



**Air pollution and cardiovascular
diseases:**

Potential biological mechanisms

Abderrahim NEMMAR

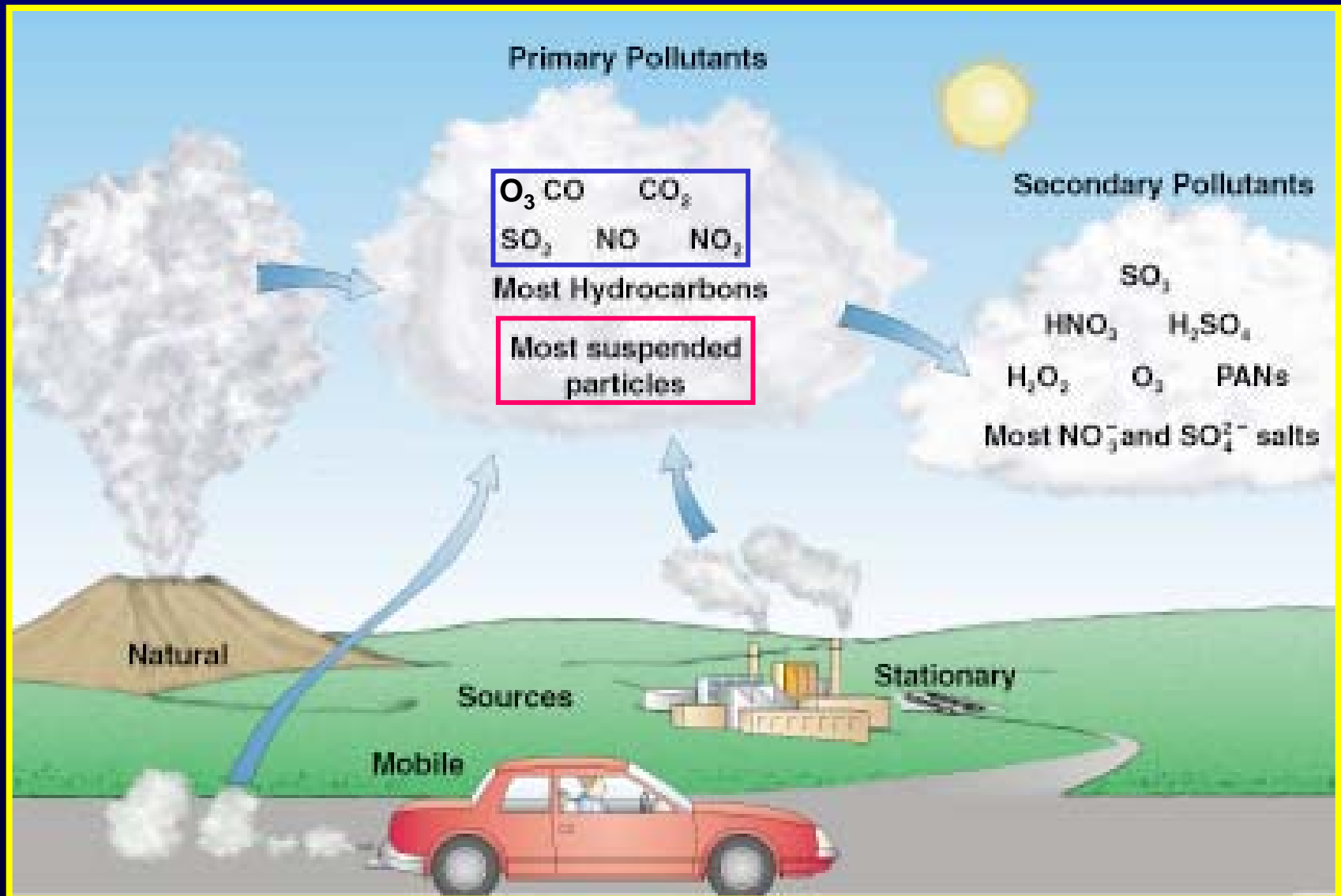
University of Leuven

Laboratory of Pneumology

Unit of Lung Toxicology

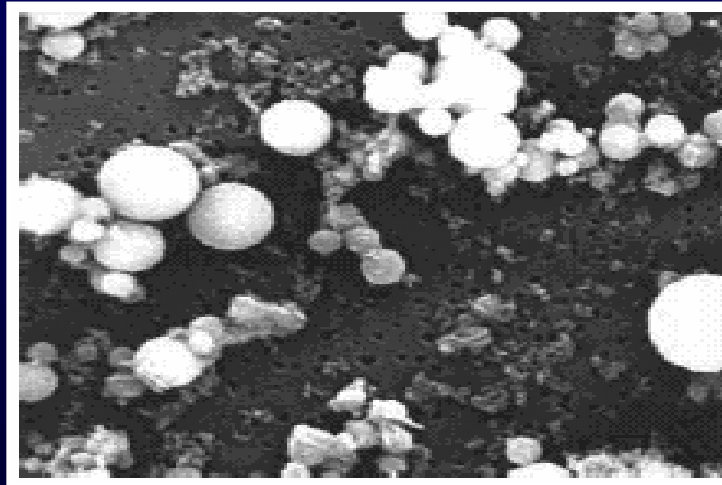
Belgium

Air pollution & health

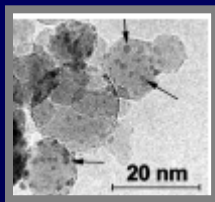
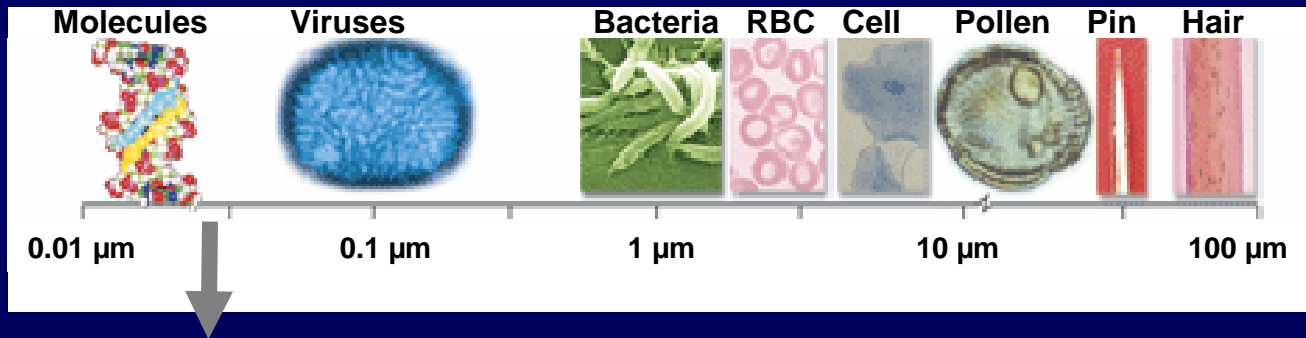


Particles & health

- Particles: **PM₁₀** “particulate matter” $\varnothing < 10\mu\text{m}$
PM_{2.5} “particulate matter” $\varnothing < 2.5\mu\text{m}$



Ultrafine particles (UFPs)



UFPs

Ultrafine particles $\varnothing < 0.1 \mu\text{m}$

- 10^5 per cm^3 in the vicinity of busy roads
- Greater surface area \longrightarrow more inflammation
- Small size \longrightarrow penetrate deeply into the respiratory tract

Cardiovascular effects of particles

Inhaled particles are significant contributor to morbidity and mortality not only with regard to the **respiratory tract**, but also the **cardiovascular system**

Cardiovascular effects of particles

- epidemiology & “clinical” studies:
 - increased particulate pollution associated with:
 - Heart rate ↑, arrhythmias without hypoxia or respiratory distress
 - Plasma viscosity ↑, C reactive protein ↑, fibrinogen ↑, factor VII ↑
 - Risk of myocardial infarction ↑, susceptibility to myocardial ischemia ↑, intima-media thickness ↑

Mechanisms

- experimental toxicology:
 - which constituents of the particles?
 - by what mechanisms?

- “biological plausibility”?



Mechanisms

Short-term increases in particulate pollution & cardiovascular morbidity

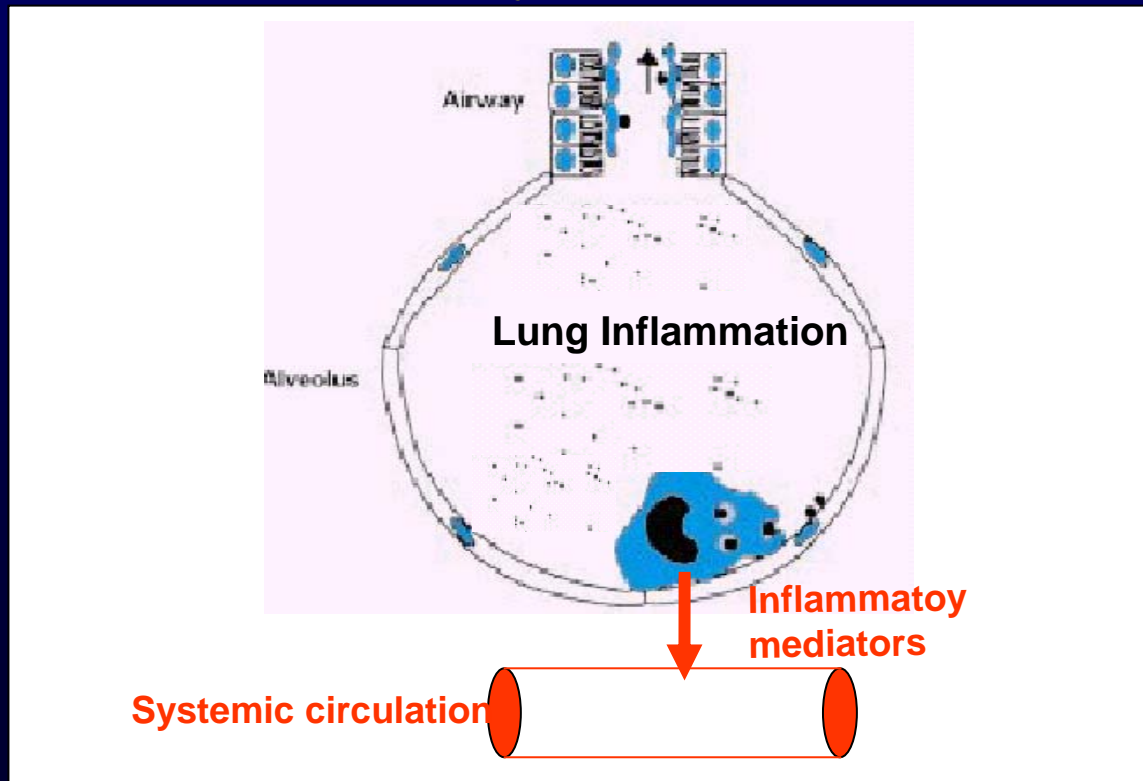
- current explanations / hypotheses:
 - inhaled particles cause
 - autonomic nervous response

