

Changes in the Patterns of Synthesis of Ribonucleic Acid Species in Immature Rat Uterus in Response to Oestradiol-17 β

By R. J. BILLING, B. BARBIROLI AND R. M. S. SMELLIE
Institute of Biochemistry, University of Glasgow, Glasgow W.2

(Received 2 December 1968)

1. After treatment of immature rats with diethylstilboestrol, the wet weight and RNA content of uterine tissue increased rapidly, reaching a peak at 40 hr. After an initial lag of a few hours, the acid-soluble ribose and protein contents also rose to maxima at 40 hr. No increase in DNA content occurred until at least 24 hr. after treatment. 2. The RNA from immature rat uterus isolated at various times up to 6 hr. after administration of oestradiol-17 β was labelled by injecting [^3H]uridine and [^3H]guanosine intraperitoneally 30 min. before the animals were killed. It was fractionated on columns of kieselguhr coated with methylated serum albumin and the radioactivity in fractions corresponding to transfer RNA, 7s RNA, ribosomal RNA, Q₁-RNA, Q₂-RNA and DNA-like RNA was determined. 3. The radioactivity of the whole RNA increased steadily for 6 hr. after hormone treatment. The earliest changes occurred in the Q₁-RNA (ribosomal RNA precursor), whereas at longer time-intervals the radioactivity of the ribosomal RNA, 7s RNA and transfer RNA increased by four- to five-fold. The radioactivity of the DNA-like RNA increased by about 50%, but only at the longer time-intervals. 4. It is concluded that one of the earliest changes in response to oestradiol treatment is a major increase in synthesis of ribosomal RNA followed later by a similar increase in synthesis of transfer RNA and by a much smaller increase in synthesis of DNA-like RNA. The change in synthesis of ribosomal RNA in immature rat uterus may represent one of the most important responses to oestradiol treatment.

It has been shown that the biological action of oestrogens in immature rat uterus is preceded by increased uptake of labelled precursors into RNA (Gorski & Nicolette, 1963) and that the DNA-dependent RNA polymerase (EC 2.7.7.6) activity of the uterus also increases (Gorski, 1964; Nicolette & Mueller, 1966). There is also evidence that increased labelling of RNA occurs in the uterus of the adult ovariectomized rat in response to oestrogen treatment (Means & Hamilton, 1966; Teng & Hamilton, 1967).

Although there is indirect evidence that the content of r-RNA* may be increased after oestrogen action (Hamilton, Widnell & Tata, 1968*b*; Hamilton, Teng & Means, 1968*a*), the changes in the full range of uterine RNA, especially the DNA-like RNA, have not been investigated in detail. In the present study, the RNA synthesized in immature rat uterus at times up to 6 hr. after hormone administration was fractionated on MAK columns and the uptake of labelled precursors into the RNA fractions thus obtained was measured.

* Abbreviations: r-RNA, ribosomal RNA; t-RNA, transfer RNA; m-RNA, messenger RNA; MAK, kieselguhr coated with methylated serum albumin; SDS, sodium dodecyl sulphate.

MATERIALS AND METHODS

[5- ^3H]Uridine (24 c/m-mole), [2- ^{14}C]uridine (53 mc/m-mole) and [8- ^3H]guanosine (4.76 c/m-mole) were purchased from The Radiochemical Centre, Amersham, Bucks.

Bovine serum albumin (fraction V) was obtained from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex. Highly polymerized yeast RNA for use as carrier was purchased from British Drug Houses Ltd., Poole, Dorset.

Biological methods. Three-week-old immature female albino rats of the Wistar strain, weighing 35–40 g., were used in all experiments. The rats were killed by cervical dislocation; the uteri were removed, cleaned of mesentery, washed with cold 0.9% NaCl and weighed after being blotted with absorbent tissue.

In those experiments in which the chemical composition of the uteri was determined, the rats received 5 μg . of diethylstilboestrol by intraperitoneal injection at various times before being killed.

