

Lessons in Science:

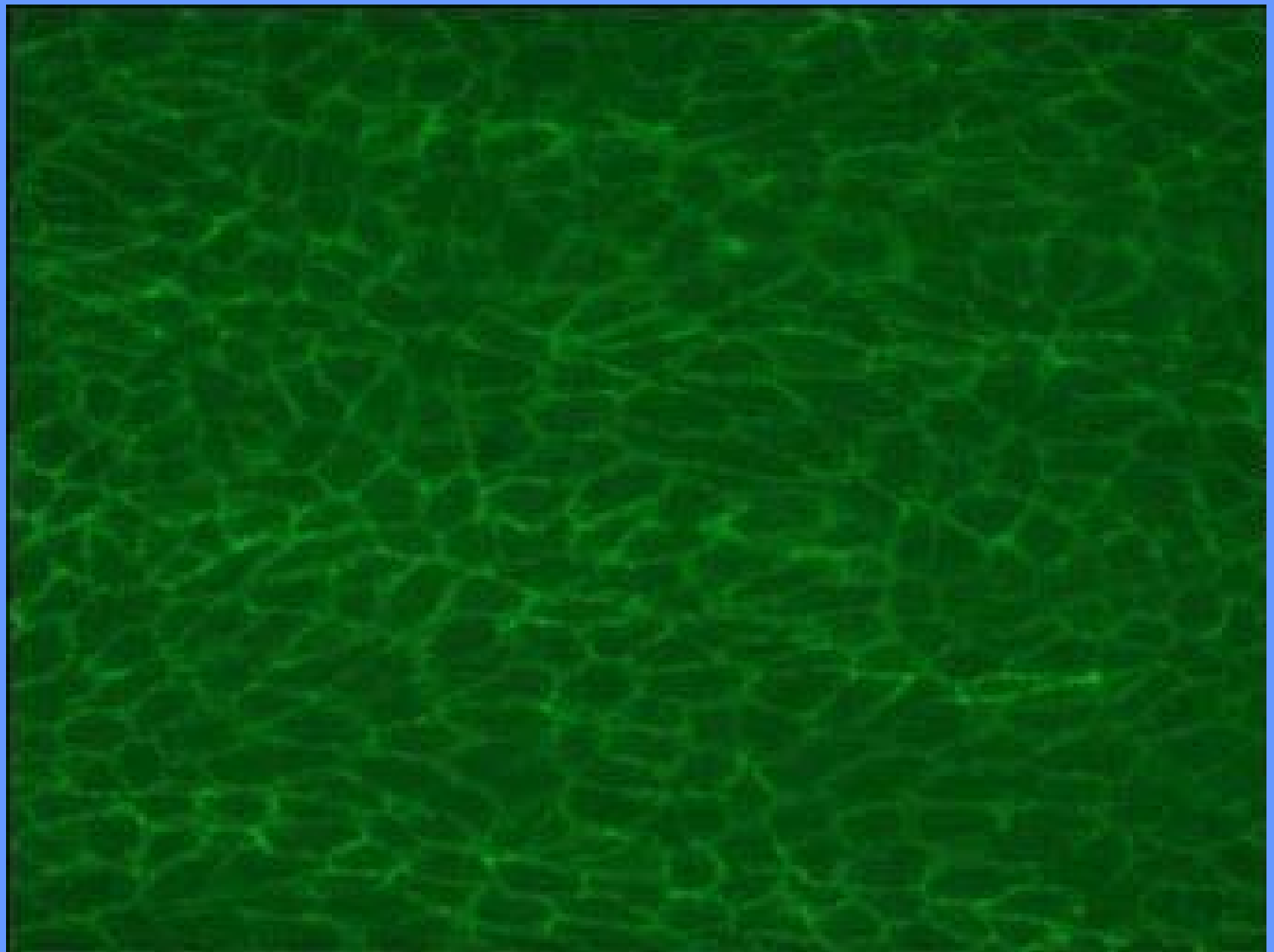


Competition In Scientific Research

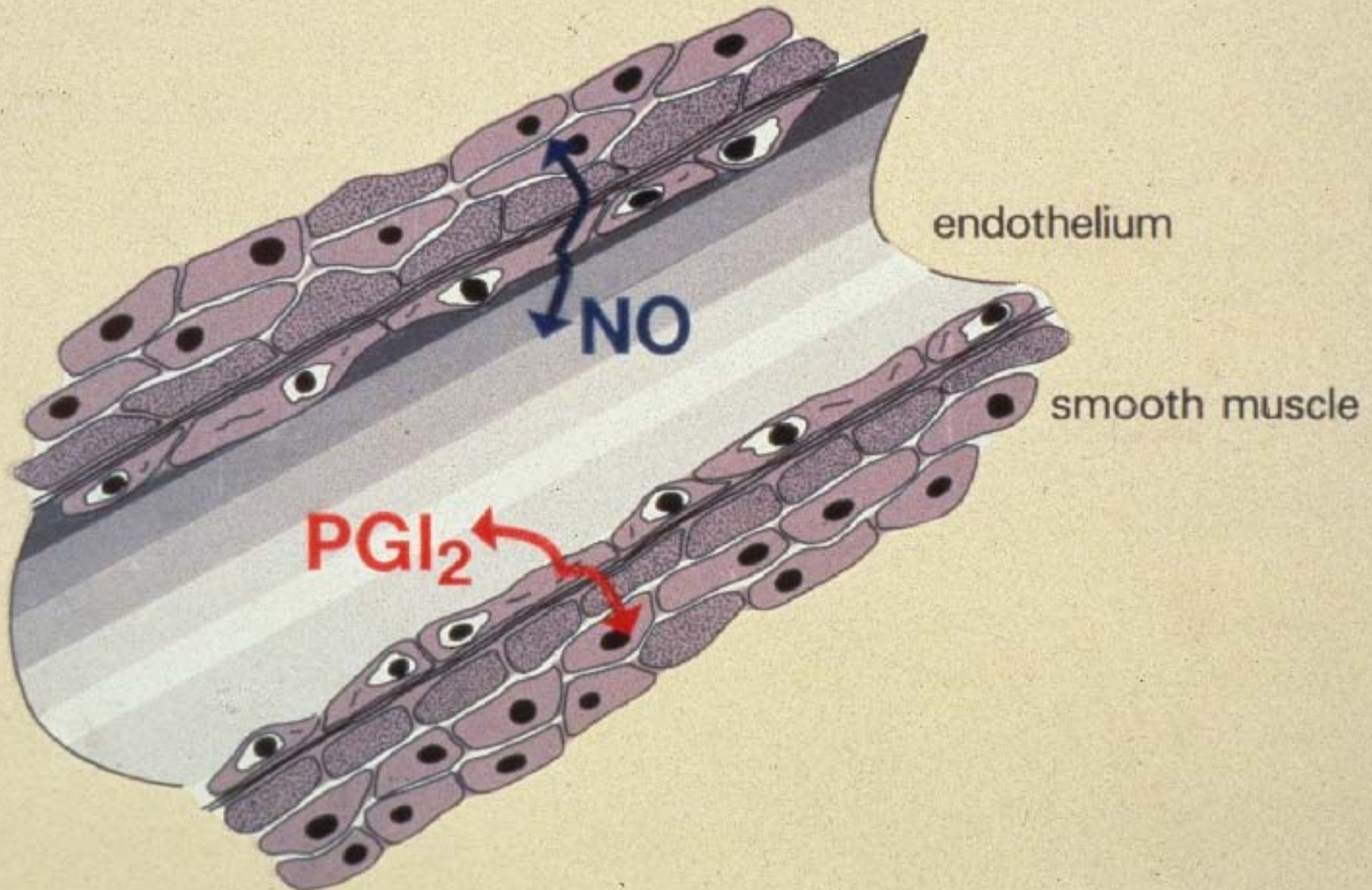
**“Competition.... inclineth
to contention, enmity, and war”**

