lecithin-cholesterol. Several alternative explanations for this fact can be offered:

(1) The tumor antigen preparations contain only one antigen which may exist in different physical states, only some of which can be conjugated to lecithin-cholesterol by the method used.

(2) The tumor antigen preparation contains multiple antigens some of which cannot be conjugated to lecithin-cholesterol particles.

(3) Excess tumor antigen was present during the conjugation procedure, and all available attachment sites on the lecithin-cholesterol particle were utilized. As obviously unagglutinated particles of lecithin-cholesterol may remain in the test solution after the conclusion of immune aggregation tests, we believe this to be improbable.

Summary. A rapid screening test for detection of antibodies to tumor antigens of papovavirus SV-40 and human adenovirus type 31 is described. The procedure is equally specific though slightly less sensitive than the CF test.

1. Habel, K., Eddy, B., Proc. Soc. Exp. Biol. & Med., 1963, v113, 1.
2. Huebner, R. J., Rowe, W. P., Turner, H., Lane, W., Proc. Nat. Acad. Sci., 1963, v50, 379.
3. Black, P. H., Rowe, W. P., Turner, H. C., Huebner, R. J., ibid., 1963, v50, 1148.
4. Rapp, F., Butel, J. S., Melnick, J. L., Proc. Soc. Exp. Biol. & Med., 1964, v116, 1131.
5. Pope, J. H., Rowe, W. P., J. Exp. Med., 1964, v120, 121.
6. Sabin, A. B., Shein, H. M., Koch, M. A., Enders, J. F., Proc. Nat. Acad. Sci., 1964, v52, 1316.
7. Shimojo, H., Yamamoto, H., Yoshikawa, E., Yamashita, T., Jap. J. Med. Sci. Biol., 1966, v19, 9.
8. Rapp, F., Kitahara, T., Butel, J. S., Melnick, J. L., Proc. Nat. Acad. Sci., 1964, v52, 1138.
9. Riggs, J. L., Takemori, N., Lennette, E. H., Proc. Soc. Exp. Biol. & Med., 1964, v120, 832.
10. Kitahara, T., Butel, J. S., Rapp, F., Melnick, J. L., Nature, 1965, v205, 717.
11. Klein, M., Chaefsky, S., Muller, M., J. Immunol., 1966, v97, 131.
12. Miller, G. L., Golder, R. H., Arch. Biochem., 1950, v29, 420.
13. Sever, J. L., J. Immunol., 1962, v88, 320.

Received March 10, 1967. P.S.E.B.M., 1967, v125.

Effect of Hypothalamic Deafferentation on Lactation in Rats.* (32163)

A. YOKOYAMA,† BELA HALÁSZ‡ AND CHARLES H. SAWYER
Department of Anatomy and Brain Research Institute, UCLA School of Medicine, Los Angeles, Calif.

Although it has been widely accepted that the suckling stimulus induces release of both prolactin from the anterior pituitary and oxytocin from the neurohypophysis, the pathway or pathways by which the suckling stimulus attains the hypothalamic areas controlling the release of these hormones has not yet been clarified. Recently considerable evidence has been reported(3,12) suggesting selective impairment of oxytocin secretion in hypothalamic lesions in lactating cats and rats. On the other hand, Averill and Purves (2) and Averill(1) reported selective blockade of prolactin secretion by lesions lateral to the paraventricular nuclei.

"Deafferentation" of the hypothalamus with a special knife designed by Halász and Pupp (7) presented itself as a good way to investigate the pathways by which the suckling stimulus causes secretion of the pituitary hormones influencing lactation. The present paper deals with preliminary observations on the effects of hypothalamic deafferentation on lactation in rats.

Methods and materials. Lactating Sprague-

* Supported by grants from Nat. Inst. Health (NB 01162) and Ford Foundation.

† Ford Foundation Fellow. Present address: Faculty of Agriculture, Nagoya Univ., Chikusa-ku, Nagoya, Japan.

