Effects of Estradiol on Uterine Ribonucleic Acid Metabolism. II. Methylation of Ribosomal Ribonucleic Acid and Transfer Ribonucleic Acid*

Theodore W. Munns and Philip A. Katzman†

ABSTRACT: Following a single intraperitoneal injection of 17β-estradiol into immature rats, the *in vitro* rates of incorporation of L-[*methyl*-^{3}H]methionine into specific uterine RNAs (methylation) were assessed by acrylamide gel electrophoresis during the initial 24 hr of the estrogen response. The radioactivity profiles of gels containing total and fractionated uterine RNA preparations demonstrated that rRNA (both 28 and 18 S) and tRNA were the principal methylated species present. Studies with actinomycin D indicated that the methylation of rRNA and tRNA occurs at the time of transcription or shortly thereafter, thereby providing evidence that methylation of these species is a reflection of their synthesis. During the early phase of the estrogen response, the methylation of rRNA and tRNA increased at similar rates, reaching values of 300% of controls at 6 hr. While the rate of tRNA methylation progressively declined after this time, that of rRNA did not become maximal until 12 hr (380% of controls). Methylation of both RNA species was still significantly above control levels 24 hr after estradiol administration. These studies represent an attempt to evaluate quantitatively the effect of estradiol on the rates of synthesis of uterine rRNA and tRNA.

Although the importance of uterine RNA synthesis for the uterotrophic action of estradiol has been suggested by the effects of transcriptional inhibitors (Ui and Mueller, 1963; Hamilton *et al.*, 1968), studies designed to characterize the selective synthesis of specific RNA species after estrogen administration have been limited and have yielded conflicting results. For example, Wilson (1963) found by sucrose gradient centrifugation that synthesis primarily of low molecular weight RNA was increased, while enhancement of all RNAs in both nuclear and cytoplasmic fractions was observed by Gorski and Nelson (1965). In the first case, the RNA in uteri exposed to the hormone *in vivo* for 4 and 24 hr was labeled *in vitro* with [^{14}C]adenine, whereas in the latter instance the RNA was labeled by short *in vivo* pulses with [^{3}H]cytidine during the initial 2 hr of the estrogen response. More recently, Billing *et al.* (1969a) utilized MAK[1] column chromatography to characterize uterine RNA synthesis during the first 6 hr of estrogen action. Following 30 min *in vivo* pulses with [^{3}H]-guanosine plus [^{3}H]uridine, major increases in rRNA and tRNA synthesis were evident 6 hr after estrogen administration, whereas mRNA synthesis was only slightly enhanced.

Since conventional precursors of the type used in the above studies are more or less uniformly incorporated into all RNA structures, a significant portion of the label would be incorporated into mRNAs during short pulses. During characterization, these polydispersed, rapidly labeled mRNAs would mask the identity of other, more discrete, RNA populations. Although MAK column chromatography offers better resolution in this regard, overlapping of mRNAs as well as their complex formation with rRNAs (Hayes *et al.*, 1966), present distinct possibilities of masking. Furthermore, even though radioactive nucleosides or bases offer a potential means of detecting the selective synthesis of various RNA species, quantitation of such species yields questionable results because of the estrogen-induced fluctuations in specific activities associated with these precursors (Mueller *et al.*, 1958; Billing *et al.*, 1969b; Oliver and Kellie, 1970; Munns and Katzman, 1971).

In the present investigation, we have attempted to overcome these difficulties by monitoring uterine RNA synthesis with L-[*methyl*-^{3}H]methionine in an *in vitro* system. Although this label is not incorporated into all RNA species, this disadvantage was offset by the apparent absence of methylation of mRNA (Srinivasan and Borek, 1966; Moore, 1966) which permitted more accurate monitoring of discrete methylated RNA species. Furthermore, since the specific activity of L-[*methyl*-^{3}H]methionine was found to be relatively constant in control and estrogen-pretreated uteri during a 2-hr *in vitro* pulse (Munns and Katzman, 1971), it seemed reasonable to assume that meaningful determinations of rates of synthesis of methylated RNA species could be ascertained by employing this system. A preliminary report of these studies was presented by Munns and Katzman (1970).

