of that liberated during the first 3 days of culture. This demonstrates that rat hypothalamus contains a factor which is capable of stimulating growth hormone release from the rat anterior pituitary *in vitro,* even after such release has ceased.

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Effects of Selected Hormones on the Closed Vaginal Membrane of the Ovariectomized Guinea Pig.* (29860)

GEORGIANA JAGIELLO (Introduced by S. R. M. Reynolds)
Department of Medicine, University of Illinois Medical Center, Chicago

Detailed descriptions have been published of the cytology and histology associated with rupture of the vaginal membrane in female guinea pigs(1,2,3). General studies of the effects of hormonal preparations have been made(4,5) and an assay for estrogen was once proposed by Hartman(6) using direct intravulvar injection. The present paper attempts to make a quantitative evaluation of the effects of parenterally administered hormones which may be active agents in this cyclic process.

Methods and materials. The estrous cycles of mature virgin female guinea pigs weighing 500-700 g were followed for 3 cycles by observation of the vaginal opening. Total oophorectomy under pentobarbital anesthesia was performed on the fourth day of the third vaginal opening through dorsal incision and observations for vaginal opening continued for 48-54 days. Only animals which failed to exhibit vulval swelling or vaginal opening were used in subsequent experiments.

Angiotensin (Hypertensin II—Ciba) was given subcutaneously in a single dose of 500 μg. Testosterone propionate, known to open the vaginal canal of the immature rat, was given in divided doses over 7 days for a total dose of 8.5 mg. Relaxin (W.C. W1164A, Lot 8360) in 5% beeswax in oil was administered subcutaneously to 4 animals in 4 daily doses for a total dose of 3750 G.P.U. Relaxin (W.C. W1164A, Lot 66) was given in the same manner for a total dose of 3500 G.P.U. Relaxin preparation P-181-1 (Frieden) was similarly administered for a total dose of 600 G.P.U. None of the 3 relaxin preparations induced vaginal cornification in ovariec-

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TABLE I. Effects of Estrogen and Progesterone.

Estradiol diproprionate (μg)	(days)	Progesterone (mg)	Interval between last dose & response (hr)	No. pigs responding	Duration (days)
10	4	0	24	3/3	18
5	3	0	24	3/3	10
1	4	0	24	4/4	13-16
.5	4	0	24	4/4	9
.1	5	0	—	0/4	0
5	1	0	96	4/4	10
1	1	0	96	8/8	9
.5	1	0	96	8/9	4-7
.25	1	0	—	0/3	0
.10	1	0	120	1(±)/8	1
0	0	5	—	0/3	7
0	0	425	—	0/3	3
5	1	—	96	4/4	10
	1	2.5	—	0/4	—
1	1		72	4/4	10
	1	2.0	48	4/4	4
1	1		72	4/4	8
	1	1	—	0/4	—

tomized adult rats examined daily for 10 days after injection of the equivalent of a single daily guinea pig dose. Similar doses of relaxin administered to 23-day-old female rats did not cause opening of the vaginal orifice.

Gonadotrophins as human chorionic gonadotrophin (HCG),† pregnant mares serum (PMS)‡ or ovine luteinizing hormone (NIH-LH-S-1)§ were injected subcutaneously. HCG, 500 I.U. daily for 4 days, was given to 4 animals. Four animals were given 50 I.U. of PMS daily for 7 days. Two to five animals were given 20-1000 μg of NIH-LH for one or two days.

Estrogen and progesterone in sesame oil were administered as detailed in Table I. Three control ovariectomized animals were treated with the appropriate vehicle for each experiment.

Results. No response was obtained from any animal given control vehicles. Angiotensin and testosterone propionate did not affect the closed vaginal membrane. All 3 relaxin preparations opened the membrane 24 hours after the fourth dose and this condition persisted for 4 days in all cases. Human chorionic gonadotrophin (HCG) and pregnant mare serum (PMS) did not affect the closed membrane, while luteinizing hormone (NIH-LH-S-1) at doses of 200 μg for one dose, or 500-1000 μg for 2 daily doses, caused only a suggestion of vulval swelling and tissue breakdown in 24-48 hours.

