

BSc in Pharmacology – Nov 2011

Pharmacology of small airways function

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Learning objectives

After the lecture (and appropriate revision) you will be able to:

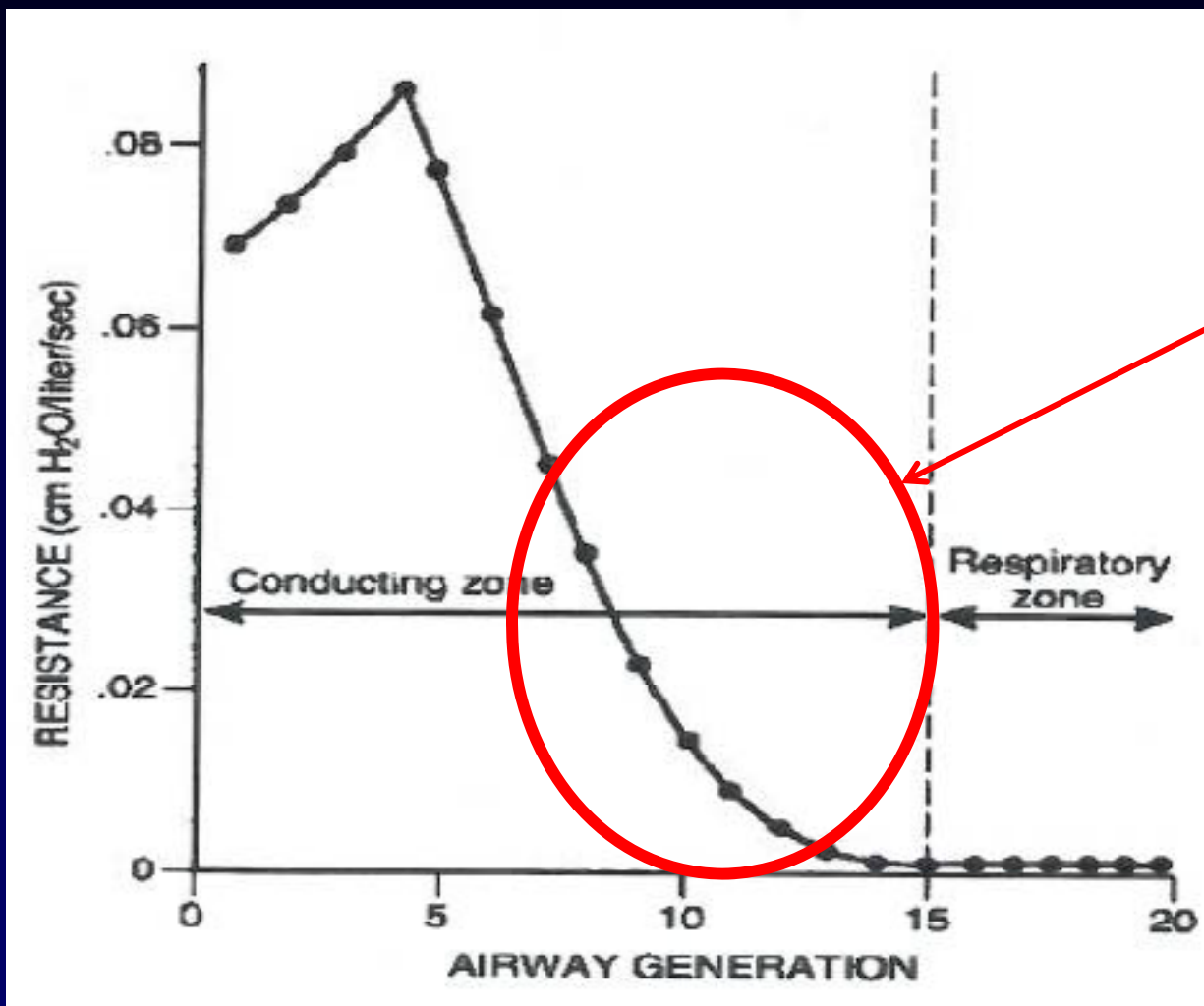
- Describe how the small airways are a critical anatomical site linking the larger (cartilaginous) airways with the alveoli
- Describe the pathophysiology of chronic obstructive pulmonary disease (COPD) and how small airways are 'vulnerable' to remodeling in COPD and become the site of greatest airflow resistance
- Describe how proteases, oxidants and other neutrophil products might effect small airways structure and function
- Describe the action of bronchodilators on small airway tone

Airway generations (human)

		Generation	Diameter, cm	Length, cm	Number	Total cross-sectional area, cm ²	
conducting zone	trachea	0	1.80	12.0	1	2.54	
	bronchi	1	1.22	4.8	2	2.33	
		2	0.83	1.9	4	2.13	
	bronchioles	3	0.56	0.8	8	2.00	
		4	0.45	1.3	16	2.48	
		5	0.35	1.07	32	3.11	
terminal bronchioles	16	0.06	0.17	6×10^4	180.0		
transitional and respiratory zones	respiratory bronchioles	17	↓			↓	
		18	↓			↓	
		19	0.0		10^5	10^3	
	alveolar ducts	T ₃	20	↓	↓	↓	↓
		T ₂	21	↓	↓	↓	↓
		T ₁	22	↓	↓	↓	↓
	alveolar sacs	T	23	0.04	0.05	8×10^6	10^4

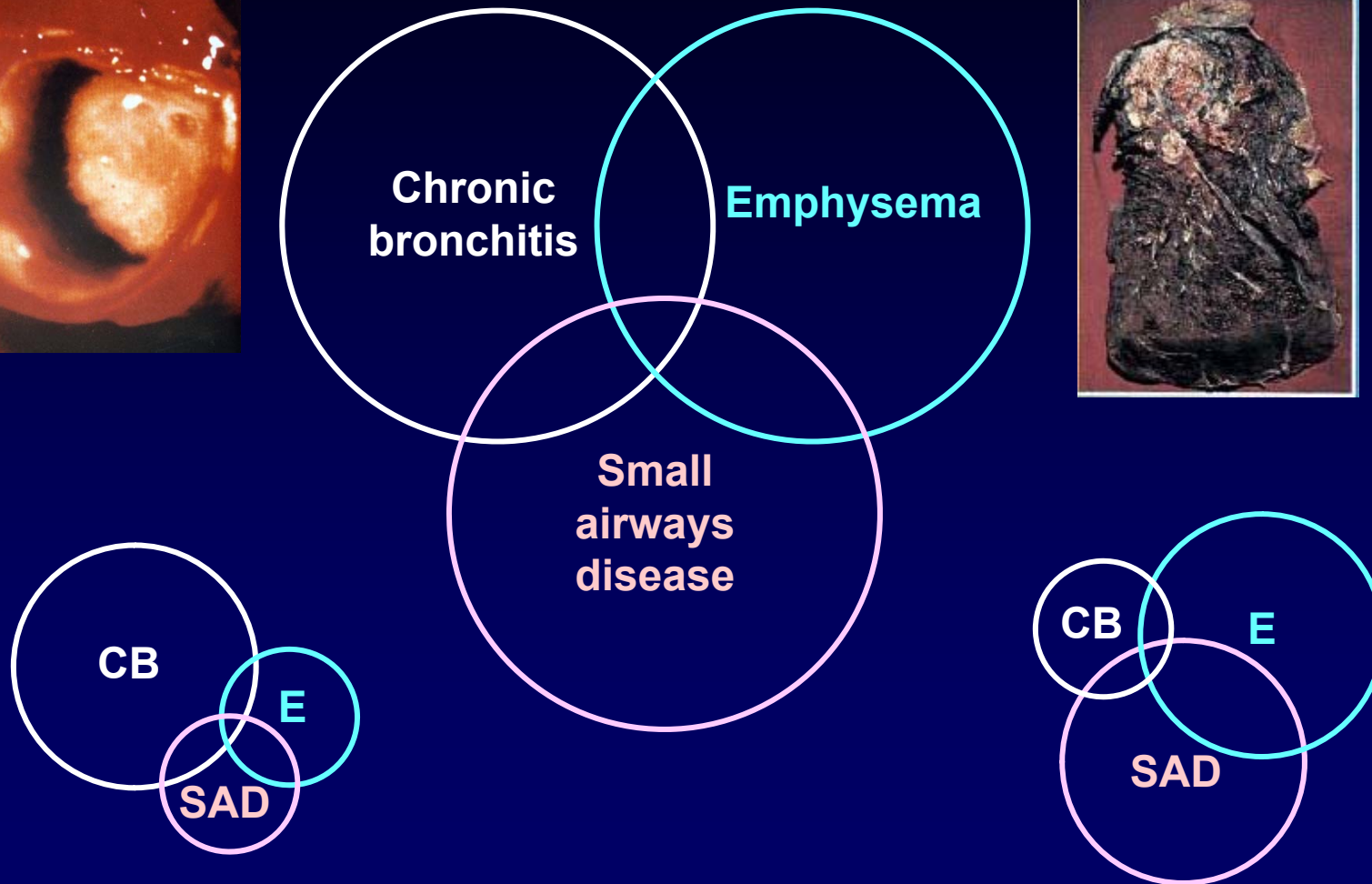
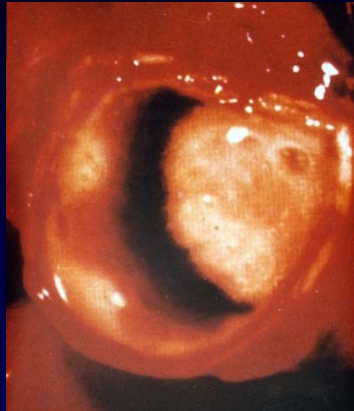
'Small airways'
(**< 2 mm**)

Airway generation and airflow resistance



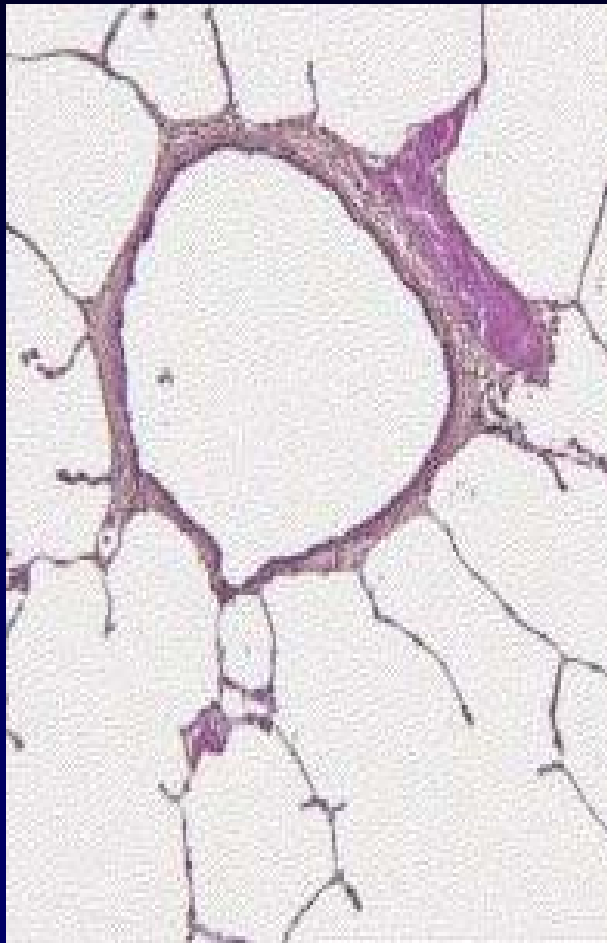
Numerous small tubes in parallel
-Increasingly reduced airflow resistance

COPD: the Venn diagram analogy

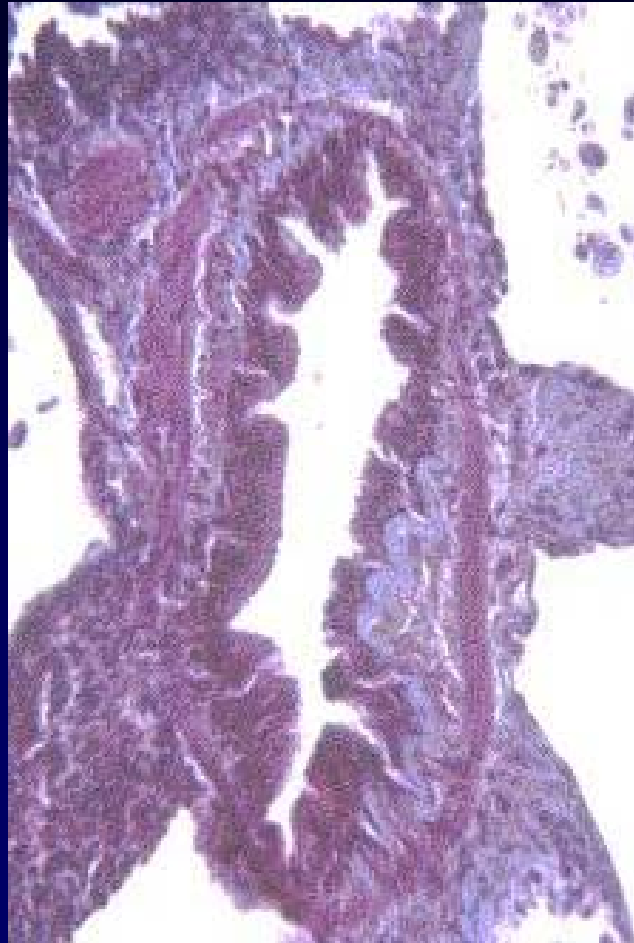


COPD: Small airways disease (bronchiolitis)

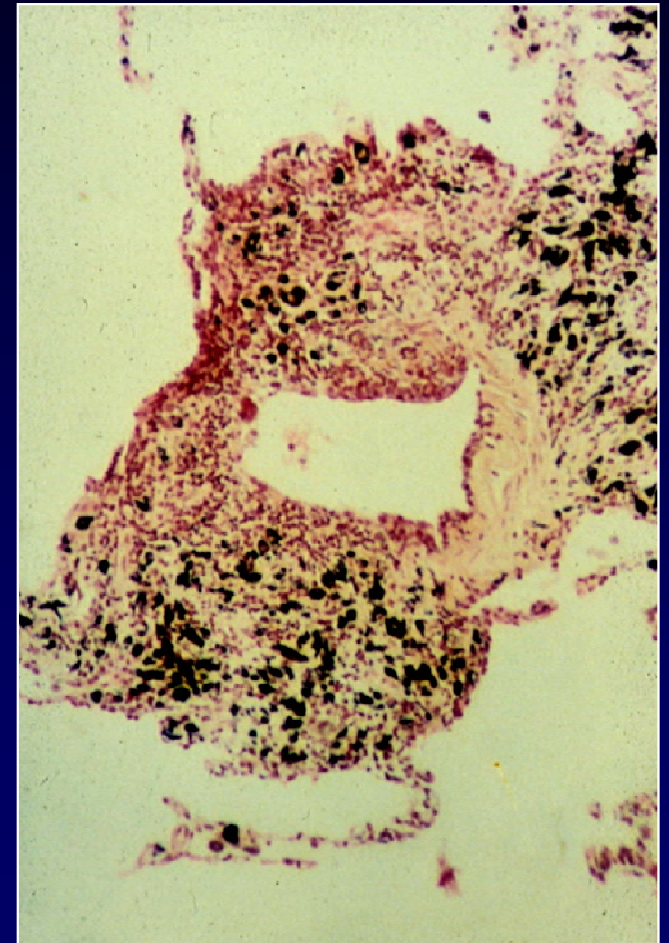
Normal small airway
(~ 0.5 mm diameter)



Similar size airway
in smoker

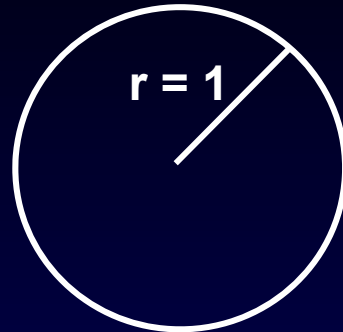


Very severe small
airways disease

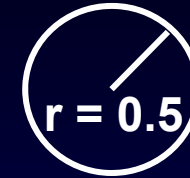


Poiseuille's law, small airways and COPD

'Normal'



