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Effect of Clofibrate on Cholesterol and DNA Synthesis in Rat Ventral Prostate (40459)

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Clofibrate (ethyl 2-(4-chlorophenoxy)-2-methyl propionate) was shown to reduce serum and liver cholesterol concentrations in rats (1). Clofibrate was also found to inhibit *in vitro* cholesterol synthesis in rat and human skin (2).

Testosterone is known to maintain the morphology and secretory activity of the prostate gland (3, 4). There is a large increase in cholesterol (5) and DNA synthesis (6) when testosterone is administered to castrated rats. Hypolipidemia caused by simfibrate (1, 3-propyl-bis (2-*p*-chlorophenoxy-2-methyl propionate) feeding for 3 weeks reduced responses of weight and nucleic acid contents to testosterone administration in the ventral prostates of castrated rats (7). This paper reports our findings of studies involving the effect of clofibrate on the cholesterol synthesis in the ventral prostate and also its consequence on DNA synthesis in the ventral prostate of normal, castrated and testosterone treated castrated rats.

Materials and methods. Animals. The normal 10 week old Wistar rats (Charles River Farms, MA) were maintained on Purina Rat Chow containing 0.3% clofibrate. The control rats were fed on standard chow. The rats were sacrificed after 2, 3, 4 and 10 weeks of clofibrate treatment.

At the end of each of the above mentioned periods, groups of eight rats each, one clofibrate treated and the other controls were anesthetized with intraperitoneal injections of sodium barbital, and the body weights were taken. The animals were sacrificed by exsanguination. The blood was collected from each rat for the preparation of serum. The two lobes of the ventral prostate gland were excised free of the fat covering. The livers were excised and sliced. The tissues were minced and weighed. Approximately 25–35 mg samples of minced tissues on wet weight basis were used to study the incorporation of radioactive precursors into cholesterol and DNA.

The effect of clofibrate on testosterone stimulated DNA synthesis in the ventral prostate was investigated by first castrating the rats fed either control or experimental diet for 15 days. The diets were continued after castration. Seven days after castration half of the control and clofibrate treated rats were sacrificed while the other half were injected subcutaneously daily for 3 days with 2 mg of testosterone propionate dissolved in sesame oil (10 mg/ml).

Analytical methods. The procedures for the *in vitro* incorporation of precursors into cholesterol and DNA were described elsewhere (5). The total amount of cholesterol in tissues and blood was determined colorimetrically by the procedure of Parekh and Jung (8). The amount of DNA was quantitated colorimetrically according to the method of Abraham *et al.* (9).

Histopathology. After 10 weeks the ventral prostate lobes were dissected from 20-week old control and clofibrate treated rats. The excised tissues were stored in 10% buffered formalin. The ventral prostate lobes were fixed with paraffin, and the thin sections were prepared with a microtome; these were stained with eosin and hematoxylin.

Results. The results presented in Table I show that after 2, 3, 4 and 10 weeks of clofibrate treatment there were no significant differences in the body weights and the prostate weights between control and clofibrate treated rats. The serum cholesterol levels in the clofibrate treated rats decreased to approximately 55–65% of the average for all control groups. The concentration of cholesterol in liver on the unit weight basis decreased to about 80% in all groups. The total cholesterol content in the prostate was reduced to approximately 70–80%. The DNA content was also reduced in the ventral prostate to 68–75% in all groups.

Table II shows the results of the effect of clofibrate treatment on the rates of synthesis of liver cholesterol, prostate cholesterol and

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DNA. Liver cholesterol synthesis was reduced to about 25–40% of its control value between 14 and 70 days of clofibrate feeding. The prostate cholesterol synthesis decreased to about 75% after 14 days of clofibrate treatment. Between 21 and 70 days of clofibrate treatment, cholesterol synthesis was reduced to approximately 35–45%. DNA synthesis in the prostate followed a similar pattern. DNA synthesis was reduced to approximately 80% after 14 days of clofibrate treatment and between 30 and 40% after 21 days of clofibrate treatment whereupon it stayed constant up to 70 days of clofibrate treatment.

