

Hemofiltration Reverses Left Ventricular Dysfunction during Sepsis in Dogs

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Depressed left ventricular (LV) contractility in sepsis has been ascribed to the presence of a circulating cardiodepressant substance (filterable cardiodepressant factor in sepsis [FCS]); however, this finding is controversial. The authors hypothesized that if a decrease in LV contractility indeed occurred due to a circulating depressant substance, then removal of this substance by hemofiltration would reverse this dysfunction. In this study, LV mechanics were examined before and after hemofiltration in anesthetized dogs during continuous intravenous infusion of live *Escherichia coli*. Left ventricular anterior-posterior and apex-base dimensions were measured by subendocardial ultrasonic crystal transducers implanted 4 weeks before the experiments. Left ventricular contractility was determined from the end-systolic pressure-dimension relationship. The slope of this relationship (E_{max}) is an index of contractility. After 4 h of sepsis, E_{max} was reduced by one half. Hemofiltration resulted in a return of E_{max} to control values. The FCS activity in the plasma was also assessed by the percent reduction in isometric contraction of electrically stimulated, isolated right ventricular trabeculae obtained from nonseptic dogs. The FCS activity reached a peak 4 h after sepsis and was reduced after 2 h of hemofiltration. The results show that during experimental sepsis, a circulating substance of less than 30,000 d produces a decrease in LV contractility and that this LV dysfunction may be improved by hemofiltration. (Key words: Heart; contractility; endotoxin; hemofiltration; myocardial depressant factor; shock.)

ALTHOUGH A DECREASE in left ventricular (LV) contractility has been implicated by some investigators as an important cause of circulatory collapse in gram-negative sepsis,¹⁻⁵ this has not been a universal finding.^{6,7} The question of whether left ventricular systolic dysfunction occurs in sepsis remains controversial. Several proponents of depressed myocardial function in sepsis have attributed this to a filterable, circulating myocardial depressant factor of small molecular weight.⁸⁻¹⁴ Lefer and others⁹⁻¹⁴ have suggested, on the basis of research using animal models

of shock, that the myocardial depressant factor is a small molecular weight peptide. However, in sepsis, the existence of such a substance is controversial and has not been substantiated by several other investigators.^{2,3}

In the current study, we hypothesized that if a decrease in LV contractility indeed occurred in sepsis due to the presence of a small molecular weight cardiodepressant substance, then removal of this substance by continuous arteriovenous hemofiltration (CAVH) might reverse LV impairment. Hemofiltration is an extracorporeal blood treatment in which blood is allowed to flow through a hemofilter primarily due to the arterial-venous pressure gradient.¹⁵ The membrane used in the hemofilter effectively permits the removal of small and medium-sized plasma solutes (see Methods) while holding back protein and blood cells. Thus, if hemofiltration improved LV dysfunction in sepsis, this would not only provide further evidence that a small molecular weight, filterable substance is a cause of myocardial dysfunction in sepsis but would provide a potentially clinically applicable method for reversing this dysfunction. In this study, we tested this hypothesis in dogs in which sepsis was induced by continuous *Escherichia coli* infusion.

Methods

PROTOCOL

These studies were performed in accordance with protocols approved by the University Animal Care Committee. Three groups of dogs (25-30 kg) were studied, a sepsis group and two control groups. The control groups included a hypotensive control group and time control group (see below). In the sepsis group ($n = 8$), measurements (see *in vivo* measurements) were made at four time periods: baseline (presepsis), 1 h postsepsis, 4 h postsepsis, and posthemofiltration. Baseline measurements were made over a ¾-h period. Sepsis was then induced by iv infusion of 10^{10} colony forming units (CFU) of live *E. coli* (0111:B4). The bacteria were suspended in normal saline solution and given over 1 h, after which measurements were repeated (1 h postsepsis). A constant infusion of approximately 5×10^9 CFU/h of *E. coli* was then maintained both for the remainder of the 4-h period (4 h postsepsis) and during hemofiltration (posthemofiltration). The total dose of *E. coli* given was one that was normally lethal.¹

In each of the above four conditions, the animal was studied at a LV end-diastolic pressure (LVEDP) of about

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15 mmHg. Six percent hetastarch in normal saline solution was used as the volume expander. Approximately 20 ml of the solution per kg body weight was used to attain LVEDP to 15 mmHg during baseline measurements. After 1-h postsepsis measurements, intravascular volume was removed to reduce LVEDP to about 5 mmHg, at which time mean systemic blood pressure (MAP) decreased to about 100 mmHg. The animal was maintained in this relatively hypotensive state for about 3–4 h without treatment, during which a small amount of iv fluid was infused at a rate of $1 \text{ cc} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Furthermore, the blood-hetastarch mixture that was removed from the animal was stored in plastic bags (Cutter, Ltd, CPDA-1 unit) for later reinfusion to maintain Hct relatively constant throughout the study.

After a period of sepsis of about 4 h, the animal was transfused with the blood-hetastarch mixture to obtain an LVEDP of 15 mmHg. Additional hetastarch solution was given if necessary. Hemofiltration (Gambro, CAVHkit AV55) was then performed for 2–3 h, during which time approximately 1.5–2 l of hemofiltrate was removed. (The hemofilter permits all solutes up to a molecular weight of about 4,000 to be removed at the same rate as the plasma water.) Molecules up to about 30,000 d were also allowed to be filtered, albeit at a slower rate. The hemofilter was placed between a femoral artery and a vein. A flow rate of 2–3 l/min was maintained with a roller pump (Cobe Perfusion System, Lakewood, CO). The animal was heparinized (3 ml: 1,000 units/ml) immediately prior to hemofiltration, and the fluid lost by hemofiltration was replaced with Ringer's solution infused intravenously. Measurements were again performed with an LVEDP of about 15 mmHg.

In the hypotensive control group ($n = 5$), hypotension was induced by the iv infusion of hydralazine, which decreases MAP primarily by arteriolar vasodilation¹⁶ (see "Discussion"). Baseline measurements at an LVEDP of about 15 mmHg were obtained in an identical manner to those in the sepsis group. Intravascular volume then was reduced so that LVEDP was again about 5 mmHg. An iv bolus of hydralazine was given to reduce MAP to about 110 mmHg, which is similar to that observed in the sepsis group. Hydralazine was then infused iv for 4 h to maintain this degree of hypotension, after which measurements were again obtained at an LVEDP of 15 mmHg. The dose of hydralazine differed between dogs and was chosen to cause hypotension equivalent to that observed in the sepsis group. Also note that hydralazine was given in the low LVEDP state (after blood had been removed following the baseline measurements) to induce hypotension in the volume-depleted state similar to that which occurred in the sepsis group. Therefore, 1-h posthypotension measurements at high hydration could not be performed without reinfusion of volume. Since we wanted the three groups to share similar blood removal

and infusion times, 1-h measurements were not obtained in this group and only three conditions were examined: baseline, 4 h posthypotension, and posthemofiltration.

In the time control group ($n = 9$), blood volume was reduced and restored at similar time intervals as in the sepsis and hypotensive groups. In this group, because we were mainly interested in the effect of time and the effect of hemofiltration, 1-h measurements were not obtained and three conditions were examined: baseline, 4 h postbaseline, and posthemofiltration.

ANIMAL PREPARATION

One month prior to the study, surgery was performed on the animal to implant pairs of subendocardial, ultrasonic crystal transducers (piezoelectric crystals, hemispherical ceramic transducers, Channel Industries, Santa Barbara, CA) along the anterior–posterior (AP) and apex–base (AB) axes of the left ventricle to be used later for recording LV dimensions.^{17–20} Implantation was performed with the animal anesthetized (30 mg/kg of pentobarbital sodium), and the lungs were mechanically ventilated with gas having an inspired oxygen concentration of 50%. Under sterile conditions, a left thoracotomy incision was made. The pericardium was split. AP and AB crystal pairs were placed in the LV subendocardially as previously described.^{17,18} The pericardium was sutured, and the chest wall incision was closed. Wires connected to the crystal transducers were brought out through the thoracotomy incision and tunneled subcutaneously to exit at the neck. The animal was treated with antibiotics for 6 days (cloxacillin and gentamicin). All animals had fully recovered at the time of study.

IN VIVO STUDY

Initial Procedures

On the day of the study, the dogs (20–35 kg) were anesthetized with pentobarbital (30 mg/kg) and paralyzed with succinylcholine (40 mg). Following tracheal intubation, the lungs were mechanically ventilated (Harvard Apparatus, S. Natick, MA). The ventilator settings were adjusted to maintain arterial P_{CO_2} at about 35 mmHg and $p\text{H}$ at 7.35. The ventilation rate was usually about 12 breaths per min, and the tidal volume was about 15 ml/kg. The rate was altered during the course of the experiment to correct for acidosis or alkalosis. All *in vivo* measurements were made at end-expiration (*i.e.*, functional residual capacity) with the animal in the supine position.

