Action of Sex Hormones on the Insulinemia of Castrated Female Rats* (33742)

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Several factors prevent the evolution of diabetes in subtotally pancreatectomized rats. Female sex hormones are among the factors known to have a protective effect from the onset of diabetes, as demonstrated by the daily administration of estrogen to castrated female rats (1). The action of endogenous sex hormones is the reason why the incidence of diabetes after subtotal pancreatectomy is higher in male than in female rats (2).

The action of these hormones on the pancreas may be relevant to the protective effect of estrogen. Rats chronically injected with estrogen showed hyperplasia and hypertrophy of the islets of Langerhans (3). This is the result of a direct stimulation of the pancreas, as demonstrated by injection of estradiol to partially pancreatectomized rats (4) and by the implantation of estradiol cristal and the subsequent hypertrophy and hyperplasia of islets around the site of the implant (5). Furthermore the insulin content of the pancreas was shown to be augmented by estradiol administration (6). An excellent review about the action of steroids hormones on pancreatic function has been published (7).

The purpose of our work was to investigate the effect of chronic treatment with sex hormones on the plasma insulin levels of ovariectomized rats.

Material and Methods. Female rats from our colony were utilized. Castration was performed via the lumbar approach when the animals had reached a body weight of 90–100 g. The rats were allowed a 3-week postoperative recovery period before the experiment was initiated. The following groups of castrated rats were studied: (i) control rats injected with olive oil (0.1 ml/day); (ii) progresterone-treated rats (500 μg/day); (iii) testosterone-treated rats (500 μg/day); and (iv) 17β-estradiol-treated rats (4 μg/day).

The treatment by subcutaneous injection lasted 7 months. Blood was withdrawn periodically by cutting the tip of the tail. Glycemia was determined by the method of Somogyi–Nelson (8) and plasmatic insulin by the immunologic method of Herbert *et al.* (9). The significance of the results was calculated by the analysis of variance.

Results. Table I shows the blood sugar values of the different group of rats. It can be seen that the 17β-estradiol-treated group is the only one which shows a significant change in blood sugar level after 6–7 months of treatment.

Table II shows that progesterone treatment for 7 months did not alter the plasma insulin level of the rats. On the other hand, testosterone propionate, at the same dose significantly augmented the plasmatic insulin level after 6 months of treatment. The elevation seen at month 7 is not statistically significant. The daily injection of 17β-estradiol increased significantly the insulinemia after 2, 5, 6, and 7 months of treatment.

Discussion. The present experiment demonstrates that chronic treatment of castrated female rats with 17β-estradiol augmented the immunologically reactive plasma insulin level, that progesterone treatment had no effect and that testosterone seemed to have increased it only after 6 months of treatment.

These results contribute to a better understanding of the protective action of female sexual hormones on experimental diabetes (1–4). This action is not related to changes in food intake, as demonstrated by forced feeding experiments (10). Castration of male

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TABLE I. Blood Glucose Levels (mg/100 ml) in Castrated Female Rats Treated with Sex Hormones (progesterone, 500 μg/day; testosterone, 500 μg/day; 17β-estradiol, 4 μg/day).

Month of treatment	Olive oil		Progesterone		Testosterone		17β-estradiol	
	($\bar{X} \pm$ SE)	(N)	($\bar{X} \pm$ SE)	(N)	($\bar{X} \pm$ SE)	(N)	($\bar{X} \pm$ SE)	(N)
First	107 ± 3.02	(14)	108 ± 3.15	(7)	108 ± 2.86	(10)	109 ± 1.81	(14)
Second	98 ± 5.73	(14)	99 ± 4.54	(7)	85 ± 4.59	(7)	94 ± 2.64	(14)
Third	115 ± 4.76	(14)	96 ± 2.31	(6)	106 ± 3.39	(7)	100 ± 3.83	(11)
Fifth	124 ± 5.37	(9)	120 ± 4.63	(6)	125 ± 1.49	(6)	129 ± 4.81	(11)
Sixth	98 ± 7.41	(10)	90 ± 3.18	(6)	99 ± 4.83	(6)	71 ± 4.05	(8)[a]
Seventh	109 ± 9.89	(8)	97 ± 5.00	(7)	102 ± 9.60	(5)	74 ± 3.51	(9)[a]

[a] Significantly different from the control group ($p < 0.05$).

TABLE II. Plasma Insulin Levels (μU/ml) in Castrated Female Rats Treated with Sex Hormones (progesterone, 500 μg/day; testosterone, 500 μg/day; 17β-estradiol, 4 μg/day).

