Diurnal Variation in Epithelial Cell Population Kinetics of Young Mouse Tongue

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The purpose of this study was to determine if there was a diurnal variation in the DNA synthesis pattern of epithelial basal cells in the dorsum of the mouse tongue between infancy and preadult ages. The pattern of DNA synthesis in the basal cells of mouse tongue in 24-hour periods for different age groups was studied by isotope labeling and radioautography. A strong diurnal variation was found in the pattern of DNA synthesis with the degree of variation dependent on the age of the experimental groups.

Irradiation of the head of several species of laboratory animals causes a mode of irradiation death called "oral radiation death." This mode was studied by Goepp and Fitch[1,2] who discovered that the initiating factor of the sequence of events leading to death was an irradiation effect in the lingual epithelium causing lack of repopulation.

In previous studies, Goepp and Fitch[3] noted that there was an apparent marked diurnal variation in oral radiation death mortality depending upon the age of the mice. It seemed that younger mice, such as those four to five weeks of age, had an appreciably lower LD_{50} than did older mice. However, the lower LD_{50} value occurred only at a certain time of day as contrasted to irradiation at other times of the day, the other times giving the same LD_{50} value as that found in older mice. The degree of diurnal variation seemed to decrease as older groups of mice were studied, and there was no apparent diurnal variation in radiation sensitivity of mice that were 14 weeks of age or older. The evidence suggested that the sensitivity to irradiation injury of the lingual epithelium was dependent upon the time of day of irradiation and that this diurnal difference apparently diminished as the mice became older and disappeared when they reached maturity at 16 weeks.

The possibility of diurnal variation in the radiation sensitivity of tissues could be of considerable importance in radiation therapy. This is hardly a new consideration, but it is still not clear because of conflicting experimental results obtained by different workers. Pizzarello, Witcofski, and Lyons[4] found considerable difference in the mortality results in the irradiation of rats depending upon the time of day. Rugh and coworkers[5] reported that their experimental results from mouse irradiation showed no diurnal dependence for radiation effect, and they adamantly refuted Pizzarello's work. These two groups of workers apparently studied normal adult experimental animals during a period of growth. Others also have studied this question, but a clear understanding of the problem has not yet been achieved.

Whitmore[6] has demonstrated that the degree of cell-culture system's sensitivity to irradiation injury can be dependent upon the (DNA) cycle. Thus, it could be inferred from the results of previous studies on oral radiation death that the lingual epithelium in younger animals may have a more synchronous DNA cycle among the progenitor population, the basal cells. Lindop and Rotblad[7] who studied the age factor in the succeptibility of man and animals to radiation also had mixed conclusions. They found that in the young mice there is a sharp peak of 40% increase in sensitivity at the age of 4 weeks. They found a steady increase in radiation sensitivity in old age, making old mice about twice as liable to irradiation injury as were younger adult mice. Von Smallmann and Grimes[8] studied diurnal rhythm in the lens epithelium of rats. The ages ranged from 1 day to 1 year, and they found

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that rats killed in the forenoon showed a reduction of ^{3}H-thymidine incorporation directly related to age. Wakonig-Vaartaja,[9] in studying the effect of urethan on mitotic cells of mice of different ages and strains, found that the mitotic index of the spleen showed a marked decline from the age of 2 to 12 days. After that time, the mitotic index maintained a steady level. Brown and Berry[10] found a diurnal variation in both the mitotic index and in the tritiated thymidine labeling index in the epithelium of the hamster cheek pouch. They concluded that the diurnal mitotic variation is the result of a partially synchronous population moving through the DNA synthetic period. Diurnal variation of mitotic activity or DNA synthesis has been investigated in the mouse, rat, and rabbit[11–14] and specifically in the oral tissues in the rat.[15–19] In addition, Glass and Goepp[20,21] demonstrated a nonrandom distribution of DNA synthesis and mitotic events in the basal cell population of mouse tongue epithelium during recovery after irradiation injury. They noted that nonrandom distribution and related synchrony seemed to be necessary for the replacement of lost epithelial layers. The observation that young mice, with presumably growing tissues, were sensitive to diurnal variations in the mortality of oral radiation death may be because of the presence of a nonrandom, synchronous occurrence of DNA synthesis which is similar to that found in the adult mouse tongues after irradiation injury.

The purpose of this study is to determine if the young mouse tongue epithelium of mice 4 to 12 weeks old has a diurnal variation in the spatial pattern and synchrony among basal cells in DNA synthesis.

