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MICHAEL HEIDELBERGER AS A CARBOHYDRATE CHEMIST

Michael Heidelberger did not begin his career as a carbohydrate chemist; he was gently seduced into it by Oswald T. Avery. Michael was born in New York City on April 29, 1888, attended public elementary school, Ethical Culture High School, did his undergraduate and graduate studies at Columbia University, and received a Ph.D in 1911 in organic chemistry under Marston Taylor Bogert. After a post-doctoral year (1911–12) in Zürich with Richard Willstätter at the Eidgenössische Technische Hochschule working on cyclooctatetraene, he returned in the fall of 1912, to the Rockefeller Institute, now Rockefeller University, as a fellow and stayed until 1927, rising to Associate Member. Nine of these years were spent with Walter A. Jacobs synthesizing a host of compounds and searching in the Paul Ehrlich tradition for magic bullets; one, tryparsamide, proved enormously successful in the treatment of trypanosomiasis, African sleeping sickness.

In the early twenties, he did some work on cardiac glycosides, and then transferred to Donald D. Van Slyke's division and worked on gas and electrolyte equilibria in blood. In the course of these studies, he developed a method for the preparation of crystalline oxyhemoglobin and had his first introduction to immunology when he and Karl Landsteiner, in two classic papers, compared the immunological and solubility methods for distinguishing hemoglobins of different species.

While he was in Van Slyke's laboratory, Oswald T. Avery and Alphonse R. Dochez were studying type-specificity among pneumococci, had used antisera to recognize the first three types, I, II, and III, and had shown, as had Hans Zinsser and Julia T. Parker, that broth culture filtrates possess type-specificity. Avery would come into the laboratory from time to time carrying a vial of dark-brown broth concentrate and shake it in front of Michael saying: "The secret of bacterial specificity is in this vial here!"—Finally, he succeeded. In 1923, a paper by Heidelberger and Avery appeared in the *Journal of Experimental Medicine* [38 (1923) 73–80], entitled "The soluble specific substance of pneumococcus", followed by one by Avery and Heidelberger, pages 81–85 on "Immunological relationships of cell constituents of pneumococcus." The polysaccharidic nature of the pneumococcal type-specific capsular substance was established. From this moment, carbohydrate chemistry played a pivotal role in his research. Until leaving the Rockefeller Institute, he and Avery, having been joined by Walther F. Goebel, continued to collaborate in purifying and characterizing the pneumococcal polysaccharides as well as the Friedländer Type B polysaccharide (now *Klebsiella pneumoniae* type B) which cross-reacts with Type II anti-pneumococcal sera [*J. Biol. Chem.*, 74 (1927) 619–629]. He and Goebel isolated an aldobiouronic acid from the type III polysaccharide [*J. Biol. Chem.*, 74 (1927) 613–618] before vast amounts of similar substances were found in plant gums.

Leaving Rockefeller in 1927, he went to Mount Sinai Hospital for a year and began some work on the typhoid bacillus, whose type-specific antigens were not at all

like those of pneumococcus. In 1928, Walter W. Palmer became Professor and Chairman of the Department of Medicine at the College of Physicians and Surgeons of Columbia University and Director of the Medical Service at the Presbyterian Medical Center and brought Michael to Columbia as Associate Professor of Medicine. His later titles were Professor of Biochemistry and Professor of Immunochemistry. Michael was responsible for two lectures in the first-year biochemistry course for medical students, one on Chemotherapy and one on Immunochemistry.

Walter Palmer provided Michael with a hard money budget which in those days was quite unique. Edward S. Harkness had established the Harkness Research Fund; the income supported the Heidelberger group which consisted of himself, Forrest E. Kendall, a technician, Check M. SooHoo, a half-time employee to wash glassware, and a laboratory assistant. I was laboratory assistant from 1933–1937, became a graduate student in Biochemistry, and his first Ph.D. The National Tuberculosis Association also provided one position, which was filled by Arthur E. O. Menzel and later by Sulo Karjala, for studying the proteins and carbohydrates of the tubercle bacillus.

It was in this environment that quantitative immunochemistry was born. Taking advantage of the absence of nitrogen in the specific polysaccharide of type III pneumococci, Heidelberger and Kendall in 1929 published "A quantitative study of the precipitin reaction between Type III pneumococcus polysaccharide and purified homologous antibody" in *J. Exp. Med.*, 50 (1929) 809–823. This paper made several major contributions—it established the value of quantitative analytical methods in the study of immunological reactions, and showed that the precipitin reaction between a single substance and its homologous antibody could be treated as an ordinary chemical reaction albeit a complex one; moreover, by the use of the micro-Kjehldahl method for analyzing washed specific precipitates for nitrogen with a nitrogen-free polysaccharide antigen, it provided strong evidence for the protein nature of antibodies, a question that was then very controversial. It also recognized the zones of the precipitin reaction, antibody excess, equivalence zone, antigen excess, and inhibition zone. The use of the quantitative precipitin curve with protein antigens became possible on the assumption, since antigen was not present in supernatants throughout the region of antibody excess and the equivalence zone, that one could obtain antibody nitrogen by subtracting the antigen nitrogen added from the total nitrogen in the washed precipitate. These findings led naturally into studies, published in the *Journal of Experimental Medicine* during the middle thirties, of the mechanism of the precipitin reaction with Forrest E. Kendall and of quantitative studies on agglutination of pneumococci with me. Heidelberger and Kendall continued to study specific polysaccharides of pneumococci. They isolated the first crystalline aldobiouronic acid from gum arabic [*J. Biol. Chem.*, 84 (1929) 639–653], made one of the earliest attempts to estimate molecular weights of polysaccharides by diffusion through a sintered-glass disc, and called attention to the difficulties in measuring molecular weights of charged molecules [*J. Biol. Chem.*, 96 (1932) 541–558].

