CHAPTER IV

DISCUSSION

Recently the change in biochemical composition of the IUD fluid have been studied extensively in rats. Yaovapolkul (3) and Jantaraniyom (8) reported the significant increase of protein in the IUD fluid. In this investigation, we also found that there was a significant increase of the total protein in the IUD fluid of about 4 fold of that found in control fluid (Table 1). Karr, et al (7) suggested that the increase in protein level in the IUD fluid could result from 2 possible causes: firstly, leaking of blood proteins into the uterine lumen through alteration in vascular permiability of the endometrium, and secondly the protein level might be generated from the lysis of cells such as the polymorphonuclear leukocyte and the denuded surface epithlial elements. The first possibility was supported by the report that the uterine fluid of the rats fitted with IUD showed the presence of a protein having the same electrophoretic mobility as autologous serum albumin (19). The second possibility required the presence of lysozomal enzymes for cells lysis or some specific protease for the cleavage of surface proteins from the epithelial lining. However, so far there was no evidence about the increase of these enzymes in the IUD fluid. Jantaraniyom (8) showed by the comparision of the protein patterns in polyacrylamide or 6% cyanogum gels of the IUD and control fluid that they were different both qualitatively and quantitatively, and the IUD contained a specific protein characteristic to the fluid but lack a minor protein normally found in the control fluid, but there was

no evidence for the de novo synthesis of that IUD-specific protein. In this study our SDS-gel electrophoresis experiment showed that there was an obvious increase in P_4-protein in the IUD fluid, which was however might not be an IUD-specific protein because P_4 was also present in the control fluid in smaller quantity. P_4 was suspected to be the protein involved in the noncovalent binding with the inorganic phosphate (P_i) to form a ^{32}P-labelled complex observed on the Sephadex G-25 column (Fig. 10 c, d, e and f).

The markedly increase of P_i in the IUD fluid previously reported by Yaovapolkul (3) was confirmed in this study (Table 1). The cause of this increase in P_i may be dued to several sources such as: it might be the by product of many energy-requiring reactions which break down ATP to ADP and P_i, or by increasing adenyl cyclase activity which resulting in the increase of cAMP and PP_i, which was subsequently broken down to P_i, or by the leaking of blood P_i into the IUD-bearing horn via alteration of vascular permeability of the endometrium. The second possibility was supported by Sim (10) who reported that there was an increase of adenyl cyclase activity and cAMP concentration in the IUD fluid of the rat. The very high concentration of P_i in the normal rat plasma is 10 ± 1 mg/ml plasma (20) also allowed the third possibility, which had been demonstrated by Karr, et al (19) to encounter for the increase of protein in the IUD fluid.

Although there were several other molecules reported to be increased in the presence of an IUD (3, 11, 21, 22), our interest was focused on P_i and protein because Yaovapolkul (3) showed that addition of P_i into control fluid until the phosphate concentration reached that found in IUD fluid

caused the antifertility effect on day 4 of pregnancy. In order to prove whether increasing P_i had any interaction with the protein in the uterine fluid, ^{32}P-P_i solely or previously incubated with the uterine fluid at various times and temperatures were separated on Sephadex G-25 column. Our investigation showed that ^{32}P-P_i specifically bound to some macromolecular components with M.W. about 5,000 dalton present in both control and IUD fluid, but did not bind to BSA (Fig. 1o a, b, c, d, e and f, Fig. 11b). Walser (23) also reported that there was a binding between plasma P_i and the plasma protein.

The results from the chasing experiment (Fig. 12a and b) and the SDS-gel electrophoresis of the ^{32}P-labelled fractions (Fig. 13a, b, c and d) indicated that the binding between ^{32}P-P_i and this protein was not occurred through phosphorylation or covalent linkage. The resistance of this binding to heating at 100°C for 10 min suggested that the protein involved in the binding was quite thermostable.

Our study on the antifertility effect of various concentrations of P_i in pregnant rats showed that P_i exerted the most drastic effect on the day of implantation (day 5 of pregnancy) as shown in Fig. 3, 4 and 5. Dubin (24), who used pregnant Sprague Dawley rats, which was a different strain used in this study, as a model illustrated that direct application of merely normal saline (0.1 ml) on day 4, 5, 6 or 7 of pregnancy decreased the fetal viability. Injection on day 6 of pregnancy was the most critical, and the volume not greater than 0.1 ml was recommended in this strain. In our case, substituting control fluid by injection into the left uterus with normal saline (Fig. 5)resulted in

milder antifertility effect of P_i (Fig. 4). These results implied that the antifertility effect of P_i required the presence of some components in the control uterine fluid, to form a biologically active complex that mediate the complete contraception. This postulation was supported by the result shown in Fig. 6 that replacement of P_i injection on day 5 with the same volume of normal saline or control fluid allowed the implantation and development of normal fetuses to occur and the antifertility effect of P_i injected into the right uterus of day 5 pregnant rats was most intensified when premixed with control uterine fluid.

