URINARY EXCRETION OF POLYAMINES IN THE ADULT RESPIRATORY DISTRESS SYNDROME

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□ *Polyamines are low molecular weight polycations that are critically important in cellular proliferation and differentiation. To investigate their potential role in acute lung injury, the polyamines spermidine, spermine, and putrescine were measured in 24-h urine collections from intubated patients with ARDS* (n = *12) or congestive heart failure with cardiogenic pulmonary edema (CHF,* n = *10) and in normal subjects* (n = *10). Mean concentrations of putrescine were similar between groups, but spermidine concentrations in patients with ARDS (52.7 ± 19.7 nmol/mg creatinine) were significantly higher than in normal subjects (4.9 ± 0.7 nmol/mg),* $p < .05$. *Mean concentrations of spermine in ARDS (270.6 ± 78.1 nmol/mg) were higher than in CHF (1.0 ± 0.5 nmol/mg),* $p < .05$, *and normal subjects (0.3 ± 0.1 nmol/mg),* $p < .05$. *Concentrations of putrescine increased significantly during the first 7 days of ARDS (241.5 ± 127.1% above baseline,* n = *6),* $p < .05$. *Urinary polyamine excretion did not correlate with severity of gas exchange or death. These results are the first to suggest a potential role for polyamines in the pathophysiology of ARDS.*

Keywords *adult respiratory distress syndrome, polyamines, putrescine, spermidine, spermine*

The adult respiratory distress syndrome (ARDS) is a diffuse inflammatory disorder of the lung that progresses from an exudative phase to a stage characterized by cellular proliferation [1]. The proliferative phase represents tissue repair that can cause in some patients further deterioration of lung function and pulmonary fibrosis. The polyamines spermidine [$H_2N(CH_2)_4NH(CH_2)_3NH_2$] and spermine [NH2(CH2)3HN-$(CH_2)_4NH(CH_2)_3NH_2$], and their diamine precursor, putrescine [$H_2N(CH_2)_4NH_2$], are low molecular weight polycations found in high concentrations in all eukaryotic cells [2, 3]. Although their exact physiologic roles are poorly defined, demonstrated cellular effects associated with polyamines suggest that they may participate in the reparative proliferative phase of ARDS. For instance, polyamines through their inter-

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actions with DNA and RNA are essential for cell proliferation and differentiation [4, 5]. Furthermore, polyamines have antioxidant [6] and lipid membrane stabilizing effects [7].

The potential importance of polyamines in regulating pulmonary function is supported by observations that increased cellular polyamine content promotes lung development [8], repair from hyperoxic lung injury [9], and the occurrence of pulmonary hypertension in animals exposed to monocrotaline [10] and hypoxia [11]. Alterations of polyamine metabolism, however, to our knowledge, have never been investigated in patients with ARDS. We hypothesized that urinary excretion of polyamines, which is a biomarker of intracellular polyamine synthesis [12, 13], would differ in patients with ARDS compared to patients with noninflammatory forms of pulmonary edema and normal subjects. To pursue this hypothesis, we measured in 24-h urine samples from patients with ARDS concentrations of putrescine, spermidine, and spermine. Values were compared with those obtained from patients intubated for congestive heart failure with cardiogenic pulmonary edema (CHF) and from normal volunteers.

METHODS

Patient Selection

The patients were entered into the study from the 46-bed intensive care units of St. Joseph's Hospital and Medical Center. All aspects of the investigations were approved by the institutional review board for human subjects.

Definition of Clinical Conditions

The adult respiratory distress syndrome was defined by the presence of widespread, bilateral pulmonary infiltrates, a $Pa_{O_2}/P_{A}O_2$ ratio <0.2, a pulmonary artery wedge pressure <18 mm Hg, a thoracic compliance <30 mL/cm H_2O, the need for intubation and mechanical ventilation, and the existence of a high-risk ARDS predisposition. Severity of hypoxemia between study groups was compared using the $Pa_{O_2}/P_{A}O_2$ ratio. Patients were followed throughout their hospitalization to determine clinical outcome.

