THE EXPOSURE EFFECT OF WATER PIPE SMOKE (WPS) ON THE RESPIRATORY TRACT OF SWISS MICE

Nada Abdulrahman F. Al-Easawi
Department of Biology, College of Science, University of Baghdad, Iraq-Baghdad.

Abstract

One of the most common means of smoking tobacco is Water Pipe (WP); it's called (Arghile or Narghile) in the Middle East countries. It represents one of the causes of indoor air pollution. During the last few year's this method of smoking spread among the young Iraqies. Some of the previous studies confirmed that WP smoking has a negative health effects more than those of Cigarettes smoking.

In this research study , mice were exposed to water pipe smoke using a special inhalation glass chamber (whole body exposure).This smoke consist of a respirable particulate matter in which are (less than 2.5µm) in diameter . Mice exposed daily to 100 puffs of WP smoke on the bases of (1hr exposure per day), the exposure experiment continued daily for the periods of 3, 5, 7 weeks consequently.

The results indicated a remarkable reduction in bronchioles diameters and an obvious increase in alveoli diameter, while the histological examination showed different degrees of hyperplasia on the epithelial layer, thickening of the parenchyma tissue and the infiltration of inflammatory cells.

The research concluded that intensity of the observed symptoms depended on the length of exposure periods, the highest levels of effects represented by the group of 7 weeks exposure in which was the longest period. The previous mentioned group suffered from hyperplasia and increased secretion of mucus leads to early signs of chronic bronchiolitis.

تأثير التعرض لدخان الشيشة في القناة التنفسية للفئران السويسرية

ندى عبدالرحمن فليح العيساوي
قسم علوم الحياة ، كلية العلوم، جامعة بغداد، بغداد - العراق.

الخلاصه

تعد الشيشة احدى العادات الشائعة في تدخين التبغ, و التسمية الشائعة لهذه الوسيلة في بلدان الشرق الاوسط هو (النرجيلة او الاركيلة), و هي واحدة من اسباب تلوث الهواء الداخلي. خلال الاعوام السابقة اصبحت هذه الظاهرة شائعة تقريبا" و على الخصوص بين فئة الشباب ضمن المجتمع العراقي. لقد اكدت بعض الدراسات السابقة بأن تدخين الشيشة له تاثيرات صحية سلبية و بشكل اخطر من تأثيرات تدخين السكائر الاعتيادية. في هذه الدراسة البحثية, تم تعريض الفئران السويسرية البيضاء لدخان الشيشة و بأستخدام حجرة تعرض استنشاقي زجاجية خاصة, و قد كان نوع التعرض هو (تعرض لكامل الجسم) داخل هذه الحجرة . ان دقائق هذا الدخان قابلة للاستنشاق, حيث يكون قطر هذه الدقائق (اقل من 2.5 مايكروميتر). عرضت الفئران يوميا" الى 100 نفخة دخان محسوبة الحجم و التركيزمن دخان الشيشة و بواقع (ساعة تعرض واحدة باليوم), حيث استمرت تجربة التعرض يوميا" على مجاميع الفئران و للفترات 3و5و7 اسابيع على التوالي. دلت نتائج هذه الدراسة البحثية على انخفاض ملحوظ في اقطار القصيبات الرئوية مع زيادة ملحوظة في اقطار الاسناخ الرئوية, في حين اظهر الفحص النسيجي المجهري درجات

متباينة من فرط التنسج في خلايا الطبقة الظهارية و تثخنا" في النسيج الحشوي فضلا" عن الارتشاح للخلايا الالتهابية. نستنتج من هذه الدراسة ان شدة الاعراض المرضية الملاحظة اعتمدت على طول فترة التعرض, اذ كانت مجموعة الحيوانات المعرضة لمدة 7 اسابيع هي الاكثر شدة" من حيث الاصابة بفرط التنسج وزيادة في افراز المادة المخاطية التي تمثل الاعراض الاولية للاصابة بالالتهاب القصبي المزمن.

Introduction

Recently a sharp increase in popularity of the water pipe use in most Arabian and European countries [1]. Mistakenly, people believes that this smoking method is less harmful and addictive than cigarettes, the observed indoor air contamination of different harmful substances during a water pipe session is high, and exposure may cause health risk for smokers [2].

