

Dihydrotestosterone in prostatic hypertrophy: *II. The formation and content of dihydrotestosterone in the hypertrophic canine prostate and the effect of dihydrotestosterone on prostate growth in the dog*

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Dihydrotestosterone in Prostatic Hypertrophy

II. THE FORMATION AND CONTENT OF DIHYDROTESTOSTERONE IN THE HYPERTROPHIC CANINE PROSTATE AND THE EFFECT OF DIHYDROTESTOSTERONE ON PROSTATE GROWTH IN THE DOG

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ABSTRACT Three types of studies have been performed in immature, mature, and hypertrophic prostate glands of the dog. First, the concentrations of testosterone and dihydrotestosterone have been measured in the three types of gland. Dihydrotestosterone was the predominant hormone recovered in all prostates studied and was present in approximately five times higher concentration in the hypertrophic as compared to the other types of dog prostate. Second, pharmacological doses of dihydrotestosterone were administered to castrated dogs for 9 months and resulted in a distinct acceleration of prostatic growth as compared to testosterone treatment. Third, the rates of formation and degradation of dihydrotestosterone were measured in normal and hypertrophic tissue and were found to be essentially the same. These observations suggest that dihydrotestosterone accumulation may be causally linked to the development of canine prostatic hypertrophy. However, the mechanism by which dihydrotestosterone accumulates in the prostate remains to be determined.

INTRODUCTION

Prostatic hypertrophy is a condition which is found commonly in only two species, man and the dog (2-4). The natural history of the condition in the dog is remarkably similar to that of the human disease. In both, prostatic hypertrophy is a common finding in the aging

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male, occurring in dogs over the age of 5 (5) and in men beginning in the 5th decade (2). In both, normal testicular function is necessary for the development of prostatic hypertrophy, and early castration uniformly prevents its occurrence (5, 6). In both, the burst of growth that results in prostatic hypertrophy usually occurs after a several year period during which prostatic size is almost constant (5, 7, 8). Finally, the enlarging gland frequently results in obstructive symptoms, growing posteriorly into the rectum and causing constipation in the dog rather than urinary tract obstruction, which is more commonly seen in man. Thus, although there are anatomical and histological differences between the processes in the two species (3, 4), the prostatic hypertrophies in man and dog have many features in common, including the fact that the pathogenesis of each is poorly understood.

Several lines of evidence have recently accrued to suggest that a continued high rate of conversion of testosterone¹ to dihydrotestosterone within the gland may be involved in the pathogenesis of prostatic hypertrophy. This evidence may be summarized as follows: first, after the administration of testosterone-³H to rats, dihydrotestosterone is the major radioactive steroid bound to the prostatic nuclear chromatin, a presumed site of action of the hormone (9, 10). Second, in 11 animal species, the rate of conversion of testosterone to dihydrotestosterone by prostate slices correlates with the ultimate size of the gland with man and dog having high rates of conversion at all times studied (11, 12).

¹ The following abbreviations are used: testosterone, 17 β -hydroxyandrost-4-en-3-one; dihydrotestosterone, 17 β -hydroxy-5 α -androst-3-one; androstenedione, androst-4-ene-3,17-dione; androstandiol, 5 α -androstane-3 α ,17 β -diol and 5 α -androstane-3 β ,17 β -diol; androstandione, 5 α -androstane-3,17-dione.

Third, in animal species with limited prostatic growth (rabbit and bull), the ability to convert testosterone to dihydrotestosterone occurs only during the active growth phase of the gland and almost completely disappears thereafter (11, 12). Fourth, dihydrotestosterone has been demonstrated to accumulate in greater concentration in hypertrophic human prostate tissue as compared to normal glands (13). Finally, Baulieu, Lasnitzki, and Robel have reported that the addition of dihydrotestosterone to prostate tissue maintained in organ culture results in marked cellular proliferation (14, 15).

The present study was undertaken to determine whether dihydrotestosterone also accumulates in the canine hypertrophic prostate, to investigate the mechanisms by which such an accumulation might occur, and to study the effect of long-term administration of dihydrotestosterone on the growth and histology of the dog prostate.

