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A "PURE" ORGANIC CHEMIST'S DOWNWARD PATH

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> We attribute to "pure chance" (automaton) all those events which are such as ordinarily admit to a telic explanation, but which happened on this occasion to have been produced without any reference to the actual result. The word "luck" (tyche), on the other hand, is restricted to that special type of chance events which (1) are possible objects of choice, and (2) affect persons capable of exercising choice.
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> Aristotle, as translated by Wheelwright (22)

I begin with this appearance of profundity because chance and luck have been frequent determinants along my way. And I add obstinacy, because I was known as an obstinate child and, having made up my mind at the age of eight that I wanted to be a chemist, though without knowing why, I stuck to it and became one.

I was born on East 127th Street in New York City on April 29, 1888. My grandparents were Jewish Germans who emigrated between 1840 and 1850, apparently because of the greater opportunities in the United States. I know little of my father's parents, except that his father, Michael, died in Silver City, Idaho. David, my father, was born in Philadelphia and left school early to earn a living. He became a partner in a firm that made carriage robes, but with the advent of automobiles, he went "on the road" almost six months of the year selling lace curtains for another manufacturer. My maternal grandparents settled in Norfolk, Virginia, and sent their oldest daughter, Fanny, my mother, to a young lady's finishing school, where she learned to play the piano. They also shipped her off to relatives in Nürnberg for a year. She and my father were married in 1884. Their first child died before I was born and I was followed in two years by a brother, Charles. My parents were devoted to each other, but the necessarily long absences of my father gave my mother much responsibility and resulted in a strictness that she herself characterized as "Spartan."

As a consequence of her year abroad, Charles and I were required to speak German at the table and were accompanied to Central Park two afternoons a week by a governess who would tolerate nothing but French. We hated both burdens, and it was only years later, when these languages became essential, that I realized what a head start I had had.

Charles and I wandered in safety all over Central Park and knew several of its policemen by name. We ranged over many more distant parts of the city with the help of one or two friendly drivers of delivery wagons, this without the knowledge of our parents. We also gravitated often to the Metropolitan Museum of Art and the Museum of Natural History. All of these excursions were possible because my mother taught us all the primary school subjects in an hour or two each day, leaving the remainder for reading (I read *David Copperfield* and was taken to opera and concerts when I was eight years old), wanderings such as those above, and playing in the street with other children just out of school.

Later, when one of my teachers at public school, a Mr. Curtis, was invited to dinner, he was told that I wanted to become a chemist. "Buy him Cooley's 'Physics,' " he said, "he ought to know physics before chemistry, and if that doesn't discourage him, maybe he will really go on to be a chemist." I skimmed rather superficially through the book and was not frightened off. The real introduction to science followed in the eighth grade at the Workingman's School of the Ethical Culture Society, with a beautiful course in botany by Dr. Henry A. Kelly, followed in the Ethical Culture High School by his tour through zoology and well-taught courses in physics and chemistry by William E. Stark, with many hours in the laboratory. These led to my first research, an unpublished venture with Mr. Stark. Late in 1904, the Seventh Avenue-Broadway subway was about to open and the newspapers contained dire predictions of mass suffocation in the soon-to-be crowded trains. During the Christmas holiday, we lugged a five-gallon demijohn of water about a quarter of a mile to the nearest subway platform, emptied it there, replacing the water with air, and bore the stoppered demijohn back in triumph to the laboratory. There we exploded a small portion of the air with hydrogen in a eudiometer. Something went wrong, however, and we found only five percent of oxygen!

