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Kinetics of Testosterone Induced-Cholesterol Synthesis in Rat Ventral Prostate (40271)

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It has been well established that under certain conditions testosterone administration may significantly affect the rates of DNA, RNA and protein synthesis in the rat ventral prostate (1–4). Testosterone also maintains the morphology and secretory activity of the prostate gland, both in *in vitro* and *in vivo* systems (5–7). On castration, there is rapid regression of the rat ventral prostate including the cessation of secretory function (8). Cholesterol has been found to be one of the major constituents of the prostate secretion (9, 10). In this paper, we are reporting the regulation of cholesterol synthesis by testosterone in the rat ventral prostate. Kinetics of cholesterol synthesis in the ventral prostate following testosterone administration to castrated rats was studied in relation to prostate weight gain, DNA and protein synthesis.

Materials and methods. Animals. Groups of six adult male intact and castrated Wistar rats (250–350 g) were maintained on Purina rat chow and water *ad libitum* and were kept under alternating 12-hr light and 12-hr dark schedule. At necropsy final body weights were determined.

Administration of testosterone to castrated animals. Castrated animals were injected subcutaneously with 2 mg of testosterone propionate, dissolved in sesame oil (10 mg/ml), at the same time every day for different periods of up to 14 days.

In vitro incorporation of radioactive precursors into cholesterol, proteins and DNA by minced prostate tissues. At various time intervals up to 14 days animals were anesthetized with intraperitoneal injections of sodium barbital and sacrificed by exsanguination. The two lobes of the ventral prostate gland were excised free of the fat covering. The tissues were minced and weighed immediately in tared Teflon test tubes and kept in ice until further use. Approximately 25–35 mg samples of minced tissues were used to study the incorporation of radioactive precursors into cholesterol, proteins and DNA.

The radioactive precursors, 2-[^{14}C]acetate (sp. activity 50.3 mCi/mmol), 4,5-^{3}H-L-leucine (sp. activity 5 Ci/mmol), and ^{3}H-methylthymidine (sp. activity 6.7 Ci/mmol) were used in these studies to determine their incorporation into cholesterol, protein and DNA, respectively. Tissues were incubated with 2 ml of Hank's Balanced Salt solution supplemented with 0.2% glucose and either 1 μCi/ml of 2-[^{14}C]acetate or 1 μCi/ml of ^{3}H-leucine or 3 μCi/ml of ^{3}H-thymidine (pregassed with 95% O_2 and 5% CO_2) at 37° for 2 hr on a constant speed shaker. At the end of the incubation period, the reaction was terminated by instant freezing of the tubes in a dry ice-acetone bath. The radioactivity of cholesterol, protein and DNA in the tissues was then determined.

Analysis of radioactivity in cholesterol. The tissues were saponified by the addition of alcoholic KOH to a final concentration of 10% KOH and 50% ethanol (95%) at 75° for 75 min. Unsaponified lipids were pooled by repeated extractions with *n*-hexane. The hexane extracts were evaporated under nitrogen and digitonin precipitation was carried out according to the procedure of Sperry (11). The cholesterol–digitonin complex was dissolved in 1 ml of methanol and 0.1 ml aliquots were counted in duplicate for [^{14}C]activity in a Packard Scintillation Counter. The rates of synthesis were expressed as counts per minute per μg of prostatic DNA.

Analysis of radioactivity in protein and DNA. The tissues were homogenized with a Brinkmann polytron and crude protein or DNA was precipitated with 5 ml of 6% trichloroacetic acid (TCA) at 0°. After 10 min, the samples were centrifuged at 4°, and the precipitates were washed twice with 5 ml of 6% cold TCA. The precipitates were then extracted repeatedly with 95% ethanol: chloroform (3:1 v/v) to remove lipids. For radioactivity counting in proteins, the ethanol–chloroform extracted precipitates were dissolved in 2 ml of 10% NaOH and 0.2

