A Dose-responsive Model of Smoke Inhalation Injury

Severity-related Alteration in Cardiopulmonary Function

TAKESHI SHIMAZU, M.D., TETSUO YUKIOKA, M.D.,* GENE B. HUBBARD, D.V.M., M.S., PAULETTE C. LANGLINAIS, M.S., ARTHUR D. MASON, JR., M.D., and BASIL A. PRUITT, JR., M.D.

From the U.S. Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas

The dose responsiveness of selected physiologic indices was studied in a sheep model of smoke inhalation injury. In this model, graded severity of injury was achieved by changing the contact time with smoke (defined by "unit"), whereas other variables were kept constant. Blood gas and cardiopulmonary indices were measured in 70 sheep, including 12 controls, either 24 or 72 hours after exposure to 3, 6, 9, 12, 15, or 18 units of smoke. A 12-unit dose of smoke was fatal within 72 hours and an 18-unit dose was fatal within 24 hours. The best correlation between smoke dose and response was observed in arterial oxygen tension 24 hours after exposure. At 24 hours, most of the cardiopulmonary indices showed significant change only after a 12-unit exposure. Although the exact shape of the dose-response curve could not be defined, sigmoid or curved linear shape was suggested, reflecting the progressive deterioration.

SMOKE INHALATION INJURY is one of the primary determinants of survival after major burns.[1] The significance of pulmonary injury due to smoke inhalation in burn patients was not widely realized until the 1970s, although antecedent work by Phillips had indicated the likelihood that such injury was of consequence.[2-4] In recent years early diagnosis of smoke inhalation and evaluation of its severity have achieved wide clinical interest, but even now the pathophysiologic mechanisms of the injury are not clear. Although several animal models have been developed to study such mechanisms, they have either been small animal models[5-8] or failed to permit control of severity.[9-12] We have developed a sheep model of smoke inhalation injury that is reproducible and dose-responsive.

Sheep are large enough to allow detailed physiologic monitoring and frequent blood sampling.[13-14] A technique for lung lymph cannulation has also been established for sheep.[15] In this model, dose responsiveness was assessed by changing the duration of smoke exposure to the lung while keeping other factors that influence severity unchanged. The severity of smoke inhalation injury is related to smoke temperature, chemical and physical composition of the smoke (gas, vapor, and particle), contact time (time exposed to smoke), and contact area (depth of breath). In the current system, the smoke was equilibrated at ambient temperature to exclude all possibility of thermal injury to the airway. The animals were exposed to a volume of smoke proportional to their body weight. The severity of injury in a spontaneously breathing animal is also influenced by hyperventilation in response to the carbon monoxide content of the smoke.[16] Spontaneously breathing animals with elongated nasal passages, with epiglottic closure, or with early bronchospasm may show further variability in severity of injury.[12] In this work, smoke exposure was done under general anesthesia using endotracheal intubation to avoid changes in the depth of breath that affect contact area. With respect to smoke

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and with the *Guide for the Care and Use of Laboratory Animals,* National Institutes of Health Publication 80-23.

* Osaka University Medical School, Department of Traumatology, Osaka, Japan.

Reprint requests: U.S. Army Institute of Surgical Research, ATTN: Library Branch, Fort Sam Houston, San Antonio, TX 78234-6200.

Correspondence: Takeshi Shimazu, M.D., U.S. Army Institute of Surgical Research, ATTN: SGRD-USM, Fort Sam Houston, San Antonio, TX 78234-6200.

Submitted for publication: December 24, 1986.

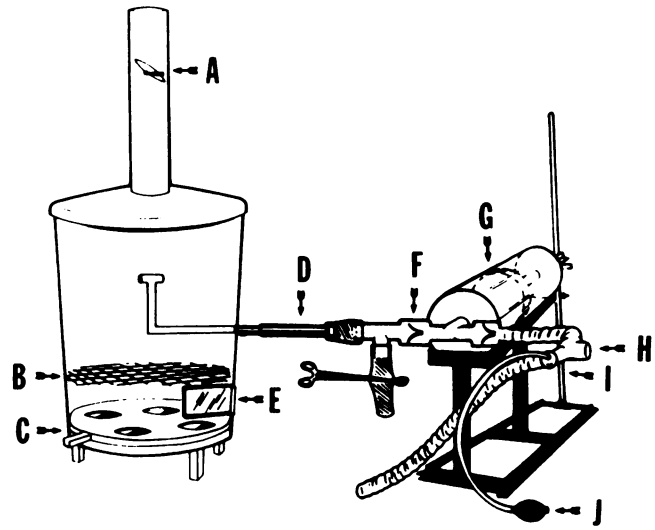

FIG. 1. Schematic presentation of smoke generator and smoke delivery system. Disposable underpads are burned in the smoke generator. Smoke is introduced through the copper pipe (D) to the volume-adjustable metal syringe (G). Either smoke or air is introduced into the pump by clamping the other rubber connection. Animals are intubated and connected to the outlet (H). Animals are insufflated with smoke when the exhalation valve (I) is closed manually by pressing the rubber bulb (J). (A) Damper; (B) Mesh; (C) Adjustable air inlet; (E) Window; (F) One-way valve.

composition, fluctuation of combustion was minimized by the relatively large size of the combustion chamber (32 gallons, 122 liters).

After smoke exposure, the sheep were extubated and allowed to breathe spontaneously to assess the natural history of smoke inhalation injury. Twenty-four or 72 hours after smoke inhalation, the sheep were reanesthetized, intubated, and catheterized for the measurement of pulmonary arterial and systemic arterial pressures. After a 2-hour stabilization period, cardiopulmonary indices were measured under anesthesia, using mechanical ventilation, a state that corresponds to the situation in patients in an intensive care unit setting.

This report describes the dose dependence of alterations in cardiopulmonary function after smoke inhalation.

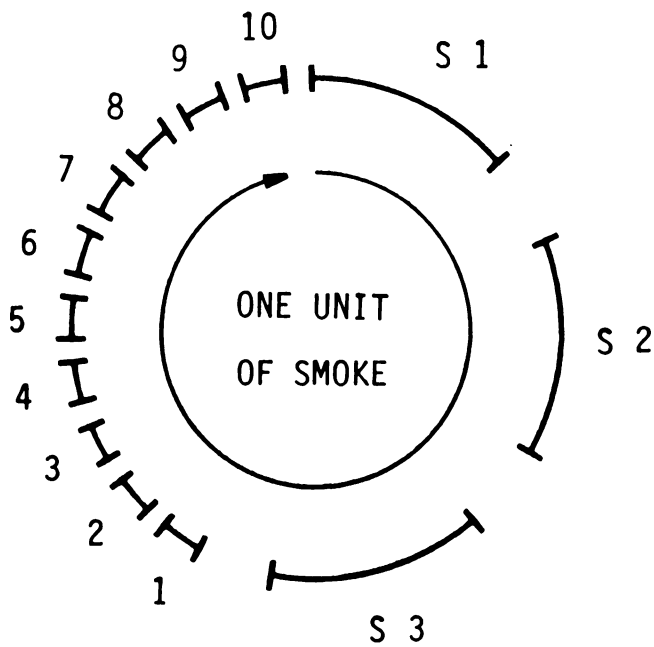

FIG. 2. Schematic representation of the "unit" of smoke. One unit is a cycle comprised of three exposures to smoke (S1–S3) followed by ten short ventilations with air. See text for details.

