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Research Article

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Departments of Medicine and Pathology, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts 02115

ABSTRACT Components of the complement system are known to play an important role in the cytolytic process and in chemotaxis of leukocytes. Cobra venom factor specifically cleaves C3 activity via activation of the alternative (properdin) complement pathway. It does not act directly on C3. If C3 is involved in tissue necrosis after ischemic injury, cobra venom factor might reduce tissue damage after acute coronary occlusion. Accordingly, in 14 control dogs occlusion of the left anterior descending artery was carried out for 24 h. Epicardial electrograms were recorded 15 min after occlusion, and 24 h later transmural specimens for creatine phosphokinase activity (CPK) and for histological analysis were obtained from the same sites. In another 14 experimental dogs, 20 U/kg cobra venom factor was given intravenously 30 min after occlusion. Serum complement levels fell within 2–4 h to <20% of normal. In the control dogs, the relationship between ST-segment elevation and CPK activity 24 h later was: log CPK = −0.06 ST + 1.48 (n = 111 specimens, 14 dogs, r = 0.77). In the experimental dogs, log CPK = −0.024 ST + 1.46 (n = 111 specimens, 14 dogs, r = 0.60), showing significantly different slopes ($P < 0.001$), i.e., less CPK depression for any level of ST-segment elevation. Histologically, 69 of 71 sites (97%) with ST-segment elevation exceeding 2 mV in the control dogs showed signs of necrosis 24 h later, whereas in the experimental group only 43 of 79 sites (54%) with abnormal ST-segment elevations showed signs of necrosis ($P < 0.0005$). At the same time, it was shown that the administration of cobra venom factor did not alter cardiac performance, collateral blood flow to the ischemic myocardium or the clotting system, but infiltration of polymorphonuclear leukocytes into the myocardium was decreased. It is concluded that cobra venom factor, by reducing the amount of C3 and C5 substrate available for chemotactic factor generation, or other as yet undefined mechanisms, protects the ischemic myocardium from undergoing necrosis, as judged by histology and local CPK activity. Hence, a new approach to limiting the extent of myocardial infarcts after experimental coronary occlusion, based upon inhibition of complement-dependent inflammatory processes, is demonstrated.

INTRODUCTION

Death in patients hospitalized with acute myocardial infarction is mainly due to the mechanical failure of the myocardium (1). Such cardiac failure is more likely the result of large noncontractile zones consequent to large myocardial infarction (2, 3). Accordingly, several investigations have been carried out in an attempt to limit the extent of the damage to the myocardium after a coronary occlusion, while this injury is still in the reversible phase, consequently limiting the size of the established myocardial infarction (4). In these experimental studies, myocardial damage was reduced with agents aimed at decreasing myocardial oxygen consumption (5–9), at increasing coronary flow either directly by reperfusion (10, 11) or indirectly by enhancing collateral flow (5, 6, 12), by enhancing anaerobic metabolism (13), and by the administration of hyaluronidase (14) and hydrocortisone (15). The application of these principles to the therapy of patients with acute myocardial infarction using some of these interventions seems to confirm these experimental conclusions (4, 6, 16–21).

Recent advances in the understanding of the role that is played by the complement system in nonspecific injury of the organism (22–25) and the possibility that the complement system may also play a role in

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Dr. C. B. Carpenter is an investigator of the Howard Hughes Medical Institute.

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production of myocardial injury caused by anoxia (26) stimulated this investigation of the effect of inhibiting the activity of C3 with cobra venom factor (24–28) on infarct size after acute experimental coronary artery occlusion.

METHODS

Animal preparation and protocols. Studies were carried out in 28 mongrel dogs anesthetized with intravenous sodium thiamylal (25 mg/kg). Respiration was maintained by a Harvard respirator (Harvard Apparatus Co., Inc., Millis, Mass.). The heart was exposed through a left thoracotomy and suspended in a pericardial cradle. The left anterior descending coronary artery or its apical branch was dissected free from the adjacent tissue and, when desired, occluded with a ligature. The left arterior descending coronary artery was not occluded at a certain distance from its origin, because this procedure, due to the anatomic variability of coronary distribution does not result in infarctions of comparable size. As previously described (5, 14), epicardial electrograms were obtained from 10 to 15 sites on the anterior surface of the left ventricle distributed in the area supplied by the occluded artery as well as areas remote from it, presumably adequately perfused. Each site selected for electrocardiographic recording was recognized by its specific relationship to the branching of the coronary arteries and veins. Sites were chosen from within the area supplied by the occluded vessel, from distant regions (and, therefore, presumably normal), and from the border zone. Inasmuch as the distribution of the branches of coronary arteries is different in each animal, sites for electrocardiographic recordings were located arbitrarily in each dog. The input impedance of the recorder amplifier was 100 Mohm, and the frequency response of the system was ±0.5 dB from 0.14 to 70 Hz. The impedance of the electrode was maintained constant, as reflected in the reproducibility of the tracings. The electrode employed was a 15 mm^2 copper cylinder with a saline-soaked wick connected to the precordial "V" lead and held by a cable perpendicular to the electrode, thus minimizing mechanical stress. Because of the large area of the electrode, small variations in location did not change the configuration of the recordings. The electrograms obtained with this system are reproducible after subsequent coronary artery occlusion (5). The site of occlusion was selected so that it might result in a moderate-sized area of infarction (i.e. with a range of 5–25 g) so that, on the one hand, enough myocardium would be available for biopsies and, on the other hand, the area would not be so extensive as to result in focal infarction block (i.e., QRS duration over 0.065 s or intrinsicoid deflection exceeding 0.040 s [29]). Electrocardiographic recordings were obtained before occlusion and 15 min after.

