Relationship of Plasma Peptides to the Myocardial Depressant Factor in Hemorrhagic Shock in Cats

By Allan M. Lefer, Ph.D., and Julian Martin, B.A.

ABSTRACT

The pathophysiological relationships of a myocardial depressant factor (MDF) present in the plasma of cats in hemorrhagic shock were studied. Aprotinin (Trasylol), an inhibitor of a variety of proteases including kallikrein and trypsin, prolonged survival of cats in postoligemic shock as well as prevented the appearance of MDF in the plasma. Trasylol did not alter the arterial blood pressure, venous pressure, or heart rate of these cats in shock, nor did it protect by exerting a positive inotropic effect. Trasylol was ineffective in preventing or reversing the negative inotropic effects of MDF in isolated cat papillary muscles. Column chromatography of plasma from control cats, from untreated shocked cats and from cats treated with Trasylol before shock showed six peptide peaks, designated A, B, C, D, E, and F in decreasing order of molecular weights. Peptides corresponding to peaks A, B, E, and F exhibited insignificant myocardial depressant activity. The peptide corresponding to peak C was present in control plasma and accounted for some of the small negative inotropic effect of control plasma. The peptide corresponding to peak D had a marked myocardial depressant effect which could account for all the activity of shock plasma. The molecular weight of this peptide was estimated to be 800 to 1000. This peptide is not bradykinin, but appears to be produced by a protease present in shock.

ADDITIONAL KEY WORDS

kallikrein papillary muscle toxic factor membrane ultrafiltration eledoisin proteases

Several investigators have reported that there is a substantial increase in the concentration of circulating peptides (1-4) or in proteolytic enzymes (2, 5) in the plasma of animals in hemorrhagic shock. However, the role of these peptides in the pathogenesis of hemorrhagic shock is not clear (6-9). Previous studies have shown that a myocardial depressant factor (MDF) present in the plasma of cats in postoligemic shock is a peptide having a molecular weight between 500 and 1000 (10). MDF accumulates in the plasma as postoligemic shock progresses, and exerts a depressant effect upon isolated heart muscle (11), upon systemic blood pressure in the intact cat (10), and upon the reticuloendothelial system (12). The purposes of this study were: (a) to determine whether MDF production could be inhibited by a protease inhibitor, and (b) to isolate MDF and determine some aspects of its chemical nature.

Methods

SHOCK PROCEDURE

Adult cats of both sexes weighing between 2.4 and 3.1 kg were used for the shock experiments. All cats were isolated for at least 10 days before use and were healthy and parasite free. They were anesthetized with pentobarbital sodium (30 mg/kg ip). Additional doses were given intravenously as needed to maintain a surgical plane of...
anesthesia but were usually not needed after hemorrhage was started. Heparin (1500 U/kg iv) was given 30 minutes before bleeding.

The cats were bled through a femoral arterial cannula into a constant-pressure reservoir. The reservoir, a Mariotte bottle, was a calibrated, clear, acrylic plastic, siliconized chamber in which the pressure was maintained constant by bubbling 95% O2-5% CO2 through the blood into the bottle at a preset hydrostatic pressure. All connecting tubing was siliconized polyethylene or Tygon. The pressure in the reservoir was set at a level which allowed the systemic arterial pressure of the cat to be maintained constant at 40 to 45 mm Hg. Rectal temperature was measured by a telethermometer and heart rate were continuously recorded on a Beckman-Offner Type RB Dynograph. When 40% of the shed blood had been taken back by the cat (120 to 160 minutes), hemorrhagic hypotension was terminated by gradually raising the pressure in the arterial reservoir to allow reinfusion of the remaining shed blood into the cat. This procedure usually took 10 to 15 minutes to insure that central venous pressure did not rise more than 2.5 cm H2O during the period of reinfusion. Ninety percent of the animals subjected to this procedure died when their mean arterial blood pressure declined to 60 mm Hg. It has previously been shown that cats in postoligemic shock rapidly reach a terminal state when their mean arterial blood pressure declines to 60 mm Hg (13). A group of five “sham shock” cats were used. In these cats, all of the surgical procedures were the same as in the shocked animals. The only difference was that the reservoir was not opened. These cats were maintained under pentobarbital anesthesia for four hours, and 30 to 40 ml of arterial blood was collected. Another group of six cats, designated controls were anesthetized and their blood collected within 20 minutes.

