contents gave an average recovery 2.5% low. The quantity of fluorine in each aliquot titrated was one-tenth the total in distillate. Four analyses each of 5 and 2γ quantities gave results 1.5% low while corresponding distillation blank determinations averaged 0.5% low.

Four analyses of a quantity of mixed sound human enamel gave the results 0.0163, 0.0154, 0.0164, 0.0160% F. Three determinations were made in which 10 micrograms F were distilled from the residue in sample. The average recovery was 10.1γ F. Three analyses in which the distillate was redistilled and one in which it was twice redistilled showed 0.0158% F in the enamel. These latter results indicate that phosphate or any other substance in enamel is not volatilized in a sufficient quantity to affect the results.

The average recovery of 5γ F added to enamel was 4.8γ. Two determinations using $NaClO_4$ showed the enamel to contain 0.0160% F. A sample of C.P. "$Ca_3(PO_4)_2$" contained 0.622% F and one of C.P. $Ca(H_2PO_4)_2$ contained 0.013% F. The method has been successfully applied to the determination of F in dentin and will be extended to other biological material.

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## Comparative Studies of Gonadotropic Hormones.* 5. Growth Response of Rat Mammary Glands in Chronic Experiments.

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A description of the changes induced in the ovaries and hypophysis of female rats following the prolonged administration of gonadotropic extracts prepared from sheep anterior pituitary glands and blood of pregnant women was given in the fourth report of this series (Fluhmann[1]). The present communication deals with the gross and microscopic effects observed in the mammary glands of these rats.

*Sheep Anterior Pituitary Extracts.* Six rats received daily injections for 63 days. Breast biopsies showed proliferation of ducts

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[1] Fluhmann, C. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 691.

with occasional branching tubules, but without colloid secretion or formation of alveolar structures. There was marked periductile fibrosis present in all animals. The injections were continued for 94 days, and 3 rats autopsied at that time were found to have the periductile fibrosis more marked than the previous series, while in 2 animals, fibroadenomatous proliferation of unencapsulated duct-containing fibrous tissue was present. In 5 rats injected for 149 days similar but more marked changes had taken place. After 179 days of injection, 5 rats showed only extension of periductile fibrosis and in 4 out of the 5 animals, fibroadenomatous areas were present as well. No cystic changes or alveolar development were noted in any of the above animals. This is in agreement with unpublished experiments carried out the previous year with acid sheep anterior pituitary extract. In previous experiments, 4 of 10 rats injected for 119 days showed, in addition to the periductile fibrosis and fibroadenomatous areas, large areas of microscopic and macroscopic cystic changes with colostrum-like secretion very similar to the cystic mastitis of women. Periductile fibrosis and fibroadenomatous areas were also found in rats given a rat anterior pituitary preparation daily for 105 and 165 days, and a human anterior pituitary extract prepared in the same manner injected for 119 days. The nipples of the rats injected with the pituitary extracts remained grey-white and non-erectile on stimulation, and showed no gross growth or elongation. No alveolar or lobule development was observed in any animals injected with acid anterior pituitary extracts. Normal virgin immature and mature female albino rats' breasts microscopically show only a fatty stroma about ducts and infrequent tubules, with only small amounts of fibrous tissues in the walls of the ducts. Consequently, periductile fibrosis and, now, non-encapsulated fibroadenomatous changes are distinct departures from the normal.

*Pregnancy Blood Extract.* The pregnancy blood extracts exerted a powerful stimulating effect on the unfolding of the alveolar mammary tree in virgin immature albino rats. Eleven rats receiving the extract for 28 days showed distinct development of alveoli and lobules, with many branching tubules and occasional areas of colloid secretion. After 59 days of such injections, 7 immature rats responded with marked lobular formation and clusters of alveoli, the lining cells of which were vacuolated and the lumen contained plentiful colloid secretion. By the end of 90 days 6 rats showed extensive lobule and alveolar development with marked vacuolation of alveolar lining cells, and plentiful colostrum and milk secretion filling

alveoli and ducts. Five rats injected for 193 days had breasts in which microscopic sections revealed extensive lobules with both advanced lactation and regressive changes present. Many inspissated concretions were found in alveoli and ducts. One true encapsulated adenoma was present. After 272 days of injection, 4 rats showed marked regressive changes in the well developed alveoli and lobules, and although milk secretion was present it was limited to local areas of gland tissue. Two of the 4 animals had definitely encapsulated adenoma, while small microscopic milk cysts were present in all animals. Concretions were present in ducts and alveoli in each instance. The injections were continued in 2 animals for 365 days. One, No. 25, revealed marked regressive changes evidenced by shrunken, compact alveoli, concretions and pigment deposits. Animal No. 37 had marked hyperplasia of the alveolar lobules with extensive secretion changes, colloid and milk production. Small cystic areas were filled with colostrum and milk. In all animals receiving human pregnancy blood extract the nipple became pink, erectile, and elongated, and remained so throughout the experiment. The alveolar and lobule growth was not obtained in castrate 20-day and mature 114-day animals after 30 days of injection. The controls for animals receiving pregnancy blood extract had only small ducts and scanty tubules in the fatty breast tissue, with no evidence of alveolar growth.

*Conclusions.* The long-continued injection of acid anterior pituitary extracts and acid extracts of human pregnancy blood produced markedly different growth effects in the female albino rat breast. The acid pituitary extract response was characterized by growth and proliferation of the duct system with increasing periductile fibrosis and the appearance of fibroadenomatous areas, but without alveolar or lobule growth proliferation. In response to extracts of human pregnancy blood there was an unfolding, growth and hyperplasia of the alveolar mammary tree with secretion changes, milk production, and after long continued injections, regressive changes, often accompanied by the appearance of adenomatous tumors of the breast.