maintain body temperature (monitored by an anal probe) close to 37 °C. All roots from $L_5$ to $S_2$ were sectioned extradurally. Bipolar platinum hook electrodes were placed on the dorsal $S_1$ root for stimulation and on the dorsal $L_7$ root for recording the dorsal root reflex (DRR). To record dorsal root potentials (DRP), a ball-tipped platinum electrode was placed close to the $L_7$ dorsal root entry into the spinal cord and the distal hook electrode used as reference. The time constant of the preamplifier was set at 1 sec to record the DRP. Supramaximal square wave stimuli were applied to dorsal $S_1$ at a rate of 0.25 Hz. Carotid blood pressure was continuously monitored and all drugs were administered through a cannulated antebrachial vein. The areas of the DRR and DRP were integrated by planimetry. After a period of 30 min when control spinal synaptic activity was recorded to ensure stability, the cats were given either pyridoxine hydrochloride (0.17 mg/kg in 2 ml saline) or an equivalent volume of saline, followed in 15 min by 0.2 mg/kg of diazepam (Valium® 1 mg/ml solution). Pyridoxine or saline had no significant effect on the recorded potentials. Diazepam consistently increased the surface area of both DRPs and DRRs. However, this increase was significantly more pronounced in the cats pretreated with pyridoxine. In pyridoxine-pretreated cats, the increase in DRP and DRR, 5 min after diazepam, averaged $33.8 \pm 4\%$ and $109.1 \pm 25\%$ respectively, whereas in saline pretreated cats, the corresponding increase was $15.7 \pm 2.9\%$ and $40.5 \pm 12.6\%$ (table).

Pretreatment with pyridoxine also prolonged the duration of action of diazepam. Thus, 30 min after diazepam in $B_6$ pretreated cats, the DRP and DRR were $22.7 \pm 4.6\%$ and $81.9 \pm 32.4\%$ above control, whereas in saline pretreated cats, the corresponding values were $9.5 \pm 3.4\%$ and $12 \pm 13.7\%$ for the DRP and DRR, respectively. Both values were significantly higher than saline controls ($p < 0.05$).

Both L-glutamic acid decarboxylase (GAD) and GABA-$\alpha$-ketoglutaric aminotransferase (GABA-T) are $B_6$-dependent enzymes. Steady state concentrations of GABA in the CNS are normally governed by the GAD activity and not by the GABA-T[6]. The activity of GAD is almost doubled by the addition of its coenzyme to the incubation medium, whereas GABA-T is not activated by pyridoxal phosphate added in vitro[7]. The activity of GAD is an almost linear function of the concentration of pyridoxal phosphate in the brain[8]. The decrease in presynaptic inhibition after depletion of GABA by semicarbazide has been found to be effectively antagonized by pyridoxine[9].

Obviously, many other neurohumoral candidates are $B_6$-dependent, but the implication of GABA in primary afferent depolarization and the obvious enhancement of presynaptic inhibition by diazepam point to the facilitation of GABA-ergic transmission by pyridoxine as a likely mechanism underlying its potentiation of diazepam's spinal actions. The potentiation of diazepam's anticonvulsant action by pyridoxine is being investigated now.

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## Uterine morphology and glycogen deposition of pregnant rats after clomiphene citrate treatment during preimplantation stages[1]

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*Summary.* Clomiphene citrate did not induce glycogen deposition in the uterine luminal epithelium of pregnant rats as it did in ovariectomized rats. However, the drug did alter the epithelial morphology which may be a factor in its postcoital contraceptive action.

Clomiphene citrate is a highly effective postcoital contraceptive agent in rats and acts either on the blastocyst or the uterus[3,4]. We have reported that clomiphene caused abundant glycogen deposition in the luminal epithelium of ovariectomized rats, an effect not caused by estradiol, and have postulated that the drug's epithelial effect may be a factor in its ability to block implantation[5]. The present experiment tests that postulate.

