is greatly reduced within 4 weeks. The length of period of hypnosis is also markedly shortened. Daily doses of 25% of the M.F.D. produce the same effects in a much shorter time. Pigs which had received 16.6 to 20.8% of the M.F.D. semi-weekly for 6 weeks showed a definite tolerance to 29.1 to 33.3% of the M.F.D. Pigs which had had several semi-weekly injections of 25% M.F.D., or several daily injections of as much as 25% to 33% of the M.F.D., and which had shown definite tolerance in both groups as measured by the duration of sleep and hypnosis, were not protected against a dose of 60 mg. (M.F.D.) per kilo.

We used 63 guinea pigs in the semi-weekly group. The results obtained from the first injection of the drug in each of the animals of this group served as controls. That is, animals which received subsequent doses were in an hypnotic state for a shorter time than they were after the first injection. Thus there were 63 controls for this group.

For the daily group 24 guinea pigs were employed. The results of the first injection in each pig served as controls, as above, so there were 24 controls for this group.

For determining the M. F. D. we sacrificed 24 normal animals.

To test the tolerance against a M. F. D. (60 mg. per kilo.) 33 pigs were used. These pigs had been treated previously as follows: (1), 4 pigs had received 19 semi-weekly injections, (2), 19 pigs had had 19 semi-weekly and 14 daily injections, and (3), 10 pigs had had 9 daily injections.

In all 111 individual guinea pigs were used.

6919

Effects of Different Anterior Pituitaries and Human Pregnancy Urine on Rat Sex Organs.*

LEO LOEB.

From the Department of Pathology, Washington University School of Medicine.

We have shown[1, 2] that anterior pituitaries of different species as

* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 642, 1128. *Endocrinology*, 1932, **16**, 129.

[2] Loeb, Leo, and Friedman, Hilda, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 741.

well as urine of pregnant women act differently on the sex organs of the guinea pig. The anterior pituitary of rat, rabbit, cat and guinea pig caused enlargement and maturation of follicles, formation of pseudo-corpora lutea and interstitial gland, and in some cases induced an ovulation. Injection of extracts as well as inoculation of pieces of anterior pituitary of cattle, hog and sheep produced an atresia of follicles so intense that it could not be attributed merely to a loss in weight of the animals; furthermore it gave rise to the formation of rudimentary interstitial gland and of usually small pseudocorpora lutea, which were essentially derived from hypertrophying theca interna and connective tissue elements, although in some cases a limited participation of granulosa could not be excluded. In exceptional cases we observed apparently an ovulation at very early stages following administration of this kind of anterior pituitary. Urine of pregnant women resembled in its action the anterior pituitaries of rabbit and rat, but differed from them in that it did not produce large mature follicles in the ovaries of the injected animals.

Since, as a test animal for the presence of anterior pituitary-like substances, the rat is commonly used, we now have studied the action of anterior pituitary of different species and of the urine of pregnant women on rats, to determine how far the results obtained were similar to those obtained in the guinea pig. For the most part young rats, weighing between 30 and 40 gm., but in some cases older animals, with initial weights of approximately 50 and 75 gm., were used. As in our former investigations, we studied microscopically in each case the ovaries in serial sections and parts of the other organs. More than 70 rats were used. We here present a summary of the results.

1. Daily inoculations for a variable number of days, of one-half rabbit anterior pituitary, of one or 2 rat anterior pituitaries and injections of one to 2 cc. of urine of pregnant women acted approximately in the same manner. They caused an enlargement of the interstitial gland, which developed as a result of the hypertrophy of the theca interna in atretic small follicles. In contradistinction to the condition found in the guinea pig, where, in addition to theca interna apparently also ordinary connective tissue cells situated around medullary vessels may change into interstitial gland, in the rat this change was limited to the theca interna cells. Also, in some cases, there was enlargement of the theca interna around certain growing follicles.

There was an increase in the number of follicles which developed into large follicles and also of large follicles the granulosa of which showed maturation, as manifested by a slight increase in the amount of cytoplasm. However, in the rat the maturation changes in the granulosa are much less evident than in the guinea pig. In certain cases when the follicles have reached this stage of development, the capillaries in the theca interna enlarge, become engorged with blood, and invade the granulosa. A layer of connective tissue begins to line the follicular cavity. The granulosa cells now enlarge and become pseudo-lutein cells, and these may divide mitotically. The egg in the meantime has undergone maturation divisions and subsequently further changes take place. These pseudolutein bodies become gradually converted into pseudocorpora lutea, as a result of organization of the central cavity by vessels and connective tissue, which later contracts. The pseudolutein tissue may further enlarge. This is the typical process which occurs in a great number of follicles. In many of these pseudolutein bodies and pseudocorpora lutea, remnants of eggs can be found, but not in every case. I have not seen any definite evidence of ovulation under these conditions, but I do not as yet wish to deny that it may sometimes occur.

