remain to be completely substantiated, it can be assumed that the pargyline-resistant enzyme activity may play a role in the cross-linking of collagen and elastin in blood vessels [3, 4, 17].

In DOCA–salt hypertensive rats, no alteration in mesenteric artery (Fig. 1) PRAO levels was detected. However, when the enzyme assay was performed when these rats were younger than SHR rats showing equivalent blood pressure readings, the difference in arterial pressure between normotensive and DOCA–salt hypertensive rats was highly significant. It should be taken into consideration that when enzyme activity was evaluated in these animals the circulating levels of the mineralocorticoid could still be high. In this regard, increased MAO activity in the rat heart has been observed after adrenalectomy [19]. On the other hand, in a study on the neuronal factors that participate in the development of hypertension, alterations of some of the enzymes involved in brain amine biosynthesis of SHR were reported [20] while, in turn, no parallel changes could be detected in DOCA–salt hypertensive rats.

In summary, compared to normotensive rats, PRAO activity was found to be enhanced in homogenates of mesenteric artery in genetically hypertensive rats. This increase in enzyme levels was correlated with the development of hypertension in this model. The reduction in blood pressure detected after administration of hydralazine, an inhibitor of cardiovascular PRAO [9], also supports the notion for a role of this enzyme in the SHR model of hypertension. The purification and further characterization of PRAO as well as the study of its distribution in different cell types of cardiovascular tissue may give an insight into the ethiology and treatment of some cardiovascular alterations.

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A study of amine oxidases in bleomycin-induced pulmonary fibrosis

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Bleomycin causes pulmonary injury and fibrosis in animals and man [1–5]. In rats, Counts *et al.* [6] showed bleomycin to increase lung lysyl oxidase activity. This amine oxidase promotes the cross-linking of collagen and elastin [7, 8]. Whether other lung amine oxidases are also affected has not been studied and forms the basis of the present investigation. In this regard, benzylamine oxidase (BzAO) is of particular interest since some workers have postulated an association between BzAO and connective tissue disorders [9, 10].

BzAO is distinct from lysyl oxidase [11] and is found in the serum, connective and cellular tissues of animals and man [8–10, 12–14]. Clinical studies show increased serum BzAO activity in patients with diagnosed fibrosis [9, 15, 16] including pulmonary fibrosis [17]. Conversely, serum BzAO is reported to be unchanged in chronic obstructive lung disease while decreased activity has been found in severely burnt patients and in cancer patients [17]. Thus, the association between serum BzAO activity and connective tissue proliferation remains tenuous and rests entirely upon assays of the serum enzyme derived from patient populations. It seemed to us that a more definitive test for a role of BzAO in connective tissue disorders could be obtained by assaying both serum and cellular BzAO in bleomycin-induced pulmonary fibrosis in rats. Monoamine oxidases (MAO) types A and B were also measured since a variety of amines have been linked to the pulmonary toxicity of bleomycin [18].

Materials and methods

Male Fischer rats (weighing 169 ± 9 g) were obtained from the Charles River Breeding Laboratories, Kingston, NY, and were barrier-maintained as described previously [4]. All animals were observed for 7 days prior to use, and bleomycin sulfate (640 μg/165 g body wt in 0.5 ml of sterile saline) or saline was quickly instilled intratracheally as described by Counts *et al.* [6].

Preparation of tissues. The animals were anesthetized

with sodium pentobarbital (4.5 mg/100 g body wt), and the thoracic and abdominal cavities were exposed. A sample of blood was drawn by cardiac puncture and allowed to coagulate, and the serum was collected by centrifugation at 5000 *g* for 10 min at room temperature. The serum was frozen in liquid nitrogen for assay of BzAO. The lungs were removed, rinsed in saline, blotted dry, weighed, and frozen in liquid nitrogen. Prior to assay, the tissue was thawed over ice, minced with scissors, and homogenized (Ultra Turrex, model SDT, for 1 min at Setting Number 4.5) at 4° using a tissue : buffer ratio (g/ml) of 1 : 10 in 1 mM phosphate buffer, pH 7.8. Crude homogenates were centrifuged at 700 *g* for 10 min, and the supernatant fraction was used for assay.

