The ability to separate intact nucleoprotein and newly synthesized density-labeled nucleoprotein on the basis of density will be useful in studies of the heterogeneity of distribution of histone on DNA and also in terms of finding out more information about the sites of histone deposition on the replicating chromosome. Current experiments underway indicate that this latter process is highly organized and occurs in a somewhat unexpected way.

Acknowledgments

The critical advice of our colleagues in this laboratory is greatly appreciated.

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Gonadotropin Stimulation of Rat Testicular Protein Synthesis. Polysome Isolation and Activity in a Cell-Free System†

Tom O. Abney,‡ James K. Skipper,§ and William L. Williams*

ABSTRACT: Follicle-stimulating hormone plus luteinizing hormone were administered in combination by intratesticular injection to mature hypophysectomized rats 14–21 days postoperatively. Within 12 hr of administration of the gonadotropins, both testicular total RNA and polysomal RNA content were increased (33 and 35%, respectively). The use of an RNase inhibitor was found to be essential to the isolation of relatively undegraded polysomes from the testis. Sucrose gradient sedimentation analysis demonstrated that the polysomal preparations each had essentially the same A_{254} profiles regardless of the testis source; intact, hypophysectomized control, or experimental animals. These findings suggest that gonadotropins induced an increase in testicular polysomal content in the absence of a shift from monomeric to polysomal aggregates. Within 12 hr of hormone administration, free testicular polysomes exhibited a significant increase in protein synthesis *in vitro;* membrane-bound polysomes did not show this response. It is concluded that increased testicular protein synthesis following gonadotropin administration results from an increase in the number of ribosomes and from a selective stimulation of protein synthesis by free testicular polysomes.

Follicle-stimulating hormone[1] administered to either immature or mature hypophysectomized male rats was reported to stimulate testicular protein synthesis *in vitro* within 30 min (Means and Hall, 1967, 1968). Further investigation revealed that administration of FSH to either immature or mature hypophysectomized rats stimulated [^{14}C]amino acid incorporation into protein by testicular polysomes *in vitro* within 1 hr (Means and Hall, 1969, 1971). Means (1971) showed that FSH enhanced *in vivo* nRNA synthesis within 15 min in the immature rat testis thus demonstrating that the hormone affects both transcription and translation in its target tissue. The present investigation was undertaken to further elucidate the role of FSH and LH in the testis. The data will demonstrate that administration of FSH plus LH to mature hypophysectomized rats results in increases in testicular total RNA and polysomal RNA and selectively enhances the capacity of free polysomes to support *in vitro* [^{14}C]amino acid incorporation into protein.

Materials and Methods

Animals. The animals used in the investigation were Sprague-Dawley male rats hypophysectomized at the time of sexual maturity, 60 days of age. They were purchased from Hormone Assay Laboratories, Chicago, Ill., and shipped to this laboratory 2–3 days after surgery. The animals were kept in a 12-hr light–12-hr dark environment and fed Purina rat chow *ad libitum* and fresh orange slices every other day. In all studies reported here the animals were used 14–21 days postoperatively. Control or experimental refers to hypophysectomized rats which were injected with saline or hormones, respectively. Intact mature male rats were used on occasion for comparative purposes and are referred to as intact.

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[1] Abbreviations used are: FSH, follicle-stimulating hormone; LH, luteinizing hormone; DOC, deoxycholate, sodium salt.

Gonadotropins. Gonadotropin hormones were received from the Endocrinology Study Section, National Institutes of Health. NIH-FSH-S9 had an activity of 1.13 NIH-FSH-S1 units/mg; NIH-LH-S17 had an activity of 1.01 NIH-LH-S1 units/mg. FSH and LH were each separately dissolved in sterile saline at a concentration of 10 mg/ml. Prior to injection, aliquots of each were combined to give a final hormone solution containing 2000 μg of FSH and 200 μg of LH/ml.

Chemicals. Biochemicals used included Na_2ATP, K_3GTP, chloramphenicol, and rabbit muscle pyruvate kinase from Calbiochem; heparin, cycloheximide, phosphoenolpyruvate (trisodium), and glutathione from Sigma Chemical Co. An L-[U-^{14}C]amino acid mixture (av 234 Ci/mol) from which tryptophan, asparagine, glutamine, methionine, and cysteine were absent, was from New England Nuclear. RNase-free sucrose and Ultra Pure Tris from Schwarz/Mann were used in all buffers.

