*Ann. Rev. Microbiol. 1982. 36:1–26*


# FIFTY YEARS OF IMMUNOLOGY

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## INTRODUCTION

From time to time when talking to young physicians or scientists, I try to recover in my thoughts what biomedical science was like in my formative days about a half a century ago. It is fairly apparent from the facial and verbal expressions of my dialogists—though they are usually discreet—that they regard the state of science of that period as belonging to a geologically distinct epoch. At such times, the same picture flashes through my mind: a conversation with a young clinical investigator in Baltimore, in about 1931. He was interested, as was I, in infectious mononucleosis and in the nature of and reason for its antibody response. His brisk, informed manner and his cognizance of the laboratory findings of the patient we were studying at the time were so akin in tenor to what such a conversation might bring forth today—with some important details added, such as the role of the Epstein-Barr virus in this disease—that I thought I would try more broadly to recall the state of knowledge at that time about those areas of immunology and related topics with which I have had experience.

This essay records personal observations about developments in the science that has been my main interest for about 50 years—immunology—and something about my experiences with students of various categories: medical, graduate, and undergraduate. Of medical practice I had only a taste during the years of World War II, and of full-time administrative duties I had a year as Acting Dean of the Stanford Medical School. Otherwise, with the exceptions—customary for midcentury scientists—of travel, committees, and the like, research and teaching have accounted for my life away from the hearth.

I came to research and teaching at a rather tender age, hence the length of exposure. My interest in immunology was sparked by a simple enough



0066-4227/82/1001-0001$02.00

stimulus, but memory of it has lasted for over half a century. I was 18 years old, a senior undergraduate at Johns Hopkins University, enrolled in a course in general biology taught by Ethan Allen Andrews. Professor Andrews was not one to pander to the interests of individual students, but he and assistants provided lectures and laboratory periods that, over the course of a year, pretty thoroughly covered the natural history of living objects, from the habits of the hydra to the anatomy of the frog and the development of the chick embryo, including, in the latter case, cutting sections of the prenatal fowl at various points in their 3 weeks of development, mounting them on slides, and executing drawings of their component parts. Of the then extant chemical understanding concerned in the workings of these and other beasts and plants, there was little word. But one day, reading one of our textbooks, I came across a few paragraphs describing antigens and their ability to induce the formation of antibodies specifically reactive with them. I thought this information was fascinating, a response sharpened considerably by a book called *Microbe Hunters,* by Paul de Kruif, which appeared at about that time. I could conceive of no more exciting way to spend my remaining years than to learn more about infections and immunity. So, at the end of that year, in 1930, I enrolled as a graduate student in the Department of Immunology at the Johns Hopkins University School of Hygiene and Public Health.

The fact that such a department existed suggests that the science had already attained a level of sophistication considerably beyond the preparation of vaccines and antisera. The Department was then small in personnel but spacious in size; it occupied an entire floor in the fairly new nine-story structure that had been erected, with the support of the Rockfeller Foundation, for research with emphasis on the training of health personnel from this and other countries. The Chairman of Immunology was Roscoe Hyde, a geneticist turned viral immunologist. Viruses were known chiefly by their effects in plants and animals, and by their filterability through porous membranes. Dr. Hyde was given to pithy aphorisms; what I recall best about his scientific teachings was his description of viruses as "genes gone wild." Another of the three faculty members of the Department was G. Howard Bailey, whose main interest at the time was the "heterophile," or Forssman antigen, a substance he and others tracked through a wide range of fauna and flora, from bacteria to man. Dr. Bailey became my mentor for 3 years, and subsequently I became his assistant for 2 years, working under a grant from the National Research Council that yielded for me $400 per annum. This was pretty scanty pay even for that period of the Great Depression, but the NIH had not yet been invented.

In those days, one leading textbook of immunology was a weighty tome by John Kolmer of the University of Pennsylvania. A more compact one

by Hans Zinsser called *Infection and Resistance* was widely used. Dr. Kolmer's book described everything then known about immunology and how to do it, from bleeding a sheep to setting up serological tests. Zinsser's book was much more concise: It dealt with immunologic events and their explanations, and only little with experimental manipulations. There were other useful texts, one especially helpful by the British author W. Topley, and a small volume by H. G. Wells of the University of Chicago on *The Chemical Aspects of Immunity.*

The tendency for inhabitants of every generation to consider that developments in their area evolved simultaneously with the kindling of their own interest has been alluded to. This is perhaps particularly so now in the era of the biologic molecule. In fact, 50 years ago, the questions that interested immunologic investigators were surprisingly similar to those that engage attention now, but of course at a much more phenomenological level.

The concept of antigenicity was well defined in chemical terms and it had been known for years that polysaccharide as well as protein could function as antigen. Landsteiner and some predecessors had provided much detailed information about the influence of simple chemical groupings in determining antigenic specificity and had devised the term "hapten" for such chemical determinants.

The antigens of many infectious agents and their subgroups were becoming known, mainly by serologic distinctions [e.g. the salmonellae, classified by Kauffmann & White (1936),] the dysentary and cholera bacilli, and others. The lipopolysaccharide antigens of Gram-negative bacilli, and the Vi (virulence) antigen of the typhoid bacillus had been characterized. The capsular polysaccharide of the pneumococcus had been isolated and purified by Avery and Heidelberger in the 1920s, and here again antigenic distinctions within the species were known and were being exploited by Felton and others to produce antibodies in horses and rabbits for the treatment of lobar pneumonia in those pre–antibiotic times (although the sulfonamides and, not long after, penicillin were about to appear).

Loss and gain of antigens by bacteria was recognized. Smooth-rough dissociation had been described by Arkwright back in 1921, and the H↔O (flagellated to non-flagellated) fluctuation in Gram-negative bacilli had been established years before by Smith & Reagh (1903) and Weil & Felix (1917). Antigenic variation was also well established. Thus, phase variation —the shift between two expressions of antigenicity in *Salmonella* flagella —had been described (Andrewes, 1925). A harbinger of things to come, premonitory of the molecular biologic era, was reported by Griffith in 1928. He manipulated the transformation of one type of pneumococcus to another by injecting into animals a noncapsulated derivative of one antigenic type with killed capsulated cells of another type, and he isolated viable cap-

sulated organisms of the latter kind. Some years after (1944) came the apocalyptic demonstration by Avery and colleagues that this could be done more directly by the transfer of nucleic acid.

A half century ago, polymorphism of antigens of animal cells was also well known. Karl Landsteiner and colleagues had defined the major human blood groups at the turn of the century, and the second human system of erythrocyte antigens (MN) was uncovered in 1922. The fact that some infectious agents could undergo changes in a major antigen during the course of infection, thus evading the specific immunity that had been acquired, had been established for *Borrelia* and for trypanosomes.

The influenza virus, which was to become the most notorious of infectious agents in this regard, was known by 1938 to show more than one antigenic form in the predominant type.