METHODS

The criteria of Berg (16) have been used to separate the prostate glands of mongrel dogs into three categories, immature (less than 5 g), mature (5-14 g), and hypertrophic glands (more than 14 g).

Measurement of tissue content of androgens. Immature, mature, and hypertrophic prostate glands were frozen at the time of removal; seven to nine glands of each type were pooled and homogenized in methanol in a Virtis homogenizer (The Virtis Co., Inc., Gardiner, N. Y.) as previously described (13). Aliquots of the methanol extracts corresponding to 15-30 g of original tissue were then processed for analysis of testosterone and dihydrotestosterone content by the double isotope derivative technique as described by Siiteri and Wilson (13).

In vitro studies of dihydrotestosterone formation. The techniques used for the measurement of the conversion of testosterone-1,2-³H to dihydrotestosterone by prostate slices have been described in detail previously (11). In brief, the prostates were removed from animals which had been bled to death and were immediately placed in ice-cold Krebs-Ringer phosphate buffer, pH 7.4 for transport to the laboratory. Tissue slices approximately 0.5 mm thick were prepared from the center portion of prostate lobes with the aid of a McIlvain tissue chopper (H. Mickle Co., Gomshall, Surrey, England). The standard incubation mixture consisted of slices (50 mg), glucose (1×10^{-2} M), testosterone-1,2-³H (6.7×10^{-8} M), and Krebs-Ringer phosphate buffer, pH 7.4, in a total volume of 2 ml. The tubes were gassed with 95% O₂-5% CO₂, capped, and incubated at 37°C for 1 hr.

For the homogenate studies, 1 g of tissue slices was homogenized in 19 ml of 0.88 M sucrose in a Dounce homogenizer (Lab Glass Inc., Vineland, N. J.) with 20 strokes of a loose plunger. The homogenate was filtered, and 0.5 ml aliquots were removed for measurement of dihydrotestosterone formation. In the experiments in which subcellular distribution of the reductase was measured, the remainder of the homogenate was centrifuged at 800 g for 10 min. The supernatant was then centrifuged at 104,000 g for 30 min and separated into particles (microsomes plus mitochondria) and soluble fractions. The sediment from the 800 g preparation was resuspended in 1.5 M sucrose, layered

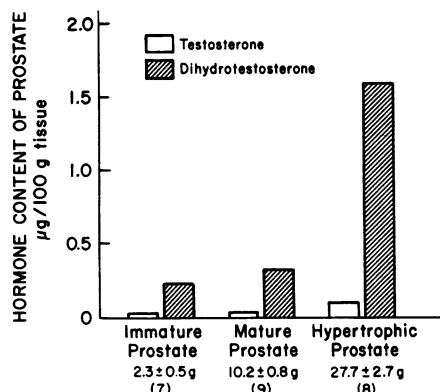


FIGURE 1 Concentration of testosterone and dihydrotestosterone in immature, mature, and hypertrophic dog prostate. The average weight \pm SEM for each pooled group is given, and the number of glands pooled is given in parentheses.

over 5 ml of 2.1 M sucrose, and centrifuged at 23,500 rpm for 90 min in a SW 20 rotor (Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif.). The pellet, which contained at least 95% clean nuclei as determined by light microscopy, was resuspended in 0.88 M sucrose. Aliquots of the whole homogenate, nuclei, particles, and soluble fraction were incubated in 0.01 M Tris buffer, pH 7.4, containing testosterone-1,2-³H, NADP (1×10^{-4} M), glucose-6-phosphate ($4 \times$

