Eur Respir J
1988, 1, 319–323

Respiratory epithelial permeability is unrelated to bronchial reactivity and small airway function in young smokers and nonsmokers

R.G. Taylor*, J.E. Agnew**, R.A. Francis***, D. Pavia*, S.W. Clarke*

Respiratory epithelial permeability is unrelated to bronchial reactivity and small airway function in young smokers and nonsmokers. R.G. Taylor, J.E. Agnew, R.A. Francis, D. Pavia, S.W. Clarke.

ABSTRACT: We studied eight young smokers and ten nonsmokers, to determine whether respiratory epithelial permeability to radiolabelled diethylenetriamine penta-acetate (^{99m}TcDTPA) was related to small airway function or bronchial reactivity. Permeability was measured in inner (containing central airways) and outer lung zones by gamma camera. Lung-to-blood half-time (LB-$T\frac{1}{2}$) was corrected for blood background. Histamine was inhaled tidally (2 min inhalations) using doubling concentrations from 2 to 64 mg·ml^{-1}. Results of small airway function tests, and of bronchial reactivity (expressed as the threshold concentration (reducing forced expiratory volume in one second (FEV_1) by 2 SD), and as the percentage reduction in FEV_1 after histamine 16 mg·ml^{-1}) were similar in smokers and nonsmokers. LB-$T\frac{1}{2}$ was shorter in smokers than in nonsmokers in both inner (median (range) 21 (5.5–33) *vs* 63.5 (41–115) min; $p<0.004$) and outer (20.5 (5.5–30) *vs* 58.5 (39–105) min; $p<0.004$) zones. Neither inner nor outer zone LB-$T\frac{1}{2}$ was related to small airway function or bronchial reactivity. Bronchial reactivity and small airway tests may be abnormal in middle-aged smokers, but neither is related to the increased respiratory epithelial permeability of young smokers, in whom it appears too sensitive an index of airway integrity.

Eur Respir J. 1988, 1, 319–323.

* Departments of Thoracic Medicine and
** Medical Physics, Royal Free Hospital and School of Medicine, London.
*** Department of Medical Physics, West Cumbria Hospital, Cumbria, England.

Correspondence to: Dr. R.G. Taylor, Thoracic Medicine, St. James's Hospital, Leeds, LS9 7TF, England.

Keywords: Bronchial provocation tests; DTPA; epithelial permeability; gamma camera imaging; respiratory function tests; smoking.

Received: March 12, 1987; accepted after revision August 17, 1987.

The reasons why chronic airflow obstruction develops in only a minority of smokers are largely unknown, and many studies have tried to identify those smokers who are particularly at risk. Sensitive tests of small airway function appear useful in this respect [31, 36]. More recently, other aspects of airway function have been examined in smokers. In particular, it has been shown that the permeability of the airways to the inhaled low molecular weight substance diethylenetriamine penta-acetate (DTPA) is increased in smokers [21], and that increased airway permeability caused by cigarette smoke is accompanied by increased bronchial reactivity in guinea pigs [20]. Furthermore, increased bronchial reactivity is present in almost a third of middle-aged smokers, in whom it is associated with a reduced forced expiratory volume in one second (FEV_1), and an accelerated rate of decline in FEV_1 [38]. These observations suggest that some relationship between different aspects of airway integrity may be relevant to the development of chronic airflow obstruction. We have looked for a relationship in smokers and nonsmokers between small airway function, bronchial reactivity, and respiratory epithelial permeability. We measured permeability selectively in inner and outer lung zones. The contribution to permeability of the conducting airways should be greater in the inner zone, so we looked here, particularly, for a relationship to bronchial reactivity. The combination of these three investigations of airway function has not been used in a single group of subjects before.

Subjects and methods

Subjects

Eighteen male hospital employees volunteered to be studied. They were of European extraction, aged 20–36 yr, and in good general health. Eight of them were regular smokers and ten were nonsmokers (had never smoked more than one cigarette a day for a year). None of the subjects took medication regularly or had had a respiratory infection within the previous eight weeks. Subjects were excluded if they had asthma. This was diagnosed by positive answers to enquiries about previous asthma or episodic wheeze, dyspnoea and tightness in the chest. All the subjects gave their written consent to be studied, and the study was approved by the hospital's ethical committee.