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ml aliquots were counted in duplicate for ^3H-activity. To measure the incorporation of ^3H thymidine into DNA, ethanol–chloroform extracted precipitates were dissolved in 2 ml of 0.3 *N* KOH at 37° for 60 min. Proteins and DNA were then reprecipitated from supernatants with 8 ml of 6% TCA. The KOH extraction and the TCA precipitation were repeated. The final TCA insoluble fraction was treated with 2 ml of 16% perchloric acid (PCA) for 20 min at 70°, followed by centrifugation. Aliquots (0.2 ml) of the acid-soluble fraction were counted in duplicate for determination of radioactivity in the DNA. All tritium determinations were made in a xylene based scintillation cocktail (aquasol-2, New England Nuclear) and counted in a Packard Scintillation Counter.

Colorimetric determinations. The amount of cholesterol was quantitated by first saponifying the tissues and the unsaponified fractions were used for digitonin precipitation. The cholesterol–digitonin complex was used for colorimetric determination by the method of Parekh and Jung (12).

DNA and proteins were extracted in similar manner described in the section above and colorimetric analyses were carried out employing the method of Abraham *et al.* (13) for DNA and the Biuret procedure (14) for protein assay.

Results. As expected, the data in Table I confirm that on castration the prostate weight declines to about 12% of the normal rat prostate weight. Body weights are not significantly affected. Amounts of cholesterol, protein and DNA in rat prostate gland, quantitated colorimetrically, also decline to 12%, 13% and 25% of their respective normal values. Rates of synthesis of cholesterol and DNA per μg prostatic DNA also decline to about 8% and 5%, respectively, in castrated animals. Contrary to the decreases in the rates of synthesis of DNA and cholesterol, the rate of protein synthesis per μg prostatic DNA remains constant in the castrated animals even though the total amount of protein present in the prostate gland of castrated rats is significantly lower than in normal animals. This may be due to the synthesis of hydrolytic enzymes that would hydrolyze the proteins present in the normal gland. The results indicate that testosterone produced by the testes is essential for the maintenance of glandular cholesterol synthesis in the prostate gland among other prostatic functions.

To examine whether testosterone would restore the prostate cholesterol levels as well as the levels of macromolecules, 2 mg of testosterone propionate in sesame oil (10 mg/ml) was injected daily subcutaneously to castrated rats for varying periods of time up to 12 days. Prostate weight as well as cholesterol, DNA and protein content in the prostate gland were quantitated and the results are presented in Fig. 1. Administration of testosterone to castrated rats increased the amount of cholesterol in the prostate gland. DNA and protein content also increased. Prostate weights and the amounts of cholesterol and protein increased more significantly after 2 days of testosterone administration. The amount of DNA remained constant up to 2 days and increased significantly after 3 days of testosterone injections. All four parameters tested increased almost equally between 2 and 5 days of testosterone treatment. The amount of protein increases sharply after 5 days of treatment which is followed after 8 days by steep increases in prostate weight and the amount of cholesterol. Contrary to increases in prostate weight and in the amount

TABLE I. EFFECT OF CASTRATION ON THE SYNTHESIS OF PROSTATE CHOLESTEROL, PROTEIN AND DNA.

			Prostate Cholesterol		Prostate Protein		Prostate DNA	
Type	Body weight (gm)	Wet prostate weight (mg)[a]	Total content (μg)[a]	Rate of synthesis (cpm)[b]	Total content (mg)[a]	Rate of synthesis (cpm)[b]	Total content (μg)[a]	Rate of synthesis (cpm)[b]
Normal	275.5 ± 11.5	116.02 ± 21.06	237.5 ± 48.1	46.62 ± 11.98	5.83 ± 0.983	108.91 ± 70.49	293.17 ± 57.97	128.83 ± 23.59
Castrated[c]	256.0 ± 16.1	17.75 ± 1.26	32.04 ± 9.46	3.816 ± 0.642	0.728 ± 0.106	102.62 ± 33.07	78.38 ± 12.43	6.53 ± 1.30

[a] All the total contents are expressed in terms of per 100 g body wt.
[b] The rate of synthesis are expressed as cpm/μg of prostate DNA isolated.
[c] Rats were castrated for 7 days.

of cholesterol, the slopes of curves for DNA and protein at 12 days of testosterone administration approached the steady state.