Aprotinin (Trasyrol,1 Delbay Pharmaceuticals, Inc.) an inhibitor of kallikrein and other proteases (10,000 kallikrein inactivator units [KIU]/kg) was administered to seven cats; an equal volume of 0.9% saline (2 to 5 ml iv) was administered to seven cats 20 to 30 minutes before bleeding. Another two cats were given 0.1 mg of thimerosal and 45 mg of NaCl as a control for the vehicle for the Trasyrol.

PROCESSING SHOCK PLASMA

When the mean arterial blood pressure of the shocked cats had fallen to 60 mm Hg (about 60 to 240 minutes after reinfusion), the carotid arterial cannula was opened, and 30 to 40 ml of blood was collected in plastic centrifuge tubes packed in ice. This blood was then directly centrifuged at 2250 × g at 4°C for 20 minutes, and the formed elements were discarded. The plasma was frozen for 24 to 48 hours and then thawed to room temperature. The thawed plasma was then placed inside dialysis tubing (Nojax tubing viscose process, Union Carbide Corp., 30 mm flat width) at 4°C under a pressure of 20 mm Hg for 40 to 48 hours. The clear solution that passes through the tubing, the plasma ultrafiltrate, contains all the myocardial depressant factor (MDF) activity of raw plasma and has the advantages of a protein-free solution (14). Ultrafiltrates of plasma were stored at −10°C until the time of the assay, when they were thawed at room temperature and gently bubbled with 95% O2-5% CO2. The pH of these ultrafiltrates was 7.2 to 7.4. The ultrafiltrates were warmed to 37°C just before assay. Some plasma samples were subjected to selective membrane ultrafiltration after conventional ultrafiltration using a Diaflo Model 10 chamber with a UM-3 membrane, and subjecting ultrafiltrate to a pressure of 2200 mm Hg for 20 to 40 minutes. The clear ultrafiltrates, which contained molecules with a molecular weight of 1000 or less, were also warmed to 37°C just before use.

BIOASSAY OF PLASMA FOR MDF ACTIVITY

Assays of myocardial depressant factor activity were performed on isolated cat papillary muscles contracting isometrically 1/sec at a temperature of 37°C, according to previously described techniques (13). All assays were performed on two papillary muscles, and the results were compared to appropriate controls; 10-ml samples were assayed in all cases. Myocardial depressant factor activity was expressed as percent decrease in developed tension of the isolated papillary muscle obtained 10 to 15 minutes after addition of the assay sample, at which time a stable contractile force was established. Contractile forces in grams were converted to developed tensions in grams per square millimeter of cross-sectional area of the muscle at the end of each experiment. Krebs-Henseleit solution as previous

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1 Donated by Delbay Pharmaceuticals Inc., Bloomfield, N. J.
**PEPTIDES IN SHOCK**

ly described (13) was used to bathe the papillary muscles and to wash out assay samples.

**INOTROPIC EFFECT OF SYNTHETIC PEPTIDES**

Synthetic bradykinin and eledoisin (Sandoz Pharmaceuticals, Hanover, New Jersey), freshly diluted in Krebs-Henseleit solution for each experiment, were added to the bath and the volume made up to 10 ml with Krebs-Henseleit solution. Readings were made at the peak of the effect on developed tension.

