Deoxyribonucleic Acid and Polyamine Synthesis in Rat Ventral Prostate

EFFECTS OF AGE OF THE INTACT RAT AND ANDROGEN STIMULATION OF THE CASTRATED
RAT WITH TESTOSTERONE, 5 α -DIHYDROTESTOSTERONE AND
5 α -ANDROSTANE-3 β ,17 β -DIOL

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The relationship between polyamine synthesis, growth and secretion *in vivo* was examined in ventral prostates from: (a) intact rats aged 3–60 weeks; (b) animals castrated for 7 days before injection with 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androstane-3-one), testosterone and 5 α -androstane-3 β ,17 β -diol for up to 10 days; (c) rats injected with the 3 β ,17 β -diol immediately after castration. Ornithine decarboxylase activity and the concentrations of putrescine, spermidine and spermine were measured. DNA-synthetic activity was monitored by measuring [¹²⁵I]iododeoxyuridine incorporation. An enhanced spermidine/spermine molar ratio reflected increased activity of the prostate. The ratio was higher (>2) in prostates from sexually immature animals, than in the intact adult (1.5), suggesting that the ratio was indicative of the proliferative activity of the tissue. However, in the androgen-stimulated castrated rat, enhanced spermidine/spermine ratios tended to correlate with hypertrophy and secretion. In both sets of experiments there was a linear relationship between protein and spermidine content. High spermidine/spermine molar ratios were the consequence of a relatively low rate of accumulation of spermine relative to spermidine and protein. The relationship between polyamine synthesis and DNA-synthetic activity was investigated in cultured prostate. A combination of insulin (3 μ g/ml) and testosterone (0.1 μ M) caused a stimulatory response in the incorporation of [¹²⁵I]iododeoxyuridine and in cell division, despite a depleted polyamine content and low ornithine decarboxylase activity in the cultured tissue.

The prostate in many mammalian species is a very rich source of putrescine and the two polyamines spermidine and spermine (Tabor & Tabor, 1964; Mann, 1964); their role in reproductive physiology has been reviewed by Williams-Ashman & Lockwood (1970). It seems that the functional significance of these compounds is related to the activity of the prostatic epithelial cells, rather than to some aspect of the physiology of spermatozoa (for leading references, see Pegg *et al.*, 1970). To obtain more information on the relationship between polyamines and growth and differentiation of the prostate, we have examined the prostatic concentrations of these amines in three experimental situations: (1) intact animals aged between 3 and 60 weeks; (2) 7-day-castrated rats injected daily with three androgens differing in their biological potencies; (3) animals injected with androgen immediately after castration. In addition, studies have been carried out on polyamine synthesis in cultured prostate. The relationship between DNA synthesis and polyamine synthesis has been investi-

gated by measurement of [¹²⁵I]iododeoxyuridine incorporation and ornithine decarboxylase activity respectively.

[¹²⁵I]iododeoxyuridine is specifically incorporated into DNA (Hughes *et al.*, 1964) and has provided a useful approach to measurements of cellular proliferation and cell loss (Micklem, 1972; Dethlefsen, 1974). Apart from the fact that it provides a very simple method for the assessment of DNA-synthetic activity in the prostate (Shipman *et al.*, 1975; Donaldson & Thomas, 1976), it has the additional advantage over [³H]thymidine that reutilization of this nucleotide is lower (Dethlefsen, 1971; Clifton & Cooper, 1973).

Ornithine decarboxylase is thought to be the rate-limiting enzyme in the synthetic pathway of the polyamines (Williams-Ashman *et al.*, 1972). Castration results in a rapid decline in ornithine decarboxylase activity, which can be restored by injection of testosterone (Pegg & Williams-Ashman, 1968b), but not by cyclic AMP (Mangan *et al.*, 1973). Further,

there is no clear correlation between DNA-synthetic activity, or cell division, and the activity of ornithine decarboxylase in the prostate (Fuller *et al.*, 1975). These findings, coupled with the fact that the enzyme is believed to have an extremely short half-life, suggest that ornithine decarboxylase is a sensitive parameter of androgen action, which is not directly related to proliferative activity in the prostate.

Materials and Methods

Materials

[5-¹²⁵I]Iodo-2'-deoxyuridine and L-[1-¹⁴C]ornithine monohydrochloride were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Pyridoxal 5'-phosphate, dithiothreitol, bovine serum albumin, calf thymus DNA, testosterone, 5 α -dihydrotestosterone and 5 α -androstane-3 β ,17 β -diol were all obtained from Sigma (London) Chemical Co., London S.W.6, U.K. Citric acid analysis kit was obtained from Boehringer (London) Corp., London W.5, U.K.

