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Myocardial and Coronary Involvment in Hemorrhagic Shock

Annual Report

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# ABSTRACT

Hemorrhagic shock is still a major therapeutic challenge for military physicians, yet understanding of pathophysiologic processes involved in this disorder remains incomplete. There are many reasons to expect that ischemic myocardial dysfunction might complicate hemorrhagic shock, causing reduced cardiac function and reserve even after volume replacement. Prior experimental studies of the heart in hemorrhagic hypotension, however, have not yielded a clear and consistent concept of the extent and duration of myocardial and coronary involvement in this disorder. In part, this relates to drastic changes in cardiac loading that accompany hemorrhage. To overcome these difficulties this research protocol evaluates myocardial contractility by quantifying the relationship between end-systolic pressure and volume (ESPVR), a technique that minimizes the effects of loading conditions. Analysis of the data from these studies should result in a more complete understanding of hemorrhagic shock and may provide a rational basis for improvement in selection of therapeutic strategies. The first year of this project has been devoted to developing the instrumentation to be used, validating the ESPVR technique in this environment, and performing a study on the effects of a ten-minute period of severe hemorrhage on contractility. Both the use of ultrasound crystals sewn on the heart and conductance catheters introduced into the left ventricle through a peripheral vessel have been explored as methods to derive the required high-fidelity left ventricular volume measures.

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# Forward

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

Project Budget, 1Nov84 - 1Nov85

Salaries: Timothy J. Gawne, Ph.D., $17,000/annum, $17,000 total

Kristen S. Gray, $14,000/annum, $10,500 total

Major Expenses:

| No. | Item | Total Cost | Justification |
|---|---|---|---|
| 80 | Domestic Pigs, 55-70 lbs. | $7200 | Model for hemorrhagic shock |
| 1 | Animal Transport Cage | $1400 | Required for transportation of chronically instrumented conscious pigs. |
| 1 | 4-Channel Sonomicrometer | $4000 | Required for measurement of global dimensions by the method of sonomicrometry |
| 16 | Hemispherical Ultra-sound Transducers | $2400 | For use with sonomicrometer listed above |
| 7 | Konigsberg Implantable Pressure Transducers | $4900 | Required for high-fidelity recordings of left-ventricular pressures |
| 1 | Millar Transducer-tip Pressure Transducer, with Control Box | $1500 | As above, for introduction via peripheral vessel |
| 1 | IBM-PC, 640K RAM, 10Meg Disk, B&W monitor, High-Speed Printer, Plotter | $4000 | Required for data analysis and generation of pressure-volume loops. |
| 2 | Tecmar Lab Master 12-bit A/D converters | $1600 | Required to transform analog into digital data for IBM-PC |
| 1 | Vetter 8-Channel FM-Tape Recorder | $8000 | Required to save record of experiments for later use and computer processing. |
| - | Assorted Surgical Instruments | $4000 | Required for performing experimental surgeries |
| - | Assorted Electronic Components and Supplies | $3000 | Required for interconnecting modifying and designing electronic equipment |
| - | Harvard Respirator | $1500 | Required for performing experimental surgeries |
| - | Mettler Balance | $1500 | Required for weighing drugs, tissue samples |

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## Background

The role of the heart in causing or perpetuating hemorrhagic shock is not fully-defined. In part, this reflects the gross distortions in cardiac loading conditions (preload, afterload, and heart rate) that occur during hemorrhage. If cardiac output is reduced during hemorrhagic shock, it has proven extremely difficult to determine if this is due to either changes in loading condions on the heart, to a loss of ventricular function, or to some combination of the two. Previous studies have been inconclusive, with some pointing to a central cardiac failure (1,2,3,4,5,6,7), and others to a failure of the periphery (8,9) as the initial decompensatory event in hemorrhagic shock.

Recently, a new measure of cardiac contractility which quantifies the slope of the End-Systolic Pressure Volume relationship, has been defined. This index of cardiac contractility has been shown to reflect changes due to inotropic drugs, and to be highly insensitive to changes in preload, afterload, heart rate, and perfusion pressure (10,11,12,13). A major purpose of this project is to use this measure of contractility to determine the role of the heart in the pathogenesis of hemorrhagic shock. Development of a means suitable for assessment of contractile function during and after hemorrhage was the first objective undertaken in carrying out the program of study.

## Acute Study

There are two kinds of ultrasound crystals that can be used to determine global dimensions, lensed flat plates and hemispheres. The lensed flat plates are quite inexpensive and readily available, but have very poor beamwidth. Thus, when lensed plates are used to detect signal transmission and, hence, to determine a dimension across the heart, they must be very carefully placed. Even so, the signal may be lost if some intervention changes either the size of the heart or its placement in the chest. Therefore lensed plates cannot be reliably used in chronic animal preparations, where the very act of closing the chest wall may cause the signal to be lost. Hemispherical ultrasound crystals do not have this problem, but are expensive and difficult to obtain, and we did not receive any until April 1985. Therefore it was decided to perform an acute study in the open chest condition, both to validate the methods used and to acquire additional data on hemorrhage. These data were presented at the meeting of the American Physiological Society in November 1985, in Niagara NY, and the abstract was published in The Physiologist.

This study investigated the effects of a ten-minute period of severe (25-35 mm.Hg mean arterial pressure) hemorrhage on the slope of the end-sysolic pressure-volume relationship, or Emax. There is now considerable evidence that Emax can provide a reliable measure of contractility that is independent of preload, afterload, or heart rate (10,11,12,13). Young (8-12 wks) domestic pigs were used as the animal model. The pigs were anesthetized with pentobarbital and instrumented with three pairs of sonomicrometer crystals (minor axis, major axis, and equatorial wall thickness) to estimate ventricular volume. This methodology has been widely used, and validated against an electromagnetic flowprobe (14,15). A sample pressure-volume loop as obtained with this setup is illustrated in Figure 1. All studies were conducted in the open chest, open pericardium condition. An inflatable occluder cuff sewn around the descending aorta provided the transient changes in afterload needed to determine Emax. This technique could reliably identify the depressant effect of 0.8 micrograms/kilogram propranolol or 40 mg/kg pentobarbital on the Emax. (See Figure 2). 12 animals had sufficient blood withdrawn over a ten-minute period to reduce the mean arterial pressure to between 25 and 35 mm.Hg. The pressure was maintained at this level for ten minutes, following which all the shed blood was reinfused. Three animals died during the hemorrhage; the remainder survived for the duration of the study. Seven control animals were used. After reinfusion Emax increased significantly (18.14 vs 10.86 mm.Hg/cc) above control for 50 minutes before returning to baseline, indicating increased contractility. We conclude that brief periods of severe hemorrhage that are survived have no short-term effect on the myocardium immediately after restoration of blood volume. Graphs of Emax, mean arterial pressure, heart rate, cardiac output, and cardiac mechanical work versus time are plotted in Figures four through eight.