Lung function

The FEV_1 was measured with a Vitalograph spirometer. Maximal expiratory flow at 50% ($\dot{V}max_{50}$) and 25% ($\dot{V}max_{25}$) of vital capacity were

measured with an Ohio 840 spirometer and Bryans 60000 X-Y recorder. An Ohio 700 nitrogen analyser was also used for the single-breath nitrogen test, to determine the slope of phase III ($\Delta N_2/l$) and closing volume as percentage of vital capacity, (CV/VC%). The largest of three values of FEV_1, and the $\dot{V}max_{50}$, $\dot{V}max_{25}$, $\Delta N_2/l$ and CV/VC% from the largest of three vital capacity tracings were each expressed as a percentage of the predicted value [5, 6, 25]. Results from the single-breath nitrogen test were not obtained from one smoker because of technical failure.

Bronchial reactivity

This was measured according to our modification [37] of an established protocol [7], using a Wright nebulizer which produced an aerosol mass median diameter (AMMD) of 3.45 (geometric SD 1.77) μm (Malvern 2600 Particle and Droplet Sizer, Malvern Instruments Ltd). Each subject inhaled nebulized solutions of histamine acid phosphate in doubling concentrations from 2–64 $mg \cdot ml^{-1}$ through a short mouthpiece during 2 min of normal tidal breathing, until the strongest solution was inhaled, or the FEV_1 dropped by 20% (PC_{20}). Bronchial reactivity was assessed by determining the threshold concentration [14], the strength of histamine which reduced the initial FEV_1 by two standard deviations, derived from the three baseline plus the three post-saline values [37], and also as the percentage reduction in FEV_1 after inhaling nebulized histamine 16 $mg \cdot ml^{-1}$ [37]. We used these indices rather than PC_{20} to measure bronchial reactivity, because between them they give recordable values in all non-asthmatic subjects [8, 37], most of whom have a PC_{20} greater than 16 $mg \cdot ml^{-1}$ [15].

Respiratory epithelial permeability

This was measured according to a modification of the protocol of JONES *et al.* [21]. An aerosol of technetium-labelled diethylenetriamine penta-acetate (^{99m}TcDTPA) was generated from an Acorn nebulizer, shielded in a lead pot and driven at a compressed airflow of 10 $l \cdot min^{-1}$. Nebulization continued until the aerosol generated filled a 25 litre reservoir bag, which was then left undisturbed for 5 min to allow large particles to settle out. The MMD of the aerosol subsequently inhaled was 0.6 (2.5) μm, with less than 5% of the particles > 2 μm in diameter [12]. Each subject inhaled the aerosol with normal tidal breathing while seated in front of a gamma camera (International General Electric MaxiCamera). Inhalation was stopped when a predetermined lung count of 1,600 counts $\cdot$ sec^{-1} had been reached. Sequential 1-min gamma camera images were then recorded, together with counts from a collimated scintillation counter positioned over the right thigh, pointing away from the bladder, to record the count as it built up in peripheral blood. After 30 min, a bolus of 8 MBq (approximately 220 μCi) of ^{99m}TcDTPA was injected intravenously to allow correction of the lung clearance curve for the contribution from vascular tissue in the lung detector

