Female Sex Hormones: Effect on the Kinetics of Cholesterol Metabolism in Rabbits[1] (37419)

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It has long been proposed that female sex hormones protect premenopausal women from developing coronary heart disease (1–3). In our previous attempt to investigate the possible mechanisms by which female sex hormones lessen the vulnerability of women to coronary heart disease, we failed to demonstrate, in rabbits, a substantial change of aortic acid mucopolysaccharides under the influence of such hormones which could account for the slower process of atherosclerosis in females (4). We did however find a definite hypocholesteremic effect of estrogen in these rabbits (4).

The hypocholesteremic effect of estrogen has been shown to be the result of shifting of serum cholesterol to tissues (5), change in serum lipoprotein patterns (6, 7), and suppression of endogenous cholesterol synthesis (8–10), but the exact mechanism is still not certain because the results are quite controversial (11–15).

The present study was designed to investigate the influence of female sex hormones on the kinetics of cholesterol metabolism in rabbits in order to better understand the mechanism of this hormonal action.

Materials and Methods. Experimental design. Forty-one adult, female New Zealand white rabbits, weighing an average of 2,805 g, were housed in individual cages and fed purina rabbit pellet *ad libitum* throughout the experiment; it contained a negligible amount of cholesterol (0.06 mg/g of chow) (16). Bilateral oophorectomy was performed under ether anesthesia in 32 of these rabbits. The remaining 9 rabbits served as normal intact female controls. Two weeks after the surgery the castrated rabbits were divided into four equal groups: A, B, C, and D (8 in each group). Group A rabbits received no sex hormone treatment. Each rabbit in Group B was given a daily injection of 2 mg of progesterone (17α-ethynyl-17-hydroxy-5(10)-estren-3-one or norethynodrel) subcutaneously. A daily dose of 0.2 mg of estrogen (17-ethynlestradiol-3-methyl-ether or mestranol) was given subcutaneously to each of 8 rabbits in Group C. The rabbits in Group D were treated alternately with a daily dose of 0.2 mg of estrogen for one week and a daily dose of 2 mg of progesterone for another week. The nine noncastrated rabbits (Group E), received no hormone treatment. The above regimen was continued throughout the rest of the experiment.

Six weeks after the commencement of the hormonal treatment, rabbits in all groups including the controls received a single dose of 25 μCi of cholesterol-7α-^{3}H intravenously. The cholesterol-7α-^{3}H solution for intravenous injection was prepared according to the method described by Chobanian and Hollander (17). Blood specimens were obtained from the central arteries of the rabbits' ears once a week throughout the entire experimental period and every other day during the first two weeks after the injection of cholesterol-7α-^{3}H. All animals were sacrificed between 90 and 100 days after the administration of cholesterol-7α-^{3}H.

Chemical and mathematical analyses. Serum cholesterol levels were determined by the Sperry–Webb method (18) and their radioactivities were measured by liquid scintillation counter techniques.

Compartmental analysis of the disappear-

[1] Supported by Public Health Service Grant HL-13612-03 from the National Heart and Lung Institute.

ance curve of serum cholesterol specific activity (SA) was carried out by a computerized program based on the combination of the kinetic analysis reported by Gurpide *et al.* (19), and the input–output analysis shown by Perl and Samuel (20). The curves of all cases uniformly fit a two-pool system with the following general equation:

$$SA_{\text{serum}(t)} = A_1 e^{-at} + A_2 e^{-\beta t}$$

Here $SA_{\text{serum}(t)}$ is the SA of serum cholesterol at time t. A_1, A_2, a, β are constants, and e is the base of the natural logarithms. The following kinetic parameters could be obtained by this program: total input (I_T), output, or turnover rate of cholesterol in the system, mean transit time of cholesterol ($\bar{t}$), total traced mass (M_T) and individual pool size (M_A and M_B) and exchange rate between compartments A and B ($K_{AB}M_A$, $K_{BA}M_B$).

