from injured or dying cells. However, in work to be published, the lysozyme activity was not elevated at 12 hours after irradiation so that release from dying cells is probably not an important factor.

The precise extent to which these various factors, individually or in combination, contribute to the observation of increased lysozyme activity in irradiation chimeras reported here must await further work for definition.

Increased weight of kidneys and liver has been noted by Frei *et al.*, in leukemia(12). In our experiments, the animals given homologous marrow had definitely increased kidney weight that became maximal at 9 days but was nearly normal by 14 days after treatment. This weight increase was not, therefore, correlated with the increase in lysozyme activity, the peak of which came later. The relationship in time after treatment, between the increase in liver weight that we have previously found(13) in mice given homologous cells and the increase in kidney weight reported here is of interest, although the mechanism involved is unknown.

Summary. Assay of lysozyme activity in homogenates of kidney of irradiation chimeras has shown that a marked increase in activity is associated with the presence of grafted homologous bone marrow. This increase began about 3 days after injection of the marrow cells, reached its peak 20-40 days and returned to normal 100-200 days after treatment. A significant increase in weight of kidney, maximal at 9 days after treatment, was observed in the animals given homologous bone-marrow cells.

Dr. D. G. Doherty and Amleto Castellani helped in setting up the assay procedure.

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Prolactin Activity in Blood During the Normal Human Menstrual Cycle.* (28404)

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Because of various difficulties in bioassay techniques there have been scanty previous data on prolactin activity in the blood or urine of normal men and women. In a previous paper mostly concerned with presenting proofs of the validity of our bioassay procedure, we(1) found no prolactin activity in the blood of children, normal young men and normal young women in the first half of the menstrual cycle. However, prolactin activity was present in the blood of normal women during the second-half of the cycle. These assays were performed on single specimens of blood obtained from different individuals

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at various times of the cycle. To gain more information on the day-to-day fluctuation in prolactin activity, it was decided to perform serial blood prolactin assays in a single subject. This report presents the results of daily blood prolactin assays in 5 normal young women, one normal young woman receiving Enovid for contraceptive purposes, and one normal young man, during the time interval of a 28-day menstrual cycle. These observations are of importance because they provide substantive data concerning the possible physiologic role of prolactin in the normal menstrual cycle.

Methods. Prolactin activity of human whole blood was concentrated by means of acid acetone extracts, using the Sulman method for extraction of chromatophorotropic activity of human blood(1,2). Extracts were prepared from 10-ml aliquots of whole blood, and the final volume of the extract was adjusted to 4 ml, so that each 1 ml of extract was equivalent to 2.5 ml whole blood.

The blood extracts were assayed by a single-injection local pigeon crop-weight technique previously described by us(1), using the increase in weight of the crop sac as the end-point. There was one important change in the assay method used for this study. Weights of both sides of the crop sac were measured instead of our previous usage of measurement of only the right side. Four birds were used per assay. Each pigeon received a single local intradermal injection of 0.5 ml of blood extract (equivalent to 1.25 ml of whole blood). Control birds received similar injections of distilled water of equal volume. In addition, 2 dose levels of ovine prolactin,† 0.05 I.U. and 0.5 I.U. were assayed with each weekly run of blood extracts tested. The end-point of the assay is the increase in weight of both sides of the crop sac compared to that of the water-injected control birds.

Results. Analysis of 45 consecutive prolactin dose-response curves in an 18-month period revealed the following features. The mean crop weight of water injected control birds was 182 mg with a range of 150 to 210 mg. The 95% confidence limits for a 4 bird assay of water are ± 15%. Water injected birds gave a response equivalent to a prolactin dose of approximately 0.0025 I.U. At the 0.05 I.U. prolactin dose level, the mean crop weight was 231 mg with a standard deviation of ± 19.44 using results from 45 assays with 4 birds per assay. This represented a mean response which was 127% ± 12.85 (S.D.) of the water injected controls. At the 0.5 I.U. prolactin dose level, the mean crop weight was 286 mg ± 26.78, or 157% ± 14.42 of the water controls. The mean lambda was 0.60 and was fairly uniform. With respect to the slope (b), a seasonal variation was found with the lowest slopes during the spring months (March through June).

