

# AUTORADIOGRAPHIC INVESTIGATION OF CELL RENEWAL IN THE GALL BLADDER EPITHELIUM

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The parameters of the mitotic cycle of the gall bladder epithelial cells and the pattern of mucus formation were determined autoradiographically. The bases of the epithelial folds are cambial, but their apex consists of more highly differentiated zones. Since the proliferative pool of epithelial cells of the guinea pig gall bladder is 2.4% and since complete renewal takes place in 32 days, this structure must be regarded as one of the slowly renewed tissue systems.

The parameters of the mitotic cycle, reflecting the pattern of cell renewal during physiological regeneration, have now been determined for nearly all epithelia of enterodermal type [4, 6, 11, 12, 14, 15, 18]. The mitotic cycle of the epithelial cells of the gall bladder, however, has not hitherto been studied.

The object of this investigation was to make an autoradiographic study of renewal of the epithelial cells of the gall bladder during physiological regeneration.

## EXPERIMENTAL METHOD

Guinea pigs (68) weighing 200-250 g were used. Thymidine- $H^3$  (specific activity 15 Ci/mmole) was injected subcutaneously into 52 guinea pigs in a dose of  $0.5 \mu\text{Ci/g}$  body weight. The animals were killed 0.5, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 14, 16, 18, 21, 24, 30, 36, 48, 60, 72, 84, 96, and 108 h after the injection of thymidine- $H^3$ . The duration of the mitotic cycle and its parameters were calculated by the methods of Quastler and Sherman [15] and Trasher [18]. The proliferative pool was determined by Mendelsohn's equation [13], and the difference between the durations of the mitotic cycle as determined by the method of Quastler and Sherman [15] and Quastler [16] also was calculated. To determine the indices of proliferative activity of the gall bladder epithelium 6000 cells were counted in each animal. To study the secretion of mucus by the gall bladder epithelium, three guinea pigs received an injection of  $\text{Na}_2\text{S}^{35}\text{O}_4$  (specific activity 5 mCi/ml) 4 h before sacrifice in a dose of  $0.9 \mu\text{Ci/g}$  body weight. The tracks above these autoradiographs were counted for each animal in 135-150 conventional squares, the area of each of which was  $260 \mu^2$ .

## EXPERIMENTAL RESULTS

The simple monolayer epithelium of the guinea pig gall bladder consists of cells in various stages of the life cycle; ordinary high-prismatic, pale postmitotic, dark degenerating, and single goblet-like cells in the region of the neck of the gall bladder.

The experiments showed a statistically significant increase in the number of mitoses between 8 p.m. and 6 a.m. (mitotic index  $\text{MI}=2.4\%$ ) and a decrease between 9 a.m. and 6 p.m. ( $\text{MI}=1.7\%$ ) (Fig. 1). Similar phenomena were observed in the epithelium of the common bile duct of rats [3] with a well-defined diurnal rhythm both of the number of cells in the period of DNA synthesis and in the number of mitoses, with a significant unimodal increase in the number of synthesizing cells (from midnight to 8 a.m.) occurring 4 h before the increase in the number of mitoses (from 4 a.m. to noon).

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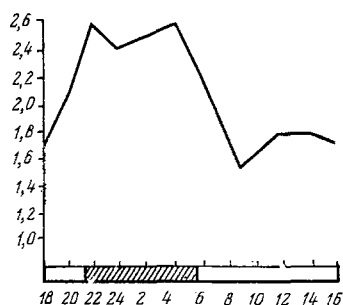


Fig. 1

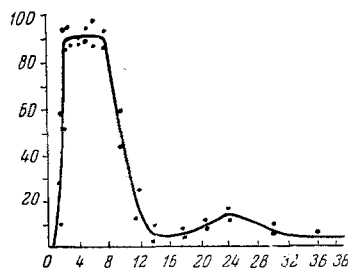


Fig. 2

Fig. 1. Diurnal rhythm of mitotic activity of epithelial cells of guinea pig gall bladder. Abscissa, time of day (dark period shaded); ordinate MI (in %).

Fig. 2. Change in percentage of labeled mitoses in epithelium of intact guinea pig gall bladder. Abscissa, time after injection of thymidine- $H^3$  (in h); ordinate, number of labeled mitoses (in %).

TABLE 1. Indices of Proliferative and Secretory Activity of Epithelial Cells of Guinea Pig Gall Bladder in Different Parts of the Mucous Membrane ( $M \pm m$ )

Area investigated	No. observed in areas of mucous membrane studied (in % of total number)			Incorporation* of Na <sub>2</sub> S <sup>35</sup> O <sub>4</sub>
	mitoses	H <sup>3</sup> -labeled cells		
		after 1 h	after 108 h	
Apex of folds	6,0±1,2	2,0±0,5	33,0±6,0	21,6±0,6
Lateral part	21,0±2,0	10,0±1,1	20,0±5,0	19,1±0,9
Base of folds	73,0±2,2	88,0±1,1	47,0±6,5	16,7±0,8

\*Number of tracks per conventional unit area.

The duration of the mitotic cycle of the epithelium of the intact gall bladder was 18 h; its parameters (in h) were:  $g_2 \text{ min} = 1.5$ ,  $M = 1$ ,  $g_1 = 8.5$ ,  $S = 7$  (Fig. 2). It must be emphasized that after 72 h this cell population included single cells with undiluted label, probable evidence that a very small proportion of the cells had left this particular mitotic cycle.

The overwhelming majority of proliferating cells, as was suggested previously [1, 7], was situated at the base of the folds (Table 1). The use of  $Na_2S^{35}O_4$  showed (Table 1) that mucous formation was more intensive at the apex than at the base of the folds ( $0.01 > P > 0.001$ ). The bases of the folds in the gall bladder must therefore be regarded as cambial and the apex as more highly differentiated zones.

The changes in the relative proportion of cells labeled with thymidine- $H^3$  with time (Table 1) reflect migration of the layer of epithelium from the base of the folds to their apex, in agreement with results obtained for the epithelium of the rabbit gall bladder [8].

Calculation of the proliferative pool of the gall-bladder epithelial cells by two indirect methods gave virtually identical results (2.3-2.4%). The time for complete renewal of the epithelial layer, calculated by Quastler's equation [16], was 32 days.

Comparison of the principles governing physiological regeneration of the epithelium of the gall bladder (the results of this and the previous investigations [2]) and of the small intestine [6, 11, 15, 17] showed agreement between the duration of the mitotic cycle and its parameters, the location of the cambial zones, and the absence of polyploid cells. However, the number of cells in the proliferative pool was several tens of times smaller in the epithelium of the gall bladder than in that of the small intestine, as a result of which its complete renewal took 32 days compared with 1-2 days for the small intestine [9, 10]. The gall bladder epithelium must therefore be regarded as a slowly renewed tissue system [5].

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\*Date omitted from Russian original — Publisher.