**SPECIAL PROCEDURES**

**Column Chromatography**

Ten-milliliter samples of plasma ultrafiltrate conventionally obtained were lyophilized to dryness and then reconstituted to 2 ml with distilled water. The 2-ml samples were centrifuged at 2250 × g for 10 minutes, and the supernatant fluid was applied to a column 90 cm by 1.5 cm (i.d.) containing Bio-gel P-2 (200 to 400 mesh) polyacrylamide gel suspended in glucose-free Krebs-Henseleit solution. Purified cat hemoglobin was used as a marker for detection of column bed irregularities. The flow rate of the column ranged from 0.15 to 0.20 ml/min, but was constant for each sample run. All column chromatography was conducted at 4°C and samples were collected by an ISCO Model 270 fraction collector at 10- to 20-minute intervals. Peptide peaks were estimated by optical density measurements at 230 μm, the optimal absorbance for peptide bonds, using quartz cuvettes in a Beckman Model DU spectrophotometer.

Samples of Krebs-Henseleit solution were taken before (column inflow) and after (column outflow) applications of a sample for bioassay for MDF activity, along with all peaks. The eluates for each plasma sample applied to the column were composed of six distinct optical absorption peaks. Samples of column inflow and outflow were periodically checked for bacterial contamination, but none was found. Nevertheless, the column was washed with Krebs-Henseleit solution containing penicillin (100 U/ml) every 2 to 3 weeks. Three to five column volumes were used to wash the antibiotic solution out of the column. Mixtures of angiotensin II (Hypertensin, Ciba Pharmaceutical Co.) and glycyl-glycine (Nutritional Biochemicals Corp.) were applied to the column for molecular weight markers.

**Protein Determination**

Five complete sets of peptides present in the column peaks (230 μμ) were assayed for the presence of protein using the ninhydrin method of Clark (15), and were read at 570 μm against a reagent blank.

**Incubation of Plasma with Kallikrein**

Twenty-milliliter samples of normal cat plasma (collected as described earlier) or Krebs-Henseleit solution were incubated at 37°C for 2 hours without added oxygen. The plasma or buffer samples contained 100 kallikrein units/ml of hog pancreatic kallikrein (50 KIU/mg). Some plasma samples also contained 1 mg/ml of 8-hydroxyquinoline, an antagonist of carboxypeptidase B and other proteolytic enzymes. All samples were ultrafiltered by conventional means for 48 hours and assayed twice on cat papillary muscles. The plasma ultrafiltrates were then lyophilized and applied to the Bio-gel column as described above. The peptides present in the peaks obtained were assayed on cat papillary muscles according to standard techniques.

**Results**

The effects of Trasylol (10,000 KIU/kg) on the hemodynamic status of cats subjected to hemorrhagic shock are shown in Table 1. Trasylol did not decrease mean arterial blood pressure when compared to the saline controls in cats prior to induction of hemorrhagic shock. The Trasylol vehicle had no effect on any variable measured. Trasylol had no detectable effects on central venous pressure, heart rate, bleedout volume, duration of hypotension, or time to peak bleedout. Thus Trasylol did not alter the cardiovascular variables measured in the cats before hemorrhage nor the intensity or duration of the oligemic period. However, it markedly prolonged the post-reinfusion survival time ($P < 0.001$).

The prolongation of survival was accompanied by a significantly lower ($P < 0.001$) titer of MDF in the plasma obtained from these cats, when compared with the depressant activity obtained from cats treated first with saline. Figure 1 shows a graph of the depressant activity of plasma from these cats as well as of the control and sham shock cats for reference points. The ultrafiltrates of plasma from sham shock cats had depressant activity that was not significantly different from that of plasma ultrafiltrates of control or of Trasylol-treated shock cats. Myocardial depressant activities in the two cats classified as survivors were −18% and −23%.