Airway collapse
and/or contraction

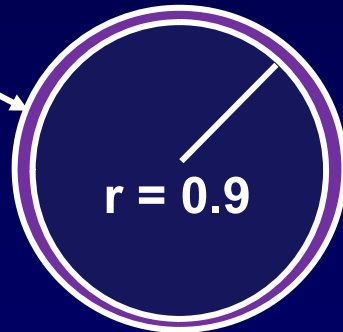


$$R \propto \frac{1}{r^4} = 1 \text{ unit}$$

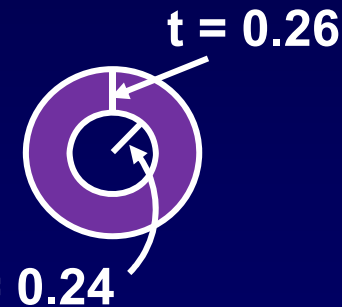
$$R \propto \frac{1}{(0.5)^4} = 16$$

COPD

Fibrosis
 $t = 0.1$



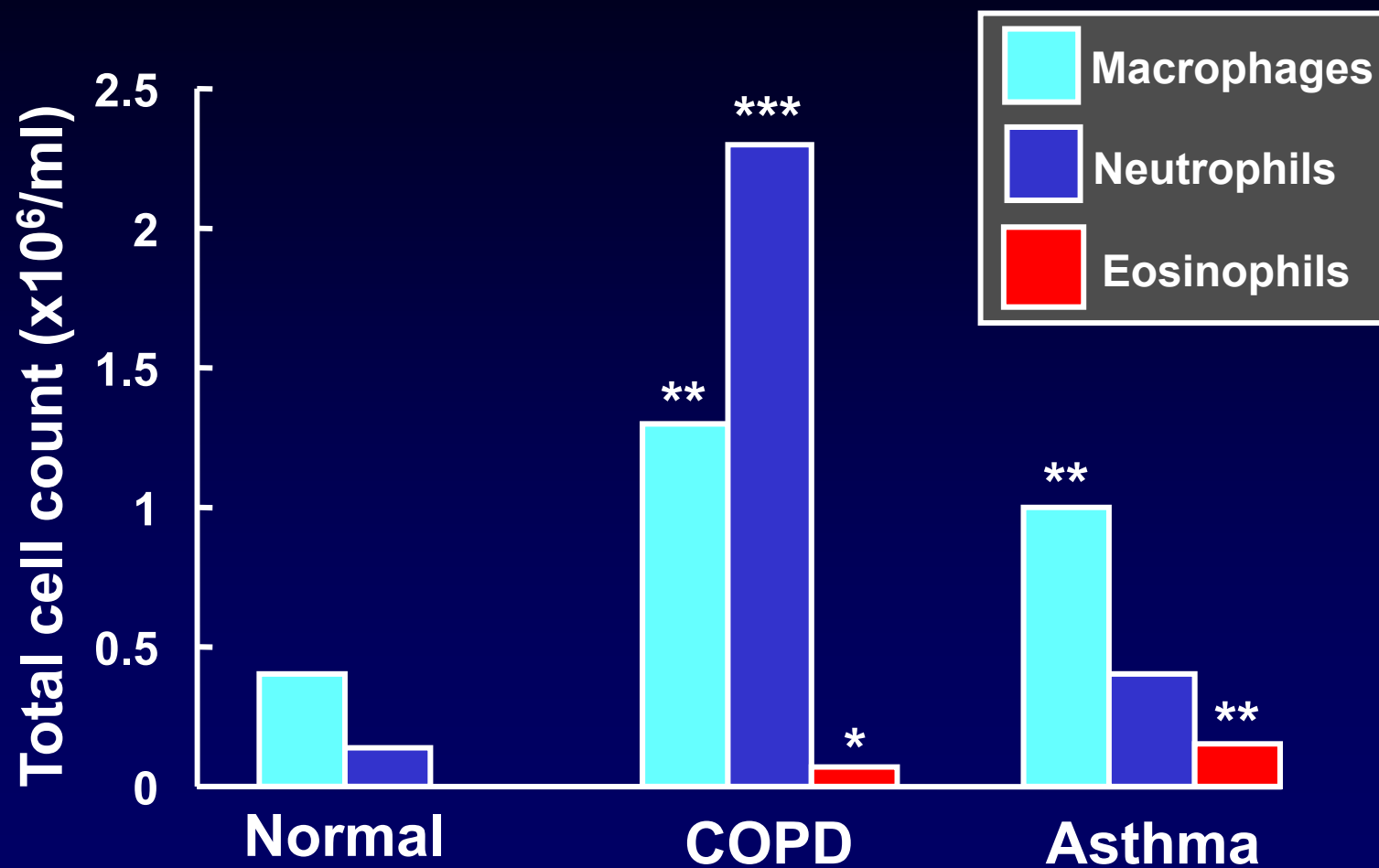
Airway collapse
and/or contraction



$$R \propto \frac{1}{(0.9)^4} = 1.5$$

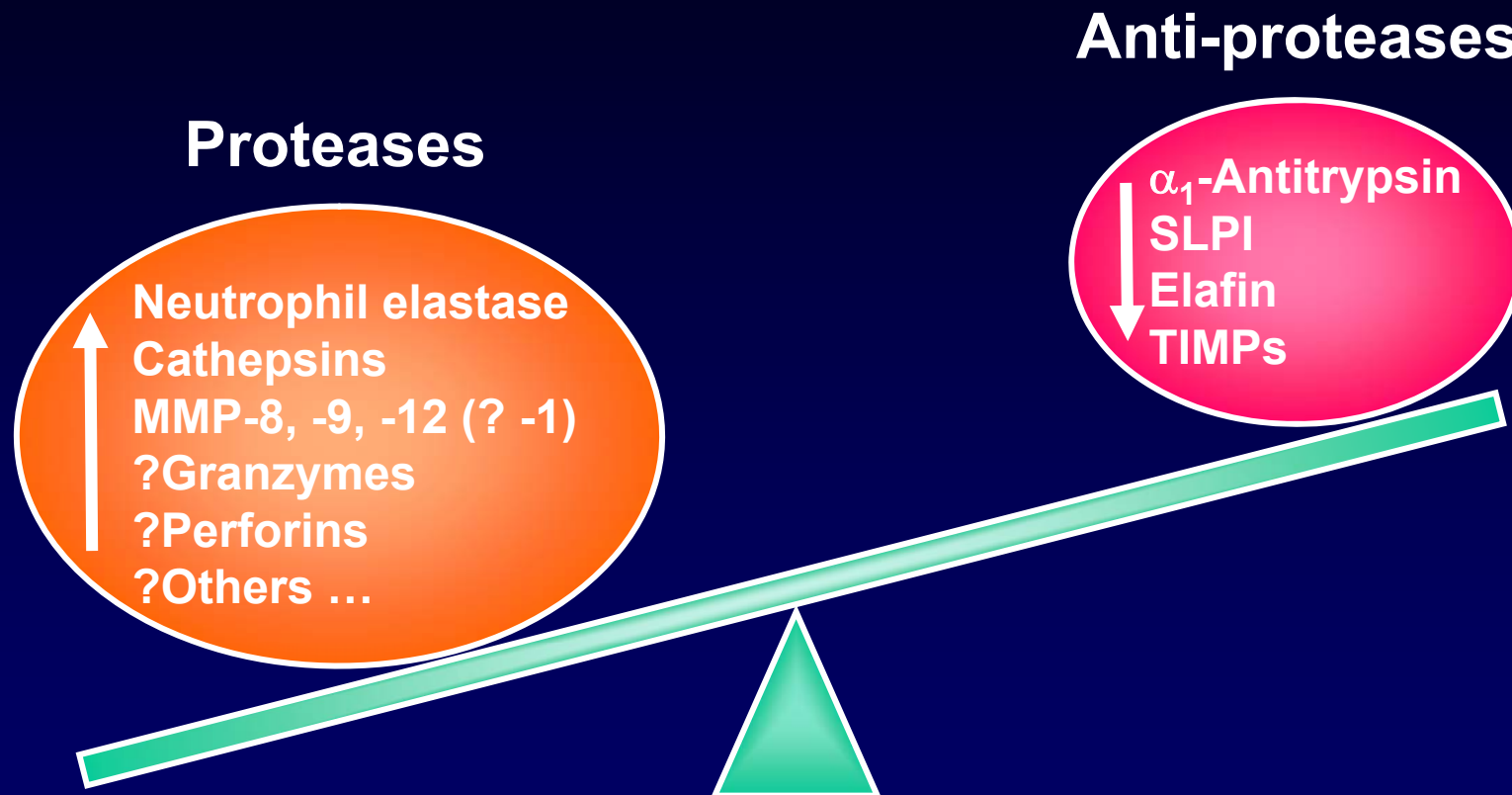
$$R \propto \frac{1}{(0.24)^4} = 300$$

COPD vs asthma: inflammatory cells in induced sputum

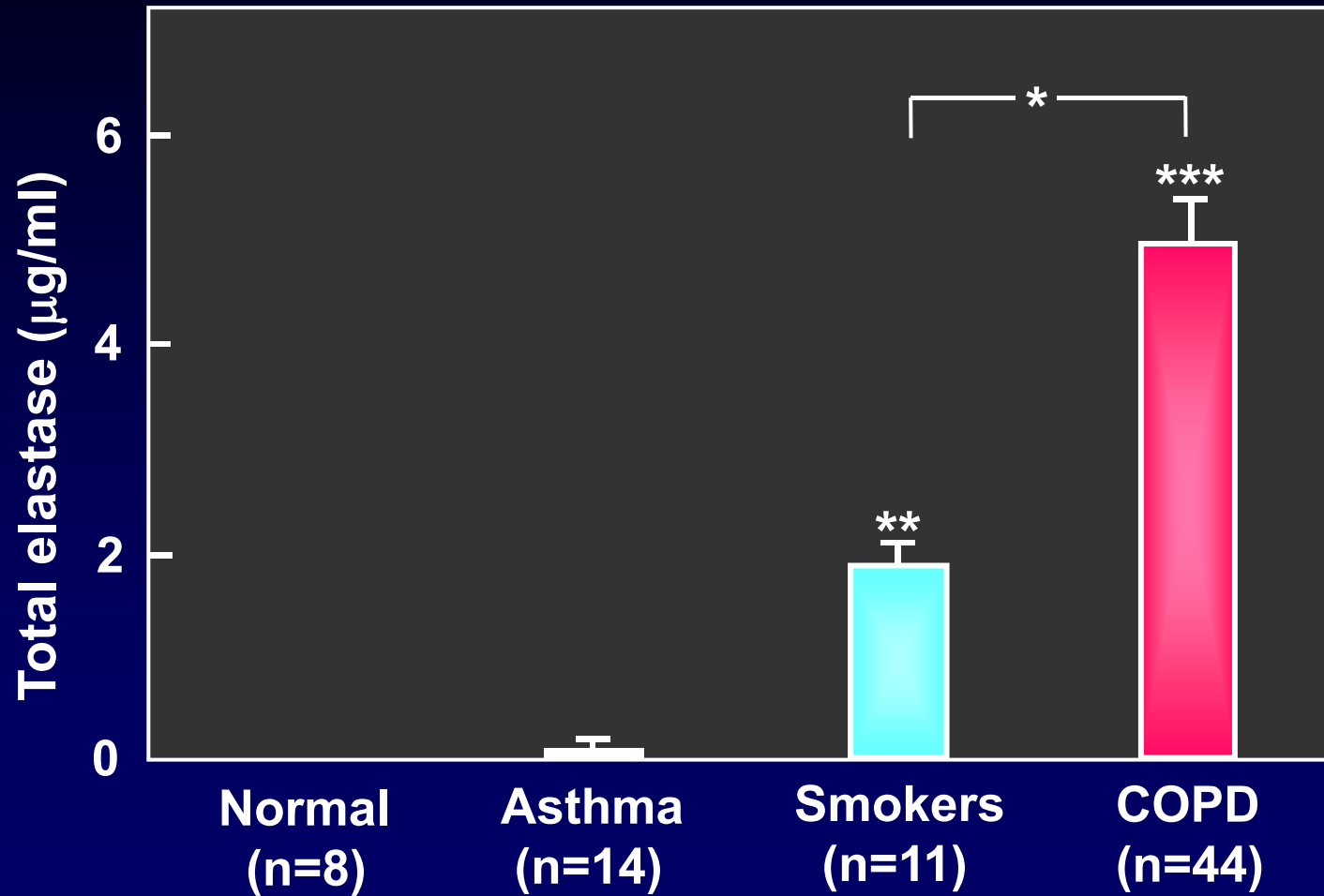


(Keatings V et al: Am J Respir Crit Care Med 1997)

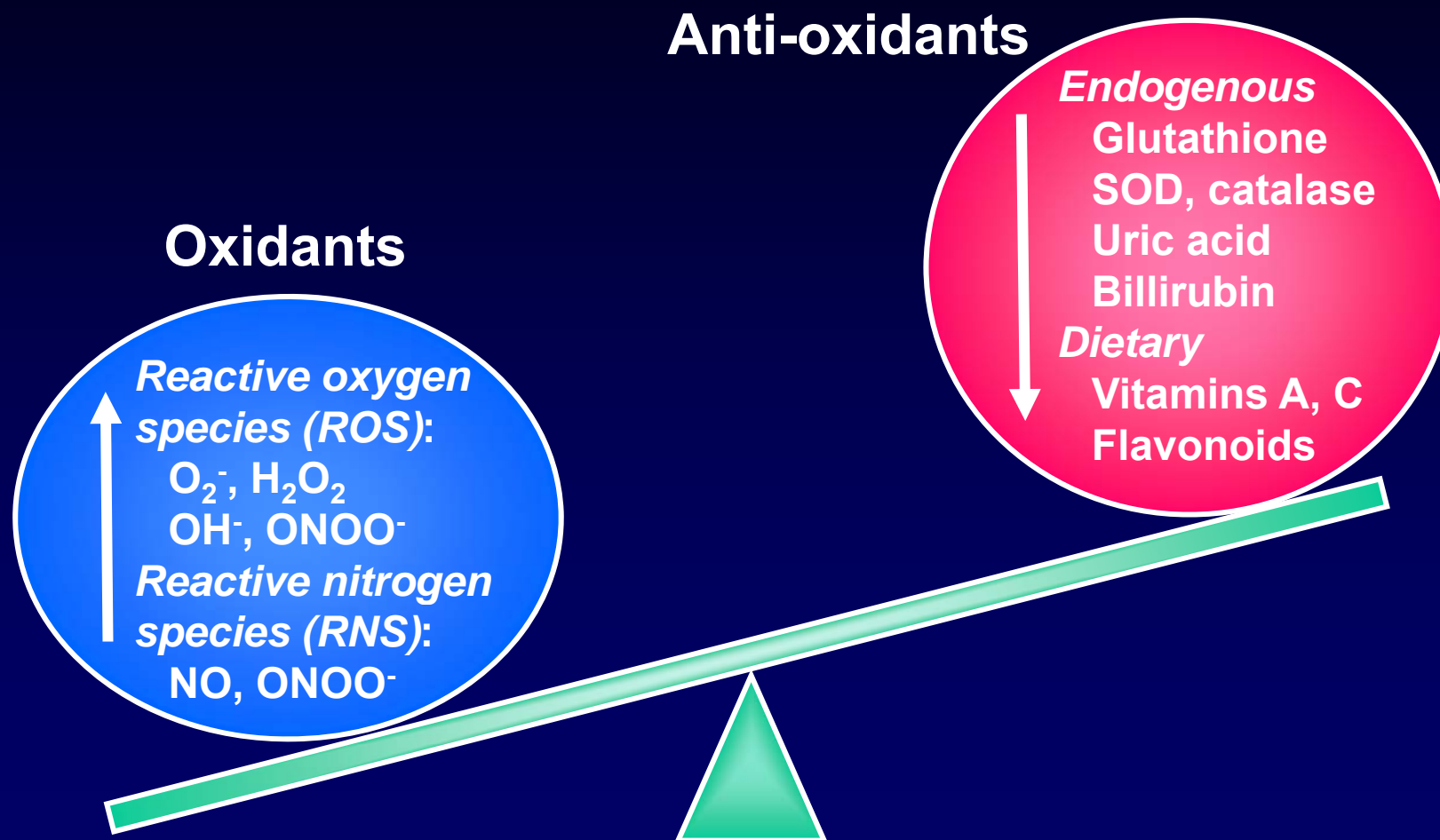
Protease-antiprotease 'imbalance' in COPD



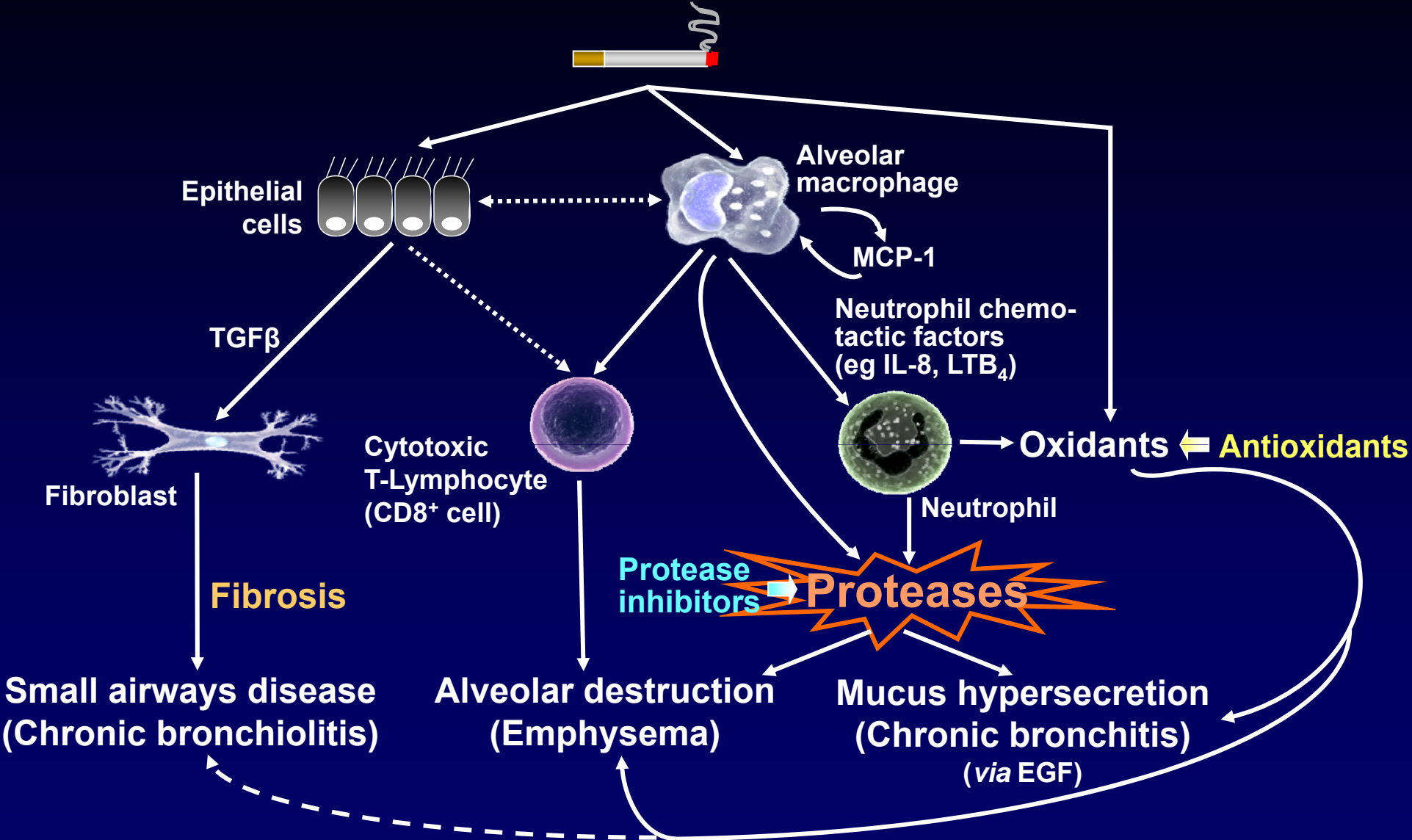
Lung elastase in respiratory diseases (induced sputum)



