EFFECT OF A COPPER IUD ON THE INCORPORATION OF [³H]THYMIDINE AND [5-³H]URIDINE INTO THE ENDOMETRIUM OF THE RABBIT DURING EARLY PREGNANCY

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Summary. The influence of a copper IUD on the incorporation of [³H]thymidine and [5-³H]uridine into the endometrium of the rabbit was studied during early pregnancy. The effect of the copper device was compared with those of polyethylene and platinum IUDs.

The comparison showed that the presence of a copper IUD resulted in a significantly lower incorporation of [³H]thymidine on the 3rd day of pregnancy and in a higher incorporation of [³H]thymidine and [5-³H]uridine on the 5th day of pregnancy. The increase in the total amount of DNA in the endometrium in early pregnancy was markedly reduced in the copper IUD-containing horn, and autoradiography showed that the labelling frequency of epithelial cells in the endometrium was significantly reduced. A possible effect of the copper IUD on the stromal cells is discussed.

These effects of copper could be due either to an altered hormonal responsiveness of the endometrium in the presence of copper or to a 'direct' effect of copper on nucleic acid metabolism.

INTRODUCTION

Several reports on the effect of IUDs on the metabolism of nucleic acids have been published (for references, see Grundsell & Håkansson, 1975). Some of the results obtained are contradictory, e.g. the discrepancy in the DNA content in the endometrium and in the endometrial cells (Hagenfeldt, 1972; Hagenfeldt & Johannisson, 1972) and the studies on the incorporation of labelled precursors (Joshi, 1967; Laumas & Yadava, 1969).

Grundsell & Håkansson (1975) studied the effect of a copper IUD and three 'inert' IUDs (two of plastic and one of platinum) on the nucleic acid metabolism in rabbits stimulated with HCG. The copper IUD inhibited the increase in DNA synthesis normally obtained 48 hr after stimulation, whereas the 'inert' IUDs had no such effect. This inhibition resulted in a lower increase in the total amount of DNA in the endometrium. The incorporation of [5-³H]uridine into the endometrium was significantly higher in the horn containing a copper IUD 120 hr after HCG stimulation; 'inert' IUDs had no such effect.

One of the possible reasons for the conflicting data reported in the literature might be differences in the hormonal stimulation of the animals. The hormonal conditions of rabbits made pseudopregnant by stimulation with HCG and those of normally pregnant animals are not necessarily identical. The present investigation studied the effect of a copper IUD on the incorporation of labelled precursors into nucleic acids during early pregnancy.

MATERIALS AND METHODS

Seventy-nine virgin rabbits of mixed breed weighing between 2 and 3 kg were used. Three types of IUDs were studied. (1) Copper IUD (Cu-IUD). This consisted of a polyethylene catheter 50 mm long and 0.5 mm in outer diameter (Clay-Adams, Parsippany, N.Y.), around which a copper wire 0.23 mm in diameter was coiled, giving a surface area of 200 mm²; (2) Platinum IUD (Pt-IUD). This was identical with the Cu-IUD except for the change of metals. (3) Plastic IUD (Pl-IUD_{1.0}). This consisted of a polyethylene catheter, 1.0 mm in diameter and 50 mm long. The IUDs were sterilized in ethanol and rinsed in sterile physiological saline before they were introduced into the uterine horns under sterile conditions through a small puncture in the antimesometrial wall of the uterus just above the uterocervical junction. The lower end of the IUD was fixed by a 4-0 silk suture at the site of the puncture. No corpora lutea were present at the time of operation. The effect of the Cu-IUD was always compared with that of one of the other IUDs. Thus, one horn was fitted with the Cu-IUD and the other with either the Pl-IUD_{1.0} or the Pt-IUD. The IUDs were randomized between the horns. Tables 1 to 3 list the experimental groups with the number of animals. On the 5th day after operation, the does were mated with fertile bucks and then killed by cervical dislocation at different times (0, 36, 48, 60, 72, 96 and 120 hr) after mating. The presence of corpora lutea in each ovary was registered. The uterine horns were opened and the endometrial surface was gently rinsed with Parker 199 medium (SBL, Stockholm, Sweden). The part of the uterine horn corresponding to the length of the IUD (the IUD-part) was carefully scraped with a sharp curette.

