BIOCHEMICAL CHANGES IN UTERINE FLUID AFTER INSERTION OF VARIOUS TYPES OF INTRA-UTERINE CONTRACEPTIVE DEVICE

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Fertilized ovum transported from the oviduct remains for several days in the uterine fluid until its nidation in endometrium. A large supply of metabolites is necessary for the growth of cellular mass and accumulation of blastocoelic fluid. Endometrial secretions provide the optimal intrauterine environment for the nutrition of pre-implantation blastocyst.

Uterine fluid is a very rich source of amino acids. Most individual amino acids are more concentrated in the uterine fluid than in the blood serum. With many amino acids a definite gradient exists between the uterine fluid and the blastocoelic fluid; thus facilitating transfer by simple diffusion.

Specific protein e.g. 'Blastokinine' in rabbit appears at a definite time before implantation and disappears after implantation; thus suggesting its role in the induction and regulation of blastocystic development and implantation. In human uterine fluid also, a similar protein fraction occupying the same position as 'Blastokinine' was obtained on immunoelectrophoresis. Electrolytes by providing optimum membrane potential induce blastocyst endometrial adhesion.

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Material and Method

The present work has been carried out on women attending the Out-patients' Department and Family Planning Centre of Patna Medical College Hospital for observing the biochemical changes in the composition of uterine fluid after insertion of Copper-T 200.

In both Lippes loop and Copper-T 200 series, the maximum number of patients had the uterine fluid collected between 3 to 6 months of use. In a very small group in Copper T-200 series it was collected after a long term use (after one year).

In all the 3 series those in proliferative phase slightly outnumbered those in the secretory phase.

Protein was found to be increased in the secretory phase as compared to the proliferative phase in all. Those with Lippes loop and Copper T-200 showed increased protein levels in proliferative phase as compared to the control, the increase being more marked with Copper T-200. Secretory phase increase was also much more marked with Copper T-200.

In the control a decrease in sodium level of uterine fluid was observed in the secretory phase, whereas in those with Lippes loop or Copper T-200 it was seen to be increased in the secretory phase.

TABLE I

Distribution of Duration of Contraceptive (I.U.C.D.) in the Test Series Before Collection of the Uterine Fluid

Duration in months	Lippes loop		Copper-T 200	
	No. of cases	Percentage	No. of cases	Percentage
3 to 6	6	60.0	24	58.54
6 to 9	4	40.0	9	21.95
9 to 12	–	—	6	14.63
>12	–	—	2	4.88
Total	10	100.0	41	100.00

TABLE II

Phase of Menstrual Cycle at the Time of Collection of Uterine Fluid in the Control and I.U.C.D. Series

Phase of menstrual cycle	Control		Lippes loop		Copper T-200	
	No. of cases	Percentage	No. of cases	Percentage	No. of cases	Percentage
Proliferative	14	63.64	6	60.00	27	65.85
Secretory	8	36.36	4	40.00	14	34.15
Total	22	100.00	10	100.00	51	100.00

TABLE III

Quantity of Protein in Uterine Fluid in Different Phases of Menstrual Cycle in the Control and Test Series

Phases of menstrual cycle	Protein in mgm/100 ml.		
	Control	Lippes loop	Copper T-200
Proliferative	267.857 ± 16.412	333.66 ± 7.527	338.333 ± 25.198
Secretory	299 ± 3.545	349.5 ± 5.972	382.857 ± 7.080

TABLE IV

Quantity of Sodium in Uterine Fluid in Different Phases of Menstrual Cycle in the Control and I.U.C.D. Series

Phases of menstrual cycle	154.805 ± 4.803		
	Control	Lippes loop	Copper T-200
Proliferative	143.428 ± 3.278	119.733 ± 2.298	138.455 ± 4.121
Secretory	123.425 ± 2.198	136.3 ± 4.912	Na + mEq/L.

TABLE V

Potassium Level of Uterine Fluid in Different Phases of Menstrual Cycle in the Control and I.U.C.D. Series

Phases of menstrual cycle	K + mEq/L.		
	Control	Lippes loop	Copper T-200
Proliferative	13.321 ± 1.522	14.0 ± 1.224	17.7 ± 2.731
Secretory	29.337 ± 2.154	32.45 ± 0.806	33.428 ± 1.892

Level of potassium in the uterine fluid was found to be slightly increased over pre-insertional level (control) with Lippes loop, and a little more with Copper T-200 in the proliferative phase. Secretory phase showed a marked rise in potassium level in both control and I.U.C.D. series.