Vascular catheters needed for the study were inserted under sterile conditions. An arterial catheter was placed in the left femoral artery for obtaining arterial oxygen samples for P_{O_2} , P_{CO_2} , $p\text{H}$, and blood pressure measurement. A 4-ml Fogarty catheter was inserted into the left femoral vein and positioned in the inferior vena cava such

that venous return was maximally reduced when the balloon was inflated. This catheter was later used for obtaining the end-systolic pressure dimension relationship (see below). The right femoral artery and vein were cannulated with polyethylene catheters (0.22 cm ID) for connecting to the hemofilter. Another polyethylene catheter was placed in one jugular vein for the infusion and removal of blood as required. A thermistor-tipped catheter was advanced into the pulmonary artery to measure mean pulmonary artery pressure (Pap), mean pulmonary capillary wedge pressure, (Pwp) and cardiac output (CO), which was measured by the thermodilution technique (Columbus Instruments, OH). Another catheter was inserted into the right atrium for injecting cold saline boluses used in the CO determination and for measuring right atrial pressure (Rap). All of the fluid-filled catheters used for vascular pressure measurements were connected to transducers (Statham, Hato Rey, PR) and were referenced relative to the left atrium. Transducer outputs and all signals were displayed on an eight-channel Hewlett-Packard recorder (Palo Alto, CA).

A high-fidelity, transducer-tipped catheter (Millar Instruments, Houston, TX) was advanced into the LV through a carotid artery for measuring LV pressures. The maximal rate of increase and decrease of the LV pressure (+ or - dp/dt) was obtained by electronic differentiation.

In Vivo Data Analysis

The ultrasonic crystal transducer method of measuring ventricular dimensions has been detailed elsewhere.¹⁷⁻²⁰ The wires from the crystal pairs were attached to a sonomicrometer (Sonomicrometer 120, Triton Technology, San Diego, CA), and the outputs displayed on a recorder. According to well-described criteria, LV end-diastolic dimension (EDD) was defined by LVEDP, which in turn was defined as the pressure at which +dp/dt increased by 150 mmHg/s with the increase sustained for at least 50 ms when no A wave was present at higher heart rates.²⁰ End-ejection dimension (EED) was defined by maximum negative dp/dt, a point when flow across the aortic valve had ceased.^{20,21}

The end-systolic pressure dimension analysis was used to define contractility in the intact dog.^{19,22,23} This analysis was used in two ways. Changes in contractility between conditions were assessed not only by the slope of the end-systolic pressure–dimension relationship (E_{\max} ; see below) but also by the change in end-systolic dimension at a given end-systolic pressure of 120 mmHg. The latter pressure was chosen because it was the highest end-systolic pressure usually obtained during sepsis. Thus, changes in E_{\max} and a single point on the relationship could be compared between conditions. Together, these two indices would provide an accurate description of the changes in ventricular function that occurred during the course of the study.²⁴

In terms defined by Sagawa,²² end-systole is the point in the cardiac cycle at which the ratio of ventricular pressure to dimension reaches its highest value (maximal elastance). From the maximal pressure–dimension coordinates examined over multiple beats of different dimensions and pressures, a linear relationship can be obtained: $Pes = E_{\max} (Des - Do)$, where Pes is end-systolic pressure, Des is end-systolic dimension, Do is the extrapolated dimensional intercept, and E_{\max} is the slope of the relationship and defines contractility. Do is not changed by the contractile state of the heart but may be influenced by changes in the resistance and capacitance properties of the peripheral circulation²⁵ (see Discussion).

Sagawa *et al.*^{22,23} indicated that at least three pressure–dimension coordinates are necessary to construct the E_{\max} relationship. Accordingly, this was the minimum number we obtained during each condition. To obtain these multiple coordinates, the balloon positioned in the inferior vena cava was rapidly inflated with normal saline, which produced transient venous occlusion with a progressive fall in left ventricular end-systolic pressure and dimension. Venous occlusion was performed during end-expiration and took about 4 s. Linear regression analysis of the end-systolic pressure–dimension coordinates that were generated was then used to determine E_{\max} . Although we used linear rather than volume dimensions to determine E_{\max} , others have shown that both pressure–dimension and pressure–volume relationships show similar changes in response to global alterations in ventricular contractility.^{23,26} Furthermore, because end-systolic relationship may be slightly curvilinear at the extremes of ventricular pressure,²⁷ end-systolic dimensions in all cases were compared over a similar range of end-systolic pressures during the respective conditions.

In the sepsis and two control groups, each full set of measurements at each study condition consisted of arterial blood gas measurements, central hemodynamics (Rap, Pap, MAP, stroke volume [SV], and systemic vascular resistance [SVR]) and LV mechanics (LVEDP and LV end-systolic, end-ejection, and end-diastolic dimensions). Stroke volume was calculated as CO/heart rate, and SVR was calculated as (MAP-Rap)/CO.

ADDITIONAL *IN VIVO* MEASUREMENTS

Following these initial studies, subsequent measurements were performed in additional groups of dogs to examine whether changes in serum ionized calcium or pentobarbital concentrations between conditions could explain the changes in E_{\max} due to hemofiltration in our initial sepsis group (see Results). In five dogs (*i.e.*, additional sepsis group), sepsis was produced in the identical manner as in our initial sepsis group, and measurements of serum ionized calcium and pentobarbital concentrations were additionally obtained at baseline, 4 h postsepsis,

and posthemofiltration conditions. Moreover, in a second group of five dogs, the changes in E_{\max} were followed in which no hemofiltration was performed between 4–6 h of sepsis (sepsis hemofiltration and time control group). This latter group would therefore control for the 4- to 6-h period in which hemofiltration was performed in our initial sepsis group. In the sepsis hemofiltration and time control group, pentobarbital and serum ionized calcium concentrations were also examined in the above three conditions.

In these two additional groups, after the initial bolus of pentobarbital was given to anesthetize the animal, supplemental pentobarbital was administered according to the following protocol to maintain relatively constant levels throughout the experiment. The elimination half-life of pentobarbital was considered to be 8 h based on the work of Frederiksen *et al.*²⁸ Since the experiment lasted about 8 h, the additional dose of pentobarbital would consist of one-half of the initial anesthetizing dose. The latter dose was administered over the time-course of the experiment and was given in three equally divided doses approximately every 2½ h from the initiation of the experiment. These additional doses were slowly infused iv (over 10 to 15 min) and were administered at least ¾ h before measurements were obtained in a given condition to ensure a steady-state level. Pentobarbital concentrations were measured by high performance liquid chromatography.²⁸

Also, in these two additional groups, serum albumin concentrations were measured to account for dilutional changes of pentobarbital between conditions that may affect interpretation of the results. Because dilutional changes occurred mainly between the baseline and 4-h postsepsis periods (see Results), the concentration of pentobarbital that was unbound to plasma constituents was also estimated in these conditions as a more precise index of the level of anesthesia. In the baseline and 4-h postsepsis conditions, plasma was passed through an Amicon filter with 1000-d cut-off, and the concentration of pentobarbital in the ultrafiltrate was measured to represent the unbound portion. The ratio of unbound to total serum pentobarbital could therefore be estimated. Otherwise, the protocol and methodology in these two groups were the same as in the three initial groups, except that an open-chest, open-pericardium preparation was used in which only the AP crystal pair was implanted to determine LV dimensions.¹⁷ Also iv fluids were slightly increased from 1 ml · kg⁻¹ · h⁻¹ to 3 ml · kg⁻¹ · h⁻¹ between the 1- and 6-h measurement periods in these additional groups.

IN VITRO MEASUREMENTS

An *in vitro* ventricular preparation was used to assess the presence of a filterable cardiodepressant factor in sep-

sis (FCS). The FCS activity was examined in the initial three groups (sepsis group, and hypotensive and time control groups). The trabecular preparation used was similar to that previously described.²⁹ Mongrel dogs (3 to 10 kg) were anesthetized with pentobarbital. The hearts were removed, flushed with 50 ml of cold Krebs-Henseleit solution (KH), and placed in ice-cold oxygenated KH. Three to four thin trabeculae (<1 mm diameter) obtained from the right ventricle were tied at each end with 6–0 silk thread. Each thin muscle was suspended in a vertical constant temperature bath (10 ml) containing KH (in mM: NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.4; NaHCO₃, 25; and dextrose, 11). The solution was gassed with 95% O₂ and 5% CO₂, and maintained at 37° C. Isometric contractions at optimum length were recorded with a force transducer (Grass FT03C) connected to a polygraph (Grass Model 7). The muscle was stimulated electrically *via* platinum punctate bipolar electrodes with rectangular pulses (1 ms duration) at an intensity of 50% above threshold delivered at intervals of 2,000 ms.

