66–6891

HUIBREGTSE, William Henry, 1936–
HYPOTHALAMIC MEDIATION OF CORTICO–
TROPHIN SECRETION.

University of Arizona, Ph.D., 1966
Physiology

University Microfilms, Inc., Ann Arbor, Michigan

HYPOTHALAMIC MEDIATION OF CORTICOTROPHIN SECRETION

by

William Henry Huibregtse

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF ZOOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1966

THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by William Henry Huibregtse

entitled Hypothalamic Mediation of Corticotrophin Secretion

be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

Robert B. Livingston — Dissertation Director — 2/8/66 — Date

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SIGNED: William H. Heinbrigtal

ACKNOWLEDGMENTS

This study was supported in part by an Institutional Research Grant from the American Cancer Society (No. 700-451) awarded to Dr. Robert B. Chiasson in 1964. The investigation was completed while the author was a pre-doctoral fellow on a U.S.P.H.S. award (No. 1-F1 GM-24, 729-01A1). I wish to express my appreciation to the above, as well as to members of the faculty and fellow graduate students whose comments and suggestions have been invaluable. Special recognition is due to Mr. Wm. C. Dewel for his technical assistance.

I am especially indebted to Dr. Robert B. Chiasson for his guidance and assistance in every aspect of my graduate career and for his example in perpetuating the highest degree of scholarship. I am thankful, above all, to my wife, Rhoda, for her example in perseverance and for her encouragement and patience during the course of this study.

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ABSTRACT

Daily rhythmical excretion of urinary free 17-hydroxycorticosteroid has been determined in guinea pigs. The effects of daily photoperiod lengths on the excretion pattern were studied as was the effect of continuous darkness. Using a six-hour mean excretion value for four consecutive periods a day, it became evident that at least a nine-hour photoperiod is necessary in order to discern a daily rhythmical pattern. Animals acclimated to continuous darkness gave suggestion of an endogenous rhythm but the results were not conclusive.

Electrolytic lesions in the median eminence area were effective in blocking the rhythmical excretion of cortisol in response to a 12-hour light - 12-hour dark photoperiod regime.

It is evident that daily photoperiod length is a factor in "setting" a rhythmical pattern of adrenal secretion and that hypothalamo-hypophyseal pathways (via the median eminence) are essential to proper maintenance of this response.

INTRODUCTION

The present study was undertaken to determine the role of photic stimulation (*via* the hypothalamus and pituitary) on circadian adrenal secretory rhythms. The study is divisible into two major parts on the basis of treatments applied to the animals: 1. The effects of photoperiod alteration on the rhythmical excretion of 17-hydroxycorticosterone (cortisol, 17-OHCS) in normal, untreated animals. 2. The effects of median eminence lesions on rhythmical cortisol excretion in animals acclimated to a fixed twenty-four hour light-dark regime of twelve hours light and twelve hours dark (12L-12D).

Polypeptide neurohumors which influence the release of some of the anterior pituitary hormones have been isolated and in part characterized (Deuben, 1965; Guillemin *et al.*, 1962; Schally *et al.*, 1960; McCann *et al.*, 1965). The role of photoperiods in regulating hormone secretion during vertebrate reproduction and migration has been studied extensively (Rowan, 1925; Wolfson, 1965; Farner, 1964). Other photo-neuro-endocrine effects on circadian rhythms of both vertebrates and invertebrates have been recently reviewed by Whipple *et al.* (1964).

Although adrenal rhythmicity has been investigated (Saba *et al.*, 1963, 1965; Galicich *et al.*, 1965; Vagnucci *et al.*, 1965; Rinne and Sonninen, 1964; McCarthy *et al.*, 1960), relatively little is known concerning this phenomenon. One limitation to research in this area is the lack of an accurate and expedient technique for measuring adrenal activity in terms of adrenocorticosteroid production. The guinea pig

(*Cavia porcellus*) is a particularly suitably experimental animal because of its unique characteristic of excreting a relatively high concentration of 17-hydroxycorticosterone in its urine (Burstein, 1952; Burstein and Dorfman, 1954). The advantages of this approach include minimal disturbance to the animals during sample collections and the repeated use of the same animals over long periods of time. The disadvantage to this technique is that steroid excretion is not recorded at the moment of its release but is represented by an average value over some period of time. This prevents precise determination of the animal's response to experimental variables as well as to the uncontrolled effects of growth and acclimation during the course of the experiment.

MATERIALS AND METHODS

Experiments were carried out with male albino guinea pigs (except for four virgin females in one group) purchased from Animal Supply Company, Napa, California. The animals were housed in community cages prior to the experiments and in individual metabolism cages during the experiments. The animals were fed Purina guinea pig chow supplemented periodically with cabbage or lettuce and allowed water *ad libitum*.

The metabolism cages were placed in a light-dark controlled room and although temperature was not controllable, it was recorded for top and bottom cage rows at each collection period. Three 100W incandescent bulbs placed in front of the cages served as a light source. Light intensity measured from 50-70 ft. candles at the exterior of the cages (the presence of water and food containers at the front of the cages made accurate determination of light intensity inside the cages impossible). The greatest temperature range in the cage room for any twenty-four hour period was 4°C, the usual variation being 2-3°C. The normal temperature variation from beginning to end of any one experiment was 3-4°C although in one case it varied 6°C. The vertical temperature gradient in the cage room had a maximum of 1.5°C.

Urine samples were collected for four consecutive six-hour periods (numbered one to four). When employing the usual 12L-12D cycle, periods two and three (0800-2000 hr) were light and periods four and one (2000-0800 hr) were dark. The light was controlled by an

on-off switch without gradation and an incandescent red safe light served for collections during dark periods. Urine samples were collected in glass flasks containing 1 cc of a 0.5 per cent solution of thymol in glacial acetic acid. Urine volume was measured at the end of each collection period and the extraction procedure was usually performed immediately although some samples were frozen for twenty-four to seventy-two hours before extraction.

The method of extraction and measurement of cortisol followed that of Porter and Silber (1950) modified to use five milliliters of urine in 30 ml. of redistilled reagent grade chloroform for the initial extraction. The first three steps of the extraction were performed in polypropylene test tubes.

Standards were prepared by adding a stock solution of grade A hydrocortisone alcohol in graded volumes to urine. Identical samples of water-diluted urine were compared with the hydrocortisone standards and the difference in per cent absorbance between the two was used in plotting a standard curve from which the sample concentrations were read. All absorbance readings were made on a Beckman DU or DU-2 Spectrophotometer at 410mμ. Samples having a concentration of 2μg cortisol/cc, or greater, were determinable within an error of ten per cent. The majority of the samples contained from 2-5μg 17-OHCS/cc.

Animals to be lesioned or sham-operated were anesthetized with a mixture of urethane (600 mg/kg) and pentobarbital (20 mg/kg) following the method of Fajer and Vogt (1963). It was later found that the animals responded much better postoperatively when the dose was 450 mg/kg urethane and 15 mg/kg pentobarbital. Lesioning was performed

under anesthesia in a slightly modified Stellar-Johnson stereotaxic apparatus. Atlases of the guinea pig brain are available for very small animals weighing 200-300g (Luparello, Stein and Park, 1964) and for fully matured animals, over 500g (Tindall, 1965). Since the animals used in this study were intermediate in size (300-500 grams), a compensatory formula for leveling the animal's head was derived by following the technique of Luparello et al. (1964), and by trial and error. It was found that by using two thirds of the distance (in millimeters) from the interaural axis to the coronal suture as one point and twice that distance (in millimeters) as another point the median eminence area lay within one millimeter on either side of the coronal suture. The exact placement of the electrode was facilitated by using the X-ray technique described by Egge and Chiasson (1962). Lesioning was produced by passing 5 ma of current for 15-20 sec. while sham-operated controls received identical treatment except that no current was passed through the electrode.

