through agar may be due to its instability at 37°(2), and to inactivation by agar or aqueous agar extracts(17).

Summary. Japanese B encephalitis virus on hamster kidney cell cultures has been studied by the plaque method, and the following results obtained. 1. The effect of some environmental variables on plating efficiency: a. Maximum adsorption was obtained by 120 minutes incubation at 35°C. b. Optimal conditions for initiating plaques were obtained by seeding the virus in a small volume of lactalbumin medium containing bovine albumin. c. Cations were required for maximum virus adsorption but plaques were produced in the absence of added Ca^{++} and Mg^{++}. d. Washing cultures with phosphate-buffered saline (pH 7.0) before and after exposure to virus was not required, and it was shown that plaques developed only from virus adsorbed on cells before agar was applied. 2. The titers obtained by plaque assay were in almost all instances similar to those of the tube culture method and varied with the mouse titer as might be expected on the basis of the passage level in mice or in HKC. 3. Hamster kidney cells proved slightly more sensitive for plaque assay than chick embryo cells, when HKC adapted virus was used.

1. Dulbecco, R., Vogt, M., *J. Exp. Med.,* 1954, v99, 167.

2. Diercks, F. H., Hammon, W. McD., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 627.

3. Rohitayodhin, S., Hammon, W. McD., *J. Immunol.,* 1962, v89, 823.

4. Melnick, J. L., in *Diagnostic Procedures for Virus and Rickettsial Diseases,* 2nd ed., p142, *Am.* Public Health Assn., 1956.

5. Rhim, J. S., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 887.

6. Puck, T. T., Garen, A., Kline, J., *J. Exp. Med.,* 1951, v93, 65.

7. Bachtold, J. G., Bubel, H. C., Gebhardt, L. T., *Virology,* 1957, v4, 582.

8. McLaren, L. C., Holland, J. J., Syverton, J. T., *J. Exp. Med.,* 1960, v112, 581.

9. Wallis, C., Melnick, J. L., *Virology,* 1962, v16, 122.

10. Rhim, J. S., Melnick, J. L., *Tex. Rep. Biol. Med.,* 1961, v19, 851.

11. Olitsky, P. K., Yager, R. H., Murphy, L. C., *U. S. Armed Forces Med. J.,* 1950, v1, 415.

12. Kanda Inoue, Y., Iwasaki, T., Kato, H., *J. Immunol.,* 1961, v87, 337.

13. Tytell, A. A., Torop, H. A., McCarthy, F. J., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 916.

14. Valentine, R. C., Allison, A. C., *Biochem. Biophys. Acta,* 1959, v34, 10.

15. Bachrach, H. L., Callis, J. J., Hess, W. R., Patty, R. E., *Virology,* 1957, v4, 224.

16. Youngner, J. S., *J. Immunol.,* 1956, v76, 288.

17. Schulze, J. T., Schlesinger, R. W., *Fed. Proc.,* 1962, v21, 455.

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Experimental "Lactational" Mammary Gland Growth in the Rat as Measured by DNA.* (28064)

DAVID R. GRIFFITH† AND C. W. TURNER
(With technical assistance of Mary E. Powell)
Department of Dairy Husbandry, University of Missouri, Columbia, Mo.

Previous studies in this laboratory have employed desoxyribonucleic acid (DNA) as a quantitative index of mammary gland development during pregnancy, lactation, and involution(1,2,3). It had been considered that mammary gland growth occurred primarily during the first ⅔ of pregnancy, followed by gradual initiation of lactation during the final trimester(4). By estimating DNA of the mammary glands, it was found that DNA increased significantly during last ⅓ of pregnancy and between day 1 and 3 of lactation indicating cellular proliferation continues during early stages of lactogenesis in

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TABLE I. Experimental Lactational Growth in Rat Mammary Gland.