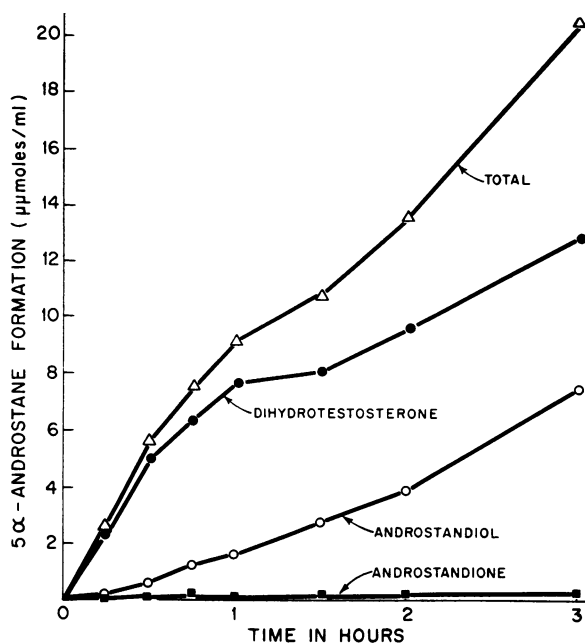


FIGURE 2 Time course of the appearance of various 5 α androstane metabolites after the incubation of prostate homogenates with testosterone-1,2-³H. Each flask contained homogenate (0.5 ml), NADP (1×10^{-4} M), glucose-6-PO₄ (4.3×10^{-3} M), glucose-6-PO₄ dehydrogenase (6.4 IU), testosterone-1,2-³H (3.5×10^{-8} M), and Tris buffer, pH 7.4 (0.01 M) in a total volume of 1.0 ml. The samples were incubated at 37°C for varying periods of time and then processed as described in the text.

TABLE 1
Dihydrotestosterone Formation by Homogenates and Slices of Dog Prostate

Type of prostate.....		Immature	Mature	Hypertrophic
Weight of prostate, g \pm SEM		1.6 \pm 0.5	8.7 \pm 1.0	29.6 \pm 3.6
Dihydrotestosterone formation				
Tissue slices	μ moles/g per hr \pm SEM	288 \pm 69 (4)	276 \pm 26 (10)	234 \pm 38 (10)
Homogenates	μ moles/g per hr \pm SEM	77 \pm 23 (4)	190 \pm 29 (10)	176 \pm 28 (10)
	μ moles/mg of protein per hr \pm SEM	2.3 \pm 0.8 (4)	2.8 \pm 0.4 (7)	2.5 \pm 0.3 (7)

The number of measurements in each experiment is given in parentheses.

10^{-3} M), and glucose-6-phosphate dehydrogenase (6.4 IU) in a total volume of 1 ml at 37°C for varying periods of time. Aliquots of the various fractions were also removed for analysis of protein (17) and desoxyribonucleic acid (18).

The incubations of slices and homogenates were terminated by the addition of 5 volumes of chloroform:methanol (2:1), and the lipids were extracted and backwashed as described by Folch, Lees, and Sloane Stanley (19). Aliquots of the chloroform:methanol solution were then chromatographed by thin-layer chromatography using the chloroform:methanol system of Gomez and Hsia (20) and analyzed for radioactivity as described previously (11).

Administration of testosterone and dihydrotestosterone to intact animals. Male mongrel dogs, judged to be less than 1 yr of age, were divided into four groups. Group 1 (three control animals) received 1 ml of triolein-benzyl benzoate (20:1) by intramuscular injection three times weekly. Group 2 (three animals) were castrated and given 1 ml of the triolein-benzyl benzoate three times weekly. Groups 3 and 4 (four dogs each) were castrated and given 25 mg of dihydrotestosterone or testosterone dissolved in 1 ml of the triolein-benzyl benzoate three times a week. 9 months later the animals were killed. The prostates were removed, dissected free of connective tissue, and weighed. Portions were taken for histological examination, and the rate of dihydrotestosterone formation was measured in 50 mg of slices as described above. Slices (25 mg) were also homogenized in duplicate for the measurement of protein (17), ribonucleic acid (21), and desoxyribonucleic acid (18) as described by Maggio, Siekevitz, and Palade (22).

RESULTS

The results of the analysis of testosterone and dihydrotestosterone content in pooled dog prostate glands are

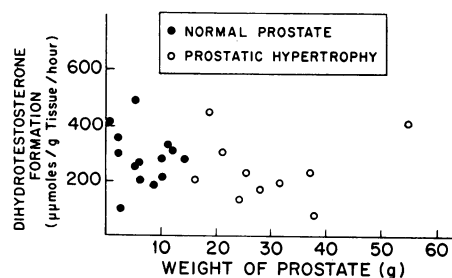


FIGURE 3 The relation between prostate weight and the rate of dihydrotestosterone formation by tissue slices. The tissue slice data from Table I have been plotted as a function of the weight of the prostate.

