agreement with this, the accelerated RRA decrease in the young saline group with a lower saline intake level was only indicated, but the different reactivity manifested itself by an inverse relation between saline intake and RRA, which was absent in the adults (table 2). This presumably reflects a higher RRA responsiveness to salt excess in immature rats. There is evidence that RRA plays a role in regulation of glomerular blood flow[11,12] and some data indicate that the vasculature of immature rats is more prone to develop hypertensive lesions[13]. It may be speculated that, in connection with the accelerated RRA decrease, renal glomeruli in weanling rats developing DOCA-saline hypertension are exposed to an elevated perfusion pressure in the period of higher vulnerability. A vicious circle mechanism[14] might thus be started more easily and lead to a more pronounced hypertensive response indicated here, which becomes more evident after a prolonged exposure to the DOCA-saline regimen[5].

1 I. Pohlová and J. Jelínek, Pflügers Arch. *351*, 259 (1974).
2 S. Solomon, A. Iaina and H. Eliahou, Proc. Soc. exp. Biol. Med. *153*, 309 (1976).
3 A. Aperia and P. Herin, Am. J. Physiol. *228*, 1319 (1975).
4 L.K. Dahl and E. Schackow, Can. med. Ass. J. *90*, 155 (1964).
5 H. Musilová, J. Jelínek and I. Albrecht, Physiologia bohemoslov. *15*, 525 (1966).
6 F. Gross, H. Brunner and M. Ziegler, Recent Prog. Horm. Res. *21*, 119 (1965).
7 R. Müller-Suur, H.V. Gutschke, K.F. Samwer, W. Oelkers and K. Hierholzer, Pflügers Arch. *359*, 33 (1975).
8 J. Jelínek and V. Krpata, Physiologia bohemoslov. *16*, 182 (1967).
9 L. Tobian, Fedn Proc. *26*, 48 (1967).
10 H.H. Bengele and S. Solomon, Am. J. Physiol. *227*, 364 (1974).
11 K. Thurau, in: Angiotensin, p.480. Ed. I.H. Page and F.M. Bumpus, Springer Verlag, Berlin 1974.
12 J.S. Kaufman, R.J. Hamburger and W. Flamenbaum, Am. J. Physiol. *231*, 744 (1976).
13 W.A.J. Crane and L.P. Dutta, J. Path. Bact. *88*, 291 (1964).
14 F.B. Byrom and L.F. Dodson, Clin. Sci. *8*, 1 (1949).

Effect of sex hormones on plasma cholesterol in castrated and noncastrated male rats[1]

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Summary. The administration of estradiol to both castrated and noncastrated male rats was associated with significantly increased plasma cholesterol levels as compared to controls, the estradiol in the noncastrated rats overriding the tendency of testosterone to lower plasma cholesterol.

The widespread clinical use of estrogen and contraceptive steroid combinations has given rise to many studies on the effect of these substances on plasma lipids. Results in both humans and experimental animals have been conflicting. Early human clinical studies suggested a hypocholesterolemic effect of estrogen[2-5], whereas more recent studies have indicated that the administration of estrogen or estrogen-progestogen combinations results in an increase in plasma cholesterol[6-8]. Experimental animal studies in the chicken and turkey[9,10] indicated a hypercholesterolemic effect of estrogen, whereas studies in the rat have indicated that estrogen decreases plasma cholesterol concentrations[11,12]. A biphasic, dose-related response to estrogen has also been suggested, high doses decreasing and low doses increasing plasma cholesterol levels[13].

Androgens, on the other hand, have in general been found to lower plasma cholesterol in both humans and experimental animals, although there are some conflicting reports[14]. The data for androgens are not so extensive as those for estrogens.

Little has been done in the way of comparison of estrogen effect in the castrated versus noncastrated male animal, and the following is a report on the effect of testosterone and estradiol on plasma cholesterol in the castrated male rat as well as the effect of estradiol on plasma cholesterol in the intact male rat.