Buffers. Buffers were designated as medium H (0.25 M sucrose, 0.005 M $MgCl_2$, and 0.1 M KCl, in 0.05 M Tris-HCl, pH 7.6, at 5°), medium K (same as medium H but made 0.025 M KCl), medium E (medium H or K minus sucrose), medium G (medium H or K made 2.3 M sucrose), and 10.2 and 34.2% sucrose gradient solutions prepared in medium H or K. Buffers were filtered through a 0.45 μ filter (Millipore Filter Corp.) and stored at −20° until used.

Gonadotropin Treatment of Animals. Rats were divided into two groups; experimental rats were injected intratesticularly with 50 μl of hormone/testis. This was equivalent to 0.1 unit/100 μg of FSH plus 0.01 unit/10 μg of LH per testis. Control rats were injected with saline *via* the same route. At designated time intervals after injection all animals were killed by decapitation and the testes were removed to cold medium H on ice. The tunica albuginea was removed and the tissue weight was determined for each group. The pituitary fossae were routinely examined to verify the complete removal of the pituitary. At the time of use all animals that did not show the expected decrease in body and testis weight were eliminated from the study since this was indicative that hypophysectomy was incomplete. Preparation of all cellular fractions was performed at 0–5°.

Preparation of the RNase Inhibitor. The rat liver RNase inhibitor described by Roth (1956) was prepared from livers of male or female rats, age 45–70 days of age. The 105,000*g* (av) supernatant containing the RNase inhibitor activity (medium I) was prepared, decanted, and stored in aliquots at −20°. Addition of medium I to a homogenate is reported to inhibit the breakdown of polysomes by either endogenous or exogenous RNase (Blobel and Potter, 1966). Inhibitor activity, as measured by the integrity of polysomal absorbance 254-nm profiles, was stable for at least 3 weeks at −20°. Consequently, medium I was used within 2 weeks after preparation.

Preparation of Polysomes. Testicular tissue was finely minced and polysomes were prepared by a modified method of Blobel and Potter (1967a). The tissue was homogenized in three volumes of cold medium H using ten strokes in a Potter-Elvehjem homogenizer fitted with a Teflon pestle (0.006-in. clearance). With the exception of several initial experiments in which no RNase inhibitor was used, one-tenth volume of medium I was next added and the homogenate was filtered through two layers of gauze cloth and centrifuged at 17,000*g* for 10 min. The resulting supernatant was decanted and made to a final concentration of 4% Triton X-100, 1% DOC. This fraction was layered over a two-layer discontinuous sucrose gradient prepared in 10-ml Oak Ridge-type centrifuge tubes. The upper 2 ml of the gradient was 1.38 M sucrose and the lower phase was 2.0 M sucrose. The samples were centrifuged at 105,000*g* for 4 hr. The polysomal pellets were gently rinsed with 0.5 ml of cold medium H and resuspended in cold medium H and homogenized using three strokes in a glass–Teflon homogenizer. Aliquots (25–50 μl) were taken to determine the ratio of absorbance at 260 and 280 nm. The remainder of the sample was either frozen and stored as 500-μl aliquots in liquid N_2 or used for sucrose gradient analysis and *in vitro* assays of amino acid incorporation. Polysomes retained the characteristic sedimentation profiles and the *in vitro* incorporation capacity for at least 2 months frozen and stored in liquid N_2. Polysomes prepared as described above contained both free and membrane-bound ribosomes and were taken to be representative of the total testicular ribosomal population.

By modification of the technique described above a second procedure for the isolation of polysomes was developed. The changes made were: the use of medium K rather than medium H as the homogenization buffer, centrifugation at 17,000*g* for 15 min rather than 10 min, and alteration of the detergent concentration to yield a final concentration of 0.25% DOC only. All other buffers were prepared using medium K. Polysomes prepared by this procedure again contained both free and membrane-bound ribosomes, but the free ribosomes as a per cent of the total preparation were increased due to the longer centrifugation time at 17,000*g* and the subsequent use of a lower detergent concentration. To obtain a fraction enriched in membrane-bound polysomes, the initial 17,000*g* pellet was resuspended in medium K containing 0.25% DOC and rehomogenized using five strokes in the Potter-Elvehjem homogenizer. This fraction was then centrifuged at 17,000*g* for 10 min and the resulting supernatant was layered on a discontinuous sucrose gradient as described above for the preparation of polysomes.

Sucrose Gradient Analysis. Linear (10.2–34.2%) 28-ml sucrose gradients were made as previously described (Means *et al.*, 1969) and allowed to equilibrate for 1–2 hr under refrigeration. Samples of 2–5 absorbance 260 units of polysomes in 1.0 ml were layered on the gradients and centrifuged at 25,000 rpm in a Beckman SW 25.1 rotor without use of the brake. Polysomal profiles were recorded at A_{254} using an ISCO fraction collector and recorder.

