Conclusions. Disturbance of normal salivary flow in the rat results in an increased incidence of experimental caries. The effect is most marked in rats with retained mucous saliva on a caries-producing diet and is in direct proportion to the amount of serous saliva removed. Caries production has even been observed in operated rats deprived of their serous saliva when fed a diet known to produce minimal dental destruction. The lower ratio of fracture to carious lesions in rats with such saliva indicates that the rate of development of carious lesions in these groups is very rapid.

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Responses of Hypophysectomized Immature Female Rats to Mare Serum Hormone.*

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Hypophysectomized mature and immature female rats have been shown to respond to mare serum hormone by a marked increase in ovarian weight.[1, 2] The ovaries of the mature rats contained stimulated follicles, corpora lutea and corpora lutea atretica.[3] However, in virtually every individual case the period of treatment, the dosage administered and the time elapsing between hypophysectomy and the start of treatment were varied.[1, 3] Due to the persistence of corpora lutea after hypophysectomy the mature rat does not afford a basis for accurate estimation of hormonal action. Recently Noble, *et al.*,[4] have shown that follicle stimulation, granulosa luteinization and interstitial cell development resulted from two different dosages of this material in hypophysectomized immature rats. The first dosage was started 24 hours after the operation and the second 10 days later.

The investigation here reported was undertaken to study the effects of varied doses of the mare serum hormone "Gonadin"† on

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1 Evans, H. M., Meyer, K., and Simpson, M. E., *Mem. Univ. Calif.*, 1933, **11**, 257.

2 Smith, P. E., *J. Am. Med. Assn.*, 1935, **104**, 553.

3 Hamburger, C., *Endokrinologie*, 1936, **17**, 8.

4 Noble, R. L., Rowlands, I. W., Warwick, M. H., and Williams, P. C., *J. Endocrinology*, 1939, **1**, 22.

† Mare serum hormone, "Gonadin," was generous'y supplied by Doctor Donald Wonder, Cutter Laboratories.

hypophysectomized immature female rats that were all treated in the same manner. An interval of 5 days was allowed to elapse after hypophysectomy in all cases. The hormone was injected subcutaneously once daily for 5 days and the animals autopsied 24 hours after the last injection.

Sixty-three immature female rats ranging from 29 to 36 days of age were hypophysectomized, at which time the vaginal orifice was closed in all cases. Body weights at the time of operation ranged between 52 and 96 g and averaged 71 g, whereas the body weight at autopsy (10 days later) ranged between 53 and 92 g and averaged 67 g. All pituitary capsules were serially sectioned, stained with Masson trichrome and examined microscopically. Only data from completely hypophysectomized animals are presented. Weights of the adrenals, ovaries and uteri were taken at autopsy, and the ovaries were examined under the binocular microscope to ascertain grossly the response obtained. The ovaries of at least half of the animals on each dose level were sectioned serially.

Mare serum hormone was administered in 8 different total doses to groups of 5 to 10 animals each. The ovarian weight was found to increase with a corresponding increase in the amount of material injected. Ovaries of hypophysectomized controls after 10 days weighed 6 to 7 mg, whereas ovaries that weighed 250 mg were obtained with the largest dose of hormone employed. A greater effect would possibly have resulted had larger doses been used. Marked variation of ovarian weight was observed in most groups. Only 3 groups had less than 100% weight variation, 2 of which were the lowest doses tested (Table I).

Although ovarian weight was significantly increased with doses of 5, 10 and 12.5 r.u. the most pronounced effect observed with these doses was excessive thecal luteinization. A varied number of small follicles with or without antra were present, but in the ovaries of only

TABLE I.
Effects of Mare Serum Hormone "Gonadin" on Hypophysectomized Immature Female Rats.

Total dose, r.u.	No. of rats	Avg ovarian wt, mg	Range of ovarian wt, mg	Avg uterine wt, mg
5.0	5	12.1	9.5-15	27.6
10.0	6	21.5	18.5-27	50.8
12.5	9	28.4	17-45	118.0
20.0	6	48.3	38-63	128.0
25.0	9	84.3	50-137	119.4
30.0	9	104.4	74-190	143.0
40.0	10	145.4	92-241	120.7
50.0	9	184.1	91-250	148.3

3 of the 15 animals that received 10 and 12.5 r.u. were there follicles of medium size. A further increase of the dosage (20 r.u.) produced normal vesicular follicles of good size as well as luteinized thecal tissue. The heaviest ovaries in this group contained a large proportion of vesicular follicles. The closest approach to pure follicle stimulation was obtained with total doses of 25 and 30 r.u. The ovaries were primarily composed of large vesicular follicles which were fairly uniform in size and in which the granulosa and ova were normal. However, luteinized thecal tissue, although occurring in a very small amount, was nevertheless always present. The first evidence of granulosa luteinization was exhibited in the ovaries of rats that had received 40 r.u. and in a few cases corpora lutea had formed. Very large normal follicles formed a large part of most of these ovaries. The largest amount of hormone administered (50 r.u.) caused the formation of corpora lutea, many of which were corpora lutea atretica, follicles with thickened granulosa and some large normal vesicular follicles.

Summary and Conclusions. Cole[5] and Cartland and Nelson[6] reported their inability to secure evidence for the presence of more than a single principle in pregnant mare serum. However, Evans, *et al.*,[7] have indicated that this material can be fractionated with ammonium sulphate into FSH and ICSH as tested in hypophysectomized rats in a 72-hour period, but the details of the tests were not reported. It is well known that a varied ovarian response can be obtained with gonadotropins by altering the experimental conditions and the present experiments indicate the importance of dosage in the response elicited by mare serum hormone in hypophysectomized immature rats. In these experiments the predominant formation of thecal luteinization was observed with low doses of this material. Larger amounts of this hormone primarily caused the development of large vesicular follicles, whereas with a further increase of the dosage corpora lutea resulted. Large amounts of castrate urine extract also produced corpora lutea but this material produced only follicle stimulation in the absence of thecal luteinization in ovaries weighing as much as 80 mg.[8] Thus a definite difference in the gonadotropic activity of mare serum hormone and castrate urine extract can be cited as both materials were tested under identical experimental conditions.

5 Cole, H. H., *Am. J. Anat.*, 1936, **59**, 299.

6 Cartland, G. F., and Nelson, J. W., *J. Biol. Chem.*, 1937, **119**, 59.

7 Evans, H. M., Korpi, K., Simpson, M. E., and Pencharz, R. I., *Univ. Calif. Publ. Anat.*, 1936, **1**, 275.

8 Tyndale, H. H., Levin, L., and Smith, P. E., *Am. J. Physiol.*, 1938, **124**, 174.