Table III shows the comparison of body weight, prostate weight, prostate cholesterol and DNA content of castrated and 3-day testosterone treated castrated rats fed either on standard or clofibrate containing diet for 22 days. In castrated rats, there was no significant difference between control or clofibrate treated rats. When the castrated rats

TABLE I. EFFECT OF ORAL CLOFIBRATE TREATMENT ON THE BODY WEIGHT, PROSTATE WEIGHT, TOTAL PROSTATE CHOLESTEROL AND THE LEVELS OF DNA, LIVER CHOLESTEROL AND SERUM CHOLESTEROL OF NORMAL RATS.

Treatment period (days)	Group	Body weight (g)	Prostate Weight (mg)*	Total prostate cholesterol (μg)*	Total prostate DNA (μg)*	Liver cholesterol conc. (mg/g of tissue)	Serum cholesterol (μg/100 μl)
14	Control	332 ± 4	105.0 ± 10.1	317.0 ± 21.7	301.0 ± 15.7	5.43 ± .30	95.5 ± 5.2
	Clofibrate	328 ± 9	95.4 ± 7.7	223.0^b ± 12.1	209.0^c ± 6.7	4.48^a ± .18	61.5^c ± 2.6
21	Control	350 ± 5	108.0 ± 5.5	326.0 ± 11.7	333.0 ± 15.4	5.11 ± .17	105.0 ± 5.9
	Clofibrate	343 ± 13	114.0 ± 5.9	288.0^b ± 2.7	265.0^b ± 9.1	4.24^a ± .09	68.3^c ± 4.7
28	Control	376 ± 6	110.0 ± 6.2	326.0 ± 10.7	359.0 ± 16.9	5.46 ± .08	105.0 ± 0.6
	Clofibrate	385 ± 9	84.2 ± 9.1	238.0^c ± 11.6	245.0^c ± 12.0	4.59^a ± .21	58.3^c ± 1.8
70	Control	475 ± 15	122.0 ± 7.4	343.0 ± 10.1	352.0 ± 22.5	5.29 ± .11	122.0 ± 1.1
	Clofibrate	490 ± 15	113.0 ± 5.9	246.0^c ± 6.2	243.0^b ± 11.4	4.32^a ± .22	85.5^c ± 2.4

* Prostate weights, prostate cholesterol and DNA contents are expressed as mean ± S.E./100 g body weight. All the values are obtained from groups of six to eight rats.
Significance: p value—$^a < .05$, $^b < .01$, $^c < .001$.

TABLE II. Effect of Oral Clofibrate Treatment on the Rate of Synthesis of Liver Cholesterol, Prostate Cholesterol and DNA in Normal Rats.*

Treatment period (days)	Group	Rate of synthesis (cpm/g tissue) × 10^5		
		Liver cholesterol	Prostate cholesterol	Prostate DNA
14	Control	0.78 ± 0.08	2.59 ± 0.09	2.31 ± 0.15
	Clofibrate	0.33 ± 0.07^a	1.90 ± 0.11^b	1.89 ± 0.18
21	Control	1.38 ± 0.16	6.47 ± 0.79	2.78 ± 0.21
	Clofibrate	0.36 ± 0.19^a	2.15 ± 0.24^b	1.19 ± 0.17^b
28	Control	1.06 ± 0.23	5.31 ± 0.44	2.79 ± 0.20
	Clofibrate	0.25 ± 0.04^a	2.51 ± 0.35^b	0.82 ± 0.14^b
70	Control	0.96 ± 0.04	6.73 ± 0.06	2.43 ± 0.16
	Clofibrate	0.36 ± 0.02^b	2.79 ± 0.22^b	0.99 ± 0.06^b

* All the values are obtained from groups of six to eight rats and expressed as mean ± SE.
Significance: p value. $^a < .01$, $^b < .001$.

