TABLE III. Specific Activities of Dose-Incorporation Regression Lines at 0 Dose of ^{32}P.

Fraction	Time after ^{32}P injection (hr)	Specific activity at 0 dose[a]	p
	Lymph nodes		
DNA	4	1.86	>.80
	48	—6.55	>.40
RNA	4	6.4	>.80
	48	—6.7	>.50
ASP	4	10.8	>.90
	48	—10.8	>.40
	Thymus		
DNA	4	—20.4	>.20
	48	17.9	>.50
RNA	4	—23.6	>.10
	48	13.2	>.70
ASP	4	—8.4	>.80
	48	3.6	>.95

[a] Specific activity at 0 dose of ^{32}P dose-incorporation regression line calculated for doses 0.191–1.91 μCi/g; p is the probability that this specific activity does not differ significantly from 0. If there were no radiation effect of ^{32}P upon its incorporation, the specific activity of the regression line at 0 dose should be 0.

1.91 μCi/g of body weight did not significantly modify the rate of DNA or RNA synthesis in the lymphoid tissues of rats. There was, however, a highly significant depression of DNA synthesis in the thymus, but not in the lymph nodes 48 hr after the intraperitoneal injection of 9.5 μCi of ^{32}P/g of body weight. It is concluded that doses of ^{32}P up to 1.9 μCi/g of body weight may be used to study nucleic acid synthesis for time intervals up to 48 hr in the lymphoid tissues of rats.

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The Effect of Cortisone on Mitotic Activity in the Rat Incisor* (33458)

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It is now well known that cortisone stimulates the rate of eruption of the continuously erupting rat incisor. The administration of this steroid has been found to result in an accelerated rate of eruption of the incisor in adults (4) and in a precocious development and eruption in fetal (1, 2) and in newborn

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rats (3–5). Hypophysectomy and adrenalectomy cause a marked reduction in the incisor eruption rate, while the subsequent injection of cortisone greatly accelerates this rate (4, 6). However, notwithstanding the fact that the stimulatory effect of cortisone has been clearly demonstrated, there is no consensus with respect to the factor or factors underlying the eruptive process.

Addison and Appleton (7) were among the first to propose the theory that cell proliferation is responsible for tooth eruption and several other investigators (8–11) have since reported evidence interpreted as supporting this concept. However, despite this fact the theory has not received general acceptance. The purpose of the present study was to determine the effect of cortisone on the rate of cell division in the tissues of the rat maxillary incisor as a possible cause of the accelerated eruption observed in such animals. We are not aware of any other investigation relating cortisone and mitotic activity in dental tissues.

Materials and Methods. Female, albino rats of the Sprague-Dawley strain (age 30 days) were permitted to acclimate to the animal quarters for a period of 7 days. During the experiments they were kept in constant light from 7 a.m. to 7 p.m. and in complete darkness from 7 p.m. to 7 a.m. All injections were subcutaneous. Body weights were recorded prior to all injections and just prior to sacrifice.

In the first of two experiments, 16 treated and 16 control rats were employed. Beginning at 7 a.m., 1 p.m., 7 p.m., and 1 a.m. four animals for each period were given a single injection of 0.5 mg of cortisone.[2] Controls received an equal volume of normal saline. At 6 hr after this injection, all animals received a single injection of colchicine[3] (1 mg/kg). They were sacrificed exactly 3 hr later.

The second experiment was conducted in the same manner, except that the dosage of cortisone administered was doubled (1 mg) and 14 instead of 16 controls were used. Three instead of 4 controls were employed at the 7 p.m. and 1 a.m. periods.

Immediately following sacrifice skulls were split midsagitally and maxillary incisors were recovered and fixed for 48 hr in a buffered neutral 10% formalin solution. The incisors were then decalcified in a sodium citrate–formic acid solution, washed in tap water for several hours, dehydrated, imbedded in paraffin *in vacuo*, sagitally sectioned at 5 μ and stained with Harris hematoxylin and eosin.

