Biol. & Med., 1938, v38, 63.
7. Hamner, C. E., Williams, W. L., J. Reprod. Fertil., 1963, v5, 143.
8. Tsuchiya, S., Seikagaku, 1962, v34, 561.
9. Lardy, H. A., Hansen, R., Phillips, P., Arch. Biochem., 1945, v6, 41.
10. Mann, T., Biochem. J., 1945, v39, 451.
11. Bomstein, R. A., Steberl, E. A., Exp. Cell Res., 1959, v18, 217.
12. Newton, A. A., Rothschild, L., Proc. Roy. Soc. B., 1961, v155, 183.
13. McElroy, W. D., J. Biol. Chem., 1951, v191, 547.
14. Morton, B. E., Lardy, H. A., Biochemistry, 1967, v6, 43.
15. ———, ibid., 1967, v6, 50.
16. ———, ibid., 1967, v6, 57.

Received April 13, 1967. P.S.E.B.M., 1967, v125.

Anti-Hypercholesterolemic Effect of Dehydroepiandrosterone in Rats. (32297)

M. BEN-DAVID, S. DIKSTEIN, G. BISMUTH AND F. G. SULMAN
Department of Applied Pharmacology, School of Pharmacy, Hebrew University, Jerusalem, Israel

Interest in the use of steroid hormones as anti-hypercholesterolemic agents was first aroused by the report of Eilert(1), who showed that estrogens reduced the serum cholesterol level. The action of estrogens in man is naturally accompanied by undesirable feminizing effects(2), but their anti-hypercholesterolemic effect is superior to that of androgens.

Hellman *et al*(3) and other workers reported that androsterone, administered parenterally, was an effective anti-hypercholesterolemic agent, with the strongest effect occurring in hypothyroid patients with their typical increased serum cholesterol level. Hellman *et al*, therefore, imputed to androsterone a "thyromimetic" activity. On the other hand, Cohen *et al*(4) showed that androsterone had no effect on the serum protein-bound iodine, and that hence its anti-cholesterolemic effect is not mediated through a thyromimetic activity.

Because of the lack of activity of orally administered androsterone, synthetic and related androgens have been investigated. A new synthetic androgen, of the same order of potency as androsterone, *i.e.*, SC-12790, taken orally, was found to be active as an anti-hypercholesterolemic agent(5). However, its thyromimetic activity was not established. It is well known that in animals as well as in man hyperthyroidism or administration of thyroxine (T-4) or triiodothyronine (T-3) causes hypertrophy of many organs in the body, especially the heart(6-8), while a decrease in heart weight was noticed in hypothyroidism or after thiouracil administration (9). In the present study, the anti-hypercholesterolemic effect and the thyromimetic activity of dehydroepiandrosterone were studied and compared with that of SC-12790.

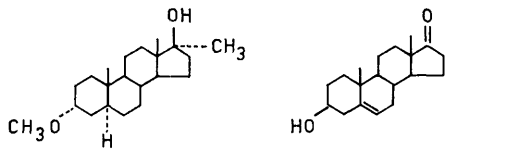
Methods and materials. Adult rats: 102 adult male rats of the "Sabra" strain of the Hebrew University were used. Experiments were carried out in normal as well as in hypercholesterolemic rats. Hypercholesterolemia was achieved by two methods: a) administration of 0.1% propylthiouracil (PTU) in the drinking water for 10 days; b) administration of both 0.1% PTU in the drinking water and 5% cholesterol in the food for 21 days. Daily oral doses of anti-cholesterolemic drugs were given together with the above hypercholesterolemic agents. Blood specimens were taken from the heart under ether anesthesia prior to and at the end of the experiment, without adding any anticoagulant. Serum cholesterol levels were measured by the method of Chiamori and Henry(10). The BMR of the hypothyroid rats was reduced by 10-20%.

SC-12790 (Fig. 1, I) and dehydroepiandrosterone (DHA) (Fig. 1, II), were suspended in 20% propylene glycol with some drops of 0.5% Tween-80 added and given orally. T-3 sodium was dissolved with a few drops of 0.1 N NaOH; H_2O was added to

TABLE I. Lack of Effect of 10 Days' Oral Treatment with Dehydroepiandrosterone (DHA) and SC-12790 on Serum Cholesterol Levels (SCL) of Normal Male Rats (170 ± 20 g).

