

# DISCOVERY OF THE DEFENSIVE SYSTEM OF THE ENDOTHELIUM, THE LINING OF THE ARTERIAL WALL

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## Introduction

Blood flows through the vessels that are tightly covered by a monolayer lining – ‘a membrane’ – endothelium. Is it really a membrane? ‘Yes, indeed, a primitive membrane’ – answered Rudolf Virchow, who first observed it at autopsy and described in 1860. A hundred years later, Sir Howard Florey expressed some doubts about this term, saying that ‘endothelium could be more than a sheet of cellophane’. In 1996 Sir John Vane called the endothelium ‘a maestro of blood circulation’ [1].

Rudolf Altschul was the first to think that the endothelium might have a secretory function. He was a political émigré to Canada from Central Europe during World War II. With help of a simple microscope he perceived that the endothelium rises like a palisade to defend arteries against an approaching catastrophe, brought about by atherosclerosis. In his book published in 1954 he wrote: ‘the secretory function of endothelium needs to be considered’. The book contains a moving dedication to ‘Anni Caroline who was very brave when the ship went down’ [2].

We know now that the endothelium is the main defensive barrier in the cardiovascular system. It achieves this goal by synthesizing several chemical compounds with powerful biological activity, of which prostacyclin and nitric oxide are most important. Other compounds, like heme oxygenase-1 [3], are also emerging, but they will not be discussed here.

## The superfamily of eicosanoids

Prostacyclin belongs to the superfamily of eicosanoids [in Greek ‘eicosa’ (εἴκοσα) stands for twenty – in that case twenty carbon atoms in a molecule]. Indeed, prostaglandins derive from eicosa-all cis-5,8,11,14-tetraenoic acid, i.e. arachidonic acid (AA). AA may be subdued to a number of enzymic manipulations. Firstly, phospholipase A<sub>2</sub> cuts it out from the cellular phospholipids stores. Next, free AA is exposed to the enzymes available in various types of cells and in various compartments. For us, the most interesting are cyclooxygenases-1 and -2. They generate prostaglandin endoperoxides (PGG<sub>2</sub>

and  $\text{PGH}_2$ ), which are substrate for both thromboxane and prostacyclin synthesis, through action of the specific enzymes (synthases). Thrombogenic thromboxane  $\text{A}_2$  is generated by COX-1 in blood platelets (Fig. 1). Aspirin at low doses is a pretty selective inhibitor of COX-1 [4] in blood platelets, hence aspirin is effective against myocardial infarction. However, in a special category of ‘aspirin-sensitive’ patients, aspirin itself and other non-steroidal anti-inflammatory drugs may precipitate asthmatics attacks interfering with COX-1 activity in the respiratory tract [5,6].

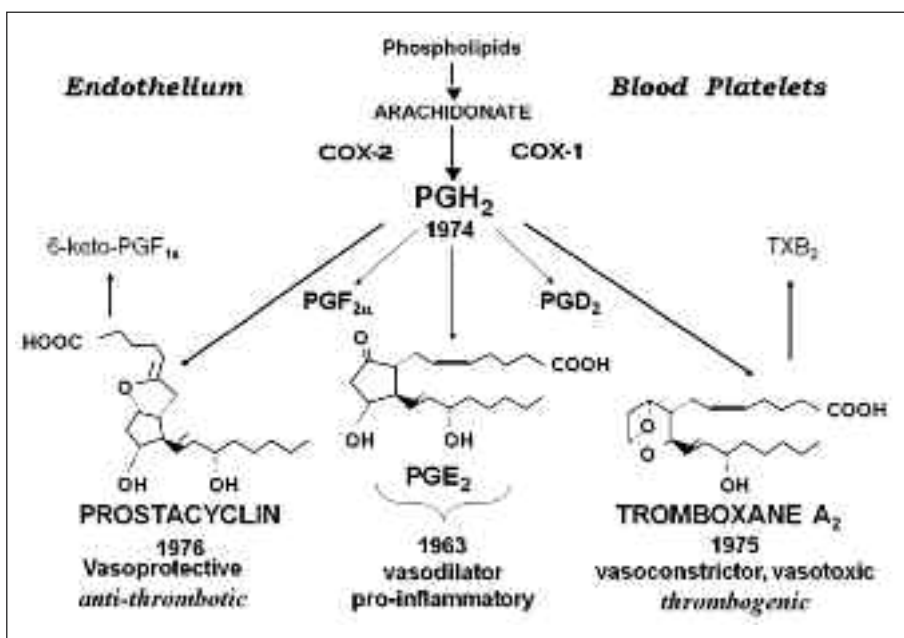


Figure 1. Arachidonate transformation via the cyclooxygenase (COX-1, COX-2) pathways.  $\text{PGH}_2$  – prostaglandin endoperoxide  $\text{H}_2$ ;  $\text{PGD}_2$ ,  $\text{PGE}_2$ ,  $\text{PGF}_2$  – prostaglandins  $\text{D}_2$ ,  $\text{E}_2$  and  $\text{F}_2$ .

