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# Modulatory Influence of Oral Contraceptive Pills Ovral and Noracycline on 3-Methylcholanthrene-induced Carcinogenesis in the Uterine Cervix of Mouse

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**The present study reports the modulatory influences of combined oral contraceptive formulations, Ovral (0.05 mg ethinylestradiol plus 0.5 mg norgestrel per pill) and Noracycline (0.05 mg ethinylestradiol plus 0.1 mg lynestrenol per pill), on methylcholanthrene (MCA)-induced carcinogenesis in the uterine cervix of Swiss albino mouse. Placement of cotton thread impregnated with beeswax containing ~300 μg of MCA yielded cervical tumors in 0.0%, 8.6% and 26% animals, respectively, in 30, 60 and 90 days. Concomitant treatments with doses $D_1$ (1/2000th of a pill), $D_2$ (1/200th of a pill) and $D_3$ (1/20th of a pill) of Ovral yielded cervical tumors in 0.0%, 0.0% and 4.5% mice at 30 days, 0.0%, 6.2% and 10% mice at 60 days and in 3.3% ($P<0.05$), 3.4% ($P<0.05$) and 47% mice at 90 days, respectively. Likewise, concomitant treatments with doses $D_1$ (1/2000th of a pill), $D_2$ (1/200th of a pill) and $D_3$ (1/20th of a pill) of Noracycline yielded cervical tumors in 0.0%, 0.0%, 16.6% mice at 30 days, 4%, 3.7% and 54% ($P<0.05$) mice at 60 days and 3.2% ($P<0.05$), 20% and 63% ($P<0.05$) of mice at 90 days, respectively. Both Ovral and Noracycline displayed biphasic action on MCA-induced cervical carcinogenesis in mice. At lower dose levels ($D_1$ and $D_2$), they were inhibitory while at the higher dose level ($D_3$) they were augmentatory in their actions. Both pills also significantly enhanced the incidence of cervical hyperplasia.**

Key words: Oral contraceptive pill — Ovral — Methylcholanthrene — Cervix — Carcinogenesis

Oral contraceptive (OC) pills, as effective means of preventing unwanted pregnancy, are being used by as many as 60 million women globally.[1] This widespread use raises the question of whether these pills have any association with initiational, promotional or inhibitory events in the processes of carcinogenesis especially in the target organs among the users. Epidemiological studies have yielded equivocal results regarding the possible association between OC pill use and risks of breast cancer,[2] cervical cancer[3–8] and malignant melanoma.[9–11] Studies have also suggested an increased risk of hepatocellular carcinoma among long-term OC pill users.[12–14] As in the case of other cancers, the possible cervical cancer risk among pill users elicits two main questions: 1) does the oral pill induce cervical cancer among users? 2) does the oral pill have any modulatory influence on the precancerous and cancerous lesions elicited by known or unknown carcinogens? While the first problem is being adequately investigated, the second one has yet to receive sufficient epidemiological as well as experimental attention.

The present study was designed to assess the modulatory influence of combined OC pills, Ovral and Noracycline, on the process of carcinogenesis induced by methylcholanthrene (MCA) in the uterine cervix of young, virgin, adult Swiss albino mouse.

## MATERIALS AND METHODS

Randomly bred, 8- to 9-week-old, virgin Swiss albino mice (Source: Animal Facility, JNU, New Delhi) were maintained in an air-conditioned animal room and provided with standard food pellets (Hindustan Lever Ltd., India) and tap water *ad libitum*.

**Chemicals** MCA was obtained from Sigma (USA), hematoxylin from Merck, Germany and eosin from BDH, England. Yellow beeswax was obtained from Mysore (India) and filtered four times in the molten state (60°C) to remove dust particles. OC pills Ovral (each containing 0.05 mg ethinylestradiol and 0.5 mg norgestrel) and Noracycline (each containing 0.05 mg ethinylestradiol and 1 mg lynestrenol), manufactured respectively by Wyeth Laboratories and Ciba-Geigy India, were purchased from a local pharmacy.

**Tumor induction** To induce tumors in the mouse uterine cervix, Murphy's string method[15] as described by Manoharan and Rao[16] was used. Under mild ether anesthesia, sterile cotton thread impregnated with beeswax containing ~300 μg of MCA was inserted into the canal of the uterine cervix by means of laparotomy.

