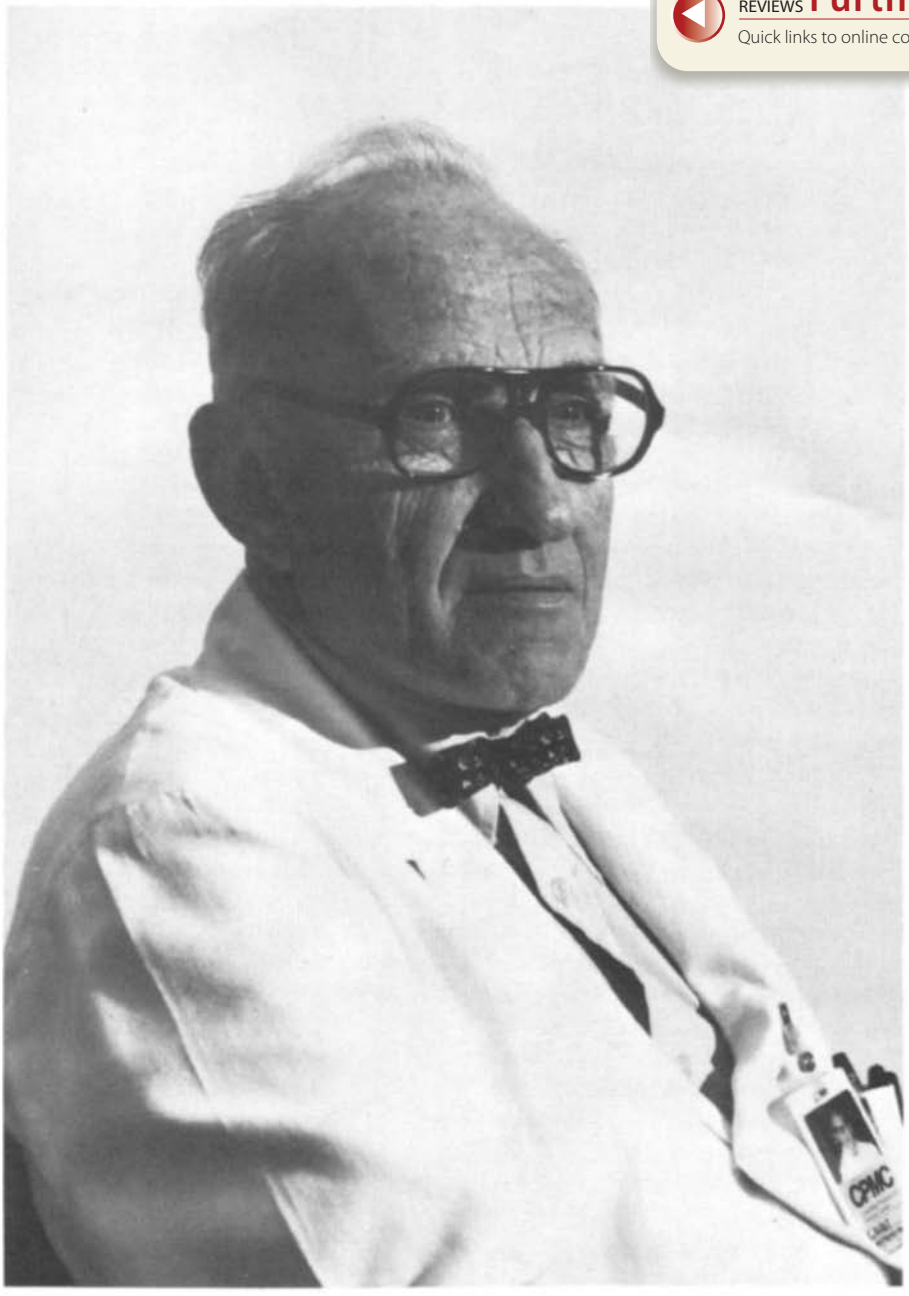

Elvin A. Kabat

Ann. Rev. Immunol. 1983. 1:1–32

GETTING STARTED 50 YEARS AGO—EXPERIENCES, PERSPECTIVES, AND PROBLEMS OF THE FIRST 21 YEARS

Elvin A. Kabat

Departments of Microbiology and Human Genetics and Development, and the Cancer Center/Institute for Cancer Research, Columbia University College of Physicians and Surgeons, New York, New York 10032; and the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

This first volume of the *Annual Review of Immunology* will appear just 50 years after I began working in immunochemistry in Michael Heidelberger's laboratory at Columbia University's College of Physicians and Surgeons. The differences between then and now in the path for a graduate student, postdoctoral, and beginning independent investigator are striking, and I thought for this prefatory chapter I would recount some of the experiences of my first 21 years in the field, considering not only the work but also the outside economic and political influences on my career, as well as World War II and its aftermath of loyalty and security investigations and the Senator Joseph McCarthy period.

On January 1, 1933, I began working in Michael Heidelberger's laboratory as a laboratory helper. The definitive paper on the quantitative precipitin method by Heidelberger and Forrest E. Kendall had appeared in the *Journal of Experimental Medicine* in 1929 and the laboratory was well on its way to providing analytical chemical methods for measurement of antigens and antibodies. This paper furnished the key to modern structural immunology and immunochemistry, which, not without strong resistance from the then classical immunologists, changed our way of thinking.

0732-0582/83/0410-0001$02.00

I had received a BS degree in September, 1932, from the City College of New York and had majored in chemistry. I was 18 years old. The country was in the depths of the depression and my family had suffered acutely. I had been walking the length and breadth of New York visiting universities and hospitals looking for a job, literally walking, since I rarely had a nickel to take the subway and if I had only a nickel I used it for lunch. My mother had started selling dresses in our apartment; by chance Mrs. Nina Heidelberger had become one of her customers and she suggested I go up to see Michael. At the end of October or early November of 1932, he offered me a job at a salary of $90 a month to begin on the first of January, and the possibility of a future for me and for my family began.

Michael had hesitated about giving me the job since I had been to college and the previous incumbent had only been a high school graduate. I assured him that I would do the routine, which involved making solutions, keeping the laboratory clean, and washing glassware when Mary O'Neill, our half-time glassware worker, was ill, etc, provided he would let me do as much technical work and research as I was capable of doing. Hans T. Clarke, the Chairman of the Biochemistry Department at P and S, had interviewed me in connection with my application to do graduate work and had accepted me as a PhD candidate. I had explained that I would be unable to begin until I found a job, but this was the norm during the depression. Many graduate students had part- or full-time jobs; some were teaching at City College or elsewhere and were doing their graduate studies part-time at Columbia. Of the $90 a month, I had to give my parents $50 toward paying the rent and I had to pay the Columbia tuition, then $10 a point per semester, for my courses, plus registration and laboratory fees.

My hours were 8:30 A.M. to 5:00 P.M., and beginning in February, 1933, I took an evening course in experimental physical chemistry with Professor Charles O. Beckman. My graduate courses were taken in the evening or late afternoon, or during the summer session, except for those given at P and S. During the summer of 1933 I took the course in medical bacteriology given by Calvin B. Coulter. We became good friends and he, Florence Stone, and I collaborated in a study of the ultraviolet absorption spectra of a number of proteins I had prepared in Michael's laboratory. Through him I met Arthur Shapiro, then a medical student, who was full of ideas. We became very close friends; he was one of the very first to see the potentialities of bacterial genetics and several years later Sol Spiegelman worked with him. One of the required courses, which was given only in the daytime at the downtown campus, was quantitative organic analysis and students had to spend lots of extra time in the lab. My job made this difficult, so Hans Clarke, who had written the classical text in the field, gave me 18 compounds to identify and I did these in Michael's lab.

The Department of Biochemistry was at its peak in reputation and productivity with Michael, Rudolf Schoenheimer, Karl Meyer, Erwin Chargaff, Erwin Brand, Sam Gurin, David Rittenberg, and Sam Graff. Graduate students worked in a large laboratory on the fifth floor and included Joe Fruton, Lew Engel, David Shemin, De Witt Stetten Jr, Sarah Ratner, Konrad Bloch, William H. Stein, Abe Mazur, and Ernest Borek, and somewhat later Seymour Cohen and David Sprinson. I got to know W. E. van Heyningen, who came to us as a postdoctoral fellow. Since I worked directly in Michael's lab my contacts with the other students were much less close. My interests also led me to be close to the Bacteriology Department, of which Frederick P. Gay was Chairman and whose members included Beatrice Seegal, Theodor Rosebury, James T. Culbertson, Claus Jungeblut, Maxim Steinbach, Calvin Coulter, Florence Stone, Sidney J. Klein, and Rose R. Feiner. I attended both the weekly biochemistry and microbiology seminars. Professor Gay, who had been closely associated with Jules Bordet and was still waging the Paul Ehrlich-Jules Bordet war of antibodies as substances rather than as vague properties of immune serum, was strongly anti-immunochemical and became very impatient with my questions at seminars. This view came across clearly to the medical students and Edward H. Reisner, P and S '39, wrote the following little ditty, which I learned about from Oscar Ratnoff.

> Pasteur inspired Metchnikoff and Metchnikoff was nuts,
> He had a pupil named Bordet who hated Ehrlich's guts,
> Now Ehrlich had the goods you know, but this we dare not say,
> For we descend from Metchnikoff through Bordet out of Gay.

The Heidelberger laboratory in 1933–37 consisted of Forrest E. Kendall, who was working on pneumococcal polysaccharides and on the mechanism of the precipitin reaction, Arthur E. O. Menzel, who was studying the proteins and polysaccharides of the tubercle bacillus, Check M. Soo Hoo, our bacteriologist, myself, and Mary O'Neill. Several visiting scientists, among whom were Henry W. Scherp, Torsten Teorell, D. L. Shrivastava, Alfred J. Weil, and Maurice Stacey, came for varying periods, the longest being 1 year. When Forrest left in 1936 to go to Goldwater Memorial Hospital with David Seegal, Henry P. Treffers, who had just completed his PhD with Louis P. Hammett at Columbia, replaced him.

