My Scientific Life and Hard Times
or, More Than You Wanted to Know

An unauthorized autobiography by Norman Radin

Warning from the Surgeon General's Office: The detailed memories in this biography may be hazardous to your health – and possibly not even accurate.

Prehistory

I practiced becoming a scientist at an early age by building many objects – rubber band-powered boats for my bathtub play, erector set projects, and model airplanes. I spent many hours designing and testing paper airplanes and ultimately achieved long straight flight paths. I was lucky that my father had so much good paper to sacrifice. While even younger, I built and crawled through multi-room lean-to houses, made out of discarded rectangular thick cardboards that my father gave me (they were used by cloth manufacturers to flat-fold bolts of cloth). Using large sheets of heavy brown paper from my father's dress manufacturing plant, I drew and laid out plans for ideal cities. This predated the current city design computer program, Sim City, by ~70 years. Around this time, I thought I would become an airplane pilot or railroad train engineer. I used to draw movies, frame by frame, of airplane adventures. Then I started to read about science.

I also started to read science fiction – H. G. Wells, Tom Swift, and Jules Verne. I was one of the first purchasers of the new science fiction magazines – *Air Wonder Stories* (later changed to plain *Wonder Stories)*, *Amazing Stories, Astounding Stories,* etc. If I had not forgotten about them years later after leaving home, or if my mother had not thrown them out, they would now be worth thousands of dollars. I have always loved these stories, despite their highly uneven quality – after all, the scientists in many of these stories were great role models. They bravely tackled incredibly difficult, fundamental scientific problems, like time travel and faster-than-light travel. No matter how complex or expensive their experiments, they usually worked the first time. However, some of these scientists, like Dr. Jekyll, made the mistake of running the experiments on themselves. Others, like Dr. Frankenstein, used poor starting materials.

My first goal as a future scientist was to become an astronomer. The beauty of the orbs hanging in space intrigued me and I had a few opportunities to actually look at them through small telescopes. Some years later I saw a pair of twin stars, one golden yellow and the other sky blue. A friend, Justin Shapiro (who later founded the chemical equipment company, LabIndustries), showed me Jupiter's moons with his reflector telescope. At about age 12 I joined the American Interplanetary Rocket Society. (This title might sound internally contradictory but it still makes sense to me.) My dad took me to the meetings and intently followed the presentations along with me. We saw movies of Goddard's rocket flights that fired up my quivering desire to join this research. I didn't anticipate that I would later spend three years during World War II developing new rocket fuels for the National Defense Research Council. And that my son, Lon, would later design and build his own rockets, using his own fuel

mixture and brown gummed packaging tape for the motor body., Our neighbors wisely put a stop to his tests despite beautiful take-offs and flights.

After a while, I realized that astronomy – at this stage of science – did not seem immediately suitable as a hands-on activity, and the drudgery of grinding telescope mirrors was unappealing. Chemical experiments seemed more realistic for this young lad.

Apparently my work habits at age 12 were not too suitable for a budding scientist as I remember my home room teacher, Mrs. Mabel Moulthrop, criticizing my sloppy notebook in front of the entire class. This public humiliation straightened me out somewhat and I became much more systematic in my organization of knowledge. She was a great prodder of my scientific and social development and I have long regretted my failure to maintain personal contact with her. Busy, busy, busy! She was a great example of the importance of good teachers and I have tried to emulate her in working with my own students.

High School

My first research experiments began at about age 13, when my dad had a plumber install a gas line in our home's basement for a Bunsen burner and gave me a long wooden table (which I think he made) near our laundry tub. I spent my meager allowance (even then a shortage of grant funds!) at Eimer and Amend, a chemical supply house just a few blocks from my school. This school, Stuyvesant High, in downtown Manhattan, specialized in science and technology and one had to take an entrance exam to get in. The school Principal, von Nardroff, used to enjoy presenting us with demonstration/lectures on various physical phenomena. I remember one lecture-demonstration on the mixing of colored lights, another on the rheological properties of sand. We had nice big chemistry and physics labs, even rats; the latter, however, were wild and scary, not experimental. Recently the city of New York replaced our even then-dilapidated school building with an incredibly expensive and elegant school. I pity the many science-minded students of today who live in cities that lack such a school.

My equipment and chemical supplier, Eimer and Amend, later became a world-wide supplier, Fisher Scientific. They had a special counter for kids like me, with cheap discontinued items no longer bought by alchemists. I made do with glass retorts (yes, actual retorts!), soft glass beakers that cracked if heated too rapidly, little porcelain cups, and discarded pots, dishes, and jars from my mother.

Those were fun years, filled with the stimulating fumes of hydrochloric and nitric acids and sulfur dioxide. With my retort and Bunsen burner, I distilled nitric acid from sodium nitrate and sulfuric acid; cooling by air served as a partial – not too efficient – condensing agent to liquefy the nitric acid vapor. I used the acids to convert metallic elements into beautifully crystalline salts that ended up in my mom's glass jars.

Having developed a love of classical music before this, aided by the excellent violin playing of my sister, Ruth, and encouraged by my father's pleasure in listening to it, I took piano lessons at about age 9. It seemed that I couldn't remember much of my teacher's directions nor could I memorize the music, and playing chords seemed too difficult for my small hands, so I became a dropout after about six months. However I continued to play without further lessons and enjoyed the results, errors and all. My sister, Ruth, and I played many violin-piano duets, which I enjoyed immensely. Ruth developed a lovely singing voice and I accompanied her in many songs. She taught herself to play piano too, so we played 4-hand piano duets; I particularly remember Moszkowski's bright Spanish Dances. When I entered high school, I naively thought that their orchestra might have a place for a pianist. After a brief

audition (for which I forgot to bring my music and tried in vain to play the piece from memory), the head of the Music Department asked me if I would like to enter the orchestra as a flutist. I had never even seen a flute, much less heard one in a non-orchestral setting. Acting on an unusually brave impulse, I agreed and was presented with a flute, a place in the school auditorium to practice, exemption from the Physical Education classes, and a volunteer flute teacher – who, by chance, happened to be a chemist. What a wonderful decision that was!

My teacher gave me additional chemical supplies, such as white phosphorus. My research slowed down when I tried to convert this to phosphoric acid by auto-combustion under an inverted beaker. I placed the phosphorus on a Petri dish floating in a pan of water and waited for it to self-ignite and use up the limited supply of oxygen under the beaker. The resultant phosphorous pentoxide should have dissolved in the water, forming phosphoric acid. Unfortunately for this plan, the wet phosphorus spattered boiling water on the inside of the beaker – not made of Pyrex – which broke and spread the pentoxide or phosphoric acid fumes throughout the house. My poor mother thought the basement was on fire.

One time I tried to make glass from its basic components by heating them together in our house coal furnace. People today have little familiarity with coal furnaces and they might be surprised to learn that they were not sealed-in units like the current gas and oil furnaces. We used to broil calves' liver on top of the glowing coals and their bright blue flames. Unfortunately for this experiment, I made the discovery that aluminum pots melt in a coal furnace, at a temperature below that of glass. This illustrated the importance of reading the literature before doing an experiment.

Another uncompleted experiment was run in our kitchen, where I attempted to make sugar from beets, following some skimpy set of instructions. I remember extracting the beets with hot water, treating the extract with charcoal, and evaporating down the solution. But I lost my nerve and didn't taste the product.

My cousin, Nat Radin (also a chemist), tells me that my basement lab was closed down after I exploded too much hydrogen in a demonstration. But that episode – if it happened – is completely lost from memory.

I do remember an attempted launching of a hot air balloon from my back yard. I made it out of a large amount of tissue paper and attached some sort of burning device underneath. Fortunately, my mother became alerted and ran out of the house just in time to stop the take-off. I had to admit this was a dumb idea.

Justice, of a sort, was wreaked on me many years later when my son tried to inflate a balloon by loading it with sodium carbonate and vinegar. I caught him at this too late to keep the mixture – too large an amount for the purpose – from spurting out of the balloon onto our bathroom mirror. The aluminum frame of the mirror shows a permanent etch-stain to this day.

In reading a biography of Thomas Edison, I felt great empathy for his early chemical experiments, which also produced some undesirable effects. His laboratory in New Jersey, which I visited much later, contained an impressive collection of large bottles of chemicals.

In high school, students were allowed to work on extra chemical experiments after school hours and to devise their own. I remember preparing a beautiful dichroic salt, chromium aluminum sulfate; these crystals had two colors, depending on which face you looked through. We also recrystallized sodium chloride with concentrated hydrochloric acid. Unfortunately for our nasal membranes, these experiments were run without a venting hood to remove the acid fumes. Colloid chemistry and

adsorption were topics of interest to me then and I built an apparatus to measure the adsorption of nitrogen by charcoal. The chemistry teacher gave me money from the petty cash account to buy some charcoal from Eimer and Amend. I didn't publish the results, which seemed to show a very low adsorptive power.

Early in my scientific career I read a book, "Creative Chemistry," written around 1918 by Edward Slosson. This book showed the many interesting things chemists did (little thought of pollution then) and urged youngsters to become chemists and make the U.S. independent of the German chemical industry. The field of plastics was described as a field of great promise. The same field was recommended many years later to the young actor, Dustin Hoffman, in the movie, "The Graduate." The book persuaded me to give up my plan to become an astronomer and become a professional chemist. Since then I have encountered several other chemists who were moved into the field by the same book.

Another book that intrigued me was Henley's "20th Century Formulas, Recipes and Processes." It contained many recipes for making things, like glue, ink, and hectograph copiers. (The latter were very common in my youth, used by teachers to assign homework or exams or furnish non-standard information. They consisted of a rectangular pan containing a gelatinous layer. The original copy was written on a special paper with a purple ink that soaked into the gelatin. Then one could make copies by laying clean sheets of paper on the top of the gelatin. A portion of the ink came out of the gelatin and soaked into the paper.) I also remember formulas for silvering sheets of glass – they really worked. I believe this book is still in press, after many updates.

In my senior year I took a biology course from an alcoholic biology teacher and recognized the tremendous future in biological chemistry. This switched me from my previous plan to go into some indeterminate career in chemistry.

Stuyvesant High was quite an unusual school. One of the most pleasant courses was on motors and generators. The lab for this course consisted of a large room, filled with large motors that could be rewired by simply plugging large connectors into a circuit board, the way telephone operators used to connect conversers. In this way, the motors could be changed in type, with different characteristics. I remember that we had 3-phase motors and generators and the elements of electronic circuits – unfortunately, I remember very little of the content now. However it must have helped as I later used many kinds of motors in the course of designing new equipment.

Stuyvesant High was a curious mixture of pre-college courses and occupational technical courses, such as metal casting. I used to admire the neat molds the students in the latter course made; they would assemble the two halves and pour molten metal into the assembly.

Later, in Chicago, I enjoyed visiting the Museum of Science and Industry, which had a wonderful exhibit showing how rotary motion (originating in knobs or motors) could be converted into a variety of other kinds of motion – linear, oscillatory, etc. I also loved to view their beautiful collection of the elements and crystalline rocks and gems. I check out mineral exhibits whenever I go to a museum or mineral/fossil show and have a few of my own. I suppose this is a rather primitive kind of enjoyment, like that of the American Indians who traded Manhattan Island for a set of colored glass beads. Probably it was a good deal for everyone concerned.

Part of my mechanical development came during the many summers during which I attended summer camp (Camp Rosemont, in Pennsylvania). There I spent much time in the arts and crafts shop, fabricating metal art objects, learning techniques of metal handling. I also worked at the camp newspaper, learning how to make mimeograph stencils (drawing, typing, lettering headlines in various

fonts, correcting typos with correction fluid). The distinctive odor of mimeograph ink has always held a special place in my list of affections. In later years, I was in charge of the camp newspaper and wrote most of it, including crossword puzzles and a gossip column attributed to Gnorman the Gnome.

In my last years in Grammar (Elementary) School, I wrote and typed up a class newspaper. I can't remember whether it was copied or simply posted on the wall for the other students.

All this practice in English composition naturally lead me later to scientific writing and editing. Students in New York State had to take state-wide pre-college exams ("Regent Exams") and I was one of two students who received a 100% grade in the English exam. The other student was I.A.L. Diamond, who later wrote all the Columbia College plays and went to Hollywood. I went with him only to Columbia College. He was a coauthor with Billy Wilder of "Some Like it Hot," which starred Marilyn Monroe. The idea of writing plays has always appealed to me but I never tried it. However I have been a member of a play-reading social group for about 40 years.

The Regent Exams earned me a scholarship, which paid for 1/4 of my college tuition – a much-valued aid for my father, who just barely managed to pay the rest of the cost on the installment plan.

I discovered, while in high school, that I liked to give expository lectures and remember presenting a lecture demonstration in the auditorium on colloid chemistry and the formation of agate (the many-layered rock). I was pleased to discover that public speaking did not make me nervous.

I also enjoyed practice on the Math Team, which generally beat the kids from other New York high schools. Our coach would give us two nickels for the round-trip subway fares to engage the competitors on their home grounds. I remember being surprised that the students in one school were locked in the school via guarded doors – I guess this is common in New York today. If you are curious to know how a math team works, it went this way: We were lined up in a row of desks and given a problem to solve in a fixed time. Our answer sheets for each problem were then rapidly gathered up for grading. No cheering section or viewers. The problems were ingenious, obviously devised and collected by puzzle-loving math teachers over a period of many years.

My cousin Nat recently dug up the first issue of *Amateur Scientist*, a magazine for high school students which he helped start. It contains a long-forgotten short-short story that I must have written for English class. It is titled "There's Always Tomorrow" and describes experiment No. 6,172 in a series of attempts to develop a polio vaccine. As you can guess from the title, this experiment also failed. This concept clearly stemmed from some failed experiments of my own. However I didn't know about the problems of grant support then – if I had, I would have lowered the experiment number to a more realistic value. Also, I was amused to note, I didn't know about the problem of statistical evaluation – the scientist in this story had used only one monkey in the trial.

College Years

I pursued the goal of becoming a biochemist very directly in Columbia College and took as many science courses as possible. I remember one elderly physics professor who lectured strictly from his notes and couldn't correct numerical or formulaic errors on the blackboard without studying his notes. One time he didn't realize he had jumped forward several pages when a draft from an open window blew them off his table. This produced some titters which must have confused this unhappy, weakening mind. Another physics professor, who later won the Nobel Prize, lectured in a ground-floor room and had to stop talking each time an attractive girl walked by on the sidewalk outside. Columbia College was a male student college then, so this problem didn't arise too often. Another professor, a physical

chemist (teaching quantum mechanics, I think), realized how unclear her lectures had been and gave us all A's. She too won a Nobel Prize later.

The inorganic chemistry course included lab demonstrations by the teacher. I quickly learned not to sit in the front of the class (my usual location when I discovered that this kept me awake). The students in the front rows endured impressive demonstrations of exploding nitrogen triiodide and the thermite reaction (powdered aluminum mixed with iron oxide). The latter reaction produced such hot, molten metal that drops splashed through their shirts.

The inorganic quantitative analysis course was very enjoyable except for the copper analysis problem. In this course, each student was given a sample of unknown percentage composition and had to turn in reports for each value determined by his lone efforts. If the value was correct within a certain acceptable range, he received an A. If it was wrong by more than this range, but still within a reasonable value (I forget the maximum allowed), he was permitted to redo the analysis and get a good grade anyway. If the error was beyond this reasonable one, he was out of luck. My copper value turned out to be in the redoable range. However instead of repeating the analysis, I did a literature search – that is, I asked my fellow students how they had made out with this method. It turned out that everyone had obtained a slightly low value in the first analysis. Accordingly I concluded the method itself was in error and, without redoing the analysis, submitted a corrected, elevated value, which hit within the acceptable range. I have never since assumed that someone's analytical method gives you the correct result. One of my recent papers gives evidence to show that previous studies of a certain enzyme have all given very low values.

Organic chemistry proved to be very attractive to me. Our first lab experiment consisted of brewing a large amount of tea and isolating the caffeine in the extract. That was the only delicious smell I ever encountered in the course but even the later, malodorous ones give me a nostalgic feeling whenever I visit an organic lab.

My professor for synthetic organic chemistry called me up for questioning when the course ended and asked why I had bought so much ethyl alcohol from the University Stores. Did I sell it or drink it? No, I was a compulsive washer who believed in washing all glassware with generous amounts of this solvent. Years later I learned that he was an alcoholic and now I wonder if I really did buy so much alcohol. I never liked keeping track of lab expenditures.

Another recollection from that chemistry course stems from the last day of school, when students were supposed to return their equipment for a credit against their deposit. Glassware that had gotten scratched during the course could not be returned, for indistinct reasons, and some students were stuck with useless expensive items. Many of them angrily threw the flasks on the floor, to express their disgust and give the janitor something to do. One poor student had planned an experiment involving evaporation in a very large porcelain dish, costing the then-monumental sum of $25 – it cracked during heating. Several years later I learned that the professor in charge of student supplies had been stealing some of the money; I now suspect that this nonreturnable policy somehow put money into his pocket.

A project I devised for "free play" in the basic organic lab course was to synthesize a new antibiotic, by coupling two well-known antibiotics, sulfanilamide and arsphenamine (the drug for syphilis treatment since its invention by Paul Ehrlich). Unfortunately the course ended before I had time to finish the synthesis and I have ever since wondered whether it might have been useful. Years later I had another idea, albeit more nebulous, for a dual-purpose drug: a contraceptive coupled to an aphrodisiac. Such a drug may be the only solution to the overpopulation problem.

Students in organic chemistry lab courses can easily get into trouble. In my experiment involving a Grignard reagent, the reaction got out of control and shot a thermometer and stream of reagents up to the ceiling. This left me unscathed but the ceiling had an unreachable deposit on it for some time. I returned to the lab to check on it a few years later and found that my mark on Columbia College had been erased and painted over. Subsequently others have reported a similar experience and I now wonder why we weren't told to start the reaction with a small amount of reagents (which wouldn't overheat), then add the rest. Later in life, when I took over the presentation of a biochemistry lab course, I learned that teachers frequently don't worry about poorly designed instructions for experiments.

A fellow student at the lab bench opposite mine was starting an experiment in which a formaldehyde polymer had to be depolymerized, then distilled into a reaction mixture. I had already finished the experiment and suddenly realized that she was just starting it. But instead of adding a few drops of concentrated sulfuric acid to catalyze the depolymerization, she was adding about 25 ml of the acid. It took me a while to assimilate the event, then I yelled Duck! and dropped to the ground myself. She too left her mark on the ceiling and escaped injury.

Another fellow student in the same lab, who later became rather famous as a biochemist, was doing an experiment involving a reaction with metallic sodium. As I turned to look at him, I saw him place the sodium-containing flask under a stream of water in the sink. There was no time for me to respond as the sodium instantly reacted with the water to form hydrogen and the flask exploded in a yellow flash. Bits of burning sodium stuck to his head and I rushed over with a towel and wiped him off. Incredibly, he was essentially undamaged! Just a fit of absentmindedness, apparently. I don't remember finding pieces of glass anywhere.

One of my teachers was Louis P. Hammett, famous for his studies of mechanisms in organic reactions. In my spare time I read his book on this subject with great interest. I think this was the first work of its type. The Chemistry Dept. in Columbia had many stars. By coincidence, he became my boss when I took time out for war research.

An interesting challenge arose when I finished the course in Qualitative Organic Analysis (with the noted organic chemist, Arthur C. Cope). He had found a metal storage cabinet that contained many large bottles of chemicals, whose labels had decomposed. Evidently fumes escaping from one of the bottles had turned the labels to dust. Instead of throwing the chemicals out, he offered some of the students money to try to identify them. The material I was given proved too difficult, given the available resources (mainly test tubes and melting point tubes). I still wonder what it was. Sometimes the same label loss problem arises in my lab; label-making has been a primitive art until recently. I now use a typewriter with plain paper and tape the label onto the container with a new clear plastic tape which looks pretty permanent.

My first two years at Columbia were marked by their novel course, "Contemporary Civilization." This consisted of the study of history, philosophy, literature, and economics. Some members of the faculty had written a special textbook for the course, but we also had to read many books and write essays on self-chosen topics. A particularly valuable book was *"The Making of the Modern Mind,"* by John Randall. This book and course really taught me a great deal about the world and how civilization had come to be what it was (in 1937, very frightening). My teacher for this course was Robert Bierstedt, a very stimulating young man. Recently, I read in the Alumni News that Columbia has discontinued the course because adequate teachers could no longer be found....an ominous sign of the times.

As a scientist I found many of the philosophers' thoughts to be low-level, amateurish attempts at scientific understanding of the world. Of course many of them lived in prescientific times and couldn't be expected to think clearly. Their thoughts on religion were so illogical as to be frustrating but amusing. The only philosophy that impressed me was operationalism, the idea that words have meaning only in terms of the operations people have to perform to define them. This automatically delists the many words in our language which simply cannot be defined and should be discarded.

Graduate School

In 1941 I graduated from college and become a grad student in the Biochemistry Dept. at Columbia University, located in the Medical School (College of Physicians and Surgeons) at 168th Street in Manhattan. Attendance required me to stand in the subway for one hour each way, since I still lived at home to save money; this was a rather tiring process which was aggravated by the fact that underarm deodorants had not yet been invented.

During this time, I became acquainted with a fellow student, Isaac Asimov, who was even then a famous writer of science fiction. As I recall, he was a student with Charles Dawson, trying to characterize the poison in poison ivy. I asked Isaac when he was going to give up writing and become a serious scientist. He said "When I get my degree and a job." What a wrong prediction! He has since written hundreds of books, not all fiction. Perhaps his one failure was coauthorship of a textbook on biochemistry. I thought it was very good, original in design, but apparently it never gained acceptance.

Not long after, I got a part-time job doing library research on the industrial uses of soap for the Association of American Soap and Glycerine Producers. I was assigned various fields of application, spent time searching *Chemical Abstracts* and the patent literature in the magnificent New York Public Library on 42nd Street, and wrote up my findings for publication in trade journals. During my job interview, the head of the organization asked me if I could explain why his silver pocket watch tarnished so fast. Previously he had kept it in his pants watch pocket (an archaic tailorism!) but when he shifted to a side pocket, it started to turn black. The hypothesis I offered was that his side pocket might be made of black cloth, like his suit, and the dye used to make it was probably a sulfur dye (information from my organic course). This seemed unlikely since – I thought – all pants pockets are made of <u>white</u> cloth. He assured me that his suit cost a great deal – $100! – and it might be different. A check of the cloth showed that it was indeed black. I got the job. <u>My</u> pockets are still white.

All of my articles were published in trade journals (such as *Rubber Age* and *Paper Industry & Paper World*) but, to my surprise, my supervisor insisted on putting her name on as the sole author except for two articles, which I now proudly claim as my first scientific publications. She collected all the articles and published them as a book – with her name as the author. This was my first and last experience as a ghost writer. Outside of the money I earned, and the practice in constructing papers, a major boon was the learning of a great number of technical processes involving fatty acids, the primary components of soap. A curious property of metallic soaps (zinc, copper, etc.), their insolubility in water and their ability to dissolve in organic solvents, stuck in my mind and led to several useful applications later in life. A course on The Phase Rule also helped kindle my interest in solubility, partitioning between immiscible liquids, and other physical properties of substances. (Curiously, the important concept of "solubility" has reached few people. Most people think that sugar "melts" in water. The topic surely deserves a few minutes of each pupil's time.)

Another thing I learned from reading many patents was that I couldn't discern the differences

between seemingly identical patents. The field seems to need special minds.

War Years

After a year in graduate school, it seemed impossible in 1942 to finish my training because of the war and I sought a chemical defense job. In those days all companies wanted to know your "Racial Descent – English, French, Irish, Hebrew, etc." This was an ominous question. They even wanted to know your mother's full maiden name, which made it harder to hide your "descent." At that time in American history, there was a distinct prejudice against hiring Jews in many industries, chemical companies included. In present times, this prejudice has no doubt continued to a lesser extent but the modern sensitivity toward the employment problems of "minorities" is also restricted. Strange how Edward Slosson's book had exhorted me to become a chemist but neglected to mention that only certain kinds of chemists were actually wanted….

(While I never complained about these questionnaires, I later – in 1950, when I was looking for a post-postdoctoral job – got more nervy and irritated. To one respondent, who had requested a photograph before considering my qualifications, I wrote "...your request for a photograph was received with misgivings as such requests are used by many as instrumentalities of racial discrimination.")

Abbott Laboratories also wanted to know if I was musical and what was my voice range! Apparently they supported choral groups and must have been anxious to hire tenors, who are always in demand in choruses. I am not a tenor although I enjoy singing. I still have their job application form, which I didn't bother to fill out.

Willard Libby, the inventor of carbon dating of ancient artifacts, interviewed me for a defense job and wanted to know why I was trying to evade the draft. He made an unpleasant impression. Another job I interviewed for was in Maryland, apparently at a poison gas facility. The odor in the air was strongly evident the moment my train stopped and I was happy to miss getting that job. I finally ended up in a rocket fuel research lab near Pittsburgh, run by the National Defense Research Council, the Univ. of Pittsburgh, and the U.S. Bureau of Mines.

Scarcely believing that rockets were actually being used by the German and Russian armies, I learned about the design of solid fuel rockets from documents captured from German soldiers. The documents described the rockets used by our allies, the Russians. That was the only way we could get this information from our ally! It told a lot about Stalin's paranoia. I started under a noted physical chemist from Princeton (Walter Kauzmann), testing the stability and physical properties of small pellets of solid rocket fuel. I made large batches of various formulations, using large mixers in an unventilated area. My significant loss of odor sensitivity might be attributed in part to the inhalation of butanol and ammonium picrate. The picrate, beautiful large orange crystals, was shipped to us in large wooden crates by a munitions factory. One crate arrived with a pair of overalls on top of the crystals. Perhaps the owner of the overalls had dissolved during the nitration process of manufacture, but he didn't spoil the crystals.

For a while I tried to develop personal rockets (micro bazookas, with tiny war heads and rocket chambers) but they all exploded without going anywhere. At least I learned the art of fuse preparation and bomb ignition, features which I taught my son some years later. When he left home, I had to destroy surprisingly large amounts of explosives, signifying that I gave him too large an allowance.

I graduated to supervision of a crew of workers, who molded the powder into various kinds of

compressed pellets. My first scientific contribution to the war effort was to introduce the idea of lubricating the press molds with a metallic soap, spread on the metal as a solution in an organic solvent – a product of my soap studies. My supervisory contribution to the war effort consisted of rewarding effective workers with kisses (just the girls). Without a car, I couldn't figure out a better reward. One young man almost blew us all up by hitting a jammed mold with a screwdriver and hammer.

In a competing lab nearby, they had the benefit of Linus Pauling as a consultant. Their goal was to develop solid fuel rockets without compressing a powdered fuel. They caught one of the workers pushing buttons at random, the buttons that controlled the handling of rocket fuel behind a concrete wall! One stormy night our powder magazine was apparently struck by lightning and exploded.

A lab adjacent to mine specialized in the development of shaped charges, the concave shells used later to penetrate tanks. At the time of the war, I knew only that they made and gave away a lot of nicely shaped sheet copper ash trays. (They tested the shaped charges for penetration power by exploding them next to a stack of copper sheets and the nonpenetrated ones took on nice concave depressions.)

Another lab, down the hill, was more secretive and I learned their goal only when the first atomic bomb exploded over Japan in 1945. They were assigned the job of designing the spherical implosion system that ignited the atomic bomb.

