orotic acid, independent of the diet, is now available for the study of fatty liver induction. The results suggest that the intestine and its contents do not play a necessary role in causing orotic acid induced fatty liver.

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Effect of Adrenalectomy and of Dexamethasone upon Circadian Distribution of Mitosis in the Cornea of Rats—I.* (32329)

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Corticosteroid participation in the general control mechanisms responsible for the distinct circadian mitotic and metabolic fluctuations, which occur in mammalians, has been established. Thus, potassium excretion by the kidneys and neutrophil and eosinophil levels in the blood have distinct diurnal changes which are related either to mineral or glucocorticoid activity(1-7).

Mitotic activity and its distinct circadian variations, with highest levels in the late morning and lowest levels at night, have been established in rats and mice, and are related to adrenal function(7-10).

RNA and enzyme synthesis can be stimulated by administration of glucosteroids(11-14), while DNA synthesis and mitosis, in general, have been reported to be inhibited by these hormones(14-17). Whether this inhibitory effect could in any way be related to the low levels of mitosis which are found in tissues of mammals, coinciding in time with periods of rising concentrations of circulating glucocosteroids(18,19), has not been established.

The well documented mitotic stimulation found in the corneal epithelium of rats, following partial hepatectomy, is apparently related to adrenal gland function, as judged by both its absence in adrenalectomized animals and by its reestablishment in adrenalectomized animals treated with dexamethasone (20,21).

Evidence has recently been found that glucosteroids have a stimulatory effect upon mitosis in the corneal epithelium of rats. Thus, the administration of dexamethasone, a potent glucosteroid, to adrenalectomized animals resulted in the appearance of a significantly greater number of cells undergoing

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mitosis in the cornea of rats, in the morning period(20,21), as compared to their respective controls, whereas, neither estrogens nor testosterone were active in this regard.

The apparent dependence of post-partial hepatectomy mitotic stimulation upon adrenal function and the clear effect which dexamethasone exerted upon mitosis in the cornea of adrenalectomized rats led us to further investigate the effect of this glucosteroid upon the circadian distribution of mitosis in the cornea of rats.

Evidence will be presented later(22), which indicates that, under our experimental conditions, the normal 11:00 h "peak" number of cells in mitosis found in normal, in adrenalectomized, and in adrenalectomized rats treated with dexamethasone in the afternoon period, is shifted to 0700 hr when steroid administration is done at 0900 hr to 1000 hr, instead of at 1500 hr to 1700 hr of the preceding day.

Evidence will be presented in this work to indicate: That the adrenal glands are important in maintaining the normal high and low levels of cells in mitosis in the cornea, as found respectively at 10:00 hr and at 23:00 hr; that the administration of dexamethasone to adrenalectomized rats, reestablished the normal day/night ratio. In addition to this fact, it appears significantly to increase the number of cells in mitosis, as found in the 10:00 AM period of the day; that the number of hours which elapse between administration of the steroid and sacrifice of the animal is critical, and depending upon the time, either decreased, elevated or unaltered numbers of cells in mitosis can be found in the cornea of dexamethasone-treated, adrenalectomized rats.

Materials and methods. Female rats of the Wistar strain, ranging in weight between 105 and 135 g, were used in all experiments. Partial hepatectomy (70%) was performed according to the technique of Higgins and Anderson(23). Subsequently, in this paper the term hepatectomy will be used to mean partial (70%) removal of the liver. Bilateral adrenalectomy was performed according to the technique of Grollman(24). Mortality rate was low and comparable to that in previous work(20). All animals were fed a complete commercial diet *ad libitum*. Physiologic saline was given, *ad libitum*, instead of water, to all adrenalectomized rats. The animals were sacrificed by decapitation at varying times as indicated under *Results*.

TABLE I. Occurrence of Mitoses in Cornea of Normal and Adrenalectomized Rats.

Groups	Mitoses/50 microscopic fields ± SE	
	Time of sacrifice	
	10:00 hr	23:00 hr
Normal	172 ± 6.9 (33)	41 ± 7.5 (14)
Adrenalectomized	133 ± 6.5 (29)	70 ± 6.0 (28)

SE ± standard error of the mean values.
( ) No. of animals in each experimental group.

The eyes were removed from the animals and fixed in alfac (a mixture containing 85 ml of ethanol 80%, 10 ml of formalin and 5 ml of glacial acetic acid). Total corneas were stained by the Feulgen reaction(25) as described previously(20). Mitoses were counted with the use of a microscope with a reticulum (8 mm × 8 mm) ocular (8×) and oil immersion lens (100×). Fifty fields (125 μ × 125 μ) were counted in each cornea.

