through C_{20}) to corn oil or coconut oil shows that while all the fatty acids used enhance cholesterol solubility, highest solubility is obtained when C_6, C_8, C_{10}, or C_{12} fatty acids are used.

We are grateful to Dr. L. D. Wright for permission to see his manuscripts before publication. We are also indebted to Drs. V. Babayan, Drew Chemical Co., F. Mattson, Proctor & Gamble Co.; D. Rathmann, Corn Products Co., J. Proctor, National Dairy Products Co.; R. Purdy, Pacific Vegetable Oil Co.; R. Vander Wal, Armour & Co.; and Dr. K. K. Carroll, Univ. of Western Ontario for gifts of some of the fats and oils used in these experiments.

1. Wilkens, J. A., deWit, H., Bronte-Stewart, B., *Can. J. Biochem. Physiol.,* 1962, v40, 1091.

2. Swell, L., Trout, E. C., Jr., Hopper, R., Field, H., Jr., Treadwell, C. R., *Ann. N. Y. Acad. Sci.,* 1959, v72, 813.

3. Beveridge, J. M. R., Connell W. F., Haust, H. L., Mayer, G. A., *Can. J. Biochem. Physiol.,* 1959, v37, 575.

4. Hashim, S. A., Arteaga, A., Van Itallie, T. B., *Lancet,* 1960, vi, 1105.

5. Kritchevsky, D., Tepper, S. A., Langan, J., *J. Atherosclerosis Res.,* 1962, v2, 115.

6. Wright, L. D., Presberg, J. A., *Fed. Proc.,* 1963, v22, 269.

7. ———, PROC. SOC. EXP. BIOL. AND MED., 1964, v115, 497.

8. Lin, T. M., Karvinen, E., Ivy, A. C., *Am. J. Physiol.,* 1955, v183, 86.

9. Wright, L. D., private communication.

10. Kritchevsky, D., Tepper, S. A., *J. Nutrition,* 1961, v74, 441.

11. Kim, K. S., Ivy, A. C., *Am. J. Physiol.,* 1952, v171, 302.

12. Swell, L., Flick, D. F., Field, H., Jr., Treadwell, C. R., *ibid.,* 1955, v180, 124.

Received February 3, 1964. P.S.E.B.M., 1964, v116.

Changes in Pituitary LH Concentration during Pseudopregnancy in the Rat.* (29173)

NEENA B. SCHWARTZ AND IRVING ROTHCHILD

Departments of Physiology, University of Illinois College of Medicine, Chicago, and Obstetrics and Gynecology, Western Reserve University School of Medicine, Cleveland, Ohio

Accompanying ovulation in the cyclic rat, pituitary LH content falls from maximal values at proestrus to minimal values at estrus(1,2). During pseudopregnancy, when ovulation is suppressed, pituitary folliculotrophin† (FTH) content, as measured by the mouse uterine weight assay, rises above levels found in mixed cyclic pituitaries(3). Since this assay method does not distinguish between FSH and LH, the question of what happens to pituitary LH content during pseudopregnancy has remained unanswered. In the present study direct measurements of pituitary LH concentration were made daily during pseudopregnancy. The ovarian ascorbic acid depletion method was used to assay LH(4); this assay method is not sensitive to FSH or other pituitary hormones(1,4, 5). Pituitary LH concentration was also measured in cyclic rats on the days of proestrus, estrus and the first day of diestrus (metestrus) to provide control data.

Materials and methods. (1) *Donor rats and pituitary collection.* All donor rats were of the Sprague-Dawley strain (obtained from Holtzman Rat Co., Madison, Wis.). They were housed 10 to 20/cage, in a room lighted with natural daylight, and allowed *ad libitum* access to Purina rat chow and water. Vaginal smears were taken daily, except Sunday, until each rat became part of an experimental group, after which smears were taken daily. Pituitaries of cyclic rats were removed on

* This work was supported by USPHS grants from Nat. Inst. of Arthritis and Metab. Diseases, from the Division of General Medical Sciences, and an institutional grant to Western Reserve University for the study of aging.

