

Maternal nicotine exposure during pregnancy and development of emphysema-like damage in the offspring

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Abstract The aim of this investigation was to determine whether nicotine exposure (1 mg/kg body mass/d) during pregnancy and lactation contributes to the rupturing of alveolar septa in the lungs of neonatal rats. These rats received nicotine only via the placenta and mother's milk. The results show that maternal nicotine exposure interferes with elastic tissue formation. It also interferes with alveoli formation and causes the development of emphysema-like lesions. It is therefore suggested that maternal nicotine intake from smoking during pregnancy and lactation may interfere with lung development and maturation to an extent that increases susceptibility to emphysema.

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Early postnatal morphogenesis of the rat lung is characterised by a number of qualitative tissue, cellular, sub-cellular, and molecular remodellings. The developmental events contribute in different ways to the transformation of an immature lung into a structurally and functionally competent organ.¹ Interference with the process of lung development may thus have an adverse influence on the metabolic, structural and functional development of the lung, its effectiveness and resistance against disease.

A study by Collins *et al.*² demonstrated that maternal smoking adversely modifies fetal lung growth. It is not known which of the many components of cigarette smoke is responsible for this. However, Luck and Nau³ clearly illustrated that maternal smoking resulted in the accumulation of considerable amounts of nicotine in fetal blood and mother's milk. Since nicotine is rapidly absorbed by the infant⁴ and since it accumulates in the respiratory tract after absorption,⁵ it is possible that this alkaloid may have a detrimental effect on lung growth and development. Maritz⁶ found that maternal nicotine

exposure during pregnancy had an irreversible adverse effect on carbohydrate metabolism in the neonatal rat lung. He also showed enhanced cellular multiplication in the lungs of these rat pups.⁷ Recently Maritz and Woolward⁸ demonstrated a change in the type I/type II cell ratio from 1,58:1 for normal rat pups to 0,22:1 for the nicotine-exposed rat pups, as well as thickening of the blood-air barrier in the latter group.⁹

Smoking is a major risk factor associated with the development of emphysema,¹⁰ a disease characterised by erosion of the alveolar walls.¹¹ This erosion is the result of deterioration of the components of the lung connective tissue framework,¹² and changes in the composition of the ground substance responsible for the stability of this framework.¹³ Since nicotine interferes with elastogenesis¹⁴ and other metabolic pathways⁷ of the neonatal lung, as well as with the cellular development thereof,⁸ it is possible that maternal nicotine exposure may induce a sequence of events which causes emphysema. The aim of this study was to determine whether maternal nicotine exposure during pregnancy and lactation, in doses comparable with the intake of habitual smokers,⁹ does induce emphysema-like lesions in the lungs of the offspring.

Materials and methods

Animals

White virgin female rats (Wistar descendants) of 200 - 250 g were used in the present investigation and were fed a stock diet (Epol rat cubes) throughout the experiment. All animals received food and tap water as required. Room temperature was kept at 22°C and day-night cycle of 12 hours was maintained. We maintained our own breeding programme for both control and experimental animals. The length of gestation averaged 22,5 days. Animals were mated overnight and were afterwards randomly assigned to control and experimental groups.¹⁴ Whether mating had occurred was determined by the presence of mating plugs and sperm in vaginal smears. The day of the appearance of the vaginal plug was designated day 0 of gestation. Nicotine exposure commenced on day 7 of gestation to avoid nicotine interference with blastocyte implantation and initial embryonic growth; it was continued until the pups were weaned 3 weeks after birth. The dams received single daily doses of 1 mg nicotine/kg body weight subcuta-

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neously until the birth of the litter, whereafter nicotine was given intraperitoneally.¹⁴ This procedure was followed to ensure that nicotine reached the fetus or suckling rats only after its absorption into the blood. Control animals received normal saline instead of nicotine. Animals were killed by decapitation on predetermined days, 24 hours after their last exposure.

Lung tissue samples of 7-, 14- and 21-day-old control and nicotine-exposed rat pups were used. Only sections of the inferior aspect of the left lung lobe were used for this investigation.

Tissue preparation

The lung tissue samples were collected for histochemical determination of elastic tissue according to Verhoeff's method as described by Culling.¹⁵ Lung tissue sections of 4 μ m were stained for 20 minutes with Verhoeff's stain. A 2% ferric chloride solution was used to differentiate elastic fibres. After rinsing in water and 95% alcohol to remove iodine, they were counterstained with Van Gieson's stain. The samples were then dehydrated, cleared and mounted in DPX for investigation of tissue elastin status. Haematoxylin and eosin (H and E) stains were performed on those tissue samples assigned for light microscopic investigation of lung alveolar structure.¹⁵ Fixation was not done under pressure in order to prevent rupture of the septa because of stress. For each age group of the control and nicotine-exposed animals, 9 rat pups from 3 different litters were used, i.e. a total of 3 pups from each litter.

