allow of a choice among these possible explanations.

Incubation with actinomycin of cells that had already developed the characteristic concentration of H^3 uridine into nucleolar RNA leads to a loss of this concentration. This would mean both that the loss of RNA from the nucleolus is not inhibited by actinomycin, and that the synthesis of nucleolar RNA is so inhibited.

Summary. Actinomycin D, in addition to preventing incorporation of uridine into cellular RNA, also prevents the "migration" of previously incorporated radioactive uridine in acid insoluble form from the nucleus to the cytoplasm of HeLa cells.

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Dose-dependent Responses to Endotoxin in the Dog.* (28522)

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The sudden arterial hypotension that develops in the anesthetized dog after intravenous injection of gram-negative bacterial endotoxin is well-documented(1-5). The primary circulatory derangement contributing to this form of hypotension in the dog is the rapid pooling of blood in the hepato-splanchnic bed and concomitant reduction in circulating blood volume(6-8). Since total peripheral resistance is not increased under these circumstances(9,10), reduction in venous return is reflected by decline in systemic arterial pressure. The evidence that this mechanism underlies the early stages of the endotoxin response is convincing(11). The initial depression in arterial pressure is generally followed by a return to near normal levels. If a sufficiently large dose of endotoxin is involved, a progressive hypotension develops that may terminate in death.

This course of events is consistently observed with a variety of gram-negative endotoxins at dose levels exceeding one mg/kg. Experiments reported here extend previous observations to include responses to small, graded doses of the purified lipopolysaccharide (LPS) from *Serratia marcescens*. Systemic arterial pressure, portal venous pressure, blood pH, hematocrit and rate of mortality were measured.

Materials and methods. Twenty-five adult mongrel dogs of both sexes were used (12-21 kg). The animals were divided into 5 equal groups according to dose given. Control group 0 received an injection of normal saline; groups 1, 2, 3, and 4 received, respectively, 0.001, 0.01, 0.10 and 1.0 mg/kg LPS in saline. LPS was prepared by the procedure of Boivin *et al.*(12) from the non-chromogenic Bizio strain (no. 264) of *S. marcescens* grown on a synthetic medium.

Animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Polyethylene catheters were placed in the abdominal aorta and inferior vena cava through the femoral artery and vein. The portal vein was catheterized through a splenic branch vein. Arterial and portal pressures were continuously monitored with Statham pressure transducers and a Sanborn recorder. One mg/kg heparin was administered as needed to maintain catheter patency.

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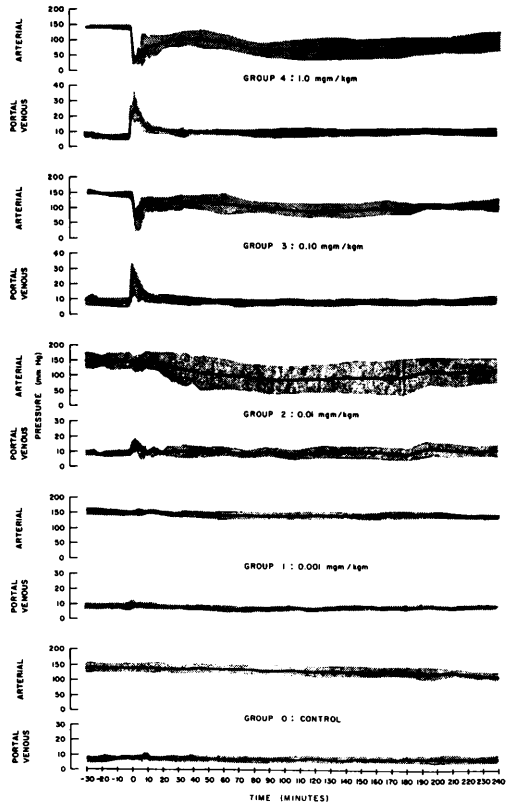

FIG. 1. Portal venous and systemic arterial pressure patterns after injection of *S. marcescens* LPS at 4 dose levels. Heavy line represents mean response of 5 animals; stippled area is ± 1 S.D. Injection made at time 0.

