Failure of Ovariectomy to Influence Drum Tolerance or Phagocytic Stimulation by Zymosan in the Rat.*† (26936)

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Several observations in recent years have suggested the possibility that physiologic titers of estrogen exert a regulatory influence upon the reticuloendothelial system (RES) of female animals. However, the results have been conflicting. For example, Halpern, *et al.*(1) have shown that female mice respond to BCG infection with a greater RES stimulation than do males, but that castration of female mice does not significantly reduce the functional hypertrophy of the RES which normally follows BCG infection in unoperated females. Similarly, gonadectomy in the female rat has no effect upon phagocytic efficiency, as revealed by an unaltered rate of clearance of colloidal particles from the circulation after castration(2.) We studied the effect of ovariectomy upon stimulation of the rat's phagocytic tissues by Zymosan, an RES-stimulating agent with effects comparable to those produced by BCG. Since the development of conditioned or tolerant states in the rat appears to be associated with enhancement of RES function(3), we also studied the effect of ovariectomy upon development of resistance to rotational trauma in the Noble-Collip drum(4).

Methods. Animals. Female albino rats of the Sprague-Dawley strain (Charles River Farms), weighing between 140 g and 160 g were used. Bilateral ovariectomy was performed under light ether anesthesia.

Zymosan treatment. Suspensions of Zymosan (Lot #5B171) were prepared in sterile, isotonic saline, at a concentration of 10 mg/ml, by rapid mixing in a mortar and pestle homogenizer. Animals were injected on the fourth, sixth, and eighth postoperative days with 5 mg intravenous doses. On the tenth day after operation, and 48 hours after the last dose of Zymosan, global phagocytic index (K) was determined according to the method of Biozzi *et al.*(5). The measurement of phagocytic index was carried out with a 16 mg/100 g dose of colloidal carbon (Pelikan c 11/1431 a, Gunther Wagner, Germany) suspended in 10% gelatin and injected intravenously.

Drum tolerance. Other animals were conditioned in the Noble-Collip drum according to the following regime. Two hundred turns were given on the 4th postoperative day, 200 turns on the 5th day, 300 turns on the 6th and 7th days, and 500 turns on the 8th day. On the 9th postoperative day, the animals were rested and the next day were challenged with 900 revolutions in the drum, a normally LD 100 procedure. Weights of all animals were recorded daily throughout the training period and up to the day after challenge.

Autopsy data. Animals were sacrificed immediately after determination of phagocytic index or 24 hours after challenge in the Noble-Collip drum. At autopsy, weights of liver, spleen, pituitary, adrenals, thymus, and strips of uterus were measured. The smaller organs were trimmed of excess fat and weighed to the nearest 0.1 mg on a Roller-Smith torsion balance.

Results. 1. *Development of drum tolerance in the spayed* vs. *sham-operated female rat.* By the 11th postoperative day, significant atrophy of the uteri and hypertrophy of the thymus glands could be observed in the ovariectomized group in comparison with sham-operated controls. These effects are shown in Table I as the changes in body wt/organ wt ratios. Both of these effects on estrogen target organs confirmed the existence of a state of estrogen-deprivation in the castrated animals during the experiment. Despite this fact, there was no alteration in ability of the castrated animals to respond to the conditioning process, as shown by their

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TABLE I. Effect of Conditioning in the Noble-Collip Drum on Ovariectomized and Control Rats.

Exp. group	Daily wt gain (g)*	Survival after challenge	Body wt (g)/organ wt (g) ratio* × 10^{-2}				
			1 cm uterus	Thymus	Spleen	Pituitary	Adrenals
Sham-operated (12)	2.7 ± .4	12/12	44 ± 2.6	4.3 ± .2	2.6 ± .3	178 ± 9.4	31 ± 1.9
Bilaterally ovariectomized (11)	3.1 ± .4	10/11	222 ± 20.0	2.9 ± .3	2.5 ± .1	178 ± 9.4	36 ± 2.6
			$p < .001$	$p < .01$			

* Mean ± stand. error of mean.

continued weight-gain during conditioning and their response to final challenge with a normally LD/100 dose of rotational trauma. Moreover, there was no statistically significant difference between experimental and control animals with regard to size of selected "stress organs" (spleen and the pituitary-adrenal system).

