

Estramustine phosphate inhibits germinal vesicle breakdown and induces depolymerization of microtubules in mouse oocyte

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Summary. The first meiotic cell division (meiotic maturation) of the dictyate stage of mouse oocytes, removed from the follicle, resumes spontaneously *in vitro*. This study indicates that estramustine phosphate reversibly blocks meiotic maturation by inhibiting germinal vesicle breakdown. Oocyte cryostat sections were stained with anti-tubulin and two antibodies (anti-MAP1 : JA2 and 5051) which decorated the metaphase spindle during meiotic maturation. It was found that (1) estramustine phosphate depolymerized microtubules *in ovo* and dispersed non-tubulin antigens associated with microtubules of the metaphase spindle at various stages of maturation, and (2) estramustine phosphate decreased the ability of taxol to induce cytoplasmic asters. These results suggest that germinal vesicle breakdown and microtubule polymerization may be linked.

Introduction.

The small number of prophase microtubules present in mouse oocyte, which contains no centriole (Szöllösi *et al.*, 1972b), are replaced during first meiotic cell division or meiotic maturation by a large number of spindle microtubules. Microtubule nucleation capacity increases at the nucleoplasm-cytoplasm interface at the onset of nuclear envelope breakdown (Calarco *et al.*, 1972 ; Szöllösi *et al.*, 1972a, b). At the same time, the critical level of tubulin polymerization decreases since taxol can then induce the formation of numerous spindle-like structures (Rime *et al.*, 1987). These observations indicate that new microtubule-organizing centers (MTOCs) are generated during the breakdown of the nuclear envelope. The role of microtubule-associated proteins (MAPs) in this process is poorly understood. It has been shown however that antigens stained by anti-MAP antibodies are present in the mitotic spindle (Izant *et al.*, 1982 ; Bloom *et al.*, 1984 ; Wiche *et al.*, 1984 ; Bonifacino *et al.* (1985). We have shown recently that

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the first and second meiotic metaphase spindles in mouse oocyte can be decorated with a monoclonal antibody raised against MAP1 from rat brain (Rime *et al.*, 1987). The immunofluorescence studies of Calarco-Gillam *et al.* (1983), Maro *et al.* (1985) and Schatten *et al.* (1986) have shown that 5051 antibody, a polyclonal antibody taken from a scleroderma patient and recognizing MTOC in somatic cells (Calarco-Gillam *et al.*, 1983 ; Brinkley, 1985), also marks the poles of barrel-shaped metaphase II of mouse oocyte. Although the mouse oocyte antigens recognized by these antibodies were not biochemically characterized, they can be considered to be spindle-associated antigens, according to cytological criteria. These antibodies have been used as a tool to analyse the cellular distribution of meiotic microtubule-associated non-tubulin proteins during germinal vesicle breakdown (GVBD) in mouse oocyte.

Estramustine phosphate, a cytotoxic anti-mitotic compound, has been shown recently to inhibit microtubule assembly *in vitro* by interacting with MAPs (Hartley-Asp, 1984 ; Kanje *et al.*, 1985 ; Wallin *et al.*, 1985). Estramustine phosphate also produces microtubule disassembly *in vivo* in different cell lines (Stearns and Tew, 1985). Estramustine may act *in vivo* by binding to MAPs in the whole cell. It was therefore of interest to know if estramustine phosphate could interact directly or indirectly with non-tubulin proteins associated with metaphase microtubules in mouse oocyte.

The present study shows that estramustine phosphate reversibly destabilized mouse oocyte metaphase microtubules and disorganized the immunofluorescence staining obtained with both the 5051 and the JA2 antibodies. Estramustine phosphate also inhibited the breakdown of the nuclear envelope and the formation of spindle microtubules.

Materials and methods.

Chemicals. — Estramustine phosphate, estradiol-3-(N,N-bis-(2 chloro-ethyl) carbamate) 17 β -phosphate, was the gift of Dr Hartley-Asp, AB Leo Research Laboratories, Helsingborg, Sweden. Taxol was the gift of Dr M. Süffness of the NIH, Bethesda, MD, USA.

Oocyte collection and culture. — Follicular oocytes were obtained from the ovaries of 8 to 14-week old Swiss albino mice (Ifracredo, France). The mice were killed by cervical dislocation and their ovaries removed and placed in a Petri dish containing M2 culture medium (Fulton and Whittingham, 1978). The antral follicles were carefully dissected and the oocytes liberated by puncturing the follicle with a needle. 20 to 40 oocytes, obtained in this way from each mouse, were cultured in M2 under paraffin oil equilibrated with the medium at 37 °C.

Antibodies. — Mouse monoclonal antibody against β -tubulin of chick brain was obtained from Amersham (N.357). Mouse monoclonal antibody against rat brain MAP1 (JA2) was the gift of Dr M. De Brabander, Janssen Pharmaceutica Research Laboratories, Beerse, Belgium (De Mey *et al.*, 1984 ; De Brabander *et al.*, 1985). Antibodies against pericentriolar material (microtubule-organizing center or MTOC) and the centriole cylinder were contained in the serum of a

human patient with linear scleroderma (Calarco-Gillam *et al.*, 1983). This antibody was the gift of Dr Calarco-Gillam, University of California, Medical Center, San Francisco, USA. An antiserum specific to BHK matrix proteins was a gift from by Dr Simard, Institut du Cancer de Montréal, Canada (Dagenais *et al.*, 1984).