Materials and Methods

A total of 54 male C57 BL/6 mice was sparated into a 4-, 8-, and 12-week-old age group. Mice were housed in plastic cages with SAN-I-CEL bedding, and were fed Teklad Mouse and Rat diet and given water ad libitum. The mice were caged in a room with a stable air-conditioned environment (70 to 74 F) without window facilities. The fluorescent lights were on continuously during the 24-hour experimental period.

The number and distribution of basal cells in DNA synthesis were determined at 4-hour intervals for 24 hours in the groups of 4-, 8-, and 12-week-old mice. Tritiated thymidine (^{3}HTdR*) was used to label cells in DNA synthesis. The isotope (specific activity of 3.0 Ci/mM) was administered intraperitoneally at a dosage level of 15 μCi per mouse. The isotope was administered for each group of mice at 400, 800, 1,200, 1,600, 2,000, and 2,400 hours. All mice were killed by cervical dislocation one hour after the administration of ^{3}HTdR.

The tongues were removed and fixed in 10% buffered Formalin for 24 hours. Then the tongues were longitudinally sectioned at the midline and the hemisections were embedded in a rosin-eskar paraffin. The first 12 sections were cut at 3 micrometers and placed on two glass slides. After being deparaffinized, the sections were stained for the Feulgen reaction. Radioautographs were done of the isotope specimens, using the coating technique described by Kopriwa and Leblond.[22] The slides were dipped in NTB 3 liquid emulsion,† air dried,

TABLE 1

PERCENTAGE OF LABELED BASAL CELLS AFTER ADMINISTRATION OF ^{3}HTDR GIVEN AT DIFFERING TIMES IN 24 HOURS IN MICE, AGE 4 WEEKS

Time (hr)	Mouse No.*	Percentage Labeled Cells	Mean	SD
400	1	33.85		
	2	20.20	26.07	± 7.02
	3	24.17		
800	4	16.56		
	5	17.50	16.99	± 0.47
	6	16.93		
1,200	7	9.27		
	8	9.74	12.9	± 5.89
	9	19.69		
1,600	10	7.65		
	11	6.15	7.80	± 1.72
	12	9.59		
2,000	13	31.25		
	14	15.68	19.39	± 10.50
	15	11.25		
2,400	16	27.82		
	17	23.34	25.82	± 2.28
	18	26.30		

* Three different mouse samples per time group.

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TABLE 2

PERCENTAGE OF LABELED BASAL CELLS AFTER ADMINISTRATION OF ^{3}HTdR GIVEN AT DIFFERING TIMES IN 24 HOURS IN MICE, AGE 8 WEEKS

Time (hr)	Mouse No.	Percentage Labeled Cells	Mean	S D
400	1	14.53		
	2	21.30	20.40	± 5.47
	3	25.36		
800	4	26.30		
	5	21.46	21.42	± 4.89
	6	16.51		
1,200	7	6.83		
	8	5.31	6.27	± 0.84
	9	6.67		
1,600	10	20.31		
	11	18.11	18.67	± 1.44
	12	17.60		
2,000	13	13.65		
	14	6.25	14.58	± 8.84
	15	23.86		
2,400	16	17.03		
	17	10.95	15.33	± 3.84
	18	18.03		

and placed in lightproof plastic slide boxes containing a drying agent, then the boxes were stored at 4 C for three weeks for radioautographic exposure. The radioautographs were developed with D19 developer,† dehydrated, and counterstained through the emulsion with fast green at a pH of 5.8 to prevent staining of the nucleoproteins.

Four hundred and eighty basal cells were counted per section and four serial sections, 1,920 cells, were counted per tongue specimen. Counts were begun at the anterior one third of the tongue and continued posteriorly toward the intermolar eminence. Only basal cell nuclei that were covered by five or more grains over the nuclear outline were counted as labeled.

To characterize the distribution of labeled cells within each specimen, each sample was divided into a series of "statistical grids." If labeled cells were, indeed, randomly distributed within the sample, the number of such cells within each statistical grid should be approximately equal for all grids; and the number of labeled cells to be expected within each grid would be equal to the total number of labeled cells within the specimen divided by the number of grids. The significance of deviations from these expectancies was tested by means of chi-square analyses,[23] using the general formula $(f_o - f_e)2/f_e$. Each statistical grid thereby yielded an index of that grid's deviation from the frequency of labeled cells expected in a random distribution. The sum of these indexes for each specimen produced for that specimen a total chi-square value greater or less than a calculated critical value dependent on a preselected level of statistical significance and the degrees of freedom inherent in the statistical procedure. For these analyses, each analysis included 31 *df*, and the 0.05 probability level was chosen as the maximum to establish nonrandomness.