Forrest Kendall worked opposite me and taught me the micro-Kjeldahl method, which in those days was the basic tool in the laboratory since all quantitative precipitin assays on the washed precipitates were finally analyzed for total nitrogen. Digestions were carried out in 100-ml flasks. The working range was between 0.1 and 1.0 mg of N. I generally was responsible for doing all of the digestions for Michael and Forrest, and after I acquired

sufficient skill I also carried out the distillations and titrations for Michael. It was not unusual to run 40 distillations a day. I also ran many quantitative iodine determinations on thyroglobulin, using the method developed by Professor G. L. Foster in the Biochemistry Department, until Herbert E. Stokinger became a graduate student with Michael and was given the thyroglobulin problem.

The laboratory was a very exciting place in which to work and I generally kept Forrest busy asking him questions, no doubt interfering often with his train of thought. He was very patient and helpful, but Michael once suggested that I had better put some of my questions to him rather than to Forrest. I was always able to get answers or was told where to find them.

After a few months, as Michael has described (2), I suggested that one might use a well-washed suspension of heat-killed bacteria of known N content to remove antibody and measure antibody N as agglutinin. Michael said that I could do this so I started with type I pneumococci. The method was very successful, provided one took care in growing the organisms to avoid autolysis. What happens when this procedure is not strictly adhered to has already been published (3).

The combined application of the quantitative precipitin and quantitative agglutinin methods permitted us to establish that the antibody to the type-specific polysaccharide of pneumococci was the same whether measured as precipitin or as agglutinin. Naturally, the bacterial suspension was able to remove antiprotein, and this was measured independently with a suspension of rough unencapsulated pneumococci. In those days we were only beginning to be aware of antibody heterogeneity, that our type-specific antibody was a complex mixture of antibodies, some of which were non- or co-precipitating, and that all of these were being measured as agglutinin or as precipitin. Michael and I also studied the course of the quantitative agglutinin reaction and showed that it followed the empirical equation he and Forrest had described for the quantitative precipitin reaction, and which they later derived from the Law of Mass Action.

The quantitative agglutinin work was supposed to be my PhD dissertation; the first two papers on the method and on the identity of agglutinin and precipitin had been published in the *Journal of Experimental Medicine* in 1934 and 1936. These, as reprints, and the third part on the mechanism of agglutination were submitted early in 1937. However, the University's rules had been changed so that the student was required to be the first author on any publication and Michael had been the first author on the two papers. I could of course have used the third part as the thesis, but I had also been studying the quantitative precipitin reaction between R-salt-azobiphenylazo-serum albumin and rabbit antibody to serum albumin. This system had certain unsuspected unique aspects since the introduction of the

haptenic group had not altered the reactivity with anti-serum albumin. Michael suggested that I write this up and use it as my PhD dissertation, which I did in about 3 or 4 weeks. It created a minor sensation at the Faculty of Pure Science office when I brought down the copies of the new dissertation and took back the other.

Arne Tiselius had suggested some time earlier that it would be nice to have someone from Michael's laboratory to study the physicochemical properties of purified antibodies at Uppsala and that the Rockefeller Foundation might provide a Fellowship. He had just developed the moving boundary method of electrophoresis with the rectangular cell, which first made possible precise quantitative estimates of purity and permitted resolution of mixtures of proteins. Michael had spent two summers in Uppsala working with Kai O. Pedersen and with Tiselius and had suggested me. This was an extraordinary opportunity to learn the newer methods of fractionating and characterizing macromolecules in the Svedberg laboratory. Frank Blair Hanson came to interview me from the Foundation and I was awarded the Fellowship.

The Fellowship paid $125 per month. On the original application there was a question as to whether one had any special financial obligations and I had noted that I gave my parents $50 a month. When I received the award, it specified $125 per month. I went to the Foundation's offices to discuss whether I could get along on $75 per month. They looked at my original application and assured me that $50 would be sent to my parents and that I would receive the full $125. I made sure that I did not spend much of my first month's stipend until several weeks later when a letter from my mother arrived (this was before transatlantic airmail) saying that the first check had been received promptly on September 1, 1937. After that I was able to live quite comfortably.

The Rockefeller Foundation also paid for cabin class transportation from New York to Uppsala. Since I was entitled to a month vacation for my work with Michael, I wanted to spend it in Europe and especially to visit the Soviet Union, to which at that time I was very favorably inclined, predominantly because of the great economic suffering of my family during the depression and also because of Russia's United Front policy in opposition to Hitler and their support of the Spanish loyalists. The Rockefeller Foundation agreed to give me what it would cost them to send me directly in cabin class and I could go anywhere I chose. In those days if one purchased a tourist class ticket on the Queen Mary, one could go by rail second class anywhere in Europe for $10 extra. I chose Leningrad, spending several days in Paris and Warsaw. For $50 one could spend 10 days in Leningrad and Moscow with all hotels, meals, travel between cities, and guided tours to places of interest included. In Paris I visited the Institut Pasteur, saw the

Pasteur Museum, and met Gaston Ramon, who invited me to lunch at his home where I met André Boivin and Lydia Mesrobeanu. In the Soviet Union I was unable to see any scientists and so I was essentially a tourist visiting museums, factories, etc, on standard guided tours. I then spent a day or two in Helsinki, took the boat to Stockholm and the train to Uppsala, and arrived at the Institute early in September.

Before leaving and in anticipation of my being able to go to Uppsala, Michael had suggested that we immunize several different species with suspensions of pneumococci, so Soo Hoo and I injected two pigs and a monkey. These immunizations were less traumatic than those we had done earlier, injecting two goats (4). Rabbit and horse antisera were available in the lab and a cow was immunized at Sharpe and Dohme. Torsten Teorell with Forrest and Michael had found that a given quantity of pneumococcal polysaccharide precipitated less antibody in 15% salt than in physiological saline, due to a shift in the combining proportions at equilibrium when the reaction was carried out in high salt. Michael and Forrest then used this finding to purify antibodies to polysaccharides by precipitating the antibody from a large volume of antiserum with polysaccharide under physiological conditions of 0°C, washing repeatedly with cold saline, and extracting the washed precipitate with 15% NaCl at 37°C to dissociate a portion of the antibody. With individual antisera as much as 15–30% purified antibody could be obtained and over 90% was precipitable by polysaccharide. This was the first time substantial amounts, tens of milligrams, of antibody could be prepared. Michael and I also extended the method by using a suspension of bacteria to remove the antibody followed by washing and elution with 15% salt. These purified antibodies plus various antisera and antigens were shipped to Uppsala and were available when I arrived.

The laboratory was at a peak in its productivity with Professor Svedberg popping in and out, with Arne Tiselius, then a docent, and with Kai O. Pedersen and Ole Lamm. Numerous visitors came for various periods, including Basil Record from Haworth's laboratory, Frank L. Horsfall from the Rockefeller Institute, whose interests were similar to mine, J. B. Sumner with crystalline urease and concanavalin A, G. Bressler from Leningrad, G. S. Adair from Cambridge, and Gerhard Schramm from Germany.

Professor Svedberg was very anxious to test his new separation cell, which had a membrane dividing the cell into an upper and a lower compartment. Using some of my horse antipneumococcal serum, he centrifuged it until the 18S peak had gone below the membrane. I tested both proteins and found all of the antibody in the lower compartment.

I had also brought some of the crystalline horse serum albumin used in my PhD thesis. Tiselius was very surprised when we examined it by electrophoresis at a concentration of 0.5 or 1% and saw only a single peak. He

said it was the first time he had seen a protein that was a single component electrophoretically. Although new techniques provide more and often better criteria for establishing purity, they frequently only show that not enough care was taken in purifying the material. The need for such care was most forcefully demonstrated years later when Knight (8) examined by paper chromatography Emil Fischer's collection of 34 synthetic peptides made half a century earlier, which his son Hermann had brought to Berkeley. All but three, which contained a trace of one of the constituent amino acids, gave a single spot.

I got to work right away with the help of Kai Pedersen learning to run the oil turbine ultracentrifuge and the diffusion apparatus to measure the molecular weights of the antibodies I had brought. Surprisingly, the horse antibodies were quite polydisperse. We were at a loss to understand this. Fortunately, the State Serum Institute had a horse that had been under immunization for a short time, and on purifying its antibody by the salt dissociation method we found a single 18S peak. All of the other antibodies gave multiple peaks, as did a second sample from the same horse after further immunization. Therapeutic use of antipneumococcal horse sera in Sweden had not been very successful, so I was able to get serum from humans recovering from lobar pneumonia who had not received antibody, through the courtesy of Jan Waldenström. I tested the serum of quite a few convalescents, found one with about 1 mg of antibody protein per ml, purified the antibody from about 40 ml of serum, and measured its molecular weight.

Arne Tiselius and I studied the electrophoretic properties of antibodies. One of the rabbit hyperimmune anti-ovalbumin sera I had brought had 36.4% precipitable antibody. We examined its electrophoretic pattern before and after removal of the antibody and found, by the decrease, 37.2%, in area of the gamma globulin peak, that we had removed the same proportion of the total serum protein. This definitely established that these antibodies were gamma globulins [now immunoglobulin (Ig) G, one of the five major immunoglobulin classes]. The electrophoretic patterns we published appear in most textbooks of immunology.