Intubated patients with cardiogenic pulmonary edema were identified in the coronary care unit and were required to have a subsequent course compatible with cardiac disease, a pulmonary artery wedge pressure >18 mm Hg, no clinical evidence of pneumonia, and no high-risk

predisposition for ARDS. Normal control subjects were nonsmoking individuals in good health without use of medications.

Polyamine Assays

A 24-h urine sample was collected in ice-cooled containers with bactericidal sodium fluoride tablets within 6 h of intubation for ARDS or CHF and from normal volunteers. Urine collections were repeated on the seventh day after intubation in surviving ARDS patients. No patient with cardiogenic pulmonary edema was available on the seventh day for analysis of urinary polyamines. Samples were coded so that personnel in the laboratory had no knowledge of the subjects' clinical conditions. One-milliliter aliquots from each collection were deproteinized by 100 μL of 50% sulfosalicilic acid (SSA) containing the internal standard (3-3'-diamino dipropyle amine [DPA], 22 pmol), and filtered through a millipore filter (0.22-μm pore size). One portion of the filtrate (300 μL) was used for determination of free polyamines. Another portion (500 μL) was hydrolyzed in an equal volume of 6 N HCl for 12 to 14 h, lyophylized, reconstituted in 500 μL of dilution buffer and filtered through another 0.22-μm filter. The amount of conjugated polyamines was obtained by subtracting the free from total in the hydrolyzed sample.

An automated amino acid analyzer method for the separation of polyamines proposed originally by Morton and Lee [14] was followed with modification [15]. A Beckman Model 7300 amino acid analyzer was fitted with a 20-μL sample metering loop and a 10-cm column prepacked with specially formulated, uniform, spherical polystyrene based cation-exchange resin, and a fluorescence detector. Fluor-R reagent (*O*-phthaladehyde) from Beckman was used and the polyamines were separated and eluted in 55 min. Three sodium citrate buffers of different ionic strengths but the same pH (5.43) were used. Buffer A was pumped through the column for 25 min to eliminate all bound and free amino acids and their related compounds. Buffer B was then used for 25 min to elute putrescine (retention time [RT] = 26.9 min), cadavarine (RT = 35.6 min), DPA (RT = 38.2 min), and spermidine (RT = 44.9 min), followed by buffer C for 5 min to elute spermine (RT = 53.3 min). The fluorometer was operated at an excitation wavelength of 340 nm and an emission wavelength of 455 nm. A calibration mixture containing 20 pmol/20 μL of each of the polyamines was used. The coefficient of variation of the internal standard area (n = 10) in the calibration mixture was within 4%. This analytical procedure resulted in 83 to 90% recovery of each of the polyamines [15]. Urinary creatinine was measured using Jeffe's picrate method in a centrifugal analyzer. Polyamine concentra-

tions were reported as total values (free and conjugated) and expressed as nmol/mg creatinine.

Statistical Analysis

Differences between urine values from different patient groups were analyzed with ANOVA and a post hoc Dunn's test. Paired groups of data were compared with a Wilcoxon signed rank test. Relations between clinical features and polyamine values were examined by linear regression. p values of .05 or less were considered to be significant. All data were expressed as means ± SEM unless otherwise stated. Statistical analyses were performed with a Macintosh computer using the statistical software Statview 4.0 (Abacus Inc., Berkeley, CA, USA).

RESULTS

Clinical and epidemiologic data regarding the study groups are shown in Table 1. Pneumonia, shock, and sepsis were the predominant predispositions for ARDS in the study patients. Deaths occurred primarily during the first 2 weeks and resulted most commonly from sepsis and multiorgan failure. Six of the ARDS patients died within the first 6 days of ARDS from intractable shock (2 patients) or hypoxemia (4 patients) before completion of the second urine collection for polyamine determination on day 7. An additional 2 ARDS patients died later during the hospitalization. The mortality rate for ARDS patients (67%) was within the range reported in recent studies. The mean value of the Pa_{O_2}/P_{AO_2} ratios at onset of ARDS demonstrated the severity of impaired gas exchange in these patients (Table 1). All CHF patients either died (n = 3) or were discharged from the hospital (n = 7) within 7 days of admission and were unavailable for day 7 urine collections.

