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Methyltestosterone Effect on Serum Cholesterol Radioactivity in Dogs Given C^{14}-Acetate.* (28492)

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The administration of androgens to healthy subjects with normal serum lipid levels diminishes the concentration of α lipoproteins (high density lipoproteins, $d>1.063$ g/ml) and usually increases the concentration of β lipoproteins ($1.019<d<1.063$ g/ml)(1,2). Because the concentrations of these two major lipoproteins change reciprocally, little change in serum cholesterol levels may be noted when androgens are given, although serum phospholipid levels diminish because of the high phospholipid content of α lipoproteins (3).

When increased serum levels of lower density ($d<1.019$ g/ml) lipoproteins are present, resulting in elevated cholesterol, phospholipid and triglyceride concentrations, the intramuscular administration of androsterone (4,5) or oral administration of C-17 alkylated derivatives, *e.g.*, methyltestosterone, may cause remarkable reduction in the concentration of these lipids(6,7).

In the dog, serum lipids are present mainly as high density lipoproteins, *i.e.*, approximately 70% of the serum cholesterol and 80% of the phospholipid can be accounted for in $\alpha(d>1.063$ g/ml) lipoprotein. Intramuscular testosterone propionate or oral methyltestosterone administration to the dog lowers the concentration of both α and lower density lipoproteins(8) and thus serum cholesterol, phospholipid and, to a lesser extent, triglyceride levels(8,9,10,11). Recently Mosbach, Abell and Kendall(10,12) reported that methyltestosterone is hypocholesterol-

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emic in rats and Raiford and Wong noted a similar effect in chickens(13).

The mechanism whereby gonadal steroids modify lipid metabolism and transport is of considerable interest and importance in respect to atherogenesis. This paper reports the influence of methyltestosterone on the radioactivity incorporated into serum cholesterol in dogs following sodium acetate-1-C^{14} administration.

Methods and materials. Four adult male mongrel dogs previously treated with vermifuge and observed for several weeks were utilized. Each weighed approximately 25 kg (except #83 whose weight was 16 kg) and was fed a diet containing 6 parts commercial dog food and 2 parts canned horse meat. This mixture was approximately 24% protein, 6% fat and 27% carbohydrate. The animals had free access to water. Intake of food was constant throughout the study and the animals remained healthy except that dog #82 gradually lost 4 kg during the 11 weeks of study.

After a 2-month control period during which serum lipids and body weight were stable, each animal received, while fasting, 500 μc of sodium acetate-1-C^{14} (approximately 8.2 mg) intravenously in 1 ml of 0.9% saline. Thereafter, blood samples were obtained at 1½, 6 and 24 hours while the dogs were kept fasting. Administration of methyltestosterone, 200 mg daily, 6 days per week, was then begun. After 2 or 3 months, by which time serum lipids had stabilized at new low levels, blood was obtained for background count, and sodium acetate-1-C^{14} injection repeated.

The digitonides of free and ester cholesterol were prepared by an adaptation for small scale work of the method of Rosenfeld *et al.*(14). After isolation and washing, the digitonides were split with boiling benzene according to the method of Kelsey(15) and ultimately were obtained in petroleum ether solution. Separate aliquots of these solutions were taken for cholesterol determination and radioactivity assay by liquid scintillation counting. Cholesterol was determined by the Sperry and Webb modification (16) of the Schoenheimer and Sperry method (17), lipid phosphorus by the wet digestion procedure of Youngburg and Youngburg(18), with phosphate measurement by the method of Fiske and Subbarow(19), and triglycerides by the method of Van Handel and Zilversmit(20). The factor 25 has been used to convert lipid phosphorus to "phospholipids." Ultracentrifugal separation of serum lipoproteins was carried out in a solvent of density 1.063 g/ml at 105,000 $\times$ g for 24 hours, utilizing a modification(21) of the method of Havel, Eder and Bragdon(22). Lipoproteins of $d>1.063$ g/ml are referred to as "α lipoproteins," those of $d<1.063$ g/ml as "β lipoproteins." (The $d<1.063$ g/ml fraction includes β and lower density lipoproteins and chylomicrons).

Results. Serum lipid levels before and during methyltestosterone administration are listed in Table I. Transitional values observed during the first few weeks of treatment are excluded. Serum total and free cholesterol and phospholipids fell significantly during methyltestosterone administration in each animal. Triglyceride levels also diminished. The degree of esterification of cholesterol remained essentially unchanged. The decrease in cholesterol was relatively greater than that in phospholipid, as evidenced by diminished serum C/P values.

