Proliferation and differentiation in mammalian airway epithelium

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The infrequency of mitotic figures led to dispute amongst early workers as to the occurrence of cell division in adult mammalian airway epithelium [30, 63, 83, 227]. It was not until 1951 that quantitative work in the respiratory tract began to concentrate on the distal (i.e. respiratory) portion of the lung (reviewed by Bertalanffy [20, 21], Kauffman [132], and Masse et al. [159]). Studies of the lining epithelium of conducting airways soon followed [11, 25, 26, 27, 32, 38, 148, 208, 230]. The present review summarizes the results of many studies of cell division and differentiation in conducting airway epithelium. It is divided into three major sections: 1) general principles and techniques used in assessment of cell kinetics; 2) normal proliferation and differentiation in conducting airways and 3) effects of irritants and carcinogens, mechanical trauma, drugs, infection and physiological factors on cell proliferation and differentiation. Proliferation and differentiation, which occur during airway development, are not discussed here: the interested reader is referred to the following papers and reviews [24, 47, 119, 129, 132, 207, 237].

At least eight epithelial cell types are recognized in the lining epithelium of conducting airways and three in the epithelium lining the alveoli (for reviews see [41, 116, 117, 118]). In man the tracheobronchial surface epithelium is pseudostratified, ciliated and columnar reducing in thickness and degree of pseudostratification with airway generation until the epithelium is simple columnar at the level of the small bronchioles [167]. In animals the surface epithelium of the trachea and bronchus normally has only two layers of nuclei, one basal and one superficial, and all cells reach the basement membrane. In human bronchial epithelium, obtained from grossly normal areas of resected lung, two or more rows of basally situated cells are often observed, indicating a degree of basal cell hyperplasia [154]. Figure 1 shows epithelial cell types distinguished according to position (basal or luminal), presence of cilia and secretory granules. The most common basally situated cell, the 'basal' cell, was described and proposed as a progenitor cell by a number of investigators [24, 27, 63, 187, 227]. In peripheral bronchioles, where basal cells are absent, the Clara cell is the progenitor cell. Clara cells may divided in response to epithelial irritation and subsequently differentiate to form mature secretory and ciliated cells [74, 78, 79, 81]. In the alveolus, the type II cell is the progenitor cell [45, 70, 76] from which the type I cell differentiates. In conducting airways, there are three types of secretory cell, distinguished both by the nature of their secretory granules and the airway level at which each occurs: serous and mucous (i.e. goblet) cells normally occur in the proximal airways only [118], while Clara cells are usually found distally, limited to the small bronchioles of most but not all species [171, 174]. Dividing luminal cells have been observed and identified as serous and mucous cells [11, 38], but until recently the

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majority of the epithelial cells in proximal airways have been thought to arise following the division and differentiation of basal cells [27, 32, 52, 187]. An alternative suggestion is that the mucous cell plays the major regenerative role in conducting airway epithelium, a hypothesis which is gaining more and more support [153, 156]. Therefore the surface epithelial cells of the lower respiratory tract which have a capacity to divide are the basal, serous, mucous, and Clara of conducting airways and the alveolar type II cells of the respiratory portion of the lung. The number and proportion of each, which contribute to the pool of dividing cells, varies with airway level and species.

1. Introduction and techniques

1.1 Mitosis

Observation and simple quantitation of mitotic figures allows three indices, which measure cell renewal, to be determined: 1) Mitotic index (MI), the number of cells in mitosis at any one time (often expressed as a ratio, i.e. divided by the total number of cells counted); 2) Rate of entry into mitosis (REM) calculated from the accumulation of mitoses during time (t) after use of stathmokinetic (i.e. mitotic-arresting) agents such as colchicine [67, 217, 221] and the vinca alkaloids vinblastine and vincristine [48, 221]. The accumulated mitotic count is divided by (t) to give the REM which represents a measure of the rate at which new cells are born; 3) Turnover time (TT), can be determined from the REM if the total number of cells present is known [3, 19, 145, 148, 217]. Implicit in this determination is the assumption that all cells in the tissue are dividing, and that they are doing so at the same rate. It should be noted, however, that the renewal rate of a tissue depends not only on the number of dividing cells (which in vivo is usually only a fraction of the population) but also on the length of time taken for each cell to divide. When the two variables are unknown or altered, the calculation of TT based on REM is inaccurate.

1.2 Cell cycle

In 1953, HOWARD and PELC [112] showed, with radioactively labelled DNA precursors, that a finite time existed between the end of synthesis of new DNA (i.e. S phase, which takes time (tS)) and mitosis.
M.M. Avers, P.K. Jeffery (M). They subsequently formulated the now familiar Cell Cycle concept, proposing that dividing cells pass through a series of complex biochemical events which precede mitosis. The interval between mitosis and $S$ phase was referred to as 'gap' 1 ($G_1$) and that between the end of DNA synthesis and the beginning of mitosis was called 'gap' 2 ($G_2$), (fig. 2). Any tissue is likely to have only a fraction of its total cell number proliferating, or cycling, at any one time and this fraction is referred to as the Growth fraction (GF). Cells in the GF can move into the non-proliferating (non-cycling) fraction of the population by three routes. They can: a) permanently leave the cycle, perform their appropriate function and subsequently die (i.e. the normal process of ageing), b) leave the cycle temporarily or permanently and enter a 'resting' state ($G_0$), yet be available to re-enter the cycle on receiving the appropriate stimulus (e.g. liver parenchymal cells in response to partial hepatectomy), or c) leave the cycle and die due to abnormality or malfunction [3, 149].

When the DNA precursor thymidine (T) or iododeoxyuridine (I UdR), labelled with tritium ($^3$H) or carbon-14 ($^{14}$C), (emitters of low-energy $\beta$ particles), is made available to a population of 'cycling' cells, those in the $S$ phase incorporate it irreversibly into their DNA, the extent to which this is done being dependent on the activity of the enzyme thymidine kinase. Thereafter, the cell is 'labelled' and can be visualized by autoradiography [189] which enables the proportion of cells synthesizing DNA, (i.e. the Labelling index (LI)) to be calculated. Usually LI is expressed as a ratio of the number of cells labelled divided by the number of cells counted (often expressed per 100 or 1000 cells counted). In a population where all the cells are cycling, the number of cells in any phase of the cycle is directly proportional to the length of that phase. Thus as $t_s$ is longer than $t_m$ [3, 145], the LI for a given population will be correspondingly higher than the MI.

Both pulse and continuous labelling with tritium- and carbon-labelled thymidine can be used to obtain information about the length of distinct phases of the cell cycle, the growth fraction and rates of cell birth and loss. Four techniques will be described in brief. Extensive discussion of procedure standardization, the problems and some of the solutions involved in the use of these techniques, is available in Wright and Allison [237] in the section on methodology. In particular, there are potential anomalies which may be introduced in long-term experiments, due to the re-utilization of $^3$H-thymidine ($^3$H-T) following the degradation of nuclei containing the label. If the latter is likely then an isotope such as $^3$H-I UdR (which is re-utilized to a lesser extent) can be used.

1) A method of assessing the fraction of labelled mitoses (FLM) was developed by Quastler and Sherman [176] which enables cell cycle time ($t_C$) and the duration of each cycle phase ($t_{G_1}$, $t_S$, $t_{G_2}$, and $t$) to be measured. Proliferating cells in a population are labelled by a single pulse of $^3$H-T and followed with time as they move through $G_2$ into mitosis to produce a cohort of labelled mitotic figures. Consecutive samples at short-time intervals after the pulse, and subsequent autoradiographic analysis of the population, allows the rise and fall in the percentage of labelled mitotic figures with time to be plotted (i.e. the FLM curve). If all cells in the population are cycling asynchronously and at the same rate, a well-defined curve is generated, rising from 0 to 100% and back to 0% of the labelled mitotic figures with each complete passage of the originally labelled cells around the cycle. The lengths of each cycle phase and $t_C$ can be

![Diagram](image_url)

Fig. 2. Diagrammatic representation of cell cycle. The growth fraction (GF) consists of cycling cells each passing through a series of complex biochemical events (e.g. $S$ and $G_2$) preceding mitosis (M). Thereafter cells may either continue to cycle or leave the cycle to die, terminally differentiate or enter a 'resting' state ($G_0$) from which they may be re-called when required. (Modified from [3]).
calculated from measurements made of the FLM curve [3, 12, 94, 108, 163, 177, 197, 211].