Treatment	No. of animals	B.W. (g) mean	DFFT (mg) mean	DNA (μg/mg DFFT)	Total DNA (mg/100 g B.W.)
Day 3 of lactation	8	269	789	37.5 ± 1.26	10.78 ± .34
1 μg E+3 mg P—19D	21	278	606	31.5 ± 1.02	6.81 ± .38
" " —24D	16	262	561	30.5 ± .79	6.37 ± .29
" " + 3D— 2 mg LGH	20	248	490	38.1 ± 1.42	6.41 ± .35
" " + 3D—250 μg HCA	7	251	659	28.0 ± 1.22	7.18 ± .39
" " + 3D 250 μg HCA +2 mg LGH	12	255	641	30.3 ± 1.20	7.25 ± .48
2 μg E+6 mg P—19D	22	292	606	29.3 ± 1.45	6.06 ± .36
" " —24D	12	277	584	29.6 ± .89	6.22 ± .31
" " + 3D 2 mg LGH	5	321	854	26.6 ± 1.56	6.63 ± .39
" " + 3D 1.5 mg GH	5	321	772	29.3 ± 1.81	9.05 ± .39*
" " + 3D+ 2 mg GH	6	241	662	25.3 ± 1.42	6.02 ± .30
" " + 3D 250 μg HCA	5	274	864	28.2 ± .77	8.37 ± .50*
" " + 3D+ 2 mg LGH 1.5 mg GH	9	305	891	28.4 ± .78	8.62 ± .26*
" " + 3D+ 2 mg GH 500 μg HCA	7	274	940	27.5 ± 1.60	9.15 ± .31*
" " + 3D+ 2 mg LGH 1.5 mg GH 250 μg HCA	6	298	862	29.1 ± 1.93	8.2 ± .17*

E—estradiol benzoate
P—progesterone
HCA—hydrocortisone acetate
LGH—lactogenic hormone
GH—growth hormone
D—days
DFFT—dry, fat-free weight
* Significant to E+P control animals P>.01.

rat, mouse, and guinea pig(1,2,5,6,7). This latter growth may be defined as "lactational" in comparison to "pregnancy" growth.

It has been shown possible to develop mammary gland DNA experimentally approximately equal to that found at end of normal pregnancy by ovarian hormones, estrogen and progesterone(8). Other studies have reported initiation of lactation, in suitably primed experimental animals, by exogenous administration of hydrocortisone acetate (HCA) growth hormone (GH) and lactogen (LGH) in various combinations(9,10,11,12,13,14).

The purpose of this study was to determine if the pituitary hormones, GH and LGH and adrenal hormone HCA, in various combinations, were capable of stimulating experimentally the post-parturient "lactational" growth of the mammary gland of normal animals.

Material and methods. Data were obtained from primiparous Sprague-Dawley-Rolfsmeyer rats which were ovariectomized 2 weeks before beginning the study. Animals received subcutaneous injection of 1 μg estradiol benzoate (EB) and 3 mg progesterone (P) or 2 μg EB and 6 mg P daily for 19 or 24 days to induce lobule-alveolar development about equal to that observed at end of pregnancy. On day 20 E + P administration was eliminated in experimental animals; they then received LGH‡ (2 mg), GH‡ (1.5 or 2.0 mg) and HCA (250 or 500 μg) alone or in combination daily for 3 days. Animals were killed on day 24, and DNA determined as previously described(1).

Results. Injection of 1 μg EB and 3 mg P for 19 and 24 days into ovariectomized rats stimulated mean DNA gland values of 6.81 and 6.34 mg/100 g BW, respectively. These DNA values for the mammary glands are somewhat lower than corresponding value for rats at end of pregnancy (DNA, 7.63 mg) (1). Mean DNA values for glands of animals injected with 2 μg EB and 6 mg P for 19 and 24 days were 6.06 and 6.22 mg/100 g BW. DNA values for animals injected for 24 days were not significantly greater than after 19 days (Table I).

