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Research Article

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LOCALIZATION OF THE BIOCHEMICAL SITE OF ACTION OF TESTOSTERONE ON PROTEIN SYNTHESIS IN THE SEMINAL VESICLE OF THE RAT *

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Although the administration of testosterone clearly causes marked diminution in nitrogen excretion (3) and results in increased protein deposition in kidney, liver, muscle, carcass, and accessory sex tissue (4), the mechanisms of this protein anabolic action are unexplained. While this effect of testosterone is probably the result of an enhancement of protein synthesis, previous attempts to elucidate this action have been complicated by two factors: first, the major enzymatic steps in the synthesis of protein have been described only in the past few years (5); and second, previous attempts to demonstrate an influence of testosterone on protein synthesis in several nonsexual tissues of the rat (6) and mouse (7, 8) have yielded differences which, although consistent, are very small.

Several observations have suggested that the rat accessory sex organs might serve as suitable tissues for an exploration of the mechanisms by which testosterone influences protein metabolism. The accessory sex organs are very responsive to the administration of testosterone (9), and in fact, Scow has reported that as much as 25 per cent of the total weight gain induced by testosterone in castrated rats occurs in the sexual tissue (10). Furthermore, Greer has demonstrated that the rat prostate rapidly and selectively concentrates testosterone-4-C^{14} (11).

The present report describes a study of the influence of testosterone administration on protein synthesis in the rat seminal vesicle. Evidence is presented that testosterone administration will enhance markedly the synthesis of protein in slices of seminal vesicles from immature rats. Furthermore, an examination of the biochemical reactions in the synthesis of protein has revealed that this effect of testosterone does not involve either amino acid synthesis or transport but is secondary to the enhancement of a specific step in protein synthesis, the conversion of soluble ribonucleic acid-amino acid complexes to microsomal ribonucleoprotein.

EXPERIMENTAL METHODS

Incubation procedure. Male rats of the Long-Evans strain, weighing from 50 to 75 g, were given, intramuscularly, 5 mg per day of testosterone propionate for periods varying from 12 hours to 3 days. In some experiments the rats were castrated under ether anesthesia at the beginning of the injections. The testosterone-treated animals and either normal or castrated controls were decapitated, and their prostates and seminal vesicles quickly excised and placed in ice-cold Krebs-Ringer bicarbonate buffer, pH 7.4. Slices, approximately 0.5 mm thick, were prepared by hand; the slices from several animals were pooled, washed gently in the ice-cold buffer, blotted, and weighed. Portions of the slices (10 to 100 mg) were placed in centrifuge tubes, and substrates and Krebs-Ringer bicarbonate buffer were added to give a constant volume; samples were incubated either in duplicate or triplicate. The tubes were gassed with 95 per cent oxygen-5 per cent carbon dioxide for 10 seconds, sealed, and incubated at a 30° angle in a Dubnoff metabolic shaker at 37.5° C for varying time intervals.

Analytical methods. At the end of the incubation period the slices were quickly washed five times with 5 ml of cold Krebs-Ringer bicarbonate buffer and homogenized in 1 ml water at 0° C by grinding with a motor-driven pestle. In experiments in which the ribonucleic acid (RNA) fractions were analyzed, the slices after incubation were quickly washed once with cold buffer and immediately homogenized at 0° C in 0.1 M Tris buffer (12) containing 0.5 M NaCl, 0.005 M $MgCl_2$, and either 0.1 M nonradioactive L-valine or L-tyrosine.[1] The homogenate

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[1] Since soluble RNA-amino acids can be synthesized at 0° C (12), a 100-fold excess of nonradioactive amino acid was added in order to prevent further incorporation of C^{14}-amino acid.

was then fractionated by differential centrifugation in the same buffer at 0° C (13), and the 105,000 G supernatant portion and the microsomal pellet were separated for further analysis.

