presumably associated with cells of lesser differentiation in a variety of loci. The presence of pepsinogen I (rather than pepsinogen II and III) in the embryologically different epithelium of the seminal vesicles is therefore not so surprising.

We have reported small but significant differences in the characteristics of different human gastric pepsins(10) which must have escaped detection by others(1,3); otherwise it is difficult to account for the reported similarity(3) between gastric and seminal pepsinogen with respect to alakli resistance and activity *vs* pH curves.

Although other proteolytic enzymes active around pH 7 are known to be present in semen, the presence of pepsinogen is, teleologically at least, somewhat puzzling. The near neutral pH of semen precludes conversion of the inactive proenzyme (pepsinogen I) to proteolytically active pepsin I. Seedorff has discussed the problem of activation of seminal pepsinogen; he speculated that activation most likely occurred on the cellular surface of the spermatozoa(3). It is, however, also possible that conversion of pepsinogen to pepsin may take place in the vagina after coitus. The weakly acidic pH (4.0-5.0) of normal adult vaginal secretions(11) would make this feasible. If this is the case, it may be that proteolytic activity from seminal pepsinogen plays a role in the complex process of reproduction.

Summary. Fractionation of human semen on DEAE (diethylaminoethyl)-cellulose has shown that the seminal proteolytic activity at low pH is eluted as a single peak of proteolytic activity. This peak of proteolytic activity exhibits no milk clotting activity unless it is first activated by acidificaton. The chromatographic mobilities of the proteolytic activity at low pH of unacidified and acidified semen are similar to those of unacidified and acidified gastric mucosal pepsinogen I, respectively. The possible conversion of seminal pepsinogen to pepsin by weakly acidic vaginal secretions posed the question of a possible role for pepsin in human reproduction.

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In vitro Reinitiation of Pituitary Somatotropin Release by an Acid Extract of Hypothalamus.*† (29859)

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Rat anterior pituitary loses most of its capacity to release somatotropin (STH) after culture for 6 days *in vitro*(1). This appears to be true for the release of all tropic hormones of the anterior pituitary except prolactin(2), which is inhibited by the hypo-

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thalamus under most conditions(3). Hypothalamic stimulation has been shown to be necessary for release of normal quantities of all tropic hormones except prolactin(1,3,4, 5,6,7).

The ability of hypothalamic extracts to reinitiate hormone secretion from the cultured pituitary after hormone production has ceased, has received little attention. Guillemin and Rosenberg(8) reported that addition of hypothalamic explants to a pituitary culture 4 days old, after cessation of ACTH release, stimulated the hypophyseal explants to resume secretion of ACTH. However, Florsheim, Imagawa and Greer(9) failed to reinitiate TSH secretion from cultured mouse anterior pituitary after addition of hypothalamus.

Recently we reported that a crude acid extract of rat hypothalamus stimulated release of 4-6 times as much STH from the cultured rat anterior pituitary as pituitary cultured without a hypothalamic extract or with a cerebral cortical extract(1). This indicated that a somatotropin releasing factor (SRF) is present in rat hypothalamus. It was of interest, therefore, to determine the effect of SRF on rat anterior pituitary which had previously been cultured for 6 days and had essentially ceased to release STH.

Materials and methods. Two similar groups of mature virgin female Carworth CRN rats, 180-210 g body weight, served as donors for hypothalamic and anterior pituitary tissues. To prepare the acid extract of hypothalamic tissue, the median eminence region was excised from decapitated rats and immediately frozen on dry ice. Twenty-four hypothalami were homogenized with a ground glass homogenizer in 12 ml of 0.1 N HCl and centrifuged at 30,000 $\times$ *g* for 30 minutes at 4°C. The homogenate was neutralized with 1.0 N NaOH.

Anterior pituitaries were removed from 24 rats and each was cut with a sterile scalpel into 8 explants of approximately equal size. The explants were divided equally among 24 culture dishes so that 2 groups had half of the explants from every anterior pituitary. Each dish contained the equivalent of one pituitary and 3 ml of synthetic medium 199. All procedures were carried out under sterile conditions. The culture was maintained in a humidified atmosphere under constant gassing with 95% O_2 - 5% CO_2 at 37°C, and the medium was changed at 3-day intervals. Details of the culture procedure have been published(10).

