Studies on the Microsomal Sodium-plus-Potassium Ion-Stimulated Adenosine Triphosphatase System in Rat Ventral Prostate

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A $Mg^{2+} + Na^{+} + K^{+}$ -stimulated adenosine triphosphatase (ATPase) preparation was isolated from rat ventral prostate by flotation of microsomal membranes in high-density sucrose solutions. The reaction medium for optimum $Na^{+} + K^{+}$ -stimulated ATPase activity was found to be: Na^{+} , 115 mM; K^{+} , 7–10 mM; Mg^{2+} , 3 mM; ATP, 3 mM; tris buffer, pH 7.4 at 38°, 20 mM. The average ΔP_i ($Mg^{2+} + Na^{+} + K^{+}$ minus $Mg^{2+} + Na^{+}$) was 9 μ moles/mg. of protein/hr., representing a 30% increase over the $Mg^{2+} + Na^{+}$ -stimulated ATPase activity. At high concentrations, K^{+} was inhibitory to the enzyme activity. Half-maximal inhibition of $Na^{+} + K^{+}$ -stimulated ATPase activity was elicited by ouabain at 0.1 mM. The preparation exhibited phosphatase activity towards ribonucleoside triphosphates other than ATP. However, stimulation of P_i release by $Na^{+} + K^{+}$ was observed only with ATP as substrate. The apparent K_m for ATP for $Na^{+} + K^{+}$ -stimulated activity was about 0.3×10^{-3} M. Ca^{2+} inhibited only the $Na^{+} + K^{+}$ -stimulated ATPase activity. Mg^{2+} could be replaced by Ca^{2+} but then no $Na^{+} + K^{+}$ stimulation of ATPase activity was noticed. The addition of testosterone or dihydrotestosterone (17 β -hydroxy-5 α -androstane-3-one) *in vitro* at 0.1–10 μ M under a variety of experimental conditions did not significantly increase the $Na^{+} + K^{+}$ -stimulated ATPase activity. The enzyme preparations from prostates of orchidectomized rats, however, exhibited a drastic decrease in the specific activity of $Na^{+} + K^{+}$ -stimulated ATPase; these changes were prevented in the orchidectomized rats by injection of testosterone propionate.

A considerable amount of evidence indicates that $Na^{+} + K^{+}$ -stimulated ATPase* systems present in most mammalian tissues, are involved in the transport of Na^{+} and K^{+} across cell membranes (see, e.g., Rothstein, 1968; Albers, 1967; Skou, 1964, 1965; Judah & Ahmed, 1964a).

The nature and hormonal control of $Na^{+} + K^{+}$ -stimulated ATPase systems in the rat ventral prostate gland is of interest for a number of reasons. First, in sexually mature rats the gland is composed largely of tall columnar epithelial cells that actively secrete a fluid which contributes extensively to the seminal plasma (Mann, 1964; Price & Williams-Ashman, 1961). $Na^{+} + K^{+}$ -stimulated ATPase activity might play an important role in ion translocations that accompany the secretory functions of ventral prostate epithelial cells. Secondly, the

development of the ventral prostate is under the strict control of androgenic hormones, which are required for the maintenance of the adult size and secretory capabilities of the gland throughout the lifetime of the rat. Orchidectomy of adult rats leads to a rapid involution of the prostate involving, among other things, cessation of secretion and a striking loss in the endoplasmic reticulum of the epithelial cells (Price & Williams-Ashman, 1961). These effects of androgen withdrawal are reversed by administration of androgenic steroids. The question arises whether $Na^{+} + K^{+}$ -stimulated ATPase systems associated with the prostatic endoplasmic reticulum are affected by androgenic hormones *in vivo* or *in vitro*.

Farnsworth (1967, 1968) reported that 0.1 μ M-testosterone *in vitro* enhanced the $Mg^{2+} + Na^{+} + K^{+}$ -stimulated ATPase activity of rat ventral prostate

* Abbreviation: ATPase, adenosine triphosphatase.

microsomes. Since $\text{Na}^+ + \text{K}^+$ -stimulated ATPase appears to be involved in cation transport across cell membranes, Farnsworth (1967) postulated that stimulation of this enzyme system and hence the overall process of cation transport 'might provide the energy required for the spectrum of bio-synthetic activities credited to testosterone'. The control of many biosynthetic processes in rat ventral prostate by androgens is well documented (Williams-Ashman, Liao, Hancock, Jurkowitz & Silverman, 1964).

The present work was undertaken to delineate the properties of $\text{Na}^+ + \text{K}^+$ -stimulated ATPase of the rat ventral prostate and to examine the effects of androgens on this enzyme system *in vitro* and *in vivo*. A preliminary report of part of this work has been given (Ahmed, 1969).

MATERIALS AND METHODS

Treatment of animals. Prostatic tissue was obtained from adult male Sprague-Dawley rats (200–300 g.) maintained on a standard laboratory diet.