Mechanisms

current explanations / hypotheses:

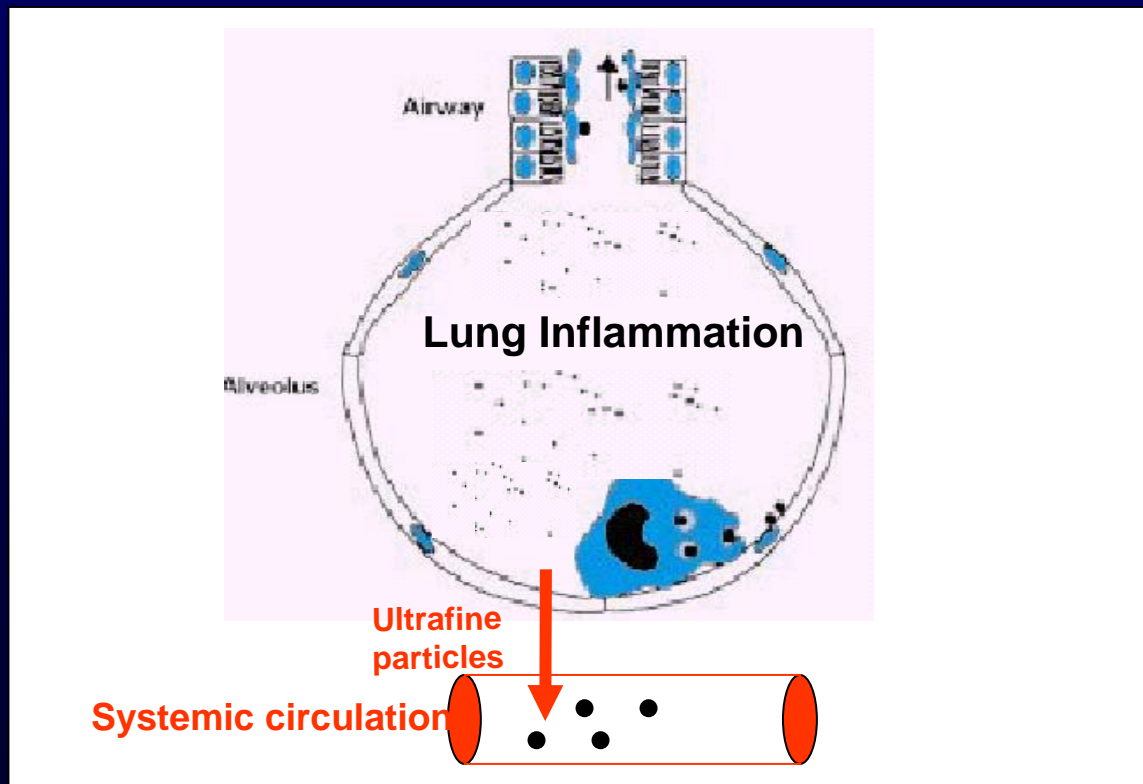
inhaled particles cause

- pulmonary inflammation with systemic release of cytokines



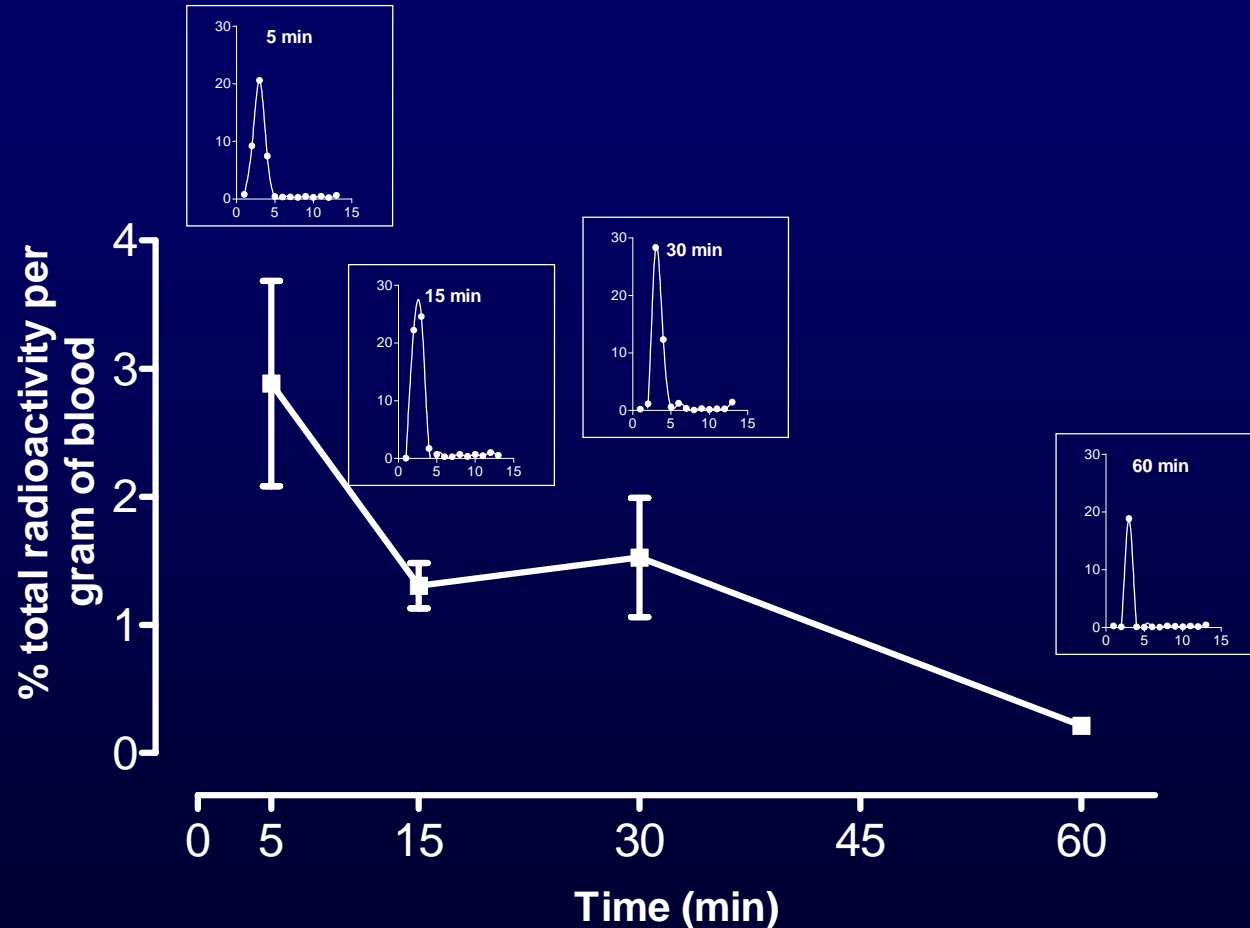
Mechanisms

- alternative / complementary hypothesis:
inhaled ultrafine particles ($\text{\O} < 0.1 \mu\text{m}$)
 - pass into the circulation
 - exert “direct” effects on cardiovascular endpoints



Our recent contributions

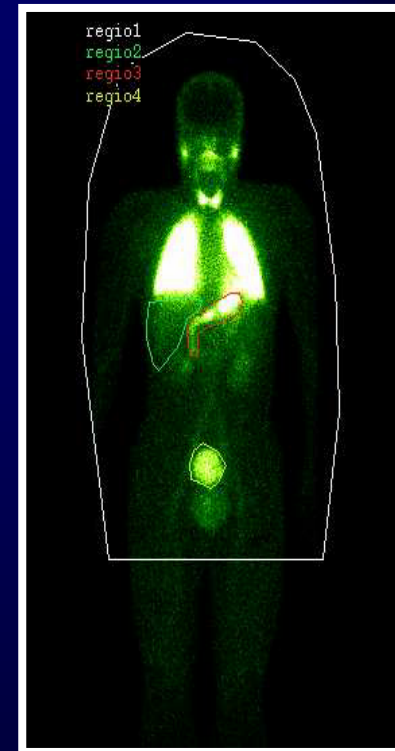
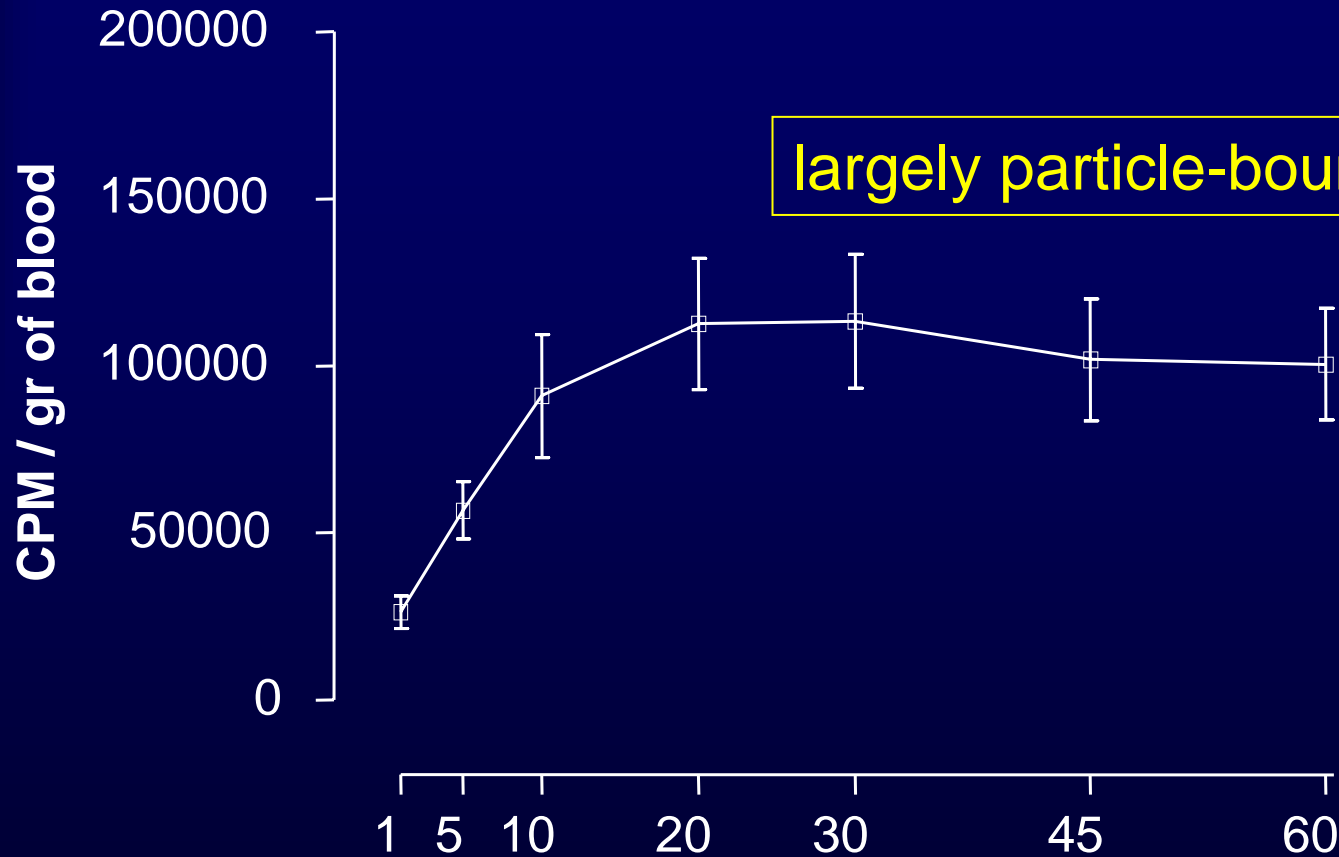
intratracheal instillation of ^{99m}Tc -albumin nanocolloid particles (80 nm) in hamsters



