and staphylococci would suggest that the end result in reference to mortality is the same in splenectomized and sham operated animals at periods varying from 4 to 255 days post-splenectomy. Whether the more fulminating illness observed at 5 days but not 8–12 days, postsplenectomy, following streptococcal infection was related to the specific organism, the absence of the spleen per se, or the more recent time interval since major surgery, i.e., removal of the spleen as compared to sham operation is not clear and bears further investigation.

Conclusions. Intravenous challenge of rhesus monkeys with T14 and S23 strains of Group A hemolytic streptococci was followed by similar clinical and laboratory evidence of infection, and the former strain appeared somewhat more virulent. Aerosol challenge with the T14 strain produced no demonstrable disease but did offer some partial protection to subsequent intravenous challenge 4 weeks later. No significant differences were noted between splenectomized and normal monkeys in reference to fatal outcome after challenge 5–12 days after surgery. However, splenectomized monkeys challenged at the 5-day period either died earlier, or if they survived had longer periods of clinical illness, than their nonsplenectomized controls.

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Effect of Pretreatment with Androgen in the Neonatal Rat on LH-Induced Ovarian Cholesterol Depletion* (32758)

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The ovarian cholesterol depletion (OCD) assay for LH reported by Bell *et al.* (1) has not been usable in this and other laboratories (2) because of the multiphasic nature of the dose–response curve. We have demonstrated that ovarian cholesterol levels in PMS-HCG treated Wistar rats exhibit a diurnal rhythm, and that this rhythm may reflect endogenous LH release (3). This could then account for the multiphasic nature of the curve obtained in the assay. We have also shown that androgen sterilization will block this diurnal rhythm (3). The present study was designed to determine the effect of LH on ovarian cholesterol levels in the androgenized rat pretreated with PMS and HCG.

Method and Materials. All rats used in this study were of the Purdue-Wistar strain. The rats were housed six per cage, and kept in a controlled environment of 72–74°F. with a relative humidity of 45–55%. The light and dark cycle consisted of 13 hours of light and 11 hours of darkness with the light cycle starting at 7:00 a.m. Food and water were given *ad libitum.*

The PMS-HCG[1] treatment consisted of subcutaneous injections of 50 IU of PMS on days 22 and 24 of age followed by 25 IU of HCG on day 26. Both hormones were dissolved in physiological saline and injected in a 0.1 ml volume. Pretreatment with androgen consisted of a single subcutaneous injection of 1.25 mg of testosterone propionate on day 2 of age.

Ten days following PMS-HCG treatment

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[1] The PMS (Equinex®) and HCG (A. P. L.®) were obtained through the courtesy of Dr. J. B. Jewell, Ayerst Laboratories, New York.

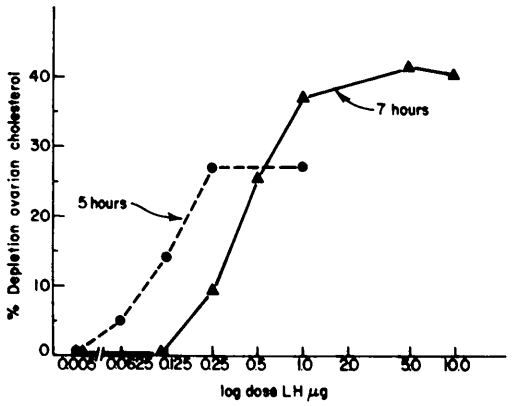

FIG. 1. Ovarian cholesterol depletion in the androgenized rat pretreated with PMS and HCG. ●- - - - ● depletion over a 5-hour period; ▲——▲ depletion over a 7-hour period.

animals were unilaterally ovariectomized by the dorsal approach under ether anesthesia, and injected ip with the test substance (injection volume 1.0 ml). After 5 or 7 hours the animals were killed by cervical dislocation and the contralateral ovary removed. Ovaries were quickly cleaned and weighed to the nearest 0.2 mg on a torsion balance immediately after removal from the animal. The gonads were then homogenized in 10 ml of glacial acetic acid, filtered through a no. 1 Whatman filter paper and analyzed for total cholesterol by the method of Zlatkis *et al.* (4). The quantities of cholesterol in the two ovaries were compared and the percentage depletion calculated for each rat.

