REFLECTIONS FROM BOTH SIDES OF THE COUNTER[1]

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Like many of my colleagues in different parts of the world, I have spent a large part of my life both as an active research worker—which, as everybody knows, also involves finding money to support one's work—and as a member of organizations which distribute funds or awards, or by other means attempt to stimulate and support scientific progress. Working in a field in which basic research frequently leads to practical applications in medicine or in industry, the question of balance between "pure" and "applied" often turns up. It has happened to me several times that these double functions have given rise to a dialogue between the two halves of a Janus face—if this rather wild parable may be permitted here.

Take for example an after-dinner speech in presence of authorities and other distinguished people who (hopefully) listen attentively to a scientist describing research as the key to the well-being of mankind. When he returns to his laboratory the following morning and takes a look at his experiments and his notes, a certain hesitation may develop (which is not necessarily a kind of hangover): "That well-being of mankind which I promised yesterday may, alas, have to wait several more years still." It is, however, fortunate, although sometimes a little embarrassing, that people nowadays understand that research, and especially fundamental research, will have to take its time before it pays dividends. Governments and private foundations are prepared to spend funds for research which must be regarded as enormous, compared to what was usual only a few decennia ago. We still have to remind them, in

[1] This chapter was written during a stay at Villa Serbelloni, Bellagio, in October 1967. I wish to express my gratitude and appreciation to the Rockefeller Foundation and to the hosts of the Villa, Mr. and Mrs. Marshall, for the hospitality and excellent facilities which I enjoyed.

most cases, that the share given to long-range fundamental work always tends to lag behind.

Being aware of the fact that an increasing amount of the national income is being spent on research in most developed countries, those who are responsible for the distribution of such funds (many of them active scientists themselves) may often feel that the background for wise decisions is rather insufficient. I shall not enter upon a discussion of this much-debated problem here but will limit myself to some personal reflections in this connection, especially concerning the promotion of basic scientific research.

This is perhaps the most difficult problem in the field. We know far too little about the optimal conditions for scientific productivity, chiefly because the decisive factor is the individual. We may try to trace the development of a great discovery by reading papers. This is done, of course, and "research about research" is becoming an active and useful discipline. Nobody believes however, that this or similar activities will result in a publication *How to Make Great Discoveries* similar to the well-known *How to Win Friends and Influence People.* To stimulate basic research, even if we have access to many more facts than at present, is not a "push-button" affair. Neither could we hope to develop a flourishing period in the arts, like the Renaissance, by some ever so well-organized campaign.

A scientific paper, however, is usually of rather limited value if we want to know how things really happened. The facts and the conclusions may be presented in a perfectly logical order and with admirable elegance, but, did it really happen in this way? The author writes his paper when he has arrived at the final results and then first realizes which way he should have gone. Probably the way he actually took was much more complicated, involving sidesteps, mistakes, disappointments, wishful thinking, and still more of that kind. Thus, a scientific paper mostly involves a certain kind of after-rationalization, naturally perfectly understandable and permissible. Scientific work is objective and its results should be devoid of the personality of the author. In a way this is a pity, since I believe that in science the human subjective factor is involved in the act of creation almost as much as in art, literature, and music.

In his book *The Sleepwalkers*, Arthur Koestler (1) has presented and commented upon several examples of how scientific progress has evolved through the ages along much more irregular paths than generally realized. One can also find in works on the history of science many stories—some of them well-known—about what I would like to call the "triggering" mechanism which releases a scientific discovery (Newton's apple, Kekule's bus-ride, etc.). And a Danish philosopher, Ludwig Feilberg, who towards the end of the last century wrote a very interesting book on such phenomena, stated that his most productive period for new ideas was when he was brushing his teeth. Probably he then felt no obligations to the rest of the world and his mind could play around freely.

We cannot go deeper here into a discussion of these psychological phe-

nomena. Neither can we recommend that the authorities encourage scientists to sit under apple trees, ride on buses, or brush their teeth. But it is well to keep in mind how essential an anti-stress atmosphere is for any creative effort, including the sciences.

More essential and easier to grasp is the situation described as "the prepared mind." The release mechanism must act upon something, and I believe that an analysis of the prepared mind, with all factors involved, provides a good deal of the kind of information we are looking for. It seems to me that one of the particularly attractive and interesting aspects of the Prefatory Chapters in this Annual Review is that they may provide excellent first-hand information in this respect, which is otherwise not easily accessible. I am proud to have been invited to join the group of distinguished authors to these chapters, and my ambition has been to try to present some experiences which might be of interest to some readers from this particular point of view, in line with what I have said above.

There is, however, always the difficulty that any kind of introspection during the creative process probably will disturb and perhaps even spoil everything. Too much observation interferes and it is even tempting to generalize the Heisenberg principle to many phenomena other than those in the world of atoms: rare birds and plants, beautiful landscapes and the genuine traditions of their inhabitants being transformed into tourist attractions, the object of interest being obscured or scared and losing its very character by too many indiscrete onlookers. Even "confessions" of a scientist about how things really happened may also involve a kind of after-rationalization. This has been emphasized to me by my friends in the field of History of Learning when I have tried to persuade them to pay attention also to today's events in science fields, for example, by personal interviews with scientists while they are still alive and active.

Early Work in Electrophoresis

To most research workers the decisive factor in preparing their minds in a general way is obviously their impressions and experiences during their university years, particularly if they have the good fortune of having a great scientist as their teacher. This was so in my case and it should be obvious to all those familiar with the work in physical and biochemistry in Sweden how much I owe to The Svedberg—a great personality and a good friend. Last year I was asked to write a prefatory chapter on "50 years of Physical Chemistry in Sweden" for the *Annual Review of Physical Chemistry* and decided to have this chapter deal with The Svedberg and his work (with his successor Stig Claesson as co-author) (2). I became research assistant to Svedberg in 1925—a very productive period at the Institute, when the ultracentrifuges were developed and this new technique started to give such significant results in the protein field. Svedberg is a fascinating mixture of a physicist and a biologist, but perhaps not too much of a chemist (as he once confessed to me). To a certain extent I believe he saw the ultracentrifuge also as an in-

strument to classify the animal and plant kingdoms according to the physicochemical properties of their macromolecular components. I remember the excitement when it was found that pure proteins sedimented as strictly homogeneous substances: a surprise to a colloid chemist but not unexpected to a chemist who regarded them as ordinary chemical substances but of very large molecular weight. I managed to make some minor contribution to the ultracentrifugation technique, introducing (with Ole Lamm) refractive index observation methods and also a theoretical treatment of the influence of charge and of electrolytes on the sedimentation, which at that time was incompletely understood. My main interest, however, became electrophoresis and my first crude experiments were a continuation of earlier work done in Madison by Svedberg and Scott. It was very stimulating to do this work against the background of the development of the ultracentrifuge. Electrophoresis appeared to me at first much less fundamental, but it turned out to have some interesting characteristics of its own which fascinated me. Svedberg was very encouraging but was, of course, too much absorbed in his own work with the centrifuge to give me much of his time. And electrophoresis appeared so much simpler from a technical standpoint, suitable for a young man to play around with more or less on his own. Moreover, the excellent instrument workshop greatly facilitated the testing of new ideas.

