Discovered in 1845

A typical endothelial cell is about 30 μm long, 10 μm wide, and 0.2 – 3 μm thick.

Accounts for 1% or less of the arterial weight.

As recently as the late 1960s it was thought of as merely “a sheet of cellophane.”
Prostacyclin and NO – fundamental mediators in the vasculature
## Bioassay profile of different vasoactive substances

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>PGE₂</th>
<th>PGF₂₀α</th>
<th>PGG₂/PGH₂</th>
<th>TXA₂</th>
<th>PGX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat stomach strip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chick rectum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat colon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat jejunum strip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit coeliac artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
One great advantage of bioassay is that it measures biological activity.
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 (acetylsalicylic acid) in the nonspecific prophylaxis of coronary thrombosis

Arachidonic acid

→ cyclic endoperoxides
  PGG₂, PGH₂

→ prostaglandin F₂α
→ prostaglandin D₂
→ prostaglandin E₂
→ C17 hydroxyacid
→ malondialdehyde MDA
→ thromboxane A₂

Metabolic pathway of arachidonic acid, 1975
Platelet aggregation induced by PGG$_2$ and TXA$_2$

Arachidonic acid

Metabolic pathway of arachidonic acid in platelets

- ASPIRIN
  - cyclic endoperoxides
    - PGG$_2$, PGH$_2$
  - thromboxane A$_2$
Is the vasoconstrictor thromboxane $A_2$ also made by the vessel wall?
Differential bioassay of PGE$_2$ and vessel wall extract

BCA

RSS

CR

RC

PGX 10ng/ml

PGE$_2$ 2ng/ml
Anti-aggregatory activity of PGE$_1$ and PGX on human platelets

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

Effect of low-dose aspirin on the metabolic pathway of arachidonic acid in platelets and the vessel wall
Effect of low and high dose aspirin on bleeding time in healthy volunteers

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

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

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
Effect of a COX-2 inhibitor on the metabolic pathway of arachidonic acid in platelets and the vessel wall.
# Cardiovascular risk of COX inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Relative risk vs nonuser</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naproxen</td>
<td>0.97</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>1.25</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1.30</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1.07</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.40</td>
</tr>
<tr>
<td>Rofecoxib (&gt;25 mg)</td>
<td>2.19</td>
</tr>
<tr>
<td>Rofecoxib (&lt;25mg)</td>
<td>1.33</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>1.06</td>
</tr>
</tbody>
</table>

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

“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 energic intervention of lightning!”
The L-arginine: NO pathway
Biology of the L-arginine: NO pathway

- Cardiovascular system
- Nervous system
- Immunology and inflammation
Publications with "nitric oxide" in the title
The action of nitric oxide in the corpus cavernosum
Effect of L-NMMA (100mg kg⁻¹) on blood pressure and heart rate

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

Oxidative stress: a most significant factor in cardiovascular disease
Oxidative stress, prostacyclin and NO

arachidonic acid → PGG₂ → prostacyclin synthase → prostacyclin

L-arginine → NO → ONOO⁻

1976

1986
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

Nitric oxide (NO)

Formation of ROS in the presence of NO

Formation of peroxynitrite (ONOO-)

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