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Detection of Pepsin in Tracheal Secretions After Forced Small-Volume Aspirations of Gastric Juice

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Abstract

Background—Detecting small-volume aspirations of gastric contents is an important but difficult task. A potentially useful method for this purpose is assaying tracheal secretions for pepsin, an expected constituent of gastric juice.

Methods—A 2-group experimental design was used. The primary subjects were 161 experimental and 21 control New Zealand white rabbits; 161 acutely ill humans provided the gastric juice used in the project. The animals were anesthetized before being intubated and mechanically ventilated. Three separate boluses of human gastric juice mixed with dye-stained enteral formula were instilled into the experimental animals' tracheas; the 21 control animals received only 0.9% sodium chloride solution. At the beginning of each experiment, 0.4 mL/kg of the substance was infused over a 30-minute period; the infusion was then stopped and 90 minutes were allowed to elapse before endotracheal suctioning was performed. This procedure was repeated at hour 2 and hour 4. After completion of the multiple aspiration portion of the study, 23 additional animals were subjected to a single aspiration of 0.4 mL/kg of a mixture of human gastric juice and dye-stained enteral formula; secretions were obtained at 2 hours, 4 hours, and 6 hours. An immunoassay was used to test for pepsin in all of the tracheal secretions.

Results—In the 3-aspiration group, pepsin was found in all of the secretions from 92.5% (149/161) of the experimental animals; in contrast, no pepsin was found in any of the secretions from the 20 control animals. In the single-aspiration group, pepsin was found in all of the tracheal secretions from the 23 animals at 2 hours and 4 hours and 21 of the 23 animals at 6 hours.

Conclusions—The immunoassay used in this animal model study was able to detect pepsin in >90% of the experimental animals' tracheal secretions after multiple or single forced aspirations of gastric juice. The extent to which pepsin can be detected in the tracheal secretions of acutely ill tube-fed humans requires investigation, as does the extent to which clinical outcomes are affected by pepsin-positive tracheal secretions.

Although a single large-volume aspiration of gastric contents is easily detected, a more common scenario in critically ill, tube-fed patients is a series of small-volume, unobserved aspirations. Repeated aspirations can lead to nosocomial pneumonia, with the potential for sepsis.[1] Early recognition of occult aspirations is important so that interventions can be implemented to prevent further aspirations. Unfortunately, small-volume aspirations are

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difficult to detect. Methods previously used for this purpose (such as adding dye to enteral formula and testing for glucose in tracheal secretions) have been shown to be unreliable.[2,3]

One potentially useful method for detecting aspiration is assaying tracheal secretions for pepsin, an expected constituent of gastric juice. The limited numbers of previous studies that are described below demonstrate that assays performed on tracheal secretions are feasible in various laboratory and clinical models.

In a laboratory study, Badellino et al[4] tested for proteolytically active pepsin in bronchoalveolar fluid lavaged from 24 rabbits after the intratracheal instillation of human gastric juice (2 mL/kg); 12 additional rabbits served as controls and had normal saline instilled in the same volume. Bronchoalveolar lavage was performed at 15 minutes, 30 minutes, or 60 minutes after fluid instillation. In the human gastric juice group, pepsin activity was detected in postaspiration lavage fluid in 8 of 8 animals at 15 minutes, 6 of 8 at 30 minutes, and 5 of 8 at 60 minutes. Pepsin activity was not detected in the lavage fluid from the control animals at any time.

Using gastric and tracheal secretions from 10 fasting preoperative patients, Ufberg et al[5] found pepsin in all of the gastric specimens, but in none of the tracheal secretions. In a later study, Ufberg et al[6] tested for pepsin in the tracheal secretions of 168 intubated patients; 148 had been intubated in the emergency department of a level I trauma center and 20 had been intubated in a prehospital setting. Pepsin was found in 22% of the 148 patients intubated in the hospital, compared with 50% of the 20 patients intubated before admission, $p = .008$. The authors concluded that patients are more likely to aspirate gastric contents when endotracheally intubated in a prehospital setting. In both studies, a fibrinogen digestion technique was used to assay for pepsin.