Orchidectomy was performed via the scrotal route under ether anaesthesia. Testosterone propionate (1 mg.) was administered subcutaneously in sesame oil (0.2 ml.); the control animals received the same volume of oil. The tissues from each group of rats (castrated animals, castrated animals treated with testosterone and normal animals) were pooled before homogenization, and the microsomal membrane preparations were isolated from each group simultaneously.

Chemicals. Baker Analyzed reagents (J. T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.) were used. Solutions were prepared in double-distilled ion-free water and were checked by flame photometry or atomic absorption spectroscopy for cross contamination of ions. If necessary, salts were further purified by recrystallization from ion-free water. EDTA, ATP, UTP, GTP, CTP and ITP were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. These were neutralized with tris base and passed through Dowex AG-50 cation-exchange resin (tris form; X8; 200–400 mesh) to remove any other cations. Final pH was adjusted to 7.4 with tris base. Tris was obtained from Calbiochem, Los Angeles, Calif., U.S.A. Sucrose and mannitol were Mann Assayed grade (Mann Research Laboratories Inc., New York, N.Y., U.S.A.). Testosterone, dihydrotestosterone (17 β -hydroxy-5 α -androstan-3-one), testosterone propionate, spermine hydrochloride and spermidine hydrochloride were obtained from Sigma Chemical Co.

Preparation of the enzyme system. Rats were killed by decapitation and the two lobes of ventral prostate were removed after any adhering fat and connective tissue had been carefully trimmed off. About 2–3 g. of prostate tissue (from six rats) was placed in a beaker kept on crushed ice and was minced with a fine scissors. To this, a medium consisting of 0.25 M-mannitol–20 mM-tris–3 mM-EDTA, pH 7.4, was added so that the final homogenate would be about 10% (w/v). The homogenate was prepared in a Duall homogenizer with a Teflon pestle (Kontes Glass Co., Vineland, N.J., U.S.A.). A small amount of tissue was not homogenized by this procedure and was discarded. The

microsomal fraction was isolated from this homogenate by the procedure described by Ahmed & Judah (1964). It was washed once in 20–30 ml. of a medium consisting of 0.25 M-mannitol–20 mM-tris–1 mM-EDTA, pH 7.4. The microsomal pellet so obtained was used to isolate a membrane fraction as described by Ahmed & Judah (1964). The microsomal membrane fraction was harvested from the top of the tubes and suspended in 30 ml. of 0.25 M-mannitol–20 mM-tris–1 mM-EDTA, pH 7.4. This suspension was centrifuged at 133000 g_{av} for 60 min. and the sedimented protein was suspended in a total volume of 2.0 ml. of the mannitol–tris–EDTA, pH 7.4, medium, described above. The suspension (referred to as microsomal membrane preparation) was stored frozen at -10° in small portions, which were removed and suitably diluted with the same mannitol medium before use. Only once-thawed material was used, as repeated freezing and thawing caused loss of enzyme activity. In a few preliminary experiments the microsomal pellet was used without further processing. All the above preparative procedures were carried out at 4° . Rat brain enzyme preparations, when used, were isolated exactly as described by Ahmed & Judah (1964).

Assay of ATPase activity. The assay of $\text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$ -stimulated ATPase was performed at 38° and the reaction mixture, in a final volume of 2.0 ml., consisted of MgCl_2 (3 mM), ATP (tris salt, 3 mM), tris–HCl buffer, pH 7.4 at 38° (20 mM), NaCl (115 mM), KCl (8.0 mM) and 30–60 μg . of prostatic microsomal membrane preparation suspended in 0.2 ml. of the mannitol–tris–EDTA medium described above. The reaction was generally started by the addition of protein. When effects of drugs were to be studied, the enzyme preparation was preincubated with the drug for 1–2 min. in the above reaction medium, and a suitable mixture of MgCl_2 and ATP was added to initiate the reaction. When testosterone or dihydrotestosterone was added *in vitro*, they were added as solutions in ethanol in volumes of 0.01–0.02 ml. and the controls contained appropriate amounts of ethanol. The 'basic' activity ($\text{Mg}^{2+} + \text{Na}^+$ -stimulated ATPase) was determined by omitting KCl from the incubation medium. Any variations from this procedure are indicated in the text. The incubation time was adjusted so that not more than 15% of the total ATP was hydrolysed. The reaction was terminated by adding 2 ml. of ice-cold 10% (w/v) trichloroacetic acid. The contents of the reaction flasks were then centrifuged in a refrigerated centrifuge for 5 min. at 5000 g and the supernatant was analysed for P_i liberated.

Chemical analysis. P_i was determined by the method of Fiske & Subbarow (1925) or that of Berenblum & Chain (1938). The method of Lowry, Rosebrough, Farr & Randall (1951) was used to determine the protein.

Expression of results. The results are expressed as μmoles of P_i liberated/mg. of protein/hr. The $\text{Na}^+ + \text{K}^+$ -stimulated ATPase activity, ΔP_i , refers to the ATPase activity in the presence of $\text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$ minus that in the presence of $\text{Mg}^{2+} + \text{Na}^+$.

