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EFFECTS OF MONOCROTALINE PRETREATMENT OF RATS ON REMOVAL OF 5-HYDROXYTRYPTAMINE AND NORADRENALINE BY PERFUSED LUNG

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1 The alkaloid, monocrotaline, causes significant pulmonary damage in many species, including the rat. We, therefore, determined whether the inactivation of biogenic amines by perfused lungs of rats was modified by prior treatment of the animals with monocrotaline.

2 Young rats (45 to 50 g) treated for 21 days with monocrotaline (22 μg/ml) in their drinking water developed right ventricular hypertrophy. Treated animals gained weight more slowly and consumed less food and water than control rats that drank tap water. Lungs from monocrotaline-treated animals were heavier and had a higher protein content than control lungs.

3 Isolated lungs from treated animals removed and metabolized 50% less perfused 5-hydroxytryptamine than did controls.

4 The diminished 5-hydroxytryptamine metabolism was probably due to impaired delivery of substrate to intrapulmonary monoamine oxidase (MAO) since MAO activity in 600 *g* supernatant fractions of homogenates of lungs from monocrotaline-treated rats was not different from control values.

5 Pulmonary removal of perfused noradrenaline was decreased about 60% by the 21-day treatment, suggesting that the effects of monocrotaline were somewhat nonspecific.

6 These effects were not caused by monocrotaline directly, since perfusion of lungs from untreated animals with this drug did not alter removal of co-perfused 5-hydroxytryptamine.

7 Reduced pulmonary removal of circulating biogenic amines following pretreatment with monocrotaline may reflect damage to capillary endothelium, which could also affect other metabolic functions of lung.

Introduction

Monocrotaline is a naturally occurring pyrrolizidine alkaloid which produces hepatic and pulmonary lesions in mammals. Grazing animals consume grasses and small plants of several families which contain these toxic alkaloids and the resulting livestock losses are of major economic importance in certain areas of the world. Furthermore, pyrrolizidine poisoning has been reported to occur in man (McLean, 1970).

Administration of monocrotaline to young rats results in damage to lung tissue (Schoental & Head, 1955; Merkow & Kleinerman, 1966; Valdiva, Lalich, Hayashi & Sonnad, 1967), including swelling of pulmonary capillary endothelial cells, thrombosis and lesions of the arterial media. Morphological alterations in pulmonary capillary endothelium produced by monocrotaline are probably associated with changes in the metabolic function of these cells. Since pulmonary endothelial cells are known to remove and metabolize a wide variety of circulating endogenous vasoactive substances as well as many drugs (Vane, 1969; Gillis, 1973; Fishman & Pietra, 1974; Junod, 1975), it was of interest to determine whether monocrotaline alters this function of pulmonary endothelium. For this purpose, we examined the effect of monocrotaline on pulmonary removal and metabolism of perfused 5-hydroxytryptamine (5-HT) and noradrenaline (NA); these amines are taken into pulmonary endothelial cells by a facilitated transport process and metabolized by the lung (Hughes, Gillis & Bloom, 1969; Strum & Junod, 1972; Iwasawa, Gillis & Aghajanian, 1973; Cross, Alabaster, Bakhle

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& Vane, 1974; Nicholas, Strum, Angelo & Junod, 1974).

Methods

Twenty-one-day monocrotaline treatment

Male Sprague-Dawley rat pups weighing 45 to 50 g were placed in plastic cages in groups of four and were allowed food (Purina Laboratory Chow) and water *ad lib.* Drinking water was prepared as follows: monocrotaline stock solution (22 mg/ml) was prepared by dissolving crystalline monocrotaline in 0.2 N HCl and adjusting the pH to 6–7 with NaOH. To make the monocrotaline drinking water, 1 ml of the stock solution was diluted to 1 litre with tap water. Monocrotaline-treated animals drank tap water containing 22 μg/ml monocrotaline, while controls were allowed tap water with the same NaCl concentration as the treated water. All animals were treated for 21 days, during which time body weight as well as food and water consumption were recorded periodically.

