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PHOTOPERIODIC CONTROL OF ANDROGEN METABOLISM AND BINDING IN ANDROGEN TARGET ORGANS OF HAMSTERS (*PHODOPUS SUNGORUS*)*

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SUMMARY

Male Dshungarian dwarf hamsters (*Phodopus sungorus*, Pallas 1776) aged 90 ± 7 days were used throughout the study. They were kept either under long photophase days of 16L:8D (group L) or under short photophase days of 8L:16D (group S). Testes and prostate weight and total radioactivity uptake into various organs 30 min. after intracardial injection of [^{3}H]-testosterone ([^{3}H]-T) were determined. Photoperiodical changes in the action of steroids were investigated by the determination of the [^{3}H]-T metabolite pattern *in vivo*, and by *in vitro* studies on the available androgen receptor of prostate cytosol.

The S-group reduced the weight of the testes to 3% and that of the prostate and seminal vesicles to 10% of the L-group values. The decrease in the weight of the accessory sex organs was accompanied by a dramatic increase in the radioactivity uptake relative to plasma. The plasma of S-hamsters retained less radioactivity than that of L-hamsters. The metabolite pattern of [^{3}H]-T in prostate and seminal vesicles *in vivo* changed towards increased inactivation with less 5α-dihydrotestosterone and more steroids of high polarity formed. An increase in the concentration of available binding sites of the prostate cytosol androgen receptor was observed in S-hamsters when compared to the L-group. Short term castration (16 h) increased the receptor in both, L- and S-hamsters, while long term castration (7 days) lowered the receptor by 60% compared to short term castrated hamsters.

The data indicate, that a photoperiodic control of androgen action exists on the level of the target organs, which is assisted by peripheral changes of androgen metabolism, as reflected in plasma, which are not directly controlled by the hypophyseal-gonadal feed back system. The peripheral changes lead to a higher elimination and inactivation of plasma T under short photoperiods. Furthermore, in the target organs also a higher inactivation and a lower formation of biologically active metabolites suppress the biological action of the remaining active androgen. The decreasing androgen levels are also accompanied by increasing available cytosolic androgen receptor concentrations. The decrease of receptor after long term castration suggests that the regressed testes are involved in the maintainance of the androgen receptor and of androgen responsiveness.

INTRODUCTION

The effects of different photoperiods on the reproductive organs of photosensitive species have been studied almost exclusively by the feed-back regulation of sex hormones by the pineal-hypothalamus-pituitary-gonadal axis. Relatively little attention has been paid to specific alterations in the androgen target organs. This paper focuses on the action of short versus long photophase days on steroid biochemical events in the androgen target organs, especially the prostate in order to provide evidence of a direct action of the photoperiod. Earlier observations in mallard drakes [1] suggested that the metabolite pattern of [^{3}H]-T *in vivo* in androgen target organs may change to more polar compounds when the animals were investigated in the autumn. Similar findings were reported from non-target organs, for example the liver of rats [2] and the testes of photorefractory birds [3]. These changes may be directly related to melatonin, the principal pineal product [4]. It could not be concluded from the *in vivo* investigation of mallards that the photoperiod itself leads to changes in the target organs. Since those birds were not castrated prior to the assay, the higher androgen uptake in the penis and other tissues could have been exclusively caused by the photoperiodical changes in the androgen supply of the tissues, leading to differing saturations of intracellular androgen binding molecules and of enzyme systems. In this investigation, we utilized males of a highly short day sensitive hamster species which were investigated either castrated or uncastrated for the biochemical assays. Since castration reduces circulating androgens of short and long day hamsters to similarly low levels, direct effects of the photoperiod on the cellular level may become detectable.

MATERIALS AND METHODS

Experimental animals. Male *Phodopus* hamsters were bred in the authors colony at 20 ± 2°C under long day conditions (16L:8D). The lights were on

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from 0600 h–2400 h. The litters were weaned on day 21 after birth and then maintained for 69 ± 7 days either under long days in the breeding room or under short days (8L:16D) in a neighbouring room of the same temperature. All animals were castrated 16 h prior to the assays except those for radioimmunological determination of testosterone and prolactin, and those determined for receptor studies without castration or after long term castration. Long term castration was performed 7 days before the experiment.