In addition, certain of the data obtained were subjected to a statistical analysis of variance, a somewhat more sensitive statistical test.[23]

TABLE 3

PERCENTAGE OF LABELED BASAL CELLS AFTER ADMINISTRATION OF ^{3}HTdR GIVEN AT DIFFERING TIMES IN 24 HOURS IN MICE, AGE 12 WEEKS

Time (hr)	Mouse No.	Percentage Labeled Cells	Mean	S D
400	1	18.23		
	2	16.83	17.02	± 1.13
	3	15.99		
800	4	11.46		
	5	8.44	9.48	± 1.73
	6	8.49		
1,200	7	10.63		
	8	13.80	12.39	± 1.61
	9	12.74		
1,600	10	19.74		
	11	13.75	17.03	± 3.04
	12	17.61		
2,000	13	7.24		
	14	9.01	9.71	± 2.87
	15	12.87		
2,400	16	10.63		
	17	14.53	17.33	± 8.46
	18	26.83		

† Eastman Kodak Co., Rochester, NY.

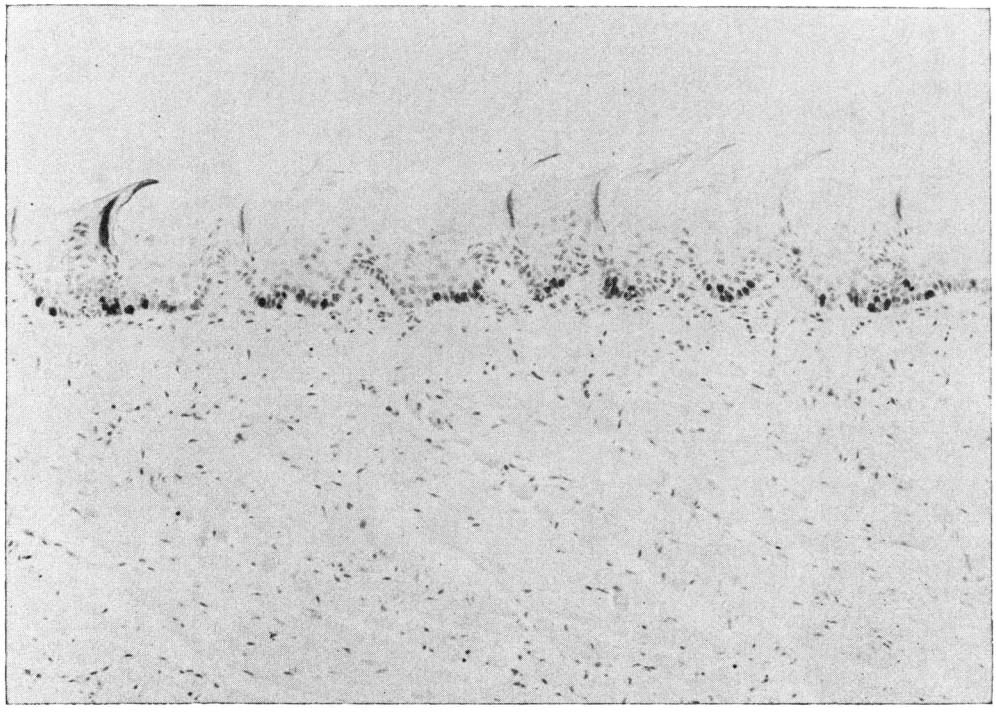

FIG 1.—Microscopic appearance of radioautograph of tongue section from 8-week-old mouse at 800 hours after administration of ^{3}HTdR one hour before sampling. Examination reveals apparent nonrandom distribution of isotope-labeled basal cells as evidenced by exposed emulsion grains seen over nuclei of basal cells (original mag × 100).

The labeling index, the percentage of basal cells labeled by ^{3}HTdR one hour after injection, was determined as follows: labeling index = number of labeled cells × 100%/ total number of counted cells in the basal layer.

Spatially related groups of isotope-labeled basal cells were described as clusters. The characteristics of the cluster were defined as two or more labeled contiguous basal cells with more than two nonlabeled contiguous basal cells on both sides of the cluster of labeled cells. If the span of nonlabeled cells between labeled was two or one, both groups of labeled cells, including the span of unlabeled cells, were counted as one cluster. The reasons for this are based on previous observations made by Glass and Goepp.[17,18] They reported that in continuous labeling experiments, the area of labeling began with two or three cells and the area enlarged along the basal cell layer by adding labeled cells. However, in flash label experiments where the isotope is available for only about 20 minutes, areas began with two or three contigously labeled cells with long spans of unlabeled cells between. In continuing sample intervals, there was a great increase in the number of labeled groups separated by only one or two unlabeled cells. This was interpreted to mean that spans of one or two unlabeled cells represented between groups of labeled cells represented cells that were actually part of a spatially related group as evidenced by the results of continuous label experiments. Cluster counts were described as the cluster factor in the statistical tests that were done. Within the scope of this report, cluster factor is identified as the number of clusters/the total number of labeled cells.