### The early days of prostaglandins [7]

Ulf Svante von Euler was the first who in 1935 used the name *prostaglandin* for a lipid factor that he extracted from *glandula prostatica*; that factor contracted smooth muscles of various organs. In 1960 Sune Bergström and his coworkers isolated prostaglandins from the biological material and determined their chemical structures (cyclic lipids). The abbreviation PGs was introduced; the first established were:  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$ . Later other prostaglandins were dis-

covered. In 1964 David Van Dorp of Unilever in Holland and Sune Bergström of Karolinska Institutet in Sweden, with their coworkers, discovered that PGs were biosynthesized from polyunsaturated fatty acids. For the physiologically important PGs of the 2 series – a specific substrate is arachidonic acid (AA).

### The discovery of prostacyclin

Prostacyclin was discovered in 1976 by Richard Gryglewski in collaboration with Salvador Moncada and a student, Stuart Bunting, in John Vane's laboratory. In the early 1970s Priscilla Piper, John Vane and Richard Gryglewski noticed that challenged, sensitized lungs release an activity which they called 'rabbit aorta contracting substance' [7]. Two years later Bengt Samuelson identified this activity as composed of prostaglandin endoperoxides (PGG<sub>2</sub>, PGH<sub>2</sub>) and thromboxane A<sub>2</sub> (TXA<sub>2</sub>) [8]. He isolated these compounds and sent a sample of PGH<sub>2</sub> to John Vane. So when Richard Gryglewski, a young Polish pharmacologist, came for his third sabbatical to Vane's laboratory, John gave him these endoperoxides and asked him to look for their conversion to PGs or TXA<sub>2</sub> by ground-up cells (homogenates or microsomes) of various organs. He used the Vane Bioassay Cascade, equipped for the detector of PGs (mainly a rat stomach strip) and for TXA<sub>2</sub> (a special assembly of rabbit aorta). John McGiff of the Valhalla New York Medical College depicted the Vane Bioassay Cascade (Fig. 2) as 'the triumph of intellect over the technology'. So, with varying results, Gryglewski tested homogenates from different animal organs. The microsomes from the most studied organs converted PG endoperoxides to prostaglandins, exclusively. Of course, blood platelets converted PGG<sub>2</sub> and PGH<sub>2</sub> to TXA<sub>2</sub>. When it came to the pig aortic microsomes – they behaved differently – since neither PGG<sub>2</sub> nor TXA<sub>2</sub> were produced and, even worse, the cascade detected no biological activity at all. At this point Gryglewski and his colleagues started to play around with their biological detectors within the Vane Bioassay Cascade (Fig. 3). They introduced alterations, incorporating rabbit celiac and mesenteric arteries as well as rat colon. Then they detected a unique set of contractions and relaxations (the unique set of fingerprints, as they called it) in response to a mixture of aortic microsomes incubated with PGG<sub>2</sub> and PGH<sub>2</sub>. The responses were, however, variant and even elusive, so the jokes of 'an invisible Polish hormone' (PGX) appeared in the laboratory where we worked. Gryglewski had a brilliant thought. Maybe something so volatile was produced that it disappeared at room temperature? So he set up a trap for that 'something' by repeating the experiment on ice. This time the detector system showed – in a reproducible way – a compound