For indirect immunofluorescence, the second antibody was directed against the species of the first antibody : anti-mouse Ig fluorescein-labeled antibody from sheep (Amersham, N.1031) and anti-mouse Ig Texas red-labeled antibody from sheep (Amersham, N.2031) for the anti-tubulin monoclonal antibody, anti-mouse IgM fluorescein-labeled antibody from rats (Biosys, N.5059) for the JA2 monoclonal antibody and anti-human IgG.AM fluorescein-labeled antibody from goats (Institut Pasteur Production, N.74511) for the anti-MTOC (5051) antibody.

Immunocytochemical procedures. — The mouse oocytes, fixed in acetic (0.5 %) ethanol (70°) for 30 min at room temperature, were progressively hydrated and washed in PBS buffer (137 mM NaCl, 2.7 mM KCl, 0.5 mM MgCl₂ (6 H₂O), 3.2 mM Na₂HPO₄ (12 H₂O), 1.5 mM KHPO₄, 0.68 mM CaCl₂, pH 7.3) before being embedded in gelatin (20 % in PBS buffer) and cooled in isopentane (– 130 °C).

For tubulin or lamin staining, cryostat sections (Frigocut 2700, Reichert-Jung) (7 µm) were hydrated and permeabilized 30 min in PBS buffer containing Saponin (0.03 %) and then incubated for 15 min in PBS buffer containing Saponin (0.03 %) and bovine serum albumin (BSA) (2 %). The sections were then incubated with the anti-tubulin or anti-lamin antibodies, diluted in PBS buffer-Saponin (0.03 %) BSA (2 %), for 2 h at room temperature (dilution 1/300 for anti-tubulin antibody and 1/100 for anti-lamin antibody). After incubation with the antibody, the sections were washed in PBS buffer-Saponin (0.03 %) and incubated for 1 h in the dark at room temperature with the fluorescein-labeled anti-mouse Ig antibody (dilution 1/40) or with the fluorescein-labeled anti-rabbit IgG antibody (dilution 1/800) recognizing, respectively, the anti-tubulin and the anti-lamin antibodies. The sections were then washed in PBS buffer-Saponin (0.03 %) and incubated for 10 sec with Hoechst dye 33258 (Calbiochem) (2.5 µg/ml in PBS) at room temperature to visualize the chromosomes. The sections were washed in PBS and mounted in glycerol (50 % in PBS buffer).

The same procedure was used with JA2 antibody but the PBS buffer was replaced by 10 mM Tris, 100 mM NaCl, pH 7.6, because staining with JA2 antibody is blocked by phosphate (De Brabander, personal communication). The sections were incubated for 1 h 30 with JA2 antibody (dilution 1/250) at 37 °C and for 1 h in the dark at room temperature with the fluorescein-labeled anti-mouse IgM antibody (dilution 1/100).

The same procedure was used for double indirect immunofluorescence, *i.e.* for anti-tubulin and anti-MTOC (5051) antibodies. After incubation with the first (anti-tubulin) and the second antibody (Texas red-labeled anti-mouse Ig antibody) (dilution 1/300), the sections were washed in PBS buffer-Saponin (0.03 %), and then incubated with anti-MTOC antibody (5051) diluted in PBS buffer-Saponin (0.03 %) BSA (2 %) (dilution 1/100) for 1 h in the dark at room temperature. These sections were then washed in PBS buffer-Saponin (0.03 %) and incubated in the dark for 1 h at room temperature with the fluorescein-labeled anti-human

IgG.AM antibody (dilution 1/100), then washed in PBS buffer and mounted as described previously.

In each experiment, the control sections were only incubated with the second antibody. Under these conditions no fluorescence was observed. The specificity of anti- β tubulin antibody was ascertained by absorption with an excess ($\times 20$) of purified tubulin isolated from rat brain, as described by Shelanski *et al.* (1973).

Observations were made in light epifluorescence (λ : 560 nm, 490 nm and 380 nm) with a Leitz Dialux 20 microscope. Micrographs were taken using Ilford HP5 film exposed at 400 ASA and developed in Microphen (Ilford).

Oocyte treatment.

With estramustine phosphate. — A stock solution of 15 mM estramustine phosphate (gift of Dr Hartley-Asp, Sweden) in absolute ethanol was used in these experiments. The solution was diluted in M2 medium at various final concentrations between 20 and 50 μ M, and the oocytes were incubated in this medium for various times before fixation. The control experiments were realized by incubating the oocytes in M2 medium containing the same concentrations of absolute ethanol alone. The nitrogen mustard moiety is known to not mimic estramustine effects (Hartley-Asp and Gunnarsson, 1982).

With taxol. — A stock solution of 10 mM taxol (gift of Dr M. Süffness, Bethesda, MD, USA) in dimethylsulfoxide was used in these experiments. The solution was diluted in M2 medium at a final concentration of 10 μ M and the oocytes were incubated in this medium for various times before fixation. The

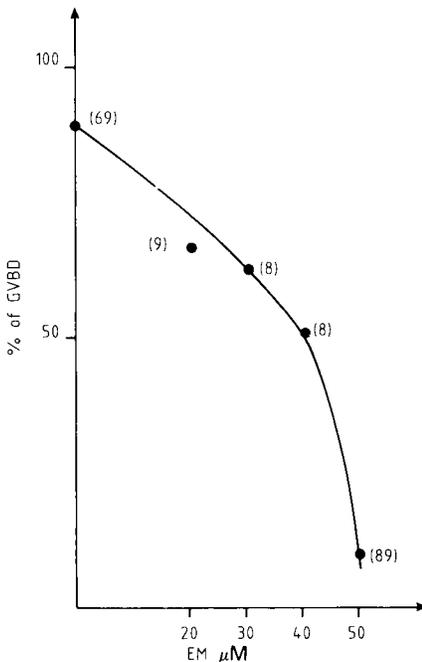


FIG. 1. — Inhibition of germinal vesicle breakdown by estramustine phosphate; dose-reponse curve. Numbers in brackets indicate the number of oocytes analyzed for each concentration of estramustine phosphate.

control experiments were realized by incubating the oocytes in the M2 medium containing the same concentrations of dimethylsulfoxide alone.

