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Immunization Attempts with Lipopolysaccharides (LS) of Gram Negative Microorganisms Against Lethal Action of LS on Rat Fetus *in utero.** (27228)

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It was demonstrated(3) that the LS of gram negative microorganisms would destroy the litters of pregnant rats when given i.p. after implantation of the fetuses.

In the present experiments, attempts were made to protect the litters *in utero* against otherwise lethal doses of LS by previously immunizing the mother animals. Strains of rats, their care, age, diet, determination of onset of pregnancy, time of sacrifice and recording of findings were the same as previously described(3). In the present experiments, the LS of *S. marcescens* and *E coli* were used.

Experiments. (Tables I and II). Groups of pregnant rats were injected daily from gestation day (g.d.) 1 to 7 or from g.d. 7 to 13 with s.c. or i.m. doses of 0.2 mg/kg of LS of *E. coli* or LS of *S. marcescens.* A part of each injected group was permitted to go to term to study the effect of the injections. The remainder were then injected on g.d. 11 and 12 or 15 and 16 with an amount of LS previously lethal to all litters.

Results with LS of E. coli. In comparison to the controls, only 16% of the fetuses could be protected when the mothers were immunized from g.d. 1 to 7 and 26% when they were treated from g.d. 7 to 13. The litters from mothers treated with LS of *S. marcescens* from g.d. 1 to 7 showed that 42% of the fetuses survived, and when treated from g.d. 7 to 14, a maximum of 26% survived. The fate of the litter of rats challenged on the 15th g.d. after immunization from g.d. 1 to 7 with LS of *S. marcescens* was worse than that of rats injected with the lethal doses on g.d. 11 and 12, in spite of 3 further days of immune body production. The findings demonstrated that antibodies formed to the LS injections were not able to protect the fetuses *in utero* completely. To study this problem further, 2 groups of 4 rats each were injected daily s.c. and i.m. with 0.2 mg/kg of LS of *E. coli* or with 0.2 mg/kg of LS of *S. marcescens.* The *E. coli* group was bled from the tail vein on the *12th* g.d. and then injected with 2 mg/kg i.p. of LS of *E. coli.* The animals were kept in metabolic cages, their urine collected, and sacrificed 6 hours after the challenging dosis of LS. Blood was collected under sterile conditions and the serum separated.

The *S. marcescens* treated group was bled from the tail vein on the *16th* g.d., then injected with 2 mg/kg of LS of *S. marcescens.* The animals were then kept in metabolic cages and sacrificed after 6 hours and blood collected sterilely. The fetuses in all 8 animals were found to be dead at sacrifice. Total serum protein estimation and electrophoresis was carried out on all 16 sera. The sera of both experimental groups showed a uniform response. Total serum protein decreased from 22% to 28% with a marked decrease in the globulin. In the animals where the albumin also sharply decreased, it appeared in the urine of the rats (Fig. 1). The findings demonstrated that a severe an-

* This investigation was carried out with Grant-in-Aid from the Population Council Inc., and U.S.P.H.S.

tibody antigen reaction took place with precipitation of the reaction product. The sera of the animals sacrificed 6 hours after the 2 mg/kg dosis of LS were then investigated for the presence of antibodies against the LS with the technic of agglutination of tanned sensitized red cells described by Boyden(1). The sera of all 8 rats of the 2 experimental groups showed the presence of antibodies in dilutions of 1:8500 by agglutination of the sensitized red cells coated with LS antibodies, within 4 hours with complete hemolysis within 24 hours in the icebox.

The investigation clearly demonstrated

TABLE I. Immunization Attempts against the Litter Destroying Dose of 2 mg/kg of Lipopolysaccharide *E. coli*.

No. of rats	Day & dose		Mother's wt gain	Uteri total implants	Fetuses			Placental remnants	Mothers with all res. litters	Litter wt	Fetus	
					Live	d Stunted	Dead or resorbed				Wt	Length
12		a		117			117		12			
	2 mg/kg i.p.	b	+11.5	9.7	0		9.7					
	12 g.d.	c					100		100			
10	1 to 7 g.d.	a		100	84	22	16					
	.2 mg s.c.	b	+40.2	10	8.4	2.2	1.5			43.1	5.1	3.6
		c			84	26	16					
14	1 to 7 g.d.	a		126	20		106	48	11			
	.2 mg s.c.	b	+19.1	9	1.4		7.6			24	5	4.2
	2 mg/kg i.p.	c			15.9		84.1	45	78			
	11 g.d.											
9		a		100			100	9	9			
	2 mg/kg i.p.	b	+ 9.7	9.1			9.1					
	15 g.d.	c					100	100	100			
13	7 to 13 g.d.	a		118	115		3	3				
	.2 mg s.c.	b	+43.7	9.1	8.9					49.5	5.6	3.8
		c			97.5		2.5	100				
15	7 to 13 g.d.	a		150	39	2	111.0	75	11			
	.2 mg s.c.	b	+10.8	10	2.6		7.4			40.1	5	3.9
	2 mg/kg i.p.	c			26		74	67.5	73.4			
	15 g.d.											

a, total; b, per mother; c, % of total; d, % of live; g.d., gestation day.

that (a) fetuses *in utero* could not entirely be protected against the LS by immunizing the mothers; (b) a strong antigen antibody reaction took place when the challenging dosis of LS was administered; (c) an excess of antibody in the serum of the experimental animals was present during and after the time the challenging dosis of the LS was given. From these facts, it is probable that the LS is the carrier of the toxic Lipoid A, against which no antibody is formed and which destroys the fetuses.