After lesioning, the animals were kept on the 12L-12D regime for five to seven days before being tested for the rhythmical excretion pattern. Postoperative recuperation time was considered to be the time required for sham-lesioned controls to reestablish a normal excretion pattern. Two or more steroid determinations were made for each of the treated groups before the animals were subjected to an ether anesthesia-abdominal laparotomy stress as an additional physiological indication of effectiveness of the lesion. Seventeen-hydroxycorticosteroid output was determined for four consecutive periods following stress. Serial sections of brains were studied to verify the location of the lesions.

RESULTS

Guinea pigs were allowed to acclimate to a 12L-12D regime for at least three days. After this period their response was fairly consistent. The normal excretion pattern, showing the variation between four consecutive six-hour collection periods, is represented in Figure 1. The day-to-day variation in excretion patterns for one group of animals is shown in Figure 2. The numerical data on which these, and all subsequent figures are based, as well as the probability values (Student's t test) for those periods which differ significantly, are given in the appendix.

The 17-OHCS excretion during the first six-hour light period is significantly lower than any of the other six-hour periods when a large sample is used (P from 0.05 to 0.001). If a smaller sample size (n=9) is used only periods 2 and 3 differ consistently from day to day (Figure 2, a-e). If the data for nine animals are combined for all five days (n=44) the pattern again resembles that of a large sample inasmuch as the excretion during the first six hours of light is significantly lower than during the other three periods (Figure 2, f). It is apparent that for untreated animals on a 12L-12D regime the six-hour mean excretion values are lowest during the first light period (period 2), rise to a maximum level during the second six hours of light (period 3), and remain intermediate and relatively constant during the twelve hours of darkness (periods 1 and 4).

FIGURE 1

Variation in urinary 17-OHCS excretion for four consecutive six-hour periods for 32 guinea pigs on a given day. Animals were on a 12L-12D regime with at least a three-day acclimation period. Periods two and three were light, periods one and four were dark. Mean values ± 95% confidence limits.

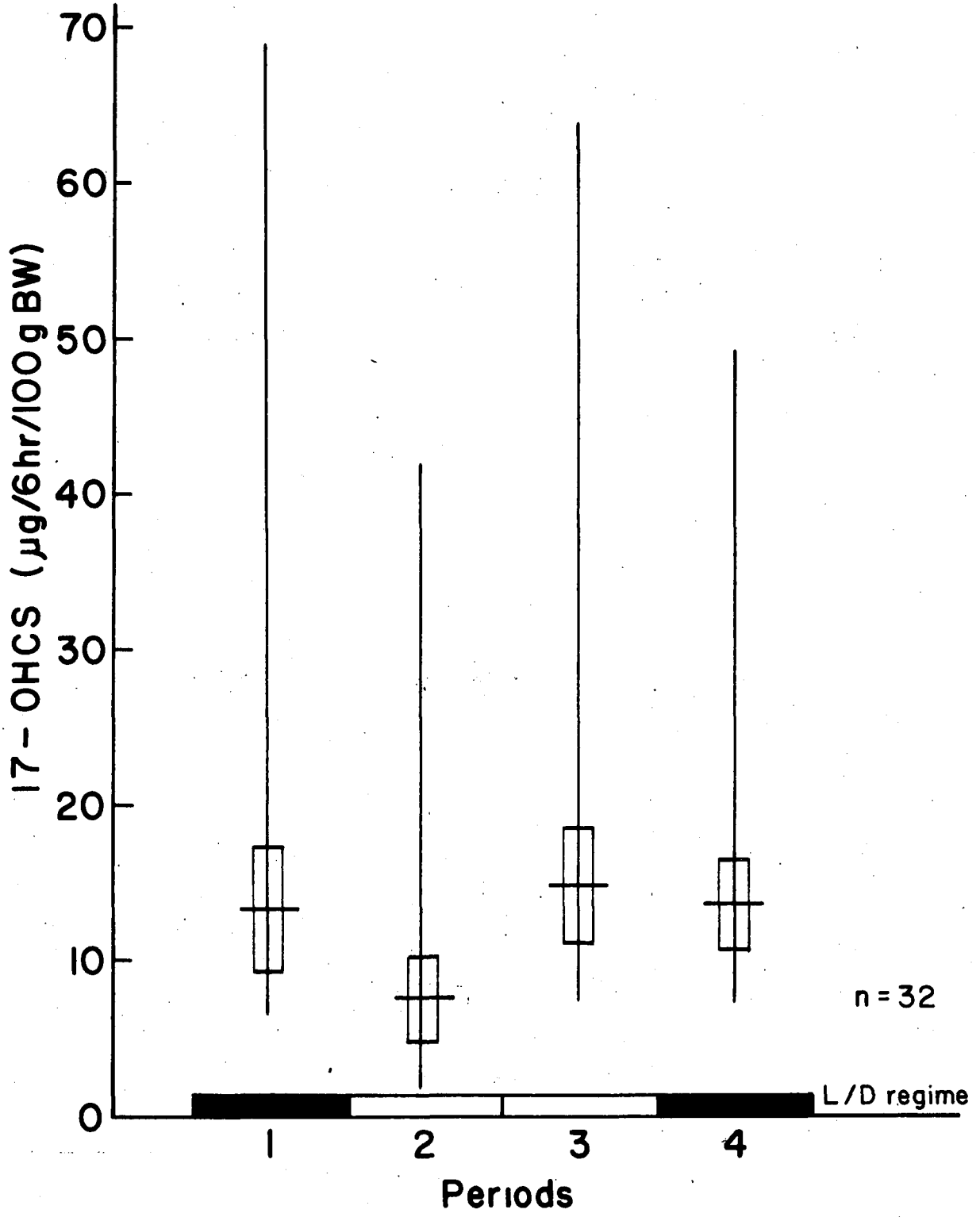
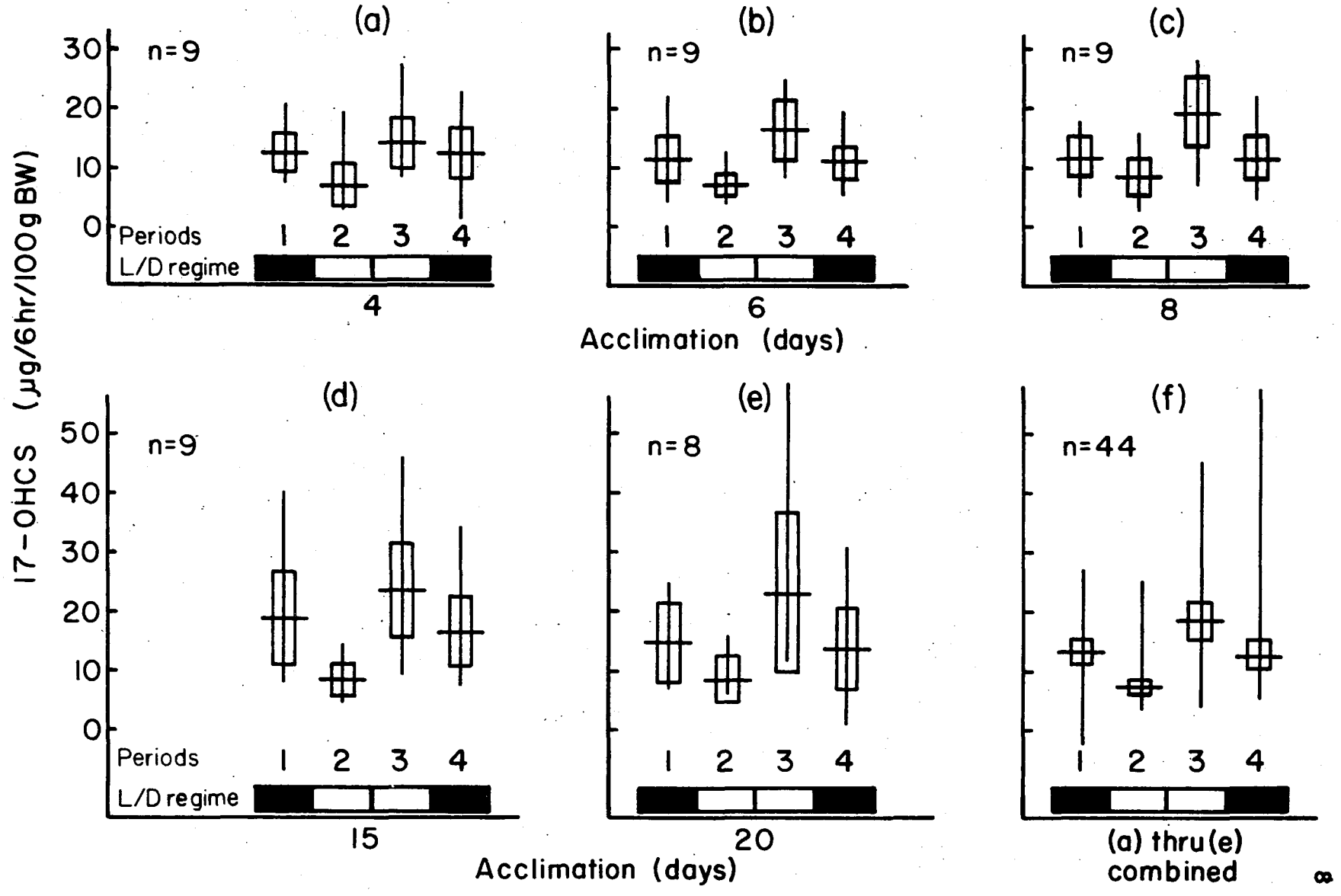

