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ANTAGONISTS OF NUCLEIC ACID DERIVATIVES AS MEDICINAL AGENTS

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INTRODUCTION

My early life was almost unmitigated joy. I was a much wanted son, and brother to two sisters twelve and fifteen years older than I. I was the son of leading citizens of Hoquiam, Washington, the son of a ship builder who had adapted the wooden hull of the lumber carriers from sail to steam. My father, George Herbert, Sr., was involved in church, YMCA, and philanthropy in general. My mother also was active in public affairs, and had been responsible for much of the financial work for the shipyard when it was her father's. I am one of the few people now who saw Halley's Comet at its spectacular appearance in 1909.

My schooling was all smooth. After the grade schools in Berkeley and San Diego, California, and Bellingham and Seattle, Washington, I attended Franklin High School in Seattle. I was Salutatorian of my class. My salutatory speech was, at the suggestion of my faculty mentor, on the life of Louis Pasteur. His service to "my fellow man" became an aspiration. I entered university with a career in medicine in mind but was diverted by attraction to chemistry. In the end, biochemistry at Harvard Medical School was a suitable synthesis of both interests. I graduated cum laude from the University of

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Washington in 1927, received an M.S. in 1928, and was elected to Phi Beta Kappa and Sigma Xi.

I spent a year 1928–9 in the Chemistry Department at Harvard, Cambridge, Massachusetts, followed by a summer in Berlin, Germany, in the Institut for Auslander in the university. I concentrated on the language but weekends in exciting regions enriched the language lessons. I and Chuck Porter had a room on Schiffbauerdam where we could watch the barges on the Spree, which in those days were the homes as well as the freight carriers of the owners. We were within a block of the Schiffbauerdam Theater where the Dreigroschen Oper (The Three Penny Opera) began its long career. After the course, Chuck and I spent several weeks touring Germany, Switzerland, and France before catching our ship home from La Havre.

My further graduate work was in the Department of Biological Chemistry at Harvard Medical School. As was his custom with beginning graduate students, Otto Folin, the Head of the Department, assigned me to his Associate Professor, Cyrus H. Fiske. I soon was too involved with the Fiske program to follow the usual termination after one year. Fiske (and Subbarow) had followed the discovery of phosphocreatine with the discovery of adenosine triphosphate. I was assigned the problem of following its metabolic products. In the end this created some analytical methods (superceded twenty years later by ultraviolet spectroscopy) but stimulated a life-long interest in nucleic acids. I received my Ph.D. in 1933, the nadir of the great depression.

BURROUGHS WELLCOME COMPANY

It was 1942 before I obtained a permanent position at Burroughs Wellcome Co., in Tuckahoe, New York. Meanwhile I had spent time as an instructor and tutor at Harvard College, a research appointment at the Huntington Hospital, and as a senior biochemist in the Department of Medicine, in Western Reserve Medical School, Cleveland, Ohio.

I was interviewed and given an appointment at Burroughs Wellcome Co. in the spring of 1942. However, by the time I joined the organization in July, there was no research director, and I was the whole biochemistry department. This allowed me freedom to follow my own interests and knowledge. The antimetabolite principle had recently been enunciated by Woods and Fildes as a result of their observation that para aminobenzoic acid reversed the inhibitory effects of sulfanilamide. The idea was that making a substance similar to constituents of nucleic acids might reveal species differences in nucleic acids, and enlightenment as to the biosynthetic pathways. I enlisted the help of Elvira Falco (who soon transferred from Bacteriology to Biochemistry). A screening test was set up with *Lactobacillus casei*. Its growth requirements were significant. It was possible to determine whether analogs of the purine and pyrimidine bases, and a growth factor not long afterwards

identified as folic acid, were growth inhibitory or growth promoting. We were able thus to obtain a substantial amount of information from 25 mg of compound. (Our colleagues in other pharmaceutical houses had to submit 25 g of a compound before any biological work would be done.) We had enough interesting results for publication. Meanwhile, we had acquired research directors on both sides of the Atlantic; Charles Kellaway in the Wellcome Foundation in England, and Erwin E. Nelson in the United States. Both felt that I was promising, and agreed to some addition to my staff. Gertrude Elion was selected in 1944. Her first assignment (with an outside investigator) involved thio compounds as antithyroid agents, but by the end of 1945 she was in full swing with the major objectives of the program and after 47 years and many experiences and honors is still with it.