In this milieu of information concerning the diversity of antigens and their expressions in infectious agents, I undertook as a neophyte in graduate work to study *Trypanosoma equiperdum,* a flagellated blood protozoan related to the agents of African sleeping sickness. This parasite multiplies vigorously in rodents, so that a few days after infection a drop of fresh blood swarms with them, lashing about and kicking aside blood cells with their flagella. Giemsa stain of a blood film shows beautifully colored crescent-shaped organisms, each with a pale membrane along its border terminating in a single flagellum. Aside from the beauty of the beast, I was intrigued by the immunologic caprice of the infection it produces. Mounting numbers in the blood over a period of several days suddenly disappear completely, simultaneously with the appearance of antibodies against them. Then a few organisms reappear and again rapidly proliferate, unaffected by the coexisting antibodies. An individual animal might experience several such crises, i.e. the sudden disappearance of trypanosomes and their subsequent reimergence. With each crisis an antibody of new specificity appears, and each relapse strain evades the series of preformed antibodies. Since the trypanosomes could not be cultivated, strains of each antigenic specificity had to be carried in mice, with transfer to a fresh animal before the first crisis occurred.

This interplay of host and parasite is a fascinating compacted version, at least in principle, of what was later found to be the case with the influenza virus: a game between host and parasite, the former responding with an almost completely protective immunity, the latter in turn responding by discarding one antigenic guise and adopting another. In the case of the trypanosome, the game is played in an individual host; in the case of the virus, it is played in a community of hosts, worldwide, so that after a particular antigenic type has run its course, i.e. has exhausted the reservoir of nonimmune subjects, a new antigenic version appears.

In the 1930s, the nature of the trypanosomal antigen participating in this game was unknown, as was the process of antigenic alteration. I found, as had others, that the number of trypanosomal variants was finite for, if relapse strains were collected from a series of infected hosts, about a dozen could be accumulated. But any individual animal was eventually fatally overcome by a relapse strain, usually after the third or fourth recurrence.

The membrane antigen is now recognized as a glycoprotein, and the variant antigens appear to result not from mutations, but from rearrangements of the DNA, as might be anticipated from the nonrandomness of the number of variations.

## HETEROGENETIC ANTIGENS

The diversity of antigens that provides distinctions among members of a species—of bacteria or of cells of higher organisms—has its obverse image in the existence of components of biologically diverse "containers" antigenically the same, or closely related. This was recognized, long before my neophytic years, as residing in a sharing of chemical structures reactive with the same antibodies. The best studied of these heterogenetic or so-called heterophile antigens was the Forssman substance, characterized back in 1911 by the investigator whose name it was given. This substance was found to be extractable by organic solvents from the tissues of various animals and other forms of life. Its lipoidal extractability did not reflect the basis for its antigencity; it contains also carbohydrate and protein, and therein resides its antibody-inducing ability.

Because of the ubiquity in nature of this Forssman antigen, antibodies to it are also widespread in those species that do not themselves carry it. For example, in man, a non-Forssman-containing creature (except those with Type A erythrocytes, whose carbohydrate moiety is similar to that of Forssman antigen), low titers of anti-Forssman antibodies occur universally.

In the early 1930s, a wide range of bacteria, plants, and animal tissues had been surveyed for possession of this antigen; my preceptor, Professor G. H. Bailey, with Mary Shorb, had carried out a considerable part of these surveys. Its presence in infectious agents was especially enticing for the immunologists interested in disease, for it evoked such visions as possible cross-immunization by environmental entities (e.g. foods containing the antigen) that might protect against pathogenic organisms, such as certain of the pneumococci, which also contain it.

During this period, infectious mononucleosis came to be recognized as a problem for young people, though it must have existed for some millenia before and is now fairly commonly seen. A pair of investigators, Paul and

Bunnell, reported that patients with this disease had increased titers of antibodies against sheep erythrocytes—cells that had become the prototype for study of Forssman-bearing antigens. The disease affects lymphoid organs and lymphocytes, inter alia, and it became of much interest to us to determine the nature of this antibody response as a possible lead to a more specific diagnostic test for the disease, as well as for providing a possible clue to its etiology.

Hence, we tested sera from cases of the disease against a wide variety of Forssman-containing tissues and bacteria and found that the antibody concerned was in fact not against antigen of the Forssman type, but rather against an entirely independent heterogenetic antigen of the sheep red blood cell that occurs also in the erythrocytes of cattle. This finding did provide a more specific diagnostic serologic approach to the disease, but at the time it gave us no lead to etiology. The question of a virus as a causative agent certainly occurred to us, and we tried vainly to use tissues or tissue debris from diseased areas to absorb the antibodies, but these efforts bore no fruit. Methods for viral cultivation were not yet available; only many years later was it found that the Epstein-Barr virus is the causative agent. This virus infects the B class of lymphocytes (the antibody-producing type), but whether the cattle cell antigen is shared by this virus is still not clear. Since the potential antibody-synthesizing lymphocytes are infected, and since a potpourri of other antibodies (such as the Wassermann antibody) as well as excessive immunoglobulins often accompany this disease, it may be that the characteristic antibody is one of the many non-specifically stimulated by the resident virus, though the fact that this particular specificity is so constant an accompaniment of the disease makes this unlikely.

## ANTIBODIES

Fifty years ago antibodies were being enthusiastically exploited, if not too well characterized chemically. They were respected for their ability to modify the courses of certain diseases, either by administration of serum from animals (usually rabbits or horses)—as in the treatment of diphtheria, lobar pneumonia, and tetanus—or through active induction by vaccination, as was the case for smallpox, typhoid fever, whooping cough, diphtheria, yellow fever, scarlet fever, plague, leptospiral disease, and some infections of domestic animals. Serologic tests for antibodies (or antigens) had been in use for decades, including the classical ones of precipitation of soluble antigens, agglutination of particulate substances such as bacteria, lysis (with complement) of certain bacteria and animal cells, particularly erythrocytes, complement fixation as in the famous Wassermann test for syphilis, opsonization for phagocytosis of particles by leucocytes and macrophages, and neutralization of bacterial and other toxins and of viral infectivity.

The insertion of markers into antibodies was being developed by Albert Coons with fluorescein isothiocyanate—a major prelude to the later localization of antibody synthesis in lymph nodes and spleen, and soon after in cells, the plasmacytes.

Exemplary studies of the protective mechanisms of antibodies in bacterial infections were carried out, during this period, by Cannon at the University of Chicago, and by Arnold Rich at Johns Hopkins University. Rich found that in rabbits infected with pneumococci and provided with antibodies, but deprived of most of their leucocytes by a bone marrow poison, the bacteria remained clumped (agglutinated) at the injection site for a time; but, in the absence of polymorphonuclear cells, they eventually broke loose to spread through the body. With these cells present, the bacteria were phagocytosed and destroyed: a beautiful demonstration of what had been inferred from more piecemeal evidence in the past.

There was also an appreciation adduced experimentally of the influence of heredity on specific antibody formation, and on the abilities of certain mice to resist particular infectious agents.

Little was known about the molecular nature of antibodies beyond the fact that they were "globular proteins" separable from other proteins of plasma by salting out or dialysis. Nothing was known about their cellular source. If anyone surmised that the lazy-looking lymphocytes might be the culprits, this was well hidden in the literature. I recall that, in a lecture in pathology in 1931, the lecturer, one of the sharpest existing exponents of his specialty, remarked that it was a constant rebuke to the intelligence of members of his profession that a function could not be assigned to those cells so ubiquitous in their distribution and in their intrusion into pathologic areas.