† The term folliculotrophin is used as a generic one for FSH and LH, to distinguish these gonadotrophins from luteotrophin (LTH), which is also a gonadotrophin. A fuller justification for the use of this term may be found in *Endocrinology,* 1960, v67, 9.

the day of proestrus, estrus, or on the first day of diestrus (metestrus). Pseudopregnancy was induced by mechanical stimulation of the cervix with a small glass rod on the days of proestrus and estrus. In our hands this method induces pseudopregnancy in at least 95% of cases. Pituitaries of the pseudopregnant rats were removed on each day of the diestrus, except day 2, until and including day 12.

All rats were killed before noon. The pituitaries were removed, gently blotted dry of blood, and weighed on a Roller-Smith torsion balance; the posterior lobe was then removed and the anterior lobe crushed between a pair of chemically clean glass slides. The latter were then separated and the tissue allowed to dry in air. All pituitaries of a particular group were collected on the same pair of slides, which were kept in a desiccator over calcium chloride between autopsies and until they were prepared for assay. From 5 to 8 pituitaries were collected for each of the 3 pools of cyclic rat pituitaries, and from 3 to 5 pituitaries were collected on each of the days of pseudopregnancy, except for day 12 when 7 pituitaries were collected and assayed in 2 separate pools. One consisted of pituitaries from 2 rats, each of which showed solid uteri and leucocytic vaginal smears. The other consisted of 5 pituitaries from rats showing predominantly nucleated epithelial cells in the vaginal smear and ballooned uteri at autopsy.

(2) *Method of assay.* Immature recipient female rats (obtained from Sprague-Dawley, Inc.) were pretreated with pregnant mares serum gonadotrophin (PMS) and human chorionic gonadotrophin (HCG) as described previously(1). Before the day of assay, the dried pituitary material was scraped from the slides, ground to a fine powder with an agate mortar and pestle and weighed. The powder was put into suspension in saline on the day of assay. On occasion, some of the solution was refrozen for replication assay in a different group of recipients. The LH standard (NIH-LH-S_1 or S_2), or the pituitary material being assayed, was injected intravenously 161 hours after the HCG injection, and both ovaries were removed 4 hours later for ascorbic acid measurement(6). Two doses of LH standard, 0.4 and 1.6 μg, and equivalent dose ratios of the pituitary material, were used. (The *higher* dose of pituitary powder ranged from 0.28 to 0.40 mg per rat.) Five recipient rats were used at each dose and 2 or 3 pituitary pools were assayed simultaneously. Pituitary potencies were calculated using standard statistical techniques(7).

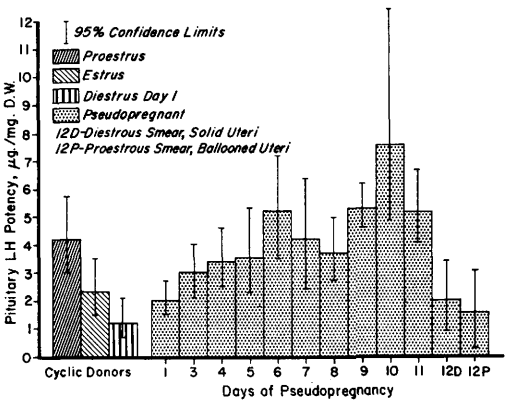

FIG. 1. Pituitary LH concentrations (μg/mg dry wt) in cyclic and pseudopregnant rats. Pituitary pools were collected and assayed separately on day 12 of pseudopregnancy, depending on the condition of the vaginal smear (proestrous or diestrous) or uterus (solid or ballooned).

Results. (1) *Concentration of LH in pituitaries of cyclic rats in relation to time of ovulation.* The concentration (μg LH/mg dry weight) of LH in the pituitary during 3 stages of the cycle is seen in Fig. 1. The concentration was maximal on the morning of proestrus, and minimal on diestrus day 1. The value found on the day of estrus was about half that found for proestrus and remained low on the first diestrus day, confirming previous observations(1) made on Sprague-Dawley rats obtained directly from Sprague-Dawley, Inc.

