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Failure of Ovarian Hormones to Cause Mating Reactions in Spayed Guinea Pigs with Hypothalamic Lesions.*

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It has been reported that female cats with small lesions in the hypothalamus in such a position as to interrupt the supraoptico-hypophysial tract were not observed to come into heat and were never bred in the laboratory.[1] It has more recently been found that the production of small lesions in a comparable part of the hypothalamus of the female guinea pig is followed by a complete lack of the mating response and in some cases also by disturbances in the ovarian cycle.[2] Although the disturbances in the sexual cycles may be attributable to a secondary disruption of hypophysial function, the majority of the animals showed regular sexual cycles which were normal so far as the physical changes in ovaries, uteri and vaginae were concerned, suggesting that their hypophyses were functioning normally. The present investigation was undertaken in order to determine whether the lack of the mating response in guinea pigs following hypothalamic lesions of the type described is due to an hormonal insufficiency or to the destruction of neural elements indispensable to the estrous or mating reflex.

Marrian and Parkes[3] have shown that vaginal estrus may be brought about by an amount of estrogen which is insufficient to induce uterine changes or copulatory behavior. On the other hand, Dempsey and Rioch[4] were unable to induce behavioral estrus in a guinea pig following removal of the brain rostral to a plane extending between the anterior limits of the superior colliculus and the posterior edge of the mammillary bodies, although the reflex arc remained intact when the ventral limit of the section was in front of the mammillary bodies. On the basis of this evidence they have postulated a sexual center located in the ventral hypothalamus at the

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1 Fisher, C., Magoun, H. W., and Ranson, S. W., *Am. J. Obstet. and Gynec.*, 1938, **36**, 1.

2 Dey, F. L., Fisher, C., Berry, C. M., and Ranson, S. W., *Am. J. Physiol.*, 1940, **129**, 39.

3 Marrian, G. F., and Parkes, A. S., *J. Physiol.*, 1930, **69**, 372.

4 Dempsey, E. W., and Rioch, D. M., *J. Neurophysiol.*, 1939, **2**, 9.

level of the mammillary bodies. Bard,[5] however, has reported that estrous responses may be elicited in cats following massive lesions in the posterior hypothalamus which destroy all known descending paths from that part of the brain, and believes that the integration of the reflex is a mesencephalic function.

A series of 27 young, adult female guinea pigs, weighing between 400 and 600 g, were ovariectomized. Following a recovery period they were each brought into full behavioral estrus several times by the subcutaneous injection of 12.5-15.0 IU of estrogen† on hours 0, 24, 48, and 60, followed by 0.2 IU of progesterone‡ on hour 72, after the method described by Collins, Boling, Dempsey and Young.[6] After the constancy of the response to ovarian hormones had been established in each animal, lesions were placed in the hypothalamus at the level of the posterior border of the optic chiasma with the aid of a Horsley-Clarke instrument bearing a unipolar electrode. Three lesions were placed in each animal, one in the midline and one on each side of the midline at a distance of one millimeter, by passing a direct current of 3.0 ma for 30 seconds. In 22 animals the lesions were placed 1 mm above the ventral surface of the brain, and in 5 animals the lesions were placed 6 mm above the ventral surface. Five of the animals with the low lesions failed to survive the operation. Gross inspection of the brains from these animals indicates that the lesion occurs just posterior to the optic chiasma. The remaining 22 animals recovered completely, grew normally, and remained in excellent condition for the duration of the experimental period. Aside from the diabetes insipidus which developed in some animals, and a transitory period of depression which lasted for approximately 12 hours after the operation, there were no criteria by which the operated animals could be differentiated from normal anestrous female guinea pigs.

At least 2 attempts have been made to induce estrus in 17 of these animals with lesions near the ventral surface of the brain, using the dose of ovarian hormones which was sufficient to alter the behavior of the animals before the lesion. None of the animals so treated showed either proestrous or estrous behavior. It was impossible to elicit the estrous reflex by manual stimulation of the vulva or the lumbo-sacral region of the back, and none of these animals would

[5] Bard, P., *Res. Publ. Assn. Res. Nerv. Ment. Dis.*, 1940, **20**, 551.

† Theelin, through the courtesy of Dr. Oliver Kamm, Parke, Davis and Co.

‡ Proluton, through the courtesy of Dr. Erwin Schwenk, Schering Corp.