I almost guessed that there was a secret project to build an atomic bomb. Before the second World War had begun, the *New York Times* published an article on the breathtaking breakthrough in Italy: ^{235}U nuclei had been cleaved by bombardment with neutrons, with the production of additional neutrons. The article pointed out the possibility of using the released energy for generating steam or electric power. Later, after the war had begun, I visited my Alma Mater, Columbia College, and happened to notice a large crane next to the physics building, lowering a very large device that looked like a centrifuge. I decided it must be a cyclotron but did not connect it then with uranium isotope separation. However I later read in *Chemical & Engineering News* that there was a severe shortage of fluorine, which seemed odd to me as fluorine at that time had very limited use. Suddenly I realized that someone was making uranium fluoride and checked the Rubber Handbook (the main databank on chemistry at the time) – it said that UF_6 was a relatively volatile compound: it looked as though someone was injecting this substance into the cyclotron (or many cyclotrons) and isolating ^{235}U. However I thought that it would be used to power warships – it never occurred to me that the reaction could be made to take place in a fraction of a second and destroy a city. It was pretty lucky that none of the German spies in the U.S. noticed that cyclotron or the article on the fluorine shortage!

One of my jobs was to act as chauffeur for some bigwigs, some of whom – I learned later – were prominent in the design of the bomb. So the atomic bomb was, to some extent, in my hands. I drove these scientists to and from Pittsburgh in a stretch limousine and still remember a moment when I went over some railroad tracks too fast; I thought the car might break in two and strew us on the road.

It was interesting to watch testing of anti-explosion systems for miners. Our lab was initially a coal mine safety lab, and this work continued during the war. We were warned before each test and could watch the entrance to the mine, down the hill. When the coal dust exploded, you could see a huge flame shoot out of the mouth and simultaneously feel the heat from the flame. Afterward, the boom! reached us. This was an impressive demonstration of the nature of radiant heat. And an impressive demonstration of the danger of working in mines. This lab developed ways of coating mine walls with a nonflammable powder which could bind the coal dust to the walls, rendering it nonexplosive. Unfortunately too many coal mine owners and workers do not take advantage of such safety measures.

Another very audible test was made with our rocket fuel pellets. The evaluation lab would load our "grains," as they were called, into a strong steel chamber with a narrow flared orifice at the open end, like the orifices one sees at the bottom of current rockets. The grains were ignited with a fuse and the pressure inside the chamber was recorded as the powder burned. The ideal burn consisted of a long "shooosh" and a brief fade-out, with a fairly constant internal gas pressure. This meant that one could safely use the grain in a relatively light steel cylinder and maximize the size of the explosive head to be hurled. All too often I could hear a brief "shHH-BOOM" and fragments of steel would fly about. As the gas pressure rose, due to accelerating burning, the remaining fuel would burn even faster and finally explode.

I advanced to the job of formulator, devising improved fuel mixes, and learned a good deal about the properties of plastics, which were mixed with the ammonium picrate to hold it together. My best formulation, which utilized carbon black in the binding plastic, proved to be very insensitive to fluctuations in the pressure of the burning gases, evidently because the carbon blocked the transmission of radiant heat into the still-unburned section of the fuel. We made a huge batch for testing in large rockets just as the war ended in the summer of 1945. I thought this was a major discovery but don't know if it was pursued.

An extra activity during and after the war for 12 years was the preparation of abstracts of new chemical articles for *Chemical Abstracts.* An editor would send me a batch of reprints, which I would then summarize. I chose German articles in an effort to keep alive my ability to read chemical German, which in those days was important. After getting nearly every abstract back from the editors with arcane changes in the writing style and abbreviation rules, which seemed to vary with the individual editor, I gave up in frustration. Their efforts to keep the abstracts short necessitated the use of strange locutions but at least they helped me learn the rules for naming chemicals (which are not well known to many chemists).

A couple of us young fellows at the labs decided to try to maintain our basic knowledge of chemistry by enrolling in an evening course at Carnegie Institute of Technology, near downtown Pittsburgh. We signed up for a course in synthetic organic chemistry but soon discovered that the teacher, the lab facilities, and our postprandial state of alertness deteriorated rapidly. The lab facilities were pretty depleted and our teacher soon vanished. I have always felt admiration for people who have the stamina to attend evening courses after a full day of work. This admiration was reinforced after the war when I took a course in neuroanatomy at night at Columbia. The teacher's slides would go up on the screen, the lights would go out, and I would fight to stay awake. I don't think I actually snored.

This battle between lights and unconsciousness has been the source of one of my pains of being a scientist. The huge mass of data enlisted by every scientific speaker forces him to utilize slides, and many lecture rooms have no middle ground between utter darkness and utter light. For some years, when I forced myself to drink coffee, caffeine alleviated the problem. In later years, when I became hypersensitive to caffeine, this aid had to be relinquished. However, I think I surprised some colleagues and speakers who thought my resting eyelids were a sign that I was asleep.

During the war period I managed to devote much time to serious reading. One book on evolution came to the amusing conclusion that Darwin was completely wrong, since "there should be nothing alive now since rocks are fittest to survive." An economics book that I read praised the capitalist system and pointed out that rich people had suffered more from the recent depression than poor people since statistics showed that they had suffered a larger *percentage* drop in income! Evidently this brilliant author thought that a 20% drop in a substandard salary was of little significance. I have since frequently

noted similar failures to appreciate the difference between percents and absolute values.

Another amusing book was *The Critical Temperature of Serum*, by a noted physical chemist at Rockefeller Institute. This scientist, whose instrument for measuring surface tension was widely used, had found that human blood serum underwent many changes in physical properties at about 56^{O}C. These changes were obviously all due to the denaturation by heat of the serum proteins, but he apparently didn't have the slightest information about this and concluded that serum must contain some intriguing component, to which he tentatively assigned a name. I wonder if the publisher had the manuscript reviewed by a biochemist before accepting it.

Another little book, by the same author, discussed the nature of life. For a man who didn't know that blood contains proteins, this was a remarkable essay. He cited an unpublished calculation by some unnamed scientist that estimated the probability that a molecule as complex as a protein could – by pure chance – form from an assortment of atoms (presumably carbon, hydrogen, etc.). The nature of the calculation, which is strictly meaningless, was not explained but the calculated probability was very small and the author concluded that life therefore could not have appeared by chance. It was shockingly clear that he did not understand why <u>any</u> atoms would combine to form anything at all – obviously atoms of sodium and chlorine, if sufficiently close, form salt with a probability of one, while atoms of helium and neon would never form a molecule (at least that was true in those days). I read many strange books in those days.

Graduate Student Days

Back to Columbia P&S in the fall of 1945, where I thought I would study under Erwin Chargaff on the nature of DNA. He had no money to offer for my support so I moved over to David Rittenberg, who was noted for his metabolic studies with heavy nitrogen and heavy hydrogen. One of Chargaff's students, Steve Zamenhof, self-supporting by virtue of his job with a scale manufacturer, eventually made the crucial analyses which helped Watson and Crick (not Chargaff or me or Steve) to solve the problem of DNA structure – and win <u>their</u> Nobel Prize.

One of my claims to fame, while a grad student, was the authorship of a spoof inspired by my departmental chairman's set of books summarizing the chemical efforts involved in the war-time work to bring penicillin to the battlefield. My shortened version of these books was "The isolation and characterization of plentisillin." This article described the chemistry and testing of a completely <u>non</u>antibiotic substance which was noted mainly for the ethanol produced as a side-product in its isolation. I thought it was pretty funny and submitted it for publication to *Science*, the editors of which decided to publish it as a special edition of *Science*, with its own cover page and imaginary volume number (). They gave it away free at an AAAS meeting. The article has subsequently been reprinted in the *Worm Runners' Digest* and a recent collection of similar articles, *Droll Science*. Perhaps I would have gained more fame if I hadn't used a pseudonym as the author, Norman Nadir. Not much of a challenge for a cryptographer.

Konrad Bloch, who was in the Biochem. Dept. at Columbia and later won a Nobel Prize for his work on lipids, stopped me in the hall and almost complimented me on my plentisillin article: "I didn't think you had it in you." I understand this reaction – my smile muscles are very weak and I have difficulty in projecting a jolly countenance. Most professional comedians smile infectiously and cue their listeners to their jokes. One of my many ambitions was to write and publish funny stories but I realized this would take time away from research.

While I was working in Rittenberg's lab, which had one of the few fan-operated hoods in the department, a man came by and tried to induce my mentor to write an article on isotopes in biochemistry for a new journal that was being started, *Nucleonics*. Rittenberg waved him over to me, apparently on the basis of my successful article "in" *Science*. This recommendation gave me more serious fame when the idea expanded itself into five articles. They earned me enough to pay for a marriage to a delightful girl, Norma, and a honeymoon in the end of 1947. And it led later to a long association with a company that sold radioisotopes and isotope equipment. Rittenberg liked my last article, a mathematical analysis of isotope data and the population-aging equation, so much that he wanted me to submit it to a more serious journal instead of to *Nucleonics*. However it really wasn't very original, mainly what seemed to me a clearer explanation than those originally published. Now, I can't understand the math.

In those days, radioisotopes and counting equipment had just become available and for my thesis I had to devise methods for synthesizing labeled compounds and counting the biological products in a manually operated Geiger counter. To count the radioactive compounds, I had a glassblower make me a set of funnels which included a removable sintered glass filter disk. The insoluble radioactive material was filtered from a suspension in liquid and formed a relatively flat, uniform coat on the disk which could be inserted into the counter.

Rittenberg taught me techniques of transferring CO_2 (the chemical form of labeled carbon then available). During one synthesis, after blowing the gaseous CO_2 into a receiver with nitrogen and freezing the gas for the next step, I noticed a liquid had condensed in the receiver together with the solid gas. My mind didn't respond quickly enough to interpret the observation and soon afterward the liquid expanded to gaseous form and popped the radioactive CO_2 out into the lab. Students didn't rate hoods in that department, so I held my breath and moved back fast, warning others to get away. I later realized that the liquid in the receiver was simply nitrogen, which had liquefied due to the use of very cold liquid nitrogen outside the vessel. In other words, I didn't realize that commercial liquid nitrogen comes in a state that is <u>below</u> its boiling point. For years I wondered if my bones had assimilated some of that ^{14}C.

For my thesis research, which was done with the help of a co-mentor, David Shemin, I had to chase and catch ducks in a bird coop on the Columbia P&S roof, hold the poor struggling bird while slitting its jugular veins, and collect the blood in a large dish. Portions of the blood were incubated overnight with labeled glycine or acetic acid or other compounds and I introduced the idea of adding penicillin and streptomycin to maintain sterility. (These were the only mold-derived antibiotics then available.) I don't know if my publication started the custom or whether others had the same idea, but 45 years later I still see papers describing the use of this mixture in cell cultures, without attribution, of course.

The residual, blood-depleted ducks were donated to various departmental members for cooking, which makes my thesis subject better than theses based on mice or rats. However I never liked inflicting pain on these poor animals.

My thesis research was based on the previous discovery by Rittenberg and Shemin that the heme in red cell hemoglobin was made from an amino acid, glycine, and acetic acid (vinegar). The question was: how did they get assembled into the complex molecule, heme? I was able to show that no other compounds were involved and that the source of the various atoms was one or the other of the starting atoms. The thesis ended up as two publications, one of which was chosen by the Journal of Biological Chemistry in 2006 to be labeled "a JBC Classic." This tribute was directed to Rittenberg, not to me or Shemin, but I should have shared the credit since most of the work and novel intellectual effort came from me. Shemin later figured out the complete process.

While a student I did odd jobs in the department and still remember how nervous Shemin was as he watched me convert $2,000 (prewar money!) worth of heavy nitrogen to glycine. Somehow, perhaps because of my general habit of emotional non-reactivity, this task did not make me fearful.

I did drop something important later, a 1-liter bottle of nitric acid mixed with sulfuric acid, which was too heavy for my single-handed grip. It narrowly missed Prof. Erwin Brand (the first man to analyze a protein for its amino acid content) but it splashed on my pants. I hastily jumped into a nearby sink – unexpectedly athletic! – and let the water dissolve my nitrated pants without harm to me. Until recently I always wore heavy woolen pants and recommend them highly to any chemist. A co-student was not so lucky when she spilled chromic-sulfuric acid on her (typically female-thin) clothes and developed a permanent red scar. That must have been very painful.

My departmental chairman (Hans T. Clarke, the founder of Eastman Kodak's organic section) told me that he too had lost his pants in a chemical accident and had since then always kept an extra pair of pants in his office. I followed this good advice for many years. Dr. Clarke also let me use his glassblowing torch – possibly the only thing used in his lab – and gave me personal lessons in glassblowing. This skill has come in very handy throughout my career for devising new apparatus and repairing broken equipment. Professional blowers are always overloaded, especially if you are not a member of the Chemistry Dept. I often wished I had a glassblower's lathe, which is needed to make really straight seals. My hands have always been too shaky to allow me to make professional-looking connections.

A small but valuable lesson that I learned from Shemin was the use of a Pasteur pipet. This simple glass-and-rubber bulb device was known then to everyone as an eyedropper or medicine dropper, but practical ones were not in chemical use or commercially available. We made our own and I continued to do this for many years until they became available commercially. But the first ones commonly available were made from "soft" glass (high in sodium content and therefore a little soluble in water). One day I was transferring a solution of a pH indicator in water and was astounded to see the indicator color change from yellow to blue inside the pipet. Evidently the glass dissolved fast enough to increase the alkalinity of the water by a significant extent. I discovered that one could now buy borosilicate (Pyrex) Pasteur pipets and promptly switched to them.

At one point in my student career, I came up with a scheme for synthesizing cholesterol from "coal, air, and water," that is, from man-made chemicals as opposed to chemicals that could be formed only by living creatures. This kind of project has long been an ambition for some organic chemists. I showed Clarke my proposed sequence of reactions and he thought I should try them. When I said that I had only thought about the possibility, rather than actually trying it, he seemed rather disgusted.

I remember also asking Clarke if he thought that it might be possible to make antibodies to antibodies. In those days, the remarkable specificity of antibody-antigen interactions was just becoming appreciated. Very little was known of the chemical nature of antibodies. My question, which he answered with a "Maybe," was just a curious thought rather than a plan to use such anti-antibodies. If only I had foreseen the proliferation of uses of this idea that were to come!

For a student seminar, which all students had to present, I chose an explanation for the new technique called column chromatography. Stein and Moore, at Rockefeller Institute, had recently greatly advanced its use, separating all the amino acids, a monumental achievement. Clarke interrupted my exposition to announce that my explanation was incomprehensible: "If I didn't know what you are saying, I wouldn't know what you are saying." New Yorkers talk like that. This cooled me off a bit but

he later gave me excellent private editing lessons for my *Nucleonics* articles, for which I was very grateful. He was a major editor of the *J. Amer. Chem. Soc.*, the predominant American chemical journal, and helped steer me into my future life as an editor. Actually, no one in those days had a clear understanding of the nature of chromatography and I have often endeavored in later years to understand it more thoroughly and teach it to others.

A similar comment I remember from those days came from my Geheimrat, as Rittenberg liked to be called. Recently a colleague next door to Columbia's P&S had discovered that D-glutamic acid made mentally retarded children brighter. This raised a stir until other researchers were unable to confirm it. Rittenberg asked him: "How much D-glutamic acid should you have eaten in order to know better than to publish such a finding?" An analogous form of this question ought to be asked of several later studies of this ilk.

At Rittenberg's suggestion, I synthesized deuterium-labeled DL-glutamic acid and ate it (with some trepidation). The idea was based on intriguing reports that tumors contained D-amino acids. It was believed that ingested D-glutamic acid (unlike the common L-isomer) was not metabolized and was simply excreted into the urine. If the concentration of the isotope was lower in the urinary amino acid than in the ingested amino acid, that would mean that the body *normally* makes some. I don't remember why this project fizzled out; maybe I couldn't isolate the glutamic acid from my urine. Or maybe L-glutamic acid contaminated it. Alton Meister told me later that he too had been unable to isolate the compound from urine.

This experiment reinforced my belief that a scientist should not use himself as an experimental subject. Some years later I read an experiment while browsing in the old book section of the medical library at the Univ. of Michigan. A short news paragraph in a medical journal reported that some chemist had synthesized a yellow-green oil and tasted it; he promptly died. Apparently his lab notes did not give any information on what he had made.

One curious item that I saw was in Rittenberg's possession: it was a flask containing pure $^{1}H_2O$. Ordinary water, as found in nature, contains a small amount of $^{2}H_2O$ (heavy water). When a deuterium factory separates out and purifies the heavy water, the process leaves behind some water that is totally (or substantially) free of heavy water. Of course it has no biochemical value.

My life's career was strongly shaped by a fellow student, Steve Zamenhof, the one who made the breakthrough analysis of DNA. He is related to the Zamenhof who invented Esperanto. Steve escaped oncoming Nazi troops by swimming a fantastic distance. He pointed out to me that virtually all the troubles in the world come from human stupidity. It is true that disease causes much trouble, but stupidity spreads disease and keeps us from discovering cures. Thus the wisest goal that a biochemist should seek is the invention of a way to make people smarter – much smarter.

Steve had already tried to do this with rats, by injecting growth hormone into pregnant rats in his home laboratory. He found that the brains of the offspring were larger than those of the control rats, a step in the right direction perhaps. I adopted this goal for my scientific career but have had difficulty in devising the correct scientific approach. In 1979, Atsushi Hara and I succeeded in producing young mice with brains that were 13% larger – a significant increase – by injecting them with a compound (conduritol B epoxide) which forced them to accumulate a normal substance, glucosylceramide. I don't know yet if they were smarter and no one seems to have followed up on this discovery.

Note inserted 1997: a former postdoc, Jin-ichi Inokuchi, tells me that a by-product compound that we

had discarded after synthesizing a new drug, D-threo-PDMP, seems to have memory-enhancing or intelligence-enhancing activity. This compound, like conduritol B epoxide, induces an elevation in tissue glucosylceramide. So perhaps my original goal will be serendipitously attained after all. Maybe there is hope for humanity after all.

Unfortunately the epoxide proved to be rather difficult to make, which discouraged me from trying to extend the study. I put in a considerable amount of effort in trying to make the synthesis more reliable and eventually published an improved method. Because of the effort required for the synthesis, I continue to hoard the epoxide instead of using it. A carbohydrate lab has followed our synthetic procedure and now offers it for sale, but the price is much too high for mouse experiments.

A picture of myself as a grad student comes to mind: I used to run, not walk, to destinations in school. It sounds strange now; I have never seen other students run and I stopped doing it after getting my Ph.D. But I still have the feeling that there is a shortage of time, the time needed to accomplish the goal that Zamenhof set for me. I frequently think of myself (and all other scientists) as being in a desperate race against the destructive and stupid people of the world, in order to prevent humanity's destruction. This feeling accounts in part for my impersonal behavior and lack of small talk – I'm in a hurry, even when I am relaxing.

All or most of the graduate students in biochemistry at P & S worked in a single large lab. We had for company the famous Dr. Zacharias Dische, the inventor of several notable color reactions for specific sugars. He had one of the few colorimeters then commercially available, the Klett. This was in the days before spectrophotometers had appeared. Dr. Dische was a kind gentleman who let me use his rare instrument. He later married a lab assistant; she became a grad student under his tutelage and obtained a Ph.D.

Another inhabitant of this lab worked next to me, an elderly lady who did something with porphyrins. She would come into the lab, shake some very large separatory funnels containing different colored solutions, then leave them in supports that covered her lab table. I was never able to worm out from her what she was doing and believe she never published anything. But the colors made the lab more interesting.

Another unusual person in our lab benzoylated amino acids. He did publish some papers on his method (a simple acylation reaction), including carefully measured melting point values. It seems that he did nothing else and was rarely in the lab. We were friends of a sort and he showed me his set of atomic models, beautifully machined steel balls which could be connected to each other, made at his own expense. Apparently they were never used. In those days, one couldn't buy realistic atomic models, so perhaps the models weren't as silly as they sound now.

One time he told me about one of the visiting or guest biochemists, an M.D. not too well trained in physics, who had filled a very large Erlenmeyer flask with rat feces. This intrepid researcher wanted to lyophilize (freeze-dry) the feces, so he connected the flask to a vacuum source. Of course an ordinary Erlenmeyer this size could not withstand a vacuum and it imploded, sending feces and glass everywhere. This happened at night and the investigator stalked out in disgust, leaving his residues. My friend kindly cleaned it up so that we students wouldn't have to smell the stuff the next morning.

My friend was somewhat of a loner and apparently did most of his work – if any – at night. Someone told me he saw him making large hand movements, somewhat like the slow-motion exercise movements we see in movies of contemporary China (Tai Chi). It turned out that he was practicing pouring liquids from one flask to another – all imaginary!

A member of the faculty, a semiretired friendly gentlemen who seemed to do nothing in his lab, showed me that one could make beautiful micro rockets from a glass melting point tube filled with a little ammonium picrolonate. You load a little of the nitro compound into the bottom, closed end of a very narrow glass tube, then let it sit in a larger glass tube (the launcher). With the picrolonate section protruding from the open end of the launcher, you place a lit match under the explosive and away goes the rocket, at tremendous speed.

Our Biochem. Dept. had quite a group of semiretired, semifunctional biochemists who eventually disappeared. I always wondered whose generosity it was that kept them alive; probably Dr. Clarke's. At least they made for a colorful group.

My wartime experience with plastics in rocket fuel tempted me, while a student, to go into business – as a part-time occupation – making laboratory equipment out of plastic. As a glassblower and dropper of glass equipment, I knew the need for an efficient way to make unbreakable equipment and designed an assortment of products to sell. It was fun thinking out solutions to the problems of working with moldable plastics, yet producing equipment that did the same thing as meltable, sealable glass. However, I soon realized that this could not be a part-time activity and reluctantly dropped the idea. Later, a few others had the same idea, more seriously, and now one can buy a remarkable assortment of chemical equipment made out of the many new – and better – plastics that subsequently became available.

Postdoc-ing

When I came near the end of my thesis lab work in 1948, I was able to win a postdoctoral fellowship from the U. S. Atomic Energy Commission. This too came from my early writings and work with radioisotopes, as well as Dr. Rittenberg's influential and enthusiastic letter of support. When my name was published in a list of AEC fellows in the *New York Times,* a religious peace organization wrote a little booklet denouncing my forthcoming work on developing another weapon of war, perhaps another atomic bomb. I wrote a letter of protest, which they published together with a new diatribe. Actually a pretty funny misunderstanding.

The AEC fellowships were given out with no strings attached, so that one needed only to find a host with whom to work. My wife, Norma, said we should go to the other end of the country, California. New York City was even then becoming very grungy. I went to work at the beginning of 1949 as a postdoc with H. A. Barker (called "Nook" for some reason) at Berkeley. He had isolated a microorganism from the mud of San Francisco Bay, which was a byproduct of the cities lining the bay. Sewage treatment plants were apparently unknown then, as one could confirm by driving and breathing along the edges of the bay. The mud was a rich source of organisms living on sewage, and Barker had waded into the muck and pulled out one that lived on uric acid as its sole organic food. (Uric acid is a major component of human urine.) I identified some of the intermediates in its utilization of uric acid and almost identified one of them, aminoimidazole carboxylic acid. This had to be left for his next postdoc. In a step needed to synthesize it nonenzymatically, for comparison with the natural substance, I had to use a cylinder of hydrogen cyanide and set up my equipment in the large open courtyard inside the Univ. of Calif. Life Sciences Bldg., protecting myself with a gas mask. I survived the reaction and still have the compound I made, but scared a lot of other people in the building. Not every biochemist has survived hazardous reactions; one noted but sloppy fellow in the same building died from phosgene when his reaction vessel exploded. Chemistry indeed has its risks, so easy to forget.

When my fellowship money ran out in the summer of 1950, I had to find another position. I.L. Chaikoff, a prominent researcher in the lipid field at Berkeley, offered me a second postdoc job. Curious coincidence: he worked on lipids and cancer, a topic that now is my major interest. What would have happened if I had stayed with him? Norma and I moved to a new apartment just as Roger Williams came to town to talk about his ideas on the chemical individuality of people ("chemical fingerprinting"). It seemed that this might point me in the direction of raising human intelligence and I quickly read his slim book on the subject. I offered my services and he started me off in Austin at the University of Texas. I felt a little guilty about deserting Chaikoff, even though I had not actually started work and Chaikoff had not chosen me over other applicants, but he assured me this was an OK move.

My first assignment was helping Lester Reed with his newly discovered vitamin, lipoic acid. I was the second person in the world, after Reed, to view crystals of the compound. My job was to discover its function and I did find out something about it, by growing bacteria on a medium deficient in lipoic acid, then testing the bacteria with various metabolites. In those non-air-conditioned days, on many a day after coming to the lab in the morning, it was necessary to add ice to the Warburg bath to cool it down to body temperature, 37^{o}C, before starting an enzyme incubation. I offered my results for publication and was promptly rejected. Williams urged me to try another journal; this time the editor accepted my paper without asking for any changes and thanked me. My first lesson in the arbitrariness of reviewers.

Williams proposed a problem: how can you estimate a person's vitamin status? How much of a particular vitamin is in a person's body? I proposed to make vitamin B2 (riboflavin) with heavy nitrogen (^{15}N), feed it to a person, let it mix with the body stores of B2, then isolate and analyze the B2 that is excreted into the urine. Since the vitamin in food and in the body contains primarily ordinary nitrogen (^{14}N), the ratio of ^{15}N to ^{14}N in the mixture of the two kinds of B2 in the body would tell you the ratio of the two weights, fed B2 vs B2 already in the body. I had to assume that the two kinds of B2 would mix quickly throughout the body.

This isotope dilution method would require the use of a microorganism to synthesize the vitamin from a medium containing ^{15}N-ammonium nitrate. The best organism for this purpose was tightly controlled because it could destroy the Texan cotton crop if let loose. After I received permission to grow the organism, it was simply mailed to me via the regular postal service. What would have happened if the postal truck had gotten into an accident and let the cells loose? In those days environmental controls existed but they were haphazard and few people thought about them.

First I had to develop a method for isolating B2 from human urine. To do this, I had to cover the lab windows with ghastly orange plastic shades, the kind used in furniture store windows to prevent sun damage. B2 is quite sensitive to sunlight (a point disregarded by milkmen, who typically left the bottled milk on your front steps). [I remember the clippety-clop of the milk horse in my youth, and the need to look down at the pavement as you crossed the street.]

To make my lab even more bizarre, I used pyridine as a column solvent since that was needed for the published method. Because of a hood lack, the columns had to be run in the open, so people shunned visiting my lab on those days. I became rather tolerant of the odor, possibly because of my wartime work with ammonium picrate, but the odor penetrated my body and clothing. I would place 5-gallon bottles, topped with a large funnel, in the men's room and leave a card requesting urine donations. As a preservative against bacterial degradation, acetic acid was left in the bottle. One day I found a note scrawled on my card – "It tastes sour!" There's a complainer in every crowd.

This project never quite made it to completion, as the man who was going to run the mass spectrometer (needed for analyzing ^{15}N) left Texas. This left the instrument – typically a very delicate device in those days – in an unusable state. I still think the idea was a good one and wonder if anyone has since tried it.

Another study was carried out on the role of D-alanine in bacteria, in collaboration with the estimable Esmond Snell. This amino acid exists in bacteria despite the belief of some philosophical scientists that God created life using only the L-amino acids. I synthesized radioactive D-alanine by a new method, of wide utility, which used a kidney enzyme, N-acylase, to separate the mixture of D- and L-alanine. I came close to proving that the bacteria made a dipeptide of D-alanine and a nucleotide containing D-alanine but couldn't prove it in the time available. The latter compound was later characterized and found to be important in the synthesis of bacterial cell walls.

Dave Metzler, who at that time was Snell's student or postdoc, needed some glyoxylic acid for an experiment. It was commercially unavailable at that time and hard to make by published methods, but I had devised a simple method while a postdoc with Barker. Dave polished the method, which involved a reaction between tartaric acid and periodic acid, and we published it together; I wonder if it is in use commercially. Dave later became quite famous in the pyridoxal field and wrote a thick biochemistry textbook.