The chest was then closed in layers and drained with an underwater catheter. The dogs were then divided alternately into two groups: a control group (14 dogs) in which no therapy was administered and an experimental group (14 dogs) in which cobra venom factor (CVF)[1] (20 U/kg) was administered intravenously in a bolus 30 min after occlusion. Otherwise the two groups were treated identically, and each dog received saline 20 ml/kg per 24 h intravenously. During this 24-h period, arterial pressure was monitored through a Statham P23Db pressure gauge (Statham Instruments Div. Gould Inc., Oxnard, Calif.), and electrocardiograms (lead aVF) were monitored continuously. 24 h after occlusion the dogs were reanesthetized, reintubated, the chests were reopened and the hearts excised. Specimens were taken from eight sites from which epicardial electrograms had previously been obtained. They were selected to include two specimens from the normal myocardium and six specimens from sites with ST-segment elevations. A specimen could include either one or two electrocardiographic sites with the same ST-segment elevations. These were transmural biopsies and were divided longitudinally in two, one specimen of 400 mg was analyzed for creatine phosphokinase (CPK) activity and the other part of the specimen (≅100 mg) was fixed in Bouin's solution for histologic examination using hematoxylin and eosin staining.

Venous clotted blood samples for measurement of complement activity were obtained from all dogs before operation, just before CVF administration and then at 1, 2, 4, 6, 8, 12, and 24 h post-CVF injection. In the control dogs, blood samples were obtained at comparable periods. Blood samples were also obtained for determination of white blood cell and platelet counts, prothrombin time, partial thromboplastin time, and fibrinogen concentration. In an additional two healthy dogs, the same amount of CVF was injected intravenously, and complement levels observed in the blood for 2 wk, to determine the duration of complement level depression.

In 10 additional dogs with coronary artery occlusions (five controls and five CVF treated), regional myocardial blood flow was measured 15 min after coronary artery occlusion (i.e. before CVF administration) and again 6 h later, by the radiolabeled microspheres method (30–32), to assess whether CVF administration altered the usual pattern of evolution of collateral flow to the ischemic zone after occlusion.

In two control dogs, biopsies were taken from normal and from infarcted tissue 24 h after occlusion and analyzed by immunofluorescent methods. The biopsies were quick frozen, and 4-μm sections were cut and stained with fluorescein-labeled sheep anti-dog IgG, C3, fibrinogen, and albumin. These antisera were monospecific by immunoelectrophoresis.

In four other dogs with a left anterior descending coronary artery occlusion, a rigid cannula was placed in the left ventricular cavity through the apex, and the effect of the intravenous CVF administration on left ventricular systolic and end diastolic pressure and dP/dt recorded, to assess whether CVF has a direct effect on myocardial function.

Myocardial CPK analysis was carried out as previously described (5) by spectrophotometric assay. CVF itself did not influence the CPK assay system as judged by experiments with purified CPK, myocardial tissue slices, and homogenate with and without added CVF.

Histological studies were carried out on 5-μm thick histologic sections of tissue that had been fixed in Bouin's solution, dehydrated in increasing concentrations of ethyl alcohol, cleared in toluene, embedded in paraffin, and stained with hematoxylin and eosin. Each specimen was coded, and thus the histologic examination was carried out by the pathologist without knowledge of the origin of the specimen. The sections were classified as normal or as showing signs of early myocardial necrosis as described in detail previously (13). The results were then analyzed using the Chi-square test.

In addition, in each slide the number of neutrophils per five high power fields (×400) were counted in the border area of the infarct which is the usual location of these cells 24 h after coronary occlusion (24-h-old infarctions and their borders can be localized by gross inspection of the heart). Thereafter, the number of neutrophils in the five fields was averaged. The number of neutrophils contained in the area of ST-segment elevation was then compared between the CVF-treated and untreated animals using Student's t test for group observations.

[1] *Abbreviations used in this paper*: CH_{50}, hemolytic complement activity producing 50% lysis of sensitized erythrocytes; CPK, creatine phosphokinase; CVF, cobra venom factor; RMBF, regional myocardial blood flow; SDS, sodium dodecyl sulfate.

Measurements of regional myocardial blood flow (RMBF). RMBF and cardiac output were measured 15 min and 6 h after coronary artery occlusion using carbonized microspheres, size 7–10 μm (Minnesota Mining & Manuf. Co., St. Paul, Minn.) labeled with gamma-emitting radionuclides ^{46}Sc and ^{85}Sr (30, 31). The microspheres, suspended in a solution of 50% sucrose with two drops of Tween 80 (ICI United States, Inc., Wilmington, Del.) to avoid aggregation, were mechanically agitated and ultrasonicated for 45 min before use. 15 min and 6 h after the occlusion, 4 ml containing 1.5×10^6 of one type of labeled microsphere was injected over 15 s through a catheter placed in the left atrium; during the next 15 s the catheter was flushed with 5 ml saline; simultaneously, a reference sample was collected through a catheter placed in the femoral artery using a 50-ml heparinized plastic syringe placed in a Harvard withdrawal pump (Harvard Apparatus Co.) operating at a constant rate of 15.3 ml/min. 6 h after occlusion this procedure was repeated with the other isotope-labeled microspheres. No changes in heart rate or arterial pressure were observed after the injection.