Experimental Procedures

The treatment of animals and tissues, *in vitro* labeling conditions, and procedures for determining RNA have been previously described (Munns and Katzman, 1971). Actinomycin D was donated by Merck and Company.

Extraction of Uterine RNA. Pools of tissues of from 5 to 20 immature rats, depending on the degree of uterine growth, were extracted by treatment with hot phenol–sodium dodecyl sulfate, based on the method of Scherrer and Darnell (1962). Glassware and solutions were autoclaved prior to use and all operations were conducted at 0–4° unless otherwise noted.

Groups of uteri were rinsed and homogenized (Duall ground glass homogenizers) in ice-cold buffer A (0.05 M NaAc–0.001 M EDTA–0.14 M NaCl, pH 5.1) which contained 1% sodium dodecyl sulfate, 10% RNase-free sucrose, and appropriate amounts of bentonite (Fraenkel-Conrat *et al.*, 1961). To the homogenates was added an equal volume of hot (65°) redis-

* From the Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri 63104. *Received May 11, 1971.* Supported by NSF GB-8778 and NASA NGT-26-006-001.

† Author to whom correspondence should be addressed.

[1] MAK represents methylated albumin kieselguhr.

TABLE I: Recovery of Uterine RNA by Hot Phenol–Sodium Dodecyl Sulfate Extraction.[a]

Estradiol pretreatment *in vivo* (hr)	Homogenate RNA/group		Extracted RNA/group		% recovered	
	μg	dpm × 10^{-4}	μg	dpm × 10^{-4}	RNA	dpm
0	604 ± 62	18.2 ± 1.7	315 ± 40	8.4 ± 0.9	52	46
6	686 ± 78	46.7 ± 5.2	389 ± 37	23.7 ± 3.0	57	51
12	947 ± 93	58.1 ± 4.8	538 ± 40	27.8 ± 2.4	57	48
24	1895 ± 158	35.7 ± 3.8	1046 ± 81	17.1 ± 1.5	55	48

[a] Triplicate groups of uterine tissues (5/group) were pulsed *in vitro* with L-[*methyl*-^{3}H]methionine for 1 hr. Aliquots from perchloric acid insoluble hydrolyzable fractions of uterine homogenates and from dialyzed preparations of hot phenol–sodium dodecyl sulfate extracted RNA were used for chemical and radioactivity determinations as described by Munns and Katzman (1971).

tilled phenol containing 0.1% 8-hydroxyquinoline and saturated with buffer A. This mixture was immediately placed in a water bath at 65°, shaken vigorously for 5 min, cooled, and then centrifuged (10,000*g*, 5 min) to separate the phenol and aqueous phases. The aqueous phase was removed and the phenol phase reextracted with an equal volume of buffer A at 4°. The combined aqueous phases were reextracted with phenol (5–10°) in the cold for 15 min and the resultant aqueous phase centrifuged at 20,000*g* for 20 min to remove remaining bentonite and denatured proteins. The supernatant was made 70% (v/v) with respect to ethanol by addition of absolute ethanol containing 2% KAc and stored at −20° for several hours prior to centrifugation (20,000*g*, 10 min). The RNA contained in the pellet was dissolved in buffer B (0.1 M NaCl–0.001 M EDTA, pH 6.2). In some cases, this solution was dialyzed against buffer B for 4–6 hr prior to electrophoresis or further fractionation.

Fractionation and Characterization of Uterine RNA. Approximately 1 mg of unfractionated RNA in 1.0 ml of buffer B was layered on preparative gradients (28-ml capacity, SW 25.1 rotor) of 5–20% sucrose (RNase-free) in buffer B and centrifuged in a Beckman Model L ultracentrifuge for 14 to 16 hr at 24,000 rpm. The gradient tubes were punctured, and the effluent was pumped into a Gilford 2000 spectrophotometer equipped with a flow cell permitting the collection of discrete fractions from which the RNA was precipitated by the addition of ethanol.

