methyl esters in either diet leave this source open to question.

There has been no evidence that methyl esters may be formed in animal tissues. However, McManus, Contag and Olson(11) have reported the presence of endogenous ethanol (but not other alcohols) in various tissues and Sato, Byerrum and Ball(12) have investigated the formation of pectinic acid methyl esters in plant tissues by transmethylation with methionine. It is possible that a similar reaction could occur in the animal body.

These studies show that more work is required to explain the source of the methyl esters reported here. However, they also point to a potentially interesting pathway and show that in experiments in which labeled fatty acids are fed as methyl esters, the separated cholesteryl ester fraction should be examined with care.

Summary. Methyl elaidate-1-C^{14} was fed to fat-deficient guinea pigs. Analysis of the body lipids by gas-liquid and by thin-layer silicic acid chromatography gave conclusive proof of the occurrence of methyl esters. The estimated activity attributed to recovered methyl elaidate compared well with the activity of the fed material. Occurrence of methyl esters in body as well as in blood lipids was conclusively demonstrated in normal pellet-fed animals. By using propanol instead of methanol and also by merely soaking the whole liver or adipose tissue in ethyl ether for extracting lipids it was further shown that the methyl esters are not formed during homogenization and extraction. Distribution of the activity in the various purified lipid components was determined.

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Effect of Lipopolysaccharides of Gram Negative Bacilli in the Rat Litter *in utero.** (27227)

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Bang(1) described abortions in cattle due to infection with gram negative micro-organisms; Zahl and Bjerknes(9) observed abortions in mice injected with endotoxins of gram negative organisms. The present paper reports systematic studies of the effect of lipopolysaccharides (LS) of gram neg. organisms on the litter of rats. The LS isolated and purified according to Boivin(2), purchased from Difco, included the LS of a gram positive streptococcus. The compounds investigated were isolated from cultures of: *E. typhosa* 901, (Difco #107832) (Table II); *S. marcescens* (Difco #902153 and USPH #Rx-B12555) (Table IV); *B. abortus* Bang (Difco #108539) (Table V); *E. coli*

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

(Difco #026B6 control 111 213 (Table III): *Streptococcus* A (Difco #108538) (gram positive) (Table VI).

Toxicity studies carried out in non-pregnant rats are listed in Table I. *Toxicity to pregnant rats.* The toxicity to pregnant rats was considerably greater than to non-pregnant animals as the effect of the compound added the burden of dead fetuses to the maternal metabolism. This was especially noted after LS administration on and after the 15th gestation day (g.d.). One-third to one-half of all experimental animals died in those groups within 4 to 24 hours. Animals which died thus are not listed in the tables in the number of rats in the experimental groups. Death occurred with extensive intra and extra uterine hemorrhages and with shock. Doses of LS destroying fetuses or litters induced usually vaginal hemorrhages within 24 to 48 hours after injection. The mother rats lost body tonus and ceased to gain weight.

Methods. Experiments were conducted with Long-Evans strain rats, bred and reared in our own colony. Only animals which had one satisfactory previous litter were used. The animals were kept on the White Breeding diet of Long-Evans, were approximately 6 months old and weighed 250 g each. *Controls.* One hundred non-pregnant rats of the same strain and age gained an average of 8% body weight in 21 days. One hundred pregnant control rats gained an average of 36.1 ± 12.6% of body weight during the first 21 g.d. This group had an average of 9.4 ± 3.4 fetuses per litter. Average fetus weight was 4.7 ± 1.3 g. Average fetus length was 4.4 ± .79 cm. The percentage of resorbed fetuses was 1.7 ± 2.4. Complete litter resorption did not occur.