^{14}C-biogenic amine removal by perfused rat lung

After 21 days of treatment, rats were anaesthetized by administration of sodium pentobarbitone (50 mg/kg, i.p.) and given 500 u of heparin intravenously to prevent clotting. The pulmonary artery and trachea were cannulated *in situ* and the lungs were carefully removed from the animal and placed in the perfusion apparatus (maintained at 37°C). During surgery and immediately after placement in the perfusion apparatus, lungs were inflated and deflated several times to prevent atelectasis. During subsequent perfusion the lungs were statically inflated with 2 ml of room air. Lungs were perfused in a single-pass (non-recirculating) system at a rate of 10 ml/min with Krebs-bicarbonate perfusion medium (Hughes *et al.*, 1969) at 37°C containing 3.5% bovine serum albumin and aerated with 95% O_2 and 5% CO_2. Perfusion pressure was monitored continuously by means of a Grass Model 7 Polygraph with P23AA Statham pressure transducer.

After 5 min of perfusion with Krebs medium, lungs were perfused with medium containing either [^{14}C]-5-HT or [^{14}C]-NA (each at 0.1 μM) for 10 minutes. Effluent perfusion medium was collected during the last 5 min of perfusion for analysis of ^{14}C-amine and metabolites. Removal of amine was calculated as the difference between the radioactivity of unchanged amine in the perfusion medium and that in the collected effluent. Percentage removal (% R) of perfused amine was calculated as:

$$\%R = \left[\frac{C_{a,i} - C_{a,o}}{C_{a,i}}\right] \times 100,$$

where $C_{a,i}$ and $C_{a,o}$ represent concentrations of ^{14}C-amine in the inflow and effluent perfusion medium, respectively.

To separate ^{14}C-amine and metabolite, 0.5 ml aliquots of lung effluent were passed through columns of Bio-Rex 70 cation exchange resin (sodium form; pH 6.0) after which the deaminated metabolite was eluted from the columns with 2.5 ml of water (Roth & Gillis, 1975). When effluents containing NA were analyzed, columns were subsequently eluted with 3 ml of 2% boric acid to remove unchanged catecholamine and then with 3 ml of 0.2 N HCl to remove *O*-methylated products. The radioactivity in water, borate and acid washes from the columns was determined by liquid scintillation spectrometry.

Preparation of tissue homogenates

Animals were anaesthetized by administration of sodium pentobarbitone (50 mg/kg i.p.). To clear organs of blood, the hepatic portal vein was cannulated and perfused with cold, heparinized (5 u/ml) isotonic saline after cutting the inferior vena cava below the kidneys to allow drainage of the perfused saline. Organs were then removed, weighed and homogenized in 8 volumes of 0.1 M phosphate buffer containing 0.25 M sucrose in a Waring blender, and the homogenates were centrifuged at 600 *g* for 10 minutes. Resulting supernatant fractions were frozen, then thawed and rehomogenized by means of a glass homogenizer when MAO activity and protein (biuret method) was measured.

Determination of monoamine oxidase activity

[^{14}C]-5-HT was used as substrate for determination of monoamine oxidase activity in 600 *g* supernatant fractions of lung and liver homogenates. Reaction mixtures contained 0.1 μmol of [^{14}C]-5-HT in a total of 2 ml of 0.05 M potassium phosphate buffer. Aliquots of 600 *g* supernatant fractions (0.2 ml liver or 0.6 ml lung) were added and the mixture incubated at 37°C for 10 minutes. Deamination of 5-HT by these volumes of tissue supernate was linear during the 10 min incubation period. The reaction was stopped by addition of 0.2 ml of 0.2 M $ZnSO_4$ followed by 0.2 ml of 0.2 M $Ba(OH)_2$. The mixture was then centrifuged at 3000 *g* for 10 min, and the supernatant fraction was analyzed by cation exchange chromatography for [^{14}C]-5-HT and its acid metabolite, [^{14}C]-5-hydroxyindoleacetic acid ([^{14}C]-5-HIAA), as described above.

Statistics

Results were expressed as $\bar{X} \pm$ s.e. mean and were analyzed statistically by Student's *t* test (Snedecor & Cochran, 1967).