Plasma samples were studied after an initial equilibration period of 60–90 min. Each muscle was used to determine the depressant activity of plasma obtained after one experimental condition. Trabeculae for studying all of the plasma samples from each control or septic dog came from the same animal. The percent change in isometric contraction amplitude from control caused by each plasma sample was calculated as an index of FCS in each condition. In each of the three groups, the plasma samples were obtained at the same LVEDP in the respective conditions to prevent changes in FCS due to dilution alone. The plasma was stored at –25° C and usually assayed within 1 to 3 days. Plasma samples from all study conditions (four study conditions in a septic dog or three conditions in a control dog) were studied simultaneously. The experimenter determining the depressant activity of the plasma sample was blinded regarding the source of a particular sample. An 0.5-ml plasma sample was used for the assay because in preliminary experiments it was observed that this quantity of plasma, obtained from animals septic for 4 h, produced a near-maximal decline in developed tension.

Statistical analyses used included a one- and two-way analysis of variance (ANOVA), the Student-Newman-Keuls (SNK) multiple comparison test, an unpaired *t* test, and Bonferroni's corrected paired *t* test.³⁰ The specific tests employed are indicated in the legends to the figures and tables.**

** In the sepsis group, two of the eight dogs (dog 1 because of equipment failure, and dog 8 because of hemodynamic instability) could not be studied posthemofiltration. Because two missing values posthemofiltration would make ANOVA analysis complicated and since we were primarily interested in the effect of hemofiltration on 4 h of

TABLE 1. Hemodynamics in Initial Sepsis Group (Mean \pm SD)

Parameter	Baseline (n = 8)	1 h Postsepsis (n = 8)	4 h Postsepsis (n = 8)	Posthemofiltration (n = 6)
MAP	155 \pm 27	152 \pm 29	120 \pm 29*†	110 \pm 12
LVESP	168 \pm 21	155 \pm 19	124 \pm 14.0*†	115 \pm 14
LVEDP	15.6 \pm 5.8	14.6 \pm 5.3	14.4 \pm 5.6	11.9 \pm 4.1
SV	41 \pm 7.0	44 \pm 10	40 \pm 11.3	45 \pm 6.9‡
HR	173 \pm 16	178 \pm 17	167 \pm 20	158 \pm 25
Pap	25.3 \pm 4.8	25.1 \pm 5.2	23.7 \pm 5.1	24.6 \pm 7.4
SVR	22.1 \pm 5.9	18.9 \pm 3.2	19.3 \pm 7.9	15.7 \pm 4.0
CO	7.0 \pm 1.3	7.8 \pm 1.5	6.4 \pm 1.6	6.9 \pm 1.6
Rap	5.6 \pm 1.2	6.0 \pm 2.1	6.7 \pm 1.1	7.5 \pm 1.4

MAP = mean systemic blood pressure (mmHg); LVESP and LVEDP = left ventricular end-systolic pressure and end-diastolic pressure, respectively (mmHg); SV = stroke volume (ml); HR = heart rate (beats/min); Pap = mean pulmonary artery pressure (mmHg); SVR = systemic vascular resistance (mmHg/l/min); CO = cardiac output (l/min); Rap = mean right atrial pressure (mmHg).

* $P < 0.01$ from baseline, † $P < 0.01$ 1 h vs. 4 h; by ANOVA and Student's–Newman Keuls (SNK) multiple comparison test. ‡ $P < 0.01$ 4 h vs. posthemofiltration by Bonferroni corrected paired t test. (See footnote **.)

Results

Table 1 shows the mean results of the hemodynamic measurements obtained in the initial sepsis group. At 4 h postsepsis, MAP decreased about 25% from baseline, despite similar LVEDP between conditions. Hemofiltration did not restore MAP. Table 2 shows the hemodynamic measurements obtained in the time control and hypotensive control groups, respectively. In the hypotensive control group, MAP was maintained at a level similar to that observed in the sepsis group at comparable conditions. There was no change in MAP in the time control group between conditions.

Figures 1 and 2 show the E_{\max} values. In figure 1, results from two representative dogs in the initial sepsis group are shown. As previously indicated, end-systole was defined as the point at which ventricular pressure to dimension reached its maximum value (*i.e.*, maximal elastance).^{19,22–23} From multiple maximum pressure–dimension coordinates obtained during venous occlusion, linear regression analysis was used to determine the slope of the relationship (E_{\max}) that defines contractility. In both dogs in figure 1, at 1 h postsepsis the slope of the relationship (E_{\max}) was unchanged compared with baseline. However, at 4 h postsepsis, E_{\max} was reduced compared with baseline. This decrease in E_{\max} at 4 h postsepsis indicates a decrease in contractility. The end-systolic relationship was shifted upward and to the left after hemofiltration, indicating an increase in E_{\max} and an improvement in contractility after hemofiltration.

sepsis, results between 4 h postsepsis and posthemofiltration were compared by Bonferroni's corrected paired t test. The first three conditions were compared by ANOVA, and a Bonferroni correction was used in the paired t test analysis. Since the 4-h postsepsis results were used twice, the critical T value was corrected for the additional comparison so that a value reported as $P < 0.05$ was less than 0.025 before the Bonferroni correction (see ref. 30).

In all conditions, the data yielded linear relationships with consistently high R^2 values (coefficient of determination, *i.e.*, square of the correlation coefficient). In the initial sepsis group, the R^2 (mean \pm SD) for the AB crystal pair was 0.95 ± 0.06 at baseline, 0.92 ± 0.11 at 1 h postsepsis, 0.90 ± 0.09 at 4 h postsepsis, and 0.86 ± 0.17 posthemofiltration. For the AP crystal pair, the respective R^2 values were 0.96 ± 0.03 vs. 0.92 ± 0.08 vs. 0.87 ± 0.12 vs. 0.90 ± 0.08 . Values of R^2 for the other two study groups were similar to these in all study conditions.

The mean E_{\max} values in the initial sepsis group are shown in figure 2 (upper panel). By 4 h postsepsis, E_{\max} decreased relative to presepsis in all dogs in both crystal pairs. After hemofiltration, E_{\max} was increased in all dogs in both crystal pairs compared with 4 h postsepsis, reaching values similar to baseline values. From the middle and bottom panels in figure 2, it can be seen that mean E_{\max} values in the two control groups were unchanged between conditions. The changes in E_{\max} from baseline to 4 h postsepsis and from 4 h postsepsis to posthemofiltration were significantly different from the changes in E_{\max} over respective intervals in the control groups.

In the respective groups, end-systolic dimensions were also compared between conditions at an end-systolic pressure of ~ 120 mmHg (the mean highest pressure obtained during sepsis). The relative changes in end-systolic volume between conditions for the three groups are shown in figure 3 (see legend to fig. 3 for calculation of end-systolic volume). As shown in figure 3, relative end-systolic volume increased after 4 h of sepsis and decreased posthemofiltration in the sepsis group, and the results in figure 3 are consistent with the E_{\max} results in figure 2.

The increase in E_{\max} in the initial sepsis group between 4 h postsepsis and posthemofiltration was not due to changes in serum ionized calcium or pentobarbital concentrations between these conditions. In the additional sepsis group, E_{\max} along the AP dimension, which was

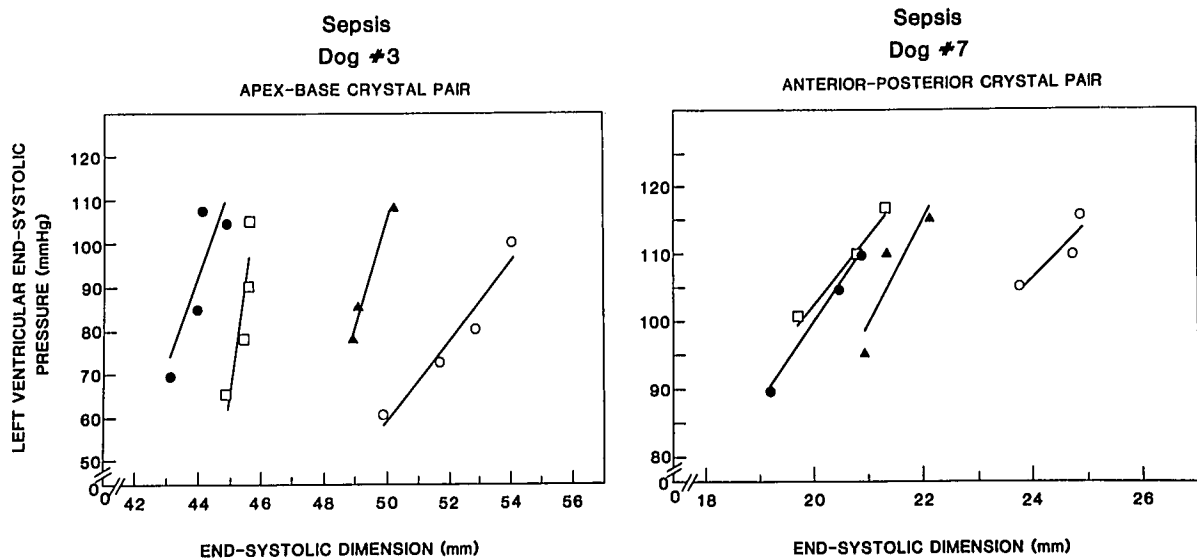


FIG. 1. Left ventricular end-systolic pressure–dimension relationships for two dogs in the initial sepsis group obtained during baseline (squares), 1 h postsepsis (filled circles), 4 h postsepsis (open circles), and posthemofiltration (triangles). Relative to baseline, this relationship was shifted downward and to the right at 4 h postsepsis and the slope (E_{\max}) was decreased. Relative to 4 h postsepsis, the end-systolic relationship was shifted upward and to the left posthemofiltration, and E_{\max} was increased. See text for discussion.

