Effects of Testosterone on Adenosine Triphosphate and Nicotinamide Adenine Dinucleotide Levels, and on Nicotinamide Mononucleotide Adenylyltransferase Activity, in the Ventral Prostate of Castrated Rats

D. S. COFFEY, RUBY R. ICHINOSE, JUN SHIMAZAKI,[1] AND H. G. WILLIAMS-ASHMAN

The James Buchanan Brady Urological Institute, The Johns Hopkins Hospital, and the Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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SUMMARY

Using different analytical methods, it was possible to confirm the observation of Ritter [*Mol. Pharmacol.* **2**, 125 (1966)] that within the first few hours after injection of free testosterone into castrated rats there occurs a large yet transient fall in the total level of ATP in the ventral prostate gland. In contrast to Ritter's findings, however, only marginal changes in the total levels of NAD and NADH in the prostate occurred over the first few hours after androgen administration. The findings presented do not support Ritter's conclusion that an increased biosynthesis of NAD represents a major early biochemical event in the action of androgens on the prostate of orchiectomized rats. Observations on the properties of prostatic nuclear nicotinamide mononucleotide adenylyltransferase (EC 2.7.7.1) and changes in the activity of this enzyme due to androgen deprival and administration are documented. An NMN-dependent polymerization of the adenylate moiety of ^{14}C-ATP by prostatic cell nuclei is also described.

INTRODUCTION

In a recent publication, Ritter (1) reported that within the first few hours after administration of testosterone to orchiectomized rats, there occurred transient fluctuations in the total ATP and pyridine nucleotide levels in the ventral prostate gland. Two weeks after removal of the testes, the concentrations of these coenzymes in the prostate were essentially in the normal range. Following intramuscular injection of free testosterone into the castrates, there was a dramatic decrease in total ATP levels, which attained a minimum value equivalent to about 25% of the castrate control at 1 hr. There was some concomitant increase in total prostatic ADP, but this could only account for about 20% of the loss in ATP. Over the following 10 hr, the ATP concentration gradually returned toward the control value. Ritter also observed oscillations in the levels of NAD, NADH, and NADPH during the first 10 hr after the androgen was administered. This was characterized by an elevation of 143% in the combined content of NAD and NADH in the first hour; this high level of NAD(H) was maintained for a further 5 hr before gradually subsiding to the norm. From the results of parallel measurements of prostatic NADP and NADPH levels, Ritter (1) concluded that

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[1] Dr. Shimazaki was supported by a Bio-Medical Research Fellowship from the Population Council, Inc. Present address: Department of Urology, Gunma University School of Medicine, Maebashi, Japan.

within the first few hours after testosterone administration there was no great enhancement of pyridine nucleotide transhydrogenase reactions in the gland. Since the testosterone-induced fluctuations in NAD were also observed in animals poisoned with puromycin or actinomycin D, it appeared that new RNA or protein biosynthesis was not required for these actions of the androgen to be manifest. Ritter (1) suggested that an increased biosynthesis of NAD might constitute an important early phase in the action of androgens on the prostate, and may reflect "a redirection of energy metabolism toward the more efficient production of ATP" (1).

Another contribution by Ritter and Paddle (2) records that addition of testosterone to explants of prostates of castrated rats in organ culture media containing 0.1% glucose was followed 40–60 min later by a gradual increase in fluorescence that was presumed to be due to reduced pyridine nucleotides. Oscillations of this fluorescence were often superimposed on the increasing steady-state fluorescence, which increased on the average by 8.5%. In the presence of lower levels of glucose (0.05%), the total increase in fluorescence was only 2%. Hydrocortisone and testosterone appeared to have synergistic effects on prostatic reduced pyridine nucleotide fluorescence. Ritter and Paddle (2) conjectured that the testosterone-induced increase in fluorescence due to reduced pyridine nucleotides "seems to be the result of an increase in the state of cytoplasmic reduction following an increase in the rate and amount of glycolysis, for it proceeds through a series of oscillations similar to those which occur when glucose is added to starved yeast cells" (*cf.* ref. 3).

