signed to prolong survival of chick embryo cultures(8). White observed beneficial effects on strain "L" mouse fibroblasts in semi-synthetic media(9). Waymouth stimulated growth of these cells in synthetic media with iron(10). Ham found iron to be essential for growth of isolated Chinese hamster cells in media containing serum albumin and fetuin(11). The current studies quantitatively confirm these findings on mammalian and avian cell cultures. Hemoglobin also has been observed earlier to stimulate growth of cell cultures(12,13,14).

A marked difference between mammalian and avian cells was the incapacity of the latter to respond to human transferrin. The toxic sera presumably already contained high concentrations of transferrin which was not utilized by the avian cells or actually was involved in the toxicity of the serum through interference with availability of minute quantities of iron.

Summary. Iron salts and iron-bearing materials replaced lactalysate in stimulation of growth of Walker carcinosarcoma 256 cells in serumless medium and replaced lactalysate and embryo extract in stimulation of chick embryo lung cultures in a similar medium with or without further addition of serum. Presumptive evidence is presented that a stimulatory activity of lactalysate is due to iron and iron-bearing materials. A specific function of lactalysate and embryo extract in overcoming toxicity of serum for embryo cell cultures was served equally well by iron or iron-bearing materials. The avian cells revealed in their incapacity to respond to transferrin a characteristic distinguishing them from the Walker tumor cells.

We wish to acknowledge excellent technical assistance of Miss Joan L. Molowski.

1. Neuman, R. E., Tytell, A. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 252.
2. ———, *ibid.*, 1961, v106, 857.
3. ———, *ibid.*, 1960, v103, 71.
4. Oyama, V. I., Eagle, H., *ibid.*, 1956, v91, 305.
5. Tytell, A. A., Neuman, R. E., unpublished observations.
6. Surgenor, D. M., Strong, L. E., Taylor, H. L., Gordon, R. S., Jr., Gibson, D. M., *J. Am. Chem. Soc.*, 1949, v71, 1223.
7. Gubler, C. J., Cartwright, G. E., Wintrobe, M. M., *J. Biol. Chem.*, 1949, v178, 989.
8. Morgan, J. F., Morton, H. J., Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.
9. White, P. R., *J. Nat. Canc. Inst.*, 1955, v16, 769.
10. Waymouth, C., Abst., Tissue Culture Assn., 1960.
11. Ham, R. G., *Fed. Proc.*, 1960, v19, 387.
12. Baker, L. E., *J. Exp. Med.*, 1929, v49, 163.
13. Ehrman, R. L., Gey, G. O., *J. Nat. Canc. Inst.*, 1953, v13, 1099.
14. Pace, D. M., Bookhardt, J. F., *Growth*, 1960, v24, 203.

Received May 22, 1961. P.S.E.B.M., 1961, v107.

Mammary Lobulo-Alveolar Growth Induced by Anterior Pituitary Hormones in Adreno-Ovariectomized and Adreno-Ovariectomized-Hypophysectomized Rats.*† (26783)

P. K. TALWALKER AND J. MEITES
Department of Physiology and Pharmacology, Michigan State University, East Lansing

Studies in ovariectomized-hypophysectomized or in adreno-ovariectomized-hypophysectomized rats have indicated that hormones from the anterior pituitary and ovaries are both essential for inducing mammary lobulo-alveolar growth(1-3). The adrenals are believed to be of secondary importance, although it is recognized that they secrete several steroids which can influence mammary growth(3). Only a small degree of mammary growth was produced by injecting anterior pituitary extracts into ovariectomized

* Published with approval of Michigan Agr. Exp. Station as Journal Article No. 2822.
† This was supported in part by NIH grants to J. Meites.

TABLE I. Induction of Mammary Lobulo-Alveolar Growth by Anterior Pituitary Hormones in Adreno-Ovariectomized and Adreno-Ovariectomized-Hypophysectomized Rats.

