7. Meerovitch, E., *Can. J. Microbiol.*, 1961, **7**, 685.
8. Diamond, L. S., Harlow, D. R. and Cunnick, C. C., *Trans. R. Soc. Trop. Med. Hyg.*, 1978, **74**, 431.
9. Sargeaunt, P. G., Williams, J. E., Kumate, J. E. and Jimenez, E., *Trans. R. Soc. Trop. Med. Hyg.*, 1980, **74**, 653.

EFFECT OF DIETHYL CARBAMAZINE CITRATE ON PRECOCIOUS PUBERTY INDUCED BY ESTRADIOL MONOBENZOATE IN IMMATURE RATS

M. S. MURTHY AND V. S. VENKATA SUBBU*
Department of Pharmacology, J.J.M. Medical College, Davangere 577 004, India.
*Department of Pharmacology, P.G. Institute of Basic Medical Sciences, Madras 600 042, India.

Abstract

Precocious puberty along with superovulation induced by administering exogenous estradiol monobenzoate in immature rats was totally blocked by concommitant administration of diethyl carbamazine citrate. This suggests that diethyl carbamazine citrate significantly blocks the central positive feedback effect of estrogen on the release of gonadotrophin or hypothalamic gonadotrophin releasing factors.

Introduction

SOME of the piperazine derivatives exhibit varying degrees of antifertility effects[1]. The mechanism of action of diethyl carbamazine citrate (DEC) as an oral contraceptive has not been studied. An extensive study was therefore undertaken to explore the possible mechanism of action of DEC as an antifertility agent in albino rats[2].

Induction of precocious puberty along with superovulation by exogenous administration of small doses of estrogen is chosen as a parameter because it can be specifically blocked by an anti-gonadotrophic agent. This paper presents the effect of DEC on superovulation in immature rats.

Materials and Methods

Immature (27-day old) female albino rats (Wistar strain), inbred in the departmental animal house and weighing between 35–40 g were used in the present study. The rats were maintained under controlled light (12 hr light:12 hr dark) and constant temperature (22 ± 2° C) and fed with standard pellet diet (Hindustan Lever) and tap water *ad libitum.*

The drugs and vehicle were administered between 12.00 and 13.00 hr everyday to avoid diurnal variation. DEC was dissolved in distilled water and the volume was kept constant at 0.1 ml per animal irrespective of dose and route of administration. Estradiol monobenzoate (EM) was suspended in sesame oil and the volume was kept constant at 0.1 ml per animal. All the rats were divided into different subgroups. Each subgroup consisted of 10 animals. The rats were treated for 3 days as shown in table 1. On the 4th day, all the animals were sacrificed by cervical dislocation, their body wights were recorded and the vaginal state was observed (if opened, smears were taken and scored after methylene blue staining). Their uteri were quickly removed, free of fat and connective tissue and their intraluminal fluid expressed by pressing between filter paper foldings. The tissues were weighed on a torsion balance.

In animals with vaginal opening, vaginal smears were taken and stained with methylene blue for 10 min and scored under microscope[3]. The oviducts of these animals were carefully separated from the ovaries and the ova within them were expelled by flushing with normal saline. The ova were counted under dissecting microscope. Evans blue was used as a vital stain after being dissolved in hyaluronidase (1%).

The ovaries were fixed in 10% formaline saline, serially sectioned at 6 μ and stained with haematoxylin and eosin and screened.

Results

The wet weight of uterus, the percentage of vaginal opening and the number of eggs per rat are given in table 1. In the control and DEC-treated rats there was no vaginal opening. Their uterine weights were significantly low when compared to that of estrogen-treated group. The vaginae of the animals treated with EM opened on the 4th day of the treatment; the vaginal smears showed plenty of cornified epithelial cells in layers (estrus). The number of ova released during this superovulatory period was counted (mean 10.20). The uterine weights were significantly increased when compared with vehicle-treated control group ($p < 0.001$). In the rats treated with EM μ DEC combination (irrespective of route of administration of DEC), there was a significant increase in the uterine weights with no sign of ovulation and the vaginae remained closed.