70
60
50
40
30
20
10
0
17-OHCS (µg/6hr/100g BW)
n = 32
L/D regime
1
2
3
4
Periods

FIGURE 2

Urinary 17-OHCS excretion patterns for one group of guinea pigs on five different days; a thru e. Combined values of the same animals for all five days; f. The length of acclimation to a 12L-12D regime is indicated. (95% confidence limits are shown).


(a)
n=9
30
20
10
0
Periods
1 2 3 4
L/D regime
4
(b)
n=9
1 2 3 4
6
(c)
n=9
1 2 3 4
8
Acclimation (days)
17-OHCS (µg/6hr/100g BW)
(d)
n=9
50
40
30
20
10
0
Periods
1 2 3 4
L/D regime
15
(e)
n=8
1 2 3 4
20
(f)
n=44
1 2 3 4
(a) thru (e) combined
Acclimation (days)

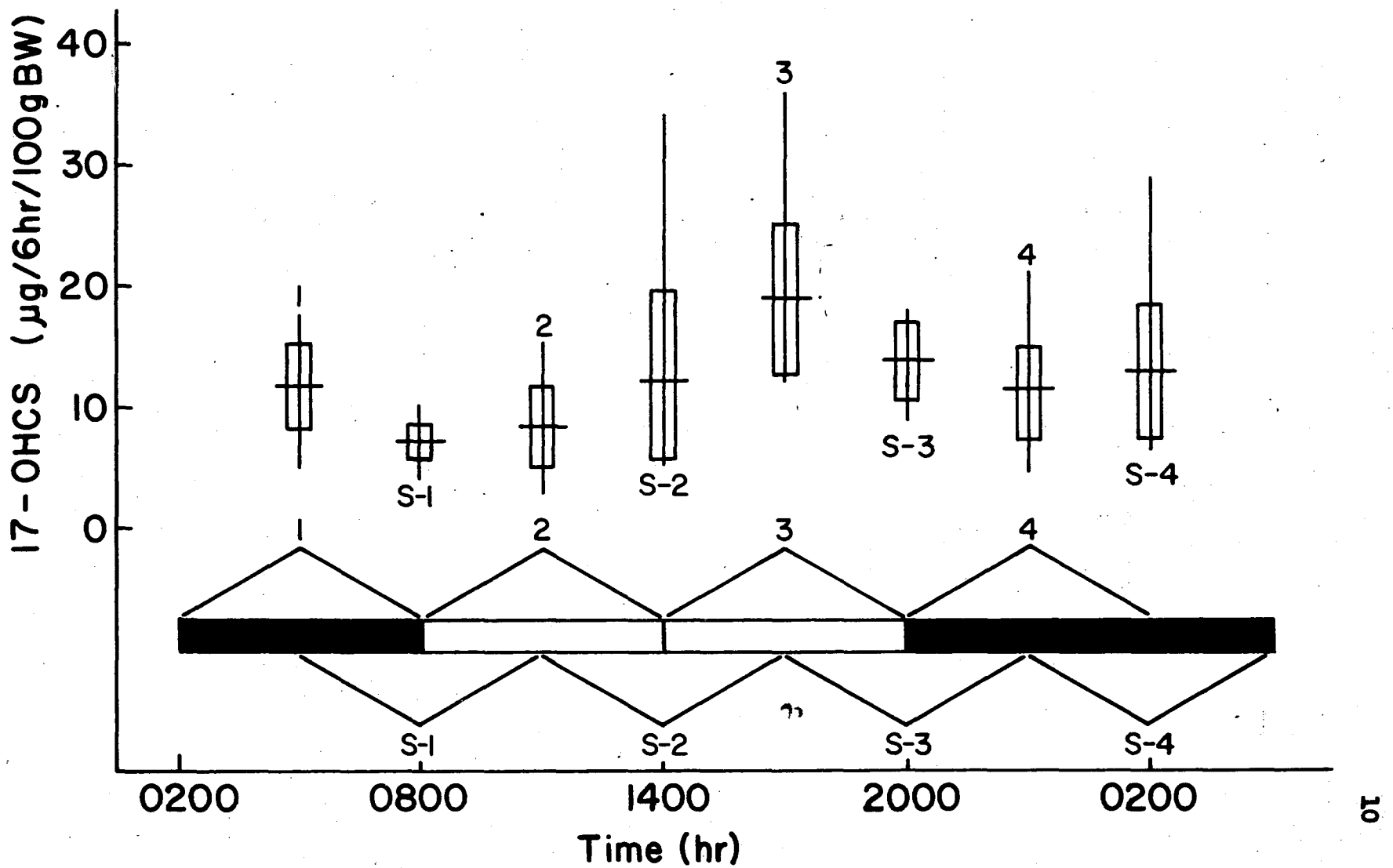
Collections of 17-OHCS excretions were also made one-half period out of phase with the original schedule overlapping the original periods by three hours. The light-dark sequence was not altered. This new collection schedule did not result in a more distinct six-hour mean excretion pattern of 17-OHCS but it did appear to delineate the gradient of cortisol excretion in a twenty-four hour period (Figure 3). The lowest excretion level of cortisol apparently occurs very close to the onset of light while the highest cortisol excretion comes after the animals have been subjected to at least nine hours of light.

