individually clamped for a period of 75 minutes. The clamps then were removed and both kidneys were exteriorized and placed underneath the skin. Within 30 minutes, the dogs received X-irradiation in the amount of 200 R's to one kidney, the other serving as a control. This irradiation was repeated in the same fashion on the 1st and 2nd post operative day. BUN's and renograms also were followed. On the 3rd post operative day, ureteral catheters were passed to both kidneys through a ureteral incision and renal function studies were performed. Diuresis was induced, if feasible, by administration of 4% urea† in normal saline intravenously. The animals then received an intradermal injection of 60 μc of radiohippuran and 3 radiohippuran clearances were determined for each kidney as previously described(5). The dogs were sacrificed following the clearance determinations.

Results and discussion. Fig. 1 gives the results of the mean of 3 clearances in all the dogs, with the radiated kidney represented by the solid black bars and the non-radiated kidney by the cross hatched bars. It can be clearly seen that the radiated kidney, in almost all instances, has greater clearance than the non-radiated kidney. (The non-radiated kidney not only had a significantly lower clearance of radiohippuran, but also was unable to concentrate the smaller volume of urine it produced during the osmotic diuresis induced.)

† urea—Ureaphil—courtesy of Abbott Laboratories.

Fig. 2 represents the mean differences in volume noted between the 2 sides.

In Fig. 3 is shown a typical renogram from a radiated kidney and the non-radiated control (B). The abnormal renogram found on the non-radiated side is similar to that found on the other side before radiation was given (A).

Summary. On the basis of the results obtained, it appears reasonable to suggest that the effect of radiation in modifying acute rejection is possibly secondary to its effect upon ischemia. Whatever the mechanism may be, it appears that a small amount of radiation has a beneficial effect upon the kidney damaged by anoxia, a finding which may have therapeutic value in a variety of conditions.

‡ Hipputope—courtesy of E. R. Squibb & Sons.

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Plasma LH in Cyclic Female Rats.* (30086)

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The usual failure to detect LH in the plasma of intact, randomly selected female rats(1,2) is probably the result of a combination of the insensitivity of methods, the 15-minute plasma half-life of LH(3) and the presumably short time span within the estrous cycle during which large quantities of LH are secreted(4-7). In two recent investigations plasma LH was measured during specific times of the estrous cycle in rats: Van der Werff ten Bosch *et al*(8) found highest values during "late diestrus"; whereas Rami-

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rez and McCann(9) found lowest values during diestrus and highest values between 5 PM and 6 PM of proestrus. In the present study blood was collected for LH assay(2,3,5) from intact rats during the day of proestrus at a time when indirect evidence indicates that the pituitary secretion of the ovulatory surge of LH occurs(4-7), thus increasing the probability that LH would be detected.

Materials and methods. Donor animals. Sixty-day-old female rats from Sprague-Dawley, Inc., Madison, Wis. were kept in a temperature controlled, light controlled (lights on 5 AM-7 PM) room for 2 weeks before the start of vaginal smearing. Previously described criteria for cycle stage were rigidly adhered to(6,7). Rats running either 4-day or 5-day cycles were used. Untreated proestrous rats (N = 47) were quickly anesthetized with ether between 2:30 and 3:50 PM, blood was collected(10) in tubes containing heparin and centrifuged. The plasma was kept frozen until the day of assay (less than 3 weeks later). The uterus was inspected for ballooning, indicative of proestrus(7), dissected and weighed. In many cases, the anterior pituitary was also removed, weighed, and quick-frozen. Pituitary LH assays were carried out as described previously(7) and potencies were calculated in the usual way (11). Eight rats which had been running 4-day cycles, autopsied on the day of expected proestrus, did not show ballooned uteri. Data from these animals ("diestrus, 5-day") are considered separately.

