

the capacity to form and store 5-HT and 5-HIAA. It is highly suggestive that 5-HT and 5-HIAA measured in this study were formed in the tumor. This was supported by an increase in the content of 5-HT and a decrease in the content of 5-HIAA in tumors after application of trancylpromine. Trancylpromine is an inhibitor of monoaminoxidase<sup>6</sup> and the changes observed after administration of this drug indicate that the enzyme exists in the neurogenic teratocarcinomas. Injection of probenecid, an inhibitor of active transport of 5-HIAA from neural tissues into the blood stream<sup>7</sup>, did not affect the content of this substance in the tumors. We were thus unable to demonstrate the existence of an active transport for 5-HIAA out of the tumor, although this does not mean that 5-HIAA does not get out of the tumors. The absence of active transport could somehow be related to the histological immaturity of the neural tissue in the tumors.

**Zusammenfassung.** Neurogene Teratocarcinome weisen 5-HT und 5-HIAA-Konzentrationen auf, die ähnlich gross oder auch höher sind als im Gehirn der erwachsenen

Maus. Die Tumoren sind reich an Monoaminoxidase. Ein aktiver Transport von 5-HIAA aus den Tumoren konnte nicht nachgewiesen werden.

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<sup>6</sup> F. TH. VON BRÜCKE and O. HORNYKIEWICZ, *Pharmakologie der Psychopharmaka* (Springer Verlag, Berlin-Heidelberg 1966).

<sup>7</sup> N. H. NEFF, T. N. TOZER and B. B. BRODIE, *J. Pharmac. exp. Ther.* 158, 214 (1967).

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## Effect of Hemiovariectomy and Strain of Rat on Serum Gonadotropin Levels

The compensatory increase in both size and function of the remaining ovary in the rat following hemiovariectomy may be due to a reduction in gonadal steroid inhibition of the hypothalamus and pituitary gland resulting in a rise in serum gonadotropin levels. Although EDGREN et al.<sup>1</sup> could not detect changes in gonadotropin levels following hemiovariectomy, BENSON et al.<sup>2</sup> observed elevated plasma FSH levels 4 days after removal of 1 ovary. Using radioimmunoassays, HOWLAND and SKINNER<sup>3</sup> were able to detect elevations in serum levels of both LH and FSH 1 day after removal of an ovary on the day of estrus. Hemiovariectomy as late as 8 PM on day 3 of the cycle (estrus = day 1) in rats with 4-day-cycles or 2 AM on day 4 in rats with 5-day-cycles leads to doubling of the number of ova shed by the remaining ovary at the next estrus<sup>4</sup>. Therefore if a rise in serum levels of gonadotropins is necessary for the increase in the ova shed by the remaining ovary at the subsequent estrus, hemiovariectomy at metestrus or diestrus might lead to a rapid elevation in serum gonadotropin levels. The following study was conducted to test this hypothesis.

**Materials and methods.** Sprague-Dawley or Long-Evans strain rats from our own colony that were 8–10 weeks old were used in this study. The rats were assigned to 1 of the 3 treatment groups (Table) on the morning that a predominantly leucocytic vaginal smear was

obtained. Early in the afternoon of the same day the surgical procedures were carried out. The rats were lightly anesthetized with ether and were hemiovariectomized or sham-hemiovariectomized. The control animals received no treatment. On the following morning (approximately 21 h after surgery) the animals were removed from their cages and decapitated. This was done quickly with care being taken to avoid exciting the animals in an attempt to minimize any possible effects of acute stress. Trunk blood was collected and allowed to clot. Serum samples were frozen until assayed for FSH and LH.

The concentration of LH in individual serum samples was determined by radioimmunoassay<sup>5</sup>. FSH concentrations were determined by a similar procedure using materials distributed by the National Institute of Arthritis

<sup>1</sup> R. A. EDGREN, A. F. PARLOW, D. L. PETERSON and R. C. JONES, *Endocrinology* 76, 97 (1965).

