[Chem. Pharm. Bull. 22(12)2830—2835(1974)]

UDC 547.91.09 : 615.322.076.9

Toxicological Approaches to the Metabolites of *Fusaria*.[1] VII. Effects of Zearalenone on the Uteri of Mice and Rats[2]

Yoshio Ueno, Noriko Shimada,[3c] :ko Yagasaki,[3b] and Makoto Enc

Laboratory of Microbial Chemistry, Faculty c :naceutical Sciences, Science University of Tokyo,[3a] and St. Mari :edical University[3c]

(Received April 17, 1974)

Zearalenone produced by the fungus *Fusarium roseum* (*Gibberella zeae*) caused a marked uterotrophic response in mice and rats. Single or repeated administrations to immature mice and rats resulted in an increase of the uterine weight several times higher than the control weight. This effect was specific to the uterus, and oral administration was more effective than other routes. Ovariectomized mice were highly sensitive to zearalenone, and the dose-response curve was linear when daily doses of 1 to 2 mg/kg were given for one week. Zearalenone promoted cellular proliferation and mitosis in the uterine muscle cells.

Zearalenone [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone] (Fig. 1) is a fungal estrogen produced by *Fusarium* spp. and is associated with food-borne diseases of farm animals. Consumption of the feeds and foods polluted by zearalenone-producing fungi such as *F. roseum* (syn. *F. graminearum* and *Gibberella zeae*) gives rise to enlarged vulvae and mammary glands and, in some cases, prolapse of the vagina and rectum in poisoned animals.[4] Administration of zearalenone to female weanling rats causes the weight of their uteri to increase.[5,6]

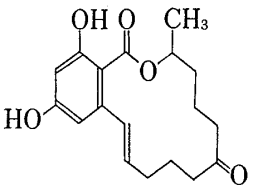


Fig. 1. Zearalenone

In an approach to the mode of action of this estrogenic mycotoxin the present authors examined the effect of zearalenone on the weight of the uterus of mice and rats in various dosages, administration routes and administration times. Estrogenic action of zearalenone on ovariectomized mice was also investigated.

Materials and Methods

Zearalenone——The mycotoxin was isolated and purified from the rice grains infected artificially by *Fusarium roseum* M-3-2 according to the method previously reported.[7]

Animals——Female ddyS mice (3 week-old) and female Wistar rats (3 week-old) were purchased from Shizuoka Agricultural Cooperations for Experimental Animals (Hamamatsu). They were injected or intubated with a single dose or repeated doses of varied amounts of zearalenone in 0.1 ml/10 g body weight of olive oil or propylene glycol solution. At the prescribed times thereafter, the animals were killed by cervical dislocation and within one minute the whole uterus was removed free of surrounding fat and connective tissue and collected on filter paper wetted with ice-cold isotonic saline to prevent dryness. Each uterus was weighed on a microbalance.

1) Part VI: Y. Ueno, K. Ishii, N. Sato, and K. Ohtsubo, *Japan. J. Exp. Med.*, **44**, 111 (1974).
2) This work was presented in part at the 93rd Annual Meeting of Japan Society of Pharmaceutical Sciences, Tokyo, April, 1973, and published in abstract form (III-179).
3) Location: *a) Ichigayara-Funagawa-machi, 12, Shinjuku-ku, Tokyo, 162, Japan; b)* Present Address: *Hyakunin-cho, 3-24-1, Shinjuku-hu, Tokyo, 160, Japan, Department of Toxicology, Metropolitan Institute of Hygienic Sciences; c) Sugao, 2095, Takatsu-ku, Kawasaki, 213, Japan.*
4) C.M. Christensen, G.H. Nelson, and C.J. Mirocha, *Appl. Microbiol.*, **13**, 653 (1965).
5) C.J. Mirocha, C.M. Christensen, and G.H. Nelson, *Can. Res.*, **28**, 2319 (1968).
6) C.J. Mirocha, C.M. Christensen, and G.H. Nelson, *Biotech. and Bioengr.*, **10**, 468 (1968).
7) K. Ishii, M. Sawano, Y. Ueno, and H. Tsunoda, *Appl. Microbiol.*, **27**, 625 (1973).