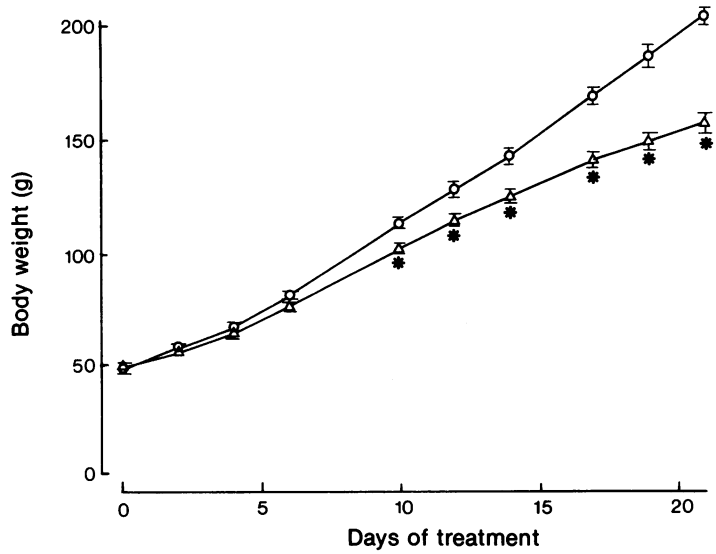

Figure 1 Effect of 21-day monocrotaline treatment on growth of rats. Treated rats (△) were allowed 22 μg/ml of monocrotaline in their drinking water *ad lib* for 21 days beginning at day zero; (○): control rats. Points represent mean values. Vertical lines show s.e. means; n = 37–39 rats per group. *Significantly different from control value of same day ($P \leq 0.05$).

Drugs and isotopes

(±)-[Carbinol-^{14}C]noradrenaline (±)-bitartrate (37 mCi/mmol) and 5-hydroxy[side chain-2-^{14}C]tryptamine creatinine sulphate (56 mCi/mmol) were purchased from Amersham/Searle Corp., Arlington Heights, Ill.; 5-hydroxytryptamine creatinine sulphate complex from Sigma Chemical Co., St. Louis, Mo.; Bio-Rex 70 cation exchange resin (100–200 mesh) from Bio-Rad Laboratories, Richmond, Ca.; bovine albumin powder (Fraction V) from Schwarz/Mann Corp., Orangeburg, N.Y. and monocrotaline from S.B. Pennick and Co., Lyndhurst, N.J.

Results

Effect of monocrotaline pretreatment on:

Growth and food and water intake As shown in Figure 1, monocrotaline-treated animals gained weight more slowly than did controls. At the end of the treatment period control rats weighed 202 ± 4 g compared to 155 ± 4 g for treated animals.

Pretreatment with monocrotaline was associated with decreased water and food consumption. During the 21 days of treatment, control rats consumed a total of 582 ± 19 ml of water, while rats drinking monocrotaline-treated water drank only 379 ± 18 ml (Figure 2a). From the latter figure it was calculated that the total amount of monocrotaline consumed during the study averaged 8.34 ± 0.39 mg per rat.

In the early days of the study, the rate of food consumption of both treated and control animals increased, but after 10 days of monocrotaline treatment food consumption remained constant, while that of control animals increased continuously (Figure 2b). During the 21 day study, controls consumed 314 ± 9 g of food per rat, whereas monocrotaline-treated rats consumed an average of 236 ± 8 g of food per animal.

Organ weights and protein content Table 1 presents the effects of monocrotaline on lung and liver weight. Although livers from treated animals weighed significantly less than controls, the liver/body weight ratio was unaltered by the treatment. The protein concentration of 600 *g* supernatant fractions of liver homogenates was not significantly altered by the monocrotaline treatment. In contrast to liver, the lung/body weight ratio of treated animals was greater than controls and was associated with an increase in the protein content of 600 *g* supernatant fractions of lung homogenates.

Table 2 depicts data from animals used in 5-HT lung perfusion experiments. It can be seen that monocrotaline treatment increased both wet and dried lung weights. Therefore, lung dry weight/wet weight ratio was unchanged. Huxtable, Laugharn & Paplanus (1977a) previously reported that this method of monocrotaline administration produces pulmonary hypertension in Wistar rats, resulting in an increased right heart mass. Data from the present study (Table 2) confirm this finding, as indicated by

