Effect of 6 Diazo 5 Oxo L-Norleucine (DON) on the Rat Litter *in Utero.** (22848)

J. B. THIERSCH

Department of Pharmacology, University of Washington School of Medicine and Institute of Biological Research, Seattle.

DON—a diazoketone—was first isolated from a streptomycelium and later synthesized. The compound gave promise of tumor inhibition in experimental tumors as well as in human leukemias by inhibiting purine synthesis *de novo,* as reported. DON was first reported by Murphy and Karnofsky to affect the chick and rat embryo adversely, in doses smaller than those of Azaserine (AZS)(4). The present experiments were carried out to determine its effect on the litter of pregnant rats.

Methods. The Long Evans strain of rats was used in the present experiments. The experiments were conducted as previously described in detail(5). Only rats which had one previous satisfactory litter were used. Vaginal smears were done daily, to determine the onset of pregnancy. The compound was given by intraperitoneal injection, dissolved in sterile water, in such concentration that the animals received 1 cc of solution for each 100 g of body weight. Due to the instability of the drug, the watery solution was frozen solid until the time of injection. Any defrosted surplus was discarded. The experimental animals were all sacrificed on the 21st day of gestation. At autopsy the internal organs were inspected, the uteri removed, and contents noted and recorded as described before. The results were listed and compared with the controls (Table I).

Results. The weight changes of the experimental animals corresponded to those of the controls and evidenced their wellbeing. The internal organs of the mother animals were found to be within normal limits—especially the liver, bone marrow and pancreas.

The first group of rats, treated with .05 mg/kg on the 7th and 8th days of gestation (Table I, 2) showed an increased number of fetal resorptions, but no malformations. The second group, treated with a single dose of .3 mg/kg on the 8th day of gestation, responded with 92% of all fetuses resorbed, and 62% of all litters destroyed. Stunting was noted in one fetus (Table I, 3). The same dosage, given at 3-hour intervals, twice on the same day, induced death in all but 1 stunted and malformed fetus, and completely destroyed the litter in all animals but 1 of a group of 21 rats (Table I, 4). The results indicated that if efficient litter destruction was to be obtained, a larger amount of drug had to be given. On raising the dosage to .5 mg/kg given on the 4th and 5th days of gestation, 39% of all the fetuses, but only 12.5% of all litters, were destroyed. Stunting and malformation increased to 10% of the surviving fetuses (Table I, 5). The single doses of .5 mg/kg, given on the 8th day of gestation, resulted in death and resorption of 96% of all the fetuses, but only in 77% total litter destruction. The surviving fetuses were all stunted and malformed (Table I, 6). The dosage of .5 mg/kg given twice on the 7th and 8th days of gestation, or on the 11th and 12th days of gestation, resulted in complete litter destruction without survivors (Table I, 7, 8).

DON and progesterone. Attempts were made to protect the fetuses against the action of DON on the 7th and 8th days of gestation, with 3 injections of 10 mg of progesterone given intramuscularly on the 6th, 7th and 8th days of the gestation period. At sacrifice, 82% of the fetuses were found to be resorbed, and all the surviving embryos were found to be stunted (Table I, 9).

DON and adenine. Another group of rats, treated on the 7th and 8th days of gestation with .5 mg DON, received 1 hour before each injection of the drug 250 mg of adenine sul-

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TABLE I. Effect of DON on Rat Litter *In Utero*.

No. of rats		Day of dose	Dose, mg/kg	Mother's wt gain, %	Total implants	Fate of litter: Live	d	Stunt	Dead	Res.	Mothers with all resorb. litters	Avg litter wt	Avg single fetus: Wt, g	Length, cm
95	a	7,8 p*	Controls		905	891			1	13				
	b		p*	+39.1	9.5	9.4			.01	.14	0	45.7	4.9	4.3
	c					98.5			.11	1.4				
25	a	7,8			217	163			2	52				
	b		.05	+35	8.7	6.5			.08	2.1	0	37.1	4.5	3.9
	c					75			.9	24				
16	a	8			135	11	1			124	10			
	b		.3	+12.4	8.4	.7	.9			7.7		8.1	4.5	3.9
	c					8.2	9.1			92	62.5			
21	a	8,8			199	1	1			198	20			
	b		.3, .3	+ 5.8	9.5	.05	.05			9.4		3.1	3.1	3.5
	c					.5	100			99.9	95.4			
24	a	4, 5			172	104	10			68	3			
	b		.5	+18.3	7.2	4.3	4.1			2.9		23.4	4.7	3.7
	c					60.5	9.9			39.5	12.5			
26	a	8			219	7	7			212	20			
	b		.5	+16.6	8.4	.27	.27			8.1		6.0	3.4	3.4
	c					3.2	100			96.8	77			
24	a	7,8			189					189	24			
	b		.5	+ 7.7	7.8					78				
	c									100	100			
25	a	11,12			184					184	25			
	b		.5	+12.3	7.5	0				7.5				
	c									100	100			
16	a	7,8			156	27	27			129	8			
	b		.5 + pro*	+17.2	9.7	1.7	1.7			8.0		10.7	3.5	3.4
	c					17.3	100			82.6	50			
7	a	7,8			62	45				17				
	b		.5 + ade*	+24	8.8	6.4				2.4	0	38	4.3	4.7
	c					72.5				27.4				