Results.

Inhibition of mouse oocyte meiotic maturation by estramustine phosphate

Oocytes liberated from their follicle spontaneously resume meiosis *in vitro* (Donahue, 1968). Germinal vesicle breakdown (GVBD) occurred in 89 % of the oocytes removed from the follicle and cultured for 4 h, as judged by examination under the light microscope (fig. 1). Less than 10 % of the oocytes cultured for 4 h in the incubation medium containing 50 μM of estramustine phosphate underwent GVBD (fig. 1). The inhibition of GVBD was dose-dependent, with an ED 50 of 45 μM . As shown in figure 2, the nuclear lamina, a protein network limiting the nucleoplasmic surface of the nuclear membrane (Gerace *et al.*, 1984), was fragmented at the time of GVBD. It was unchanged after estramustine phosphate action, indicating that the lamina did not depolymerize in the presence of the drug. This observation suggests that estramustine is a potent inhibitor of the first meiotic cell division of mouse oocyte.

The reversibility of estramustine phosphate action was tested by culturing the oocytes for 4 h in the presence of the drug (50 μM) immediately after their release from the follicle. The estramustine phosphate was then washed out and the oocytes placed in the control medium. GVBD occurred 4 h later and was followed by the extrusion of the first polar body. The inhibition of GVBD by estramustine phosphate was therefore reversible in these experimental conditions. At the concentrations tested, estramustine phosphate inhibition depended on the combined effects of the hydrophobic steroid and the hydrophilic mustard moieties since neither the steroid nor the nitrogen mustard alone inhibit maturation (Hartley-Asp and Gunnarsson, 1982).

Effects of estramustine phosphate on microtubules and microtubule-associated protein during meiotic maturation

We analysed estramustine phosphate *in vivo* effect on the cellular distribution of microtubules and of an antigen recognized by an antibody raised against a microtubule-associated protein (MAP1), as well as on the localization of microtubule-organizing centers (MTOCs). Three antibodies were used in this immunofluorescence study on cryostat sections. The first antibody was a monoclonal antibody against MAP1 (antibody JA2, the gift of Dr M. De Brabander) (De Mey *et al.*, 1984 ; De Brabander *et al.*, 1985). The second antibody was a polyclonal autoantibody from a scleroderma patient and recognizes MTOC in somatic cells (Calarco-Gillam *et al.*, 1983) and mouse oocytes (Maro *et al.*, 1985). The third antibody, anti-tubulin antibody from Amersham, was a monoclonal antibody against β -tubulin.

1) *Prophase oocytes.* — The oocytes were stained by double indirect immunofluorescence immediately after removal from the follicles. Anti-tubulin antibody produced a light background staining of the whole cytoplasm together