Materials and Methods

Animals

One- to two-year-old neutered male, commercially available, random source sheep weighing 30.8 ± 4.3 kg (mean ± SD) were used in this study. The animals were housed in covered outdoor runs, treated for parasites, and fed commercial chow and water *ad libitum*. Baseline complete blood counts, total proteins, and blood chemistries were done 3 weeks before experimental use. The animals were fasted for 24 hours before smoke exposure and experimental use. Twelve sheep were used as controls and 58 were exposed to smoke; 41 sheep were studied 24 hours after smoke exposure and 17 sheep were studied 72 hours after exposure. Immediately after smoke inhalation, the sheep were housed in climate-controlled facilities at 74–76 F (24–25 C) and a relative humidity of 40–50%.

Smoke Generation and Exposure

Smoke was produced by burning 10 disposable underpads (45 × 52.5 cm, 40 g each, Hosposable, Inc., Bound Brook, NJ) made of polyethylene, wood pulp, and nonwoven cellulose fabric. The smoke generator was a 32-gallon metal container equipped with an air inlet, a dampered chimney, a window, and smoke outlet (Fig. 1). The smoke passed through a copper pipe (30 × 1.6 cm) into a volume-adjustable metal syringe, which permitted insufflation of either smoke or air. The smoke reached ambient temperature during passage through the pipe and delivery system and contained 10–14% oxygen, 3–8% carbon dioxide, 0.7–2.2% carbon monoxide, and other combustion products (methane, ethylene, propylene, and acetaldehyde), but no cyanide.

The experimental sheep were individually exposed to 3, 6, 9, 12, 15, or 18 units of smoke. One unit of smoke consisted of three successive exposures to smoke with a tidal volume of 30 mL/kg and breath hold of 5 seconds followed by 10 successive ventilations with room air (Fig. 2). The time required per unit was about 50 seconds. Before smoke insufflation, the sheep were intubated, anesthetized with methohexital sodium (9 mg/kg,

Brevital® Sodium, Eli Lilly and Company, Indianapolis, IN) and paralyzed with succinylcholine chloride (0.7 mg/kg). The sheep were extubated after smoke inhalation. Of the 41 sheep studied 24 hours after smoke exposure, six sheep were exposed to 3 units of smoke, eight sheep to 6 units, nine sheep to 9 units, seven sheep to 12 units, seven sheep to 15 units, and four sheep to 18 units. For the 72-hour studies, seven, six, and four sheep were exposed to 6, 9, and 12 units of smoke, respectively.

Monitoring

Sheep were studied 24 or 72 hours after smoke inhalation. On the day of measurement, arterial and central venous lines, a Swan-Ganz catheter (7F, American Edwards Laboratories, Irvine, CA), a lung water catheter (American Edwards Laboratories), and an esophageal balloon were inserted after general anesthesia and intubation. Anesthesia was induced with methohexital sodium (9 mg/kg) and maintained with alpha-chloralose (0.05 g/kg, Calbiochem®, La Jolla, CA) and the sheep were paralyzed with pancuronium bromide (0.03–0.04 mg/kg, Pavulon®, Organon Pharmaceuticals, West Orange, NJ). After the placement of catheters, the animals were positioned prone and artificially ventilated. A volume-limited ventilator (Bear 2™, Bear Medical Systems, Inc., Riverside, CA) with a tidal volume of 15 mL/kg was used at a respiratory rate of 12 per minute. Sigh ventilation with a tidal volume of 21 mL/kg was applied every 3 minutes to prevent atelectasis. Lactated Ringer's solution was continuously infused at a rate of 1 mL/kg per hour.

Central venous pressure and pulmonary artery pressure (PAP) were monitored with Statham P23Db transducers (Statham Instruments, Oxnard, CA) and systemic arterial pressure was monitored with a Hewlett-Packard 1290A quartz transducer (Hewlett-Packard Company, Waltham, MA). These pressures were recorded on a Hewlett-Packard four-channel recorder (Model 7754A). Respiratory indices were monitored with a pneumotachograph (Model 17212, Gould, Inc., The Netherlands) for flow rate and tidal volume and a differential transducer (MP-451, Validine Engineering Corporation, Northridge, CA) for transpulmonary pressure, with the pulmonary variables recorded on another Hewlett-Packard four-channel recorder (Model 7754A). Cardiac output was measured in triplicate by thermodilution technique (cardiac output computer, Model 9520A, American Edwards Laboratories) and lung water was measured by thermal-dye double indicator-dilution method (lung water computer, Model 9310, American Edwards Company). Blood gas analysis was performed using an IL 1303 pH/blood gas analyzer and an IL 282 CO-Oximeter (Instrumentation Laboratories, Inc., Lexington, MA). Cardiopulmonary indices and blood gas levels were measured every 30 minutes, and the values measured after 2 hours of stabilization were taken as representative values.

Necropsies were performed on all sheep dying spontaneously or sacrificed at the end of the experiments. A complete set of tissues was fixed in 10% neutral buffered formalin and processed by standard methods. The locations of the tissue sample collection sites were midtrachea, tracheal bifurcation, right and left proximal and distal bronchi, apical and diaphragmatic lobes, and any other morphologically significant foci.

Data are displayed as mean ± SE. Regression analysis by least-square technique was used to examine the linear relationship between physiologic variables and the number of units of smoke. Linear regression analyses included the control sheep unless otherwise indicated. Survival rate was analyzed by stepwise logistic regression. Multiple comparisons were done by one-way analysis of variance (Tukey studentized range method and the Student-Newman-Keuls multiple range test). Differences were considered significant at $p < 0.05$.[17]

Results

Sheep resumed spontaneous breathing soon after smoke exposure, and breathing was supported by an ambu-bag until they were extubated. The sheep usually started walking within 10 minutes after smoke exposure. Sheep exposed to smoke showed symptoms such as coughing, wheezing, and frothing beginning 4–6 hours after smoke exposure. All sheep in which physiologic measurements were made could breathe spontaneously and stand unassisted, although sheep exposed to 12 units or more showed distinct respiratory distress.

