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A "PURE" ORGANIC CHEMIST'S DOWNWARD PATH: CHAPTER 2—THE YEARS AT P. AND S.*

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> The World of Science . . . is the purest democracy on earth, a brotherhood from which poverty bars no one—neither the color of skin nor religious belief. Intelligent devotion to the pursuit of scientific truth, and competent effort, or support of effort, toward this end, automatically enrolls one in the great company of the "Fifth Estate." One may find in this real "League of Nations" some of the most inspiring of human associations . . . Here may be encountered some of life's greatest opportunities for service to humanity and certainly some of its greatest compensations. Happy are its devotees!
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> Charles Fuller Baker,
> Dean of the College of Agriculture,
> University of the Philippines
> In *The Philippine Agriculturalist,* 14:455 (1926)

In 1928 the Department of Practice of Medicine of the College of Physicians and Surgeons of Columbia University moved with the Presbyterian Hospital from 70th Street and Park Avenue to the newly built Medical Center on the site of the Yankees' baseball park at 168th Street and Broadway. Walter W. Palmer was, at that time and for many years later, chairman of the department. One of his close friends and advisors was Henry D. Dakin, an ex-English chemist of "chloramine T" and Dakin pad fame, and they had collaborated in a study of thyroidal function. Partly as a result of

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Dakin's influence and accomplishments, Palmer was determined to have a chemist in his newly expanded department and succeeded in obtaining one-half million dollars for that purpose from Edward S. Harkness, a principal benefactor of the Center. Thus was the Harkness Research Fund established, and my lucky earlier acquaintance with "Bill" Palmer in Van Slyke's laboratory at the Rockefeller Institute led to my choice as the first full-time chemist in a Department of Medicine. With the appointment as Associate Professor of Medicine went the title, Chemist to the Presbyterian Hospital, with purely consultative duties.

Medicine at the Center was taught and practised by a closely knit group of clinicians with widely divergent interests in research. Its leaders were Palmer himself, who studied the thyroid gland with Alexander Gutman and Ethel Benedict (later, Mrs. Gutman); Alphonse R. Dochez, concentrating at that time on streptococcal infections with Alvin F. Coburn, Franklin M. Hanger, and David Seegal; Robert F. Loeb, interested in a wide range of metabolic disorders; and Franklin M. Stevens, an allergist. Every Tuesday, after lunch, there was a departmental meeting in Palmer's spacious office at which he opened the proceedings with a discussion of problems of general interest or with medical gossip when there were no problems. Visitors were presented and anyone was free to voice suggestions or complaints or to bring up a topic of interest. Interactions with other departments were frequent; for example, Fordyce (Johnny) St. John, a Professor of Surgery, came in one day to protest the burden placed on his department by operations which could have been prevented by earlier medical intervention. The weekly meetings were continued by Robert Loeb when he succeeded Palmer on the latter's mandatory "retirement" to become Director of the Public Health Research Institute of the City of New York. I stress these meetings because, in another department at the Center, there was only one departmental meeting in the entire twenty-seven years I spent at P. and S. We also had afternoon teas in Dochez's and Hanger's offices, during which stimulating ideas were tossed about. For these teas we contributed small sums or a monthly cake.

I was given two laboratories at the northwestern end of the department's (8th) floor. Offered an office in the main stem of the hospital, near Dr. Palmer's, I chose instead a narrow architectural afterthought leading off from my personal laboratory past the powerhouse stack and designed to bring that feature out to the main building line. Although intolerably hot in summer, it was cozy in winter and saved long walks back and forth. I was soon joined by Forrest E. Kendall, who had just received his PhD in organic chemistry at the University of Illinois with Professor W. A. Noyes. It was a surprise, when Kendall arrived, to find that he had only one hand. The other had been cut off by a threshing machine on the family farm, but

in spite of his handicap he had earned enough to put his younger brothers and sisters through college. In the eight years that we worked together, the absent hand was never missed. I had brought from Mt. Sinai Hospital, as technician, Check M. Soo Hoo, a California-trained Chinese who served faithfully and admirably with me for thirty-five years at P. and S. and at the Institute of Microbiology, until a series of heart attacks ended his life. The Harkness Fund also provided us with a laboratory helper and assistance for washing glassware.

During the last years at the Rockefeller Institute and the year at Mt. Sinai Hospital I had been reading Jules Bordet's huge, novelesque *Traité d'Immunologie* and Arrhenius and Madsen's *Immunochemistry,* a brave attempt in 1907 to apply principles of physical chemistry to the immunology of the time. It appeared to me that there was a crying need to determine the true nature of antibodies and that until this was done there could be no end to the polemics and uncertainties that were plaguing immunology. It was evident, also, that the purely relative methods in use, giving titers or their reciprocals, were inaccurate, often misleading, and incapable of permitting a decision as to whether antibodies were really globulins or substances of unknown nature adsorbed to these proteins. To a chemist, it appeared reasonable to assume that the principles of analytical microchemistry could be applied to the estimation of antibodies in units of weight, a prerequisite to the determination of their nature. This had been attempted by Hsien Wu et al (1a) but not carried to a conclusion.

Actually, the time was propitious for the development of the absolute method we envisaged. Felton had just devised a simple procedure for partial purification of antibodies in antipneumococcal horse sera by precipitation with the so-called euglobulins on dilution with large volumes of water and solution of the sediment in physiological saline (2). And if antibodies should indeed be proteins, the ideal initial antigen for their estimation would be the nitrogen-free, specifically precipitating capsular polysaccharide of type II or type III pneumococcus, which I had isolated with Oswald T. Avery at the Rockefeller Institute several years before (1b). Any nitrogen in a specific precipitate, therefore, would be derived from the antibody if conditions could be found to eliminate nonspecific, adsorbed proteins.