Fig. 1 shows results of all of the assays for prolactin activity of blood extracts obtained almost daily from 5 normal young women throughout the duration of a 28-day menstrual cycle. Blood was drawn from each woman daily with the exception of weekends and holidays. Inspection shows that significant prolactin activity (equivalent to 0.05 I.U. per 1.25 ml whole blood) was found in a majority of blood extracts during the first 5 days and last 10 days of the cycle. On the other hand, only 2 of 40 blood extracts assayed between days 6 and 18 were found to have significant prolactin activity. Prolactin activity was demonstrable in the blood of 4 of the 5 subjects during the first 4 to 7 days of the cycle. In the remaining subject no activity was found during the first week of the cycle. Prolactin activity reappeared in the blood of all 5 subjects between the 19th and 23rd day of the cycle and persisted until time of menstruation.

For comparison, Fig. 2 shows serial assays of blood extracts obtained from a 30-year-old normal male during a time interval of 31 days. Only 2 of 24 extracts yielded positive prolactin responses, the remaining assays showing absence of prolactin activity. It is not clear whether the 2 positive assays in this subject indicate transient prolactin secretion by the pituitary, or merely reflect bird instability.

Discussion. The data reported here sug-

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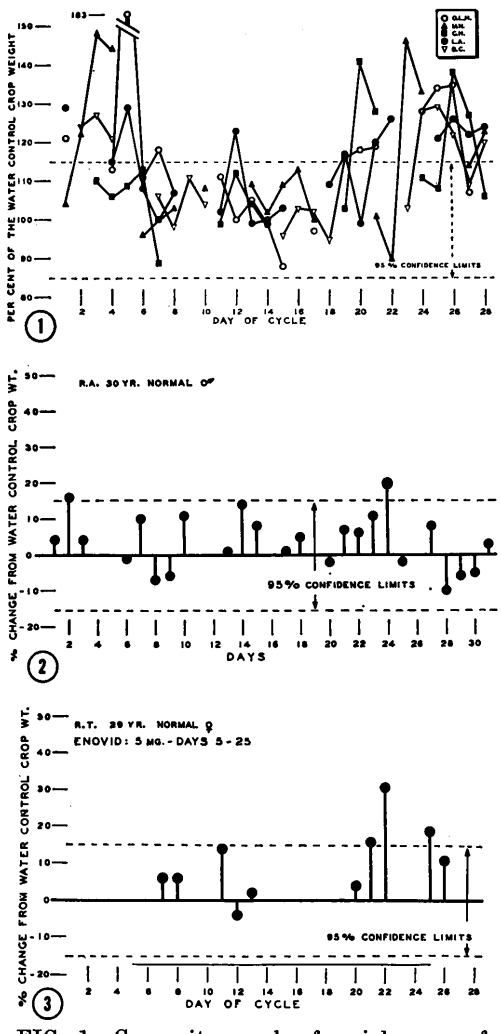

FIG. 1. Composite graph of serial assays for prolactin activity of blood extracts obtained almost daily from 5 normal young women (age range 21 to 29 yr) throughout a 28-day menstrual cycle.

FIG. 2. Serial assays for prolactin activity of blood extracts obtained from a 30-yr-old normal man during a period of 31 days.

FIG. 3. Serial assays for prolactin activity of blood extracts obtained from a normal 29-yr-old woman receiving Enovid as an oral contraceptive agent in a dose of 5 mg daily from days 5 to 25 of the menstrual cycle.

gest a reevaluation of the role of prolactin in the normal human menstrual cycle. The time of its peak concentrations in blood differs from that found for the other 2 known pituitary gonadotropins(3,4), follicle stimulating hormone (FSH) and luteinizing hormone (LH), which is at midcycle (the time of ovulation). There has now been ample documentation that FSH and LH are the gonadotropins responsible for the entire process of human ovulation including the postovulatory development of the corpus luteum (5). Enovid is a hormonal preparation consisting of norethynodrel and ethynyl-estradiol, which has been widely employed as a contraceptive agent because of its ability to prevent ovulation by inhibiting the pituitary release of FSH and LH(6). Several blood prolactin assays were performed during a single menstrual cycle in one young woman receiving Enovid for contraceptive purposes in a dose of 5 mg daily from days 5 to 25 of the cycle. Although we were unable to secure daily blood specimens from this patient with consequent gaps in the cycle, the assay data illustrated in Fig. 3 suggest the presence of prolactin activity during the latter part of the cycle despite administration of the gonadotropin inhibitor, Enovid.