Sloughing of the airway epithelium occurred consistently in sheep exposed to smoke (Fig. 3). This pseudomembrane formation was typically seen in major airways and became progressively thicker in sheep exposed to higher doses of smoke. At autopsy, almost complete occlusion of the trachea was seen in sheep that died spontaneously 12 or more hours after exposure. Histologic changes of the airways included disruption, swelling, and loss of cilia (Fig. 4A). Increased numbers of inflammatory cells were common in the laminae propriae, respiratory mucosae, and airway lumina. Surface accumulation of mucocellular debris, often with obstruction, was associated with parenchymal congestion, edema, and atelectasis (Fig. 4B). These parenchymal changes were most prominent in the dependent areas of the lung.

Mortality at 24 and 72 hours is shown in Figure 5. Six units or less of smoke was not fatal by 72 hours, but 12-unit doses were fatal by 72 hours. Therefore, 15- or

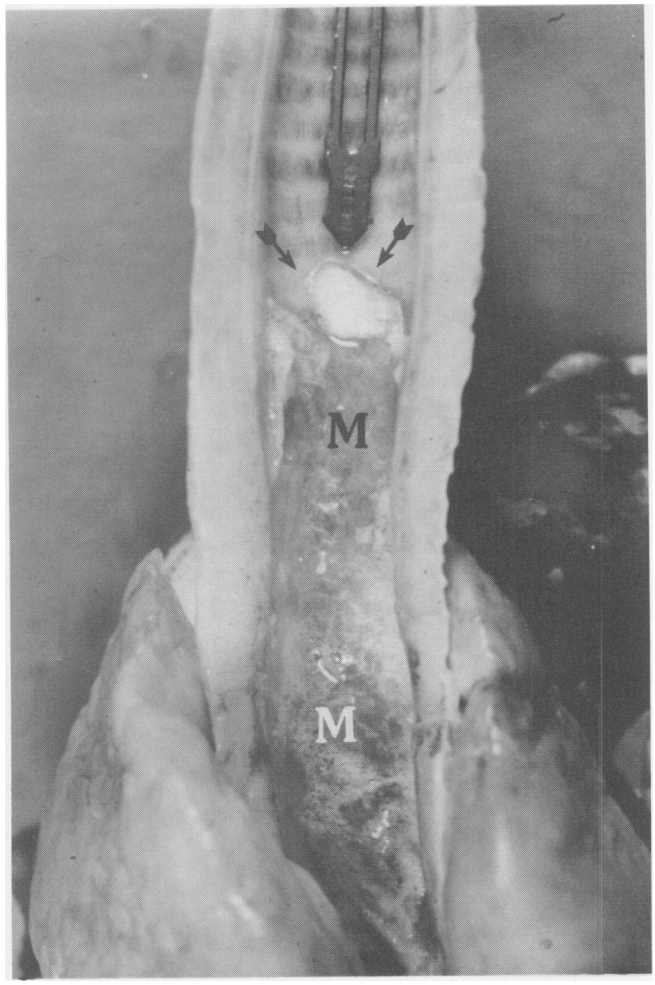

FIG. 3. Trachea of a sheep 24 hours after exposure to 9 units of smoke. This photo illustrates the formation of a pseudomembrane (M) in the trachea and bronchi. The membrane is separating from the underlying epithelium (arrows) and partially occludes the trachea and lower respiratory tract.

18-unit smoke exposures were not studied for survival or physiologic response at 72 hours. Eighteen-unit doses were fatal by 24 hours; one of the four sheep receiving an 18-unit dose died within 1 hour after exposure and three others died between 5 and 15 hours after exposure. Mortality at 24 hours was well predicted by the logistic model (mortality = $\exp(y)/(1 + \exp(y))$, where $y = 0.535x - 7.865$ and x = number of smoke dose units). According to this equation, the best logistic regression estimation of LD_{50} at 24 hours was 14.7 units of smoke. Data were insufficient for statistical analysis at 72 hours.

Carboxyhemoglobin levels immediately after smoke exposure are shown in Figure 6. Even 3 units of smoke raised the carboxyhemoglobin level over 40%. Eighteen units of smoke elevated the mean blood carboxyhemoglobin level to 85.4 ± 2.6%.

The arterial oxygen tension (PaO_2) of each group is shown in Figure 7. Control sheep had an average PaO_2 of 88.9 ± 1.2 torr (N = 12, electrode at 37 C). Sheep exposed to 9 units or more had significantly lower PaO_2 than the controls at both 24 and 72 hours after smoke exposure. At 24 hours after smoke inhalation, PaO_2 (y = torr) was negatively related to the number of units of smoke exposure (x = units): $y = 90.0 - 3.24x$ ($r^2 = 0.646$, N = 44, $p < 0.01$). The data were insufficient for statistical analysis at 72 hours.

At 24 hours, none of the sheep exposed to 9 units or less of smoke showed significant depression of plasma

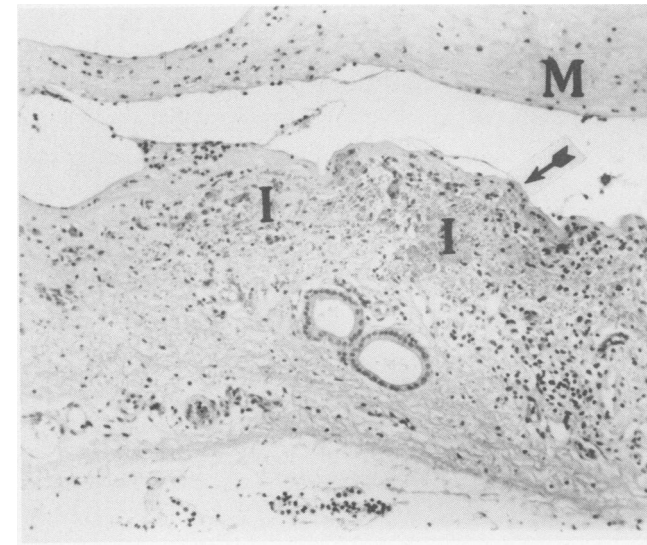

FIG. 4A. Histologic changes at 24 hours after exposure to 9 units of smoke. Note the sloughing of necrotic tracheal epithelium (arrow), the inflammatory cell infiltration (I), and the presence of a thick pseudomembrane (M). (Hematoxylin and eosin, original magnification ×370.)