The brilliant quantitative methods and deductions by Heidelberger in this country and Marrack in England had demonstrated the mutual multivalence of antibodies and antigens in their reactions in varying proportions. But the precise number of combining sites per antibody molecule was not known, and some that precipitated poorly with soluble antigen were considered univalent, hence incapable of forming the lattice of precipitate resulting from mutual multivalence. Only some years later did studies with simple antigens (those possessing only one determinant group) establish in the ultracentrifuge and by equilibrium dialysis that antibodies have two combining sites, unless polymerized.

There were indications that all the antibodies made against a particular antigen were not the same in a number of respects: in their "avidity" for antigen, their capacity to activate complement, their ability to aggregate antigen, their protective capacities against infection, and their location among the globulins precipated by different concentrations of salt.

I found intriguing the notion that the association of antibody with differ-

ent globulins might be related to their variable activities, and that this association might change with progressive immunization. Charles Pait, then a recent medical school graduate, was interested in these questions; an instructorship was available for him, so we collaborated in a study that eventually answered some of our questions.

The entities we used as antigens were in fact complex mosaics of antigens: sheep erythocytes, horse serum, and the bacterium *Proteus vulgaris*. The antisera obtained from rabbits at different stages in immunization were dialyzed against distilled water for prolonged times, with periodic removals of globulin precipitates. These were quantified as to nitrogen content and then were tested for antibody activity by various methods intended to indicate the changing quality of antibody per unit of nitrogen: for example, the ability of anti-erythocyte serum to promote lysis of the cells as compared with agglutination.

At this time, Arne Tiselius in Sweden had devised an apparatus for characterizing proteins by their migration in an electrical field, and a colleague, Eloise Jameson of the Stanford University Department of Chemistry, had journeyed to his laboratory to learn something of the new technology. She brought back one of the earliest models of the electrophoresis apparatus; this employed the schlieren mode of photographing moving protein boundaries. She and her young co-worker, Claudio Alvarez-Tostado, kindly turned their time and expertise to making photos of our antibody-containing fractions; the areas under the peaks I then laboriously measured by planimetry. But the basic knowledge required for interpretation, or perhaps the precision of the measuring techniques, were not adequate for informing our project beyond the point obtainable with Kjeldahl determinations and serologic assays.

The structures of antibody molecules and their globulin associations and functional differences had to await the work in the late 1940s of Porter, and subsequently Edelman and many other immunochemists, for precise definition. But, at the time, it was exciting to add to the evidence that all antibodies are by no means functionally the same, and that their activities and globulin associations shift with repetition of an antigenic stimulus and with time.

## IMMUNOLOGIC HYPERSENSITIVITY

Fifty years ago immunologic hypersensitivity was a well-advanced concept among researchers, in both the laboratory and the clinic. The recognition of anaphylaxis was several decades along, dating from Portier and Richet's discovery back in 1902, when Prince Albert of Monaco, weary of being stung by Portugese Men-of-War while bathing in his coastal waters, impor-

tuned these scientists to find out what could be done about these poisonous hydrozoans. They injected extracts of these creatures into dogs and found them to be not only toxic, but also sensitizing to a shock syndrome on reinjection. At about the same time, in the US, Theobald Smith saw a form of respiratory shock on re-exposure of guinea pigs to diphtheria toxoid. Other manifestations of antibody-associated reactivities had also been described in the early years of the century: between 1903 and 1906 the Arthus reaction, human allergic reactivity or atopy, and the serum sickness syndrome in man. Furthermore, efforts were being made to do something about these; the concept and application of methods of desensitization were being discussed by Weil and others by 1913. In the mid-1930s, Cooke and Loveless reported the appearance in people undergoing desensitization of antibodies of the same specificity for antigen as the sensitizing ones, but differing from these in that they did not cause reactions; instead, they blocked the sensitizing antibodies from combining with antigen. Again, this was evidence for populations of functionally diverse antibodies, long before knowledge existed of their different molecular classes.

Histamine had been known for some years as a probably important mediator of hypersensitive reactions. Its origin from the basophil granule was to be uncovered later. In this period another, and what eventuated as the most important mediator of blood-vessel dilatation and smooth muscle constriction in hyperactive man, was found by Kellaway and associates. It was called slow reacting substance (SRS), in current parlance a leucotriene; like prostaglandin, it is a derivative of arachidonic acid.

Antibody-mediated immunologic hypersensitivity, or "immediate hypersensitivity," has been of recurring interest to me beginning in the mid-1930s, when Bailey and I worked with the pneumococcal polyaccharide capsular antigen. In those times, horses were commonly used by pharmaceutical companies for producing antibodies against pneumococci of different antigenic types for the specific treatment of pneumonias. Hypersensitive reactions to equine serum were frequent, and sometimes fatal, so that enlightenment about the conditions that might modify these occurrences was eagerly sought. Horse antibodies were known to be incapable of sensitizing guinea pigs for anaphylactic shock, whereas antibodies made by rabbits were very adept in this regard.

Our hope was to get some insight into these functional differences. Since molecular structures were unknown, our efforts were directed at phenomenological characterizations. If we used very small quantities of horse serum we found we could sensitize guinea pigs fairly regularly. Without knowledge of the structure of antibody molecules, or of the cells to which such molecules need to be anchored to combine with antigen and release the mediators of anaphylaxis, we could go no farther at the time.

Years later, in the mid-1960s, and in a different context, I collaborated with Alfred Amkraut, Leon Rosenberg, Oscar Frick, and others to learn something about the requirements for eliciting anaphylaxis with antigens possessing single reactive sites for antibody. By now, the knowledge that only some classes of antibody could attach to appropriate cell membranes was well established, and it had been found that—for the cell to be provoked into excreting anaphylactic mediators—it was necessary that the antigen link two adjacent antibody molecules at the cell surface. For this purpose, antigens with single combining sites would obviously not do. But we found that anaphylactic reactions could be induced with certain single-determinant compounds.

I believe that a thread links these findings separated by thirty years: the inability of horse antibody to sensitize subjects unless provided in very small amounts, and the ability of certain monovalent antigens to provoke the anaphylactic reaction despite the general requirement for antibody linkage by antigen. I think both point to an alternate path by which hypersensitive reactions can come about. Antibody-antigen complexes formed in the circulation may activate the formation of substances, e.g. bradykinin, that like the products of the mast cell constrict smooth muscle and dilate small vessels. This view was reinforced by the later finding with my postdoctoral fellow Baldwin Tom that isolated rabbit antibody of a molecular subclass known to be non-membrane attaching, when combined with antigen in vitro, would induce hypersensitive reactions upon injection into guinea pig skin, and with Frick and Liacoupoulis that very excessive doses of antigen injected into sensitized animals or added to lung strips from such animals would not elicit reactions. Both findings can be ascribed to the occurrence of non-membrane-associated activities by complexes of antigen with antibody in appropriate ratio, so that complement is activated, bradykinin is released, or both.

## AUTOIMMUNITY

Autoimmunity was a well-developed concept a half century ago in all its aspects—theoretical, experimental, and clinical. The fertile mind of Paul Ehrlich envisioned the body reacting immunologically against itself and, with his aptness for the Latin bon mot, had dubbed this "horror autotoxicus." By 1904, Landsteiner and Donath had found a clear-cut clinical instance of this: Patients with paroxysmal hemoglobinuria destroyed their own red cells by means of antibodies and excreted hemoglobin in the urine. These rather unusual antibodies attach themselves to erythocytes at low temperatures, as when the subject immerses a limb in cold water. On return to the usual body temperature, the attached antibodies activate comple-

ment, and the cells are lysed. All of these conditions were revealed by the investigators seventy-five years ago, and no clearer example of autoimmunity has since been described, although many instances are now known or suspected.

