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SERENDIPITY IN IMMUNOLOGY

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PART I: TRAINING IN IMMUNOLOGY

This is an essay about the role of luck, in one guise or another, in immunology. Since the Editors intend that the introductory essays should also be in some degree personal, before considering luck (as serendipity in a philosophical and historical context) I shall write about the good fortune that has enabled me to live through and even to take part in the adolescence of immunology as it grew to its present flourishing adulthood.

In last year's *Annual Review of Immunology,* Elvin Kabat discussed getting started 50 years ago. My beginning was 5 years later. I had been reading preclinical medicine at Cambridge, in the naive but currently accepted belief that having a medical qualification would qualify anyone to do medical research. Even more naively I believed that medical research was bound to be of some good to mankind—that it could escape perversion by the war which, even in the mid-1930s, many students feared would be inevitable unless the governments of the time were prepared to realize that *Mein Kampf* was to be taken literally. It was possible to take a year to do an advanced course (so-called Part II) in Biochemistry in a lively department that included not only Gowland Hopkins, but Joseph and Dorothy Needham, N. W. Pirie, Hans Krebs, Ernest Baldwin, Marjory Stephenson, Malcolm Dixon, Frank Young, and a group of research students among whom were two future Nobel Prize winners (R. L. M. Synge and L. F. Leloir). The message from this course was that enzymes and their specificity were the most exciting objects of study. Antibodies were never mentioned, but on reading J. R. Marrack's remarkable monograph on

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The Chemistry of Antigens and Antibodies (27a) I realized that antibodies had specificity comparable to enzymes and appeared much easier to obtain at will. Marrack's book, and W. W. C. Topley's section on "Immunity" in *Principles of Bacteriology and Immunity* (45), convinced me that immunology was what I wished to study. In order to do the three clinical years required for a medical qualification I went to University College Hospital Medical School (UCHMS). The Professor of Bacteriology was Ashley Miles—a superb teacher. Miles was working at the time on the antigens of *Br. melitensis*. He subsequently became Deputy Director and Head of the Biological Standards Division at the National Institute for Medical Research (NIMR), and later Director of the Lister Institute for Preventive Medicine. The Professor of Chemical Pathology at the Medical School was Charles Harington, later the Director of the NIMR. Also at UCHMS were Sir Thomas Lewis and George Pickering, interested in histamine as well as in heart disease, and the versatile experimental pathologist Roy Cameron, under whom was working Leonard Glynn. It was a small school by modern standards, but lively and friendly; and it did something quite exceptional at the time—namely, allow students who were keen enough to take part in the work of the departments. In this atmosphere it was possible to learn some of the techniques of immunology for which at the time there were no textbooks [the first edition of Kabat & Mayer's *Experimental Immunochemistry* (22a) appeared in 1948, and Landsteiner's revised classic *The Specificity of Serological Reactions* (26a) in 1945].

Harington, who had earlier synthesized thyroxine and glutathione, had become interested in immunochemistry and was testing the possibility of controlling the effects of hormones and some pharmacologicals by antisera raised against their protein conjugates. As Elvin Kabat pointed out in his chapter last year, accurate quantitative studies were possible even though it was not until 1939 that antibodies were recognized as being γ-globulins. Harington and his colleagues were able to show that rabbit antisera to thyroxine could prevent the metabolic effects of thyroxine in rats, and antisera to aspirin could prevent the antiphlogistic action of aspirin. He and Gordon Butler (later Director of the Chalk River Laboratory) were investigating antisera against stilbestrol when in 1939 the Second World War put an end to such esoteric research. It was almost 20 years later that research on antisera to hormones was taken up again by Erlanger (10a). A puzzle at the time was why some proteins, such as insulin and gelatin, were apparently not immunogenic. The concept of immunological tolerance had not been formulated, and Ehrlich's notion of "horror autotoxicus" was forgotten or not considered relevant. As an explanation for this non-immunogenicity Harington suggested that insulin lacked carbohydrate and gelatin lacked tyrosine. He assigned me the project of attaching carbobenzyloxyglucosidotyrosyl groups to these proteins and testing their immunogenicity. Without realizing what a privilege it was, I was accepted as a

member of the team. By neglecting clinical studies and working mainly at night, I was able to show that the subject rabbits made detectable antibodies against the attached groups but not against the parent proteins (18a). Not a very surprising result, but splendid training!

When war finally broke out UCH was closed, except for a few beds in the basement. Students had to find places wherever they could until the teaching could be reorganized.

Having scraped through the final examination in 1940 I got a post as intern in the Department of Medicine at the Postgraduate Medical School at Hammersmith, in quick succession under John McMichael, Edward Sharpey Shafer, and Eric Bywaters—all able scientists as well as good clinicians. Soon, however, I came down with pulmonary TB. In 1941, when back on my feet again, I went to work with Douglas McClean on hyaluronic acid and hyaluronidase at the Lister Institute of Preventive Medicine at Elstree. Although I knew little about polysaccharide chemistry, I obtained advice from Walter Morgan, also at the Lister Institute, who was working at the time on *Shigella* antigens and beginning his classic studies on human blood group antigens. I thus gained some insight into complex polysaccharides and the enzymes that hydrolyzed them at a time when few people were studying them.