Thomas Hobbes, Leviathan, 1651





Prostacyclin and NO – fundamental mediators in the vasculature

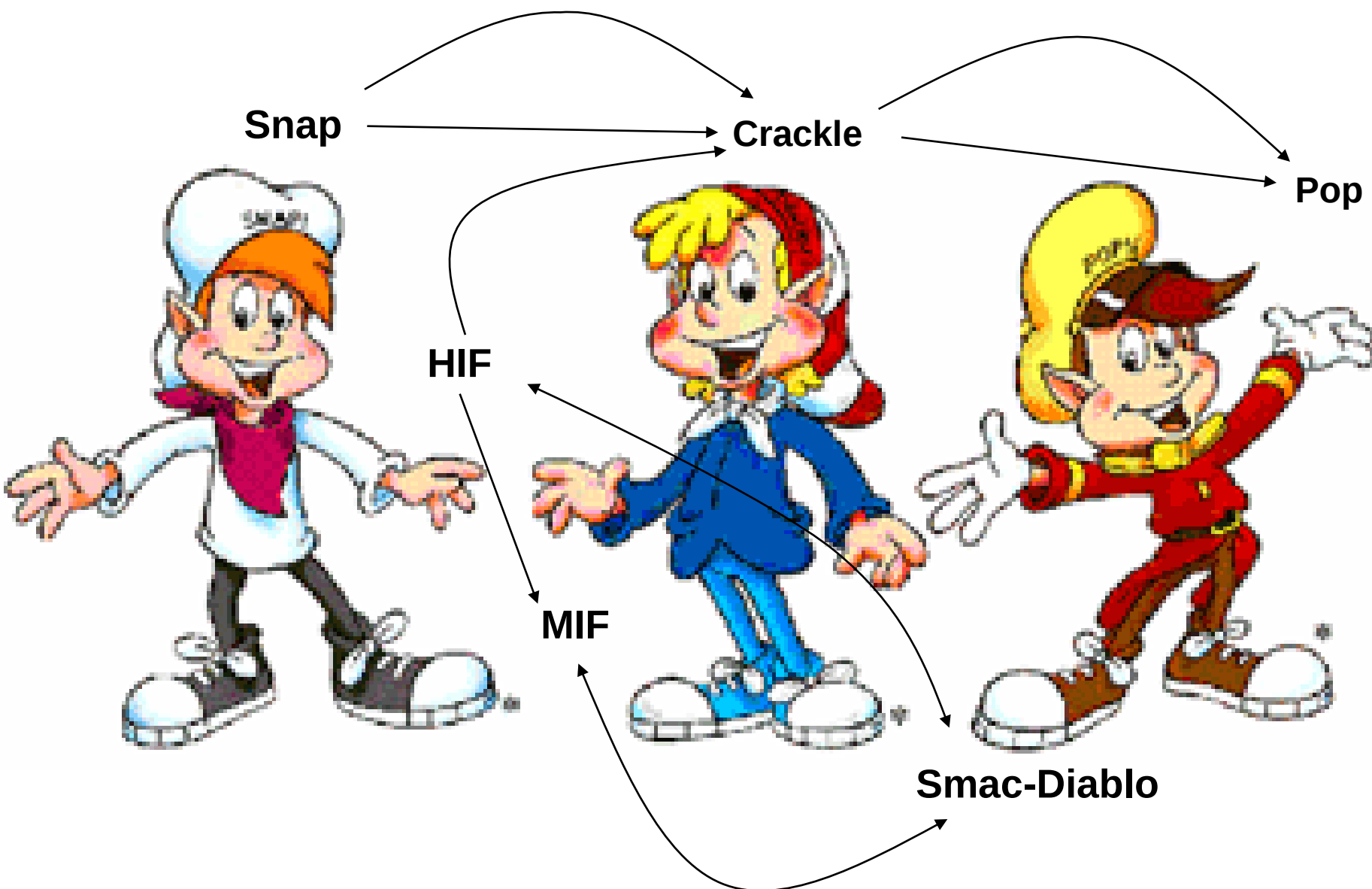




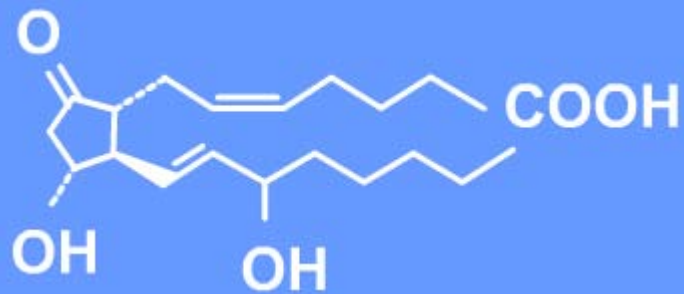
Bioassay profile of different vasoactive substances

TISSUE	PGE ₂	PGF _{2α}	PGG ₂ PGH ₂	TXA ₂	PGX
Rat stomach strip					
Chick rectum					
Rat colon					
Cat jejunum strip					
Rabbit aorta					
Rabbit coeliac artery					

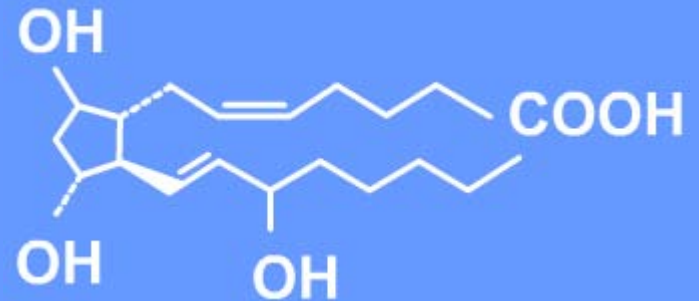
**One great advantage
of bioassay is that it
measures biological
activity**



Arachidonic acid



Prostaglandin $F_{2\alpha}$



Prostaglandin E_2

Metabolic pathway of arachidonic acid, 1971

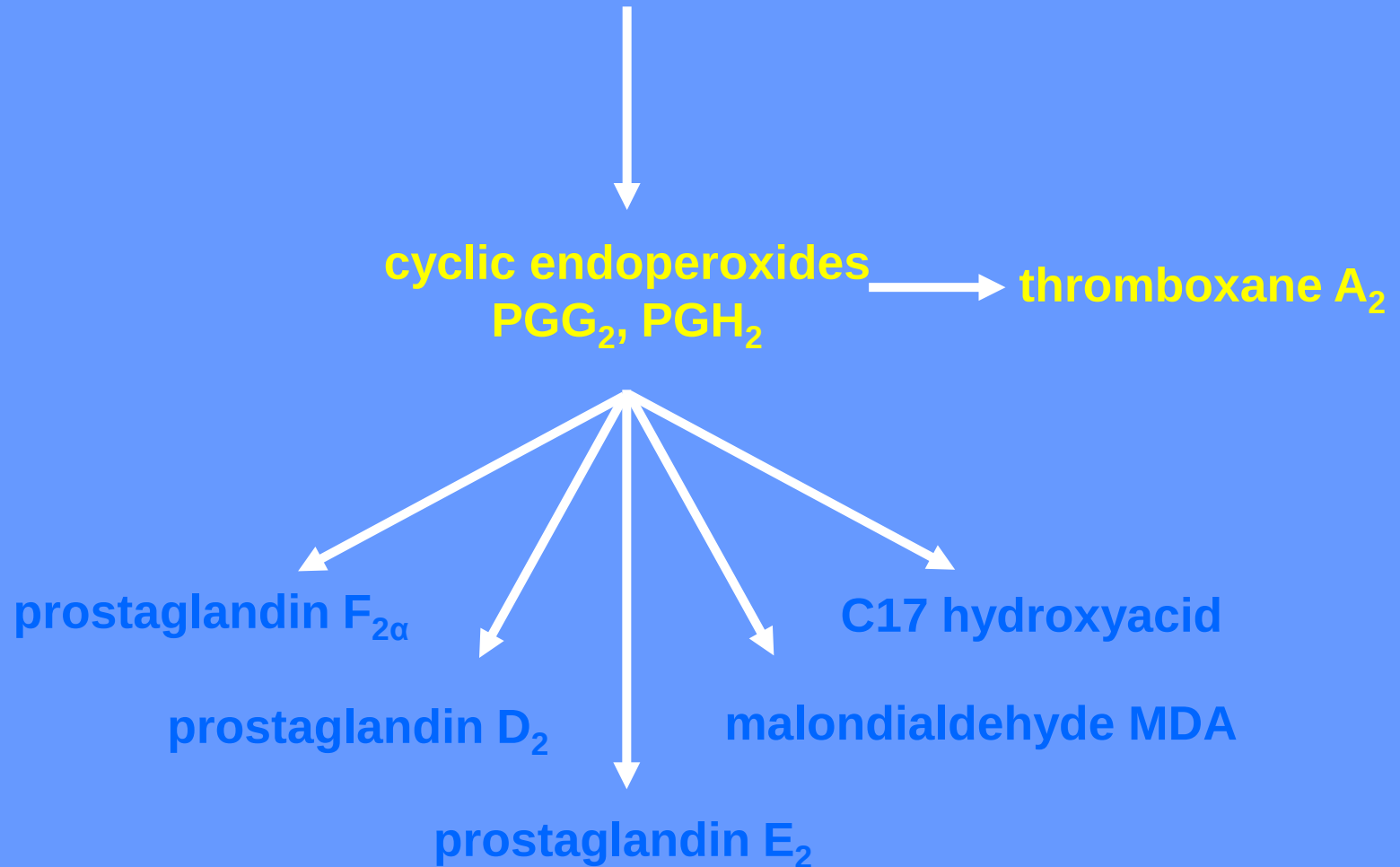
**This explained the mechanism
of action of aspirin-like drugs**

