Biochem. J. (1968) **109**, 705
Printed in Great Britain

The Early Effects of Oestradiol-17β on Ribonucleic Acid Synthesis in Rat Uterus

By R. J. Billing, B. Barbiroli and R. M. S. Smellie
Institute of Biochemistry, University of Glasgow

(*Received* 6 *August* 1968)

It has been shown that the biological action of oestrogens in immature rat uterus is preceded by increased labelling of RNA (Gorski & Nicolette, 1963) and that the DNA-dependent RNA polymerase (EC 2.7.7.6) activity of the uterus also increases in response to oestrogen treatment (Gorski, 1964; Nicolette & Mueller, 1966). Although there is evidence that the species of RNA formed in response to oestrogens is primarily of ribosomal origin (Hamilton, Widnell & Tata, 1968; Hamilton, Teng & Means, 1968), the nature of the changes in the pattern of RNA synthesis has not been investigated in detail.

In the present studies RNA was isolated from the uteri of oestradiol-treated and untreated immature rats, after the injection of labelled precursors of RNA. Three-week-old female albino rats of the Wistar strain, weighing 35–40g., were used. The isolated RNA was fractionated on columns of kieselguhr coated with methylated serum albumin and the distribution of radioactivity in the various RNA fractions was determined.

In one series of experiments the animals were divided into two groups and 1hr. before they were killed the test group received 1μg. of oestradiol-17β in 0·1ml. of 0·9% NaCl intraperitoneally; the control group received 0·1ml. of 0·9% NaCl. At 30min. before they were killed both groups received a mixture of 25μc of [^{3}H]uridine (specific radioactivity 2·7c/m-mole) and 25μc of [^{3}H]guanosine (specific radioactivity 4·76c/m-mole).

Animals were killed by cervical dislocation and the uteri from the test and control groups were pooled separately for the isolation of RNA.

In other experiments, in which the dual-labelling procedure of Ellem (1967) was used, one group of six rats received 1μg. of oestradiol in 0·1ml. of 0·9% NaCl intraperitoneally 90min. before they were killed; the controls received 0·1ml. of 0·9% NaCl. At the same time the control group were given 100μc of [^{3}H]uridine (specific radioactivity 212mc/m-mole) and the hormone-treated animals were given 25μc of [^{14}C]uridine (specific radioactivity 53mc/m-mole).

The uteri from all 12 animals were pooled for the extraction of RNA. In calculating the radioactivity of the RNA fractions, allowance was made for the factor 4 between the specific radioactivities of the ^{3}H- and ^{14}C-labelled precursors.

RNA was extracted from the pooled uteri by the hot-phenol method of Warner, Soeiro, Birnboim, Girand & Darnell (1966) and fractionated on columns of kieselguhr coated with methylated serum albumin as described by Mandel & Hershey (1960), Ellem (1966) and Muramatsu, Hodnett & Busch (1966). Fractions (2ml.) were collected and their extinctions at 260mμ were measured. The RNA in each fraction was precipitated by the addition of 0·5ml. of 50% (w/v) trichloroacetic acid and collected on 25mm. Millipore filter membranes of 0·45μ pore size. After a washing with 5% (w/v) trichloroacetic acid the membranes were dried at 50° and placed in scintillation vials with 10ml. of toluene-based scintillator [0·5% (w/v) 2,5-diphenyloxazole and 0·03% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)benzene].

Radioactivity was measured with a Nuclear–Chicago model 725 or a Packard series 4000 liquid-scintillation spectrometer and corrections for quenching were made by using the channels-ratio method.

Fig. 1(*a*) shows the elution pattern for RNA from a control group, and Fig. 1(*b*) shows the pattern obtained from animals treated with oestradiol. No significant difference occurs in the distribution of ultraviolet-absorbing material from the two groups. However, there is an increase in total radioactivity of RNA recovered from the test group of 72% above that in the controls. The greatest change is evident in the Q1 peak, which shows an increase of 150% over the controls, and the ribosomal RNA peak shows an increase of 67%. Smaller increases of about 30% occur in the radioactivity of peak Q2 and of the peaks eluted with sodium dodecyl sulphate at 35° and 70°.

Somewhat similar results were obtained in the dual-labelling experiment (Fig. 1*c*), where the hormone and labelled precursors were administered simultaneously 90min. before the animals were killed. The total ^{14}C recovered was 63% greater than the total ^{3}H and the largest increase (156%) was again found in peak Q1. In this experiment, however, the ribosomal RNA peak was 130% and component Q2 was 58% higher in the test than in the control animals.

