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CHOLINESTERASE - FROM BASIC SCIENCE TO CLINICAL PRACTICE

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Rektor, members of the Council of the University, Ladies and Gentlemen: It is with great pleasure and with a sense of honour that I stand before you today.

I would like to tell you a little about the process of discovery in science as I have been fortunate to experience it. The topic I have chosen is: "Cholinesterase - from basic science to clinical practice". What I will try to show you is how experiments carried out to answer basic questions of science can lead, in time, to a contribution to the practice of medicine. The experiments on cholinesterase that I shall describe were not carried out with any practical application in mind; at the time they were done I was not even aware of the problem in clinical medicine to which they were eventually to provide a solution.

I would like to illustrate four of the many factors that contribute to the path of discovery:

first, the primacy of experimental observation over current dogma;
second, the dedication and determination of the young scientist;
third, curiosity, without which life in science is barren;
fourth, the chance encounter with a colleague.

First, let me illustrate how experimental observation can challenge current dogma. I expect that everyone here will have heard of the chemical transmitter acetylcholine, which is secreted from certain nerves in the brain and in the peripheral nervous system. Its best known role is as the transmitter at the junction between the motor nerve and the muscle fibre: it is the agent that causes contraction of the muscles in your legs and arms. In order to stop the muscle from going into a spasm it is obviously necessary to get rid of the acetylcholine as soon as the muscle has contracted: that is the role of cholinesterase, an enzyme which hydrolyses the acetylcholine to inactive products.

Cholinesterase exists in the body in two different forms: the form that destroys acetylcholine after it has been released from nerves is called acetylcholinesterase and is present in the membranes of the muscle and nerve. The second form is called non-specific cholinesterase and is present free in the blood plasma. No one knows the function of the cholinesterase in the blood, but it is known that it is in the blood because it is secreted from the liver. By the early 1970's it was established that the two forms of cholinesterase were separate proteins and we now know that they are coded

by genes on different chromosomes. A dogma developed that stated that the two forms of cholinesterase were not only different proteins but that the acetylcholinesterase of nerves and muscle was an enzyme that was permanently fixed to the cell membranes, in contrast to the soluble form of cholinesterase in the blood plasma. You can find this dogma in many textbooks of the time and, indeed, in textbooks today. How did this dogma arise? Perhaps it was because people assumed that because an established function of acetylcholinesterase was to destroy acetylcholine after it had acted, the enzyme that did so must be fixed to the cell membrane near the nerve ending from which the acetylcholine was released. In other words, there was no need to consider any additional or alternative role for acetylcholinesterase.

Just 20 years ago, in 1973, IAN CHUBB and I were trying to isolate the synaptic vesicles that store acetylcholine from the nerves that innervate the adrenal gland. In line with current dogma, we were using the enzyme acetylcholinesterase as a marker for the fragments of membranes of the nerves in the different fractions we obtained after centrifugation of an homogenate of the nerve. We were very surprised when we found that in fact only about half of the acetylcholinesterase was attached to the membranes, the rest was soluble in the homogenate. Because of the prevailing dogma, we assumed that the soluble acetylcholinesterase was some sort of artefact and that it had come off the membranes during the experiment. However, we did many experiments that convinced us that this was not the case: the soluble acetylcholinesterase was different from the membrane-bound enzyme in several respects.

What was the role of this soluble acetylcholinesterase? We assumed that it was soluble in the homogenate because it had been trapped inside a cell particle that was broken open during homogenisation. It seemed unlikely that it was anything to do with the destruction of acetylcholine and so we wondered whether it had another function. A few years earlier, LILIANA LUBINSKA in Warsaw had shown that acetylcholinesterase was one of the proteins that is rapidly transported along the nerve trunk from the cell body towards the nerve endings. It had been assumed that this transported acetylcholinesterase was attached to the membranes. We wondered whether the soluble form of acetylcholinesterase might in fact be being transported towards the nerve ending in order for it to be secreted from the nerve. We were, in fact, able to show that this is the case: acetylcholinesterase can be secreted from the nerve endings upon stimulation of the nerve. In other words, nerve cells not only secrete small molecules that are transmitters but also a large molecular weight protein.