I also spent a considerable amount of time studying specific precipitates of ovalbumin rabbit anti-ovalbumin and horse serum albumin–rabbit antiserum albumin dissolved in excess antigen both by ultracentrifugation and by electrophoresis, calculating the composition of the soluble complexes. This was all done by the Lamm scale method, which was very tedious and especially hard on the eyes. Before my return to the US I left a manuscript with Arne Tiselius, which, because of the war, never was published, although he cited our work and an electrophoretic pattern showing the soluble complexes as a schlieren band migrating behind the ovalbumin band

appears in Tiselius' Harvey Lecture (10). It remained for Jonathan Singer and Dan Campbell to do the definitive study. By that time, the automated Schlieren method had replaced the laborious scale method and work was much easier.

I have already described my experiences at the Nobel Ceremony in December 1937 (6).

The terms of the Rockefeller Foundation Fellowship required that the sponsor permit the Fellow to return to a position in the laboratory from which he came and Michael had assured the Foundation he would do so. However, he told me he would do his best to find me a more independent position, and some months after I had arrived in Uppsala I received a letter from Jacob Furth offering me a position as Instructor in Pathology at Cornell University Medical College at $2400 per year beginning September 1, 1938, to work on viruses causing tumors and leukemias in chickens. Michael advised me to accept and I did.

I believed it was important for me to visit certain laboratories before returning to the US and discussed this with Harry Miller of the Rockefeller Foundation. They tended to discourage such travel but finally agreed that I could leave Uppsala about July 15 to visit Linderstrom-Lang and the State Serum Institute in Copenhagen; J. D. Bernal, I. Fankuchen, and John Marrack in London; F. G. Hopkins in Cambridge; W. N. Haworth and Maurice Stacey in Birmingham; Hans Krebs in Sheffield, where I spent 4 days learning to do some enzyme reactions in the Warburg apparatus; Gorter in Leyden; and den Dooren de Jong in Amsterdam, finally going to Zurich to attend the International Physiological Congress before returning to the US. All of these visits established lasting friendships and contacts with junior as well as senior investigators, and in my report to the Rockefeller Foundation I emphasized the desirability of providing similar opportunities to their other fellows.

In Zurich in August, 1938, everyone was very disturbed about the situation in Spain where the Franco forces were about to or had already cut Catalonia from the rest of loyalist Spain. Many of the members of the Loyalist government were physiologists, including Juan Negrin, the Prime Minister, and Cabrera, the Foreign Minister, and a good-sized delegation attended the Congress. A group of us felt we should do something to express our support and organized a dinner at the Congress, which was very well attended and raised a considerable sum. I was anxious to go to Spain to find out what people in the US could do to help, and it was arranged for me and Lew Engel to go to Spain for a few days before sailing for the US. My US passport was not valid for travel to Spain so Dr. Cabrera wrote on a sheet of paper that all border patrols should permit me to enter and leave Spain without making any marks in my passport. We took a train from Zurich

to Perpignan, France, and to Cerbère, the usual crossing point, thinking it would be easy to get across. I very soon had a French soldier with a bayonet prodding me along until I got on the train back to Perpignan. Wondering what to do we decided to visit the Spanish consul in Perpignan. When he saw the Cabrera note, he told us to go out and get pictures taken, and when we returned he affixed them to a Spanish Carte d'Identité on the back of which he had written the contents of the Cabrera letter (Figure 1), summoned his car and chauffeur, and had us driven from Perpignan to the Hotel Majestic in Barcelona. The Hotel Majestic was the center for foreign correspondents and there I met Herbert L. Matthews, the *New York Times* correspondent. Food was very scarce and I was hungry all the time, most especially after the rationed meals. Indeed, when I left a French dining car, on my way back, I emptied the bread tray into my pockets. Most of my time was spent visiting hospitals where the wounded Americans of the Lincoln Brigade and other International Brigade volunteers were being treated. I vividly remember speaking to one wounded anti-fascist German soldier who felt his problems were not being understood because he spoke only German; I was able to translate his wishes into English to one of the doctors.

On returning to New York, I immediately began to work at Cornell with Jacob Furth. Eugene Opie, the Chairman of the Pathology Department, had built up an important department with Jacob, Murray Angevine, Robert A. Moore, and Richard Linton, who had been at Columbia and with whose work in India on cholera antigens I had become familiar. Vincent du Vigneaud was Chairman of Biochemistry and permitted me to attend their weekly seminars. I became close friends with W. H. Summerson and soon collaborated in studies with Dean Burk, Otto K. Behrens, Herbert Sprince, and Fritz Lipmann, who had left Germany and arrived shortly thereafter from Denmark. Cornell Medical School was not anxious to have too many Jews on its staff and du Vigneaud, who had made a definite offer to Lipmann, indicated that he would resign if the appointment did not go through.

At Cornell the main problem Jacob Furth wished me to work on was to purify the virus of one of his chicken strains that caused leukosis if injected intravenously and tumors like the Rous sarcoma if injected subcutaneously. The approach was essentially to centrifuge crude extracts in the Pickels air-driven ultracentrifuge, following the biological activity by assays in baby chicks, as well as by nitrogen to estimate the extent of purification. I wanted to buy a micro-Kjeldahl apparatus, but a previous postdoctoral fellow with Jacob had purchased a Dumas apparatus. Jacob, considering me a chemist and wishing to save money, wanted to get the Dumas working. I had to insist that this was not a suitable method if one had to run many determinations and he finally consented to buy a micro-Kjeldahl apparatus; he was

REPUBLICA ESPAÑOLA

CONSULADO DE ESPAÑA
EN
PERPIÑAN

EL CONSUL DE ESPAÑA EN PERPIÑAN

CONCEDE AUTORIZACION para volver en ESPAÑA y regreso

por la frontera de La Junquera a Elvin Kabat

nacido el 1 de septiembre de 1914

en Nueva York Provincia de Estados Unidos

de estado soltero y profesión Químico -

Motivo del viaje Particular.

y por tanto ruega á las Autoridades civiles y militares, nacionales y extranjeras, que no le pongan impedimento en su viaje, antes bien le presten todo el favor y ayuda que necesitare.

Dado en Perpiñan á 20 de agosto de 1938

EL CONSUL DE ESPAÑA,
p. d. EL CANCILLER

Firma del portador,

Elvin Kabat

Y Campy

CONSULADO DE ESPAÑA

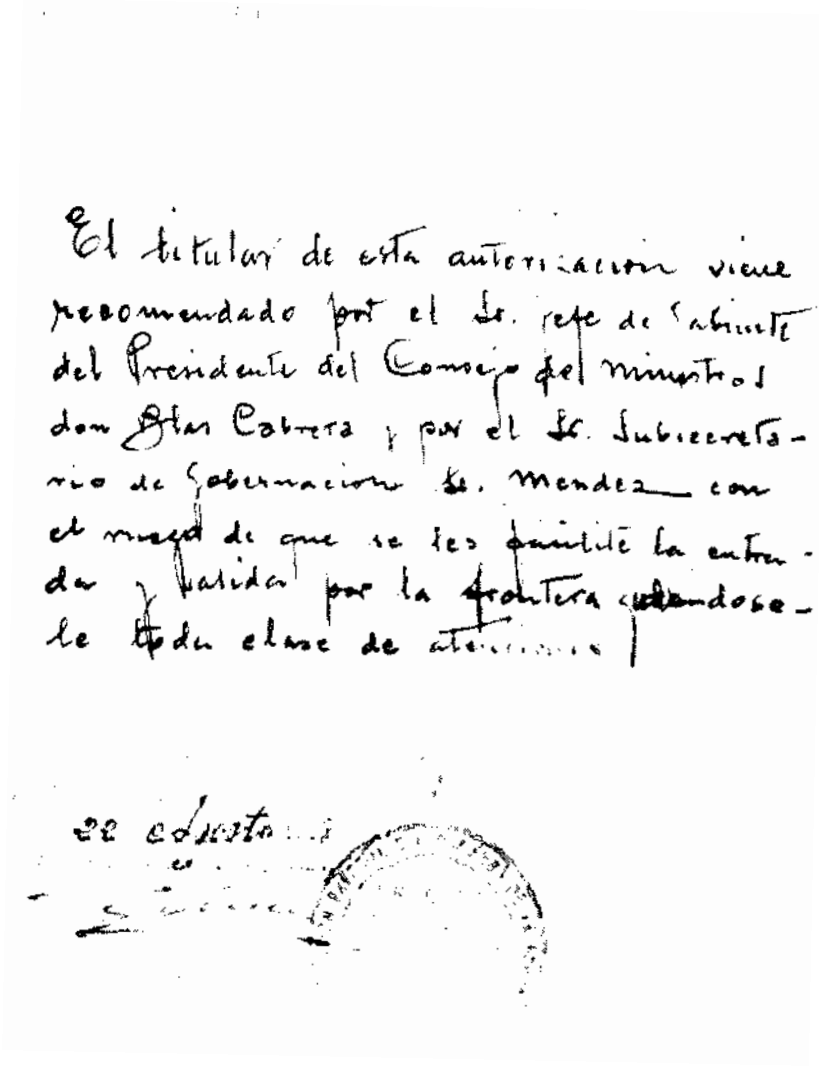
El titular de esta autorización viene recomendado por el Sr. jefe de Gabinete del Presidente del Consejo de ministros don Blas Cabrera y por el Sr. Subsecretario de Gobernación Sr. Mendez con el ruego de que se les facilite la entrada y salida por la frontera [illegible] de toda clase de atenciones

Figure 1 Spanish Carte d'Identité, with the Foreign Minister's letter.

very happy when he saw 40 to 50 analyses a day coming out immediately and told me that they had tried to use the Dumas for a whole year without a single successful result.

