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Effect of Cortisone, Properdin and Reserpine on *Neisseria gonorrhoeae* Endotoxin Activity. (23910)

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In connection with studies on endotoxin derived from *Neisseria gonorrhoeae*(1), we were interested in determining whether certain therapeutic agents would afford a measure of protection against, or enhancement of, toxic properties of endotoxin. We used as therapeutic agents cortisone, reserpine and properdin. Cortisone has been shown by workers to afford some measure of protection against toxic effects of certain bacterial toxins (2). Reserpine and properdin were included since to our knowledge they have not been investigated previously in conjunction with any endotoxin. It was hoped that reserpine and properdin might exert a protective action through interference with possible neurotoxic effect of endotoxin. It has been recently suggested that the properdin system may be a factor involved in natural resistance(3).

Materials and methods. Male CF1 mice weighing 16-18 g, were kept (about 10 animals/cage) in an air-conditioned room and had free access to water and Puritan Mouse Ration. Endotoxin was prepared according to our recent procedure(1). The culture medium, however, has been slightly modified. The nutrient broth has been omitted from the medium and proteose peptone concentration has been increased to 8 g/l. This change resulted in more rapid growth of gonococcus, harvesting being possible in 96 hours. Acetone powder was dissolved in a small volume of 0.05 N sodium hydroxide. The resulting solution was adjusted to pH 7.0-7.4 with 0.25 N acetic acid, diluted to desired concentration with sterile distilled water and administered intraperitoneally. Cortisone (cortone acetate) and reserpine (Serpasil) were diluted with sterile saline to appropriate concentrations and administered intramuscularly. Karber's procedure was used for calculating LD_{50} values and interpreting their statistical meaning(4).

Experiments were conducted as follows: each group of mice was sub-divided into control-challenge and treated-challenge groups. The control-challenge group received only endotoxin, whereas, the treated-challenge group received endotoxin plus one of the 3 agents. Endotoxin controls (LD_{50} 2-3 mg) were run with each experiment since LD_{50} values varied somewhat with each new group of mice. Mice

were observed for a 5-day period.

Results. Cortisone, One Hour After Challenge. In a preliminary experiment a single injection of 1 mg of cortisone 1 hour after challenge, using 5 different endotoxin concentrations with only 6 mice in each group, gave cortisone LD_{50} 3.36 mg/control LD_{50} 2.38 mg, a relative increase in resistance of 41%. This increase was not significant because of small number of mice at each level ($0.05<P<0.1$). To confirm the magnitude of this increase the experiment was repeated with 10 mice for each concentration of endotoxin. The relative increase in resistance of the 10 mice to each of 5 different endotoxin concentrations, based on cortisone LD_{50} 4.29 mg/control LD_{50} of 2.93 mg, was equal to 46%. This 46% increase in resistance to endotoxin was statistically highly significant ($P<0.01$).

Cortisone, One Hour Before Challenge. With 1 single injection of 1 mg of cortisone 1 hr before challenge using 6 mice for each of 5 different concentrations, the relative resistance, based on cortisone LD_{50} 5.33 mg/control LD_{50} 2.38 mg, was equal to 2.24 or 224%. This increase in LD_{50} for cortisone was highly significant even with only 6 mice in each group at each dose ($P<0.01$).

Properdin. The potency of properdin was 9 units/mg. Properdin solutions (reconstituted to isotonicity in sterile distilled water) were directly added to endotoxin solutions just before injection. Five different endotoxin concentrations with 36 to 50 mice/group were used in each of 3 experiments. Listed in Table I are properdin units injected together with endotoxin, LD_{50} values for these mixtures, the ratios of LD_{50} values for these mixtures/LD_{50} of controls. The fairly large dose of 50 units of properdin produced a highly significant increase of resistance of mice to the endotoxin ($P<0.01$). With 25 units and 12.5 units of properdin the resistance was less significant. The 3 groups of experiments were conducted with 3 different lots of endotoxin and mice.

Reserpine. Reserpine was injected 1 hr before challenge. There were 5 different endotoxin concentrations in Exp. 1, and 10 mice in each group. In Exp. 2 there were 6 different endotoxin concentrations and 6 mice/group, except in 3rd and 4th group, in which there were 10 mice/group. In the control there were 5 different endotoxin concentrations and 10 mice/group. It is seen in Table I that 2 μg of reserpine had no effect on endotoxin activity. Five μg of reserpine, however, increased toxicity of endotoxin by 56% which is significant ($P<0.05$). There was no mortality in controls given reserpine, properdin, or cortisone only (not listed in Table I).

TABLE I. Response of Endotoxin to Therapeutic Agents.

Agent	Exp. No.	Schedule of treatment	LD_{50} of mixture, mg	Mixture LD_{50}/control LD_{50}
Cortisone	1	1 mg 60 min. after challenge	3.36	1.41
		Control	2.38	
	2	1 mg 60 min. after challenge	4.29	1.46
		Control	2.93	
	3	1 mg 60 min. before challenge	5.33	2.24
		Control	2.38	
		Units		
Properdin*	1	6.25	3.22	.95
		25	3.80	1.12
		Control	3.40	
	2	12.50	3.36	1.35
		25	4.00	1.61
		Control	2.48	
	3	50	5.10	1.62
		Control	3.14	
		μg		
Reserpine†	1	2	2.93	.97
	2	5	1.68	.56
		Control	3.02	

* Directly added to endotoxin solution.
† Inj. 60 min. before challenge.

Summary. Cortisone significantly protected mice from the lethality of *Neisseria gonorrhoeae* endotoxin. Similar results were obtained with properdin, provided relatively large doses were employed. Reserpine, under certain conditions, significantly enhanced the toxicity of endotoxin.

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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.

* This work performed with grant from U.S.P.H.S. and Population Council.

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