measured as 33 ± 16.5 mmHg/mm at baseline, decreased to 16.2 ± 13.9 mmHg/mm ($P < 0.05$ vs. other conditions by ANOVA and SNK) at 4 h postsepsis and increased back to initial values posthemofiltration (43 ± 13 mmHg/mm). Corresponding serum ionized calcium concentrations (mmol/l) were unchanged between conditions: 1.17 ± 0.1 at baseline, 1.10 ± 0.1 at 4 h postsepsis, and 1.13 ± 0.06 posthemofiltration. Serum pentobarbital concentrations (mg/l) were nearly the same between 4 h postsepsis and posthemofiltration (17 ± 2 and 16 ± 4 , respectively; $P < 0.05$ by ANOVA and SNK) and were slightly decreased from baseline (24 ± 3.7) as serum albumin was also decreased from 6.2 ± 1.9 g/l at baseline to 3.6 ± 1.34 g/l at 4 h postsepsis and to 3.0 ± 1.4 g/l posthemofiltration (significantly different, $P < 0.05$). Even though pentobarbital concentrations were decreased from baseline, unbound pentobarbital was nearly the same at baseline and 4 h postsepsis, and measured 8.5 ± 1.7 mg/l ($n = 4$) and 8.3 ± 1.6 mg/l ($n = 3$), respectively; the percentage of unbound to total serum pentobarbital slightly increased from $33.5 \pm 10.8\%$ at baseline to $40.0 \pm 6.4\%$ at the 4-h period. Expressed relative to the baseline albumin concentration, moreover, the respective serum pentobarbital concentrations were slightly higher posthemofiltration ($n = 5$; 24.1 ± 4 vs. 29.7 ± 4 vs. 38.1 ± 17) but were not significantly different between conditions.

In the sepsis hemofiltration and time control group, E_{\max} (mg/mm) measured along the AP dimension decreased from baseline to 4 h postsepsis and remained decreased between the 4- to 6-h interval of sepsis when he-

mo-filtration would ordinarily be performed (57 ± 39 vs. 22.7 ± 5.7 vs. 19.7 ± 9.8 ; $P < 0.05$ baseline vs. other conditions by ANOVA for the latter two values). There were no changes in serum ionized calcium concentrations (mmol/l) between conditions (baseline vs. 4 h postsepsis vs. posthemofiltration: 1.21 ± 0.11 vs. 1.14 ± 0.18 vs. 1.12 ± 0.26 , respectively) or between groups. Pentobarbital serum concentrations (mg/l) at 4 h postsepsis (20.8 ± 5.5) and at 6 h postsepsis (22.5 ± 6.4) were not different and were slightly decreased from baseline (26.8 ± 5.7 ; $P < 0.05$ by ANOVA and SNK for former two values), and serum albumin (g/l) was also decreased from 8.2 ± 0.8 at baseline to 5.2 ± 1.5 after 4 h and to 5.2 ± 1.5 ($P < 0.05$ by ANOVA and SNK for latter two values) after 6 h of sepsis. Expressed relative to baseline albumin concentration, there were no differences in pentobarbital levels between the three conditions.

In table 1, a reduction in MAP in the initial sepsis group was not associated with increased stroke volume (SV). At 4 h postsepsis, ventricular end-ejection dimensions and fractional shortening, respectively, were not different from baseline (baseline vs. 4 h postsepsis, 22.1 ± 5.8 mm vs. 21.2 ± 5 mm and $23.8 \pm 7.6\%$ vs. $20.4 \pm 6\%$ in the AP crystal pair; 42 ± 5.9 mm vs. 45.3 ± 6.8 mm and $11.9 \pm 3.5\%$ vs. $9.9 \pm 5.4\%$ in the AB crystal pair). After hemofiltration, however, SV increased compared with 4 h postsepsis (table 1). Relative to 4 h postsepsis, increases in fractional shortening ($20.4 \pm 6\%$ vs. $25.5 \pm 6.4\%$ in AP crystal pair; $9.9 \pm 5.4\%$ vs. $12.8 \pm 6.6\%$ [$P < 0.05$] in the AB crystal pair) and decreases in end-ejection dimensions (21.2 ± 5 mm vs. 19.0 ± 4.5 mm [$P < 0.05$] in

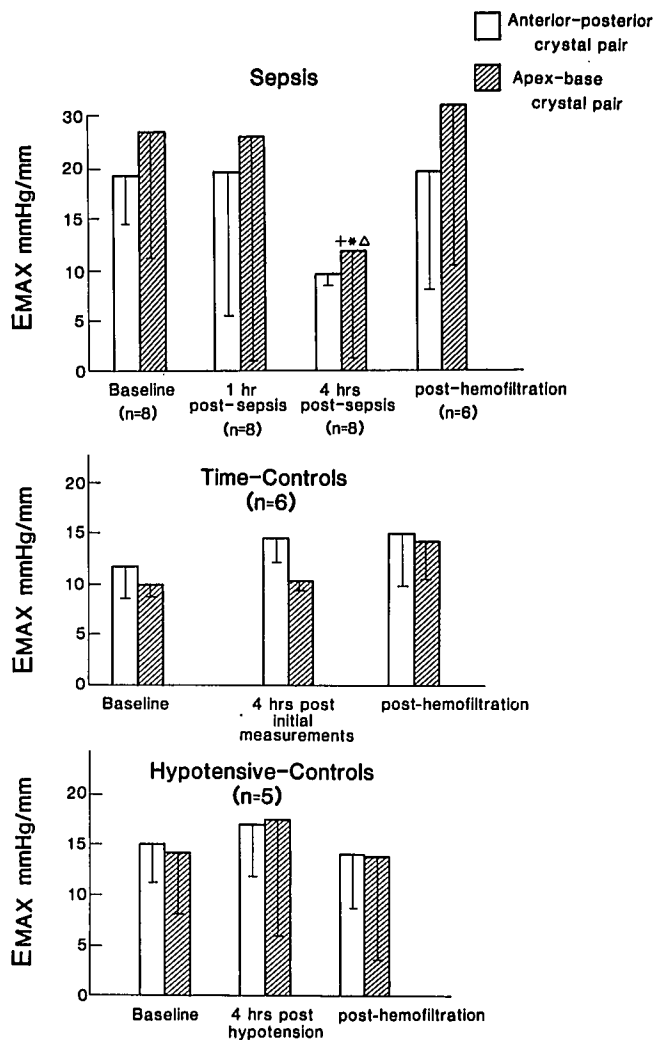


FIG. 2. The mean (\pm SD) slopes of the end-systolic pressure dimension relationship (E_{max}) obtained at the different measurement periods are shown for the initial sepsis group, hypotensive-control group, and the time-control group. In the sepsis group, compared with baseline and 1 h of sepsis, E_{max} was significantly decreased in both crystal pairs at 4 h postsepsis ($*P < 0.05$ by ANOVA and SNK). Posthemofiltration, E_{max} was increased compared with 4 h postsepsis in both crystal dimensions ($+ = P < 0.006$, by Bonferroni corrected paired t -test). There were no changes in the control groups. The respective changes in E_{max} between baseline and 4 h and between 4 and posthemofiltration were significantly different between the sepsis and control groups by unpaired t test ($\Delta = P < 0.025$).

AP crystal pair; 45.3 ± 6.8 mm vs. 44.2 ± 7.3 mm in the AB crystal pair [$P < 0.05$]) tended to follow the increase in SV and E_{max} posthemofiltration.

In the hypotensive control group, however, the results observed during the hypotensive and posthemofiltration periods were different from the findings in sepsis. The average dose of hydralazine that was given over the 6-h period was 169 ± 112 mg. In the hypotensive control group, from baseline to 4 h posthypotension, MAP was

reduced by about 33% by design (table 2). Associated with this decrease in MAP was an increase in SV of about 50% (table 2). Between baseline and 4 h posthypotension, SV increased from 64 to 90 ml, and end-ejection dimensions decreased from 44.2 ± 9.1 mm to 41 ± 9.2 mm ($P < 0.05$) in the AB crystal dimension and from 19.1 ± 8.6 mm to 16.3 ± 9 mm ($P < 0.05$) in the AP dimension. Since end-diastolic dimensions were unchanged between conditions (fig. 4), the fractional shortening of each dimension increased with hypotension, from $14 \pm 5.6\%$ to $18.7 \pm 5.4\%$ in the AB crystal pair ($P < 0.05$) and from $30.4 \pm 6.6\%$ to $41.6 \pm 6.4\%$ ($P < 0.05$) in the AP pair. After hemofiltration, SV remained increased compared with baseline, but there were no further changes in ventricular mechanics between 4 h posthypotension and posthemofiltration. Fractional shortening and end-ejection dimensions remained at $40.8 \pm 15.5\%$ and 16.6 ± 8.4 mm, respectively, in the AP crystal pair and $16.7 \pm 0.4\%$ and 41.9 ± 8.4 mm in the AB crystal pair posthemofiltration.