To the homogenized tissue preparation was then added 4 ml cold 0.4 N perchloric acid. The tubes were centrifuged, and the supernatant liquid containing the free amino acids and the precipitate, which contained both RNA-amino acids and protein, were analyzed as follows. One portion of the supernatant was added to 10 ml of dioxane-methanol-ethyleneglycol-naphthalene (100:10:2:6) containing 0.4 per cent 2,5-diphenyloxazole and 0.02 per cent 1,4-*bis*-2 (5-phenyloxazolyl)-benzene and assayed for C^{14} in a Packard Tri-Carb liquid scintillation counter as described by Bray (14). In some experiments free tyrosine was determined on another portion of the supernatant by the spectrophotofluorometric method of Waalkes and Udenfriend (15). In the experiments utilizing acetate-2-C^{14} as substrate the perchloric acid supernatant was adjusted to pH 7, diluted to 50 ml with water, and passed through small columns of Dowex 50-X4 hydrogen (5 × 1 cm). The columns were washed four times with 10 ml of water and eluted with 25 per cent NH_4OH. The eluate was taken to dryness and dissolved in 1 ml water. One aliquot was assayed for C^{14} as before. When the product remaining after Dowex-50 chromatography was rechromatographed on paper with butanol-acetic acid-water (50:12:50) and assayed for radioactivity in an Atomic Accessories Scanogram II chromatogram scanner, 80 to 90 per cent of the radioactivity was found in the ninhydrin-reacting region corresponding to glutamic acid (Rf standard 0.17: Rf unknown 0.18). The remainder of the radioactivity was located in a single ninhydrin-reacting spot which was not further identified (Rf 0.06).

RNA-amino acids and protein were separated from the perchloric acid-insoluble precipitate by a modification of the method of Hoagland and co-workers (12). The precipitate was washed twice with cold 0.2 N perchloric acid and then washed at 5° C, first with ethanol-0.2 N perchloric acid (5:1) and finally with ethanol. The precipitate was next suspended in ethanol-ether (3:1), incubated at 47° C for 20 minutes, and centrifuged. The RNA was extracted from the precipitate with 10 per cent NaCl at 100° C for 30 minutes. The mixture was centrifuged and the supernatant liquid decanted. RNA was precipitated from the NaCl extract with 60 per cent ethanol at − 10° C, separated by centrifugation, and dissolved in 1 ml water. One aliquot was assayed for C^{14} as described by Bray (14), and RNA was determined on another portion by the orcinol method (16).

The protein precipitate which remained after NaCl extraction was suspended in 5 ml 1 N NaOH at 40° C for 30 minutes (17). The protein was reprecipitated by the addition of 2 ml 10 N HCl and washed once with 5 ml of 10 per cent trichloroacetic acid and twice with acetone. The acetone-washed precipitates were then dissolved in 1 ml 1 N NaOH. The protein content was determined on one portion by the method of Lowry, Rosenbrough, Farr and Randall (18), and C^{14} was assayed in another aliquot by the method of Bray (14). In contrast to a previously described liquid scintillation method of assaying proteins for radioactivity (19), these protein preparations did not exhibit spontaneous phosphorescence after exposure to light.

TABLE I

Effect of testosterone administration on protein synthesis in slices of accessory sex tissue of castrated and normal rats *

Pretreatment of rat	Tissue	Protein-C^{14} synthesized from: L-valine-1-C^{14}	L-tyrosine-U-C^{14}
		cpm/mg	*cpm/mg*
Castrated	Seminal vesicle	1,151	339
Normal	Seminal vesicle	2,113	472
Testosterone-treated	Seminal vesicle	3,088	818
Castrated	Prostate	2,759	825
Normal	Prostate	2,752	737
Testosterone-treated	Prostate	2,104	680

* Male rats (50 to 75 g) were either castrated or injected with 5 mg testosterone propionate 2 days prior to death. Slices (25 mg) were incubated for 1 hour in 0.8 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, glucose (6.2×10^{-3}M), and either L-valine-1-C^{14} (5×10^{-4}M containing 1.01×10^{5} cpm) or L-tyrosine-U-C^{14} (5×10^{-4}M containing 1.06×10^{5} cpm) in a total volume of 1.0 ml.