A strange event occurred while I was in Texas, when Fritz Lipmann (a very famous biochemist) came to the University to present a talk. I was invited to join the faculty members and Lipmann for dinner – a nice honor for a mere postdoc. After dinner, everyone went home and threw out the off-hand suggestion that I entertain Lipmann for the rest of the evening! I was totally nonplussed at this impoliteness and unprepared to act on the suggestion – my small apartment, with its wife and baby Lon, seemed inappropriate for a prominent visitor. We walked around town for a while in what must have been a boring period for Lipmann (I have never been a small talk entertainer, socially or scientifically), then I took him back to his motel. For these many years I have reworked that evening in my mind, wondering what I should have done.

Actually I was myself treated even worse when I went to the University of Connecticut to give a talk. After dinner out with some hosts I was dumped in a rooming house, which served as a motel. Unfortunately I had not thought to bring emergency reading rations and there was no TV set in the house for desperate watching. However there was a piano in the main living room, on which I improvised for several hours at the expense of the house owner's mind. (Luckily there were no other guests.) A pianist with a good memory could no doubt get a lot of productive playing done under this condition, but my memory for music is virtually zero so I have to approximate and improvise.

My First Real Job

In 1952 I moved to my first independent position, at the Hines V.A. Hospital near Maywood, IL. I was the biochemical head of a small Radioisotope Facility which treated patients with isotopes or used radioisotopes for diagnosis. I got this job because of my past experience with radioisotopes and fame from the *Nucleonics* articles. Actually I had no connection with the clinical operations and simply did my own research.

In my effort to follow Steve Zamenhof's advice to make people smarter, I consulted my notebook filled with potential projects and chose to study a substance which seemed to occur primarily in the brain. This was a substance in the category of lipids called, very appropriately, cerebroside. Virtually

nothing except its structure was known then but I felt that it must be very important in brain function and, perhaps, in intelligence (or God would not have put so much of it there). We later showed that about 1% of the intact mature brain consists of cerebroside. This is a lot for one substance, considering the fact that the brain consists mostly of water and contains many thousands of substances.

First I applied what I had learned in Lester Reed's lab – the chromatographic use of a little-known adsorbent, Florisil – and my experience in Columbia with ion exchange chromatography (the technique I had failed to describe adequately to H.T. Clarke). This led to one of the first uses of chromatography in the lipid field, a way of isolating pure cerebroside in nearly 100% yield and an analytical method for measuring its concentration in brain. This approach was probably the first for a single lipid and I still remember Jordi Folch's generous praise for the method at a meeting where I described it. I thought these were the first steps needed to follow my future attempts to manipulate its level in brain.

Florisil – a well-named silicon-containing material made in Florida – had some modest popularity among lipid researchers after this publication and I had the satisfaction of attending a meeting session during a major biochemical meeting: an entire morning or afternoon devoted to papers describing uses for the stuff. However, the manufacturer of Florisil never became interested in modifying their product to make it more effective and it was soon displaced by a new product from another company, a silica gel called Unisil.

My isotope counting was done in a narrow room which contained our windowless Geiger counter, built for efficient tritium and ^{14}C counting. Since one had to count each sample for a specified time interval, write down the accumulated counts, and replace the sample manually with a new one, I spent a lot of time reading there, leaning back against the wall. Later I realized that the wall was covered with ugly brownish green glazed tile – the color of some uranium salts, said a nook in my memory stores. A check with a portable Geiger counter showed that the wall was hot as a (discharged) pistol and I quickly changed my work habits. Why didn't I publish a warning or have the wall covered? I keep wondering if cancer will ever strike at my back. Much later, a benign tumor indeed formed in the myelin sheath of my balance nerve, next to the brain, close to the radioactive wall tiles. This tumor – called an acoustic neuroma in those days – cost me hearing in one side and a damaged facial nerve that caused many years of pain in one eye. I had to give up flute playing and whistling because of poor muscle control.

The tumor, in its early, unrecognized stages, also cost me some dangerous falls. Growing on the balance nerve, it produced an unappreciated poor sense of balance. One time, while attending a meeting in New York City, I slipped in the hotel bathtub, slammed into the metal tub, and broke open the skin on my forehead. The hotel offered to put me into a cab, bloody towel and all, to send me – unaccompanied – to their official doctor. Luckily, Bob Glew was also attending the meeting, waiting in the hotel lobby as I walked through, and volunteered to go with me in case I had received a concussion. He stayed with me while a doctor sewed up the cut and I now have a thin scar to remind me of the event and Bob's kind concern. By coincidence, Bob and I were studying the same enzyme and we collaborated later on two papers. He has led an interesting life as a doctor/teacher in Africa and I hope to read his autobiography some day.

My sense of balance, based on only one set of semi-circular canals, is still a bit unreliable and the hearing in the affected ear is nil. I tend to drift while walking but no one has arrested me for being drunk. If a wall is nearby, I try to walk close to it, as a guide. I had several other dangerous falls, before and after the tumor was removed, and must try to remember that my sense of balance is now permanently unreliable.

The job in Maywood was temporary, until the new V. A. Research Hospital in Chicago could be finished. I moved there and worked closely with John A. D. Cooper, who later became the head of the Assn. of Amer. Medical Colleges. John got us Lyle Packard's second scintillation counter, a working lemon but a great replacement for Geiger counters. It got me started in scintillation counting and led me to meet Bernie Agranoff in a more serious way. Occasionally the building power would go off and, when it came on again, the counter would blow an expensive bank of tubes. No transistors then. After a while Packard added a circuit to prevent this. Other problems aided his learning process and he finally made a tremendous business out of making scintillation counters.

Life in the V. A. Hospital had its humorous moments. The man who designed our lab installed a room with light-tight black window shades, in the belief that Geiger counters were harmed by light. I never figured out the origin of this idea. This wasn't as silly as a newspaper story about some American scientists who had supposedly come to study a fallen UFO. They threw Geiger counters at it, said the report. In those days, counters were very expensive, which shows how governments always manage to waste big bucks, even in news fiction.

One time I was trying to make a toluene-soluble product from galactose, in order to render it countable by liquid scintillation, and succeeded in making efficient conversion to the thio ether acetate, which dissolved quite nicely. However it was impossible to buy small amounts of ethyl mercaptan, the starting reagent. (For the nonchemist reader: this is one of the smelliest chemicals known.) The mercaptan was sold in a sealed bottle which couldn't be resealed. So I tried storing it inside a larger bottle but the odor came through anyway. Then I placed the bottle in a freezer, which should lower the rate of vapor escape. This only succeeded in stinking up my freezer. Pouring the material down the sink seemed too dangerous for the sewage system, not to mention all the open street sewers in Chicago. The Occupational Safety and Health Administration (OSHA) did not exist in those days to help solve the problem. I finally oxidized the mercaptan with potassium permanganate in a hood, which converted it to odorless, biodegradable (I hoped) ethylsulfonic acid. Halfway though this process, a desperate man raced into the lab – the hospital kitchen had been cleared of its staff because of a mysterious horrible odor. A trip up to the roof disclosed a strange design by the architect or duct installer: the vent from my lab hood directly faced the vent from the kitchen. The kitchen hood fan was off, and the vapors from my hood vent were sucked down into the kitchen. Previously the vapors were too dilute for the kitchen workers to notice, and I hate to think of how much radioactivity entered the kitchen at other times.

The V.A. Hospital was built to send vented air out the top of the building, but no provision had been made for an air inlet. All the windows were carefully sealed so they wouldn't let in fresh air (not a bad idea for Chicago winter days, which were black from all the soft coal furnaces). As a result, the building operated under a vacuum and many a glass entrance door smashed shut after people forced their way in or out and let go of the door. This smashing problem was solved (!?) by installing a revolving entrance door, which maintained the vacuum more effectively than before. Of course the air outlets (the fans venting the air at the roof) could not operate at full efficiency since air could not freely enter the building.

Another curious feature of this construction attitude was the inability to turn off the heat in the spring. I begged the chief engineer to tell me where the thermostat was located. He finally relented and revealed that it was inside the hot air ducting, not in the rooms! Thus the thermostat made sure that the heated air entering the rooms always had the same temperature, no matter what the room temperature was – too hot for springtime and fall and sun-facing rooms. I was told that the V.A. Administration in Washington DC gave the orders, when to turn off the heat and when to turn it on. The hospital's

latitude or other climatic variable was irrelevant. The thermostat had no function with regard to comfort or need or fuel bills.

The V.A. also had some quaint ideas about purchasing techniques. I was warned that one must specify every aspect of a desired piece of equipment, such as the number of switches or meters. The model number and name of the manufacturer were not enough. Silly as this sounded, I nevertheless followed this advice. One time, I ordered an expensive balance according to this rule. A more skeptical colleague also wanted the same balance but neglected to specify every detail and found that he had actually ordered an Italian imitation of the same balance. This lower bid saved the V.A. $5 and left him with a distinctively inferior balance.

It was initially aggravating to try to order some chemical or small piece of equipment in a hurry. "Why can't you plan ahead?" was the response. Obviously the Purchasing Dept. head had no conception of the nature of research. After all, hospital supplies get consumed at a steady pace and there is never (?) a need for something new, in a hurry. Finally I realized that it was necessary for me to reply: "I just had a good idea for curing cancer and want to try it out as soon as possible." This reply worked every time, but the bureaucrats must have wondered why my ideas didn't really work.

Around this time, Alexander Geiger, in Chicago, was trying to keep dog heads alive without the dog's body. He used a heart-lung apparatus as a substitute for the rest of the body, to oxygenate and circulate the blood, and was able to maintain brain wave patterns and blood flow for several hours. He felt that absence of the liver prevented longer survival and that the liver must normally supply some important nutrients to the brain. On the basis of my discovery that brain cerebroside was probably made from uridine diphosphogalactose, and Bernie Agranoff's discovery that lecithin synthesis requires cytidine, he added uridine and cytidine to the circulating blood and obtained considerably longer survival.

In response to *his* discovery, I concluded that patients in a coma might also be lacking uridine and cytidine and convinced Morris Lipton, at the V.A. Research Hospital, to try this with a patient. In those days committee approval did not seem to be needed for such trials. With some trepidation, I prepared a sterile-filtered isotonic solution of the two compounds which we injected into a patient who was in a coma due to terminal liver cancer. He promptly woke up and was quite lucid. After a while – I forget how long – he returned to his unconscious state. The next day we repeated the test and obtained the same results. This seemed like a striking response and I wanted to try more patients but Lipton lost his interest or nerve and I neglected to pursue the idea with other physicians. Uridine was quite expensive so I wasn't sure whether this was a practical idea. I have since mentioned our experience to several physicians but none seem to have followed it up.

However, many years later (2006) R. J. Wurtman, apparently without knowing my work or Geiger's work, tried feeding uridine phosphate to rats and found a definite improvement in memory. He published additional papers on the subject, showing also that the inclusion of omega-3 fatty acids produced additional improvement. The number of connections between different nerves increased. suggesting the brains had become more "sophisticated." This seems like a patentable discovery to me, with great potential for mankind, but I haven't detected such a patent.

John Cooper, after we obtained our scintillation counter, pointed out that scintillation counting was limited to samples which were members of a limited class of biological substances (compounds soluble in toluene). He asked me to think of a way tp measure the radioactivity in more types of substances. Since any ^{14}C compound could be burned to CO_2, the problem reduced itself to devising a way to

dissolve CO_2 in a substance that was soluble in toluene. My student-day studies of metallic soaps for the Soap and Glycerine Producers came to the rescue again and it was apparent that a strongly basic organic, toluene-soluble amine would form a toluene-soluble salt with CO_2.

Several trials failed to produce a suitable substance but a perambulatory discussion with Bill Holland, one of my students (I taught a night course in biochemistry for Northwestern Univ.), brought out a suggestion: a commercially available amine chloride salt called Hyamine 10-X. This was sold as a general kind of germicide. It took me, together with a postdoc, John Passmann, almost a year to devise a convenient method of converting it to the free base, which could then react with CO_2. Ion exchange worked well on a small scale but I felt that we should use silver oxide for large scale preparation. Unfortunately this yielded a yellow solution of Hyamine hydroxide, which did indeed dissolve CO_2 but gave low counting efficiency. I finally realized that the color must be due to colloidal silver, the result of impurities in the starting material, and eliminated it by treating it like photographic silver – that is, by exposing the solution to sunlight. This precipitated the silver in dense metallic lumps and yielded a colorless solution. I was pleased with this neat solution but learned many years later, to my chagrin, that manufacturers who were selling my Hyamine hydroxide solution were using ion exchange for the synthesis, the first method I had used and discarded.

We also had to devise a way to transfer the CO_2 formed by the combustion process into the 2-oz screw cap jar that was then placed inside the counter.

The Journal of Biological Chemistry rejected the whole idea, saying that it was of little interest to biochemists, even though they had previously published a clumsy, impractical method – by one of their chief editors – for counting radioactive CO_2 in gaseous form. I published the description in Analytical Chemistry instead and have ever since regretted my failure to patent the substance and its method of synthesis. Lyle Packard offered to sell it if I would furnish large amounts but I rejected this idea because it seemed that it would consume too much time. The fellow who did accept the offer became a millionaire and 34 years later it is still being sold. I think that this work helped to popularize the use of liquid scintillation counters but never anticipated that so many counters would be sold. Congress appropriated large sums of research money for a few subsequent years, allowing many labs to purchase counters of this sort.

I found that Hyamine hydroxide could also dissolve radioactive acids or any substances, such as proteins, which could be readily converted to acids. Others later found that it would dissolve whole tissues. These applications and the basic idea were followed up by others and several other amines were developed with similar properties. I had the gratification of seeing many published citations to my method but after a few years they decayed into references in the text to “the classical method” without citing my publication. Poor John Passmann, my postdoc who worked on the problem, suffered the additional insult of having his name frequently misspelled in what were obviously serially-derived citations. Now papers mention its use but offer no reference or “classical” adjective. That’s the usual history of scientific discoveries. Bunsen was lucky for a long time but now people refer only to a lower-case bunsen burner and don’t even know that there was a Dr. Bunsen.

I developed some other counting methods and for a while one of my other substances, Primene, was also commercially available for this purpose.

John Cooper introduced me to Nuclear-Chicago Corp., a Chicago-based supplier of Geiger counters and radioactive chemicals. This led to a long, very pleasant, and lucrative association with some very capable, hard-working people. As a public service, and to gain recognition for their company, they

asked me to write a series of didactic articles on specific ways isotopes could be used. In essence, this was an extension of the writing I had done for the soap producers and *Nucleonics* magazine. This appealed to my basic enthusiasm for exposition and was a lot of fun. I got some glimpses of the life that advertising agencies led, since one of them produced the articles (art work and printing). I particularly remember seeing a gift one of the staff there had received for his birthday: a life-size photograph of a beautiful nude model, taken or printed through a micro-screen pattern. The model had been hiding behind the photo in the same pose when it was presented, I was told. Do biochemists have such celebrations?

These articles were distributed widely and gave me some fame. I think they must have been helpful to many people who were just getting into the use of radioisotopes. Nuclear-Chicago also wanted to publish a lab manual which described radioisotope-based experiments that could be done in all levels of chemistry and biochemistry courses in colleges. They wanted to sell a kit of equipment and chemicals which was economical and educational, yet used a minimal amount of radioactivity (not enough to require control by the Atomic Energy Commission). The goal was a considerable but intriguing challenge to which I addressed myself in spare time, rounding up several people who agreed to devise several experiments each, following my basic plan. However, it turned out that all the experiments needed rewriting and even some lab work on my part, to correct errors or make the experiments more practical.

I devised a stainless steel filtration device (similar to the one I had made as a grad student) which could be used by students to rapidly prepare radioactive samples for counting with a simple Geiger counter. The lab work needed to develop the experiments was somewhat more than I had anticipated, but interesting and thought-provoking. We sent each experiment out to an independent college lab for testing. I wrote a separate guide book for the college teachers who were to supervise the experiments. Nuclear-Chicago published the lab manual and sold some of our kits, but I think many college teachers did not want to bother with such unfamiliar methods. A weakness of the whole idea is that it expected the different teachers in the different fields of college chemistry to join together to buy the equipment. At any rate, the proceeds from writing this book helped pay for my first home.

When I moved to the Univ. of Mich. in late 1960, together with Amiya Hajra (who was still working on his Ph.D. thesis), Nuclear-Chicago asked me to develop a combustion apparatus that would automatically burn radioactive organic compounds and transfer the $^{14}CO_2$ and $^{3}H_2O$ to scintillation solvents. Hajra and I worked on this for a while and came very close to a practical device but we couldn't figure out how to speed up the process of transfer of the gases to the scintillation vials. At least I learned a lot about solenoid valves, timers, and process automation which helped me in future experiments. Ultimately Lyle Packard's company solved the problem – but not very elegantly or reliably. It is still a difficult problem.

A little before this, in 1957, I published the first radioactive study of the metabolism of cerebrosides and a related substance, cerebroside sulfate. The data showed that cerebrosides are not inert structural materials, as had been assumed before, but undergo enzymatic breakdown as well as conversion to cerebroside sulfate. The cerebroside sulfate, once formed, however, appeared to be inert and I suggested that it was the stuff of which memories are made, since both seem to be permanent. The J. of Biol. Chem. actually accepted this suggestion and let me publish it without forcing me to hedge on it. I can't believe that the current breed of reviewers, almost all hypercritical, would allow such an idea to be printed. "Too hypothetical...do the experiments needed to prove it first...wild idea..." Unfortunately for my suggestion, a researcher repeated my study over a longer time period and found that cerebroside

sulfate does eventually break down to some extent. Nevertheless, I still feel that a single molecule could act like a "memocule" to store information just as a simple material, black ink, stores all the information in a book. Joyce Benjamins tells me that I once suggested – in jest – that the ink is written on the lamellae of myelin, as the myelin tongue wraps itself around axons. This would yield a micro version of the spiral scrolls that were used as books in ancient times. As I think of this idea now, it has a certain plausibility: perhaps the axon can read the ink deposits back, like the magnetic pickup on a computer disk drive – but without the rotating motor!

Now a Real Academic

The V.A. Research Hospital was affiliated with Northwestern Univ. Med. School and in 1957 I moved full-time as an Asst. Prof. to the Biochem. Dept., just 2 blocks away. I was given a large lab whose drawers were full of cockroaches. The roaches loved to nest or rest in my test tubes. The lab tables were made of wood, covered with warped sheets of Masonite, a material made from glue and sawdust. The lab wasn't air-conditioned, but the cooling compressor for an adjacent lab was mounted in my lab and transferred the heat from the other lab into my lab. That struck me as a highhanded design decision. The windows had no screens, so bugs would enter the lab in the summer, sniff the vapors from the organic solvents in my test tubes, collapse, and drown. I had to cover every open container with aluminum foil to keep them out.

In keeping with their general attitude toward researchers, the Medical School administrators insisted on the use of a central animal facility, in which the mice were stored in the same room as the cats. The perpetually barking dogs were kept in the adjacent room. When I ran the medical student biochemistry lab, I had to keep the mice for the students in a small room, heated (non-thermostatically) by hot pipes traversing the room; one time, the mice died from the heat and spoiled the students' experiment. I also had the bad luck of having the departmental water still in my lab. While it made my lab even hotter in the summer, it was comfortable in the winter.

I set up a polyethylene pipet soaker in a sink and filled it with chromic/sulfuric acid. One morning I discovered that this plastic, despite the manufacturer's opinion, really cannot handle that acid mixture. The night before, the acid formed a crack in the plastic jar, dissolved the sink's trap, and ran down to a floor drain, etching a permanent groove in the floor. This experience did not prevent me from having many later unhappy experiences with the limitations of plastic stability.

For example, some years later, 1967 or 1968, when Liselotte Hof was in my lab in Ann Arbor, we bought plastic measuring cylinders made of a new plastic (polypentane) that was touted as being resistant to organic solvents. When Liselotte (who had bravely come from Germany to the wilds of Ann Arbor) began using them, we discovered that her thin layer chromatography plates exhibited eight unexpected lipid spots. Tracking backwards, we found these were chemicals inside the plastic, extracted from the cylinders by our solvent. When I wrote about this in a complaint to the manufacturer of the cylinders, the answer was "There is no extractable lipoidal material in our...cylinders!" With greater than usual stubbornness, I sent them a copy of the TLC plate photo and insisted they try the experiment themselves. This time their reply was more subdued. Apparently all plastics except the fluorinated ones contain the catalysts needed for polymerization, antioxidants to protect them from air, ultraviolet absorbers to protect them from sunlight, and mixtures of alkylated phenols with a sulfur compound. Some contain mold release lubricants to speed the forming process. These, together with incompletely polymerized plastic, are all small molecules, extractable by solvents. Hof and I published a warning note

to this effect. We had found that the plastic was "resistant" to solvents but it gave in easily.

Similar warnings have been published by others about Tygon tubing and plastic centrifuge cups. The oil from vacuum pumps is another source of lipid contaminants in lab air (preventable by attaching a filter trap to the pump vent). An interesting point about Tygon, a very widely used kind of tubing, is that the plasticizer used to keep it soft leaches out slowly even into water (not only into organic solvents) and patients connected to i.v. intakes in hospitals were accumulating the plasticizer in their blood and tissues. Moreover, the plasticizer is so commonly used in plastics that its *vapors* have permeated virtually every person in the USA and it is now a "naturally-occurring" chemical. Remember this when you slide into a hot car: hold your breath and open the windows fast! Several scientists have been embarrassed after reporting its existence in tissues, thinking it is a biological substance. Its use in medical tubing is now forbidden.

The still in my lab at Northwestern University, for purifying water, utilized Tygon tubing which carried the hot condensed water to a reservoir. Thus everyone in the department had been using "plasticized water." I had wondered why the tubing became stiff after a while, not realizing that it had lost its plasticizer. How many experiments did this impure water affect? In my travels I have seen this bad practice in many labs. The still manufacturers only gradually reacted to this problem.

When I was at Northwestern University, the water in Chicago came from Lake Michigan without any intervening filtration step. Thus one could often see floating particles of potentially disturbing origin in a glass of water or in the rinsing water used to rinse acid from washed pipets. I learned plumbing skills and installed a water filter in the rinsing line. Ever since then I have installed filters or had filters installed in glassware washers and pipet washers. It seems strange that others have not noticed the presence of suspended particles in rinse water or the danger of contaminating cleaned glassware, even in cities having a more civilized water system. Dirt often enters water feeder pipes, through the joints buried in the ground, and ends up in the little, coarse filter traps many of us have at the tip of sink faucets.

Thinking of water brings up many memories, out of chronological order. In my early scientific days I used distilled water without any thought about its purity. Then I began to notice advertisements from manufacturers claiming that "their" water was purer. I guess scientists became interested in the subject and new ideas about stills and how to purify water appeared. The process of distillation, which uses a 1-plate distillation column when run on a large scale, requires that there be no spray carryover in the steam and that the steam be condensed only partially, to allow low boiling point impurities to escape through a vent, uncondensed. In other words, the distilled water that forms in the condenser should be quite hot. Moreover, the boiling water that remains in the pot should be discarded before its impurities become too concentrated, to prevent the carryover of highly contaminated spray.

These two principles, I learned, were unknown to most chemists and – more importantly – to the men who controlled the stills. As the city tap water boils in the pot, salts and organic chemicals become insoluble and gradually coat the walls of the heating coil, which slows down the rate of boiling. Thus men from the Plant Department must come in periodically and wash the pot with strong hydrochloric acid and other substances whose nature is unknown to us users. We assume that the washers rinse the washing agents out of the still pot and steam condenser before refilling the still with tap water and restarting the boiling process. To my despair, I discovered that these principles cannot be relied on.

When I moved to the Mental Health Research Institute (MHRI) in Ann Arbor, I found also that the flow rate of the cooling water (that converts the steam back to distilled water) fluctuated, possibly due to

temperature changes in the tap water or attic, where the still was housed. Thus, the temperature of the condensate would vary too, overly cooled one day, insufficiently cooled another day. The rate of water production could not be checked since there was no flow meter in the condenser. In other words, you could not tell when the boiler needed "descaling" (removal of the insulating mineral deposits on the heating coil). The low level of technology at the still manufacturers was incredible.

Additionally, I discovered that the air inside the reservoir in the attic, where the distilled water was stored before we drew down on it, was hardly clean. As we drew down on the reservoir, faster than the still could make new water, attic air entered the tank and the chemicals in the air dissolved in the "clean" water. Thus we discovered that molds were growing inside the reservoir. Perhaps they were a source of useful contaminants. I solved the problem of erratic water flow by installing a pressure controller in the cooling water line, but discovered later that the men who serviced the still increased the water flow in order to make the condensate nice and cold.

I thought I solved that problem by moving into a newly renovated building, the Neuroscience Lab. Bldg. I stipulated that this new still would be of the latest type, which utilized a thermostat in the condensate line to make sure it was always hot. Moreover the boiling pot was to be emptied and refilled periodically by a timer so that impurities could not accumulate too much. The still manufacturers had now come to appreciate the problem of air contamination, so we were able to buy a reservoir that was airtight except for a "breather tube" filled with charcoal and a small-pore filter that would keep microorganisms out. (I have such a breather in my lab reservoir too.) As further protection, I ordered a water softener to be installed on the inlet pipe leading to the boiler; I thought this would reduce the amount of material that would bind to the boiler lining and lessen the frequency of pot cleaning.

Stipulating and getting are two different things ("Man proposes, Plant Maintenance disposes"). After we moved in to the new labs, over the course of several years, I came to discover that (1) the automatic features of the still had never been installed and (2) the water softener had never been filled with salt for the ion exchange process. This is like the very funny movie, *Mr. Blandings Builds His Dream House*. Cary Grant and his wife stipulated the room colors they wanted to the painter – very specific <u>shades</u> of pink, blue, and green. As they walked away, the painter passed on the orders to his assistant: "pink, blue, and green." We consumers are mites who annoy the real powers.

After I registered these complaints, the Plant Maintenance people solved the problems by changing the lock on the still room so that I couldn't check on what they had done. We could detect the more egregious errors quite easily: twice my lab bottle of deionizers was acidified by the distilled water, another time some detergent penetrated the deionizers, so that our reagent solutions foamed on shaking. I don't know if researchers in other buildings at the U-M noticed these problems, but eventually the school administration threw out the stills in <u>all</u> the buildings and replaced them with complex systems based on an initial reverse osmosis filter. These units are serviced by an outside company and they appear to be reliable; at any rate, they are so complex that I have no way of checking on their performance by simply looking. So I am back to my former carefree days of not thinking about water purity. Many researchers now install a separate water purifier in their labs to ensure good cleanliness.

This reminds me, again out of chronological order, of a time I spent as a guest in someone else's lab. To my amazement, I found that the glassware and pipets in the cabinets – supposedly clean – were visibly dirty. This did not bother the other people in the lab, who mainly used disposable plasticware. Apparently it is possible to publish good work even with dirty glassware. It seemed to me that the dishwashing person needed some retraining, so I volunteered to talk to her. However, no one ever saw her; she worked at night when no one was around. The room holding the dishwashing machine was

always locked, but eventually a key was found and I studied the machine. It was obviously broken; all it could do was spray some of the glassware with hot water. It took quite a while to repair the machine but no one complained about the delay.

The same problem exists in my present location, the Division of Nephrology in the Univ. of Michigan. For over two years many of the water sprayers in the Divisional dishwasher have been clogged, so that many items of glassware do not receive a water jet. Despite my entreaties, the machine has not yet been repaired. This problem is a reflection of the great use of plastic apparatus nowadays, which reduces the amount of dirt encountered in many experiments. Everyone assumes that packaged plastic equipment is surely clean. However plastics fabricators must use many of the same impurities that I mentioned above.

