defined and the pigment was present in the form of coarse dense granules.

The amount in the liver appeared to have decreased to a greater extent than in the spleen and bone marrow. Few Kuppfer cells containing pigment were found, but many large phagocytic cells containing pigment were seen in the perivascular tissue, especially in the portal triads, a fact suggesting migration of pigmented cells in the lymph. Whether any of the pigment presumably originally present in the Kuppfer cells had been eliminated in the bile could not be told by histological methods.

The pigment was not found in the kidneys, lungs, myocardium, adrenals, alimentary tract, generative organs or central nervous system, but was present in considerable amount in lymph nodes.

Blood counts on animals injected with large amounts of sodium salt of chlorin-e-rhodin-g did not show any alteration from the normal blood picture in serial examination during the course of administration of the pigment, although much pigment was found in the bone marrow of these animals at necropsy.

Summary. Chlorin-e-rhodin-g was very slowly eliminated from the tissue of rabbits after intravenous injection. The major portion of that deposited in the spleen and bone marrow after a month of administration was still present in phagocytic cells 3 months later. Somewhat greater elimination occurred from the liver. The deposit in the hemopoietic organs had no evident effect on their function.

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Effect of Stilbestrol on Lactogenic Content of Pituitary and Mammary Glands of Female Rats.*†

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Estrogenic treatment of male and female rats has been shown to cause an increase in the lactogenic content of the pituitary (Reece and Turner[1]). This increase amounted to 45% after treatment with

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 781.

† This study has been aided in part by a grant of the International Cancer Research Foundation.

1 Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 266, 1937, 64.

estrone and as much as 229% after estradiol benzoate administration. Milk secretion was initiated, at least in some cases, from lobule-alveolar glands.

Reece and Mixner[2] found that 200 γ of testosterone propionate injected per day for 15 days into mature spayed rats caused an increase of 41% in lactogen content per pituitary. Pituitary weight was not changed. The treatment induced an extensive development of the lobule-alveolar system of the mammary glands and initiated secretion.

Androsterone, which does not cause mammary growth,[3] was found to cause no significant change either in pituitary weight or pituitary lactogen content in mature spayed female rats.[4] Desoxycorticosterone, which does cause mammary hyperplasia,[5] nevertheless caused no increase in pituitary lactogen content in male and female guinea pigs in spite of a significant increase in size.[6]

Stilbestrol has been shown to be at least as active as estradiol benzoate and more active than estrone on the hyperplasia of the mammary glands of mice[7] and genital organs of rats.[8] It was of interest to compare its effects with that of the natural estrogens on the lactogen content of the pituitary and on the mammary glands of mature spayed rats. It was also thought that a substantial increase in pituitary lactogen content on stilbestrol‡ administration might help to explain the copious lactation which has been shown to occur from the use of this drug in the virgin and dry goat.

Methods. Mature, multiparous rats were spayed and allowed to recover from the operation for 2 weeks. They were then given 10 daily subcutaneous injections of stilbestrol (4:4 dihydroxy $\alpha\beta$ diethyl stilbene) dissolved in oil. On the eleventh day they were sacrificed and the pituitaries removed and weighed. The mammary glands were fixed in Bouin's fluid and prepared as whole mounts. Representative glands were sectioned. The lactogenic content of the pituitaries was assayed by the minimum-micro technic and calculated

2 Reece, R. P., and Mixner, J. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 66.

3 Nelson, W. O., and Merkel, C. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 823.

4 Reece, R. P., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 265.

5 Van Heuverswyn, J., Folley, S. T., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 389.

6 Turner, C. W., and Meites, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 234.

7 Lewis, A. A., and Turner, C. W., *Cancer Research*, 1941, **1**, 55.

8 Sondern, C. W., and Sealey, J. L., *Endocrinology*, 1940, **27**, 670.

‡ Stilbestrol was kindly supplied by Dr. D. F. Green of Merck & Co., Rahway, N. J.

to international units on the basis of the work of Meites, *et al.*[9]

Results. The weight of the pituitaries from the multiparous control rats was considerably higher than that found by Reece and Turner[1] in mature virgin(?) rats (14 mg and 7.5). Lactation has been shown to cause a permanent increase in pituitary size in rabbits[10] and cattle.[11]

A dosage of 3.2 γ per day of stilbestrol caused a 21% increase in pituitary size and 29% increase of lactogen per 100 g body weight over that found in the untreated females (Table I). The content

TABLE I.
Lactogenic Content of Pituitaries of Stilbestrol Treated Multiparous Spayed Rats.

No. of animals	Wt autopsy, avg, g	Days treated	Daily dosage, γ	Pit. wt		I.U. of lactogen					
				mg	increase, %	per pit.	increase, %	per 100 g body wt	increase, %	per mg pit.	increase, %
12	238	0	0.0	14.0		.29		.122		.028	
12	223	10	3.2	16.9	21	.35	21	.157	21	.028	0
6	214	10	10.0	20.1	44	.74	155	.346	184	.059	111
5	226	10	20.0	28.7	105	.90	210	.398	226	.050	79

per mg of pituitary was not changed. A dosage of 10 γ per day was still more effective causing an increase of 44% in pituitary weight and 155% in i.u. of lactogen per pituitary. The content per mg of pituitary was increased 111% and per 100 g body weight, 184%. A dosage of 20γ per day was still more effective in increasing pituitary size (105%), content per pituitary (210%), and per 100 g body weight (226%) but gave a lower increase in content per mg of pituitary (79%). The pituitary lactogen in the groups of rats given 100 γ and 200 γ of stilbesterol was considerably higher than that found by Reece and Turner,[1] and Reece, *et al.*,[12] in the pituitaries of normal lactating rats soon after parturition.