In the 1930s, there was considerable speculation about an autoimmune basis for sympathetic ophthalmia, in which injury to the uveal tract of one eye sometimes results in destruction of this tissue in the other. In 1933, came the initial experimental demonstrations that injections into monkeys of nerve tissue—even that derived from the same species—would eventually induce an immunologic response with damage to their own myelin sheaths, a syndrome called "experimental allergic encephalomyelitis" or EAE.

The fact of such occurrences, spontaneously or experimentally induced (and, in the case of EAE, this may perhaps occur in humans after repeated doses of rabies vaccine prepared from animal nerve tissue), suggested a number of interesting points regarding the nature of an immunologic responding system that could countenance such gaffes as reacting against one's own tissues, and the nature of the tissue antigens that would permit such responses. Obviously, these must be organ-specific antigenic determinants, a thought not precisely apocalyptic even in those days before knowledge of the DNA code. A number of examples of such tissue specificities were known.

My entrée into these considerations started during research with antipneumoccoccal antibodies in the early 1930s, with G. H. Bailey. The bacteria were cultivated in beef muscle infusion broth and, in our experiments on anaphylaxis alluded to earlier, the fluids from the culture medium were sometimes used for eliciting shock in animals passively sensitized with antibodies against the bacteria. We were surprised to find that when we used sterile broth as an experimental control, anaphylaxis occurred, as with the bacterial soup. It was evident that the bacteria used to vaccinate rabbits for the production of antibacterial antibodies were simultaneously inducing antibodies to constituents of the broth itself, and we questioned whether or not this might be specific for the tissue from which the broth was prepared. Indeed, these antibodies were specific, directed against a thermostable antigen in the muscle. Broths made from other organs had their own specificities, as did tissues from a variety of species, including man.

We were unable to induce autoimmune disease, e.g. by injecting rabbits with vaccines cultured in rabbit muscle broth, or by administering antitissue antibodies produced in another species. This inability is of course a frequent finding in studies of autoimmunity: In many instances the presence of circulating antibodies cannot be correlated with pathologic effects, possibly because of the inaccessibility of the antigens involved.

Allergic encephalomyelitis has been of recurrent interest to me and col-

leagues, one of whom, Elizabeth Roboz-Einstein, was among the initial discoverers that a basic protein isolated from myelin carries the antigenic determinant that induces the disease. Antibodies were produced in rabbits against this protein and were labeled with a fluorescent
Rauch in our group was able with this procedure to localize the antigen in the myelin sheath and, interestingly, to show that the antibodies induced by bovine myelin protein reacted as well with the nerve sheaths of humans and other species. This underscored the well-known fact that encephalomyelitis could be induced as well by heterologous as by homologous nerve tissue or its protein.

The central role of lymphocytes in cellular immune responses was still novel at this time, and we wanted to find whether or not cells from the immunized animal might combine with the protein antigen. Attachment of the labeled protein to some cells was evident, but at the time there was no distinction of antibody-producing (B) from cellular-reactive (T) lymphocytes; hence we were unable to make a final assessment of this central question. However, we tentatively interpreted our observations to suggest a direct lymphocyte-antigen combination.

## INFECTION AND VACCINATION

A half century ago, the art of prophylactic vaccination was well advanced; after all, it began, almost simultaneously with the origin of the United States, with Jenner's vaccine for smallpox. It is difficult to pinpoint the true beginning of almost anything, but it seems that earlier in that century Lady Mary Wortley Montagu brought back from Turkey to England the idea that a crust of smallpox lesion itself could be given in "small" doses to induce a milder-than-usual case of the disease which would be protective. If one wished to pursue the origin of prophylaxis further, it is recorded that Mithridates of Pontus, a king whose thirst for power led him to kill every conceivable pretender to his throne, including his mother and children, dosed himself with small amounts of the regicidal poisons then in vogue by drinking the blood of ducks that had imbibed these in sublethal quantities. This was about 2100 years ago. It is unlikely he thought in terms of acquired immunity, but he certainly had in mind adapting himself to these noxious substances.

By the mid-1930s, the vaccines—as all active immunizing agents came to be called—included toxins or toxoids of diphtheria, scarlatina, clostridia and staphylococci, not all yet suitable for application, but one of them, diphtheria toxoid, had altered the visage of childhood. Although viruses had not yet been seen, vaccines containing modified viral agents (smallpox, rabies) had long been in use, and yellow fever vaccine was on the verge of

a highly successful career. Influenza vaccine was also about to take off with the American and British demonstrations of the immunogenicity in humans of cultivated live virus. Bacterial prophylactics for typhoid, the two paratyphoid fevers, whooping cough, plague, and tuberculous and hemorrhagic fever (leptospirosis), as well as vaccines for some predominately animal diseases (anthrax, fowl cholera), were well established. This was in addition to the therapeutic use of preformed antibodies to treat disease already in progress by passive immunization, i.e. diphtheria and tetanus antitoxin and antipneumoccal serum for lobar pneumonia. It was fully appreciated in the case of bacteria that certain surface antigens were required to induce protective immunity. As mentioned before, the pneumococcal type-specific capsular polysaccharides had been isolated, and successful efforts to use these for active vaccination were reported in 1935.

Immunization to rickettsial diseases, because the causative agents were not cultivable outside living tissues, was approached with attempts to exploit the microbes in their insect vectors. The fertile egg yolk sac was not discovered as a good pabulum for these organisms until 1938, and the culture of tissues, although long useful for other studies, had not been developed as a medium for growth of viruses or rickettsias. But ingenuity was not to be denied; for typhus fever, an imaginative vaccine had been devised by Weigl in Poland in the first years of the 1930s that entailed infecting lice per rectum, and a week later harvesting rickettsias by a kind of enema. The germs were then inactivated with alcohol or formalin; this vaccine was administered to almost 200,000 people. A few years later, Herald Cox discovered that the typhus bacteria would grow in yolk sacs of fertile eggs.

When one considers the array of vaccines that have arrived on the scene since those days of fifty years ago, some for major plagues of mankind, the status of prophylasis in those times seems modest. But from the point of view of principles, a great deal had already been established. Aside from the fact of the efficacy of immunization per se, toxins had been detoxified, the cellular requisites for effective bacterial vaccines had been clarified, in one case a chemical derivative of a bacterium had been readied for trial as a vaccine, and viruses modified from their original virulence were in use. I think no other chapter in man's ascent from the ooze has contributed comparable benefit to his physical welfare—and to that of other species—without exacting the customary price of progress: danger to the individual and disruption of his surroundings. This triumph is epitomized by the declaration that appeared in the Bulletin of the World Health Organization in May, 1980: It proclaimed that, consequent to an effort that began twenty years ago, the world is now free of smallpox. This conquest of a plague that had decimated mankind for millenia is without parallel in the history of

human affairs; it deserves blasts of trumpets and an international official holiday as an annual observance.

