domestic rabbit induced by the Shope papilloma virus was cultured *in vitro* and a continuously growing cell line, designated as SP-8, was established. Among the two types of cells, i.e., polygonal and columnar cells, which grew in the primary culture and which both showed the presence of SPV antigen by immunofluorescence as previously reported, the latter type (columnar cells) grew predominately even in the first subcultivation and subsequently composed the main cellular constituent of the SP-8 cell line. The morphological feature of the SP-8 cells was fibroblast-like in appearance. By inoculation into rabbits, however, the cells were occasionally observed to form epithelial arrangements. The modal number of chromosomes in SP-8 cells was found to be 44 and those cells had a characteristic male rabbit karyotype which showed no difference in appearance. Deviation of chromosome numbers from the modal number was observed. The incidence of tetraploid cells at the twentieth passage was higher than in the earlier passages. Although the SP-8 cells exhibited neither specific fluorescence for SPV antigen nor elevated arginase activity, the possibility of persisting viral genome in these cells was discussed.

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Evidence for the Release of Ovulating Hormone in PMS-Treated Immature Rats* (32932)

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In 1962, Strauss and Meyer (1) demonstrated that a critical time period for ovulation existed in immature rats induced to precocious puberty with pregnant mare serum gonadotropin (PMS). On the basis of their experiments involving carefully-timed hypophysectomy or injection of barbiturates, they hypothesized, as Everett and Sawyer (2) had done years earlier, that the 2–4 p.m. "critical period" represented the time during which sufficient pituitary ovulating hormone (OH)[2] was released to induce ovulation. In 1964, Schwartz (3) demonstrated that an

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[2] The term ovulating hormone will be used to represent whatever gonadotropin(s) is (are) released to cause ovulation.

injection of pentobarbital before the critical period on the day of proestrus prevented the usual decrease in pituitary LH potency which occurs on the night of ovulation. Thus the blockade of ovulation by pentobarbital was associated with a blockade of LH release. Since it has been suggested that immature rats induced to precocious puberty with PMS constitute a valuable preparation for the study of the role of the hypothalamus and pituitary in controlling ovulation (1), it seemed pertinent to establish whether or not barbiturate blockade of ovulation in these rats was also associated with a blockade of the release of the ovulation-inducing hormones. Inasmuch as it is not yet known whether ovulation is associated with release of LH alone or a combination of LH and FSH, it was decided to employ an assay in which ovulation, itself, was the end point. The primary tenant of the approach would then be that if OH is depleted during the critical period for ovulation, the ovulation-inducing potential of the pituitary, regardless of which gonadotropic hormones constitute this potential, would be decreased on the morning ova are first present in the oviducts as opposed to the previous day. The OH potency would, however, remain high in rats in which ovulation had been blocked by an injection of PB before the critical period.

Materials and Methods. Donor rats. In each of 4 replicates of the donor experiment, at least 19 immature female albino rats, 25–27 days old, weighing 58–70 gm were obtained from the Holtzman Co. The rats were maintained on Rockland rat diet and water *ad libitum* and were exposed to 14 hours of light and 10 hours of darkness at a room temperature of 26 ± 2°C. All times mentioned in this report are "colony time." In this convention of Everett and Sawyer (2), midnight, colony time, is the midpoint of the dark period. At 30 days of age, (day 30), between 9 and 10 a.m., the rats were given a single subcutaneous injection of 0.4 Cartland-Nelson unit of PMS (Gonadogen)[3] in 0.5 ml of 0.9% NaCl (saline).

At 1:30 p.m. on day 32, five of these rats were killed by decapitation while under ether anesthesia; 5 more were killed at 4:30 p.m. The pituitary glands were removed, the neurohypophyses were discarded, and the adenohypophyses were frozen until assayed a few days later. The absence of ruptured ovarian follicles and the presence of fluid-filled uterine horns indicated that ovulation had not occurred in any of these animals. The remaining 9 (or more) rats were divided into 3 treatment groups. One group received a subcutaneous injection of 10.0 mg of phenobarbital sodium (PB) in 0.25 ml of water at 1:30 p.m.; a second group received the same dose of PB but at 4:30 p.m.; the remaining group was injected with 0.25 ml of saline at 1:30 p.m. The animals weighed 85–100 gm at this time. These 3 groups were killed by decapitation on the following morning (day 33); the pituitary glands were removed and frozen. The ovaries were examined for freshly ruptured follicles and the oviducts for ova. The entire above-described procedure was repeated 4 times during 4 separate weeks.