The actions of testosterone on prostatic pyridine nucleotides described by Ritter (1, 2) are considerably more rapid than many previously described effects of androgen treatment on respiratory and glycolytic mechanisms in male genital glands of orchiectomized mammals. In 1944, Barron and Huggins (4) first demonstrated that orchiectomy or estrogen treatment resulted in a decline in the respiration (but not the glycolysis) of dog prostate tissue slices. Later it was repeatedly demonstrated that administration of androgens to castrates enhanced the oxygen consumption (5–9) and respiration-coupled synthetic reactions (5) of slices of various male accessory glands of reproduction, as well as the activity of certain respiratory enzymes in these organs (9–12). Yet these effects of testosterone, which could not be mimicked significantly by addition of the hormone to suitable tissue preparations *in vitro*, were manifest only after a lag period of some 15–30 hr. It was concluded (*cf.* refs. 13, 14) that such actions of androgens on prostatic respiratory processes were contingent on prior increases in the synthesis of respiratory enzymes and possibly of carriers such as cytochrome *c*. The much swifter changes in pyridine nucleotides and adenosine polyphosphates that Ritter (1, 2) found in the prostate of castrates after androgen administration are therefore of considerable interest. It is noteworthy in this connection that changes in the pulse-labeling of RNA (15–17) and of nuclear RNA polymerase activities (18–20) in male accessory glands of orchiectomized rodents have been reported to occur within 1 hr after the injection of testosterone.

The experiments described in this paper confirm, with the aid of different analytical methods, the swift yet transient androgen-induced decline in prostatic total ATP levels described by Ritter (1). An examination of the levels of total prostatic NAD concentrations, however, revealed only marginal changes in this coenzyme under the same conditions. Attention was also given to certain enzymic processes which may be involved in NAD biosynthesis in relation to androgen-induced prostatic growth. Several groups of investigators (21–23) have concluded that a low NAD content is characteristic of many rapidly growing normal and malignant tissues. Further studies (24–26) indicated that these low tissue concentrations of NAD could be correlated with the activity of the nuclear enzyme nicotinamide mononucleotide adenylyltransferase (EC 2.7.7.1). Preiss and Handler (27) suggested that the latter

enzyme also catalyzes the transformation of nicotinic acid mononucleotide to deamido-NAD. While there is evidence that reactions involving both NMN and nicotinate mononucleotide may be involved in the biosynthesis of NAD (28–30), the exact pathways and regulatory mechanisms responsible for maintenance of NAD levels in various tissues are still unclear, as is evident from the excellent recent discussions by Chaykin (31) and Greenbaum and Pinder (32). Although NMN adenylyltransferase is involved only in the terminal steps of the complex pathways of pyridine nucleotide biosynthesis, its apparently unique nuclear localization, and the reported association of the enzyme with chromatin-like material (33) and intranuclear structures rich in RNA (23), suggest that nuclear regulatory mechanisms are important in the control of NAD formation. Accordingly, we investigated the properties of NMN adenylyltransferase in the prostates of normal and orchiectomized rats during the early responses of this organ to testosterone administration. In the course of these studies, it became desirable also to examine the NMN-dependent polymerization of labeled ATP to acid-insoluble products, which appeared to reflect the synthesis by prostatic nuclei of poly ADP-ribose from NAD, a reaction recently reported to occur in a number of other mammalian tissues (34–38).

MATERIALS AND METHODS

Animals. Adult male albino rats of the Sprague-Dawley strain (250–350 g) were purchased from Huntingdon Farms. They were maintained on a diet of Purina laboratory chow and water ad libitum under a constant 12-hr-light–12-hr-dark lighting schedule. Orchiectomy was performed via the scrotal route 2 weeks before the intramuscular injection of 0.1 ml of water containing a suspension of 150 μg of free testosterone (Testryl, Squibb). Control rats received injections of 0.1 ml of water.