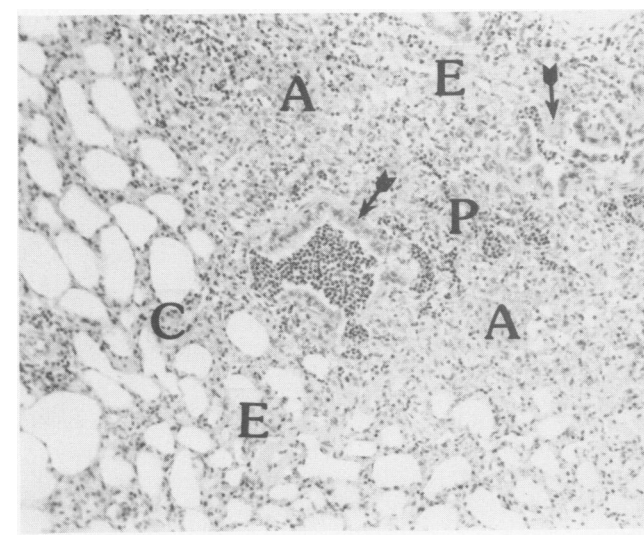

FIG. 4B. Parenchymal changes are shown. Occlusion of bronchioles (arrows) by inflammatory cells and necrotic debris is noted with concomitant atelectasis (A), congestion (C), edema (E), and pneumonia (P). (Hematoxylin and eosin, original magnification ×370.)

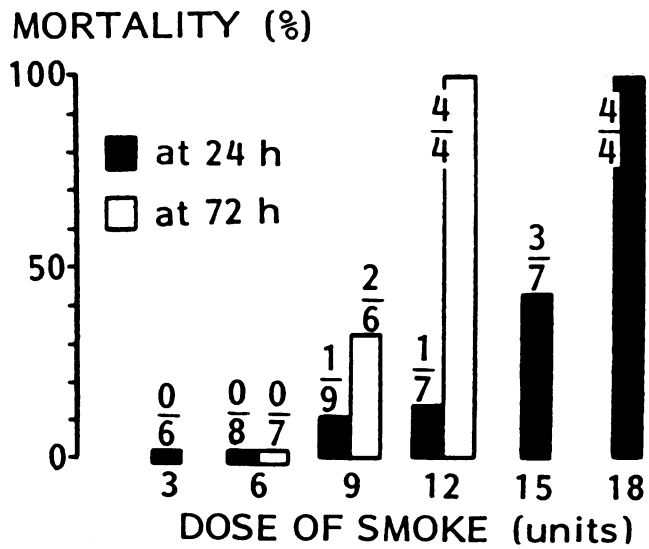

FIG. 5. Mortality at 24 hours (solid bars) and 72 hours (open bars) after smoke exposure. Mortality is shown as percentage. Fractions on the bars are actual number of dead sheep (numerator)/number of sheep studied (denominator). Mortality at 72 hours for 15 or 18 units of smoke exposure was not studied because 12 units of smoke was already fatal by 72 hours.

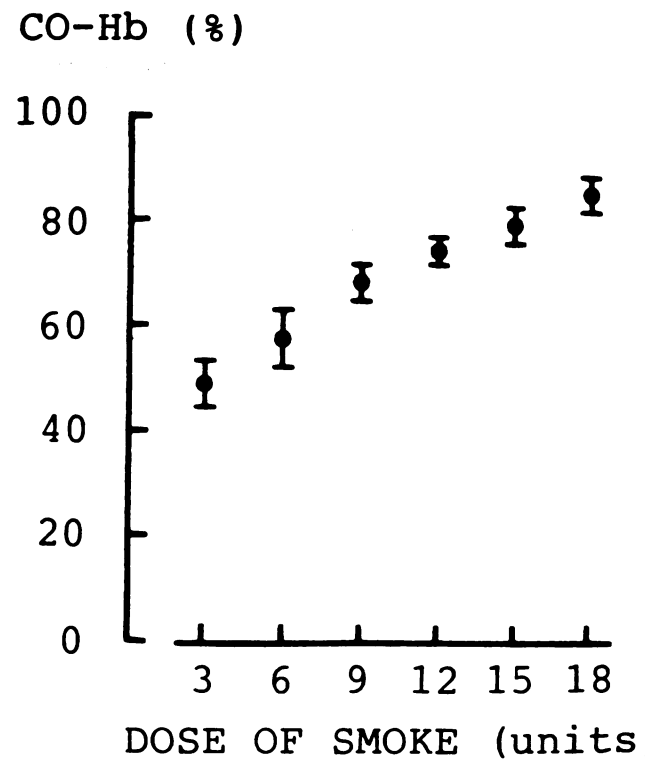

FIG. 6. Peak levels of carboxyhemoglobin (CO-Hb). Blood samples were taken immediately after smoke exposure from the external jugular vein.

pH (Table 1). Some of the sheep exposed to 9 units or more had elevated plasma $PaCO_2$ at 24 hours, but this was not a consistent finding (Table 1). At 72 hours, surviving sheep exhibited no acidosis, but one had hypercapnea ($PaCO_2$ = 42 torr).

Mean blood pressure (mBP, y = torr) also showed dose responsiveness (Fig. 8) between 3 and 15 units of smoke (x = smoke units) at 24 hours: $y = 136.0 - 3.55x$ ($r^2 = 0.439$, N = 32, $p < 0.01$). Sheep exposed to 3 units of smoke showed highly elevated blood pressure and those exposed to 12 units or more had significantly lower blood pressure than others. The data at 72 hours were not sufficient for such analysis. Sheep exposed to 12 units or more also showed significantly increased PAP at 24 hours (Table 1).

Cardiac index (CI) was higher in the 3-unit and 6-unit groups than in controls at 24 hours, but the difference was not statistically significant (Table 1). At 9- and 12-unit doses, CI decreased progressively, but the CI of the 15-unit group did not differ significantly from control. A significant difference of CI was seen only between the 6-unit and 12-unit dose groups at 24 hours. At 24 hours, the left ventricular stroke work index (LVSWI) of the 12-unit group was significantly lower than the preinjury level. No significant differences in total peripheral resistance index (TPRI) were noted in any group.