When animals were injected with 1 μg EB + 3 mg P for 19 days, then with 2 mg LGH, 250 μg HCA or combination of these 2 hormones daily for 3 days, mean DNA values of glands were 6.41 mg, 7.18 mg and 7.25 mg/

‡ LGH and GH kindly supplied by NIH.

100 g BW. These values were not significantly different from animals injected with EB + P alone.

Groups of animals injected with 2 μg EB + 6 mg P daily for 19 days, then for 3 days with (a) 250 μg HCA, (b) 1.5 mg GH, (c) 2 mg LGH + 1.5 mg GH, (d) 2 mg GH + 500 μg HCA, (e) 2 mg LGH + 1.5 mg GH + 250 μg HCA stimulated increased gland growth as indicated by DNA/100 g BW of 8.37 mg, 9.05 mg, 8.62 mg, 9.15 mg and 8.2 mg, respectively. These DNA values were all increased significantly in comparison with E + P values at 19 or 24 days.

Administration of 2 mg LGH alone was ineffective in stimulating increased DNA (6.63 mg/100 g). No reason can be advanced for the ineffectiveness of the 2 mg level of GH in comparison to 1.5 mg unless the group contained a number of rats with reduced capacity for mammary gland growth (see frequency distribution of DNA of rat mammary glands(8)).

It will be noted that DNA values of all treatment groups were significantly less than the DNA value observed on day 3 of lactation ($P<.01$). However, DNA values on day 19 or 24 of EB + P were also lower than day 19 of pregnancy. Injection of 2 mg GH + 500 μg HCA, stimulated mean DNA of 9.15 mg/100 g BW. This value is 47% greater than EB + P at day 24 and should be compared with an increase of 40.7%(1) during comparable post-parturient period. It is only 15% less than DNA value at day 3 of lactation.

Mammary glands of rats at end of EB + P treatment show little evidence of milk secretion. All hormones used induced various degrees of lactation. GH, however, was less effective than HCA or LGH alone or in combination.

Discussion. Previous data have indicated mammary growth continues during early lactation(1). Mammary gland DNA increased approximately 40.7% from days 18-20 of pregnancy to day 3 of lactation. Although normal day 3 DNA lactating value of 10.78 mg was not attained experimentally, a comparable increase in DNA of 47% above the 2 μg EB + 6 mg P value occurred in the present study with injection of 500 μg HCA + 2 mg GH. Further, 1.5 mg GH alone was quite effective.

While the two levels of ovarian hormones appear equally effective in stimulating mammary gland of pregnancy, only the higher level appeared to condition the glands to respond to GH and HCA. Lactogenic hormone which is secreted in increased amounts postpartum and is necessary for initiation and maintenance of milk secretion did not influence "lactational growth." The two key hormones in stimulating mammary gland growth post-partum appear to be GH and HCA separately and together.

Milk secretion was observed in all mammary glands upon gross inspection after the 3-day treatment. Exogenous lactogenic hormone could be expected to have such effect. HCA has been shown to stimulate lactation (13) possibly by stimulating the release of endogenous lactogenic hormone. GH appears to have a similar but less marked effect.

Ovariectomy on day 2 of lactation(15,16) does not prevent "lactational growth" of mammary gland. The present study with ovariectomized animals also indicates that ovarian hormones are unnecessary.

It is concluded that ovarian hormones (EB + P) play a dominant role in the preparturient growth of the mammary gland of the intact rat, whereas GH and HCA play dominant roles in "lactational growth" of these glands.

It should be emphasized that these results were obtained with rats with intact pituitaries and conclusions drawn apply only to such animals.

Summary. Mammary gland growth comparable to that occurring during normal pregnancy was induced in ovariectomized rats by daily injection of 2 μg EB + 6 mg P for 19 or 24 days. GH, LGH and HCA were then injected separately and in combination for 3 days. These hormones stimulated significant increases in mammary gland growth as measured by DNA/100 g BW. 1.5 mg GH increased DNA to 9.05 mg/100 g while 2 mg GH + 500 μg HCA increased DNA to 9.15 mg/100 g. This latter value is 47% greater than EB + P at day 24. It is only 15%

less than DNA value at day 3 of lactation. All hormones stimulated lactogenesis but GH was less effective than the other 2 hormones. It was concluded that ovarian hormones (EB + P) play a dominant role in preparturient growth, whereas GH and HCA play dominant roles in "lactational growth" of rat.