Preparations of total and fractionated RNA were characterized by acrylamide and acrylamide–agarose (composite) gel electrophoresis. The EC-470 preparative slab electrophoretic unit (EC Corporation, Philadelphia, Pa.) was used exclusively. Electrophoretic procedures, including buffers, staining solution, preparation of gels, etc., were carried out according to the procedures of Peacock and Dingman (1967) and Dingman and Peacock (1968), unless otherwise noted. Appropriate quantities of RNA, dissolved in 0.1 ml of buffer B containing 5% RNase-free sucrose, were carefully layered into gel slots and after several minutes, the initial amperage of 50 mA was increased to 90 mA (constant) for the duration of electrophoresis. Migration of RNA was monitored with a tracking dye (bromothymol blue), whose migration was similar to that of tRNA; electrophoresis was discontinued after the tracking dye had migrated 7–10 cm.

Identification of discrete electrophoretic bands from unfractionated preparations of RNA was based on comparative migrations of specific RNAs isolated from subcellular preparations. The RNA extracted from a 100,000*g* supernatant identified tRNA (4 S), whereas extraction of polysomes isolated by the method of Wettstein *et al.* (1963), identified rRNAs (28 S and 18 S). The 5S species was identified by its selective release during warm (25°) phenol–sodium dodecyl sulfate extraction of the above polysome fraction (Pene *et al.*, 1968).

A slight contamination of RNA with DNA was detected by minor, purple-staining bands migrating slightly ahead of 18S rRNA. Incubation with DNase (Peacock and Dingman, 1967) resulted in their removal as well as establishing their identity. Lack of significant quantities of released radioactivity during enzyme hydrolysis indicated that methylation of DNA during the initial 24 hr of the estrogen response was negligible.

Assessment of Techniques. The data in Table I show that 50–60% of the RNA orginally present in uterine homogenates (based on chemical determinations) was extracted by hot phenol–sodium dodecyl sulfate treatment. The somewhat smaller recovery (45–50%) of RNA based on radioactivity measurements was anticipated since only 85–90% of the radioactivity released during selective hydrolysis of the perchloric acid insoluble fraction of homogenates was derived from RNA; the remainder representing "hydrolyzed protein" (Munns and Katzman, 1971). On the other hand, contamination of phenol-extracted RNA due to labeled proteins and/or aminoacylated tRNA was quite small, accounting for less than 2% of the extracted radioactivity, as determined by similar recovery studies in uteri pulsed with L-[*carboxyl*-^{14}C]-methionine. The recovery of RNA from uterine tissues was not significantly influenced by estrogen pretreatment (Table I).

The radioactivity associated with RNA in gels was determined by the method of Mayol and Thayer (1970). The gel was cut into equal slices (approximately 1.7 mm) and after incubating each slice at 25° for 24–48 hr in a scintillation vial with 10 ml of (1:10) NCS–organic scintillator, the radioactivity was determined. This treatment completely recovered the isotope applied to the gel (Table II).

Results and Interpretations

Methylation of Uterine RNA. In attempting to identify those RNAs which become methylated during a 2-hr *in vitro* pulse, unfractionated RNA obtained by hot phenol–sodium dodecyl sulfate extraction was electrophoresed on low per cent composite gels. Figure 1 illustrates the resultant RNA stain-

TABLE II: Recovery of Labeled RNA from Acrylamide Gels.[a]

Isotope	dpm applied (10^{-4})	Efficiency (%)	dpm recovered (10^{-4})	Recovery (%)
^{3}H	23.60	32.3	23.34	98.9
^{3}H	4.72	32.3	4.93	104.4
^{14}C	4.34	43.6	4.41	101.7
^{14}C	2.17	43.6	2.09	96.4

[a] After electrophoresis of suitable aliquots of radioactive RNA (labeled with L-[*methyl*-^{14}C or -^{3}H]methionine), the gel was sliced into 1.7-mm sections and the sum of the radioactivity contained in the slices compared to the total radioactivity applied to the gel.