Our target was rapidly growing cells, our objective was selectivity. Ongoing results with bacteria showing major differences in response by different species, and low toxicities in mice and rats convinced us and our superiors that we had a promising program. So Peter Russell and Norman Whitaker came from England. By 1947 the word in the laboratory was "now we have the chemotherapeutic agents, we have only to find the diseases they will cure." I arranged with C. P. Rhoads for tumor testing at Sloan Kettering Institute, and Kellaway arranged with Brigadier Boyd and Len Goodwin in England to conduct malaria testing. Soon Stanley Bushby joined in with antibacterial work, first in England and then in the USA after we had moved to North Carolina.

2,6-diaminopurine

Among our first compounds for Sloan Kettering was 2,6-diaminopurine, also submitted by George Brown of the Chemistry Department of that institute. This gave one spectacular remission in a young woman—in retrospect almost a cure—and cemented our relationships with cancer research, with Sloan Kettering Institute, and with Joseph H. Burchenal, the physician in charge. Rhoads induced the Charles F. Kettering Foundation to supplement our meager budget. This help became critical a few years later when the Wellcome Foundation found itself in financial distress because Henry Wellcome had failed to provide for the Inland Revenue Service of Great Britain in his will.

6-mercaptopurine

Falco synthesized thioguanine, but was unable to purify it (probably simply a matter of particle size of the starting material). The experiment did introduce the direct method of thiation with phosphorus pentasulfide to purine chemistry. Elion followed, first with 6-mercaptopurine (6-MP) and then with a successful preparation of thioguanine. These substances gave moderate results in the routine sarcoma 180 trials; but Don Clarke, who ran the testing

program, held several groups of mice, which had been treated as usual for one week, for an additional week without treatment. A spectacular finding with 6-MP was that 40% of the tumors regressed during the second week. (This is difficult to explain simply because it was found that the rejection was immunological and that 6-MP is immunosuppressive.)

Burchenal quickly took the compound into the clinic, obtained remissions in leukemics, and precipitated a major program sponsored by Sloan Kettering Institute. Rhoads enlisted 12 centers to conduct comparative clinical trials. When he found the feedback exciting, he gave a story to Walter Winchell (THE pundit of radio) to the effect that Sloan Kettering had an exciting new antileukemic agent that had been submitted by George Hitchings, of Tuckahoe, New York.

In the next few days, I was inundated with calls from doctors and patients, and heard a whole range of sad stories in over 600 of these calls. The Medical Department of BW was caught unaware of this new development and was urgently briefed. Supplies of 6-MP were extremely limited at that time and so it was full steam ahead to scale up the synthesis of this drug. In the meantime, Don Searle of BW appealed to the FDA to expedite the commercial release of 6-MP. At FDA, a Dr. King undertook to evaluate the 6-MP data himself, and to visit all the investigators who were working with this compound; thereafter, he was satisfied that useful clinical remissions were being obtained. There followed the unique happening that the commercial release of the drug occurred in August, 1953, even before the official data were reported in the literature at a symposium in 1954. The other unique aspect of the marketing of the drug involved the US Patent Office. The problem was that to obtain a patent for an anticancer drug, it was necessary to have "five-year cures." This was clearly not feasible for the newly discovered antileukemia drug. The examiner who was petitioned by BW appealed to his superior and the two of them investigated the situation. They also set aside their usual rules and awarded the patent. Even with the commercial release, the development group was still not able to scale up the synthesis of this drug to commercial quantities, and had to continue with the painstakingly slow laboratory synthesis. That year some of us spent the Christmas holidays making enough 6-mercaptopurine to give to patients. I spent New Year's Day in the laboratory, going off in the morning with a sandwich. At the end of the day, my wife Beverly said to me, "George, did you know this was New Year's Day?"

Azathioprine

A new lead soon appeared. Robert Schwartz, working with William Dameshek at Tufts University, had established a test for immunosuppression using bovine γ-globulin in rabbits and measuring the antibody response. He had tried a variety of antitumor agents but found that he had tried no antimetabolite. I responded to his appeal with a generous supply of 6-MP. It

gave exciting effects, and in due course, a paper was published. This came to the attention of Roy Calne who was transplanting kidneys in dogs, at St. Mary's Hospital, with the purpose of identifying the cells involved in rejection (inevitable when donor and recipient were unrelated). Calne thought 6-MP deserved a chance in his system, obtained the drug from Wellcome, Beckenham, England, and found that the drug did indeed prolong survival of the transplanted kidney (up to 43 days in one case). Calne received a Commonwealth Fund Fellowship that permitted a year in the United States and he chose the Peter Bent Brigham Hospital and Joseph E. Murray to pursue transplantation. Murray, because he had devised all the surgical techniques for renal transplants in man, showed that a genetic difference between donor and recipient led inevitably to rejection. (Murray's skills were honored by a Nobel Prize in 1990.) Calne stopped in Tuckahoe, New York, to see us, proceeded to Boston with a pocket full of compounds (mostly related to 6-MP) to try on the canine renal transplantation he proposed to carry out. Soon he found 56-322 (azathioprine, IMURAN® "not uninteresting". It eventually was repeatedly confirmed as an immunosuppressant in dogs, was taken to the clinic by Murray, and became the major drug (supported by prednisone or equivalent) permitting transplantation where donor and recipient were unrelated. It was used with steroids but it was perhaps two decades before other agents were added to the regimen.