Nemmar *et al.* Am. J. Respir. Crit. Care Med. 2001;164:1665-8.

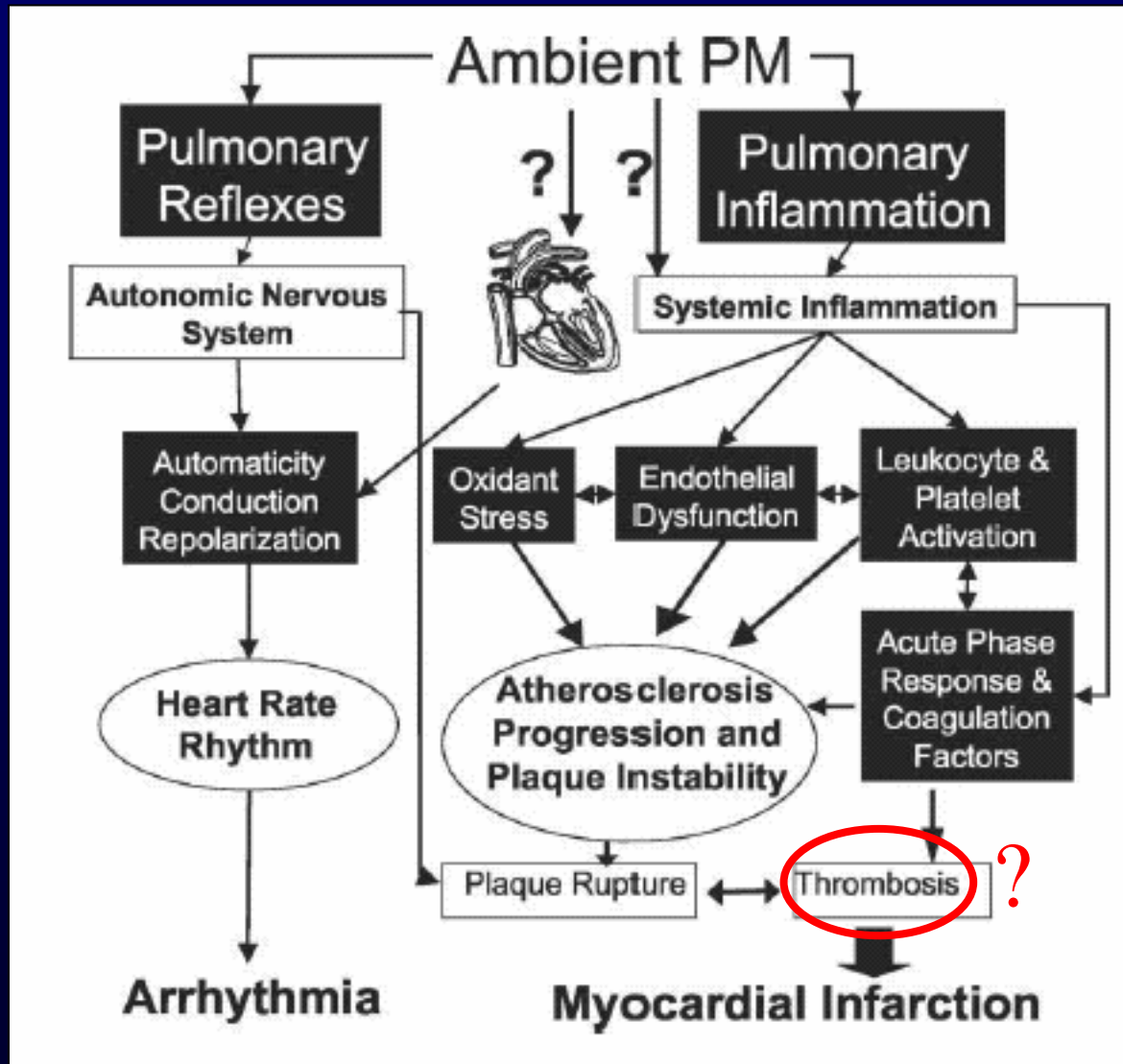
Our recent contributions

Inhalation of ^{99m}Tc -labelled, ultrafine carbon particles **in humans** (Technegas)



Nemmar *et al.* *Circulation*. 2002;105:411-4.

Brook RD *et al.* Air pollution and cardiovascular disease. A statement for health-care professionals from the expert panel on population and prevention science of the American Heart Association. *Circulation* 2004; 109: 2655-71

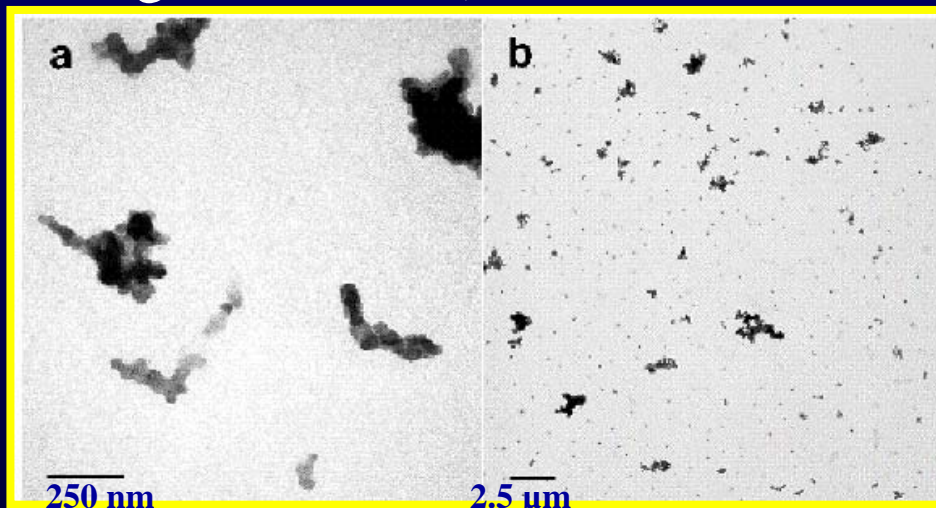


Diesel exhaust particles in lung acutely enhance experimental peripheral thrombosis

Nemmar et al. *Circulation*, 2003,107, 1202-8

Methods (1)

- Diesel exhaust particles (DEP) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA)



sonicated + vortexed immediately before administration

Methods (2)

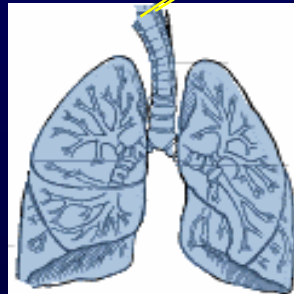
Pulmonary inflammation

- DEP 5 - 500 $\mu\text{g}/\text{animal}$ or vehicle were i.t. instilled to hamster

1 hour

Bronchoalveolar lavage (BAL)