Assay rats. Twenty or 21-day-old female rats weighing 45–50 gm were obtained from the Holtzman Co. The lighting, temperature and food were identical to that described above for the donor rats. On day 22, between 7:30 and 8:30 a.m., 0.75 Cartland-Nelson unit (15 IU) of PMS in 0.5 ml of saline was injected subcutaneously. At 1:30 p.m. on day 24, 7.5 mg of PB in 0.25 ml of water was injected subcutaneously. The animals weighed 55–65 gm at this time. Homogenates equivalent to ¼ or ½ of a donor rat pituitary gland, 0.5 or 1.0 IU of HCG[4] or 0.25 ml of saline were injected intraveneously (i.v.) between 2:10 and 4:00 p.m. The assay rats were sufficiently sedated from the PB so that with the use of some restraint the i.v. injections were given without additional anesthetization. On the following day (day 25) between 8:00 a.m. and 2:30 p.m., the rats were

[3] Gonadogen was supplied through the courtesy of the Upjohn Co. According to the Upjohn Co., "one Cartland-Nelson unit" is equivalent to approximately 20IU.

[4] HCG—courtesy of the Upjohn Co. The stated potency agreed quite well with the actual potency when assayed against the International Standard Preparation of HCG with the ovulation assay described above.

TABLE I. Effect of Timed Injections of Phenobarbital on Ovulation in Donor Rats.

Treatment on day 32		No. of rats[a] ovulating/no. of rats receiving that treatment				
		Donor replicate 1	Donor replicate 2	Donor replicate 3	Donor replicate 4	Totals
Saline	1:30 p.m.	2/2	4/4	3/3	6/6	15/15
Phenobarbital	1:30 p.m.	1/3	1/3	0/4	1/6	3/16[b]
Phenobarbital	4:30 p.m.	3/3	3/3	4/4	6/6	16/16

[a] Autopsied on the morning of day 33.

[b] PB 1:30 p.m. vs saline 1:30 p.m. ($p < .01$). PB 1:30 p.m. vs PB 4:30 p.m. ($p < .01$). Probability computed from the tables of Mainland and Murray (11).

killed with an overdose of ether. The oviducts were examined for ova; if ova were present, they were removed and counted under a dissecting microscope. The ovaries were examined for the presence of freshly ruptured follicles.

This assay procedure was repeated 4 times for the assay of the donor pituitary glands removed before and after the critical period on day 32. Four assay rats were given each dose of pituitary homogenate or HCG. The assay procedure was repeated an additional 3 times for assay of pituitaries from donor rats treated with PB or saline on day 32 and autopsied on day 33. In each of these 3 assays, 3 or 4 rats were given each dose of pituitary homogenate or HCG.

Preparation of the pituitary homogenates. For each replicate of the donor experiment, the frozen pituitaries within each treatment group were pooled; therefore, each pool contained at least 3, but no more than 6, pituitaries. Each pool was weighed and then homogenized in a ground glass tube in saline to a concentration of ½ or ¼ pituitary per 0.25 ml.

At autopsy on day 33 of the donor rats, it was observed that in each of the first two replicates of this experiment, 1 out of 3 of the rats injected with PB at 1:30 p.m. on day 32 had ovulated (see Table I). As it was thus likely that in these 2 rats, the critical period had started early, it did not seem justifiable to include the pituitaries of these non-blocked rats in a pool of pituitaries from rats in which ovulation had been blocked. The exclusion of 1 pituitary from each of the first 2 replicates of the donor experiment left only 2 pituitaries in each of these 1:30 p.m.–PB pools. As this did not leave sufficient tissue in this treatment group for separate assays, all pituitaries removed on day 33 in the first 2 replicates of the donor experiment were pooled. Thus only 3 (instead of 4) separate assays were performed on pituitaries removed on day 33.

Results. Ovulation response of the donor rats autopsied on day 33. If changes in the pituitary content of OH associated with the critical period and ovulation are to be studied, it is essential that it be determined whether the donor rats are actually displaying a critical period for ovulation. The design of our experiment made it possible to do this, as ovulation data was obtained at the time of pituitary collection on day 33. Moreover, since the rats that were killed at 1:30 or 4:30 p.m. on day 32 were chosen randomly from the same population of rats that were not killed until day 33, it could be assumed that the ovulation data from the rats treated with PB at 1:30 or 4:30 p.m. reflected the state of OH secretion of pituitaries removed from other rats at corresponding times on day 32. The data in Table I demonstrate that a critical period for the release of OH was occurring between 1:30 and 4:30 p.m. on day 32, in that injection of PB at 1:30 p.m. blocked ovulation in most rats, whereas injection of PB at 4:30 p.m. did not.