2) The double labelling technique enables $t_S$ and the rate of entry into DNA synthesis to be calculated by using both $^{3}$H- and $^{14}$C-labelled thymidine. The energies, and thus 'path lengths', of each isotope are sufficiently different to enable cells, labelled with either isotope, to be distinguished by autoradiography [3, 195, 233]. A cell cohort is given a single pulse of $^{3}$H-T (short path length), and after a known time interval ($t$), which must be shorter than $t_S$, a single pulse of $^{14}$C-T (long path length). The distinctly labelled cell populations can be subsequently differentiated and counted by having two superimposed layers of autoradiographic emulsion, one of which is too distant to be affected by the short path length of $^{3}$H-T. Assuming that the rates of entering and leaving the S phase are equal (i.e. the cell population is in a steady state), $t_S$ can be calculated [3].

3) Continuous labelling enables the growth fraction $t_S$, the rate of entry into DNA synthesis and $t_c$ to be calculated [3, 93]. A cell population in vivo or in vitro is 'continuously' exposed to a low level of $^{3}$H-T, such that each cell in the growth fraction, as it comes into the S phase, becomes labelled. This can be done in 

**Proliferation and Differentiation in Airways**

In the respiratory tract there is a continuous turnover of epithelial cells, which may or may not be preceded by division. Differentiation is sometimes associated with a decreasing capacity for division [148].

2.1 Proliferation

For earlier discussion on cell proliferation in the respiratory tract the reader is referred to BOREN and PARADISE [38], BOWDEN [40], KAUFFMAN [132], and WRIGHT and ALLISON [237].

2.1.1 Mitotic index (MI) and rate of entry into mitosis (REM). Dividing tracheal epithelial cells were first found in the latter part of the 19th century [30, 44, 63]. The results of contemporary studies of MI and REM in the respiratory tract are summarized in table I. To enable comparison, the data have all been recalculated and expressed as the number of cells.
Table I. - Rate of entry into mitosis (REM')

<table>
<thead>
<tr>
<th>Airway level</th>
<th>Animal</th>
<th>Sex</th>
<th>Age (wks)</th>
<th>REM'$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>mouse</td>
<td>?</td>
<td>8</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>?</td>
<td>12</td>
<td>0.16</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>♂</td>
<td>8</td>
<td>1.7</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>♂</td>
<td>5</td>
<td>2.6</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>♀</td>
<td>5</td>
<td>0.9</td>
<td>32</td>
</tr>
<tr>
<td>Trachea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Basal Cell</td>
<td>hamster</td>
<td>♂</td>
<td>9 - 13</td>
<td>0.25</td>
<td>38</td>
</tr>
<tr>
<td>- Mucous Cell</td>
<td>hamster</td>
<td>♂</td>
<td>9 - 13</td>
<td>0.18</td>
<td>38</td>
</tr>
<tr>
<td>Bronchi</td>
<td>mouse</td>
<td>?</td>
<td>8</td>
<td>0.3</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>?</td>
<td>12</td>
<td>0.15</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>0</td>
<td>9 - 12</td>
<td>0.4</td>
<td>127</td>
</tr>
<tr>
<td>Alveolar Epithelium</td>
<td>guinea pig</td>
<td>♂</td>
<td>adult</td>
<td>1.5</td>
<td>20, 21</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>♂</td>
<td>adult</td>
<td>1.5</td>
<td>20, 21</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>♂</td>
<td>12</td>
<td>0.7</td>
<td>203</td>
</tr>
</tbody>
</table>

REM: No. mitoses per 1000 total cells counted per h mitotic arrest.

entering mitosis per 1000 cells counted per colchicine hour (i.e. REM). The REM of airway and alveolar epithelia is indeed small, the calculated range for the respiratory tract is 0.14 - 3 mitoses per 1000 cells per hour. By contrast REM has been estimated for rat duodenal epithelium as 30 per 1000 cells per hour [150] and for oesophageal epithelium of 2 month old mice as 11 per 1000 cells per hour [28].

MI and REM have been shown by numerous workers to vary with the airway level, animal strain and the sex and age of the animal studied:

a) airway level: MI decreases as the airways examined descend from the trachea to the distal bronchiolus, particularly in young and growing animals. In older animals the same trend is present but it is less pronounced [26, 32];

b) animal strain: given the same sex, age and airway level, MI varies between strains of mice [203] and rat [27], although this difference may lessen with age [203];

c) sex differences: SIMNETT and HEPPESTON [203] suggest a trend of increasing MI with age in females not found in male mice. In 33 day old rat airways, MI is, however, higher in males than females: by 93 days the difference disappears. Interestingly, the alveolar epithelial and migratory cells of the older rats show a higher MI for females than males and in this respect is similar to data for mice [32];

d) age: MI shows changes with age. SIMNETT and HEPPESTON [203] compared young adult mice (3 months) with mice at the end of their natural lifespan (18 - 24 months of age). Whereas one strain of male mice showed a 60 - 90% reduction of their 3 month value, another showed no significant difference. Female mice showed an increase of MI with age. The studies of BORK and HARKONEN [26] and BOLDUC and RUD [32] have concentrated only on the early phases of growth (i.e. to 3 months) where the decrease in MI coincided with the plateau of the growth curves for the animals.

2.1.2 Labelling index (LI). Since $^3$H-thymidine ($^3$H-T) is taken up during $t_S$, which is longer than $t_M$ [3, 50, 164], a relatively larger proportion of the cycling population is labelled by $^3$H-T (and thus is available for quantitation) than is seen by mitotic count (MI). However, the observations of changes in LI with airway level, animal strain and age, parallel those described for MI and are summarized in table II.

a) airway level: LI decreases from proximal (central) to distal airways [27, 32, 59, 200, 201];

b) animal strain: for the same age (or weight) and sex, significant differences in LI in the trachea have been reported between strains of rat [27] and, in the alveolus, between strains of mice [203];

c) sex differences: no differences in LI have been found at any airway level in adult rats and mice [32, 59, 201, 203], a finding confirmed in older rats (93 days old) [32]. However, in younger rats (33 days old), males have twice the number of labelled cells than females [32];

d) age: SIMNETT and HEPPESTON [203] have examined three strains of mice at ages 3, 12 and 24 months and shown a significant decrease in LI with age. This trend is confirmed by EVANS et al. [73].

Extrinsic factors may also influence cell division:

e) diurnal variation: diurnal variations in mitotic and labelling indices have been well demonstrated in tissues such as epidermis and small bowel [4, 49] and also in the respiratory tract. In the latter, a peak of
Table II - Labelling index

<table>
<thead>
<tr>
<th>Airway level</th>
<th>Animal</th>
<th>Sex</th>
<th>Age wks</th>
<th>LI labelled cells per 1000 cells counted</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>rat</td>
<td>♂</td>
<td>adult</td>
<td>4</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>♀</td>
<td>adult</td>
<td>5-12</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>♂</td>
<td>adult</td>
<td>0.4-0.6</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>rat: conventionally derived</td>
<td>♂</td>
<td>5</td>
<td>20</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>rat: minimal disease</td>
<td>♂</td>
<td>5</td>
<td>7</td>
<td>230</td>
</tr>
<tr>
<td>Trachea</td>
<td>mouse</td>
<td>♂</td>
<td>adult</td>
<td>5-17</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>♀</td>
<td>adult</td>
<td>10-40</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>♂</td>
<td>8</td>
<td>10</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>♀</td>
<td>adult</td>
<td>16</td>
<td>59</td>
</tr>
<tr>
<td>Trachea</td>
<td>hamster</td>
<td>♂</td>
<td>adult</td>
<td>4</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>- basal</td>
<td>♂</td>
<td>adult</td>
<td>5</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>- mucous</td>
<td>♂</td>
<td>adult</td>
<td>2</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>- basal</td>
<td>♂</td>
<td>adult</td>
<td>8</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>- mucous</td>
<td>♂</td>
<td>adult</td>
<td>4</td>
<td>102</td>
</tr>
<tr>
<td>Bronchus</td>
<td>rat</td>
<td>♂</td>
<td>adult</td>
<td>10</td>
<td>238</td>
</tr>
<tr>
<td></td>
<td>rat</td>
<td>♀</td>
<td>adult</td>
<td>2-4</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>hamster</td>
<td>♂</td>
<td>adult</td>
<td>0.02-0.03</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀</td>
<td>adult</td>
<td>1-2</td>
<td>102</td>
</tr>
<tr>
<td>Bronchus</td>
<td>mouse</td>
<td>♂</td>
<td>adult</td>
<td>8</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>♀</td>
<td>adult</td>
<td>4-10</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>♂</td>
<td>adult</td>
<td>16</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>♀</td>
<td>adult</td>
<td>3</td>
<td>59</td>
</tr>
<tr>
<td>Bronchiole</td>
<td>human in-vitro</td>
<td>?</td>
<td>adult</td>
<td>2-7</td>
<td>201</td>
</tr>
<tr>
<td>Alveolar Epithelium</td>
<td>mouse</td>
<td>♂</td>
<td>12</td>
<td>7</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>♀</td>
<td>52</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>♂</td>
<td>78</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>hamster</td>
<td>♂</td>
<td>adult</td>
<td>11</td>
<td>102</td>
</tr>
</tbody>
</table>