Saline

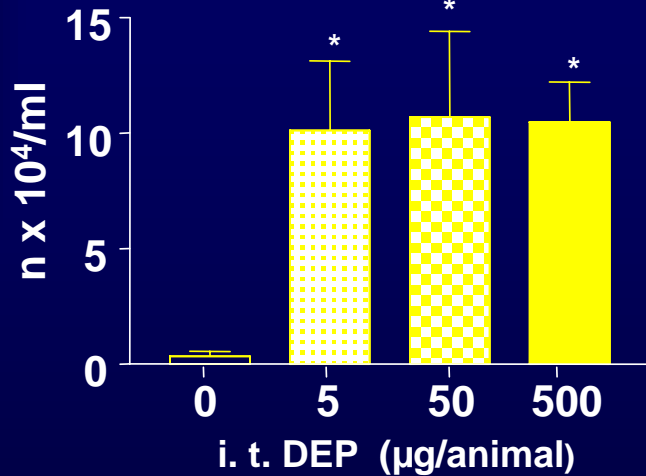


- Cells
- Proteins
- Histamine

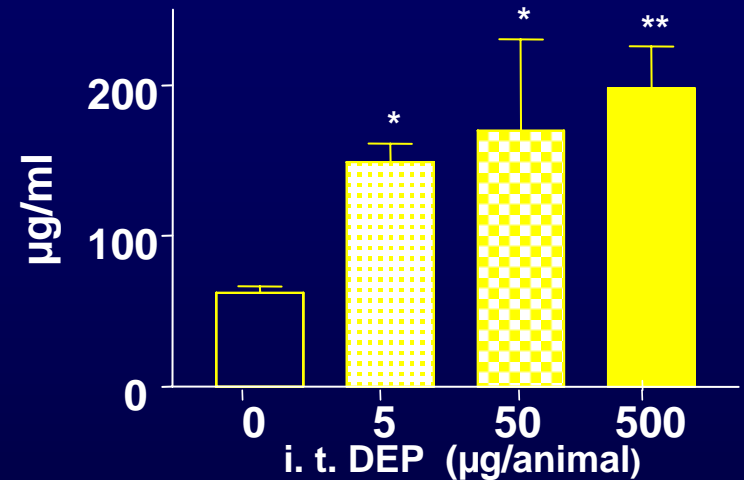
Results (2)

Pulmonary inflammation (BAL)

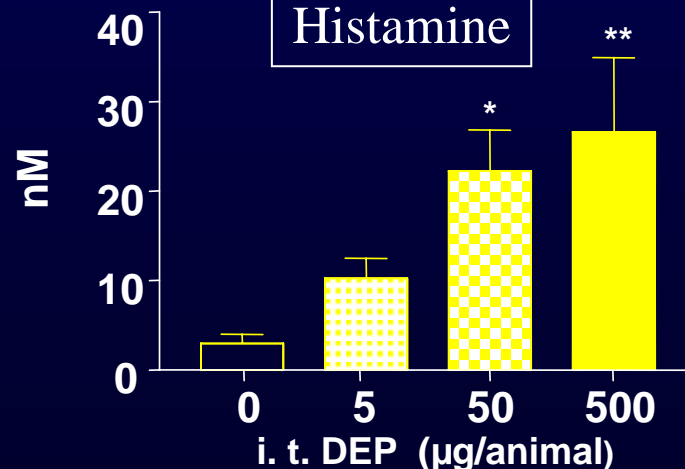
Neutrophils



Proteins

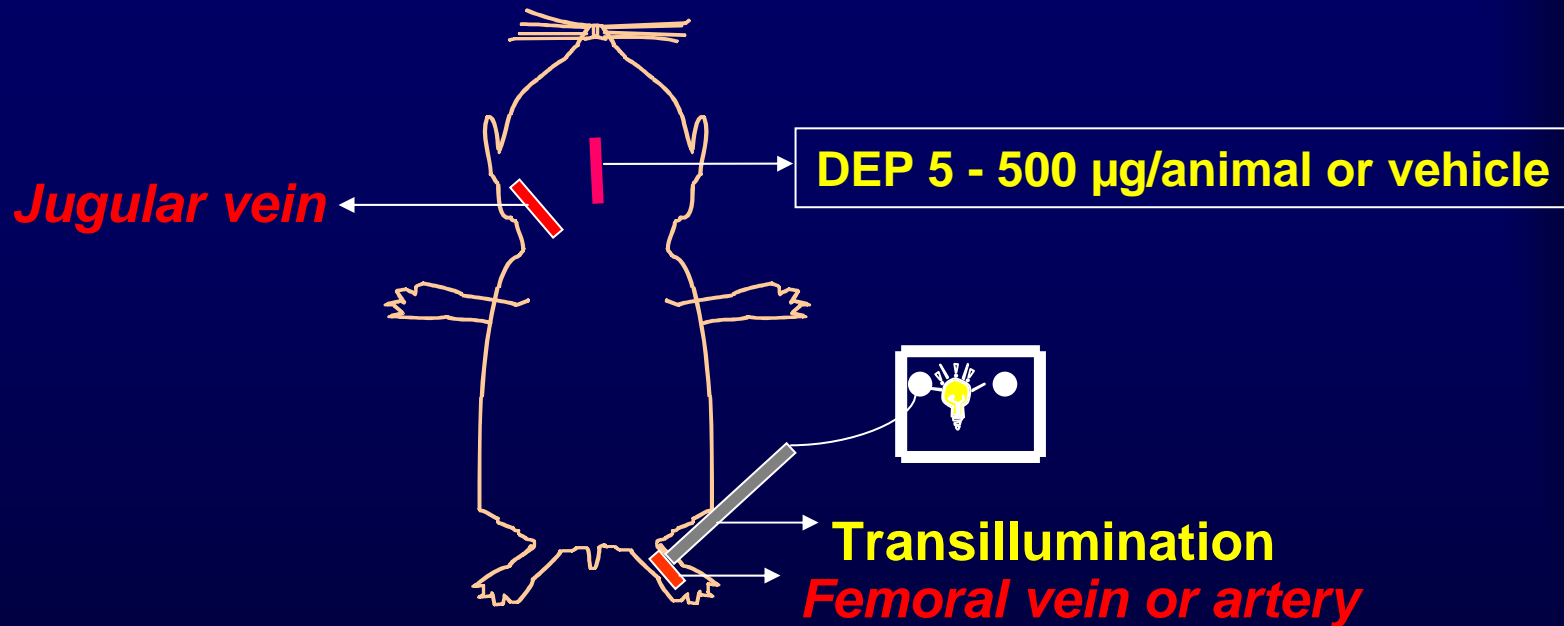


Histamine



Methods (2)

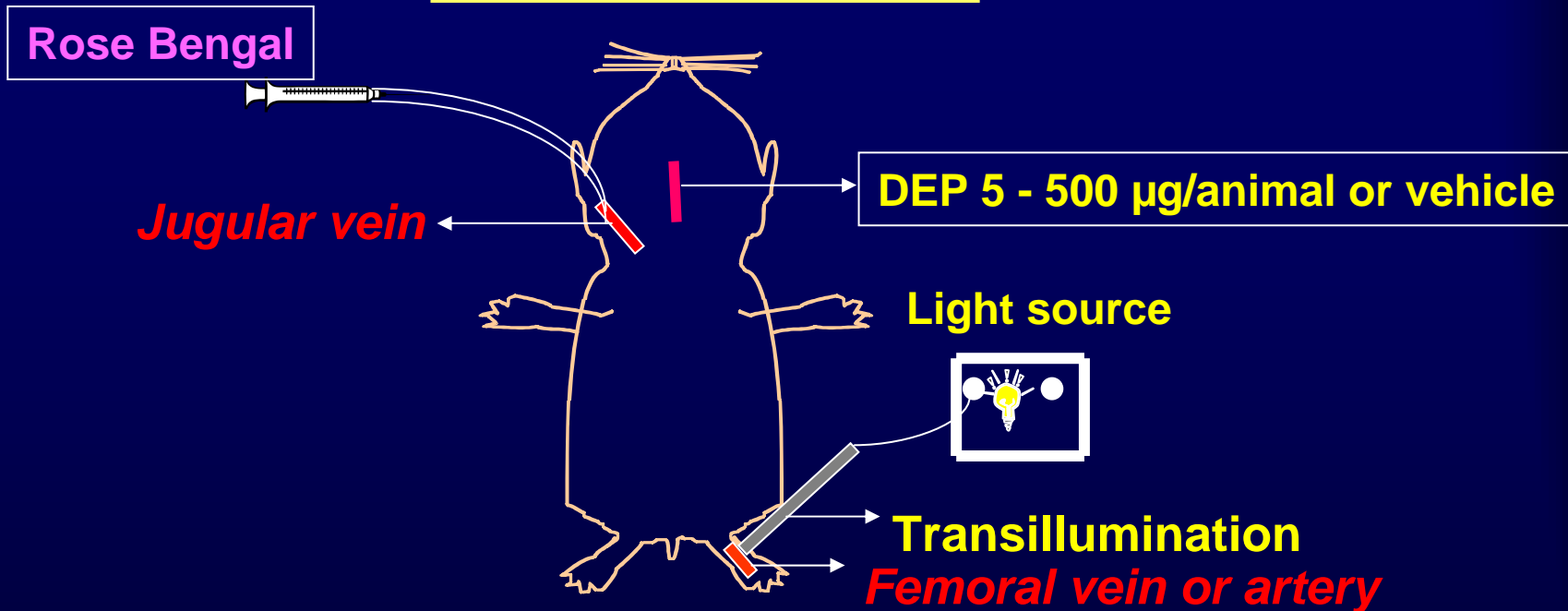
In vivo experiments



1. inject DEP (or vehicle) i.t.

Methods (2)

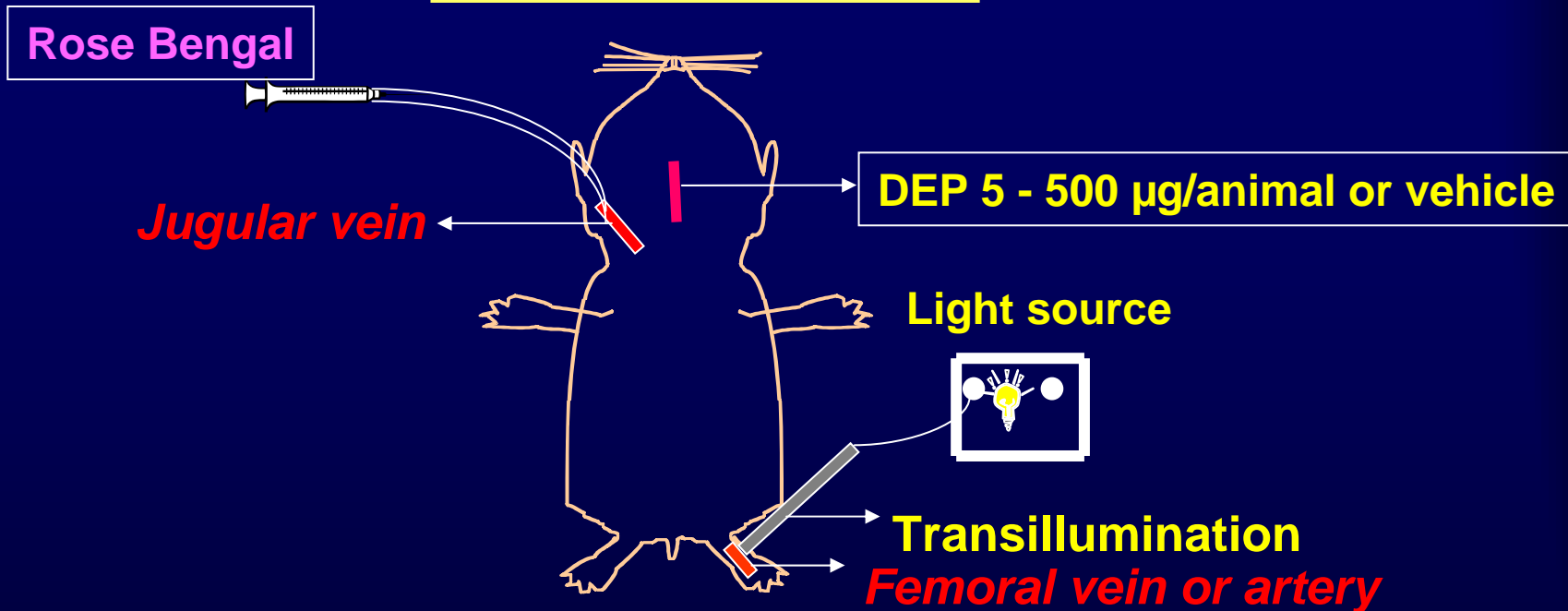
In vivo experiments



2. 10 min later, inject Rose Bengal i.v.