Preparation of animal tissue

Male Wistar rats were used. Castration was performed via the scrotal route under ether anaesthesia. Androgens were injected subcutaneously in propylene glycol. The control animals received propylene glycol alone. Animals were killed by cervical dislocation. The ventral prostatic lobes were dissected free of fat and capsule *in situ* and excised. They were then blotted quickly, weighed and maintained on ice. Some tissue was teased into 2mm³ explants for incubation with [¹²⁵I]iododeoxyuridine. A weighed amount was homogenized and portions taken for the following assays: (1) ornithine decarboxylase; (2) polyamine and amino acid analysis; (3) protein; (4) DNA; (5) citric acid.

[¹²⁵I]Iododeoxyuridine incorporation

Portions of tissue (2mm³; approx. 20mg) were incubated for 6h with 2 μ Ci of [¹²⁵I]iododeoxyuridine in 3ml of Eagle's minimum essential medium as described by Shipman *et al.* (1975). The explants were removed, blotted and weighed. The tissue was fixed in ethanolic Bouin's solution for 24h and then washed five times in 70% (v/v) ethanol to remove free precursor. This procedure allowed histological examination of the tissue after it had been counted in a Gamma Set 500 radioisotope counter. The results were expressed as d.p.m./ μ g of DNA or /mg wet weight.

Ornithine decarboxylase assay

Weighted tissue samples were homogenized in 20vol. of ice-cold distilled water, containing 5mm-dithiothreitol, with a Teflon/glass pestle and mortar; 5mg of tissue was used per determination. The assay medium contained 5mm-dithiothreitol, 0.1mm-pyrid-

oxal phosphate and 50mm-Na₂HPO₄/NaH₂PO₄ buffer, pH7.2. In the earlier experiments, 0.1 μ Ci of L-[1-¹⁴C]ornithine monohydrochloride (15mCi/mmol) was used as substrate. The method of assay is described by Fuller *et al.* (1975). Since the assay was carried out under non-saturating conditions of substrate, results were expressed as d.p.m. of ¹⁴CO₂ liberated/h. The amount of ¹⁴CO₂ released was linear over the range 5–10mg of tissue, but there was occasionally some divergence from linearity at 25–40mg of tissue/assay. Later assays were carried out under saturating conditions of substrate, with 0.5 μ Ci of L-[1-¹⁴C]ornithine per assay tube in the presence of 150nmol of carrier ornithine. Results were expressed as nmol of ¹⁴CO₂/h per mg of protein. The amount of ¹⁴CO₂ released was linear over the range 5–35mg of tissue. Ornithine decarboxylase activity was in the range 0.8–2.3nmol of ¹⁴CO₂/h per mg of protein for prostates from animals aged 13–24 weeks, compared with 3.0nmol of ¹⁴CO₂/h per mg of protein reported by Mangan *et al.* (1973) for rats aged 8–10 weeks. Pegg *et al.* (1970) gave the activity of their normal controls (age not specified) as 0.34nmol of ¹⁴CO₂/h per mg of protein. However, in the latter study, the thiol-protecting agent dithiothreitol, as used by Jänne & Williams-Ashman (1970), was not included. The activity and stability of the enzyme is markedly increased by dithiothreitol. We found that tissue stored at –21°C in the presence of 5mm-dithiothreitol lost 20% activity over 6 days. Tissue homogenized and assayed in the absence of dithiothreitol had only 16% of the normal activity; no activity was detected after storage for 2 days at –21°C. In view of the possible inhibitory effects of polyamines on ornithine decarboxylase activity (Pegg & Williams-Ashman, 1968a; but see also Jänne & Williams-Ashman, 1971) we have investigated the effects of adding to the assay mixture putrescine (1.5, 3 and 15nmol), spermidine (40, 80 and 400nmol), spermine (30, 60 and 300nmol) and citric acid (35, 70 and 350 μ g). Even the highest concentrations (representing about a 10-fold increase over the normal concentration in the assay tube for 5mg of tissue) did not affect the enzyme activity by more than 10%.

Polyamine and amino acid analysis

Samples of the homogenate prepared above were agitated with an equal volume of aq. 10% (w/v) trichloroacetic acid, left on ice for 30min and then centrifuged at 800g at 4°C. The supernatant was then removed and stored at –20°C before analysis.