Oxidant-antioxidant 'imbalance' in COPD



COPD pathophysiology: simple 'schema'



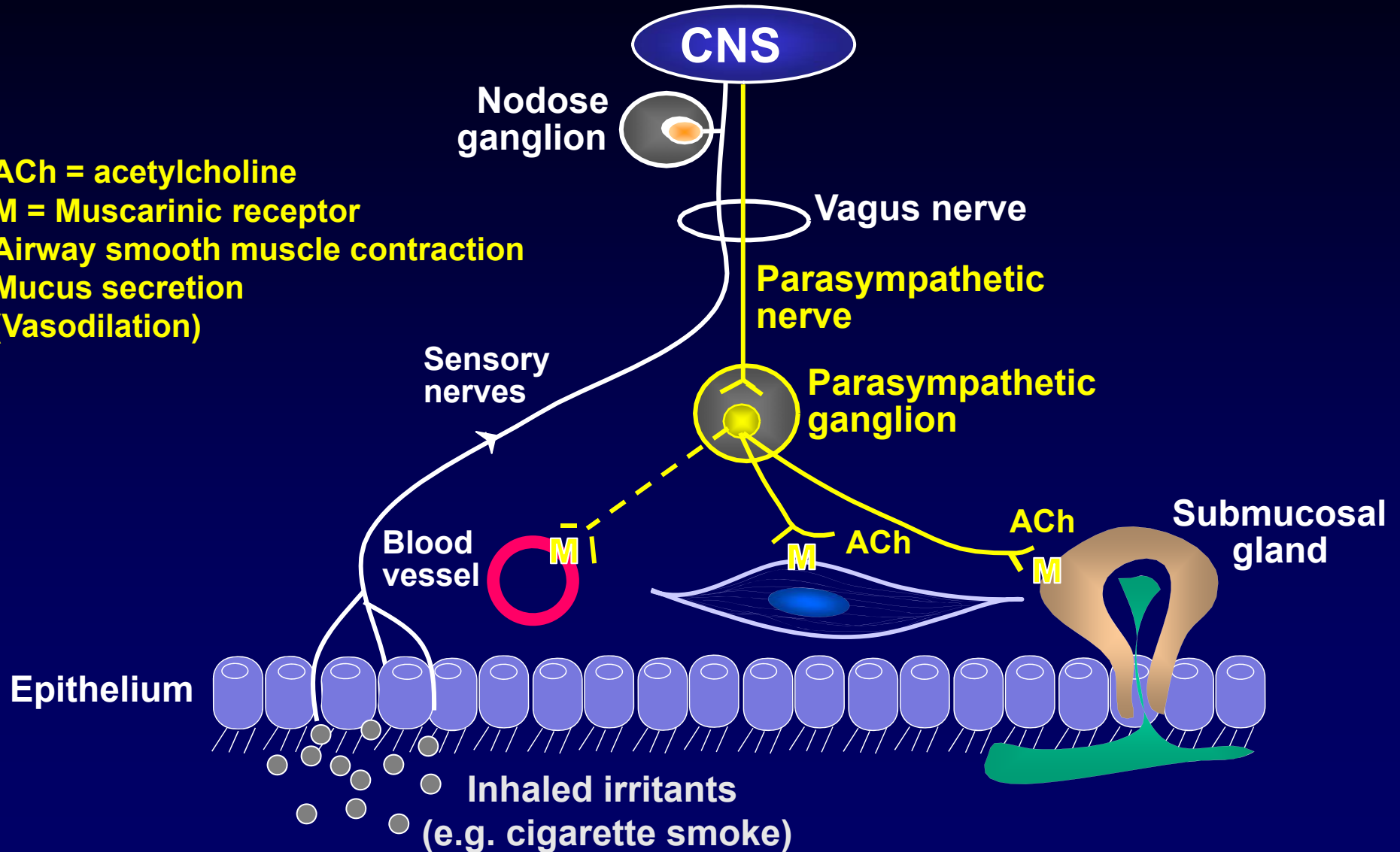
Cholinergic mechanisms in the airways

ACh = acetylcholine

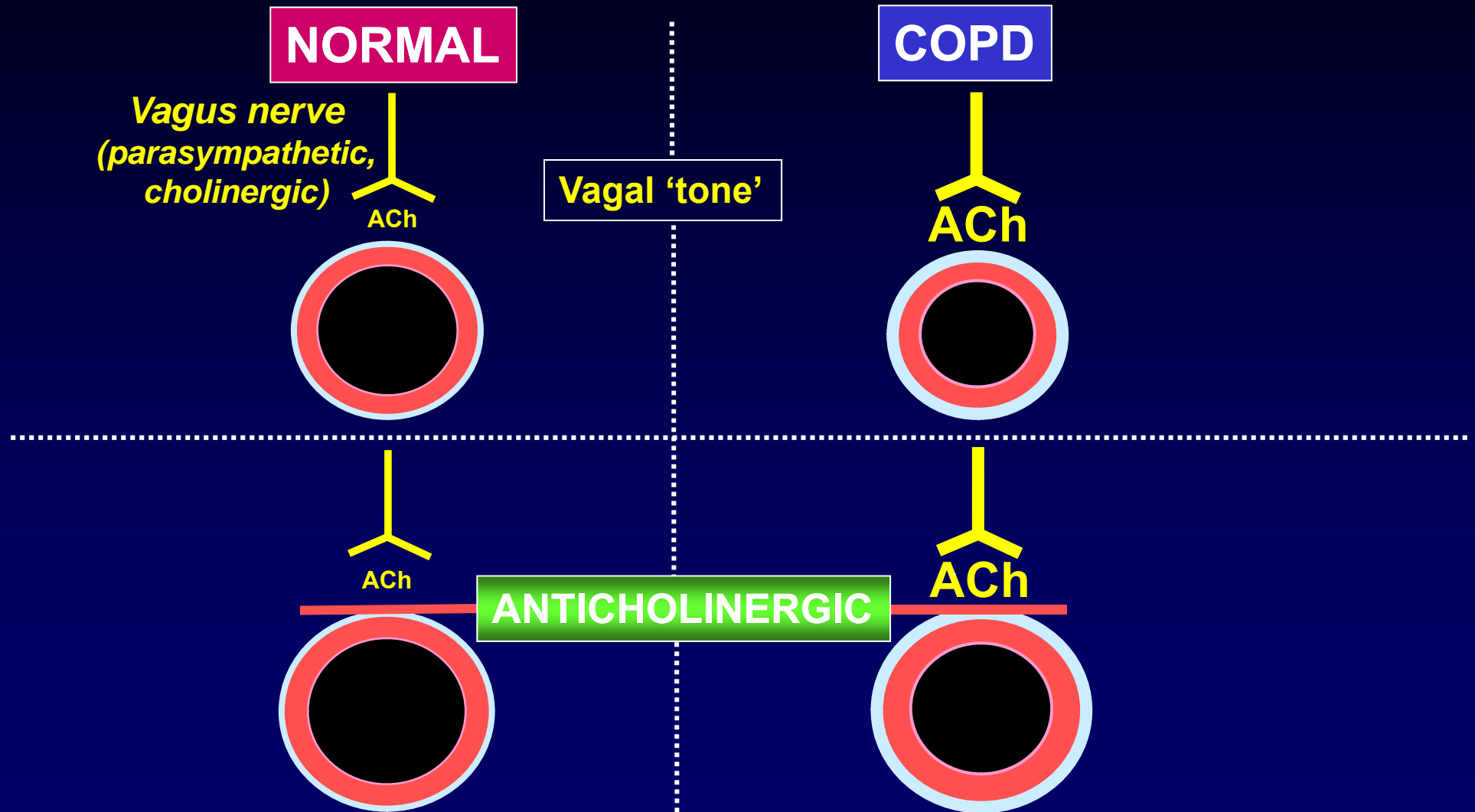
M = Muscarinic receptor

Airway smooth muscle contraction

Mucus secretion
(Vasodilation)



Cholinergic nerves and anticholinergics in COPD airways



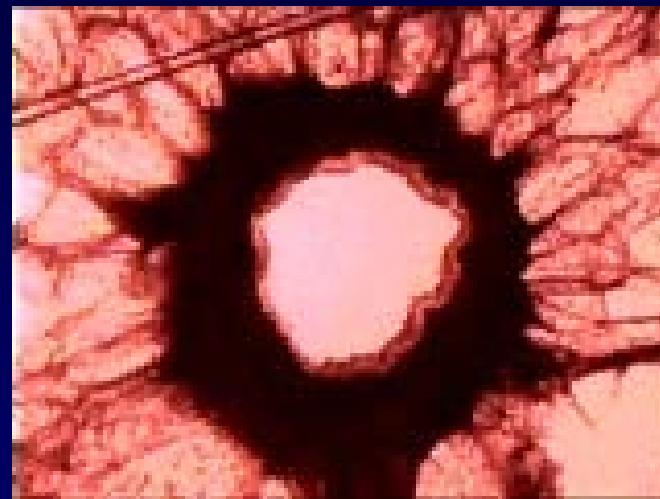
Cholinergics vs anticholinergics in human small airways

Cholinomimetic = carbachol (CCh), 10 nM – 1 μ M, image every 10 sec
Anticholinergic = tiotropium bromide (TB), 0.3 nM

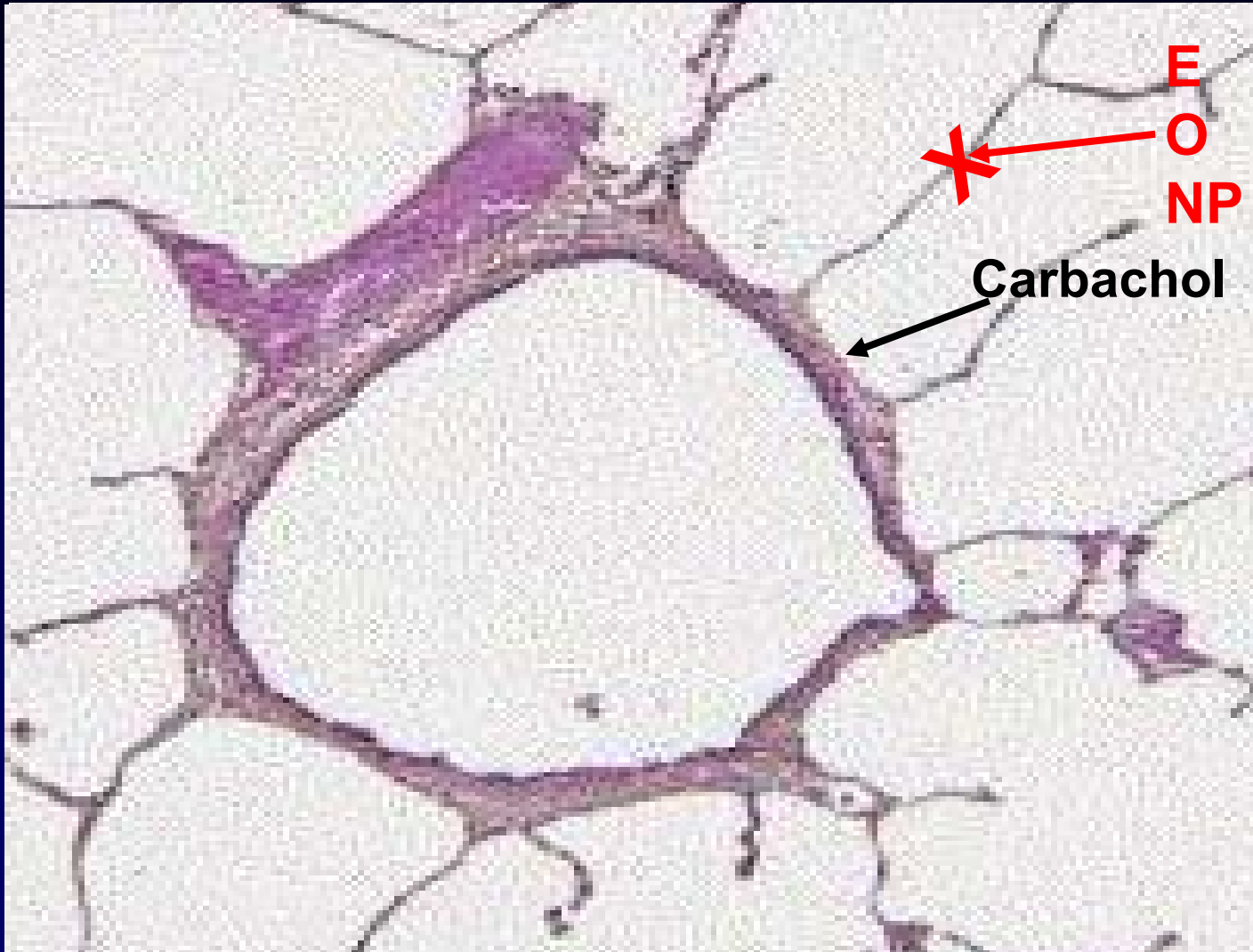
CCh



TB + CCh



Effect of elastase (E), oxidants (O) or neutrophil products (NP) on small airway calibre?



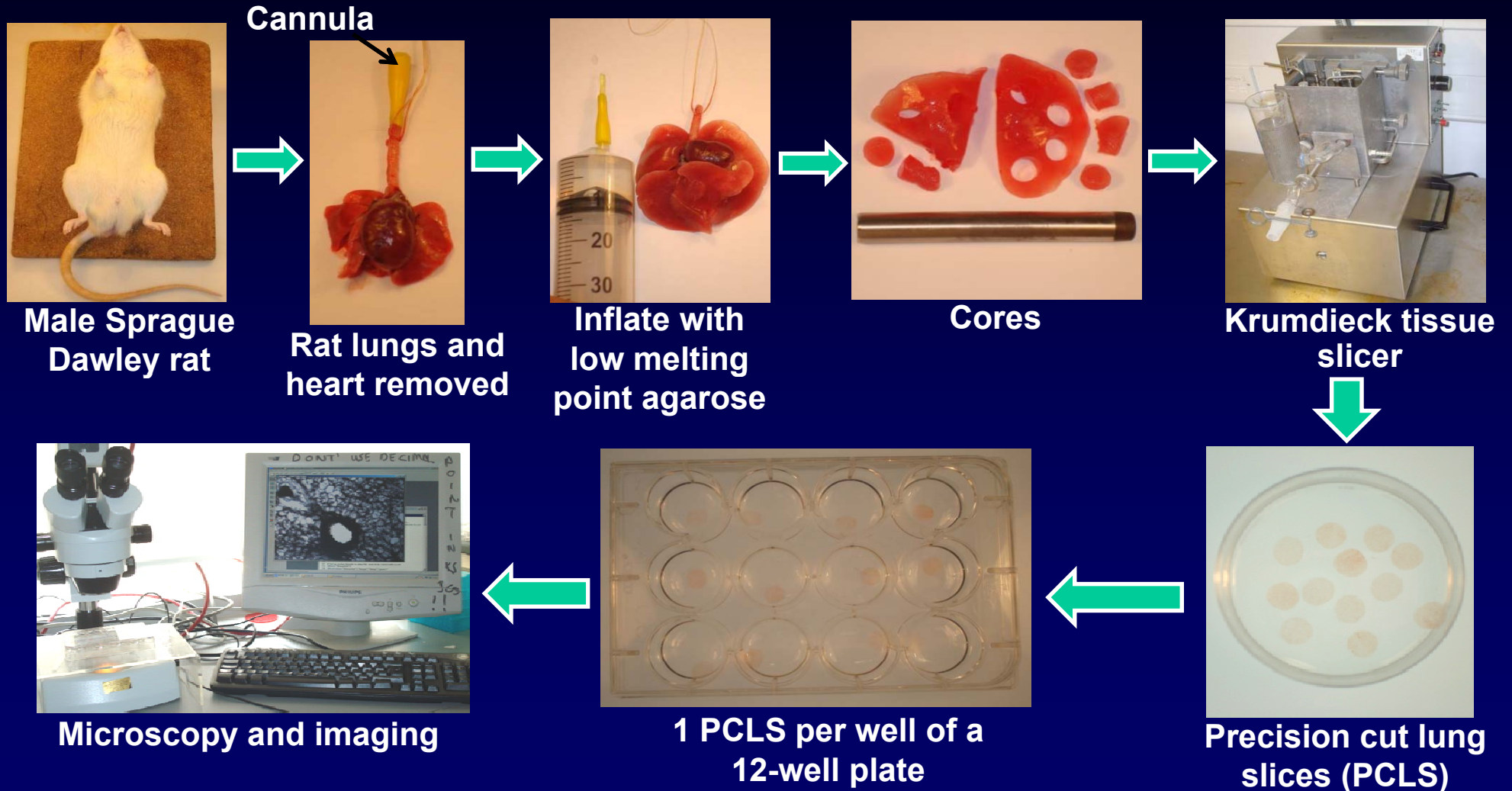
Hypothesis

**Elastase, oxidants and neutrophil products alter the contractile response of small airways
(? more 'easily' contracted)**