Radial alveolar count

The radial alveolar count method of Emery and Mithal¹⁶ as adjusted by Cooney and Thurlbeck¹⁷ was used. The lung tissue of 3 rats from 3 different litters was used and counts were made of 10 fields per animal to give a total of 30 fields for each age group.

Statistical analyses

Statistical analyses of differences between the groups were carried out by means of the unpaired *t*-test. A probability level of $P < 0,05$ was designated significant in this study. Experimental data were compared with control data only. Results were recorded as means \pm standard deviations.

Results

Elastic tissue

When Figs 1 and 2 are compared, it appears as if Fig. 2 is more magnified and therefore represents a smaller cross-section of the lung than Fig. 1 does. This, however, is because of structural differences between the control and nicotine-exposed neonatal rat lungs. The fact that the nuclei of the cells in Figs 1 and 2 are the same size is a clear indication that these figures are of the same magnification ($\times 1\,000$) and thus represent the same cross-sectional area in both lungs.

Only data on 14-day-old animals were included as no further observable changes in elastic tissue occurred between days 14 and 21.

The results show apparently equal quantities of elastic tissue in the lungs of the both the 14-day-old control and nicotine-exposed rat pups. However, more elastic tissue fibres occur in the septa of the control lungs and these are more evenly distributed throughout the lungs of the control rats than in the lungs of the nicotine-exposed rat pups.

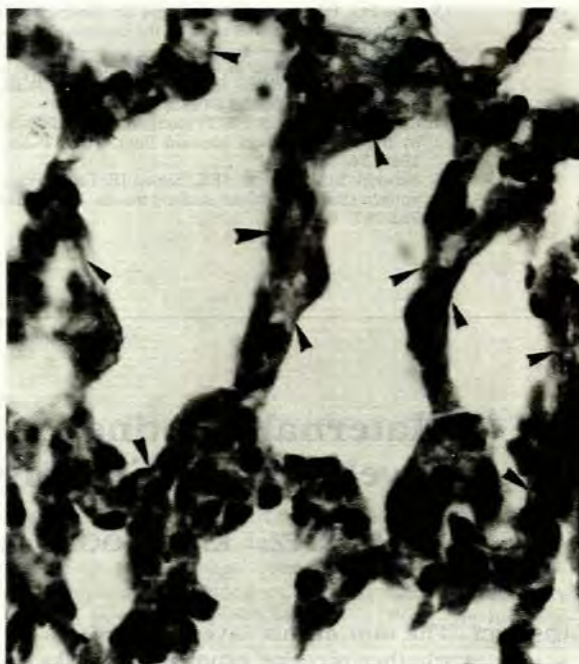


FIG. 1. Section of lung tissue ($\times 1\,000$) of 14-day-old control rat pups illustrating elastic tissue distribution. Elastic fibres (arrowhead) occur in all alveolar septa.

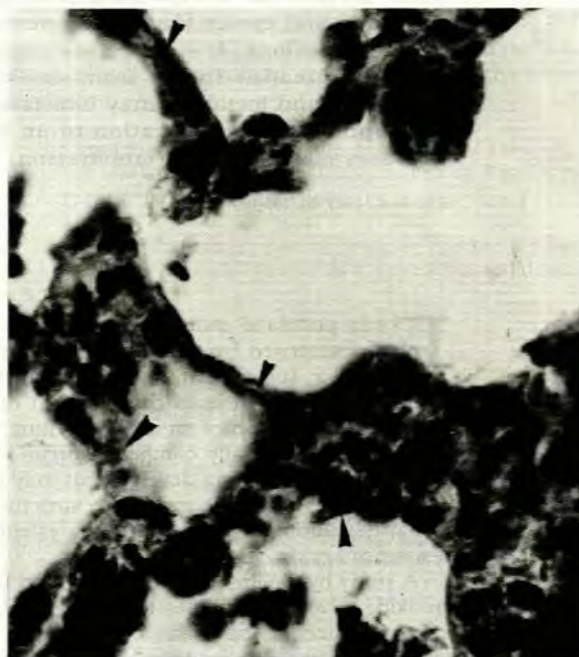


FIG. 2. Section of lung tissue ($\times 1\,000$) of 14-day-old nicotine-exposed rat pups illustrating elastic tissue distribution. Elastic fibres (arrowhead) occur, but not in all alveolar septa.

Alveolar structure

No damage to the lungs of the 7-, 14- and 21-day-old control rat pups occurred (Figs 3, a, b and c). Alveolar damage was not evident in the lung samples of the 7-day-old nicotine-exposed rat pups (Fig. 4 a).