Group & No. of animals	Treatment* (3× daily; 10 days)	Mammary rating†					Avg body wt (g)		
		I	II	III	IV	V	Initial	Final	Difference
	Adreno-ovariectomized rats								
1 (9)	.1 ml saline	3	6	—	—	—	222	225	+ 3
2 (7)	2 mg STH	—	2	5	—	—	217	260	+43
3 (7)	30 I.U. prol.	—	—	4	3	—	208	222	+14
4 (7)	1.33 mg STH + 20 I.U. prol.	—	—	—	1	6	220	261	+41
5 (10)	20 mg A.P.	—	—	2	5	3	224	244	+20
	Adreno-ovariectomized-hypophysectomized rats								
6 (9)	.1 ml saline	6	3	—	—	—	211	212	+ 1
7 (9)	1.33 mg STH + 20 I.U. prol.	—	—	4	5	—	182	225	+43

* STH = Growth hormone; prol. = prolactin; A.P. = anterior pituitary powder.
† See text for mammary rating system.

or adreno-ovariectomized rats(4). Recently, Clifton and Furth(5) observed complete lobulo-alveolar development in adreno-gonadectomized male rats of the Fischer strain after implanting "mammotropic" pituitary tumors intramuscularly. No data were given as to completeness of adrenalectomy in these rats. Since these pituitary tumors apparently secrete both STH and prolactin(5), it was of interest to determine whether frequent injections of large doses of these 2 hormones or of whole anterior pituitary powder could induce lobulo-alveolar mammary growth in adreno-ovariectomized or adreno-ovariectomized-hypophysectomized rats.

Methods. Mature virgin female Carworth rats of the CFN strain, weighing 180-240 g each, were used in this study. Rats in Groups 1-5 (Table I) were adreno-ovariectomized (doubly operated) while rats in Groups 6 and 7 were hypophysectomized by the parapharyngeal approach and 3 days later were adreno-ovariectomized (triply operated). Following each operation, the rats were injected once subcutaneously with 30,000 units of penicillin G. Completeness of adrenalectomy was assessed in 2 ways: (a) after killing the rats at end of experiment, they were carefully examined with a magnifying lens for adrenal remnants; and (b) 17 rats of the same strain, age and weight were adreno-ovariectomized and supplied with tap water instead of 1% saline. Death occurred in 16 rats on the following days after surgery: one rat on day 7, 2 on day 9, one on day 10, 3 on day 11, 3 on day 12, 2 on day 13, and one each on days 14, 15, 17 and 19. The remaining rat was killed on day 24, and one adrenal was found which had not been completely removed. The sella turcica of all hypophysectomized rats was examined for pituitary fragments at end of experiment. Only rats with complete pituitary removal are included in the results.

Twenty-four hours after adreno-ovariectomy, all rats were treated for 10 days as shown in Table I. Hormones were injected thrice daily at intervals of approximately 6 hours between 9 A. M. and 9 P. M. Bovine STH‡ and ovine prolactin‡ were dissolved in distilled water and anterior pituitary powder§ was suspended in physiological saline. All rats were fed *ad libitum* on Wayne Lab-Blox pellets supplemented with canned Armour's Dash dog food, and were given 1% saline to drink. Slices of oranges and 5% glucose in drinking water were made available to all hypophysectomized rats. Body weights of all rats were recorded at beginning and end of each treatment.

The rats were killed on the day following last injection, and both inguinal mammary pads were dissected free, spread flat on cork and fixed in Bouin's fluid. One mammary pad from each animal was stained by a standard procedure(6), and rated for development according to the following scale:

I = Few ducts; few or no end buds
II = Moderate duct growth; moderate

‡ Donated by Endocrinology Study section, NIH.
§ Supplied through courtesy of Dr. J. D. Fisher, Armour Pharmaceutical Co., Kankakee, Ill.

