Effects of Taurine on Bleomycin-Induced Lung Fibrosis in Hamsters[1] (42868)

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Abstract. **Four groups of hamsters were assigned as saline + saline, taurine + saline (TS), saline + bleomycin (SB), and taurine + bleomycin (TB). The animals were treated with either saline or taurine (500 mg/kg ip) for 1 week and just prior to intratracheal instillation of bleomycin (7.5 units/kg) or saline on the eighth day. Thereafter, taurine administration was continued ip (250 mg/kg) and in drinking water (1%) for another 14 days. Bleomycin-induced increases in lung collagen were significantly inhibited in TB hamsters. Plasma taurine concentration in the TS group was significantly higher than that of the other groups. Lung lavage (bronchoalveolar lavage fluid) taurine in the SB group was significantly higher than the saline + saline and TS groups. Bronchoalveolar lavage fluid supernatant protein and acid phosphatase levels in the SB and TB groups were significantly increased over the saline + saline and TS groups. Although the total numbers of cells recovered in bronchoalveolar lavage fluid was not different among the four groups, there were significantly fewer neutrophils in the TB as compared with SB hamsters. Morphometric analysis revealed less than half as much lesion (diffuse mononuclear alveolitis and multifocal fibroplasia) in TB as compared with SB hamsters. Also, consolidated foci were less frequent and smaller in TB as compared with SB hamsters. Taurine may attenuate bleomycin-induced inflammation and fibrosis by scavenging reactive oxygen metabolites.** [P.S.E.B.M. 1989, Vol 190]

Bleomycin, an effective antitumor drug, is used clinically to treat a variety of tumors in man. Although bleomycin's antineoplastic activity is advantageously accompanied by little, if any, cytotoxic effect on bone marrow (1), its use in clinical chemotherapy is greatly limited by its association with the development of pulmonary fibrosis (2, 3). One approach which could help in harnessing the maximum benefits of bleomycin therapy is its combined use with other agents in order to minimize its pulmonary toxicity without compromising its antineoplastic activity. Some attempts have been made toward this goal, but the results produced have been far from satisfactory (4, 5).

The precise mechanism of bleomycin-induced lung toxicity remains unclear. However, it appears that under aerobic conditions an intracellular bleomycin-Fe^{2+} complex generates oxygen-free radicals (6, 7) and causes lung injury which eventually progresses to fibrosis (8, 9). Therefore, it is possible that an effective elimination or blockade of the action of reactive oxygen species (ROS) may offer protection against bleomycin-induced lung injury (10, 11).

Taurine, 2-aminoethanesulfonic acid, is a naturally occurring sulfur-containing amino acid present in high concentrations in most mammalian tissues (12). Taurine is not a constituent of mammalian proteins and its main function in mammals appears to be in the formation of conjugated bile salts (13, 14). It is well documented that taurine plays an important role under physiologic and pathologic conditions in both the cardiovascular and nervous systems (15, 16). The beneficial effects of this amino acid have usually been attributed to its detoxifying, antioxidant, and membrane-stabilizing properties (17–19). Administration of exogenous taurine has been used to protect against oxidant damage in many tissues including the lung (20–22). In the present study, we have investigated the possible protective effects of taurine against bleomycin-induced pulmonary toxicity in the hamster.

Materials and Methods

Male Golden Syrian hamsters (102–135 g) were purchased from Simonsen, Inc. (Gilroy, CA). Bleomy-

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cin sulfate (Blenoxane) was the generous donation of Bristol Myers Co. (Syracuse, NY). Taurine and hydroxyproline were obtained from Sigma Chemical Co. (St. Louis, MO). [^{3}H]Hydroxyproline was obtained from New England Nuclear Research Products (Boston, MA). All other chemicals were obtained from standard commercial sources.

Treatment of Animals. The hamsters were housed in groups of four in facilities approved by the American Association for the Accreditation of Laboratory Animal Care and were allowed to acclimate in the facilities for 1 week prior to all treatments. A 12 hr light/12 hr dark cycle was maintained and hamsters had access to water and Rodent Laboratory Chow 5001 (Purina Mills Inc., St. Louis, MO) *ad libitum*. Animals were randomly divided into four experimental groups: 1, saline + saline (SS); 2, taurine + saline (TS); 3, saline + bleomycin (SB); and 4, taurine + bleomycin (TB). TS and TB hamsters were pretreated by intraperitoneal injection of taurine (500 mg/kg) daily for 1 week. SS and SB hamsters were pretreated with saline vehicle. Following 1 week of pretreatment, the hamsters were placed under pentobarbital anesthesia (75–85 mg/kg) and received either 0.5 ml of bleomycin sulfate solution (0.75 units/100 g body wt) (SB and TB) or 0.5 ml of sterile isotonic saline (SS and TS) intratracheally by the transoral route (23). For the next 2 weeks, TS and TB hamsters received a taurine solution (250 mg/kg ip) once a day plus 1% taurine in drinking water. The SS and SB hamsters received an equivalent volume of sterile isotonic saline (ip) for the same length of time. Bleomycin sulfate and taurine were freshly dissolved in sterile isotonic saline prior to injection.