Histological studies of uteri, placentas, and fetuses showed early intra-decidual and intra-

TABLE II. Immunization Attempts against the Litter Destroying Dose of 2 mg/kg of Lipopolysaccharide *S. marcescens*.

No. of rats	Day & dose		Mother's wt gain	Uteri total implants	Fetuses			Placental remnants	Mothers with all res. litters	Litter wt	Fetus	
					Live	d Stunted	Dead or resorbed				Wt	Length
13	2 mg/kg i.p. 12 g.d.	a		106	3		103	63	12			
		b	+12.2	8.1			7.9	4.8		16	5.3	4.5
		c			2.7		97.3	61	92			
8	1 to 7 g.d. .2 mg s.c.	a		77	75		2	2				
		b	+46.5	9.6	9.4				0	48.1	5.1	4.2
		c			97		3	100				
9	1 to 7 g.d. .2 mg s.c.	a		75	32	12	43	28	3			
		b	+17.1	8.3	3.5	1.3	4.8			26	4.9	3.7
	2 mg/kg i.p. 11 & 12 g.d.	c			42.6	32.6	57.4	65	33.3			
10	2 mg/kg i.p. 15 g.d.	a		86			86	15	10			
		b	− 2.2	8.6	0		8.6	1.5				
		c					100	17.4	100			
11	1 to 7 g.d. .2 mg s.c.	a		105	18		87	77	6			
		b	+20	9.5	1.6		7.9	7.0		14.1	3.9	3.9
	2 mg/kg i.p. 15 g.d.	c			17.1		82.9	90	54.6			
10	7 to 14 g.d. .2 mg s.c.	a		88	87		1	1				
		b	+44.6	8.8	8.7				0	50.3	6	4.4
		c			99		1	100				
10	7 to 14 g.d. .2 mg s.c.	a		94	16		73	59	5			
		b	+14.1	9.4	1.6		7.3	5.9		15.3	4.8	3.7
	2 mg/kg i.p. 15 & 16 g.d.	c			17		83	80.7	50			
13	7 to 14 g.d. .2 mg s.c.	a		144	37	4	107	76	8			
		b	+17.7	11.1	2.8		8.3	5.8		18.6	3.1	3.2
	2 mg/kg i.p. 16 g.d.	c			25.7	10.8	74.3	71	61			

a, total; b, per mother; c, % of total; d, % of live; g.d., gestation day.

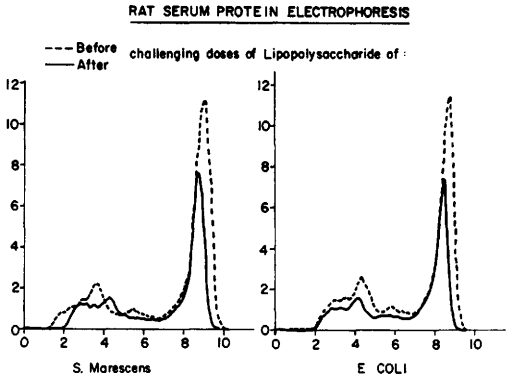

FIG. 1

placental hemorrhages, varying in size from 1 to 5 mm, with nuclear changes in the endothelial lining of decidual and placental venous sinuses. The nuclei showed a crenated membrane with folding and an abnormal density. Some of the fetuses were edematous but no specific lesion was found on sectioning and staining with H. and E.

Discussion. The present experiments confirm the difficulties encountered in attempting to immunize cows against *B. abortus* Bang (2) to prevent abortions. The findings suggest that the toxic factor of the LS is Lipoid A, which is not antigenic but is carried in the larger molecule of the LS.

Summary. Groups of rats immunized with doses of LS of *E. coli* or *S. marcescens* from g.d. 1 to 7 or 7 to 13, and then injected with a single dosis of LS otherwise lethal to the litters, showed 15% to 43% of fetuses surviving. The serum protein of the experimental animals showed the presence of a globulin antibody which was much reduced within 6 hours after the challenging dosis of LS. Sufficient circulating antibodies against the LS, however, were present 6 hours after the challenging dosis was given, demonstrated by agglutinated and hemolyzed sensitized red cells in dilutions of 1:8500.

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Effect of Lipoid A of the Lipopolysaccharide (LS) of *E. coli* on the Rat Litter *in utero.** (27229)

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It was shown(1) that the LS of gram neg. microorganism given parenterally to pregnant rats after implantation would destroy the litters *in utero*. According to Westphal(2) the toxicity of LS depends on the amount of Lipoid A in the molecule of the LS. It was, therefore, of interest to compare the effect of the extracted lipoid from the LS without the carrier molecule with the effect of the original LS on the rat litter *in utero*. The material, supplied by Westphal, consisted of LS *E. coli* CO111-3116-S4, and Lipoid A-11 preparation CO111-L2, 10 mg/ml in 0.5% dextran methiolate 1:10,000. Toxicity: The single LD_{50} for the LS was 20 mg/kg and for the Lipoid A more than 200 mg/kg. The methods used were identical with those described previously(1).

Experiments. The first group of pregnant rats were injected i.p. with 2 mg/kg of LS on the 12th gestation day (g.d.), the second group with 2 mg/kg of Lipoid A-11, and the third and fourth group with 20 mg/kg on the 12th g.d. The results are listed in Table I. All litters treated with 2 mg/kg of the LS died. The 2 mg/kg of the Lipoid A had only a stunting effect, but did not destroy a single litter. Four per cent of all fetuses were resorbed which is the upper normal limit. Group 3 and Group 4 treated with 20 mg/kg

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