Decreases in serum cholesterol and phospholipid values were the result of losses of these lipids from both α and β lipoprotein fractions (Table II). The loss was usually relatively greater from the α than from the β lipoprotein fraction. The C/P ratios diminished in both α and β fractions.

Cholesterol specific radioactivity prior to and during methyltestosterone administration is plotted for each dog in Fig. 1. The specific radioactivity of serum free cholesterol was depressed by methyltestosterone in all animals. Cholesterol ester specific radioactivity, on the other hand, was unaltered by methyltestosterone in 2 animals (dogs 74 and 80), increased in one (dog 82) and diminished in the other (dog 83).

Mean values for free and ester cholesterol total radioactivity per 100 ml of serum in

TABLE I. Serum Lipids During Methyltestosterone Administration; Mean Values ± Standard Error.

Serum lipids, mg/100 ml

Dog No.		Cholesterol Total	Cholesterol Free	Phospholipid	C/P	Triglyceride
74	Control	166 ± 3 (18)	37 ± 2 (10)	358 ± 5 (18)	.43	52 ± 13 (3)
	Methyltestosterone	73 ± 3 (9)	20 ± 1 (7)	201 ± 4 (9)	.36	32 ± .1 (2)
	Decrease:	93 (p <.001)	17 (p <.001)	157 (p <.001)		20 (ns)
80	Control	147 ± 2 (14)	34 ± 1 (8)	314 ± 5 (14)	.47	46 ± 3 (3)
	Methyltestosterone	62 ± 3 (9)	14 ± 1 (7)	176 ± 6 (9)	.35	25 ± 1 (2)
	Decrease:	85 (p <.001)	20 (p <.001)	138 (p <.001)		21 (p <.01)
82	Control	106 ± 2 (11)	26 ± 3 (6)	244 ± 6 (11)	.43	36 ± 3 (3)
	Methyltestosterone	18 ± 2 (9)	4 ± .4 (7)	68 ± 6 (9)	.26	8 ± .5 (2)
	Decrease:	88 (p <.001)	22 (p <.001)	176 (p <.001)		28 (p <.01)
83	Control	136 ± 4 (8)	30 ± 3 (6)	312 ± 6 (8)	.44	45 ± 7 (4)
	Methyltestosterone	47 ± 2 (9)	14 ± 1 (7)	128 ± 6 (9)	.37	25 ± 13 (2)
	Decrease:	89 (p <.001)	16 (p <.001)	184 (p <.001)		20 (ns)

Figures in parentheses are numbers of samples; ns = not statistically significant.

TABLE II. Decrease in Lipoprotein Cholesterol and Phospholipid Content During Methyltestosterone Administration; Mean Values ± Standard Error. n = number of samples. Since not all sera were subjected to ultracentrifugation the sums of the lipids in the lipoprotein fractions do not necessarily agree with mean serum values listed in Table I.

Lipoprotein lipid content, mg/100 ml

Dog No.		α (d >1.063 g/ml) lipoprotein Cholesterol	Phospholipid	C/P	β (d <1.063 g/ml) lipoprotein Cholesterol	Phospholipid	C/P	n
74	Control	112 ± 2	273 ± 8	.41	42 ± 5	67 ± 6	.63	3
	Methlytestosterone	52 ± 4	148 ± 9	.35	23 ± 3	45 ± 3	.51	3
	Decrease	60 (p <.001)	125 (p <.001)		19 (p <.05)	22 (p <.05)		
80	Control	112 ± 1	237 ± 12	.47	40 ± 3	62 ± 4	.64	2
	Methyltestosterone	57 ± 6	140 ± 8	.41	18 ± .7	36 ± 2	.50	3
	Decrease	55 (p <.01)	97 (p <.01)		22 (p <.01)	26 (p <.02)		
82	Control	69 ± 5	181 ± 1	.38	27 ± 4	51 ± 6	.53	2
	Methyltestosterone	12 ± 1	39 ± 1	.47	9 ± 1	21 ± 1	.43	3
	Decrease	57 (p <.01)	142 (p <.001)		18 (p <.02)	30 (p <.02)		
83	Control	107 ± 3	227 ± 9	.47	37 ± 4	58 ± 7	.63	3
	Methyltestosterone	31 ± 5	90 ± 7	.35	13 ± 3	25 ± 2	.48	3
	Decrease	76 (p <.001)	137 (p <.01)		24 (p <.01)	33 (p <.01)		

the 4 animals as a group are plotted in Fig. 2. The control curves are characteristic of C^{14}-acetate incorporation, with peak activity in free cholesterol occurring within 1½ hours after acetate injection, followed by a gradual rise in ester cholesterol activity to a level exceeding that of free cholesterol after 5 to 6 hours. The average total radioactivity present in ester cholesterol during methyltestosterone administration was increased in relation to that in free cholesterol and equalled it as early as 1½ hours after C^{14}-acetate injection. Mean free cholesterol activity between 1½ and 6 hours post-injection diminished more slowly during methyltestosterone administration.