Discussion

Increase in protein level in uterine fluid of control cases in the secretory phase occurred in accordance with the findings of Kar *et al* (1968). Higher level of protein in endometrium in the secretory phase has also been reported by Rosado *et al* (1972). Moyer and Mischell (1971) could not find any significant difference in the two phases either in control or in I.U.C.D. series.

This increase in the secretory phase of the control could be explained by the appearance of specific protein fractions, probably manufactured in the endometrium and secreted into the uterine fluid as described by Kunitake and Wells (1965). On the basis of qualitative analysis of protein fractions in which out of 9 fractions present in the uterine fluid, 4 fractions were found to be similar to serum and 5 native to the uterine fluid. Appearance of a specific protein 'Blastokinine' has been reported by Krishnan and Daniel (1967) in rabbit at 3 days post-coitus, reaching a maximum at 5 days post-coitus, and disappearing by 8 days post-coitus. Gerald *et al* (1971) found that uterine washings in women contained protein fractions with similar electrophoretic mobility as Beta-globulin not present in human serum on immunoelectrophoretic examination, thus testifying the secretory contribution of endometrium. However, they could not ascertain any relationship between the number of protein bands and the phase of menstrual cycle.

Increase in protein content of uterine fluid occurred in the presence of I.U.C.D. in both phases. This is in accordance with the findings of Kar *et al* (1968) in women using Lippes loop. Moyer and Mischell (1971) also reported similar effect with Lippes loop, but they did not find any significant difference between the 2 phases. Kar *et al* (1964) also reported similar results in rats with intrauterine silk suture. Joshi and Tejuja (1969) also reported increased protein content in endometrium of women with I.U.C.D.

More marked increase in the protein level of uterine fluid with copper T & I.U.C.D. is similar to the findings of Hagenfeldt (1972). Protein composition of uterine fluid has been reported to be changed by the presence of Copper T by Johnson (1972). High level of protein in the uterine fluid in the presence of I.U.C.D. has been observed by Peplow *et al* 1973).

Increased level of protein could be explained on the basis of increased permea-

bility of vessels of endometrium in the presence of I.U.C.D. and hence consequent leakage of protein through it. Protein of similar electrophoretic mobility as autologous serum albumin has been found in the uterine fluid of rat horn fitted with I.U.C.D. The same fraction was found to be present from the horn not fitted with I.U.C.D. Thus, vascular source of protein in the presence of I.U.C.D. is established. Another source could be lysis of cells, polymorphs and denuded surface epithelium as suggested by Kar *et al* (1968).

Increased protein has been suggested by Kar (1969) as disturbing the osmotic tension and consistency of fluid and thus interfering with histotrophic transport and viability of pre-implantation blastocyst.

A recent development has been more frequent finding of large molecular weight protein Ig^G, Ig^M and Ig^A in the uterine fluid in I.U.C.D. series than in control by Chandra *et al* (1974). These immunoglobulins secreted locally may be responsible for increased protein level and its blastocidal activity. Peplow *et al* (1973) have reported much greater albumin and gamma-globulin activity on I.U.C.D. side in pre-oestrous rats.

However, the quantitative analysis of protein does not seem to be of much help in ascertaining the mechanism of action, as increase is found to occur in both the situations favouring the one where blastocyst implantation is favourable i.e. secretory phase of the control and the one where it is unfavourable i.e. in the presence of I.U.C.D. The answer to the problem lies in the qualitative analysis of the protein fractions whereby, those fractions which are favourable for blastocyst implantation could be differentiated from those which are inimical to it. However, the quantitative analysis does provide the clue that same change is occurring in the protein composition and it may have adverse effect on the implantation.

More marked increase in the level of protein in the presence of Copper I.U.C.D. appears to be paradoxical on a superficial glance in view of the reported fact of disruption of protein synthesis machinery (polysomes) in the presence of cupric ions (Hernendez *et al*, 1974). This could be explained on the basis of formation of abnormal proteins rather than on diminished formation. Appearance of specific proteins changing the uterine milieus, unable to support blastocyst, has also been suggested by Arther (1974) by the administration of synthetic steroids during pre-ovulatory period. Besides denaturation of protein by urea present in uterine fluid in the presence of I.U.C.D. has also been suggested by Kar *et al* (1968).