RESULTS

Na^+ and K^+ requirement of prostatic ATPase. Farnsworth (1967) has reported the presence of cation-activated ATPase in prostate microsomes. In the present work, a membrane fraction isolated from microsomes was used because the results were

Fig. 1. Requirement of Na^+ and K^+ for $\text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$ -stimulated ATPase of microsomal membrane preparation of rat ventral prostate. The composition of the incubation medium was the same as described in the Materials and Methods section except for changes in Na^+ and K^+ as shown. The reaction was initiated by addition of the enzyme system. ΔP_i refers to ATPase activity in the presence of $\text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$ minus that in the presence of $\text{Mg}^{2+} + \text{Na}^+$ or $\text{Mg}^{2+} + \text{K}^+$.

Fig. 2. Effect of K^+ on the $\text{Mg}^{2+} + \text{Na}^+$ -stimulated ATPase of prostatic microsomal membrane preparation. The composition of the incubation medium was the same as described in the Materials and Methods section except that the concentration of K^+ was varied in the range 0–20 mm as shown. ΔP_i refers to ATPase activity in the presence of $\text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$ minus that in the presence of $\text{Mg}^{2+} + \text{Na}^+$.

considerably more reproducible than when crude microsomes were employed as a source of the enzyme. Thus in the latter case the activity of the ATPase in the presence of $\text{Mg}^{2+} + \text{Na}^+$ was usually considerably higher when compared with $\text{Mg}^{2+} + \text{choline}$ (choline chloride being substituted for sodium chloride at equimolar concentration) and addition of K^+ to the $\text{Mg}^{2+} + \text{Na}^+$ system in these cases often produced no further increase in ATPase activity. A possible explanation for these findings is that enough K^+ was bound to the crude microsomes, so that the presence of Na^+ caused some activation of the ATPase activity. Aldridge (1962) has commented on this problem in working with brain microsomes. When prostate microsomal membrane preparation was used, ATPase activity in the presence of $\text{Mg}^{2+} + \text{Na}^+$ was generally the same as that in the presence of $\text{Mg}^{2+} + \text{choline}$ and addition of K^+ to the $\text{Mg}^{2+} + \text{Na}^+$ system invariably resulted in further increase in ATPase activity. The specific activity of the $\text{Na}^+ + \text{K}^+$ -stimulated ATPase activity of prostatic microsomal membrane preparation was some 50% higher than with crude microsomes. No attempt was made to localize the enzyme system quantitatively in subfractions of prostate homogenates. However, a greater part of the activity of the homogenate sedimented in the so-called nuclear fraction (residue 5 min. at 1085g). This could be due to the presence of unbroken cells and cell membranes adhering to nuclei and debris. The activity recovered in the present preparation represents about 5–10% of that in the total homogenate.

The $\text{Na}^+ + \text{K}^+$ -stimulated portion of the ATPase activity or ΔP_i (as defined in the Materials and Methods section) was generally 9 μmoles/mg. of protein/hr. (varying from 4 μmoles to 12 μmoles with different preparations). The ratio of the ATPase activities in the presence of $\text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$ and in the presence of $\text{Mg}^{2+} + \text{Na}^+$ was generally 1.3 (range 1.2–1.5). The enzyme system gradually lost activity on storage, resulting in about 50% loss of activity in 4 weeks. The preparations were used for several days but were discarded when about half of the activity was lost or if ΔP_i values fell below 4 μmoles/mg. of protein/hr. In general, the properties of the $\text{Na}^+ + \text{K}^+$ -stimulated ATPase did not appear to alter during this period.

As observed with other tissues (see, e.g., Skou, 1965), it was also found that the activity ratio $\text{Mg}^{2+} + \text{Na}^+ + \text{K}^+ / \text{Mg}^{2+} + \text{Na}^+$ could be increased considerably by using 0.1% deoxycholate in the homogenizing medium, with further loss of total yield of the enzyme activity (K. Ahmed, unpublished work). However, use of deoxycholate or other detergents in the preparation of prostate enzyme was avoided as our major interest was to study the effects of androgens on the enzyme system *in vitro* as well as *in vivo*.

Fig. 1 shows that the presence of both Na^+ and K^+ in suitable proportions is necessary for maximum ATPase activity of prostatic microsomal membrane preparation. The activity is due to the combined effect of Na^+ and K^+ , as shown by maintaining the total Na^+ and K^+ concentration at

150 mM. When the concentration of Na^+ was fixed at 115 mM and the K^+ concentration was varied, maximum activation was observed when K^+ was present at 7–10 mM (Fig. 2).

Linearity of reaction. Under standard conditions of incubation and with the test system as described in the Materials and Methods section, the $\text{Na}^+ + \text{K}^+$ -stimulated ATPase activity was linear for 1 hr. or over the time studied. Varying the amount of protein in the range 20–60 μg . in the reaction mixture resulted in a linear rate of P_i release from ATP over a period of 60 min. provided that no more than 15% of the total ATP was hydrolysed during the course of reaction.

