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Antifertility Activity of DMA in Hamsters: Protection with a Luteotropic Complex (41046)

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Abstract. Administration of *N,N*-dimethylacetamide (DMA) terminated pregnancy in hamsters given the compound around nidation (Days 4–5 of pregnancy). DMA was 100% effective at a sc dose of 2.2 g/kg which was, however, about one-third of the lethal dose. This effect was reversible after about 10 days. Oral activity was significant but less effective compared with sc (67% at 2.2 g/kg). Daily injections of progesterone (5 mg/hamster/day—4 consecutive days) blocked the antifertility effect of DMA as did injections of a "luteotropic complex" (1 mg/hamster/day prolactin plus 5.1 IU/hamster day of gonadotropin activity in pregnant mare serum for 4 consecutive days). Microscopic examination of ovarian tissue from DMA-treated hamsters showed extensive degeneration and hemorrhage of corpora lutea. The luteotropic complex greatly improved the histological conditions of the ovaries.

During a routine survey of chemical structures thought to have antifertility activity in pregnant hamsters, *N,N*-dimethylacetamide (DMA) was used as one of the vehicles to dissolve certain test compounds. It was soon discovered that DMA alone interrupted pregnancy under conditions of the test. Subsequently, a review of the literature disclosed a report on the abortifacient effects of DMA on pregnant rats (1). When given before implantation (Days 2, 3, 4, or 5) DMA altered normal blastocyst development, if given up to the 14th day of gestation. Several materials such as amino acids, folic acid, thymidine, and thymine were given in an attempt to antagonize DMA effects. All were without satisfactory results and only thymine had a transitory protective effect from Day 2 to 7 of gestation. An additional report has also mentioned toxic effects of DMA on pregnancy in both rats and women as well (2).

The present study reports DMA termination of pregnancy in the hamster. It also describes the DMA-induced histological changes in the ovary as well as the protective effect of a hamster hormonal "luteotropic complex" (3, 4) given concurrently with DMA.

Materials and Methods. Golden hamsters (*Mesocricetus auratus*)[2] were maintained in screen-bottom cages and fed fresh cut apples twice a week and Purina lab chow and water *ad lib*. A constant supply of males were kept for breeding while females were purchased constantly for experiments. They were housed for at least 2 weeks to establish regular ovulatory cycles and nutritional stability previous to breeding. On cycle Day 4 (proestrus), a single female was housed overnight with two males. Early the next morning, vaginal smears containing sperm served as evidence for pregnancies. This procedure has proven effective in predicting pregnancy in many laboratories as well as in our laboratory (5). On Day 1 of pregnancy (day of sperm), test hamsters were weighed (80–100 g) and placed in groups of six, housed three per cage.

Administration of compounds. Test materials[3] shown in Fig. 1 were given sc or orally in 0.5 ml volume when possible. They were dissolved or diluted with either deionized sterile water, 95% ethanol or used directly without a vehicle. Compounds were usually injected in the AM on Day 4 of pregnancy; in special tests DMA was given on different days of pregnancy.

For luteotropic complex treatment, phys-

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iological saline solutions of ovine prolactin (PRL)[4] and pregnant mare serum (PMS)[5] were injected separately in 0.25-ml volumes. The PMS was used for follicle stimulating hormone (FSH) activity; it should be noted that luteinizing hormone (LH) activity was probably present as well. The PRL single dose was 118 IU/kg or about 10 IU/hamster (0.5 mg) twice daily, 10 AM and 2 PM and the gonadotropin activity in PMS was injected as a single dose of about 30 IU/kg or about 2.5 IU/hamster, twice daily as above. Progesterone was dissolved in 95% ethanol and diluted with sterile water to 50% ethanol; 0.6-ml volume given sc was equivalent to 59 mg/kg (about 5 mg/hamster) once a day. All hormonal materials were injected on Days 4, 5, 6, and 7 of pregnancy (see Table II). Control animals were given either deionized sterile water, physiological saline, 50% ethanol, or 95% ethanol vehicles, on respective days of pregnancy for a given protocol and they did not alter pregnancy.