The endometrium was put into Parker 199 medium and brought into a suspension of small tissue fragments and single cells by the method of Borell (1952). This suspension was then incubated for 1 hr at 37°C, either with [³H]-thymidine (methyl-[³H]thymidine, sp. act. 1.9 Ci/mmol: Schwarz/Mann Inc., Orangeburg, N.Y.) at a final concentration of 10 µCi/ml, or with [5-³H]uridine (sp. act. 27.8 Ci/mmol: NEN, Frankfurt a.M., West Germany) at a final concentration of 20 µCi/ml. After incubation, the cells were washed with phosphate-buffered physiological saline (pH 7.1), extracted in 5% trichloroacetic acid (TCA) at 4°C for 30 min and washed once in TCA and once in absolute ethanol. The samples were stored at -20°C until they were further processed. The incubations were set up within 90 min of the animals being killed. All tests were carried out in duplicate.

Determination of DNA and radioactivity

The cells were dissolved in 0.6 or 1 ml sodium hydroxide for 1 hr at 37°C.

This solution was used for determination of DNA according to the method of Ceriotti (1952) as modified by Bonting & Jones (1957). A standard curve was made for each series of determinations. The extinction value, OD_{490} , was divided by the angle coefficient of the standard curve. This value was called the adjusted DNA value.

To determine the amount of radioactivity, 100 μ l sodium hydroxide solution was transferred to a scintillation vial. To this was added 1 ml Soluene 100 (Packard Ltd, Stockholm, Sweden) and 14 ml scintillation solution (300 mg dimethyl-POPOP, 5 g PPO, 1 litre toluene). The determinations were then carried out in a Packard TriCarb spectrometer 3310. The efficiency was almost constant and was continuously checked with external standardization.

Preparation of autoradiographic slides and counting of cells

Cell suspensions were incubated with [3 H]thymidine at 37°C for 60 min, as described above. After three washings in phosphate-buffered physiological saline (pH 7.1), pellets were resuspended and fixed for at least 20 min in 60% acetic acid, made 0.1 N with respect to hydrochloric acid, then stained in orcein (2% in 60% acetic acid). Slides were prepared by means of the squash method employed for chromosomal preparations. The autoradiographic procedure was carried out according to the method of Schmid (1963). Kodak AR 10 stripping film was used. The film was exposed for 3 days and developed in Kodak D 19 developer and fixed in Kodak X-ray fixative. The technical background was consistently very low. Only technically good preparations with a fairly equal distribution of cells were used for the registrations.

One slide from each horn was examined, and 400 to 600 randomized microscopic fields were counted. As a measure of the labelling frequency of stromal or epithelial cells, the total number of labelled cells in the microscopic fields was divided by the total number of cells situated along a diameter of the microscopic fields. This diameter was given by a line inserted in the eyepiece of the microscope. The number of cells registered along this diameter was proportional to the total number of cells and was used as it was more easily counted. Epithelial and stromal cells were assessed separately. No differences were found between duplicate slides analysed in this way.

Statistical considerations

The ct/min/DNA values are not normally distributed (Nordqvist, 1969), but a near normal distribution can be obtained by conversion to logs. Thus, as a measure of the incorporation of the different labelled precursors, the following expression was used:

$$a = 100 \times \log_{10} \frac{\text{ct/min} \times 10^4}{\text{AES} \times (\text{DNA})}$$

where (DNA) was the adjusted DNA value in OD_{490} units and AES the value of the automatic external standard. As the adjusted DNA value in OD_{490} units was proportional to mg DNA, the a values were related to $\log \text{ct/min/mg DNA}$. The statistical calculations were performed on these a values (mean of double values for each horn). In the Text-figures, the mean values of the

incorporation of labelled nucleosides have been expressed as ct/min/DNA (a values divided by 100 and converted to anti-logs), which permits a direct estimate of the differences. The difference was calculated between a values for the two horns of each animal. The deviation from zero was tested by an analysis of variance where all sub-groups (different times after mating of the animals) in each experimental group were included. The analysis of variance was performed according to the following formula:

$$F = \frac{(Q_A + n\bar{x}^2)/n}{Q_O/(N-n)}$$

where Q_A was the sum of squares between sub-groups, $\bar{x}$ was the mean value of all differences in the group, Q_O was the sum of squares within sub-groups, N was the total number of animals in the group, and n was the number of sub-groups. If the differences of a group were found to deviate significantly from zero, the deviation of the mean differences of each sub-group from zero could be analysed by means of a two-tailed t test (Armitage, 1971).

$$t = \frac{\bar{x}_i}{\sqrt{Q_O/(N-n)} \cdot \sqrt{1/n_i}}$$

where $\bar{x}_i$ was the mean value of the differences of the sub-group, n_i was the number of animals of the sub-group, and Q_O , N , and n were as above.