It was difficult to relate working on hyaluronidase and hyaluronic acid—interesting though they were—to what was going on in the war. Despite lacking any special training in pathology (owing to the disruption of medical studies at UCH I had rarely attended and never conducted an autopsy), I applied for a post as assistant pathologist at a large (900 bed) general hospital in North London. Ashley Miles agreed to act as a referee on the condition that if I got the job I would spend two months in the wartime Sector Bacteriology Laboratory he was running, so as to learn some real bacteriology. To my astonishment I got the job, and in the specified two months of intensive training I was taught enough practical bacteriology to cope. The morbid anatomist was Walter Pagel, an authority on tuberculosis and a noted medical historian, who had an enquiring mind and a splendid sense of Jewish humor. There was no other pathologist (the head of department being a prisoner of the Japanese), and I found myself in charge of the hematology, biochemistry, and bacteriology sections of the laboratory. Each section had a well-trained technician in charge, with whom I reached an amicable agreement: Each knew more about the job than I did; I would learn from them, but in the end I would probably know more than they; when innovation was needed, or novel problems arose, we would tackle the situation together. This arrangement worked well. Of all possible trainings for biomedical research, I can think of none better than being Lord High Everything Else in a busy general hospital during wartime! We were in close touch with an able clinical staff and had constantly to introduce new techniques and take on ad hoc research problems.

There were no central reference laboratories to which problems or specimens could be referred. One solved problems by asking friends and acquaintances and/or looking up the literature and getting on with the job oneself. Medical students, seconded from the Middlesex Hospital, were pressed into helping and it was possible to get a surprising amount done. Since Walter Pagel was liable to asthma and severe bronchitis, we both knew that sooner or later he would become ill and I would have to do the autopsies and make the histopathology reports. He taught me to cope with the commoner problems, though I jibbed at reporting on frozen sections of brain in mid-operation, and got the neurosurgeon, who was quite competent to do so, to look at these himself. When Walter Pagel did fall ill, and the dreaded moment came in which I had to perform the autopsies, I made an arrangement with the mortuary attendant whereby I kept the staff and students busy with talk while he inconspicuously indicated on the corpse where I was to cut next. Our first such autopsy was on an elderly man who had died from type III pneumococcal pneumonia. (We typed the strains at the time as part of a study of the etiology of pneumonias, and of their response initially to sulphonamides and later to penicillin.) When I lifted the knife after opening the lung, long viscous strands of type III pneumococcal polysaccharide hung from it, and I was able to discourse on why type III was the commonest cause of severe pneumococcal pneumonia in the elderly. In the course of another 400–500 autopsies (almost all patients who died were autopsied) I realized what a lot can be learned from them. For example, there is no clearer demonstration of normally invisible lymphatics than the spread of carcinoma along them.

When the war ended, the former head of laboratory was due to return. I thought he was bound to regard me as a usurper, so I resolved to return to full-time research. Antibiotics promised to eliminate many diseases, but it was evident that cancer, old age, and rheumatic diseases would remain serious problems. There had been many admissions for rheumatic fever during my time as pathologist, and although the connection between rheumatoid arthritis and streptococcal infection was obvious, in 1946 the causal relationship was not. I wrote a proposal to investigate why and how streptococcal infection might stimulate autoantibody production against cardiac muscle and vascular endothelium. My initial hypothesis was that streptolysin S might complex with cell surfaces and that interaction between the complex and antistreptolysin S would lead to vasculitis and valvulitis. The Medical Research Council gave me an appointment on its external staff to work in the Department of Bacteriology at UCH under Wilson Smith, a virologist from the team responsible for isolating influenza virus. Rheumatic fever promptly became uncommon in the UK, and even regular visits to the isolation hospitals to which cases of scarlet fever were sent failed to reveal fresh cases. Furthermore, since I had no clinical status at UCH I was not allowed to see patients, take blood samples,

or even swab throats. I ended up trying unsuccessfully to reproduce streptococcal tonsillitis in rats and rabbits, discovering that antistreptolysis S was not an antibody at all but a property of plasma lipoproteins.

Partly because of the difficulties of doing immunological research on patients, and partly because progress had resumed in basic immunology, I decided that application of immunology to the understanding of disease processes (as opposed to prophylactic immunization) would require an insight into immunopathology. I began to work on the Arthus reaction and on anaphylaxis.

The best-equipped and most prestigious center of biomedical research in Britain at the time was the National Institute for Medical Research at Hampstead. Its director was Harington (now Sir Charles), who had succeeded Sir Henry Dale; the head of the Division of Biological Standards was Ashley Miles. The Division studied, and prepared national and international standards for, the control of biological materials for therapeutic use. These included the newly discovered antibiotics penicillin and streptomycin, and others as they came along. Work on antibiotics had been assigned to Bruce White (best known for the Kauffmann-White classification of Enterobacteriaceae), but he died from leukemia in 1949. Although my work with antibiotics was confined to some therapeutic trials conducted when they were first released for civilian use—trials in which I had taken part while still a pathologist—I was invited to succeed Bruce White.

