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Effects of Hormonal Pretreatment of Cardiac Necrosis In the Japanese Quail (38797)

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The myocardial necrosis induced with massive doses of the synthetic catecholamine isoproterenol is influenced by numerous environmental factors. Thus, temperature (1), altitude acclimation (2), muscular exercise (3), pretreatment (4) with isoproterenol, nutritional status (5), and species, age, and strain of the animals (6, 7) have all been shown to affect the severity of the lesion induced by this drug. Rona *et al.* (7) and Wexler (9) have shown that the male rat is more susceptible than the female to the necrogenic actions of this drug and it has been suggested that the increased susceptibility is due to the greater body weight of the male rat at a given age (7). Previously, we reported (10) that isoproterenol-induced myocardial necrosis is more severe in the male Japanese quail (*Cotournix cotournix*) even though the female of this species is significantly heavier at any age.

This study was undertaken to determine if the sex difference in susceptibility to isoproterenol necrosis in the Japanese quail could be altered by hormonal treatment.

Methods. Male and female Japanese quail hatched and raised in New Brunswick, NJ were used in these experiments. All males were castrated or sham operated at 4 wk of age and allowed to recover for 1 wk prior to hormonal treatment. All birds were injected with 0.2 cc cotton seed oil in which either 1.0 mg β-estradiol-3-benzoate or 1.0 mg testosterone propionate had been dissolved. The groups were identified and treated according to the following schedule (Table I). CE-castrated, estrogen-treated, males; CT-castrated, testosterone-treated, males; C-castrated, no treatment, males; MS-sham operated, no treatment, males; FT-testosterone-treated, females; F-no treatment, females. Hormonal treatment was continued for 3 wk and all birds were the same age when injected with isoproterenol.

After 3 wk the animals were weighed and blood was drawn from a wing vein to determine microhematocrit ratios.

Isoproterenol (80 mg/kg of body weight) was injected subcutaneously on 2 consecutive days at 24-hr intervals. On the third day the animals were sacrificed, the hearts removed, and the degree of damage evaluated macroscopically. The extent of the lesions was graded using a scheme similar to that devised by Rona *et al.* (11) and used by us previously (5, 10). (0, no necrosis; 1, diffuse paling of the tip; 2, limited necrosis of the tip; 3, necrosis involving one-third of the left ventricle; 4, more than one-half of the left ventricle effected by necrosis).

Results. Body weights and hematocrit ratios for the six groups of birds at the end of the treatment period but before isoproterenol injection are shown in Table I. The rate of body weight gain in the castrated males was increased by estrogen treatment and depressed by testosterone treatment. The estrogen-treated castrates (CE), the testosterone-treated females (FT), and the untreated females (F-) all weighed significantly more than the sham-operated males (MS).

Hematocrit ratios were depressed by estrogen treatment in the castrated males while they were increased by testosterone treatment in male castrates and in the intact female. The hematocrit ratios of the sham-operated males (MS) were significantly less than the testosterone-treated castrates (CT), and the testosterone-treated females (FT) but significantly greater than the estrogen-treated castrates (CE). In Japanese quail, as in several species of birds, the normal hematocrit ratio is higher in the male (12).

TABLE I. MEAN BODY WEIGHTS AND HEMATOCRIT RATIOS IN JAPANESE QUAIL AFTER HORMONAL TREATMENT. C = CASTRATES, E = ESTROGEN, T = TESTOSTERONE, − = NO TREATMENT, M = INTACT MALES, S = SHAM OPERATED, F = INTACT FEMALES. (STUDENT *t* TEST USED TO TEST SIGNIFICANCE.)

