it is probably desirable to take an electrocardiogram if giving high doses. A few patients reported throbbing headache at the height of the blood pressure rise. The cardiovascular effects of tyramine are similar to those reported for norepinephrine(8), which also causes increase in blood pressure and TPR, but inconsistent changes in CO and pulse rate. The ECG changes following tyramine are also similar to those reported with norepinephrine by Miller *et al.*(9).

The use of the blood pressure response to a small dose of tyramine as a test of the adequacy of tissue catecholamine stores is complicated by the considerable variability between patients. The response would have to be quite small to differ significantly from the normal group. Since at a dose of 0.4 mg/kg the response is more marked and still on the linear part of the dose-response curve but with a similar variation, the response at this dosage would probably provide a more sensitive indication of reactivity than that at the smaller dose. Determining a dose-response curve should be more informative than administration of any single dose. The peak rise in systolic and mean pressures are equally satisfactory statistics, and both are superior to the area of the mean pressure curve.

The variation from time to time in the same patient is much smaller than that between patients. Thus repeated testing of a patient should be more satisfactory in detecting change in reactivity than a single test would be in classifying a patient as normal or deficient. Tyramine has been used in this way in man to display the effect of reserpine on the tissue norepinephrine stores(4).

Summary. 1. Intravenous injection of tyramine in man causes elevation of blood pressure and TPR, but inconsistent effects on CO and pulse rate. Changes in the site of impulse formation and conduction were seen in the ECG. 2. The reproducibility of the tyramine response was studied in 18 subjects to assess its value as a test of tissue catecholamine stores. 3. The dose-response relationship of the pressor effect of tyramine was determined.

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Effect of Adrenalectomy on Mitotic Proliferation of Gastric Epithelium.* (27777)

RAY H. CLARK† AND BURTON L. BAKER
Department of Anatomy, University of Michigan Medical School, Ann Arbor

Mitotic activity in the gastric epithelium of rats occurs almost exclusively in 2 cell types, the non-differentiated forms of surface mucous cells and the mucous neck cells. We have found that mitotic activity in both cell types follows a circadian periodicity. The peak of activity occurs at about 10 a.m. and the low point between 8 p.m. and 12 midnight (to be published). The objectives of this investigation were to determine (a) the effect of adrenalectomy on mitotic activity, (b) the relationship of possible changes to circadian periodicity, and (c) how the influence of adrenalectomy is modified by standardization

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of lighting and other disturbances to which the rats are subjected. Rasanen(1) reported that adrenalectomy increases mitotic activity in the gastric mucosa but considered neither the presence of a circadian periodicity nor the possible complication of non-standardized environmental factors.

Materials and methods. Two experiments were carried out to observe the influence of adrenalectomy, the first dealing with rats kept under standardized conditions and the second with rats kept under the usual laboratory conditions.

Experiment I. Young, adult, female Sprague-Dawley rats weighing 180-190 g were arranged in pairs, one member of each pair being adrenalectomized and the other sham-operated. For one week before operation and until termination of the experiment, the rats were kept under standardized conditions which consisted of exposure to light from 6 a.m. to 6 p.m., isolation in individual cages away from other animals and human beings except at one feeding time per day, and maintenance of room temperature at approximately 73°F. The rats were fed Purina Laboratory Chow *ad libitum* and the adrenalectomized rats were given 0.9% NaCl for drinking fluid. Seven days after the operation each group of 5-10 pairs of adrenalectomized and sham-adrenalectomized rats were killed at 2-hour intervals during the day and night, each group having been undisturbed for at least 48 hours prior to time of sacrifice. The terminal mean body weight was 191 ± standard deviation 11 g for the sham-operated controls and 204 ± 13 g for the adrenalectomized animals.

Experiment II was carried out similarly to Experiment I except that the rats were kept in a large animal room with no control of lighting conditions or other disturbances. Control rats were pair-fed against adrenalectomized rats and 7 days following operation all animals were starved 12 hours prior to termination of the experiment. The mean terminal body weight was 193 ± 6.9 g for controls and 193 ± 8.6 g for adrenalectomized rats.