Realization of such a quantitative analytical micromethod became the immediate task for Kendall and me. I felt, though, that I had to justify Palmer's insistence on having a chemist in his department, so that we were soon collaborating with Bill on a study of thyroglobulin (3) and with Dochez's group on fractionation of the antigens of hemolytic streptococci (4, 5). In addition, the novel presence of organic chemists in a medical department often brought microbiologists, surgeons, and others to our laboratory to borrow chemicals or obtain suggestions on overcoming un-

foreseen difficulties. Our horizons were immeasurably widened and there was scarcely a day without excitement of one kind or another.

Antibodies to type III Pneumococcus, purified according to Felton, gave precipitates with the nitrogen-free type III pneumococcal capsular polysaccharide which were centrifuged down. The supernatants were analyzed for nitrogen, and this value, subtracted from the original amount of total nitrogen taken, gave a measure of antibody nitrogen by difference. Varying proportions of antigen and antibody were studied and we attempted to explain the results according to the law of mass action, much as other chemical precipitations could be accounted for quantitatively. Shortly after our publication (6), John R. Marrack, whose *Chemistry of Antigens and Antibodies* (7) soon attracted wide notice, came to the laboratory, having made the trip from England as ship's physician on a small Cunard liner. He objected to our assumptions that there were only two antigen-antibody complexes and that equilibria existed, because we had made no study of the kinetics. This resulted in an enduring friendship and caused Kendall and me to shift our emphasis, without abandoning the mass law, in the final development of a quantitative theory of the precipitin reaction (8).

Realizing that analyses by difference were applicable only to solutions of purified antibody, we added normal serum and began analyzing the precipitates. When well-washed in the cold with 0.9% saline, these precipitates yielded results identical to those previously obtained. We could then use whole sera and determine their content of precipitable antibody in units of weight.

This was all very well for antibodies to polysaccharides, but what about those elicited by the vast numbers of protein antigens? To solve that problem we had to distinguish between antigen nitrogen and antibody nitrogen. ^{15}N had not yet been discovered and radioactive isotopes had not been tamed for use in biology. We therefore had recourse to dyes. By coupling tetrazotized benzidine on one side with R-salt, a naphtholdisulfonic acid, and on the other side with crystalline hen's egg albumin, we produced an Easter-egg-like purplish protein antigen. This was treated rather drastically until it no longer precipitated anti-egg albumin. Injected into rabbits, it gave rise to antibodies that formed pink to red precipitates with the antigen, depending upon the proportions used. Washed precipitates were dissolved with alkali, rinsed into a colorimeter cup and compared, visually, with alkaline solutions of the dye. Photoelectric colorimeters were not yet developed. The solutions of the precipitates were rinsed quantitatively into micro-Kjeldahl flasks. Total nitrogen minus antigen-dye N gave antibody N (9). When we had worked out the course of this reaction, we were ready to study that of a colorless protein, crystalline egg albumin (10). Later, with H. P. Treffers, now Professor of Pathology at Yale, and a young B. Davis,

whose short career was ended by poliomyelitis, we went on to study the immunochemical effects of phosphorylating the protein. The denaturation of egg albumin was also investigated with Catherine F. C. MacPherson (11) (who helped develop the method for estimation of antibodies in human sera, then studied the polysaccharides of several types of *Hemophilus influenzae* at Babies Hospital, went on to E. G. D. Murray's department at McGill, and is now an authority on proteins of the brain at the University of Western Ontario), the deamination of egg albumin with Paul H. Maurer (12a, 12b) (now for many years Professor of Biochemistry at Jefferson Medical), and the cross-reaction between hen's egg and duck's egg albumins (13) with Abraham G. Osler (who became Professor of Microbiology at Johns Hopkins, spent many years in research at the Public Health Research Institute of the City of New York, and has just gone to the University of California at San Diego).

Quite reasonably, some of our colleagues were worried about soluble antigen-antibody complexes in the egg albumin system, but as we had shown that they did not occur measurably and as we claimed only the estimation of precipitable antibody, our data appeared valid. Most welcome confirmation of our analyses came by an entirely independent physical-chemical method (14). Even this did not convince all "hard-core" immunologists, for as late as 1939 a new edition of a standard text insisted that we were "doubtless measuring non-specific nitrogen" instead.

Development of a quantitative theory of the precipitin reaction taxed our ingenuity, but the experiments we had done led inevitably and inexorably toward the interaction of multivalent antigen with multivalent antibody (8). We had partially hydrolyzed the type III polysaccharide and shown that the larger fragments could precipitate portions of the antibodies, so that the intact substance was obviously multivalent. From the proportions of antibody to antigen in precipitates from protein systems, it seemed reasonable to assume that a protein such as egg albumin must have at least five or six combining groups, so that our theory was not limited to polysaccharide antigens. As for the antibody, we knew from the beginning that precipitates in the region of antibody excess could combine with more antigen; therefore, two or more combining groups did not seem unreasonable. My friend, Felix Haurowitz, however, with true scientific skepticism, insisted for many years that antibodies need not be more than univalent, but he finally capitulated. We always remained friends in spite of this disagreement as to theory, and we argued in our correspondence and at meetings with as much relish as we experienced when playing sonatas together, for Felix was both a superb scientist and an excellent pianist. For many years, Waldo E. Cohn organized and played cello at evening sessions of chamber music at the annual meetings of the Federation of American Societies for Experimental

Biology. Felix and I frequently took part in these. Another critical friend was Linus Pauling, who believed that the valence of antibodies could not exceed two. However, I attended a lecture of his at Brooklyn Polytechnic and, with great glee, detected a trivalent antibody on one of his lantern slides, inserted to give a specific precipitate the proper degree of compactness. Linus also, with Dan Campbell and David Pressman, criticized our assumptions as "arbitrary and unlikely," which surely was their privilege. With a different set of assumptions, however, they arrived at an equation practically identical with ours (15). This, of course, pleased us immensely. Later, our simple equation for a quantitative theory was shown to be a special case of a more general theory (16) and this, in turn, was found to be a special case of a still more general theory requiring calculations by a computer (17). This was progress, but for immunologists needing simple, quantitative explanations and calculations in many, but not all, precipitating systems, our formulation and equation remained practical and useful. Moreover, the theory permitted a number of valid predictions, as appears later.