Substrate specificity. Although the preparation exhibited the ability to hydrolyse various ribonucleoside triphosphates in the presence of $\text{Mg}^{2+} +$

Na^+ , only the hydrolysis of ATP was stimulated by the presence of both Na^+ and K^+ . Thus, in a typical experiment, the phosphatase activities with ATP as substrate were 10.3 and 14.3 μmoles of P_i formed/mg. of protein/hr. in the presence of $\text{Mg}^{2+} + \text{Na}^+$ and $\text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$ respectively. The phosphatase activity values for GTP, UTP, CTP and ITP as substrates (each at 3 mM concentration) were respectively 12.8, 14.3, 10.7 and 14.3 μmoles of P_i formed/mg. of protein/hr. in the presence of $\text{Mg}^{2+} + \text{Na}^+$ or $\text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$. The apparent K_m for ATP (derived from Fig. 3) for the $\text{Na}^+ + \text{K}^+$ -stimulated ATPase activity was about $0.3 \times 10^{-3} \text{ M}$. Maximal rates of P_i liberation were observed in the presence of 3 mM-ATP and 3 mM- Mg^{2+} .

Effect of ouabain. The $\text{Na}^+ + \text{K}^+$ -stimulated ATPase of prostatic microsomal membrane preparation was inhibited by ouabain, which produced a 50% inhibition at a concentration of about 0.1 mM without any effect on the $\text{Mg}^{2+} + \text{Na}^+$ -stimulated ATPase. The nature of ouabain interaction with the prostatic preparation appeared to be similar to that in preparations from erythrocytes (Dunham & Glynn, 1961), liver (Judah & Ahmed, 1964b) and brain (Ahmed & Judah, 1965; Ahmed, Judah & Scholfield, 1966) in that the inhibitory effect of ouabain was partially reversed when K^+ concentration in the incubation medium was increased (Table 1).

Effect of testosterone and dihydrotestosterone in vitro. Farnsworth (1968) reported that testosterone (0.1–1.0 μM) when added *in vitro* to microsomes from rat ventral prostate enhanced the $\text{Na}^+ + \text{K}^+$ -stimulated ATPase by about 30%. We attempted to repeat these observations with the present preparation as the source of the $\text{Na}^+ + \text{K}^+$ -stimulated enzyme system. The effect of testosterone at 0.1, 1.0 and 10 μM final concentration in the reaction medium was investigated. No stimulatory effect of testosterone at these concentrations was found on the $\text{Mg}^{2+} + \text{Na}^+$ - or the $\text{Mg}^{2+} + \text{Na}^+ +$

Fig. 3. Effect of varying the ATP concentration on $\text{Na}^+ + \text{K}^+$ -stimulated ATPase activity. ATP concentration in the incubation medium was varied as shown. All other experimental details were the same as described in the Materials and Methods section. ΔP_i refers to ATPase activity in the presence of $\text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$ minus that in the presence of $\text{Mg}^{2+} + \text{Na}^+$.

Table 1. Partial reversal of ouabain inhibition of prostatic microsomal membrane $\text{Na}^+ + \text{K}^+$ -stimulated ATPase by altering the concentration of K^+ in the incubation medium

Ouabain was present in the incubation medium at a concentration of 0.1 mM. The reaction was initiated by adding the enzyme system. All other details were exactly the same as described in the Materials and Methods section except that K^+ was present in concentrations shown. $\text{Mg}^{2+} + \text{Na}^+$ -stimulated ATPase activity, in the presence or absence of ouabain, was 12.3 μmoles of P_i formed/mg. of protein/hr.

K^+ (mM)	$\text{Na}^+ + \text{K}^+$ -stimulated ATPase activity (ΔP_i , $\mu\text{moles/mg. of protein/hr.}$)		
	Controls	Ouabain	Inhibition (%)
2	3.3	0.4	88
4	3.8	1.4	63
8	4.8	2.0	58

K⁺-stimulated ATPase activities. In fact, a small inhibition of the Na⁺+K⁺-stimulated ATPase was observed. In view of the suggestion of Bruchovsky & Wilson (1968) and Liao & Anderson (1968) that dihydrotestosterone might be an 'active form' of testosterone, we also tested the effect of dihydrotestosterone at 1.0, 10 and 100 μ M concentration. No significant effects were observed.

Experiments were repeated with ventral prostate microsomes from the rat in exactly the same test system as described by Farnsworth (1968).

