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Intravascular and peritoneal coagulation and fibrinolysis in horses with acute gastrointestinal tract diseases

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Summary: *Components of the coagulation and fibrinolytic cascades, prothrombin and activated partial thromboplastin times, endotoxin activity, and albumin concentration were measured in blood and peritoneal fluid from 20 healthy horses and from 153 horses with acute gastrointestinal tract diseases at admission. Overall, 77% (117/153) of affected horses survived to discharge from the hospital, and 85% (82/97) of horses discharged were reported to be normal 9 to 14 months later. Significant differences in hemostatic factors were more common in peritoneal fluid than in blood. Tissue plasminogen activator, plasminogen, protein C, antithrombin III, and α_2-antiplasmin activities and concentrations of fibrinogen and fibrin degradation products were significantly ($P < 0.05$) greater in peritoneal fluid from horses with colic, and, with the exception of fibrinogen concentration, were associated with detection of endotoxin. Higher values for these variables, except tissue plasminogen activator activity, were significantly ($P < 0.05$) associated with survival. Plasminogen, antithrombin III, and α_2-antiplasmin activities were significantly ($P < 0.05$) greater in peritoneal fluid from horses with inflammatory or strangulating lesions, compared with those in horses with simple colic. Plasminogen-activator inhibitor type 1 activity, fibrin degradation products concentration, and prothrombin time were significantly ($P < 0.05$) greater in the blood of horses with colic. Survival was inversely associated with significantly ($P < 0.05$) greater intravascular concentrations of fibrin degradation products and fibrinogen and prothrombin time. This study revealed marked contrasts between peritoneal and intravascular coagulation and fibrinolysis in horses with colic, indicating that inferences regarding the peritoneal environment, particularly with respect to fibrinolytic capacity, should not be made on the basis of factors measured in blood.*

Abnormal hemostasis has been implicated in the pathogenesis of gastrointestinal tract diseases accompanied by colic in horses over the past decade.[1-8] Although those studies have provided considerable insight into the pathogenesis of colic, 2 specific aspects of the role of hemostasis in colic have not been addressed. First, changes in the fibrinolytic regulatory proteins that control the degradation of polymerized fibrin have not been investigated in horses with gastrointestinal tract diseases, primarily because of the lack of specific assays. Second, studies of hemostasis in horses with colic have focused on evaluation of intravascular factors, rather than on examination of coagulation and fibrinolysis in peritoneal fluid, the site where the disease process originates.

Interest in the regulation of hemostasis in gastrointestinal tract diseases in horses arises from the clinical observation that complications potentially related to hemostatic dysfunction, such as jugular vein thrombosis and laminitis, develop frequently in horses with colic. A severe clinical complication associated with ischemic gastrointestinal tract lesions is the development of intra-abdominal adhesions. Twenty percent of horses that survive surgery for small intestinal lesions have another episode of colic attributable to intra-abdominal adhesions, which often necessitates a second laparotomy or leads to euthanasia.[9] In human beings, in which intra-abdominal adhesions are the most frequent cause of intestinal obstruction,[10] the development of adhesions is attributed to insufficient fibrinolytic activity.[11,12] It has been hypothesized that fibrin, deposited on serosal surfaces within the inflamed abdomen, is not degraded, and provides a scaffold for the ingrowth of vessels and fibroblasts, thereby forming permanent fibrous adhesions.[13]

The inflammatory process and associated changes in blood and peritoneal fluid that develop in gastrointestinal tract disease are initiated by endotoxin, the toxic lipopolysaccharide component of gram-negative bacteria. Translocated from the bowel lumen into the peritoneal cavity and from there into the circulation,[14] endotoxin profoundly affects intravascular hemostasis by initiating coagulation and inhibiting fibrinolysis.[15-18] Although the net effect of endotoxin on intra-abdominal coagulation and fibrinolysis is not known, fibrinolysis has been assumed to be inhibited, reflecting intravascular conditions.

The purpose of the study reported here was to investigate coagulation and fibrinolysis in blood and peritoneal fluid from healthy horses and from horses with naturally developing gastrointestinal tract diseases. Because 89% of horses that develop adhesions after surgery for small intestinal diseases develop related clinical signs within 210 days,[9] follow-up evaluation to determine the long-term outcome for each horse was conducted no sooner than 9 months after the date of admission to the hospital.