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FIGURE 1



A: PIG439A1.DAT
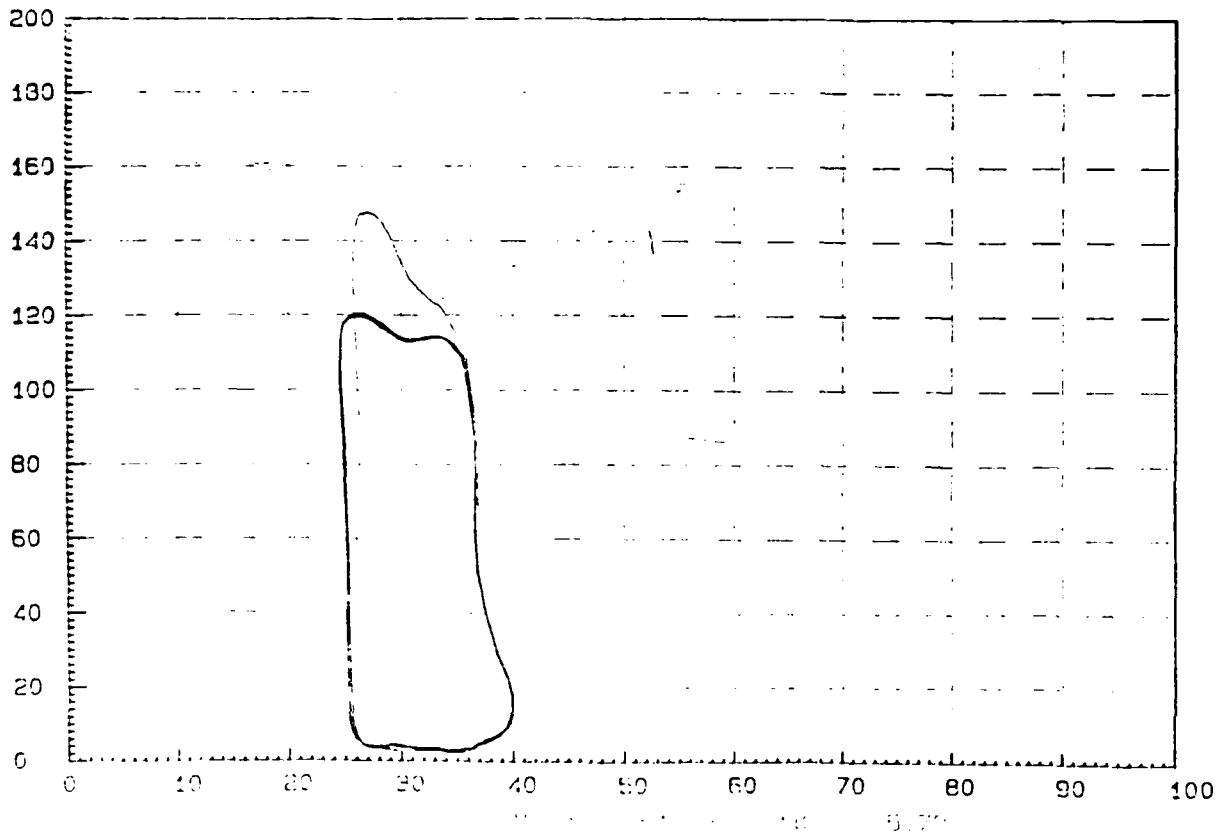
Sample plot of a left-ventricular pressure-volume loop.
Volume was estimated from three channels of sonomicrometer data
(Triton Instruments) and pressure was measured via a high-
fidelity pressure-tip catheter (Millar Co.). The last beat of
this series had afterload transiently increased by an inflatable
occluder sewn aroung the descending aorta. Data was sampled at a
rate of 200/second with a Tecmar lab Master on an IBM-PC, and was
stored on floppy disks. Scale is 0-200 mm.Hg. y-axis, 0-100 cc.
x-axis.

