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# EFFECT OF D-PENICILLAMINE ON THE FETAL LUNG OF RATS: A MODEL FOR CONGENITAL EMPHYSEMA*

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The pathogenesis of pulmonary emphysema is characterized by permanent abnormal enlargement of the distal air spaces due to destruction of their walls, the etiology of which is yet unclear. Since the structural and elastic properties of the lung depend primarily on the connective tissues, collagen and elastin, they may play an important role in the pathogenesis of emphysema[1].

Copper is an essential component of lysyl oxidase which is important in the cross-linking of collagen and elastin. It has been demonstrated that the cross-linking of collagen and elastin in mice is impaired by penicillamine, and that the lung structure and function of these mice also reveal abnormalities[2,3]. The aim of this study is to evaluate the effects of penicillamine on the development of lung tissue in the fetal rat.

## Material and Methods

Pregnant albino rats, with a mean weight of 250 g (range between 220 to 300 g) were used in this study. Their gestational ages were determined by the presence of a vaginal plug on the first day of gestation, and abdominal palpation after the tenth day.

Six animals received a daily dose of either 300 mg (low-dose) or 400 mg (high-dose) of D-penicillamine (DPA) in their drinking water during their last six days of gestation. Two rats were used as controls and received no drug. Between the twentieth to twenty-first days of gestation the fetuses in the four animals of the study group (two high-dose and two low-dose) and in one of the controls were delivered by cesarean section. The remaining two animals, who received low-dose DPA during their gestation, and one control, were given a normal diet containing no DPA for three weeks after delivery, which included the lactation

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period. At the end of this period their offspring were sacrified and evaluated in the same manner as the newborn animals. The serum copper levels were determined in all the animals, and it was demonstrated that these levels had decreased in the study group (83 and 87 mg/dl in low-dose; 69 and 71 mg/dl in high-dose) more than in the control group (95 and 98 mg/dl). However, the serum copper levels were the same both in the study group and the control group three weeks after delivery (92.93 and 95 mg/dl).

The offspring were weighed and then killed by decapitation. Their lungs were removed, weighed, embedded in paraffin blocks, stained with hemotoxylin-eosin and examined using light microscopy.

All the tissues were evaluated for the presence of bronchomegaly, cystic alveoli, atelectasis, vascular aneurism, perivascular loose connective tissue and pulmonary hemorrhages. The findings were evaluated statistically according to Student's *t* test.

## Results

It was observed that D-penicillamine treatment during gestation caused marked growth retardation in the offspring. Although the lung weight to body weight ratio in the newborn animals did not differ between the low-dose and control groups, it was significantly decreased in the high-dose group (Table I).

TABLE I: Effect of D-Penicillamine on Lung Tissue (Macroscopic Findings)

| | DPA During Gestation | Body Weight (gm) | Lung Weight (gm) | LW / BW × 100 |
|---|---|---|---|---|
| At the end of gestation | 1. None (n:7) | 9537 ± 390 | 258 ± 64 | 2.69 ± 0.57 |
| | 2. Low-dose (n: 10) | 8378 ± 483 | 233 ± 56 | 2.78 ± 0.63 |
| | 3. High-dose (n: 8) | 4897 ± 579 | 94 ± 15 | 1.92 ± 0.29 |
| In the third week | 4. None (n: 8) | 15083 ± 1482 | 356 ± 53 | 2.43 ± 0.48 |
| | 5. Low-dose (n: 6) | 13034 ± 1614 | 226 ± 42 | 1.75 ± 0.34 |
| | Statistical comparisons | | | |
| | 1-2 | $p < 0.001$ | $p > 0.05$ | $p > 0.05$ |
| | 1-3 | $p < 0.001$ | $p < 0.001$ | $p < 0.01$ |
| | 2-3 | $p < 0.001$ | $p < 0.001$ | $p < 0.005$ |
| | 4-5 | $p < 0.05$ | $p < 0.001$ | $p < 0.01$ |

LW : Lung weight

BW. Body weight

The hematoxylin-eosin stained histologic sections of the lungs in the study group showed bronchomegaly, cystic alveoli, atelectasis, vascular aneurism, perivascular loose connective tissue, and small hemorrhagic foci. Resumption of a normal diet in the animals of the study group for a period of three weeks did not restore the lung tissue to normal (Table II; Figs. 1, 2).

