shaken and washed by repeated centrifugations in physiological saline until the supernatant fluid was clear. The latter was poured off and the remaining material weighed. A few drops of 25% NaOH were then added to the centrifugate and the material was arbitrarily standardized in saline so that 1 cc. contained a certain number of milligrams of wet weighed material, the amount depending upon the experiment. The entire centrifugate was ground in a ball mill for from 2 to 3 hours, and its pH brought up to 7.2; it was then bottled. The resulting mixture was not a true solution, but a finely precipitated substance which had the appearance of ground glass. When 0.1 cc. of a 1/100 dilution of this substance was injected into human subjects, it gave a localized inflammatory reaction that started in 6 and reached its maximum in 24 hours (50 individuals in all). When a 1/100 dilution was boiled for from 10 to 15 minutes, it lost its power to cause a reaction in human subjects (10 observations).

When this same dilution was concentrated to a powder *in vacuo* it lost from 90 to 95% of its wet weight (averages from 6 specimens). For example, the amount obtained from 250 cc. of culture media was 300 mg. of dried powder. The wet weight of the centrifugate in this case was 4.375 gm.

When this powder was injected intravenously into rabbits in the manner and doses described previously, it produced agglutinins specific for old strains of *H. pertussis*.

7751 C

The Effect of Gonadotropic Hormones During Gestation and Lactation.

H. SELYE, J. B. COLLIP AND D. L. THOMSON.

From the Department of Biochemistry, McGill University, Montreal.

Many authors have studied the effect of gonadotropic extracts, prepared from pregnancy urine or pituitary tissue, on the course of gestation, and it is well known now that small doses do not interfere with the development of the embryos, but large doses cause abortion or intra-uterine fetal death.[1-4]

Our previous experiments[5, 6] convinced us that the development

[1] Katzman, P. A., Levin, L., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 873.

and maintenance of the corpus luteum of gestation cannot entirely be explained by the action of the known gonadotropic hormones in themselves, and that some other factor must be present during this period in order to produce the typical pregnancy ovary. It seemed of interest, therefore, to compare the reaction of the ovary to gonadotropic hormones during pregnancy with that obtained in the non-pregnant condition. We have, therefore, performed the following experiments:

Eight pregnant and 6 non-pregnant adult female rats received daily 20 rat units of the anterior pituitary-like hormone of pregnancy urine (A.P.L.) on 4 consecutive days by subcutaneous injection. They were killed on the fifth day. The ovaries of the non-pregnant animals were only slightly enlarged, averaging 80 mg. They contained numerous relatively small corpora lutea, and there was little luteinization of theca cells. The ovaries of the pregnant animals, on the other hand, were larger—averaging 113 mg. They showed marked signs of theca luteinization and contained numerous unusually large cystic corpora lutea. This atypical ovarian reaction to A.P.L. was still more marked in a second series in which 8 pregnant and 4 non-pregnant rats were given 50 units of A.P.L. over a period of 5 days. These animals were sacrificed on the sixth day. While the ovaries of the pregnant animals averaged 191 mg., those of the non-pregnant controls averaged 113 mg. Here again the presence of unusually large cystic corpora lutea, and the marked luteinization of theca cells distinguished the ovaries of the pregnant animals from those of the controls.

In another series of 6 hypophysectomized pregnant rats treated with 20 units of A.P.L. daily for 5 days and killed on the sixth day, we saw no increase in the weight of the ovaries as compared with uninjected pregnant controls (average 77 mg.). There was no follicle maturation and no corpus luteum formation, the only result of the treatment being a luteinization of theca cells as described in previous publications on non-pregnant rats treated with A.P.L after hypophysectomy.[7, 8]

From these experiments we conclude that during pregnancy

[2] Bourg, R., *Compt. rend. Soc. de biol.*, 1931, **108**, 216.

[3] Martins, T., and Fabiao, M., *Compt. rend. Soc. de biol.*, 1930, **105**, 791.

[4] D'Amour, F. E., D'Amour, M. C., and Gustavson, R. G., *J. Pharmacol.*, 1933, **49**, 146.

[5] Selye, H., Collip, J. B., and Thomson, D. L., *Anat. Rec.*, 1934, **58**, 139.

[6] Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 488.

[7] Collip, J. B., Selye, H., and Thomson, D. L., *Nature*, 1933, **131**, 56.

[8] Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 262.

A.P.L. leads to an unusually marked ovarian reaction with the formation of very large corpora lutea, and that this change in the effect of A.P.L. is conditioned by a change in the function of the hypophysis and not directly by the developing ova.

In this connection we should like to mention another experiment in which a gonadotropic extract, prepared from pig pituitary glands, was given to 6 pregnant and 6 non-pregnant adult female rats daily for 4 days. The dosage was approximately comparable to that of the urinary preparation used in the above experiment. The animals were killed on the fifth day. While the ovaries of the pregnant rats averaged 152 mg., showing again large cystic corpora lutea, those of the controls averaged only 91 mg. and consisted mainly of relatively small corpora.