Getting back to my three years at Northwestern, an unusual sequence of events brought me to a new aspect of lipid metabolism. My mother had developed multiple myeloma, a fatal and horrible form of cancer, and my desperate searches of the literature disclosed a claim that eating a high level of dry, defatted protein had helped a patient. From reading the ads in journals, I had known that the VioBin Corp., near the University of Illinois in Urbana, sold defatted tissues. I wrote to the company and ordered a can of defatted pig liver, the only animal protein they had. As might be expected, this material did not help my mother, but the owner of the company (Ezra Levin) wrote to me and asked why I wanted it. Our correspondence led to a consultantship for me and interesting interactions at his plant and in my office.

Levin had invented and patented a method for extracting lipids from tissues – a topic close to my heart – based on azeotropic distillation of the tissue homogenate together with dichloroethane. The water in the tissue distilled off together with part of the solvent; the latter was easily recovered for reuse and the lipids in the distillation pot, largely dissolved in the remaining solvent, were simply filtered off from the protein-rich residue. VioBin used this process to extract wheat germ and was a major supplier of the nonlipid residue (wheat germ powder) for cereal manufacturers and wheat germ oil. Actually this process did not dissolve or extract all of the lipids, especially the sphingoglycolipids, but few people knew of them then.

Levin sold a great deal of the oil not only to health food stores, but also to farmers, who used it to pep up the sexuality of their male and female breeder horses and cattle. He showed me unsolicited letters to this effect from farmers, which I thought were pretty convincing. “My stallion was uninterested in the mares until I started to dose him with your oil...” It seemed to Levin that there must be some kind(s) of sex hormones in wheat germ oil and he was able to show androgenic activity in roosters, using measurements of the rooster’s comb as an assay. He convinced an Urbana biochemist, George Wolf, to try to isolate the sex factor. Wolf came up with crystals of a simple long chain aliphatic alcohol, octacosanol. Apparently tests of this material in people were not convincing to pharmaceutical companies but additional tests by an athletic director at the school (Univ. of Illinois) seemed to show that the alcohol increased the stamina of his athletes (a forerunner of the currently illegal steroids!). The alcohol is now sold in health food stores as an “endurance factor.” Perhaps it does improve sexual endurance.

Levin had an additional belief that many of the early reports on sexual enhancement by wheat germ oil, ascribed to its vitamin E content, were actually due to the mistaken belief among many nutritionists that the two materials were identical. Of course wheat germ oil is a complex mixture of lipids. After vitamin E was found in the oil, researchers used the oil only because it was cheaper than pure vitamin E. As the latter became cheaper and the optically active isomer became available, the experimenters

dropped the use of the oil and the sexual discoveries disappeared. It appeared to students of the subject that the earlier studies must have been in error. Levin asked me to search the literature and see if these impressions were indeed true. In looking at these articles, I was struck by the carelessness of the authors, some of whom spoke of the various materials as being interchangeable; others did not even specify the source of their material (pure vs natural mixture). My conclusion is that Levin was probably correct and that nutritionists have missed the boat on this important question. This was an additional reason for my low opinion of nutrition researchers. Unfortunately, no one seems to have carried on the study of octacosanol metabolism and function.

Levin had an even more innovative, bolder idea: a way to eliminate protein-deprivation among the poor people of the world. Of the various kinds of food that poor people lack, protein is probably the most important. For those who live near the oceans, sea creatures ought to be a major protein source but many of the creatures are not ordinarily considered edible and, in any event, a significant part of any fish is discarded in the process of food preparation. Levin had the idea that he could build large lipid extraction systems (dichloroethane azeotropic distillation) near the coast and the plant would extract the lipids from all the sea creatures captured in the nets of fishermen. To keep the cost even lower, he planned to skip the usual dissection process and extract whole organisms: intestines, bones, scales, and all. He succeeded in developing an economical extraction step, after lipid removal, to take out the fishy odor, which is apparently due to some amines or amine oxides.

Levin actually built a pilot plant in Massachusetts and invited me to try the powder. With some queasiness, I made and ate a hamburger made from a mixture of beef and the fish powder. It wasn't bad and I certainly did not get sick from it. Levin said he had tried it in bread and other foods and had found it quite edible.

Acceptance and growth tests in Mexican children looked good. A publication by a Mexican group, presenting the data, pointed out that "...millions of economically weak families...have been suffering from malnutrition in our country. They hardly ingest 60% of the calories an adult needs to produce an average work...large groups of our people ostensibly tired, gloomy, stolid, without sense of responsibility...enduring their nutritional misery..." Of course one can multiply that tale by a thousand. It's easy to see why Levin made this new project his primary one.

He next tried to get the United States government to build similar factories along the coast of India, which was at that time suffering from a distinct food shortage. I tried to talk him out of this idea on the basis that, if the plants were successful, they would only raise the population even further and produce starvation on an even larger scale when the seas became completely fished out. This fish depletion is already visibly happening, with consequent unpleasant disputes over fishing areas and quota assignments. My hard heart chose small-scale misery over large-scale misery.

These two antithetical ideas invoke an important ethical dilemma for all scientists. If we "do good" and help people beat disease and famine and prolong their life span, and if they don't change their beliefs regarding the importance of procreation, we simply increase the scale of the suffering that comes from overpopulation. I don't mean only food shortages, as per Malthus' warning, but also the rapid depletion of the earth's mineral reserves (including water), increasing pollution of air, soil, and water, wars over territory, and the psychological craziness that comes from excessive population density. Scientists and the industrial revolution have already made these problems severe – should we accelerate them by further discoveries and inventions? I think the only way out of this dilemma is to advance science to the point where we can make people smart enough to reduce the population. Or develop my hypothetical aphrodisiac-contraceptive pill.

[It is time for a radical stoppage of conception. Suppose no more children were born in the next 20 years! The world population would decrease by ~2,000,000,000 people (this figure is based on a crude estimate of death rates – I am no population statistician). There are obvious difficulties in this idea, even if people were sensible enough to adopt it.]

As luck would have it, Levin's proposal was rejected by our government, which suddenly became very sensitive to the supposed views of the Indian people. "Why," they responded, "should we support the eating of intestines, scales, bones and other fish parts when our own people would reject them as being distasteful." This was very considerate, but I think they didn't ask the people who would be offered the fish meal. Moreover, I believe that Americans and most other people do eat repulsive parts when they eat canned sardines and anchovy paste, as well as invertebrates of all types.

The last word on this idea came from some nutrition studies, which compared Levin's fish powder with other lipid-extracted proteins. It turned out that fish bones are high in fluoride, which inhibited growth in test animals. I don't know whether this is a realistic factor, since it depends on how much fish powder people would eat, but the report seemed to close out the possibility of offering poor people a chance to raise their protein intake.

Reviewing my copious correspondence with Ezra Levin, I see many forgotten ideas and projects. Levin's defatting technique was applied to many animal organs, as well as wheat germ, and he sold a variety of products, some of which I used in my lab. He offered powdered duodenum, pancreas, thyroid, kidney, etc. The duodenum and pancreas had some clinical use for disorders of the digestive system. He had a wide-ranging imagination and correspondence with researchers and clinicians. He would write, or visit me in Chicago, or call me up, asking for literature studies, or names of researchers to contact, or suggestions for uses of his products. He could have been a productive research team leader but didn't have a Ph.D. or M.D degree. I greatly appreciated our stimulating interactions.

The chairman of biochemistry at Northwestern University Medical School, Smith Freeman, belonged to the fraternal society, Rotary International, and he was approached by mail by a fellow Rotarian in Japan. This fellow wanted to send one of his young faculty members to the States for additional biochemical training for 2 years. Freeman knew I had received a grant and passed the letter to me. That is how Yasuo Kishimoto – my first top-notch postdoc – came to my lab. We have enjoyed a very productive and friendly association ever since. Kishimoto was an exquisitely accurate worker, with an inventive mind, and we produced many an innovative, efficient technique for studying the fatty acids in cerebrosides and related brain lipids. I wish we had published each technique as a separate paper instead of combining them and including the results of the study. This would have given them more visibility and use.

Amiya Hajra also played a major role in my scientific life. He joined me as a grad student in Northwestern Univ. and stayed with me when I moved to the far-more-stimulating Mental Health Research Institute in Ann Arbor. Hajra helped devise new gas chromatographic techniques, particularly a way to measure the radioactivity of separated fatty acids and microtechniques for localizing the radioactive atoms in biologically labeled acids. That allowed him to study the cerebroside acids that were formed from radioactive acetic and propionic acids. We confirmed the previously published reports that propionic acid is a precursor of the odd-numbered fatty acids. When we injected animals with [^{14}C]carboxyl-labeled acetic acid and isolated the major odd-numbered cerebroside hydroxy fatty acid (h23:0), we were puzzled to find relatively little radioactivity in the carboxyl carbon. A preprint from James Mead reported similar results and we quickly clarified the situation by analyzing the second carbon atom of h23:0. This atom contained a relatively high amount of radioactivity and it was

immediately obvious that we had discovered a new oxidative pathway for the very long fatty acids. This was an exciting moment. This pathway, published in 1967, was a 1-carbon degradation system, whereby the fatty acids are broken down one carbon atom in each step, in contrast to the better-known, more common 2-carbon system. This explained our previous discovery, the occurrence of a relatively high amount of 23- and 25-carbon fatty acids in cerebrosides.

This interpretation of the data was strengthened by doing a time study, that is, by killing animals at different times after the initial injection. The radioactivity distribution followed the mathematics of turnover, worked out 20 years earlier by Entenman and Zilversmit but forgotten by many biochemists. Mead published a paper on the 1-carbon pathway but his reasoning was flawed because he (and the reviewers of the manuscript) did not remember the mathematical analysis that applied to this approach. By sheer luck, he killed his animals at a single time point which fortuitously supported the 1-carbon pathway. If he had killed them earlier, he would have missed it.

As a matter of fact, I have read many a paper and manuscript in which the authors had misread the analyses of Entenman and Zilversmit and had therefore come to the correct or incorrect conclusion, depending on when they killed their animals. I was on a thesis committee at Northwestern (assigned to me too late) for which the graduate student's and thesis adviser's understanding of the principles had led to a completely pointless study which could not be published. One major basis of this misunderstanding, I am convinced, comes from a deep-set, built-in human aversion to *ratios* (long division). Instead of measuring the ratio, total radioactivity divided by total weight of material, these scientists measured only the total activity.

This kind of aversion appears throughout scientific and nonscientific thought, as illustrated by the popular use of the term "light" instead of "low density." "Light" refers to weight when actually the user ordinarily means "weight divided by volume." I tried straightening out the editors of Science on this subject once, and they agreed with me, but the error frequently appears in their pages anyway. Another good example of this human frailty is the frequently cited "Gross National Product," which has little meaning if one does not divide the value by the number of people in the country. If the GNP rises 4% and the population rises 4%, there has been no significant change. The only ratio that seems to come to mind naturally is the measurement of speed, which is apparently a survival essential.

For visa reasons, Kishimoto had to return to Japan for two years; then he was allowed to rejoin me in Ann Arbor. Among the facts we discovered: the fatty acids in brain cerebrosides had an unexpectedly high content of the odd-numbered acids (23 and 25 carbon atoms); the proportion of these acids increased with age; the concentration of cerebrosides in brain increased with age for an unexpectedly long time (far longer than the popular idea of the so-called adult brain); human and rat cerebrosides were rather similar in composition; the hydroxy acids resembled the common, nonhydroxy acids in chain lengths except for a lack of 18-carbon hydroxy acids. The hydroxy acids were found to be major brain components, suggesting they play a special role there. In a survey of the hydroxy acids outside the brain, we found that there were none in liver and stored fat, while the peripheral nerves and spleen had reasonable concentrations. Skin had some and it was unusual in that its hydroxy acids were rather short, as short as 10 carbon atoms. We missed the discovery, made many years later in another lab, that omega-hydroxy fatty acids are important components of skin.

Looking at the brain sphingosine-linked fatty acids, we discovered the existence of 25 previously unknown fatty acids containing one or more double bonds. Fifteen previously unknown hydroxy acids were also discovered. It seemed likely from the structural similarities between hydroxy and nonhydroxy acids that the former were made from the latter by some kind of oxygen-inserting enzyme. We tried to

demonstrate this by incubating radioactive lignoceric acid with brain subcellular fractions, and found that a small amount of conversion did indeed occur. Somehow, when the summer arrived, the enzyme activity left and we gave up the problem. I believe that no one else has yet clarified the enzymatic reaction although Kishimoto, in his own lab, made a number of additional observations and found some unexpected cofactors.

Looking at the positions of the double bonds in the fatty acids, we concluded that (1) the brain contains several different enzymes that insert a double bond at specific, different locations and that (2) these acids underwent chain lengthening and shortening by steps of two carbon atoms at a time. That is, oleic acid, with a double bond between carbons 9 and 10 (counting from the methyl end of the molecule), must be elongated by two carbon atoms by addition to the other end of the molecule. Hajra and I demonstrated this reaction with labeled fatty acids, especially acetic acid, injected into rats. It was evident that a great flux occurs constantly in these acids, release from the sphingolipids by an enzyme of the amidase type, attack of the released acid by two chain-shortening systems, elongation of the released acid by an elongation system, and reutilization of the products to make new sphingolipids. Why so much modification? Perhaps this is necessary to keep the brain responsive to the changing world, our changing food intake and our changing activities.

Before we had made these findings, there was a general belief among biochemists that brain lipids were rather inert – once made, they simply stayed there. Even after we published our findings, some papers appeared claiming the authors could not detect a dynamic state. One such paper utilized rabbits, but only one rabbit was used to furnish each time point, and the variability between individual rabbits (as well as experimental variability) was so great that a statistically significant change with time could not be established. This was simply a case of thoughtlessly poor experimental design. It was just one step higher than the experiment I described in my high school short story, with only a single experimental animal. We always used several animals per time point and applied the standard statistical treatments.

Hajra reminds me of an element of luck that helped us clarify the dynamic nature of fatty acid metabolism. We did our earlier experiments with ^{14}C-labeled acetate but some of the data were difficult to interpret because so much of the degraded fatty acid, after synthesis from the acetate, was reutilized to make new ^{14}C fatty acids. At this point we learned that Richard Kowalczyk (a member of the Dept. of Biological Chemistry at U-M) had been buying much tritium-labeled acetic anhydride for some study and was willing to give us the unused portion of this material. Hajra (with some reluctance) converted the leftovers to pure [^{3}H]acetic acid which was converted by the animal's body to tritium-labeled fatty acids. These acids were subsequently degraded by the body to radioactive water, which was diluted with the total amount of water in the body so that the tritium could not be reused to an appreciable extent to make new molecules of labeled fatty acids. This contrast with ^{14}C-labeled acetic acid gave us crucial additional information.

Subsequent work in other laboratories confirmed most of our conclusions but I am not sure whether all of the postulated desaturating enzymes have been found.

Much of this work involved the use of a then-new method for analyzing fatty acids, gas chromatography. We went through a lot of agony devising methods for modifying the primitive commercial gas chromatographs to make them work with the very long fatty acids from the sphingolipids. I was pleased to see that one manufacturer adopted my idea of connecting the chromatographs to the glass columns by means of Kovar seals, which I had learned about as a grad

student when studying Rittenberg's mass spectrometer. Indeed, an unpleasantly large portion of my research career has involved figuring out ways to connect one tube to another and trying to outwit the many suppliers who manufacture incompatible connectors.

Looking at the other fatty acids in brain, those not bound to sphingosine, we discovered 25 previously unknown fatty acids. A big surprise was the discovery that much of the so-called oleic acid of brain was really cis-vaccenic acid, previously rarely observed because of the difficulty in separating the two acids. It appeared that these fatty acids could not be attacked by the oxygenating and 1-carbon degrading enzymes.

Another interesting discovery we made, with Martha Wajda's help, was the chemical structure of the cerebroside esters. The existence of this class of lipids had been noted by two other labs, but their chemical characterization had not been done. We showed that the fatty acid in ester linkage is attached to cerebroside at the end of the galactose portion of the molecule. Fatty acid analyses indicated that it was formed from cerebroside by a fatty acid transfer reaction. Two other labs later came out with different structures but the contradictions have never been clarified as everyone decided not to study the matter any more. I would love to return to this question and settle it with new methodology. Very recently some experimental evidence has been offered to support the belief that the fatty acid is transferred from a still-unidentified source to the cerebroside molecule.

A New Life

In 1960, three years after my move to Northwestern, Bernard Agranoff invited me to join him in a move to the U-M's recently built Mental Health Research Institute ("MHRI"). This move would involve giving up my tenured position as Assoc. Prof. to become a nontenured "Research Scientist" but it had many merits and I jumped at the chance. Agranoff showed me Ann Arbor, which I found to be very charming, a wonderful contrast to The Windy City. He also gave me a taste of a local (Detroit) beverage, Vernor's ginger ale. This clearly needed to be imprinted on a child in order to make it palatable (Bernie had grown up in Detroit). The move to Ann Arbor proved to be one of my very best decisions. I like the idea of working in a city whose very name (in abbreviated form – A^2) is scientific.

One merit of moving away from Chicago is that there are far fewer salesmen here. In Chicago, several salesmen from lab supply houses had taken a liking to my conversation and it was proving difficult to get them to move on to some other victim.

One of my first useful collaborations was with Bernie Agranoff, in the development of a simple way to detect and measure cerebroside. This took advantage of an enzyme that had (by coincidence) been developed by my former boss, John Cooper: galactose oxidase. In 1961 it was reported that the enzyme acted on carbon 6 of galactose and galactose compounds; this meant that it might well be active against brain cerebroside. The insolubility of cerebroside in water blocked our efforts once more but Bernie – working in his lab with his own hands – found that the enzyme could work in a solution that contained much organic solvent, enough to dissolve the lipid. Apparently some enzymes, especially from molds, are remarkably stable to organic solvents. The enzyme produced hydrogen peroxide too, which is easily measured.

Six years later, in my lab, David Bowen applied the reaction to make labeled cerebroside. After the enzyme oxidized the lipid, we reduced it back to cerebroside with radioactive borohydride. This very useful reaction gave us much tritium-containing galactocerebroside suitable for all sorts of studies. Liselotte Hof applied the method in my lab to make radioactive lactosylceramide and used this too to

measure enzyme activity. Many other labs followed our procedure to make these and other galactose-labeled lipids. Some time later, Gregory Evangelatos, a visitor from Greece, examined the nature of the galactose oxidation reaction and we found that the speed of the reaction could be distinctly increased. I think our evidence supported the idea that a super-oxidized form of copper, Cu^{3+}, is the active component of the enzyme. However, this modification seems to have been missed by users of galactose oxidase.

One small job I was given by the MHRI Director, together with Bernie, was to review the biochemical aspects of the Director's large Schizophrenia Project, which had been going on mainly in a mental hospital in the nearby city of Ypsilanti. A potentially interesting subproject was an attempt to isolate a "schizophrenia factor" from the urine of patients. The supposed biochemist who worked on this collected urine and let it evaporate down to a small volume in large dishes, open to the air. The smell due to bacterial putrefaction must have been awful! He then injected the concentrate into the brains of mice and discovered that they leaped into the air, presumably from the pain and possible direct chemical action on the nerves. He thought that the height of the leap was different when normal urine was used. This cruel, stupid project was quickly closed down. It obviously had not been approved by the University Animal Care Committee, perhaps because it was done in the hospital.

Around 1958 I attended a Gordon Conference at which David Green described his experiments showing that fatty acid synthesis required the presence of CO_2. Salih Wakil had already published similar information and had shown that radioactive CO_2 was not incorporated into the fatty acids, so it was evident that it entered the pathway only in a metabolic intermediate. At that time there were reports of the existence of malonic acid in urine and I mentioned this, as well as the known condensation reactivity of malonic acid esters, suggesting to Green that the CO_2 combined with acetic acid to form malonic acid. He said that the thought had occurred to him but it seemed too unlikely. Two of the meeting's attendants, Roscoe Brady and Wakil, returned to their labs after the meeting and quickly confirmed my suggestion. This was an important breakthrough in the study of fatty acid synthesis. I should have done the experiment myself but felt reluctant to drop my current experiments. Later, I was surprised to read Feodor Lynen's retrospective account of this meeting, in which he stated that *he* had made this suggestion. Curiously, I don't remember that. Perhaps Lynen had made the suggestion at a different time, to a smaller group.

Hajra and I spent a summer in Jim Mead's lab, next to UCLA, and extended his demonstration that hydroxy acids could be converted to nonhydroxy acids via an intermediate keto acid, which was then decarboxylated to form the 1-carbon shorter acid. This visit had an unexpected complication. I arrived in California ahead of Hajra and found an empty apartment nearby. That first night I received a telephone call, despite my assumption that the phone had been disconnected. It was from a threatening man, ordering me to pay my debt to him. Evidently the previous tenant had skipped out without paying up. I tried to convince the caller that I was not that person but was called again in the middle of the night with further threats. I called the phone company to disconnect the phone but learned they don't do that at night. I did not sleep well.

Back home in Ann Arbor, we studied the enzyme that catalyzed the second step and showed that several trace-level substances (cofactors) were needed by the enzyme. One factor was a small acidic, unstable substance which had the properties of vitamin C. We tested the vitamin but found it to be inactive. Later, when we were stumped as to its chemical nature, Bernie Agranoff offered the suggestion that it might be vitamin C. At this moment, my painfully erratic memory failed me – I forgot that we had already tested it. This time, a flash of common sense made me try it at several different

concentrations and we found that it was indeed the enzyme's cofactor. But it inhibited the enzyme when added at a higher concentration – the concentration that we had inadvertently used in our first trial. This is one of the few functional roles known for vitamin C. The discovery is of special interest in view of Linus Pauling's claim that high intakes of this vitamin are good for you. If this is so, the benefit may come from its ability to block certain enzymes. Another explanation, I might suggest, is that it catalyzes several nonenzymatic reactions that could be helpful.

Krystyna Kopaczyk joined our group and tried injecting radioactive cerebrosides directly through the skull of young rats, into the brain. This crude approach has been adopted by others, also with surprisingly useful results. We found that labeled lignoceric acid in lignoceric acid-containing cerebroside was liberated from its linkage and converted to acetic acid and that it also entered into linkage with glycerol, in the ester-type lipids. In fact, we then found that lignoceric acid is a natural component of the ester-type lipids. This was a little surprising since it had always been assumed to be a component only of sphingolipids. We also showed that some of the cerebroside was converted to ceramide, evidently due to the existence of a galactosidase, and that the ceramide was converted to sphingomyelin. At that time it wasn't evident how the latter conversion occurred and it wasn't until nine years later that we identified the enzyme.

From Kopaczyk's and Kishimoto's work, it seemed very likely that a galactosidase must exist in brain. Hajra attacked this problem and eventually, in 1965, showed that the enzyme does exist, that it is greatly stimulated by fatty acids plus a bile acid, and that it exists outside the brain too, despite the low levels of cerebroside outside the brain. We had a problem in getting the cerebroside into a physical form that would make it accessible to the enzyme molecules. We succeeded by emulsifying the lipid in a mixture of two detergents (I had learned about the merits of using dual detergent mixtures from literature furnished by Rohm & Haas Co., a major detergent manufacturer). Evidently the galactosidase did not object to the presence of detergents, not knowing the common belief that detergents spoil enzymes.

A new postdoc from England, David Bowen, was able to purify the enzyme to some extent, but the complete purification eluded us. We used some desperate methods, including digestion of brain membranes with trypsin, which did not harm our enzyme. I remember David sturdily carting around 5-gallon bottles filled with enzyme extracts and Sephadex, which he used in large columns for purification. His bottle of Sephadex still sits in our cold room; I haven't the heart to discard it. [Now, 1994, I had to throw it out to liberate space in the cold room. And two labs have just purified the enzyme and even cloned its gene.]

The partially purified enzyme acted also on lactosylceramide, a cerebroside containing glucose and galactose. Later, Kuni Suzuki showed that this enzyme is defective in children with Krabbe's disease, a genetic error. Very recently, a lab has shown that "our" enzyme is really two different, but similar enzymes. Actually, we had separated our preparation into three active fractions. We found that sphingosine inhibited the enzyme; this discovery is one of the first to show that sphingosine can inhibit enzymes. Sphingosine is now a popular subject as a potential biological control agent, particularly for protein kinase C.

I suppose many scientists have found themselves misquoted in someone's paper. Sometimes the author has attributed a good discovery to you, sometimes not. One amusing error occurred after Kishimoto and I published a paper in 1967 on the lipids found in human brains from patients who died from multiple sclerosis. In this disorder, of unknown cause, patches of white matter lose much lipid and thus lose their normal white appearance. Wallace Tourtellotte, a former investigator of lipids in these

patients, had developed – and still maintains – a bank of sliced brains from such patients. He dissected out the delipidated regions ("plaques"), as well as normal-appearing white matter from nearby; also supposedly normal white matter from individuals who had died of other causes. Among our findings was the discovery that the glycerophospholipids of the plaques contained a rather high concentration of a minor fatty acid, C22:6. This acid is relatively abundant in fish oil and brain and is now of great interest as a possible preventer of heart attacks or strokes. (We were the first to report its high occurrence in brain.) Shortly after we published this paper, Joseph Bernsohn and Leo Stephanides published a speculative paper on multiple sclerosis in which they cited our data as supporting their idea. We had found, they said, that the level of C22:6 was *half the normal level*, in direct contradiction to our data. They proposed that M.S. was due, in part, to a shortage of this fatty acid. This interesting idea gained some support for a while, but we can't claim credit for the "supporting data." Another lipid biochemist, Hugh Sinclair, had suggested the year before that children fed cow milk (who would be expected to be deficient in C22:6), might form white matter that is abnormally sensitive to some degenerative influence. Bernie Agranoff extended this idea later by showing a striking geographical correlation in the U.S., state by state, between the incidence of M.S. and the production of milk. Maybe there is something to the matter...

Pierre Morell's coming to my lab started us off on the enzymes that <u>form</u> cerebroside, rather than degrade it. Only one paper on the subject existed in the literature, a brief note stating that stearoyl CoA reacts with psychosine to form cerebroside. There was no information in the paper to suggest that a blank had been run (with dead enzyme) or that a time study had been done. Despite the flimsiness of the report, its finding was assimilated by all biochemistry textbooks and review articles. No further work on this enzyme had appeared for some time. Morell wisely decided this was the time to study the enzyme further. He soon found that the reaction did indeed take place, but it was slow and an enzyme was not involved. We therefore looked for another reaction and made the discovery that UDP-galactose reacted <u>enzymatically</u> with ceramide. To our surprise, the reaction went far better with ceramide containing a hydroxyl group than with ordinary ceramide. It took many years for textbook authors to mention our enzyme system and for years after people referred to a "controversy" over the two pathways. I think the newer books have stopped mentioning that initial, erroneous report. As a sad and irritating matter of fact, <u>several</u> of the enzyme reactions we discovered went through the same slow process of displacing incorrect literature reports and citations. Some biochemists seem to have no trouble with critical, careful reviewers in getting their work published.

A paper appeared from another lab claiming that cerebrosides containing hydroxy acids could not be formed from ceramide in vivo, although we had established the existence of the process in vitro. This was based on an experiment in which labeled serine was injected into rats and ceramides and cerebrosides were isolated from the brain. As expected, ^{14}C was found in the two kinds of cerebroside (hydroxy fatty acid and nonhydroxy fatty acid) but ^{14}C was found only in ceramides formed from nonhydroxy fatty acid. This finding, which puzzled the authors, was interpreted by them to mean that the brain did not really make ceramides containing hydroxy acids. However, the authors made the unstated assumption that one should find radioactivity in a labeled compound even if the amount present is too small to see. In fact, the hydroxy ceramides are evidently converted to cerebrosides so fast that only negligible amounts of the labeled intermediate accumulate.