* p = placebo; pro = progesterone; ade = adenine.
a = total No. b = per mother rat. c = % of total implants. d = % of live.

fate. At sacrifice, only 20% of all the fetuses were found to be resorbed, and not a single litter was entirely destroyed. The mothers showed typical adenine kidneys(3). The results indicated that adenine antagonized the action of DON. The surviving fetuses were found to be normal (Table I, 10).

Action of DON. At sacrifice, the maternal internal organs were found to be normal. The mammary glands were lactating, the ovaries presented corpora lutea of pregnancy, the livers, bone marrow and pancreas were found to be normal. From this and the survival of numerous placentas to term, one had to conclude that DON, like AZS, acted directly on the fetuses. The malformations observed corresponded to those described for folic acid antagonists and AZS, consisting predominantly of skeletal defects(2).

Placentas. In many instances, the placentas survived the resorbed fetuses. Many uteri were studded with placentas of varying sizes without a single fetus. The largest number of surviving placentas without fetuses was noted in Group 3, Table I, where 75% of all the placentas were found at sacrifice. Group 5 and Group 9 followed, with 44% and 53%. Group 7 and Group 8 in Table I, however, showed only a minimum of 2% of surviving placentas.

Effect of repeated litter destruction with DON on fertility and subsequent offspring of the rat (Table II). After the litter-destroying doses of DON (2 times .5 mg/kg) (Table

TABLE II. Reproductive Performance of Female Rats after Previous 4 Abortions with .3 mg/kg DON on 7 + 8 Day of Gestation.

No. of rats		Mother's wt gain, %	Total implants	Fate of litter: Live	Fate of litter: Res.	Avg litter wt	Avg single fetus: Wt, g	Avg single fetus: Length, cm
9	a		68	68				
	b	+25.3	7.5	7.5	0	47.5	6.3	5.0
	c			100				

a = total No. b = per mother rat. c = % of total implants.

I, 7) was determined, it was of interest to study the effect of multiple consecutive litter destruction in the same mother animals. For this reason, a group of mature rats who had previously had normal litters were aborted 4 consecutive times, on the 7th and 8th days of gestation, with DON. Several of the rats died during the experiment from infection and other complications such as pyometra, uterine hemorrhage, and pneumonitis. Delay was noted in re-mating of the female rats following litter destruction. It occurred at the natural end of term, between 23 and 30 days after sperm was found in the vagina, and 14 to 20 days after DON administration. Failure to detect sperm in the vagina of the experimental rats led in 2 instances to the production of litters after only 1 previous abortion, and in 4 instances after 2 previous abortions. The litters of these rats appeared to be normal in all respects. In 7 instances, rats delivered stunted small litters with skeletal malformations in spite of DON administration, during the 3rd period of gestation. This was due to disintegration of DON in the watery solution at room temperature.

After 4 consecutive abortions, the rats were re-mated and permitted to go untreated to term (Table II). The litters appeared to be normal. Neither the ovarian cycle nor fertility was adversely affected by previous DON administration.

Discussion. In comparison to AZS, DON is a less stable compound, especially in solutions with a pH of less than 6.7. It is therefore more difficult to preserve in a watery solution. On the other hand, the compound appears 10 times more active in total litter destruction than AZS(4), while offering a greater margin of safety between toxic doses to the mothers and fatal doses to the litters. Similarly to AZS, DON also permits complete litter destruction from the time of implantation to mid-term(4). Unlike AZS, adenine protected a large number of fetuses against the action of DON, which supports the theory of DON's inhibition of the *de novo* synthesis of purine bases(1).

Summary. DON affects adversely the fetus when given from time of implantation to mid-term, and two doses of .5 mg/kg during this time will result in complete litter destruction. The action of DON appears to be directly on the fetus and not on placenta, ovary or pituitary. Progesterone given prior to DON administration did not protect the litters. Large doses of adenine sulfate given prior to DON administration protected the litters to a large degree. Repeated complete litter destruction in the same animals gave no evidence of cumulative toxicity, nor did DON adversely affect the fertility of the mother rats, or subsequent offspring.

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