Treatment (oral)	Daily dose, p.o. (mg/kg)	No. of animals	Serum cholesterol level (mg %) mean ± S.E. Before treatment	After 10 days' treatment
Control (saline)	0.1 ml	6	58.3 ± 2.5	64.2 ± 1.3
DHA	5	6	65.8 ± 4.3	60.3 ± 2.7
SC-12790	5	6	67.3 ± 4.5	61.8 ± 2.0

achieve the desired volume; the solution was neutralized by a few drops of 0.1 N HCl to pH-7 and given s.c.



SC-12790 I

Dehydroepiandrosterone (D.H.A.) II

FIG. 1. I. SC-12790: 3α-methoxy-17α-methyl-5 α-androstane-17α-01. II. Dehydroepiandrosterone: DHA.

Juvenile rats: Experiments were carried out on 6 groups of 9 female 21-day-old albino rats each. The animals were treated for 10 days with the following substances: PTU, SC-12790 and D.H.A. per os; TSH, T-3 and saline by s.c. injection. Blood specimens were taken from the heart 24 hours after the last injection. Then the animals were sacrificed, heart and thyroid removed and weighed.

Results. Adult male rats: Oral treatment with DHA and SC-12790 (5 mg/kg/d) for 10 days did not significantly reduce the serum cholesterol level in normal animals (Table I). However, in PTU-treated rats, DHA (5 mg/kg/d), given for 10 days, counteracted the increase in serum cholesterol level achieved in the control rats treated with PTU alone, while SC-12790 failed to do so at the same dose level (Table II). In animals made hypercholesterolemic (Fig. 2) by combined treatment with PTU (0.1% in drinking water) and cholesterol (5% in the diet), neither steroid had any effect on the high cholesterol level during the first 10 days of treatment, but after 21 days the serum cholesterol level was significantly reduced ($P < 0.01$) in the DHA-treated animals, while it still remained high in those treated with SC-12790.

Table II illustrates also the effect of PTU (0.1% in drinking water), PTU + DHA (5 mg/kg/d p.o.), PTU + T-3 (100 μg/kg/d p.o.) and PTU + SC-12790 (5 mg/kg/d p.o.) on serum cholesterol levels (SCL) and thyroid weights of the rats. PTU administration resulted in goiter production and an increase in SCL. Combined treatment with T-3 and PTU completely prevented the ef-

TABLE II. Effect of Dehydroepiandrosterone (DHA) and T-3 per os on Serum Cholesterol Levels (SCL) and Thyroid Weights of Adult Male Rats Which Had Been Given Propylthiouracil (PTU) (0.1% in Drinking Water) for 10 Days.

Treatment	Dose per day	No. of animals	B.W. (g) mean ± S.E. Before treatment	After treatment	Thyroid wt (mg/100 g B.W.) mean ± S.E.	Serum cholesterol level (mg %) mean ± S.E.* Before treatment	After 10 days' treatment
Control (saline)	.1 ml	6	150 ± 4	189 ± 3	6.7 ± .4	52.5 ± 2.0	55.0 ± 3.1
PTU	.1% in water	12	200 ± 4	182 ±10	14.5 ±1.7	51.1 ± 3.2	75.7 ± 3.4
PTU and DHA	.1% in water 5 mg/kg per os	12	199 ± 2	191 ± 5	15.8 ± .1	51.7 ± 1.8	60.2 ± 1.4
PTU and T-3	.1% in water 100 μg/kg s.c.	6	191 ± 3	185 ± 5	7.0 ± .3	55.6 ± 1.9	59.7 ± 5.7
PTU and SC-12790	.1% in water 5 mg/kg	12	180 ± 4	185 ±10	14 ± .8	52.0 ± 3.0	83.0 ± 7.0

* $P < 0.01$

Effect of SC-12790 and of dehydroepiandrosterone on the serum cholesterol level of male rats (170 ± 20 g), given 0.1% propyl thiouracil in drinking water and 5% cholesterol in diet