Materials and Methods

Study design—Blood and peritoneal fluid were obtained from 153 adult horses within 30 minutes of admission to the university veterinary teaching hospital for evaluation of colic. Blood and peritoneal fluid also were collected, using identical techniques, from 20 horses ranging in age from 1 to 15 years and determined as healthy on the basis of physical examina-

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tion. Healthy horses were housed in paddocks and fed grass hay and a balanced concentrate ration.

Horses with colic were allotted retrospectively to 1 of 4 groups, according to diagnosis. Simple colic (group A) included all nonstrangulating, noninflammatory diseases such as large-colon impactions, large-colon displacements without ischemia, and colic without definitive diagnosis. Strangulating diseases (group B) included surgical lesions requiring intestinal resection. Inflammatory diseases (group C) included peritonitis, colitis, and proximal enteritis. Group D included those horses that were admitted with a ruptured viscus and abdominal contamination with ingesta. All horses also were grouped for analysis on the following bases: outcome (survival or nonsurvival), surgical or nonsurgical treatment, detection of endotoxin in blood or peritoneal fluid, and the development or absence of complications including laminitis, jugular vein thrombosis, and intra-abdominal adhesions.

A follow-up survey was conducted by telephone 9 to 22 months after horses were admitted. The results of the survey were used in the following analysis: all abnormal horses as a group, as well as each diagnostic group, were compared with respect to long-term survival or nonsurvival, cause of death, recurrence of colic, and development of intra-abdominal adhesions after discharge from the hospital. Horses were considered to have adhesions if such a diagnosis was made at surgery or necropsy, as reported by the owner, or if the owner reported that the horse had had 4 or more episodes of colic requiring veterinary attention since discharge from the hospital and the horse had undergone surgery during the initial hospitalization.

Sample collection—Blood was obtained by direct jugular venipuncture. Peritoneal fluid was collected by insertion of a blunt teat cannula into the peritoneal cavity through a stab incision made after aseptic preparation of a clipped site on the ventral midline, 4 cm caudal to the xiphoid process.[19] Blood or peritoneal fluid was collected into glass tubes without anticoagulant, and into 3 sterile polypropylene tubes, each containing a different solution: 3.8% sodium citrate (1:9, citrate: sample), an acidic (pH 4.5) 3.8% sodium citrate solution (1:9, citrate:sample),[20] or bovine thrombin and aminocaproic acid. Sample aliquots were prepared within 1 hour of collection and stored at −70 C until assayed in duplicate.

Hemostasis and albumin assays—Prothrombin time, activated partial thromboplastin time, and fibrinogen concentration were determined, using a photoelectronic clot-timing device[a] and commercial kits.[b] Concentration of fibrin degradation products was determined by latex agglutination.[c] Activities of protein C,[21] α_2-antiplasmin,[21] antithrombin III,[21] and plasminogen[22] were measured by previously described chromogenic assays adapted to a computer-assisted centrifugal analyzer.[d] Albumin concentration was measured using a standard bromcresol green assay, and was used as a marker of passive protein flux and dehydration.

Assay of tissue plasminogen activator (tPA) activity—A standard tPA/plasminogen-based chromogenic assay,[23] using commercial reagents,[e] was used to measure tPA activity in plasma and peritoneal fluid collected in an acidic citrate anticoagulant. Citrated plasma or peritoneal fluid was centrifuged at 800 × g for 20 minutes at 4 C within 1 hour of harvesting. Activity of tPA was measured on the basis of its ability to activate plasminogen, which in turn cleaves a chromogenic substrate, with specificity conferred by the addition of partially digested fibrin. Samples (25 µl) or standard[f] were incubated with 100 µl of 1*M* sodium acetate (pH 3.9) at 37 C for 20 minutes, then diluted with 2 ml of sterile water (final dilution of 1:85). Twenty microliters of sample or standard (120, 60, 30, 15, 7.5, 3.25, 1.625, or 0 IU/ml) was added to wells in duplicate, followed by 200 µl of a solution containing 0.5 mg of human glu-plasminogen[e] and 5 µ*M* chromogenic substrate[e] per 12.5 ml final volume in TRIS buffer. Finally, 10 µl of a partially digested fibrin solution[e] (2.67 mg/ml in TRIS buffer) was added to each well, and the plate was incubated for 3.5 hours at 37 C, followed by spectrophotometric analysis in a microtitration plate reader at 405 nm. Because the relationship of activity of equine tPA and human Bowes melanoma cell line-derived tPA is not known, results were reported as U/ml, rather than IU/ml.