Results. The LS was given i.p., and in 2 instances intragastrically, to groups of rats in different stages of pregnancy. Intragastric doses of the LS had little effect on the litters of pregnant rats. In contrast to a single intraperitoneal 2 mg/kg dose which killed all the fetuses, a single i.p. 10 mg/kg dosis of LS of *E. typhosa* (Table II) given on the 12th g.d. to 10 rats permitted survival of 85% of all fetuses without stunting of the survivors and a single 50 mg/kg dosis on the 9th g.d., given to 8 mother rats, had no effect on the fetuses—only 1 being resorbed. Onset of gestation (g.d.) was determined by massive sperm findings in the vagina. Pregnant animals were kept in single stainless steel cages to avoid urinary or fecal contacts. Mother rats were sacrificed under ether anesthesia on the 21st g.d. Uteri were removed and contents recorded, weighed and fixed. The fetuses were sectioned and cleared to study skeletal development. Maternal internal organs and placentas were inspected macroscopically, fixed in formalin and studied in H and E paraffin sections. *White blood counts.* The peripheral WBC and differential WBC of 6 pregnant rats treated on the 12th g.d. with 1 mg/kg of LS *E. coli* was examined. Before injection of the LS, an average WBC of 17,700 was found with a differential count of polymorphonuclears, 76%; lymphocytes, 20%; monocytes, 2%; and eosinophiles 2%. Counts 1, 2, 3, 4, 5, 6 and 12 hours after injection showed a marked dip in WBC to an average of 9,000 after 2 hours and a rebound to an average of 32,000 with a reversal of the differential counts to an average of 89% polymorphonuclears, 9% lymphocytes, 1% monocytes and 1% eosinophiles. After 12 hours, the counts had all returned to pre-injection figures. All animals showed on sacrifice complete litter destruction. *Fever.* Following injection of LS *S. marcescens,* rectal temperature recordings were kept on 15 rats. The animals showed a rise of .4°C for 24 hours, then returned to their normal pre-injection temperature.

In the tables, under "Mothers Weight Gain", is listed the average percentage of body weight gain of the entire group measured from 0 to 21st g.d. before sacrifice. Under "Fetuses Dead" are listed all dead and partly resorbed fetuses, still recognizable as separate entity from the surviving placenta. The fetuses often consisted of shapeless lumps. Under "Surviving Placentas" are listed all the organs which were found *in situ* in uterus at time of sacrifice. The organs varied greatly in structure and size depending on days of LS administration and fetal death. In the tables, the effect of the LS on

TABLE I. Toxicity of Lipopolysaccharides Given Intraperitoneally to Non-pregnant Female Rats.

Micro-organism	Dose in mg/kg	Rats		LD_{50} (mg/kg)
		Dead	Total	
E. typhosa	1	0	6	
	5	2	6	
	10	5	6	6
E. coli	50	5	6	
	20	5	6	
	10	3	6	10
S. marcescens	20	2	5	
	30	4	5	25
	40	5	5	
	50	5	5	
B. abortus Bang	1	0	6	
	5	0	6	
	10	0	6	
	25	0	6	
	50	2	6	
	100	6	6	60
Streptococcus A	50	0	6	
	100	0	4	
	200	0	3	
	500	1	3	over 500

single fetuses and the total litter is recorded separately. This is regarded as important.

It was difficult to establish the LD_{50} for LS *Streptococcus* A due to lack of sufficient quantities of compound (Table VI). Limited toxicity studies with 100, 300, 500 mg/kg indicated an LD_{50} of over 500 mg/kg. From the limited experiments, it was evident that this LS was also most toxic to the litter at midterm and, that by comparison to the LS of the other microorganisms tested, it was far less effective in fetus and litter destruction.