Bronchoalveolar Lavage Fluid (BALF). Two weeks after the intratracheal injection, the hamsters were "killed" under pentobarbital anesthesia (90–120 mg/kg ip). The abdominal cavity was opened and blood collected in a heparinized syringe from the caudal vena cava. The plasma was separated by centrifugation at 1500*g* for 20 min at 4°C and frozen at −20°C until assayed for taurine content. Immediately thereafter, the lungs were lavaged *in situ* according to the method of Giri *et al.* (24). Total cell count in BALF was estimated by Coulter Counter and slides for differential cell counts were prepared according to the method of Willcox *et al.* (25). Cell differentials were based on a minimum of 250 cells counted per slide. The remaining BALF was centrifuged at 1500*g* for 20 min at 4°C, the resulting supernatant aliquoted, and then stored at −80°C. Acid phosphatase activity and protein content in BALF supernatant were determined according to the methods of Ignarro (26) and Lowry *et al.* (27), respectively. After being lavaged, the lung tissue was prepared for either biochemical or histologic analysis.

Biochemistry. For biochemical studies, the lungs were perfused *in situ* from the right side of the heart with ice-cold isotonic saline. The lung lobes were dissected free of nonparenchymal tissue, and rinsed in ice-cold saline to wash off blood, immediately frozen in liquid nitrogen, and stored at −80°C. Later, the frozen lungs were thawed and homogenized in isotonic saline by a Polytron (Brinkmann). The homogenate was thoroughly mixed by repeated inversions and the final homogenate volumes (9–10 ml) were recorded. The samples were aliquoted and stored at −20°C. For collagen assay, a 1-ml aliquot homogenate was precipitated with 0.25 ml of 50% trichloroacetic acid, centrifuged, and the precipitate hydrolyzed in 2 ml of 6 *N* HCl overnight at 110°C. [^{3}H]Hydroxyproline (1×10^5 dpm) was added to each sample to determine recovery and the hydroxyproline content was measured by the technique described by Woessner (28). The recovery of [^{3}H] hydroxyproline ranged from 82 to 97% and was used to correct the final amount of hydroxyproline for each sample.

Measurement of Taurine in Plasma, BALF, and Lung Tissue. The samples were analyzed for taurine concentration by means of an amino acid analyzer (model 121 M amino acid analyzer; Beckman Instruments, Palo Alto, CA) according to the methods of O'Donnell *et al.* (29).

Histopathologic and Morphometric Analysis. For histologic studies, the lungs were prepared according to the methods outlined by Giri and Hyde (30). Briefly, the lungs were fixed via the trachea at a pressure of 30 cm of water with a cacodylate-buffered glutaraldehyde-paraformaldehyde fixative. The right cranial and caudal lobes and the left lobe were later blocked, embedded in paraffin, cut in 7-μm sections, and stained with hematoxylin and eosin. A single section from each of the three sampled lobes was used to evaluate the lesions.

Parenchymal lesions were defined as thickening of interalveolar septa due to edematous swelling, inflammatory cells or fibrosis associated with hyperplastic epithelial cells, and some airway inflammatory cells remaining after lung lavage. The volume of parenchymal lesion within the lung was estimated using point counting techniques and a square lattice test system at a final magnification of ×160. Every field on the section (from about 20 to 40) was evaluated because of the patchy distribution of lesion. The volume of parenchymal lesion was calculated from the product of the volume of the lung, the volume of parenchyma per lung, and the volume lesion per parenchyma as described previously (31).

Statistical Analysis of Data. The biochemical data on BALF supernatant were expressed on the basis of total lavage fluid volume recovered per lung. Tissue levels of hydroxyproline and taurine, lung volume, and volume of parenchymal lesion were also expressed per total lung. Expression of the data on a per lung basis avoids the artifactual lowering of the values in treated animals (32). All values were reported as the mean ± 1 SE and were analyzed by a one-way analysis of variance

and Duncan's multiple range test (33). A value of $P \leq 0.05$ was considered significant.

Results

General trends in body weight were noted at the time of sacrifice (Table I), with the saline-treated (SS and TS) animals having gained weight while bleomycin-treated (BS and TB) groups lost weight. It was also noted that animals in the TS group gained significantly less weight than the control hamsters in the SS group. The initial body weight of all animals was 120.6 ± 1.1 g (SE). No significant difference in mean initial body weight was noted between groups.

Lung Collagen. The effect of different treatments on the total lung collagen content is shown in Figure 1. Bleomycin treatment significantly elevated lung hydroxyproline levels of SB and TB groups above their respective control groups. However, the hydroxyproline content of the TB group was significantly less than that in the SB group. There was no significant difference in collagen content between the SS and TS groups.