Around this time, a biochemist published a confirmation of the original erroneous report. When I confronted him at a meeting with the question – "Did you run a no-enzyme blank to see if the reaction was truly enzymatic?" – he hesitatingly consulted his memory and then assured me that he had. A few

months later, he published a new report, stating that the reaction was a simple non-enzymatic one, without mentioning our prior publication to this effect, making it seem as though this was not a retraction of his first paper. An irritating attitude for a scientist.

Very recently, with the aid of PDMP, one of my inhibitors for the enzyme that makes glucosylceramide (the glucose analog of the much more prevalent than brain galactosylceramide), some researchers showed that there are two enzymes that transfer galactose to ceramide. One reacts with hydroxy acid-containing ceramide and is insensitive to the inhibitor. The other reacts with nonhydroxy acid ceramide and is (like the glucose-transferring enzyme) inhibited by our compound. I think the latter enzyme is closely derived through evolutionary mutation from the glucose utilizer.

Not long ago, the existence of another nonenzymatic reaction was found in other labs, the glucosylation of proteins by glucose. This reaction goes on all the time and very probably results in accumulation of glycosylated structural proteins during one's lifetime. Now I wonder if the nonenzymatic acylation of psychosine that we discovered is simply an example of a similar nonspecific reaction that gradually forms N-acylated proteins and lipids from the many acyl-CoA compounds in cells. It would be easy to look for such compounds.

Around 1969, Peter Braun came to me from the lab of Esmond Snell, my former postdoctoral mentor, and we decided to follow up on Braun's characterization of the enzyme system which makes sphingosine, the third component of cerebroside that we studied. With Pierre's help, we succeeded in showing that a similar enzyme makes the C_{20} isomer of sphingosine, which is the one found in gangliosides, not in cerebrosides. I think we moved that field forward a bit, but there has been little further progress except for Meir Lev's later discovery that cycloserine, an antibiotic, was a good inhibitor of the first enzyme step. In the last few years, however, some good discoveries have been made in this field and the recent upswelling in interest will surely clarify the many questions still left.

We also made the interesting finding that the ketonic intermediate in sphingosine synthesis was readily acylated to form a ketonic ceramide. It is possible that this amide is the true intermediate in the synthesis of the sphingolipids.

Braun also studied the ability of a myelin protein to bind lipids. This was the protein originally described by Jordi Folch, a strange material that could dissolve in a mixture of chloroform and methanol. (Proteins are normally insoluble in such a solvent mixture.) In analyzing the preparation we made, we were surprised to find a significant amount of bound fatty acid which appeared to be chemically linked to the molecule. This was the first report of a such a protein; it was confirmed later by Folch, whose paper subsequently received all the citations to the discovery. In recent years, many such proteins have been discovered; apparently it is a trait of many membrane proteins. We found that the protein could combine with cerebroside and other lipids and acidic lipids precipitated the protein from solution. This ability supports the self-assembly idea of myelin synthesis: the brain simply makes the components and they form a complex in the myelin sheath by themselves.

Jung Hyun, in my lab, tried to characterize the fatty acid-containing region in the protein but all our attempts led to precipitation of this unusual protein. This question has been answered only recently.

We next turned to the enzyme which combines fatty acids with sphingosine. Pierre Morell did a beautiful job of making the labeled fatty acid CoA compounds and showed that our previous interpretations of our findings were correct. The speed of reaction depended on the particular fatty acid; this explained the observed relative proportions of these acids in sphingolipids. Later, M. David Ullman and I extended these observations and obtained good evidence which indicated that several different

enzymes were involved, each with its own fatty acid preferences. This topic, too, has not been followed up much by anyone.

Shimon Gatt had previously reported the synthesis of ceramide from free fatty acid and sphingosine, but this was clearly an example of the reversible action that one typically sees in special conditions in vitro with hydrolytic enzymes (ceramidase, in this case). In the genetic disease, ceramidosis, there is no shortage of ceramide synthesis despite the lack of ceramidase in these patients. It is evident that ceramidase may produce some "reshuffling" of ceramide fatty acids but is not involved in net synthesis.

When Morell and Braun left my lab, they continued their productive association and in 1972 published a fine review of sphingolipid metabolism that received a surprising number of citations (for a review article). Their thank-you in the end of the article was much appreciated!

Around 1968, Martha Wajda, Bernie Agranoff, and I collaborated on a study of fish brain lipids, since he was doing so many interesting experiments on fish memory. (This was an example of a true "fishing expedition" – a type of experiment regularly denounced by snooty reviewers of grant proposals.) To our surprise we found a substantial amount of triglyceride – simple fat – which does not normally occur in mammalian brain. (The term "fat head" is a surprisingly accurate way to describe a person whose brain does not work well.) When the analysis was repeated several times with additional fish, the amount of triglyceride decreased with each run. Finally, in one of my closer involvements in lab work, I peered over Martha's shoulder to watch the dissection and noticed tiny shiny globules in the gelatinous substance that surrounds fish brains. This was the source of the fat, which apparently had been washed off more and more thoroughly with each successive experiment. We should have published this interesting observation – why is fat stored <u>there</u>? – but somehow we dropped the whole project.

I also became interested in the problem of extracting lipids from tissues, because of various technical problems, and published two long articles on the subject. This area of interest developed also from my early readings on metallic soaps and development of methods for dissolving radioactive materials in organic solvents. When I saw a report that our major solvent, chloroform, was carcinogenic, Atsushi Hara and I developed a new solvent system, hexane plus isopropyl alcohol, which is less toxic and much cheaper. It turned out to have several better qualities than the popular mixture, although it is not good for ganglioside extraction. Several papers from other labs later confirmed its superiority, but habit dies hard and many people – even postdocs in my own lab – do not follow or remember our paper. While I question the importance of the claim that chloroform is carcinogenic, there is the fact that it is more expensive than the solvents we introduced. Our mixture also dissolved much less protein than the "classical" chloroform-methanol mixture, making further processing more efficient.

A variation of this area of interest was the development of liquid/liquid partitioning systems which could result in efficient purification of biological materials, such as the products of enzyme reactions. Readers of these memoirs who have made salad dressings are familiar with liquid/liquid systems, which consist of two liquids that do not dissolve one another. Such systems were studied by some prominent biochemists in my early scientific days and they even devised machines that could carry out such fractionations automatically, on a large scale. I tried to invent improved devices but gave up after doodling many hours away. One of my friends in graduate school, Herb Meltzer, developed solvent mixtures which produced three layers, instead of two, and even built an expensive – and clumsy – machine for automatically moving the various layers in a 3-dimensional fractionator.

(Another of his ideas involved a way to make copies of pictures by a dry, electrostatic method. We spent an afternoon trying to make it work but failed. Later, someone else invented the Xerox machine and made it work – after many years of tinkering. Too bad it is still an unreliable method.)

I lowered my sights from automation to produce a convenient liquid/liquid mixture that made the analytical determination of cerebroside sulfate very easy. Another system, which included castor oil (!), proved effective to assay the enzyme that cleaves cerebroside into galactose (or glucose) and ceramide. Recently, Girja Shukla and I developed a partition system using newly-available solvents for isolating radioactive cerebroside after its enzymatic formation. It uses a solvent recently introduced into gasoline, methyl *t*-butyl ether.

Around 1969, when Antoinette Brenkert was here, the Animal Care Service at the U of M gave us samples of brain and liver from a cat which had died from a disease that looked like a genetic human lipid storage disorder. We identified the stored lipid as sphingomyelin, making this the first known cat with Niemann-Pick disease. (This is a rare human genetic error which proves fatal at an early age.) Unfortunately the cat was of unknown origin and we could not breed more cats with the trait. That could have made it possible to follow the changes in the tissues as the disease developed, something that cannot be done well with humans. I gave up on this idea but was later taught a lesson about persistence by David Wenger, in Denver. Wenger also received such a cat, but he devoted much effort to tracking down the cat's relatives. He found the dealer who had sold the cat, then traced through the dealer's garbage can that he called a file to find who had sold <u>him</u> the cat. It turned out that other cats in this litter had been sold to various people and he tried to track them down. Two or so of the cats had been run over – a common fate of city cats – but one owner could not be traced. Wenger learned that this owner was sought by the police but managed to convince the necessary people that he had only a scientific motive in finding the cat. This owner agreed to give up the cat, for the sake of science, and Wenger found another litter member of the opposite sex. After all this effort, it turned out that the cats bred poorly and I believe he gave up the project.

Brenkert continued Morell's study of the enzyme that makes cerebroside, as well as the one that makes glucocerebroside (discovered by Subhash Basu and Saul Roseman across the street from my lab!). We determined the properties of the enzymes and found marked changes with age in rat brain. It was unnerving to me, however, to realize that Brenkert's handwriting in her laboratory notebook could be read only by her. An interesting discovery, made with Brenkert, Ramesh Arora, Otto Sellinger, and Art Flangas, was the finding that the enzyme making glucocerebroside (the precursor lipid for the synthesis of over 300 known lipids) occurs in neurons but not in oligodendroglia, the cells that make myelin. Yet the latter cells were found by others to contain some of those lipids. I think one could conclude that oligos get their glucose-containing lipids from adjacent nerve cells. This observation later led me to study the transfer process, which utilizes a twin pair of proteins. More of that later.

Twelve years later, M. J. Brammer confirmed our results, using glia and neuronal preparations isolated by different methods. However he did a more thorough study, showing the presence of UDP-glucose epimerase in glia, and suggested that glia do make some glucocerebroside and that the epimerase kept us from observing this small amount of synthesis. He agreed with us in suggesting that this difference is "relevant to the understanding of brain cell function."

With this enzyme assay method in hand, I started a long-term effort to devise chemicals which could inhibit this and related enzymes. This effort is still going on, after about 19 years [now, in 2000, 29 years], and I think some of our inhibitors will prove to have very important therapeutic effects. When I applied for a grant to hire an organic chemist to make the new compounds, the application received a

low rating and was not funded. The reviewers explained the weakness in my proposal: "perhaps the attempt to devise an inhibitor will fail." In other words, they wanted a guarantee that I would succeed or else, "forget it." Somehow the notion of risk in research had not permeated the reviewers and I was a bad risk, despite a good record of new organic and biochemical discoveries. Luckily, NIH discovered a little extra cash at the end of the budget year – unbelievable nowadays! – and the project was funded.

One unexpected finding from this approach was the discovery that Ramesh Arora and I could make compounds which stimulated cerebroside galactosidase. We proposed that such compounds might have therapeutic value for patients with Krabbe's disease, who suffer from only a partial lack of the enzyme. The stimulators did indeed stimulate the defective enzyme from such patients, furnished by James Austin, in Denver. This is a rare disease, which may explain why no one took me up on the suggestion.

One compound, made by John Erickson in 1972 or 3, proved to be a very efficient inhibitor of the glucosidase that degrades glucocerebroside. This was N-hexyl glucosylsphingosine ("HGS"), closely related in structure to the enzyme's substrate. Several other laboratories utilized HGS to characterize the enzyme, even quite recently. Glyn Dawson and Allen Stoolmiller, at the Univ. of Chicago, used HGS to produce a model form of Gaucher's disease in several kinds of cells of neural origin. The cells not only accumulated glucocerebroside, like the cells of individuals with the disease, but also showed impaired uptake of the lipid when it was added to the cell medium. The uptake phenomenon supported our previous conclusion that glial cells have to get their glucocerebroside from other cells, and that some kind of specific mechanism is involved, which could be inhibited by HGS. Another interesting but still-perplexing observation was the discovery that the concentration or activity of glucuronidase, a little-studied enzyme, decreased considerably in the "Gaucher cells."

Kenneth Warren and I tried to produce model Gaucher's disease in rats with radioactive HGS. We found that the inhibitor is also a substrate for glucosidase, changing into N-hexyl sphingosine, itself an inhibitor of the enzyme. An interesting finding is that HGS was absorbed by the spleen relatively specifically; the spleen is the site of most of the storage of glucocerebroside in the human disorder. Perhaps this finding indicates that the spleen not only forms glucocerebroside in the course of its normal demolition of white and red blood cells, but also scavenges the blood for glucocerebroside that is liberated by other body organs.

The effect of HGS on cultures of human fibroblasts was studied by Warren and Irwin Schafer's laboratory, at Case Western Reserve. I had become friendly with Schafer while we were on the same USPHS Mental Retardation review committee. (He had a slightly irritating but constructive habit of trying to find something good to say about every otherwise weak grant proposal.) These cells accumulated glucocerebroside, as expected, as well as a lipid formed from glucocerebroside, apparently galactosylgalactosylglucosylceramide. This was not unexpected since the lipid had been found in some Gaucher patients. An important observation is that the "Gaucher fibroblasts" grew faster than control cells. This matched the well-known increased growth of the spleen and liver in Gaucher patients. It supported my hunch that glucocerebroside is a growth stimulator, a hunch that I confirmed in several later studies. Walt Holleran and Peter Elias, in 1995, reported the same relationship in skin.

During the period of these studies, David Ullman examined the question: how is sphingomyelin made? Several previous papers had offered differing enzyme routes, but all were based on questionable-looking data, and we decided to try our hands at it. A paper had just appeared, based on a turnover study of virally-transformed fibroblasts with labeled choline. The conclusion, which looked reasonable, was that lecithin might be a precursor of sphingomyelin. Our previous study with labeled cerebroside injected into rat brain had indicated that ceramide was the precursor of the sphingolipid portion. Ullman

therefore tried incubating liver microsomes with labeled ceramide and lecithin and conclusively proved that an enzyme transferred the phosphocholine portion of lecithin to the ceramide molecule. (A subsequent paper from a prominent German lipid biochemist reported that our work could not be confirmed, but the authors made the inexplicable decision to include diglyceride in their incubation medium, despite our observation that diglyceride is a strong inhibitor of the enzyme. This foolish report delayed acceptance of our finding and probably gave the skeptics in NIH Study Sections an additional reason to reduce my grant support.)

Our paper, which appeared in 1974 and which was corroborated in two other labs, had to fight its way for many years against the many incorrect pathways which had been uncritically published in journals and textbooks. However, it now looks from the work of others as though the reaction goes on in all organs despite our conclusion that brain was different. Twenty years later, a laboratory showed that the enzyme worked in both directions: it converted sphingomyelin and diglyceride to ceramide and lecithin. This enzyme has been generally disregarded but it may be found to play an important role in controlling cell growth and differentiation. [In 2000, it sure has!]

We also obtained preliminary evidence for a second route of synthesis, a reaction between ceramide and phosphatidylethanolamine. Ullman later, in his own lab, showed that this product is methylated to form sphingomyelin. There are many nice studies waiting to be done on this reaction.

I decided to try synthesizing an inhibitor of the enzyme that makes galactocerebroside, in the belief that this could help patients with Krabbe's disease. These people have a low level of the enzyme that degrades cerebroside, so I reasoned that slowing down the synthesis of cerebroside – to match the rate of hydrolysis – would alleviate their symptoms. Obviously this hypothesis assumes that the accumulated stores of the lipid could be destroyed or reutilized for the formation of myelin or cerebroside sulfate. We did succeed in producing some compounds with a modicum of inhibitory activity but nothing that looked therapeutically useful.

While walking out of a meeting on sphingolipidoses, held behind guarded gates (because of neighborhood crime) in a New York City hospital in 1971, I overheard a mother asking her physician if there was any possible way he could help her child who was suffering with Gaucher's disease. This genetic disorder is somewhat more prevalent than Krabbe disease and I suddenly realized that it was much more amenable to my therapeutic approach than Krabbe's. Accordingly we switched over to the enzyme which makes glucocerebroside.

A succession of organic chemists in my lab – Ramesh Arora, Radhey Misra, and Ranga Vunnam – synthesized well over 100 compounds related in structure to ceramide and glucocerebroside. Unexpectedly, some of the compounds acted as substrates for the enzyme, producing abnormal glucocerebroside. This raises the possibility of producing interesting effects by administering the abnormal substrates. One inhibitor acted by binding to the enzyme in a chemical, permanent reaction, which raises the question: how would the body dispose of such a chemically modified enzyme? Animals injected with this compound showed signs of excitement, so we accordingly tested it against monoamine oxidase. Robert Schatz (then working for Otto Sellinger) and N. Narasimhachari (at the Medical College of Virginia) found that the inhibitor was pretty effective against monoamine oxidase, especially type B, and we were hopeful that a pharmaceutical company would consider this a useful drug. However that hope did not pan out.

Our best inhibitor was a compound, D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol ("PDMP"). The effectiveness of the compound made it a promising candidate for therapeutic action and

we have put much more effort into the material.

Vunnam developed an improved assay for the glucocerebroside-synthesizing enzyme and noted that the enzyme in brain and liver seemed to have different properties. Recent data from experiments by Girja Shukla seem to show that kidney also has a different version of the enzyme. This makes the problem more complicated.

In 1977 Marvin McMaster and I worked out a neat way of making the lipid in radioactive form, making it easy to assay the enzyme that hydrolyzes glucocerebroside. The method was adopted without difficulty by New England Nuclear Corp. but they later withdrew offering it because of slow sales of the compound. I see that the method is still being used in some labs.

Along a different tack a little later, Atsushi Hara prepared conduritol B epoxide ("CBE"), which had been shown by Günter Legler and Julian Kanfer to inactivate the enzyme that hydrolyzes glucocerebroside. CBE thus seemed like a good inhibitor for generating model "Gaucher mice." Hara followed the changes in the enzyme's level after a single injection of CBE into mice. He found that the enzyme was inactivated very quickly but the excess CBE was rapidly excreted (or metabolized) and the normal process of enzyme synthesis regenerated new enzyme molecules fairly quickly. About half the previous number of enzyme molecules appeared to accumulate by 6 to 9 days. It was apparent that we would have to inject the mice every day to maintain a low level of the enzyme.

A second enzyme, which could hydrolyze an unnatural glucose-containing material, was apparently unaffected by the inhibitor, showing the presence of two kinds of glucosidase in the liver. (The function of the second enzyme is still unknown, and I suppose it acts on dietary glucosides that escape digestion in the intestine.) Robert Glew later utilized this finding to develop a simple assay for the enzyme acting on glucocerebroside without using labeled glucocerebroside.

Another interesting discovery coming from this work was the finding that the glucuronidase level in liver, spleen, and kidney dropped after CBE injection, just as we had seen before in neuroblastoma cells treated with our other glucosidase inhibitor, HGS. But in brain, the activity of the enzyme rose! From experiments Hara did to explain this, it appears likely that the loss of glucocerebroside-cleaving enzyme causes a leakage of glucuronidase from <u>all</u> cells, into the extracellular space, but the blood-brain barrier keeps the enzyme from leaving the brain. If such a situation applies to other proteins, the increases in other brain proteins that have been observed in other laboratories should be reexamined.

Perhaps the most important finding in this study, mentioned earlier in this review, was the increase in brain size produced by eight daily injections of CBE. The liver also grew significantly faster (9%). This strengthened my hunch that glucocerebroside is a growth stimulator.

I mentioned before that Hara and I devised an improved solvent for extracting most lipids (not gangliosides). In order to isolate the glycolipids, it was customary to degrade the glycerol-type lipids by removing the tissue water from the extract, then treating the extract with alkali for at least an hour. We tried this with our hexane-isopropyl alcohol tissue extracts by directly adding a concentrated solution of sodium hydroxide in methanol. Three of the major lipids in tissues, neutral fat, lecithin, and phosphatidylserine, were cleaved in only two minutes or less. Other glycerol-based lipids took a few minutes longer. It wasn't necessary to remove the water from the extract, a useful saving of time. This elegant improvement does not seem to have been noted by many others; it needs more publicizing.

Hara's sharp eyes noticed that the lipid extract, after treatment with sodium hydroxide, contained a small amount of a second liquid. Investigation showed that the liquid contained the alkali, as well as virtually all the cerebroside sulfate, much of the cerebroside that contains hydroxy acids, and small

amounts of other alkali-stable lipids. This fortuitous discovery – like so many others – was too good to forget and it dragged me along a side-path. Thus we developed a very efficient method of isolating cerebroside sulfate, a hitherto little-available brain lipid. Wiser now to the need for commercialization, I suggested to the U-M Intellectual Properties Office that we patent this method but was told the patent would be useless since there would be no way to enforce it against companies that were secretly using it. So there is more to an invention than invention.

In 1978 or 1979, while I was on a site visit for NIH, I heard a talk at Johns Hopkins by Saul Brusilow on a patient whose genetic illness he had recently characterized. The patient was going blind due to a lack of a particular enzyme involved in the urea cycle. Brusilow had tried several treatments, based on dubious rationales, and there seemed no hope for therapy. While listening to the talk, I suddenly remembered several enzyme pathways that I had learned about while in graduate school 30 years before and which had been used in metabolic isotope studies by my professors there. One of these pathways converted glycine and benzoic acid to hippuric acid, another converted glutamine to a similar product, both of which were excreted in the urine. Since the nitrogen atoms of most amino acid can be converted to glycine and glutamine, it seemed likely that these pathways could bypass the previously considered "essential" urea pathway of nitrogen excretion. I told Brusilow about the enzymes after his talk. He promptly confirmed my guess and quickly normalized the patient with large doses of sodium benzoate. It was evident that much of the urea excretion pathway could be replaced by these strange, apparently useless enzyme systems. (Benzoate does occur in some foods, as well as soda pop, and it might be toxic to people lacking the enzymes.)

Brusilow, with a collaborator, Mark Batshaw, offered to make me a coauthor on their paper in *The Lancet* but I foolishly felt at the time that my contribution to their elegant study was too small and settled for a thank-you. They made up for this a bit by including my name as a co-patentee when they patented the treatment method, which could have wide usefulness in any disorder involving a problem with ammonia metabolism or the urea cycle. They sent me a release form stating that I gave up my financial rights to Johns Hopkins Medical School, an altruistic gesture that I agreed to. A company later gave the school about $50,000 for the right to test the treatment further; the school kept much of this and gave most of the rest to Brusilow and Batshaw, who squeezed out $900 to me. Well, anyway I had the satisfaction of knowing that my treatment has saved a number of lives, according to Hugo Moser. Unfortunately, an error by the Johns Hopkins pharmacy overdosed one of the patients by a factor of ten; he died.

Recently I read that the method has also been useful for patients with portal systemic encephalopathy, a liver disorder that damages the brain by its failure to control the level of ammonia in the blood. Thus I can honestly now claim, after writing grant applications with promises to this effect for many years, that I did something good for people with brain disorders even though glycolipids had nothing to do with it.

Another project that proved interesting was the one carried out by Ray Metz, one of my three grad students. A few years before, 1977-1978, Krishna Mallia (now Asst. Director of Research at Pierce Chemical Co.) had shown the existence of soluble brain protein(s) with the ability to bind radioactive cerebrosides. We made little progress on the purification and gave up when our preliminary paper was rejected. Ray took on the project and unswervingly pursued it to a much higher level. The protein (actually two very similar proteins that could be separated) may be a carrier protein that might function to carry the lipids to different cells or parts of a cell. This is the process that we had postulated to explain the lack of ceramide glucosyltransferase in glia. We developed a very convenient assay for the

protein that used liposomes made from radioactive glucocerebroside, cholesterol, and lecithin as the source of unbound lipid, and red cells or red cell membranes as the acceptor. Only an extremely small amount of the "cerebroside uptake protein" was needed to catalyze the transfer of a measurable amount of cerebroside. This protein was also detected in brain soon after by a Japanese lab, which has continued its study.

I wonder now if this transfer protein is involved in the shedding of glycolipids from tumor cells, which Stephan Ladisch has shown to occur to a significant extent. He demonstrated that the lipids thus released block the immunological defense reaction that the cancer patient's immune system ought to develop against tumors. If our glycolipid transfer protein is truly required for the shedding process, it is possible that antibodies to the protein would have therapeutic value. Ladisch later showed that cancer cells treated with our inhibitors to block their release of the lipids were no longer able to interfere with immunological reactions. This makes our inhibitors of great potential value in cancer therapy.

Around the same time, another grad student, Susan Berent, devised a way to isolate a protein that stimulates the glucosidase that degrades glucocerebroside. This protein, which was stable to boiling water (!), had been discovered in a Gaucher patient's spleen about 10 years before by Mae Wan Ho and John O'Brien but they had been unable to isolate it in a pure state. We decided to start first with normal spleen, then compare it with the protein from a Gaucher's spleen. The protein turned out to have some odd properties, which made it difficult to purify, but Sue, working long hours together with her inspired assistant, overcame these obstacles and isolated several milligrams from bovine spleen. It is quite active: only 30 μg are needed for an assay of its stimulatory power. Its effectiveness was improved by including phosphatidylserine and a trace of detergent in the test tube. We showed that the protein is quite small, which apparently helps explain why it is stable to heat. We also showed that it acted by forming a complex with the enzyme and phosphatidylserine. It was clearly a mixture of similar proteins, each of which had similar activity.

Comparison by electrophoresis of the normal stimulator and the one from a Gaucher patient's spleen showed that the latter was an even more complex mixture and I attributed this to nonenzymatic changes that occurred over the period of many years during which the Gaucher substance accumulated in the spleen. The idea was based on the growing evidence that proteins can undergo simple chemical changes in the body, given enough time. Unfortunately we never proved the hypothesis; it should be relatively easy to do with the methods now available.

About 7 years later, Akira Sano, a biochemical psychiatrist, came to my lab from his laboratory in Japan and revived the project. He improved the isolation method, starting with guinea pig liver, and showed that the mixture of activator proteins differed in containing different carbohydrate groups attached to a single point in the protein. The carbohydrates were removed, producing a single protein band, and were shown to be unnecessary for the stimulatory activity. We determined the complete sequence of the amino acids in the protein chain and noticed that some of the molecules in the purified preparation were missing one or two of the amino acids from one end of the chain. This end of the protein chain contains lipoidal and alkaline amino acids, forming a region that may bind the acidic phospholipid. The region that contains the carbohydrate group probably does not bind to the enzyme or substrate, since removal of the carbohydrate has no effect on the protein's activity.

In comparing other proteins by a computer program, operating from a large data base, we found that our protein strongly resembles one of the lung surfactant proteins. This protein differs primarily in lacking the carbohydrate side chain and is thus highly insoluble in water. It does, however, have the ability to bind a particular kind of lecithin and is essential for lung expansion during breathing. I believe

it is now sprayed into the lungs of premature infants to help them breathe. Another version of the stimulator appears in sperm cells; it possesses a sulfate group attached to the carbohydrate group. We found that this protein stimulates glucosidase as well as the non-sulfated activator, and I wonder if it plays a role in egg cell fertilization. Perhaps glucocerebroside in the gel around the egg (vitellin) or the egg plasma membrane has to be hydrolyzed before the sperm can penetrate.

While Sano was here, other laboratories also began working with the activator protein and related stimulating proteins. John O'Brien, in particular, had found a precursor protein which contained the glucosidase-stimulator as part of its structure. The precursor protein also contained a sphingomyelinase stimulator along its chain. The two proteins turned out to be remarkably similar in certain features and it is evident that they are derived by evolution from a single gene. When we studied O'Brien's proposed sequence for the entire precursor protein, Sano realized that there were two critical errors in the data and that the precursor protein actually contained four sequences that were similar to our protein's sequence. It was evident that the precursor protein is made first, then split by a peptidase into four equal parts, each differing in a few details, enough to make them specific for different enzymes. O'Brien has now corrected the error, using our method of isolation, and reported that two of the four segments stimulate glucosidase and cerebroside galactosidase, and the other two stimulate other sphingolipid hydrolases. I was greatly irked by the very slow publication process that the Journal of Biological Chemistry adopted for our manuscript, so that O'Brien's paper appeared well before ours. What price priority?