Albert Claude had essentially done a study with Rous sarcoma virus similar to what we were planning and had found that high-speed sedimentable materials were present in normal tissues. This was all in the days before subcellular organelles, mitochondria, microsomes, etc, and it took me a long time to convince Jacob that one could not get a pure virus just by centrifugation of tumor extracts. Among our interesting findings at that time were the demonstration that alkaline phosphatase and the Forssman and Wassermann antigens were associated with these high-molecular-weight particles. We also prepared rabbit antisera, which we used to try to characterize these materials.

Jacob also suggested that I try to work out some histochemical methods for localizing enzymes in tissues. I was just getting started when George Gomori published his elegant method for localizing alkaline phosphatase in paraffin sections of alcohol-fixed tissues. We adopted it and carried out a study of the distribution of alkaline phosphatase in normal and neoplastic tissues. The stained sections were very impressive; colored lantern slides were just coming into use and Jacob thought we should have a colored plate for our paper in the *American Journal of Pathology,* which would cost $400. This was considered too expensive. It was finally arranged that the Department of Pathology would pay $100, the research fund would pay $200, and Jacob and I would each pay $50. It was a very worthwhile investment because it stimulated much work on the subject.

In Europe I had hoped to visit Kögl's laboratory at Utrecht; he had startled the world by announcing that malignant tumors had large amounts of D-amino acids instead of L-amino acids. Much of his data could be accounted for by racemization, but from one tumor several grams of D-glutamic acid had been isolated. Naturally, everyone began to try to check this and Jacob got me some tumors, thinking that after I established that I could isolate D-amino acids I could then work on leukemic cells, which were available in much smaller amounts. However, I worked up a number of tumors but only found L-glutamic acid. Two confirmations of Kögl's work were soon reported and it seemed clear that I was not a very good chemist. The problem was solved by the genius of Fritz Lipmann, who suggested that after hydrolysis, one could add D-amino acid oxidase and estimate D-amino acid concentrations by using the Warburg apparatus. He, Dean Burk, Otto Behrens, and I soon showed that there were no significant amounts of D-amino acids. David Rittenberg at Columbia applied the isotope dilution method and also failed to find D-amino acids. This turned out to be one of the instances of falsification of data, someone having apparently

added D-glutamic acid to Kögl's hydrolysates. It led to my coining an aphorism: "Every incorrect discovery has always been independently verified."

In our efforts to purify the tumor viruses, we frequently found that saline extracts of tumors were extremely viscous, and from one chicken with a tumor, we were able to get a considerable quantity of this very viscous fluid. The viscosity reminded me of the pneumococcal polysaccharides I had prepared. I added some sodium acetate to the fluid and then two volumes of ethanol and got an extraordinarily stringy precipitate, almost all of which adhered to the stirring rod and could be removed. Karl Meyer at P and S had reported the isolation of hyaluronic acid as behaving similarly and I said to Jacob, who was watching me precipitate the polysaccharide, that it looked like hyaluronic acid. He became somewhat upset that I could make such a statement from so little evidence, but when I added a little hyaluronidase provided by Karl Meyer, the viscosity disappeared. We learned it was essential to treat our tumor extracts with hyaluronidase before ultracentrifugation. David Shemin, who had been at City College with me, had received his PhD in biochemistry at Columbia, and had taken a job there in the Department of Pathology with James Jobling, the Chairman, working on Rous sarcoma, had similar findings.

Dean Burk was also very interested in tumor metabolism and he, Jacob, Herbert Sprince, Janet Spangler, Albert Claude, and I carried out studies in the Warburg on chicken tumors.

On the international scene my stay at Cornell was the period of the Munich agreement, the conquest of Czechoslovakia by Hitler, the Nazi-Soviet pact, the partition of Poland between Germany and Russia, and the Finnish-Soviet war. These events shook me and I began to worry about my political views.

About the spring of 1940, Tracy J. Putnam, Director of the Neurological Institute at Columbia, wanted an immunochemist to work on multiple sclerosis and asked Michael Heidelberger to suggest someone. Michael gave him a choice, assuring him that if he picked me he would have a strong personality on his hands. He invited me for an interview and said that he understood that I had lots of my own ideas and that I could work on whatever I wished except he hoped I would not discriminate against neurological problems. The salary was $3600 per year and Hans Clarke had agreed that I would have an appointment as Research Associate in Biochemistry (assigned to Neurology). Funds were available for a technician; two laboratories were available in the Neurological Institute, which had formerly been intern's bedrooms, plus some space shared with the clinical laboratory. It took little thought on my part to decide to accept. Robert F. Loeb, for whom I had the greatest admiration and affection, was then

Associate Director of the Neurological Institute and was a great attraction for me. I was especially thrilled when I met him at Woods Hole in the summer of 1940 and he came over to me saying, "Elvin, we need you, we can hardly wait until you get to Neuro."

I arranged to leave Cornell at the end of May, 1941, taking June as my terminal vacation. I started at P and S and the Neurological Institute on June 1, 1941, and took my vacation later in the summer.

My laboratories were temporarily occupied by Norman Weissman, a friend who had gotten his degree in biochemistry, and Murray Glusman, with whom I was to collaborate years later when he returned from a Japanese prison camp. Harold Landow, a resident in neurology, was interested in collaborating. We decided to look at the electrophoretic patterns of cerebrospinal fluid in the Tiselius electrophoresis apparatus. Dan H. Moore, who had come from Lederle laboratories where he had studied horse antisera electrophoretically, had set up a laboratory for electrophoresis and had an air-driven ultracentrifuge. For the Tiselius electrophoresis, the cerebrospinal fluids had to be concentrated by pressure dialysis to a small volume and had to be run in a 2-ml micro cell. Large amounts of fluid were available from pneumoencephalograms and we arranged to obtain these. We readily obtained good patterns on concentrated cerebrospinal fluid and showed that patients with multiple sclerosis often had substantial increases in the gamma globulin fraction, as did patients with neurosyphilis, and that positive colloidal gold tests correlated with increased gamma globulin. This led naturally to a study of serum proteins with Franklin M. Hanger, which showed high levels of gamma globulin to be responsible for positive cephalin flocculation tests in various sera. These findings provided some insight into the mechanism of these two clinical diagnostic tests.

At the same time, Harold Landow and I began a quantitative study of passive anaphylaxis in the guinea pig. Oddly enough, although the antibody N content of rabbit antisera could be determined by quantitative precipitin analysis, nobody had measured how little antibody was required to sensitize a guinea pig so that fatal anaphylaxis would result on subsequent administration of antibody. Our data showed, with anti-ovalbumin and anti-pneumococcal serum that 30 μg of antibody N sensitized 250-g guinea pigs, so that fatal anaphylaxis would result if 1 mg of ovalbumin or of pneumococcal polysaccharide were given 48 hr later. This was the beginning of a series of studies on quantitative aspects of allergic reactions. In the summer of 1940, while still at Cornell, Mary Loveless and I had worked at Woods Hole, trying to measure uptake of skin-sensitizing antibody (later recognized as IgE) in sera of ragweed-sensitive patients, using a suspension of formalinized pollen with negative results. The skin sensitizing power of the sera was removed, but no increase in N in the washed pollen was detectable.

As we now know from the work of the Ishizakas and Bennich, IgE is so much more active per unit weight than rabbit IgG that the method was not suitable. For similar reasons I also obtained negative results trying to measure uptake of bacteriophage by suspensions of bacteria.

Also while at Cornell Professor du Vigneaud had given me permission to use the Tiselius electrophoresis apparatus he had just purchased. I examined numerous leukemic sera for changes in protein pattern, but significant changes were not seen. However, a Bence Jones protein he also provided gave a beautiful homogeneous peak.

During the first weeks after my return to Columbia in June, 1941, I stopped in to see Alexander and Ethel Gutman, whose laboratories were opposite Michael's and from whom I had received advice while with Michael. They were studying myeloma sera in the Tiselius apparatus and showed me their patterns with their sharp peaks moving between β_2 and gamma globulin, the mobility of the peaks differing among individual sera. I asked them why they were not studying Bence Jones proteins since the mobility of my peak had also been between that of β_2 and gamma globulins. Al took out a pattern of a Bence Jones protein he had sent to someone several years earlier. There was a beautiful sharp peak like the one I had observed—it was labeled "boundary disturbance." We decided to add Bence Jones proteins from a myeloma patient to normal serum and compare the pattern with that of the patient's serum by itself; we found we could reproduce the patient's serum pattern in a number of instances by using Bence Jones proteins of different mobility. We then used a rabbit antiserum to urinary Bence Jones protein from a myeloma patient to measure the levels in the serum of the same patient.

I also continued the histochemical localization of enzymes, studying normal and neoplastic tissues of the nervous system with Harold Landow and William Newman, who came to us as a technician, later studied medicine, and became Professor of Pathology at George Washington University. Abner Wolf, who was Professor of Neuropathology, also was interested in this field. We collaborated closely on histochemical studies on acid and alkaline phosphatases and especially on producing and studying disseminated encephalomyelitis in monkeys by injection of brain tissue emulsified in Freund adjuvants, until the grant for this work was summarily terminated in 1953 during the hysteria generated by Senator Joseph McCarthy.

On the evening of June 22, 1941, I was at a party—everyone was discussing the rumors that Germany was going to attack Russia. I argued vigorously that Hitler would not, and when I had just about convinced everyone, we turned on the radio. This marked my retirement as a political prognosticator. It was far more tragic for mankind that Stalin was taken in than

that I was. I joined with many others to set up a group for Russian War Relief at the Medical Center. The doubts generated by the Nazi-Soviet pact were stilled.

