Interview with Prof. Ryszard Gryglewski

A Polish Hormone

PAP/Teodor Walczak

Prof. Ryszard Gryglewski receiving the Medicus Magnus Gold Medal as one of two Poles during the 7th International Symposium organized by the Polish Medical Academy. Warsaw, 1995

Academia: What's the story with the discovery of prostacyclin?

Ryszard Gryglewski: *Prostacyclin is a significant part of my life, and scientifically the most important. The discovery of prostacyclin would not have been possible without earlier discoveries made by the Nobel Laureate Professor John Vane, member of the Royal College of Surgeons. John devised a bioassay technique combining biochemistry, biology, and pharmacology. It was based on a very complex biological system, which allowed us to study various biologically-active substances. Cells or tissues (in our case it was cultured endothelial cells) were placed in solution in columns. The solution was passed through the column allowing us to analyze specific biologically-active substances originating from the cells we were studying. The most important element of the system was a cascade now known as Vane's cascade. It was composed of various smooth muscle organs (five, six, seven) placed under the generator. The cascade danced under the influence of the generated active substance. Some organs contracted, others relaxed. They were all sorts of organs: a strip from a rat's stomach, a chicken's rectum. It was rather comical. John was searching for more and more organs which would dance under the influence of biological substances such as acetylcholine or adrenaline. The more organs he tried, the more answers he got to contribute to the overall picture – the ability to discern the given substance. With prostacyclin they danced like never before...*

Did you suspect straight away that it was a special and important substance?

The image we saw on the recording was nothing like we'd ever seen before. We used various well-known chemical mediators as controls.

Nothing fit. On top of that prostacyclin is very unstable. In the cascade we can mark the half-life by adjusting the distance between the detectors. We set one for a period of 5 seconds, the other for 5 minutes, and saw that the half-life is somewhere in between, allowing us to set an increasingly narrow interval. We used substances with well-known modes of action as controls: acetylcholine, bradykinin, adrenaline. Depending on the organ's response, we were able to find a fingerprint for each substance. When at certain conditions we found something that didn't fit, we knew it was something new. But the story is even more complicated. In the 1970s – long before we started working on it in London – Sune K. Bergström and Bengt I. Samuelsson (later Nobel laureates themselves) at the Karolinska Institutet in Stockholm had already discovered a very important group of chemicals: prostanoids. These compounds consisted of groups containing various fatty acids, such as the 20-carbon arachidonic acid with four double bonds. The unsaturated bonds are sensitive locations – they tend to become cyclical, forming ring compounds. The name „prostanoid" reflects the fact that large quantities of those substances are synthesized from arachidonic acid in the prostate.

Did they realize that it was something extraordinary?

These are very interesting compounds. They contract and relax various organs. Bergström and Samuelsson later discovered intermediate compounds between prostacyclin and arachidonic acid – prostaglandin cyclic peroxides. In contrast to prostaglandin E2, which was rock-stable, they quickly disintegrated (their half-life was 4 minutes). The unstable bridge cracked to form prostaglandin C2. Following the discovery of prostaglandin cyclic peroxides, the Swedes started studying various organs to find out whether prostaglandin is synthesized everywhere, and it turned out that it is present in the majority of organs. However, Samuelsson discovered that thromboxane A2 is synthesized in blood platelets following cyclization from prostaglandin cyclic peroxides. And thromboxane A2 is a powerful vasoconstrictor. This makes sense, because if there's a hole in a blood vessel, a plug isn't sufficient to plug stem the bloodflow – the vessel also needs to be constricted. Prostaglandin A2 was discovered in 1975. Its precursor was discovered a year earlier, so that means its source was identified even before its existence was known. The discovery that this precursor also forms thromboxane followed soon after.

Did you notice this before – this dual action?

No, others noticed it later... Great discoveries come from various scientists working together, at different locations, through different sources, at the crossover. Samuelsson couldn't send us thromboxane, because it is too unstable. However, the peroxides are relatively stable, especially when they are dissolved in toluene (they disintegrate quickly in water without enzymes). He gave both the prostaglandins – PGG2 and PGH2 – to John Vane. He knew John and knew that he was good at his stuff. Meanwhile I'd just arrived in London from Kraków. I'd been there twice already; John trusted me.

And so he simply gave them to you?