Subhash Datta and Shankar Iyer prepared antibodies in rabbits to our glucosidase activating protein and we used them to measure the concentration of the protein in various tissues. The concentration changed with age and paralleled the changes in glucosidase activity. When Datta injected glucocerebroside into mice, the liver absorbed it rapidly and the level of activator in the liver rose rapidly. It seems that cells must react quickly to an elevated level of glucocerebroside by trying to speed the lipid's hydrolysis. In accordance with our other findings, we noted that the mouse liver grew as the result of the injection. Within one day, the liver grew 13 to 37%! This growth was not simply an accumulation of water since the weight of protein, lipids, and DNA also rose. Two enzymes that are involved in DNA synthesis, thymidine kinase and ornithine decarboxylase, also rose rapidly. The growth phenomenon was stimulated by including CBE with the glucocerebroside, no doubt because the CBE protected the injected lipid from breakdown by the liver's glucosidase. The spleen size was not increased (as in Gaucher's disease), evidently because most of the injected lipid ended up in the liver. This work strengthened my belief that glucocerebroside is a growth stimulator.

Datta, together with Michael Snider, an independent investigator in the other side of my lab building, showed that cultured neuroblastoma cells could absorb the stimulator protein from the cell medium (measured with the antibody assay). The uptake could be blocked by including mannose phosphates in the medium, showing that the cells absorbed the protein via mannose phosphate binding receptors. This also showed that the carbohydrate portion of the protein contained mannose phosphate or a similar group; that may explain the role of the carbohydrate portion of the protein – it is needed for uptake from the extracellular fluid.

The cells also absorbed glucosidase but this process did not involve the mannose phosphate receptors, a discovery confirmed soon after in Greg Grabowski's lab. Phosphatidylserine uptake was aided by the enzyme, and vice versa, showing that they could form a complex without the activator. However, all three were rapidly absorbed as a ternary complex, via the mannose phosphate system. The ternary complex rapidly hydrolyzed glucocerebroside in cells previously loaded with the lipid.

(Many years earlier I had submitted a paper to the J. of Theoretical Biology proposing that ternary

complexes were common active forms of enzymes or other proteins. I had accumulated published data showing examples of the need for an acidic lipid, together with a neutral lipid, to bind to a specific protein and thereby make it functional. The paper was sent back for revision, apparently accepted, but through some strange quirk I completely forgot about it and discovered the unrevised manuscript much later. What a memory!)

When Akira Sano returned to his own lab in Japan, he continued study of the glucosidase activator and recently published a paper showing that cell nuclei contain a good deal of the activator (probably without the carbohydrate part) while other cell regions contain the precursor protein and intermediates in the formation of the active form. This discovery suggests to me that glucosylceramide acts as a growth stimulator by virtue of its ability to induce the activator protein. The latter, by entering the nuclei, somehow causes cells to grow and divide.

Sano, in the course of checking out the specificity of our glucosidase activator, discovered the existence of a new activator, one that acts on α-glucosidase. This is one of the enzymes responsible for the conversion of stored glycogen into glucose. People who have a low level of this enzyme suffer from Pompe disease and die from muscle deterioration. Together with new arrivals from India, Arti and Girja Shukla, we purified the activator protein to some extent and showed that it has the ability to both activate and stabilize the enzyme. We developed a very simple assay for the activator, only a few nanograms of which produce a distinct effect on the enzyme. The protein occurs in urine, and less than one microliter of urine is sufficient for evaluation. That must be important but I don't know why.

Through some line of reasoning, I decided that this activator protein might be involved in cystic fibrosis, the genetic disease involving excessive glycoprotein formation in the lungs. With the aid of a pediatrician at the U-M, we obtained urine samples from cystic fibrosis and other children and assayed them for the concentration of activator. We found no correlation, although one child produced a very high level and should have been studied further.

When Jin-ichi Inokuchi came to my lab in 1986, we decided to see if my glucosyltransferase inhibitor, PDMP, could work in intact mice to lower the levels of glucocerebroside. This was a prelude to proposing its use in treating Gaucher's disease. It did work, producing about a 35% decrease in liver glucocerebroside within 5 hours after injection. Evidently this lipid is under active control, exhibiting a high turnover rate. Since PDMP was a mixture of four isomers, we separated them and found that the enzyme inhibitory power resided in just one isomer, which we established as being the D-threo isomer. At this point I made a distinct switch in my research (a last gasp before my schwannoma tumor surgery and beckoning retirement).

Many studies had accumulated indicating that glycolipids formed from glucocerebroside play a special role in cancer cells. Maurice Rapport was a voice in the wilderness for many years with this kind of discovery, but in recent years Sen-itiroh Hakomori, Lars Svennerholm, and others (especially Japanese biochemists) have utilized modern techniques to discover more of these connections. Novel glycolipids, not detectable in normal cells, had been isolated from tumors. It seemed to me that these novel lipids might be responsible for the pathological features of tumors and that a block in their normal destructive metabolism (acting on trace concentrations in normal cells) would cause a dangerous accumulation of the "cancer lipid." The block – which could be called a micro-sphingolipidosis in a single cell – could be caused by any of several mechanisms, the sole requirement being that both alleles (matching genes in a pair of chromosomes) must be defective. This pair of genes might code for a protein required for the breakdown of the lipid, such as a specific glycosidase or a cohydrolase needed by the hydrolase. However I suppose, as Hakomori has suggested, the accumulation of the lipid might

be due to a synthase gene (which forms the lipid) becoming activated by some gene defect.

PDMP, by blocking the formation of the cancer glycolipid, should act to cure cancer. Inokuchi jumped at the idea and enthusiastically began an intense year of research with mouse cancer cells, the Ehrlich ascites carcinoma. My research grant, which had been approved for a seven year period for the study of nervous system glycosphingolipids, was diverted to this new cause – with some qualms. We showed that the drug permanently cured 30 to 40% of the mice that had been inoculated with the cancer cells and prolonged the life of the others. Moreover injection of glucocerebroside stimulated the growth of the tumor, as predicted. We published this finding, applied for a patent on the drug via the U-M, and wrote a self-invited review article (a "commentary") on the subject for *Biochemical Pharmacology*. Inokuchi had to return to his position in Japan and has continued study of the roles of glycolipids in tumors and brain.

A paper from Inokuchi's lab, which included my name because of significant input on my part, was published in late 1989. Its main conclusion is that melanoma cells need glycolipids in order to bind to laminin and collagen IV. PDMP exposure greatly reduced their ability to bind to these matrix proteins. This explains part of the antitumor activity of PDMP in vivo: cancer cells grow in groups, like normal cells, but some break loose and start a daughter tumor at any site they can latch onto. If they are unable to bind to laminin or collagen IV, they cannot metastasize. Inokuchi has subsequently shown this directly with mouse Lewis lung tumor cells, using PDMP. [A similar paper from another laboratory appeared in 2000.]

An important paper from Sen-itiroh Hakomori's lab, derived from work with PDMP and cultured T cells, has shown that the glycolipid-depleted cells cannot proliferate when they are exposed to interleukin 2. While the mechanism of this interference is still unclear, despite a large amount of work, they were able to show that the PDMP-treated cells formed three specific phosphotyrosine-containing proteins which may block cell proliferation. The appearance of these proteins was probably due to the accumulation in the treated cells of ceramide, the precursor of glucosylceramide. PDMP forces cells to accumulate ceramide since it does not inhibit its synthesis. Some years later, it was discovered (mainly by Yusuf Hannun, I believe) that ceramide somehow stimulates the attachment or removal of phosphate groups from proteins. I now believe this is a major role for ceramide and, in my latest paper in 2003, have proposed a mechanism by which it acts. It explains some of the remarkable variety of biological effects exerted by the sphingolipids.

Hakomori's cells also accumulated N,N-dimethylsphingosine, a previously unknown lipid which may be derived from ceramide when the ceramide cannot be utilized to make glucocerebroside. This new compound was shown to be a strong inhibitor of protein kinase C, an enzyme believed to be important in cell proliferation. These findings open up the possibility that PDMP would be useful in patients not only with cancer, but also with T cell problems, such as rheumatoid arthritis, transplanted organ rejection, juvenile diabetes, and lupus.

Arti Shukla made radioactive D-threo-PDMP and studied its disposition by mice. We now know that it is rapidly converted to other compounds and excreted rapidly. From the properties of the new compounds, it appeared likely that a liver enzyme complex, involving a family of P450 enzymes, attacks PDMP. We tested this idea by injecting an inactivator of cytochrome P450, piperonyl butoxide. This compound had been introduced into spray-type insecticides many years before for the same purpose: to protect the poison against degradation by the insect's cytochrome P450. It did indeed protect our drug from degradation and I hope we will be able to enhance the effectiveness of the drug that way.

Apparently no one has tried this approach for prolonging the effectiveness of the many drugs used in humans that are degraded by P450, and I hope that this work will lead to more studies of this sort. Actually the medical literature treats the phenomenon (which is seen also with cimetidine, a stomach acid reducer) as a dangerous nuisance, since it necessitates adjusting drug doses downward. After all that work that went into figuring out the correct dose levels for patients with average P450 activity, you can see that pharmacologists don't want to repeat it with patients whose P450 has been deliberately weakened. Yet the approach would put all patients on an even footing so dosages could be chosen more reliably for every patient.

James Shayman, in whose lab I am now a guest, has been studying the effects of PDMP in cultured glomerular kidney cells, the MDCK cells. PDMP slows their proliferation, while CBE and glucocerebroside stimulate their proliferation and block the effect of PDMP. The glycolipid-depleted cells are now more sensitive to bradykinin, which acts very rapidly to cause the cells to form inositol trisphosphate. This effect is consistent with the idea that PDMP inhibits protein kinase C by at least one mechanism. The depleted cells also seem to produce prostaglandin more rapidly, suggesting that phospholipase a activity is normally repressed by glycolipids. Shayman has obtained a grant to pursue these findings in greater depth, with me listed as co-investigator. So (real) retirement might still get postponed yet again.

Note in 1997: retirement gained a stronger hand in August 1996, when I left Jim, the U of M, and Ann Arbor and moved to Menlo Park, near Stanford Univ. and my son, Lon. Norma was diagnosed as having multiple myeloma – my mother's cancer – the year before and it was evident that she would need much more care. Now I can only nudge researchers into trying PDMP or my newer inhibitors, especially for the study of myeloma.

When I applied for a grant to pursue the cancer chemotherapy idea further, the reviewers concluded that I was too inexperienced to do this adequately despite my success with the Ehrlich ascites carcinoma mice. When I reapplied a year later, taking pains to clarify their many previous misunderstandings (that looked like deliberate misreadings), the reviewers began their comments with the following: "Dr. Radin, a 69-year-old biochemist,...." What more need be said when an applicant is over the hill?

I am particularly interested in kidney glycolipids, as Jin Inokuchi found that PDMP in normal mice produced a small but distinct decrease in kidney size. This was the organ most affected. Accordingly, Girja Shukla and I embarked on a study of the glucocerebroside-forming enzyme in kidney. Pierre Morell had once published assays of this enzyme in kidney and found very low activity, which seemed unlikely. We confirmed the observation but discovered that the low activity is due to rapid destruction of the labeled substrate used in the enzyme assay, UDP-glucose. After a considerable effort we were able to block that destruction somewhat (by including NAD or NADP in the incubation medium) and now find good activity. To improve the reproducibility and speed of the enzyme assay, we devised a new liquid/liquid partitioning system that effectively and conveniently separates the radioactive lipid formed from its radioactive precursor. The solvent system chosen has a low density so that the labeled cerebroside floats to the top and is easily transferred for counting.

We also found that incubating the tissue homogenate with the incubation medium in an ultrasonic bath, maintained thermostatically at 37^{o}C, yielded high and uniform activities. Such baths are not commercially available and it took some experimentation to design one that worked well. I tried to publish this design, believing that it ought to replace the ordinary shaking incubator baths that were introduced many years ago by Dubnoff. However, reviewer after reviewer – including my own

colleagues – said that ultrasonic baths destroy enzymes and therefore cannot be used to measure enzymes. They did not find it necessary to document this belief. If this strange preconception were true, we would not be able to measure our activities! Eventually I found a small journal, with a low circulation, that accepted the idea. I wonder if anyone will utilize my idea.

Bruce Fenderson, in Hakomori's lab, has shown that cells depleted of their glycolipids by PDMP are normal with respect to glycoproteins (i.e., the drug does not affect glycoprotein synthesis). With a laser spot bleaching technique, he showed that the mobility of glycoproteins and glycolipids in the plasma membrane is unaffected by the loss of glycolipids. However there is an increased proportion of highly mobile lipids, so it seemed likely that glycolipids normally act to keep some lipid molecules in islands of low mobility. A few years later, it was discovered that the glycolipids, together with some other lipids and enzymes, occur largely in special small regions in the outer cell membrane, called caveolae. Other aggregates, called GEMS, have also been found to be rich in glucosylceramide.

A major development came about 1994 when Jim Shayman and I applied for a Small Business Innovation Research grant from NIH. These grants were set up to foster the practical application of laboratory discoveries. Ours involved collaboration with a small business, a chemical company called BIOMOL Research Labs., run by Bob Zipkin. The application yielded the very modest sum of $50,000 and Bob undertook to make analogs of PDMP (variants of the molecular structure in which different sections of the molecule were replaced with similar sections). This is a standard procedure to follow when one discovers a "lead" compound, a promising drug that can "lead" the way to better ones. We evaluated the different compounds for activity against the glucosyltransferase that converts ceramide to glucosylceramide.

As a result, we found a much more potent analog of PDMP, PPPP. This compound led to more interest in our work and – later yet after I left Jim – to a still more active inhibitor, ethylenedioxyPPPP. Later, some researchers published imporoved methods of making the drugs.

After this successful grant project finished (in the allowable 6 [*six*!] months), we applied for a stage II grant, in which it would be possible to do much more work with the new inhibitor. This was rejected because we had not accomplished enough in those 6 months. Obviously this time limitation was enforced by a non-researcher.

Miscellaneous

For several years, we were purifying some of our radioactive lipids by thin layer chromatography, using large glass plates coated with thick layers of silica gel. It was quickly apparent that placing the mixtures (in solution) on the silica gel in a uniform, narrow band was difficult to do. We tried various devices but the flow rate of the liquid, as it soaked into the powder, was difficult to control. Finally I hit upon the idea of forcing the liquid out of a syringe by pushing the syringe plunger down by means of an inclined plane. That is, we pushed the syringe in a vertically held slider in a line above the line on which the solution was to be applied as a streak. A long metal bar, lower at one end than at the starting point, forced the plunger down at a rate that matched the rate at which we pushed the syringe. Bill Strauch, our shop man in MHRI at the time, made the device and it worked like a charm after a few modifications.

In 1964, I showed it to Nick Pelick, who was on the staff of Applied Science Corp., a major supplier of thin layer chromatography supplies. Nick wanted to sell the streak applicator and offered me $500 for the "rights," such as they were. (I didn't try to patent it as it seemed so obvious – once you

thought about it.) I asked for a royalty instead but couldn't budge him. Pelick neatened up the design and sold it as the Radin-Pelick Streaker. He gave me one of the first models, which I still have.

Unfortunately for this source of fame, Pelick decided to form his own company, so Applied Science took his name off the product. "Why take my name off too?" I asked. Their response was that the revised design should not be attributed solely to me. It now sells for about $1000 and I understand that several thousand have been sold. A 1% royalty would have come to....oh, well.

I played a smaller role in the invention of a device that allows one to dispense a specified volume of liquid repetitively, from a reservoir. Through an unusual connection – my cousin Nat had dated a girl who subsequently married an inventor – I became acquainted with her husband, Justin Shapiro. We became very good friends and I used to propose new devices to him for manufacture. At one point I bought a dispenser, the only one then available, and realized it had several design flaws. It was a simple pump, basically, consisting of a syringe whose plunger was elevated by a spring, so that the plunger was always outside the syringe barrel except when you pushed it down to dispense the liquid. This let the solid material in the solution dry out on the plunger and jam it. While the plunger was in the up position it picked up dirt from the air. Another defect is that it utilized two little rubber valves in the pump mechanism; these could not be used with organic solvents (my favorite kind of solvents).

I pointed out the need to change these features and Shapiro devised an all-glass system that solved these problems and started a small revolution in the liquid handling field. He invited me to name it; I chose "Repipet," a contractive juxtaposition of *repetitive* and *pipet*. He has been selling this device and various elaborations on it for many years, and several companies have come out with variations on the design. The irony of this success story is that they all jam up anyway. Even though the plunger is normally down inside the barrel, the solutions dry out when they are not in use. I have a drawer-full of the broken parts resulting from these jam-ups, the products of strong assistants and postdocs.

I described a new method of organizing animal-drug studies based on an old statistical idea that random selection of animals is a mistake and that one can get more meaningful results by matching groups of animals in such a way that the groups have similar mean weights and spread in weights (that is, the standard deviation in weights within the different groups should be as similar as possible). A particular merit of the idea is that one need not analyze each individual animal but can work with the pooled organs of the animals within each matched group. Chemical analyses are usually tedious and expensive, so there is much merit in pooling organs instead of analyzing each animal separately.

Paul Klinger, Agranoff's fish trainer and programmer, wrote a computer program for matching the animals, but his initial attempts ran very slowly since they were based on random sets of values that were evaluated to see how closely they fit the desired distribution. Many sets of values had to be set up and evaluated, so the program took hours to run on our Apple //e. In a remarkable stroke of insight, my wife Norma pointed out a simple way to make up a preliminary set of weight groups which could then be evaluated. A series of systematic switches of values from one group to another quickly gave us the best possible assortments. I tried to publish a paper describing the rationale and the program but was rejected by four journals. Each statistician reviewer thought the idea was bad, but their explanations (when furnished) were so obscure that I could not deal with them. They all disregarded my citations of earlier statisticians who recommended the method but did not at the time have computers. Finally I consulted J. E. Keith Smith, a Statistics Prof. at the U-M who used to be associated with MHRI. He pointed out that my approach reduced the ability of the experimenter to detect small but statistically significant differences between the groups. I argued that in most animal studies there is little value in finding a significant <u>small</u> effect and Keith furnished some statistical jargon for me to use. This won

approval for the manuscript but I am not sure that anyone is following my approach.

At one point, in 1982, the U-M decided to encourage University members to invent new devices or processes that might earn money for the University. My submission was the idea that ball point pens should be filled with *two* colors of ink. The second color should occupy only the top 1/4 inch or so, so that the user would be alerted to the impending emptying of the pen and hasten to replace it as soon as possible...but without running dry at an awkward moment. This idea won me a monetary reward and a terrible dinner at the Michigan League, but no attempt was made to patent it or offer it to a pen manufacturer. To those who use rolls of paper in printing calculators or voltage recorders, the idea of a warning colored streak is quite familiar – why not with pens?

Over most of my research career I have been making sorties into the problem of measuring the concentration of lipids in tissues. The approach I finally adopted was to separate the lipids by thin layer chromatography, then blacken (char) the lipid spots to make them visible, and measure the degree of blackness. Our first method of measurement, worked out by Ramasamy Selvam and, later, by Ashok Hospattankar and Gayatri Deshmukh, was somewhat bizarre. We scraped the blackened silica gel off the glass backing plate and suspended it in a radioactive gel. This lowered the apparent radioactivity, as measured with a scintillation counter, so we could measure 5 to 40 μg of lipid.

This method required a lot of handling, which meant that only very intent and careful workers could get accurate results. When Tom Ford-Holevinski and Bernie Agranoff started discussing the combination of a video camera with an Apple computer (around 1982), we decided that this approach might work with the blackened spots. We simply mounted the camera so that it could look at one spot at a time, then let the computer convert the picture to numerical values derived from each "pixel" (microregion) in the screen image. The programming effort involved in this basically simple concept was considerable but today we are still using Tom's system. It underwent many refinements as improved equipment became available. Now we can measure 0.8 to 2.4 μg samples rather accurately. Unfortunately the optical system we use has a limited range of linearity so some preliminary analyses are sometimes necessary to get the right amount of sample on the plate. Since we published these methods, two commercial instruments of a similar nature have come onto the market; they are very expensive and I haven't seen any studies of their usefulness.

In the course of developing these quantitative thin layer analyses, we went through many developments to improve the chromatographic procedures. Mike VanRollins struggled with these problems for some time, then Inez Mason and I. We ended up with a rather complex streak applicator, which applies three samples at a time to the chromatographic plate. One motor moves the plate while another motor drives the three syringe plungers to apply three solutions in the form of short streaks. It uses my daughter's discarded hair dryer to evaporate off the solvent during the streaking.

The availability of an Apple computer and the insufficiencies in available programs led me to try my hand at programming. Bits of knowledge were picked up from Ford-Holevinski, Paul Klinger, a lab assistant (Dan del Vecchio, now a professional programmer), and my son (who started programming while in high school). They often helped me out when I got stuck in the strange subtleties of the BASIC language. I even published a program for printing bottle labels. Nowadays, when I rely much more on the very sophisticated programs available for the Macintosh, I wish the programs could be modified, but this is entirely beyond me.

The Lipid Center

In 1965 I tried to set up a Lipid Center, where scientists could come for a little while to prepare large amounts of specific lipids, radioactive or other. At that time, there were many lipids being discovered but which could not be studied because so few people have facilities and equipment suitable for large-scale preparative isolations. This idea was inspired by the Enzyme Center at Tufts University, which was set up for similar problems involving enzyme isolation. Not only natural lipids needed large-scale workup, but unnatural ones which might be useful as enzyme inhibitors. Many nutritional studies involving lipids were (and still are being) run in an inadequate way because of the unavailability of pure lipids. The Center ought to have a permanent staff who could guide the visiting scientist in the scaling up of his methods and the use of the equipment. Later I enlarged the idea to include a facility at the Center for training biochemists in lipid methodology.

After corresponding with various people, I managed by the next spring to enlist the active interest of Herb Carter, one of the leading American lipid chemists. He suggested expanding the scope of the Center and gave it the name, Biological Preparations and Isolation Center. The University of Illinois offered the use of a large building and a grant application was submitted to NIH, asking for almost $900,000 the first year.

A group of interested people visited the proposed site, which looked very practical to me, although Urbana, IL is not on a direct airplane route. Unfortunately, this was the year that President Nixon started to impound (divert) research funds so the project never materialized. Despite the greater commercial availability of lipids today, I still think it is a good idea. But money for basic research is even scarcer now.

Editing, Reviewing, Writing

My experiences as a reviewer of manuscripts from other scientists, and later as an Executive Editor for *Analytical Biochemistry,* gave me a store of memories of a scientific nature. I was (and remain) amazed at the number of scientists who make serious scientific errors in their experiments and writing. Some of these papers get published anyway and I know of several in my own field. Basic rules of biochemistry get broken often – the use of a buffer to prepare a solution in a pH region outside its buffering action, the failure to run controls and blanks, wrong chemical structures! One lab bought psychosine sulfate and used it to prepare an affinity column, which apparently had some use. But they thought that they had bought the sulfate ester of psychosine (not commercially available); actually it was the sulfate *salt*, an entirely different substance.

One paper that was published, presumably approved by the editor and the reviewers, stated that the authors had added catalase and/or peroxidase in order to <u>hydrolyze</u> hydrogen peroxide, which was formed by galactose oxidase. Neither of these well-known enzymes can be considered a hydrolase.

Several authors have sought to describe the shortcomings of a particular published method, but when I examined the details I found that they had actually examined <u>their</u> modified version of the original method, for reasons unstated. They did indeed show that their modified method was no good, but why publish it and attribute it to the previous author? I hope I caught all of these totally weird studies.

One manuscript described the synthesis of an optically active compound, starting with reagents that were not optically active! The rejected and dejected author called me on the phone for a long discourse on how he and fellow scientists who had reviewed his paper saw nothing wrong with this. He even confirmed the existence of optical activity by testing the compound with a specific enzyme, which did

not attack the molecule. I pointed out that he had not tested the enzyme to see if it was able to act on the *racemic* form of the molecule. No control experiment, no physical measurement of optical rotation. After a month or so he called back to say that I – and the history of science – were right. What would have happened to his and the journal's reputations if the paper had been published?

More than one such conversation took place over long distance wires, in which I tried to teach basic science to various would-be authors. Generally my efforts failed. It seems likely that some students, once they get their Ph.D.'s, become impervious to reminders of their former lessons. For a while I handed out boiler plate advice to authors, taken from my computer's store of paragraphs covering common errors. Even simple information, such as listing a few rules of chemical nomenclature, proved useless to authors who were revising their papers. When I listed specific errors in typing abbreviations, their secretaries proved unteachable. Almost no secretary or author uses the spelling checker in his word processor despite the simplicity of the checking programs. Careful editing took up a lot of time and probably antagonized many people. Eventually I quit decision-editing but still do a lot of reviewing for other editors.

I am not the only one who has done detailed editing. I remember one of my papers that had been accepted for *J. Neurochem*. I was astounded to see the proofs come back with my original manuscript on which Donald Tower, the chief editor, had written innumerable changes. Somehow, presumably with the aid of a huge thesaurus, he had inserted a synonym for almost every word. As far as I could tell, this well-meaning and important person at N.I.H. had spent a tremendous amount of time making changes which seemed largely pointless. I wrote Don a letter suggesting that he not work so hard at this.

Many people get upset when their writing is changed and I am afraid that I am one of them. However I try to keep this reaction under control as I know some changes are really improvements. One series of changes made me actually angry, when I was writing my lab manual describing experiments that illustrated the different uses of radioisotopes. Nuclear-Chicago Corp., for whom I was doing the writing, had assigned one of their people to check over my manuscripts. She too, like Tower, made unilateral changes which – in this instance – actually introduced scientific errors that could have damaged my scientific reputation. It cost the company money to correct the errors (already typeset) and I threatened to quit the project if she didn't leave the text alone. I have rarely or never gotten this angry.

Recently I sent a short paper to the British journal, *The Biochemical Journal*, via the FAX system, which seemed like a nice way to save time, especially for international communications. The paper was approved and set into type and published without sending any proofs. Somehow they couldn't spare the 1-day time delay to send me a FAX copy of the proofs. The editor who had retouched the manuscript had changed the name of a vital compound on which the study was based; it was a completely different compound now. What leads people to make such changes?

I was one of the founding editors of the *Journal of Lipid Research,* which first appeared in October 1959. Together with Howard Newman and Don Zilversmit, I originated a nice service for lipid biochemists. For each issue, we prepared a collection of recent publication references that described methods for studying lipids. After a few years, Dave Bowen took over Zilversmit's role, then he replaced me. Another of my postdocs, Peter Braun, later took over David's part. This public service continued for many years; I don't think any other journal offers such a listing.

Scientific Begging

A substantial portion of my scientific life was spent preparing for and attending Study Section meetings for N.I.H. I was recruited into the Physiological Chemistry S.S. by Saul Roseman, who was then at the U of M and retiring from his stint on the S.S. This kind of service, which is after all a duty of scientists, seemed likely to help me improve my own grant renewal applications. That hope turned out to be a mistake; my own applications suffered the same misreadings and hasty evaluations that other applications suffered, whatever the quality of the applications.

I learned that there are psychological dynamics at work in the review meetings: a forceful opinion from a charismatic scientist – right or wrong – could outweigh a gentler expression of opinion when it came to swaying the opinions of the less-involved reviewers. However I learned to manage my counterpart in the reviews of some lipid projects. When he would firmly state that "this proposal is a good one but the same thing is already being done by someone else" I would ask him to name the other investigator. This sort of insistence on specifics gradually cooled off his self-assurance.

