added coenzyme and .61 with coenzyme, from which it was inferred that a great loss in coenzyme accompanied the lesions in the mouse (1). Thus it appears that loss of coenzyme in the dystrophic chick muscle is proportionately not as large as in the dystrophic mouse muscle. Normal white chick muscle contains more G-1-6-diphosphate than rat or mouse muscle. Repeated experiments showed that saturation of the PGM system with this coenzyme was not possible with additions equivalent to 8-16 mg of boiled extract prepared from rodent muscle whereas saturation was readily achieved when white chick muscle was employed.

Phosphorylase activity, as well as PGM, is considerably greater in white than in red muscle of the normal chick(3). It is only in white muscle that phosphorylase is decreased in the vit. E-deficient chick. The sensitivity of white muscle to the vitamin deficiency might be correlated with the initially high levels of phosphorylase and PGM, and a longer period of feeding the deficient diet might possibly affect the red muscle as well. PGM activity in the back muscles of rabbits fed a vit. D-deficient diet was lower than normal but the results were not statistically significant(9).

Summary. Phosphoglucomutase activity determined with and without added coenzyme (glucose-1-6-diphosphate) was increased in white muscles of chicks fed a vit. E-deficient diet, but was unaffected in red muscle. Muscle lesions were more extensive in the white muscle. Normally white muscle contains more PGM than red muscle. The coenzyme for PGM is much more plentiful in white chick muscle than in rat or mouse muscle so that direct determination of maximum PGM activity by saturation of the system with added coenzyme is possible, thus eliminating the necessity of extrapolating to infinite coenzyme concentration.

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Estrogen Therapy in Immature Female Rats with Posterior Hypothalamic Lesions. (26495)

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Recent findings(1-5) support the concept of hypothalamic control of the hypophyseal-gonad axis. Corbin and Schottelius(6) have further implied a dual capacity of the hypothalamus, as regards puberal initiation and sexual maturation. Failure of the puberty-parturition continuum may be attributed to ovarian hypofunction, representing a secondary effect of posterior hypothalamic damage.

Gale(7) tested the effectiveness of replacement therapy in hypothalamically lesioned, pregnant rats, by administering progesterone and estradiol benzoate and preventing gestational impairment and abortion. Information is relatively scarce, however, relating estrogen therapy to reproductive organ maturation in immature female rats with discrete hypothalamic lesions. The response of the reproductive organs to estrogen replacement in prepuberal female rats with posterior hypothalamic lesions is the object of this study.

TABLE I. Means and Standard Errors of Body Weights, Hypophyses, Reproductive Organs, Vaginal Opening and Cycling in Normal, Sham-Operated, Lesioned Untreated, Lesioned Estrogen-Treated, and Lesioned Oil-Treated Female Albino Rats. No. of animals per group in parentheses.

	Body wt (g) (21 days)	Body wt (g) (75 days)	Hypophysis wt (mg) (75 days)	Reproductive organ wt (mg) (75 days)	Day vagina opened	Cycling began, days post vaginal opening	Vagina opened days post treatment	Cycling began, days post vaginal opening
Group 1, normal control (13)	39.1 ± 2.6	178.3 ± 3.7	6.41 ± .51	450.7 ± 10.4	37 ± .51	7 ± .25		
Group 2, sham-operated control (11)	41.6 ± 2.3	170.8 ± 5.7	6.60 ± .46	490.6 ± 29.1	36 ± .54	7 ± .25		
Group 3, lesioned untreated control (8)	42.5 ± 1.0	142.8 ± 10.3	2.70 ± .20	173.0 ± 23.0	60 ± 5.7	Diestrus		
Group 4, lesioned estrogen-treated (8)	42.0 ± 1.2	152.3 ± 18.1	3.60 ± .27	443.2 ± 24.7	45 (2 animals)	"	53 ± 1.9 (6 animals)	Constant estrus
Group 5, lesioned oil-treated control (4)	42.0 ± .84	144.5 ± 12.9	3.60 ± .49	166.6 ± 30.1			69 ± 2.9	Diestrus

