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Inhibitory Effect of 3-Methylcholanthrene on Induction of Massive Necrosis of Adrenal Cortex by 7,12-Dimethylbenz(a)Anthracene.* (28282)

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Evidence has been reported(1) indicating that the mechanisms by which 7,12-dimethylbenz(a)anthracene (DMBA) and 3-methylcholanthrene (3MC) inhibit corticosterone synthesis in the adrenal gland are not the same. It was suggested that depletion of adrenal corticosterone in DMBA-treated rats is secondary to DMBA-induced acute necrosis of the adrenal cortex, since dead cortical cells cannot synthesize hormones. The mechanism by which 3MC suppresses corticosterone synthesis was believed to be an inhibition of the biosynthetic pathway leading to corticosterone. The exact nature of this mechanism has yet to be elucidated.

Currie *et al.*(2) recently reported that 2-methyl-1, 2-bis (3-pyridyl)-1-propanone (Metopirone, or Ciba SU 4885) given prior to DMBA inhibited DMBA-induced acute necrosis and hemorrhage of the adrenal cortex in rats. Metopirone is known to interefere with the synthesis of cortisol and corticosterone by inhibition of 11β-hydroxylation(3). This interesting observation led us to believe that since 3MC inhibits corticosterone synthesis, it should exert an inhibitory effect on production of adrenal necrosis by DMBA. In the present investigation, it was shown that 3MC given to rats prior to DMBA could indeed prevent the induction of acute necrosis and hemorrhage by DMBA. Furthermore, the mechanism by which 3MC inhibited corticosterone synthesis in the adrenal cortex appeared to be similar to the one by which Metopirone inhibited it.

Materials and methods. The experimental animals were female Sprague-Dawley rats 55 to 60 days old and weighing 165 to 185 g. The carcinogens were dissolved in olive oil in a concentration of 30 mg/ml for 3MC, and 20 or 30 mg/ml for DMBA. They were fed to the rats through a stomach tube in all experiments. Metopirone was dissolved in olive oil in a concentration of 10 mg/0.1 ml, and was injected into the rats intraperitoneally.

There were 10 groups of experimental animals, each containing 10 rats: (a) controls, (b) rats receiving 20 mg/ml of DMBA, (c) rats receiving 30 mg/ml of DMBA, (d) rats receiving 10 mg of Metopirone intraperitoneally every 4 hours for 3 doses, (e) rats given 30 mg/ml of 3MC daily for 2 consecutive days and then a single dose of 20 mg/ml of DMBA 48 hours later, (f) rats given 30 mg/ml of 3MC daily for 2 consecutive days and then a single dose of 30 mg/ml of DMBA 48 hours later, (g) rats given 10 mg of Metopirone every 4 hours for 3 doses and then a single feeding of 20 mg/ml of DMBA 4 hours

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TABLE I. Effect of 3MC and Metopirone on Inhibition of Induction of Adrenal Necrosis by DMBA.

Groups	No. of rats	Mean adrenal wt, mg/100 g body wt	Adrenal necrosis	Mortality
Control	10	27.9 ± 2.8	0/10	0/10
DMBA 20 mg × 1	10	54 ±11	9/10	0/10
3MC 30 mg × 2 + DMBA 20 mg × 1	10	27.6 ± 3.2	0/10	0/10
DMBA 30 mg × 1	10	56.2 ±11	10/10	6/10
3MC 30 mg × 2 + DMBA 30 mg × 1	10	28.8 ± 3.8	0/10	0/10
Metopirone 10 mg × 3	10	32.9 ± 4.5	0/10	0/10
Metopirone 10 mg × 3 + DMBA 20 mg*	10	32.2 ± 2.9	0/10	0/10
Metopirone 10 mg × 3 + DMBA 30 mg × 1*	10	32.5 ± 1.9	0/10	0/10
Metopirone 10 mg × 3 + DMBA 30 mg × 1†	10	35.2 ±13.4	2/10	0/10
Olive oil 1 ml × 2 + DMBA 20 mg × 1	10	47.6 ± 7	10/10	4/10

* DMBA was given 4 hr after last dose of Metopirone.
† DMBA was given 16 hr after last dose of Metopirone.
± Standard deviations.

after the last dose of Metopirone, (h) rats given 10 mg of Metopirone every 4 hours for 3 doses and then a single feeding of 30 mg/ml of DMBA 4 hours after the last dose of Metopirone, (i) rats given Metopirone 10 mg every 4 hours for 3 doses and then a single feeding of 30 mg/ml of DMBA 16 hours after the last dose of Metopirone, and (j) rats receiving 1 ml of olive oil daily for 2 days and then 20 mg/ml of DMBA 48 hours later.