In Fig. 2 the ratios of cholesterol, protein and DNA content of the prostate glands from testosterone treated castrated rats are presented. The ratios of both cholesterol/DNA and protein/DNA increase on the administration of testosterone. This would be expected since de novo synthesis of enzymes for cholesterol synthesis pathway would be required for an increase in cholesterol content.

Figure 3 shows the rate of synthesis of cholesterol, DNA and proteins at various periods of testosterone administration up to 14 days. The rates of synthesis of protein and cholesterol peak 2 days after testosterone injection, whereas DNA synthesis peaks after 4 days of treatment. The two peaks in protein and cholesterol synthesis after 2 days and again after 5 days might indicate the synthesis of structural components followed by synthesis of secretory components. After 5 days, synthesis of DNA, protein and cholesterol decreases and remains at a steady state for the remainder of the 14 days of testosterone treatment. Despite the fact that the rate of cholesterol synthesis per microgram of prostatic DNA reaches a steady state, the sharp increase in cholesterol content upon testosterone administration at day 12 can be accounted for by the increased prostate weight as seen in Fig. 1.

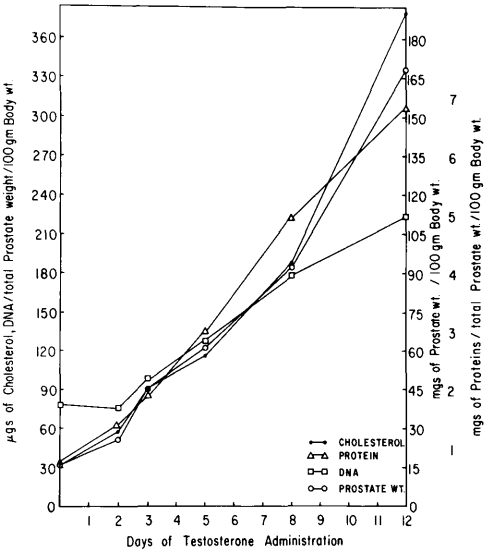

FIG. 1. The effect of testosterone administration to the castrated rats on prostate weight and the contents of cholesterol, DNA and protein in the ventral prostate. The mean values are obtained from groups of six rats.

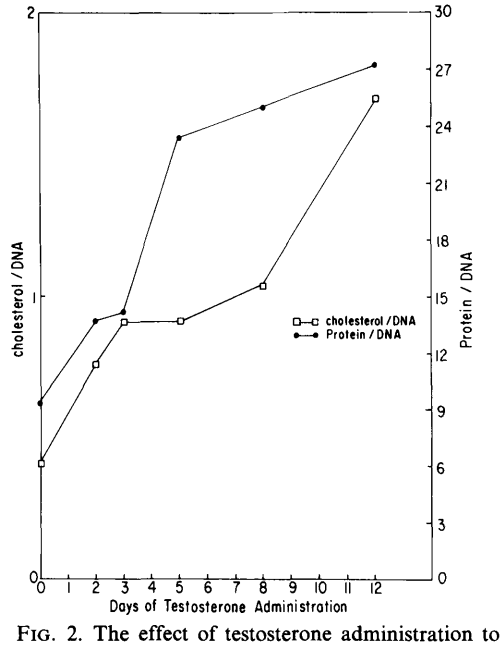

FIG. 2. The effect of testosterone administration to the castrated rats on the content ratios of cholesterol/DNA and protein/DNA in the ventral prostate.

