EFFECT OF IUD ON UTERINE CYCLIC AMP AND THE ACTIVITIES OF ADENYL CYCLASE AND PHOSPHODIESTERASE DURING THE OESTROUS CYCLE AND EARLY PREGNANCY IN RATS

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Oestrogen administration to spayed rats produces biochemical changes in the uterus. Wrenn, Wood & Bitman (1969a) found that oestrogen increased the glycogen and nucleic acid content and the weight of the uterus and Szego & Davis (1967) noted that oestrogen raised the level of cyclic AMP in the uterus. The presence of an IUD has been shown to cause similar changes (Wrenn *et al.*, 1969b). There thus appears to be a similarity between the action of oestrogen and the effect of an IUD. The effect of an IUD on uterine cyclic AMP was investigated in rats during the oestrous cycle and early pregnancy. The activities of adenylyl cyclase and phosphodiesterase were also studied.

Batches of about ten female Sprague-Dawley rats about 10 weeks old and weighing from 170 to 200 g were used. The stage of the oestrous cycle was determined from vaginal smears. Nylon IUDs were made and inserted through the cervical os into one uterine horn at dioestrus as described by Wrenn *et al.* (1968). The rats were kept for 2 weeks and were observed to have regular cycles before their use in experiments. Rats at pro-oestrus were placed with males of proven fertility. The vaginal smears were examined daily and the day on which spermatozoa were found was designated Day 1 of pregnancy.

Rats were anaesthetized with 30 mg pentobarbital/kg given intraperitoneally. The uteri were removed and immediately dropped into liquid air. Fat was removed from the frozen uteri. The IUD-bearing and contralateral control horns were weighed separately and ground in 10 vols of 6% trichloroacetic acid in an all-glass homogenizer and the suspension was centrifuged. The supernatant was extracted four times with ten times its volume of peroxide-free ether. A 50- μ l vol. of the trichloroacetic acid-free supernatant was then transferred to a fresh test-tube, freeze-dried and mixed with 50 μ l tris buffer supplied with the cyclic AMP kit (Code TRK 432, The Radiochemical Centre Ltd., Amersham, England). The assay for cyclic AMP was carried out according to the instructions given with the kit. A toluene/triton X-100 based scintillant (2 vols toluene containing 0.4% w/v PPO and 0.01% w/v POPOP and 1 vol. triton X-100) was used and radioactivity was measured in a Packard Liquid Scintillation Counter. The recovery of cyclic AMP by this extraction process was

determined using tritium-labelled cyclic AMP issued with the assay kit. A mean recovery value of $98 \pm 1.7\%$ was obtained.

Rats were killed by cervical dislocation and their uteri were immediately removed and trimmed free of fat. The IUD was removed and the 'experimental' and contralateral 'control' horns were weighed separately. Each horn was then minced into small pieces and homogenized in six times its weight of 20 mM-tris-HCl buffer, pH 7.4, containing 10 mM-KCl, 4 mM-MgCl₂ and 10 mM-theophylline. A Sorvall Omni-mixer with a 5-ml microchamber attachment was used. The homogenate was kept at 0 to 4°C and used on the same day.

The enzyme reaction was started by adding 100 µl of the homogenate to 0.2 ml tris buffer containing, in the final volume of 0.3 ml, 2 mM-ATP, 4 mM-MgCl₂, 10 mM-theophylline, 10 mM-NaF, 10 mM-phosphoenol pyruvic acid and 10 units pyruvate kinase. The mixture was incubated for 15 min at 30°C after which the enzyme reaction was stopped by immersing the tube in boiling water for 1 min. Evaporation was minimized by sealing the tube with Parafilm 'M' paper (American Can Company, Wisconsin). The tube was then cooled in ice and after centrifugation, 100 µl supernatant were transferred to a fresh test-tube and freeze-dried. Assay of cyclic AMP was carried out as described above. Production of cyclic AMP in the above assays was linearly related to time for the first 35 min when 2 mM-ATP was used. When 1 mM-ATP was used, the linearity lasted only 15 min but the rate of cyclic AMP production was unchanged.

The homogenate of rat uterus was prepared as described for extraction of adenylyl cyclase except that the buffer did not contain theophylline and a w/v ratio of 10 instead of 6 was used. The activity of phosphodiesterase was assayed as described by Sim & Chantharaksri (1973).