*Materials and methods.* Virgin Holtzman rats (220–230 g) were housed with males of proven fertility. Clomiphene citrate (0.5 mg/kg by gavage) was administered at 08.00 h of days (D) 2–4 of pregnancy (D 0 = sperm +). Control and treated rats were killed at 08.00 h of D 5 and 8. 4 rats were bilaterally ovariectomized under ether anesthesia, rested 10 days, treated with clomiphene (0.5 mg/kg) on 3 consecutive mornings, and sacrificed 24 h later. The uteri were fixed in an 80% alcoholic solution of cold picric-acid formalin, and embedded in paraffin. Serial sections were made of all uteri. 3 successive sections were: 1. stained with H and E, 2. treated by the PAS procedure and 3. treated with 1% malt diastase (30 min at room temperature) prior to the PAS technique. Uteri from the rats killed on D 8 were stained with H and E only. The slides were read without knowledge of treatment, and glycogen grades recorded as previously described[5].

*Results.* None of the 4 clomiphene-treated rats killed on D 8 any gross or microscopic evidence of implantation. The 5 control rats killed on D 8 had normal implantation sites. All controls had microscopic evidence of decidualization on D 5, but decidual cells were not observed in the uteri of the clomiphene-treated rats on D 5.

The uteri of the control rats killed on D 5 had a straight lumen with parallel epithelial surfaces. The blastocysts and a few of the decidual cells contained glycogen, but none was observed in the low columnar luminal epithelium. The myometrium at the implantation site contained abundant glycogen (table) in the inner circular layer. Sections from intersite regions of the uterus had much less glycogen in the inner circular layer, and the lumen was more convoluted than at the nidation sites. The nuclei of the luminal epithelium were basally located in both site and intersite regions (figure 1, A).

The uteri of the clomiphene-treated rats had 4 distinguishing characteristics. The nuclei of the luminal epithelial cells were centrally located with prominent vacuoles present (figure 1, B) above and below the nuclei. Less peripheral stromal edema was noted. The general morphology of the lumen was more convoluted in the treated animals. Glycogen accumulation in the inner circular layer was clearly less than at the nidation sites. Glycogen was not observed in the luminal epithelium of the clomiphene-treated pregnant rats (figure 2, B), but large amounts of glycogen were observed in nearly every luminal epithelial cell of the treated ovariectomized rats (figure 2, A).

Blastocysts were observed in different areas of the lumen of clomiphene-treated pregnant rats killed on D 5 and D 8 (figure 3). They were scattered throughout the lumen with irregular orientation of the inner cell mass. The blastocysts were never attached to the luminal epithelium.

*Discussion.* The absence of glycogen in the luminal epithelium of the treated rats at the time of implantation was a marked contrast to the response in ovariectomized rats. Since little or no glycogen is observed in the cells of intact animals[5,9–11], we postulated that clomiphene's alteration of epithelial morphology and biochemistry may be a factor in

Histochemical analysis of uterine glycogen in pregnant (Day 5) and ovariectomized rats treated with 3 consecutive daily dosages of clomiphene

| Treatment | Rats | Glycogen grade | | |
|---|---|---|---|---|
| | | | Myometrium | |
| | | Luminal epithelium | Inner circular | Outer longitudinal |
| Pregnant | | | | |
| Control Site | 10 | – | 4+ | 3+ |
| Intersite | | – | 2+ | 2+/3+ |
| Clomiphene | 11 | – | 2+ | 2+ |
| Ovariectomized | | | | |
| Control | 4 | – | – | 1+ |
| Clomiphene | 4 | 4+ | 1+ | 3+ |