A Locarte automatic analyser was used. Separation of the amino acids and polyamines was achieved by ion-exchange chromatography on a cation-exchange resin of 8%-cross-linked sulphonated polystyrene beads in the sodium form, contained in a 12cm column. Citric acid/sodium citrate buffers were used

(buffer 1, 0.35 M, pH 5.28; buffer 2, 1 M, pH 6.65). Flow rates for reagent and eluate were respectively 30 ml/h and 72 ml/h. The following elution regime allowed the separation and on-line (Nova 1220) estimation of ornithine, arginine, putrescine, spermidine and spermine, by using the ninhydrin colour reaction: buffer 1, 35 min, no NaCl present, 35°C; buffer 2, 20 min, no NaCl, 65°C; buffer 2, 50 min, 1 M-NaCl, 65°C; buffer 2, 130 min, 2 M-NaCl, 65°C. Our results for prostates of animals aged between 20 and 60 weeks (Table 1) are similar to those cited by Tabor & Tabor (1964) (spermidine, 7.7 $\mu\text{mol/g}$; spermine, 5.7 $\mu\text{mol/g}$) and Moulton & Leonard (1969) (spermidine, 11 $\mu\text{mol/g}$). Pegg *et al.* (1970) determined the normal concentrations to be: putrescine, 0.36 $\mu\text{mol/g}$; spermidine, 6.20 $\mu\text{mol/g}$; spermine, 6.94 $\mu\text{mol/g}$. The relatively low value for their spermidine concentration is reflected in the spermidine/spermine ratio of 0.89, compared with 1.5 in the present work and 1.35 for the results of Tabor & Tabor (1964). The difference is not due to the procedures used for the initial extraction of the homogenate. Essentially the same results were obtained when the tissue was homogenized with 0.01 M-HCl as described by Pegg *et al.* (1970).

Other assays

Citric acid was measured by using a Boehringer food analysis kit. Protein was determined by the method of Geiger & Bessman (1972), which corrects for the presence of dithiothreitol. Bovine serum albumin was used as standard. DNA was measured by the method of Merchant *et al.* (1964), with calf thymus DNA dissolved in 5% (w/v) HClO_4 as standard. All spectrophotometry was performed in a Gilford 240 spectrophotometer fitted with rapid-sampling device.

Organ culture

Prostates were teased into 2 mm³ portions. Explants were placed on lens tissue supported on a stainless-steel grid in a Petri dish containing Eagle's minimum essential medium (3 ml) supplemented with benzylpenicillin (3 $\mu\text{g/ml}$) and streptomycin (7 $\mu\text{g/ml}$). The dishes were placed in a McIntosh and Fildes jar and

gassed in humidified $\text{O}_2 + \text{CO}_2$ (95:5). The medium was changed every 2 days. Mitotic activity of the cultured tissue was determined as described by Shipman *et al.* (1975). A 0.02% solution of vincristine sulphate (20 μl) was added to the culture medium, and the explants were harvested after 6 h.

Results

Changes with age of animal

The concentrations of ornithine, putrescine and polyamines were compared in ventral prostates from sexually immature and two groups of mature rats (Table 1). The total polyamine content of the prostate at 3 weeks was about 80% that of the adult value. The small increase in total polyamine concentration with age was due mainly to a progressive rise in the spermine concentration (Table 1, Fig. 1a); the spermidine concentration did not alter much between 3 and 60 weeks. The ratio spermidine/spermine was high in the immature animal, decreased with age until about 15 weeks and then remained at about 1.5 (Fig. 1b).

Relationship between proliferative activity and polyamine synthesis in cultured prostate

Ornithine decarboxylase activity was examined in rat prostate cultured in the presence and absence of insulin and testosterone (Figs. 2a and 2b). Even in the presence of the two hormones, activity in the explants fell after 1 day of culture to 7% of that of the fresh tissue; in the absence of hormone supplements the activity was even lower. During the first 3 days of culture the tissue was clearly metabolically viable in other respects, since pyruvate kinase and lactate dehydrogenase activities were maintained. The tissue also showed the expected stimulatory response to androgen both in terms of incorporation of [¹²⁵I]-iododeoxyuridine and increased mitotic activity. Fig. 3 shows the changes in the concentrations of putrescine, spermidine and spermine in the tissue. Even in the presence of testosterone and insulin, the concentrations of both polyamines were markedly decreased after 1 day of culture, spermidine being lost preferentially. Despite the fact that the medium was

Table 1. *Effect of age on the concentration of ornithine, putrescine and polyamines in rat ventral prostate*
Each determination was carried out on pooled material from three adult rats or five immature animals. Results are means $\pm$ S.E.M.

