bovine kidney, Chang liver, human amnion, monkey kidney, human conjunctiva, and "L" cells.

Summary. Cytological changes occurring in cultures of feline renal cells infected with virus of feline viral rhinotracheitis are described. The features of cytopathogenicity of this virus are (1) development of intranuclear inclusion bodies; (2) formation of giant cells; and (3) a slight reduction in metabolic rate of infected cells.

1. Crandell, R. A., Maurer, F. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 487.
2. Madin, S. H., Andriese, P. C., Darby, N. B., *Am. J. Vet. Res.*, 1957, v18, 932.
3. Dulbecco, R., Vogt, M., *J. Exp. Med.*, 1954, v99, 167.
4. Robbins, F. C., Enders, J. F., Weller, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 370.
5. Fisher, T. N., Ginsberg, H., *ibid.*, 1957, v95, 47.
6. Cheatham, W. J., Crandell, R. A., *ibid.*, 1957, v96, 536.

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Effect of Midbrain Lesions on Ovulation and Adrenal Response to Stress in Female Rats.* (24994)

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As part of a continuing study attempting to identify brain areas essential for normal pituitary function effects of localized brain lesions on stress-induced corticosterone release and adrenal ascorbic acid decrease have been investigated. Earlier experiments revealed a dichotomy between adrenal ascorbic acid decrease and corticosterone release following surgical stress in rats with electrolytic lesions placed in different parts of the hypothalamus(1). In the present study adrenal responsiveness has been measured in rats with lesions in the diencephalon, midbrain and at the level of rostral pons. In addition, since lesions in the midbrain have been shown to inhibit ovulation(2) changes in ovulatory cycle were investigated in some animals.

Materials and methods. Coagulating brain lesions were made in 28 adult female Sprague-Dawley rats (225-300 g) from our breeding colony. The coagulating current (2.7 milliamperes/sec.) was delivered for 5-10 sec. from a Birtcher Blendtome surgical unit through paired stainless steel insect pin electrodes 1 mm apart, insulated except for 1 mm at tip. Midline lesions were made at various levels between posterior border of mammillary body and posterior end of the interpeduncular nucleus 1 to 3 mm from base of brain. In control rats electrodes were inserted but no current applied. Rats were maintained postoperatively in individual cages on Purina lab chow or force-fed 3-5 times daily with milk-5% glucose solution. Constant room temperature (37-38°C) and controlled lighting conditions were maintained(2). Four days after lesioning, the left adrenal vein was cannulated and blood collected for 30 minutes. Pre- and post-stress adrenal ascorbic acid levels were determined immediately after cannulation ("resting" levels) and at end of collection(3), using the method of Roe and Kuether(4). Corticosterone was analyzed as noted earlier(5). When necessary, anesthesia was induced by pentobarbital sodium (3 mg/100 g, i.p.). Nine rats were studied for changes in ovulatory cycles by method described by Critchlow(2). In these rats, unilateral ovariectomy was performed on first or second day post-lesioning. No rat was included in the series if microscopic examination of pituitary revealed damage from lesioning.

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TABLE I. Effect of Lesions on Adrenal Ascorbic Acid Decrease, Corticosterone Release and Ovulation in Female Rats.

Group	No. rats	Corticosterone, total μg	Adrenal Ascorbic acid — Resting level, mg %	Adrenal Ascorbic acid — % change
A Controls: no lesion	7	33 ± 2*	424 ± 28	−24 ± 2
B Midbrain lesion: central area between lesion Groups C and D	7	40 ± 1	431 ± 26	−27 ± 3
	2	*37*† *(43, 30)*	*325* *(313, 336)*	*−6* *(0, −12)*
C Lesion: level of posterior midbrain and rostral pons	3	55 (52, 54, 58)	461 (427, 438, 518)	−23 (−21, −22, −26)
	4	*68* *(56, 61, 61, 92)*	*302* *(281, 288, 317, 320)*	*−18* *(−7, −10, −17, −37)*
D Lesion: posterior diencephalon, rostral midbrain	2	19 (19, 19)	387 (369, 405)	−15 (−15, −15)
	3	*18* *(15, 20, 20)*	*376* *(352, 381, 394)*	*−6* *(0, −5, −12)*

* Mean ± S.E. of mean. † Italicized data: ovulation blocked; unilateral ovariectomy.

Animals were divided into 3 groups on the basis of location of brain lesion and corticosterone secretion rates (Table I).

Results. Lesioned areas. The central core of midbrain was extensively damaged in all lesioned rats. The lesioned area common to rats exhibiting depressed corticosteroid levels (Table I, Group D) involved posterior diencephalon (subthalamus and dorsal hypothalamus) and extended into rostral midbrain (Fig. 1). In contrast, the lesioned area common to rats exhibiting high corticosterone levels (Group C) involved posterior midbrain and extended into the level of rostral pons. In the animal of this group exhibiting highest release of corticosterone (92 μg), the most extensive lesion was observed. Only fibers of the lateral lemniscus connected anterior and posterior portions of the central nervous system. Midbrain lesions (Group B) not involving either the area of posterior diencephalon damaged in Group D animals or posterior midbrain and rostral pons level destroyed in Group C animals did not alter corticosterone levels. Brains of rats in which ovulation was shown to be blocked were damaged in region of mammillary peduncle as described previously by Critchlow(2). However, this area did not appear to exert any control on corticosterone secretion since it was undamaged in some rats of each group.