TABLE III. EFFECT OF ORAL CLOFIBRATE TREATMENT ON THE TESTOSTERONE DEPENDENT INCREASE IN PROSTATE WEIGHT, PROSTATE CHOLESTEROL AND DNA CONTENT IN 7-DAY CASTRATED RATS.*

	Group	Body Weight (gm)	Prostate Weight (mg)	Prostate Cholesterol (μg)	Prostate DNA (μg)
Untreated	Control	309 ± 7	20.8 ± 2.2	71.6 ± 5.5	135 ± 5.9
	Clofibrate	318 ± 7	15.7 ± 1.9	61.2 ± 3.3	119 ± 9.3
3-day Testosterone treated	Control	344 ± 13	39.2 ± 0.9	103.0 ± 1.8	159 ± 1.5
	Clofibrate	327 ± 8	31.1 ± 1.2^a	72.7 ± 1.8^b	127 ± 3.8^b

* All values are expressed as mean ± SE/100 g body weight.
Significance: p value. $^a < .01$, $^b < .001$.

were injected with testosterone for 3 days, the prostate weight, cholesterol and DNA contents were lower by 20, 30 and 22%, respectively, in the clofibrate treated animals as compared to the rats on the standard diet.

Table IV shows the comparison of the rate of synthesis of cholesterol and DNA in castrated and 3-day testosterone treated castrated rats fed either on standard or clofibrate containing diet. In the castrated rats there was no significant difference between the clofibrated treated or control rats. In testosterone treated animals, rates of synthesis of cholesterol and DNA were reduced 60 and 65%, respectively, in clofibrate treated rats as compared to controls.

TABLE IV. THE EFFECT OF ORAL CLOFIBRATE TREATMENT ON THE TESTOSTERONE DEPENDENT INCREASE IN PROSTATE CHOLESTEROL AND DNA SYNTHESES OF 7-DAY CASTRATED RATS.*

		Rate of synthesis (cpm/g tissue) × 10^5	
	Group	Prostate cholesterol	Prostate DNA
Castrated	Control	0.40 ± .02	0.91 ± 0.12
	Clofibrate	0.36 ± .02	1.08 ± 0.08
3-day Testosterone treated	Control	9.76 ± .58	30.4 ± 1.4
	Clofibrate	5.71 ± .32[a]	20.2 ± 1.8[a]

* All the values are obtained groups of six to eight rats and expressed as mean ± SE.
Significance: p value. [a] < .001.

Figure 1 is a photomicrograph of a normal ventral prostate stained with eosin and hematoxylin taken from a 20-week old control rat while Fig. 2 shows the histopathological effect of 10 weeks of clofibrate treatment on the ventral prostate of a 20-week old normal rat. In comparison with the normal control prostate, there is a large reduction in the infoldings within the acini in the ventral prostate of the clofibrate treated rat. Figure 3 illustrates a more extreme effect of clofibrate treatment on the infolding of the acini in clofibrate treated rats.

Discussion. An increase in the cholesterol content was reported in the benign prostate hyperplasia (10) and in prostate carcinoma (11) in humans. The hypocholesterolemic drugs such as the polyene macrolides (12), cholestyramine (13), colestipol (14) and β-sitosterol (15), by oral route of administration, have been shown to decrease the size of the enlarged prostate. Simfibrate, another hypolipidemic drug, was found to reduce the increase in the nucleic acid content of the