These statistical methods were also used to analyse differences in the total amount of DNA in horns fitted with different IUDs. The amount of DNA was expressed in OD₄₉₀ units, as described above. To get equal variances in the different sub-groups, the DNA values were converted to logs. Anti-logarithmic values were used to construct the Text-figures (see above).

RESULTS

Effect of IUDs on the incorporation of [³H]thymidine

The effect of the Cu-IUD on the incorporation of [³H]thymidine was compared with that of the PI-IUD_{1.0} in a pilot series. After mating, the incorporation increased in the endometrium of horns fitted with both types of IUDs. A maximum was reached about 48 hr *post coitum* (*p.c.*). This peak seemed to be lower in the Cu-IUD horn. At 0, 36, 96 and 120 hr *p.c.*, the incorporation of [³H]thymidine seemed to be higher in the Cu-IUD-containing horn. When all sub-groups of this series were tested together, a significant difference in the incorporation of [³H]thymidine in horns fitted with the PI-IUD_{1.0} and the Cu-IUD was obtained ($0.01 < P < 0.025$), but t tests on each sub-group showed a statistically significant difference only at 120 hr ($0.001 < P < 0.01$). Another experimental series was devised to find out whether these effects of the Cu-IUD could be reproduced. From Table 1 (Group I) and Text-fig. 1a, the results of this series were seen to be almost identical with those of the pilot series, and the reduced incorporation of [³H]thymidine in the Cu-IUD-containing horn at 60 hr *p.c.* was statistically significant ($0.01 < P < 0.02$).

The effect of the Cu-IUD was also found to differ significantly from that of a Pt-IUD ($P < 0.001$; Table 1, Group II, Text-fig. 1b). The incorporation of

Table 1. Effect of IUDs on the incorporation of [^3H]thymidine into the DNA in endometrial cells of pregnant rabbits

Group	Combination of IUDs	F value	Significance (P_s)	Sub-groups (hr)	No. of animals	Mean difference	S.E.	Significance (P)
I	Pt-IUD _{1.0} and Cu-IUD	6.76 at 3 and 18 d.f.	0.005 < P < 0.01	0 60 120	7 7 7	-7.83 20.50 -27.49	7.81 7.81 7.81	N.S. 0.01 < P < 0.02 0.005 < P < 0.01
II	Pt-IUD and Cu-IUD	11.68 at 2 and 15 d.f.	P < 0.001	0 60 120	6 6 6	-24.65 43.18 -26.87	9.55 9.55 9.55	0.01 < P < 0.025 P < 0.001 0.01 < P < 0.02

The differences in incorporation between differently treated horns in each group were tested in an analysis of variance. The table shows the variance ratios (F values) and the significance levels (P_s). The deviation of the mean differences of each sub-group from zero was analysed by means of a t test. The table gives the significance levels (P). A negative mean difference implies that the incorporation is lower in the Pt-IUD_{1.0} or Pt-IUD-containing horns.

TEXT-FIG. 1. Mean incorporation of [^3H]thymidine into DNA in endometrial cells of PI-IUD_{1.0}- (hatched columns), Pt-IUD- (solid columns) and Cu-IUD- (open columns) containing horns in pregnant rabbits at 0, 60 and 120 hr after coitus.

[^3H]thymidine was found to be significantly higher in the Cu-IUD horn at 0 hr ($0.01 < P < 0.025$) and at 120 hr ($0.01 < P < 0.02$), but was significantly lower at 60 hr ($P < 0.001$).

Effect of IUDs on the incorporation of [^3H]uridine

In a pilot series, the incorporation of [^3H]uridine into the endometrium of horns fitted with the PI-IUD_{1.0} or the Cu-IUD increased after mating, reaching

TEXT-FIG. 2. Mean values of the incorporation of [^3H]uridine into RNA in endometrial cells of PI-IUD_{1.0}- (hatched columns) and Cu-IUD- (open columns) containing horns in pregnant rabbits at various times after coitus.