Results

Preliminary microscopic examination of the radioautographic tongue specimens revealed apparent spatial groupings of labeled basal cells similar to those previously described by Glass and Goepp.[13] These groupings or clusters appeared to be most evident in the sample of 8-week-old mice (Figs 1, 2). All samples were counted and the labeling index was determined. The results are given in Tables 1 through 3.

For the purpose of comparison, the labeling index values (percentage of labeled cells) were plotted against the sample age and time of day (Fig 3).

After determinations of labeling index, the basal cell population was divided into previously described statistical grids. Probability values were determined for all samples and are given in Table 4.

Cluster factor determinations were made through use of the formula already described. Tables 5, 6, and 7 give the values that were obtained.

The cluster factor values, multiplied by 100 to demonstrate percentage cluster factor, were plotted in a histogram against the time of day for each age group in the total samples (Fig 4). In addition, for purposes of comparison, label values and the cluster factor percentage values were plotted together against time of day for each age sample group (Fig 5).

The mean numbers of thymidine-labeled cells for each of the age groups and at each sampling time are given in Tables 8 and 9.

The significance of deviations of the various group means from their respective row and column means and of these from the grand mean was tested by a two-way analysis of variance as described previously in the Method section. A summary of the results of this analysis is given in Tables 10 and 11.

Discussion

Examination of mean label indexes given in Table 1 (4-week-old mice) reveals a significant ($P < .01$, based on analysis of variance of labeled cell counts) drop in the label index value at 1,600 hours, which is less than one third of the values seen in the peaks of labeling that occurred at 400 and 2,400 hours. Similarly, in 8-week-old mice (Table 2), there was a

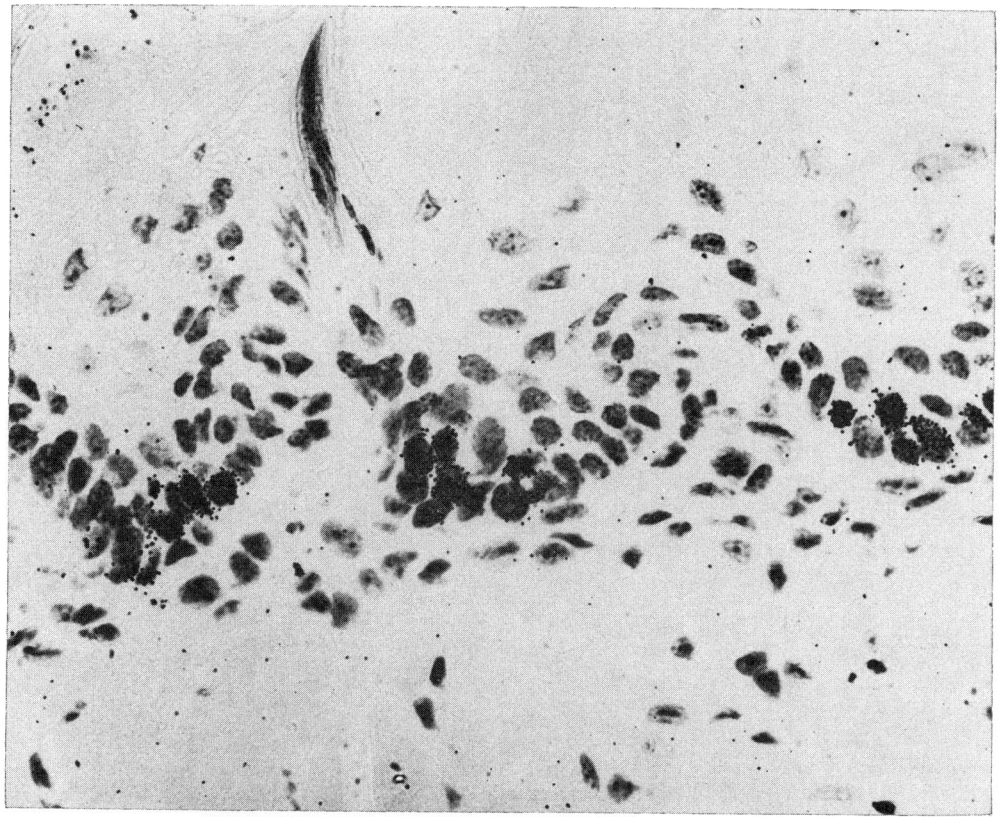

FIG 2.—Higher magnification of Figure 1 showing distribution of exposed emulsion grains over labeled basal cells that appear to have a nonrandom distribution in basal cell layer (original mag × 400).