Methods (2)

In vivo experiments

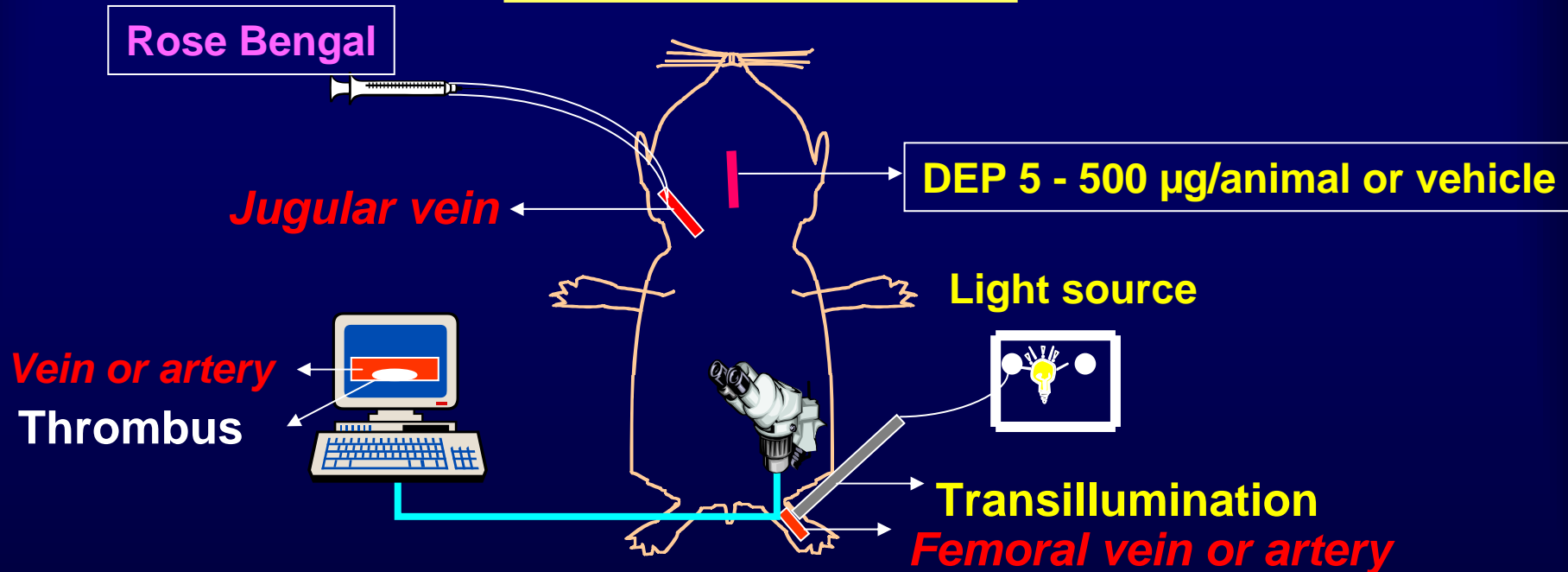


3. 2 min green light exposure (540 nm)

oxidative damage to endothelium

Methods (2)

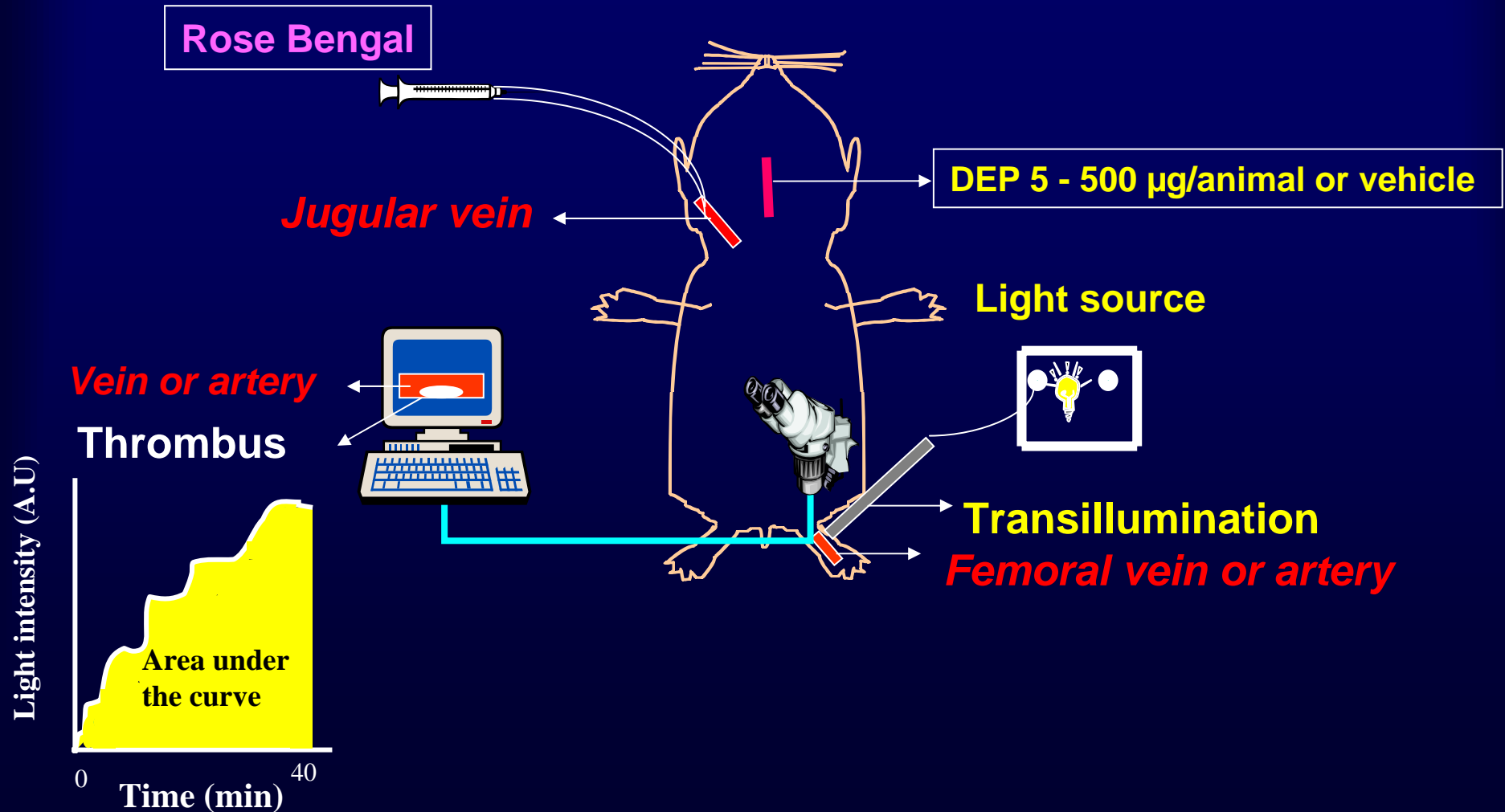
In vivo experiments



4. follow thrombus formation during 40 min

Methods (2)

