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## Hypocholesterolemic Action of 17α-Methyltestosterone (MeT) in Hypophysectomized Rats.* (28741)

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Administration of large doses of either naturally-occurring or synthetic androgens produces a significant lowering of the serum cholesterol concentration in several species of laboratory animals(1,2,3,4). The size of the dose required suggests that the effect of these substances is indirect and is not a result of their normal hormonal activity. The present study explores the possibility that the cholesterol-lowering effect of the androgens may be mediated via the pituitary gland.

*Experimental.* Normal and hypophysectomized male Sprague-Dawley rats were obtained from the Charles River Breeding Laboratories. Two weeks after hypophysectomy the animals were divided into 4 groups of 8 animals each and were housed in individual cages. Water and the pertinent diets (Table I) were available *ad libitum* throughout the experimental period (4 weeks). During the first 2 weeks of the experiment all hypophysectomized animals received 5% sucrose in their drinking water. The unoperated control groups (Table I) consisted of 3 animals per group. They were included in the present study to ascertain that Sprague-Dawley rats exhibit the same response to methyltestosterone as the CFN Wistar rats reported on in detail previously(3).

The stock diet consisted of Rockland laboratory chow; cholesterol and methyltestosterone in the indicated amounts were added to this diet in ether solution(3). The rats were weighed and bled weekly. Blood was obtained under ether anesthesia from the capillary blood vessels around the eye. At the end of 4 weeks the animals were killed under anesthesia by exsanguination from the heart, and testes, adrenals and thyroid of the operated rats were examined to determine whether hypophysectomy had been complete.

Serum total cholesterol concentrations and cholesterol content of the "high density" (α) and "low density" (β) lipoprotein fractions

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TABLE I. Serum Cholesterol Patterns of Intact and Hypophysectomized Rats.

| Dietary group | No. of animals | Body wt, g | Serum cholesterol conc, mg % | % of serum cholesterol in α-lipoprotein | % of serum cholesterol in β-lipoprotein |
|---|---|---|---|---|---|
| **Hypophysectomized rats (2 wk)** | | | | | |
| I Basal | 8 | 170 ± 3.8* | 110 ± 4.0* | 44.8 | 55.2 |
| II " + .05% MeT† | 8 | 169 ± 6.3 | 82 ± 2.9 | 53.6 | 46.4 |
| III " + 1% cholesterol | 6 | 166 ± 5.6 | 539 ± 84 | 6.4 | 93.6 |
| IV " + 1% cholesterol + .05% MeT | 6 | 176 ± 4.9 | 373 ±163 | 11.8 | 82.2 |
| **Hypophysectomized rats (4 wk)** | | | | | |
| I Basal | 8 | 159 ± 3.6 | 106 ± 6.2 | 48.2 | 51.8 |
| II " + .05% MeT | 8 | 153 ± 8.6 | 47 ± 2.8 | 56.1 | 43.9 |
| III " + 1% cholesterol | 5 | 152 ± 8.4 | 579 ± 93 | 8.5 | 91.5 |
| IV " + 1% cholesterol + .05% MeT | 6 | 167 ± 3.3 | 361 ±174 | 8.6 | 91.4 |
| **Intact rats (2 wk)** | | | | | |
| V Basal | 3 | 347 (295-395)‡ | 88 (86- 91)‡ | 71.2 | 28.8 |
| VI " + .05% MeT | 3 | 315 (305-321) | 63 (58- 65) | 79.6 | 20.4 |
| VII " + 1% cholesterol | 3 | 354 (323-371) | 107 (92-117) | 48.6 | 51.4 |
| VIII " + 1% cholesterol + .05% MeT | 3 | 338 (322-352) | 68 (65- 70) | 42.1 | 57.9 |
| **Intact rats (4 wk)** | | | | | |
| V Basal | 3 | 369 (305-402) | 93 (86- 99) | 71.2 | 28.8 |
| VI " + .05% MeT | 2 | 309 (275-342) | 41 (36- 46) | 71.4 | 28.6 |
| VII " + 1% cholesterol | 3 | 379 (330-420) | 100 (72-122) | 50.8 | 49.2 |
| VIII " + 1% cholesterol + .05% MeT | 3 | 351 (330-368) | 68 (60- 82) | 52.6 | 47.4 |

* Standard error of mean. † MeT = Methyltestosterone. ‡Range.

of the serum were assayed as described previously(3). The weekly serum samples of each group were pooled for lipoprotein analysis.