Table 1 Clinical characteristics of study population

Group	n	Age (years)	Gender	Predispositions for ARDS	Pa_{O_2}/P_{AO_2}	Mortality rate
ARDS	12	50 ± 23	4 F, 8 M	Pneumonia 3 Pneumonia + sepsis 3 Aspiration 3 Hypertransfusion 1 Shock 2	0.13 ± 0.02	8/12
CHF	10	70 ± 16	3 F, 7 M		0.28 ± 0.05	3/10
Normal	10	47 ± 10	5 F, 5 M			—

Note. Values are means ± SEM except for age values, which are means ± SD.

Mean urinary putrescine concentrations were similar in patients with ARDS (332 ± 135 nmol/mg creatinine) compared to patients with CHF (226 ± 38 nmol/mg creatinine) and normal subjects (354 ± 63 nmol/mg creatinine) (p = NS, Figure 1). Mean urinary spermidine concentrations in patients with ARDS (52.7 ± 19.7 nmol/mg creatinine) were significantly higher than values measured in normal subjects (4.9 ± 0.7 nmol/mg creatinine, $p < .05$), but not those observed in patients with CHF (21.4 ± 10.3 nmol/mg creatinine, p = NS, Figure 2). Mean urinary spermine concentrations were increased in patients with ARDS (270.6 ± 78.1 nmol/mg creatinine) compared to patients with CHF (1.0 ± 0.5 nmol/mg creatinine, $p < .05$) and normal subjects (0.3 ± 0.1 nmol/mg creatinine, $p < .05$, Figure 3).

Percent increases of day 7 urinary excretion of polyamines compared to baseline day 1 values in the 6 patients who survived to the second urine

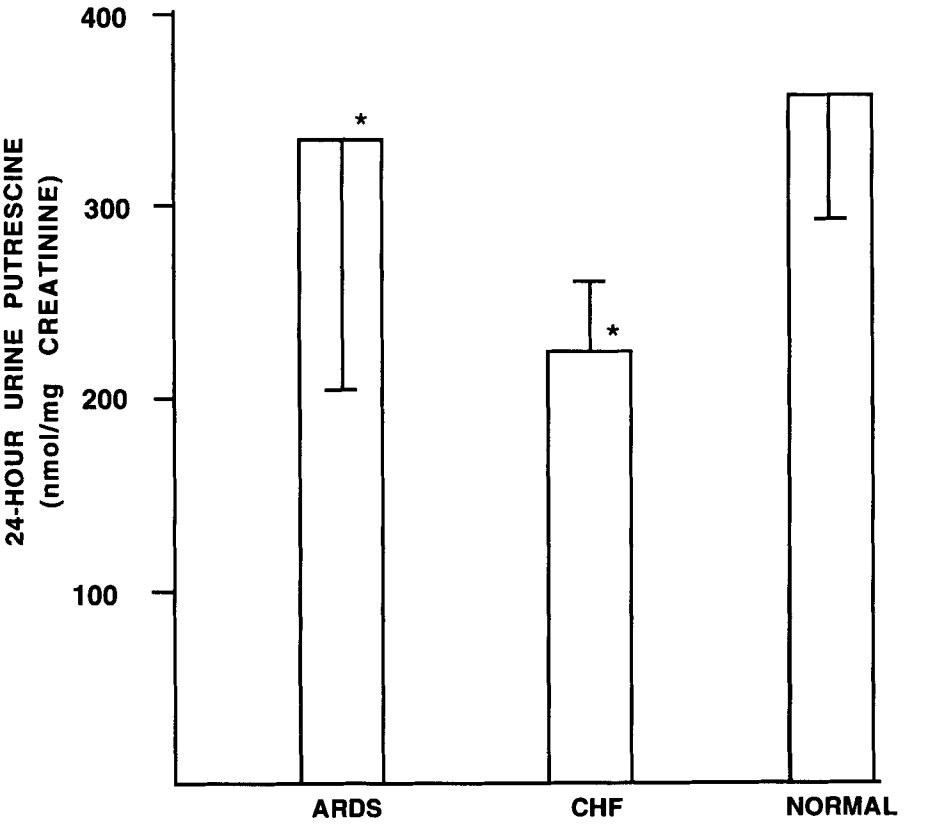

Figure 1. Concentration of putrescine indexed to urinary creatinine in 24-h urine collections from patients with adult respiratory distress syndrome (ARDS), congestive heart failure (CHF), and normal subjects. *p = NS compared to normal subjects.

