

Cytodynamics in the respiratory tract of the rat¹

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The knowledge of cytodynamics in the respiratory tract is still in its infancy. Since Bertalanffy and Leblond (1953) described their findings in a study of the alveolar macrophage cells in the lung of the albino rat, few investigations have been reported in the literature (Koburg, 1962; Spencer and Shorter, 1962; Meyer zum Gottesberge and Koburg, 1963; and Shorter, Titus, and Divertie, 1964b), and these have indicated that the findings may vary greatly in the epithelia in the various parts of the respiratory tract, even between areas in close relationship to one another. The purpose of this paper is to report the findings obtained in a study of the cytodynamics in the trachea, bronchi, and pulmonary alveoli of the normal adult Sprague-Dawley rat by means of the technique of nuclear labelling with tritium-labelled thymidine combined with autoradiography.

Tritium-labelled thymidine (³HT) is incorporated specifically by the nuclei of cells synthesizing deoxyribonucleic acid (DNA) (Taylor, Woods, and Hughes, 1957). An assumption is made that labelled cells behave in a fashion identical to that of normal cells. Since it has been shown by Krause and Plaut (1960) that the incorporation of ³HT modifies DNA synthesis in root cells of the bean and enhances the incorporation of thymidine, and since other possible toxic effects of ³HT on various cell systems have been described (Samuels and Kisieleski, 1963), this assumption may not be wholly valid, but its use is accepted generally, at present, in studies of cytodynamics in cell populations.

MATERIALS AND METHODS

Adult Sprague-Dawley rats² of both sexes were used. The animals weighed 150 to 160 g. at the start of

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the experiment. A dose of 200 μ c. of ³HT (specific activity 7.2 c./mM.; Volk Radiochemical), in a total volume of 0.5 ml. of isotonic saline solution, was injected intraperitoneally into each animal at 8.00 a.m. on the first day of the experiment. Subsequently the animals were maintained in free-run cages at 72 to 74° F. with free access to food (Rockland rat diet R4 pellets; A. E. Staley) and water. The animals were killed by ether anaesthesia at intervals (two males and two females at each interval) of 15 and 30 minutes; 1, 2, 4, 6, 12, and 24 hours; 2, 3, 4, 5, 6, 7, and 8 days; and 2, 3, and 4 weeks after injection.

The lungs, with the trachea and bronchi attached, were removed immediately after death and 10% neutral formalin was instilled into the trachea, after which the organs were placed in the same fixative. They were embedded in paraffin wax, and histological sections of the organs were cut at a thickness of 3 μ in a frontal plane to obtain sections of the trachea and main bronchi and full sections of the entire pulmonary parenchyma. Autoradiographs were prepared both by the stripping-film technique (Kodak AR 10) and by the use of nuclear-track liquid emulsion (Kodak NTB 3). After exposure for three to four weeks and development, the slides were stained with haematoxylin and eosin and examined by light microscopy.

The percentages of labelled cells in the trachea, large and small bronchi, and the alveolar macrophage cells were estimated from counts of 1,000 nuclei in each tissue. The nucleus was considered to show labelling when at least 5 grains were observed. The number of mitotic figures showing labelling was also counted and expressed as a percentage of the total number of mitotic figures found in the counts of 1,000 nuclei. Counts were made by means of an oil immersion lens with a final magnification of 1,250.

RESULTS

Because no differences were observed between the animals of either sex, the results in both sexes will be described together. Fifteen minutes after the injection of ³HT, nuclei of epithelial cells lining

the trachea showed nuclear labelling, and the labelled cells were confined to basal situations in the pseudo-stratified, columnar epithelium (Fig. 1). Individual variations were seen in the percentage of cells showing nuclear labelling, but the average was 0.5%. In the large bronchi labelling was seen in 0.4% of basally situated, pseudo-stratified, columnar epithelial cells. In small bronchi the percentage of labelled cells was 0.2 in the single-layered low columnar or cuboidal epithelium. Labelled alveolar macrophage cells were found in the walls of pulmonary alveoli (Fig. 2), but it was not possible to differentiate between the vacuolated and non-vacuolated forms described by Bertalanffy and Leblond (1953). These labelled cells were distributed at random in alveoli throughout the parenchyma of the lung, and the average percentage was 1.2. No labelled mitotic figures were seen in the trachea, bronchi, or alveolar macrophages. No mitotic figures were identified in the epithelium of the small bronchi.