Assay of plasminogen-activator inhibitor type 1 (PAI-1) activity—This assay was based on measurement of residual tPA activity after a known amount of exogenous tPA was added to the sample.[24] The assay was identical to the tPA activity assay, with an additional initial incubation step in which a tPA standard (40 IU/ml) was added to 25 µl of sample for 15 minutes at 25 C. Duration of assay incubation was 2.5 hours.

Endotoxin assay—Blood and peritoneal fluid samples were collected aseptically into 3.8% sodium citrate (1:9, citrate:sample); plasma and peritoneal fluid were diluted 1:10 with pyrogen-free water, boiled for 10 minutes, then frozen at −20 C until assayed for endotoxin. A *Limulus* amebocyte lysate assay[g] was used according to the manufacturer's instructions.[5] Sometimes, in horses with severe hemoconcentration, insufficient plasma or peritoneal fluid was obtained to allow completion of the endotoxin assay. Endotoxin quantitation therefore was completed in plasma from 145 horses and in peritoneal fluid from 142 horses.

Statistical analysis—Comparisons were made by use of ANOVA, with mean values compared by use of the Scheffe test, or by χ^2 analysis. Differences were considered significant when $P < 0.05$. Values are expressed as the mean ± SEM. All data for fibrin degradation products were transformed for statistical analysis, as described previously.[8]

Results

Significant findings were not obtained when horses were grouped by surgery or long-term survival and the data were analyzed. Complications such as laminitis, jugular vein thrombosis, and intra-abdominal adhesions were identified too infrequently to allow relevant statistical analysis. One horse with inflammatory disease developed laminitis, and 1 with inflammatory disease developed jugular vein thrombosis; 2 horses, 1 with inflammatory disease and 1 with a strangulating lesion, developed both laminitis and jugular vein thrombosis. Intra-abdominal adhesions were discovered at surgery in 1 horse and at necropsy, following a short hospitalization, in 2 other horses. During surgery for a nonstrangulating lesion in 1 horse, intra-abdominal adhesions were identified; this horse had not had recurrence of colic 1 year later. One horse with enteritis that underwent laparotomy had recurrent colic after discharge that was suspected, but not proven, to be attributable to adhesions. Horses did not develop confirmed intra-abdominal adhesions after surgery for strangulating lesions.

Activity of PAI-1, concentration of fibrin degradation products, and prothrombin time were significantly greater in blood samples from horses with colic, compared with those from healthy horses (Table 1). In peritoneal fluid, tPA, plasminogen, protein C, antithrombin III, and α_2-antiplasmin activities and concentrations of fibrinogen and fibrin degradation products were significantly greater in horses with colic, compared with values in healthy horses.

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Table 1—Hemostatic factors (mean ± SEM) measured in blood and peritoneal fluid samples from 20 healthy horses and from 153 horses with gastrointestinal tract diseases

Sample Group	Factor									
	PAI-1 (U/ml)	tPA (U/ml)	PLSG (%)	PROTC (%)	ATIII (%)	α_2AP (%)	FIB (mg/dl)	FDP	PT (sec)	APTT (sec)
Blood										
Healthy	6.68	4.70	113.4	89.9	204.0	203.9	182.3	1.8	9.6	40.7
	±0.61	±0.63	±3.1	±3.8	±6.7	±8.8	±8.6	±8.6	±0.1	±1.6
Colic	28.38*	2.24	105.3	89.7	197.0	215.9	192.1	3.5*	10.0*	46.8
	±4.74	±0.60	±1.5	±6.7	±4.8	±3.0	±4.9	±0.2	±0.1	±1.7
Peritoneal fluid										
Healthy	NM	10.5	9.1	21.4	27.7	12.29	10.1	1.9	NM	NM
		±6.49	±0.9	±1.2	±2.8	±2.10	±1.1	±0.2		
Colic	NM	29.30*	37.7*	33.7*	75.3*	55.9*	29.2*	5.0*	NM	NM
		±2.88	±9.1	±1.4	±3.4	±3.2	±1.9	±0.4		

*Significantly different ($P < 0.05$) from the corresponding values for healthy horses for that sample type.

