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S-Adenosyl-L-Methionine Decarboxylase Activity in the Rat Epididymis: Ontogeny and Androgenic Control

MARCELO A. DE LAS HERAS* AND RICARDO S. CALANDRA*†

*From the *Laboratorio de Esteroides, Instituto de Biología y Medicina Experimental and the *†Cátedra de Endocrinología, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Buenos Aires, Argentina.*

ABSTRACT: The authors describe the occurrence of high levels of S-adenosyl-L-methionine decarboxylase (SAMDC) activity in the rat epididymis, and its ontogeny and androgenic control. As early as 15 days of age, SAMDC activity exists, although a peak of activity is observed at 25 days. Bilateral orchidectomy resulted in a decline of epididymal SAMDC activity. However, an androgen-independent fraction, accounting for 34% of total activity, appears to exist in the epididymis. In 45-day-old orchidectomized rats, SAMDC activity was stimulated by testosterone treatment in a dose-dependent manner. However, treatment of 45-day-old intact animals with a high dose of the androgen failed to modify SAMDC activity, indicating that, at this age, the enzyme is maximally stimulated by endogenous androgens. The observed effect of testosterone on castrated rats was completely abolished by concomitant treatment with the antiandrogen flutamide. This compound was ineffective on the androgen-insensitive fraction. To assess the contribution of circulating and luminal androgens to the maintenance of epididymal SAMDC, rats were unilaterally orchidectomized and activity was determined in both epididymides after 7 days. The SAMDC activity was identical in epididymides from both sides, suggesting that circulating androgens suffice to maintain normal levels of activity. It was concluded that androgens regulate epididymal SAMDC activity, although an androgen-independent fraction appears to exist.

Key words: S-adenosyl-L-methionine, androgen, flutamide, polyamines.

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The polyamines putrescine, spermidine, and spermine, organic cations found in all cell types, are considered to play an important role in cellular structure and function (Tabor and Tabor, 1984). Increases in intracellular polyamine levels are closely related to nucleic acid and protein synthesis, and the activities of the key enzymes in polyamine biosynthesis are stimulated during processes leading to growth and differentiation (Jänne et al, 1978; Russell, 1985; Pegg, 1986).

The initial and rate-limiting enzyme in polyamine biosynthesis, ornithine decarboxylase (ODC), is readily induced by growth-promoting stimuli in resting cells, making its activity a highly sensitive marker of the action of trophic hormones and growth factors on their target tissues (Russell, 1985). S-adenosyl-L-methionine decarboxylase (SAMDC) catalyzes the conversion of S-adenosyl-L-methionine (SAM) into a species (decarboxylated SAM) that provides the aminopropyl moiety for the synthesis of spermidine from putrescine and of spermine from spermidine. As a consequence, both activities are necessary for the synthesis of these latter compounds, which are considered to be more active than their precursor.

Several studies have focused on polyamine metabolism in the male reproductive organs. In some species, the high polyamine levels found in seminal plasma are derived mainly from prostate secretion (Williams-Ashman et al, 1972; Piik et al, 1977). In the prostate, androgens markedly stimulate ODC and SAMDC activities (Pegg and Williams-Ashman et al, 1968; Moulton and Leonard, 1969; Pegg et al, 1970) by an increase in their mRNA content (Berger et al, 1984). The seminal vesicles also show androgen-dependent ODC and SAMDC activities (Piik et al, 1977; Fjøsne et al, 1988). In contrast, little attention has been paid to polyamine biosynthesis in the epididymis. In previous reports (de las Heras and Calandra, 1987; de las Heras et al, 1988), we have shown that rat epididymal ODC activity is strictly androgen-dependent. In the current study, we describe the occurrence of high levels of SAMDC activity in the rat epididymis, and its ontogeny and androgenic control.

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Correspondence to: Dr. Ricardo S. Calandra, Laboratorio de Esteroides, Instituto de Biología y Medicina Experimental, Obligado 2490, 1428 Buenos Aires, Argentina.