In spare time I read some biochemistry (which at that time was not included in the chemistry curriculum in Uppsala). I remember being fascinated by the enormous variability and above all the specificity of biochemical substances, so new and so strange to a physical chemist. My daily worries in the electrophoresis work were connected with impure or badly defined materials. Even those substances that had the blessing of the ultracentrifuge as being homogeneous did not always behave well in my apparatus. This was particularly true with the serum proteins. Gradually I became convinced that the definition and the purification were all-important problems not only for the substances in my hands, but for the whole of biochemistry. Thus, separation became the key problem and I became convinced that this would require a number of alternative methods, considering the multitude of substances one would have to deal with. Not only ultracentrifugation, not only electrophoresis, but other methods would also have to be explored, preferably those which depended on physicochemical phenomena, as these are more likely to be gentle. When working with biological materials I had learned to have the deepest respect for their sensibility to drastic treatments. I remember speculating much about further development of chromatographic and adsorption methods but, fortunately for myself, decided to pursue the exploration of electrophoresis technique first. This work led to my doctoral dissertation (3), which was published and defended in late 1930. Although it was very well received by the Faculty and by The Svedberg himself and led to appointment as "docent," I remember very vividly that I felt disappointed. The method was an improvement, no doubt, but it led me just to the point where I could see indications of very interesting results without being

able to prove anything definite. I can still remember this as an almost physical suffering when looking at some of the electrophoresis photographs, especially of serum proteins. I decided to take up an entirely different problem, but a scar was left in my mind which some years later would prove to be significant.

Diffusion and Sorption in Zeolite Crystals—a Deviation

I had read about the unique capacity of certain zeolite minerals to exchange their water of crystallization for other substances such as ethyl alcohol, bromine, mercury, etc., the crystal structure remaining intact even if the crystal was evacuated to remove water. The "empty" crystal served as a kind of molecular sieve. Much work on the diffusion of proteins in solution had been going on for some time at the institute, making use of optical methods for the observation. I collected some particularly high-quality specimens of zeolites during a visit to the Färöe Islands, experiencing the joy of field work. Sometimes I was left alone for a whole day among freshly weathered rock on waterfront shelves at the foot of very steep mountains, sea puffins my only company until fishermen came with their boat to fetch me in the evening. I remember well asking them what would happen if the sea became too rough for their boat. They replied, pointing upwards: "We will take you that way with a rope," an experience which fortunately did not prove necessary.

I doubt if my work on zeolites has left any traces in the scientific literature and I am not sure to what extent it was inspired from my interest in separation, thus being a forerunner of work on molecular sieving done at the Institute of Biochemistry 30 years later. I knew, however, that I had very little chance of getting a permanent position in my country in those fields which attracted my interest: biochemistry and biophysics. There were simply no chairs available, but there was one in inorganic chemistry to be available in 1938, and might not zeolites be counted as inorganic chemistry?

Anyhow, I enjoyed this work, particularly when I could follow the diffusion by observing the crystal in a polarizing microscope in a specially constructed vacuum chamber (4). The anisotropy of diffusion came out beautifully. I believe that the most important outcome of this work was that it prompted me to go to Princeton to work with H. S. Taylor on adsorption phenomena. I was fortunate enough to be granted a Rockefeller Foundation fellowship for this purpose and thus spent September 1934 to August 1935 in the U.S.A. This turned out to be a most stimulating year of decisive influence on my career. The atmosphere in the Frick Chemical Laboratory was very inspiring; I remember particularly Henry Eyring's seminars and the frequent discussions by groups of people from many different fields, with a frankness and informality which I had not experienced before. Of equal, or perhaps even greater, importance for my future were the frequent contacts with the Rockefeller Institute, both in Princeton and in New York, which led to lasting and inspiring friendship with J. Northrop, W. Stanley, M. Anson, and many

others, as well as opportunities of meeting men like Landsteiner, Michaelis, and (later) Heidelberger. They all expressed to me a strong belief in the future of "experimental biology." They knew my work in electrophoresis and when I told them about my difficulties they nevertheless greatly encouraged me to carry on. In discussions with them I found that many times their problems needed something which had been in my mind for years, but which, so far, I had failed to realize. Thus, while still in the U.S.A. I started to make plans for a systematic investigation of disturbances and sources of error in electrophoresis; even though this would take several years, I was now convinced that it was worthwhile.

A New Attempt at Electrophoresis; the Resolution of the Main Protein Components of Serum

Actually this now took less time then I had expected, although it involved both experimental work and theoretical calculations. I learned something which I later tried to teach others, namely that if difficulties arise one should not look for an alternative procedure too quickly, but should try to go deeper into the subject first. One of my most enjoyable experiences during this work was to learn how useful it was that Nature has arranged the density maximum of water at +4°C, just where I needed it. The greatest difficulty in electrophoresis of solutions of high conductivity (e.g. serum) is the risk of convections caused by the heat produced by the electric current. By choosing a temperature where the density varies very little with temperature this risk is largely eliminated, and one can apply much stronger currents, giving much better resolution. By using alternating current instead of direct current heat convection could be studied separately.

The introduction of a refractive index method of observation, the schlieren method, also meant a great advantage as compared to the ultraviolet absorption used earlier in both ultracentrifugation and electrophoresis. It was soon considerably improved by Longsworth, Philpot, and Svensson and was also refined to a high degree by Lamm in his "scale" method.