Pulmonary resistance (PR) was significantly elevated at 24 hours in sheep exposed to 9 units or more and at 72 hours in sheep exposed to 9 units of smoke (Fig. 9). Lung static compliance changes were the reciprocal of

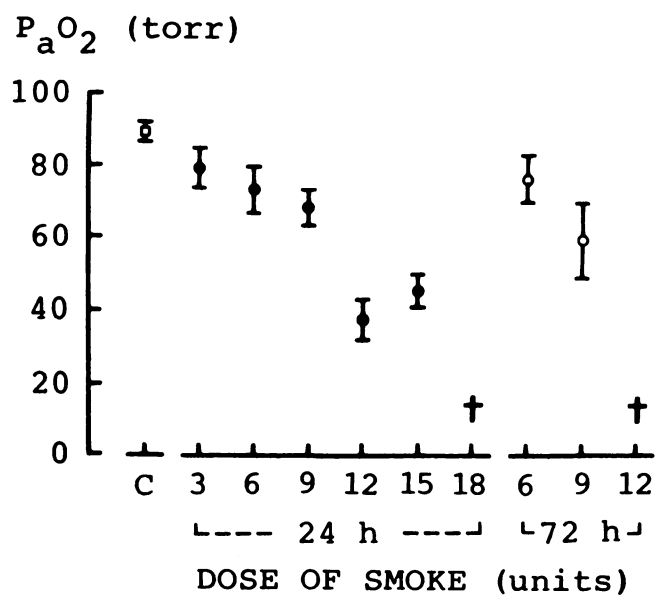

FIG. 7. Arterial oxygen tension (PaO_2) of the control (C) and smoke-exposed groups. Crosses (+) show that all the sheep in that group had died by the time of physiologic measurement. At 24 hours after smoke inhalation, PaO_2 (y = torr) was negatively related to the number of units of smoke expsoure (x = units): $y = 90.0 - 3.24x$ ($r^2 = 0.646$, N = 44, $p < 0.01$). The data at 72 hours were not sufficient for statistical analysis. Note that the values shown here are direct readings from the electrode set at 37 C. The actual PaO_2 in the sheep is 7–12 torr higher after temperature correction for their body temperature (39.5–40.0 C).

TABLE 1. *Other Cardiopulmonary Indices in Each Group (Mean ± SE)*

	Control	24 Hours after Exposure						72 Hours after Exposure		
Smoke (units)	—	3	6	9	12	15	18	6	9	12
Number of sheep	12	6	8	9	7	7	4	7	6	4
Arterial pH	7.49	7.50	7.52	7.49	7.35	7.46	—	7.54	7.44	—
	0.02	0.01	0.02	0.02	0.05	0.05	—	0.01	0.03	—
$PaCO_2$ (torr)*	30.3	30.2	29.1	30.0	40.0	36.8	—	29.6	32.5	—
	1.2	0.3	0.9	1.4	3.2	1.9	—	1.5	3.6	—
mPAP (torr)	9.3	12.0	10.3	14.7	19.1	20.5	—	9.7	11.6	—
	1.1	1.7	1.4	2.1	2.1	1.4	—	1.4	0.7	—
CI (L/min/m^2)	3.4	3.7	4.2	3.7	2.9	3.2	—	3.5	3.5	—
	0.2	0.2	0.2	0.3	0.4	0.2	—	0.2	0.4	—
LVSWI	33.3	41.2	35.0	32.3	17.8	31.1	—	24.9	30.2	—
	3.4	3.3	1.8	2.5	2.7	2.9	—	2.7	4.4	—
TPRI	2627	2704	2110	2516	2519	2151	—	2835	2395	—
	145	163	98	248	375	376	—	256	260	—
EVLWV (mL/kg)	9.2	12.6	11.4	12.3	18.0	13.4	—	11.7	10.9	—
	0.5	1.1	0.5	1.3	1.5	1.8	—	0.5	1.4	—

* $PaCO_2$ values are direct readings from the electrode set at 37 C.

mPAP = mean pulmonary artery pressure; LVSWI = left ventricular stroke work index (g-m/m^2); CI = cardiac index; TPRI = total peripheral resistance index (dynes sec m^2/cm^5); EVLWV = extravascular lung water volume.

those in PR; compliance was significantly lower at 24 hours in sheep exposed to 12 units or more and at 72 hours in sheep exposed to 9 units (Fig. 10).

At 24 hours, extravascular lung water was significantly elevated in sheep exposed to 12 units compared with the controls or to those sheep exposed to 9 units of smoke or less. No significant difference was found between the 12-unit and 15-unit groups (Table 1).

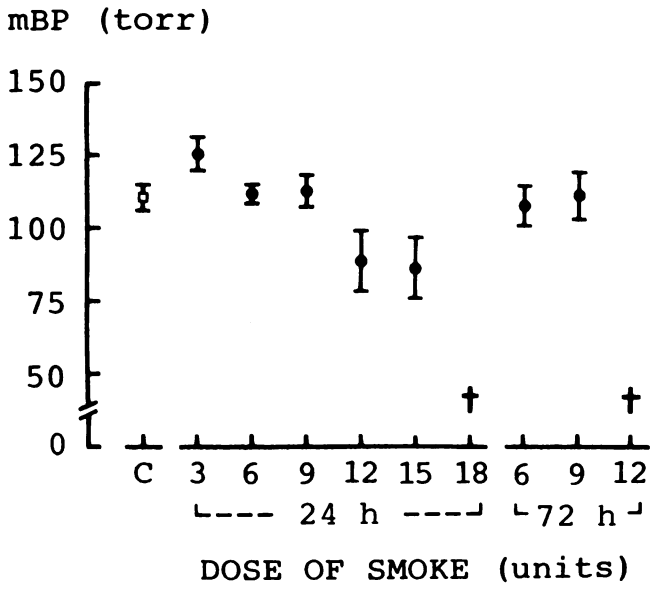

FIG. 8. Mean blood pressure (mBP) of each group. At 24 hours, mBP (y = torr) showed similar relationship to PaO_2 between 3 and 15 units of smoke (x = units): $y = 136.0 - 3.55x$ ($r^2 = 0.439$, $N = 32$, $p < 0.01$). The data at 72 hours were not sufficient for such analysis.

Discussion

In this model, anesthetized sheep were insufflated with smoke, using endotracheal intubation, to inflict a consistent reproducible injury to the lung. After smoke exposure, the sheep were extubated and allowed to breathe spontaneously. Twenty-four or 72 hours after smoke inhalation, the sheep were reanesthetized, intubated, and catheterized for physiologic evaluation. After a 2-hour stabilization period, cardiopulmonary indices were measured under general anesthesia and mechanical ventilation. We used alpha-chloralose as the anesthetic agent because this agent exerts only minimal effects on cardiopulmonary function.[18] We returned the sheep to the prone position as soon as the catheters were placed to avoid any adverse effects of the supine position. Prolonged supine positioning causes congestion in the lung, frothing, and cyanosis.[19] Sigh ventilation was used to prevent atelectasis, which is likely to develop in sheep, even in the prone position. In exposing the sheep to smoke, a tidal volume of 30 mL/kg was used. This tidal volume did not present a problem for the 24–40-kg sheep used in this study, but such a volume-to-weight ratio might produce hyperinflation of the alveoli in larger sheep.