In vivo *uptake and metabolism.* 16 h castrated hamsters, either L or S, were injected with 25 μCi [^{3}H]-testosterone (85–100 Ci/mM, NEN) in 100 μl of saline. After 30 min, the animals were sacrificed by cervical dislocation. Blood was collected in heparinized tubes and centrifuged at 3,200 rev./min for 2 min. in an Eppendorf centrifuge. Prostates, seminal vesicles, levator ani and quadriceps femoris muscles were quickly removed, cleaned of connective and fatty tissue and then homogenized at 0°C with 3 vol. of 0.88 M sucrose in an all glass homogenizer. Aliquots of the homogenates were counted for total radioactivity content, and the remainder extracted twice with 4 vol. of diethyl ether and once with chloroform. The dried solvent extract was partitioned in a system of 70% ethanol–*n*-heptane (1:1, v/v) and the dried ethanol phase subjected to thin layer chromatography (t.l.c.) or radiogaschromatography (RGC). TLC was carried out using three different solvent systems which allowed the reliable separation of at least 8 androgens [6]. The fractions were visualized, using unlabelled carrier steroids, by U.V.-light or iodine vapour. The t.l.c. fractions of 8 androgens (5α-androstane,3α,17β-diol, 5α-androstane 3β,17β-diol, testosterone, 5α-dihydrotestosterone, androsterone, epi-androsterone, 5α-androstanedione and 4-androstenedione) were scraped directly into scintillation vials and measured in a Packard Tri-carb scintillation spectrometer, model 3390 with external standardization and 544 absolute activity analyzer.

Available prostate cytosol androgen receptor. The receptor was measured by agar gel electrophoresis [7, 8] after homogenization of prostatic or muscular tissue in liquid nitrogen. The tissue was suspended in 2 vol. of Tris–EDTA–mercaptoethanol buffer (pH 7.4) and incubated with labelled 5α-DHT or with labelled and unlabelled 5α-DHT (100-fold excess). The specific activity was 40 Ci/mmol (NEN). After completion of the electrophoresis, 3 mm blocks of gel were counted for radioactivity content.

Radioimmunoassays. Plasma testosterone was determined without chromatography [9]. Prolactin was measured utilizing an anti-human prolactin antibody (IDW, Germany) which in dilution experiments with hamster plasma showed curves parallel to human standard curves.

Protein measurement. Total soluble proteins were determined by using 1N Folin phenol reagent [10] and a calibrated human serum sample as a standard.

RESULTS

After 10 weeks of short day exposure, the weight of testes, and of accessory sex organs, plasma testosterone and prolactin were significantly lower when compared to the L-group. All changes were statistically significant (Table 1). The decrease in the testis weight from 793 to 24 mg was the most obvious change. The weight of the prostate was also much lower under S-conditions (5.1 g) when compared to the L-group (48.5 g). Similar changes were observed in the weight of the seminal vesicles in the bulbocavernosus/levator ani muscle and in the ventral gland. The latter two organs were well developed in the L-group, but due to the high degree of involution could not be excised from the surrounding tissues in the S-group. Therefore, data cannot be given. Plasma testosterone decreased almost by factor 10 from 3.3 to 0.4 ng/ml, and plasma prolactin was lowered from 5.8 to 1.4 ng/ml under S-conditions.

All organs measured (Table 2) took up relatively and absolutely more labelled material under short day conditions. The relative uptake above plasma level increased to a factor of 8.6 in the prostate and 8.3 in the seminal vesicles, compared to factor 2.0 in the L-group. Levator ani and ventral gland could be measured only in the L-hamsters. The quadriceps femoris muscle also increased the uptake of radioactivity against plasma, increasing to plasma level in the S-group, while the L-group showed up with only 0.5 times plasma level. Table 3 shows the absolute uptake in fmol/ml or fmol/g. It was assumed that the radioactive material measured had the molecular

Table 1. Organ weight, plasma prolactin and testosterone levels

Group	Body weight (g)	Testis weight (paired, mg)	Prostate weight (mg)	Sem. ves. weight (mg)	Plasma prolactin (ng/ml)	Plasma testosterone (ng/ml)
L	35.5 ±5.1	792.7 ±85.0	48.5 ±12.7	35.1 ±5.9	5.8 ±1.3	3.3 ±1.6
S	31.8* ±3.9	24.0* ±6.9	5.1* ±3.1	2.9* ±2.7	1.4* ±0.5	0.4* ±0.1

* $P < 0.001$ compared to L.

Male *Phodopus* hamsters were kept after weaning for 10 weeks under long (L) or short (S) photophase day conditions. On day 90 ± 7 days the animals were sacrificed, bodies and organs weighed, and immunoreactive plasma prolactin and testosterone measured. All values were significantly lower in the S-group.