Methods and materials. Forty-four, 21-day-old female albino rats (Holtzman) were divided into the following groups: Group 1 (13 animals) was the normal control group; Group 2 (11 animals) was the sham-operated control group; Group 3 (8 animals) consisted of the lesioned, untreated subjects; Group 4 (8 animals) contained the lesioned, estrogen-treated rats, and Group 5 (4 animals) represented the lesioned, oil-treated control group. Bilateral destruction of the mammillary body (MB) region was performed on day 21. Lesioning technic was a modification of that described by Corbin and Schottelius(6). Animals were allowed Rockland rat diet and water *ad lib.* Body weights were recorded every 5 days. From the time of vaginal opening, smears were examined daily up to time of autopsy to determine presence and length of cycles. On day 52, Group 4 received 0.5 μg estradiol valerate in 0.1 ml of sesame oil, I.M. (a repository dose, effective for approximately 3 weeks), and Group 5 received 0.1 ml of the vehicle. All rats were observed for behavioral changes, and were sacrificed on day 75. Fresh weights of pituitaries and reproductive organs (total weight of ovaries + oviducts + uteri + vagina) were recorded. Brains were fixed in 10% formalin for 5 days, sectioned at 10 μ and stained in H and E. Data were statistically analyzed by Students' "t" test. A probability of $\leqq$ 0.05 was considered to be significant.

Results. Results are summarized in Tables I and II. Histological examination of lesioned brains revealed damage limited to areas at the level of the MB. Animals with posterior hypothalamic damage showed definite inhibition of reproductive organ development, their organs being less than ½ the weight of the normal controls. Groups 3 and 5 had the smallest reproductive organ and pituitary weights. These animals exhibited thin, unvascularized, atrophied sex organs. Moreover, the ovaries were pale, showing retarded follicular and luteal development. Pituitaries of animals in Groups 3, 4, and 5 were significantly smaller than those of the normal and sham-operated controls, indicating a direct correlation between hypophyseal

TABLE II. Table of "t" Values for Normal, Sham-Operated, Lesioned Untreated, Lesioned Estrogen-Treated and Lesioned Oil-Treated Female Albino Rats at 75 Days.

Groups compared	Body wt	Hypophysis wt	Reproductive organ wt	Day of vaginal opening
1 & 2	1.10	.28	1.33	1.35
1 & 3	3.25*	4.56*	11.0 *	4.03*
1 & 4	1.42	4.0 *	.24	
1 & 5	2.52†	4.18*	8.90*	10.3 *
2 & 3	3.58*	3.60*	8.27*	4.21*
2 & 4	.97	5.80*	1.24	
2 & 5	1.37	4.50*	7.76*	10.8 *
3 & 4	.43	2.72†	8.31*	1.16
3 & 5	.10	1.90	.17	1.40
4 & 5	.33		7.13*	4.57*
4 & 3 + 5				2.25‡

* $P \leq .01$ † $P \leq .02$ ‡ $P \leq .05$

weight and hypothalamic integrity.

Vaginae of Groups 1 and 2 opened between days 36 and 37, with a cycling pattern being established 7 days later. In contrast, Groups 3 and 5 (which are theoretically identical) did not exhibit vaginal opening until days 60 and 69, respectively, and thereafter gave continuous leucocytes smears. Thus, sesame oil alone had no effect upon Group 5. Group 4 included 2 animals whose vaginae opened on day 45 which, nevertheless, remained in constant diestrus.

The vaginae of the six estrogen-treated rats of Group 4 opened on day 53, one day after treatment. The reproductive organs of Group 4 were equivalent in weight to those of the normal and sham-operated controls, at least by the time of sacrifice.

While cannibalism and viciousness were present in some lesioned subjects, there were no indications of hyperphagic behavior or obesity. Estrogen was ineffective in relieving the hostile attitude of such animals. Although a significant difference existed between body weight of the normal controls and the lesioned untreated animals, this may be attributed to the fact that the lesioned animals reduced their food intake for about 2 weeks after operation.