Male rats of approximately 5 weeks of age were obtained from Charles River Breeding Laboratories (CD strain) and divided into 5 groups. Rats in 3 of these groups were castrated, using pentobarbital anesthesia (40 mg/kg i.p.), and all rats were maintained on a diet of Purina rat chow. Weekly i.m. injections were initiated the day after operation and were given to all rats for 3 weeks according to the following schedule. In each case the injection volume was 0.1 ml.

Group I. Castrated. Cottonseed oil.
Group II. Castrated. Depotestosterone cypionate (Upjohn) in cottonseed oil, 1 mg.
Group III. Castrated. Depoestradiol cypionate (Upjohn) in cottonseed oil, 10 µg.
Group IV. Noncastrated. Cottonseed oil.
Group V. Noncastrated. Depoestradiol cypionate (Upjohn) in cottonseed oil, 10 µg.

After 3 weeks of treatment rats were killed instantaneously by cervical dislocation. Blood was drawn immediately from the heart into tubes containing EDTA and plasma was separated by centrifugation and was frozen for storage. Plasma total cholesterol levels were subsequently determined by the method of Pearson et al.[15] as used by Kritchevsky et al.[16].

The mean plasma total cholesterol levels for the 5 groups are given in the table. In both castrated and noncastrated male rats the administration of estradiol was associated

Total cholesterol concentrations in plasma of hormone treated rats

Group	Manipulation and therapy	Number of animals	Total cholesterol mg/dl plasma (Mean + SE)
I	Castrated, cottonseed oil	19	93.8 ± 2.7*
II	Castrated, testosterone	19	89.4 ± 4.1
III	Castrated, estradiol	17	117.0 ± 3.1*
IV	Noncastrated, cottonseed oil	14	92.1 ± 3.5**
V	Noncastrated, estradiol	13	113.1 ± 4.5**

* $p < 0.0005$, ** $p < 0.005$.

with a significantly increased plasma cholesterol ($p < 0.0005$ in the castrated rats and $p < 0.005$ in the noncastrated rats). The administration of testosterone to castrated male rats resulted in lower levels of plasma cholesterol, but the difference was not significant.

Our results are consistent with other recent reports in other experimental animals. Kudzma et al.[9] administered diethylstilbesterol to 5-day-old chicks of undetermined sex and found an increase in plasma cholesterol. Neilson and Simpson[10] administered a single dose of diethylstilbesterol to male noncastrated turkeys and found an increase in plasma cholesterol. Our results conflict with those of Fewster et al. who found that estradiol decreased plasma cholesterol[12]; however their dosage was 1.7 mg estradiol benzoate daily, and the decrease they found with this high dose would be consistent with the biphasic effect described by Uchida et al.[13].

The finding that estrogen was associated with a rise in plasma cholesterol in the presence as well as the absence of naturally occurring testosterone indicates that the estrogen effect was overriding in comparison to the effects of testosterone on plasma cholesterol. These effects of estrogen on plasma cholesterol in male rats would appear to be contrary to the proposed protective role of estrogen in the development of atherosclerosis. In this connection a recent study by Phillips[17] revealed a significantly increased plasma estrogen level in a sample of 15 men who had had a myocardial infarction between the ages of 32 and 42 as compared to control subjects. The findings of our study as well as those of others indicate that the role of sex hormones in cardiovascular disease is a very complex one and that probably there are many factors other than lipid metabolism which play a role in the protective effect of estrogen. Thus the mechanism by which estrogen purportedly protects the female remains obscure.

The study reported herein was done in conjunction with a long-term study in our laboratory on the effect of sex hormones on vascular connective tissue[18]. This long-term study, as well as an earlier one[19], indicates that estrogen decreases collagen and elastin accumulation in rat aorta. Since collagen and elastin are increased in atherosclerotic lesions, the effect of estrogen to decrease their accumulation could be hypothesized to be 1 factor contributing to the protective role of estrogen in the female.