Age (weeks)	No. of determinations	Concentration ($\mu\text{mol/g}$)				
		Ornithine	Putrescine	Spermidine	Spermine	Putrescine + polyamines
3-8	7	0.16 $\pm$ 0.06	0.58 $\pm$ 0.10	8.0 $\pm$ 0.4	2.9 $\pm$ 0.2	11.6 $\pm$ 0.5
11-19	7	0.14 $\pm$ 0.06	0.27 $\pm$ 0.07	7.3 $\pm$ 0.4	4.6 $\pm$ 0.3	12.2 $\pm$ 0.5
20-60	8	0.24 $\pm$ 0.06	0.34 $\pm$ 0.07	9.0 $\pm$ 0.4	5.9 $\pm$ 0.3	15.2 $\pm$ 0.5

Fig. 1. Age-related changes in (a) spermine concentration and (b) spermidine/spermine molar ratio in rat prostate

changed on the second day of culture, the concentrations of the two polyamines were no different on day 4 from on day 2. It is possible that these tissue concentrations are a measure of the intracellular content of the prostatic cells, once the stored secretory material has been leached out.

Effect of androgen stimulation on castrated rats

Androgens were injected daily into 7-day-castrated rats, and tissues were examined on days 0, 2, 3, 4 and 10 of androgen treatment. This approach permitted a comparison of the initial stimulatory response to androgens, with the effects seen later when mitotic activity had desynchronized (Tuohimaa & Söderström, 1974; Lesser & Bruchovsky, 1973, 1974).

Table 2 shows the concentration of putrescine and polyamines in prostates of castrated rats (aged 24 weeks) injected with 5 α -dihydrotestosterone for 2, 3, 4 and 10 days. The spermidine concentration increased rapidly, and normal values were obtained by day 4 of treatment. The spermine concentration had not returned to normal by day 10 of treatment. In

Fig. 2. Properties of rat prostate cultured (a) in the absence of hormone supplements and (b) with insulin (3 μg/ml) and testosterone (0.1 μM)

Animals were aged 12 weeks. ▲, Ornithine decarboxylase; □, lactate dehydrogenase; ○, pyruvate kinase; ■, incorporation of $[^{125}\text{I}]$ iododeoxyuridine; ●, number of arrested cells/6h per 10^3 cells on incubation with vincristine sulphate.

fact, the spermine value on day 10 was lower than in the 7-day-castrated rat, and in animals injected with testosterone. Similar results were obtained with rats aged 14 weeks.

Fig. 3. Changes in the concentration of putrescine (■), spermine (▲) and spermidine (●) in rat ventral prostate maintained in organ culture for up to 4 days. The medium was changed on day 2. Animals were 12 weeks old. About 80mg of tissue was used/determination. Essentially the same results were obtained when the medium was supplemented with insulin (3 μg/ml) alone and in combination with testosterone (0.1 μM).

Fig. 4 shows the change in the spermidine/spermine ratio, and the change in weight of the prostate, both for the 5α-dihydrotestosterone-stimulated animals and for 7- and 17-day-castrated controls. The regressed prostate had a much lower spermidine/spermine ratio than tissue from the intact animal.

Fig. 4. Comparison of the effects of castration and daily injection of 5α-dihydrotestosterone (400 μg/100g body wt.) on the wet weight and spermidine/spermine molar ratio. Rats were 24 weeks of age. Pooled material from two to three animals was used/determination. ●, Spermidine/spermine molar ratio; ▲, mg wet weight of tissue/prostatic lobe. ----, Changes in prostates of control animals which were not injected with 5α-dihydrotestosterone after the 7th day after castration.

Table 2. Effect of daily injection of androgen (400 μg/100g body wt.) on polyamine concentrations in ventral prostates from castrated rats

Injectons were started 7 days after castration. Animals were killed 24 h after the last injection. Pooled material from two to three animals was used/determination.

Treatment	Concentrations (μmol/g)		
	Putrescine	Spermidine	Spermine
Animals aged 24 weeks			
Intact control	0.22	6.45	4.32
7-day-castrated	0.10	3.93	3.21
7-day-castrated + 2 days of 5α-dihydrotestosterone	0.27	3.87	3.08
7-day-castrated + 3 days of 5α-dihydrotestosterone	0.34	5.29	3.06
7-day-castrated + 4 days of 5α-dihydrotestosterone	0.40	6.95	3.59
7-day-castrated + 10 days of 5α-dihydrotestosterone	0.58	6.54	2.21
7-day-castrated + 10 days of testosterone	0.59	8.63	3.56
Animals aged 14 weeks			
Intact control	0.42	7.63	4.74
7-day-castrated + 10 days of 5α-dihydrotestosterone	0.60	10.15	2.78
7-day-castrated + 10 days of testosterone	0.57	8.68	3.19

This is in better agreement with the observations of Pegg *et al.* (1970), who found that spermidine was lost preferentially from the tissue, than with the observations of Caldarera *et al.* (1968), who found that both polyamine concentrations declined at the same rate. Both the wet weight and the spermidine/spermine ratio increased between days 3 and 10 of treatment with 5α -dihydrotestosterone.