At termination of both experiments each animal was anesthetized with ether, weighed, and the stomach filled with 4.5 ml of Zenker-formol fixative within 2 minutes after removal of each animal from its cage. Six hours later a strip of the gastric wall extending from the transverse ridge to the pyloroduodenal junction was excised along the greater curvature. It was placed in Zenker-base for 18 hours, then postchromated in 3% $K_2Cr_2O_7$ for 2-3 days. The tissue was embedded in paraffin, sectioned at 5 μ, and stained with a combination of periodic acid-leucofuchsin (PAS) and Bensley's copper chrome hematoxylin. This procedure made possible a differentiation of the surface mucous cells and the mucous neck cells on the basis of the morphology of their contained mucus when they were in division. The mucus appeared clumped, granular, and densely stained with PAS in the surface cells but in the mucous neck cells it was foamy in appearance and less intensely stained. Mitotic figures and interphase nuclei in the 2 cell types were counted at 0.5-mm intervals in areas 0.02 mm^2 beginning at the transverse ridge and continuing to the beginning of the pyloric glands. Thirty-five to 40 fields were counted per stomach. In the sham-operated rats a mean of 2415 surface cells and 1633 mucous neck cells were counted per animal. In the adrenalectomized rats a mean of 2448 surface cells and 1581 mucous neck cells were counted per animal. The mitotic index was expressed as number of mitoses per 1000 cells. The probability of error was determined with the Student t formula except when the variance ratio was significantly high, in which case the Welch formula(2) was used.

Results. Circadian periodicity. When the data obtained from rats kept under standardized conditions (Exp. I) were grouped according to operative treatment without regard to the specific time of day when the tissues were taken, both the surface and mucous neck cells exhibited a higher mitotic index during the day than night in both control and adrenalectomized rats (Table I). The mitotic indexes were approximately twice as high during the day, all differences being significant at less than the 0.1% level. In the controls the mitotic index of the *surface cells* (Table II), reached a peak of 10.8 ± 2.0 at

TABLE I. Summation of Data on the Influence of Adrenalectomy on Mitotic Index under Standardized Conditions (Experiment I).

	No. of rats		Surface cells			Mucous neck cells		
Period	Control	Adx*	Control	Adx	P†	Control	Adx	P
Day‡	35	33	7.2 ± .5§	11.0 ± .7	<.001	5.7 ± .6	6.9 ± .5	NS‖
Night	40	36	3.5 ± .3	5.0 ± .4	<.01	3.0 ± .3	4.2 ± .4	<.05
		P¶	<.001	<.001		<.001	<.001	

* Adx = adrenalectomy.
† Comparison between values for control and adrenalectomized rats.
‡ Day = 6 A.M. to 4 P.M.; night = 6 P.M. to 4 A.M.
§ Stand. error of mean.
‖ NS = not significant at 5% level.
¶ Comparison between values for day and night.

10 a.m. after which it declined and reached its lowest level of 2.8 ± 0.8 at 12 midnight. The difference between these means was significant at less than the 0.1% level. The initiation of the subsequent daily rise was evident at 2 a.m. The diurnal rhythm was present after adrenalectomy, the peak of 15.3 ± 1.8 being reached somewhat later at 12 noon, with the low of 3.8 ± 0.5 occurring also at 12 midnight.

The highest mitotic index of 9.5 ± 2.3 for the *mucous neck cells* (Table II) occurred at 10 a.m. in the control animals, followed by a decrease in the index to a low of 1.4 ± 0.3 at 10 p.m. The difference between these means was significant at less than the 0.1% level of confidence. After 10 p.m. the index began to rise again. An increase occurred at 4 p.m. which was not, however, significantly above the index at 2 p.m. or 6 p.m. Following adrenalectomy the highest index for mucous neck cells occurred at 8 and 10 a.m. but the subsequent rate of decline was slower. Thus, adrenalectomy had little influence on the rhythmic mitotic proliferation of gastric epithelial cells.

Influence of adrenalectomy. Grouping of all of the data into values obtained from night or day without regard to specific time (Table I) showed that adrenalectomy increased the mitotic index for surface cells both during night and day. Although the mean index for mucous neck cells was higher in adrenalectomized than non-adrenalectomized rats during both night and day, only the difference for night was significant.