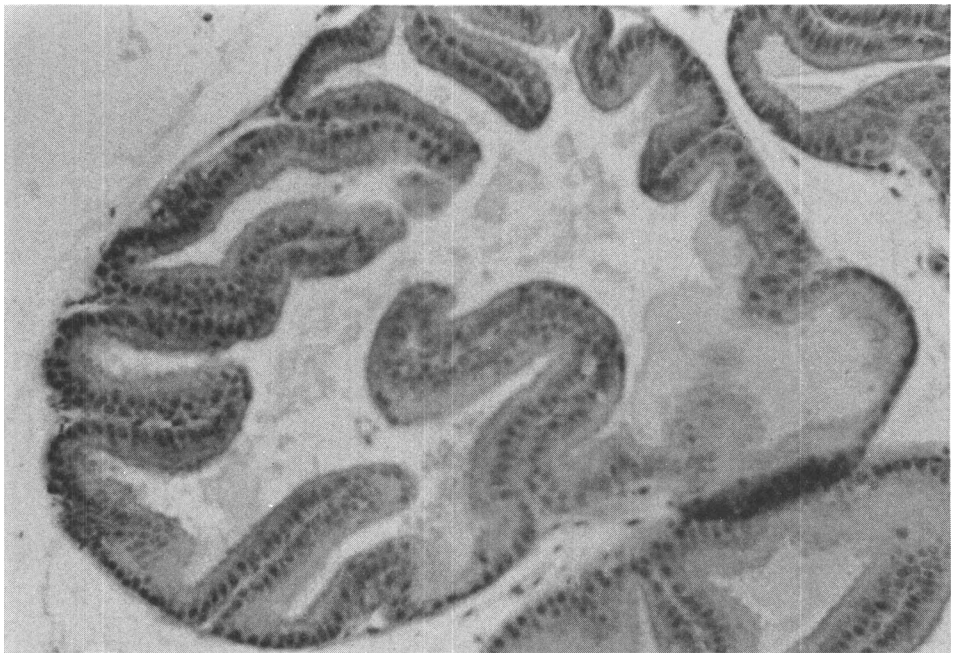

FIG. 1. Microscopic section of a prostate gland from 20-week-old normal rat (hematoxylin and eosin, ×160). Note the presence of excessive infoldings within the acinus of epithelial cell.

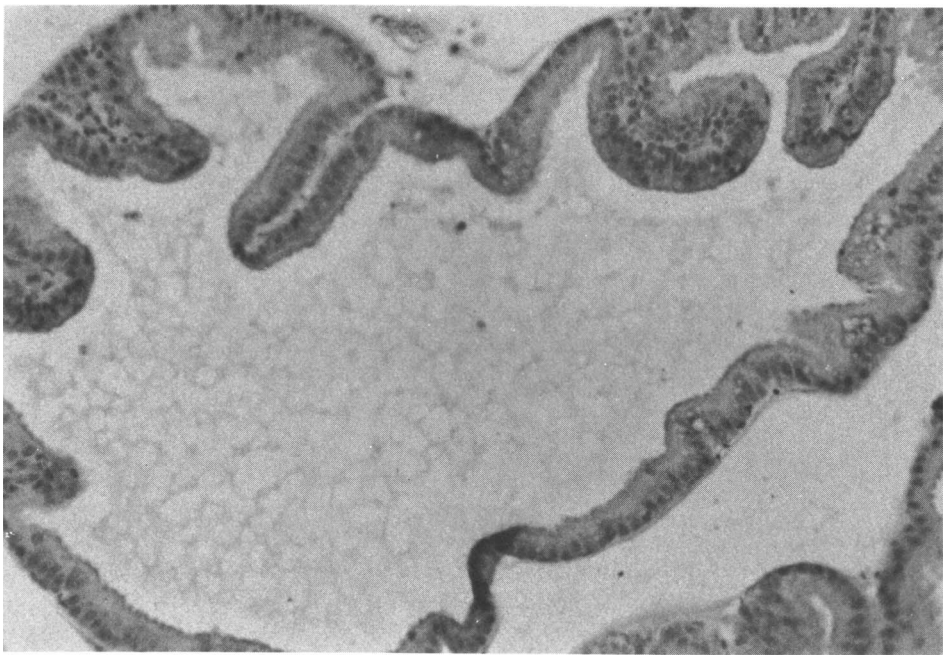

FIG. 2. Microscopic section of a prostate gland from 20-week-old normal rat, after oral administration of clofibrate for 10 weeks (hematoxylin and eosin, ×160). Note the reduction of infoldings within the acini.