For these experiments rat ventral prostate microsomes were isolated and suspended in the test media described by Farnsworth (1968). Under these conditions all of the ATP in the reaction was hydrolysed. To ensure that no more than 15% of the total ATP was hydrolysed, the protein contents in each test system were adjusted to 24 μ g./0.5 ml. of suspension while keeping the reaction time after addition of ATP-tris mixture at 4 min. Under these conditions a small Na⁺+K⁺ stimulation of ATPase activity in controls was observed, as reported by Farnsworth (1968). The mean values for Mg²⁺+Na⁺+K⁺-stimulated and Mg²⁺-stimulated samples without testosterone were 188.7 $\pm$ 3.1 and 182.4 $\pm$ 0.7 μ moles of P_i/mg. of protein/hr. With testosterone present, they were 188.5 $\pm$ 2.5 and 180 $\pm$ 3.5 respectively. The ratios of P_i formed in the presence and the absence of Na⁺+K⁺ were calculated for the testosterone and control experiments; the average difference of these paired values (with and without testosterone) was 0.015 $\pm$ 0.021 (s.e.m.) ($P < 0.5$). This difference is clearly not significant.

Effect of spermine and spermidine on ATPase. Spermine and spermidine are present in large amounts in ventral prostate (Rhodes & Williams-Ashman, 1964). One of the physiological actions of these polyamines appears to be their ability to stabilize various cellular and subcellular particles (Tabor & Tabor, 1964; Jellink & Perry, 1967). deMeis (1968) reported that spermine and spermidine (at concentrations of 0.5–2 mM) activated Ca²⁺ uptake by muscle microsomes. It was also reported that spermine (4 mM) inhibited heart muscle microsomal ATPase by 50%. We therefore studied the effect of spermine and spermidine on Na⁺+K⁺-stimulated ATPase of the present preparation. Spermine inhibited the Na⁺+K⁺-stimulated ATPase by some 15% at a concentration of 1 mM, whereas spermidine at the same concentration had no significant effect.

Effect of Ca²⁺ on ATPase. Na⁺+K⁺-stimulated ATPase systems are inhibited by Ca²⁺ (Skou, 1964; Epstein & Whittam, 1966). At 0.1 mM-Ca²⁺ the Na⁺+K⁺-stimulated ATPase of the present preparation was inhibited by about 50% whereas 1 mM-Ca²⁺ completely abolished the Na⁺+K⁺-stimulated activity. At these or higher Ca²⁺ concentrations no effect, or only a small effect, was observed on the Mg²⁺+Na⁺-stimulated portion of the activity (Table 2). When Ca²⁺ was substituted in place of Mg²⁺, this enzyme preparation exhibited a high ATPase activity, the effect of Ca²⁺ being maximal at 2 mM. These results are somewhat different from those obtained with rat brain enzyme system, where a marked inhibition of Mg²⁺+Na⁺-stimulated ATPase activity was observed in the

Table 2. *Effect of Ca²⁺ and Mg²⁺ on microsomal membrane ATPase of rat prostate and brain*

Mg²⁺ or Ca²⁺ or both were present in the concentrations indicated. The reaction media were otherwise as described in the Materials and Methods section. The reaction was initiated by the addition of the enzyme system. In experiment (b) the conditions used for study of the brain enzyme were the same as for the prostate system.

Additions (mm)		ATPase activity (μ moles of P _i formed/mg. of protein/hr.)		Δ P _i (μ moles) (B – A)
Mg ²⁺	Ca ²⁺	Na ⁺ (A)	Na ⁺ + K ⁺ (B)	
(a) Prostate enzyme				
3.0	—	18.2	24.3	6.1
3.0	2.0	17.2	17.2	0
3.0	1.0	18.2	18.2	0
3.0	0.1	18.2	21.3	3.1
—	1.0	18.8	17.2	0
—	2.0	22.2	20.8	0
—	3.0	21.8	20.8	0
(b) Brain enzyme				
3.0	—	3.5	52.0	48.5
3.0	1.0	1.9	1.9	0
—	1.0	1.2	1.2	0
—	2.0	1.0	—	—

Table 3. *Effect of castration and testosterone treatment on cation-stimulated ATPase activities of rat ventral prostatic microsomal membrane preparations*

Castrated rats received subcutaneous injections of 0.20 ml. of sesame oil for 5 days after orchidectomy; the testosterone-treated castrated rats each received 1 mg. of testosterone propionate, dissolved in 0.2 ml. of sesame oil, daily for the same period. At the end of 5 days the rats were killed and the prostates from each group were pooled for isolation of the microsomal membrane preparations. In three different experiments the wet weights of prostate tissue from 23 castrated animals, 12 testosterone-treated castrated animals and 12 normal control animals were 4.3, 7.1 and 4.5 g. respectively. The yields of the microsomal membrane protein in the same order were 8.8, 15.1 and 10.8 mg. $Mg^{2+} + Na^{+}$ and $Mg^{2+} + Na^{+} + K^{+}$ incubation media were the same as described in the Materials and Methods section. In $Mg^{2+} + K^{+}$ incubation medium, 58 mM-KCl was present. The ATPase assays on the three types of preparations were performed within 3 days after isolation of the enzyme systems. Values for ATPase activity in each series of experiments represent the means of at least four determinations. The variations were less than $\pm 4\%$. The ΔP_i values in parentheses show the ranges observed in three different experiments.