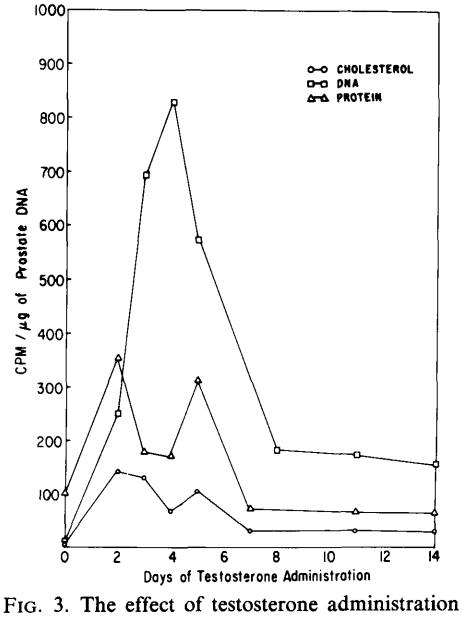

FIG. 3. The effect of testosterone administration to the castrated rats on the rates of synthesis of cholesterol, protein and DNA in the ventral prostate. The mean values are obtained from groups of six rats.

Discussion. Swyer (15) reported an increase in the cholesterol content of the adenomatous portion of enlarged human prostate glands as compared to normal glands. Braunstein (16) reported the presence of refractile and doubly refractile crystals as well as a positive Schultz reaction in the cytoplasm of human prostatic carcinoma cells indicative of a high content of cholesterol. Leav and Ling (17) reported the similar findings on tissues derived from neoplastic canine prostate gland.

Since the discovery that the hypocholesterolemic polyene macrolides (18) by the oral route decreased the size of the enlarged prostate glands of dogs (19) and hamsters (20), there has been increasing clinical evidence (21–27) that these drugs affect the symptoms of prostatism caused by benign prostatic hyperplasia. Other hypocholesterolemic agents such as cholestyramine (28), colestipol (20), simfibrate (29) and β-sitosterol (30) have now also been reported to affect the prostate gland. Considering that hypocholesterolemic drugs in general appear to affect the cholesterol-containing enlarged prostate gland and realizing the importance of cholesterol in this organ, it became necessary to study cholesterol metabolism in the prostate gland and its possible regulation by testosterone, a recognized mediator of other prostatic functions.

It is very evident from these current studies that testosterone is a major factor in the synthesis of cholesterol in the prostate gland. On testosterone administration to castrated rats the amount of cholesterol increases before an increase in DNA content. Liao *et al.* have shown that the RNA polymerase activity from the prostate of castrated rats is enhanced within a few hours of single injection of testosterone. This may mean that the initial increase in cholesterol content is more likely due to increased RNA and protein synthesis. Following the initial cholesterol curve there is an increase in DNA content and then another increase in cholesterol content.

The observed two different phases in the amount of prostate cholesterol, the first of parallel increase with protein from day 0 and the second of a sharp increase in cholesterol between day 8 and 12 can be explained on the basis of cholesterol having a dual function in the gland. In the first phase, it is likely that only structural or membrane cholesterol is synthesized. After 8 days of testosterone administration when the gland approaches the normal state, since cholesterol is an important secretory product of the prostate gland, greater amounts of cholesterol-synthesizing enzymes might be produced as indicated by the large increase in protein content. This would be followed by the synthesis of a large amount of secretory cholesterol. Prostate weight rises in parallel with the amount of cholesterol.

The sharp increases in the synthesis of cholesterol, DNA and protein is followed by a sharp decrease on continuous testosterone administration. This may be due to a shift in testosterone metabolism in the prostate gland where testosterone may be converted to inactive or less active metabolites as compared to the conversion to a highly active metabolite such as dihydrotestosterone (31). This indicates that testosterone may be acting both as a positive and negative regulator of cholesterol synthesis in the prostate gland.

Summary. The absolute cholesterol content and rate of cholesterol synthesis was compared in rat ventral prostates obtained from adult normal and castrated rats. Cholesterol content and synthesis reduces to about 8–12% in the ventral prostate of castrated animals as compared to normal rats. Daily testosterone injections to castrated rats elicits a sharp increase in cholesterol content which correlates with an increase in prostate weight. The rate of cholesterol synthesis per microgram of prostatic DNA increases steeply 2 days after testosterone administration and then goes down and reaches a steady state after 5 days.