In the mid-1930s, poliomyelitis remained one of the fearsome infections for which there was no prevention; laborious and expensive efforts were tried to limit its disastrous consequences. Because President Franklin Roosevelt had suffered this disease, he instigated the formation of the National Foundation for Poliomyelitis, which became a source of support for treatment and research. The Department of Bacteriology and Experimental Pathology at Stanford University was one center of laboratory investigation of this disease; this effort was headed by Professor Edwin W. Schultz, the Department's chairman. In 1935, he invited me to migrate from Baltimore for a year of postdoctoral fellowship under the aegis of the Foundation. In those days, such a transcontinental junket was quite an adventure. Although rail transportation was as fast and comfortable as it was ever to be, the trip was not lightly undertaken, in part because of the just subsiding depression. So the prospect of the journey, of a year in California, and of the opportunity for research on an interesting disease was an exhilirating one.

The department in 1935 was housed in a building that had survived the earthquake of 1906. It had been part of a large quadrangular museum of which intervening portions had collapsed, and hugh chunks of sandstone and stuccoed brick remained in the field between the department and the residual museum for many years after my arrival. This building had been assigned to the department in 1911 shortly after Hans Zinsser arrived from Columbia to become the first head of bacteriology as a Division of the medical school; for several preceding years, a course in bacteriology for medical students had been taught by Professor Robert Swain of the Chemistry Department. The quarters were intended to be temporary, a status terminated in June, 1981, when the department moved to handsome new quarters in the medical school complex.

Despite its rough treatment by natural forces and its primary design for a purpose very different from biomedical research, the old structure had lent itself well to modification, and by the time of my arrival there were a number of laboratories as well as teaching facilities accommodating a class of 65 medical students and about 30 graduates and undergraduates. On the lower of its two floors, some of the space housed animals; this facility was supplemented by an outside structure to take care of several hundred rhesus monkeys (a few of which occasionally escaped into neighboring Palo Alto) in addition to the more usual experimental animals.

The research laboratories contained the full range of current technical instruments, and also facilities unusual at the time. The outstanding example of this was a collection of air-driven ultracentrifuges. These were then

being developed in this country by Professor Beams, a physicist at the University of Virginia. A local mechanician, Louis Grebmeir, then and for a number of succeeding years, manufactured ultracentrifuges with rotors of varying capacities and of different alloy compositions for the department in his tireless search for the ultimate machine. The rotors, whether large or small, were all air driven, and some attained speeds of up to 90,000 rpm. On one occasion a larger one tore loose from its mooring, which consisted of a thin wire shaft suspended from a vane that whirled on a bed of compressed air. The rotor sheared the lid bolts of the surrounding thick steel casing and tore a section out of the wall across the room. The assistant who customarily shepherded runs of ultracentrifugation was fortunately not in position at the moment, directly in the line of fire.

A room was maintained at constant temperature and humidity for the manufacture of cellulose membranes of predetermined average pore diameters. These were used for obtaining approximate dimensions of viruses.

At a somewhat later time Professor Schultz was among the sponsors of a University invitation to Professor L. Marton of Belgium, a pioneer in the development of the electron microscope. Professor Marton remained, I believe, for two years and assembled one microscope in the department. I recall well the photographs of *E. coli* bacteriophage made with this instrument, and the discussion about the figure-8 object in its head. I am not certain whether the question of chromosomal identity was seriously raised at the time; my recollection may be distorted by hindsight.

The department was a small one. In addition to Edwin W. Schultz, William Manwaring was a Research Professor with major interest in immunology. At the time of my arrival, Michael Doudoroff was a graduate student, as was Ryland Madison, who worked with Dr. Manwaring in the then new area of fibrinolysin, the streptokinase that causes fibrin to dissolve. Charles Clifton, who in 1945 became the first editor of this Annual Review, had preceded me in the department by a few years and researched and taught in the area of bacterial physiology. Paul Beard held a joint appointment in the Department of Engineering; he was concerned with sanitary bacteriology. Byron Olsen was a new instructor, charged primarily with teaching medical bacteriology.

This small group encompassed a good variety of research interests, and the department was a lively place for a young fellow. Dr. Schultz's interest in viruses extended to the bacteriophages, then relative newcomers to the field. In addition to trying to learn something about their nature, he set up a laboratory to determine whether or not these bacterial predators might be useful therapeutically. He advertised to physicians that the laboratory would try to adapt a bacteriophage—of which there was a large collection —to any bacterium isolated from a patient. This was done at cost to the

laboratory, then about $2.00. The physician was expected to report briefly on the use of the phage and the subsequent fate of the patient. After several years the project was abandoned for lack of adequate reporting and because of the advent of sulfonamides.

The main effort in the department was in poliomyelitis research, including studies of the virus itself, of methods for detecting it or an immunologic response to it that would obviate the need for neutralization tests in monkeys, of establishing the infection in smaller animals, and of seeking an effective vaccine or some other way to ward off infection.

I participated in these activities along with Louis Gebhardt, who was then a graduate student, eventually to become head of the Department of Bacteriology at the University of Utah. Another young colleague was medical student Harold Pearson, later an instructor in the Department and subsequently Professor in various departments of the medical school of the University of Southern California.

Efforts to establish poliomyelitis in smaller animals failed, but about this time other investigators found that certain strains of the virus could be transmitted to small mammals—the cotton rat, and eventually hamsters and mice.

We had some success in characterizing the virus. Elford cellulose membranes had already "sized" it in the correct range of about 25 mM, and we were eventually able to sediment it in the air-driven ultracentrifuge. I have a vivid memory of Harold Pearson standing by the centrifuge with a pitch-pipe, which, in the earliest days, was used for estimating speed. But reference to our publication indicates that during the course of this study, in 1936, we had become more sophisticated; Mr. Grebmeier had devised a stroboscopic light that picked up a marker on the rotor. In any case, we found that about 30,000 rpm for two hours sedimented pellets containing the virus.

We had been trying to arrange a serologic or other immunologic test for the presence of virus or of reactivity to it—to no avail. But, several years later, Hubert Loring of the Biochemistry Department sedimented larger amounts of virus, and together we demonstrated complement fixation with the sera of immunized rats that had resisted challenge infection. This protection of rats was premonitory of Salk's conquest of the disease some years later. It has been puzzling to me why our similar efforts in monkeys, which we had attempted many times to immunize with variously inactivated viral preparations, were never sufficiently convincing to presage Salk's later success. We worked with a single type of virus; antigenic distinctions among types were beginning to be appreciated, but we used the same viral preparations for vaccination and challenge. Perhaps the viral mass was not sufficient, or the strain employed was not sufficiently immunogenic.

It was thought by some investigators at that time, from work with mon-

keys, that the route for poliomyelitis infection was via the olfactory bulbs. There was a good deal of painstaking histological evidence to track the progress of virus to the central nervous system by that path in monkeys, which could readily be infected by lavaging their nasal passages with virus suspension. As fate would have it, this is not true for humans, who acquire their infections by the gastrointestinal route. But the rhesus monkey was considered so faithful a surrogate for man in poliomyelitis studies that this divergence that later became very apparent was not fully appreciated at the time.

Dr. Schultz devised a procedure intended to block entry of the virus into the olfactory nerves by spraying the nasal passages with tannic acid or zinc sulfate. Monkeys treated in this way subsequently resisted infection by this route, but the few hardy human volunteers who received the treatment succeeded only in losing some of their ability to smell.

