PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, **147** 407-411 (1974)

The Antifertility Effect and Improved Tolerance of Copper Bearing Hydrogel Coated Intrauterine Devices in Rabbits[1] (38352)

R. H. DAVIS, H. SCOTT, G. A. KYRIAZIS, AND H. BALIN

Department of Obstetrics and Gynecology, Division of Reproductive Biology, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania 19102; and Materials and Physical Sciences Department, Franklin Institute Research Laboratories, Philadelphia, Pennsylvania 19102

Polyethylene I.U.D.'s wrapped with copper wire have good contraceptive effectiveness, but some anxiety still exists about fragmentation and systemic toxicity (1). A copper-bearing I.U.D. coated with hydrogel according to our findings appears to lend itself to the minimization of expulsions, composition changes, bleeding and pain (2–17). We wish to report our obtaining an improved tolerance and contraceptive effect with unsutured rabbit I.U.D.'s made from polyethylene rods covered with a strong coating of water-swellable hydrogel covalently grafted onto the polyethylene and over spiral-grooved-seated copper wire.

Materials and Methods. The uteri from 20 rabbits were measured and the average uterine corpus length was found to be 109.5 ± 4.5 mm with a lumen diameter of 1.9 ± 0.2 mm. Lumen casts verify these measurements and under high power magnification we observed uniform round minute indentations due to the endometrial glandular crevices over the uterine lumen casts. From these data we constructed rod-shaped I.U.D.'s (3 × 38 mm) in which a 0.5 mm thick coating of water-swellable poly-2-hydroxyethylmethacrylate hydrogel was covalently grafted onto $BaSO_4$-pigmented polyethylene rods and over copper wire seated in spiral-grooves as shown in Fig. 1.

To test for anti-fertility effectiveness, radiation sterilized polyethylene, hydrogel-coated polyethylene and hydrogel-coated copper bearing polyethylene I.U.D.'s (3 × 38 mm in dry state; 108 mm^2 copper) were installed without suture fixation into both uterine horns of 3–5 kg New Zealand white rabbits (lumen casts (18) provided accurate I.U.D. dimensions). Control animals were sham-operated. All animals were rested for 32 days to permit the rabbits to come out of possible pseudo-pregnancy (persistent corpora lutea) and to recovery from surgery. Each rabbit was double mated with proven bucks and cesarean sectioned on day 29 of an assumed pregnancy. The uncoated polyethylene I.U.D. bearing animals were not cohabited with males because of early I.U.D. expulsions. Records were kept on maternal body weight, percent fertility, percent expulsions, percent increase in I.U.D. weight, and average litter size and weight. When an I.U.D. was expelled the peritoneal cavity, vagina and cage were searched. At autopsy the I.U.D. condition, any gross inflammation of the uterine cavity containing the device as well as the ovarian condition were noted. Portions of empty uterine control horns as well as various I.U.D.-bearing uterine horns were placed in 10% formalin, sectioned and stained with hematoxylin-eosin, PAS and Trichrome stains (19).

In an attempt to explore the mechanism of action of the hydrogel-coated I.U.D. with or without copper, the uterine horns of New Zealand white rabbits (3–5 kg) containing I.U.D.'s over 30 and 61 days were washed out with 1.0 ml physiologic saline (0.95% sodium chloride) and the measured volume was brought up to the WBC pipette mark with 1.0% glacial acetic acid (20). The number of cells counted per four

[1] This work was supported by the National Institute of Child Health and Human Development – Center for Population Research (NIH-71-2231).