Table 2. Effect of IUDs on the incorporation of [5-³H]uridine into RNA in endometrial cells of pregnant rabbits

Group	Combination of IUDs	F value	Significance (Ps)	Sub-groups (hr)	No. of animals	Mean difference	S.E.	Significance (P)
I	PI-IUD _{1.0} and Cu-IUD	3.49 at 3 and 16 d.f.	0.025 < P < 0.05	0 60 120	5 7 7	-0.99 7.17 -25.70	9.76 8.25 8.25	N.S. N.S. 0.005 < P < 0.01
II	Pt-IUD and Cu-IUD	8.02 at 3 and 14 d.f.	0.001 < P < 0.005	0 60 120	5 6 6	-31.66 11.13 -39.85	11.27 10.29 10.29	0.01 < P < 0.02 N.S. 0.005 < P < 0.01

A negative mean difference implies that the incorporation is lower in the PI-IUD_{1.0} or Pt-IUD-containing horns.

a peak at about 48 hr *p.c.*, and the incorporation of [5-³H]uridine seemed to be higher in the Cu-IUD-containing horn at 96 and 120 hr (Text-fig. 2). An analysis of variance including all sub-groups gave an *F* value of 2.73 (at 7 and 13 d.f.), which was almost significant ($P \approx 0.05$). These results were reproduced in another experimental series (Table 2, Group I, Text-fig. 3a). An analysis of variance including all sub-groups gave a statistically significant *F* value ($0.01 < P < 0.05$) and a *t* test showed that only the mean difference at 120 hr *p.c.* was statistically significant ($0.001 < P < 0.01$).

When the effect of the Cu-IUD was compared with that of the Pt-IUD (Table 2, Group II, Text-fig. 3b), the results were found to be almost identical with those obtained when the Cu-IUD was compared with the Pl-IUD_{1.0}. In this group, however, the incorporation of [5-³H]uridine was higher in the Cu-IUD-containing horn at 120 hr *p.c.* ($0.001 < P < 0.01$) and at 0 hr ($0.01 < P < 0.02$).

TEXT-FIG. 3. Mean values of the incorporation of [5-³H]uridine into RNA in endometrial cells of Pl-IUD_{1.0}- (hatched columns), Pt-IUD- (solid columns) and Cu-IUD- (open columns) containing horns in pregnant rabbits at 0, 60 and 120 hr after coitus.

Effects of IUDs on the DNA content in the endometrium

The total amount of DNA in the endometrium of the IUD-part of each uterine horn was determined. The DNA content in the endometrium was markedly increased during early pregnancy in the pilot series and in Groups I and II (Table 3). This increase was less in the presence of the Cu-IUD (Text-figs 4, 5a and b). Table 3 gives the results of the analysis of variance for the three groups separately (pilot series and Groups I and II). There were significant differences in DNA content between differently treated horns in all groups. In the pilot series, the DNA content was significantly decreased at 72, 96, and 120 hr *p.c.* In the other two groups (when the Cu-IUD was compared to the Pl-IUD_{1.0} and the Pt-IUD), the amount of DNA was significantly lower at 60 and 120 hr (Table 3).

Autoradiographic analysis of endometrial cells

The effect of the Cu-IUD on the epithelial and stromal cells was studied by means of autoradiography. The endometrial cell suspensions from three of the

Table 3. Effects of IUDs on the total amount of DNA in endometrial cells of pregnant rabbits

Group	Combination of IUDs	F value	Significance (Ps)	Sub-groups (hr)	No. of animals	Mean difference	S.E.	Significance (P)
Pilot series	Pl-IUD _{1.0} and Cu-IUD	7.63 at 7 and 13 d.f.	$P < 0.001$	0	2	-0.0449	0.1944	N.S.
				36	2	0.1346	0.1944	N.S.
				48	3	0.0553	0.1588	N.S.
				60	2	0.1007	0.1944	N.S.
				72	5	0.4682	0.1229	$0.005 < P < 0.01$
I	Pl-IUD _{1.0} and Cu-IUD	6.03 at 3 and 21 d.f.	$0.001 < P < 0.005$	96	3	0.4636	0.1588	$0.01 < P < 0.02$
				120	3	0.8622	0.1588	$P < 0.001$
				0	10	-0.0581	0.0650	N.S.
				60	7	0.2153	0.0778	$0.01 < P < 0.02$
				120	7	0.2419	0.0778	$0.005 < P < 0.01$
II	Pt-IUD and Cu-IUD	8.27 at 3 and 19 d.f.	$0.001 < P < 0.005$	0	10	0.1200	0.0779	N.S.
				60	6	0.2832	0.1006	$0.01 < P < 0.02$
				120	6	0.3832	0.1006	$0.005 < P < 0.01$

A negative mean difference implies that the total amount of DNA is lower in the Pl-IUD_{1.0}- or Pt-IUD-containing horns (see also Table 1).

TEXT-FIG. 4. Mean values of the total amount of DNA expressed in OD₄₉₀ units for the PI-IUD_{1.0}- (hatched columns) and Cu-IUD- (open columns) containing horns of pregnant rabbits at various times after coitus.