After 30 minutes the findings were similar to those seen after 15 minutes, and the average per-

centages of cells showing labelling were 0.4 in the trachea, 0.4 in the large bronchi, 0.2 in the small bronchi, and 1.1 in the alveolar macrophages. No mitotic figures were seen to be labelled in the epithelia of the trachea and main bronchi or in alveolar macrophages. No mitotic figures were seen in the epithelium of small bronchi.

After one hour labelled nuclei of epithelial cells in the trachea and large bronchi were still confined to basal situations in the epithelium, and the average percentages of cells showing labelling were 1.2 in the trachea and 0.4 in the large bronchi. In the small bronchi the average percentage of labelled cells was 0.2, and in the alveolar macrophage cells it was 1.2. No labelled mitotic figures were seen in the epithelial cells of the trachea or large bronchi or in alveolar macrophage cells. No mitotic figures were noted in the epithelium of small bronchi.

Similar distributions of labelled cells were found after two hours. However, at this interval, labelled mitotic figures were seen for the first time in the epithelia of the trachea and large bronchi and in

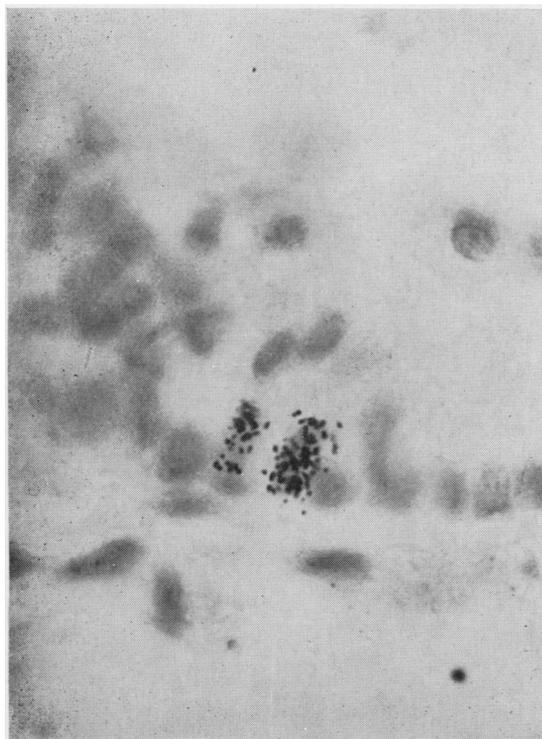


FIG. 1. Section from rat trachea 15 min. after parenteral injection of ^3HT showing labelled nuclei confined to basal situations. Autoradiograph. H. and E., $\times 1,280$.

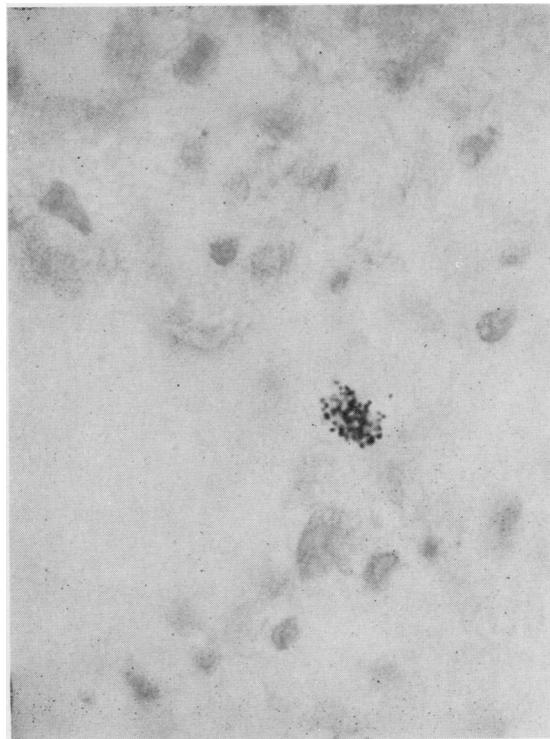


FIG. 2. Section from rat lung 15 min. after parenteral injection of ^3HT , showing labelled alveolar macrophage. Autoradiograph. H. and E., $\times 1,280$.

