Incorporation of [³H]Uridine into the Ribonucleic Acid of Rat Uterus during Pseudopregnancy and in the Presence of I.C.I. 46474 [*trans*-1-(*p*- β -Dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene]

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1. The incorporation of [³H]uridine into RNA of rat uterine tissue has been measured during pseudopregnancy and in rats receiving different doses of an anti-implantation compound [*trans*-1-(*p*- β -dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene, I.C.I. 46474]. 2. In the uterus of the pseudopregnant rat uridine incorporation into RNA increased markedly on day 3 of pseudopregnancy, remained high until day 5 and decreased sharply by day 6, rising again by day 9. 3. This pattern of change was similar to, but not identical with, that previously found in the pregnant rat. 4. Rats receiving I.C.I. 46474 at a dose concentration below that preventing implantation showed a pattern of RNA synthesis similar to that found in untreated control rats. 5. Rats treated with doses of I.C.I. 46474 sufficient to inhibit implantation revealed a totally different pattern of incorporation of [³H]uridine into uterine RNA. 6. The results are discussed in terms of previous findings with the normally pregnant rat. It is concluded that the increasing uterine RNA synthesis found on day 3 in the pregnant rat is an important requirement for the occurrence of the subsequent implantation.

It has been shown (Heald & O'Grady, 1970) that during the earliest stages of pregnancy in the rat a marked increase occurs in the rate of synthesis of uterine RNA on day 3 of pregnancy. It has been further shown that this period is also marked by an increased rate of protein synthesis (Reid & Heald, 1970) and an increased rate of utilization of C-1 of glucose (M. A. H. Surani & P. J. Heald, unpublished work). The cause of these changes, particularly in RNA metabolism, is not known. It has been suggested that the increased RNA synthesis could be due to a release of oestrogen from the ovaries commencing on day 2 of pregnancy (Miller, Owen & Emmens, 1968), though direct estimation of oestrogen in the peripheral blood of the pregnant rat failed to show such an increase (Yoshinaga, Hawkins & Stocker, 1969).

The physiological significance of the increase in the rate of RNA synthesis is also an open question and it was suggested (Heald & O'Grady, 1970) that the increase was an essential event in the sequence leading to the subsequent successful implantation of the blastocyst. To examine this concept further the incorporation of [³H]uridine into uterine RNA of the rat was determined during early pseudopregnancy and in animals treated with an anti-implantation compound [*trans*-1-(*p*- β -dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-

ene, I.C.I. 46474] presumed to act by nature of its anti-oestrogenic activity (Walpole, 1968).

The pseudopregnant animal was chosen since it has been well established (see Psychoyos, 1966) that the uterus of pseudopregnant rats permits the implantation and normal development of blastocysts transferred to it from a pregnant rat. Thus it could be expected that the metabolism of uterine RNA should be similar to that of the pregnant animal in this preparation where the possible effects of the blastocyst had been eliminated. Use of I.C.I. 46474 was made in the expectation that doses effective in preventing implantation might prevent the rise in RNA synthesis on day 3 of normal pregnancy, whereas doses permitting implantation would have little or no effect.

MATERIALS AND METHODS

The rats were a Holtzman-derived Sprague-Dawley strain maintained in windowless rooms at a mean temperature of 22°C under a lighting schedule of 6.00 a.m.–8.00 p.m.

Pseudopregnancy was induced by caging virgin females singly with vasectomized males, day 1 of pseudopregnancy being the day on which copulation plugs were found in the vagina or on the floor of the cage. Pregnant animals were obtained by mating virgin females with normal

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males, day 1 of pregnancy being the morning on which sperm were found in the vaginal smear.

Rats in which the effect of I.C.I. 46474 on RNA metabolism was being studied received a single oral dose by 10.00 a.m. on day 2 followed by an intravenous injection of [^3H]uridine (100 μCi , specific radioactivity 26.7 Ci/mmol by 11.00 a.m. on days 2, 3, 4 and 5). The I.C.I. 46474 was administered as a suspension in aqueous 0.05% Tween 80 and controls dosed with Tween 80 alone were included in the series.

The injection of [^3H]uridine and procedures for the analysis of RNA and DNA in the uterine tissue were as described by Heald & O'Grady (1970).

Statistical analysis was carried out by standard procedures as described by Snedecor & Cochran (1967).