In the experiments with radioactive precursors, 1 μg . of the sodium salt of oestradiol-17 β (Roberts & Szego, 1947) in 0.1 ml. of 0.9% NaCl was administered intraperitoneally at zero time to test animals, and the controls received 0.1 ml. of 0.9% NaCl. At 30 min. before death all rats received 25 μC of [5- ^3H]uridine and 25 μC of [8- ^3H]guanosine intraperitoneally in 0.2 ml. of 0.9% NaCl. Experiments were also performed with doses of oestradiol-17 β down to 0.1 μg ./rat, when the changes in labelling of RNA were the same as those observed with 1 μg . of oestradiol. The main

series of experiments was conducted with the larger amount of hormone, since other workers in this field have used doses of 5 μg . (Gorski & Nicolette, 1963; Nicolette & Mueller, 1966) or 10 μg . (Hamilton *et al.* 1968b).

Determination of RNA, DNA, protein and acid-soluble ribose. In these experiments individual uteri were homogenized in 4 ml. of cold water at full speed for 1 min. in a Silverson Vortex homogenizer (Silverson Machines Ltd., London S.E.1). Then 0.45 ml. of 2 M-HClO₄ was added to the homogenate, giving a final concentration of 0.2 M, and after standing on ice for 15 min. the mixture was centrifuged at 600 g for 5 min. The supernatant fluid was decanted into a clean tube and designated the acid-soluble fraction. The sediment was suspended in 2.0 ml. of water, and 1.0 ml. of the suspension was taken for the measurement of protein. Then 1.0 ml. of 1 M-HClO₄ was added to the remaining 1 ml. of suspension and the mixture was heated at 70° in a water bath for 1 hr. After centrifugation at 600 g for 5 min. the supernatant fluid was taken for the determination of DNA and RNA.

The ribose content of the acid-soluble fraction and of the hot-HClO₄ extract was determined by the method of Kerr & Seraidarian (1945). Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). DNA was determined by the method of Burton (1956).

RNA preparation. The method of RNA extraction was essentially that of Warner, Soeiro, Birnboim, Girand & Darnell (1966), with hot phenol and SDS, but the following modifications were employed. The uteri from 14 animals were pooled and homogenized in 6 ml. of 0.05 M-sodium acetate buffer, pH 5.1, containing EDTA (0.01 M) and bentonite (0.66%), with a Silverson Vortex homogenizer. The final volume of the uterine homogenate was 20 ml., which contained 0.2% bentonite and 1% (w/v) SDS. This was deproteinized with 20 ml. of 90% (w/v) redistilled phenol. The period of shaking at 60° in a water bath was increased from 3 min. to 5 min., so that the final temperature of the emulsion was approx. 58°. The RNA was precipitated from the pooled aqueous fractions by the addition of sodium acetate to give a final concentration of 2% (w/v), followed by 2 vol. of cold ethanol. The mixture was kept at -20° for at least 30 min., after which time the precipitate was centrifuged at 1360 g for 30 min. at -5°, dissolved in a small volume of buffer and extracted with ether three times. The recovery of RNA from 14 uteri by this procedure was always the same to within $\pm 5\%$.

Chromatography on MAK columns. The MAK column used had internal diameter 1.5 cm. and consisted of three layers as described by Mandel & Hershey (1960). It was surrounded by a jacket so that the temperature could be controlled. The column was eluted with a linear 0.4-1.5 M-NaCl gradient in 0.02 M-sodium phosphate buffer, pH 6.7, at 35° at a flow rate of 60 ml./hr. After all the RNA that could be eluted with salt had been removed, the tenaciously bound RNA was removed by eluting first at 35° and then at 80° with 0.2% SDS in 0.4 M-NaCl in phosphate buffer (Muramatsu, Hodnett & Busch, 1966; Ellem, 1966). Fractions (1 ml.) were collected and the E_{260} of alternate tubes was measured.

Radioactivity. Fractions from the columns were pooled in pairs, 50 μg . of carrier RNA was added and 50% (w/v) trichloroacetic acid was added to give a final concentration of 10% (w/v). The RNA was collected on Millipore filter membranes (0.22 μm . pore size) and washed with 5 ml. of

10% trichloroacetic acid. The membranes were placed in scintillation vials and dried at 50°. Then 10 ml. of toluene-based scintillation fluid [0.5% (w/v) 2,5-diphenyloxazole and 0.03% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)benzene] was added and the radioactivity was measured with a Philips liquid-scintillation analyser. Corrections for quenching were made by using the channels-ratio method. The average recovery of radioactivity from the MAK columns was 98%.

