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The Synthesis of Ribonucleic Acid in Immature Rat Uterus Responding to Oestradiol-17β

By J. T. KNOWLER and R. M. S. SMELLIE
Institute of Biochemistry, University of Glasgow, Glasgow W.2, U.K.

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Stimulation of incorporation of labelled precursors into the RNA of immature rat uterus is an early result of oestradiol-17β action. However, the extent of the increased incorporation varies with the mode of administration of the labelled precursors and with the weight of the rat. At the age and weight range normally used response is maximal at ten times control incorporation, 4h after the administration of 0.3μg or more of oestradiol-17β. Under these conditions the stimulation of incorporation into the acid-soluble fraction is only 2–2.5-fold. When the purified RNA is separated on polyacrylamide gels the major increase in incorporation of radioactive precursor is found in rRNA and 4S RNA; the formation of the former has been followed from the 45S precursor. Preceding these events by at least 30min, however, is an increase in the incorporation of precursor into RNA species of very high molecular weight, which remained in the first few slices of the gel. The possible significance of these findings is discussed. The increased synthesis of rRNA in response to oestradiol-17β is more strongly inhibited by actinomycin D than the synthesis of other RNA species. Cycloheximide, depending on time of administration and dosage, inhibits either RNA synthesis or the maturation of rRNA.

It is well known that RNA synthesis is stimulated in the responsive tissues of immature or ovariectomized animals after the administration of oestradiol-17β (Gorski, Noteboom & Nicolette, 1965; Hamilton, 1968, 1971; Billing, Barbiroli & Smellie, 1969*b*; Ui & Mueller, 1963). The information accumulated to date has, however, failed to provide a clear picture of the inter-relationship between oestrogen action and RNA synthesis, and workers in the field, together with those studying other steroid hormones, are divided in their opinions. On the one hand there are those who postulate that control by steroid hormones is at the level of the new generation of ribosomes produced or in their specific localization within the cytoplasm (Tata, 1970). Earlier work, however, suggested that the primary action of steroid hormones was the induced synthesis of specific mRNA species and that all other events were secondary to this (Karlson, 1963; Sekeris, 1965). Some evidence to support these ideas continues to accumulate (Hahn, Schjeide & Gorbman, 1969; O'Malley & McGuire, 1969; Church & McCarthy, 1970; Segal, Davidson & Wada, 1965). There is in fact no reason why both postulates should not be valid. Thus the primary response to a steroid hormone could be the synthesis of specific mRNA species, the translation of which might result in the synthesis of specific proteins as observed by Notides & Gorski (1966). These proteins, which might include enzymes such as RNA polymerase, could promote the synthesis of new ribosomes, which in turn could contribute to the control of subsequent differentiation in the responding tissue. The further elucidation of these possibilities requires a more refined investigation of the nature of the RNA species synthesized in response to hormone, and for this reason the present study was begun. Preliminary reports of some of this work have been presented (Knowler & Smellie, 1971*a*,*b*).

MATERIALS AND METHODS

Reagents. Oestradiol-17β, cycloheximide, ribonuclease and deoxyribonuclease (ribonuclease-free) were obtained from Sigma (London) Chemical Co. Ltd., London S.W.6., U.K. Actinomycin D and Pronase were purchased from Calbiochem Ltd., London W1H 1AS, U.K.: the Pronase was self-digested at 37°C for 2h before use. Acrylamide and *NNN'N'*-tetramethylethylenediamine were obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K., and the former was purified as described by Loening (1967). Ethylene diacrylate was purchased from Kodak Ltd., Kirkby, Lancs., U.K. Sodium dodecyl sulphate (specially pure), and bentonite were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K., and bentonite was purified by the method of Fraenkel-Conrat, Singer &

Tsugita (1961). [5-^{3}H]Uridine (5Ci/mmol), [8-^{3}H]-guanosine (500mCi/mmol), [*methyl*-^{14}C]methionine (53.6mCi/mmol) and [4,5-^{3}H]lysine (360mCi/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Toluene scintillator was 0.5% 2,5-diphenyl-oxazole in toluene and in counting gel slices this was mixed with 2-methoxyethanol (3:2, v/v). Dioxan scintillator was 0.7% 2,5-diphenyloxazole and 10% naphthalene in scintillation-grade dioxan.

Experimental animals. The rats, which were derived from the Wistar strain and bred at Glasgow University, were 18–21 days old and in all later experiments were limited to a weight of 25–30g. Unless otherwise noted oestradiol-17β was given in a single intraperitoneal injection in 0.1ml of 0.9% NaCl ('saline') containing 0.5% (v/v) ethanol. Control animals received saline–ethanol only, and when inhibitors were given these were administered in saline without ethanol. Except where stated, radioactive precursors were given intravenously via the lateral tail vein in 0.2ml of saline.

Incubations in vitro. In one experiment RNA was methylated *in vitro*. Uteri were removed from four rats, dissected free of connective tissue and incubated in a 5ml conical flask in 2ml of methionine-free Eagle's medium containing 5μCi of [*methyl*-^{14}C]methionine/ml and 20mM-sodium formate. The flask and media were flushed with O_2+CO_2 (95:5) and incubated for 30 or 60min at 37°C in a shaking water bath. After incubation, the uteri were washed twice in cold saline, blotted dry, frozen and RNA was prepared from them as described below.

Preparation of acid-soluble and acid-insoluble fractions. Acid-soluble and acid-insoluble fractions were prepared by a modification of the method of Billing, Barbiroli & Smellie (1969*a*) from the uteri of rats that had received 10μCi of each of [5-^{3}H]uridine and [8-^{3}H]guanosine 30min before death. Uteri were removed from the animals, washed in saline, placed individually in universal containers and rapidly frozen in a solid-CO_2 bath. They were then either stored for up to 3 days at −60°C or used immediately.