**Experimental design** The animals were sorted into different control and experimental groups (see Tables I, II and

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III). Three different doses of the OC pills Ovral and Noracycline used in the present study were as follows. Ovral: (a) dose $D_1$ (1/2000th of a pill) containing 0.025 $\mu$g of ethinylestradiol and 0.25 $\mu$g norgestrel; (b) dose $D_2$ (1/200th of a pill) containing 0.25 $\mu$g of ethinylestradiol and 2.5 $\mu$g norgestrel; (c) dose $D_3$ (1/20th of a pill) containing 2.5 $\mu$g of ethinylestradiol and 25 $\mu$g norgestrel. Noracycline: (a) dose $D_1$ (1/2000th of a pill) containing 0.025 $\mu$g of ethinylestradiol and 0.5 $\mu$g lynestrenol; (b) dose $D_2$ (1/200th of a pill) containing 0.25 $\mu$g of ethinylestradiol and 5 $\mu$g lynestrenol; (c) dose $D_3$ (1/20th of a pill) containing 2.5 $\mu$g of ethinylestradiol and 50 $\mu$g lynestrenol. OC pill treatments at all 3 dose levels were given by the oral route daily for the periods of 30, 60 and 90 days to the mice with or without intracervical thread insertion.

Animals of groups 1, 2 and 3 (Table I) were treated with vehicle (distilled water) only, for 30, 60 and 90 days respectively. Beeswax-impregnated threads containing 300 $\mu$g of MCA each were inserted intracervically in the animals of groups 4, 5 and 6 (Table I), which were then treated with vehicle only for 30, 60 and 90 days, respectively. Mice of groups 7, 8 and 9 (Table I) were given intracervical wax-only impregnated threads and then treated with vehicle for 30, 60 and 90 days, respectively. Groups 1–9 served as controls for those experimental groups which received different doses of the pill plus/minus carcinogen (see Tables II and III).

Animals of groups 1, 8 and 15 (Tables II and III) were treated respectively with doses $D_1$, $D_2$ and $D_3$ of pills for 30 days while animals of groups 2, 9 and 16 (Tables II and III) received respectively doses $D_1$, $D_2$ and $D_3$ of pills for 60 days. Likewise the animals of groups 3, 10 and 17 (Table II and III) were treated respectively with doses $D_1$, $D_2$ and $D_3$ of pills for 90 days. Animals of all these groups (i.e. 1, 2, 3, 8, 9, 10, 15, 16 and 17) did not have intracervical thread insertions.

Carcinogen-thread-inserted animals of groups 4, 11 and 18 (Tables II and III) were treated for 30 days respectively with doses $D_1$, $D_2$ and $D_3$ of pills. Similarly the carcinogen-thread-inserted animals of groups 5, 12 and 19 (Tables II and III) were treated respectively with doses $D_1$, $D_2$ and $D_3$ of pills for 60 days and animals of groups 6, 13 and 20 (Tables II and III) were treated respectively with doses $D_1$, $D_2$ and $D_3$ of pills for 90 days following carcinogen thread insertion.

Animals of groups 7, 14 and 21 (Tables II and III) were given intracervical wax-only impregnated threads and treated respectively with doses $D_1$, $D_2$ and $D_3$ of pills for 90 days.

The animals were killed after 30, 60 and 90 days and their uterine cervices were fixed in Bouin's fluid. The animals with threads missing or displaced were discarded at autopsy. The uterine cervices embedded in paraffin wax were sectioned serially at a thickness of 5–6 $\mu$m in a plane parallel to the long axis of the organ. The sectioned slices of the cervices were stained with Harris hematoxylin and eosin. The precancerous and cancerous lesions screened in serial sections were classified as described elsewhere.[17] Chi-square test was used for determining the statistical significance of differences in the tumor incidences between control and experimental groups.

Table I. MCA-induced Precancerous and Cancerous Lesions in the Uterine Cervix of Mouse

| Gr. | Treatments | | | Effective no. of mice | Body weight (g) [mean±SEM] | | Number of mice with cervical lesions | | | | | | | Tumor incidence (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Intracervical route | Oral route | Period (days) | | Initial | Final | Hyperplasia (%) | Dysplasia + | Dysplasia ++ | Dysplasia +++ | Squamous cell carcinoma (invasive) Diff. | Poorly diff. | Undiff. | |
| 1 | Nil | Vehicle | 30 | 15 | 21±0.3 | 25±0.3 | 0 (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0/15 (0) |
| 2 | Nil | Vehicle | 60 | 15 | 20±0.2 | 28±0.2 | 0 (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0/15 (0) |
| 3 | Nil | Vehicle | 90 | 15 | 20±0.4 | 30±0.4 | 1 (6.6) | 0 | 0 | 0 | 0 | 0 | 0 | 0/15 (0) |
| 4 | MCA+wax thread | Vehicle | 30 | 20[a] | 20±0.2 | 25±0.2 | 13 (65) | 5 | 1 | 0 | 0 | 0 | 0 | 0/20 (0) |
| 5 | MCA+wax thread | Vehicle | 60 | 23[a] | 21±0.2 | 29±0.3 | 10 (43) | 3 | 3 | 2 | 1 | 1 | 0 | 2/23 (8.6) |
| 6 | MCA+wax thread | Vehicle | 90 | 23[a] | 20±0.3 | 30±0.5 | 10 (43) | 3 | 3 | 1 | 3 | 1 | 2 | 6/23 (26) |
| 7 | Wax thread | Vehicle | 30 | 13[a] | 19±0.3 | 24±0.3 | 1 (7) | 0 | 0 | 0 | 0 | 0 | 0 | 0/13 (0) |
| 8 | Wax thread | Vehicle | 60 | 13[a] | 20±0.2 | 27±0.2 | 0 (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0/13 (0) |
| 9 | Wax thread | Vehicle | 90 | 25[a] | 20±0.3 | 29±0.3 | 3 (12) | 4 | 0 | 0 | 0 | 0 | 0 | 0/25 (0) |

