Renal and Pulmonary Alterations Induced in Rats by a Single Injection of Monocrotaline.* (31748)

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Platelet thrombosis of alveolar capillaries was observed in rats 24 hours after a single injection of monocrotaline(1). It was further shown that one injection of the alkaloid could produce right ventricular hypertrophy and thickening of the pulmonary artery in association with alterations of the alveolar structure such as hyaline thrombosis of the capillaries, edema, hemorrhage and necrosis(2). These findings suggest that administration of monocrotaline can predispose to pulmonary hypertension in rats by altering the alveolar capillaries, which eventually causes cor pulmonale.

The present study was undertaken to establish 1) if one administration of monocrotaline would produce a series of pulmonary arterial lesions which might be associated with long-standing pulmonary hypertension, and 2) whether or not this alkaloid can affect the vascular system in other organs.

Materials and methods. Thirty-four male suckling rats of Sprague-Dawley strain were selected from 6 litters for experimentation. They were weaned to a commercial diet† on the 21st neonatal day. Two hundred mg of crystalline monocrotaline (196-8°C melting point) was dissolved in 0.6 ml of 1 N HCl. Five ml of distilled water was added, the pH adjusted to 7.0 and finally diluted to a volume of 10 ml. The solution was administered by subcutaneous injection into the interscapular region.

Twenty-two rats 14 days of age were injected with a dose of 120 mg of monocrotaline/kg of body weight. The rats were permitted to die or become moribund before sacrificing so that the autopsies could be performed. In the control group, 12 rats were injected with 0.1 ml of saline and sacrificed on the 40th day.

The weight gains and gross alterations of the viscera were recorded in each case. Samples of the lung, heart, liver, kidney, spleen, pancreas and lymph nodes from the mediastinum, mesentery, renal pelvis and hepatic hil-

TABLE I. Survival and Gross Findings of Rats After a Single Injection of Monocrotaline.

Survival, days	No. examined	Heart: Weight gain, g Maximum	Heart: Weight gain, g Final	Heart: Weight g	Heart: Weight %/B.W.*	Heart: Cross-sectional area in %† L. vent.	Heart: Cross-sectional area in %† R. vent.	Heart: Cross-sectional area in %† Septum	Pulmonary hemorrhage Massive	Pulmonary hemorrhage Petechial
20-28	7	73.7 ±17.6	64.1 ±17.8	.75 ±.17	.75 ±.07	39.6 ±5.1	29.9 ±4.3	30.4 ±3.7	2	5
31-34	4	93.3 ±14.5	71.8 ±7.9	.89 ±.20	.75 ±.16	38.7 ±4.9	31.0 ±3.8	30.5 ±1.9	0	4
41-47	10	121.8 ±25.8	107.6 ±26.5	1.06 ±.16	.77 ±.09	37.5 ±5.0	34.6 ±4.4	31.5 ±3.6	0	10
40 (control)	12	221.3 ±18.3	221.3 ±18.3	.74 ±.04	.35 ±.02	54.5 ±3.9	15.5 ±2.7	29.2 ±2.8	0	0

* Maximum body weight.

† Hearts were cut transversely at their widest dimension. After processing and staining slides with the heart specimens, the sections were inserted into a "Filmac 100 Reader Printer" so that the myocardium was magnified 13 times. Outlines of ventricles and septum were then traced onto paper of uniform thickness. Approximation of the cross-sectional area of both ventricles and septum was estimated by weighing the paper of the ventricular silhouettes.

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† Lab-Blox, Allied Mills Inc., Chicago, Ill.

um were saved for histological examination.

Results. Twelve control rats grew satisfactorily for 40 days and did not show any changes on gross inspection. Twenty-one of 22 test rats either died or became moribund 20 to 47 days after injection. Toxic signs in these rats were characterized by labored breathing, a decrease in body weight and hypothermia. At autopsy, every rat exhibited some evidence of pulmonary hemorrhage (Table I). Pleural effusion was seen in 4 cases and ascites in 6 instances.

TABLE II. Histological Response in Lungs and Kidneys to a Single Injection of Monocrotaline.

Survival, days	No. examined	Lung						Kidney			
			Arterial								
		Capillary thrombosis	Intimal hyalinization	Med. musc. hypertrophy*	Thrombosis	Necrotizing arteritis	Glomerular alteration† +	++	+++	Arterial thickening	Arterial thrombosis
20-28	6	6	5	5	1	0	6	0	0	2	0
31-34	4	4	4	4	2	0	2	1	1	2	1
41-47	10	10	10	10	9	6	0	7	3	9	7
40 (control)	10	0	0	0	0	0	0	0	0	0	0

* Medial muscular hypertrophy.