‡ Ford Foundation Fellow. Present address: Inst. of Anatomy, Pecs Univ., Pecs, Hungary.

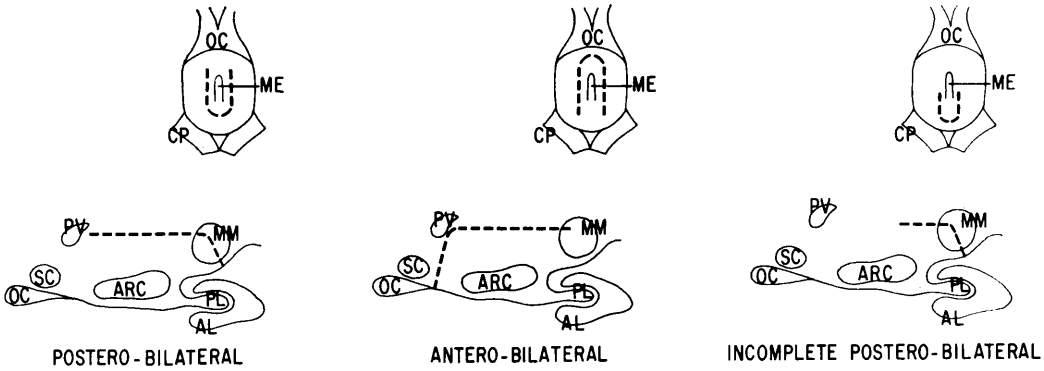

FIG. 1. Diagrammatic representation of the surgical procedures performed in 3 groups of rats. Broken line indicates extent of the knife cut. AL:anterior lobe of hypophysis; ARC: arcuate nucleus; CP: cerebral peduncle; ME: median eminence; MM: medial mammillary nucleus; PV: paraventricular nucleus; SC: suprachiasmatic nucleus; OC: optic chiasm; PL: posterior lobe of hypophysis.

Dawley rats weighing 260-360 g were used. They were housed in individual cages, each with 8 pups. Deafferentation was carried out on the 9th-11th day of lactation by the procedure of Halász and Pupp(7) under methohexital sodium (Brevital sodium, Eli Lilly & Co., Indianapolis) anesthesia.

In all rats the neural connections to the medial basal hypothalamus were partially interrupted. Three different kinds of deafferentation were performed: (a) In 4 animals the medial basal hypothalamus was left in contact with the anterior hypothalamus, but all of its other afferents were severed (postero-bilateral cut). The knife cut started just behind the optic chiasma and ended in the medial mammillary nuclei; laterally it passed through the ventromedial nuclei. The ventro-dorsal extent of the deafferented region was from the base of the brain to the level of the paraventricular nuclei. (b) The posterior connections of the media basal hypothalamus were left intact and all the others were interrupted (antero-bilateral cut, 3 animals). The extent of the deafferented region was the same as in the previous group. (c) In 2 rats only the very posterior part of the medial basal hypothalamus (premammillary - mammillary region) was deafferented bilaterally (incomplete postero-bilateral cut). The extents of the cuts in the 3 groups of rats are shown diagrammatically in Fig. 1.

Before and after the operation the weight of each mother rat and that of her pups were recorded daily. Nursing behavior was inspected and mammary glands were palpated every morning after the operation. When a decrease in litter weight was observed for 2-3 days after the operation in spite of vigorous suckling and good nursing behavior, twice daily injections of oxytocin (Syntocinon, Sandoz) (morning and evening, 2 i.u./rat, i.p.) were begun and continued for 6-7 days. Immediately after injection each mother rat was returned to her own cage to allow her to nurse her pups. The difference in the pups' weights before and 30 minutes after the injection of oxytocin was recorded, and the sum of the morning and evening increases in weight was regarded as the daily milk yield of a mother rat. After 5-6 days of the oxytocin regimen, injections were stopped for 2-3 days to reconfirm the decrease in weight of pups suckling the operated animals. The water intake of the animals was measured every day after the operation in the antero- and postero-bilateral groups and before and after operation in the incomplete group. Animals were weaned on the 21st day of lactation. Vaginal smears were taken daily during lactation and for several cycles after weaning.

