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The 1984 Nobel Prize in Medicine

The 1984 Nobel Prize in Medicine was awarded to three immunologists: Cesar Milstein, 57 years old, born in Argentina, of the British Medical Research Council's laboratory in Cambridge; Georges J. F. Köhler, 38, a German, of the Basel Institute of Immunology in Switzerland; and Niels K. Jerne, 72, London-born but educated in Denmark, professor emeritus at the Basel Institute. Immunologists were confident that Milstein and Köhler would eventually receive this award, and the inclusion of Jerne brings special satisfaction to many immunologists who believe that his extraordinary impact on the field deserved earlier recognition. The choices reflect an interesting blend: recognition to Milstein and Köhler for a methodological breakthrough that has profound practical significance, and in the case of Jerne for theoretical advances that have shaped our concepts of the immune system.

Köhler and Milstein were cited for developing the technique of monoclonal antibody formation that has had a revolutionary impact on immunology itself and on many other areas of biology. Before their studies, antibodies were prepared by injecting animals with foreign macromolecules, bleeding the animals, and separating the antiserum from the blood. The technique was laborious and presented major limitations. One problem is that the immune system can respond to tiny amounts of antigen. Therefore, even with the purest antigens, it is commonplace for antibodies to be produced against trace amounts of contaminating proteins. These antibodies have to be identified and removed to obtain antiserum specific for the antigen in question. Antibodies specific to a given antigen, however, still constitute a very heterogeneous mixture: they differ with regard to their specificity for different antigenic determinants on the same antigen molecule, their affinity for the determinant, and the class of antibody produced (there are at least ten different classes of heavy or light immunoglobulin chains in mice). It is not surprising, therefore, that the properties of antisera can differ among identically immunized animals or even between bleedings from the same animal. Also, such antibodies are contaminated with many other serum proteins unless they are purified by difficult and time-consuming procedures that require large amounts of pure antigen. As a result, serological assays were frequently misleading and not readily reproducible, and chemical studies of antibodies were hampered by their lack of homogeneity. In addition, the immune system has complex regulatory mechanisms that permit only a small fraction of the B-cell clones (the precursor cells of the antibody secretors) to be expanded and eventually to secrete antibody. These are the immunodominant clones; the vast majority of antigen-reactive clones are not expressed to a detectable degree in the hyperimmune sera that are used as reagents. Hence, only the tip of the iceberg of the B-cell repertoire is observed in conventional immunization.

Fusion of Antibody-Forming Cells with Myeloma Cells

At the time that Köhler and Milstein began their studies, no techniques had been developed for the long-term growth of specific antibody-forming plasma cells in culture. Michael Potter, at the National Cancer Institute in 1962, had induced plasma-cell tumors (called myelomas) in mice, and others had adapted such tumors to grow indefinitely in culture. In 1974, Köhler went to Milstein's laboratory in Cambridge as a postdoctoral fellow, and these two investigators decided to immortalize antibody-forming cells by fusing them with a myeloma line. Their primary objective was to use such fused cells to study the genetic basis of antibody diversity.

The fusing of two different cell types had been performed earlier by several investigators as a means of studying intracellular regulation and somatic cell genetics. The novel idea of Köhler and Milstein was to use such a technique to immortalize the antibody-forming cell, which only lives for several days in culture, by fusing it with a myeloma cell line, which can divide indefinitely in culture. It was hoped that the tumor cell line would endow the hybrid cell, called a hybridoma, with the capacity for survival. It was assumed that specific antibody formation would not be inhibited by the tumor cells because Richard G. Cotton and Milstein had just shown that when two myeloma lines were fused, immunoglobulin from each parental cell was expressed. Hence the myeloma cell line would also continue to secrete its own immunoglobulin (of unknown specificity) but it would not have the specific antibody activity in question.