**...and at least one of their side
effects – the gastric damage**

Experiences with aspirin (acetyl salicylic acid) in the nonspecific prophylaxis of coronary thrombosis

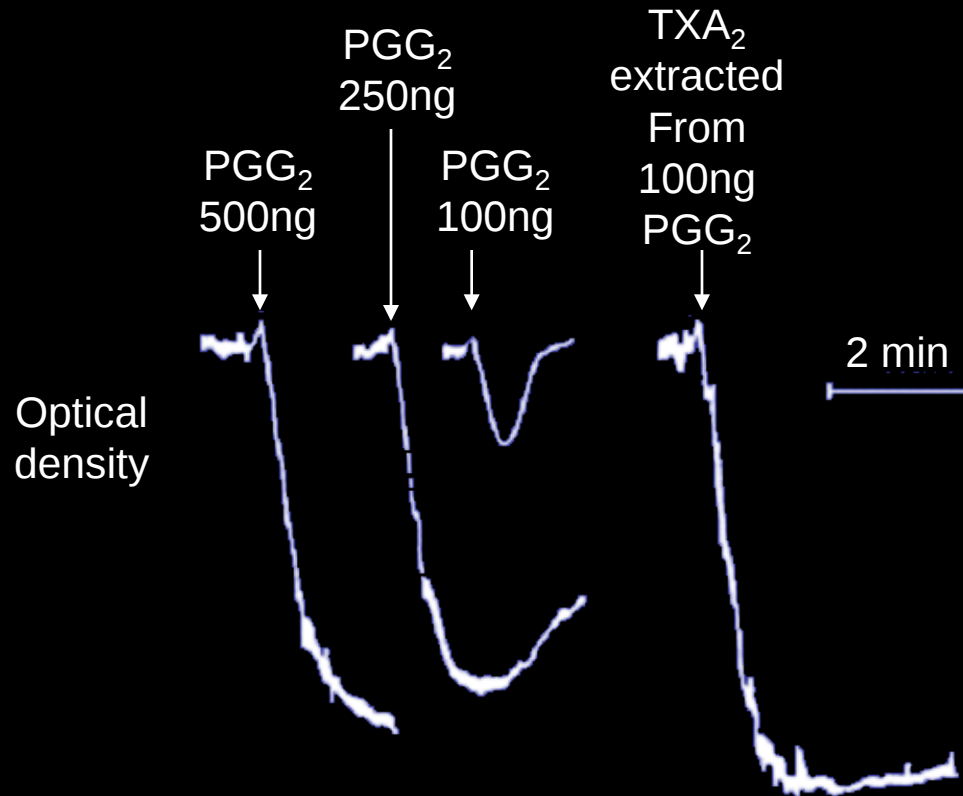
Craven (1953) Mississippi Valley Med. J. 75: 38 - 44