† Magnitudes of glomerular alterations: (+) Necrosis was seen in less than 20% of total estimated glomerular area.
(++) More than 20%, but less than 40%.
(+++) More than 40%.

The histological alterations observed in the lungs are listed in Table II. The pulmonary vessels were found to be normal in 10 control rats. Alveolar capillary thrombosis associated with edema, hemorrhage and focal necrosis appeared in all test rats. Lymphocytes, mast cells and siderophages were noted in the swollen alveolar walls. Peribronchial lymphatics were dilated and filled with proteinaceous material which contained extravasated erythrocytes. Mediastinal lymph nodes were grossly discolored with blood and found on histological inspection to have hemosiderosis and erythrophagocytosis. It was noticed that the arterial lesions occurred more frequently in arterioles and small to medium muscular arteries and appeared to be greater in rats which survived for longer intervals. Necrotizing arteritis was seen in 6 of 11 rats which survived more than 41 days.

The heart was significantly enlarged in all test rats except in one animal which died on the 28th day (Table I). The hypertrophy and dilatation interestingly was limited to the right ventricle (Fig. 4). The magnitude of ventricular hypertrophy was evaluated by estimating the cross sectional areas of ventricles and septum at the widest cardiac dimension (Table I). By these criteria it can be seen that all 3 groups exhibited some evidence of right ventricular hypertrophy. Histological examination revealed small foci of myocardial degeneration in the right ventricle or septum of 3 cases and focal lymphocytic infiltration in 5 instances. These histological lesions were estimated to occupy less than 5% of the total right ventricular and interventricular septal areas.

Kidneys of test rats were discolored brown and petechial hemorrhage appeared in the cortex. Ferrocyanide reaction disclosed deposits of ionizable iron in the proximal convoluted tubules. Glomeruli were swollen and occasionally contained proteinaceous material positive for ferric iron. Thrombosis associated with subendothelial hyalinization or even necrosis was noted in the glomerular capillaries and afferent arterioles. The epithelial