shown in Fig. 1. The seven glands classed as immature weighed 2.3 ± 0.5 g, the nine mature glands averaged 10.2 ± 0.8 g, and the eight hypertrophic glands averaged 27.7 ± 2.7 g in weight. The testosterone concentration of the hypertrophic glands was slightly increased to $0.03 \mu\text{g}/100$ g in the immature group, $0.03 \mu\text{g}$ in the mature glands, and $0.10 \mu\text{g}$ in the hypertrophic prostates. In each group the concentration of dihydrotestosterone was greater than that of testosterone, $0.23 \mu\text{g}$ in the immature tissue, $0.33 \mu\text{g}$ in the mature glands, and $1.60 \mu\text{g}/100$ g in the hypertrophic glands. This striking increase in the content of dihydrotestosterone in hypertrophic as compared to the normal glands is similar to the changes observed in the hypertrophic prostate of man (13). Furthermore, this increase in dihydrotestosterone content constitutes both a sevenfold increase in concentration and a doubling in the ratio of dihydrotestosterone to testosterone from 8:1 to 16:1 as one compares the immature to the hypertrophic group; and if one takes into account the increased size of the glands, the total amount of dihydrotestosterone accumulation is even more striking. It is also of interest that the content of dihydrotestosterone is greater in the dog prostate than in that of man (13). The reasons for these differences are as yet unclear.

In order to determine whether the accumulation of dihydrotestosterone in the hypertrophic prostate was the result of increased transport of the substrate (testosterone) into the cell or increased amounts of enzyme and/or cofactors required for the conversion of testosterone to dihydrotestosterone, the formation of dihydrotestosterone was compared in homogenates and tissue slices from immature, mature, and hypertrophic prostate glands. In the first such experiments the optimal conditions for the measurement of the rate of conversion of testosterone to dihydrotestosterone in homogenates were established.

The relationship between the testosterone concentration in the incubation media and the rate of formation of dihydrotestosterone by prostatic homogenates was first investigated. The rate was roughly linear at all concentrations studied between 2×10^{-8} and 5×10^{-7} M.

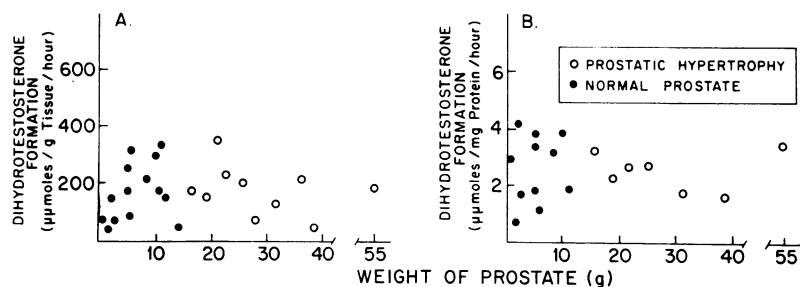


FIGURE 4 The relation between prostate weight and the rate of dihydrotestosterone formation by homogenates. The homogenate data from Table I have been plotted as a function of the weight of the prostate. A. Dihydrotestosterone formation per unit weight of tissue. B. Dihydrotestosterone formation per milligram of protein.

For all subsequent studies the concentration of testosterone used was 6.8×10^{-8} M, a level which is above the blood concentration of testosterone in man (23) and which was chosen in order to minimize the effects of endogenous substrate on the assay.

The time course of the appearance of various 5α -androstane metabolites after the incubation of homogenates with testosterone is shown in Fig. 2. In these preparations, which contained the NADPH₂ generating system, the only significant 5α -androstane derivatives recovered were dihydrotestosterone and androstandiol. The rate of formation of these two derivatives was almost linear for 1 hr, and all subsequent incubations were terminated at this time. In contrast, as is true in slices of rat prostate (11, 12), the major metabolite observed in the tissue slices was androstandione.

The relation between the concentration of NADP and the rate of dihydrotestosterone formation was also examined. In the absence of NADP, very little dihydrotestosterone was formed; and at all concentrations of NADP tested between 1×10^{-5} M and 3×10^{-4} M, the rate was constant. In all subsequent studies, a NADP concentration of 1×10^{-4} M was used.