either mitotic or labelling indices has been found in the morning in rats [164, 193, 205] and the afternoon/evening in mice and hamsters [57, 138, 140]. Studies carried out under conditions of constant light by BOREN [36] and BOREN and PARADISE [38] show no significant diurnal variation in LI with time. In normal (SPF) male rat main bronchial epithelium, AYERS and JEFFERY [11] found that the total number of labelled cells in the morning (08:00) is almost double the value found 12 hours later. Although the labelled cell population is made up of basal, serous and cells of indeterminate morphology, the observed change is due to a significant difference only in the numbers of labelled serous cells; b) vitamin A status; retinoids have been shown to play an important role in the control of epithelial proliferation and differentiation including that of the respiratory tract [39, 236]. WOLBACH and HOWE [234] demonstrated excessive cellular proliferation in respiratory epithelium of vitamin A-deficient animals which subsequently underwent squamous keratinizing metaplasia. CONDON [52], in a study of regeneration in normal and vitamin A-deficient rat tracheal epithelia, has observed rapid epithelial proliferation: 8
hours after colchicine administration, 60% of epithelial cells are in mitosis compared with ‘a few scattered mitoses’ present in normal epithelium. Harris et al. [106] have confirmed the observations, finding large increases in both MI and LI in vitamin A-deficient hamster trachea. However, Shermann [198], in a comparison of tracheal, cornal and epidermal epithelia from vitamin A-deficient rats, has found a decreased MI in all the epithelia studied. MI returns to normal levels after local or oral application of vitamin A acetate. Lane and Gordon [147] observe foci of squamous metaplasia in vitamin A-deficient rat tracheal epithelium, but report no significant difference between the LI of rats on a normal diet and those deficient in the vitamin. Chopra [46] has examined the change in LI of basal and mucous cells in newly established tracheal explants from normal and deficient hamsters. Mucous cells from deficient epithelia show only an initial transient increase in LI, basal cells maintain a high proliferative activity throughout the life of the explant and subsequently give rise to squamous metaplasia.

An extensive study of vitamin A-deficiency and restoration in hamster tracheal epithelium by McDowell et al. [156, 157] has shown that the primary effect of deficiency is a decrease in the proliferation rate of basal cells and (to a much greater extent) mucous cells, with minimal morphological change. They also described the development of squamous lesions made up of epidermoid cells containing PAS positive granules and where LI was raised above control levels. The authors conclude that: 1) vitamin A is necessary for maintenance of normal rates of cell proliferation and that mucous cells are particularly responsive to its absence; 2) prolonged vitamin A-deficiency caused cell death which resulted in a reparative response; 3) mucous cells, which actively proliferate during repair, are capable of expressing keratin-manufacturing or mucous secretory phenotypes, but vitamin A-deficiency results in a predominance of the former, i.e. epidermoid lesions rather than mucociliary.

In a study of the effect of restoration of vitamin A over seven days, the same authors [156] found that basal cell proliferation remained consistently less than that of controls, but mucous cell proliferation began to rise two days after restoration, as did the numbers of preciliated cells 24 hours later. Within seven days a restored pseudostratified epithelium with normal proportions of basal, mucous and ciliated cells was present. The results suggest that the mucous cell plays a primary role in normal development and repair of tracheobronchial epithelium.

2.1.3 Turnover time. The turnover times (TT) of respiratory epithelia have been calculated for a variety of species, airway levels and ages in both sexes (table III). Two approaches have been taken. Firstly, in the studies of Spencer and Shorter [208], Shorter et al. [200, 201] and Divertie et al. [59] the time for each labelled cell type to migrate (i.e. ‘migration time’) to the epithelial surface and disappear (‘slough’) was observed and used to give an estimate of TT for distinct cell populations. However, the estimate was made without accounting for differences between cell types in respect of tM, tS or their distinct contributions to the growth fraction (see table III). Secondly, TT has been calculated from counts of either the mitotic or labelling indices [31, 203]. Again no account was taken of differences either of tS or the growth fraction (table III). Bleskin and Fopp [27], assuming a value for tS of 8 hours and using LI data, calculated that the TT for different airway levels in two strains of rats of varying ages ranged from between 67 and 111 days.

Wells [230] has also calculated TT of rat tracheal epithelium using data obtained from pulse labelling with $^3$H-T. With time, labelled basal cells migrate into the superficial differentiating layer, thus the ratio of labelled basal cells to total labelled cells in the epithelium decreased. From the rate of decrease of the ratio, the TT has been calculated for two ‘grades’ (of microbiological status) of five week old rats, 11–12 days, for animals suffering from chronic respiratory disease and 37–42 days for relatively clean animals. TT has been shown to vary between species and strains [27, 230], and to increase with both increasing age and descending airway level [27]. No differences have been reported between males and females.

2.1.4 Cell cycle and phase times. There are relatively few studies which determine the length of the cell cycle and its phases for distinct cell types (table IV). Results vary widely; e.g. tC of hamster tracheal basal cells is reported as 28 h by one group and 159 (± SEM, 11 h) by another and that of mucous cells is 25 h or 97 (± SEM, 4 h) respectively [37, 38]. Use of colchicine-induced metaphase block and $^3$H-T labelling in mice gives a value of 380 h for tC of tracheal basal cells [28]. Table V shows the findings for different phases of the cell cycle for the tracheal basal cell: tG from 6–12 and tG from 12–44 h [37, 38]. Comparison of tS of mouse alveolar wall cells in males and females of three strains at three ages shows that: a) tS is longer in males than females (two out of three strains); b) tS becomes longer with age (one strain only) and c) tS varies between strains [203]. Similar comparisons for other species and airway generations have yet to be made. The reported values for tG are relatively constant and lie between three and four hours for both basal and superficial (including mucous) cells (table V). For tM, there are only two reports for the respiratory tract (trachea): findings for both basal and superficial cells are similar and range from 12 to 36 min [38]. The reason for the variation is not clear. In other tissues, tM has been reported to last between 1 and 2 h in mouse forestomach epithelium [235], 2 h in mouse ear epidermis [173], and 1.7 h in adult (8 weeks old) rat liver [175].