Doris Lorz had been testing selected compounds as inhibitors or substrates for xanthine oxidase. She had had two in vitro systems, but was frustrated because animal metabolism of uric acid ended in allantoin, the estimation of which would have been too time consuming for routine work. The finding that 6-MP gave prominently 6-thiouric acid (not metabolized further in animals) opened the door for investigation of her inhibitors. R. Wayne Rundles was not slow in taking the most promising compound (allopurinol, 4-hydroxypyrazolo (3,4-d) pyrimidine) to the clinic. First it was shown (in collaborative papers) to inhibit the oxidation of 6-MP, and, before long its action in secondary hyperuricemia and gout were reported. Today allopurinol (ZYLOPRIM®) is the drug of choice for treatment of gout and hyperuricemia. It should not be considered negligible that this drug in common with the antineoplastic agents has contributed to saving the lives of uncounted gouty individuals who might have died of kidney failure resulting from deposits of uric acid.

VIRUS INHIBITION

This account would not be complete without mention of a foray into virus inhibition. In collaboration with Randall Thompson, studies supplemented later by our colleague John Bauer, supplied evidence that vaccinia virus, first in vitro, and then in smallpox, was destroyed by our derivatives of isatinthiosemicarbazone. The disease disappeared, but the finding that virus

infections could be controlled by medication possibly encouraged further studies. This laboratory, much later, produced the antiherpetic acyclovir (ZOVIRAX®) and the anti-AIDS drug zidovudine (RETROVIR®).

So far, an accompanying main route of investigation (and useful products) had been postponed for extended pursuit of the purine-analog track. One of the earliest products of this program, diaveridine [5-(3,5-dimethoxybenzyl)-2,4-diaminopyrimidine is still viable as a veterinary product and recently as an anti-inflammatory analgesic. It was an early member of the 2,4-diaminopyrimidine group that was identified using *L. casei* as having an antifolic acid activity. Early studies proceeded in two directions. Goodwin took up the challenge of antimalarial activity, and the leading product of these studies, pyrimethamine (DARAPRIM®) soon evolved. This compound had rather high activity on its own. It was approved and marketed as monotherapy, rather than in combination with a sulfonamide, due to prejudice by authorities against combinations. Resistance to it was developed through misuse. It eventually was issued as a combination with sulfadoxine (FANSIDAR®) and is still in use in many countries.

The diaminopyrimidines had a somewhat checkered history. Early on my colleagues took heroic doses of several of the compounds, experienced side effects (mostly confusion), and recommended return to the drawing board. Our objective was soon 5-(3,4,5-trimethoxybenzyl)-2,4-diaminopyrimidine (trimethoprim). The efforts of Falco & Roth were successful in making enough for in vitro bacteriological work, and finally in developing a productive method. We found our administrative, and even medical, colleagues, reluctant to believe that we had indeed synthesized an antifolic acid compound so highly selective that it would kill bacteria but was nontoxic at enormous doses. We combined it, at first, with sulfadiazine (a double blockade of the biosynthesis of purines and thymidine). It languished for two or three years, during which time it was known in combination with sulfadiazine as "Daddy's magic medicine" because it cured my wife when her annual recurrences of bronchopneumonia began. The languishing tapered off when I gave a paper about TMP and its uses in Toronto. The meeting was attended by Gerhardt Zbinden, then Vice President for RD&M for Hoffmann-La Roche in Nutley, New Jersey. Soon a proposal for a license came. Out of that, Wellcome London made contacts with Roche Basel, and a proposal for license or collaboration was received. Our Chief Executive Officer, William N. Creasy, was unconvinced. He expressed the desire to sell the whole thing to Roche for $1,000,000 (vetoed by London). By that time development of trimethoprim/sulfamethoxazole was well under way by both pharmaceutical houses (SEPTRA® and BACTRIM®). Eventually, more than 5 billion dollars of drug was sold and it is credited with saving over one million lives. It is still the preferred antibacterial in the third world for its broad spectrum, stability, and moderate cost.