RESULTS

Pseudopregnant animals. The incorporation of [^3H]uridine into the total uterine RNA over the first 9 days of pseudopregnancy is shown in Fig. 1. The results are presented both in terms of specific radioactivity of RNA and in terms of specific radioactivity corrected to a standard count in the acid-soluble fraction of the tissue to minimize any variation that might occur due to differing sizes of the intracellular uridine pools (Heald & O'Grady, 1970). The patterns of uptake (which were similar in both instances) follow in general the pattern found by Heald & O'Grady (1970) in the pregnant rat uterus, except that the marked change in uptake on day 4 (see also Fig. 4) did not occur. In both Figs. 1(a) and 1(b) there was statistically no difference between the uptake on days 3, 4 and 5, but a significant decrease ($P < 0.05$) occurred by

day 6. Thereafter uptake remained low until day 8, when a further increase in uptake occurred continuing to day 9. Experiments were not continued beyond this point, since first, comparison with previous results (Heald & O'Grady, 1970) on pregnant rats was desired and secondly, by days 10–11 some animals began to return to the normal oestrous cycle.

Variations in RNA content/mg dry matter are shown in Table 1. Although there was an initial decrease in RNA content between days 1 and 2 of pseudopregnancy the difference was barely significant. Thereafter no significant changes were noted throughout the remaining period. Thus the lowered values on days 7 and 8 were not significantly different from values on days 6 or 9, partly because of the considerable scatter in the results. On these values therefore, it is not possible to conclude that any major change in RNA content occurred during this period.

More clearly defined changes occurred in both total uterine wet weight and in the RNA/DNA ratios (Table 1) between days 1 and 2 and during days 6–9. Thus, following an initial decrease by day 2 the uterine wet weight and the RNA/DNA ratios remained constant until day 7, when values decreased and remained low during the ensuing 2 days.

The dry matter content of the pseudopregnant uterus also changed markedly (Fig. 2), the initial variation commencing by day 5 when there was a decrease in dry matter content, reaching a minimum on day 6 and then increasing sharply by day 9.

Effects of I.C.I. 46474. Examination of a range

Fig. 1. Incorporation of [^3H]uridine into RNA of rat uterine tissue during pseudopregnancy. (a) Specific radioactivity; (b) specific radioactivity of RNA corrected to a standard count in the acid-soluble fraction of the tissue extract (see Heald & O'Grady, 1970).

Table 1. *RNA content/unit dry matter, total uterine wet wt. and RNA/DNA ratios of pseudopregnant rats*

Rats were mated and uterine tissue was removed and analysed as described by Heald & O'Grady (1970). Values are given as means $\pm$ S.E.M.

Day of pseudopregnancy	No. of animals/group	RNA (μ g/mg dry wt.)	Total uterine wet wt. (mg)	RNA/DNA ratio
1	3	20.7 $\pm$ 0.41	419 $\pm$ 21.8	342 $\pm$ 15.1
2	4	16.51 $\pm$ 1.55	329 $\pm$ 6.4	261 $\pm$ 17.6
3	4	19.43 $\pm$ 1.34	298 $\pm$ 17.99	285 $\pm$ 10.6
4	5	20.45 $\pm$ 1.20	323 $\pm$ 7.39	318 $\pm$ 21.1
5	3	19.8 $\pm$ 0.48	288 $\pm$ 2.51	258 $\pm$ 14.7
6	3	19.26 $\pm$ 2.35	344 $\pm$ 19.1	302 $\pm$ 15.1
7	3	16.03 $\pm$ 0.48	260 $\pm$ 8.1	218 $\pm$ 7.8
8	3	17.26 $\pm$ 0.95	220 $\pm$ 23.3	179 $\pm$ 10.6
9	3	19.65 $\pm$ 2.95	215 $\pm$ 20.8	211 $\pm$ 32.5

Fig. 2. Change in dry matter content of the uterus of the pseudopregnant rat. Vertical bars represent the standard error of each point.