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Materials and Methods

Chemicals

(Carboxyl-^{14}C)-S-adenosyl-L-methionine (60 mCi/mmol) was purchased from New England Nuclear (Boston, MA). Testoster-

one propionate (TP) was a gift from Laboratorios Gador (Buenos Aires, Argentina). Flutamide (4′-nitro-3′-trifluoruro-ethylisobutyranilide) was supplied by Schering Canada Ltd. (Quebec, Canada) through the courtesy of Dr. F. Labrie. All other reagents were obtained from Sigma Chemical Co. (St Louis, MO).

Animals and Treatments

Male Sprague–Dawley rats were used for all experiments. Animals were maintained in an air-conditioned room (22°C) with a 12-hour light: 12-hour dark cycle. They received laboratory chow and water *ad libitum*. Unilateral orchidectomy was performed via a scrotal incision under ether anesthesia, avoiding damage to the epididymides. Testosterone propionate (0.02 to 2 mg in 100 µl of corn oil) was injected subcutaneously every 2 days for 7 days. Flutamide (5 mg) was injected subcutaneously (suspended in 200 µl of 0.9% sodium chloride solution containing 1% gelatin) twice a day for 7 days. In all cases, treatments began 24 hours after the day of orchidectomy, referred to as day 0. Animals were decapitated in the morning of day 8, and epididymides were kept for SAMDC assay at −20°C for less than 2 weeks.

Preparation of Supernatants

Epididymides were thawed, cut, and homogenized in cold 50 mmol/L phosphate buffer (pH 7.4) containing 2 mmol/L β-mercaptoethanol (5 ml/g tissue) using an Ultraturrax homogenizer (Janke & Kunkel, IDA Werk, Staufen, West Germany) at 0°C to 4°C. Homogenates were centrifuged at 20,000 g for 30 minutes, and the supernatants were used immediately for SAMDC assay and protein determination. For the assessment of the Michaelis constant (Km) and dependence on putrescine, the supernatants (1 ml) were freed of endogenous substrate and putrescine by filtration through a 8-ml column of Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden). Protein-containing fractions were pooled and concentrated by filtration through a Centricon-10 microconcentrator (Amicon Division, Danvers, MA) to a final volume of 1 ml.

SAMDC Assay

The SAMDC activity was assayed according to the method of Pegg and Williams-Ashman (1969) with some modifications. Aliquots (200 µl) were incubated with 200 µl of a buffer composed of 50 mmol/L sodium phosphate, 2.5 mmol/L dithiothreitol, 3 mmol/L putrescine, 0.4 mmol/L SAM p-toluenesulphonate, and 1 µCi/ml ^{14}C-SAM. Incubations were carried out for 60 minutes at 34°C in 10-ml glass vials with rubber stoppers. Reactions were stopped by injecting 500 µl of 40% (p/v) trichloroacetic acid. Incubation continued for 30 minutes at 34°C. The released carbon dioxide was trapped in polypropylene wells containing 150 µl of hyamine (Packard Instruments Co. Inc., Downers Grove, IL). At the end of incubations, wells were dropped into 2.5 ml of scintillation mixture (0.4% 2,5-diphenyloxazole [PPO] and 0.004% phenyloxazolylphenyloxazolylphenil [POPOP] in toluene) and radioactivity was counted with 90% efficiency. The results are expressed as picomoles of $^{14}CO_2$ released per hour per milligram of protein.

Other Methods

Protein concentration was measured by the method of Lowry et al (1951) using bovine serum albumin (BSA) as the standard. Statistical analysis was performed using Student's t-test when two groups were compared or using analysis of variance and Duncan's test for multiple comparisons (Li, 1964).