	No. of rats	ATPase activity (μ moles of P_i formed/mg. of protein/hr.)			ΔP_i (μ moles)	
		$Mg^{2+} + Na^{+}$	$Mg^{2+} + K^{+}$	$Mg^{2+} + Na^{+} + K^{+}$	$Mg^{2+} + Na^{+} + K^{+}$ minus $Mg^{2+} + Na^{+}$	$Mg^{2+} + Na^{+} + K^{+}$ minus $Mg^{2+} + K^{+}$
Castrated rats	23	92.9	97.7	96.1	3.2 (1.2-6.1)	0 (0-0.6)
Castrated rats treated with testosterone	12	24.0	23.9	30.0	6.0 (5.2-7.1)	6.1 (5.8-6.6)
Normal rats	12	35.6	36.6	44.3	8.7 (6.2-11.8)	7.7 (6.0-9.8)

presence of 1 mM- Ca^{2+} , and when Ca^{2+} was substituted for Mg^{2+} the ATPase activity was greatly decreased. However, with the enzyme systems from both prostate and brain, no $Na^{+} + K^{+}$ -stimulated activities were demonstrable when Mg^{2+} was replaced by Ca^{2+} .

Effects of testosterone on prostatic ATPase in vivo. Since testosterone *in vitro* appeared to have no effect on the cation-stimulated ATPase of rat ventral prostate, it was decided to test whether the enzyme system was influenced by androgen deprival or administration *in vivo*. The effect of castration and injection of testosterone to castrated rats on the wet weight of ventral prostates and yield of microsomal membrane protein is described in the legend to Table 3. Orchidectomy resulted in drastic decrease in the size of the ventral prostate, whereas injections of testosterone to castrated animals produced an increase in prostate weight. The yield of microsomal membrane protein was correspondingly affected.

In preliminary experiments on the enzyme preparations from castrated rats, a two- to three-fold increase in $Mg^{2+} + Na^{+}$ -stimulated ATPase activity was observed compared with normal or testosterone-treated rats. It is well known (Price & Williams-Ashman, 1961) that after orchidectomy there is a gradual increase in the relative proportion of fibromuscular cells in the rat ventral prostate concomitantly with involution of the epithelium. It is therefore possible that the large increase in

$Mg^{2+} + Na^{+}$ -stimulated ATPase activity was derived from a contractile (myosin-type) ATPase originating from the fibromuscular cells of the prostates of castrated rats. A true estimate of the $Na^{+} + K^{+}$ -stimulated ATPase activity obtained by subtracting $Mg^{2+} + Na^{+}$ activity from the $Mg^{2+} + Na^{+} + K^{+}$ activity in this case would not necessarily be a correct estimate of the $Na^{+} + K^{+}$ -stimulated ATPase portion of the prostatic epithelium of castrated animals, since it has been reported that myosin ATPase is activated by K^{+} more than by Na^{+} (Kielley, Kalckar & Bradley, 1956). It was therefore decided to compare the $Mg^{2+} + Na^{+} + K^{+}$ -stimulated ATPase activity with both the $Mg^{2+} + Na^{+}$ -stimulated and $Mg^{2+} + K^{+}$ -stimulated ATPase activities of the enzyme preparations from castrated, testosterone-treated castrated and normal rats. It is clear from Table 3 that when ΔP_i ($Na^{+} + K^{+}$ -stimulated ATPase activity) was calculated by subtracting $Mg^{2+} + K^{+}$ from $Mg^{2+} + Na^{+} + K^{+}$ -stimulated activities there was almost a 95% loss of the $Na^{+} + K^{+}$ -stimulated ATPase activity in the castrated animals. This change was prevented by treatment of castrated animals with testosterone. These observations were further corroborated by determining the ouabain-sensitive (and hence $Na^{+} + K^{+}$ -stimulated) portions of the $Mg^{2+} + Na^{+} + K^{+}$ -stimulated ATPase activities of each of the three types of enzyme preparation (Table 4). Ouabain at a concentration of 1.25 mM, which would completely inhibit the $Na^{+} + K^{+}$ -

Table 4. *Ouabain-sensitive Na⁺ + K⁺-stimulated ATPase of prostatic microsomal membrane preparations isolated from castrated rats with and without testosterone treatment*

Details of castration and testosterone treatment were as described in Table 3. The enzyme systems were incubated in the Mg²⁺ + Na⁺ + K⁺ medium (as described in the Materials and Methods section) with or without 1.25 mM-ouabain. The reaction was started by addition of the enzyme preparation. Each ATPase value is the mean of triplicate determinations. P₁ values in parentheses refer to the ranges of values observed in two different experiments.