Similar studies were carried out to investigate changes in ornithine decarboxylase activity and DNA-synthetic activity. 5α -Dihydrotestosterone was compared with 5α -androstane- $3\beta,17\beta$ -diol. The $3\beta,17\beta$ -diol has only a very low androgenic potency as assessed by the change in prostatic weight in the androgen-stimulated castrated rat (Hilgar & Hummel,

1964; Liao & Fang, 1969). Incorporation of [^{125} I]-iododeoxyuridine increased over the first 3 days of treatment with 5α -dihydrotestosterone (Fig. 5a). In the 5α -androstane- $3\beta,17\beta$ -diol group, there was an increase in incorporation on day 2 (equivalent to that of 5α -dihydrotestosterone), but by day 3 the incorporation was only 8.2 d.p.m./mg wet weight, compared with 52.9 d.p.m. for 5α -dihydrotestosterone. However, on day 10 there was no significant difference between the two treatments.

The corresponding results for the activity of ornithine decarboxylase per mg of protein are shown in Fig. 5(b). After 5α -dihydrotestosterone treatment, there was an initial peak in activity, which coincided with the peak in DNA-synthetic activity. Activity remained high at day 10. For 5α -androstane- $3\beta,17\beta$ -diol, there was an apparently linear increase in ornithine decarboxylase activity between days 2 and 10 of treatment. Thus, although the pattern of response to 5α -dihydrotestosterone and $3\beta,17\beta$ -diol were distinctly different during the early stimulatory phase, the tissue responses were similar on day 10. In fact, on day 10 ornithine decarboxylase activity, expressed per mg of protein, was rather higher for 5α -androstane- $3\beta,17\beta$ -diol than for 5α -dihydrotestosterone treatment. However, these assays were carried out under non-saturating conditions with respect to ornithine (see the Materials and Methods section) and a change in the ornithine concentration could have influenced the apparent activity of the enzyme. In all subsequent experiments, activities were measured by using a saturating concentration of carrier ornithine.

The responses on day 10 of treatment were examined in greater detail in an experiment in which testosterone (400 μ g/100g body wt. per day), 5α -dihydrotestosterone (400 μ g/100g body wt. per day and 40 μ g/100g body wt. per day) and 5α -androstane- $3\beta,17\beta$ -diol (400 μ g/100g body wt. per day) were compared. Results are given in Table 3. The wet weights, total DNA and total protein per prostatic lobe reflected the expected order of potency: 5α -dihydrotestosterone (high dose) > testosterone > 5α -dihydrotestosterone (low dose) > 5α -androstane- $3\beta,17\beta$ -diol > castrated control. Citric acid was not a particularly useful parameter of response, since a significant amount was retained by the regressed control tissue (0.42 mg/g, compared with 0.65 mg/g for fresh tissue). In general, the products involved in polyamine synthesis, except for spermine, were restored by daily administration of testosterone, and by both doses of 5α -dihydrotestosterone. 5α -Androstane- $3\beta,17\beta$ -diol (400 μ g/100g body wt. per day) was less effective than the lower dose of 5α -dihydrotestosterone (40 μ g/100g body wt. per day), the total polyamine concentration being only 36% of the fresh tissue concentration. The spermidine/spermine ratio for the $3\beta,17\beta$ -diol treatment (1.6) was

Fig. 5. Effects of daily injection of 5α -dihydrotestosterone (▲) and 5α -androstane- $3\beta,17\beta$ -diol (●) on (a) [^{125} I]iododeoxyuridine incorporation and (b) ornithine decarboxylase activity

Rats, aged 24 weeks, were castrated 7 days before injection with 400 μ g of each steroid/100g body wt. per day. Pooled tissue from three animals was used for determination.

Table 3. *Effects of androgen on prostatic activity*

Animals were castrated, left for 7 days, and then injected with androgen for 10 days; 17-day-castrated controls were injected with propylene glycol alone. Pooled material from two to three animals was used/assay. The results are means of two separate experiments with 14- and 15-week-old animals. N.D., Not detected.