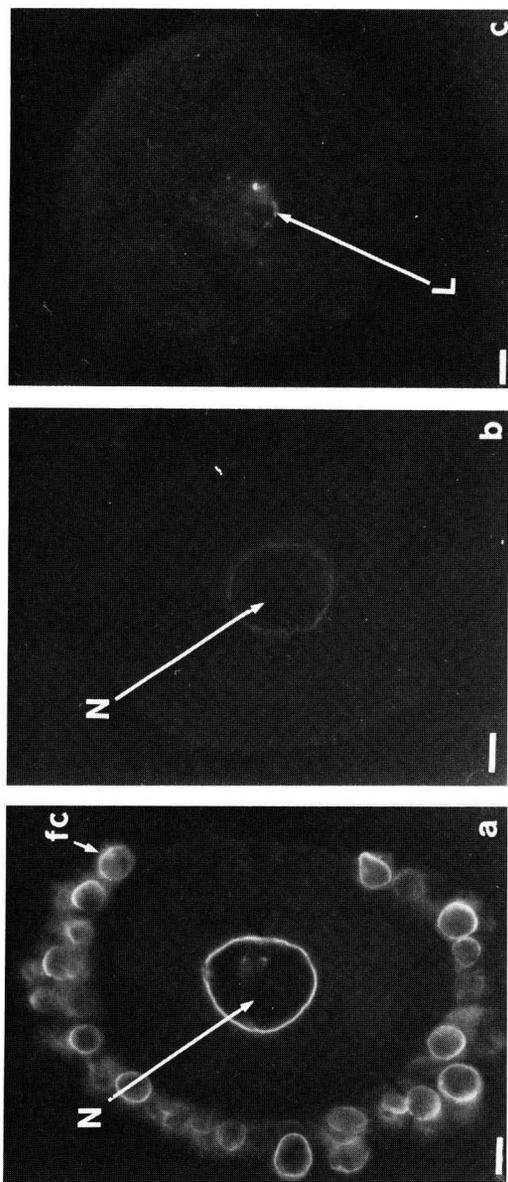
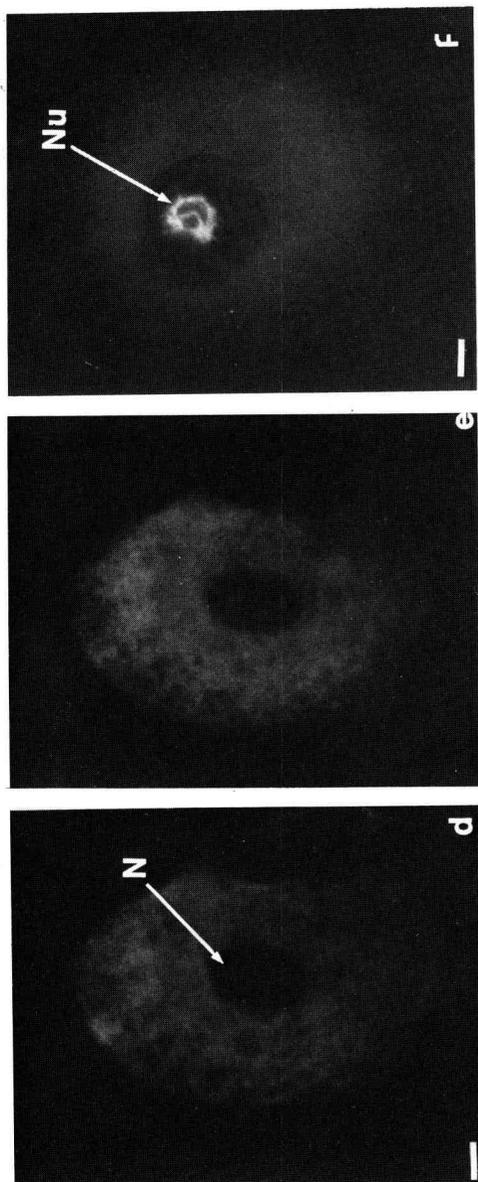
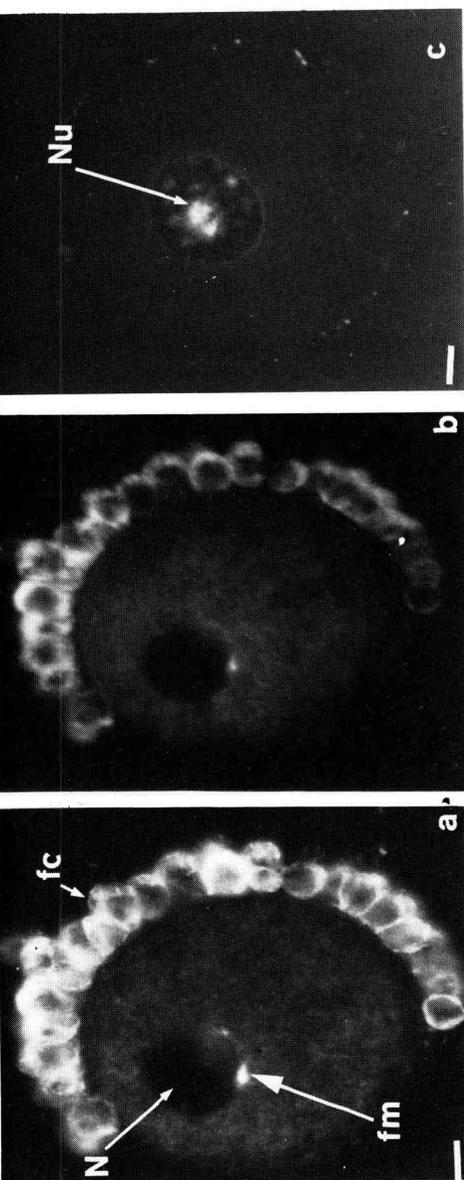


FIG. 2. — Immunofluorescence staining of control and estramustine phosphate-treated oocytes with anti-lamin antibody. Oocytes were fixed immediately after their removal from the follicle (a). Oocytes after 4 hr treatment with 50 μ M estramustine phosphate (b). Control oocytes after 4 hr in estramustine phosphate free medium (c).
N = nucleus ; L = lamina fragmented ; Bar = 10 μ m.

FIG. 3. — Immunofluorescence staining of control and estramustine phosphate-treated oocytes at germinal vesicle stage with anti-tubulin, JA2 and 5051 antibodies. Control oocytes were fixed immediately after their removal from the follicle. Sections of control oocytes incubated with anti-tubulin antibody (a), 5051 antibody (b), and JA2 antibody (c). Anti-tubulin (d), 5051 (e) and JA2 (f) antibody patterns for sections of estramustine-treated oocytes (50 μ M, 4 hrs).
N = nucleus ; Nu = nucleolus ; Em = foci of microtubules ; Fc = Follicular cells ; Bar = 10 μ m.



with discrete areas of higher fluorescence near the cytoplasmic surface of the nuclear envelope (fig. 3a). 5051 antibody also decorated this region of the same section (fig. 3b). No fluorescence was seen inside the nucleus. The immunofluorescence pattern of JA2 antibody was strikingly different in oocytes fixed at the GVBD stage, since JA2 antibody decorated only a trabecular structure in addition to the nucleolus inside the germinal vesicle (fig. 3c); no fluorescence was seen associated with the perinuclear microtubules.

The germinal vesicle did not break down in oocytes removed from the follicle and cultured for 4 h in the presence of 50 μ M estramustine phosphate. Cryostat sections of these oocytes stained with either anti-tubulin or 5051 antibody showed no fluorescence around the germinal vesicle, only a light background staining of the whole cytoplasm (figs. 3d, e). This indicates that both antigens were dispersed in the cytoplasm after estramustine phosphate treatment. In contrast, the JA2 antibody immunofluorescence pattern was unchanged (fig. 3f).