Results

Partial Characterization of Epididymal SAMDC Activity

Under our assay conditions, the release of $^{14}CO_2$ from ^{14}C-SAM was linear with the incubation time up to 60 minutes and protein concentration up to 10 mg/ml (data not shown). For the assessment of Km for SAM, aliquots of a pooled supernatant from 45-day-old rats, which were free of endogenous substrate, were incubated with a constant concentration of ^{14}C-SAM and increasing concentrations of unlabeled SAM. Data were analyzed by the method of Lineweaver-Burk. As shown in Figure 1, epididymal SAMDC reaction had a Km value for SAM of about 50 µmol/L.

When supernatants were not filtered through Sephadex, SAMDC activity was detected in the absence of exogenous putrescine. Negligible values were obtained in filtered samples, whereas a significant activation was observed by the addition of putrescine (Fig 2).

Epididymal SAMDC was found to be unevenly distributed throughout the organ. In 45-day-old rats, higher values of activity were found in the corpus and cauda compared with the caput (mean ± SD: 118 ± 5, 202 ± 29, and 194

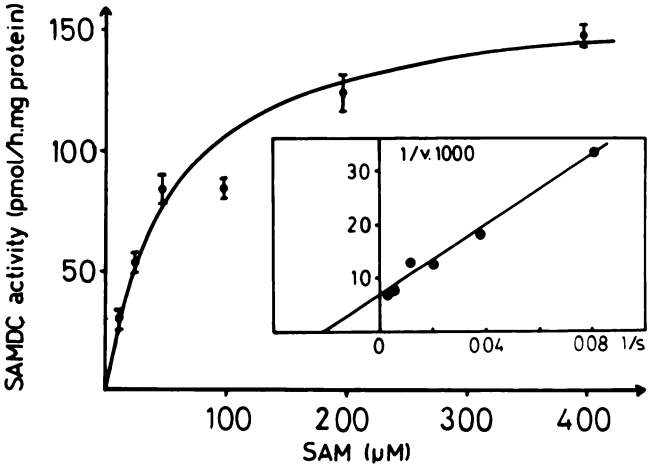

FIG. 1. Lineweaver-Burk analysis of epididymal SAMDC activity. Supernatant was prepared from a pool of epididymides from five animals. Values are presented as the mean ± SD for three replicates. This figure is representative of three experiments that gave essentially the same results. Values obtained in the experiment were 49.7 µmol/L for Km and 0.99 for correlation coefficient.

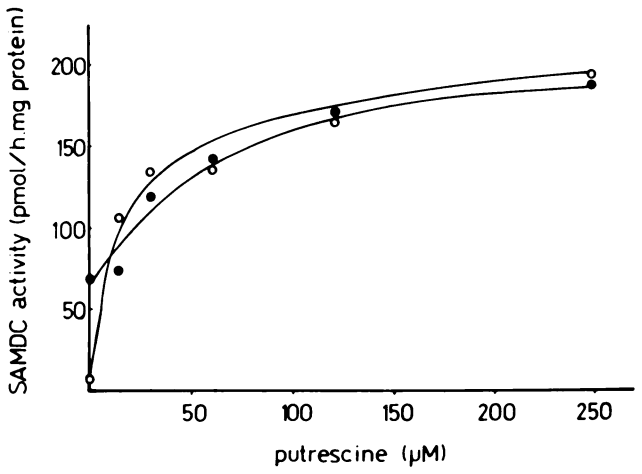

FIG. 2. Effect of putrescine on epididymal SAMDC activity. Supernatant was prepared from a pool of epididymides from five animals and divided into two fractions, one of which was previously filtered through Sephadex G-25 (open circles) or used directly (filled circles) in the enzyme assay.

± 24 pmol CO_2/h/mg protein for caput, corpus and cauda, respectively; $P < 0.05$ for values from corpus and cauda compared with caput). Analysis of epididymal SAMDC from rats at different ages showed that this activity exists as early as 15 days, although a peak of activity is clearly seen at 25 days (Fig 3). All subsequent experiments were carried out on 45-day-old rats, since SAMDC is readily detected at this age and spermatozoa have not yet entered the organ (Robaire and Hermo, 1988).