Rapid freezing of tissues. Animals were anesthetized with an intraperitoneal injection of 200 mg of urethane in 1 ml of 0.15% NaCl. Laparotomies were performed in an environmental chamber at constant temperature, 37°, with 100% humidity. Ventral prostates were rapidly dissected free from fat and connective tissue. With the glands *in situ*, the tissue was rapidly frozen by grasping it with aluminum Wollenberger tongs which had been cooled with liquid nitrogen. The solidly frozen prostate tissue was broken free and placed in liquid nitrogen. This technique, described by Burch, Lowry, and Von Dippe (39), has been demonstrated to minimize the rapid postmortem changes in tissue nucleotide levels. Tissue from three to five rats were pooled and pulverized in a mortar to a fine powder while being maintained under liquid nitrogen. The tissue for extraction was weighed at −20° in a desiccated balance and assayed immediately.

Assay of ATP. The powdered tissue (25 mg) was homogenized in 1 ml of cold 0.75 N perchloric acid. The system was neutralized to pH 7.4 with cold K_2CO_3 and cleared by centrifugation at 10,000 × *g* for 10 min. The pellet was extracted with 1 ml of cold water, and the combined supernatant fluids were assayed for ATP by the specific luciferin-luciferase reaction (40) by recording the light emission with a Farrand fluorometer. One-half milliliter of the extract was assayed at 25° in a final volume of 2.0 ml at pH 7.4 containing 53 mM glycine, 10 mM $MgSO_4$, 10 mM Na_2HAsO_4, and 0.2 ml of luciferin-luciferase solution prepared as described by Strehler and Totter (40). Internal standards were run with each preparation and indicated recoveries varying between 93 and 100%.

Assay of NAD. Frozen powdered prostate (25 mg) was rapidly plunged into 1.0 ml of 0.1 N HCl in a boiling water bath. After 5 min, the solution was rapidly cooled to 4° and the system was neutralized with cold NaOH. The system was cleared by centrifugation at 10,000 × *g* for 5 min. The pellet was re-extracted with 1 ml of cold water, and the NAD was assayed in the combined supernatant fluids by the fluorometric technique described by Lowry *et al.* (41), involving conversion to NADH by the action of ethanol and yeast alcohol

dehydrogenase. The recovery of internal standards varied between 89 and 95%.

Determination of NADH. The frozen tissue (25 mg) was extracted with 1.0 ml of 0.1 N $NaHCO_3$, containing 1 mg of cysteine hydrochloride, in a boiling water bath for 5 min. After neutralization and re-extraction, the NADH was determined with the technique of Lowry *et al.* (41) by measurement of decrease in fluorescence after addition of yeast alcohol dehydrogenase and acetaldehyde. The recovery of internal standards varied between 91 and 99%.

Preparation of cell nuclei. Prostatic cell nuclei were isolated by a modification of the procedure of Chaveau *et al.* (42). Fresh ventral prostate tissue was placed in an iced vessel and minced thoroughly with scissors. The mince was homogenized at 2° with 10 volumes of 2.2 M sucrose containing 1 mM $MgSO_4$ and 5 mM β-mercaptoethanol in a glass homogenizer equipped with a Teflon pestle. After filtration of the homogenate through silk cloth, the material was centrifuged for 1 hr at 50,000 × *g* in a swinging bucket rotor of a Spinco model L-2 ultracentrifuge at 2°. The cell nuclei, recovered in the pellet at the bottom of the tube, contained 58–72% of the total cellular DNA of the homogenate in various experiments (the rest of the total cellular DNA was recovered quantitatively in the original supernatant fluid obtained after ultracentrifugation of the homogenate, largely as a result of some fragmentation of the cell nuclei during homogenization). The RNA:DNA ratio of the isolated nuclei varied between 0.23 and 0.28. The material in the pellet was suspended in 0.25 M sucrose containing 1 mM $MgSO_4$. Light microscopy of these preparations revealed well-preserved cell nuclei with conspicuous nucleoli.

Determination of NMN adenylyltransferase activity. The nuclear suspension (0.2 ml, equivalent to 50 μg of nuclear DNA) was added to a 0.5-ml assay system containing a final concentration of 2 mM NMN, 2 mM ATP, 8 mM $MgSO_4$, 100 mM nicotinamide, and 20 mM Tris-HCl of pH 7.7 (37°). The reaction was terminated by dilution with 1.0 ml of a cold solution containing 1.5 M ethanol, 50 mM sodium pyrophosphate, and 200 mM Tris-HCl, pH 10.1. The system was centrifuged at 4° for 5 min at 10,000 × *g*, and 1.0 ml of the supernatant was removed and combined with 0.1 ml of a solution containing 0.1 mg of crystalline yeast alcohol dehydrogenase. After 10 min at room temperature, the NADH was determined fluorometrically. The reaction rate was linear with respect to time and enzyme concentration over the ranges reported.