The vessels in the medulla of the ovary are usually enlarged, and there may develop an edematous condition in the ovary which may lead to injury of the interstitial gland and even of growing follicles. Thus, gradually, the degeneration of granulosa of such follicles may become quite prominent, when the stage of full development of many pseudocorpora lutea has been reached. It is perhaps also due to this hyperemia that in full-sized follicles the intrafollicular fluid may increase considerably, dilate the follicles and exert a pressure on the granulosa.

The vagina, which in these cases usually begins to open on the third or fourth day of the experiment, undergoes proliferation and keratinization. Subsequently. the keratin is cast off and the squamous epithelium may be reduced in thickness. The uterine epithelium becomes usually high cylindrical at a certain stage and the mammary gland shows increased proliferation. These changes are more pronounced in rats injected with pregnancy urine, on account of the presence of follicular hormones in this urine.

2. Daily inoculation of one or 2 guinea pig anterior pituitaries shows effects much less marked in the ovary of the rat. We find merely growing follicles of various sizes—exclusive of large mature follicles—as well as follicles in various stages of degeneration.

After 8 days of inoculation we encounter follicles which seem to approach maturation. The vagina may show a limited proliferation, but does not usually form typical squamous epithelium. The uterus is in a resting state or shows only slight signs of stimulation. The mammary gland remains relatively inactive. We find then in the rat, as well as in the guinea pig, that the effects of guinea pig anterior pituitary are much less marked than those of rabbit and rat anterior pituitary. In the rat this difference between the effects of these types of anterior pituitaries seems to be perhaps still more pronounced than in the guinea pig. However, we have a right to assume that it is of a quantitative rather than of a qualitative character.

3. Daily injections into rats of quantities of acid extracts of anterior pituitary of cattle varying between 0.1 and 1 cc. do not lead to the development of large, mature follicles. The follicles seem on the whole to correspond to the average type of those observed in normal ovaries. There may be seen, however, some fairly large follicles. Granulosa degeneration of follicles of medium and somewhat larger size is usually prominent. From the fourth day on, pseudocorpora lutea appear which are mostly of small size, corresponding in character to hypertrophic atretic follicles, in the wall of which the cells in certain areas assume the character of pseudolutein cells. These pseudolutein cells are usually surrounded by capillaries. In some cases such pseudocorpora lutea are somewhat larger. One has the impression that one has to deal in these cases with a transformation of theca interna elements and of connective tissue cells in pseudolutein tissue, a process which occurs essentially during the atresia of follicles. However, in a few ovaries, examined 3 or 4 days following the beginning of injections, I could see an ingrowth of some capillaries from the theca interna into the granulosa in follicles in which the cavity was lined by a layer of connective tissue. This ingrowth was quite localized and often accompanied by degeneration of granulosa cells nearby. In view of these observations, which we intend to test further, we do not wish to exclude the possibility that granulosa cells may participate in the formation of some of these small pseudocorpora lutea.

The interstitial gland around quite atretic follicles in the medulla does not enlarge as it does in the ovaries of animals treated with rat or rabbit anterior pituitary or with pregnancy urine. The vagina does not change into typical squamous epithelium and shows at best only a very slight mitotic activity. In the large majority of

cases, the vagina remained closed during the period of injections. Also the uterus is in a resting condition, or it may show signs of only a mild stimulation. The mammary gland is likewise mostly in a relatively resting condition or is slightly proliferating.

We see then in the rat, as in the guinea pig, a marked difference between the effects of extract of cattle anterior pituitary and the effects of rabbit and rat anterior pituitary or of pregnancy urine. The pseudolutein bodies differ in character, in the latter cases, very markedly from those found after injection of extract of cattle anterior pituitary and resemble the structures seen in corresponding cases in the guinea pig. The behavior of the vagina, uterus and mammary gland likewise corresponds to the findings in the guinea pig.

4. However, in the rat the atresia of follicles, which we find in the guinea pig after injection of extract of cattle anterior pituitary, is lacking, presumably due to a greater power of resistance to injurious influences possessed by the granulosa cells of the rat follicles as compared with those in the guinea pig ovary. This greater resistance in the rat is also indicated by the lack of degeneration of these granulosa cells at the time of ovulation. This characteristic feature enables the rat to have such a short sexual cycle and the ovulation of so many follicles at the beginning of this cycle. The greater sensitiveness of the granulosa cells in the guinea pig explains certain differences in the effect of pregnancy urine which are lacking in the rat. On the whole, the ovary of the guinea pig seems thus to be a finer reagent for the effects of anterior pituitary preparations than the more resistant ovary of the rat.

5. Moreover the rat as a whole seems to be more resistant to the action of anterior pituitary preparations than the guinea pig. We have noticed this greater resistance formerly in the case of the thyroid gland, and we notice it now in the case of the sex organs, where pro unit weight of the animal we applied greater quantities of anterior pituitary material in the rat than in the guinea pig, in order to obtain the typical effects. This difference in the responsiveness between rat and guinea pig is especially evident in the case of the inoculation of guinea pig anterior pituitary.