Use of histopathology in pharmacology models for respiratory diseases: Case studies for ARDS, anti-asthmatics and measle virus pneumonia

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Merck KGaA, Global Head of Chemical and Preclinical Safety, Darmstadt, Germany for the Colleagues of the ISTP, 26-28 OCTOBER 2018

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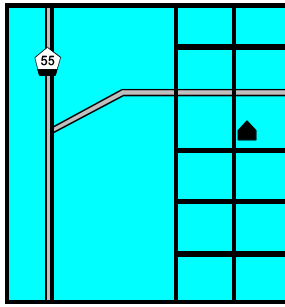

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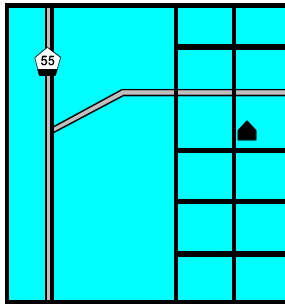

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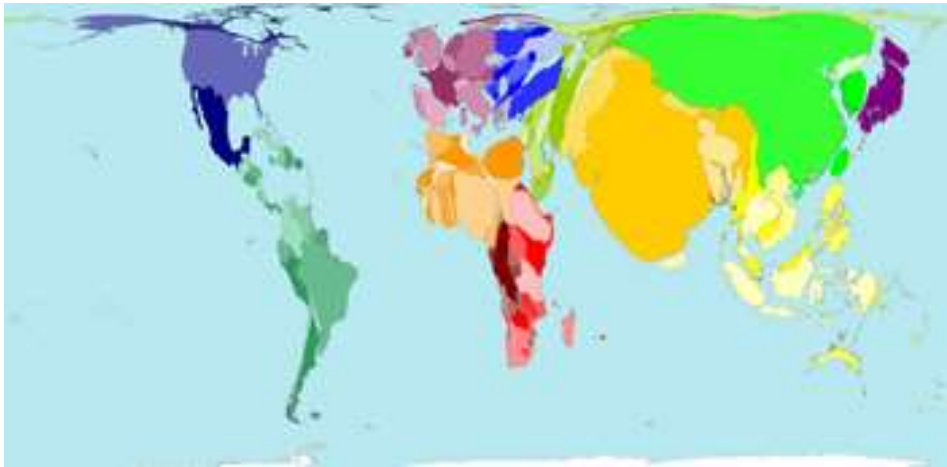
Why are respiratory diseases important ?

The Worldmapper

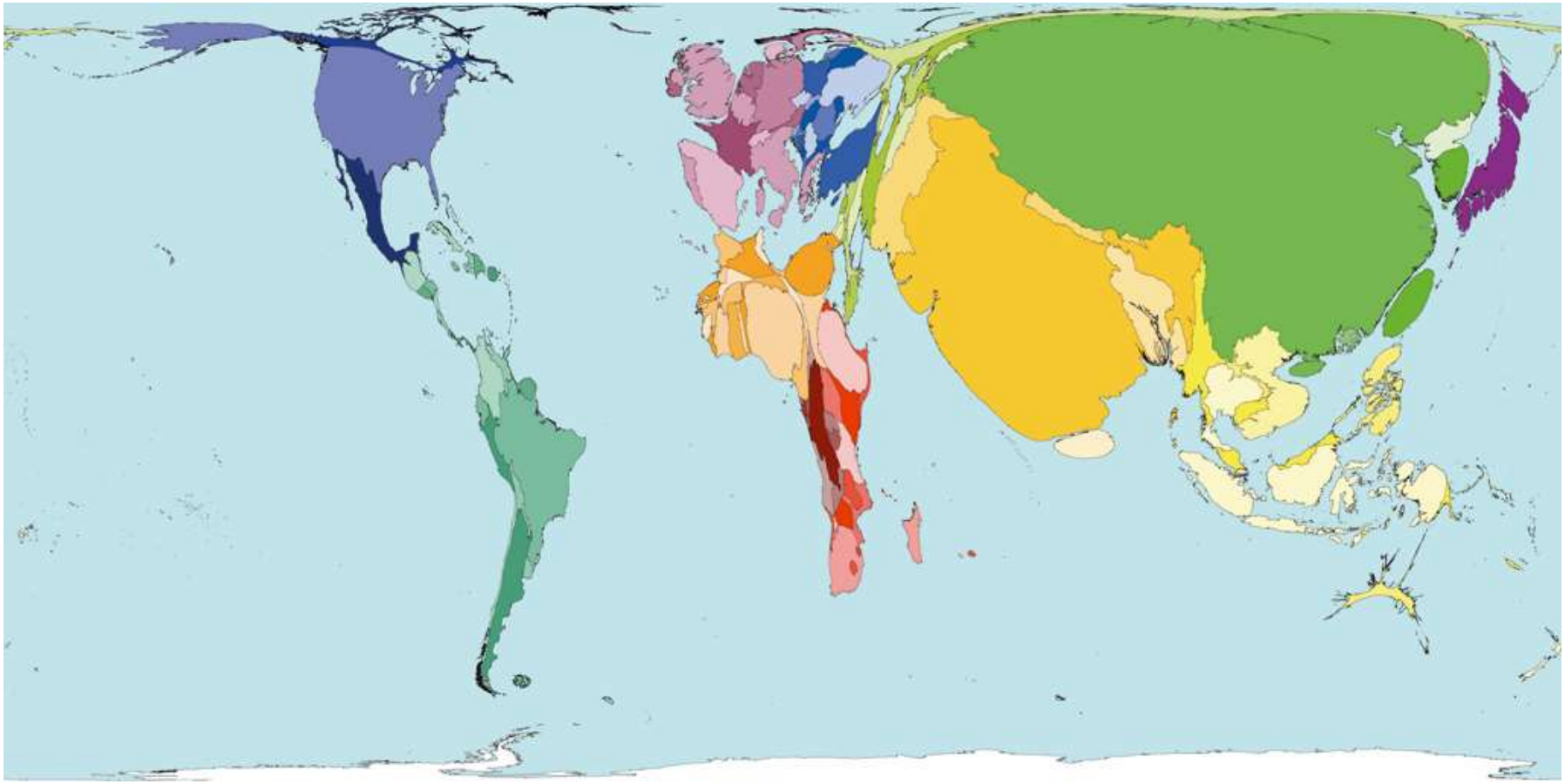
This website contains 696 maps, with associated information and PDF 'poster' file. Each map relates to a particular subject. Click on the 'Thumbnail Index' which gives thumbnail previews of the maps, 'Map Categories' which is classified to see the choice, or a new option 'A-Z Map Index', and view a map and associated information. There is also a Site Map and Help page.
The country cartograms contain 171 maps showing a population grid for each covered territory/region projected on a cartogram. More information on the country cartograms are explained on this info page.

Land Area Map

Population Map



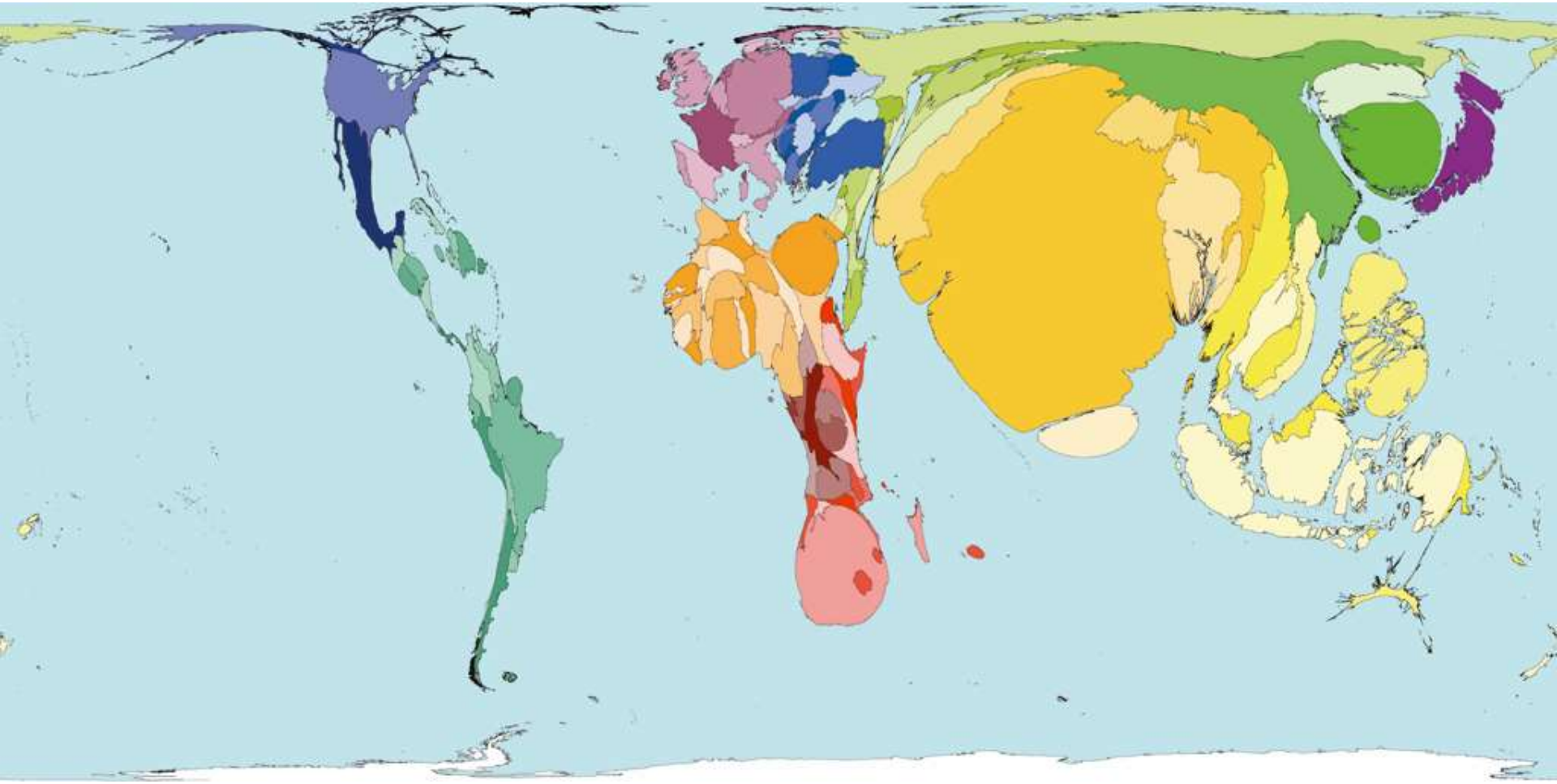
Deaths from respiratory diseases



Respiratory diseases caused 6.48% of all deaths worldwide in 2002, an average of 595 deaths per million people per year. Respiratory infections are counted separately.

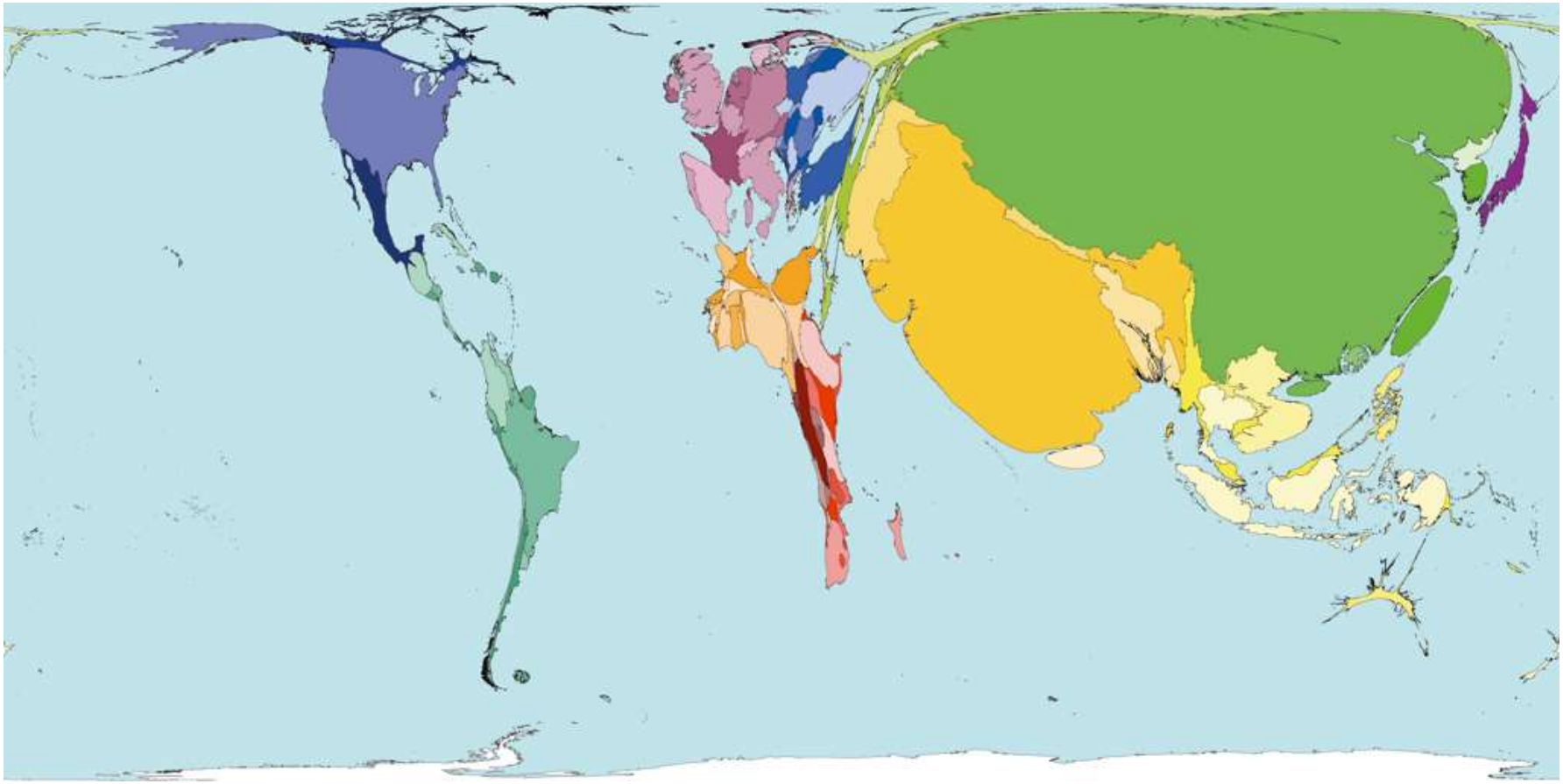
1. Chronic obstructive pulmonary disease (74% of deaths).
2. Chronic bronchitis and Asthma, (7% of deaths).
3. Other respiratory diseases, (19% of deaths).

Asthma deaths



Asthma caused 0.42% of all deaths worldwide in 2002, an average of 39 deaths per million people per year.

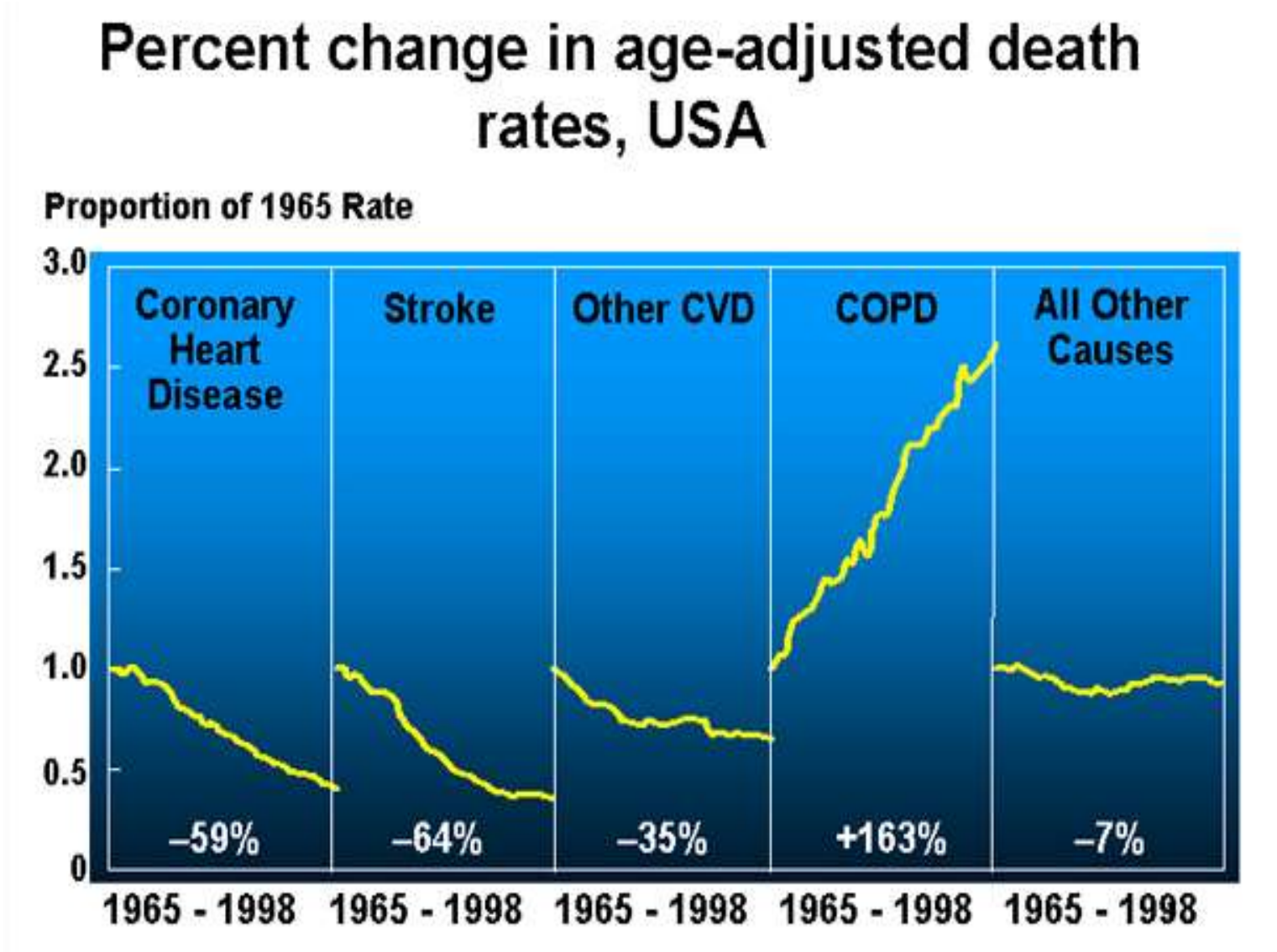
Deaths from chronic bronchitis



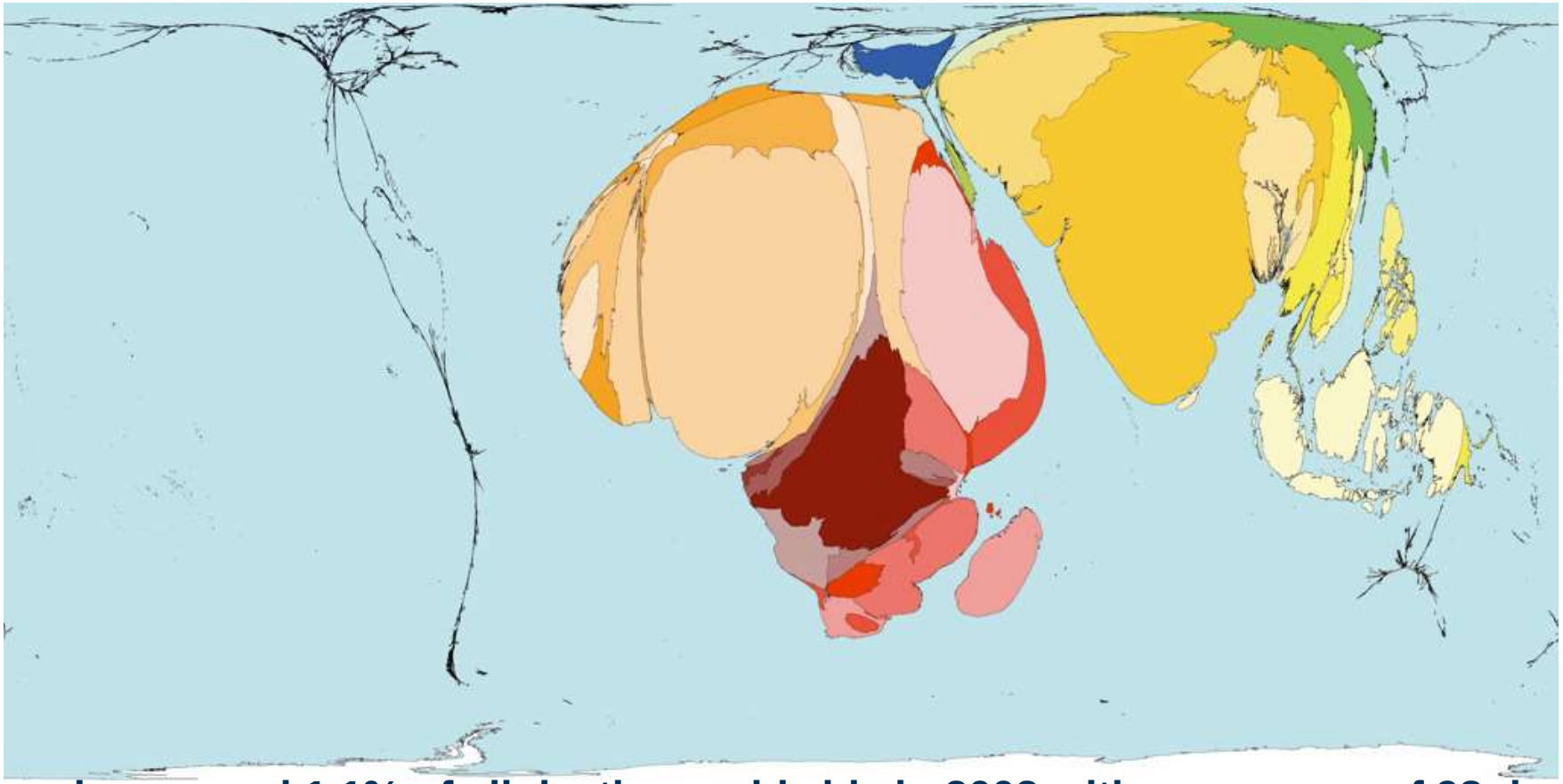
In 2002 Chronic bronchitis caused 8.3% of deaths in people over 60 years old, 3.2% of all deaths in rich territories, 9.5% of all deaths in poor territories and 2.8% of all deaths in very poor territories.

Global Burden of Disease estimated in 2002 Chronic bronchitis to cause 2.0% of all Male, 1.7% of all Female, 2.7% of all Rich territory and 3.1% of all Poor territory burden of disease (Disability Adjusted Lost Years).

COPD : 4th leading cause of death



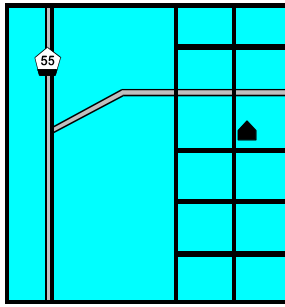
Measles deaths



Measles caused 1.1% of all deaths worldwide in 2002 with an average of 98 deaths per million people; Due to measles vaccination: between 1999 and 2005, there was a 60% reduction in annual measles deaths worldwide, from 873,000 to 345,000. Africa, where children were most prone to die when they caught measles because of poor nutrition and other infections had a 75% drop in deaths. In 1999, 506,000 African children died; **90% aged under five. By 2005, the figure had fallen to 126,000...**

Primitive indoor stoves like this one in Koluha, India, create smoke that causes lung & heart diseases & low birth weight, killing 1.9 million people annually, the United Nations says

This is your road-map you can enjoy !!