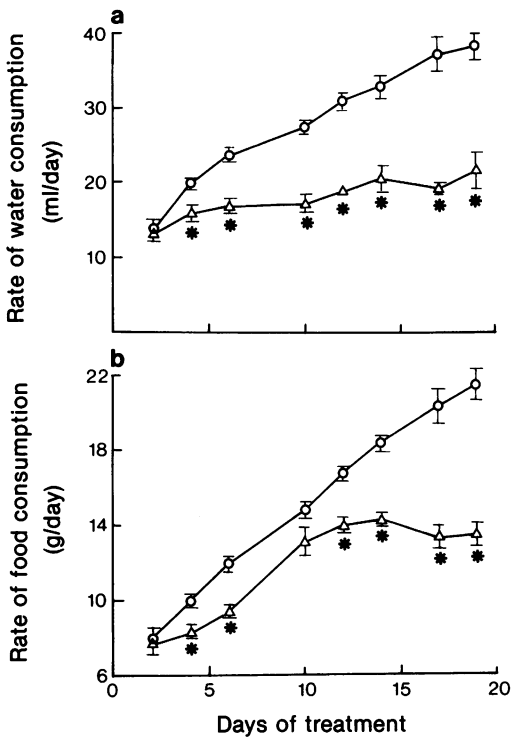

Figure 2 Effect of 21-day monocrotaline treatment on (a) rate of water and (b) food consumption by rats. Rats were allowed tap water (control, ○) or tap water containing 22 μg/ml monocrotaline (treated, △) for 21 days *ad lib.* beginning on day zero. Data points represent mean results from 11 cages containing 1–4 animals per cage. Values are given as food consumption and water consumption; Vertical lines show s.e. mean. *Significantly different from control value of same day ($P \leq 0.05$).

the 70% increase in right ventricle/body weight ratio. In contrast, the weight of the remainder of the heart (left ventricle and septum) was unaffected by monocrotaline treatment (Table 2).

Pulmonary removal of perfused biogenic amines To determine whether pulmonary changes produced by monocrotaline treatment were associated with impaired removal and metabolism of biogenic amines, these functions were assessed in lungs of rats pretreated for 21 days. Mean perfusion pressure of isolated lungs from monocrotaline-treated rats (9.1 ± 1.0 mmHg) did not differ significantly ($P > 0.05$) from that of control lungs (6.1 ± 0.7 mmHg). Removal of 5-HT was decreased in lungs from monocrotaline-treated rats as indicated by increased amounts of unchanged 5-HT appearing in effluent (Figure 3); simultaneously, less 5-HIAA was recovered in the effluent of lungs from treated animals. Table 3 indicates that impairment of 5-HT removal was evident whether data were expressed on a whole organ basis or per g of lung tissue. In fact, the decrease in removal was more marked when expressed per g of tissue, since the lung weight of monocrotaline-treated animals was elevated.

Since both NA and 5-HT may be transported into lung endothelial cells at separate transport sites (Iwasawa & Gillis, 1974), we determined whether monocrotaline pretreatment also influences intrapulmonary NA removal. Data presented in Table 4 indicate that pretreatment of rats with monocrotaline decreased the pulmonary removal of perfused [^{14}C]-NA. Indeed, removal of [^{14}C]-NA is more sensitive to this treatment than that of 5-HT, since, on a whole organ basis, [^{14}C]-NA removal was decreased by 51% (Table 4) whereas removal of [^{14}C]-5-HT decreased by 27% (Table 3).

Figure 3 suggests that lungs from monocrotaline-treated rats metabolize 5-HT more slowly than controls. To determine if this reflected decreased enzyme activity or, alternatively, diminished uptake of 5-HT into lung tissue, we measured the deamination of [^{14}C]-5-HT by 600 *g* supernatant fractions of lung homogenates from rats pretreated with monocrotaline. It can be seen (Table 5) that enzyme activity per mg protein was lower than in similar preparations of control lung. When enzyme activity was expressed

Table 1 Effect of 21 day monocrotaline pretreatment on organ weight and protein content* of rat lung and liver

	Lung		*Liver*	
	Control	*Treated*	*Control*	*Treated*
(Organ wt/body wt) × 10^2	0.59 ± 0.01	1.10 ± 0.12†	4.61 ± 0.20	4.47 ± 0.12
Protein (mg of 600 g supernate protein per g tissue)	58.4 ± 2.4	86.7 ± 5.8†	127.4 ± 6.6	119.5 ± 2.9

* Treated rats drank water containing 22 μg/ml monocrotaline for 21 days beginning at weaning. Results are expressed as $\bar{X}$ ± s.e. mean.; $n = 5$ animals per group. Data from same animals as Table 5.
† Significantly different from control value ($P \leq 0.05$, two-tailed Student's *t* test).