Table 2. Radioactivity uptake *in vivo* relative to plasma after [^{3}H]-testosterone injection

Group	Prostate	Seminal vesicles	Levator ani bulbocavernosus muscle	Quadricep femoris muscle
L	2.0 ± 0.5	2.0 ± 0.6	1.7 ± 0.5	0.5 ± 0.2
S	8.6 ± 4.7*	8.3 ± 5.7*	n.m.	1.0 ± 0.1*

* $P < 0.001$; n.m. not measured.

Male *Phodopus* hamsters, kept either under long (L) or short (S) photophase days were castrated for 16 h when 25 μCi of [^{3}H]-testosterone were injected intracardially. 30 min after the injection, the organs were measured for total radioactivity content. All organs took up more radioactivity under short photoperiods.

Table 3. Retention in plasma and uptake into various organs of [^{3}H]-testosterone (fmol/ml or fmol/g tissue)

Group	Plasma	Prostate	Seminal vesicles	Levator ani bulbocavernosus muscle	Quadr. fem. muscle
L	7.4 ±2.2	14.8 ±4.4	14.7 ±5.4	12.6± ±4.6	3.7 ±1.5
S	3.3* ±0.9	24.0** ±16.3	21.4 ±13.4	n.m.	3.3* ±0.6

* $P < 0.001$; ** $P < 0.01$; n.m. not measured.

Male *Phodopus* hamsters were treated as described in Table 2. The data, however, were calculated in fmol per gram or fmol per ml under the assumption that the radioactive material had the molecular weight of testosterone.

Table 4. Metabolites of [^{3}H]-tesosterone *in vivo*

Group	Tissue	3α	3β	T	5α-DHT	Epi-A	A	4-Dion	Dion
	Plasma	2.9	2.1	44.6	0.8	0.3	1.5	1.9	0.8
	Prostate	2.8	1.1	21.5	39.6	1.1	0.8	0.6	5.8
L	Sem. ves.	2.7	1.9	16.2	35.2	1.0	0.8	0.5	1.0
	Lev. ani	4.8	2.6	17.2	6.0	1.0	1.6	0.4	0.8
	Quadr. m.	4.7	4.1	20.8	0.7	1.0	5.0	1.5	0.7
	Plasma	2.0	1.7	17.1	0.4	0.3	1.3	1.2	1.3
S	Prostate	0.9	0.6	9.6	21.5	0.4	0.2	0.7	2.7
	Quadr. m.	3.8	2.7	14.1	0.5	0.8	3.5	1.5	0.6

Metabolites were measured in 16 h castrated male hamsters 30 min. after intracardial injection of [^{3}H]-testosterone. Separation of androgens was achieved in three t.l.c. systems. The existence of metabolites was confirmed by radiogaschromatography. The amounts of tissue obtained under short photophase days were too low to be investigated in the sem. ves., and in the lev. ani. Symbols: Sem. ves.: seminal vesicles; lev. ani: bulbocavernosus/levator ani muscle; quadr. m.: quadriceps femoris muscle; 3α:5α-androstane 3α,17β-diol; 3β:5α-androstane-3β,17β-diol; T: testosterone; 5α-DHT: 5α-dihydrotestosterone; Epi-A; epiandrosterone; A: androsterone; 4-Dion: 4-androstenedione; Dion: 5α-androstanedione. (Mean values of 3 experiments on 6-12 L- or 25-28 S-hamsters each).

weight of testosterone. The values were higher in all organs under short photoperiods, except in the muscle. The plasma of S-hamsters, on the contrary, took up only one half of the radioactivity seen in L-hamsters.

The metabolite pattern of both L- and S-hamsters, showed several quantitative differences. Although in both groups 5α-DHT was the main product, in the prostate and seminal vesicles, the percentage of this androgen was much lower under short day conditions. While in the prostate of L-hamsters 40% was found, the prostate of S-hamsters contained only 22% 5α-DHT in the solvent extractable radioactivity fraction (Table 4). On the other hand, less testosterone remained unchanged in the S-group in the prostate, in the skeletal muscle and in plasma, so that the total turnover of testosterone was increased in the S-group. The equilibrium between T and 5α-DHT, however, remained unchanged. The androstanediols were also lower in plasma, prostate and muscle of S-hamsters. The portion of unidentified, mostly high polar steroids was higher in the S-group. These compounds were found to have mobilities on t.l.c. similar to 5β-androstanediols, of androstanetriols and others. No attempt was made to exactly identify these steroids. Most other androgens showed also a reduction under S-conditions, but due to low percentages, which sometimes approached the discrimination limit from background (0.4%), these figures must be considered with care. Impurities originating from the administered testosterone did not influence the data, since they were found not to migrate with any of the metabolites investigated here. This was confirmed by RGC.

