General Anesthesia

Atypical plasma cholinesterase. A personal discovery story: a tale of three cities

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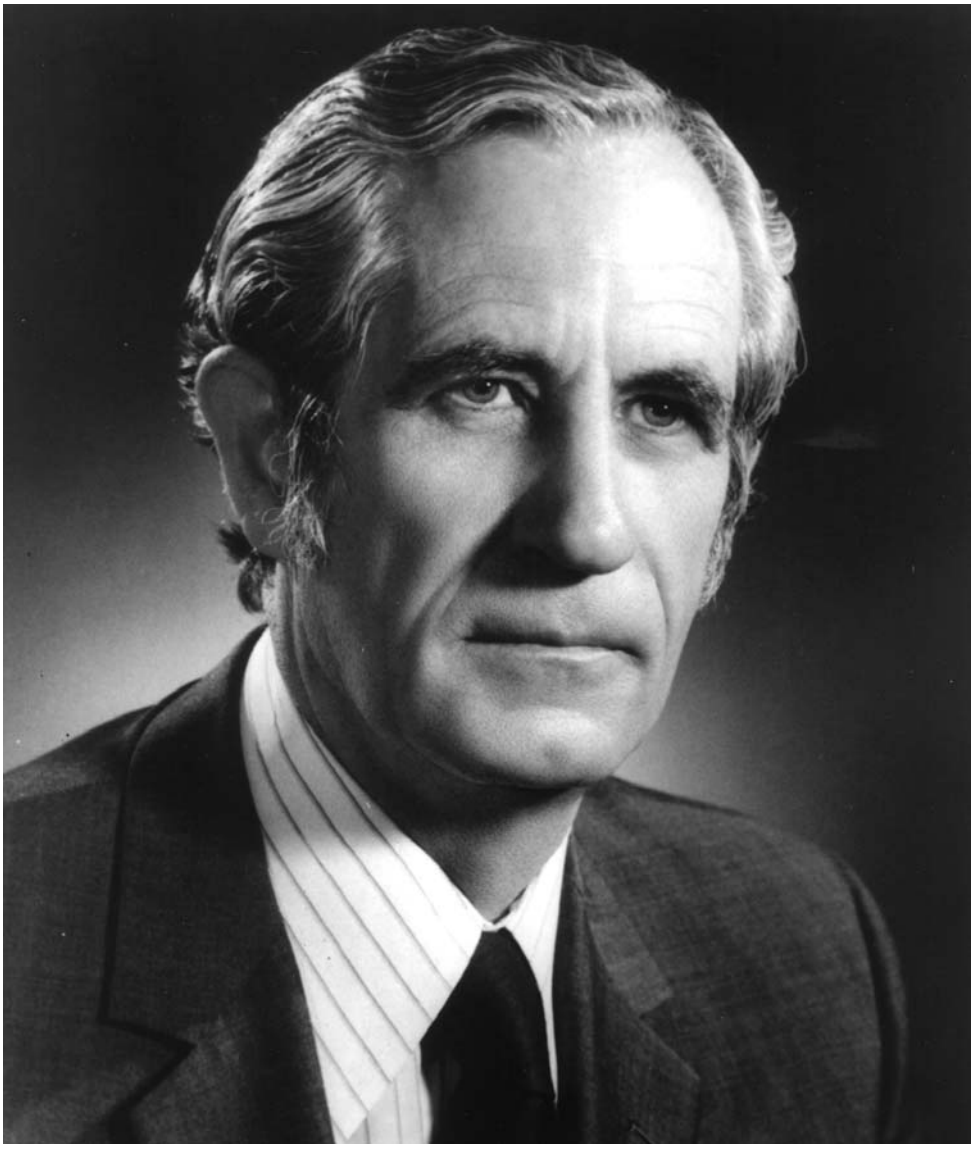

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THIS is a historical review of my personal role in the identification of a genetic variant of butyrylcholinesterase which unfolded over a series of events during the 1950's. The investigations started in post-war Germany, but its principal lessons are not time-or location-dependent; this review is meant to show that the way to discovery is often not straightforward. There may be a role of luck, circumstances, and help of many persons, leading to new development. It is hoped that this report will serve as a stimulus to young investigators to be on the lookout for new scientific possibilities and pathways.

In the 1950's, practically nothing was known about any genetic alteration of drug effects, although such occurrences had been predicted by Garrod[1] and by Haldane,[2] and a few cases had been observed. At the present time, pharmacogenetics has become a worldwide recognized field of study, supported by the newly developed and still developing methods of genomic testing. The medical importance of pharmacogenetics has lately been increasing dramatically, and further increases are on their way. Many young scientists confronted by their research tasks will take the current status of the subject matter as a given fact, and they may not have a full appreciation of its origin.