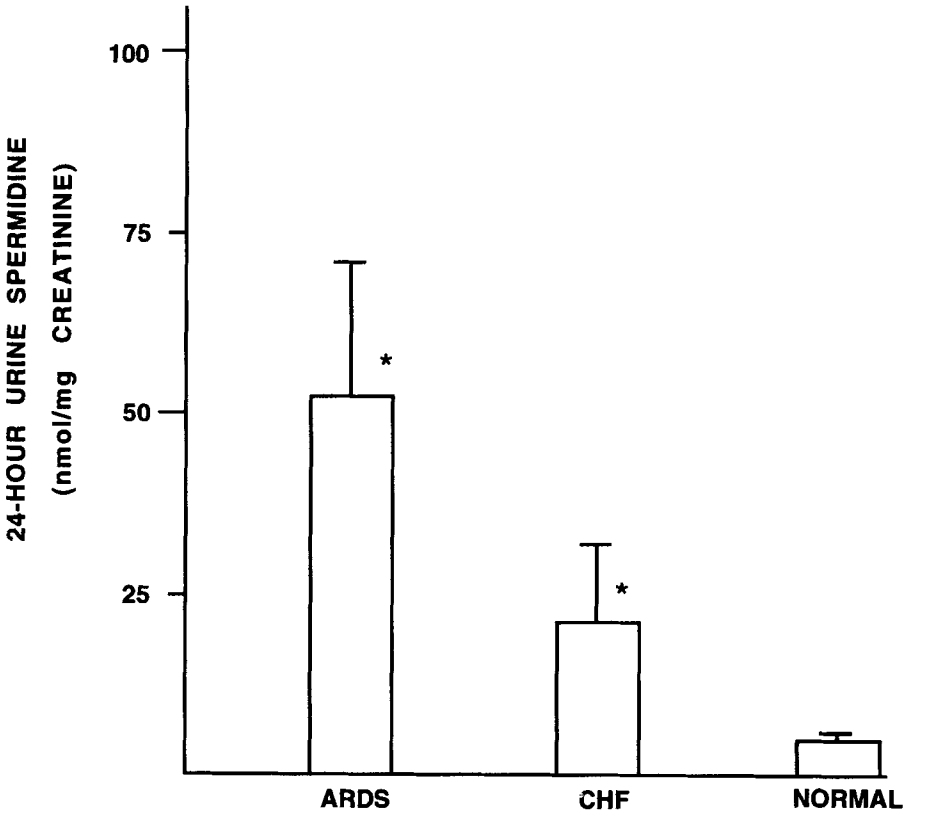

Figure 2. Concentration of spermidine indexed to urinary creatinine in 24-h urine collections from patients with adult respiratory distress syndrome (ARDS), congestive heart failure (CHF), and normal subjects. *$p < .05$ compared to normal subjects.

collection are shown in Figure 4. Although all three measured polyamines increased during the first 7 days of ARDS, only the rise in urinary excretion of putrescine reached statistical significance. No correlations existed between individual values for urinary polyamine excretion and those measured for Pa_{O_2}/P_{AO_2} ratios. Ratios of acetylated putrescine and acetylated spermidine to total putrescine and total spermidine respectively were not different between groups or between values measured on the first and seventh days of ARDS (data not shown).

DISCUSSION

The subacute phase of ARDS is characterized by intense cellular proliferation within the lung that may contribute to impaired gas exchange and persistent lung injury despite being a reparative process [1]. Sub-