After a 30 minute control period, saline or LPS was injected intravenously and pressure responses were recorded for the following 4 hours. Hematocrit and blood pH determinations were made at specific intervals during control and experimental periods. At the end of experiment catheters were withdrawn and the abdominal incision sutured. No antibiotics were administered. If the animal died within 48 hours, the dose given was considered lethal for that animal.

Results. Systemic arterial and portal venous pressures for each group of animals are illustrated in Fig. 1. Within 1-4 minutes a typically severe portal pressure elevation and arterial pressure depression developed in animals that received 1.0 and 0.1 mg/kg LPS. Pressure changes in animals receiving 0.01 mg/kg were less pronounced, while at the lowest LPS dose (0.001 mg/kg), early circulatory responses were not elicited. These data indicate that the magnitude of the early circulatory responses (portal venous and arterial pressure) after endotoxin depends on the relative quantity of material injected. Log-dose plots describing this relationship are shown in Fig. 2. At all dose levels, however, the pressure alterations were transient. Within 30 minutes after LPS, portal and arterial pressures were at or near levels considered normal for the dog (Table I).

Other alterations which accompany shock induced by endotoxin also depended on dose level. Data describing changes in heart rate reduction (during initial hypotension), acidosis and hemoconcentration, relative to LPS dosage, are given in Table II. Rate of mortality was not consistent, but appeared to parallel dosage. Because no sham-operated animals died, all deaths were attributed to effects of LPS.

Discussion. There are few data available for the dog with which to compare the dose-dependent responses we report. Egdahl(13) has stated that 0.01 mg (total quantity) of purified *E. coli* endotoxin(14) elicited adrenal cortical response, fever, and hypotension

TABLE I. Portal Venous and Systemic Arterial Pressure Alterations Following LPS Administration.

Group No. & LPS dosage (mg/kg)	Portal venous pressure (mm Hg)				Systemic arterial pressure (mm Hg)			
	T_0	Maximum	T_{30}	T_{240}	T_0	Minimum	T_{30}	T_{240}
0; Control	8 ± 1*	9 ± 2 (8)†	7 ± 2	8 ± 2	138 ± 15	137 ± 16(12)	137 ± 17	112 ± 13
1; .001	8 ± 2	10 ± 2 (1)	8 ± 2	9 ± 1	152 ± 8	151 ± 6 (1)	145 ± 8	139 ± 7
2; .01	9 ± 2	15 ± 4 (3)	10 ± 4	11 ± 4	148 ± 27	136 ± 25 (2)	125 ± 52	120 ± 40
3; .10	8 ± 3	28 ± 6 (1)	10 ± 3	10 ± 3	142 ± 13	61 ± 33 (3)	119 ± 26	110 ± 21
4; 1.0	7 ± 2	26 ±10 (4)	10 ± 2	10 ± 3	145 ± 8	28 ± 4 (2)	101 ± 24	95 ± 21

* Values are means ± 1 standard deviation; 5 animals per group.

† Numbers in parentheses are min after injection at which maximum or minimum pressures were recorded.

TABLE II. Changes in Parameters Typically Associated with Endotoxin Shock According to LPS Dose Level.

Group No. & LPS dosage (mg/kg)	Heart rate (beats/min)				Blood pH				Hematocrit (%)				Mortality (deaths /total)
	T_0	Minimum (0-15 min)	T_{30}	T_{240}	T_0	Minimum (0-240 min)	T_{30}	T_{240}	T_0	Maximum (0-240 min)	T_{30}	T_{240}	
0; Control	174 ± 35*	172 ± 37	176 ± 42	176 ± 44	7.33 ± .04	7.33 ± .04	7.34 ± .04	7.35 ± .06	46 ± 5	49 ± 4	46 ± 6	49 ± 4	0/5
1; .001	182 ± 31	182 ± 28	180 ± 24	188 ± 29	7.30 ± .04	7.30 ± .04	7.30 ± .04	7.31 ± .05	46 ± 9	47 ± 8	45 ± 8	47 ± 8	1/5
2; .01	176 ± 27	174 ± 29	172 ± 40	148 ± 59	7.34 ± .03	7.21 ± .12	7.32 ± .06	7.22 ± .15	48 ± 5	52 ± 3	49 ± 5	51 ± 2	3/5
3; .10	182 ± 27	144 ± 20	162 ± 26	188 ± 32	7.28 ± .05	7.21 ± .04	7.21 ± .04	7.23 ± .09	45 ± 10	48 ± 8	46 ± 9	47 ± 8	2/5
4; 1.0	138 ± 34	88 ± 40	124 ± 32	155 ± 48	7.32 ± .05	7.15 ± .11	7.15 ± .11	7.21 ± .05	38 ± 8	52 ± 6	43 ± 6	52 ± 6	4/5

* Values are means ± 1 standard deviation; 5 animals per group.