1. Coffey, D. S., Shimazaki, J., and Williams-Ashman, H. G., Arch. of Biochem. Biophys. **124,** 184 (1968).
2. Liao, S., Leininger, K. R., Sagher, D., and Barton, R. W., Endocrinology **77,** 763 (1965).
3. Liang, T., and Liao, S., Proc. Nat. Acad. Sci. U.S.A. **72,** 706 (1975).
4. Williams-Ashman, H. G., Liao, S., Hancock, R. L., Jurkowitz, L., and Silverman, D. A., Recent Prog. Horm. Res. **20,** 247 (1964).
5. Baulieu, E. E., Lasnitzki, I., and Robel, P., Nature (London) **219,** 1155 (1968).
6. Robel, P., Lasnitzki, I., and Baulieu, E. E., Biochimie **53,** 81 (1971).
7. Baulieu, E. E., Le Goascogne, C., Groyer, A., Feyel-Cabanes, T., and Robel, P., Vitamins Horm. **33,** 1 (1975).

8. Huggins, C., and Clark, P. J., J. Exp. Med. **72,** 747 (1940).
9. Mann, T., 'The Biochemistry of Semen', p. 130, Methuen and Co., London (1954).
10. Mann, T., Natl. Cancer Inst. Monogr. **13,** 235 (1963).
11. Sperry, W. M., J. Lipid Res. **4,** 221 (1963).
12. Parekh, A. C., and Jung, D. H., Anal. Chem. **42,** 1423 (1970).
13. Abraham, G. N., Scaletta, C., and Vaughan, J. H., Anal. Biochem. **49,** 547 (1972).
14. Layne, E., Methods Enzymol. **3,** 450 (1957).
15. Swyer, G. I., Cancer Res. **2,** 372 (1942).
16. Braunstein, H., The Amer. J. Clin. Pathol. **41,** 44 (1964).
17. Leav, I., and Ling, G., Cancer **22,** 1329 (1968).
18. Schaffner, C. P., and Gordon, H. W., Proc. Nat. Acad. Sci. **61,** 36 (1968).
19. Gordon, H. W., and Schaffner, C. P., Proc. Nat. Acad. Sci. **60,** 1201 (1968).
20. Wang, G. M., and Schaffner, C. P., Invest. Urol. **14,** 66 (1976).
21. Casella, G., Policlinico Sez. Med. **77,** 285 (1970).
22. Casella, G., La Chirurgia Generale **19,** 1 (1970).
23. Kljucharev, B. V., Berman, N. A., Ivanov, N. M., Margolin, A. M., and Mikhailets, G. A., Voprosy Onkologii (Leningrad) **18,** 36 (1972).
24. Keshin, J. G., Int. Surg. **58,** 116 (1973).
25. Kljucharev, B. V., Mikhailets, G. A., Berman, N. A., Ivanov, N. M., and Margolin, A. M., Urologiia i Nefrologiia (Moscow) **38,** 40 (1973).
26. Orkin, L. A., Urology **4,** 80 (1974).
27. Sporer, A., Cohen, S., Kamat, M. H., and Seebode, J. J., Urology **6,** 298 (1975).
28. Addleman, W., New England J. Med. **287,** 1047 (1972).
29. Yamanaka, H., Shimazaki, J. Koya, A., Mayuzumi, T., Imai, K., Yoshikazu, I., and Shida, K., Endocrinol. Japan **24,** 213 (1977).
30. Effinghaus, K. D., and Baur, M. P., Z. Allg. Med. **53,** 1054 (1977).
31. Bruchovsky, N., and Lesser, B., *in* "Cellular Mechanisms Modulating Gonadal Hormone Action", (R. L. Singhal and J. A. Thomas, eds.) p. 1, University Park Press (1976).

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