Fig. 3. Effects of I.C.I. 46474 on the number and site of implantation sites in rat uterus in early pregnancy. ●, No. of implantation sites/horn; ○, mean wet wt. of implantation sites.

of doses of I.C.I. 46474 revealed that the strain of rats used in these experiments was more sensitive to the compound than that used by Harper & Walpole (1967). Thus a dose of 0.1 mg/kg given orally on day 2 of pregnancy diminished the number of implantation sites found on days 9–10 to a mean of 2/horn (Fig. 3) (this value is derived largely from the inclusion of a single rat that had the normal number of implantation sites); whereas a dose of 0.5 mg/kg was required by Harper & Walpole (1967) to prevent implantation. Doses of 0.05 mg/kg, although decreasing the mean weight of the implantation sites by days 9–10, did not significantly alter the mean number/horn. In separate experiments, groups of 12 rats dosed with 0.05 mg/kg on day 2 of pregnancy, produced normal litters, the weaning weights and period of gestation

of which were the average for the colony, whereas rats dosed with 0.1 mg/kg on day 2 did not produce litters.

The effect of these two dose concentrations of I.C.I. 46474 on the uptake of [3 H]uridine into the uterine RNA is shown in Fig. 4. The pattern of change of the control group was identical with that previously found in pregnant rats from this colony (Heald & O'Grady, 1970) and was paralleled closely by the pattern of uptake into animals receiving 0.05 mg of I.C.I. 46474/kg on day 2. Animals receiving 0.1 mg/kg, however, showed a different pattern. Thus uptake into RNA did not show a peak value on day 3 nor was a decline observed on day 4.

Fig. 4. Incorporation of [³H]uridine into RNA in uteri from rats receiving different doses of I.C.I. 46474 orally on day 2 of pregnancy. (a) ●, Tween control group; ○, group receiving 0.05 mg of I.C.I. 46474/kg; (b) ●, group receiving 0.1 mg of I.C.I. 46474/kg. All values are expressed relative to a standard count in the acid-soluble fraction.

Table 2. RNA content/unit dry matter, total wet wt. and RNA/DNA ratios in the uteri of rats receiving I.C.I. 46474

Rats were treated with I.C.I. 46474 at 0.05 and 0.1 mg/kg on day 2 of pregnancy, and analyses were performed as described in the Methods section and by Heald & O'Grady (1970). Values are given as means $\pm$ S.E.M., with numbers of animals/group in parentheses.

Day of pregnancy	RNA (μ g/mg dry matter)			Uterine wet wt (mg)			RNA/DNA ratio		
	Dose concentration			Dose concentration			Dose concentration		
	Controls	0.05 mg/kg	0.1 mg/kg	Controls	0.05 mg/kg	0.1 mg/kg	Controls	0.05 mg/kg	0.1 mg/kg
2	18.7 $\pm$ 0.60 (3)	19.36 $\pm$ 0.27 (7)	16.12 $\pm$ 0.59 (5)	318 $\pm$ 18.23 (3)	287 $\pm$ 14.6 (7)	282 $\pm$ 9.1 (5)	281 $\pm$ 11.5 (3)	246 $\pm$ 10.1 (7)	245 $\pm$ 15 (5)
3	19.69 $\pm$ 1.25 (4)	24.5 $\pm$ 1.69 (7)	20.56 $\pm$ 1.36 (6)	248 $\pm$ 18.8 (4)	282 $\pm$ 9.5 (7)	293 $\pm$ 10.7 (6)	388 $\pm$ 60.9 (4)	304 $\pm$ 16.0 (7)	331 $\pm$ 32 (6)
4	20.61 $\pm$ 1.47 (3)	24.6 $\pm$ 0.82 (4)	19.05 $\pm$ 0.59 (4)	282 $\pm$ 28.5 (4)	308 $\pm$ 19.5 (4)	256 $\pm$ 21.0 (4)	297 $\pm$ 21.3 (3)	344 $\pm$ 8.8 (4)	344 $\pm$ 17.3 (4)
5	22.43 $\pm$ 1.92 (3)	25.10 $\pm$ 0.99 (4)	23.58 $\pm$ 1.95 (3)	—	310 $\pm$ 21.5 (4)	283 $\pm$ 32.4 (3)	335 $\pm$ 39.1 (3)	325 $\pm$ 11.3 (4)	309 $\pm$ 22 (3)

Statistical analysis of the data showed that differences in specific radioactivities between days 2 and 3 and days 3 and 4 of the Tween control group and in the groups receiving I.C.I. 46474 at 0.05 mg/kg were significant ($P < 0.01$ for control group; $P < 0.05$ for the I.C.I. 46474-treated group) whereas no significant changes were detected in the group receiving 0.1 mg of I.C.I. 46474/kg. These conclusions were confirmed by analysis of variance of the results on days 2–4 in all cases, both the Tween control group and the groups receiving

0.05 mg of I.C.I. 46474/kg, showing a variance with a significance by $P < 0.05$, whereas the group receiving 0.10 mg/kg showed no significant variance.