Metheny et al[7] found proteolytically active pepsin in 94% of the gastric secretions collected from 343 acutely ill adults; in contrast, pepsin was detected in only 2 of 148 tracheobronchial secretions collected from the same population. The 2 respiratory secretions that were positive for pepsin were found in patients with multiple risk factors for aspiration.

To increase the probability of finding low pepsin concentrations, Metheny et al used an immunoassay to detect pepsin in 136 tracheal secretions from 30 mechanically ventilated, tube-fed patients.[8] Multiple samples were obtained from 26 of the 30 patients (range, 2 to 11 per patient). Fourteen of the 136 specimens, collected from 5 different patients, contained low pepsin concentrations (1.9 to 17.8 μg/mL); 13 of the 14 pepsin-positive secretions were obtained from patients lying flat in bed. Meert et al[9] used the same immunoassay to test for pepsin in 100 tracheal secretions from 37 critically ill, mechanically ventilated children (ranging in age from 2 weeks to 15 years). Pepsin was detected in 9 of the 100 tracheal secretions. Five of the 37 children had at least 1 pepsin-positive secretion; those with positive-pepsin secretions were more likely to have clinical evidence of gastroesophageal reflux than were those with pepsin-negative secretions ($p < .01$).

Using an assay for proteolytically active enzyme, Krishnan et al[10] found pepsin in the tracheal secretions of 31 of 37 children with a history of gastro-esophageal reflux and chronic respiratory symptoms; in contrast, pepsin was not detected in the tracheal secretions of 26 children without a history of these conditions.

Objectives

1. An experimental animal model study was designed to determine:
    a. the extent to which a pepsin immunoassay can detect pepsin in tracheal secretions after 3 forced small-volume aspirations of enteral formula mixed with human gastric juice; and

   b. the extent which a pepsin immunoassay fails to detect pepsin in tracheal secretions after 3 forced small-volume aspirations of 0.9% sodium chloride solution.

2. The animal study was also designed to examine the extent to which the concentration of pepsin in the suctioned tracheal secretions after 3 forced small-volume aspirations was affected by:

   a. volume of the secretions at each of the 3 data- collection periods (2 hours, 4 hours, and 6 hours); and

   b. visible blood in the secretions at each of the 3 data-collection periods (2 hours, 4 hours, and 6 hours).

3. In addition, the study was designed to determine the extent to which a pepsin immunoassay can detect pepsin in tracheal secretions after a single small-volume aspiration of enteral formula mixed with human gastric juice.

MATERIAL AND METHODS

For the first 2 objectives, a 2-group experimental design was used to determine the efficacy of pepsin as a marker for pulmonary aspiration of gastric contents. The primary subjects were 161 experimental and 21 control New Zealand white rabbits that weighed approximately 3 kg each. In addition, gastric juice was collected from 161 acutely ill humans for use in the project. Approval for the inclusion of human subjects was obtained from the St. Louis University Human Subjects Committee; the animal experiments were approved by the St. Louis University Animal Care Committee and conducted according to institutional animal care and utilization committee standards.

Gastric juice was collected from the nasogastric tubes of hospitalized acutely ill adults on the day preceding each experiment. All of the patients had been fasting for at least 4 hours, and no medications had been received by nasogastric tube or mouth within the preceding hour. The gastric juice was assayed for pepsin by the Anson[11] method and then refrigerated until the following morning, when it was mixed half and half with 1 of 8 enteral formulas. Our plan for using a mixture of half gastric juice and half enteral formula was based on an assumption that the volume of gastric juice in the typical tube-fed patient is roughly equivalent to the hourly formula infusion rate. We opted to use gastric juice from a variety of critically ill patients at random; our rationale was to simulate the concentrations of pepsin found in the gastric juice of patients who are at highest risk for aspiration. In so doing, we had a wide range of gastric juice pepsin concentrations represented in the study. Another reason for using unaltered gastric juice from critically ill patients (rather than pepsin standard solutions) was to determine if other constituents in human gastric juice might interfere with the assay. We measured trypsin and bilirubin concentrations in each of the gastric samples and found that neither of these substances interfered with the immunoassay.