Now I was convinced that my new apparatus would work and I was impatient to demonstrate this. Thus, instead of trying a sample of a reasonably homogeneous protein, I picked out a sample of serum from the refrigerator, dialyzed it against a buffer solution and put it into the machine. If it worked with serum, it should work with almost anything else. After about two hours I observed four distinct schlieren bands, indicating the migration of albumin and three globulin components which were named α, β, and γ. This was a great surprise to me, although there had been some indications of this in my earlier work. I wanted to share my enthusiasm with somebody, and I brought my old friend K.O. Pedersen into the room, who after a thorough inspection muttered: "Maybe there is something there." (He has always been a very critical and careful investigator.)

This work soon led to a publication describing in some detail the new apparatus and the background for its construction, as well as the discovery of the main serum components. To my surprise it was not accepted in a bio-

chemical publication to which it was sent first, being too "physical." Thus, in 1937 it appeared in the *Transactions of the Faraday Society* (5). The reaction was immediate and extremely positive; as my American friends had predicted, I was flooded with letters and requests for reprints and even a telegraphic order (from Edwin S. Cohn) for an apparatus to be built in our workshop. I remember particularly a very kind letter from Dr. Harvey Cushing, asking me for a reprint for his library of the classics of medicine, a very memorable event, indeed, for a young scientist. Svedberg, who was just about to leave for a lecture tour in the U.S.A., took with him some of my results, which no doubt contributed to the rapidly developing interest in the new method. I was particularly happy when I saw a paper by Landsteiner (who had been so encouraging and kind to me) which demonstrated the electrophoretic differentiation of duck and hen egg albumin in the new apparatus. The Rockefeller Institute was engaged very early in the new technique under the very able guidance of Longsworth, who introduced some important improvements, above all the automatic scanning of the schlieren diagrams and the theoretical interpretation of the results. A close contact between our groups was established involving also a highly appreciated and lasting friendship. Frank Horsfall, Jr. came to Uppsala for a year to study the method, and we had great fun together in "crossing snails" as we put it. We demonstrated that the reversible dissociation-association of hemocyanins from *Helix pomatia* and *Helix nemoralis*, achieved by shifting the pH, led to the formation of hybrid molecules, whereas the same experiment with *Helix pomatia* and the more distant species *Litorina litorea* appeared to give no or very little hybridization (6). In 1939 I received an invitation from Dr. Walter Bauer to spend a year at the Rockefeller Institute Hospital to continue our collaboration. Unfortunately, my stay was limited to only two months because of the war. The fruitful contacts and the exchange of information (even of unpublished results) has, however, continued ever since. I wish to mention here particularly also the names of Moore and Stein, and Kunkel, who spent a year in Uppsala from 1950 to 1951.

Today, when exchange of scientists between countries (even those who are rather distinctly apart politically and geographically) is promoted by governments and academies by different forms of bilateral cultural agreement, it is well to remember that an absolute reciprocity in the number of scholars who are sent from each country is not essential. A scientist coming to a laboratory to learn something will usually contribute experience and many new ideas which more than compensates for the expense of the host institution or the host country. This has, at least, been our experience and I believe it is generally so, at least with countries of similar scientific standards.

The Research Professorship: Continued Work on Electrophoresis

In 1937, mainly on the initiative of Svedberg, a research professorship was established at the University of Uppsala by a generous donation of Major Herbert Jacobsson and Mrs. Karin Jacobsson of the well-known Gothenburg

shipping family Broström. I was the first to be appointed to this chair, intended "for research and teaching in those fields of chemistry and physics, which are of importance for the processes of life." Thus, being in a secure position and with the promise of support from the Rockefeller and Wallenberg Foundations, I could plan for the future. Eight years later, in 1946, a department of biochemistry in the Faculty of Science was officially established with its own budget and personnel, and in 1952 we moved into a new building, having had until then only a few rooms in the Institute of Physical Chemistry at our disposal. In the meantime, however, many things had happened.

Looking back, I believe there has been a tendency in my mind not to stick to a subject when convinced that it is in good hands with other people. This applies to my scientific work as well as to administrative functions of different kinds. I may wish to continue by giving advice and stimulation as much as I can, also perhaps criticism, without really doing anything with my own hands. I have often felt the desire to try something new, as I have already indicated above in connection with my early speculations about the significance of separation in general—not only by electrophoresis.

People have sometimes asked me why we did not study at an early stage the many clinical applications which electrophoretic analysis of pathological sera seemed to promise. It is true that we did some work, for example, the demonstration (with Kabat) of an antibody as a separate electrophoretic component in the gamma globulin group. But I felt that with our particular background and our experience it would be better to concentrate upon a further improvement of the method in different directions and on applications to problems which were closer to our field of interest. I must admit, however, that I was a bit skeptical about the great expectations raised by my medical colleagues. I remember, however, how one of my many good friends among them once mentioned that some people already had investigated sera from mental patients by electrophoresis. He added: "Tiselius, what I particularly like about your new method is that so many patients will have to be released, but still more, that quite a number will have to be taken in."

Even though early work in Uppsala and, above all, elsewhere pointed the way for the clinical application of electrophoretic analysis in certain pathological conditions, the real breakthrough in this field came with the introduction of the much simpler method of filter paper electrophoresis. In 1927 I made some experiments in this direction and separated red phycoerythrin from blue phycocyanin by electrophoresis in a slab of gelatin, obtaining beautiful narrow migrating zones. I did not pursue this work and never published anything. I was above all interested in the quantitative aspects and disliked introducing an ill-defined medium such as gelatin. This showed a lack of foresight on my part; in many cases, the separation is the all important issue, even if one has to abstain from the quantitative aspects and thus the possibility of predicting what is going to happen. Much later (in the early sixties) Hjertén demonstrated that in weak agar gels there is hardly any influence of the medium, and before that Smithies in his beautiful experi-

ments in concentrated starch gels obtained extremely good resolution, to which the medium itself contributes essentially by a sieving mechanism.