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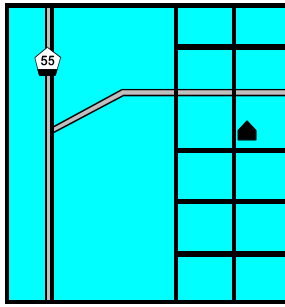

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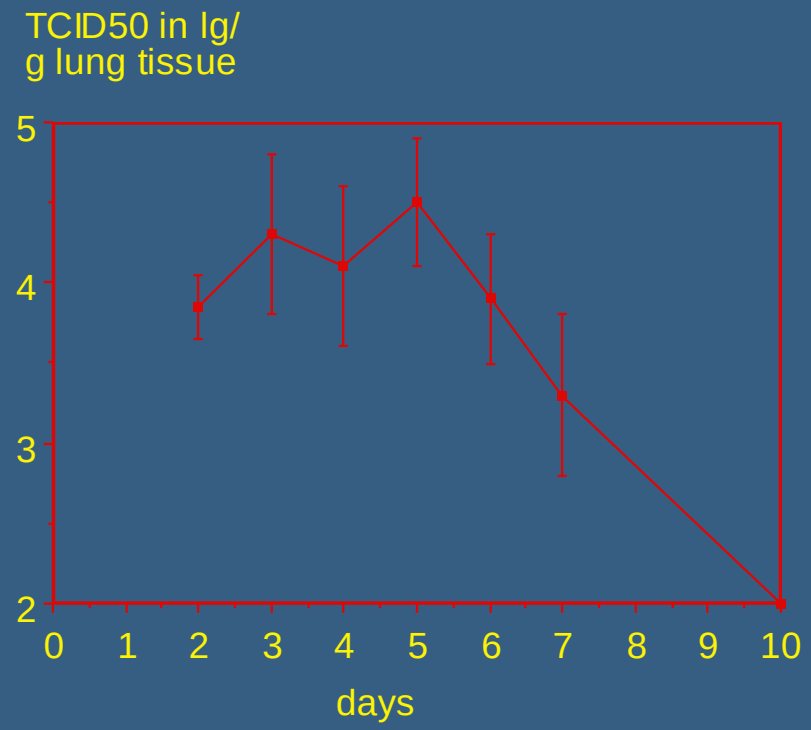
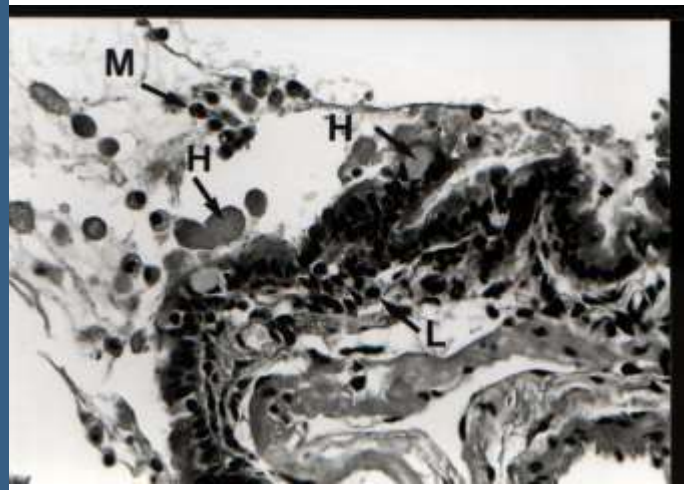
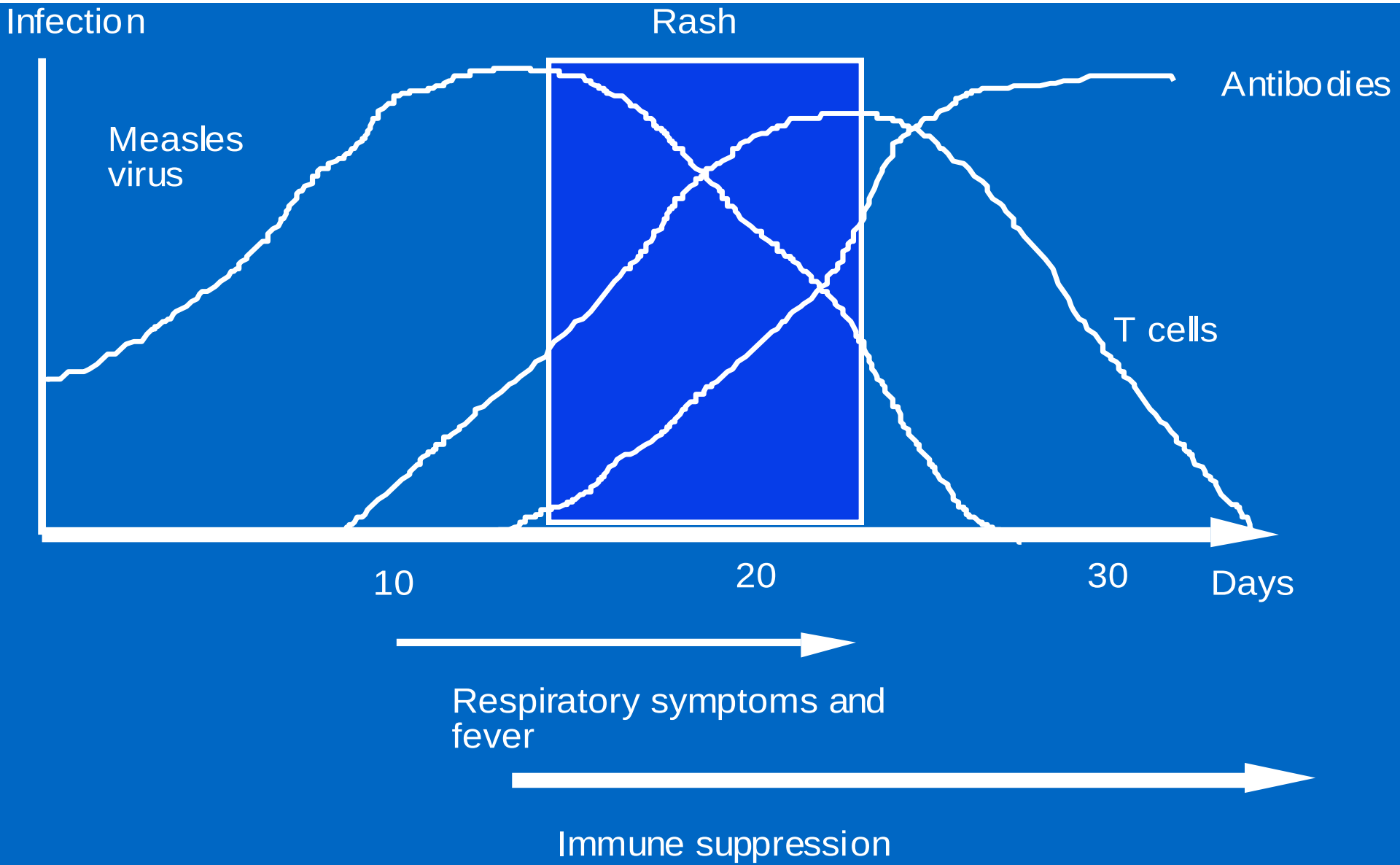
Cotton rats are susceptible to measles virus infection

-Immune suppression
-Vaccination studies

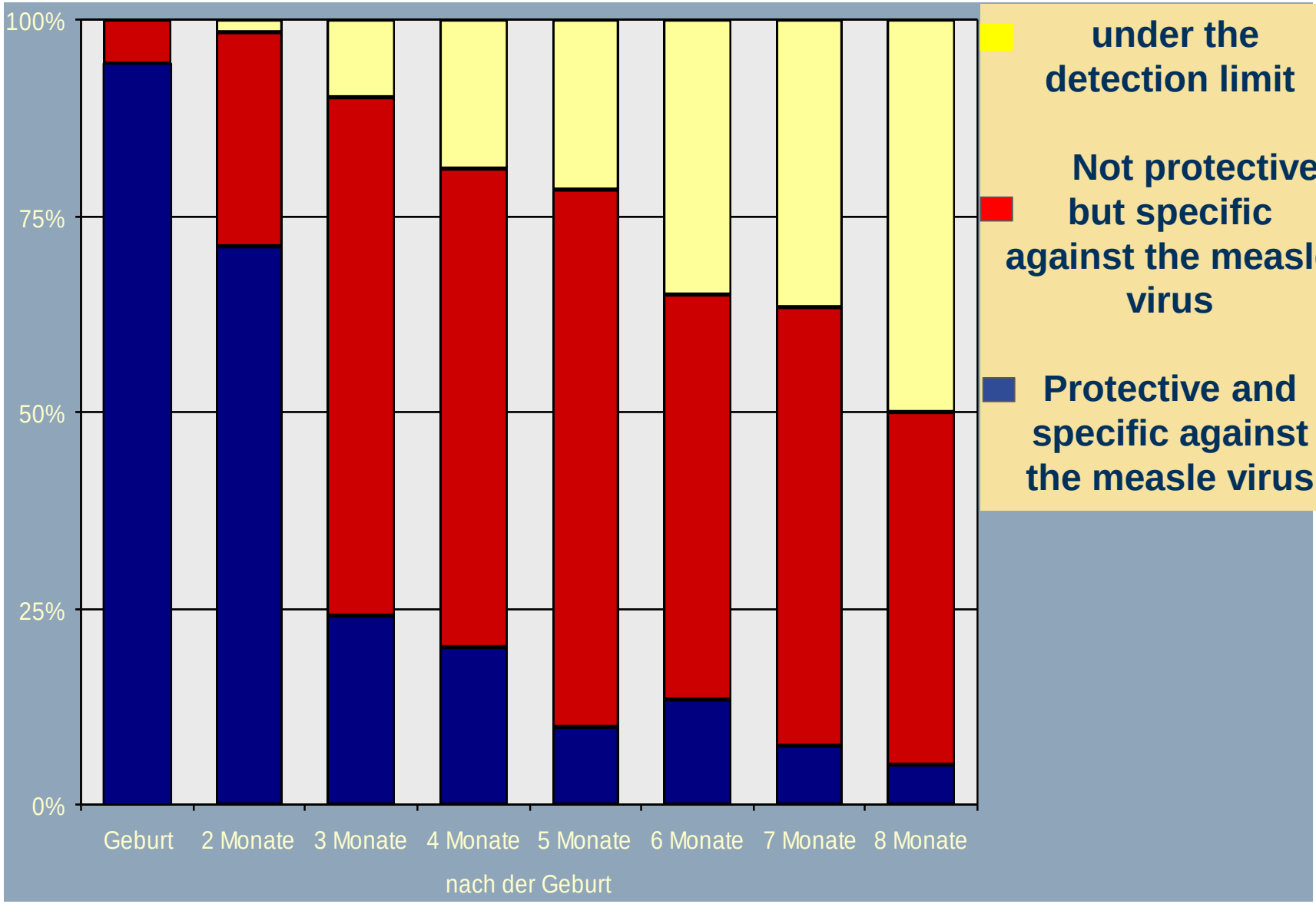




Measles virus infection

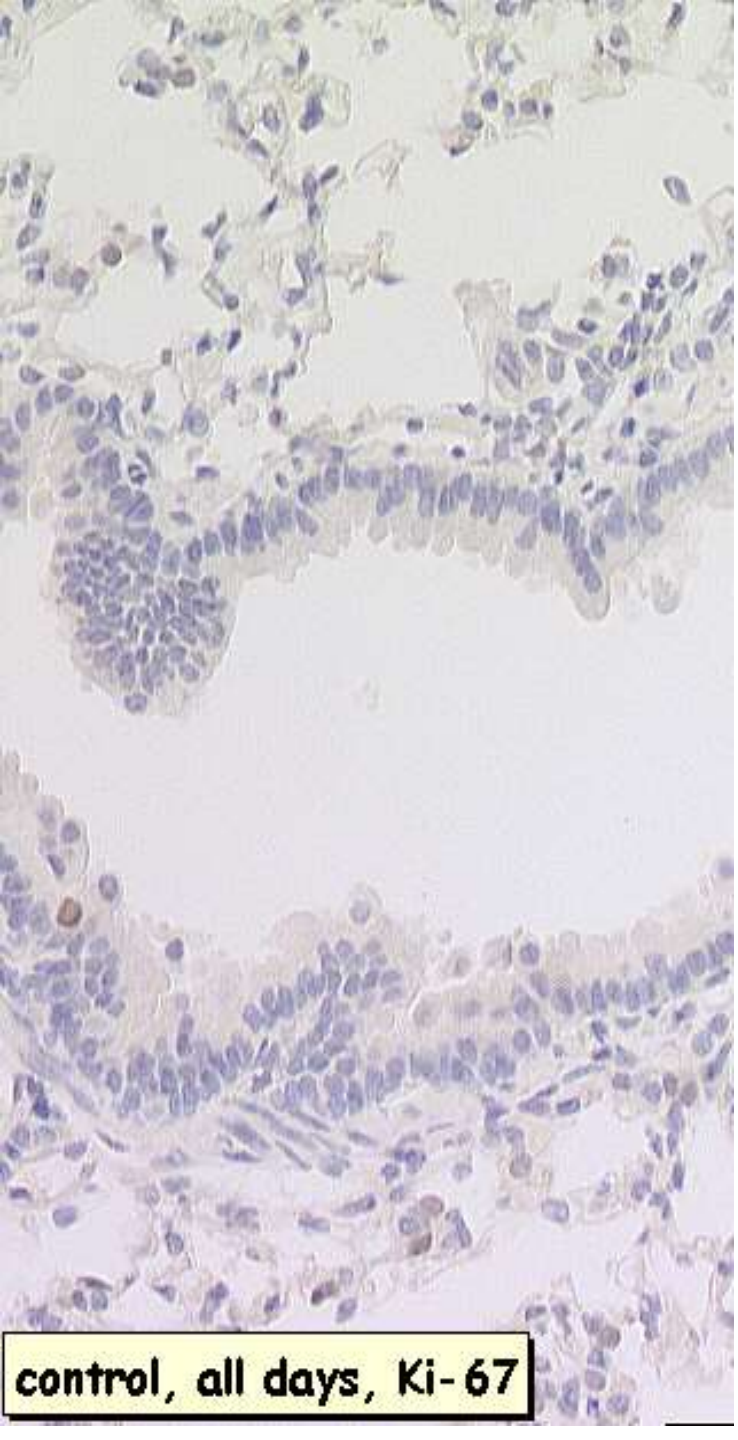


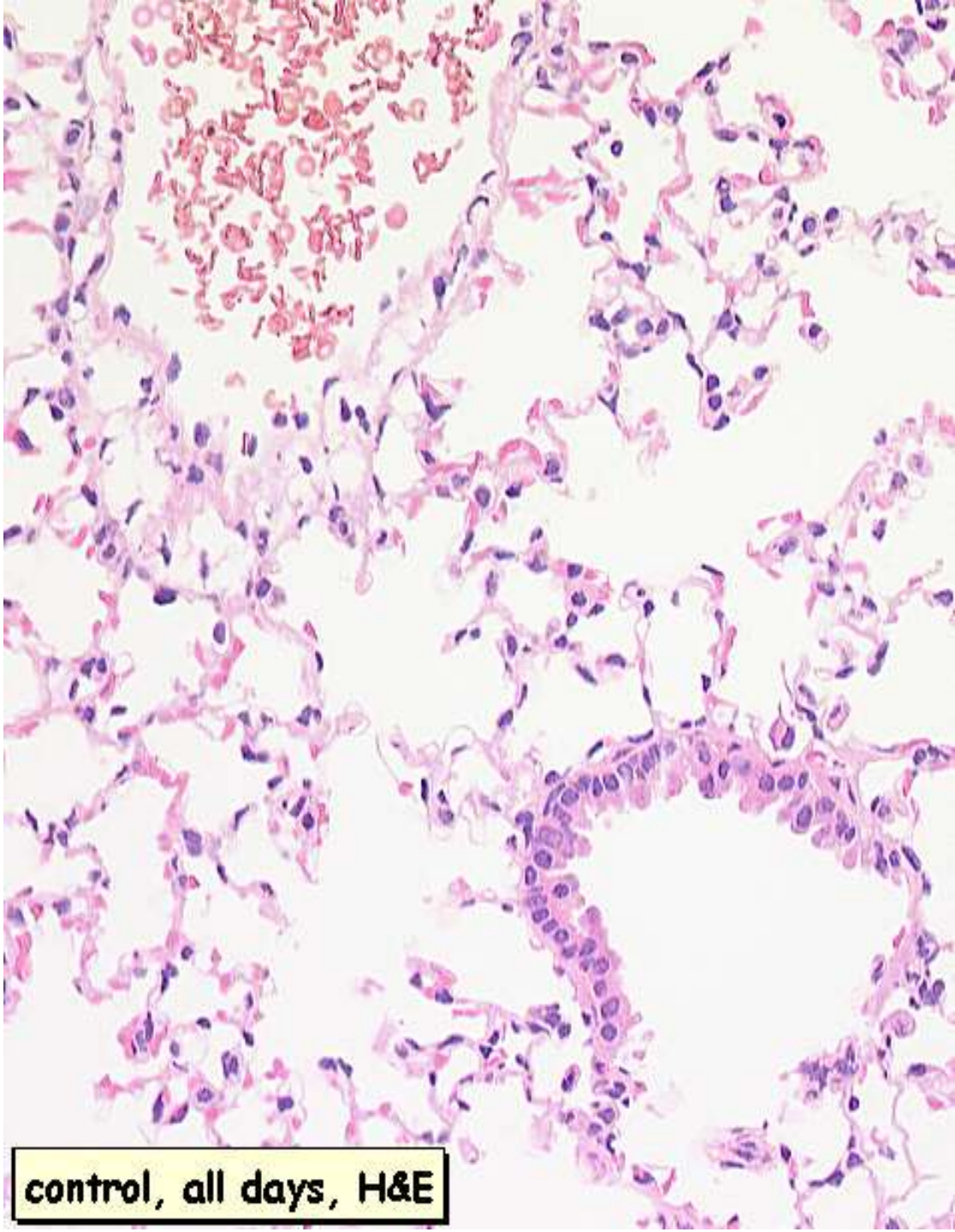
Time course and type of antibodies against the measle virus



Material & Methods

- Female, 6 weeks old inbred Cotton rats (cotton N/Ico) were obtained from Iffa Credo, France.
- For intranasal (i.n.) infection MV was given to ether anesthesised cotton rats in a volume of not more then 100µl. 2, 4, 6, 8, 14 and 26 days post infection, the three right lung lobes were prepared for histological examination. Slides were coded and evaluated blindly.


control, all days, Ki-67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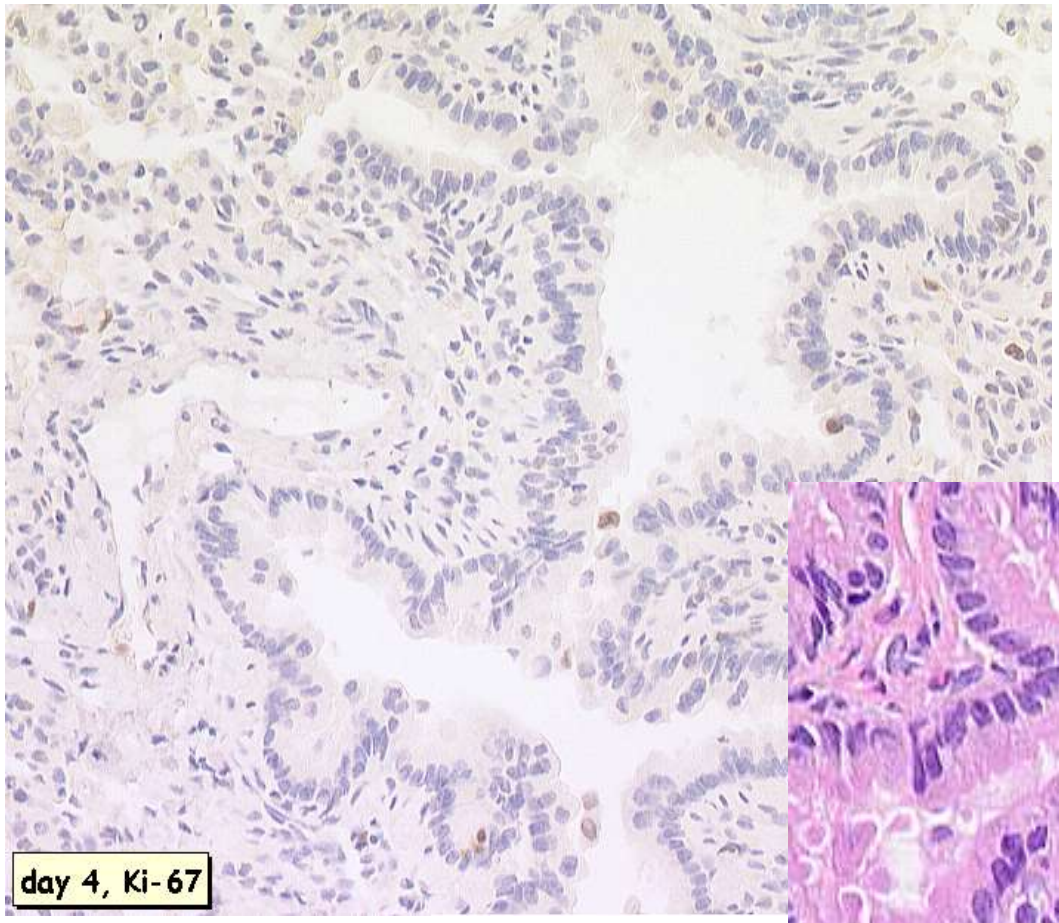
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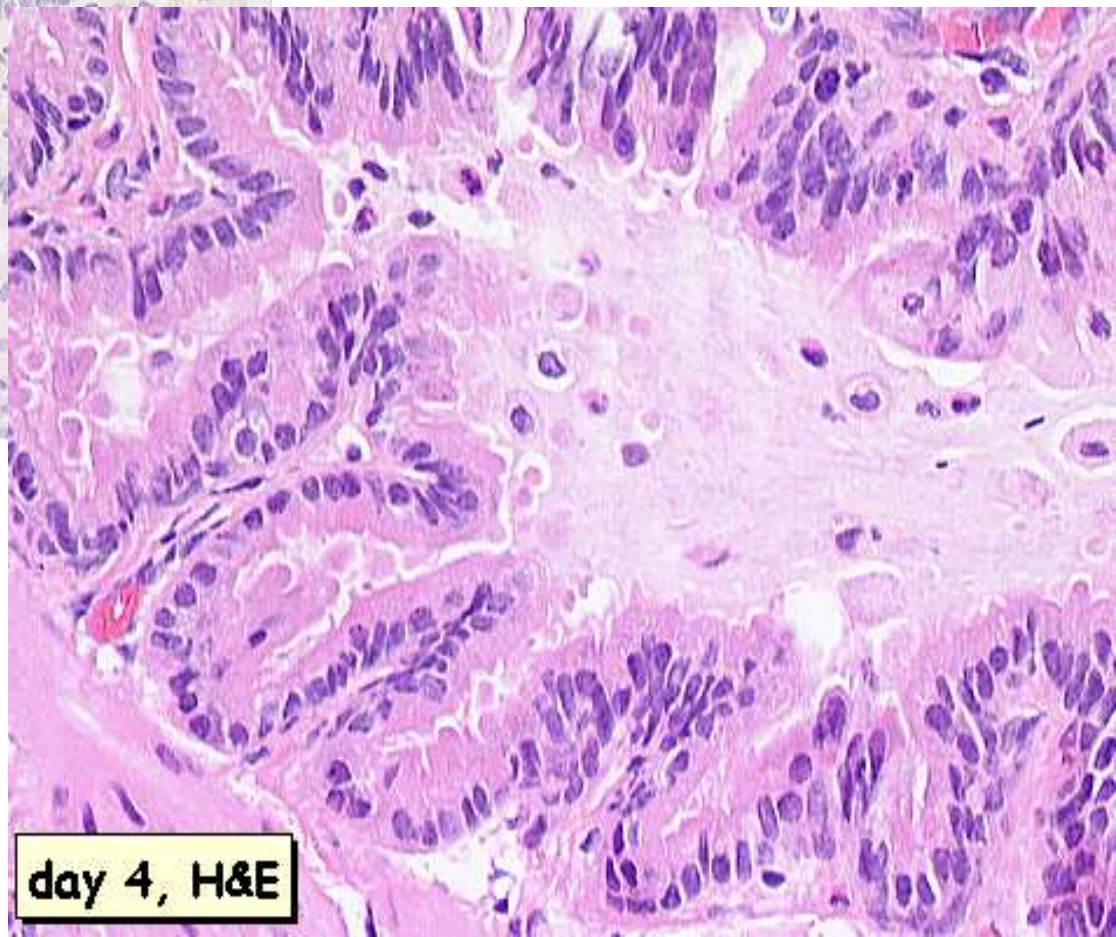
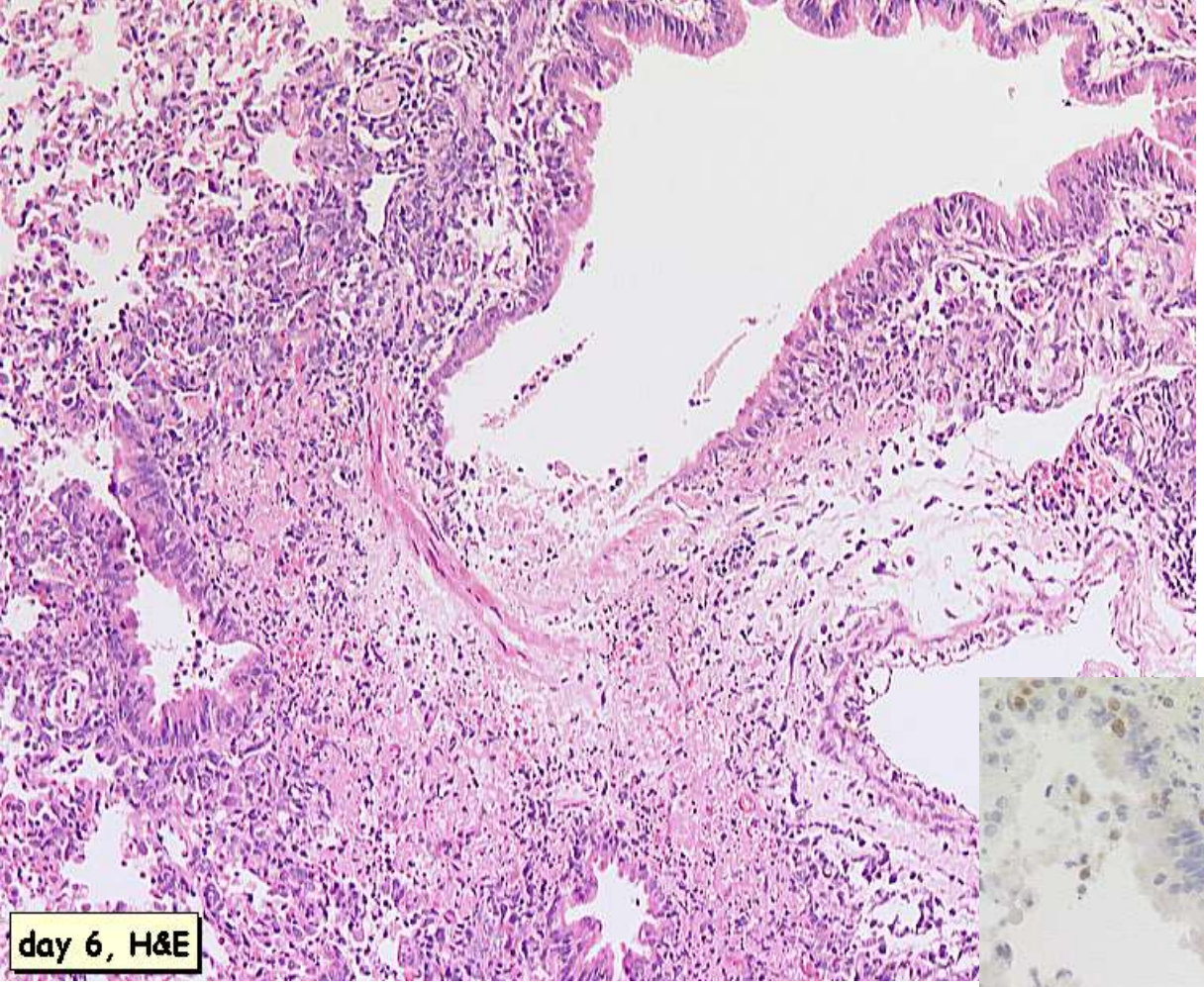