Main aims

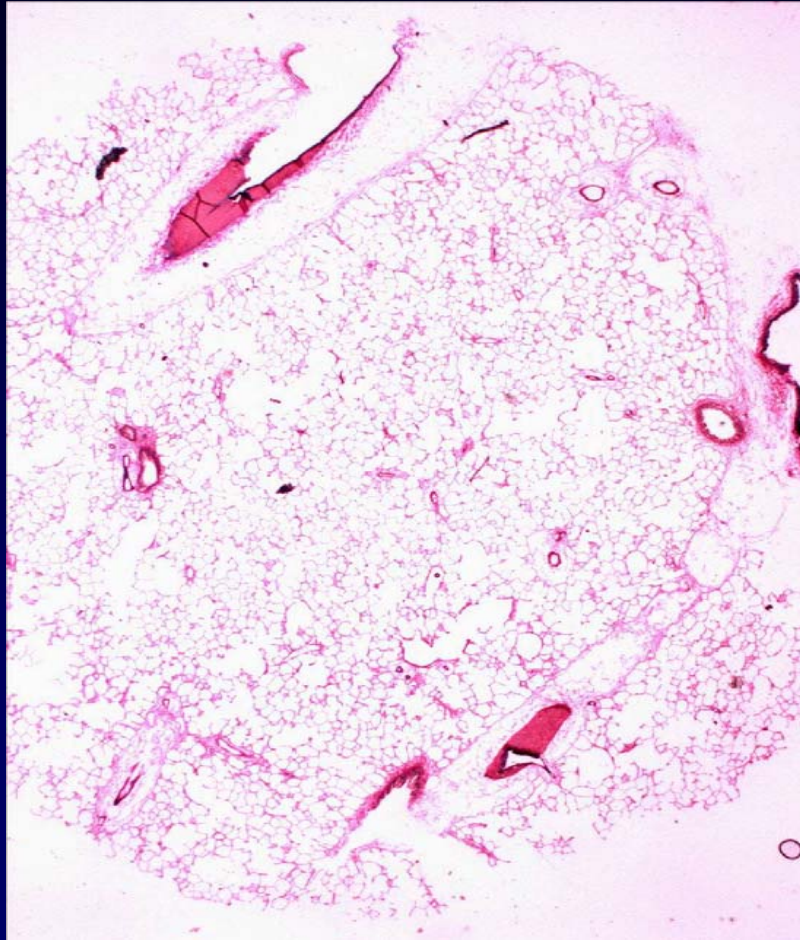
- **Examine the effects of elastase, oxidants and neutrophil products on structure of rat small airways and surrounding parenchyma**
- **Investigate effect of elastase, oxidants and neutrophil products on small airway contraction to carbachol (CCh – stable analogue of acetylcholine)**

Preparation of rat precision cut lung slices (PCLS)

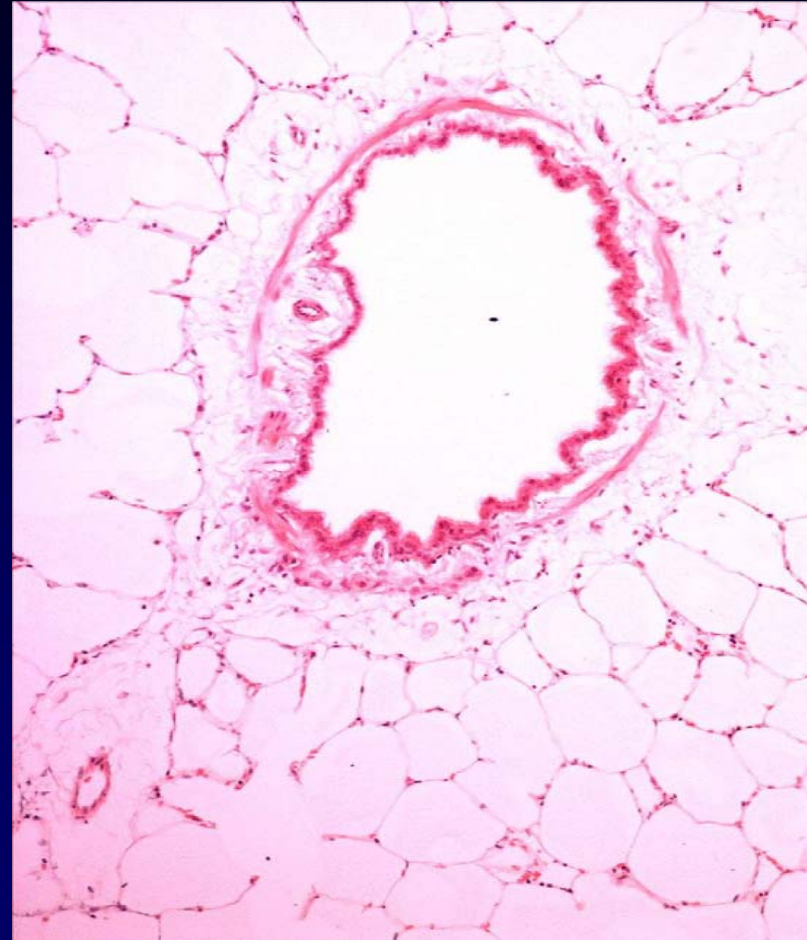


Histology of rat PCLS

Low power - entire lung slice

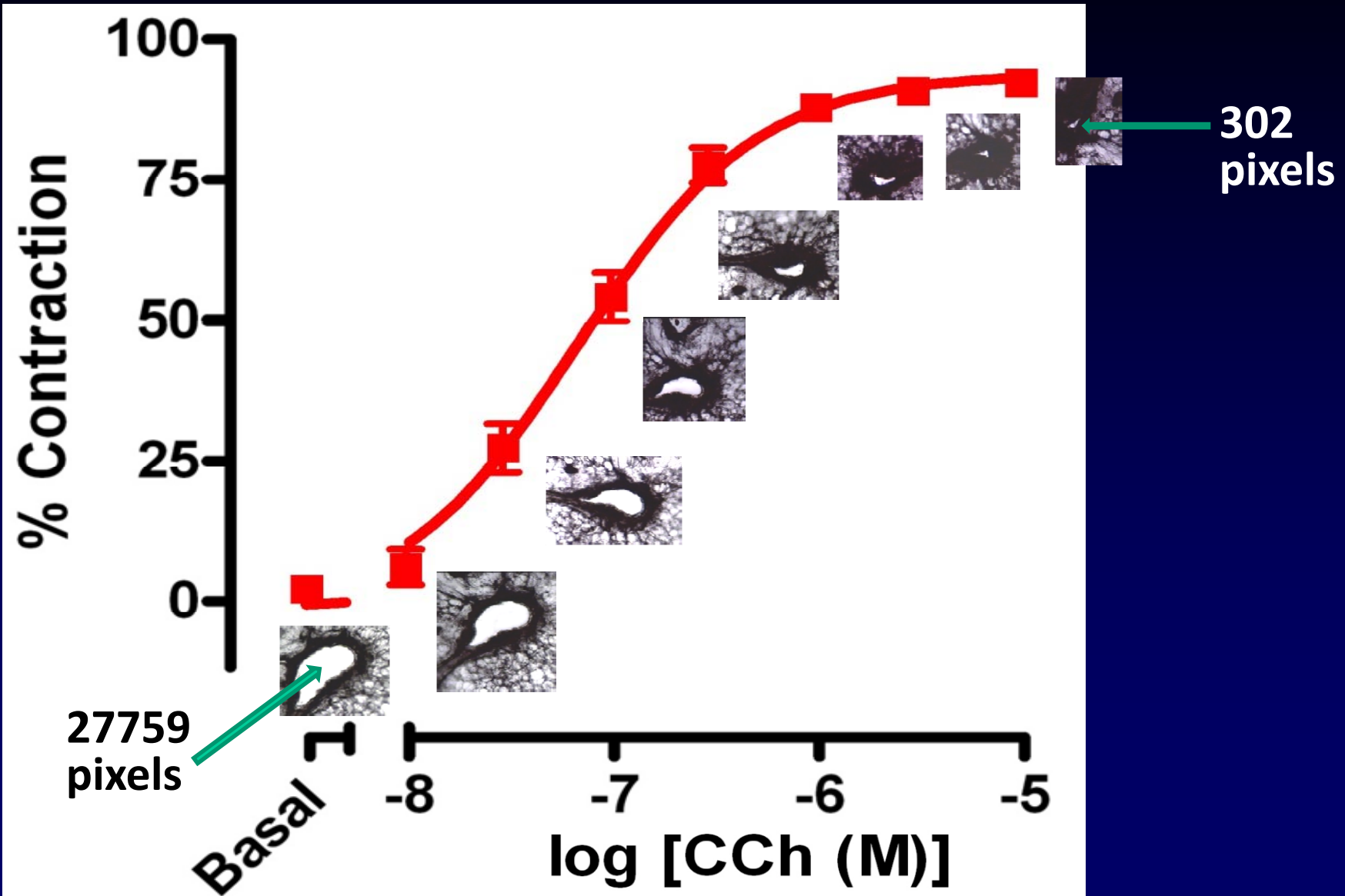


Higher power – small airway

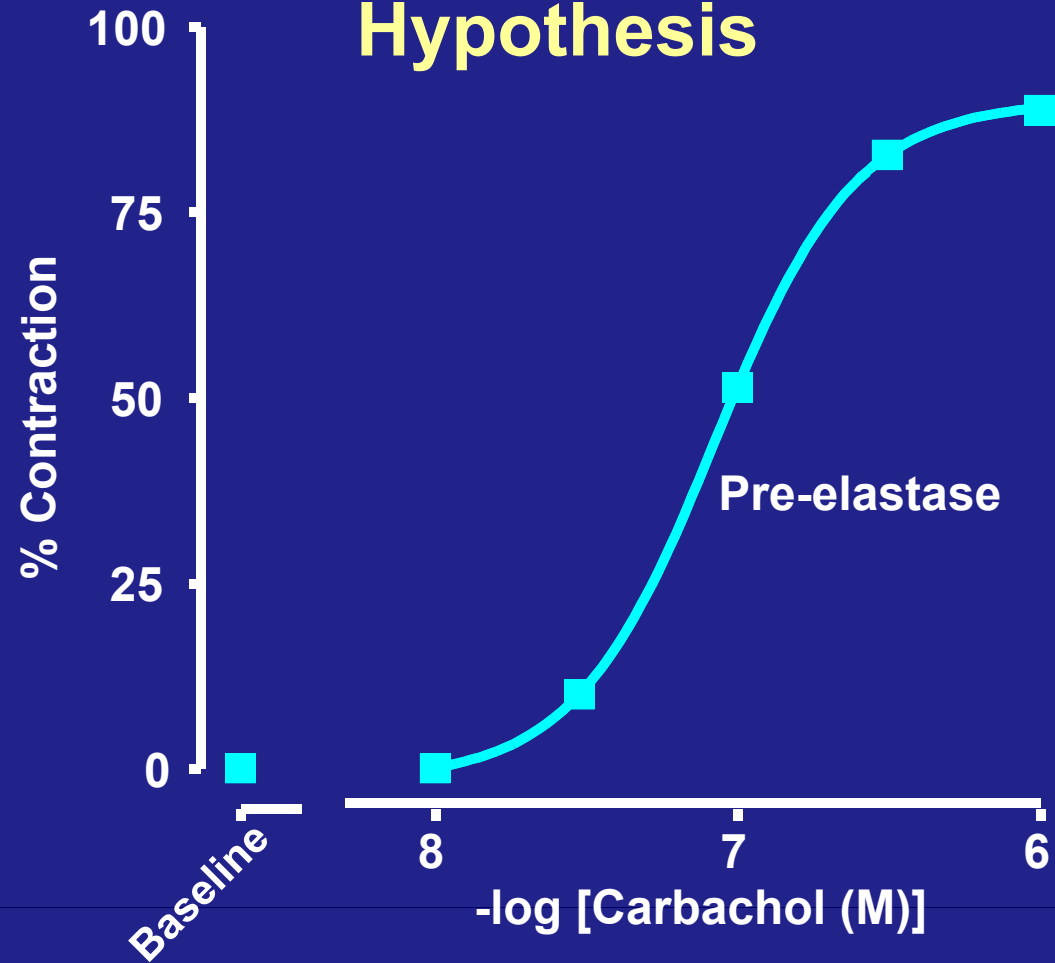


H&E stain

Observing and quantifying CCh contraction

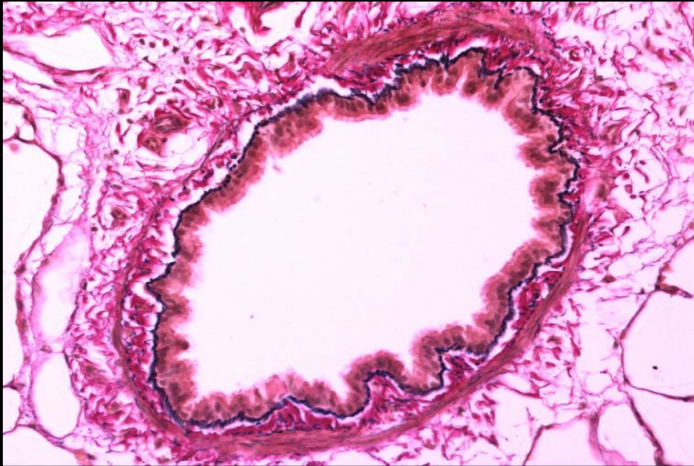


Hypothesis

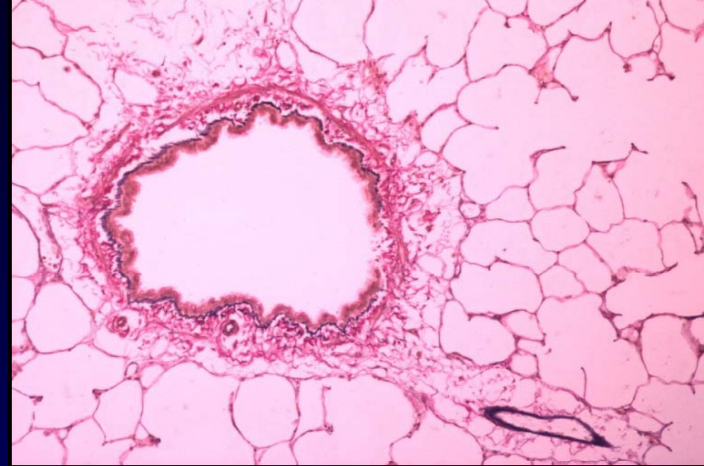


Effect of elastase on lung slices (and elastin content)

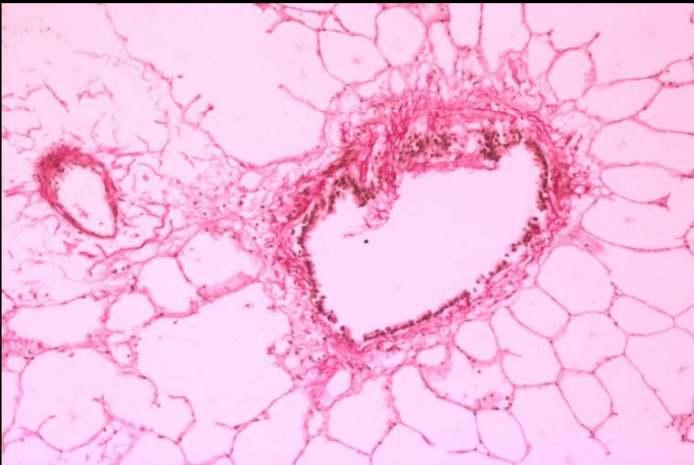
Control (higher mag.)