Month of treatment	Olive oil		Progesterone		Testosterone		17β-estradiol	
	($\bar{X} \pm$ SE)	(N)	($\bar{X} \pm$ SE)	(N)	($\bar{X} \pm$ SE)	(N)	($\bar{X} \pm$ SE)	(N)
First	24.6 ± 2.34	(14)	20.3 ± 2.16	(6)	29.7 ± 3.53	(9)	29.6 ± 3.86	(14)
Second	20.0 ± 2.35	(14)	14.0 ± 4.60	(7)	30.1 ± 4.56	(7)	48.1 ± 10.0	(14)[a]
Third	24.5 ± 2.97	(14)	19.4 ± 2.04	(7)	35.8 ± 4.92	(6)	39.1 ± 5.45	(11)
Fifth	26.8 ± 4.64	(7)	18.3 ± 3.20	(6)	35.0 ± 5.01	(5)	44.8 ± 6.26	(8)[a]
Sixth	24.1 ± 3.42	(10)	23.5 ± 4.89	(7)	55.0 ±11.9	(6)[a]	47.5 ± 4.13	(8)[a]
Seventh	34.0 ± 5.08	(9)	17.5 ± 4.83	(6)	51.3 ± 9.73	(6)	81.5 ± 11.3	(10)[a]

[a] Significantly different from the control group ($p < 0.05$).

rats, weighing between 70 to 80 g diminished the incidence of diabetes due to partial pancreatectomy while ovariectomy has the opposite effect. When castration is performed before 24 hr of life the evolution of diabetes is alike in both sexes (11). Estrogen treatment augmented the insulin content of rat pancreas. Evidences exist suggesting that this action is mediated through the hypophysis (12). As a result of our experiment it would seem likely that estrogen treatment, besides increasing the biosynthesis of insulin also stimulates its secretion, although we cannot rule out a diminished insulin catabolism induced by estrogen treatment. To our knowledge no studies have been done to elucidate the possible action of estrogen on insulin metabolism.

Summary. Castrated female rats received daily subcutaneous injections of progesterone (500 μg/day), testosterone (500 μg/day), and 17β-estradiol (4 μg/day) for 7 months. Lower fasting blood sugar values were found in the 17β-estradiol-treated group after 6 and 7 months of treatment. The plasma immunoreactive insulin level was augmented by the sixth month of treatment in the testosterone treated group and by the second, fifth, sixth, and seventh months of treatment in the 17β-estradiol group.

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Hypocholesterolemic Activity of *N*-(Ferrocenylmethyl)piperidine (33743)

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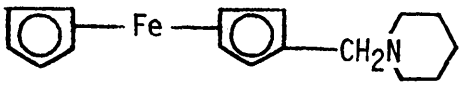
The search for new hypocholesterolemic agents has resulted in the discovery of many active compounds in the last few years. It is highly probable that the majority of these affect cholesterol biosynthesis in some way. In particular, several have been reported which inhibit one of the later reactions in the sequence (1–3).

The purpose of the present paper is to report the investigation of a member of a series of new hypocholesterolemic agents having ferrocene as a component of their structure. The results of this study show that this compound, *N*-(ferrocenylmethyl)piperidine,



causes abnormal quantities of a compound resembling 7-dehydrocholesterol to acumulate in serum and liver, and probably acts by inhibiting 7-dehydrocholesterol reductase. Very little work has been reported on the pharmacological activity of ferrocene compounds, although it has been suggested that ferrocene or ferrocene derivatives could be used as hematinic agents (4, 5).

Experimental Methods. Animal studies. The chemical was adsorbed on silica gel from an acetone solution and mixed with ground rodent mash on a roller mill to give a concentration of 0.007%. Groups of six male weanling rats (Harlan Wistar strain, Harlan Industries, Cumberland, In.) were maintained on the experimental diet for 1 week, then killed and blood and liver samples were collected.

Analytical. A modification (6) of the method of Sperry and Webb (7) was used for the determination of "fast-acting" sterols and cholesterol. This method takes advantage of the fact that the so-called "fast-acting" sterols give full color development in 12 min at 0°, whereas cholesterol does not react at all in this time. After standing at 25° for an additional 30 min, the absorbancy is again determined. After a suitable correction for the contribution of "fast-acting" sterols to the latter reading, the amount of cholesterol can be determined. Materials absorbing at 281.5 mμ were determined spectrophotometrically by measuring the absorbancy in ethanol of nonsaponifiable material from serum and liver. Correction for extraneous absorption was made using a three-point procedure (8) with selected wavelengths of 276.5, 281.5, and 284.5 mμ. The reference material for "fast-acting" sterols and the 281.5 mμ determination was 7-dehydrocholesterol.

A spectrophotometric assay (9) was used to determine inhibition of the enzymatic reduction of 7-dehydrocholesterol *in vitro*. Each flask contained 13,000*g* rat liver supernate equivalent to 0.25 g of liver, 0.75 μmoles of 7-dehydrocholesterol, 1.7 μmoles of NADP, 30 μmoles of nicotinamide, 15 μmoles of glucose-6-phosphate, and 70 μmoles of Tris–maleate buffer, pH 7.2. The total volume was 1.0 ml and the incubations were carried out in duplicate at 37° for 2 hr under nitrogen.