2.1.5 Growth fraction (GF) and ‘resting’ cells (G0). The growth fraction can be determined with either the
Proliferation and Differentiation in Airways

Table III. - Migration/Turnover time

<table>
<thead>
<tr>
<th>Airway Level</th>
<th>Animal</th>
<th>Sex</th>
<th>Age weight g</th>
<th>Migration time days</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>mouse</td>
<td>♂️♀️</td>
<td>adult</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td></td>
<td>adult</td>
<td>5-7</td>
<td>200</td>
</tr>
<tr>
<td>Trachea</td>
<td>rat</td>
<td>♂️♀️</td>
<td>adult</td>
<td>6-7</td>
<td>201</td>
</tr>
<tr>
<td>Bronchus</td>
<td>mouse</td>
<td>?</td>
<td>4</td>
<td>3 &amp; 21</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>?</td>
<td>4</td>
<td>7-10</td>
<td>208</td>
</tr>
<tr>
<td>Bronchus</td>
<td>rat</td>
<td>♂️♀️</td>
<td>adult</td>
<td>7-8</td>
<td>201</td>
</tr>
<tr>
<td>Alveoli</td>
<td>mouse</td>
<td>?</td>
<td>4</td>
<td>7</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>mouse</td>
<td>?</td>
<td>4</td>
<td>21</td>
<td>208</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Turnover Time</th>
<th>days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td></td>
</tr>
<tr>
<td>rat</td>
<td></td>
</tr>
<tr>
<td>- S.D.*</td>
<td>adult</td>
</tr>
<tr>
<td>- B.H.*</td>
<td>200 g</td>
</tr>
<tr>
<td>- B.H.*</td>
<td>330 g</td>
</tr>
<tr>
<td>rat</td>
<td></td>
</tr>
<tr>
<td>- C.D.*</td>
<td>5</td>
</tr>
<tr>
<td>- M.D.*</td>
<td>5</td>
</tr>
<tr>
<td>rat</td>
<td></td>
</tr>
<tr>
<td>♂️♀️</td>
<td>5</td>
</tr>
<tr>
<td>Trachea</td>
<td></td>
</tr>
<tr>
<td>rat</td>
<td></td>
</tr>
<tr>
<td>♂️♀️</td>
<td>7</td>
</tr>
<tr>
<td>rat</td>
<td></td>
</tr>
<tr>
<td>♂️♀️</td>
<td>15</td>
</tr>
<tr>
<td>Trachea</td>
<td></td>
</tr>
<tr>
<td>hamster</td>
<td>♂️</td>
</tr>
<tr>
<td>Bronchus</td>
<td></td>
</tr>
<tr>
<td>- main</td>
<td>rat B.H.*</td>
</tr>
<tr>
<td>- small</td>
<td>rat B.H.*</td>
</tr>
<tr>
<td>Bronchiole</td>
<td></td>
</tr>
<tr>
<td>rat B.H.*</td>
<td>♂️</td>
</tr>
<tr>
<td>Alveolar Epithelium</td>
<td>mouse - A/Gr₁</td>
</tr>
<tr>
<td></td>
<td>mouse - A/Gr₁</td>
</tr>
<tr>
<td></td>
<td>mouse - A/Gr₁</td>
</tr>
<tr>
<td></td>
<td>mouse - A/Gr₁</td>
</tr>
</tbody>
</table>

*S.D.: Sprague Dawley; *B.H.: Black Hooded; *C.D.: Conventionally Derived; *M.D.: Minimum Disease.

Continuous labelling or double labelling techniques. The only such determinations for respiratory tract that have been reported are those of Boren and Paradise [37, 38] (table VI). Their studies of hamster trachea show that the GF consists of 2-4% of basal cells and 1% mucous. Studies in rats by Ayers and Jeffrey [11] and Donnelly et al. [61] have shown that serous and indeterminate (also referred to as 'intermediate') cells contribute to the GF in rat tracheal epithelium.

Boren and Paradise [38] have observed, among the proliferating basal and mucous cell populations, a
very small proportion of cells that do not show grain halving with time after a pulse label with $^3$H-T. This suggests the presence of small numbers of both basal (0.3%) and mucous (0.1%) cells which were either resting (i.e. in $G_0$) or, at least, proliferating more slowly than other labelled cells.

To summarize proliferative activity in the respiratory tract: 1) normally very few cells are dividing, resulting in a 'long-lived' epithelial surface (i.e. slow turnover); 2) the numbers of dividing cells vary between species and strains; they decrease from proximal to distal airway levels and they vary with increasing age; 3) there is diurnal variation in the number of proliferating cells; 4) vitamin A is an important influencing dietary factor; 5) there is very little information about cell kinetics in airway epithelium, with most from animal data and virtually nothing known about human lungs.
2.2 Cell differentiation

The process of epithelial cell renewal is seen as the passage of cells through proliferative, maturation and functional compartments in response to death or sloughing of previously existing cells. In 1881, DRASCH [63] suggested that dividing respiratory epithelial basal cells differentiated first into mucus cells and then, in time, these became ciliated, without an intervening division. On the other hand, the suggestion that daughters of dividing basal cells matured first into ciliated columnar and then into mucus cells arose from observations of respiratory epithelia from asthmatic patients and experimental injury and repair in rabbit and rat tracheae [52, 111].

The sequence of morphological changes (i.e. 'pathways') during differentiation has been studied by pulse labelling a cohort of cells synthesizing DNA with $^3$H-T and observing changes in the pattern of labelling, with time. Using this method BINDREITTER et al. [24] has studied proliferation and differentiation in normal young rats and suggests that differentiation proceeds from basal to mucous and then to ciliated cells. A continuous labelling study of rat airway epithelium has led BLENKINSOPP [27] to suggest that basal cell division produces one basal and one superficial cell, i.e. there is an asymmetric division where one daughter cell is committed to differentiation and the other remains in the basal compartment. The former then produces two daughter cells, one of which is lost by sloughing.

AYERS and JEFFERY [11] have studied differentiation in main bronchial epithelium of normal specific pathogen free rats following a single pulse of $^3$H-T. With time, a significant decrease in the proportion of cells containing label occurs, suggesting a gradual continuous loss of cells from surface epithelium. Examination of the change in percentage label in each cell type suggests that basal cells differentiate to both mucus and ciliated cells, and that the pathway includes an 'intermediate' cell type, which acts as an uncommitted cell stage through which differentiating cells pass. Detailed quantitative light and electron microscopic studies of hamster tracheal epithelium [155] have, however, established that true intermediate cells are very rare and cells, so designated at the light microscope level, are a heterogenous population including secretory cells and 'tall' basal cells which are difficult to classify (i.e. indeterminate) [177, 155].

A cell kinetic study of hamster tracheal epithelium, [38] has suggested a 3-compartment model of cell renewal: the first comprises a self-renewing one of proliferating basal cells which gives rise to a compartment consisting of mucus cells. Some mucus cells retain the ability to divide while others lose it and become fully differentiated. The third compartment consists of fully differentiated cells only, which do not divide, and have a finite life span.

A different proposal has been suggested by DONNELLY et al. [61], who observed changes in both the numbers of each labelled cell type and the combinations of labelled 'adjacent cell pairs' which, in theory, represent the recent progeny of a single cell's division. No significant changes occur in labelling indices over a ten day period, following a pulse of $^3$H-T. However, changes in labelled pairs of adjacent cells suggest that: a) ciliated cells can develop from cells of intermediate (indeterminate) morphology within 24 h and b) that both superficial mucous (goblet) and ciliated cell development is preceded by two divisions, a basal cell division followed by an intermediate cell division. The cell type produced depends on the balance of environmental influences. The studies of McDOWELL and TRUMP [153] on human tissue and parallel studies of epithelial injury in animals, indicate that secretory cell hyperplasia, stratification, epidermoid metaplasia and 'carcinoma in situ' are closely related histogenetically. The authors believe that the lesions arise mainly from hyperplastic proliferation of mucus-secreting cells associated with phenotypic changes. Cell quantification and labelling experiments with $^3$H-T indicate that 'pre-ciliated' cells are the progeny of secretory cell divisions and that many of the former still contain secretory granules carried over from parent cells following their division. The authors propose that secretory cells play the major role in genesis of ciliated cells during foetal and neonatal development, during adult epithelial cell turnover and during epithelial regeneration.

In summary, several studies have shown that in

<table>
<thead>
<tr>
<th>Tracheal cell type</th>
<th>Animal</th>
<th>Sex</th>
<th>Age wks</th>
<th>Method of measurement</th>
<th>Growth fraction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>hamster</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>1.8</td>
<td>37</td>
</tr>
<tr>
<td>Mucous</td>
<td>hamster</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>1.4</td>
<td>37</td>
</tr>
<tr>
<td>Basal</td>
<td>hamster</td>
<td>♂</td>
<td>9-13</td>
<td>C.L.</td>
<td>3.9</td>
<td>37</td>
</tr>
<tr>
<td>Mucous</td>
<td>hamster</td>
<td>♂</td>
<td>9-13</td>
<td>C.L.</td>
<td>1.3</td>
<td>37</td>
</tr>
</tbody>
</table>

C.L.: Continuous labelling.
tracheo-bronchial epithelium, mucous-secreting and indeterminate cells comprise the proliferating fraction and that there is basal cell differentiation to both mucous and ciliated cells. Mucous cells not only proliferate but can differentiate into ciliated cells with the latter a poorly dividing end stage but highly functional cell. 'Compartments' serving different kinetic and functional roles have been suggested but their exact components and quantitative relationships and the factors which influence their functional relationships are as yet unknown.