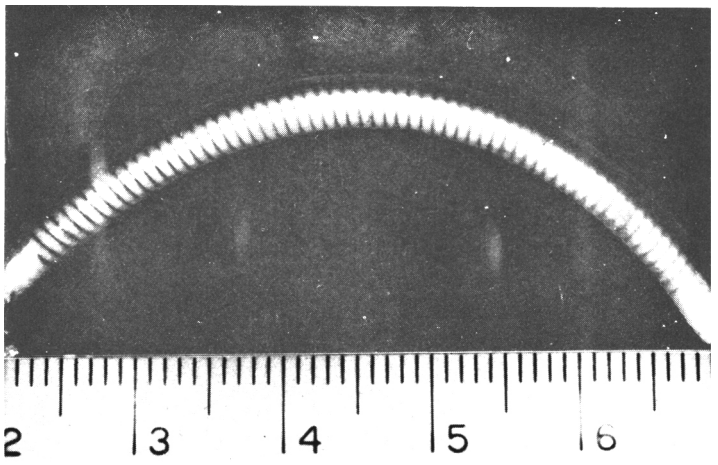

FIG. 1. The copper hydrogel-coated I.U.D. after 61 days in the New Zealand white rabbit.

squares of the hemocytometer × 50 equals WBC/mm^3.

Preliminary semi-quantitative measurements of the total amount of Cu^+ and Cu^{2+} ions which leach out through the hydrogel-coating and into saline solutions from submerged copper bearing I.U.D.'s was determined with copper-specific color—indicating Quantofix sticks (Gallard–Schlesinger, Chemical Manufacturing Corporation). Seven short (39 mm long with 108 mm^2 copper surface) and three long (76 mm long with 216 mm^2 copper surface) I.U.D.'s were placed for 19 days in 10 and 20 ml double distilled physiologic saline solutions, respectively. The copper content in μg/ml was read on alternate days by dipping the indicator sticks into the saline solutions and matching the resulting colors with those of a precalibrated Cu-concentration color chart. The mean values obtained from the sets of seven short and three long I.U.D.'s were recorded. The standard error was calculated using the formula $(\Sigma d^2/N(N-1))^{\frac{1}{2}}$ and the probability value P by Student's t test for significance.

Results. Adult female rabbits containing I.U.D.'s were successfully double mated with proven bucks and cesearean sectioned on day 29 of an assumed pregnancy (Table I). The percent fertility for sham operated controls was 86.6% as compared to 38.5% for hydrogel-graft coated polyethylene I.U.D.'s without copper and 0% for copper-coated I.U.D. bearing animals. Unsutured polyethylene rods without hydrogel were coated with a purulent slippery-like material so that all of them were expelled to the outside whereas 59% of the hydrogel-coated without copper and 50% of the hydrogel-coated with copper were retained. The latter I.U.D.'s swelled and became rubbery within the uterine walls to minimize expulsion. The I.U.D. length was such that some free lumen (about one-third) was observed at both ends of the uterus. No fragmentation of either copper or hydrogel was observed and the hydrogel was not covered with purulent material. The I.U.D. weight increased from 7.2% for the polyethylene alone to 37.8% for the copper-coated I.U.D. In rabbits containing hydrogel-coated rods without copper the average number of fetuses per litter was slightly reduced when compared to sham operated controls. Eight fetuses were recorded in the presence of the hydrogel-coated I.U.D. without copper and they were located towards the cervix with the rod residing toward the uterine fundus. No teratogenic effect was observed and fetal weights were similar to controls. The I.U.D. has little or no effect on maternal body weight.

No hydrogel or copper fragments were found in histologic examination of uteri containing hydrogel-coated or copper hydrogel-coated I.U.D.'s. No leukocytic infiltration into endometrium or myometrium was noted, as might be expected, in a foreign body inflammatory reaction. In general, the uterine histology was not markedly altered or distorted by hydrogel-coated copper bearing I.U.D.'s.

The copper-bearing hydrogel-coated I.U.D. caused a 68.1% higher total white blood cell count in uterine fluid over 61 days (not seen at 30 days) above control horns without an I.U.D.

TABLE I. Antifertility Influence of Hydrogel-Coated Copper (108 mm²) Polyethylene Intrauterine Device in New Zealand White Rabbits.