Berlin: the initial stimulant for the study

During the war, I served the German navy as a ship surgeon and ended up as a prisoner of war in Arizona, USA. After my release in 1947, I went to Berlin in order to be near my parents, and in the hope of becoming a medical scientist. I received an offer to work in two departments, Pathology and Pharmacology. The downtown pharmacology building had been bombed out during the war, and the Department had moved into what was then the American-occupied sector of Berlin; Pathology was still in the Russian-occupied part. Therefore I chose to become a pharmacologist.

In one of the hospitals in Berlin in 1948, a patient had died after injection of the local anesthetic drug procaine. Procaine was an old drug and known to be very safe.[3] After a pain-free period at the site of injection, it was metabolized in the body by an enzyme in the blood called "procaine esterase".[4] Professor Hans Herken from the Department of Pharmacology knew of many changes that occur in blood after poor nutrition.[5] This was easy to investigate in Berlin, since nutrition was poor for many people after the war. Herken wondered whether the patients who had died from procaine might have had a nutritional decrease of this procaine esterase activity. He wanted to see whether poor nutrition lowered the activity of this esterase sufficiently so that procaine - not being metabolized - could suddenly become toxic. Dr. Herken decided to investigate the metabolism of procaine by procaine-esterase, and he asked me to work with him.

Herken thought of using ultraviolet spectrophotometry to follow the metabolism of procaine. That is, ultraviolet light is made to shine through a procaine solution which will absorb some of the light; the absorbency is measured and is proportional to the concentration of the drug. Thus, the diminution of the drug's concentration by its metabolism is indicated. It is a fundamentally simple method, but at that time in Berlin, its use was complicated. In the first place, the help of a physicist with sophisticated equipment was needed to produce ultraviolet radiation of the proper wavelength. For measuring this radiation, we received the gift of a photomultiplier from the occupying American Army. The results of our efforts were beautiful: the destruction of procaine by procaine-esterase in a bit of human blood serum could be precisely measured.[6] However, this task in Berlin ended there for me. Why?

A group of American medical scientists had come to Berlin on a visit organized by the Unitarian Church in the USA. Most of the visitors spoke some German; for instance, one visitor was a former Professor of Pharmacology of the University in Freiburg who had left Hitler Germany prior to the war and had become a Professor at Harvard. Only one visitor, Professor Lyman C. Craig of Rockefeller University in New York, did not know any German at all. His talk in English on a new chemical separation method called "counter-current distribution" before the Faculty of Medicine aroused much interest but could not be understood by a large part of the audience. I volunteered to interpret his talk and was asked to step forward, standing beside him. His talk became a success. A few weeks later, I received from Professor Carl F. Schmidt, Chair of the Department of Pharmacology at the University of Pennsylvania in Philadelphia, an invitation for a year's study.

Philadelphia: development of my method of study

I arrived at the Department of Pharmacology late in 1949. There was usually one big cardiovascular experiment per week (e.g., Aviado *et al.*).[7] In addition, I sometimes had to help with the teaching of young students. However, there was also much free time, and I looked around to see what other investigators were doing. Thus I came to know that the Department had a "Beckman Spectrophotometer", a little longish box

sitting on one of the benchtops in the laboratory. I realized that with this little box, one could measure the metabolism of procaine by the method initiated by Dr. Herken in Berlin, which had required in Berlin a whole room with special equipment and the help of a physicist. I got permission to use this little box for experiments with procaine. I did most of the experiments at night because then nobody else wanted to use this equipment, and because the results which I obtained at night were better than day-time measurements because of fewer fluctuations of the electrical current in the laboratory.

I experimented not only with procaine but with various other chemicals. Thus I happened to use benzoylcholine, a compound known to be destroyed by cholinesterase.[8] My important observations were that the destruction of benzoylcholine could also be seen in the spectrophotometer, and that it was also being metabolized by the enzyme which metabolized procaine. This meant that procaine-esterase was actually plasma cholinesterase, an enzyme well described in the published literature. When I tried to publish these points of information in a biochemical journal, the manuscript was rejected because the reviewers were convinced that blood plasma contains only one kind of esterase, so that the identity of procaine-esterase and cholinesterase was to be taken for granted; thus my paper was thought to report something obvious. This was a ridiculously mistaken criticism; even then as now, several different esterases in human plasma were known. Dr. Schmidt helped me to get my findings published in another Journal.[9]

I met in Philadelphia another young scientist who was a member of the Johnson Foundation, a University-affiliated research unit of biochemistry and biophysics. I visited him there and thus met Dr. Britton Chance, the famous head of the Johnson Foundation. When Dr. Chance learned of my experiments with the destruction of procaine, he offered help whenever I might need it. I ended up having excellent instructions in enzyme kinetics, a topic unfamiliar to most pharmacologists.