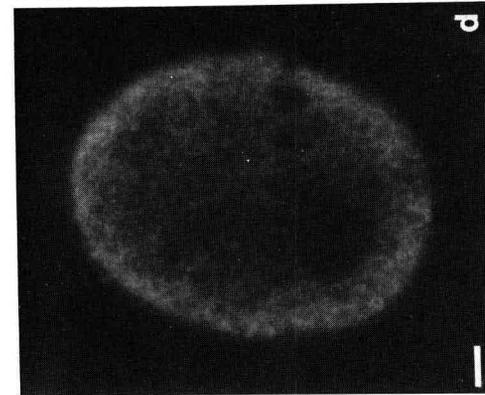
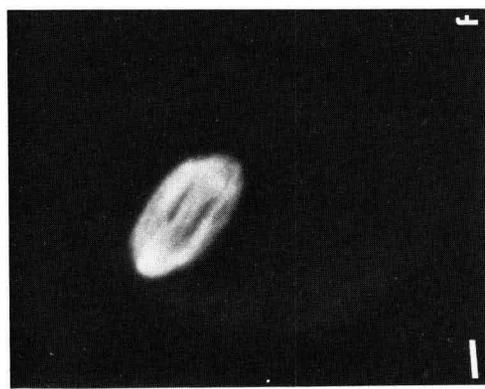
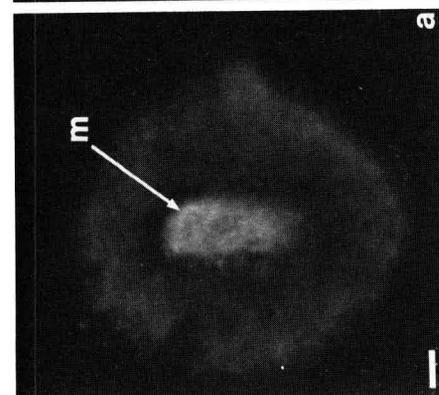
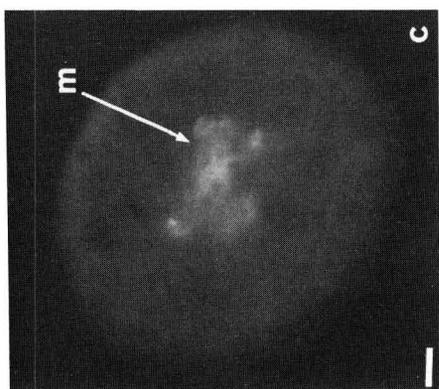
2) *Germinal vesicle breakdown.* — Numerous microtubules were stained with anti-tubulin antibody where the nucleus had broken down in oocytes in which GVBD was complete, as judged by light microscopy (after about 4 h in culture) (fig. 4a). Condensed chromosomes were located inside the area where microtubules were seen. On the same section, the 5051 antibody also decorated the same region (fig. 4b). JA2 antibody also stained this region of a different oocyte section (fig. 4c). The immunofluorescence staining patterns of the anti-tubulin, 5051 (not shown) and JA2 antibodies totally disappeared (figs. 4d, e) in oocytes treated with 50 μ M of estramustine phosphate for 15 min at the time of GVBD. Under these conditions, the background staining of the cytoplasm by all three antibodies slightly increased.

3) *Metaphase oocytes.* — The metaphase I spindle (9 to 10 h of culture) and metaphase II spindle (10 to 15 h of culture) were the only structures decorated by anti-tubulin and 5051 antibodies (figs. 5a, b); they were associated with condensed chromosomes (fig. 5c). 5051 antibody decorated the whole metaphase spindle on cryostat sections of *in vitro*-matured oocytes, as reported earlier for JA2 antibody (Rime *et al.*, 1987).

Estramustine phosphate (in standard conditions: 15 min, 50 μ M) abolished the staining patterns of anti-tubulin and 5051 antibodies of metaphase I and metaphase II spindles. But small foci of microtubules could be seen in some cases (figs. 5d, e). The chromosomes remained condensed and aligned in the metaphase plane (fig. 5f).

FIG. 4. — *Immunofluorescence labelling of control and estramustine phosphate-treated oocytes at the time of GVBD with anti-tubulin, 5051 and JA2 antibodies.* Control oocytes were fixed at the time of GVBD, as judged by light microscopy (4 hr of culture). Sections of control oocytes incubated with anti-tubulin (a), 5051 (b) and JA2 (c) antibodies. Anti-tubulin (d), and JA2 (e) antibody patterns for sections of estramustine phosphate-treated oocytes (50 μ M, 15 min) at GVBD as judged by light microscopy. Anti-tubulin antibody pattern (f) of oocyte treated 15 min with estramustine phosphate at the time of GVBD, and then incubated 2 hr without the drug: a metaphase I spindle is formed.

m = microtubules; Bar = 10 μ m.



The reversibility of estramustine phosphate action was tested by incubating oocytes at GVBD (4 h of culture) for 15 min in the presence of 50 μM of estramustine phosphate. All the microtubules disappeared (fig. 4d). The oocytes were washed and transferred to estramustine phosphate-free medium and cultured for 2 h. They were then fixed and the cryostat sections stained with anti-tubulin antibody. Microtubules reappeared in the oocyte (fig. 4f), as in control oocytes. The microtubules which were induced to depolymerize by estramustine phosphate were therefore capable of repolymerizing after the drug was removed.