Arachidonic acid



Metabolic pathway of arachidonic acid, 1975

Platelet aggregation induced by PGG₂ and TXA₂



Needleman *et al*, (1976) Nature 261: 558 - 560

Arachidonic acid

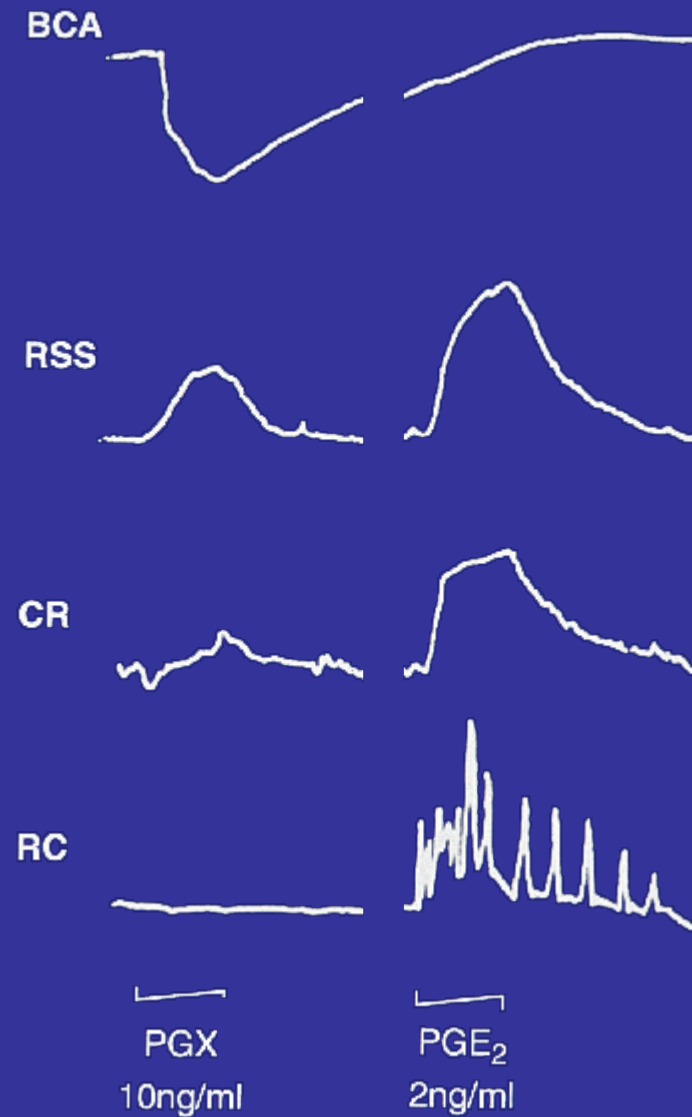


cyclic endoperoxides
PGG₂, PGH₂ → **thromboxane A₂**

Metabolic pathway of arachidonic acid in platelets

**Is the vasoconstrictor
thromboxane A_2 also
made by the vessel wall?**

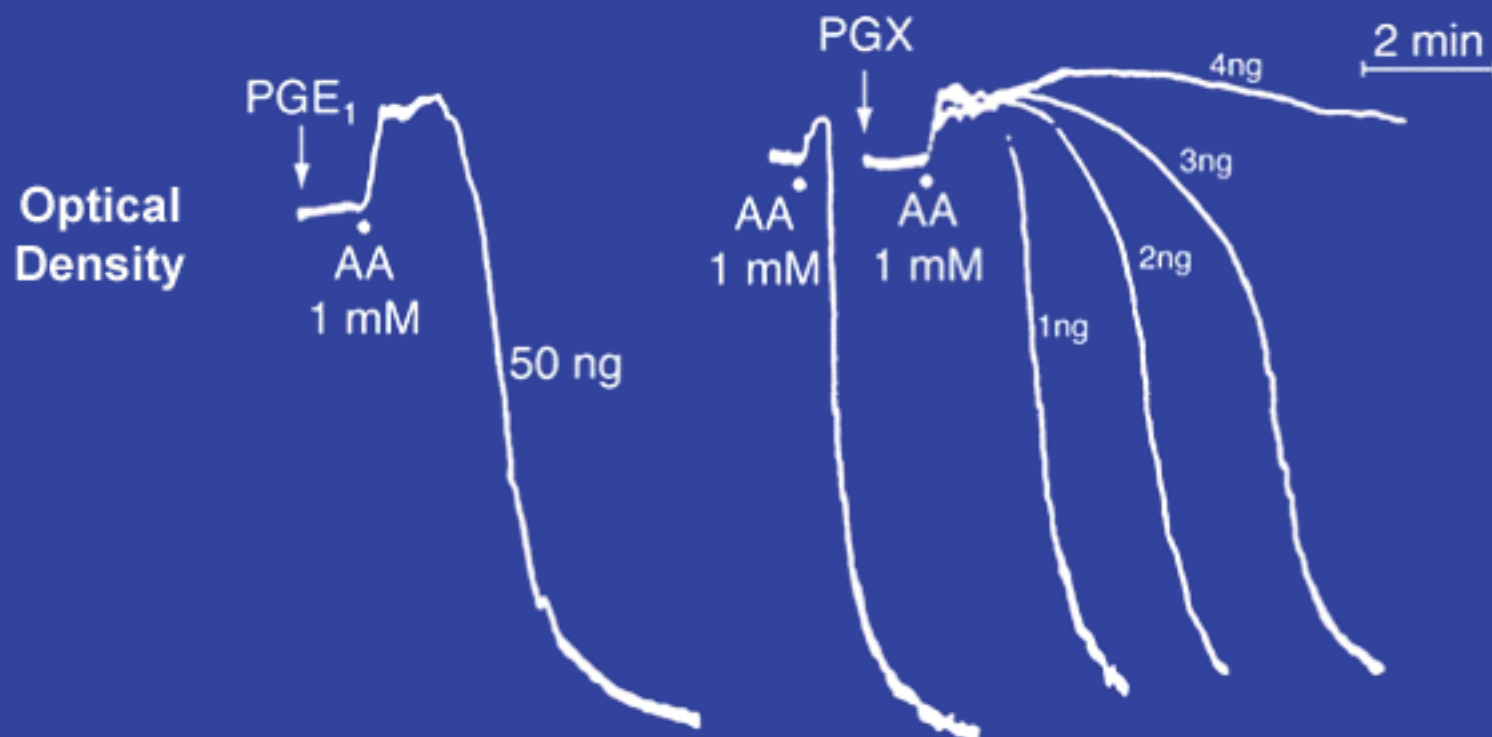
Differential bioassay of PGE₂ and vessel wall extract



The birth of a discovery



Anti-aggregatory activity of PGE₁ and PGX on human platelets



Moncada et al. (1976) Nature 263: 663-665

Arachidonic acid



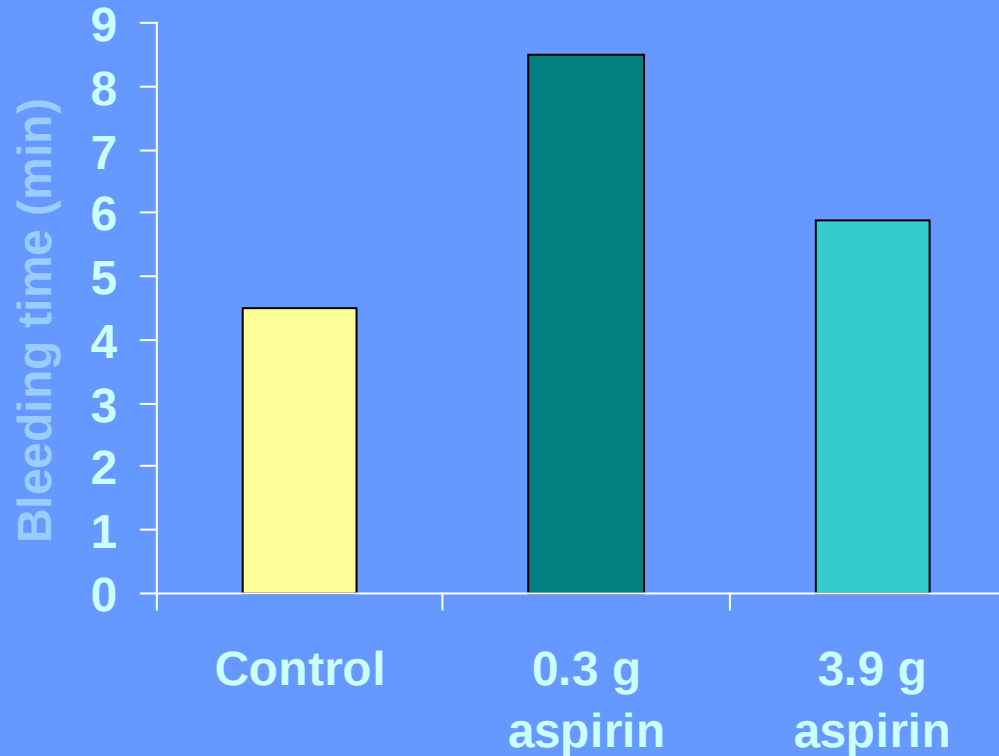
Metabolic pathway of arachidonic acid
in platelets and the vessel wall

Aspirin selectively inhibits platelets

- **platelet cyclooxygenase is very sensitive to aspirin***
- **inhibition of platelet cyclooxygenase lasts for the whole lifetime of the platelet**

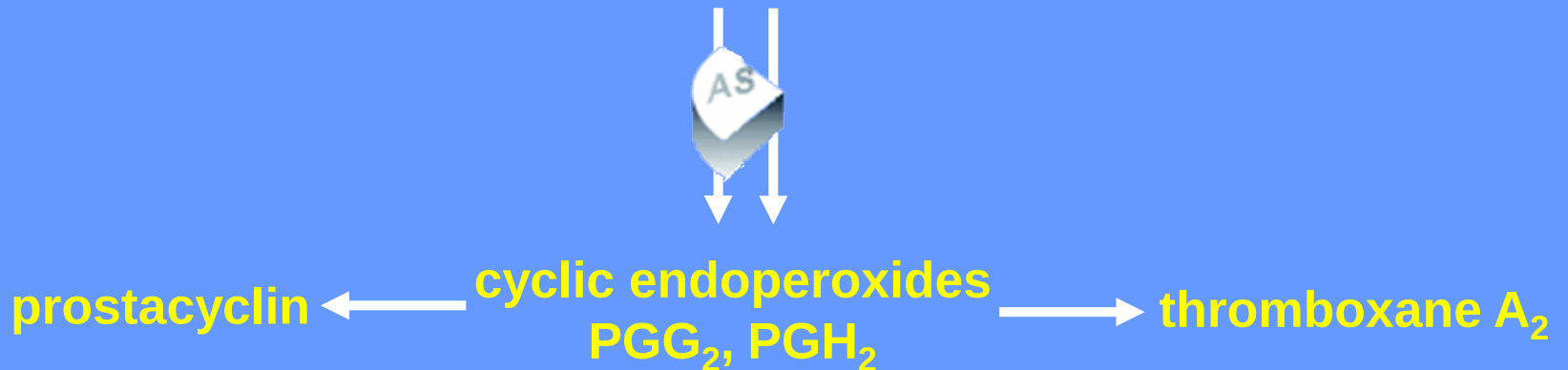
***Burch et al (1978) J. Clin. Invest. 61: 314-319**

Effect of low and high dose aspirin on bleeding time in healthy volunteers



O'Grady and Moncada (1978) Lancet 312:780

Arachidonic acid



Effect of low - dose aspirin on the metabolic pathway of arachidonic acid in platelets and the vessel wall

Clinical trials show that aspirin:

- prevents stroke in patients with atherosclerosis or TIA
- reduces risk of myocardial infarction in unstable angina
- reduces mortality in acute myocardial infarction
- prevents occlusion of vein grafts
- reduces risk of metastasis in cancer patients

**Don't use aspirin
for primary prevention
of cardiovascular disease**

Barnett et al (2010) Brit. Med. J. 340: 920 - 922

**It will be difficult
to beat “old aspirin”**

Cyclooxygenases (1990)

COX-1: physiological processes

COX-2: inflammatory responses

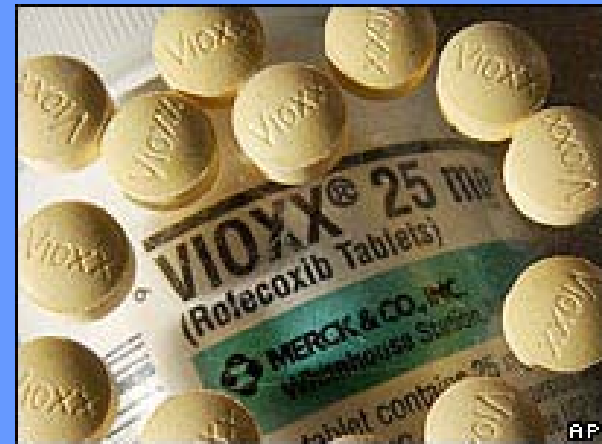
Inhibition of COX-2 results in:

- **inhibition of prostacyclin**
- **cardiovascular side effects**

**McAdam *et al.* (1999) Proc. Natl. Acad. Sci. USA
96: 272 - 277**

Vioxx settlement to total \$4.85bn

The maker of Vioxx has agreed to pay \$4.85bn to settle legal claims that the controversial drug caused many users to suffer strokes and heart failure.