NMN-dependent polymerization of ATP-^{14}C. Purified prostatic nuclei were lysed by suspension for 30 min in 0.05 M Tris, pH 7.5. After homogenization, each 0.1-ml aliquot contained 1 mg of nuclear protein, which was assayed for NMN-dependent ATP polymerase activity by a modification of the method of Chambon *et al.* (34) in a 0.5-ml system containing 0.1 M Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 60 mM KCl, 2 mM NMN, 1 mM β-mercaptoethanol, 20 mM KF, and 2 mM (1 μC/μmole) ATP-8-^{14}C (Schwarz BioResearch). The reactions were allowed to proceed for 30 min at 37° and were terminated by addition of 5 ml of ice-cold 6% trichloracetic acid containing 0.01% (w/v) ATP. After thorough mixing, the acid-insoluble material was separated by centrifugation at 10,000 × *g* for 15 min. Three cycles of redissolving the precipitate in 2 ml of 0.05 N NaOH were performed, followed by immediate reprecipitation with 5 ml of trichloracetic acid–ATP solution. The final, washed, acid-insoluble residue was dissolved at room temperature in 0.5 ml of 97% (w/v) formic acid. To this were added 15 ml of a scintillation medium of the following composition: xylene–dioxane–absolute ethanol (1.0:1.0:1.0, by volume) containing 7.5% (w/v) naphthalene, 0.45% (w/v) 2,5-diphenyloxazole (Pilot Chemicals), and 0.0045% w/v) 1,4-bis(5-phenyloxazol-2-yl)benzene (Pilot Chemicals).

Radioactivity was assayed with a Nuclear-Chicago Unilux I liquid scintillation system. The background counts were in the range of 30–40 cpm. The average of two 10-min counts minus a back-

ground was obtained for each sample.

Other methods. RNA was determined by the orcinol reaction with purified yeast RNA as standard; DNA was measured by the diphenylamine method with calf thymus DNA as the reference material (43). All pH measurements were made with a glass electrode and refer strictly to the stated temperatures. Protein was determined by the biuret method (44).

RESULTS

Effects of testosterone on ATP, NAD, and NADH levels of rat ventral prostate. In agreement with Ritter (1), it was observed that the concentration of total ATP, NAD, and NADH in the ventral prostate was within the normal range in rats that had been castrated 7 days previously (Table 1). Our values for the absolute levels of ATP and NADH are in good agreement with those reported by Ritter (1). However, the values shown in Table 1 for normal prostatic NAD contents are only about 60% of those found by Ritter (1). Since in our experiments the recoveries of internal standards for ATP, NAD, and NADH (added in suitable amounts to the extraction media just prior to addition of the powdered frozen tissue) were invariably greater than 89% (see above), the reason for the discrepancy between our

TABLE 1

Early effects of testosterone on ATP, NAD, and NADH levels in rat ventral prostate

Each value represents the average of a duplicate determination on pooled tissue from three to five rats.

	Ventral prostate tissue concentration *mμmoles/g tissue, wet wt*									
	ATP				NAD			NADH		
Animals	Expt. 1	Expt. 2	Expt. 3	Mean	Expt. 1	Expt. 2	Mean	Expt. 1	Expt. 2	Mean
Normal	873	860	1006		54	47		11	7	
	914	710			41	52	48	12	14	
		920		880					13	11
Castrate	910	905	965		58	40		12	7	
	1190	952	840		48	38		5	10	
		495		893		53	47		12	9
Castrates + 150μg testosterone										
1 hr postinjection	651	640	476		44	34		13	14	
	420	755	474		52	36		6	8	
		251		524		35	40		11	10
3 hr postinjection	682	805	740		63	59		20	9	
	737	640	584		75	79		12	9	
		633		689		67	69		15	13
8 hr postinjection	726	915	893		51	46		11	10	
	811	802	915		43	51		16	7	
		747		830		39	46		8	10
Castrates + control injections[a]										
1 hr postinjection			1012							
			915	963						
8 hr postinjection	920				56			19		
	861			890	52		54	14		16

