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Cholesterol and Hormone Levels in Sera of Juvenile Female Rhesus Monkeys[1] (40027)

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Many investigators have reported that estrogens and androgens may affect blood and tissue lipids (1, 2). Estrogen administration has been shown to increase serum high density (α) lipoproteins and decrease low density (β) lipoproteins (3, 4). Barr *et al.* (5) first noted the inverse relationship between the ratio of α/β lipoproteins and susceptibility to coronary heart disease. This relationship has been given considerable publicity as newer means of measuring the α/β lipoprotein ratio have become available (6–8).

Aitken *et al.* (9) reported an increase in serum cholesterol levels following oophorectomy, and Mann and Thorogood (10) have suggested that ovarian hormones may protect premenopausal women from Type IV hyperlipoproteinemia.

Many of the data on the effect of estrogens and androgens on serum lipids and lipoproteins concern middle-aged men and women; little is known about these effects in early life. Since the rhesus monkey is reported to have a menstrual cycle and hormone levels comparable to those in women (11), the effects of 17β-estradiol and testosterone propionate on the plasma lipid levels of juvenile female monkeys were tested.

Materials and methods. Fifteen 5-month-old prepubertal female rhesus monkeys (*Macaque mulatta*) weighing 1.1 ± 0.4 kg were fed a laboratory monkey ration *ad libitum*. The diet was supplemented with fruit and vitamins. The monkeys were weighed weekly and their stage of sexual development and evidence of menses recorded. Five monkeys were given subscapular implants of Silastic capsules containing 10 mg of 17β-estradiol. Five other monkeys were given similar implants containing 15 mg of testosterone propionate. Capsules were prepared according to the method of Karsch *et al.* (12) and were replaced every month over the course of the study.

Blood samples were taken monthly until menarche, which occurred when the body weight of the monkeys reached 3.4 kg. Many of the initial menses were anovulatory.

Blood was drawn from the femoral vein using heparinized syringes. Plasma estradiol and testosterone levels were measured by radioimmunoassay (RIA), the former by the method of England *et al.* (13) and the latter according to Furuyoma *et al.* (14). Plasma cholesterol and triglyceride levels were determined by standard Autoanalyzer methodology as described by Schwartz and Hill (15). The low (β) and high (α) density lipoproteins were separated by dextran sulfate fractionation (16) and the cholesterol content of the fractions was determined (17). The lipoprotein phospholipid content was determined by the method of Kraml (18). Livers, ovaries, and adrenal glands were weighed when the animals were killed. Statistical differences were computed using Student's *t* test.

Results and discussion. There were no significant differences in weight gain between treated and untreated monkeys (Fig. 1). Cholesterol levels of all groups showed a type of seasonal variation which had been seen previously in monkeys (19); however, cholesterol levels were reduced in both groups of hormone-treated monkeys. The estrogen-treated monkeys exhibited the lowest cholesterol levels throughout (Fig. 1). Phospholipid levels were significantly lower in the two hormone-treated groups and were also consistently lower in the