Group	*N*	Body weight (g)	*P* value vs MS group	Hematocrit (%)	*P* value vs MS group
CE	10	104.7[a] ±2.4[a]	$P < 0.05$[a]	26.5 ±1.9	$P < 0.05$
CT	13	92.9 ±1.5	NS[b]	49.1 ±0.9	$P < 0.05$
C-	12	100.3 ±2.9	NS	47.0 ±1.1	NS
MS	11	95.8 ±2.4	—	44.8 ±1.4	—
FT	10	108.3 ±1.5	$P < 0.05$	49.2 ±1.2	$P < 0.05$
F-	10	121.2 ±2.9	$P < 0.05$	42.5 ±1.1	NS

[a] Mean ± SE.
[b] Not significant.

All groups lost body weight in response to the first and second doses of isoproterenol (Fig. 1). Body weight loss was particularly severe in the untreated (F-) and testosterone-treated (FT) females and least in the estrogen-treated castrates (CE). As in our previous experiments the loss of body weight was due in part to a reduction in food intake and in part to a severe diuresis induced by the drug.

Hearts of estrogen-treated castrates (CE) were significantly more resistant than hearts of sham-operated males (MS) to isoproterenol-induced necrosis (Fig. 2). Myocardial necrosis was least severe in the estrogen-treated castrates (CE); moderately severe in the testosterone-treated castrates (CT), untreated castrates (C-), and untreated females (F-); and most severe in the sham-operated males (MS) and testosterone-treated females (FT).

Discussion. In a previous publication we reported that female Japanese quail are more resistant than males to isoproterenol-induced cardiac necrosis (10). Since the males weighed significantly less than females of comparable age, the male's increased susceptibility to this type of necrosis could not be attributed to greater body weight.

This work indicates that castrated males treated with estrogen for 3 wk are significantly heavier and have lower hematocrit ratios than sham-operated males. In addition, they are more resistant to cardiac necrosis elicited by isoproterenol. A similar estrogen-induced resistance to cardiovascular disease after 2 yr of estrogen replacement therapy has been reported in castrated human females. Higano *et al.* (13) reported that significantly less cardiovascular disease developed in estrogen-treated castrated women than developed in untreated castrated women controls. It has also been demonstrated in human males surviving acute myocardial infarction that estrogen treatment significantly prolonged survival and decreased 5-yr mortality rates by 50% (14).

The mechanism by which estrogen may exert its protective effect is unclear. It has been demonstrated that a decrease in uterine, renal, and peripheral vascular resistance accompanies pregnancy and that these resistance changes can be reproduced experimentally by the administration of estrogen (15). Brown *et al.* (16) have further demonstrated that peaks in uterine blood flow during the estrous cycle coincide with the cyclic surges of estrogen secretion, and that these blood flow changes can be reproduced experimentally by the administration of estrogen and progesterone. Thus, estrogen has been shown to be a potent vasodilator in many vascular beds but its specific action on myocardial blood flow has not yet been described.

Since increased body weight gain has been associated with increased susceptibility to isoproterenol necrosis in rats (8), it is unclear how estrogen can increase cardiac resistance while increasing the rate of body weight gain. Moreover, Fleckenstein *et al.* (17) have shown that isoproterenol acts to flood the contractile machinery with calcium, and procedures which tend to reduce blood calcium levels (18) tend to reduce the severity of cardiac necrosis.