In the time control group (fig. 3), although there were no changes in E_{max} between conditions, there were slight increases in the dimensional intercept of the end-systolic pressure–dimension relationship (D_0) in both crystal pairs between baseline and 4 h, and a tendency for this finding to occur in the hypotensive control group (9 of 10 observations; table 3). There were no further changes in D_0 posthemofiltration. In the sepsis group, an increase in D_0 was predominantly observed between 4 h postsepsis and posthemofiltration. This occurred in all experiments along the AP dimension and in five of six experiments along the AB dimension (see "Discussion").

Furthermore, there was wide variability in baseline SV and E_{max} between groups. Baseline SV (tables 1 and 2) and baseline E_{max} (fig. 2) along the AP dimension were not different between groups. Along the AB dimension, baseline E_{max} in the time control group was less than in the sepsis group ($P < 0.05$ by unpaired t test), but baseline E_{max} between the sepsis and hypotensive control groups were not different (see "Discussion").

In figure 4, EDD are shown for the initial three groups. Left ventricular end-diastolic dimensions were the same during all conditions (fig. 4), and no changes in ventricular diastolic compliance were observed between conditions in any of the groups.

The results of the contractility measurements performed on isolated right ventricular trabeculae are shown in figure 5. The FCS activity could be detected by this assay as early as 1 h postsepsis, and there was a further increase in plasma FCS activity at 4 h postsepsis. After hemofiltration, FCS activity decreased to about baseline levels. In the two control groups, there was no change in FCS activity over the measurement period. Baseline plasma taken from an individual dog often affected the

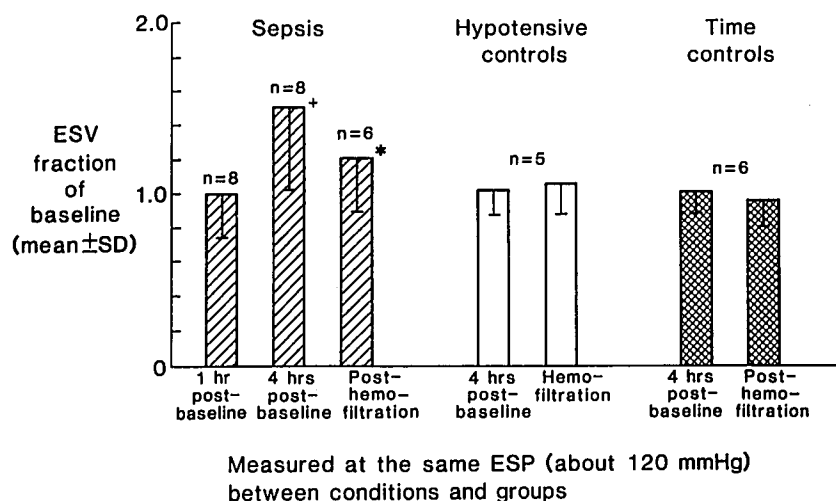


FIG. 3. For each of the three groups in figure 2, the fractional change in end-systolic volume (ESV) in the various conditions relative to that observed at baseline is shown. Results are plotted as mean \pm SD and were obtained at the end-systolic pressure of about 120 mmHg. Relative ESV was calculated from the ratio of the end-systolic dimensions in the respective conditions relative to the baseline value ($ESV_{relative} = AP \times AP \times AB$ end-systolic dimensions obtained in the various conditions/baseline value). The anterior-posterior end-systolic dimensions were used twice, and the apex-base end-systolic dimension used once, since the anterior-posterior dimension approximates the septal-to-lateral dimension. In the sepsis group, ESV was increased at 4 h postsepsis and reduced by hemofiltration ($+P < 0.01$ at 4 h between groups by ANOVA. $*P < 0.01$ hemofiltration vs. 4 h postbaseline in the sepsis group by Bonferroni corrected *t*-test).

isolated trabecula to a variable extent, and all results are reported relative to the baseline measurement. The mean change in developed tension of isolated ventricular muscle when baseline plasma was placed into the bath was $-1 \pm 17\%$ in the sepsis group, $-12 \pm 13\%$ in the hypotensive control group, and $-23 \pm 16\%$ in the time control group, differences that did not achieve statistical significance (see "Discussion").

In figure 6, for each dog in the initial sepsis group, the changes in E_{max} from baseline to 4 h postsepsis and from 4 h postsepsis to posthemofiltration are plotted against the respective changes in the *in vitro* assay. In every dog

studied, both positive and negative changes in E_{max} were inversely associated with changes in plasma FCS activity. The correlation was significant for both crystal pairs.

In the sepsis group and hypotensive control group, the hemofiltration filtrate was also examined for the presence of FCS activity. In the sepsis group, filtrate significantly reduced isometric tension compared with baseline plasma ($-18.6 \pm 15\%$; $P < 0.05$); thus, FCS was present in the filtrate in the sepsis group. In the hypotensive control group, however, there was no increase in FCS activity in the filtrate relative to baseline plasma ($0.2 \pm 28\%$).

In table 4, arterial blood gas and Hct results are shown

TABLE 2. Hemodynamics in Hypotensive Controls and Time-Control Dogs

Group	Parameter	Baseline	4 h Posthypotension	Posthemofiltration
Hypotensive Controls	MAP	143 \pm 13	118 \pm 10*	121 \pm 8*
	LVEBP	150 \pm 11	133.5 \pm 18*	133 \pm 10*
	LVEDP	11.2 \pm 4.0	12.4 \pm 3.5	16 \pm 3.7
	SV	58 \pm 21	90 \pm 18*	92 \pm 11.4*
	HR	154 \pm 20	182 \pm 24	162 \pm 13
	SVR	13.9 \pm 3.1	6.9 \pm 1.7*	7.3 \pm 1.5*
Time-Controls	Parameter	Baseline	4 h Post-Initial Measurements	Posthemofiltration
	MAP	145 \pm 16.9	153 \pm 17.7	142 \pm 19.8
	LVEBP	153 \pm 11.4	159 \pm 13	158 \pm 67
	LVEDP	11.4 \pm 6.7	11.4 \pm 4.0	11.1 \pm 3.5
	SV	50 \pm 15.8	51 \pm 19.7	58 \pm 19.8
	HR	166 \pm 32	167 \pm 19	166 \pm 20
SVR	18.7 \pm 6.1	17.4 \pm 4.35	15.0 \pm 3.0*	

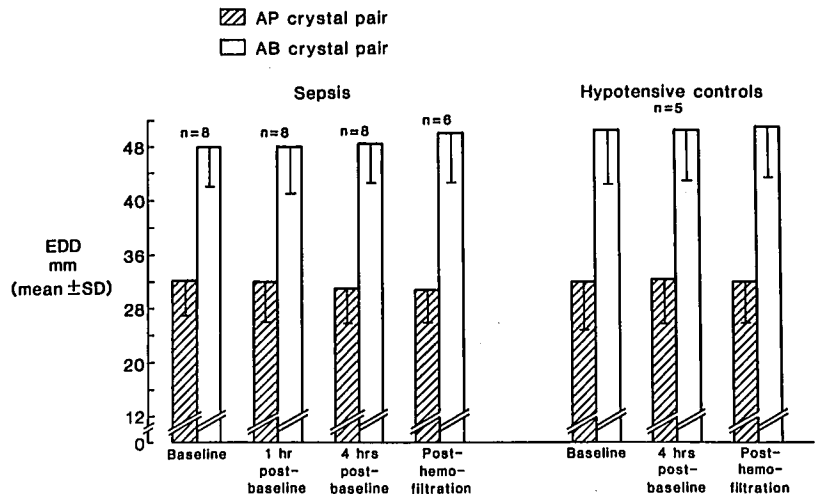
Values are means \pm SD.

MAP = mean blood pressure (mmHg); LVEDP and LVEBP = left ventricular end-diastolic and end-systolic pressures (mmHg), respectively; SV = stroke volume (ml); HR = heart rate (beats/min); SVR

= systemic vascular resistance (mmHg/l/min).

* $P < 0.05$ from baseline by the ANOVA and SNK multiple-comparison test.

FIG. 4. Left ventricular end-diastolic dimensions (EDD) were unchanged in the three initial groups (see fig. 2). The sepsis group and the hypotensive-control group are shown.



for the initial three groups. Note that hematocrit decreased after baseline measurements in all groups because of the initial blood volume expansion with 6% hetastarch in normal saline solution but changed little the following 4 h. A slight further decline was seen in all groups after hemofiltration. Hematocrit was about the same for the three groups during similar conditions. The pH was decreased slightly in the sepsis group from baseline to 4 h postsepsis, but there were no further changes posthemofiltration. Arterial P_{O₂} decreased slightly in the sepsis and hypotensive groups between 4-h measurements and post-hemofiltration.