At the end of the experiment, 6 h after the occlusion, the heart was rapidly excised, and eight transmural biopsies (weighing 2.25±0.06 g) were obtained. They contained all the ischemic tissue as well as normal portions from the anterior and posterior ventricular walls. The biopsies were then divided into endocardial and epicardial layers. The radioactivity of the reference blood sample and of the tissue specimens was counted in a gamma scintillation well counter (model 4233 Nuclear-Chicago Corp., Des Plaines, Ill.). Calculations of RMBF and cardiac output (CO) were made as described by Utley et al. (32).

Endocardial, epicardial, and transmural RMBF of the normal and of the ischemic sites were compared separately, considering each site where the flow was <60 ml/min per 100 g, 15 min after occlusion, to be ischemic. The comparison of the flows was made between the values obtained after the control period of 15 min and those collected after 6 h, using the Student's paired *t* test.

Purification of the CVF. Lyophilized *Naja naja* venom (Ross Allen's Reptile Institute, Inc., Silver Springs, Fla.) was reconstituted and chromatographed on DEAE-cellulose and Sephadex G-200 (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) according to the method of Ballaw and Cochrane (33). After dissolving 1 g of venom in 65 ml sodium phosphate buffer, 0.03M, pH 7.4 at 37°C, the sample is applied to a 2.5 × 40-cm column of equilibrated DEAE-cellulose. The nonbound proteins, mostly phospholipase, are washed through at 50 ml/h for 16–18 h. A linear salt gradient to 0.5 M NaCl is then begun, and the anticomplementary peak is concentrated and applied to a G-200 column in isotonic saline phosphate buffer, pH 7.4. The final peak is a homogenous band on polyacrylamide electrophoresis (34) (Fig. 1), and when assayed according to the hemolytic complement activity producing 50% lysis of sensitized erythrocytes (CH_{50}) inhibition method of Ballow and Cochrane (33) has a specific activity in the range of 4 μg protein/U. A very faint second band, close to the main band, was seen on the sodium dodecyl sulfate (SDS) gel. Because this apparent contaminant disappears upon treatment, it consists of subunits, and may therefore represent a structural variant or breakdown product of the major component. The nature of this additional band, present in all CVF purified by present methods (33), has not been further investigated. In no instance was there any apparent toxic effect of CVF administration in dogs or rats, suggesting that phospholipase or endotoxin contamination, if present at all, was minimal. In purification of CVF, care must be taken to avoid contact with unsiliconized glass, as we have found it to aggregate upon storage in other than plastic or siliconized containers.

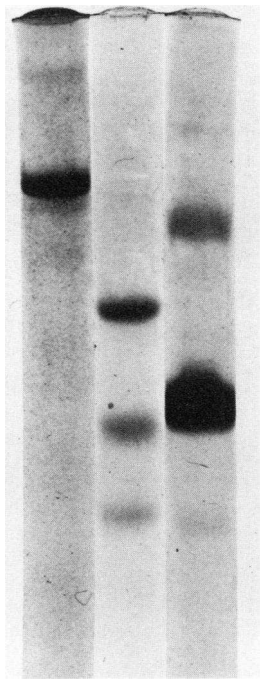
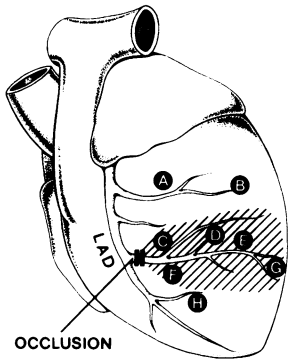


FIGURE 1 Sodium dodecyl sulfate (SDS)-polyacrylamide electrophoresis of CVF. Purification of CVF is based upon separation by charge (DEAE-cellulose) and molecular radius (Sephadex G-200). Both polyacrylamide and SDS-polyacrylamide electrophoresis show the final product to be homogeneous. Shown on the left is 200 μg of the CVF preparation in SDS-polyacrylamide, with a faint second band below the major protein. In the presence of β-mercaptoethanol (center) the 135,000 dalton molecule is reduced to subunits of 80,000 and 51,000. An additional 34,000 dalton band appears to represent further breakdown into subunits. The faint second band is no longer present after reduction. Bovine serum albumin is shown on the right for reference.

Hemolytic complement activity in the serum. Hemolytic complement activity was measured by calculation from the dilution producing 50% lysis of optimally sensitized sheep erythrocytes by the method of Kent and Fife (35).

RESULTS

Group I-occlusion alone. The predictive value of ST-segment elevation for CPK activity and histological structure 24 h later was established in this group (14 dogs, 111 specimens). Sites remote from the region dependent upon a blood supply from the occluded artery with normal ST segments 15 min after occlusion (0–2 mV elevation) showed normal CPK activity 24 h later (34.9±0.9 [mean±SEM] 1U/mg of protein [14 dogs, 32 specimens]). The log of the CPK activity 24 h after occlusion was inversely proportional to ST-segment elevation 15 min after occlusion: log CPK = −0.060 ST + 1.48 (r = 0.77, n = 111 specimens). Therefore, higher ST-segment elevation 15 min after occlusion was predictive of lower CPK activity 24 h later (Figs. 2 and 3).