While Forrest and I were polishing up the precipitin reaction, our first graduate student, Elvin A. Kabat, arrived. He had received his BS *cum laude* at City College and was to work as a laboratory helper while studying for the PhD degree. Within a few weeks, this young whirlwind had read H. Gideon Wells's and Arrhenius's books and all of our papers and wanted to know why we hadn't adapted our quantitative method to bacterial agglutination. "We've been too busy," we said: "You go ahead with it," and he did.

Studies of the agglutination of microorganisms had been hampered, not only by the sole use of titers for measurement, but also by overemphasis of the effects of electrical charge and by lack of recognition that the combining ratios of bacteria and antibodies could vary greatly in different systems. Because of such differences, standard textbooks contained statements like "anti-*Salmonella* sera (titers up to 1:1,000,000) are stronger than antipneumococcal (titers up to several thousand)." Had this comparison been valid, most anti-*Salmonella* animals would have died of heart failure owing to the viscosity of their blood. Our plan was to wash killed type-specific pneumococci until the washings no longer gave tests for capsular polysaccharide. This was to avoid confusion of agglutinins with precipitins. A measured volume of the washed cells could then be added to a known volume of homologous antiserum. Agglutination occurred, as sufficient type-specific polysaccharide remained on the cells, and the added antibody nitrogen could be measured. Elvin worked out the proper conditions for accuracy, and bacterial agglutination was found to be a precipitin reaction at the cellular surface and subject to the same theory and similar quantitative expression (18).

An immediate practical result was the termination of the still ongoing dispute as to whether agglutinins and precipitins were identical or different. Though not as virulent as that over unitarian or pantheism, the ineffectual thought and effort given to it retarded progress. With our genuinely quantitative methods it was easy to show that, with respect to a single antigen and its homologous antibodies, precipitation and agglutination were functions of the same fraction of antibody (19). As protection had already been found to parallel the antipolysaccharide, three independent manifestations of the action of antibodies were shown to be due to the same protein molecules.

There was another intensely practical result when Hattie E. Alexander, microbiologist of the Babies Hospital at the Center, asked for help to improve the potency of the rabbit anti-*H. influenzae b* sera with which she wished to treat influenzal meningitis in infants. Quantitative agglutinin estimations that precisely measured the results of various dosages and routes of injection of the bacilli into rabbits soon resulted in sera with up to ten times the former content of agglutinins and precipitins for the polysaccharide of type *b*. Injection of the new sera into infants with influenzal meningitis cured most of them (20).

Confident of our methodology, we turned to our original quest: to find out whether or not antibodies were actually modified globulins. Again, luck favored us, for we were joined for several months by Torsten Teorell, presently (1978) Emeritus Professor of Physiology at the University of Uppsala. Torsten had been working with W. J. V. Osterhout at the Rockefeller Institute and had a consuming interest in the biological functions of electrolytes. We put him to work on the effects of varying concentrations of salts on the precipitin reaction with the aid of his own modification of the micro-Kjeldahl technique. An increase in sodium chloride diminished precipitation, but all added polysaccharide was still precipitated in the region of antibody excess. This, we soon realized, offered an approach to the isolation of pure antibody: a thoroughly washed precipitate formed in the region of antibody excess in 0.9% saline should liberate only antibody when equilibrated in 0.5M or M NaCl. This was all very well on a micro-Kjeldahl scale, at which nonspecific proteins could easily be washed out within the limit of error of the method. Our first large-scale preparation, however, was only about 70% pure, and it took Kendall, Kabat, and me two years before the first batch of analytically pure antibody was obtained (21a, 21b). This was typical globulin and provided the last link in the chain of evidence that antibodies were actually globulins. With this secure knowledge of the chemical nature of antibodies, one could now proceed to the study of their biosynthesis, cellular origin, and control. Visiting Denmark the following summer, I tried to interest August Krogh, a leading physiologist, in undertaking such a study, but he was fully occupied with other problems. Other Scandinavians, notably Mogens Björneboe and Astrid Fa-

graeus, however, stimulated by the new findings, soon implicated the plasma cell in the formation of antibodies (22a, 22b). These workers not only started cellular immunology on its prodigious expansion but also contributed much to its development.

While the quantitative studies were in progress, Florence Rena Sabin came to the Rockefeller Institute with radical ideas and experiments on the nature of tuberculosis. A warm friendship soon developed, and Florence was often at our home, particularly when we also invited Oswald T. Avery. My first wife, Nina Tachau, and I also went to the Cosmopolitan Club as her guests or to her home, where Florence was almost as proud of her skill in broiling steaks as of her scientific accomplishments. At her urging and with a grant from the National Tuberculosis Association, the complex mixtures of polysaccharides and proteins of various strains of mycobacteria were studied by means of fractionations meticulously carried out by Arthur E. O. Menzel (23a, 23b) and later by Sulo A. Karjala.