Effects of estramustine phosphate and taxol on in ovo microtubules

Taxol has two effects on maturing oocytes immediately following GVBD : it causes a considerable increase in the bundling of microtubules around the chromosomes and induces the formation of asters in the cytoplasm (Rime *et al.*, 1987). The whole region near the chromosomes was stained by anti-tubulin antibody (fig. 6a) 5051 and by JA2 antibodies, as in control oocytes (figs. 6b, c). The cytoplasmic asters were also decorated by the three antibodies. Using indirect double immunofluorescence staining under the light microscope, we could not discern whether JA2 and 5051 antibodies decorated the microtubules themselves or the perimicrotubular regions, although the anti-tubulin and 5051 antibody patterns were very similar (figs. 6a, b). The capacity of taxol (known to induce microtubule polymerization *in vitro* : Schiff *et al.*, 1979 and *in vivo* : Heidemann and Gallas, 1980) to induce microtubule assembly in estramustine phosphate-treated oocytes was determined by incubating oocytes at the time of GVBD with 50 μM of estramustine phosphate for 1 h ; taxol was then added and its effect analysed 15 min later. As shown in table 1 and figures 6d and e, estramustine phosphate decreased the efficiency of the induction of cytoplasmic asters by taxol.

TABLE I

Effect of prior exposure to estramustine phosphate upon taxol-induced asters in denuded oocytes.

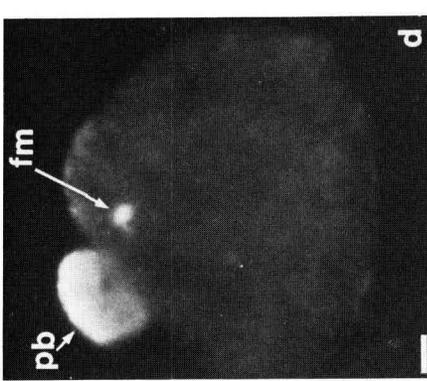
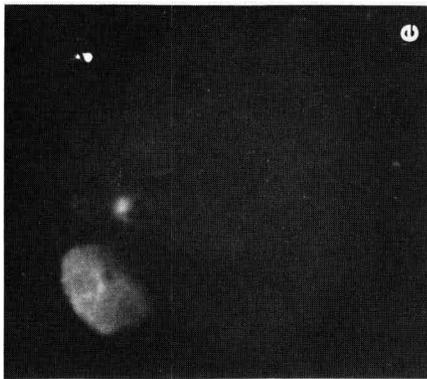
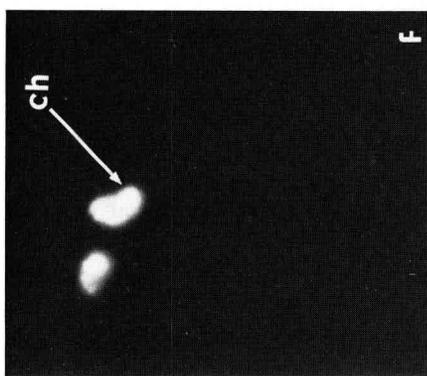
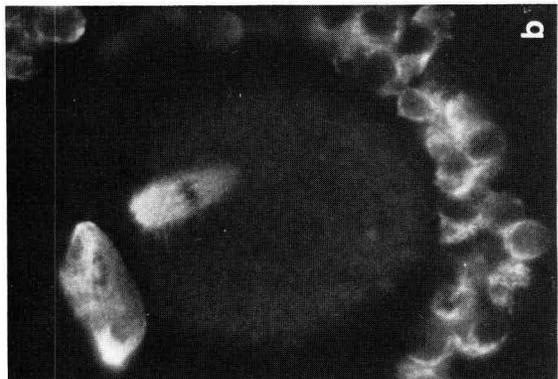
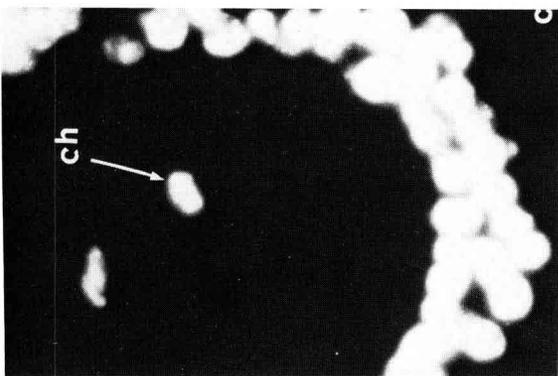
Taxol		Estramustine phosphate plus taxol	
Number of oocytes analyzed	Number of cytasters per oocyte (mean \pm SD)	Number of oocytes analyzed	Number of cytasters per oocyte (mean \pm SD)
14	3.42 \pm 1.4	15	1.7 \pm 1.35 ^a