TABLE 4

PROBABILITIES ASSOCIATED WITH CHI-SQUARE VALUES OF THE DISTRIBUTION OF LABELED BASAL CELLS AT VARYING TIMES AND AGES

Time (hr)	Mouse No.	Chi-Square Probability Values		
		4 Wk	8 Wk	12 Wk
400	1	< 0.275	< 0.012	< 0.028
	2	< 0.050	< 0.032	< 0.215
	3	< 0.010	< 0.003	< 0.175
800	4	< 0.338	< 0.001	< 2.206
	5	< 0.157	< 0.026	< 0.194
	6	< 0.338	< 0.094	< 0.062
1,200	7	< 0.251	< 0.113	< 0.036
	8	< 0.256	< 0.134	< 0.280
	9	< 0.313	< 0.106	< 0.025
1,600	10	< 0.300	< 0.017	< 0.020
	11	< 0.017	< 0.100	< 0.020
	12	< 0.375	< 0.360	< 0.030
2,000	13	< 0.750	< 0.090	< 0.030
	14	< 0.100	< 0.030	< 0.090
	15	< 0.300	< 0.530	< 0.150
2,400	16	< 0.120	< 0.325	< 0.750
	17	< 0.312	< 0.001	< 0.017
	18	< 0.300	< 0.001	< 0.163

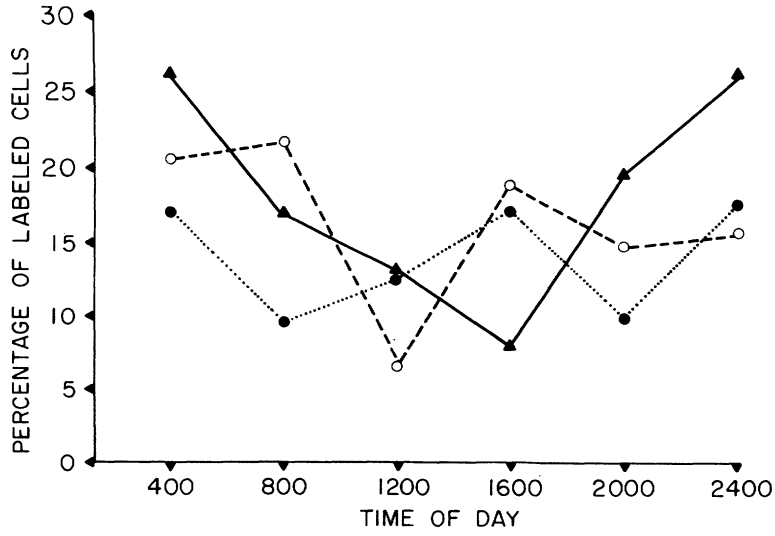

FIG 3.—Mean percentages of isotope-labeled basal cells found in each of three different age groups as compared to time of day sampling intervals. *Solid triangles joined by solid line,* 4-week-old mice; *open cicrcles joined by dashed line,* 8-week-old mice; *solid circles joined by dotted line,* 12-week-old mice.

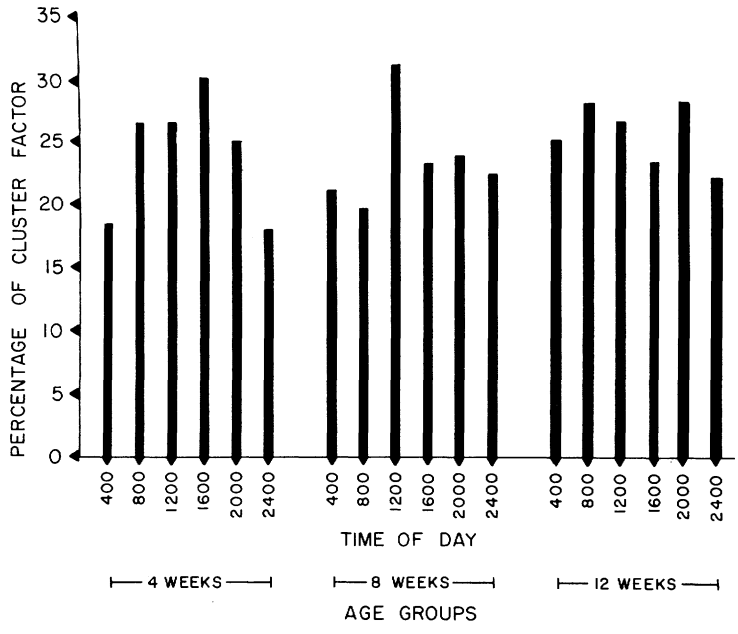

Fig. 4

FIG 4.—Histogram showing mean cluster factor percentages in each of three different age groups as compared to time of day sampling intervals.