To study the effect of the LS on the mothers, placentas and litters, groups of 6 pregnant rats were treated the 11th and 12th and 15th and 16th g.d. with LS of *E. coli, Abortus* Bang, *E. typhosa* and *S. marcescens*. The animals were sacrificed 6, 24, 48, and 72 hours after the LS administration, the internal organs, placentas and fetuses fixed in Bouin's solution, and studies in paraffin sections stained with H and E. *Histological Findings*. The maternal organs showed within 6 to 24 hours an acute lymphadenitis of the mesenteric lymph nodes with enlargement, edema and sinus catarrh. The sternal and femoral bone marrow responded within 48 hr with an over-production of cells of the myeloid series. Occasionally, scattered small areas of liver necrosis were seen within 24 hours, located in the periphery and mid-zone of the liver lobules. These foci were characterized by the enlargement of groups of 10 to 25 liver cells with pyknosis of the nuclei. Occasionally, a few leukocytes and erythrocytes were found scattered throughout from capillary hemorrhages. Rats treated on the 11th and 12th g.d. showed an enormous dilatation of the blood vessels of the decidual and maternal placenta within 6 hours. After 12 hours, small and larger hemorrhages were noted from the dilated blood vessels, partly destroying the decidua and maternal placenta, often leading to destruction of the organs within 48 hours. At 96 hours, many placental remnants were pushed by large hemorrhages off the uterine wall and became necrotic. Great variation was seen in the placentas. Completely necrotic organs, with shadows of a few giant cell remnants were found in the same litter with organs relatively well preserved. Here, one still could identify the decidua, maternal placenta and rudimentary allantoic placenta, as well as giant cell layers. Well preserved surviving organs found at sacrifice corresponded to those described by Pritchard and Huggett(4) following crush injury to the embryo at different gestation ages. Malformations were rarely noted in surviving fetuses. General retardation of growth with general stunting was often found but no single or multiple skeletal defects were apparent. Results are listed in Tables II and VI.

Discussion. Zahl and Bjerknes(9) reported decidua-placental hemorrhages occurring within 6 hours in mice and rabbits after injection with endotoxins of *Shigella paradysenteriae* Flexner, *Salmonella typhimurium* and *Rhod. rubr.* The lesions induced from the 12th to 18th g.d., resulted in abortion or resorption of the embryos. In the present experiments intraplacental hemorrhages were noted in many animals within 12 or 24 hours after LS administration. However, the impression was gained that the death of the fetuses was not caused by placental hemorrhages alone but to action of the LS on the fetus. The placental hemorrhages often ex-

TABLE II. Lipopolysaccharide *E. typhosa* 901.

No. of rats	Day of dose	Dose, mg/kg		Mother's wt gain	Uteri total implants	Fetuses: Live	Fetuses: d Stunted	Fetuses: Dead	Fetuses: d Gross malf.	Fetuses: Resorb.	Surviving placentas	Mothers with all res. litters	Litter wt	Fetus: Wt	Fetus: Length
14	8	1	a		124	102	3			22	7	1	42		
			b	+32.2	8.8	7.3				1.7				5.3	4.3
			c			82.0	1.2			18	31.9	7.1			
12	12	1	a		106	3	3		1	103	103	11	10		
			b	+10.0	8.9					8.6	8.6			3.3	3.1
			c			2.8	100			97.2	100	91.5			
14	15	1	a		133	25				108	85	10	27.9		
			b	+18.9	9.5	1.8				7.7	6.0			4.5	4.3
			c			18.8				81.2	79	71.5			
16	19	1	a		138	63		74		1	75	7	live 41.6	5.9	4.6
			b	+30.9	8.6	3.9		4.7			4.7				
			c			45.7		54.3			100	43.8	dead 24.8	2.3	3.0
9	8	2	a		92	87		3		2	5	0			
			b	+38	10.1	9.7							49.4	5.1	4.3
			c			94.6		3.2		2.2	100	0			
11	12	2	a		99	1				98	10	10			
			b	+ 3.7	9.0					8.9			4	4	4
			c			1.1				99	9.8	90			
18	15	2	a		164	14		127		23	66	14			
			b	+12.3	9.1	.78		7.0		1	3.6		15.7	4.2	4.2
			c			8.5		77.5		14	44	78			
10	19	2	a		92	11	11	81			81	7	live 13.3	3.6	3.2
			b	+12.7	9.2	1.1	1.1	8.1			8.1				
			c			12	100	88			100	70	dead 16.7	1.4	1.7
8 oral	9	50	a		68	67				1					
			b	+42.2	8.5	8.3				3	0		45.5	5.4	4.2
			c			97									
10 oral	12	10	a		81	69		6		5	7				
			b	+36.5	8.1	6.9		.6				0	30	5.0	4.3
			c			85		7.4		6.2	63				

a, total; b, per mother; c, % of total; d, % of living

TABLE III. Lipopolysaccharide *E. coli* (Difco O26B6).