[a] Intramuscular injection of 0.1 ml of water.

values for prostatic NAD levels and those of Ritter (1) is not apparent. Two differences between the procedures of Ritter (1) and this paper could conceivably be of significance in this regard: (a) urethane was used as an anesthetic in our experiments, whereas Ritter employed CO_2–O_2 mixtures for this purpose; (b) we used Sprague-Dawley rats of relatively greater age and body weight.

One hour after injection of 150 μg of free testosterone into the castrates, there occurred an average decrease of 42% in the total ATP levels, as determined by the luciferin-luciferase luminescence method. This large decrease of about 370 mμmoles of ATP per gram of prostate, wet weight, was slowly restored to nearly normal levels within 8 hr. These findings confirm those of Ritter (1), who estimated ATP by different enzymic procedures. However, we did not observe any large changes in NAD and NADH levels at 1, 3, and 8 hr after injection of the hormone. Table 1 shows that the only significant change over this period was a small and transient rise in NAD content at 3 hr. This average increase of 22 mμmoles of NAD per gram of prostate, wet weight, was considerably smaller than the corresponding earlier maximal alteration of ATP levels. Ritter (1) observed much larger changes in prostatic NAD content at 3 and 5 hr and in NADH levels at 1 and 2 hr after administration of testosterone. It is worth noting that in our experiments, the prostates were frozen very rapidly *in situ* by a procedure similar to that employed by Ritter (1) in order to diminish artifactual postmortem changes in the tissue content of various acid-soluble nucleotides.

Nuclear NMN adenylyltransferase. Table 2 illustrates the properties of an NMN adenylyltransferase associated with isolated rat ventral prostate cell nuclei which catalyzes the synthesis of NAD from ATP and NMN. The enzyme is fairly stable; less than 6% of the activity was lost when the nuclei were frozen, or kept at 4°, for 3 days in 0.25 M sucrose containing 1 mM $MgCl_2$.

NMN adenylyltransferase is firmly bound to prostatic cell nuclei, and less than 10% of the total enzyme activity could be removed by washing the nuclei three times with 0.01 M Tris-HCl of pH 7.4. However, increasingly higher salt concentrations extracted the enzyme in "soluble" form from prostatic cell nuclei (Fig. 1) in a manner comparable to that previously reported for liver nuclei by Traub, Kaufman, and Ginzburg-Teitz (23), who concluded that the NMN adenylyltransferase is associated with nuclear particles rich in RNA. Numerous efforts to demonstrate an association of prostatic nuclear NMN adenylyltransferase with nuclear ribonucleoprotein particles were unsuccessful; however, extraction of the enzyme with solutions of fairly high ionic strength resulted in a concomitant solubilization of nuclear RNA but not of nuclear DNA (Fig. 1). Although the enzyme was released together with RNA, it did not appear to remain associated with RNA in the salt solutions. When the prostatic nuclear material solubilized in 0.5 M NaCl solutions was passed through a column of Bio-Gel P-300 (previously equilibrated against 0.5 M NaCl), the NMN adenylyltransferase activity behaved as if the enzyme had a molecular weight in the

TABLE 2
Properties of NMN adenylyltransferase in prostatic nuclei

System	Relative rate[a]
Complete[b]	100
−$MgSO_4$	11
−ATP	0
−NMN	0
+ATP, 6 mM	97
+NMN, 6 mM	51
+Nicotinamide, 100 mM	98
+β-Mercaptoethanol, 20 mM	123
+NaCl, 0.5 M	109
+KCl, 0.5 M	100
+Testosterone, 10 μM	96

[a] 100 = 52 mμmoles of NAD formed per 100 μg of nuclear DNA per hour.

[b] ATP, 2 mM; $MgSO_4$, 8 mM; NMN, 2 mM; 20 mM Tris-HCl, pH 7.7 (37°); and nuclei equivalent to 100 μg of DNA.