in vivo experiments



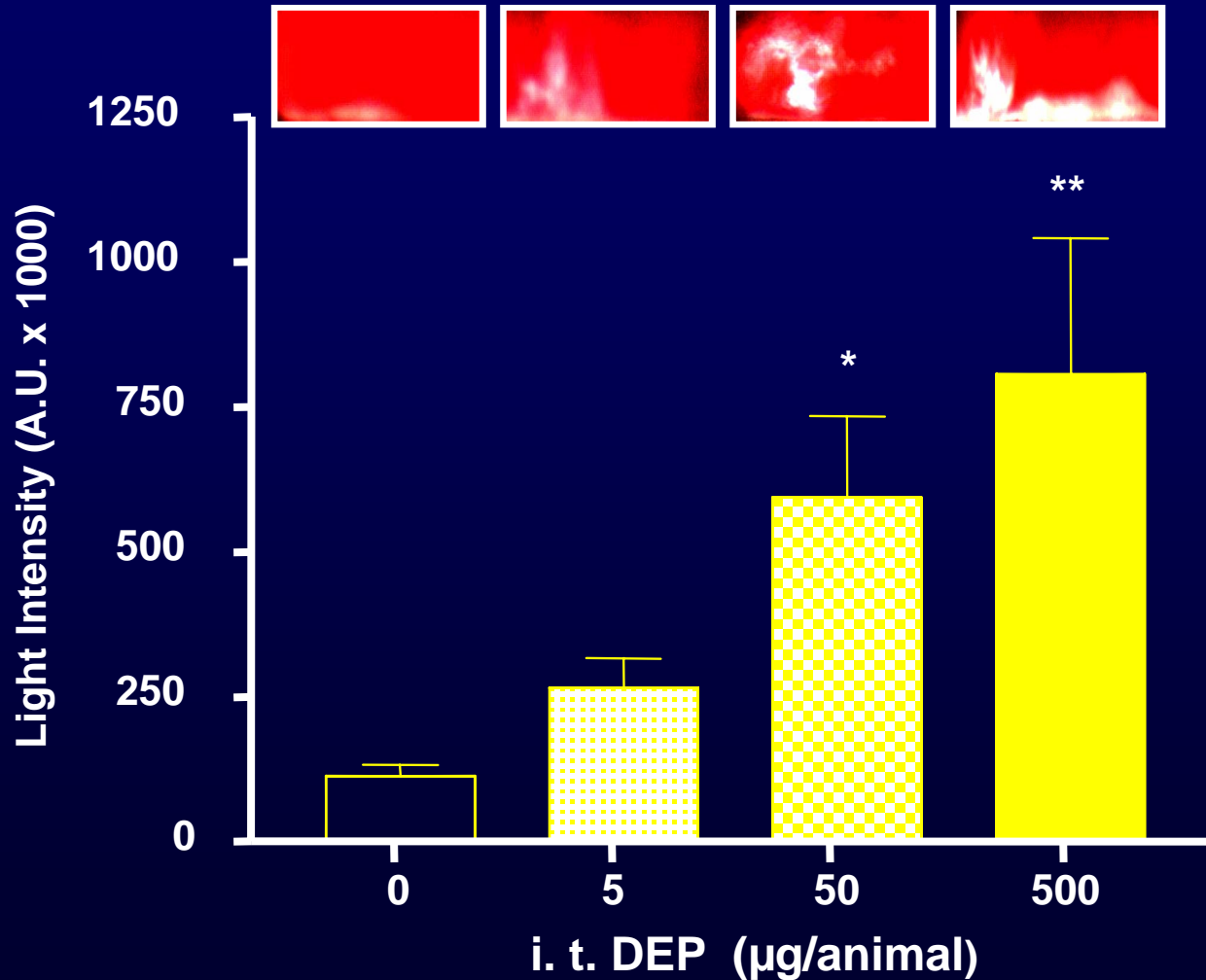
Methods

in vivo experiments



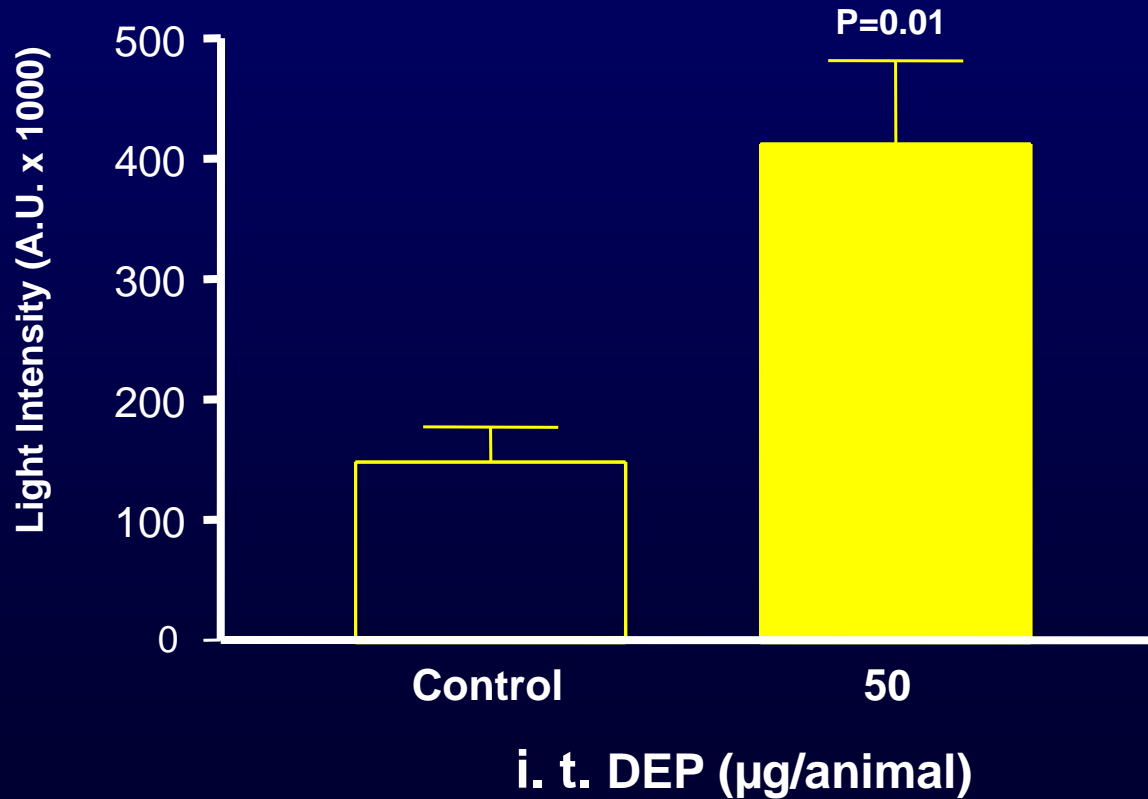
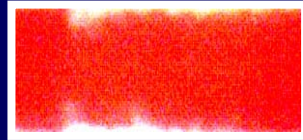
Results (2)

Peripheral venous thrombosis



Results (2)

Peripheral arterial thrombosis



Methods (3)

Platelet function analysis PFA-100[®]