At the Ethical, I also learned to write good English from Percival Chubb and was taught higher mathematics, even trigonometry, by Matilda Auerbach, a superb but very strict teacher. As a freshman at Columbia, I started with an exciting course in qualitative chemical analysis, given for the first time according to the ionic theory by a young instructor, Hal T. Beans, against the wishes of the old-fashioned head of the department, a Professor Wells. He had Beans brought up on charges before the president, Nicholas Murray Butler, but Butler said Beans was right to teach in the most modern way possible. Another memorable course was Charles F. Chandler's Industrial Chemistry, which consisted mainly of personal anecdotes ranging far and wide. Quantitative analysis, the next year, with Floyd J. Metzger, led eventually to an offer of a fellowship if I would go on to a Ph.D. with him, but by that time I had tasted the joys of organic chemistry and decided on that for my doctoral work. I did get a master's degree, with Metzger, and we published two small papers together (17, 18). The second was rejected by W. A. Noyes, Senior, at that time the

peppery editor and sole referee of the *Journal of the American Chemical Society,* on the ground that we did not know how to standardize potassium permanganate. Metzger, who had been watching me like a hawk, sent the paper back, saying that if Noyes didn't take it he would send it to the *Zeitschrift für analytische Chemie* with a note explaining why. Noyes took the paper.

Organic chemistry was taught by Marston Taylor Bogert and John M. Nelson (affectionately called Pop Nelson), both excellent teachers. After the preliminaries, Bogert would write two unrelated compounds on the board and ask you to synthesize one from the other. All around the room, a handful of us at each session would be racking our brains at the blackboard, while the rest of the class looked on in grim amusement, knowing their turn would come, too. I became interested in organophosphorus compounds as a subject for my thesis and Bogert wisely let me have a fling at the ones in the chemical museum. However, they were difficult to handle and sometimes caught fire in the air, and before long I was ready to join the other grads and work on quinazolines, with which one could figure out everything on paper and all compounds were nicely crystalline. An earlier student had made a quinazolone phthalone as a brown powder with hopefully useful properties as a yellow dye, and this topic was assigned to me. The more I purified the phthalone, the paler it became, so I added bromine to a derivative of it, hoping to intensify the color. Uncooperatively, the last traces of color disappeared. This intrigued me, and with Nelson's coaching I found that oxidative splitting had occurred (1). But this took time and I obstinately stuck to it, and at the end of two years I had only twenty new compounds instead of the forty traditionally required. Incidentally, one of my fellow students had his forty new compounds but was never able to do a C and H combustion correctly, also a requirement. Both of us were finally recommended for the Ph.D. degree, and guess to what eminence my colleague rose: a Professorship of Organic Analysis at a large, well-known university!

As for me, the question was "what next?" A part-time, older, German-American laboratory instructor named Hoffman had often pointed out the deficiencies of chemical, especially organic chemical, teaching and insisted that at least a year in a European laboratory under one of the recognized masters was a prerequisite to a successful career. My parents were willing to stake me to this, and I was selfish enough to accept what I knew was a sacrifice. But first they wished me to seek advice from old Dr. Samuel Meltzer, who had listened with his massive head against my chest as our family physician throughout my boyhood and had then become the distinguished head of Physiology at the Rockefeller Institute for Medical Research. He tried, wisely, I think, to discourage me, saying a scientific career was nothing for a poor man's son, but he gave it up and turned me over to the chemists, who would know the best person under whose direction to work. Thus, I had the luck to meet P. A. Levene, Walter A. Jacobs, and D. D. Van Slyke at tea that same afternoon. I had thought of going to Emil Fischer, with whom all three had worked, but they said he was aging and assigning his students to his assistants. On the other hand, Richard Willstätter was much younger, very able, and doing outstanding research on alkaloids and chlorophyll. Again, luck was with me, for Willstätter was willing to have me come.

