1954, v33, 540.
3. Wardlaw, A. C., Pillemer, L., *Ann. N. Y. Acad. Sci.*, 1956, v66, 244.
4. Described by Cornfield, J., Mantel, N., *J. Am. Statistical Assn.*, 1950, v45, 181; Irwin, J. O., Cheeseman, E. A., *J. Hygiene*, 1939, v39, 574.

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Effect of Alazopeptin (A) on Litter and Fetus of the Rat *in utero*. (23911)

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A. was first isolated and identified from a culture of *streptomyces griseus planus* by DeVoe and collaborators(1). It has the formula of $C_{15}H_2N_7O_6$ with ultra violet and infrared absorption bands characteristic of Diazoketones; it is apparently a peptide consisting of 1 mole of Alpha-alanine and 2 moles of C_6-diazoketoamino acid. A. is very soluble and quite stabile in alkaline media. It inhibited growth of some bacteria as well as Sarcoma 180 in test mice. In previous experiments, 2 other diazoketones(2,3) were extremely toxic to the rat fetus *in utero*. It was, therefore, of interest to examine also the effect of A. on the rat fetus.

Methods. Mature Long-Evans strain rats were used exclusively. The animals were kept on White breeding diet of Long-Evans. Only rats with previous satisfactory breeding performance were employed and mated. The day of onset of gestation was determined by vaginal smear. The morning of massive vaginal sperm findings, females were separated in single cages and so kept until 21st day of gestation to avoid urinary or fecal contact. The day before littering, the animals were sacrificed under ether anesthesia, the uteri removed, placentas, fetuses, sites of implantation noted and recorded. Surviving fetuses were measured, weighed and fixed and later cleared to study malformations. A. was freshly prepared, dissolved in sterile water and injected intraperitoneally without delay. The results of the treatment of three groups of rats are listed in Table II.

TABLE I. Toxicity: Alazopeptin Aa223 Lederle.

	100 mg/kg	150 mg/kg	200 mg/kg
LD_{50}	3/8	6/8	6/8
% wt loss	13	16	17
	.25 mg/kg	.5 mg/kg	1.0 mg/kg
5 LD_{50}	2/6	6/6	6/6
% wt loss	17	15	20

The single LD_{50} for mature Long-Evans rat was 150 mg/kg; the 5 LD_{50} between .25 and .5 mg/kg. All intoxicated rats showed watery diarrhea and fatty livers. Animals dying from effect of compound were dehydrated and lost 20% of initial body wt. Surviving rats recovered quickly within 5 days from single or multiple doses.

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Results. In *Exp. 1*, .3 mg/kg given on 7th and 8th day of gestation, *i.e.* time of implantation, destroyed all litters completely. 67.5% of the placentas showed remnants of organs in the uteri at sacrifice—usually measuring only 2 to 3 mm in diameter. In *Exp. 2*, two doses of .5 mg/kg, given also on 7th and 8th day of gestation, destroyed 271 of 273 fetuses, with a single fetus surviving in 2 different litters. Both of these fetuses were stunted and would not have survived. Total litter destruction occurred in 26 out of 28 rats, or in 93%. In this series, placental remnants were found in 37% of the resorbed fetuses. In the *third experiment*, .5 mg/kg was given on the 11th and 12th day of gestation, that is at mid term. All litters were completely destroyed and resorbed but, in 81%, surviving placental remnants were found at sacrifice.

The *mother animals*, sacrificed on 21st day of gestation, showed macro- and microscopically no detectable lesions in their internal organs; particularly none in bone marrow, liver or intestinal tract. Practically all animals of the 3 experimental groups showed secretory activity in the mammary glands, corresponding to normal pregnant controls. The two

TABLE II. Alazopeptin; Lederle Aa223.

No. of rats	Day of dose, i.p.	Mother's wt gain		Uteri total implants	Fetuses: Live	Fetuses: Stunted	Resorbed	Surviving placentas	Mothers with total resorbed litters	Litter wt	Fetus: Wt	Fetus: Length
14	7 & 8 .3 mg/kg	+8.3	a	126			129	85	14			
			b	9	0		9					
			c				100	67.5	100			
28	7 & 8 .5 mg/kg	+8.6	a	273	2	2	271	102	26			
			b	9.75			9.7			2.25	2.7	2.8
			c		.6	100*	99.4	37.4	93			
16	11 & 12 .5 mg/kg	+6.75	a	162			162	132	16			
			b	10.1	0		10.1	8.25				
			c				100	81.5	100			

a = Total b = Per mother c = % of total * % of live.

surviving fetuses of Group 2 were stunted and showed on clearing a retarded skeletal development, evidenced by absence of ossifying centers of supraoccipital and nasal bones, as well as metacarpal diaphysis 2, 3, 4 and 5.

Discussion. The present observation significantly demonstrates the sensitivity of the early embryo to A., a fact to be considered in chemotherapeutic efforts using this compound as antibiotic or antitumor agent in man. Furthermore, A. like O-Diazo-Acetyl-L-serine and 6-Diazo-5-oxo norleucine(2,3) permits the control of reproduction by either preventing proper implantation of the fertilized ova or destruction of the implanted embryos in the early phases of gestation without severely intoxicating the mothers.

Summary. Alazopeptin had the following toxicity in the adult Long-Evans rat: (1) Single LD_{50} of 150 mg/kg, the 5 LD_{50} of .3 mg/kg. (2) Alazopeptin destroyed *in utero* entire litters of Long-Evans rats (at time of implantation) when given i.p. on 7th and 8th day of gestation in doses of .3 mg/kg. (3) And in doses of .5 mg/kg i.p. (at mid term) when given on 11th and 12th day of gestation. (4) Placental remnants frequently survived the fetuses and were found at term. (5) The lethal dose of A. to the fetus of .5 mg/kg was approximately 1/30 of the LD_{50} for the mother animal.

1. DeVoe, S. E., Rigler, N. E., Shay, A. J., Martin, J. H., Boyd, T. C., Backus, E. J., Mowat, J. H., Bohonos, N., Alazopeptin: *Antibiotics Annual,* 1956-1957, *Medical Encyclopedia,* N. Y., 730-735.

2. Thiersch, J. B., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 27.

3. ———, *ibid.,* 1957, v94, 22.

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Cardiovascular Responses to Norepinephrine in Acute Adrenal Insufficiency.* (23912)

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Since Elliot's observations(1) on responses of adrenalectomized cats to various pressor agents, numerous investigators have concerned themselves with the problem of cardiovascular reactivity in adrenal insufficiency. A summary of the work on cardiovascular responses to sympathomimetic stimuli in adrenal insufficiency appears in the review by Ramey and Goldstein(2). An impairment in the vas-

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