Dexamethasone 0.4 mg (generously supplied by Merck Sharp and Dohme of Brasil), was administered by the intraperitoneal route at different times as described under *Results*.

Results and discussion. Normal Wistar rats were sacrificed at 10:00 hr and 23:00 hr in several groups, over a span of 7 months. A total of 33 rats were obtained for the 10:00 hr period and 14 for the 23:00 hr period. The same was done for the group of adrenalectomized rats and a total of 29 and 28 animals were obtained, respectively, for the 10:00 and 23:00 hr periods. The results with all groups are presented in Table I.

In the group of normal rats, as indicated in Table I, the number of cells in mitoses, as found in the 10:00 hr period of the day, is approximately 4 times greater than that which was found in the 23:00 hr period, giving a morning/night (M/N) ratio = 4. On the other hand, while the number of cells in mitoses is still higher in the morning than at night, in adrenalectomized rats, the M/N

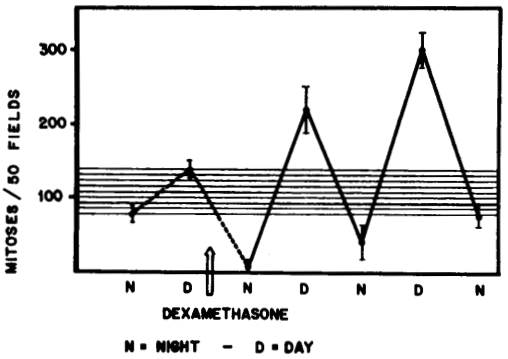

FIG. 1. Effect of Dexamethasone upon diurnal and nocturnal levels of mitoses in the cornea of rats. Rats were sacrificed at either 10:00 hr (D) or at 23:00 hr (N). The number of cells in mitoses in each group ± standard error of the mean values is presented for untreated adrenalectomized rats and for Dexamethasone-treated adrenalectomized rats; Dexamethasone was administered at 14:00 hr, as indicated in the graph by the arrow.

ratio is significantly smaller (M/N = 1.9) than that found in normal rats. It is also interesting to note that a significantly lower number of mitoses is found in the morning period in adrenalectomized animals as compared to that which is obtained in normal animals (172×133, $P < 0.001$). The opposite effect occurred in the night period when the animals without adrenals had a significantly greater number of cells in mitoses as compared with the normal controls (70×41; $P < 0.001$). These data suggest a synchronizing effect exerted by the adrenal glands in normal animals and, consequently a distinctly different day/night distrubution of mitoses.

This hypothesis is strengthened by the response demonstrated in Dexamethasone-treated adrenalectomized animals. In Graph I is presented the data obtained in 3 separate experiments with adrenalectomized animals that received, on the 4th day after bilateral adrenalectomy, a single 0.4 mg dose of Dexamethasone, by the intraperitoneal route, at 14:00 hr. In each experiment, 5 groups of 3 to 5 rats were sacrificed in sequence, starting on the same day of Deamethasone administration at 23:00 hr, and followed by groups sacrificed at 10:00 hr and 23:00 hr of the following two days.

In Graph I, the pre-Dexamethasone treatment phase was ploted with the average numbers as presented in Table I. The results indicate two important facts: 1st:—an apparent return to a normal morning/night mitotic ratio, as M/N mitotic ratios of 5.5 and 3.8 in the first and second days, respectively, following treatment with Dexamethasone, are closer to the normal 4, than to that found in untreated adrenalectomized rats, 1.9; 2nd:—an apparent overall stimulation of mitoses in the first and second days after treatment with the steroid, as their respective night levels are either similar (39 mitoses/50 fields) or higher than normal (79 mitoses/50 fields), with both morning levels (217 and 304 mitoses/50 fields, respectively, for the first and second days after Dexamethasone administration) significantly ($P<0.001$) greater than that of either normal rats (172 mitoses/50 fields) or that of adrenalectomized rats not treated with the hormone (133 mitoses/50 fields).

As these studies did not go beyond the second day post Dexamethasone, additional experiments were conducted with the use of adrenalectomized rats with and without partial hepatectomy, to determine the total duration of this period of increased mitotic activity. Hepatectomized animals with intact adrenal glands were used to determine the length of the post partial-hepatectomy period of increased mitotic activity and, at the same time, compare it with that of Dexamethasone-treated adrenalectomized-hepatectomized animals.

The results obtained with hepatectomized animals are presented in Table II.

