in a thermal death point experiment on *Cl. botulinum* 72 months after heating. However, his *Cl. botulinum* spores may have been injured by heating while those of *Cl. acetobutylicum* and *Cl. Pasteurianum* picked directly from stock cultures should be of unimpaired vitality.

Why should some single spores, always a low percentage of those picked, germinate in the end. Shall we believe that the others were not viable when picked, died during incubation, or are still alive and dormant? We have no way of answering, but what we do know of dormancy in single spore isolations should make us very cautious about making definite statements that a culture is killed by a certain treatment or that a substance is free from anaerobic spores.

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Inhibition of Ovulation and Associated Histological Changes.*

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The idea that the corpus luteum is responsible for the inhibition of ovulation seems to have been first elaborated by Beard[1] and by Prenant.[2] The experimental proof of the inhibitory action of the corpus luteum has been attacked in many different ways. The two most general methods have been based on anatomical and physiological modifications of the reproductive tract, correlated with the presence or absence of a functional corpus luteum, and, changes produced by the injection of extracts of lutein tissue. Corner and Hurni[3] reported negative results in an attempt to inhibit ovulation by injecting corpus luteum extracts in normal white rats. Loeb[4] failed to obtain consistent positive results in the guinea pig, while Papanicalaou[5] recently reported inhibition of ovulation in the same animal. Pearl and Surface[6] were able to stop hens from laying by injections of water extracts of a dried commercial preparation.

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1 Beard, J., Jena, 1897.

2 Prenant, A., *Rev. Med. de l'Est.*, 1898, xxx, 385.

3 Corner, G. W., and Hurni, F. H., *Am. J. Phys.*, 1918, xlvi, 483.

4 Loeb, L., *Biol. Bull.*, 1914, xxvii, 1.

5 Papanicolaou, G. N., *J. Am. Med. Assn.*, 1926, lxxxvi, 1422.

6 Pearl, R., and Surface, F. M., *J. Biol. Chem.*, 1914, xix, 236.

Kennedy[7] described positive results on the rabbit for extracts made from a desiccated commercial preparation which were injected for a period of time before mating, resulting in the inhibition of ovulation. Parkes and Bellerby[8] reported inhibition of ovulation in mice by injections of an emulsion of an ether extract of the corpus luteum of the cow. They noted that not all of their extracts were potent, and that those that were soon deteriorated. Many different kinds of extracts have been employed by various investigators but the results obtained through their use are so contradictory that they cannot be discussed in this brief report.

An acid alcohol extract of the fresh corpus luteum of the sow has been prepared in this laboratory and refined to the extent that very little protein remains. This preparation is capable of sensitizing the uterus of spayed rats and guinea pigs so that placentomata may be produced (Weichert).[9] It will relax the pubic ligaments of virgin guinea pigs in oestrum (Hisaw)[10]; and will inhibit ovulation and oestrum in the normal female albino rat. Inhibition of oestrum was best effected by subcutaneous injections of an amount equivalent to 3 gm. of fresh tissue given every 4 or 5 hours from the beginning of oestrum until the experiment was ended. Rats injected in this manner did not come into oestrum for 6 to 12 days, the length of time for which ovulation was inhibited depending upon the amount of the extract injected. Attempts are now being made to inhibit ovulation for a greater period and to determine at what stage of the dioestrous interval injections may be most effective.

A histological study was made of the ovaries, uterus and vagina of the animals in which ovulation had been inhibited. The ovaries of these animals showed many involuting corpora lutea with the presence of large mature follicles in cases where oestrum had been inhibited for 12 days. The uterus presented the picture found in pregnant and pseudopregnant rats. The outer layers of the vaginal mucosa were composed of tall, columnar, vacuolated cells, the amount of vacuolation and the growth of the vaginal mucosa being directly proportional to the length of time for which the ovulation had been inhibited. Long and Evans[11] described similar histological pictures in the reproductive tract of the albino rats during

[7] Kennedy, W. P., *Quart. J. Exp. Phys.*, 1924, xv, 103.

[8] Parkes, A. S., and Bellerby, C. W., *J. of Phys.*, lxiv, 232.

[9] Weichert, C. K., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 490.

[10] Hisaw, F. L., reported at the meeting of the A. A. A. S. at Nashville, Tenn., 1927.

[11] Long, J. A., and Evans, H. M., Memoirs of the University of California, vi, 1922.

pregnancy and pseudopregnancy. The only difference between the normal conditions as reported by these authors and that produced by the injection of corpus luteum extracts is one of degree. The height of the vaginal mucosa and the vacuolation of the same was greater, when ovulation had been inhibited by extracts of the corpus luteum, than is normally found during pregnancy and pseudopregnancy.

In order to determine whether the corpus luteum, the follicular hormone or the interaction of both of these substances was responsible for the histological condition just described, these hormones were used separately and in combination on castrated female rats. The results of this study demonstrated that neither the corpus luteum nor the follicular hormone alone was responsible for the picture of pregnancy as found in the vaginas of normal rats, in which ovulation had been inhibited by injection of corpus luteum extracts. The experiment did show, however, that the changes occurring in the vaginas of such animals were due to the action of first, the follicular hormone, and, second, the subsequent effect of the corpus luteum hormone. The data at hand seem to indicate that the proliferation of the cells of the vaginal mucosa is produced by the follicular hormone while the corpus luteum hormone is responsible for the vacuolation of these cells.

These results agree with the physiological relationship of these 2 hormones, described by Hisaw,[10] who found that the pelvic ligaments of the virgin female guinea pig must first come under the influence of the follicular hormone before the corpus luteum hormone could produce relaxation of the symphysis pubis. A similar relationship was described by Weichert[9] for the production of placentomata in castrated female rats and guinea pigs. Thus it seems that the inhibition of oestrum and ovulation is due to the corpus luteum hormone alone while the histological changes produced in the uterus and vagina are brought about through a cooperation of the follicular and the corpus luteum hormones in which the former must act before the latter can function.