*Results.* Table I presents average body weights and serum cholesterol pattern of the animals after 2 and 4 weeks on experiment. As previously observed in animals of the Wistar strain(3), oral administration of 0.05% MeT reduced the serum cholesterol concentration of intact rats to well below normal levels within a period of 2-4 weeks. The relative distribution of this cholesterol between the $\alpha$ and $\beta$ lipoprotein fractions was unchanged at the end of 4 weeks.

The hypophysectomized rats (Group I) had higher serum cholesterol concentrations than the intact controls (Group V). Addition of 0.05% MeT to the diet of hypophysectomized rats produced a highly significant fall in serum cholesterol levels from 110 mg% (overall average of Group I) to 47 mg% at the end of 4 weeks ($p < 0.0005$)(5). The hypocholesterolemic effect of MeT in hypophysectomized animals (Group II) was already apparent after 2 weeks on experiment ($0.01 > p > 0.005$).

The serum cholesterol pattern of the hypophysectomized rats differed from that of the intact controls. The ratio of the cholesterol concentration of the $\alpha$ and $\beta$ lipoprotein fractions in the operated animals on the basal diet was approximately 1:1, whereas this ratio in the normal controls was 2.5:1. When MeT alone was administered to both groups, there was a small change in this ratio in the normal group after 4 weeks (4:1). The ratio in the hypophysectomized group remained at about 1:1.

Addition of 1% cholesterol to the diet of the hypophysectomized animals (Group III) induced an intense hypercholesterolemia, the serum cholesterol concentration reaching an average value of 579 mg% at the end of 4 weeks. In contrast, the unoperated controls (Group VII) developed a very mild hypercholesterolemia with average serum cholesterol concentrations of 100 mg%. In the cholesterol-fed hypophysectomized rats more than 90% of the serum total cholesterol

was found in the β-lipoprotein fraction whereas in the intact rats only about 50% of the serum cholesterol was present in this fraction. Both the intact and hypophysectomized animals receiving the high cholesterol diet responded to MeT administration. In the intact group the serum cholesterol levels returned to the normal range, although the lipoprotein pattern did not. In the hypophysectomized group fed cholesterol plus MeT (IV) the average serum cholesterol concentration remained consistently lower than in comparable animals not receiving androgen (III). At the end of 4 weeks Group III had an average serum cholesterol concentration of 579 mg% while Group IV averaged 361 mg%. Similar differences were observed throughout the experimental period but the statistical significance was not high ($p = 0.053$ at 4 weeks) because of large individual variations in serum cholesterol values. The distribution of cholesterol between the 2 lipoprotein fractions was very similar in the 2 operated groups (III and IV).

*Discussion.* Administration of massive doses of 17 α-methyltestosterone and of other naturally-occurring or synthetic androgens produces a marked lowering of serum cholesterol concentrations in dogs(1,2), rats(3), and rabbits on high cholesterol intakes(4). The mechanism underlying this hypocholesterolemic action of androgens is unknown. Data obtained so far suggest that in some species, particularly the rabbit, androgenic substances prevent the tissue storage of cholesterol and 5α-cholestanol(6), but balance studies in the dog do not provide convincing proof of this assumption(7). Since the androgens are effective whether administered orally or parenterally(1,2) it seems unlikely that they act by direct competition with the intestinal absorption mechanism of sterols.