On Sunday, December 7, 1941, I was working in the laboratory and heard the news about Pearl Harbor. No one knew what was happening but we decided to move acids and hazardous chemicals to a storeroom in the basement. It was clear that medical scientists, immunologists, and immunochemists were going to make important contributions to the war effort. Michael Heidelberger, who I saw very frequently since my return to Columbia, had been working for the Pneumonia Commission of the Board for the Control of Epidemic Diseases, US Army, on immunization against lobar pneumonia, using pneumococcal polysaccharides. I proposed that the type I (now group A) meningococcal polysaccharide, which had been purified by Geoffrey Rake and Henry W. Scherp, might be used for immunization in man. Meningitis had been a serious problem during recruiting in World War I. The Meningitis Commission under the Chairmanship of J. J. Phair was centered at Johns Hopkins and gave me an initial subcontract for $1000, renewed for a year at $2500, to purify type I meningococcal polysaccharide and study its antigenicity in man. I needed to order some equipment, which I did before going on a trip. When I returned I found that the order had not been processed. I phoned Dean Willard C. Rappleye, who said they were having a problem about whether my subcontract would receive overhead. I asked how much the overhead was and when he said $40, I shrieked into the telephone, "And for forty dollars you are holding up my work on immunization against meningitis with a war going on!" There was a moment of deathly silence and I expected to hear that I was no longer employed by Columbia. Instead, he said, "I'm sorry. I guess you are right. We'll order it right away."

From today's perspective it is easy to see how the Universities' attitudes on getting the incredible amounts of money they demand in indirect costs developed, to understand what happens to the reputation of the investigator who cannot bring in what they consider his share, and to marvel at the unanimity administrators exhibit in lobbying against any proposed reductions when they can agree on almost nothing else.

I hired Hilda Kaiser, whose husband, Samuel Kaiser, had been dismissed from Brooklyn College as a consequence of the New York State Legislature's Rapp Coudert Committee and later suggested to Michael that he hire Sam for the pneumonia work, which he did. All of the individuals fired from the City Colleges during that period were reinstated with apologies 40 years later, many posthumously.

We began to immunize medical student volunteers with type I meningococcal polysaccharide following Michael's schedule for pneumococcal

polysaccharides. In these and all my later studies on blood group substances, dextrans, levans, etc, I generally injected myself first with any new materials unless they were expected to cross-react with antibodies to antigens I had already received. We used Michael's precipitin assay with about 4 ml of serum per test and with Hilda Kaiser and Helen Sikorski were able clearly to demonstrate that precipitins were formed; C. Philip Miller and Alice Foster at the University of Chicago assayed the sera for protective antibody and we demonstrated that the polysaccharide was antigenic. Only 4 of 38 individuals, however, produced a significant antibody response and this seemed poor by comparison with the pneumococcal polysaccharides, so immunization was not considered promising. When immunization with meningococcal polysaccharides was taken up years later, radioimmunoassay was available and detection of an antibody response became thousands of times more sensitive. We too would have found detectable antibody by radioimmunoassay in a substantial proportion of the 38 subjects.

My laboratory was involved in two other projects during the war: one with the Office of Scientific Research and Development and the Committee on Medical Research on false-positive serological tests for syphilis; and the other for the National Defense Research Committee on the plant toxin ricin, a possible chemical-biological warfare agent, with a view toward developing methods of detecting it and protecting against its toxic effects. Our experiences in buying and immunizing two horses as part of this work have already been described (5).

Bernard D. Davis was assigned to the laboratory by the US Public Health Service to work on serological tests for syphilis. With Ad Harris and Dan Moore we studied the anticomplementary action of human gamma globulin and succeeded in purifying Wassermann antibody by absorption on and elution from lipid floccules.

For the studies on ricin, which were classified secret, Michael Heidelberger and I joined forces as co-responsible investigators. Ada E. Bezer came to work in my laboratory on this problem and we began an association that was to last over 20 years until she went to work with WHO in Ibadan, Nigeria. We were able to demonstrate by immunochemical methods that the toxic and hemagglutinating properties of ricin were due to different substances. This was later confirmed by Moses L. Kunitz and R. Keith Cannan, who independently succeeded in crystallizing ricin. One very puzzling observation was that the protective power of anti-ricin sera could be assayed readily in animals but did not correlate with the amount of precipitable antibody. We now know of course that ricin has a combining site for carbohydrates and therefore was reacting with and precipitating non-antibody gamma globulin and probably other serum glycoproteins, as well as antibody.

During the war years Manfred Mayer had replaced me in Michael's laboratory. He continued the study of the cross-reaction of types III and VIII antipneumococcal antibodies Michael, D. L. Shrivastava, and I had begun earlier. We became very close friends.

The war years had seen important changes in my personal life. Harold Landow, a brilliant neurologist and close friend, had committed suicide in 1940 because of his parent's objections to his marrying outside of his religion. In 1940 at a literary meeting I had been introduced to a young Canadian named Sally Lennick. She had come to New York from Toronto to study painting. We met again a year later. Then in the summer of 1942 we met at a party, began seeing one another frequently, and were married on November 28, 1942; our oldest son, Jonathan, was born on June 5, 1944.

It had become clear at this time that a book outlining the thinking and methodology of quantitative immunochemistry was sorely needed. Most workers using the quantitative precipitin method had learned it directly from Michael or from someone who had learned it from Michael and this was limiting growth of the field. I had been invited to write a review entitled "Immunochemistry of the Proteins" for the *Journal of Immunology* by Alfred J. Weil; it appeared in December, 1943, and I was astonished at the hundreds of requests for reprints. Manfred Mayer and I decided to write a text called *Experimental Immunochemistry,* which would not only give the methods in detail but would also outline principles and concepts so that it would be useful to students and workers who might think immunochemistry of value in attacking their problems. It would also include the preparation of materials needed for work in immunochemistry. We prepared an outline and submitted it to John Wiley, who did a survey and assured us that it would never sell even a thousand copies. Fortunately, Charles C Thomas came to visit Michael and immediately agreed to take it and invited Michael to write a preface.

We set right to work by dividing up the chapters and beginning to write. We usually met on Saturday or Sunday at one or another's apartment and read aloud what had been written, correcting, revising, and reordering sections. Our wives were left to entertain each other or to be bored by listening to what we had written. The book was sent to the publisher at the end of 1945, but because of paper shortages after the war, and various other delays, it did not appear until 1948. Fortunately, we were aware of many studies during the war years that were just being prepared for publication and insisted on doing extensive revisions in proof so that the book was up to date. It had a substantial influence on the field and the first edition went through four printings, the last being in 1958. The second edition, which appeared in 1961, also went through four printings.

After Pearl Harbor, a group of us at Columbia who were members of the

American Association of Scientific Workers devoted a considerable amount of time to considering what could be done to aid the war effort, especially with respect to defense and improving methods of immunization. The use of bacterial and viral warfare agents by the Nazis was considered a possibility and Theodor Rosebury and I undertook to prepare from the literature a report of the agents that might be used; we were assisted by Martin H. Boldt, then a medical student. We spent several months preparing the review, and Alphonse Dochez, who was an adviser to the Secretary of War, gave it to him. Naturally, it was withheld from publication by us voluntarily during the war. The Army was also very concerned about the possible military use of infectious agents and set up Camp Detrick near Frederick, Maryland, as a large-scale military research facility. Ted Rosebury started working there full time and I became a consultant, spending several days there each month. Our report was among the first items new personnel at Fort Detrick were given to read.

At the end of the war, when the Smythe Report on the Atomic Bomb was published, we felt it was in the public interest that our report on bacterial warfare should also be published and we requested clearance from the War Department. Several incidents arose. In our original request for clearance we stated in a footnote that we had withheld the report from publication during the war but that it would now be published with the approval of the War Department. In their letter stating that we could submit the review for publication, they requested that the words with the approval of the War Department be changed to "in view of the removal of war time restrictions" (Figure 2). We naturally complied. The *Journal of Immunology* indicated it would consider publishing it. In accordance with University policy we then gave the report for approval to Dr. Dochez, who was Chairman of the Bacteriology Department. Although he had originally taken the report to the Secretary of War, he said it had nonscientific implications and that he would have to take it up with Dean Rappleye. Rosebury and I were called to the Dean's office and were told the report might offend religious groups and that if we insisted on publishing it we should write our resignations on the spot. Since we were in no position to do this, and since our appeals about Freedom of Speech and the Press were unavailing, we put the report in a drawer. We also went to see Osmond K. Frankel, a leading civil liberties attorney, who told us essentially that we had unlimited freedom to publish but having done so we did not have the right to work for Columbia University. About 6 months later, Dean Rappleye came to Ted Rosebury's office and said that he had made a mistake, that the University did not intend to limit our freedom to publish, and we could submit the report for publication. We did, and it was published in the May, 1947, issue of *Journal of Immunology* (9), which also arranged to sell reprints. The report created a world-wide sensation (Figure 3).

[illegible] (Of a photostated letter, as follows:)

ARMY SERVICES FORCES
Camp Detrick
Frederick, Maryland

30 March 1946

Dr. Theodor Rosebury
Department of Bacteriology
Columbia University
630 West 168th Street
New York, New York

Dear Ted:

I took up with Captain Foy the matter of publication of your review on bacterial warfare.

It is his opinion that you are free to publish this in view of the fact that all restrictions on publication have been removed, that is to say, insofar as there is no direct relation to the War Department. Although you and your associates were identified with the War Department program in this field after preparation of the manuscript, it is very obviously unrelated to those experiences and might as well have been written by any scientist of equal competency and interest.

If it is to be submitted for publication, the footnote on the first page should be revised to exclude reference to the War Department. The note should, however, indicate the date of the original preparation, the fact that it was withheld from publication at that time, and that only minor editorial changes have been made in the text. In other words, the footnote should not imply that the War Department has reviewed the article and that the article has been revised to exclude material which still remains classified.