Bronchoalveolar Lavage Fluid Data. BALF recovery from SS and TS hamsters averaged 93%, but was only 79% in SB and TB hamsters. The number of total cells and the absolute number of different cell types in the BALF of the four treatment groups are shown in Figures 2 and 3. Although the total number of BALF cells was not different among different groups, the bleomycin-treated groups had significantly fewer alveolar macrophages and more neutrophils as compared with the SS and TS groups. BALF from TB animals contained significantly fewer neutrophils, but more monocytes than SB hamsters. SB hamsters had significantly more lymphocytes in BALF than SS and TS groups whereas lymphocytes were only marginally increased in TB hamsters.

The protein content and acid phosphatase activity of BALF supernatant in the four groups of hamsters are shown in Table II. Fourteen days after bleomycin treatment, both acid phosphatase activity and protein content in the BALF supernatant were significantly increased. Taurine treatment did not significantly alter these two parameters from the respective control values.

Taurine Levels in Plasma, BALF, and Tissue. The concentration of taurine was determined in the plasma, BALF supernatant, and lung tissue of all groups (Table III). The plasma taurine level in TS hamsters was significantly higher than that in any other group (SS, SB, and TB). However, the taurine content in BALF supernatant from the SB group was significantly greater than that from SS and TS hamsters. Taurine levels in BALF from TB hamsters were elevated, but not significantly. There was no significant difference in the lung tissue levels of taurine among all four groups, although there was a general trend toward increased taurine in all bleomycin-treated groups.

Histopathology and Morphometry. Lung tissue from control hamsters (SS and TS) had parenchyma which appeared to be normal (Fig. 4). Hamster lungs from the SB group had multifocal lesions that comprised about 45% of the total parenchyma (Table I). The lesions contained accumulations of alveolar and interstitial mononuclear inflammatory cells, most of

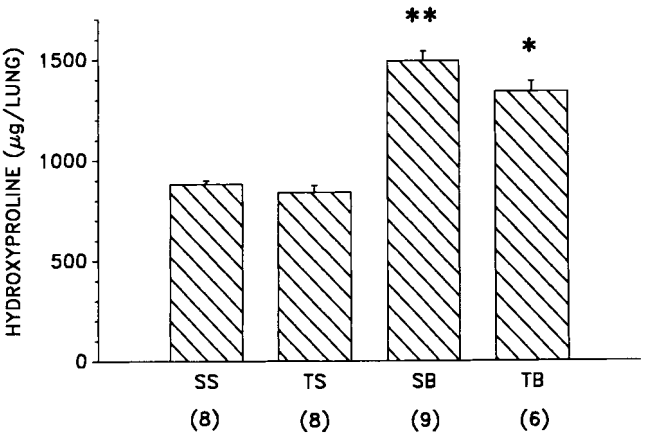

Figure 1. Lung hydroxyproline content at 14 days after intratracheal instillation of either saline or bleomycin in four different groups: SS, TS, SB, and TB. *Significantly higher ($P < 0.05$) than SS and TS groups but lower ($P < 0.05$) than SB group. **Significantly higher ($P < 0.05$) than all other groups.

Table I. Effects of Intratracheal Administration of Bleomycin with or without Taurine Treatment on Body Weight, Lung Volume, and the Volume of Parenchymal Lesion in the Hamster[a]

Treatment[b]	Weight gain (%)	Lung volume (cm^3)	Volume of lesion (cm^3)
SS	118.4 ± 1.0 (11)[c]	5.59 ± 0.28 (4)	0 (4)
TS	106.3 ± 1.9 (11)	5.38 ± 0.26 (4)	0 (4)
SB	82.3 ± 3.3 (15)[d]	4.28 ± 0.17 (6)[d]	1.95 ± 0.12 (6)[c]
TB	80.8 ± 5.6 (10)[d]	4.56 ± 0.54 (4)[d]	0.80 ± 0.26 (4)[d]

[a] Values expressed as mean ± SE with number of animals in parentheses. Weight gain was expressed as a percentage of the initial body weight (120.6 ± 1.1 g) at the start of the study.
[b] Four groups of hamsters were assigned as SS, TS, SB, and TB. The animals were treated with either saline or taurine (500 mg/kg ip) for 1 week and just prior to intratracheal instillation of bleomycin (7.5 units/kg) or saline on the eighth day. Thereafter, taurine administration was continued ip (250 mg/kg) and in drinking water (1%) for another 14 days.
[c] Significantly different ($P < 0.05$) from all other groups.
[d] Significantly different ($P < 0.05$) from SS and TS groups.