I have already mentioned above that right from the beginning of this period I had the good fortune to have very able collaborators to whom I could entrust the fulfillment of the electrophoresis work. Thus, Harry Svensson considerably improved the optical system of the moving boundary method and made fundamental contributions, with experimental verifications, to the theory of electrophoretic migration. This resulted in the first dissertation from the Institute [in 1946 (7)]. Svensson became docent of biochemistry and also later, after having left us, continued such work in Stockholm, in the U.S.A., and later as professor of physical chemistry in Gothenburg. A number of other collaborators working with serum proteins and enzymes provided us with many questions about methods and gave us a solid ground of practical applications to use in our planning for the continued methodological work. I firmly believe (as I have often stated) that in such work it is very essential to have a constant exchange of ideas and experiences between those who develop methods and those whose prime interest is to apply them—if possible under the same roof. The latter do not always seem to have the patience to go deeper into methods if there is trouble, and the former run the risk of becoming perfectionists or gadgeteers if they forget that methods should work not only in model experiments. The best proof of the usefulness of a new method is that it is applied by others without any persuasion.

The War Years: Chromatography of Colourless Substances

As Sweden was fortunately not involved in the World War of 1939 to 1945, work in the Institute went on almost as usual, although with reduced personnel (many were called up for military service). I served for a time as a member of the Board of Defence Research. We tried to make ourselves useful by contributing some work of immediate importance, as our country was suffering from shortage of many essential commodities. The feeling that we were for a time being almost completely cut off from a large part of the world with which we always had enjoyed friendly relations, was depressing. The great tragedy which was going on all around us and the fear that we ourselves might become involved made pure scientific work for one's own pleasure sometimes appear out of place. During this time, however, I became strongly interested in chromatography and studied some of the earlier literature on the subject. I felt that electrophoresis was hardly specific enough for separation of the multitude of substances occurring in materials of biological origin. I was surprised to find that very little use had been made of such methods, except for carotenoids and some other coloured substances. And not even Willstätter, who made such extensive use of adsorption in the purification of enzymes, and who applied chromatography to some of his other problems, came to use this method to any considerable extent.

I decided to attempt "chromatography of colourless substances" or, as I preferred to call it, "adsorption analysis" by observing the separation, not on

the column but in the eluate. Influenced by my previous experiences, I decided to use optical methods for continous determination of the concentration of the separating substances as they were leaving the column. With the very able help of Stig Claesson (8) (who later became Svedberg's successor as professor of physical chemistry) I built a microrefractometer for this purpose, and started extensive investigations on the adsorption analysis of sugars, amino acids, peptides, and many other colourless substances. I learned a great deal from this work, becoming aquainted with the phenomena behind the separation. I distinguished between frontal analysis, elution analysis, and displacement analysis, and, as far as I know, this had not been done before (9). Especially the displacement phenomena must have been observed many times before with coloured substances, as one can see it when a mixture is applied to a column, even before any eluant has been added. I could account for this phenomenon theoretically and this work gave me a great deal of satisfaction. The key problem in chromatography by elution is to eliminate the "tailing" of the migrating zones, which are due to the fact that the adsorption isotherms are curved, giving rise to a stronger binding at low concentrations than at high. In displacement analysis, the zones form a procession where any tailing material from one particular zone will be displaced to where it belongs by the following zone. The drawback of the method is, however, that the zones emerge in immediate contact with each other. Thus the elution analysis, with well-separated zones, is superior in most cases (with the possible exception of large scale preparative work, e.g., in the separation of rare earths on ion exchangers).

In most of this work we used active carbon as adsorbent and we tried many methods to rectify the adsorption isotherms, e.g. by pretreatment of the carbon with small amounts of strongly adsorbed materials. Some of this work led to useful results, and the methods came into practical application. In general, however, I must say that it never led as far as I had hoped. The reason was simply that these problems were solved in a superior way by others: above all by Martin & Synge in their partition chromatography and by Moore & Stein by introducing fraction collectors and ionic exchange resins for amino acid analysis. I still believe, however, that this early work of ours came to play a role in much of the subsequent and more successful work in our laboratory. We became aware of the key problems and our wishful thinking was influenced accordingly. This was also a great period for research workers in separation. It was now generally realized that separation is not only essential for substances, but it is also of the utmost importance as a tool in determining the structure of large molecules, demonstrated in a most striking way by Sanger in his insulin work and by many who followed in his footsteps.

The Dextran Story

My account of what we were doing during the war years and immediately afterwards would be rather incomplete if I did not mention dextran. Not only

did this substance come to play an important role in our activities (even up to this day), but the dextran story may also be of interest as an example of how things may happen in research.

We had been asked to do some work on the freeze-drying of plasma for use in military medicine, where our experience in that field with our own protein materials was considered to be of value. We had also been approached by the Swedish Sugar Manufacturers Corporation, who were having trouble with some of their beet extracts becoming contaminated with slimy substances which obstructed the filters used in the raffination. The problem was essential as there was a shortage of coal and fuel in general. We found or rather confirmed that the slime was due to dextran produced by bacterial infection (*Leuconostoc mesenteroides*).

We decided to look for a sensitive and specific reaction for dextran and hence, tried to produce an antiserum by injecting dextran into rabbits. To our surprise no reaction was observed and the animals seemed to be quite indifferent to even large doses. The two young biochemists who were active in both the plasma and the dextran projects, being aware of the importance of finding a substitute for plasma, now asked themselves: why not try dextran? [Grönwall (10) and Ingelman (10, 11)]. Earlier substitutes (gum arabic, etc.) had proved unsatisfactory and even dangerous, as they produced reactions or accumulated in the kidneys or in the liver. Dextran of a suitable molecular weight could be obtained, it did not appear to give rise to reactions, and was likely to be gradually broken down in the body. They tried (and successfully) to introduce large quantities of dextran, first in rabbits, then in dogs, and finally in human patients. The pharmaceutical company, Pharmacia, which had just moved from Stockholm to Uppsala, became interested and started to produce dextran ("Macrodex") which is today one of their most important products. Thus, a close collaboration started between Pharmacia and the Institute of Biochemistry which I believe has been of great mutual benefit. There is an open exchange of information in seminars and private discussions and some research workers in Pharmacia had their earlier training with us. It was a particular pleasure to be able to tell the Sugar Manufacturers that instead of trying to prevent the formation of dextran in their extracts they should now build a factory in order to produce it in large quantities and sell it to Pharmacia for further treatment.

This story has always seemed to me to be interesting and significant since it demonstrates how a prepared mind (in this case, the awareness of a need) and a cross-fertilization of ideas may lead to useful results in a very unexpected direction. And many, perhaps most, significant results are unexpected.