Studies on the effect of high salt-concentrations on the precipitin reaction with

Kendall and with Torsten Teorell, who came as a Rockefeller Foundation Fellow [*J. Exp. Med.*, 63 (1934) 819–826], showed that the capacity of a given quantity of specific polysaccharide to bind antibody was decreased in 15% sodium chloride solution, and this led to the use of this solution in purifying antipneumococcal antibody [*J. Exp. Med.*, 64 (1936) 161–172; 67 (1938) 181–199] and isolating it in a state of analytical purity.

During this period, indications were appearing that the pneumococcal polysaccharides were being degraded as a consequence of evaporating broth filtrates on a steam-bath and, as Avery and Goebel [*J. Exp. Med.*, 58 (1933) 731–755] and John F. Enders and A. M. Pappenheimer, Jr. [*Proc. Soc. Exp. Med*, (1933) 31–37] showed, by the use of strong alkali or acid in their preparation. Henry W. Scherp, who had come from the University of Rochester as a postdoctoral fellow after having studied meningococcal polysaccharides, was put to work on this problem and, together with Michael Heidelberger and Forrest Kendall, showed [*J. Exp. Med.*, 64 (1936) 559–572] that, if the broth was concentrated *in vacuo* at room temperature or by ultrafiltration and if alkali and acid were avoided, products of very high viscosity were obtained that reacted to a much greater extent with rabbit antisera, although their reactivity with horse antisera was not significantly different. With the Type I polysaccharide, alkali also caused deacetylation and substantially reduced its ability to precipitate antibody with both horse and rabbit antisera.

In 1934 and 1936, Michael took short sabbatical leaves as a Guggenheim Fellow and worked in Uppsala with K. O. Pedersen, A. Tiselius, and T. Svedberg making the earliest measurements on the sedimentation constant of purified antibodies [*J. Exp. Med.*, 65 (1937) 393–414] and demonstrating a difference in size between horse and rabbit antibodies, and also measuring the molecular weight and isoelectric point of thyroglobulin.

Together with D. L. Shrivastava, a Rockefeller Foundation Fellow, Michael and I made a quantitative study of the cross-reaction between Types III and VIII pneumococcal polysaccharides [*J. Exp. Med.*, 65 (1937) 487–496]. These studies were continued with Manfred M. Mayer [*J. Exp. Med.*, 75 (1942) 35–47] and provided some insight into the role of structural differences among polysaccharides on serological reactivity. Mayer and Heidelberger [*J. Biol. Chem.*, 143 (1942) 567–574] also studied the ability of a homologous polysaccharide to displace a cross-reacting polysaccharide from a specific precipitate. Cellulose oxidized on C-6 to give a soluble cellobiouronic acid polymer was shown to cross-react with Types III and VIII antipneumococcal horse sera [G. L. Hobby and M. Heidelberger, *Proc. Nat. Acad. Sci. U.S.*, 28 (1942) 516–518]. This reaction enabled surgeons, who used oxidized cellulose as a packing for wounds, to tell how long it remained in the body, as it slowly seeped into the blood and urine.

A series of investigations was initiated during this period on the quantitative measurement of complement by washed antigen–antibody precipitates with the participation of Manfred M. Mayer, Alfred J. Weil, Mauricio Rocha e Silva, A. J. Osler, and M. Leon. Suspensions of stroma were used by Henry P. Treffers to

measure hemolysin, and attempts to induce antibodies in heterologous species to washed, polysaccharide–antipolysaccharide specific precipitates were also made by Treffers, R. C. Krueger, and A. J. Weil. Differences between antigenic determinants of horse antitoxins and horse antipneumococcal antibodies were seen, but the extraordinary complexity of immunoglobulins was not yet recognized, and the problem could not be fully resolved. With Rudolph Schoenheimer, David Rittenberg, and Sarah Ratner, the incorporation of dietary amino acids into newly formed antibody was demonstrated.

