

# AUTORADIOGRAPHIC STUDY OF THE MITOTIC CYCLE OF THE ACINAR EPITHELIUM OF THE MOUSE SUBMANDIBULAR SALIVARY GLAND

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The duration of the  $G_2$  and S periods of the mitotic cycle was determined from the percentage of labeled mitoses curve in acinar cells of the submandibular salivary gland of male mice. When labeled thymidine was injected at 6 AM the first labeled mitoses appeared after 1.5 h. The duration of the  $G_2$  period plus half of mitosis was 3-4 h. The duration of the S period varied from 10.5 to 11.5 h.

Factors determining the different levels of cellular renewal in rapidly as opposed to slowly renewed organs are unknown. One characteristic feature of the low proliferative activity of the cells of slowly renewed organs is considered to be the substantial prolongation of the duration of the individual phases of the mitotic cycle. However, experimental data on this question are contradictory. The duration of the S-period of the mitotic cycle in hepatocytes and in the renal epithelium of rats is 1.5-2 times longer than in the intestinal epithelium [2, 4, 7, 8]. Meanwhile, the duration of the S-period in the acinar cells of the mouse pancreas is not significantly different from that of the intestine [3, 5].

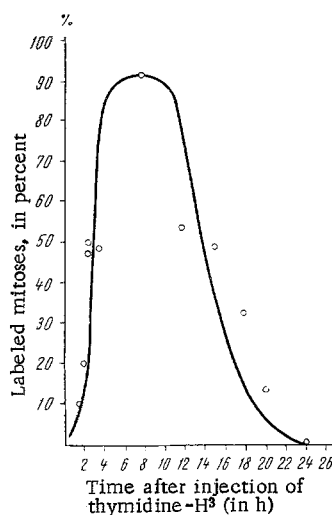


Fig. 1. Change in percentage of labeled mitoses in acinar cells of mouse submandibular salivary gland at different times after single injection of thymidine- $H^3$ .

It was decided to study this problem in relation to another slowly renewed organ — the salivary gland. The level of cellular renewal of the secretory epithelium of the salivary glands is extremely low [1]. The index of labeled nuclei for the acinar epithelium of the submandibular salivary gland of rats and mice after administration of thymidine- $H^3$  varies from 1 to 1.5% [1, 6].

The duration of individual periods of the mitotic cycle for the acinar epithelium of the salivary glands was studied from the curve of labeled mitoses, and changes in the labeling index were studied at different times of the 24-h period after injection of thymidine.

## EXPERIMENTAL METHOD

The experimental animals were 31 male C57Bl/6J mice with a mean weight of 20 g. The mice were kept singly or in pairs in jars with natural daylight for four days before the experiment began. The animals received natural food and drink ad lib. at 10-11 AM.

Thymidine- $H^3$  with a specific activity of 8.6 Ci/mmol was injected intraperitoneally in a single dose of  $0.7 \mu\text{Ci/g}$  body weight at 6 AM. The animals were sacrificed singly or in pairs at different times during the 24-h period after injection of the isotope. The submandibular salivary gland was fixed in Carnoy's mixture. Paraffin sections,  $5 \mu$  in thickness,

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TABLE 1. Changes in Percentage of Labeled Nuclei and Labeled Mitoses in Acinar Epithelium of Mouse Submandibular Salivary Gland

Index	Time of day															
	6:30 AM	7:00 AM	7:30 AM	8:00 AM	8:30 AM	9:00 AM	9:30 AM	10:00 AM	11:00 AM	12 noon	2:00 PM	6:00 PM	9:00 PM	12 mid night	6:00 AM	10:00 AM
Time after injection of thymidine (in h)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	5.0	6.0	8.0	12.0	15.0	18.0	24	28
Percentage of labeled nuclei	1.18	0.006	0.53	0.05	0.40	0.01	0.18	0.64	0	0.04	1.24	0.04	0.04	0.43	0.28	0.13
Percentage of labeled mitoses	0	0	10	20	50	0	49	0	0	0	97	0	46	39	0	0
	(22)	(6)	(29)	(10)	(48)	(50)	(29)	30	(24)	(3)	(96)	53	(30)	(13)	(10)	(10)

Note. Total number of mitoses relative to which the percentage of labeled mitoses was determined is given in parentheses.

were coated with diluted (1:3) type R (NIKFI) emulsion and exposed for 50 days. The autographs were stained with Mayer's hematoxylin. At least 300 fields of vision were counted for each mouse. The percentage of labeled mitoses was calculated for those cases in which between ten and 100 mitoses were found. Mitoses were regarded as labeled if there were at least two grains of silver above them. In addition, the index of labeled nuclei was determined at different times after a single injection of the isotope.

## EXPERIMENTAL RESULTS

The results of the counts are given in Table 1. The index of labeled nuclei in the acinar cells of the submandibular salivary gland varied considerably among individual animals despite the fact that the period of DNA synthesis was fairly prolonged. This indicates marked asynchronism in the time of onset of this period in individual mice during the 24-h period. During the first few hours after a single injection of thymidine- $H^3$  at 6 AM the index of labeled nuclei varied from its maximum to virtually zero. The maximum value of the labeling index in the acinar cells of the submandibular salivary gland of the mice was 1.2%, in agreement with data in the literature [6]. The mean index of labeled nuclei for the 24-h period was 0.4%. The sharp fluctuations in the labeling index created considerable difficulty when the percentage of labeled mitoses was analyzed. Because of the very low labeling index and the virtually zero mitotic activity, the results for some animals were omitted when the curve of the percentage of labeled mitoses was plotted. As Fig. 1 shows, the first labeled mitoses appeared 1.5 h after injection of the isotope, which evidently corresponds to the minimal duration of the  $G_2$ -period. About 50% of mitoses were labeled 3 h and 4 h after injection of thymidine- $H^3$ . According to the literature, this time corresponds to the mean duration of the  $G_2$  period plus half the duration of mitosis. The percentage of labeled mitoses reached its maximum (97) 8 h after injection of the isotope. Immediately after, the number of labeled mitoses began to fall, and reached 50% again between 12 and 14 h after injection of the thymidine- $H^3$ . Determination of the duration of the S period of the mitotic cycle from the 50% level of the curve of labeled mitoses accepted in the literature gives a value of approximately 10.5–11.5 h. The salivary glands were taken from mice in which I. V. Markelova [9] investigated the pancreas and showed that the first labeled mitoses appeared 2 h after injection of the isotope, the mean duration of the  $G_2$  period plus half the duration of mitoses was 4 h, and the S-period was 7 h. It is thus clear that the duration of the S period in cells of different but slowly renewed organs in the same animal varies. In the submandibular salivary gland of the mouse the duration of the  $G_2$ -period is about equal to, but that of the S period is rather longer than its duration in the pancreas, and more than 1.5 times greater than its duration in a rapidly renewed tissue such as the intestinal epithelium [7]. The results confirm the results of other experiments [3–6] which showed that the duration of the S-period is not constant for the somatic cells of higher animals.

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