One group of seven animals (including four virgin females) was studied for rhythmical 17-OHCS excretion patterns following acclimation to a 6 hour light-18 hour dark regime (6L-18D). Urine collections were made following acclimation for five, eight, and fourteen days. After fourteen days the regime was changed to nine hours light and fifteen hours of darkness. The animals were acclimated to this new (9L-15D) regime for six days. The six-hour mean values for cortisol excretion indicate no response to a 6L-18D regime even after fourteen days acclimation (Figure 4). On the other hand, acclimation for six days on the 9L-15D regime (Figure 4) reveals a distinct pattern, identical to that of animals acclimated to a 12L-12D regime.

The rhythmical cortisol excretion pattern was further examined in intact animals by placing a group of nine guinea pigs in continuous darkness. The response of this group was measured following acclimation to continuous darkness for two, seven and eleven days. The six-hour mean excretion values for four consecutive periods on those days have been graphed in Figure 5. Following two days acclimation to total

FIGURE 3

Urinary 17-OHCS excretion patterns for nine guinea pigs on a 12L-12D regime with the collection periods shifted one-half period from usual. Mean values ± 95% confidence limits.


17-OHCS (µg/6hr/100gBW)
40
30
20
10
0
1
2
3
4
S-1
S-2
S-3
S-4
0200
0800
1400
2000
0200
Time (hr)

darkness, it appears that there is still adherence to the 12L-12D pattern (to which this group had previously been acclimated). After seven and eleven days of continuous darkness, no pattern can be discerned for six-hour mean cortisol values (Figure 5). After eleven days of continuous darkness the animals were again acclimated (for five days) to a 12L-12D regime. However, the light and dark periods were reversed from the original regime; that is, the light periods occurred during the natural night hours and the dark periods during natural daylight hours. A pattern similar to that of animals acclimated to the original 12L-12D regime appeared; that is, the second six hours of light yielded a significantly higher mean cortisol value than the first six-hour light period. The pattern differed from that of animals on the original 12L-12D regime in having much lower cortisol values during the two dark periods (Figure 5).

The original 12L-12D regime was employed for studies of rhythmic excretion involving mediation through the hypothalamus. Sham-operated controls resumed rhythmic excretion of 17-OHCS when placed on a 12L-12D regime for four or five days. The data for nine sham-operated animals, measured for a combined total of twenty-one days, was used as an indication of rhythmic 17-OHCS excretion in control animals. These data are graphed in Figure 6 and show that sham-operated animals resumed a "normal" pattern, the 17-OHCS values during the first six hours of light being significantly lower than the values during the other three collection periods ($P<0.02$). Two or three sham-operated controls were included in each group of lesioned animals.

FIGURE 4

Urinary 17-OHCS excretion patterns for seven guinea pigs acclimated for 14 days to a 6L-18D regime followed by five days acclimation to a 9L-15D regime. Four animals were virgin females. Mean values ± 95% confidence limits.

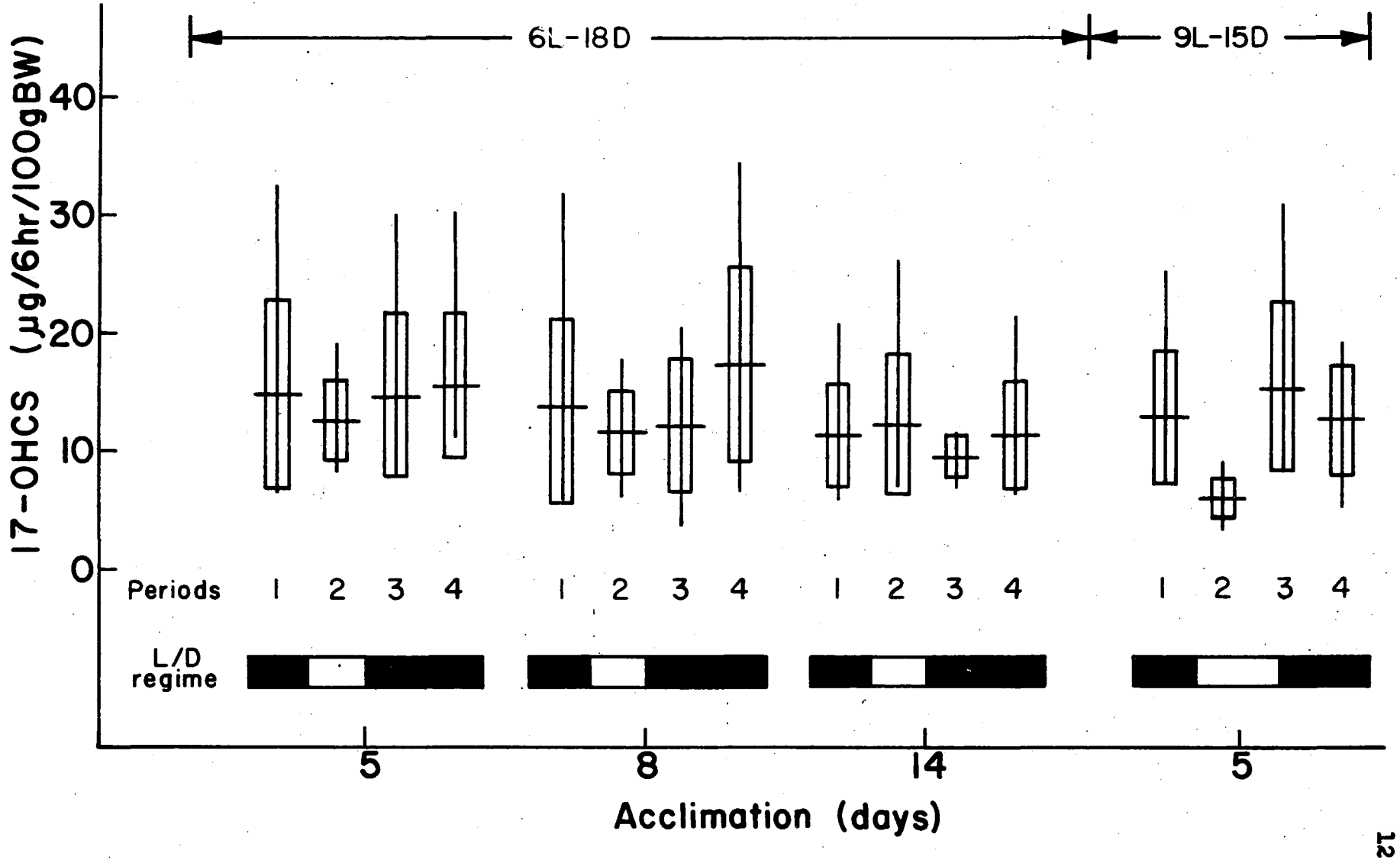
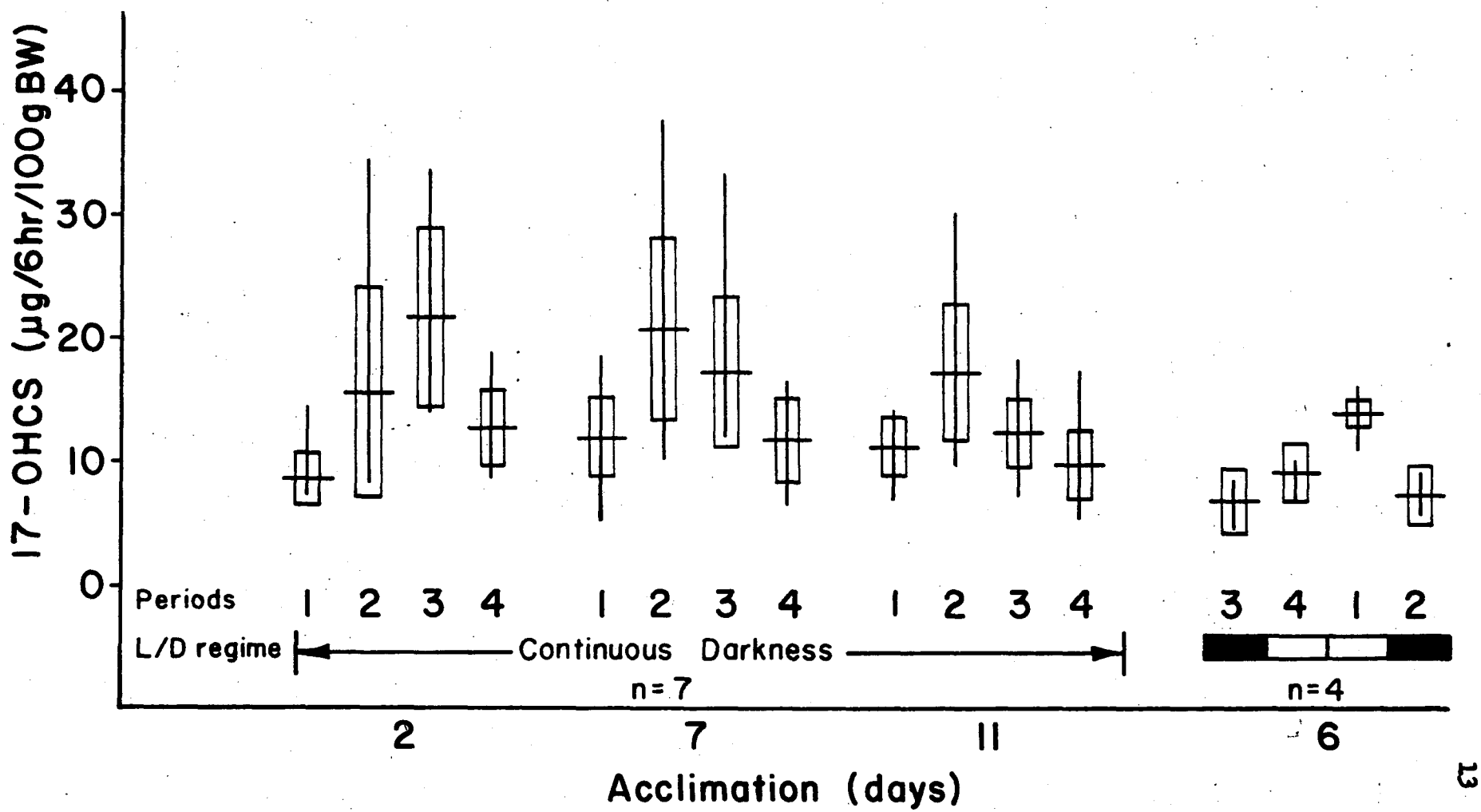