## TUBERCULOSIS

In the early 1930s, tuberculosis was still a serious problem, although a number of the more health-oriented countries of the world showed a progressive decline in incidence. A vaccine had been introduced in France in the early 1920s, which consisted of a living attenuated strain of bacilli of bovine origin, isolated for use as vaccine after long cultivation, and named for its originators, Bacille Calmette-Guérin, or BCG.

BCG has had a peculiarly checkered career in the annals of prophylactic immunology. There has never been question about its efficacy in experimental animals, but its usefulness for humans, although demonstrated many times in some controlled trials, has been found wanting in others. To confound the issue of use further, a major disaster occurred in Lübeck owing to a confusion of cultures, so that a group of infants was dosed with virulent bacteria by mouth. The fear of such a consequence was never completely overcome, though the negligence that led to it had been amply documented. In the US, an additional objection on the part of some pediatricians was that wide-spread use of the vaccine would obviate the diagnostic value of the tuberculin test, which depends on allergic reactivity to the bacillus. Hence, fifty years ago the US needed a broadly acceptable prophylatic agent, and a therapy more useful than the rest and mountain air then available to those with adequate financial resources.

My interest in tuberculosis evolved from a course in pathology at Johns Hopkins University with Arnold Rich, a man endowed with an exceptional ability to stimulate students. He was then writing *The Pathogenesis of Tuberculosis*—a thousand-page volume of information and concepts couched in an unusually attractive style.

In the early 1930s, much was known about this disease both clinically and

pathologically, and there was a considerable store of information and conjecture about its immunologic features. Some of those conjectures are still with us. The fact that infection with the mycobacterium led to delayed hypersensitivity, as evidenced by reactivity to the bacilli, or to tuberculin, a concentrate of the medium in which they had grown, had been found by Robert Koch in the 1880s. There were those who equated this cellular hypersensitivity with immunity to the bacterium, i.e. with cellular- rather than antibody-mediated resistance. Rich could not believe nature would be so profligate as to evolve two different entities, cells and antibody molecules, both with the ability to recognize specific antigens. This conviction led him to try to abolish the cellular reactivity of delayed hypersensitivity in vaccinated guinea pigs by dosing them with increasing amounts of tuberculin, a process known to immunologists as desensitization. Such animals without sensitivity retained the ability to resist challenge infection, ergo, he believed his thesis was substantiated. But in fact, his share in the ultimate truth of this matter proved to be only partial: There is a cellular immunity to the tubercle bacillus as well as to other infectious agents and many tumors; the subsets of cells (lymphocytes) that participate in this are probably different from those responsible for delayed hypersensitivity. But the recognition units for antigen on these cells partake of antibody-like character, although their final definition has not yet been made.

I found Rich's lectures on this and related topics to be very stimulating and, subsequently, I thought a good deal about possible approaches to some of the immunologic facets of the disease. I surmised that if one could isolate from the bacillus its protection-inducing antigen(s), this might unravel the confusion regarding immunity and cellular reactivity while providing a vaccine of unimpugnable safety.

The California Tuberculosis Association at about that time, in the late 1930s, was in a state of nascent activation. A number of clinicians, among them Harold Trimble, Reginald Smart, Buford Wardrip, and Corwin Hinshaw, had a scholarly enthusiasm about the nature of tuberculosis along with their clincial interest in it. They had begun to assert their views in eastern meetings of the National Tuberculosis Association and succeeded in obtaining larger slices of funds to support research. They were good enough to provide my efforts with some of this.

I began a long continuing effort to disassemble *Mycobacterium tuberculosis* into components I hoped might be correlated with the body's responses to it. A number of my predecessors had devised procedures for fractioning the bacilli: notable among them were Anderson at Yale, Florence Sabin at the Rockefeller Institute, and Florence Seibert at the Phipps Institute of the University of Pennsylvania. They had prepared lipid, protein, and polysaccharide derivatives and had learned a good deal about responses to them,

which led, in Seibert's work, to preparation of the widely used PPD (purified protein derivative) of the bacillus to supplant tuberculin for diagnostic skin testing.

I cultivated about a pound of virulent *M. tuberculosis* cells in a synthetic medium and began to extract these with organic solvents, intending to test each extractive and the bacillary residue for its capacity to induce protective immunity, delayed hypersensitivity, and antibodies to bacterial proteins.

The tubercle bacillus and its close relatives have the interesting ability to induce cellular delayed hypersensitivity not only to their own protein antigens, but also to any other extraneous antigen mixed with them for injection, particularly if the mixture is enclosed in an oily vehicle. Such mixtures also markedly promote antibody production to the extraneous antigens. These adjuvant abilities, first disclosed by French investigators in the 1930s, were brought to focus by Jules Freund in the early 1940s. Thenceforth, the bacillary-oil mixture has been known as Freund's adjuvant.

After a good deal of labor, we found that bacteria deprived of a group of chloroform-soluble lipids lost the ability to induce cellular delayed hypersensitivity. The extractive, furthermore, could replace the bacillus in this activity in association with a variety of antigens, with tuberculoproteins as well as with such extraneous substances as picryl chloride and egg albumin. Extraction of the lipids also deprived the bacteria of their immunizing ability, and of their antibody-stimulative capacity as constituents of Freund's adjuvant.

At this juncture in the research, in 1949, a Guggenheim Fellowship opened the way for a year of experience in Europe. I look back on that adventure as my closest brush with heroism, abetted by my wife's resolution and endurance. At the time, our five daughters were between ages ten and two. In 1950, airplanes flew, but hardly as family conveyances, at least not in our circles. It was boat and train all the way, replete with eighteen pieces of hand luggage and a number of auxiliary trunks and cartons that came along in their own time.

My scientific intinerary called for several months in Basel with Professor Josef Tomcsik at the Hygienic Institute of Basel University, and in Sweden with Dr. Gösta Widstrom, a colleague in tuberculosis research. These plans broadened in the event to include a short stay in Paris and several weeks in a fishing village between Nice and Cannes, where I settled my wife and daughters for a several-month period while I went off to Basel, to which I eventually fetched them. There I spent some months extracting lipids from various bacteria, writing a book on immunity, and enjoying the warm hospitality of our hosts, Josef and Olga Tomcsik,

The Tomcsiks had come from Budapest to succeed Robert Doerr, the well-known virologist and immunologist, who had headed the department.

Professor Doerr still came to his office daily; he was engaged in revising his monographs on the viruses, and—since I had shipped in cartons of current reprints on immunologic subjects as grist for my own textbook, and gave him full access to these—after a time we established a warm relationship. This overcame his general coolness toward America and its inhabitants. He had visited the US several years earlier, at a rather advanced age and without the benefit of conversational English or a companion. Nonetheless, he had crossed the country, and he told me in his forthright way that he found little to admire about it, with the exception of the Pacific Ocean, which he found to be first rate.

Professor Doerr spanned the immunologic era from Paul Ehrlich to the 1950s. He had worked with many of the contributors to the origins of immunology, and conversations with him were illuminating and entertaining. The word "dummkopf" was never far from his descriptive armamentarium.

We went then to Copenhagen for several months at the State Serum Institute, for an equal time to Stockholm, then to Britain, and finally to Paris and Le Havre for the return home.