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2 J. Marmorston, O. Magidson, J.J. Lewis, J. Mehl, F.J. Moore and J. Bernstein, N. Engl. J. Med. *258*, 583 (1958).
3 M.F. Oliver and G.S. Boyd, in: Hormones and atherosclerosis, p.403. Ed. G. Pincus. Academic Press, New York 1959.
4 H.A. Eder, in: Hormones and atherosclerosis, p.335. Ed. G. Pincus. Academic Press, New York 1959.
5 R.W. Robinson and R.J. Lebeau, J. Atheroscl. Res. *5*, 120 (1965).
6 V. Wynn, J.W.H. Doar, G.L. Mills and T. Stokes, Lancet *2*, 756 (1969).
7 S. Rossner, U. Larsson-Cohen, L.A. Carlson and J. Boberg, Acta med. scand. *190*, 301 (1971).
8 M.E. Molitch, P. Oill and W.D. Odell, J. Am. med. Ass. *227*, 522 (1974).
9 D.J. Kudzma, P.M. Hegstad and R.E. Stoll, Metabolism *22*, 423 (1973).
10 J.T. McL. Neilson and C.F. Simpson, Atherosclerosis *18*, 445 (1973).
11 R.B. Alfin-Slater and L. Aftergood, Lipids *6*, 693 (1971).
12 M.E. Fewster, R.E. Pirrie and D.A. Turner, Endocrinology *80*, 263 (1967).
13 K. Uchida, M. Kadowaki, K. Miyata and T. Miyake, Endocr. jap. *16*, 211 (1969).
14 A. Solyom, Lipids *7*, 100 (1972).
15 S. Pearson, S. Stern and T.H. McGavack, Analyt. Chem. *25*, 813 (1953).
16 D. Kritchevsky, S.A. Tepper, H.K. Kim, D.E. Moses and J.A. Story, Exp. molec. Path. *22*, 11 (1975).
17 G.B. Phillips, Lancet *2*, 14 (1976).
18 G.M. Fischer and M.L. Swain, Am. J. Phys.; Heart Circulation Physiol. *1*, H617 (1977).
19 G.M. Fischer, Endocrinology *91*, 1227 (1972).

Gastrin stimulated H^+ secretion in amphibian gastric mucosa: Effect of tetrodotoxin[1]

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Summary. The role of neural mechanisms in gastrin stimulated H^+ secretion was studied using amphibian gastric fundic mucosa. Spontaneously secreting mucosae were converted to resting state (zero H^+ secretory rate) using Burimamide. Following removal of burimamide, 3×10^{-6} M tetrodotoxin did not block gastrin stimulation of H^+ secretion indicating that neural mechanisms are not required.

A strong interrelationship between neural and hormonal control of gastrointestinal secretory and motor function has been suggested by in vivo and in vitro experiments. In intestinal muscle tetrodotoxin blocks the effect of gastrin on acetylcholine release and motor activity[2] suggesting a similar mechanism may exist for secretory stimulation by gastrin. This paper reports studies into the requirement of neural mediation for gastrin stimulation of gastric H^+ secretion. Tetrodotoxin, a compound which blocks neural Na^+ channels, and the non-secreting bullfrog mucosa form the model system for this investigation.

Materials and methods. All chemicals used in preparation of solutions were reagent grade, Mallinkrodt Chemical Co.; pentagastrin was a gift from Ayerst Pharmaceutical Co., and tetrodotoxin was purchased from Sigma Chemical Co. Potency of the tetrodotoxin was confirmed by lethality in rat following i.p. injection.

Adult bullfrogs were killed by severing the spinal cord in the neck and pithing. The abdomen was quickly opened and the gastric fundus removed. It was placed in amphibian Ringer's solution bubbled with 95% O_2/5% CO_2, and the outer muscle layer was stripped from the underlying mucosa. The mucosal tube was opened, stretched, and mounted between the halves of a lucite chamber. The composition of solutions bathing the mucosal and serosal surfaces have been reported previously[3]. The solutions were