6L-18D
9L-15D
17-OHCS (µg/6hr/100gBW)
40
30
20
10
0
Periods
1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4
L/D regime
5
8
14
5
Acclimation (days)

FIGURE 5

Urinary 17-OHCS excretion patterns for guinea pigs acclimated to continuous darkness followed by six days acclimation to a reversed 12L-12D regime. Mean values ± 95% confidence limits.


17-OHCS (µg/6hr/100g BW)
40
30
20
10
0
Periods
1 2 3 4 1 2 3 4 1 2 3 4 3 4 1 2
L/D regime
Continuous Darkness
n=7
n=4
2
7
11
6
Acclimation (days)

Three criteria were used to determine the effectiveness of hypothalamic lesions: 1. Verification of the lesioned area by histological examination. 2. Presence or absence of a rhythmical excretion pattern of urinary 17-OHCS similar to the "normal" pattern. 3. Response of the animal to a stress produced by ether anesthesia and abdominal laparotomy.

Brain lesions were verified histologically in twelve animals. Eight of the twelve animals had lesions which effectively blocked the rhythmical excretion of cortisol eighteen of the twenty-two days measured (Figure 7). The four other animals had ineffective lesions. The locations of lesions are illustrated in Figure 7. The caption for Figure 7 includes data on the effect of the lesion on the animals daily rhythmical cortisol excretion.

Each animal's response to stress was determined by comparing the 17-OHCS values during the six-hour period immediately following stress application with the 17-OHCS values of the same six-hour period on a day preceding (or following) the day of stress. The sham-lesioned control animals had a significantly higher cortisol level following stress ($P=0.001$) while the eight effectively lesioned animals showed no significant response (Figure 6).

Those lesions which included a major portion of the median eminence area (areas 2, 3, and 4 in Figure 7) were effective in blocking the animals' response to stress and their rhythmical response to a 12L-12D regime. The lesion in animal No. 51 was centered in the infundibular tract and may have extended into the median eminence. The only data on this animal was obtained two days post-operatively and no

stress response data was obtained. Lesions in the tractus infundibularis and the nucleus arcuatus were also effective in blocking rhythmical excretion. In animals No. 53 and No. 55 the lesion was not 100 per cent effective in blocking the rhythm and these animals also showed a weakly positive stress response. The lesioned area in these two animals, however, included less than half of the cross sectional area of the tract. Animal No. 61 was totally ineffectively lesioned in the tractus infundibularis and responded positively to stress. In this case the electrode penetrated the basisphenoid bone and only the most ventral portion of the tractus infundibularis was lesioned. Lesions behind the median eminence in the infundibulum (Animal No. 28) and in a paraventricular nucleus (Animal No. 32) were not effective in blocking the animals' rhythmical cortisol excretion.

FIGURE 6

Urinary 17-OHCS excretion patterns of guinea pigs acclimated to a 12L-12D regime following sham-operation or median eminence lesions; a-b. Responses of sham-operated and effectively lesioned animals to ether-laparotomy stress; c-d.