The adrenals of rats and rabbits treated with large doses of androgens atrophy, presumably because of the suppression of ACTH secretion by the anterior pituitary gland. This suggests the possibility that the hypocholesterolemic action of androgens is mediated by this gland since androgens do not prevent adrenal enlargement caused by exogenous ACTH(8). If this is so, treatment with methyltestosterone would not produce a reduction of serum cholesterol levels in hypophysectomized animals. The results of the present study indicate, however, that the hypocholesterolemic action of methyltestosterone can be observed in hypophysectomized rats as well as in intact animals. It is concluded, therefore, that the action of methyltestosterone and of other androgens upon serum cholesterol and lipoprotein concentrations is not mediated via the pituitary.

It was found, in confirmation of 2 other recent studies(9,10) that serum cholesterol levels of hypophysectomized rats are significantly higher than those of intact controls. This was observed on low cholesterol stock diets and on diets containing 1% cholesterol. Wells and Ershoff(9) administered desiccated thyroid to hypophysectomized Sprague-Dawley rats and found that on a basal diet the serum cholesterol levels of these animals did not drop to that of their intact counterparts. In hypophysectomized rats on a 1% cholesterol diet, whose serum cholesterol levels reached an average of 507 mg% desiccated thyroid reduced these levels to an average of 147 mg%, which is only slightly higher than the normal value for operated animals. Administration of thyroid hormone to intact rats on a basal diet produced a slight elevation of serum cholesterol levels. The results of the present study, with methyltestosterone, differ from those obtained with desiccated thyroid: In hypophysectomized rats maintained on a low cholesterol diet, administration of methyltestosterone reduced serum cholesterol concentrations to well below the control values, while in the cholesterol-fed group the drug did not restore normal serum cholesterol levels; in the intact rats the synthetic androgen likewise produced a very definite hypocholesterolemia. These differences make it rather unlikely that the action of methyltestosterone is related directly to its influence upon thyroid activity.

Although the serum cholesterol concentration of intact rats is only slightly lower than that of hypophysectomized animals, the latter carry a considerably larger proportion of their serum cholesterol in the low density (β) lipoprotein fraction. This abnormal lipopro-

tein-cholesterol pattern was not restored to normal by androgen treatment, although there was a tendency for the cholesterol to return to the high density ($\alpha$) fraction. In the cholesterol-fed dog the administration of large doses of androgen preferentially lowers the cholesterol content of the $\alpha$-lipoprotein fraction of the serum(2). In the rat this differential effect of methyltestosterone upon serum lipoproteins is much less pronounced or absent. This finding does not exclude the possibility, however, that the hypocholesterolemic effect of methyltestosterone is related to changes in the biosynthesis of the protein moiety of the serum lipoproteins since the androgens are known to have protein-sparing activity.

*Summary.* 1. Hypophysectomized rats receiving a low cholesterol stock diet had higher serum cholesterol concentrations and carried a larger proportion of their serum cholesterol in the low density ($\beta$) lipoprotein fraction than intact controls. 2. In hypophysectomized rats fed the stock diet plus 0.05% MeT serum cholesterol concentration was reduced to about the same extent as that of unoperated controls. In the hypophysectomized rats the cholesterol content of the low density ($\beta$) lipoprotein fraction remained elevated whereas the intact controls maintained a normal cholesterol pattern. 3. When the basal diet supplemented with 1% cholesterol was fed, the hypophysectomized animals attained serum cholesterol concentrations averaging 579 mg% at the end of 4 weeks. Ninety-two per cent of this cholesterol was found in the $\beta$-lipoprotein fraction. Addition of 0.05% of methyltestosterone to this diet produced a lowering of serum cholesterol levels: the average cholesterol concentration was 361 mg% at the end of 4 weeks. In both groups more than 90% of the serum cholesterol was present in the $\beta$-lipoprotein fraction. 4. It is concluded that the hypocholesterolemic action of methyltestosterone in the rat is not mediated via the pituitary.

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