Dr. Schmidt invited me to stay in Philadelphia, and he prepared to help me become an American citizen. While these preparations went on, there was a large scientific meeting in Cleveland. I gave a talk, describing my findings. The Chairman of the session was Dr. Ken Ferguson, the Chair of Pharmacology in Toronto who invited me to visit him in Toronto. Since I was in Philadelphia with a student visa, I had to leave the USA so that I could properly immigrate. Thus I decided to travel to Toronto. During my visit, Dr. Ferguson invited me to accept a university position in his Department. After many contemplations and discussions, I accepted the invitation.

Toronto: discovery of a genetic variant of cholinesterase

I arrived in Toronto late in 1951. I was given various research tasks. But then came an opportunity to use my experiences from Berlin and Philadelphia to study plasma cholinesterase. There was a double history to this opportunity. First, Otto Loewi in Austria had received the Nobel prize for his work during 1921–1926; his discoveries included cholinesterase, a protein in blood which could destroy the messenger substance acetylcholine. Next, during the Second World War, there was in Toronto the biochemist Dr. Bruno Mendel who had immigrated to Canada from Holland before the German occupation of Holland. He found that cholinesterase consisted of an entity in red blood cells which he called "true cholinesterase", and one in plasma which he called "pseudo-cholinesterase".[8] He thought that only true cholinesterase functioned to destroy the transmitter acetylcholine and thereby terminated its action - as Otto Loewi had discovered. He wondered about the physiological role of pseudocholinesterase in plasma (today it is still unknown). Although Dr. Mendel had left Toronto to go back to Holland, there was still an investigation of pseudocholinesterase going on in Toronto.

The measurements of the activity of pseudocholinesterase (or "plasma cholinesterase" as I liked to call it) were performed by using the then traditional gasometric method: it involved measurement of the CO_2 gas which was formed by the reaction. The method was labour-intensive and complicated, since one had to know with precision the amount of gas produced; thus, one had to know the volume of the reaction vessels. In order to know this volume, the vessels were filled prior to the test with mercury, which then were put onto a scale and weighed. When I learned that these studies were going on in the Department of Biochemistry, I declared that I had a much better method to measure plasma cholinesterase activity, an ultraviolet method using a spectrophotometer. I recommended adoption of my method. I was asked to prove my point.

Thus, I set up a comparative study. I asked the students of my class for a small blood donation, and I explained the purpose; most of the students allowed me to take a sample of their blood. I separated the plasma from the red blood cells, and measured cholinesterase activity in plasma, once with the gasometric method, and once with my ultraviolet method. The results obtained with the two methods agreed

very well.[10] However, the biochemists were still critical, saying that all the student's cholinesterase activities were in the normal range; would my test be good in blood plasma with low cholinesterase activity?

Dr. Ferguson knew how to help. He knew a physician in one of the psychiatric hospitals who had patients with established low plasma cholinesterase activity. Again, there was a story involved. Dr. Donald Gunn from the Ontario Hospital in New Toronto had to treat patients with schizophrenia. There being no effective drugs at that time, he treated them with electroshock. This treatment, which often had good therapeutic successes, also had the big drawback that it sometimes stimulated skeletal muscles to maximal contracture, so much that some patients' bones were broken. To avoid these excessive muscle contractions while still treating the brain with the beneficial electricity, Dr. Gunn injected succinylcholine which he knew to act for only a few minutes after injection because it was rapidly destroyed by plasma cholinesterase.[11]

Dr. Gunn had two patients who did not respond normally to succinylcholine. When he injected this drug, its paralyzing effect was not over in a few minutes as usual, but lasted for almost an hour. Since electroshock treatment usually needs to be given a few times before the patient improves, Dr. Gunn also knew that the response of these patients to succinylcholine was always prolonged. He therefore had the plasma cholinesterase of these patients tested in a government laboratory, and he knew that they had a low cholinesterase activity. He gave me blood from these two patients to try out my method.

When I tested the plasma from these two patients in my spectrophotometer for their cholinesterase activity, a strange thing happened: both had activity to start with but the activity became less and less during the minutes of my observation. I had never seen this before; usually the activity is steady and slows down only just before it stops. I had learned enough enzyme kinetics from Dr. Chance in Philadelphia to understand the meaning of my observation. It could only be that the drug being metabolized by the patient's plasma had a low affinity for the destroying cholinesterase. Indeed, one could calculate that in the living body, succinylcholine would not be metabolized by the particular cholinesterase of these patients; the binding between drug and esterase was too poor.

The change of enzyme behaviour in these patients could only mean a change of structure of the enzyme and that was likely genetic. I asked Dr. Gunn for blood from relatives of these patients. The cholinesterase in the plasma of the patients' parents was not normal but one could not call it abnormal with any certainty. With two helpers, I worked for almost a year to clearly pinpoint the abnormality in these parents' blood.[12,13] We found that a drug called "dibucaine", when added to plasma, inhibited the normal cholinesterase but hardly touched that of the patients; the parents' cholinesterase was partly inhibited. Thereby, we could clearly distinguish between the esterases of the patients, the parents, and the normal. Genetically speaking, the patients were homozygous with two abnormal genes for cholinesterase, the parents heterozygous with one normal and one abnormal gene, and both contrasted with ordinary people who had two normal genes.