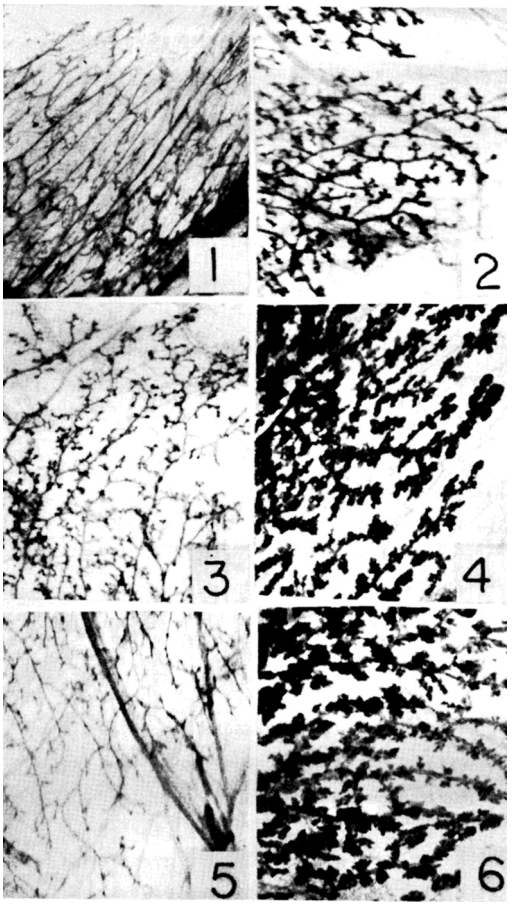

FIG. 1-6: Photographs of whole mounts of mammary glands from rats treated 3× daily for 10 days. × 7.5.

Fig. 1-4: Adreno-ovariectomized rats.

FIG. 1. 0.1 ml saline. Moderate duct development and few end buds.

FIG. 2. 2 mg STH. Extensive duct growth with many end buds.

FIG. 3. 30 I.U. prolactin. Moderate duct growth with many end buds.

FIG. 4. 1.33 mg STH and 20 I.U. prolactin. Extensive duct and dense lobulo-alveolar development.

Fig. 5-6: Adreno-ovariectomized-hypophysectomized rats.

FIG. 5. 0.1 ml saline. Few ducts with little branching.

FIG. 6. 1.33 mg STH and 20 I.U. prolactin, Extensive duct and lobulo-alveolar development.

number of end buds

III = Numerous ducts and branches; many end buds

IV = Numerous ducts and branches; moderate lobulo-alveolar (L-A) growth

V = Numerous ducts and branches with dense L-A growth, as in mid or late pregnancy.

Results. The data are summarized in Table I. In doubly operated rats, mammary glands from saline treated controls (Group 1) consisted of moderately developed ducts with few end buds (Fig. 1). Treatment with STH (Group 2) induced extensive duct growth with many branches and end buds but no L-A growth (Fig. 2). Prolactin (Group 3) also induced duct growth with many branches and end buds (Fig. 3), and 3 out of 7 rats showed limited L-A development. Combined treatment with STH and prolactin (Group 4) induced extensive duct growth and dense L-A development comparable to that seen in rats after mid or in late pregnancy (Fig. 4). Mammary growth produced by anterior pituitary powder (Group 5) did not equal that produced by combined STH and prolactin treatment, but most rats showed moderate L-A development. In triply operated rats, mammary glands from control animals (Group 6) consisted essentially of small ducts with little branching and few or no end buds (Fig. 5). Combined treatment with STH and prolactin (Group 7) produced extensive duct growth and branching and moderate L-A development (Fig. 6).

Doubly or triply operated controls gained very little in average body weight during treatment, while rats given STH or STH and prolactin gained an average of 41-43 g each. Prolactin and anterior pituitary powder produced average body weight gains of 14 g and 20 g, respectively.

Discussion. In previous studies with triply operated rats, administrations of estrogen and progesterone or adrenal corticoids were needed in addition to STH and prolactin to induce lobulo-alveolar (L-A) growth(2). The results of the present experiment show that in doubly or triply operated rats combined treatment with bovine STH and ovine prolactin, or bovine anterior pituitary powder alone, can induce L-A development. Our results are therefore in agreement with those of Clifton and Furth(5) who found good L-A development in adreno-gonadectomized male rats implanted with "mammotropic" pituitary tumors. The procedures used here are

believed to account to a large degree for the present findings. Thus, prolactin and STH or whole anterior pituitary powder were injected thrice instead of only once daily; injections were begun the day after surgery; and relatively large doses of these hormones were used. Also, our rats were mature females, 3 to 4 months old, whereas Lyons *et al.*(2) employed mainly young rats.