SITE	ST (mV)	CPK (I U /mg prot)	HISTOLOGY
A	0	34.9	NORMAL
B	0	29.1	NORMAL
C	3	23.3	ABNORMAL
D	6	8.9	ABNORMAL
E	5	8.5	ABNORMAL
F	4	9.4	ABNORMAL
G	7	11.2	ABNORMAL
H	0	31.3	NORMAL

● BIOPSY SITES
▨ AREA OF ST SEGMENT ELEVATION

FIGURE 2 The relationship of ST-segment elevation 15 min after occlusion and CPK activity and histological structure 24 h later in an experiment from group I (occlusion alone). Left: A schematic representation of the anterior surface of the heart and its arteries. The shaded area represents the area of ST-segment elevation 15 min after occlusion. The circles represent sites where biopsies were taken. LAD, left anterior descending coronary artery; sites of occlusion, sites where the left anterior descending coronary artery was occluded. Right: Comparison between ST-segment elevation 15 min after occlusion and CPK and histological appearance 24 h later in the same sites.

Each of the 14 dogs exhibited a similar pattern in the relationship between ST-segment elevation 15 min after occlusion and CPK activity 24 h later. Thus, when this relationship was analyzed in each dog separately, the average slope was 0.072±0.005 (Table I).

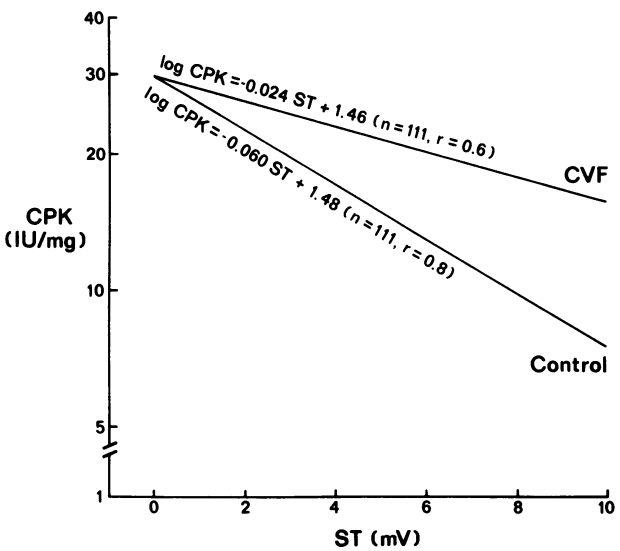


FIGURE 3 Relationship between epicardial ST-segment elevation 15 min after occlusion and myocardial CPK activity 24 h later in the same sites. Control line, control group (14 dogs): log CPK = −0.06 ST + 1.48 (n = 111 specimens, r = 0.77). CVF line, group II; 14 dogs which received CVF 30 min after occlusion: log CPK = −0.024 ST + 1.46 (n = 111 specimens, r = 0.60). The difference between the slopes is highly significant ($P < 0.001$).

TABLE I
Relationship between ST-Segment Elevation 15 min after Occlusion and CPK Activity 24 h Later in Each Site

Control				CVF			
Dog	Slope	Inter-cept	r	Dog	Slope	Inter-cept	r
1	0.064	1.45	0.76	1	0.052	1.31	0.75
2	0.117	1.57	0.96	2	0.022	1.53	0.82
3	0.072	1.54	0.94	3	0.035	1.65	0.81
4	0.016	1.41	0.63	4	0.040	1.61	0.71
5	0.080	1.40	0.94	5	0.025	1.37	0.79
6	0.074	1.54	0.94	6	0.019	1.36	0.50
7	0.085	1.49	0.90	7	0.042	1.50	0.88
8	0.108	1.57	0.97	8	0.051	1.58	0.57
9	0.107	1.54	0.94	9	0.027	1.52	0.60
10	0.033	1.46	0.74	10	0.034	1.46	0.85
11	0.030	1.50	0.74	11	0.018	1.43	0.65
12	0.091	1.49	0.92	12	0.026	1.45	0.71
13	0.055	1.52	0.79	13	0.019	1.45	0.89
14	0.096	1.59	0.94	14	0.039	1.49	0.68
Mean	0.072	1.50	0.86		0.032*	1.48	0.73
±SE	0.009	0.016	0.03		0.003	0.026	0.03

Analysis was carried out in each dog separately.
* $P < 0.001$, in comparison to control.

97% (29/30 specimens) of sites with normal ST-segment elevation (0–2 mV elevation) showed normal myocardial structure (Fig. 2). 97% (69/71) of sites with ST-segment elevation exceeding 2 mV 15 min after occlusion exhibited early signs of myocardial infarction. These findings included a more pronounced eosinophilic appearance, loss of myofiber cross-striation, karyolysis, and karyorrhexis (Figs. 2 and 4). The number of polymorphonuclear neutrophils in each slide from a specimen that exhibited ST-segment elevation averaged 27.0±2.2 (SEM). Serum complement levels, as measured by CH_{50}, in 35 normal dogs were 190±48 U (±1 SD). In dogs in the control group CH_{50} either was unchanged or fell slightly; however, it always remained within normal limits.

Examination of infarcted tissue, including the healthy margins from two control dogs, by immunofluorescent microscopy revealed no deposition of IgG, C3, or albumin. In areas of marked necrosis, focal areas of fibrinogen were seen interspersed between the myofibrils.