Preparation of the pH 5 Enzyme Fraction. Using the procedure of Campagnoni and Mahler (1967), a pH 5 precipitate was prepared from the 105,000*g* supernatant fraction of the testis. Aliquots were taken to determine the protein concentration and the final pH 5 enzyme preparation was adjusted to 5–10 mg of protein/ml. The enzyme fraction was either used immediately in an *in vitro* incorporation assay or frozen and stored as 500-μl aliquots in liquid N_2. The *in vitro* activity remained unchanged for at least 4 months when stored at −196°.

Cell-Free Protein Synthesis. The system for *in vitro* protein synthesis was a modification of the procedure described for the rat testis (Means *et al.*, 1969). The components of the reaction were: ATP, 1.0 mM; GTP, 0.4 mM; P-enolpyruvate, 10 mM; pyruvate kinase, 25 U; glutathione (GSH), 6.0 mM; $MgCl_2$, 6.4 mM; KCl, 10 mM; sucrose, 100 mM; Tris-HCl, 50 mM; L-[U-^{14}C]amino acid mix, 1.0 μCi; five [^{12}C]amino acids, 0.05 mM each; pH 5 enzyme fraction, 500–1000 μg of protein and 2–4 A_{254} units of polysomes. The final volume was 0.5 ml (pH 7.0) and the reactions were run at 32°, the optimum temperature for rat testicular protein synthesis (Davis, 1969). Stock solutions of all the reaction components, except the cellular fractions, were prepared, filtered through a 0.45 μ filter and frozen at −80° until used. Glassware was acid washed and autoclaved to eliminate contamination. The filter paper disk method of Mans and Novelli (1961) was employed to measure [^{14}C]ami-

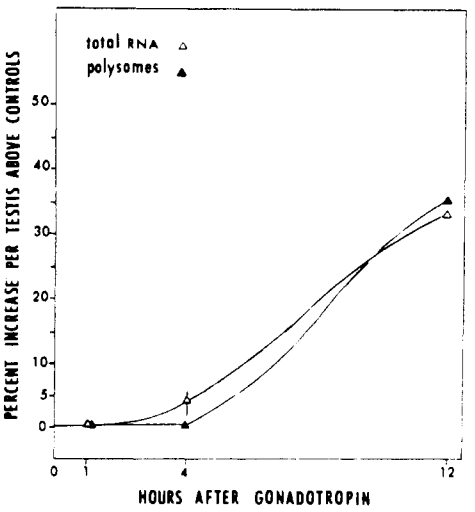

FIGURE 1: Gonadotropin-induced increase in total RNA (△) and polysomal RNA (▲) content in the rat testis. FSH plus LH were injected intratesticularly and the increases were determined and expressed as per cent above controls. Total RNA increased from a mean of 29 to 39 A_{260} units/testis at 12 hr with a mean difference of 10 ($n = 5$, $p < 0.01$). Polysomal RNA increased from a mean of 4.0 to 5.58 A_{260} units/testis at 12 hr with a mean difference of 1.58 ($n = 8$, $p < 0.01$). Each point is the mean of five to eight determinations using four to ten animals each time.

no acid incorporation. At timed intervals from 0 to 60 min, 100-μl aliquots from each reaction tube were spotted on numbered paper disks. The disks were acid precipitated, washed, and extracted to remove unincorporated radioactivity. Following the washing procedure, the disks were transferred to glass scintillation vials containing 5–10 ml of scintillation fluid (8 g of 2,5-diphenyloxazole and 200 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene in 2 l. of toluene). Incorporation of [^{14}C]amino acids into protein was determined using a Packard 3380 scintillation counter. Counting efficiency for ^{14}C on the disk varied from 50 to 60%. Incorporation is expressed as pmol of [^{14}C]amino acid incorporated/A_{260} unit of polysomes; 1 pmol of the [^{14}C]amino acid mix is equivalent to 519 dpm of radioactivity.

Determination of RNA and Protein. The method of Fleck and Munro (1962) was used to measure testicular RNA A_{260} units and the method of Lowry *et al.* (1951) was employed for protein determination.