That September, 1911, I boarded the French cabin liner "Chicago" (off-season rate, $50, for an outside cabin to myself, good food, and 11 days to Le Havre) and soon found myself fighting hard not to fall in love, for I had been brought up with the now strange notion that that was taboo until one could support a wife. My struggle was a result of a remarkable, attractive, and intelligent girl on her way to the Sorbonne for a Ph.D. (which she won) and who could read a whole page at a glance. Anyhow, we enjoyed being together and even renewed our friendship forty years later when we were both living alone and she was Professor of English Literature at a leading women's college. But I did fall in love with Paris during a week with as few hours as possible in a little back room on the top floor of a small, still existent hotel. Shortly after, Willstätter received me kindly and hopefully at the Federal Polytechnic Institute in Zurich. I became one of about twenty students to whose laboratory tables the Professor came twice a day. His first assistant was Arthur Stoll, who became the guiding spirit of the Sandoz Company of Basel, and his second assistant was Laszlò Zechmeister, with whom an abiding friendship developed and who came to the United States many years later as Professor of Organic Chemistry at the California Institute of Technology. I wanted to work on chlorophyll, but Willstätter said that would take two years—one to learn methods and another to penetrate further into its structure. But he had a one-year problem, a study of cycloöctatetraene, which had been synthesized in the laboratory by Ernst Waser, who then left for a job in industry. It would be an expensive problem, starting with a rare alkaloid, pseudopelletierine, and requiring much silver nitrate to remove nitrogen atoms by the Hofmann degradation (24). One had to pay for all materials, and when I doubted that my father, who was footing the bill, could afford it, Willstätter proposed that he and I should alternate in buying, whereupon he presented me with 500 grams of alkaloid and saw to it that it was always his turn when we had to buy silver nitrate and my turn for acetic acid or the like. I started with a practice degradation of a less-valuable alkaloidal derivative, and while I was watching the steam distillation, the mixture suddenly crystallized but fortunately remained fluid. Watching this unexpected happening intently, I did not hear Willstätter come up behind me. Suddenly, an arm with a pointing finger shot past my head and a voice hissed in my ear: "Was-s-s is-s-st das-s-s?" I was so startled I could not answer, and only later did I realize that sodium hydroxide had crystallized temporarily from the hot alcoholic solution. I am sure this made Willstätter realize that his new American student was very inexperienced in the handling of the unexpected or of a difficult problem, but that was precisely why I had come to him. Cycloöctatetraene, as made from the alkaloid, had the unpleasant habit of throwing bridges across the 8-carbon span, so that in addition to the final steps of preparation one had to do all necessary experiments with the product as rapidly as possible. This meant starting at 8 AM and working steadily until 3 the next morning. Not only did all details have to be thought out and all apparatus have to be readied for the appointed time, but there were difficulties unknown to the modern research worker. I mention one: the only way to obtain solid CO_2 was to invert a tank of the compressed gas on a diagonal wooden frame and blow the gas into a leather bag. Yields were low, and around midnight it was necessary to cross the street to the

convenient pub, Café Friedegg (still there), and shoulder one of the tanks supplying the beer pumps. I had three of these nineteen-hour workdays, and the next day I was fit only for cleaning up.

There were also diversions. I played clarinet in the student orchestra, and in the middle of the overlong annual concert we played the Mozart quintet—the only time I have had a musical criticism in a daily paper, the "Züri Zietig," as the Neue Zürcher Zeitung was popularly called. Then there were fine operas and also concerts, for which one could borrow a regular student's card and be admitted for almost nothing. At these I heard Fritz Kreisler and Percy Grainger for the first time, and even the clarinetist, Mühlfeld, for whom Brahms wrote so superbly. I also had long Sunday walks with Hugh Clark, my English neighbor in the lab, a ten-day memorable trip at Easter to Italy, and, at the end of the academic year, the Professor's excursion up the lake to a fine restaurant, where, after dinner, I played a few selections and Zechmeister, with great seriousness, read a beautiful paper on "hippopotamuric acid."