Dual-labelling experiments in vitro. Five 3-week-old female rats were each given 1 μg . of oestradiol-17 β intraperitoneally 3½ hr. before they were killed. Then the uteri were removed and washed in N.C.T.C. medium 109, placed in a 25 ml. conical flask containing 2 ml. of the same medium, 0.2 ml. (10 μC) of [¹⁴C]uridine and 5 μl . (5 μC) of [³H]-guanosine. The flask was gassed with O₂+CO₂ (95:5), sealed and shaken in a water bath at 37° for 45 min. The uteri were removed, placed in ice-cold saline, washed several times with fresh saline and finally blotted with absorbent tissue. RNA was extracted by the method described above.

RESULTS

Fig. 1 shows the changes that occurred in the chemical composition of immature rat uterus after the intraperitoneal injection of 5 μg . of diethylstilboestrol. Similar results were obtained in a less complete series of experiments using 1 μg . of oestradiol-17 β . The RNA content and wet weight of the uterus began to rise shortly after administration of diethylstilboestrol and both continued to increase for about 40 hr., although the overall change in RNA content was relatively greater than the change in wet weight. Acid-soluble ribose

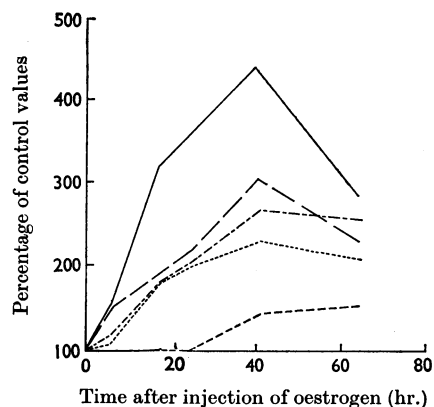


Fig. 1. Changes in the composition of immature rat uterus after oestrogen treatment. Diethylstilboestrol (5 μg .) was injected intraperitoneally at various times before the rats were killed. For details see the Materials and Methods section. Results are expressed as percentages of control values. —, RNA; - - -, wet wt.; · · ·, acid-soluble ribose; - · - ·, protein; — — —, DNA.

content began to increase rather more slowly than RNA content and wet weight, and reached a plateau at about 40hr. The protein content showed little change over the first 6hr., but thereafter it rose to a maximum at about 40hr. No change was observed in the DNA content of the uterus until about 24hr., and this then rose slowly by about 50% over the period between 24 and 40hr.

Fig. 2 shows the elution patterns obtained when RNA from control and oestradiol-treated rat uteri were fractionated on MAK columns. The control pattern is shown in Fig. 2(a) and the patterns obtained from animals given oestradiol 30min. and 1, 2 and 6hr. before being killed are shown in Figs. 2(b), 2(c), 2(d) and 2(e) respectively. No major changes in the E_{260} values were observed at the various time-intervals, although at 6hr. there was evidence of a slight increase in the total content of r-RNA.

The total radioactivity of RNA extracted from the uteri increased with time after administration of oestradiol. The increase over the control value was 22%, 48%, 85% and 144% at 30min. and 1, 2 and 6hr. respectively. The earliest increases occurred in the ribosomal precursor (Q_1) peak (Muramatsu *et al.* 1966; Ellem, 1966), which rose to 54% above the control value in the first 30min. and to 145% above the control value at 1hr. Thereafter the amount of radioactivity in this fraction appeared to stabilize. The mature r-RNA fraction showed an increase of 40% above the control value at 30min. and 1hr., but after 2hr. and 6hr. there was a marked rise in the radioactivity in this fraction with increases of 115% and 400% respectively above the control.

The Q_2 fraction showed a slight increase of 24% above the control value after 30min., rising slowly to 71% above the control at 6hr.

The total radioactivity in the fraction eluted with 0.2% SDS showed no increase at 30min., but a slight increase of 24% at 1hr., rising to 52% above the control value after 6hr.

No increase occurred in the t-RNA fraction until 1hr., by which time it had risen to 92% above the control t-RNA value. Thereafter the radioactivity in this fraction rose rapidly until at 6hr. it was 400% above the control value.