<sup>2</sup> B. BENSON, S. SORRENTINO and J. S. EVANS, *Endocrinology* 84, 369 (1969).

<sup>3</sup> B. E. HOWLAND and K. R. SKINNER, *J. Reprod. Fert.* 32, 501 (1973).

<sup>4</sup> R. D. PEPPLER and G. S. GREENWALD, *Am. J. Anat.* 127, 1 (1970).

<sup>5</sup> G. D. NISWENDER, A. R. MIDGLEY JR., S. E. MONROE and L. E. REICHERT JR., *Proc. Soc. exp. Biol. Med.* 128, 807 (1968).

Serum levels of LH and FSH (mean  $\pm$  S.E.M.) in control, sham-hemiovariectomized and hemiovariectomized rats of 2 strains

Strain	Treatment	No. of rats	Serum LH <sup>a</sup> (ng/ml)	Serum FSH <sup>b</sup> (ng/ml)
Long-Evans	Control	11	23.6 $\pm$ 2.6	191 $\pm$ 20
	Sham	13	20.0 $\pm$ 3.8	204 $\pm$ 24
	Hemi	15	20.7 $\pm$ 2.8	289 $\pm$ 22
Sprague-Dawley	Control	16	17.0 $\pm$ 1.8	213 $\pm$ 21
	Sham	15	11.8 $\pm$ 1.2	174 $\pm$ 7
	Hemi	15	14.7 $\pm$ 1.3	350 $\pm$ 26

<sup>a</sup> Strain  $P < 0.01$ . <sup>b</sup> Treatment  $P < 0.01$ .

and Metabolic Diseases, NIH. The standards used for LH and FSH, respectively, were NIAMD rat-LH-RP-1 and NIAMD rat-FSH-RP-1.

**Results and discussion.** Analysis of variance indicated that the mean serum LH concentration in rats of the Long-Evans strain was higher than that in the Sprague-Dawley rats. The effect of treatment, when considered over both strains, as well as the interaction of strain with treatment were not significant. However, a separate analysis of LH values for Sprague-Dawley rats showed that animals subjected to sham surgery had lower ( $P < 0.05$ ) LH levels than controls. In contrast to LH, serum FSH concentration did not differ between the 2 strains but a significant ( $P < 0.01$ ) treatment effect was observed. Hemiovariectomized rats had elevated serum FSH levels when compared with either the controls or the rats subjected to sham surgery. These data extend the findings of HOWLAND and SKINNER<sup>3</sup> and suggest that hemiovariectomy, not only on the day of estrus, but also at later stages of the cycle causes a prompt rise in serum FSH levels. The lack of an effect of hemiovariectomy on serum LH in this study may indicate that a rise in the level of this hormone is not required for compensatory ovarian changes to occur. Our data also provide evidence that the LH levels as well as the changes in LH level in response to stress may vary with strain of rat<sup>6</sup>.

**Résumé.** Des rates de lignées Sprague-Dawley ou Long-Evans ont subi une hémio-variectomie ou une hémio-variectomie simulée. Les niveaux sériques de LH et de FSH ont été mesurés sur des échantillons obtenus 21 h après le traitement. Une hémio-variectomie entraîne une augmentation des niveaux sériques de la FSH, mais pas de LH. Ils sont plus élevés chez les Long-Evans que chez les Sprague-Dawley. Chez ces dernières, une opération simulée abaisse les niveaux sériques de la LH.

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## Neurosecretion in the Pineal Gland of *Macaca rhesus*<sup>1</sup>

It is well known that the mammalian pineal gland synthesizes and releases indoleamine hormones<sup>2</sup>. In addition, a group of polypeptides have been extracted from the pineal gland<sup>3-8</sup>. These peptide compounds have biological activity equivalent to or greater than that of the indoleamines and the peptides also may be pineal hormones. Speculative information only is available on the possible secretion site of the polypeptides<sup>9</sup>. Among mammals, neurosecretory fibres have heretofore been identified only in the pineal gland of the hedgehog<sup>10-12</sup>.