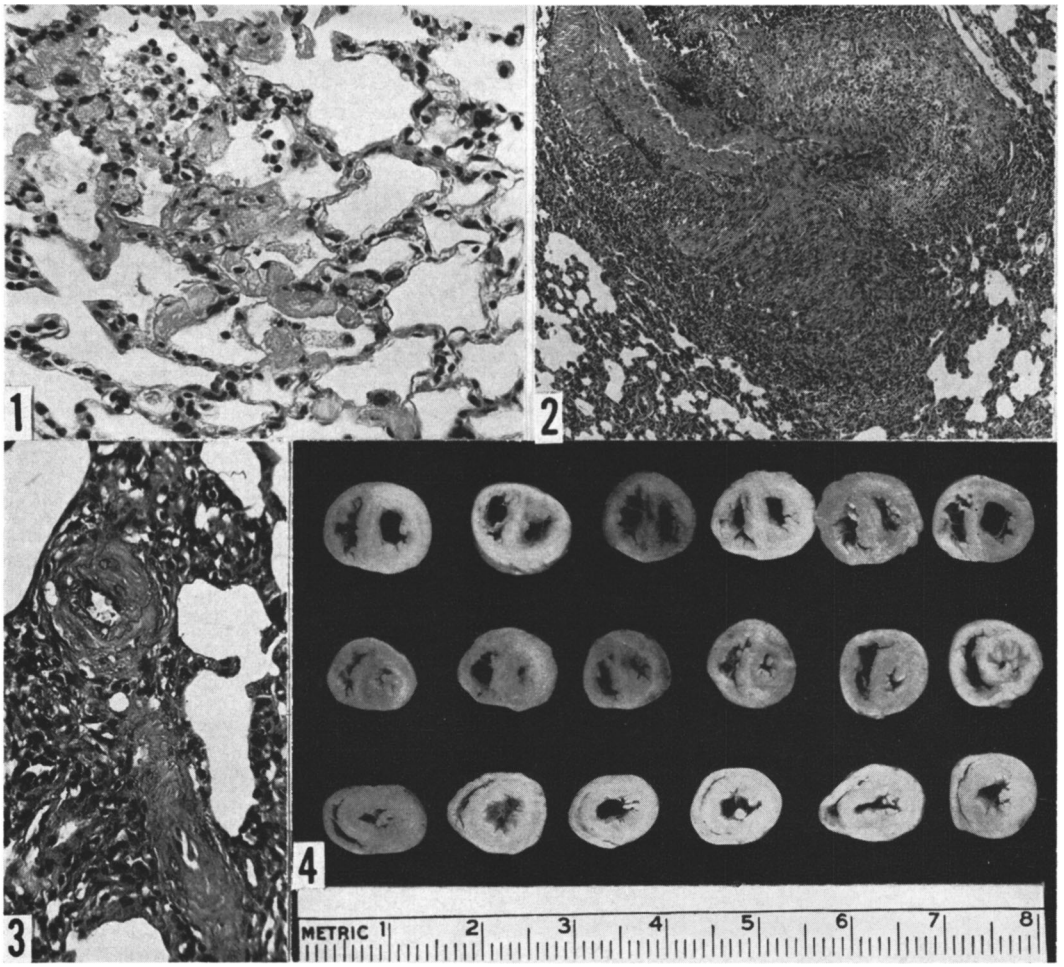

FIG. 1. An area of the lung from a rat that died 28 days after injection of monocrotaline. Capillary thrombosis and focal necrosis of alveolar walls are seen. Hematoxylin-eosin: 140 ×.

FIG. 2. Necrotizing arteritis in a rat 43 days following a single injection of monocrotaline. Hematoxylin-eosin: 45 ×.

FIG. 3. An area of the lung from a rat 46 days following a single injection of monocrotaline. Intimal thickening and medial muscular hypertrophy of muscular arteries are seen. Hematoxylin-eosin: 150 ×.

FIG. 4. Various grades of right ventricular hypertrophy in transverse sections of hearts. All the specimens are oriented with right ventricle on left side. Six specimens on top row are from test rats 41 to 47 days after an injection of monocrotaline; 6 in middle row are from test rats 28 to 34 days after injection and 6 at the bottom are from control rats on the 40th day.

and mesangeal cells of the tufts underwent either cytoplasmic vacuolation or karyolysis. Leukocytic infiltration of glomeruli was rare. Thickening of the interlobular arteries with intimal hyalinization and medial hypertrophy was seen in 13 cases, 8 of which were associated with thromboses. Hemosiderosis and erythrophagocytosis could also be seen in the renal lymph nodes. Mesenteric arteritis was seen in one rat and thickening of the intima and media in 2 other instances. Similar arterial lesions have been observed in rats with generalized hypertension(3).

There were 6 examples of hepato-nodular regeneration and 7 of megalocytosis as described by Bull(4). Various degrees of anisonucleosis of hepatocytes and hemosiderosis of Kupffer cells were seen in most instances. The hepatic hilar lymph nodes were positive for hemosiderin.

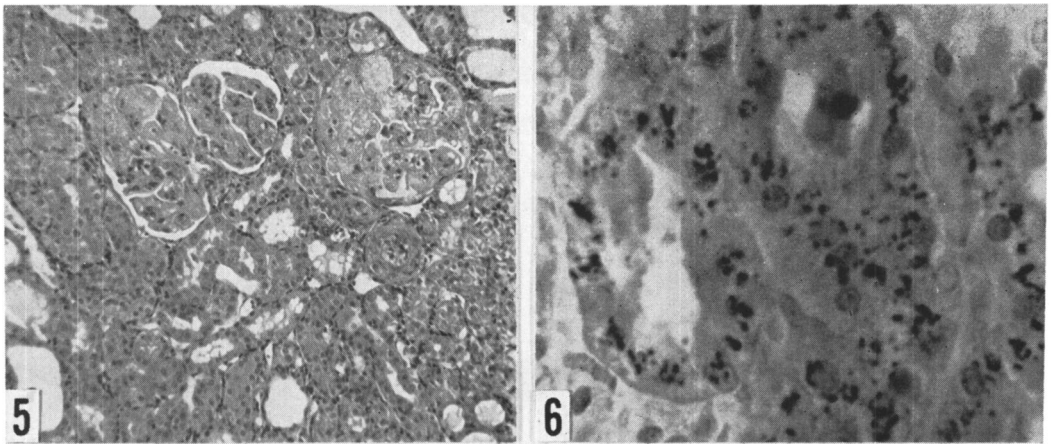

FIG. 5. An area of the kidney from a rat 46 days following a single injection of monocrotaline. The 3 glomeruli show necrotic changes in various grades. Hematoxylin-eosin: 100 ×.

FIG. 6. A microscopic section of the kidney from a rat 46 days following a single injection of monocrotaline. Hemosiderin granules are demonstrated in proximal tubular epithelial cells. Ferrocyanide reaction: 380 ×.

