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.

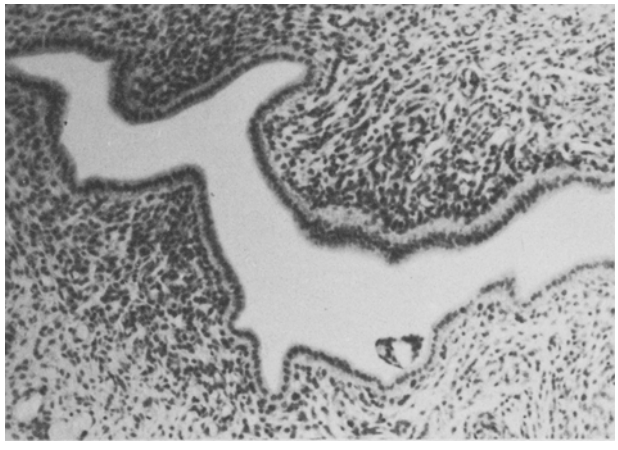

Fig. 3. Endometrium of clomiphene-treated pregnant rat on D 8 showing the convoluted morphology of the lumen and the presence of a blastocyst in the lumen. H and E. × 81.

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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

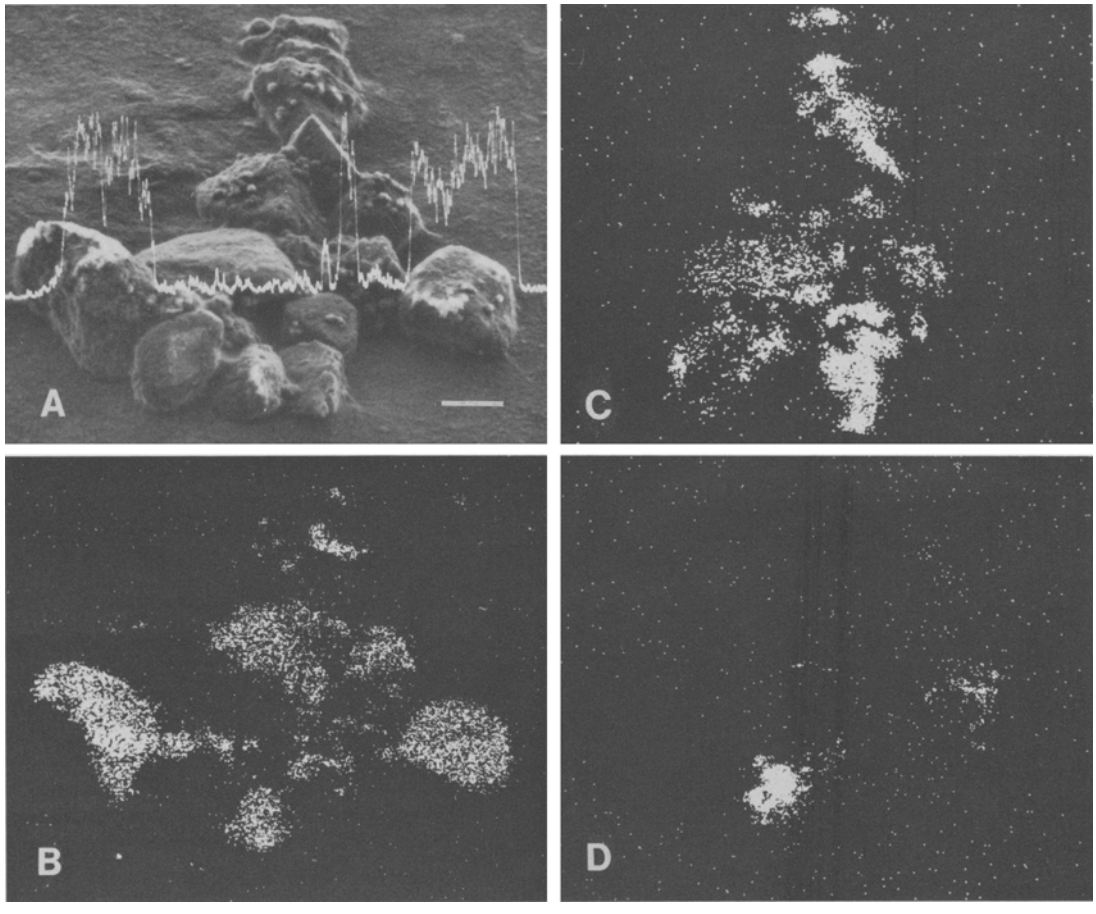
with a Philips 501 scanning electron microscope, operated at 15 KV with a 100 nm spot, and equipped with an EDAX model 711 electron dispersive X-ray analyzer.