The uteri were thawed, finely chopped with scissors and homogenized in 2.5ml of ice-cold water by using a glass homogenizer with a motor-driven Teflon pestle (Jencons, Hemel Hempstead, Herts., U.K.). All subsequent steps were performed at 0–4°C. The homogenate plus a further 2ml of water used to wash the homogenizer were added to 0.5ml of 50% (w/v) trichloroacetic acid and mixed. After standing for 15min, one-quarter of the homogenate was separated from the remainder and both portions were sedimented at 800*g* for 5min. The supernatant from the smaller fraction was discarded and the pellet put to one side for DNA determination. The supernatant fluid from the larger fraction was retained and the pellet washed with a further 2ml of 5% (w/v) trichloroacetic acid. The washings were added to the supernatant and this constituted the acid-soluble fraction. The pellet was suspended in a small volume of 5% trichloroacetic acid and mixed with 2ml of 2% (w/v) kieselguhr in 5% trichloroacetic acid. A further 2ml of kieselguhr suspension was added to a Millipore filtration unit and the kieselguhr collected as a pad on a 2.5cm-diam. Whatman no. 1 filter-paper disc. The acid-insoluble pellet bound to kieselguhr was collected as a second layer on this pad and washed with 3×15ml portions of 5% trichloroacetic acid, 1×15ml portion of ethanol and 2×15ml portions of diethyl ether. The pad was extracted with 0.5ml of Hyamine hydroxide for 10min at 60°C and the radioactivity measured by scintillation counting in 10ml of toluene scintillator, with efficiencies of 16–18%. The radioactivity in 0.4ml samples of the acid-soluble fraction was counted in 10ml of dioxan scintillator with efficiencies of 18–21%. The pellet put aside for DNA assay was digested in 0.5M-$HClO_4$ at 70°C for 1h and the DNA estimated by the method of Burton (1956).

Preparation and electrophoresis of RNA. RNA was prepared from the uteri of four to eight animals, each of which had previously received an intravenous injection of 125μCi of each of [5-^{3}H]uridine and [8-^{3}H]guanosine at different times before death. For the isolation of high-molecular-weight RNA species the method of Joel & Hagerman (1969) was used, except that the precipitations, originally in the method of Di Girolamo, Henshaw & Hiatt (1964) and which were designed to remove DNA oligonucleotides, were shortened to 1½h. In our hands, these precipitations, whether for 1½ or 16h, resulted in substantial losses of 4S RNA. In preparing RNA for separation of the smaller species of RNA on 7.5% polyacrylamide gels, therefore, these precipitations, together with the deoxyribonuclease treatment, were omitted. Instead the initial ethanol precipitate was dissolved in 1mM-$MgCl_2$ and the high-molecular-weight nucleic acids were precipitated with 1M-NaCl in the presence of 1mg of bentonite/ml at 0°C for 4–6h. The precipitate was removed by sedimentation at 800*g* and the RNA remaining in the supernatant was recovered by precipitation with 2vol. of ethanol at −20°C overnight. The removal of high-molecular-weight RNA and DNA from low-molecular-weight RNA by this procedure was not very efficient, but was sufficient to avoid overloading the gels.

Purified RNA was separated on 2.7 and 7.5% polyacrylamide gels as described by Loening (1967, 1969), except that bisacrylamide was replaced by ethylene diacrylate at 0.25 and 0.125% in 2.7 and 7.5% gels respectively. After electrophoresis at 5mA/gel the gels were scanned at 260nm with a gel-scanning attachment for the Gilford 240 spectrophotometer. They were then sliced into 1mm sections by using a Mickle gel slicer and the individual slices were digested in vials with 0.5ml of aq. 2M-NH_3 soln. at 50°C. After evaporation to dryness, the residues were taken up in 0.3ml of water and left for 30–60min to allow the gel residue to swell and the RNA digest to dissolve. Radioactivity was then counted in 10ml of toluene 2-methoxyethanol-based scintillator with efficiencies of 16–18%.

RESULTS

Effects of injection route and weight of rats on response to oestradiol. In most of the experiments that have been reported on the stimulation of RNA synthesis in rat uterus both hormone and radioactive precursor have been injected intraperitoneally and large variations in the responses have been found in replicate animals (Hamilton, 1964; Greenman, 1970). We have compared the responses of animals receiving precursor and hormone by various injection routes and have found that the

mode of administration of the labelled precursors can exert a marked influence on their incorporation into uterine RNA, although the route of administration of hormone has little effect upon the response obtained.

When the labelled ribonucleosides were injected intraperitoneally, the response to oestradiol given 2h previously was comparatively low (2–3.5-fold increase in incorporation into RNA) and very variable, and the increase in labelling of the acid-soluble fraction was of the same order (2–2.5-fold). Intravenous injection of the precursors gave slightly lower values for total radioactivity in both the acid-soluble and acid-insoluble fractions but oestradiol treatment consistently produced an 8–11-fold increase in incorporation into RNA whereas the increase in labelling of the acid-soluble fraction remained at 2–2.5-fold. Furthermore variation between duplicate animals was greatly diminished.

When the precursors were administered subcutaneously, the total uptake into RNA was low, and although the response to oestradiol (approx. 4-fold) was greater than that obtained by using the intraperitoneal route, the variation between animals was high. Similar results have been obtained in mouse uterus by Miller & Emmens (1967) and, since the completion of the present work, Greenman (1970) has compared subcutaneous and intraperitoneal administration of the RNA precursors without, however, demonstrating any response to hormone.

By using the intravenous route for administration of labelled ribonucleosides we have observed that the maximum increase in labelling of uterine RNA in response to oestradiol occurs at a dose of approx. 0.3 μg/rat, and that even at concentrations as low as 0.01 μg/rat there is a fourfold increase in uptake into the acid-insoluble fraction.

We have found that variations in rat weight also have a marked effect on the response to oestradiol and this can be important because, depending on litter size, an 18–21-days-old rat can weigh from 20 to 40g. In experiments with 18–21-days-old rats selected into groups according to weight the uptake of precursors into RNA was measured at various times after the injection of 1 μg of oestradiol. The greatest response in all groups occurred approx. 4h after treatment and amounted to 12-fold for 20–25g rats, 10-fold for 25–30g rats, 7.5-fold for 30–35g rats and 6-fold for 35–40g rats. For all weight ranges the increase in labelling of the acid-soluble fraction in response to oestradiol was similar, with a peak at 2–2.6 times the control value at 4h.

