sumably also its utilization.

Summary. The effect of epinephrine and norepinephrine on the individual free fatty acids of plasma was investigated in anesthetized dogs. The increased arterial FFA after administration of catecholamines was due to a rise in concentration of most major fatty acids. The percentage contribution of oleic and linoleic acids to total arterial FFA was also increased. A direct relationship was found, both in normal and in diabetic dogs, between the resting arterial FFA level and percentage of oleic acid in FFA. An attempt was made to relate these findings to the selective release of FFA from adipose tissue that occurs in the normal animal.

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Quantitative Response of Rat Mammary Glands to Mammogens. I. Estrogen Alone and with Progesterone.* (27605)

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Silver(1) reported that 17 β-estradiol dipropionate (0.25 and 1.0 μg) induced no extension of the mammary duct system of suckling, ovariectomized, hooded Norway rats. In our laboratory these doses of estrogen have stimulated mammary growth in suckling rats of 3 strains, including hooded Norway rats. Lyons *et al.*(2), Kirkham and Turner(3), and Smith(4) have shown that the optimal estrogen to progesterone ratio for lobule-alveolar growth is 1:400, 1:3000-5000, and 1:5000, respectively. Since the ratio of estrogen to progesterone is important in lobule-alveolar growth it was of interest to determine whether estrogen and progesterone, when injected at various ratios, were more effective in stimulating mammary duct growth than estrogen alone.

Methods. Two or 3 pups per litter were taken at random for ovariectomy at 8 days of age from litters of 10 pups divided as equally as possible by sex. Hormone injections (sbc) were begun on the 11th day of age and were continued through the 19th day with sacrifice on the 21st day.

Eleven ovariectomized pups received no treatment and served as controls. Additional ovariectomized pups were injected with: either 1.0 μg (10), 2.0 μg (12), or 4.0 μg (11) of estradiol dipropionate (ED) every other day alone, or with 0.5 μg ED every other day and 1.0 mg progesterone (P) daily (6); 0.5 μg ED plus 2.5 mg P every other day (8); 1.0 μg ED every other day plus 1.0 mg P on 11th and 15th day of age (11); or 1.0 μg ED plus 1.0 mg P every other day (12). This provided ED:P ratios of: 1:4000, 1:5000, 1:400, and 1:1000.

After sacrifice on the 21st day, right abdominal mammary glands were removed, stained, and mounted in balsam. Mammary gland area was determined by the method of Macdonald and Reece(5) and expressed as mm^2 per 100 cm^2 of body surface area ($W^{2/3} \times 10$).

Results. Mammary areas of pups which received 1.0, 2.0, or 4.0 μg ED were 37.5,

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TABLE I. Mammary Growth Response of Suckling, Ovariectomized Rats to Estrogen Alone and with Progesterone.

Treatment*	No. of animals	Mammary area†
Control	11	17.4 ± 1.1
1.0 μg ED	10	37.5 ± 3.1
2.0 " "	12	51.8 ± 2.7
4.0 " "	11	51.7 ± 2.3
.5 μg ED + 1.0 mg P daily	6	64.5 ± 1.5
" " + 2.5 mg P/2 days	8	49.7 ± 2.4
1.0 μg ED + 1.0 mg P day 11 & 15	11	45.2 ± 2.2
" " + 1.0 mg P/2 days	12	45.2 ± 2.6

* ED, 17 β-estradiol dipropionate/2 days. P, progesterone.

† mm^2 per 100 cm^2 of body surface area ($W^{2/3} \times 10$) with S.E.

51.8, and 51.7, respectively. These areas were significantly greater ($P<0.01$) than the mammary area of ovariectomized controls (17.4). Mammary gland areas of pups receiving 2.0 and 4.0 μg of estrogen were not statistically different; however, they were statistically greater than those of animals receiving 1.0 μg of estrogen.

The mammary area of pups injected with 0.5 μg ED every other day plus 1.0 mg P daily was 64.5, which was significantly greater ($P<0.01$) than that of all other treatments. The mammary areas of pups receiving 0.5 μg ED plus 2.5 mg P every other day, 1.0 μg ED every other day plus 1.0 mg P on 11th and 15th day of age, or 1.0 μg ED and 1.0 mg P every other day were 49.7, 45.2, and 45.2, respectively. Although these mammary areas approached those of rats injected with 2.0 μg ED alone, they were nevertheless not significantly different from glands stimulated with 1.0 μg ED alone (Table I).