	Intrauterine devices (no fixation suture)[a]			
	Sham operated controls	Polyethylene alone	Hydrogel coated polyethylene	Copper hydrogel polyethylene[c] coated
No. of animals	15	9	16	16
Body weight (kg)	4.1 ± 0.1	3.7 ± 0.4	3.9 ± 0.2	4.2 ± 0.3
% I.U.D. expulsions	—	9/9 (100%)[d]	19/32 (59%)	16/32 (50%)
% Fertility[a]	86.6	—	38.5[e]	0.0
Aver. no. fetuses/litter	6.8 ± 0.5	—	0.6 ± —	—
% increase I.U.D. wt	—	7.2±2.4	34.9±5.1	37.8±5.2
Aver. fetal weight (g)	36.9±1.8	—	34.0±6.5	—

[a] % fertility in I.U.D. containing uterine horns.

[b] No fixation sutures used as opposed to other workers in field — to avoid the antifertility artifacts introduced by such procedures.

[c] No copper or hydrogel fragmentation was observed.

[d] Evidence of gross purulent slippery material on polyethylene I.U.D. surface which was not seen on hydrogel coated I.U.D.'s.

[e] No teratogenic effect of fetuses; ± = standard error.

However, the copper did not evoke a massive mobilization of leukocytes nor the formation of a slippery purulent material on the hydrogel surface such as found to form on polyethylene surfaces (Table II).

Our preliminary results showed copper released from the I.U.D. into *in vitro* saline on day 3 recorded at 2.8 ± 0.5 μg/ml which rose to 22.0 ± 2.0 μg/ml saline on day 11. A plateau of 37.5 ± 4.2 μg/ml was reached on day 13. The hydrogel maintained its composition over the entire period (Table III).

Discussion. The hydrogel-coating grafted to polyethylene I.U.D.'s bearing copper wire under the hydrogel contributes to contraceptivity and retention (tolerance) in rabbits and if ultimately applied to I.U.D.'s for women might be expected to lead to I.U.D.'s clinically superior to the plastic and copper-bearing I.U.D.'s. The percent fertility of our sham-operated control rabbits of 86.6% compared to 38.5% for hydrogel-coated I.U.D.'s without copper and 0.0% for coated I.U.D.'s with copper suggests that we may have improved on current pregnancy rates of uncoated copper polyethylene devices. The expulsion of uncoated polyethylene devices with and without copper (an observation with two rabbits each having two I.U.D.'s) suggests that we can further diminish expulsions with hydrogel coating (4). The limited experiment whereby two rabbits were instrumented with copper-bearing I.U.D.'s without hydrogel revealed expulsion in both horns of two rabbits without suture fixation. The slippery purulent

TABLE II. Total White Blood Cell Count in Uterine Fluid Obtained from Adult New Zealand White Rabbits Containing Hydrogel-Coated Polyethylene Intrauterine Devices With and Without Copper for 30 and 61 days.

	Control uterine horn (no I.U.D.)		Hydrogel-coated I.U.D. horn		Copper-hydrogel-coated I.U.D. horn	
	30 days	61 days	30 days	61 days	30 days	61 days
No. of animals	7	13	6	10	7	13
W.B.C./mm³	9000±2280	9090±920	—	8205±363	8986±2040	15283±760*

± = standard error; W.B.C./mm³ = cell count × 50.

*$P < 0.001$ when compared to controls (+68.1% increase).

TABLE III. The Rate of Diffusion of Copper Ions from Copper Hydrogel-Coated Polyethylene Intrauterine Devices into Saline[a].

Time (days)	Copper ions Cu^{+}/Cu^{++} (μg/ml)
1	0.0±0.0
3	2.8±0.5
5	6.5±0.8
7	9.0±0.7
9	20.5±2.3
11	22.0±2.0
13	37.5±4.2
15	37.5±4.2
17	37.5±4.2
19	37.5±4.2

[a] Copper coated IUD in 10 ml double distilled saline. Ten I.U.D.'s per time period; ± = standard error.

material covering the polyethylene surface was absent on the hydrogel surface which may explain the significantly better retention values recorded with hydrogel-coated I.U.D.'s. The hydrogel slightly swelled within the uterine lumen to produce an optimal physiologic "snug fit" without uterine distention even in the presence of copper. The polyethylene I.U.D. complications based on an inflammatory response to a foreign body I.U.D., cited earlier, and expulsion may be eliminated by the hydrogel-coating procedure.