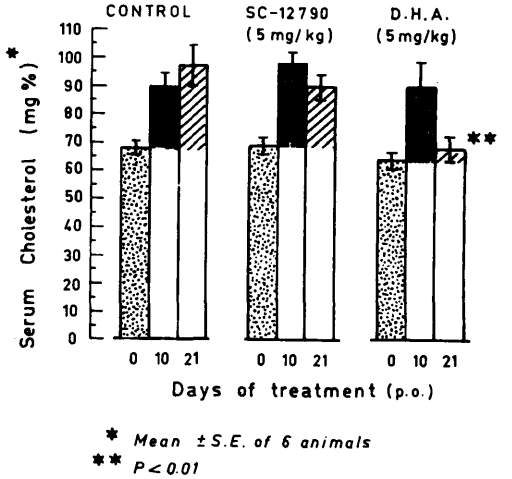


FIG. 2. Comparison of anti-cholesterolemic effects of DHA and SC-12790 in adult rats which received propylthiouracil and cholesterol.

fects engendered by PTU alone, since no change occurred in either the cholesterol values or in the thyroid weights. On the other hand, combined treatment with PTU and DHA failed to prevent thyroid hypertrophy induced by PTU treatment, but significantly reduced the high SCL ($P<0.01$).

Immature female rats. TSH injections (3 IU/kg/d s.c. for 10 days) produced a 50% increase in thyroid weight (Fig. 3). However, no significant change was found in either heart weight or in SCL. T-3 (50 μg/kg/d s.c.) caused an increase of 25% in the heart weight and a significant decrease in the SCL ($P<0.01$); thyroid weight showed no change. In the PTU-treated animals (0.3% in the diet), the thyroid weight was considerably increased (over 330%) and an increase was also found in the SCL ($P<0.01$), while heart weight was decreased. SC-12790 (5 mg/kg/d p.o.) did not produce any relevant changes in the parameters examined. DHA (5 mg/kg/d p.o.) did not significantly influence the thyroid weight; the heart weight was significantly increased (7%) and no change in the SCL was observed.

Discussion. It is well known that it is difficult to cause artificial hypercholesterolemia in rats(11,12). Ranney and Saunders(5) obtained an increase of 15% in SCL of rats by administration of 0.02% PTU in the drinking water. However such treatment did not influence the SCL in our rats. The concentrations of PTU required to induce a 35% increase in SCL in our rat strain was 0.1% in the drinking water or 0.3% in the food. Greater increase in SCL (+45%) was achieved by combined treatment with 5% cholesterol in the food and 0.1% PTU in the drinking water for 10 days. Another combined treatment—1% cholic-acid and 2% cholesterol in the food(12,13)—was not found useful for our experiments since it increased the SCL to more than 4 times its initial values, an unphysiological level which did not react to DHA or SC-12790. SC-12790, reported by Ranney and Saunders(5) to be anti-hypercholesterolemic, was not effective in our experiments. The lack of efficacy of SC-12790 here seems to be due to the higher levels of SCL obtained in this study (35-50%) as against the 15% increase used by Ranney and Saunders. On the other hand, DHA significantly prevented the increase in

Change in % of serum cholesterol level, heart and thyroid weights of treated juvenile rats as compared to control. Results after 10 days administration.

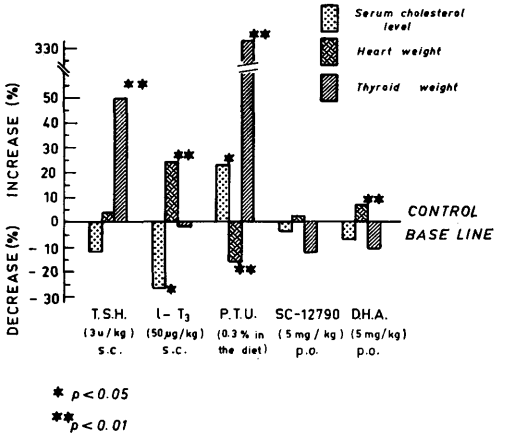


FIG. 3. Comparison of anti-cholesterolemic effects, heart weights and thyroid weights in juvenile rats which received TSH, T-3, PTU, SC-12790 and DHA.