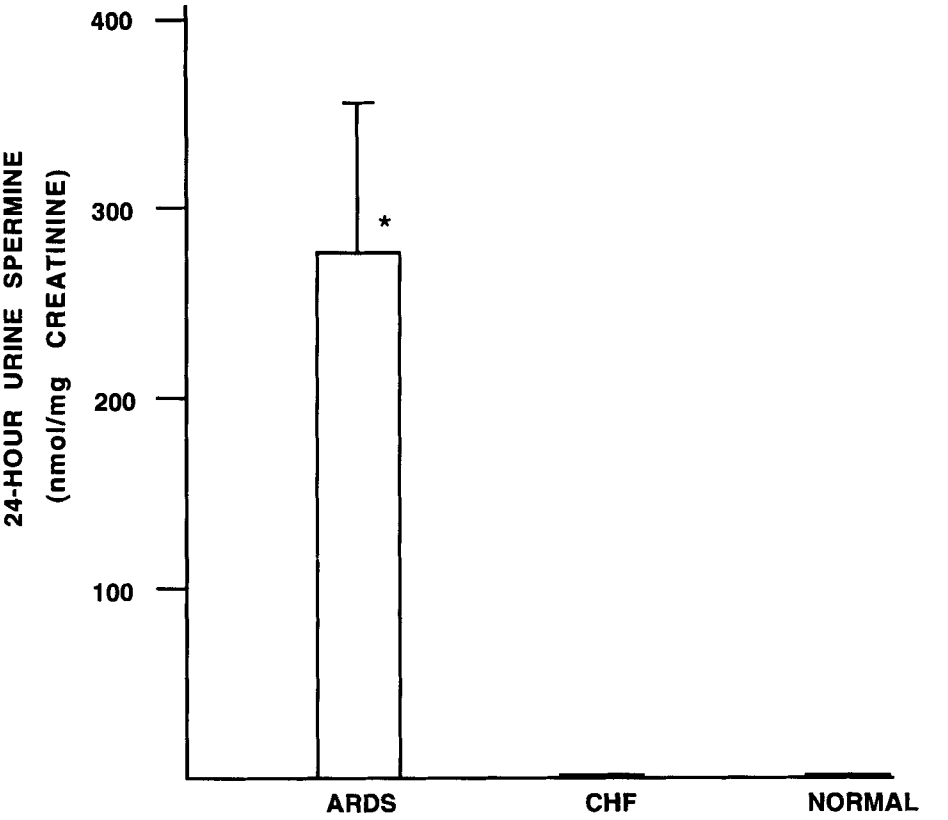

Figure 3. Concentration of spermine indexed to urinary creatinine in 24-h urine collected from patients with adult respiratory distress syndrome (ARDS), congestive heart failure (CHF), or normal subjects. *$p < .05$ compared to CHF and normal subjects.

populations of patients during this stage of the disorder may develop intense fibroblast stimulation and deposition of collagen that further increase mortality and morbidity. Because no drug therapy exists to control these features of the disease, identification of central mediators of cellular proliferation is important for the development of rational therapeutic approaches to ARDS.

Polyamines are low molecular weight compounds that are ubiquitous in eukaryocytes and are critically important in cell proliferation and differentiation [2, 3]. Their polycationic structure at physiologic pH promotes bonding to the negatively charged phosphate groups on numerous cytosolic enzymes and macromolecules, such as DNA and RNA. Cellular synthesis of polyamines usually begins before the onset of DNA synthesis, and low polyamine concentrations inhibit trophic responses in many tissues [16]. Polyamines promote the translation of a variety of messenger