In 1934 and 1936 I was given partial John Simon Guggenheim Memorial Fellowships to work with The Svedberg's ultracentrifuge in Uppsala for periods of about six weeks, as I did not wish to be absent longer from the laboratory. The first time, I took samples of purified thyroglobulin along and prepared others with Swedish material. To our great surprise, this protein secreted by the thyroid gland was found to be of high molecular weight (24). On the second trip, purified equine antibodies to pneumococcal polysaccharides were centrifuged and also found to have high molecular weight (25), confirming Kendall's and my findings with the Northrop cell and in accord with independently arrived at results by ultrafiltration (26a, 26b). This time Nina and I were there in May and part of June, when we could enjoy the explosive Swedish Spring and its twenty-four hour shades of brightness. When there were not too many calculations to be done in the evening we would drive along an east-west road, enjoying colorful sunsets toward the north and watching them merge into equally beautiful sunrises. Svedberg had assigned his codeveloper of the ultracentrifuge, Kai O. Pedersen, to most visitors, to teach them the many precautions, controls, and actual use of the complicated, oil-driven machine with its terrifyingly numerous gauges and its optical system perfected by Ole Lamm. Kai, a gentle, self-effacing man of immense competence, had been nursing numerous visiting scientists along for some years, to the detriment of his own research. The many papers published with his help contained only brief acknowledgments for his efforts. As my time was limited, he practically gave up his work while I was there. We and our wives became fast friends and have often exchanged visits on both sides of the Atlantic. For his essential part in the work, I naturally made him a co-author of the resulting publications, a precedent which happily encouraged subsequent visitors to do the same.

By 1937 Elvin Kabat had completed two projects worthy of the PhD awarded for one of them. Thanks to a fellowship from the Rockefeller Foundation, he, too, went to Uppsala to work with Arne Tiselius and Kai Pedersen on the electrophoretic and ultracentrifugal properties of antibodies, including purified samples from various species of animals[1] which we sent over (27a, 27b). This research confirmed our analytical data on antibody content, as already noted, and even more firmly established the protein nature of antibodies and demonstrated their variations in molecular size.

Goodner & Horsfall had also shown that antipolysaccharide in rabbit antipneumococcal sera was of relatively low molecular weight, and had reported good clinical results with these sera (28). This led us to immunize rabbits with type I, II, or III pneumococci, and, with Joseph C. Turner of our department as clinician, to devise a simple method of partial purification of the antibodies for use in the Presbyterian Hospital (29). Cases of pneumonia due to I, II, or III received an intravenous infusion of 400–600 mg of the appropriate antibody. Nurses were instructed to bring me a few cc of the patient's blood half an hour later. Serum was drawn off and tested with homologous capsular polysaccharide. A positive precipitin reaction showing excess antibody usually resulted, but one severely infected type III patient required five bottles of antibody solution before an excess was established and recovery ensued. Under this regime deaths from these types of pneumonia were practically abolished at the Hospital as was also serum sickness. Soon afterward, the sulfa-drugs and penicillin came into use and typing and serum therapy were abandoned.

Having quantitated specific precipitation and agglutination, we took on the problem of complement (alexin), so important for diagnosis and immune lytic action. Was it a substance, as Ehrlich and his followers maintained, or was it merely a colloidal state of freshly drawn serum, as the school of Bordet insisted? Interest in this question was stimulated by the arrival of Alfred J. Weil from Germany and Otto G. Bier from Brazil, both of whom were familiar with the peculiarities of complement. An adequate series of controls soon made it possible to show that complement-containing sera added appreciable weight to specific precipitates formed with rabbit antipolysaccharide or antiprotein (30). Complement was therefore a substance or a series of substances (four components were then known) and Ehrlich's ideas were vindicated. Simultaneously, Pillemer, Ecker, Oncley, and Cohn purified the first of the four components (31), and arrived at the same conclusion. Further quantitative studies in the laboratory at P. and

[1] A turkey that had received injections of formalinized pneumococci was ultimately roasted by a Hospital chef and became the main dish of a laboratory luncheon. The formalin and pneumococci did not affect its flavor.

S., carried out with Manfred M. Mayer, Graciela Leyton of Chile, and Abraham Osler, established optimal conditions for the estimation and more efficient use of complement, and showed that, in many immune systems, its "fixation" ran parallel to specific precipitation.

Quantitative immunochemical methods also provided a check on metabolic studies with heavy nitrogen. Because of the rapid entry of ^{15}N into proteins in intact animals, Schoenheimer concluded that preformed peptide bonds opened and closed easily (32). Collaborating with his group, Treffers and I injected an ^{15}N-fed rabbit with killed type III pneumococci and antibodies to type I polysaccharide from another rabbit. Quantitative analyses of the circulating actively forming and passive antibodies at varying intervals showed that only the anti-III molecules being synthesized were capable of taking up ^{15}N. The simultaneous presence, with ^{15}N, of independent, immunologically specific markers thus settled an important aspect of the metabolism of proteins in the living animal (33). The central idea for this work, also quantitative studies of hemolysins, of antibodies to proteins raised in horses (in part with Jules Freund and Robert C. Krueger), and friendly assistance to foreign visitors, were contributions made by Henry P. Treffers during four postdoctoral years in the laboratory.

A foreign visitor not previously mentioned was Pierre Grabar, who arrived in 1937 as a Rockefeller Foundation Fellow, at the suggestion of André Boivin of Strasbourg. Pierre, a physical chemist, had only shortly before become interested in immunochemistry but developed into its leading European exponent on returning to the Pasteur Institute in Paris. Then there was Bertil Josephson, of Stockholm, whose interest in immunochemistry grew out of his specialization in the functions of the kidney. Still another was Sverre Dick Henriksen, a microbiologist from the laboratory of T. Thjötta in Oslo. Sverre could not return to the Rikshospital because of the invasion of Norway by the Germans, but was helped by a Rockefeller Foundation fellowship until he and his wife, Aase, departed for medical service in the Little Norway camp in Canada. Lifelong friendships developed with all of these visitors and their families.

