Tritiated Thymidine Labeling of Normal and NDV-Infected Chick Nasal Epithelial Cells (35772)

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Upper respiratory tract infection in man and experimental animals is accompanied by destruction of specialized and other cells, replacement by undifferentiated cells, reorganization of the different cell layers, and differentiation in some of these. Little information is available about cell dynamics in upper respiratory mucosae of any animal (1, 2), little more for trachea and bronchi (3, 4). Replacement of cells in chicken nasal mucosae has not been studied by tritiated thymidine labeling. In the present study, the proliferative compartment (5) of the ciliated cell system has been delineated, labeling indices were determined for the inner and outer surfaces of the middle turbinates, and the effect of an acute viral infection on the labeling index was ascertained.

Materials and Methods. Day-old hatchery chicks were laboratory raised for 3 weeks (wt, 140–190 g), then divided in two groups. Twenty-seven controls were injected intraperitoneally with 0.5 μCi/g of body weight of tritiated thymidine (TdR-CH_3-^3H); two were decapitated at intervals from 1 to 48 hr and from 3 to 18 days. Another 27 were inoculated intranasally with 1 drop/nostril of an undiluted saline preparation of mesogenic ("B") strain Newcastle disease virus (NDV) and 24 hr later each was thymidine-injected; two per time interval were then decapitated at 2-hr intervals from 1 to 57 hr. Each head was fixed in 10% formalin; both maxillary turbinates were cut out, dehydrated, paraffin embedded, cut serially at 5–6 μ, mounted on glass slides, deparaffinized, and dip-coated with bulk photosensitive emulsion (Eastman Kodak NTB_2 melted at 40–42°). Slides were dried for 2 hr at room temperature, then stacked in dry sealed plastic boxes, refrigerated upright at 4° for 3 weeks, developed in Dektol (1:1), fixed in Kodak P-5, washed in running tap water for 15 min, and stained with toluidine blue. Injections were initiated at 12:00 noon and continued through midnight to avoid diurnal effects.

In recording labeled cells, sections of both conchae of each pair of chicks were examined and the technically best was selected. In controls, one turbinate per time interval was counted; in the infected group, two or all four. The labeling index (% of labeled cells) in each epithelial cell system, ciliated and acinar, was calculated separately for the outer and inner surface of each turbinate tip area at each time interval encompassing 10 to 12 acini plus the interacinar ciliated cells on each surface (Figs. 1, 2). This was done in both control and infected specimens. The total number of labeled lymphocytes and the percentage of lymphocytes in the same areas were also recorded. In controls, it was often necessary to go beyond the first 10 acini to find 100 lymphocytes. Relative percentages of labeled nuclei in the acinar and ciliated systems, whether in a basal stem, intermediate proliferative, or surface end cell position, were ascertained for each time interval. Finally at five time intervals the percentage of labeled cartilage cells in one entire turbinate from a control and an infected chick was determined.

Results. Figure 3 shows percentages of labeled cells in ciliated and acinar cell systems in the *outer* mucosal surface (orient in Fig. 1) of control turbinates from 1 hr to 18 days after a single pulse of thymidine, and in infected birds from 1 to 57 hr after injection

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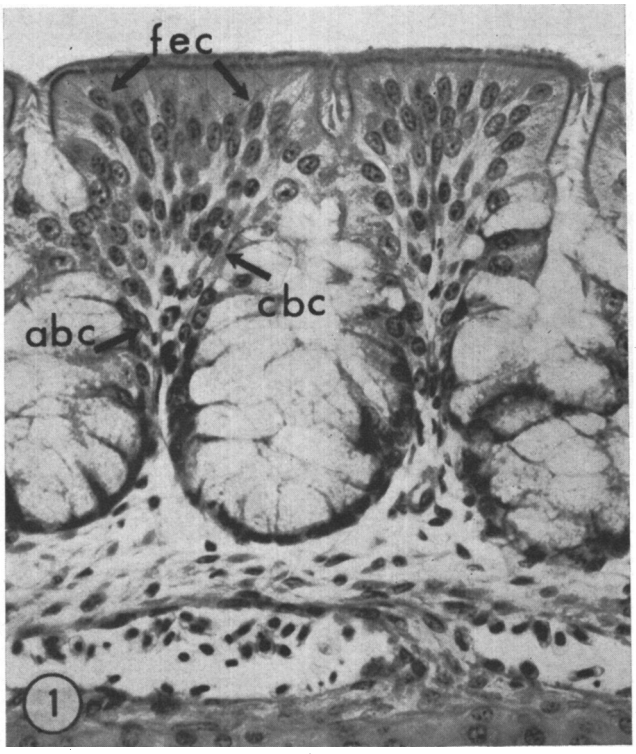