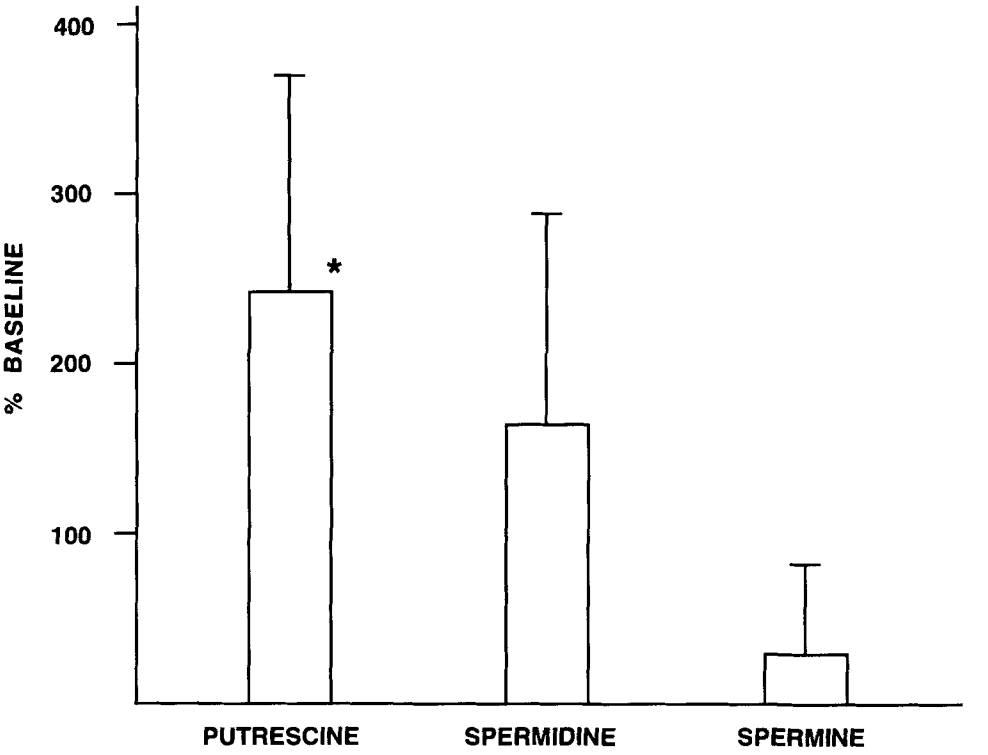

Figure 4. Percent increase of day 7 compared to day 1 (baseline) urinary excretion values for putrescine, spermidine, and spermine indexed to urinary creatinine in the 6 patients with adult respiratory distress syndrome who survived the initial 7 days of respiratory failure. *$p < .05$ by paired analysis.

RNAs [16] and can affect the tertiary structure of messenger RNA [18]. In cell-free systems, polyamines also affect DNA structure [19] and stimulate DNA transcription and replication [20]. The complexity of the mechanisms that rigorously regulate intracellular polyamine concentrations suggests important roles for these compounds in cellular growth, differentiation, and proliferation [2].

To assess the potential role of polyamines in patients with ARDS, we measured their content in 24-h urine samples collected during the onset of respiratory failure and compared the results with those from patients with cardiogenic pulmonary edema and normal volunteers. Assays of the urinary excretion of polyamines have been used extensively to measure tissue content of these compounds [4] and appear not to be affected by changing rates of glomerular filtration [21]. At entry into the study, all 12 of the ARDS patients had severe impairment of oxygen exchange and diffuse pulmonary infiltrates. None of the patients were successfully ex-

tubated within the first 14 days of ARDS. During this period, previous studies have reported the onset of progressive proliferative alterations of pulmonary structure [1].

All study groups had similar urinary excretions of putrescine, and no statistically significant differences existed between groups. Urinary excretion of spermidine was higher for patients with ARDS compared to normal subjects but similar to values observed in patients with CHF. Spermine concentrations, however, were markedly increased in patients with ARDS compared to values from the other study groups. Of note was the observation that all patients with ARDS who survived to the seventh day increased their urinary excretion of putrescine during this time period. No correlation existed between individual polyamine concentrations and the severity of hypoxia or clinical outcome in the patients with ARDS.

The absence of increased urinary excretion of putrescine at the onset of ARDS does not exclude the possibility that tissue synthesis of putrescine may have been increased. Putrescine can undergo rapid conversion to spermidine, which can then be used to form spermine [4], thereby lowering the plasma concentration of putrescine available for urinary excretion. The elevated levels of spermidine and spermine in patients with early ARDS compared to normal subjects supports this possibility. By the seventh day of acute lung injury, the observed increase in urinary putrescine concentrations in ARDS patients may have resulted from further stimulation of ornithine decarboxylase (ODC), which decarboxylates ornithine to form putrescine, or competitive inhibition by putrescine of other polyamine pathways, as has been described for spermine synthase [22]. In any event, the pattern and timing of ODC activity and the tissue content of the various polyamines has been shown to vary between organ systems and the stimulus involved [4]. We anticipate that animal models of acute lung injury will be required to elucidate these complex interactions through timed assays of polyamines and polyamine synthetic enzymes in lung tissue, the alveolar space, plasma, and urine.