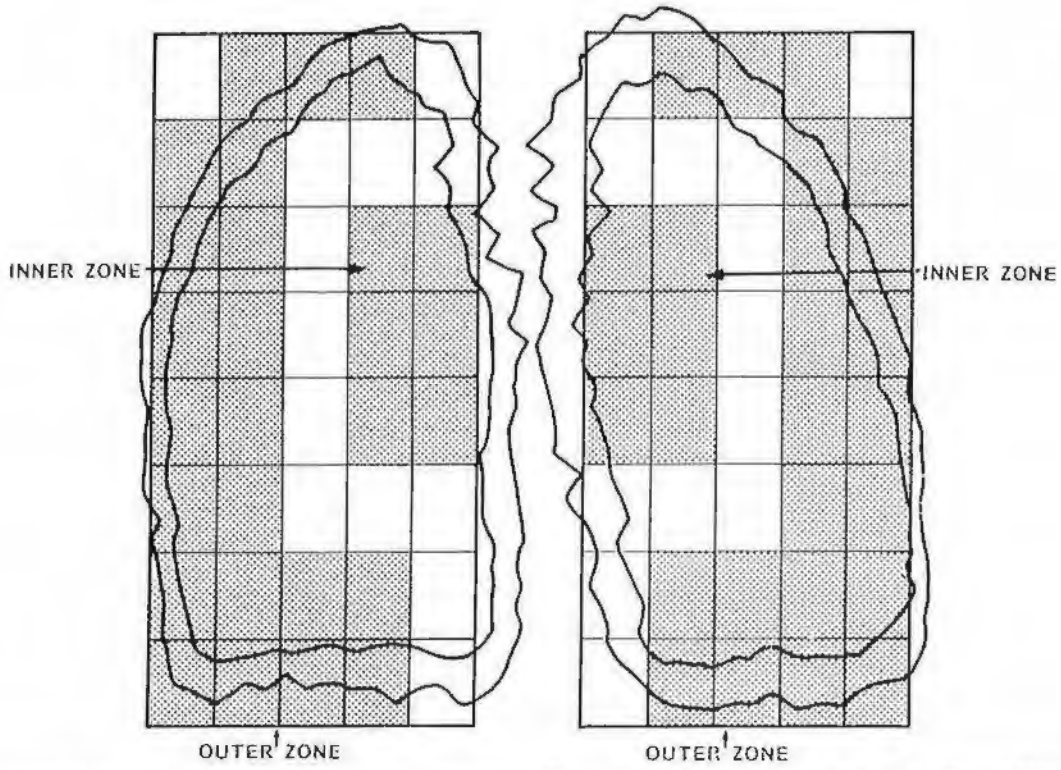

Fig. 1. Inner and outer lung zones determined by gamma camera used for measuring respiratory epithelial permeability to ^{99m}TcDTPA. The outer margins of the lungs are defined by the 15% and 30% contours of a ventilation image obtained with ^{81m}Kr.

field [21, 23]. The corrected counts were plotted semilogarithmically, and a half time (LB-$T\frac{1}{2}$) was determined for the clearance of DTPA from lung to blood, using inner and outer zones for each lung [2] (fig. 1).

Statistical analysis was performed using Student's t-test, Wilcoxon's rank sum test, and Spearman's rank correlation coefficient. A p value of <0.05 was regarded as significant.

Results

There were no differences between the smokers and nonsmokers in age or baseline lung function (table 1). In the smokers, the median (range) duration of smoking was 9 (6–20) yr, and daily consumption 20 (10–30) cigarettes.

The corrected lung to blood half time (LB-$T\frac{1}{2}$) was significantly shorter in smokers than in nonsmokers in both inner and outer lung zones, but within each group of subjects, the LB-$T\frac{1}{2}$ was similar in the inner and outer zones (fig. 2).

In both smokers and nonsmokers, neither inner nor outer zone LB-$T\frac{1}{2}$ was significantly related to any index of small airway function ($\dot{V}max_{50}$, $\dot{V}max_{25}$, $\Delta N_2/l$, CV/VC%) or bronchial reactivity (threshold concentration, percentage reduction in FEV_1 with nebulised histamine 16 $mg \cdot ml^{-1}$).

Representative illustrations of the results obtained from smokers are shown in figures 3–5.

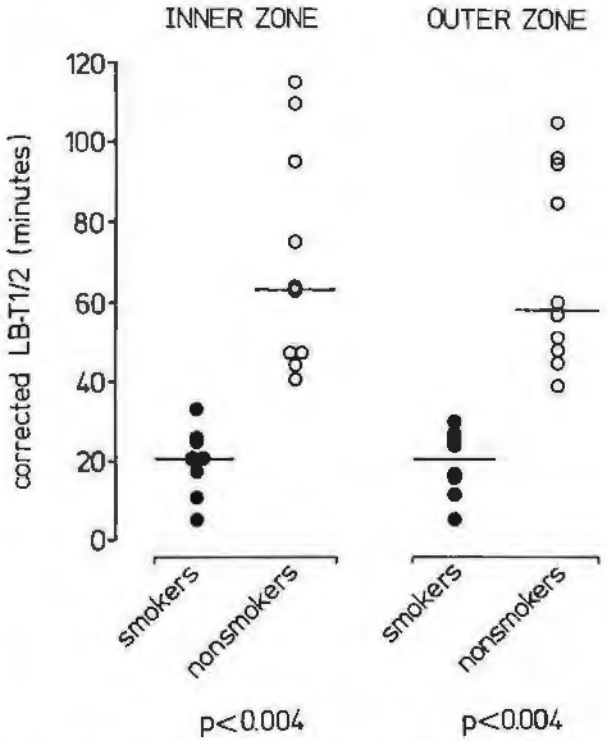

Fig. 2. Lung-to-blood half-time (LB-$T\frac{1}{2}$) for clearance of inhaled ^{99m}TcDTPA from inner and outer zones of lung (corrected for blood background) in smokers and non-smokers.