FIGURE 2

## — CARDIAC OUTPUT

### EM FLOWPROBE VS SONOMICROMETER
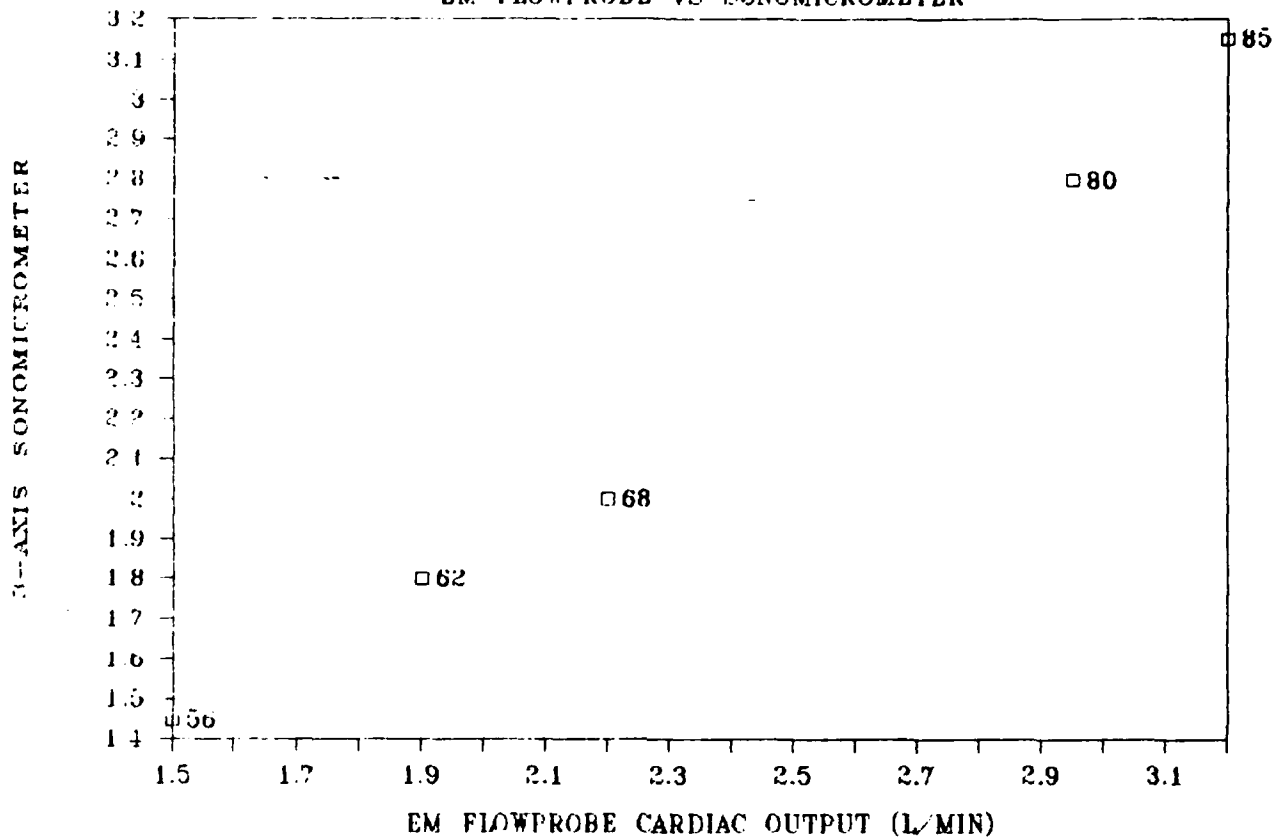


Graph of cardiac output as derived from an electromagnetic flowprobe (Carolina Instruments) versus cardiac output derived from three channels of sonomicromter data on one pig. Mean arterial pressure at which the readings were taken are indicated at each data point.

Figure 3

# EMAX VS PROPRANOLOL
## PERCENT OF CONTROL VALUE
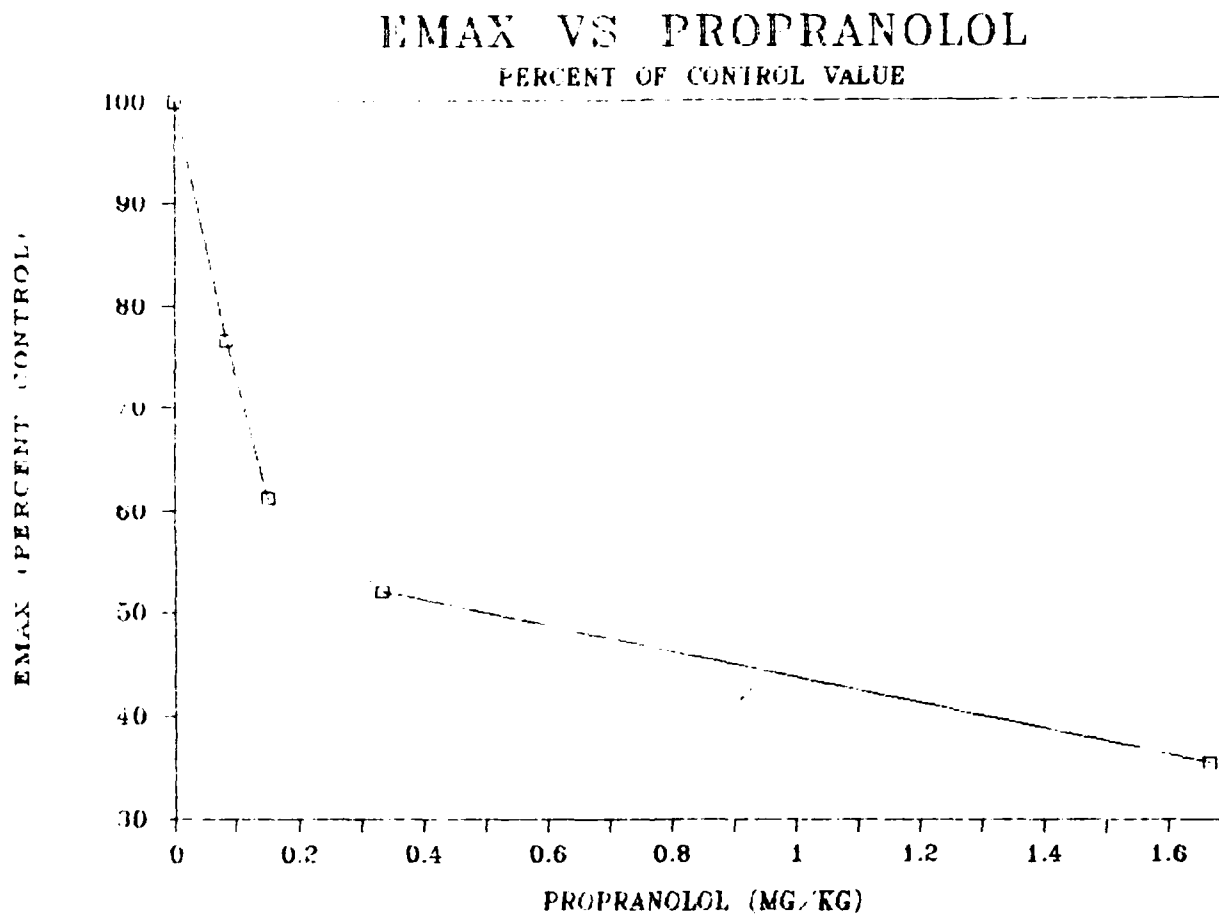


Graph of slope of the end-systolic pressure-volume relationship
(Emax) versus amount of propranolol in three pigs.