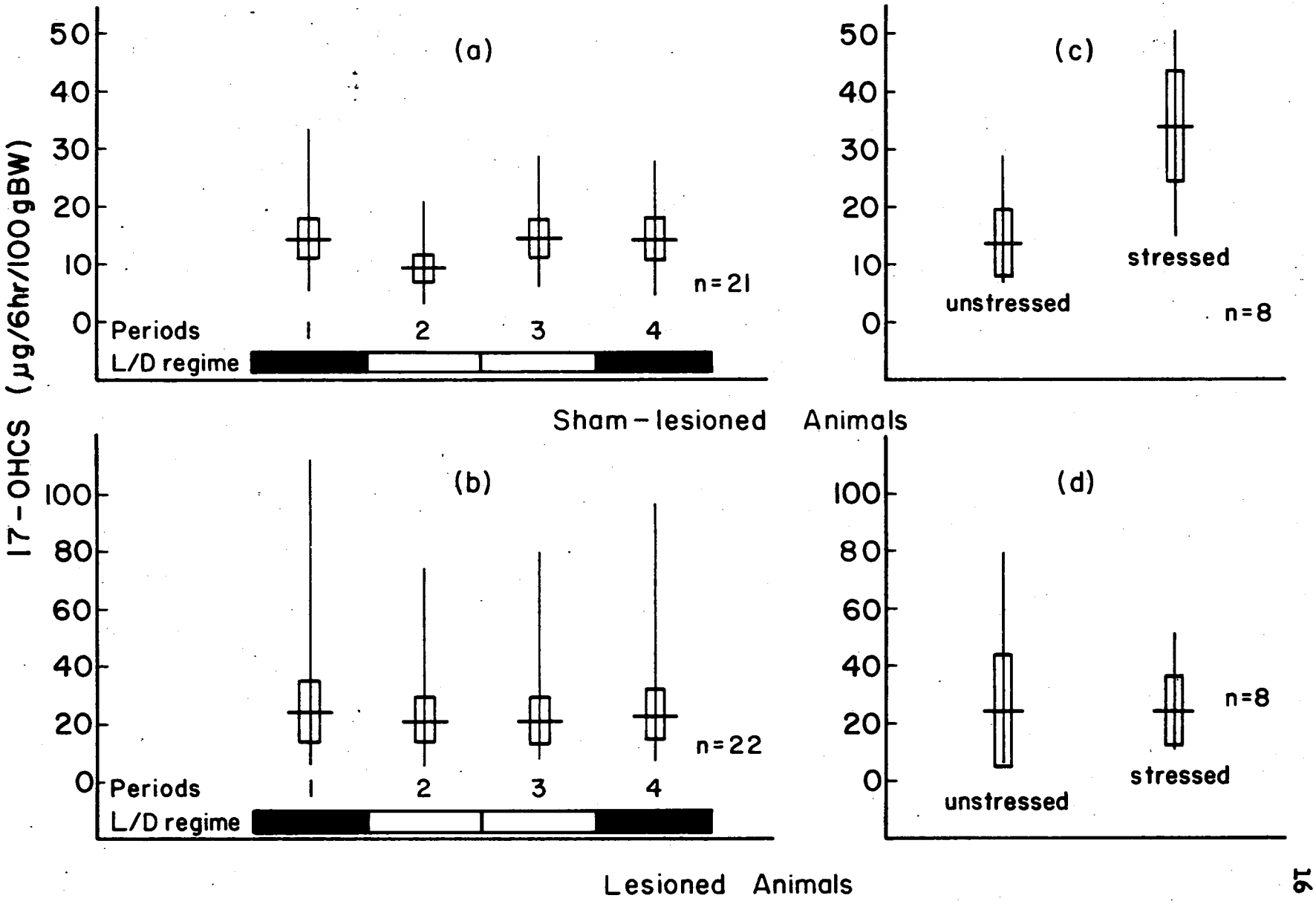
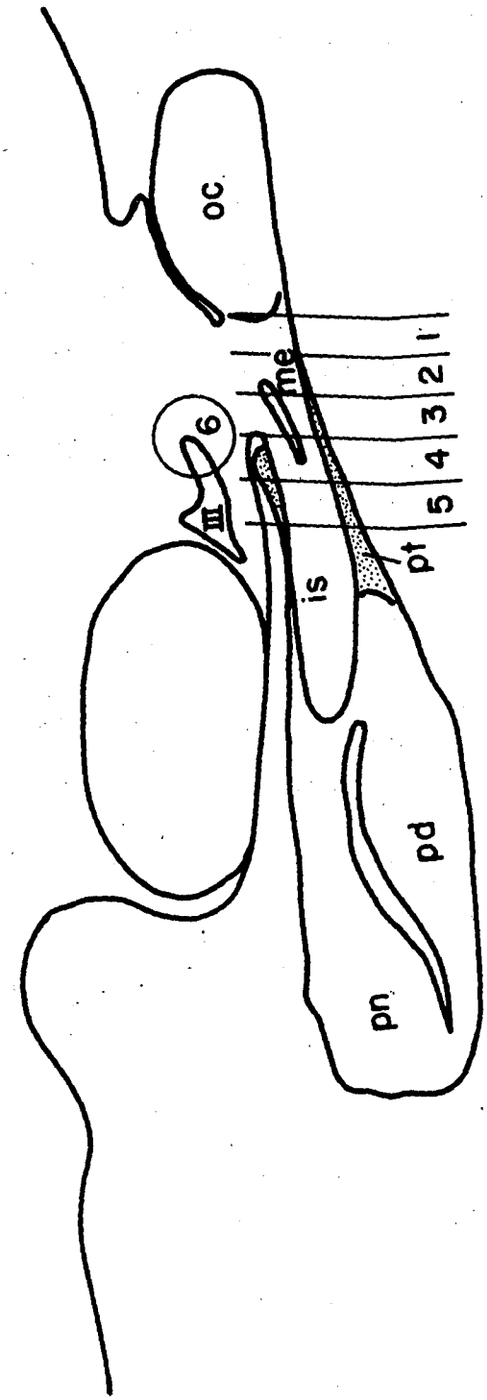

17-OHCS (µg/6hr/100gBW)
(a)
(b)
(c)
(d)
Periods
L/D regime
1
2
3
4
n=21
n=22
n=8
unstressed
stressed
Sham-lesioned Animals
Lesioned Animals

FIGURE 7

Diagram of parasagittal section through guinea pig brain illustrating areas lesioned. The data given below correlate the animal with its lesion and indicate the presence or absence of rhythmical cortisol excretion for each day measured and the animals' response to stress.

	Animal No.	Area Lesioned	Rhythm Present (Days)	Rhythm Absent (Days)	Stress Response
Effective Lesions	17	4	0	2	neg.
	44	2-3	0	2	neg.
	45	2	0	2	neg.
	47	1-2	0	2	neg.
	48	2-3	0	2	-
	53	1	2	2	weak
	55	1	1	3	weak
	59	1	0	4	neg.
Ineffective Lesions	28	5	2	0	-
	32	6	1	-	-
	51	1-2	1	-	-
	61	1	4	0	pos.

Legend: oc, optic chiasma; pn, pars nervosa; pd, pars distalis; pt, pars tuberalis; is, infundibular stalk; me, median eminence; III, third ventricle.


oc
me
6
III
is
pt
pd
pn
1
2
3
4
5

DISCUSSION

In order to extrapolate a hypothalamic mediated photoperiod effect from a urinary excretory rhythm, a correlation must be established between the metabolism of cortisol and the hypothalamo-hypophyseal axis. It has been shown that renal clearance of steroid compounds is an accurate indication of plasma levels (Cope and Black, 1959; Tait and Burstein, 1964; Beisel et al., 1964). A rhythmical pattern of urinary corticoid excretion can safely be said to imitate, with a slight phase lag, the rhythm of plasma corticosteroid. Adrenal secretory rhythm has been demonstrated in both intact rats (Saba et al., 1963a) and mice (Halberg et al., 1959) as well as for incubated adrenal glands (Andrews et al., 1965). However, Saba (with rats) and Halberg (with mice) have found that the adrenal secretory rhythm does not coincide with the plasma or urinary steroid level. No literature of this nature is available for guinea pigs. It must be pointed out, however, that the nature of cortisol-binding by the liver differs considerably between rats and guinea pigs. Wyngaarden et al. (1955), using C^{14} labeled hydrocortisone, demonstrated that in guinea pigs 69-74% of the injected radioactive steroid appeared in the urine within twenty-four hours while in rats only 24-29% appeared in the urine in the same length of time. Conversely, the feces of rats contained 50-60% of the injected radioactive steroid in twenty-four hours while guinea pigs yielded only 6-24% in their feces. This is an indication of the enterohepatic and/or

intestinal reabsorption difference between the two species. It could possibly, though certainly not conclusively, be postulated from this data that adrenal secretory rhythm more nearly parallels plasma corticoid levels in guinea pigs than in rats.