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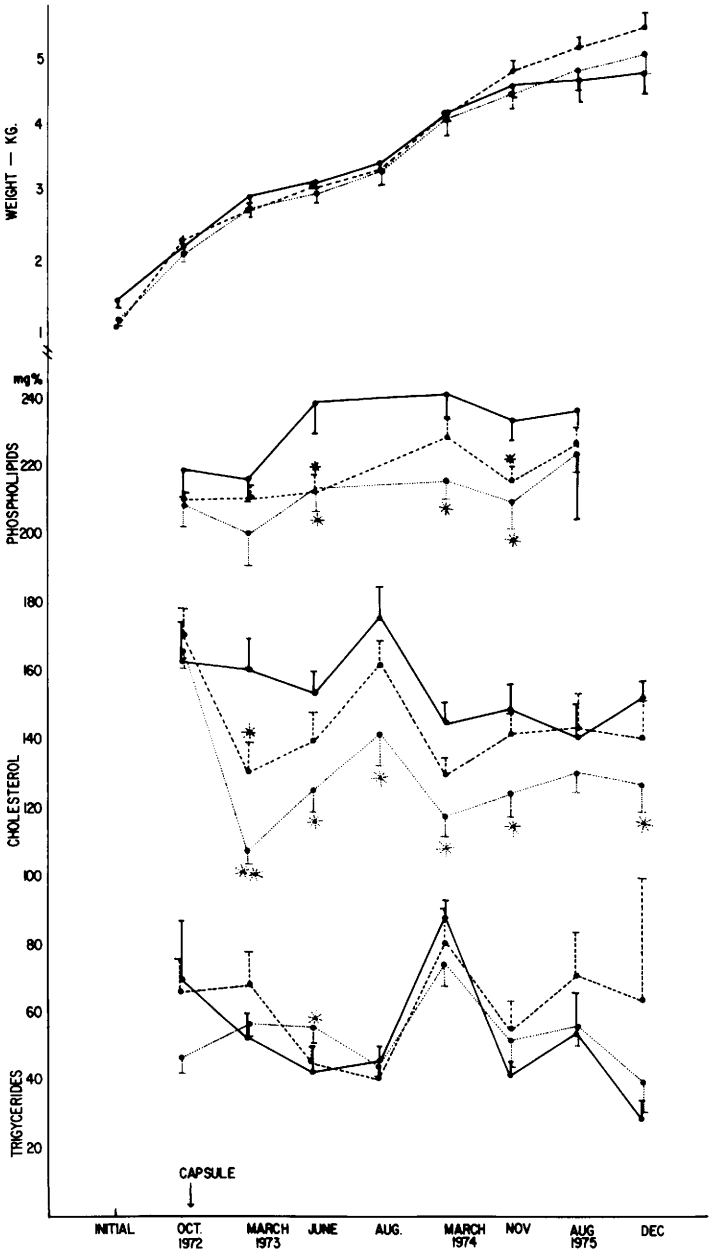

FIG. 1. Changes in body weight and plasma lipids in untreated monkeys (●——●) and in monkeys bearing implants of testosterone proprionate (●- - -●) or 17β-estradiol (○· · · ·○). (*)$P < 0.05$; (**)$P < 0.01$.

estradiol group (Fig. 1). Triglyceride levels were generally comparable for all groups. The plasma cholesterol levels and the α/β lipoprotein cholesterol ratios are shown in Table I. The estradiol group exhibited the lowest levels of plasma cholesterol and of α/β lipoprotein cholesterol ratio.

Implantation of 17β-estradiol or testosterone resulted in significantly elevated levels of the respective hormones (Figs. 2 and 3). Delayed development of sexual characteristics such as sex skin and clitoral hypertrophy were evident in the testosterone-treated monkeys.

TABLE I. TOTAL PLASMA CHOLESTEROL, α LIPOPROTEIN CHOLESTEROL, AND α/β LIPOPROTEIN CHOLESTEROL LEVELS IN MONKEYS AFTER 2 YEARS OF ESTROGEN OR ANDROGEN TREATMENT.

Group	No.	Plasma cholesterol (mg/dl)	α Lipoprotein cholesterol (mg/dl)	α/β Lipoprotein cholesterol ratio
Control	5	152 ± 4.2[a]	57 ± 5.3	0.60 ± 0.07
Testosterone propionate	5	140 ± 11.6	48 ± 2.9	0.55 ± 0.08
17β-Estradiol	5	126 ± 7.9*	40 ± 2.7**	0.49 ± 0.05

[a] Standard error.
* $P < 0.02$ vs control.
** $P < 0.05$ vs control.