Treatment ...	Intact control	Testosterone (400 µg/100 g body wt./day)	5α-Dihydrotestosterone		5α-Androstane-3β,17β-diol (400 µg/100 g body wt. per day)	17-day-castrated control
			(400 µg/100 g body wt. per day)	(40 µg/100 g body wt. per day)		
Wet wt. (mg/lobe ± S.E.M.)	175 ± 2	174 ± 3	225 ± 4	126 ± 2	42.0 ± 1	27.8 ± 9
DNA (µg/lobe ± S.E.M.)	369 ± 3	290 ± 4	367 ± 5	227 ± 3	96 ± 2	64.5 ± 1
Protein (mg/lobe ± S.E.M.)	18.6 ± 0.7	16.4 ± 1.0	28.7 ± 1.0	11.8 ± 0.6	2.4 ± 0.3	1.7 ± 0.2
Ornithine decarboxylase activity (nmol of ¹⁴ CO ₂ /h per mg of protein)	1.40	0.82	0.54	0.31	0.41	N.D.
10 ² × Ornithine decarboxylase activity (nmol of ¹⁴ CO ₂ /h per µg of DNA)	7.0	4.6	4.2	1.6	1.0	N.D.
Protein (µg/µg of DNA)	50	57	78	52	26	26
[¹²⁵ I]iododeoxyuridine (d.p.m./h per µg of DNA)	—	19.2	24.5	23.3	15.8	4.7
Citric acid (mg/g)	0.65	0.86	0.79	0.74	0.65	0.42
Ornithine (µmol/g)	0.14	0.78	1.45	1.63	0.27	0.24
Arginine (µmol/g)	3.06	5.93	5.28	7.36	1.85	1.45
Putrescine (µmol/g)	0.42	0.57	0.60	0.56	0.26	0.05
Spermidine (µmol/g)	7.63	8.68	10.15	8.26	2.74	0.54
Spermine (µmol/g)	4.74	3.19	2.78	3.91	1.72	0.50
Spermidine/spermine ratio	1.5	2.7	3.6	2.1	1.6	1.1

similar to that for fresh tissue (1.5), whereas the other androgen treatments led to ratios (2.1–3.6) that were more typical of the immature prostate (Fig. 1).

5α-Androstane-3β,17β-diol tended to compare rather more favourably with the other treatments when iododeoxyuridine incorporation and ornithine decarboxylase activity were examined. The incorporation of [¹²⁵I]iododeoxyuridine for the four androgen treatments was in the range 15.8–24.5 d.p.m./h per µg of DNA, compared with 4.7 d.p.m./h per µg of DNA for the castrated control. When ornithine decarboxylase activity was expressed per mg of protein, the result for the 3β,17β-diol treatment was also comparable with that of 5α-dihydrotestosterone. A different pattern of response was seen when ornithine decarboxylase activity was expressed per µg of DNA. The activities then followed the same order of potency as the changes in prostatic weight and DNA. The apparently high activity per mg of protein in the 3β,17β-diol-treated group suggests that, although the androgen was capable of supporting ornithine decarboxylase activity, albeit less effectively than 5α-dihydrotestosterone at a dose of 40 µg/100 g body wt.

per day, it was ineffective in stimulating synthesis of cellular proteins in general. This can also be seen by comparing the mg of protein/lobe and µg of protein/µg of DNA for the 3β,17β-diol treatment with the values for the castrated control.

Relationship between polyamine and protein content in the prostate

The relationship between the concentration of protein/prostate and the concentration of the two polyamines/prostate was examined, by using values for intact animals aged 7–36 weeks (Fig. 6a) and the data for the six treatments shown in Table 3 (Fig. 6b). Both sets of results showed a linear relationship between protein and spermidine content of the prostate. Androgen treatments, which led to a marked stimulation in protein synthesis, also led to an equivalent enhancement in spermidine synthesis without concomitant increase in spermine synthesis, thus giving a high spermidine/spermine ratio. Similarly, prostates from young rats, which showed a high spermidine/spermine ratio, had a lower rate of accumulation of spermine than of spermidine.

Fig. 6. Relationship between protein and polyamine content/prostate in ventral prostates from (a) intact animals aged 7-36 weeks and (b) after the six treatments shown in Table 3
■, Spermidine; ●, spermine.

Maintenance of prostatic activity by androgen treatment

Starting immediately after castration, animals were injected daily with 5α -androstane- 3β , 17β -diol, and killed on days 2, 4 and 7. The changes in biochemical properties of the tissues were compared with those of castrated animals of the same age injected with propylene glycol alone. Results are expressed as a percentage of the activity of the intact animal (Figs. 7 and 8). In the castrated control group, prostatic DNA and protein concentrations and the incorporation of [^{125} I]iododeoxyuridine fell with time (Fig. 7a). The results for the 3β , 17β -diol group (Fig. 7b) tended to support the impressions gained from the data in Table 3 that this androgen was more effective in maintaining DNA than protein synthesis.