RESULTS AND DISCUSSION

In the initial studies an attempt was made to ascertain whether testosterone administration does, in fact, influence protein synthesis in the rat accessory sex tissue under *in vitro* conditions. Immature rats were injected with 5 mg testosterone propionate per day for 2 days. The prostate and seminal vesicles from these rats and from normal and castrated controls were removed, sliced, and incubated with either L-valine-1-C^{14} *or* L-tyrosine-U-C^{14}. The results of such an experiment are shown in Table I. Testosterone pretreatment increased protein synthesis two- to threefold in the seminal vesicle from both valine-C^{14} and tyrosine-C^{14} and did not influence protein synthesis in the prostate. Although the enhancement of protein synthesis was more marked when the treated animals are compared with castrated rather than normal controls, noncastrated controls were used in the subsequent experiments in order to avoid any possible secondary degenerative effects of castration (20). And, although the degree of enhancement varies from experiment to experiment, testosterone pretreatment always resulted in accelerated protein synthesis in slices of seminal vesicles from young rats.

TABLE II

Effect of time of testosterone administration on protein synthesis in slices of rat seminal vesicle *

Time of testosterone administration	Radioactive precursors	Amino acid-C^{14} converted to: Intracellular amino acids		Protein
		cpm/mg protein	*cpm/10 mμmoles*	*cpm/mg*
Control	L-valine-1-C^{14}	8,833		669
12 hours		6,987		1,178
1 day		7,280		2,058
2 days		10,448		3,398
3 days		8,228		3,103
Control	L-tyrosine-U-C^{14}	10,028	7,129	117
12 hours		7,671	7,040	254
1 day		11,774	7,522	724
2 days		11,472	8,030	682
3 days		12,524	8,031	792

* Slices were incubated for 1 hour in Krebs-Ringer bicarbonate buffer, pH 7.4, glucose (6.2×10^{-3}M) and either L-valine-1-C^{14} (5×10^{-4}M containing 5.00×10^{5} cpm) or L-tyrosine-U-C^{14} (5×10^{-4}M containing 5.03×10^{5} cpm) to make a final volume of 0.6 ml.

The effect of varying the time of testosterone pretreatment from 12 hours to 3 days is illustrated in Table II. In this experiment protein specific activity from both valine-C^{14} and tyrosine-C^{14} was doubled within 12 hours and reached a maximum of a five- to sixfold increase within 1 or 2 days after commencing testosterone therapy. It is of particular interest that the increase in protein synthesis occurred before any difference in washed wet weight could be demonstrated between the normal seminal vesicles (average weight 3.10 mg) and the seminal vesicles from rats given one injection of testosterone 12 hours prior to death (average weight 2.70 mg); within 24 hours the average weight of the seminal vesicles had increased to 6.09 mg, and in the 3-day rats the average weight was 9.98 mg. This doubling of the rate of protein synthesis within 12 hours is particularly noteworthy in view of the slow absorption and rapid excretion of testosterone (21).

The demonstration of an enhancement of protein synthesis of this magnitude by testosterone administration made it possible to evaluate some of the biochemical mechanisms by which this effect might be mediated. Figure 1 summarizes the major steps in protein synthesis. Free amino acids within the cell may arise from one of two sources. First, they may be transported into the cell from the extracellular fluid (22), or second, amino acids may be synthesized by the fixation of ammonia with α-ketoglutarate to form glutamic acid (23), which can subsequently undergo transamination to form any of the nonessential amino acids (24). Amino acids arising from either of these two sources are then activated in a reaction requiring adenosine triphosphate (ATP) to form amino acid adenylates (25), which are then bound to soluble RNA, forming the soluble RNA-amino acid complexes (12). In a reaction requiring guanosine triphosphate (GTP), these RNA-amino acids are then transferred into the ribosomes of the microsomes. At this step peptide bonding occurs, resulting in the formation of ribonucleoproteins (26). The completed protein is subsequently stripped off the ribosome particle and released into the supernatant portion of the cell (27). In an attempt to identify the precise locus of testosterone action, this pathway of synthesis has been studied at five critical sites: amino acid transport (step 1), amino acid synthesis (step 2), RNA-amino acid formation (step 4), microsomal ribonucleotide formation (step 5), and the final stripping off of the completed protein (step 6).