the alveolar macrophage cells, and approximately 25% of the mitotic figures were labelled in each of these cellular populations. No mitotic figures, either labelled or unlabelled, were seen in the epithelium of small bronchi. After four and six hours there was no significant increase or decrease in the percentages of labelled cells. The percentage of labelled mitotic figures in the tracheobronchial epithelia and alveolar macrophages was 50% at four hours and 100% at six hours. No mitotic figures were identified in the small bronchi. After 12 hours labelled nuclei were seen for the first time in intermediate as well as basal situations in the epithelia of the trachea and large bronchi. No changes were found in the average percentages of labelled cells; the figures remained at 1.2% and 0.4% in the epithelia of the trachea and main bronchi, respectively. The percentage of labelled epithelial cells in the small bronchi was 0.2, and the percentage in alveolar macrophages was 1.2. The percentage of labelled mitotic figures in the tracheobronchial epithelia and the alveolar macro-

phage cells was approximately 25% at this time interval. No mitotic figures were seen in the epithelium of small bronchi.

From these results it can be concluded that the G_2 (premitotic gap) phase for epithelial cells in the trachea and large bronchi and for the alveolar macrophages was a minimum of two hours and that the S phase (phase of synthesis of deoxyribonucleic acid) of these cells was approximately eight hours. This latter figure was estimated by measuring the interval between the mid-points on the curve of the rise and fall of the percentages of labelled mitotic figures plotted against the time in hours elapsing after the injection of ^3HT (Lipkin, Sherlock, and Bell, 1963; Shorter, Moertel, Titus, and Reitemeier, 1964a).

Twenty-four, 48, 72, and 96 hours after administration of ^3HT , the labelled nuclei in the epithelial cells of the trachea and large bronchi were situated in basal and intermediate positions (Fig. 3) in percentages of 1.1, 1.1, 1.0, and 1.4 in the trachea and 0.3, 0.6, 0.4, and 0.5 in the large

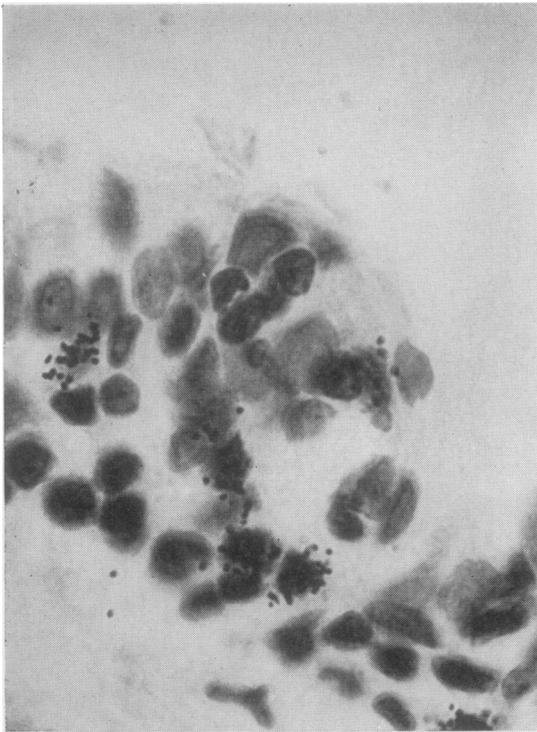


FIG. 3. Section from large bronchus of rat 72 hours after parenteral injection of ^3HT , showing labelled nuclei in basal and intermediate positions of epithelium. Autoradiograph. H. and E., $\times 1,440$.

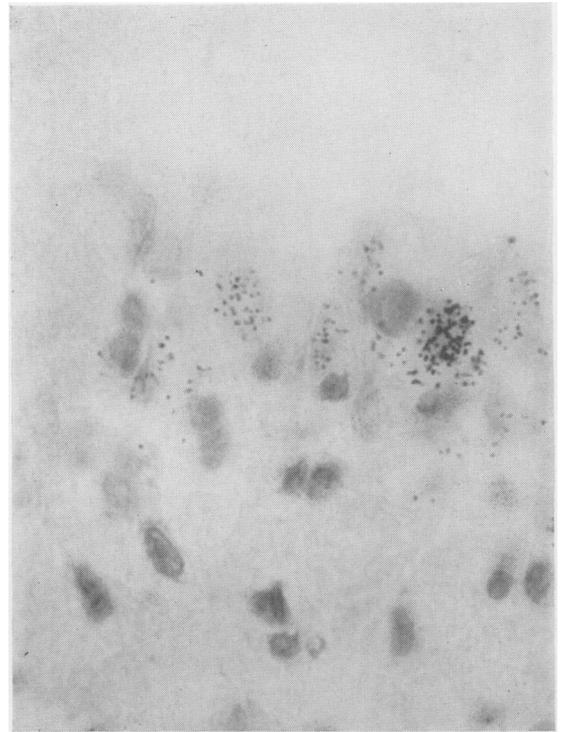


FIG. 4. Section from large bronchus of rat five days after parenteral injection of ^3HT , showing labelled nuclei in superficial and intermediate positions. Autoradiograph. H. and E., $\times 1,200$.