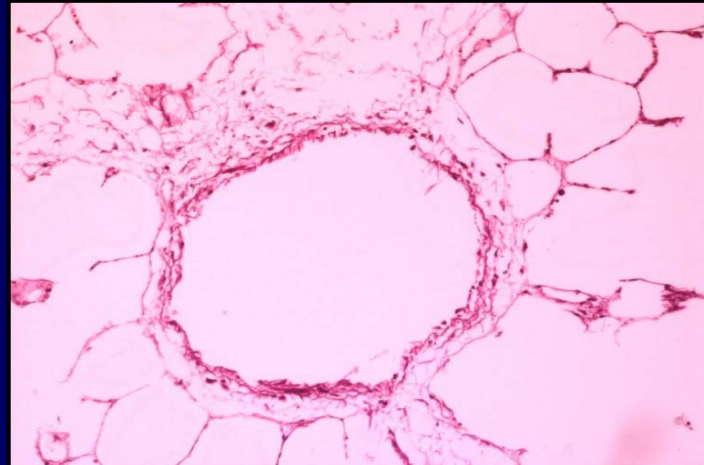
0.01 U/ml elastase



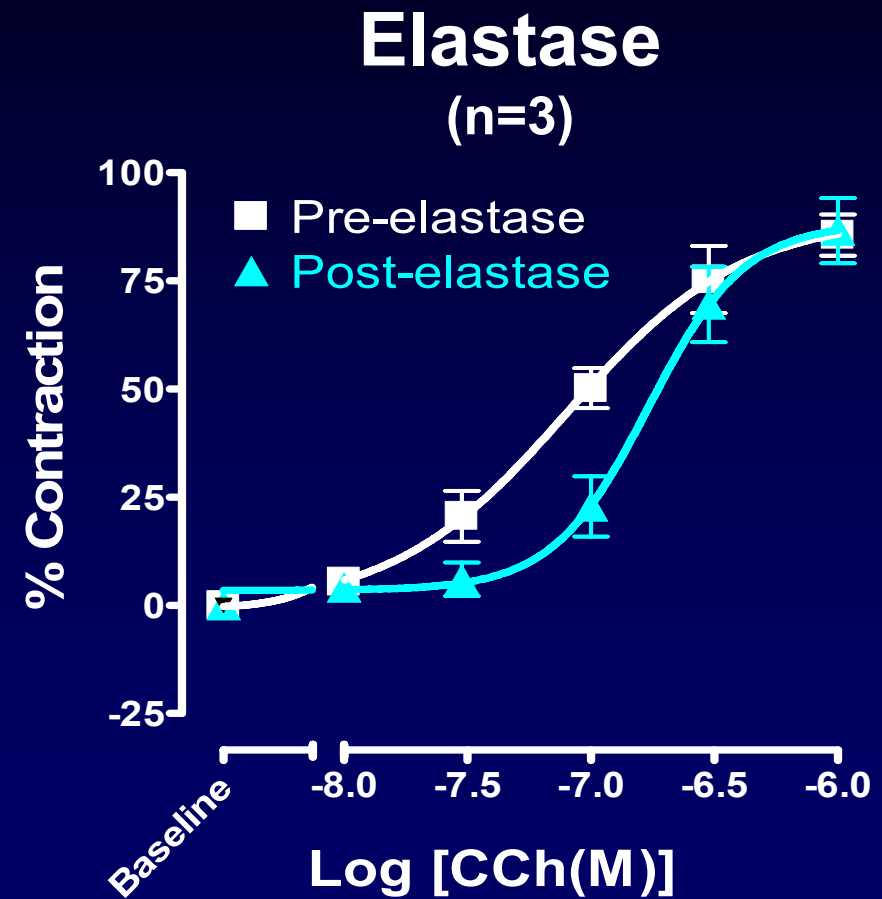
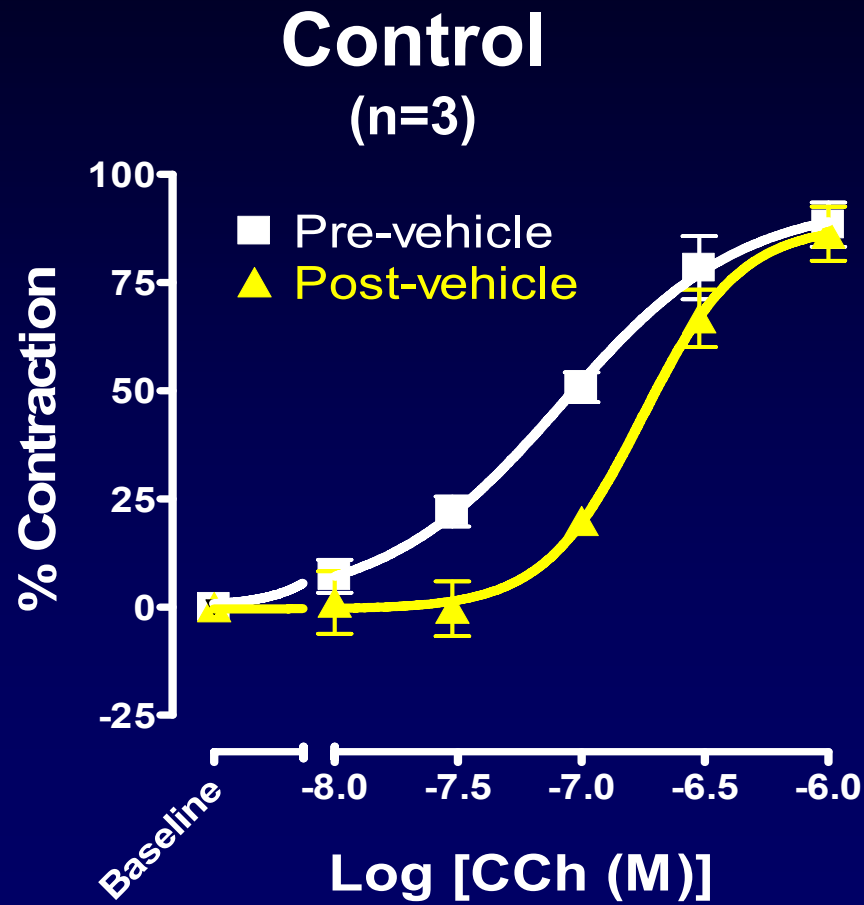
0.1 U/ml elastase



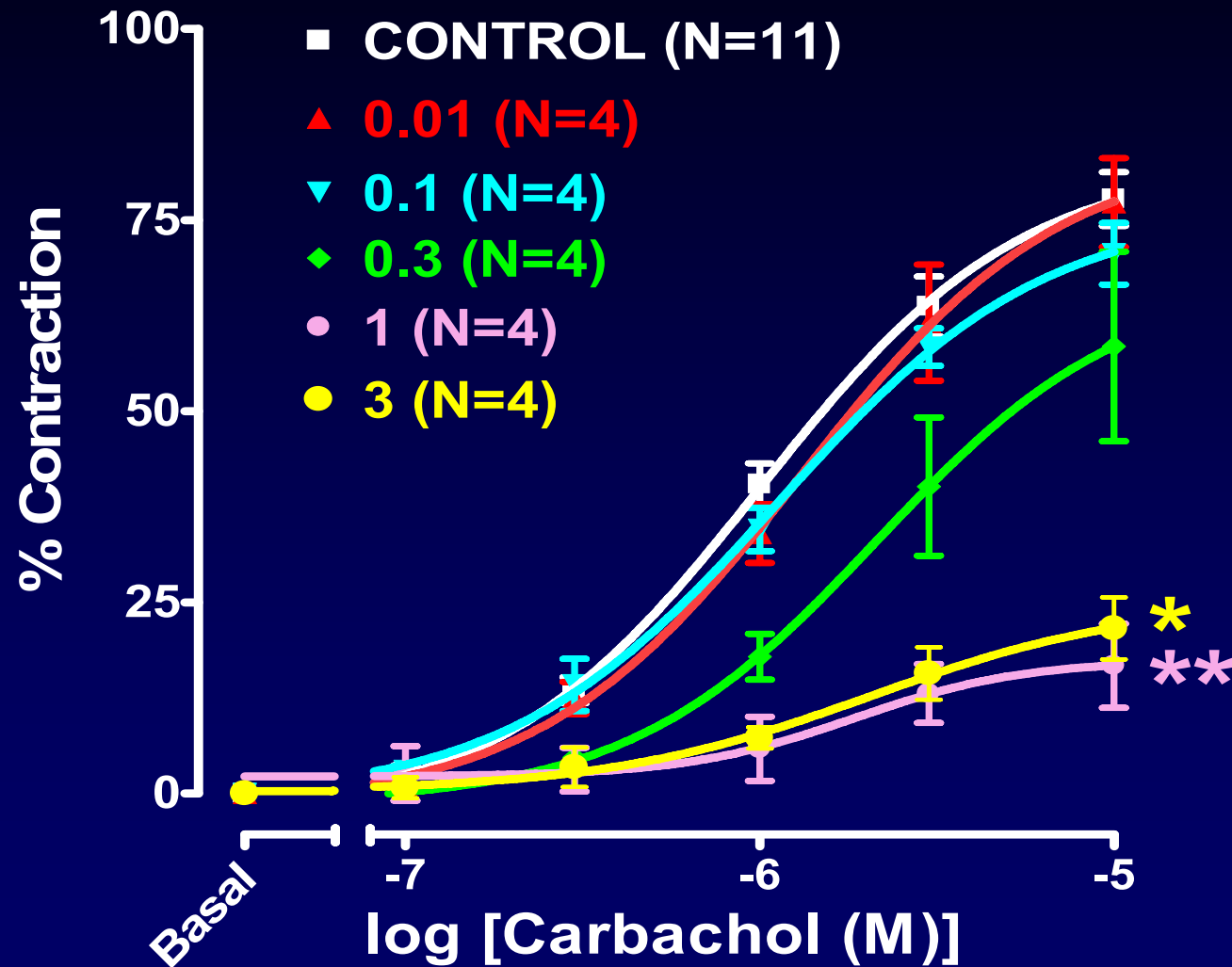
1 U/ml elastase



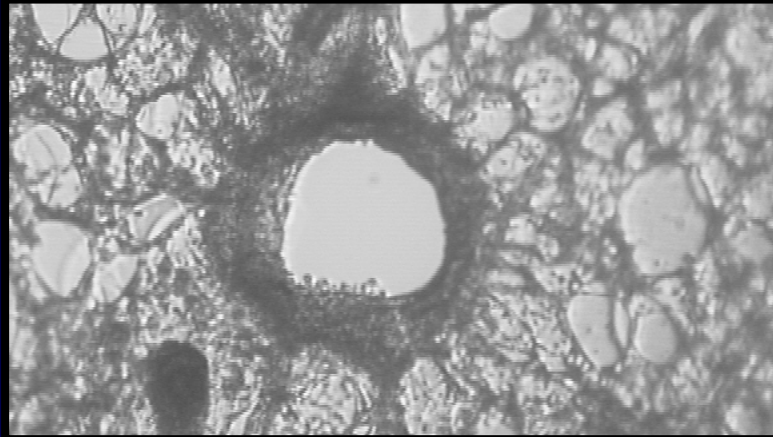
Effect of elastase (1 U/ml) for 1 hour on rat small airway contraction



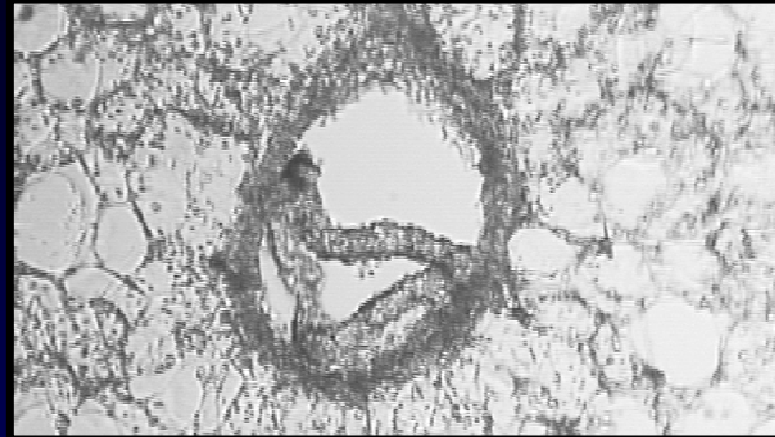
Effect of elastase overnight (~16 h)



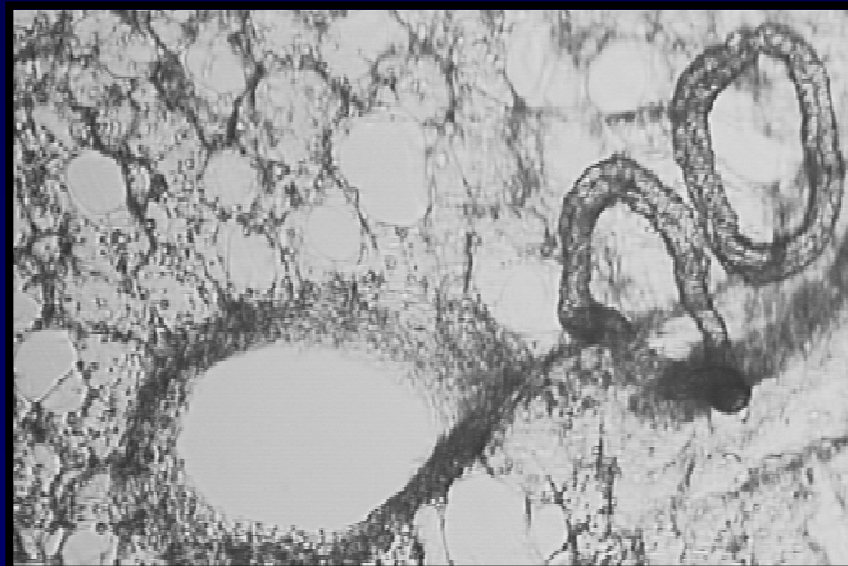
Effect of higher concentrations of elastase on small airway morphology (camera images)



Control

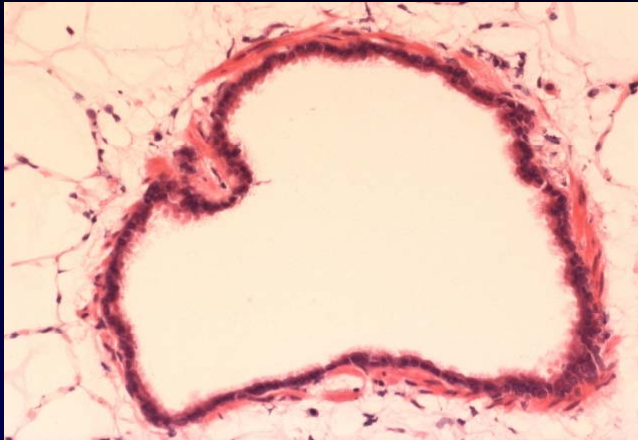


**10 U/ml
elastase**

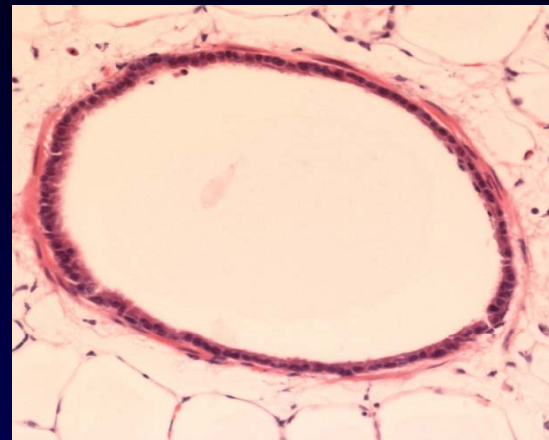


**30 U/ml
elastase**

Effect of H_2O_2 and cigarette smoke medium (CSM) on histology of rat small airways

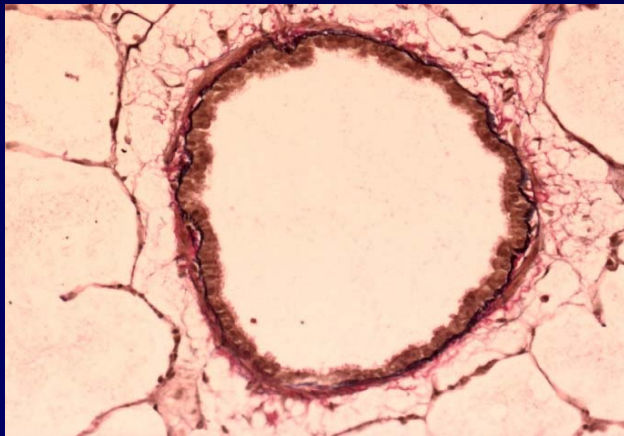


Control

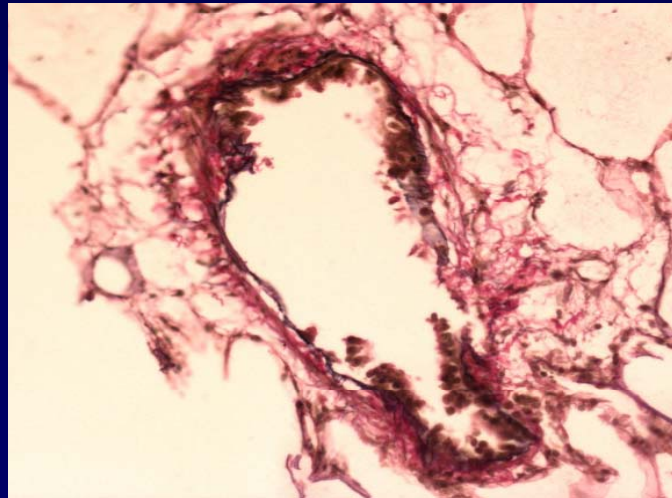


50% CSM

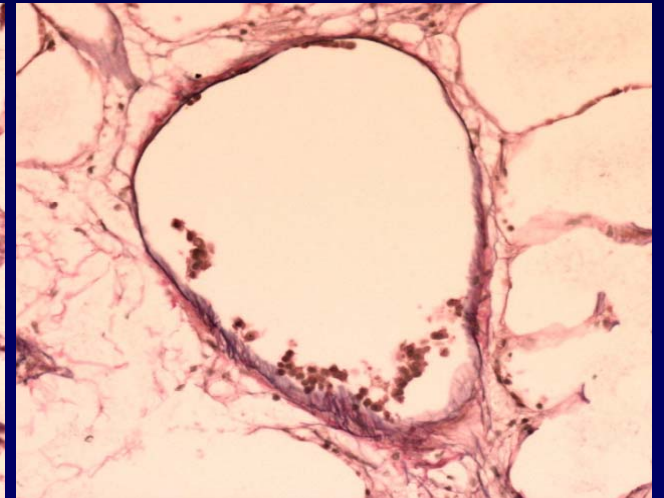
CSM: smoke from one cigarette bubbled through incubation buffer and diluted appropriately