Because the studies of Noall, Riggs, Walker and Christensen (28), and Metcalf and Gross (29) have indicated that the intracellular penetration of the nonutilizable amino acid, α-aminoisobutyric acid, is enhanced by several hormonal agents including testosterone, it was important to determine whether testosterone might stimulate protein synthesis by enhancing the intracellular transport of a naturally occurring amino acid (step 1). After incubation for 1 hour the intracellular levels of both valine-C^{14} and tyrosine-C^{14} in seminal vesicle slices did not appear to be influenced by testosterone administration (Table II). It was necessary, however, to exclude the possibility that testosterone might accelerate the rate of amino acid transport at early time intervals in this tissue. Therefore, the intracellular penetration of L-tyro-

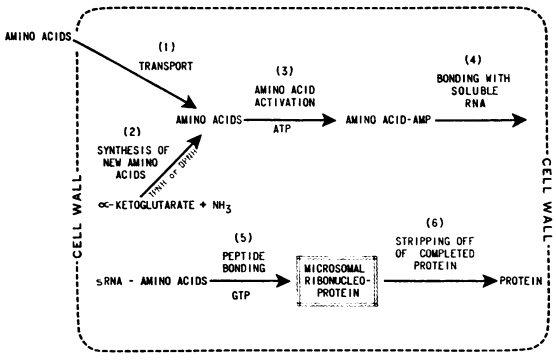

FIG. 1. MECHANISM OF PROTEIN BIOSYNTHESIS.

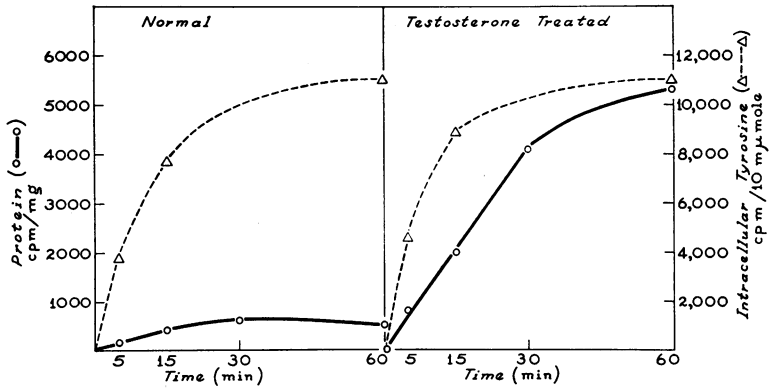

FIG. 2. TIME COURSE OF TRANSPORT AND INCORPORATION INTO PROTEIN OF L-TYROSINE-C^{14} BY SLICES OF RAT SEMINAL VESICLE. Slices of seminal vesicle from normal and testosterone-treated rats (3 days) were incubated for 1 hour in Krebs-Ringer bicarbonate buffer, pH 7.4, containing glucose (6.2×10^{-3}M) and L-tyrosine-U-C^{14} (5×10^{-4}M containing 1.06×10^{6}cpm) in a final volume of 1.0 ml.

sine-U-C^{14} was studied at intervals varying from 0 to 60 minutes (Figure 2). The specific activity of intracellular tyrosine is shown by the dotted line. Despite a profound difference in the specific activities of the intracellular protein-C^{14}, at no time was there a significant difference in the specific activity of the intracellular tyrosine.

To ascertain whether an increase in the amino acid pool size, undetectable by an examination of the specific activity of tyrosine and valine alone, might be responsible for the accelerated protein synthesis, the intracellular pool of tyrosine in the seminal vesicle was measured at varying time intervals (Figure 3). No significant difference was observed in the level of either intracellular tyrosine-C^{14} or tyrosine between the tissues from the normal and testosterone-treated animals despite a twofold acceleration in protein synthesis in the slices from the testosterone-treated rats. Thus, it was concluded that the enhancement of protein synthesis in this tissue cannot be secondary to an acceleration of amino acid transport.