TABLE 1

Effect of estradiol monobenzoate and DEC on the reproductive system of immature rats

Group	Treatment and dose Schedule/ rat/day for 3 days	Final body weight (g)	Wet weight of uterus (mg)	% Vaginal opening	Number of ova/rat
I.	Vehicle (sesame oil) 0.1 ml, S.C.	37.40 ± 0.52	20.90 ± 0.69	0	0
II.	Estradiol monobenzoate 0.1μ g, S.C.	37.70 ± 0.71	77.80 ± 1.53*	100	10.20 ± 0.98@
III.	DEC. 20 mg/kg b.w. oral	35.40 ± 1.18	20.80 ± 0.84	0	0
IV.	Estradiol monobenzoate (0.1μ g) S.C. + DEC. (20 mg/kg b.w.) oral	38.30 ± 0.58	67.20 ± 1.94*	0	0
V.	Estradiol monobenzoate (0.1μ g) S.C. + DEC. (20 mg/kg b.w.) I.P.	37.90 ± 0.60	75.90 ± 2.06*	0	0

* $P \leqslant 0.001$ *vs* Control. @Ovulation occurred in all rats (10). b.w.= body weight
(Values are mean ± SEM based on 10 animals in each group).

HISTOPATHOLOGY OF THE OVARY

Under dissecting microscope the ovaries of EM treated group were enlarged when compared to the EM + DEC combination-treated group.

H and E stained sections of EM + DEC treated ovaries showed different stages of follicular development with no sign of ovulation; *corpus luteum* was not present; perifollicular fibrosis was an added feature in this group of ovaries (figure 1). The ovaries of the EM-treated group showed a number of Graafian follicles with ova and a number of *corpora lutea* (figure 2).

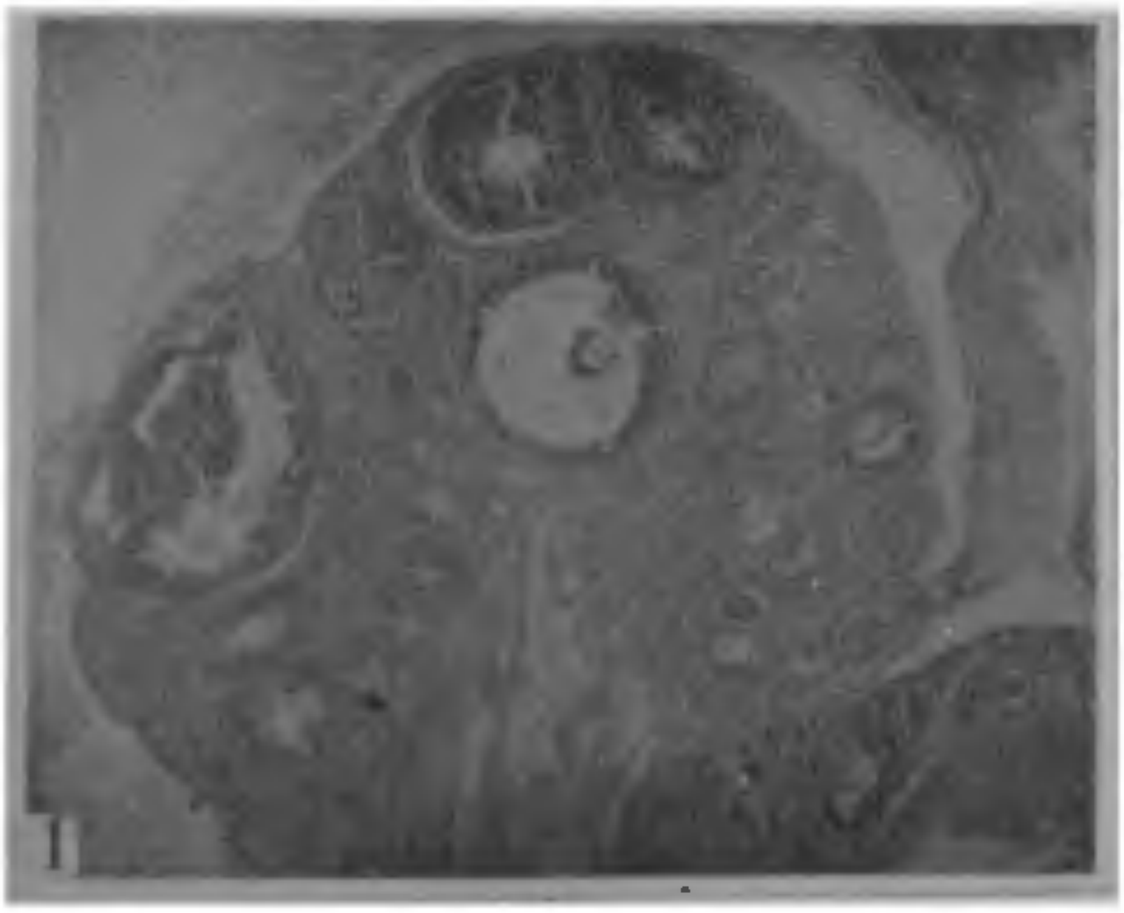

Figure 1. Estrogen-DEC treated immature ovary—H & E section (× 100).