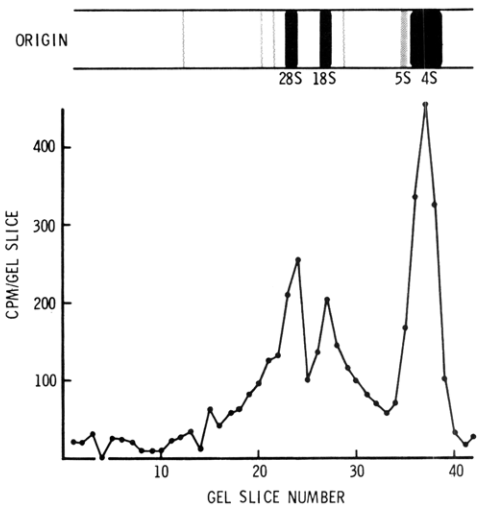

FIGURE 1: A representative radioactivity profile of unfractionated uterine RNA pulse-labeled with L-[*methyl*-^{14}C]methionine for 2 hr *in vitro* and electrophoresed in 1.25% composite gels. The staining pattern of the gel associated with this profile is illustrated at the top of the figure. The RNA was extracted with hot phenol–sodium dodecyl sulfate from unprimed uterine tissues.

ing pattern and accompanying radioactivity profile of such an electrophoregram. Major peaks of radioactivity coincided with each of three major bands of RNA identified as 28 S, 18 S, and 4 S. Smaller amounts of dispersed radioactivity, located on either side of the rRNA bands, were presumed to be either methylated rRNA precursors and/or their breakdown products.

Since low molecular weight RNAs were not adequately resolved in low per cent composite gels, the staining pattern and radioactivity profile of these species were determined on 5% acrylamide gels (Figure 2) after fractionation by sucrose gradient centrifugation. Although a number of discrete bands were present, only the tRNA band (4 S) contained significant amounts of radioactivity. In agreement with the results of others, the low amount of radioactivity contained in the other regions of this gel (Figure 2) suggested that mRNA (Srinivasan and Borek, 1966; Moore, 1966) as well as 5S RNA (Knight and Darnell, 1967; Forget and Weissman, 1968) were not methylated, thus indicating, that the entry of isotope into RNA *via* "1-carbon" metabolism was not appreciable. In support of the above views, preliminary studies of the methylated base patterns of uterine RNA in our laboratory have indicated that the quantity of radioactivity incorporated into unmethylated purines ("1-carbon" metabolism) was insignificant with regard to that incorporated into methylated bases (methylation).

These studies indicate that the methylation of uterine RNA, with respect to both the extent and types of RNA which become methylated during short *in vitro* pulses, is similar to that found in other mammalian systems (Brown and Attardi, 1965; Cooper, 1969a,b). Based on numerous radioactivity profiles of gels of unfractionated uterine RNA obtained after 2-hr pulses *in vitro* (and *in vivo;* unpublished), we have estimated that methylation was more or less equally distributed between rRNA and tRNA (approx 30:20:50 for 28 S:18 S:4 S). Small quantities of other low molecular weight methylated RNA species, although detected, contributed little to the overall methylation profile due to their extremely small populations (Weinberg and Penman, 1968; Zapisek *et al.*, 1969).

Since a number of investigators have reported that newly synthesized RNAs are particularly sensitive to nucleases during extraction (Henshaw *et al.*, 1965; Lazarus and Sporn, 1967), it was necessary to determine a suitable pulse period that would permit the majority of methylated RNAs undergoing synthesis to become processed into more nuclease-resistant, mature forms. To do this, the effect of duration of the *in vitro* pulse on the methylation profile of unfractionated RNA was investigated. Figure 3 illustrates the radioactivity profiles of RNA prepared from uterine tissues pulsed *in vitro* with L-[*methyl*-^{3}H]methionine for 30, 60, and 120 min. The resolution of label associated with rRNA species in preparations obtained after longer incubation was greatly increased, whereas after a 30-min pulse the resolution was insufficient for adequate determination of rRNA radioactivity. Resolution of tRNA, on the other hand, was satisfactory, yielding a prominent tRNA peak of radioactivity in each instance (suggesting that at the time of methylation, precursor tRNAs are more resistant to nuclease activity). The background of dispersed radioactivity which appeared predominately between the 18S to 4S region of the gel and was believed to contain precursor rRNA breakdown products, did not appre-