Implantation sites and tissue preparation. Animals were sacrificed by cervical dislocation on Day 8 and each uterus observed for implantation sites. Ovaries were removed from special groups of animals, fixed in 10% Formalin, embedded in paraffin, sectioned (10 μm), and stained with hematoxylin, phloxine, and eosin.

Results and Discussion. Data shown in Table I outline antifertility potency of DMA given at various times and doses. As much as 2.2 g/kg sc had to be injected for 100% inhibition on Day 4 of pregnancy. This dose could be given up to Day 6 of pregnancy and still be effective (Table I, footnote *b*). Since the lethal dose was found to be about 6 g/kg sc, DMA interrupted pregnancy at a low activity potency compared to toxicity (one-third lethal dose) and was most effective just before and a short time after nidation on Day 5. Oral activity of DMA was significant (67%) at the 100% effective sc dose. The diethyl analog was more effective orally than DMA (Table I, footnote *c*), and at the same time somewhat less toxic (LD_{100} of 11 mg/kg sc). Experiments are in progress to determine whether DMA acts primarily on the hypothalamus, anterior pituitary, or the ovary.

Table II lists data obtained in antifertility mechanism studies wherein certain hormone preparations were found protective in maintaining pregnancy in the presence of DMA. Progesterone given daily during gestation Days 4, 5, 6, and 7 protected quite well against effects of DMA, since implantation sites were visible in most hamsters on Day 8 inspection (Group III). The luteotropic complex of prolactin and PMS (3) was completely protective against the luteolytic effect of DMA (Group VI). Single injections of either PMS or prolactin showed significant but not complete protection to DMA (Groups IV and V). In a special series, groups of pregnant hamsters were treated with sc DMA to reverse pregnancy but were not sacrificed. Mating trials 10 days after the original DMA exposure (most animals gave evidence of ovarian cycle function) indicated significant regeneration of reproductive components since once-treated hamsters again mated and showed uterine implantation sites after sacrifice (footnote *b*, Table II).

The antifertility potencies of a series of analogs of DMA are shown in Fig. 1. Certain substitutions on the amide nitrogen appeared necessary for antifertility activity since acetamide was inactive.

Ethyl groups appeared to enhance activity (Table I, footnote *c* and Fig. 1, compounds 1, 8 and 9). Larger butyl or phenyl groups decreased activity (compounds 4 and 5, respectively). A single phenyl group for compound 6 (also called acetanilide, the well-known antipyretic analgesic) allowed 100% activity. Increasing the chain length did not alter potency (compound 7) but decreasing the chain length 1-carbon lowered antifertility activity (compound 8). These potencies are most likely a result of both specific tissue responses and differential metabolic disposition of compounds. It can not be assumed, however, that all compounds acted at the same site until the pri-

[4] Lactogenic hormone (20 IU/mg), Calbiochem, San Diego, Calif. 92112.

[5] Gonadotropin (1970 IU of gonadotropin/mg), Calbiochem.

TABLE I. ANTIFERTILITY POTENCY OF DMA IN PREGNANT HAMSTERS

Dose of DMA (g/kg)	No. of trials	Route	Treatment day of pregnancy	Percentage inhib. of pregnancy[a]
2.2	5	sc	4[b]	100
1.8	2	sc	4	67
1.4	2	sc	4	50
1.1	2	sc	4	17
0.9	2	sc	4	0
2.2	2	sc	3	67
2.2	2	sc	2	33
2.2	2	sc	1	17
2.2	4	Oral[c]	4	67

[a] Percentage inhibition of pregnancy did not change for trials of six animals each for a given dose. Control groups run with 0.9% saline vehicle remained pregnant for all treatment days.
[b] Single injections of 2.2 mg/kg DMA on pregnancy Days 5, 6, 7, or 8 gave percentage inhibition of pregnancy, respectively, of 100, 100, 67, or 67.
[c] *N,N*-diethylacetamide given orally at 2.2 mg/kg was 100% effective; 1.1 mg/kg was ineffective.

mary site of action for DMA is known and comparisons made.