Selection of Fused Cells

Milstein's laboratory represented an optimal choice by Köhler. Milstein, a leader in research on the molecular aspects of antibody formation, was known to be imaginative and receptive to new ideas. He had made seminal observations at the protein and nucleic acid levels of the intracellular mechanisms underlying immunoglobulin synthesis, had pioneered in the study of genetic variation among immunoglobulin molecules, and had developed his own myeloma lines. A critical element in the planned experiments was the need for a selective technique to recover only fused cells. Since antibody-forming cells die quickly, it was only necessary to delete the myeloma cells. A mutant myeloma cell line was used that was deficient in

the enzyme hypoxanthine phosphoribosyltransferase. Without this enzyme the cells die in medium containing a mixture of hypoxanthine, aminopterin, and thymidine (HAT). Thus, it was possible to select hybrid cells that could grow in the presence of HAT because of the contribution of the wild-type enzyme by the normal antibody-forming plasma cells.

Successful formation of such hybridomas was reported in 1975 in *Nature*. The dividends were far-reaching. It now became possible to obtain antibodies that not only are specific to a single antigenic determinant but are also a homogeneous population of molecules and, therefore, represent a chemically pure reagent. Such antibodies can be obtained in unlimited amounts either in tissue culture or by injecting the hybridoma cells into the peritoneal cavity of mice. The injected neoplastic cells proliferate and secrete large amounts of antibody resulting in antibody-rich ascites. It is a relatively simple matter to purify these monoclonal antibodies, particularly if they have a binding site for *Staphylococcus aureus* Protein A, which can be used to separate them.

Diverse Applications of Monoclonal Antibodies

These antibodies proved to be ideal reagents for the radioimmunoassays that play increasingly important roles in medicine and the basic science laboratory. It is possible, using this new technique, to generate antibodies with specificities that are not detectable by conventional immunization. Indeed, the entire B-cell repertoire, which had not been observed or fully appreciated in the past, could now be examined. It was as though the entire iceberg could now be lifted out of the water, turned into different positions, and scrutinized in detail. All that is needed is to set up a screening procedure to detect a clone of predetermined specificity; it is possible by appropriate radioimmunoassays or enzyme assays to screen several thousand B-cell clones to find the desired one.

The power of the technique was quickly appreciated, and immunology journals were soon flooded with reports of monoclonal antibodies to new antigens that had hitherto escaped detection. Many were differentiation antigens on lymphocytes that helped to distinguish different stages of cell development and to define subsets that perform discrete functions. It became possible to phenotype the surface antigens of all cell lineages. These reagents opened up new avenues for dissecting molecular aspects of immunoglobulins, such as the nature of the antigen-combining site and the three-dimensional structure of the antibody molecule. New gene products were also detected. As an example, the class II major histocompatibility complex antigens in the human, which are associated with increased susceptibility to such diseases as rheumatoid arthritis, juvenile diabetes, and multiple sclerosis, were poorly defined before monoclonal antibodies were available. The different loci and alleles for these antigens are still being enumerated in the human and, as a result, new disease associations are being described.

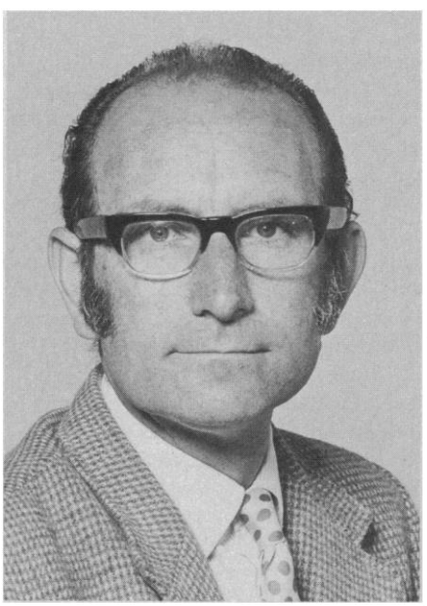
Monoclonal antibodies have the potential to improve the diagnosis and treatment of cancer. Thus, antibodies against tumor-associated antigens are being developed for imaging small tumors and have been administered clinically to treat tumors. Such antibodies have also been conjugated to toxins or cytotoxic drugs and have been used to treat experimental tumors.

In addition to their importance to immunology and medicine, monoclonal antibodies have had a major impact on other areas of biological investigation. Many recent advances in molecular biology were made possible by the use of monoclonal antibodies for the detection of recombinant clones synthesizing proteins of interest. Monoclonal antibodies can be used to isolate a protein from a mixture of macromolecules and to examine the function of particular domains of the protein. Such antibodies against plant proteins have also been prepared and used to determine an auxin transport site in plants.