Captain Foy believes it is likely that the journal to which you submit the article may feel it necessary to submit it to the Bureau of Public Relations for clearance, in which case it would probably be referred back to us, but we would not recommend that you suggest such action to the journal or make any reference to clearance other than such as I have indicated.

I trust that this is clear and satisfactory to you and I hope that you will be able to find a publisher for the article. Frankly, I believe that it is good propaganda for us at this time. Perhaps I should not even call it propaganda since I feel that in general it is a fairly factual and well-balanced presentation of the subject matter. We both understand, of course, that were you given complete liberty you would on the basis of your recent experiences, amend and supplement considerably, but that of course is not permissible.

[illegible] draft of the footnote as revised before [illegible] publication.

[illegible]

COPY (Of a photostated letter, as follows:)

ARMY SERVICES FORCES
CAMP DETRICK
Frederick, Maryland

4 April 1946

Dr. Theodor Rosebury
Department of Bacteriology
Columbia University
630 West 168th Street
New York, New York

Dear Ted:

I have looked over the revised copy in connection with your proposed article on bacterial warfare and believe that it is now in conformity with Captain Foy's suggestions. You may, therefore, proceed to submit it for publication.

While in the Washington office yesterday I saw a draft of the article which Sidney Shalett has prepared for publication in Colliers. It appears to me to be permissible under our security classification, but as is true of most of the articles that are coming out now, perhaps makes for a rather further "bulge in the line". I suppose that is to be expected. The references therein to you, as to certain others, are perhaps slightly on the distasteful side, but I suppose that the press must be permitted some freedom in its own field, and has to work in a little "local color" in order to dilute the otherwise heavy fare of scientific data. I passed the paper along for General Loucks and General Waitt to have a look at it. You mentioned that you would like to see the copy before publication and I believe that this might be possible for you to arrange through the New York Times office there.

Sincerely yours,

(Signed) Oram

ORAM C. WOOLPERT, M.D.
Technical Director

OCW:pkm

EMPLOYEE'S EXHIBIT #16

Figure 2 Letters from the War Department.

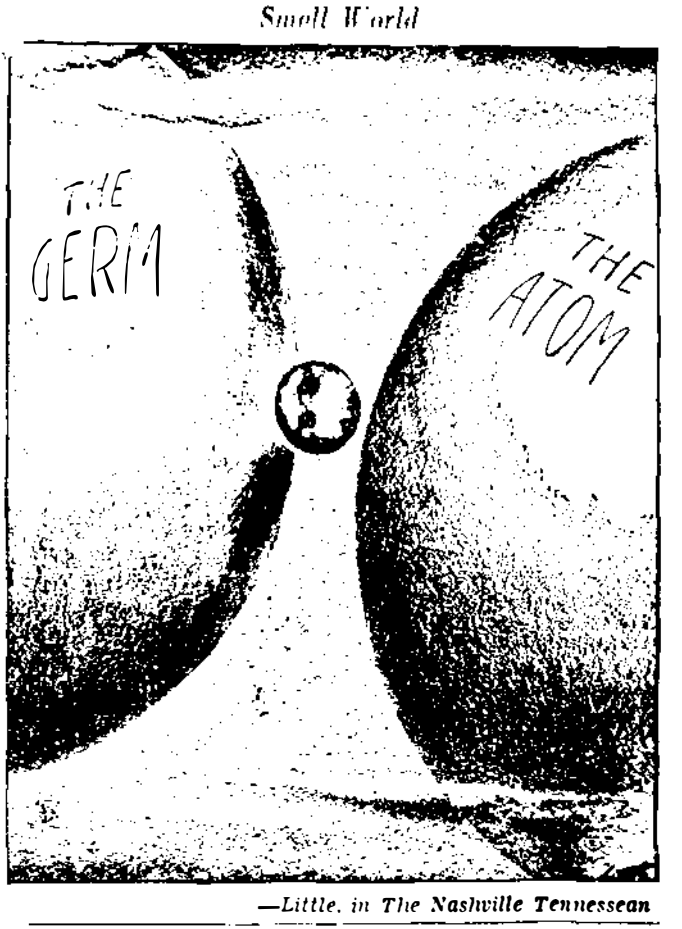

Figure 3 Political cartoon (by Tom Little, in *The Tennessean*) resulting from the published bacterial warfare article. (Reprinted with permission.)

When it appeared, Time magazine implied that we were procommunist and interpreted the statement in the footnote to indicate that we had not obtained clearance but had published it on our own "in view of the removal of wartime restrictions." Michael Heidelberger wrote a letter supporting our loyalty, which they published. At the same time Howard J. Mueller, Professor of Bacteriology and Immunology at Harvard, who had also been a consultant at Camp Detrick, wrote Dean Rappleye suggesting that Rosebury and Kabat should be fired because they had timed the publication of their report to appear when Congress was considering the War Department's budget so that they would get more money for biological warfare, although the war was over. Dean Rappleye knew that if any one had

determined if and when the report would appear it was he and not Rosebury and Kabat. However, we learned that our clearances for Camp Detrick were cancelled just after the report was published. The FBI was assigned to investigate us and the following episode with my landlord was told to me.

Sally and I were leaving for Europe on June 20, 1947, to attend the International Cytology Congress in Stockholm and the International Microbiology Congress in Copenhagen. I wrote my landlord, giving him the dates we would be away and enclosing two rent checks dated July 1 and August 1. We left our two children, Jonathan, age 3, and Geoffrey, born May 11, 1946, with my parents. Shortly after our departure, an FBI agent visited the landlord. The conversation as recounted to me went about as follows. Did you know the Kabats were leaving for Europe? Do you think they are ever coming back? He showed them the letter and the checks. The questions continued. If you were planning to leave the country wouldn't this be a good way to hide your intentions? My landlord naturally was very surprised when we returned on August 20.

In Stockholm at the Cytology Congress, there was substantial interest in the report on bacterial warfare and a group of Swedish microbiologists invited Sally and me to an elegant lunch at the Operkellaren; several of the guests were evidently assigned to entertain Sally while the others turned the topic of conversation to my report. Since Rosebury and I had been involved in research subsequent to our report, all of which was still secret, it would have been almost impossible for me to discuss anything without creating the impression that it represented the report plus additional classified information. Fortunately, suspecting what the lunch was about, I had put a reprint of the report in my pocket and in reply to each specific question, I merely read the pertinent sections written in 1941 and finally gave the reprint to one of the microbiologists sitting next to me.

At the end of the war, I took up two major lines of investigation. One, on the immunochemistry of blood group A and B substances, was to continue to the present day. The background and details of work in this area and the personal aspects have been described by me recently (7). The second was an attempt, together with Abner Wolf and Ada Bezer, to produce acute disseminated encephalomyelitis in monkeys because of its possible relation to multiple sclerosis. The use of the Pasteur treatment for rabies had been known to result in what were termed paralytic accidents, which involved disseminated demyelinating lesions in the brain and spinal cord. This had been shown by Tom Rivers and Francis Schwenkter to be due to the nervous tissue and they succeeded in producing the disease in monkeys by injecting suspensions of spinal cord tissue. Many injections were needed and the disease did not appear for a long period. Tracy Putnam had always been most interested in multiple sclerosis and was delighted when we got back

to this problem. Jules Freund had shown that one could get a very enhanced and protracted antibody response, as well as delayed type hypersensitivity, by incorporating antigens with mineral oil and killed mycobacterium by using an emulsifying agent, and I was anxious to see if this procedure could be used to produce disseminated encephalomyelitis in monkeys more rapidly and reproducibly. We found that after only one to three injections of brain tissue emulsified in the Freund adjuvant, the monkeys developed the disease and we sent a short note to *Science* early in 1946. While having dinner with Isabel Morgan during the Federation meetings I learned that she had made similar observations in monkeys she had been injecting with spinal cord tissue containing poliomyelitis virus with Freund adjuvants and then had omitted the virus with similiar results. She was to present her work at the Society of American Bacteriologists meetings in May. We decided that we would write up our detailed papers completely independently, then send them to one another to criticize and request that they be published side by side. Since her paper arrived a few days before ours had been typed, I gave the unopened envelope to Robert Loeb for safekeeping until I had mailed our manuscript. The results were completely concordant and both papers appeared together in the *Journal of Experimental Medicine* in January 1947.

The National Multiple Sclerosis Society had just been founded and sometime after our paper appeared I received a phone call from them saying they wanted me to apply for a grant. I said that I really was not interested in applying and didn't need the money. They replied that I "just have to" since they had received an anonymous contribution on condition that they used it to support our work. When I inquired as to the amount of the contribution and was told it was $10,000, I said that this was not really sufficient to make the more intensive effort they wished. I drew up an application for $64,350 for 3 years of support, which they showed the anonymous donor who generously provided the entire sum. This was the first grant made by the National Multiple Sclerosis Society. I was able to increase the size of my monkey colony from about 10 or 12 to 40. In 1950, the Public Health Service gave me a grant to continue this work, but it was cancelled in 1953.

By the end of the war my salary had increased to $4500 per year, but with the inflation following the removal of price controls, plus my growing family, I had to take a part-time job teaching elementary chemistry at City College. The multiple sclerosis grant provided an equivalent sum and made it possible for me to give up this outside work.

We spent the summer of 1948 in Woods Hole where we first met Fred and Sally Karush and their children. This was the beginning of a life-long friendship between the two families and Fred spent 6 months in my laboratory in 1950. The laboratory was then so crowded that he had to work a second shift beginning in the late afternoon.