Table 2 Effect of 21-day monocrotaline treatment on total body, lung and heart mass in rats*

	Control	*Monocrotaline-treated*
Body weight (g)	191 ± 7	162 ± 13
Lung wet weight (g)	1.42 ± 0.09	2.26 ± 0.25†
Lung dry weight (g)	0.234 ± 0.016	0.333 ± 0.028†
Lung dry weight/wet weight	0.159 ± 0.004	0.150 ± 0.006
[Rt. vent/bw] × 10^4	7.59 ± 0.41	12.90 ± 2.02†
[(Lt. vent + septum)/bw] × 10^4	24.29 ± 1.42	25.77 ± 1.66
[Rt. vent/(Lt. vent + septum)]	0.318 ± 0.026	0.508 ± 0.048†

* Treated rats drank water containing 22 μg/ml monocrotaline for 21 days beginning at weaning. Lungs from these animals were perfused for 10 min with 0.1 μM [^{14}C]-5-HT prior to weighing. Amine removal data from same animals are presented in Figure 3. Results are expressed as $\bar{X}$ ± s.e. mean; n = 6–7 rats per group.
† Significantly different from control value ($P \leq 0.05$, two-tailed Student's t test, group analysis).

per g of lung or on a whole organ basis, however, no effect of monocrotaline was observed.

Although the liver has been commonly viewed as the primary target organ with regard to monocrotaline toxicity, the ability of liver homogenates to metabolize 5-HT was unaffected by the 21 day monocrotaline regimen we employed (Table 5).

Effects of acute monocrotaline treatment on 5-hydroxytryptamine removal by rat lungs Table 6 presents data from experiments designed to test the effect of acute monocrotaline treatment on pulmonary biogenic amine removal. Perfused lungs from animals injected with monocrotaline 24 or 48 h before perfusion, removed [^{14}C]-5-HT to a degree similar to those of animals injected with saline, regardless of the route of monocrotaline administration. The highest dose used (175 mg/kg) was equal to the LD_{50} (72 h) for monocrotaline in rats (Bull, Culvenor & Dick, 1968). A dose of 300 mg/kg killed all of the animals within 24 h, regardless of route of administration. Livers from animals injected with 100 mg/kg or more monocrotaline, 24 h earlier, consistently appeared very darkened and grossly haemorrhagic. In contrast, livers from rats treated for 21 days with 22 μg/ml monocrotaline in their drinking water were only slightly darker than controls but otherwise appeared normal.

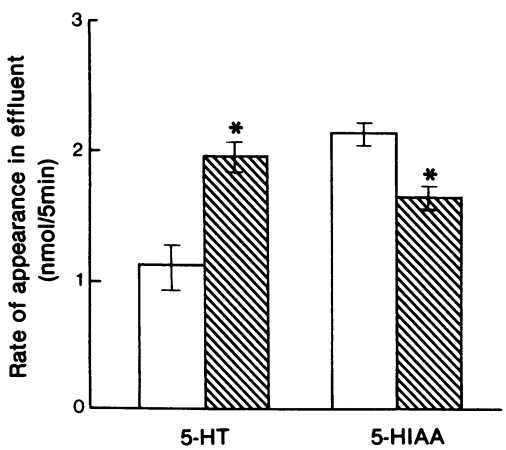

Figure 3 Effect of 21 day monocrotaline pretreatment on removal of perfused [^{14}C]-5-hydroxytryptamine by isolated rat lung. Rat lungs from untreated rats (open columns) or 21-day monocrotaline pretreated rats (arched columns) were perfused with 0.1 μM [^{14}C]-5-hydroxytryptamine (5-HT) as described in Methods. 5-HIAA refers to 5-hydroxyindoleacetic acid metabolite. Vertical lines show s.e. means.; n = 7 for controls, 6 for treated group. *Significantly different from control value ($P \leq 0.05$).

Perfusion of lungs from untreated rats simultaneously with up to 1 mM monocrotaline and 0.1 μM [^{14}C]-5-HT did not alter removal of the amine.