Discussion

This study shows that, in young smokers with normal lung function, there is no relationship between respiratory epithelial permeability and either bronchial reactivity or the results of sensitive tests of small airway function. Previous studies have looked for such relationships, but have not compared permeability with both reactivity and small airway tests in the same subjects [10, 11, 24, 32, 33].

The lack of association between these three aspects of airway integrity is initially surprising, because smoking can certainly affect all three. Young smokers, whose spirometry is normal, may have small airway disease, the extent of which correlates with the functional abnormality expressed as the increase in $\Delta N_2/l$ and CV/VC% [9]. The primary lesion in such cases is a progressive inflammatory reaction in the

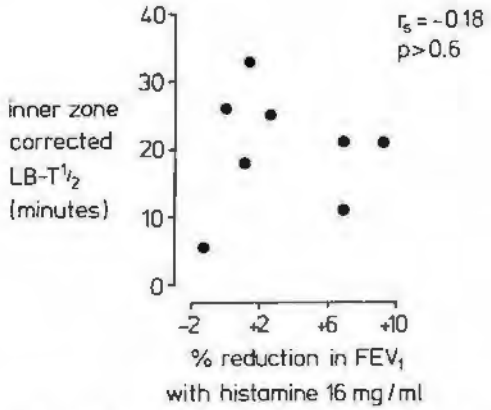

Fig. 3. Relation in smokers between inner zone corrected LB-$T\frac{1}{2}$ and percentage reduction in FEV_1 after inhaling nebulised histamine 16 $mg \cdot ml^{-1}$.

Table 1. - Age and results of baseline lung function tests in smokers and non-smokers. Values are median (range); lung function values are percent predicted.

	Age	FEV_1	$\dot{V}max_{50}$	$\dot{V}max_{25}$	$\Delta N_2/l$	CV/VC%
Smokers	27 (20-36)	99 (83-121)	99 (49-114)	75 (36-87)	143 (106-187)	104 (66-159)
Non-smokers	23 (20-29)	102 (77-114)	87 (61-112)	85 (53-107)	144 (84-198)	86 (38-149)

Smokers v. non-smokers: all p values >0.05.

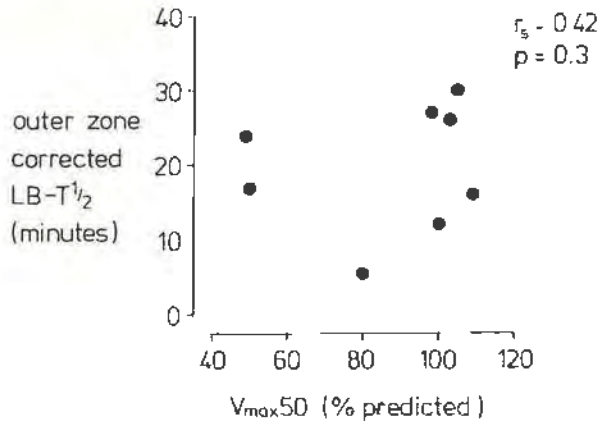

Fig. 4. Relation in smokers between outer zone corrected LB-T½ and $\dot{V}max_{50}$ (as percent predicted).