Generally water pipes consist of (head , body , water bowl and hose) the most common type of tobacco that placed in the head of water pipe is called *Ma'assel* contains approximately 30% tobacco and 70% honey or molasses . Usually flavored with (apple, grape, Strawberry, mint …etc). [3] Tobacco smoke is an aerosol that contains both gaseous and suspended particulate material. The particles are largely liquid droplet containing a wide variety condensed organic compounds [4]. Studies conducted by researchers at the American university of Beirut showed that Water Pipe Smoke (WPS) contains a significant quantities of the same chemicals which make cigarette smoke harmful . The results showed that collected smoke of 10gm of common *Ma'assel* used in water pipe head contains:

Nicotine 2.5 mg , Tar 242 mg and carcinogenic heavy metals (part of the Tar) like : Arsenic 165 ng , Beryllium 65ng , Nickel 990 ng , Cobalt 70 ng , Chromium 1340 ng , lead 6870 ng. [5].

In WP the uptake of tobacco nicotine is equivalent to 2-12 cigarettes per portion of tobacco used. [6] ,While a single 100 puff of water pipe smoke session produces as much

Tar as 20 or more than cigarettes.[4] moreover a single water pipe smoking session yields 20 times the amount of polycyclic aromatic hydrocarbons (PAH) found in main stream cigarettes as well as formaldehyde , acetaldehyde and acrolein [7].

Carbon monoxide concentration may also elevate because of the charcoal used to burn water pipe tobacco [8].

Water pipe smokers may absorb higher concentrations of these substances because of higher concentrations in the smoke itself or because of the mode of smoking including frequency of puffing, depth of inhalation and length of smoking session [9].

Smoke particles deposition percentage varies from one person to another depending on many variables including depth of inhalation hold time in lungs, puff volume and exhalation volume and particle diameter and growth [10], [11].

Pipes are major emitters of respirable suspended particles less than 2.5 microns (PM2.5) in diameter (range between 0.01µ - 0.2 µ) [12].

In a US College campus water pipe lounge, measurement of PM2.5 concentration levels at two different dates were found to be 1.1 and 2.7 times higher than the National Ambient Air Quality Standard (NAAQS) for 24 hours [13].

The respirable particulate matter of (PM 2.5 µ) in diameter are easily inhaled deep in to the lungs. [(12)] also its responsible for increases the histological damage in respiratory tract .Tar and Nicotine are considered to be the primary causes of smoking – related diseases , which include cancer of respiratory tract and reduction of lung function [5].

Long term exposure to compounds found in WPS such as: Co, Cyanide, responsible for pulmonary damages and lose of elasticity in the alveoli, leading to emphysema [14]. Furthermore, water pipe use increases the risk of bronchogenic carcinoma as well as lung [15].

In Four studies in Rats, Mice, exposure to whole tobacco smoke lead to modest increase in the occurrence of malignant and / or benign lung tumers [16].

However, there are little researches on water pipe at the laboratory level.

This research is interested in WPS as a result of this phenomena existence in the Iraqi community. A domestic WPS set attached to a mice inhalation chamber in order to explore the precise damage on mice respiratory tracts in a calculated manner. Thus, the mice are an experimental model for human.

Material and Method

The study was carried out on (18) un-exposed laboratory bread Swiss male mice (balb/c strain) of age (7-10 weeks). Water and food were available ad-*libitum*, except during inhalation exposure. Animal room temperature was

maintained at (25°C) and light /dark was set at 12 hr intervals.
Whole body inhalation exposure was carried out using a locally made glass chamber of 16Lt. capacity. Figuer.1 designed as a static system according to the specifications of WHO [17] [18].

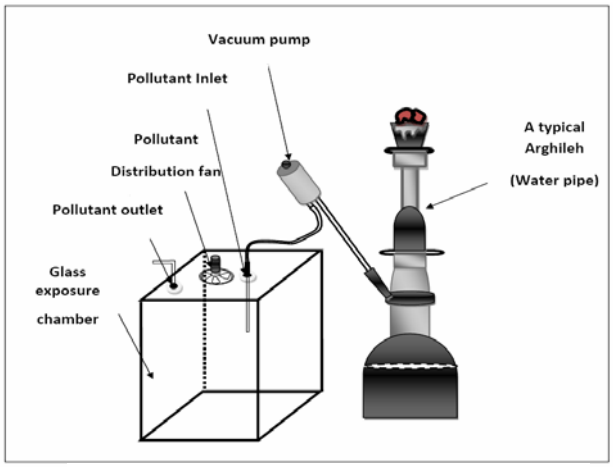


Figure 1: Inhalation Exposure Chamber.