[^{3}H]-5α-DHT was bound to prostate cytosol androgen receptors with high affinity and limited capacity. Figure 1 shows a typical graph of electrophoretic separation of binding proteins under L-conditions. The binding component (fractions 11–15) resembled closely that of the rat prostate cytosol, was heat labile and displaceable by a 100-fold excess of unlabelled 5α-DHT. The receptor was well demonstrable in short term castrated L-hamsters (Fig. 1A) in which 106 fmol/mg soluble cytosol protein were measured, but was visible only as a small shoulder in uncastrated L-hamsters (Fig. 1B). This shoulder was equal to only 6 fmol available receptor per mg soluble protein. Muscle (Fig. 1C) showed no such receptor under the conditions used, although a small elevation of radioactivity was seen in the receptor fractions 11–15. This radioactive 5α-DHT, however, was not displaceable by excess 5α-DHT and was considered being unspecifically bound.

Figure 2 shows the same experiment performed on S-hamsters. Short term castrates showed a large receptor peak, which exceeded that of the L-hamsters by factor 2.5 (250 fmol/mg protein, Fig. 2A). In contrast to uncastrated L-hamsters, the prostates of uncastrated S-hamsters (Fig. 2B) showed a relatively large receptor peak which was lower than that of castrated S-hamsters but 8 times higher than that of the respective uncastrated L-group. In muscle (Fig. 2C) again, no specific binding was measured. L-animals which were castrated 7 days before the experiment (Fig. 3) had a lower receptor concentration than those castrated 16 h before (42 fmol/mg protein).

DISCUSSION

The reduction in the weight of the accessory sex organs observed under short photophase days was

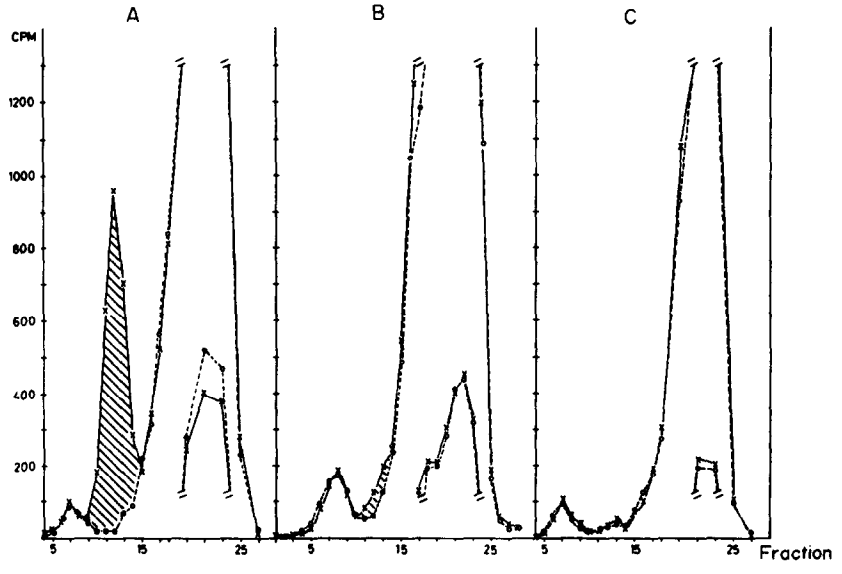

Fig. 1. Homogenates of L-hamster prostates were incubated with labelled 5α-DHT (×——×) or with labelled and a 100-fold excess and unlabelled (O——O) 5α-DHT. 105000 *g* cytosols were prepared and binding proteins separated by agargel electrophoresis. A: prostate of short term castrated animals; B: prostate of uncastrated animals; C: muscle of short term castrated animals.