Through all this I was worried about the future, and though offered an assistantship by Professor Eugen Bamberger at the Polytechnic, I wrote letters to a score of universities in the United States, proffering my services as instructor in organic chemistry. Not only was I turned down by all but the very last, but most of the answers bore only two cents postage instead of the then required five. The Swiss imposed a double penalty, so that being refused was expensive as well as discouraging. In the end, I received an appointment at the University of Illinois. After writing up the year's work with Willstätter for publication (23) (I asked him for a letter of recommendation and he said "Shall I write it the way I think, or the way you wish?"), I started for home, intending to see something of Germany and England on the way.

At Nürnberg, where I stayed a few days with relatives of my mother, a cable came from my father stating that I could have a job at the Rockefeller Institute if I returned at once and was approved by its director, Dr. Simon Flexner. As I had liked the Institute's chemists who had given me such good advice, I took an upper berth, second class, on the steamer that could get me back the fastest, the "Provence." Here, pure chance, as defined by Aristotle, was operative, or was it luck, since it aided in the choice? Getting into a compartment at random on the boat train from Paris to Le Havre, I found myself in the company of two professors from the University of Illinois and their wives, pleasant and interesting people, but with a lack of enthusiasm for their university that made me all the more inclined to accept the appointment at Rockefeller if I passed muster. This position was to assist Dr. W. A. Jacobs in synthesizing drugs for the cure of poliomyelitis, as part of the study of the disease by Dr. Flexner himself, and I started in September, 1912, as a Fellow of the Institute at a salary of $1200 a year.

One of the first things my father insisted upon after my return was for me to add up my expenses. I wondered at that, for he was not interested in details not mentioned in my letters, only in the total. I soon found out, for when I had calculated it as somewhat over $800, he immediately wrote out a check to my brother for the same amount. Alas, this benefitted Charles very little, for a sequel to an earlier attack

of rheumatic fever, endocarditis, caused his death the following spring. Early in his illness, the Health Department of the City of New York immunized a horse with the strain of *Streptococcus viridans* isolated from Charles' blood. This first contact with immunology was not encouraging, for large injections of the horse's serum failed to help.

The Rockefeller Institute was rather small in 1912, with two buildings containing laboratories and another for the hospital. There were a few tables in a small lunchroom, a very necessary adjunct, as the nearest eating places were quite distant. Jacobs proved to be a very shy, kindly person and a keen organic chemist with original ideas. He made it clear that I was an essential part of the laboratory and that my name would be on every paper published, as my work would give him time for additional syntheses. He also taught me, when, for example, a reaction had to be refluxed for several hours, to start something else so that I could carry forward a number of syntheses simultaneously, keep working steadily, and not get things mixed up, all by his own example. He and his wife, Laura, were very hospitable and I was often at their home in Mt. Vernon, where Walter played the pianola very temperamentally. He had perforated rolls of all of Beethoven's piano works and of arrangements of the symphonies for the piano. At first we went to the lunch room of the Institute together, but as he always led me to the table at which the other chemists sat, I soon realized that I was missing much of the life of the Institute. After a month or so of this, I plucked up enough courage to ask if he would mind if I went to lunch at my own convenience. He did not, and then I began to meet men such as Jacques Loeb, Peyton Rous, Alexis Carrel, Hideyo Noguchi, and John Auer, all of whom would linger at the table and talk with young, unknown beginners like myself. I became an authority on Jacobs, often being asked questions about him, as he was scarcely known to the rest of the Institute because of his shyness and his habit of always lunching with the chemists.

For the chemotherapy of polio, Jacobs chose as his lead the slight therapeutic effect of hexamethylenetetramine, several quaternary salts of which had been made. He foresaw that a large number of such salts could be prepared from the almost limitless choice of chloro, bromo, and iodo compounds, both aliphatic and aromatic. We bought what we could, synthesized large amounts of others, and combined them with hexamethylenetetramine (11, 11a, 14, 15). Some of the new salts were highly bactericidal. One or two even seemed to delay the death of monkeys infected with the virus, but this was found to be the result of a loss of virulence of the virus itself.

