animals of other species that have been used for this purpose.

In the porcine thoracic aorta, endothelium commenced to cover the crimped dacron prosthesis soon after its implantation. The entire length of a dacron prosthesis, 4.5 cm long, was completely covered with endothelium in 7 days.

Summary. Prostheses of knitted, crimped dacron, some with and some without gelatin impregnation, were placed in the thoracic aortas of 17 young pigs. The prostheses replaced excised segments of aortas, 4 to 6 cm long, and were left in place for 7 to 139 days. "En face" and pellicle silver preparations revealed complete endothelial covering of the surface of a prosthesis after 7 days.

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Ovulating Hormone Release in Gonadotrophin Treated Immature Rats.* (27511)

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It has been reported that the gonadotrophin of pregnant mare serum (PMS) is capable of producing ovulation in the immature rat (1-3). Evidence exists that the ovulation brought about by PMS is due to a certain inherent luteinizing property of the PMS molecule, and to the ability of PMS to cause release of endogenous gonadotrophin(3). Recently Strauss and Meyer, working in this laboratory, obtained data which supports the concept that ovulation caused by a single injection of PMS in 30-day-old immature rats is due to release of ovulating hormone from the animal's own pituitary. Their data, obtained from barbiturate injection and hypophysectomy, strongly indicates that ovulating hormone release occurs between 2:00 and 4:00 p.m. on the afternoon of the second day after PMS injection.

In attempting to use the 21-day-old female rat to assay luteinizing hormone by the superovulation method of Zarrow(4), considerable difficulty was encountered because the priming dose of PMS often caused ovulation. It became of interest to determine whether the ovulation was due to release of pituitary gonadotrophin during a precise period such as Strauss and Meyer had found in the 32-day-old rat.

Materials and methods. Female rats 21 days of age and weighing 45-50 g were obtained from Holtzman Co. and Sprague-Dawley, Inc., Madison, Wis. On the following day between 7:30 and 8:00 a.m. a single dose of 1.375 Cartland-Nelson Units of PMS (Upjohn Gonadogen) was injected subcutaneously. The animals were killed 3 days later between 8:00 a.m. and 1:00 p.m. The oviducts were removed and the ova counted by the method of Rowlands(5), except that the ova in the egg mass were separated mechanically.

In the experiments involving barbiturate blockage of ovulation, 167-250 mg/kg of an aqueous solution of sodium barbital was injected intraperitoneally at 1:30 or 5:30 p.m. on the day before autopsy. In one experiment, human chorionic gonadotrophin

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(HCG)‡ was injected intravenously at 2:00 p.m., one-half hour after injection of barbital.

Hypophysectomy was performed by the parapharyngeal approach between 1:00 and 1:30 p.m. and between 5:00 and 5:30 p.m. on the afternoon of the day before autopsy. Completeness of hypophysectomy was checked by examining the sella turcica under magnification; rats in which pituitary fragments were found were not included in the results.

The rats were maintained on Rockland rat diet and water *ad lib.*, and were kept in a room where the temperature remained between 78 and 80°F. The lights were automatically turned on at 6:00 a.m. and off at 8:00 p.m. Central Standard Time, thus producing a 14-hour light period and a 10-hour dark period. Employing the convention of Everett and Sawyer(6), unless otherwise stated, all times in this report are "colony time"; "midnight" colony time is the midpoint of the dark period. Therefore, midnight colony time occurs at 1:00 a.m. Central Standard Time.

Results. Five minutes after administration of barbital a limited ataxia was apparent in the rats. Approximately 20 to 30 minutes, however, was required for complete sedation to develop. The rats never became completely anesthetized since stimuli such as pinching of the tail elicited reflex movements. Signs of recovery, such as spontaneous movement, were apparent by 4:30 p.m. Mild sedation, however, was observed as late as 12 hours after injection.

Inhibition of ovulation was achieved by administering barbital at 1:30 p.m. on the day before autopsy (Table I). In Holtzman rats ovulation occurred in only 10 of the 31 rats given this treatment, while 27 out of 33 saline-injected controls ovulated. Even in the barbital-treated animals which did ovulate, the average number of ova was significantly reduced ($P<.01$). If barbital administration was delayed, however, until 5:30 p.m., the percentage of rats ovulating and the average number of ova per rat ovulating was like those of the saline controls.