SCL ($P<0.01$) effected in the control animals which had been treated with PTU alone.

To determine whether or not the anti-hypercholesterolemic effect of DHA is due to thyromimetic activity, its effects on thyroid and heart weights and the SCL were simultaneously measured, as these parameters were found to be sensitive to changes in the pituitary-thyroid axis. DHA did not influence thyroid weight, as did exogenous TSH (Fig. 3). It is reasonable to assume, therefore, that DHA did not influence either stimulation of endogenous TSH or thyroglobulin release, and that its anti-hypercholesterolemic property is not mediated through a thyromimetic effect.

DHA decreases SCL only in hypothyroid animals. It is also known that in myxedema levels of 17-ketosteroids are low and treatment with thyroid hormones causes an increase in 17-KS secretion in urine only in secondary myxedematous conditions(14). It is, therefore, possible that thyroid hormones influence the metabolism of endogenous DHA, so that in hyperthyroid conditions it is the lowering of DHA which causes hypocholesterolemia. This would explain why exogenous addition of DHA decreases high SCL.

DHA is a weak androgen. It shows only 3% of the testosterone activity on the prostate and seminal vesicle of infantile rats(15). According to Ranney and Saunders(5), SC-12790 has the same androgenic activity as androsterone which has only 15% of the androgenic activity of testosterone. It is possible that DHA whose androgenic activity is 1/5 that of SC-12790 is a more potent anti-hypercholesterolemic agent because it has a lesser androgenic effect.

Summary. Dehydroepiandrosterone (DHA) and 3α-methoxy-17α-methyl-5α-androstane-17α-01 (SC-12790) were studied for their anti-hypercholesterolemic and thyromimetic activities. The following findings were established: 1. DHA (5 mg/kg/d per os for 10 days) was found to be an anti-hypercholesterolemic agent which prevented increase in the serum cholesterol level (SCL) of rats made hypercholesterolemic by propylthiouracil (PTU) treatment. It was also found that the same dose of DHA given over a period of 21 days prevented an increase in SCL in rats made hypercholesterolemic by combined PTU and cholesterol feeding. DHA, however, did not reduce the SCL of normal rats. 2. SC-12790, when given under the same experimental conditions as DHA, did not reduce the SCL of hypercholesterolemic rats. 3. DHA did not produce any change in thyroid weight as did exogenous TSH and T-3. It seems that the anti-hypercholesterolemic effect of DHA cannot be explained by a thyromimetic activity. These findings are of clinical importance since DHA, which is a weak endogenous androgen, is orally active as an anti-hypercholesterolemic agent. The possible mechanism of its action is discussed.

We are indebted to the Robert Szold Center for Applied Research for their invaluable assistance; our appreciation is due to Mr. K. Khazen, for devoted help. Our thanks are due to G. D. Searle & Co., Chicago for the supply of SC-12790; Ikapharm, Israel for dehydroepiandrosterone; Smith, Kline and French Labs., Philadelphia for sodium liothyronine (Cytomel); Nat. Inst. of Health, Bethesda, Md. for TSH.

1. Eilert, M. L., Metabolism, 1953, v2, 137.

2. Oliver, M. F., Boyd, G. S., in: Hormones and Atherosclerosis, Pincus, G., ed., Academic Press, N.Y., 1959, p403.

3. Hellman, L., Bradlow, H. L., Zamoff, B., Fukushima, D., Gallagher, T. F., J. Clin. Endocrinol. & Metab., 1959, v79, 936.

