

## EXPERIMENTAL BIOLOGY

### RENEWAL TIME, PROLIFERATIVE POOL, AND DURATION OF MITOTIC CYCLE OF EPITHELIAL CELLS OF THE COMMON BILE DUCT

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The duration of the mitotic cycle and its periods ( $T=56.6$  h,  $M=0.9$  h,  $G_1=48.5$  h,  $S=6$  h,  $G_2=1.2$  h), the proliferative pool (7.5%), and the renewal time (754.5 h) of the epithelium of the common bile duct were determined in rats by the use of thymidine- $H^3$  and colchicine. This epithelium must be regarded as one of the slowly renewed tissue systems.

KEY WORDS: epithelium; DNA synthesis; mitotic cycle; bile duct.

Many investigations of physiological regeneration and renewal of mammalian cells [4, 5, 8, 10] have provided no information about the epithelium of the biliary tract. Only one or two papers on the renewal of cells of the gall bladder have been published [1, 2, 6].

The object of this investigation was to study renewal of the epithelial cells of the common bile duct of intact rats.

#### EXPERIMENTAL METHOD

Experiments were carried out on 37 male albino rats weighing 200-250 g from the "Rappolovo" Nursery. The animals of group 1 (eight) received an injection of colchicine (0.1 mg/100 g body weight) and were killed 6 h later. The duration of mitosis was determined by the formula  $(MI_{cont} \times t_{col}) / MI_{col}$  [7] and the mitotic activity (the percentage of cells dividing per hour) by the formula  $MI_{col} / t_{col}$ , where  $MI_{cont}$  is the mitotic index for intact animals,  $MI_{col}$  the mitotic index for animals receiving colchicine, and  $t_{col}$  the period of action of colchicine. If in the course of 1 h  $n\%$  of cells divide, the renewal time of the whole epithelium will be  $100/n$ .

The rats of group 2 (13) received a subcutaneous injection of thymidine- $H^3$  in a dose of  $0.5 \mu\text{Ci/g}$  body weight (specific activity 15 Ci/mole). The animals were killed 40 min and 1, 1.5, 2, 2.7, and 3.5 h after the injection of the isotope. The duration of mitosis was determined by Thrasher's method [13], the duration of the  $G_2 + \frac{1}{2}M$  periods from the ascending limb of the curve of labeled mitoses (the time of appearance of 50% of labeled mitoses), the duration of the S-period, and the renewal time by Quastler's formula [12], and the proliferative pool by Mendelsohn's equation [9].

Having calculated the renewal time by Quastler's formula [12] and the colchicine method and also the proliferative pool, the duration of the mitotic cycle could be determined.

Five and six times respectively, the animals of groups 3 and 4 received subcutaneous injections of thymidine- $H^3$  in a dose of  $0.5 \mu\text{Ci/g}$  body weight (specific activity 4.1 Ci/mole) with intervals of 6 h between injections. The rats were killed 1 h after the last injection of thymidine. From the results of these experiments it was possible to determine the number of cells participating in the proliferative pool.

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## EXPERIMENTAL RESULTS

Rats have no gall bladder [11] and for that reason their common bile duct is relatively large (about 10 mm). The third of the duct next to the liver was studied. The mucous membrane of the common bile duct in rats, with its uneven surface, forms crypt-like depressions. The epithelial lining consists of a single row of simple prismatic epithelium. In the floor of the crypt-like depressions 57.7% of all epithelial mitoses and 65.3% of all DNA-synthesizing cells were found, in the sides of the crypts there were 18.5 and 12.3% respectively, and at the top 23.8 and 22.4%. As was shown previously [3], in the 24-h period the number of cells synthesizing DNA and the number dividing by mitosis changes in accordance with a certain rhythm: the number of DNA-synthesizing cells is greatest in the period from midnight to 8 a.m. and the number of mitoses reaches a maximum between 4 a.m. and 12 noon. The duration of mitosis and the renewal time were thus determined at times when the number of mitoses was at high and low levels and the results were averaged. The duration of mitosis, calculated by the colchicine and autoradiographic methods, was 0.9 h. The renewal time of the epithelium was 754.5 h (800 h by the colchicine method, 709 h by Quastler's formula [12]).

On the basis of the mean diurnal number of DNA-synthesizing and mitotically dividing cells determined previously [3] (8.45% and 1.27% respectively) the duration of the S-period was calculated (6 h).

The proliferative pool calculated by the indirect (7%) and direct method (fivefold saturation 8.2%, sixfold 7.8%) gave similar results.

If 7.5% of cells participate in the proliferative pool and the epithelium is renewed in 754.5 h, the duration of the mitotic cycle will be 56.6 h. The duration of the  $G_2$  period was found to be 1.2 h, so that the  $G_1$  period ( $T - G_2 - M - S$ ) was 48.5 h.

On the basis of these observations on physiological regeneration, the epithelium of the common bile duct in rats must be regarded as a slowly renewed tissue system [4].

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