Commercial "*Muessel*" or *Ma'assel*, flavored with strawberry (Arabic origin), used. It's composed of approximately 30% Tobacco and 70% Honey or Molasses.
Water pipe smoke was generated daily by burning 2gm of "*Ma'assel* " using a commercial charcoal then introduced as puffs into the chamber using a manual vacuum pump , each puff capacity is 50cc /2sec.
Mice exposed to 100 puffs /day, one puff / 2 sec., the exposure operation took 1hr/day for 3, 5, 7 weeks consequently.
The control group animals (n=9), followed the same system but they exposed to fresh air only.
Animal's kept together during exposure, precise labeling was so helpful to carry out this operation in order to avoid any confusion that could happen during the experiment.
Animals were sacrificed after the exposure periods by cervical dislocation, then a mid line incision making up to the peritoneum which was open in to the line of the skin incision. The lungs of each animal were infused in situ- with 0.5 ml (Formalin 10%) using a 1ml syringe in order to expand the lungs to a near normal physiological volume ,thus facilitate histological examination , in addition to its benefit in rapid fixation of the delicate pulmonary tissue.
Lungs – after fixation – were dissected out and kept in formalin .Tissue preparation for light microscopic examination and paraffin – embedded; sections (5µm) were stained with Eosin –Haematoxylin [19].
Detailed histopathological studies of control and exposed animal's lung sections were examined using a light microscope with oculometer.
Slide examination maintained by choosing five slides for each animal. A section had been selected from every slide and from the same positions. That means a 45 sections for the control animal group had been studied and another 45 sections had been studied for the experimental group.
The diameter of 15 bronchioles had been measured in each section for a 75 bronchioles in each animal; simultaneously 15 alveoli had been measured in each section for a 75 alveoli in each animal.
Bronchioles and alveoli diameter measurements maintained by taking the vertical and horizontal diameters mean using an ocular meter in which is calibrated by a stage micrometer.

Statistical Analysis

The histological results of the treated groups bronchioles and alveolar diameter were compared with control group and among the groups using F- test for analysis of variance (ANOVA) and Least Significant Test (L.S.D.) [20].

Results

The main results of this research showed Respiratory (bronchioles, Alveoli) diameters variation and Histo-pathological changes in respiratory tracts. The bronchioles diameter average of control and exposed animals are shown in (Table -1).
The bronchioles diameter average of animals exposed to WPS reduced gradually from (93.4 ±2.76µm) at the end of the third week to (73.1±2.50 µm) at the end of the seventh week. The bronchioles diameter average of the control animals exposed to fresh air ranging between (131.82–134.68µm).A significant decrease ($P < o.o1$) was observed between the bronchioles diameter average in the control group and exposed group.
Variation in alveolar diameter average is demonstrated in (Table-2).

Table 1: Average bronchiolar diameter of experimental group as compared to the control mice group.

Animal group	Average diameter in micrometer ± Standard deviation		
	3 weeks	5 weeks	7weeks
Control	131.82±2.41	129.87±3.33	134.68±2.63
Experimental	93.49 ± 2.76	89.50 ± 1.68*	73.14 ± 2.50**

*** significant at p<0.05**
**** Significant at P < 0.01**

Table 2: Average alveolar diameter of experimental group as compared to control mice group.

Animal group	Average diameter in micrometer ± Standard deviation		
	3 weeks	5 weeks	7weeks
Control	29.2 ± 2.30	30.0 ± 1.87	28.6 ± 2.60
Experimental	33.96 ± 1.59*	35.00 ± 1.0*	38.93 ± 1.81*

*** Significant at P < 0.01**

Figure (2) shows the normal picture of pulmonary tissue. While figure (3) shows lung tissue of animals exposed by inhalation of WPS for 3 weeks.