Results. Changes in the body weights. The average body weight of the rabbits at the beginning of the experiment was 2805 ± 403 g (Mean ± SD). They were considered as full-grown adult rabbits. However, their body weights still kept increasing during 22 weeks of the experimental period. The body weights of Groups A, B, C, D, and E rabbits at the end of the experiment were 3636 ± 510 (Mean ± SD), 3731 ± 320, 3431 ± 379, 3719 ± 369 and 3167 ± 260 g, respectively. All castrated rabbits gained more weight during the experimental period than the normal intact rabbits ($p < 0.01$). Although the mean body weight of the castrated rabbits treated with estrogen was lower than that of the other castrated rabbits; statistically the difference was not significant. On the average, each of the castrated rabbits gained 5–6 g per day, while each normal intact rabbit gained only 3 g/day.

Changes in serum cholesterol levels. The mean serum cholesterol level of all rabbits before any special treatment was given was 49 ± 17 mg/100 ml (Mean ± SD). The serum cholesterol levels of the normal intact rabbits stayed in this normal range throughout the experiment (Fig. 1).

After castration, the serum cholesterol levels increased rapidly reaching the level of 88 ± 36 mg/100 ml (Mean ±SD), and 92 ± 39 mg/100 ml at the end of the first and second week, respectively. The cholesterol levels of the castrated rabbits without supplementary hormones remained at this high level (95 ± 40 mg/100 ml) throughout the experiment (Fig. 1).

Treatment of the castrated rabbits with progesterone alone did not bring down the elevated serum cholesterol level to the pre-castrated range. Their mean serum cholesterol level (91 ± 33 mg/100 ml) was not different from that of the non-hormone-treated castrated rabbits ($p > 0.1$).

The serum cholesterol levels of Groups C and D at any time were essentially the same (68 ± 23, and 72 ± 30 mg/100 ml, respectively). The elevated serum cholesterol levels

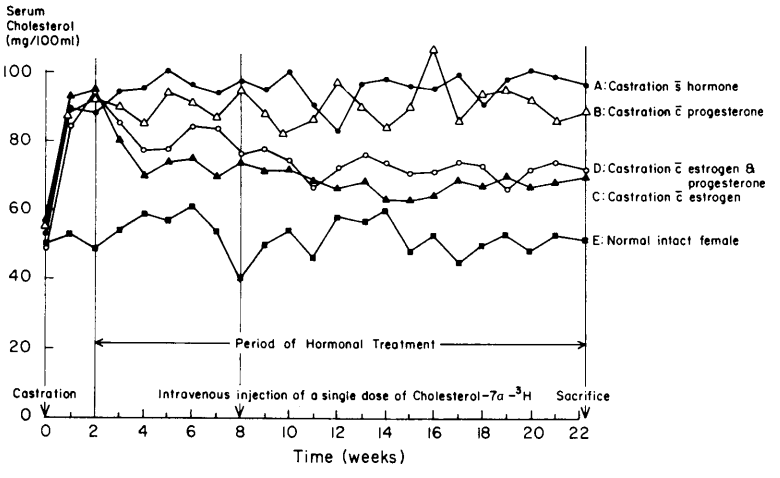

FIG. 1. Changes of serum cholesterol levels during 22 weeks of experiment.

after castration, declined rapidly after estrogen administration. The levels fell toward the precastrated value, but were still significantly higher than the latter ($p < 0.01$).

Kinetic analysis of cholesterol metabolism. The specific activity of serum cholesterol of all rabbits declined rapidly during the first week after intravenous administration of cholesterol-7α-^{3}H; the curves then became less steep and finally reached a straight line when plotted in a semilogarithmic scale. Compartmental analysis revealed that these curves best fit a general equation for a two-pool system:

$$SA_{\text{serum}(t)} = A_1 e^{-\alpha t} + A_2 e^{-\beta t}$$

The mean equation for each of the five groups of rabbits is shown in Table I. The mean values of the various kinetic parameters of such a two-compartment model are also included in the table.