2. *Stimulation of phagocytic index with Zymosan in the spayed* vs *sham-operated female rat.* Animals were treated surgically as before, and on the 10th postoperative day autopsy revealed a significant atrophic degeneration of the uterine horns of the castrated animals (Table II). Despite this state of estrogen-deprivation, RES phagocytic function in the castrated animals was increased by Zymosan to the same extent as in controls. Table II shows the changes in both groups in global phagocytic index (K), unit phagocytic index (alpha) and relative spleen and liver weights. According to Benacerraf(2), the mean normal values for K and alpha in the rat, at the dose of carbon employed in our experiments, are 0.013 and 4.5 respectively; while ratio of body weight to combined liver and spleen weights in the untreated rat generally varies about a mode frequency of 18. Zymosan treatment in both experimental and control groups resulted in a greater than 10-fold increase in K and a significant increase in alpha. The large change in global phagocytic efficiency was associated in both groups with a significant hypertrophy of liver and spleen, especially of the latter organ which increased as much as 300% in weight.

An additional observation was made with respect to the thymus gland of the Zymosan-treated rat. The data presented in Tables I and II show that Zymosan treatment prevented the thymic hypertrophy associated with ovariectomy and produced normal-sized glands in the ovariectomized-Zymosan treated group; whereas, in sham-operated rats treated with Zymosan, there was frank atrophy of thymic structures.

Discussion. The original observation of Nicol(6,7) of an increase in number and activity of phagocytic cells of guinea pig uterine endometrium during the follicular phase of the estrous cycle prompted the suggestion that estrogenic hormones may be involved in regulation of the RES of female animals. Subsequently, it was shown that treatment of mice with large doses of synthetic or natural estrogens causes hypertrophy of liver and spleen and an accelerated rate of phago-

TABLE II. Effect of Zymosan Treatment on Ovariectomized and Control Rats.

Exp. group	Global phagocytic index (K)*	Body wt (g) /liver + spleen wt (g) (W/Wls)*	Unit phagocytic index (α)*	Body wt (g) /1 cm uterus wt (g)	Body wt (g)/ thymus wt (g)
				Ratio* × 10^{-2}	
Sham-operated (8)	.186 ±.024	13.1 ±.5	7.4 ±.5	54 ± 3.0	7.8 ± 1.4
Bilaterally ovariectomized (11)	.199 ±.015	13.0 ±.3	7.5 ±.2	172 ± 12.0	4.8 ± .3
				$p < .001$	

* Mean ± stand. error of mean.

cytosis by these organs(8-10). Synthetic estrogen (diethylstilbesterol) has a similar effect upon the RES of the rat, although estradiol does not affect phagocytosis in the rat as it does in the mouse(9). Our results clearly show that ovariectomy in the rat does not interfere with the hypertrophic response of RES organs to Zymosan. This finding is in accord with that made by Halpern in his study of phagocytic stimulation by BCG in ovariectomized mice(1). It thus seems unlikely that physiologic titers of estrogenic hormone exert a significant regulatory effect during hyperfunctional states of the RES in the mouse or rat, despite the ability of certain of these steroids to cause profound stimulation of this system when administered in greater than physiologic doses.

Benacerraf has suggested that the enhanced susceptibility of Zymosan-treated mice to bacterial endotoxin results from an exhaustion of adrenal cortical function after Zymosan(11). Assuming that this phase of adrenal cortical exhaustion follows an initial phase of adrenal cortical hypersecretion brought on by the effects of Zymosan treatment, the concomitant atrophy of thymus structures in Zymosan-treated animals can be explained on the basis of the known inhibiting action of corticosteroid hormones on this organ.

Hruza(12) has reported that castration of adult female rats does not affect their resistance to single doses of rotational trauma. In our study we were concerned with the possible effects of ovariectomy upon the ability of animals to become adapted to trauma during the course of conditioning. Our finding that gonadectomy does not interfere with development of resistance is therefore in accord with results obtained with unconditioned rats.

Summary. Bilaterally ovariectomized and sham-operated rats were compared with respect to their conditioning responses in the Noble-Collip drum, and their responses to Zymosan, a RES-stimulating agent. Estrogen-deprivation in castrated animals was confirmed by the presence of atrophic uteri and hypertrophic thymus glands. Gonadectomized animals gained weight during the conditioning process and survived final challenge as well as did controls. There was no difference in the effect of conditioning upon weights of spleen, pituitary or adrenals in the 2 groups. Zymosan treatment resulted in equivalent hypertrophy of liver and spleen, and an equivalent increase in phagocytic activity per unit of tissue in the 2 groups. It was concluded that ovariectomy does not inhibit the response of the RES of the rat to stress or to stimulation with Zymosan. It is suggested that physiologic titers of ovarian hormone are not required during the development of hyperfunctional states of the RES in female rats.

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