To become a member of the staff of the NIMR, especially under Harington and Miles, seemed a dream come true. It was understood that persons working in the Standards Division should have at least half their time free to do research on whatever they chose, and that members of other Divisions would help and advise them when needed. Furthermore, much of the work of the Division concerned antisera and prophylactic vaccines. Once the antibiotics were under control there would be a chance to return to the study of immunology in an ideal environment. I jumped at the chance and spent the next eight years in charge of antibiotic (and later some enzyme) standards. The work itself was far from dull, and it drove home the possibility of doing really accurate bioassays; the importance of linearity and parallelism of dose response curves became clear. Above all there was the opportunity to introduce immunological techniques and immunological problems to colleagues in various Divisions and to obtain their help or to cooperate with them. Projects included application of the newly available radioisotopes of iodine and carbon to studies of immunoglobulin metabolism and the synthesis of antibodies in vivo and in vitro, pharmacological studies of cutaneous anaphylaxis and of platelet involvement in allergic reactions, and demonstration of the role of granulocytes in Arthus reactions. I mention these not because they were important advances in immunology but to illustrate how widespread were the possibilities of cooperation. So many of the technical staff wanted to learn simple immu-

nological methods that for a while I ran lunch-hour seminars to which increasing numbers of the scientific staff also came. By 1957 immunology had caught on sufficiently at the NIMR for the Director to suggest that I be relieved of duties in the Biological Standards Division in order to set up a small Immunology Division composed of the persons with whom I had been working most closely—Brigitte Askonas, Walter Brockhurst, and Brigid Balfour. This was the first formal immunological post created in Britain (though of course much first-class research in immunology was done under other titles). From then on we were in the remarkably fortunate position to do what we chose, at our own pace, in a multidisciplinary Institute staffed with able and friendly scientists. At that time, the Institute was also as well equipped as any biomedical research center in Britain. When three years later Sir Peter Medawar succeeded Sir Charles Harington as Director, and brought with him his group from University College to take over under Avrion Mitchison the Division of Experimental Biology, the NIMR became a center that for many years attracted immunologists as visiting workers from all over the world.

This all happened before immunology reached adulthood around 1960—i.e. before the basic structure of immunoglobulin was proposed and lymphocytes were shown to be the immunocompetent cells. I have written in this egocentric vein to illustrate both my luck in the able people who were willing to teach and tolerate a beginner, and the devious route by which in those early days it was possible to become an immunologist. By present criteria, when specialized training and a PhD are needed before a candidate will be considered even for a temporary position, to gain such wide experience would be almost impossible.

PART II: SERENDIPITY IN IMMUNOLOGY

Grants committees are duty bound to see that money made available for biomedical research is spent to the best advantage—i.e. to further the discovery of how biological processes work and how to control them in the interests of better health. As a member of various such committees, I have come to realize that the manner in which grant applications must be formulated leaves little scope for discovering something new. A well-presented grant application demonstrates, as it should, that the applicant knows the field in which he wishes to work, understands why it interests him, and is familiar with the relevant literature. He or she is then expected to define a precise problem in this field, set out in considerable detail how it is to be tackled experimentally, and state what sort of findings are expected. It is useful to add a few hopeful sentences about how the findings will advance knowledge or have some practical impact in medicine, veterinary science, agriculture, etc. It is better still

if the applicant has already done enough work on the project to know that it will succeed. The committee, most of whom have no special knowledge of the field, feel that they understand the proposal and are therefore more deeply impressed when the application is clearly set out. Such a procedure, which compels the applicant to show expertise, clear thinking, good judgment, and the ability to distinguish a wood as something more than the trees that grow in it, provides, when accompanied by favorable reports from referees who know the subject well, a means of distributing grants sensibly. It enables the grant-giving body to allot funds, which are not inexhaustible, according to its idea of priorities. The committee decides whether the applicant's project may make practical contributions to research on cancer, skin disease, dentistry, virology, population control, etc, or whether—as at least such bodies as the British Medical Research Council intend—it may contribute fundamental knowledge in an area where this is lacking.

The sort of application I have in mind is illustrated in the instructions for Project Grant Applications to the British Medical Research Council.

> The purpose of the Council's scheme of project grants is to provide support . . . for single projects—pieces of work designed to seek the answer to a single question or to a small group of related questions. . . . It is the Council's policy that the duration of support under this scheme should normally not exceed three years; a single clearly defined project may be expected to have been completed (or to have failed) in this time and the need for extension should be exceptional.

The work funded according to such requirements may well be worth doing, but if it is carried out as set forth in an application like the one just described it cannot discover anything fundamentally new. It can test an hypothesis and extend it, or show that the original reasoning was incorrect, but expectation of the general outcome is implicit in the application. On the other hand, if the application were to state that "these are the lines along which I expect to begin my experiments, but I really hope an unforeseen observation will prompt an unexpected idea," it would need an unusually enlightened committee to award the grant. Yet this process of following up surprises is how a good many important (and unimportant) discoveries are made.

This brings me to the subject of serendipity. This strange word, which literally means a property of Ceylon (Sri Lanka), is used rather commonly nowadays. It was coined by an eccentric minor English writer, Horace Walpole, 4th Earl of Oxford, in a letter to Sir Horace Mann, written in 1754. Walpole mentions a fairy tale, "The Three Princes of Serendip" (probably by the Italian Bocci), in which the princes "were always making discoveries, by accidents and sagacity, of things they were not in quest of." His own example was that "Lord Shaftesbury happening to dine at Lord Chancellor Clarendon's, found out the marriage of the Duke of York and Mrs. Hyde by the respect with which her mother treated her at table." The importance of serendipitous

discoveries obviously depends upon circumstances. The word, in any event, filled a gap in the English language and rapidly became part of the accepted vocabulary.

To discuss the role of serendipity in immunology—by which I mean the unexpected observation seized upon and turned to advantage by the prepared mind—could be invidious. Only someone getting on in years, who has lived through some of the important growing stages in immunology and has worked in several of the areas involved, would have the impudence to try.