800 μl blood

- 40 mbar

Opening: \varnothing 150 μm

Membrane coated with
collagen & epinephrine

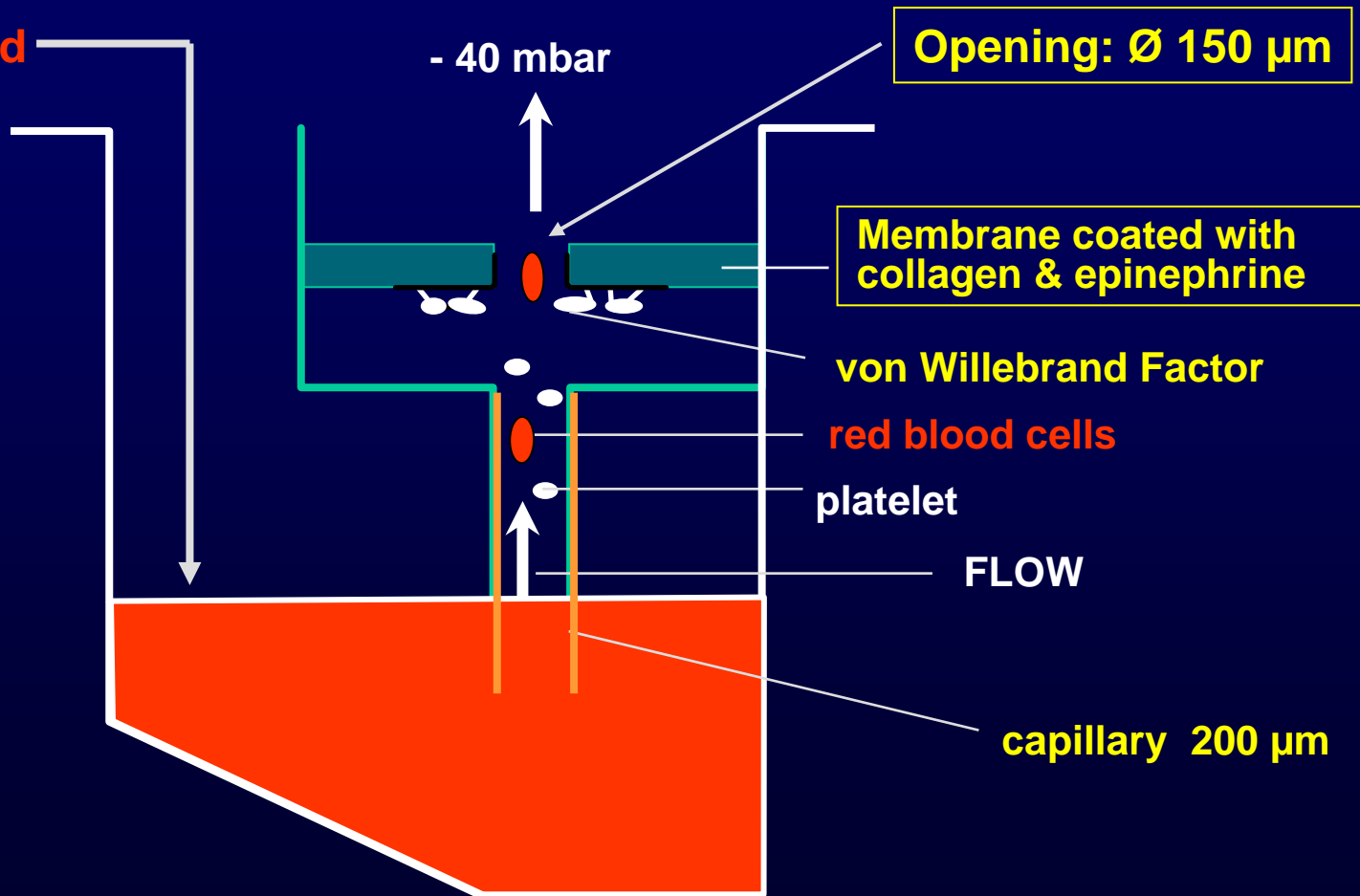
von Willebrand Factor

red blood cells

platelet

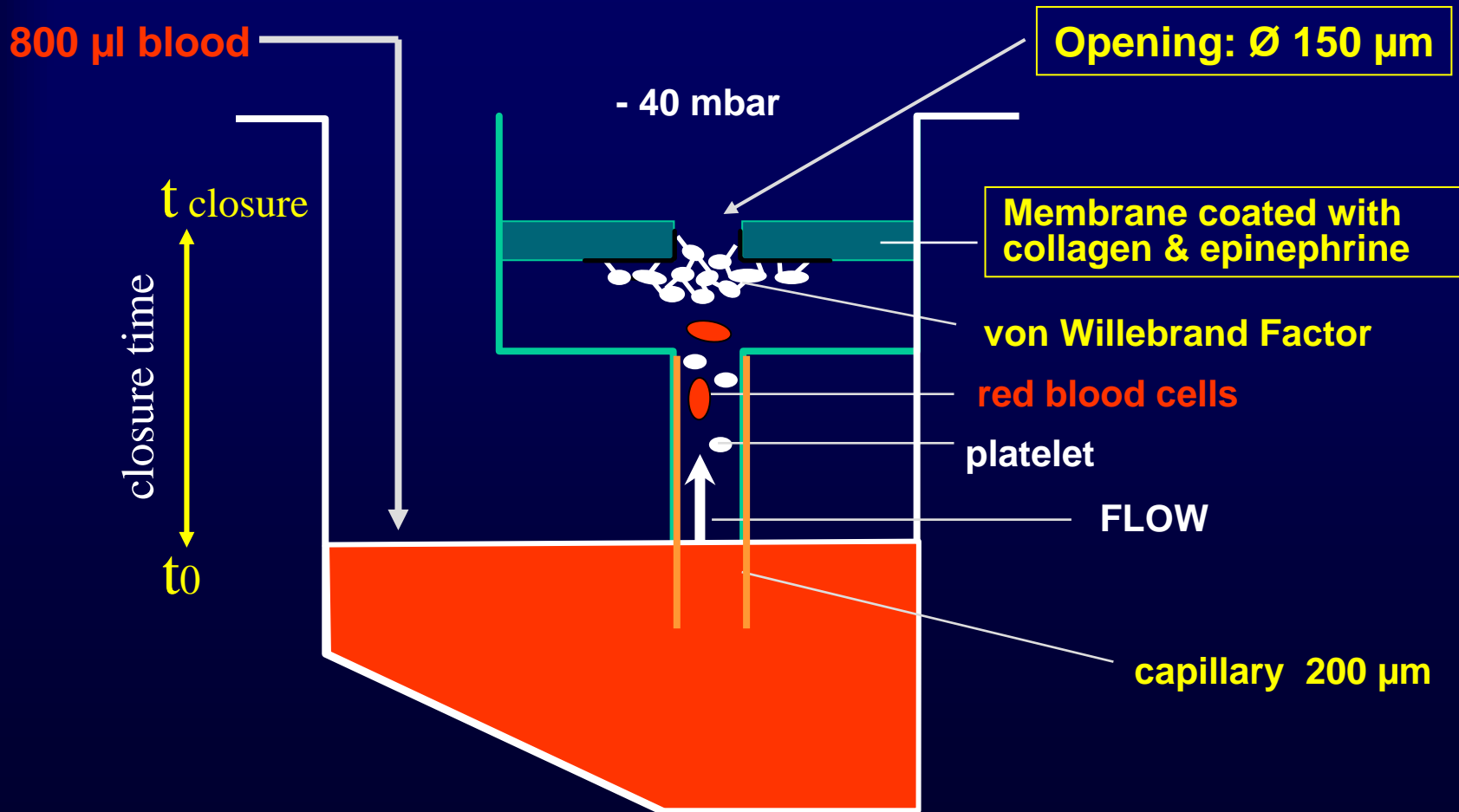
FLOW

capillary 200 μm



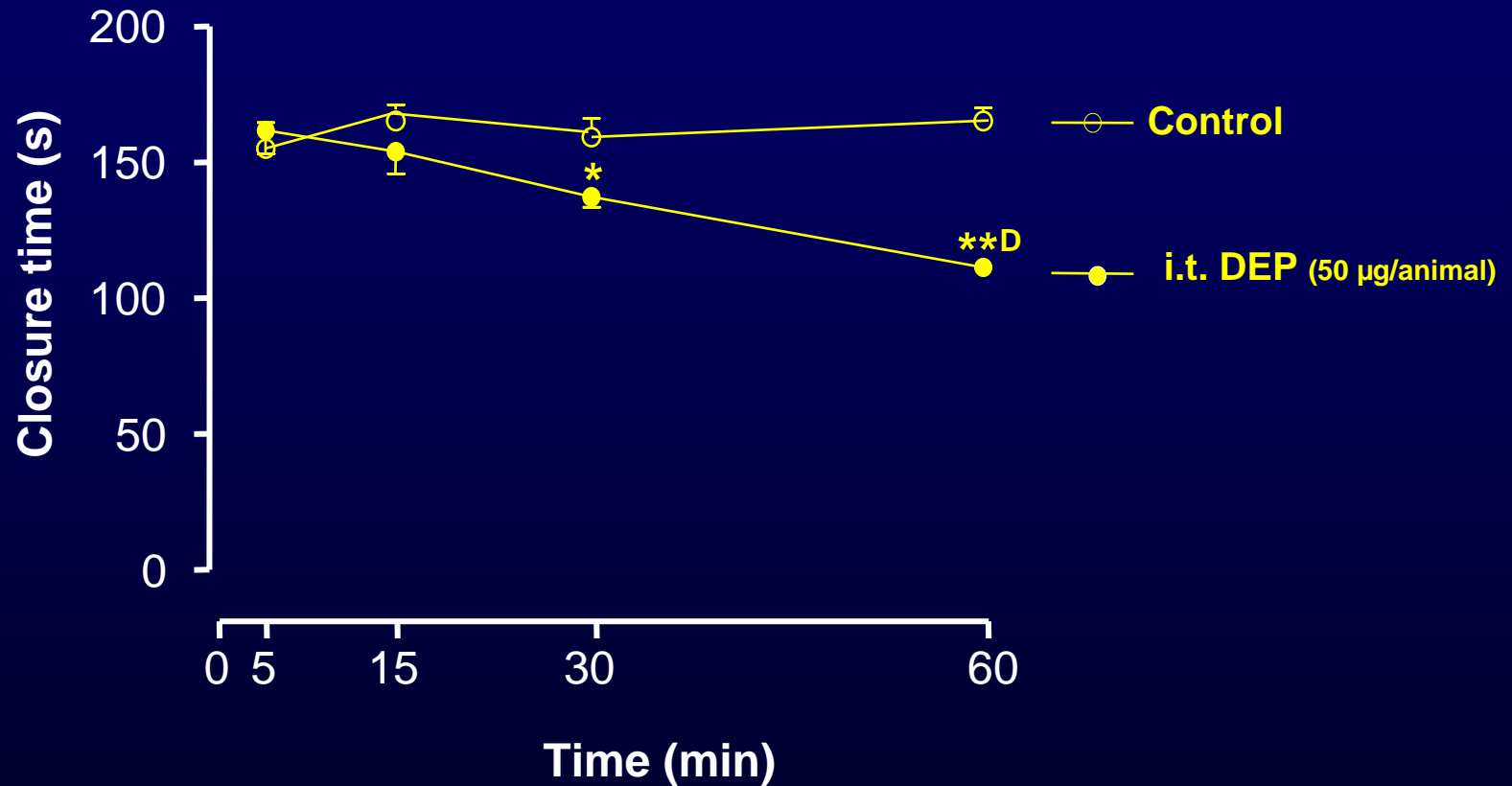
Methods (3)

Platelet function analysis PFA-100[®]



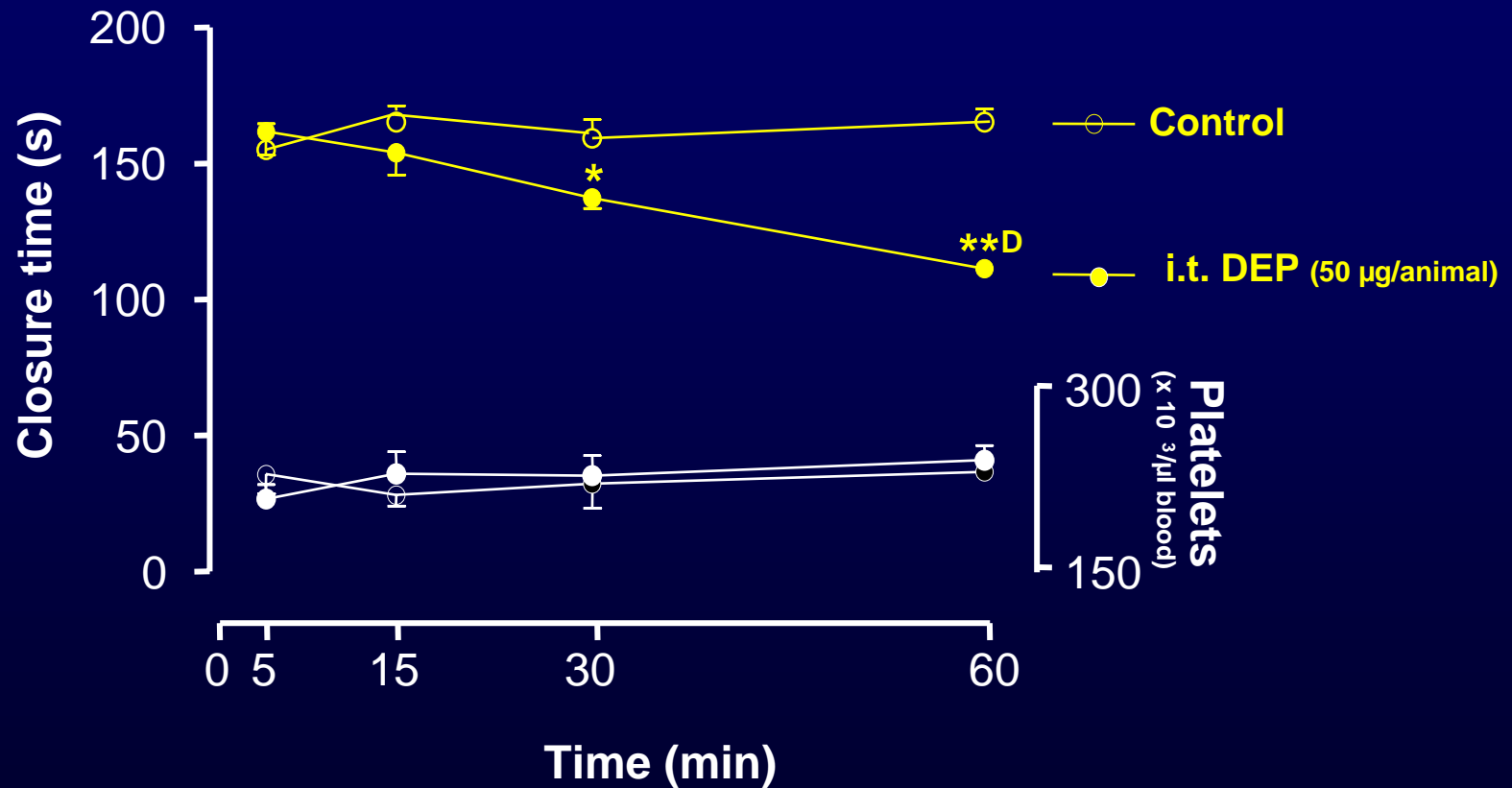
Results (3)