Perhaps you can imagine the skepticism that greeted our findings. After all, we had challenged two accepted dogmas: first that acetylcholinesterase is invariably bound to membranes and second that nerve cells can only secrete small molecules. I am pleased to say that the experimental evidence finally overcame both these dogmas, but it was surprisingly difficult to convince some people!

Now I will illustrate the second factor on the path to discovery: the dedication and determination of the young scientist.

In this case, the young scientist in question is Hungarian: his name is PÉTER SOMOGYI and two years ago you honoured him with Doctor Honoris Causa. The story begins in 1970 with a visit for one year by ISTVÁN BENEDECZKY to my laboratory in Oxford. István is well known to all of you because he has just retired as your Professor of Zoology. István is a wonderful teacher and I was fortunate to learn much from him during his period in Oxford. Not long after he returned, he wrote to me about a young student who had first been taught by his wife Rózsa in school; she had spotted his talents, and then PÉTER SOMOGYI had worked with István on a project during his time as an undergraduate. István wrote that Péter would benefit from a period in another University. I was impressed by István's recommendation and I managed to find a small scholarship for Péter, who by then had already won a national prize in Hungary for an essay he had written on the mechanism of secretion from nerves.

We had just the project for Péter. István had taught him how to use the electron microscope and we wanted to find out where in the nerve cell the soluble form of acetylcholinesterase was located. One of the pioneers of the electron microscopic localization of acetylcholinesterase was PÉTER KÁSA of the Medical University here in Szeged and so I asked Péter if he would be able to learn the method from Kása before he came to Oxford. Péter did just that: he traveled from Budapest to Szeged each day to carry out experiments and, because he was a poor student, the only way he could travel was by hitch-hiking along the road. Now Péter is not one to waste any time: rather than spend hours waiting in Szeged while the samples were incubating, he carried the reagents with him in his rucksack and changed the solutions on the way back to Budapest. Of course, I knew nothing about this before Péter arrived in Oxford but I was tremendously impressed that he had already mastered the necessary technique before he had arrived. Within a short time he had done the critical experiments that gave a clue as to where soluble acetylcholinesterase is stored in nerves and how it could be secreted from them. Furthermore, before he left Oxford he had written an excellent paper describing the results, that was accepted without change in the Proceedings of the Royal Society.

Dedication and determination: these are two invaluable qualities for a scientist and Péter's discoveries led quite naturally to the next part of the story.

The third factor that contributes to the path of discovery is curiosity, something no scientist should be without. Now that we had shown that acetylcholinesterase was a secretory protein in the peripheral nervous system, I could at last satisfy my curiosity about a fact that had long puzzled me. The cerebrospinal fluid that baths the brain and spinal cord contains much less protein that does blood plasma but the dogma of the time was that this protein was derived by filtration from the blood. You may recall that the cholinesterase of blood plasma is the non-specific form. On the other hand, the cholinesterase activity in cerebrospinal fluid is largely due to the presence of acetylcholinesterase and the amount of the non-specific cholinesterase is very small. These facts were puzzling: could it be, I wondered, that the acetylcholinesterase present in cerebrospinal fluid is in fact derived from them nerves in the brain and that these nerves secrete acetylcholinesterase just as we had found nerves outside the brain

to do? There was only one way to satisfy my curiosity about this question: do an experiment. This I did with SALLY GOODMAN and IAN CHUBB: we were able to show that stimulation of sensory nerves in the periphery, a procedure that was known to activate certain pathways in the brain, led to an increase in the concentration of acetylcholinesterase in the cerebrospinal fluid. Shortly afterwards SUSAN GREENFIELD joined me and showed that electrical stimulation applied to certain parts of the brain itself also led to an increase in the concentration of acetylcholinesterase in the cerebrospinal fluid. We were then able to demonstrate secretion of acetylcholinesterase from isolated brain slices, so establishing acetylcholinesterase as a neurosecretory protein in the brain. Incidentally, although this is another story, the curiosity of SUSAN GREENFIELD has led her to demonstrate that the secretory form of acetylcholinesterase has unusual modulatory actions on nerve cells in the brain that are totally unrelated to its ability to destroy acetylcholine.

