Effect of O-Diazo Acetyl-L-Serine on Rat Litter.* (22847)

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O-Diazo acetyl-l-serine (AZS), a serine analogue first isolated from culture broth of a streptomyces and later synthesized(2) was reported to affect adversely the chick and rat embryo(4,7,8). According to the first report(7), pregnant rats treated with a single dose of 5 mg/kg or 2.5 mg/kg doses failed to produce live litters when injected on the critical 8th day of gestation. Doses on the 10th and 11th days induced fetal resorption and malformations(7,8). The main object of the present experiments was to determine not so much the effect of the drug on the single fetus but on the total litter, its effect on repeated use in the same animal and its effect on subsequent fertility and litter.

Methods. Long-Evans strain of rats was used as previously described(9,10). The single LD_{50} for Wistar rats of 200 g weight was reported to be 70 mg/kg intraperitoneally and the LD_{50} of 5 doses on 5 successive days 25 mg/kg(7). The doses of AZS administered in the present experiments to the pregnant mothers varied from 2.5 to 10 mg/kg. Experimental groups of rats, which all had only one previous litter, were injected intraperitoneally on the 4th and 5th, the 8th, or 7th and 8th, or 11th and 12th, or 15th and 16th days and sacrificed on the 21st day of gestation (the day before littering), counting the morning of massive sperm findings in the vagina as day 0. At autopsy no abnormalities were detected in the mother rats. The entire uterus with tubes and ovaries was removed, then opened longitudinally, opposite the site of the placental implantations. The placentas, fetuses and resorptions were counted, weighed, and recorded. The fetuses were inspected, measured, weighed and then fixed in 10% formalin. All fetuses were x-rayed and the cranium incised for evidence of hydrocephalus.

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Results. Weight changes. Normal, nonpregnant control groups of rats of the same age as the experimental animals gained 8%, and pregnant controls 36%, of their initial body weight during a period of 21 days. The experimental groups of rats with their litter intact (Table I, 1,2,9) gained weight like the control rats, but groups of animals with their entire litter destroyed following drug administration on the 8th day gained an average of 18% (Table I, 6) and with drug administration on the 11th and 12th days, an average of 11.5% of total initial body weight (Table I, 8). This weight gain indicated that the doses of AZS administered were not toxic to the mothers.

Effect on litter. Two doses of 5 mg/kg AZS given intraperitoneally on the 4th and 5th days of gestation failed to show an effect on the litter (Table I). The single 2 mg/kg intraperitoneal dose which is equivalent to a 2.5 mg/kg oral dose on the 8th day of gestation, led to stunting of 11% of all the living fetuses, and to resorption of 17% of all implants (Table I, 2). The 2.5 mg/kg intraperitoneal dose permitted only 13% of all fetuses to survive, with stunting and malformation of 34% of the survivors (Table I, 3). Thirteen out of 28 mothers, or 41%, were found at sacrifice to have lost their entire litter, (Table I, 3). The same dose given on the 7th and 8th days of gestation showed no significant difference in fate of the litters at sacrifice (Table I, 4). The single 5 mg/kg intraperitoneal dose resulted in the survival of only one single malformed fetus in the entire group of 26 rats treated, the others being completely resorbed (Table I, 5). The 10 mg/kg intraperitoneal dose killed all fetuses without exception (Table I, 6). Two doses of AZS, given on the 11th and 12th days of gestation, or at midterm for rats, were found to be equally fatal to the litters. The 5 mg/kg doses permitted the survival of 8 stunted

TABLE I. Effect of AZS on Rat Litter.