The present report deals with neurosecretory material in the pineal glands of 9 adult male and female monkeys (*Macaca rhesus*). Our studies have established the following facts. The monkey pineal, 1. contains neural processes which stain for neurosecretion with a variety of staining and histochemical procedures; 2. this stainable material is also found in perivascular spaces and in the walls of blood vessels; 3. a similar stainable material is found primarily within the outer layer of the multilayered corpuscles, the so-called brain sand.

The neurosecretory material was most obvious in pineal glands that had been fixed in a mixture of glutaraldehyde and picric acid. The neurosecretion was found in nerve fibres in the vicinity of the pineal recess and in fibres lying between groups of pinealocytes. The fibres contained single (Figure A) or numerous globules (Figure B) of neurosecretory material. The presence of this material within neuronal processes sometimes caused localized dilation of the fibres resembling Herring bodies of the posterior pituitary. In some areas, fibres containing neurosecretory material were found to enter the perivascular space of a capillary. The stained fibres were usually associated with droplets of similarly stained material within the perivascular space and within nearby multilayered corpuscles (Figure C). It should be emphasized that the substance within the nerve fibres, and that in the perivascular spaces and in the outer layer of the multi-

layered corpuscles, stained identically with Gomori's chromium hematoxylin phloxine method.

The character of the stained material differs from that seen in the hypothalamo-hypophyseal neurosecretory system. The pineal neurosecretory substance was characteristically purple-red after the Gomori technique with the droplets in the perivascular space containing slightly darker cores. Tintorially, the material resembles that found in the hypendymal cells of the subcommissural organ<sup>13</sup>. The pineal neurosecretion was also stainable with methods used to demonstrate endocrine polypeptide cells (see Pearse<sup>14</sup>). The substance was stained with aldehyde fuchsin after either permanganate oxidation or, more selectively, after hydrolysis in warm 0.2 N HCl. When aldehyde fuchsin was diluted with ethanol-McIlvaine

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<sup>2</sup> R. J. WURTMAN, J. AXELROD and D. E. KELLY, *The Pineal* (Academic Press, New York 1968).

<sup>3</sup> S. M. MILCU, S. PAVEL and C. NEACSU, *Endocrinology* 72, 563 (1963).

<sup>4</sup> S. PAVEL and S. PETRESCU, *Nature, Lond.* 212, 1054 (1966).

<sup>5</sup> D. W. CHEESMAN and B. L. FARISS, *Proc. Soc. exp. Biol. Med.* 133, 1254 (1970).

<sup>6</sup> I. EBELS, A. MOSZKOWSKA and A. SCAMAMA, *C. r. Acad. Sci., Paris* 260, 5126 (1965).

<sup>7</sup> B. BENSON, M. J. MATTHEWS and A. E. RODIN, *Life Sci.* 10, 607 (1971).

<sup>8</sup> R. BENSINGER, M. VAUGHAN and D. C. KLEIN, *Fedn. Proc.* 32, 252 Abs (1973).

<sup>9</sup> S. PAVEL, *Endocrinology* 89, 613 (1971).

<sup>10</sup> W. BARGMANN, *Anat. Anz.* 100, 30 (1954).

<sup>11</sup> P. SUOMALAINEN, *Bull. Mus. comp. Zool. Harv.* 124, 271 (1960).

<sup>12</sup> A. OKSHE, *Progr. Brain Res.* 10, 3 (1965).

<sup>13</sup> I. TEICHMANN, in *Neurosecretion* (Ed. F. STUTINSKY; Springer, Berlin-Heidelberg-New York 1967), p. 92.

<sup>14</sup> A. G. E. PEARSE, *J. Histochem. Cytochem.* 17, 303 (1969).