Gr.=group; Dysplasia: +=mild, ++=moderate, +++=marked; Diff.=differentiated, poorly diff.=poorly differentiated, Undiff.=undifferentiated

*a*) Animals with the threads remaining intact in the cervical canal at autopsy.

Table II. Modulatory Influence of Ovral on MCA-induced Precancerous and Cancerous Lesions in the Uterine Cervix of Mouse[a)]

| Gr. | Treatments | | | Effective no. of mice | Body weight (g) [mean±SEM] | | Number of mice with cervical lesions | | | | | | | Tumor incidence (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Intracervical route | Oral route | Period (days) | | Initial | Final | Hyperplasia (%) | Dysplasia + | Dysplasia ++ | Dysplasia +++ | Squamous cell carcinoma (invasive) Diff. | Squamous cell carcinoma (invasive) Poorly diff. | Squamous cell carcinoma (invasive) Undiff. | |
| 1 | Nil | Pill ($D_1$) | 30 | 15 | 19±0.2 | 24±0.3 | 13 (86)[e)] | 0 | 0 | 0 | 0 | 0 | 0 | 0/15 (0) |
| 2 | Nil | Pill ($D_1$) | 60 | 19 | 20±0.2 | 26±0.2 | 17 (89)[f)] | 0 | 0 | 0 | 0 | 0 | 0 | 0/19 (0) |
| 3 | Nil | Pill ($D_1$) | 90 | 15 | 20±0.3 | 27±0.3 | 7 (46)[g)] | 2 | 0 | 0 | 0 | 0 | 0 | 0/15 (0) |
| 4 | MCA+wax thread | Pill ($D_1$) | 30 | 24[b)] | 21±0.2 | 24±0.2 | 22 (91) | 2 | 0 | 0 | 0 | 0 | 0 | 0/24 (0) |
| 5 | MCA+wax thread | Pill ($D_1$) | 60 | 22[b)] | 21±0.2 | 28±0.2 | 0 (0) | 6 | 1 | 0 | 0 | 0 | 0 | 0/22 (0) |
| 6 | MCA+wax thread | Pill ($D_1$) | 90 | 30[b)] | 20±0.3 | 29±0.3 | 12 (6.6) | 4 | 2 | 0 | 1 | 0 | 0 | 1/30 (3.3)[c)] |
| 7 | Wax thread | Pill ($D_1$) | 90 | 20[b)] | 20±0.2 | 27±0.2 | 11 (55) | 2 | 0 | 0 | 0 | 0 | 0 | 0/20 (0) |
| 8 | Nil | Pill ($D_2$) | 30 | 10 | 20±0.3 | 24±0.3 | 7 (70)[h)] | 1 | 0 | 0 | 0 | 0 | 0 | 0/10 (0) |
| 9 | Nil | Pill ($D_2$) | 60 | 21 | 22±0.2 | 28±0.2 | 18 (85)[i)] | 1 | 0 | 0 | 0 | 0 | 0 | 0/21 (0) |
| 10 | Nil | Pill ($D_2$) | 90 | 14 | 20±0.3 | 29±0.3 | 9 (64)[j)] | 1 | 0 | 0 | 0 | 0 | 0 | 0/14 (0) |
| 11 | MCA+wax thread | Pill ($D_2$) | 30 | 25[b)] | 20±0.2 | 24±0.2 | 20 (80) | 0 | 0 | 0 | 0 | 0 | 0 | 0/25 (0) |
| 12 | MCA+wax thread | Pill ($D_2$) | 60 | 16[b)] | 20±0.2 | 28±0.2 | 12 (75) | 1 | 1 | 0 | 0 | 0 | 1 | 1/16 (6.2) |
| 13 | MCA+wax thread | Pill ($D_2$) | 90 | 29[b)] | 22±0.2 | 30±0.2 | 16 (55) | 4 | 1 | 1 | 1 | 0 | 0 | 1/29 (3.4)[d)] |
| 14 | Wax thread | Pill ($D_2$) | 90 | 20[b)] | 20±0.3 | 28±0.3 | 14 (70) | 1 | 0 | 0 | 0 | 0 | 0 | 0/20 (0) |
| 15 | Nil | Pill ($D_3$) | 30 | 11 | 20±0.2 | 23±0.2 | 10 (91)[k)] | 0 | 0 | 0 | 0 | 0 | 0 | 0/11 (0) |
| 16 | Nil | Pill ($D_3$) | 60 | 17 | 20±0.3 | 26±0.3 | 15 (88)[l)] | 2 | 0 | 0 | 0 | 0 | 0 | 0/17 (0) |
| 17 | Nil | Pill ($D_3$) | 90 | 13 | 20±0.3 | 28±0.3 | 9 (69)[m)] | 1 | 1 | 0 | 0 | 0 | 0 | 0/13 (0) |
| 18 | MCA+wax thread | Pill ($D_3$) | 30 | 22[b)] | 21±0.2 | 24±0.2 | 20 (91) | 1 | 0 | 0 | 0 | 0 | 1 | 1/22 (4.5) |
| 19 | MCA+wax thread | Pill ($D_3$) | 60 | 20[b)] | 20±0.2 | 28±0.2 | 14 (70) | 2 | 2 | 0 | 1 | 0 | 1 | 2/20 (10) |
| 20 | MCA+wax thread | Pill ($D_3$) | 90 | 17[b)] | 20±0.3 | 25±0.3 | 8 (47) | 0 | 0 | 0 | 8 | 0 | 0 | 8/17 (47) |
| 21 | Wax thread | Pill ($D_3$) | 90 | 18[b)] | 20±0.3 | 27±0.3 | 17 (94) | 0 | 0 | 0 | 0 | 0 | 0 | 0/18 (0) |