Discussion. The present experiment demonstrates that a single injection of monocrotaline can induce pulmonary arterial injury in rats equal to that seen after long-term feeding or repeated injections of this alkaloid(5-7). The histological character of the arterial lesions and the association of right ventricular hypertrophy suggests the presence of pulmonary hypertension. Capillary thromboses, focal necrosis and edema of the alveolar walls might be responsible for the increased resistance to blood flow. The focal degeneration and inflammation of the myocardium which appeared in 8 of 21 test rats did not seem to be the primary cause of right ventricular hypertrophy because the area of involvement was considered to be minimal and it occurred in a minority of test animals.

It was shown that monocrotaline can also injure the kidneys in addition to the liver and lungs. Glomerular necrosis has been observed following the feeding of *Crotalaria spectabilis* to adult rats(8). In agreement with previous observations, the glomerular lesions consisted of hyaline thrombosis, swelling and focal necrosis of the tufts. This shows that monocrotaline can induce a somewhat similar histological injury in widely separated tissues, in which a transfer of gases or solutes is known to occur. The consistent appearance of renal hemosiderosis in test rats implies that intravascular hemolysis is associated with monocrotaline intoxication. The thromboses in the lung and kidney may be a factor which contributes to erythrocyte destruction.

The mode of action of monocrotaline in producing the pulmonary and renal alterations is not clear. However, it should be pointed out that a single injection of alkaloid induces histological alterations after a specified latent period. Peculiarly, a latent period of several months elapsed before a single oral administration of Senecio-alkaloids produced a chronic hepatic injury(9). Culvenor *et al* have reported that heliotrine, an allylic pyrrolizidine ester can react with nucleophilic reagents, such as benzyl mercaptan *in vitro* (10). This suggests that monocrotaline as well as allied pyrrolizidine alkaloids probably injure in target cells by interacting with the nucleophilic portions of the nucleic acids, proteins or some other cellular components.

Summary. A single subcutaneous injection of monocrotaline produced various grades of pulmonary arterial injury including necrotizing arteritis in rats. The arterial lesions were presumed to be sequelae of long standing pulmonary hypertension as manifested by right ventricular hypertrophy. It was noted that monocrotaline could also produce a renal hemosiderosis, as well as glomerular necrosis in association with hyaline thrombosis in the glomerular capillaries and afferent arterioles.

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Attachment of Modified Erythrocytes to Phagocytic Cells in Absence of Serum.* (31749)

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Phagocytosis by macrophages *in vitro* requires in most cases the presence of serum in the medium or pretreatment of the particulates with serum or serum fractions(1,2,3). The overall phagocytic process can be shown in some instances to involve discrete attachment and ingestion steps(3,4). The first step embodies the "recognition" of "foreign" particulates or of endogenous aged or damaged body constituents, while "self" components are spared(5). Because of the large amount of prior knowledge concerning erythrocytes as well as their size and availability, these particles were chosen for the study of this early phagocyte-particle interaction. It was found that erythrocytes modified by various procedures attached to macrophages, but not to granulocytes, in the absence of added serum factors. This interaction was temperature dependent and was blocked by prior trypsinization of the macrophages. Preliminary communications of this work have appeared previously(6,7).

Materials and methods. Macrophages were obtained by washing the peritoneal cavity of NCS mice (Rockefeller University colony) with 4 ml buffered saline (9 vol 0.85% NaCl solution plus 1 vol M/15 potassium phosphates pH 7.3), and permitted to attach to coverglasses(8). Mice of both sexes weighing 20-25 g were used.

Horse peripheral blood leucocytes (70-80% polymorphs) were obtained after sedimentation of the erythrocytes and centrifugation of the leucocyte-rich plasma. Heparin or citrate were used as anticoagulants. The resulting leucocyte pellets were washed twice and resuspended in buffered saline before attachment to coverglasses.

Sheep red cells were obtained from the Animal Blood Centre, Syracuse, N. Y. Mouse, horse, rabbit and human cells were collected from heparinized blood. Erythrocytes were washed 4-6 times in buffered saline before and after the following treatments. Each of the agents was dissolved in buffered saline pH 7.3. Except when otherwise noted the conditions given apply to mouse erythrocytes at 22°C. *Glutaraldehyde:* 10 volumes of 1-5% glutaraldehyde were added to red cells and incubated for 24-48 hours at 5°C. Red cells obtained from the horse, mouse, rabbit and human were processed in the same fashion. These cells were stable at 5°C for at least 2 months. *Tannic acid*(9) was used at 25 μg/ml for 20 minutes. *Periodate*(10) M/500 was used at pH 6.1 for 10 minutes in the dark. *Polylysine*(11) obtained from Yeda, Rehovot, Israel, was applied at 5-10 μg/ml for 10 minutes. *Colloidal silica* ("Syton 200," lot no. 5039, obtained from Mon-

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