Cell kinetics and tumour formation

Cell turnover in the lungs of mice with hereditary lung tumours¹

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Cell turnover time varies from one tissue to another but is reproducible as a hereditary characteristic for a given cell line. The present study indicates that it is prolonged in the lungs of mice congenitally predisposed to developing primary lung tumours. Primary malignant tumours occur more frequently in areas of relatively slow cell replacement in the human gastrointestinal tract than in those where cell turnover is rapid. On the basis of these observations it is suggested that postmaturity of a cell may be a significant factor in the development of neoplastic change.

The genetic information in a cell is ordered and controlled by the macromolecules of the nucleic acids—deoxyribonucleic acid (DNA) and the several kinds of ribonucleic acid (RNA). In animals, DNA is contained largely in the cell nucleus and determines the morphological and functional characteristic of a species or a tissue. When cells have become differentiated the change is irreversible and under normal circumstances they do not revert to a simpler form. This intrinsic stability in the early stages of development depends on interaction between the nucleus and the cytoplasm. The feedback mechanisms responsible for this orderly development have not been fully elucidated. They are complex and appear to involve RNA, proteins, and histones (Allfrey, Littau, and Mirsky, 1963).

There is general agreement that the carcinogenic process involves an unknown alteration in the heritable characteristics of the cells from which the tumour arises (Walpole, 1959). The initiation of the process is an irreversible chemical interaction with previously stable genetic receptors. This results in genetic mutation in the cell which occurs without recognizable histological change. Subsequently, a gradual and potentially reversible stage of promotion occurs, permitting the process of malignancy to become morphologically obvious. Hyperplasia is present at this stage but may not be an important factor in the production of the tumour but simply an accompaniment of it.

Biological systems are characterized not only by a balance and integration of the function of individual units but by cyclical behaviour, one form of which is cell division, and in recent years an increasing amount of attention has been given to the kinetics of cellular proliferation, in both experimental animals and man (Spencer and Shorter, 1962; Shorter, Titus, and Divertie, 1966; Lipkin, 1965).

Up to the present time it has been inferred that a tissue is more likely to give rise to malignancy from cell mutation if rapid cell division occurs in it. In our opinion, it is at least as reasonable to suggest that a tissue characterized by rapid cell turnover and consequently by frequent shedding of mature cells in a renewing cell population may be relatively resistant to the development of malignancy by limiting the time necessary for mutant or newly malignant cells to become established in it. Cell turnover times vary from one tissue to another but remain constant within reasonable limits for a specific type of cell and may be regarded as a heritable characteristic of it. More than 75% of inbred strain A/J mice develop spontaneous pulmonary adenomas of alveolar origin by the age of 18 months. Since this is also an inherited characteristic, we considered it would be of interest to study the cell turnover time in adult strain A/J mice at a time before the anticipated appearance of these tumours to determine whether this would be faster or slower than in other strains of mice not predisposed to tumour formation in the lungs.

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MATERIALS AND METHODS

Ninety-six adult strain A/J mice¹ of both sexes, weighing between 15 and 20 g., were used in the study. At the time of injection the mice were 14 months old. One microcurie of tritium-labelled thymidine (³HT) (specific activity 3 mc./mM)² per gram of body weight in a total volume of 0.2 ml. of isotonic saline solution was injected at 7.30 a.m. by the intraperitoneal route into each animal. The animals were kept in free-run cages in an air-conditioned environment at a temperature of 72 to 74° F. with free access to food³ and water. Two male and two female mice were killed by ether anaesthesia at 15-minute intervals from 15 to 105 minutes from the time of injection. Further sacrifices were made at 4, 6, 8, 12, and 24 hours; 2, 3, 4, 5, and 7 days; and 2, 3, 4, 5, 6, 7, and 8 weeks from the time of injection.

The lungs, with trachea and bronchi attached, were removed quickly and 10% neutral formalin was instilled into the trachea, after which the organs were placed in the same fixative. After embedding in paraffin, histological sections were cut at a thickness of 3 microns in a frontal plane so that full sections of the trachea, main bronchi, and entire pulmonary parenchyma were obtained. Radio-autographs were prepared using Kodak nuclear track emulsion (NTB3). After exposure for three weeks and development in Kodak D19, the slides were stained with haematoxylin and eosin and examined by light microscopy. All counts were made using an oil immersion objective (magnification $\times 1,250$).

The percentages of labelled cells in the trachea, bronchi, and alveoli 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.

RESULTS

No significant difference appeared in the study between male and female mice, and the results for these will be described together. In animals sacrificed 15 minutes after the intraperitoneal injection of ³HT, nuclei of epithelial cells lining the trachea showed labelling predominantly in basal positions in the columnar epithelium and to a lesser extent in superficial and intermediate positions. There was individual variation among the animals in the number of cells labelled, but the average was 1.6% based on counts of one

thousand cells. Larger bronchi showed the same frequency of nuclear labelling with a similar distribution in the epithelium. In the smaller bronchi the cuboidal or low columnar epithelium was single layered, and 0.3% of the nuclei were labelled. Cell nuclei were labelled in random fashion in alveolar walls throughout the lungs, but whether these were in pulmonary surface epithelium or alveolar cells could not be established with certainty. The incidence of labelling in these cells averaged 1.1%.