3. Factors influencing proliferation and differentiation

Numerous extrinsic agents, shown to affect cell division and thought to alter patterns of differentiation in airway epithelium, have been implicated in the aetiology of respiratory disease. Foremost are airborne irritants such as tobacco smoke and industrial or domestic environmental pollutants (e.g. oxides of sulphur, nitrogen, ozone and vehicle exhaust fumes), chemical carcinogens and infective agents. Studies of the sequence of reparative changes after mechanical trauma, and administration of certain therapeutic drugs which may change proliferative activity provide further insights into the extent to which respiratory tract epithelial cells can divide and differentiate.

3.1 Irritation

Tobacco smoke is a complex organic mixture and may damage each airway level in distinct ways, whereas simpler inorganic substances often have a predilection for one particular airway level or another, depending on their physical characteristics, such as solubility in water [104].

i) Tobacco smoke (TS)

Tobacco smoke comprises the combustion products of well over 1500 components [17]. Morphological and proliferative changes may be induced by whole TS and both its particulate and gaseous phases contribute [5, 18, 22, 51, 188]. Tobacco substitutes may also be harmful [18].

Whole tobacco smoke, when given daily to experimental animals for short periods of up to twelve weeks, causes an increase in epithelial thickness, and mucous (goblet) cell hyperplasia in proximal airways [22, 109, 115, 121, 125, 127, 144, 160, 225, 226]. There is also an increase in the number and size of alveolar macrophages [225, 226]. Continued exposure may lead to loss of ciliated and goblet cells, basal cell proliferation, and focal squamous cell metaplasia which, with cell atypia, may be regarded as 'carcinoma in situ' [8, 9, 10, 18, 55, 58, 62, 105, 139, 151, 182]. Similar findings are described in man [7].

a) Proliferation – The effects of TS on MI and LI have been studied only after short-term exposure (table VII). In all species studied, MI increases in response to TS in both extrapulmonary and intrapulmonary airways, but mainly in the former [31, 34, 127]. Nearly all studies have utilized male animals and although sex differences have been reported in the mucous cell (hyperplastic) response [109, 113], no comparison of MI or LI has been made. Both Lamb and Reid [144] and Wells and Lamerton [229] have found a dose-related response to TS, the highest number of cigarettes smoked producing the greatest number of mitotic divisions. The peak of the mitotic rise is at 24 h which, in spite of continued exposure, falls to control levels by about two days following commencement of exposure [229]. If, however, there is a break in TS exposure, a second peak is generated on recommencement [34, 121].

The effect of TS on LI has been studied by Born [36] and Ayers and Jeffery [11]. In the former study on hamsters there is a significant increase in LI (up to four-fold) in the airways of 400 μm or less, after only 8 h of TS. A second peak in LI is found at 40 h of exposure, in the alveolus but not in the intrapulmonary conducting airways. Trachea and main bronchi were not examined in the study. Ayers and Jeffery [11] have examined the LI in rat main bronchus at selected time intervals of up to 14 days of TS exposure and find a significant rise at 24 h, which remains at 3 days, falls to control levels by 7 days and is unchanged at 14 days. Labelled basal cells produce the increase seen at 24 h, whereas at 3 days both labelled indeterminate (intermediate) and secretory cells, containing a mixture of serous and mucous granules (i.e. transitional), are the main dividing cells. Although epithelial LI returned to control levels by seven days, the distribution of dividing cells is altered. In control animals, 72% of labelled cells are basal and 17% serous; no mucous cell is labelled. In contrast, after seven days of TS exposure, 37% of labelled cells are basal and 40% mucous. This finding suggests that the population of mucous cells can respond to irritants by proliferation, and indicates one important mechanism by which the TS-induced increase in mucous cell number may be brought about.

b) Differentiation – Jeffery and Reid [115, 121] suggest that the increase in mucous cell number seen in the rat is initially due to transformation of existing serous cells as cells with transitional features are found. Ayers and Jeffery [11] have labelled cohorts of proliferating cells with a single pulse of 3H-T and monitored differentiation by recording changes in the proportions of each cell type labelled with time of TS exposure. Compared with unexposed controls, there is no marked change in the pathways of differentiation; the ciliated cell still forms the major and product of differentiating (labelled) basal cells. However, newly occurring mucous cells do not arise as a consequence of basal cell differentiation, but rather from the transformation of pre-existing serous cells and subsequent division of newly-formed mucous cells.

ii) Sulphur dioxide (SO2)

Sulphur dioxide is associated with environmental pollution (both domestic and industrial) where it may act synergistically with other particulate pollutants [14, 114]. It is highly water soluble and ionisable and
after short-term exposure appears to affect central (large) more than distal, airways [56, 120, 124, 143]. Studies of the morphological changes induced by SO₂ are plentiful [6, 114, 160, 168, 209]. Only two studies, however, have reported quantitative data about SO₂-induced changes in respiratory epithelial cell kinetics (Table VIII). The first of these by Rem [178] has used SO₂ to 'model' human bronchitic changes. Specific pathogen free rats are exposed to 400 parts per million (ppm) of SO₂ three hours daily for up to six weeks, sufficient to induce mucous cell hyperplasia. In the trachea and central intrapulmonary airways, MI increases from 0.15% in controls to about 1.4% at four days of exposure. Thereafter, it falls slightly by three weeks but is maintained, for up to six weeks, at a level significantly higher than that of controls. In central airways, the early rise in MI is followed by a rise in mucous cell number. In distal bronchioli, MI is not significantly raised, yet a rise in mucous cell number also occurs.

Okuyana et al. [170] have examined the response in chickens to doses of SO₂ given daily for fourteen days. There is mucosal hypertrophy and a 2–10 fold increase in MI, an increase in the numbers of mucous

<table>
<thead>
<tr>
<th>Animal</th>
<th>Exposure time</th>
<th>Airway level</th>
<th>Change in MI</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>2 hours (48)</td>
<td>tracheo-bronchial</td>
<td>↑10 x (24 h)</td>
<td>229</td>
</tr>
<tr>
<td></td>
<td></td>
<td>epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>48 hours (32)</td>
<td>bronchi bronchioli</td>
<td>↑3 x</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>alveoli</td>
<td>↑4 x (8 h)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑4 x</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>4 days/week for 6 weeks (25)</td>
<td>trachea</td>
<td>↑7 x (6 weeks)</td>
<td>34, 127</td>
</tr>
<tr>
<td>Rat</td>
<td>14 days (25)</td>
<td>main bronchus</td>
<td>↑7 x (24 h)</td>
<td>11, 121, 122</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>↑12 x (7 days)</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>24 hours</td>
<td>tracheo-bronchial</td>
<td>↑10 x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 days (25)</td>
<td>epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td></td>
<td>↑9 x</td>
<td>229</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td></td>
<td>↑2 x</td>
<td></td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>100 puffs</td>
<td>tracheal basal cells</td>
<td>↑14 x (24 h)</td>
<td>241</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal</th>
<th>Exposure time</th>
<th>Airway level</th>
<th>Change in MI</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>5 days/week</td>
<td>trachea</td>
<td>↑12 x*</td>
<td>143</td>
</tr>
<tr>
<td>Rat</td>
<td>5 days/week</td>
<td>hilar airway</td>
<td>↑</td>
<td>)</td>
</tr>
<tr>
<td></td>
<td>45 days</td>
<td>hilar airway</td>
<td></td>
<td>168</td>
</tr>
<tr>
<td>Dog</td>
<td>4 hours/week</td>
<td>trachea</td>
<td>↑</td>
<td>)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bronchi</td>
<td></td>
<td>242</td>
</tr>
<tr>
<td>Chicken</td>
<td>14 days</td>
<td>trachea</td>
<td></td>
<td>170</td>
</tr>
</tbody>
</table>

*max. seen at 4 days.
and peptide-secreting cells (epithelial and glandular),
macrophages, and recruitment of inflammatory cells.

iii) Nitrogen dioxide (NO₂)
Extensive studies by Evans and his colleagues have shown that the primary site of damage by NO₂ is at the level of terminal and respiratory bronchioli and adjacent alveoli (see [42]). Short-term exposure (e.g. daily for 40 days), induces bronchial and bronchiolar hyperplasia with tall columnar epithelium and frequent mitotic figures. Longer exposure (i.e. for the animals' lifetime) induces emphysema.