According to these results, stilbestrol appears to be more effective than estrone per gamma in increasing the lactogen content of the pituitary in the rat for Reece and Turner[1] secured a 45% increase per pituitary with a dosage of 50 γ per day of estrone. With 100 γ daily of estradiol benzoate they found a 73% increase in lactogen per pituitary after 18 days' treatment. After 6 to 14 days' treatment with 20 γ daily of estradiol benzoate the increase per pituitary was 229% which is slightly higher than we secured with stilbestrol

[9] Meites, J., Bergman, A. J., and Turner, C. W., *Endocrinology*, 1941, **28**, 707.
[10] Bergman, A. J., and Turner, C. W., *Endocrinology*, in press.
[11] Lewis, A. A., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.* 310, 1939.
[12] Reece, R. P., Hathaway, I. L., and Davis, H. P., *J. Dairy Sci.*, 1939, **22**, 1.

(210%). However, Meites and Turner[13] secured a 410% increase in pituitary lactogen content of guinea pigs given a total of 3000 i.u. of estrone in 15 days. They also secured a comparable increase on stilbestrol administration.

The mammary glands from the control group of rats consisted, in half the cases, of fully developed duct systems. Several of these had only main and secondary ducts while others had interlobular ducts well developed. The other control rats had from slight lobule development to, in two cases, fairly well developed alveolar lobules. In the latter cases the alveoli were unhypertrophied.

In the group of rats given 32 γ of stilbestrol there was also considerable variation in the development of the mammary glands but the range was from early to heavy lobule development. In 6 cases alveolar lobules were extensively developed. These alveoli were hypertrophied, indicating fair secretion.

The group of rats given 100 γ of stilbestrol did not have any greater development of lobules but the alveoli in all cases were hypertrophied with secretion. The mammary glands from rats given 200 γ of stilbestrol were more variable in regard to alveolar hypertrophy, resembling the group given 32 γ.

Reece and Turner[1] reported the secretion of milk in mammary glands from spayed female rats treated with estradiol benzoate. In this study all of the rats given 100 γ of stilbestrol were secreting milk at autopsy. In these cases there were many alveoli well hypertrophied and filled with milk. In other cases serous secretion had been initiated.

Discussion. The administration of stilbestrol by injection[14] or percutaneous application to the udder[15] has been shown to readily cause copious lactation in virgin and dry goats. This treatment was also shown to cause lobule-alveolar development in virgin goats.[16] In this study 10 days' treatment with stilbestrol was shown to cause milk secretion in some mature spayed female rats. This lactation was of slight extent compared with that found after parturition, however, perhaps because of the short period of treatment and incomplete development of the mammary glands. As an explanation of this milk secretion, it was found that the lactogenic content of the pituitaries of these rats was increased as much as 226% over that found in non-lactating untreated rats.

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13 Meites, J., and Turner, C. W., unpublished.

14 Lewis, A. A., and Turner, C. W., *Proc. Am. Soc. An. Prod.*, 1940, p. 63.

15 Folley, S. J., Watson, H. M. S., and Bottomley, A. C., *J. Physiol.*, 1940, **98**, 1.

16 Lewis, A. A., and Turner, C. W., *J. Dairy Sci.*, 1941, **24**, 845.

Summary. Stilbestrol treatment of mature spayed rats for 10 days caused serous or milk secretion from partially developed lobule-alveolar glands. The lactogenic hormone content of the pituitaries per 100 g body weight was increased as much as 226% through an increase in size of the pituitary and content per milligram.

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Influence of Environmental Temperature on Growth of Mammary Lobule-Alveolar System.*†

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Many studies have been reported indicating that variations in such environmental factors as light, temperature, and diet influence the secretion of the anterior pituitary hormones. In several instances it has been shown these same factors do not adversely influence the ability of the end organs such as the gonads and the thyroid to respond to their growth stimuli. Thus in the case of underfeeding, the testes[1] and the thyroid[2] are responsive to less gonadotropic and thyrotropic hormone respectively than the glands of animals on a normal ration.

Recently, Trentin and Turner[3] showed that the amount of estrogen required to stimulate the growth of the mammary duct system increased as the food intake was decreased. In the light of previous work it was suggested that in this case also the inanition might decrease the responsiveness of the pituitary to estrogen stimulation resulting in the production of a much smaller amount of the pituitary mammogenic duct growth factor. The responsiveness of the pituitary would thus be involved rather than the responsiveness of the end organ, the mammary gland.

During periods of high environmental temperature in the summer of 1941, it was observed that progesterone alone and with estrogens failed to stimulate the same degree of mammary lobule-alveolar de-

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1 Breneman, W. R., *Endocrinology*, 1940, **25**, 1094.

2 Stevens, D. J., *Endocrinology*, 1940, **26**, 490.

3 Trentin, J. J., and Turner, C. W., *Endocrinology*, in press.