In five control dogs RMBF was measured 15 min and 6 h after coronary artery occlusion (Table II). In the nonischemic sites, the transmural, endocardial, and epicardial flows were 96.6±4.0, 97.3±4.0, and 98.6±4.6 ml/min per 100 g and 114.6±6.2, 111.7±5.9, and 115.6±8.3 ml/min per 100 g, respectively. In the ischemic sites, the RMBF fell between 15 min and 6 h after occlusion. The transmural, endocardial, and epicardial flows were 24.2±4.3, 19.2±3.7, and 26.0±4.1

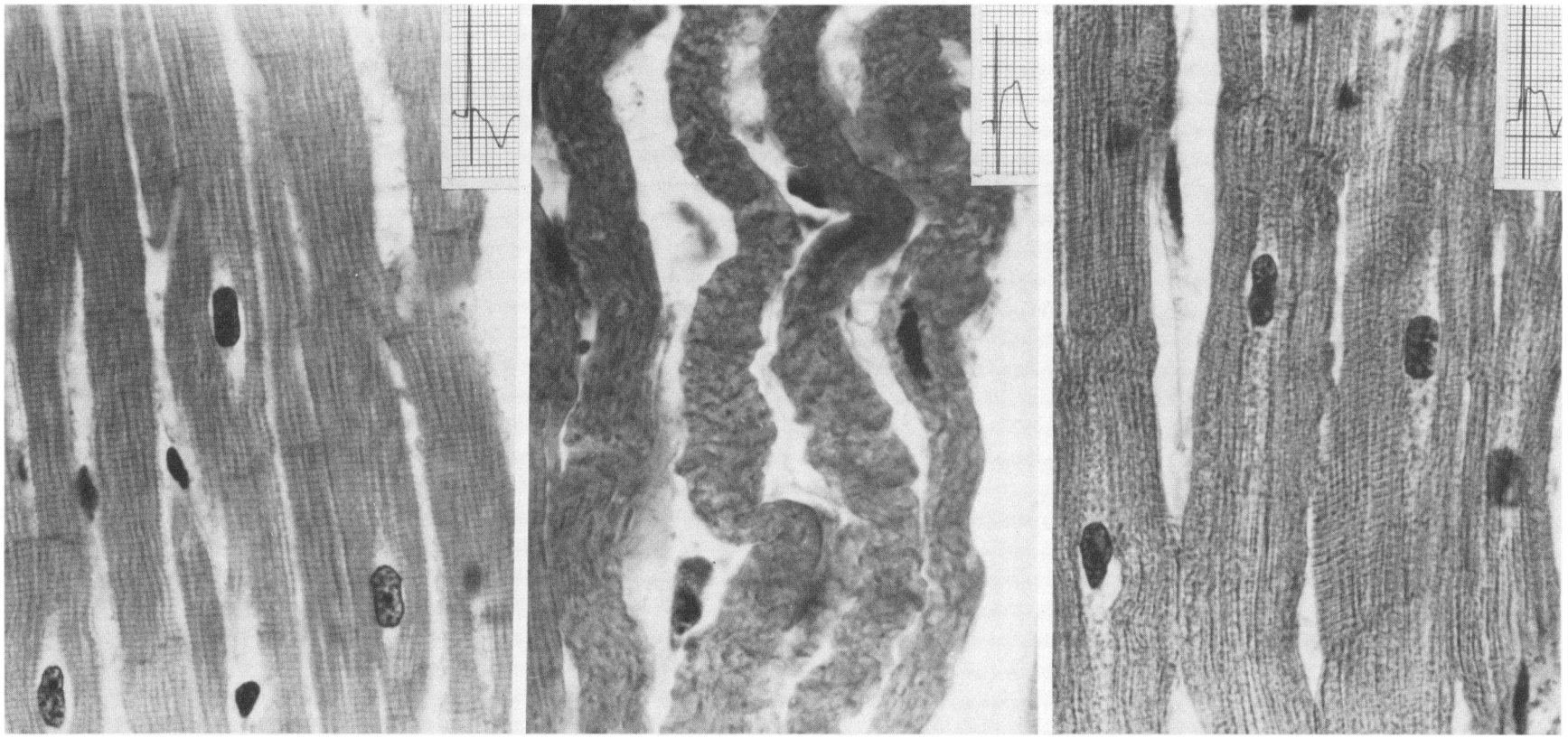


FIGURE 4 Left panel: histology from sites without ST-segment elevation. Epicardial electrocardiogram 15 min after occlusion and hematoxylin- and eosin-stained sections obtained from the same sites 24 h later. Note the normal myocardial structure. Middle panel: histology from a site with ST-segment elevation from the control group (group I). Epicardial electrocardiogram 15 min after occlusion and hematoxylin- and eosin-stained section from the same site 24 h later. Note ST-segment elevation on the electrocardiogram and histological abnormality as described in text. Right panel: histology from a site with ST-segment elevation of the same magnitude as that in the middle panel in a dog from the group treated with CVF. Note the preservation of normal myocardial histological appearance.