Ornithine decarboxylase activity fell markedly in both the control (Fig. 8a) and androgen-treated groups (Fig. 8b). In the latter group there was an increase in activity between days 2 and 7, although on day 7 the activity was still only 14% of that of fresh tissue. Despite the poor maintenance of ornithine decarboxylase activity, the 3β , 17β -diol was very effective in preventing a rapid loss of spermidine and maintaining a high concentration of spermine (Fig. 8b). Citric acid content was also maintained by the 3β , 17β -diol (0.78 mg/g on day 7 of treatment, compared with 0.27 for the 7-day-castrated control and 0.85 for the intact animal).

Fig. 7. Maintenance of prostatic activity with 5α -androstane- 3β , 17β -diol
DNA (●) and protein (■) content and [^{125} I]iododeoxyuridine incorporation/mg wet wt. (▲) are compared in ventral prostates from (a) castrated rats and (b) castrated rats injected with the 3β , 17β -diol (400 μ g/100 g body wt. per day). Pooled material from three animals, aged 13 weeks, was used per determination. Results are expressed as percentages of the values in tissue from intact rats of the same age.

Fig. 8. Maintenance of prostatic activity with 5 α -androstane-3 β ,17 β -diol

Spermine (●) and spermidine (■) concentrations ($\mu\text{mol/g}$) and ornithine decarboxylase activity (▲), expressed as nmol of $^{14}\text{CO}_2/\text{h}$ per μg of DNA, are compared in ventral prostates from (a) castrated rats and (b) castrated rats injected daily with the 3 β ,17 β -diol (400 $\mu\text{g}/100\text{g}$ body wt. per day). Pooled material from three animals, aged 13 weeks, was used per determination. Results are expressed as percentages of the values in tissue from intact rats of the same age.

Discussion

Between days 1 and 5 of treatment of castrated rats with testosterone and 5 α -dihydrotestosterone, the primary cellular response is proliferation; after about the fifth day the response changes from proliferation to differentiation (Coffey *et al.*, 1968; Lesser & Bruchovsky, 1973, 1974; Tuohimaa & Niemi, 1974; Rennie *et al.*, 1975). Pegg *et al.* (1970) have shown that, during the testosterone-induced growth phase, ornithine decarboxylase activity and spermidine concentration increase; the spermine concentration starts to rise about the time when there is a switch-over from proliferation to secretory function. Similarly, during treatment of castrated rats with 5 α -dihydrotestosterone (400 $\mu\text{g}/100\text{g}$ body wt. per day), the spermidine concentration increases (Table 2) during the period of enhanced DNA-synthetic activity (Fig. 5a). The spermine concentration, however, is low after 10 days of treatment, but higher concentrations of spermine are found in the 7-day-castrated rat prostate (Table 2), and in the prostates from animals injected with a low dose of 5 α -dihydrotestosterone (40 $\mu\text{g}/100\text{g}$ body wt. per day; Table 3) and with testosterone (400 $\mu\text{g}/100\text{g}$ body wt. per day;

Table 2). These results, when taken together with the change in the spermidine/spermine ratio during the time-course of treatment with 5 α -dihydrotestosterone (Fig. 4), suggest that a high ratio is indicative of hypertrophy and secretion. This appears to be at variance with the results in Fig. 1(b), which show that the spermidine/spermine ratios are higher in the prostates of young rats than in those of mature animals. Cell division and DNA-synthetic activity are high in the immature prostate, and these activities decline markedly with age (Price, 1936; Donaldson & Thomas, 1976). Thus, although the above results agree with the general principle expressed by Russell & McVicker (1972) that an enhanced spermidine/spermine ratio indicates enhanced biosynthesis, the ratio does not differentiate between predominantly mitotic and secretory activities. Pegg *et al.* (1970) have discussed the possibility that there may be a specific requirement for spermidine during proliferative activity in the prostate. However, it is also possible to have accumulation of polyamines under conditions where RNA synthesis is stimulated without a concomitant change in proliferative activity, as in the hypertrophied heart (Russell, 1973).