The time constants for the first and second exponential functions of these equations, $1/\alpha$ and $1/\beta$, were significantly greater in castrated rabbits treated with no hormone or progesterone alone than in the normal intact female rabbits. It is also true for the mean transit time, $\bar{t}$, of the tracer cholesterol. Treatment of the castrated rabbits with estrogen shortened all these time constants which was no longer different from those of the intact animals. Weekly alternation of estrogen and progesterone resulted in values that were lower than those of castrated rabbits without hormone but higher than those of normal rabbits.

The rate constants of the input of cholesterol into compartment B from outside the system and of the output from compartment B to outside the system were very small and negligible, and were omitted from the table. The rate constants for interexchange of cholesterol between two compartments, K_{AB} and K_{BA}, showed no significant statistical difference among all groups except for lower values observed in the castrated rabbits treated with progesterone. On the contrary, a distinct difference was found in the rate constants of output of cholesterol from compartment A to outside the system, K_A; lower in castrated rabbits treated with no hormone or progesterone alone, and higher in the other three groups.

A uniform statistical relationship existed among these groups of rabbits as to the sizes of compartment A, B, and total pool (M_A, M_B, and M_T), and the production rate of compartment A: (a) these values were lowest in the normal rabbits, (b) castrated rabbits treated with no hormone or progesterone alone gave similar values to one another which were higher than those of the other three groups except for the PR_A of castrated rabbits treated with both hormones, (c) treatment with estrogen alone or alternating with progesterone lowered these values which, however, were still higher than those of the normal rabbits.

Discussion. Steady state of equilibrium. A steady state of equilibrium of body cholesterol should be attained and maintained throughout the period of kinetic study of cholesterol metabolism in order to obtain precise, valid results. This is the reason why cholesterol-7α-^{3}H was given only six weeks after the commencement of hormonal therapy which was presumably the period required to obtain a new steady state of equilibrium under the controlled hormonal influences The rather stable serum cholesterol levels in each group of rabbits throughout the experimental period provided good evidence of existence of such steady states of equilibrium.

In spite of the stability of serum cholesterol levels, however, the body weights of these rabbits increased gradually during the period of study, indicating a slow expansion of body cholesterol pool, for the amount of cholesterol in the body increased with the increase of tissue mass. Therefore, the body cholesterol of these rabbits was not exactly in a steady state. The slow increase of body weight might introduce some errors in the measurements of the kinetic parameters. For the purposes of intergroup comparison, the possible errors were negligible since the same errors might be introduced to a similar extent in all groups.

Effects of castration. After bilateral oophorectomy, the serum cholesterol levels almost doubled the precastration levels within two weeks (Fig. 1). A similar phenomenon has been observed in humans (3). The data ob-

TABLE I. Least Square Equations for Changes of Serum Cholesterol Specific Activity and Various Kinetic Parameters of Cholesterol Metabolism in Five Groups of Rabbits.[a]

