may mediate the action of physalaemin.

Summary. Electrolytes and amylase concentrations of rat saliva which is evoked by physalaemin, are virtually identical to those found in saliva evoked by stimulation of postganglionic cholinergic nerve fibers. Mean Na concentrations of parotid and submaxillary saliva were found to be respectively, 139 $\pm$ 4 and 32 $\pm$ 5, and K, 20 $\pm$ 4 and 46 $\pm$ 2; submaxillary flow rate was higher than flow rate from parotid, but both exhibited the high rates characteristic of cholinergic nerve stimulation; amylase concentration of parotid saliva was about 20 mg/mg. The secretion was, however, neither blocked nor modified in any way when the cholinergic blocking agent atropine was present. Furthermore, it was not modified by the presence of either α or β adrenergic blocking agents, or all three blocking agents simultaneously. An indiscriminate action on all receptor sites is thus ruled out. These data provide further evidence for the conclusion previously indicated (1–3) that this agent causes secretion by a direct action on gland cells that differs from that usually produced by autonomic transmitter substances.

The authors wish to thank Farmaceutici Italia, Milan, Italy, for generously providing the Physalaemin, and Dr. Sahagian-Edwards of Ayerst Laboratories, N. Y. through whose courtesy Inderal was provided.

1. Bertaccini, G., Cei, J. M., and Erspamer, V., Brit. J. Pharmacol. **25**, 363 (1965).

2. Bertaccini, G., and DeCaro, G., J. Physiol., (London) **181**, 68 (1965).

3. Emmelin, N. and Lenninger, S., Brit. J. Pharmacol. **30**, 676 (1967).

4. Schneyer, C. A. and Hall, H. D., Proc. Soc. Exptl. Biol. Med. **121**, 96 (1966).

5. Baxter, H. J. Biol. Chem. **102**, 203 (1933).

6. Yoshida, Y., Sprecher, R. L., Schneyer, C. A., and Schneyer, L. H., Proc. Soc. Exptl. Biol. Med., **126**, 912 (1967).

7. Schneyer, C. A., and Schneyer, L. H., Am. J. Physiol. **199**, 55 (1960).

8. Myers, V. C., Free, A. H., and Rosinski, E. E., J. Biol. Chem. **154**, 39 (1944).

9. Schneyer, C. A., and Hall, H. D., Am. J. Physiol., **209**, 484 (1965).

10. Emmelin, N., Acta Physiol. Scand. **34**, 29 (1955).

11. Leeman, S. E. and Hammerschlag, R., Endocrinology, **81**, 803 (1967).

Received January 2, 1968. P.S.E.B.M., 1968, Vol. 127.

Relation of Local Circulation between Ovaries and Uterus to Lifespan of Corpora Lutea in Rats* (32921)

JAMES A. CLEMENS,[1] HIROSHI MINAGUCHI,[2] AND JOSEPH MEITES
Department of Physiology, Michigan State University, East Lansing, Michigan 48823

The finding by Loeb (1) that removal of the uterus results in persistence of the corpora lutea in the guinea pig was confirmed in many other species (2). The part of the uterus which appears to influence the lifespan of the corpora lutea is the endometrium (3–5). Partial hysterectomy prolongs maintenance of corpora lutea in direct proportion to the amount of uterine tissue removed (4–6). Uterine transplants in hysterectomized rats were reported to shorten the duration of pseudopregnancy (7).

A substance apparently is produced by the endometrium which exerts a local luteolytic effect on the corpora lutea of the ipsilateral side, as recently demonstrated in swine (6), guinea pigs (8), sheep (9), and pseudopregnant rats (10). The pathway involved in this local control mechanism has not been well elucidated. Inskeep and Butcher (9) recently reported that ligature of the blood vessels supplying the anterior portion of the uterine horn increased luteal lifespan in sheep. The present

* Supported in part by NIH research Grant No. AM 4784.

[1] NIH predoctoral trainee (GM-1121).

