samples of AZT to the National Cancer Institute, where researchers had developed a method of testing compounds for activity against HIV, growing the virus in immortalized human T4 cells.\textsuperscript{52}

\textsuperscript{50} Elion. “The purine path to chemotherapy” (ref. 26), 462–463; Sneader, Drug Discovery (ref. 18), 259.
\textsuperscript{51} Sneader, Drug Discovery (ref. 18), 260–261.
\textsuperscript{52} Sneader, Drug Discovery (ref. 18), 260–261.
Within two weeks of receiving AZT, the NCI researchers had concluded that it was highly effective against HIV. By June 1985 the findings of the NCI investigators had been confirmed by others at Duke University. The FDA gave approval for a Phase I clinical trial in July 1985, and January 1986 a randomized, double-blind clinical trial in 282 patients had begun. The trial was interrupted after sixteen weeks because of distinctly lower mortality among patients receiving AZT.53

Even in brief outline, the AZT story exemplifies three fundamental characteristics of the Hitchings-Elion program. One of these is the continuity and coherence of the antimetabolite research program over more than four decades and many changes in the research environment. The Burroughs Wellcome researchers had long experience in synthesizing and testing compounds, including nucleosides and nucleoside analogs, as potential antimetabolites against a variety of cells (microbial pathogens, cancer cells) and viruses. The prior testing of AZT as an antibacterial by them and the accumulated information about it that was already available in consequence were results of this program. Their understanding of AZT as an antimetabolite that inhibited an enzyme, later identified as reverse transcriptase, specific to the pathogen, was a natural extension of the antimetabolite concept.

The work on AZT also exemplifies the joining of rational and empirical elements in the Hitchings-Elion program and its extension. Rational, because AZT was a member of a defined class of compounds, nucleosides and nucleoside analogs, considered to be potential antimetabolites with selective activity on different kinds of pathogens, but especially investigated by Elion and her colleagues as antiviral agents. Empirical, because only screening could determine which members of this class had selective action on specific pathogens. Within such an approach, specific results could not be predicted, and in this sense AZT is an instance of the group’s strategy as defined by Hitchings in one compact formulation: “choose a promising field of work and remain untargeted but opportunistic so that the accumulated knowledge dictates the target.”54

Finally, the effort from which AZT emerges embodies the character of the Burroughs Wellcome program as industrialized research. This means not simply that the research is located in and paid for by industry, but also that the work is done in a research organization in which the primary goal is to produce viable chemotherapeutic agents, that is entities that are at the same time medical technologies and commercial products. To this end the research is organized not to advance knowledge within a particular discipline, although new knowledge is produced, but in a collaborative and interdisciplinary process.

53 Sneader, Drug Discovery (ref. 18), 261.
that selects and coordinates the knowledge and techniques needed to advance the goal of producing new medicines. In the course of this work outside individuals and institutions, including clinicians and research laboratories, are enlisted as needed.

The beginnings of this organization are already visible in the small group that Hitchings assembled in the mid-1940s. Although larger and more articulated by the 1980s, its essential features were still in place. By the time they went to Stockholm both Hitchings and Elion had retired. The research group and program they had created, and that had enjoyed so many successes, would continue.