I arrived after I'd also made an important discovery of the mechanism of action of glucocorticosteroids – hydrocortisone. My studies showed that hydrocortisone inhibits the synthesis of pro-inflammatory prostaglandins, explaining its anti-inflammatory properties. This happens at a very high level of the cellular membrane, where prostaglandins are released from cellular membrane phospholipids. When phospholipids are unfolded, they release arachidonic acid. At the time we didn't know about prostacyclin. I demonstrated that when hydrocortisone is delivered to a system containing cellular membranes, it inhibits the release of arachidonic acid – the forefather of the entire prostanoid family.

But when I arrived in London, John said he didn't believe a word of it, even though I'd already published it. Still, John told me I had to sit down and repeat everything. So I said that I had not published my work just to have to do it all over again as soon as I arrived in London, and that he could read the article, find some researchers and repeat the experiment himself. He got really offended at that for a few days. In

Archiwum prywatne

Prof. Gryglewski's scientific achievements have received the highest accolades. He has held numerous important functions. During the 1980s he was the Rector of the Medical Academy in Kraków

the end he came over and said, "Here's what I got from Samuelsson. I have no idea what to do with it, but I have to write back to him. Do something with it." Anhydrous prostaglandin peroxides PGG2 and PGH2 were dissolved in toluene at -20°C. Each time we tried doing something with them, we had to quickly pipette off the toluene. To start with we got prostaglandins, of course; they don't need enzymes. This was not a major discovery. But later I started wondering what would happen if I put those peroxides through various organs. I tried platelets as well. In platelets I got thromboxane, which has very powerful vasodilatory properties. This could be observed using John Vane's method. So I took other organs – liver, kidney, stomach. I got prostaglandins again – nothing new. I started thinking that no-one ever studied arteries in those organs, mainly because they are very small in rats and chickens. They couldn't be isolated. I had to find a bigger artery.

Hmm, this sounds very serious...

I went to a slaughterhouse and ordered some pigs' arteries. They were huge tubes. They needed to be homogenized somehow to isolate the microsomes, where cellular enzymes are located. They were crushed and centrifuged. This was the most difficult stage of the experiment. The tubes were just like snakes – they coiled, slithered, it was impossible to cut them. I had no idea how to chop them. Fortunately help was at hand from a charming Brazilian. Mr. Uba Tuba had a luxuriant moustache and was an expert at homogenizing various tissues. He suggested that I freeze the arteries and crush them to powder while frozen. It was really thanks to this elderly gentleman that I was able to take the experiment further. We froze the arteries in liquid nitrogen and broke them up with hammers. I added Samuelsson's peroxides. It was rather complicated technically. I put it through the organs in our system, and got nothing. Absolutely nothing. The cascade didn't even twitch. To start with I thought that perhaps the stuff deteriorates quickly and loses its activity. But I thought I'd try changing the composition of the cascade just in case. This was my Eureka moment. I changed the reactive composition of the organs. It turned out that if you place a spiral-cut blood vessel in the cascade, you observe a major relaxation of the vessel.

So the whole cascade didn't respond, but the artery relaxed?

And it kept repeating. This had to be something completely new. I tried it on platelets as well. It turned out that this new "something" had an antagonistic effect on thromboxane. It was quite striking. To begin I was being teased by everyone else in the lab: "Oh, Richard's discovered a Polish hormone!" And yet in subsequent experiments everything suggested that we found something with a powerful dilating effect on blood vessels, something that was neither thromboxane nor prostaglandin E2. John turned to his friends in the US, from the team at the Upjohn Company, asking them to help elucidate the structure of our substance. It took them six months. Then we decided to announce the structure of prostacyclin at a conference in the US. When John and I were there, we experienced one of the most extraordinary events.

Do tell!

On the evening before announcing the structure, we were joined by a gray-haired, elderly man of Polish-Jewish extraction. He was behaving quite strangely, constantly nagging the Americans to show him the structure. "All in good time", was their reply. The elderly man (I didn't know him at the time, but he was Jo-

sef Fried, one of the most outstanding American scientists) was getting quite irritated. So, the next day we present our structure. At the discussion stage he raises his hand and asks if he can speak. He gets up, puts his own slide on the projector, switches it on to reveal... an identical structure. And he had derived it two months earlier. There was an almighty fuss! The Americans were terribly mad at him. I met him a different time and asked, "Prof. Fried, you are a great theoretical chemist – how did you get that structure?" And he replied, "When I read in your paper that you discovered something physiologically opposite to thromboxane, I had the structure within a couple of hours."

He solved it theoretically?

Yes, he didn't need to conduct any experiments. You only have to look at the structure – it is an isomer of thromboxane. It couldn't have been anything else – it is simply an isomer with the opposite properties.