It is well established that the production and secretion of adrenocorticosteroids in normal animals is a function of the amount of corticotrophin elicited from the pars distalis of the pituitary gland (Ungar, 1964). It is also well known (Ganong, 1963) that neurosecretory material(s) from hypothalamic centers affect the synthesis and release of corticotrophin. There is some evidence of rhythmical production and release of neurosecretory material from hypothalamic nuclei and fiber tracts (Rinne and Sonninen, 1964). It is generally known that each of the above-mentioned anatomical areas or physiologically described rhythms are in some way controlled and/or regulated by photostimulation (see Whipple, 1964). Photoperiodic control of guinea pig adrenal rhythm is probably mediated through the hypothalamus.

Normal guinea pigs acclimated to a twenty-four hour light-dark cycle (12L-12D) showed a definite and consistent rhythmical excretion of urinary 17-hydroxycorticosterone. The pattern established in this study is represented by mean values of cortisol excreted during four consecutive six-hour periods. The 17-OHCS values were always lowest during the first light period (period 2) and rose to a peak during the second six hours of light (period 3). During the two dark periods (periods 1 and 4) the steroid level was usually intermediate between the high and low. Although there was individual deviation, this pattern was consistently present in groups with as few as nine animals. In some

individuals the greatest deviation occurred during the dark periods, the light-period patterns of individual animals invariably followed the group pattern. Thus, for lesioned animals, the light period excretion values of cortisol were considered to be more indicative of the animals' responsiveness to the 12L-12D regime.

A more precise picture of the rhythmical cortisol excretion pattern was obtained by shifting the six-hour collections one-half period out of phase with the 12L-12D regime. That is, the collection periods were now shifted to overlap the original periods by three hours. This procedure made it clear that the period of low cortisol excretion occurs very close to the onset of light while the high cortisol excretion period occurs after at least nine hours of light (Figure 3). These phase relations are in general agreement with results from other studies on adrenocorticoid rhythm for guinea pigs (Burstein et al., 1964), rats (Saba et al., 1963 a, b) and mice (Halberg et al., 1959).

Different light-dark regimes were established in order to determine the role of photofraction in the rhythmical cortisol excretion pattern. There was no discernible excretion pattern in response to a 6-hour light-18-hour dark cycle. In contrast, in response to a 9-hour light, 15-hour dark regime seven animals showed a rhythmical excretion pattern identical with that of animals kept on a 12L-12D regime (Figure 4). This is interpreted as evidence that at least nine hours of light are necessary for a rhythmical excretion pattern of cortisol.

Guinea pigs on a continuous darkness schedule displayed definite fluctuation of cortisol levels both individually and collectively (Figure 5), but there were no discernible rhythms. An attempt to

establish the existence of a phase shift by regression analysis proved unsuccessful. The possibility of a shift in phase is crudely evident on inspection of Figure 5, however, the use of six-hour mean values as well as discontinuous sampling may be responsible for the negative result.

When four animals which had been on total darkness were again placed on a 12L-12D regime they showed a "normal" pattern for the two consecutive light periods. The two dark period values were distinctly lower than would normally be expected. The reason for these lower values is not evident.

The results of the photofraction and continuous darkness experiments make it evident that there is a fluctuation in cortisol excretion levels which appears to be independent of photic stimulation. The present technique is not precise enough to elucidate the periodicity of any such cycle. What is evident with the present technique is that there appears to be a nine-hour photoperiod required for "setting" the daily rhythmic excretion of cortisol.

Effectively lesioned animals should respond in the same way as the non-lesioned group maintained in continuous darkness. The grouped data for lesioned animals shows no fluctuation in steroid level (Figure 6, b). In spite of this data each effectively lesioned animal showed a fluctuation in cortisol excretion for each day measured. The fluctuation was random and was not influenced by the light-dark regime. The randomness of fluctuation served to equalize the data when it was grouped. Saba *et al.* (1963b) had similar results for groups of rats with median eminence lesions, but their technique did not reveal the

fluctuation for individual animals. In further contrast to the results of Saba et al. (1963b) the effectively lesioned guinea pigs in the present study showed an overall elevation in urinary steroid levels. This urinary steroid rise may, however, be the result of an undetected infection, a dietary vitamin C deficiency, or a combination of both.

It appears, therefore, that lesions or total darkness eliminate the rhythmicity in urinary 17-OHCS levels. This rhythmicity is also eliminated if the animal is subjected to less than nine hours of light during a twenty-four hour period. On an individual basis, even without photic stimulation, there is a fluctuation in urinary cortisol levels in dark acclimated and lesioned animals. A different method of analysis may clarify these individual rhythms. The results on median eminence lesions indicate that an intact hypothalamo-hypophyseal tract is necessary for the establishment of a photoregulated adrenal rhythm.

SUMMARY AND CONCLUSIONS

The rhythmical excretion of urinary 17-hydroxycorticosterone by guinea pigs determined by four consecutive six-hour mean values has been found to be dependent on a minimum of nine hours of photostimulation in every twenty-four hours. Animals acclimated for two weeks on six hours of light - eighteen hours of dark did not give evidence of periodicity in cortisol excretion. All animals acclimated to a 12L-12D regime showed a definite and consistent excretory cortisol rhythm. The lowest steroid values occur close to the onset of light and the peak excretion of steroid requires at least nine hours of light.

Animals in continuous darkness for eleven days did not show a rhythmical excretion as a group but showed individual fluctuation in steroid levels which may possibly be evidence of an endogenous rhythm.

Effective median eminence lesions in guinea pigs prevented adherence to a rhythmical excretion pattern when the animals were kept on a 12L-12D regime. Individual lesioned animals showed random fluctuation which may also have been indicative of an endogenous rhythm. Sham-operated control animals yielded a "normal" excretory cortisol rhythm on a 12L-12D regime.

The present method did not detect any endogenous, free running cycles of urinary cortisol excretion in individual animals. There is a suggestion of a free running cycle which can be set by an appropriate daily photoperiod but the evidence to support this hypothesis is not conclusive.

A P P E N D I X

TABLE 1

Data used to plot standard curve (micrograms steroid/ml = 0.2 + 0.058 x % absorbance) for hydrocortisone alcohol (grade A) in guinea pig urine using the technique of Silber and Porter (1954).

g/cc of Hydrocortisone	Number of Days Replicated	Mean Absorbance Value ± 95% conf. lim.
0.5	5	0.007 ± 0.003
2.0	5	0.030 ± 0.003
5.0	4	0.078 ± 0.007
10.0	3	0.173 ± 0.009
20.0	3	0.427 ± 0.037

TABLE 2

Urinary 17-OHCS excretion patterns for guinea pigs acclimated to a 12L-12D regime. Thirty-two animals determined for four consecutive six-hour periods after minimum of five days acclimation. Values are μg 17-OHCS/6hr/100g BW. These data are for Figure 1.