Platelet function analysis *ex vivo*



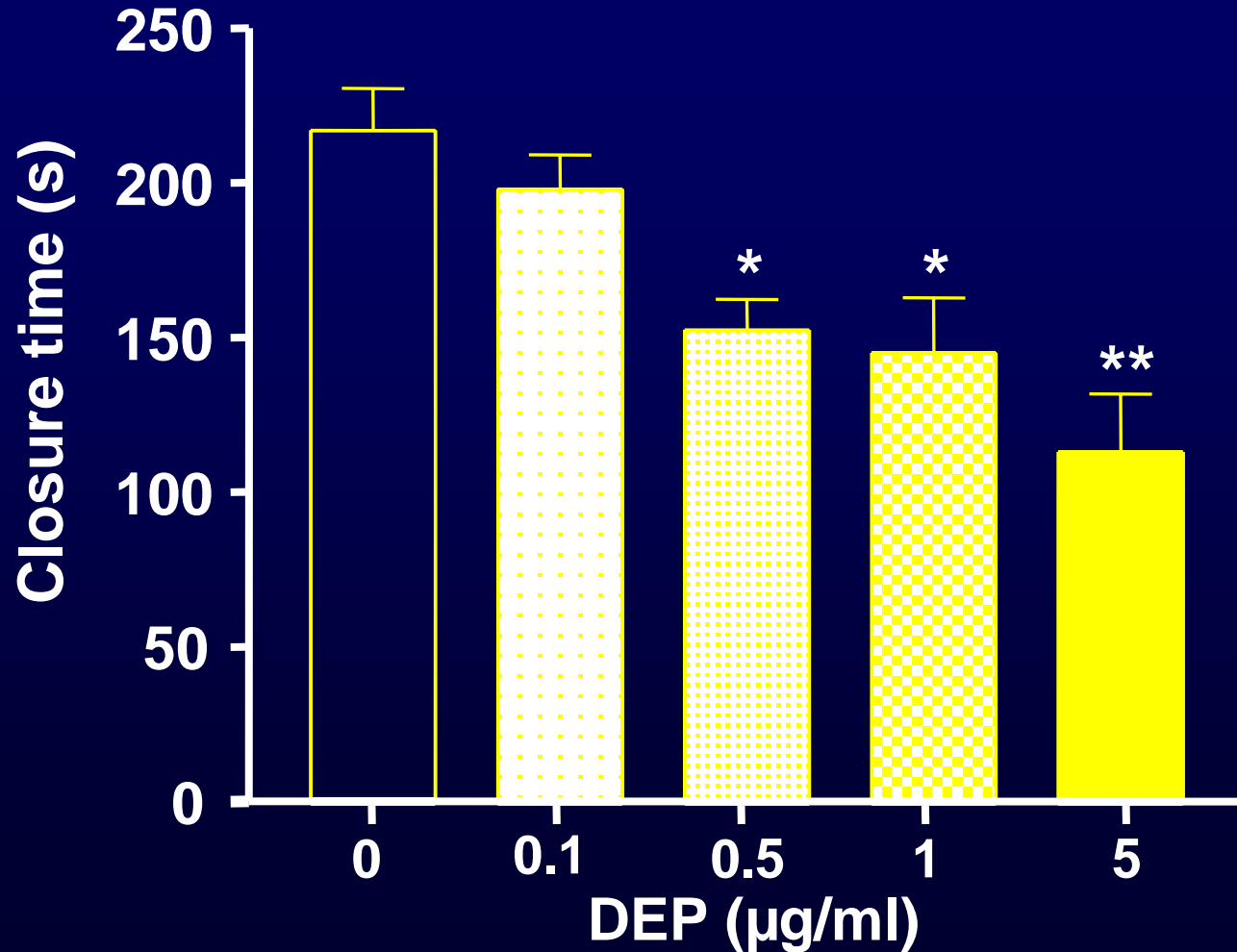
Results (3)

Platelet function analysis *ex vivo*



Results (3)

Platelet function analysis *in vitro*



Summary & conclusion

- Within 1 hour after their deposition in the lungs, DEP :
 - ✉ cause pulmonary inflammation
 - ✉ aggravate thrombosis, as substantiated *in vivo*, *ex vivo* and *in vitro*.

Further experimental studies

- DEP cause lung inflammation and aggravate thrombosis at 1, 6 and 24 h.
- Pre-treatment of hamsters with:
 - Diphenhydramine, a histamine H1-receptor antagonist:

	<u>Lung inflammation</u>	<u>peripheral thrombosis</u>
1 h	inhibition	no change
6 h	inhibition	inhibition
24 h	inhibition	inhibition

- Pulmonary inflammation and peripheral thrombosis are correlated at 6 h and 24 h.
- At 1h, the prothrombotic effect does not appear to result from pulmonary inflammation but possibly from the blood penetration of DEP (or their associated components).

Nemmar *et al.* Am. J. Respir. Crit. Care Med. 2003; 168:1366-72.

Further experimental studies

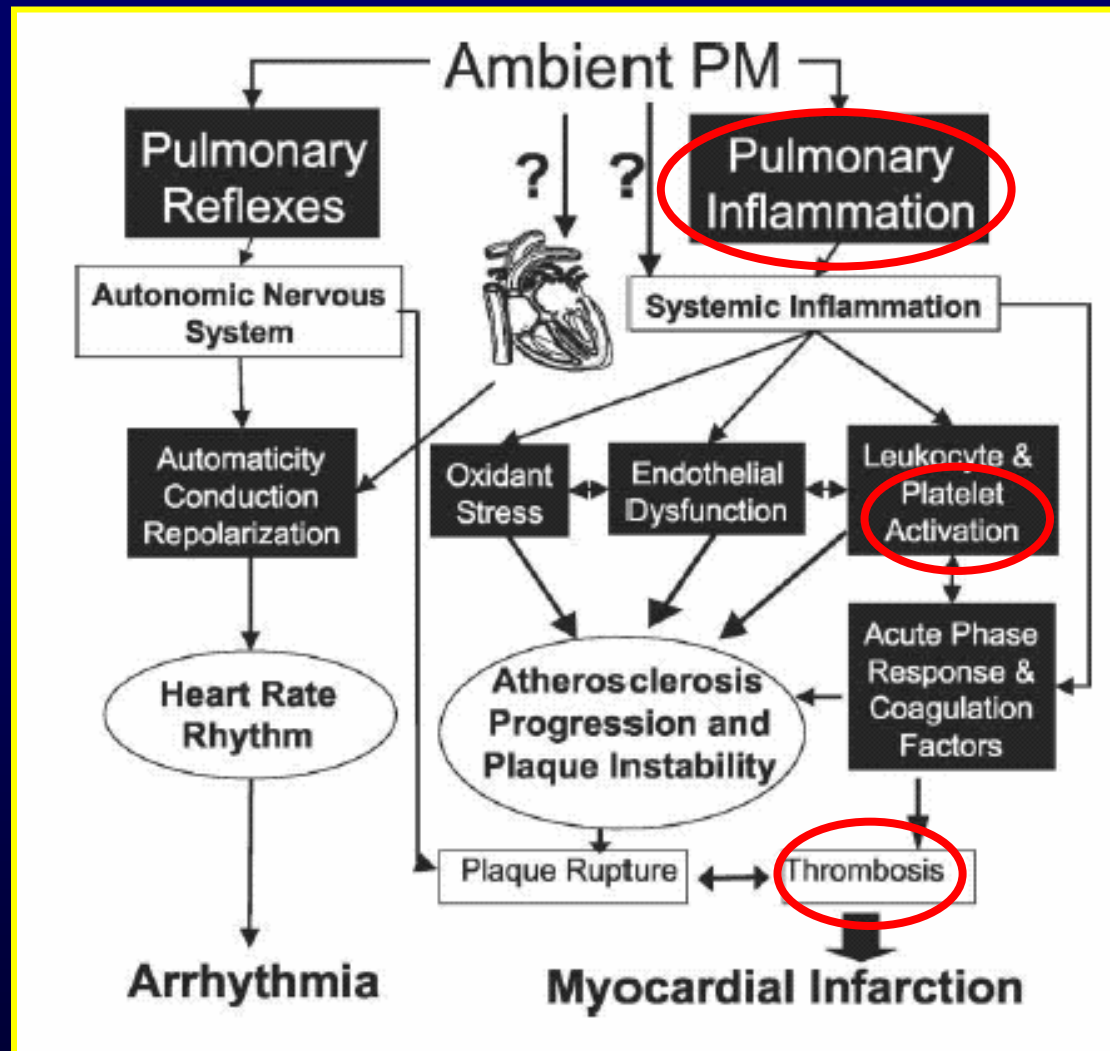
- Diesel exhaust particles (DEP) cause lung inflammation and aggravate thrombosis at 24 h.
- Pre-treatment of hamsters with:
 - Dexamethasone or cromoglycate:

	<u>Lung inflammation</u>	<u>thrombosis</u>	<u>histamine release</u>
24 h	inhibition	inhibition	inhibition

- Mast cells stabilization and anti-inflammatory pretreatment:
 - abrogate the histamine production in the lung.
 - reduce the inflammation-induced peripheral thrombotic events in the circulation.

Nemmar *et al.*, *Circulation*. 2004; 110:1670-7.

General conclusion



Brook RD *et al.* Air pollution and cardiovascular disease. A statement for health-care professionals from the expert panel on population and prevention science of the American Heart Association. *Circulation* 2004; 109: 2655-71

Acknowledgements

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(Toxicology Unit)

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Air pollution and cardiovascular diseases: **Potential biological mechanisms**

Thank you for your attention

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