[2] Postdoctoral Fellow, The Population Council, New York, New York. Present address: Department of Obstetrics and Gynecology, Tokyo University Medical School, Tokyo, Japan.

study was undertaken to determine the role of the local circulation from the uterus to the ovary on the lifespan of the corpora lutea in the rat.

Materials and Methods. In 3 experiments, 85 female Sprague-Dawely rats weighing 200–250 gm were used. The rats were obtained from Spartan Animal Farms, Haslett, Michigan for Expt. 1, and from Hormone Assay Lab., Chicago, Ill., for Expts. 2 and 3. The rats were kept in a constant temperature room (25 ± 1°C) with automatically controlled lighting (14 hours light daily), and were fed Wayne Lab-Blox pellets (Allied Mills Inc., Chicago, Ill.) *ad libitum*. Each of the rats had demonstrated at least two regular 4 or 5 day estrous cycles before the beginning of treatment. Rats were stimulated uniformly on the day of proestrus or estrus with a glass rod to induce pseudopregnancy. A diestrous-type smear on the day after estrus was designated as day 1 of pseudopregnancy.

In the first experiment, 3 groups of rats were treated as follows: group 1, bilateral ligature of both arterial and venous circulation joining the uterus to the ovary and oviduct on the fifth day of pseudopregnancy; group 2, removal of bilateral uterine horn on the fifth day of pseudopregnancy; group 3, laparotomy (sham operation) on the fifth day of pseudopregnancy. After termination of pseudopregnancy, the rats were followed for at least two cycles and then stimulated again to induce a second pseudopregnancy. All surgery was done under ether anesthesia.

In the second experiment, 3 groups of rats were operated on the fifth day of pseudopregnancy as follows: group 1, bilateral section of both arteries and veins joining the uterus to the ovary and oviduct; group 2, removal of bilateral uterine horns; and group 3, sham operation (laparotomy). Two estrous cycles were followed after termination of pseudopregnancy and the rats were again stimulated to induce a second pseudopregnancy.

In the third experiment, 3 groups of rats were treated on the first day of diestrus of the estrous cycle as follows: group 1, bilateral ligature of the blood vessels joining the uterus to the ovary; group 2, removal of the bilateral uterine horns; group 3, sham operation (laparotomy). The rats were stimulated on the day of proestrus or estrus after the operation to induce pseudopregnancy. The day of termination of pseudopregnancy was determined by finding a proestrous or estrous type of vaginal

TABLE I. Effects of Ligature of Uterine–Ovarian Blood Vessels or Hysterectomy on Duration of Pseudopregnancy.

Expt. no.	Group	Treatment	No. of rats	First pseudopregnancy[a] (length in days)	Second pseudopregnancy[a] (length in days)
I	1	Ligature of both oviducts and blood vessels joining the uterus to the ovary	6	17.0 ± 1.0[bd]	14.2 ± 0.5[d]
	2	Hysterectomy	6	20.2 ± 1.4[bd]	18.2 ± 0.8[e]
	3	Sham operation	6	12.7 ± 0.2[be]	13.3 ± 0.5[d]
II	1	Section of both oviducts and blood vessels joining the uterus to the ovary	11	19.6 ± 0.9[bd]	16.6 ± 0.7[d]
	2	Hysterectomy	11	21.6 ± 1.0[bd]	19.9 ± 0.6[e]
	3	Sham operation	11	14.6 ± 0.6[be]	12.9 ± 0.4[f]
III	1	Ligature of blood vessels[c] joining the uterus to the ovary	11	17.7 ± 0.7[d]	
	2	Hysterectomy[c]	11	20.4 ± 0.8[e]	
	3	Sham operation[c]	12	14.4 ± 0.4[f]	

[a] Mean ± SE.

[b] Operation was performed on the fifth day of pseudopregnancy.

[c] Operation was performed on the first day of diestrus of the estrous cycle.