Group	No.	Least square equation for changes of serum cholesterol specific activity	$\frac{1}{\alpha}$[c]	$\frac{1}{\beta}$[c]	$\bar{t}$ Day[f]	K_{AB}[g]	K_{BA}[g]	K_A[g]	M_A[h] mg/rabbit (mg/kg)	M_B[h]	M_T[h]	PR_A[i] mg/day (mg/kg/day)
A. Castration without hormone	8	$60{,}995e^{-0.465t} + 9732e^{-0.01871t}$	$2.15 \pm 0.21^{b,c}$	53.6 ± 4.6^{c}	43.0 ± 5.6^{c}	0.331 ± 0.063	0.085 ± 0.063	0.116 ± 0.021^{c}	797 ± 181^{c} (218 ± 31)	3040 ± 446^{c} (841 ± 100)	3837 ± 606^{c} (1059 ± 119)	89.2 ± 10.0^{c} (24.9 ± 4.3)
B. Castration with progesterone	8	$60{,}046e^{-0.380t} + 9180e^{-0.0193t}$	2.63 ± 0.55^{c}	51.9 ± 6.4^{c}	40.6 ± 6.1^{c}	0.231 ± 0.049^{c}	0.071 ± 0.010^{c}	0.111 ± 0.030^{c}	861 ± 271^{c} (229 ± 63)	2725 ± 686^{c} (727 ± 148)	3587 ± 832^{c} (956 ± 170)	88.8 ± 8.5^{c} (23.8 ± 1.4)
C. Castration with estrogen	8	$64{,}804e^{-0.650t} + 13{,}400e^{-0.0201t}$	1.54 ± 0.17^{d}	38.3 ± 5.6^{d}	31.6 ± 4.3^{d}	0.450 ± 0.243	0.098 ± 0.023	0.171 ± 0.033^{d}	$419 \pm 37^{c,d}$ (123 ± 17)	$1820 \pm 376^{c,d}$ (531 ± 89)	$2239 \pm 358^{c,d}$ (654 ± 86)	$71.1 \pm 9.0^{c,d}$ (20.8 ± 2.5)
D. Castration with estrogen and progesterone	8	$75{,}462e^{-0.241t} + 11{,}329e^{-0.0217t}$	$1.77 \pm 0.29^{c,d}$	46.1 ± 9.9	34.2 ± 4.5^{d}	0.547 ± 0.252	0.106 ± 0.030	0.177 ± 0.046^{d}	$515 \pm 130^{c,d}$ (140 ± 42)	$2429 \pm 214^{c,d}$ (656 ± 58)	$2944 \pm 283^{c,d}$ (796 ± 89)	86.9 ± 10.6^{c} (23.6 ± 3.8)
E. Normal intact female rabbits	9	$54{,}635e^{-0.714t} + 10{,}544e^{-0.0204t}$	1.40 ± 0.32	37.5 ± 5.4	30.5 ± 3.5	0.466 ± 0.185	0.115 ± 0.039	0.169 ± 0.035	323 ± 80 (101 ± 20)	1270 ± 194 (401 ± 61)	1593 ± 201 (502 ± 56)	52.7 ± 8.0 (16.7 ± 2.4)

[a] Mean of the group.

[b] Mean $\pm$ SD.

[c] Significant difference as compared with normal intact rabbits (Group E), $p < 0.01$.

[d] Significant difference as compared with castrated rabbits without hormonal treatment (Group A), $p < 0.01$.

[e] $\frac{1}{\alpha}$, $\frac{1}{\beta}$: Time constants of first and second exponential function, respectively.

[f] $\bar{t}$: Mean transit time of cholesterol.

[g] K_{AB}, K_{BA}, K_A: Rate constants for transport of cholesterol from Pool A to B; B to A and from Pool A to outside the system, respectively.

[h] M_A, M_B, M_T: Sizes of rapidly, slowly and total exchangeable pools respectively.

[i] PR_A: Production rate or turnover rate of rapidly exchangeable pool.

tained from kinetic studies revealed that, in addition to the elevation of serum cholesterol levels, the sizes of pools A and B also expanded to 2.5 and 2.4 times that of the normal control rabbits, respectively (Table I).

The possible reasons for such increase of body cholesterol were either increase of its input or decrease of its output or both. The increase of the production rate of pool A to 1.7 times the normal control indicated an increase of input of cholesterol from outside the system, rather than a decrease of output from the system. There were two sources of cholesterol input into compartment A from outside the system; one was dietary cholesterol absorbed by intestine, the other was the newly synthesized cholesterol in compartment A. Since the rabbits were fed a diet containing a very small amount of cholesterol, the production rate of compartment A could be considered as the amount of cholesterol synthesized daily in this compartment.

In the steady state, PR_A may also represent the output or excretion of cholesterol in compartment A to outside the system. Therefore, the cholesterol excretion was also enhanced in the castrated rabbits. The mean transit time of the tracer cholesterol, however, was 1.4 times that of normal controls. The prolonged transit time resulted in a relatively greater expansion of compartments A and B as compared with the increase of PR_A.

In short, removal of ovaries and hence the ovarian hormones, apparently removed the inhibitory effect on endogenous cholesterol synthesis in compartment A and consequently stimulated its synthesis. The excretory mechanism was also proportionally enhanced to meet the increase of synthesis. However, the mean transit time of the tracer cholesterol was prolonged after castration, resulting in the expansion of both compartments A and B in the same proportions. Elevation of serum cholesterol levels in such animals was the reflection of expansion of the body cholesterol pool.