The following elements were found in the general spectrum: calcium, iron, magnesium, titanium, silicon, and aluminum. We found that silicon and aluminum were present in the graphite paste and mounts used to hold samples, but that other elements in the general spectrum were localized in specific particles in the samples. A typical result from saccular otoconia is shown in the figure. Particles showing high concentrations of calcium (figure, A, B) were identified as endogenous otoconia because of their morphological similarity to otoliths known to be calcium carbonate in other species[9,10]. Iron, titanium and magnesium were found in particles which were distinct from the endogenous otoconia (figure, C, D).

Magnetite (Fe_3O_4) is one of a class of iron oxides that is black and ferrimagnetic. The fact that the particles in question contain major Fe, Mg, and Ti components suggests at least two possibilities. First, that they are a titanian magnesian magnetite, since there exist solid solutions between magnetite and ulvospinel (Fe_2TiO_4) and between magnetite and magnesioferrite ($MgFe_2O_4$). Second, that they are magnetite and ilmenite ($FeTiO_3$) intergrown on a microscopic or submicroscopic scale. In the latter case, magnesium will substitute for the iron in ilmenite and geikielite ($MgTiO_3$), or for the Fe^{2+} in magnetite, or both. Because our elemental concentration maps show a segregation of the titanium into more or less distinct regions, this would favor the second hypothesis (Rouse, personal communication). A similar analysis on samples from the utricular otoconia showed similar results.

We assume that the magnetic otoconia are exogenous in origin, entering along with sand particles through the external orifice of the endolymphatic duct of this bottom feeding ray[11]. It is unlikely that guitarfish from the Spanish Atlantic coast contain unusually large amounts of ferrites as a result of local environmental factors, since a closely related guitarfish *(Rhinobatos productus)* from southern California has similar black magnetic particles (O'Leary, unpublished observations). However, they are spatially organized within the receptors. The black particles in *R. productus* are not distributed uniformly in all receptor regions, but form a conspicuous curved band among the endogenous otoconia in the ventral region of the sacculus adjacent to the lagena (Dunn, unpublished observations).

Elasmobranchs (sharks, skates and rays) have relatively large vestibular systems that have been used in fundamen-



Scanning electron micrograph and specific element concentration densities in a sample of saccular otoliths. *A* A micrograph of otoliths is shown superimposed with results from an electron microprobe line scan for calcium along the horizontal position indicated by the baseline. The peaks indicate regions along the baseline containing significant amounts of calcium. *B* A two-dimensional concentration map for calcium is shown as obtained from the same sample as that shown in *A*. Calcium concentration is proportional to the density of white dots. *C*, *D* 2-dimensional concentration maps for iron and titanium are shown in *C* and *D*, respectively, similar to that shown for calcium in *B*. The white dots indicating relative concentration densities in *B*, *C*, and *D* can be identified with specific particles in *A*. Points corresponding to iron and titanium are concentrated in particles which are, in general, different from the clusters of calcium. Small trace concentrations of magnesium and other metals were also found in certain particles. Scale bar represents 50 µm. This work was performed in the laboratories of the Centro de Investigacion Josefina y Manuel Pascual, Cadiz, Spain in August, 1979.

tal studies of the afferent responses and physiological mechanisms of the semicircular canals[12-15] and inertial receptors[16-19]. The presence and distribution of magnetic otoconia might indicate an additional functional role based on either the increased density of magnetic otoconia, or a magnetic sensitivity of the sensory cells. Whether or not the magnetic otoconia are functionally useful to the animal is presently unclear and requires further investigation.