TABLE 5

CLUSTER FACTOR OF LABELED BASAL CELLS AT DIFFERING TIMES IN 24 HOURS IN MICE, AGE 4 WEEKS

Time (hr)	Mouse No.	Cluster Factor	Mean	SD
400	1	0.152		
	2	0.207	0.183	± 0.028
	3	0.190		
800	4	0.343		
	5	0.226	0.264	± 0.069
	6	0.222		
1,200	7	0.310		
	8	0.261	0.264	± 0.044
	9	0.222		
1,600	10	0.306		
	11	0.334	0.299	± 0.039
	12	0.257		
2,000	13	0.158		
	14	0.253	0.252	± 0.094
	15	0.345		
2,400	16	0.169		
	17	0.206	0.179	± 0.024
	18	0.161		

TABLE 6

CLUSTER FACTOR OF LABELED BASAL CELLS AT DIFFERING TIMES IN 24 HOURS IN MICE, AGE 8 WEEKS

Time (hr)	Mouse No.	Cluster Factor	Mean	SD
400	1	0.226		
	2	0.215	0.211	± 0.017
	3	0.193		
800	4	0.179		
	5	0.195	0.196	± 0.018
	6	0.215		
1,200	7	0.323		
	8	0.295	0.309	± 0.020
	9	0.334		
1,600	10	0.193		
	11	0.285	0.229	± 0.049
	12	0.209		
2,000	13	0.223		
	14	0.273	0.237	± 0.031
	15	0.216		
2,400	16	0.265		
	17	0.239	0.225	± 0.048
	18	0.172		

TABLE 7

CLUSTER FACTOR OF LABELED BASAL CELLS AT DIFFERING TIMES IN 24 HOURS IN MICE, AGE 12 WEEKS

Time (hr)	Mouse No.	Cluster Factor	Mean	SD
400	1	0.227		
	2	0.276	0.250	± 0.025
	3	0.247		
800	4	0.294		
	5	0.270	0.282	± 0.012
	6	0.281		
1,200	7	0.258		
	8	0.273	0.266	± 0.011
	9	0.265		
1,600	10	0.226		
	11	0.217	0.233	± 0.022
	12	0.248		
2,000	13	0.292		
	14	0.251	0.283	± 0.029
	15	0.306		
2,400	16	0.238		
	17	0.241	0.221	± 0.033
	18	0.183		

monophasic pattern of labeling with a significant difference, the lowest value seen at 1,200 hours as contrasted to the peak value occurring at 800 hours. The 12-week-old group of mice displayed a biphasic pattern of isotope labeling. The lowest values of labeling occurred at 800 to 2,000 hours. These values are significantly different from the times of highest labeling index values occurring at 400, 1,600, and 2,400 hours. Of additional interest is the change in the ratio between high and low values in the differing age groups. Table 1 gives a 3.3:1 ratio between high and low values, and Table 2 gives a 3.4:1 ratio between high and low values, whereas Table 3 gives a ratio of mean values of 1.8:1 between the two high and two low values. The relationships between the values and comparison of the experimental groups can be seen in Figure 3. The curves generated from the values obtained from the 4-week-old and 8-week-old groups of mice are roughly similar in frequency and amplitude. However, the 12-week-old group has a curve that demonstrated double the frequency and half the amplitude of the two previous curves.

The data given in Tables 1, 2, and 3 and seen in Figure 3 clearly demonstrated that there in a diurnal variation in DNA synthesis activity in basal cells of mouse tongue epithelium. The fact that the 12-week-old group curve exhibits

TABLE 8

MEAN NUMBERS OF LABELED CELLS FROM ALL EXPERIMENTAL GROUPS

Age (wk)	Time (hr) 400	800	1,200	1,600	2,000	2,400	Row Means
4	125.3	81.7	62.3	40.7	93.3	124.0	87.9
8	98.0	102.7	29.5	69.7	72.7	74.0	74.4
12	82.0	45.7	58.5	80.5	46.7	89.3	67.1
Column means	101.8	76.7	50.1	63.6	70.9	95.8	76.5