Ovarian tissue changes which occurred after administration of DMA, with and without pituitary hormonal support, are shown in photographic plates of Figs. 2 and 3. Control ovaries represented in Fig. 2, Plate A showed all stages of follicular development and were dominated by mature corpora lutea which rose above the ovarian surface. Follicular development, maturation, and atresia, as well as luteal maturation and degeneration followed a normal pattern. The ovaries of hamsters given DMA (Fig. 2, Plate B) showed normal follicular development and maturation. Corpora lutea of DMA treated hamsters, however, showed congestion, frank hemorrhage, and pyknotic nuclei prior to attaining mature size. Because corpora lutea were undergoing degenerative changes prior to maturation, one gained the impression of overall atrophy.

When PMS was administered daily after DMA, pyknotic nuclei and luteal cell degeneration was less severe than in Plate B and more diffuse within the corpora lutea (Fig. 3, Plate C). However, even though

TABLE II. DMA ANTIFERTILITY MECHANISM STUDIES IN PREGNANT HAMSTERS

Group	Subcutaneous treatment on days 4, 5, 6, and 7 of pregnancy[a]	Percentage inhibition of pregnancy
I	Control + daily vehicle injections only	0
II	DMA[b] + daily vehicle injections only	100
III	DMA + progesterone[c]	17
IV	DMA + PMS[d]	67
V	DMA + prolactin[e]	67
VI	DMA + prolactin + PMS	0

[a] Two trials of six hamsters per group gave identical results.
[b] Single dose of 2.2 g/kg DMA on Day 4. Two additional sets of six hamsters treated with DMA and vehicle, but not sacrificed on Day 8, were observed for possible recovery of ovarian cycle function. Consequent mating trials, 10 days post DMA, yielded 83% incidence of pregnancy, indicated by implantation sites.
[c] 59 mg/kg once/day (about 5 mg/hamster).
[d] 60 IU/kg/day PMS (about 5.1 IU/hamster).
[e] 236 IU/kg/day total (about 20 IU or 1 mg/hamster).

	STRUCTURE	COMPOUND	PERCENT INHIBITION OF PREGNANCY
1.	$H_3C-C(=O)-NH_2$	Acetamide	0
2.	$H_3C-C(=O)-N(CH_3)_2$	N,N-dimethyl-acetamide (DMA)	100
3.	$H_3C-C(=O)-N(C_2H_5)_2$	N,N-diethyl-acetamide	100
4.	$H_3C-C(=O)-N(C_4H_9)_2$	N,N-dibutyl-acetamide	67
5.	$H_3C-C(=O)-N(C_6H_5)_2$	N,N-diphenyl-acetamide	0
6.	$H_3C-C(=O)-NH(C_6H_5)$	N-phenyl-acetamide	100
7.	$H_3C-H_2C-C(=O)-N(CH_3)_2$	N,N-dimethyl-proprionamide	100
8.	$H-C(=O)-N(CH_3)_2$	N,N-dimethyl-formamide	33
9.	$H-C(=O)-N(C_2H_5)_2$	N,N-diethyl-formamide	66

FIG. 1. Comparative potencies and chemical structures of analogs of DMA tested for antifertility in hamsters at 2.2 g/kg sc.

corpora lutea were comprised of fairly mature appearing cells, they failed to extend appreciably above the ovarian surface. Most corpora lutea contained foci of leukocytic infiltration, having perivascular orientation. Many follicles were seen in all stages of development. After the prolactin treatment ovaries showed corpora lutea which lacked the obvious alterations seen after DMA effects alone or following PMS injections (Fig. 3, Plate D). There did appear to be fewer developing follicles in relationship to the interstitial tissue; the ovaries were regarded as atrophic. As can be seen in Fig. 3, Plate E, the combination of both hormone preparations was much more effective in protecting the ovary against DMA exposure. Corpora lutea were mature and protruded above the surface of the ovary at several loci. Some were lightly infiltrated with PMN leukocytes with a perivascular distribution. A small amount