For comparison blood was also collected during the same time of day from (a) untreated estrous rats (N = 24); (b) proestrous and estrous rats injected with pentobarbital (3 mg/100 g) at 2 PM (N = 21); (c) ovariectomized rats (N = 19); (d) hypophysectomized rats (N = 15). The above dose of pentobarbital blocks ovulation in virtually 100% of rats running 4-day cycles(4, 7). Of the ovariectomized rats, 10 were ovariectomized between 10 and 11 AM on the morning of proestrus and were autopsied 5 or 7 days later; 9 were ovariectomized at random cycle stages and autopsied 3 months later. Cyclic or post-partum rats were hypophysectomized by Hormone Assay Labs., Chicago. Daily vaginal smears were taken until autopsy (19 to 35 days later) with plasma being assayed only from those rats showing ovarian, adrenal and thyroid atrophy, diestrous smears and a sella turcica with no trace of pituitary tissue.

Plasma assays. Recipient rats were pretreated with PMS (50 IU) and HCG (25 IU) as described previously(5-7) and were used for assay on the seventh day (161 hours) following HCG injection. One ovary was removed prior to intravenous administration of saline, 0.1 μg LH (NIH-LH-S_2 or S_5) or 2 cc of plasma. One hour after the injection the second ovary was removed. Ascorbic acid was measured by the method of Mindlin and Butler(12). In most cases plasma from a single donor was injected into a single recipient (*i.e.*, plasma pooling was infrequent).

Results. The OAA (ovarian ascorbic acid) depleting activity of plasma for the various experimental groups is summarized in Table I, along with the results for saline and LH (0.1 μg) (injected into each recipient assay group). Only in experiment C was the effect of saline injection alone significant. LH exerted a significant depleting effect in every experiment (Table I). Plasma from ovariectomized rats showed significant depleting activity 5-7 days and 3 months after surgery. The plasma from hypophysectomized rats also contained a small but significant OAA depleting activity (Table I).

Plasma from untreated proestrous and estrous rats (Table I) demonstrated, by an analysis of variance: (a) a significantly higher depleting activity in plasma from rats running 5-day cycles than rats running 4-day cycles ($P<.05$); (b) no significant differences in depleting activity between proestrous and estrous plasma ($P>.05$); (c) no significant interaction between cycle stage and cycle length on plasma depleting activity. Pentobarbital injection eliminated all differences in OAA depleting activity (Table I) (combining all of the pentobarbital data: $N = 22$, $6.5\% \pm 2.3$, $P<.02$). The rats which were autopsied in the expectation that they were proestrous animals running 4-day cycles, but which did not show ballooned

TABLE I. Plasma Ovarian Ascorbic Acid Depleting Activity.

Exp	Substance injected: Source	No. plasma donors	Recipients: No.	OAAD* % ($\bar{x} \pm$ S.E.M.)	P† t test
(A)	Saline	—	10	6.0 ± 4.6	>.05
1 assay	LH, .1 μg	—	11	10.0 ± 3.9	<.05
	OVAX, 3 mo	9	9	14.3 ± 2.3	<.01
(B)	Saline	—	11	−1.0 ± 2.8	>.05
2 assays	LH, .1 μg	—	11	11.5 ± 4.0	<.02
	OVAX, 5-7 days	10	9	13.1 ± 2.0	<.01
	HYPOX, 19-35	15	14	6.3 ± 2.5	<.05
(C)	Saline	—	47	5.1 ± 1.8	<.01
7 assays	LH, .1 μg	—	52	11.7 ± 2.0	<.01
	PRO 4–	28	27	9.3 ± 3.3	<.01
	PRO 5–	19	15	16.9 ± 2.4	<.01
	EST 4–	16	16	4.9 ± 1.6	<.01
	EST 5–	8	8	11.5 ± 4.2	<.05
	PRO+PENT 4–	7	7	5.3 ± 3.6	>.05
	PRO+PENT 5–	6	7	7.7 ± 5.5	>.05
	EST+PENT 4–	3	3	6.6 ± 8.2	>.05
	EST+PENT 5–	5	5	6.7 ± 4.1	>.05
(D)	Saline	—	6	−.2 ± 3.0	>.05
1 assay	LH, .1 μg	—	6	15.5 ± 5.3	<.05
	"DIEST" 5–	8	8	15.0 ± 4.1	<.01

PRO = proestrus; EST = estrus; 4– or 5– = cycle length; PENT = pentobarbital, 3 mg/100 g given at 2:00 PM; OVAX = ovariectomized; HYPOX = hypophysectomized; "DIEST" = rats autopsied at time of supposed proestrus, but without ballooned uteri.