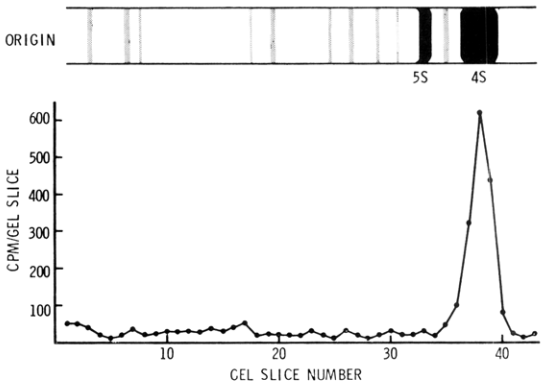

FIGURE 2: A representative radioactivity profile of low molecular weight uterine RNAs pulse-labeled with L-[*methyl*-^{14}C] methionine for 2 hr *in vitro* and electrophoresed on 5% acrylamide gels. The staining pattern of the gel associated with this profile is illustrated at the top of the figure. The labeled RNA was extracted from unprimed uterine tissues by hot phenol–sodium dodecyl sulfate treatment, fractionated by sucrose gradient centrifugation and the low molecular weight RNAs (associated with the 4S peak of the sucrose gradient) electrophoresed.

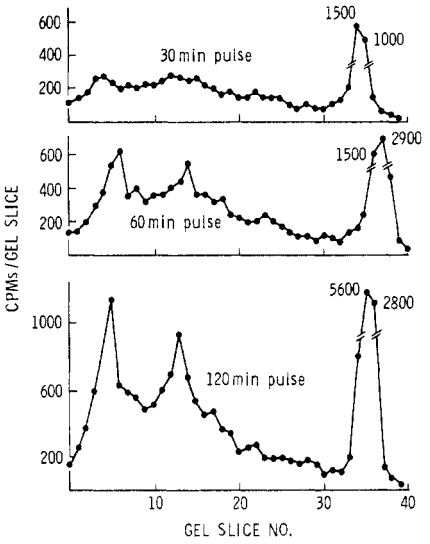

FIGURE 3: The effect of duration of *in vitro* pulse on the methylation profile of total RNA. Unprimed groups of uteri were incubated with L-[*methyl*-^{3}H]methionine for the indicated times and extracted with hot phenol–sodium dodecyl sulfate, and equivalent amounts of the isolated RNA were electrophoresed on 2.8% composite gels. The sum of the radioactivity contained in gel slices 1 through 18 and 32 through 37 were used in determining the extent of methylation of rRNA and tRNA, respectively.

TABLE III: Effects of Actinomycin D on the Methylation of Uterine RNA.[a]

Preincubation (min)[b]	Actinomycin D	Treatment of tissues (% of controls)			
		Unprimed		E_2 6 hr (*in vivo*)	
		rRNA	tRNA	rRNA	tRNA
0	—[c]	100	100	100	100
0	+	8.2	19.8	7.8	24.1
15	+	<1.0	14.1	<1.0	22.0
30	+	<1.0	6.8	<1.0	15.2

[a] Groups of unprimed and 6-hr estrogen-pretreated tissues (5/group) were incubated in the presence (and absence) of 50 μg of actinomycin D for prescribed periods of time prior to the addition of L-[*methyl*-^{3}H]methionine. At this time incubation was continued for an additional 60 min. RNA was isolated by hot phenol–sodium dodecyl sulfate extraction, electrophoresed on 2.8% composite gels and the incorporation of isotope into rRNA and tRNA determined from the appropriate gel slice. The extent of methylation of rRNA and tRNA from groups preincubated with antibiotic is compared to control groups not exposed to antibiotic and expressed as percent controls. Control dpms were similar to those recorded in Table IV. [b] Preincubation period with actinomycin D prior to addition of L-[*methyl*-^{3}H]methionine. [c] Control.

ciably increase during longer *in vitro* pulses. On the other hand, the radioactivity associated with rRNA and tRNA bands progressively increased. Since Greenberg and Penman (1966) have reported that the 45S rRNA precursor becomes methylated at the time of transcription and requires approximately 30 min to be processed into 28S and 18S rRNAs, a considerable portion of the label incorporated into uterine RNA during a 30-min pulse would represent methylated rRNA precursors. During longer *in vitro* pulses, however, proportionately more of the radioactivity would be processed into mature—more nuclease-resistant—ribonucleic acids.