TABLE II
RMB Flow in Control and Treated Dogs

		RMBF in normal sites			RMBF in ischemic sites			Cardiac output
		Trans*	Endo‡	Epi§	Trans	Endo	Epi	
		ml/min/100 g			*ml/min/100 g*			
CVF treated	15 *min*	96.9 ±5.3‖	102.1 ±7.3	101.0 ±5.2	26.2 ±4.2	26.1 ±4.1	27.8 ±3.5	2.063 ±0.270
	6 *h*	100.1 ±13.6	111.4 ±14.4	103.5 ±13.3	20.1 ±5.8	11.6 ±2.9	24.9 ±8.4	1.742 ±0.210
Control	15 *min*	96.6 ±4.0	97.3 ±4.0	98.6 ±4.6	24.2 ±4.3	19.2 ±3.7	26.0 ±4.1	2.569 ±0.168
	6 *h*	114.6 ±6.2	111.7 ±5.9	115.6 ±8.3	15.1 ±3.6	13.7 ±6.3	24.1 ±5.8	2.272 ±0.275

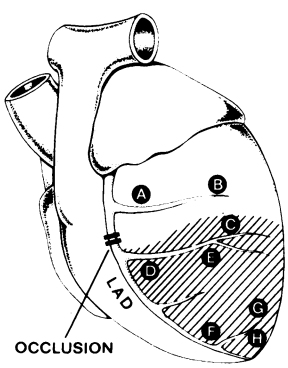
* Transmural flow.
‡ Flow in endocardial specimens.
§ Flow in epicardial specimens.
‖ Mean±1 SE.

at 15 min. 6 h later they fell to 15.1±3.6, 13.7±6.3, and 24.1±5.8 ml/min per 100 g.

Group II-occlusion plus CVF. In this group of 14 dogs, the CVF (20 U/kg) was administered 30 min after occlusion, i.e., after completion of epicardial mapping. Sites remote from the occluded area that showed normal ST segments (0–2 mV elevation) showed normal CPK activity which was not different from that in the control group, showing that CVF by itself did not change CPK activity (34.0± 1.1 as compared to 34.9±0.9 1U/mg of protein). However, CPK activities in many sites that did show ST-segment elevation over 2 mV, and thus were expected to have CPK depression, were within normal limits. In sites in which CPK was depressed this was less marked than in the control group (Figs. 3 and 5). Histologically, several sites with ST-segment elevations exceeding 2 mV, which were expected to show histological signs of early myocardial necrosis, were completely normal (Figs. 4 and 5). In all dogs, the reduced depression of CPK activity subsequent to the administration of CVF 30 min after occlusion was reflected in a significantly ($P < 0.001$) less steep slope: $\log \text{CPK} = -0.024\,\text{ST} + 1.46$ ($n = 111$, $r = 0.060$; Fig. 3). This relationship was different from that which existed in the control dogs in each of the animals treated with CVF, with an average slope of 0.032±0.003, which is significantly less than in the individual control dogs (0.072±0.009, $P < 0.001$; Table I). Histologically, all (29/29) sites with normal (0–2 mV) ST-segment elevation exhibited normal structure, as expected. However, 46% (36/79) of sites that showed ST-segment elevation exceeding 2 mV at 15 min after occlusion and thus before CVF administration, exhibited normal histologic appearance 24 h later, showing preservation of cell integrity as a consequence of CVF administration ($X^2 = 33.9$, $P < 0.0005$).

The infiltration of polymorphonuclear neutrophils was reduced in the CVF-treated dogs. The average number of neutrophils in sites with ST-segment elevation exceeding 2 mV was 6.8±0.9 which is significantly lower than in the control series (27.0±2.2, $P < 0.001$).

The levels of total complement in the serum, as expressed by its hemolytic activity, dropped sharply



SITE	ST (mV)	CPK (I U /mg prot)	HISTOLOGY
A	0	37.8	NORMAL
B	0	36.0	NORMAL
C	4	16.5	ABNORMAL
D	10	15.4	ABNORMAL
E	11	26.6	NORMAL
F	8	25.0	NORMAL
G	5	24.0	NORMAL
H	12	15.8	ABNORMAL

● BIOPSY SITES
▨ AREA OF ST SEGMENT ELEVATION

FIGURE 5 The effect of CVF on the relationship of ST-segment elevation (before CVF administration) to CPK activity and histological structure 24 h later in an experiment from group II. Left: schematic representation of the left anterior surface of the heart and its arteries. LAD, left anterior descending coronary artery; site of occlusion, site of coronary artery occlusion; shaded area, area of ST-segment elevation 15 min after coronary occlusion (before CVF administration). Right: comparison between ST-segment elevation 15 min after occlusion, i.e. before CVF administration, and CPK activity and histological structure 24 h later.

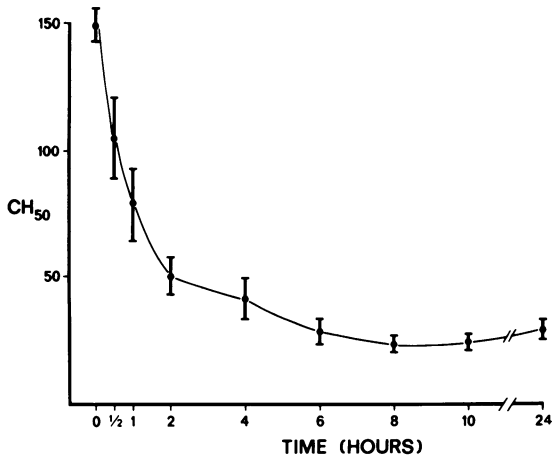


FIGURE 6 Fall in the complement hemolytic activity CH_{50} after the administration of CVF in seven dogs. Note the sharp drop in CH_{50} reaching a minimal level after 4–6 h. Bars represent SEM.

after CVF administration (Fig. 6). It was one-third of its initial activity 2 h after the injection and reached very low levels 4–6 h later, maintaining the minimal levels for the entire 24 h. In fact, in the two additional normal dogs in which the same dose of CVF was injected, CH_{50} continued at reduced levels for 5–9 days.