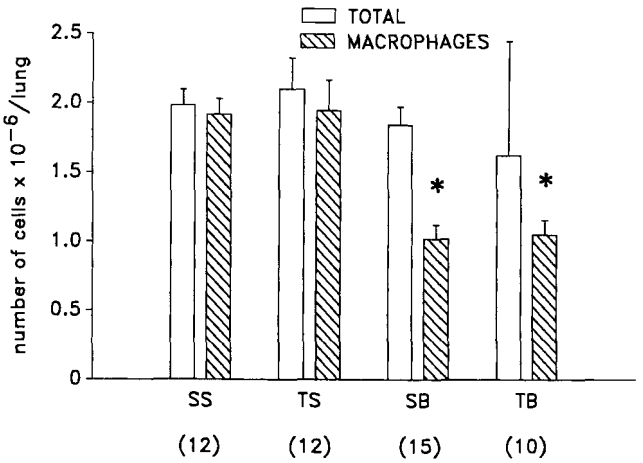

Figure 2. Total cells and macrophages in bronchoalveolar lavage fluid at 14 days after intratracheal instillation of either saline or bleomycin in four different groups: SS, TS, SB, and TB. *Significantly lower ($P < 0.05$) than SS and TS groups.

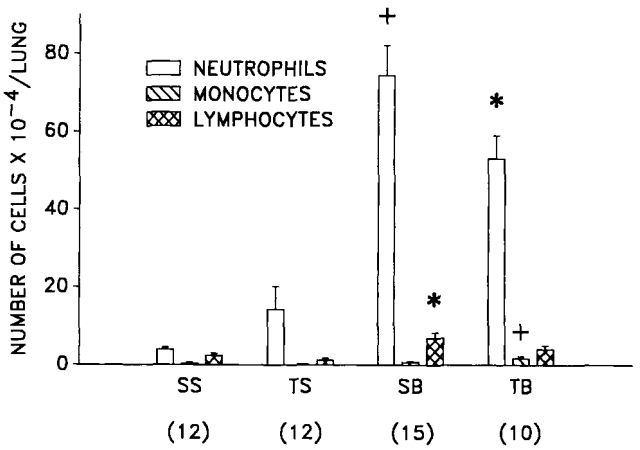

Figure 3. Differential cell counts in bronchoalveolar lavage fluid at 14 days after intratracheal instillation of either saline or bleomycin in four different groups: SS, TS, SB, and TB. *Significantly higher ($P < 0.05$) than SS and TS groups. +Significantly higher ($P < 0.05$) than all other groups.

which were macrophages. Some of these foci were organized interstitial cells with a fibroblast appearance surrounded by extracellular fibers (Fig. 5). In areas lacking organized connective tissue, but where alveolitis was still present, interalveolar septa appeared to be thicker than those of controls (SS and TS). About half of the SB hamsters also had peribronchiolar and periarteriolar fibroplasia. Although mononuclear alveolitis and multifocal organized interstitial cells were still present in TB hamsters (Fig. 6), the organized foci were less frequent and smaller than those seen in SB hamsters. Lungs from the TB group had significantly less parenchymal lesion (nearly 60%) than the SB-treated group (Table I).

Discussion

Bleomycin-induced lung injury in hamsters has been used as a model to study possible mechanisms for the development of pulmonary fibrosis and to test the anti-inflammatory and antifibrotic effects of other agents (2–5, 23, 24, 34–37). The exact mechanism of bleomycin-induced pneumotoxicity which eventuates in fibrosis is not known. However, under aerobic conditions a bleomycin-Fe^{2+} complex in the cell may undergo a cyclic oxidation-reduction reaction and generate ROS, in particular superoxide anion and hydroxyl radical (6, 7). ROS may injure the lung and initiate inflammation in the lungs, which may result in fibrosis (8, 9).

In the present study, bleomycin-induced fibrosis was significantly inhibited by daily treatments with exogenous taurine as determined by biochemical, histologic, and morphometric techniques. In accordance with data previously reported (23, 34), bleomycin alone significantly increased the total lung collagen (hydroxyproline) relative to control animals. However, in animals treated with taurine and bleomycin, the hydroxyproline level was significantly lower than that of animals treated with bleomycin alone. This is also consistent with our histologic observation of reduced fibroplasia and the significant reduction in the volume of lesion in these animals. These results indicate that taurine partially ameliorates bleomycin-induced pulmonary fibrosis.

Taurine also affected the bleomycin-induced inflammation as determined by BALF analysis. Significantly fewer neutrophils were found in the TB group when compared with SB animals. Although not significant, a similar trend was noted for the lymphocyte population in lavage. When considered in conjunction with the histopathology results, this suggests that taurine may have partially blocked the initial bleomycin-mediated cell injury, which in turn resulted in less inflammation. Since alternative interpretations of these results are possible, further studies are necessary to define the effect of taurine on the levels of chemotactic

Table II. Effects of Intratracheal Administration of Bleomycin with or without Taurine Treatment on Acid Phosphatase Activity and Protein Content of Bronchoalveolar Lavage Supernatant in the Hamster

Treatment[a]	Protein[b] (μg/lung)	Acid phosphatase[b] (nmol/hr/lung)
SS	1378 ± 124 (11)	114 ± 12 (10)
TS	1459 ± 73 (12)	120 ± 13 (11)
SB	4830 ± 454 (15)[c]	352 ± 34 (15)[c]
TB	4286 ± 705 (10)[c]	390 ± 49 (10)[c]

[a] Four groups of hamsters were assigned as SS, TS, SB, and TB. The animals were treated with either saline or taurine (500 mg/kg ip) for 1 week and just prior to intratracheal instillation of bleomycin (7.5 units/kg) or saline on the eighth day. Thereafter, taurine administration was continued ip (250 mg/kg) and in drinking water (1%) for another 14 days.