Lest attributing importance to serendipity be regarded as trivializing discovery, an important point must be made. Immunology, being a branch of biology, is concerned with mechanisms that operate in, and have operated to produce, living creatures as they have evolved on earth. There is no guarantee that similar mechanisms function anywhere else in the universe; and although they can certainly fascinate us inasmuch as they shed light upon the mystery of Life, immunological discoveries have no *cosmic* significance. In contrast to physics and chemistry, whose generalizations when apply throughout the universe, generalizations in biology are—so far as we know—limited to the past, present, and future behavior of particular elaborate organisms, whose rules we try to discover. Of course these organisms do not disobey the laws of physics and chemistry, though they probably transcend them; but even the discovery of something as exciting as the genetic code in DNA concerns a particular device that has permitted living organisms to survive and evolve. Especially since the subsequent discovery of introns and exons, it is difficult to conceive of another device that would perform as well as this code; but if self-replicating entities capable of independent existence and combining certain of the other properties we associate with life had arisen in quite another way, different devices would presumably have evolved.

The process of scientific discovery has occupied minds much abler than mine—including that of Peter Medawar; see, for example, his essay on *Induction and Intuition in Scientific Thought* (32). Although it may be possible for great minds in mathematics or physics to arrive at verifiably valid generalizations by purely mental processes—thereby showing that the processes of mental logic conform in some fascinating way with causality as it operates in the physical world—in biology we can only proceed by observation and experiment. Medawar stressed the importance of hypothesis (or if not so clearly formulated as to be dignified by this term, of "hunch") in the design and choice of experiments. He separated experiments into four kinds:

1. Baconian. "I wonder what would happen if . . . ." Noncritical experimental play.
2. Deductive or Kantian. Examination of the consequences of varying the axioms or presuppositions of a scheme of deductive reasoning.

3. Critical or Galilean. Actions carried out to test "a hypothesis or preconceived opinion by examining the logical consequences of holding it."
4. Demonstrative or Aristotelian. Intended to illustrate a preconceived truth and to convince others of its validity.

"A good methodology," writes Medawar, "must, unlike inductivism, provide an adequate theory of the origin and prevalence of error . . . and it must also make room for luck."

Few people, I suppose, literally follow Francis Bacon's advice about how to probe the secrets of Nature. The Baconian approach implies that one might try rubbing two sticks vigorously together, not to test whether they would generate enough heat to ignite them but simply to see what would happen. This is certainly one way of discovering something entirely original; and when a subject is in its infancy, the Baconian approach may be the only one possible. In general, however, we know too much nowadays to proceed without some sort of hypothesis. We are also aware that in science an hypothesis is only useful if its consequences can be tested. Since any hypothesis stands a good chance of being overthrown or modified in the light of later knowledge, the hypothesis must be capable of refutation rather than of verification.

Medawar's second kind of experiment involves a motivation that I suspect is not uncommon—namely, such irritation at the certainty with which some hypothesis is promulgated that one designs experiments or formulates an alternative or contradictory hypothesis out of a sheer sense of devilment. It also includes a formula once put forward for gaining a Nobel Prize: Open a standard textbook at random, choose the most dogmatic statement on any page, and test rigorously its validity. I doubt, however, whether this advice is generally applicable!

Nevertheless, when able people turn a currently acepted hypothesis upside down the result can be enlightening. The trouble is that the mental effort involved is likely to be worthwhile and the arguments are likely to be accepted seriously by others only if there are at least some hints that currently accepted hypotheses are deficient or susceptible to modification. As an example I give Niels Jerne's early suggestion (21) that the body normally contains a population of cells already able to produce specific antibodies against almost every immunogen, and that the function of an immunogen is to take the antibody back to the cell that made it—wrong, but not hopelessly wrong! Jerne would probably not have produced the hypothesis had he not been studying antibodies against viruses, especially bacteriophage. Because the methods used in these studies were extremely sensitive he could find antibodies against the different viruses in all normal sera (20).

I could list other examples where received wisdom was challenged and superseded, noting that in each case the challenge followed clear indications

that then-current hypotheses were incapable of accounting for well-attested observations. These examples would include the clonal section hypothesis of Talmage & Burnet (5, 44), elaborated in 1959 by Burnet (6) and Lederberg (27); the demonstration (43) that a single monoclonal antibody may have combining sites capable of binding more than one distinct epitope (an observation whose relevance to the question of the size of the antibody repertoire is not always appreciated); the multigene control of the synthesis of single polypeptide chains in Ig and other proteins; and the discovery of idiotypes and anti-idiotypes by Oudin (41). This is not to state that immunologists never produce hypotheses based on the purely logical consequences of varying the axioms in a scheme of deductive reasoning, in the absence of a strong hint that such rethinking was needed. I regard Niels Jerne's (22) formulation of the network theory of the immune system as an example of this mental feat, which requires an unusually bold and clear mind.

Experiments in Medawar's fourth category are the stuff of a good many PhD and MD theses, which fill the libraries of universities and the pages of journals without adding greatly to scientific knowledge.

Most research workers do experiments that belong in Medawar's third category. They formulate an hypothesis—however limited—and carry out experiments to test whether or not it correctly predicts their results. The hypothesis provides the justification for designing the experiments. Cynics claim that the *sole* justification of any hypothesis is to make people do experiments. They above others must be aware of the importance of luck!