Oestradiol stimulation of the synthesis of high-molecular-weight RNA species. The initial extraction of RNA by the method of Joel & Hagerman (1969) gave an 80–85% recovery but it was heavily contaminated with DNA, which interfered with the separation of RNA on polyacrylamide gels. The purification procedure employed completely removes all DNA detectable by the diphenylamine reaction but also results in losses of RNA, particularly of low-molecular-weight species. Comparison of polyacrylamide gel separations of the high-molecular-weight RNA before and after purification, however, showed similar patterns of radioactivity distribution, though the purification appeared to result in the loss of some of the RNA of very high molecular weight, which remained at the gel origin. It could be, however, that the higher rate of labelling in this peak in impure preparations reflects non-specific binding of RNA to the DNA remaining at the gel origin.

RNA from the uteri of 18–21-days-old rats weighing 25–30g was separated for 5h on 2.7% polyacrylamide gels. Fig. 1 shows the effect of oestradiol on the incorporation of tritiated ribonucleosides into the RNA species at various times after oestradiol treatment and 15min after administration of labelled ribonucleosides. Incorporation of precursor into control animals was low and confined to species of very high molecular weight. At 1h after hormone treatment there was a marked increase in the incorporation into the RNA species that remained in the first two to three slices of the gel. This early response was also observed after 30 and 45min but not after 15min of hormone treatment. At 2 and 4h after hormone treatment the incorporation into these species was greatly increased; there was also a strongly labelled peak corresponding to 45S rRNA precursor and a pronounced peak corresponding to the 32S rRNA precursor. The former of these two peaks was not always clearly separated from the radioactivity peak corresponding to higher-molecular-weight species.

When the labelled ribonucleosides were administered 30min before death a similar sequence of results was obtained except that after the longer pulse much of the radioactivity had moved from the 45S rRNA precursor into the 32S species, and incorporation was also apparent in the RNA species of the ribosomal subunits. The results of some of these experiments, performed 4h after hormone treatment, are included in Fig. 3, in which the 45S and 32S rRNA precursor peaks are particularly well defined, and in Fig. 5(*a*). With this pulse length, too, the earliest increases were found in the RNA species close to the gel origin.

Fig. 2 shows distribution of radioactivity in RNA species of rat uterus 1h after administration of the precursors. Again after 1h of hormone treatment the increased incorporation is apparent in the RNA species remaining in the first few slices of the gel.

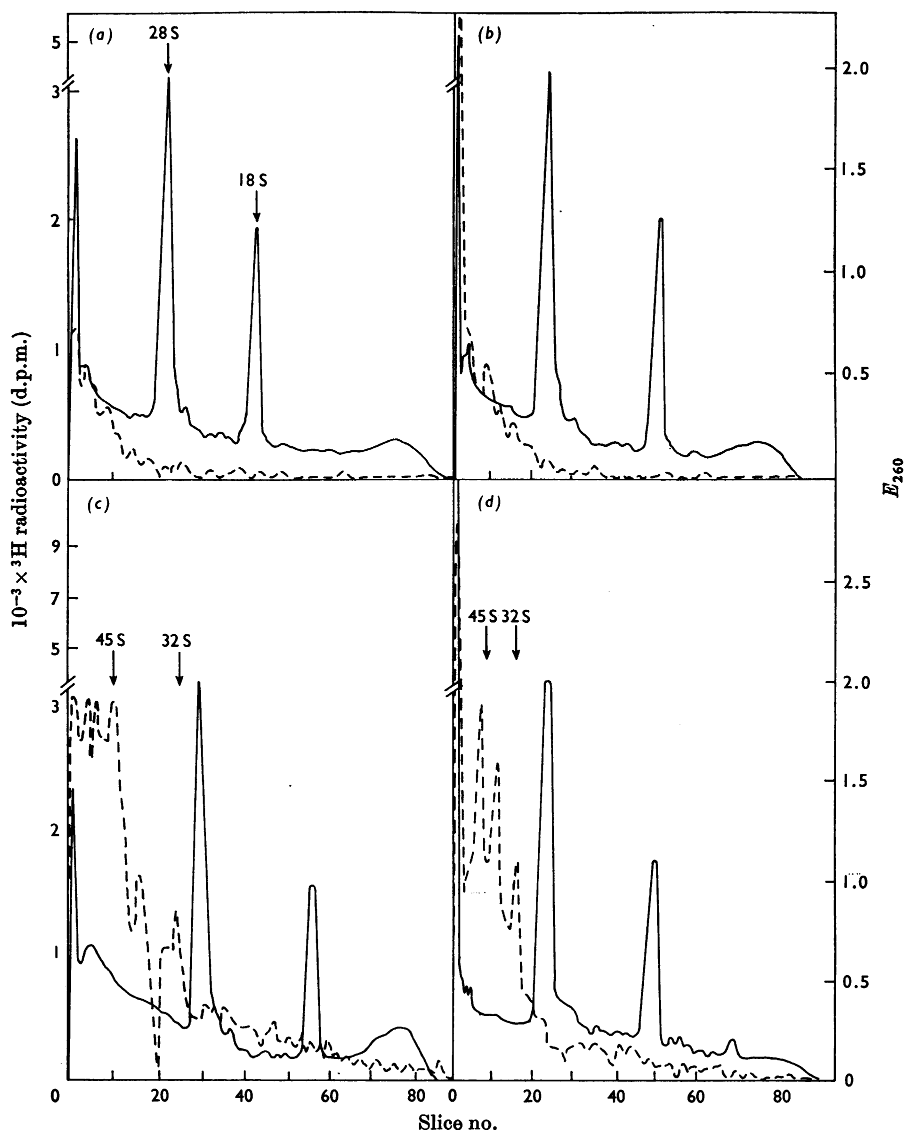

Fig. 1. Incorporation into RNA of radioactive precursor administered 15 min before death. To 18–21-days-old rats weighing 25–30 g 1 μg of oestradiol was given by intraperitoneal injection and 125 μCi of each of [5-^{3}H]-uridine and [8-^{3}H]guanosine was injected intravenously. Purified uterine RNA was separated for 5 h in 2.7% polyacrylamide gels. (*a*) Control; (*b*) 1 h after oestradiol-17β; (*c*) 2 h after oestradiol-17β; (*d*) 4 h after oestradiol-17β. ——, E_{260}; ----, radioactivity per slice (d.p.m.).