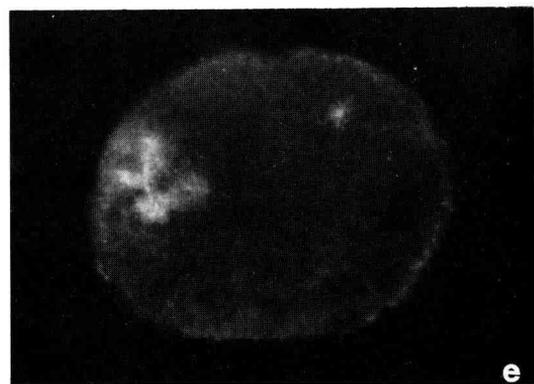
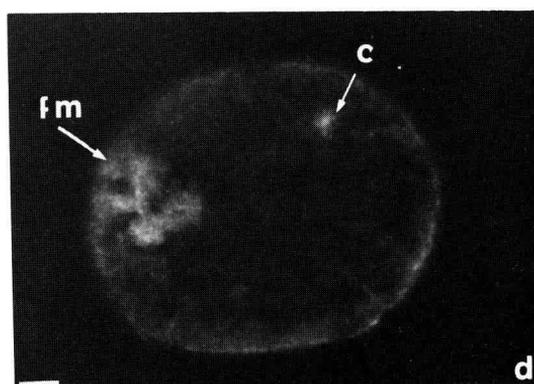
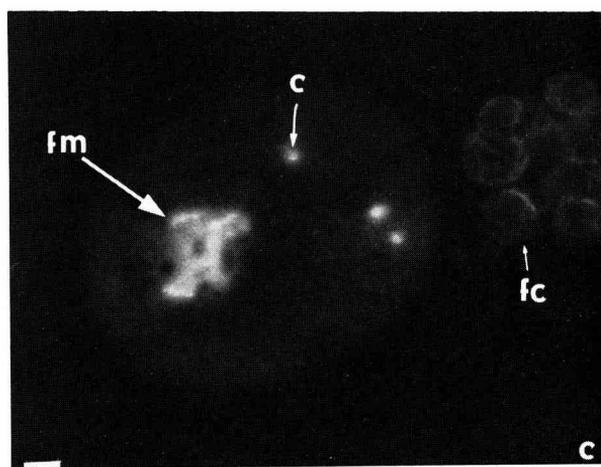
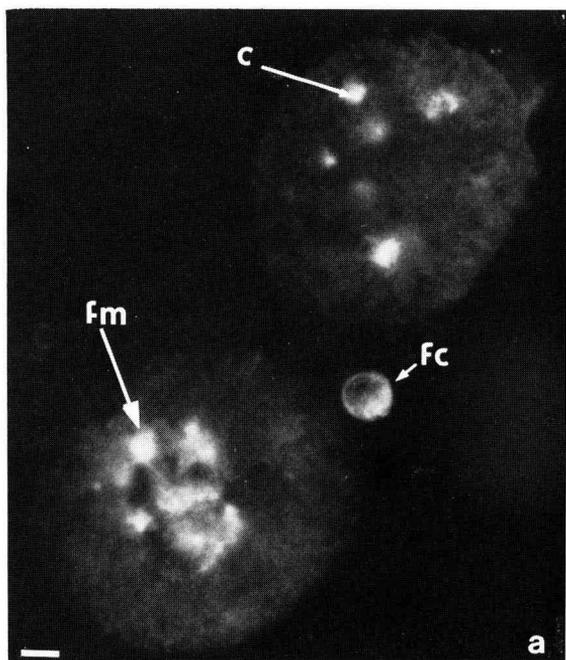
a : P < 0.01 (t-test).

In both series of experiments, taxol (10 μM , 15 min) was added after 4 hr of culture. In estramustine phosphate-treated oocytes, the drug (50 μM) was added 1 hr before taxol treatment.

FIG. 5. — *Immunofluorescence labelling of control and estramustine phosphate-treated metaphase II oocytes with anti-tubulin, 5051 and JA2 antibodies.* Control oocytes were stained at metaphase II by anti-tubulin (a) and 5051 (b) antibodies. Hoechst dye (c). Anti-tubulin (d) and 5051 (e) antibody and chromosome (f) patterns for sections of estramustine phosphate-treated metaphase II oocytes (50 μM , 15 min).

fm = foci of microtubules ; pb = polar body ; fc = follicular cells ; ch = condensed chromosomes ; Bar = 10 μm .





Discussion.

Estramustine phosphate (50 μM), an estradiol mustard, was found to have two major effects on mouse oocytes : it inhibited the breakdown of the nuclear envelope and provoked the reversible depolymerization of metaphase microtubules. It also partially inhibited the induction of asters by taxol in maturing oocytes after GVBD.

The block of oocyte maturation by estramustine phosphate was reversible and very similar to the inhibition of the maturation of mouse and rabbit oocytes induced by isopropyl-N-phenylcarbamate (IPC), an antimitotic agent which particularly affects cells lacking centrioles (Crozet and Szöllösi, 1979). Estramustine consists of estradiol-17 β linked to nitrogen mustard by a carbamate ester bond. It is therefore a plausible hypothesis that the carbamate moieties of both drugs act at the same level to inhibit GVBD. However, the effective inhibiting dose of IPC is higher (200 μM) than that of estramustine phosphate (50 μM) suggesting that the steroid moiety amplifies the effect of the estramustine molecule. To induce its action, the lipophilic steroid may control the targeting of the drug to a hydrophobic site, perhaps an intracellular membrane such as the nuclear envelope. Interestingly, it was recently shown that fluorescent dansylated estramustine accumulates in human prostatic tumor cells around the nuclear envelope (Stearns *et al.*, 1985). However, the way in which these drugs interact with the nuclear envelope remains unknown. Van Blerkom and Bell (1986) reported that nocodazole, a drug which depolymerizes microtubules by binding to the molecule of tubulin (Hoebeke *et al.*, 1976), does not inhibit GVBD in mouse oocyte but does inhibit spindle formation. We have successfully repeated this experiment. Thus, intact microtubules are not necessary for the breakdown of the nuclear envelope. In contrast, estramustine phosphate, which does not interact with the tubulin molecule itself but probably with microtubule-associated proteins (Kanje *et al.*, 1985 ; Wallin *et al.*, 1985), inhibits GVBD and also induces the depolymerization of oocyte microtubules. Therefore, estramustine may not inhibit GVBD by acting on the microtubules, but by other mechanism implicating the interaction of putative MAPs and nuclear envelope breakdown.