Dysplasia: + = mild, ++ = moderate, +++ = marked; Diff. = differentiated, Poorly diff. = poorly differentiated, Undiff. = undifferentiated.

*a)* Compare with the control values given in Table I.

*b)* Animals with the threads remaining intact in the cervical canal at autopsy.

Significance level: *c)* $P<0.05$ [Gr. 6 (Table II) vs. Gr. 6 (Table I)]; *d)* $P<0.05$ [Gr. 13 (Table II) vs. Gr. 6 (Table I)]; *e)* $P<0.001$ [Gr. 1 (Table II) vs. Gr. 1 (Table I)]: *f)* $P<0.001$ [Gr. 2 (Table II) vs. Gr. 2 (Table I)]; *g)* $P<0.05$ [Gr. 3 (Table II) vs. Gr. 3 (Table I)]; *h)* $P<0.05$ [Gr. 8 (Table II) vs. Gr. 1 (Table I)]; *i)* $P<0.001$ [Gr. 9 (Table II) vs. Gr. 2 Table I)]; *j)* $P<0.001$ [Gr. 10 (Table II) vs. Gr. 3 (Table I)]; *k)* $P<0.005$ [Gr. 15 (Table II) vs. Gr. 1 (Table I)]; *l)* $P<0.001$ [Gr. 16 (Table II) vs. Gr. 2 (Table I)]; *m)* $P<0.001$ [Gr. 17 (Table II) vs. Gr. 3 (Table I)].

## RESULTS

The findings of the present study are summarized in Tables I, II and III. Administration of the OC pill did not affect the body weight gain except in the animals of the group treated with carcinogen and the highest dose ($D_3$) of the pill, in which a slight fall in the body weight gain was noted. A small number of animals died in each group, most likely due to post-operative complications.

The animals which received intracervical MCA-plus wax threads and were killed after 30, 60 and 90 days (Gr. 4, 5 and 6) displayed 0.0%, 8.6% and 26% tumor incidences, respectively (Table I), while animals with wax-only threads and treated with vehicle (Gr. 7, 8 and 9) and animals treated with vehicle only but without intracervical thread insertion (Gr. 1, 2 and 3) did not display any incidence of tumor (Table I).

**Influence of oral contraceptive pill Ovral** The findings of the present study are shown in Table II. Animals without intracervical thread insertion did not show any tumor incidence when treated with any of the three doses ($D_1$, $D_2$ and $D_3$) of the pill for 30, 60 and 90 days (Gr. 1, 2 and 3; 8, 9 and 10; 15, 16 and 17). When animals with wax threads were exposed to the three different doses ($D_1$, $D_2$