During our stay in Basel, I received a note from Edgar Lederer of the Centre National de la Recherche Scientifique in Paris, suggesting a collaboration in working out the chemical characterization of the mycobacterial wax. Dr. Lederer was a pioneer in chromotographic analysis, and he hoped we might be able to distinguish which components in our lipoidal mixture were responsible for the biological effects. This collaboration continued for a number of years, during which Lederer's group chromatographed extractives, and we tested them in guinea pigs for their biologic capacities. For a time, these isolations made it appear that the salient substance concerned in the induction of cellular hypersensitivity was composed of an unsaturated fatty acid peculiar to mycobacteria, and called mycolic acid, esterified with a saccharide. But subsequent chemical studies by others showed that mycolic acid extracted from the bacillus is linked to muramic acid, which in turn is joined to four peptides. Eventually the important ingredient was identified as muramyl dipeptide—a very simple and ubiquitous component of bacterial cell walls. One wonders why a wider array of bacterial cells do not show the striking adjuvant and delayed hypersensitivity-inducing properties of mycobacteria if the simple muramyl-dipeptide is at the root of these. The possibility that mycolic acid may, after all, participate in the biologic events stimulated by these bacteria still intrigues some investigators.

The ignorance of the mid-1930s regarding the protection-inducing immunogen of the tubercle bacillus is not entirely relieved today. Perhaps the

most promising recent lead into the solution of this has been proposed by my former student, Alfred Crowle, now at the Webb-Waring Institute of the University of Colorado Medical School. With great persistence Dr. Crowle has pursued evidence that he first uncovered as a student over twenty five years ago, that a trypsin extract of the bacillus contains a proteinacous protection-inducing substance. Recently, after many successful tests for protection in animals, this antigen has been administered to human subjects in whom it has shown immunogenicity. The World Health Organization is contemplating broader-scale tests at this writing.

With analogous persistence, another student of that era, Ivan Kochan, has elaborated evidence of mycobacterial stasis in animal sera to the point of defining a siderophore called mycobactin, which abstracts iron from the environment for the bacterium, and which may vary in effectiveness or quantity with the virulence of the organism.

Long experience with the striking immunologic events occasioned by the mycobacterium turned my attention to more general questions about cellular immunity.

Fifty years ago, cellular immunity and delayed hypersensitivity were well-developed concepts in respect to infectious diseases—such as tuberculosis, lymphogranuloma venereum, smallpox, and syphilis—for all of which diagnostic skin test materials were available. A lively investigational interest was also well developed in regard to the cellular hypersensitivity induced by plants and chemicals, such as poison oak, poison ivy, and various chemicals used in industry. But notions about the nature of these reactivities was obscure; they were definable mainly by the fact that reactivity could be demonstrated in the absence of antibodies, as for example in the demonstration back in 1910 by Bail that hypersensitivity to tuberculin in animals could be transferred via cells from the peritoneal cavity or the spleen, but not by antibody-containing serum.

The great problem besetting those times was, of course, that no one knew which cells were responsible for immune reactivities, either for antibodies or for cellular immunity. It was not until the 1950s that lymphocytes emerged as the central actors in the immunologic drama, and the unwinding of the different functional populations of these umbiquitous cells has been going on ever since, so that we know now that a subset of thymus-derived lymphocytes (T cells) are involved in delayed hypersensitivity, but that these may be different from another subset concerned with protective immunity, whereas another major population of cells derived from the bone marrow (B cells) are destined to be synthesizers of antibodies. But some of the important questions that plagued the immunologic generation of the 1930s are still unclarified, e.g. what precisely is the nature of the receptor

that recognizes antigen on the delayed hypersensitive cell, and exactly how does the macrophage actived by Freund's adjuvant encourage cellular reactivity?

My interest in these questions led to a series of studies over some years, aimed mainly at the central one: What kind of stimulus determines whether or not an immunologic response will be predominately cellular or humoral and, once uncovered, can the stimulus be patterned to accommodate various situations in which one or the other kind of response would be desirable? An example would be the case of tumors, against which protective immunity is frequently of cellular type.

Experiments with students Margot Pearson, Michael Brunda, and Judith Britz showed that macrophages exposed to Freund's adjuvant and then mixed with an antigen in vitro for injection into animals gave rise to a predominately cellular immunity to the contained antigen. I hypothesized that this might be a result of degradation of antigen by the lysosomal enzymes of activated macrophages. This idea was to me a very attractive hypothesis because it fell in so neatly with reported examples of simple chemical antigenic entities that stimulate only cellular responses. However, Judith Britz showed rather convincingly that the effect depends upon a soluble factor from activated macrophages that in some way stimulates the appropriate T lymphocytes into responsiveness, a substance of the group now referred to as interleukins.

A good share of my professional time at Stanford University was spent in teaching—mostly of medical and graduate students, and episodically of undergraduate majors in microbiology. I was myself periodically a classmate of my own students in some of my earlier years. I came to the Department as a Fellow in 1935, but I was soon enthralled by the California ambience, the opportunities for research, and the University's public health nurse, Yvonne Fay, who later became a Raffel, and much to my joy has remained so for over forty years. Fortunately, Professor Schultz fell in with my views, and in 1937 I was made an Instructor. He further acceded to my developing notion that I be allowed periodic leaves for taking a medical degree. Some of the preclinical courses I had already completed at Johns Hopkins University as part of my doctoral requirements, so that between Stanford University and Duke University, which offered summer clinical clerkships, I was able to graduate from Stanford with the medical class of 1942.

Aside from the educational benefits of this training, I had the interesting experience of being cohorts with two classes at Stanford and with three at Duke University, and I formed some valued friendships while savoring my double role. Consequently, I felt an especially close rapport with medical students for many years, until the mid-1960s when some slippage occurred,

occasioned by the prevalent student unrest, our own revision of teaching programs attendant upon a shift of teaching hospital from San Francisco to the University campus at Palo Alto, and probably my own changing outlook. In any case, for a quarter century or more I felt a warm association with medical students and thereon hangs the tale that follows. These events began about twenty years ago and unraveled over a period of more than two years.

One day in a lecture to the medical class about the anthrax bacillus and its relatives, I happened to mention that I had recently received a letter from Professor Ascoli, then a retired microbiologist living in Italy, in which he complained that in my recently published book on immunity, in a chapter devoted to anthrax, I had failed to take account of his report that extracts of the tissues of buried cattle could be used for the retrospective serologic diagnosis of anthrax. This reaction was ascribed to bacterial polysaccharides and was considered to be helpful for the diagnosis of herd infections.

I promptly forgot having mentioned this mild anecdote, nor did I associate it with what follows until a long time later.

One day a letter in scripted hand arrived from London. Its content was this:

My Dear Dr. Raffel,

I read with great interest your recent book *Immunity* and was greatly pleased to see a person of such youth make a fine contribution to the study of micro-organisms and serums.

I would like to protest, however, the very scanty reference to my research and especially the Jenner-Adams test (Proc. Royal Acad., 1799) for type III cowpox. I trust that in future editions, you will give credit where credit is due.

I remain

Yr. humble servant
Edw. Jenner

Some weeks later, a letter in German arrived from that country, posted from Kloster Lechfeld. Again, after pleasant introductory remarks, the writer went on: "Leider muss Ich aber zugeben, das Ich etwas enttaüscht bin, da Sie nichts über mein 'Serum gegen die Seminaria morbi' noch von meinem 'Gegen-Teufel Toxoid' geschrieben haben." This bore the signature of Fracastorius. Apparently he used German to express displeasure.