day 4, H&E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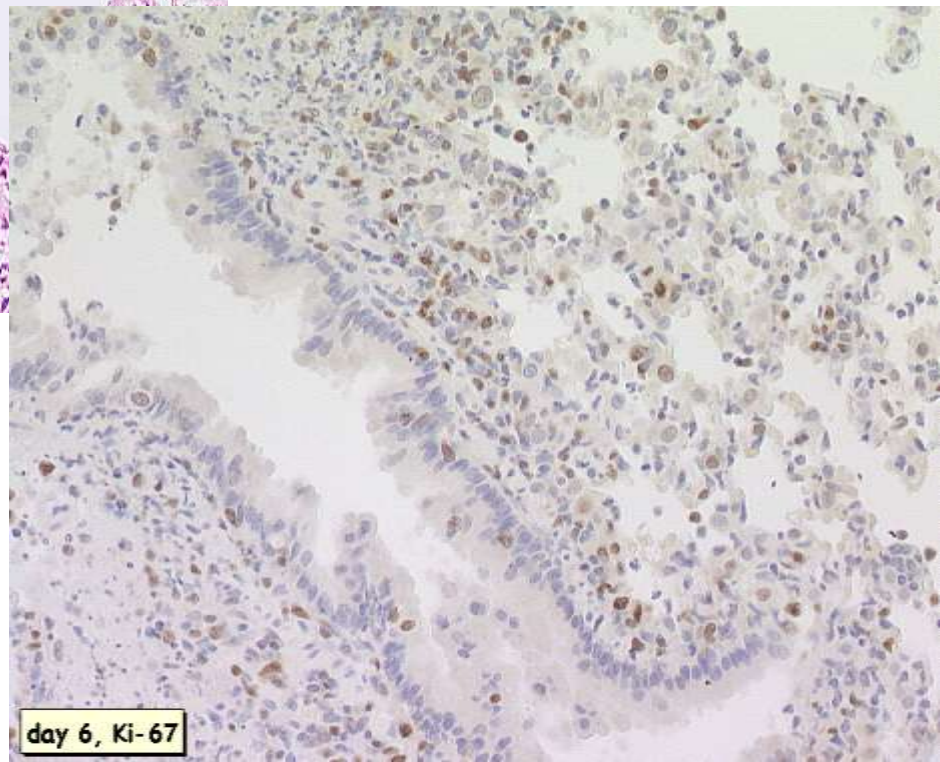
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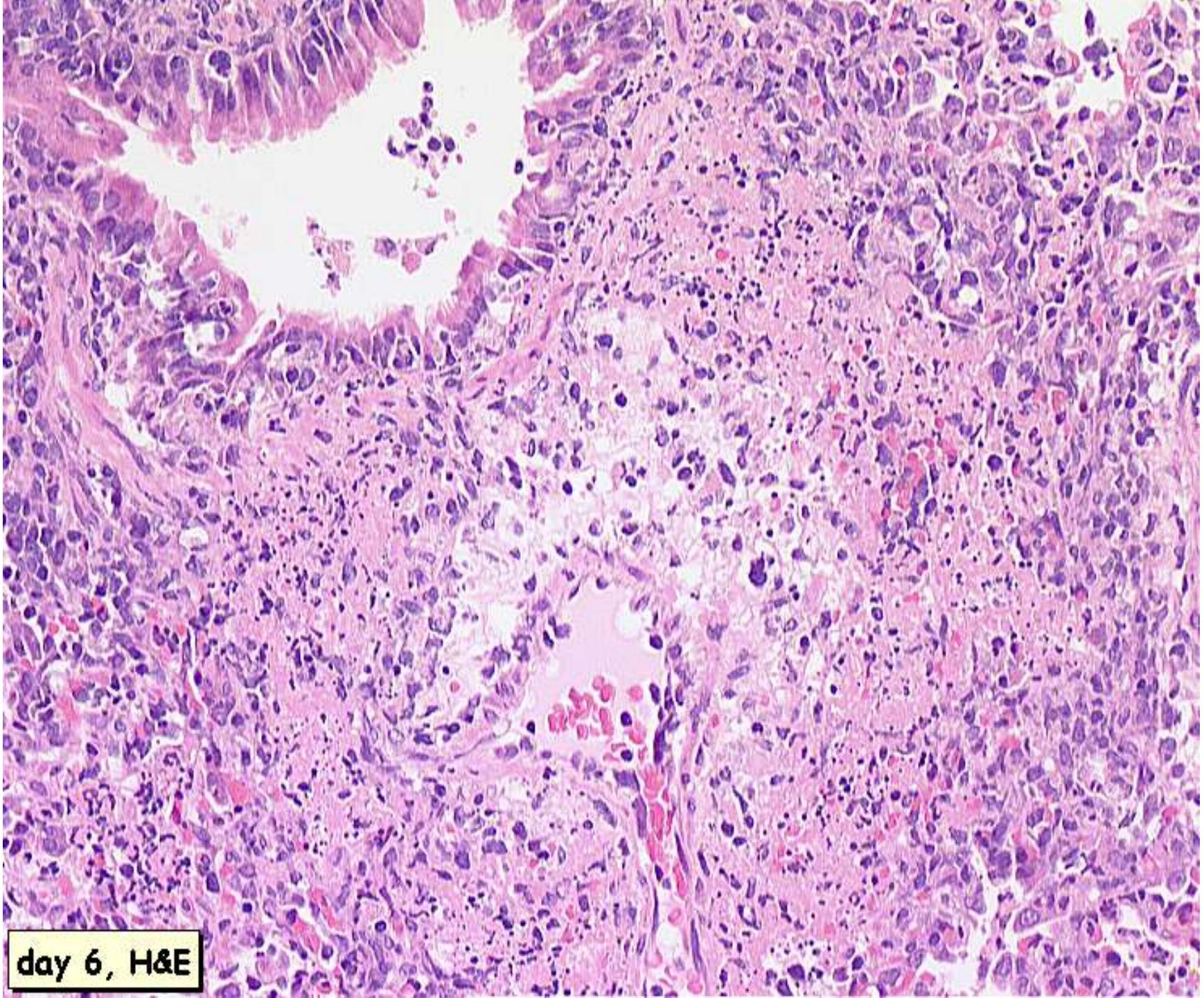
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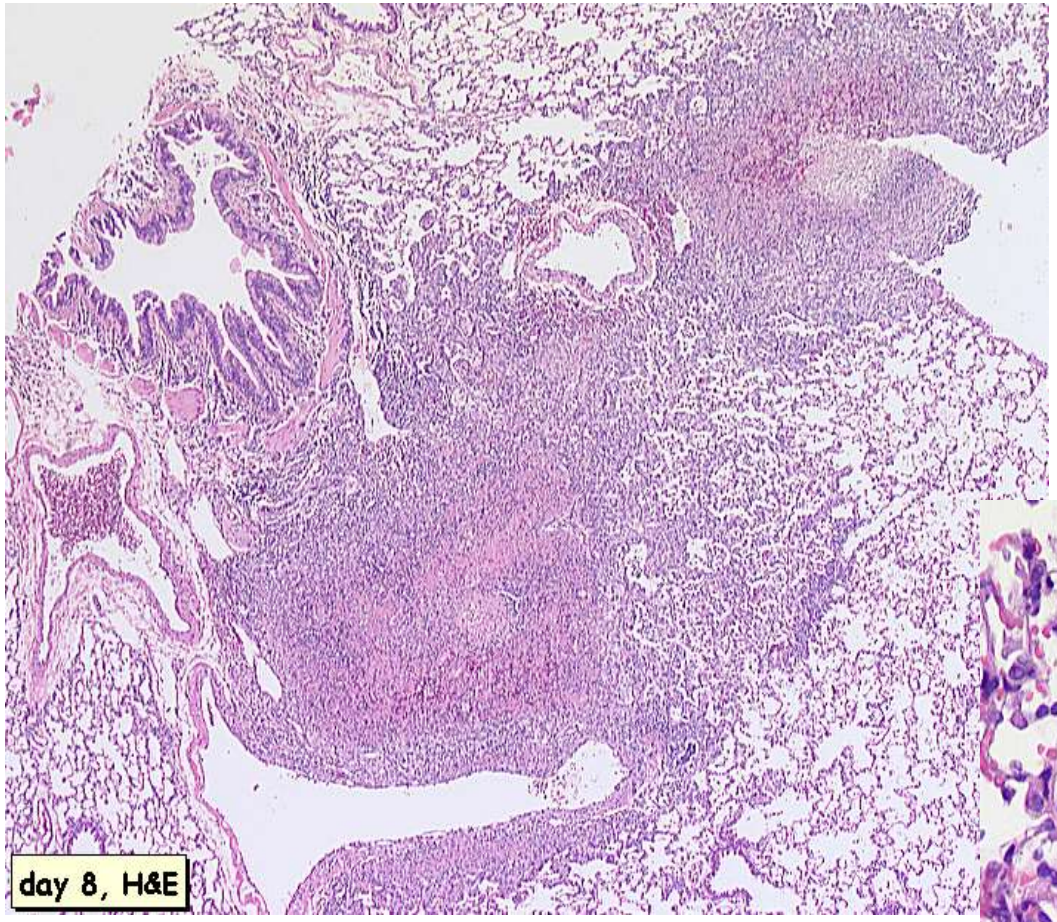
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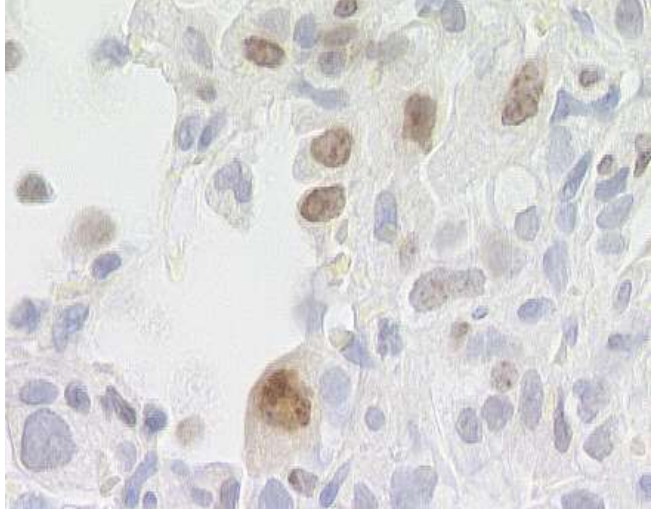

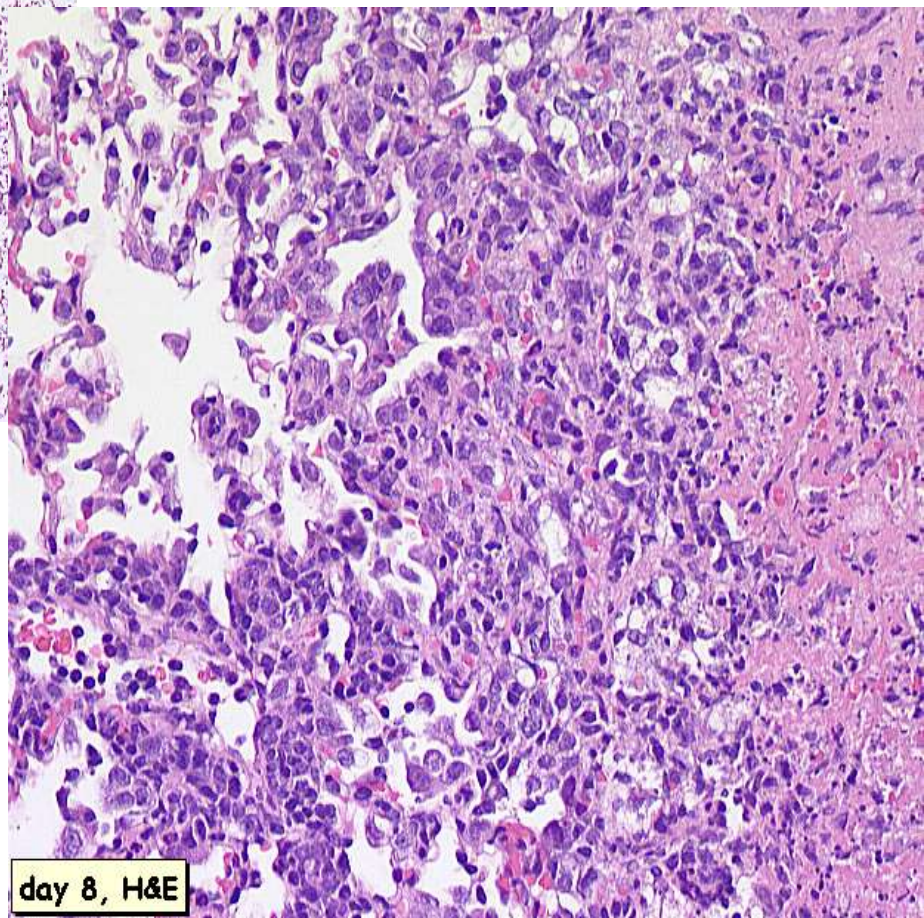
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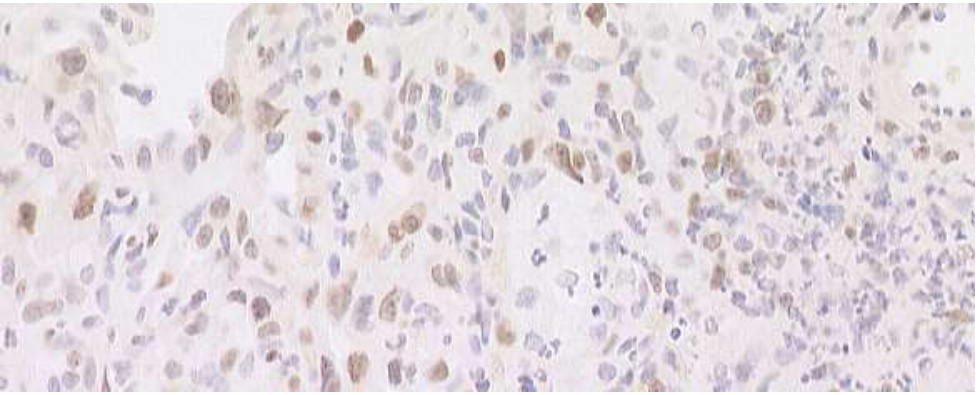