Effect of Actinomycin D on Uterine RNA Methylation. Studies with actinomycin D were conducted to ascertain the relationship between synthesis and methylation of RNA (Table III). If uteri were incubated in the presence of both antibiotic and L-[*methyl*-^{3}H]methionine for 1 hr, the amount of isotope incorporated into rRNA was less than 10% of that which was incorporated into control tissues incubated in the presence of isotope alone. If tissues were preincubated with antibiotic for 15 and 30 min prior to a 1-hr pulse, incorporation of isotope into rRNA was almost totally absent (<1% of controls). Under the same labeling conditions, the incorporation of isotope into transfer ribonucleic acid was markedly reduced, although not completely suppressed. Actinomycin D was equally effective in both estrogen-treated and control uteri.

These results support the views of Greenberg and Penman (1966) and Zimmerman and Holler (1967) that the methylation of rRNA takes place during or immediately after transcription. The small amount of rRNA which was methylated when isotope and antibiotic were added concommitantly, presumably was due to the time required for complete inhibition of rRNA transcription. Reduction in the methylation of tRNA, which was not as immediate as that of rRNA, suggested that an interval existed between tRNA transcription and methylation. Similar results have been reported by Bernhardt and Darnell (1969) for methylation of precursor tRNA in HeLa cells during actinomycin D chases. These results are not unexpected since the tRNA methylases have been shown to reside in the cytoplasm (Burdon *et al.*, 1967; Burdon and Clason, 1969; Mowshowitz, 1969).

Thus, from the above studies it seems reasonable to assume that methylation of uterine RNA occurs on recently synthesized species and that L-[*methyl*-^{3}H]methionine may be a useful precursor for minotoring the synthesis of rRNA and tRNA.

Effects of Estradiol on Uterine rRNA and tRNA Methylation. To assess the effects of estradiol on the rates of methylation of these RNAs, both single- and double-label isotope techniques were employed. The RNA was extracted from uteri which had been primed with estradiol *in vivo* for varying periods of time prior to being pulsed for 2 hr *in vitro* with L-[*methyl*-^{14}C or -^{3}H]methionine. As shown in Table IV and Figure 4, estradiol profoundly affected the rates of methylation of both rRNA and tRNA. The extent of methylation of rRNA and tRNA was assessed by determining the quantity of radioactivity, or ^{3}H/^{14}C ratios, in the appropriate regions of the gel (see Figure 3) and compared to control values as presented in Table IV. These data, as summarized in Figure 4, illustrate the rates of rRNA and tRNA methylation during the course of estrogen action. Wet weight, RNA accumulation, and total RNA methylation (Munns and Katzman, 1971) are included for comparison.

These results indicate that the rates of methylation of rRNA and tRNA increased to the same extent during the first 6 hr after injection of hormone. While the rate of tRNA methylation began to decline after this time, that of rRNA did not become maximal until 10–12 hr, and thereafter declined. The results obtained in the studies utilizing single and double iso-

TABLE IV: Effect of Estradiol on the Methylation of Uterine tRNA and rRNA.[a]

	Single label (^{3}H)				Double label (^{3}H and ^{14}C)			
	tRNA		rRNA		tRNA		rRNA	
Treatment (hr)	dpm[b]	% control	dpm[b]	% control	Ratio	% control	Ratio	% control
E_2 (0)	1.8	100	1.7	100	1.8	100	1.9	100
E_2 (2)	2.6	145	2.7	160				
E_2 (4)	4.5	250	4.2	245				
E_2 (6)	6.0	335	5.9	345	6.2	340	6.7	350
E_2 (10)	5.0	275	6.6	390				
E_2 (12)	4.4	245	6.5	385	4.9	270	7.9	415
E_2 (24)	2.3	130	3.9	230	2.6	145	4.9	260

[a] Following the prescribed estradiol pretreatment *in vivo*, groups of uteri (5–10/group) were incubated in the presence of L-[*methyl*-^{3}H]methionine for 2 hr, extracted with hot phenol–sodium dodecyl sulfate and the RNA electrophoresed on 2.8% composite gels as described in Experimental Procedures. In the double-label experiment, 6-hr estrogen-pretreated tissues (4 groups with 10 uteri/group) were incubated under the same conditions with L-[*methyl*-^{14}C]methionine and combined with various groups of tissues incubated with the ^{3}H isotope prior to RNA extraction (10 [^{14}C]uteri + 5 [^{3}H]uteri). The amount of label or ratio of labels associated with tRNA and rRNA, separated during electrophoresis, was determined from the appropriate gel slices and expressed in terms of dpms/uterus (^{3}H), ratios (^{3}H/^{14}C), and per cent of controls. [b] dpm $\times 10^{-4}$.

topes were in excellent agreement, as was also the case with regard to accounting for the methylation of total RNA by the sum of the methylation of the major methylated species.