The nature of the peaks between t-RNA and r-RNA is not clearly known. The first one could be 7s RNA (Pene, Knight & Darnell, 1968), which is produced from 28s r-RNA by the method of RNA preparation with hot phenol and SDS. The second peak is probably DNA, of which only a small amount is extracted by this method of RNA preparation. The radioactivity in these fractions increased in a similar way to that in the r-RNA fraction.

The changes in radioactivity of the principal

RNA components with time are shown in Fig. 3. The labelling of the ribosomal RNA complex (r-RNA and Q_1 -RNA) rose steadily over the 6hr. period from the time of administration of oestradiol to about four times the control value. The t-RNA fraction, after an initial delay of 30min., rose to about five times the control value, whereas the RNA fractions eluted with SDS showed a much more gradual increase in radioactivity to a value about 50% higher than that in the controls.

r-RNA contains higher proportions of guanine and cytosine than of adenine and uracil, whereas in other RNA species these differences tend to be smaller. It is therefore possible to obtain some information about the base composition of RNA species by comparing the incorporation of [^3H]-guanosine and [^{14}C]uridine into RNA molecules. This procedure was employed as a means of assessing the guanine and uracil contents of the various species of RNA obtained from rat uteri after oestradiol treatment, and the results of such an experiment are shown in Fig. 4. It is evident that the $^3\text{H}/^{14}\text{C}$ ratio is similar in the t-RNA, 7s-RNA, r-RNA and Q_1 -RNA peaks, but that this ratio is different in the Q_2 -RNA fraction and in the RNA eluted with SDS. These results indicate that the Q_2 -RNA and the RNA eluted with SDS have a base composition more like that of DNA than have t-RNA, 7s-RNA, r-RNA and Q_1 -RNA.