Toward the end of that summer I saw a note on the bulletin board at the MBL that a house was for sale by the Professor of Microbiology at Washington University, Jacques Bronfenbrenner, whom I knew quite well. It took only a few minutes to arrange to buy the house. Our children became very attached to it and we spent part of each summer there during the years when they were growing up, except when I was on sabbatical. I would either work in the library or rent a laboratory bench. In 1949, I rented a laboratory bench, which then cost $50 for the summer, so that I could prepare an enzyme from snail hepatopancreas that split blood group substances. The business office at Columbia refused to pay the bill, saying that grants stipulated that the institution was to provide laboratory facilities. I assured them that the $50 entitled me to order and receive snails collected by the MBL boats and that if they would put snails on my desk in 48 hr I would do the work in New York—they paid the $50. Woods Hole was, of course, a marvelous place for making scientific contacts. In 1949, Shlomo Hestrin of the Hebrew University had the laboratory bench opposite me and we became very close friends.

Around this time a problem developed in my relationship to the Biochemistry Department at Columbia. For several years Tracy Putnam, the Professor of Neurology, had been asking Hans Clarke to promote me to Assistant Professor, but to no avail. This was in no way directed towards me personally, nor did it reflect any doubts about my work. Hans Clarke was a very fine person, but he was completely unaware of the need to promote people. He did not consider persons in other departments with titles in biochemistry as real members of his department, although he did not promote the regular members either. A substantial number of Research Associates had been in these positions for years, requests of the chairmen of the departments in which they worked being unavailing. Indeed, Walter W. Palmer, the Professor of Medicine, told me he had asked Hans Clarke to promote Michael Heidelberger to full Professor for 17 years before he agreed.

I broke the logjam of Research Associates by transferring to the Department of Bacteriology. The Chairman, Dr. Dochez, was very anxious to introduce immunochemistry into the medical teaching. In discussing my situation he suggested that he would be willing to give me an Assistant Professorship. The appointment was delayed for a year by the objections of Hans Clarke, who evidently didn't want to promote me or lose me, but it finally took effect on July 1, 1946. Louis Levin, who for about 10 years had been Research Associate in Biochemistry assigned to Anatomy, an appointment similar to mine, had left a year earlier for the University of Chicago because the Anatomy Department could not arrange his promotion. He was brought back as Assistant Professor of Anatomy and later went to the Office of Naval Research and the National Science Foundation.

After this, Hans Clarke agreed to several promotions of other Research Associates.

As Assistant Professor of Bacteriology, I was entitled to have graduate students and my first was Sam M. Beiser, who had been in the Navy and came to Columbia under the GI Bill. He was recommended by Arthur Shapiro. His PhD dissertation was on blood group substances from bovine gastric mucosa. The Academy of Allergy was just setting up a fellowship program (1). Even though Beiser was not working in allergy, at my suggestion that the fellowship might stimulate his interest in allergy, he received it and later devoted some time to work in this area. After two postdoctoral years with Bernard Davis, he returned to Columbia, rising to Professor and was Acting Chairman at the time of his death from cancer in 1972. Among his most important contributions were studies on antibodies to nucleotides with Bernard F. Erlanger. His bovine blood group glycoproteins have remained important standards and Michael and I are still using the pneumococcal C-polysaccharide he prepared.

My position in the Department of Bacteriology, the title of which was changed to Microbiology, and in the Neurology Department was eminently satisfactory over all these years. Tracy Putnam left in 1946 and was followed by Edwin G. Zabriskie, and in 1948 by H. Houston Merritt. Professor Dochez retired, and Beatrice Seegal became Acting Chairman of the Department of Bacteriology until Harry Rose was appointed in 1951. Drs. Zabriskie and Dochez had recommended my promotion to Associate Professor in 1947 and I was promoted in 1948. Harry Rose was also very supportive. Indeed, when he was offered the position as Chairman of Microbiology in 1951 he laid down two conditions—that I be promoted to full Professor and that my salary, which until then had come from grants, be paid out of departmental funds. This took effect on July 1, 1952.

By 1947, the laboratory had three major lines of investigation—immunochemistry of blood group substances (see 7), acute disseminated encephalomyelitis, and quantitative studies on allergic reactions. Edward E. Fischel, who was in the Department of Medicine, and I measured the amounts of antibody needed to produce Arthus reactions passively in the rabbit, and Grange Coffin, a medical student, and David M. Smith, a dental student, continued studies on passive anaphylaxis. Baruj Benacerraf came to see me in 1946, having completed medical school and a 1-year internship before serving in the Army and wanting to spend a postdoctoral year in the laboratory. There were no funds, but he told me he had independent means and came, as he later put it, on the "Benacerraf Fellowship." He was a very intense and dedicated worker and we studied quantitative aspects of the latent period in passive anaphylaxis and also the passive Arthus reactions in the guinea pig. John H. Vaughan spent two postdoctoral years in the

laboratory studying the skin-sensitizing properties of rabbit antibodies to ovalbumin and conalbumin in human skin.

We had found increases in the gamma globulin in the cerebrospinal fluid of patients with multiple sclerosis by electrophoresis, but this was impractical for diagnostic purposes. Murray Glusman, who had returned to the Neurological Institute after the war, Vesta Knaub, and I decided to measure the amounts of gamma globulin by using a microquantitative precipitin reaction. We also measured albumin immunochemically at the same time. It became clear that the method would give more informative results than the colloidal gold tests, and it became possible to follow gamma globulin levels in cerebrospinal fluid in patients over prolonged periods. David A. Freedman, Jean Murray, and Vesta Knaub measured the albumin and gamma globulin levels in 100 cases of multiple sclerosis and found 85 with elevated gamma globulin levels. In carrying out such a study it was essential not to report our findings until the diagnosis had been made in the absence of these data, otherwise clinicians who avidly grasp for objective data would soon gain a clinical impression of the value of the test and would not make their diagnosis until the test was in the patient's chart. Subsequent studies were carried out with Melvin D. Yahr and Sidney S. Goldensohn, and the quantitative precipitin method for determining gamma globulin in the cerebrospinal fluid of patients at the Neurological Institute became one of my routine responsibilities for 30 years, until it was superseded by more sensitive and automated immunochemical methods. I had many close friends and other colleagues at the Neurological Institute during this period, including Saul R. Korey, Harry Grundfest, and David Nachmansohn.

At the end of the war Abner Wolf had become an attending consultant in Neuropathology at the Bronx Veterans Administration Hospital. The Veterans Hospitals were encouraging research and were setting up substantial laboratory facilities. William Newman had received his MD and had gone there, as had another MD, Irwin Feigen, and Abner suggested that we continue studies on histochemical localization of enzymes there. I was appointed attending consultant and spent one afternoon a week there for several years.

While I was at the VA, President Truman issued an Executive Order initiating loyalty and security investigations of Federal employees with the following criterion: "Reasonable grounds must exist for the belief that one is disloyal to the United States." James B. Sumner, with whom I had been very friendly at Uppsala in 1937–1938 and who I had later met briefly on only one occasion in the US, went to the FBI to tell them that I had been a communist while in Uppsala. This initiated a series of FBI investigations on the basis of which I was presented with charges about the various organizations to which I had belonged or contributed during the late 1930s.

I had a hearing before the VA loyalty board, which dismissed me. I appealed finally to the Presidential Loyalty Review Board, which reversed the decision and reinstated me. I returned to the VA, but it was obvious that there were pressures to reduce further the rigidity of the criteria and the quality of the evidence upon which an individual could be dismissed, so I decided to resign. This essentially led to my giving up work on the histochemical localization of enzymes in tissues. The Presidential Loyalty Review Board was abolished by President Eisenhower, responding to pressures that it had been too lenient. Indeed, it was the only board whose members were not Federal employees and thus was not subject to pressures from Congress and from within the Executive branch. I was very pleased that I had carried out all my appeals without a lawyer.

The Bronx VA Hospital Loyalty Board that dismissed me had also written a letter to the passport office telling them that I should not be allowed to travel, and so my passport was cancelled; it was not returned when I was reinstated. I had been invited to address the First International Congress of Allergists in Zurich in the summer of 1950 but was unable to obtain a passport. I went to the passport office to discuss the matter and was told that if I gave the names of anyone I knew or thought to be a communist I could get a passport. Needless to say I was unable to travel until the decision of the US Supreme Court in 1955 that every American citizen had an unlimited right to travel, when I attended the International Congress of Allergology in Petropolis, Brazil. Baruj Benacerraf brought my situation to the attention of the Zurich Congress in his address.

I received the invitation to the Allergology Congress while in Woods Hole. It provided $1000 for travel expenses. I wrote a letter saying that I could not accept because I could not get a passport. I walked into town and dropped the letter in the box and walked across the street to the drug store to buy the New York Times. The headline announced the US Supreme Court Decision. I rushed to the Post Office and was able to retrieve my letter.

Sanford Elberg, with whom I became friendly at Camp Detrick, invited me to teach two courses at the University of California at Berkeley during the summer of 1950. Sally, Jonathan, Geoffrey, and I drove across country, stopping in Springfield, Illinois, to see the Lincolniana, in Denver, in Salt Lake City, and at Boulder (now Hoover) Dam. I had a very enjoyable time teaching—among the students or auditors were A. A. Benedict, Mel Herzberg, Fred Aladjem, and Keith Smart. I met and became friends with Ed Adelberg, Roger Stanier, Mike Doudoroff at Berkeley, and K. F. Meyer, Professor of Bacteriology at San Francisco. While in Berkeley I was invited to speak at the Naval Biological Laboratory, the Navy's equivalent of Camp Detrick. In this highly classified institution and at a time of considerable

hysteria, Keith Smart, in trying to shorten an obituary-like, overly long introduction, brought down the house by saying, "Dr. Kabat is a member of a large number of organizations, which I had better not mention." Of course he then felt obligated to list them.