[b] Values expressed as mean ± SE with number of animals in parentheses.

[c] Significantly greater ($P < 0.05$) than SS and TS groups.

Table III. Effects of Intratracheal Administration of Bleomycin with or without Taurine Treatment on Taurine Content in Plasma, Bronchoalveolar Lavage Supernatant, and Lung Tissue

Treatment[a]	Taurine Concentration[b]		
	Plasma (nmol/ml)	Lavage fluid (nmol/lung)	Lung tissue (nmol/lung)
SS	167 ± 22 (11)	197 ± 67 (12)	5700 ± 250 (8)
TS	236 ± 23 (11)[c]	145 ± 35 (12)	6100 ± 320 (8)
SB	158 ± 18 (15)	421 ± 50 (15)[d]	8020 ± 560 (8)
TB	175 ± 19 (10)	290 ± 45 (10)	7770 ± 720 (8)

[a] Four groups of hamsters were assigned as SS, TS, SB, and TB. The animals were treated with either saline or taurine (500 mg/kg ip) for 1 week and just prior to intratracheal instillation of bleomycin (7.5 units/kg) or saline on the eighth day. Thereafter, taurine administration was continued ip (250 mg/kg) and in drinking water (1%) for another 14 days.
[b] Values expressed as mean ± SE with number of animals in parentheses.
[c] Significantly higher ($P < 0.05$) than all other groups.
[d] Significantly higher ($P < 0.05$) than SS and TS groups.