No. of rats		Day of dose	Dose, mg/kg	Mother's wt gain, %	Total implants	Fate of litter: Live	Fate of litter: d Stunt	Fate of litter: Dead	Fate of litter: d Malf.	Fate of litter: Res.	Mothers with all resorb. litters	Avg litter wt	Avg single fetus: Wt, g	Avg single fetus: Length, cm
18	a				166	162		1		4				
	b	4, 5	5.0 i.p.	+36.5	9.2	9.0				.2	0	49.0	5.0	4.6
	c					97.6		.6		2.4				
21	a	8	2.5 oral	+37.9	164	135	16		49	29		29.8	4.6	4.0
	b				7.8	6.4	.75		2.3	1.4	0			
	c					82.3	11.8		37.3	17.7				
28	a	8	2.5 i.p.	+21.2	227	29	9		1	198	13	7.4	3.8	3.6
	b				8.1	.97	.32		.36	7.1				
	c					12.8	31.0		3.5	87.3	41.4			
23	a	7, 8	2.5 i.p.	+20.8	199	30	13			169	13	8.2	2.5	3.5
	b				8.7	1.3	.56			7.4				
	c					15.1	41.3			85.0	56.5			
26	a	8	5.0 i.p.	+28.0	232	1		1		231	25	4.2	4.2	4.1
	b				8.9	.38		.38		8.9				
	c					.44		100.0		99.6	96.2			
37	a	8	10.0 i.p.	+18.3	350					350	37			
	b				9.5					9.5				
	c									100.0	100.0			
24	a	11, 12	5.0 i.p.	+14.7	240	8	5		3	232	22	13.2	3.3	3.4
	b				10.0	.33	.21		.12	9.7				
	c					3.3	62.5		37.5	96.7	91.6			
23	a	11, 12	10.0 i.p.	+11.5	223					223	23			
	b				9.7					9.7				
	c									100.00	100.0			
26	a	15, 16	10.0 i.p.	+34.8	221	207		2		9		37.1	4.7	4.0
	b				8.5	8.0		.1		.4	0			
	c					93.7		.9		4.4				

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

fetuses, all present in only 2 litters, out of a total of 240 implantations in this group of 24 rats. The other 22 mother rats had lost their entire litters. The 8 live fetuses found were immature, and 3 also grossly malformed and doubtfully viable (Table I, 7). The 10 mg/kg doses induced 100% litter destruction in 23 rats, while the mothers gained an average of 11.5% of their initial body weight (Table I, 8). Experiments with AZS given to pregnant rats on the 15th and 16th days gave entirely different results. The 10 mg/kg doses had only little effect on the litters, inducing only 5% fetal death, and not destroying a single entire litter in any mother animal (Table I, 9). At autopsy it was evident that neither ovaries nor the corpora lutea of pregnancy nor the placentas were primarily affected by AZS—nor was the pituitary inhibited as the typical lactation changes of the mammary glands indicated. From this one has to conclude that AZS acted directly on the fetus.

Gross malformations of fetuses consisted in hydrocephalus, malformed heads, harelips, skeletal defects, and massive general edema. Stunting consisted in the production of fetuses less than 3.5 cm in length and 3.5 g in weight (See means of normal control litters) (10). Livers and sterna of all mothers were examined histologically and found to be normal.

Placentas. The parasitism of the placentas independent of that of the fetus as first emphasized by Ballantyne(1) was evident in the present experiments. In the experimental rats, approximately one-third to one-half of all the placentas survived the fetuses. Even with complete resorption of the embryos, it was quite usual to find placentas at the site of implantation varying in size from a few millimeters to almost normal size. Three giant placentas were observed in animals where only one or 2 of the fetuses of the litter survived. In 2 instances, the placentas with fetuses weighed 1.7 g each; in the third instance, 1.2 g. The histological structure of the placentas without fetuses varied. Frequently, very small surviving placentas consisted of a central hemorrhagic area surrounded by a rim of giant cells only. When the fetuses were destroyed on the 7th and 8th days of gestation, the surviving placentas failed to show the typical penetration of allantoic vessels into the trophoblast with good labyrinth formation. Surviving fetuses destroyed on the 11th and 12th days of gestation showed abortive labyrinth formation and normal disintegration of the reticularis. In short, changes were noted in the placentas which corresponded to those previously reported by Huggett and Pritchard(6) after crushing of the fetus and hormone administration to the mothers.