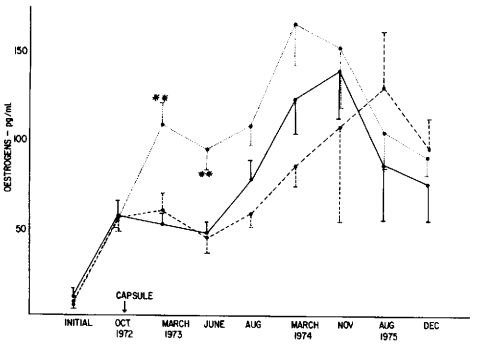

FIG. 2. Plasma estrogen levels in untreated monkeys (●——●) or in monkeys bearing implants of testosterone propronate (●- - - -●) and 17β-estradiol (○· · · ·○) during puberty. (**)$P < 0.01$.

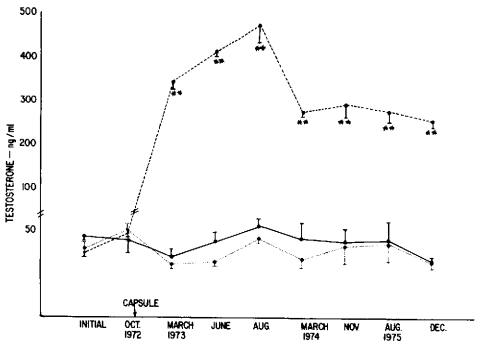

FIG. 3. Plasma testosterone levels in untreated monkeys (●——●) or in monkeys bearing implants of testosterone propionate (●- - - -●) and 17β-estradiol (○· · · ·○) during puberty. (**)$P < 0.01$.

There were no significant changes in the weights of livers, ovaries, or adrenal glands (Table II).

In man, serum cholesterol increases rapidly after birth and then usually increases progressively with age. An increase in serum cholesterol early in life has also been reported for nonhuman primates (20). In man the lipoprotein level and distribution of lipids between the lipoprotein classes appear to be established prior to puberty (21).

Estradiol levels in the rhesus monkey increase during puberty (22), but few data are available on lipoprotein levels in prepubertal monkeys (23). Methyl testosterone and other testosterone analogs have been reported to lower serum lipids in hyperlipidemic individuals (24), although their effect on the α and β lipoproteins may be diet dependent (25). In this study prolonged elevation of testosterone levels did not effect significant lowering of cholesterol levels. Testosterone propionate treatment did not influence weight gain, although marked signs of virilization were evident.

The lower cholesterol levels observed in monkeys bearing estradiol implants may reflect the effects of raising plasma estrogen levels before puberty to the level found in early follicular and late luteal phases of the menstrual cycle. Estrogen has been reported to transfer cholesterol from serum to liver (26) and to alter catabolism of the very low density lipoproteins (27) and the α lipoproteins (28). Plasma lipids are elevated in oophorectomized women (9) and the risk of coronary heart disease in women increases after menopause (29). The higher levels of plasma estradiol in African girls, as compared to their Caucasian counterparts, has been correlated with a lower risk of coronary heart disease (30).

It would be of interest to determine to what degree serum hormone levels in prepubertal rhesus monkeys can be modified by diet and other environmental factors.

Summary. Juvenile female rhesus monkeys were treated with either 17β-estradiol or testosterone propionate (by implanta-

TABLE II. ORGAN WEIGHTS IN FEMALE JUVENILE RHESUS MONKEYS (g).

	Weight (kg)	Adrenal glands	Ovaries	Liver	Liver as % body wt
Untreated control (5)[a]	4.82 ± 0.29	1.00 ± 0.12	0.35 ± 0.07	91.6 ± 2.3	1.90
17β-Estradiol capsule (5)	5.08 ± 0.26	1.05 ± 0.07	0.42 ± 0.09	89.3 ± 7.5	1.76
Testosterone propionate capsule (5)	5.23 ± 0.22	0.93 ± 0.11	0.39 ± 0.06	99.2 ± 12.7	1.90

[a] Number of animals given in parentheses.

tion) and the hormonal levels were kept elevated to puberty. There was no effect on weight gain in either group. Estradiol lowered plasma cholesterol and phospholipid levels significantly. Neither treatment had a significant effect on plasma α/β lipoprotein cholesterol ratios or on the weights of liver, ovaries, or adrenals.

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