[def] Mean lengths of pseudopregnancies with different superscripts are significantly ($p>0.05$) different from each other.

smear, followed by a diestrous type of smear on the next day. The duration of pseudopregnancies was analyzed by Duncan's (11) and Kramer's (12) multiple range tests and the *t* test.

Results (Table I). When both oviducts and blood vessels between the uterus and ovaries were ligated on the fifth day of pseudopregnancy (group 1) in the first experiment, pseudopregnancy was significantly ($p<0.01$) prolonged when compared with the sham-operated rats (group 3). The rats (group 2) in which the bilateral uterine horns were removed also showed a significantly longer period of pseudopregnancy ($p<0.01$) than the sham-operated rats (group 3). The differences in length of pseudopregnancies between groups 1 and 2 were not significant ($p>0.05$). These rats resumed normal estrous cycles after termination of pseudopregnancy. When the rats were stimulated again, a significantly prolonged pseudopregnancy ($p<0.01$) was observed in the hysterectomized rats (group 2). However, pseudopregnancy in the rats with ligated blood vessels and oviducts between the uterus and ovaries was not significantly ($p>0.05$) longer than the sham-operated rats (group 3). The duration of the second pseudopregnancy in group 1 was significantly ($p<0.05$) shorter than that of the first pseudopregnancy.

In the second experiment, both the oviducts and blood vessels joining the uterus to the ovary were sectioned on the fifth day of pseudopregnancy. Again, removal of the bilateral uterine horns (group 2) significantly ($p<0.01$) prolonged luteal maintenance during the first and second pseudopregnancies. The rats in which the blood vessels joining the ovaries to the uterus and oviducts were sectioned (group 1), showed a longer pseudopregnancy ($p<0.01$) than the sham-operated rats. The duration of the second pseudopregnancy in group 1 was significantly ($p<0.05$) shorter than the first pseudopregnancy, but longer ($p<0.01$) than that of the sham-operated rats (group 3).

In the third experiment, all rats were operated on the first day of diestrus of the estrous cycle and stimulated on the next proestrous and estrous day following operation. Following ligation of only the blood vessels joining the uterus to the ovaries, pseudopregnancy in group 1 was significantly prolonged ($p<0.01$) as compared with the sham-operated rats (group 3). However, the length of pseudopregnancy in group 1 was shorter ($p<0.05$) than in the hysterectomized group (group 2).

Discussion. The results of the first experiment show that ligature of both oviducts and the blood vessels joining the uterus to the ovary prolonged maintenance of corpora lutea in the first pseudopregnancy. However, when these rats returned to proestrus or estrus and were stimulated again, they failed to show a prolonged pseudopregnancy. In the second experiment, when the blood vessels and the oviducts were sectioned in the broad ligament, pseudopregnancy was longer than in sham-operated rats, both in the first and second pseudopregnancies. Furthermore, ligature of only the blood vessels joining the uterus to the ovary significantly prolonged the lifespan of corpora lutea in the third experiment. None of these operations affected the length of the estrous cycle. Removal of the bilateral uterine horns confirmed the reported prolongation of luteal function by this means in rats (13,14).

Barley *et al.* (10) recently reported that unilateral hysterectomy combined with removal of the ovary on the same side did not affect length of pseudopregnancy. However, unilateral hysterectomy in combination with unilateral ovariectomy on the side opposite the removed uterine horn extended the lifespan of the corpora lutea. When the oviduct and mesosalpinx were ligated and cut, pseudopregnancies were also longer than those of the controls or of rats with only the oviduct ligated and cut.