Discussion

Although previous studies have shown that a depression in LV contractility in sepsis may be associated with the presence of a circulating myocardial depressant factor,⁹⁻¹⁴ the existence of such a factor remains highly con-

troversial.^{2,3} To further address this issue, we designed this study to test whether the removal of a possible depressant factor by hemofiltration would result in a reversal of cardiac depression in sepsis. Not only would a positive finding further support the contention that myocardial depression in sepsis is caused by a filterable substance of small molecular weight (<30,000 d), but it would demonstrate that this impairment can be reversed acutely by removal of the substance from the circulation. Our results show that LV contractility was in fact significantly impaired after approximately 4 h of bacteremia and that this decrease in contractility was reversed by hemofiltration. Hemofiltration caused plasma FCS activity to decrease to baseline levels, and this change was both temporally and quantitatively associated with restored ventricular contractility.

Although FCS activity could be detected at 1 h post-sepsis when LV contractility was not yet decreased, this

TABLE 3. The Extrapolated Intercept (D₀) in the Three Groups (means ± SD, mm)*

Group		1 h			
		Baseline	Postsepsis	4 h Postsepsis	Posthemofiltration
Initial sepsis	Anterior-posterior crystal pair	10.5 ± 6.1	10.3 ± 5	7.6 ± 4.5	12.4 ± 7‡
	Apex-base crystal pair	33.3 ± 7.7	29.9 ± 12	30.7 ± 9.8	36.3 ± 14
Time-control		Baseline	4 h Post-Initial Measurements		Posthemofiltration
	Anterior-posterior crystal pair	11.54 ± 14.14	13.86 ± 13.30†		12.84 ± 12.35†
	Apex-base crystal pair	30.25 ± 7.01	32.71 ± 7.03†		34.09 ± 6.50†
Hypotensive control		Baseline	4 h Posthypotension		Posthemofiltration
	Anterior-posterior crystal pair	7.1 ± 9.6	6.0 ± 2.8		7.2 ± 7.9
	Apex-base crystal pair	29.4 ± 9.3	33.4 ± 8.0		31.2 ± 8

* The extrapolated dimensional intercept (D₀) was obtained by linear regression analysis.

† P < 0.05 from baseline by ANOVA and SNK.

‡ P < 0.05 change in D₀ between 4 h postsepsis and posthemofiltration versus the change in D₀ during the same interval in combined control groups by unpaired t test.

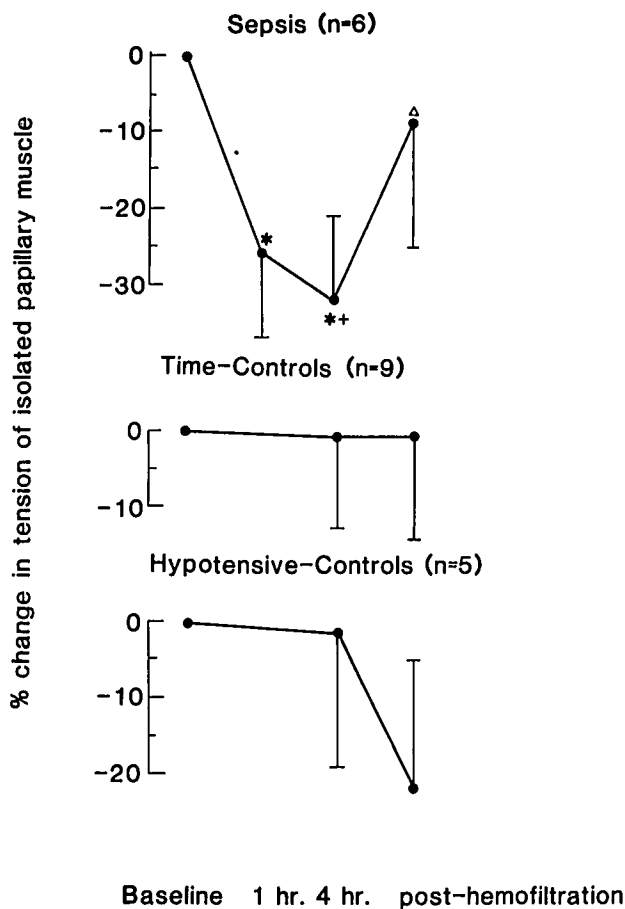


FIG. 5. Isolated right ventricular trabeculae obtained from nonseptic dogs were used to assess *in vitro* the filterable cardiodepressant substance (FCS). One hour, 4 h, and posthemofiltration measurements are shown for the individual groups and are reported relative to baseline measurements. In the initial sepsis group, plasma obtained at 1 h and 4 h postsepsis caused a decrease in isometric tension (mean \pm SD) with the peak decrease occurring at 4 h postsepsis. Posthemofiltration, this depressant effect was reduced compared with 4 hrs postsepsis ($*P < 0.01$ from baseline, $\Delta P < 0.05$ from 4 h postsepsis by ANOVA and SNK). In the time-control and hypotensive-control groups, there were no changes over time or with hemofiltration. The respective changes in isometric tension from baseline to 4-h measurements and from 4-h measurements to posthemofiltration were significantly different between sepsis and control groups ($+P < 0.02$ by unpaired *t* test).

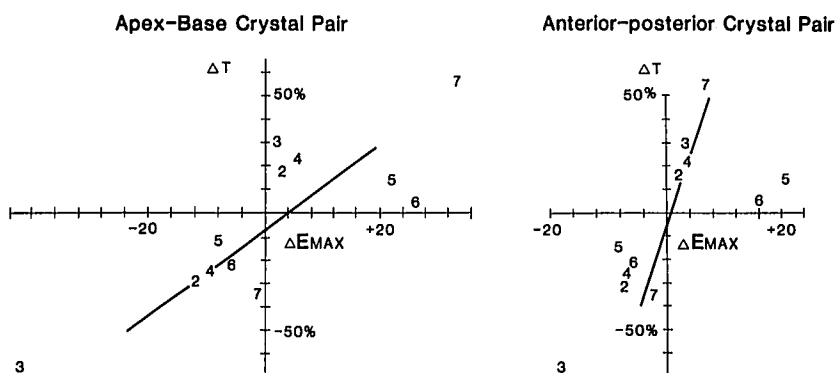
is not surprising. Parker and Adams⁴ showed that it takes about 2 h before myocardial dysfunction occurs in endotoxin shock in guinea pigs. It appears likely that after FCS appears in the circulation, time or sufficient quantities are required before depression of contractility. Alternatively, the effect FCS produces on myocardial cells may be a metabolic or other change that requires time to develop. Either of these possibilities would be consistent with our data, since our FCS *in vitro* assay in all cases involved exposure of the right ventricular trabeculae to the test plasma for a period of longer than 1 h, and the myocar-

dium *in vitro* would be exposed to FCS for increasing lengths of time as the experiment progressed. Another possibility is that the ventricular muscle *in vitro* is more susceptible to FCS effects than the myocardium *in vivo* since metabolic substrates or counteracting homeostatic and repair mechanisms are now likely to be operative *in vivo*. Among these latter possibilities, one important one is the probable presence of increased circulating catecholamines that may be released early in the course of sepsis. In support of this, Cho³¹ showed that when *E. coli* endotoxin was administered in dogs, the initial cardiac response was increased contractile force, which in turn could be blocked by beta-adrenergic receptor blockade or by catecholamine depletion by reserpine.

Brand and Lefer⁹ initially detected circulating myocardial depressant factor (MDF) activity in plasma from cats in irreversible postoligemic shock and suggested that MDF was dialyzable, had a molecular weight of 500–1,000 d, and was a peptide.^{9–13} On the other hand, in a subsequent study³² in which plasma of septic dogs was passed through filters (Amicon) of different pore sizes, we found that the molecular weight of FCS was between 10,000 and 30,000 d and that FCS was heat labile. The reason for the different findings may be related to the different types of shock studied. Different mechanisms or factors may be involved in hemorrhagic and septic shock. We used the term "FCS" rather than "MDF" because the pathophysiology of cardiac depression in sepsis, and hence the cardiodepressant substance, may be different than that found in hemorrhagic shock. Furthermore, in our *in vitro* measurements, baseline plasma depressed isometric tension to some extent when it was added to the tissue bath (see "Results"). This finding was previously described by Lefer and co-workers,^{11,33,34} although the mechanism is not clear. The depression caused by baseline plasma was not different in the three groups in figure 5 and occurred to the same degree when reported in the literature. Thus, the results in our study not only support the notion that a circulating substance may cause LV dysfunction in sepsis but also extend this further. The major new finding in the current study is that LV dysfunction in sepsis can be readily reversed by removal of FCS by hemofiltration.