PAI-1 = plasminogen-activator inhibitor type 1; tPA = tissue plasminogen activator; PLSG = plasminogen; PROTC = protein C; ATIII = antithrombin III; α_2AP = α_2-antiplasmin; FIB = fibrinogen; FDP = fibrin degradation products; PT = prothrombin time; APTT = activated partial thromboplastin time; NM = not measured.

Table 2—Hemostatic factors (mean ± SEM) in peritoneal fluid samples from horses with gastrointestinal tract diseases, grouped by detection of endotoxin in blood or peritoneal fluid samples

Sample Endotoxin detected	Factor									
	PAI-1 (U/ml)	tPA (U/ml)	PLSG (%)	PROTC (%)	ATIII (%)	α_2AP (%)	FIB (mg/dl)	FDP	PT (sec)	APTT (sec)
Blood										
No (n = 128)	27.8	2.4	106.0	87.4	198.4	215.5	194.0	3.6	10.1	47.8
	±4.5	±0.7	±1.7	±2.1	±5.1	±3.3	±5.2	±0.2	±0.1	±1.9
Yes (n = 17)	39.0	1.8	102.3	88.2	196.6	213.6	182.7	3.0	9.9	39.8
	±25.5	±0.8	±3.6	±6.2	±14.9	±9.6	±16.1	±0.4	±0.3	±3.6
Peritoneal fluid										
No (n = 101)	NM	24.5	33.8	31.2	68.4	51.4	27.5	4.4	NM	NM
		±3.4	±2.5	±1.4	±3.5	±3.7	±2.1	±0.4		
Yes (n = 41)	NM	42.4*	47.0*	38.7*	90.2*	65.8*	33.4	7.2*	NM	NM
		±6.3	±4.4	±3.3	±7.4	±6.5	±4.2	±1.1		

*Significantly different ($P < 0.05$) from the corresponding value for the group in which endotoxin was not detected for that sample type. *See* Table 1 for key.

The activity of tPA in peritoneal fluid from horses with colic and from healthy horses exceeded the tPA activity in corresponding plasma samples. In addition, tPA activity was higher in peritoneal fluid from horses with colic, compared with that from healthy horses, although it was lower in blood from horses with colic, compared with that from healthy horses. Activity of PAI-1 in peritoneal fluid samples was detected only in 35% (7/20) of healthy horses. In virtually all peritoneal fluid samples from horses with colic, activity of PAI-1 was undetectable.

Significant differences were not found when blood values from horses with colic were compared on the basis of endotoxin detection either in blood or in peritoneal fluid (Table 2). However, activities of tPA, plasminogen, protein C, antithrombin III, α_2-antiplasmin, and concentration of fibrin degradation products in peritoneal fluid samples were significantly greater in horses with colic and endotoxin in their peritoneal fluid, compared with that in horses with colic, but undetectable endotoxin in peritoneal fluid.

Intravascular concentrations of fibrin degradation products and fibrinogen and prothrombin time were significantly greater in horses that did not survive to discharge. Nonsurviving horses also had significantly greater activities of plasminogen, α_2-antiplasmin, protein C, and antithrombin III, and concentration of fibrin degradation products in their peritoneal fluid, compared with those from horses that survived to discharge (Table 3).

Significant differences were not detected in intravascular hemostatic values when horses with colic were grouped by diagnosis. In peritoneal fluid samples, horses with inflammatory or strangulating diseases had significantly greater plasminogen, antithrombin III, and α_2-antiplasmin activities, compared with those from horses with nonstrangulating/noninflammatory diseases (Table 4). Fibrinogen concentration in peritoneal fluid samples was significantly greater in horses with inflammatory lesions, compared with that from horses with nonstrangulating/noninflammatory diseases. Concentration of fibrin degradation products in peritoneal fluid samples from horses with strangulating lesions was significantly greater, compared with that from horses with nonstrangulating/noninflammatory diseases. Using χ^2 analysis, strangulating lesions or ruptured bowel was significantly associated with endotoxin detection in peritoneal fluid samples.