FIG. 1. Enlargement of acini and interacinar ciliated cell system: (abc) acinar basal cell; (cbc) ciliated system basal cell; (fec) functional end cell. Proliferative compartment is between basal cells and end cells; H & E, × 450.

of tritiated thymidine. Infection had been initiated 24 hr before thymidine injection.

On *inner* surface mucosae of the same turbinates at the same time intervals the percentage of labeled cells showed a mean of 1.5% for controls and a mean of 0.1% for infected. Together the data indicate that normal interphase labeling is roughly twofold greater on the outer than on the inner surface of the turbinates in both ciliated and acinar systems. They also show that synthesis of DNA was sharply reduced in infected turbinates during the 57 hr postinfection covered by the study period. In one chick in which infection happened to be unilateral, averaged indices (*i.e.*, ciliated + acinar cells) for the normal turbinate were well within the control range (2.0%), while those for the infected turbinate were 0.5%.

In controls, the percentage of labeled lymphocytes also showed a peak at 12 hr; in infected turbinates, the *percentage* of labeled cells stayed somewhat below control values until about 35 hr, but the actual *numbers* of labeled lymphocytes showed sharp peaks and drops which correlated with the varying intensity of lymphocyte infiltration in a given specimen. Lymphocytes may well recirculate locally within the tissues involved in the infection.

Table I indicates the level in the mucosa, whether at a basal stem cell, central proliferative, or surface end cell position, at which labeled cells were found in control turbinates at intervals between 1 hr and 18 days following thymidine injection (orient on Fig. 1). It seems clear that in the ciliated system the percentage of labeled cells progressed steadily from the basal to the proliferative area, then to the surface. In the acini, the relative positions of labeled cells showed no patterned placement.

Table II shows an average 0.6% of labeled cells in the turbinate cartilage in 4 normal

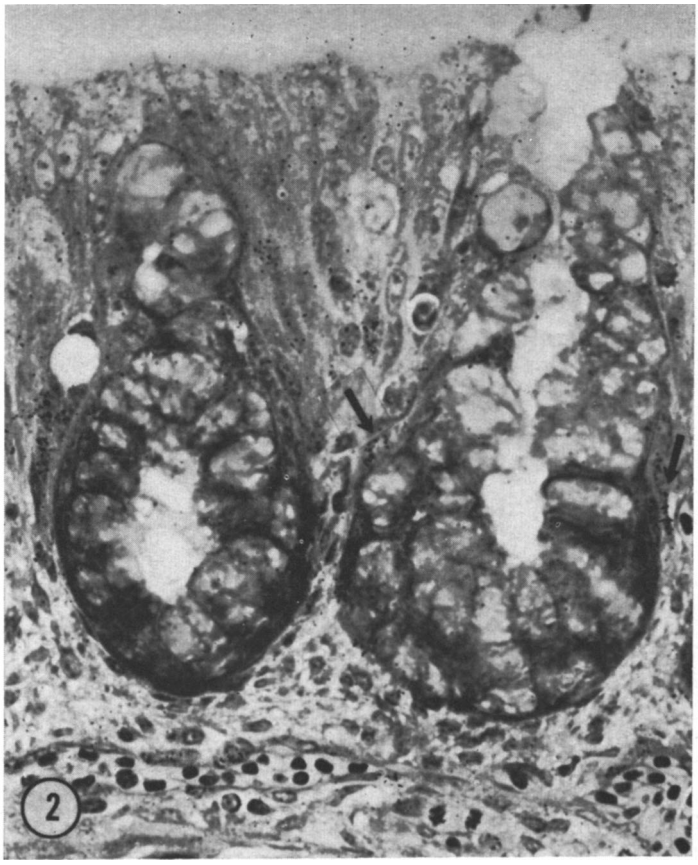

FIG. 2. Labeled ciliated and acinar (arrows) cell nuclei 12 hr after tritiated thymidine injection, control; minor background label; toluidine blue, ×675.

chickens, in contrast with an average of 0.1% in 4 infected chickens.