4. Cohen, W. D., Higano, N., Robinson, R. W., Lebeau, R. J., J. Clin. Endocrinol., 1961, v21, 1208.

5. Ranney, R. E., Saunders, F. J., Proc. Soc. Exp. Biol. & Med., 1964, v116, 596.

6. Gross, J., Pitt Rivers, R., Biochem. J., 1953, v53, 652.

7. Sandler, G., Wilson, G. M., Quart. J. Exp. Physiol., 1959, v44, 282.

8. Boyd, G. S., Montgomery, G. L., J. Atheroscler. Res., 1961, v1, 470.

9. Guthbertson, W. F. J., Elcoate, P. V., Ireland, D. M., Mills, D. C. B., Shearley, P., J. Endocrinol., 1960, v21, 45.

10. Chiamori, N., Henry, R., Am. J. Clin. Path., 1959, v31, 305.

11. Furman, R. H., Howard, R. P., Metabolism, 1962, v11, 76.

12. Kritchevsky, D., in: Animal and Clinical Pharmacologic Techniques in Drug Evaluation, No-

dine, J. H., & Siegler, P. E., ed., Yearbook Medical Publ., Chicago, 1964, p193.
13. Walker, J. B., Emerson, G. A., J. Nutrit., 1964, v82, 311.
14. Engstrom, W. W., Mason, H. L., J. Clin. Endocrinol., 1944, v4, 517.
15. Dorfman, R. I., Shipley, R. A., Androgens, J. Wiley & Sons, N. Y., 1956.

Received April 13, 1967. P.S.E.B.M., 1967, v125.

Alteration of Endogenous Catecholamine Metabolism by Ethanol Ingestion.* (32298)

VIRGINIA E. DAVIS, JESSE L. CASHAW, JAMES A. HUFF, HAROLD BROWN, AND NICKIE L. NICHOLAS
Metabolic Research Laboratory, Veterans Administration Hospital; Moody Clinical Research Unit, Methodist Hospital; and Department of Medicine, Baylor University College of Medicine, Houston, Texas

Recent work in this laboratory has demonstrated that ethanol ingestion in man evoked marked alterations of C^{14}-norepinephrine metabolism. Following the ingestion of ethanol, a significant portion of intravenously administered C^{14}-norepinephrine was diverted from the major oxidative route of metabolism which results in the formation of C^{14}-3-methoxy-4-hydroxymandelic acid to a reductive route as evidenced by an increased excretion of C^{14}-3-methoxy-4-hydroxyphenylglycol(1, 2).

In this study chemical determinations of 3-methoxy-4-hydroxymandelic acid (MHMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were performed on human urine collected under the same experimental design used in the isotope study. These assays were necessary to determine if the excretion of endogenous 3-methoxy-4-hydroxymandelic acid and 3-methoxy-4-hydroxyphenylglycol derived from the metabolism of both epinephrine and norepinephrine was also markedly altered by ethanol ingestion. Administration of moderate amounts of ethanol has been reported to result in increased urinary excretion of epinephrine and norepinephrine in man and experimental animals(3, 4,5). Therefore, total 0-methylated catecholamine (metanephrine and normetanephrine) levels were also assayed in order to determine if ethanol ingestion resulted in increased excretion of the total 0-methylated catecholamine metabolites.

TABLE I. General Outline of Experimental Program.

	Collection period	Time of collection	Treatment
Day 1	I	8 AM– 4 PM	Ethanol*
	II	4 PM–12 MN	—
	III	12 MN– 8 AM	Ethanol*
Day 2	I	8 AM– 4 PM	—
	II	4 PM–12 MN	Ethanol*
	III	12 MN– 8 AM	—

* Each subject ingested 60 ml of absolute ethanol diluted with orange juice at the beginning of these collection periods.

Materials and methods. The study was conducted using 2 normal adult males weighing 75 and 87 kg. Urine was collected for 48 hours using six 8-hour collections which were stored in the refrigerator. At the end of each 8-hour collection period the urine was diluted to 1000 ml with deionized water and stored frozen until analyzed. At the beginning of every other collection period each subject drank 60 ml of absolute ethanol diluted in orange juice. Thus, during the 2-day study there was a urine collection for a given 8-hour period with and without ethanol treatment. Details of the time of ethanol administration and urine collections are given in Table I.

The 0-methylated acid, glycol, and amine metabolites of the catecholamines were isolated from urine, oxidized with periodate to vanillin, and the vanillin was determined spec-

* This study was supported by USPHS Training Grant AM-5063 and Research Grants FR-00134 and HE-05435 from Nat. Inst. Health.