Of course it is impossible to sway everyone to your side every time. One investigator had published only one brief note on his subject, a provocative but improbable finding. This paper seemed insufficient to support a renewal. Nevertheless, one or two members of the S.S. stated plaintively and vehemently "But he is so highly regarded at the University. Everyone consults him for advice. He taught me how to use a Beckman!" I didn't think that learning to use a Beckman spectrophotometer was so difficult that the teacher deserves a grant but I was outvoted.

Three years later he came back for a renewal again, this time without <u>any</u> publications at all. The same argument was produced and we decided to make a site visit. (These days, the overload on the reviewers and the general desire to reject applications have destroyed the idea of a personal evaluation on site.) A team went to the lab and we discovered that it was thickly coated with dust and unpopulated. It turned out that the entire discovery on which the application was based had been the work of an East European assistant – now gone – whose lab notes could not be deciphered. The assistant had written the paper. This time the application was rejected.

Our S.S. Executive Secretary, who accompanied us in the joint dinners that followed a long day of arguing, was an enthusiastic eater. He would remind us that "alcohol is a food" so we could legitimately charge dinner drinks to our food bill. Since I don't drink alcohol, and since the restaurant bill was divided into equal shares, I helped redistribute the alcohol bill. Our Exec. Sec., as an N.I.H. employee in Bethesda, could not charge his food bill to N.I.H. so we would take up the cost of his meal into our shares.

My second review committee service was in a Mental Retardation Research and Training Committee. I was invited into this group by virtue of my interdisciplinary exposure at MHRI. We spent much time on trips, visiting large groups of interdisciplinary scientists who had devised large, complex grant applications. This had its interesting points and I learned things that never would have entered my ken as a biochemist. However, the time spent at each site was becoming burdensome and, after a 3-day stay at the University of Washington, I wrote a letter of resignation. Sitting still for such long periods, listening to speaker after speaker, was hard on my bottom and top. However I let myself be talked into finishing the 4-year stint. I was impressed by the dedication of the N.I.H. staff, who accompanied us on the site visits and tried hard to maintain a high level of discussion.

Of course these complex grants consumed much money in each approval and I often thought about whether the individual projects could have been funded if they had not been part of a program project. Somehow I could never bring myself to take part in such an application at the U of M.

One of my chores at MHRI was the administration of our Training Grant. I had to fill out the grant application renewal forms, assemble proposals from our various senior staff members, handle site visitors, write annual progress reports, correspond with postdoctoral applicants, sit with my colleagues to evaluate the applications, and write the award letters. There was some work involved in reassigning funds for equipment and supplies. Since this was an Institute-wide grant, I tried to apportion out the awardees on some kind of even-handed basis. I was often reminded of the lament of the Lord Chancellor in Gilbert & Sullivan's *Iolanthe*. He was in charge of female orphans who were ready for matrimony. He sang:

...And everyone who'd marry a ward
Must come to me for my accord,
And in my court I sit all day
Giving agreeable girls away.
With one for him and one for he,
And one for you and one for ye,
But never, oh never, a one for me.

Well, it wasn't that bad, but I did feel that some of the apportionments were ill-advised, especially the ones assigned to pursue Jim McConnell's finding (?!) that some memories could be transferred by injection or ingestion. None of those postdoctoral fellows actually worked on that topic, no doubt for good reasons.

Professorial Life

While at Northwestern Medical School I was a real "professor," teaching medical students and participating in endless committee meetings that were supposed to produce improved learning. The medical student course that I inherited involved many lectures and lab periods, the latter requiring many hours of planning, experiment testing, and sheer attendance. Each lab period, by tradition, lasted three hours so the number of student contact-hours was appreciable.

I put a lot of effort into making the experiments interesting, a particularly difficult problem since most medical students really want "medical action," not basic biochemical knowledge. One experiment required the students to go on a special diet for a week, analyzing their blood cholesterol before and after. The medical biochemists in the Department drew the blood samples and we got gifts of various items for the students to try eating. They enjoyed this kind of experiment, which had a lot of health relevance, but I wonder if now we would be allowed to tamper with the health of innocent students.

One of my colleagues, Jim Garvin, assigned his section of students to eating Serutan, the popular treatment for constipation. These students, despite the unpleasant fecal softness, obtained impressive declines in their blood cholesterol levels. Garvin's rationale was that the gelatinous Serutan particles might block the reabsorption of intestinally-excreted cholesterol, which normally accounts for a large part of the cholesterol in blood. In recent years, possibly because of this pathbreaking experiment, the use of similar food "fibers" has attained some fame.

My lectures were presented with my then-youthful verve and I had the satisfaction of receiving student applause at the end of some courses. This was not to happen again at U-M.

When I moved to U-M, the new position was strictly that of a non-tenured researcher, as with most of the staff members of MHRI. However, a time came when the growing Vietnam war effort seemed

more important to our best and brightest leaders than biochemical research, and non-tenured researchers at the U-M started to lose their jobs. Several members of the Institute began urging our Director, Gardner Quarton, to seek teaching positions for us in the various departments here. Eventually he was moved to accede and I was offered the chance to join the Dept. of Biological Chemistry, then under the leadership of Jud Coon. The new position would not involve a transfer of actual money, only the pleasure of teaching and the chance of inducing a graduate student to work in my lab. The Department, however, would not offer tenure for this contribution, but the Dept. of Psychiatry agreed to cover that contingency.

Thus, around 1973, I began an 11-year stint as a professor again. This brought two excellent students to my lab, Sue Berent and Ray Metz, so I guess it was worth it. The Department paid their stipends only for the first two years and my grant had to support them afterward. This requirement, which may well be peculiar to the U-M Department, was a source of some pain for me as I felt that research grants were not supposed to support students. I don't remember why I had this impression but eventually was induced to believe that this was legitimate.

My primary teaching assignment was one semester a year of biochemistry lab experiments, about eight contact-hours per week. I found that the previous teachers of this course had concocted a mess of unworkable experiments and ill-advised scientific statements in a lab manual which must have left many students confused, misinformed, and frustrated. Much effort was needed to straighten out the mess, which took up considerable time.

Complicating the problem was Jud Coon's insistence on assigning first-year graduate students the job of preparing the reagents and equipment needed by the students. These poor innocents had themselves just come from colleges with only the minimum experience obtained by taking college courses in chemistry. Few had studied biochemistry or had more than a preliminary knowledge of the field. Moreover they were themselves entering a new and demanding environment in which they had their academic studies as their primary activity. The net result was that many experiments were prepared incorrectly and only frustrated our tuition-paying, somewhat anxious students. Another problem arose because faculty members had access to the student supply storeroom and lab (and used them as a supply depot), so that teaching assistants had to scour the Department for missing equipment and supplies. Several times I saved the day by furnishing the required reagents from my own lab.

One of my T.A.'s forgot to order chloroform for a lipid experiment, so he stole some from an adjacent research lab. Unfortunately, the bottle he stole was labeled on *two* sides – one side said it contained chloroform and the other side revealed the true contents. Of course this is a dreadful way to store reagents but in his haste my student failed to notice the switch. The entire experiment failed because of this but perhaps our students learned the lesson – read all labels on a bottle! At least there was no poison in the bottle.

Each year, as I uncovered the misunderstandings and errors made by the T.A.'s, I rewrote the instructions for them and eventually preempted most of the errors that could be made. I think my instructions must have had much educational value for these graduate students.

The students who took our course were not graduate students, which disappointed me, but premedical, predietician, and premicrobiology students. Many of them were not enthusiastic worshippers of biochemical knowledge and suffered a good deal. One poor student that I remember did so badly that I decided she could not have possibly taken the prerequisite courses. She assured me that she had even taken a biochemistry course, the so-called Keller plan course. This is a course given at the

U-M which requires students to simply cram a segment of a textbook and pass periodic tests. A failure to pass a test could not affect the grade since the student could recram for the exam and try again. Like most students I met who had taken this course, her memory of the course contents was exactly zero. She couldn't remember the teacher's name either. I strongly feel that this kind of course is a big mistake.

One year I received respite from the lab course and was assigned a small group of first-year medical students to help them learn the content of their biochemistry course. The way the course was organized, a few departmental professors presented lectures in a large hall, which we sub-teachers were supposed to audit. These teachers prepared the exams, which we could not see before or after. Perhaps knowledge of the exam contents would bias our discussions. My job, as I understood it from a very brief orientation, was to "help" the students. Initially I asked the students if there was any point that seemed unclear despite their attendance at the lecture and perusal of their textbook. This was an unpopular question, so I tried rewording points from the lecture that seemed to need elaboration. Unfortunately, I did not take the time to prepare formal presentations and found myself losing attendees as the weeks wore on. The students clearly did not want short quizzes on the lecture contents. Somewhat later I learned that the most popular sub-faculty members were simply giving complete lectures on all the topics covered by the primary lecturers. Considering the fact that all the students bought copies of the lecture summaries, our jobs seemed superfluous. When my group dropped almost to zero I simply stopped coming in and was surprised to learn later that my students were pretty disgruntled with me. That kind of teaching was not in my job description.

After 11 years, I quit the lab teaching job. There was little reward in it and, I felt, little recognition of my time-consuming efforts. The lab course clearly was a low-priority item in the curriculum; it was impossible to get more experienced teaching assistants. Moreover I felt sorry for many of the students who were forced into taking a course for which they had no aptitude or interest. I kept wondering whether the "regular" faculty members (the ones housed in the Medical School building) worked this hard at teaching. Fortunately I was able to maintain my tenured status by being granted a new title – Prof. of Neurochemistry in Psychiatry. Robert Friedel, then chairman of Psychiatry, approved this. By coincidence, he has himself published papers on lipid biochemistry.

Ironically, the members of the Institute who had nagged Quarton into snagging departmental affiliations for us were unable to convince any department that this was a good idea.

Hired Help

I suppose my scientific life story should mention some of the unusual people who worked for me. When I first decided to become a scientist, the thought that I might be hiring people to help me never entered my mind. But I saw, as a grad student, that the professors each had a helper or two (not the crowds that one sees now) and realized that this was a normal way of doing research. When I started my first independent job, at Hines V.A. Hospital, in Illinois, the job came with assistants. One of them used some strange, foolish techniques and when I sought to query her, she explained that her previous boss had taught her to do these things. I concluded that her boss must have been a pretty poor scientist until she started telling me that ***I*** had told her to do some new silly things. That is when I realized that there was a loose connection somewhere.

Another aide, a postdoc, nearly set the lab on fire by smoking while handling ether. I attributed this to absentmindedness until he disappeared one day and I received a call from his psychiatrist. He had

had another one of his "spells" and had to be locked up for a while. Apparently he had been hallucinating while at work. He returned to work after a while and then had another episode of detention. When I asked a friend of mine, who had recommended him to me, if he had exhibited this problem before, the answer was "Yes, but I thought he should have a second chance." After all, what are friends for? Of course this is a tragic story and I wonder if some kind of chemotherapy ever helped him.

Another postdoc worked very odd hours, so he said. When I asked to see his lab results, he reported them from his notebook, which was carefully turned so that I couldn't look over his shoulder. I never got a written report or anything useful from him.

Another unfortunate choice was a young Japanese postdoc whose experiments never seemed to work. Closer inspection showed that he was doing important steps incorrectly and we had a few mutually unhappy interchanges on the subject. The result was that I asked him to quit. Bernie Agranoff decided that he was likely to cause me bodily harm, and attempted to defuse his feelings by hiring him to work on a new project. Perhaps Agranoff did save my life. I left town for a stay in another lab and learned that the postdoc did poorly on the new project and locked himself up in his apartment. He was coaxed into seeing a psychiatrist, who quickly arranged for him to return to Japan. It seemed likely that there had been a severe cultural shock; he had failed to socialize with anyone.

Still another postdoc appeared only rarely at his bench. I received only evasive explanations and finally removed this headache. Years later he applied for a grant, which I was asked to review. His c.v. didn't mention me at all; he stated that he had been a postdoc in another lab at MHRI! This seemed pretty improbable as he had been paid from my grant, but I can easily understand how he could have misremembered his stay here.

One postdoctoral associate, an M.D. from Poland, came to me by an odd chain of circumstances. Project Hope, a charitable organization that sends physicians to backward countries to treat their patients, also had a project to bring Polish physicians to the U.S. for a few months of specialized training. One doctor, in charge of a clinical lab in Krakow, wanted to get experience in analytical lipid techniques and applied for study in the laboratory of a noted specialist in that field, in Texas. For some reason the latter couldn't accommodate the M.D. and suggested to the organization that they send him to me. When the Project Hope representative phoned me for an okay, I agreed to the idea and was told that it would be a while before the mystery individual would arrive.

Suddenly, about a week later, I received a call telling me to meet the visitor at the airport. Somehow the schedule had been moved up. I met the hapless exhausted visitor, who spoke reasonably good English, and took him back to Ann Arbor. It turned out that he thought he was to land in Texas, to study gas-liquid chromatography at the other lab – he hadn't been told of the change in plans. The Project Hope had given him a total of $40 in cash in New York City; this was his total startup fund, to be supplemented later by checks. Of course I advanced him a considerable sum and found him an apartment, etc.

The poor man was disappointed further to discover that I wasn't using gas-liquid chromatography at the time. I tried to interest him in a problem in lipid analysis using liquid-solid chromatography (an approach somewhat different from the other approach, despite the similarity in names), but he seemed somewhat dispirited. The culture shock must have been considerable and the language barrier made things more difficult. He did not spend much time in the lab.

A few years ago, someone's assistant – no doubt not mine – decided to vandalize my lab in a gentle

way. This person turned on our hydrogen and nitrogen gas cylinders, letting them vent into the building. Fortunately the hydrogen was dissipated fast enough to prevent an explosion. Another little trick consisted of putting acid into Akira Sano's buffer bottle; fortunately he figured out the problem before spoiling much work. Another bottle, which supposedly contained pure solvent, turned out to contain some white material dissolved in the liquid. I don't know how many experiments that person spoiled. It seemed that the University Security Division could install a monitoring video camera and recorder to catch the vandal, but they somehow couldn't bring themselves to install one. The attacks apparently ceased but we may still have solutions around that have been modified. I had heard of a similar situation in another lab, which was solved by moving each lab person into an empty lab on another floor, one at a time. When the vandalism appeared in the new lab, they figured out who was responsible. But this technique wasn't available to me and, besides, how many vandals are dumb enough to get caught this way?

The above list of unfortunate associates sounds, on rethought, as though I had no good scientists working with me. Of course this would be an incorrect conclusion. and I owe most of my scientific discoveries to the hard, sometimes intense efforts of many individuals. Inez Mason, my assistant for a remarkably long time, has been an invaluable aide whose memory about the lab and its contents have frequently saved me much time. She helped teach many individuals about our technology. One of her very useful ideas was that we should take over control of a little-needed hall near my lab and convert it to a storage space by bricking in one doorway. I promptly named it *Mason Hall,* which, by coincidence, is the name of a major U-M building. A few of my associates, such as Sue Berent, had the ability to inspire their own assistants (especially Janet Selmek); she was outstanding in this regard.

Not the least credit must go to the many people who supported my research, especially Bernie Agranoff. He brought me out of slavery in Chicago, like Moses, and enthusiastically supported my work financially and intellectually. I wish we had had more time for interactions.

Of course, as I review my career, I think of many scientific friends in various cities and countries who gave me good advice, hospitality, congenial company during meetings, and technical information, as well as – in some cases – generous collaboration. My coming to the U of M was a lucky break, for this University has been run by administrators who appreciated the value to scientists of a strong scientific infrastructure (computer availability, libraries, reasonable parking space, etc.).

The comment on lab contents above reminds me that I have for many years maintained a computerized list of all purchases, that I periodically update and print out. This makes it easy to reorder items. Every drawer and shelf in the lab is numbered and the storage sites are listed in the printout too. This is a vital aid in finding supplies. I am surprised that other labs do not seem to use this approach, but maybe other investigators have a much better memory than me. Or a Rolodex set of cards is sufficient.

Drowning under Data

A topic that I haven't touched on is the "literature pit." When I was a grad student in 1948, I had the impression that I knew a large fraction of the important biochemical data then available. It may sound incredible, but in 1946 there just didn't seem to be all that much known. New articles were appearing, of course, and it was certainly necessary to monitor a few journals and search *Chemical Abstracts* and *Beilstein*, but there was none of my current feeling that new research findings are entering a bottomless pit that can be stirred up safely only in small portions. At that time I kept my reading notes in a loose-

leaf notebook divided into a few topics. As the flood broadened, I tried storing information on 3x5 cards, also divided into a few topics, constantly growing in number.

Of course everyone else was starting to reel under the load and Agranoff and I decided (I don't remember who talked whom into it) to buy a cute literature recovery device: a small highspeed drill, mounted over a board in such a way that it could drill a hole in sheets of plastic in any one of 10,000 positions (i.e., in a matrix of 100 x 100 positions). Each sheet of plastic was assigned a code number, corresponding to a particular subject. Each position number, 1 to 10,000, was sequentially assigned to each new journal article that we wanted to store. Thus, an article might be assigned the number 256 and it might cover topics 2, 7, and 45, (identified by a code record). Then we would lay three sheets, numbers 2, 7, and 45, on the drilling board and drill one hole in position 256. This allowed us to locate all the articles in our possession that were relevant to those three topics, since the light in the light box below the three sheets could shine through only hole 256 (and any other article with a common hole). One would then search through the file cabinets, which contained all our collected articles in numerical order, to study the relevant ones. A little experience with this method showed us what common sense should have predicted – it was simply too time-consuming to use.

I remember an unusual moment concerning this technique: I had gone to a biochemistry meeting at which one of the speakers was supposed to give a talk on the method. At the last minute the audience was told that the speaker was ill and the talk had been canceled. I volunteered to describe it and gave, I thought, a lucid description which probably wiped out anyone's thoughts of buying the system. (This was the closest I came in my life to satisfying the fantasy of being a last-minute substitute for a sick flutist in a large professional orchestra – "Is there anyone in the audience who can...?")

In 1966, before or after trying this drilled hole retrieval device (I don't remember when), I tried a similar system which was more popular. This was the Termatrex system, which used 5 x 7 cards edged on all four sides by rows of holes. Each hole was numbered and could be coded to any topic, just as with the 10,000 hole plastic sheets. By punching out a bit of the cardboard between a hole and the edge of the card, one could make the card fall out of a stack when shaken with a long pin inserted through the hole. Only the edge-punched cards would fall down. The nice thing about these cards is that one could write the bibliographic reference and a summary of the paper right on the card.

Unfortunately, it turned out that the punched cards fell out only occasionally, due to bending of the cardboard around the punched-out sections. Typing notes on the cards also warped them and kept them from falling out. Moreover, only a few cards could be shaken on the pin at any one time, making it tedious to search a large number of cards. I think others found the same problems and believe the system is no longer sold.

Another time, I convinced Gardner Quarton, our Director of MHRI then, to buy a microfilm camera and fiche reader. This would allow us to store reprints in much more compact form and find them more quickly than by a filing cabinet system. Unfortunately I did not realize until it was too late that the sharpness of the photos with this system was too poor to make reading practical. The demonstration films furnished by the salesman had been derived from unusually large print, which was quite clear. It was embarrassing to be fooled so badly.

After these retrieval debacles I turned to Xeroxing the first page of each new article, storing each sheet in a specific folder whose number was assigned the topic number previously assigned to the plastic sheets. Thus it was possible to find the original copy by perusing a folder on some topic of interest. I still have these folders, the list of which now takes up almost 13 computer-printed pages.

Those early Xerox copies are pretty pale, a reminder of how marginally effective the early machines were. The folders, together with the original articles and the Xerox first-page copies, now occupy 12 filing cabinet drawers and hold an estimated 12,000 articles.

This method of cross-indexing obviously had the disadvantage of covering too few topics per article and I was fortunate enough to work in an Institute whose directors and staff saw the great potential in computers. A hard disk in a multiple disk drive of a Prime computer was assigned to me, so I rented a printing terminal (a Diablo) and bought an acoustic modem to make the connection by phone. I had to learn the primitive word processing program then available (a difficult line-oriented system in which each line was numbered) and started entering the bibliographic references for each article, together with a list of key words and comments. Later I hired part-time students and other itinerants to type these, and found myself spending much time in correcting typos. As I recall, the rental for this terminal ran to about $1200 for one year – imagine what one can get for $1200 now! – and it was financially very helpful when simple terminals and dot-matrix printers became available. The word processing program did not include a word searching program, making it somewhat useless, but Paul Klinger wrote a search program for us.

Meantime I subscribed to *Current Contents* and several journals, but scanning them was a time-consuming operation. Fortunately some of my postdocs and a student, Ray Metz, brought me articles that I had missed. Some thoughtful colleagues in other labs kept sending me copies of their publications. It is grimly amusing to read of the efforts by people opposed to animal experimentation to force scientists to do a thorough review of the literature before embarking on a new project or research proposal involving animal work. Non-scientists (and non-biochemists) don't have any idea of the magnitude of the task. Even when you find an article that might be relevant, it is in many cases difficult to assimilate and memorize all the important findings and ideas.

A few years later, the Prime computer started to die of age and abandonment by the manufacturer. MHRI bought someone else's Prime and cannibalized it to keep our machine working. The warning finally came, "Get your files out of the computer or lose them." I bought an Apple //e with a 5 megabyte hard disk and Klinger helped me transfer the files. This was a rather tedious job and I believe part of the data evaporated into electronic limbo then. Later, due to several breakdowns of the Apple hard disk and malfunctions of the disk operating system, additional files evaporated. While I used a floppy disk backup program, this only stored the newly depleted files, a useless kind of backup (there was no easy way to recognize when data had been lost).

Searching the remaining files, stored in Apple Writer format, was pretty tedious because each file could hold only 64 kilobytes. As time went on, I ended up with 46 files. The Apple Writer word processing program is interesting and unusually useful because it contains within it a sophisticated macro program which allows one to write complex procedures for handling text files. Among other macros I wrote with this system was a searching program which automatically went through all 46 files, looking for any desired word or fragment of a word. I tried to publish my macro programs but somehow the Apple magazines were not interested. I was even able to write an alphabetizing program and banners of any length, all with the word processing program combined with a BASIC program.

A useful fast-searching program became available which replaced my slow program. The seller of the program (Fast Data) advertised it as a program so useful that the U.S. government refused to let him sell it to Russia! It is certainly a remarkably effective program but how could Russia use it with Apple computers to destroy the free world? No doubt this was the work of a bureaucrat expert in computer matters.

When the Macintosh SE with a 20 Mb disk became available, I was able to buy one and transferred my Apple files to the Mac disk with the aid of a grad student. I was surprised to discover that even with 1 Mb of RAM in this much-faster computer, contrasted with 0.128 Mb of RAM in the Apple IIe, the length of a useful file was only doubled. A fast search program, Gofer, became available for the Mac and it is now possible to search through all my reprint files in just a few minutes, looking for several topics at a time, even while working on some other project.

Now I have given up all journal subscriptions and *Current Contents* and rely instead on *Reference Update*, a weekly list of recently published articles in over 2000 journals. The list arrives on a computer disk every week and the Macintosh automatically searches for all the names and words in my "strategy" list. It prints out the address of the author who has the reprints, making it easy to write for a copy. The "finds" of interest are stored in my Mac's hard disk for subsequent searching with Gofer.

Even with the computerized search technique, the work of searching, choosing which articles to get, reading the reprints, storing the citations in my disk, and filing away the reprints in selected folders takes a significant amount of time, at least 3 hours a week. If you fall behind, the flood (or whatever simile you prefer) piles up. What is the use of all this? It seems inefficient but I guess it is essential, especially to a person with a weak memory.

Science and Architecture

My first years at MHRI were in a basement laboratory, windowless and pretty cramped. Spacewise, it was a substantial reduction compared to my lab at Northwestern U. Luckily, the Food Service Bldg. at U-M was vacated and, partly through Agranoff's intercession, it was designated to be an interdisciplinary Neuroscience Lab. Bldg. One floor was to be assigned to MHRI, another to animal psychology, and the third to Neurology. This building was a nice solid structure, consisting partially of huge cold rooms (for storing meat, etc.) The floors were very thick, in order to support heavy baking and food mixing machinery. The University architects recommended tearing out everything except the floors, outer walls, and supporting pillars. Bernie and I were given the privilege of designating what was to go in our labs.

This was an exciting challenge, an opportunity to correct all the bad designs we had been living with. One catch was that our budget was unknown: "You ask for it, we'll see if we can afford it." Another catch was that the floors were so thick that drilling holes for sink drains and other utility plumbing would be very expensive.

We looked into the idea of installing sliding glass doors, so that the expensive, 100% fresh lab air could be separated from the cheaper, recirculated inner building air. No – very expensive! We finally settled on a number of innovations, which I later described in a paper published by *American Laboratory.* Our lab benches were made to order, much shallower than standard benches, in order to minimize the storage of unneeded lab equipment. Clean glassware was stored on open shelves in a dustfree room, kept clean by a large sterile filter (HEPA) system that blows air through the room constantly. Lab shelves were simple, very shallow wooden shelves that reduced the storage of bottles behind bottles (lost from view). Each pair of lab benches was separated from one another by a lattice-work of Unistrut supports and aluminum rods, making the use of the common portable support stand less necessary. One section of the lab floor was left open to allow the use of open steel shelving, making electronic equipment accessible from the back. (Manufacturers of lab equipment want to make their devices look neat and pretty, so they put the electric cables and adjustment knobs on the back,

where you can't see what you are turning or pulling.)

We had special fume hoods built with white vinyl plastic instead of the usual dismal, dirt-storing gray cement board sheets. We connected the hood lights to the room lights so that no one could work in a dark hood, or leave the hood lights on when going home. Iconoclasts to the end, we restricted the gas outlets to the hoods, noting that lab bench manufacturers had not noticed the nearly complete demise of the Bunsen burner. Cup sinks, those tiny sinks found at the back of all the standard lab benches – which are used mainly to store rubber stoppers and scraps of paper – were eliminated except in the hoods.

I saved a surprising amount of money by reducing the number of wall switches, replacing them with a circuit breaker box. One needs such a box anyway and the breakers can be used as switches by placing them near the doorway. However, this money-saving idea backfired on me when one of my assistants couldn't remember how to turn off the room lights (the circuit breakers for which were clearly labeled) and turned off the main power breaker instead. All the freezers and refrigerators warmed up before this oddity was discovered.

The University architects came up with the ingenious idea of bringing in electricity, air, gas, sink drain pipes, and water through vertical rectangular channels added to the outside of the building. It was easier to break access holes in the walls than to drill holes through the floors. Unfortunately they forgot about the Ann Arbor winter and we had some disastrous pipe freezeups on weekends. Electric heaters were installed to prevent this but now we sometimes get very hot water coming out of the cold water pipes.

(A separate problem arose more than once because the U-M sends extremely hot water to each building, via underground pipes, and blends the water at the entrance to each building with cold water, using a mixing valve. This brings the hot water down to a safer temperature. One night the blending valve broke and hot water entered the cold water line so that the cold water was hot too. I had an ultracentrifuge running at about 100,000 rpm all night when this happened, and the cooling water for the motor bearings stopped cooling, allowing the bearings to melt. I think that failure cost at least $500 for the new motor and I subsequently installed a thermostatic switch to turn off the motor if the cooling water got too hot. This disaster has happened to others in the university but I think the centrifuge manufacturer never installed a thermostatic cutoff on any of the newer models.)