	No. of rats	ATPase activity (μmoles of P ₁ formed/mg. of protein/hr.)		Ouabain-sensitive ATPase activity (μmoles of P ₁ formed/mg. of protein/hr.) (A - B)
		Mg ²⁺ + Na ⁺ + K ⁺ (A)	Mg ²⁺ + Na ⁺ + K ⁺ plus ouabain (B)	
Castrated rats	15	117.0	116.1	0.9 (0.4-1.4)
Castrated rats treated with testosterone	8	28.8	21.8	7.0 (5.8-8.2)
Normal rats	8	49.6	40.2	9.3 (7.5-11.1)

stimulated ATPase, was used in these experiments. Table 4 shows that the ouabain-sensitive portion of the Mg²⁺ + Na⁺ + K⁺-stimulated ATPase activity of the enzyme preparation from castrated rats was decreased by at least 90% when compared with the values for normal or testosterone-treated castrated rats. Addition of testosterone *in vitro* to the test system with enzyme preparation from castrated rats produced no changes in these ATPase activities.

DISCUSSION

The above experiments indicate that a Mg²⁺ + Na⁺ + K⁺-stimulated ATPase is associated with rat ventral-prostate microsomal membranes. This prostatic enzyme system has certain properties in common with Na⁺ + K⁺-stimulated ATPase systems previously described in other mammalian tissues, e.g. a requirement for Na⁺ and K⁺ in definite proportions, inhibition of the Na⁺ + K⁺-stimulated component by high concentration of K⁺, inhibition by ouabain *in vitro* (and reversal of ouabain inhibition by high K⁺ concentrations) and specificity for ATP as substrate.

The preparations derived from rat ventral prostate microsomes behave rather differently towards ribonucleoside triphosphates, other than ATP, from comparable brain Na⁺ + K⁺-stimulated ATPase systems. In the latter case the hydrolysis of GTP, UTP, CTP and ITP in the presence of Mg²⁺ + Na⁺ or Mg²⁺ + Na⁺ + K⁺ was the same and proceeded at a rate less than 5% of that found with ATP (K. Ahmed, unpublished work). The prostatic preparation described here, on the other hand, vigorously hydrolysed GTP, UTP, ITP and CTP. It is possible, however, that this is due to contamination of the prostatic preparation by quite different enzyme(s) from the Na⁺ + K⁺-stimulated ATPase.

The Na⁺ + K⁺-stimulated ATPase activities of both the brain and prostatic preparations are inhibited by low concentrations of Ca²⁺. However, at higher concentrations of Ca²⁺, the Mg²⁺ + Na⁺-stimulated component of the brain ATPase system is strongly inhibited, whereas the Mg²⁺ + Na⁺-stimulated ATPase component of the prostatic enzyme is not significantly affected. When Ca²⁺ is substituted for Mg²⁺ in the reaction, the Ca²⁺ + Na⁺-stimulated ATPase activity of brain preparation is only about one-third of that observed in the presence of Mg²⁺ + Na⁺. In the prostatic preparation, there is, in fact, a greater Ca²⁺ + Na⁺-stimulated ATPase activity compared with that in the presence of Mg²⁺ + Na⁺. However, the increased activity due to the presence of K⁺ is seen only when Mg²⁺ + Na⁺ are present. Schwartz, Laseter & Krantz (1963) have observed similar effects of Ca²⁺ on the enzyme system isolated from dog parotid gland.

Ouabain is known to inhibit the Na⁺ + K⁺-stimulated ATPase activities of all the tissues that have been investigated so far (Schatzmann, 1953; see also, e.g. Skou, 1964; Rothstein, 1968). The concentrations of ouabain required to produce half-maximal inhibition of Na⁺ + K⁺-stimulated ATPase activities vary according to the tissue and a wide range of tissue sensitivity has been reported. It is noteworthy that the concentrations of ouabain required for 50% inhibition of the dog parotid gland enzyme system (Schwartz *et al.* 1963) are of the same order of magnitude as those required for the rat ventral prostate preparations (i.e. 0.1 mM).

Our experiments show that within 5 days after castration of adult rats there is a considerable increase in the hydrolysis of ATP by prostatic microsomal membrane preparations incubated in the presence of Mg²⁺, and either K⁺ or Na⁺ alone. However, androgen deprivation results in a virtual

obliteration of the extra liberation of P_i due to addition of both Na^+ and K^+ . The Na^++K^+ -stimulated hydrolysis of ATP is restored by injection of testosterone into the castrated rats. In marked contrast with the reports of Farnsworth (1967, 1968), we were unable to demonstrate any effect of testosterone (or dihydrotestosterone) *in vitro* on the Mg^{2+} -dependent hydrolysis of ATP in the presence of Na^+ or Na^++K^+ by a number of different prostatic microsomes or microsomal membrane preparations under a variety of experimental conditions, including the use of test systems with the same composition as those used in Farnsworth's (1967, 1968) experiments. This suggests that the profound effects of testosterone *in vivo* on the microsomal Na^++K^+ -stimulated ATPase of rat ventral prostate that we observed may reflect changes in the biosynthesis of this enzyme system, which may decline rapidly after orchidectomy with the loss of the endoplasmic reticulum membranes from the epithelial cells. It is noteworthy that after castration there is a gradual increase in the relative proportion of fibromuscular cells in rat ventral prostate (Price & Williams-Ashman, 1961). Moreover, it is well known that actomyosin from smooth-muscle cells exhibits vigorous ATPase activity (Needham & Calkwell, 1956). Thus it is conceivable that the enhanced Mg^{2+} -dependent ATP hydrolysis of the prostatic preparations in the presence of Na^+ or K^+ that occurs after orchidectomy may be due to actomyosin or other enzymically active proteins derived from fibromuscular cells.