All protein hormones used in this study were dissolved in saline and kept frozen until used. Pituitary extracts were prepared by grinding the anterior pituitary in 2.0 ml of saline, centrifuging the homogenate, and using the appropriately diluted supernatant fluid for assay. The LH[2] used as a standard was NIH-LH-B1. Relative potencies were expressed in terms of NIH-LH-S1 (1 unit/mg).

Results. Figure 1 is a graph of the composite dose-response curves obtained at either 5 or 7 hours after injection of LH. A linear relationship between the log-dose of LH and the response was obtained between 0.0625 μg and 0.25 μg LH at 5 hours and between 0.25 μg and 1.0 μg LH at 7 hours. The dose-response curve was monotonic in all instances. The results of nine different assays are shown in Table I. The average λ for the nine assays was 0.32 and the average slope was 37.9.

Other pituitary hormones have an effect on ovarian cholesterol levels in this animal preparation only to the extent to which they are contaminated with LH (Table II). The relative potencies of FSH, ACTH,[3] and TSH were 0.015, 0.0078, and 0.008 units/mg, respectively. A dose of 500 μg of prolactin failed to elicit a response. Injection of 250 μg of prolactin to animals that were also treated with two dose levels of LH (0.5 μg and 1.0 μg) did not significantly affect the relative potency of the LH. Pitocin and pitressin both failed to produce a significant response. The average LH content of two different anterior pituitary glands from male rats (body weight, 600 gm) was 4.0 μg of LH/mg of wet wt.

TABLE I. The λ and Slope Characteristics of Bioassay Results Using the Androgenized PMS-HCG Treated Immature Rat.

Type (hours)	Design	No. of animal/point	(λ)	Slope
5	2	5	0.50	34.0
5	2	6	0.34	39.3
5	3	5	0.25	35.6
5	2	5	0.30	34.0
5	2	5	0.28	38.5
7	2	5	0.29	40.6
7	3	6	0.15	46.2
7	2	5	0.47	32.0
7	2	5	0.35	41.0
			$\bar{X}=0.32$	$\bar{X}=37.9$

Discussion. Androgen sterilization not only blocks the diurnal rhythm in ovarian cholesterol levels (3) but also produces an animal which will respond in the OCD assay in a predictable fashion. The multiphasic nature of

[2] The LH, FSH, and prolactin were obtained through the courtesy of the Endocrine Study Section, NIH.

[3] The ACTH was obtained through the courtesy of Dr. J. Bastian, Armour Pharmaceutical Co., Illinois.

TABLE II. Effects of Various Pituitary Hormones and Male Pituitary Extracts on the OCD Bioassay Using Androgenized PMS-HCG Treated Rats.

Test substance	Design (hours)	(λ)	Relative potency[a]	95% confidence interval
FSH (NIH-FSH-S3)	2 × 2 (5)	0.29	.015	.007 –.029
ACTH (Armour)	2 × 1 (7)	0.26	.0078	.0045–.0123
TSH (Mann)	2 × 1 (5)	0.29	.008	.003 –.021
Prolactin (NIH-P-S7)	2 × 1 (5)		—[b]	
Pitocin	2 × 1 (7)		—[c]	
Pitressin	2 × 1 (7)		—[c]	
LH + 250 μg Prolactin	2 × 2 (5)	0.25	1.15	0.78 –1.25
LH + 250 μg Prolactin	2 × 2 (5)	0.30	1.01	0.76 –1.18
Anterior pituitary ♂	2 × 2 (5)	0.22	.0044	.0028– .0070
Anterior pituitary ♂	2 × 2 (5)	0.24	.0036	.0019– .0064

[a] Relative potency in terms of NIH-LH-S1 (1 unit/mg).
[b] A dose of 500 μg gave no significant response.
[c] A dose of 0.1 IU gave no significant response.

the dose-response curve (2) is abolished; however, the sensitivity is also reduced. The sensitivity of the OCD assay using the androgenized rat is approximately equal to that of the ovarian ascorbic acid assay for LH (5).