It is interesting to note that the metaphase spindle was stained by both the anti-tubulin and the 5051 antibody. This is in contrast to the observations of Calarco-Gillam *et al.* (1983), Maro *et al.* (1985) and Shatten *et al.* (1986) who, after carrying out indirect immunofluorescence of oocytes *in toto*, found that only the poles of the metaphase were stained by 5051 antibody. The non-metaphase

FIG. 6. — *Effects of estramustine phosphate and taxol on the microtubules of the oocyte at GVBD.* Sections of oocytes treated by 10 μM taxol for 15 min at GVBD, and stained by anti-tubulin (a), 5051 (b) and JA2 (c) antibodies. Sections of oocytes treated by 50 μM of estramustine phosphate for 1 hr, and then in estramustine phosphate plus taxol (10 μM) for 15 min and stained by anti-tubulin (d) and 5051 (e) antibodies.

fm = foci of microtubules ; fc = follicular cells ; c = cytasters ; Bar = 10 μm .

pericentriolar material foci described by Maro *et al.* (1985) and Shatten *et al.* (1986) were never observed in the present study. This discrepancy may be due to our immunofluorescence technique on cryostat sections that allows a better diffusion of the antibody, and then responsible for labelling of the antigen along the whole spindle. This unusual observation raises the question of whether the 5051 serum revealed an antigen which was not limited to the spindle poles but distributed throughout the metaphase spindle. Only an electron microscope immunofluorescence study can determine if this antigen is localized on the microtubules or in the cytoplasm around the microtubules. This particular distribution of the antigen, known to recognize the MTOCs of somatic cells, could be a particularity of the meiotic metaphase spindle which remains stable until sperm penetration.

A major question therefore is : is the *in vivo* inhibitory effect of estramustine phosphate on oocyte microtubules caused by interaction with MAPs ? Although our results do not answer this question directly, they show that :

1) Estramustine induced the disappearance of the MTOCs, as revealed by 5051 antibody (figs. 3e, 4e, 5c), since no MTOCs can be observed after estramustine phosphate treatment of oocytes at different stages of maturation (GV, GVBD and metaphase).

2) Estramustine dissociates the co-localization (observed by immunofluorescence) of microtubules and antigens revealed by both the anti-MAP1 and the 5051 antibodies after GVBD. Since estramustine phosphate did not bind to tubulin, the antigens recognized by the JA2 and 5051 antibodies may be the target for estramustine phosphate, in agreement with the hypothesis that the drug preferentially acts on MAPs (Kanje *et al.*, 1985 ; Wallin *et al.*, 1985).

As noted above, the antigens recognized by JA2 and 5051 antibodies remain unknown. Although non-tubulin protein antigens recognized by both the 5051 and the JA2 antibodies are associated with microtubules after GVBD, their cellular distribution is strikingly different in prophase-maintained oocytes (figs. 3b, c). In these oocytes the nuclear localization of MAP1-analog antigen was unchanged by estramustine phosphate treatment (fig. 3f). Since MAP1-analog became associated with the microtubules after GVBD, it could be involved, as already suggested, in the genesis of new MTOCs when the nuclear envelope breaks down (Sato *et al.*, 1983 ; Bloom *et al.*, 1984 ; Bonifacino *et al.*, 1985 ; Ohno *et al.*, 1985 ; Jesus *et al.*, 1986 ; Sato *et al.*, 1986).

The observation that estramustine phosphate decreased the *in vivo* bundling action of taxol may be significant, suggesting that estramustine phosphate counteracts the *in vivo* taxol effect in mouse oocyte. In an earlier study (Rime *et al.*, 1987), we showed that JA2 antibody decorated asters induced by taxol in maturing oocytes, indicating that non-tubulin proteins might participate in the production of taxol-induced asters. A specific MAP, which remains to be identified, may thus be involved in the formation of asters after taxol treatment.

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Résumé. *Le phosphate d'estrामustine inhibe la rupture de l'enveloppe nucléaire et induit la dépolymérisation des microtubules de l'ovocyte de souris.*

L'ovocyte de Souris bloqué en prophase de première division méiotique (maturation méiotique), libéré de son follicule, reprend spontanément sa méiose *in vitro*. Ce travail montre que le phosphate d'estrामustine bloque de manière réversible la maturation méiotique en inhibant la rupture de l'enveloppe nucléaire. Les coupes à congélation d'ovocytes de Souris sont marquées par l'anticorps anti-tubuline et par deux anticorps qui décorent le fuseau de métaphase (anti-MAP1 : JA2 et le sérum 5051) pendant la maturation méiotique. Il a été montré que : (1) le phosphate d'estrामustine dépolymérise les microtubules *in ovo* et disperse les antigènes associés aux microtubules du fuseau de métaphase à différentes étapes de la maturation. (2) le phosphate d'estrामustine diminue la capacité du taxol à induire des asters cytoplasmiques. Ces résultats suggèrent que les protéines associées aux microtubules soient impliquées dans la rupture de l'enveloppe nucléaire.

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