Discussion. The decrease in serum lipid (lipoprotein) levels during methyltestosterone administration is consistent with that previously reported by this laboratory utilizing refractometric ultracentrifugation(8) and by Mosbach, Abell and Kendall(9,10,11).

Although liver lipid concentrations were not determined in these animals, the previously reported study(8) indicated that liver cholesterol and phospholipid concentrations do not change in association with the marked reduction in serum lipid levels induced by methyltestosterone. Thus it may be concluded that the cholesterol pool (liver plus plasma) diminished in size during methyltestosterone administration.

The diminished specific activity of free cholesterol and the decrease in cholesterol

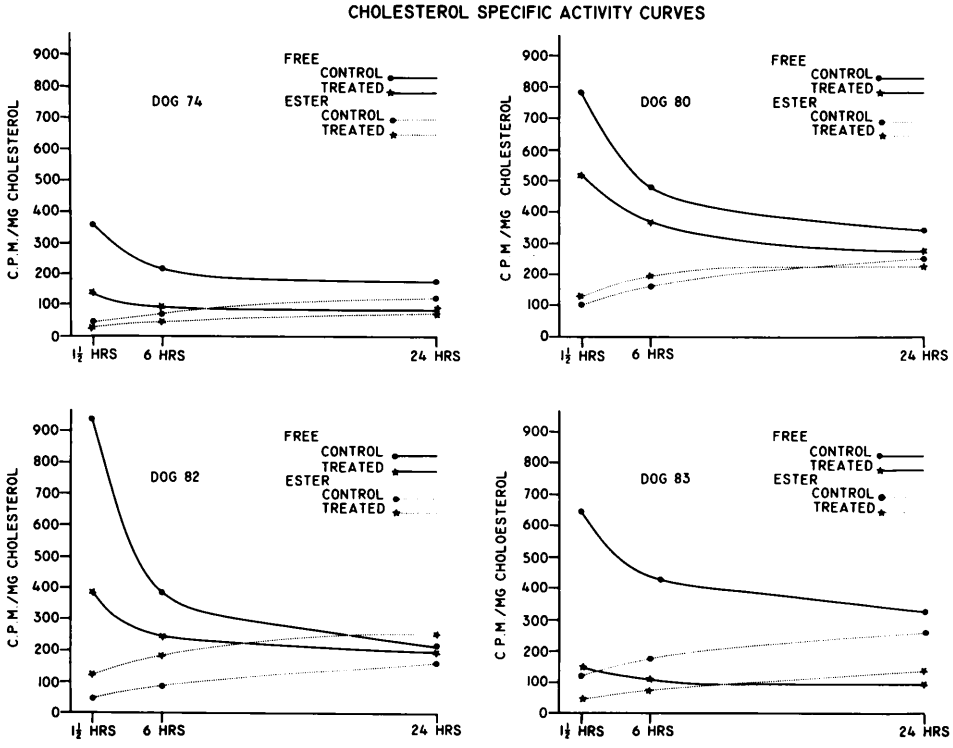

FIG. 1. Cholesterol specific radioactivity, 1½, 6 and 24 hr after C^{14}-acetate injection, before and during methyltestosterone administration.

pool size suggest reduced synthesis of free cholesterol. However, liver cholesterol specific activity data would be required to establish this possibility. The slower rate of decline of plasma free cholesterol radioactivity between 1½ and 6 hours post-injection in the treated animals suggests a diminished uptake of free cholesterol by the liver.

The data suggest the possibility that ester cholesterol is derived from a pool different from that of plasma "free cholesterol," or that newly synthesized cholesterol is preferentially esterified.