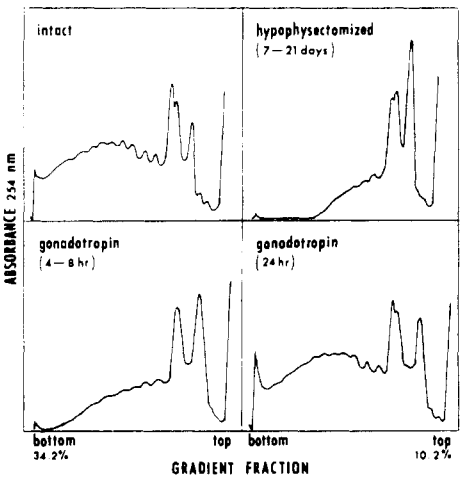

FIGURE 2: Sucrose gradient profiles of rat testicular polysomes prepared in the absence of RNase inhibitor. Aliquots of 2–5 A_{260} units of each were applied to the gradients. Sedimentation was from right to left.

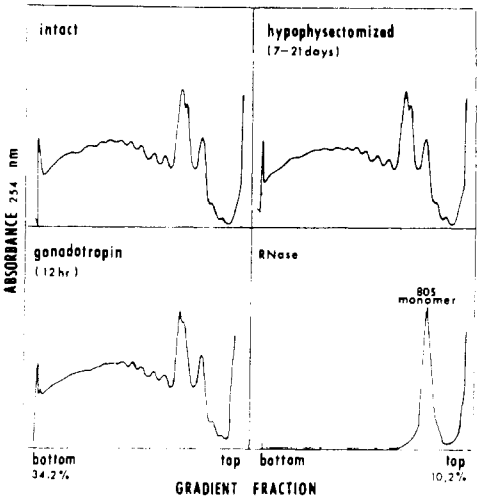

FIGURE 3: Sucrose gradient profiles of rat testicular polysomes prepared in the presence of an RNase inhibitor. Aliquots of 2–5 A_{260} units of each were applied to the gradients. Sedimentation was from right to left. These profiles are representative of the total ribosome population.

Results

As shown in Figure 1, within 12 hr of gonadotropin administration, increases were recorded in testicular total RNA and polysome RNA A_{260} units: 33% (range, 17–37%) and 35% (range, 15–37%), respectively. These data represent the mean per cent increase above controls taken from five to eight experiments and are considered substantial increases for the relatively short experimental period of 12 hr. It should be noted that the animals were used 14–21 days postoperatively, a period in which the testes were undergoing postoperative degeneration. All cellular constituents, including RNA and polysomes, were therefore decreasing on a per testis basis during that period. Statistical analysis of the data was therefore performed using the paired variable Student's t test. The mean differences ($\bar{d}$) between control and experimental values were calculated to be significant ($p < 0.01$) for all data reported here. Since testicular RNA and polysome content were increased by 12 hr most of the studies on the properties of testicular polysomes and protein synthesis were performed at that time.

Polysomes were initially isolated without medium I as described in the first procedure in Methods. The sedimentation profiles of these polysome preparations are presented in Figure 2. Using this procedure it can be seen that the profiles of intact rat testicular polysomes showed a high polymer region with relatively little degradation. The profiles of testicular polysomes from hypophysectomized controls exhibited a high monomer-dimer region and relatively little polymer, characteristic of RNase degradation. Following gonadotropin treatment for 4–8 hr the polymer region had increased and by 24-hr postinjection the profiles resembled those from the intact testis. These initial data led to the premature conclusion that gonadotropins had enhanced testicular polysome assembly. Such a response to gonadotropins could occur as a result of RNA synthesis, both ribosomal and messenger, or as a result of transport of RNA from the nucleus to the cytoplasm. On the other hand, the difference might also be attributed to a greater lability of control polysomes during the isolation process. To clarify this point a repetition of that work was carried out using the same procedure with the addition of medium I as an RNase inhibitor. The use of medium I was found to yield undegraded polysomal preparations from all testicular tissues studied as demonstrated in Figure 3. The polysomal preparations had essentially the same sedimentation profiles regardless of the testis source: in-

TABLE I: Properties of Rat Testicular Polysomes.[a]

Source of Polysomes	Polysomes as % of Total RNA	A_{260} Units/ Testis	A_{260} Units/g	% Polysome[b]	% Monomer + Dimer
Intact mature	24.5	23.0	17.0	81	19
Hypophysectomized control[c]	16.7	4.0	10.0	76	24
Hypophysectomized 12-hr FSH plus LH	18.6	5.58	9.35	77	23

[a] All values are the mean of three or four determinations. [b] % polysomes and % monomer plus dimer refer to the absorbance 254 profiles from sucrose gradient analysis. Polysomes refer to the trimer and larger ribosomal aggregates. [c] Values for the hypophysectomized control and the FSH plus LH experimental group are data from rats hypophysectomized 17 days.

tact, hypophysectomized control or 12-hr experimental animals.