In some experiments, immature female mice of 22—24 days of age were ovariectomized and used for experiments 10—12 weeks later.

The organs were fixed in 10% formalin, sliced and stained with hematoxylin and eosin for histological examination.

Results

Effect of a Single Administration of Zearalenone on Normal Immature Mice

Effects of a single administration of zearalenone on the body weight and uterine weight of female immature mice were determined in 3 doses with various intervals after injection. Zearalenone produced increases in the uterine weight proportional to the doses from 1 to 10 mg per kilogram, and the maximum increase was observed one day after injection. With the high doses of 5 and 10 mg/kg, the uterine weight was still higher than the control even 5 days after administration (Fig. 2).

A time course of the increase in the uterine weight after a single oral dose of 10 mg/kg is shown in Table I. A significant increase of the uterine weight was already detected 6 hours after administration, and the maximum increase was attained 18 hours after administration, when the uterine weight was 2.5 times greater than the control. As for the body weight, no statistically significant change was detected in the above two experiments.

Effect of zearalenone on the uterine weight was examined by various routes of administration (Table II). Administrations of 5 and 10 mg/kg of zearalenone by oral, subcutaneous, and intraperitoneal routes produced an increase in the uterine weight of immature mice. With the dose of 10 mg/kg, no difference in the degree of the increase was detected among the three routes of administration. With the lower dose of 5 mg/kg, statistical analysis showed that the oral route was the most effective.

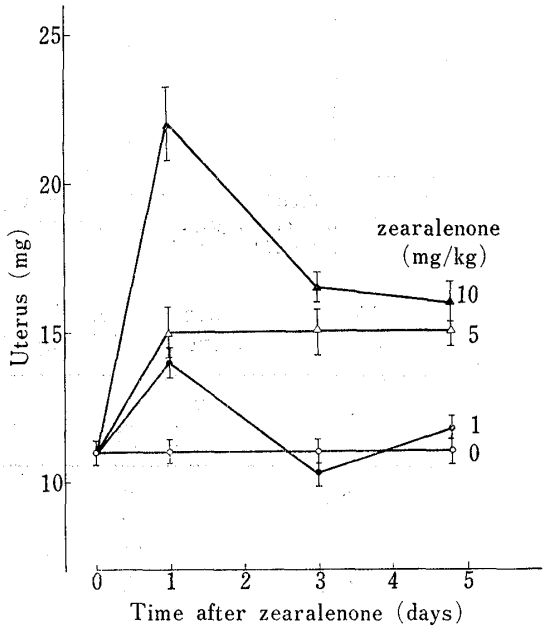


Fig. 2. Changes of the Weight of Uterus in Immature Female Mice after a Single Injection of Zearalenone

Zearalenone dissolved in propylene glycol was orally administered to 3 week-old mice and the weight of uteri was determined 1, 3 and 5 days after injection. Each point in the figure represents an average of 5 mice.

—○—: control
—●—: zearalenone 1 mg/kg
—△—: zearalenone 5 mg/kg
—▲—: zearalenone 10 mg/kg

Effect of Repeated Administrations of Zearalenone on Immature Mice and Rats

Immature virgin mice and rats were given orally 0.5 to 2.0 mg/kg of zearalenone once daily for one week, and changes in body weight as well as the weights of four organs, *i.e.*, uterus, liver, kidneys and spleen, were measured 24 hours after the last injection. As illustrated in Table III, no detectable change of the body weight was demonstrated in the treated mice and rats. Among the four organs, however, the uteri of both mice and rats became about 3 times larger than the control and the other three organs exhibited no statistically significant change in weight.

Histopathological observation of the uteri of the mice and the rats treated with zearalenone revealed a hypertrophy of myometrium and an increase of mitosis in the uteri of rats (Photo 1) and mice (Photo 2). No noticeable changes were observed in the liver, kidneys, spleen and small intestine.