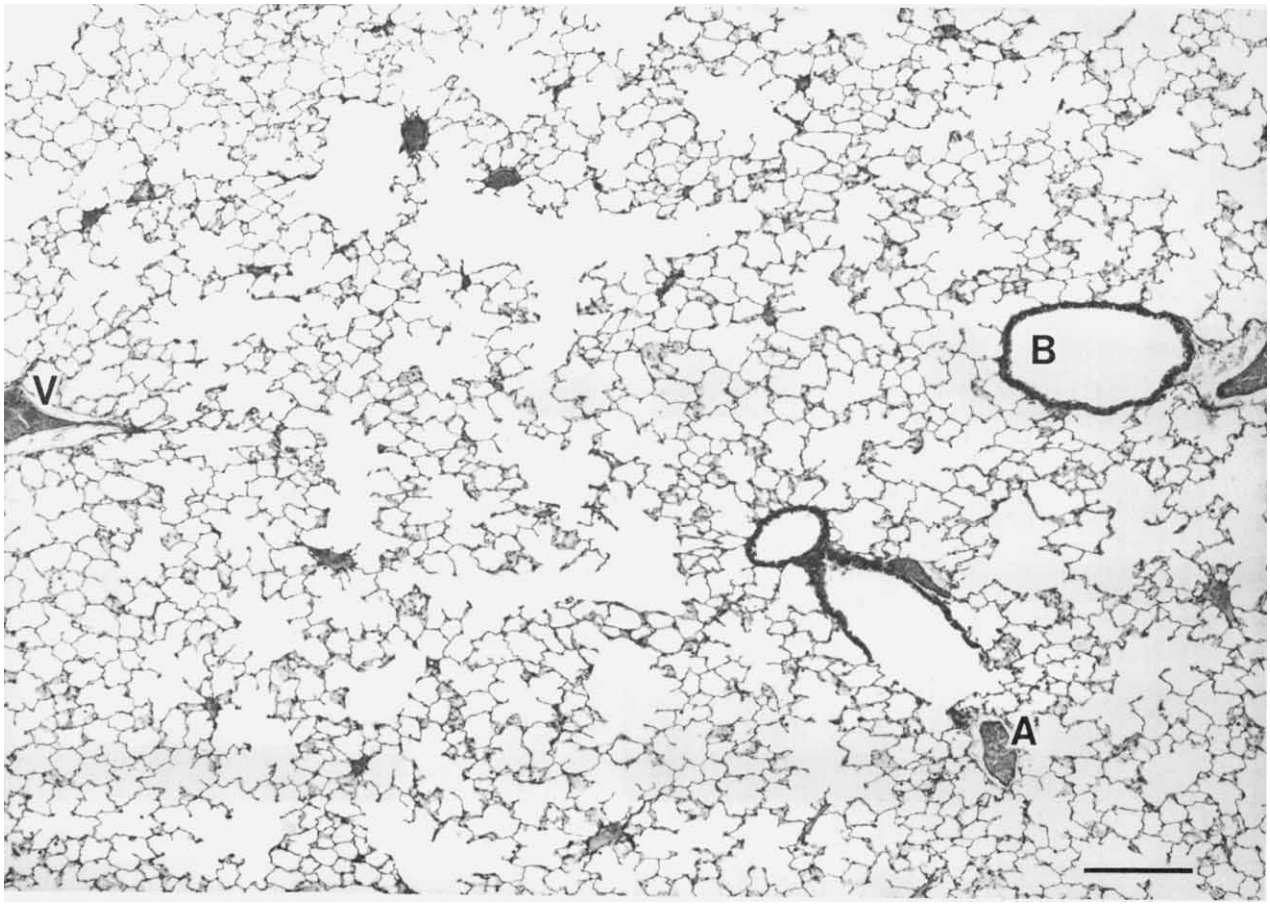

Figure 4. A light micrograph of parenchymal tissue from a hamster treated with taurine and saline. Note the normal appearance of interalveolar septa, small airway, and vessels. A, arteriole; B, small bronchus; and V, small vein. Bar = 0.2 mm.

mediators in bleomycin-injured lungs. Although it has been shown that selected lymphocyte populations apparently modulate bleomycin-induced fibrosis in the rat and certain mouse strains (35, 36), our data suggest that the effect of taurine in the hamster model is not mediated by an immunosuppressive mechanism. This is consistent with other work done in this species which has shown that a decrease in BALF lymphocytes does not necessarily correlate with decreased fibrosis (37).

Although the exact role of taurine in this partial protection is not known, the antioxidant property of taurine may be involved. Taurine has been reported to react with hypochlorous acid, a product produced by both neutrophils and monocytes, to form both mono- and dichloramines, the so-called "long-lived" oxidants (38). These chloramines can damage a variety of cellular components, are actively taken up by cells such as the erythrocyte, and are apparently detoxified by glutathi-