The findings presented in this paper do not lend support to Farnsworth's (1968) hypothesis that a primary site of direct action of androgens on the prostate is the Na^++K^+ -stimulated ATPase system associated with the endoplasmic reticulum of this organ. Moreover, our experiments make it unlikely that the transient decrease in the total ATP concentration in rat ventral prostate that occurs within an hour or so after injection of free testosterone into castrated rats (Ritter, 1966; Coffey, Ichinose, Shimazaki & Williams-Ashman, 1968) can be ascribed to any direct activation of microsomal ATPase systems. Nevertheless it is clear that ATP hydrolysis by prostate microsomes is profoundly influenced by androgens *in vivo*, and prostatic cation-activated ATPase systems certainly merit much further investigation, considering the present lack of understanding of secretory mechanisms in male accessory reproductive glands (Mann, 1964; Williams-Ashman *et al.* 1964).

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REFERENCES

- Ahmed, K. (1969). *Fed. Proc.* **28**, 833.
 Ahmed, K. & Judah, J. D. (1964). *Biochim. biophys. Acta*, **93**, 603.
 Ahmed, K. & Judah, J. D. (1965). *Canad. J. Biochem.* **43**, 877.
 Ahmed, K., Judah, J. D. & Scholefield, P. G. (1966). *Biochim. biophys. Acta*, **120**, 351.
 Albers, R. W. (1967). *Annu. Rev. Biochem.* **36**, 275.
 Aldridge, W. N. (1962). *Biochem. J.* **83**, 527.
 Berenblum, I. & Chain, E. B. (1938). *Biochem. J.* **32**, 295.
 Bruchovsky, N. & Wilson, J. D. (1968). *J. biol. Chem.* **243**, 2012.
 Coffey, D. S., Ichinose, R. R., Shimazaki, J. & Williams-Ashman, H. G. (1968). *Molec. Pharmacol.* **4**, 580.
 deMeis, L. (1968). *J. biol. Chem.* **243**, 1174.
 Dunham, E. T. & Glynn, I. M. (1961). *J. Physiol.* **156**, 274.
 Epstein, F. H. & Whittam, R. (1966). *Biochem. J.* **99**, 232.
 Farnsworth, W. E. (1967). *Abstr. 49th Meet. Proc. Endocrin. Soc., Bal Harbor, Fla.*, p. 46.
 Farnsworth, W. E. (1968). *Biochim. biophys. Acta*, **150**, 446.
 Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
 Jellink, P. H. & Perry, G. (1967). *Biochim. biophys. Acta*, **137**, 367.
 Judah, J. D. & Ahmed, K. (1964a). *Biol. Rev.* **39**, 160.
 Judah, J. D. & Ahmed, K. (1964b). *J. cell. comp. Physiol.* **64**, 355.
 Kieley, W. W., Kalcekar, H. M. & Bradley, L. B. (1956). *J. biol. Chem.* **219**, 95.
 Liao, S. & Anderson, K. M. (1968). *Nature, Lond.*, **219**, 277.
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
 Mann, T. (1964). *The Biochemistry of Semen and of the Male Reproductive Tract*, pp. 194-200. New York: John Wiley and Sons Inc.
 Needham, D. M. & Calkwell, J. M. (1956). *Biochem. J.* **63**, 337.
 Price, D. & Williams-Ashman, H. G. (1961). In *Sex and Internal Secretion*, p. 366. Ed. by Young, W. C. Baltimore: The Williams and Wilkins Co.
 Rhodes, J. B. & Williams-Ashman, H. G. (1964). *Med. exp.* **10**, 281.
 Ritter, C. (1966). *Molec. Pharmacol.* **2**, 125.
 Rothstein, A. (1968). *Annu. Rev. Physiol.* **30**, 15.
 Schatzmann, H. J. (1953). *Helv. physiol. pharmacol. Acta*, **11**, 346.
 Schwartz, A., Laseter, A. H. & Kraititz, L. (1963). *J. cell. comp. Physiol.* **62**, 193.
 Skou, J. C. (1964). *Progr. Biophys.* **14**, 131.
 Skou, J. C. (1965). *Physiol. Rev.* **45**, 596.
 Tabor, H. & Tabor, C. W. (1964). *Pharmacol. Rev.* **16**, 245.
 Williams-Ashman, H. G., Liao, S., Hancock, R. L., Jurkowitz, L. & Silverman, D. A. (1964). *Recent Progr. Hormone Res.* **20**, 247.