With respect to specific times of day, the mitotic index for surface cells at 12 noon and 2 p.m. was significantly higher than in the non-adrenalectomized controls (Table II). Also, at 10 p.m. a significant difference was obtained. Although at all other times but

TABLE II. Influence of Adrenalectomy on the Mean Mitotic Index under Standard Conditions (Experiment I).

	No. of rats		Surface cells			Mucous neck cells		
Time	Control	Adx*	Control	Adx	P†	Control	Adx	P
6 A.M.	5	4	5.9 ± 1.2‡	8.2 ± .2	NS§	5.6 ± 1.3	5.1 ± 1.0	NS
8 A.M.	5	5	8.6 ± .5	11.0 ± 1.0	"	7.0 ± 1.1	8.4 ± 1.4	"
10 A.M.	5	5	10.8 ± 2.0	9.8 ± 1.3	"	9.5 ± 2.3	8.4 ± .6	"
12 N.	6	6	8.2 ± .6	15.3 ± 1.8	<.01	5.8 ± 1.2	7.2 ± .4	"
2 P.M.	10	9	4.8 ± .4	8.6 ± 1.1	<.01	3.3 ± .6	6.5 ± .9	<.05
4 P.M.	4	4	7.3 ± .9	12.9 ± 3.3	NS	5.6 ± .6	5.2 ± 1.4	NS
6 P.M.	8	5	3.5 ± .4	4.7 ± 1.0	"	3.2 ± .6	4.2 ± .9	"
8 P.M.	5	7	3.3 ± .6	5.1 ± .8	"	3.6 ± 1.0	5.7 ± .9	"
10 P.M.	6	4	3.6 ± 1.0	7.6 ± .5	<.05	1.4 ± .3	2.8 ± .9	"
12 M.	6	7	2.8 ± .8	3.8 ± .5	NS	2.3 ± .7	2.9 ± .5	"
2 A.M.	9	8	3.0 ± .4	4.4 ± .8	"	3.4 ± .4	4.6 ± .7	"
4 A.M.	6	5	5.4 ± 1.1	5.4 ± .7	"	3.8 ± .8	4.3 ± .5	"

* Adx = adrenalectomy.
† Comparison between values for control and adrenalectomized rats.
‡ Stand. error of mean.
§ Difference between control and adrenalectomized animals not significant at 5% level.

TABLE III. Influence of Adrenalectomy on Mean Mitotic Index of Gastric Mucosa under Non-standardized Conditions (Experiment II).

	No. of rats		Surface cells			Mucous neck cells		
Time	Control	Adx*	Control	Adx	P†	Control	Adx	P
2 P.M.	9	9	3.3 ± 1.0‡	13.1 ± 2.5	<.01	.9 ± .4	3.9 ± .9	<.01
6 P.M.	8	11	5.2 ± 1.4	6.8 ± 1.1	NS§	3.5 ± 1.2	4.4 ± .6	NS

* Adx = adrenalectomy.
† Comparison between values for control and adrenalectomized rats.
‡ Stand. error of mean.
§ Difference between control and adrenalectomized animals not significant at 5% level.

10 a.m. a higher index was obtained for adrenalectomized rats, the differences were not significant at the 5% level. When the values from 4 a.m. to 10 a.m. were considered as a group, little difference was found, the mitotic index for the controls being 7.4 ± 0.5 as compared with 8.6 ± 0.7 following adrenalectomy. With respect to mucous neck cells, although the mean index was usually higher in adrenalectomized rats than in their controls, only at 2 p.m. was this difference significant at less than the 5% level of confidence. During the period from 4 a.m. to 10 a.m. the mitotic index for controls was 6.4 ± 0.8 and for adrenalectomized rats 6.6 ± 0.6. Thus, the greatest stimulating influence of adrenalectomy on surface and mucous neck cells appeared to occur shortly after the normal peak of proliferative activity.

Experiment II. The environment affects the mitotic index since the values obtained for control rats under non-standardized conditions at 2 p.m. were lower for both cell types (Table III) than those obtained under standardized conditions (Table I). Under non-standardized conditions the mitotic rate following adrenalectomy was increased at 2 p.m. over that of the controls in both the surface and mucous neck cells (Table III). This result was similar to that obtained at 2 p.m. under standardized conditions. At 6 p.m. no significant change occurred following adrenalectomy, which was also similar to the result obtained under standardized conditions.