1mM H_2O_2



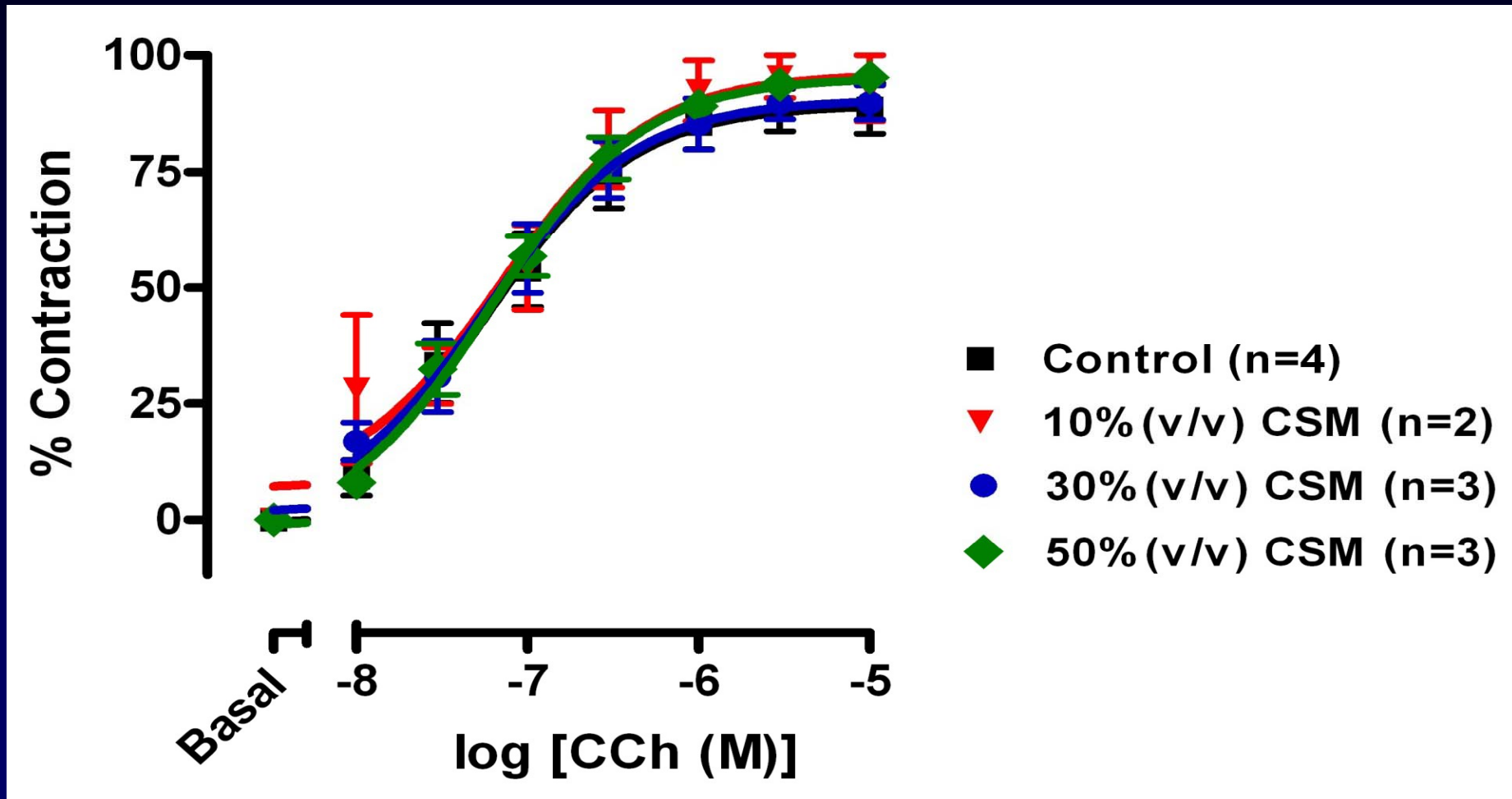
2mM H_2O_2



3mM H_2O_2

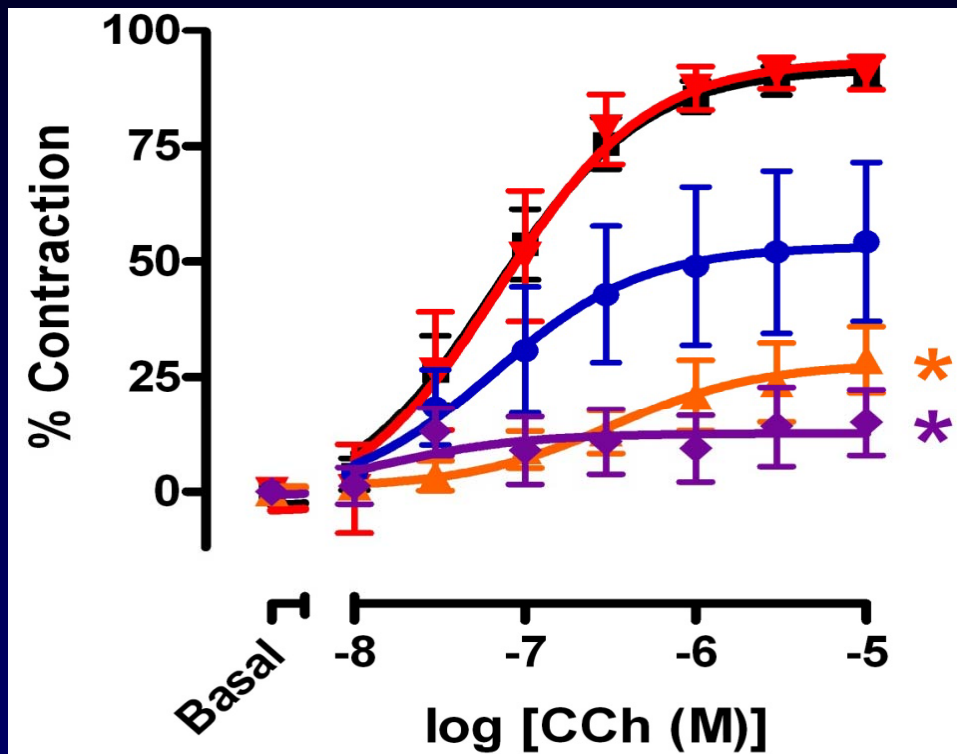
H&E stain

Effect of overnight CSM on contraction of rat small airways



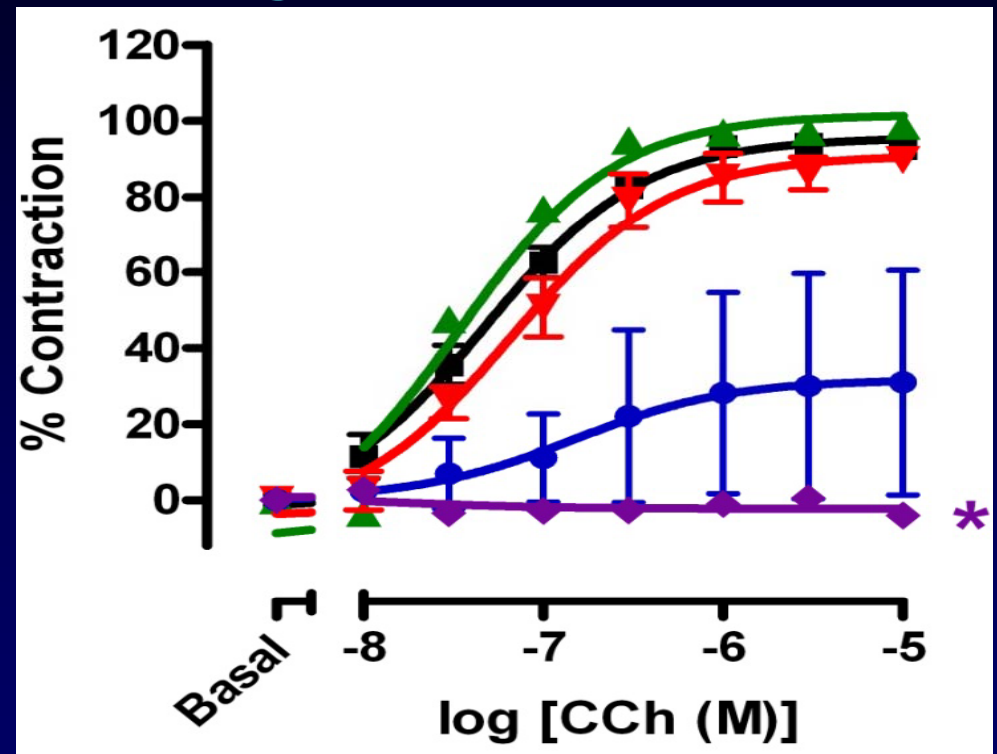
Effect of H₂O₂ on contraction of rat small airways

3h incubation



- Control (n=8)
- ▼ 1mM H₂O₂ (n=4)
- 2mM H₂O₂ (n=5)
- ▲ 3mM H₂O₂ (n=4)
- ◆ 10mM H₂O₂ (n=3)

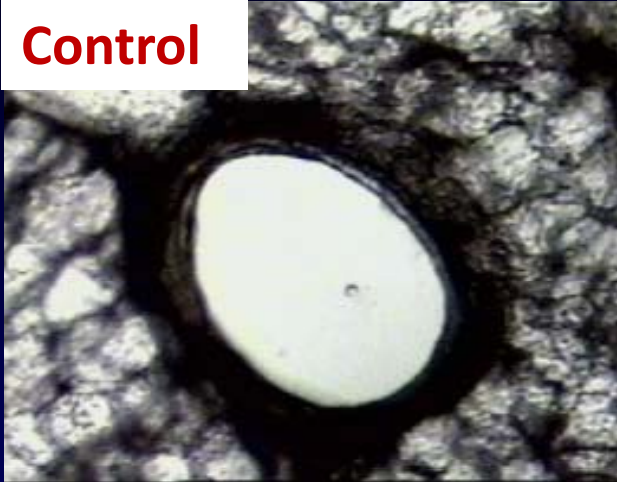
Overnight incubation



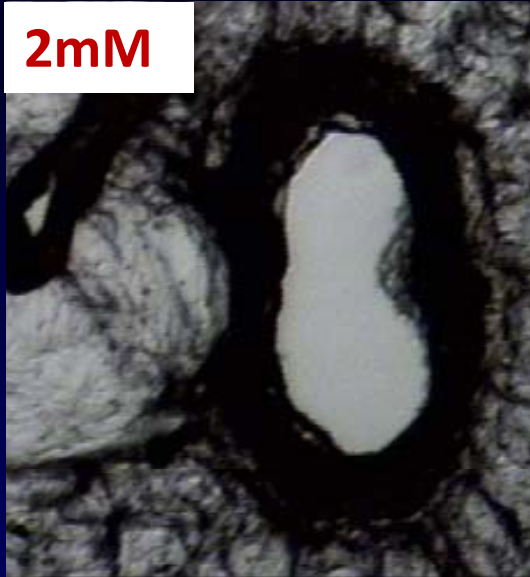
- Control (n=5)
- ▲ 0.1mM H₂O₂ (3 airways)
- ▼ 1mM H₂O₂ (n=3)
- 2mM H₂O₂ (n=3)
- ◆ 10mM H₂O₂ (n=3)

Effect of H₂O₂ (3 h) on morphology of rat small airways (camera images)

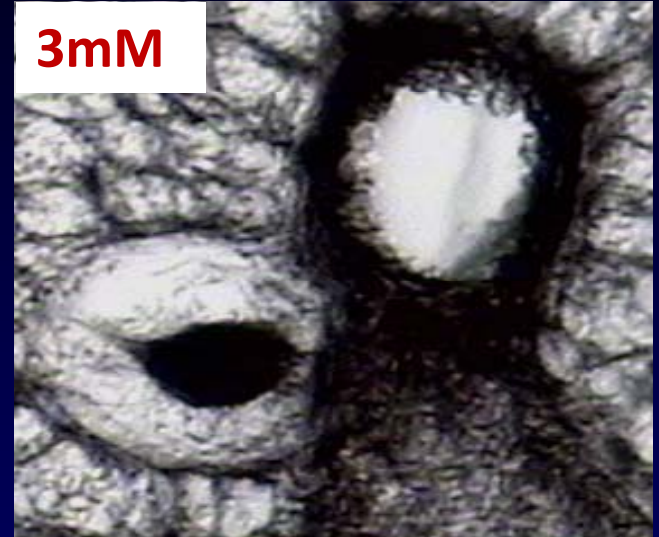
Control



2mM



3mM



1mM



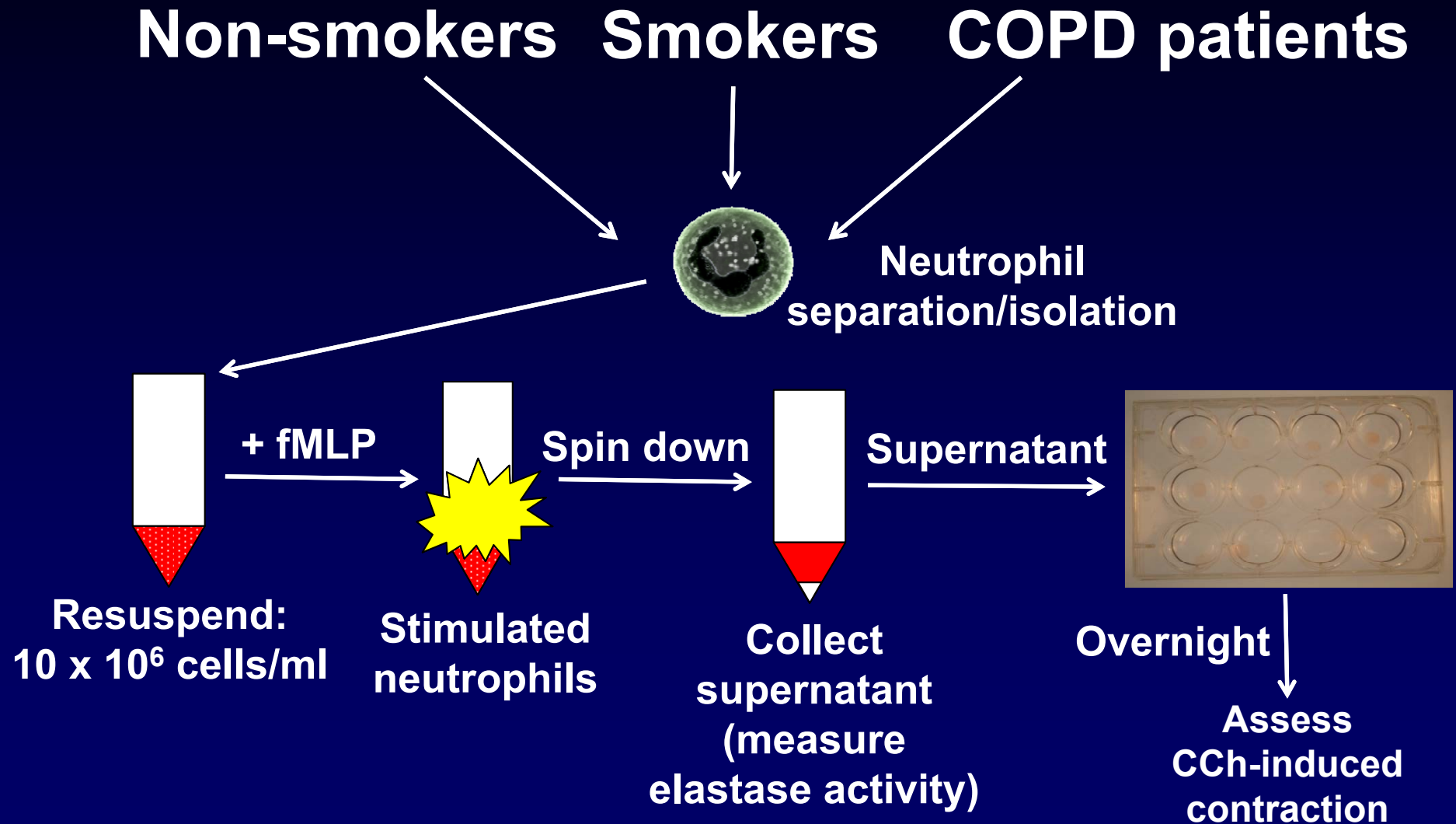
2mM

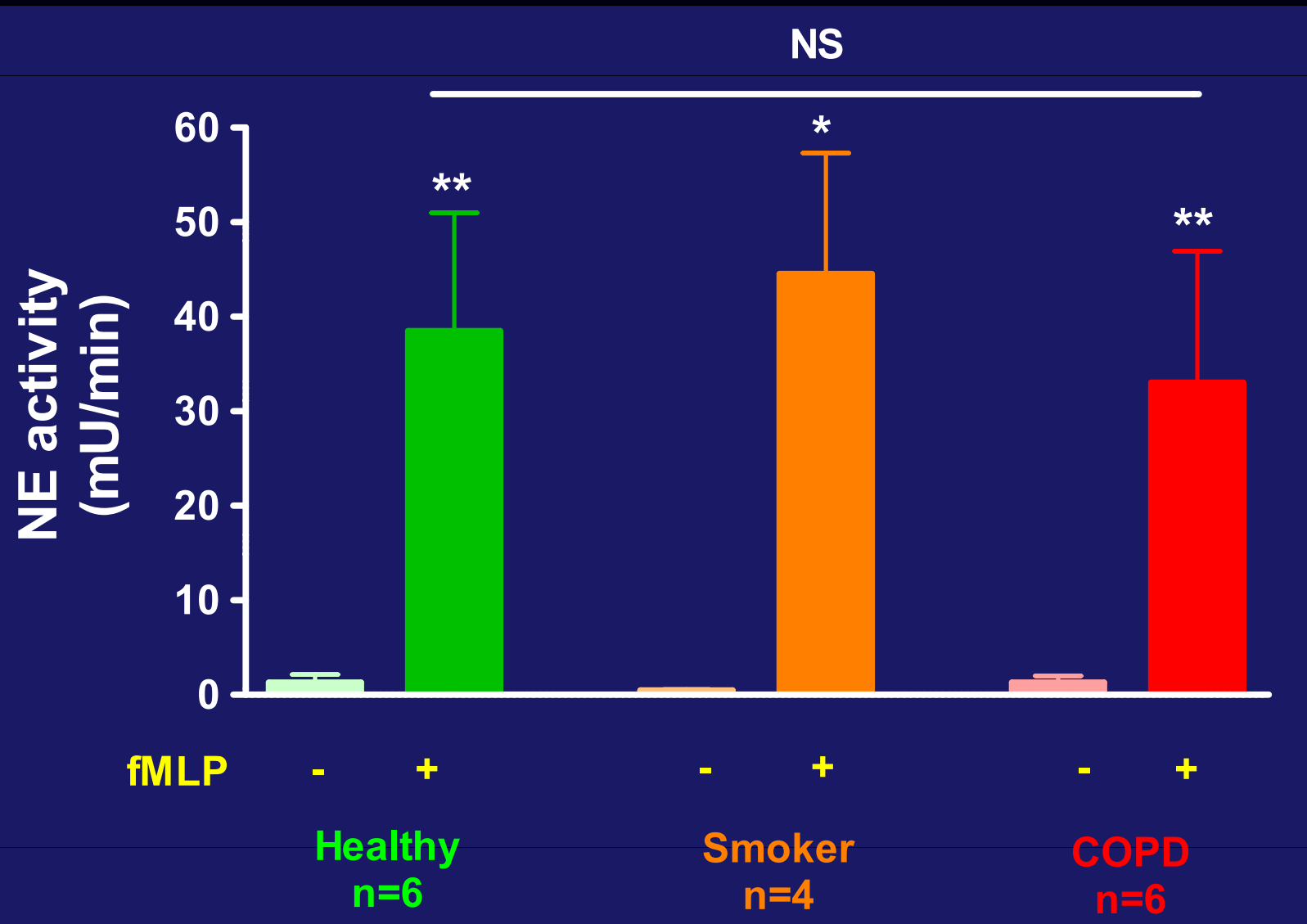


10mM



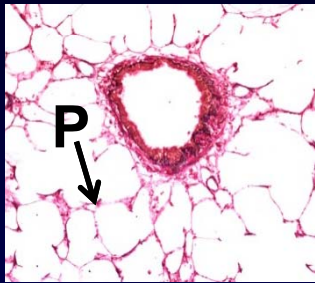
Effect of supernatants from stimulated neutrophils?