To understand the significance and limits of the results of this study, several physiologic characteristics of sheep need be pointed out. In sheep, a major difference from humans is seen in the erythrocytes. Sheep not only have low Na-K pump activity, but also have relatively lower blood hemoglobin concentrations, higher P-50

values, lower 2,3-DPG levels, and a higher body temperature.[20-21] These differences must be taken into account when analyzing and comparing hemodynamics and oxygen transport in sheep and humans.[22] For example, actual PaO_2 at the sheep's body temperature (39.5–40 C) is usually 7–12 torr higher than the values obtained from blood gas analyzers with the electrode set at 37 C. Sheep also have very low plasma cholinesterase activity.[23] Interestingly, succinylcholine chloride did not have a prolonged effect in the study sheep.

Stephenson et al.[12] have suggested that the severity of smoke inhalation injury is dose-related, but physiologic measurements of dose responsiveness have not been made except for a mortality study in rats.[6] The sheep model of smoke inhalation injury used in these studies is reproducible and manifests dose responsiveness of hemodynamic and pulmonary variables that are most distinct at 24 hours. The lesser number of sheep studied at 72 hours and the narrower range of smoke doses used in the sheep studied at 72 hours may account for the poorer correlations noted at that time.

Mortality at 24 hours was well approximated by the logistic model (mortality = exp(y)/(1 + exp(y)), where y = 0.535x − 7.865 and x = number of smoke dose by units). This logistic model predicts mortality of 0.19% and 0.94% for 3 and 6 units of smoke exposure, respectively. Although all sheep given those doses of smoke were sacrificed after physiologic measurements at 24 or 72 hours, the clinical condition of all sheep was consistent with recovery from the inhalation injury and survival. In the sheep exposed to 6 units of smoke, none of the physiologic variables measured at 72 hours showed further deterioration from values measured at 24 hours after smoke exposure. Histologically, tracheal epithelium examined 72 hours after 6 units of smoke exposure showed metaplasia, suggesting early healing. These physiologic and histologic findings suggest that damage from 6 units of smoke is reversible. On the other hand, a 9-unit or greater dose of smoke caused progressive changes resulting in much higher mortality and further deterioration in lung function by 72 hours after exposure. Histologic changes in those sheep were also progressive; the airway epithelium showed further deterioration and congestion and atelectasis of the lung were more severe at 72 hours. The parenchymal changes were most distinct in dependent areas of the lung. This accumulation of occlusive exudate in dependent airways is probably related both to the effect of gravity and to the loss of effective normal elimination of exudate by ciliary action and coughing.

The dose-response patterns of selected physiologic indices were analyzed using the data at 24 hours after smoke exposure. Four patterns were observed (Fig. 11): (1) No apparent smoke dose effect, as exemplified by

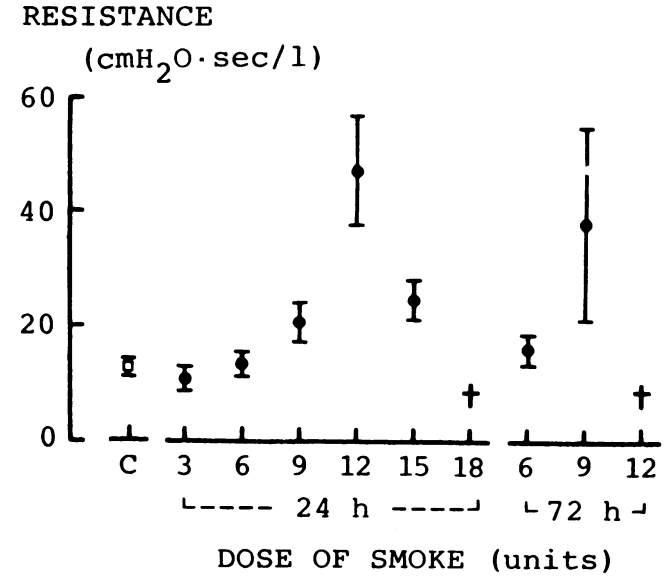

FIG. 9. Pulmonary resistance (PR) of each group. PR was calculated by the following equation: PR = (mouth pressure − intrapleural pressure)/flow rate. Mouth pressure means pressure at the connection of respiratory circuit and tracheal tube. PR includes airway resistance plus pulmonary tissue resistance.[28]

TPRI. (2) An apparent threshold effect, *i.e.*, poor linear correlation with dose ($r^2 < 0.20$), but significant change at 12-unit and/or 15-unit doses and no significant difference between those two groups: pH, peripheral vascular resistance index (PVRI), and right ventricular stroke work index (RVSWI). (3) A linear relationship (0.20

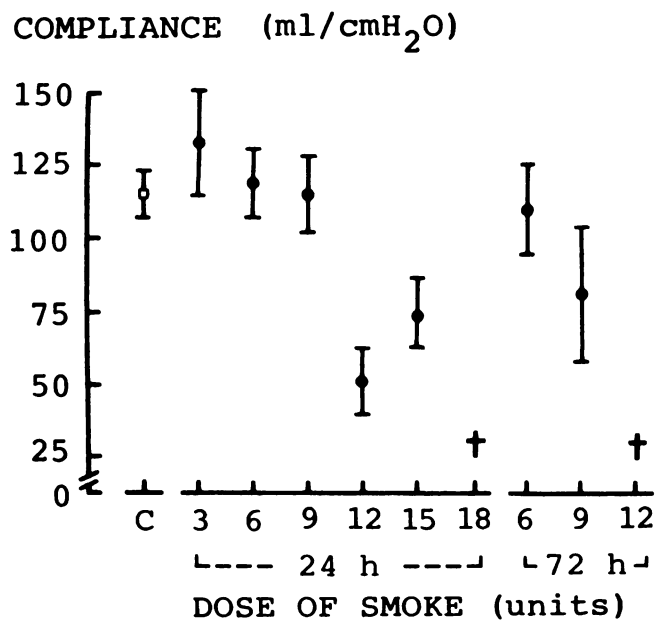

FIG. 10. Static compliance of each group. Static compliance was calculated from the following equation at end-inflation: Compliance = volume change/transpulmonary pressure. Change in compliance was a mirror image of that of pulmonary resistance.