The Nobel Prize

In 1948 I was awarded the Nobel prize in chemistry for work on electrophoretic and adsorption analysis, especially for the discovery of the heterogenous nature of serum proteins. One year earlier, at the 1947 Nobel banquet,

I had the privilege of speaking to the Nobel laureates of that year. I will quote from this speech (12):

Nobel Laureates:

In science and in medicine your work in search for the truth has disclosed new laws of Nature and opened up new and vast fields for research of the utmost importance for the welfare of mankind. And, in literature, new truths of a different kind have been brought to light in a way of which only art in its highest and most subtle forms is capable. When a new thought is born, or when one of the deep secrets of Nature yields to the searching scientist—in this very act of creation—there is a pure and primitive happiness deeper than anything of this kind which can ever be granted a human being to experience.

This view has been formulated in one simple sentence by the great Swedish chemist Carl Vilhelm Scheele, who some 150 years ago wrote 'Det är ju sanningen vi vilja veta, och vad är det väl icke för en ljuvlighet att få tag på den.' (It is the truth we are searching for, and what a delight it is to find it.)

Nobel Laureates: Like all creative work, your achievements must have given you many moments of that sublime happiness which Scheele had in mind when he wrote these words. Probably your work has also many times, perhaps even more frequently, involved disappointments. In any case, we do not believe that to you even the highest awards and the most whole-hearted recognition can be more than a faint reflection of the deep satisfaction you must have experienced in your work. We do believe, however, that the Nobel prizes afford us all a suitable way of expressing our indebtedness—an indebtedness from the whole civilized world—to those pioneers in science, medicine, and literature who have by their deeds enriched this civilization and pointed the way for its further development. We would like you to consider your awards for what they should be and what they are: an expression of the gratitude of mankind.

And then, in 1948, again at the Nobel banquet, I expressed my feelings of gratitude on being myself awarded the Nobel prize in a speech from which I quote the following (13):

Alfred Nobel was a great idealist, who thought in international terms. The Foundation which bears his name was created with the intention of furthering achievements which he considered to be of great benefit to mankind. That he awarded prizes for these achievements shows that he believed individual endeavour to be an important part of progress in all cultural fields.

This banquet, which is dedicated to the memory of Alfred Nobel and serves as the ceremonial background for the distribution of the prizes, may well prompt one to ask: To what extent is progress in literature and science linked with the personality of poets and scientists? That this is indeed the case is beyond all doubt, but there is a difference between these two forms of endeavour—one cannot judge them by the same standards. Poets, like all creative artists, impress the stamp of their own personality on their work far more than scientists can. The work of the poet bears his individual mark. Thus, as an individual, he is indispensable for the development of culture and civilization. The scientists, however, seeks objective truth, which is and must be completely free from all traces of his individual personality.

It can be said with certainty of all scientific discoveries that if they are not made by one scientist, they will sooner or later be made by another. Naturally there is a strong emphasis on the 'sooner or later.' The enormous progress made

in science and in medicine seems today to demonstrate a process of organic growth which proceeds according to its own laws, and in which it is frequently difficult to distinguish between individual achievements. It not infrequently occurs that the same discovery is made in different parts of the world at more or less the same time, with perhaps a few days or weeks between them. Rapid communication of all new discoveries and intensive correspondence between scientists in all parts of the world has contributed to the advancement of science in a spirit of team work in numerous fields. This can only be of benefit to science and thus to civilization as a whole. These thoughts should serve as a reminder for the individual scientist when considering his own role in assisting development, and should deter him from any false conception of his own importance. These were just a few ideas which occurred to me on being myself the recipient of the greatest honour which can be awarded to a scientist.

There is more to be said about Nobel awards and the Nobel Foundation, but I will return to this below.

An Expanding Program: Not Only Separation

The Nobel prize had some consequences. One was that the plans for a building for the Institute of Biochemistry were accelerated. I remember paying a visit to the Undersecretary for Education and Research a few weeks after the announcement of the award, asking him if now the outlook was not much brighter. He agreed, but added: "If you have done all these nice things we read about in the papers with only three rooms at your disposal, I have some difficulties in understanding why you need a whole new building." One of the rooms was originally intended to be a pantry, and a Stockholm newspaper one day showed a caricature of myself as the man who won a Nobel prize working in a kitchen. Such things may be helpful nowadays, and we got the building and moved to the new premises in 1952.

Biochemistry now attracted an increasing number of students and more advanced research workers. We could organize a more diversified program often based upon the interest shown by particularly able people who gradually became leaders of groups: Thus Porath (14) constructed columns for zone electrophoresis, also for preparative use, and applied them in the study of certain pituitary hormones. He also continued work on serum fractionation, later continued by Bennich, who recently discovered a new γ-globulin of considerable interest. Porath inspired and successfully led many other investigations and gradually became my second in command, now director of the Institute. Malmström brought with him a strong interest in metal-combining enzymes and after some years published a dissertation on enolase. He organized an "enzyme group" which attracted some very able students. He carries on this work as professor of biochemistry in Gothenburg. Boman introduced molecular biology in the institute after a very profitable year with Lipmann at the Rockefeller Institute. He is now professor of microbiology in the University of Umeå in north Sweden. Roos (15) made a very penetrating study of great clinical interest on the purification of certain pituitary hormones. His dissertation was published in October 1967. Weibull studied the purification and some of the properties of bacterial flagellae (16). His con-

tinued work on the lysis of bacteria led to remarkable results and he is now professor of microbiology in the University of Lund.

Our orientation towards microbiology made us feel very hampered by the fact that there was no chair for general microbiology in Sweden. We decided to work for this, with much support from colleagues in related fields. We started a teaching course in 1957 and found in von Hofsten a very enthusiastic and devoted teacher. The authorities were gradually convinced that this subject needed support and today there are chairs in the faculties of science in Uppsala, Lund, and Umeå, and more are coming.

It would lead too far to go into further detail about all these new activities, even if I would have liked to mention many more names, also among our foreign guests who have contributed so much. In the literature references at the end of this chapter the reader will find a list of dissertations in biochemistry from this Institute up to the end of 1967. A review of recent work at the Institute on separation is found in (17). It seems appropriate in this chapter that I limit myself to work where I was more directly involved. I just want to add that in most of the cases our experience in separation and our equipment for such work provided a platform for new ventures of a different kind. It is mostly not easy to start something new, especially not today when international competition is more severe than ever.