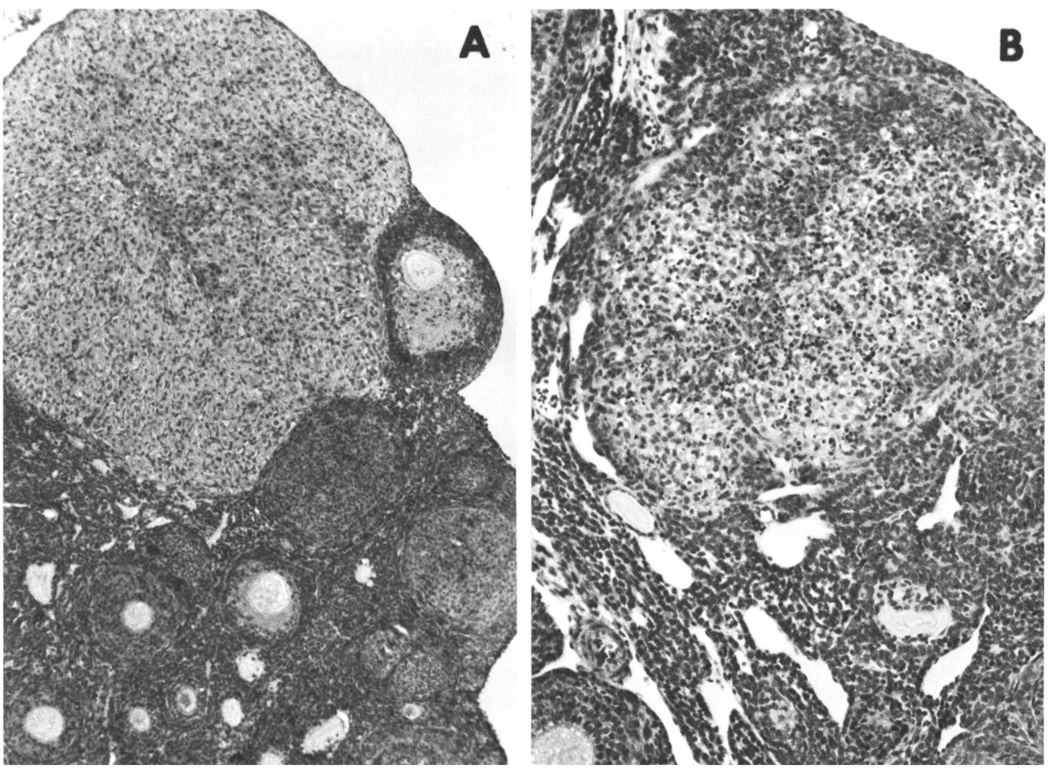

FIG. 2. Microscopic comparison of typical ovaries taken from control (A-30× magnification) and DMA-treated (B-48× mag.). Magnification includes 3× for photographic enlargement in addition to microscopic.