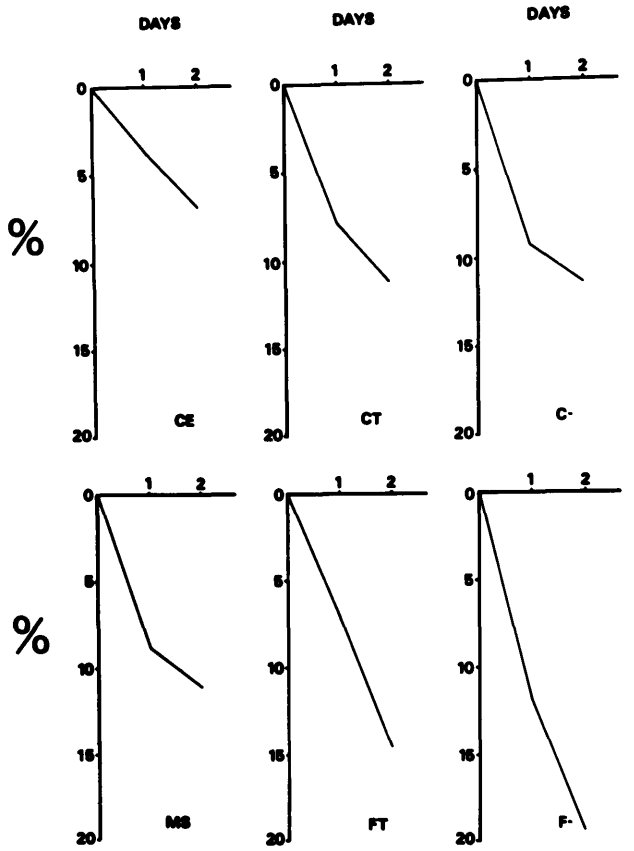

FIG. 1. Severity of myocardial infarcts in Japanese quail after hormonal treatment: (abscissa) Groups as identified in Table I; (ordinate) infarct score. SE marked off for each mean. Student t test used to test significance. * $P < 0.05$.

Estrogen treatment in birds is associated with elevated blood calcium levels and decreased medullary bone formation (12). Thus, it would seem that estrogen treatment by its effect on blood calcium levels in birds would exacerbate the cardiac necrosis elicited by isoproterenol. However, our results indicate that estrogen treatment has a definite protective effect on the heart.

A possible explanation is that estrogen treatment may increase the efficiency of the mechanisms by which isoproterenol is inactivated or eliminated from the body. Since one avenue of isoproterenol metabolism is conjugation in the liver, and since it has been shown that estrogen acts to increase liver weight in birds by increasing the number and size of liver cells (12), it might be possible that estrogen-treated birds have more efficient livers. However, this explanation appears unlikely because Campbell (19) has demonstrated that estrogen may, in fact, impair liver function as measured by clearance of sodium bromsulphthalein (BSP).

Summary. Male Japanese quail castrated and treated with β-estradiol-3-benzoate for 3 wk were more resistant than sham-operated males to the necrogenic effects of massive doses of isoproterenol.

The estrogen-treated castrate also weighed significantly more and had significantly lower hematocrit ratios than either the sham-operated male or the intact male. These results indicate that there is a definite sex-related protective effect against this

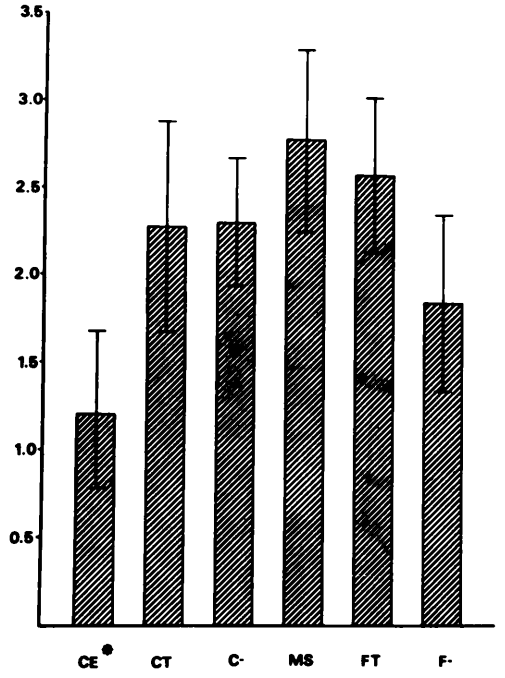

FIG. 2. Loss of body weight in response to isoproterenol injection: (abscissa) days after injection; (ordinate) percent body weight change. Groups as identified in Table I.

type of cardiac necrosis which cannot be explained on the basis of differences in body weight.

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