As indicated by the data in Table I the increased yield in polysomal A_{260} units after 12-hr gonadotropin treatment was due to an increase in ribosome content per testis, a mean of 4.0 *vs.* 5.8 A_{260} units of polysomes/testis from control and experimental animals, respectively. A comparison of the A_{260} units of polysomes/g of tissue reveals that the yield from control tissue was equal to or greater than that from experimental testis, a mean of 10.0 *vs.* 9.35 A_{260} units/g of tissue from control and experimental testes, respectively. The increase in polysomal A_{260} units/testis after gonadotropin treatment was thus an increase in polysome content rather than a difference in extraction during the tissue fractionation process. The proportion of ribosomes in the monomer-dimer region *vs.* the polymer species was not greatly altered either by hypophysectomy or gonadotropin treatment as shown by the results in Table I. Analysis of the A_{254} profiles of each polysomal preparation revealed that 81% of the ribosomes from the intact rat testis were associated as polysomes as compared to 76 and 77% in the control and 12-hr experimental testes, respectively.

Having previously demonstrated that gonadotropin administration increased testicular protein content *in vivo* within 12 hr (Abney and Williams, 1974) the mechanisms that modulate this synthesis were investigated. Assays of the pH 5 enzyme fractions from intact, hypophysectomized control and experimental rat testes were performed in the cell-free system using intact rat testicular polysomes as a common RNA preparation. All the results were comparable and indicated that no difference existed in the various pH 5 enzyme fractions in regard to *in vitro* amino acid incorporation. The activity for each pH 5 enzyme fraction was in the range of 43–47 pmol of [^{14}C]amino acid incorporated/A_{260} unit of polysomes. Assays of the polysomes from intact, hypophysectomized control and experimental rat testes were done in the cell-free system using a common pH 5 enzyme fraction. It should be emphasized that the polysomal fractions under consideration were prepared as described in Methods using medium H (0.1 M KCl) plus Triton X-100 and DOC. The *in vitro* activities of these various polysomal preparations were equivalent. These data suggested that the increase in protein synthesis *in vivo* was due to the increased polysome content and not to an alteration by gonadotropin action of the polysome activity (amino acid incorporation/A_{260} unit).

The procedure for isolating polysomes was then altered and a study was made relating the effects of salt and detergent concentrations to polysome yield, A_{254} profiles, and *in vitro* activity. Using medium K (0.025 M KCl) and a final concentration of 0.25% DOC, polysomes were isolated from control and experimental rat testes. It was noted that changes in the salt and detergent concentrations did alter the ratio of absorbance at 260 and 280. The most pronounced alteration of ratio was caused by varying the KCl concentration. Lower K^+ (0.025 M) resulted in ratios near 1.50 regardless of the detergent concentration. Higher K^+ (0.1 M) resulted in values near 1.65 and 0.1 M K^+ plus high concentrations of detergents increased the ratio to 1.70.

RNA and polysome distribution during preparation was determined at each stage of tissue fractionation. From each sample, aliquots were taken to determine the RNA concentration; the RNA in the homogenate was taken as 100% of the total RNA. The per cent RNA in each fraction was found to be the same for both control and experimental testes. A large portion of the RNA was lost during the first centrifugation, yielding only 37% in the supernatants. Rehomogenization of the 17,000*g* pellet and centrifugation solubilized approximately 14% of the RNA. It was assumed that the remaining 49% which was discarded contained tightly membrane-bound RNA plus the nuclear RNA which is only a small fraction of the total RNA. If, as in liver (Blobel and Potter, 1967b), testicular RNA is 80% rRNA, 15% nonsedimentable, and 5% nRNA then the free polysomes and membrane-bound polysomes represented approximately 22 and 58% of the cellular RNA, respectively. Thus, approximately 65% of the free polysomes were recovered while the major portion of the membrane-bound ribosomes was lost during fractionation, specifically in the 17,000*g* pellet.

Sucrose gradient analysis of these polysomes revealed that the A_{254} profiles were comparable to those isolated previously (Figure 3). These profiles were indicative of relatively little RNase degradation based on the average value of 71% polymer in the free polysome profiles. The membrane-bound preparations exhibited similar patterns with a slightly higher per cent of polymer. No appreciable difference in profiles was observed between the control and experimental polysomes. This was true for both free and membrane-bound preparations.