Tissue plasminogen activator was the only factor with peritoneal fluid sample values that consistently exceeded those in blood. In healthy horses, tPA activity was 2.2-fold greater, and in horses with gastrointestinal tract diseases, 6.2-fold greater, in peritoneal fluid samples compared with blood samples. Among peritoneal fluid sample values, 2 other components of fibrinolysis, plasminogen and α_2-antiplasmin, were 4.1- and 4.5-fold greater, respectively, in peritoneal fluid samples from horses with colic, compared with that in healthy horses. In contrast, all other measured proteins, including al-

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Table 3—Hemostatic factors (mean ± SEM) in blood and peritoneal fluid from 153 horses with gastrointestinal tract diseases, grouped as survivors (n = 117) or nonsurvivors (n = 36)

	Factor									
Sample Survival	PAI-1 (U/ml)	tPA (U/ml)	PLSG (%)	PROTC (%)	ATIII (%)	α_2AP (%)	FIB (mg/dl)	FDP	PT (sec)	APTT (sec)
Blood										
Survivors	24.4	1.7	105.6	88.3	199.2	215.9	185.5	3.3	9.9	46.3
	±4.9	±0.3	±1.5	±2.2	±5.4	±3.4	±4.7	±1.7	±0.1	±2.1
Nonsurvivors	43.4	4.4	106.2	79.9	188.7	217.4	217.2*	4.3*	10.5*	48.7
	±14.0	±2.8	±4.8	±4.7	±12.3	±7.8	±15.8	±0.4	±0.3	±3.5
Peritoneal fluid										
Survivors	NM	29.3	34.2	32.2	70.7	50.1	27.7	4.3	NM	NM
		±3.1	±2.3	±1.5	±3.7	±3.3	±2.1	±0.3		
Nonsurvivors	NM	31.9	55.0*	41.2*	98.2*	84.8*	37.6	8.2*	NM	NM
		±8.3	±5.6	±3.9	±7.3	±7.8	±5.2	±1.4		

*Significantly different ($P < 0.05$) from the corresponding values for survivors for that sample type. *See* Table 1 for key.

Table 4—Hemostatic factors (mean ± SEM) in blood and peritoneal fluid samples from 153 horses with acute gastrointestinal tract diseases, grouped by diagnosis

	Factor									
Sample Group*	PAI-1 (U/ml)	tPA (U/ml)	PLSG (%)	PROTC (%)	ATIII (%)	α_2AP (%)	FIB (mg/dl)	FDP	PT (sec)	APTT (sec)
Blood										
A	23.9	1.8	105.1	89.1	200.1	213.3	180.8	3.4	10.0	47.3
	±6.6	±0.4	±1.8	±2.4	±6.2	±3.6	±5.2	±0.2	±0.1	±2.4
B	28.0	2.0	100.8	86.5	180.4	226.0	203.0	3.8	10.0	48.5
	±7.5	±0.6	±3.9	±3.7	±11.5	±7.9	±12.5	±0.4	±0.2	±4.2
C	41.7	4.1	107.6	84.4	200.8	218.0	218.2	3.5	10.0	46.9
	±12.4	±2.7	±3.8	±5.7	±10.8	±7.5	±15.1	±0.4	±0.1	±3.6
D	27.9	1.1	113.4	72.6	195.2	195.3	183.1	3.6	10.2	34.1
	±6.3	±0.5	±8.5	±7.1	±32.8	±16.4	±15.4	±0.7	±0.3	±1.1
Peritoneal fluid										
A	NM	26.1	29.5[a]	29.7	60.5[a]	40.6[a]	23.6[a]	3.9[a]	NM	NM
		±3.3	±2.5	±1.2	±3.3	±3.2	±2.0	±0.3		
B	NM	40.0	48.1[b]	40.2	100.3[b]	73.9[b]	32.4[a,b]	8.0[b]	NM	NM
		±7.9	±4.5	±4.6	±8.8	±7.0	±4.8	±1.5		
C	NM	31.2	51.3[b]	40.2	97.4[b]	83.5[b]	42.2[b]	6.6[a,b]	NM	NM
		±8.4	±5.3	±4.0	±8.1	±8.6	±5.4	±1.1		
D	NM	20.5	56.5[a,b]	37.4	84.4[a,b]	86.2[a,b]	41.8[a,b]	4.1[a,b]	NM	NM
		±6.9	±16.1	±9.4	±12.3	±10.0	±11.4	±1.6		

*A = Simple colic; B = strangulating diseases; C = inflammatory diseases; D = ruptured viscus.
Means marked with different letters are significantly ($P < 0.05$) different between groups. *See* Table 1 for key.

bumin, differed from 1.6- to 2.9-fold in peritoneal fluid samples from horses with colic, compared with that in healthy horses.