Vioxx was withdrawn from sale in 2004

BBC News Channel, Monday March 12th 2007

Cardiovascular risk of COX inhibitors

Drug	Relative risk vs nonuser
------	--------------------------

Naproxen	0.97
Meloxicam	1.25
Indomethacin	1.30
Ibuprofen	1.07
Diclofenac	1.40
Rofecoxib (>25 mg)	2.19
Rofecoxib (<25mg)	1.33
Celecoxib	1.06

From: White (2007) Hypertension 49: 408-418

Arachidonic acid

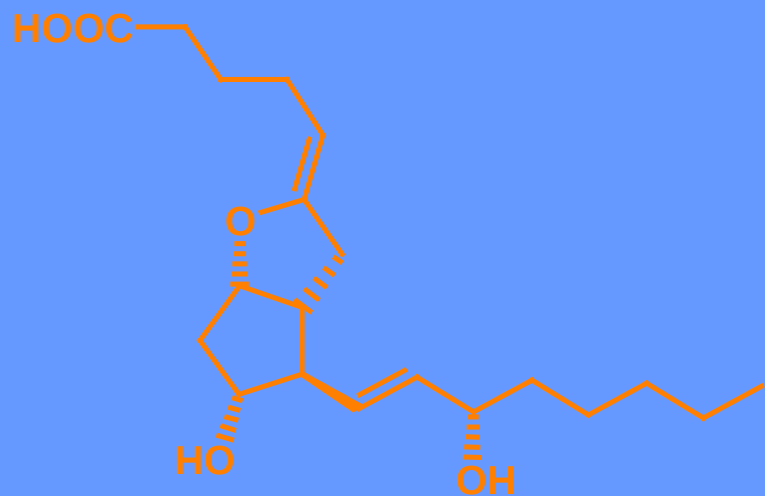


prostacyclin ← cyclic endoperoxides
PGG₂, PGH₂ → thromboxane A₂

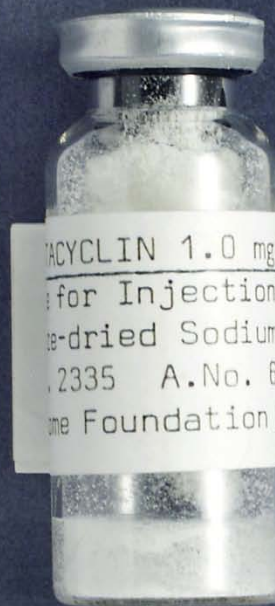
Effect of a COX-2 inhibitor on the metabolic pathway of arachidonic acid in platelets and the vessel wall

COX-2 inhibitors may be beneficial in:

- **cancer – colon, breast, prostate, lung**
- **Alzheimer's disease**
- **Parkinson's disease**
- **schizophrenia**
- **major depression**
- **ischaemic brain injury**
- **diabetic peripheral nephropathy**



Prostacyclin

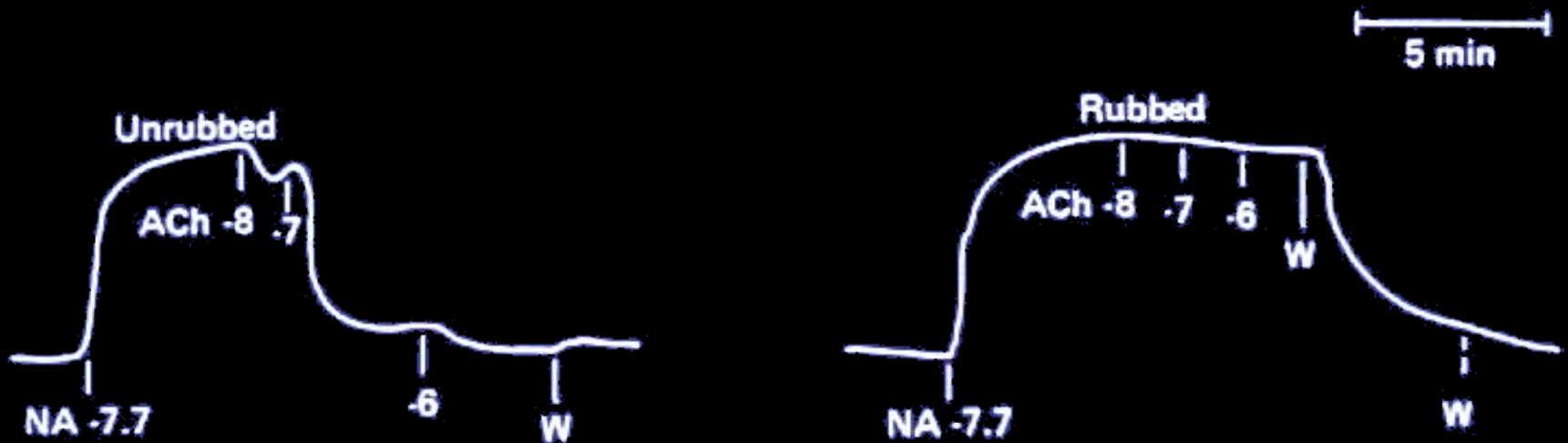


PROSTACYCLIN
First Experimental Batch
1978

Clinical uses of prostacyclin:

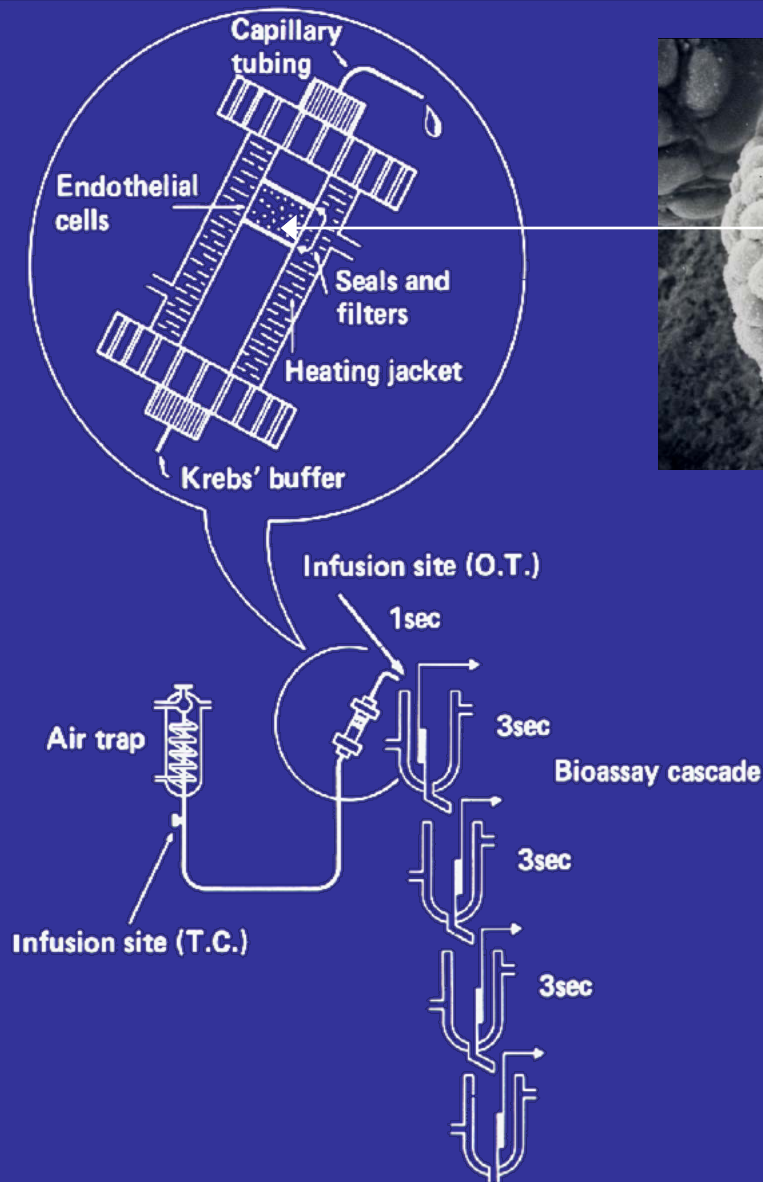
- Primary pulmonary hypertension
- Peripheral arterial disease
- Cardiopulmonary bypass
- Organ transplantation