As indicated in Table II, increased mitotic activity was present for up to 48 hours post partial hepatectomy; on the 3rd day post hepatectomy both the 10:00 hr and the 23:00 hr periods had returned to approximately normal levels both in hepatectomized animals with intact adrenals and in the adrenalectomized-hepatectomized, Dexamethasone-treated rats.

On the other hand, the most interesting finding in this series of experiments was the contrast between the number of cells in mitoses in the Dexamethasone-treated adrenalectomized-hepatectomized animals and those in Dexamethasone-treated adrenalectomized rats

TABLE II. Effect of Partial Hepatectomy upon Mitoses in Cornea of Normal and Dexamethasone-Treated Adrenalectomized Rats.

	No. of cells in mitoses/50 microscopic fields ± SE						
	Hours after hepatectomy						
	12	24	36	48	60	72	84
Groups	N*	D*	N	D	N	D	N
Controls	67 ± 12.6 (5)	122 ±12 (5)	108 ± 16.2 (5)	247 ± 11.0 (19)	64 ± 10.2 (5)	178 ± 19.4 (5)	67 ± 11.2 (5)
Adrenalectomized	43 ± 11.6 (8)	68 ± 5.7 (8)	78 ± 18.5 (8)	381 ± 34 (9)	76 ± 13 (4)	156 ± 12.7 (8)	64 ± 11.4 (6)

* D and N: periods of the day, respectively 10:00 and 23:00 hr.
( ) No. of animals in each group. SE; standard error of the mean values.

with intact liver (Graph I). As can be seen (Table II), 68 ± 5.7 cells in mitoses were found per 50 microscopic fields 24 hours post hepatectomy, while in the adrenalectomized animals with intact liver, in the corresponding period, 217 ± 17 cells in mitoses/50 fields were found. Evidently, the severe stress decreased food intake, and related metabolic alterations, which occur in the adrenalectomized-hepatectomized rats, were not present in the group of rats which underwent adrenalectomy only. However, it was conceivable that the hour of drug administration, which varied in the two groups, could in part be responsible for the difference in the number of cells in mitoses. Since mortality is severe in adrenalectomized animals(20) in the immediate period which follows partial hepatectomy, it has been our practice to administer the steroid one to one and a half hours before surgery, usually around 08:00 hr; Dexamethasone administration was effected at 14:00 hr in the adrenalectomized rats with intact livers. Therefore, to verify whether this contrast could possibly account for the striking difference between the two groups, additional experiments were done, both with adrenalectomized-hepatectomized rats and with adrenalectomized animals with intact livers. The results obtained with 2 groups of adrenalectomized rats in which 0.4 mg of Dexamethasone was administered at 09:00 hr and 14:000 hr, respectively, are presented, along with those of adrenalectomized untreated controls, in Table III.

TABLE III

Groups	No. of cells in mitoses/50 microscopic fields ± SE
Adrenalectomized controls	136 ± 22 (5)
Adrenalectomized:	
Dexamethasone-treated at 09:00	48 ± 12.4 (5)
Dexamethasone-treated at 14:00	201 ± 21.7 (10)

As seen, the expected results were obtained; at 10:00 hr of the day following steroid administration a markedly decreased number of cells in mitoses was found in the group of rats treated at 9:00 hr and a significantly increased number of cells in mitoses was encountered in the group of rats treated in the afternoon, at 14:00 hr, when compared to the control level of untreated adrenalectomized rats.

For a study of adrenalectomized-hepatectomized rats, it was decided that a time should be selected distant enough from hepatectomy to avoid the major metabolic disturbances of the surgical trauma and from the immediate influence of the dose of Dexamethasone, given to prevent the high mortality which would otherwise occur in these animals.

Thus, the time chosen for these experiments was the morning of the 3rd day after partial hepatectomy which, as can be seen on Table II, satisfies the above metioned criteria. The results obtained with the treatment of Dexamethasone, 0.4 mg as a single I. P. dose, at 30, 24, 18, 12 and 4 hours before sacrifice, 72 hours after hepatectomy and 73 hours after the first dose of Dexamethasone are presented on Table IV.