Discussion. Basic information on the intestinal epithelial system has accrued from combined data on rates of turnover, migration, and multiplication (DNA synthesis and mitosis) of cells under varying conditions. It is to be expected that nasal mucociliated surface cells would also be constantly replaced. Mouse nasal mucosal cells have been shown to have labeling indices of 0.66% in septum, 1.1% in turbinates, 8.9% in nasopharynx, and 1.6% in trachea (2). Rat mucosae showed rates of 1.2% in trachea and 0.4% in large bronchi (3). Our mean indices of about 3% and 1.5% in mucosae of outer and inner surfaces of chicken turbinates also indicate slow rates of synthesis. Migration of cells to the surface seemed quite slow: only 7% of the originally labeled cells had reached the surface by 48 hr, and at all periods between 7 and 18 days only about 40% of labeled cells were in the end cell position.

The mitotic rate could not be determined in the present study. Colchicine was not used and visible mitotic figures in controls were very rare; serial sections of entire turbinates may be required to collect significant values. Also, the cell cycle time could not be determined in the 18-day period.

The study has shown that, in chick turbinate mucosae, the proliferative compartment is between basal stem cells and the functioning end cells at the lumen surface. The relative percentage of labeling in the proliferative compartment stayed at about 60% from 12 hr to 4 days. The rise in the labeling index on the outer surface at 12 hr

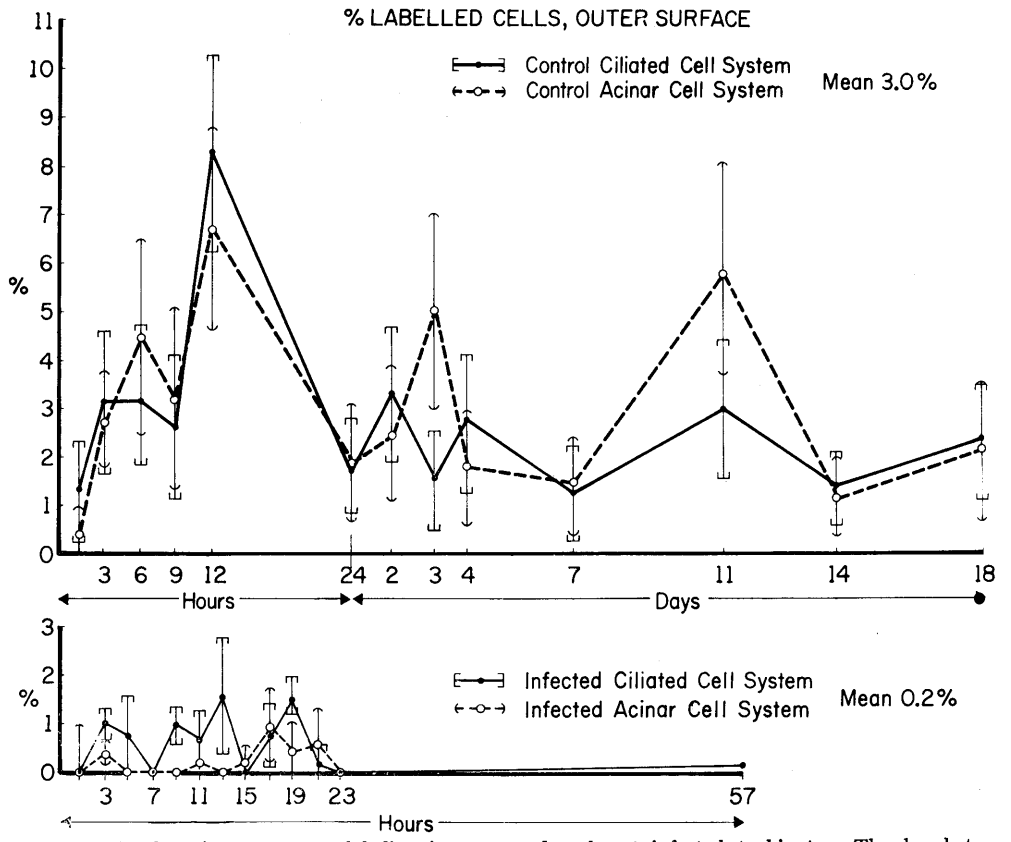

FIG. 3. Each point represents labeling in 1 control and 2–4 infected turbinates: The brackets represent the 95% confidence limit.

probably represents continued division of cells that were labeled within 1 hr of thymidine injection.