Mitotic cell counts were made on four cell layers of the labial (basal or proliferative) loop of the incisor. These layers[4] were the stratum intermedium, the ameloblast, the odontoblast and the adjacent pulp. Successive circumscribed fields were counted beginning at the base of the labial loop and continuing to the point where cell division ceases, i.e., at the beginning of predentin formation. The microscopic field was defined by means of an 8 $\times$ 8-mm square, etched onto an ocular micrometer disc, inserted into the ocular (10 $\times$) of the microscope. The area of each oil immersion field was 6400 μ^2. Special care was taken so that overlapping areas were not counted more than once.

Counts were made using a Leitz binocular microscope with an oil immersion lens (100$\times$). At least 4 sagittal sections were counted for each tooth and only every fourth section was counted in order to avoid duplication. The average number of mitotic cells per tissue section was then calculated for each cell layer of each incisor. The data were statistically analyzed employing the t test or the F test (12). Differences were considered significant at the 5% level of probability.

Determination of the presence or absence of a cyclic cell proliferation in each of the cell layers for both treated and control groups was made by means of the Analysis of Variance Test (F test). When a significant variance ratio was established between the means of the four periods of any cell layer,

[2] Cortisone (Cortone acetate) generously supplied by Sharp and Dohme, Division of Merck and Company, Inc., Philadelphia, Pa.

[3] Colchicine alkaloid USP purchased from Fisher Scientific Co., New York, N.Y.

[4] Actually the "ameloblasts and odontoblasts" are preameloblasts and preodontoblasts in this area of the incisor, however, for the sake of brevity we will not include the prefix.

TABLE I. Average Number of Mitoses per Tissue Section in the Incisor of Rats Treated with 0.5 mg of Cortisone and of Controls.[a]

Time colch. adm.	No. of rats[b]	No. obs	Stratum intermedium	Ameloblasts	Odontoblasts	Pulp
7 a.m.	4T	28	19.68 ± 4.65[c]	41.32 ± 6.43[d]	9.46 ± 3.00[c]	16.43 ± 4.11[c]
	4C	32	16.72 ± 4.41	37.50 ± 4.72	7.91 ± 2.65	13.94 ± 4.05
1 p.m.	4T	28	18.14 ± 4.36	34.21 ± 8.23	9.18 ± 3.77[c]	16.57 ± 6.04[c]
	4C	28	19.00 ± 4.34	37.86 ± 9.30	7.04 ± 3.51	20.46 ± 6.02
7 p.m.	4T	32	26.63 ± 4.94[d]	46.13 ± 7.74	11.34 ± 4.44[c]	21.56 ± 5.73
	4C	32	23.31 ± 4.99	46.56 ± 7.53	9.16 ± 3.05	22.72 ± 8.74
1 a.m.	4T	28	24.00 ± 5.55[c]	43.29 ± 5.64	9.21 ± 2.92	20.21 ± 6.08
	4C	24	20.04 ± 5.92	40.50 ± 7.66	10.25 ± 3.02	17.79 ± 4.35

[a] Mean values per tissue section ± SD.

[b] T = treated; C = control.

[c] $p<0.05$; [d] $p<0.01$; [e] $p<0.001$ = differences between the means of cortisone treated and control rats.

the pooled sum of the squares of the two periods having more mitotic figures were tested against the pooled sum of the squares of the other two periods to determine if a significant variance of means existed between these two groups.

Results. At 7 a.m. the incisors of rats receiving 0.5 mg of cortisone revealed a significant increase in the number of colchicine arrested cells in each of the four cell layers studied (ameloblast, $p<0.01$; other cell layers, $p<0.05$). However, at 1 p.m. treated rats revealed less cell division in the stratum intermedium and ameloblast layers ($p>0.10$), and in the pulp ($p<0.05$). The reverse was true for the odontoblast layer ($p<0.05$). The treated animals of the 7 p.m. period revealed significantly more mitoses in the stratum intermedium and the odontoblast layers while virtually no difference was noted in the ameloblast layer and the pulp. At 1 a.m., all layers of treated animals disclosed an increase with the exception of the odontoblast layer. However, only the stratum intermedium showed a statistically significant ($p<0.05$) difference (Table I).