Meanwhile, in part with D. L. Shrivastava, another Rockefeller Foundation Fellow and later Assistant Director of the Central Drug Research Laboratory at Lucknow and an expert on cholera, we did a quantitative study of homologous and cross-precipitation in the pneumococcal type III and type VIII systems (34a, 34b). This not only disclosed five different kinds of heterogeneity in the equine antibodies used, but also enabled us to predict the most likely arrangement of the sugars in the type VIII substance. There was also a practical sequel. A paper on oxidized cotton made it obvious to me that this linear polymer of cellobiose had been converted into analogs of types III and VIII polysaccharides by conversion of many $-CH_2OH$

groups to –COOH. One could predict from our quantitative theory that substances with multiple identically or similarly linked sugars or amino acids should give cross-precipitation in appropriate antisera. I wrote for samples of oxidized cotton and found at once that their neutralized solutions precipitated antipneumococcal III and VIII sera heavily (35). Our surgeons were then using oxidized cotton pads as a hemostatic and as a packing for wounds, for they could safely be left inside and would slowly disappear. By means of a rapid precipitin test of a patient's serum or urine with equine antipneumococcal type III serum, I was able to tell the surgeons, almost to the hour, how much time elapsed before the pads were wholly absorbed.

Then came the Second World War and theoretical problems were slowed or shelved and immediate concerns of the military were given priority. Two of our projects were highly secret and both illustrated the damaging effects of scientific secrecy. The first, with Forrest Kendall and L. A. Julianelle, was to protect and cure animals infected with anthrax, an agent our enemies were supposed to be planning to use. Julianelle found that for mice, penicillin, just then available, was the answer. Secrecy prevented Julianelle from publishing this and also lost him the benefits of priority when microbiologists at the Mayo Clinic, working outside of governmental auspices, announced the same finding in *The New York Times.* The second secret project concerned ricin, the enormously toxic principle of the castor bean which, it was feared, might also be used as a weapon. Elvin Kabat and I further purified samples of ricin, and colleagues in two other laboratories actually crystallized it. Suddenly, secrecy as to the chemical studies was lifted because of an incautious general's speech. I telephoned my friends in the project, proposing that we all publish our data in the same number of the same journal. They agreed, so we quickly wrote our paper, in which their independent work was mentioned, and sent it to Washington. Clearance was speedily granted, but alarming reports caused secrecy to be clamped down again before our colleagues' papers were written. This created the ridiculous situation that our publication (36) disclosed results that those who had obtained them were forbidden to mention.

Two nonsecret projects, however, proceeded normally. Because of a continuing large series of pneumococcal pneumonias during several years at a training camp for aviators at Sioux Falls, South Dakota, it became advisable to find out whether or not human subjects could be protected by immunization with purified capsular polysaccharides of the causative types. Felton had attempted this during the Great Depression by immunizing many thousands in camps of the Civilian Conservation Corps. His massive experiment was ill-fated, however, because healthful, outdoor living conditions led to almost no pneumonia in any of the camps, unimmunized or immu-

nized, and mouse protection tests of sera of vaccinated persons varied from titers (alas!) of 0–2,000,000, an uninterpretable spread. I asked for volunteers from the entering classes at P. and S. in 1942 and 1943. Most of the students agreed to be injected with the polysaccharides of types I, II, and V and were wonderfully faithful in appearing for injections and bleedings, even though the latter often meant going without lunch during the hectic wartime compression of the medical course into three years. For a few exquisitely sensitive individuals it was fortunate that we first injected only about one third (15–20 μg) of the total dose, waiting 24–48 hours before adding the remainder if no reaction, or only a slight one, occurred. Strangely enough, in no case was a marked reaction due to known previous infection with pneumococci of types I, II, or V. Maximal values of precipitin in serum were reached in two to six weeks and a good response to one type did not guarantee an equal result with the others. The antibodies, unlike those to diphtheria toxoid, for example, were evoked at remarkably permanent levels, diminishing only slightly during months and even years (37). But were the volunteers protected against types I, II, and V? Our busy students would not have enjoyed being challenged with virulent pneumococci, but luck favored us. A paper by Barry Wood had appeared opportunely (38), in which rats sprayed 12 hr before with virulent type I pneumococci were saved by 0.02 cc of a rabbit antipneumococcal I serum but not by 0.002 cc. We analyzed this antiserum and calculated that 0.2 cc gave a concentration of antibody in the blood of Wood's rats about three times that of the average of our volunteers. Assuming that if three times our average could cure, one-third as much should protect, I recommended to the Surgeon General that vaccination be tried at Sioux Falls. We decided to inject polysaccharides of types I, II, V, and VII, as the resident microbiologists had found these types in about 60% of the pneumonias. The camp's population was randomly marshaled into two lines of 8,500 each. Those in one line were injected with 1 cc of saline as control subjects. Those in the other line were given 1 cc containing 50–70 μg of each of the polysaccharides. These had been prepared by us, by E. R. Squibb and Sons under Tillman D. Gerlough's direction, and by Augustus C. Wadsworth and Rachel Brown. As seen in Table 1, within two weeks there were no more pneumonias caused by the four types among those vaccinated. Even the nonvaccinated were partially protected because, as the microbiologists found, the vaccinated personnel no longer carried these pneumococci in their noses and throats. Pneumonias due to pneumococcal types not in the vaccine remained equal in both groups, which showed the strict specificity of the protection (39). The entire study, so beautifully organized and monitored in the field under Colin M. MacLeod's direction showed that epidemics of pneumococcal pneumonia in closed populations could be ter-

Table 1 Interval between injection and the development of pneumonia in immunized and nonimmunized subjects[a]