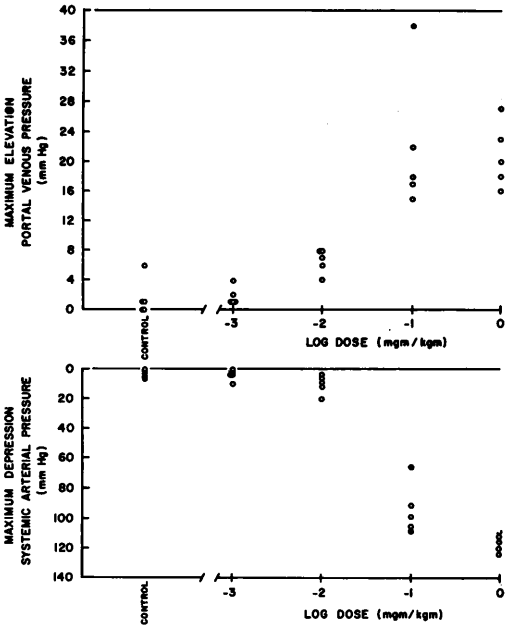

FIG. 2. Maximum portal pressure elevation (upper) and systemic arterial pressure depression (lower) as functions of log-dose *S. marcescens* LPS. Each point represents maximum response for one animal during the first 15 min following injection.

without death, and that 0.2 mg (total quantity) represented the maximum dose tolerated without a fatality greater than 10%. The quantities of endotoxin used in his experiments correspond to approximately 0.0007 and 0.013 mg/kg respectively. Although we detected no hypotension with 0.001 mg/kg of *S. marcescens* LPS, 0.01 mg/kg was fatal in 3 of 5 animals. Davis *et al.* (15) reported a maximum portal pressure rise of 145% and a minimum value for arterial pressure of 40% of the control level in dogs given one mg/kg *E. coli* endotoxin also prepared according to Spink(14). With *S. marcescens* LPS at the same dose level, we observed a maximum rise in portal pressure of 370% and an arterial pressure decline to 20% of the control value. Although these comparisons suggest that *S. marcescens* LPS is more potent than the endotoxin prepared from *E. coli*, differences observed may result from other factors. Specific strain of organism, culture medium, method of isolation and purification procedure may well contribute to the potency of the final product.

Although acute hypotension was induced regularly with 0.10 mg/kg LPS, only 2 of 5 animals died, suggesting that the reduction in arterial pressure *per se* does not involve irreversible processes. Death probably results from processes occurring after the initial phase of the reaction. A current hypothesis for which considerable evidence has been presented maintains that the endotoxin response is mediated predominantly through the action of both endogenous and newly-synthesized histamine(16-19). The present data suggest that the endotoxins can exert a mass action effect with respect to liberation of histamine. It has been demonstrated that histamine is able to induce a transient hypotension related to dose level(20). Small quantities of endotoxin may effect a low-level release of histamine. Larger quantities may stimulate both a generalized release and histidine decarboxylase activation(17,19). The latter condition combined with the metabolic impairments induced by the endotoxins at the cellular level(21,22) may represent the factors constituting its toxicity.

Summary. Dogs injected with *S. marcescens* lipopolysaccharide at graded dose levels between 0.01 and 1.0 mg/kg responded with characteristic sudden portal pressure elevations and arterial hypotension. The magnitude of these alterations and of the acidosis and hemoconcentration which ultimately develop in endotoxin treated animals were positively related to dose level. At doses less than 1 mg/kg no immediate relationship was evident between initial circulatory response and mortality rate. This suggests that the initial circulatory alterations which may result from the histamine-releasing properties of endotoxin can be reversed. The lethal property of endotoxin may reside in the secondary irreversible impairments it induces.

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