EAT tolerate skin grafts until the death of the animal(1). Patients with malignant disease have been shown to tolerate transplantations better than those without neoplasia (13). Southam has also shown an abnormal leukocytic response by the Rebuck skin window technic in patients with malignancy(15). Thus, it seems apparent that a subdued leukocytic reaction in the presence of malignant disease reduces the defenses of the host to various types of challenge. If neoplastic cells do inhibit the host leukocyte response, then the malignant tissue must possess the special property of induction of this phenomenon, thus aiding in its propagation and metastasis. Further studies are under way to clarify these observations.

Summary. A method has been described to demonstrate leukocytic responses in the peritoneal cavity of mice to challenge substances, which uses quinacrine to label the leukocytes for fluorescent microscopy. The leukocyte reaction to intraperitoneally inoculated Ehrlich ascites tumor cells was observed to be decreased in the presence of an already established ascites tumor. Massive tumor inoculation (6 cc EAT intraperitoneally) appears to suppress the outpouring of leukocytes in an immediate fashion, although a response does occur when turpentine is administered concomitantly. One explanation for these observations is the alteration of host defenses by the neoplastic cells.

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Effect of Purified Luteinizing Hormone-Releasing Factor (LH-RF) on Plasma LH Activity at Various Stages of the Estrous Cycle of the Rat.* (31309)

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An LH-releasing factor (LH-RF) has been found in hypothalamic extracts from several mammalian species(1-5). This factor has been purified and separated from the other hypothalamic neurohormones such as follicle stimulating hormone-RF, growth hormone-RF, corticotrophin-RF, and melanocyte stimulating hormone-RF which appear to control adenohypophyseal secretion(2,3,6). The factor is a small, basic polypeptide dissimilar

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from vasopressin and oxytocin(2,3,5,6). A physiological role for the LH-RF is suggested by several observations. For example, the content of stored LH-RF has been shown to vary in several situations associated with altered secretion of LH(7,8) and evidence for a circulating LH-RF in the blood of hypophysectomized rats(9) and chickens(10) has recently been presented.

Crude hypothalamic extracts are capable of elevating plasma LH in a variety of circumstances. An elevation in plasma LH follows injection of these extracts into ovariectomized rats in which the release of LH has been inhibited either by hypothalamic lesions or by injection of gonadal steroids such as estrogen or a combination of estrogen and progesterone. An elevation in plasma LH was also observed following injection of hypothalamic extracts into normal female rats which were selected without regard to the stage of their estrous cycle(11).

It was of considerable interest to determine if the hypophysis would respond to the LH-RF with an outpouring of LH at all stages of the estrous cycle and if there was any change in sensitivity to the factor at different stages of the cycle. The present experiments show that an elevation in plasma LH occurs after injection of purified LH-RF at all stages of the estrous cycle.

Materials and methods. Adult, virgin female rats of the Sherman strain were caged individually in a room with constant temperature (72-76°F) and lighting (lights on 7 A.M. to 7 P.M.) and were fed Purina laboratory chow. Only rats which had exhibited 3 or more regular estrous cycles as determined by vaginal smears were used. At the time of experiment each rat was anesthetized with ether and an equal volume of ammonium acetate (0.1 M, pH 5.5) diluent or Sephadex-purified LH-RF was injected into the external jugular vein during 30-60 seconds. Ten minutes after beginning the injection, the animals were bled from the external jugular vein while still anesthetized with a heparinized syringe. Blood from 3 or more donors was pooled and centrifuged. The pooled plasma was injected intravenously at a dose of 2 ml/100 g into immature female Sherman rats which had been pretreated with gonadotrophins. The two ovary, one hour, modification of the ovarian ascorbic acid depletion test of Parlow(12) was performed as previously described(13). The percentage ovarian ascorbic acid depletion was calculated for both experimental and control plasmas which were tested on the same day to avoid the effect of any day-to-day fluctuations in sensitivity of the test animals. Significance of differences between the two groups was calculated by Student's t test.