Effects of progesterone alone. The treatment of castrated rabbits with daily administration of progesterone did not alter any kinetic parameters or serum cholesterol levels in these animals. They were essentially the same as those of the castrated rabbits with no hormonal treatment.

Effects of estrogen alone. The elevated postcastration serum cholesterol levels were remarkably reduced by administration of estrogen for two weeks. Although their serum cholesterol levels, thereafter, were still above those of the normal control rabbits, this demonstrated a definite hypocholesteremic effect of estrogen in the castrated rabbits.

The kinetic study showed the value of PR_A in these animals fell between that of normal controls (135%) and the castrated rabbits with no hormonal treatment (80%). In other words, the endogenous cholesterol synthetic activity in estrogen-treated rabbits was only 80% of that of the castrated rabbits with no hormonal treatment, representing a 20% reduction, or suppression. However, such synthetic activity was still higher than that of the normal rabbits.

Another remarkable effect of estrogen on the cholesterol metabolism to be emphasized was the normalization of the mean transit time of the tracer cholesterol, which was prolonged after castration. The shortening or normalization of mean transit time prevented further expansion of the body cholesterol pool. As a matter of fact, compartments A and B increased in the same proportion as PR_A in these rabbits (1.3–1.4 times normal).

The reason for the absence of total normalization of PR_A in the estrogen-treated, castrated rabbits was not quite certain. One possible explanation was the insufficient dose of estrogen given, another explanation was that the effects of mestranol might not be exactly the same as the natural occurring estrogens in rabbits.

Effects of cyclic alternation of estrogen and progesterone. The changes of the serum cholesterol levels of the castrated rabbits treated with weekly alternation of estrogen and progresterone followed the same pattern as those treated with estrogen alone. Although the mean values of all the kinetic parameters were higher in the former than in the latter, no statistically significant differences were observed except for the sizes of compartment B and the total pool.

Since administration of progesterone alone was shown to have no effect on cholesterol metabolism, the changes of various kinetic

parameters in these rabbits treated with both hormones were apparently due to pure estrogenic influence and indeed followed a pattern similar to those treated with estrogen alone. The higher absolute values of the kinetic parameters observed in these animals were conceivably the result of smaller amounts of estrogen administered (daily for every other week instead of daily throughout the study). The slowly exchangeable pool or compartment B seemed to be more vulnerable than compartment A to expansion when the dose of estrogen given was insufficient.

Conclusions. There were two primary effects of castration on cholesterol metabolism in female rabbits: (a) removal of the inhibitory effect of estrogen on cholesterol biosynthesis, and (b) prolongation of the mean transit time of the tracer cholesterol. As a consequence of the first effect, endogenous cholesterol synthesis was increased. A proportional increase of its excretion compensated for such overproduction and maintained a steady state of cholesterol in the body. As a consequence of the second effect, a new steady state of equilibrium was attained in which both slowly and rapidly exchangeable pools were expanded including an elevated serum cholesterol level.

Administration of estrogen alone to the castrated rabbits suppressed the stimulated cholesterol biosynthesis and shortened the prolonged mean transit time of the tracer cholesterol to normal, whereas the administration of progesterone showed no such effects.

Summary. Thirty-two castrated adult female rabbits were divided equally into four groups treated with no hormone, progesterone alone, estrogen alone, and weekly alternation of estrogen and progesterone, respectively. An additional nine intact female rabbits served as normal controls; 25 μCi of cholesterol-7α-^{3}H was given to each rabbit intravenously six weeks after the commencement of hormonal treatment. The subsequent disappearance curves of serum cholesterol specific activity were analyzed and various kinetic parameters were obtained.

The results indicated that castration removed the inhibitory effect on cholesterol biosynthesis and prolonged its mean transit time with consequent expansion of body exchangeable cholesterol pool size. Such effects could be reversed by administration of estrogen alone or with progesterone but not by administration of progesterone alone.

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Received Mar. 14, 1973. P.S.E.B.M., 1973, Vol. 143.