2Donated by Farbenfabriken Bayer, Wuppertal-Elberfeld, West Germany.
TABLE 1
Effect of 10,000 KIU/kg Trasylol (Aprotinin) on Cardiovascular Function in Hemorrhagic Shock in Cats

<table>
<thead>
<tr>
<th>Hemodynamic variable</th>
<th>Shock + saline (n = 6)</th>
<th>Shock + Trasylol (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure</td>
<td>Control (mm Hg)</td>
<td>155 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>After drug (mm Hg)</td>
<td>145 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>After reinfusion (mm Hg)</td>
<td>146 ± 3.7</td>
</tr>
<tr>
<td>Central venous pressure</td>
<td>Control (cm H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>After reinfusion (cm H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>Heart rate</td>
<td>Control (beats/min)</td>
<td>212 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>After reinfusion (beats/min)</td>
<td>210 ± 6.9</td>
</tr>
<tr>
<td>Bleedout volume (ml/kg)</td>
<td></td>
<td>35.2 ± 3.4</td>
</tr>
<tr>
<td>Duration of hypotension (min)</td>
<td></td>
<td>122 ± 18.8</td>
</tr>
<tr>
<td>Time to peak bleedout (min)</td>
<td></td>
<td>54 ± 9.9</td>
</tr>
<tr>
<td>Survival time (min)*</td>
<td></td>
<td>78.4 ± 7.2</td>
</tr>
</tbody>
</table>

All values are means ± SE; n = number of cats in each group.  
*Defined as the time from completion of reinfusion to the time at which MABP declined to 60 mm Hg. †P < 0.001 when compared to shock + saline controls.

The nature of the protective effect of Trasylol on survival of cats in postoligemic shock was investigated. Figure 2 shows data clearly indicating that Trasylol did not exert an inotropic effect in cat papillary muscles, nor did it modify the inotropic effect of control or shock plasma ultrafiltrates when added to papillary muscles along with the ultrafiltrates. Furthermore, Trasylol did not reverse the negative inotropic effects of ultrafiltrates plasma from shock cats. Figure 3 represents the depressant effect of a sample of shock plasma ultrafiltrate containing MDF. Trasylol failed to reverse this depressant effect in four such experiments. The change in developed tension after addition of Trasylol was less than 5% in all four experiments. Trasylol (10,000 KIU/kg) was administered to three additional cats in which a strain gauge arch was sutured to the right ventricle. The segment of myocardium directly under the gauge was stretched to 25% of its end-diastolic length. Trasylol did not exert any significant change in developed tension over a 2-hour period. Thus, the beneficial effects of Trasylol are unrelated to a positive inotropic effect of the drug.
Since Trasylol is an antagonist of kallikrein and other proteases (16, 17), and since it appeared to block the production of MDF in shock, MDF was thought to be a peptide produced by proteases. In an attempt to obtain further data on this point, synthetic bradykinin and eledoisin were added to cat papillary muscles to determine whether these peptides possessed any negative inotropic activity. Table 2 shows that bradykinin has a moderate positive inotropic effect at both 1 and 10 µg/ml, whereas eledoisin has a marked negative inotropic effect at these concentrations. The small positive inotropic effect of 10 µg/ml of bradykinin was not statistically different from that of 1 µg/kg.

In an attempt to isolate, purify, and determine the molecular weight of MDF, column chromatography (gel filtration) was carried out on ultrafiltrates of plasma from

**TABLE 2**

<table>
<thead>
<tr>
<th>Peptide concentration (µg/ml)</th>
<th>Percent change in developed tension</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Bradykinin</th>
<th>Eledoisin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.0 ± 0.0</td>
<td>-1.2 ± 1.1</td>
</tr>
<tr>
<td>1</td>
<td>+4.2 ± 0.7</td>
<td>-26.2 ± 4.6</td>
</tr>
<tr>
<td>10</td>
<td>+13.3 ± 3.8</td>
<td>-77.8 ± 2.9</td>
</tr>
</tbody>
</table>

All values are means ± SE in response to addition of each dose to six papillary muscles. Both bradykinin and eledoisin were diluted in Krebs-Henseleit solution from a concentrated stock solution of peptide which was made up in distilled water.
Peak contractile force (upper line) and resting contractile force (lower line) of a typical papillary muscle response to Trasylol 11 minutes after addition of an ultrafiltrate of plasma containing myocardial depressant factor. The peak contractile force minus the resting contractile force is equal to the developed contractile force. This graph is representative of four such experiments. The concentration of Trasylol in the bath (200 KIU/ml) was calculated to be that in the blood, assuming all of the Trasylol was retained in the intravascular compartment.