We randomly selected the enteral formula used during each experiment until we had achieved an even distribution of formulas among the experimental animals. (A variety of enteral formulas were included so that we could test the amount of glucose in tracheal secretions to evaluate the efficacy of glucose oxidase reagent strips in detecting aspiration; glucose findings are not included in the current paper.) Half of the experimental animals had FD&C Blue Dye Number 1 added to the instilled formula in a concentration of 0.8 mL/L and the remaining half had dye added in a concentration of 1.5 mL/L. (We tested 2 concentrations of dye to determine the efficacy of dye in detecting repeated small-volume aspirations; we found that neither concentration was effective.)[12] The mean pepsin concentration in the 161 human gastric juice specimens was 352 μg/mL (range 13.6 to 925.2 μg/mL). Over three-fourths (77.6%) of the 161

patients from whom gastric juice was collected were receiving either an H_2 receptor antagonist or a proton pump inhibitor.

The animals were anesthetized, intubated with a 3.5-mm uncuffed endotracheal tube, and mechanically ventilated (VIP BIRD Infant Pediatric Ventilator with an attached warm air respiratory humidifier; Fisher & Paykel Healthcare, Auckland, New Zealand). Continuous monitoring for hemodynamic and acid-base status was performed. A tidal volume of 50 mL with a positive end-expiratory pressure of 2 cm H_2O was used in all animals, with the frequency altered to control acid-base balance.

A volumetric infusion pump (Harvard Syringe Pump, Model 4400; Harvard Apparatus, Inc., Holliston, MA) was used to instill 3 separate boluses of human gastric juice mixed with enteral formula via a 1.22-mm polyurethane catheter introduced through the endotracheal tube into the mainstem bronchus of the 161 experimental animals. The 21 control animals received 0.9% sodium chloride solution by the same method.

At the beginning of each experiment, 0.4 mL/kg of the substance was infused over a 30-minute period; the infusion was then stopped, and 90 minutes were allowed to elapse before endotracheal suctioning was performed with a 6.5 French catheter attached to a 20-mL pediatric mucus trap. A 90-minute period was selected to allow the dilution of the infused substance by local respiratory secretions. The suctioned material was visually inspected for blood before being taken to a research laboratory where its pepsin concentration was measured by an immunoassay.

At hour 2, an additional 0.4 mL/kg of the appropriate substance was infused over a 30-minute period. Again, the infusion was stopped and 90 minutes were allowed to elapse before endotracheal suctioning was performed. At hour 4, this process was repeated (see Fig. 1). Thus, by the end of the 6-hour experiment, each animal had received intratracheally a total volume of fluid (either gastric juice mixed with enteral formula or 0.9% sodium chloride solution) equivalent to 1.2 mL/kg body weight.

We opted to do 3 infusions of the mixture of dye-stained formula and gastric juice to determine the effect of multiple aspiration events on the ability to detect pepsin, dye, and glucose in the tracheal secretions. Our hypothesis was that successive aspirations would be easier to detect than would be a single aspiration.

After completion of the multiple-aspiration portion of the study reported above, we infused a mixture of half gastric juice and half dye-stained enteral formula (0.4 mL/kg) at baseline into the tracheas of 23 experimental rabbits and suctioned secretions at 2 hours, 4 hours, and 6 hours to test for pepsin. Our rationale was to determine how long pepsin could be detected in tracheal secretions after a single aspiration event.