Experiments were conducted to further standardize the conditions for cell-free protein synthesis. As illustrated in Figure 4, [^{14}C]amino acid incorporation increased linearly with increasing amounts of pH 5 enzyme fraction. These results were obtained using intact rat testicular pH 5 enzyme and 2.0 A_{260} units of polysomes per assay tube; the polysomes were isolated with the use of medium K and 0.25% DOC. Incorporation increased linearly up to 1000 μg of protein and approached a plateau at 1500 μg of protein/2.0 A_{260} units of polysomes at 60 min. Inhibition of incorporation is reported to occur by addition of the pH 5 fraction or cell sap above the level of saturation (Flessel, 1971). For this reason 375–500 μg of pH 5 protein/A_{260} unit of polysomes was used in all later experiments to avoid the problem of inhibition. The optimum Mg^{2+} concen-

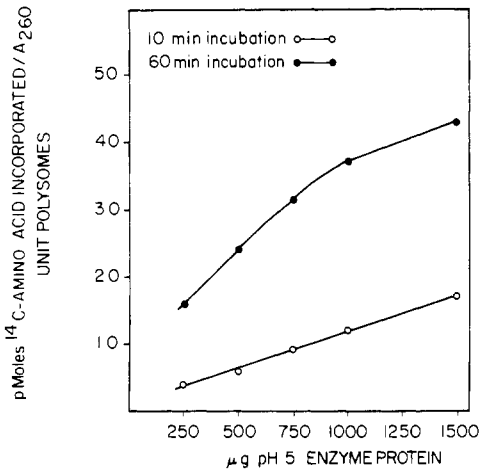

FIGURE 4: Amino acid incorporation in the cell-free system as a function of the pH 5 enzyme fraction concentration. The polysomes and the enzyme fraction were both prepared from intact rat testes. Incorporation after 10 min (○) and after 60 min (●) was determined as described in Methods using 2 A_{260} units of polysomes per assay tube.

tration for testicular polysome activity *in vitro* is reported to be 6.4 mM (Means *et al.*, 1969). In light of the fact that the concentration reported by Means *et al.* (1969) was determined at 30 min under slightly different reaction conditions, data obtained in this investigation confirm an optimum Mg^{2+} concentration of approximately 6.4 mM.

Considering the data which suggest that the conditions of isolation affect the properties of polysomes it became of interest to study the *in vitro* activities of polysomes from control and experimental testes prepared using medium K (0.025 M KCl) and 0.25% DOC. Figure 5 illustrates that gonadotropins administered *in vivo* resulted in a stimulation of *in vitro* amino acid incorporation by free polysomes. A mean increase of 35% in the initial rate (10 min) was recorded and the extent of incorporation (60 min) was 15% above controls. This stimulation was determined to be significant ($p < 0.01$). Incorporation was linear during the first 15–20 min and continued to increase but at a reduced rate during the last 40–45 min. Initiation of polypeptide synthesis occurs only minimally in cell-free systems of

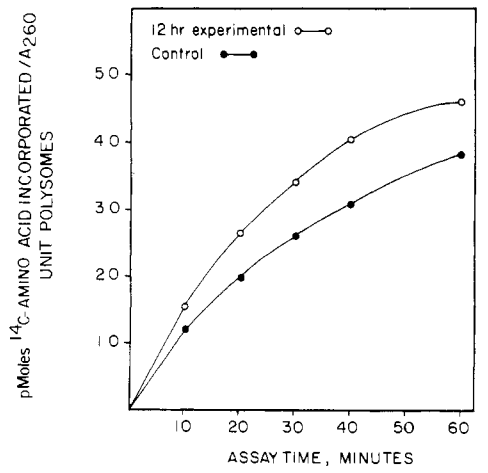

FIGURE 5: The effect of *in vivo* gonadotropin administration on [^{14}C]amino acid incorporation by rat testicular polysomes in the cell-free system. Free polysomes were isolated from groups of control animals (●) or from animals injected 12 hr prior with FSH plus LH (○). The reaction was run for 60 min with aliquots being removed at the time intervals indicated. Incorporation by experimental polysomes was enhanced above that of the controls at each time point ($n = 4$, $p < 0.01$ at 10, 20, and 30 min, < 0.05 at 40 and 60 min).

TABLE II: Characteristics of the *in Vitro* Protein-Synthesizing System from the Mature Hypophysectomized Rat Testis.[a]

System	pmol of [^{14}C]Amino Acid Incorp/A_{260} Unit of Polysomes	% of Complete System
Complete	46.0	100
Omissions		
Polysomes	6.8	15
pH 5 enzyme	23.0	50
ATP	1.4	3
GTP	11.0	24
Additions		
Cycloheximide (1 mg/ml)	10.0	22
Chloramphenicol (1 mg/ml)	46.0	100
RNase	2.3	5