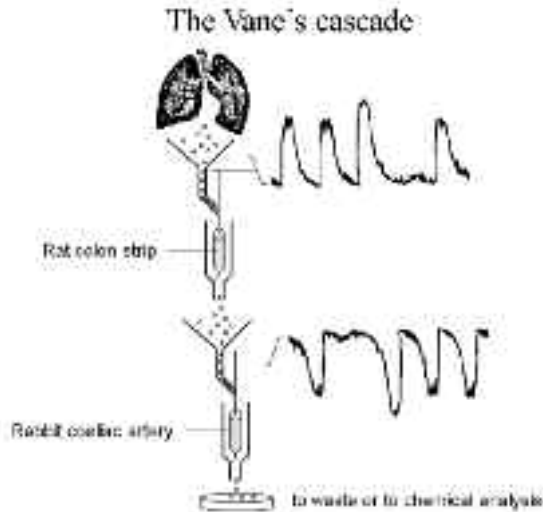


Figure 2. The Vane Bioassay cascade. The effluent from challenged lungs superfuses strips of various experimental organs. The registration system records contraction of rat colon and relaxation of rabbit coeliac artery.

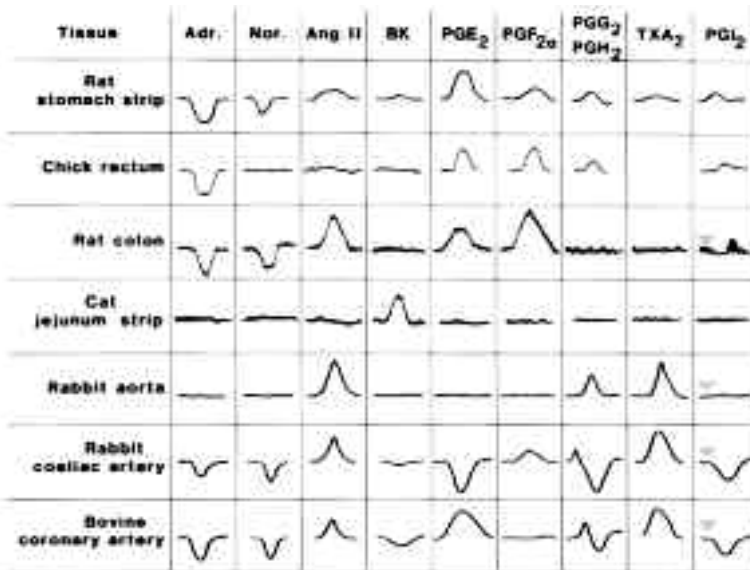


Figure 3. A slide presented by Sir John Vane during his Nobel Lecture in Stockholm 1982 shows set of 'fingerprints' for various biologically active compounds as they are registered in the bioassay cascade. (Adr = Adrenaline, Nor = noradrenaline, Ang II = angiotensin, BK = bradykinin).

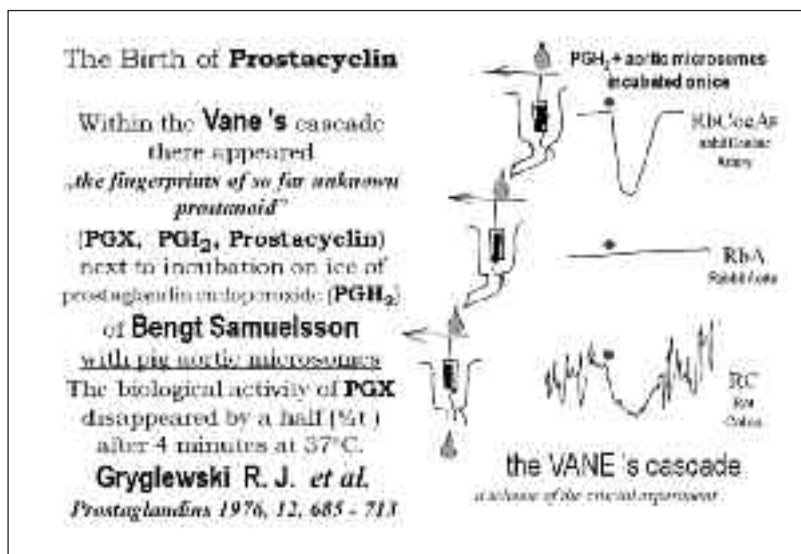


Figure 4. The crucial experiment showing generation of prostacyclin.

that was unknown (Fig. 4). It was prostacyclin (PGI<sub>2</sub>). In a series of quick, ingenious experiments Gryglewski and his colleagues provided proof of the existence of prostacyclin [9]. Early studies [10,11] demonstrated that low density lipoproteins (LDL) inhibit prostacyclin biosynthesis, while high-density lipoprotein (HDL) exert an opposite, protective effect; results of these studies were later confirmed by several authors. A concept of inhibition of PGI<sub>2</sub> biosynthesis by lipid peroxides as a hypothetical step in development of atherosclerosis was proposed [12] (Fig. 5).