A time course of the increase in the uterine weight in mice after the start of repeated administrations of zearalenone is represented in Fig. 3. The mice received orally a daily

dose of 2 mg/kg of zearalenone for 8 days from the 21st day after birth. The control received propylene glycol only, and at each point in the figure 5 mice were sacrificed and their uteri were weighed. In the control mice the uterine weight was constant for about 10 days, *i.e.*, about 30 days after birth, and it then increased sharply to about 50 mg. In the treated mice, the uterine weight increased up to the 5th day of the experiment and remained constant or

Table I. Time-dependent Changes of the Weight of Uterus in Immature Mice

Time after zearalenone administration (hours)	No. of mice	Body weight (g)	Uterine weight (mg)
0	8	12.4±0.8	10.5±2.5
6	8	12.9±1.3	13.6±2.3
12	8	12.7±0.9	16.9±3.5
18	8	12.5±1.1	24.9±6.1
24	8	12.5±0.8	21.6±5.6
36	8	12.5±0.8	20.6±2.8
48	8	12.6±0.6	17.6±3.5

Zearalenone dissolved in propylene glycol was administered orally to 3 week-old mice in a dose of 10 mg/kg. The average body weight and the uterine weight in 8 control mice at 48 hours of the experiment were 12.4+0.9 g and 12.5+3.4 mg, respectively.

Table II. Effect of a Single Administration of Zearalenone on the Uterus of Immature Mice by Different Routes of Administration

Route	Zearalenone (mg/kg)	No. of mice	Body weight (g)	Uterine weight (mg)
p.o.	0	5	12.0	11.1(1)[a]
	5	5	11.8	19.9(1.8)
	10	5	12.4	17.9(1.6)
s.c.	5	5	12.2	17.4(1.5)
	10	5	12.7	18.6(1.7)
i.p.	0	5	12.0	11.2(1)
	5	5	12.2	13.6(1.2)
	10	5	11.9	18.4(1.7)

a) ratio to the control
Zearalenone dissolved in olive oil (*i.p.*) or propylene glycol (*p.o.* and *i.p.*) was administered to 3 week-old female mice, and the body weight and the uterine weight were measured 20 hours after injection.

Table III. Changes of the Weight of Organs in Immature Mice and Rats by Daily Administration of Zearalenone

Animals	Zearalenone (mg/kg)	No. of animals	Body weight (g)	Organ weight (mg)			
				Uterus	Liver	Kidneys	Spleen
Mice	0	8	11	5± 3.1(1)[a]	502±106	151± 24	46±21
	0.5	9	10	6± 2.5(1.2)	518± 73	157± 23	40±18
	1.0	8	11	11± 1.0(2.2)	553± 75	164± 25	55±20
	2.0	10	11	18± 1.1(3.6)	547± 72	166± 23	56±20
Rats	0	5	66	35±20.3(1)	3160±213	738± 56	152±39
	1.0	5	65	89±34.8(2.5)	3176±738	732±203	154±48

a) ratio to the control
Zearalenone dissolved in propylene glycol was daily injected to 3 week-old mice for 7 days or to 3 week-old rats for 8 days.

even decreased thereafter in spite of the additional administrations for 3 days. After the termination of administration, the uterine weight decreased further to the starting level on the 15th day of the experiment, and then it recovered to the normal weight by the 25th day.