Pepsin Assay Used for Gastric Secretions

Pepsin assays on the human gastric juice were performed in a research laboratory using a UV/VIS Spectrometer, Lambda 2 (Perkin Elmer Corp., Analytical Instruments, Norwalk, CT). All samples were centrifuged before analysis. The Anson assay for pepsin depends on the estimation of products of hydrolysis of hemoglobin, which is denatured at the low pH (2) of the assay; the estimation uses the UV absorbance (280 nm) of the products.[11] We elected to use an active enzyme assay for gastric juice because it is faster and easier to perform. The active enzyme assay takes <30 minutes to complete, whereas the immunoassay requires 24 hours; also, an active enzyme assay was suitable because we tested the gastric juice shortly after it was collected. To assure that assays by both methods (active enzyme and immunoassay) yield similar results, we tested 50 split samples of gastric juice by both methods. The results

were highly correlated, $r = .915$, $p < .000$. The mean pepsin concentrations were almost identical (188.4 *vs* 180.9 μg/mL); paired *t* test analysis found no significant difference in the split sample values ($t = .746$, $p = .460$).

Pepsin Assay Used for Tracheal Secretions

An immunoassay with rooster polyclonal antibodies to purified human pepsin was used to detect pepsin in tracheal secretions. To develop the assay, we began by purifying human pepsin; a column containing 3 mL of agarose beads conjugated with pepstatin was first equilibrated with a solution containing 0.1-M HC1 and 0.15-M NaCl. Next, 10 mL of human gastric juice containing >400 μg/mL of pepsin was loaded onto the column, followed by extensive wash with a solution containing 0.1-M Hcl and 0.15-M NaCl, until the OD_{280} (optical density at a wavelength of 280 nanometers) of the flow-through was <0.01. The bound pepsin was then eluted with 10 mL of 6-M urea and dialyzed extensively against PBS. Purity of pepsin was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis; 2.8 mg was obtained. About 400 μg of purified human pepsin and complete Freund's adjuvant mixture were injected subcutaneously into 3 roosters as a primary injection. The animals were boosted at 28 days with antigens in incomplete Freund's adjuvant; boosts were repeated 5 times and blood was collected for future use. Blood for the analyses was obtained from the roosters 10 days after the secondary booster; a 1:1000 dilution of the serum was used for the immunoassay.

By testing increasingly dilute standards of pepsin solutions, we established that the immunoassay could detect pepsin in a concentration as low as 1 μg/mL. Before the study reported here, we pilot-tested the assay in 10 rabbits before and after gastric juice was instilled into their lungs; pepsin was not detected in any of the secretions before the instillations but was detected in all afterward. We also tested for cross-sensitivity with other possible gastric constituents, namely, trypsin and bilirubin; none was found.

An immunoassay was selected because it is capable of detecting partially degraded pepsin, an important consideration when gastric juice is exposed to the alkaline environment of the lung. For the assay, 150 μL of each tracheal specimen was mixed with an equal volume of 1:2 Laemmli Sample Buffer (Bio-Rad Laboratories, Hercules, CA) containing 2-mercaptoethanol (Bio-Rad Laboratories) and boiled for 5 minutes. The treated sample was then resolved by using sodium dodecyl sulfate polyacrylamide gel electrophoresis and was transferred onto nitrocellulose membrane (Bio-Rad Laboratories). After the membrane was blocked overnight in 50 mL of phosphate-buffered saline (pH 7.5; containing 0.2% Tween-20 [PBST] and 10% nonfat dry milk), it was incubated at room temperature for 2 hours with the rooster polyclonal antibodies to human pepsin diluted 1000-fold in PBST. The membrane was then washed 3 times in PBST and incubated at room temperature for 2 hours with the alkaline-phosphatase–conjugated rabbit antichicken IgG (Sigma, St. Louis, MO) diluted 1:20,000 in PBST. Finally, after the membrane was rinsed 3 times in PBST, pepsin was visualized by using the Alkaline Phosphatase Kit (Bio-Rad) according to the manufacturer's procedures. Standard curves for the assay were created by assaying samples containing pepsin at concentrations of 2, 5, 10, 25, and 50 μg/mL.

Although the pepsin immunoassay can detect pepsin in very small quantities, it cannot detect the minuscule amount of pepsinogen (<0.1 μg/mL) present in blood (presumably after direct absorption from gastric mucosal cells).[13,14] Therefore, the possibility of a false-positive pepsin reading in tracheal secretions because of a transudative process is negligible. Secretions that contained visible blood were assayed in the same manner as non-bloody secretions.