During the course of the following two years, at intervals of one to several months, there came a further series of letters. The next was mailed from Paris by Louis Pasteur:

Très cher et honoré confrère, . . .J'ai trouvé vos explications d'une très grande clarté et j'admets qu'une grande partie de la recherche enterprise par vous ne m'était pas connue. Je trouve pourtant que vous avez commis un péché d'oubli et si vous voulez bien me pardonner ma suggestion, je pense que vous devriez accorder plus de place à l'étude de la rage . . .

The series now took a different tack: A letter from Ferdinand Magellan from Spain, after the customary felicitous introduction in his language, took me to task for giving insufficient consideration to the intriguing disease syphilis. He countered my impression that the disease had been introduced to Europe by Columbus on his first return from America. According to this informant, syphilis was brought from the Philippines—he himself has had the disease ("no joking matter") for 400 years.

There followed a communication from J. P. Higginbottom of the Hertfordshire and Bedfordshire Archeological Society accompanied by a box of Assyrian clay tablets "recently brought to light, in which an early dynasty immunologist, . . .although he favours your book as a whole, takes exception to your ideas on the acquired hemolytic anemia."

Then came a letter from Hong Kong, written in Chinese by Confucius, pointing out that in 498, when the letter was written, K. K. Chen had already found an herb that stimulated the pituitary gland and in turn the adrenals. Why had I not acknowledged this in speaking of cortisone and ACTH?

Next came an admonishing epistle from Mary Baker Eddy who did not think much of the book or of medicine in general. A subsequent note from Oa Mook in a completely foreign tongue (Lunarese, fortunately interlined with some translation) took umbrage at the fact that I had failed to refer to any lunar microbiologists and trusted that this omission would be remedied in future editions.

The final two letters became seriously scientific. The first from British Columbia—written by"E. Power Blake-Nutting, Director of the Spring Island Test Station"—stated that he had been struck by my discussion of cellular antigens and immunologic kidney disease. He in turn had some unpublished observations of studies undertaken with the Sooty Tern (*Aecleptis nigra*) and the Tufted Puffin (*Naris Wellanderi*). He injected macerated puffin kidney into the tern and derived an antiserum that on injection into the puffin, produced kidney damage. The injected puffin, in turn, revealed, in ultracentrifuged extracts of its kidneys, the presence of "a puffin antitern antipuffin kidney antibody." This was injected into a tern, and after two weeks, its globulin "plus previously prepared puffin antitern antipuffin kidney antibody" produced no damage in the puffin, "suggesting that the puffin antitern antipuffin kidney antibody had been neutralized by a tern antipuffin antitern antipuffin kidney antibody. We believe that this is the first demonstration antianti-antibody. . . . I hope that this may be of some help in clearing up the subject of cellular antigens."

Finally, Albert Smudge, PhD, wrote from Hawaii, from the Institute of Marine Biology, concerning homologues of the human atopic allergies seen in fish. These observations were based on studies of the homohomo-

nukunukuapaoua, which, though it "serves as food for scores of larger fish, is strongly avoided by the Kualueluilui. When the two are placed in the same tank, about 80% of the Kualueluilui will show signs of distress: convulsive gill movements, increased body slime secretion, and in 24 hours a patchy necrosis of the skin . . . The Schultz-Dale reaction is negative . . . There seems to be a homohomonukunukuapaoua anti-Kualueluilui beta globulin antibody . . . " which "is the antibody of piscine atopic allergy."

This was the last of the epistolary series, and it happened to coincide with the graduation of that class of medicine that had been sophomores at the time of initiation of the series, when our course in microbiology and immunology was given. The author of these well-informed letters, and his operational methods in the use of several languages and always appropriate sites of posting, remained enigmas. At the seniors' farewell picnic of that year, the author was pointed out to me by a classmate, but the designated individual blandly refused either to acknowledge or disavow this claim.

Thus ended what was for me a delightful sequence, extending for more than two years, of first-rate wit and humor, and a warm feeling than at least one student had been moved by interest in the subject to have engaged in this protracted tour de force.

The conclusion of my vocation in formal teaching came far from home, in Iran, in 1977, the year before the Shah's approaching end had become obvious to all. But, in the first three months of that year, my wife and I lived in Shiraz, about 450 miles south of Teheran, and we had no hint of brewing troubles, nor for that matter apparently did the CIA.

The University of Shiraz was regarded as a symbol of the country's participation in modern education and scientific thought. It was a relatively large institution, second to the University of Teheran in size and prestige, and its special character lay in the fact that the teaching in all its branches was conducted in English. Since many of the faculty, at least in the School of Medicine with which I was associated, had spent considerable time in training in the US, and a few had done so in Britain, English presented no problem to most of them. The difficulty lay in the audience. The medical students, of whom there were only about 65 per class, were not equipped to cope with spoken English, virtually to a man—or woman, of whom there was a good representation, about a third of the total. The Iranian lecturers improved the situation by speaking slowly, putting on the board each point as it was made. But, most importantly, they were able to throw in a word or phrase of Farsi (i.e. Persian) at critical junctures.

My first lecture was to be an overview of immunology and, as it happened, this talk had to be delivered in the anatomy dissecting room. Perhaps fifty cadavers occupied the farther reaches of this spacious chamber. After

about ten minuties of what I considered a spritely monolog, it occurred to me that students and cadavers were absorbing it with about equal avidity. Following this introductory effort of mine, one student confided to a faculty associate that I must have come from Texas (a state that I have visited only briefly on a few occasions) because he had heard cowboys on television, and I sounded just like them. This presumably related to form, not content.

During the remainder of my stay I spent a good part of my time in reducing my remaining seventeen lectures to a sprinkling of more easily conveyed concepts and bits of information. I should say that the students were courteous and attentive through all this, and they were probably entirely capable of grasping what I wished to say; we simply did not share a common medium of communication.

Thus concluded my years of professing and researching. I tasted the satisfactions of telling others about what interested me, and occasionally I had the thrill of fitting together notions in the laboratory. All this was done against the backdrop of a happy family life, a felicitous ambience in which to live it, and opportunities for extensive travels and stays in a number of other countries. As the run of destinies go, a fortunate one.

My association with the Annual Review of Microbiology was part of the background that contributed to the enjoyment of this destiny. In the days of my youth at Stanford, in 1945, I was invited to join its editorial staff as an associate editor to Charles Clifton, a faculty colleague who eventually wrote the first of these remembrances-of-things-past. The other first associate editor was Albert Barker and, among the three of us, we shared the reading of manuscripts for many volumes to come. The first editorial board, which met annually for a day with the editors to generate lists of topics and potential authors, was a particularly stimulating one—including as it did William Taliaferro, C. B. van Niel, M. D. Eaton, J. M. Sherman, E. C. Stakman, and W. E. Herrell—and succeeding boards have perpetuated a happy balance of accomplished men and women.

The early days with their associations were heady ones for me, and, over the years, this facet of my activities, and the friendship with Murray Luck and his wife Edoe, have been for my wife and me among the happiest of our relationships. Although my formal retirement from the *Annual Review of Microbiology* came with volume 33 in 1979, I am delighted that my affiliations with the Editorial Board continues, and apparently will until, as current editor Mort Starr puts it, I have lost my marbles or become otherwise disqualified.