No. of rats	Day of dose	Dose, mg/kg		Mother's wt gain	Uteri total implants	Fetuses: Live	Fetuses: d Stunted	Fetuses: Dead	Fetuses: d Gross malf.	Fetuses: Resorb.	Surviving placentas	Mothers with all res. litters	Litter wt	Fetus: Wt	Fetus: Length
14	15	.5	a		145	17	17	128			116	10			
			b	+19.1	10.3	1.2	1.2	9.1			8.3		15.3	2.1	2.3
			c			11.7	100	88.3			91	71.5			
10	8	1.0	a		74	43	28			31	31	2			
			b	+32.8	7.4	4.3	2.8			3.1	3.1		34.1	3.4	2.6
			c			58	65			42	100	20			
15	15	1.0	a		149			118		31	118	15			
			b	+ 7.9	9.5			7		2.1	7				
			c					79		21	79	100			
10	19	1.0	a		123	12	12	111			111	8			
			b	+49.4	12.3	1.2	1.2	11.1			11.1		41.1	3.3	2.8
			c			9.7	100	90.5			100	80 toxic			
12	12	2.0	a		117					117		12			
			b	+11.5	9.7					9.7					
			c							100		100			
9	15	2.0	a		100					100	100	9			
			b	+ 9.7	9.1					9.1	9.1				
			c							100	100	100 toxic			

a, total; b, per mother; c, % of total; d, % of living

TABLE IV. Lipopolysaccharide *S. marcescens* (Lot #902153).

No. of rats	Day of dose	Dose, mg/kg		Mother's wt gain	Uteri total implants	Fetuses					Surviving placentas	Mothers with all res. litters	Litter wt	Fetus	
						Live	d Stunted	Dead	d Gross malf.	Resorb.				Wt	Length
14	7 & 8	1	a		116	108	1			8	4				
			b	+38	8.3	7.7				.57	.5	0	39.9	5.1	4.4
			c			93				7	50				
12	11 & 12	1	a		126	18	18			108	54	10			
			b	+10.5	10	1.5	1.5			9.4	4.5		16.3	2.4	2.8
			c			14.3	100			85.7	50	83			
10	15 & 16	1	a		90	11	11			79	60	8			
			b	+ 4.9	9	1.1	1.1			7.9	6		19.5	3.5	3.3
			c			12.2	100			87.8	76	80			
10	18 & 19	1	a		82	8		74			53	7			
			b	+10.4	8.2	.8		7.4			5.3		12.7	4.7	3.9
			c			9.7		90.3			71.5	70			
16	8	2	a		116	90	13			26	12	3			
			b	+29.5	7.2	5.6	.8			1.6			28.4	5.0	3.8
			c			77.5	14.5			22.5	41	18.7			
13	12	2	a		106	3				103	103	12			
			b	+12.2	8.1					7.9	7.9		16	5.3	4.5
			c			2.7				97.3	100	92			
10	15	2	a		86					86	15	10			
			b	- 2.2	8.6					8.6	1.5				
			c							100	17.4	100			

a, total; b, per mother; c, % of total; d, % of living

TABLE V. Lipopolysaccharide *Br. abortus* Bang.