The present results point to a role for the blood circulation from the uterus to the ovary in the local control of luteal lifespan. The vascular supply of the ovary is derived from two sources: principally from the ovarian, and to a lesser extent, from the uterine blood vessels. The uterine blood vessels originate from the hypogastric artery and give off many branches to the uterus, and anastomose in the broad ligament with the terminal branch of the ovarian blood vessels. These communicating blood vessels between the uterus and the ovary presumably provide the means by which

a luteolytic substance produced by the endometrium reaches the ovary. Failure to prolong pseudopregnancy in the second trial of the first experiment apparently was due in part to regeneration of blood vessels, as an extensive adhesion was observed which macroscopically revealed the presence of regenerated blood vessels. An increased level of the uterine luteolytic substance in the general circulation by the time of the second induction of pseudopregnancy may also have contributed to luteal regression. Ligature of the communicating blood vessels between the ovary and the uterus apparently does not completely eliminate luteolytic substances in the general circulation, since the duration of the first pseudopregnancy in this group was shorter than in the hysterectomized rats. A lymphatic circulation connecting the ovaries and uterus has not been established for the rat, but this possibility cannot be ruled out at this time.

Summary. The effects of sham operation, bilateral ligature of blood vessels joining the uterus to the ovary, and hysterectomy were determined on the length of pseudopregnancy in rats. Hysterectomy or bilateral ligature of blood vessels joining the uterus to the ovary significantly increased the length of pseudopregnancy when compared to sham-operated controls. These results indicate that the circulation between the uterus and ovaries is important in local control of luteal lifespan in the rat.

1. Loeb, L., Proc. Soc. Exptl. Biol. Med. **20**, 441 (1923).

2. Anderson, L. L., Bowerman, A. M., and Melampy, R. M., in "Advances in Neuroendocrinology," Nalbandov, A. V., ed. p. 345. Univ. of Illinois Press, Urbana, Illinois, 1963.

3. Bradbury, J. T., Brown, W. E., and Gray, L. A., Recent Progr. Hormone Res. **5**, 151 (1950).

4. Anderson, L. L., Butcher, R. L., and Melampy, R. M., Endocrinology **69**, 571 (1961).

5. Butcher, R. L., Chu, K. Y., and Melampy, R. M., Endocrinology **71**, 810 (1962).

6. Du Mesnil Du Buisson, R., Ann. Biol. Animal, Biochim., Biophys. **1**, 105 (1961).

7. Hochter, O., Fraenkel, M., Lev, M., and Soskin, S., Endocrinology **26**, 680 (1940).

8. Fisher, T. V., Anat. Record (abstract) **151**, 350 (1965).

9. Inskeep, E. K. and Butcher, R. L., J. Animal Sci. **25**, 1164 (1966).

10. Barley, D. A., Butcher, R. L., and Inskeep, E. K., Endocrinology **79**, 119 (1966).

11. Duncan, D. B., Biometrics **11**, 1 (1955).

12. Kramer, C. Y., Biometrics, **12**, 307 (1956).

13. Silber, M. and Rothchild, I., Acta Endocrinol. **43**, 521 (1963).

14. Melampy, R. M., Anderson, L. L., and Kragt, C. L., Endocrinology **74**, 501 (1964).

Received Nov. 8, 1967. P.S.E.B.M., 1968, Vol. 127.

The Precipitin Band as a Mechanical Barrier Revealed by Gel Diffusion Studies of Gonadotropic Hormones* (32922)

BEI-LOO CHEN[1] AND CHARLES A. ELY

Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, New York 10032

That all components which do not cross-react in a multiple precipitating antigen/antibody system behave independently as if each were present alone in agar gel double diffusion preparations is a widely accepted point of view (1–4). However, in the course of a study in our laboratory of the antigenic relationship between gonadotropic hormones where a hyperimmune rabbit antiserum to ovine luteinizing hormone (AOLH) was utilized, it was observed that a heavy precipitin line sometimes acted as a nonspecific mechanical barrier, interfering with the free diffusion of heterospecific antigens as well as unrelated antibodies. In this study AOLH completely absorbed with sheep tissues ($AOLH^{st}$) re-

*This study was supported in part by Grant HD-01143-02 from the USPHS and Grant T-296A from the American Cancer Society.

[1] Research Associate, Department of Anatomy.