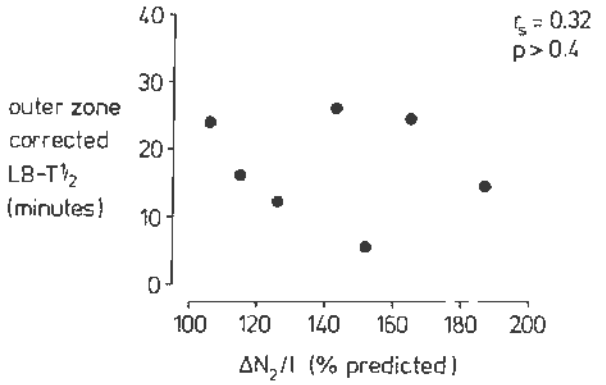

Fig. 5. Relation in smokers between outer zone corrected LB-T½ and $\Delta N_2 \cdot l^{-1}$ (as percent predicted).

small airways [9]. Cigarette smoke causes a dose-dependent inflammatory reaction in the airways of guinea pigs. This is matched in time and extent by an increase in respiratory epithelial permeability, and also in bronchial reactivity, perhaps caused by the exposure of nerve endings lying within the epithelium [19, 20, 35]. Intraepithelial nerves may be similarly affected in man [26].

Several studies which used the inhaled ^{99m}TcDTPA method have confirmed the original observations of JONES *et al.* [21] that respiratory epithelial permeability is greater in symptomless smokers than in nonsmokers [18, 24, 28, 32]. There is good evidence that the increase in permeability is closely related to smoking [17, 23, 28–30].

Although bronchial reactivity measured using FEV_1 is not increased in symptomless young smokers [4, 16, 27, 37], their small airways do show abnormal reactivity (as assessed by partial expiratory flow-volume curves), even when the smokers are similar to nonsmokers in pre-challenge function and in reactivity measured using FEV_1 [27]. However, the degree of bronchial reactivity measured using FEV_1 was not related to either the normal values of $\Delta N_2/l$ or CV/VC% in young smokers [24, 37], or to the abnormal values of middle-aged smokers [13].

Even though smoking causes abnormalities of small airway tests, respiratory epithelial permeability and bronchial reactivity, there are several theoretical explanations for our observation that these indices of airway function are not related to one another in individual subjects. Firstly, the measurement of permeability may reflect events taking place predominantly in the alveoli, whereas that of bronchial reactivity reflects changes in the conducting airways. Current methods of imaging cannot distinguish precisely where aerosol is deposited in the respiratory tract, the planar image being only two-dimensional [1] and acquisition time for tomographic images long, compared with the expected LB-T½. Commonly used techniques employ particle sizes and modes of inhalation which cause the DTPA aerosol to be deposited in the alveoli and small conducting airways, and large airway labelling is not seen [21, 22, 28]. In addition, the surface area of the respiratory tract increases enormously distal to the terminal bronchioles, so the alveolar influence on permeability predominates.

It is not certain if the permeability of the conducting airways is the same as that of the alveoli. In one study [11], subjects inhaled labelled DTPA aerosol, of aerodynamic mass median diameter 6.3 μm, rapidly to accentuate deposition on the central airways, and its subsequent rate of disappearance was similar to that reported by others who used 2 μm particles [21]. However, recent work suggests that mucociliary clearance, rather than epithelial permeability, may account for removal of much of the aerosol from the central airways [3]. We tried to allow for any regional difference in permeability and the fact that the particle size of the DTPA aerosol was smaller than that of the histamine aerosol by measuring permeability in an inner lung zone, which contained the central airways. Despite this, no relationship to reactivity emerged, and others have found similiar results [32]. However, although the inner zone provided counts from the central airways, it also included alveoli lying in front of and behind them, because the image was only two-dimensional. So even though permeability appeared to be similar in the inner and outer zones within each of our two groups of subjects, this may not actually be the case. It is unlikely that the larger particle size of the histamine aerosol influenced the bronchial reactivity results, because reactivity is similar when smaller particles are used [34].

References

1. Agnew JE, Pavia D, Clarke SW. – Airways penetration of inhaled radioaerosol: an index to small airways function? *Eur J Respir Dis*, 1981, 62, 239–255.
2. Agnew JE, Bateman JRM, Pavia D, Clarke SW. – Radionuclide demonstration of ventilatory abnormalities in mild asthma. *Clin Sci*, 1984, 66, 525–531.
3. Barrowcliffe M, Jones JG, Agnew JE, Francis RA, Clarke SW. – The relative permeabilities of human conducting and terminal airways to ^{99m}Tc-DTPA. *Eur J Respir Dis*, 1987, 71, (suppl 153), 68–77.