Finally, the relation between the amount of homogenate added and the rate of dihydrotestosterone formation was studied. In this study the amount of dihydrotes-

tosterone formed was proportional to the amount of homogenate added between 0.1 and 0.8 ml. Therefore, for the comparative studies 0.5 ml of a 1:20 homogenate was incubated for 1 hr in a total volume of 1 ml of Tris buffer, pH 7.4, containing testosterone-1,2-³H (6.8×10^{-8} M), glucose-6-PO₄ (4.3×10^{-3} M), NADP (1×10^{-4} M), and glucose-6-phosphate dehydrogenase (6.4 IU). This assay system meets reasonable criteria for an enzymatic analysis except for the fact that it is not zero order with respect to substrate concentration. The measurements in tissue slices were done at identical concentrations of testosterone-1,2-³H (6.8×10^{-8} M) and under similarly linear time and weight conditions (11).

The results of such measurements in 24 dog prostates are given in Table I. The weights of the prostates used here are similar to those in Fig. 1, averaging 1.6, 8.7, and 29.6 g in the immature, mature, and hypertrophic groups. No difference was observed in the rate of dihydrotestosterone formation by the tissue slices in these experiments, 288 ± 69 , 276 ± 26 , and 234 ± 38 μmoles/g per hr in the three groups, respectively. In the homogenate studies, there was an apparent increase in dihydrotestosterone formation from 77 ± 23 μmoles/g per hr in the immature glands to 190 ± 29 in the mature prostates and 176 ± 28 in the hypertrophic glands, when calculated on a wet weight basis. Since

TABLE II
Intracellular Distribution of 5α -Reductase in Dog Prostate

Type of prostate.....	Mature (4)	Hypertrophic (4)
Weight of prostate, g \pm SEM	8.3 \pm 1.2	31.0 \pm 4.1
5α -reductase concentration, μmoles/g per hr	134 \pm 33	177 \pm 43
Per cent 5α -reductase recovery in:		
Washed nuclei	45 \pm 8	27 \pm 7
Microsomes plus mitochondria	53 \pm 9	72 \pm 8
104,000 g supernatant	1 \pm 1	2 \pm 1

The recovery in nuclei has been corrected for the percentage of recovery of the total homogenate DNA in the washed nuclei.

TABLE III
Catabolism of Dihydrotestosterone by Slices of Dog Prostate

Dihydrotestosterone conversion to	Prostate		
	Immature	Mature	Hypertrophic
		% \pm SEM	
Androstandione	14.8 \pm 4.5	31.0 \pm 2.9	25.0 \pm 2.6
Androstandiol	3.0 \pm 0.6	3.2 \pm 0.9	3.3 \pm 0.6

The data from the tissue slice experiment of Table I have been calculated as the per cent of total 5 α -androstande derivatives recovered by thin-layer chromatography.

the immature glands contain large amounts of stroma and are more difficult to homogenize than the other glands, it was thought that this apparent increase in enzyme activity might be the result of this technical difficulty; consequently, the homogenate data were corrected for the protein actually recovered in the filtered homogenate, and it can be seen that the rates of formation of dihydrotestosterone by these three types of homogenates were almost identical, 2.3 \pm 0.8, 2.8 \pm 0.4, and 2.5 \pm 0.3 μ moles/mg of protein per hr. On the basis of these measurements in tissue slices and broken cell preparations, it was concluded that there is probably no difference in the concentration of the reductase enzyme, in the cofactor(s) required for the reduction, or in the intracellular transport of the hormone between immature, mature, and hypertrophic dog prostates.

Since the hypertrophic group includes a wide spread of glands from near normal in size to some very large prostates, the data in Table I have been plotted in relation to the size of the individual glands (Figs. 3 and 4). Although there is some scatter of the data, no significant change in dihydrotestosterone formation was observed with increasing size of the glands either in the slice studies (Fig. 3) or the homogenates (Fig. 4). These findings are in keeping with the previous conclu-

sion that the increased concentration of dihydrotestosterone in the hypertrophic dog prostate is not due to an increased rate of dihydrotestosterone formation in this tissue.