Table III. Modulatory Influence of Noracycline on MCA-induced Precancerous and Cancerous Lesions in the Uterine Cervix of Mouse[a]

| Gr. | Treatments | | | Effective no. of mice | Body weight (g) [mean±SEM] | | Number of mice with cervical lesions | | | | | | | | Tumor incidence (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Intracervical route | Oral route | Period (days) | | Initial | Final | Hyperplasia (%) | Dysplasia + | Dysplasia ++ | Dysplasia +++ | Squamous cell carcinoma CIS | Squamous cell carcinoma (Invasive) Diff. | Squamous cell carcinoma (Invasive) Poorly diff. | Squamous cell carcinoma (Invasive) Undiff. | | |
| 1 | Nil | Pill ($D_1$) | 30 | 10 | 20±0.3 | 24±0.4 | 8 (80)[f] | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0/10 | (0) |
| 2 | Nil | Pill ($D_1$) | 60 | 15 | 20±0.3 | 26±0.2 | 12 (80)[g] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/15 | (0) |
| 3 | Nil | Pill ($D_1$) | 90 | 12 | 21±0.3 | 29±0.2 | 11 (91)[h] | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0/12 | (0) |
| 4 | MCA+wax thread | Pill ($D_1$) | 30 | 25[b] | 18±0.2 | 21±0.3 | 15 (60) | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0/25 | (0) |
| 5 | MCA+wax thread | Pill ($D_1$) | 60 | 24[b] | 20±0.3 | 27±0.4 | 10 (38) | 3 | 2 | 8 | 0 | 0 | 0 | 1 | 1/24 | (4) |
| 6 | MCA+wax thread | Pill ($D_1$) | 90 | 31[b] | 21±0.2 | 30±0.2 | 19 (61) | 6 | 3 | 2 | 0 | 0 | 0 | 1 | 1/31 | (3.2)[c] |
| 7 | Wax thread | Pill ($D_1$) | 90 | 19[b] | 19±0.2 | 30±0.2 | 13 (68) | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0/19 | (0) |
| 8 | Nil | Pill ($D_2$) | 30 | 12 | 21±0.2 | 24±0.3 | 7 (58)[i] | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0/12 | (0) |
| 9 | Nil | Pill ($D_2$) | 60 | 13 | 20±0.3 | 27±0.3 | 11 (84)[j] | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0/13 | (0) |
| 10 | Nil | Pill ($D_2$) | 90 | 15 | 21±0.3 | 30±0.3 | 11 (73)[k] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/15 | (0) |
| 11 | MCA+wax thread | Pill ($D_2$) | 30 | 25[b] | 20±0.2 | 25±0.2 | 17 (68) | 2 | 3 | 1 | 0 | 0 | 0 | 0 | 0/25 | (0) |
| 12 | MCA+wax thread | Pill ($D_2$) | 60 | 27[b] | 20±0.2 | 28±0.4 | 8 (29) | 5 | 6 | 5 | 0 | 1 | 0 | 0 | 1/27 | (3.7) |
| 13 | MCA+wax thread | Pill ($D_2$) | 90 | 20[b] | 19±0.3 | 31±0.4 | 6 (30) | 4 | 4 | 2 | 0 | 3 | 1 | 0 | 4/20 | (20) |
| 14 | Wax thread | Pill ($D_2$) | 90 | 14[b] | 20±0.3 | 30±0.4 | 10 (71) | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0/14 | (0) |
| 15 | Nil | Pill ($D_3$) | 30 | 15 | 18±0.2 | 22±0.3 | 9 (60)[l] | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0/15 | (0) |
| 16 | Nil | Pill ($D_3$) | 60 | 10 | 20±0.3 | 28±0.3 | 7 (70)[m] | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0/10 | (0) |
| 17 | Nil | Pill ($D_3$) | 90 | 10 | 21±0.2 | 30±0.3 | 8 (80)[n] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/10 | (0) |
| 18 | MCA+wax thread | Pill ($D_3$) | 30 | 24[b] | 18±0.2 | 21±0.2 | 7 (29) | 5 | 6 | 2 | 1 | 1 | 2 | 0 | 4/24 | (16.6) |
| 19 | MCA+wax thread | Pill ($D_3$) | 60 | 24[b] | 20±0.2 | 27±0.5 | 5 (21) | 2 | 1 | 3 | 0 | 6 | 3 | 4 | 13/24 | (54)[d] |
| 20 | MCA+wax thread | Pill ($D_3$) | 90 | 19[b] | 21±0.2 | 26±0.5 | 3 (15) | 1 | 3 | 0 | 1 | 7 | 1 | 3 | 12/19 | (63)[e] |
| 21 | Wax thread | Pill ($D_3$) | 90 | 14[b] | 20±0.3 | 28±0.4 | 10 (71) | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0/14 | (0) |

Dysplasia: + = mild, ++ = moderate, +++ = marked; Diff. = differentiated, Poorly diff. = poorly differentiated, Undiff. = undifferentiated. CIS = carcinoma *in situ*.

*a*) Compare with the control values given in Table I.

*b*) Animals with the threads remaining intact in the cervical canal at autopsy.