After 2 and 4 h of hormone treatment, incorporation into the ribosomal subunit species is strongly stimulated with incorporation into the pre-ribosomal species still apparent.

Further characterization of the RNA species of higher molecular weight. From Figs. 1 and 2 and from the ^{3}H radioactivity profile in Fig. 3 it can be seen that with increasing time after precursor administration the radioactivity in the RNA appears to move from a peak, assumed to be 45 S RNA, through a second peak assumed to be 32 S RNA and finally into the rRNA species. The identities of the 45 S and 32 S RNA peaks have been confirmed by methylation experiments and by

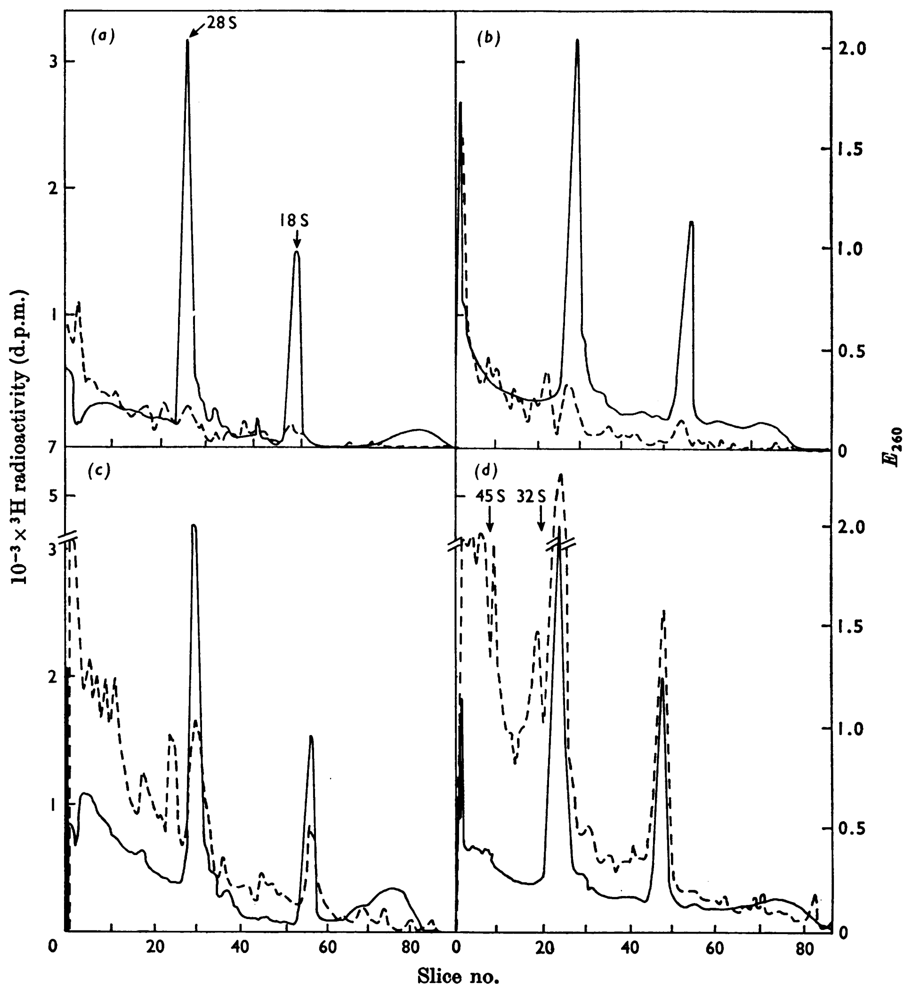

Fig. 2. Incorporation into RNA of radioactive precursor administered 1h before death. For experimental details see the legend to Fig. 1.

cofractionation with known samples of ^{14}C-labelled 45 S and 32 S RNA prepared from HeLa cell nucleoli, kindly supplied by Dr B. E. H. Maden.

In the methylation experiments one sample of ^{3}H-labelled RNA was prepared in the usual way, 30min after precursor administration, from rats treated with oestradiol 4h before killing. Another sample of RNA was prepared after labelling of the uteri with [*methyl*-^{14}C]methionine *in vitro* as described in the Materials and Methods section, 4h after oestradiol treatment of the rats.

The ^{3}H- and ^{14}C-labelled RNA samples were mixed and fractionated on 2.7% polyacrylamide gels for 5h. Fig. 3(*a*) shows the distribution of ^{3}H and ^{14}C in the various RNA species when the labelling with [*methyl*-^{14}C]methionine *in vitro* had been carried out for 30min and Fig. 3(*b*) shows the results obtained after 1h of labelling with [^{14}C]-methionine *in vitro*. In Fig. 3(*a*) the RNA in the 45 S, 32 S and 18 S peaks is methylated whereas the 28 S peak contains little ^{14}C. Fig. 3(*b*) shows that after 1h a substantial amount of methylation of 28 S RNA has taken place and that the 18 S peak is still more heavily labelled. This sequence of methylation fits in with the rate of rRNA synthesis observed in these experiments and with the now accepted sequence of events in the formation of rRNA (Scherrer, Latham & Darnell, 1963; Soeiro, Birnboim & Darnell, 1966; Greenberg & Penman, 1966). The high-molecular-weight RNA close to