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FIGURE 4

## ESPVR
### CORRECTED FOR CONTROL
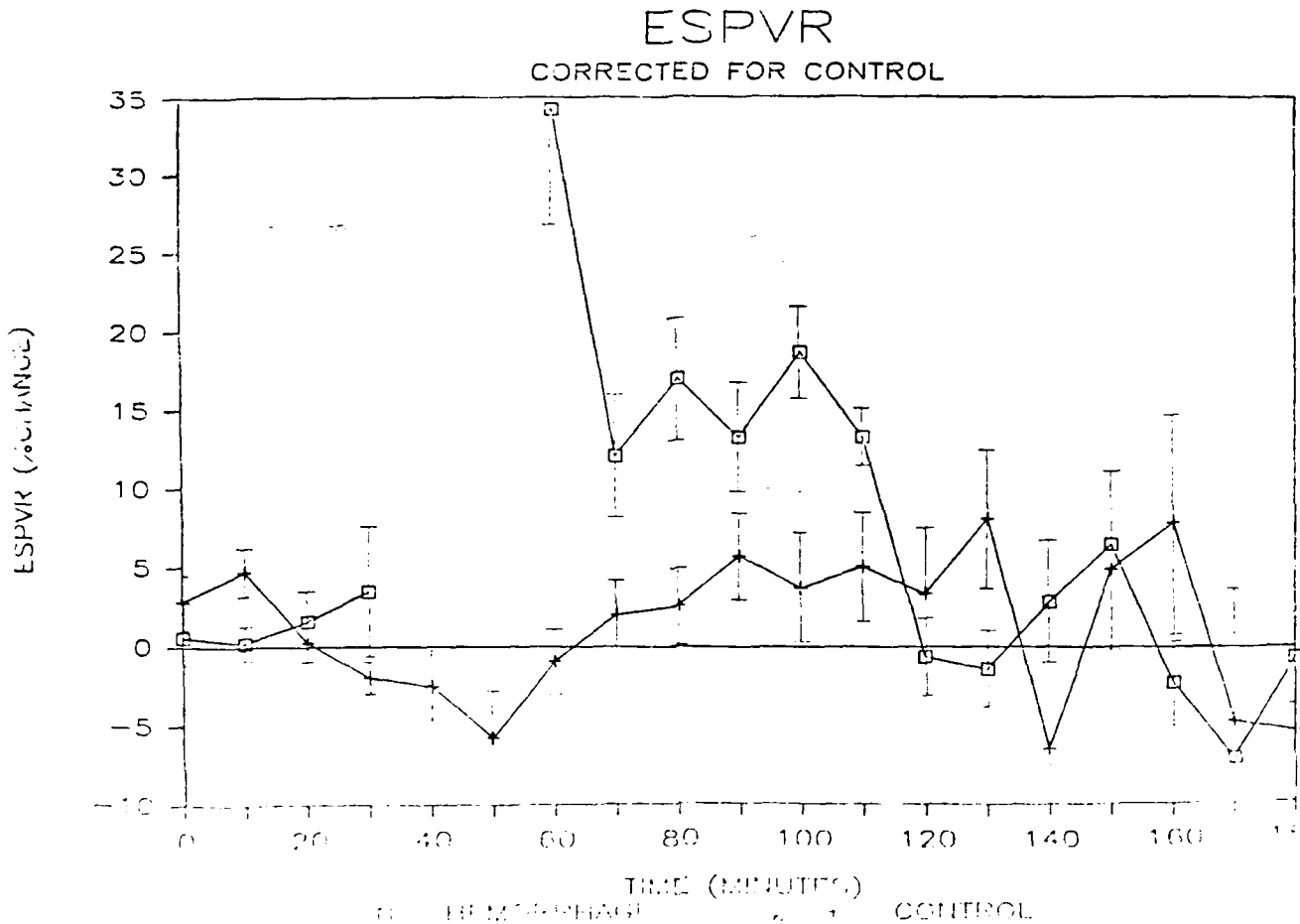


Graph of the End-Systolic Pressure-Volume relationship versus
time. No data points were taken for the 30 minutes of severe
hemorrhage. Data was normalized for percent change from control.
There was a strong correlation between ESPVR for the control
animals and time into the study ($r=0.72$, average of -0.098%
change from control/minute). Correcting for this linear decline
with time, a one-way ANOVA with repeated measures yielded
significant results for the hemorrhage group, with Dunnet's test
giving significant changes from control at 60,70,80,90,100, and
110 minutes. Not correcting for the decline in control values
with time yielded significant results only at 60 minutes. In
this and all other graphs significance is defined as $P<0.05$.

Figure 5

# - MEAN ARTERIAL PRESSURE
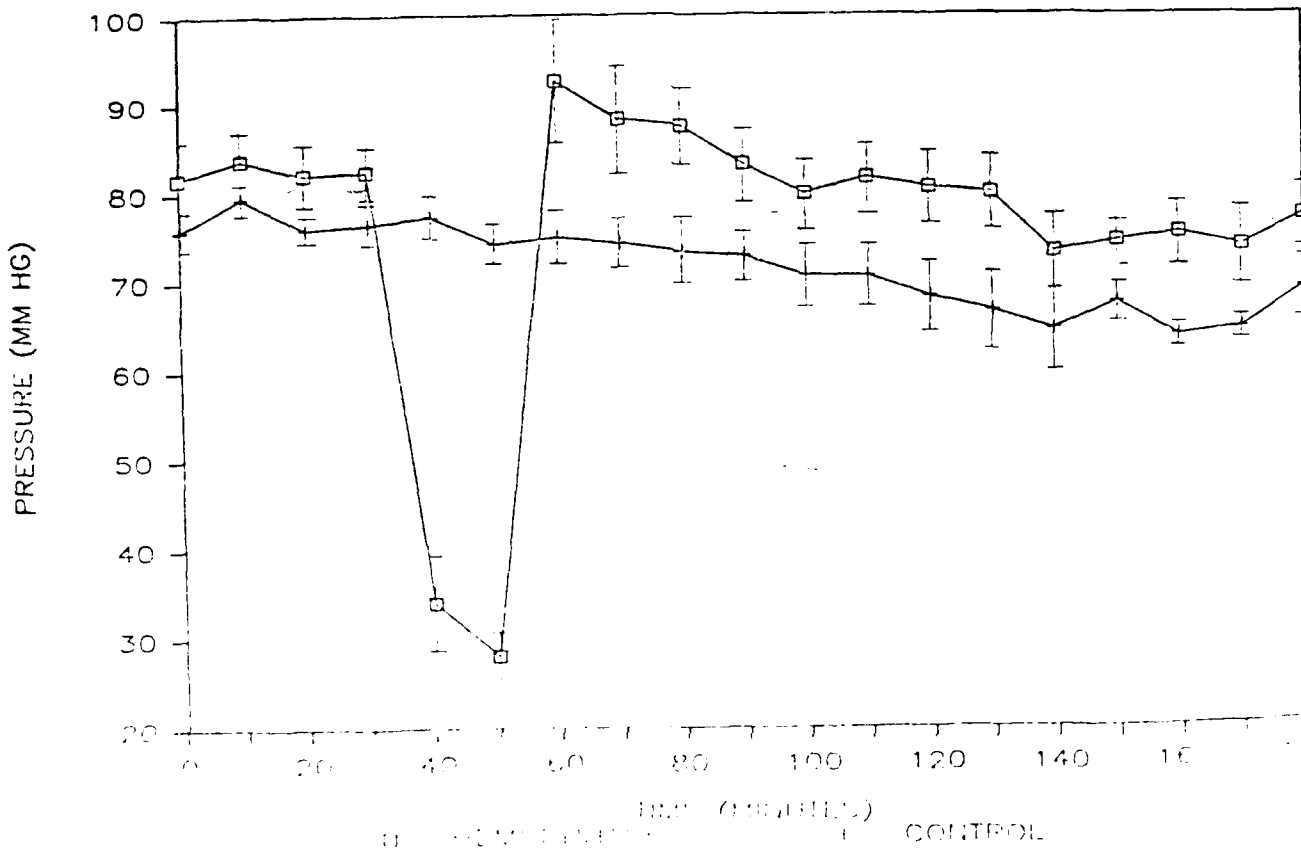


Graph of mean arterial blood pressure versus time. For the
hemorrhaged animals blood was withdrawn over a ten minute period
until the mean arterial pressure fell to 30 mm Hg. This was
maintained for ten minutes, after which all the removed blood was
reinfused. The control animals demonstrated a decline in
arterial pressure with time (r=0.731). Correcting for this in
the hemorrhaged animals, a one-way ANOVA with repeated measures
on the hemorrhage data showed significant results, with Dunnet's
test showing differences from control at 30,40,50,60, and 70
minutes.