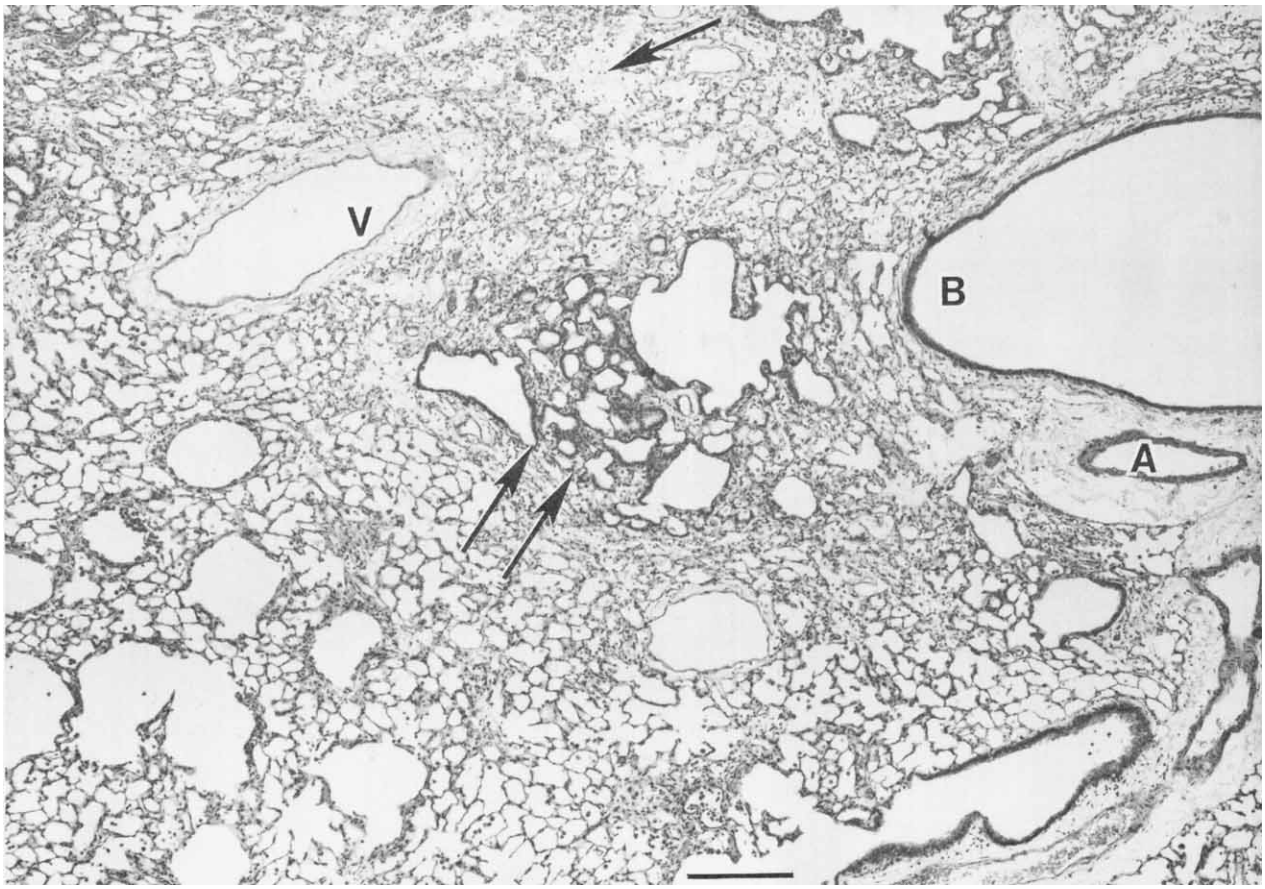

Figure 5. A light micrograph of parenchymal tissue from a hamster treated with saline and bleomycin. Note that almost half the micrograph is involved in lesion that is composed of organizing fibroblasts in alveolar airspaces (arrow) and hyperplastic epithelial cells in other airspaces (double arrows). A, artery; B, large bronchus; and V, vein. Bar = 0.2 mm.

one-dependent processes (39). In hamsters exposed to toxic levels of NO_2, taurine prevented both acute inflammatory and morphologic alterations in the lung (22). In rabbit spermatozoa, taurine was found to scavenge superoxide radicals and reduced their formation (20). There is some evidence that this may be due to scavenging of hydroxyl radicals by the sulfinate moiety of hypotaurine, 2-aminoethanesulfinic acid (40). In addition, administration of taurine to rats intoxicated with CCl_4 has been shown to decrease malondialdehyde formation *in vivo* in the liver and in hepatic microsomal fractions treated *in vitro* (21). Thus, ROS generated by both phagocytes and bleomycin may be potentially scavenged by taurine or taurine precursors.

A second possible mechanism for taurine's protective action may involve bleomycin-induced inflammation and the potential reduction/oxidation properties of the bleomycin-Fe^{2+}-oxygen complex (41). There is some evidence that reducing agents such as glutathione and their precursors may exacerbate bleomycin-induced cytotoxicity by regenerating Fe^{2+}, which would potentially allow the cyclic oxidation and reduction of the complex and continuous production of superoxide (42, 43). Neutrophils, attracted by the initial bleomycin injury, produce hypochlorous acid, which can react with the amine moiety of taurine and form chloramines (38). Chloramines can enter the cell and deplete glutathione (39), leaving fewer reducing equivalents available for reducing the bleomycin-Fe^{2+} complex and reducing formation of ROS.

In contrast to the above evidence, pretreatment with taurine prior to CCl_4 administration has also been shown to exacerbate hepatic lipid peroxidation *in vivo* (21) in a calcium-dependent manner. Taurine treatment also either enhanced or suppressed the rise in hepatic free calcium levels which follows CCl_4 administration (21), depending on whether it was administered before or after the CCl_4. The authors suggested taurine treatments modulated intracellular calcium levels and the subsequent calcium-dependent lipid peroxidation. Bleomycin lung injury is likewise accompanied by elevated tissue levels of calcium (32). Thus, taurine may also act to stabilize microsomal membranes following bleomycin-induced injury, resulting in reduced intracellular levels of free calcium ion, which may prevent calcium-mediated inflammatory and fibroproliferative events.