[a] Polysomes were prepared using medium K (25 mM KCl) plus 0.25% DOC. The complete system contained all components as described in Methods and included 1000 μg of pH 5 enzyme and 2 A_{260} units of polysomes from hypophysectomized gonadotropin-injected (12-hr experimental) rat testes. Polysomes from the control testis exhibited the same characteristics as those described above. The values for the complete system represent the mean of four or more determinations at 60 min.

this nature, so the increase in activity of polysomes from experimental testes presumably reflects an increased rate of elongation. The properties of this cell-free system are presented in Table II. The system showed a complete dependence on polysomes and the requirement for a pH 5 enzyme fraction. Omission of ATP resulted in 97% inhibition, whereas the lack of GTP inhibited incorporation by 76%. Addition of cycloheximide inhibited the system by 78%; however, chloramphenicol had no effect on incorporation. As expected, addition of RNase to the reaction resulted in a loss of polysome activity. Thus, as a result of gonadotropin injection *in vivo*, both the rate and extent of [^{14}C]amino acid incorporation *in vitro* by free polysomes were enhanced. The *in vitro* activities of the membrane-bound polysomes from control and experimental testes were also examined. Gonadotropin injection did not effect a stimulation of bound polysome activity *in vitro*. These data are evidence that gonadotropins selectively stimulated free polysome activity.

The time required for gonadotropins to induce this stimulation of free polysome activity was examined; 1 hr after hormone injection the *in vitro* activity of the polysomes was equal to that of the controls. At 4 hr, an apparent stimulation of activity had occurred but was shown to be insignificant ($p > 0.05$). Therefore, between 4 and 12 hr after gonadotropin administration, testicular protein biosynthesis was effected by an increase in free polysome activity. It appears that one of the mechanisms that modulate an increase in testicular protein synthesis following gonadotropin administration is an increase in amino acid incorporation per A_{260} unit of free polysomes.

Discussion

It is generally accepted that LH (Hall and Eik-Nes, 1962) and FSH (Means and Hall, 1967) each act in the testis to stimulate protein synthesis. Further, there is evidence, though final

proof is lacking, that one gonadotropin augments the action of the other in stimulating testosterone synthesis (Connell and Eik-Nes, 1968; Lostroh, 1969; Johnson and Ewing, 1971). The present study was designed in an attempt to elicit a more physiological response by injecting both FSH and LH as opposed to administering only one gonadotropin. Though admittedly unorthodox, the intratesticular route of injection was chosen since other investigators have successfully used this method in gonadotropin studies (Squire *et al.*, 1963; Murphy, 1965). Results have demonstrated that within 12 hr FSH and LH induced increases in both testicular RNA and protein content *in vivo* (Abney and Williams, 1974).

Many growth promoting hormones stimulate the rate of RNA synthesis *in vivo* and it is known that this RNA is important in the regulation of growth and protein synthesis in the target organ (Tata, 1965, 1967; Hamilton *et al.*, 1968). An increase in total RNA and polysome content suggests that the gonadotropins stimulated the rate of testicular RNA synthesis *in vivo*. The observation that polysome content per testis was increased in the absence of a shift from monomers to polysomes would suggest that all populations from monomeric to polysome aggregates had increased following gonadotropin administration. FSH was reported to enhance nRNA synthesis in the immature rat testis within 15 min (Means, 1971). These early synthetic events could presumably lead to an increase in testicular RNA and protein within 12 hr.

The use of a specific RNase inhibitor in the preparation of polysomes is considered essential to the recovery of relatively undegraded polysomes from the testis, especially from hypophysectomized animals. Preparations isolated with the use of an inhibitor are more representative of polysomal integrity *in vivo* than those prepared in the absence of the inhibitor. Gonadotropins increased the stability of polysomes as is evident from the A_{254} polysomal profiles prepared in buffers containing no medium I (Figure 2). The possibility exists that an RNase inherent to the polysomes was less active after gonadotropin injection. These results might be interpreted to mean that the testis of the hypophysectomized control rat exhibits a higher RNase activity than that of either the intact or experimental animals. The presence of an RNase inhibitor during the isolation process revealed that hypophysectomy and gonadotropin administration did not alter the polysomal A_{254} profiles in comparison to those from the intact rat testis. Many hormones stimulate protein synthesis by enhancing polysome activity in the target tissue (Tata, 1967; Means and Hall, 1969; MacDonald and Korner, 1971; Schwartz, 1972) but few hormones are reported to stimulate the conversion of monomers into polysomes (Palmiter *et al.*, 1970; Means *et al.*, 1971).