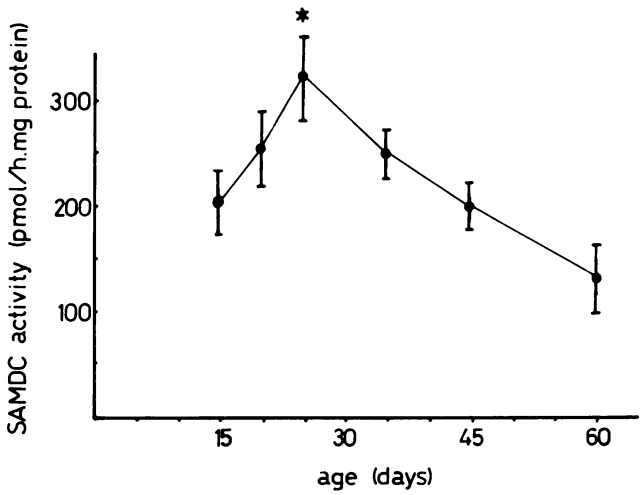

FIG. 3. Ontogeny of epididymal SAMDC activity. Values are presented as the means ± SD for five samples. Samples from 15- to 25-day-old rats were prepared by pooling epididymides from two animals. For 35- to 60-day-old rats, individual determinations were carried out. *P < 0.05 compared to values at 15 days.

Androgenic Control of Epididymal SAMDC Activity

Bilateral orchidectomy resulted in a decline of epididymal SAMDC activity (Fig 4A). This effect was statistically significant 3 days after orchidectomy. After 7 days, values of SAMDC activity were about 34% of those of intact controls. At 14 days, values were not significantly different from those obtained at 7 days (not shown). In orchidectomized rats, SAMDC activity was increased by androgen treatment in a dose-dependent fashion. As seen in Figure 4B, a dose of 0.5 mg every 2 days completely restored the activity, whereas supraphysiologic levels were attained at the dose of 2 mg. While epididymal SAMDC was restored to normal levels in castrated rats, treatment of intact animals with a high dose of androgen (0.5 mg TP every 2 days) failed to modify SAMDC activity (Table 1), suggesting that, at this age, endogenous androgens are in excess to maintain maximal levels of the enzyme.

The stimulatory effect of TP on epididymal SAMDC activity in castrated rats was completely abolished by con-