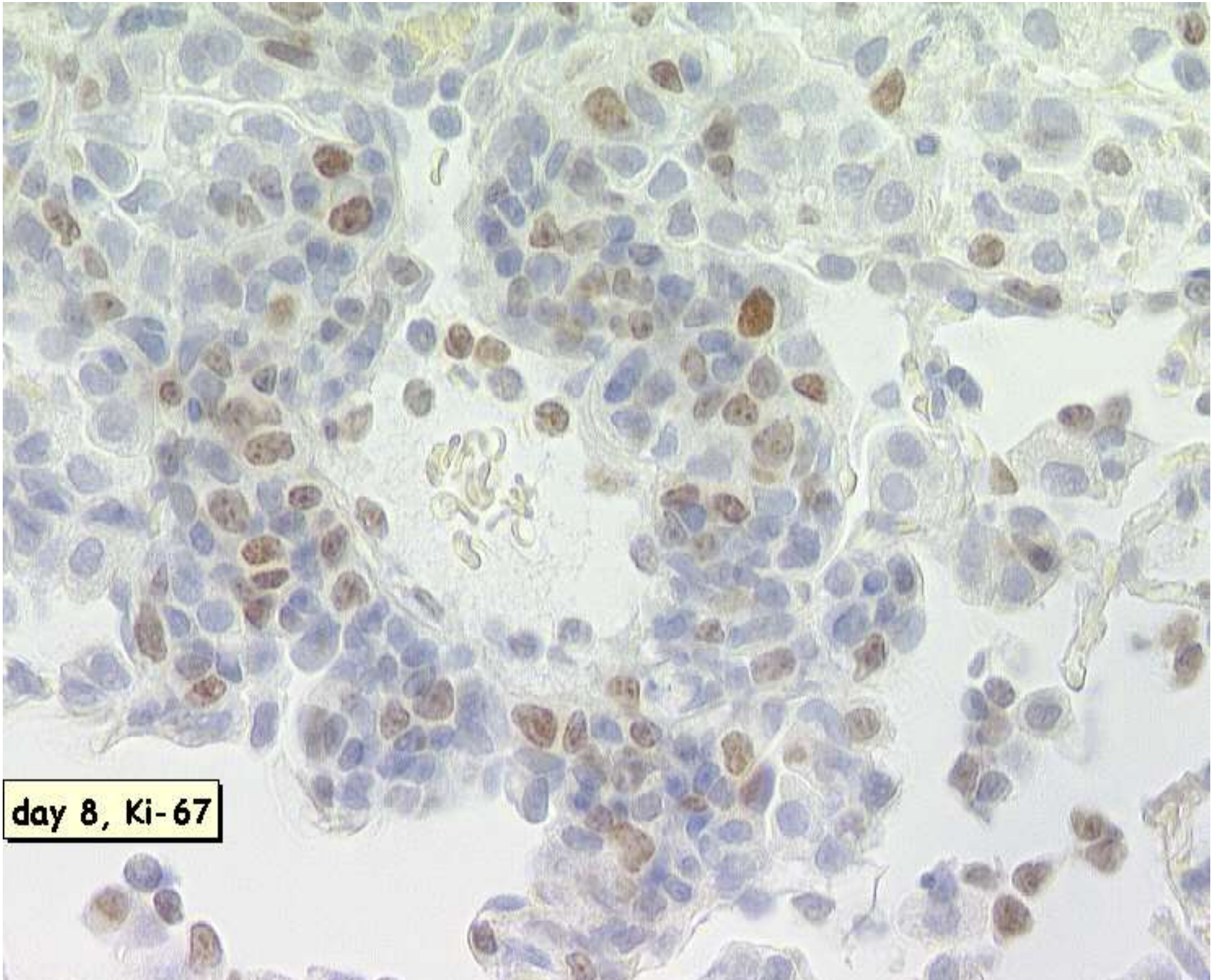
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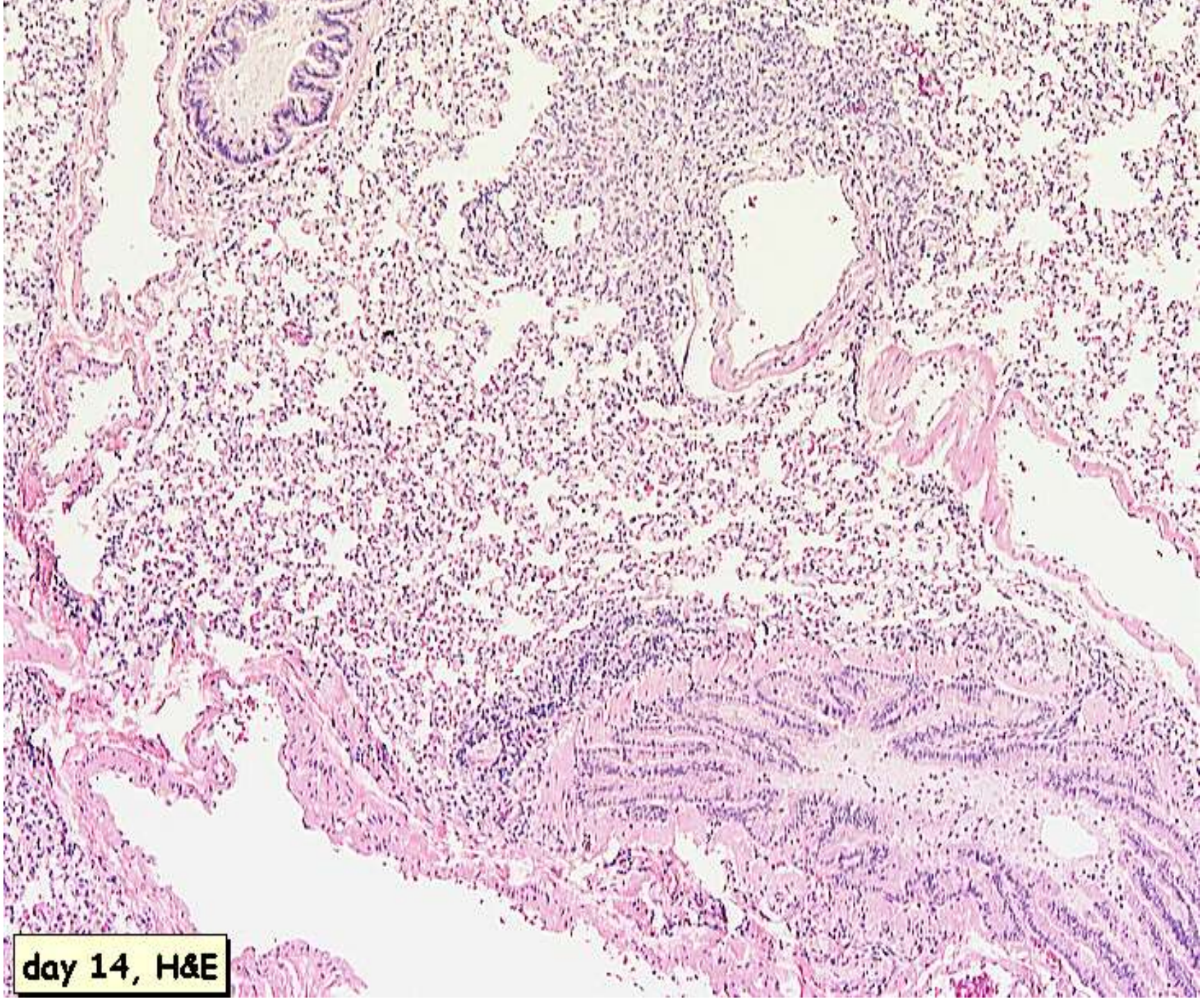
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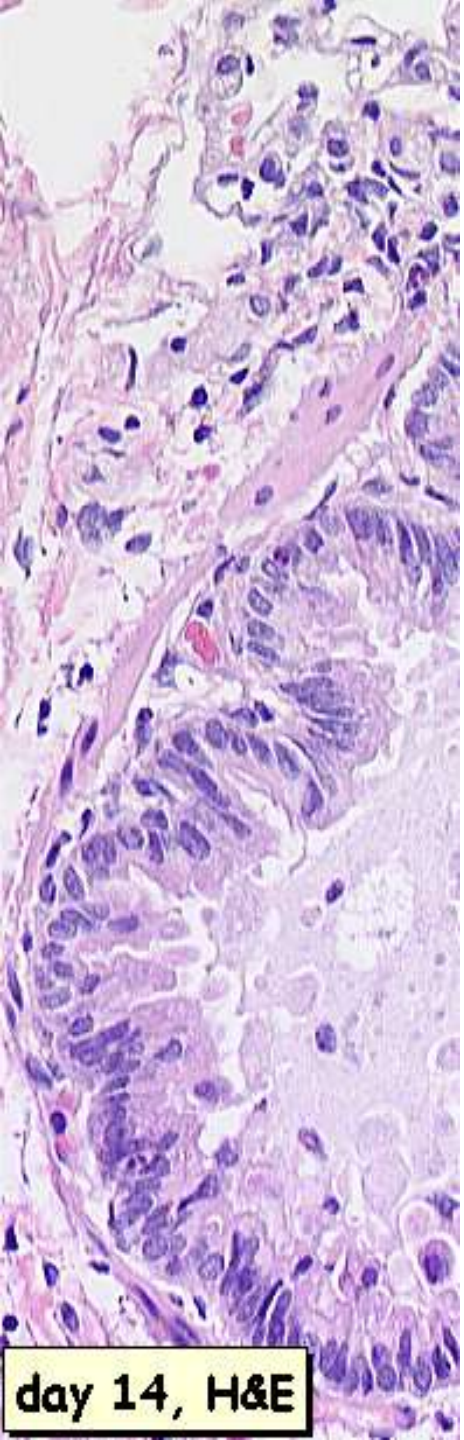
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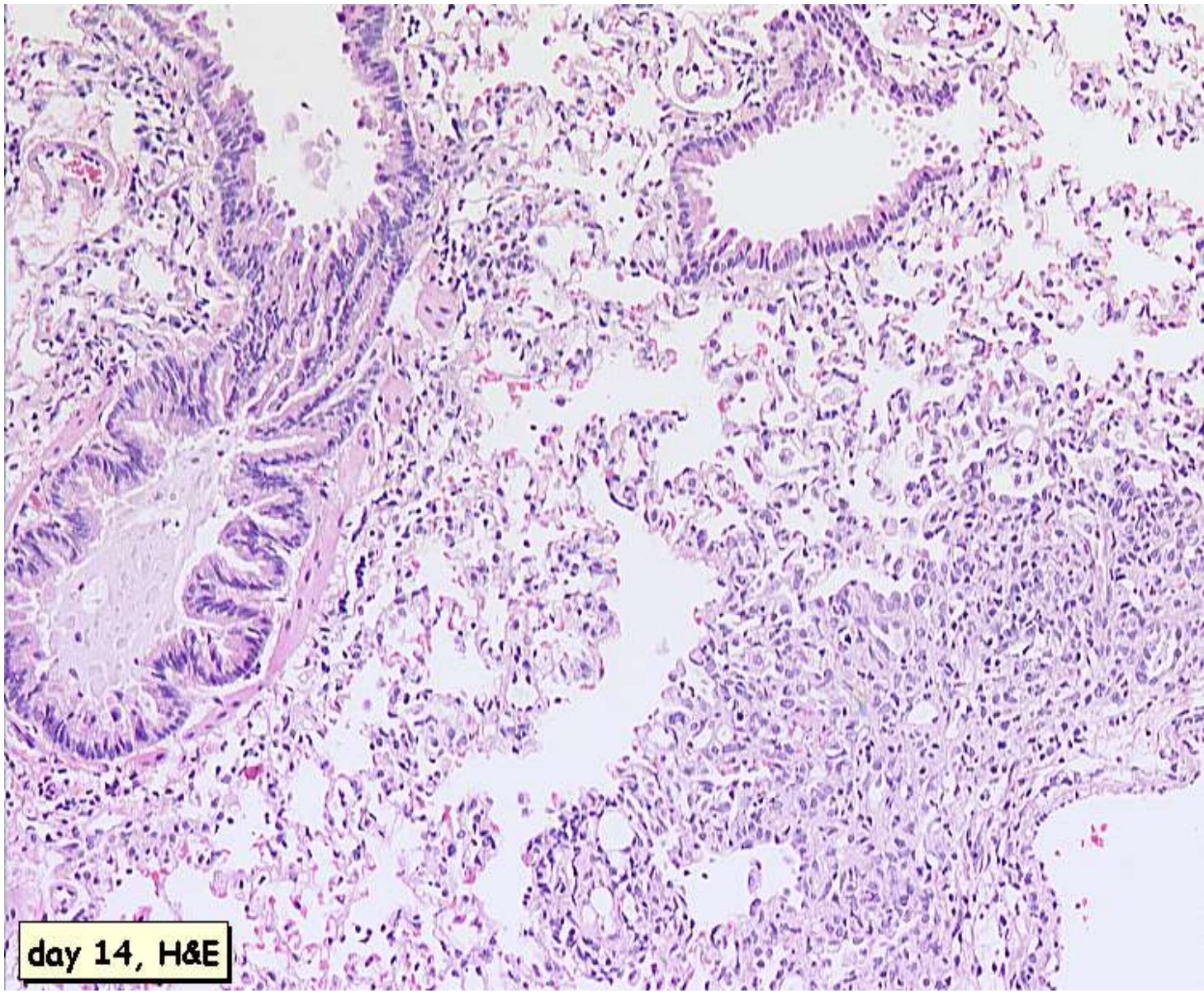
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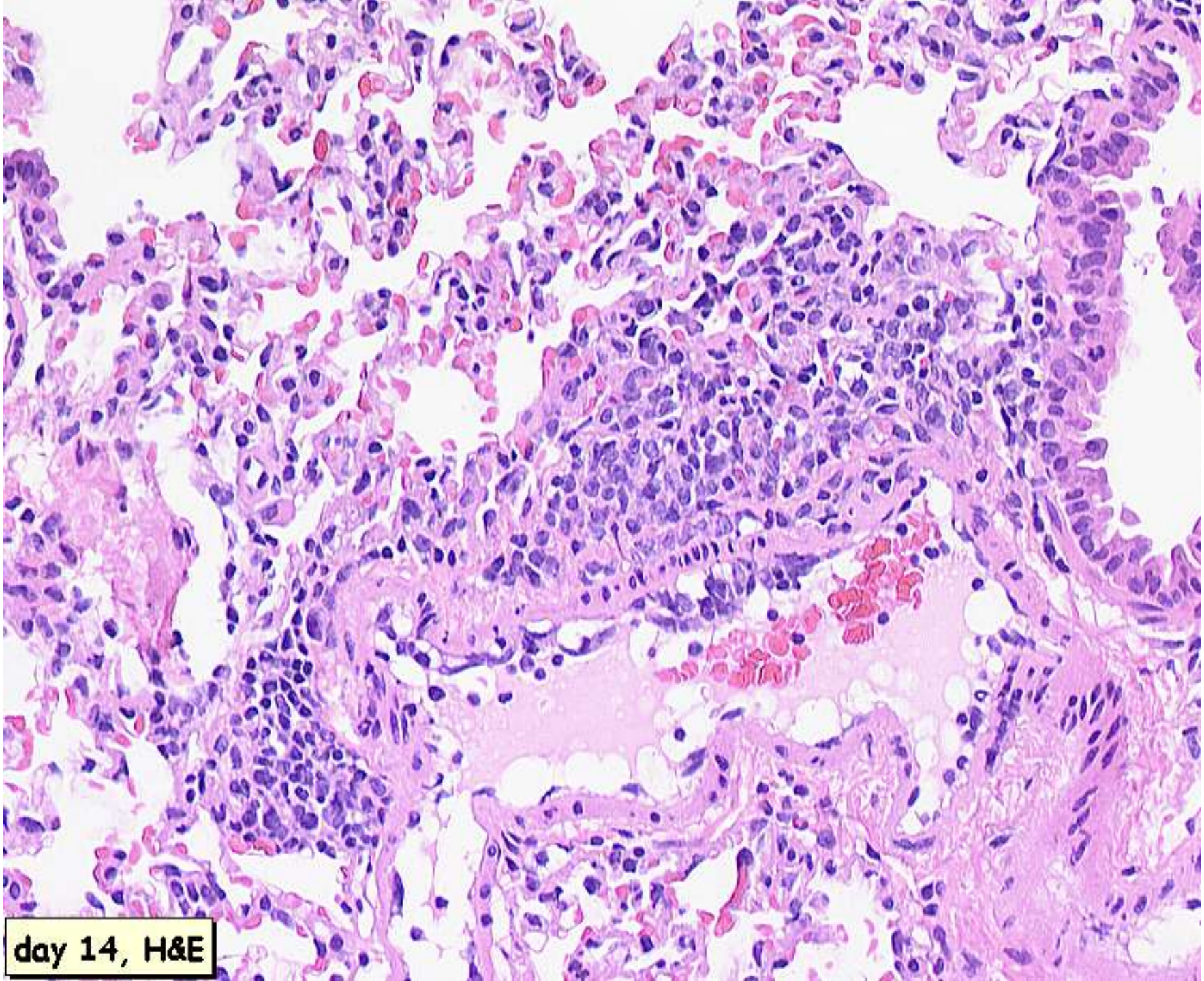
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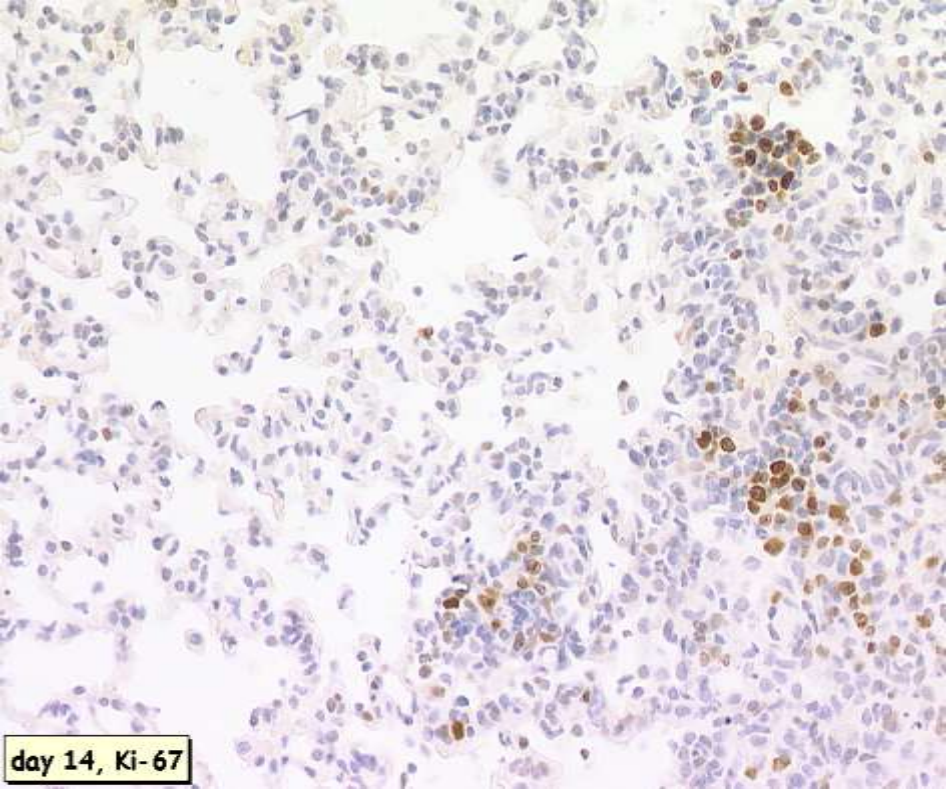
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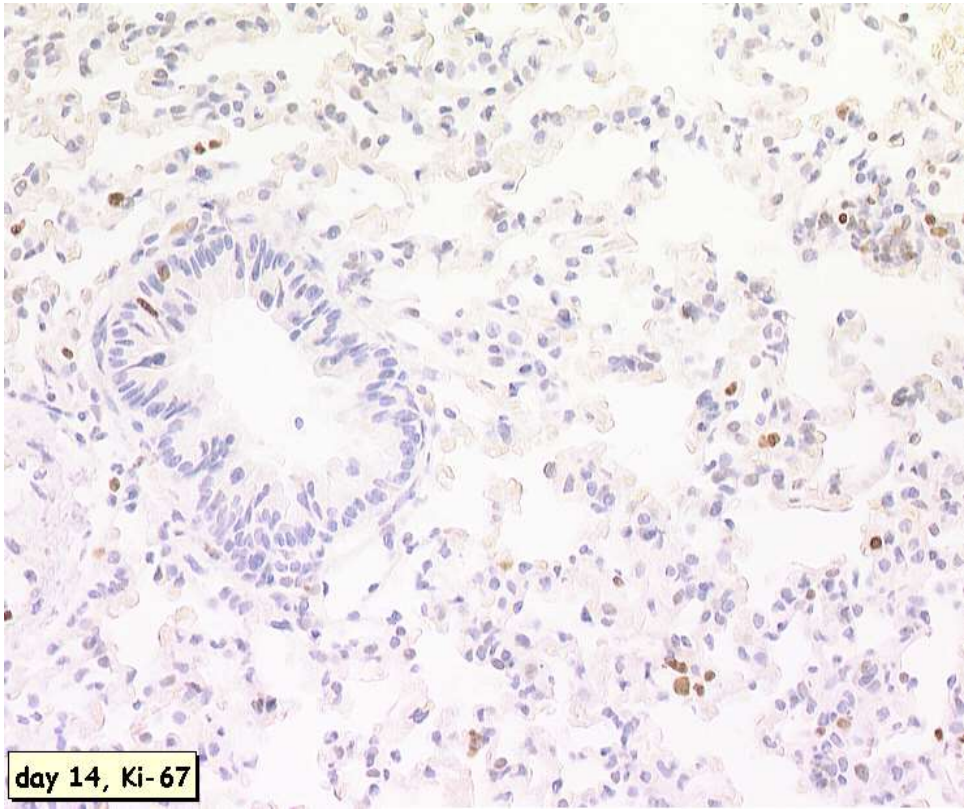
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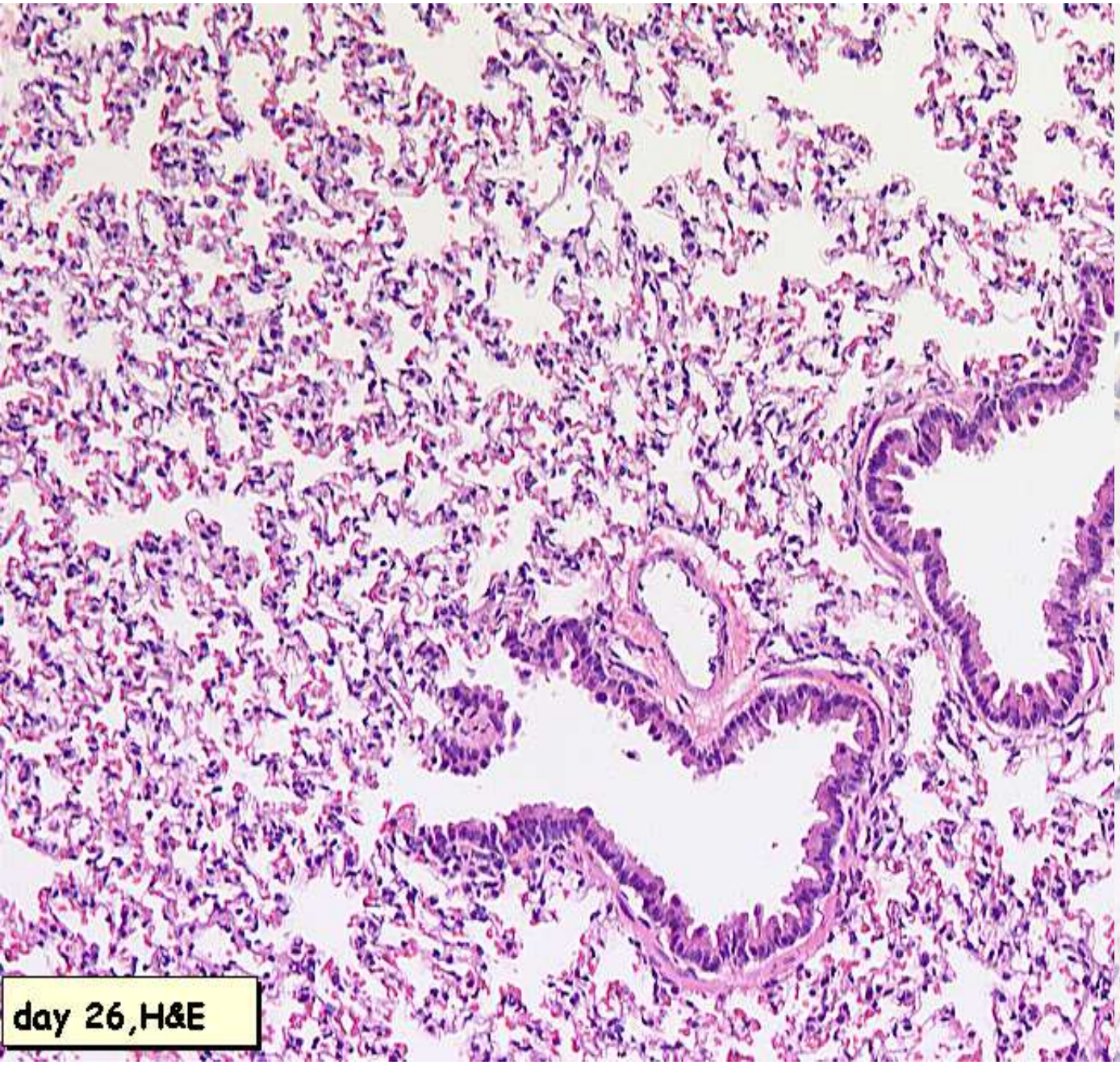
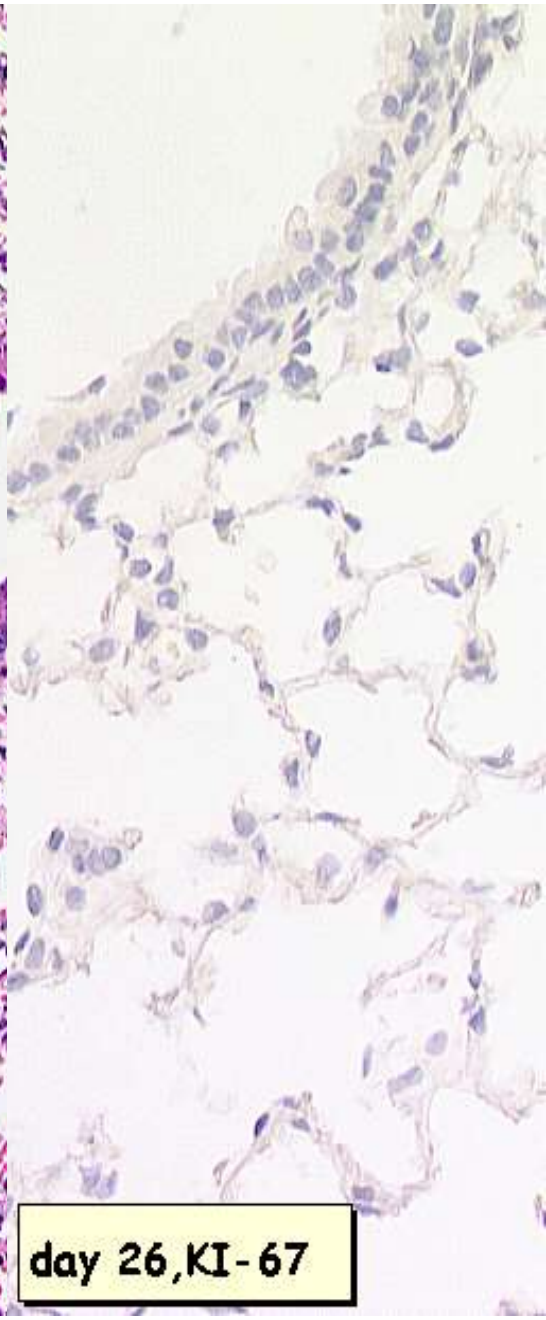
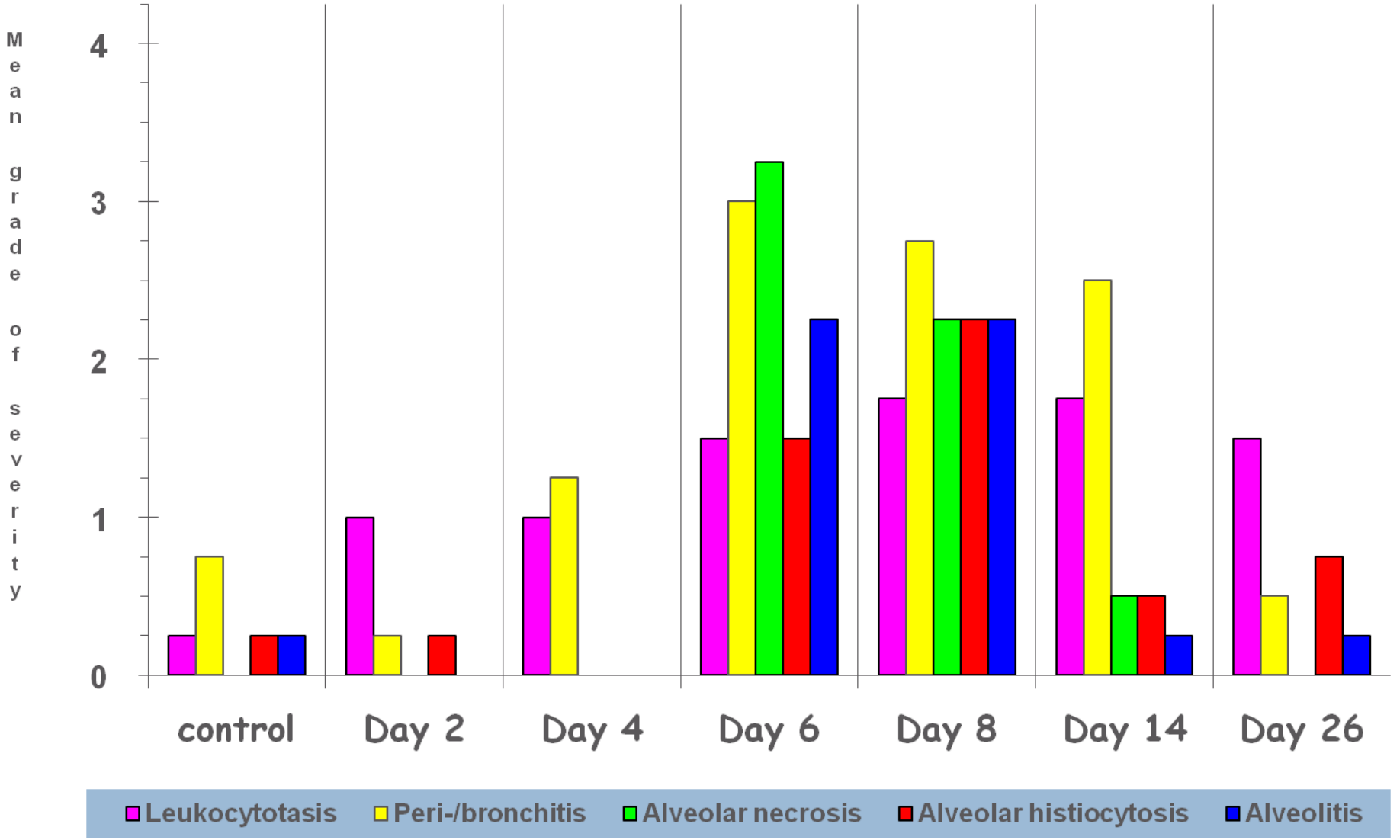

day 26,H&E


day 26,KI-67

Time course of lung lesions after intra-nasal measle virus infection in Cotton rats



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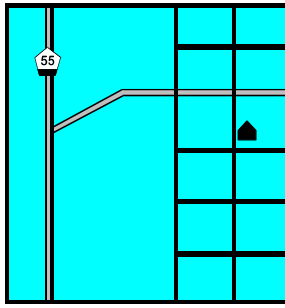


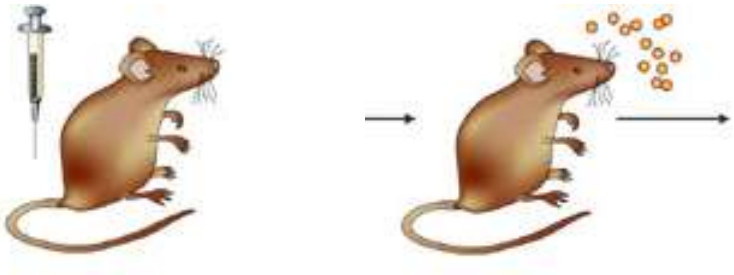
Brown Norway rat asthma model

Animal biology:

- Brown Norway (BN) rat has a high capacity for IgE production
- Airway hyperresponsiveness upon exposure to allergens or some chemicals
- Used as model of allergic respiratory diseases such as asthma