In 1952 Congress appropriated $100,000 for research in immunochemistry in the budget of the Office of Naval Research (ONR). To evaluate what problems were worthy of support, William V. Consolazio, whom I had never met, and Louis Levin called a conference at P and S. About 10 or 15 people were present, including myself, Michael Heidelberger, and Dan Campbell. Everyone around the table indicated what problems he thought were important. I stressed use of immunochemical criteria of purity of proteins and polysaccharides. The meeting lasted until lunch time. When it was over and everyone was leaving, Bill Consolazio said to me, "You are staying here," and then asked, "To whom would you give money on the basis of the suggestions?" I outlined the programs I thought should be supported. He then said, "You have to take some money." I responded, "I don't need any money, I'm loaded with money." He gave me a yellow pad, saying, "You are not leaving here until you write out a proposal. You can hold on to the money and you can activate the contract any time within the next five years." I wrote out a title, "Immunochemical Criteria of Purity of Proteins and Polysaccharides," my name, the University's name and address, a short abstract, and a budget that provided for a postdoctoral fellow and supplies. When I came to overhead I asked, "What are you going to do about overhead?" At that time ONR was having a battle with Columbia—they would only pay 10% overhead, believing that the investigator was interested in doing the work anyway. The Dean was very dissatisfied with 10% and had threatened to throw ONR out completely. Consolazio replied, "Oh, put down 25% overhead—you don't want the money and I had to twist your arm." Several weeks later two Navy contract negotiators came to see Dean Rappleye with the piece of paper containing my almost illegible handwriting saying, "We've come to give you this contract." The Dean said, "What contract?" They replied, "Its all written on this piece of yellow paper." I was in the process of having it typed. The Dean then said, "What about overhead?" They replied, "Twenty-five percent." My stock at Columbia rose enormously. I only activated the contract a year later when the Public Health Service cancelled my grants at the height of the McCarthy hysteria. It was like having money in the bank. ONR supported me for 17 years. Consolazio and Levin left ONR when the National Science Foundation was set up and Bill suggested that I apply for a grant. I received one of the first awards-$60,000 for 3 years, appropriated out of the first year's molecular biology budget; it represented about 8% of the total. This replaced my blood group grant, which the Public Health Service cancelled in 1953. NSF has been the main support of my laboratory since then.

In 1951 I was asked to serve on the National Research Council's Subcommittee on Shock, which was concerned about the severe allergic reactions being found on administration of dextran, which had been developed as a plasma expander in Sweden. It was generally believed that the reactions were due to contamination with bacterial protein. I naturally suggested that the dextran itself, like the pneumococcal polysaccharides, might be antigenic in man. Although clinicians were prepared to inject 30 g of dextran intravenously, no one on the committee would inject 1 mg to see if it was antigenic. I did an initial skin test, had a blood sample taken, and gave myself two injections of 0.5 mg of dextran a day apart. A second skin test 3 weeks later showed a typical wheal and erythema, and quantitative precipitin assays on the pre- and post-immunization sera showed that my antidextran level had risen from 1.5 to 25 μN/ml. At the next meeting of the Subcommittee on Shock, I demonstrated my precipitates and also did a skin test on myself and on Doug Lawrason, the Secretary of the Committee, as a control.

Deborah Berg and I studied the quantitative precipitin reaction of dextran and antidextran produced in medical student volunteers. I suggested to the National Research Council that Paul Maurer be asked to do a study to confirm our findings, which he did. To prove that the antibodies were indeed antidextran we used biosynthetically labeled [^{14}C]dextran to precipitate the antibody, and ^{14}C analyses by David Rittenberg, Laura Pontecorvo at P and S, and Leon Hellman and Maxwell Eidinoff at Sloan-Kettering established that the dextran was precipitated by the antibody.

The antigenicity of dextran made possible studies probing the size of the antibody combining site. One of the dextrans, B512, developed at the Northern Regional Research Laboratory of the Department of Agriculture at Peoria, Illinois, was built of 96% α1→6- and 4% α1→3-linked glucoses and so had very long stretches of α1→6-linked glucoses. Allene Jeanes at Peoria and Turvey and Whelan in England were isolating the series of α1→6-linked isomaltose oligosaccharides. The system, α1→6 dextran and human antidextran and the α1→6 oligosaccharides, essentially provided a molecular ruler, since they permitted one to compare the potency on a molar basis of the various oligosaccharides in inhibiting precipitation of antidextran by dextran. This became a fourth area of interest of the laboratory, which continues to the present day.

In the summer of 1952, Sally and I again drove across country with our three sons (David, born August 7, 1951). I wished to learn more carbohydrate chemistry and decided to work with Herman O. L. Fischer at Berkeley, having become very attracted to the Bay Area from my earlier visit. Herman asked me what I wished to do and when I said something about learning methylation of sugars, he took out a sample of galactinol, an

α-galactoside of inositol, and said that methylation and hydrolysis would show where galactose was linked to the inositol. Clinton E. Ballou and Donald L. MacDonald collaborated on the problem and taught me the technics; fortunately, the methylated compound crystallized. The sabbatical was a wonderful experience and we were able to renew old friendships and make many new ones that have lasted. One unexpected consequence of the methylation study was that galactinol turned out to be one of the best inhibitors of the blood group B-anti-B reaction until the disaccharide DGal $\alpha 1 \rightarrow 3$DGal and larger oligosaccharides were isolated.

My sabbatical ended in February, 1953, and we drove back to New York via the southern route, visiting various friends at universities and doing some sightseeing, arriving in New York early in March. It was hard to adjust to the rest of the New York winter after Berkeley. The laboratory was thriving; the allergic encephalitis, spinal fluid gamma globulin, blood group substance, and dextran problems were all going well. I had been cleared by the top Presidential Loyalty Review Board and presumably could carry on with my activities normally. However, rumors of grants being cancelled were becoming more frequent. Linus Pauling had his Public Health Service Grants cancelled and had to appoint others as Responsible Investigator so that the work could continue. My 3-year grant for the monkey studies was running out and I had naturally applied for a renewal. I received a letter from Frederick L. Stone, Chief, Extramural Programs, National Institute of Neurological Diseases and Blindness, dated December 14, 1953, which read,

> Dear Dr. Kabat:
>
> Your application for a research grant, identified as B-9(C4), and entitled "Immunochemical Studies on Acute Disseminated Encephalomyelitis and on Multiple Sclerosis" has now had a final review. I regret to report that this request falls in the group of applications for which grants cannot be made.

This was followed by a visit to Houston Merritt saying that this was because of the political climate, that they didn't know exactly why, etc. He then made the suggestion that the work could go on if someone else's name was substituted as Responsible Investigator. Although many others in this situation complied, I refused, responded with the appropriate four-letter words, and began a boycott of the US Public Health Service, which had imposed the policy on NIH. Anyone wishing to work in my laboratory had to agree not to accept any funds from any unit of the USPHS for the period he was working with me, nor could any employee of the USPHS set foot in my laboratory unless he was coming for some unrelated purpose. I received much support from scientists at NIH, but the top administration did nothing.

The reactions of my colleagues were varied. Most expressed support. Columbia University through Dean Rappleye, Houston Merritt, and Harry Rose supported me unequivocally. The scientific societies fought the policy vigorously. Michael and many others refused to review grant applications. Unfortunately, not all actions were of this type: One person, whom I had considered one of my closest friends, avoided me and on one occasion even hid in the stacks of the Woods Hole library when he saw me coming toward him.

Abner Wolf, Ada Bezer, and I had to kill off the only monkey colony in the world then being devoted to the multiple sclerosis problem. Shortly thereafter I was informed that the tenth committed year of my Blood Group Grant was cancelled.

I shall have to leave the story at this point. The suspense will not be dreadful. You all know that I survived and continued to work actively, that I spent a year at NIH as a Fogarty Scholar, and that I have been spending 2 days a week at NIH for the past 7 years. The rest of the story must wait for another occasion.

ACKNOWLEDGMENT

I thank Daniel M. Singer and Harvey N. Bernstein of Fried, Frank, Harris, Shriver, and Kampelman, Washington, D. C., for their advice, assistance, and direction in obtaining various government files about me under the Freedom of Information/Privacy Act.

Literature Cited

1. Cohen, S. D. 1979. The American Academy of Allergy—An historical review. *J. Allergy Clin. Immunol.* 64:332–466
2. Heidelberger, M. 1979. A "pure" organic chemist's downward path: Chapter 2—The years at P and S. *Ann. Rev. Biochem.* 48:1–21
3. Kabat, E. A. 1978. Life in the laboratory. *Trends Biochem. Sci.* 3:N87
4. Kabat, E. A. 1978. Close encounters of the odoriferous kind. *Trends Biochem. Sci.* 3:N256
5. Kabat, E. A. 1979. Horse sense? *Trends Biochem. Sci.* 4:N204
6. Kabat, E. A. 1980. Wrong door at the Nobel banquet. *Trends Biochem. Sci.* 6:VI
7. Kabat, E. A. 1982. Contributions of quantitative immunochemistry to knowledge of blood group A, B, H, Le, I, and i antigens. *Am. J. Clin. Pathol.* 78:281–92
8. Knight, C. A. 1951. Paper chromatography of some lower peptides. *J. Biol. Chem.* 190:753–62
9. Rosebury, T., Kabat, E. A. 1947. Bacterial warfare. *J. Immunol.* 56:7–96
10. Tiselius, A. 1939–1940. Electrophoretic analysis and the constitution of native fluids. *Harvey Lectures* 35:37–70