Control adrenalectomized-hepatectomized rats that received a single 0.4 mg dose of Dexamethasone before partial hepatectomy

TABLE IV

Groups		Cells in mitoses/50 microscopic fields ± SE*
Controls		154 ± 7.9 (5)†
Treated with 0.4 mg of Dexamethasone (hr before sacrifice)	30	113 ± 15 (8)
	24	76 ± 12 (15)
	18	208 ± 16 (8)
	12	172 ± 11 (8)
	4	124 ± 18 (8)

* SE; standard error of the mean values.
† ( ) No. of animals each group.

were sacrified 73 hours later, at 10:00 AM; these animals had in their corneas 154 ± 7.9 mitoses per 50 microscopic fields. This result is a clear return to pre-hepatectomy levels. The second dose of Dexamethasone, administered to the animals at different time intervals before sacrifice, either altered or radically changed the period of the day in which maximum mitotic activity occurred (Table II). Since a period of the day in which maximum mitotic activity was naturally occurring, was chosen to serve as the control, it was clearly possible to influence the level of mitotic activity. Thus, in the groups in which Dexamethasone was administered either at 11:00 hr or 17:00 hr of the day, a depression (76 mitoses per 50 fields), or a stimulation (208 mitoses per 50 fields), respectively, was observed in the number of cells in mitoses as seen the next morning at 11:00 hr. Additional work is being done by the authors, in which, following the administration of Dexamethasone at 09:00 hr, in one group, and 15:00 hr, in another group of animals, the 24 hour period post drug administration is being studied with the different groups of animals being sacrificed at 4 hour intervals(*22*). It is believed that synchronization, with the period of highest mitotic activity placed at an earlier time of the day, is the most likely explanation for the depressed levels of mitoses found in the group of rats treated at either 09:00 or at 11:00 hr, and presented in Table III and IV. As judged by the results already obtained, the peak number of cells in mitoses should be expected at 17 to 20 hours after Dexamethasone administration. Therefore, a group of animals receiving Dexamethasone at 11:00 hr of the day should have a period of high mitotic activity around 07:00 hr of the following day. Whether the above presented results, in which depressed mitotic activity was found in the group of rats receiving steroid in the morning period of the day, are in any way related to those found 24 hours post hepatectomy, both in controls and adrenalectomized animals treated at 9:00 hr, is being investigated.

Additional experiments are also underway to investigate:

First, whether any possible relationship exists between the steroid effect upon mitoses in the corneal epithelium of rats and the effect of these agents upon the synthesis of RNA;

second, to verify whether a stimulation of DNA synthesis rather than an inhibitory effect, is produced in the time period between the onset of the maximum steroid effect upon RNA synthesis and that of maximum effect upon the final event, mitoses.

Summary. The effect of adrenalectomy and of Dexamethasone treatment in adrenalectomized rats upon diurnal distribution of mitoses in the cornea of rats, was investigated. Evidence is presented to demonstrate that either increased or decreased numbers of cells in mitoses can be found in the cornea of rats in the late morning period of the day in Dexamethasone-treated adrenalectomized rats. Data are presented to indicate that these differences are related to the time interval between Dexamethasone administration and the time at which the animals were sacrificed.

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Renal Hemodynamic Response to Osmotic Diuresis in Man.* (32330)

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The rate of glomerular filtration in normal man remains essentially constant under most circumstances, even with marked changes in renal blood flow(1). In a study of the urinary concentrating mechanism it was observed unexpectedly that filtration rate decreased during osmotic diuresis(2). Depression of filtration rate in the dog has been reported during infusion of hypertonic glucose and glucose with mannitol(3). This response has also been described during infusion of hypertonic solution of mannitol in the anaesthetized dog following laparotomy and has been attributed to increased medullary blood flow(4). In the present study in man no evidence for shunting of blood was obtained, since depression of filtration rate during osmotic diuresis occurred without a significant change in either renal blood flow or extraction ratio of p-aminohippurate. It is suggested rather that an increase in proximal intratubular pressure is responsible, at least in part, for the depression of glomerular filtration.

Methods. Patients were selected from the wards and outpatient clinics of the Third and Fourth (New York University) Divisions of Bellevue Hospital. Observations were made in 12 normotensive subjects without evidence of cardiovascular or renal disease. All subjects were maintained on regular hospital diets. Fluid restriction was imposed for periods ranging from 16 to 24 hours prior to the test. Pitressin® (20 units of vasopressin) was injected intramuscularly. After urethral catheterization, priming doses of inulin and para-aminohippurate were administered, followed by sustaining infusions at 2 ml per minute, delivering Pitressin at a rate of 1 mU (milliunit) per kg per hour.

Blood and urine specimens were collected over 30 to 45 minutes for determination of "basal" (control) rates of glomerular filtration (GFR), renal plasma flow (RPF) and osmolal clearance (C_{osm}) and during an infusion of 10% mannitol solution at 20 ml per minute for 47 to 98 minutes. In 3 subjects (T.S.,

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