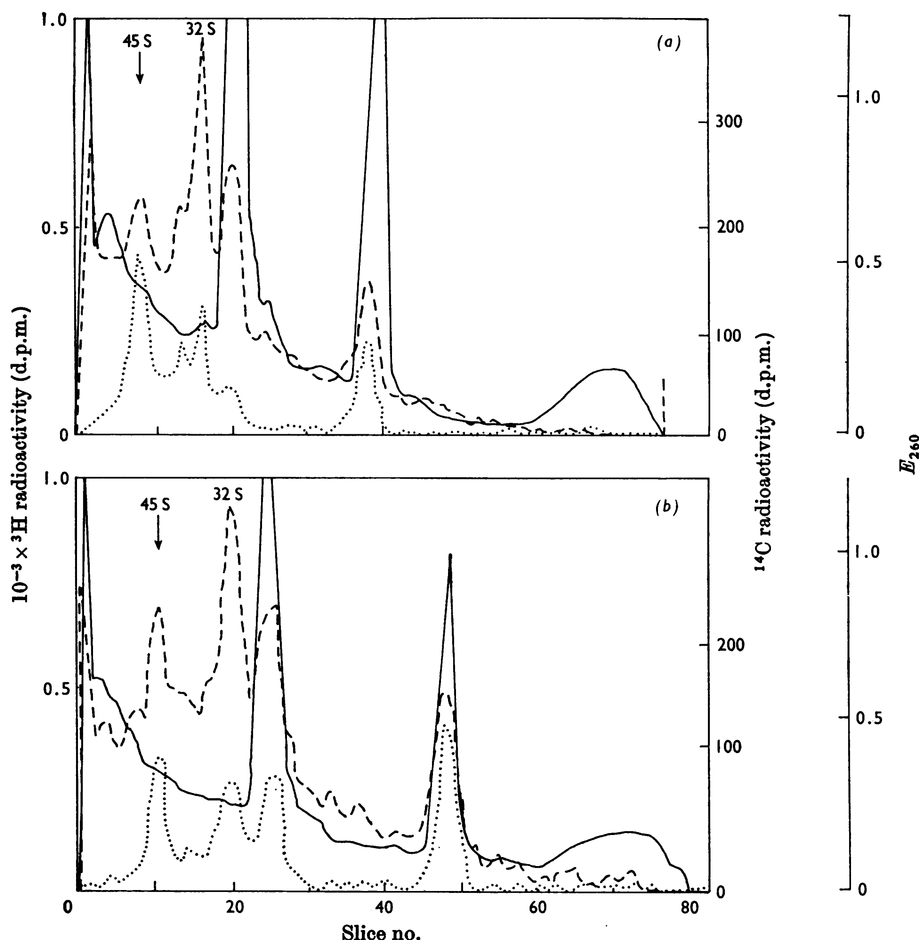

Fig. 3. Methylation of uterine RNA. To 18–21-days-old rats weighing 25–30g 1 μg of oestradiol was given by intraperitoneal injection 4h before death. The uteri were either labelled *in vivo* with a 30min pulse of 125 μCi of each of [5-^{3}H]uridine and [8-^{3}H]guanosine or were incubated *in vitro* with 5 μCi of [*methyl*-^{14}C]methionine/ml. The purified RNA samples were mixed and separated on 2.7% acrylamide gels for 5h. (*a*) Labelled *in vitro* for 30min; (*b*) labelled *in vitro* for 60min. ——, E_{260}; ----, ^{3}H radioactivity; ·····, ^{14}C radioactivity.

the gel origin does not appear to be methylated either at 30min or 1h and it should be noted that, although these species are highly susceptible to attack by ribonuclease, they are not affected by Pronase.

In the cofractionation experiments, ^{3}H-labelled uterine RNA and ^{14}C-labelled 45S and 32S RNA samples from HeLa cell nucleoli were mixed and separated on 2.7% polyacrylamide gels. The ^{14}C-labelled 45S and 32S RNA peaks corresponded with the ^{3}H-labelled peaks from the uterine RNA.

The effect of treatment with actinomycin D on the oestrogen-stimulated incorporation of radioactive ribonucleosides into immature rat uterus is shown in Fig. 4. At a dose of 15 μg the inhibitor caused partial inhibition of synthesis of rRNA species and rather less-marked inhibition of synthesis of the high-molecular-weight RNA. At a dose of 30 μg/rat rRNA synthesis was almost totally eliminated, whereas the synthesis of the RNA of very high molecular weight remaining near the gel origin was only partially inhibited and even at doses of actinomycin D as high as 120 μg/rat there was still some incorporation into this fraction although rRNA synthesis was completely suppressed.

Fig. 5 shows the effect of cycloheximide (600 μg/rat) on synthesis of high-molecular-weight RNA promoted by oestradiol. Depending on the time-interval between administration of inhibitor and