(2) *LH concentration in the pituitary during pseudopregnancy.* The LH concentration in the pituitaries of the pseudopregnant rats is also shown in Fig. 1. On the first day of diestrus, LH concentration resembled that seen on the first day of diestrus or estrus in the cyclic rats, indicating that cervical stimulation had not changed this initial low value. By the 4th day of diestrus, the concentration had reached a value similar to that of the cyclic rat's pituitary during

proestrus; but instead of the characteristic fall that would have occurred on the next day in *cyclic* rats, the concentration in the pseudopregnant rats continued to rise through the 11th day of diestrus. This rise was confirmed statistically by means of a regression analysis of LH concentration expressed as a function of day of pseudopregnancy (eliminating day 12): LH, μg/mg = 1.72 μg/mg + 0.404 $\times$ day of pseudopregnancy. The slope, 0.404 (with dimensions of μg/mg/day), is significantly greater than 0 ($P < 0.01$), indicating that the apparent rise during the course of pseudopregnancy (Fig. 1) is real. The intercept (hypothetical day 0) of the line, 1.72 μg/mg, is within the confidence limits for estrous values (Fig. 1). LH concentrations on the 9th-11th day have a combined value of 5.4 μg/mg (95% conf. lim. = 4.7-6.2) which is suggestively higher than the cyclic proestrous levels, 4.2 μg/mg (95% conf. lim. = 3.0-5.7).

The drop in LH concentration on day 12 is highly significant, since there was no overlap of confidence limits between day 11 and day 12 values. Although both groups of day 12 rats seemed to be in a preovulatory rather than an immediately postovulatory condition (by vaginal smear and uterine criteria) their pituitary LH concentrations were in the estrous range (Fig. 1).

Discussion. The fall in pituitary LH concentration between the mornings of proestrus and estrus (Fig. 1) is critical to an interpretation of the data on pituitary LH concentration in pseudopregnant rats and resembles in Sprague-Dawley derived Holtzman rats what has been observed for 3 other strains. For Sprague-Dawley rats obtained directly from the original breeder pituitary LH content at proestrus is 8-9 μg and at estrus is 4 μg(1,2) for pituitaries weighing 10.3 mg (wet); for Sprague-Dawley derived rats raised in California the change in LH content from proestrus to estrus is 19.9 μg to 9.8 μg for pituitaries weighing 9.0 mg(8); for Sprague-Dawley derived Charles-River rats, whose pituitaries weigh 14.6 mg, proestrus levels are 25.3 μg (95% conf. lim. = 14.9-43.0) and estrous levels are 12.3 μg (95% conf. lim. = 9.6-15.7) (N. B. Schwartz, unpublished observations). The wet weights of the pituitaries in this study were quite constant regardless of cycle stage, 9.6 mg (SD = 1.1) for all cyclic rats and 9.1 mg (1.9) for all pseudopregnant rats. Thus in 4 different strains of Sprague-Dawley rats, regardless of *absolute* levels of LH in the pituitaries, the change from proestrus to estrus is essentially a 50% drop in LH concentration, which undoubtedly reflects the discharge of LH associated with ovulation.

By contrast, in the pseudopregnant rats, LH concentration increases from the low diestrus day 1 value up to proestrous levels by the 3rd or 4th day, as it does in cyclic rats (Fig. 1; 1,2), but then does not drop. Instead there is a significant trend of pituitary LH concentration upward through the 11th day of pseudopregnancy, probably a reflection of the delay in ovulation. The effect of pseudopregnancy is thus similar to the effect of pentobarbital, which, in blocking ovulation in cyclic rats, also prevents the drop in LH concentration that would occur on the day after proestrus(2).

This tendency for LH concentration in the rat's pituitary to be elevated during pseudopregnancy has also been observed by Van Rees and de Groot (personal communication), and resembles the changes previously described for total FTH(3). The tendency for the pituitary concentration of total FTH, or FSH and LH specifically to rise during homologous luteal phase conditions has also been noted in the guinea pig(9), rabbit(10, 11), sheep(12,13,14) and pig(15). It seems most likely that this effect is in some way due to progesterone, since progesterone is of course secreted during the luteal phase, can block ovulation, and can be shown to increase the concentration in the pituitary of total FTH(3) as well as LH specifically(16) when administered to cyclic rats. We have also found that treatment of pseudopregnant rats for 28 days with progesterone maintains the high level of LH seen during untreated pseudopregnancy (N. B. Schwartz and I. Rothchild, unpublished studies). The mechanism of this effect of progesterone, aside from its obvious relation to the inhibition of ovulation, remains to be determined.