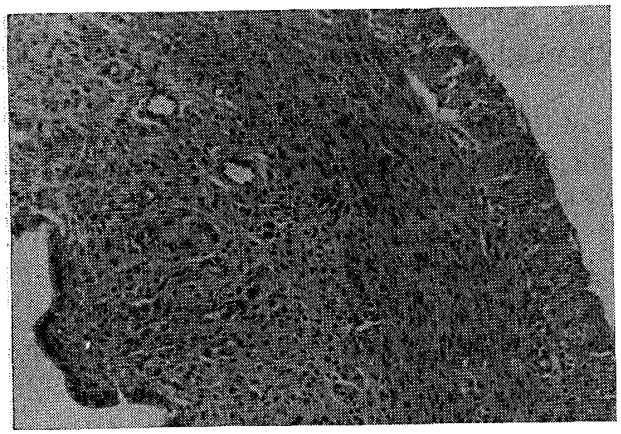
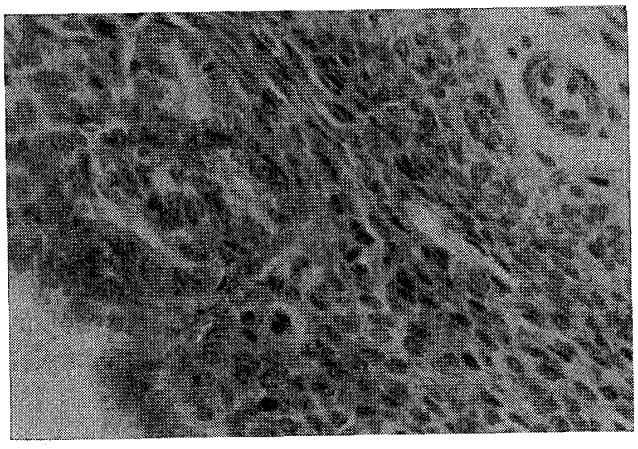


Photo 1. Histological Examination of the Uterine Tissue of Rat

Zearalenone dissolved in propylene glycol was orally administered for 8 days to 3 week-old rats in a dose of 1 mg/kg/day. (H.E. ×100).



Ehoto 2. Histological Examination of the Uterine Tissue of Mice

Zearalenone dissolved in propylene glycol was orally administered for 7 days to 3 week-old mice in a dose of 2 mg/kg/days. (H.E. ×400).

TABLE IV. Comparison of the Effects of Zearalenone on Normal and Castrated Mice

Mice	Zearalenone (mg/kg)	No. of mice	Uterine weight (mg)	Ratio
Normal	0	5	90±27	1
	5	5	93±14	1
Castrated	0	5	12± 0.9	1
	2	5	41±17	3.4
	5	5	54±22	4.5

Adult female (8 weeks old) and castrated female mice were administered orally with zearalenone and the weight of uteri was determined 18 hours after the administration.
Zearalenone was dissolved in propylene glycol.

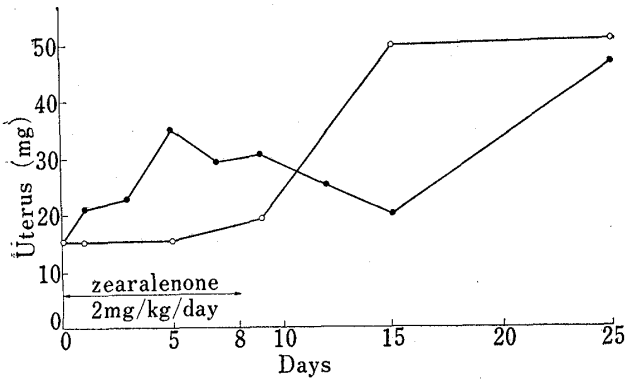


Fig. 3. Time-Course of the Uterine Weight in Immature Mice after Daily Administrations of Zearalenone

Zearalenone dissolved in propylene glycol was orally administered for 7 days to 3 week-old mice in a dose of 2 mg/kg/day. Control mice received the solvent.
Each point in the figure represents an average of 5 mice.
—○—: control —●—: zearalenone

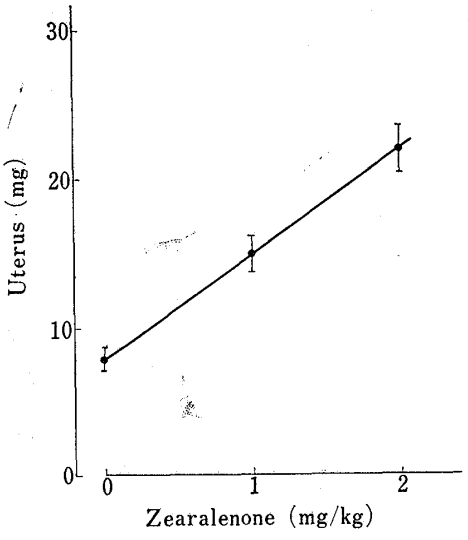


Fig. 4. Dose-Reseponse Curve of Uterine Weight in Ovariectomized Mice

The data were quoted from Exp. (B) in Table V.