TEXT-FIG. 5. Mean values of the total amount of DNA expressed in OD₄₉₀ units for PI-IUD_{1.0}- (hatched columns), Pt-IUD- (solid columns) and Cu-IUD- (open columns) containing horns of pregnant rabbits at 0, 60 and 120 hr after coitus.

Table 4. Labelling frequencies in stromal and epithelial cells of uterine horns containing PI-IUD_{1.0} or Cu-IUD in pregnant rabbits

Rabbit no.	PI-IUD _{1.0} horn		Cu-IUD horn		χ^2	P
	Unlabelled	Labelled	Unlabelled	Labelled		
Stromal cells						
I	452	13	334	7	0.44	—
II	553	15	283	1	5.30	—
III	1053	9	345	2	0.24	—
Epithelial cells						
I	68	110	53	49	5.00	0.01 < P < 0.025 P < 0.001 P < 0.001
II	90	165	45	36	10.50	
III	61	59	48	14	12.03	

animals studied at 72 hr *p.c.* in the pilot series (where the effect of the Cu-IUD was compared with that of the PI-IUD_{1.0}) were used to prepare autoradiographic slides. Table 4 shows that the labelling frequency of the epithelial cells was lower in the Cu-IUD-influenced horns. This reduction in labelling frequency was statistically significant in the three does studied (Table 4). A tendency towards a lower labelling frequency of the stromal cells in the Cu-IUD-containing horn was noted. As the figures obtained from each doe were fairly low, the χ^2 values obtained from all animals were added: $\chi^2=5.98$ at 3 d.f. ($P>0.05$).

DISCUSSION

The effects of the Cu-IUD on the incorporation of [³H]thymidine into endometrial cells in pregnant rabbits agree well with those reported earlier in rabbits made pseudopregnant by stimulation with HCG (Grundsell & Håkansson, 1975), i.e. the incorporation of [³H]thymidine was depressed in the Cu-IUD-influenced endometrium at 60 hr *p.c.*, whereas it was significantly increased in the Cu-IUD-containing horn at 120 hr *p.c.* Almost identical results were obtained when the Cu-IUD was compared with the effect of either the PI-IUD_{1.0} or the Pt-IUD. Similar results have also been reported by Prager (1969) who found a decreased incorporation of [¹⁴C]thymidine into the endometrium of the rat in the presence of a copper IUD. It is possible, however, that Prager's results do not reflect an effect on DNA synthesis (Grundsell & Håkansson, 1975).

The results in Table 4 show that the labelling frequency of epithelial cells influenced by copper was significantly lower than that of those influenced by a plastic IUD; a slight tendency towards a lower labelling frequency of the stromal cells influenced by copper was observed. If the differences in the total amount of DNA were due only to an effect on the epithelial cells, the ratio of epithelial cells to stromal cells should be markedly higher in the control-IUD horns than in the Cu-IUD horns. No such difference was found, however, and thus copper also seems to have an influence on the stromal cells.

Although the Cu-IUD led to a greater incorporation of [5-³H]uridine into the endometrium than did the PI-IUD_{1.0} or Pt-IUD at 120 hr, and the Pt-IUD at 0 hr *p.c.* no difference between the Cu-IUD and PI-IUD_{1.0} was observed at 0 hr. Thus the type of 'inert' control IUD is not without importance in studies of the effects of 'active' IUDs. The effect of the Cu-IUD is almost identical in pseudopregnant (Grundsell & Håkansson, 1974) and pregnant animals. There seems to be some difference, however, in the stimulation, as the incorporation of [5-³H]uridine into the endometrium increases during the first days of pregnancy, whereas it gradually decreases during early pseudopregnancy.

At the time of maximum proliferation, i.e. 48 to 72 hr *p.c.* or after HCG injection (Lloyd, 1937; Lee & Dukelow, 1972; Davies & Hoffman, 1973), a copper IUD caused a lower incorporation of [³H]thymidine into the endometrium of the rabbit. The endometrial proliferation following ovulation is the result of progesterone acting on oestrogen-primed endometrium (Allen, 1930;

Hisaw & Leonard, 1930; Leonard, Hisaw & Fevold, 1932; Courrier, 1950; Leroy, 1974). A reduced endometrial proliferative response in the presence of a Cu-IUD, as demonstrated in the present study and in a previous study (Grundsell & Håkansson, 1975), may be due either to an altered hormonal responsiveness of the endometrium in the presence of copper or to a 'direct' effect of copper on nucleic acid metabolism.

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