Since cholesterol, phospholipid and triglyceride levels were all diminished by methyltestosterone administration, it is necessary in explaining these observations to search for factors, capable of modification by gonadal steroids, which are common to the metabolism or transport of all 3 classes of lipids. The protein anabolic effect of testosterone derivatives may modify the availability of protein for combination with lipid to form lipoprotein, *e.g.*, by increasing amino acid incorporation into muscle protein, thus altering plasma lipid transport. Of relevance in this respect is the observation in human subjects that dietary protein deprivation without caloric restriction diminishes serum lipoprotein levels and modifies the lipoprotein re-

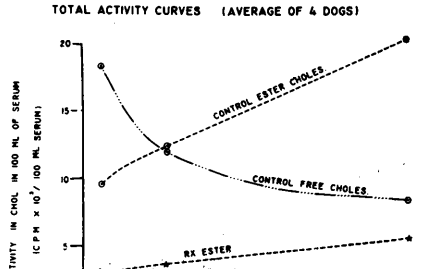

FIG. 2. Mean total radioactivity of free and ester cholesterol in sera of 4 dogs after C^{14}-acetate injection before and during methyltestosterone administration.

sponse to methyltestosterone administration (21). The effect of methyltestosterone administration on the incorporation of amino acids into the protein moiety of lipoproteins is being investigated.

There are certain similarities in the results reported here in dogs given methyltestosterone and previous studies in human subjects with obstructive jaundice: (a) diminished incorporation of radioactivity from C^{14}-acetate or glucose into free cholesterol(23), (b) diminished rate of disappearance of serum free cholesterol radioactivity(23), (c) normal or increased specific radioactivity of ester cholesterol(23), and, (d) reduction in high density lipoprotein levels(24). Thus the possibility that testosterone, especially methyltestosterone, interferes with the transport of conjugated bilirubin from the liver cell into the bile must be considered. We have infrequently observed minor histochemical changes, such as spheroidal lipoidal inclusions in cells about the central veins, or scattered small foci of lipid-laden hepatic or adipose cells, in dogs receiving methyltestosterone(8).

Electron microscopic studies by Schaffner, Popper and Perez(25) revealed dilatation of bile canaliculi and shortening and reduction in number of microvilli, with normal liver cell structure, in specimens from human subjects and rats receiving norethandrolone. Serum bilirubin levels were normal at the time of liver biopsy and light microscopic studies revealed no abnormalities. These drug-induced alterations in hepatic ultrastructure and those associated with extrahepatic biliary obstruction are basically similar(26).

Gass and Umberger(27) observed no changes in sulfobromophthalein (BSP) retention or clearance during administration of methyltestosterone or norethandrolone to dogs in dosages of 10 to 100 mg per day for as long as 50 days. However, androgenic steroids alkylated at the C-17 position are potentially icterogenic and methyltestosterone, 17α-methyl-Δ^4-androsten-17β-ol-3-one, provides the classic example. This subject has been reviewed recently by Sherlock(28).

Evidence that methyltestosterone influences hepatic sterol and bile acid metabolism is also provided by the studies of Mosbach and Bevans(29) who found that large doses of methyltestosterone (25 to 50 mg/kg/day) suppressed the formation of gall stones (composed of cholesterol and dihydrocholesterol) and diminished the intensity of the inflammatory reaction in gall bladders of rabbits fed diets containing 0.25 to 1.0% dihydrocholesterol. The concentrations of dihydrocholesterol in serum, liver and intestines were reduced to values one-fourth to one-half those noted in the animals not given methyltestosterone.

Thus it is possible that the lipid and lipoprotein effects of testosterone derivatives are related to alterations in sterol and bile acid metabolism and the accompanying changes in hepatic ultrastructure which they may induce.

Although the changes in cholesterol specific radioactivity during methyltestosterone administration indicate an effect on cholesterol metabolism *per se,* they do not explain the marked decrease in all 3 lipids, cholesterol, phospholipids and triglycerides. One must consider the possibility that qualitative and/or quantitative changes in the pattern of the plasma bile acids may, through surfactant effects capable of disrupting various interactions between lipid or protein moieties (30,31), alter micelle structure and transport of these lipids.

Summary. Incorporation of radioactivity into serum free and ester cholesterol of 4 dogs was studied before and during administration of methyltestosterone 200 mg/day, 6 days/week. Methyltestosterone administration caused diminution of serum total and free cholesterol, phospholipids, triglycerides and C/P ratios. Degree of esterification of cholesterol remained essentially unchanged. Both high density (α) and lower density (β) lipoprotein concentrations decreased. The specific radioactivity of free cholesterol was depressed by methyltestosterone in all animals while ester cholesterol specific activity was unchanged in 2, increased in one and diminished in the other. The average total radioactivity present in ester cholesterol dur-

ing methyltestosterone administration was increased in relation to that in free cholesterol and equalled it as early as 1½ hours after C^{14}-acetate injection.

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