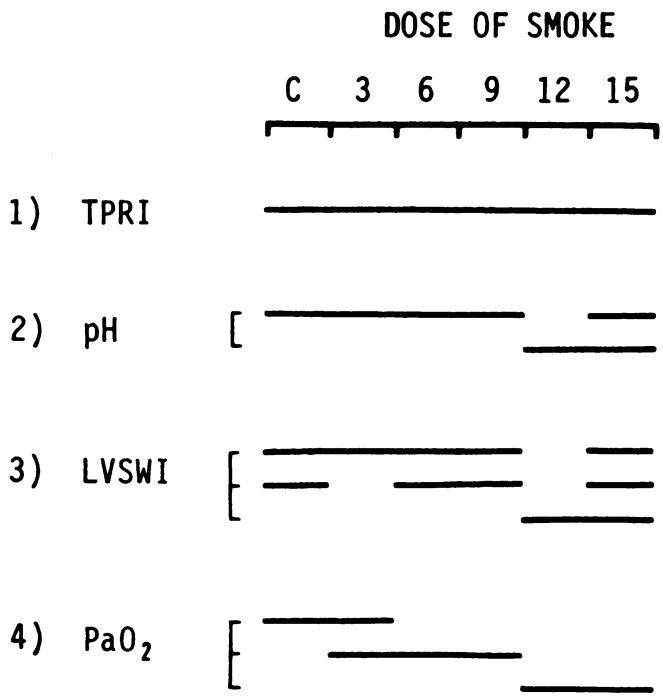

FIG. 11. Four patterns of response in physiologic indices at 24 hours after smoke exposure. Multiple comparisons by the Student-Newman-Keuls multiple range method are described by the underlining method. Groups underlined by the same line are not distinguishable from one another. Typical examples of the four patterns are shown. (1) No apparent smoke dose effect. (2) An apparent threshold effect. (3) A linear relationship ($0.20 < r^2 < 0.40$) over at least a portion of the smoke dosage range. (4) A good linear relationship ($r^2 > 0.40$) over the entire range of doses of smoke. See text for details.

$< r^2 < 0.40$) over at least a portion of the smoke dosage range: $PaCO_2$, PR, static compliance, lung water, LVSWI, and CI, where LVSWI = between 3 and 15 units and CI = between 6 and 15 units. (4) A good linear relationship ($r^2 > 0.40$) over the entire range of doses of smoke: PaO_2 ($r^2 = 0.646$), mBP ($r^2 = 0.439$), and mPAP ($r^2 = 0.407$), where mBP = between 3 and 15 units.

TPRI was maintained within the normal range in all surviving sheep, suggesting that the TPRI is well protected by homeostatic mechanisms and is not an index of the severity of inhalation injury. Indices showing threshold changes (pH, PVRI, and RVSWI) also appear to be well maintained until a severe insult is incurred. Changes in these indices are indicative of a significant smoke inhalation injury. Once a threshold level was exceeded, no significant further change in these physiologic indices was noted in the 15-unit group compared with the 12-unit group. Indices that manifested the third or fourth patterns showed moderate to good correlation between the number of units of smoke and magnitude of change.

For some variables, a significant difference from the control level was not seen (CI) nor was observed only after exposure to 12 units of smoke ($PaCO_2$, LVSWI, PR, static compliance, lung water, mBP, and PAP) when assessed by analysis of variance. PaO_2 showed the highest correlation coefficient ($r^2 = 0.646$), but a significant difference from the control level was observed only in groups exposed to 9 units of smoke or more. The dose-response curve of PaO_2 (Fig. 7) and the fact that changes of most of the cardiopulmonary indices became significant only after 12-unit exposure suggest that the dose-response curve is sigmoid in shape rather than linear, and that the steep portion of the curve is located between 9 and 12 smoke units (Fig. 12). The steep portion of the curve might also be approximated by curved linear (polynomial) functions, reflecting the progressive nature of such injury. Stephenson et al. pointed out that, by inference from the literature, there is no variable to reliably predict lung injury due to smoke inhalation.[12] That failure may be related to the sigmoid nature of the dose-response curve suggested by this study. To describe the sigmoid curve precisely, it will be necessary to assess the physiologic response over a wide range of inhalation injury severity. Several of the indices examined in this

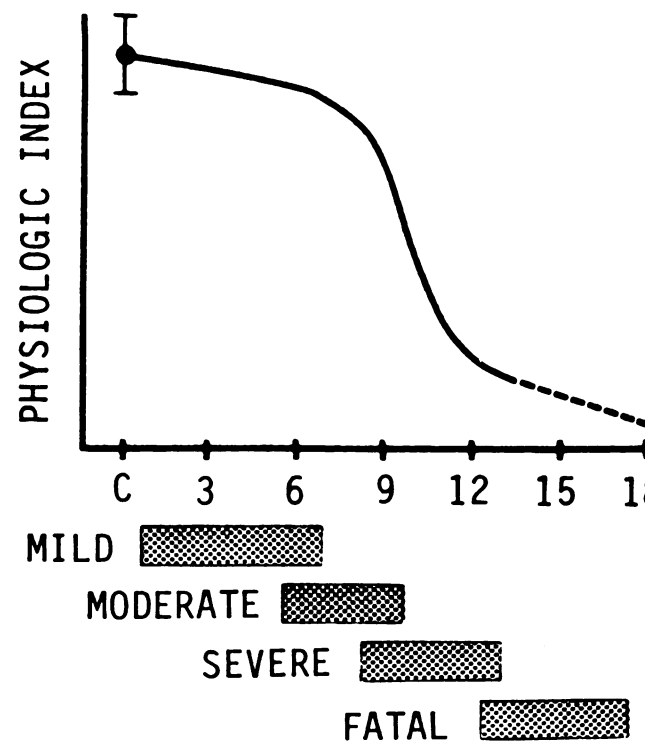

FIG. 12. Schematic presentation of dose-response curve of sigmoid nature and grading of smoke inhalation injury. Ordinate represents physiologic indices and abscissa shows doses of smoke in units. Statistical significance is not detectable at doses below 9 units. Substantial change occurs between 9 and 12 units. The plateau on the right side (shown as an interrupted line) is not clearly observed because those higher doses are fatal. The bars indicate the grading of smoke inhalation injury. See text for details.

study do not appear to be sensitive indicators of injury severity since they were not significantly altered by nonlethal doses of smoke.

Although others have reported no significant changes in PAP after smoke inhalation and have attributed pulmonary edema in such models to increased capillary permeability,[11] we observed a significant increase in PAP in sheep exposed to high doses (12 and 15 units) of smoke (Table 1). Judging from the mortality in the sheep studied by Herndon et al.[11] (13 of 27 at 72 hours), that severity of inhalation injury corresponded to little more than that caused by 9 units of smoke in our study. The change in PAP that we observed was modest until the smoke dose exceeded 9 units, above which it increased greatly. The parallelism of changes in PAP and extravascular lung water volume in the current study suggests that elevation of intravascular pressure may contribute to pulmonary edema after smoke inhalation injury.

The effect of inhalation injury on extravascular lung water volume is unclear. Markedly different thermal-dye measurements of extravascular lung water volume have been reported.[24,25] As an explanation of the discrepancies, Prien et al. have recently reported that pulmonary edema of smoke inhalation was undetectable by indicator-dilution technique in a sheep model.[26] That finding may reflect differences between patients with burns and inhalation injury and experimental models with smoke inhalation alone. In laboratory studies, including our study, the animals did not have cutaneous burns and they did not receive large volumes of resuscitation fluid. Since the composition of administered fluids affects lung water accumulation in burn patients,[27] differences in resuscitation regimens may account for some of the reported differences in lung water measured in burn patients. We showed in this study that lung water change was not significant until the severity of inhalation injury reached a certain level, *i.e.,* 12 units of smoke, which caused 100% mortality within 72 hours. This indicates that, apart from any insensitivity of the indicator-dilution methods, extravascular lung water volume may not be increased in less severe inhalation injury.