Luck, or in this context serendipity, has contributed to more fundamental observations in immunology—and of course in other branches of biomedical science (e.g. physiology)—than the published accounts of the observations might indicate. It seems appropriate here to mention examples from my own experience—not because my experiments were of great importance but because I know what really happened and can relate the facts without shame or risk of giving offense. It will become obvious that discoveries of mine that involved a concurrence of chance observations with what may be termed "a prepared mind" would sooner or later have been made by others.

I begin with two examples, minor and unpublished, from my years as a pathologist.

Pseudomucinous Cysts of the Ovary as a Source of Blood Group Substances

I thought that these cysts might be derived from ovarian granulosa cells and might contain hyaluronic acid. Since the viscous cyst fluid was unaffected by hyaluronidase, this was obviously wrong. However I had seen volunteers in Walter Morgan's laboratory chewing rubber bungs and thinking of lemons in order to produce saliva as a source of blood group substance, and cyst fluid

was not unlike saliva. When the next cyst came along I checked the blood group of its owner (group A) and took the fluid to Walter Morgan. It was almost pure A substance! The role of luck is shown by the fact that the next ten cyst fluids were unidentifiable as blood group substances (they were H or Le, which had not yet been characterized). The eleventh was B substance. If the first had not been identifiable Morgan would not have kept the rest, and would have continued to rely on saliva.

Procaine Penicillin

When it first became available I was responsible for issuing penicillin to patients. At that time penicillin was impure (400 units/mg), and injections were often painful. If any batch, tested on myself, proved severely painful, I mixed it with procaine. After an hour or two a fine amorphous precipitate appeared, which could be easily resuspended and painlessly injected. I showed in rabbits that it would protect against streptococcal infection, and that most of the penicillin appeared in the urine within 24 hours. Bottles of procaine penicillin mixture were issued to the wards with instructions to resuspend before injection. A penicillin manufacturer's representative to whom I recommended the procedure was uninterested at the time, but when I met him again four years later he told me that procaine penicillin had been patented and that $1 million in royalties were owed to the patent holder! By then penicillin was almost pure, and on mixing with procaine it rapidly crystallized as large needles that would not pass through a syringe unless pretreated in a micronizing mill. It would have been impossible to issue such crystals in suspension to the wards. Although I had no intention of preparing long-acting penicillin, the fact that it had been used sufficed to prevent the patent's being enforced and saved the British penicillin manufacturers $1 million!

Platelets and Granulocytes in Arthus Reactions

During the period when I thought I was doing experiments relevant to rheumatic fever, I used to elicit reversed passive Arthus reactions (i.e. inject known amounts of antibody intracutaneously, followed later by antigen intravenously) that were reproducible, convenient, and measurable quantitatively. The intensity of such reactions was reduced by cortisone, but the only obvious histological difference between treated and control animals was that the granulocyte infiltration was diminished when cortisone had been administered. Since at that time (1952) little was known about mediators of inflammation other than histamine, I supposed that histamine must somehow be involved and that perhaps it came from granulocytes. It was not difficult to show that rabbit blood contained quite a lot of histamine (and serotonin) but that it was virtually all in platelets and not in granulocytes. The obvious experiment was to see whether interaction of antigen and antibody in plasma would cause release of

histamine and serotonin from platelets—which it did (17), though the relevance of this finding to any phenomenon but anaphylaxis in the rabbit is questionable. More interesting was the observation that selective removal of neutrophil granulocytes in vivo, by nitrogen mustard in rabbits or specific antiserum in guinea pigs, prevented the inflammatory response so long as granulocytes were almost absent from the blood (13a). These experiments were valid, but they were based on a chance observation stimulated by a quite erroneous assumption about the role of histamine.

Antilymphocyte Antibodies

Erroneous assumptions about histamine also led to the first demonstration that antilymphocyte antibodies would prevent delayed-type hypersensitivity reactions. Theo Inderbitzin and my colleague Walter Brocklehurst had observed that when cutaneous delayed-type reaction was induced in guinea pigs, skin histamine level rose markedly at the test site. Our technique of histological fixation did not preserve guinea-pig mast cells—a fact of which we were unaware; otherwise we might have stumbled upon a role for mast cells revealed much later by Philip Askenaze (2)—so we thought perhaps infiltrating lymphocytes were the conveyors of the histamine. I prepared specific rabbit antisera against guinea-pig platelets, granulocytes, and lymphocytes and tested their effect on delayed-type response in vivo. Much to our surprise, antilymphocyte antibodies abrogated the response. This occurred whether or not lymphocytes were eliminated from the circulation. We were too unsure of what this meant to publish the findings, though they were published by Inderbitzin (19). When Medawar and Levy used antilymphocyte sera later, for much better reasons, the immunization schedule that worked was supplied from my notes.

Complement 'Holes'

Bob Dourmashkin (then with the Imperial Cancer Research Fund) had found by electron microscopy, using negative staining, that saponin-treated erythrocyte membranes apparently contained a beautiful pattern of hexagonal channels. When it was pointed out by Alec Bangham that these were simply due to arrangement of cholesterol molecules in the surface lipids around the solvated saponin, this was a disappointment. I had purified various hemolysins (streptolysins S and O, staphylolysin, *Cl. welchii* α-toxin), and I gave them to Dourmashkin to see whether he could demonstrate more interesting lesions with these. As a last-minute thought I added a complement hemolytic system. The toxins produced characteristic lesions (10), but the most regular and interesting were those produced by complement. Tibor Borsos was enlisted to prove that the lesions actually corresponded to those predicted theoretically, and to convince us that complement could be studied even by novices (4). A wholly unforeseen line of work was initiated (15).