Figure 4 shows a graph of the column elution pattern from a shock plasma ultrafiltrate placed on a Bio-gel P-2 column. The original plasma ultrafiltrate had a myocardial depressant activity of 60%. There were six peaks in the sample, designated peaks A through F. Two markers, angiotensin and glycyl-glycine, were placed on the column after the shock sample was eluted. Thus it can be seen from Figure 4 that the substances represented by peaks A, B, and C have molecular weights larger than 1050, whereas those in peaks D, E, and F have molecular weights between 130 and 1050. The only two peaks with any pronounced depressant activity were peaks C and D. The activity in peak D could account for all the original activity in the plasma ultrafiltrate (Table 4).

Figure 5 is a column elution pattern for a control plasma ultrafiltrate. Only peak C is of significant size, and since it is present in control plasma, it cannot be MDF.

Figure 6 shows the elution pattern from a plasma sample obtained from a shocked cat treated with Trasylol before bleeding. Prominent are peaks A, C, and E. Peaks D and F are almost entirely absent. Peak B is about the same in all samples and has so small an area that it may not be a separate peptide. Peak C again can account for all the activity of the original ultrafiltrate.

Figure 7 shows the elution pattern for plasma from a shock cat; the sample was filtered through a selective membrane that allowed only molecules of 1000 M. W. or smaller to pass through the membrane. This ultrafiltrate gave activity similar to the conventional ultrafiltrate used in Figure 4. It is evident from Figure 7 that only peaks D, E, and F are present in this sample. The entire
activity can be accounted for by the activity present in peak D.

Table 3 summarizes the data relevant to the substances present in the six peaks eluted from the Bio-gel P-2 column. MDF was found in peak D. The other peaks are not completely identifiable at the present time.

Table 4 summarizes the inotropic activity of peptides corresponding to the six identifiable peaks. The eluant (Krebs-Henseleit solution minus glucose passed through a Bio-gel P-2 bed) demonstrated a 12 to 13% average depression when assayed against normal Krebs-Henseleit solution. If one subtracts this activity from all the assay values, it is evident that peaks A, B, E, and F exerted no

**TABLE 3**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Approximate molecular weight</th>
<th>Ninhydrin test</th>
<th>Presence in shock plasma</th>
<th>Presence in control plasma</th>
<th>Prevented by Transylol</th>
<th>Myocardial depressant activity</th>
<th>Probable identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&gt;1600</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>no</td>
<td>0</td>
<td>Large peptides, proteins</td>
</tr>
<tr>
<td>B</td>
<td>1300</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>no</td>
<td>0</td>
<td>Unknown</td>
</tr>
<tr>
<td>C</td>
<td>1100</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>no</td>
<td>slight</td>
<td>Normal component of plasma</td>
</tr>
<tr>
<td>D</td>
<td>800-1000</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>yes</td>
<td>very great</td>
<td>MDF</td>
</tr>
<tr>
<td>E</td>
<td>500-700</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>no</td>
<td>0</td>
<td>Small peptide(s)</td>
</tr>
<tr>
<td>F</td>
<td>200-300</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>yes</td>
<td>0</td>
<td>Very small peptide(s)</td>
</tr>
</tbody>
</table>

**FIGURE 4**

Graph of optical density at 230 μm vs. elution volume in milliliters (column elution pattern) of a typical sample of ultrafiltrate of plasma from a shock cat placed on a Bio-gel P-2 column. The original ultrafiltrate assayed at a 60% depression of developed tension. The assay of each peak is shown above the peak. The positions of angiotensin II and the dipeptide glycylglycine are shown at the bottom for markers. Peak D contained all the myocardial depressant factor activity of the original sample.