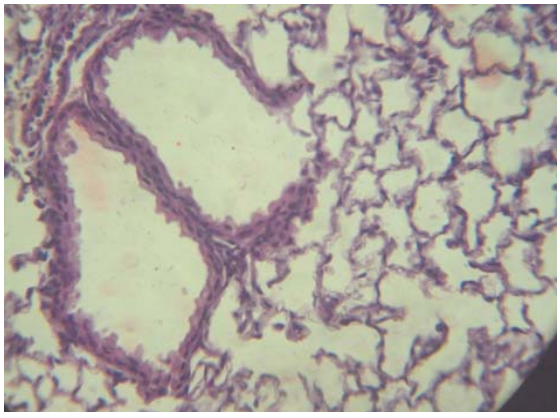
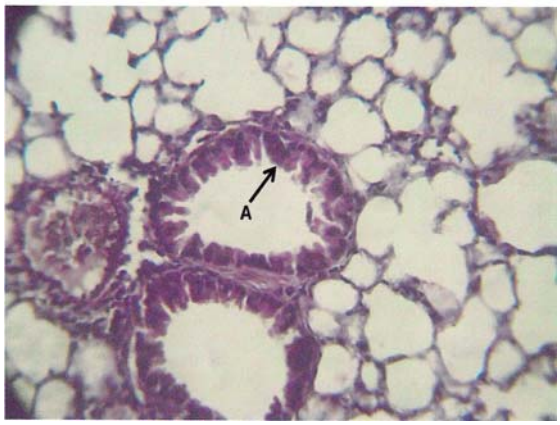


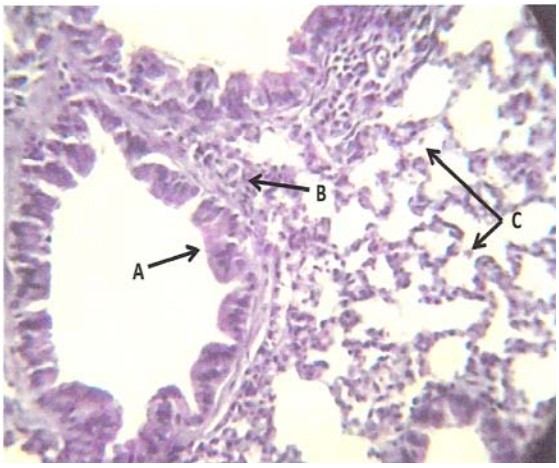
Figure 2: Normal tissue picture of the lung in mice.H. &E.40 X.

There was a progressive increase in alveolar diameter average after WPS exposure between three and seven weeks. The alveolar diameter increase from (33.96± 1.56 µm) at the end of the third week to (38.93± 1.81 µm) at the end of the seventh week. This increase was statistically significant (P< o.o1).



Figuer 3: Lung tissue of animals exposed to WP smoke 3 weeks.
A: Mild Hyperplasia; H. &E.40 X .

The section through the lung of exposed mice for three weeks showed some degrees of infiltration of inflammatory cells, as well as bronchioles underwent mild intensity of hyperplasia. On the other hand lung parenchyma appears thickened particularly in alveolar septa. Lungs of animals exposed to 5 weeks showed similar – but more prominent – signs of these histopathological changes appear in figure (4).



Figuer 4: Lung tissue of animals exposed to WP smoke 5 weeks.
A: Moderate intensity Hyperplasia;
B: infiltration of inflammatory cells;
C: Alveolar Macrophages, H. &E.40 X.

In figure (5), More sever changes could be seen in lungs of animals that exposed for 7 weeks, with more sever (higher intensity) hyperplasia of the epithelial tissues of bronchioles, with an increased amount of mucous secretion and infiltration of inflammatory cells.

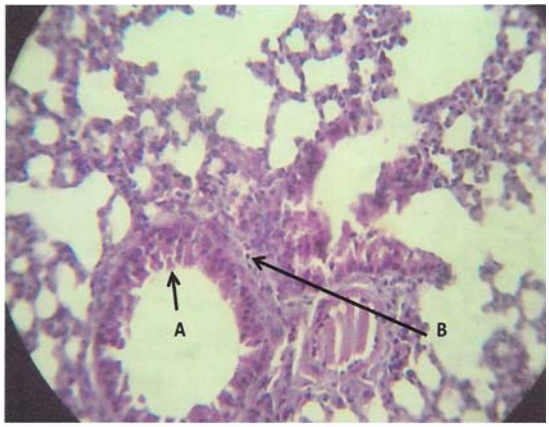


Figure 5: Lung tissue of animals exposed to WP smoke 7 weeks.
A: High Intensity Hyperplasia;
B: infiltration of inflammatory cells; H. &E.40 X.