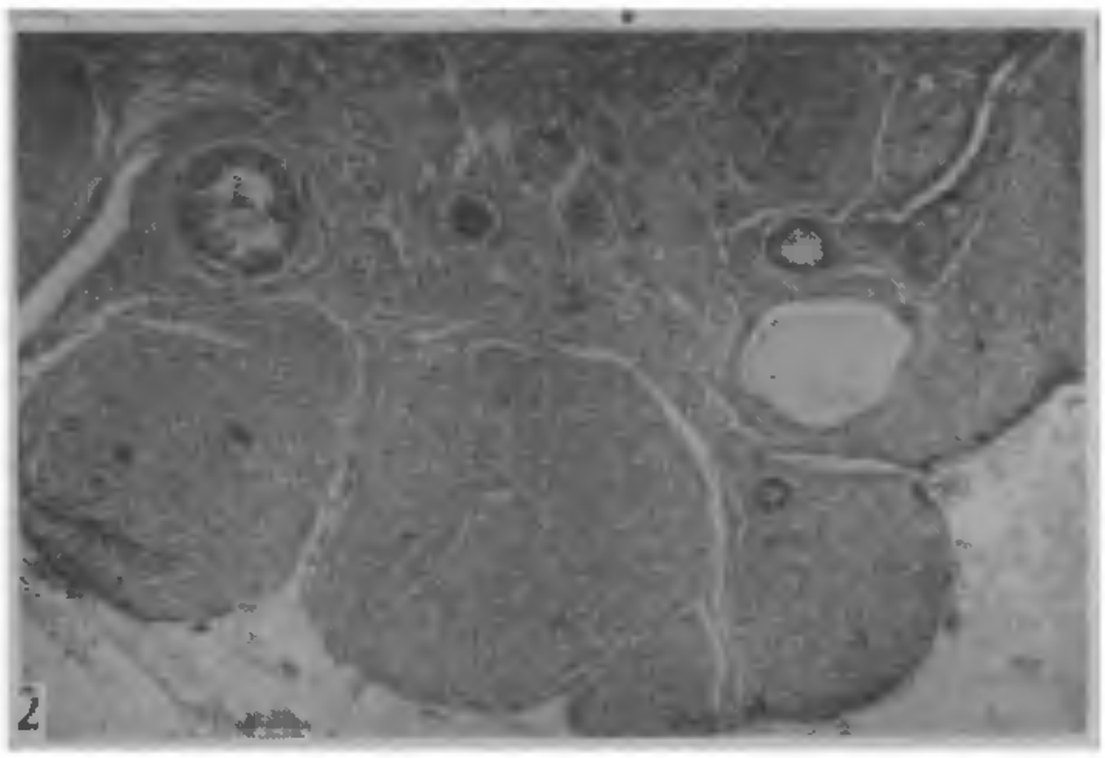

Figure 2. Estrogen treated immature ovary H & E section (× 100).

DISCUSSION

The effect of small doses of estrogen on vaginal opening is identical with the onset of puberty, since precocious vaginal opening, first estrus and ovulation were followed by regular estrus cycle[4-10]. This is further confirmed by our finding that EM induced the same effects in immature rats.

Since the onset of puberty involves the first ovulation and is followed by normal cyclicity thereafter, the mode of action of estrogen must be through central nervous system, by increasing the secretion of gonadotrophin which precipitates puberty[11-13]. There are two possible mechanisms for the ovulation advancing effect of estrogen; one is a stimulation of pituitary to release the ovulating hormone (leutinizing hormone) (LH)[14], and the other is to increase the sensitivity of pituitary to endogenous hypothalamic gonadotrophin

releasing factor, resulting in greater gonadotrophin release for a given amount of releasing factor[15].