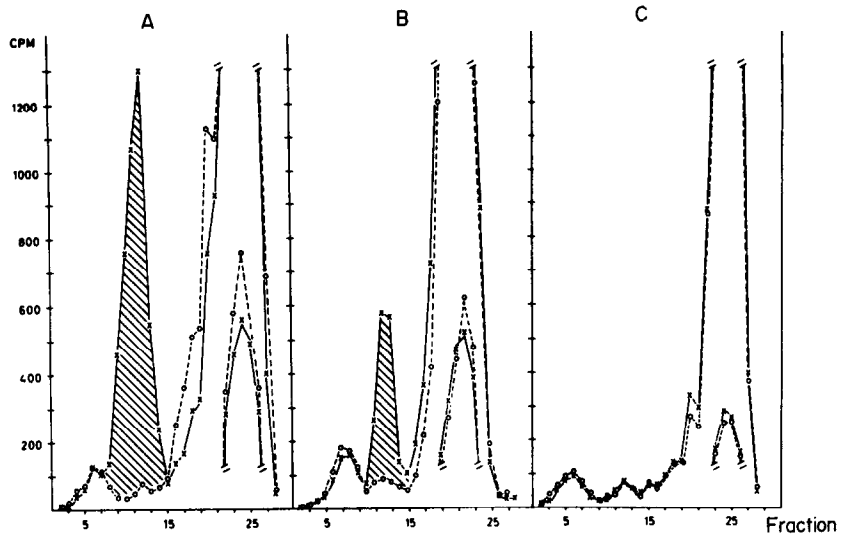

Fig. 2. Homogenates of S-hamster prostates were incubated with labelled or labelled and unlabelled 5α-DHT and prepared as described in Fig. 1. A: prostate of short term castrated animals; B: prostate of uncastrated animals; C: muscle of short term castrated animals.

shown to be accompanied by an increase in the radioactivity uptake relative to plasma. It is conspicuous that this increase is almost as high as the decrease in the organ weights. However, when the uptake was calculated on a molar base, the increase was less pronounced. Considering the plasma values, it becomes clear that the relative uptake increased partly because of the lower radioactivity content of the S-hamster plasma. The lower radioactivity was caused by a higher clearance of the injected compound from plasma. It has been also shown, that there remains a prostate specific increase in the radioactivity uptake by the S-hamster prostate even after correction for the lower plasma radioactivity.

We have speculated that this increase may have been caused by an increasing concentration of available binding sites of the cytosolic androgen receptor, since the availability of receptor proteins is inversely related to ligand concentration. S-hamsters have a lower androgen concentration in plasma, hence, at a constant receptor production, the available binding sites should be increased. On the other hand, the androgen receptor synthesis in rats was androgen dependent. Castration for 7 days was followed by a heavy, or even total, loss of receptor. The receptor concentration in S-hamster showed, that the long term androgen deficiency under short photoperiods does not cause the receptor to decrease, but it even increased. Long term surgical castration of L-hamsters, however, was followed by a loss of about 60%. From this point of view, our S-hamsters were not comparable to long term castrates. Moreover, since short term castration of short photoperiod maintained hamsters led to a further increase in the available receptor concentration, there is evidence that the testes still produce ligands which occupy most of the receptor binding sites. If one assumes that the 16 h castrates exhibited approximately the total receptor concentration, or the amount of receptor synthesized during these 16 h of castration, then about 80% were still occupied by ligands supplied by the testes. This is in accordance with the observation that testosterone is still measurable in S-hamster plasma and that this testosterone may be 5α-reduced by the prostatic 5α-reductase activity seen under both L- and S-conditions. It is in accordance with the higher plasma androgen levels, that 95% of the total receptor may have been occupied in the L-group.

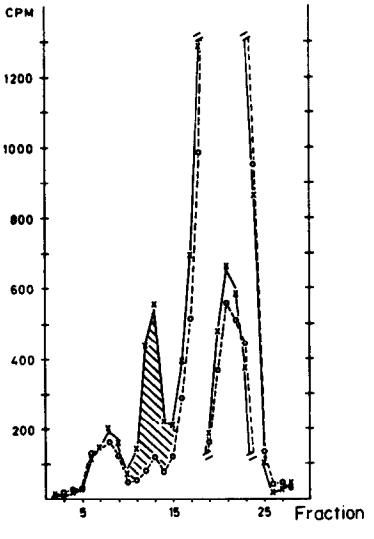

Fig. 3. Homogenates of long term castrated L-hamsters were incubated with labelled or labelled and unlabelled 5α-DHT and prepared as described in Fig. 1.