Also results refers, Alveolar macrophages and lymphocytes were very frequently seen in alveolar lumen.

Discussion

This study resulted that exposure to WPS cause a high significant decrease in bronchioles diameter average in mice lungs. This observation is analogous to the results of two studies [21][22]. In which bronchiolar constriction had been observed as a result of exposed to tobacco fume for (1 month) in lung of rat. Bronchiolar constriction had most commonly been attributed to the effect of the Tobacco smoke – evoked inflammatory response as observed by Wright and Harrison[23].Other results reported by Filia *et al*.[24]. Indicated that male Guinea pigs treated with cigarette smoke by inhalation twice a day for 28 days developed lung lesion, including bronchial hyperplasia that leads to constriction .As well as structural changes of small airways is a leading cause of morbidity and mortality in the world [25].

On the other hand alveolar diameter was found to increase, leading to a condition of enlargement, in which increased by increasing exposure period. a number of studies reported the development of air space enlargement as an effect of chronic exposure to NO_2 , in which represented an important gaseous component of WPS, which clearly demonstrated that air spaces enlargement in rat lungs exposed to NO_2 could be associated with accelerated lung growth instead of lose of alveolar septa walls[26]. This finding was also noticed by Matulionis and Yokel[27], after exposing male mice to Tobacco smoke by chronic inhalation for (3 – 8.5 months).The alveolar macrophages perform their role in engulfing particles that could reach to alveolar region ,facilitate destruction of these cells and consequently the release of their photolytic enzymes thus causing autolysis and dilation of these walls.

In this research study increased lymphocyte is one of the indicators of inflammation as well as macrophages because of increased of surfactant. Many lesions including bronchioles hyperplasia, metaplesia, arising in an area with macrophages infiltration [28,29]. Histo-pathological changes show increase of mucous secretion. Reid and Jones[30] . Indicated that rapid increase in cell division of Goblet cells as a response to particulate of tobacco smoke attributed hyperplasia, which manifests itself in the increase of mucous secretion as a first step.Both hyperplasia and increased secretion of mucous leads to a condition of bronchio - constriction which might be considered as the early signs of chronic bronchiolitis.