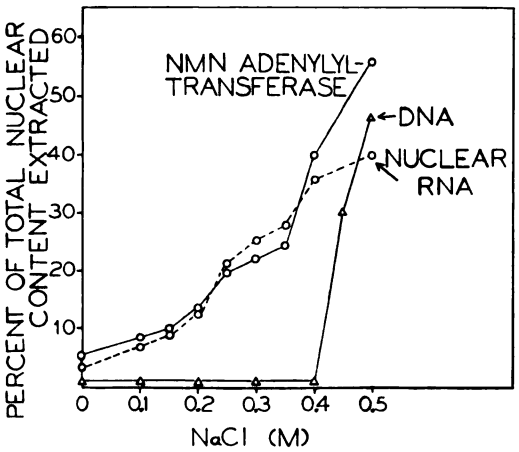

FIG. 1. *Association of nuclear RNA extraction with solubilization of NMN adenylyltransferase*

Purified prostatic nuclei equivalent to 100 μg of DNA per milliliter were subjected to the stated NaCl concentrations at 0° for 1 hr. The extracts were centrifuged at 22,000 × *g* for 10 min, and the soluble fractions were assayed for DNA, RNA, and NMN adenylyltransferase. The results are expressed as the percentage of the original nuclear content solubilized. Assays of extract and nuclear pellet indicated greater than 90% recovery of all components.

vicinity of 200,000, assuming a roughly spherical shape of the protein.

Effects of castration and androgen treatment on prostatic NMN adenylyltransferase. It is evident from Table 3 that 7 days after orchiectomy there is a substantial decrease in prostatic nuclear NMN adenylyltransferase activity, even though the tissue NAD, NADH, and ATP levels (Table 1) are essentially normal under these conditions. The rapid changes in prostatic total ATP levels 1 hr after injection of testosterone into the castrates, and the small changes in NAD content observed at 3 hr (Table 1), are not accompanied at these early intervals by any significant alteration in nuclear NMN adenylyltransferase activity (Table 3). At 3 and 6 hr after testosterone administration, the NMN adenylyltransferase activities were not enhanced, but they were significantly increased within 24 hr. Daily injection of testosterone for 6 days restored the prostatic NMN adenylyltransferase activities of the castrates to normal values.

NMN-dependent polymerization of ATP-^{14}C by prostatic nuclei. The assay system used for measurement of NMN adenylyltransferase involved the enzymic determination of NAD formed from the precursors NMN and ATP. If the prostatic nuclear extracts transformed the NAD so produced into other products during the course of the reaction, then artificially low values for NMN adenylyltransferase activity might be obtained. Chambon and his co-workers (34) discovered an enzymic process associated with liver nuclei which promoted the incorpo-

TABLE 3

Effects of castration and testosterone treatment on ventral prostate NMN adenylyltransferase activity

Each value represents the average of a duplicate determination on a pooled nuclear preparation obtained from three to five rats.

Condition	Nuclear NMN adenylyltransferase[a]	Relative activity (mean value)
Normal	56	100[b]
	47	
	59	
Castrate	31	57
	34	
	28	
Castrate + 150 μg testosterone daily (time post-injection)		
3 hr	30	59
	33	
	26	
6 hr	31	61
	34	
	30	
1 day	37	72
	39	
	41	
2 days	50	89
	45	
	46	
6 days	60	104
	53	
	55	

[a] mμmoles NAD formed/100 μg nuclear DNA/hr.

[b] Equivalent to 1.18 μmoles of NAD formed per gram of tissue, wet weight, per hour.

ration of the adenylate moiety of ATP into acid-insoluble products which exhibited a complete requirement for NMN. The ribose portion and the ester phosphate groups of NMN and ATP were incorporated into the products in equimolar amounts, and it was shown that NAD was an obligatory intermediate in the formation of the acid-insoluble reaction product. The over-all reaction was shown to involve (a) the synthesis of NAD from NMN and ATP, catalyzed by NMN adenylyltransferase, and (b) a transglycosidation reaction, in which linkages were formed between the C-1 carbon atoms of the ribose moieties adjacent to the nicotinamide group and the C-2 of the adenosine ribose, with the simultaneous elimination of free nicotinamide, so that the acid-insoluble product was a poly ADP-ribose (34, 37). This polymerization reaction was later shown to occur in the nuclei of a number of mammalian tissues (35–38) and appears to be catalyzed by an NAD glycohydrolase (EC 3.2.2.5) associated with the nuclei (38).