* $\dfrac{(\text{OAA, } \mu\text{g/mg, 1st ovary}) - (\text{OAA, } \mu\text{g/mg, 2nd ovary}) \times 100}{(\text{OAA, } \mu\text{g/mg, 1st ovary})} = \text{OAAD \%}$

For LH or plasma, % depletion for each recipient was corrected for mean depletion of saline-injected rats of the same assay.

† Probability that OAA depletion as defined in * is zero $\left(\dfrac{t = \bar{x} - 0}{\text{S.E.M.}}\right)$

uteri, are presumed to have switched spontaneously to a 5-day cycle(7). Plasma from these "diestrous, 5-day" rats had a high depleting activity (Table I).

Pituitary LH contents and uterine weights are summarized in Table II. Although pituitary LH contents were different between proestrous and estrous rats at 2:30-3:50 PM (Table II), these pituitaries showed no differences in LH content from their respective 10 AM controls(6,7). Pentobarbital treatment had no effect on pituitary LH content (Table II). Uterine weights showed the expected differences between proestrous and estrous rats(7). Ovariectomy of 5 days duration did not raise pituitary LH content above proestrous levels; however, by 7 days LH content is high (Table II). After 3 months there was a very large increase in LH content (Table II).

Discussion. Previous indirect evidence indicating that a surge of LH secretion occurs on the afternoon of proestrus is that: (a) pituitary LH content drops slightly but significantly between 10 AM and 4 PM of proestrus(5) and drops by 50% between the mornings of proestrus and estrus when fresh ova are found(6,7,13); (b) pentobarbital administration at 2 PM of proestrus, but not at 4 PM, blocks ovulation in the 4-day rat (4) and also prevents the estrous drop in pituitary LH content(7). That LH secretion also occurs on the day before proestrus is suggested by the maximal estrogen secretion which begins at that time(7). The direct evidence on plasma LH levels in the present

TABLE II. Pituitary LH Content and Uterine Weight.

Group	No.	Uterine wet wt, mg ± S.D.	No. pools	Pituitary LH content, μg/pit‡ (95% conf lim)
OVAX 3 mo	—	—	1	57.2 (31.4–104.4)
OVAX* 5 days	3	205 ± 24	2	6.6 (4.5– 9.7)
OVAX* 7 days	7		3	12.4 (8.3– 18.5)
PRO†	45	569 ± 113	7	7.8 (5.7– 10.8)
EST†	24	412 ± 76	4	4.4§ (2.2– 8.6)
PRO† + PENT	13	495 ± 54	5	8.4 (6.5– 10.8)
EST† + PENT	8	378 ± 52	3	4.3 (3.1– 6.0)
"DIEST"	8	389 ± 88	—	—

Abbreviations as in Table I.

* No differences were seen in the uterine data related to time after ovariectomy (5 or 7 days); data were pooled.

† No differences were seen in data between 4- or 5-day cyclic rats; data were pooled.

‡ Combined potency(11).

§ Significant heterogeneity among pools; confidence limits include variance among pools(11).

study (Table I) and in two recent studies(8, 9) supports the above indirectly obtained conclusions: plasma LH levels on the afternoon of proestrus are higher than on the morning of proestrus(9) or on the morning (9) or afternoon of estrus† (Table I); pentobarbital administration reduces proestrous plasma LH levels (Table I); and plasma LH levels on the afternoon of diestrus are high (8, Table I).