Anoter suggestion for the significance of increased protein in interfering with the implantation of blastocyst and death of pre-implantation blastocyst could be interference with the supply of amino-acids to blastocyst from the uterine fluid due to increased viscosity of uterine fluid containing greater amount of protein. Upto 7th day, the rabbit blastocyst membrane is impermeable to protein and hence nourishment is supplied in the form of amino acids. Our values of protein are in accordance with those of Gerald *et al* (1971) who reported it to be 3.6 mgm. per ml. (control). Discrepancy of value in the present series from that of Kar *et al* (1964) could be due to different method of collection. Kar *et al* (1964) used pooled samples from 6 patients, whereas in the present series analysis of uterine washing from each patient was done separately.

Increase in potassium level in luteal phase of control in our series is in accord-

ance with Rosado *et al* (1972). We also observed a slight rise in potassium with I.U.C.D. over pre-insertional level in accordance with Rosado *et al* (1972). Similar finding is reported by Kar *et al* (1964) in rats in the presence of a foreign body. Rise in potassium level could be explained by the increased permeability of vessels seen in the secretory phase (at the time of nidation) as well as in the presence of I.U.C.D. Besides, depressed metabolic activity in the presence of I.U.C.D. due to inactivation of certain enzymes could be responsible for the leakage of potassium from endometrial cells. As it is a well established fact that potassium has a tendency to leak out of cell unless activity is held or drawn back by the energy supplied by metabolism. Proliferative phase has higher metabolic rate and hence must be capable of retaining more of potassium in cells. Therefore, it will lead to a lower level of potassium in uterine fluid during the proliferative phase as compared to the secretory phase.

Sodium was found to be decreased in the secretory phase of the control. This is in accordance with the observations of Clemetson (1973). This decrease in sodium level could be explained on the basis of inadequacy of sodium pump mechanism. Normally sodium has a tendency to accumulate in the cells but it is continuously being pumped out of the cells by active energy, in contrast to potassium which is being held inside the cell by active energy. Depressed metabolism in the secretory phase and hence consequent lack of energy must be responsible for the accumulation of sodium inside the endometrial cells. This is also supported by the histological finding of stromal and cellular oedema in the secretory phase which could be explained as being secondary to sodium retention.

In the present series, a fall in sodium level was observed in the proliferative phase of those with I.U.C.D. This could be due to depressed metabolism in the presence of I.U.C.D. The metabolism has been shown to be depressed in the presence of copper I.U.C.D. as evident by decreased thymidine uptake in the endometrial cells (Prager, 1969). Sodium was found to be increased in the secretory phase as compared to the proliferative phase; more marked rise occurring with Copper T than with the Lippes loop. This increase in luteal phase is similar to the observations of (Hegenfeldt, 1972). Although he could not find any significant alteration between proliferative phase sodium level of control and the test series. Kar *et al* (1964) reported contrary findings in rats with I.U.C.D., but this could be due to species difference. However, Kar *et al* (1968) could not find any significant alteration in human beings with I.U.C.D.

Increased level of sodium in the secretory phase of I.U.C.D. could be speculated to be due to increased vascular permeability in I.U.C.D. group which might counterbalance the effect of depressed metabolism retaining sodium. Thus, sodium escaping from the vessels might be responsible for increased sodium level in the uterine fluid.

The values obtained for both sodium and potassium in the present series are similar to those of Clemetson *et al* (1973). It is higher than that obtained by Kar *et al* (1968). This could be due to similar method of collection as adopted by Clemetson (1973).

These abnormal electrolyte levels might interfere with electrolyte levels of blastocoelic fluid disturbing its water balance, or they may evoke changes in the cellular electrical membrane potential

which may interfere with endometrial blastocyst adhesion preventing nidation.

Summary

Biochemical analysis of uterine fluid for Protein, Sodium and Potassium was conducted in 51 healthy women with Copper-T 200 (41 cases) and Lippes loop (10 cases); with 20 cases without pill or I.U.C.D. serving as control.

Protein was found to be significantly raised ($p = < 0.01$) in the presence of I.U.C.D.; the increase being much more marked with Copper I.U.C.D. than with the Lippes loop. Potassium was found to be significantly raised ($p = < 0.01$) over the pre-insertional level in the presence of Copper I.U.C.D. in both the phases. Sodium showed significant decrease ($p = < 0.01$) in the presence of Lippes as well as Copper loop in proliferative phase.

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