The chemical structure of prostacyclin was determined shortly after its discovery, followed by successful chemical synthesis. Soon it was given to man. Ryszard Gryglewski and the author of this paper were the first men to receive intravenous infusions of prostacyclin in 1977. These exciting experiments on ourselves, full of unexpected adventures, have been described [13]. Having established the safety of the procedure on ourselves and colleagues from our Department, we continued the observations on the action of prostacyclin on the volunteers, both healthy and patients. By the end of 1979 over 70 subjects had received PGI<sub>2</sub> either intravenously or by inhalation. These studies led to the following conclusions on the actions of prostacyclin in man:

1. PGI<sub>2</sub>, administered either i.v. or by inhalation, exerted powerful anti-platelet effects. It prolonged bleeding time, suppressed platelet aggrega-

tion, dispersed circulating platelet aggregates and prevented formation of thrombin. On the contrary, it did not affect such plasma coagulation indices as prothrombin time or partial thromboplastin time [14,15].

2. PGI<sub>2</sub> produced profound circulatory effects [16]. Flushing of the face, spreading down to the neck in the form of a collar, were the first clinical symptoms which appeared in all the subjects after only a few minutes of the infusion at the low dose (2-5ng kg<sup>-1</sup> min<sup>-1</sup>). Erythema of the palms and feet was also observed in the majority of patients receiving PGI<sub>2</sub> by inhalation.

There was a distinct fall in peripheral and total pulmonary vascular resistances. This was accompanied by a drop in intra-arterial blood pressure, and the acceleration of heart rate. Stroke volume, cardiac output, mean right arterial pressure, and left ventricular end diastolic pressure showed no significant change. Prostacyclin appeared to act predominantly on resistance vessels (Fig. 6).

3. Prostacyclin simulated fibrinolysis without systemic degradation of fibrinogen [17].
4. PGI<sub>2</sub> and its stable analogs affected glucose metabolism, leading to a moderate hyperglycaemia upon i.v. infusion [18] and modulation of insulin secretion in isolated pancreatic islets [19].
5. Lung function studies revealed no changes following i.v. or inhaled administration of PGI<sub>2</sub> to healthy subjects and patients with asthma [14,15].

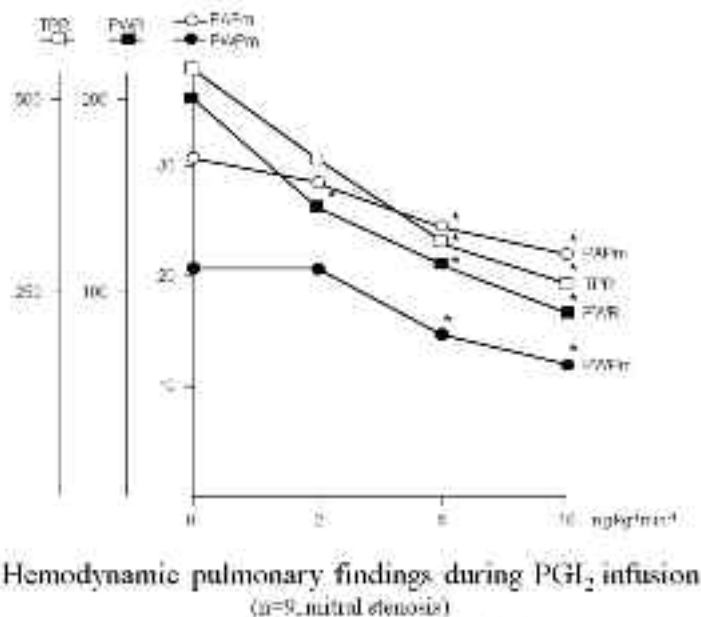


Figure 5. John Vane and Richard Gryglewski (at right) in 1976.

These insights into action of PGI<sub>2</sub> led to the following early clinical applications:

1. Pulmonary hypertension. In 1980, based on our pilot studies in primary and secondary pulmonary hypertension [16,20], we proposed that prostacyclin, administered for an extended period of time either i.v. or by inhalation, may be a new useful therapy in these conditions.
2. Advanced peripheral artery disease, affecting middle- and low-caliber arteries (e.g. peripheral vasculopathies) [21,22].
3. Prinzmetal angina pectoris [23,24].