TABLE 9

MEAN NUMBERS OF CLUSTERS FROM ALL EXPERIMENTAL GROUPS

Age (wk)	Time (hr) 400	800	1,200	1,600	2,000	2,400	Row Means
4	26.3	17.3	14.0	8.0	20.7	26.7	18.8
8	21.7	21.7	5.5	15.7	15.3	17.0	16.2
12	19.3	11.3	14.0	18.0	11.0	20.7	15.7
Column means	22.4	16.8	11.2	13.9	15.7	21.5	16.9

considerable change in frequency and amplitude needs interpretation. A somewhat similar type of study done by Goepp[3] with the same type of mice but of a mature age (16 weeks) demonstrated no significant diurnal difference in DNA synthesis activity between various times during a 24-hour period. Also, Goepp[3] found that 16-week-old mice had no significant diurnal difference in mortality from oral radiation death. However, he also found that 12-week-old mice did show a diurnal difference in such radiation sensitivity, and the degree of difference was increasingly greater in 8-week- and 4-week-old mice. The findings in this present study probably parallel the radiation sensitivity differences seen by Goepp. The 4-week- and 8-week-old groups of mice have a relatively high ratio of labeling index between peak and nadir and, thus, these groups should have a diurnal variation in radiation sensitivity since, as Whitmore[6] had shown, radiation sensitivity is related to the DNA cycle. The 12-week-old group of mice had a smaller ratio between peak and nadir. Thus, one could conclude that diurnal variation is greater in degree in younger

TABLE 10

ANALYSIS OF VARIANCE FOR LABELED CELLS

Source	S S	df	F
Time (T)	15,360.46	5	4.37*
Age (A)	3,772.16	2	2.68
T × A	14,953.84	10	2.13†
Error	23,164.87	33	
Total	57,251.33	50	

* $P < 0.05$.
† $P = 0.05$.

TABLE 11

ANALYSIS OF VARIANCE FOR CLUSTER FACTOR PERCENTAGE

Source	S S	df	F
Time (T)	771.13	5	6.34*
Age (A)	87.75	2	1.80
T × A	682.00	10	2.80†
Error	801.87	33	
Total	2,342.75	50	

* $P < 0.005$.
† $P < 0.005$.

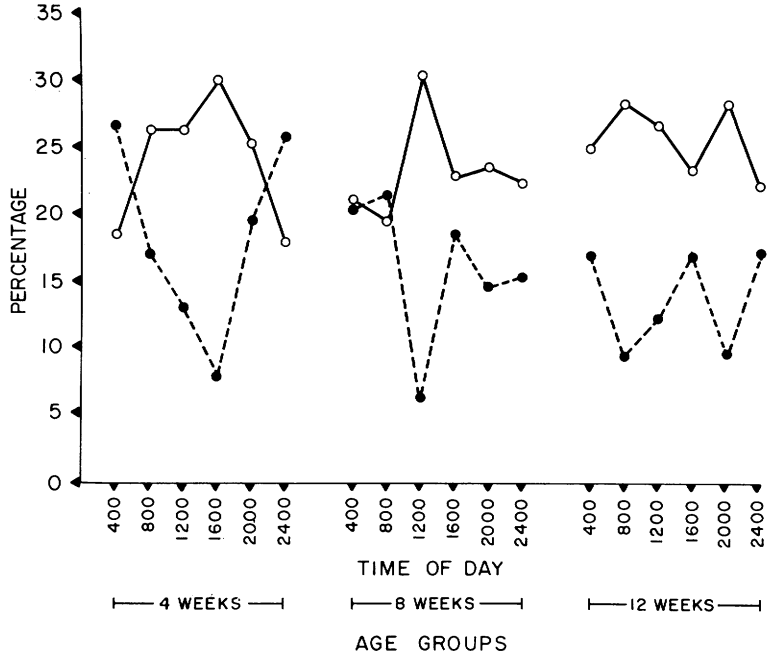

FIG 5.—Graph to contrast curves of means of percentage of labeled basal cells as opposed to means of cluster factor percentage in three different age groups of mice as compared to time of day sampling intervals. *Open circles joined by solid line*, cluster factor percentage; *solid circles joined by dashed line*, labeling index percentage.

mice, and that this variation tends to diminish with aging until maturity, at 16 weeks, when there is no diurnal variation in DNA synthesis. Although such a conclusion could be made, it should be noted that there may be intersample variations as a function of intervening growth spurts and plateaus. These could produce unrecorded fluctuations in an apparent quasilinear relationship between labeling index and age.

The chi-square analyses of the distributions of labeled basal cells (Table 4) provide evidence that generally there was a random distribution of labeled basal cells in the groups of mice and at most sampling times. Several groups did demonstrate a statistically significant nonrandom distribution of labeled basal cells. Glass and Goepp[20,21] reported nonrandom distributions of labeled basal cells in epithelium having relatively high labeling indexes and when this epithelium was replacing lost cell layers after irradiation injury. In the present study, both the 4-week-old and the 8-week-old group of mice had their peak labeling index time at 400 hours. These groups also demonstrated nonrandom distributions of labeled basal cells (Table 4). The 12-week-old group showed nonrandom distributions at 1,600 hours. This time was the same as one of the biphasic peaks of the labeling index curve of this group.