The obligatory role of endothelium in ACh-induced vascular relaxation



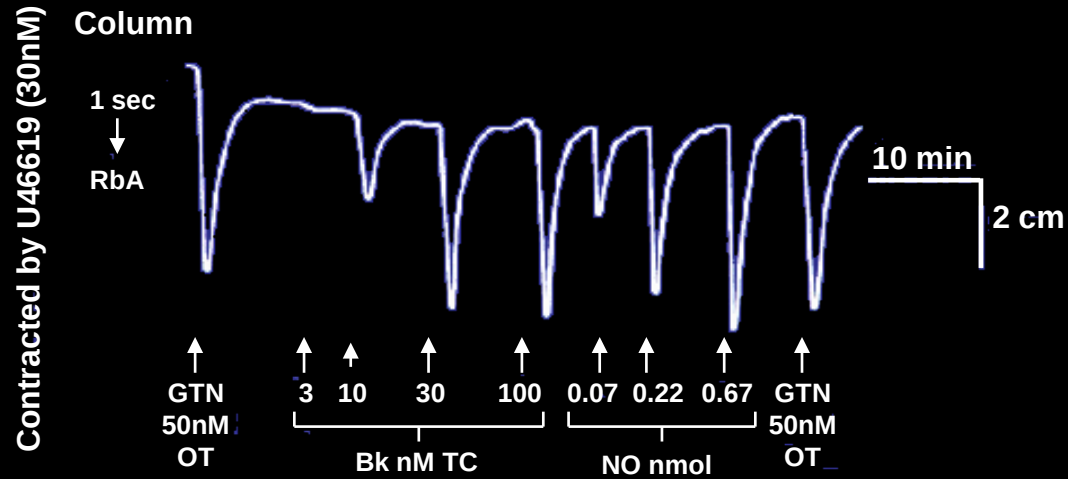
Furchgott and Zawadzki, Nature 288, 373-376, 1980

Bioassay of EDRF released from endothelial cells

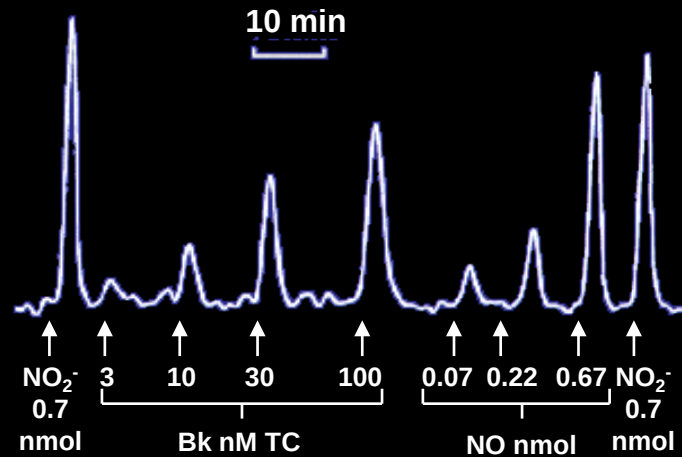


Detection of endogenous and exogenous NO

A Bioassay



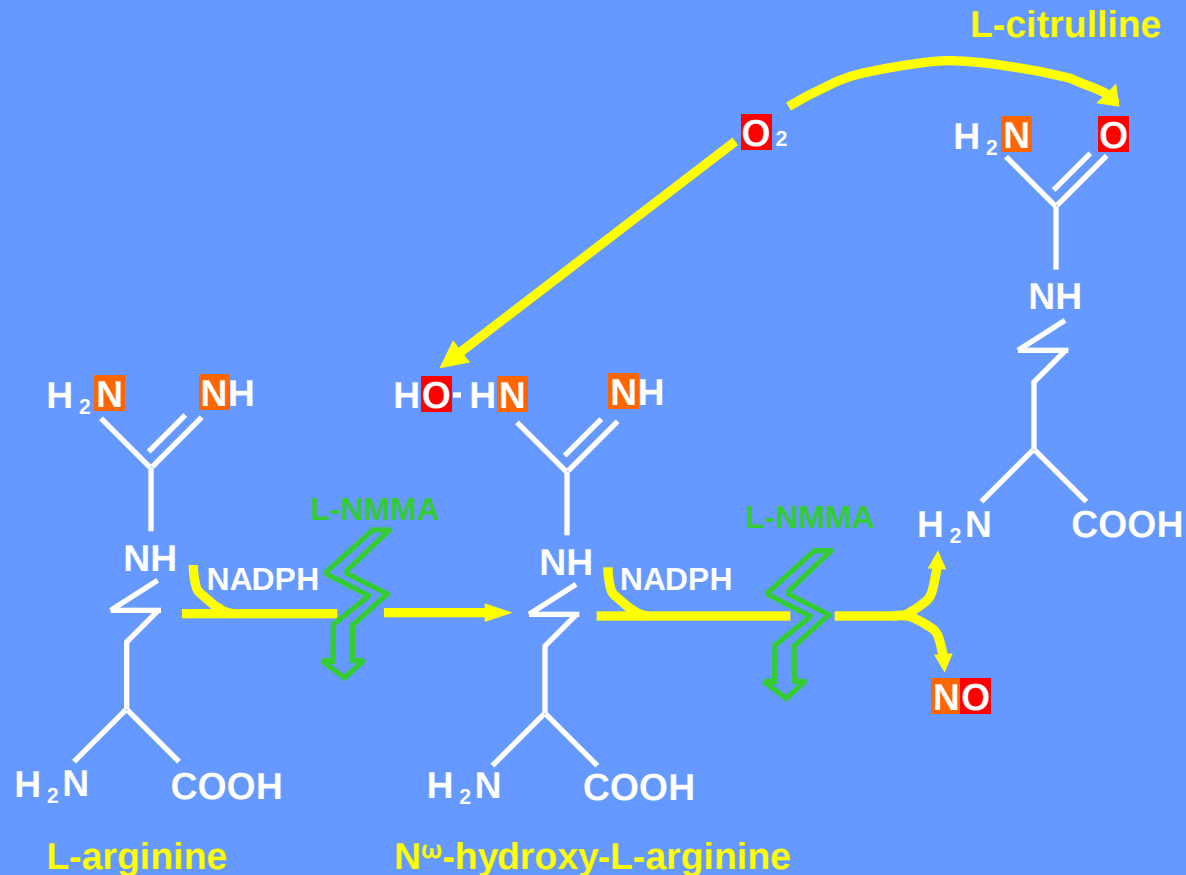
B Chemiluminescence



Palmer *et al* (1987) *Nature* 327: 524 - 526

“You were very persuasive; but unconvincing! I am sceptical for the simple reason that the formation of nitrogen oxides demands some pretty heavy thermodynamic considerations. Nitric oxide is produced in the upper atmosphere through the energetic intervention of lightning!”