Fig. 1(*c*) also shows the ratio of ^{14}C to ^{3}H in the various fractions. The greatest deviations from unity occur in the ribosomal RNA (3·2); the ratio

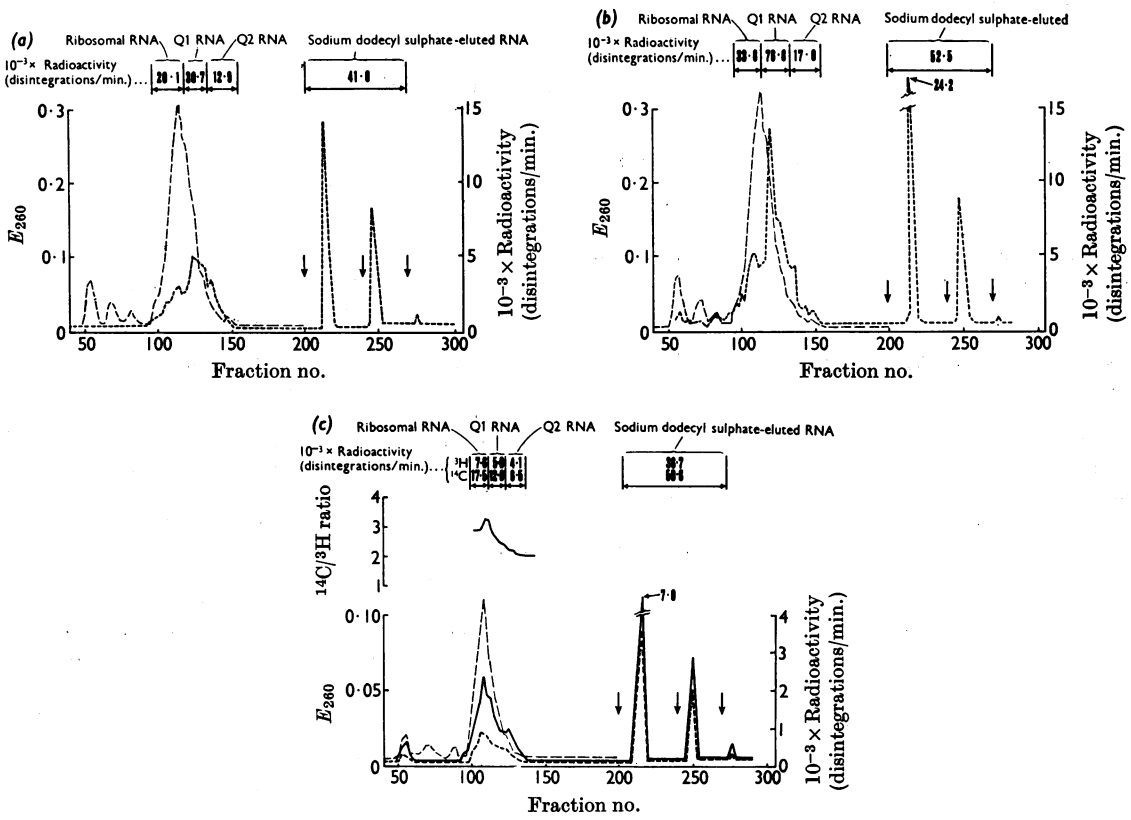

Fig. 1. Chromatography of rat uterus RNA on columns of kieselguhr coated with methylated serum albumin. (*a*) Elution pattern of E_{260} and of ^{3}H radioactivity for the RNA from the uteri of 14 control rats; 40 E_{260} units were applied to the column. Controls received 0·1 ml. of 0·9% NaCl 1 hr. before they were killed and 25 μc of [^{3}H]uridine plus 25 μc of [^{3}H]guanosine 30 min. before they were killed. (*b*) Elution pattern of E_{260} and of ^{3}H radioactivity for the RNA from the uteri of 14 oestradiol-treated rats; 40 E_{260} units were applied to the column. Controls received 1 μg. of oestradiol-17β in 0·1 ml. of 0·9% NaCl 1 hr. before they were killed and 25 μc of [^{3}H]uridine plus 25 μc of [^{3}H]guanosine 30 min. before they were killed. (*c*) Elution pattern of E_{260} and of ^{3}H and ^{14}C radioactivity obtained on chromatography of ^{3}H-labelled RNA from the uteri of six control animals mixed with ^{14}C-labelled RNA from the uteri of six oestradiol-treated rats; 15 E_{260} units were applied to the column. Control animals received 0·1 ml. of 0·9% NaCl and 100 μc of [^{3}H]uridine 90 min. before they were killed. Test animals received 1 μg. of oestradiol-17β in 0·1 ml. of 0·9% NaCl and 25 μc of [^{14}C]uridine 90 min. before they were killed. The ratio of ^{14}C to ^{3}H for the major components is plotted in the upper section of (*c*). ——, E_{260}; – – – –, ^{3}H radioactivity; ——, ^{14}C radioactivity.

for component Q1 is in the range 2·5–3, and for the other fractions the ratio is about 1·6.

According to Muramatsu *et al.* (1966) and Ellem (1966) fraction Q1 represents a ribosomal precursor RNA, and the present results indicate that among the early events that follow oestradiol treatment is an increase in the synthesis of all RNA fractions, and particularly in the synthesis of ribosomal precursor.

B.B. is a N.A.T.O. Research Fellow.

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