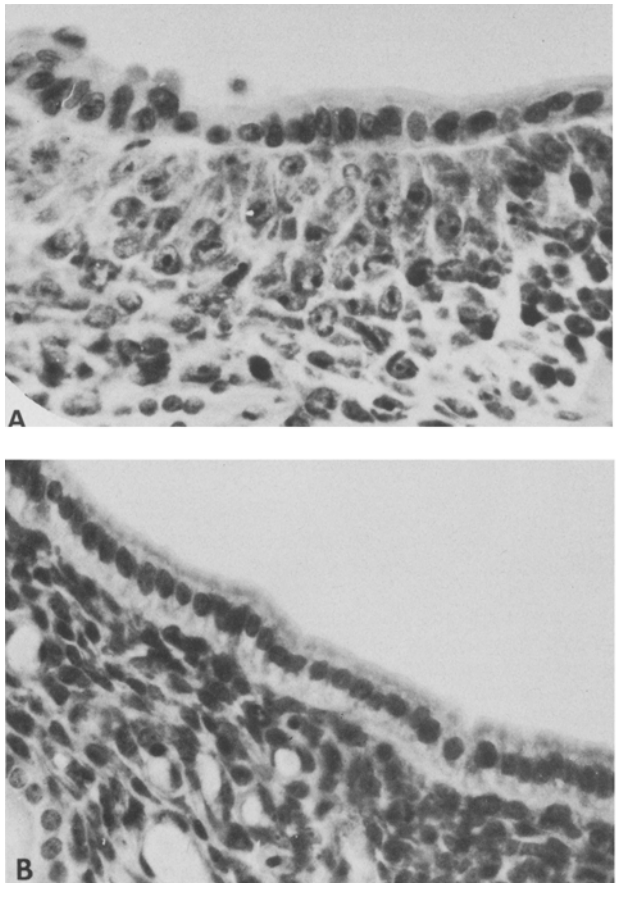


Fig. 1. *A* Implantation site of a D 5 control rat showing luminal epithelium and adjacent decidual cells. Note basal position of epithelial nuclei. H and E. ×326. *B* Luminal epithelium and adjacent stromal cells from clomiphene-treated rat on D 5. Note central position of nuclei and clear spaces related to them. H and E. ×326.

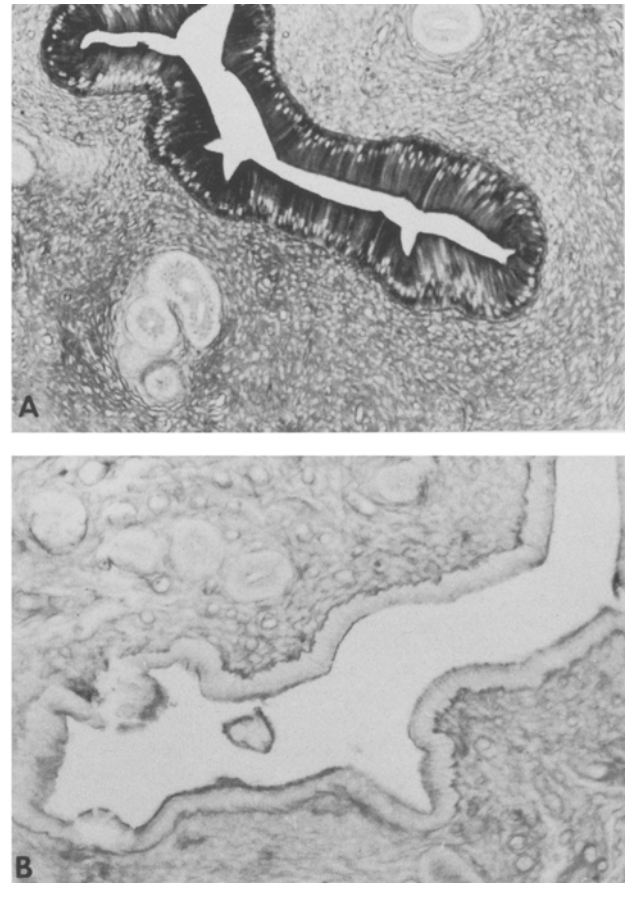


Fig. 2. *A* Endometrium of clomiphene-treated ovariectomized rat. Note the very tall columnar luminal epithelial cells containing a great amount of glycogen. No glycogen is present in the glandular epithelium. PAS×81. *B* Similar view of clomiphene-treated pregnant rat. No glycogen is observed in the luminal epithelium and the cells are much lower than above. A blastocyst is observed in the lumen. PAS.×81.

the drug's ability to block implantation. Despite the absence of the expected effect, the difference in nuclear location and presence of vacuoles was quite different from the controls and is associated with lipid deposition in these cells[12,13]. Therefore, clomiphene treatment did alter the morphology and possibly the chemical balance of these cells whose importance in implantation has been reported[14], as has positional change of the nuclei[15]. Clomiphene may be delaying the preparatory stages for the attachment reaction and may be altering the hormonally controlled uterine events leading to the attachment stage. Since blastocysts were observed in the lumen of the treated rats on D 8, the drug may be inducing delayed implantation.