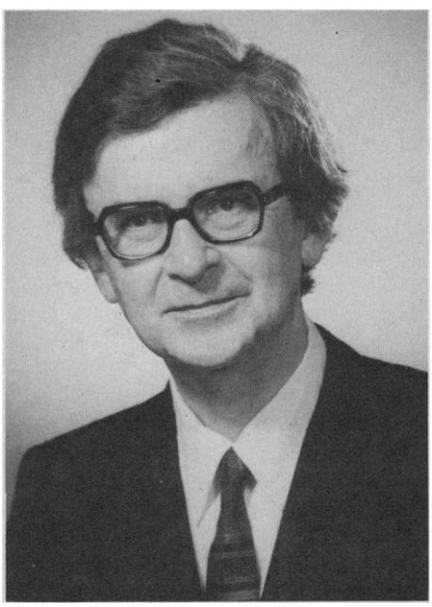
The Milstein-Köhler experiments demonstrate the power and unpredictability of basic science. The goal of the initial experiments was relatively narrow. It is doubtful that these investigators fully appreciated all the ramifications of the new method; rather they pursued their interests unburdened with mission-oriented restraints and their results had significance far beyond their expectations.

The Influence of Niels Jerne

It is unlikely that the studies of Köhler and Milstein would have taken place without the accomplishments and influence of Niels Jerne. He is regarded by many immunologists as the scientist who, along with Sir MacFarlane Burnet, has had the greatest impact on contemporary immunology. Jerne's creativity and style were already apparent when he was a medical student. He planned incisive and simple experiments. Thus, he used the diphtheria toxin–antitoxin system as a model of antigen-antibody interaction and of the antibody response because antitoxin can be easily measured by its capacity to neutralize the ability of toxin to cause an inflammatory response in rabbit skin. Jerne observed that the antitoxin made in the primary response could be more readily dissociated from toxin than the antitoxin formed in the



Cesar Milstein



Niels K. Jerne

secondary response. Jerne coined the term "avidity" to describe the ease of dissociating antibody from antigen. This experiment provided the first demonstration that, during immunization, the quality of antibody improves.

Jerne probably spent no more than 1 or 2 days injecting rabbit skin with different doses of toxin, measuring the size of the inflammatory response, calculating the volume of epidermal cells damaged and the concentration and volume of injected toxin, and arriving at the conclusion that one diphtheria toxin molecule was capable of killing a single cell. It would be many years later when more sophisticated tissue culture techniques confirmed this conclusion. It was Jerne's approach to conceive experiments that had testable predictions; if the predictions were easily tested, Jerne did the experiments; if not, he let others do the testing. The "others" eventually became the majority of cellular immunologists who are still testing several of his major hypotheses.

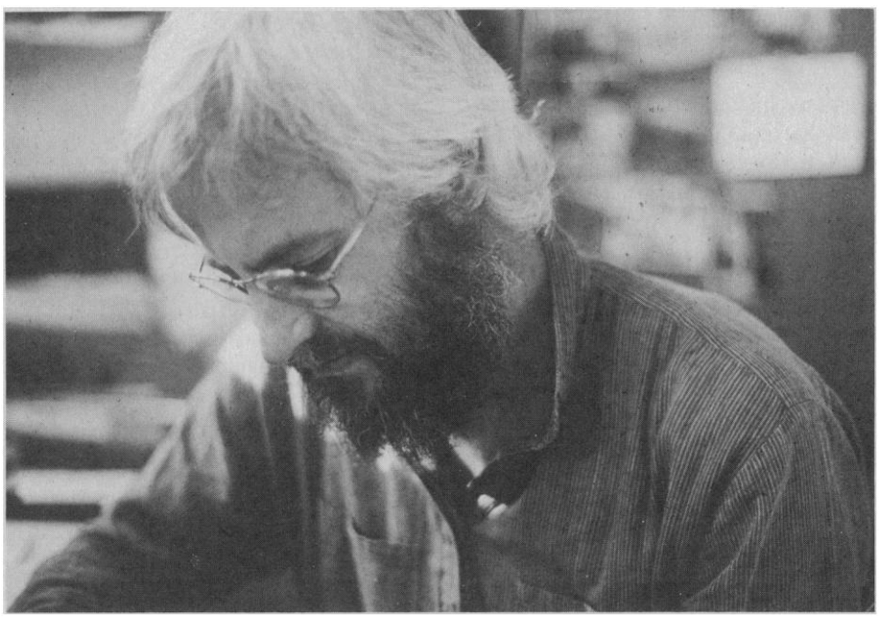
In 1963, cellular immunology was in need of an assay to measure individual antibody-forming cells. At the time, it was only possible to measure serum antibody and speculate about the preceding cellular events. In that year, Jerne and Albert Nordin described a simple method for enumerating antibody-forming cells by exploiting the ability of antibody and complement to lyse red blood cells. Thus, using antibody-forming cells to red blood cells, they developed a plaque-forming technique that represented an important new tool for cellular immunologists. This methodological advance initiated a major new investigative thrust toward understanding the cellular events underlying the antibody response.