At autopsy the brain was fixed in 10% formalin and serial frontal histological sections were made to check the completeness of the deafferentation microscopically.

Results and discussion. The results are summarized in Table I. After the operation, rats in both the postero-bilaterally and antero-

TABLE I. Effects of Deafferentation of the Hypothalamus in Lactating Rats on Daily Gain in Weight of Pups and on Daily Water Intake of Mothers.

Group (operation)	No. of animal	Avg daily gain in wt of pups (g) Pre-operation	Post-operation without oxytocin	With oxytocin	Changes in mothers' body wt (from the day of op. to weaning (g)	Avg daily water intake (g) Pre-operation	Operation to weaning	After weaning
Postero-bilateral	DO-1	5.1	—9.9	12.2	+ 4.0		86.6	63.5
	DO-3	12.3	—5.9	10.0	+18.0		90.5	106.0
	DO-6	11.9	—8.6	16.6	+60.0		159.4	175.5
	A-72*	9.2	—7.6	6.9	+38.0		no severe diabetes insipidus	
Antero-bilateral	DO-2	5.1	—8.5	6.1	+55.0		91.9	116.2
	DO-4	10.6	—9.9	11.8	—35.0		204.0	219.0
	DO-9	15.6	—7.4	11.2	—85.0		225.9	235.0
Incomplete postero-bilateral	DO-13	12.8	5.4†	13.1 (without OX)	—15.0	67.6	48.4	36.0
	DO-14	11.9	7.1‡	6.8 (without OX)	+15.0	60.7	57.1	31.6

* Operated on 7th day of lactation.
† Pups' weight began to increase from 2 days after operation.
‡ Pups' weight began to increase from 6 days after operation.

bilaterally deafferented groups failed to rear their pups although they revealed very good nursing behavior and the pups suckled them very vigorously. Without exogenous oxytocin supplied to the mother each litter of pups lost between 5-10 g weight per day. The mammary glands of the animals which failed in nursing were full of milk which could be easily expressed manually. An increase in pups' weight was observed after recovery from surgical shock in the 2 animals of the incomplete postero-bilateral group. Two out of 3 animals deafferented antero-bilaterally showed severe diabetes insipidus, and they drank more than 200 ml of water per day. However only one out of 4 postero-bilaterally deafferented animals showed even a moderate diabetes insipidus. In this rat the lateral cut was slightly more anterior than in the other animals. It can be assumed that the supra-optico-hypophysial tract was partially interrupted in this case.

With oxytocin replacement (2 i.u. × 2/day) the pups could obtain as much milk as before deafferentation. Therefore, it is likely that the milk obtained was uninterruptedly synthesized by the gland during the period of replacement. When the replacement regimen of oxytocin was stopped, the pups lost weight again. This finding shows that only the milk ejection reflex was impaired by either type of deafferentation.

Since the amount of milk produced was as great as that during the period before operation, the secretion of ACTH, which is believed to be one of the hormonal complex necessary to maintain lactation in the rat, seemed not to be impaired by the deafferentation. However, it is not clear from the present results whether ACTH secretion was enhanced by the deafferentation as Halász and Pupp(7) reported.

Prolactin, another hormone necessary to maintain lactation in the rat, was apparently also secreted continuously in the deafferented animals but only as long as the suckling stimulus was applied. Animals both in the postero-bilateral group and the antero-bilateral group showed vaginal estrus 3 or 4 days after weaning, and this was followed by fairly regular cycles. Neither the occurrence of ovulation nor the histology of the ovaries was studied. Halász and Gorski(8) found that the animals are not able to ovulate after the interruption of the anterior connections of the medial basal hypothalamus. In DO-4 the first estrus after weaning was followed by a persistently diestrous smear. The suckling stimulus, therefore, must have reached the hypothalamic area regulating prolactin secretion in the rats used in the present experiments. The suckling stimulus appears to activate the release of prolactin by blocking temporarily the chronic inhibitory mecha-

nism that holds prolactin secretion in check (11). Everett and Quinn(5) suggested that the area involved in the induction of pseudopregnancy (prolactin secretion?) was located over the tuber cinereum and was distinct from the region responsible for ovulation. It is not clear from the present experiments, however, by what route the suckling stimulus reached the specific hypothalamic area. Further observations are needed in the completely deafferented lactating animals although Halász and Pupp(7) reported two different types of ovarian change in completely deafferented animals.