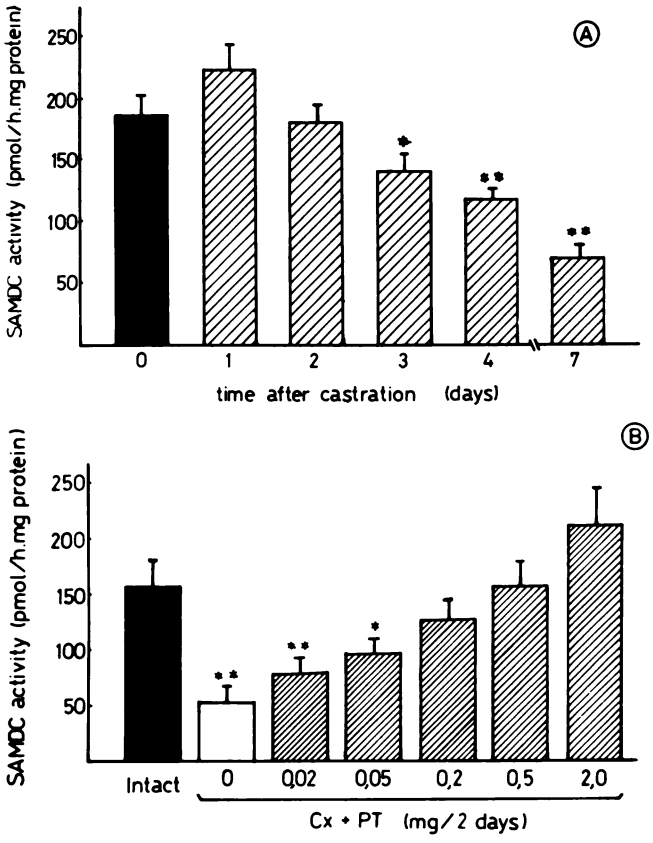

FIG. 4. Effect of bilateral orchidectomy and testosterone replacement on epididymal SAMDC. (A) Time course of SAMDC decline after bilateral orchidectomy. (B) Dose-response relationship after testosterone treatment. Values are presented as the means ± SD for four animals. *P < 0.05 and **P < 0.01 compared to values from intact animals.

Table 1. *Effect of testosterone on SAMDC activity in intact and castrated rats and abolishment by flutamide*

Treatment	SAMDC activity (pmol/h per mg protein)
Intact	
Vehicle	133 ± 5
TP, 0.5 mg/2 days	134 ± 8
Castrated	
Vehicle	56 ± 13*
TP, 0.5 mg/2 days	145 ± 25
TP, 0.5 mg/2 days + flutamide, 5 mg/day	71 ± 7*

In all cases, treatments began 24 hours after orchidectomy (day 0). Animals were sacrificed in the morning of day 8. Values are presented as means ± SD for four animals.

* $P < 0.01$ compared to values from intact controls.

comitant treatment with flutamide, an antiandrogen known to act as a specific blocker of androgen receptors. When 5 mg of this compound was injected daily with a stimulatory dose (0.5 mg) of TP, the same values as those from castrated animals were found (Table 1).

Effect of Unilateral Orchidectomy on Epididymal SAMDC

To assess the contribution of circulating and luminal androgens to the maintenance of epididymal SAMDC, rats were unilaterally orchidectomized, and SAMDC activity was determined in both epididymides after 7 days. The SAMDC activity was identical in epididymides from both sides (121 ± 8 pmol/h/mg protein for the intact side and 121 ± 11 pmol/h/mg protein for the orchidectomized side), suggesting that there were enough circulating androgens to maintain normal levels of activity.

Discussion

The reaction catalyzed by SAMDC is necessary for the synthesis of spermidine and spermine from putrescine. In some organs (ie, the prostate and seminal vesicles of androgen-treated castrated rats; Kapyaho et al, 1980), the production of decarboxylated SAM occurs at a lower rate than that of putrescine, thereby limiting the rate of polyamine synthesis. In this study, we examined the occurrence of SAMDC in the rat epididymis and its androgenic control using the same experimental models as those for the analysis of epididymal ODC (de las Heras and Calandra, 1987; de las Heras et al, 1988).

The Km value for SAM found in the epididymis is comparable to that found in the rat prostate (Pegg and Williams-Ashman, 1969), although epididymal SAMDC activity in the epididymis is about six times lower than that measured in the prostate on a basis of picomoles per hour per milligrams of protein.

When supernatants were freed of endogenous putrescine, as assessed by fluorometric assay of its dansylated derivative (data not shown), SAMDC activity exhibited a marked dependence on this diamine (Fig 2). This effect of putrescine is similar to that found for the enzyme from rat and human prostate (Pegg and Williams-Ashman, 1969; Zappia et al, 1972). Although SAMDC is detectable in crude preparations in the absence of exogenous putrescine, this does not imply that this mechanism operates *in vivo* under normal conditions because the intracellular concentrations of putrescine available for the enzyme are not known.