Although individual accumulations of rRNA and tRNA were not determined, their increases, as judged by the density of the stain (methylene blue) of their respective bands in gels after electrophoresis, appeared to parallel the accumulation of total RNA (the latter shown in Figure 4).

General Discussion

The results of the present study strongly support the evidence presented by others that an important feature of estrogen action in the rat uterus (Hamilton *et al.*, 1968; Billing *et al.*, 1969c) and the chick oviduct (O'Malley *et al.*, 1968; Dingman *et al.*, 1969) is the increasing rate of synthesis and accumulation of rRNA and tRNA, presumably required to support the increased rate of protein synthesis occurring somewhat later in the response. Whether the rate-limiting step in general and/or specific protein synthesis is associated with alterations in the quantity and/or quality of these RNAs is at present unknown. In this regard, Billing *et al.* (1969a,c) reported only slight increases in mRNA synthesis relative to that of rRNA and tRNA during the first 6 hr of the estrogen response.

It is perhaps significant that the maximum rates of methylation of total RNA and/or rRNA which we have observed to occur approximately 9–12 hr after the estrogen response is initiated, parallel the findings of Teng and Hamilton (1968) with regard to maximum binding of estradiol to uterine chromatin as well as the extent of this binding throughout the initial 18 hr of the response. In other related studies, comparable increases in uterine RNA polymerase activity whose product is rRNA in nature (Hamilton *et al.*, 1968), as well as template activity (Barker and Warren, 1966; Teng and Hamilton, 1968; Church and McCarthy, 1970), have also been reported.

Of considerable interest in this investigation were the observed differences in the rates of methylation of rRNA and tRNA, *i.e.*, maximum methylation of rRNA occurred 4–6 hr later than that of tRNA (Figure 4). We have assumed that these patterns of methylation are primarily the result of differences in rRNA and tRNA synthesis. This assumption is supported by the present study with actinomycin D, indicating that the bulk of RNA methylation occurs on newly synthesized RNA in contrast to hypermethylation of preexisting RNA. However, these studies do not provide information with regard to possible changes in the extent of methylation of newly synthesized RNA. The latter could be brought about by relative shifts in tRNA populations and/or the synthesis of "new" tRNA species possessing different degrees of methylation. In this regard, estrogen-induced alterations in tRNA populations (Yang and Sanadi, 1969; Sharma and Borek, 1970), tRNA methylase activities (Hacker, 1969; Lipshitz-Weisner *et al.*, 1970), and methylase inhibitor levels (Sharma and Borek, 1970) have been reported.

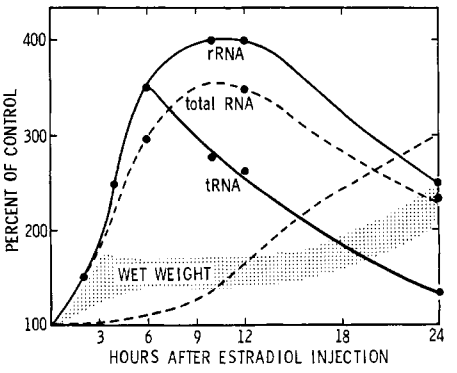

FIGURE 4: A summary of the effects of estradiol administered *in vivo* on the methylation of uterine rRNA, tRNA, and total RNA as determined *in vitro*. The extent of methylation of rRNA and tRNA was determined by acrylamide gel electrophoresis (see Table IV), whereas total RNA methylation was determined by selectively hydrolyzing the perchloric acid insoluble fractions of uterine homogenates as described by Munns and Katzman (1971). Wet weights (hatched area) and RNA accumulation (undesignated dashed line) are presented for reference.

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