The fact that androgen sterilization abolishes the multiphasic nature of the response to LH supports the contention that in the nonandrogenized animals either endogenous LH release is involved or the manner in which the ovary responds to LH is quite different. That the injection of testosterone propionate in the neonatal rat can abolish the release of the ovulatory surge of LH is indicated by reports that this treatment results in the production of a persistent estrus condition which resembles the persistent estrus rat produced by anterior hypothalamic lesions (6, 7). Furthermore, treatment with a high dose of androgen at 2 days of age is considered to have the effect of a physiologic lesion in the same area of the hypothalamus (8). Normal ovulation and corpora lutea formation do not occur in these androgenized rats (8, 9); however, the finding that normal ovulation and corpora lutea formation do occur when the ovaries are transplanted to normal rats (10) indicates that the ovaries have not become permanently altered by androgen treatment. These facts indicate strongly that endogenous LH release is involved in producing the multiphasic dose-response curve obtained in nonandrogenized animals.

Ovaries of androgenized rats also appear to be affected by the androgen treatment. This can be seen in the change in glucose metabolism in the ovary (11), cholesterol levels in the ovary following PMS and HCG (3) and ovarian steroidogenesis (12). Therefore the ovarian physiology of these two animals appears to be quite different and probably alters their ability to respond to LH. The reduced sensitivity to LH following androgen treatment is probably due to this direct effect of androgen on the ovaries. Other studies also indicate that the ovaries of androgenized rats are less sensitive to exogenous gonadotropins (13, 14).

The OCD assay using the androgenized animal is specific for LH and only gives a response to other pituitary hormones when LH is present in the extract. The relative potencies for LH activity in the ACTH, TSH, and FSH tested in this study are in good agreement with the amount of LH present as reported by the Endocrine Study Section (NIH). A dose of 500 μg of prolactin gave no significant response, which indicates the LH contamination of this preparation is less than 0.02%. Vasopressin has been reported to cause ovarian ascorbic acid depletion (15); however, pitressin (0.1 IU) in this assay gave no significant response. Likewise, pitocin had no effect. The quantities of pituitary LH observed in the male rat were in good agreement with previous investigations using the

ovarian ascorbic acid technique (16).

Conclusions. Androgen sterilization results in the elimination of the multiphasic dose-response curve obtained with the OCD bioassay for LH. The dose-response curve in the androgenized animals is highly reproducible, but the sensitivity of the assay is considerably reduced. The abolition of the multiphasic curve by androgen treatment suggests that endogenous LH release is responsible for the multiphasic type of response in the non-androgenized rat. A linear log dose-response relationship for LH is obtained between 0.0625 μg and 0.25 μg using a 5-hour assay time interval, and between 0.25 μg and 1.0 μg using a 7-hour assay time interval. This response is reproducible and is specific for LH.

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Absorption of Lipids from Mixed Micellar Bile Salt Solutions* (32759)

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It is well established that cholesterol, fatty acids, and monoglycerides can be solubilized in bile salt and lecithin micelles (1–3), and that these lipids are normally present in micellar form in the intestinal lumen (4–6). It has been suggested that bile salts act as intraluminar transport vehicles for lipids, and that the absorption of specific lipids is dependent on their solubility in micellar media (5–7). Simmonds *et al.* (8) recently reported marked differences in the rate of absorption of the individual components of mixed micelles from the intestinal lumen in humans, and suggested that micellar lipids are not absorbed as intact aggregates. However, Johnston and Borgstrom (9, 10) reported that intestinal slices incubated in bile salt micelles containing oleic acid-^{14}C and monopalmitin-^{3}H removed the two labeled components in the same ratio as their concentrations in the incubation media.

The present report provides further evidence that mixed micelles are not absorbed as intact aggregates but rather that the individual components of micelles are absorbed independently by the intestinal mucosa. The basic approach employed was to incubate everted rat intestinal sacs in micellar solutions of bile salt and monoolein, plus cholesterol, oleic acid, and lecithin in various combinations. Each compound, except lecithin, was labeled with either ^{14}C (sodium tauro. etc, monoolein and oleic acid) or ^{3}H (choles-

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