bronchi for each of the time intervals, respectively. The percentage of labelled cells in the epithelium of small bronchi was 0.2 at each time interval. The average percentages of nuclear labelling in alveolar macrophages at these intervals were 2.0, 2.1, 2.0, and 1.6, respectively. No labelled mitotic figures could be identified in the epithelia of the trachea or large bronchi or in the alveolar macrophage cells. No mitotic figures were seen in the small bronchi.

Five days after injection of ^3HT , labelled epithelial nuclei were seen in superficial situations in the epithelium of the trachea and large bronchi in addition to intermediate positions (Fig. 4), and by six days labelled nuclei were seen only on the surfaces of these epithelia. The average percentages of labelled nuclei in the trachea and large bronchi at these time intervals were 1.2 and 1.4, respectively. In the small bronchi the percentages were 0.2 at five days and 0.0 at six days. The average percentage of labelled alveolar macrophages was 1.6 at each time interval. No labelled mitotic figures were seen.

After seven days labelled cells were not seen in the epithelium of the trachea; thus, the migration of labelled tracheal epithelial cells had taken place in approximately six to seven days. Four-tenths per cent. of the cells in the epithelium of the large bronchi still showed labelling, but these labelled nuclei were confined to the superficial part of the epithelium. No labelled cells were seen in the small bronchi, whereas 0.4% of alveolar macrophages were labelled at this time. No labelled mitotic figures could be identified.

After eight days labelled nuclei were not seen in the epithelia of the trachea or the large or small bronchi. It was concluded that the migration of the labelled cells in the epithelium of the large bronchi had occurred in approximately seven to eight days. The percentage of alveolar macrophages showing nuclear labelling was 0.4. No labelled mitotic figures were seen.

After two weeks 0.3% of alveolar macrophage cells still showed nuclear labelling, but after three weeks no labelling was seen in any of the sections examined. The overall findings suggest that two populations of alveolar macrophage cells existed, one with a life span of approximately seven days and the other with a life span of two to three weeks.

Histological evidence of purulent tracheobronchitis was found in two animals, one a female examined 24 hours after, and the other a male killed 48 hours after, the injection of ^3HT . These animals were excluded from the study detailing

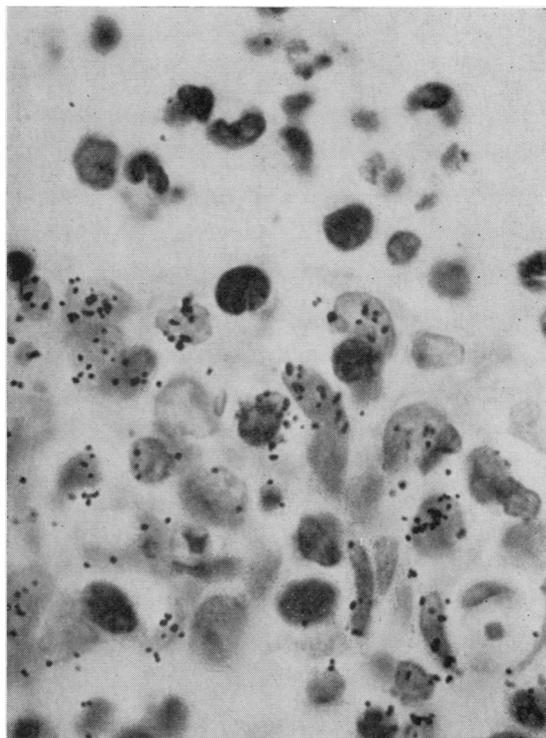


FIG. 5. Section from main bronchus of rat 24 hours after parenteral injection of ^3HT in animal with purulent tracheobronchitis, showing presence of large numbers of labelled epithelial cells. Autoradiograph. H. and E. $\times 1,360$.

normal findings. In each case an average of 8% and 10% of epithelial cells showed nuclear labelling in the trachea and large bronchi, respectively, and the labelled cells were distributed throughout the epithelia in superficial, intermediate, and basal situations (Fig. 5). No labelled mitotic figures were seen in the tracheobronchial epithelia, and the numbers of labelled cells were not increased in the small bronchi or lung parenchyma.