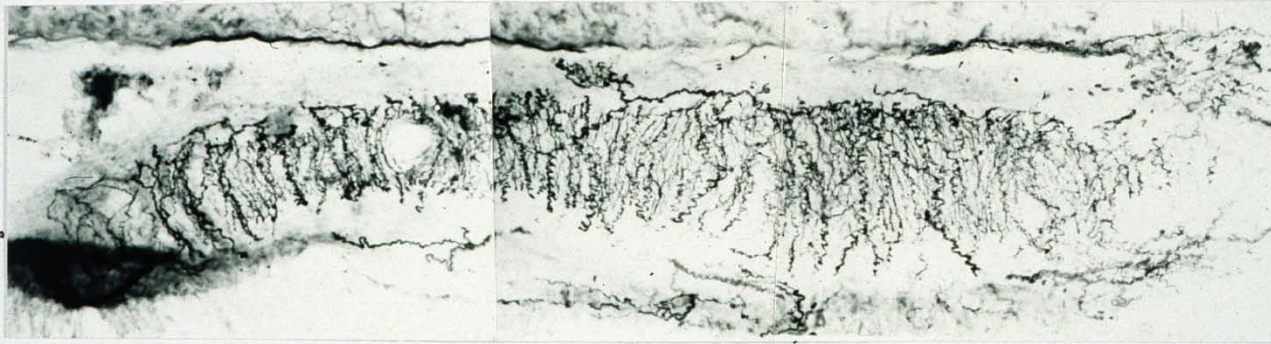
The L-arginine:NO pathway



Biology of the L-arginine: NO pathway



**nNOS around an arteriole
in the human corpus cavernosum**



H
800μm

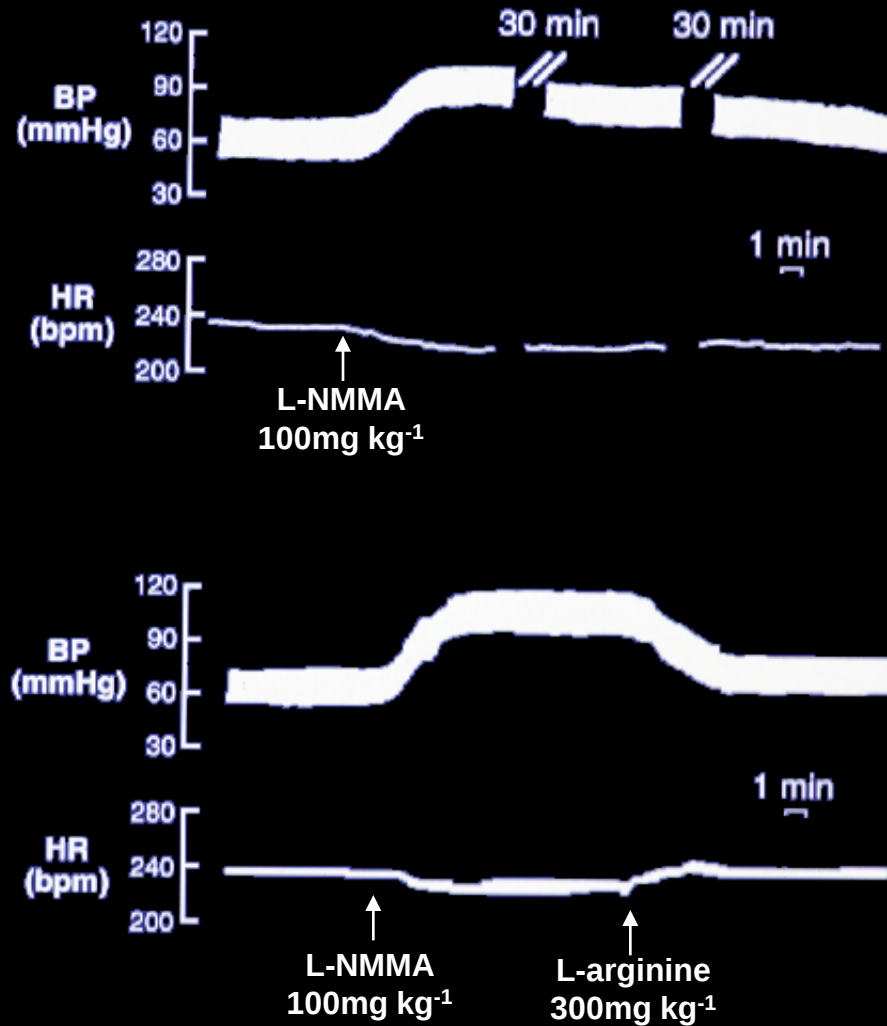
**The action of nitric oxide
In the corpus cavernosum**

NO NO



NO erection

Effect of L-NMMA (100mg kg⁻¹) on blood pressure and heart rate



Rees *et al*, (1989) Proc. Natl. Acad. Sci. USA 86: 3375 - 3378

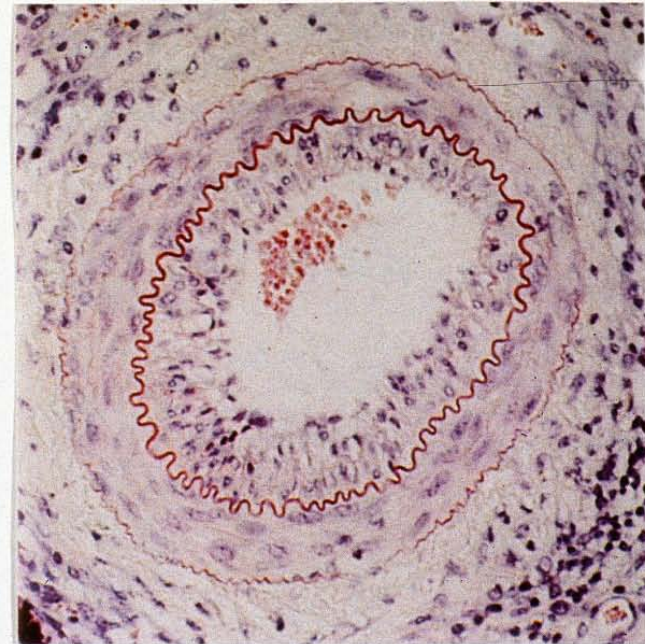
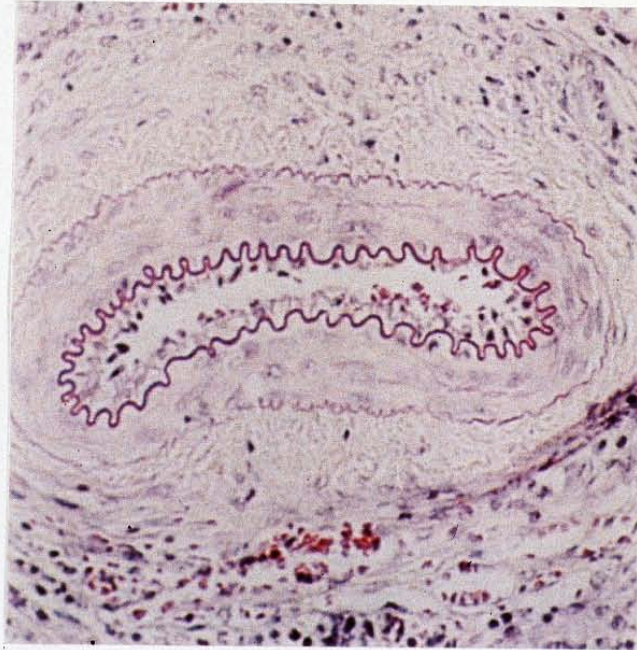
**The cardiovascular
system is in a state
of active
vasodilatation**