Various studies [53, 70, 75, 77, 79, 85, 86, 102, 137] have each used ³H-T labelling to elucidate which airway level and epithelial cell is most profoundly affected by NO₂ and which cells take part in the proliferative response. Ciliated cells of the terminal bronchioli and type I cells of adjacent alveoli are destroyed by NO₂ [212]. Subsequently, nonciliated bronchiolar (Clara) cells and alveolar type II cells proliferate within 24 h, reaching a maximum at 24–28 h [102, 212]. They return to control levels by 2–4 days [82, 215], (table IX). The proliferating Clara and type

<table>
<thead>
<tr>
<th>Table IX. - Effect of nitrogen dioxide on proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animal</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Hamster</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Hamster</td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Rat</td>
</tr>
<tr>
<td>Rat</td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Rat - 1 month old</td>
</tr>
<tr>
<td>Rat - 25 months old</td>
</tr>
</tbody>
</table>
II cells differentiate into ciliated and type I cells respectively [70, 76, 79, 81]. Older animals show the same pattern of response, but the onset of proliferation is slower, more tissue damage occurs, and the magnitude of the proliferation is greater than that observed in younger animals [73]. Evans et al. [82] have shown that the proliferative response is dose related, and that once the LI has returned to control levels, it remains so despite continued exposure, irrespective of age. Thus it appears that the newly repaired epithelial cell populations become resistant to the toxic effect of the irritant [73, 82].

iv) Ozone (O₃)
Ozone is a highly toxic component of photochemical smog: its sources and effects have been reviewed by Stokinger [218], Dungworth et al. [64] and Bils and Christie [23]. It causes injury in proportion to the exposure dose [75, 78, 196], the trachea is severely affected, and terminal and respiratory bronchioi as well as adjacent alveoli are subject to more damage than more distal alveoli [86, 213, 214, 216]. Exposure conditions vary and the doses used in a number of species range from 0.1 to 3.5 ppm. Quantitative observations of MI have been made by Schwartz et al. [196], quantitative studies of LI reported by Evans and co-workers [75, 78, 80] and Castleman et al. [45], and studies of differentiation have been made by Stephens et al. [215] and Castleman et al. [45] (table X). The prime targets for damage are the ciliated cells of conducting airways and type I cells of the alveolus [29, 35, 45, 75, 78, 152, 161, 172, 194, 196, 213, 214, 215]. The nature of the proliferative response and the resultant sequelae appear to be the same whether exposure is continuous or not [215]. Penha and Worthheimer [172] have reported a mitotic response in tracheal basal cells with subsequent hyperplasia and squamous metaplasia, after daily 2 hour exposure of young mice to 2.5 ppm for 45 days. In rats and monkeys proliferation of Clara cells reach a peak after 2–3 days [45, 75, 78, 152, 213, 214, 215]. High doses (15 ppm) produce an increase in LI in all epithelial cells, which peaks at 2–3 days [199]. When exposure is prolonged beyond 3 days, LI returns to control levels by about 4 days in spite of continuing exposure [75, 78], although Lum et al. [152] have observed a raised LI throughout the 168 h of exposure. Associated with the increase in LI is a change in the relative proportions of bronchiolar epithelial cells. Clara cells increase in number and ciliated cells decrease [45, 75, 78, 152]. Evans and colleagues [75, 78] have found that Clara cells constitute all of the originally labelled population, but by 4 days, 33% of labelled cells are ciliated and 68% are Clara cells, and the authors suggest that the proliferating Clara cells differentiate into ciliated. Grain counts indicate that Clara cells divide at least once before their subsequent differentiation. Following type I cell necrosis, type II cell proliferation results in alveolar repair within 48 h [45, 75, 78, 215], indicating that the latter is the alveolar stem cell.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Exposure time [O₃ ppm]</th>
<th>Airway level</th>
<th>Change in MI time after O₃</th>
<th>Change in LI</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>24 h (0.7)</td>
<td>terminal bronchiole</td>
<td>-</td>
<td>↑ 9 x non-ciliated secretory (24 h)</td>
<td>78</td>
</tr>
<tr>
<td>Rat</td>
<td>168 h (0.8)</td>
<td>terminal bronchiole</td>
<td>-</td>
<td>↑ 30 x (72 h)</td>
<td>152</td>
</tr>
<tr>
<td>Rat</td>
<td>48 h (0.5, 0.9)</td>
<td>alveoli - type II cells (24–30 h)</td>
<td>-</td>
<td></td>
<td>214</td>
</tr>
<tr>
<td>Mouse</td>
<td>2 h/day (2.5)</td>
<td>trachea</td>
<td>↑ basal cells</td>
<td>-</td>
<td>) 172</td>
</tr>
<tr>
<td>Mouse</td>
<td>8 days (0.5–0.8)</td>
<td>bronchioli</td>
<td>↑ Clara cells</td>
<td>-</td>
<td>) 223</td>
</tr>
<tr>
<td>Mouse</td>
<td>6 h (0.5–3.5)</td>
<td>alveoli</td>
<td>-</td>
<td>↑ (24 h)</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ (72 h)</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>50 h (0.8)</td>
<td>respiratory bronchiole</td>
<td>-</td>
<td>↑ cuboidal bronchiolar cells (50 h)</td>
<td>45</td>
</tr>
</tbody>
</table>
Kultchitsky cells have also been estimated to have an LI of 0.5% in control animals and show a response to ozone, with an increase in LI to 9% after 36 h of exposure [45]. There is also evidence of the development of tolerance to ozone [35, 152, 213, 215]. After 48 h exposure, the epithelial proliferative response in the terminal bronchioli stabilizes and repair begins to return epithelial morphology to normal [214]. LI decreases to near control levels within 4 days and in spite of continued exposure (maintained at 0.4 and 0.5 ppm), does not show a further rise [78]. If the dose is increased, there is a second rise and subsequent fall in LI, with a corresponding new level of tolerance [75, 78]. STEPHENS et al. [215] have shown that, prior to weaning, bronchial epithelium of rats is resistant to oxidant-induced damage. Oxidants produce little or no injury until 35 days of infancy.

EVANS et al. [80] have also shown, in a study using ageing rats (18-20 months), that LI is initially lowered in response to a single 6 h exposure (0.5-3.5 ppm), then increases to control levels or above by 72 h. Thus age influences the proliferative response to ozone.

v) Oxygen (O₃)

Oxygen in high concentrations (40-100%) is toxic to lung tissue, and induces: 1) an exudative phase comprising alveolar oedema, haemorrhage, fibrinous exudate, hyaline membrane formation and alveolar necrosis; 2) a subacute proliferative phase leading to alveolar epithelial (type II) hyperplasia, interstitial fibrosis and partial resolution [23]. Experimental studies using rats, mice and monkeys have shown that the severity of the damage to proximal airways and alveoli depends on concentration [169, 223]. Most experimental studies have concentrated on the alveolus where oxygen induces type I cell necrosis followed by type II cell proliferation. In response to 90-100% O₂, EVANS and colleagues [71, 72] and ADAMSON and BOWDEN [1] found an initial decrease in alveolar cell proliferation followed by hyperplasia, resulting in a cuboidal alveolar lining (see Table XI). LUM et al. [152] have compared the effects of ozone (0.8 ppm) and 80% O₃ on the LI of terminal bronchioli and found that the O₃-induced proliferative response is delayed and is smaller than that initiated by O₃. Differential cell counts show that the nonciliated (Clara) secretory cell is the most important proliferating cell in the response to both oxidants. Oxygen also appears to affect the proliferative component of repair. Following initial damage by ozone, exposure to oxygen at concentrations higher than 60% inhibits the proliferation by which repair to ozone normally proceeds [103].

vi) Chemical carcinogens

Of the aromatic hydrocarbons, benz(a)pyrene (BP) and dimethyl-benz-anthracene (DMBA) have been widely studied, although few papers have examined the early proliferative changes induced in the respiratory tract. Binding of tritiated BP occurs with DNA of ciliated, mucous and basal cells, and is dependent on enzyme activation [107, 219]. During Vitamin A deficiency, which has been linked to increased risk of developing carcinoma [13], binding is increased and appears to be greatest in areas of squamous metaplasia (HARRIS et al., unpublished observation quoted in [91]).