Rats running 5-day cycles have higher plasma LH levels than rats running 4-day cycles at proestrus and estrus (Table I), but there are no differences between them in pituitary LH content on the morning or afternoon of proestrus or estrus(6,7, Table II).

† The apparent discrepancy between this study and a previous one(9) regarding the statistical significance of the difference between OAAD activity of proestrous and estrous plasma is probably the result of our using plasma samples from individual donors, not pooled samples(9); this increases variation in response because it provides an estimate of donor variability in addition to recipient variability.

Pentobarbital reduced plasma LH activity in 5-day rats to minimal values (Table I), but does not block ovulation in rats running 5-day cycles, probably because the "critical period" for LH release lasts longer than the duration of action of the pentobarbital(14). The higher plasma LH levels (Table I) in the 5-day rat might be related in some way to this altered duration of the "critical period"(14).

Pituitary LH content during the "critical period" of LH release on the afternoon of proestrus has not dropped from 10 AM levels(6,7, Table II); even by 4-5 PM(6,7) pituitary LH content is not as low as on the morning of estrus(6,7), suggesting that LH release continues after 5 PM. This is supported by two observations: (a) high plasma LH levels are seen between 5 and 6 PM of proestrus(9); and (b) although 4-day rats given atropine at 4 PM have ovulated by the following morning, such treatment prevents the drastic changes in ovarian cholesterol usually seen by that time(15). These data suggest that when the rapid LH release starts at 2 PM of proestrus, synthesis is speeded up to the same extent; only after 4 PM does release continue in the absence of matching synthesis.

Plasma from the hypophysectomized rats contained a substance which caused a significant OAA depletion (Table I). It is unlikely that this is LH, which should have disappeared by this time(3), or that it is vasopressin(2). It could conceivably be luteinizing hormone releasing factor (LRF)(2), since the recipients were not hypophysectomized. At least the same degree of depletion was also induced by plasma from 4-day estrous rats and from all pentobarbital-treated rats (Table I). On the basis of the present data it cannot be decided whether the activity is due to LRF or some other substance, and whether the same substance is responsible for the minimal 5-7% depletion seen in non-hypophysectomized rats (Table I).

Summary. Plasma LH was measured by the one-hour OAA depletion assay in cyclic, ovariectomized and hypophysectomized rats. Plasma collected between 2:30 and 3:50 PM of proestrus contained as much circulating

LH as is seen following ovariectomy; not as much LH was in plasma at the same time on the afternoon of estrus. The 5-day cyclic rats had significantly more LH in plasma at both proestrus and estrus than was found in 4-day cyclic rats. Pentobarbital administered at 2 PM (at a dose level which blocks ovulation in the proestrous 4-day rat) reduced plasma OAA depleting activity to the minimal amount found in hypophysectomized rats.

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Factors Influencing Uptake of Triiodothyronine-I^{131} by Bull Spermatozoa.* (30087)

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Radioactive labeled glucose(1,2), fructose (3), phospholipids(4), glycerol(5), and amino acids(6) have been previously used to study the metabolic activity of bull spermatozoa. An earlier report(7) from this station indicated that there was an active *in vitro* uptake of radioactive triiodothyronine-I^{131} (T_3-I^{131}) by bull spermatozoa. It was found that uptake of T_3-I^{131} was related to viability of the sperm cells and the presence of seminal plasma and egg yolk in the suspending medium and that it was independent of the amount of triiodothyronine added to the diluted semen. The present report is a further investigation of the factors affecting uptake of T_3-I^{131} by bull spermatozoa.

Materials and methods. Semen from dairy bulls was used in 4 different trials. After diluting the semen to the desired sperm cell concentration the spermatozoa were washed 3 times by centrifugation (1140 *g*). The solutions used for washing and suspending the spermatozoa were 2.9% sodium citrate and a more complex ionic medium, the Norman and Johnson (N-J) solution(8) containing 500 mg% of glucose. Stock solutions of commercial preparations of radioactive triiodothyronine (Squibb) were diluted to 50 ml with psysiological saline solution and were used

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