4. Brown NE, McFadden ER, Ingram RH. – Airway responses to inhaled histamine in asymptomatic smokers and nonsmokers. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1982, 52, 508–513.
5. Buist AS, Ross AB. – Predicted values for closing volumes using a modified single breath nitrogen test. *Am Rev Respir Dis*, 1973, 107, 744–752.
6. Buist AS, Ross AB. – Quantitative analysis of the alveolar plateau in the diagnosis of early airway obstruction. *Am Rev Respir Dis*, 1973, 108, 1078–1087.
7. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. – Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy*, 1977, 7, 235–243.
8. Cockcroft DW, Berscheid BA, Murdock KY. – Unimodal distribution of bronchial responsiveness to inhaled histamine in a random human population. *Chest*, 1983, 83, 751–754.
9. Cosio M, Ghezzo H, Hogg JC, Corbin R, Loveland M, Dosman J, Macklem PT. – The relations between structural changes in small airways and pulmonary-function tests. *N Engl J Med*, 1977, 298, 1277–1281.
10. Dolovich M, O'Byrne PM, Dirks R, Newhouse MT. – Lung epithelial permeability in normal subjects, asthmatics and smokers. *Chest*, 1982, 82, 253.
11. Elwood K, Kennedy S, Belzberg A, Hogg JC, Paré PD. – Respiratory mucosal permeability in asthma. *Am Rev Respir Dis*, 1983, 128, 523–527.
12. Francis RA, Agnew JE, Sutton PP, Pavia D, Clarke SW. – Ventilation imaging with easily prepared ^{99m}Tc aerosols. *Nuclear Med Commun*, 1981, 2, 203–208.
13. Groth S, Lindell SE, Kabiraj MU, Bülow K, Arborelius M, Simonsson BG. – Bronchial reactivity and small airway dysfunction in subjects with intermediate alpha$_1$-antitrypsin deficiency. *Bull Eur Physiopathol Respir*, 1984, 20, 279–284.
14. Habib MP, Paré PD, Engel LA. – Variability of airway responses to inhaled histamine in normal subjects. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1979, 47, 51–58.
15. Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF, Dolovich J. – Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol*, 1981, 68, 347–355.
16. Higenbottam TW, Hamilton D, Clark TJH. – Changes in airway size and bronchial response to inhaled histamine in smokers and nonsmokers. *Clin Sci*, 1978, 54, 11P.
17. Higenbottam TW, Chamberlain AT, Borland CD. – The relationship between small airways dysfunction and lung epithelial permeability in cigarette smokers. *Am Rev Respir Dis*, 1984, 129 (part 2), A177.
18. Huchon JG, Russell JA, Barritault LG, Lipavsky A, Murray JF. – Chronic air-flow limitation does not increase respiratory epithelial permeability assessed by aerosolized solute, but smoking does. *Am Rev Respir Dis*, 1984, 130, 457–460.
19. Hulbert WC, Walker DC, Jackson A, Hogg JC. – Airway permeability to horseradish peroxidase in guinea pigs: the repair phase after injury by cigarette smoke. *Am Rev Respir Dis*, 1981, 123, 320–326.
20. Hulbert WC, Paré PD, Armour C, Wiggs B, Hogg JC. – The relationship between airway permeability and hyper-reactivity. *Am Rev Respir Dis*, 1982, 125, 226.
21. Jones JG, Minty BD, Lawler P, Hulands G, Crawley JCW, Veall N. – Increased alveolar epithelial permeability in cigarette smokers. *Lancet*, 1980, 1, 66–68.
22. Jones JG, Minty BD, Royston D. – The physiology of leaky lungs. *Br J Anaesth*, 1982, 54, 705–721.
23. Jones JG, Minty BD, Royston D, Royston JP. – Carboxyhaemoglobin and pulmonary permeability in man. *Thorax*, 1983, 38, 129–133.
24. Kennedy SM, Elwood RK, Wiggs BJR, Paré PD, Hogg JC. – Increased airway mucosal permeability of smokers. *Am Rev Respir Dis*, 1984, 129, 143–148.
25. Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. – Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am Rev Respir Dis*, 1983, 127, 725–734.
26. Laitinen A. – Autonomic innervation of the human respiratory tract as revealed by histochemical and ultrastructural methods. *Eur J Respir Dis*, 1985, 66, (suppl 140), 1–42.
27. Malo JL, Filiatrault S, Martin RR. – Bronchial responsiveness to inhaled methacholine in young asymptomatic smokers. *J Appl Physiol: Respir Environ Exercise Physiol*, 1982, 52, 1464–1470.
28. Mason GR, Uszler JM, Effros RM, Reid E. – Rapidly reversible alterations of pulmonary epithelial permeability induced by smoking. *Chest*, 1983, 83, 6–11.
29. Minty BD, Jordan C, Jones JG. – Rapid improvement in abnormal pulmonary epithelial permeability after stopping cigarettes. *Br Med*, J 1981, 282, 1183–1186.
30. Minty BD, Royston D, Jones JG, Hulands G. – The effect of nicotine on pulmonary epithelial permeability in man. *Chest*, 1984, 86, 72–74.
31. Nemery B, Moavero NE, Brasseur L, Stanescu DC. – Significance of small airway tests in middle-aged smokers. *Am Rev Respir Dis*, 1981, 124, 232–238.
32. O'Byrne PM, Dolovich M, Dirks R, Roberts RS, Newhouse MT. – Lung epithelial permeability: relation to nonspecific airway responsiveness. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1984, 57, 77–84.
33. Rees PJ, Shelton D, Chan TB, Eiser N, Clark TJH. – Effects of histamine on lung permeability in normal and asthmatic subjects. *Thorax*, 1985, 40, 603–606.
34. Ryan G, Dolovich MB, Obminski G, Cockcroft DW, Juniper E, Hargreave FE, Newhouse MT. – Standardization of inhalation provocation tests: influence of nebulizer output, particle size, and method of inhalation. *J Allergy Clin Immunol*, 1981, 67, 155–161.
35. Simani AS, Inoue S, Hogg JC. – Penetration of the respiratory epithelium of guinea pigs following exposure to cigarette smoke. *Lab Invest*, 1974, 31, 75–81.
36. Tattersall SF, Benson MK, Hunter D, Mansell A, Pride NB, Fletcher CM, Peto R, Gray R, Humphreys PRR. – The use of tests of peripheral lung function for predicting future disability from airflow obstruction in middle-aged smokers. *Am Rev Respir Dis*, 1978, 118, 1035–1050.
37. Taylor RG, Clarke SW. – Bronchial reactivity to histamine in young male smokers. *Eur J Respir Dis*, 1985, 66, 320–326.
38. Taylor RG, Joyce H, Gross E, Holland F, Pride NB. – Bronchial reactivity to inhaled histamine and annual rate of decline in FEV_1 in male smokers and ex-smokers. *Thorax*, 1985, 40, 9–16.