Although the above accomplishments are impressive, it was Jerne's three major hypotheses that were cited by the Nobel committee. One can predict certain stages after the publication of a Jerne hypothesis: (i) incredulity, anger, or amusement; (ii) reports that dismiss the hypothesis because it does violence to the facts; (iii) a second reflection (is there really anything to this concept or is it as absurd as one originally thought?); (iv) reports that verify one or more of its predictions; (v) a new "bandwagon" with fanatic recruits; and (vi) an army of immunologists that proceeds to evaluate and debate the subject in great depth for the next decade or two. Surely, these are the stigmata of an important hypothesis.

Jerne was the first modern scientist to propose a selective theory of antibody formation; that is, he suggested that the information for generating antibody molecules of different specificities is present in the host before it encounters antigen. This hypothesis represented a great intellectual leap from the instructive theories proposed by the prominent immunochemists of the day who believed that antigen served as a template on which antibodies were formed. The new concept defied common sense because it was already known that a single animal could produce an apparently unlimited number of antibody specificities, that is, an animal could respond to injection of any foreign macromolecule by synthesizing highly specific antibodies. Without knowledge of the genetic basis of antibody formation it appeared unreasonable to assume that such a massive amount of information could be contained in the genes for the immune system. The particulars of Jerne's hypothesis needed correction, however. Burnet exploited this selective concept and properly suggested that the selection process takes place between antigen and clones of lymphocytes, each one of which produces antibody of a single specificity. The hypothesis has since been proved and provides the theoretical foundation for the cellular basis of immunity.

In 1971, Jerne described a theory to explain the development of the T cell's specificity for antigen. He postulated that an individual's major histocompatibility complex antigens expressed in the thymus gland were the major driving force for stimulating thymocytes to divide and mutate at a high rate. The result was the generation of new specificities and an enlarging repertoire. This theory antedated the discovery that T cells recognize foreign antigens only in association with self major histocompatibility complex antigens and that restriction to these self molecules is imprinted in the thymus.

The idiotype network theory of Jerne has also influenced the field profoundly. Jacques Oudin and Henry Kunkel had previously shown that antibodies themselves possess unique antigenic determinants (idiotypes) associated with their antigen-combining sites. Jerne proposed that antibody to such idiotypes could be the key regulatory force of the immune system. Thus, he envisioned that in unimmunized animals there is a balanced production of idiotypes and anti-idiotypes. After immunization there is a marked increase in the serum concentration of specific antibody molecules and, therefore, their idiotypes. These molecules then stimulate an antibody response to their idiotypes (an anti-idiotype response) that limits the first response and returns the immune system to a new steady state. Jerne visualized that the immune response is a network of idiotypic–anti-idiotype cells and molecules that keep the system in dynamic balance unless perturbed by the introduction of antigen, idiotype, or anti-idiotype. There is now considerable data to support this idea and the concept has profound implications regarding the physiological regulation of the immune system and the alterations underlying pathological states such as autoimmunity. The theory has practical implications



Georges J. F. Köhler

as well. For example, it has been possible experimentally to stimulate a protective antibody response to an infectious agent by immunizing with an anti-idiotype that stimulates a clone of B cells that produces the specific antibody. Hence, a new method of immunization is possible that does not employ specific antigen!

