between rate of respiration and per cent concentration of heavy water. It will be observed that concentrations below 20% had no inhibitory effect, 45% caused a 5% inhibition, 60% about 13%, 72% heavy water about 20%, 86% heavy water about 27%, and 97-98% heavy water about 50% inhibition after one hour. The effect tends to increase after 2 hours. If respiration is to be reduced to zero in 100% heavy water the curve must be very steep between the values 80% and 100%, a point difficult to test on account of the hygroscopic properties of D_2O.

7391 C

Effect of Combination of Two Antagonistic Anterior Pituitary Hormones on Sex Organs of Guinea Pig.*

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It has been shown that 2 antagonistic hormones are present in the anterior pituitary gland, one causing growth and maturation of follicles and the other atresia of follicles in the ovary of the guinea pig; the former predominates in the anterior pituitaries of rabbit, rat and guinea pig and the latter in the glands of cattle, sheep and hog, at least in the state in which these latter are accessible to us for experimentation.[1, 2, 3] When as the result of the action of the first hormone the follicle has reached full size and matured, either ovulation and formation of true corpora lutea may take place or there may be ingrowth of vessels and connective tissue into the granulosa and formation of pseudocorpora lutea not associated with ovulation.[2] There occurs a third effect common to the substances in which both of these hormones are found, a transformation of theca interna, and probably also of connective tissue cells into strands of interstitial gland and into interstitial gland bodies, which transformation is found in the course of follicular atresia.[4] Tran-

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

2 Loeb, Leo, *Ibid.*, 1933, **30**, 1335.

3 Loeb, Leo, *Ibid.*, 1932, **29**, 642.

4 Loeb, Leo, *Endocrinology*, 1932, **16**, 129.

sitions from the interstitial gland bodies to small pseudocorpora lutea occur and such pseudocorpora lutea may be seen especially after several injections of extract of cattle anterior pituitary into the guinea pig. Smaller amounts of pseudolutein tissue may furthermore be produced in the ovary of the guinea pig in follicles undergoing connective tissue atresia as a result of cattle anterior pituitary extracts. However, we do not wish to exclude the possibility that under certain conditions also granulosa may participate in the formation of these structures, at least in a limited way.

Under these conditions it was of interest to study the combined effect of these 2 antagonistic principles in the guinea pig. Such investigations were begun several years ago; at that time we combined injections of extract of cattle anterior pituitary with implantations of guinea pig anterior pituitary and in one case with implantation of anterior pituitaries of rabbit.[4] We found an inhibiting effect of the action of the extract on the growth promoting action of rabbit and guinea pig anterior pituitary. Continuing these experiments we wished to determine whether the atresia-causing hormone of the anterior pituitary would still be able to counteract the hormone causing follicular growth and maturation, in case the extract of cattle anterior pituitary containing the former principle were given in a first period and this were followed in a succeeding period by daily inoculations of either guinea pig, rat or rabbit anterior pituitary glands. In control experiments, the guinea pigs in a first period were underfed so that they lost weight like those animals which had been injected with cattle anterior pituitary extract. In addition the effects of inoculation of various types of anterior pituitary glands in well fed guinea pigs, as well as the effect of underfeeding alone and of extract not accompanied by implantation of anterior pituitary glands, were tested. The numerous experiments, which we carried out were varied as to the quantities of both kinds of anterior pituitary material used and as to the length of time during which these hormones were applied.

We used in our experiments guinea pigs, because, as we have pointed out previously, the ovary of this animal represents the most delicate reagent for the 2 principles present in the anterior pituitary glands of various species, the one causing atresia of follicles and the other promoting the development of mature follicles; in the rat, which has usually served as test animal for investigators who studied the effect of combinations of different anterior pituitary preparations or of anterior pituitary-like preparations, the atresia producing effect of certain anterior pituitary preparations cannot

be discovered or it can be distinguished from other effects only with great difficulty.[5]

The initial weight of the female guinea pigs, used in our experiments, varied between 175 and 220 gm. We used the acid extract of cattle anterior pituitary to which reference has been made in our earlier publications[6] and which has served for the large majority of our experiments; however, as we have pointed out previously, alkali extract, and as we may add, extract prepared with 0.9% NaCl solution, act in principle in the same way. The anterior pituitary glands which we employed for implantation were those of male animals (rabbits weighing between 1500-2000 gm., rats weighing about 150 gm., and guinea pigs weighing 350-420 gm.).

In a first period of 5 days, the guinea pigs received daily injections of 1 or 2 cc. of extract; or they were underfed, or still others were kept under normal conditions. In a subsequent period varying between 4 and 7 days, the same animals received daily implantations of the anterior pituitary glands, others which did not receive these implantations, served as controls. Fourteen guinea pigs received anterior pituitary glands of guinea pigs (1 or 2 glands daily); 27 guinea pigs were daily implanted with rabbit anterior pituitaries (1/3 to 1 gland each time) and 18 guinea pigs received daily implantations of rat anterior pituitaries (1-2 glands each time).