Assessment of epididymal SAMDC at different stages of sexual maturation provided the first suggestion of an androgenic control of this enzyme. The SAMDC activity peaked around day 25 (Fig 4). Between days 20 and 25, the synthesis of testicular androgens is increased (Podestá and Rivarola, 1974; Moger, 1977), epididymal dihydrotestosterone (DHT) content and androgen receptors become detectable (Calandra et al, 1974), and androgen-binding protein (ABP) appears in the caput (Hansson et al, 1974). According to Danzo and Eller (1985), ABP is detected in the rat epididymis on day 25. These data are indicative of an increase in the androgens available in the epididymis. In relation to this, Setty and Jehan (1977) showed that the phase of most rapid growth of the epididymis, as assessed by histologic examination, begins around day 21, which is in agreement with the increase in thymidine incorporation of DNA found at day 25 (De Larminat et al, 1981). Unlike ODC, SAMDC is detected as early as 15 days, an age at which the epididymis is not under the influence of testicular androgens. This would imply that a population of androgen-independent enzymes exists in the epididymis of immature animals. The mode of regulation of this activity and the physiologic implications of its occurrence at this age are unknown and need further investigation. The decline in SAMDC activity, expressed per milligrams of protein, observed from 25 days onward is mostly due to an increase in the protein content of the organ and not to an inactivation of the enzyme. In fact, the total content of enzyme activity, expressed as picomoles per hour per organ, remained essentially constant between 30 and 60 days.

Similar to ODC activity, SAMDC activity displayed the characteristics of an androgen-dependent enzyme. However, it does not appear to be as sensitive a marker of androgen action as ODC. First, as stated above, an androgen-independent enzyme appears to exist in the epididymides of immature rats. Second, even though its activity is decreased on orchidectomy, this effect is not evident after 2 days. According to Aafjes and Vreeburg (1972), a considerable decrease in epididymal androgen content occurred at this time. Three possible explanations are: 1) SAMDC has a longer half-life than ODC, contradicting the values (about 1 hour) described for the enzyme previously (Tabor and Tabor, 1984); 2) the enzyme activity is maintained by nontesticular factors or testicular factors other than androgens

with a low turnover rate; or 3) both orchidectomy and flutamide treatment of androgen-maintained orchidectomized rats failed to decrease SAMDC to undetectable values. Taken together, these results support the idea that at least one form of the enzyme not regulated by androgens exists in the epididymis.

Our data do not allow us to evaluate the presence of isoforms since they have a different regulation or distribution within the cellular types of the organ. It is accepted that the principal cells of the epithelium display a much higher sensitivity to androgens than other cells (Moore and Bedford, 1979a, 1979b). It is tempting to speculate that ODC is confined to the principal cells of the epididymis, whereas SAMDC is active in other cells as well. Alternatively, the coexistence of isoforms within the same cell would guarantee the synthesis of spermidine and spermine in the absence of androgenic stimulus, eg, from putrescine taken up from blood. In summary, we conclude that high levels of SAMDC activity are present in the rat epididymis and show an androgenic control, although it is not as strict as that of ODC.

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References

Aafjes JH, Vreeburg JTM. Distribution of 5α-dihydrotestosterone in the epididymis of bull and boar, and its concentration in rat epididymis after ligation of efferent testicular ducts, castration and unilateral gonadectomy. *J Endocrinol.* 1972;53:85–93.

Berger FG, Szymanski P, Read E, Watson G. Androgen-regulated ornithine decarboxylase mRNAs of mouse kidney. *J Biol Chem.* 1984; 259:7941–7946.

Calandra RS, Podestá EJ, Rivarola MA, Blaquier JA. Tissue androgens and androphilic proteins in rat epididymis during sexual development. *Steroids.* 1974;24:507–518.

Danzo B, Eller BC. The ontogeny of biologically active androgen-binding protein in rat plasma, testis and epididymis. *Endocrinology.* 1985; 117:1380–1388.

De Larminat MA, Cuasnicú PS, Blaquier JA. Changes in trophic and functional parameters of the rat epididymis during sexual maturation. *Biol Reprod.* 1981;25:813–819.

de las Heras MA, Calandra RS. Androgen-dependence of ornithine decarboxylase in the rat epididymis. *J Reprod Fertil.* 1987;79:9–14.

de las Heras MA, Suescun MO, Calandra RS. Ornithine decarboxylase activity as a marker of androgen and antiandrogen action in the rat epididymis. *J Reprod Fertil.* 1988;83:177–183.