On the 7th day of culture an acid extract of 2 rat hypothalami was incorporated into the medium of each of the 12 dishes in group B (Table I). An equivalent amount of 0.1 N HCl and 1.0 N NaOH solution was added to each of the dishes in group A on the 7th day of culture. Since rat cerebral cortical extract had previously been shown to be inactive for STH releasing activity(1), it was not used in this experiment. The culture was terminated at the end of 9 days. The medium from each 3 days of culture was dialyzed for 20 hours in running tap water at 4°C, lyophilized and stored in a freezer.

Growth hormone activity was measured by the standard tibia test in young hypophysectomized rats(11). The lyophilized medium was diluted to 3 dose levels with distilled water and injected i.p. into hypophysectomized rats (Hormone Assay Laboratories, Chicago). Regression lines were calculated by the method of least squares. Differences between slopes were determined by analysis of covariance, and relative potencies by the method of Irwin as described by Pugsley (12).

Results. Significant amounts of growth hormone were released during the first 3 days of culture by the rat pituitary (Table I, Groups A1 and B1). After the first 3 days of culture, growth hormone activity in the medium was essentially insignificant (Table I, Groups A2 and B2). It has been stated that at least a 40 μ increase in epiphyseal cartilage width above hypophysectomized controls must be obtained to be considered indicative of STH activity in pituitary tissue (13), and we have adopted this end point. In control group A, STH release remained insignificant during the 3rd three days of culture (Group A3). Addition of an extract of rat hypothalamus to the cultured pituitary during the third 3 days of culture reinitiated release of growth hormone (Group

TABLE I. Release of STH by Rat Anterior Pituitary Cultured With and Without Rat Hypothalamic Extract.

Group	No. of assay rats	Dose AP equivalent assay rat/ 4 day	Response ($\mu \pm$ SE)	% of control (P = .05)
	5	.2	158 ± 8	—
A1 1st 3 days AP culture (control)	5	.6	186 ± 12	—
	5	1.6	210 ± 11	—
	5	.2	153 ± 6	
A2 2nd 3 days AP culture	4	.6	138 ± 13	NS
	4	1.6	151 ± 7	
	4	.2	132 ± 2	
A3 3rd 3 days AP culture	5	.6	159 ± 5	NS
	5	1.6	154 ± 4	
	5	.2	150 ± 8	
B1 1st 3 days AP culture (control)	5	.6	170 ± 15	100
	5	1.6	228 ± 5	
	5	.2	145 ± 5	
B2 2nd 3 days culture	5	.6	157 ± 12	NS
	4	1.6	164 ± 3	
	4	.2	149 ± 5	
B3 3rd 3 days of culture—with hypothal extract	3	.6	177 ± 7	78
	5	1.6	199 ± 6	
Hypophysectomized assay controls	5	—	131 ± 4	—

B3). In fact, these pituitary explants released 78% as much STH as was released during the first 3 days of culture (B1). The lack of greater stimulation can probably be attributed to a progressive reduction in viable tissue which we observed in this system(2).

Discussion. These results provide cogent evidence for the existence of SRF in acid extracts of rat hypothalamus. They demonstrate that SRF is capable of reinitiating release of somatotropin by rat anterior pituitary *in vitro* after release has declined to insignificant levels. We have previously established that the tibia assay measures growth hormone activity released by cultured rat anterior pituitary and that acid extracts of hypothalamus or cerebral cortex are not responsible for introducing exogenous somatotropin(1). Furthermore, work by Courrier *et al*(14) has demonstrated that while saline extracts of hypothalamus possess significant amounts of ACTH, FSH, and LH activity, acid extracts are void of these factors.

Pecile, Müller, Falconi and Martini(15) recently reported that intracarotid injections into rats of acid extracts of rat hypothalamus markedly decreases the pituitary content of somatotropin, and we have confirmed this observation(1). These reports, together with the evidence that transplantation(16,17) and massive lesions of the median eminence(18) depress anterior pituitary growth hormone secretion, comprise the *in vivo* evidence for a hypothalamic influence on somatotropin secretion. The data presented here provide additional evidence for the existence of an SRF in rat hypothalamus, and indicate that the cultured rat pituitary cannot long secrete STH without hypothalamic stimulation.

Summary. Two groups of rat anterior pituitaries were cultured on synthetic medium 199 at 35°C in an atmosphere of 95% O_2 - 5% CO_2 for 9 days. The medium was changed every 3 days. At the end of the 9-day culture period, the medium from each 3-day period was assayed for growth hormone by the standard tibia test. Both groups of anterior pituitaries released substantial amounts of growth hormone during the first 3 days of culture, but no significant amounts during the second or third 3 days of culture. Addition of a rat hypothalamic extract at the beginning of the third 3 days of culture, reinitiated growth hormone release to 78%

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)

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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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