Evidence was obtained that isolated prostatic nuclei catalyze the incorporation of ATP-^{14}C into acid-insoluble products by an NMN-dependent reaction which appears to involve the aforementioned reaction sequence in which NAD is an intermediate. Table 4 shows that the NMN-dependent polymerization of ATP-^{14}C by prostatic nuclei occurs at a slower rate than with liver nuclei, but that the properties of this multienzyme system are in full accord with the data obtained with liver nuclei by Chambon *et al.* (34). As expected, addition of unlabeled NAD to the reaction mixture diminished the NMN-dependent incorporation of labeled ATP into the acid-insoluble product. Nicotinamide also inhibited the reaction. It is noteworthy that although the entry of radioisotope from ATP-^{14}C into the reaction product was proportional to the amount of nuclear extract protein added to the reaction mixtures, the incorporations were not linear with respect to time over prolonged periods (Fig. 2). The extent of this polymerization reaction by the prostatic nuclear extracts was not sufficient to cause significant interference with the NMN adenylyltransferase assays. Moreover, the presence of large concentrations of nicotinamide in the NMN adenylyltransferase assay system would diminish markedly any loss of NAD

TABLE 4
NMN-dependent polymerization of ATP-^{14}C by prostatic nuclei

System	Incorporation of ATP-^{14}C[a]
	μμmoles/mg protein/30 min
Complete[b]	399
−NMN	22
+Nicotinamide, 10 mM	39
+NAD, 10 mM	1
+Pancreatic RNase, 100 μg	517
+$(NH_4)_2SO_4$, 150 mM	13
+$(NH_4)_2SO_4$, 300 mM	5

[a] One milligram of nuclear protein is equivalent to 265 μg of nuclear DNA.

[b] Nuclear protein, 1 mg; 100 mM Tris-HCl, pH 7.5; $MgCl_2$, 10 mM; KCl, 60 mM; KF, 20 mM; β-mercaptoethanol, 1 mM; NMN, 2 mM; and ATP-8-^{14}C, 2 mM (1 μC/μmole).

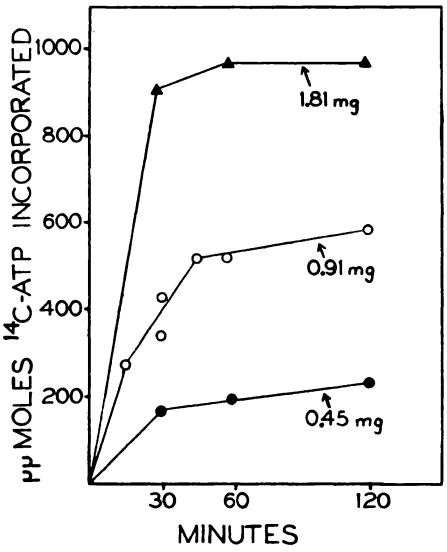

FIG. 2. *The extent of the NMN-dependent ATP-^{14}C polymerization reaction with time and with the amount of nuclear protein in the assay system*

formed as a result of further polymerization to form poly ADP-ribose.

DISCUSSION

The foregoing experiments confirm Ritter's (1) findings that (a) the total ATP level of the regressed ventral prostate glands of adult orchiectomized rats is within the normal range, and (b) 1 hr after injection of testosterone into the castrates, there occurs a precipitous fall in prostatic total ATP levels, which later rise again toward normal values. This transitory androgen-induced decline in prostatic ATP is similar to the marked lowering of uterine ATP levels which Aaronson *et al.* (45) observed 1 hr after treatment of oöphorectomized rats with estrogen. In both instances, the decline in tissue ATP levels during the early phases of action of these gonadal hormones represented a very considerable loss (20–50%) of the total cellular ATP. Ritter (1) has stated that the early testosterone-induced fall in prostatic ATP levels can by no means be accounted for by a stoichiometric increase in ADP levels. The swift diminution of ATP therefore raises the question as to pathways which may be involved in the utilization of this nucleotide. The data presented in this paper make it clear that the concomitant changes in NAD(H) content of the prostate are very small in comparison with alterations in the ATP levels.