The present investigation was not designed to identify the organ sites where increased polyamine synthesis occurred. However, investigations have demonstrated that exposure of animals to 100% oxygen or ozone results in acute lung injury and increased lung content of polyamines [9, 23, 24]. It is possible, therefore, that the lungs are the primary source of polyamines that are excreted by patients with ARDS. It is more likely, however, that other sites of increased polyamine synthesis exist, considering that ARDS represents a systemic disorder with diffuse endothelial cell injury and an eventual organ expression that is not confined to the lung. Vascular injury and repair in multiple tissues most probably con-

tributed to some degree to the alterations in polyamine synthesis observed in this investigation. Subsequent studies with analyses of bronchoalveolar lavage samples from patients with ARDS will be required to determine the pulmonary contribution to increased polyamine synthesis in acute lung injury. The sources of increased spermidine synthesis in patients with CHF is uncertain considering that to our knowledge no studies have been performed on the effects of pulmonary edema or myocardial infarction on polyamine metabolism.

Several mechanisms exist whereby the alterations in polyamine metabolism observed in this study may have physiologic significance in ARDS. Polyamines may not contribute to the initial lung injury but may participate in tissue repair. This paradigm is supported by stress-induced damage models in intestinal mucosa wherein inhibition of polyamine synthesis does not alter the injury but prevents healing of the microscopic cellular damage [25]. Addition of exogenous spermine to the model reconstituted healing despite the persistence of polyamine synthesis inhibition [26]. Exogenous polyamines in models of ischemic neurotrauma also exert a protective effect [27]. Polyamines, therefore, may promote an influx of reparative cells and a division of existing cells to replace those lost by injury. Conversely, an overly exuberant proliferation of reparative processes in the lung induced by polyamines may contribute to impairment of lung function.

Polyamines may further support lung function through interactions with toxic oxygen metabolites, which are considered important components of the pathophysiology of ARDS [28]. Polyamines have an antioxidant capacity to reduce hydroxyl radical and superoxide anion that increases from putrescine to spermine [6]. Furthermore, spermine is 30 times more efficient than α-tocopherol in preventing lipid peroxidation and also stabilizes the antioxidant activity of α-tocopherol [29, 30]. Additionally, polyamines have direct lipid membrane stabilizing effects that may result from their cationic interactions with negatively charged residues of membrane-bound proteins or acid phospholipids [7]. The polyamine-induced alterations of surface charges may influence some of the membrane-bound enzymes as well as the biosynthesis of membrane lipids and glycoproteins [7].

Mechanisms exist by which polyamines may be injurious to cell function. Excessive concentrations of intracellular polyamines promote degradative metabolism of spermidine and spermine to putrescine by way of N^1-acetyltransferase, which is rapidly induced by tissue stress [31]. This metabolic sequence produces ammonia [32], hydrogen peroxide, and aminoaldehydes as toxic by-products [33]. As suggested in models of neural injury [34], rapid induction of N^1-acetyltransferase converts a rise

in all three polyamines (which is considered neuroprotective) to an isolated increase in intracellular putrescine (which is associated with cellular damage). In this context, it is notable that ARDS patients in the present investigation increased their urinary excretion of putrescine but not spermidine or spermine after 7 days of respiratory failure. Although this observation is compatible with the metabolism of spermidine and spermine to putrescine with resultant generation of toxic by-products, the elimination of these polyamines may also have occurred through their oxygen radical scavenging actions [23, 24].

In conclusion, this pilot investigation is the first to our knowledge to detect an elevated urinary excretion of spermine in patients with ARDS compared to normal controls and intubated patients with cardiogenic pulmonary edema and an increased excretion of spermidine compared to controls. Although the urinary excretion of all measured polyamines increased during the first 7 days of ARDS, only the rise in urinary putrescine reached statistical significance. Although these data suggest the potential importance of polyamines in mechanisms that underlie the pathophysiology of ARDS, further studies will be required to determine if increased polyamine excretion correlates with the onset or progression of ARDS or occurs as a stress response to the underlying critical conditions. Laboratory investigations using blockers of polyamine synthesis may be of value in animal models of acute edematous lung injury and postinflammatory tissue repair.

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