The effect of testosterone administration on the *de novo* synthesis of amino acids from acetate-C^{14} (step 2) was then examined in rat seminal vesicle (Table III). There was no significant difference

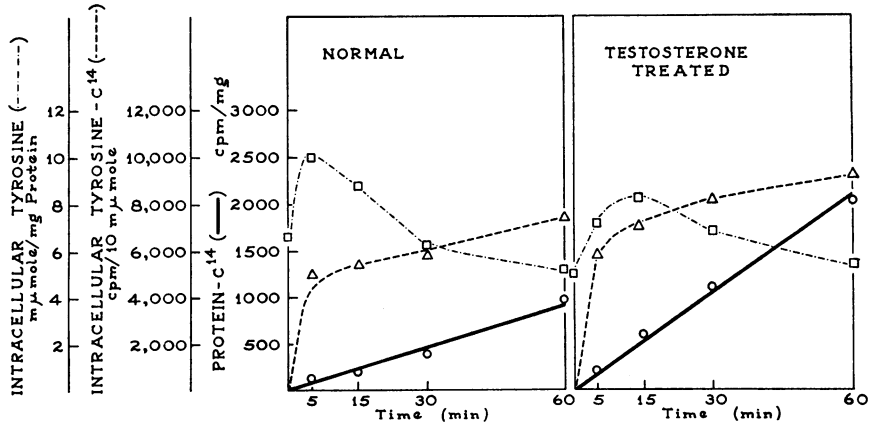

FIG. 3. TIME COURSE OF TRANSPORT AND INCORPORATION INTO PROTEIN OF L-TYROSINE-U-C^{14} AND L-TYROSINE BY SLICES OF RAT SEMINAL VESICLE. Slices of seminal vesicle from normal and testosterone-treated rats (3 days) were incubated for 1 hour in Krebs-Ringer bicarbonate buffer, pH 7.4, containing glucose (6.2×10^{-3}M) and L-tyrosine-U-C^{14} (5×10^{-4}M containing 5.03×10^{5}cpm) in a final volume of 0.65 ml.

between the rates of amino acid synthesis before and after testosterone administration, despite the fact that in these experiments protein synthesis from both acetate-C^{14} and tyrosine-C^{14} was increased in the testosterone-treated slices. These findings are in accord with the report by Kochakian, Endahl and Endahl (30) that the concentrations of both glutamic dehydrogenase and transaminase in several tissues of the rat or mouse are unaffected by testosterone administration. In addition, these findings exclude the possibility that testosterone might influence the rate of the glutamic dehydrogenase reaction in this tissue by alteration of the level of cofactors utilized in reaction (31). It is clear, therefore, that the anabolic effect of testosterone occurs at some step after the synthesis and intracellular transport of amino acids.

As shown in Figure 4, step 4 in protein biosynthesis, the formation of soluble RNA-amino acid, was then examined in slices of seminal vesicles from normal and testosterone-treated rats. The specific activity of soluble RNA-tyrosine at varying time intervals is shown by the dotted line, and the specific activity of microsomal protein is demonstrated by the solid line. Despite the marked effect of testosterone on the specific activities of the microsomal protein, at no time was there a significant difference in the specific activity of soluble RNA-tyrosine. This experiment rules out the possibility that any of the preceding steps in protein synthesis—amino acid transport, amino

TABLE III

Influence of testosterone administration on the conversion of acetate-2-C^{14} to amino acids and protein by slices of rat seminal vesicle *

Exp.	Pretreatment	Precursor	C^{14} recovered as: Intracellular amino acids	C^{14} recovered as: Protein
			cpm/mg protein	*cpm/mg*
1	None	L-tyrosine-U-C^{14}	4,590	339
	Testosterone (1 day)	L-tyrosine-U-C^{14}	6,108	728
	None	Acetate-2-C^{14}	11,625	1,368
	Testosterone (1 day)	Acetate-2-C^{14}	15,386	1,914
2	None	L-tyrosine-U-C^{14}	1,263	143
	Testosterone (2 days)	L-tyrosine-U-C^{14}	1,075	353
	None	Acetate-2-C^{14}	7,161	359
	Testosterone (2 days)	Acetate-2-C^{14}	9,639	1,214