One of the earliest known consequences of the actions of androgens and estrogens on the prostate gland and uterus, respectively, is an enhancement of nuclear RNA synthesis (15–20, 46–50). The extent to which the hormone-dependent early decline in ATP levels in these organs reflects the synthesis of ribonucleic acids (or other macromolecules) and their precursors cannot be estimated even roughly from the very limited experimental evidence presently available. Other possible pathways for utilization of ATP, such as the formation of cyclic 3′,5′-adenylate, would also merit study in this connection.

Our findings do not support the hypothesis of Ritter (1) that an increased biosynthesis of NAD represents a major early biochemical event following stimulation of the prostate of castrated rats by exogenous androgen. The fluctuations in prostatic NAD(H) levels we observed over the first few hours after administration of testosterone are smaller than those reported by Ritter (1), and even the largest changes in the content of these nucleotides we found at 3 hr after injection of the hormone are of marginal significance. Moreover, during these early phases of the action of the androgen, there was no change in the activity of nuclear NMN adenylyltransferase (*cf.* refs. 32, 51). It is noteworthy that the values we obtained for the content of NAD and NADH in the ventral prostate before and at various intervals after administration of testosterone to castrated rats (*cf.* Table 1) were subject to considerably less variation from one experiment to another than those reported by Ritter (1). For example, Ritter reported that the prostatic NADH content rose 7-fold during the first hour after testosterone treatment in four experiments, in which the means of duplicate analyses were 175.8, 23.8, 26.4, and 53.6 mμmoles per gram of fresh tissue.

Much further work will have to be performed to evaluate Ritter's (1) conclusion that within the first few hours after administration of androgen to orchiectomized rats there occurs a redirection of prostatic energy metabolism toward a more efficient production of ATP as a result of activation of respiratory rather than glycolytic processes, and at a time when the over-all respiration of the tissue and the levels of various respiratory enzymes have not yet changed. It may be worthwhile emphasizing that measurements of the total levels of ATP and pyridine nucleotides in tissues may not be very enlightening in this regard. The distribution of these nucleotides between various cell compartments is well known to be very uneven, and estimations of nucleotide levels of the type reported by Ritter (1) and in this paper do not differentiate between free and protein-bound nucleotides within any region of the cell. Indeed, as Krebs (52) has pointed out so incisively, calculations of tissue levels of

the ratio of oxidized to reduced *free* pyridine nucleotides in various cell compartments (made on the basis of measurements of various oxidized and reduced substrates for enzymes that are localized in discrete regions of the cell) may give values which are vastly different from those obtained from "direct" estimations of NAD:NADH and NADP:NADPH ratios. It may also be pointed out that increases in tissue pyridine nucleotides may not only reflect an enhanced rate of synthesis of the coenzymes, but also may result from a decrease in their rate of degradation. Finally, the relative increase in the fibromuscular stroma of the rat ventral prostate which occurs after orchiectomy (53) further complicates interpretation of the type of measurements of total tissue content of various nucleotides discussed by Ritter (1) and in this paper.

It is shown in this paper that isolated prostatic cell nuclei catalyze an NMN-dependent incorporation of ^{14}C-ATP into acid-insoluble products which probably result from polymerization of the adenosine diphosphate ribose moiety of NAD, formed from ATP and NMN by the action of an NMN adenylyltransferase in the nuclear preparations. After these experiments were completed, Nishizuka *et al.* (54) reported that the polymerization of the ADP-ribose moiety of NAD by liver cell nuclei involves an initial transfer of ADP-ribose to nuclear histone, with the simultaneous release of nicotinamide. A successive transfer of the ADP-ribose moiety results in formation and elongation of a homopolymer with repetitive ADP-ribose units; both the polymer and the monomer ADP-ribose appear to be joined to histone(s) by covalent linkages. Further examination of this reaction may be of considerable interest from the standpoint of the possible regulation by histones of the control of RNA transcription in prostatic cell nuclei (14).

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