The investigation into the effects of salts and detergent concentrations on polysome properties was prompted by the fact that absorbance ratios at 260 and 280 and other characteristics are influenced by the procedures used during isolation (Zomzely *et al.*, 1966; Coleman, 1969; Means *et al.*, 1971; Palmiter, 1971). It is concluded that the absorbance ratios of testicular polysomes are more sensitive to changes in K^+ than to variations in Mg^{2+} concentration. This study suggests that protein, of which the nature and function are unknown, was removed from the polysomes by higher K^+ concentrations.

The polysomes prepared in the present study were representative of the whole testis. The capacity of testicular polysomes to support [^{14}C]amino acid incorporation *in vitro* was similar to that described for other tissues. The lack of gonadotropin influence on the activity of the pH 5 enzyme fraction confirms the results of Means and Hall (1969). Results from the initial studies of *in vitro* protein synthesis had indicated that the activity of polysomes was not increased as a result of *in vivo* gonadotropin administration. Further investigation revealed that the activity of polysomes was greater if the isolation was done in the presence of lower concentrations of K^+ and detergent. Those results suggested that the higher ionic environment and more rigorous conditions of isolation removed from the polysomes factors essential for enhanced protein synthesis. The similarity in the A_{254} profiles and the addition of equal concentrations of RNA to the *in vitro* assay ruled out the possibility of a quantitative difference in the RNA preparations. It is reasonable to assume that the lower activity of medium H polysomes was not a result of contaminating detergents since the isolation procedure should have eliminated these substances. Further, each polysomal pellet was rinsed carefully after centrifugation to remove contaminants. Means *et al.* (1969) reported no difference in activity between testicular polysomes isolated with or without detergent.

One of the most interesting findings presented in this study was the *in vitro* activities of free and membrane-bound polysomes from both experimental and control animals. The 35% increase in the initial rate of incorporation observed with free polysomes indicates that gonadotropins had acted at the ribosomal level. The lack of gonadotropin effect on the bound fraction further indicates that this stimulation was selective for free polysomes only. MacDonald and Korner (1971) reported results of a similar nature for growth hormone action in rat liver. These investigators found, however, that growth hormone stimulated the synthetic capacity of the membrane-bound fraction but had no effect on free polysomal activity. Other investigators had demonstrated that in the immature rat testis, FSH enhanced the synthetic capacity of polysomes within 1 hr (Means and Hall, 1969, 1971). Analysis of the polysome isolation procedure employed suggests that these investigators were studying primarily the free polysomes. In the present study the fractionation into free *vs.* bound led to an enrichment of gonadotropin-responsive polysomes in the free fraction. As a result the free polysomes reflected *in vitro* an increase in protein synthesis. The rate of turnover of mRNA associated with free polysomes in the rat liver is reported to be greater compared to mRNA associated with bound polysomes (Murty and Sidransky, 1972). This might also be the case in testicular tissue; thus gonadotropin-induced mRNA synthesis would be reflected to a greater degree in the free polysomes.

The distribution of RNA in experimental and control testes did not differ appreciably indicating that gonadotropins had no effect in regard to interconverting membrane-bound and free polysomes. The finding that free and membrane-bound polysomes represented approximately 22 and 58%, respectively, of the total testicular RNA is similar to the distribution of hepatic polysomes as reported by Blobel and Potter (1967b). A partial purification of the polysomal population was achieved by preparing both free and membrane bound and it is logical to assume, based on the data presented here, that the hormone-responsive polysomes were fractionated primarily as free polysomes. The free fraction was calculated to represent approximately 80% of the recoverable ribosomes in this study.

Since the rate of testicular protein synthesis is regulated by gonadotropins through changes in both ribosome content and alterations of polysome activity, attempts were made to isolate from the polysomes a salt extracted fraction which, when added back *in vitro*, would stimulate amino acid incorporation. All attempts to date have failed, thus further investigations are required to elucidate the molecular mechanism involved. The 35% stimulation in polysome activity can be viewed as only a fraction of the real increase in hormone-responsive polysome

activity. This would be the case since whole testicular tissue which contains many different cell types was used. Further investigation may require the use of isolated cell fractions, *i.e.*, interstitial tissue or tubule preparations to facilitate a greater purification of polysomes from gonadotropin responsive cells. In conclusion, the data obtained here reveal for the first time several important effects of gonadotropin action in the testis. Within 12 hr of administering FSH plus LH, testicular total RNA and polysomal RNA content were increased. Secondly, the concentrations of KCl and detergent used during polysome isolation were critical in obtaining preparations which showed the gonadotropin-induced increase in [^{14}C]amino acid incorporation. Finally, but of great importance, the gonadotropins selectively enhanced the protein synthetic capacity of the free polysome fraction within 12 hr.

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