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appreciable depression. Only peaks C and D possessed significant negative inotropic activity. If one compares these corrected values with the original ultrafiltrates, the activity in control and Trasylol pretreated shock samples can be accounted for from the activity in peak C, and one can account for all the MDF activity in shock samples from the activity in peak D.

The conventional plasma ultrafiltrates of the

*Column elution pattern of an ultrafiltrate of control cat plasma. The original ultrafiltrate assayed at a 24\% depression of developed tension. Little or no myocardial depressant factor activity was present in this sample after elution from the column. Peak C contained all the activity present in the original ultrafiltrate.*

*Column elution pattern of an ultrafiltrate of plasma obtained from a shock cat treated with Trasylol (10,000 KIU/kg) before shock. Note the lack of myocardial depressant factor activity and the similarity of the original assay (20\%) and the assay of all the peaks with that of the control sample shown in Figure 5.*
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3.0 -
2.5 -
2.0 -
1.5 -
1.0 -
0.0 -

BIOASSAY RESULTS
-10% -13% -17% -62% -5% -10%

PLASMA UF
ORIGINAL ASSAY = -53%

FIGURE 7

Column elution patterns of an ultrafiltrate of shock plasma refiltered through a Diaflo UM-2 membrane with a cutoff at a molecular weight of 1000. Note the almost complete absence of peaks A, B, and C, all of which contain compounds with molecular weights above 1,000. All the myocardial depressant factor activity of the original ultrafiltrate was retained and was accounted for in peak D.

TABLE 4

Inotropic Activity of Peptide Peaks (Percent Decrease in Developed Tension)

<table>
<thead>
<tr>
<th>Assays of original ultrafiltrates</th>
<th>Control (4)</th>
<th>Shock + saline (6)</th>
<th>Shock + Trasylol (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20.2 ± 3.7</td>
<td>58.1 ± 3.3</td>
<td>22.2 ± 3.5</td>
</tr>
<tr>
<td>Peak</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>11.5 ± 4.5</td>
<td>11.1 ± 3.5</td>
<td>18.3 ± 7.1</td>
</tr>
<tr>
<td>B</td>
<td>17.3 ± 5.8</td>
<td>13.7 ± 1.9</td>
<td>17.2 ± 1.1</td>
</tr>
<tr>
<td>C</td>
<td>28.1 ± 3.5</td>
<td>31.4 ± 2.9</td>
<td>32.8 ± 2.7</td>
</tr>
<tr>
<td>D</td>
<td>16.5 ± 1.8</td>
<td>68.8 ± 3.1</td>
<td>19.1 ± 4.3</td>
</tr>
<tr>
<td>E</td>
<td>12.0 ± 4.6</td>
<td>13.2 ± 2.3</td>
<td>21.5 ± 4.1</td>
</tr>
<tr>
<td>F</td>
<td>12.4 ± 1.3</td>
<td>16.7 ± 3.1</td>
<td>17.5 ± 2.8</td>
</tr>
</tbody>
</table>

Column controls (Krebs-Henseleit solution): Column inflow = —12.8 ± 2.7% (6 samples); column outflow = —13.5 ± 3.1% (6 samples).

All values are mean percent decreases in developed tension ± ș. Number in parentheses is number of cats in group.

Kallikrein incubation experiments could not be accurately assayed because 8-hydroxyquinoline produced a positive inotropic effect of about 60 to 80% when added to the cat papillary muscles. However, when the samples were eluted from the column, four peaks appeared, corresponding to peaks A, C, D, and E of the previously cited samples. The peptide present under peak D assayed at —46% in three samples; no other peak exhibited an average activity greater than —12%. The column controls showed activities of —10% in these experiments. The average activity of peak D in two control incubates of plasma was —21%. Thus a substantial activity was found in kallikrein-incubated plasma samples,
but this degree of myocardial depressant activity is not as high as that found in the plasma of cats in late postoligemic shock.