Radioactive Suicide

Many years ago, before the clonal selection hypothesis, it seemed relevant to ask whether any antigen molecules were present in a cell stimulated to make antibody. With Hugh McDevitt we had shown that the number of antigen molecules in an antibody-secreting cell would not be more than 15 (30). Nossal had gone further and shown that the number was less than three. However, we had examined (T,G)-A-L partially labeled with ^{125}I as the antigen, in cells making anti-(T,G)-A-L, and theoretically the radiolabeled molecules might not have been those relevant. The only way to meet this criticism was to iodinate totally the tyrosines in (T,G)-A-L, converting it to TIGAL, and to examine cells making antibody against the iodinated form. In this case the radiolabel would be part of the immunogenic determinant. Hans Uli Keller and I set out to reexamine the question using ^{125}I TIGAL with specific activity about 2000 μCi/μg. This was a somewhat academic exercise, since there was by now general agreement that antigens did no more than trigger predetermined B cells, but we had done all the spadework and decided to go ahead.

To our surprise, although mice responded by making antibody against TIGAL perfectly well, they failed to make any against highly radioactive TIGAL. Yet the same mice responded to H-pertussis antigen injected, as an adjuvant, at the same time. Only when we sought an explanation did it occur to us that the B cells with receptors for TIGAL must have been selectively killed by weak β-emission from the ^{125}I (18). By a rather better reasoning process Ada & Byrt (1) had reached similar conclusions in respect of mouse spleen cells treated with highly radioactive flagellin. These experiments provided at the time the best evidence for the validity of clonal selection.

Inhibition of Antihapten Responses by Hapten-Conjugated Polysaccharides

Intrigued by the problem of why thymus-independent (T1) immunogens appeared to be incapable of receiving T-cell help, I thought that if a suitable hapten were attached (e.g. DNP onto pneumococcus type III capsular polysaccharide, S3) and this were administered to mice sensitized by application of DNCB to the skin, the DNP-reactive T cells would enable DNP-S3 to behave as a thymus-dependent immunogen. In fact it turned out that quite small amounts of DNP-S3 not only failed to increase the response to S3 but almost completely prevented mice primed against DNP-conjugated proteins from making anti-DNP on rechallenge with the same conjugate (38). This observation, the opposite of what was expected, led to a series of experiments with my colleagues Gerry Klaus and Abul Abbas to determine the mechanism by which B cells could be switched off specifically by antigens (23).

It also led indirectly to the observation that T1-1 and T1-2 immunogens are retained in different macrophage populations in distinct compartments in

lymphoid tissues. Because any possible therapeutic applications of selective suppression of antihapten responses were unlikely to involve using S3, I tried out conjugates of a variety of polysaccharides readily available commercially. In order to study their metabolism at the same time, I also conjugated small amounts of tyramine so as to permit trace labeling with radio-iodine. Again quite unexpectedly I found out that whereas conjugates of some polysaccharides were potent suppressors of secondary antihapten responses, others were poor suppressors but potent stimulators (14). All the polysaccharides had prolonged half-lives in the body but the T1-1 and T1-2 conjugates became located quite differently in different tissues. Autoradiography revealed that T1-2 conjugates were confined to a subset of macrophages (16), whose functions are still being studied.

Follicular Dendritic Cells and B Memory Cell Generation

It had been proposed by Dukor and his colleagues that activation of C3 could be a necessary and sufficient second signal to stimulate B cells with receptors for an antigen to secrete specific antibody (9). For various reasons this seemed unlikely. We thought it could be tested by seeing whether thymus-deprived mice could make an antibody response to Cobra venom factor (CVF), a naturally occurring form of activated C3, which had already been shown to be a potent immunogen. It turned out that the response to CVF was completely thymus dependent, which was inconsistent with Dukor's hypothesis (42). But this observation also made it possible to keep thymus-deprived mice with undetectable C3 levels for weeks on end.

Evidence had been produced that in mice treated with CVF, aggregated Ig—and by inference antigen-antibody complexes—failed to become localized on follicular dendritic cells (f.d.c) in germinal centers. It was also known that B memory cells could be generated in thymus-deprived mice. Knowing that such mice could be chronically depleted of C3 with CVF, we could test whether deposition of antigen-antibody complexes on f.d.c. were important or even essential for the generation of B memory cells. This proved to be the case (24) and led to a series of interesting experiments by Gerry Klaus that have emphasized the importance of antigen presentation in special microenvironments in determining the outcome in immune response (25).

Not every experiment I undertook was based on an hypothesis that proved false, but most of those that led to anything novel or interesting arose because of some unexpected or chance observation that I was fortunate in being able to follow up.

Having revealed my own dependence on serendipity, I may now describe briefly the origin of some more significant discoveries in immunology made by others, for which the importance of unexpected or chance observations has

been revealed by the discoverers themselves or by colleagues, or of which I know the actual sequence of events at first hand.