However, these studies reflect what is taking place in the whole cell. Since dihydrotestosterone formation in prostate occurs both in the nucleus (9, 12) and in the particulate fraction of the cytoplasm (12), the possibility was then investigated that the intracellular site for dihydrotestosterone formation might change with age (Table II). In this study the intracellular distribution of the enzyme was compared between four mature prostates and four hypertrophic glands. There was no striking difference between the amount of enzyme in nuclei (45 and 27%) or in the particulate fraction (53 and 72%). This intracellular distribution is almost identical to that of the rat prostate; and as has previously been reported for rat prostate (12), almost no 5 α -reductase activity was demonstrable in the 104,000 *g* supernatant fraction of either type of gland. As the result of these studies it was concluded that neither the concentration nor the intracellular distribution of the enzyme changes with age in the dog prostate.

Since the formation of dihydrotestosterone does not increase with age, the next possibility examined was whether the rate of catabolism of dihydrotestosterone might be diminished in the hypertrophic prostate. The following two principal metabolites of dihydrotestosterone have been recovered from the dog prostate: 5 α -androstande-3,17-dione, the major metabolite observed in tissue slices, and a mixture of 5 α -androstande-3 β ,17 β -diol and 5 α -androstande-3 α ,17 β -diol, the principal metabolites recovered from homogenates. The conversion of dihydrotestosterone to these metabolites were compared in the tissue slice studies of the three types of prostate (Table III). The data from the slice studies of Table I have been expressed as the amount of androstandione and androstandiol formed as a per cent of the total 5 α -

TABLE IV
Effect of Testosterone and Dihydro-

Group	Operation	Treatment	Number of animals	Average weight	Prostate weight	Dihydrotestosterone formation in prostate slices
				kg	g \pm SEM	μ moles/g per hr \pm SEM
A	None	Triolein	3	8.3	2.8 \pm 0.4	309 \pm 41
B	Castration	Triolein	3	10.8	1.8 \pm 0.5	200 \pm 32
C	Castration	Dihydrotestosterone	4	8.0	6.6 \pm 0.6	221 \pm 24
D	Castration	Testosterone	4	7.8	3.6 \pm 1.1	332 \pm 65

Young males (<1 yr in age) were injected either with 25 mg of testosterone or dihydrotestosterone dissolved in triolein-benzyl benzoate (20:1) or with the triolein-benzyl benzoate alone three times weekly. At the end of 9 months the animals were killed, and the prostates were removed and treated as described in the text. Photomicrographs of representative histological preparations from each group are shown in Fig. 5.

androstane derivatives recovered. In no instance was a decreased rate of catabolism by either pathway observed; indeed the rate of formation of androstandione doubled between the immature and mature glands. Since these metabolites account for approximately 90% of the recovered radioactivity, it was concluded that there is no decrease in the catabolism of dihydrotestosterone in the hypertrophic prostate.

The final question that was asked was whether dihydrotestosterone could be the cause of prostatic hypertrophy in this species. As is shown in Table IV, the effect of the administration of pharmacological amounts of testosterone and dihydrotestosterone on the growth of the immature dog prostate was studied in castrated dogs. The average prostate weight was 2.8 g in the control group and fell to about 1.8 g 9 months after castration. In the testosterone-treated castrates the average weight increased to about 3.6 g. In contrast, in the dihydrotestosterone-treated animals the average weight was 6.6 g, a distinct acceleration of growth but not true prostatic hypertrophy. If dihydrotestosterone accumulation is a causal factor in the development of prostatic hypertrophy in the dog, a much longer time period than the 9 months chosen for this study may be required for exogenous dihydrotestosterone to cause prostatic weights in excess of 14 g. That such a cause-effect relationship may in fact exist is suggested by the results of the histological studies of these prostate glands (Fig. 5). In panel *a* of Fig. 5 is shown a control gland; the acini are lined with columnar epithelium and surrounded by thin fibrous septae. After castration (Fig. 5, panel *b*), virtually all glandular elements have disappeared, leaving only undifferentiated stroma. The testosterone-treated tissue (Fig. 5, panel *d*) appears normal, whereas the prostate from the dihydrotestosterone-treated animal contains large, irregular-shaped alveoli (Fig. 5, panel *c*), a finding characteristic of canine prostatic hyper-

trophy (5, 15). Thus, the production by dihydrotestosterone of growth and of microcyst formation in dog prostate are compatible with a possible role for this hormone in the production of prostatic hypertrophy in this species.