Significance level: *c*) $P<0.05$ [Gr. 6 (Table III) vs. Gr. 6 (Table I)]; *d*) $P<0.05$ [Gr. 19 (Table III) vs. Gr. 5 (Table I)]; *e*) $P<0.05$ [Gr. 20 (Table III) vs. Gr. 6 (Table I)]; *f*) $P<0.001$ [Gr. 1 (Table III) vs. Gr. 1 (Table I); *g*) $P<0.001$ [Gr. 2 (Table III) vs. Gr. 2 (Table I)]; *h*) $P<0.001$ [Gr. 3 (Table III) vs. Gr. 3 (Table I)]; *i*) $P<0.05$ [Gr. 8 (Table III) vs. Gr. 1 (Table I); *j*) $P<0.001$ [Gr. 9 (Table III) vs. Gr. 2 (Table I)]; *k*) $P<0.001$ [Gr. 10 (Table III) vs, Gr. 3 (Table I)]; *l*) $P<0.05$ [Gr. 15 (Table III) vs. Gr. 1 (Table I)]; *m*) $P<0.005$ [Gr. 16 (Table III) vs. Gr. 2 (Table I)] and *n*) $P<0.001$ [Gr. 17 (Table III) vs. Gr. 3 (Table I)].

and $D_3$) of the pill for 90 days (Gr. 7, 14 and 21), no tumor incidence was observed during the study period. Animals with intracervical insertion of MCA-plus-wax threads and treatments with OC pill dose $D_1$ for 30 and 60 days (Gr. 4 and 5) did not show any tumor incidence, while animals similarly treated for 90 days (Gr. 6) displayed 3.3% tumor incidence [($P<0.05$) Gr. 6 (Table II) vs. Gr. 6 (Table I)]. When animals were treated intracervically with MCA-plus-wax threads and exposed to OC pill dose $D_2$ for 30, 60 and 90 days (Gr. 11, 12 and 13) the tumor incidences were 0.0%, 6.2% and 3.4% [($P<0.05$) Gr. 13 (Table II) vs. Gr. 6 (Table I)] respectively. On the other hand, MCA-plus-wax thread-inserted animals when treated with OC pill dose $D_3$ for 30, 60 and 90 days (Gr. 18, 19 and 20) displayed tumor incidences as high as 4.5%, 10.0% and 47.0%, respectively. The tumors were squamous cell carcinomas (Fig. 1) of differentiated, poorly differentiated or undifferen-

Fig. 1. Photograph showing squamous cell carcinoma of the uterine cervix of mouse (×400).

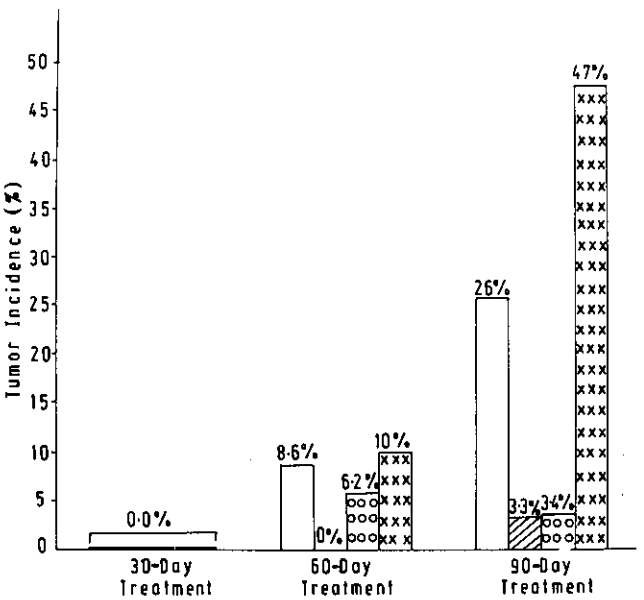


Fig. 2. Percentage tumor incidence following the treatment with MCA (~300 $\mu$g) and different doses of the OC pill Ovral. (□) Only MCA, (▨) MCA + Ovral ($D_1$), (□ooo) MCA+Ovral ($D_2$), (□xxx) MCA+Ovral ($D_3$).