Data Analysis

Descriptive statistics were used to report the data. The incidence of pepsin-positive tracheal secretions in the experimental and control animals is presented in Table I. Multiple regression was used to predict the concentration of pepsin in tracheal secretions of the experimental animals from the following variables: concentration of pepsin in gastric secretions, visible blood in the tracheal secretions, and volume of the tracheal secretions at 2 hours, 4 hours and 6 hours. An independent samples *t* test was used to compare pepsin concentrations in the tracheal secretions according to the concentration of dye added to the enteral formula instilled into the animals' lungs.

RESULTS

We were able to suction sufficient tracheal fluid for pepsin testing in 89% (56/63) of the attempts in the control animals, and in 99% (479/483) of the attempts in the experimental animals. The mean ± SD volume of secretions was 0.21 ± 0.18 mL at 2 hours, 0.31 ± 0.26 mL at 4 hours, and 0.41 ± 0.31 mL at 6 hours. Blood was visually observed in 27% of the secretions at 2 hours, 34% at 4 hours, and 45% at 6 hours. Mucus was not visualized in any of the secretions.

Pepsin was found in 94.8% (454/479) of the tracheal secretions from the experimental animals but was absent in all 56 secretions from the control animals. The secretions' pepsin concentrations decreased by approximately 10% from the 2-hour to the 4-hour data-collection point and by 9% from the 4-hour to the 6-hour data-collection point. See Table II.

Detection of pepsin in the tracheal secretions at the 2-hour, 4-hour, and 6-hour data-collection points is presented in Table I. Pepsin was detected in >93% of the experimental animals' tracheal secretions but in none of the control animals' secretions.

The 25 pepsin-negative tracheal secretions from the experimental group occurred among 12 animals; 5 had negative pepsin readings at all 3 data collection points, 3 had negative readings at 2 points, and 4 had only 1 negative reading. The mean predilution gastric pepsin concentration infused into the animals with no pepsin in their tracheal secretions was significantly lower than that in the animals with positive tracheal pepsins at all 3 data-collection points (82.9 μg/mL *vs* 365.7 μg/mL, $p < .001$).

Descriptive statistics and intercorrelations for the dependent and predictor variables are presented in Table III. As shown in Table IV, the major predictor of pepsin concentration in the animals' tracheal secretions at the 2-hour data-collection point was the pepsin concentration in the human gastric juice instilled into their lungs. Results at the 4-hour and 6-hour data-collection points were highly similar to those at the 2-hour point. Although statistically significant at all 3 data-collection points, the contribution of visible blood was small in comparison to the influence of gastric pepsin concentration. Controlling for the other 2 predictors in the model (gastric pepsin and volume of the secretions), pepsin concentration in the secretions was 32 to 36 μg/mL lower when visible blood was present. Volume of the tracheal secretions did not contribute significantly to variation in tracheal pepsin concentrations at any of the 3 data-collection points.

In the single-aspiration portion of the study performed on 23 rabbits, pepsin was detected in 100% of the tracheal secretions at 2 hours and 4 hours and in 91.3% of the secretions at the 6-hour data-collection point. The mean pepsin concentration in the tracheal aspirates decreased from 245.4 μg/mL at 2 hours, to 172.5 μg/inL at 4 hours, and 77.2 μg/mL at 6 hours.

DISCUSSION

Findings from this controlled animal study indicate that the pepsin immunoassay described above was highly effective in detecting 3 forced small-volume aspirations of gastric juice. Especially noteworthy was that it was able to detect pepsin in >93% of the experimental animals' tracheal secretions over the 3 data-collection points. This is in contrast to previously reported findings of increasingly diminished sensitivity of the blue dye method in detecting multiple aspirations in the same group of animals, $p < .05$.[12] Although the concentration of pepsin in the tracheal secretions decreased by about 10% after the second and third aspiration events, these changes did not affect the pepsin assay's ability to detect pepsin. As anticipated, the strongest predictor of the tracheal secretions' pepsin concentration was the amount of pepsin in the gastric juice instilled into the animals' lungs. Volume of the tracheal secretions and visible blood in the tracheal secretions had little impact on their pepsin concentrations. Although the difference was small, visibly bloody secretions contained less pepsin than did those that did not contain blood; possibly this was because blood in the secretions diluted the pepsin concentration.