Acclimation (Days)	Periods	1	2	3	4
	L/D regime	D	L	L	D
5 (minimum)	Range	6.4-68.9	1.9-42.2	7.4-64.1	7.4-49.4
	Mean	13.2	7.6	14.8	13.7
	± SE	1.97	1.34	1.90	1.42
n=32	Periods differing significantly		2/1 P<0.05	2/3 P<0.01	2/4 P<0.01

TABLE 3

Urinary 17-OHCS excretion data for one group of guinea pigs acclimated to a 12L-12D regime. Data are for five separate days collected during four consecutive six-hour periods. Values are μg 17-OHCS/6hr/100g BW. These data are for Figure 2, a-e.

Acclimation (Days)	Periods	1	2	3	4
	L/D regime	D	L	L	D
4	Range	7.8-20.4	3.0-19.2	8.6-27.2	6.3-22.3
	Mean	12.4	7.0	14.1	12.3
	± SE	1.35	1.63	1.82	1.86
n=9	Periods differing significantly		2/1 P<0.05	2/3 P<0.02	2/4 P<0.05
6	Range	4.9-21.8	4.2-12.4	8.3-25.8	5.2-19.4
	Mean	11.7	7.3	16.2	11.1
	± SE	1.74	0.88	2.20	1.51
n=9	Periods differing significantly		2/1 P<0.05	2/3 P<0.01	2/4 P<0.05
8	Range	5.2-17.6	2.9-15.7	12.2-36.0	4.6-21.4
	Mean	11.8	8.5	19.0	11.6
	± SE	1.53	1.48	2.83	1.57
n=9	Periods differing significantly			2/3 P<0.01	
15	Range	8.1-39.6	4.5-14.2	9.1-45.6	7.4-34.0
	Mean	18.8	8.6	23.3	16.5
	± SE	3.41	1.09	3.45	2.43
n=9	Periods differing significantly		2/1 P<0.02	2/3 P<0.001	2/4 P<0.01
20	Range	7.8-25.0	6.2-16.3	12.3-58.0	6.2-31.3
	Mean	15.4	8.9	23.6	14.2
	± SE	2.86	1.71	5.72	2.96
n=8	Periods differing significantly			2/3 P<0.05	

TABLE 4

Urinary 17-OHCS excretion values for guinea pigs on a 12L-12D schedule. Each six-hour period represents the combined data for nine animals determined five times (one animal determined four times). Values are μg17-OHCS/6hr/100g BW. These data are for Figure 2, f.

	Periods	1	2	3	4
	L/D regime	D	L	L	D
Nine animals collected for 5 days n=44	Range	4.9-39.6	2.9-19.2	8.3-58.0	4.6-34.0
	Mean	13.0	8.1	19.1	13.1
	± SE	1.02	0.57	1.54	0.95
	Periods differing significantly	2/1	2/3	2/4	3/1	3/4
			P<0.01 for each			

TABLE 5

Urinary 17-OHCS excretion for seven guinea pigs (4 virgin females) following acclimation to either six or nine hours of light in twenty-four hours. Values represent μg17-OHCS/6hr/100g BW. These data are for Figure 4.

Acclimation (Days)	Periods	1	2	3	4
	L/D regime	D	L	D	D
5	Range	6.4-32.6	8.2-18.0	8.1-30.3	10.9-30.3
	Mean	14.9	12.6	14.7	15.5
	± SE	3.27	1.44	2.83	2.55
n=7	Periods differing significantly		None		
8	Range	5.7-31.8	5.9-17.8	3.7-20.5	6.7-34.4
	Mean	13.9	11.7	12.2	17.4
	± SE	3.39	1.48	2.32	3.33
n=7	Periods differing significantly		None		
14	Range	5.6-20.9	6.8-26.1	6.5-11.5	6.2-21.5
	Mean	11.3	12.1	9.5	11.2
	± SE	1.80	2.46	0.75	1.87
n=7	Periods differing significantly		None		

	Periods	1	2	3	4
	L/D regime	D	L	½L-½D	D
6	Range	7.4-25.3	3.2-9.0	8.2-31.1	5.1-19.4
	Mean	12.9	5.9	15.4	12.6
	± SE	2.28	0.70	2.98	1.96
n=7	Periods differing significantly		2/1 P<0.02	2/3 P<0.01	2/4 P<0.01

TABLE 6

Urinary 17-OHCS excretion of guinea pigs acclimated to continuous darkness for 11 days followed by six days acclimation to a reversed 12L-12D regime. Values are μg17-OHCS/6hr/100g BW. These data are for Figure 5.

Acclimation (Days)	Periods	1	2	3	4
	L/D regime	D	D	D	D
2	Range	7.2-14.3	8.2-34.4	14.0-33.7	8.4-18.7
	Mean	8.4	15.3	21.5	12.5
	± SE	0.94	3.45	2.99	1.28
n=7	Periods differing significantly		1/3 P<0.01	1/4 P<0.05	3/4 P<0.02
7	Range	7.5-18.4	10.1-37.6	11.7-33.1	6.2-16.1
	Mean	11.7	20.5	17.0	11.5
	± SE	1.39	3.15	2.64	1.51
n=8	Periods differing significantly		1/2 P<0.05	2/4 P<0.05	
11	Range	6.5-13.9	9.3-29.8	6.8-18.2	5.2-17.1
	Mean	10.9	16.9	12.1	9.5
	± SE	1.01	2.40	1.17	1.22
n=8	Periods differing significantly		1/2 P<0.05	2/4 P<0.02	

Acclimation (Days)	Periods	1	2	3	4
	L/D regime	L	D	D	L
5	Range	10.7-15.9	5.4-8.8	4.2-8.2	6.6-9.9
	Mean	13.7	7.0	6.6	8.9
	± SE	1.24	0.78	0.84	0.77
n=4	Periods differing significantly		1/2 P<0.01	1/3 P<0.01	1/4 P<0.02

TABLE 7

Urinary 17-OHCS excretion data for guinea pigs acclimated to a 12L-12D regime following lesions or sham-operation. Each group consisted of eight animals for which urine samples were collected on a combined total of 21 or 22 days. Values are μg17-OHCS/6hr/100g BW. These data are for Figure 6, a-b.

Treatment Group	Periods	1	2	3	4
	L/D regime	D	L	L	D
Sham-operated	Range	5.6-32.8	3.3-20.7	6.2-28.8	4.8-27.1
	Mean	14.4	9.3	14.5	14.3
	± SE	1.57	1.16	1.56	1.66
n=21	Periods differing significantly		2/1 P<0.02	2/3 P<0.02	2/4 P<0.02
Effectively Lesioned	Range	7.1-112.0	5.8-73.0	7.7-79.4	7.7-95.6
	Mean	24.1	21.3	21.0	23.4
	± SE	4.91	3.51	3.80	3.88
n=22	Periods differing significantly		None		

TABLE 8

Urinary 17-OHCS excretion data for guinea pigs before and after ether-laparotomy stress. Response of sham-operated and lesioned animals are shown (μ g17-OHCS/6hr/100g BW). These data are for Figure 6, c-d.

		Unstressed	Stressed	Probability Value
Sham Lesioned	Range	7.0-28.8	15.2-50.3	
	Mean	13.9	34.1	P$\leq$0.001
n=8	$\pm$ SE	2.52	4.09	
Lesioned Animals	Range	8.3-79.4	11.8-50.2	
	Mean	24.6	24.4	NS
n=8	$\pm$ SE	8.23	4.99	

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