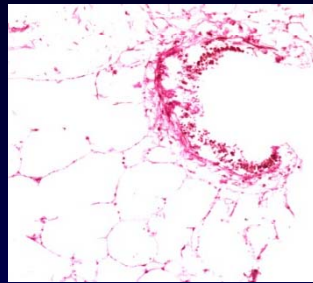


Effect of supernatants on morphology of rat small airways

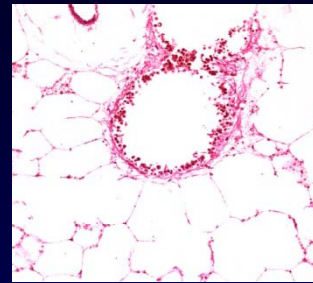
Untreated



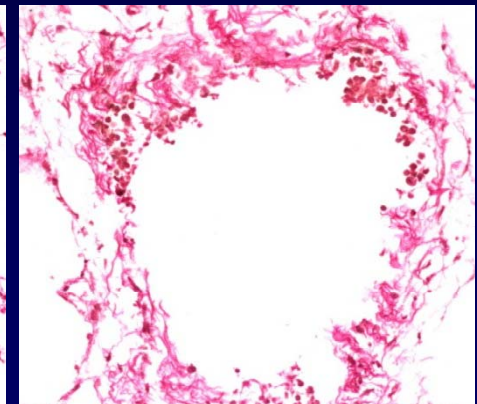
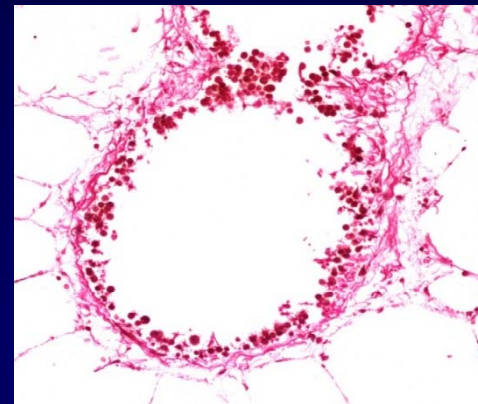
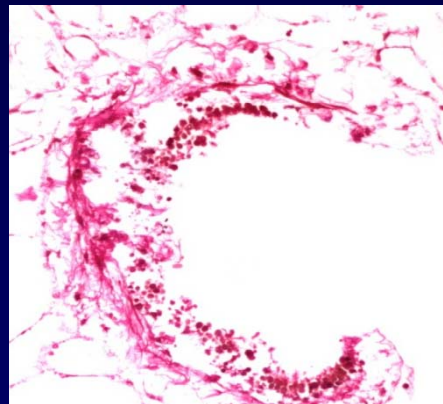
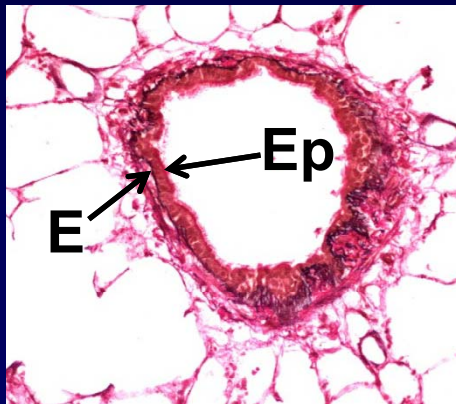
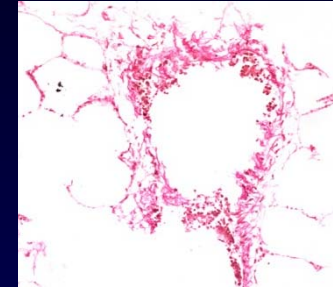
Healthy



Smoker



COPD



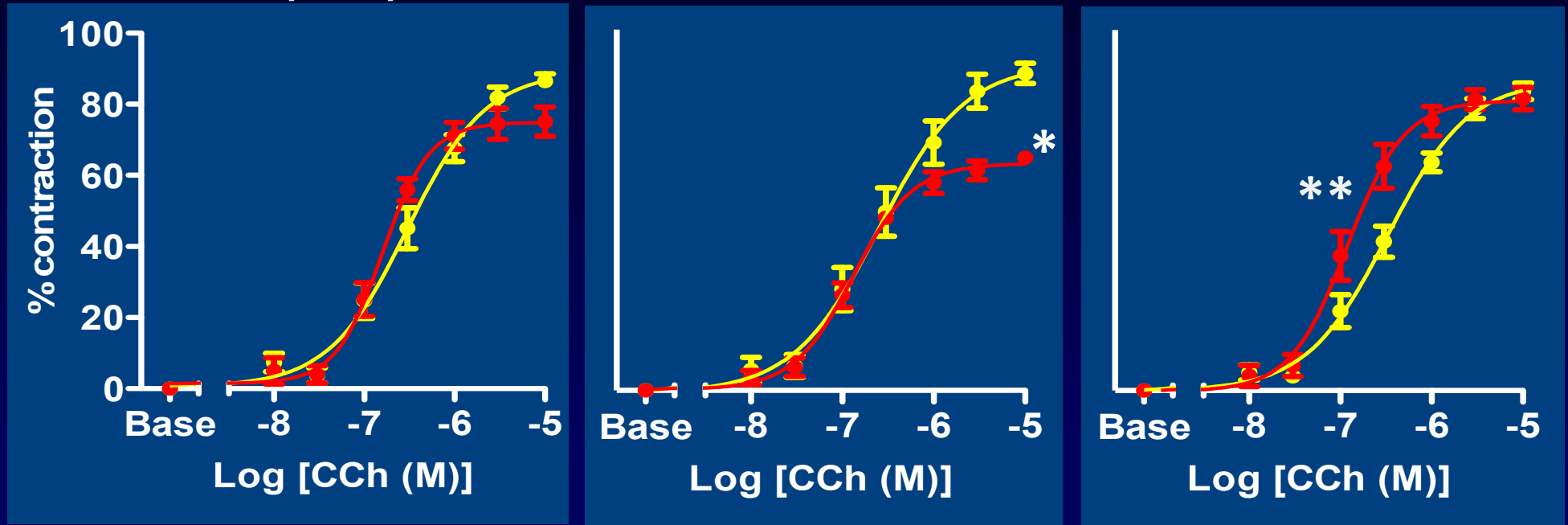
P = parenchyma, E = elastin (dark staining), Ep = epithelium
(↓ elastin in parenchyma, ↓ elastin in airway wall)

Effect of supernatants on contraction of rat small airways

Non-smokers
(n=6)

Smokers
(n=4)

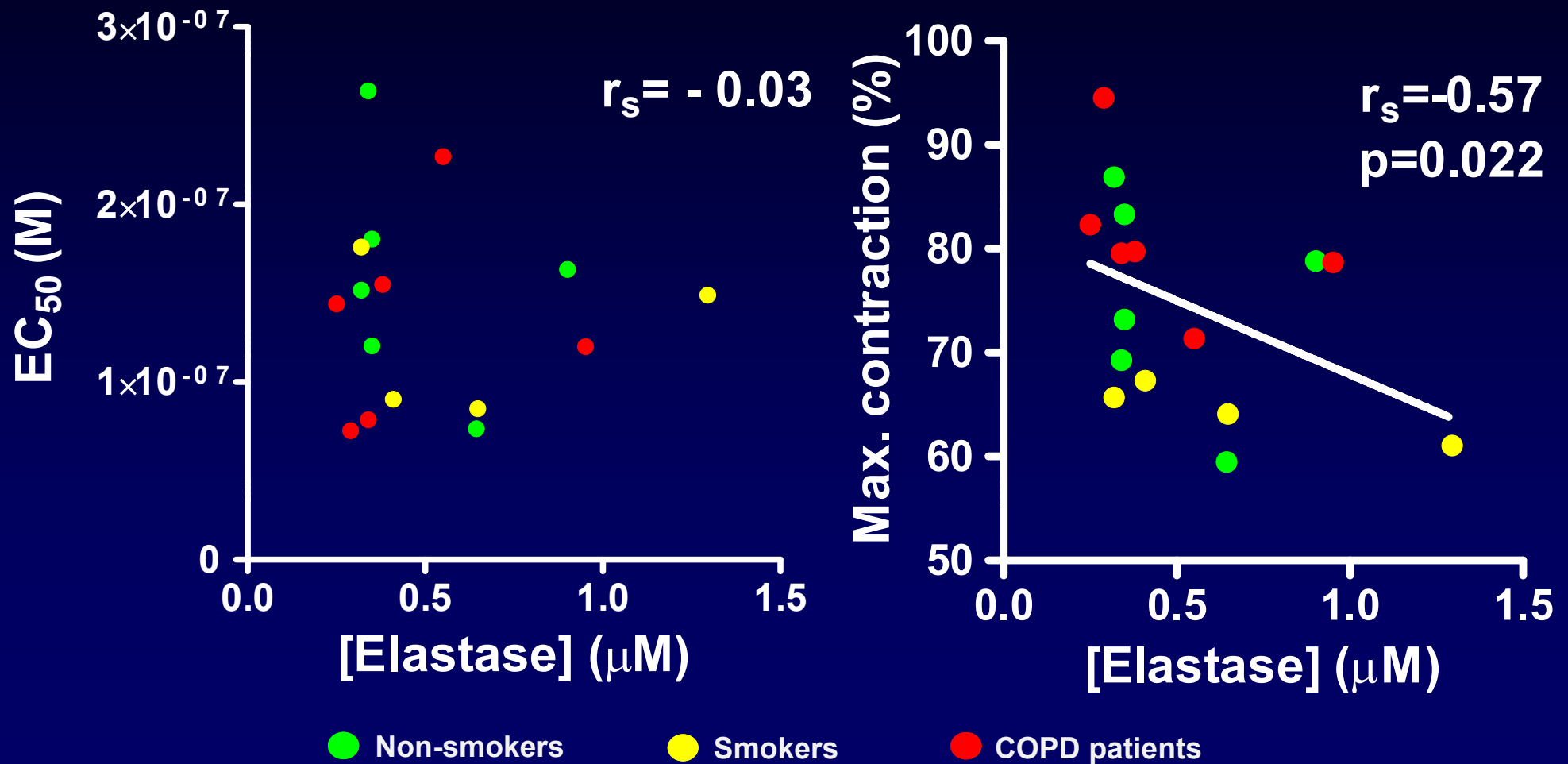
COPD
(n=6)



— Control — Supernatant

*p<0.05 (max contraction), **p<0.01 (EC₅₀)

Supernatant [neutrophil elastase]: correlations with contractility



What dose this all mean?

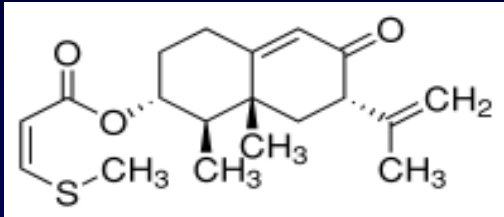
Elastin content markedly diminished in parenchyma and airway wall of PCLS incubated with stimulated neutrophil supernatants – no obvious difference between non-smokers, smokers and COPD

Parameter	REDUCED EC ₅₀	REDUCED MAXIMAL CONTRACTION
NON-SMOKERS	×	×
SMOKERS	×	✓✓
COPD PATIENTS	✓✓	×
CORRELATION WITH [NE]	×	✓

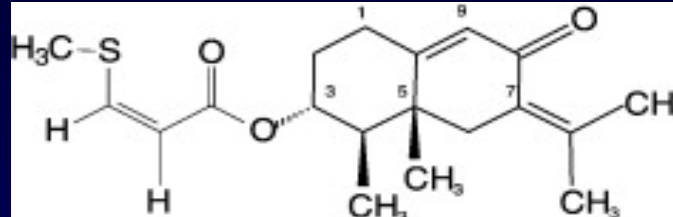
Neutrophil activation releases elastase in equal amounts from non-smokers, smokers and patients with COPD. Therefore, additional, or other, 'reactive mediators' must cause differing effects on small airway contractility between the three subject groups

Petasins

- Derived from *Petasites hybridus* (Butterbur, UK)
- Different petasins based on different isomers



S-petasin

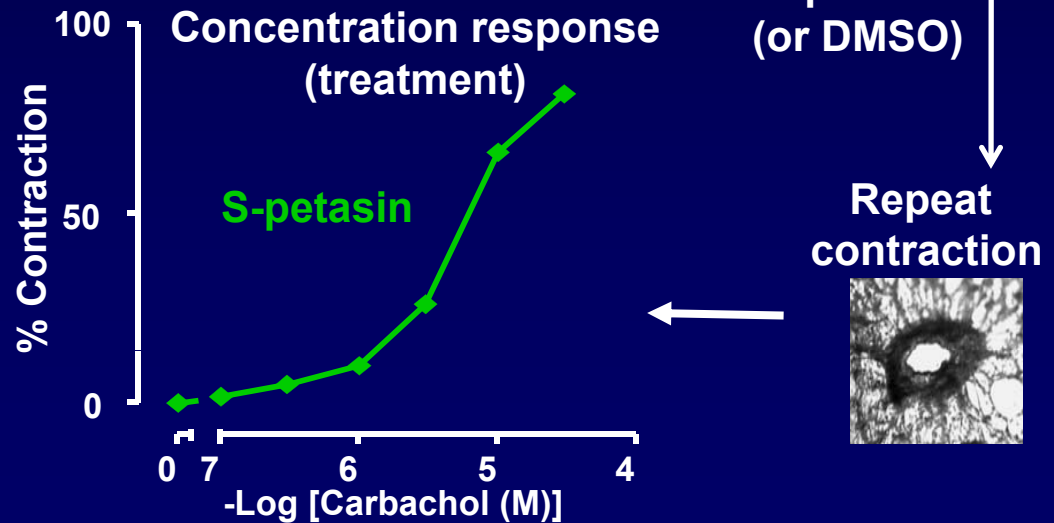
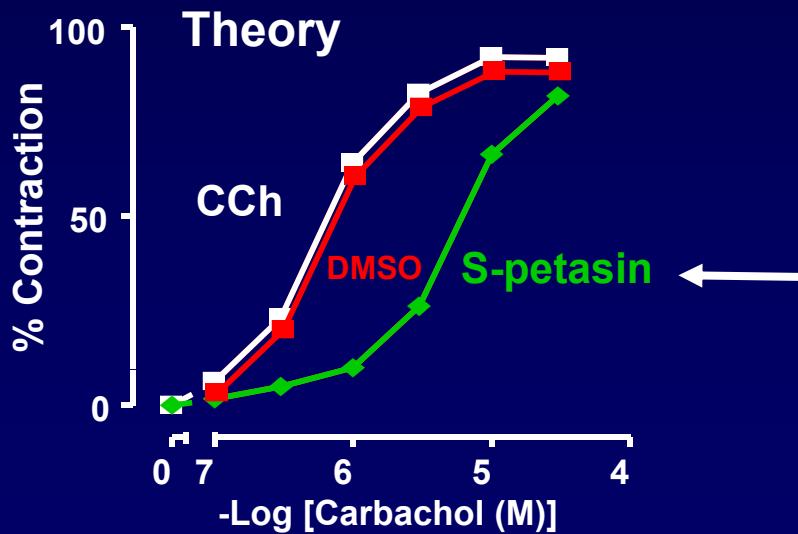
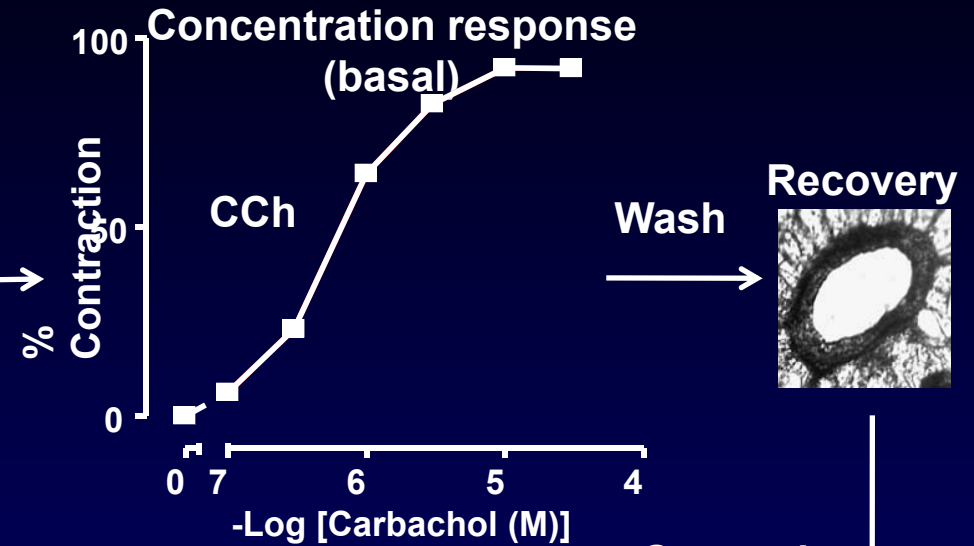
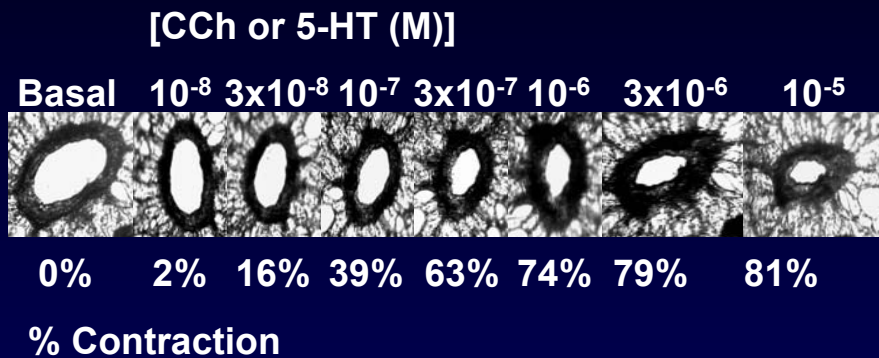