During the whole course of this experiment, the growth of the control and experimental mice were found to be similar to each other.

Effect of Zearalenone on the Uterine Weight of Ovariectomized Mice

Three week-old virgin female mice were ovariectomized and used 10—12 weeks after operation for the test of estrogenic activity of the mycotoxin.

In Table IV, effect of zearalenone on the uterine weight was compared between the normal and castrated mice.

In the normal 14 week-old females, no change of the uterine weight was observed 18 hours after receiving 5 mg/kg of zearalenone orally. In contrast, the castrated mice responded to the administration of 2 and 5 mg/kg zearalenone and their uterine weights were 3.4 and 4.5 times greater, respectively, than that of the castrated control.

Repeated administrations of zearalenone to the castrated mice for 5 or 7 days caused increase in the uterine weight that were proportional to the dosage (Table V), and as represented in Fig. 4, the dose-response curve was completely linear when the mice received 1 and 2 mg/kg of the mycotoxin for 7 days.

TABLE V. Effects of Oral Daily Administrations of Zearalenone on the Weight of Uterus in Castrated Mice

Exp. No.	Zearalenone (mg/kg)	Days	No. of mice	Body weight (g)	Uterine weight (mg)
(A)	0	5	7	24.3	12.6±0.9(1)[a]
	1		9	24.4	19.6±0.9(1.6)
	2		9	24.6	31.5±5.0(2.5)
	5		9	24.3	39.6±5.1(3.0)
(B)	0	7	8	26.5	8.0±0.6(1)
	1		7	26.2	14.9±1.3(1.9)
	2		6	26.3	21.8±1.8(2.7)

a) ratio to the control

Zearalenone dissolved in olive oil was administered orally to castrated mice daily for 5 (Exp. A) or 7 days (Exp. B), and the weights of body and uterus were determined 18 hours after the last injection.

Discussion

Zearalenone has a resorcylic nucleus conjugated with a macrocyclic lactone. Although it lacks a steroidal structure, this agent exhibits a potent uterotrophic activity in several animals. With an aim to study the mode of action, the authors examined the effect of zearalenone on the uterine weight of normal and castrated mice and rats in different routes and times of administration.

In immature mice, single administrations of zearalenone in the oral doses of 1 to 10 mg/kg were found to cause significant increase in the uterine weight (Fig. 1), and this effect was observable even when the mycotoxin was administered by the subcutaneous or intraperitoneal route. This finding indicates that zearalenone is easily absorbed from the injected site. It appears that zearalenone is more active when administered orally (Table II) and this suggests that its activity may be due to some of its metabolites formed *in vivo*.

In the case of repeated administrations of zearalenone to immature mice, a dose-response curve was obtained when the cumulative doses ranging from 3.5 mg to 14 mg/kg were administered over a period of seven days (Table III), and a similar curve was obtained with castrated mice (Table IV, V and Fig. 4).

In the normal immature mice and rats, zearalenone caused increase in the weight of the uterus only and not in those of the other organs examined (Table III). In the adult mice, the agent exhibited its activity only when the mice were ovariectomized (Table IV). These findings strongly support the assumption that zearalenone exhibits a high affinity to

uterine tissue. The histo-pathological findings also indicated that the administration of zearalenone induced proliferation and mitosis in the uterine muscle cells, and this promotion of cellular growth by zearalenone resulted in the increase of the uterine weight. Results of biochemical analysis of the mechanism of cellular growth will be reported elsewhere.

As reported in Fig. 3, the normal increase of the uterine weight was temporarily depressed after termination of the continuous administration of zearalenone to immature mice. This result suggests that the mycotoxin depressed the normal growth of ovary. In this respect, effect of zearalenone on ovarian tissue remains to be investigated.

Acknowledgement This work was supported in part by a grant from Grant-in-Aid for Cancer Research from the Ministry of Welfare (1973).