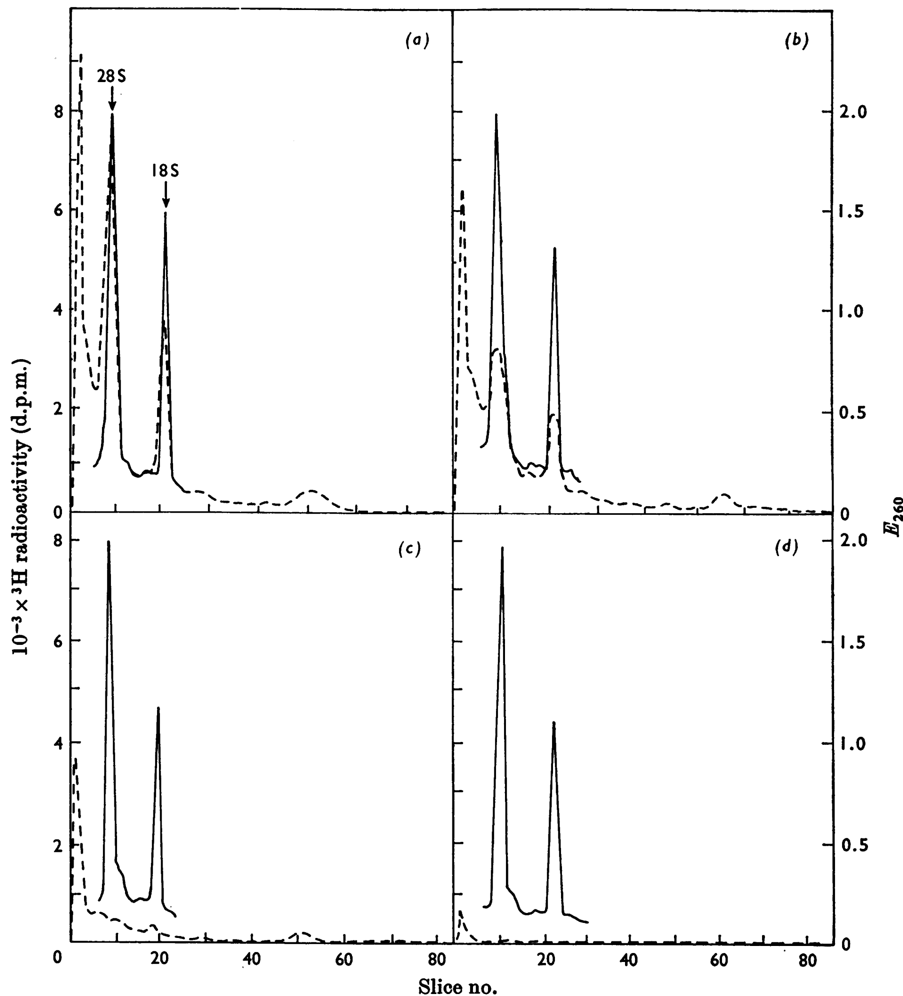

Fig. 4. Effect of actinomycin D on oestradiol-17β-stimulated RNA synthesis. To 18–21-days-old rats weighing 25–30g 1μg of oestradiol-17β and actinomycin D were administered simultaneously 4h before death, and 125 μCi of each of [5-^{3}H]uridine and [8-^{3}H]guanosine was administered 1h before death. Purified RNA was separated on 2.7% polyacrylamide gels for $2\frac{1}{4}$h. (*a*) No actinomycin D; (*b*) 15μg of actinomycin D/rat; (*c*) 30μg of actinomycin D/rat; (*d*) 120μg of actinomycin D/rat. ——, E_{260}; ----, radioactivity per slice (d.p.m.). For reasons of clarity the E_{260} trace is limited to that corresponding to the ribosomal subunit species.

precursor this concentration of cycloheximide brought about a 96–98% inhibition of the incorporation of [4,5-^{3}H]lysine into acid-insoluble protein. When given 1h before oestradiol, cycloheximide severely curtailed the incorporation of radioactivity into all RNA species. Administration of cycloheximide simultaneously with oestradiol strongly inhibited incorporation into rRNA and the 45S and 32S RNA peaks but the material of very high molecular weight at the gel origin was affected much less. When the inhibitor was given 1h after oestradiol, the principal effect appeared to be inhibition of maturation of rRNA. Thus after a 30min pulse of radioactivity, when the 32S RNA peak should be most strongly labelled and the 28S and 18S RNA peaks should also be labelled, only the 45S RNA peak showed marked incorporation.

When 60μg of cycloheximide/rat was used the effect on ribosome maturation was observed at all the above-mentioned administration times. The inhibition by cycloheximide of the maturation of ribosomes has been recorded by Willems, Penman & Penman (1969) in HeLa cells and by Craig & Perry (1970) in L cells.

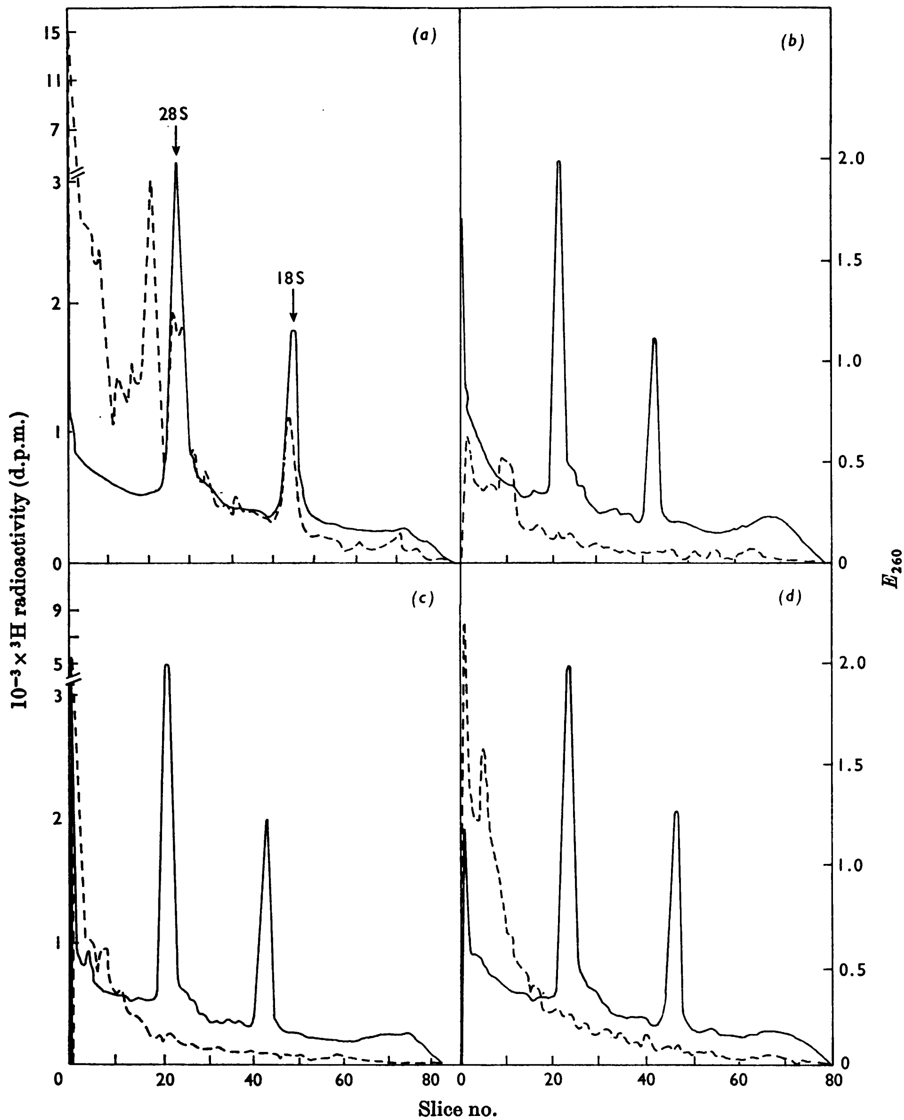

Fig. 5. Effect of cycloheximide inhibition of protein synthesis on oestradiol-17β-stimulated RNA synthesis. To 18–21-days-old rats weighing 25–30g were administered 1μg of oestradiol 4h before death and 600μg of cycloheximide at various times. Both were given by intraperitoneal injection but 125μCi of each of [5-^{3}H]-uridine and [8-^{3}H]guanosine was injected intravenously 30min before death. (*a*) No cycloheximide treatment; (*b*) cycloheximide given 1h before hormone; (*c*) cycloheximide given simultaneously with hormone; (*d*) cycloheximide given 1h after hormone. ——, E_{260}; ----, radioactivity per slice (d.p.m.).