For the most part, these findings tend to confirm a suggestion made by Glass and Goepp[20,21] that a higher than normal labeling index seems to be directly related to nonrandom distributions of labeled basal calls..

The histogram in Figure 4 shows a marked diurnal variation in cluster factor percentage in the 4-week-old and 8-week-old groups. The 12-week-old group shows a small, biphasic diurnal variation. It should be noted that the determination of cluster factor results in lower values for samples that contain more labeled cells per cluster than higher cluster factor values that, conversely, contain fewer cells per cluster. Thus, the relatively low values of cluster factor are seen at 2,400 and 400 hours in the 4-week-old age group. As seen in Figure 5, the plot of the cluster factor curve is almost an identical reciprocal of the plot of the labeling index. Hence, when the labeling index values are high and the cluster factor values are low, there is a smaller number of clusters with relatively more labeled cells per cluster. After that time, the labeling index values drop and the cluster factor index rises, meaning that there are more clusters with smaller numbers of labeled cells per cluster. Comparing this finding with the chi-square analysis, it is apparent that the time of larger cluster size is also the time of highly significant nonrandom distribution of labeled basal cells. The finding of increasing cluster size is consistent with the characterization of growth of clusters as described by Glass and Goepp[21] in their continuous isotope labeling studies.

The definition of cluster, as previously given, allows for the identification of clusters in experimental groups whether or not the chi-square analyses showed random distributions in those groups. The definition of cluster takes into account the "doughnut effect" that must be considered in the light of previous continuous labeling experiments. However, the chi-square analysis is straight-line without consideration for the doughnut effect. The previous continuous labeling experiments of Glass and Goepp[21] revealed that, initially, clusters appear as small groups. The small clusters continue to add labeled cells at the edge of the cluster and, thus, grow to larger cluster size. However, in flash label experiments, such as this study, the isotope label is available for only about 20 minutes. Thus, if two small clusters are seen close to each other and have long nonlabeled spans of basal cells on either side of the two closely spaced clusters, the two probably represent one cluster in which there has been a "fallout" in the center because the center cells were in DNA synthesis just before labeling and, thus, are not labeled.

The results of the analysis of variance tests are given in Table 10. The resulting values demonstrate that the incidence of isotope labeling of basal cells is clearly time-dependent, and inspection of the Means in Table 8 demonstrates a strong and definite diurnal rhythm that ebbs at midday and peaks in the early morning hours. Incidence of these labeled cells as a function of the animal's age is not statistically significant, yet there seems to be a regular diminution with increasing age. Similarly, although again this effect does not attain statistical significance in this test (largely because of the relatively small numbers of mice sampled), the diurnal ebbs and peaks of incidence are most strongly noted in the younger mice and consistently diminish with age.

A similar analysis was done with data of the cluster factor values (Table 9). Results of the two-way analysis of variance are given in Table 11. Here again, a strong diurnal rhythm is clear, the ebbs and peaks of which are, as be-

fore, at midday and in the very early morning hours, respectively. Also as before, this rhythm is most sharply delineated in the youngest mice and seems to become progressively less distinct with age; although age by itself is not statistically significant, the interaction of age with a sampling time achieves the levels of statistical significance.

Conclusions

The purpose of this study was to determine if there was a diurnal rhythm in the DNA synthesis pattern of basal cells in the squamous epithelium of the dorsum of mouse tongue in the time between youth, just weaned, and maturity. In addition, it was proposed to determine if DNA synthesis occurred in spatially related groups of basal cells as had been reported previously in mouse tongue epithelium after injury.

It was found that there are strong diurnal rhythms in the pattern of DNA synthesis in epithelial basal cells. Furthermore, there was considerable evidence that the degree of this diurnal rhythm tended to decrease with the older age group of mice. It was also found that at the time of greatest DNA synthesis activity, basal cells in DNA synthesis tended to be found in nonrandom distribution in the basal cell layer, that is, they were found in clusters.

It is apparent, then, that studies of epithelial population kinetics in growing animals, in tissues recovering from injury, and perhaps in neoplastic tissues should take into account a possible diurnal rhythm of DNA synthesis. Furthermore, the results of this study and previous ones suggest that the occurrence of clusters is directly related to periods of growth or recovery from injury to epithelium. In addition, the occurrence of diurnal variation in DNA synthesis patterns, the degree of which is apparently related to animal age, may help to provide a reason for the previous conflicting claims as to the existence of diurnal variation in radiation sensitivity in experimental animal groups.

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