At this juncture, with Dr. Flexner's encouragement, we turned to African sleeping sickness, or trypanosomiasis, which was making whole regions of that continent uninhabitable. Atoxyl, para-acetaminophenyl-arsonate, widely used by Thomas in its treatment (20), was dangerously toxic and not very effective. As Jacobs and I began to synthesize new and hopefully better arsenicals, we were joined by Drs. Wade H. Brown and Louise Pearce, who were to test our compounds for efficacy against trypanosomes in animals. Paul Ehrlich of Frankfort, the great pioneer of modern chemotherapy, had started on the same quest but it had led to "606" or Salvarsan, a cure for syphilis, instead. His most active synthetic against

trypanosomiasis was the trivalent, highly toxic, and therefore unusable para-arsenophenylglycine. Jacobs thought that the inactivity against trypanosomes of the less poisonous pentavalent para-phenylglycine arsonic acid might be a result of its highly reactive carboxyl group, which could combine with many components of tissues rather than with the parasites. He proposed masking the –COOH by converting it into $-CONH_2$, the amide, a grouping common to tissues, and this substance, sodium para-phenylglycinamide arsonate, our first and simplest arsenical, was more active than the many others that we synthesized in attempts to improve upon it. Dr. Flexner named it "Tryparsamide" and it was patented for purposes of control (10). A large batch was prepared by Powers, Weightman, and Rosengarten, a Philadelphia pharmaceutical concern, and when World War I was over, Louise Pearce conducted a field test in the Belgian Congo. She found Tryparsamide to be superior to the previously used drugs, and tests in this country showed it to be useful in the treatment of tertiary syphilis as well.

The value of Tryparsamide was eventually recognized by the Belgian government by bestowing upon us (and Thomas, who had introduced Atoxyl) the Order of Leopold II and monetary awards. Louise Pearce was given the lion's share deservedly, for she had risked her life and handled the field tests beautifully. I was angry, though, that Brown, who had merely tested our drugs on animals, received twice as much money as Jacobs, whose brilliant idea led to Tryparsamide, while I, the lowest in the group, was given as much as Jacobs. I immediately protested to the Belgian consul general and to the ambassador, but they claimed that nothing could be done after the king had signed the decree. Walter never complained, but my blood pressure still goes up as I write this.

Supplies of Salvarsan were very meager after the onset of World War I, and such difficulties were encountered in attempts to manufacture it here that Ehrlich was accused of deliberately omitting some essential step in his application for a patent. This was not true, however, for Jacobs and I, who were used to handling arsenicals, had no trouble in making the substance. By this time, I was a 1st lieutenant in the Sanitary Corps of the Army, assigned to U.S. Laboratory No. 1, the Rockefeller Institute, much of which was given over to training Army physicians in laboratory techniques. Jacobs and I thought we could improve upon Salvarsan and actually synthesized an analogue that was at least as active and less toxic. After exhaustive tests in animals by Brown and Pearce, it was tried with good results on about 100 human cases of syphilis. However, a second batch inexplicably resulted in several dangerous cases of dermatitis and we withdrew the drug.

In the early summer of 1915, when it appeared that we might soon be at war, I enrolled in a six-week officer's training course at Plattsburg, New York, to learn something of military life and soon found out how easy it was to acquire the art of killing. After that, armed with a Marksman's button and a commission as 1st lieutenant in a volunteer army that was quickly found to be utterly useless, I went cross-country to a summer camp at Center Lovell, on Lake Kezar in Maine, where I had my own canoe. The first evening, while I was playing Pergolese's "Nina" with my friend Stanley Ries at the piano, the door opened and a startlingly lovely girl