In an in vitro study, using neonatal rat tracheas, CROCKET et al. [54] have found that exposure to BP, DMBA or methyl-cholanthracene (MCA) at a range of doses and for a maximum of 14 days, causes loss of differentiated (superficial) cells and early increases in both MI and LI in the remaining basal cells. The increases in MI and LI are not always proportional, nor are they alike for each agent. Continuation of exposure with the higher doses of each agent results in the production of squamous metaplasia. Intratracheal administration of BP, repeated weekly for up to twenty weeks, gives rise to early cell proliferation of either basal [179, 180] or mucous cells [16]. In either case, these changes precede the development of squamous metaplasia, keratin formation and premalignant changes.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Exposure time [O₂ %]</th>
<th>Airway level</th>
<th>Change in MI LI time of max. change</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>4 days (20-95%)</td>
<td>terminal bronchiol e</td>
<td>↑ 8 x (48 h)</td>
<td>103</td>
</tr>
<tr>
<td>Mouse</td>
<td>60 days (90%)</td>
<td>alveoli</td>
<td>↑ 3 x type II (36 h)</td>
<td>1</td>
</tr>
<tr>
<td>Mouse</td>
<td>168 h (80%)</td>
<td>alveoli</td>
<td></td>
<td>72</td>
</tr>
<tr>
<td>Rat</td>
<td>168 h (80%)</td>
<td>terminal bronchiol e</td>
<td>↑ 15 x Clara (168 h)</td>
<td>152</td>
</tr>
</tbody>
</table>
The action of nitrosamines, in particular diethyl nitrosamine (DEN) on airway epithelium has been studied by Reznik-Schuller and co-workers [181, 183-186]. In hamster trachea, DEN induces basal cell proliferation, mucous cell differentiation and epithelial cell hyperplasia, followed by development of papillary polyps made up of mucous and 'intermediate' cells. In hamster bronchi, several nitrosamines are found to specifically affect Clara and APUD (Kultchitsky-like) cells, inducing their proliferation and the subsequent production of squamous metaplasia [186].

A single exposure to urethane can elicit bronchiolar nonciliated (Clara) cell and type II alveolar cell hyperplasia with multiple adenoma formation in mice [131, 132]. Dyson and Heppleston [66] have given urethane as a single injection, which causes a rise in LI of alveolar cells, peaking at two weeks after urethane and returning to control levels after two months. Subsequent tumour formation is first observed four weeks later. In another study by Kaufman [131], mice were continually exposed to urethane in drinking water, resulting in an early decrease in LI and type II cell necrosis. This is followed by an increase in LI which peaks at six weeks, together with a doubling of the number of type II cells. Over the next sixteen weeks, LI returns to control levels, type II and type I cell numbers decrease and tumours develop.

These observations are in accord with the multi-stage concept of carcinogenesis, i.e. two distinct phases following a carcinogenic stimulus: 1) a nonspecific response in which there is cell proliferation and 2) later development of atypical and malignant changes (often in the absence of continuous carcinogen exposure).

3.2 Mechanical injury

The healing following mechanical trauma of tracheal epithelium has been studied as an experimental model of airway cell proliferation and differentiation. Two techniques have been used: 1) gentle stroking with cotton swabs, which removes superficial cells only, leaving basal cells essentially intact [95-99, 146, 147] and 2) curettage, which removes all epithelial cells and aims to leave a denuded basement membrane [15, 52, 133-136, 158, 231].

1) Stroking – Lane and Gordon [146, 147] have used the gentle stroking technique on the ventral (i.e. anterior) surface of rat trachea and have given colchicine and H-T to visualize the proliferative response. The authors found that immediately after injury, there is a small early wave of dividing cells which peaks at seven hours post-injury and comprises 4% of the epithelial cell population: the 4% represents mostly superficial mucous cells [96, 97]. Subsequently, there is a larger peak of LI at 22 h, made up of 42% of the total cell population. A large peak in MI then occurs at 32 h (also comprising 42% of cells), which is followed by a smaller peak in LI at 39 h, made up of 7% of the population [98].

The authors used double labelling and grain counting to distinguish between different epithelial cell populations. Double labelling has shown that the early minor wave of dividing cells (7 h, MI peak) is a different population from that which is synthesizing DNA at 22 h [97]. Grain counts show that the cells synthesizing DNA at 22 h go on to enter mitosis at 32 h, forming the second and major mitotic peak. The authors suggest that the last mentioned population of cells is normally at 'rest' in the G1 phase of the cell cycle. Grain counts also show that the cells synthesizing DNA at 39 h do not enter mitosis thereafter, and thus come to rest in the G2 phase of the cell cycle.

The results suggest, therefore, three separate epithelial cell populations, one cycling and two 'resting', one in G1 and the other in G2. The response to mild injury is a single cycle of synchronous cell division in the two 'resting' cell populations. The G1 population is thought to be basal and the G2 population mucous [97, 147].

Study of the subsequent differentiation and reconstruction of a functional mucociliary epithelium [146] reveals that the percentage of labelled basal cells decreases and that of labelled mucous cells increases, without changes in grain counts, indicating that during this process mucous cells differentiate from basal cells without prior division [98]. Cell cycle phase times have been determined by stimulating another wave of DNA synthesis with a second injury at the original site: tS for basal cells is 8-9 h, tG2 is 2.5-3.5 h and tC is 28 h. tG1 is then calculated as 14.5-16.5 h [96, 97].

2) Curettage – Initially, curettage induces cell migration [52, 231] followed by mitosis at 24-48 h post-injury in both basal and mucous cells [158]. The result is a squamous epithelium which covers the wound area. Mitosis continues among the newly-migrated cells, producing immature 'indifferent' daughter cells which subsequently differentiate over 72-96 h post-injury into functional ciliated and mucous cells [158].

Keenan and colleagues [133-136] have quantified the kinetic responses in hamsters. Control values of MI and LI in the trachea of untreated animals were found to be 0.19 and 0.63% respectively, made up of dividing mucous and basal cells in the ratio of approximately 2:1 respectively. The authors emphasize the importance of the secretory cell in the regenerative process. As evidence they cite the following observations: 1) secretory cells form the greatest proportion of the dividing cell population in control epithelium and 2) in response to the stimulus of injury, a greater percentage by far of secretory cells than basal cells, proliferate. When labelled cells were each taken as discrete populations, a larger proportion of labelled secretory cells than basal cells were found in metaphase (arrested by colchicine), at a given time interval after wounding, suggesting that the movement of the former through the cycle was more rapid. In addition, epidermoid cells covering the wound area contain PAS-positive material. Therefore, the authors consider that the resulting epider-
mepithelium is mainly the result of dividing secretory cells. By continuous infusion, nearly all the epidermoid cells become labelled as they continue to proliferate. The proliferation rate returns to normal between 60-120 h and epidermoid metaplasia gives way to a nearly normal mucociliary epithelium.

CONDON [52], WILHELM [232] and LANE and GORDON [147] have examined proliferation and repair in airway epithelium of vitamin A-deficient animals. Deficient epithelia develop multiple foci of keratinizing stratified squamous metaplasia. CONDON [52] has found that many more epithelial cells than normal divide, whereas LANE and GORDON [147] find no difference between LI in deficient and normal epithelium. In vitamin A-deficient animals, curettage results in the same sequence of cellular events, as in normal repair, but the wound area is restored with a keratinizing squamous metaplasia whether the original surface is squamous or columnar and mucociliary in type. The repair time is also shorter (i.e. 6-8 days) than in normal animals (i.e. 12-14 days) [232]. Interestingly, the wave of mucous cell proliferation reported in normal repair after gentle stroking [98] does not occur in vitamin A-deficient animals [147].