Figure 6

# HEART RATE
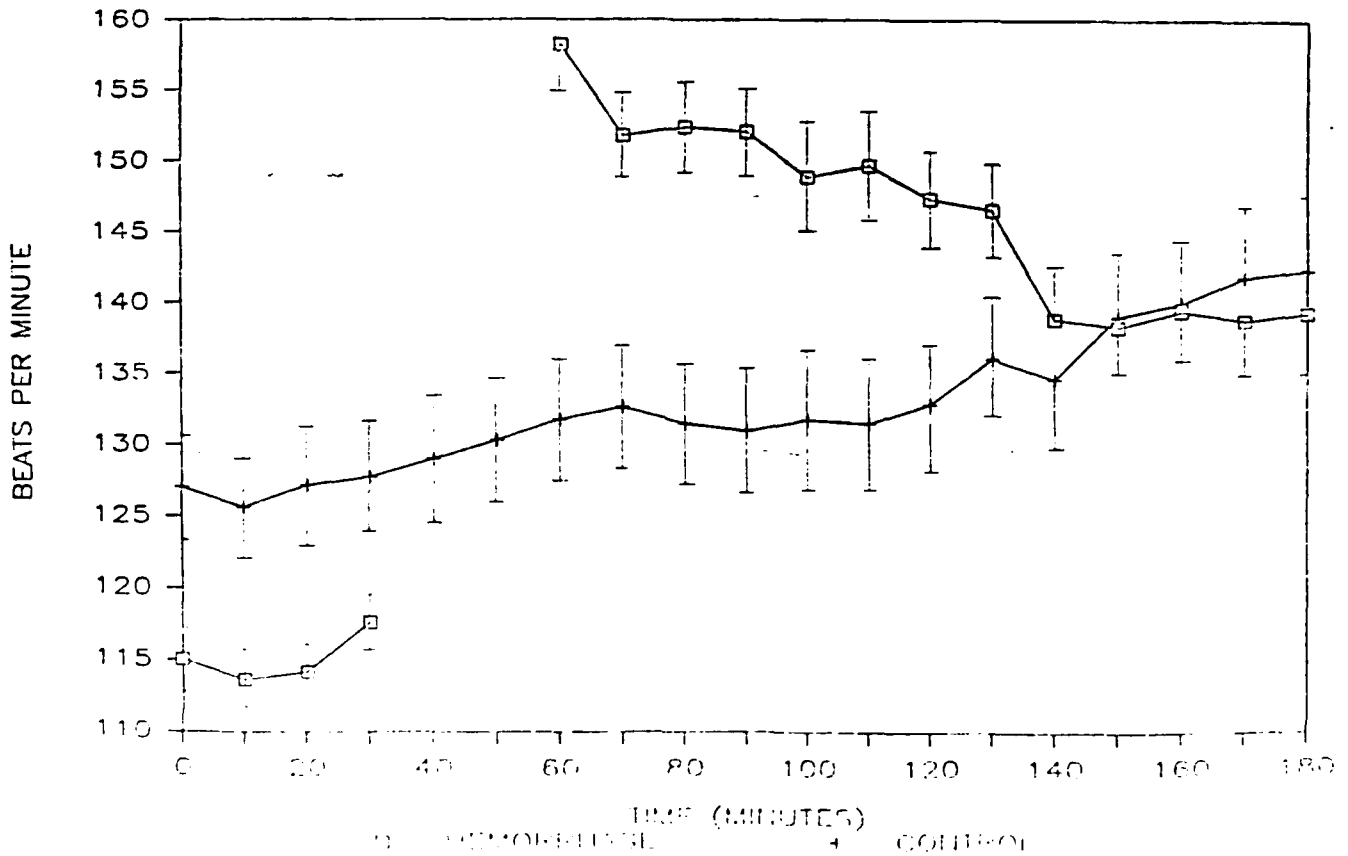


Graph of average heart rate versus time. The heart rates
for the control animals showed a strong rise with time
(r=0.74). Correcting for this in the hemorrhaged animals,
a one-way ANOVA with repeated measures showed significant
results, with Dunnet's test showing significant differences
from control for all the post-reinfusion times. A simple
t-test on the zero-time values for both hemorrhage and
control animals showed significance.