walked in. Stanley stopped playing and said, "Meet Nina!" Would Aristotle have called this "chance" or "luck?" Companionship with Nina Tachau was a joy, and the following June we were married. Our son, Charles, was born in 1920; his mother died of cancer in 1946: we had been comrades for 31 years and had helped each other in our respective careers, for Nina was a writer and later became a frequent speaker for the American Association for the United Nations and chairman on foreign policy for the New York City League of Women Voters. While on this personal note, I might as well tell of living alone for the next nine years, bouyed by the excitement of the laboratory, the encouragement of warm friends, and visits to my son and his growing family. A good friend, Nellie Doogan, who had been in the family for years, came in daily to cook breakfast and clean; another, Anna Greene, cooked dinners two or three times a week. Then, one evening, I was invited to play the Mozart trio for clarinet, viola, and piano by Vally Weigl, widow of Karl Weigl, the composer. The violist was Charlotte Rosen, an outstanding musician and a cheerful, outgiving woman who had been a concert violinist in her native Germany and whose husband, a dermatologist, had died several years before. Though she lived in the same apartment house I did, I had never seen her, as she went to the second floor via the stairs while I used the elevator to the tenth. We walked home together, and again chance or luck and music brought companionship that ripened into marriage. With two happy marriages in one lifetime, I count myself among the most fortunate.

One of my duties as 1st Lieutenant at U.S. Laboratory No. 1 in 1917–18 was to march the visiting medical captains, majors, and lieutenant-colonels up and down while teaching them the Sanitary Detachment Drill, which I had learned at Plattsburg. This was also the year of a severe epidemic of influenza, at that time thought to be caused by an influenza bacillus. Dr. Martha Wollstein, of the Institute, prepared a vaccine from this bacillus and I persuaded all of my close relatives to receive injections of it. None of us caught the viral disease! Chance, once more!

At the close of the war, Jacobs and I decided that we had had enough of pure synthetic organic chemistry, but Dr. Flexner, an ardent believer in chemotherapy, insisted that we tackle bacterial infections, notably pneumococcal and streptococcal diseases. Lloyd D. Felton joined us for the testing in animals, and we started to synthesize increasingly active bactericides, including a number of cinchona alkaloidal derivatives (8–8b) more potent in vitro than Optochin (12), which had been used with some success against local pneumococcal infections. However, the combination "drug and bug" usually killed the test mice more quickly than the drug alone. One of the intermediates that we converted into such useless substances was para-aminophenyl sulphonamide, or Sulfanilamide, which the Tréfouels, Nitti & Bovet (21) found to be the active portion of the purple dye for which Domagk received the Nobel Prize in 1939. That so simple a substance could cure bacterial infections by a mechanism other than direct killing of the microorganisms never occurred to us. If it had, we might have saved hundreds of thousands of lives in the twenty years before Domagk, the Tréfouels, Nitti, and Bovet made their discoveries. I always told this story in lecturing on chemotherapy in the course on biochemistry at the College of Physicians and Surgeons of Columbia University and begged the students never to allow themselves to become slaves of an idea.

After nine and a half years of chemotherapy, Walter and I were still good friends but were eager to go on to something else, and, we agreed, independently. Dr. Flexner finally consented to our dropping out of chemotherapy, Felton went to the U.S. Public Health Service, and Walter went ahead with structural studies on cardiac-active glycosides, which we had started together (13), and related alkaloids. I wanted to find the active principles of several ancient Chinese drugs that had attracted notice in Western medicine, but Dr. Flexner did not consider that worth equipping a new laboratory and sent me over to Van Slyke, who was then chemist to the hospital of the Institute, to learn some biochemistry "so we can find you a job more easily somewhere else."