Discussion

The depressed growth rate of rats drinking water containing monocrotaline (Figure 1) agrees with results of other workers using different means of administration (Turner & Lalich, 1965; Merkow & Kleinerman, 1966; Kajihara, 1970). Associated with the observed depression of growth rate was diminished food and water consumption. The effect on water consumption may have been a toxic manifestation of monocrotaline, but could also be explained by the bitter taste and consequent unpalatability of the alkaloid (McLean, 1970). If so, the depressed water intake may have caused the diminished food consumption. However, the results argue against this possibility, since

Table 3 Effect of 21-day monocrotaline pretreatment on removal of perfused [^{14}C]-5-hydroxytryptamine by isolated lung of rat*

	5-Hydroxytryptamine removal (nmol/5 min)		
	Control	*Treated*	*% decrease*
per g (wet) lung	2.57 ± 0.22	1.22 ± 0.14†	53
per g (dry) lung	15.76 ± 1.41	8.11 ± 0.84†	49
per whole organ	3.56 ± 0.17	2.60 ± 0.12†	27
n	7	6	

* Treated rats drank water containing 22 μg/ml monocrotaline for 21 days beginning at weaning. Lungs were perfused at a flow of 10 ml/min with 0.1 μM [^{14}C]-5-HT as described in Methods. Results are expressed as $\bar{X}$ ± s.e. mean.
† Significantly different from control value ($P \leq 0.05$, two-tailed Student's *t* test).

although intake of water was greatly depressed very early in the study, food intake was not severely affected until the tenth day of treatment (Figure 2).

Huxtable *et al* (1977a) studied the effects of this method of monocrotaline administration after various times. Right ventricular hypertrophy was first apparent after 12–14 days of treatment, and this paralleled a sharp increase in protein synthesis (as measured by leucine incorporation) in the right ventricle. Figure 2 indicates that during the initial latent period, there is a decreased rate of food intake, which returns to normal immediately before the initiation of cardiac hypertrophy. In the later days of treatment, when protein synthesis in the heart has been shown to be greater than that of controls (Huxtable *et al.*, 1977a) food intake in the present study was severely depressed.

When placed in the perfusion apparatus, control lungs routinely cleared completely of blood and appeared uniformly white. In contrast, the surface of lungs from monocrotaline-treated rats remained slightly pink after initial perfusion and, in addition,

Table 4 Effect of 21-day monocrotaline pretreatment on removal of perfused [^{14}C]-noradrenaline by isolated lung of rat*

	Noradrenaline removal (nmol/5 min)		
	Control	*Treated*	*% decrease*
per g (wet) lung	0.712 ± 0.026	0.299 ± 0.051†	58
per g (dry) lung	4.60 ± 0.21	1.77 ± 0.29†	62
per whole organ	0.985 ± 0.075	0.480 ± 0.078†	51
n	6	3	

* Treated rats drank water containing 22 μg/ml monocrotaline for 21 days beginning at weaning. Lungs were perfused at a flow of 10 ml/min with 0.1 μM [^{14}C]-noradrenaline as described in Methods. Results are expressed as $\bar{X}$ ± s.e. mean.
† Significantly different from control value ($P \leq 0.05$, two-tailed Student's *t* test).

Table 5 Effect of 21-day monocrotaline pretreatment on monoamine oxidase activity of 600 *g* supernatant fractions of organ homogenates of rats*

	[^{14}C]-*5-HT (nmol) metabolized per 10 min*			
	Lung		*Liver*	
	Control	*Treated*	*Control*	*Treated*
per mg protein	3.15 ± 0.08	2.00 ± 0.15†	7.10 ± 0.67	6.07 ± 0.52
per g organ	185 ± 11	174 ± 17	920 ± 134	722 ± 56
per whole organ	223 ± 29	292 ± 28	8954 ± 1353	5178 ± 707

* Treated rats drank water containing 22 μg/ml monocrotaline for 21 days beginning at weaning. Results are expressed as $\bar{X}$ ± s.e. mean.; $n = 5$ animals per group.
† Significantly different from control value ($P \leq 0.05$, two-tailed Student's *t* test).