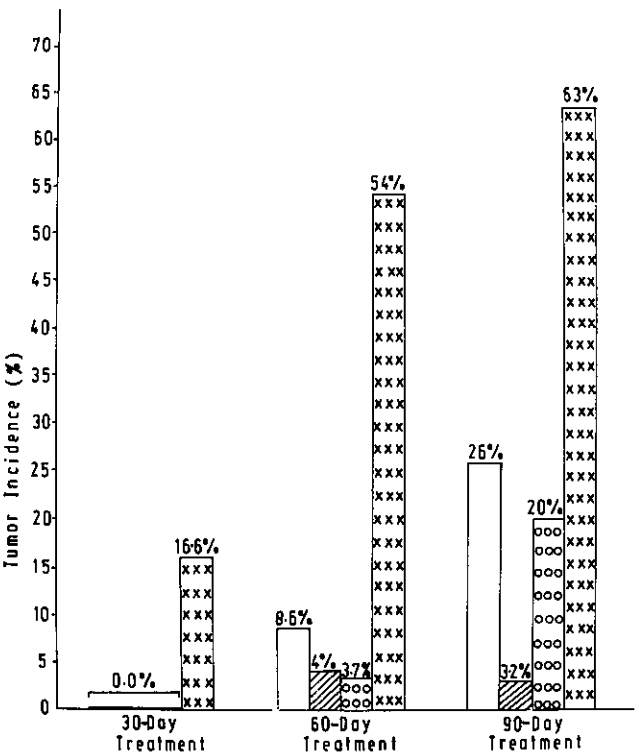


Fig. 3. Percentage tumor incidence following the treatment with MCA (~300 $\mu$g) and different doses of the OC pill Noracycline. (□) Only MCA, (▨) MCA+Noracycline ($D_1$), (□ooo) MCA+Noracycline ($D_2$), (□xxx) MCA+Noracycline ($D_3$).

tiated types. Fig. 2 shows the percentage tumor incidences following treatment with MCA and different doses of Ovral.

Besides displaying an interesting modulatory influence on the incidence of squamous cell carcinoma, all three doses (i.e., $D_1$, $D_2$ and $D_3$) of the OC pill significantly enhanced the incidence of hyperplasia: $P<0.001$, Gr. 1 (Table II) vs. Gr. 1 (Table I); $P<0.001$, Gr. 2 (Table II) vs. Gr. 2 (Table I); $P<0.05$ Gr. 3 (Table II) vs. Gr. 3 (Table I); $P<0.05$, Gr. 8 (Table II) vs. Gr. 1 (Table I); $P<0.001$, Gr. 9 (Table II) vs. Gr. 2 (Table I); $P<0.001$, Gr. 10 (Table II) vs. Gr. 3 (Table I); $P<0.005$, Gr. 15 (Table II) vs. Gr. 1 (Table I); $P<0.001$, Gr. 16 (Table II) vs. Gr. 2 (Table I) and $P<0.001$, Gr. 17 (Table II) vs. Gr. 3 (Table I). Incidences of different dysplastic conditions show no definite correlation with the pill treatments.

**Influence of oral contraceptive pill Noracycline** The findings of the present study are shown in Table III. Animals without intracervical thread insertion did not show any tumor incidence when treated with any of the three doses ($D_1$, $D_2$ and $D_3$) of the pill for 30, 60 and 90 days (Gr. 1, 2 and 3; 8, 9 and 10; 15 16 and 17). When animals with wax threads were exposed to the three different doses ($D_1$, $D_2$ and $D_3$) of the pill for 90 days (Gr. 7, 14 and 21), no tumor incidence was observed during the study period. Animals with intracervical inser-

tion of MCA-plus-wax threads and treatments with OC pill dose $D_1$ for 30, 60 and 90 days (Gr. 4, 5 and 6) displayed the tumor incidences of 0.0%, 4.0% and 3.2% ($P < 0.05$), respectively. When animals were treated intracervically with MCA-plus-wax threads and exposed to OC pill dose $D_2$ for 30, 60 and 90 days (Gr. 11, 12 and 13) the tumor incidences were 0.0%, 3.7% and 20%, respectively. On the other hand, MCA-plus-wax thread-inserted animals when treated with OC pill dose $D_3$ for 30, 60 and 90 days (Gr. 18, 19 and 20) displayed tumor incidences as high as 16.6%, 54% ($P<0.005$) and 63% ($P<0.05$), respectively. The tumors were squamous cell carcinomas of carcinoma *in situ* or infiltrating type. Fig. 3 shows the percentage tumor incidences following treatment with MCA and different doses of Noracycline.

Treatment with all three different doses (i.e., $D_1$, $D_2$ and $D_3$) of the OC pill significantly enhanced the incidence of hyperplasia in the animals without intracervical thread insertion [$P<0.001$, Gr. 1 (Table III) vs. Gr. 1 (Table I); $P<0.001$, Gr. 2 (Table III) vs. Gr. 2 (Table I); $P<0.001$, Gr. 3 (Table III) vs. Gr. 3 (Table I); $P<0.05$, Gr. 8 (Table III) vs. Gr. 1 (Table I); $P<0.001$, Gr. 9 (Table III) vs. Gr. 2 (Table I); $P<0.001$, Gr. 10 (Table III) vs. Gr. 3 (Table I); $P<0.05$, Gr. 15 (Table III) vs. Gr. 1 (Table I); $P<0.005$, Gr. 16 (Table III) vs. Gr. 2 (Table I) and $P<0.001$, Gr. 17 (Table III) vs. Gr. 3 (Table I)]. Incidences of different dysplastic conditions show no definite correlation with the pill treatments.