Except for the above-mentioned work by Porath and his collaborators on preparative-zone electrophoresis in columns packed with different kinds of inert stabilizing materials (especially modified cellulose), extensive investigations on different types of zone electrophoresis in gels were now performed by Hjertén & Jerstedt. Hjertén was the first to discover the advantages of using gels of agarose (that is, agar from which the agaropectin has been removed) and worked out convenient methods for preparing it in bead form, easy to pack into columns. Such suspension columns (also of polyacrylamide) are often convenient in electrophoretic analysis also of large molecules or even particles which do not migrate easily in a compact gel. Hjertén's main contribution was, however, the development of the "free-zone electrophoresis" where the zones separate in a horizontal quartz tube, slowly revolving around its long axis. Convections due to gravity are thus eliminated and no stabilizing medium is required. This micro method gives high resolution and accurate values of mobilities. A complete account of this method is given in Hjertén's dissertation (18).

In separation work, one's ambition is mostly to obtain substances as pure and homogeneous as possible. It is a common experience, however, that certain materials will resist purification beyond a certain level, even if different methods are attempted. This may be disappointing, but such a result may be extremely valuable from an entirely different point of view. An "impurity" which sticks tenaciously to the substance we wish to isolate may do so because in the original biological material it belongs to this substance in a way which is significant from the structural or functional point of view. Thus, an "impure" substance may represent a very valuable piece of information. It is tempting in such cases to formulate the distinction between the organic

chemist and the biochemist by saying that the latter should also devote himself to the study of impure substances.

Submicroscopic particles or more generally fragments of a biological structure are of course "impure" from a strictly chemical point of view but represent just that kind of information. It is therefore natural that they are being investigated both from the point of view of structure and of function. I have often speculated about the possibility of a systematic study of fragments, which should be obtained by some kind of successive dispersion of some biological material (19). Given methods for the separation of such fragments according to size and some other properties, it ought to be possible to reconstruct on paper the original structure in a way somewhat analogous to sequence determination of amino acids in a protein molecule by analysis of fragments obtained by hydrolysis. Such structure analysis by successive dispersion might be useful in conjunction with electron microscopy.

Nothing like this has been achieved so far, but considerations of this kind have stimulated our interest in the study of the separation of particles, submicroscopic and even microscopic. In addition to Hjertén's work already referred to above, the very interesting partition method of Albertsson, described in his dissertation (20), should be mentioned. It can be demonstrated that the larger the molecular or particle size, the more closely similar properties are required for two phases if a defined and not too one-sided distribution of the molecules between these two phases is to be possible. Moreover, with biochemical material, the phases should consist mainly of water. Albertsson demonstrated that it was possible to obtain such two-phase systems by dissolving small quantities of certain high polymers, such as polyethylene glycol or dextran in water. One will then observe a sharp boundary separating two solutions, one containing mainly dilute polyethylene glycol, the other dilute dextran, and both containing 95 to 98 per cent water. Low molecular weight substances will distribute equally between such phases, but as the molecular weight goes up the material will go preferentially into one or the other phase, or sometimes will form an interphase. The method is not sensitive to temperature changes (as with the lower glycols used by Martin & Synge in their partition chromatography of proteins). By changing such parameters as the electrolyte composition or the molecular weight of the (commercially available) phase-forming polymers, it is often possible to change the partition in one direction or the other. This interesting method (which also can be operated easily on a very large scale) has proved very useful, for example, in virus purification. Albertsson, who is now professor of biochemistry in the university of Umeå, has stepped up the separation efficiency by constructing a counter-current apparatus adapted for this type of work.

Further Advance in Chromatography: Gel Filtration

Adsorption on calcium phosphate gels has long been used for purification of enzymes. We felt the need of a greater range of specificity in chromatography of proteins, nucleic acids, etc., than was available with the commonly

used materials for separation of such substances. When trying columns of calcium phosphate we ran into great trouble, which was difficult to account for (work with Hjertén and Levin). It was gradually found that the phosphate changed its properties during the experiment and we decided to try the most stable modification of calcium phosphate, namely hydroxyl apatite, which is easy to prepare. This has worked well and is now often used in various connections. Application to nucleic acids and nucleotides appears particularly interesting.

During many years the desirability of working out a chromatographic method based upon differences in molecular weight or size had been the subject of much discussion at the Institute. I made some attempts at a kind of zone ultrafiltration—a logical extension of zone electrophoresis and zone ultracentrifugation. I used gel columns but they always cracked when I applied high pressure to get the liquid through. Synge and I discussed these experiments and concluded that the only way of getting the liquid through a compact gel column was to apply electroosmosis. Synge and Mould continued such experiments and could demonstrate the separation of uncharged substances on strips of nitrocellulose when an electric current was sent through.

A great step forward was when Porath & Flodin (21) demonstrated that columns of gel particles could act as a kind of molecular sieve, the smaller molecules being retarded by penetrating more or less into the gel, the larger being less affected and therefore appearing first in the filtrate. It was again our old friend the dextran which came to our help. I have been told that all this started by an unexpected observation. Research workers at the Institute tried to use particles of dextran gel (made by cross-linking of dextran) as a filling material in electrophoresis columns. They forgot to turn on the current but observed nevertheless a beautiful separation when buffer solution ran through the column. A study of this phenomenon was taken up by Porath & Flodin and gradually led to the almost explosive development of the field. At an early stage we sent some dextran gel to Stanford Moore at the Rockefeller University, together with an account of our first results. We got a most enthusiastic reply. Flodin was invited to a Gordon conference in the U.S.A. and the new method spread almost epidemically.

It is true that similar attempts were made in other laboratories at the same time or somewhat earlier, but it seems that dextran gels were so superior that the method came into general practical application first when this material was introduced.

A very fruitful collaboration with the Pharmacia company was again established. They now produce dextran gels of various degrees of cross-linkage under the commercial name of Sephadex, recently used also for work in certain organic solvents.

Gel filtration seems to be essentially an exclusion process with no affinities involved (except in certain cases). The absence of "tailing" is remarkable even at fairly high concentrations and also in many cases where some ad-

sorption affinities appear to be involved. It seems that our dream of chromatography with linear isotherms has come true at last; the physico-chemical background is not yet quite clear, however, especially not in the behaviour of larger molecules. Other gels have now also come into use for the same purpose (agarose, polyacrylamide) largely through the work of Hjertén. The most recent development in this field [by Porath and his group (22)] involves the introduction of specific groups or even proteins on the gel matrix which makes it possible to use highly specific columns which are of great interest in, for example, immunochemistry and enzymology.