One of our innovations in lab building design was the specification that an extra fine air filter be installed in the lab air supply. This was a $30,000 filter in the shape of a huge belt, that moved slowly in front of the air blower. As I recall, the unused part of the belt was to be agitated to get rid of the dirt, readied for the active part of the cycle. This seemed like a good idea for our predicted need to do chemical work at higher and higher sensitivities, at levels that would be limited by dirt in the air. We were very pleased that the U-M accepted this expense but were puzzled on moving into the building to find that the air was unusually dirty. After a series of queries and assurances that we were dreaming, we finally learned that workmen were cutting up insulation (asbestos?) in an unfinished basement room whose air return bypassed the air filter for the whole building! How does one design such a system? We were a bit unnerved when the air-conditioning specialist kept referring to the air carrying ducts as "ducks."

Some years later we discovered that the expensive super-filter had never been turned on. I still don't know if it is operating – the Plant Department changed the lock on the utility room to keep me out. Foiled again!

The air inlet for the building was placed at ground level next to the parking lot. This allowed workers who parked their trucks next to the inlet – and left the motor running to save turning the ignition key – to send in remarkably obnoxious exhaust gases into the air supply. At those times we gagged on the mysterious poisonous vapors. Eventually we found the explanation and moved the air inlet up one floor.

These tribulations remind me of a story about a university chemistry building that soon, after opening, started to deliver rusty distilled water. It turned out that the plumbers had installed iron pipes for the distilled water line, instead of tin-coated or aluminum piping. What an expense for tearing out all the walls in front of the pipes! One always hopes that architects monitor the builders, but there is no way of proving this occurs as ordinary mortals are prevented "by the insurance company" from watching them at work.

Interdisciplinary Thinking

My presence at MHRI had the beneficial effect of teaching me a modest amount of behavioral psychology, some of which was quite interesting. This was part of my primary goal of making people smarter. (I also picked up some of this kind of information from my wife, a social psychologist.) One summer a psychology student agreed to work with me and we built a maze in an attempt to measure learning ability in rats. In reading about this subject, I was struck by the lack of interest on the part of animal psychologists in measuring learning ability. They seemed to be much more interested in memory, the ability to remember what the animals had finally learned. The animals were "trained to criterion" – the speed of learning was irrelevant.

For various reasons, we settled on a water maze, which consisted of a large water-tight box with stainless steel divisions. The rat was placed in the water at one end and was expected to swim to a resting platform hidden at another point. There we would pick him up and dry him off, presumably a reward. What I didn't expect is that some rats enjoyed swimming and were in no hurry to get out. We made the water colder, with a refrigerated circulator, which helped a bit. Another unexpected problem was counting decision errors. If a rat turned back while swimming along a lane, and then turned back again, continuing in the same direction, was that an error? If a rat turned his head to the right at an intersection, but then turned left (correctly), was that an error? If he turned more of his body to the right and then swam left, was that a bigger error? (I've had similar problems in marking student exams.) The learning curves we obtained were pretty erratic and we gave up.

To encourage interdisciplinary thinking, the senior staff at MHRI was induced to eat lunch together. The founding fathers of the Institute, in drawing up plans for the building, managed to insert a private lunchroom in the top floor (The Floor of the Thinkers). We had a private cook prepare meals with a modest degree of variety, at a subsidized price. For some years, this worked and there were many interesting discussions. However, the number of senior staff people eating lunch there gradually dwindled and these cross-level discussions vanished. I guess people got tired of the same cook's techniques; as we aged, some had to shift to dietary restrictions; we ran out of things to discuss; some people just got angry with the ideas that were expressed (mine, for example) and disappeared.

Some unusual research was done at MHRI (now called (now called Molecular & Behavioral Neuroscience Institute). One project was a thesis project in which rats were bandaged into immobility with adhesive tape and then tumbled in a rotating drug (lined with baffles to hurt the rat). The student's research required him to check the state of the rat at intervals and determine how many drum rotations

were needed to kill it! His more scientific input showed that surgical removal of a specific part of the rat's brain prolonged its life. Well, that does show something, presumably an effect on induction of the shock reaction, but the experiment was nevertheless a cruel and crude one. When I asked why the rat was not anesthetized during the tumbling, the answer was "The results might be different." I complained about this project to our Institute's Assoc. Director, Ralph Gerard, and I believe that he put a stop to it.

The student's thesis adviser, whose name was ironically that of a wild animal, also indulged in another kind of torture. He immobilized cats with a paralytic drug, keeping them alive and conscious by mechanical means, and showed that open-brain surgery produced certain effects. The eyes of the cat were open and dry and the head undoubtedly felt pain at the cutting and probing. "But what is pain?" asked this scientist philosophically. This kind of research was popular among physiological psychologists for a while but I believe that journals no longer accept such studies. That's scientific progress!

Seminars that Kept Me Awake

A scientific biography ought to mention some outstanding talks that were heard. I remember one at a national convention in Atlantic City in which the speaker showed a movie illustrating his tremendous discovery. He had placed a live little frog in a metal cage and lowered it – or forced it – down the gullet of a large dog. After leaving it in the dog's stomach for a while, he pulled it back up and revealed the frog hippety-hopping away when it was freed. The control experiment was done with a dead frog, which not only did not hippety-hop but was also partially digested. This showed that live tissue is harder to digest, at least by stomach juice. The whole experiment seemed bizarre to me and I asked the speaker why he did not at least anesthetize the live frog. The answer: "Because the experiment is more spectacular this way!" This inhumane report encouraged me to join and support the Animal Welfare Institute, which does many good things to protect live animals.

At MHRI, we heard some pretty fantastic reports from visiting speakers. One psychologist from Columbia told us of a project that he was planning to do. First he described his rationale, an algebraic equation that sought to describe why alcoholics eventually stopped drinking after a binge. As I recall, the variables to be measured were time, volume of alcohol, and satiety. This last variable looked like a difficult one to measure, but he planned to take blood samples periodically from the alcoholic volunteers and analyze them for every chemical known to occur in blood. I asked him how much blood he expected to draw from these subjects; it turned out that he had not bothered to calculate the amount needed for each analysis. Nor, of course, the cost of such an extensive set of analyses.

A talk by a famous astrophysicist (at MHRI!) proved an embarrassing disaster. The speaker became more and more confused and finally walked away, with regrets. We were later told that he was suffering from a terrible hangover. That topic wasn't in the psychologist's alcoholism study.

To strengthen the interdisciplinary nature of our Institute, we were required to present talks to one another about our research. One biochemical speaker mentioned, among other chemicals, ATP, a very common body component. One of the unfortunate staff members, a sociologist I believe, asked "What is ATP?" Ralph Gerard angrily interjected, "If you don't know what it is by now, you'll never know!" Pretty harsh words for a senior staff member but I suppose he deserved them – for the foolishness of *admitting* he didn't have even the dimmest idea of what was being presented. He should have kept quiet and endured. He left the Institute not long after, helping to change the composition of the staff to a more

biological average.

One time I gave one of those talks, after which a famous mathematician member of MHRI criticized the whole project. “What is the use of studying the chemical makeup of the brain, rather than its mechanism? A mechanical calculator works the same way whether it is made of metal or wood.” This comment was a perfect example of how a distinguished nonbiochemist, even after many hours of exposure to biochemical talks, can fail to have even the foggiest idea of what life is about. My sole response to this rhetorical dinosaurism was “Try adding alcohol to a calculator and see if it has any effect!” In those days, I should add, there were no electronic calculators, which might have confused his mechanical understanding of addition and subtraction.

A speaker, from Bell Labs, I believe, showed us a little black box that had a few blinking lights on it. It resembled a nerve cell, he claimed, but in order to support this claim he had to disregard almost every known characteristic of a nerve cell. Anyway, he couldn’t get the lights to blink correctly.

Another pseudo-mathematical talk was presented by a member of our Institute, a retired famous mathematical biologist who had moved to Ann Arbor. I had skipped listening to his earlier talks as the result of reading his abstracts, which were kindly presented in the announcements (more speakers should do this and save listeners’ time!). However, I couldn’t resist listening to his talk on the nature of schizophrenia. He presented an algebraic equation which connected the probability of becoming a schizophrenic and the number of traumas encountered in the course of a person’s life. He didn’t explain how to measure the traumas or their number but, after a long series of mathematical manipulations, produced an equation that predicted that the chance of becoming a schizophrenic increased with age. Of course this is an obvious conclusion from the initial premise. Its naive disregard of all the known data on schizophrenia was astounding but the capacity audience listened respectfully. One listener, at the end, asked him if there was some way that psychiatric practitioners could benefit from his analysis. The speaker stood there for a while, stroking his beard to help him respond to this novel question, then he finally said: “You will have to do more research.”

That advice applies to all of us.

GRAND SUMMARY

Looking back over my scientific life now, I see several categories of research (listed in no particular order):

1) Analytical studies -

Data on the amounts of cerebrosides, sulfatide, ganglioside, fatty acids of the sphingolipids, brain and other tissues and subcellular fragments.

Data on the activity of several enzymes, particularly those involved in the synthesis and degradation of the simple sphingolipids.

Changes in the above amounts as a function of age or as the result of treatment with drugs or a correlation with sex of animal.

2a) Development of new equipment or adaptation of available equipment for new applications -

Melting point apparatus (my first innovation as a grad student 42 years ago).

Equipment for vacuum evaporation, still sold but not under my name (the Nutator). This utilized a wrist-action shaker, with the "fingers" (liquid-containing test tubes) pointing down.

Dispenser for highly radioactive solutions.

Via a friend, I played an important role in originating and naming the Repipet, which led to the many valve-type dispensers now available.

GLC devices for collecting samples, connecting columns. The use of a metal-glass seal was adopted by a British chromatograph manufacturer for several years.

A reservoir microburet, sold by American Instrument Co. for a while.

Apparatus for applying large amounts of solution as a long streak on TLC plates for preparative TLC, sold by Applied Science, starting 1965. Still selling, after 25 years, now sold by Alltech for $995.

Apparatus for filtering radioactive samples for use in Geiger counters, sold by Nuclear-Chicago Corp. for a while.

Double-flask apparatus for oxidizing organic samples in liquid reagent and transferring the resultant CO_2 to scintillation vial.

Use of pipetting dispensers for lab classes.

An O-ring reaction tube for low pressure reactions (methanolysis).

Use of blueprint paper for recording TLC separations.

Modification of a popular fraction collector for use with large fractions. I still use the apparatus, after ~32 years.

Advice on the design of laboratories.

Effluent detector, collecting a portion of each column fraction on a paper strip for later analysis.

Device for applying multiple portions of solution on a single spot on a TLC plate.

Computer-operated video densitometer for measuring intensity of TLC spots.

Computer programs for label printing and animal sorting/matching.

Ultrasonic bath as a substitute for shaking type incubator baths.

2b) Development of new methods for chemical procedures -

New reagents for measuring radioactivity – Hyamine, Primene, both still sold.

Chromatographic purification and colorimetric determination of cerebroside. Modernized for quantitative TLC.

New methods for lipid extraction.

New methods of lipid liquid/liquid partitioning, for use in enzyme assays, sulfatide determination, lipid purification or isolation.

New methods of making radioactive materials (substrates, lipids, drugs) – galactose oxidase, chemical oxidation of glucosylceramide.

New or improved reactions for making known compounds – conduritol B epoxide, fatty acid methyl esters (using dimethoxypropane), glyoxylic acid (from tartaric acid).

Reactions for the identification of fatty acids and structure determination (mercuric acetate adducts to

separate on a column according to double bonds, micro-scale ozonolysis).

3) Synthesis of new compounds -

Inhibitors for enzymes.

4) Protein studies -

Discovery of new enzymes and proteins: galactocerebrosidase, galactocerebroside synthase, α -glucosidase stimulating protein, glycolipid transfer protein.

Improved ways of isolating known proteins (β-glucosidase activator).

Characterization of new and known proteins – properties, amino acid sequence of β-glucosidase activator, discovery of first fatty acid-linked protein.

Lipid-binding properties of proteins – myelin, myelin proteolipid protein.

5) Inhibitor studies -

Development of inhibitors acting against several sphingolipid enzymes.

Therapy of cancer – PDMP.

Blockage of pathogens to tissues.

6) Metabolic pathways -

Discovery of the enzymatic pathways for the synthesis of galactocerebroside and sphingo-myelin, clarification of the synthesis of ceramide (demonstration of the existence of several different ceramide synthases), demonstration of the elongation and shortening of sphingolipid fatty acids, discovery of the 1-carbon degradation pathway.

Discovered the first cat with Niemann-Pick disease.

7) Teaching -

I ran the medical student biochem course in Northwestern Univ., devising many new experiments.

I ran a section of the U-M undergraduate biochem lab course, devising many new experiments and revising old ones.

Wrote lab manual for experiments. illustrating the uses of radioisotopes in different fields of chemistry, printed by U.S. Printing Office.

Wrote didactic articles on isotope applications for *Nucleonics* and Nuclear-Chicago Corp.

Wrote many "small manuals" for local use – how to write papers, use of word processing programs, use of graph plotting programs, etc.

8) Public and University service -

Member of an NIH Study Section 4 years.

Member of a Mental Retardation Research and Training Committee (like a Study Section, with more site visiting) 4 years, for the Natl. Institute for Child Health and Human Development.

Advisory Board for the Delta Regional Primate Research Center. Saw a lot of monkeys in huge cages.

Divisional Board for Biological and Health Sciences, Rackham Graduate School, University of Michigan, reviewing U-M grant applications 2 years.

Editing boards for 4 journals and Executive Editor for *Anal. Biochem.* I saved many an author from publishing dumb claims or incomprehensible language.

Reviewer of grant applications and manuscripts for many journals. Ad hoc site visits and grant review sessions.

Directed the MHRI Training Grant for 18 years.

Contributed abstracts for *Chemical Abstracts* for 12 years.

Collected references on recent published articles describing lipid techniques for the *Journal of Lipid Research*.

9) Honors -

NIH Predoctoral Fellow.

Atomic Energy Commission Postdoctoral Fellow.

Michigan Quest for Technology Award.

U-M Senior Research Scientist Lectureship Award 1990.

I was among the first group of investigators to receive the Jacob Javits award for neuroscience research. The primary effect of the award was that it extended my current 5-year grant into 7 years.

Listed in *Who's Who in America* and *American Men and Women of Science.*

Addendum seven years and ~34 publications later (1997): Things have been going on as above but I am slowing down now. After open head surgery (resulting in loss of hearing and balance in my right side and damage to my right eye) and heart surgery, plus two hernia operations, my energy production rate is clearly slower. A significant fraction of my time now goes to medical checkups and body maintenance procedures. Also looking for things I just laid down somewhere. However I still followed the new literature with Reference Update (until a year ago) and distributed "finds" of relevance to glycolipids. Even now, I nag other researchers and try to talk them into doing experiments with my glucosyltransferase inhibitors, which are now commercially available. At the moment, I think three labs are actually responding to my suggestions, maybe more. I keep track of publications that mentioned or utilized the inhibitors; there are now at least 85 [over 160 now in 2000] so my hope that they will finally find practical use is growing.

One exciting discovery coming out of the inhibitor work, from Myles Cabot's lab, was the observation that drug-resistant tumor cells make a much greater amount of glucocerebroside than "wild-type" tumor cells. This is a crucial point since most people die from cancer when the tumor cells mutate to develop drug resistance. Myles showed that one of my inhibitors, as could be predicted, blocked this lipid accumulation and caused loss of the drug resistance. In another study, Richard Pagano found that a drug-resistant strain of tumor cells was much more sensitive to growth blockage by our first inhibitor, PDMP.

Stephan Ladisch has, for many years, been furnishing evidence that gangliosides are excreted by

cancer cells at a high rate and these glycolipids protect the cancer cells from immunorejection by the patient's immune system. (It is generally considered odd that cancer cells are not ordinarily antigenic and several papers from various labs have indicated that the tumors excrete some kind of protective material.) Ladisch has now shown that one of our inhibitors can block this excretion phenomenon (what else would you expect!?) and I am looking forward to direct proof that this blockage enables the body to generate immunological attack on the tumor in vivo.

Actually our first paper on PDMP with mouse cancer showed this but the proof was incomplete. We showed that the mice that had been cured could no longer be re-"infected" with new tumor cells, evidently because they were now immunized against them. If we had had the time and financial support, we would have examined the immunoglobulins in the cured mice.

In another direction, it looks as though the inhibitors should prove useful in fighting infections of all sorts. There are many papers showing that pathogens – microbial and viral – bind to the victim's cells via the glycolipids in the cells. Presumably they would not be able to proliferate and hurt the victim if the latter is depleted of glycolipids with our inhibitors, even after the infection has established itself. A paper from C. Svanborg's lab has shown this to be true for a pathogenic form of E. coli and human kidney and intestine cells, in vitro. If only someone would try this in vivo…

An unexpected twist to this story is the discovery from K. Haldar's lab that our inhibitors prevent malarial parasites from developing in red blood cells. The mechanism is via interference with the parasite's enzyme that makes sphingomyelin, not glucocerebroside.

So I can't stop thinking about science and watching the literature now!

Addendum April 2003 – I am now almost 83 but miraculously still alive and functioning scientifically. My body has revealed further signs of aging: prostate cancer (apparently cured by radiation and anti-testosterone drugs), glaucoma, a lens transplant, and an aortic aneurism (treated with a stent). Since the above update, my wife Norma has died of multiple myeloma – as did my mother many years before! We moved to California to try to get better therapy and got involved with an elderly doctor at Stanford Univ. Hospital who seemed to know about the disease. Unfortunately he refused to try any recently published treatment approach – "They are crazy," he said of some authors. Our son and family lived nearby, which was a pleasant plus in terms of helpfulness and cheer, but we should have moved to Los Angeles, where several research-oriented doctors treated myeloma patients. I was certain that it would be impossible to try treating Norma with one of my inhibitors, which I am certain would have helped greatly. I discovered that a non-physician cannot even request a blood analysis of his own blood! (In California, anyway.) Thus you cannot test your own drug. Norma died very unpleasantly, thanks to medical failure to act on her very low platelet count. When I asked about the blood analysis shortly before this, I was told that the values were normal. There were other important faults with the hospital.

During and after these bitterly disappointing events, I continued my study of the current scientific literature, aided greatly by the U of M Medical Library. This wonderful library sent (and continues to send) me abstracts of recent publications, and now allows me to download copies of many publications, so I manage to struggle along without easy access to paper copies. (This reminds me of the very different attitude at the Stanford Medical Library, which has a gate at the entrance and requires a bar code card for entry. When I moved to Menlo Park, CA, I wrote to the chairman of the Biochem. Dept. and requested a card, as well as notifications of coming seminars. The latter never arrived but eventually I was grudgingly accorded a pass card – for one year. Luckily, the librarian kept renewing my card

without waiting for reauthorization. What a contrast with the U-M, not to mention every other school I visited!

As the result of much free time to read and think about the field of sphingolipid medicine and biochemistry, I developed some interesting new ideas which I was able to publish, albeit with much resistance at first. These latest papers are:

196. Treating glucosphingolipid disorders by chemotherapy: Use of approved drugs and over-the-counter remedies. J. Inherited Metabolic Dis. 23:767-777, 2000.
197. Killing cancer cells by poly-drug elevation of ceramide levels: a hypothesis whose time has come? Eur. J. Biochem. 268:193-204, 2001.
198. Apoptotic death by ceramide: will the real killer please stand up? Med. Hypotheses 57:96-100, 2001.
199. The development of aggressive cancer - a possible role for sphingolipids. Cancer Invest. 20:779-786, 2002.
200. Cancer progression in the kidney and prostate: vital roles of sphingolipids in chemotherapy. Urology 60:562-568, 2002.
201. Designing anticancer drugs via the Achilles heel: ceramide, allylic ketones, and mitochondria. Bioorganic & Medicinal Chem. 11:1511-1529, 2003.
202. Killing tumors by ceramide-induced apoptosis: critique of available drugs, Biochem J. 371: 243-256, 2003.
203. Infections and glycolipids. [Letter to Editor] Postgrad. Med. J. 79:185, 2003.

No. 198 was the suggestion that ceramide produces cell death by undergoing oxidation in mitochondria to form a ketone. The product should be very reactive and should produce new ceramide molecules, like the nuclear reactions in an atomic bomb. In 2007, H. Azuma confirmed my guess.

No. 199 is a detailed hypothesis that explains the dangerous aspects of cancer. I am particularly pleased with No. 201, which grew out of No. 196 and examination of the structures of anticancer drugs. If it is taken seriously, many new anticancer drugs should become available. No. 202 is a list of drugs that – **on a rational basis** – should be used in cancer therapy. No. 203 explains findings reported with a Gaucher disease patient who developed a serious infection.

I am very pleased with the recent advances in sphingolipid research, which hold great promise for treating a wide variety of disorders. More and more researchers have been entering the field, discovering an unexpectedly wide range of biological effects. Over 300 papers have been published in which our drugs have been used. This research owes much to the vision of Michael Seidel, the venturesome owner of a small chemical company, Matreya, Inc. He undertook to offer some of our drugs for sale to researchers, eliminating the hassle of my supplying the materials. This gamble paid off for him financially, and allowed many experimenters to elucidate the roles of the glucosphingolipids in living cells.

A large pharmaceutical company, Genzyme, has purchased rights to the seven or so patents that Jim Shayman and I applied for, covering uses of our drugs in the PDMP "family." They have been making good progress in evaluating one of them and have been sending the U-M checks as each evaluation milestone was achieved. It is lucky for everyone that Jim successfully fought to finance the patenting process, since the U-M Patent Office (now the Technology Management Office) refused to pay the expense. The "vision thing" has to be defended by people with genuine vision. Fortunately, the

chairman of Jim's department (Internal Medicine) had the vision thing and put up much of the required money. They are being repaid many-fold for this venturesome attitude. My primary affiliation, the Mental Health Research Institute, financed my first patent (which I owe to Bernie Agranoff's faith in me), and that money has also been substantially repaid.

I can't help complaining about the low caliber of patent lawyers, who made me waste much time trying to educate them and hold on to the novel features of the ideas. Even worse was the patent examiner of the U. S. Patent Office, who willfully discarded claims and made petty demands on us in order to increase the fees earned by the Office. I will be pleasantly surprised if no competitor infringes upon the gravely weakened patents that we were eventually awarded.

Addendum Sept. 2009 – I am now over 89 but miraculously still alive and have made a little more progress. Here are the latest papers I thrust onto the incredulous scientific world:

204. Poly-drug cancer therapy based on ceramide. Eksp. Onkol. 26:3-10, 2004. An invited article in a Russian journal (in English).

205. Sphingolipids as coenzymes in anion transfer and tumor death. Bioorg. Med. Chem. 2004, 12:6029-6037.

206. Preventing the binding of pathogens to the host by controlling sphingolipid metabolism. Microbes Infect. 8:938-945, 2006.

207. Meta-analysis of anticancer drug structures – significance of their polar allylic moieties. Anti-Cancer Agents in Medicinal Chemistry 7:209-222, 2007.

208. Allylic structures in body and cancer drugs that control cell life and death. Expert Opinion on Drug Discovery 2:1-13, 2007. Invited article.

209. Drug design: hiding in full view. Drug Develop. Res. 69:15-25, 2008.

No. 204 presents the not-entirely original idea that cancer therapy requires a cocktail of drugs that attack as many as possible of the enzymes processes that produce ceramide, while blocking the formation of ceramide products that *stimulate* cancer growth. It has become clear from recent discoveries that ceramide and its products are engaged in a constant rebalancing act to control the growth of cells, especially cancer cells.

No. 205 is a radical concept which, if correct, explains how sphingolipids produce the large number of biological effects being uncovered. I am waiting for scientists to confirm my theory. The editor of the journal liked it so much that he featured it on the cover page. The basic idea is that sphingolipids attach to enzymes and serve as temp-orary reactants with acidic substances. For example, a protein phosphate ester forms a diester in which the second alcohol group is the allylic hydroxyl group of the sphingolipid (mainly ceramide). This phosphate diester is then hydrolyzed so that the protein loses its phosphate and the sphingolipid now is a phosphate ester. The latter is then hydrolyzed to reform the original lipid.

No. 206 is an update of an idea published by Karlsson over 20 years ago. He pointed out that microbes and their toxins bind to the glucosphingolipids on our cell surfaces, then they enter the cells to do their dirty work. I listed many of the recent findings that support the idea and listed ways to counteract the binding – including the use of my P-drugs.

Nos. 207 and 208 are updates of my allylic hypothesis that had been proposed in paper 201. I discerned a number of fine points about the use of allylic groups in cancer drugs, which ought to help in designing improved drugs. I am pretty pleased with the papers and hope they will make a dent.

No. 209 extends my allylic hypothesis to other drugs besides anticancer drugs and lists a lot more

examples. I went to great pains to explain how to recognize the presence of an allylic group in a molecule and give examples of the allylic hypothesis that were recognized (and proved) by earlier writers who failed to recognize the generality of the concept. This article has been somewhat rewritten after publication, and amplified with more examples; this is the version I distribute to other researchers.

I found that writing the last three papers was very difficult, partly because they incorporated a huge number of findings, partly because I found my concentration of thought more difficult to maintain. I would start to look up something, then get distracted by some other thought, forgetting the original goal. Also my eyesight has been deteriorating, making it difficult to read or hit the correct keyboard key. Moreover, the reviewer assigned to my final manuscript was an unusually ignorant scientist who was especially irritating. The field of sphingolipids seems especially prone to criticism by people who are pretty ignorant – I call this sphignorance – inexplicable ignorance about all things sphingolipoid. I have suffered enough from such people and have decided to quit the whole writing business.

This is a shame because there is a need for more teaching about the roles of sphingolipids in life. It looks as though they are important in aging and it should be possible to slow aging by controlling sphingolipid metabolism. I have over 200 pages of thoughts and references on the subject. PubMed, the huge collection of published bio-science articles at the National Library of Medicine, lists 28,200 articles. My guess is that aging is due to too rapid a formation of ceramide and inadequate attachment of glucose to make glucosylceramide. This seems to cause degeneration and fatigue, major aging problems. The trick for staying younger is reducing ceramide formation, which could be done with low doses of L-cycloserine and N-acetylcysteine (the latter slows hydrolysis of sphingomyelin to form ceramide).

Florence is annoyed that I didn't really quit and start a normal life. We spend much time in the mechanics of our senior life style, maintaining health, visiting doctors, keeping track of medicines and the Medicare-imposed book-keeping, etc. The good part is that I don't have to worry about money, the result of the University of Michigan's generosity and perspicacity of TIAA/CREF, plus Norma's hard work and earnings, and Bernie Agranoff's appreciation of my work.

My hopes that my P-drug research would be enabled by Genzyme Corp. look limited beyond Gaucher disease. My visit to their staff was very unpromising and I have to hope that other scientists will carry the P-drugs further. In 2009, Genzyme started a Phase III test, which may result in medical use at last in 2012. Apparently the patients are responding well to the drug.

Around the end of 2010, Genzyme had been stuck for some time with a viral contamination of their fermentation process for making human GlcCer glucosidase, so they had to use our P-drug to extend the remaining enzyme. Some companies have already begun selling the generic version of the enzyme in Europe so Genzyme is now facing serious competition. One would expect them to speed the testing of Eliglustat, but there is a scarcity of Gaucher patients! The expected rapid approval of the orphan disease drug has proved to be an illusion. I am not the only one upset by the poor performance of this company. The Icahn group of investors almost kicked out the people running Genzyme, Perhaps because of their influenced the latter agreed to sell out to a large French pharmaceutical company, Sanofi-Aventis. I may have helped the sale by writing to the head of the French company, telling him of the gold mine in Eliglustat hidden in our patents for cancer and infection therapy. He responded favorably and raised the offered price, which clinched the deal.

Mentime, Jim Shayman has demonstrated other benefits of Eliglustat and a homolog that penetrates into the brain more rapidly. The latter should help those children whose genetic error attacks them while

in the mother.