Brown Norway rat asthma model

Sensitization i.p. **Challenge twice a day i.n**



BAL

Day 1, 14

- **i.p.: ovalbumin adsorbed on alum**
- **i.n.: ovalbumin in PBS**

Day 26

Organ harvesting

Cell suspension

Density gradient centrifugation

Histology

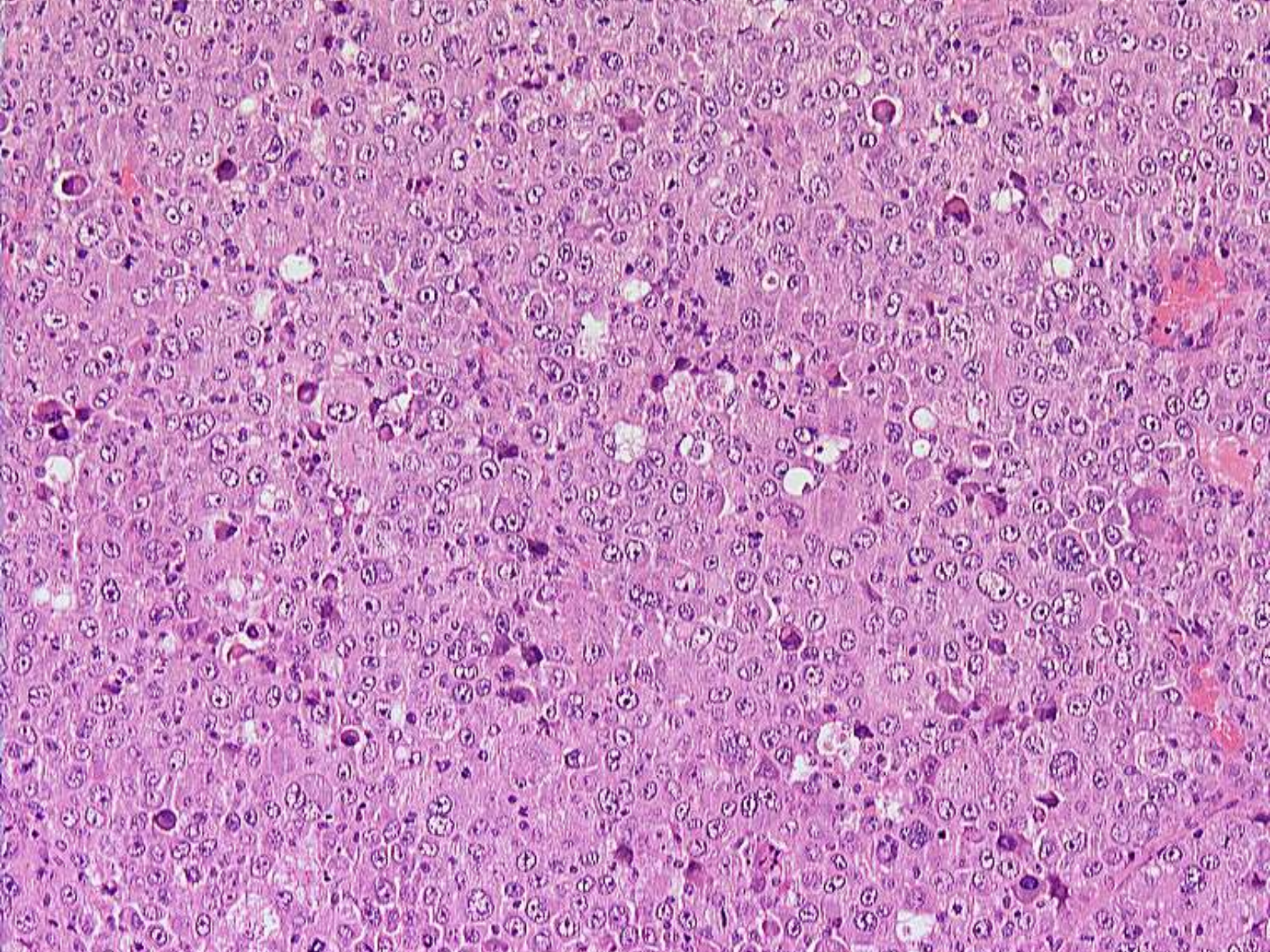
FACS

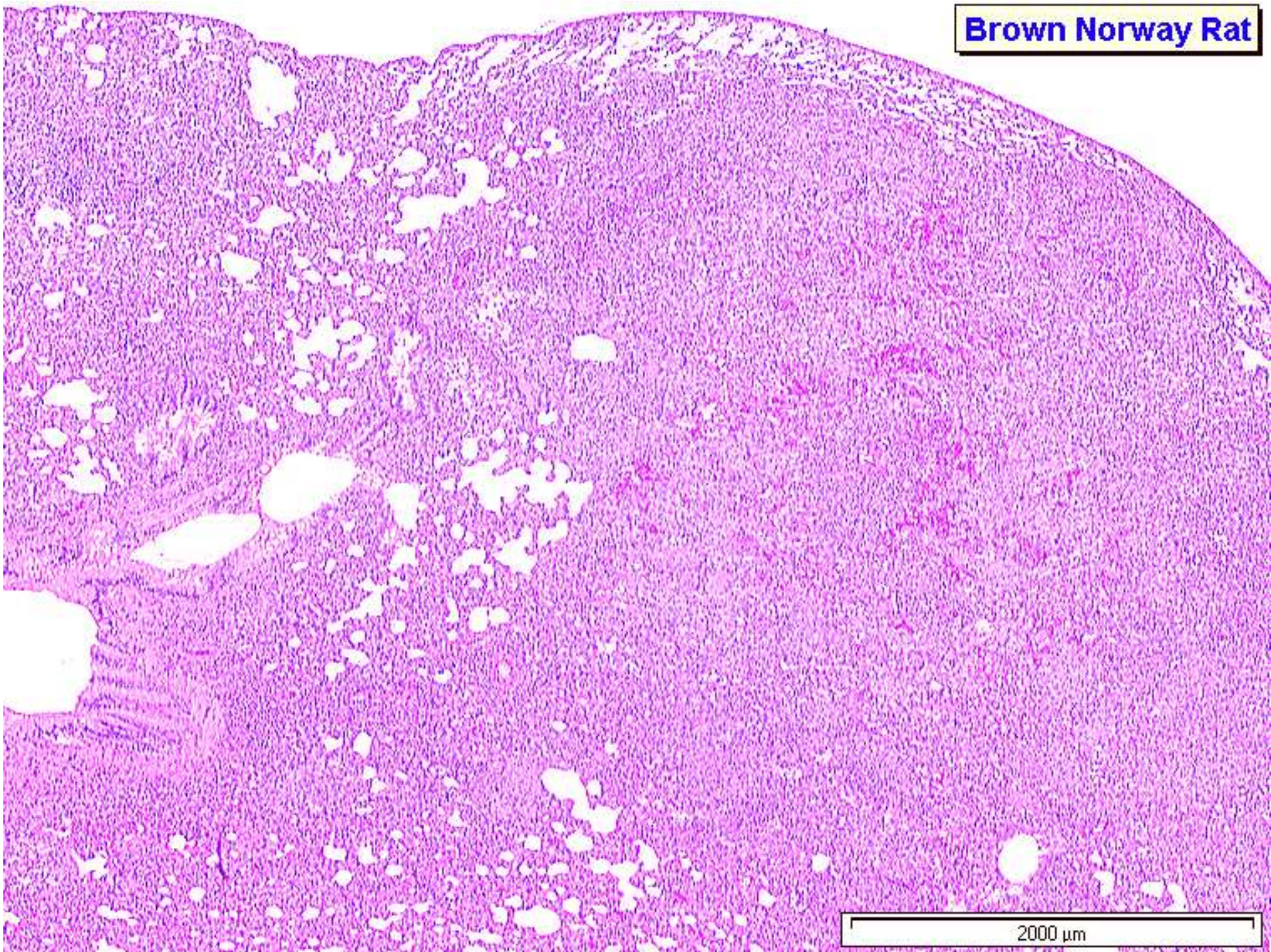
Cell culture

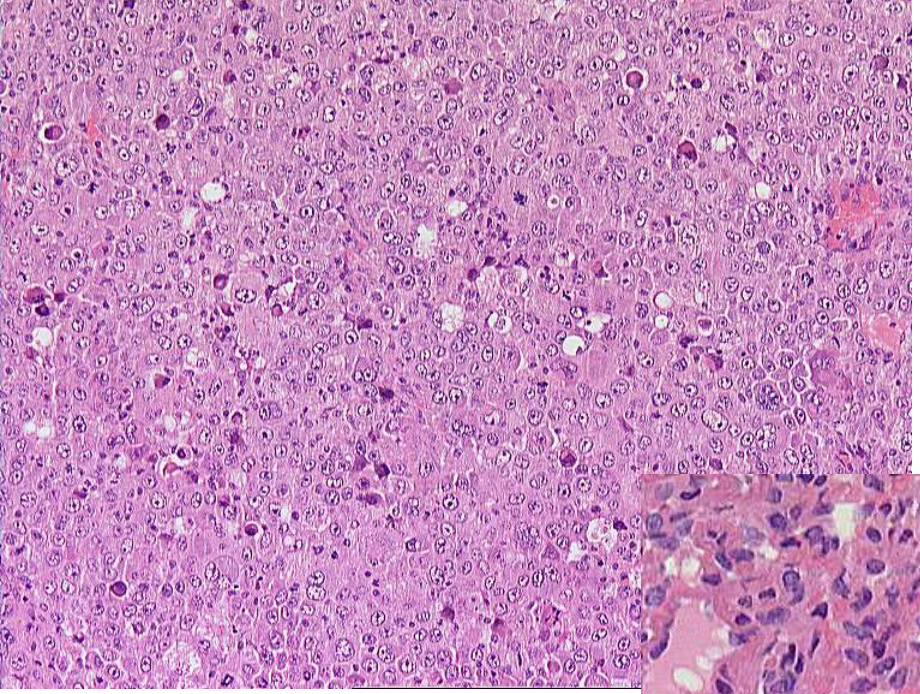
RT-PCR

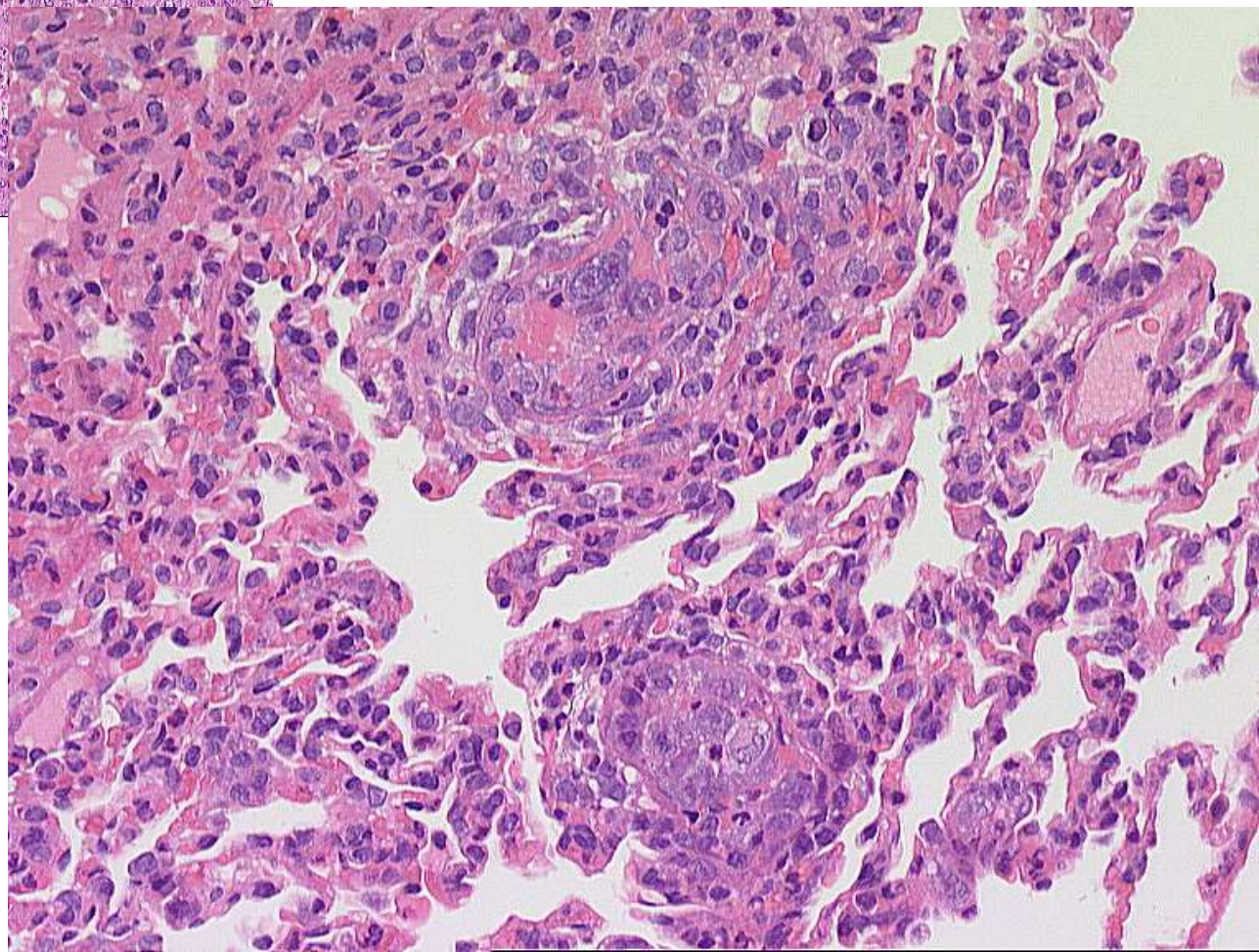
Study design: oncology research

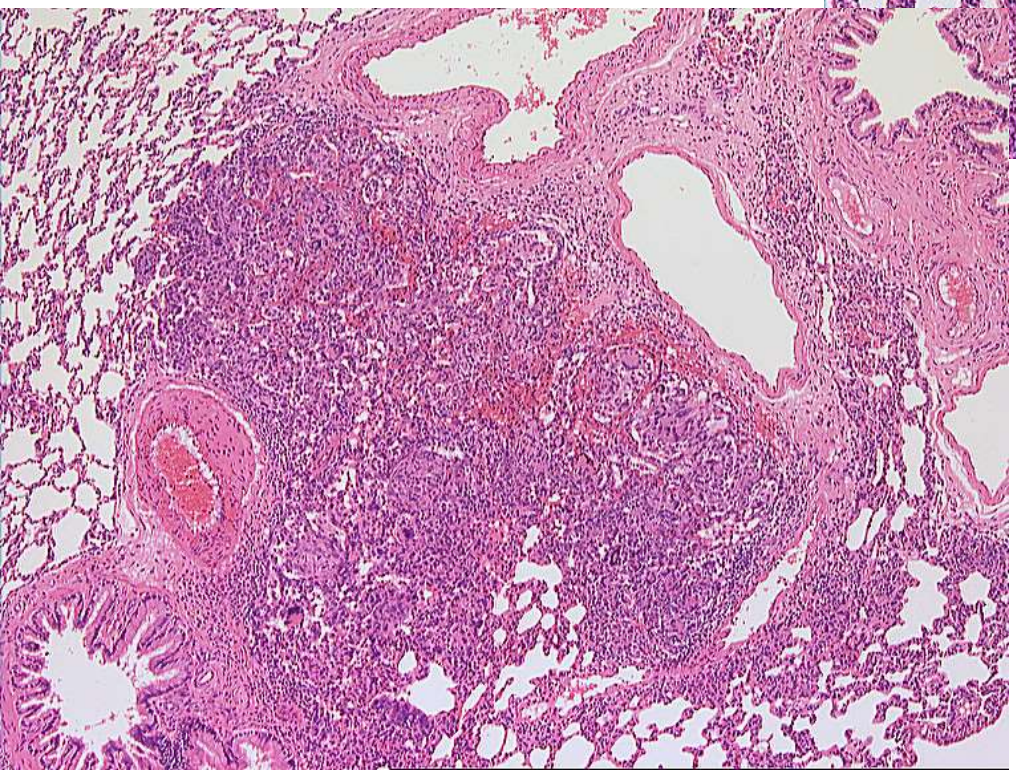
- Animal: Brown Norway rats, female
- Treatment: Matrix metalloproteinase inhibitors (MMP), oral, for 3 weeks
- Age: 9 weeks at time of necropsy
- Animal status: sacrified 21 days after tumour implantation
- Organ/Tissues: **subcutaneous tissue** (implanted mammary tumour, allograft BN 472) and **lung for counting macroscopic metastasis number and size**

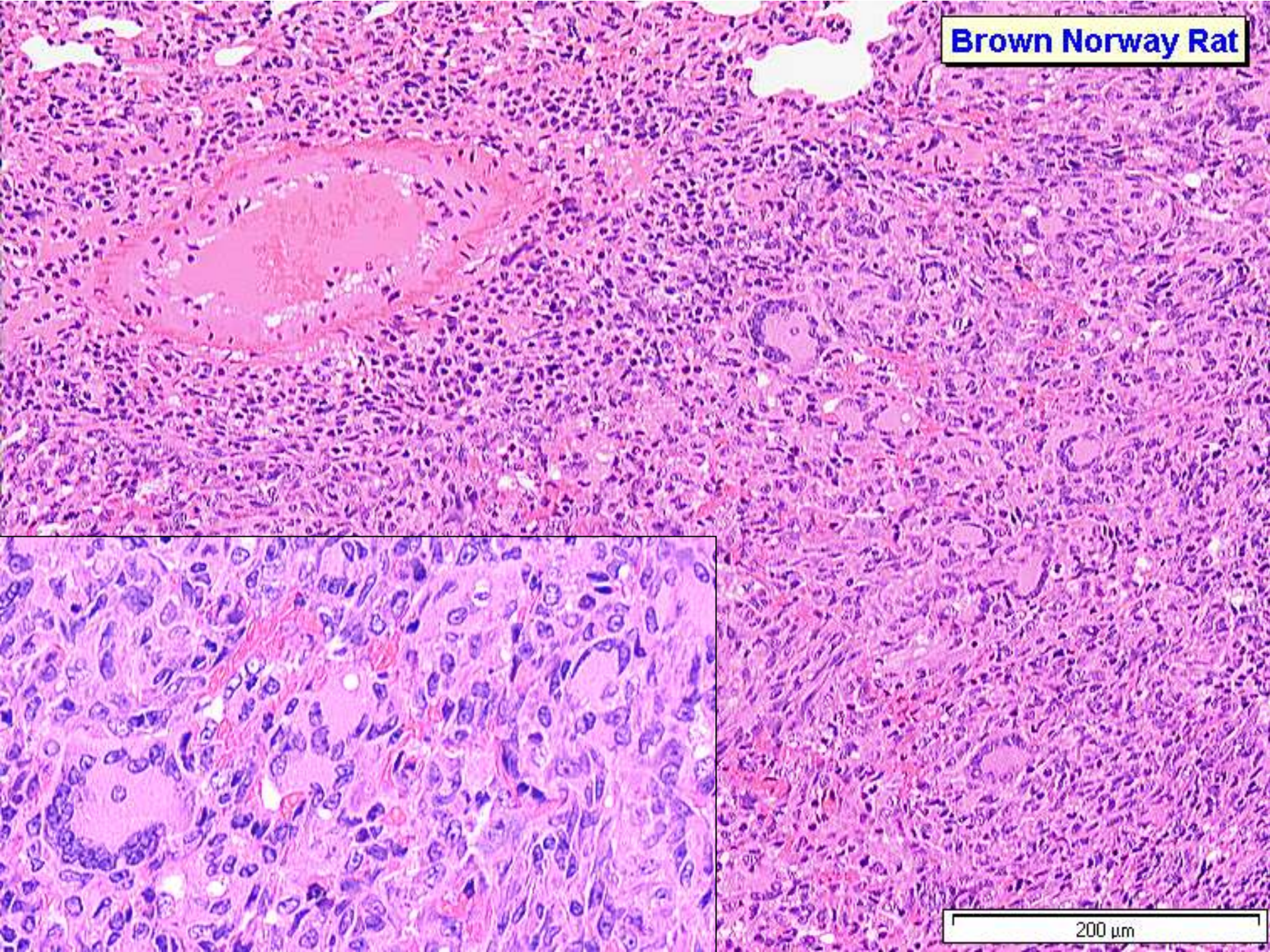


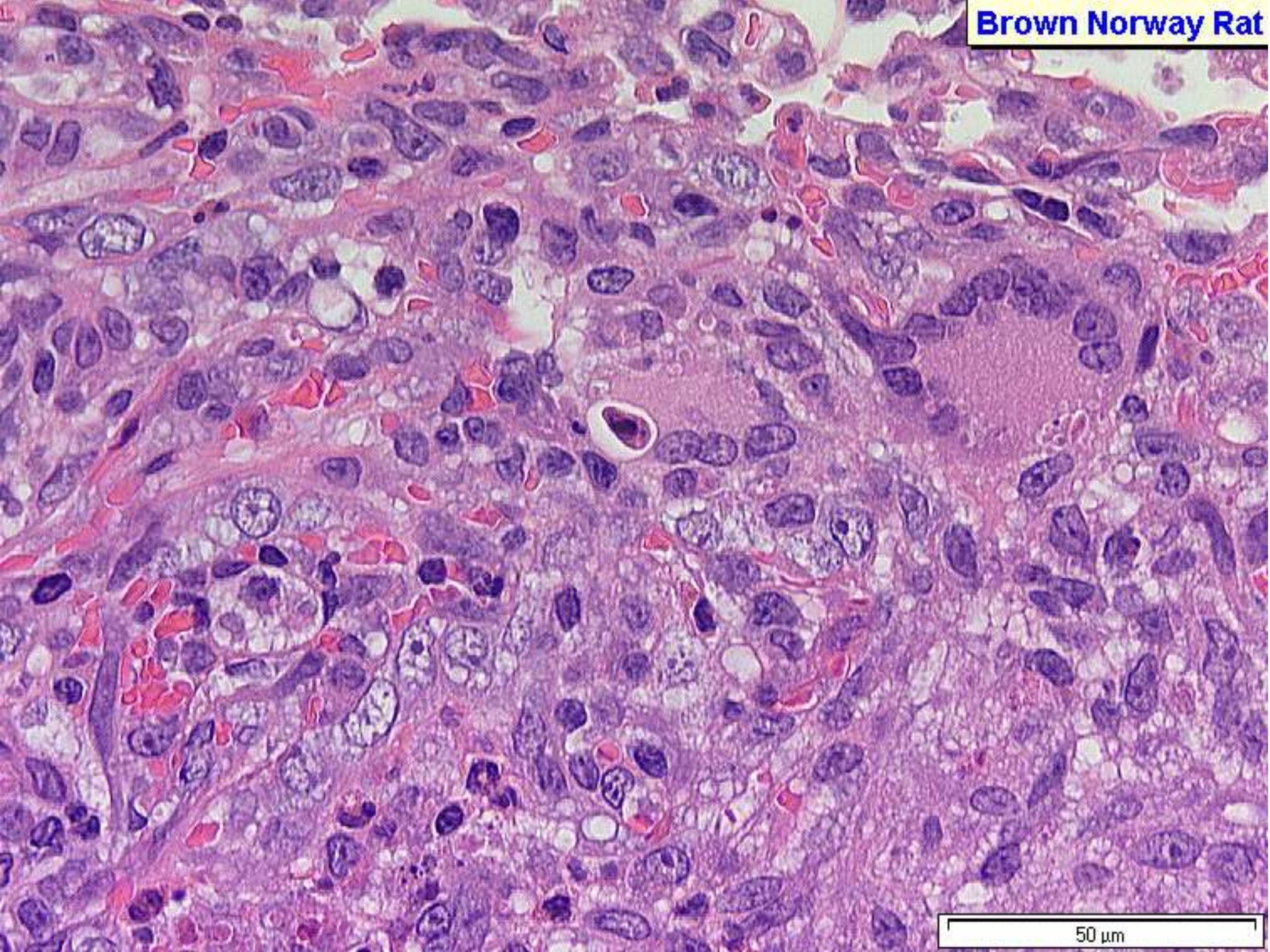

Brown Norway Rat
2000 µm

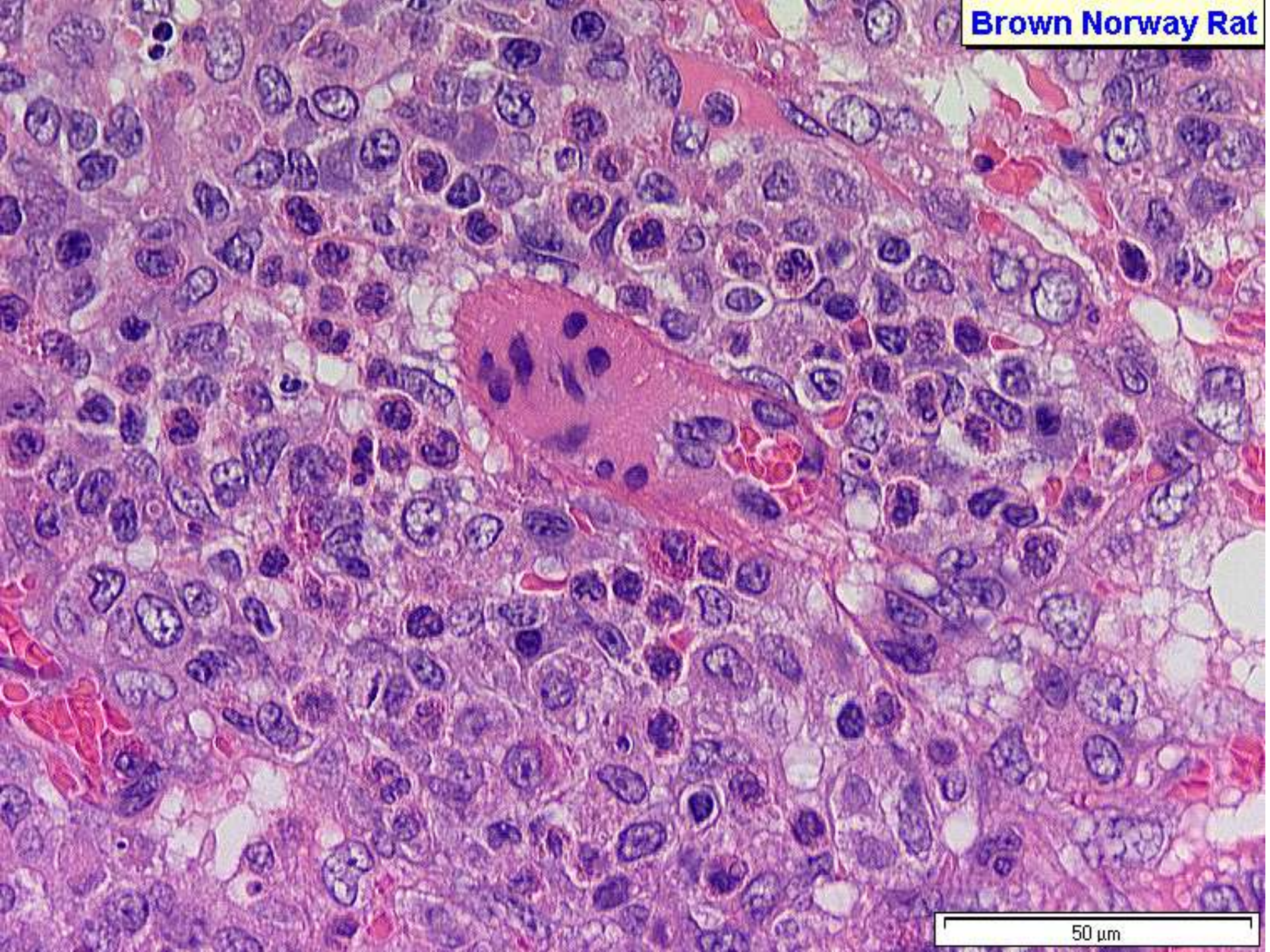







Brown Norway Rat
200 µm


Brown Norway Rat
50 µm


Brown Norway Rat
50 µm

Diagnosis

Granulomatous bronchopneumonia

Granulomatous pneumonia in rats from different breeders

Table 1. Incidence and mean severity grade of granulomatous pneumonia and eosinophilic infiltration depending on breeder and state of Brown Norway rats.

breeder	country	sex	weeks	rats with pneumonia/ examined rats	severity grade of granulomatous pneumonia	severity grade of eosinophilic infiltration
Chales River	Germany	m	6	11/11	2.6	2.2
Charles River	France	m	6	4/5	2.2	2.3
Charles River	USA	m	6	2/5	0.8	0.9
Harlan	USA	m	6	5/5	2.5	1.9
Harlan	**UK**	**m**	**6**	**0/6**	**0.0**	**0.8**
Moellgard	Denmark	m	6	4/5	1.6	1.5

pneumonia – granulomatous pneumonia; eosinophils – infiltration with eosinophilic granulocytes, * mena of semiquantiative gradings; no (0), mild (1), moderate (2) to severe (3) for pneumona and eosinophilic infiltration.

