Other dog viruses, infectious canine hepatitis, canine herpesvirus and reovirus did not produce giant cells in DL cultures. The conclusion was made, therefore, that giant cell formation was induced by CDV and the method for assay of virulent virus in dog tissues had been demonstrated. Rapidity of giant cell formation in cultures prepared from infected dogs indicated that this process may occur *in vivo* and giant cells have been reported associated with distemper.

Summary. Cell cultures prepared from dog lungs without trypsinization contained viable lung macrophages. When such cultures were inoculated with CDV, giant cells were seen 2 to 6 days after inoculation. When virulent CDV was titrated in DL cell cultures and in dogs, comparable endpoints were obtained. Giant cell formation in lung macrophage cultures, therefore, permitted demonstration and assay of virulent CDV.

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Experimental Canine Endotoxin Shock: Failure to Correlate Outcome With Persistent Endotoxemia.* (32510)

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Although studies on experimental canine endotoxin shock have yielded valuable information on the pathogenesis of this form of shock there is a lack of knowledge on the distribution of endotoxin in the tissues and body fluids during the course of shock. The present investigation was concerned with correlating the persistence of endotoxemia with recovery or death. Observations in man have suggested that the detection of endotoxin in the circulating blood can have diagnostic and prognostic values(1,2).

It was previously observed upon rapidly injecting a bolus of endotoxin intravenously into adult dogs that a precipitous drop in systemic blood pressure occurred within 30 to 60 seconds(3). Within the following 60 minutes partial recovery of the blood pressure occurred and hemodynamic stability often lasted up to several hours before death ensued. A fatal outcome was related to renal failure and metabolic acidosis. Since no information was available on the duration of endotoxemia in the dog serial blood determinations for endotoxin were carried out using a bioassay, postulated as specific for endotoxin.

Materials and methods. Adult mongrel dogs were anesthetized with 30 mg/kg sodium pentobarbital and maintained lightly anesthetized during the course of the experiments. A catheter was placed in the lower aorta of each of 10 dogs through the femoral artery, and coupled to a transducer for continuous monitoring and recording of blood pressures. All injections were made into a catheter in-

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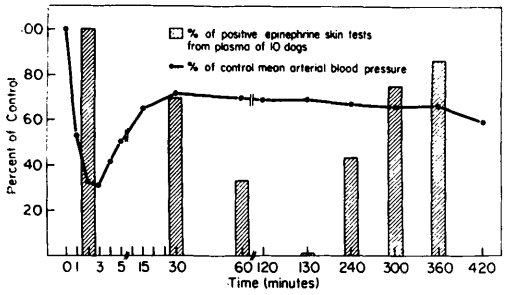

FIG. 1. Results of epinephrine rabbit skin tests during course of canine endotoxin shock.

troduced into the inferior vena cava through the femoral vein.

Escherichia coli endotoxin of the Boivin type was prepared in our laboratory and injected rapidly in a dose of 0.75 mg/kg(4). Plasma samples from whole aortic blood in heparin were collected prior to the injection of endotoxin; during the initial period of hypotension (1-3 minutes post-endotoxin); during recovery from hypotension (30-60 minutes post-endotoxin); hourly during hemodynamic stability; and during the terminal hypotensive period (6 hours post-endotoxin).

Endotoxin in the plasma samples was detected by means of the rabbit epinephrine skin test(5). Five to 6 ml of canine plasma was injected intravenously into New Zealand albino rabbits, and simultaneously, 50 μg of epinephrine was introduced intradermally. The test was considered positive for endotoxin if an area of hemorrhagic necrosis of 20 mm or more was observed within 24 hours at the site of the epinephrine injection. Each rabbit was used only once for a single specimen of plasma.

Results. The mean arterial blood pressures over the course of 7 hours for all 10 dogs are presented graphically in Fig. 1. The pressure declined to 31% of the control value within 3 minutes after injection of endotoxin and then gradually rose to 72% in 30 minutes, remaining at 66-72% for 6 hours. The pressure then gradually declined to 59% of the control value. The mortality rate was 60%, 4 of the 10 dogs surviving longer than 72 hours.