Experiences from the Other Side of the Counter, 1946 to 1967

World War II demonstrated in a very convincing manner what scientific research can contribute and what great potentialities may be involved not only in the military field but also—and this now became imminent—in peaceful activities for the restoration of human welfare and for our continued existence as a whole. Governments approached scientists for advice. Some of them preferred to remain in a corner of the laboratory, others were more willing to contribute, even if they were somewhat embarrassed by this sudden benevolence and startled by the limelight of public attention.

In 1945 I was asked by the Government to become a member of a committee which was to recommend measures for improving conditions for research in the field of sciences, especially basic research. I accepted although I had no previous experience of committee work. I found to my surprise that people listened to me and often followed my advice. The committee was quite successful and practically all its recommendations were approved by the Parliament—something very unusual in our early experience. Thus among other things, a research council for sciences was established (similar councils for technological and medical research had already been organized during the war period). I was asked to become the chairman of the new council. This was rather unexpected because for obvious reasons one usually prefers to have in the chair a person who is not actively engaged in the field and may be suspected of representing local interests. I learned later that my name had been submitted by the rectors of the Universities of Stockholm and Lund who evidently felt confident that I would not speak unduly for Uppsala. Anyhow, I accepted a four years' appointment, being flattered and feeling that I might be able to help the new council fulfill the hopes of my fellow scientists and also to gain the confidence and even the appreciation of the authorities and of the general public—all this is very essential for its future existence and expansion. Many people had expressed doubt that it was wise to give a large sum of money (at that time 1 million Swedish crowns, today 25 million Swedish crowns per year) to a group of scientists to play around with, with very little or no government control.

I remember with particular pleasure how the council soon found that speaking too much for local interests was considered unstylish and the support of promising research, wherever it was done, was the all-important goal

we were aiming at. I remember also the excellent collaboration with our secretary general, Dr. Funke, who still has this function today, and is also secretary of our Atom Energy Council and head of the Administrative Council of CERN.

I had hardly left the council (in 1951) when I was asked to join a committee for cancer research, which was to plan for an organization where activities were to be financed mainly by raising private contributions. The Government offered to pay a sum equal to what we could collect. This committee worked very slowly and I was often disappointed as it appeared so difficult to come to an agreement. Differences between representatives of clinical and basic research was the main source of disagreement. When finally the "National Society for Cancer Research" was formed, I was asked to be chairman of the research committee which was to deal with applications and recommend grants. Surprisingly enough there were few disagreements. Conflicts between "pure" and "applied" faded into the background as there were comparatively few applications for clinical work. This was not the first time that I had seen prestige arguments loose some of their weight when it came to actual practice.

In 1947 the first International Congress of Chemistry after the war was held in London under the auspicies of the International Union of Pure and Applied Chemistry (IUPAC). I had been asked to give one of the plenary lectures about our work on electrophoresis and separation in general. It was a very memorable reunion of scientists from many countries. Our English hosts did their utmost, despite all the hardships and sufferings they had just come through, and were very successful indeed. There were many discussions about future international collaboration in a spirit of general optimism. I was elected as one of the vice presidents in charge of the Section for Biological Chemistry. Four years later, at the IUPAC Congress and Conference in Washington in 1951 I was elected president of the Union for a four year period, succeeding H. R. Kruyt.

My work in the Union involved a great deal of travelling (perhaps too much) but brought me into contact with international affairs and personalities. At this time IUPAC was run almost as a family affair; Raymond Delaby as General Secretary and Leslie Lampitt as Honorary Treasurer were the dominant figures. Both were very forceful personalities and highly devoted to the Union, but they sometimes disagreed. I had to be the mediator and was usually successful. I believe the International Unions through their Commissions and Conferences do very useful and necessary work and act as clearance centers for much valuable information and many interesting personal contacts. Some of the work, for example questions of nomenclature or standardization, may not appear very fascinating but has to be done. I often hoped that IUPAC could play a more active rôle in organizing international research projects in chemistry, but perhaps time was not yet ripe for this. I believe, however, that such collaboration will become necessary in many fields. With the present expansion of scientific research, we will soon find that

no single country can afford to do everything which appears possible or even promising. There tends to be a waste of effort by unnecessary duplication, by secrecy, and competition based upon prestige. It is to be hoped that the international Scientific Unions will play their part in guiding the development in organized collaboration, such as we have seen in the International Geophysical Year, and which seems to be coming in the International Biological Program now being launched.

A difficult problem during my presidency of IUPAC was that many biochemists wanted to form a Union of their own instead of remaining in the Chemistry Union as a Section for Biological Chemistry. I was surprised to find that there existed in some countries (especially in Britain) conflicts and disagreements between biochemists and chemists, and that these feelings came to expression in our discussions. I remember saying once that any biochemist who goes sufficiently deep into his problem runs the risk of becoming a chemist. But enforced collaboration never works. The Biochemistry Union was started and I believe that a very good and increasing collaboration has been established. The tendency will probably be towards an increasing number of international unions as science continues to become more and more differentiated. Just because of this, it becomes increasingly important that a closer collaboration between the unions be established directly or through the International Council of Scientific Unions.

Two of my later functions "on the other side of the counter," namely as one of the initiators and members of the Science Advisory Council to the Swedish Government and as a member of the board of the Wallenberg Foundation—Sweden's most important private foundation for the support of science and culture—are both too recent to be viewed in retrospect. I shall, therefore, not deal with them here. In the latter case, I have been given an opportunity to practice some of the viewpoints I have expressed in Warren Weaver's recently published book on U.S. Philanthropic Foundations (23). It has been a great satisfaction in both cases to be able to do service to those who have given me support and encouragement throughout my scientific career.