The sharp depression in the labeling index in the NDV-infected chicks raises the interesting question of mechanism. Relatively few cells were actually destroyed and most of these were in the inner-surface epithelium. Yet nearly all mucociliated cells were altered morphologically during the phase of acute infection. Perhaps at the time of labeling—24 hr after virus inoculation—some substance had been released from the infected portion of cells, which inhibited DNA synthesis in the normal cells. Dulbecco and Johnson (6) showed that in tissue cultures, purified interferon produced by NDV inhibited uptake of thymidine in normal cultured cells in the absence of the virus itself. In the intact chicken, the inhibition evidently lasts only a few days, since in related NDV experiments we found increased numbers of mitotic

TABLE I. Percentages of Labeled Cells Found in Basal Stem, Proliferative Compartment, and Surface End Cell Positions in Control Chickens at Sequential Times from 1 hr to 18 Days After Thymidine Labeling.

After labeling (hr)	Basal (%)	Prolif. (%)	End cell (%)	No. of cells labeled
1	87	13	0	8
3	73	27	0	22
6	55	45	0	20
9	47	53	0	15
12	42	58	0	54
24	40	60	0	22
Days				
2	33	60	7	28
3	23	59	18	17
4	18	64	18	11
7	18	41	41	17
11	26	32	42	19
14	25	33	42	12
18	28	29	43	21

TABLE II. Percentages of Labeled Cells in Control and Infected Cartilage Cells at 5 Periods After Labeling, Based on 3000 Cells/Turbinate Cartilage (30 fields, ± 100 cells/field).

	(hr)	No. of cells labeled
Normal	6	19
	12	20
	48	20
	72	16
	96	14
Av		18 cells or 0.6%
Infected	55	2
	55	2
	56	2
	57	5
	57	5
Av		3.5 cells or 0.1 %

figures at 4–5 days when virus infection was decreasing. In the study of Shorter *et al.* (4) two rats which had spontaneous tracheobronchitis showed 8–10% of the epithelial cells labeled at all levels (4).

The depressed rate in the labeled infected system was not a fault in thymidine administration or an artifact; total numbers of labeled lymphocytes peaked and fell focally in accord with histologically demonstrable focal infiltration. However, it might represent a delayed G_1 phase of cell division. In infected birds, the low number of cells available for counting at a few of the time intervals precluded more specific definition of relative rates at those times.

Finally the relatively reduced rate of DNA synthesis in mucosal cells in the turbinate under surface in contrast to those in the outer (main airway) surface may be germane to the selective initial susceptibility of under-surface mucosae to both laryngotracheitis virus (LTV) (7) and NDV (8) infections.

Summary. The tritiated thymidine labeling index in nasal turbinate epithelia was determined in chicks which had been infected 24 hr with Newcastle disease virus and in control chicks. Control indices were calculated at periods from 1 hr to 18 days, indices in infected epithelia at two hr intervals 1–57 hr after thymidine injection. Mean control values were consistently higher in outer-surface epithelia than in the shallower inner-surface system (3.0:1.1); peak labeling was at 12 hr. The proliferative compartment in the ciliated cell system was between basal stem cells and superficial end cells; 7% of labeled cells were in the end cell position at 48 hr; about 40% were in this position at all periods between 7 and 18 days. Indices were sharply reduced in infected turbinates in all compartments in all mucociliated epithelia at all times between 25 and 81 hr postinoculum. Lymphocyte control indices peaked at 12 hr, fell somewhat below control values for 35 hr postinfection. Indices in turbinate cartilage cells were 0.6% in controls, 0.1% in infected chicks.

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