Discussion. Comparison of the reproductive organs of a normal control, sham-operated control, lesioned untreated, lesioned estrogen-treated and lesioned oil-treated rat is shown in Fig. 1.

FIG. 1. Comparison of reproductive organs of normal control (A), sham-operated control (B), lesioned untreated (C), lesioned estrogen-treated (D) and lesioned oil-treated (E) rat at 75 days. Redrawn from photograph.

Ovarian failure in the lesioned rats not receiving estrogen therapy is evidenced by delayed puberty, reproductive organ immaturity and persistent diestrus state. Pituitary weights are compatible with such gonadal hypofunction; but, as is generally conceded, weight alone is not a reliable index of hypophysial secretory function. It appears evident that posterior hypothalamic damage prevents elaboration or release of hypophysial gonadotropins, thus inhibiting ovarian estrogen secretion; hence, the atrophy of the reproductive complex.

Those animals receiving estradiol valerate showed vaginal opening within one day of injection, and exhibited reproductive organ growth comparable in weight to those of normal and sham-operated controls, and in considerable excess of Groups 3 and 5. The hormone treated animals lacked developing ova; they exhibited a constant estrous state, as evidenced by continuous cornified smears. Absence of estrual cycling in these animals strengthens the concept of the dependence of gonadotropin release upon hypothalamic function. The lack of ova production and the state of impaired estrual cycling in all the lesioned animals (indicating absence of hypophyseal-ovarian feedback) strongly imply failure of the FSH-LH mechanism whose maintenance is, in turn, dependent upon normal hypothalamic integrity(8-11).

Statistically, Groups 3 and 4 did not show a significant difference in day of vaginal opening which is misleading due to the fact that the opening was induced by estrogen treatment. Since the day of vaginal opening in Groups 4 and 5 is significantly different, and since Groups 3 and 5 are essentially identical, the latter groups were combined for statistical analysis. Consequently, upon com-

parison with Group 4, a significant difference was seen in day of vaginal opening.

Summary. Twenty, 21-day-old female albino rats were subjected to bilateral lesions of the region of the mammillary bodies. Within one day of receiving a repository dose of 0.5 μg of estradiol valerate, all 6 recipients showed vaginal opening with subsequent constant estrus and reproductive organ growth. Four animals receiving sesame oil and 8 receiving no treatment exhibited definite signs of delayed puberty and reproductive organ immaturity. These results indicate the inability of the prepuberal hypothalamus to elaborate or secrete those neurosecretory substances essential for the functional sexual state of the mammal, and the dependence of estrogen secretion upon normal hypothalamic activity as mediated *via* the hypothysis.

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Adaptation of Fluorescent-Microscopy to Determination of Genetic Variations in Mouse Mammary Tumor Agent. (26496)

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It was previously found that fluorescent microscopy could be adapted for studying the antigenic mosaic make-up of tissues containing the mouse mammary tumor agent (MTA) (1). While fluorescent microscopy, as Hughes(2) indicated, has definite limitations in differentiating neoplastic from normal tissues, we have found that this method offers certain advantages over the routine serologic and neutralization tests used for such studies.

"Incidence" and "age of occurrence" of mammary tumors in the mouse, have been shown to be influenced by certain inherited factors(3). Especially implicated have been the hormonal make-up of the animal and the genes responsible for natural protection or susceptibility to the MTA. Certain inbred stocks of mice have been developed that not only have a high incidence of tumors at an early age, but can pass the agent to succeeding generations by nursing. Inbred genetically pure mice have also been selected which are highly resistant to the MTA.

Little is known about the antigenic changes occurring within the MTA when it is passed from one strain of mice to another. In part, this is due to inability to isolate and purify a single entity which might be termed the "agent" and in part due to the time period necessary for neutralization tests. Inhibition or neutralization tests are the only reliable method so far available for determining the effect of antisera on the MTA. Unfortunately, it requires a period of 6 months, in

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