termediates for amino acid synthesis.

Summary. Data suggest that major pathway of glucose oxidation in growing cultures of *A. niger* was *via* gluconate. The citric acid cycle played a minor role in young mycelium and inhibitory action of DNB appeared to involve further depression of formation of citric acid cycle intermediates with consequent decrease in rate of amino acid biosynthesis.

1. Higgins, E. S., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 527.

2. Higgins, E. S., Richert, D. A., Westerfeld, W. W., *ibid.*, 1956, v92, 509.

3. Smith, I., *Chromatographic Techniques,* Interscience Publ., N. Y., p178-188, 1958.

4. Foster, J. W., *Tex. Rep. Biol. Med.,* 1958, v16, 70.

5. Nelson, N., *J. Biol. Chem.,* 1944, v153, 375.

6. Barker, S. B., Summerson, W. H., *ibid.,* 1941, v138, 535.

7. De Moss, R. D., in S. P. Colowick and N. O. Kaplan, eds., *Methods in Enzymology,* vIII, Acad. Press, N. Y., pp 232-234, 1957.

8. Entner, N., Doudoroff, M., *J. Biol. Chem.,* 1952, v196, 853.

9. Cleland, W. W., Johnson, M. J., *ibid.,* 1956, v220, 595.

10. Behal, F. J., *Arch. Biochem. Biophys.,* 1959, v84, 151.

11. Caughey, W. S., Smiley, J. D., Hellerman, L., *J. Biol. Chem.,* 1957, v224, 591.

Received August 30, 1960. P.S.E.B.M., 1960, v105.

Effects of Androsterone on Atherogenesis and Plasma Lipids in Cockerels. (26185)

DONALD L. COOK

Division of Biological Research, G. D. Searle and Co., Chicago, Ill.

Testosterone propionate has been reported to inhibit coronary atherogenesis with simultaneous reductions in plasma cholesterol, phospholipid and cholesterol/phospholipid ratios in cholesterol-fed pullets(1). In the male bird Katz *et al.*(2) observed no effects on serum lipids and atherogenesis with small doses of testosterone. Higher doses significantly inhibited hypercholesterolemia, although aortic and coronary atherogenesis were not influenced(3). The effect on blood lipids, but not on atherogenesis, was in agreement with previous workers(4,5). The latter investigators reported decreased atherogenesis in testosterone-treated cockerels. The recent report by Hellman *et al.*(6) on hypocholesterolemic effects of androsterone in hyper- and normo-cholesterolemic patients stimulated further studies with androgens in experimentally-induced atherosclerosis. The present report compares effects of androsterone and testosterone propionate on atheroma and blood lipid shifts induced by cholesterol feeding in cockerels.

Methods. Three groups of Hy-line cockerels, 6½ weeks of age, were used for the study. Group 1 (control) was fed an atherogenic diet consisting of mash containing 2% cholesterol and 5% cottonseed oil. Daily subcutaneous injections of corn oil were given in volumes equivalent to those given drug treatment. Group 2 was fed similarly and injected daily with 3 mg/kg of androsterone dissolved in corn oil; Group 3 received the diet and 3 mg/kg of testosterone propionate in the oil solvent. Dosage of both compounds was adjusted each week to the changing weight of the birds. Plasma cholesterol and phospholipid concentrations, comb indices and food consumption were determined at 2-week intervals during the experimental regimen. Total cholesterol was determined by the method of Zlatkis *et al.*(7). The lipid phosphorus, determined by Sperry's method (8), was multiplied by the factor 25 to give the amount of total phospholipids. All birds were sacrificed and autopsied at the end of 8 weeks. The aortas were cut open longitudinally, fixed in 10% Formalin and stained with Sudan IV. Degree of aortic atherosclerosis was graded visually into 5 groups(0-4), according to the area of intima involved by le-

TABLE I. Effects of Androsterone and Testosterone Propionate on Plasma Lipids and Atheroma in Cholesterol-Fed Cockerels in an 8-Week Study.

Group	Plasma lipids		Atheroma	
	Cholesterol	Phospholipid		
	mg %		Aorta (0-4)	Coronary,† %
Control	1573 ± 134	594 ± 34	1.6 ± .2	45.1 ± 2.6
Androsterone	1353 ± 91	466 ± 26*	1.5 ± .2	25.9 ± 3.0*
Testosterone propionate	1376 ± 160	477 ± 44*	1.7 ± .1	30.6 ± 2.6*

* $P < .05$ that treat = control; values represent mean response (± stand. error) of 10 chicks /group.

† Proportion of arteries (in %) showing intimal lipid deposition; observations based on 23 to 84 arteries/heart.

sions. The hearts were fixed in Formalin and 4 equally spaced blocks were cut from each heart between the coronary groove and the apex. One frozen section was then cut from each block and stained with Sudan IV and hemotoxylin for histological examination of the coronary arteries. The index of coronary atherogenesis was based on the proportion of arteries showing presence of lipid in the intima. All evaluation for lesions in the aorta and coronary arteries was done under blind experimental conditions. The statistical significance of differences between mean values was determined by Student's t test.

Results. The group mean values for plasma lipids were not significantly different at 2, 4 or 6 weeks, hence only values for 8 weeks along with data on atherosclerotic lesions are shown in Table I. Plasma cholesterol levels for the androsterone and testosterone-treated groups were slightly lower but not significantly different from that of the control group. Both compounds, however, appeared to be approximately equipotent in reducing plasma phospholipid concentrations ($P<0.05$). By a primary effect on phospholipids, both steroids elevated the cholesterol/phospholipid (C/P) ratio, a metameter frequently used for description of relative shifts of the lipids in plasma. C/P ratios (mean ± S.E.) for control, androsterone and testosterone propionate were 2.60 ± 0.11, 2.90 ± 0.07 and 2.84 ± 0.19, respectively.