* Slices were incubated in Krebs-Ringer bicarbonate buffer, pH 7.4, glucose (6.2×10^{-3}M), and either L-tyrosine-U-C^{14} (5×10^{-4}M containing 5.03×10^5 cpm) or acetate-2-C^{14} (5×10^{-4}M containing 5.00×10^5 cpm) in a total volume of 1.0 ml.

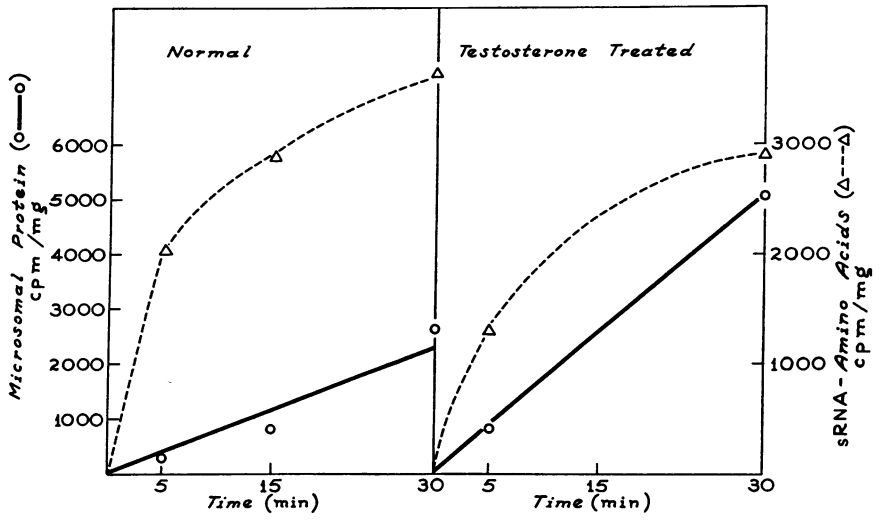

FIG. 4. TIME COURSE OF INCORPORATION OF L-TYROSINE-U-C^{14} INTO SOLUBLE RNA-AMINO ACID AND MICROSOMAL PROTEIN OF SLICES OF RAT SEMINAL VESICLE. Slices of seminal vesicle from normal and testosterone-treated rats (1 day) were incubated for varying time periods in Krebs-Ringer bicarbonate buffer, pH 7.4, containing glucose (6.2×10^{-3}M) and L-tyrosine-U-C^{14} (5×10^{-4}M and 5.03×10^5cpm) in a final volume of 0.65 ml. At the end of the various incubations the slices were homogenized and the microsomes and soluble fractions separated by differential centrifugation, as described in the text.

