duction of rat anti-rabbit globulin antibodies in the new born, such as is known to be a factor in the development of disease in adult rats(8). (d) The failure to produce clinical disease in newborn rats injected with nephrotoxic serum may be due to the fact that new glomeruli open as the animal matures.

Summary. Rabbit antibodies against adult rat kidney were found to be fixed in the kidneys of new-born rats. Since new-born rats do not develop clinical disease when injected with anti-rat kidney antibodies, this suggests either that the antigens giving rise to cytotoxic antibodies are not present at this early date or the new-born rats are deficient in complement. Six weeks after new-born rats are so injected they still have the rabbit globulin (antibody) in the glomeruli. However, rabbit globulin was demonstrated in the glomeruli on the medullary side of the cortex and not in those of the periphery indicating that new glomeruli in the peripheral region become functional as the animal matures. The increase in functional glomeruli may be the reason for the observation that new-born rats injected with anti-rat kidney serum do not develop clinical disease.

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Hormonal Control of Mammary Gland Involution in the Rat.* (27557)

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Previous studies in this laboratory have employed desoxyribonucleic acid (DNA) as a quantitative index of normal and experimental growth of rat and mouse mammary glands(1,2,3,4,5). Present investigation was undertaken to study effects of oxytocin (OXT), lactogen‡ (LGH), and hydrocortisone acetate (HCA) on mammary gland involution in the rat. Morphological methods (6) have demonstrated that all these hormones will retard retrogression of lobule-alveolar system of rats. The question arises whether the hormones actually retard cellular degeneration or only maintain milk synthesis in remaining secretory cells. Since DNA is an index of cellular number, it is possible to answer this question by its determination.

Materials and methods. Primiparous Sprague-Dawley Rolfsmeyer rats were employed in present experiments. Young were separated from control and experimental animals on day 3 of lactation, and dams were injected for a 10-day period. Experimental animals received (a) 2 USP units of OXT, either intraperitoneally (ip) or subcutaneously (sc), 3 × daily (b) 1 and 2 mg LGH sc (c) 100, 500 and 1000 μg of HCA sc from day of weaning. Animals were killed on day

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‡ Lactogenic hormone, ovine, kindly supplied by Nat. Inst. Health.

TABLE I. Effect of Lactogen, Oxytocin, and Hydrocortisone Acetate upon Mammary Gland Involution in Rat.

No. of animals	Days of lactation or involution	Treatment	Final BW† (g)	DFFT* (mg)	DNA (μg/mg DFFT)	Total DNA (mg/100 g BW†)
			Mean		± S.E.	
8	Day 3 of lactation	—	269	789	37.5 ±1.26	10.78 ±.34‖
38	3-day lactation, 10-day involution	—	270	342	29.9 ± .59	3.77 ±.14
20	*Idem*	OXT‡	258	290	29.3 ± .61	3.25 ±.11
14	"	OXT§	264	330	27.6 ± .96	3.46 ±.24
19	"	1 mg LGH sc	285	439	28.2 ± .62	4.43 ±.23[2]
21	"	2 mg LGH sc	278	529	29.5 ±1.05	5.58 ±.24[1]
9	"	100 μg HCA sc	264	327	30.1 ± .79	3.67 ±.83
18	"	500 μg HCA sc	238	374	29.6 ±3.1	4.61 ±.15[1]
14	"	1000 μg HCA sc	217	389	27.6 ±1.2	4.85 ±.26[1]

* Dry, fat-free tissue. † Body wt. ‡ OXT—2 USP units 3×/day ip. § OXT—2 USP units 3×/day sc. ‖ From Ref. 4. OXT = oxytocin. LGH = lactogen. HCA = hydrocortisone acetate. [1] Probability <.01 from control. [2] Probability <.05 from control. sc = subcut. ip = intraper.

11, and 6 abdominal-inguinal mammary glands were removed from each rat. DNA was determined as previously described(4).

Results. Mammary gland DNA/100 g body weight (BW) on day 3 of lactation has been determined as 10.78 mg(4). After 10 days of involution, control DNA values were 3.77 mg/100 g BW. Mean DNA or OXT injected animals, either ip or sc, was 3.24 and 3.46 mg/100 g BW. LGH, 1 or 2 mg, DNA values were 4.43 and 5.58 mg/100 g BW. HCA at 100, 500, and 1000 μg levels gave DNA values of 3.67, 4.61 and 4.85 mg/100 g BW, respectively. DNA levels following OXT by either method of administration were not significantly greater than in control animals. DNA values after both levels of lactogen, 1 mg ($P<.05$) and 2 mg ($P<.01$) were significantly greater in comparison with controls. Based on final BW, HCA increased DNA markedly when 500 or 1000 μg were employed ($P<.01$), but not at the lower level (100 μg). Mean weight loss of 500 and 1000 μg HCA groups was 20 and 48 g respectively. Other groups maintained their BW.