The average weight of the anterior pituitary glands for each treatment group was calculated. The greatest percentage difference in weight between any 2 groups of pituitaries was 18%; [Av wt. of a pituitary removed at 4:30 p.m. on day 32 was 3.70 mg; av wt. of a pituitary for the group given PB at 1:30 p.m. on day 32 (pituitaries removed on day 33)

TABLE II. Ovulation Response of Recipient Rats to Homogenates of Donor Rat Pituitaries.

Treatment of donor rat on day 32	Time of removal of donor rat pituitary (day)	Dose of pituitary injected	Number of recipient rats	Percentage of recipient rats ovulating	Av no. ova /ovulating rat
Killed 1:30 p.m.	32, 1:30 p.m.	¼	16	81.3	9.0
		½	16	100.0	31.3
Killed 4:30 p.m.	32, 4:30 p.m.	¼	16	56.3	8.0
		½	16	100.0	21.3
Phenobarbital 1:30 p.m.	33, 7 a.m.–12 noon	¼	9	77.7	12.4
		½	9	100.0	28.9
Phenobarbital 4:30 p.m.	33, 7 a.m.–12 noon	¼	9	33.3	11.3
		½	10	100.0	20.0
Saline 1:30 p.m.	33, 7 a.m.–12 noon	¼	9	0.0[a]	—
		½	10	30.0	2.3

[a] The proportion of recipient rats ovulating in response to pituitary homogenate from saline-treated donors (pituitaries removed on day 33) differs significantly ($p < .05$) from the response obtained from each of the other pituitary homogenates. The probability was computed from the tables of Mainland and Murray (11).

was 4.35 mg]. Inasmuch as no claims will be made for the significance of small differences in pituitary OH potencies, the pituitary weight data are not included in the tables.

Consideration of the pertinent ovulation parameters. When determining the extent to which ovulation was induced in the recipient rats by a test substance, two parameters can be considered, namely, the percentage of recipient rats that ovulate, and secondly, the average number of ova per ovulating rat. In this study, the two parameters paralleled one another very closely, in that as the percentage of rats that ovulated increased, the average number of ova per ovulating rat also increased. For example, 0.5 IU of HCG induced ovulation in only 2 out of 24 rats, (one rat ovulated in two of the seven assays) with an average of 2.5 ova per ovulating rat; one IU of HCG induced ovulation in 23 out of 23 rats with an average number of 26.4 ova per ovulating rat. Likewise, with regard to the response obtained with pituitary homogenates, invariably an increase in dosage produced an increase in the percentage of rats ovulating and an increase in the average number of ova per ovulating rat. Thus, both parameters will be considered when discussing the data in Table II. Since in our assay rats only the 1.0 IU dose of HCG produced ovulation consistently, a dose response curve for our standard was not established. Hence, while including these 2 doses of HCG in each assay did provide information on the consistency of the sensitivity of the ovulation response, it did not make it possible to state the activity of the pituitary glands in units of a standard. None of the 23 assay rats injected with saline ovulated; thus it can be assumed that all of the ovulation observed in the assay rats was resulting from the injection of exogenous gonadotropin. In earlier studies in which PB was not injected, this same priming dose of PMS (0.75 CNU) produced ovulation in 16.7% of 223 rats (4). It seems likely that the total absence of ovulation in the similarly treated rats of this present study was due to a PB blockade of the endogenous OH release.

Ovulation response of the assay recipient rats. In Table II, the data are presented as the average of the results of 4 separate assays of pituitaries removed on day 32, and the average of the results of 3 assays of pituitaries removed on day 33.

Little difference, if any, existed in the ovulation-inducing potency of pituitaries removed at 1:30 p.m. on day 32 as opposed to 4:30 p.m. Likewise, for the pituitaries removed on day 33, there was only slight evidence of a difference in pituitary OH

potency between rats treated on day 32 with PB at 1:30 p.m., and those given PB at 4:30 p.m. In spite of the similar OH potencies of the above-mentioned groups, the data in Table I indicates that sufficient OH secretion had occurred between 1:30 and 4:30 p.m. to induce ovulation. If no treatment was given on day 32 other than an injection of saline, the pituitary OH potency on day 33 was markedly diminished as compared to all of the other treatment groups. Note that in contrast to all of the other treatment groups, homogenate of pituitary glands from the saline-treated rats equivalent to ¼ of a pituitary produced no ovulation, and homogenates equivalent to ½ of a pituitary only minimal ovulation. These data are suggestive that even though enough OH is released between 1:30 and 4:30 p.m. to induce ovulation, this represents only a portion of the total OH release on the night of ovulation in non-PB-treated rats. Thus it would seem that PB blocks OH release regardless of when it is administered, and that OH release normally extends beyond the minimum time period essential to induce ovulation, i.e., beyond the critical period.