As described earlier, about 5% (25/479) of the tracheal secretions from the experimental animals that sustained 3 aspirations tested negative for pepsin, despite the intra-tracheal instillation of pepsin-containing gastric juice. Although likely not the only reason for this occurrence, negative pepsin values were observed more often when the instilled gastric juice had a pepsin concentration <210 μg/mL ($p < .05$). It is possible that pepsin was absent from some of the tracheal secretions because our suctioning attempts did not reach pockets of fluid contaminated with gastric juice.

Our study differs from that reported by Badellino et al[4] in several ways. First, we used an immunoassay, whereas Badellino et al used an active enzyme assay. Second, our study used a mixture of half gastric juice (0.2 mL/kg) and half enter al formula (0.2 mg/kg) to infuse into the animals' tracheas, whereas Badellino et al used pure gastric juice (2 mL/kg). In so doing, we infused only one-tenth of the amount of gastric juice. Another difference is that our study tested for pepsin over a period of 6 hours, whereas Badellino et al tested for a maximum of 1 hour. We used a larger sample (161 experimental animals that sustained 3 aspiration events and 23 that sustained a single aspiration event); in contrast, Badellino et al tested for pepsin in 24 experimental animals. At the end of 6 hours, we found evidence of pepsin in 94.3% (149/158) of the secretions collected from the multiple aspiration animals and in 91.3% (21/23) of the secretions from the single aspiration animals. In contrast, Badellino et al detected pepsin in 62.5% (5/8) of the animals after a period of 1 hour. Last, Badellino et al used a bronchoalveolar lavage technique, whereas we did not use saline during our suctioning attempts. Thus, the secretions tested in our study were not as diluted as those tested by Badellino et al.

A major strength of the study was our ability to control the time of the aspiration event, the amount of fluid aspirated, and the interval of secretion retrieval from the animals' lungs. Also, we knew the concentration of pepsin in the gastric juice instilled into the animals' lungs and thus were able to make comparisons with pepsin found in the retrieved tracheal secretions.

The extent to which the findings from our animal model study are applicable to humans is unknown. A major limitation was the absence of visible mucus in the secretions suctioned from the animals' lungs. Although rabbits have goblet cells that are capable of secreting mucus, they do not do so to the extent that is found in intubated humans (who can form mucus freely after irritation of the lungs by foreign substances). Because the secretions were not diluted with mucus, as one would expect to find in humans, it is probable that the pepsin concentrations in secretions retrieved from the animals' lungs were much higher than what would be observed

in clinical situations. As reported earlier, using the identical assay, we found much lower pepsin concentrations (1.9 to 17.8 μg/mL) in 14 of 136 tracheal secretions retrieved from 30 mechanically ventilated, tube-fed patients at high risk for aspiration.[3]

Another potential limitation of the study was the possibility that the pepsin concentration in the instilled gastric juice diminished slightly during overnight storage before its use in the experiment the following morning. Also, whereas one-third of the mixture of gastric juice and enter al formula was used for the first instillation, the rest was refrigerated until used for the second and third instillations (2 or 4 hours later). If pepsin in the mixture deteriorated further during these time periods, the pepsin concentrations in the 4-hour and 6-hour tracheal specimens may have been lower than that found at 2 hours. A slight decrease in the tracheal secretions' pepsin concentrations was observed over time; however, we were unable to ascertain the cause of this change.