All rats except those in experiment (d) were killed by decapitation on the 3rd day after administration of DMBA. Rats in experiments (d) were killed 4 hours after the last dose of Metopirone. The adrenals were examined grossly, then trimmed and weighed. All adrenal glands were fixed in formalin and sections were stained with hematoxylin and eosin.

Results. The results obtained with the 10 groups of experimental animals are summarized in Table I. It is again demonstrated that 20 mg of DMBA induced massive adrenal necrosis and hemorrhage in 80-90% of the rats on the third day after the solitary feeding of DMBA. When 30 mg of DMBA was given, adrenal apoplexy developed in 100% of the animals and 50 to 60% of the rats died on the 3rd day after carcinogen administration. Grossly the adrenal glands were dark red, greatly enlarged and engorged with blood. Microscopically, the necrosis of zonas fasciculata and reticularis was extensive. There was diffuse karyolysis throughout the inner 2 zones of the cortex, but eosinophilic granular debris of the cells remained. Feeding 1 ml of olive oil prior to DMBA seemed to accelerate and intensify the necrosis and hemorrhage of the adrenal cortex. The mortality on day 3 after feeding of 20 mg of DMBA in the rats previously fed olive oil was greatly increased.

The weight of the adrenal gland as calculated in mg per 100 g of body weight is shown in Table I. Adrenal weights in DMBA treated rats are significantly higher than those in the control and 3MC pretreated rats. Marked increase of adrenal weight is the result of massive hemorrhage and necrosis.

The present experiment clearly demonstrated that 3MC, although without effect on the adrenal cortex so far as morphology was concerned, remarkably protected the adrenal cortex from the necrosis-inducing action of DMBA. Grossly the adrenal gland in rats pretreated with 3MC prior to administration of 20 mg of DMBA appeared bright yellow in color with no evidence of hemorrhage. Their weights were the same as those in the control rats. Histologically, the adrenal cortex in rats pretreated with 3MC prior to administration of 20 mg of DMBA showed no evidence of necrosis or any degenerative changes (Fig. 1 and 2). In rats receiving 30 mg of DMBA after pretreatment with 3MC, the adrenal cortex again showed no hemorrhage or necrosis despite the increase of dose of the carcinogen.

In an exploratory experiment to determine the dose of Metopirone (ditartrate), it was discovered that doses larger than 10 mg/0.1 ml (approximately 6 mg/100 g body weight)

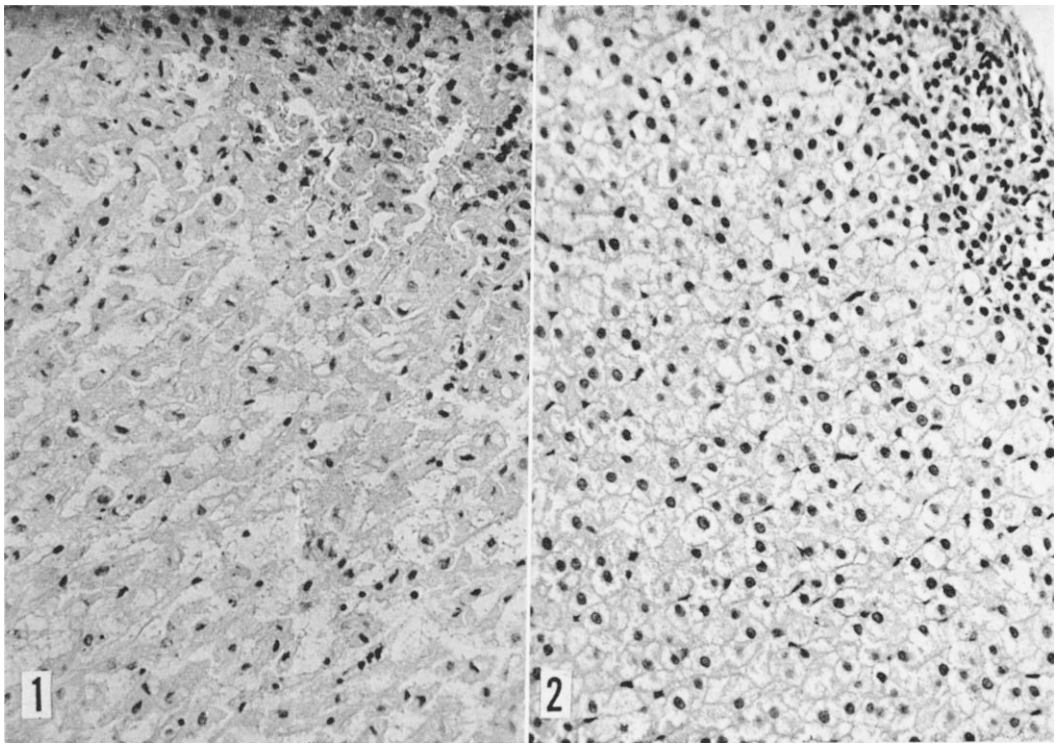

FIG. 1. Massive necrosis involving zona fasciculata on 3rd day after DMBA treatment. Right upper corner shows normal cells of zona glomerulosa. × 256.