During World War II, Michael Heidelberger and Manfred Mayer made extensive but unsuccessful attempts to immunize humans with formalinized sporozoites of *Plasmodium vivax* and *P. falciparum* to prevent relapses. Most important, however, was his participation in large-scale volunteer and army field trials on immunization with pneumococcal polysaccharides, carried out under the leadership of Colin MacLeod [*J. Exp. Med.*, 82 (1945) 445–465]. This study clearly demonstrated the effectiveness of purified pneumococcal polysaccharides, and these were manufactured commercially for a time. There is now a renewed, widespread interest and use of polysaccharide vaccines, especially in combatting pneumococcal, meningococcal, and hemophilus infections, especially in developing countries, and commercial production has resumed. The antigenicity of various pneumococcal polysaccharides and the duration of the immune response in man was also evaluated. Across the street at the Neurological Institute, I was engaged in a parallel study showing the antigenicity in man of Type I (group A) meningococcal polysaccharide. We also collaborated in a study on ricin and obtained highly purified preparations, but did not recognize that its receptor site is specific for oligosaccharides having a nonreducing terminal D-galactosyl group, and that this was the reason for its reactions with many glycoproteins. The specific polysaccharides of five groups of *Hemophilus influenzae* were isolated in collaboration with C. F. C. MacPherson, H. Alexander, and G. Leidy.

The post-war years were spent partly in studying components of complement, with O. J. Plescia and K. Amiraian, the effects of structural changes on immunological specificity, antibody purification, and in efforts to purify, from mouse milk, the virus of mammary tumor. However, the pneumococcal polysaccharides and their antisera were not neglected, and the foundations were being laid for Michael's research program for the next almost three decades. Glycogens, limit dextrins, glucans, and galactans were all studied, and the relationship between structure and specificity explored. One could readily use cross-reactions with antibodies to polysaccharides of known structure generally to identify a given terminal sugar group in an unknown polysaccharide, and often to obtain many more structural insights. Collaborators working in the laboratory included S. Adams, B. Björklund, and F. Cordoba.

From the chemical side, structures of Types XVIII and VI polysaccharides were elucidated in collaboration with H. Markowitz and P. A. Rebers, respectively; from the Type VI polysaccharide a crystalline phosphate-free repeating unit, D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-ribitol was isolated [*J. Amer. Chem. Soc.*,

83 (1961) 3056–3059]. The structure of the type XIV polysaccharide was studied with S. A. Barker, M. Stacey, and D. J. Tipper and found to contain D-glucose, as well as D-galactose and 2-acetamido-2-deoxy-D-glucose; a partial structure was proposed from methylation data.

Michael retired officially from Columbia University in 1956, but while on terminal sabbatical leave he became, in 1955, Visiting Professor of Immunochemistry at the newly opened Institute of Microbiology at Rutgers University, where he stayed for nine years, and then moved back to New York as Adjunct Professor of Pathology at the New York University College of Medicine where he is as active and productive as ever. While at Rutgers University, his tenth graduate student, Sergio Estrada-Parra from Mexico, completed his Ph.D. dissertation on the structure of the type XVIII pneumococcal polysaccharide. Sergio, now Professor of Immunology in the Escuela de Ciencias Biologicas, Mexico City, has named his department "The Michael Heidelberger Department of Immunology".

Since 1956, excluding papers appearing later but on work done at Columbia University, Michael has published about 70 papers and is still going strong. The names of more than 100 co-authors appear on his 350-odd publications. Although his last Ph.D. student finished at Rutgers University, he continues to attract and train post-doctoral fellows at New York University. Studies have concentrated on correlating structure and activity of various polysaccharides from quantitative estimates of their cross-reactivity and with the stocks of horse anti-pneumococcal sera prepared during the thirties for therapeutic purposes. He continues to publish review articles summarizing these studies. He takes delight in predicting from cross-reactions what his colleagues in sugar chemistry will find after months of laborious study.

Michael has received thirteen honorary degrees, including five from French, and one each from Swedish, Norwegian, Italian, and Mexican universities, and four from the United States, the most recent from his Alma Mater, Columbia University, in 1974. He has received many awards and medals, among others the Légion d'Honneur, the Leopold II award (Belgian), the von Behring and Lasker Awards, the Pasteur Medal of the Swedish Medical Society, and the National Medal of Science.

Working side-by-side with Michael as I did for four and a half years, as his assistant and as a graduate student, was an exciting experience. One had to learn to be extremely skillful and precise. I can well remember being watched out of the corner of his eye and how our qualitative precipitin and agglutination tests were read by him while I recorded the results. In 1936, while I was still a graduate student, Butenandt came to visit the laboratory and wanted to see some quantitative precipitin and agglutinin determinations. Michael brought him to my desk and left him to watch me carry out the procedure—I knew then that my technic was considered satisfactory. His laboratory remains an ideal place for a young post-doctoral fellow to learn quantitative carbohydrate immunochemistry.

He has truly founded a school of quantitative immunochemistry, in large part growing out of his studies on immunologically specific polysaccharides. One can

trace his influence as a line of descent of five or six successive generations of Ph.D. students, and even farther with post-doctoral fellows.

Outside his old laboratories on the eighth floor of the College of Physicians and Surgeons a plaque reads: "In these laboratories 1928–1956 Doctor Michael Heidelberger guided the creation of a new scientific discipline: quantitative immunochemistry. This plaque commemorates the celebration of his eightieth birthday, 29 April 1968."

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