Discussion and Conclusion

- Macroscopic evaluation of lung is not sufficient to assess the presence of metastases in this organ
- To confirm the presence of metastases, the histopathologic examination is mandatory

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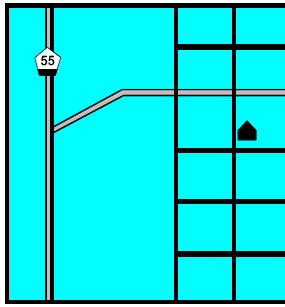
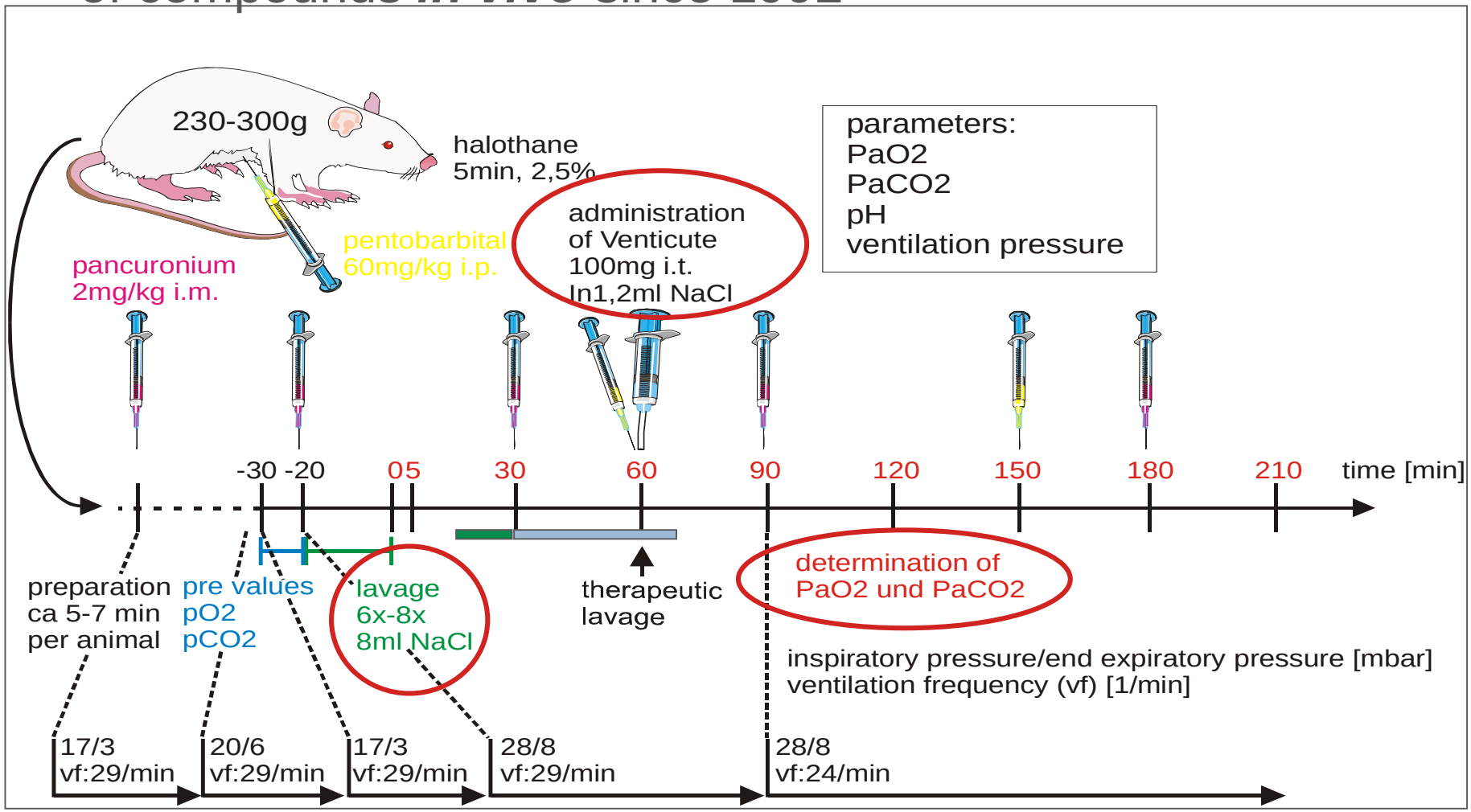
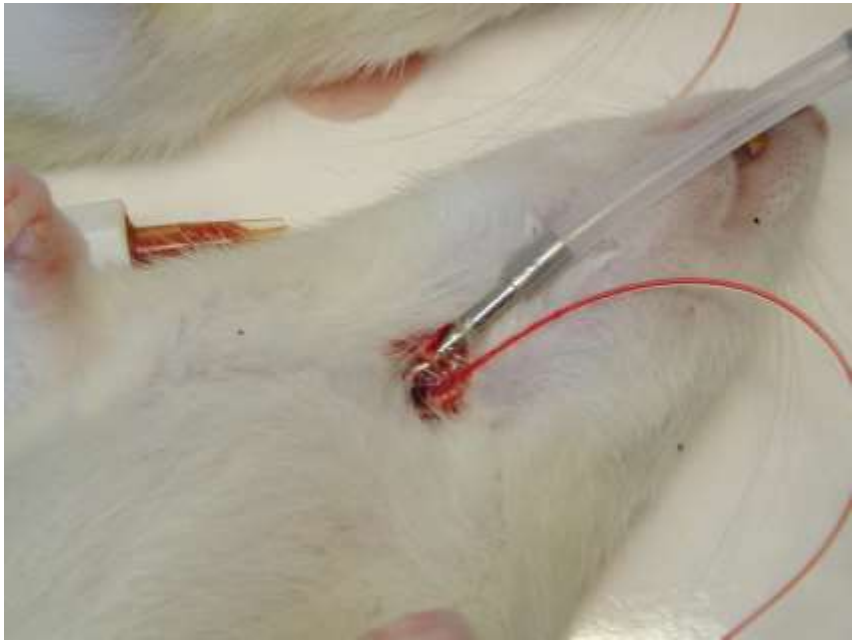


Rat lung lavage model

The **R**at **L**ung **L**avage model is used to measure activity/potency of compounds ***in vivo*** since 1992

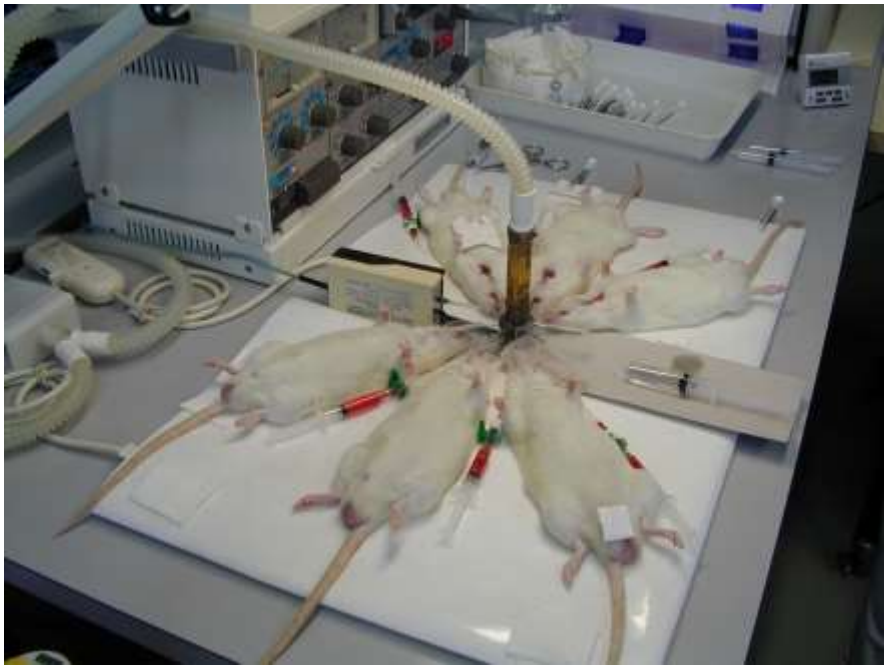


Rat lung lavage model

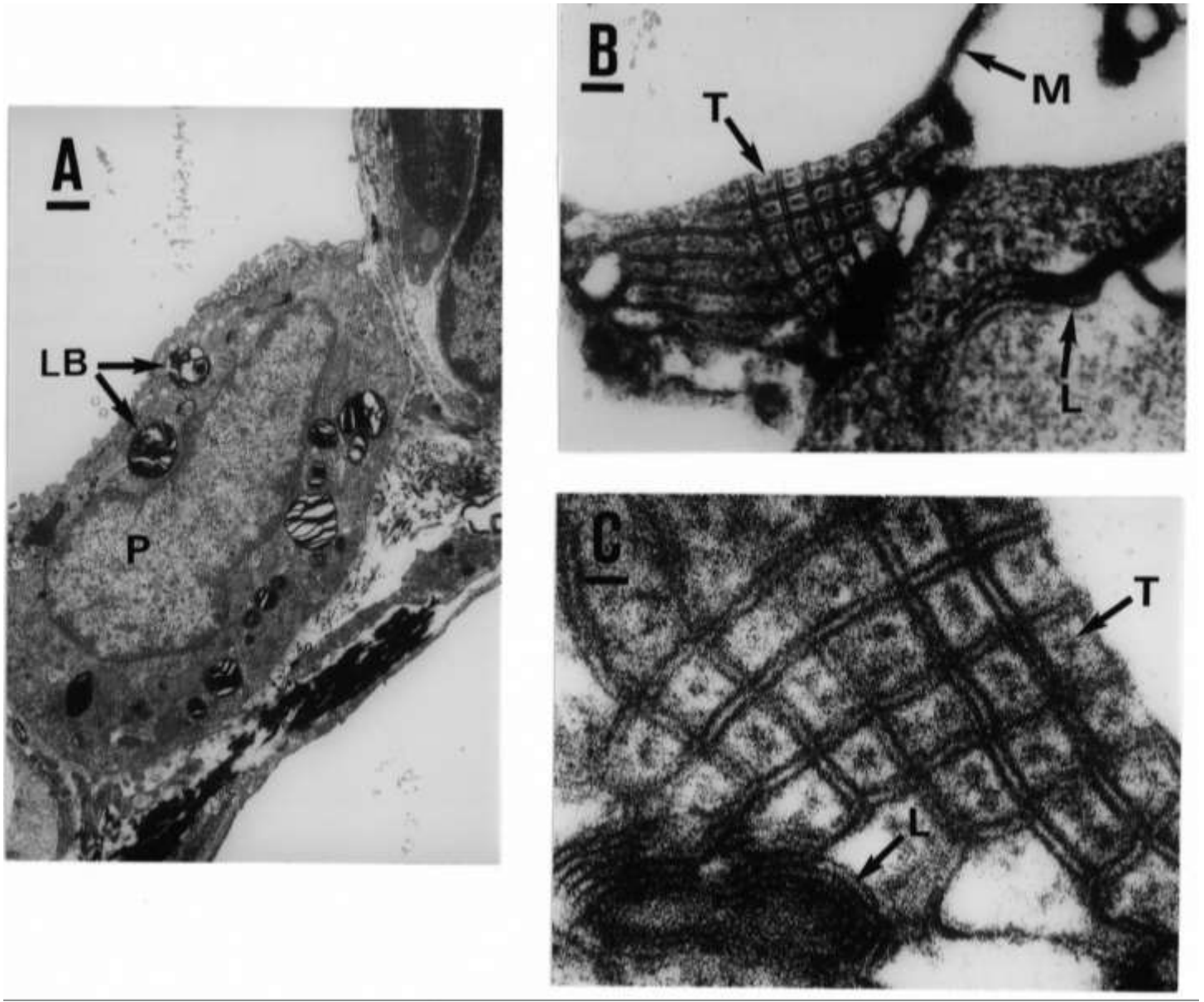


Tracheotubus und
Arterienkatheter

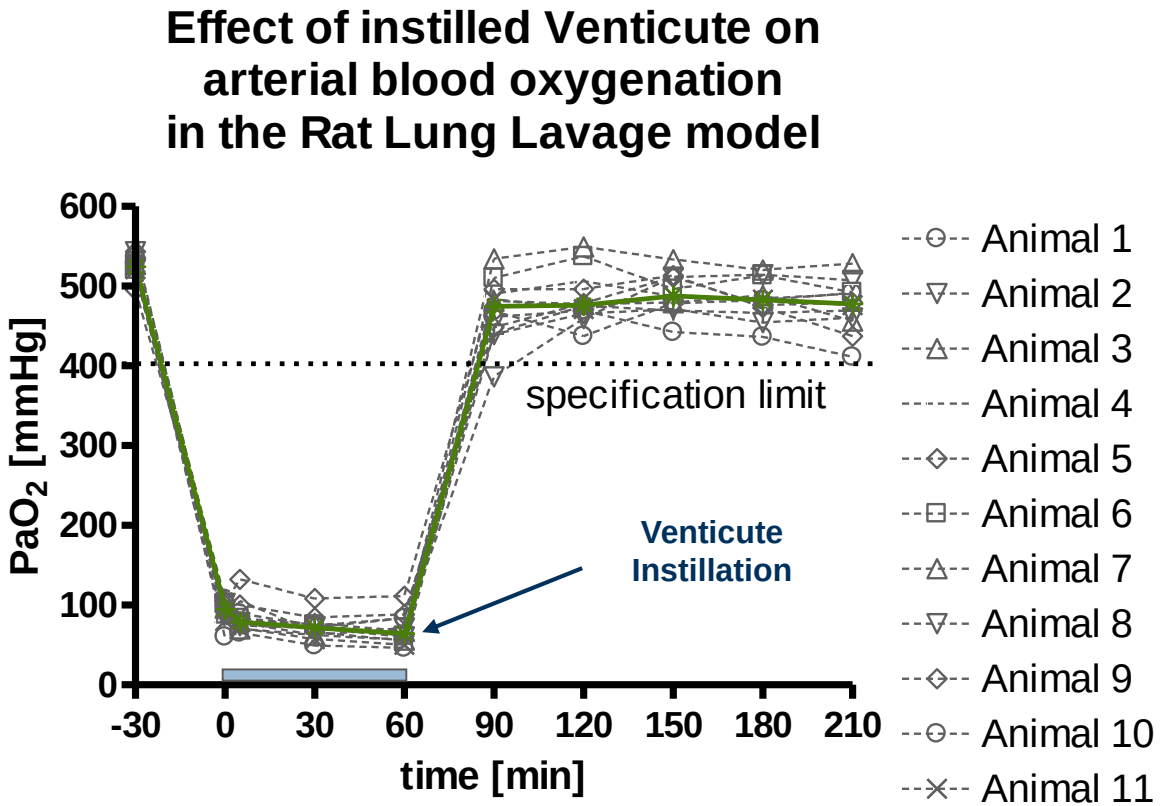
Ratten am Beatmungsgerät



Surfactant ultrastructure

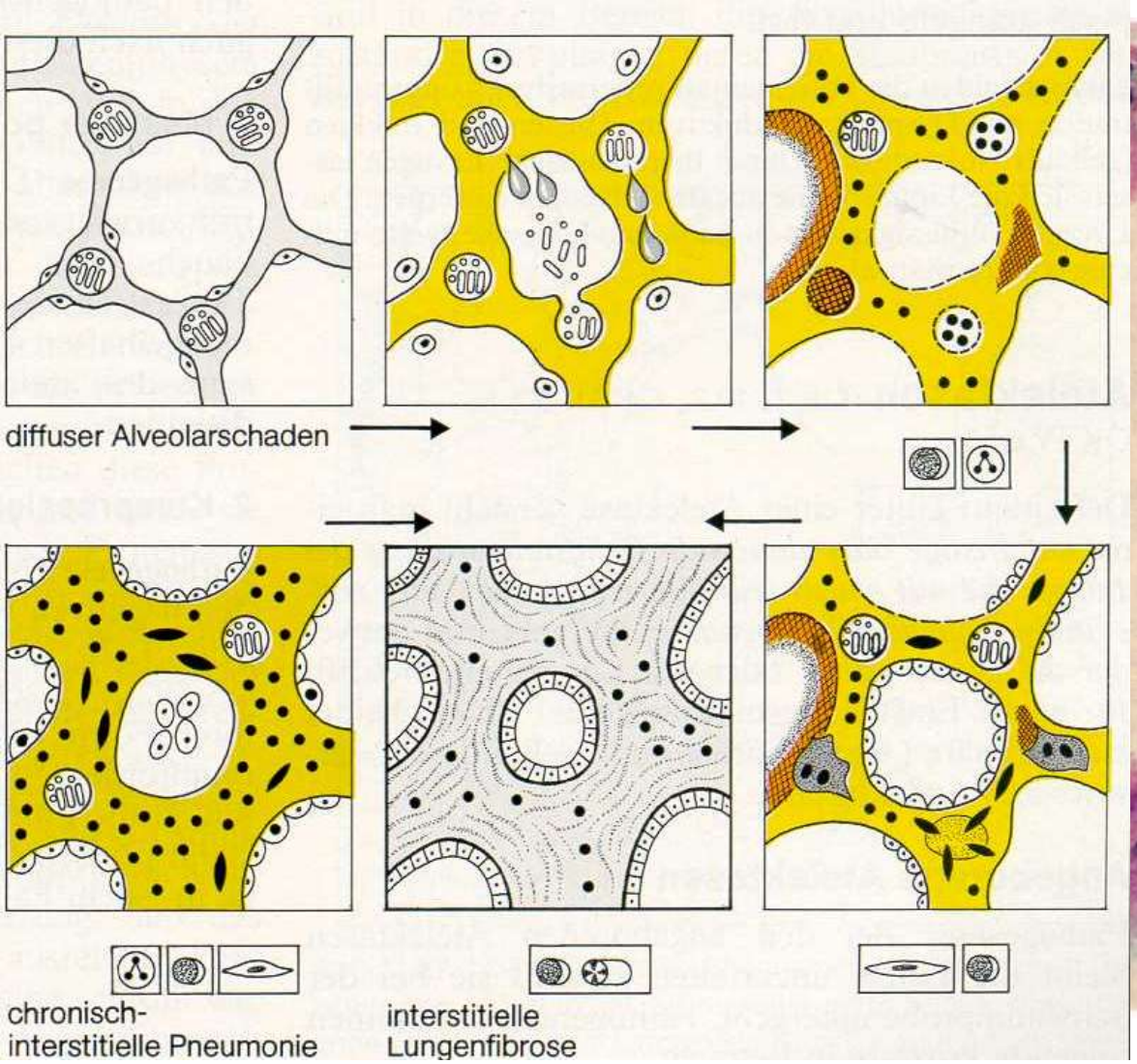


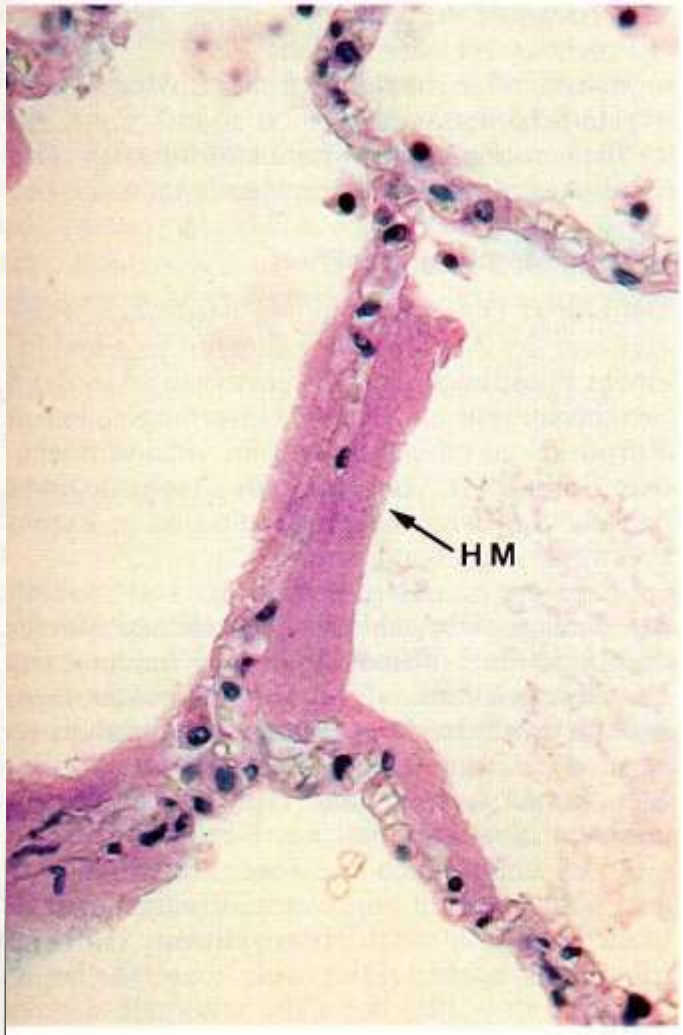
Background Standard Setup / Normal Response



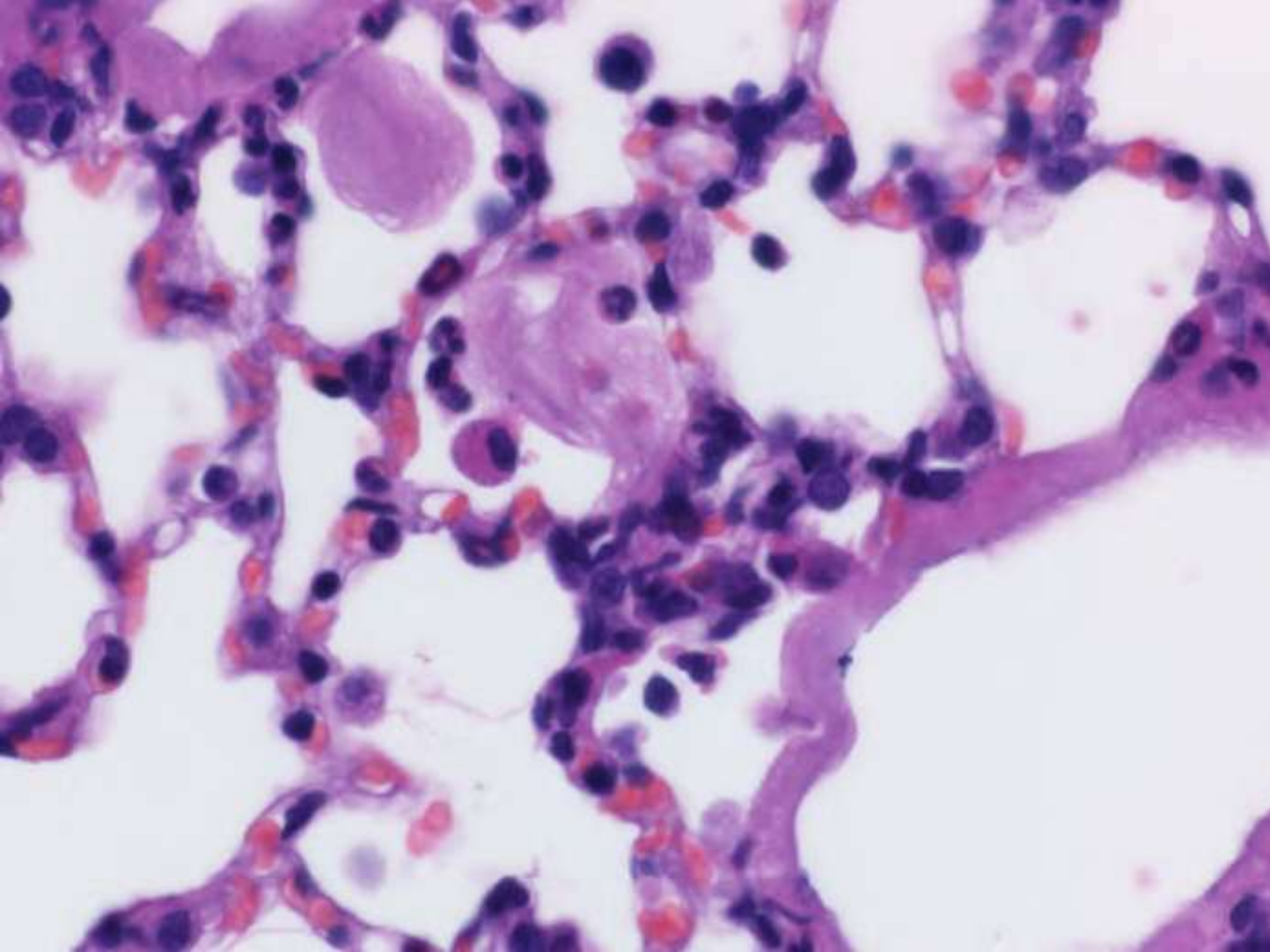
Male Spraque dawley rats (256-265g) were undertaken multiple saline lavages (8 - 10) at t = -30 to 0 min. Venticute (0440520000 5/36M) was instilled in a dose of 100mg/kg at t=60min i.t.