In two cats, graded doses of kallikrein were injected intravenously and mean arterial blood pressure recorded. Doses of less than 0.2 mg were ineffective, and a dose of 0.6 to 1.0 mg elicited a peak depressor response of 50 to 75 mm Hg. These depressor responses were transient, the arterial pressure returning to control pressure in 5 to 15 minutes. Samples of material from peaks A and D were injected intravenously in three other pentobarbital-anesthetized cats. A transient decrease in mean arterial blood pressure of 30 to 50 mm Hg resulted only with addition of 5 ml of peak D. Peak A exerted a slight increase in mean arterial blood pressure.

**Discussion**

The data presented in this paper illustrate a close relationship between plasma protease systems and the production of MDF. For example, MDF formation has been shown to be prevented by cortisol (10), and in this study by Trasylol. Both of these agents are known to inhibit the formation of a variety of proteases which can release peptides (16-19). MDF is a peptide with a molecular weight in the range of the known peptides released by proteases, such as kinins (about 800 to 1000). MDF has a hypotensive effect (10) similar to that induced by kallikrein in the intact animal (20), and it has a negative inotropic effect. However, no known naturally occurring mammalian kinin exerts a large negative inotropic effect. Incubation of normal plasma with kallikrein produced moderate MDF activity, but since the kallikrein preparation was not pure, the activity could represent the cleavage product of several proteases other than kallikrein, such as trypsin.

The precise mechanism, including the stimulus, for the release of MDF in hemorrhagic shock is not clearly understood. Presumably ischemia, hypoxia, or both are the initiating events in triggering the MDF-producing system. Whether lysosomal disruption is an intermediate step in the production of peptides such as MDF, as postulated by Sutherland et al. (21) and Lefer and Martin (10) cannot be evaluated at this time.

MDF accumulates in the plasma of cats in postoligemic shock probably because the usual means of clearance of this substance are impaired. The kidney and the reticuloendothelial system are likely sites of MDF removal, and both are severely depressed in postoligemic shock (12, 22). In the normal animal, MDF is probably cleared rapidly, thus explaining the transient nature of the depressor effect of MDF in shocked animals not in shock (10).

MDF appears to be a peptide in the molecular weight range of 800 to 1000. Furthermore, it has previously been shown to be destroyed by the nonspecific protease pronase (10). MDF has some characteristics in common with kinins—it is a peptide of the same molecular weight as kinins and is released by proteases—and may represent a kinin not previously described. However, there is not sufficient proof that MDF is a kinin. It certainly is not bradykinin, because it is a smaller molecule than bradykinin and bradykinin is not a negative inotropic agent.

A normally occurring peptide (peak C) in plasma that has a moderate negative inotropic effect is slightly larger than MDF and may act on the same receptor as MDF. If it does, it has a weaker affinity for the receptor, since its effect is not seen in shock plasma; that is, the effects of peaks C and D are not additive, but when both are present, only the effect of peak D is seen. Another possibility is that the effects of the peptides in peaks C and D are additive, but that the peptide in peak D saturates the available receptors and prevents the effect of the peptide in peak C from being expressed.

None of the other peptides (peaks A, B, E, and F) is of any significance in the myocardial depressant activity in shock. However, these other peptides may exert other effects, some of which are unknown; some may represent other known peptides (e.g., angiotensin, vasopressin). In this regard, MDF itself is known to be a reticuloendothelial depressant agent (12), and its hypotensive effect in the
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intact cat (10) may be due to a direct effect on vascular smooth muscle. MDF may in fact have a wide spectrum of biological effects.

Acknowledgment

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