The hospital, devoted to research on a limited number of diseases and to the care and treatment of patients with these diseases, was directed by Dr. Rufus Cole, who also headed the team studying pneumonia. Van Slyke had a group actively probing the functions of the kidneys, and in 1921, when I started work with him, he had just begun a study of the equilibrium between oxygen and hemoglobin with Dr. A. Baird Hastings. They were having trouble obtaining enough purified oxyhemoglobin with intact oxygen-carrying power for their tonometric experiments. It devolved upon me to eliminate the trouble, and as this was primarily an organic chemical problem, I was soon making many grams at a time of crystalline equine oxyhemoglobin with virtually 100% oxygen-carrying power (4). This involved keeping the materials at low temperatures, so we put a No. 2 International centrifuge into a cold room. Eventually, my laboratory helper was threatened with a nervous breakdown as a result of the many colds caught from the necessary sudden changes in his working temperatures. Accordingly, I asked the president of the centrifuge company, then a frequent visitor to laboratories using his centrifuges, to build me one with an insulated brine coil around it, as the hospital had circulating brine to cool the banks of refrigerators and the cold rooms. The new instrument fulfilled its purpose and, thus, was the first refrigerated centrifuge invented and operated. Many of the visitors to the laboratory immediately ordered copies, as the wonder was how such a machine, so essential to the development of modern biochemistry, microbiology, and, indeed, molecular biology, had not been devised earlier. Thousands of refined, improved models are now in use throughout the world—my financial profit was $50 for writing the International Company's first descriptive booklet.

During the two years that I was making oxyhemoglobin and learning biochemistry, several more instances of good luck occurred. Dr. Karl Landsteiner, the famous Austrian pathologist and immunologist, became a Member of the Rockefeller Institute, and as he was interested in the immunological properties of hemoglobin, we were soon collaborating (9, 16) and I was learning my first immunological techniques from a great master. Then, Dr. Walter W. Palmer, head of the Department of Medicine at the Presbyterian Hospital, spent some months working with Van Slyke. Acquaintance with him was later to prove of crucial importance to me. And last, but not least, Dr. Oswald T. Avery, microbiologist of the pneumonia team, would come in from time to time with a small vial of brownish powder to say, "When can you work on this, Michael? The whole secret of bacterial specificity is in this vial." Eventually, the need for oxyhemoglobin ended and I was transferred

to the pneumonia group, although "Van" generously let me continue to use the same laboratory. Thus began my career as a microbiologist.

In 1917, Dochez & Avery had published their discovery of the serologically type-specific "soluble specific substance" of pneumococcus (3), and five years had elapsed before a chemist undertook its characterization. In the meantime, Dochez had left the Institute, leaving Avery, or "the Fess," as he was affectionately called, to carry the study forward. At that time, there were only three "fixed types" of Pneumococcus, type I, Neufeld's "typical Pneumococcus," and types II and III, all other serological variants having been relegated to "group IV." Fess said we had better not start with type III, which formed the largest capsules, because some people called it *Streptococcus mucosus,* nor was type I a favorable prospect because its capsules were very small. That left only type II with its capsules of intermediate size. I needed type II antiserum with which to identify serologically active fractions, so Fess took me down to the cold room where there were shelves of large bottles of therapeutically used, type-specific horse sera. I spied a bottle of type II serum half filled with mold and, to Avery's horror, took it along for the chemical tests, sterility being unnecessary for rapid, qualitative precipitin tests. Another innovation that he was dubious about was centrifuging weak precipitin tests that did not settle rapidly by themselves, but he was soon convinced by a series of adequate control tubes.

In our initial studies, we concentrated batches of meat-infusion broth cultures on a large steam bath in a hood until we had accumulated the concentrate from 300 liters. Precipitation with alcohol and centrifugation gave a three-layer separation, with the active material in the gummy middle layer. The more we purified this, the less nitrogen it contained, which surprised us, as all immunologically active substances were supposed to be proteins. Finally, when it was virtually nitrogen free, Fess said, "Could it be a carbohydrate?" So I boiled a bit with acid and got a strong test for reducing sugars, one of which was shown to be glucose (5, 5a). An acidic component was recognized, but its identification as D-glucuronic acid and the finding of L-rhamnose were accomplished much later (2, 19). One must remember that our work was done before the introduction of paper chromatography, when one had to isolate the sugar itself or a characteristic derivative and the relatively large amounts of purified material required were not easy to obtain. The conclusive test that we had the "soluble specific substance" was to precipitate some with two liters of outdated antiserum and recover essentially the same polysaccharide from the washed, proteolytically digested precipitate. To test the resistance of the type II substance (SII) to carbohydrate-splitting enzymes, Fess suggested that we write to Peterson and Fred in Wisconsin for some of their thermophilic cellulose-splitting bacteria. These microbiologists obligingly sent us a culture, and we seeded it into a slurry of filter-paper pulp made up in a 1:20,000 nutrient broth solution of SII and put it into an incubator at 55°C to stew overnight. Next morning the filter paper was gone, but the culture was still 1:20,000 as to SII!