Experiments with AZS Given Before Mating. AZS was given to a group of 6-months-old female rats in 2 doses of 10 mg/kg before mating. This amount would have been sufficient to destroy all litters *in utero* any time during the period of implantation to midterm (7th to 12th day). All rats tolerated the drug well and were subsequently mated. Four animals conceived within 2 days, 7 within 3 days and 2 within 4 days, the rest later—a fact illustrating that the drug did not interfere with the ovarian cycle. The rats were found to be pregnant at sacrifice. The weight gain of the pregnant experimental rats was within normal limits; so were the mean number of 9.6 implantations per rat. Of 239 implanted fetuses, 223 or 93.4% were found to be alive. Of these 3 were less than 3.4 cm long, that is, stunted. One fetus was found dead in a litter where 2 other fetuses were completely resorbed. The total number of resorbed fetuses in the 25 experimental rats was 15, or 6.3%, with a standard deviation of $\pm$ 2.4. The mean fetal weight of the experimental animals was 5.3 g, the mean length 4.1 cm, the mean litter weight 47.8 g —all within normal control limits. Most fetal resorptions occurred in litters where the mating had been successful within 3 ovarian cycles from the time of drug administration. The results demonstrated that not sufficient AZS was incorporated in the ova to render them non-viable. However, a small increase in resorbed fetuses over the controls, and also the appearance of a few stunted embryos, occurred (Table II).

TABLE II. Effect of AZS on Rat Litter.

No. of rats		Day of dose	Dose, mg/kg	Mother's wt gain, %	Total implants	Fate of litter: Live	Fate of litter: d Stunt	Fate of litter: Dead	Fate of litter: Res.	Mothers with all resorb. litters	Avg litter wt	Avg single fetus: Wt, g	Avg single fetus: Length, cm
25	a	2 ×	2 × 10 AZS		239	223	3	1	15				
	b	before		+38.9	9.6	8.9	.12	.04	.6	0	47.8	5.3	4.1
	c	mating				93.4	1.3	.4	6.3				
12	a	7,8 pro-	5 AZS + pro-		103	5			98	9			
	b	gesterone	gesterone	+21.6	8.6	.4			8.1		6.5	3.5	3.5
	c					4.8			95.0	75			
5	a	7,8 adenine	5 + adenine		53				53				
	b			+11.2	10.3				10.3	100			
	c				100				100				
28	a	7, 8	6MP AZS		246	17			229	26			
	b			+13.8	8.8	.7			8.2				
	c		5.0 1.0			6.9			86.0	93			
26	a	7			248	1	1		247	25	4.0	3.4	4.0
	b		5.0 2.5	+18.9	9.5	.04	.04		9.5				
	c					.4	100		99.5	96.2			
29	a	7, 8			261	2	2		259	28			
	b		5.0 2.5	+14.4	9.0	.1	.1		8.9		6.0	3.0	3.1
	c					.8	100		99.4	96			
21	a	11, 12			192	2	2		190	20			
	b		5.0 5.0	+16.2	9.2	.1	.1		9.1		2.0	1.0	1.0
	c					1.0	100		99.0	95.2			

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

TABLE III. Reproduction Performance of Female Rats after Repeated Complete Litter Destruction with AZS on A—7th and 8th, or B—11th and 12th Days of Gestation.

No. of rats		No. of abortions	Mother's wt gain, %	Uteri total implants	Fetuses: Live	Dead	Resorb.	Avg: Litter wt, mg	Avg Single fetus: Wt, mg	Length, cm
37	a			352	346		.3			
	b	0	+36.1	9.5	9.4	—	.08	44.6	4.7	4.4
	c	control			99.1		.8			
12	a			108	108					
	b	1*	+36.0	9.0	9.0	—	—	58.8	6.5	4.5
	c				100					
9	a			74	7.4					
	b	2*	+38.0	8.2	8.2	—	—	45.7	5.6	4.2
	c				100					
10	a			73	73					
	b	3*	+37.3	7.3	7.3	—	—	48.5	6.2	4.2
	c				100					
10	a			94	82	9	.3			
	b	4*	+38.0	9.4	8.2	.9	.3	50.3	5.4	4.1
	c				87	11 ‡	3.2			
9	a			79	79					
	b	5*	+38.0	8.6	8.6	—	—	57.6	6.0	4.6§
	c				100					
10	a			81	81					
	b	1†	+37.0	8.1	8.1	—	—	52	5.4	4.1
	c				100					
30	a			239	216	18	5			
	b	2†	+35.0	7.9	7.2	.6	.16	48	6.1	4.2
	c				90.4	8.4‡	2.1			
6	a			59	52	3	4			
	b	3†	+36.0	9.8	8.6	.5	.6	52.4	5.8	4.2
	c				88.2	5.8‡	6.8			
10	a			73	73					
	b	4†	+37.0	7.3	7.3	—	—	49	6.7	4.5§
	c				100					