It is important to identify the severity as well as the presence of inhalation injury. In this study, PaO_2 showed the best dose-response relationship, and although it appears to be the most reliable index for estimating the severity of smoke inhalation injury, PaO_2 changes are not specific to such injury. It may be more useful to grade smoke inhalation as mild (3–6 units), moderate (6–9 units), severe (9–12 units), and invariably fatal (over 12 units) (Fig. 12). Mild smoke inhalation injury did not cause death by itself and all the physiologic indices remained normal. In some animals with mild injury, no tracheal pseudomembrane formation was evident and when present was minimal. Diagnosis of smoke inhalation injury of this degree would be difficult without histologic examination at an appropriate time. Moderate injury was associated with a mortality of 30% by 72 hours. Pseudomembrane formation in the major airways was always evident but many physiologic indices still did not change significantly. Severe inhalation injury caused 10–14% mortality by 24 hours and 30–100% mortality by 72 hours. A thick pseudomembrane formed in the airways and physiologic indices showed significant changes. The variability of changes noted in the various indices may be attributable to the sigmoid shape of the dose-response curve. Invariably, fatal injury caused total loss of airway epithelium and 100% mortality. Although this grading is based on an experimental model of smoke inhalation without cutaneous burns, a similar grading for smoke inhalation in burn patients may be useful in evaluating the severity of smoke inhalation and assessing treatment outcome. This dose-responsive sheep model can now be used to determine the pathogenesis of inhalation injury, to assess the effect of associated burn injury, and to evaluate the effectiveness of therapeutic interventions.

Acknowledgments

The authors gratefully acknowledge Staff Sergeant Frederick J. Nangle, III, and Rebecca Bexar, MLT (ASCP), for their assistance in conducting the experiments and Christine C. Davis for preparation of the manuscript.

References

1. Moylan JA Jr. Inhalation injury—a primary determinant of survival following major burns. JBCR 1981; 2:78–84.
2. DiVincenti FC, Pruitt BA Jr, Reckler JM. Inhalation injuries. J Trauma 1971; 11:109–117.
3. Moylan JA Jr, Wilmore DW, Mouton DE, et al. Early diagnosis of inhalation injury using ^{133}Xenon lung scan. Ann Surg 1972; 176:477–484.
4. Phillips AW, Cope O. Burn therapy: III. Beware the facial burn! Ann Surg 1962; 156:759–766.
5. Potkin RT, Robinson NB, Hudson LD, et al. An animal model of smoke inhalation. Am Rev Resp Dis 1980; 121:178.
6. Zawacki BE, Jung RC, Joyce J, et al. Smoke, burns, and the natural history of inhalation injury in fire victims: a correlation of experimental and clinical data. Ann Surg 1977; 185:100–110.
7. Thomas WC Jr, O'Flaherty EJ. A system for exposing animals to smoke generated in a steady-state fashion. Environmental Research 1980; 23:326–333.
8. Dressler DP, Skornick WA, Kupersmith S. Corticosteroid treatment of experimental smoke inhalation. Ann Surg 1976; 183:46–52.
9. Walker HL, McLeod CG Jr, McManus WF. Experimental inhalation injury in the goat. J Trauma 1981; 21:962–964.
10. Nieman GF, Clark WR Jr, Wax SD, et al. The effect of smoke inhalation on pulmonary surfactant. Ann Surg 1980; 191:171–181.
11. Herndon DN, Traber DL, Niehaus GD, et al. The pathophysiology of smoke inhalation injury in a sheep model. J Trauma 1984; 24:1044–1051.

12. Stephenson SF, Esrig BC, Polk HC Jr, et al. The pathophysiology of smoke inhalation injury. Ann Surg 1975; 182:652–660.
13. Borrie J, Mitchell RM. The sheep as an experimental animal in surgical science. Br J Surg 1960; 47:435–445.
14. Halmagyi DFJ, Colebatch HJH. Some cardiorespiratory parameters in anesthetized sheep. J Appl Physiol 1961; 16:45–47.
15. Staub NC, Bland RD, Brigham KL, et al. Preparation of chronic lung lymph fistula in sheep. J Surg Res 1975; 19:315–320.
16. Watanabe K, Makino K. The role of carbon monoxide poisoning in the production of inhalation burns. Ann Plast Surg 1985; 14:284–295.
17. Dixon WJ, ed. BMDP Statistical Software. Berkeley: University of California Press, 1983.
18. Booth NH. Hypnotics and analgesics. *In* Jones LM, ed. Veterinary Pharmacology and Therapeutics. Ames: Iowa State University Press, 1965; 182–207.
19. Hecker JF. Experimental Surgery on Small Ruminants. London: Butterworths, 1974; 29–32.
20. Ellory JC, Tucker EM. Cation transport in red blood cells. *In* Agar NS, Board PG, eds. Red Blood Cells of Domestic Animals. New York: Elsevier Science Publishers, 1983.
21. Huisman THJ, Kitchens J. Oxygen equilibria studies of the hemoglobins from normal and anemic sheep and goats. Am J Physiol 1968; 215:140–146.
22. Parer JT, Jones WD, Metcalfe J. A quantitative comparison of oxygen transport in sheep and human subjects. Respir Physiol 1967; 2:196–206.
23. Hazelwood JC, Heath GE. A comparison of cholinesterase activity of plasma, erythrocytes, and cerebrospinal fluid of sheep, calves, dogs, swine, and rabbits. Am J Vet Res 1976; 37:741–743.
24. Peitzman AB, Shires GT 3d, Corbett WA, et al. Measurement of lung water in inhalation injury. Surgery 1981; 90:305–319.
25. Tranbaugh RF, Lewis FR, Christensen JM, et al. Lung water changes after thermal injury: the effects of crystalloid resuscitation and sepsis. Ann Surg 1980; 192:479–490.
26. Prien T, Traber DL, Herndon DN, et al. Pulmonary edema with smoke inhalation undetected by indicator dilution technique. Proceedings of the American Burn Association (Abstract 144), 1986.
27. Goodwin CW, Dorethy J, Lam V, et al. Randomized trial of efficacy of crystalloid and colloid resuscitation on hemodynamic response and lung water following thermal injury. Ann Surg 1983; 197:520–531.
28. Comroe JH. Physiology of Respiration. Chicago: Year Book Medical Publishers, 1974; 124.