S-isopetasin

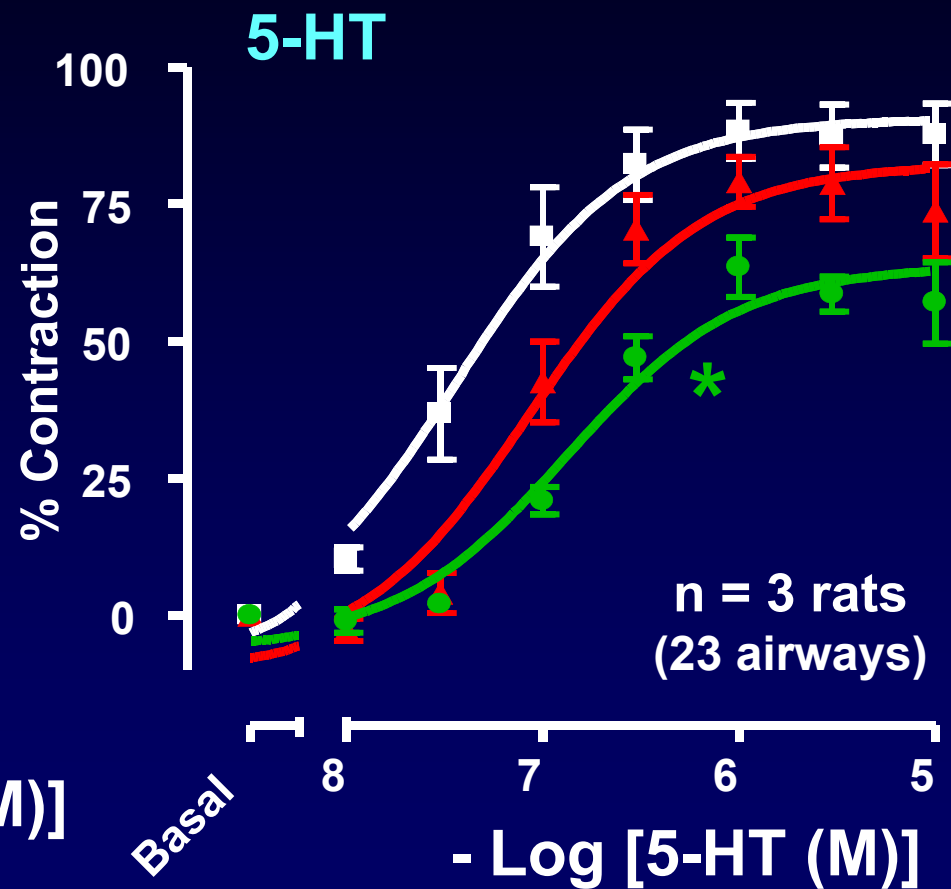
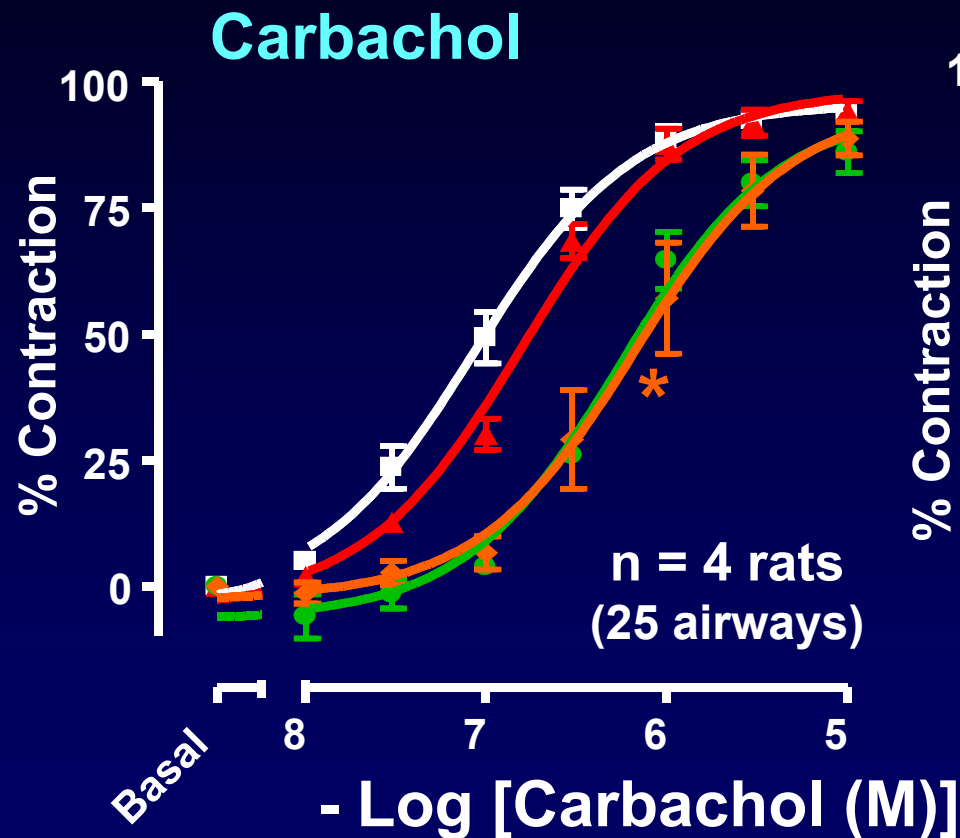


- Anti-inflammatory activity (e.g. inhibit cytokine release from inflammatory cells)
- Relax smooth muscle (e.g. guinea-pig trachea)
- Effect of S-petasin on small airways unknown

Measurement of effect of S-petasin on small airways

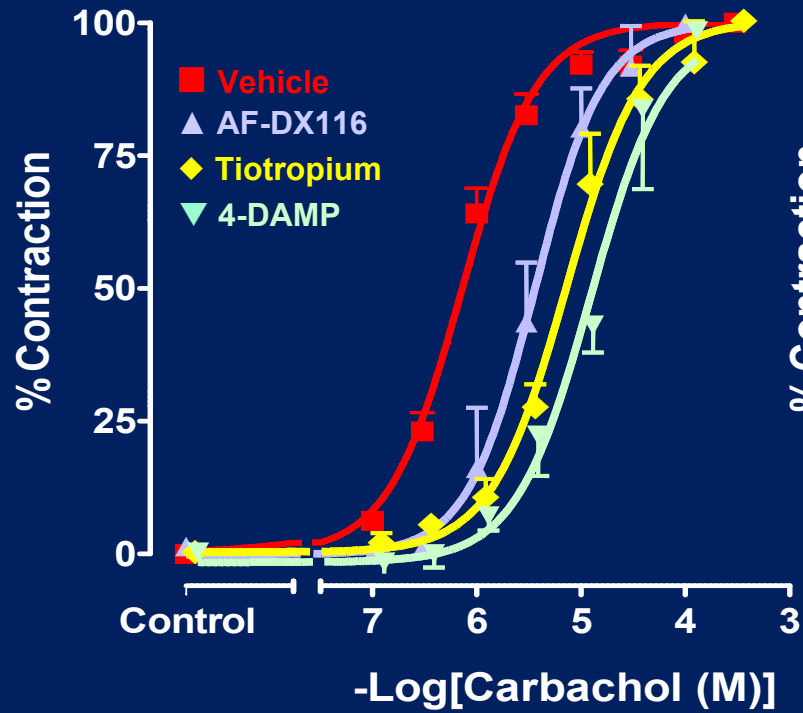


Effect of S-petasin on contractions of rat small airways ('bronchoprotection')

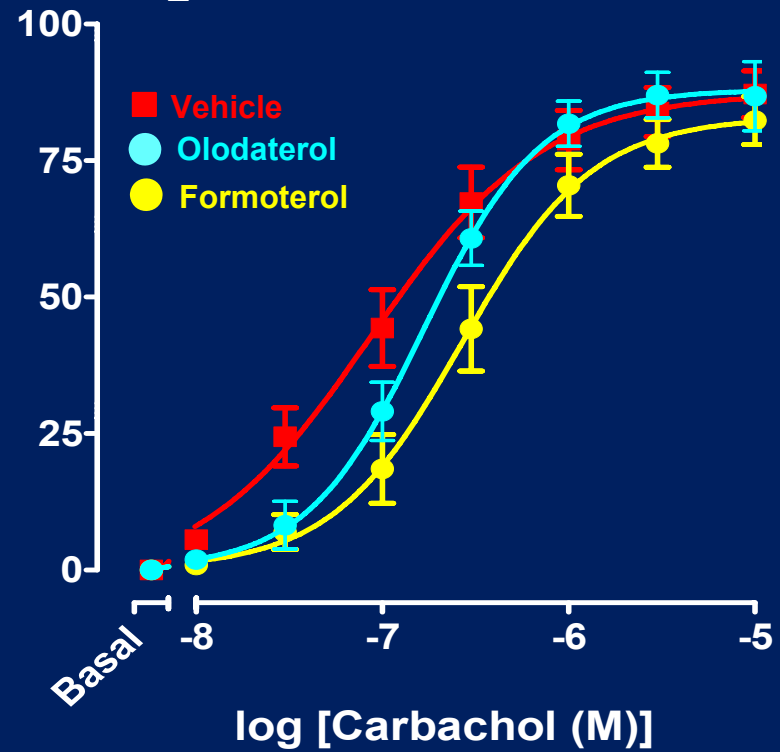


■ Carbachol/5-HT ▲ DMSO ● 10 μM S-petasin ◆ 30 μM S-petasin

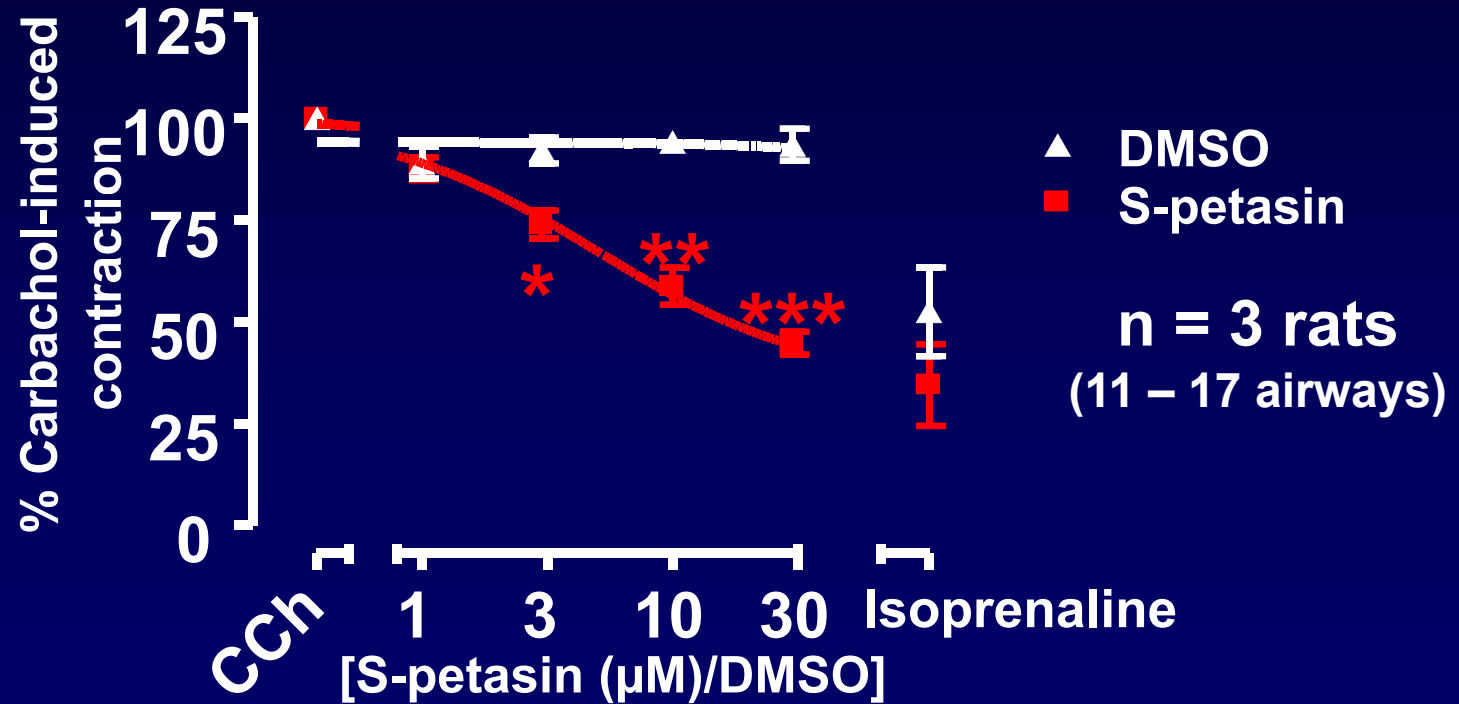
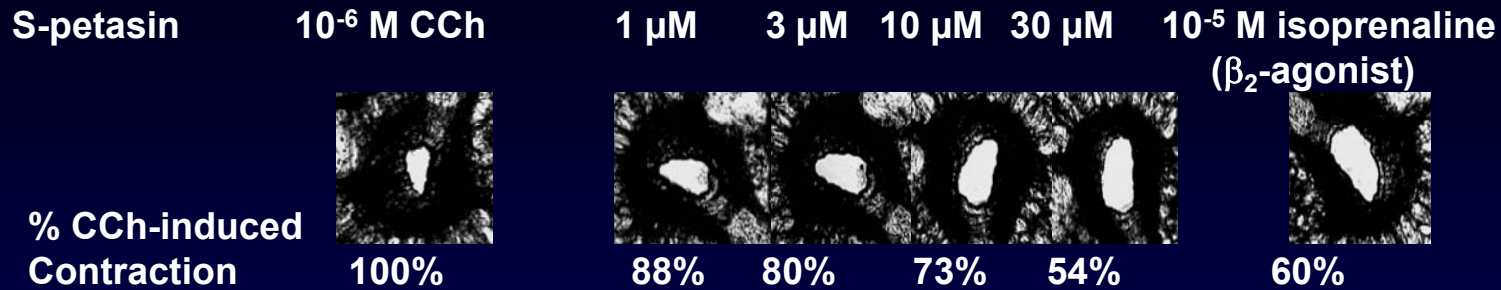
Antimuscarinics



β_2 -agonists

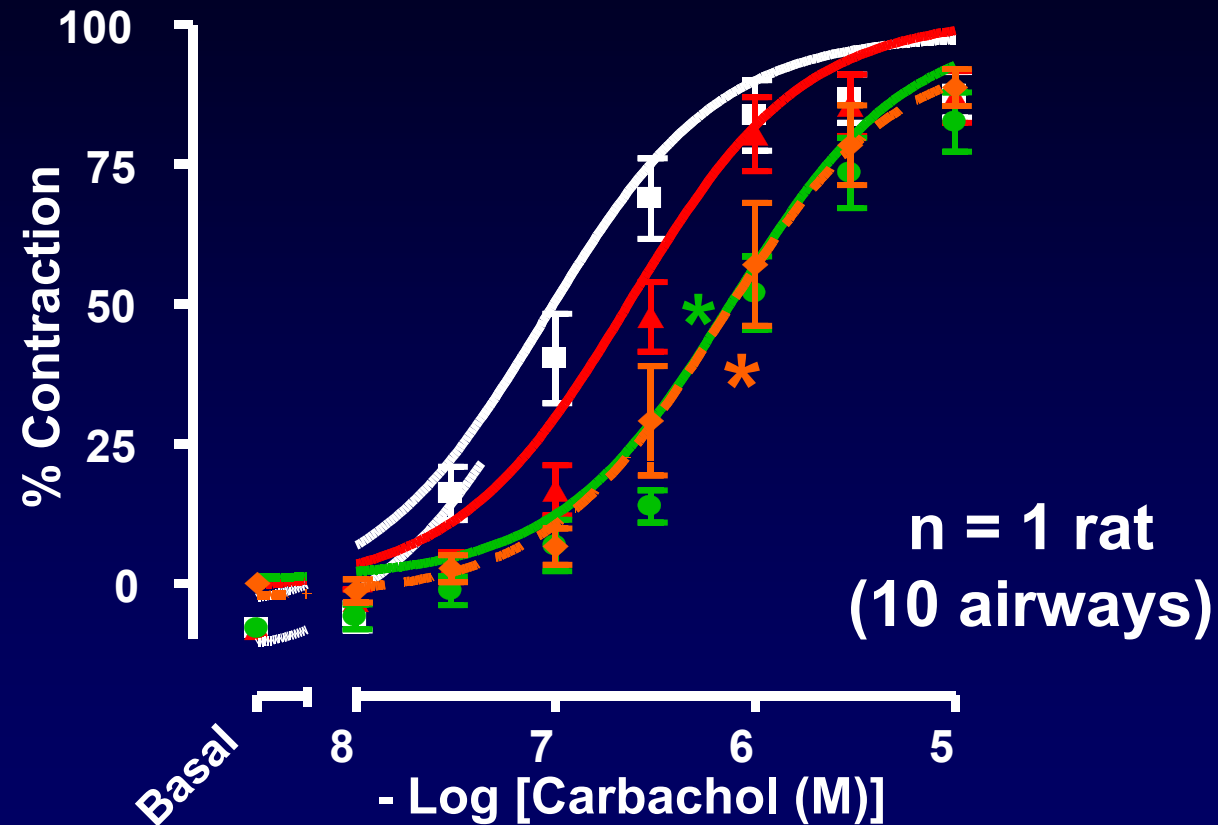


Effect of S-petasin on CCh pre-contracted rat small airways ('bronchodilation')



Effect of heated S-petasin on contraction of rat small airways

- 50°C for five hours in methanol (isomerisation?)



n = 1 rat
(10 airways)

- Carbachol
- heated 30 μM S-petasin
- ▲ heated 0.15% Methanol
- ◆ Non-heated 30 μM S-petasin

Summary: small airways

- Critical anatomical site linking larger (cartilaginous) airways with alveoli (gas exchange) – near zero airflow resistance
- ‘Delicate’ structure, dependent on alveolar attachments for patency
- ‘Vulnerable’ to remodelling in COPD (+ effects of alveolar damage)
 - become site of greatest airflow resistance
- Elastase, H_2O_2 and neutrophil products damage PCLS but have very different effects on small airway contractility – why?
- What about cigarette smoke medium?!
- PCLS useful in evaluating bronchodilators (e.g. S-petasin)
- Many unanswered questions!