Discussion

With the addition of tPA and PAI-1 to the array of hemostasis assays available, we could evaluate the regulation of fibrinolysis in horses with gastrointestinal tract diseases. Detection of circulating fibrin degradation products reflected concurrent activation of coagulation and fibrinolysis (indicating deposition and subsequent degradation of fibrin). With respect to activity of tPA and PAI-1, the regulatory proteins that control plasmin mediated fibrinolysis, PAI-1 activity predominated in plasma. The enzymatic activity of any factor measured in such a complex system is influenced by many conditions, including the rates of production, utilization, clearance, and degradation. In addition, the PAI-1 activity measured in plasma reflects the balance between circulating tPA and PAI-1. The information collected in this study was insufficient to allow more detailed analysis; however, the higher PAI-1 activity in plasma from horses with colic suggested that the net effect on fibrinolysis was inhibitory in these horses.

The significantly greater plasma PAI-1 activity and lower tPA activity in horses with colic, compared with that in healthy horses, was similar to findings reported in people with gram-negative septicemia.[25,26] The increase in plasma PAI-1 activity documented in those human patients is attributed to the influence of endotoxin on fibrinolysis, and has been interpreted as a poor prognostic indicator in septic shock.[27] In the study reported here, mean plasma PAI-1 activity was greater in nonsurviving horses, and in horses with endotoxin detected in plasma samples.

The association in this study of high blood concentration of fibrin degradation products and prolonged prothrombin time with increased risk of death supported the previous findings of Johnstone and Crane[2] and Henry and Moore.[5] Decreased plasma antithrombin III activity has been identified by Darien et al[4] and Johnstone and Crane[2] as a poor prognostic indicator in horses with colic. Although nonsurvivors in the study reported here had lower mean plasma antithrombin III

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activity, this difference was not significant. These values were consistent with a hypercoaguable state in which the extrinsic coagulation cascade is activated in horses with gastrointestinal tract diseases. Under these circumstances, prothrombin time is prolonged because of consumption of factor VII,[7] and antithrombin III is consumed in an effort to control thrombin generation.[4] In response to this accelerated coagulation, fibrin degradation products, which normally are not found in blood, appear as deposited fibrin is degraded.

The results of our study suggested that coagulation is activated in the face of inhibited fibrinolysis in the blood of horses with colic. Because less is known about the response of the abdominal cavity to injury, coagulation and fibrinolysis values measured in peritoneal fluid samples from the horses with colic in this study were interpreted on the basis of an understanding of the mechanisms controlling peritoneal fluid production and composition. Fluid dynamics in the normal peritoneal cavity are governed by hydrostatic and oncotic pressures in capillaries lining the visceral peritoneum and in the interstitial space separating these capillaries from the mesothelial lining of the peritoneal cavity.[28] Peritoneal fluid leaves the abdomen through lymphatics, primarily located along the ventral part of the diaphragm. With inflammation, fluid dynamics and membrane permeability are altered, and there is nonspecific leakage of plasma proteins into the peritoneal fluid.[28]

In addition to nonspecific changes in peritoneal fluid protein constituents that may develop with intra-abdominal inflammation, perturbation of peritoneal macrophages by inflammatory stimuli such as endotoxin results in increased protein synthesis and secretion.[29] For example, mouse peritoneal macrophages elaborate several clotting factors, including factors V,VII, IX, X, and prothrombin.[29] Activated macrophages also produce plasminogen-activator and express surface procoagulant activity,[29] and elaborate proinflammatory substances, such as tumor necrosis factor and interleukin-1, which serve as intercellular messengers that alter the rate and composition of protein production by cells.[30]

Dehydration, hemoconcentration, and the inflammatory effects of endotoxin all likely contributed to the greater values of protein constituents in peritoneal fluid of horses with colic in the study reported here. For instance, protein C and antithrombin III are similar in size to albumin, and the difference in these factors in peritoneal fluid between healthy horses and horses with colic was similar to that for albumin. However, the greater activities of plasminogen and α_2-antiplasmin in peritoneal fluid from horses with colic exceeded those of protein C and antithrombin III by more than 200%. Plasminogen is the precursor of plasmin, the protease responsible for inducing fibrinolysis by cleaving polymerized fibrin,[31] whereas α_2-antiplasmin is the primary inhibitor of activated plasmin in blood.[31] Thus, the difference observed in these 2 proteins seemed to be caused by a specific event, potentially directed by activated peritoneal macrophages, and resulted in the influx of fibrinolytic proteins into the peritoneal fluid of horses with colic.