Forty guinea pigs received injections of acid extract either alone or in combination with subsequent implantations of rabbit, rat or guinea pig anterior pituitary glands. Forty guinea pigs were underfed and a number of these guinea pigs received subsequently implantations of anterior pituitary glands.

Altogether 93 guinea pigs were studied in this way. At the conclusion of the experiments in each case both ovaries were cut into complete serial sections and many sections through uterus, vagina and cervix and mammary gland were prepared.

These investigations led to the following conclusions: 1. The hormone causing atresia of follicles, administered in a first period, prevents the full effect of the hormone causing growth and maturation of follicles administered in a subsequent period.

2. This inhibiting effect is in the same direction as the effect exerted by underfeeding, but it is much more pronounced.

3. The pseudocorpora lutea, which may be produced in the

[5] We shall discuss the literature concerning the effects of the interaction of various sex hormones on the sex organs in another connection.

[6] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860; and 1930, **27**, 490.

first period of extract injection, may still remain well preserved through the second period, during which the follicular growth hormone acts, but in other cases they may perhaps undergo degenerative changes similar to those observed in true corpora lutea during the process of retrogression.

4. There is no antagonism between the effects of the atresia causing hormone and the process leading to the transformation of atretic follicles into interstitial gland and into pseudocorpora lutea, as far as the latter develop at the expense of the theca interna; these changes are not noticeably inhibited by the first named hormone.

5. This result confirms the previous conclusion that the 2 kinds of lutein tissues, one developing from the granulosa and the other from theca interna and probably also from connective tissue cells situated in the medulla and in atretic follicles may owe their development to different kinds of hormone action. Both the extract of cattle anterior pituitary and the anterior pituitary gland substance of rabbit, rat and guinea pig seem to have in common this effect on theca interna and connective tissue transforming them to interstitial gland tissue.

6. We may assume that the atresia causing hormone fixes itself to the follicles, remains combined with them for some time and thus inhibits the full development and maturation processes in the follicles, which otherwise would have been induced by the follicular growth hormone, even after the completion of the period of the extract injections.

7. The hormone causing follicular atresia induces indirectly a state of rest in vagina, uterus and mammary gland; it is probably closely associated or identical with the thyroid stimulating hormone.[7] However, in case pseudocorpora lutea develop in the ovaries under the influence of this hormone, we often, though not always, note changes in the mucosa of the uterus indicating a mild state of stimulation. The follicular growth producing hormone induces the release of the follicular hormone and thus causes proliferation of vagina and mammary gland and an oestrous state of the uterus.

8. We could confirm our previous observation that the growth processes elicited in the vagina by the implantation of anterior pituitaries of rabbit and rat remain usually incomplete, although the number of mature follicles and of lutein bodies developing under

[7] Loeb, Leo, and Friedman, Hilda, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 172.

those conditions in the ovaries may be much greater than in normal ovaries of guinea pigs at the time of ovulation. One single mature follicle present at the time of oestrous in the guinea pig ovary rapidly causes marked proliferation and keratinization of the epithelium of the vagina.

7392 C

Relationship Between Pharmacological Action and Chemical Structure of Barbituric Acid Derivatives.

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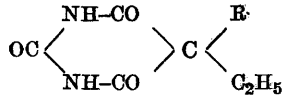
To elucidate the relationship between the chemical structure and the pharmacological action in the barbital series, over 50 derivatives were investigated. These compounds were all 5,5-substituted barbituric acids, having the general formula:



wherein R-alkyl radical (normal or secondary with 2 to 9 C-atoms). Several of them were new compounds synthesized for the first time.[1]

Albino rats weighing from 71 to 126 gm. (average 96 gm.) were employed in this study. Solutions of the sodium salts of the compounds were injected intraperitoneally. The minimal anesthetic dose (M.A.D.), the duration of action, and the minimal lethal dose (M.L.D) were determined by using 5 animals for each dose level.

Since space does not permit a presentation of detailed results, only a few salient points will be discussed here. In Figs. 1 and 2, the "primary alkyls" refer to those compounds having a normal alkyl group. It should be noticed from Fig. 1 that with the increase in number of C-atoms in the alkyl group, either normal or secondary, both the M.A.D. and M.L.D. grow relatively smaller, but when the alkyl radical is longer than 5 C-atoms, the amount required to anesthetize or kill rats again increases. The therapeutic index, or the ratio between the M.L.D. and M.A.D., appears to be gradually greater as the alkyl chain lengthens.

[1] Shonle, H. A., Waldo, J. H., Keltch, A. K., and Coles, H. W., Read at the Am. Chem. Soc. Meeting, St. Petersburg, Florida, Mar. 25-30, 1934.