DISCUSSION

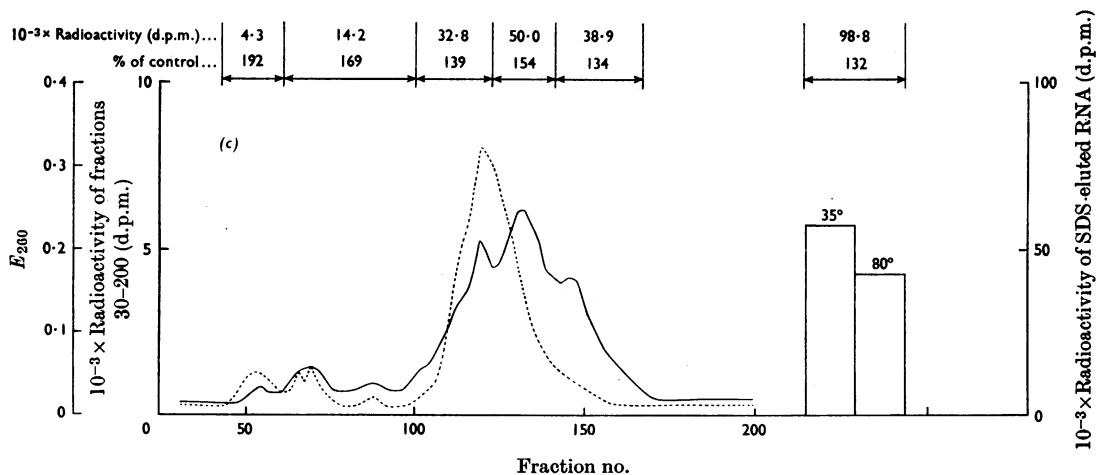
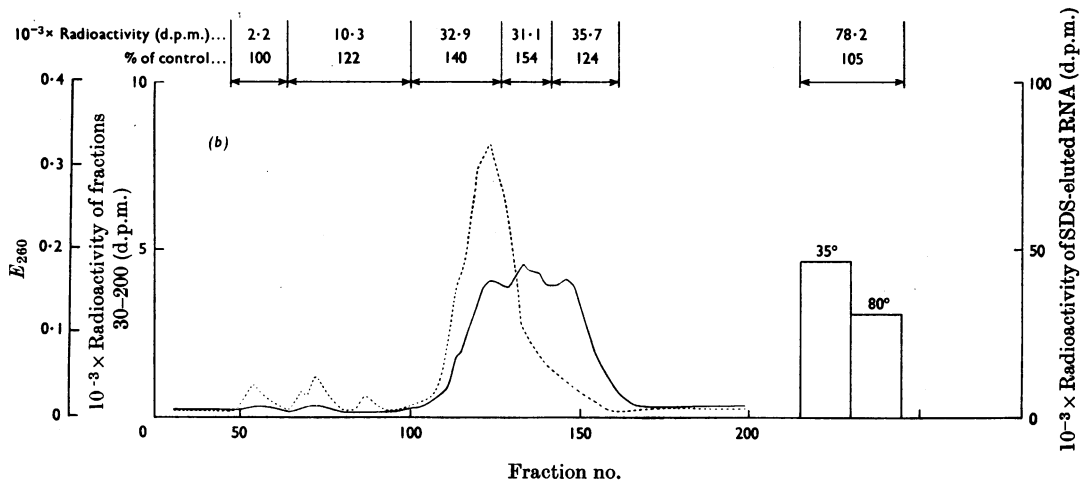
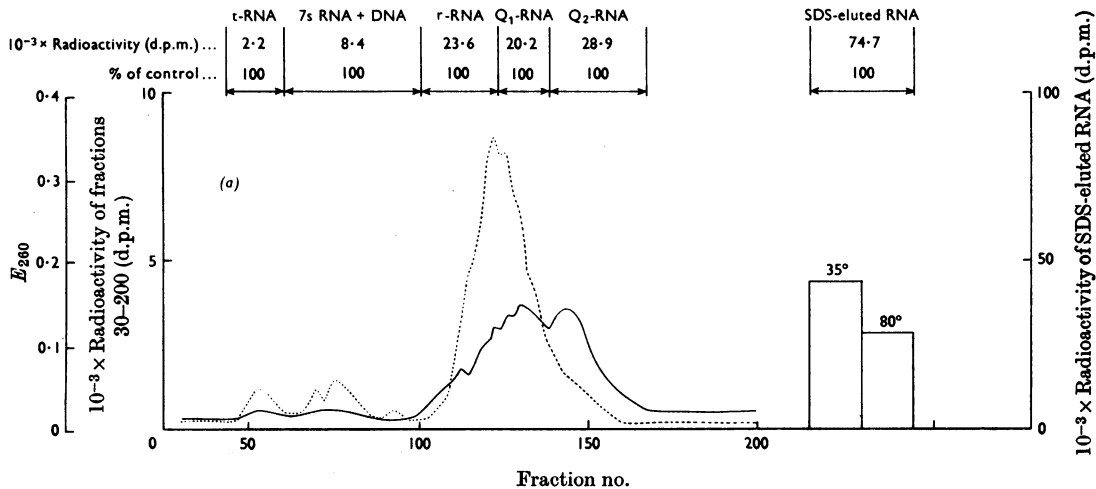
These results demonstrate that one of the earliest responses of immature rat uterus to oestradiol is an increase in the total amount of RNA in the tissue, and that changes in the pattern of incorporation of labelled precursors into RNA occur within 30min. of administration of the hormone.

Q_1 -RNA is believed to represent a precursor of r-RNA (Muramatsu *et al.* 1966; Ellem, 1966), and it is therefore relevant that the earliest increase in labelling occurred in the Q_1 -RNA, whereas at longer time-intervals after hormone treatment large increases occurred in the labelling of r-RNA.

Since (Fig. 1) the RNA content of the uterus continued to rise for about 40hr. after the administration of oestrogen, and labelling of the r-RNA and Q_1 -RNA complex (Fig. 3) was still increasing sharply at 6hr., it seems likely that further increases in labelling of r-RNA occur at time-intervals longer than 6hr.

Our results suggest that, among the earliest events after oestradiol treatment, one of the most important quantitative changes is an increase in the synthesis of r-RNA and that this is achieved through increased formation of Q_1 -RNA, which is then transformed into r-RNA.