FIG. 2. Cortical cells of zona fasciculata are unaffected by DMBA in rats pretreated with 3MC. Right upper corner shows cells of zona glomerulosa. × 256.

intraperitoneally for 3 doses were highly toxic. The toxic symptoms were ataxia, drowsiness and finally deep narcosis. A single injection of Metopirone 16.5 mg/0.1 ml of olive oil intraperitoneally produced immediate toxic reactions. The rat fell on the floor of the cage 2-3 minutes after injection of 16.5 mg of Metopirone and subsequently went into deep narcosis lasting for 4-8 hours.

Metopirone at a dose of 30 mg inhibited the necrosis-inducing effect of DMBA. Histologically, the adrenal cortex in rats pretreated with Metopirone showed no evidence of necrosis after subsequent administration of 20 mg of DMBA. In 4 of 10 rats pretreated with Metopirone, but subsequently (4 hours later) were given 30 mg of DMBA, extensive necrosis was not observed microscopically, but isolated areas of necrosis affecting only several cells were seen in the inner 2 zones of the adrenal cortex. This observation indicates that Metopirone only partially protected the adrenal cortex from necrosis when 30 mg of DMBA was given. If DMBA was fed to the rats more than 4 hours after the 3rd dose of Metopirone, 20% of the animals developed gross evidence of hemorrhage and necrosis. It is known that the optimal effect of Metopirone in suppressing the enzymatic effect on 11β-hydroxylation is within 4 hours after administration of the compound(3). It should be mentioned that adrenal weights in Metopirone treated groups were uniformly higher than those of the controls. The significance of this increase cannot be ascertained.

Discussion. Recently Morii and Huggins (4) reported that induction of acute necrosis of the adrenal cortex was dependent on the presence of "adequate" amount of corticosterone in the adrenal gland. These authors showed that whereas the adrenal cortex in immature rats was refractory to DMBA, it became susceptible to the effect of DMBA when ACTH was given to these young ani-

mals. This finding was also true of hypophysectomized rats.

Our observations are in agreement with the report of Morii and Huggins(4) that the concentration of corticosterone is critical for induction of adrenal necrosis by DMBA. Thus 3MC, which is capable of suppressing corticosterone synthesis(1), provides a remarkable protective effect on the adrenal cortex against the "necrotizing" effect of DMBA. In addition, our results also confirm the earlier observations by Currie *et al.*(2) that Metopirone partially inhibited necrosis-inducing effect of DMBA. Whatever the mechanism is, it becomes evident that it is the synthetic capability of the adrenal gland that governs the action of DMBA on the cortical cells from which the hormone is synthesized.

Summary. Successive feeding of 3MC 30 mg/ml for 2 days, 48 hours before DMBA administration inhibited completely the necrosis-inducing action of DMBA. The adrenal cortex in rats receiving 3MC prior to DMBA treatment appeared normal histologically. This remarkable protective phenomenon was also observed in rats pretreated with Metopirone, a compound which is known to suppress adrenal corticosterone synthesis by inhibiting 11β-hydroxylation. The mechanism by which 3MC inhibited the necrosis-inducing action of DMBA was believed to be similar to that by which Metopirone inhibited it.

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Relationships Between Infectious Virus, Hemagglutinin and Complement-Fixing Antigen of ECHO Virus, Type 19.* (28283)

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Some earlier observations suggested that ECHO 19, one of the hemagglutinating enteric viruses, has properties separating it from some other viruses within the group. In contrast with the separability of infectivity and CF antigen of some enteric viruses, reported also by others(1,2,3) our earliest studies indicated a close association of hemagglutinin and complement-fixing antigen with infectious virus. In more recent studies the CF antigen, and to a much lesser extent hemagglutinin were dissociated from fully infectious virus. The separation of these antigenic fractions is described, and their interrelationships discussed here.

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Materials and methods. Tissue culture (TC). Cells cultivated from kidneys of rhesus *(Macaca mulatta)* monkeys (MKTC) were used for obtaining virus stocks and for the titrations of infectious virus. Cells were maintained in a modified Eagle's medium with high cystine as described previously(4).

Viruses. Prototypic viruses of ECHO types 13, 19, 20, and 26 which have been plaque purified and have had 3 or more passages in MKTC in these laboratories were used.

Virus assay. Infectivity titrations were carried out in MKTC by inoculating serial 10-fold virus dilutions made in phosphate buffered saline (PBS). Inoculated cultures were incubated at 37°C and observed for 7 days. Infectivity endpoints (TCD_{50}) were calculated by the method of Reed and Muench(5).