The effect of these hormonal manipulations on the rate of formation of dihydrotestosterone by prostate slices and on the content of DNA, protein, and RNA is also summarized in Table IV. Dihydrotestosterone formation in the slices was the same in the control animals (309 ± 41 $\mu\text{moles/g}$ per hr) as in the prostates studied in Table I. The rate was somewhat lower per gram in the castrate and dihydrotestosterone-treated groups (200 and 221 $\mu\text{moles/g}$ per hr) and was unchanged in the testosterone-treated group (335 ± 65 $\mu\text{moles/g}$ per hr). The results of the chemical analyses are of interest. The total DNA (milligrams per gland) was 7.8 in the control group, 4.3 in the glands from the castrated animal, 15.2 in the dihydrotestosterone-treated glands, and 13.0 in the testosterone-treated group. Thus, testosterone treatment results in almost as much cell proliferation as does dihydrotestosterone. In contrast the RNA concentration was highest in the dihydrotestosterone-treated animals; this difference is even more apparent if the RNA is expressed per milligram of DNA—1.4 in the controls, 0.6 in the castrates, 2.0 in the dihydrotestosterone-treated group, and 0.9 in the testosterone-treated animals. The protein concentrations are similar to those of the RNA. Thus, the finding of an increased total content of DNA and of an increased RNA per unit of DNA suggests that dihydrotestosterone treatment has caused both hyperplasia and hypertrophy in the dog prostate as compared to the control group.

DISCUSSION

Two conclusions appear warranted from these studies. First, dihydrotestosterone rather than testosterone itself

testosterone on the Dog Prostate

Protein		DNA		RNA	
<i>mg/g</i> \pm SEM	<i>avg. mg/gland</i>	<i>mg/g</i> \pm SEM	<i>avg. mg/gland</i>	<i>mg/g</i> \pm SEM	<i>avg. mg/gland</i>
128 \pm 2	358	2.8 \pm 0.2	7.8	3.9 \pm 0.8	10.9
97 \pm 4	175	2.4 \pm 0.3	4.3	1.5 \pm 0.2	2.7
136 \pm 6	897	2.3 \pm 0.2	15.2	4.7 \pm 0.7	31.0
122 \pm 3	439	3.6 \pm 0.2	13.0	3.4 \pm 0.4	12.2