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Highly irritant ingenane type diterpene esters from *Euphorbia cyparissias* L.

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Summary. From the roots of *Euphorbia cyparissias* L. a highly irritant diterpene ester fraction was isolated and further resolved into its constituents. Together with some less active and some inactive isomers, the pure Euphorbia factors were characterized as new diesters of 13-hydroxyingenol and as triesters of the new 13,19-dihydroxyingenol. The Euphorbia factors Cy_6, Cy_{11} and Cy_{14} are the strongest irritants of the ingenane ester type hitherto known, Cy_{11} being at least as active as the standard diterpene ester type irritant 12-O-tetradecanoylphorbol-13-acetate (TPA).

The cypress spurge (*Euphorbia cyparissias* L.), a small herb with narrow leaves, is indigenous to Europe, except for the extreme Northern and North-Eastern parts. It has been introduced into North America[3] and New Zealand[4]. Since the 4th century BC the use of the latex or other parts of the plant has been known in traditional medicine[5], especially against warts and other cancerous conditions[6]. Even today an ethanolic extract of the plant is recommended in homeopathy as a purgative and for treatment of psoriasis, diarrhoea, inflammations and rheumatic diseases[7].

In our research program on the distribution of co-carcinogens (i.e. tumor promoters[8]) in the plant kingdom[9], at the beginning the large succulent Euphorbiaceae of the tropical regions of the world were studied for obvious reasons (e.g. Opferkuch and Hecker[18]). With the experience accumulated we started to investigate the rather small herbaceous, European *Euphorbia* species, such as *Euphorbia lathyris* L.[15] and *Euphorbia cyparissias* L. Investigation of the latter species was stimulated by reports indicating that it contains a toxic and irritant principle, which causes acute poisoning of cattle if the plant occurs in hay[3,10]. In acute or chronic intoxication of this kind a transfer of the irritant principle into milk may take place[3,10]. Also, from the closely related[3] herbaceous *Euphorbia esula* L. the isolation of the antileukemic 3,20-di-O-benzoylingenol[11] and aliphatic O-acylated esters of ingenol[12] has been reported. Evidence has been presented[13] that the biologically active diterpene esters contained in *Croton flavens* L. may be environmental risk factors for esophageal cancer, in connection with its local use on Curacao for a popular 'bush tea'. Hence, considering the background of its reported utilization, it appeared well worth while to describe more accurately the diterpene constituents of *E. cyparissias* L.

Diterpene ester fraction from roots. The extract of fresh roots of *Euphorbia cyparissias* L., obtained by exhaustive extraction with methanol at room temperature, is an irritant as measured by the irritant dose 50 (ID_{50}) on the mouse ear[14]. The methanolic extract is distributed between dichloromethane and water. From the organic phase, by 2 subsequent O'Keeffe distributions[14] in different solvent systems and by subsequent column chromatography of the active portion on silica gel, a diterpene ester fraction is prepared. From this fraction containing all the irritant activity pure diterpene esters can be isolated by multistage Craig distribution[14] followed by TLC of the active fractions. All pure compounds isolated are colourless resins, resisting all attempts at crystallization, so far. Altogether, according to their partition coefficients, they comprise 2 groups of polyfunctional diterpenes.

Di-O-acylates of the polyfunctional diterpene parent 13-hydroxyingenol. (**1**). The main component of the 1st group of polyfunctional diterpenes is the irritant Euphorbia factor Cy_{14} (table, **1a**): MS: 644 (M^+), 626 (M^+-18), 510 (M^+-134), 426 (M^+-218), 310 (base peak); IR (CH_2Cl_2): ν_{max}: 3580, 3550, 3510, 3420 (OH), 2925, 2850 (CH), 1722 cm^{-1} (C=O); UV (CH_3OH): $\lambda(\varepsilon)$: 193 nm (17180); $\lambda_{max}(\varepsilon)$: 291 nm (220); 90 MHz ^{1}H-NMR ($CDCl_3$, $\delta_{TMS}=0.00$):