‡ HCG and PMS, courtesy Upjohn Co., Kalamazoo, Mich.

Even though the average number of ova was consistently lower in Sprague-Dawley rats given the same treatment as Holtzman rats, the same type of response to 1:30 and 5:30 p.m. injections of barbital was obtained; both percentage of animals ovulating and average number of ova per rat ovulating were reduced in the 1:30 p.m. group (Table I).

Ovulation occurred when HCG was administered to a group of rats given barbital at 1:30 p.m. but did not occur in animals receiving saline and barbital (Table II). Hypophysectomy prior to 1:30 p.m. was also effective in blocking ovulation (Table III). Rats hypophysectomized between 5:00 and 5:30 p.m., however, did ovulate.

Discussion. Sodium barbital was shown by Everett and Sawyer(6) to be an effective inhibitor of ovulation in cycling adult rats. In work done at our laboratory, Strauss and Meyer have found that sodium barbital is also very effective in preventing the ovulation which occurs in 33-day-old rats treated with a single dose of PMS at 30 days of age. Purshottam, Mason and Pincus(7), found that another compound of the barbital series, phenobarbital, caused a significant reduction in the number of eggs ovulated by PMS-treated immature mice given HCG 42-43 hours later. Purshottam *et al.* did not postulate a mechanism of action for the barbiturate blockage, and their experiments were not comparable to those reported here since they administered HCG. In our experiments, removal of the pituitary before 1:30 p.m. prevented ovulation, but did not if removed after 5:00 p.m. It, therefore, seems probable that the inhibition produced by barbital was due to a blockade of endogenous ovulating hormone.

The fact that 1.0 I.U. of HCG was effective in inducing ovulation in blocked rats makes it seem unlikely that the barbital was inhibiting the action of endogenous ovulating hormone. The numbers of ova ovulated by HCG in the blocked animals were typical of the number usually observed in this laboratory when 1.0 I.U. of the same preparation of HCG is administered to non-blocked PMS primed immatures.

Everett and Sawyer(6), using cycling adult rats demonstrated a 2:00-4:00 p.m.

TABLE I. Effect of Sodium Barbital Administration on Ovulation in Rats Given PMS.

	Exp. No.	Saline 1:30 P.M. No. rats	No. ovul.	Avg No. ova/ovul. rat	Barbital 1:30 P.M. Dose, mg/kg	No. rats	No. ovul.	Avg No. ova/ovul. rat	Barbital 5:30 P.M. No. rats	No. ovul.	Avg No. ova/ovul. rat
Holtzman	1	7	6	21.0	167	7	3	4.7			
	2	7	6	29.2	200	6	1	1.0			
	3	5	4	32.5	250	6	1	8.0	5	4	24.2
	4	4	4	29.8	250	5	0	—	5	5	22.4
	5	5	3	27.3	250	4	4	10.8	5	4	27.5
	6	5	4	14.8	250	3	1	1.0	4	2	20.0
Totals		33	27	25.6 ± 3.7*		31	10	6.7 ± 1.5†	19	15	24.1 ± 3.8
Sprague-Dawley	1	6	5	12.6	250	2	1	2.0	2	2	7.0
	2	6	5	9.2	250	6	2	1.5	6	4	12.8
Totals		12	10	10.9 ± 2.1		8	3	1.6 ± .3 ‡	8	6	10.8 ± 3.1

* Plus or minus stand. error of mean.

Probability that such a difference in avg No. of ova per ovulating rat would occur by chance alone.

† Holtzman totals—Barbital 1:30 *vs* Saline 1:30, $P < .01$. Barbital 1:30 *vs* Barbital 5:30, $P < .01$.

‡ Sprague-Dawley totals—Barbital 1:30 *vs* Saline 1:30, $P < .01$. Barbital 1:30 *vs* Barbital 5:30, $P < .05$.

"critical period" on the afternoon of proestrus in which ovulating hormone is released. Strauss and Meyer in our laboratory have shown that this occurs at essentially the same time in PMS-primed 32-day-old rats. A similar time has been found in even younger animals as described in this report. In all these studies, the light regimen was alike, 14 hours light—10 hours dark. However, the role that such a lighting schedule plays in development of a 2:00-4:00 p.m. critical period has not been adequately studied. It is interesting, however, that rats obtained from Sprague-Dawley were exposed until 21 days of age to 24 hours of light per day, while rats obtained from the Holtzman Co. had been exposed to only 9 hours of light each day. Rats purchased from both companies, however, exhibited the same critical period even though they had been under the lighting schedule of our laboratory for only 3 days.