In the analysis of cyclic cell proliferation, inspection of the means showed more mitotic figures at 7 p.m. and 1 a.m. In the cortisone treated rats, all layers revealed a significant ($p<0.001$) difference between the means of the four periods with the exception of the odontoblast layer. This finding was interpreted as indicating that a rhythmicity was present in these layers. Further testing of the higher combined averages of the 7 p.m. and 1 a.m. periods, against the 7 a.m. and 1 p.m. periods revealed a significant difference ($p<0.001$) for the three layers (stratum intermedium, ameloblast, and pulp) indicating significantly more cell division during the former two than during the latter two periods.

All layers of the control rats revealed a significant ($p<0.001$) variance between the means of the four periods. Furthermore, when the data were pooled, a significant difference ($p<0.01$ pulp; $p<0.001$ other layers) was noted. Thus, the circadian rhythm in the controls appears to be of the same nature as that established for the treated rats.

In the second experiment, where rats had received 1 mg of cortisone, the incisors showed an increase at 7 a.m. in all layers except the pulp with the stratum intermedium ($p<0.01$) and ameloblast ($p<0.001$) disclosing a significant difference. However, at 1 p.m. the controls revealed more mitoses in all layers (stratum intermedium, $p<0.01$; pulp, $p<0.001$; ameloblast, $p>0.05$; odontoblast, $p>0.10$). The 7 p.m. period revealed higher averages in all layers studied in the cortisone treated rats. In the ameloblast layer and the pulp, this difference was significant

TABLE II. Average Number of Mitoses per Tissue Section in the Incisor of Rats Treated with 1 mg of Cortisone and of Controls.[a]

Time colch. adm.	No. of rats[b]	No. obs	Stratum intermedium	Ameloblasts	Odontoblasts	Pulp
7 a.m.	4T	24	24.08 ± 6.50[d]	43.58 ± 6.86[e]	9.71 ± 3.58	19.38 ± 7.03
	3C	24	19.29 ± 3.99	36.04 ± 5.31	8.04 ± 3.25	20.33 ± 6.90
1 p.m.	3T	24	20.25 ± 4.69[d]	40.80 ± 6.12	10.25 ± 3.35	14.80 ± 4.32[e]
	4C	28	24.96 ± 7.10	44.86 ± 8.41	11.04 ± 3.79	21.64 ± 3.83
7 p.m.	3T	24	23.88 ± 5.03	43.54 ± 6.91[d]	10.17 ± 3.89	23.33 ± 7.47[e]
	4C	32	21.78 ± 5.50	36.75 ±12.25	9.78 ± 3.42	19.47 ± 5.31
1 a.m.	4T	27	22.82 ± 5.10[d]	41.07 ± 4.59[e]	10.82 ± 3.24	22.41 ± 5.57
	3C	24	26.46 ± 4.45	45.38 ± 4.77	10.13 ± 3.70	20.67 ± 9.01

[a] Mean values per tissue section ± SD.

[b] T = treated; C = control.

[c] $p<0.05$; [d] $p<0.01$; [e] $p<0.001$ = differences between the means of cortisone treated and control rats.

but not so in the stratum intermedium and odontoblast layers. At 1 a.m. the experimental animals revealed more mitoses in the odontoblast layer and the pulp, but the reverse was true for the stratum intermedium ($p<0.01$) and ameloblast ($p<0.001$) layers (Table II).

In this experiment, inspection of the means showed that treated animals generally had more mitoses at 7 a.m. and 7 p.m. and controls more at 1 p.m. and 1 a.m. The treated rats revealed a significant ($p<0.05$) difference between the averages of the combined periods of the stratum intermedium and ameloblast layers. No significance could be established for the odontoblast layer or pulp.