Since the nervous stimulus of suckling is also capable of modifying the structure and function of the ovary,[9, 10] we have also studied the effect of gonadotropic hormones on lactating rats. Twenty-four animals, 12 lactating and 12 non-lactating adult controls, were used. Half of each group received 50 units of A.P.L. daily for 9 days, while the other half were treated for the same length of time with a comparable dosage of the gonadotropic extract prepared from pig pituitary. The macroscopical appearance of the ovaries and the increase in their weight (average 95 mg.) were very nearly the same in the 4 groups. Upon histological examination, however, we saw marked signs of thecal luteinization as well as granulosa luteinization in the ovaries of the A.P.L. treated lactating rats. The thecal luteinization in this group, just as in those previously mentioned, was particularly striking in the so-called "theca-nests"; that is, in those groups of theca cells that persist after the rest of the follicle becomes atretic. These theca-nests show no signs of luteinization in the A.P.L. treated, non-lactating animals or in any of these rats—lactating or non-lactating—which received the pituitary extract.

From these experiments we conclude that the effect of A.P.L on the ovary is modified during lactation, while that of the pituitary gonadotropic extract is the same in the lactating and in the non-lactating animal.

It seems, therefore, that the ovary of the lactating rat, like that of the hypophysectomized animal, shows a qualitatively different reaction to the gonadotropic preparations obtained from pregnancy urine and those prepared from the pituitary gland.

---

[9] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinol.*, 1934, **18**, 237.

[10] Selye, H., and McKeown, T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 683.

Although in the 4 groups of this last experiment the increase in ovarian weight was approximately the same, the appearance of the uterus was quite different in the lactating and non-lactating animals. While both the urinary and the pituitary extracts led to a great increase in the thickness of the uterus in the non-lactating animals, neither of these preparations had any marked effect on the thickness of the organ during lactation. Histologically the uterus of the non-lactating animals showed a marked increase in the connective tissue fibers of the mucosa. The epithelium was ciliated and its free surface was very irregular; mitotic divisions were rarely observed. The appearance of the organ is similar to that seen in the sterile horn in the case of unilateral pregnancy and may therefore justly be described as "progestational". The usual progestational proliferation was not produced by either the urinary or the pituitary preparation in the lactating animals, although the increase in ovarian weight was not markedly interfered with by lactation. In the lactating animals the uterine connective tissue fibers were poorly developed and the stroma of the mucosa contained more cells and fewer fibers. The epithelium was not ciliated and had a very regular free surface. Mitotic divisions of the epithelial cells were frequent. The general appearance of the organ was similar to that of the uterus of the untreated lactating rat. This inhibition of the effect of gonadotropic preparations on the uterus during lactation is probably largely independent of the ovarian response.

If one considers the thecal reaction obtained after A.P.L. administration during lactation as a result of an inhibition of hypophyseal function by nursing, it is remarkable that the weight increase of the ovary after the administration of this extract is not more distinctly inhibited. We thought that the administration of too large doses of A.P.L. might be responsible for this lack of inhibition in our animals.

We have, therefore, repeated these experiments with smaller doses. Our animals were divided into 3 groups. The first contained 6 lactating females; the second, 6 females weaned on the day of delivery. Injections were started in these 2 groups on the second day after delivery. In the third group we had 6 normal cyclic adult females. Twenty units of A.P.L. were given daily for 4 days and the animals were sacrificed on the fifth day. The weight of the ovaries averaged 85 mg. in the first group, 114 mg. in the second, and 59 mg. in the third.

This experiment shows that if adequately small doses of A.P.L. are given one may demonstrate an inhibiting effect of nursing on the

action of this hormone on ovarian weight. It shows, furthermore, that the unusually marked responsiveness of the ovary to A.P.L. which we observed during the course of pregnancy is not lost at parturition, and is still demonstrable during the first days post-partum if nursing is not allowed. A study of the uteri of these animals confirmed the findings reported above.

Summary. 1. Both the gonadotropic hormone of pregnancy urine and that prepared from pituitary tissue lead to a more marked ovarian response in the pregnant than in the non-pregnant rat. This increased responsiveness to gonadotropic hormones continues for some time post-partum if nursing is not allowed. 2. In hypophysectomized rats urinary preparations lead only to thecal luteinization, even when given during gestation. 3. Similarly in lactating and pregnant rats, the urinary preparation leads to thecal luteinization, but granulosa luteinization also occurs at the same time. Pituitary preparations do not lead to thecal luteinization during lactation. 4. Neither the urinary nor the pituitary preparation is able to produce the usual uterine reaction when given during lactation.

7752 P

A Crystalline Iron Chloride Molecular Compound of Urobilin and Stercobilin.*

C. J. WATSON. (Introduced by H. A. Reimann.)

From the Department of Medicine, University Hospital, Minneapolis.

Evidence was presented previously[1] for the identity of crystalline urobilin and stercobilin as isolated from human urine[2] and feces,[3] respectively. These crystalline substances unfortunately do not have sharp melting points, nor is the "urobilin" absorption spectrum specific,[4] hence it was desirable to provide further means of positive identification, particularly for the study of substances which may be

* Aided by a grant from the research fund of the Graduate School of the University of Minnesota.

1 Watson, C. J., *Z. Physiol. Chem.*, 1933, **221**, 145.

2 Watson, C. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1207.

3 Watson, C. J., *Z. Physiol. Chem.*, 1932, **208**, 101.

4 Fischer, H., *Ueber Blut, Blatt, u. Gallenfarbstoff. Oppenheimers Handb. d. Biochem. des Menschen u. d. Tiere. II Auflage. Ergänzungsband.* G. Fisher, Jena, 1930.