However, on days 14 and 21 after birth, the septa ruptured in the lungs of the nicotine-exposed rat pups. Irregular and enlarged air spaces were also seen in these

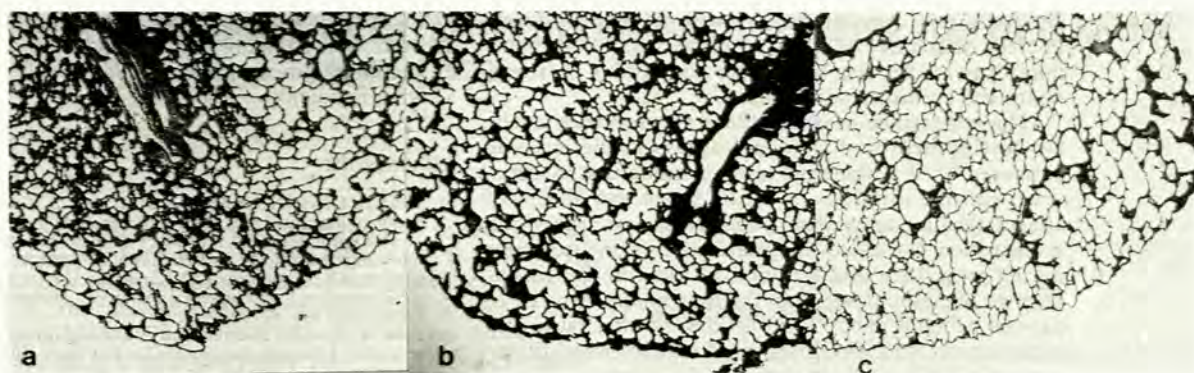


FIG. 3. Lung tissue sections (x 100) of (a) 7-day-old, (b) 14-day-old and (c) 21-day-old control rat pups.

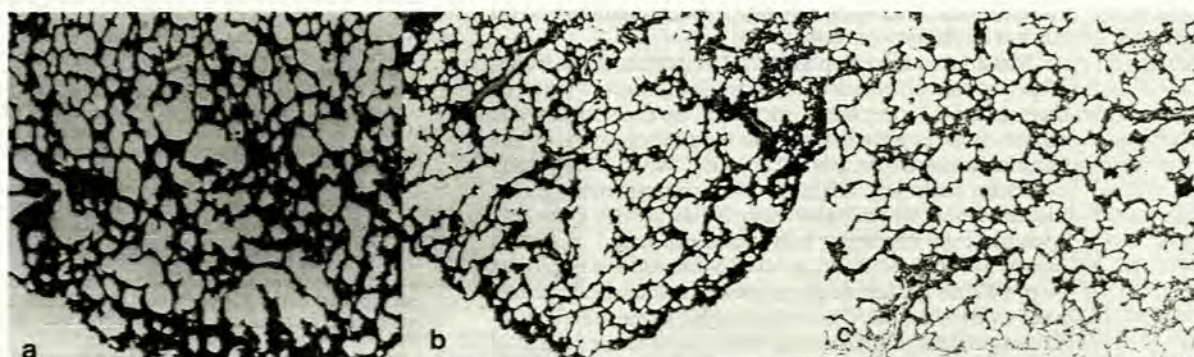


FIG. 4. Lung tissue sections (x 100) of (a) 7-day-old, (b) 14-day-old and (c) 21-day-old control rat pups.

neonates (Fig. 4, b and c). Since the lungs were not fixed under pressure, the rupturing of the septa was not due to physical stress exerted on the lungs.

Radial alveolar count

The radial alveolar count of the control and nicotine-exposed animals increased significantly between days 14 and 21 ($P < 0,05$) (Table I). However, the radial count of the lung tissue of the neonatal rats exposed to nicotine was always lower than that of the control animals ($P < 0,001$). On day 7 the count of the control lungs was 47,44% higher than that of the nicotine-exposed lungs of animals the same age. On day 14 the difference was 43,85% and on day 21 it was 46,16%.

TABLE I. Effect of maternal nicotine exposure on the radial alveolar count of neonatal rats

Age (d)	Control* (N = 30)	Nicotine† (N = 30)	P (control v. nicotine-exposed)
7	7,23 ± 0,20	3,80 ± 0,10	<0,001
14	7,23 ± 0,13	4,06 ± 0,31	<0,001
21	8,60 ± 0,10	4,63 ± 0,076	<0,001

* Control: day 14 v. day 21: $P < 0,001$.

† Nicotine: day 14 v. day 21: $P < 0,05$.

The effect of maternal nicotine exposure was the same for all lungs from the various litters under investigation.