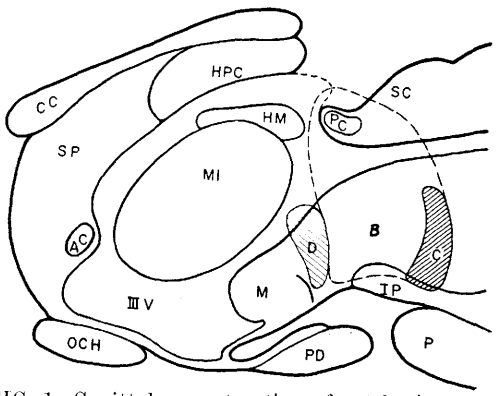

FIG. 1. Sagittal reconstruction of rat brain showing lesioned area common to all rats exhibiting inhibition of adrenal ascorbic acid and corticosterone response to stress (D), area resulting in increased corticosterone release (C) and area resulting in normal corticosterone release (B). Dashed outline representative of largest lesion evoking increased corticosterone release. Abbreviations: AC, anterior commissure; CC, corpus callosum; HM, N. habenularis medialis; HPC, hippocampal commissure; IP, interpeduncular nucleus; M, mammillary body; MI, massa intermedia; OCH, optic chiasm; P, pons; PC, posterior commissure; PD, pars distalis; SC, superior colliculus; SP, septum pellucidum; III V, third ventricle.

Corticosterone levels. The left adrenal gland of 7 control rats released an average of 33 μg corticosterone into adrenal venous effluent during the first 30 minutes post-cannulation (Table I, Group A). Comparable amounts were released in 9 lesioned rats (Group B). In contrast, 7 lesioned rats exhibited significantly elevated levels of corticosterone (Group C),—in one instance 3

times (*i.e.* 92 μg) that noted in controls. Significantly depressed levels of corticosterone were recovered from 5 lesioned rats (Group D).

Adrenal ascorbic acid (AAA). AAA response varied from normal to complete inhibition in groups B and C and was uniformly low in Group D (Table I). Resting levels of AAA were generally depressed in the unilateral ovariectomized rats in each of the lesioned groups. These animals in most cases exhibited a lesser AAA response to stress than unovariectomized members of the same group. These results in ovariectomized animals were probably due to additional stress of this operation.

Ovulatory response. In 9 of the lesioned rats the effect of midbrain damage on ovulation was checked; in each instance ovulation was observed to be inhibited. The inhibition of ovulation appeared independent of either adrenal response (Table I).

Conclusions. Since gross lesions involving posterior portion of midbrain and area at level of rostral pons resulted in increased corticosterone recovery it would appear that these structures may normally exert an inhibitory effect on adrenocorticosteroid response to stress. These findings are consistent with observation of Newman, *et al.*(6) that cortisol secretion was increased in 3 of 4 cats with brain stem lesions involving rostral pons. Other areas previously implicated in possible neural inhibition of adrenal cortical function include rhinencephalon(7) and, specifically, amygdala(8). In contrast, lesions of posterior diencephalon and rostral midbrain resulted in depressed corticosterone levels following stress, similar to those found earlier after dorsal posterior hypothalamic lesions(1). These data suggest that certain structures in posterior diencephalon and rostral midbrain must be intact for corticosteroid response to stress. The present studies confirm the previous finding that AAA and corticosterone responses to stress may vary independently following brain lesioning(1). The same brain stem areas do not appear to be involved in control of the 2 indices of adrenal function.

In addition, the data presented indicate that the midbrain area influencing the ovulatory cycle is distinct from centers concerned with ACTH regulation.

Summary. Coagulating lesions in posterior portion of midbrain and level of rostral pons of female rats resulted in increased corticosterone secretion into adrenal venous effluent in response to surgical stress. Lesions in posterior diencephalon and rostral midbrain structures resulted in depressed corticosterone secretion. Adrenal ascorbic acid response to stress was not correlated with corticosterone response. Ovulation was blocked in rats with brain lesions in any of these areas where concomitant mammillary peduncle damage was present.

1. Slusher, M. A., *Endocrinology,* 1958, v63, 412.
2. Critchlow, V., *ibid.,* 1958, v63, 596.
3. Slusher, M. A., Roberts, S., *ibid.,* 1957, v61, 98.
4. Roe, J. H., Kuether, C. A., *J. Biol. Chem.,* 1943, v147, 399.
5. Beigelman, P. M., Slusher, M. A., Slater, G. G., Roberts, S., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 608.
6. Newman, A. E., Redgate, E. S., Farrell, G., *Endocrinology,* 1958, v63, 723.
7. Porter, R. W., *Am. J. Physiol.,* 1953, v172, 515.
8. Martin, J., Endröczi, E., Bata, G., *Acta Physiol.,* 1958, vXIV, 131.

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