In animals sacrificed 30, 45, 60, 75, 90, and 105 minutes after administration of ³HT there was no significant difference in the percentage of labelling present in the lungs from the values found at 15 minutes. The average values remained at 1.4% in the trachea and 1.2% in the large bronchi, and the distribution of labelling was unchanged. In the small bronchi and alveoli the figures were 0.2% and 0.9% respectively.

At 4, 6, 8, and 12 hours after injection further counts of nuclear labelling were made with results in numbers and distributions which were unchanged from those already noted. Subsequent counts were made at daily intervals of sacrifice from one to seven days after the isotope had been given. By 48 hours (Fig. 1) superficial labelling had disappeared from the epithelia of the trachea and large bronchi, and by the four-day interval nuclear labelling was also absent from the deep layer of epithelial cells in these locations but was present in superficial and intermediate cells. The percentage of labelled nuclei was 1.2% in the trachea and 0.9% in the large bronchi. In the small bronchi it was 0.3% and in the alveoli 1.0%. At the end of five days (Fig. 2) no labelling could be seen in the trachea, and by seven days the large bronchi were free from labelled cells also. At this time the percentage of cells in the alveoli which showed nuclear labelling had fallen to 0.3% and in the small bronchi to 0.2%. With individual variations these levels persisted (0.2% in cells of the alveolar wall and 0.1% in the walls of small bronchi) to the conclusion of the experiment eight weeks after the ³HT had been administered (Fig. 3). No labelled mitotic figures were identified.

When tracheitis, bronchitis, or pneumonitis was present nuclear labelling was more frequent and occurred in 7% to 12% of the tissue cells counted.

DISCUSSION

Tritium-labelled thymidine is incorporated specifically by the nuclei of cells synthesizing DNA (Taylor, Woods, and Hughes, 1957). An assumption

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²Volk Radiochemical Company, Skokie, Illinois

³Rockland mouse diet

FIG. 1. Nuclear labelling in the wall of a small bronchus 48 hours after injection of ^3HT . (H. and E. $\times 1,500$.)

FIG. 2. Labelling of a nucleus in a superficial position in the epithelium of a large bronchus 5 days after injection of ^3HT . (H. and E. $\times 1,500$.)

FIG. 3. Labelled nuclei in the alveolar walls of mouse lung (left) 4 weeks and (right) 8 weeks after the injection of ^3HT . (H. and E. $\times 1,500$.)

tion is made that labelled cells behave in a fashion identical to that of normal cells, although it has been suggested that the DNA molecule may be altered by the incorporation of ^3HT in it (Krause and Plaut, 1960). However, its use is generally accepted in studies of cytodynamics in cell populations and values obtained by us in a previous investigation of cell turnover in rat lung using this method of isotopic labelling corresponded closely to those of Bertalanffy and Leblond (1953), who used the method of colchicine arrest and mitotic counting. In a previously reported study using ^3HT as a label, we found the cell turnover times in the lungs of adult albino mice to be comparable to those in the rat (Shorter, Titus, and Divertie, 1964). Since the method of administration, the body weight dosage, and the technique of development of the radioactive label have been identical in all these experiments, any differences in the results of the present study from those already established would seem to be due to specific characteristics in the lungs of strain A/J mice.

In spite of the relatively low incidence of labelling of cells in the epithelium of the tracheobronchial tree and lungs of these animals, the apparent migration of labelled cells from basal to superficial positions and the loss of labelled cells indicates that this is a renewing rather than an expanding cell population. In the trachea and large bronchi the different times of disappearance of labelled cells from superficial and deep positions suggests that two cell populations may exist in these sites. The persistence of labelled cells in the small bronchi and alveoli of strain A/J mice up to eight weeks after the intraperitoneal injection of ^3HT indicates a much slower turnover time in the lungs of this strain than has been noted previously in albino mice (Shorter *et al.*, 1964) or rats (Shorter *et al.*, 1966). More than 75% of inbred strain A/J mice have been shown to develop spontaneous pulmonary adenoma in the alveoli by the age of 18 months (Heston, 1942; Grady and Stewart, 1940), and it is in this tissue that cell renewal is so much slower than in rats and mice not congenitally predisposed to tumour formation.

In the gastrointestinal tract of man the most rapid cell renewal occurs in the small intestine, where primary malignant epithelial tumours are extremely rare. The proliferative cycles of mucosal cells of the stomach and colon are of longer duration than in the small intestine, and in these organs primary carcinoma is relatively common (Lipkin and Quastler, 1962). In the colonic mucosa of

patients with active or quiescent chronic ulcerative colitis the renewal time of epithelial cells is six days in contrast to three days in the normal individual (Shorter, Spencer, and Hallenbeck, 1967). In these patients primary malignancy occurs with increased frequency in the areas involved by the disease. It has also been shown that carcinoma cells of the human stomach, colon, and rectum proliferate less rapidly than normal epithelial cells in these areas (Baserga, Henegar, Kisielecki, and Lisco, 1962; Clarkson, Ota, Okhita, and O'Connor, 1964).

In view of these observations and the results of the present study, it appears that tumour formation is more common in areas of slower cell replacement than in epithelial surfaces characterized by rapid cell turnover. The present observations lead us to believe that in strain A/J mice the genetic abnormality predisposing to tumour formation is associated with prolonged cell survival in the lungs. It might be speculated that deletion of the control mechanisms of cellular homeostasis occurs more readily in individual cells of advanced age, permitting disease to develop and, in specific circumstances, dedifferentiation to occur and a tumour to appear.

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