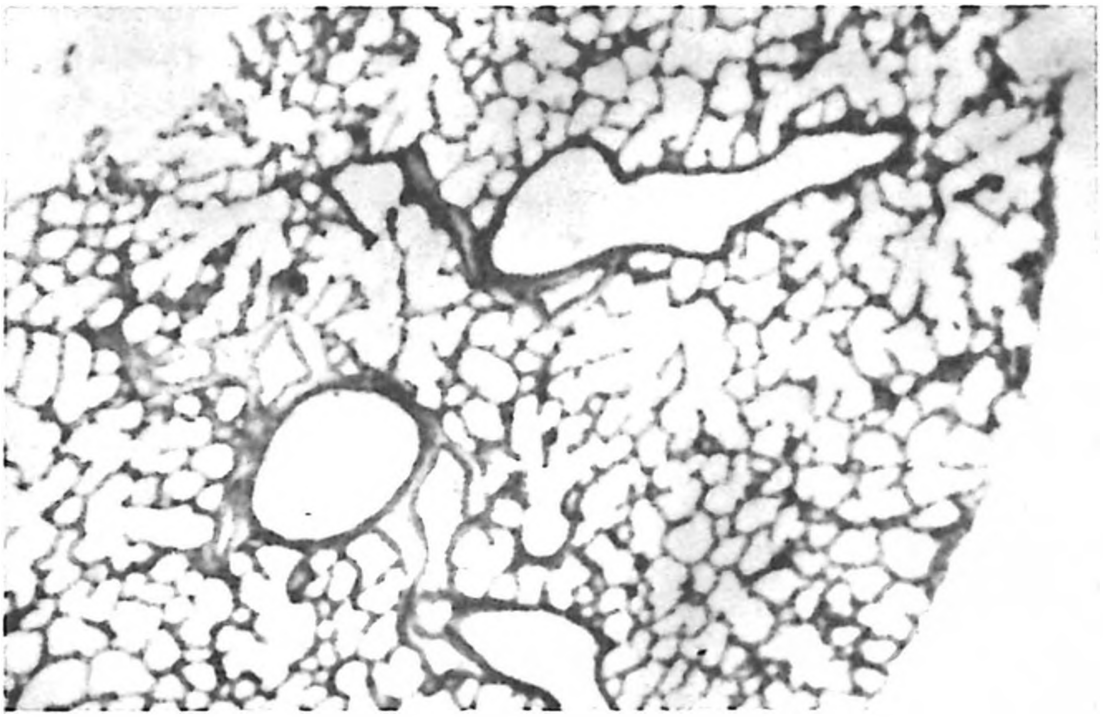

Fig. 1: Bronchomegaly, large irregular alveolar ducts and alveoli in DPA treated animals.