Interval	Number of cases of pneumonia			
Weeks	Types I, II, V, VII in immunized subjects	Types I, II, V, VII in nonimmunized subjects	All other types in immunized subjects	All other types in nonimmunized subjects
1	2	0	1	1
2	2	3	5	3
3	0	3	7	5
4	0	2	8	12
6	0	2	6	7
8	0	2	3	4
10	0	1	4	4
12	0	0	2	4
14	0	2	2	1
16	0	3	2	4
16+	0	8	16	14
Total	4	26	56	59

[a]Reprinted from *J. Exp. Med.* 1945. 82:453.

minated within two weeks after vaccination with the polysaccharides of the causative types. As many of the protected subjects had shown levels of antibody too low for our relatively insensitive precipitin technique to detect, it was evident that very little antibody could prevent the droplet infection by a few pneumococci that presumably initiates the disease.

Our fourth wartime project was carried out with Manfred M. Mayer and Myron A. Leon. The latter came from the Bronx High School of Science at the age of 16, was drafted into the Navy at 18, was reassigned to the project through the good offices of Admiral H. W. Smith, and is now Professor of Immunology and Microbiology at Wayne State University in Detroit. We had earlier prepared malarial vaccine from the hemolyzed red cells of volunteers among the malaria-infected sailors who had been admitted to the Marine Hospital on Staten Island. Manfred's wife, Elinor, a pianist, suggested that we try curing victims of malaria between relapses with vaccines made from their own parasites. This was done in about fifteen patients with mildly encouraging results. As the hospitals of the armed forces were filling up with malarial patients from the Solomon Islands and elsewhere in the Pacific, I proposed a trial of our vaccine to the Surgeon General of the Army. Better proof of efficiency was demanded, however, so I went to the Bureau of Medicine of the Navy. An entire ward of about 200 patients was quickly assigned to the project at St. Albans Hospital in Queens, New York, under the medical supervision of Commander W. A.

Coates. He explained the need for, and use of, blood from the most highly parasitized cases during relapses, and these men gave their blood liberally in spite of their discomfort. During the six months of the test our laboratory did little else than prepare malarial vaccine, and at the end the vaccine-treated and two control groups showed exactly the same rate of relapses (40). By-products of scientific value, however, were an electromagnetic method of concentrating the relatively few parasitized red cells in infections due to *Plasmodium malariae* (41) and an understanding of the triple nature of complement-fixation in malarial blood (42).

Postwar inflation soon made the Harkness Research Fund inadequate and recourse was had to the Rockefeller Foundation and the Office of Naval Research (ONR), precursor of the National Science Foundation (NSF). When I asked how the Navy could possibly be interested in one of our projects on an ONR grant, the reply was: "Do the research as you think best and the Navy will look out for its own interests." This liberal attitude was continued in the NSF when Alan Waterman left ONR to be Director of NSF.

During all these years I had been making sporadic attempts to learn more about the capsular polysaccharides of pneumococci: for example, that of type IV with Kendall, and types I, III, V, and XVIII with Harold Markowitz, now with the Mayo Clinic. The polysaccharide of type I, Neufeld's "typical" pneumococcus, was taken up several times, but the instability of its hydrolytic products proved baffling. Happily, I have interested Bengt Lindberg in this unusual amphoteric polygalacturonate. Now that commercial quantities are being prepared one may expect that he will solve its fine structure, as he has with types II and XIV.

Unlike the problem with type I, another major investigation had been slowly expanding: the use of cross-reactions to clarify relations between chemical structure and immunological specificity. This had begun (see 1b) with Avery's intuitive feeling that analogs of pneumococcal polysaccharides must exist "free in Nature." His hunch was quickly justified by the cross-reaction of gum arabic and its products of partial hydrolysis in anti-pneumococcal type II serum and the analogous precipitation by the polysaccharide of Friedlander's bacillus B (*Klebsiella* K2). The cross-reaction of pneumoccal types III and VIII has already been mentioned. Also, during a consultantship with Merck and Co., of which Per K. Frohlich was Director of Research, I had looked into their synthetic polyglucoses as possible blood-extenders and found that, like the dextrans, certain fractions precipitated heavily with antipneumococcal sera of several types. Then glycogens of various origins were found to react in this way (43). To our astonishment, the paper describing this was rejected by the *Journal of Biological Chemistry* as not being biochemical! Since many gums of plants

and other polysaccharides with nonreducing lateral end-groups of D-galactose precipitated antipneumococcal type XIV serum, one could predict that the capsular polysaccharide of this type would also contain this structural feature. I spent a summmer in the organic chemical laboratories at the University of Birmingham, England, learning techniques of chromatography and methylation from Maurice Stacey (who had been sent on a wedding trip mini-sabbatical to my laboratory in 1936 by Sir Norman Haworth) and S. Alan Barker. We verified the prediction (44). Other early uses of cross-reactions were the demonstration that an acidic impurity in the galactan of bovine lungs contained D-glucuronic acid (45) and that gum arabic was a mixture from which a fraction of quite different composition could be precipitated by antipneumococcal type II serum (46). Samples began to come in from many countries and it was often possible, by means of rapid cross-precipitation, in which the initial qualitative tests were frequently followed by quantitative estimations, to inform the sender of the nature, position, and/or linkage of one or more sugars in the product—information which might require weeks or months by purely chemical means.

In 1938 I first met Selman A. Waksman. We served on a planning committee for the International Congress of Biochemistry held in New York in 1939. My first wife, Nina, mother of my son, Charles, was on the Women's and Hospitality Committees, and many lasting friendships, especially with the foreign visitors, resulted from her activities as well. The hurried departure of the English, French, and German biochemists on the outbreak of World War II cast a heavy pall over the last days of the Congress.