CONCLUSIONS

The immunoassay used in this animal model study was able to detect pepsin in >93% of the tracheal secretions after 3 forced small-volume aspirations. It also was able to detect pepsin in 21 of 23 animals 6 hours after a single aspiration event. However, it is probable that the concentration of pepsin found in the animals' tracheal secretions greatly exceeds what would be observed in humans after gastric juice aspiration. Partly this is because humans have the capacity to dilute aspirated materials to a greater extent than did the animals used in our study. Also, it is likely that the volume of aspirated gastric contents (gastric juice and dye-stained enteral formula) was considerably higher in our animal model than what typically occurs during small-volume aspirations in acutely ill humans; for example, an equivalent single aspiration volume in a 70-kg person would be 28 mL (0.4 mL/kg). Nevertheless, these animal data are encouraging and pave the way for further clinical study of the utility of pepsin in detecting aspiration.

Although the presence of pepsin in tracheal secretions is a reasonable signal of aspirated gastric juice, the clinical significance of this finding is unknown. As indicated by Bartlett and Gorbach,[15] even healthy persons may aspirate small volumes of gastric contents with apparently no measurable *sequelae*. Thus, the extent to which clinical outcomes are affected by pepsin-positive tracheal secretions needs to be investigated.

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Table I

Incidence of pepsin in tracheal secretions from the experimental and control animals

	Tracheal secretions positive for pepsin	Tracheal secretions negative for pepsin
	2-hour	
Experimental animals	155/160 (96.9%)	5/160 (3.1%)
Control animals	0/17 (0.0%)	17/17 (100.0%)
	4-hour	
Experimental animals	150/161 (93.2%)	11/161 (6.8%)
Control animals	0/19 (0.0%)	19/19 (100.0%)
	6-hour	
Experimental animals	149/158 (94.3%)	9/158 (5.7%)
Control animals	0/20 (0.0%)	20/20 (100.0%)

Table II

Description of pepsin concentrations in tracheal secretions from experimental and control animals according to time of data collection (2 hours, 4 hours, and 6 hours)

Tracheal secretion pepsin concentration, μg/mL	Experimental animals* Time of data collection			Control animals Time of data collection		
	2-hours	4-hours	6-hours	2-hours	4-hours	6-hours
Mean ± SD	140.7 ± 99.3	126.6 ± 98.7	115.5 ± 91.6	0.0	0.0	0.0
Range	(0–422.8)	(0–422.3)	(0–413.0)	—	—	—
N	160	161	158	17	19	20

* In the experimental group, pepsin was found at all 3 data collection times in 149 of the 161 animals.

Table III

Means, SDs, and intercorrelations for dependent and predictor variables used in the 2-hour multiple regression analyses

Dependent and predictor variables	Mean (SD)	Range	Intercorrelations		
			DV: Pepsin in secretions	1. Gastric pepsin	2. Visible blood
Dependent variable: pepsin concentration in tracheal secretions (μg/mL) at 2 hours	140.7 (99.3)	0.0–422.8	1.00		
Predictor 1. Pepsin concentration of instilled gastric fluid (μg/mL)	352.2 (203.9)	13.6–925.2	.60***	1.00	
Predictor 2. Visible blood in secretions (0 = no, 1 = yes) at 2 hours	.27 (.44)	0–1	−.27***	−.18*	1.00
Predictor 3. Volume of tracheal secretions (mL) at 2 hours	0.24 (0.22)	0.005–2.000	−.10	−.07	.08

* $p < .05$,

*** $p < .001$;

DV, dependent variable.

Table IV

Regression analysis of predictors of pepsin concentration (μg/mL) in tracheal secretions at 2-hour (N = 160) data collection point

Step	Variable entered	R^2	R^2_{Change}	Final model coefficients	
				Unstandardized (SE)	Stabdardized (β)
1	Pepsin concentration in gastric secretions (μg/mL)	.36***	.359***	0.28 (0.03)***	0.57***
2	Visible blood in secretions (0 = no, 1 = yes)	.39***	.027**	−36.4 (14.3)*	−0.16*
3	Volume of tracheal secretions (mL)	.39***	.002	−20.9 (28.2)	−0.05
	$R^2_{Adj(final\ model)}$ = .38			58.1 (15.4)	—
				Constant	

* $p < .05$;

** $p < .01$;

*** $p < .001$.