Adenosine 5'-triphosphate, phosphoenol pyruvic acid, pyruvate kinase (sp. act. 425 units/mg protein) and adenylyl deaminase (sp. act. 30 to 60 units/mg protein) were obtained from Sigma.

The tissue cyclic AMP levels and the activities of the adenylyl cyclase and phosphodiesterase at the various stages of the oestrous cycle were compared in experimental and control horns of the uterus (Text-fig. 1a). Significant increases in the cyclic AMP level and adenylyl cyclase activity were observed in the IUD-bearing horn at early and late metoestrus ($P < 0.05$). The phosphodiesterase activity measured in the IUD-bearing horn was generally greater than in the control horn but the differences were not statistically significant ($P > 0.05$).

Similar comparisons of the same parameters during early pregnancy are presented in Text-fig. 1(b). The cyclic AMP level and the activities of adenylyl cyclase and phosphodiesterase were increased in the experimental horn compared to those in the contralateral pregnant horn. No pregnancy occurred in any of the IUD-bearing experimental horns. Significant differences were observed from Day 3 with peak differences occurring during Days 4 and 5 ($P < 0.001$).

The results presented in this paper indicate that the presence of an IUD is associated with increased levels of cyclic AMP in the uterus during the oestrous cycle and during early pregnancy.

The increase in cyclic AMP in the IUD-bearing horn of the uterus during the

oestrous cycle shows correlations with a similar increase in adenylyl cyclase activity. The phosphodiesterase activity was also increased but not significantly. These changes favour an increased cellular turnover of cyclic AMP. Hechter, Yoshinaga, Halkerston & Birchall (1967) have shown cyclic AMP to be able to initiate many biosynthetic processes in the rat uterus, e.g. synthesis of glycogen, RNA, DNA and protein. Intrauterine devices have also been observed to induce similar biosynthetic processes in the uterus at the period of expected implantation (Wrenn *et al.*, 1969b). It is likely that the increased

TEXT-FIG. 1. Comparison of cyclic AMP, adenylyl cyclase and phosphodiesterase activity in IUD-bearing (○) and control (●) horns of rat uteri at different stages of the oestrous cycle (a) and early pregnancy (b). Each point represents the mean $\pm$ S.E. of determinations on a minimum of three and a maximum of seven animals. PE = pro-oestrus; ES = oestrus; EM = early metoestrus; LM = late metoestrus; DE = dioestrus.

biochemical activity is mediated through an increased turnover rate of tissue cyclic AMP. Whether the biosynthetic processes induced by an excess of cyclic AMP interfere with the normal uterine biochemistry and produce conditions unfavourable to implantation is not known.

In the rat, Doyle & Margolis (1964) have shown that the presence of an IUD prevents implantation, which normally occurs between Days 4 and 5, i.e. the days in the present study on which the presence of an IUD induced peak differences in cyclic AMP levels. This finding tends to support the view that IUD action is mediated through biochemical processes.

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REFERENCES

- DOYLE, L. L. & MARGOLIS, A. J. (1964) Intrauterine foreign body. 1. Effect on reproductive processes in the rat. *Fert. Steril.* **15**, 596.
- HECHTER, O., YOSHINAGA, K., HALKERSTON, I. D. & BIRCHALL, K. (1967) Oestrogen-like anabolic effects of cyclic 3'-5'-adenosine monophosphate and other nucleotides in isolated rat uterus. *Archs Biochem. Biophys.* **122**, 449.
- SIM, M. K. & CHANTHARAKSRI, U. (1973) Rat uterine contractility and the activities of uterine adenylyl cyclase and phosphodiesterase during the oestrous cycle. *Biochem. Pharmacol.* **22**, 1417.
- SZEGO, C. M. & DAVIS, J. S. (1967) Adenosine 3',5'-monophosphate in rat uterus: acute elevation by oestrogen. *Proc. natn. Acad. Sci. U.S.A.* **58**, 1711.
- WRENN, T. R., WOOD, J. R. & BITMAN, J. (1968) A new technique for introducing IUDs into rat uteri. *J. Reprod. Fert.* **16**, 515.
- WRENN, T. R., WOOD, J. R. & BITMAN, J. (1969a) IUDs and the biochemical responses of the uterus to estrogen in ovariectomized rats. *Biol. Reprod.* **1**, 234.
- WRENN, T. R., WOOD, J. R. & BITMAN, J. (1969b) Alterations in the uterine environment produced by IUDs. *J. Reprod. Fert.* **19**, 511.