Histologic sections of uterine horns bearing hydrogel-coated I.U.D.'s with and without copper revealed no inflammation as measured by the normal counts of WBC's in uterine fluid and the absence of marked tissue alteration without WBC tissue infiltration (4). The hydrogel or copper did not fragment and we saw no gross deposits, (6–8) composition changes, (9,10) or other complications (11–17). All animals were observed daily and the behavior of I.U.D. bearing animals was similar to controls.

These data point to an enhanced tolerance of the uterus by the hydrogel coating of polyethylene I.U.D.'s in rabbits. Polyethylene I.U.D.'s without hydrogel causes a marked drop in uterine fluid pH possibly due to the disruption of uterine cells with massive liberation of lytic enzymes from lysosomes. Hydrogel-coated I.U.D.'s did not contain purulent material and produced no leukocyte response without copper. The mucosa exposed to uncoated polyethylene rods became completely devoid of PAS stainable material again suggesting the liberation of lytic material.

The observation of Doyle and Margolis (21) as well as others that silk suture by itself piercing the uterine lumen may be contraceptive leads us to avoid suture fixation. Contrary to previous observations wherein polyethylene I.U.D.'s were sutured in place near the cervix producing no contraceptive effect upstream towards the uterine fundus (22), we observed eight normal fetuses in our hydrogel-coated noncopper I.U.D. bearing rabbits all found toward the cervix with the I.U.D. located nearer to the fundus end of the uterine horn. This does not seem to fit in with the current concept that the primary contraceptive effect of I.U.D.'s can be accomplished by gametotoxic changes in the uterine fluid. In fact, one wonders about the production of a toxic substance at all which increases WBC's at the interface between the protective hydrogel and the endometrium. The eight fetuses appeared normal without teratology or degeneration.

The current use of copper I.U.D.'s on a world-wide scale has created great interest in the mode of action of copper. Zipper (4) has clearly shown that the copper-T-polyethylene I.U.D. was much more effective in preventing pregnancy. Possibly the cupric ions slowly diffuse out altering the uterine environment modifying enzymatic protein (1) to inhibit sperm motility (24, 25) preventing cervical penetration, to prevent ova implantation and to suppress uterine motility (1, 23). On removal of the copper device after several months of use the copper appears dark and weighs less. The weight loss amounts of 60 μg /day for a 200 mm^2 copper surface (1) would result in a 1.0%/day increase of copper in blood provided it does not accumulate in blood. Since our experiments revealed that the hydrogel coating inhibits the inflammatory response induced by polyethylene as evidenced by the lack of increase of WBC count and results in a less than satisfactory antifertility rate of 61.5%, we feel that the 68.1% increase in WBC count with copper-coated I.U.D.'s and a complete fertility inhibition was due to the presence of copper or an action of copper on enzymatic protein within the uterine cavity. The diffusion of copper from the hydrogel-coated device of 37.5 ± 4.2 μg/ml in our *in vitro* study seems to agree, in general, with the literature on this point. The contraceptive influence of the copper-hydrogel-coated I.U.D. probably depends on a slow release of

copper ions locally into uterine fluid. Future studies will center on testing the copper-coated I.U.D. in subhuman primates.