Enzyme assays. The activities of BzAO, MAO-B and MAO-A were determined as described previously by Andree and Clarke [19] for rat lung. [^{14}C]Benzylamine (20 μM; 10 μCi/μmole) was used to assay BzAO after inhibition of MAO with pargyline (2×10^{-4} M). MAO-B was assayed using [^{14}C]benzylamine (600 μM; 2 μCi/μmole) after inhibition of BzAO with semicarbazide (2×10^{-4} M). [^{14}C]5-Hydroxytryptamine (400 μM; 2 μCi/μmole) was used for the assay of MAO-A. Bleomycin (1500 μg/ml) failed to alter the recovery of the deaminated products which was calculated to be in excess of 90%.

Protein. The protein content of the 700 *g* supernatant fraction of tissue homogenate was measured by the microbiuret method of Goa [20] with bovine serum albumin (Fraction V) as standard.

Chemicals. [^{14}C]Benzylamine hydrochloride was purchased from ICN Pharmaceuticals, Inc., Irvine, CA (12.5 μCi/mmole), and [^{14}C]5-hydroxytryptamine binoxolate fromethe New England Nuclear Corp., Boston, MA (5.15 μCi/mmole).

Benzylamine hydrochloride was purchased from ICN Pharmaceuticals, Inc., Plainview, NY, and 5-hydroxytryptamine binoxolate from the Sigma Chemical Co., St. Louis, MO. Pargyline hydrochloride was purchased from the Regis Chemical Co., Morton Grove, IL, and semicarbazide from the Fischer Scientific Co., Fair Lawn, NJ. Bleomycin sulfate was obtained from the Bristol Laboratories, Syracuse, NY, as Blenoxane. This preparation is a mixture of thirteen bleomycins [18].

Statistical tests. The significance of differences between mean values was determined using a Student's *t*-test. P values are expressed as two-tailed.

Results

Bleomycin produced a significant increase in lung weight at 3, 7, 14 and 28 days (Fig. 1). Maximal increases occurred at days 7 and 14 with evidence for a return toward control values at day 28.

The effects of bleomycin on the specific and total lung activities of BzAO, MAO-B and MAO-A are shown in Fig. 2. Bleomycin reduced the mean specific activities of all three amine oxidases when expressed on a unit weight basis (left-hand column). Although not shown, a similar but more accentuated pattern of changes was obtained on the basis of protein content. The outcome was different, however, when total lung activities were calculated (right-hand column). Total lung activities of BzAO and MAO-B were not altered significantly by bleomycin, with the exception of a significant drop in MAO-B activity at day 3. A similar but non-significant decline in total BzAO activity was also evident at day 3. Interestingly, total lung MAO-A activity was increased significantly by bleomycin at days 7 and 14 with no change at days 3 and 28. The differences between the specific and total activity measures in Fig. 2 reflect the bleomycin-induced increases in lung weight (Fig. 1).

Serum BzAO activity was not altered significantly at any time-point. The specific activity of serum BzAO, expressed on either a weight or protein basis, was over 1000-fold lower than that found in lung. MAO-A and MAO-b activities were not detectable in rat serum.

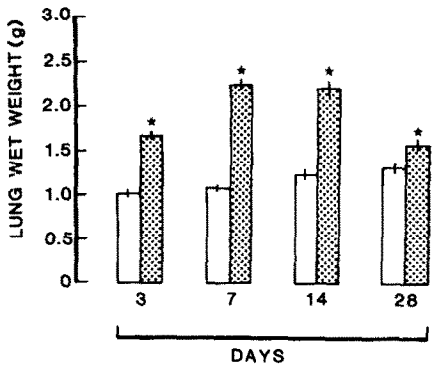

Fig. 1. Effect of bleomycin on the wet weight of rat lungs. Rats were given a single administration (day zero) of either 0.5 ml sterile saline (open columns) or 640 μg bleomycin sulfate in 0.5 ml sterile saline (dotted columns) per 165 g body weight into the trachea and were killed on the days shown. Each column is the mean ± S.E. for three to six rats. Key: (*) significantly different from control, $P < 0.05$.

Discussion

The present experiment replicated the experimental protocol of Counts *et al.* [6] who demonstrated increased rat lung lysyl oxidase activity with bleomycin as early as 3 days after intratracheal administration. Maximal elevation ocurred between 5 and 14 days. These and other findings, both histological and biochemical [1–5], support the occurrence of pulmonary fibrosis with bleomycin. The important outcomes of the present study are that total lung and serum BzAO activities were not changed significantly by bleomycin, whereas MAO-A activity was elevated at days 7 and 14 (Fig. 2).