In five dogs treated with CVF 30 min after occlusion, RMBF determined 15 min after occlusion (i.e. before CVF administration) was similar to that of the control dogs (Table II). The transmural, endocardial, and epicardial flows in the nonischemic zone were 96.9±5.3, 102.1±7.3, and 101.0±5.2 ml/min per 100 g and in the ischemic zone they were, respectively, 26.2±4.2, 26.1±4.1, and 27.8±3.5 ml/min per 100 g. 6 h after occlusion (i.e. with CVF influence) the flows in the nonischemic zone were 100.1±13.6, 111.4±14.4, and 103.5±13.3 ml/min per 100 g. The flow in the ischemic zone fell in a manner similar to that in the control group. Transmural, endocardial, and epicardial flows were 20.1±5.8, 11.6±2.9, and 24.9±8.4 ml/min per 100 g, respectively.

Heart rate, mean arterial pressure, and the time of appearance of multifocal arrhythmias characteristic of this canine model were not different in the two groups (Table III), showing that changes in the rhythm and heart rate are not indicative of infarct size (13). Four dogs in the control group and three in the CVF group died in this period as a result of these arrhythmias. CVF did not have a direct effect on cardiac output, left ventricular pressures, and left ventricular dP/dt. Cardiac output determined 15 min after occlusion and 6 h later showed that both the CVF-treated and untreated dogs showed a fall of ≅300 ml/min (Table II). Left ventricular dP/dt recorded before CVF administration and 30 min after showed no significant changes (2,447±176 and 2,382±209 mm Hg/s, respectively). Left ventricular systolic and end diastolic pressures also did not change (140±3/4±1–139±4/4±1 mm Hg). Also, there were no differences between the untreated and CVF-treated groups in respect to hematocrit, white blood cell count, platelet count, prothrombin time, partial thromboplastin time, and fibrinogen level before and 2, 6, and 24 h after coronary artery occlusion.

DISCUSSION

Recent advances in the understanding of the complement system have offered new insights to several physiological and pathological processes (22–28, 36). Of special interest is the existence of leukotactic (chemotactic) factors derived from C3 and C5, and the enhancement of phagocytosis, changes in capillary permeability, and increase of nonspecific injury to cell membrane (cytolytic activity) mediated also by components of the complement system acting in sequence from C3 to C9. Flexner and Noguchi (37) first described the anticomplementary activity of cobra venom; Klein and Wellensiek (38) demonstrated that the activities of C3 and C5 were abrogated by the cobra venom. In 1966 Nelson (39) showed that the primary effect of CVF was on C3. The isolation of CVF from venom and the mode of action were described in detail by Muller-Eberhard and Fjellstrom (27). CVF activates components of the alternative pathway of complement activation, generating C3 activator which specifically cleaves C3. CVF appears to be the C3 of the cobra, and exerts its potent effect in mammals because of resistance to the action of

TABLE III
Hemodynamic Observations

		Hours				
		0	3	6	12	24
Heart rate, *beats/min*	Control	136±4	143±6	154±6	169±6	170±6
	CVF	135±6	145±7	164±10	180±8	164±6
Mean arterial pressure, *mm Hg*	Control	120±4	118±4	116±2	110±5	89±6
	CVF	110±5	119±5	125±3	120±8	86±9

C3 inactivator (40), C3 acts as a triggering stimulus in formation of the alternative pathway C3 convertase made up of activated factor B and D. The purified factor is free of neurotoxin (phospholipase) and does not affect the clotting system when administered in vivo, although guinea pigs, but not rabbits, may have some reduction in fibrinogen levels (41). No CVF-induced alterations of dog fibrinogen levels were found in the present studies. These properties, and CVF effects on other models of complement and neutrophil-mediated injury (41), clearly distinguish this venom derivative from the anticoagulant arvin, a venom product prepared from a different genus of snake (42).

We have investigated the possibility that consequent to an acute coronary artery occlusion the damage to myocardial tissue will be reduced if the amount of C3 available in vivo is reduced. This hypothesis was based on the observation of Hill and Ward (26) regarding the importance of complement in nonspecific injury to tissues in general, and to the ischemic myocardium in particular. They demonstrated that C3-split products which are responsible for chemotaxis exist in the infarcted rat myocardium, that they are generated by the release of proteolytic enzymes from damaged muscle, and that CVF, by reducing C3 concentration, greatly reduced the chemotactic effect. On histologic examination, they noted reduction in the polymorphonuclear infiltrate, but were unable to assess the effects of CVF in protecting the ischemic myocardium because it was difficult to evaluate decreases in myocardial infarctions without a prediction of the extent of myocardial necrosis. We took advantage of a method that can evaluate changes in the size of infarction by alteration in histology and in CPK activity in sites where electrocardiograms were recorded before CVF administration. Also, CPK activity is completely independent of histological appearance and of leukocyte infiltration because leukocytes do not exhibit CPK activity. Using this technique, in which each myocardial site's fate is predictable by early ST changes, it was shown that over 40% of sites expected to show signs of early myocardial infarction did not show them when CVF was administered 30 min after coronary occlusion. These results were also paralleled by observations that myocardial CPK activity was much less depressed than expected in dogs that received CVF; in fact, many acutely damaged sites had completely normal CPK activity. Therefore, both by histological and enzymatic criteria, the extent of tissue damage at 24 h was limited by the administration of CVF.