The present award heightens the growing sense of excitement among immunologists that the immune system will be understood in large measure during the next decade or two. This is impressive when one considers two facts: (i) 30 years ago, the means by which a specific immune response is generated to an antigen was a complete mystery; and (ii) only the brain can outdo the immune system with regard to the amount of information that is received, processed, stored, and expressed. The payoff will be a clear view of immunoregulation, the means by which cells communicate with one another, and the signaling mechanisms for cell activation and suppression. These insights will generate new approaches for the prevention and treatment of autoimmune diseases, transplantation rejection, infections in which immunity is poor (such as herpes), and cancer.—JONATHON W. UHR

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Electron Transfer Between Metal Complexes: Retrospective

Henry Taube

This will be an account in historical perspective of the development of part of the field of chemistry that I have been active in for most of my professional life, the field that is loosely described by the phrase "electron transfer in chemical reactions." In the short time available to me for the preparation of this paper, I can't hope to provide anything significant in the way of original thought. But I can add some detail to the historical record, especially on just how some of the contributions which my co-workers and I have made came about. This kind of information may have some human interest and may even have scientific interest of a kind which cannot easily be gathered from the scientific journals. For publication there, the course of discovery as it actually took place may be rewritten to invest it with a logic that it did not fully acquire until after the event.

Simple electron transfer is realized only in systems such as Ne + Ne^+. The physics already becomes more complicated when we move to N_2 + N_2^+, for example; and with the metal ion complexes which I shall deal with, where a typical reagent is $Ru(NH_3)_6^{2+}$, and where charge trapping by the solvent, as well as within the molecule, must be taken into account, the complexity is much greater. Still, a great deal of progress has been made by a productive interplay of experiment, qualitative ideas, and more sophisticated theory, involving many workers. Because of space limitations, I will be unable to trace all the ramifications of the field today, and will emphasize the earlier history of the subject, when some of the ideas basic to the field were being formulated. This choice of emphasis is justified because, by an accident of history, I was a graduate student at the University of California, Berkeley, about the time the first natal stirrings of the subject of this article occurred and at a place where these stirrings were most active. As a result, I may be in a unique position to deal knowledgeably and fairly with the early history of the subject. The emphasis on the early history is all the more justified because most of the topics touched on in this article, and also closely related topics, are brought up to date in a very recent volume of the series *Progress in Inorganic Chemistry* (*1*).

Chemical reactions are commonly classified into two categories: substitution or oxidation-reduction. The latter can always be viewed as involving electron transfer, though it is agreed that when we consider the mechanisms in solution, electron transfer is not as simple as it is in the Ne + Ne^+ case. Rearrangement of atoms always attends the changes in electron count at each center, and these must be allowed for. I will, however, simplify the subject by considering only processes of simple chemistry: those in which electron transfer leaves each of the reaction partners in a stable oxidation state. While substitution reactions can be discussed without concern for oxidation-reduction reactions, the reverse is not true. The changes that take place at each center when the electron count is changed are an essential part of the "electron transfer" process, and may be the dominating influence in fixing the rate of the reaction. Moreover, most of the early definitive experiments depended on exploiting the substitution characteristics of the reactants and of the products. Thus, the attention which will be devoted to the substitution properties of the metal ions is not a digression but is an integral part of the subject.

An appropriate place to begin this account is with the advent of artificial radioactivity. This enormously increased the scope of isotopic tracer methods applied to chemistry, and made it possible to measure the rates of a large number of oxidation reduction reactions such as:

$$*Fe^{2+}(aq) + Fe^{3+}(aq) = *Fe^{3+}(aq) + Fe^{2+}(aq) \quad (1)$$

[The first demonstration of a redox exchange was made by von Hevesy and co-workers (*2*), who used naturally occurring isotopes to follow Pb(IV)/Pb(II) exchange in acetic acid.] Because chemists there were involved in the discovery of many of the new isotopes (*3*), an early interest in this kind of possibility devel-

Henry Taube is Marguerite Blake Wilbur Professor of Chemistry at Stanford University, Stanford, California 94305. This article is the basis of the lecture he delivered in Stockholm on 8 December 1983, when he received the Nobel Prize in Chemistry. The article is published here with permission from the Nobel Foundation and will also be included in the complete volume of *Les Prix Nobel en 1983* as well as in the series *Nobel Lectures* (in English) published by Elsevier Publishing Company.