The possibility suggested by Grosvenor (6), that secretion of prolactin might result from such exteroreceptive stimuli as smells, sights, and sounds as well as from suckling, has not been excluded in the present experiments.

Severe diabetes insipidus was observed in 2 out of 3 animals deafferented antero-bilaterally. It has been widely accepted that supra-optico-hypophyseal and paraventriculo-hypophyseal tracts convey antidiuretic and oxytocic hormones to the posterior pituitary gland. Therefore, impairment of oxytocin secretion and appearance of diabetes insipidus resulting from the antero-bilateral deafferentations seem to be attributable to blockade of these tracts. The fact that postero-bilaterally deafferented animals which failed in nursing showed little or no diabetes insipidus suggests selective impairment of the tract related to oxytocin secretion. It is now commonly accepted that production and release of oxytocic substances are related to the nucleus paraventricularis(4,9,10). Therefore the tract originating in the paraventricular nucleus appears to merge with the hypothalamo-hypophysiotrophic area at a point slightly posterior to that from the supraoptic nucleus. An anterolateral locus of this merger is indicated by the incomplete group in which neither impairment of oxytocin nor diabetes insipidus was found.

Summary. The effects of deafferenting the hypophysiotrophic area were studied in lactating Sprague-Dawley rats to trace the pathway or pathways by which the suckling stimulus affects hypophyseal function. Postero-bilateral and antero-bilateral deafferentation was carried out in 10 rats by the procedure described by Halász and Pupp. In 2 rats deafferented postero-bilaterally, bilateral deafferentation was started 0.5 mm posteriorly, so that the lateral deafferentation was incomplete. The operated animals (with the exception of these 2 rats deafferented incompletely) failed to rear their pups. This failure is ascribed to the blockade of milk ejection since pups could obtain milk when the mother was injected with exogenous oxytocin. Severe diabetes insipidus was observed in 2 out of 3 animals deafferented antero-bilaterally. Since only one of the 4 rats deafferented postero-bilaterally showed any diabetes insipidus, yet all showed loss of reflex discharge of oxytocin, the pathways of the latter mechanism to the hypophysiotrophic area must enter laterally, and they cannot be identical to the supra-optico-hypophyseal tract.

1. Averill, R. L. W., J. Endocrinol., 1965, v31, 191.

2. Averill, R. L. W., Purves, H. D., ibid., 1963, v26, 463.

3. Beyer, C., Mena, F., Am. J. Physiol., 1965, v208, 585.

4. Brooks, C. McC., Ishikawa, T., Koizumi, K., Lu, H-H., J. Physiol., 1966, v182, 217.

5. Everett, J. W., Quinn, D. L., Endocrinology, 1966, v78, 141.

6. Grosvenor, C. E., ibid., 1965, v76, 340.

7. Halász, B., Pupp, L., ibid., 1965, v77, 553.

8. Halász, B., Gorski, R. A., ibid., v80, 608.

9. Nibbelink, D. W., Am. J. Physiol., 1961, v200, 1229.

10. Olivecrona, H., Nat., Lond., 1954, v173, 1001.

11. Tindal, J. S., in Reproduction in the Female Mammal, G. E. Lamming, ed., Butterworth, London, in press 1966.

12. Yokoyama, A., Ota, K., Endocrinol. Japon., 1959, v6, 268.

Received March 10, 1967. P.S.E.B.M., 1967, v125.