In my experience, I have come to this conclusion (also expressed by many others in similar activities): in the support of fundamental research, the individual research worker is more essential than the research project, when judging priorities. In a small country like my own this may cause the research front to advance in a somewhat uneven manner. There may be strong and active schools in certain fields with a fine tradition, and none at all in other no-less-essential domains. I believe this has to be accepted. I have even been a spokesman for giving priorities to fields where we are particularly strong ("You mean of course biochemistry" our Prime Minister Erlander once remarked). The main argument would be that in such fields we are likely to have a number of young research workers of great promise. Any investment in them is likely to prove fruitful. In applied research, where one has to pay more attention to immediate needs of the society, of industry, public

health, agriculture, etc., priorities must be given on a somewhat different basis and one may have to accept a certain amount of government direction. Even so, personalities mean a great deal also here; but if for some reason there is no "nucleus," it has to be created, for example, by sending some young people abroad to study and to do research under some leading personality in the field.

And last, I would like to add a few words about my activites in the Nobel Foundation, with which I have been connected in various functions since 1947 when I became member of the Nobel Committee for Chemistry. To act as a member of jury which has to choose those who have contributed most to human progress is very difficult and hardly enviable. No wonder that hesitation was expressed by some members of those institutions which were entrusted in Alfred Nobel's will with the responsibility of awarding the prizes which were to carry his name. The tremendous growth of science and other human activities since the will became known (in 1897) has not made this task easier. However, we have been encouraged in the mostly positive reaction in the world, especially as regards the scientific prizes. Another kind of moral support comes from the experience that there seems to be fairly evident international opinion, in that certain candidates are nominated year after year. This despite the fact that nominations (by invitation only) come from many different countries and many different university professors and other leading personalities, invited according to a plan which aims at considerable variation and a world-wide coverage over a period of several years. Often the problem is to select one or a few among a group of let us say five to ten obviously highly worthy candidates (the total number of candidates proposed being perhaps 50 to 100). Here one often faces the problem of comparing the incomparable, and in order to reach a decision one may have to resort to a natural desire to pay attention also to a fair distribution among different important regions within each prize field.

Obviously the members of the prize-awarding institutions can not fulfill the task entrusted to them if they are not themselves in close contact with the international development in the five Nobel prize fields of human endeavour. Some of them should be actively engaged in such work. This requires a high overall level of activity in these fields. The statutes of the Nobel Foundation provide the possibility of organizing Nobel Institutes for this purpose, also by investigating results which have been proposed for an award. This somewhat unrealistic idea has to my knowledge never been realized, even though the Nobel Institutes indirectly have been of great importance as offering a possibility of providing especially deserving personalities a secure position and good facilities. It would, of course, be far beyond the resources of the Nobel Foundation to assume even a partial responsibility for the high level of research in, for example, physics, chemistry, or medicine in our country. As a matter of fact, the Nobel Institute of Medicine is today largely financed by the State and the Nobel Institute for Physics and Chemistry was taken over completely by the State a few years ago. I suggested to

the Foundation that we ought to find other means of serving the original purpose of those institutes, namely to aid the work of the prize-awarding institutions by arrangements which should facilitate international contacts. Thus, we have recently been able to start some new and—as I believe—promising activities, such as inviting Nobel guest professors and Nobel guest lecturers, and organizing Nobel Symposia. The latter are a kind of informal roundtable conferences, in limited areas of great actual interest, to which leading personalities from different parts of the world may be invited. We believe that these new initiatives may be of great value also to the Nobel countries (Sweden and Norway) as a whole. After all, they are situated in a corner of the world. The symposia were made possible by a generous grant from the recently established Bank of Sweden 300th Anniversary Fund.

The president of the Nobel Foundation has to address the distinguished audience assembled in the Stockholm Concert Hall for the prize-awarding ceremony on December 10th every year. I found this interesting and a good platform to express some personal views of the distinctions in general. Naturally, I felt somewhat hampered in my attempts to hail the laureates by being a laureate myself. Thus I came to stress the significance of the prize more as a challenge than as a personal distinction. I believe this is justified, as it seems to agree with Alfred Nobel's own intentions. In the light of recent discussions of the sometimes embarrassing consequences of a Nobel prize for the recipient (24), I would like to quote here from my last presidential address (1964) the following (25):

> While it is true that the Nobel prize is today primarily regarded as a personal award and distinction, it is nevertheless dubious whether this was Alfred Nobel's main intention. He was himself, to say the least, indifferent to worldly honours. It is also said that he wished his prizes to help and support unworldly pioneers and dreamers who were not shrewd enough to profit financially from the results of their work. He wanted to make it possible for such people to continue their work without financial worry. This conception of the prizes has of course, for obvious reasons, increasingly faded into the background. But a Nobel prize is still regarded as having a purpose and value beyond the immediate personal distinction it confers.
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> It is obviously impossible to distinguish between the work and the man behind the work in this connection. But we can refuse to let the one overshadow the other. The honouring of some outstanding achievement can often bring real support in the completion of a valuable piece of work, not merely by helping the prizewinner himself, but also by encouraging those who will follow in his footsteps. There are areas within the domains of scientific research and literature, not to mention work for peace, where such support for a good cause can be of the greatest value. The institutions responsible for awarding the Nobel prizes have, I believe, often been guided by considerations of this kind when they have taken decisions designed to realize Alfred Nobel's intentions. There are fundamental discoveries which, because of their theoretical character for example, do not attract the attention they deserve if long-term developments are to be taken into account. It is primarily immediate practical results which are looked for by the general public, the authorities, and others on whose support writers and scientists depend. And the subdued

kinds of literature, not least when they have what Nobel called an "idealistic" orientation, may require help and support to make their voices heard.

Among the Nobel prize-winners there are many, of course, who do not need this extra limelight, whose personalities and work are already well known and widely appreciated. But they may even so be willing to use their fame and achievement to draw attention to others to whom this can mean a great deal. And this is not only the case with the unworldly dreamers in whom Alfred Nobel believed and whose work he wished to forward. It is, after all, the prize-winners who make the prize and it is their achievements which ultimately form the basis for the prestige of the Nobel prize in the world.

I believe that all this could be condensed in a kind of "code of conduct" once formulated by a very prominent colleague of mine who in a discussion of such questions exclaimed: "I have my prestige to spend it." And this applies not only to Nobel laureates but to scientists in responsible positions in general, on either or on both sides of the counter.

While this is being written, the plans for a new building and a new organization of the Institute of Biochemistry in Uppsala are well on their way. They will involve an intensified collaboration above all with other biomedical research, a special center for research and service in the field of separation, and arrangements for an extensive cooperation with industrial research. I have tried to prepare the ground for all of this and I believe that the future development is in good hands.

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