Although total lung activity of BzAO was not altered significantly by bleomycin, the specific activity of the enzyme was reduced. This is because bleomycin increased lung weight (Fig. 1). MAO-B was affected similarly but total activity was reduced significantly at day 3. This reduction most probably reflects the reported cellular toxicity of bleomycin [1, 2, 4, 5, 21, 22] with subsequent recovery from the initial insult at day 7. BzAO may undergo a similar fate. In this instance, however, the decrease at day 3 did not attain significance.

The present findings with lung and serum BzAO do not support a role for the enzyme in bleomycin-induced pulmonary fibrosis. It might be argued that bleomycin induces a unique or atypical fibrotic condition in lung. This is not the case, however, since lung pathology with bleomycin appears similar to that resulting from multiple etiologies [22, 23]. Furthermore, the present data are consistent with two previous studies concerning cellular BzAO. Hayakawa *et al.* [24] and, more recently, Hayes *et al.* [25] failed to find an association between connective tissue proliferation and BzAO activity in human skin [24] and atherosclerotic aortae [25]. The pronounced and prolonged decreases in serum BzAO in post-burn patients [17] also challenge the concept of an integral reltionship between proliferating connective tissue and elevated serum BzAO activity. In addition, there is evidence that serum BzAO activity is raised in diabetic children and adults [26]. Until the source of serum BzAO is identified, it will be very difficult to offer a global explanation for these diverse findings. Currently, many more questions than answers exist with regard to the possible physiological and pathological importance of BzAO [19, 27].