Table 6 Effect of acute monocrotaline pretreatment on removal of perfused [^{14}C]-5-hydroxytryptamine by rat lung*

	Treatment			
Route of administration	i.p.	i.p.	i.v.	i.v.
Hours after dose	24	48	24	48
	% removal of [^{14}C]-5-hydroxytryptamine			
Monocrotaline dose (mg/kg)				
0	73.9	64.7	78.3	76.3
100	69.7	67.4	80.7	80.7
175	75.8	59.9	82.9	82.9

* Lungs of rats (120–230 g) injected with monocrotaline as indicated were perfused with 0.1 μM [^{14}C]-5-HT as described in Methods. Control rats were injected with saline. Values each represent data from one set of lungs perfused on the same day as other lungs pretreated similarly.

often had numerous small, dark red foci. This observation is consistent with previous reports of congestion and petechial haemorrhage following monocrotaline administration (Schoental & Head, 1955; Turner & Lalich, 1965; Merkow & Kleinerman, 1966). Interstitial alveolar oedema has also been reported as a result of monocrotaline treatment (Merkow & Kleinerman, 1966; Valdiva *et al.*, 1967; Hayashi & Lalich, 1967). In our study, oedema fluid was not routinely visible upon gross examination of lungs before or after perfusion. Since lung dry weight/wet weight ratio was not altered by monocrotaline treatment, the observed weight increase (Table 2) may be due to increased cell mass resulting from one or more previously described monocrotaline-induced cellular alterations, such as congestion, giant pneumocyte formation, epithelialization of alveoli, thickening of arterial media, increase in mast cell population and cytoplasmic thickening of endothelial cells (Schoental & Head, 1955; Turner & Lalich, 1965; Merkow & Kleinerman, 1966; Valdiva *et al.*, 1967; Stötzer, Herbst, Reichl & Köllmer, 1972).

Monocrotaline in a single dose, similar to the total amounts of drug consumed by rats in the present study, has been shown to produce marked hepatic alterations, including severe haemorrhagic discolouration and mottling (Schoental & Head, 1955). In our study, however, there was no change in liver/body weight ratio (Table 1) and the liver surface of treated rats appeared only slightly darker than controls. Furthermore, hepatic protein concentration (Table 1) and monoamine oxidase activity (Table 5) were unaffected by the administration of monocrotaline in drinking water. Huxtable *et al.* (1977a) have shown that a slightly lower dose of monocrotaline (20 μg/ml) in the drinking water produces a time-dependent medial hypertrophy of the pulmonary arteries of male Wistar rats. Associated with this is a doubling of right ventricular and pulmonary arterial systolic blood pressures. These authors pointed out that chronic administration of monocrotaline in the drinking water has the advantages of producing mild right ventricular hypertrophy without detectable inflammatory changes and without marked hepatic changes. This method, therefore, offers a convenient tool for the study of the lung lesions produced by pyrrolizidine alkaloids.

Lungs from control rats in this study removed 76.3 ± 3.7% of perfused 0.1 μM 5-HT in a single pass through the pulmonary vasculature. Endothelial cells from capillaries and small vessels, as mentioned earlier, are probably the site of this removal process (Hughes *et al.*, 1969; Strum & Junod, 1972; Iwasawa *et al.*, 1973; Cross *et al.*, 1974) and probably also contain the MAO which oxidatively deaminates circulating 5-HT (Iwasawa *et al.*, 1973). Ingestion of monocrotaline, which causes marked alterations in pulmonary capillary endothelium, diminished by about 50% the capacity of intact lung tissue to remove perfused 5-HT (Table 3). Elevated circulating 5-HT has been shown to increase pulmonary arterial pressure *in vivo* (Ozdemir, Kusajima, Wax & Webb, 1972). Therefore, the impaired ability to remove 5-HT and perhaps other vasoconstrictor substances from the circulation may contribute to the pulmonary hypertension produced by monocrotaline.

Electron micrographic studies have demonstrated monocrotaline-induced proliferation of endothelial cells, the cytoplasmic content of which can increase until endothelium markedly protrudes into the lumen of pulmonary vessels (Merkow & Kleinerman, 1966). Thus, the decrease in pulmonary 5-HT removal in monocrotaline-treated rats probably cannot be explained simply on the basis of a decreased endothelial surface area available for removal.

We measured monoamine oxidase activity in 600 *g* supernatant fractions of lung homogenates to deter-

mine whether the decreased conversion of 5-HT to 5-HIAA by lungs from monocrotaline-treated rats (Figure 3) resulted from diminished enzyme activity. The specific activity (activity per mg protein) of this enzyme was reduced because of the increased protein concentration in lung (Table 1). However, the total metabolizing capacity of lung remained unaffected (Table 5). Thus, the diminished metabolism of 5-HT by monocrotaline-treated lungs (Figure 3) probably results from impaired transport of substrate to the site of metabolism, rather than decreased enzyme activity.