In the sepsis group, the decrease in E_{max} after 4 h of sepsis was not due to systemic hypotension or decreased coronary perfusion pressure *per se*, because in the hypotensive control group, E_{max} was unchanged and SV was greatly increased at the 4-h period compared with baseline (table 2 and figure 2). Thus, the changes in cardiac mechanics in sepsis were clearly different from those that would result if the pathophysiology of sepsis reflected only systemic vasodilation. Although differences in coronary flow between the sepsis group and the hypotensive group would likely exist during hypotension, we consider it unlikely that a relative decrease in coronary flow in sepsis

FIG. 6. In the individual dogs in the initial sepsis group (dogs 2-7), the respective changes in the slope of the end-systolic pressure-dimension relationship (E_{max}) between baseline and 4 h postsepsis and between 4 h and posthemofiltration are plotted against corresponding changes in tension (ΔT) obtained in the *in vitro* right ventricular trabeculae measurements (see fig. 6.) From baseline to 4 h postsepsis, there was a decrease in E_{max} ($-\Delta E_{max}$) and a decrease in developed tension of isolated muscle ($-\Delta T$). From 4 h to posthemofiltration, E_{max} increased ($+\Delta E_{max}$), and developed tension increased ($+\Delta T$). The correlation (regression lines are shown by solid lines) was slightly greater in the apex to base crystal pair ($r = 0.868, P < 0.01$) than in the anterior-posterior pair ($R = 0.675, P < 0.05$).



could explain the different findings in E_{max} between the two groups. In this regard, Archer *et al.*³⁵ have shown that myocardial dysfunction could be observed in dogs given *E. coli* endotoxin even though coronary flow was well-maintained compared with presepsis values. Investigators such as Parker and Adams^{4,5} have examined cardiac function in sepsis in which muscle was placed in a tissue bath so that abnormal systolic function in sepsis was not dependent on considerations of coronary flow. Moreover, Spitzer *et al.*³⁶ found no evidence of myocardial hypoxia as indicated by diminished lactate use following endotoxin administration in dogs when MAP was reduced to an extent similar to that observed in the current study.

In figure 7, to more clearly illustrate the different changes in LV mechanics between the sepsis and hypo-

tensive control groups at the 4-h period, we plotted LV pressure-dimension diagrams for an individual dog in the sepsis group and one dog from the hypotensive control group. In the left-hand panel, a pressure-dimension loop of a hypotensive control dog is shown during baseline and after hypotension was induced by arteriolar vasodilation. At baseline, the ventricle fills to an end-diastolic dimension of about 24 mm at which LVEDP is about 12 mmHg. Isovolumic contraction then occurs followed by ejection, after which the ventricle relaxes. The end-ejection dimension (about 18 mm) reflects the amount of volume of the heart after contraction has ceased. Fractional shortening is given by (EDD-EED)/EDD. Stroke-volume is proportional to the difference between ventricular end-diastolic (*i.e.*, 24 mm) and end-ejection dimensions (*i.e.*,

TABLE 4. Arterial Blood Gas Variables in the Three Groups (means \pm SD)

Group	Value	Baseline	1 hr Postsepsis	4 h Postsepsis	Posthemofiltration
Initial sepsis	P_{aO_2}	107.4 \pm 24.8	102.6 \pm 23.7	102.1 \pm 15.5	80.17 \pm 19*
	P_{aCO_2}	36.0 \pm 1.07	38.9 \pm 4.05	34.3 \pm 2.92†	29.8 \pm 5.3
	pH	7.40 \pm 0.042	7.35 \pm 0.085	7.32 \pm 0.046‡	7.31 \pm 0.08
	Hct	43.0 \pm 7.25	29.1 \pm 7.62¶	31.4 \pm 5.45¶	23.3 \pm 8.6
Hypotensive controls	Value	Baseline	4 h Posthypotension		Posthemofiltration
	P_{aCO_2}	94.6 \pm 8.08	96.2 \pm 5.02		87.6 \pm 10.4‡§
	P_{aCO_2}	38.8 \pm 1.92	37.0 \pm 4.00		31.4 \pm 4.93‡§
	pH	7.40 \pm 0.047	7.35 \pm 0.032		7.39 \pm 0.076
Hct	44.0 \pm 14.9	33.6 \pm 6.19		25.6 \pm 8.02¶	
Time controls	Value	Baseline	4 h Post-Initial Measurements		Posthemofiltration
	P_{aCO_2}	93.2 \pm 4.09	95.6 \pm 7.23		101.6 \pm 23.9
	P_{aCO_2}	37.1 \pm 3.30	34.2 \pm 3.07		33.0 \pm 2.74‡
	pH	7.36 \pm 0.064	7.38 \pm 0.029		7.38 \pm 0.023
Hct	40.0 \pm 6.69	32.3 \pm 5.50¶		25.3 \pm 8.19§¶	

Hct = hematocrit.

* $P < 0.05$ from 4 h postsepsis by Bonferroni corrected paired *t* test by ANOVA and SNK; † $P < 0.01$ from 1 h postsepsis; ‡ $P < 0.05$;

§ $P < 0.05$ from 4 h measurements; ¶ $P < 0.01$, respectively, from baseline.

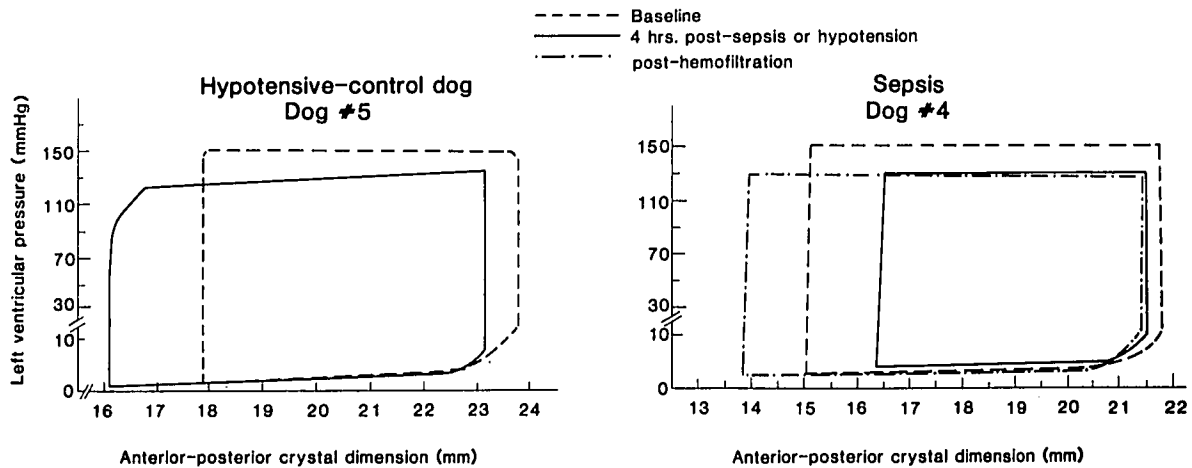


FIG. 7. Left ventricular pressure–dimension loops obtained over one beat in a hypotensive-control dog (left) and a sepsis dog (right) to illustrate the findings obtained in each group. As the ventricle fills during diastole (lowest horizontal portion of loop, from left to right), ventricular dimensions and pressures increase. Isovolumic contraction occurs with little change in dimensions. With ventricular ejection, there is a reduction in dimensions followed by isovolumic relaxation. See text for discussion.

18 mm). In contrast to end-ejection, end-systole is defined as maximal ventricular elastance, that is, the point at which the ratio of ventricular pressure to dimension is maximal.²² In the figure, end-systole would be represented by the pressure–dimension coordinate in the left upper corner of the loop. End-systolic and end-ejection dimensions may or may not coincide. End-ejection dimensions can be smaller than end-systolic dimensions if flow from the aorta to the periphery is rapid enough to keep aortic pressure less than intraventricular pressure after the ventricle has reached maximal elastance. In this case, the ventricle can still eject beyond end-systolic dimensions, although at a rate less than at E_{\max} . In this particular example, at the baseline measurement, end-ejection and end-systolic dimensions were about the same (18 mm).

In the hypotensive group, SV was increased at 4 h post-hypotension relative to baseline (table 2), while end-diastolic dimensions were unchanged between the various conditions. The increase in SV with hypotension could therefore only be due to a decrease in end-ejection volume, and such a decrease was indeed observed (see “Results”). In terms of the pressure–dimension analysis in figure 7, this decrease in end-ejection dimensions can be ascribed to two factors. The first is that during hypotension, end-systolic pressure is lower than at baseline, and because of this, the ventricle ejects to a lower end-systolic dimension along an unchanged E_{\max} relationship. The magnitude of the reduction in end-systolic dimensions is therefore dependent on the decrease in end-systolic pressure and the slope of E_{\max} (see below). Secondly, a slight further decrease in dimensions may occur during hypotension because of increased discharge of blood from the

aorta to the periphery. As described in the preceding paragraph, this increased discharge would allow the ventricle to eject past end-systolic dimensions, further lowering end-ejection dimensions compared with baseline.