Ovariectomy and adrenalectomy appeared to be complete in these rats. The 16 adrenalectomized rats not given saline died within 19 days. Since injections were begun 24 hours after removing the ovaries and adrenals, the possibility cannot be completely excluded that small amounts of steroid hormones may have been present during the first few days of treatment. It is doubtful however, that this would have been sufficient to synergize with the anterior pituitary hormones to induce full L-A growth. Clifton and Furth(5) implanted pituitary tumors in rats 4 to 16 days after adreno-gonadectomy and still obtained good L-A development.

The present results cannot be interpreted to mean that ovarian and adrenal cortical hormones have no role in normal mammary development in rats. Estrogen and progesterone have been shown to induce full mammary growth in intact or ovariectomized rats (1) and to synergize with prolactin and STH in triply operated rats(2). However, the anterior pituitary appears to be of primary importance in mammary growth in the rat, since anterior pituitary hormones alone induced full mammary development in the absence of ovaries and adrenals, whereas ovarian or adrenal cortical steroids have little or no effect on mammary growth in the absence of the anterior pituitary(1-3).

Summary. 1. Mature virgin female Carworth rats of the CFN strain were adreno-ovariectomized and were then injected thrice daily for 10 days with saline, 2 mg bovine STH, 30 I.U. ovine prolactin, 20 mg bovine anterior pituitary powder, or with a combination of 1.33 mg bovine STH and 20 I.U. ovine prolactin. Treatment with STH and prolactin together or with anterior pituitary powder induced moderate to extensive mammary duct and lobulo-alveolar growth, while treatment with prolactin and STH alone induced mainly duct and end bud development. 2. Two groups of rats were hypophysectomized and 3 days later were adreno-ovariectomized. They were then injected thrice daily for 10 days with saline or with a combination of 1.33 mg STH and 20 I.U. prolactin for 10 days. The latter treatment induced extensive duct and moderate lobulo-alveolar growth. Completeness of ovariectomy, adrenalectomy and hypophysectomy was checked in all rats upon autopsy. Sixteen additional adreno-ovariectomized rats which were given tap water failed to survive more than 19 days. 3. It is concluded that under conditions of this experiment, full mammary duct and lobulo-alveolar growth was induced by combined treatment with bovine STH and ovine prolactin in the absence of ovaries and adrenals.

Addendum. Histological examination of stained mammary sections from adreno-ovariectomized rats treated with prolactin and STH failed to reveal any secretory activity.

The authors are indebted to Mr. T. F. Hopkins for performing the hypophysectomies and to Mr. C. S. Nicoll for the photographs.

1. Turner, C. W., in *Sex and Internal Secretions* (E. Allen, Ed.), 1939, Williams and Wilkins, Baltimore, Md.

2. Lyons, W. R., Li, C. H., Johnson, R. E., in *Recent Progress in Hormone Research* (G. Pincus, Ed.), 1958, v14, 219, Academic Press, N. Y.

3. Meites, J., chap. 16 in *Reproduction in Domestic Animals* (H. H. Cole & P. T. Cupps, Eds.), 1959, Academic Press, N. Y.

4. Cowie, A. T., Folley, S. J., *Endocrinology,* 1947, v40, 274.

5. Clifton, K. H., Furth, J., *ibid.,* 1960, v66, 893.

6. Nandi, S., *Hormonal Control of Mammogenesis and Lactogenesis in the C3H/He Crgl Mouse* (Univ. of California publication in Zoology), 1959, v65, 4, Univ. of California Press, Berkeley.

Received May 29, 1961. P.S.E.B.M., 1961, v107.