With estradiol dipropionate a dose of 0.5 μg for 4 days opened the membrane for 9 days. The maximum dose tested, 10 μg for 4 days, caused persistence of opening of the vaginal orifice for a total of 18 days. Experiments with single doses of 0.1 μg to 5.0 μg yielded openings at dose levels of 0.5 μg, 1.0, and 5 μg with shorter durations than an equal dose injected chronically. The latent period between the single injections and the response was similar at all dose levels and approximated the length of treatment for chronically administered doses. Attempts to effect vaginal opening with 5 mg and 425 mg of progesterone were not successful. The length of time the vaginal orifice remained patent was reduced from 10 to 4 days in animals given a single dose of 1 μg of estradiol dipropionate and 2.0 mg of progesterone on the second day of opening. The duration of estrogen-induced openings was not affected in 4 animals each given one dose of 5 μg of estradiol dipropionate and 2.5 mg of progesterone.

Discussion. A quantitative relation between the minimum effective dose of estradiol dipropionate given parenterally and the duration of its effect in opening the otherwise

† Antuitrin-S, Parke-Davis Co.

‡ Gonadogen, Upjohn Co.

§ NIH-LH-S-1 ovine, generously supplied by Dr. A. Wilhelmi.

sealed vaginal membrane of the ovariectomized female pig was established. One-half microgram given as a single dose can induce the first histologic changes in the vaginal membrane described by Kelly *et al*(1). This dose is far greater than the amount shown by Hartman(6) to break down the membrane of the immature guinea pig when administered directly into the vulva. As noted, 2.0 mg of progesterone given to an animal primed with 1.0 μg of estradiol dipropionate reduces the duration of vaginal opening to 4 days. This approximates the natural state in cycling guinea pigs. The questionable effect of LH may be coincident with, and be masked by the effect of estrogen in the intact animal.

Demonstration of relaxin as an effective agent for opening the closed vaginal orifice has not been reported previously. Approximately 9 times more of the Warner-Chilcott preparation was required than of the Frieden preparation, a finding compatible with heterogeneity. It has been noted by Zarrow (7) that the maximum concentration of relaxin in the serum of the pregnant female guinea pig is reached around day 28 of gestation. The present findings suggest that this increase could account for the opening of the sealed vaginal membrane reported by Ford(8) to occur most commonly around day 26 or day 27 of gestation and attributed by those authors to a deficiency of progesterone.

Summary. The minimum effective dose of estradiol dipropionate, 0.5 μg as a single subcutaneous injection, which opened the closed vaginal membrane of the ovariectomized guinea pig has been determined. Vaginal opening occurred approximately 96 hours later and persisted for 4-7 days. Estrogen induced vaginal openings of 9 days duration can be shortened to the normal 4 days with 2 mg of progesterone. The effectiveness of several relaxin preparations in opening the closed vaginal membrane is reported. It is suggested that relaxin may be involved in the opening of the vaginal membrane observed in the pregnant guinea pig during the fourth week of gestation.

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Circadian Rhythmic Pituitary Adrenocorticotropic Activity, Rectal Temperature and Pinnal Mitosis of Starving, Dehydrated C Mice.* (29861)

F. HALBERG, J. H. GALICICH,† F. UNGAR AND L. A. FRENCH

Departments of Pathology, Neurosurgery, and Biochemistry, University of Minnesota Medical School, Minneapolis, and Cambridge State School and Hospital, Cambridge, Minn.

Certain circadian rhythms are described in terms of amplitude and timing in mice deprived of all food and water. Phase relations among these rhythms at different levels of organization are demonstrated as being similar to those recorded on comparable fully-fed animals. Such information was sought on the functions under study with a view

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† Present address—Neurosurgical Research Laboratory, Children's Hospital Med. Center, Boston, Mass.