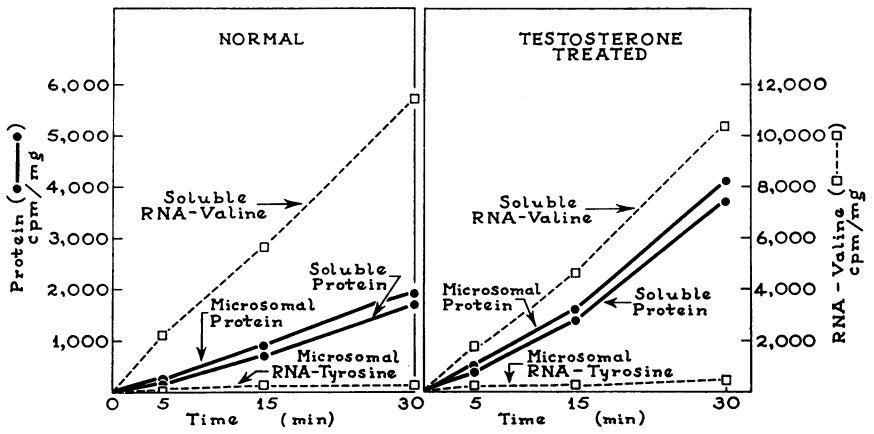

FIG. 5. TIME COURSE OF INCORPORATION OF L-VALINE-I-C^{14} INTO THE SUBCELLULAR FRACTIONS OF PROTEIN AND RNA BY SLICES OF RAT SEMINAL VESICLE. Slices of seminal vesicle from normal and testosterone-treated rats (1 day) were incubated for varying time periods in Krebs-Ringer bicarbonate buffer, pH 7.4, containing glucose (6.2×10^{-3}M) and L-valine-l-C^{14} (5×10^{-4}M and 5.0×10^{5}cpm) in a final volume of 0.65 ml. At the end of the various incubations the slices were homogenized and the microsomes and soluble fractions separated by differential centrifugation, as described in the text.

acid synthesis, amino acid activation, or soluble RNA-amino acid formation—can be rate limiting in the nontestosterone-treated tissue. It clearly indicates that testosterone action must occur between the formation of soluble RNA-amino acids and the peptide bonding of soluble RNA-amino acids to form the microsomal ribonucleoprotein (step 5).

TABLE IV

Influence of incubation temperature on protein synthesis in slices of rat seminal vesicle *

Exp.	Pretreatment	Incubation temperature	L-tyrosine-U-C^{14} converted to: Intracellular amino acids	L-tyrosine-U-C^{14} converted to: Protein
		°C	*cpm/mg protein*	*cpm/mg*
1				
	None	37	3,450	798
	None	31	4,788	153
	Testosterone (2 days)	37	4,776	515
	Testosterone (2 days)	31	4,348	657
2				
	None	37	9,306	2,800
	None	27	5,190	259
	Testosterone (3 days)	37	9,456	1,803
	Testosterone (3 days)	27	9,882	1,883

* Slices were incubated for 1.5 hours in Krebs-Ringer bicarbonate buffer, pH 7.4, containing L-tyrosine-U-C^{14} (5×10^{-4}M containing 1.06×10^{6} cpm) to make a final volume of 1.0 ml.

It was possible, however, that testosterone might also accelerate the final step in protein synthesis, the release of the completed protein from the microsome. This possibility was ruled out in the experiment shown in Figure 5. Although the synthesis of both microsomal and soluble protein from valine-C^{14} was increased at each time interval examined in the testosterone-treated slice, at no time was there a difference between the two preparations in the ratio of microsomal protein to soluble protein (1.1/1.2), indicating that the stripping off of the completed protein from the microsome in this preparation is independent of testosterone action. It is of interest that in this experiment the synthesis of soluble RNA-valine again was normal in the testosterone-treated slices. Furthermore, in this experiment the levels of both microsomal RNA (normal, 0.32 mg per mg protein; testosterone-treated, 0.38) and soluble RNA (normal, 0.028 mg per mg protein; testosterone-treated, 0.034) were comparable, thus excluding the possibility that the acceleration in protein synthesis might be secondary to an increased availability of ribonucleic acid for peptide bond formation.

The enhancement of protein synthesis in the seminal vesicle by testosterone administration, therefore, appears to be secondary to the acceleration of the conversion of soluble-RNA amino acids to microsomal ribonucleoprotein. This conclusion is supported by the experiments shown in Table IV. Hoagland and colleagues (12) and later Moldave (32) demonstrated that the transfer of the amino acid of the soluble RNA-amino acid complex to microsomal protein is sensitive to slight lowering of the incubation temperature, whereas the other steps in protein synthesis appear to proceed quite rapidly at relatively low temperature. The influence of incubation temperature on protein synthesis was evaluated in the seminal vesicle of normal mature rats (100 to 150 g). When the slices from normal and testosterone-treated rats were incubated at 37° C, only slight differences were seen in the rate of protein synthesis. Lowering the incubation temperature to either 31° or 27° C, however, had a greater depressing effect on protein synthesis in the normal than in the testosterone-treated tissue and, in eight of nine such experiments performed on slices from older animals, markedly different rates of protein synthesis occurred only upon incubation at temperatures lower than 37° C. This evidence further substantiates the profound influence of testosterone on the protein synthetic pathway.