Summary. Rod-shaped rabbit I.U.D.'s (3 × 38 mm) were constructed in which a 0.5 mm thick coating of water swellable poly-2-hydroxyethylmethacrylate hydrogel was covalently grafted onto $BaSo_4$-pigmented polyethylene rods and over copper wire seated in spiral-grooved polyethylene rods. Uncoated polyethylene I.U.D.'s, hydrogel-coated polyethylene I.U.D.'s and copper-bearing hydrogel-coated I.U.D.'s were surgically inserted into uteri of New Zealand white rabbits without suture fixation procedures. I.U.D. retention in rabbit uteri (a normally high rejection model for unsutured I.U.D.'s) were 0% (no hydrogel coating), 41% (with hydrogel coating) and 50% (with copper wire and hydrogel coating). Observed fertilities in 2-mo long experiments were 86.6% for sham-operated control rabbits, 38.5% with I.U.D.'s having only hydrogel coating and 0% with I.U.D.'s bearing copper wire and a hydrogel coating over the wire. Evidence was found of gross purulent material on uncoated polyethylene I.U.D.'s whereas inflammatory reactions to hydrogel coated I.U.D.'s (with and without copper) were comparatively minor, and no teratogenic effects were observed with hydrogel-coated I.U.D.'s. No evidence was found for either copper or hydrogel fragmentation in rabbits.

1. Oster, G. E., Fertil. Steril. **23,** 18 (1972).

2. Davis, H. J., "Intrauterine Devices for Contraception. The I.U.D.," p. 154. The Williams and Wilkins Co., Baltimore, MD (1971).

3. Zipper, J., Medel, M., Pastene, L., and Rivers, M., Amer. J. Obstet. Gynecol. **105,** 1274 (1969).

4. Zipper, J. A., Tatum, H. J., Medel, M., Pastene, L., and Rivera, M., Amer. J. Obstet. Gynecol. **109,** 771 (1971).

5. Douglas, C. D., Acta Cytol. **2,** 156 (1969).

6. Dasgupta, P. R., Kar, A. B., and Engineer, A. D., Amer. J. Obstet. Gynecol. **110,** 593 (1971).

7. Highman, W. J., Acta Cytol. **15,** 473 (1971).

8. Yang, T., and Yang, W., Amer. J. Obstet. Gynecol. **109,** 664 (1971).

9. Janakiraman, K., Gadgil, B. A., and Buck, N. C., Ind. J. Med. Res. **59,** 499 (1971).

10. Provost, R. W., and Golditch, J. M., Obstet. Gynecol. **36,** 484 (1970).

11. Guttorm, E., Gynecol. Scand. **50,** 9 (1971).

12. Westron, L., and Beggtsson, L. P., J. Reprod. Med. **5,** 41 (1970).

13. Ishiama, A., and Hiroyuki, W., Yokohama Med. Bull. **20,** 83 (1969).

14. Lebech, P. E., Steen, E., Berggreen, K., and Newmann, K., Danish Med. Bull. **18,** 146 (1971).

15. Wilson, E. A., and Dilts, P. V., Amer. J. Obstet. Gynecol. **112,** 237 (1972).

16. Ober, W. B., Sobrero, A. J., Kurman, R., and Gold, S., Obstet. Gynecol. **32,** 782 (1968).

17. Echenberg, R., and Ledger, W. J., Obstet. Gynecol. **31,** 795 (1968).

18. Batson, O. V., Demonstrations of Corrosion Specimens Prepared by a New Material. American Association Anatomists 68th Session, Philadelphia (1955).

19. Luna, L. G., Manual of Histologic Staining of Methods of the Armed Forces Institute of Pathology. ed. 3 (1960).

20. El. Sahwi, S., and Moyer, D. L., Fertil. Steril. **22,** 398 (1971).

21. Doyle, L. L., and Margolis, A. J., Science **139,** 833 (1963).

22. Parr, E. L., Schaedler, R. W., and Hirsch, J. G., J. Exp. Med. **126,** 5230 (1967).

23. Michael, D. K., and Reinke, D. A., Amer. J. Obstet. Gynecol. **107,** 188 (1970).

24. Ullman, G., and Hammerstein, J., Contraception **6,** 71 (1972).

25. Zbuzkova, V., and Kincl, F. A., Acta Endocrinol. **66,** 379 (1971).

Received February 12, 1974. P.S.E.B.M., 1974, Vol. 147.