The LH-RF used in the experiments was purified by preliminary extraction followed by gel filtration on Sephadex G-25(6). All of the tests were performed using solution from 2 adjacent tubes of one fractionation on Sephadex. These tubes had equivalent LH-releasing activity as determined by ovarian ascorbic acid depletion induced upon injection directly into immature test rats.

Results. The Sephadex-purified LH-RF produced an elevation in plasma LH as indicated by increased ovarian ascorbic acid depletion when it was injected at doses of 0.3 or 0.1 ml which corresponds to 90 and 30 μg of peptide, respectively, as determined by the Folin-Lowry reaction (Table I). The responses were of approximately equal magnitude regardless of the stage of the estrous cycle at which the injection was performed. The 0.3 ml dose was inactive when injected into hypophysectomized rats.

When the dose was reduced to 0.02 ml, equivalent to 6.0 μg of peptide, little or no effect was observed. A significant response was seen only in one group of animals injected on the morning of proestrus. When this experiment was repeated, no significant response occurred, and no response was observed in the 2 tests performed in the afternoon of proestrus.

Discussion. The present results indicate that the adenohypophysis maintains its responsiveness to exogenous LH-RF at all stages of the estrous cycle. Since no response was seen in hypophysectomized rats, it is clear that the observed results in intact animals can not be caused by contamination of the extract with LH. It is also apparent from the lack of effect in hypophysectomized rats

TABLE I. Increase in Plasma LH Activity (% Ovarian Ascorbic Acid Depletion) from Various Doses of LH-RF at Varying Times of Estrous Cycle.

Stage of cycle	Dose (ml)	No. of exp	% Ovarian ascorbic acid depletion: Control	Experimental	Difference	P
Diestrus—day 1	.3	1	7.2 ± 2.4 (6)*	18.1 ± 2.2 (6)	10.9	<.02
" —day 2	.3	2†	6.0 ± 1.8 (12)	19.1 ± 1.6 (11)	13.1	<.001
" —day 3	.3	1	3.3 ± 1.4 (6)	18.6 ± 1.7 (6)	15.3	<.001
Proestrus—late	.3	2	12.4 ± 1.2 (12)	24.7 ± 2.0 (12)	12.3	<.01
Estrus	.3	3	6.1 ± 1.0 (16)	16.1 ± 1.7 (16)	10.1	<.001
Hypophysectomized	.3	1	—.4 ± 1.9 (6)	—.6 ± 2.2 (6)	.2	n.s.
Diestrus—day 1	.1	1	.1 ± 2.5 (6)	11.0 ± 3.7 (6)	10.9	<.01
" —day 2	.1	2	2.2 ± 1.2 (12)	16.0 ± 1.6 (11)	13.8	<.001
" —day 3	.1	1	—2.7 ± 1.6 (5)	16.6 ± 1.9 (6)	19.3	<.001
Proestrus—early	.1	1	4.2 ± .8 (5)	14.5 ± 2.7 (7)	10.3	<.001
Estrus	.1	1	8.8 ± 1.0 (6)	16.7 ± 2.8 (6)	7.9	<.05
Diestrus—day 1	.02	2	3.2 ± 2.2 (12)	5.8 ± 2.1 (12)	2.6	n.s.
" —day 2	.02	1	2.7 ± 2.2 (6)	2.1 ± 1.1 (6)	—.6	n.s.
" —day 3	.02	1	1.5 ± 2.1 (6)	2.4 ± 3.0 (6)	1.0	n.s.
Proestrus—early	.02	2‡	.6 ± 3.0 (6)	11.9 ± 1.9 (6)	11.3	<.01
			9.0 ± 1.7 (7)	13.9 ± 2.0 (7)	4.9	n.s.
Proestrus—late	.02	2	6.4 ± 1.3 (10)	6.9 ± 2.0 (11)	.5	n.s.
Estrus	.02	1	9.7 ± 2.8 (6)	11.9 ± 3.2 (7)	2.2	n.s.

* Mean ± SEM (No. of test rats).

† If results of replicate experiments did not differ significantly, they were pooled for presentation.