Oestradiol stimulation of the synthesis of RNA species of low molecular weight. Fig. 6 shows the effect of oestradiol-17β treatment of rats on the incorporation of tritiated ribonucleosides into 4S and 5S RNA separated on 7.5% polyacrylamide gels. Compared with controls, incorporation is slightly enhanced after 1h and strongly stimulated after 2 and 4h of oestradiol treatment.

It was found that the synthesis of 4S and 5S RNA was considerably more resistant to inhibition by actinomycin D than was the synthesis of rRNA; some incorporation into 4S RNA occurred at inhibitor concentrations of 120μg/rat.

Cycloheximide at 600μg/rat markedly inhibited the incorporation of radioactive ribonucleosides into 4S and 5S RNA separated on 7.5% gels. When

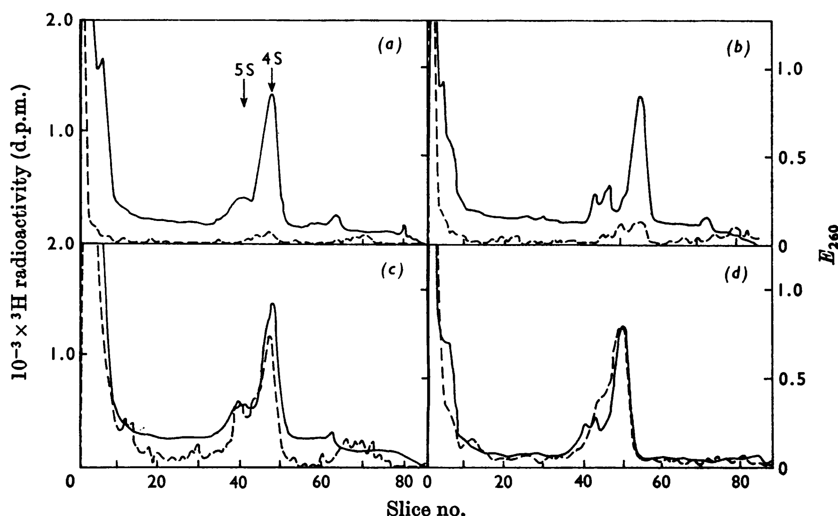

Fig. 6. Electrophoretic separation of low-molecular-weight RNA species on 7.5% polyacrylamide gels. RNA was isolated as described in the Materials and Methods section from 18–21-days-old rats weighing 25–30g. Oestradiol-17β (1μg) was injected intraperitoneally and 125μCi of each of [5-^{3}H]uridine and [8-^{3}H]guanosine was given intravenously 30min before death. (a) Control; (b) 1h after oestradiol-17β; (c) 2h after oestradiol-17β; (d) 4h after oestradiol-17β. ——, E_{260}; ----, radioactivity per slice (d.p.m.).

given 1h before or 1h after hormone, incorporation was decreased to 16 and 27% of the uninhibited value respectively.

DISCUSSION

The results in Figs. 1, 2 and 6 show that the incorporation of radioactive precursors into all species of RNA from immature rat uterus, detected by electrophoresis in 2.7 and 7.5% polyacrylamide gels, is stimulated by the intraperitoneal injection of oestradiol-17β at 1μg/rat. When the intravenous route for injection of labelled precursors is employed the increase in labelling of RNA species is very much greater than the increase in labelling of the acid-soluble nucleotide pools and it seems likely therefore that the increased incorporation into RNA represents a real increase in RNA synthesis.

The time-course of the response is different for different species of RNA. Thus increases in synthesis of rRNA and even of the 45S and 32S rRNA precursors is not striking until a relatively late stage (2–4h) after hormone treatment, and this finding is in agreement with the observations of Teng & Hamilton (1967) on the accumulation of ribosomes in the cytoplasm of ovariectomized rats after treatment with oestradiol and with our own previous observations (Billing *et al.* 1969*b*).

The increased synthesis of tRNA (Fig. 6) may precede slightly the increase in rRNA synthesis (Figs. 1 and 2), since the labelling of tRNA is clearly elevated even at 1h after hormone treatment. Synthesis of both 4S and 5S RNA, however, is even more sharply increased at 2 and 4h, as is the case with rRNA and the 45S and 32S RNA species.

Preceding these changes in rRNA and tRNA synthesis by at least 30min, however, is a very marked increase in the synthesis of RNA species of higher molecular weight than the 45S rRNA precursor. These species of RNA do not appear to be methylated and their fractionation behaviour is not affected by Pronase. In view of this it seems likely that they correspond to heterogeneous nuclear RNA species, but this remains to be confirmed.

The function of heterogeneous nuclear RNA is not fully understood, but some evidence exists that, although much of it never leaves the nucleus, it does contain sequences that eventually function as messengers. The hybridization studies of Soeiro & Darnell (1970) and particularly those of Lindberg & Darnell (1970), showing that virus-specific RNA is found in both the heterogeneous nuclear RNA and the cytoplasmic mRNA of cells transformed by the oncogenic virus SV40, support this conclusion. It should be mentioned, however, that contradictory evidence does exist in the kinetic experiments by Penman, Vesco & Penman (1968) and in the studies of cordycepin inhibition by Penman, Rosbash & Penman (1970).