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# Figure 7

## —CARDIAC OUTPUT
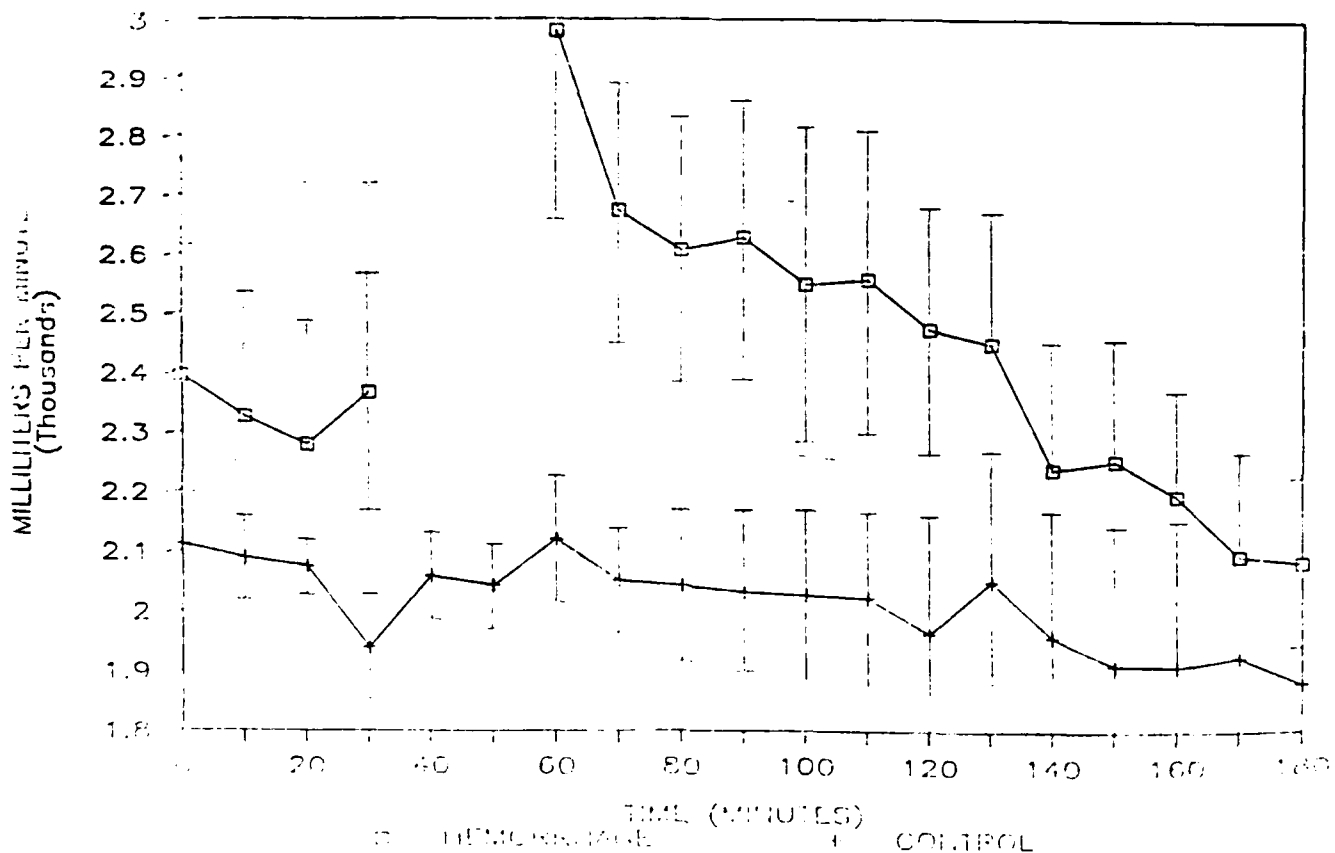


Graph of cardiac output versus time, as determined by the estimation of volume from three channels of sonomicromter data. There was a slight correlation of cardiac output versus time for the control group (r=0.69). Correcting for this in the hemorrhaged animals, a one-way ANOVA with repeated measures showed signicant results, with Dunnet's test indicating significant changes from control at 60,70,80, and 90 minutes. A simple t-test on the control versus hemorrhaged data at time = 0 did not show signicance, although it would have if applied at time = 30.

## Figure 8

# CARDIAC MECHANICAL WORK
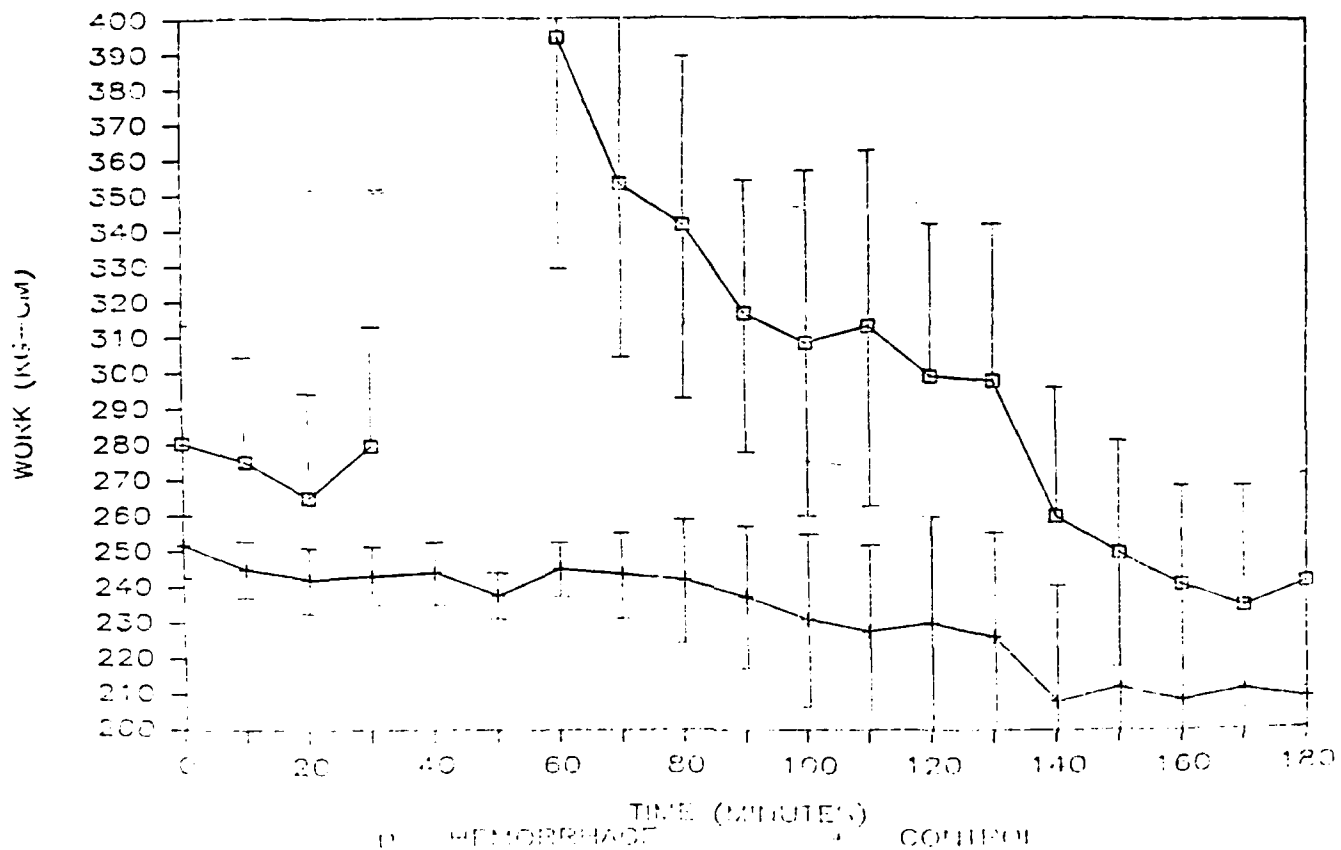


Graph of cardiac external mechanical work (neglecting
inertial work) versus time, per minute. The correlation between
work and time for the control animals was r = 0.77.
Correcting for this in the hemorrhaged animals, a one-way
ANOVA with repeated measures showed significant results,
with Dunnet's test showing significant differences from
control at 60 and 70 minutes only.  A simple t-test between
the hemorrhage and control groups at time = 0 did not
show significance at the P = 0.05 level.