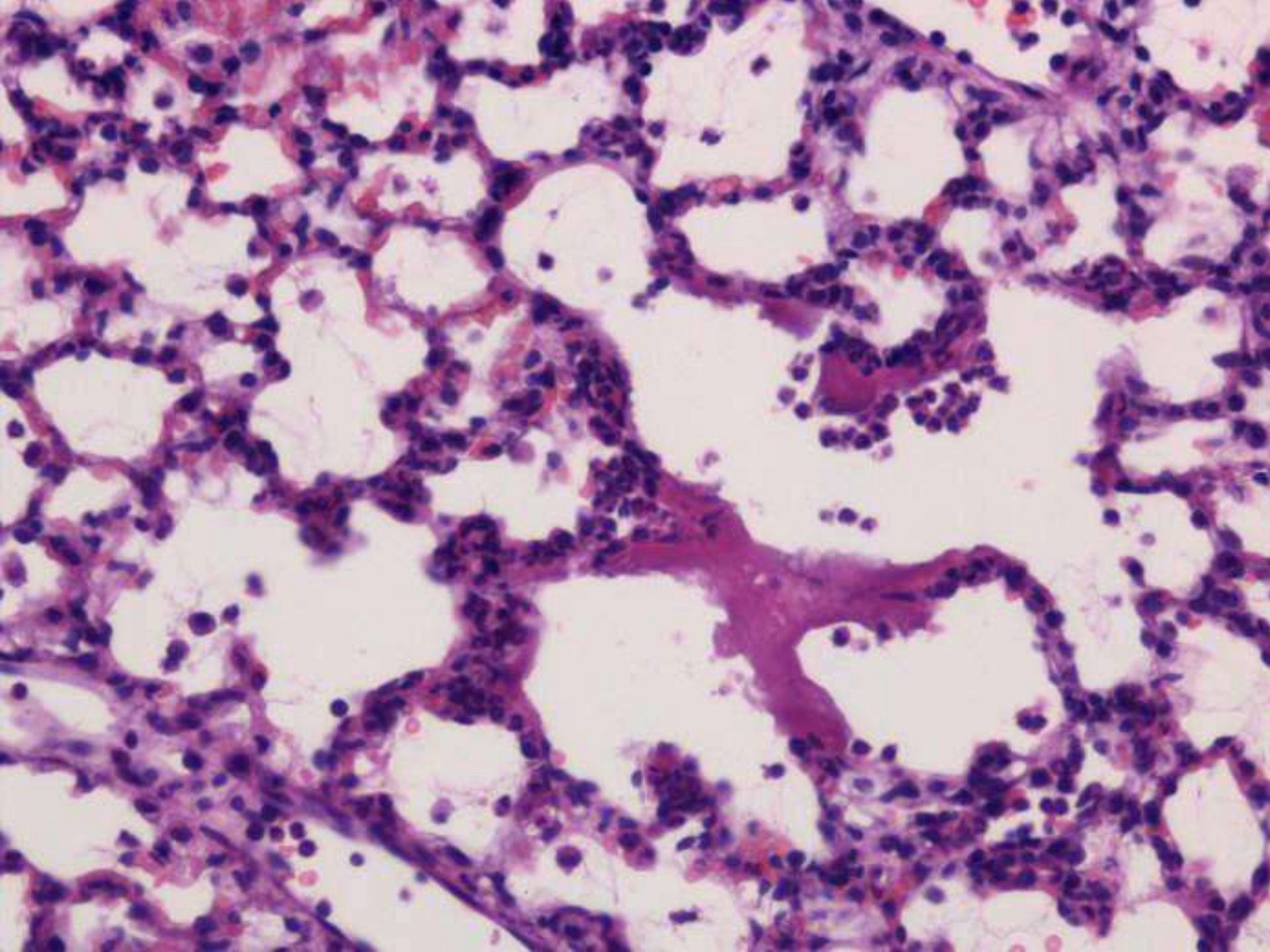
Pathology

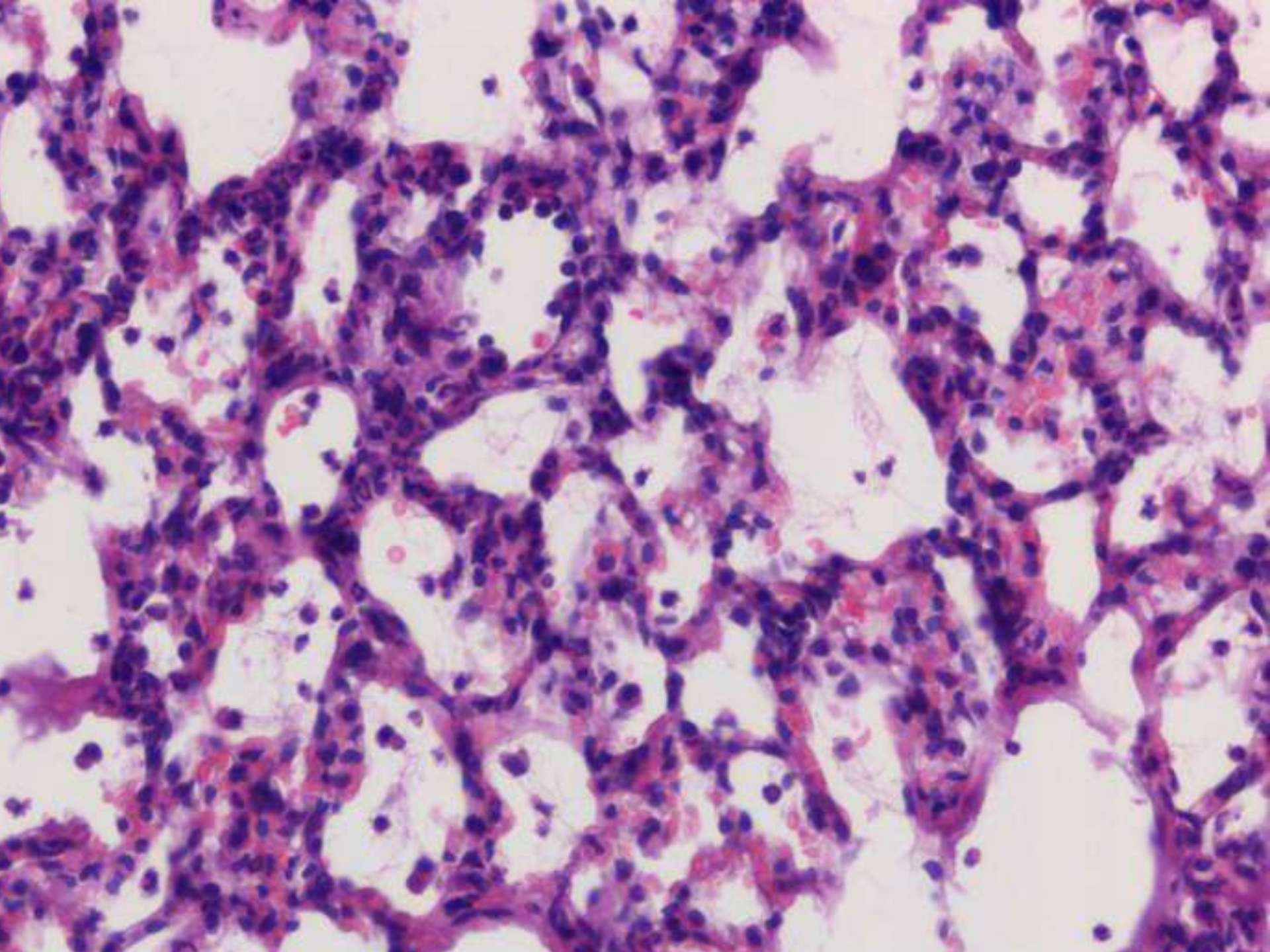


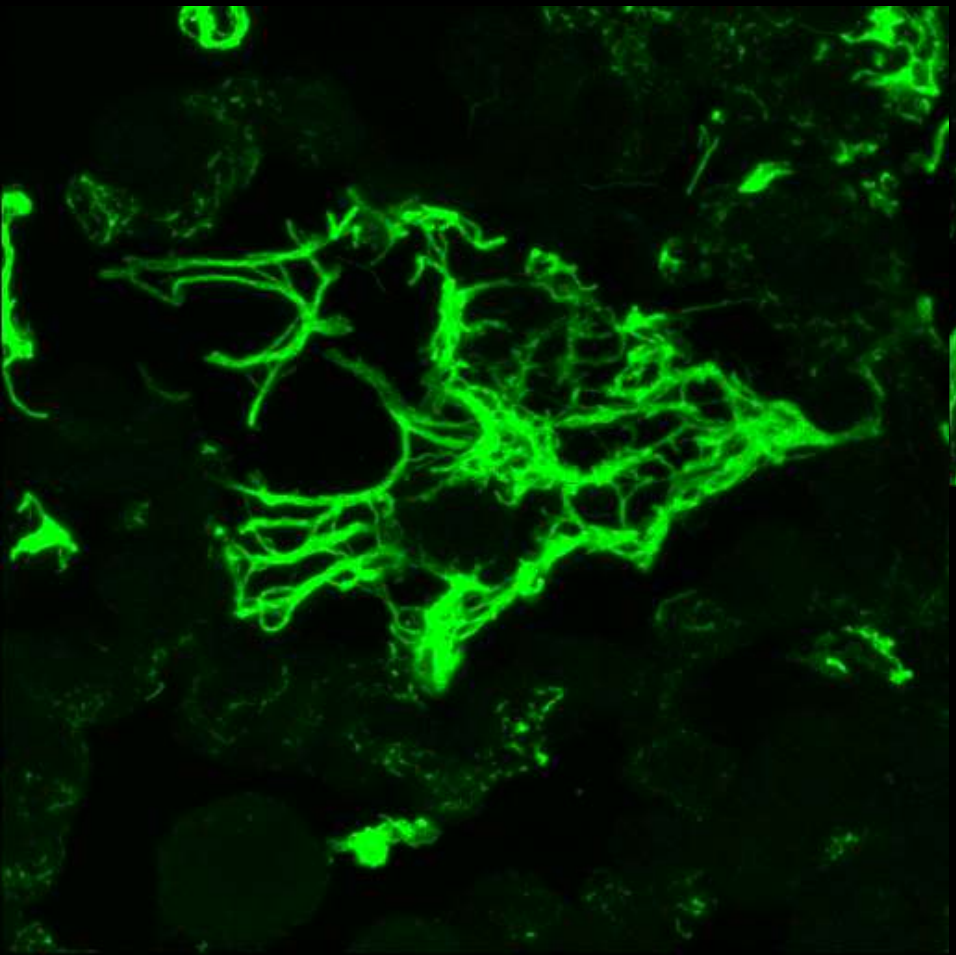


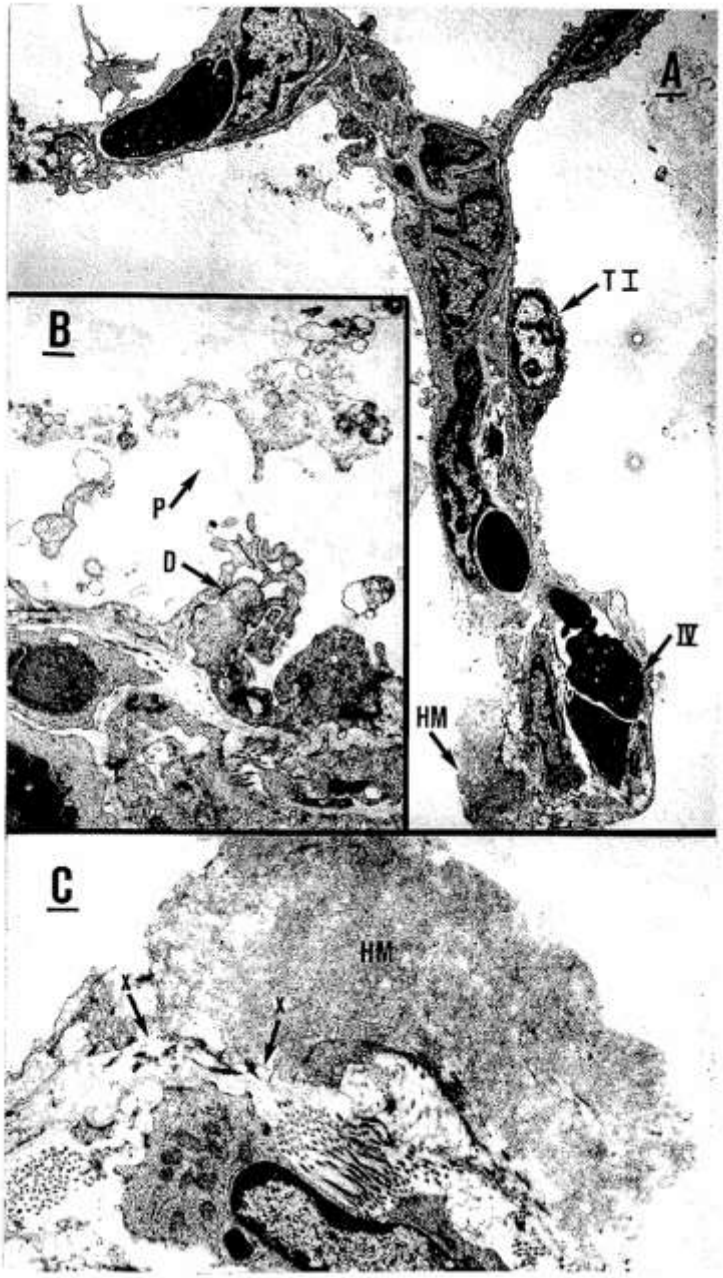
From Riede/Schaefer/Wehner: Allgemeine & Spezielle Pathologie, Georg Thieme Verlag Stuttgart, 1989







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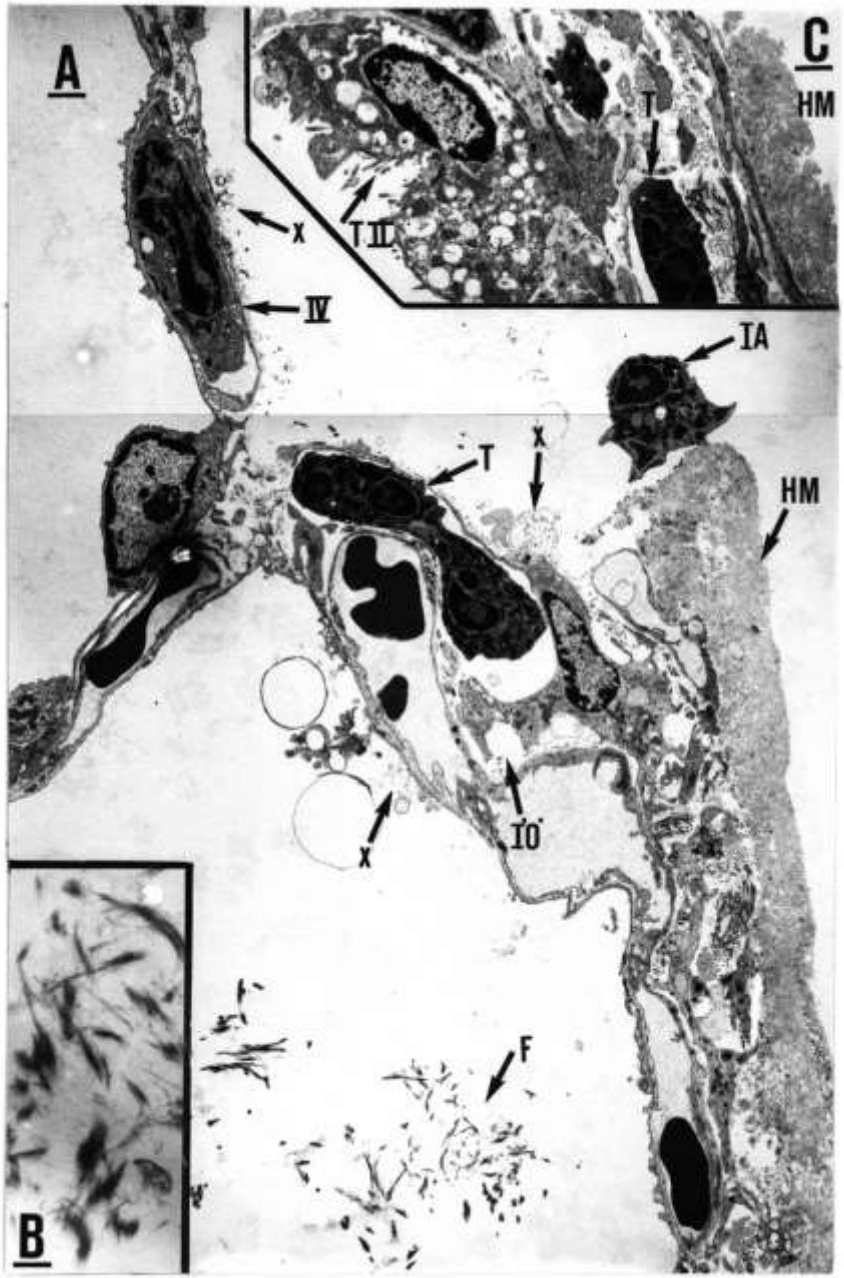
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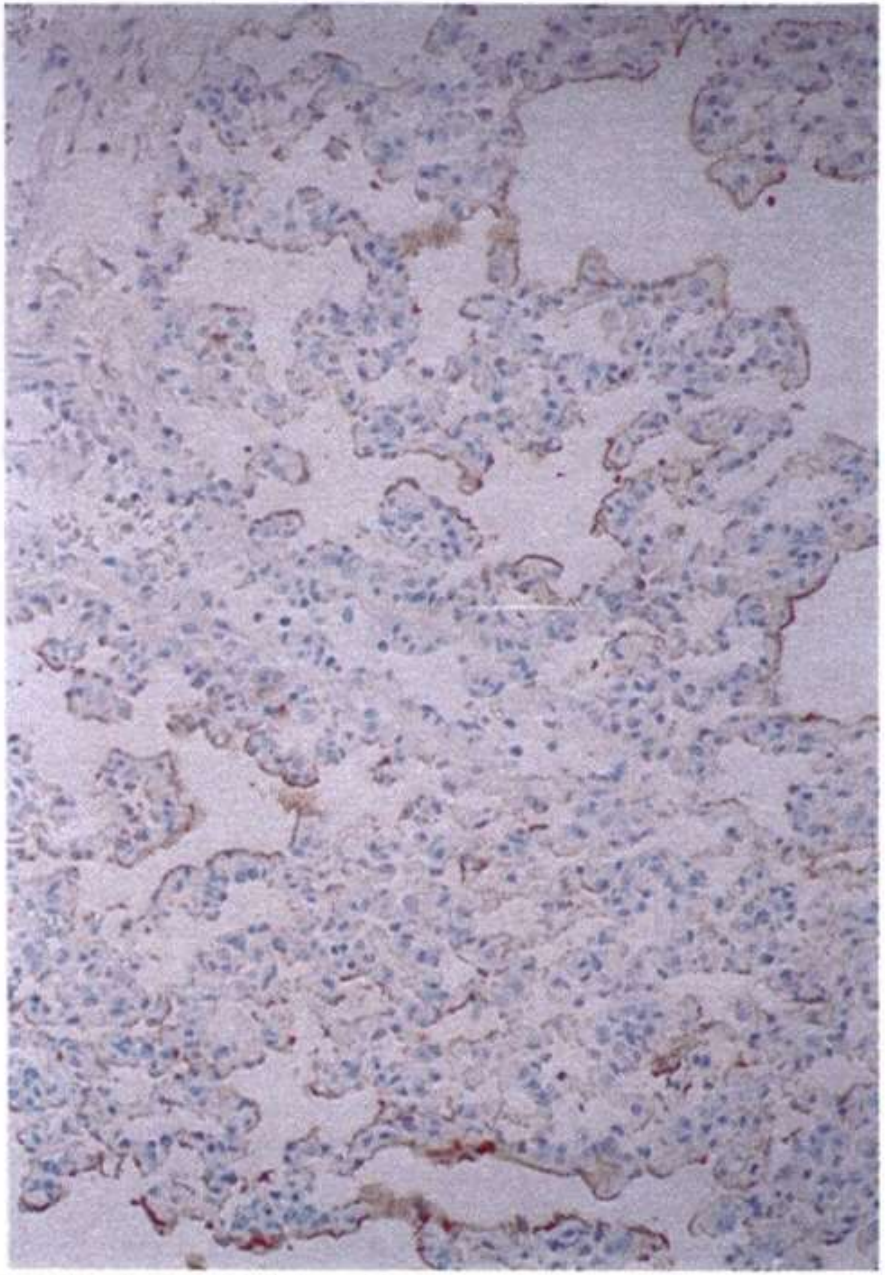
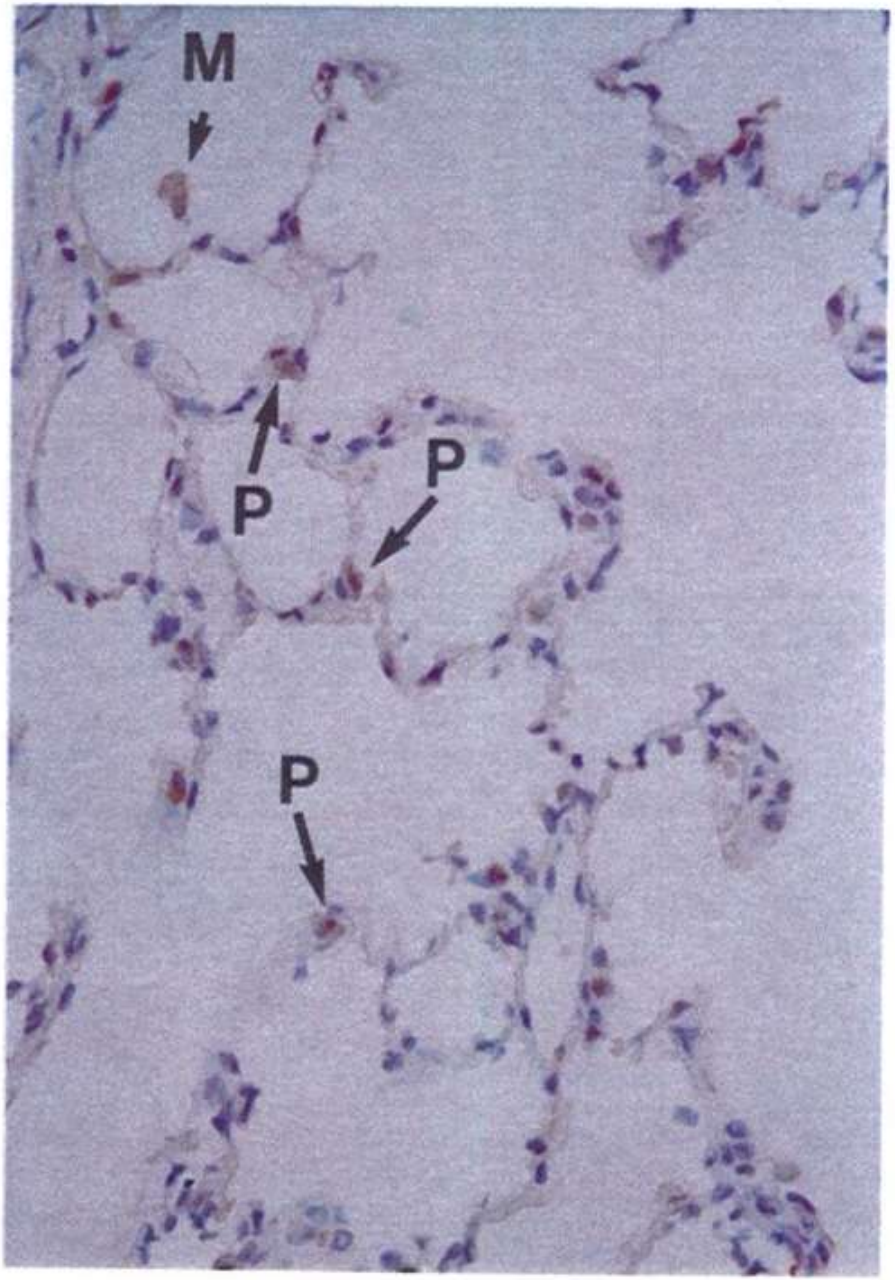
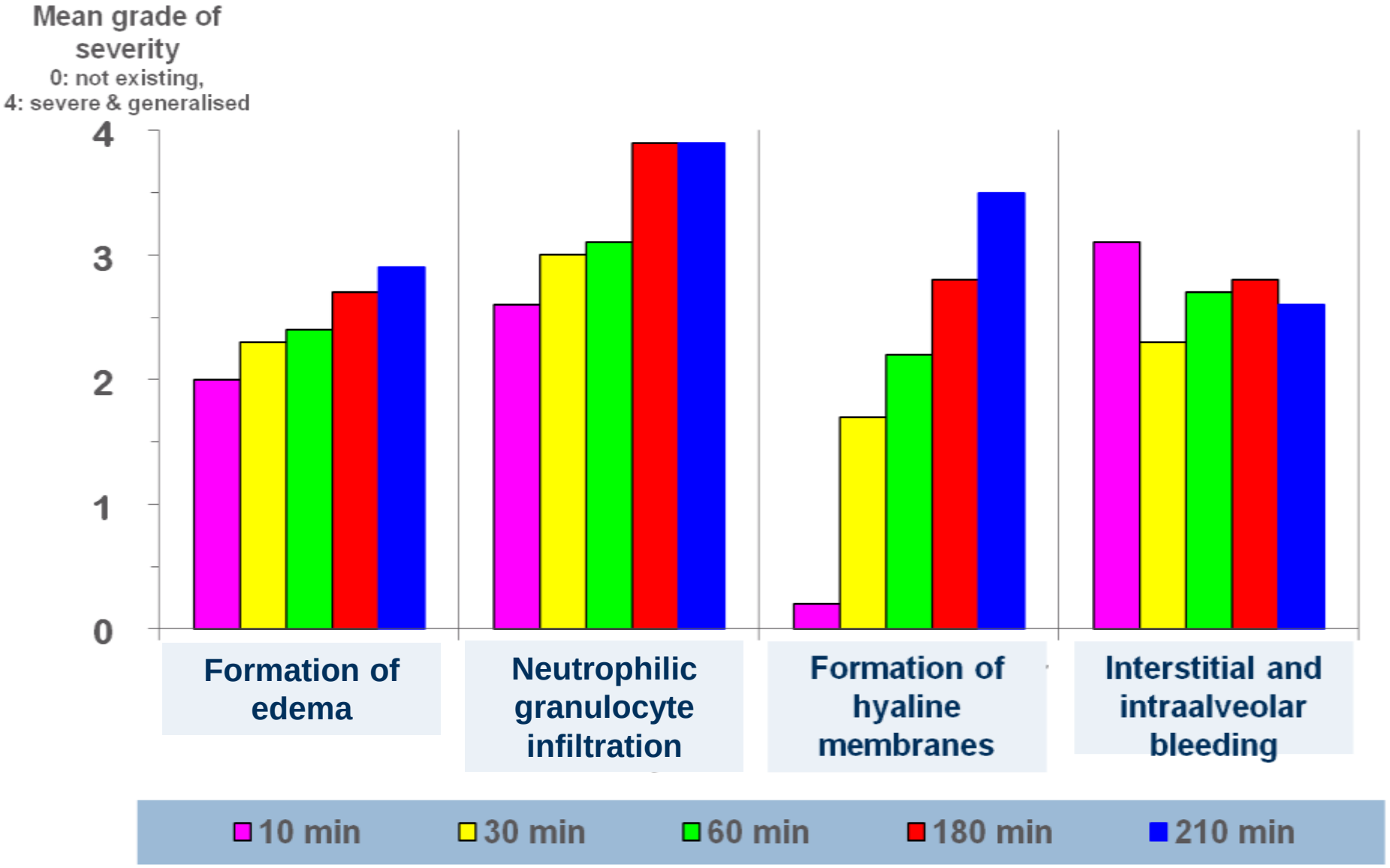


Publikation II, Abbildung 6

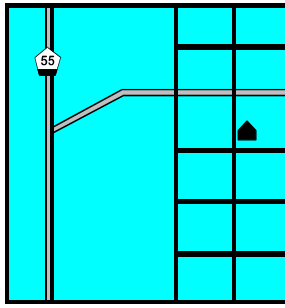


Publikation II, Abbildung 8

Time course of histological changes during the early phase



This is your road-map you can enjoy !!