From 1943 to 1946 Warren Weaver was organizing some eighty scientific talks sponsored by the US Rubber Company and transmitted over the Columbia Broadcasting System during the intermissions of Sunday concerts of the Philharmonic Orchestra of New York. It was, I am sure, Weaver's insistence that resulted in the rare circumstance of scientists being paid entertainers' fees—$500 for an eleven-minute talk. My subject was Resistance to Infectious Disease. Although my wife, Nina, was dying of cancer, as a writer she worked with me over every word, insisting on simplicity and clarity and helping shorten long sentences. The vivid memories of her aid required intense self-control when I gave the talk several months later, nervous as I also was with the realization that a million or more listeners might hear me. Perhaps they did, for this last message with her help, so frequent with earlier papers as well, brought letters from as far away as Los Angeles and Saskatchewan.

In December 1946, I was invited to the first postwar international scientific symposium, to be held in Paris to commemorate the 50th anniversity

of the death of Pasteur. The twenty-one hour flight in a nonpressurized DC-4 across the Atlantic, via Goose Bay and Shannon, was a true adventure. The hotel in Paris was allowed to have heat for only an hour in the morning and another in the evening, but we were given an adequate supply of food stamps. The resurgent spirit of the French, after their enormous losses, was inspiring, and when a minister of education spoke proudly of French science, he was rebuked by Jules Bordet in an impassioned address stressing its international rather than national quality. Another early postwar international congress on microbiology was held in Copenhagen in the summer of 1947 and I was on our delegation to it. Selman Waksman was given an award and donated the quite large monetary portion for a Danish-American exchange fellowship. At the "banquet," by chance I sat next to Gabrielle Hoerner, a statuesque Alsatian pathologist who had come along as secretary to J. E. Morin, Professor of Microbiology at Laval University in Quebec. (I was much touched when, the following winter, Professor Morin and a carful of students drove through a heavy snowstorm to attend my French lecture in Professor Armand Frappier's Department at the University of Montreal.) The cursory table talk with Gabrielle eventually narrowed down to music and my impending visit to Paris, where Gaby was intending to visit a "musical sister." An invitation resulted, and I was embarrassed, being only an amateur, to find that the "musical sister" was the leading Wagnerian soprano of the Paris opera, Germaine Hoerner. The three of us, and Gaby's eventual husband, Robert Vogt, became good friends and I owed many fine sessions at the Opéra and participation in chamber music with French musicians to these accidentally acquired friends.

Through pure chance I was twice President of the American Society of Immunologists. The society's original constitution strangely provided that the elected president assume the vice-presidency *after* his presidential year. Elected president in 1947, I was vice-president in 1948 when the membership decided to change to the more usual progression. This automatically made me president again in 1949. My presidential addresses were: "Science, Freedom, and Peace," and its variant, "Ivory Pawn in the Ivory Tower," stimulated by activities novel for me up to that time.

Nina had been Chairman on Foreign Policy for the New York City League of Women Voters and active in the Speakers' Bureau of the American Association for the United Nations. After her death, her friends in the latter, lacking funds to hire experts and knowing I was going to Europe, asked me to try to carry on her work in the US delegation to the World Federation of United Nations Associations. There were extraordinary people in our group: among them, its leader, Clark Eichelberger, the UNA's executive secretary, Charles Marburg of Baltimore, who had been in the State Department, and Cyril Bath of Cleveland, who had begun as a laborer,

had reorganized the Mexican railroads into a viable system, and in 1946 was a manufacturer of machine tools with an intensely loyal force of skilled workers. At the meeting in Prague, I acquired two warm friends in the Norwegian group, and because I knew French fairly well I was able, at a meeting of the French, English, and US delegations, to eliminate differences in meaning that had prevented agreement on two resolutions. These actually were voted on by the General Assembly of the United Nations that winter, and although they were too international to get enough votes from the nationalistic delegates, it was thrilling to know that my first dive into international politics had created even a ripple. Another year at the plenary sessions, I sat next to a pharmacist, Asare, one of two Ewe tribesmen from Togoland who had traveled through the native villages, talking about the United Nations and obtaining small sums for the journey to Geneva. They told an affecting story of a village that had spent years collecting money to build a bridge, but gave them the entire amount, considering their mission even more useful. Asare became an important official after Ghana's independence and sent a young student, Samuel Essandoh, to the United States for premedical studies and the medical course at P. and S., during which he was president of his class for one year. At the same meeting, in Geneva, were Evelyn Fox and Mary Dingman, who had been active there in the YWCA during the war, and we became fast friends. And later still, in order to finance a trip to the first meeting of the UNAs in Asia, I lectured in Hong Kong, Tokyo, Osaka, Kyoto, Hokkaido, and Bangkok, aided by the Rockefeller Foundation. Eleanor Roosevelt was a member of our delegation at the meeting in Bangkok, and I learned from her how to prepare a resolution on world peace that would be acceptable to our UNA. It was a joy for me and for my second wife, Charlotte Rosen, to be accepted by her as friends.

In the meantime, Duncan A. MacIness, of the Rockefeller Institute, unable to get the New York Academy of Sciences to agree to convene small meetings of experts to consider unsolved problems in the sciences, had been holding several highly successful midweek meetings of that type at an inn on Long Island under the auspices of the National Academy of Sciences. As the knowledge of complement was in a rather primitive state, Duncan was willing to add a conference on it to his series, with about twenty participants. All of us contributing to the study of complement were there in the autumn of 1950. We spend the first evening telling the others, among them physical chemists and enzymologists who had scarcely heard the word, what we knew, or thought we knew, of complement. The remaining two days were devoted to discussing the things that needed to be known and done, with highly stimulating and useful results (47).