As soon as we started to purify our first batch of the "soluble specific substance" of type III, I knew it would be different chemically from SII, as it formed dense fibers when precipitated by alcohol. Moreover, SII was dextrorotatory whereas SIII was

levorotatory. Both were almost nitrogen free and contained glucose, but obviously in a different combination. SII was a weaker acid than SIII.

From the products of hydrolysis of the latter, Dr. Walther F. Goebel, who had joined us, and I isolated a glucuronoglucose, which we termed an "aldobionic acid" (7, 7a) instead of the more correct "aldobiouronic acid." Such acidic bioses were later found to occur in enormous tonnage in the hemicelluloses and gummy exudates of trees and plants, but the first discovery of this combination of sugars was in the microscopic capsule of Pneumococcus type III.

Only after isolating SII and SIII did we turn our attention to type I, which immediately confirmed Fess's wisdom and unerring intuition in not working on it first, as its number, I, would have suggested. Not only were the yields small, but SI turned out to be an amphoteric polyelectrolyte and I threw the first batch down the sink, thinking it was nucleoprotein, while Zinsser and Mueller actually recorded their analogous "residue antigen" from type I as a polypeptide. It soon developed, however, that SI contained a large proportion of galacturonic acid, defining it as a third immunologically active, chemically different, type-specific polysaccharide (7b).

By this time, Avery's imagination, which always ranged far and wide, led him to the belief that if bacteria possessed immunologically reactive polysaccharides others should exist "free in nature." We accordingly bought the few plant gums then available, and lo!, gum arabic precipitated our type II antisera. While purifying a batch, I let it stand overnight in fairly strong hydrochloric acid. When I precipitated it next day with alcohol, pentose had been split off, exposing more chemical groups common to SII, so that the degraded substance reacted at a 150-fold higher titer than the native gum (6).

During all this period, I had to keep my eyes open for a job elsewhere, for although I was happy and satisfied, Dr. Flexner probably wisely insisted that as long as I stayed my work would be known as someone else's and that I should go elsewhere and stand on my own feet. Accordingly, when Dr. Samuel Bookman retired as Chemist to Mt. Sinai Hospital in New York after forty years of faithful service and the post was offered to me, I accepted it.

The year 1927–28 at Mt. Sinai was a very busy, interesting, and instructive one, with its close contact with medicine. It was necessary to reorganize the chemical laboratories, introduce new methods of analysis, and eliminate parallel determinations yielding the same information. This sometimes led to friction with the Medical Board. Although my associate, Dr. David J. Cohn, provided able assistance with the supervision of the vast amount of chemical routine, there was little sustained time for research. Accordingly, when Dr. Walter W. Palmer, Professor of Medicine at the College of Physicians and Surgeons of Columbia University, proposed that I join him as the first full-time chemist in a department of medicine, I was glad to accept. With me, from Mt. Sinai, went my technician, Check M. Soo Hoo, who was to serve ably and faithfully in that capacity for thirty-five years.

This brings me to the end of one epoch and to the start of others: twenty-seven years of ideal working conditions at P. and S. and "retirement," the story of which I hope to relate elsewhere.

Editors note: The story of Michael Heidelberger's career will be continued in the prefatory chapter of Volume 48 of the Annual Review of Biochemistry, scheduled for publication in July 1979.

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