The H-2 System in Mice

In his early studies on the genetics of mice, to be followed by studies of genetic factors in resistance to transplanted tumors, Peter Gorer observed that sera from rabbits immunized with blood from 3 strains of mice, maintained for 25 or more generations by brother-sister mating, could distinguish two heritable markers on the mouse erythrocytes (11). He later (12) cross-immunized the mouse strains with blood or leukemic cells—which proved more potent immunogens—and tested for iso-antibodies by specially sensitive tests involving agglutination of erythrocytes. These showed that blood and tumor cells evoked antibodies with similar specificities, and that one of the specificities corresponded with that of antibody II previously obtained in rabbits (hence the name H-2). At this stage he could distinguish only three separate specificities, but their genetic association was such as to enable him to propose that "normal and neoplastic tissues contain iso-antigenic factors which are genetically determined. Iso-antigenic factors present in grafted tissue and absent in the host are capable of eliciting a response which results in destruction of the graft. Antigenic differences between normal and neoplastic tissues are not normally capable of stimulating a defensive reaction."

These observations do not so much illustrate serendipity (for he was seeking what he found) as luck. It happens that mouse erythrocytes, unlike those of humans and many other species, express small amounts of what are now termed class I major histocompatibility antigens, and do not express different conventional blood group antigens. But for this, his erythrocyte agglutination tests, which made multiple analyses possible in those days, would not have revealed the H-2 system.

The Role of the Thymus in Immunity

While Robert Good and his colleagues were moving on clinical and evolutionary grounds toward the idea (which they could not prove) that the thymus was crucially involved in some kinds of immune response, Jacques Miller was studying leukemia in mice at the Institute of Cancer Research. Leukemia in AKR mice commonly arises in thymus, and Miller was examining the effect of thymectomy on the development of leukemia. He tried removing the thymus at various ages, including from newborn mice. The neonatally thymectomized mice developed an unexpected disease syndrome—wasting, hunched backs, loss of hair, and eventually death—and were prone to infection. Their blood contained fewer lymphocytes than that of sham-thymectomized controls. Miller accordingly tested the capacity of neonatally thymectomized mice to reject

allogeneic skin grafts and to respond to *Salmonella typhi* H antigen. He found that many mice retained the grafts for long periods and that the antibody response was minimal or absent (33, 34). The fact that allograft rejection could be restored by syngeneic thymocytes constituted the sought-for proof that the thymus was essential for the development of the capacity to reject allografts and to make antibodies against certain common antigens. The observation has since been brilliantly exploited by Miller and by many other workers. A minor piece of luck was the use of *Salmonella* H-antigen to reveal immune deficiency in thymectomized mice. Had he used certain other antigens (e.g. *Salmonella* O antigen or pneumococcal capsular polysaccharide) that are now known to be thymus-independent, the evidence for the role of the thymus would not have been so clear.

T-B Cell Cooperation

The first demonstration that antibody responses to sheep erythrocytes required cooperation between bone marrow– and thymus-derived cells came from experiments by Claman et al (7). These investigators were testing the capacity of thymocytes to give rise to antibody-producing cells by a technique involving intravenous injection of thymus-cell suspensions and sheep erythrocytes into lethally irradiated mice, and later enumeration of foci of cells making hemolysin in the spleens. Thymocytes were known not to restore erythro- and granulopoiesis after lethal irradiation, so Claman and his colleagues added bone marrow cells in some mice in the hope that these mice would survive better. In the event, mixtures of bone marrow and thymus cells resulted in many more hemolytic foci than either cell suspension on its own. Claman et al rightly concluded that thymocytes must somehow cooperate with bone marrow–derived cells to enable the latter to secrete antibody. This unexpected finding was subsequently exploited and analyzed more fully by Miller & Mitchell (35, 37, 38).

Genetic Control of Immune Responses

At a time when it was not unreasonable to consider instructive hypotheses of antibody stimulation (i.e. that antigen molecules directly influenced uncommitted potential Ig-producing cells to make specific antibody), it seemed important to determine whether there were any molecules of antigen in a cell making antibody. Michael Sela and I discussed how this could be done and concluded that if the polypeptide (T,G)-A—L were synthesized from radioactive amino acids, themselves synthesized using tritium, it might be possible to detect a single molecule. Israel Schechter undertook and accomplished the synthesis, but the end product proved to be insoluble and we eventually used the new ^{125}I label. Meanwhile I set out to make anti-(T,G)-A—L in rabbits so as to detect antibody-containing cells by the sandwich immunofluorescence

technique. The sandylop rabbits at Mill Hill, immunized according to a schedule that was regularly successful in Israel, made no detectable antibody. Eventually we tried immunizing other breeds of rabbit and found in contrast that Dutch or Himalayan rabbits responded perfectly well. When Hugh McDevitt joined us to work on the project the first thing he did was to test all available strains of mice for responsiveness to (T,G)-A—L. Some made antibody regularly and others did not, and we then did the experiments (mentioned earlier) in F1 hybrids between two responsive strains (30).

McDevitt had realized that the strain differences were potentially important. By studying responses in F1 and F2 generations between responsive and unresponsive strains he had concluded that a single major genetic factor was responsible. He consulted Michael Sela about using another synthetic polypeptide to test whether the phenomenon was generally applicable, and they chose a similar molecule in which tyrosine was replaced by histidine. When this was tested they again found responsive and unresponsive strains, but the strains were different (28, 29). Examination of the H-2 specificities of a large number of strains and recombinants (recently worked out by Donald Shreffler) to three different synthetic polypeptides revealed that responsiveness was controlled by a gene or genes termed *Ir-1* lying between H-2K and H-2D (31). Having brought the researchers so far, the signposts to further progress were clear and the rest has followed!