## DISCUSSION

Spontaneous tumors do not occur in the uterine cervices of the mice of Swiss albino strain maintained at JNU. However, placement of MCA-plus-beeswax impregnated cotton thread inside the cervical canal readily induces precancerous and cancerous lesions and the frequency of their occurrence depends upon the duration of exposure. Our present study reveals that the induction of these lesions can be modulated by concomitant treatment with oral contraceptive steroidal formulations present in Ovral and Noracycline. Exposure for 30 days to any of the three doses ($D_1$, $D_2$ and $D_3$) of either kind of oral pill appears to be too short a period to elicit any appreciable modulatory influence on the cervical carcinogenesis process. When the exposure period is increased to 60 and 90 days, the two lower doses ($D_1$ and $D_2$) of both formulations display inhibitory action on the cervical carcinogenesis. On the contrary, the highest dose ($D_3$) of either kind of oral pill used in the present study is augmentatory in action.

A review of the literature on estrogen action leaves no doubt that estrogen acts as an effective carcinogen in animals. Topical application of stilbesterol for a prolonged period produces cervical cancer in mice.[18] Estradiol benzoate when injected at the dose level of 10,500 IU over a period of 319 days into C3H mouse, produces carcinoma of uterine cervix with metastases to the lumbar lymph nodes.[19] Subcutaneous diethylstilbesterol (DES) treatment of C3Hf and BALB/c mice on the day of birth produces cervical tumors after 13 to 26 months.[20] DES treatment has also been shown to produce mammary tumors in C3H mice.[21] DES alone or synergistically with N-nitrosobutylurea has been shown to induce hepatic tumors in castrated male WF rats.[22] Estrogen-induced renal tumors have also been reported in Syrian hamster.[23] Different estrogens have also been shown to have promotional effect on chemically induced tumors in mice. Intramuscular treatment with 0.1 mg of estradiol benzoate, twice a week for 4 months, in castrated C3H mice increases MCA-induced cervical cancer.[24] Subcutaneous implantation of 10, 5, 2.5 and 1.25 mg pellets of ethinylestradiol for 15 and 30 weeks has been reported to promote MCA-induced cervical carcinogenesis in WLO mice.[25] Intermittent administration of low doses of estradiol monobenzoate has been found to promote cervical carcinogenesis in rats.[26] Treatment with high doses of exogenous estrogen has tumor-inhibitory action in mice. Intramuscular 17$\beta$-estradiol treatment has been found to inhibit MCA-induced cervical carcinogenesis in the uterine cervix of mouse.[17] Simultaneous implantation of mestranol inhibits the increased percentage of cervical tumor following progesterone administration in B6AF mouse.[27] 3-Hydroxy-6$\alpha$-methyl-17-acetoxyprogesterone (a potent synthetic progestational compound) promotes the process of carcinogenesis in the uterine cervix of mouse.[27, 28] The long-term administration of relatively high doses of synthetic progestin, like estrogen, has produced malignant tumors in breast in beagle dogs.[29] Subcutaneous implants of Norethindrone and Norethynodrel have resulted in ovarian tumors in mice.[30] A number of experimental results have also shown that use of progestogens can prevent or delay the development of tumors. Corporin treatment has been found to inhibit the action of estrin in evoking squamous metaplasia in the uterine cervix of monkey.[31] Subcutaneous implantation of progesterone has been shown to induce 50% reduction in DMBA-induced cervical carcinogenesis in WLO mice.[25]

Treatment with progesterone along with stilbestrol or estradiol is known to inhibit estrogen-induced tissue growth in the genital tract of New-Hampshire red chick.[32] A similar antagonism between estrogen and progesterone was demonstrated in the uterus of rabbit.[33] Simultaneous implantation of mestranol, along with the administration of progesterone or a synthetic progestin, 3$\beta$,17$\alpha$-diacetoxy-6$\alpha$-methylpregn-4-en-20-one (BL-141), inhibits the tumor-promoting action of the latter.[27]

Besides these antagonistic actions, low-dose combinations of estrogens and progestins display synergistic action in a number of animal models.[33-35]

It is not clear how the oral contraceptive formulations used in the present study display a biphasic effect on chemically induced cervical carcinogenesis in mice. It remains to be seen whether the ultimate effects of the various combinations of estrogens and progestogens present in the contraceptive pills are mediated through their antagonistic and synergistic actions. Also our present study was not designed to provide information on specific action(s) of contraceptive formulations (or their components) on different stages of chemical carcinogenesis. Further studies in this area employing different doses of individual components of the contraceptive steroidal formulations should help us in solving the riddle. Moreover, it is also necessary to know whether the steroids present in these pills modulate the MCA-induced tumor incidence either by affecting the drug-metabolizing enzymes or by competitive inhibition.

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