The severity of aortic atherosclerosis as judged by gross observation of the stained intima was similar for the 3 groups (Table I). The hearts appeared grossly normal. On microscopic examination, however, the hearts from both treatments exhibited significantly fewer arteries showing intimal lipid deposition.

Mean testicular weight of the androsterone-treated group did not differ significantly from the control group (Table II). As a contrast, treatment with testosterone induced a marked reduction in weight of the testes. Comb growth was stimulated to the same extent by androsterone and testosterone. No effect on body weight was observed with androsterone relative to the controls. Dosage of testosterone propionate used in this study, however, significantly depressed growth rate. Body weights (mean ± S.E.) for control, androsterone and testosterone propionate were 1.66 ± 0.03, 1.60 ± 0.05 and 1.39 ± 0.02, respectively. Measurements of food consumption revealed only negligible differences in intake.

Discussion. The most significant finding in the present study is that androsterone produced effects similar to those of testosterone propionate on plasma lipids and atherogenesis but without causing a reduction in testicular weight. The inhibition of cholesterol-induced *coronary atheroma* in the cockerel is apparently not related to lower plasma cholesterol levels since the treatments had no

TABLE II. Effects of Androsterone and Testosterone Propionate on Testicular Weight and Comb Growth in Cholesterol-Fed Cockerels.

Group	Testes, g	Comb growth,† mm
Control	9.1 ± 1.09	+ 89 ± 5.9
Androsterone	8.6 ± .83	+116 ± 3.7*
Testosterone propionate	.4 ± .04*	+117 ± 2.3*

* $P < .05$ that treat = control; values = mean response (± stand. error) of 10 chicks/group.

† Determined by change in length + height.

significant effect on this measurement. Such a relationship has been reported in pullets where testosterone propionate produced inhibition of coronary atheroma associated with a marked hypocholesterolemic response(1). The inhibitory effect of estrogenic agents has been reported to be associated with a decrease in C/P ratio; this reduction being due to a marked phospholipidemia with relatively little effect on cholesterol(9). It is therefore of particular interest that the inhibitory effects of the androgens used in this study were associated with decreases in plasma phospholipid and increases in C/P ratio. Recently Campbell *et al.*(10) reported that the weak androgenic agent, 19-norandrostenolone phenylpropionate, could produce significant regression of coronary atheroma without affecting plasma cholesterol or phospholipid levels in cholesterol-fed cockerels. These investigators postulated either a direct effect on the vessel wall or the anabolic (protein-conserving) activity of the compound may be playing a role in the protective action against experimentally-induced atheroma in the chick since increased dietary protein has been reported to have such a protective action(11. 12). Whatever the explanation may be, the present data support the concept that protection against coronary atheroma can be obtained with variable cholesterol and phospholipid changes in cholesterol-fed cockerels.

Summary. The effects of androsterone and testosterone propionate on atheroma and plasma lipid shifts induced by cholesterol feeding in cockerels were compared. Both compounds produced significant inhibition of coronary arterial atheroma. Aortic atherogenesis was not affected. The effects on plasma lipids were similar; non-significant reduction in cholesterol (C), significant decrease in phospholipid (P) with elevated C/P ratios. Testosterone propionate, but not androsterone, caused a significant reduction in testicular weight; both compounds stimulated comb growth. Further experimentation with androgens should provide useful information on the relationship between plasma lipid changes and inhibition of coronary atherogenesis.

The technical assistance of Astrida Kalnmalis and Margot Turner is gratefully acknowledged.

1. Cook, D. L., Edgren, R. A., Harris, T. W., *Circulation Res.,* 1957, v5, 54.

2. Katz, L. N., Stamler, J., Pick, R., Rodbard, S., *Circulation,* 1952, v6, 474.

3. Pick, R., Stamler, J., Rodbard, S., Katz, L. N., *Circulation Res.,* 1959, v7, 202.

4. Wong, H. Y. C., Simmons, R. L., Hawthorne, E. W., *Fed. Proc.,* 1956, v15, 203.

5. Wong, H. Y. C., Johnson, F. B., Wong, A. K., Anderson, J., Liu, D., *Circulation,* 1957, v16, 501.

6. Hellman, L., Bradlow, H. L., Zumoff, B., Fukushima, D. K., Gallagher, T. F., *J. Clin. Endocrinol. and Metab.,* 1959, v19, 936.

7. Zlatkis, A., Zak, B., Boyle, A. J., *J. Lab and Clin. Med.,* 1953, v41, 486.

8. Sperry, W. M., *Ind. Eng. Chem., Anal. Ed.,* 1942, v14, 88.

9. Pick, R., Stamler, J., Rodbard, S., Katz, L. N., *Circulation,* 1952, v6, 858.

10. Campbell, R. S. F., Lawrie, T. D. V., Maclaurin, J. C., Pirrie, R., *Circulation Res.,* 1960, v8, 78.

11. Stamler, J., Pick, R., Katz, L. N., *ibid.,* 1958, v6, 447.

12. Nishida, T., Takenaka, F., Kummerow, F. A., *ibid.,* 1958, v6, 194.

Received August 31, 1960. P.S.E.B.M., 1960, v105.