The results of the epinephrine skin tests in each of the rabbits receiving plasma from dogs before and after the injection of endotoxin are shown in Table I. All rabbits infused with canine plasma obtained before the administration of endotoxin yielded negative epinephrine skin tests, whereas the plasma of all 10 dogs gave positive reactions within 3 minutes after the injection of endotoxin. Plasmas drawn from 10 dogs during the recovery period (30 minutes) gave 7 positive tests and 3 negative. Thereafter there was a steady decline in the number of dogs with plasmas giving positive tests. Three hours post-endotoxin 7 dogs still surviving had plasmas with negative reactions. However, at 4 hours, 3 of 7 canine plasma samples gave positive rabbit skin tests; at 5 hours, 3 out of 4 were positive; and at 6 hours, 6 out of 7 produced positive reactions. The positive epinephrine rabbit skin tests are correlated with the mean arterial blood pressure of the dogs over a period of 7 hours in Fig. 1.

All plasma samples obtained from 6 of the animals just before death showed positive tests. Three of the 4 animals surviving 72 hours or longer had plasmas that gave a positive test 6 hours post-endotoxin.

Discussion. The intradermal epinephrine

TABLE I. Results of Rabbit Bioassay for Endotoxin in Canine Plasma.

Dog No.	Time post-endotoxin (min) 0	1	2	3	30	60	180	240	300	360	Length of survival (hr)
1	0	+			0	0	0			0	>72
2	0			+	+	+		+			5
3	0		+		0		0	+			4½
4	0		+		+	0		+			>72
5	0	+			+	0	0	0		+	>72
6	0		+		+	0	0		+	+	>72
7	0			+	0	+	0		+	+	<24
8	0			+	+	+	0	0	+	+	<72
9	0			+	+	0		0	0	+	<48
10	0		+		+	0	0	0		+	8¾

reaction in rabbits demonstrated the presence of endotoxin in the plasmas of all 10 experimental dogs during the period of hypotension immediately following the injection of endotoxin. It has been demonstrated previously that there was a marked rise in plasma epinephrine at the nadir of the initial hypotension(6). It is possible that this initial rise in plasma catecholamine might have augmented the intensity and uniformity of the dermal necrosis in rabbits following the local injection of epinephrine. As the arterial blood pressure became stabilized following this initial period of hypotension there was a decline in the incidence of detectable endotoxin in the plasma of surviving animals so that none was demonstrated at 3 hours post-endotoxin. It is at this point that endogenous epinephrine recedes also. There was a reappearance of endotoxin between 4 and 6 hours, at the end of which time there was a fall in systemic blood pressure. Terminal hypotension is also associated with a rise in plasma catecholamine. However, there was no correlation between the reappearance of endotoxin and the ultimate recovery or death of the animals. It is unlikely that the levels of plasma epinephrine have any relationship to the results of the rabbit skin tests.

The specificity of epinephrine dermal necrosis for endotoxin has been open to some question although the present results appear to confirm its value as a biological assay. Persistent endotoxemia has been demonstrated in the rabbit using Cr^{51}-labelled endotoxin(7). For 6 hours following a single injection of labelled endotoxin, a toxic factor compatible with endotoxin was identified in plasmas(8). In studies from this laboratory to be reported elsewhere(9), Cr^{51}-labelled endotoxin persisted in the blood of dogs, and neither the duration of the endotoxemia nor the concentrations of endotoxin in the plasma could be correlated with recovery or death.

Endotoxemia in human patients should be explored more intensively. Other possible biological assays for circulating endotoxin include the use of the 10-day chick embryo. We have confirmed the highly lethal effect of small amounts of endotoxin injected into the embryo(10). The basic significance of endotoxemia in the pathogenesis of shock should be more readily defined when the toxic factor of endotoxin has been elucidated chemically.

Summary. Endotoxin was detected serially in the circulating blood of dogs with a bioassay by injecting canine plasma intravenously into New Zealand albino rabbits and simultaneously 50 μg epinephrine intradermally. The presence of rabbit dermal necrosis 24 hours later indicated the presence of endotoxin in the canine plasma. Prior to the injection of endotoxin all canine plasmas gave a negative reaction. Within 3 minutes post-endotoxin all plasmas showed a positive test. At 3 hours when the blood pressures had stabilized in 7 surviving animals no endotoxin was in the plasmas. However at 6 hours endotoxin was detected in the plasma of 6 out of 7 surviving animals. The persistence of endotoxin in the circulating blood could not be correlated with survival or death.

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