Discussion. The data presented emphasize the importance of considering the diurnal rhythm in evaluating the influence of hormones on mitotic activity. An experiment in which the animals are killed at one time of day may give misleading results. Thus, in our experiments killing of the rats at any time of day but from 12 noon to 2 p.m. would have shown adrenalectomy to be without effect on cell proliferation whereas at 2 p.m. a great increase in activity was observed. Although grouping of the data obtained from various times of day and night revealed the over-all accelerating influence of adrenalectomy, this would not have occurred if all rats had been killed in late afternoon or evening. Rasanen (1) was probably fortunate in having killed his animals at a time of day when the state of the diurnal rhythm was propitious for revealing the accelerating influence of adrenalectomy.

The mitotic activity of surface and mucous neck cells was not comparably affected by adrenalectomy. Considering individual times of day both types of cells were affected most during the afternoon hours, *i.e.*, when the mitotic index for control rats was decreasing from its morning peak. Two p.m. was the only time of day when the mitotic index for mucous neck cells increased significantly, while the mitotic index for surface cells was significantly higher at 12 noon, 2 p.m. and 10 p.m. Considering mean mitotic indexes for the 12-hour daylight period and 12-hour night period, adrenalectomy increased mitotic activity in the surface cells during both periods, while mitotic activity in the mucous neck cells was increased only during the night with little change occurring during the day. Thus, the surface cells were considerably more responsive to adrenalectomy than were the mucous neck cells.

Although the general physiologic response to adrenalectomy is one of growth inhibition (3), it is remarkable that certain tissues of the body respond by accelerated growth. Among these tissues in the rat are hair(4,5),

epiphyseal cartilage(6), epidermis(1,7), forestomach epithelium(8) and possibly lymphoid tissue(9). In contrast, elevation of the glucocorticoid concentration in tissues either by systemic or local administration leads to growth inhibition which is made manifest by the entire body as well as by the tissues which normally proliferate rapidly. These include hair(5), epiphyseal cartilage, lymphoid tissues and proliferating connective tissue(10). Decreased mitotic rates have been demonstrated quantitatively in the epidermis of mice(7,11,12) and rats(13). Thus, with respect to certain tissues, glucocorticoids appear to act as growth suppressing agents at homeostatic levels. Gastric epithelial cells may be classed with these responsive tissues.

This concept is supported further by the relationship of the rhythm in gastric mitotic activity to that in corticosterone blood levels. These levels in the rat are higher at 2-3 and 9-10 p.m. than at 2-3 and 9-10 a.m.(14). Likewise, in the mouse the highest level occurs at about 4 p.m. and the lowest in early morning(15). Thus, during the morning hours when the corticosterone level is lowest, the mitotic indexes are most nearly identical in control and adrenalectomized animals. This relationship indicates that during the morning corticosterone in the normal rat has little influence on the mitotic activity of gastric epithelium. On the other hand, when the corticosterone level is high, especially during the afternoon and evening, mitotic activity appears depressed in the control animals as compared to the adrenalectomized animals.

Evidence is conflicting regarding the essential role of the adrenal gland in maintaining circadian rhythms. It is reported to be necessary for maintenance of periodicity in the level of eosinophils in the blood, of phospholipid in the liver, and of mitotic activity in the mouse epidermis(16). On the other hand, periodicity in mitotic activity in the epidermis, in epithelium of the cornea, tongue and esophagus, and in the thymus of the rat is independent of adrenal control(17). The gastric epithelium is to be grouped with the latter tissues, since the periodicity of its mitotic activity is not dependent on the adrenal gland.

Summary. Circadian periodicity in the mitotic activity of the surface and mucous neck cells of the gastric mucosa of rats persisted following adrenalectomy. Also, adrenalectomy caused a significant increase in the mitotic index of surface cells during both the day and night, while the mitotic index of mucous neck cells was increased only during the night. The most significant elevation in proliferative activity occurred during the afternoon, the least during the morning.

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