Conclusion - Cotton Wool Rat - Measles

It was demonstrated that Cotton Wool rats develop an atypical (interstitial) pneumonia with necrotic lesions after intranasal infection resembling findings from human fatal cases of measles virus pneumonia. In the absence of superinfection, *restitutio ad integrum* is observed after 26 days.

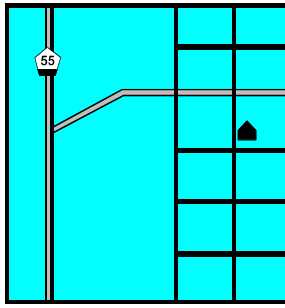
Conclusion – Brown Norway Rat - Asthma

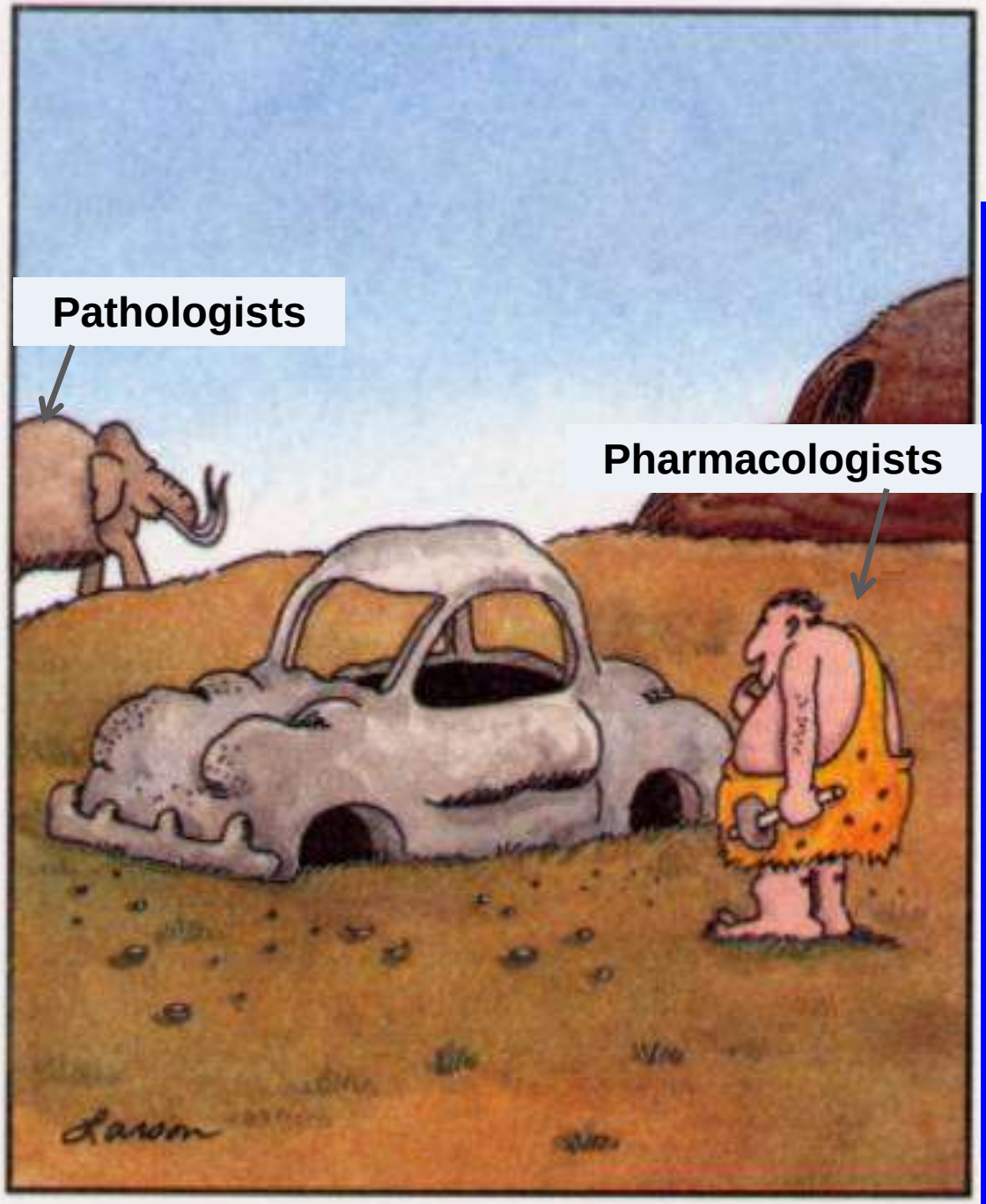
- The Brown Norway rat is not the proper model for experimental tumour growth and formation of metastases
- The immune system status of this animal strain and the high lung susceptibility to inflammatory reactions indicate that it is not suitable to be used as an experimental model in oncology.
- Suitable for respiratory research ??????

Conclusion – Rat lung lavage model

- The early exsudative phase of ARDS in the rat lavage model shows histologically a time course and sequence of events for its main features:
  - Edema formation, infiltration with neutrophils and hyaline membrane formation.
  - The severity and onset of these hallmarks is dependent to the number of lavages, the lavage volume and the therapeutic treatment.
- Bleeding is considered to be a mechanical induced artefact.

This is your road-map you can enjoy !!

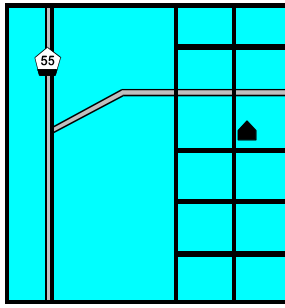




Take Home Message

Morphological & functional working scientists should liase very closely their expertise areas for pharmacology models. This improves the quality of animal models and of the obtained results for a better selection of drug candidates. Model validation is key.

This is your road-map you can enjoy !!



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Dietrich Häffner, Thomas Grebe, Ewald Benediktus
and many many others

for the excellent contribution and support

Histopathology is beautiful !!!

Thank you very much for your attention

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D. Häfner, P.-G. Germann, D. Hauschke. Drug Research 48 (3): 14-16, 1998;

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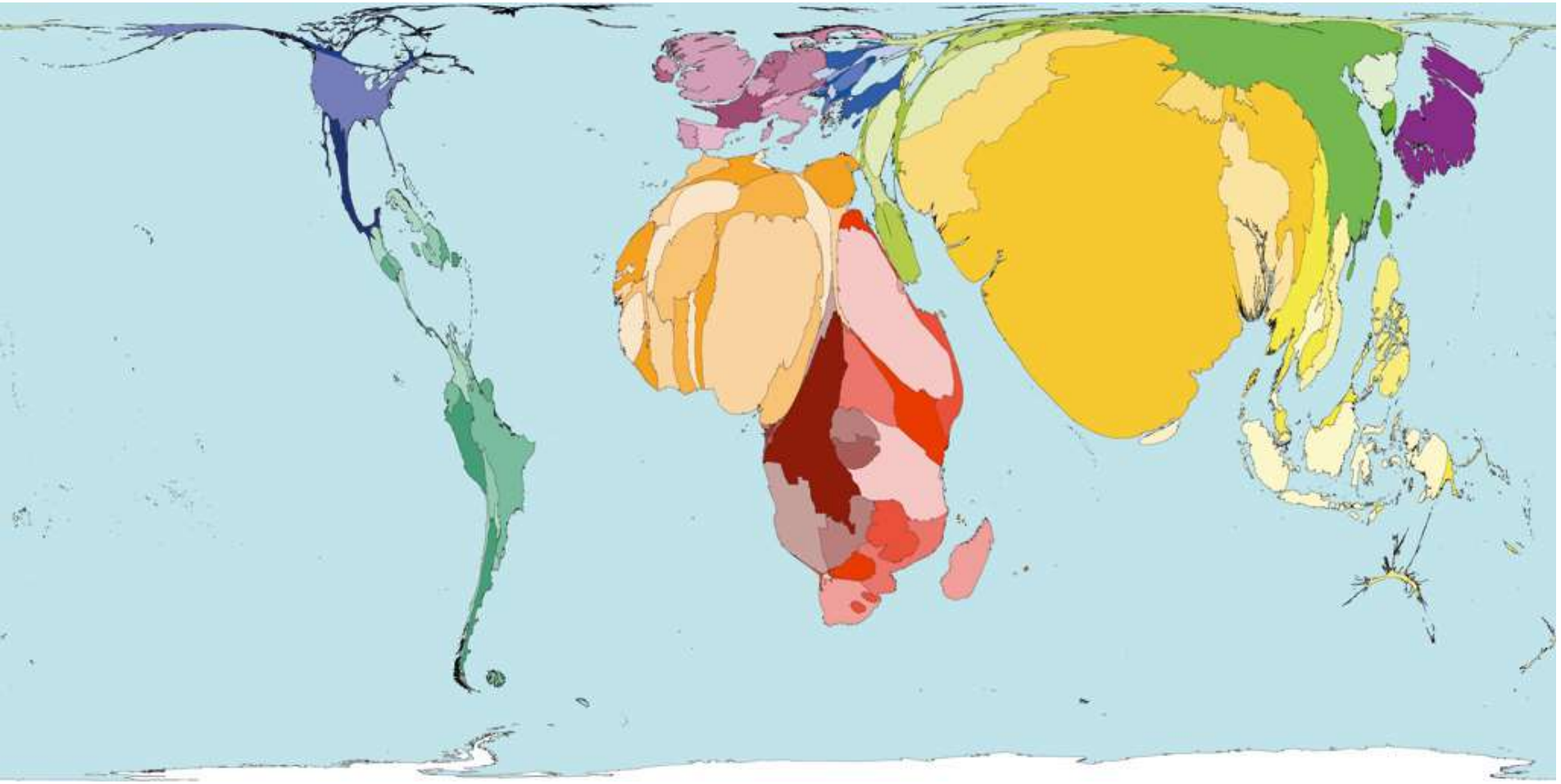
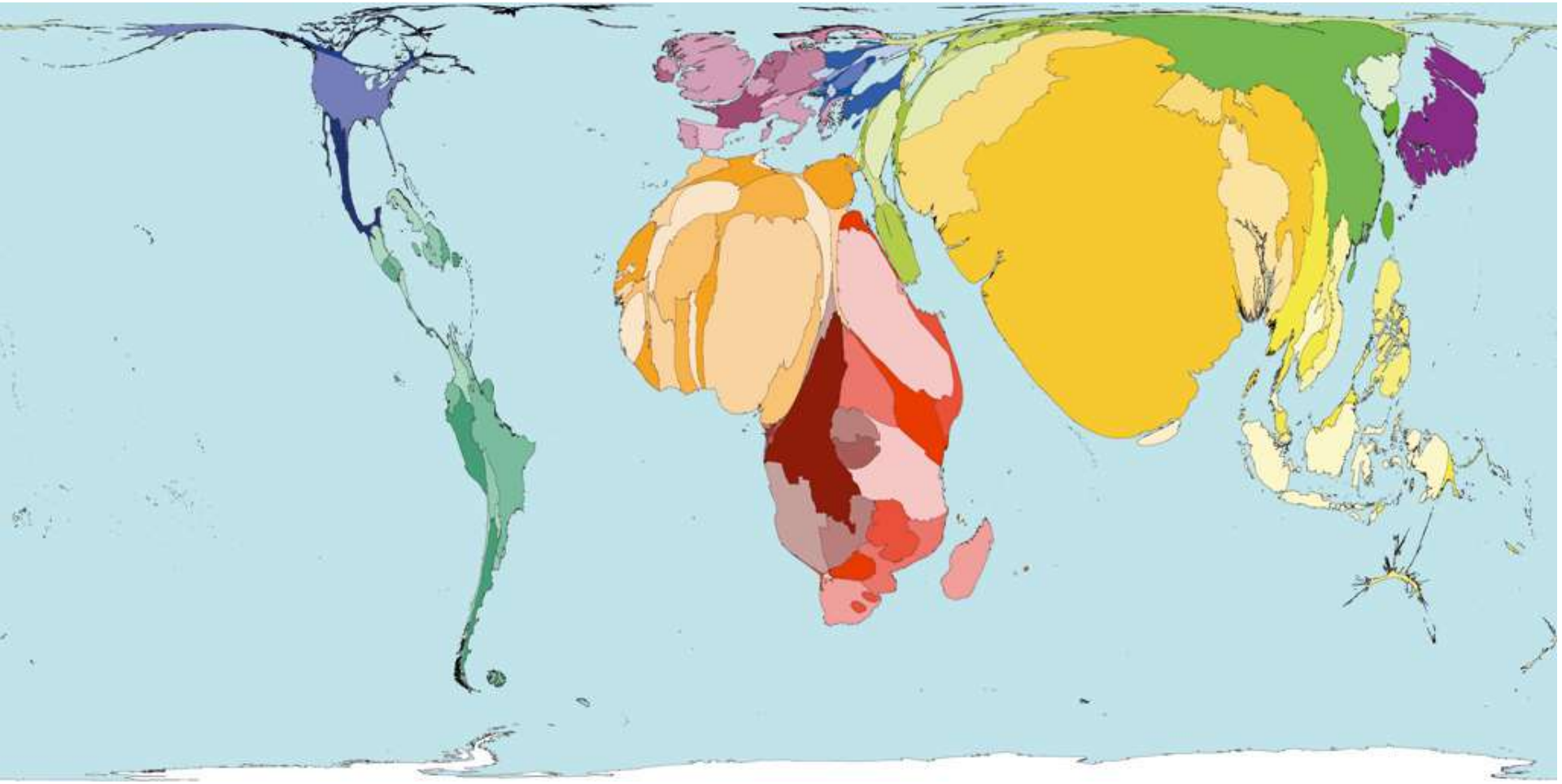
Back Up Slides

Pneumonia Deaths

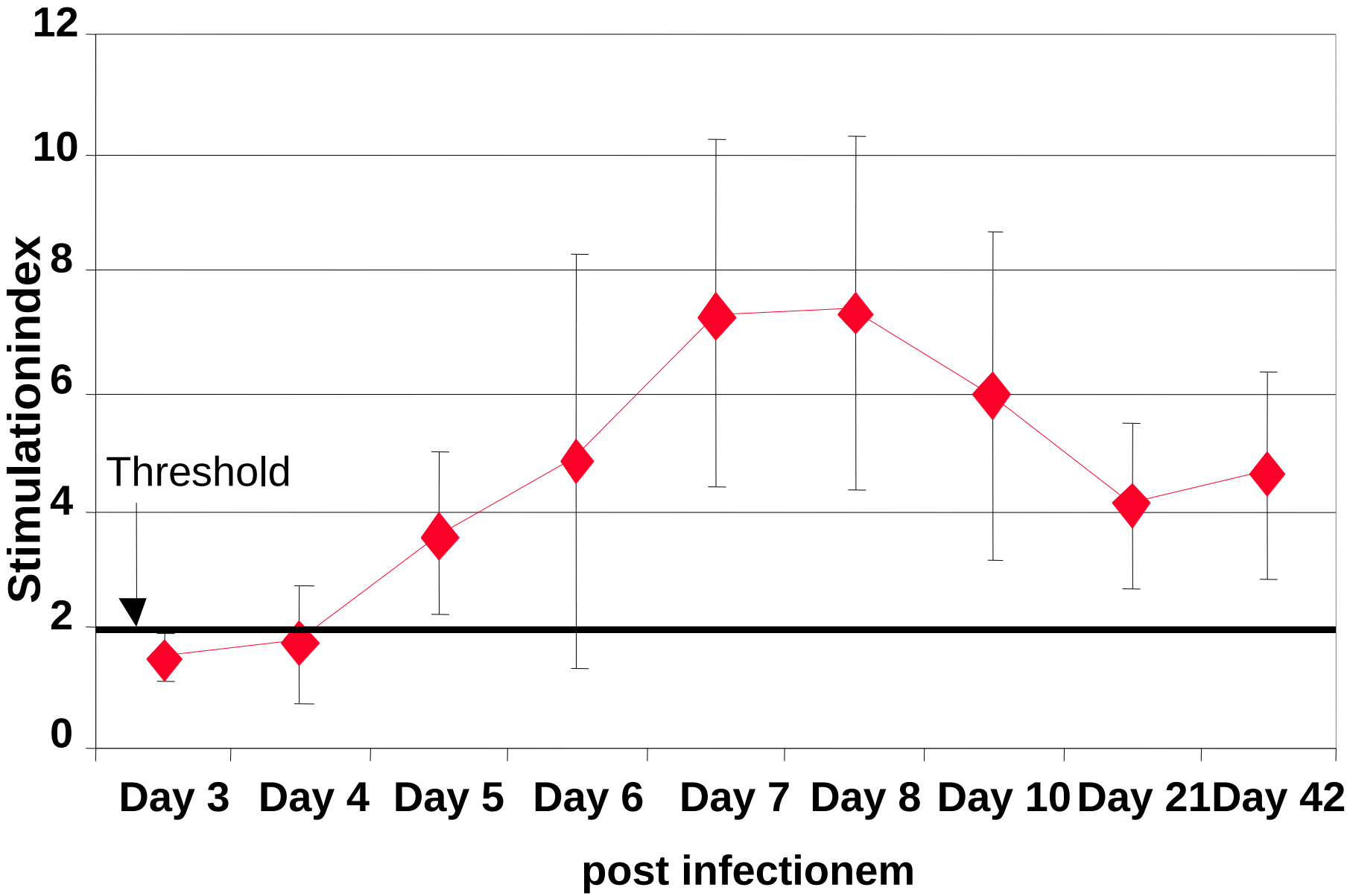


Deaths from respiratory infections



Respiratory infections caused 7.0% of all deaths worldwide in 2002, an average of 647 deaths per million people per year.

Immune cell reactivity



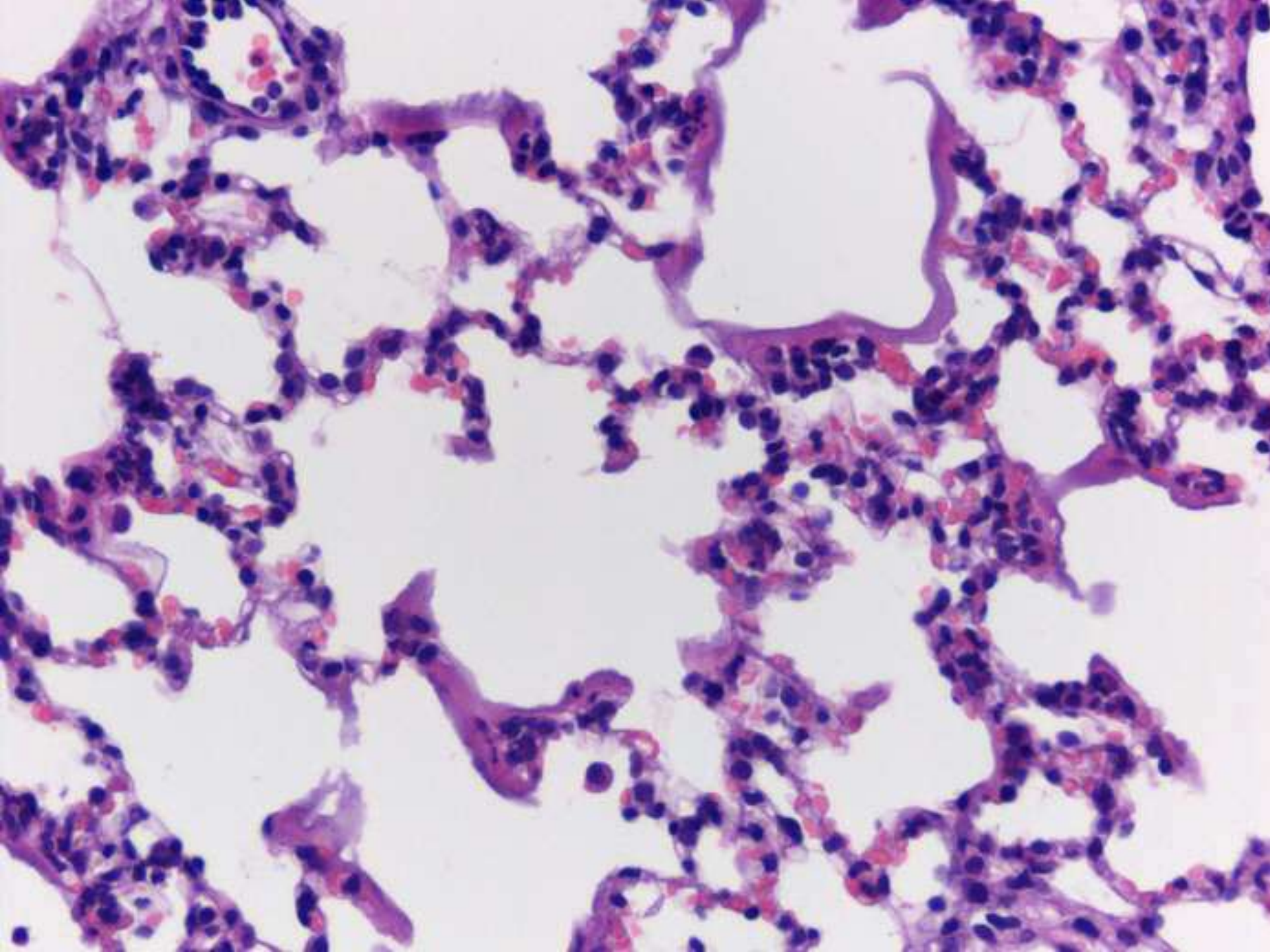


Table 2. Results of microbiological analysis of Brown Norway rat lungs in the veterinary research laboratory in Aulendorf (5 rats) and the School of Veterinary Medicine in Hannover (3 rats).

infectious agent	used method	rats: infected/analysed	
		Aulendorf	Hannover
Pasteurella multocida	Bacterioscopic, Methylen Blue strain, Gassner's blood agar	0/5	0/3
Pasteurella pneumotropica	Bacterioscopic, Methylen Blue strain, Gassner's blood agar	0/5	2/3
Listeria monocytogenes	Palcam Agar	0/5	0/3
Erysipel. rusiopathiae	Gassner's blood agar	0/5	0/3
Streptococcus spp.	Thalliumsulfate-Cristal Violett-Toxin-agar	0/5	3/3
Staphylococcus aureus:	Blood agar; blood agar with clumping factor coagulase	0/5	2/3
Bordetella bronchoseptica	Selective agar according to Gassner	0/5	0/3
Salmonella spp.	Enrichment in Rappaport-Cassiliadis-Boullion; Brillant-Green-Phenol-red-lactose agar	0/5	0/3
Escherichia coli	Selective agar according to Gassner	0/5	2/3
Mycoplasma spp. with cholesterol	Bacterioscopic, Giemsa's stain; serum agar	–/–	0/3
Leptospira spp.	Microagglutination test	0/5	0/3
Penicillium spp.	"Hamburger" test agar	0/5	0/3
Candida spp.	"Hamburger" test agar	0/5	0/3
Aspergillus spp.	"Hamburger" test agar	0/5	0/3
Mucor spp.	"Hamburger" test agar	0/5	0/3
Ectromelie-Virus	Haemagglutination	0/5	–/–