Although ovulation was not looked for in the day 12 rats, it seems possible from the low LH concentrations in these rats that it had already occurred. We have in fact observed a tendency for ovulation to occur at the end of normal pseudopregnancy even before the vaginal smear pattern showed the characteristic sequence of changes seen in cyclic rats. Furthermore, the ovulation that occurs following cessation of progesterone treatment of cyclic rats is not necessarily preceded by a proestrous smear or a ballooned uterus (J. C. Hoffmann and N. B. Schwartz, unpublished observations). On the other hand, a drop in pituitary gonadotrophin concentration occurs on day 12 of pregnancy in rats(17) and on day 12 post partum (compared with day 6) in lactating mice(18), under circumstances in which ovulation probably does not take place. The point obviously needs to be settled in the case of pseudopregnancy by direct observation for ovulation.

Summary. The LH concentration (measured by the ovarian ascorbic acid depletion assay) in the pituitaries of pseudopregnant rats showed a fairly progressive rise from day 1 of the diestrus through day 11. On day 1, the level (2.0 μg/mg dry wt) was equivalent to that seen in cyclic rats in estrus or on day 1 of diestrus. The levels virtually equalled or slightly exceeded those characteristic of proestrus (4.2 μg/mg dry wt) from day 4 through 8. On days 9 through 11, the mean potency (5.4 μg/mg dry wt) somewhat exceeded proestrous levels. On day 12 the LH concentrations (1.5 to 2.0 μg/mg dry wt) were again typical of estrous levels in cyclic rats.

The authors gratefully acknowledge generous supplies of NIH-LH from Endocrinology Study Section, Nat. Inst. Health; and technical assistance of William Talley, Jack Thiel and William Van Zant.

1. Schwartz, N. B., Bartosik, D., *Endocrinology,* 1962, v71, 756.
2. Schwartz, N. B., *The Physiologist,* 1963, v6, 270.
3. Rothchild, I., *Endocrinology,* 1962, v70, 303.
4. Parlow, A. F., *Fed. Proc.,* 1958, v17, 402.
5. McCann, S. M., Taleisnik, S., *Am. J. Physiol.,* 1960, v199, 847.
6. Mindlin, R. I., Butler, A. M., *J. Biol. Chem.,* 1937, v122, 673.
7. Bliss, C. I., *Drug. Stand.,* 1956, v24, 33.
8. Gorski, R. A., Barraclough, C. A., *Acta Endocrinol.,* 1962, v39, 13.
9. Schmidt, I. G., *Endocrinology,* 1937, v21, 461.
10. Hill, R. T., *J. Physiol.,* 1935, v83, 129.
11. Makepeace, A. W., *Am. J. Obstet. and Gynec.,* 1937, v37, 457.
12. Kammlade, W. G., Welch, J. A., Nalbandov, A. V., Norton, H. W., *J. Animal Sci.,* 1952, v11, 645.
13. Santolucito, J. A., Clegg, M. T., Cole, H. H., *Endocrinology,* 1960, v66, 273.
14. Robertson, H. A., Hutchinson, J. S. M., *J. Endocrin.,* 1962, v24, 143.
15. Robinson, G. E., Nalbandov, A. V., *J. Animal Sci.,* 1951, v10, 469.
16. Hoffmann, J. C., Schwartz, N. B., *Fed. Proc.,* 1964, v23, in press.
17. Zeiner, F. M., *Anat. Rec.,* 1952, v113, 255.
18. Greenwald, G. S., *Gen. and Comp. Endocrinol.,* 1962, v2, 453.

Received February 3, 1964. P.S.E.B.M., 1964, v116.

Exacerbation of Glycosuria by Partial Hepatectomy in the Partially Depancreatized Rat.* (29174)

DWIGHT J. INGLE
Department of Physiology, University of Chicago, Chicago, Ill.

These experiments show that removal of 70% of the liver in mildly or moderately diabetic rats is followed by increased glycosuria for several days. The glycosuria of severely diabetic rats was not increased by partial hepatectomy.

Methods. 108 male rats of the Sprague-Dawley strain weighing approximately 300 g were partially depancreatized. Six to 8 weeks later each rat was placed in a metabolism

* This investigation was supported by USPHS Research Grant.