It is noteworthy that in the 7s-RNA fraction the



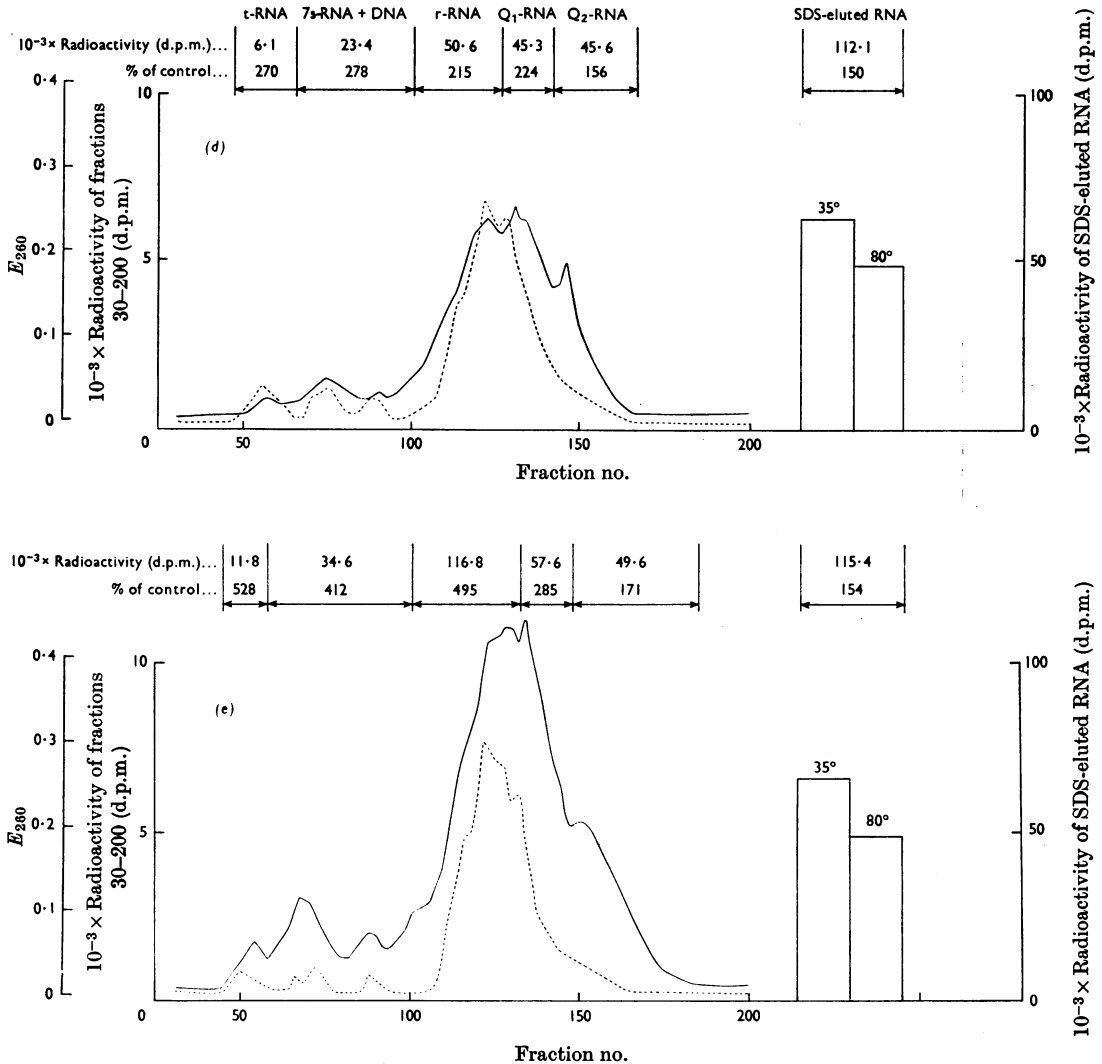


Fig. 2. Chromatography of RNA from rat uterus on MAK columns. In each experiment RNA from 14 uteri was pooled and 40 E_{260} units were applied to the column. All animals received 25 μC of [^3H]uridine and 25 μC of [^3H]guanosine 30 min. before being killed. Controls (a) received 0.1 ml. of 0.9% NaCl 3 hr. before being killed, and the test animals received 1 μg . of oestradiol-17 β in 0.1 ml. of 0.9% NaCl at the following times before death: (b) 30 min.; (c) 1 hr.; (d) 2 hr.; (e) 6 hr. -----, E_{260} ; —, ^3H radioactivity.

pattern of increased labelling was not unlike that of r-RNA; this is expected, since this species of RNA is likely to be derived from r-RNA during the extraction procedure.