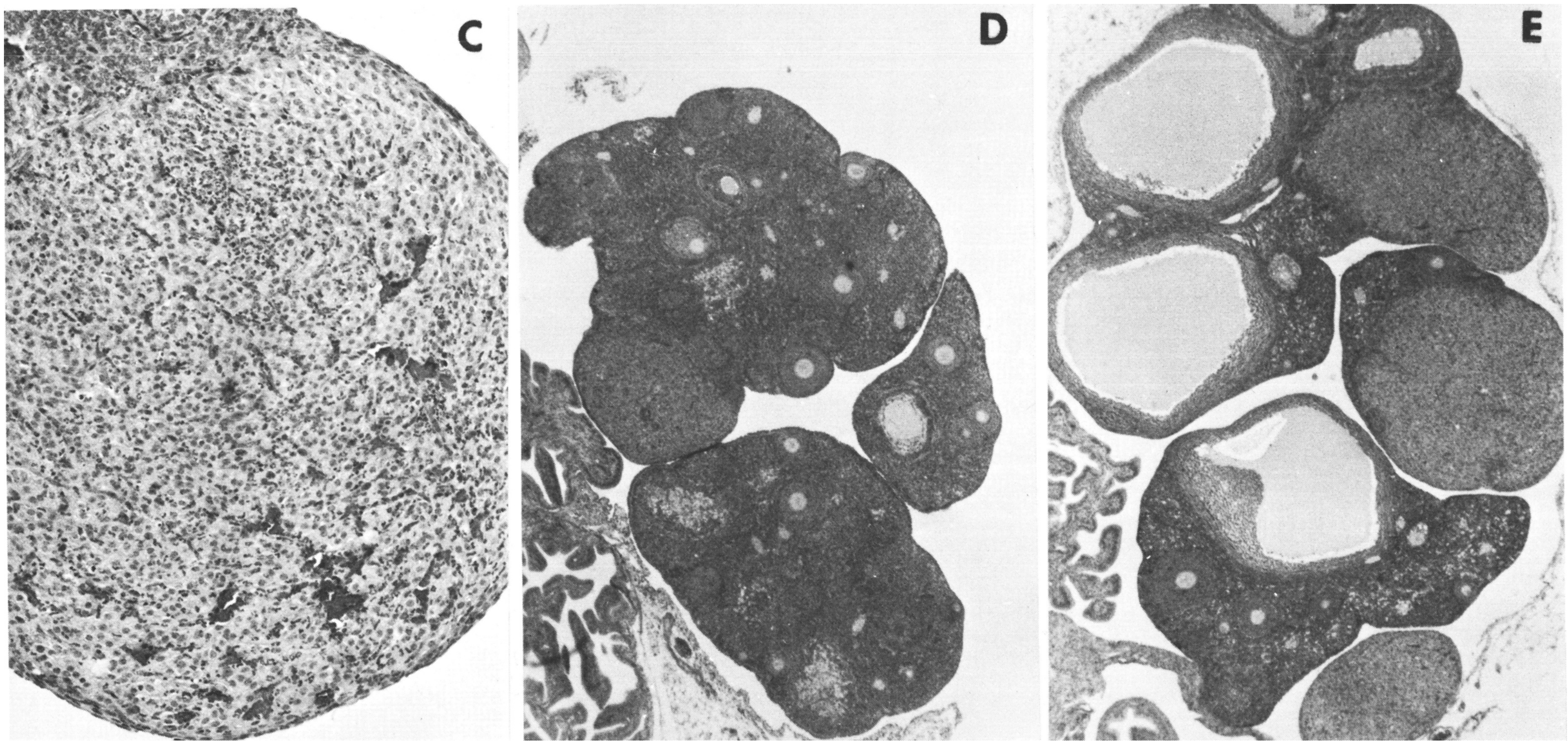

FIG. 3. Microscopic evidence of ovarian cellular protection after treatment of DMA-dose pregnant hamsters with either PMS (C-120× mag.) or prolactin (D-30× mag.); additive effect of both hormone preparations (E-30× mag.). Magnification includes 3× for photographic enlargement in addition to microscopic.

of karyorrhexis was present in the corpora lutea; several ovaries surveyed contained large Graafian follicles lacking ova. Data published to date (3, 4) indicate that the luteotropic complex preserves the corpora lutea, allowing progesterone synthesis to support early pregnancy in the hamster. In addition, since corpora luteal tissue once damaged by DMA was improved so extensively by the hormonal complex, it appeared likely that total effects were expanded over and above insuring progesterone synthesis alone.

1. Thiersch, J. B., J. Reprod. Fert. **4**, 219 (1962).
2. Nakoryakova, M. V., Gig. Tr. Prof. Zabal. **3**, 53 (1978).
3. Greenwald, G. S., Endocrinology **80**, 118 (1967).
4. Greenwald, G. S., Endocrinology **92**, 235 (1973).
5. Weeks, J. R., DuCharme, D. W., Magee, W. E., and Miller, W. L., J. Pharmacol. Exp. Therap. **186**, 67 (1973).