The studies on testosterone metabolism *in vivo* revealed that only half as much 5α-DHT was formed in the prostate under S-conditions. Since not more, but even less, testosterone remained unmetabolized, and the portion of unidentified, probably biologically inactive, metabolites increased, the prostate itself obviously posseses a capacity to change the utilization of androgens under changing photoperiods. The data show, that the low prostatic androgen utilization under S-conditions is supported by a higher peripheral androgen inactivation as reflected by the plasma, where the recovery of active metabolites and the portion of unchanged testosterone was also lower. The data on weight, uptake and metabolism in the seminal vesicles were very similar to those of the prostate and may be considered, under certain limitations, as to confirm the data in the prostate. The receptor and the *in vivo* metabolism could not be determined in seminal vesicles of S-hamsters. The same was true for the androgen dependent levator ani muscle, which was also heavily regressed under S-conditions. The metabolite pattern of the levator ani differed from the former two organs in that much less 5α-DHT was formed which is in accordance with the view that the androgen receptor of this muscle may respond to testosterone rather than DHT. It should be noted, however, that some 5α-DHT was found in this organ under L-conditions and that this may be of importance to its normal function. In the quadriceps femoris muscle, only background levels of 5α-DHT were found which were not higher than that of plasma. The higher uptake of radioactivity and the lower recovery of biologically active androgens in the quadriceps femoris muscle, in addition to the data in plasma, may be another indication that the peripheral tissues are involved in the inactivation of androgens and that these tissues influence the androgen supply of the prostate.

From the data obtained in this study it is concluded that the regression of the prostate gland and possibly of other androgen dependent structures is not simply caused by a decreasing testicular function due to the antigonadotrophic action of centrally released agents but is also the consequence of a higher peripheral androgen inactivation, as reflected in the plasma, and a higher intracellular inactivation in the prostate itself. These mechanisms may be at least partly independent of the pituitary-gonadal feed-back system. In spite of a decreasing androgen supply, the higher available receptor concentration maintains androgen responsiveness, which is supported by a persisting 5α-reductase activity. The total and/or the available binding sites are possibly regulated by testicular function. Even under S-conditions, the testes provide ligands which occupy about 80% of the binding sites and stimulate receptor synthesis. The increase in the total and/or available receptor concentration enables the prostate to respond sensitively to androgens, reappearing under the stimulation of long photoperiod days, or in response to androgen injections.

REFERENCES

1. Horst H.-J. and Paulke E.: Comparative study of androgen uptake and metabolism in domestic and wild mallard drakes (*Anas platyrhynchos* L.) *Gen. comp. Endocr.* **32** (1977) 138–145.
2. Colas A. E. and Schafer S. J.: Hepatic hydroxylation of 3β-hydroxy-5α-androstane-17-one in orchidectomized or adrenalectomized rats exposed to a reversed light cycle, constant light, or constant dark. *Endocrinology* **99** (1976) 72–78.
3. Kerlan J. T., Jaffé R. B. and Payne A. H.: Sex steroid formation in gonadal tissue homogenates during the testicular cycle of the redwinged blackbird (*Angelaius phoeniceus*). *Gen. comp. Endocr.* **24** (1974) 352–363.
4. Cardinali D. P. and Rosner J. M.: Effects of melatonin, serotonin and N-acetylserotonin on the production of steroids by duck testicular homogenates. *Steroids* **18** (1971) 25–37.
5. Moore R. J. and Wilson J. D.: Localization of the reduced nicotinamide-adenine-dinucleotidephosphate: 4-3-ketosteroid-5α-reductase in the nuclear membrane of the rat ventral prostate. *J. biol. Chem.* **247** (1972) 958–967.
6. Burić L., Becker H., Peterson C. and Voigt K. D.: Metabolism and mode of action of androgens in target tissues of male rats. 1. Metabolism of testosterone and 5α-dihydrotestosterone in target organs and peripheral tissues. *Acta endocr., Copenh.* **69** (1972) 153–164.
7. Wagner R. K.: Characterization and assay of steroid hormone receptors and steroid binding serum proteins by agargel electrophoresis at low temperature. *Hoppe-Seylers Z. physiol. Chem.* **253** (1972) 1235–1245.
8. Krieg M. and Voigt K. D.: Biochemical substrate of androgen actions at a cellular level in the prostate, bulbocavernosus/levator ani muscle and in skeletal muscle. *Acta endocr., Copenh.* **85** (1977) Suppl. 214, 43–89.
9. Bosch A. M. G., den Hollander F. C. and Woods G. F.: Specificity of antisera against testosterone linked to albumin at different positions (C_3, C_{11}, C_{17}). *Steroids* **23** (1974) 699–711.
10. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.