Fjøsne HE, Strand H, Ostensen MA, Sunde A. Ornithine decarboxylase and S-adenosylmethionine decarboxylase activity in the accessory sex organs of intact, castrated and androgen-stimulated castrated rats. *Prostate.* 1988;12:309–320.

Hansson V, Trygstad O, French FS, McLean WS, Smith AA, Tindall DJ, Weddington SC, Petruz P, Nayfeh SN, Ritzen EM. Androgen transport and receptor mechanisms in testis and epididymis. *Nature.* 1974; 250:387–391.

Jänne J, Poso H, Raina A. Polyamines in rapid growth and cancer. *Biochim Biophys Acta.* 1978;473:241–293.

Li CC. *Introduction to Experimental Statistics.* New York: McGraw-Hill; 1964.

Kapyaho K, Poso H, Jänne J. Role of propylamine transferases in hormone-induced stimulation of polyamine biosynthesis. *Biochem J.* 1980;192:59–63.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193:265–275.

Moger WH. Serum 5α-Androstane-3α-17β-Diol, androsterone, and testosterone concentrations in the male rat. Influence of age and gonadotropin secretion. *Endocrinology.* 1977;100:1027–1032.

Moore HDM, Bedford JM. Short-term effects of androgen withdrawal on the structure of different epithelial cells in the rat epididymis. *Anat Rec.* 1979a;193:293–312.

Moore HDM, Bedford JM. The differential absorptive activity of epithelial cells of the rat epididymis before and after castration. *Anat Rec.* 1979b;193:313–328.

Moulton BC, Leonard SL. Hormonal effects on spermidine levels in the male and female reproductive organs of the rat. *Endocrinology.* 1969;84:1461–1465.

Pegg AE. Recent advances in the biochemistry of polyamines in eukaryotes. *Biochem J.* 1986;234:249–262.

Pegg AE, Williams-Ashman HG. Biosynthesis of putrescine in the prostate gland of the rat. *Biochem J.* 1968;108:533–539.

Pegg AE, Williams-Ashman HG. On the role of S-adenosyl-L-methionine in the biosynthesis of spermidine by rat prostate. *J Biol Chem.* 1969;244:682–693.

Pegg AE, Lockwood DH, Williams-Ashman HG. Concentrations of putrescine and polyamines and their enzymic synthesis during androgen-induced prostatic growth. *Biochem J.* 1970;117:17–31.

Piik K, Rajamaki P, Guha SK, Jänne J. Regulation of L-ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase in rat ventral prostate and seminal vesicle. *Biochem J.* 1977;168:379–385.

Podestá EJ, Rivarola MA. Concentration of androgens in whole testis, seminiferous tubules and interstitial tissue of rats at different stages of development. *Endocrinology.* 1974;95:455–461.

Robaire B, Hermo L. Efferent ducts, epididymis, and vas deferens: structure, functions and their regulation. In: Knobil E, Neill J, eds. *Physiology of Reproduction.* New York: Raven Press; 1988:999–1080.

Russell DH. Ornithine decarboxylase: a key regulatory enzyme in normal and neoplastic growth. *Drug Metab Rev.* 1985;16:1–88.

Setty BS, Jehan Q. Functional maturation of the epididymis in the rat. *J Reprod Fertil.* 1977;49:317–322.

Tabor CW, Tabor H. Polyamines. *Ann Rev Biochem.* 1984;53:749–790.

Williams-Ashman HG, Jänne J, Coppoc GL, Geroch ME, Schenone A. New aspects of polyamine biosynthesis in eukaryotic organisms. In: Weber G, ed. *Advances in Enzyme Regulation.* Oxford: Pergamon Press; 1972;10:225–245.

Zappia V, Carteni-Farina M, Della Pietra G. S-adenosylmethionine decarboxylase from human prostate. *Biochem J.* 1972;129:703–709.