These observations suggest that prolactin acts in opposition to the other 2 pituitary gonadotropins, FSH and LH. A luteotropic function of prolactin has been conclusively demonstrated in only one animal species, the rat(7), but this, with one exception, has never been confirmed in man. Fried and Rakoff(8) found that chorionic gonadotropin and prolactin synergistically exhibited a luteotropic action in man, which neither compound alone was able to do. In view of these facts and the time of appearance of prolactin activity in the blood of normal young women, it is suggested as a working hypothesis that prolactin has an antigonadotropic action in the normal human menstrual cycle (as originally suggested by Riddle and Bates in the pigeon(9)), and that in the event of conception prolactin is available in synergism with chorionic gonadotropin (which has been detected in blood as early as the 9th day after conception(10)) to exert a luteotropic function in the early phases of pregnancy.

Summary. Prolactin activity appeared in the blood of normal young women during the first 5 and last 10 days of a 28-day menstrual cycle, whereas no activity was demonstrable during days 6 to 18 of the cycle. Blood prolactin activity appeared to be present in the latter part of the cycle of a normal woman receiving the gonadotropin inhi-

bitor, Enovid. No blood prolactin activity was found in a normal male subject during a 31-day period. These observations suggest that prolactin acts in opposition to the other 2 pituitary gonadotropins, FSH and LH, in the normal human menstrual cycle.

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Delay in Ovulation in the Hen Following Stimulation of the Preoptic Brain. (28405)

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Ovulation in the hen is delayed following electrical stimulation of the anterior median eminence with stainless steel electrodes(1). The response is attributed to the delay in, or blockade of, release of the ovulation-inducing hormone (OIH) resulting from the prolonged irritative action of metallic elements electrolytically discharged from the electrodes. Everett and Radford(2) demonstrated earlier that similarly induced irritative foci in the brain of the rat facilitate ovulation. Stimulation was found to be effective in several brain sites, including areas contiguous with the median eminence, but the most consistent results were obtained by stimulation of the preoptic hypothalamus.

The preoptic region has been shown to participate in the release of OIH in the hen (3,4,5). Destruction of a specific ventral portion of the preoptic paraventricular nucleus by electrolytic lesion blocks both normal and progesterone-induced ovulation(3, 4). Microinjection of progesterone directly into the preoptic region regularly forces ovulation(5). In our previous investigation(1) electrical stimulation of the preoptic brain in a limited number of hens had no immediate effect on ovulation. A more thorough examination, however, has shown that delays in ovulation can be evoked by stimulation at this site.

Methods. All hens were one- or two-year old White Leghorns individually caged in batteries with free access to feed and water and under electric lights from 6:00 A.M. to 8:00 P.M. Hourly records of lay, maintained from 8:00 A.M. to 4:00 P.M., allowed selection of hens ovulating in recurrent sequences of 2 or 3 eggs(1). The stimulus was invoked in the conscious hen at about 14 hours prior to time of ovulation of the first (C_1) follicle of a sequence. Ovulation of the C_1 follicle can be readily advanced or delayed when the appropriate agents are administered at this time. Significant deviations from the time of normal ovulation are easily detected by digital palpation of the oviducal egg at 8:30 A.M. and 2:30 P.M. on the following day. In the present experiments, estimates based on palpation were confirmed by autopsy at 24 hours after stimulation.

Electrodes were placed systematically throughout the preoptic region at 0.5, 1.0, 1.5, and 2.0 mm bilaterally. A single unit