This was a beautiful example of the kind of inheritance described first by Gregor Mendel who had studied peas: except for blood groups, there were at that time not many human characteristics so clearly controlled by a gene. Thus, what I had found was a genetic difference between people that would make them respond differently to a given drug! I became excited: a gene had altered the response to a drug.

My finding had some immediate clinical utility;[14] succinylcholine was often given to patients during anesthesia and surgery for good but brief muscle paralysis in order to start artificial ventilation; the occasional occurrence of a prolongation of action was known. Even some cases of death were on record, usually when the physician got desperate and tried other drugs to terminate the paralysis. Through our finding, it became clear that no particular action was required; all one had to do was to wait until the paralysis of the patient disappeared so that the patient could again breathe without help. The prolonged effect of succinylcholine did not endanger the patient as long as the patient was given oxygen and artificial ventilation.

My long search for the means to identify the cholinesterase abnormality in the heterozygous parents of Dr. Gunn's patients delayed the immediate publication of my striking findings in the patients' plasma. It happened that during that time, other investigators observed the occurrence of low esterase activity in members of a family, indicating its genetic control,[15] and they immediately published their observation. This stimulated me to briefly describe my work, and I had it published two months later.[16] Still, such a little incidence can be damaging to a scientist who thereby loses his priority in public view - even if not in his own mind. Lesson to a scientist: always publish as fast as you can. Today, about 20 different mutations of this esterase are known.[17]

I could not help but often think of my observation that a genetic variant in a patient's blood could cause a drastic alteration of a drug response. I considered

this an observation which represented a new medical principle. Dr. Norma Ford-Walker, the local Professor of Genetics with whom I often came to talk, agreed with me. Dr. Ford-Walker was a woman and Professor, at that time a rare combination. She stimulated me to read, and I thereby learned about two other examples of genetically altered drug responses: first, American soldiers during the Second World War in some tropical areas were given a drug called "primaquine" as a prevention against malaria; some soldiers taking this drug developed hemolysis. As reported recently,[18] investigators in Chicago discovered after the war that there was a genetic reason for this primaquine sensitivity, and they described the genetic defect of glucose-6-phosphate dehydrogenase also in blood.

Second, the drug isoniazid was introduced in 1952. This drug was revolutionarily useful in the treatment of tuberculosis but, in some people, it caused tingling in the hands and feet and other signs of nerve damage. It turned out that these people metabolized isoniazid at an exceptionally slow rate, and that this slowness ran in families.[19,20] Thus, the cholinesterase deficiency was not the only genetic defect affecting drug response.

I decided to search for more examples and to write a book about drugs and genetics. With my teaching duties and with continuing research, it was a slow process. Finally, I negotiated with a publisher. In the spring of 1961, I agreed to submit my manuscript on September 15 of that year, just before the new teaching duties would start. During much of 1961, I slept only three to four hours a night, working on this book. My last chapter was on renal function. I had read that in some, but not all persons, smoking a cigarette cuts down on urinary flow since nicotine may release the anti-diuretic hormone. At the end, I felt under so much time pressure that I did not finish this chapter; the manuscript went to the publisher without my renal chapter. It is strange to me that to this day, nobody seems to have looked at nicotine, genetics and the kidney.

Having taken a long time to write this book, I again lost my priority. Using the case of cholinesterase variation among other examples, Arno Motulsky in Seattle had written in 1957[21] a beautiful and crucial paper on genes and drugs, and the geneticist Vogel[22] in Germany published in 1959 the descriptive word "pharmacogenetics". What does that matter? My book on pharmacogenetics[23] came out in 1962, and this event was followed by many discoveries relating genes and drugs.

Today pharmacogenetics is more important than ever. New methodologies tend to change it increasingly into pharmacogenomics, the same kind of science but using a broader methodology. This will, in the long run, change medicine: the choice of a drug for a given patient will depend less and less on the patient's appearance, and more and more on the patient's genes.

I hope to have shown that the development of a new concept can be a different matter than the discovery of a new reaction in the laboratory. Both may depend on some luck. Finding a new reaction may require good observation, proper interpretation, and usually hard personal work and effort. A new concept will probably never arise without some knowledge of past events, or without influence of colleagues and friends.

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In a recent personal discussion that I had with Dr. Arno Motulsky, he made the suggestion which stimulated me to tell this personal discovery story as I had never done before. I also wish to thank my colleague Dr. Harold Kalant for his greatly appreciated comments.

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