That is quite a story...

I later asked my Polish colleagues, also theoretical chemists, and they also knew straight away that this had to be the structure.

This line of research really won two Nobel Prizes: for John Vane for the discovery of prostacyclin, and for Bengt Samuelsson for the discovery of thromboxane. You had a brush with at least one if not two Nobel Prizes...

Let's not exaggerate. John Vane fully deserved the prize, and I never harbored any hard feelings about it. We could have only made the discovery using his method. We had no idea at the time that it was an isomer of thromboxane... Following the discovery, John came to me and said that since I claim it is an enzymatic transformation, then it clearly must have its own inhibitor; all enzymes have them. And he asked me to find it. I asked how I should search for it, and he said he had no idea. "Start from the beginning and keep going – take the alphabet: A, B, C..." I thought to myself that if this was his idea of a joke, I decided I would show him; so I started from A, taking arachidonic acid – the forefather of the whole lot. First day – nothing. Second day – I was feeling lazy and just used the arachidonic acid I left on the lab bench the previous day. This time it worked: it inhibited synthesis. It must have oxidized sitting there in the aqueous solution. I repeated the experiment the next day. I ran a control using the acid dissolved in toluene – it didn't work. And so I took the acid straight from the toluene again, and saturated it with oxygen. Now it worked really well! It became clear that the synthesis of prostacyclin is inhibited by lipid peroxides, rather than arachidonic acid itself. We studied various fatty acid peroxides; they all worked, albeit to various degrees. We posited a theory that eating potato chips may cause atherosclerosis, since the frying process creates peroxides, which inhibit the synthesis of prostacyclin.

Together with Prof. Andrzej Szczeklik you were the first to administer prostacyclin to humans...

After the incident with Josef Fried – and I didn't hide my admiration and respect for him – the Americans from the Upjohn team took great offence. When I asked them for prostacyclin to study, they refused. Josef Fried did not want to take it on himself to make a larger quantity of the substance. He was a theoretician after all, and retired to boot. At the time I travelled quite a bit lecturing about prostacyclin. I got to know a chemist in Milan; a confirmed bachelor with a passion for brass music. He had a beautiful Venetian chest where he kept all the concert programs. He synthesized prostacyclin for us. But he was a bit peculiar really; he worked in some tiny laboratory at the university. Unfortunately it later turned out that the prostacyclin wasn't pure. Andrzej Szczeklik and I both got very ill. Of course we had a dilemma – which one should administer prostacyclin to himself first? It ended up being me, as I was the discoverer. We used a pump to inject the drug. I remember lying there and wondering how long I would have to wait or whether it would work in humans at all. After that I remember nothing: I passed out. Andrzej went through the same thing, but we had a better idea of the dose by then.

That's very brave...

But so interesting! What can I say – we wanted to be the first. And we did it.

This was the beginning not only of friendship, but also of a research collaboration.

Together we studied the effect of aspirin on a certain subtype of bronchial asthma. Andrzej is an excellent clinician. He has been working with patients with aspirin-induced asthma (AIA) for years. He had a large group of such patients. We corrected the asthma using a drug from the cyclooxygenase inhibitor group.

You have been described as a blood pharmacologist.

Each cell is a major factory with a specific task in the system. I must say that that's what fascinates me most as a biochemist: how resourceful the organism is; how well it is able to cope. I've always been enthralled by that. It is the joy of life. In my profession, curiosity is the most important.

Was that your attitude when you started?

I started with the curiosity about how my own body works. I studied medicine rather than biology, because I was interested in the humans rather than animals. This was naïve: to find out how the human body works, you really need to study animals first...

According to a ranking by Prof. Jerzy Pilc, you have been one of the most active scientists in Poland.

Everything has its own place. I was young, then I matured, worked hard, and now – what can I say – I'm retired. This doesn't mean I'm not still fascinated by it. I always keep up to date, I read a lot, check the Internet, look at what's going on in my areas of study. I'm a bit like a scent dog – my ears prick up. It's great being able to share my knowledge.

Interviewed by Patrycja Dołowy, Kraków, 21 July 2010

Prof. Ryszard Gryglewski, scientist and humanist, doctor and pharmacologist. The most frequently cited Polish scientist. One of the most outstanding originators of modern pharmacology and clinical pharmacology of the circulatory system; discoverer of prostacyclin. Laureate of the „Polish Nobel" and numerous other prestigious awards. Holder of honorary degrees from seven universities across the globe; member of scientific associations and academies.