COMMENT

We have found that nuclear labelling in the respiratory epithelia of the adult albino rat after the parenteral administration of ^3HT was limited to scattered cells, and this was similar to the findings in mice (Spencer and Shorter, 1962; Shorter *et al.*, 1964b). We did not confirm the observation made by Schultze and Oehlert (1960), who, in a study of the incorporation of ^3HT into cells of

the adult rat, noted early labelling in superficial as well as basally situated epithelial nuclei in the bronchi, a finding which has also been made in mice (Shorter *et al.*, 1964b). Despite the low incidence of labelling of cells in the epithelia of the trachea and main bronchi and in alveolar macrophages, the occurrence of a small but definite wave of labelled mitotic figures, the apparent migration of labelled cells from basal to superficial positions in the epithelia of the trachea and large bronchi followed by their disappearance, and the loss of labelled cells from the populations of alveolar macrophages indicated that these cell populations may be included in the general classification of 'renewing cell populations' (Leblond and Walker, 1956; Messier and Leblond, 1960). The classification of the population of epithelial cells in the small bronchi is more difficult, but tentatively it, too, might be included in the group of 'renewing cell populations' rather than 'expanding populations' (Leblond and Walker, 1956; Messier and Leblond, 1960) because of the disappearance of labelled cells within a relatively short time. Messier and Leblond (1960), in a definition of expanding cellular populations, have emphasized the persistence of nuclear labelling in such tissues for a prolonged time as part of the requirements for inclusion in such a classification.

The value for the duration of S phase that we have found in respiratory epithelial cells conforms to that described for most mammalian cellular systems (Bertalanffy, 1964). The migration times of the labelled cells in the trachea and large bronchi (six to seven and seven to eight days) differed widely from the figures of 4.7-6 and 26.7 days, respectively, which were described in adult rats by other workers (Leblond and Walker, 1956; Bertalanffy and Lau, 1962) using different techniques, but they were similar to those found in mice by Shorter *et al.* (1964a, b) and Spencer and Shorter (1962) using ^3HT and autoradiography. The duration of the persistence of cells showing nuclear labelling in the epithelium of small bronchi was similar to that described in the mouse by one group of workers (Shorter *et al.*, 1964b) using the same technical methods. Comparable figures are not available in the rat.

The findings suggestive of the existence of two populations of alveolar macrophages may be compared with those made in the rat by Bertalanffy and Leblond (1953). These authors described non-vacuolated and vacuolated alveolar macrophage cells and, using colchicine and mitotic counting, speculated that their life spans were eight and 29 days, respectively. The life spans of

seven days and two to three weeks for two populations of alveolar macrophage cells found in our study are similar to these results, with allowances for differences in technique, but we were unable to distinguish the two populations on morphological grounds. It has also been suggested that there are two populations of alveolar macrophages in the mouse, one with a life span of eight days and the other 35 days (Shorter *et al.*, 1964b).

As Lipkin *et al.* (1963) have indicated, the definitions of normal rates of renewal in cell populations are important since they provide base lines for studies of disease. Any abnormalities of these rates that may be found subsequently in disease processes may contribute to our understanding of the morphological changes which occur in the various stages of such diseases and may enhance our understanding of their pathogenesis.

SUMMARY

The cytodynamics of cells in the respiratory tract have been studied in normal adult Sprague-Dawley rats after the parenteral injection of tritium-labelled thymidine combined with autoradiography. The findings in the epithelia of the trachea and large bronchi showed an apparent migration time of labelled cells of six to seven and seven to eight days, respectively. Nuclear labelling in the epithelial cells of the small bronchi persisted for five to six days. The results indicate that two populations of alveolar macrophages exist, one with a life span of seven days and the other two to three weeks. The phase of synthesis of deoxyribonucleic acid (S phase) for the tracheo-bronchial epithelial cells and alveolar macrophages was eight hours. The classification of the cell populations into 'renewing cell populations' was discussed. Differences in labelling were seen in bronchi showing purulent bronchitis.

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