Discussion. Grosvenor and Turner(7) demonstrated nembutal blocks oxytocin and lactogen release from the pituitary in response to nursing stimuli. OXT at levels of .1, 3 and 6 USP units/kg failed to bring about lactogen release in anaesthetized lactating rats. These data supplied direct evidence that OXT is not the humoral link involved in lactogen discharge.

Present data demonstrated that OXT had no effect upon retarding mammary gland cellular regression during a 10-day involution period. These data also contradict hypothesis that OXT directly stimulates release of LGH from anterior pituitary. If LGH release was influenced by OXT, DNA values of OXT groups should be equal to those receiving lactogen. OXT, however, appeared to maintain some secretory activity in remaining cells. Milk was observed to flow from glands in some animals during removal. This observation may aid in explaining the discrepancy observed between morphological technics and DNA method of estimating mammary gland involution. OXT may maintain some secretory activity but not cellular integrity of mammary gland.

DNA values of animals administered LGH (1 and 2 mg) were approximately 15 and 48% greater than controls. This hormone apparently maintained mammary glands by both retarding cellular degeneration and prolonging secretory activity of cells. Milk was observed to flow from the majority of glands during removal.

HCA also appeared to maintain both cellular integrity and secretion of glands if based on final BW. However, if DNA is calculated on initial BW, the values for the 500 and

1000 μg groups would be 4.27 and 4.01 mg/100 g BW respectively. On this basis the DNA level in the 500 μg HCA group was 13% greater than controls ($P<.05$).

Summary. Effect of oxytocin, lactogen, and hydrocortisone acetate upon mammary gland involution was studied in rat. LGH retarded involution to a significant degree, by maintaining DNA 48% above control group. HCA at levels of 500 and 1000 μg retarded involution significantly when DNA was related to final BW. OXT by either subcutaneous or intraperitoneal administration was not effective in retarding involution. These hormones, however, appear to maintain some secretory activity of involuting mammary glands. Data also suggest OXT has little influence upon release of lactogen from anterior pituitary.

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Adaptation of Equine Abortion Virus to Earle's L Cells in Serum-Free Medium with Plaque Formation.* (27558)

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One of us(1) has previously reported the adaptation of equine abortion virus (EAV) to HeLa cells grown in monolayers. Repeated attempts in the past to infect Earle's original or 929 clone cells with wild type virus from infected horse tissue or with hamster-adapted virus met with failure, and were abandoned until recently. The L cell strain adapted to serum-free medium by Merchant(2) has been used in this laboratory for metabolic studies. The usefulness of this system prompted repetition of efforts to adapt EAV. The following report deals with the successful attempt to adapt this agent to L cells utilizing virus which had been adapted to another tissue culture system (HeLa).

Materials and methods. Virus. The 109th passage of EAV in HeLa cells sonicated for 4 minutes was used as the original inoculum. Precautions were taken to centrifuge the inoculum for 10 minutes at 10,000 rpm to sediment thoroughly any cells that might have escaped the process of disruption. Passage material was routinely stored at −40°C until ready for use. After freezing and thawing 2-3 times and centrifugation to deposit the gross cellular debris, monolayer cultures were inoculated with 1 ml of undiluted material.

Tissue culture systems. The tissue culture cells used in this study were obtained from Dr. Donald J. Merchant, University of Michigan, and were designated L-M 929 which originally was a clone of Earle's 929 clone cells. The cells were routinely maintained in log phase suspensions, being agitated at 120 rpm in an Eberbach rotary shaker kept at 37°. Monolayers were obtained by seeding 4-oz Brockway glass bottles with 5 ml of cell suspension diluted with fresh medium to a concentration of approximately 2×10^5 cells per ml. The medium for all cultures was serum free and is referred to as YELP by Merchant(2). Excellent monolayers were obtained in 1-3 days.

Histological procedures. For histological studies 1 ml containing approximately 200,000 cells was introduced into Leighton

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