apparently due to a decrease in solubility caused by the passage of electrolytes into the lateral chambers containing the electrodes.

Further purification of the liquid in the central compartment was attempted by putting fresh distilled water in the outer chambers and passing the current through again. A further precipitate then settled out which on testing proved to be inactive. The supernatant fluid had retained its full activity. On evaporation of the supernatant fluid a solid was obtained which represented only 1/10 of the original material and contained only a very small percent of ash. Elementary analyses of this material are in progress.

Conclusion. Electrodialysis appears to be a very promising method for the purification of the Reynals spreading factor.

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Inhibition of Estrous Cycle in the Rodent with Post-partum Urine and Commercial Prolactin.

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In attempting to ascertain the effect of urine from lactating women upon mammary glands of rats and mice, it was observed that there was an inhibition of estrus in these animals. Animals with normally recurring estrus were used, and these had daily vaginal smears for at least 3 consecutive cycles as a means of control. Injections of post-partum urine were then started. It was found that at least one, usually 2, and occasionally 3 cycles were suppressed, after which the animal resumed its normal estrous rhythm in spite of continuation or increase in the amount of injected urine. Controls, injected with the urine of normal human males and females had no alteration of the rhythm. The rodent's ovaries during this period of induced diestrus contained active corpora lutea which were similar to the ovaries of lactating animals. If injections were discontinued during this period of induced diestrus, a vaginal smear indicative of estrus was obtained within 48 hours. These findings suggested that a sub-

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stance, possibly prolactin, was present in the urine of lactating women, which accounted for the amenorrhea and which could produce analogous changes in rats and mice. Since Lyons and Page[1] reported the isolation of prolactin from the urine of lactating women, extracts were made from such urine, using their method. Only suggestive inhibition was obtained with this preparation.

Commercial prolactin prepared according to the method of Riddle was obtained through the courtesy of E. R. Squibb and Sons. Normal adult female mice in which the rhythm of the cycle was determined for at least 3 periods of estrus were used. These preparations were also capable of inhibiting the estrous cycle but for a longer time than did the post-partum urine. This confirmed the work of Dresel[2] and Lahr and Riddle,[3] which was reported during the course of these experiments. The former obtained suppression of the estrous cycle in mice whereas the latter investigators found a similar phenomenon in rats after the injection of prolactin.

The following are the preliminary observations from this laboratory: (1) Three bird units of prolactin given in divided doses twice daily was the minimal amount which would inhibit the estrous cycle for at least 3 periods. This was a constant finding although Dresel reported the suppression with smaller amounts. Smaller dosages as a rule had no effect upon the rhythm. (2) Larger doses seemed to suppress the cycle in proportion to the size of the dose, *e. g.,* 8 B.U. divided and given twice a day inhibited 4 or 5 cycles and 16 B.U. given in the same manner generally prolonged the period of diestrus for at least 40 days. With the latter dosage, the animal not infrequently failed to resume its estrous cycles even though injections were discontinued at the end of 40 days. (3) Lahr and Riddle[3] observed that about 15 days of diestrus were sometimes followed by estrus and then another period of prolonged diestrus. They further ascertained that discontinuance of injections resulted in estrus within 48 hours. These findings were confirmed in these experiments. It is possible that the former phenomenon is similar to the period of pseudo-pregnancy seen in the rodent. It was further determined that the appearance of estrus after the injections were stopped corresponded to the time it should have occurred

[1] Lyons, W. R., and Page, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **32**, 1049.
[2] Dresel, I., *Science,* 1935, **82**, 173.
[3] Lahr, E. L., and Riddle, O., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 880.

during the normal rhythm of the animal if it were left untreated. (4) Sections of the ovaries after a short period of inhibition (1 to 3 weeks) revealed active corpora lutea, which were also found by Lahr and Riddle. Sections after prolonged diestrus (4 to 8 weeks), however, showed atrophy and degeneration of the ovary. This probably explains the failure of an animal to resume its rhythm even though injections were discontinued. In view of the presence of corpora lutea a search was made for a substance other than prolactin such as the luteinizing hormone (LH) of the hypophysis which could be responsible for the changes described. Consequently, the commercial preparations were assayed by one of us (H.L.F.) and sufficient LH was found to initiate luteinization of the follicles. A substance similar in its action to LH was then isolated from the urine of lactating women (10 days to 3 months post-partum). Injections of LH (prepared by H.L.F.) containing only small quantities of lactogenic hormone produced more striking suppression of the cycles. The ovaries, microscopically, contained many more active corpora lutea than those found after prolactin injections. It is interesting that recently an extract of LH entirely free from prolactin has been obtained for the first time (H.L.F.). Experiments being carried out at present with the new preparation will be reported later.

It seems, as a result of the aforementioned experiments, that the causative agent in the inhibition of the estrous cycle in the rodent is LH and not prolactin although this needs conclusive proof. These experiments suggest also that LH or a similar substance produces the amenorrhea of lactation rather than prolactin. This would correspond more closely with the so-called corpus luteum of lactation.