Doyle and Margolis (14) reported that an IUD exerted a contraceptive effect unilaterally only in the uterus inserted that IUD. In the present study, when free P_i was injected into the right uterus, the transferred antifertility effect was observed also in the left uterus (Fig. 4 and 5). It was suggested that insertion of IUD caused an increase of P_i and protein which formed a complex molecule immediately and therefore could not diffuse into the other uterus, but free P_i injected into the right uterus could migrate to the left uterus and then bound with the component in that left uterus. In the case that control fluid was injected into the left uterus, the complex can be formed thus mediated the antifertility effect of P_i in both uteri. This postulation was supported in the experiment that ^{32}P-P_i was injected *in vivo* into the right uterus of day 5 pregnant rats and the radioactive ^{32}P was detected in every fraction of both uteri, especially in the muscular tissue of both uteri within 10 min after injection. The radioactive ^{32}P-P_i was even retained there at a constant level up to 4 days after injection (Fig. 7, 8 and 9).

The possible explanation exist for the antifertility effect of IUD which takes into account all the observations in this study is that insertion of an IUD in the rat uterus caused the increasing of P_i and accumulation of some proteins in that uterus. The increased P_i should bind specificly to some proteins and form a complex which was taken up and retained by the muscular tissue.

Doyle and Margolis (25) suggested that an IUD in the mouse inhibited implantation by alteration the tubal transport of ova. This tubal transport might be occurred by abnormal motility of the uterine muscle (26). Our investigation suggested that the complex between P_i and protein in IUD fluid might exerted its antifertility effect on the muscular contraction of the rat uterus.

Since the hydrolysis of ATP is known to be the source of immediate chemical energy in muscle (27). This ATP will bind to the myosin head and then is hydrolysed by myosin ATPase to ADP and P_i which bound to myosin and forming an "activated complex" readily bound to actin molecule. The combination of the activated myosin with actin is beleived to promote the release of ADP and P_i and a conformational change of the myosin cross-bridge which pulls the bound thin filament of actin toward the center of sarcomere and cause contraction. This phenomena is known as the sliding filament model of muscle contraction (28). In 1941, Lynen and Johnson independently suggested that P_i may be a key substance in controlling the rate of glycolysis which is the pathway generating ATP to muscle contraction. P_i is a reactant in the conversion of glyceraldehyde-3-phosphate to 1,3-diphosphoglyceric acid. Hence, it is suggested that P_i concentration that

increased in the IUD fluid might or might not accerelate ATP forming by glycolysis and increased the muscular contraction in a high manner.

Ca^{++} is another regulator in muscular contraction, ATP bound to myosin head would not split unless Ca^{++} was provided at a concentration in excess of 10^{-7} M. At this concentration, Ca^{++} will bind to troponin and causes tropomyosin to move deep into the groove on the thin filament, allowing actin and myosin to interact and producing muscular contraction (29). If a greater concentration of Ca^{++} was provided, splitting of ATP took place at agreater rate. Ca^{++} that enters all through the plasma membrane plays an important role in excitation contraction coupling in many smooth muscle, in such tissues, contractiors are diminished if the extracellular Ca^{++} concentration is lowered and are increased in the presence of elevated extracellular Ca^{++} (30). Yaovapolul (3) reported that, in rat, there was an increase of Ca^{++} in IUD fluid to 7 fold of that in control fluid. This increasing Ca^{++} might serve as the extracellular Ca^{++} and caused increased contraction of smooth muscle in the IUD-bearing uterus and resulted in the antifertility effect on the implantation of the blastocyst.

Moreover, Chaudhuri (22) found that prostaglandin(s) was released from the rat uterus inserted with an IUD. Prostaglandins E_2 and $F_{2\alpha}$ are now established as powerful oxytoxic agents and are used for induction of labour or abortion (31, 32). Prostaglandin and P_i may function together to mediate the antifertility effect of an IUD or may not.

Since the incorporation of ^{32}P-P_i was also retained in the endometrial lining of both uteri in the same manner as observed with the muscular

tissue (Fig. 9), although the level was always lower, the possibility that the endometrium was another target tissue of P_i could not be excluded. In rabbit, Halbert(33)reported that a mixture of ciliary activity of the endometrium and muscular contractions were both important for the ovum transport down the oviduct to the implantation site. Ovum transport in mice, however appeared to differ from that of the rabbit as shown by Talo (34) that the myoelectrical activity of the muscular tissue extended from the ampullary region through the isthmus was needed for this feature. These findings at least suggested that interaction of P_i with either endometrium or myometrium or both might disturb ovum transport and resulted in defected implantation.

This investigation might open a new approach to understand more about the mechanism of action of an intra-uterine device.