The observation that CVF administration caused a rather abrupt fall in complement levels within 2 h and that these levels were <20% of normal after 4–6 h shows that the depression of complement level was accomplished within the time frame in which the damage is still in a reversible phase (4, 6, 10, 11, 15, 32, 43). The effects of a single dose of CVF, representing ≅1 mg of protein, decreased complement levels in normal dogs for more than 5 days; however, it is not known whether the tissue would become necrotic when recovery of the complement system occurs after 5 days. Studies with reperfusion of coronary arteries 3 h after occlusion have shown that a similar amount of tissue was spared from undergoing necrosis when analyzed at either 24 h or at 7 days (10, 11), suggesting that sparing of tissue at 24 h can be representative of changes at 1 wk after occlusion. However, the possibility that CVF administration induces a delay but not a reduction in myocardial necrosis cannot be ruled out.

The precise mechanism of action of CVF in this study is unknown. Standard fluorescent antibody techniques showed that C3 was not normally deposited in infarcted tissue; however, in the absence of antigen-antibody complexes or C3 receptors on the tissues, complement "fixation" would not be expected. Fluid-phase activation, on the other hand, could still result in generations of complement-split products that play an important role in inflammation. In particular, activation of C3 produces a chemotactic factor for polymorphonuclear leukocytes, and further activation of the complement sequence produces the C5 chemotactic factor. CVF is known to activate C3 via alternative pathway components, resulting in depletion of C3 and C5 in vivo (40), while sparing C1 and C4 (38). In this way, further generation of chemotactic factors is markedly reduced, because of depletion of the natural substrates from which they are produced. Indeed, in this study there was markedly less polymorphonuclear infiltration in the CVF-treated dogs than in the dogs with control occlusions, which is in accord with previous observations in the rat (24). If myocardial injury in the dog releases proteolytic enzymes that activate C3 (23), one would expect that systemic depletion of C3 by CVF would prevent generation of chemotactic or vascular permeability factors in the areas of injury.

Another nonimmunological means by which the complement system may be activated in myocardial infarction is via C1 fixation to mitochondrial membranes, a mechanism known to activate the classic complement pathway, at least as far as C3 (44). These in vitro findings were in part supported by observations of transient depressions in serum C1, C4, and C3 functional activities in six patients undergoing myocardial infarction (44). The CH_{50} was insensitive to these changes, as in our dogs. Regardless of the mechanism for C3 activation, the question of biological importance in our model is whether vascular permeability factors (such as C3a, anaphylatoxin) or chemotactic factors play

an inflammatory, and deleterious, role in the marginal regions of infarcted myocardium. The simplest hypothesis is that reduction in infarct size and polymorphonuclear infiltration are related phenomena and are secondary to a reduction in chemotaxis, as shown by Hill and Ward (26). It is, of course, possible that some unknown property of CVF is protective, and that cellular infiltration is reduced because tissue damage is less. This hypothesis would also require invocation of noncomplement-dependent chemotactic factors, but such do exist. The trace contaminant, representing 15–20 mg protein per dog, is a candidate for such an unknown factor. Other mechanisms through which the CVF preparation may influence myocardial damage were explored. Thus, CVF did not alter heart rate, preload (end diastolic pressure), afterload (systolic pressure), or contractility (dP/dt), excluding the possibility of an effect through alteration in myocardial oxygen requirements. On the other hand, CVF did not change collateral blood flow to the ischemic myocardium, suggesting that this essential mechanism of alterations of the damaged zone is not effective in this case. Because the measured clotting parameters did not show modifications after CVF, it also was considered that important effects on the hemostatic system would be unlikely.

In conclusion, the administration of CVF 30 min after coronary artery occlusion decreased the size of infarction as noted histologically and decreased the amount of necrosis as evaluated by myocardial CPK activity. Considering the known specific mode of action of CVF, it is postulated that these results, based on C3 depletion, are a consequence of a decreased generation of leukotactic factors, thus reducing injury which may be produced by the released lysosomal enzymes from polymorphonuclear leukocytes. Other complement-derived activities, such as those affecting vascular permeability, could also play a role. These results illustrate the important role that complement components can play in the generation of an inflammatory response, even when activated "nonspecifically," that is, not by an antigen-antibody interaction.

The extrapolation of these results to the clinical setting must be with extreme caution because the canine model does not mimic fully the clinical conditions. There are also species differences in the distribution of the coronary arteries. Furthermore, it is conceivable that species differences in myocardial response to complement activity and its inhibition could exist, and it is not known whether infarcts modified in this way will heal normally. Nevertheless, this manner of limiting infarct size differs from those previously suggested (4–21) because the latter are attempts to reduce it by physiologic or pharmacological means, whereas CVF acts by changing the reaction of the organism to a nonspecific injury, thus offering a new approach to the reduction of myocardial necrosis after acute coronary occlusion.

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