Clinical use of prostacyclin opened new revolutionary therapeutic possibilities. In pulmonary arterial hypertension it is now the treatment of choice. The synthetic stable analogues of prostacyclin, such as iloprost, treprostinil, epoprostenol, beraprost or cicaprost altered the approach to pulmonary arterial hypertension, especially when combined with sildenafil (an inhibitor of phosphodiesterase-5) or bosentan (an antagonist of endothelin



**Figure 6.** First demonstration of vasodilatory action of prostacyclin on pulmonary circulation. The graph represents mean values in 9 patients with mitral stenosis and moderate pulmonary artery hypertension, TPR = total pulmonary resistance, PWR = pulmonary wedge pressure, PAPm = mean pulmonary artery pressure, mPWP = mean pulmonary wedge pressure.



Figure 7. Robert Furchgott when visiting Cracow in 1994.

ET-1 receptor) [25]. Prostacyclin and its stable analogues proved to be a valuable therapeutic improvement in critical limb ischemia, where they show efficacy in rest-pain relief and ulcer healing; they also show favorable results regarding major amputations [26]. Finally, there are some well known drugs, which apart from their principal mechanism of action, perform also as ‘pleiotropic’ releasers of prostacyclin from the endothelium. The best known are lipophilic angiotensin converting enzyme inhibition (ACE-1, e.g. quinapril, perindopril, ramipril) and statins (e.g. atorvastatin). The long list of prostacyclin releasers include also some  $\beta$ -adrenoreceptor blocking agents (nebivolol, carvediolol), antiplatelet thienopyridines (ticlopidine, clopidogrel) and anti-diabetic drugs (e.g. glipizide, metformin) [27].

### Discovery of nitric oxide

In the late 1970s and early 1980s, at the time when prostacyclin was an absolute hit, a scientist started to appear at medical meetings, claiming that the endothelium produces another biologically active compound, different from prostacyclin, which also dilates arteries. He called it Endothelium-Derived Relaxing Factor (EDRF) and not many of us, fascinated by prostacyclin, believed his story. But he was right. His name was Robert Furchgott (Fig. 7) and, in contrast to many self-promoting hyper-ambitious scientists, he was self-effacing with an ever mild manner, and generous to a fault. His



daughter called him ‘a real Southern gentleman’ (he was born and raised in Charleston, S.C.). He was professor of pharmacology in New York and spent most of his time studying in vitro the effects of acetylcholine (an important neurotransmitter) on strips of blood vessels of experimental animals. Acetylcholine was a well-known vasodilator in intact organisms. Furchgott was an expert on the arterial strips responses to acetylcholine. He noticed, for instance that one of his preparations, which sat beneath a sunlit window, dilated much more than preparations in a darker part of the laboratory (in retrospect, it seems that photorelaxation reflected the release of NO by blood vessels in response to light).

Furchgott showed, quite unexpectedly, that relaxation of blood vessels to certain substances depended on whether the endothelium was present or not. He made his clinical discovery in 1978 [28], when a technician failed to follow a standard protocol for preparing the rabbit aorta strips, and instead of contraction to acetylcholine, Furchgott saw relaxation. He was eager to troubleshoot this ‘accident’ and after several weeks realized that gentle rubbing of blood vessels transformed relaxation into contraction. One explanation was that acetylcholine acts on receptors on endothelial cells (removed by rubbing) to trigger the release of a substance with a relaxing activity – EDRF. Furchgott received direct evidence of this by making a ‘sandwich’ of a ring of aorta freed of endothelial cells to which he applied an endothelium of another aortic strip; the procedure transformed constriction into relaxation [28,29]. In the following years EDRF was shown to be nitric oxide (NO<sup>0</sup>) [30] and in 1998 R. Furchgott, together with Ferrid Murrad and Louis Ignarro, received the Nobel Prize ‘for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system’ (Fig. 8).

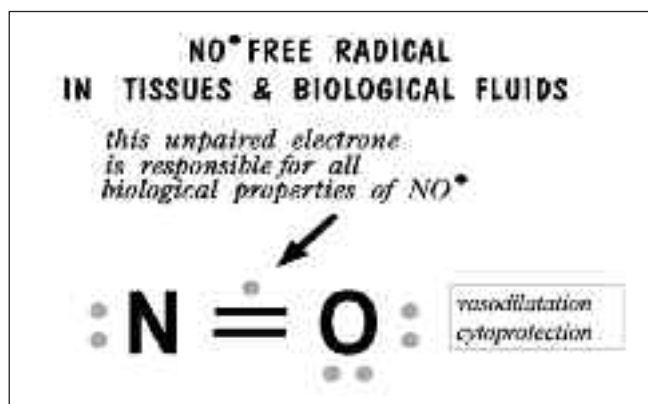


Figure 8. Nitric oxide.