Discussion

Although various factors can cause pulmonary emphysema, smoking is recognised as a major risk factor in most

emphysema patients.¹⁸ Certain biochemical links between cigarette smoking and pulmonary emphysema were suggested. It is widely believed that the destructive changes associated with emphysema are mediated largely by unrestrained proteolytic activity in lung connective tissue.¹² It is also argued that the increased elastolytic activity is partly due to the release of elastolytic proteases by cells in the lungs.¹⁹ Smoking causes several cell types, known to contain or synthesise elastolytic proteases, to increase in number in the lungs. These include pulmonary alveolar macrophages²⁰ and neutrophils.²¹ Tobacco smoke is also a rich source of oxidising agents which could inactivate protease inhibition.¹¹ These findings therefore imply that the lung protease-antiprotease balance may be impaired by smoking and thereby induce pulmonary emphysema. The component in smoke, and the mechanism by which this substance induces emphysema, is, despite all the research done, not yet known. However, the one compound most probably responsible for the harmful effects on the developing organism is the alkaloid, nicotine.²² Animal experiments that used nicotine instead of tobacco smoke show that exposure to nicotine results in a lower fetal weight and increased perinatal mortality; nicotine is therefore a major factor in the cause of recognisable perinatal manifestations.^{23,24}

The most important primary cause of generalised emphysema is, in all probability, the loss of mechanical stability of the connective tissue framework in lung parenchyma.¹⁵ The differentiation of the interstitial fibroblasts is furthermore intimately associated with alveolar formation through the synthesis and secretion of connective tissue elements.²¹ Elastin, in particular, plays an important role in alveolarisation.²⁵ It is also known that collagen is required for the development of the normal pattern of airway branching during morphogenesis of embryonic lungs,²⁶ and it is possible that there is a similar requirement for the branching of new alveo-

lar structures.²⁷ Any interference with the normal development or stability of this connective tissue framework may thus render the lung more susceptible to stress injury and eventually emphysema.

The appearance of elastic fibres also precedes alveolar development and these are always at the alveolar entrances.^{28,29} It is therefore not surprising that interference with the synthesis of elastic tissue also adversely affects alveolar formation.³⁰ The results of a previous investigation illustrated that maternal nicotine exposure during pregnancy and lactation resulted in the virtual absence of elastic tissue from the lung parenchyma of 7-day-old neonatal rats.¹⁴ Even on day 14 after birth, the organisation and distribution of elastic tissue fibres appeared different from those of the controls (Figs 1 and 2). This clearly indicates that maternal nicotine exposure during fetal and neonatal lung development interferes with the ability of the fibroblasts to synthesise elastic tissue and thus develop a stable framework for further development to maturity.

Maturation also involves alveolarisation of the lungs. The fact that alveolarisation of rat lungs occurs between days 4 and 13 after birth, more or less the period when elastic tissue is absent from the lung parenchyma of the nicotine-exposed rat pups,¹⁴ implies that alveolarisation could be adversely affected. The present investigation illustrates that the radial alveolar count, which correlates with the total number of alveoli in the lung,¹⁷ is significantly lower for the lungs of the nicotine-exposed rat pups (Table I). Although no clear structural differences were evident on day 7 after birth, the lower radial alveolar count clearly implies that there were fewer alveoli in the nicotine-exposed lungs than in the lungs of the control rats pups. It is therefore conceivable that this delay in and uneven distribution of elastic tissue synthesis and deposition by the fibroblasts at a phase of lung development which corresponds with alveoli formation, initiated the development of the emphysema-like lesions observed in the 7- (Table I), 14- and 21-day-old nicotine-exposed rat pups (Figs 4, b and c).

However, emphysema is not always associated with a decrease in the quantity of connective tissue in the lungs.²⁶ It is associated rather with impaired quality of the elements of the connective tissue framework.³¹ Although this investigation offers no direct evidence in support of this, the deterioration of the lungs between days 8 and 21 after birth, despite the presence of elastic tissue in the lung parenchyma on day 14, implies that the quality thereof was lower than in the lungs of the control animals.

Although it is not possible directly to extrapolate findings from rats to humans, the response of the rat lung to maternal nicotine exposure cannot be ignored. It has been demonstrated that maternal smoking results in a much higher incidence of lung disease in offspring. Epidemiological studies seem to suggest that maternal smoking may make the offspring more susceptible to emphysema.²² The nicotine intake as a result of smoking may influence the development of the lungs of the human fetus and neonate.

In conclusion, it appears that maternal nicotine exposure during pregnancy and lactation interferes with rat lung development to such an extent that emphysema-like lesions occur in the lungs of the offspring. This also implies that maternal nicotine intake during pregnancy may affect fetal and neonatal lung development to such an extent that the lungs of the offspring offer less resistance to foreign substances and are thus more susceptible to respiratory diseases such as emphysema. Furthermore, these results imply that the chewing

of nicotine-containing gum during pregnancy in an effort to quit smoking may also have an adverse effect on the development of neonatal lungs and contribute to a higher incidence of respiratory diseases.

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