COMMENT

Bernelli-Zazzera, Bassi, Comolli and Lucchelli have reported that protein synthesis in the regenerating rat liver is accelerated by testosterone administration (6), and Frieden and co-workers have demonstrated accelerated protein synthesis in the kidney of the testosterone-treated mouse (7, 8). The experiments reported here clearly demonstrate that testosterone also enhances protein synthesis in the seminal vesicles of immature rats. Furthermore, these experiments constitute strong evidence that the enhancement of protein synthesis is due neither to an acceleration of amino acid transport nor to synthesis but, rather, is secondary to the enhancement of a specific step in protein biosynthesis—the conversion of soluble RNA-amino acids to microsomal ribonucleoprotein.

These studies do not furnish evidence, however, as to the mechanism by which this effect is mediated. The formation of peptide bonds in protein biosynthesis is a complex reaction requiring, in addition to the soluble RNA-amino acids and the ribosome acceptor, the cofactor guanosine triphosphate, a transfer fractor, and magnesium (32). Furthermore, this reaction in some preparations is markedly accelerated by sulfhydryl compounds (33, 34). Thus, there are several mechanisms by which this enhancing action of testosterone might be mediated. Preliminary observations in this laboratory have suggested that the availability of guanosine triphosphate may be rate limiting in protein biosynthesis in the non-testosterone-treated seminal vesicle (35), and this possibility is now under further investigation.

These studies also do not yield insight into whether this enhancement of protein synthesis is a primary action of testosterone or is rather the remote consequence of an involved chain of reactions. Although protein synthesis was enhanced at the first interval studied after the administration of testosterone (12 hours), this enhancement was slight in comparison with the effects seen after longer periods of pretreatment, suggesting that this effect may be secondary. Further elucidation of this question is complicated by the slow absorption of testosterone when injected in an oil base (21); consequently, delay in reaching a maximal effect could be due either to slow accumulation of the hormone within the gland or to the fact that the enhancement of protein synthesis is secondary to other effects of testosterone. And, in fact, the enhancement of protein synthesis does occur much earlier than the previously reported testosterone effects on DNA and RNA content (36) and amino acid activation (37) in the rat seminal vesicle. This question can be settled only by evaluating the effect of water-soluble testosterone derivatives in this system.

The question then arises as to whether there is any relationship between the enhancement of protein synthesis in the rat seminal vesicle and the anabolic and androgenic effects of testosterone in the intact animal. It is logical that any generalized influence of testosterone on protein synthesis would be most marked in the seminal vesicle. First, testosterone-C^{14} is selectively concentrated in the rat accessory sex tissue (11), and second, the rat seminal vesicle is a protein secretory or-

gan. And while protein synthesis is enhanced in other tissues (6–8), changes in the specific activities of newly synthesized protein after testosterone administration are much less under circumstances in which only structural protein is formed. Therefore, while the obvious limitations of using the results of *in vitro* experiments to explain *in vivo* phenomena clearly apply to this problem, it is possible that the generalized effects of testosterone on protein anabolism in the intact animal are in fact secondary to a generalized enhancement of a specific step in protein synthesis—the peptide bonding of soluble RNA-amino acids to form microsomal ribonucleoprotein.

SUMMARY

The influence of testosterone administration on protein biosynthesis from L-valine-1-C^{14} and from L-tyrosine-U-C^{14} has been examined in slices of seminal vesicles from immature rats. Protein synthesis doubled 12 hours after testosterone administration and reached a maximal level (five- to sixfold) within 1 to 2 days. Evidence has been presented that this enhancement of protein synthesis is independent of either amino acid transport or synthesis but is secondary to the acceleration of a specific step in protein synthesis, the conversion of soluble ribonucleic acid-amino acid complexes to microsomal ribonucleoprotein. The possible relationship between these observations and the effects of testosterone in the intact animal are discussed.

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