RÉSUMÉ: Huit jeunes fumeurs et dix non fumeurs ont été étudiés pour déterminer la relation éventuelle entre d'une part la perméabilité épithéliale au 99m TcDTPA et d'autre part la fonction des petites voies aériennes ou la réactivité bronchique. La perméabilité était mesurée par une gamma camera dans les zones centrales (incluant les voies aériennes centrales) et périphériques. La demi-vie poumon-sang (LB-$T_{\frac{1}{2}}$) était corrigée pour le bruit de fond d'origine sanguine. L'histamine était inhalée pendant 2 minutes de respiration normale, avec un doublement des concentrations de 2 à 64 mg/ml. Les tests explorant les petites voies aériennes et la réactivité bronchique (réduction du FEV_1 de 2 SD et réduction du FEV_1 pour la dose d'histamine de 16 mg/ml, exprimée en % de la valeur initiale) étaient semblables chez les fumeurs et les non fumeurs. Par contre LB-$T_{\frac{1}{2}}$ était plus court chez les fumeurs que chez les non fumeurs tant dans les zones centrales (médiane étendue des variations) 21 (55-33) versus 63.5 (41-115) min; $p < 0.004$) que périphérique (20.5 (5.5-30) versus 58.5 (39-105) min; $p < 0.004$). LTB$_{\frac{1}{2}}$, qu'il concerne les zones centrales ou périphériques, n'est pas lié aux petites voies aériennes ni à la réactivité bronchique. La réactivité bronchique et les tests explorant les petites voies aériennes peuvent être anormaux chez les fumeurs d'âge moyen, mais ni l'une ni les autres ne sont liés à l'augmentation de la perméabilité épithéliale observée chez les jeunes fumeurs qui apparaît dès lors être un index trop sensible d'intégrité des voies aériennes.