Nitric oxide inhibits

- **Platelet aggregation**
- **Smooth muscle cell proliferation**

**Lack of vascular nitric oxide
contributes to
hypertension, vasospasm
and atherosclerosis**

Response of wild-type (L) and eNOS mutant (R) mice to cuff injury



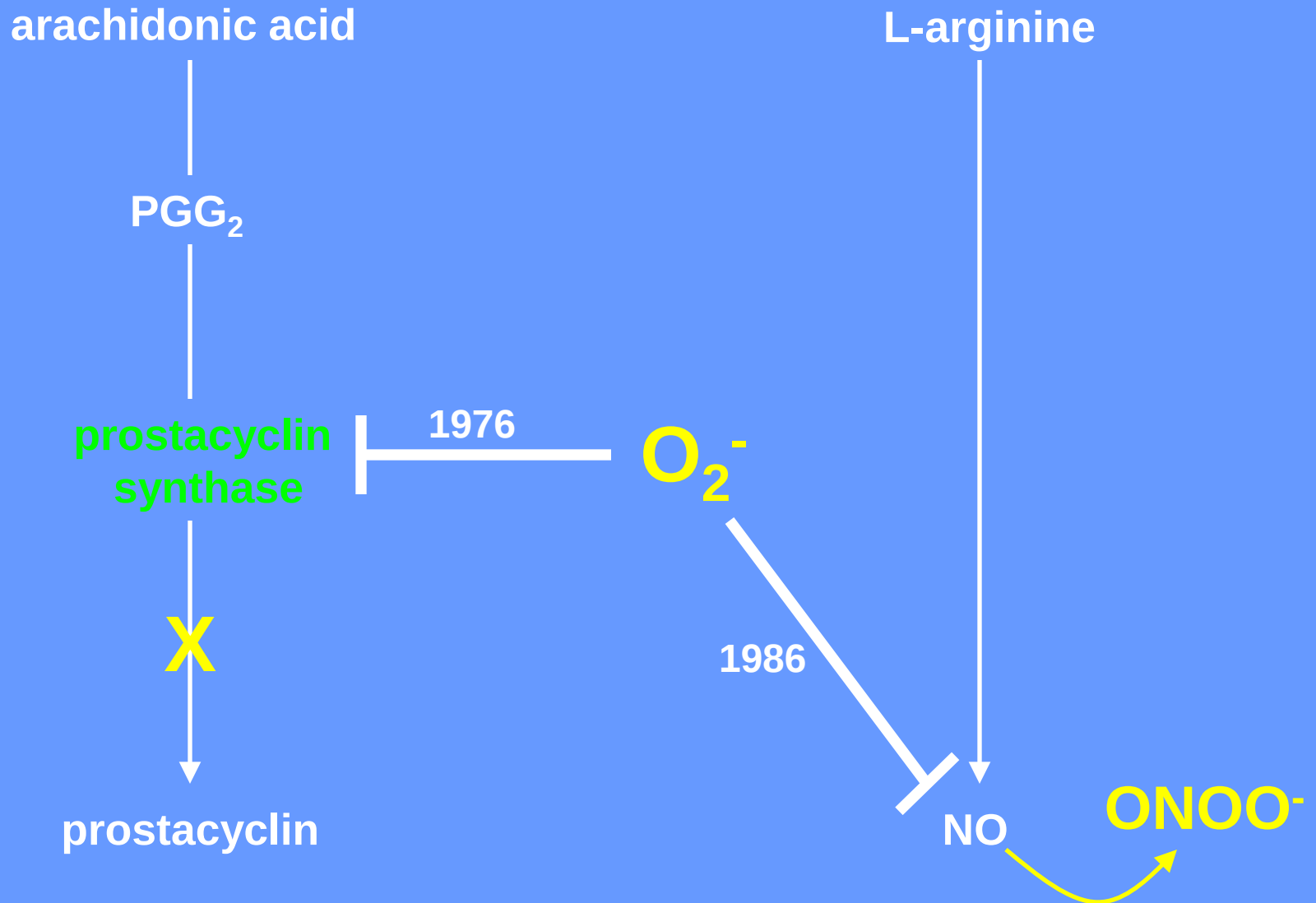
Endothelial dysfunction :
predicts disease in patients with
a family history of essential hypertension
or risk factors for atherosclerosis

Taddei *et al* (1996) Circulation 94: 1298 - 1303

Reddy *et al* (1994) J. Am. Coll. Cardiol. 23: 833 - 843

**Oxidative stress: a most
significant factor in
cardiovascular disease**

Oxidative stress, prostacyclin and NO



Conditions in which ONOO^- has been implicated

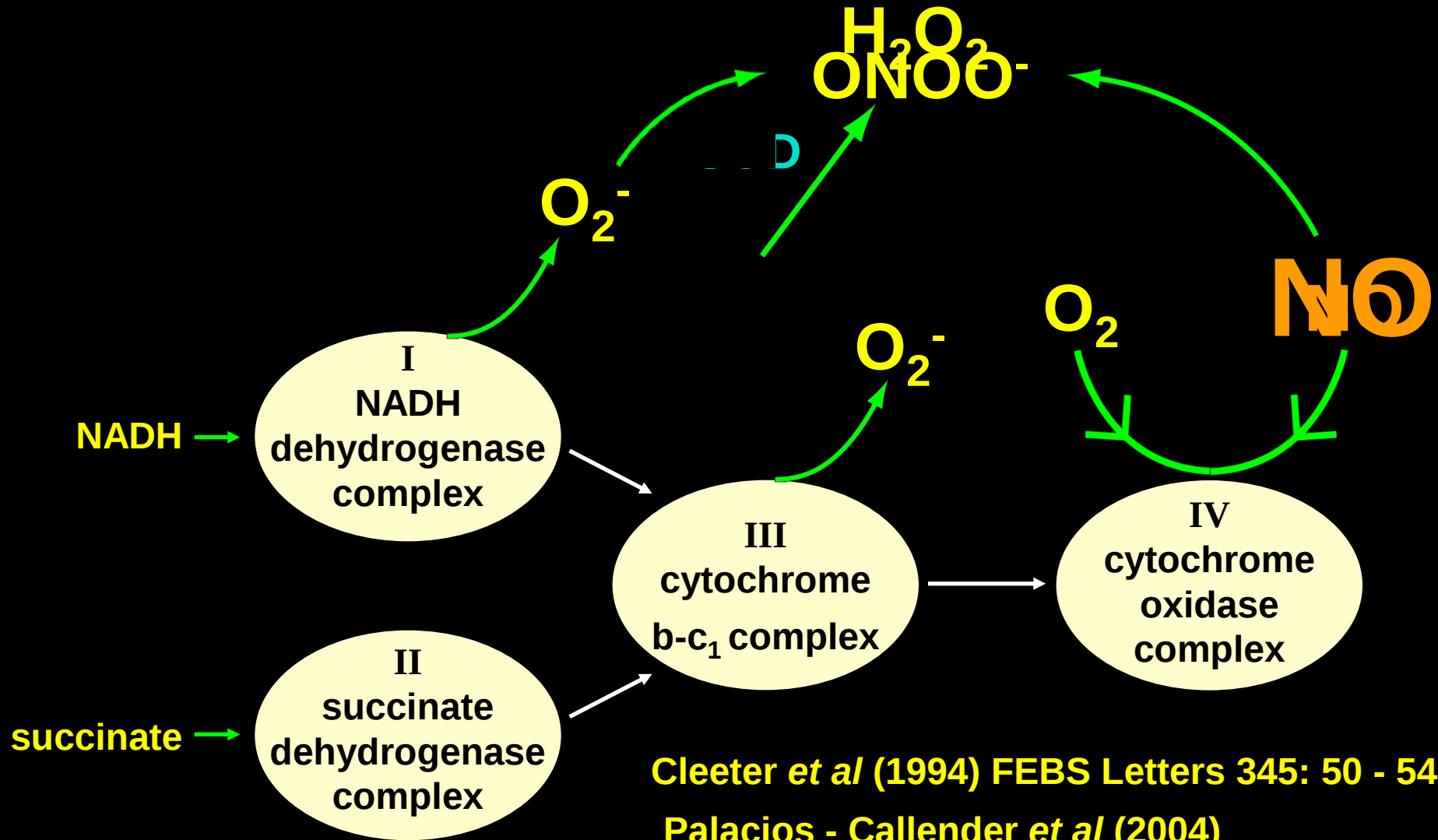
- atherosclerosis
- hyperlipidaemia
- hypertension
- myocarditis
- chronic renal failure
- septic shock
- diabetes
- angiotensin II-mediated vascular disorders
- cigarette smoking

Where do the reactive oxygen species come from?

- NADPH oxidases
- xanthine oxidase
- uncoupled endothelial NO synthase
- **mitochondrial electron transport**

Nitric oxide and the respiratory chain of NO

Formation of peroxynitrite



Cleeter *et al* (1994) FEBS Letters 345: 50 - 54

Palacios - Callender *et al* (2004)

Proc. Natl. Acad. Sci. USA 101: 7630-7635

**Hypotheses are nets:
Only he who casts will
catch (Novalis)**

**The quality of the question
defines the quality of the project**

Questions

- discard most of them ruthlessly
- sharpen the remainder
- rank them

and above all.....

simplify them!



Don't complicate matters !





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