No. of rats	Day of dose	Dose, mg/kg		Mother's wt gain	Uteri total implants	Fetuses					Surviving placentas	Mothers with all res. litters	Litter wt	Fetus	
						Live	d Stunted	Dead	d Gross malf.	Resorb.				Wt	Length
12	11 & 12	10	a		102	31	1	1		70	22	4			
			b	+15.5	8.5	2.6				5.9	1.8		19.2	4.9	4.3
			c			30				68.5	31.4	33.3			
11	7 & 8	20	a		92	92									
			b	+41.0	8.4	8.4							43.7	5.4	4.3
			c			100									
15	11 & 12	20	a		136	15	6			126	76	10			
			b	+15.8	9.0	1				8.6	5.0		12.5	4.1	3.3
			c			11	40			89.0	60	66.6			
10	15	20	a		80	24	1	1	1	54	45	3			
			b	+20.4	8	2.4				5.4	4.5		11.7	4.9	3.8
			c			30				70	83.5	33.3			
12	19	20	a		107	70	27	37			37	1		live 3.7	4.0
			b	+31.2	8.9	5.8	2.2	3.1			3.0		28.0		
			c			65.5	38.5	34.5			100	8.3		dead 2.6	2.5
13	8	30	a		117	11	11			106	19	12			
			b	+ 4.7	9.0	.8	.8			8.0	1.4		30	2.7	3.2
			c			9.5	100			90.5	17.9	92.5			
12	11	30	a		105	0				105	13	12			
			b	+ .7	8.8					8.8	1				
			c			0				100	12.3	100			
10	15	30	a		94	13	13	45		31	45	8			
			b	+ 9.9	9.4	1.3	1.3	4.5		3.1	4.5		19.2	2.9	3.3
			c			13.8	100	48		38.2	50.5	80			

a, total; b, per mother; c, % of total; d, % of living

TABLE VI. Lipopolysaccharide Streptococcus 100 mg/kg i.p.

No. of rats	Day of dose	Dose, mg/kg		Mother's wt gain	Uteri total implants	Fetuses					Mothers with all res. litters	Litter wt	Fetus	
						Live	d Stunted	Dead	d Gross malf.	Resorb.	Surviving placentas		Wt	Length
10	8	100	a		93	93								
			b	+46.5	9.3	9.3					0	49.5	5.3	4.1
			c			100								
15	12	"	a		132	44	13			88	72	6		
			b	+31.2	88	2.9				5.9	48		4.3	3.6
			c			33.2	29.5			66.8	82	40		
13	15	"	a		103	55	12			48	39	2		
			b	+30.0	7.9	4.2				3.7	3		4.8	3.8
			c			54.5	22			45.5	81	15.4		

a, total; b, per mother; c, % of total; d, % of living

ceeded those present after other antimetabolite administration resulting in fetal death. The present experiments demonstrate that the LS of gram negative bacilli can induce fetal death in small doses not affecting the mothers severely. The mode of action is unknown. According to Westphal and Luederitz(5,6,7 and 8), the LS of many gram negative bacteria are similar in composition but differ in the amounts of Lipoid A. This would explain the great variation in toxicity of the LS of the gram negative bacilli and the streptococcus employed here. The present experiments confirm previous observations that *B. abortus* Bang infections(1) can be lethal to the embryos of mammals and explain the recent observation of Kass(3) that untreated *E. coli* bacteriuria in pregnant women results in premature delivery and deaths of fetuses.

Summary. The LD_{50} for rats of LS of *E. typhosa* was 6 mg/kg, for *E. coli* 10 mg/kg, *S. marcescens* 25 mg/kg, *B. abortus* Bang 60 mg/kg, and *Streptococcus* A more than 500 mg/kg. The ratio of lethal dosages of the LS for the litters to the single LD_{50} for the mothers was for *E. typhosa* 1:3, *E. coli* 1:10, *S. marcescens* 1:12, *B. abortus* Bang 1:2, but could not be determined for the gram positive streptococcus. The LS of the above gram negative bacilli led to intraplacental hemorrhages and destroyed the entire litter of rats *in utero* in doses not lethal to the mothers when administered after implantation. The doses employed had a peak of fetus destruction between mid term and the 15th g.d. Surviving fetuses were frequently stunted but gross malformations were rarely detected. Placentas frequently survived the fetuses and stayed *in utero* until term. Large intragastric doses of LS of *E. typhosa* had no effect on mothers and litters, which, if given i.p. would have been lethal.

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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-

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