Luck (converted to serendipity by McDevitt and Sela) was involved at four points: the use of (T,G)-A—L, which had very homogeneous epitopes so that the response was largely confined to these; the initial screening, for other reasons, of mouse strains; the choice of (H,G)-A—L, which behaved differently as a second immunogen; and the fact that the H-2 specificities had already been worked out, so that scrutiny of these could immediately suggest the association between responsiveness and H-2.

Lectins as Mitogens

When P. C. Nowell was culturing leukemic cells in vitro to study their chromosomes, using a technique described by Osgood (40) he employed phytohemagglutinin (PHA) to agglutinate and remove the erythrocytes. PHA had been chosen as a nontoxic lectin, as opposed to some others such as ricin. Unexpectedly, not only did leukemic cells proliferate but mononuclear cells from normal blood regularly underwent mitosis after a few days of culture (39). From this observation originated the exploitation of lectins as polyclonal mitogens, which has greatly advanced cellular immunology.

Australia Antigen (Hepatitis B)

In his Nobel Prize address, Baruch Blumberg (3) has described how he and A. C. Allison, interested in genetic polymorphism, decided to test the hypoth-

esis that patients who received a large number of blood transfusions might develop antibodies against putative polymorphic proteins that they had not inherited, but that the blood donors had. They tested sera against one another for precipitin formation by the agar gel diffusion technique and found such a polymorphism in low-density lipoproteins. They also found a different antibody in a hemophilic patient that reacted with an antigen present in the serum of an Australian aborigine but in that of very few normal Caucasians in the United States. It was relatively common in sera from individuals in some tropical countries and in sera of patients treated for leukemia with blood transfusions. Blumberg considered the hypothesis that the presence of Australia (Au) antigen was somehow correlated with susceptibility to leukemia and investigated its presence in sera of patients with Down's syndrome, who have a much increased tendency to develop leukemia. In Blumberg's study about 30% of the sera from children with Down's syndrome contained Au antigen. The serum of one child, originally negative, later became positive; this coincided with the development of chronic anicteric hepatitis. Further study of hepatitis patients showed that many had Au antigen in their blood early in the disease but that the antigen usually disappeared within a few days or weeks. This was the clue to recognizing that the Au antigen was part of the elusive hepatitis virus. Blumberg readily acknowledged the role of serendipity (and of excellent collaboration with other colleagues).

Monoclonal Antibodies from Hybrid Myelomas

Cesar Milstein had set out to answer the question whether amino acid sequence alone controls antibody specificity and, if so, how this control is achieved. Having completed a survey of the structure and evolution of immunoglobulins (36), he began a study of some of Michael Potter's mouse myeloma cell lines cultured in vitro. He intended to investigate whether they would show an unusually high rate of detectable mutation in their Ig product (they did not) or whether there might be evidence for scrambling between the variable and constant regions. To test the latter hypothesis he used an established means—namely Sendai virus—to fuse a mouse myeloma Adj PC5 with an 8-azaguanine resistant rat myeloma line S 210, and examined whether hybrid molecules of Ig were produced (8). Hybrid molecules containing light and heavy chains derived from either parent were detected, but there was no evidence of V-C gene scrambling. For further study he wanted myeloma cell lines that produced Ig with an identified antibody specificity and would grow in continuous culture, but none of his lines combined both properties. When Georges Köhler joined him they decided to try a long shot: Would spleen cells from a mouse immunized with sheep erythrocytes fuse with a well-established 8-azaguanine resistant mouse myeloma line P3, possibly secreting some antibody molecules specific for sheep erythrocytes? The experiment succeeded

beyond their best hopes. Not only were antibody-secreting hybrid cells produced, which could be cloned, but also a quite unexpectedly high proportion of the hybrids secreted specific antibody (26). From then on, hybridoma-derived monoclonal antibodies have become exquisitely sharp and popular tools for the identification and preparation of specific antigens. The concept has been extended to fusion of T cells with T-cell lymphomas, with equally important consequences. I have included this as an example of serendipity because the inital fusion experiments were done with no conscious intention to produce monoclonal antibodies and with no clear idea of what the implications of the availability of such antibodies would be. This in no way detracts from their importance or the brilliance of the follow up!

The list could be extended—and readers could surely add examples from their own experience—but it is long enough already to make the point that in immunology, as in other branches of biology, unexpected observations *and the prepared mind* are among the most potent stimulators of important advances. I would add four more, rather obvious points. One is that unless an individual with a prepared mind carries out the experiment personally or is at least closely involved in its execution, the unexpected may not be observed, or if observed may be dismissed as irrelevant. A second is that as many controls as possible need to be done if a plausible but erroneous hypothesis is to be refuted. Third, if the experimental data are largely derived from automated instruments that measure single parameters, only data the instruments are programmed to supply will be available. (This is a warning rather than an argument against instrumentation, since some instruments can provide more information than even the eye can detect.) The fourth, and most important, is that finance for research should always contain a substantial proportion of funds to provide scientists with the security and facilities that will allow them go to in some agreed general direction, but to follow their noses wherever the trail may lead. There was a period, during much of which I was lucky enough to be at work, when in some of the more prosperous countries this was accepted wisdom; but in the current financial climate it may be worth restating.

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