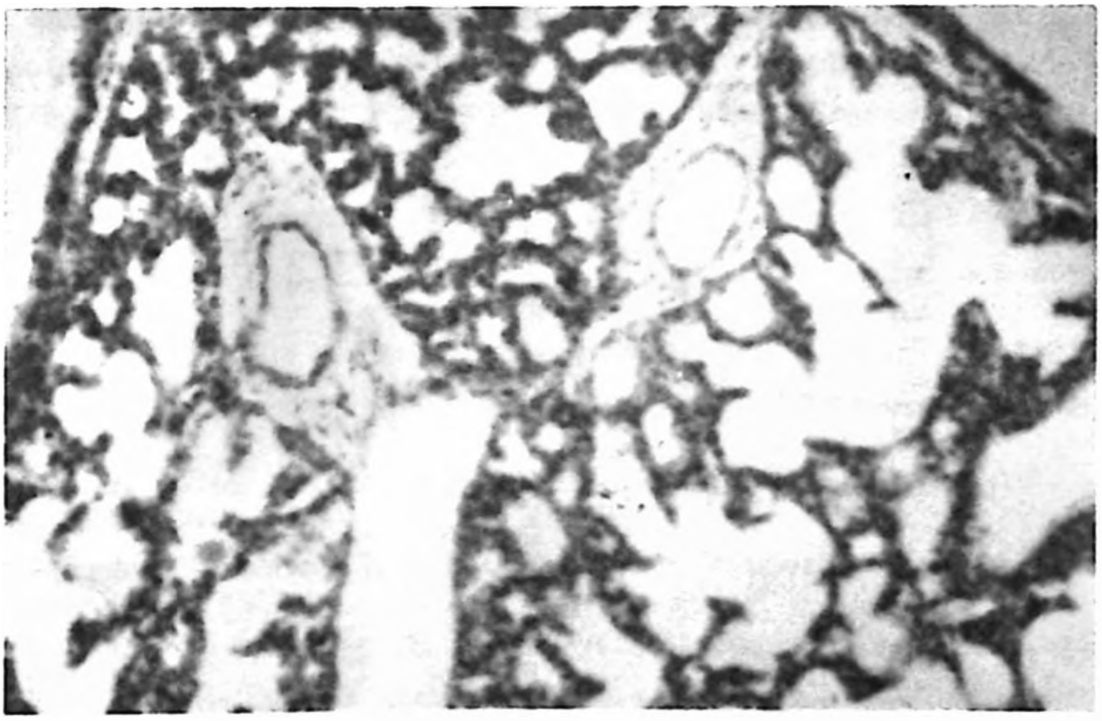

Fig. 2: Perivascular loose connective tissue in the lung.

TABLE II: Effect of D-Penicillamine on Lung Tissue (Microscopic Findings)

| | DPA During Gestation | Bronchomegaly | Cystic Alveoli | Atelectasis | Vascular Aneurism | Perivascular Loose Connective Tissue | Hemorrhage |
|---|---|---|---|---|---|---|---|
| At the end of gestation | 1. None (n: 7) | 0% | 0% | 0% | 0% | 0% | 0% |
| | 2. Low-dose (n: 10) | 40% | 40% | 40% | 20% | 35% | 50% |
| | 3. High-dose (n: 8) | 80% | 60% | 50% | 85% | 90% | 80% |
| In the third week of gestation | 4. None (n: 8) | 0% | 0% | 0% | 0% | 0% | 0% |
| | 5. Low-dose (n: 6) | 20% | 40% | 20% | 20% | 20% | 30% |
| Statistical comparisons | | | | | | | |
| | 1-2 | $p < 0.05$ | $p < 0.05$ | $p < 0.05$ | $p < 0.05$ | $p < 0.05$ | $p < 0.005$ |
| | 1-3 | $p < 0.001$ | $p < 0.001$ | $p < 0.005$ | $p < 0.001$ | $p < 0.001$ | $p < 0.001$ |
| | 2-3 | $p < 0.05$ | $p < 0.05$ | $p > 0.05$ | $p < 0.005$ | $p < 0.005$ | $p < 0.05$ |
| | 4-5 | $p < 0.05$ | $p < 0.05$ | $p < 0.05$ | $p < 0.05$ | $p < 0.05$ | $p < 0.05$ |

## Discussion

This study demonstrates that copper-deficient rats born of dams fed with DPA added to their diet during gestation had enlarged alveolar ducts and alveoli. Resumption of a normal diet, free of DPA, fed to the animals during the first three weeks of life did not restore the alveolar structure to normal.

Copper is an essential component of lysyl oxidase, the enzyme which catalyses the oxidative deamination of lysine residues in the precursor proteins of elastin and collagen[4]. Lysyl oxidase plays a key role in the cross-linking of collagen and elastin[4]. Thus, one would expect a deficiency of copper to result in a metabolic defect such as a decrease of elastin or collagen concentration. The main pathological feature in the lung tissues of the fetal rats was an alteration in the structure of the alveoli which we observed, and which has been reported by other investigators. The cause of this abnormality might result from lysyl oxidase deficiency[2-5]. Since the addition of copper appeared to restore the structure of parenchymal elastin, but did not reverse the structural abnormality of the lung, one must conclude that copper exerts a critical metabolic function in the development of the lung.

## Summary

D-Penicillamine administered orally in daily doses of 300 mg or 400 mg to pregnant rats during the last six days of gestation caused marked growth retardation, a decrease in the lung weight to body weight ratio and histological features characteristic of pulmonary emphysema in the newborn animals studied. It was also demonstrated that a normal diet given to the dams for a period of three weeks after delivery did not restore the lung tissue of the offspring to normal.

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