In 1951 Linus Pauling, presently still very much alive, was forbidden by his physician to go to the Indian Science Congress to which he had been invited. As his substitute, I departed for India by air on Christmas eve and

spent six busy weeks in that fascinating country as part of an international group that also included Erwin Brand, a biochemist of our Medical Center who was wise in the ways of amino acids and proteins and who stimulated the development of a photoelectric polarimeter for their study. Another invited foreigner was Arthur Stoll, of Basel, who had been Willstätter's first assistant when I studied in Zurich, and whose company, Sandoz, was equipping a new, air-conditioned animal house at the Central Drug Research Laboratory in Lucknow. In Calcutta, where the congress was held, and wherever we went afterward on our tour through India, Stoll was met by a Swiss consul general or commercial attaché and was usually housed in a governor's mansion. By contrast, our government left the amenities of hospitality entirely to our hosts, who did their very best under the direction of a young man named Gonçalves from the Ministry of Science who hailed from Goa, looked very Indian, and said his family had been Christians for 800 years. The general lectures of the Congress were held in an enormous open "pandal," or tent, which seated 8000 because of complaints that the general public was excluded in the original plans. As a result, entire families, from grandparents to small children, listened patiently to our technical talks. These were made difficult by the belated reverberation of our words from loud speakers at the back of the tent. I was greatly impressed by the fine research our Indian colleagues were doing, often with not-too-good facilities, but with a slowly increasing supply of excellent locally manufactured apparatus. Impressive, too, was a hospital in Bombay run by Col. A. A. Bhatnagar, with its high standards of sanitation and care of patients. In Calcutta we foreigners were given a dinner in the governor's palace and the privilege of individual talks with Nehru, who had actively furthered the development of the sciences in India. He and I, however, did not talk science but found a congenial topic in criticism of the policies of John Foster Dulles. During our tour we were kept so busy giving lectures that when we reached Delhi I refused to give any more until I had seen the Taj Mahal. It was arranged that the J. B. S. Haldanes, Brand, and I would be taken early in the morning to Agra by car, but at the appointed time the Haldanes had gone off birding and were left behind after much delay. The sad story of Shah Jahan and his beloved Mumtaz, and the glorious beauty of the monument to her were a tremendous emotional experience.

After the Congress I stayed on to complete a pet project. In the old days, Avery would say: "If we only had an elephant instead of rabbits, we'd have plenty of antiserum." I did not want to miss this chance to study the immunological properties of elephants, potential suppliers of huge quantities of antisera. But first, at Lucknow, my friend, D. L. Shrivastava, was having trouble with the supply of guinea pig complement for diagnostic tests. I suggested elephant complement instead, which apparently had never

been tried. Serum was obtained from the nearest available elephant, a day's trip away, and we set up tests with great excitement, only to be disappointed. As for elephants and antibodies, chance and luck had intervened again. Back in New York, the same Mrs. Vally Weigl, through whom I met my present wife (see 1b), had been giving piano lessons to the wife of a former maharajah. A young friend of the maharani, sister of the first secretary of the Maharajah of Mysore, came to hear us play, and we became friends through our interest in the United Nations. When the younger lady learned of my approaching visit to India and my desire to immunize elephants, she notified her brother. The result was that in Mysore and Bangalore I was quartered in the Maharajah's No. 1 guest house and given an appointment to play one of the clarinet sonatas of Brahms with the Maharajah, an excellent pianist. However, only a brief, cordial interview resulted, owing to the Maharajah's sudden violent toothache. When he heard that I could not stay for the usual weekend illumination of the fountains in the beautiful park below the massive dam that was bringing electricity to the farms of Mysore, he ordered a special midweek illumination and throngs came for the unusual event. It was difficult to imagine that Thomas E. Dewey, then Governor of New York State, would do the same for a troublesome visiting scientist. The Maharajah also made two animals available, one of which, a work elephant at the edge of the jungle, was willing to lie down on command of his mahout. After several attempts the elephant was injected through its massive ear vein and the thin skin behind the ear with a few grams of human gamma globulin which I had brought along, thinking an antiserum to it might be of use in India. When we went for a bucket of blood some days later, the mahout and forester in charge would only let us draw about 5 cc, fearing we might kill the animal and the mahout would be jobless! This, although it was customary to calm obstreperous bull elephants by taking ten liters of blood. Anyhow, the 5 cc sufficed to show that one elephant, at least, was a good producer of anti-human globulin. Whether or not this has ever been followed up I do not know. What did follow, however, was a practical joke played by my young Indian friend who had been so helpful. Because of the limited success of the project, she said, she had arranged to have three elephants sent from Mysore. Seriously alarmed because of her previous efficiency, I was able to get the New York Zoo in the Bronx to agree to receive them. When the three elephants arrived, it was in a cardboard box, and they were exquisitely carved out of rosewood!

This brings me nearly to the end of my twenty-seven happy and busy years at P. and S. Mandatory retirement from Columbia in 1956 was looming ahead, and for some time Selman Waksman, who had founded and funded the Institute of Microbiology at Rutgers University with his royal-

ties from streptomycin, had been asking me to start an immunochemical group in his institute. From 1954 on, my associate, Dr. Otto J. Plescia, and I had been giving occasional lectures at the Institute of Microbiology. "Retirement" to the laboratories there was humanely facilitated by Columbia in 1955, at Professor Elvin Kabat's suggestion, by giving me a year's terminal leave, with salary, so that I would not have to resign and could receive the proud title of Emeritus Professor of Immunochemistry. As no one wanted our specialized equipment or antigens and antibodies, we assembled a truckload and with it my small staff and I transferred to New Brunswick. Thus ends Chapter 2; within two weeks we were working again as smoothly as if we had never moved.

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