It is well established that rat prostate undergoes

regressive changes when cultured in the absence of androgen (for leading references, see Lasnitzki, 1974) and that these changes can be prevented if the medium is supplemented with testosterone alone (Lasnitzki, 1970) or in combination with insulin (Lostroh, 1968; Santi & Johansson, 1973; Fuller *et al.*, 1974). Studies in this laboratory (Shipman *et al.*, 1975; Donaldson & Thomas, 1976) have centred on the use of [^{125} I]iododeoxyuridine and the metaphase-arrest agent vincristine sulphate to monitor proliferative activity in cultured prostate. A combination of insulin (3 μ g/ml) and testosterone (0.1 μ M) is effective in stimulating DNA-synthetic activity. Thus organ culture offers an approach for investigating the relationship *in vitro* between proliferative activity and polyamine synthesis. Of particular note in this context is the observation by Lasnitzki & Lucy (1961) that arginase activity of mouse prostate is elevated after 9 days of culture, leading to an increase in ornithine concentration in the medium. These observations suggested to us that there might be a block in the utilization of ornithine in the polyamine-synthetic pathway. This has proved to be the case since, irrespective of whether or not the medium is supplemented with insulin and testosterone, ornithine decarboxylase activity is rapidly lost (Figs. 2a and 2b). Nevertheless, cultured prostate is able to respond to testosterone, both in terms of an increase in DNA synthesis and cell division (Fig. 2b). It appears, therefore, that an androgen-induced growth response can occur in the rat prostate despite a depleted polyamine content and low ornithine decarboxylase activity. Human benign prostatic tissue behaves similarly (Fuller & Thomas, 1975). The present work does not exclude the possibility that relatively low intracellular concentrations of polyamines may be necessary to sustain androgen-induced growth.

The effects of the 5 α -androstane-3 β ,17 β -diol were examined to obtain more information on the relationship between polyamine synthesis and prostatic function. It has been suggested that this compound may differ qualitatively, as well as quantitatively, in the nature of its effects on the prostate. Testosterone is metabolized in the rat ventral prostate both *in vivo* and *in vitro* to a variety of compounds, which include both 5 α -dihydrotestosterone and 5 α -androstane-3 β ,17 β -diol (see Baulieu & Robel, 1970). Although the 3 β ,17 β -diol is less effective than either testosterone or 5 α -dihydrotestosterone in the stimulation of organ weight in the castrated rat (Hilgar & Hummel, 1964; Liao & Fang, 1969), studies on the effects of these androgens on cultured rat prostate indicate that the 3 β ,17 β -diol may have a specific function in the maintenance of secretory activity (Lasnitzki, 1970). The ability of the 3 β ,17 β -diol to promote growth and secretion in the regressed

prostate, and its ability to maintain pre-existing activities immediately after castration, has been examined. The latter approach seems to be more closely related to the organ-culture situation used by Lasnitzki (1970). In neither study did the results unequivocally substantiate the suggestion that the 3 β ,17 β -diol has a specific role in secretory activity as distinct from cell division. Like Schmidt *et al.* (1973), we found that the androgen is able to support DNA synthesis *in vivo*. The major difference between the diol and the more potent androgen, 5 α -dihydrotestosterone, is seen during the first 4 days of treatment of the castrated rat (Fig. 5). Proliferative activity during the later stages of androgen stimulation is not dependent on the dose or potency of the androgen administered (Table 3). These results tend to support the conclusions of Tuohimaa & Söderström (1974) that it is the initial pulse in mitotic activity after androgenic stimulation that is dose-dependent.

The 3 β ,17 β -diol is also not effective in stimulating or maintaining protein synthesis, although it is able to stimulate ornithine decarboxylase activity. It appears that ornithine decarboxylase is very sensitive to androgenic stimulation, and the threshold of response is lower than for protein synthesis in general.

The data accumulated in the present work show that there is a well-defined relationship between spermidine and protein content of the prostate. This relationship holds not only for castrated animals injected with testosterone, 5 α -dihydrotestosterone and the 3 β ,17 β -diol, but also for the intact animals. A similar general relationship is not found for spermine. The spermine content is relatively depleted and in consequence the spermidine/spermine ratio is elevated under conditions where the young prostate is actively undergoing cell replication or where androgenic stimulation of the adult regressed prostate leads to excessive hypertrophy and secretion. It is possible, therefore, that spermine may be converted into spermidine during periods of high metabolic demand.

Although the 3 β ,17 β -diol has been shown to delay some of the regressive changes seen on withdrawal of hormonal support, no claim is made that this is a specific property of the 3 β ,17 β -diol, rather than of its metabolites. The peripheral conversion of 5 α -androstane-3 β ,17 β -diol into 5 α -dihydrotestosterone has been demonstrated in the rat by Becker *et al.* (1973) and Kreig *et al.* (1975). In view of the fact that 5 α -dihydrotestosterone at 40 μ g/100g body wt. per day is more effective than 5 α -androstane-3 β ,17 β -diol at 400 μ g/100g body wt. per day (as measured by responses on day 10; Table 3), it is distinctly possible that part or all of the stimulatory effects seen with the latter compound are due to its peripheral conversion into the more potent androgen.

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