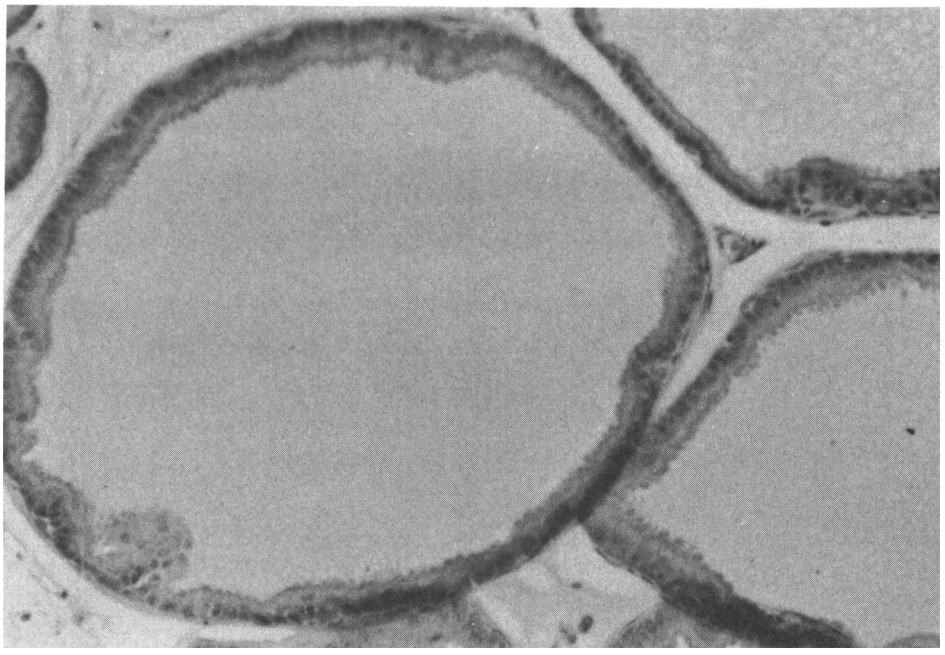

FIG. 3. Microscopic section of a prostate gland from 20-week-old normal rat, after oral administration of clofibrate for 10 weeks (hematoxylin and eosin, ×160). Note the absence of infoldings within the acini.

ventral prostate induced by testosterone administration to the castrated rats (7).

It is evident from the present studies that the treatment with the hypocholesterolemic drug, clofibrate, lowered the amount and the rate of synthesis of cholesterol in the ventral prostate (Tables I and II). The rate of synthesis and the amount of DNA were also lowered

in parallel with the cholesterol content of the prostate. The only known mode of action of clofibrate is the alteration of lipid metabolism including the inhibition of cholesterol synthesis. This would indicate that cholesterol synthesis in the prostate is essential for DNA synthesis.

The cells of the ventral prostate show a high degree of infoldings in the acini of 20-week old rats (Fig. 1). There is no significant infolding in the acini of young adult rats. It is well known that the lumen of the prostate is the reservoir of prostatic secretion which is rich in free cholesterol. In the present study, after clofibrate treatment for 10 weeks, the content of cholesterol in the lumen of the prostate is reduced which is accompanied by a reduction in the infoldings in the acini of cells (Fig. 2 and 3). Therefore it appears possible that a correlation exists between the cholesterol content in the lumen and the extent of infoldings in the acini of the prostate.

Kandutsch *et al.* (16) have shown by using the oxygenated sterols as inhibitors of sterol biosynthesis that the *de novo* sterol synthesis is required for DNA synthesis and cell division by some cultured cells. Clofibrate did not cause any significant decrease in the synthesis or content of cholesterol or DNA in the ventral prostate of castrated rats but when clofibrate treated castrated rats were administered testosterone, the prostate weight, the content and the synthesis of cholesterol and DNA were not restored to levels equal to those seen in rats fed on control diet. This indicates that the inhibition of cholesterol synthesis interferes with the testosterone dependent proliferation of the prostate gland in the castrated rat.

Summary. Clofibrate, a known inhibitor of cholesterol synthesis in the liver, was found effective in inhibiting cholesterol synthesis in the ventral prostate of rats after 2, 3, 4 and 10 weeks of clofibrate treatment by oral route. Blockage of cholesterol synthesis in prostate also inhibited the DNA synthesis in the ventral prostate. When clofibrate treated castrated rats were injected with testosterone, the prostate weight, the content and the synthesis of cholesterol and DNA were not restored to levels equal to those seen in control rats. Clofibrate feeding to normal rats for 10 weeks also caused histopathological changes in the ventral prostate of rats.

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