In addition to the major quantitative changes in labelling of r-RNA, there is also evidence of a considerable increase in t-RNA synthesis, occurring between 1 hr. and 6 hr. after hormone treatment, and this is somewhat later than the changes in labelling of r-RNA.

m-RNA may occur in the Q₂-RNA or SDS-eluted RNA fractions. Although there was increased labelling in these fractions, the changes were very small at short time-intervals, and even after 6 hr. the radioactivity in them was only increased by about 50% compared with nearly 500% in the r-RNA and t-RNA. Although the increase in labelling of the m-RNA-containing fractions was relatively small, it might nevertheless be highly significant, since it might represent the synthesis of one or

more species of m-RNA coding for the synthesis of one or a small number of proteins that have an essential role at a later stage in the hormone response.

Up to this point these results have been considered on the assumption that no changes occur in the specific radioactivities of the ribonucleotide pools in uterine tissue in response to hormone

treatment. Although changes probably occur in the specific radioactivities of the ribonucleotide pools, it is a major undertaking to investigate these changes in such a way as to obtain meaningful results, and this is beyond the scope of the present paper.

Although we cannot be certain that all species of RNA are synthesized from the same pool of ribonucleotide precursors, it is reasonable to assume this in the absence of evidence to the contrary.

If this assumption is made it is evident that any changes in ribonucleotide pools would affect the labelling of all species of RNA to a similar extent, and that whatever happens to the pools there are differential increases in the synthesis of Q_1 -RNA, r-RNA, 7s RNA and t-RNA relative to the changes in the DNA-like RNA. It is possible that one of the primary effects of oestradiol in this system is to promote massive synthesis of r-RNA, accompanied by a slightly later but equally massive increase in synthesis of t-RNA. The quantitative changes in DNA-like RNA do not appear to occur until some time after the increase in r-RNA synthesis and seem to reach a plateau at 2hr., whereas the changes in r-RNA and t-RNA continue for at least 6hr. Thus these changes in DNA-like RNA may be secondary to earlier events. However, there could be early qualitative changes in m-RNA synthesis, not detectable by the methods we employed, that are required to initiate the synthesis of new r-RNA.

At 30min. after administration of labelled precursors to control animals, by far the largest proportion of radioactivity was found in the DNA-

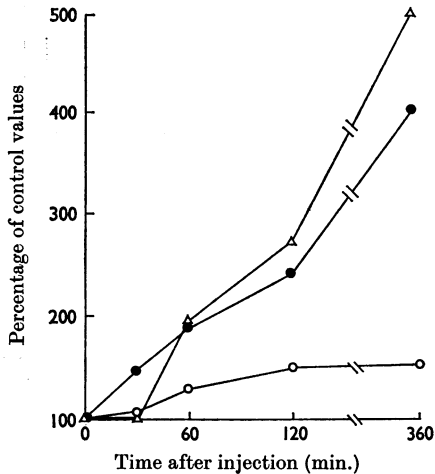


Fig. 3. Changes with time in the labelling of various RNA species from immature rat uterus after the administration of oestradiol. Experimental conditions were as given in Fig. 2. ●, Ribosomal RNA complex (r-RNA + Q_1 -RNA); Δ, t-RNA; ○, DNA-like RNA.