There is a considerable body of evidence to

support the view that oestrogen first stimulates the synthesis of specific mRNA species, that these then bring about the synthesis of a small number of proteins and that only then are the general increases in ribosome synthesis and the subsequent total protein synthesis and cell growth brought about. Thus Hamilton, Widnell & Tata (1965) found that a very early response to hormone in ovariectomized rats was the increased synthesis of uterine nuclear RNA. The kinetics of the formation of this RNA did not indicate that it was ribosomal precursor. Notides & Gorski (1966) observed the synthesis of specific protein in the immature rat uterus 40 min after oestradiol administration. This response clearly precedes the increased synthesis of ribosomes and it had already been shown that the oestrogen-dependent increases in the synthesis of rRNA and in the activity of RNA polymerase were dependent on protein synthesis (Noteboom & Gorski, 1963; Gorski & Axman, 1964). The cycloheximide-inhibition experiments presented above further underline the importance of protein synthesis, though not necessarily oestradiol-induced protein synthesis, for the increased production of ribosomes in response to the hormone.

De Angelo & Gorski (1970) have shown that the synthesis of the specific protein of Notides & Gorski (1966) is dependent on RNA synthesis and it is noteworthy that they found that very high concentrations of actinomycin D were necessary to inhibit its synthesis totally. Treatment with 4 mg of inhibitor/kg only decreased synthesis to 23% and it has been seen that a similar dosage in our experiments (120 μg in 25–30 g rats) did not completely inhibit the synthesis of the RNA of very high molecular weight remaining at the gel origin.

It is tempting then to speculate that the early increase in synthesis of species of RNA of very high molecular weight (>45 S) in rat uteri responding to oestradiol could reflect synthesis of new mRNA species, and that the translation of these into protein could be a prerequisite of increases in rRNA synthesis.

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REFERENCES

Billing, R. J., Barbiroli, B. & Smellie, R. M. S. (1969*a*). *Biochim. biophys. Acta*, **190**, 52.

Billing, R. J., Barbiroli, B. & Smellie, R. M. S. (1969*b*). *Biochem. J.* **112**, 563.

Burton, K. (1956). *Biochem. J.* **62**, 315.

Church, R. B. & McCarthy, B. J. (1970). *Biochim. biophys. Acta*, **199**, 103.

Craig, N. C. & Perry, R. P. (1970). *J. Cell Biol.* **45**, 554.

De Angelo, A. B. & Gorski, J. (1970). *Proc. natn. Acad. Sci. U.S.A.* **66**, 693.

Di Girolamo, A., Henshaw, E. C. & Hiatt, H. H. (1964). *J. molec. Biol.* **8**, 479.

Fraenkel-Conrat, H., Singer, B. & Tsugita, A. (1961). *Virology*, **14**, 54.

Gorski, J. & Axman, M. C. (1964). *Archs Biochem. Biophys.* **105**, 517.

Gorski, J., Noteboom, W. D. & Nicolette, J. A. (1965). *J. cell. comp. Physiol.* **66** (Suppl. 1), 91.

Greenberg, H. & Penman, S. (1966). *J. molec. Biol.* **21**, 527.

Greenman, D. L. (1970). *Endocrinology*, **87**, 716.

Hahn, W. E., Schjeide, D. A. & Gorbman, A. (1969). *Proc. natn. Acad. Sci. U.S.A.* **62**, 112.

Hamilton, T. H. (1964). *Proc. natn. Acad. Sci. U.S.A.* **51**, 83.

Hamilton, T. H. (1968). *Science, N.Y.*, **161**, 649.

Hamilton, T. H. (1971). In *The Biochemistry of Steroid Hormone Action*, p. 49. Ed. by Smellie, R. M. S. London: Academic Press (Inc.) Ltd.

Hamilton, T. H., Widnell, C. C. & Tata, J. R. (1965). *Biochim. biophys. Acta*, **108**, 118.

Joel, P. B. & Hagerman, D. D. (1969). *Biochim. biophys. Acta*, **195**, 328.

Karlson, P. (1963). *Angew. Chem.* **2**, 175.

Knowler, J. T. & Smellie, R. M. S. (1971*a*). *Biochem. J.* **123**, 33 P.

Knowler, J. T. & Smellie, R. M. S. (1971*b*). *Biochem. J.* **123**, 33 P.

Lindberg, U. & Darnell, J. E. (1970). *Proc. natn. Acad. Sci. U.S.A.* **65**, 1089.

Loening, U. E. (1967). *Biochem. J.* **102**, 251.

Loening, U. E. (1969). *Biochem. J.* **113**, 131.

Miller, B. G. & Emmens, C. W. (1967). *J. Endocr.* **39**, 473.

Noteboom, W. D. & Gorski, J. (1963). *Proc. natn. Acad. Sci. U.S.A.* **50**, 250.

Notides, A. & Gorski, J. (1966). *Proc. natn. Acad. Sci. U.S.A.* **56**, 230.

O'Malley, B. W. & McGuire, W. L. (1969). *Endocrinology*, **84**, 63.

Penman, S., Rosbash, M. & Penman, M. (1970). *Proc. natn. Acad. Sci. U.S.A.* **67**, 1878.

Penman, S., Vesco, C. & Penman, M. (1968). *J. molec. Biol.* **34**, 49.

Scherrer, K., Latham, H. & Darnell, J. E. (1963). *Proc. natn. Acad. Sci. U.S.A.* **49**, 240.

Segal, S. J., Davidson, D. W. & Wada, K. (1965). *Proc. natn. Acad. Sci. U.S.A.* **54**, 782.

Sekeris, C. E. (1965). In *Mechanism of Hormone Action*, p. 149. Ed. by Karlson, P. Stuttgart: G. Thieme, Verlag.

Soeiro, R., Birnboim, H. C. & Darnell, J. E. (1966). *J. molec. Biol.* **19**, 362.

Soeiro, R. & Darnell, J. E (1970). *J. Cell Biol.* **44**, 467.

Tata, J. R. (1970). In *Biochemical Actions of Hormones*, p. 89. Ed. by Litwack, G. New York: Academic Press Inc.

Teng, C.-S. & Hamilton, T. H. (1967). *Biochem. J.* **105**, 1101.

Ui, H. & Mueller, G. C. (1963). *Proc. natn. Acad. Sci. U.S.A.* **50**, 256.

Willems, M., Penman, M. & Penman, S. (1969). *J. Cell Biol.* **41**, 177.