[6] Collins, V. J., Boling, J. L., Dempsey, E. W., and Young, W. C., *Endocrinology*, 1938, **23**, 188.

accept the male. All gave good avoiding responses to such stimulation, after the manner of a normal anestrous female. In subsequent trials, 8 of the animals were injected with double the usual dose of hormones and failed to show estrus, while 4 of the animals were injected with quadruple the usual dose of hormones and also failed to come into heat.

That the failure of the ovarian hormones to induce estrus in these animals is not due to a non-specific effect of destruction in the central nervous system is shown by the experiments of Bard,[7] Bard and Rioch,[8] Brooks,[9] Dempsey,[10] and Davis.[11] In addition, the 5 animals which have had lesions placed 6 mm instead of 1 mm above the ventral surface of the brain have been brought into heat with the same dose of hormone which induced estrus before the lesion was made.

These results differ from those of Dempsey and Rioch[4] and Bard.[5] Dempsey and Rioch's localization of the sexual center is based primarily upon the results of acute experiments on one guinea pig and one cat. In their chronic experiments failure to induce estrus following the removal of the anterior hypothalamic region is attributed to the debilitating effect of the operation on the animal. In their acute experiments successive transections were made in the same animal at various levels of the brain stem either with a blunt spatula or with a small sucker. In such experiments the accuracy of the localization of a "center" depends entirely upon the accuracy with which the location of the destruction to the central nervous system can be determined. We believe it may be significant that out of the 5 cats reported upon by Bard, the lesion in the one animal which failed to come into heat extended farther forward than in the other 4 animals. Although the main body of the lesion in the cats involved all known descending tracts from the hypothalamus, the possibility of the conduction of descending impulses by other paths has not yet been ruled out.

Summary. Following appropriately placed lesions at the level of the posterior border of the optic chiasma, ovariectomized guinea pigs failed to respond to previously effective dosages of estrogen and progesterone. The results reported here indicate that the failure of these animals to show estrous behavior is not due to a lack of ovarian hor-

7 Bard, P., *Am. J. Physiol.*, 1936, **116**, 4.

8 Bard, P., and Rioch, D. M., *Bull. Johns Hopkins Hosp.*, 1937, **60**, 73.

9 Brooks, C. M., *Am. J. Physiol.*, 1937, **120**, 544.

10 Dempsey, E. W., *Am. J. Physiol.*, 1939, **126**, 758.

11 Davis, C. D., *Am. J. Physiol.*, 1939, **127**, 374.

mones. It is possible that the lack of response to the hormones is a result of the destruction of a portion of the central nervous system which is indispensable to the integration of a complex behavior pattern. If further control experiments prove this to be the case, then the possibility must be considered that the integrating mechanism involved is located in the midventral portion of the anterior hypothalamus instead of the region of the mammillary bodies or the mesencephalic tegmentum.

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Selective Localization of Evans Blue (T1824) in Subplacental Portions of Entoderm in the Rat.*

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The dye, Evans blue (T1824), a non-toxic, azo-compound, isomer of trypan-blue, has been observed following intravenous injection to localize selectively in and about malignant neoplasms in animals[1] and in man[2] but does not localize selectively in and about benign tumors in man or animals.[2] In and about the malignant neoplasms it accumulates in the macrophages and fibroblasts of the stroma. It does not penetrate into the neoplastic cells themselves, whether they be carcinoma or sarcoma.

During the course of experiments to observe its localization in tumor-bearing rats a pregnant animal (about mid-term) was inadvertently employed. At necropsy 24 hours after intravenous injection of 4 mg of the dye dissolved in 1 cc distilled water, it was noted that in addition to the sarcoma there was marked selective concentration of the dye in that portion of the visceral entoderm subjacent to the disc-shaped placenta. The remainder of the visceral entoderm did not appear grossly to have localized the dye (Fig. 1). The uterine musculature appeared tinged lightly blue as did the placenta; the embryo, and the amniotic fluid contained no dye grossly visible.

To confirm these observations 7 pregnant female white rats were

* This work was carried out under a grant from the Cancer Research Institute of the Chicago Woman's Club, Chicago, Illinois.

1 Duran-Reynals, F., *Am. J. Cancer*, 1939, **35**, 98.

2 Brunschwig, A., and Clarke, T. H., *Am. J. Path.*, in press.