‡ If results of replicate experiments differed significantly, they were not pooled.

that the plasma level of exogenous LH-RF in the injected rats did not reach a sufficient value so that we were merely measuring the injected exogenous LH-RF.

Using the present methods, there appears to be little difference in sensitivity to exogenous LH-RF at various stages of the estrous cycle. There is a suggestion in the data that the sensitivity may be slightly greater in early proestrus.

The variations in LH secretion during the estrous cycle can be explained in two ways. One could postulate a constant release of LH-RF and a variation in the hypophyseal response to the factor conditioned by changing steroid titers(14). With this view pituitary responsiveness to LH-RF would rise during proestrus, and this would be the cause for the ovulatory surge of LH secretion which occurs at this time in the estrous cycle. According to the other hypothesis, pituitary responsiveness to the LH-RF would remain constant and variations in LH output would be brought about by variations in the release of LH-RF. The present data appear to be more consistent with this latter hypothesis, since no clear-cut alteration in sensitivity to LH-RF could be ascertained at different stages of the cycle. Also in support of this latter hypothesis is the decline in stored LH-RF content which has been reported to occur in proestrus, at a time when LH release is elevated(7,8). Presumably the changing rates of LH-RF release during the various stages of the cycle are conditioned by altered levels of ovarian steroids which act on the hypothalamus to change the secretion rate of the LH-RF. Rising titers of estrogen and progesterone at proestrus presumably evoke an increased release of LH-RF which in turn triggers LH release.

Summary. Sephadex-purified LH-RF was injected intravenously into female rats at various stages of the estrous cycle. The effect on plasma LH activity was estimated by the ovarian ascorbic acid depletion technique. LH-RF evoked an increase in plasma LH activity at all stages of the estrous cycle. No definite changes in sensitivity to the LH-RF could be detected, although there was a suggestion that the sensitivity might be enhanced on the morning of proestrus. The experimental results are consistent with the hypothesis that varying rates of release of LH-RF are responsible for the changing rates of release of LH during the estrous cycle.

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Further Studies on Multiplication of Dengue Viruses in Various Host Systems.* (31310)

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Tissue culture systems susceptible to dengue virus infection offer additional methods for studies of the biological properties and the antigenic and immunogenic characteristics of this group of viruses. Cytopathic effects (CPE) are produced by selected dengue strains in primary cultures of rhesus monkey kidney(1,2) and hamster kidney(3) and in serial passage cultures of KB(4,5), BHK-21 (6), HeLa(7), human skin(8), porcine kidney(9), and stable rhesus(9). Plaque formation by these viruses has been described for only very few culture systems, namely KB (4), BHK-21(10), and primary rhesus monkey kidney(11). This report further characterizes the multiplication of the known dengue serotypes in mice and in certain susceptible culture systems.

Methods. Viruses. The following dengue virus strains were used: Type 1 (Hawaii), 124 infant mouse passages; Type 2 (Trinidad), 57 mouse passages; Type 2 (NG-B), 3 mouse passages; Type 3 (H-87), 24 mouse passages; Type 4, (H-241), 24 mouse passages; and provisional Types 5 (TH-36), 15 mouse passages, and 6 (TH-S-man), 15 mouse passages. Virus stocks were prepared as 20% infected mouse brain in borate buffered saline, pH 9, with 0.75% bovalbumin.

Tissue cultures. Primary monkey kidney cell monolayers were prepared and grown in 3 oz flint glass prescription bottles according to the methods of Hsiung(12). Roux bottle stocks of the continuous lines of rhesus monkey kidney(13) and porcine kidney(14) were grown in Eagle's minimal essential medium (15) fortified with 5-10% fetal calf serum and antimicrobials. Tryptose phosphate broth (1%) was added to the growth medium of the porcine kidney line. Cells of stock cultures were dispersed using 0.7% pronase (16) or 0.125% trypsin in serum-free growth medium and were planted at a concentration of 2.0-5.0 $\times$ 10^5 cells per milliliter. Medium changes were made at 3-5 days using growth medium but decreasing the serum concentration to 1.0% and doubling the bicarbonate concentration. Cultures were used for virus inoculation and overlay between 5-12 days after seeding.

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