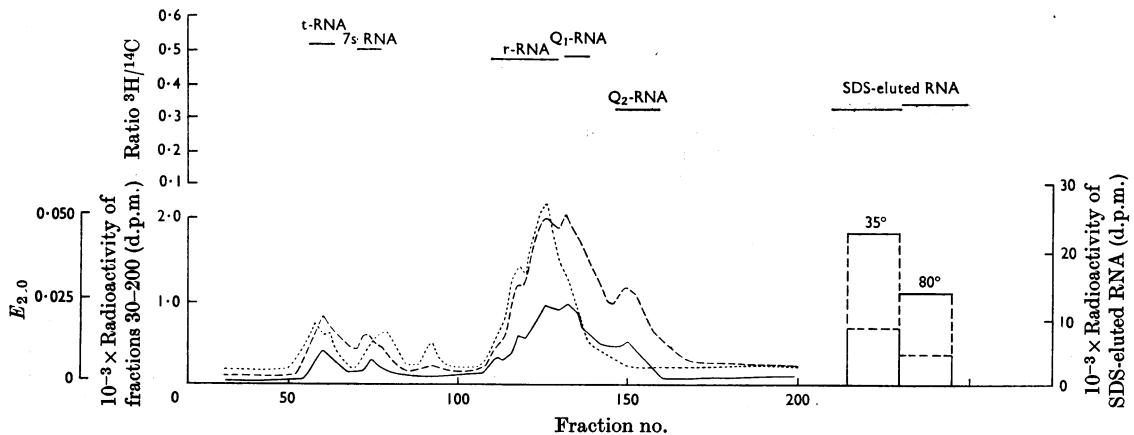


Fig. 4. Chromatography of RNA from rat uterus on a MAK column. Rats were injected with 1 μ g. of oestradiol-17 β , 3 $\frac{1}{2}$ hr. before they were killed. Isolated uteri were incubated *in vitro* with 10 μ C of [14 C]uridine and 5 μ C of [3 H]guanosine for 45 min., and the RNA was extracted and applied to the column. ----, E_{260} ; ----, 14 C radioactivity; —, 3 H radioactivity.

like RNA. Preliminary experiments showed that after longer time-intervals the radioactivity in the DNA-like RNA declined rapidly, and apparently at least some of this species of RNA undergoes rapid turnover.

Nevertheless the magnitude of the increases in synthesis of r-RNA and t-RNA, and the very early time at which the change in r-RNA synthesis occurs, strongly suggest that these two species of RNA play an essential part in the hormonal response. These changes seem to be quantitatively more important than the changes in DNA-like RNA synthesis, and possibly control of increased protein synthesis in the uterus lies to a greater extent in the formation of new ribosomes or poly-ribosome complexes or in the transport of m-RNA from the cell nucleus to the cytoplasm than in the synthesis of large amounts of m-RNA. These conclusions agree with the view put forward by Tata (1966, 1968) that increased synthesis of r-RNA is a major feature of hormone action.

This work was supported by grants from the British Empire Cancer Campaign for Research, the Medical Research Council and the Rankine Fund of the University of Glasgow, all of which are gratefully acknowledged. R. J. B. is a Science Research Council Scholar and B. B. is a N.A.T.O. Fellow.

REFERENCES

- Burton, K. (1956). *Biochem. J.* **62**, 315.
Ellem, K. A. O. (1966). *J. molec. Biol.* **20**, 283.
Gorski, J. (1964). *J. biol. Chem.* **239**, 889.
Gorski, J. & Nicolette, J. (1963). *Arch. Biochem. Biophys.* **103**, 418.
Hamilton, T. H., Teng, C. S. & Means, A. R. (1968a). *Proc. nat. Acad. Sci., Wash.*, **59**, 1265.
Hamilton, T. H., Widnell, C. C. & Tata, J. R. (1968b). *J. biol. Chem.* **243**, 408.
Kerr, S. E. & Seraidarian, K. (1945). *J. biol. Chem.* **159**, 211.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
Mandel, J. D. & Hershey, A. D. (1960). *Analyt. Biochem.* **1**, 66.
Means, A. R. & Hamilton, T. H. (1966). *Proc. nat. Acad. Sci., Wash.*, **56**, 686.
Muramatsu, M., Hodnett, J. L. & Busch, H. (1966). *J. biol. Chem.* **241**, 1544.
Nicolette, J. A. & Mueller, G. C. (1966). *Biochem. biophys. Res. Commun.* **24**, 851.
Pene, J. J., Knight, Z. & Darnell, J. E. (1968). *J. molec. Biol.* **33**, 609.
Roberts, S. & Szego, C. M. (1947). *Endocrinology*, **40**, 73.
Tata, J. R. (1966). *Progr. Nucleic Acid Res. molec. Biol.* **5**, 191.
Tata, J. R. (1968). *Nature, Lond.*, **219**, 331.
Teng, C. S. & Hamilton, T. H. (1967). *Biochem. J.* **105**, 1101.
Warner, J. R., Soeiro, R., Birnboim, H. C., Girand, M. & Darnell, J. E. (1966). *J. molec. Biol.* **19**, 349.