There are several possibilities which might explain the

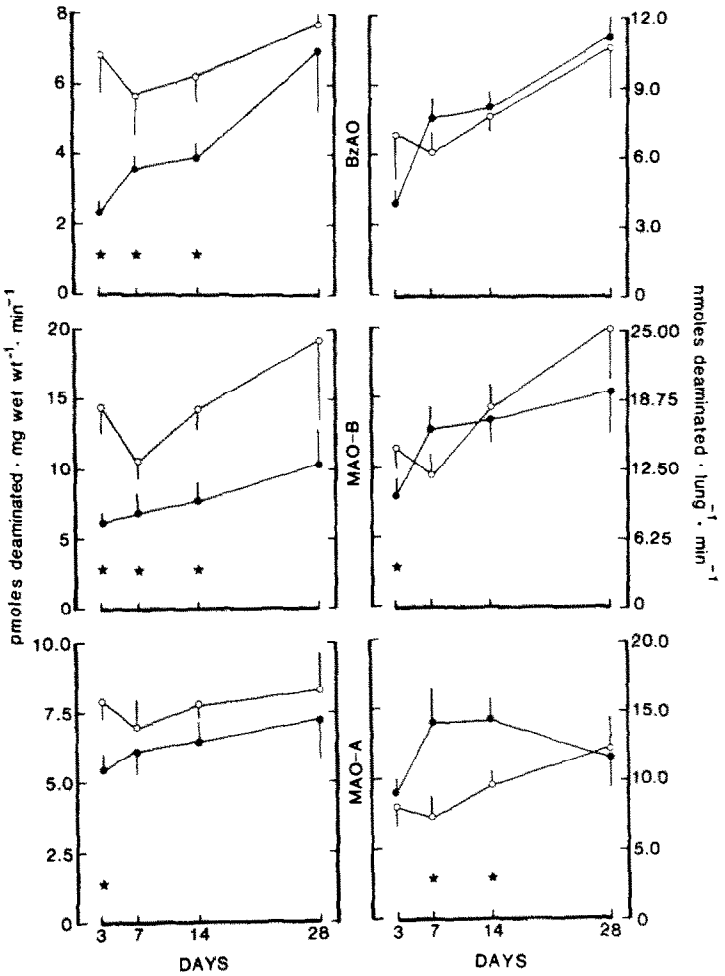

Fig. 2. Effects of bleomycin on the specific activities (left-hand panels) and total activities (right-hand panels) of benzylamine oxidase (BzAO) and monoamine oxidases (MAO-A and MAO-B) in rat lung. Homogenates from control (○——○) and bleomycin-treated (●——●) lungs were first incubated at 37° for 30 min with pargyline (2×10^{-4} M) for BzAO, semicarbazide (2×10^{-4} M) for MAO-B, and an equivalent volume of water for MAO-A, followed by cooling on ice. Remaining activity was then assayed, in triplicate, for 15 min at 37° with benzylamine (20 μM) for BzAO, benzylamine (600 μM) for MAO-B and 5-hydroxytryptamine (400 μM) for MAO-A. Given are mean values ± S.E. Control values were derived from three rats at each point. Belomycin-treated values were derived from four rats (day 3), six rats (day 7), four rats (day 14) and three rats (day 28). Key: (*) significantly different from control, $P < 0.05$.

increase in total lung activity of MAO-A. A likely cause, however, may stem from the fact that bleomycin, like other fibrotic agents, produces an inflammatory condition in lung [1–3, 5], with a change in cell population. For instance, the number of fibroblasts increases [1, 3] and type II pneumocytes proliferate [2, 22, 23]. Fibroblasts are reported to be extremely rich in MAO-A activity [28] and type II pneumocytes contain well defined and numerous mitochondria [29]. Thus, the increase in total MAO-A activity may reflect these or other dynamic changes in the cellular composition of bleomycin-treated lungs. Such an event may not be singular to MAO-A. The reported increases in lung lysyl oxidase [6] showed a similar time-course and may, therefore, be reflective of the same generalized process.

In conclusion, the present work does not support a relationship between BzAO activity of lung and serum with fibrotic lung disease induced by bleomycin. However, total lung MAO-A but not MAO-B activity is elevated in bleomycin-treated rats, although the significance of this observation is, as yet, unknown.

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The reactions of aminobutyrate aminotransferase and ornithine aminotransferase with analogues of ethanolamine *O*-sulphate

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Ethanolamine *O*-sulphate (2-aminoethyl sulphate) is an effective 'suicide' (enzyme-activated irreversible) inhibitor of 4-aminobutyrate aminotransferase (EC 2.6.1.19) [1]. By inactivating the brain enzyme *in vivo* it raises the concentration in brain of the inhibitory neurotransmitter that is the enzyme's substrate. As a result, treated animals are protected against convulsions [2–4]. Ethanolamine sulphate is very polar and would, thus, be expected to penetrate poorly to the target enzyme in brain cells. Oral administration of the compound in drinking water requires large doses and the degree of inhibition of the enzyme and consequent increases in aminobutyrate concentration are not so large as those produced by direct intracerebroventricular injection. We, therefore, wished to prepare analogues of the compound having decreased polarity but retaining the structural elements that make it an effective 'suicide' inhibitor. The inactivation mechanism requires that several features of the parent molecule be retained. The sulphate group performs two functions in mimicking the carboxyl group of aminobutyrate by furnishing an appropriately placed negative charge for binding, and in providing a good leaving group whose elimination generates the inactivating electrophile at the enzyme's active site. The amino group is essential for imine formation with the enzyme's coenzyme pyridoxal phosphate. Of the two protons on the 4-carbon of aminobutyrate only one, the pro-*S*, is labilized in the critical catalytic step [6, 7]. The inhibitory mechanism requires that the corresponding hydrogen in a suicide inhibitor be retained. The pro-*R* 4-hydrogen of aminobutyrate is not labilized and the enzyme's effective use of L-glutamate as substrate shows that bulky groups can be accommodated in this position [8]. Furthermore, compounds with acetylenic and vinyl groups in this position are good inhibitors of the enzyme [9, 10]. Thus, we chose to synthesize analogues of ethanolamine *O*-sulphate substituting the pro-*R* hydrogen with hydrophobic groups (Fig. 1).

The compounds D-alaninol *O*-sulphate (R = CH_3) and D-methioninol *O*-sulphate (R = $-CH_2-CH_2-S-CH_3$) fulfil these requirements and the present paper describes their synthesis by reduction of the appropriate D-amino acid and sulphation of the resulting alcohol. The reactions of these compounds with aminobutyrate aminotransferase are described. As a test of specificity, the reactions of these compounds with ornithine aminotransferase (EC 2.6.1.13) were also examined on the grounds that this enzyme cat-