Discussion. The decline in pituitary OH potency which we observed on the night of the first ovulation was also reported by Rennels and OSteen (5) in immature rats treated with a larger dose (25 IU) of PMS. They measured the pituitary content of FSH and LH by separate specific assays, and found no difference between the amount of LH in pituitary glands removed before (2:00 p.m.), and those removed after the critical period (7.00 p.m., which occurred on the second day after PMS treatment. The pituitary FSH content did decrease during this 5-hour interval, and pituitary LH was decreased by 2:00 p.m. on the following day. Moore (6) found in his rats that the pituitary content of LH decreased during a several-day period during which signs of puberty were becoming apparent; he associated this decreased pituitary LH content with increased estrogen secretion by the ovary. Ramirez and Sawyer (7) have reported that the pituitary content of LH drops abruptly at the time of puberty (day of vaginal opening) to a half or a third of its prepuberal level, whether puberty was natural or estrogen-induced. Rats treated with 0.4 CNU of PMS on day 30 usually have open vaginae by the evening of day 32 and always by the morning of day 33. The rats in our study could best be considered as having been induced into precocious puberty by PMS stimulation of endogenous estrogen (and or progesterone) secretion.

Our experimental results also confirm the observation of Schwartz (3) in which she noted that the high proestrous pituitary content of LH drops markedly on the night of ovulation, and furthermore, that this drop in pituitary LH is prevented by administration of pentobarbital at 2:00 p.m. on the day of proestrus.

Recent experiments of Everett (8) involving the induction of ovulation in rats by preoptic (hypothalamic) stimulation with platinum electrodes suggest that release of pituitary LH is coextensive with the hypothalamic secretion of LH-releasing factor. This present study, on the basis of continued high levels of pituitary OH even after the critical period supports the contention advanced by others (9, 10) that OH secretion, (and thus the secretion of LH and FSH releasing factor) continues long after the secretion of the minimum amount necessary for full ovulation.

Summary. The ovulation inducing potential of anterior pituitary glands of immature rats induced to precocious puberty with PMS was assayed by a method employing ovulation in other (recipient) immature rats as the end point. Although timed injection of phenobarbital (PB) demonstrated the existence of a 1:30–4:30 p.m. critical period for ovulation in the donor rats, the pituitaries of these rats failed to exhibit a definitive decrease in ovulating hormone (OH) content by the end of the critical period. If the pituitaries were not removed until the day after the critical period, pituitaries from rats injected with saline at 1:30 p.m. on the day of the critical period contained significantly less OH than rats injected at this same time or at 4:30 p.m. with PB. The results of these experiments are interpreted to mean that though sufficient OH is released during the critical period to induce

ovulation, OH release normally extends after the critical period; whenever administered, PB blocks subsequent release of pituitary OH.

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Pituitary Content of Somatotropin, Gonadotropin, and Thyrotropin in Rats with Stunted Linear Growth following Head X-Irradiation* (32933)

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X-irradiation limited to the head of the neonatal rat will result in stunted growth (1,2). We have reported that the degree of stunting is related to the size of dose and that the administration of bovine growth hormone and/or thyroxine failed to correct the stunting (2). Inasmuch as the pituitary and the central neuroendocrine system is in the path of the X-ray beam, we have carried out further studies of the pituitary hormonal system to clarify the role of endocrine systems in this form of stunted growth. In this report we give results of assays for pituitary content of somatotropin, gonadotropin, and thyrotropin in the irradiated stunted rats and consider the significance of the findings in the overall problem of the cause of stunting of the head-irradiated rat.

Materials and Methods. All of the irradiated rats and controls were produced in our colony of Long-Evans rats begun in July of 1964; additional rats of the same strain were added in September, 1965. The colony was isolated in a room with a sound-absorbing ceiling. The air was fresh, filtered and maintained at 45–55% relative humidity and 72–78°F. Lighting was set for a 14-hour day. Purina mouse breeder chow and tap water were given *ad libitum*. Lettuce was provided once weekly to nursing mothers.

A description of the caging, breeding technique, and physical data pertaining to the irradiation are given in our previous report (2). At 2 days of age the animals were restrained in teflon holders and placed beneath a ¼-inch thick lead shield. Those to be irradiated were placed with their heads exposed to the X-ray beam past the edge of a cut out portion in the center of the shield. Control rats were placed completely under the shield. At 23, 42, and 121 days of age 600R head X-irradiated and control rats were killed by ether fumes and the pituitaries immediately removed, weighed, and frozen in vials surrounded by dry ice. The pooled glands were stored at —70°C until assays

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