1. Griffith, D. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 448.
2. ———, *ibid.*, 1961, v107, 668.
3. Brookreson, A. D., Turner, C. W., *ibid.*, 1959, v102, 619.
4. Kon, S. K., Cowie, A. T., *Milk: The Mammary Gland and Its Secretion,* 1961, vI, 163.
5. Welson, W. L., Heytter, P. G., Ciaccia, E. I., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 373.
6. Greenbaum, A. L., Slater, T. F., *Biochem. J.,* 1957, v66, 155.
7. Smith, T. C., Richterich, B., *Arch. Biochem. Biophysiol.,* 1958, v74, 398.
8. Moon, R. C., Griffith, D. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 788.
9. Nandi, S., Bern, H. A., *Gen. and Comp. Endocrin.,* 1961, v1, 195.
10. Cowie, A. T., Lyons, W. R., *J. Endocrinol.,* 1959, v19, 29.
11. Chadwick, A., Folley, S. J., *ibid.,* 1962, v24, xi.
12. Johnson, R. M., Meites, J., *Endocrinology,* 1958, v63, 290.
13. Talwalker, R. K., Nicoll, C. S., Meites, J., *ibid.,* 1961, v69, 802.
14. Cole, R. D., Hopkins, T. R., *ibid.,* 1962, v70, 375.
15. Griffith, D. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 619.
16. ———, *ibid.,* 1962, v110, 862.

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Role of THAM in Protecting Mice Against Convulsive Episodes Caused by Exposure to Oxygen Under High Pressure. (28065)

SHELDON F. GOTTLIEB AND R. V. JAGODZINSKI (Introduced by A. P. Rinfret)
Research Laboratory, Linde Co., Division of Union Carbide Corp., Tonawanda, N. Y.

Recently Sanger *et al.*(1) and Bean(2) have shown that tris (hydroxymethyl)aminomethane (tris or THAM) protects rats and mice against the toxic effects of oxygen under high pressure (OHP). Both groups of investigators ascribe the protective properties of THAM to its buffering capacity and the maintenance of the cerebral pCO_2 and acid-base balance. To support this view, Sanger *et al.*(1) reported that decreasing the buffering capacity of THAM by neutralization with HCl decreased its protective properties against the toxic effects of OHP.

However, data from microbial experimentation(3-6) suggest possible alternate mechanisms for the protective action of THAM. Using *Lactobacillus arabinosus* MacLeod and Onofrey(3) showed that K^+ competitively reversed the antibacterial action of various amines including THAM. Decreasing the pH of triethanolamine completely eliminated its toxic action; these data imply that a similar effect would occur also if the pH of the other amines was decreased. Provasoli *et al.*(4,5) reported the involvement of K^+ in reversing THAM toxicities in marine and fresh water algae. It should be mentioned that the toxicity of THAM for *Volvox globator* could not be counteracted by Ca, Mg, Na, K, or trace metals(5). Packer *et al.*(6) concluded that these latter data imply that in some organisms THAM might interfere with certain organic metabolites.

The above data are interpreted to mean that the protective action of THAM against the toxic effects of OHP may not be due entirely to its buffering abilities but to its capacity to interfere with K^+ and/or organic metabolites. This investigation was designed to study the possible reversing effects of K on THAM protection against the toxic action of OHP, and possibly to gain new insights into the mechanism of oxygen toxicity.

Method. Young female Swiss mice 20-30 g were used as experimental animals. The test animals were administered 1 cc, i.p., of the appropriate test solution. The animals were placed in a "mouse house"—a multi-