3.3 Enzymic injury

Elastase given as a single intratracheal injection increases MI and LI in three cell types, e.g. the LI of 1) non-ciliated, non-secretory bronchiolar cells increases from 1 to 8% at 24 h, 2) type II alveolar cells increases from 0.5 to 15% at 2 days and 3) endothelial cells increases from 0.2 to 10% at 4 days [228].

3.4 Drugs

A variety of pharmacologically active chemicals, either in use clinically or experimentally, affect proliferation in the respiratory tract. Ioprenaline sulphate, a potent stimulator of DNA synthesis, increases MI at three extra- and two intrapulmonary airway levels when given daily at high dose [33, 115]. The effect is seen in male but not female rats. More cells are found in division in the superficial than as normal in the basal zone of the epithelium. Pilocarpine nitrate (also daily at a high dose) causes less of an increase than ioprenaline, significant only in the trachea and proximal intrapulmonary airways. Daily administration of either drug increases the number of airway mucous cells [128, 220]. Oestradiol (ethanylestradiol) given by mouth to guinea pigs, produces an initial increase of mucous cell numbers, which is later replaced by foci of squamous epithelia with a high MI [68, 69].

Conversely, several nonsteroidal and steroidal drugs have been shown to inhibit the increase in mucous cell numbers due to irritation by cigarette smoke [101, 122, 126, 190, 191]. As it is known that one component of the cigarette smoke-induced increase in secretory cell number is cell division [11, 122], it is possible that these drugs produce their effect by inhibition of proliferation. Supportive evidence comes from other organ systems which show that the nonsteroidal anti-inflammatory drug, indomethacin, inhibits increases in the LI of rat epidermis induced by promoting agents such as 12-O-tetradecanoylphorbol-13-acetate (TPA) and also of kaolin-induced granulomas and methylcholanthrene-induced tumours [84, 89]. Interestingly, the proliferative response to TPA is restored by addition of prostaglandin (PG) E2 but not PGF2a [84, 88-90]. In the respiratory tract, the anti-tussive agent, Phenylmethoxyxodiazole (PMO), present as 2% by weight of tobacco, partially inhibits increases in MI seen at two weeks of exposure to cigarette smoke [121, 127]. PMO delays (by 1 day) the mitotic response seen during the first 24 h after exposure, but, paradoxically, the delayed response is almost twice the amplitude of the earlier response without drug [34]. Apart from the inhibitory effect of Dexamethasone on LI of foetal (16-18 day) mouse lung tissue [130], little is known of the effects of steroids on pulmonary cell division. Early administration of Methyl-prednisolone to adult mice inhibits butylated hydroxytoluene-induced type II cell proliferation (as measured by LI), providing the degree of lung injury is 'mild' [206].

Colchicine and the vincor alkaloids, vinblastine and vinorelizine, are classic inhibitors of cell division arresting mitosis at metaphase [48, 67]. In this context it is interesting that vinblastine sulphate (given subcutaneously at 0.05 mg·kg^-1 daily for 21 days) prevents cigarette smoke-induced mucous cell proliferation in the upper trachea and proximal airways of the lung. However, at the dose used, vinblastine does not appear to inhibit cell division as there is no accumulation of metaphase arrests with time (EVANS et al., unpublished; [126]) suggesting an alternative mechanism of action such as inhibition of mucous synthesis [2].

Lastly, there are some findings regarding a novel action of a mucolytic drug N-acetylcysteine (NAC). NAC has now been shown both to inhibit cigarette smoke-induced secretory cell increase [123, 191] and to attenuate, but prolong, the early proliferative response [123]. The early proliferative response to tobacco smoke is formed by both basal and mucous cell division and NAC particularly inhibits the former. However, orally administered NAC also causes degeneration and sloughing of rabbit tracheal goblet cells after 20 min [142]. Nonsteroidal anti-inflammatory agents, including NAC, also appear to speed recovery after experimental cessation of cigarette smoke [192].

3.5 Infection

The MI, LI, TS and turnover time have been determined in rats with minimal disease and compared with those of conventionally derived rats, many with chronic respiratory disease [229, 230]. MI and LI are highest and turnover time shortest in the small number of diseased animals, while the values for TS
are similar in both [230]. An anecdotal finding is the report of one infected control animal in a study by BOLDUC and REID [33], which showed that both mucous cell numbers and MI are increased at each airway level examined. In another study by SHORTER et al. [201], two rats which showed evidence of purulent tracheo-bronchitis had the LI at three levels greatly increased with respect to the controls (without disease).

3.6 Neural control

As certain neuromimetic drugs alter the rate of cell proliferation, it would seem reasonable to hypothesize that the nervous system is one component which may control or, at least, affect cell division. There is some evidence from other body systems, e.g. in the intestine it has been suggested that autonomic nerves may have the capacity to respond to changes in the rate of cell loss and, in a highly localized manner, contribute to the balance of maintaining cell production and loss [222]. In addition, STENSTROM et al. [202] have shown that vagotomy is invariably associated with an increase in the fraction of cells in the S phase in the dog duodenum and jejunum six weeks postoperatively. Immunosuppression in rats also greatly increases MI (i.e. decreases turnover time) of jejunal epithelium [63]. Since gut and lung epithelia share a common origin, it might be of interest to look for a neural component in the control of airway epithelial proliferation. Indeed, the requirement of both intact motor and sensory nerves for effective epithelial repair and regeneration (albeit in amphibia) has already been well described [204].

3.7 Immune system

The mucosal immune system (gut and bronchus-associated lymphoid tissue) has recently received much attention as a first line of defence against invading micro-organisms. In this context MILLER and NAWA [165] and MILLER et al. [166] have studied the intestinal epithelial response to infection by Nippostrongylus brasiliensis. There is an increase in the mucous cell number, a thymus-dependent phenomenon in which thoracic duct lymphocytes, either directly or indirectly, appear to regulate the differentiation of intestinal mucous cells. Thus, the regulatory role of the lymphocytes, present in bronchus-associated lymphoid tissue, on cell division and differentiation may well be a fruitful area for future investigation.

4. Summary

The proliferative potential of the various cell types present in airway epithelium have been described. The techniques used to assess their proliferative activity (MI and LI), growth fraction and length of each phase of the cell cycle have been given in outline. By use of a variety of techniques, there is ample capacity to recruit non-cycling cells rapidly into the cell cycle. Under certain circumstances (e.g. cigarette smoke exposure and mechanical trauma) the mucous (goblet) cell is proliferative and its role in epithelial repair has been clearly underestimated. Factors contributing to the variation seen in baseline proliferation include species, sex, hormonal status, airway level, age, diurnal rhythm and pathogen-free status. A variety of stimuli increase proliferation as part of a reparative process: 1) irritation by oxidants, chemical carcinogens or enzymes, 2) mechanical injury, 3) infection and 4) certain drugs, some of which stimulate division whilst others inhibit the proliferative response to irritation and may be useful in controlling the response.

Increases in the numbers of some cell types may be due to differentiation rather than proliferation and the 3-compartment model can be applied to airway epithelium comprising: 1) the self renewing/proliferating compartment, made up of basal and secretory cells in the large airways, non-ciliated bronchiolar (Clara) cells in the small airways, and type II cells in the alveolus; 2) the differentiation compartment containing mature functional cells, some of which retain the capacity to divide, e.g. serous, mucous (goblet), Clara, type II cells; 3) the fully-mature end stage cells (e.g. ciliated cells) which do not normally divide.

The nervous system and lymphocytes present in epithelium and bronchus-associated lymphoid tissue may also have a part to play in controlling cell division and their roles should be investigated further. However, the lung remains relatively unexplored with regard to the factors which control the proliferation and differentiation of its many distinct cell types, both in health and disease.

Acknowledgements: We warmly acknowledge the support given to M.M. Ayers by the Cystic Fibrosis Research Trust (England) and the patience shown by J. Billingham for help in preparing the manuscript.

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