* Litter destruction on 7th and 8th days of gestation with 5 mg/kg.
† " " " 11th and 12th " " " " 10 " .
‡ Percentage of live.
§ Reared and inbred.
a = total. b = per animal. c = % of total.

Effect of progesterone and adenine with AZS on rat litter. In addition to the 5 mg/kg AZS on the 7th and 8th days of gestation a group of animals received on the 7th, 8th and 9th days, one hour before the AZS medication, 10 mg of progesterone intramuscularly. At sacrifice no protective effect of progesterone was noted; 95% of all the fetuses were resorbed, and the 5 survivors small (Table II, 2). An attempt to prevent the action of AZS on the litters by giving large doses of Adenine also failed. Five rats treated with 5 mg/kg AZS on the 7th and 8th days of gestation received 250 mg of adenine sulfate 1 hour before the AZS. At sacrifice all litters were completely resorbed. The kidneys of the mother animals showed the typical "adenine kidneys" previously described (Table II, 3).

AZS in combination with 6 mercaptopurine (6MP) (Table II, 4,5,6,7). 6MP was previously shown to destroy the rat litter in doses not less than 10 mg/kg. In combination with AZS it was more efficiently destroying the entire litters when given as listed on Table II, than either compound alone. All survivors in these groups were stunted and not viable.

Repeated litter destruction with AZS (Table III). After it was shown that AZS

as described would destroy efficiently the entire litters of rats, groups of pregnant animals were exposed to repeated consecutive abortion with the drug. One group was repeatedly aborted at the time of implantation, the other at midterm. The rats were then permitted to become pregnant again and go untreated to term. It was striking to observe that all the aborted rats would not mate until the natural term of their interrupted pregnancy would have arrived (22 days); the rats then ovulated and mated. The offspring of rats thus aborted were normal in all respects, in numbers, size and weight. Gross abnormalities were not seen nor were they detected on x-ray photography of the grown-up litter. Subsequent inbreeding of litters out of mothers thus aborted 4 to 5 times produced again normal offspring in all respects.

Discussion. The present experiments have again demonstrated that 1 or 2 doses of AZS representing only 1/18 of 1/7 of the single LD_{50} for the adult mother rat destroyed the entire litters during the first half of gestation period (7th to 12th day)(7,8). Because of the great differential action between doses lethal to the litter and toxic to the mothers, AZS is an efficient and also safe compound for the purpose of litter destruction. Furthermore, the extended action of the drug covering the time from implantation to midterm is remarkable. Other drugs have failed to have such drastic effects on the litter except for the first 48 hours following implantation(9). The malformations observed in the present experiments corresponded to those previously recorded following administration of this and other antimetabolites (7,8).

Summary. 1. The observation that AZS affects the rat fetus *in utero* is confirmed and greatly extended. 2. AZS acts on the fetus directly—and not on the ovary, placenta or pituitary. 3. AZS efficiently destroys the entire litter of rats in doses of: (a) 5 mg/kg at the time following implantation (7th and 8th days of gestation); (b) 10 mg/kg at midterm (11th and 12th days of gestation); without affecting the mothers adversely. 4. This drug effect is sharply limited in time. 5. Combination of 6MP and AZS was effective in litter destruction in smaller doses than either compound alone. 6. Neither progesterone nor adenine protected the litter against AZS. 7. AZS given before mating did not delay it, nor did it affect the subsequent litter. 8. Rats aborted 4 and 5 consecutive times with AZS showed no accumulative toxicity nor impairment of their fertility. 9. Litters raised from rats aborted 4 or 5 times were normal in all respects and produced normal litters.

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