Simultaneous administration of DEC with a dose of 20 mg kg body weight irrespective of route of administration significantly blocked the precocious, puberty, vaginal opening and superovulating effects of EM in immature rats. It can be considered the DEC blocked the central feedback effect of estrogen on the release of gonodotrophins (anti-gonadotrophic). Since there was no ovulation in DEC-treated rats primed with estrogen, it cn be surmised that DEC inhibits the production/or release of the ovulating hormone, LH or alters the sensitivity of the pituitary to endogenous hypothalamic gonadotrophin releasing factor.

In animals treated with EM and EM + DEC combination, there was a very significant increase ($p < 0.001$) in the uterine weight (*vs* control and DEC-treated animals) confirming the non-interference of DEC on the peripheral action of estrogen *i.e.*, uterus.

ACKNOWLEDGEMENT

The authors wish to thank Burroughs Wellcome & Co., Bombay for generous gift of pure diethyl carbamazine citrate.

1. Sareen, K. N., Mistra, N., Varma, D. R., Amma, M. K. P. and Gujral, M. L., *Indian J. Physiol. Pharmacol.*, 1961, **5**, 125.
2. Murthy, M. S., Ph.D. Thesis, University of Madras, Madras, February 1980.
3. Long, J. A. and Evans, H. M., *Mem. Univ. Calif.*, 1922, **6**, 1.
4. Smith, E. R. and Davidson, J. M., *Endocrinology*, 1968, **82**, 100.
5. Motta, M., Frachini, F., Giulliani, G. and Martini, L., *Endocrinology*, 1968, **83**, 1101.
6. Weick, R. F. and Davidson, J. M., *Endocrinology*, 1970, **87**, 693.
7. Weick, R. F., Smith, E. R., Dominquez, R., Dhariwal, A. P. S. and Davidson, J. M., *Endocrinology*, 1971, **88**, 293.
8. Everett, J. W., *Physiol. Rev.*, 1964, **44**, 373.
9. Haldeman, K. S., *Anat. Record.*, 1921, **21**, 60.
10. Wilson, E. D. and Zarrow, M., *J. Reprod. Fertility*, 1962, **3**, 148.
11. Frank, R. T., *J. Am. Med. Assoc.*, 1925, **85**, 1558.
12. Hohlweg, W., *Klin. Wschr.*, 1934, **13**, 92.
13. Ramirez, V. D. and Sawyer, C. H., *Endocrinology*, 1965, **76**, 1158.
14. Piacsek, B. E. and Meites, J., *Endocrinology*, 1966, **79**, 432.
15. Docke, F. and Dorner, G., *J. Endocrinol.*, 1965, **33**, 491.

EFFECT OF MAGNESIUM ON THE TOXICITY OF CHROMIUM AND LEAD TOWARDS *ESCHERICHIA COLI* AND *AEROBACTER AEROGENES*

U. S. BAGDE AND A. K. VARMA
School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India.

ABSTRACT

The effect of magnesium on the toxicity of chromium, and lead towards *E. coli* and *A. aerogenes* was studied. In the absence of any heavy metal in the growth medium magnesium alone enhanced the growth of organisms to a great extent. Increasing concentration of magnesium increased the growth and diminished the toxicity effect of chromium both in *E. coli* and *A. aerogenes*. A similar result was obtained during the studies on the influence of increasing concentrations of magnesium on the toxicity of lead in *E. coli* as well as *A. aerogenes*.

INTRODUCTION

THERE have been some reports regarding the interaction of nontoxic metals with toxic metals diminishing or enhancing the toxicity of the latter by the former. The ion antagonism has been studied but not sufficiently. In *Lactobacillus arabinosus* overcoming of Zn toxicity by Mn was reported[1]. Subsequently the improvement of the growth and the elimination of toxic effect of copper by EDTA were reported[2]. The removal of the lethal effect of Cd and Zn by EDTA, citrate, aspartate and pyruvate in *Pseudomonas* and *A. aerogenes* was also observed[3,4]. Also the toxicity of Hg was reported to be decreased by Na_2S addition in mixed rumen bacteria[5]. However the inefficiency of ions such as $MgCl_2$, $CaCl_2$, $MnCl_2$ and NaCl in abolishing or diminishing toxic effect of Hg salts in *Staphyllococcus aureus* was observed[6] and the elimination of the killing effect of $CdCl_2$ with the addition of $CaCl_2$ was noted. Babich[7] noted that the high concentration of NaCl unaffected, increased or decreased the toxicity of Zn in different organisms. In