References

1. AL-Numair, K.; Barber – Heidal, K.; AL-Assa, A. and EL-Desoky, G. **2007**. Water pipe (Shisha) smoking influences total anti oxidant capacity and oxidative stress of healthy Saudi males, *Food Agricul.Environ*. **5**:17-22.
2. Fromme, H. ; Dietrich, S.; Heitmann, D.; Dressel , H. ; Diemer, J.; Schulz, T.; Jorres ,R. A.; Berlin, K. and Volkel, W. **2009**. Indoor air contamination during a water pipe (narghile) smoking session. *Food and Chemical Toxilogical*, **47**(7): 1636 – 1641.
3. Maziak, W.; Ward, K. D.; Afifi Sowed, R. A. and Eissenberg, T. **2004**. Tobacco smoking using a water pipe: are – emerging strain in a global epidemic. *Tob. Control*,**13**:327– 333.
4. Pankow, J. F. **2001**. A consideration of the role of gas / particle partitioning in the deposition of nicotine and other tobacco smoke compounds in the respiratory tract. Chem. *Res. Toxicol.* , **14**:1465 – 1481.
5. Jabbour, S.; Geahchan, N.; Harfouch, S.; Saade, G. and Shihadeh, A. **2003**. New research: narghile smokers exposed to dangerous chemical. *Food and Chemical Toxology*, **18**:49-52.
6. WHO. **2006**. Tobacco use in Shisha: studies on water pipe smoking in Egypt. *Center of Tobacco Control Research and Education*. Paper SHISHA.
7. Sepetdjian, E.; Shihadeh, A.; and Saliba, NA. **2008**. Measurement of 16 polycyclic aromatic hydrocarbons in narghile water pipe tobacco smoking. *Food Chem. Toxicol*., **46**:1582 – 1590.
8. Shihadeh, A. **2003**. Investigation of mainstream smoke aerosol of the argileh water pipe. *Food Chem. Toxicol*.,**41**:143– 152.
9. Sharma, R. N.; Deva, C.; Behera, D. and Khanduja, K. L. **1997**. Reactive Oxygen species formation in peripheral blood neutrophils in different types of smokers. Indian *J. Med. Res*.,**106**: 475 – 480.
10. Barker, R. R. **2006**. The retention of tobacco smoke constituents in the human respiratory tract. *Inhal. Toxicol*. **17**:255– 294.
11. Bernstein, D. M. **2004**. A review of the influence of particle size, puff volume, and inhalation pattern on the deposition of cigarette smoke particles in the respiratory tract. *Inhal. Toxicol*. **16**:675 – 689.
12. Momen, M.; Loffredo, C.; Setouhy, M.; Hyland, A.; Trvers, M.J. and Mohamed, M. K. **2006**. Measurement of PM 2.5 as a marker for passive exposure to water pipe in cafes in Egypt [abstract]. :12-15.
13. Buchholtz, C. **2008**. Indoor air pollution due to hookah smoking: particulate concentrations in Lexington, Kentucky hookah lounge .*APHA 136th USA:*25 – 29 [abstract] .
14. Shafagoj, YA. and Mohammed F. **2002**. Levels of maximum end – expiratory carbon monoxide and certain cardiovascular parameters following Hubble – bubble smoking. *Saudi Med. J.*, **23**: 953 – 958.
15. Maziak, W.; Ward, K. D.; Afifi Soweid, R.A. and Eisserberg T. **2004**. Tobacco smoking using a water pipe: a re-emerging strain in a global epidemic. *Tobacco control*, **13**:327-333.
16. WHO. **2002**. *Tobacco Smoke and Involuntary Smoking* .Volume **83**.
17. WHO. **1978**. *Principles and methods of Evaluating the toxicity of chemicals part 1.EHC#6 Chap*. 2 and 6.
18. Hinners, R. G. **1966**. Animal exposure chambers. *Arch. Environ. Health*., **13**:609-615.
19. Humason, G. L. **1976**. *Animals Tissue Techniques* 2nd ed.Freemand, W.H. and company .San Francisco and London, P.269.
20. Watt, T. A. **1997**. *Introductory statistics for Biology Students* .2nd Ed. Chapman and Hall \ CRC .London p: 253.
21. Wang,Y.; Zhang, Z.; Yan, Y.; Lemon, W.J.; LaRegina, M.; Morrison, C.; Lubet, R. and You. M. **2004**. A Chemically induced model for squamous cell carcinoma of the lung in mice. *Cancer Research*, **64**:1647-1654.
22. Joshi, R. and Rana, K. S.**2009**. Effect of tobacco as fumigant on lung and Intestine of Rattus rattus. *Asian J. Exp. Sci.*, **23**(1):185-188.
23. Wright J.L. and Harrison N.**1990**. Cardiopulmonary effects of brief exposure to cigarette smoke in the guinea pig. *Resp*., **57**:70-76.
24. Fiala, E. S.; Sohn, O. S.; Wang, C. X.; Seibert, E.; Tsurutani, J.; Dennis, P. A.; Ei-Bayoumy, K. Sodum, R. S.; Desai, D. and Aliga, C. **2004**. Induction of preneoplastic lung lesions in guinea pigs by cigarette smoke Inhalation and their exacerbation by

high dietary levels of vitamin C and E. *Carcinogenesis,* **26**(3):605-612.

25. Yoshida, T. and Tuder, R. M. **2004**. Pathology cigarette smoke –induced chronic obstructive pulmonary disease. *Eur. J. bichem*., **271**(9):1757-1767.
26. Frhenbach, H. **2006**. Animal models of pulmonary emphysema: astereologist's perspective. Respiratory Medicine , article
27. Matulionis, D.H. and Yokel, O.A. **1988**. Murine lung response to kaolin conveyed by cigarette smoke .*Virchows Archive,* **413** (3):227-237.
28. Auerbach O., Stout A. P., Hammond E. and Garfinkel, L. **1961**. Change in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. *N.Engl.J.Med.,* **265**:253-267.
29. Coggins, C.R. E. **1998**. A review of chronic inhalation studies with mainstream cigarette smoke in rats and mice .*Txicol .Pathol*., **26**:307-314.
30. Reid, L. and Jones, R. **1983**. Experimental chronic bronchitis. *Int. Rev. Exp. Patho*., **24:** 335 – 382.