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## Chronic Study

After the hemispherical crystals were obtained, the focus shifted to performing studies on chronically instrumented, lightly sedated conscious pigs. The protocol was to tranquilize the pigs with an intramuscular injection of ketamine, intubate the animal and then maintain on pentobarbital anesthesia. A left lateral thoracotomy was performed, and the left internal mammary artery and vein were cannulated. An inflatable occluder cuff was sewn around the descending aorta. At this point a lidocaine drip was started, the pericardium was opened, and additional lidocaine was applied directly on the surface of the heart. Three pairs of ultrasound crystals were sewn onto the heart as decribed for the acute open-chest study (minor axis, major axis, equatorial wall thickness), and an implantable high-fidelity pressure transducer was implanted into the left ventricle through a stab incision near the apex. All wires and catheters were tunneled subcutaneously to a point between the scapulae, and then exteriorized. A chest tube was inserted, the chest wall was then closed, and the pig was allowed to recover for seven to 10 days before data was taken. Intramuscular injections of Bicillin (Penicillin) were given prophylactically every two days post surgery.

For data collection, the pigs were lightly sedated with 10 milligrams of diazepam given through the venous catheter. Additional diazepam was given as needed to provide adequate sedation. The pigs were held at a level such that they did not actively get up and walk, but could be aroused and would respond to touch. Thus the equipment could be hooked up and data collected without disturbing or otherwise restraining the animal.

Several problems developed in the course of this protocol. First, the mortality rate at outset was extremely high, exceeding 50 percent for the first two months of the study. We were never able to get all three pairs of ultrasound crystals to function at the same time in an animal that survived. The animals would either die in surgery, or would appear to recover, but die suddenly after they appeared to be otherwise healthy. We hypothesize that these animals died of arrythmias secondary to the irritation caused by the large number of transducers sewn onto the heart. We were able to significantly improve the yield to over 80 percent by using only one pair of crystals to measure minor axis. While having only one pair of crystals precludes estimating absolute volumes, it has been shown to be a reasonably accurate method of measuring Emax (16,17,18). This single-pair approach was, in fact, the method originally proposed for these studies.

In those animals that did survive, the incidence of adhesions in the thoracic cavity proved to be a serious obstacle. The use of ultrasound crystals to estimate left ventricular volume assumes that the ventricle is reasonably symmetric, but if part of the heart is stuck to the chest wall this will no longer be true, especially if ventricular volumes are changed from

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control.  Figure 9 illustrates several pressure-diameter loops
taken from one animal at two different mean arterial pressures.

Notice that at the low pressure the curve is highly distorted,
with isovolumic phases highly deviated from the straight vertical
lines they should be and which were always the case in the acute
open-chest studies.  On autopsy significant amounts of fibrous
material was found on and around the heart and the transducers,
which was generally not associated with any infection.  The
surving animals had healthy appetites, were active, had normal
temperatures for pigs (102.5 degrees F), and blood gases in the
normal range (pO2 >80 mm.Hg, 34<pCO2<42, 7.35<pH<7.42).  End
diastolic pressures were not significantly elevated (8.2+2.9
mm.Hg.), indicating that the heart and pericardium had not fibrosed
to the point of interfering with diastolic filling.

Attempts were made to avoid the interference due to the
development of adhesions by studying the animals three days after
surgery, instead of seven to 10.  Autopsy revealed minimal
adhesions at this time, and the pressure-diameter loops had an
appropriate shape at a wide range of mean arterial pressures.
However, while the animals appeared to be alert and healthy,
their blood gases were extremely poor and unstable, indicating
incomplete recovery and unsuitability for experimentation.