Available evidence (Hughes *et al.*, 1969; Iwasawa *et al.*, 1973; Nicholas *et al.*, 1974) indicates that NA, like 5-HT, is also taken up by lung by a facilitated transport mechanism but probably at a site on the endothelial cell surface different from that of 5-HT (Iwasawa & Gillis, 1974). It was of interest, therefore, to determine if monocrotaline treatment specifically affected the site of removal of 5-HT, or if the site of NA transport was also affected. Table 4 indicates that monocrotaline administration decreased the removal of NA and 5-HT (per g of lung) to about the same degree. These data suggest that the damage to endothelium produced by monocrotaline is somewhat nonspecific.

Young rats treated with monocrotaline develop pulmonary hypertension. This has been demonstrated by direct measurement of pulmonary arterial pressure (Stötzer *et al.*, 1972; Huxtable *et al.*, 1977a) as well as by the observation of associated right ventricular hypertrophy (Turner & Lalich, 1965; Hayashi & Lalich, 1967; Kajihara, 1970). In our study, rats drinking water containing 22 μg/ml monocrotaline developed right ventricular hypertrophy within 21 days of treatment, indicating the presence of pulmonary hypertension. Thus, we infer that pulmonary hypertension was associated with significantly decreased pulmonary removal of biogenic amines. In contrast, pulmonary hypertension resulting from cardiac valvular disease in man is associated with elevated removal of 5-HT and NA by lung (Gillis, Cronau, Greene & Hammond, 1974). However, monocrotaline produces histologically demonstrable damage to pulmonary endothelium, whereas endothelial cell injury does not necessarily accompany pulmonary hypertension secondary to valvular disease. In addition, there are many experimental differences between the present study and the earlier study in man.

Acute administration of monocrotaline leads predominately to liver damage in rats (McLean, 1970), whereas little lung involvement has been reported. To determine if acute treatment produces changes in the capacity of lung to remove perfused biogenic amines, rats were given a single dose of monocrotaline (i.p. or i.v.) and killed 24 h or 48 h later. While livers from these rats were darkened and mottled, the lungs were normal in appearance although slightly heavier than controls. Removal of 5-HT by perfused lungs from these animals was similar to controls (Table 6). Thus, acute monocrotaline administration, which does not produce severe alterations in pulmonary endothelial cells, also fails to alter biogenic amine disposition.

The toxic effects of pyrrolizidine alkaloids are thought to be due to their pyrrole metabolites (McLean, 1970) which are thought to be produced primarily by the liver and reach the lung in venous blood. Indeed, such metabolites have been shown to cause pulmonary histological lesions similar to those produced by monocrotaline administration to rats (Butler, Mattocks & Barnes, 1970; Chesney, Allen & Hsu, 1974). Since toxicity probably depends on hepatic metabolism, it is not surprising that perfusion of untreated lungs with medium containing monocrotaline did not alter pulmonary 5-HT removal.

In addition to their ability to alter circulating levels of biogenic amines, lungs from many species are active in the removal and/or metabolism of prostaglandins, peptide hormones, drugs and environmental pollutants (Orton, Anderson, Pickett, Eling & Fouts, 1973; Gillis & Roth, 1976; Bingham, Niemeier & Dalbey, 1976; Roth, Roth & Gillis, 1977). It is important, therefore, to evaluate the effect of monocrotaline on these other functions of lung.

Recent reports document the first cases of pyrrolizidine poisoning in humans in the United States (Stillman, Huxtable Consroe, Kohnen & Smith, 1977; Huxtable, Stillman & Giaramitaro, 1977b). Two individuals, in separate incidents, were exposed to large quantities of pyrrolizidine alkaloids. In one individual, there was resultant gastrointestinal bleeding secondary to portal hypertension, and the other developed veno-occlusive disease of the liver and portal hypertension. It has been suggested that in the United States, pyrrolizidine poisoning may be a public health problem, and may be an 'iceberg disease' in that continual consumption of small quantities may lead to compromised liver or lung functions which nevertheless remain subclinical (Huxtable *et al.*, 1977b). A recent report on the occurrence of pyrrolizidines in certain samples of honey (Deinzer, Thomson, Burgett & Isaacson, 1977) reinforces this suggestion.

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