When the pooled periods of controls were statistically compared, the stratum intermedium, ameloblast ($p<0.001$) and odontoblast ($p<0.05$) layers differed in their averages while the pulp ($p>0.10$) did not. The controls, therefore, showed a rather pronounced rhythmicity which differed from the less pronounced cyclic activity of the treated rats.

In addition to the tissues studies, colchicine arrested cells were found in the stellate reticulum, outer enamel epithelium, periodontal ligament, labial alveolar periosteum and in the walls of blood vessels.

Discussion. The stimulation of mitotic activity, observed following the administration of cortisone, would seem to indicate that this may be one of the factors involved in the mechanism by which this steroid accelerates the rate of eruption of the rat incisor. If this is true, then the results of these experiments lend support to the theory that cell proliferation is one of the causative agents in tooth eruption. Although we are not aware of any previous investigations relating cortisone and cell division in the incisor, a number of studies have dealt with the long-term effect of this hormone on related oral tissues. Goldsmith and Ross (2) observed an increase in the growth of alveolar bone and a disorganization in the arrangement of the fibers of the periodontal ligament following daily administration of cortisone in postnatal rats. However, resorption of premaxillary bone and inhibition of new bone formation has been reported in rabbits following a series of cortisone injections (13) while a decrease in the growth of mandibles of young rats maintained on a diet to which cortisone acetate had been added has also been observed (14). Short-term effects on mitotic activity of nonoral tissues have been reported. A decrease in cell division in the ear epidermis of mice at 5 hr (15) and at 2 hr (16) was observed following a subcutaneous injection of cortisone. A similar effect was reported in the bone marrow of hamsters at 4 hr (17).

Since studies on the effect of cortisone on the proliferative activity of a variety of tis-

sues have revealed stimulation, inhibition or no effect at all (18), one is lead to conclude that the tissues of the body vary widely in their reaction to cortisone. The present study has shown that the proliferating tissues of the incisor tend to react in a positive manner to this adrenal steroid. Furthermore, our study shows that this response to cortisone is cyclic in nature since we found no increase in proliferative activity at 1 p.m.

Mitotic periodicity has been reported for a wide variety of tissues, however, we know of no previous study with respect to this phenomenon in the incisor. A number of investigators have reported the occurrence of cyclic cell division in other oral tissues. Halberg *et al.* (19) observed a mitotic periodicity in the retromolar epithelium and in the periodontal ligament of rats. Rhythmicity has also been reported in the oral epithelium (20) and in the epithelium of the hard palate, cheek, and the gingiva of the rat (21).

The rhythmicity observed in our study was not of the same nature when the two experiments were compared. The higher dosage of cortisone (1 mg) may have altered and minimized the cyclic activity in relation to the first experiment. The rhythmicity of the controls was observed to differ in the two experiments. Possibly a more stringent standardization of the animal quarters environment would correct this condition. However, within each experiment the rhythmicity of the individual cell layers shows a steady consistence for both experimental and control animals.

Summary. The administration of cortisone in young rats resulted in an increase in the number of colchicine arrested cells in the basal (proliferative) loop of the maxillary incisor during certain periods of the day. A single injection of 0.5 or 1 mg in normal rats resulted in an increase in colchicine arrested cells at 7 a.m., 7 p.m., and to some extent at 1 a.m. with no effect at 1 p.m. A cyclic cell division was evident in both experimental and control animals. In rats receiving 0.5 mg more mitoses were observed at 7 p.m. and 1 a.m. Controls revealed the same rhythmicity. Animals injected with 1 mg showed a less pronounced circadian rhythm with more mitotic figures at 7 a.m. and 7 p.m. The controls showed significantly more activity at 1 p.m. and 1 a.m. It is concluded that these results provide additional evidence in support of the theory that cell proliferation in the rat is one of the causative factors in incisor eruption.

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