Only tPA activity and fibrin degradation products concentration were equal or greater in peritoneal fluid than in blood in healthy horses. Significantly greater values in these factors were associated with the detection of endotoxin in peritoneal fluid samples from horses with colic. Tissue plasminogen activator is synthesized and released from endothelial cells and from macrophages.[32,33] The cell-free fraction of peritoneal fluid seems to have a relative excess of tPA with respect to its inhibitor, PAI-1, in direct contrast to the situation in blood.[34] Apparently, high endogenous tPA activity in peritoneal fluid in all horses interfered with the indirect chromogenic assay used to measure PAI-1 activity, which depends on low endogenous tPA for accuracy. Because the quantitation of PAI-1 activity in peritoneal fluid was considered unreliable with the current assay, further analysis was not pursued.

The results of this study suggested that in healthy horses, and, to a greater degree, in horses with colic, fibrinolysis is favored in the cell-free fraction of peritoneal fluid. This conclusion was based on the observations that tPA normally is found in excess in peritoneal fluid samples from healthy horses, and increases in response to gastrointestinal tract diseases, as do plasminogen and α_2-antiplasmin. Although α_2-antiplasmin inhibits fibrinolysis by binding to plasmin, the increase in its activity may reflect an attempt to restrict plasmin activity to the site of fibrin deposition, thereby preventing indiscriminate proteolytic activity. This theory was supported by greater concentration of fibrin degradation products in peritoneal fluid of horses with colic, which exceeded that found in blood; these values were thus unlikely to have simply reflected passive movement across an inflamed peritoneal lining, but were more likely to have been indicative of activated intra-abdominal fibrinolysis.

Intraperitoneal fibrinolytic activity has been reported to be inhibited in response to gastrointestinal tract injury, in contradiction to our findings. Although samples of cell-free fraction of peritoneal fluid are readily obtainable, making this fluid useful in prospective clinical studies such as ours, fibrinolytic activity in this cell-free fluid sample may not accurately reflect conditions on the surfaces of cells and intestinal viscera. For instance, in studies in which mesothelial biopsy specimens were obtained from the peritoneal cavity of people with naturally developing inflammatory diseases, decreased plasminogen-activator activity was reported.[35] In addition, endotoxin-stimulated peritoneal macrophages also express procoagulant activity,[36] which may stimulate the deposition of fibrin on macrophages and adjacent intestinal serosa.[37] However, macrophages harvested from the peritoneal cavity of rabbits after small-intestinal resection have been shown to express increased tPA activity,[38] and enhanced peritoneal fibrinolytic activity, similar to that reported here, was observed in the cell-free fraction of peritoneal fluid from women with pelvic inflammatory disease.

Clearly, the regulation of fibrinolysis, and specifically the expression of tPA by intra-abdominal cell populations, is not yet understood. The role of peritoneal fibrinolysis in the development of intra-abdominal adhesions also remains to be elucidated. On the basis of the results of the study reported here, a specific activation of fibrinolysis seems to develop in cell-free peritoneal fluid collected from horses with gastrointestinal tract

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diseases. This increase in fibrinolytic activity is associated with endotoxemia, and develops in direct contrast to the concurrent inhibition of intravascular fibrinolysis.

The distribution of diagnoses and survival rates in the group of horses with gastrointestinal tract diseases studied here resembled those of other large case studies.[39,40] Phillips and Walmsley[41] reported 72% survival to discharge and 66% long-term survival for horses with surgical diseases, compared with 71 and 62% in our study. Unfortunately, intravascular and intra-abdominal complications that might have been related to abnormal hemostasis developed too infrequently in our study to allow meaningful analysis.

[a]Coag-A-Mate XC, Organon Technika Corp, Durham, NC.

[b]General Diagnostic Simplastin, Automated APTT, and Fibriquik, Organon Technika Corp, Durham, NC.

[c]Thrombo-Wellcotest, Burroughs Wellcome, Chapel Hill, NC.

[d]Cobas-Fara, Roche Diagnostic Systems, Nutley, NJ.

[e]American Diagnostica Inc, Greenwich, Conn.

[f]Human melanoma cell-line derived tissue plasminogen activator, American Diagnostica Inc, Greenwich, Conn.

[g]QLC-1000, Whittaker Bioproducts Inc, Walkersville, Md.

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