The mechanism by which the release of ovulating hormone is controlled in immature rats is still unknown. However, since carefully timed barbiturate administration can block ovulation, the concept is strongly favored that, as in the adult, the central nervous system is involved. Studies are now underway to determine whether the central nervous system mechanism is already "mature" in the rat at 21 days of age, or whether the exogenous gonadotrophin, acting directly, or indirectly through the ovarian steroids, accelerates this "maturation."

Summary. The incidence of ovulation and number of ova shed in 25-day-old rats induced to ovulate by PMS injection at 22 days of age was significantly reduced by administration of sodium barbital at 1:30 p.m. on the 24th day of life. However, when barbital administration was delayed until 5:30 p.m., ovulation occurred more frequently and the number of ova resembled that of non-barbiturate

TABLE II. Effects of HCG Administration on Barbital-Treated Rats.

Dose of HCG, I.U.	No. rats	No. ovulating	Avg No. ova per ovulating rat
.0 (saline)	4	0	—
.5	4	1	19.0
1.0	3	3	62.0
2.0	3	3	70.3

TABLE III. Effect of Hypophysectomy on Ovulation in Rats Given PMS.

	No. rats	No. ovul.	Avg No. ova per ovul. rat
Hypophysectomy between			
1:00 and 1:30 P.M.	7	1	2.0
5:00 and 5:30 P.M.	6	5	12.0

treated controls. Similarly, hypophysectomy before 1:30 p.m. inhibited ovulation while hypophysectomy between 5:00 and 5:30 p.m. did not. These data are interpreted to mean that ovulating hormone is released from the pituitary gland between 1:30 and 5:30 p.m. on the 24th day of life.

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Effect of Malnutrition on Liver Copper in the Rat. (27512)

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Low plasma copper levels have been reported in malnourished infants and in kwashiorkor(1,2,3). The latter disease is associated with a protein carbohydrate dietary imbalance and it is possible experimentally to produce a syndrome in rats similar to kwashiorkor, by feeding animals a low protein high carbohydrate diet(4). It therefore seemed of interest to see what effects such a "kwashiorkor-like" diet would have on the liver copper of malnourished rats.

Methods. Rats of Wistar strain and with initial weight between 40-73 g were randomly divided into 2 groups. One group was placed on diet 41B(5) while the other was placed on a low protein diet which consisted of cassava plus vitamin and mineral supplements(6).

The animals were killed between the 25-27th day and their livers were perfused with Ringer Locke solution to remove blood, using the technic described by Deegan and Maegraith(7). The livers were then weighed, dried, reweighed and homogenized. Aliquots of the homogenate were taken for estimation of copper using the method of Williams and Morgan(8). In general quadruplicate estimations were done and the mean results of the 4 estimations noted. In some instances due to small size of the liver only duplicate estimations were done.

Results. Table I shows that animals kept on a standard rat cube diet gained weight while those on the cassava diet lost weight. The total liver copper was significantly different for both the groups. However there was no significant change in the liver copper/100 g liver or in the liver copper/g body weight.

Discussion. The finding shows that in these animals the fall in liver copper is correlated with fall in liver weight and body weight. It is probable however, since changes in body weight may reflect loss of tissue and possible gain in fluid leading to oedema, that in the later stages of malnutrition this corre-

TABLE I. Effect of Feeding Diet 41B or Cassava Diet on Liver Copper. (Mean ± S.E. of 20 animals on cassava diet, and 18 animals on control diet.)

Initial wt (g)	% wt change	Total liver copper, μg	Copper, μg/100 g liver	Copper, μg/g body wt
53.7 ± 1.9	+127.3 ± 14.2	28.2 ± 1.8	544.5 ± 42.0	.26 ± .05
48.5 ± 2.2	− 27.8 ± 7.3	8.2 ± .8	547.2 ± 94.6	.21 ± .06
Not significant*	Significant	Significant	Not significant	Not significant

* Considered significant if $P < 0.01$.

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