There appears to be no mention in the literature of

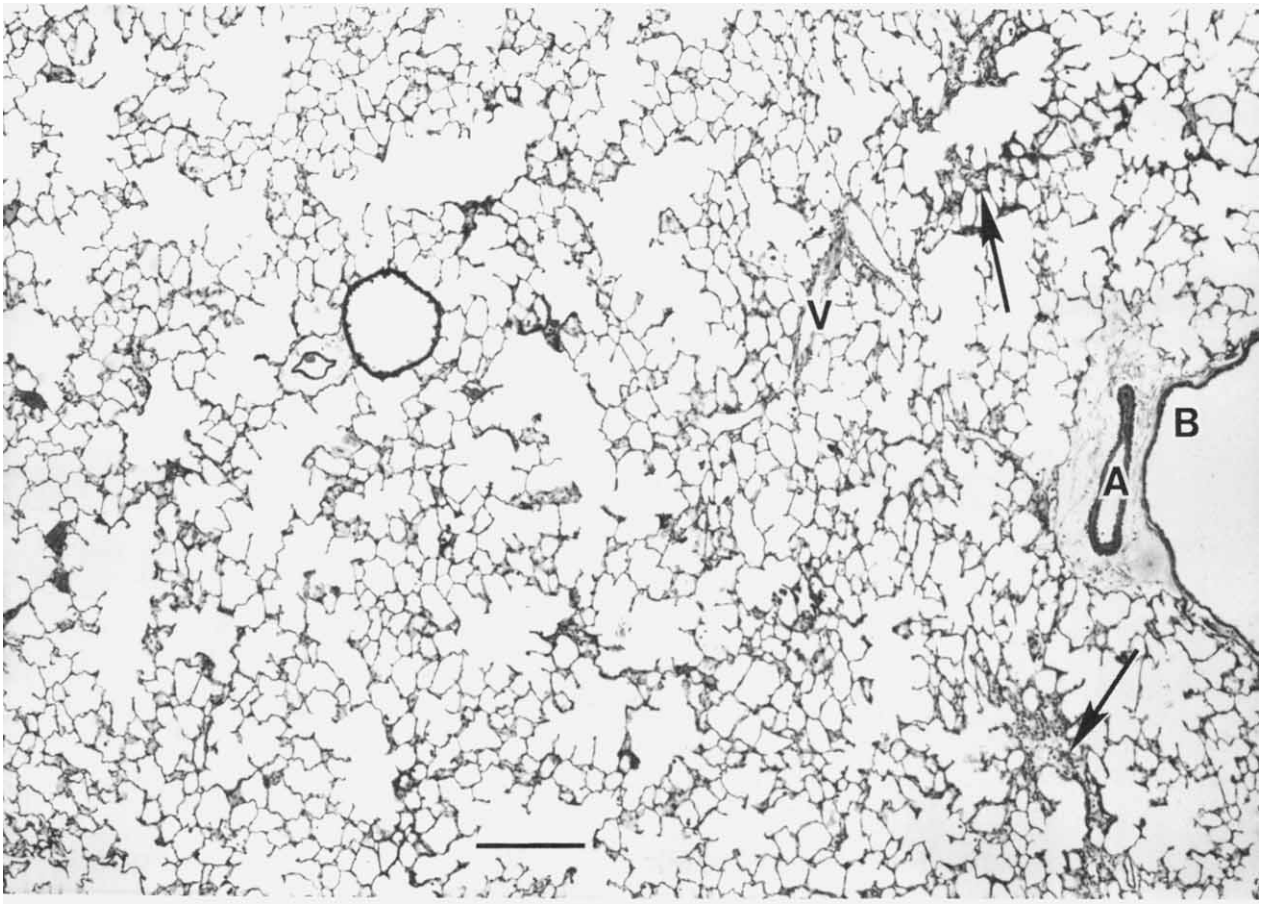

Figure 6. A light micrograph of parenchymal tissue from a hamster treated with taurine and bleomycin. Note the limited amount of lesion and interstitial fibrosis (arrows). A, artery; B, bronchus; and V, small vein. Bar = 0.2 mm.

a metabolic pathway for converting taurine to a sulfhydryl-containing antioxidant such as glutathione (44). Any such synthetic pathway would require a six-electron reduction of sulfur, making the process energetically "expensive." There is also no evidence of a pathway for the reduction of taurine to the antioxidant hypotaurine (45). Thus, although such pathways are theoretically possible, their actual existence would appear to be highly unlikely.

We observed that plasma taurine concentrations were greater than control values in TS hamsters. This strongly suggests that intraperitoneal administration of exogenous taurine can effectively elevate circulating taurine levels. The fact that a similar increase was not observed in TB animals may be due in part to peroxidase-catalyzed oxidation of taurine that probably occurs during inflammation (38, 39). A variety of conditions associated with ROS such as burn or surgical trauma, infection, and irradiation can cause hypertaurinuria (17), which suggests that bleomycin-generated ROS could also induce hypertaurinuria, resulting in excretion of excess taurine. A third mitigating factor is the anorexic action of bleomycin in this species (34), which may substantially compromise the nutritional status of the animals. Rats fed diets deficient in protein or sulfur-containing amino acids retain significantly more taurine than do animals receiving a normal diet (46, 47). Thus, both injured and non-target tissues may play a role in regulation of taurine during bleomycin lung injury.

Despite daily administration of taurine and the subsequent elevation of plasma levels of this amino acid, lung levels remained at control levels in this study. Taurine enters the cell by both active transport and diffusion (17, 39), but is apparently not incorporated into mammalian protein (12). At best, these data suggest that the uptake and/or cellular concentrations of taurine may be regulated, preventing accumulation in pulmonary tissue. If intracellular levels of taurine are regulated, it seems likely that the factors, as discussed above, which affect taurine levels in plasma may also affect intracellular concentrations. All animals treated with bleomycin tended to have higher tissue levels. This trend probably reflects the inflammatory and proliferative processes that occur in the injured lung rather than a specific protective response to oxidant injury (23, 48).

It is noteworthy that the total taurine in BALF from SB hamsters was significantly increased compared with control. The underlying mechanism for the in-

crease in BALF taurine levels in bleomycin-treated hamsters is not clear. Possibly, the direct cytotoxic action of bleomycin and the accompanying inflammation could damage epithelial and interstitial cell populations (24, 48). Injured cells may subsequently leak cytosolic constituents such as taurine into the airways. The fact that BALF levels of taurine from TB animals, although elevated, did not differ significantly from control levels may simply be another reflection of taurine's amelioration of bleomycin-induced cytotoxicity.

The results from this study indicate that taurine treatment partially, yet significantly, protected the lung against bleomycin-induced lung fibrosis. Possibly, taurine may offer an even greater level of protection if delivered directly to the lung as an aerosolized solution. Further studies are planned to answer this and other questions related to the observed antifibrotic effect of taurine.

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