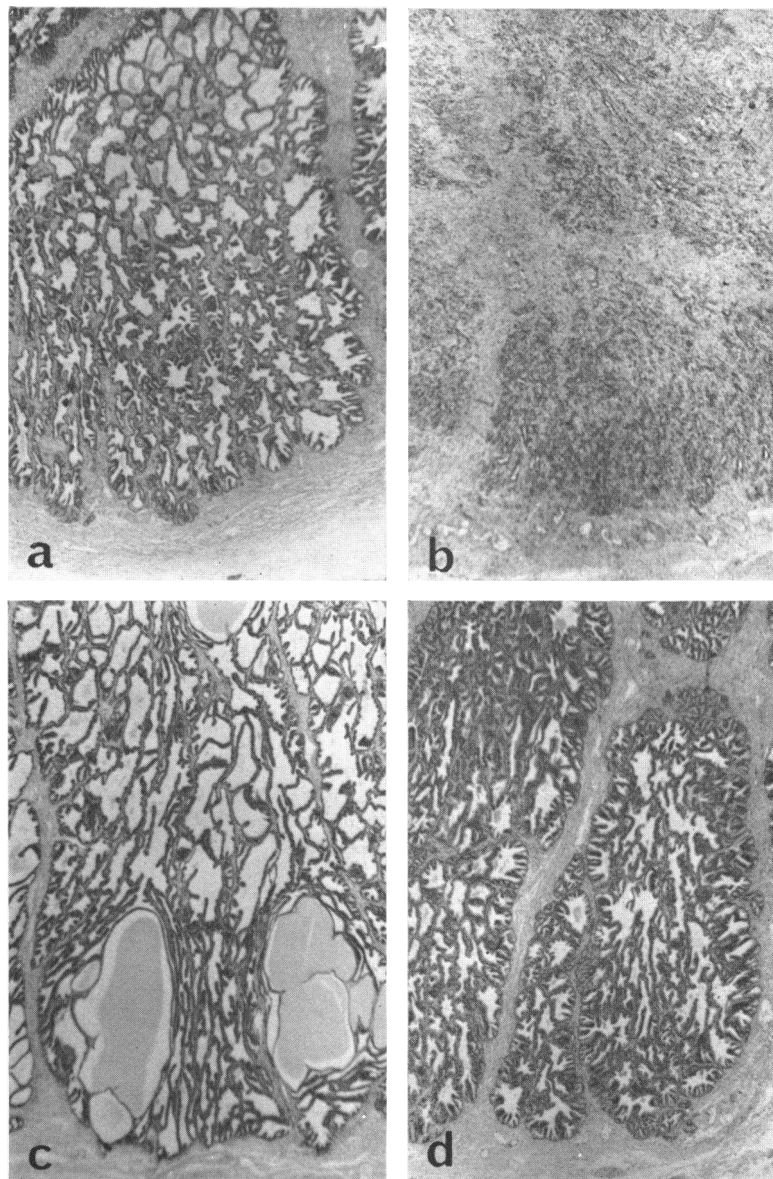


FIGURE 5 The effect of dihydrotestosterone and testosterone on the histology of the dog prostate gland. Representative sections from the experiments in Table IV have been stained with hematoxylin and eosin. (a) Control prostate, (b) prostate from a castrated dog, (c) prostate from a castrated dog treated with dihydrotestosterone, and (d) prostate from a castrated dog treated with testosterone. 40 \times .

is the major hormone recovered at all ages from the dog prostate, and both the absolute content and the ratio of dihydrotestosterone to testosterone are increased in the hypertrophic as compared with the normal immature and mature glands. Second, in view of the fact that dihydrotestosterone administration causes prostatic growth and histologic changes reminiscent of prostatic hypertrophy, it is conceivable that the accumulation of di-

hydrotestosterone over a period of several years may be involved in the pathogenesis of prostatic hypertrophy in this species. Clear-cut proof for such a relationship would require the production of distinctive prostatic hypertrophy by dihydrotestosterone administration, a phenomenon which did not occur in the 9 month study described here and which may require administration of the hormone for much longer periods of time.

The present studies leave totally unexplained, however, the mechanism(s) by which the accumulation of dihydrotestosterone occurs. In studies both in tissue slices and in homogenates, it has not been possible to demonstrate either an increased rate of dihydrotestosterone formation or a diminished rate of dihydrotestosterone catabolism in the hypertrophic dog prostate. It is most likely that dihydrotestosterone accumulation over such an extended period may be due to subtle changes in either formation or catabolism which are not demonstrable by the *in vitro* techniques used here. Second, it is also possible that the rate of dihydrotestosterone formation exceeds its rate of catabolism and turnover at all ages, that continued growth is the result, and that striking accumulation of dihydrotestosterone eventuates only as the capacity for cell division and growth decreases in the hypertrophying glands. A third alternative is that the critical factor in determining the rate of dihydrotestosterone turnover is not catabolism to other metabolites but rather excretion from the gland or some other aspect of turnover which decreases with age. Finally, it is also possible that dihydrotestosterone in the hypertrophic gland may arise from some source other than the direct reduction of prostatic testosterone, either arising directly or indirectly from a hitherto undescribed pathway.

If dihydrotestosterone accumulation is a common factor in the pathogenesis of human and canine prostatic hypertrophy, it is not clear why the histological patterns are so different in the two species. The disorder in man starts in the periurethral region and results ultimately in a fibronodular proliferation (2, 3), whereas in the dog a diffuse hyperplasia of the glandular elements predominates (3, 4, 5, 10). These striking histological differences might be due to variations in the response of different species to the same stimulus or may be the result of some more fundamental difference.

On the basis of the evidence assembled in these reports, it may be concluded that dihydrotestosterone accumulation in the prostate may be involved in the pathogenesis of prostatic hypertrophy in dog and man but that a number of unanswered problems must be solved before such a relationship can be considered as definite.

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