In the example in figure 7 (left-hand panel), the slope of the E_{\max} line was about the same during baseline condition and at 4 h posthypotension, *i.e.*, about 16 mmHg/mm in both conditions. In the figure, this E_{\max} relationship can be approximately described by a line that runs between the pressure–dimension coordinates located in the left upper sharp corner in the two conditions (*i.e.*, at coordinates of 150 mmHg, 18 mm and 122 mmHg, and 17 mm). During hypotension, end-systolic pressure decreased from 150 mmHg during baseline condition to 122 mmHg during hypotension. A decrease in end-systolic pressure of about 28 mmHg with an E_{\max} of 16 mmHg/mm would predict a decrease in end-systolic dimensions of about (28/16) or 1.75 mm, *i.e.*, close to the 1.2 mm measured. A slight further decrease in dimensions (about 0.8 mm) was observed because of continued ventricular ejection after the end-systolic dimension was reached.

With respect to the changes in the septic animals, note that unlike the hypotensive controls, end-ejection dimensions in the sepsis group did not change between baseline and 4 h postsepsis (see “Results”). In terms of the analysis in figure 7, the absence of a decrease in end-ejection dimensions despite a decrease in pressure is consistent with impaired LV function. In the sepsis group, end-systolic pressure at baseline averaged 168 ± 21 mmHg, and end-ejection and end-systolic dimensions for the AP crystal pair were about the same (22.1 ± 5.8 mm *vs.* 21.6 ± 5.7 mm, respectively). Since E_{\max} for the AP crystal pair av-

eraged 18.6 mmHg/mm during baseline (fig. 2), the observed 41 mmHg fall in mean end-systolic pressure (end-systolic pressure was decreased to 124 ± 14 mmHg at 4 h postsepsis) would have resulted in a 2.2 mm decrease in the AP dimension (41 mmHg/18.6 mmHg/mm), if contractility, *i.e.* E_{\max} and D_0 (see further below), were unchanged. Thus, the absence of a decrease in ventricular end-ejection and end-systolic dimensions between baseline and 4 h postsepsis is consistent with a decrease in ventricular contractility at 4 h postsepsis. In the example in figure 7 (right-hand panel), after 4 h of bacteremia, the end-systolic pressure–dimension coordinate (*i.e.*, represented by the left upper corner) was displaced downward and to the right of baseline.

E_{\max} was improved after hemofiltration in the sepsis group, and there was also improved ventricular emptying (see table 1 and "Results"). Mean end-systolic pressure averaged 115 ± 14 mmHg, and in the AP crystal pair, mean end-ejection and end-systolic dimensions decreased to 19.0 ± 4.5 mm and 19.1 ± 4.4 mm, respectively. Thus, posthemofiltration, end-systolic dimensions were appropriately reduced for the reduction in end-systolic pressure as ventricular dysfunction was reversed by removal of FCS. In the example shown in the right-hand panel of figure 7, the posthemofiltration end-systolic pressure dimension coordinate is shifted to the left of the baseline in a manner similar to that observed when hypotension was produced by hydralazine (fig. 7, left).

In terms of the end-systolic pressure–dimension relationship, moreover, table 3 shows that there were slight changes in the intercept (D_0) between conditions. Maughan *et al.*²⁵ showed that while the slope of the end-systolic pressure–volume relationship is insensitive to a wide range of changes in afterload impedance, its volume intercept was dependent on arterial resistance and characteristic impedance. In the study by Maughan *et al.*,²⁵ a reduction in arterial resistance and characteristic impedance shifted the curve in a parallel manner to the right, while increases in these parameters had the opposite effect. The mechanism may be related to calcium-mediated changes in the interaction between actin and myosin filaments, reflecting how the ventricle perceives the alterations in afterload impedance between conditions.

The significance of a shift in intercept is that even if contractility were unchanged between conditions, a parallel shift in the end-systolic pressure–dimension relationship to the right (*e.g.*, due to a decrease in SVR) would result in a reduction in SV for a given end-systolic pressure and EDD. In the sepsis group (table 1), D_0 was increased in almost all (11/12) observations between 4 h postsepsis and posthemofiltration, possibly reflecting the relative decrease in SVR found in this condition. As a result, the increases in SV and CO observed from 4 h postsepsis to

posthemofiltration were probably less than if D_0 had not changed. In the hypotensive group, there was a decrease in SVR between baseline and 4 h, so that a shift in D_0 in most experiments between these conditions could be explained by a decrease in resistance. The reason for the shift in the time control group between these conditions is less clear, since SVR changed only marginally. Moreover, D_0 must be obtained by extrapolation from relatively high end-systolic pressures, because during venous occlusion, coronary perfusion pressure must be maintained above 60 mmHg to prevent myocardial ischemia. It is recognized, therefore, that such an extrapolation could lead to errors in the intercept. While this factor remains a consideration in the interpretation of D_0 and although the mechanisms of how changes in afterload impedance alter D_0 remain unknown, it almost certainly appears that changes in D_0 do not reflect changes in contractility.^{22,25} Moreover, during hemofiltration, the mechanism of the decrease in SVR and, hence, the rightward shift in D_0 observed in the sepsis group are not clear. One possibility is that due to this treatment, vasoactive substances were released which in turn caused the reduction in SVR. (In fig. 7, there were only slight changes in D_0 in the two examples shown to modify the analysis.)

Furthermore, when the measurement of E_{\max} is considered, there is wide variability between animals, since this measurement is not indexed to heart size and is further dependent upon the relative distance between crystal pairs. Among animals of all sizes, LV systolic pressures are fairly uniform. On the other hand, for given changes in end-systolic pressures, the respective changes in end-systolic dimensions would be dependent upon the size of the animal and the exact crystal placement in addition to the contractile state of the heart. In a small heart, end-systolic dimensions would move less than in a large heart for a given contractile state of the muscle. Along the AP crystal dimension at the baseline measurement, there were no differences in E_{\max} between the groups, while along the AB dimension, baseline E_{\max} in the time control group was lower than in the sepsis group. However, since both the AP and AB crystal dimensions showed similar changes between conditions in the respective groups, we do not think that this baseline difference in E_{\max} along the AB crystal dimension in the control group affected the conclusions of the study. Indeed, because of the wide variability in baseline E_{\max} , SV, FCS, etc, between the animals in the present study, to analyze our findings, we examined changes in these parameters between conditions in the respective groups and compared the different responses between groups.

Our study is unique because it relates changes in *in vivo* and *in vitro* indices of contractility in sepsis. The major contribution of this study is that it shows that removal of

a filterable substance can acutely reverse depressed LV contractility in sepsis. With respect to our study, however, it should be recognized that it was not our goal to develop a model of sepsis to mimic the human condition that may have a more variable and prolonged time course than that observed in the current study. Hemodynamics in animal models of sepsis are, in general, hypodynamic or normodynamic rather than the usual hyperdynamic state observed in the clinical situation.¹ In dogs, pentobarbital anesthesia is associated with hypertension and tachycardia relative to the conscious condition,^{18,37} but the pentobarbital concentrations were almost exactly the same between the 4-h postsepsis and posthemofiltration conditions. Furthermore, in our study, severe hypotension was not produced during sepsis, only hypotension relative to baseline measurements.

The term "septic shock" is a somewhat arbitrary one, and the degree of hypotension we chose was based on our observation that the animal would remain stable at the pressure used over the interval of the present study. Moreover, to control for the effect of hypotension observed during the 4-h and posthemofiltration periods in the sepsis groups, we administered hydralazine in the hypotensive control group and, of course, recognize that alternative agents may have been used. Although all of these aspects of our study must be considered, it is important to note that this study was performed specifically to address the issues of whether a circulating cardiodepressant substance developed in sepsis and whether hemofiltration by reducing FCS could restore LV contractility. Although other models of sepsis may be examined in the future, within the framework of the objective of the current study, we feel that our model was appropriate and useful and that it aptly addressed these issues.

Could hemofiltration be used to treat myocardial dysfunction from sepsis in clinical medicine? Our study demonstrates several limitations that must be addressed before this potential can be realized. Although E_{max} improved with hemofiltration, because of accompanying increases in Do , the increase in SV was not as great as one would have liked in the clinical setting. Furthermore, since systemic hypotension was not affected, this suggests that factors responsible for hypotension and cardiac dysfunction in sepsis may be different. Thus, combined approaches to these two problems will have to be tested.¹⁰ Also, there are the potential problems associated with hemofiltration itself, among them the need for systemic anticoagulation. Continuous arteriovenous hemofiltration has been used extensively in clinical medicine as an acceptable alternative to conventional methods of acute dialytic therapy.³⁸ Other investigators^{39,40} have also used CAVH as adjunct therapy to the treatment of sepsis and have shown that CAVH may be useful clinically. Although the number of studies

in which CAVH has been used is relatively small, our data offer a rationale for further exploration of the potential of hemofiltration to improve myocardial function in sepsis.

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