Alfred Nobel was a chemist who, in 1866, discovered an explosive, dynamite, composed of nitroglycerin and a stabilizing absorbent. This discovery brought him fortune, which he used to create the famous award. In his last years of life, his physician prescribed Nobel nitroglycerin for angina pectoris. Nobel then wrote to a friend: 'It sounds like the irony of fate that I should be ordered by my doctor to take nitroglycerin *internally*'. Over a hundred years later Robert Furchgott, Louis Ingarro and Ferrid Murrad were recognized for showing that nitroglycerin produces long-lasting relaxation of cardiac muscle, because it breaks down, yielding a steady stream of NO. 'Today – said Furchgott, receiving the Nobel Prize in Stockholm – it seems like fate, but not the irony of fate' [31].

### Nitric oxide and its relationship to prostacyclin

Over the last decades, appreciable knowledge has been acquired on the biological importance of nitric oxide. It is synthesized in the body from the amino acid l-arginine by the action of NO synthase enzymes (NOS). Nitric oxide is a gaseous free radical that serves multiple functions in human physiology (Fig. 9). It causes vasodilatation and inhibits platelet aggregation, when it is secreted from endothelial cells. It exerts antioxidant, antiproliferative and anti-inflammatory properties, thus playing an important role in inhibiting the atherosclerotic process. It modulates many reactions in the immune system. Produced by macrophages it combats bacteria directly and also signals other immune responses. Furthermore, it functions as a neurotransmitter by diffusing into surrounding cells rather than activating receptors. It also plays a role in reproduction, functioning as a vasodilator during penis erection [7].

	PGI <sub>2</sub> PROSTACYCLIN	(NO) NITRIC OXIDE
half-life.....	4 min.....	6 sec.....
substrate.....	AA.....	L-ARG.....
key enzyme.....	COX.....	NOS.....
destruction by.....	LOO* & ONOO.....	O <sub>2</sub> <sup>-</sup>   inducers of endothelial dysfunction
2-nd messenger.....	c-AMP.....	c-GMP.....
main action.....	thromboresistance Cardiovascular protection	vasodilation

Figure 9. Prostacyclin vs. nitric oxide.

In pathological conditions a methylation of arginine to asymmetric dimethylarginine (ADMA) may occur. The latter inhibits eNOS. A toxic peroxynitrate ( $\text{ONOO}^-$ ) is generated in a reaction between  $\text{NO}^\circ$  and superoxide [32-34]. It selectively blocks the enzymatic activity of prostacyclin synthase, promoting development of atherosclerosis. Prevention of these disastrous processes opens a new avenue in cardiology (Fig. 10).

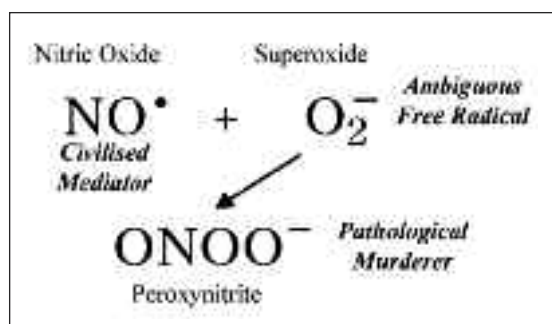


Figure 10. Reaction of nitric oxide with superoxide.

When measuring the value of scientific work, nothing is better than time. Time separates the wheat from the chaff. But we refuse to wait, we simply cannot, because we won't be here any more when the truth is revealed. We want it all here and now. However, there are no recipes for scientific discovery or for success. Max Delbrück, a brilliant physicist who introduced quantitative thought to biology, reckoned that in performing an experiment we should admit a certain degree of freedom, some flexibility, in order to perceive the unexpected, the surprise that is worth more than the expected result. He called this 'the principle of limited sloppiness'. To this principle, so well illustrated by discoveries of prostacyclin and nitric oxide, one may add a sentence: 'Never ignore the unexpected'.

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