Discussion. These results show that mammary glands of suckling, ovariectomized rats are responsive to physiological doses of estrogen (Fig. 1). The response was evidenced by an extension of the mammary duct system, an unexpected finding since Silver(1) was unable to stimulate duct extension in suckling rats with estrogen. Four μg of estrogen were more effective than one but no more effective than two. Since the mammary glands of hypophysectomized rats are nonresponsive to estrogen alone it is possible that

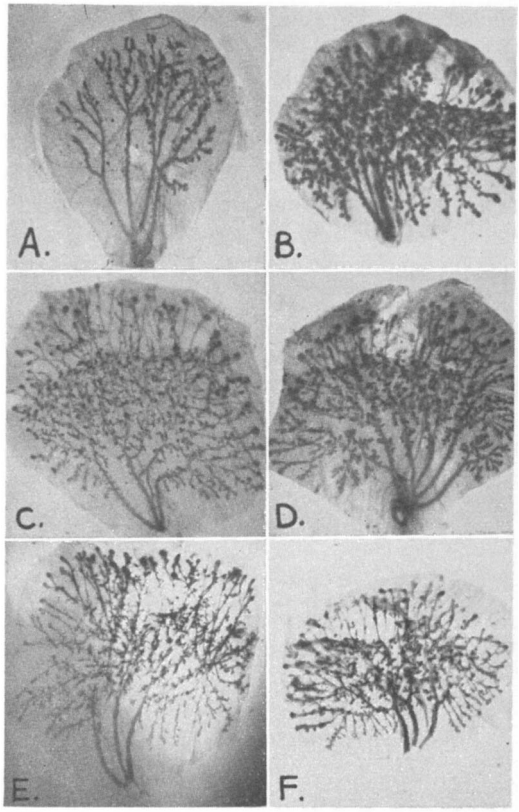

FIG. 1. Whole mounts of right abdominal mammary gland of rats ovariectomized on day 8 and sacrificed at 21 days of age. A. Ovariectomized control. B. Inj. with 1 μg estradiol dipropionate (ED) days 11, 13, 15, 17, and 19. C. Inj. with 2 μg ED, as B. D. Inj. with 4 μg ED, as B. E. Inj. with 0.5 μg ED, as B, + 1.0 mg progesterone daily. F. Inj. with 1 μg ED + 2.5 mg P, as B.

estrogen doses of 2 μg stimulate the secretion and release of pituitary mammogen. If so, the pituitary mammogen is not ACTH, since ACTH does not increase mammary response to estrogen(6). The results of extensive work have shown the mammary duct stimulating properties of estrogen, and the finding that estrogen plus progesterone were more effective than estrogen alone was somewhat unexpected.

Summary. Estrogen induced extension of the duct system of mammary glands of suckling, ovariectomized, hooded Norway rats. Two μg of 17 β-estradiol dipropionate stimulated greater mammary growth than did 1.0 μg but 4.0 μg were not superior to 2 μg. Estrogen (0.5 μg) every other day plus progesterone (1.0 mg) daily stimulated greater

mammary duct growth than did 2.0 μg of estrogen alone.

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Histidine Decarboxylase Activity in the Walker Carcinosarcoma 256* (27606)

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The hypothesis of Kahlson(1) that the increased rate of formation of histamine associated with some rapidly growing tissues may mean that histamine metabolism plays a role in growth, has led to investigation of the histidine decarboxylase activity of certain tumors. A transplantable hepatoma, F-hep, in rats of the August strain has been found to be rich in histidine decarboxylase activity, and as the tumor grows in females the quantity of histamine excreted in the urine increases(2,3,4). When the tumor was removed, the rate of excretion of histamine returned to normal. A mouse mastocytoma has been found to possess active histidine, 5-hydroxytryptophane, and desoxyphenylalanine decarboxylases(5). The present experiments were done to learn whether the Walker carcinosarcoma 256 in the Sprague-Dawley rat has the ability to form histamine *in vitro* and whether its presence causes increased excretion of histamine in the urine.

Methods. Female Sprague-Dawley rats were used. *Determination of excretion of histamine in the urine.* Four rats were kept in metabolism cages designed to permit separate collection of urine and feces. Two milliliters of normal HCl was added daily to each collecting flask to keep the pH of the mixture low enough (1.8 to 3.0) to preserve histamine.

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Urine was collected during successive periods of 4 days and was pooled and refrigerated. The female rat, in contrast to the male, excretes free histamine almost exclusively (rather than conjugated histamine) in the urine(6,7). For bioassay, such urine requires only neutralization and dilution with Tyrode's solution and may then be introduced directly to the guinea pig gut. The assay procedure used was a modification(8) of the method of Barsoum and Gaddum. Urinary excretion of histamine was measured in 4 rats before and after the Walker tumor was implanted and during recurrence after an excision.

Management of tumors. Portions of fresh Walker carcinosarcoma 256 were ground with saline solution, using a mortar and pestle; 0.3 ml of the resulting suspension of tumor particles was injected subcutaneously into 6 rats. On the twelfth day after implantation, the animals were anesthetized with ether and the tumors were removed. In the 4 animals in which urinary excretion of histamine was being measured, the operative wounds were closed and excretion of histamine in the urine was measured for 8 more days, during which time the tumors recurred.

Determination of histidine decarboxylase activity of tumors. The method used was essentially that of Waton(9). Preliminary tests indicated production of more histamine at pH 6.5 than at pH 7.2 (Table I); pH 6.5 was used in subsequent experiments. A pre-