The absence of glycogen in the luminal epithelium of the clomiphene-treated rats correlates well with our previous study[16] showing that progesterone inhibits clomiphene-induced epithelial glycogenosis. Since progesterone titers are known to be high during early pregnancy[17], this study indicates that endogenous progesterone also inhibits the clomiphene-induced epithelial effect.

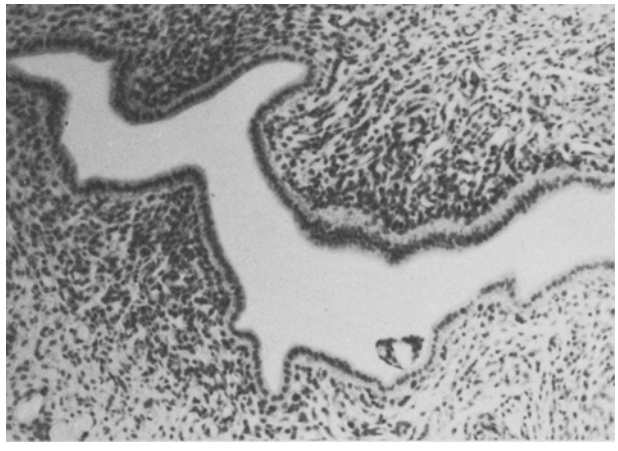

Fig. 3. Endometrium of clomiphene-treated pregnant rat on D 8 showing the convoluted morphology of the lumen and the presence of a blastocyst in the lumen. H and E. × 81.

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## Magnets in guitarfish vestibular receptors

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*Summary.* Black magnetic particles are intermixed with white crystalline otoconia in the inner ear gravity receptors of a saltwater ray. Their size and composition suggest that they are multidomains of magnetite-ilmenite.

The utricle and saccule of vertebrates are vestibular receptors for gravitational and linear inertial forces. Shearing forces on sensory hair cells result from the settling of white crystaline otoconia (calcium carbonate) toward the direction of a resultant force vector. Information from these receptors is combined with that from 3 semicircular canals in each inner ear, and used for central neural control of posture and coordinated motion[4]. We found black particles, strongly attracted to magnetic fields, interspersed among otoconia in the guitarfish utricle and saccule. When placed in water on a watchglass over a stir plate containing a rotating magnet, the black particles first rotated individually and then attached together to form a rod-shaped aggregate about 4 mm in length. The aggregate rotated in synchrony with the stir plate magnet at rotational frequencies up to 3 Hz. Other colored particles were also observed that were not magnetic. The presence of magnetic particles in vestibular receptors is particularly interesting in the light of recent evidence that pigeons[5], honeybees[6], and many species of mud bacteria[7,8] possess single domains (tiny unit magnets) of magnetite in non-vestibular regions. We therefore examined the elemental composition of the particles.

*Materials and methods.* 3 guitarfish *(Rhinobatos rhinobatos)*, ranging in length from 76–120 cm, were obtained from Atlantic coastal waters near Cadiz, Spain. The vestibular otolithic masses were removed and placed in 10 ml of distilled water. The otoconia and black particles from all 3 animals were combined; however, saccular and utricular samples were maintained separately. The gelatinous layer in which the otoconia were embedded was denatured by heating the samples at 60 °C for 15 min. After removal of distilled water, the particles were rinsed in absolute ethanol and acetone, and then dried in air on a watchglass. Samples of 5–20 black and white particles were lifted with a sharpened wooden probe and placed on graphite coated mounts for elemental analysis. The samples were analyzed