## Figure 9
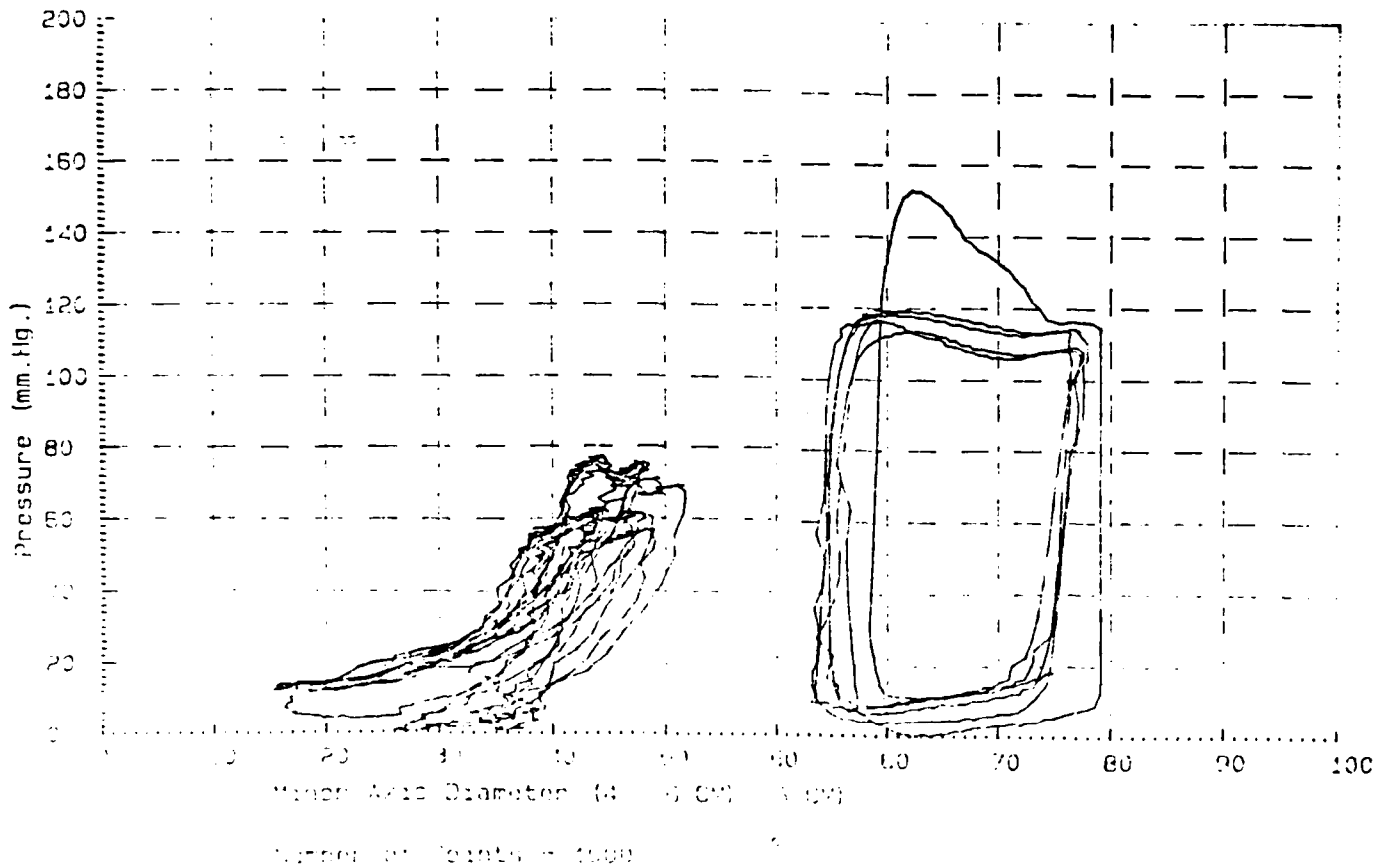


Plot of pressure-major axis diameter loops at two different mean arterial pressures in one of the chronically instrumented pigs.

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Given these problems with using ultrasound crystals in chronically instrumented pigs, we have worked on getting high-fidelity pressure-volume loops in lightly sedated pigs by introducing pressure and volume-measuring conductance catheters into the left ventricle via cutdowns on peripheral vessels. The conductance catheter technique has recently been validated against both electromagnetic flowprobe data and plethysmographic data from isolated hearts (19,20,21). Our animals are initially sedated with intramuscular ketamine, and an ear vein is catheterized. Alpha-chloralose is then used as the anesthetic agent, with lidocaine being injected subcutaneously for the cutdowns. While this is not a conscious animals preparation, the use of chloralose does not cause the depressant effects on the cirulation that pentobarbital or other anesthetics required for open-chest surgery do (22), and clearly there will be much less surgical trauma involved. A sample series of pressure-volume loops is shown in Figure 10. The change in preload was provided by transiently increasing intrathoracic pressure to occlude systemic venous return. This is accomplished with a ventilator ("ambu") bag and face mask. The lungs are quickly inflated and this volume held for five seconds. This maneuver will reduce venous return to the heart and cause the family of pressure-volume loops to move downwards and to the left.

Present plans call for documentation of the ability of conductance catheter technique to detect decrease in contractility in response to a clinically employed negative inotropic agent, verapamil. Such documentation seems very likely since this technique has been very successful in other laboratories (18,19,20). Once confirmation of our methods is obtained, we shall return to the assessment of the contractile effects of hemorrhage on closed chest animals.

## Figure 10

### ST73M35.DAT
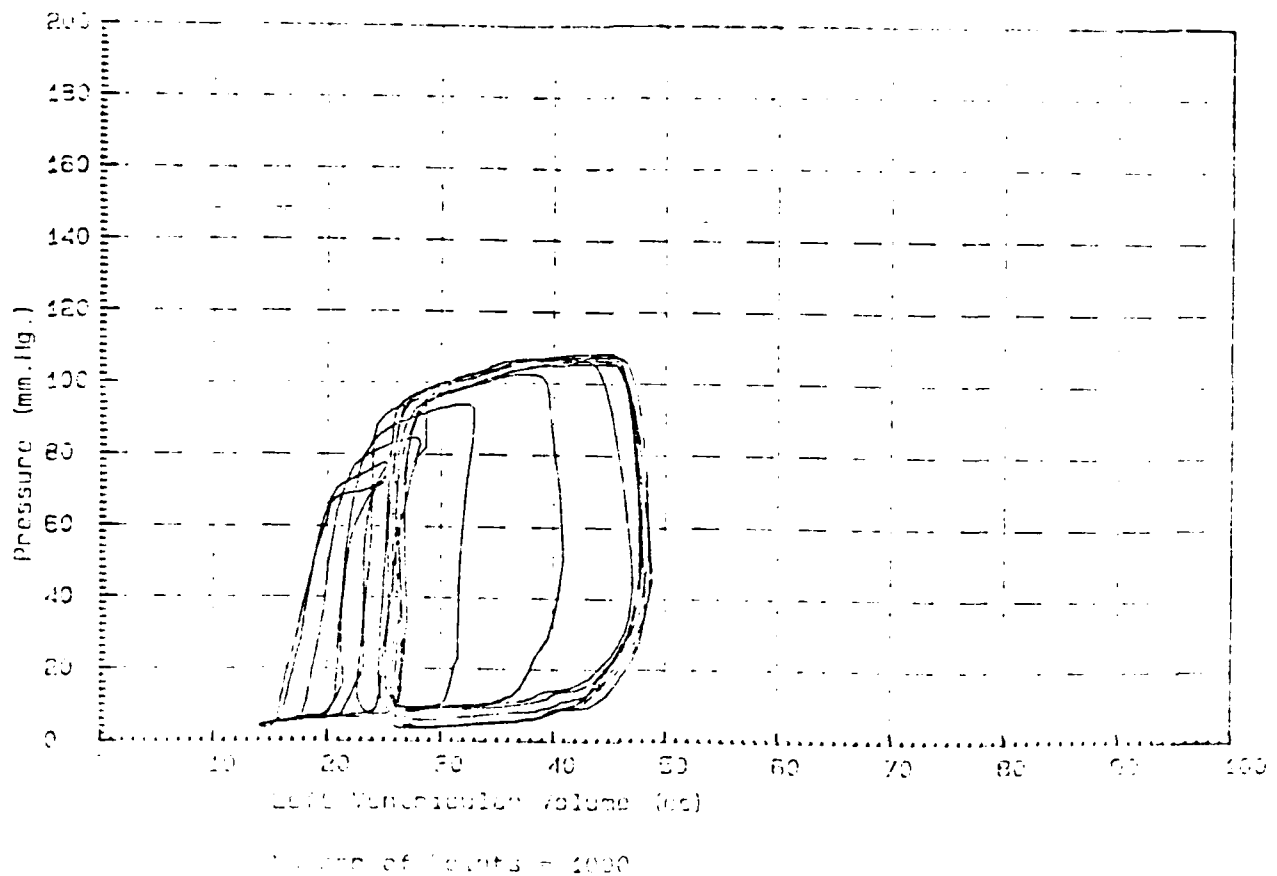


Family of pressure-volume loops recorded from a Millar catheter-tip pressure transducer and a conductance catheter introduced into the left ventricle via a cutdown on the carotid artery. THe animal was anesthetized with alpha-chloralose. The transient decrease in preload resulting in the shift of the end-systolic point downwards and to the left was caused by transiently hyperinflating the lungs.

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