Although the mean corrected specific radioactivities of the uterine RNA in the groups receiving 0.05 mg and 0.1 mg of I.C.I. 46474/kg were higher than those of the corresponding control group on day 2 the differences barely reached statistical significance ($0.1 > P > 0.05$) presumably owing to the wide scatter of the values in the treated groups. On the other hand, the specific radioactivity of the

RNA in the groups receiving 0.1 mg/kg on day 2 was, on day 5, significantly higher ($P = 0.05$) than that of the control groups on day 5.

Treatment with either dose of I.C.I. 46474 did not significantly affect either the quantity of RNA/unit dry matter, the total uterine wet weight or the RNA/DNA ratios on any day studied (Table 2) nor did treatment with the highest dose produce any increase in total uterine weight or in RNA concentration during the ensuing 2 days.

DISCUSSION

Comparison of the present results with those previously found for the uterus of the pregnant rat gives substantial support to the view that an increased synthesis of RNA by day 3 is essential for the subsequent implantation of the blastocyst. It is clear that an anti-implantation compound (I.C.I. 46474), when administered at a dose concentration sufficient to prevent implantation, produced a pattern of change completely different from that found in the normally pregnant animal (Fig. 4). The same compound administered at a slightly lower dose concentration, which did not prevent implantation and normal pregnancy, did not alter the pattern of uptake of [^3H]uridine significantly from that of the control animals.

The basis of this effect of I.C.I. 46474 is obscure. The compound, in addition to being a weak oestrogen (Harper & Walpole, 1967; Walpole, 1968) and inhibiting the oestrogen-induced release of pituitary luteinizing hormone (Labhsetwar, 1970) is presumed to act as an anti-implantation compound partly by its ability to antagonize the uterine effects of the oestrogen released on day 4 of pregnancy, and which is essential for implantation to occur (Psychoyos, 1966).

In the current experiments no stimulating effect on uterine growth or on the total RNA content were observed (Table 2) with the highest dose concentration. It was clear, however, that the compound directly affected uterine RNA metabolism and that if this effect was sufficient to distort the pattern of change normally found, implantation did not occur.

In the pseudopregnant rat previous investigations have shown that the patterns of glucose metabolism and oxygen uptake (Saldarini & Yochim, 1967; Yochim & Mitchell, 1968) and of [^3H]uridine incorporation into the total uterine RNA of the rat (Miller *et al.* 1968) are similar during the first 5 days of pseudopregnancy to those in the pregnant uterus.

The results for RNA metabolism have in general been confirmed in the present study where measurement was made in terms of specific radioactivities rather than uptake/whole uterus. It is further to be

noted that incorporation after day 5 of pseudopregnancy closely follows that found by Heald & O'Grady (1970) for the non-implanted areas of the uterus of the pregnant rat. The results in Fig. 1 therefore show that the incorporation of [^3H]uridine into RNA of the pseudopregnant rat uterus is very similar to that found in the un-implanted uterus of the normally pregnant rat. A difference appears to exist, however, in that the marked decrease in uptake on day 4 of pregnancy (see Fig. 4) was not found on day 4 of pseudopregnancy, and at present no satisfactory explanation for this can be offered.

Taken together, the results support the proposal that the peak of increased RNA synthesis found on day 3 in normal pregnancy is a necessary development for the subsequent implantation of the blastocyst in the uterus of the rat.

Since this work was completed our attention has been drawn to the work of Vittorelli, Harrison & Lutwak-Mann (1967), who showed that there was an increased incorporation of ^{32}P into the nucleic acids in the endometrium of the pregnant rabbit between days 3 and 5 of pregnancy, and that this was accompanied by the appearance of a new, stable, RNA component with a sedimentation coefficient of about 10S. These results complement our own findings and suggest that the phenomenon may be of general fundamental importance.

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