So I come to the fourth and final example of factors that contribute to discovery: the chance encounter with a colleague. There must always a place for chance in scientific discovery, be it a chance observation in the laboratory or the chance remark of a colleague and we should keep our minds open for such occasions.

The year was 1978: my wife and I were entertaining for dinner a Swedish doctor, HUGO LAGERCRANTZ, whom, 7 years earlier, I had examined for his MD thesis in Stockholm. He had moved into clinical medicine and was telling us the sad story of how, within the previous 3 months they had terminated the pregnancies of two women who were suspected of carrying a fetus with spina bifida only to find that the fetus was perfectly normal. Now in spina bifida as I expect you know, the spinal canal does not close up properly and so cerebrospinal fluid leaks from the spinal cord into the amniotic fluid. I asked him how they had diagnosed spina bifida and he told me that it was by measuring alpha-fetoprotein in the amniotic fluid. I had never heard of this procedure, but I said: 'Why on earth do you use a liver protein to diagnose spina bifida, when what you want is a protein that is present in normal cerebrospinal fluid. We have just such a protein: it is acetylcholinesterase'. He agreed with me and within a few weeks he had returned to Oxford with some samples of amniotic fluid which I analyzed blindly. I found the neurosecretory form of acetylcholinesterase to be present in some of the samples; in every case it turned out that the samples with acetylcholinesterase were from pregnancies where the fetus had spina bifida. Within a few months we had collaborated with NICK WALD and his colleagues in Oxford and had been able to establish a new test for spina bifida in early pregnancy.

Now a diagnostic test is only of value if it is better than the previous test. In fact, the previous alpha-fetoprotein test was very good in comparison with tests for other diseases. Nevertheless, it had a false positive rate of about 1%. What does this mean in human terms?

In the United Kingdom there are about 500,000 births each year and the incidence of spina bifida is about 0.5%; so, about 2,500 children each year could be born with spina bifida. For this reason, there is a national screening programme to detect spina bifida and to offer the mother the opportunity to terminate the pregnancy.

The detection rate of the screening programme is very good indeed, being 99%, but the problem is that, with a false-positive rate of 1% as many as 5,000 perfectly normal pregnancies each year could be terminated.

What, then is the false-positive rate of the acetylcholinesterase test? There have been two international studies and one very detailed study in Denmark: the most recent conclusion is that the acetylcholinesterase test has a false-positive rate one tenth of that of the alpha fetoprotein test. In human terms that means that, because of the use of the acetylcholinesterase test, up to 4,500 normal children are born each year in Britain who might otherwise have not lived. Although the prevalence of spina bifida is not high in other countries as in Britain, it is likely that the introduction of the acetylcholinesterase test in 1979 has saved the lives of tens of thousands of unborn children across the world.

So, my friends, I hope you will now understand the title of my talk. When we began these studies on cholinesterase in 1973

we were led by simple curiosity,
aided by the dedication of a young Hungarian student,
given courage to challenge accepted dogma,
and fortunate to hear from a colleague of a clinical problem that our findings could go a long way towards solving.

Finally, I should like to take this opportunity, Rektor, to thank the University most warmly for the honour they have bestowed upon me. As a token of my gratitude and that of my Department, we would like to offer to the József Attila University a scholarship so that you can send one of your students to Oxford University for a period of one year. The student would be able to work in one of the laboratories in the Department of Pharmacology or in its associated Medical Research Council Anatomical Neuropharmacology Unit. We would cover the costs of travel from Szeged and provide funds for living expenses in Oxford and for travel to a scientific meeting in Britain. I hope, Rektor, that this scholarship will help to build up even closer relations between our two Universities and to foster the pursuit of natural science.

Thank you very much.