Histol Histopath (1988) 3: 215-222

Histology and Histopathology

Maternal smoking during pregnancy affects neuroendocrine cells in the neonate hamster lung

Ingegerd M. Keith[1] and John R. Cary[2]

[1]Department of Comparative Biosciences, School of Veterinary Medicine and [2]Department of Wildlife Ecology, University of Wisconsin, Linden Drive, Madison, WI., USA

Summary. Primigravid Syrian golden hamsters were exposed in a Walton smoking machine to the smoke from either weak or strong cigarettes for 10 minute periods, 4 times a day from the 3rd to 14th (2nd last) day of pregnancy. Control hamsters were either similarly restrained in a Walton machine equipped with an unlit cigarette, or were not placed in the machine or restrained.

Examination of the progeny in the first 6 days of life showed changes in density indices of grouped pulmonary neuroendocrine (NE) cells (neuroepithelial bodies, NEB) that were related to *in utero* exposure to maternal smoking. Argyrophil NEB were more numerous, larger, and contained more cells at birth among neonates whose mothers smoked the strong cigarette (2.45 mg nicotine and 36.8 mg tar) during pregnancy. This suggests a dose-related effect as the weak cigarette (0.37 mg nicotine and 33.8 mg tar) group did not show such changes. However, some of the changes described did not last through 3 or 6 days of age. The stress resulting from restraint alone also appeared to increase argyrophil NEB indices. Lung tissue volume fraction was increased in the weak cigarette group over all other groups at birth and 3 days; this suggests that low nicotine has the strongest pharmacological effect on lung tissue growth.

The medial thickness of pulmonary arterioles was unchanged by either treatment; this provides morphometric evidence that chronic pulmonary hypertension was not present.

We could not determine whether the increased NEB indices were caused by increased stainability, by activation of resident reserve cells, or by actual mitosis.

Key words: NEB - APUD - Lung - Neonate hamster - Smoking

Offprint requests to: Ingegerd M. Keith, Ph.D., Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive West, Madison, WI 53706, USA

Introduction

In utero exposure to maternal smoking in humans has been linked to increased cancer risk during adult life (Everson, 1980). The risk of developing lung tumors appears to be significantly increased with maternal smoking, particularly among smoking males (Correa et al., 1983). In mice transplacental exposure to benzo-a-pyrene, a highly mutagenic component of cigarette smoke, induces lung and other cancers (Nikonova, 1977) and transplacental exposure of hamsters to cigarette smoke condensate likewise increases tumor incidence (Nikolov and Chernozemsky, 1979). Furthermore, children of mothers who smoked during and after pregnancy have a higher incidence of respiratory disorders (Harlap and Davies, 1974; Rantakallio, 1978; Fergusson et al., 1981), whereas no significant correlation was found with paternal smoking (Fergusson et al., 1980). Among the few favorable responses to maternal smoking is a report on reduced incidence of neonate respiratory distress syndrome (Curet et al., 1983). The present report examines transplacental effects of maternal smoking throughout pregnancy on pulmonary neuroendocrine (NE) cells in neonate hamsters. This cell type (or its stem cell) is likely the precursor of the highly malignant small-cell carcinoma of the lung and the less malignant bronchial carcinoid (Bensch et al., 1968). The former occurs predominantly among smokers, and shows increased incidence with cigarette dosage (Auerback et al., 1975). The pulmonary NE cells are classified as being of APUD (amine precursor uptake and decarboxylation) type (Pearse, 1969). They furthermore belong to the diffuse neuroendocrine (peptidergic) system (Polak and Bloom, 1979). This constitutes a third portion of the autonomic nervous system, which is intimately involved in local regulation of bodily functions via its biogenic amines and polypeptide hormones. We wished to test the hypothesis that *in utero* exposure to maternal smoking affects the pulmonary NE cell system in the progeny. Extensive

reviews of the endocrine lung (Becker, 1984) and the NE cells of the lung (Cutz, 1982) have been recently published.

Materials and methods

Cigarette smoke inhalation

Primigravid Syrian golden hamsters (Harlan Sprague Dawley strain) were placed under moderate restraint in a Walton horizontal smoking machine (Guerin et al., 1979) 4 times daily from the 3rd to the 14th (2nd last) day of pregnancy. Test subjects inhaled mainstream smoke containing 0.37 or 2.45mg nicotine and 33.8 or 36.8 mg tar per cigarette respectively (filterless standardized cigarettes: 3A1 and 2R1, Tobacco and Health Research Institute, Kentucky). Subjects in one control group "sham smoked" using an unlit cigarette, whereas subjects in another control group were unrestrained and did not sham smoke. Water and food were given ad libitum and all hamsters were maintained under the same constant environmental conditions throughout the experiment. At 0.5-5 hr, 3 days and 6 days, neonates were sampled from each litter (n = 108), killed with intraperitoneal pentobarbitol sodium, and weighed. Experimental groups and sample sizes are outlined in Table 1. The time period 0.5-5 hr post partum will hereafter be referred to as "at birth", the low nicotine cigarette (3A1) as "weak" and the high nicotine cigarette (2R1) as "strong".

To determine if the adult hamsters were appropriately dosed by the smoking machine, blood nicotine levels were measured in 8 additional sham controls and in 2 groups of 6 hamsters each, smoking the 2 cigarette strenghts. Nicotine levels within 20 min of smoking were dose dependent (Keith, 1988), and similar to those reported in human smokers (Isaak and Rand, 1972; Seyler et al., 1984).

Histological Methods

The lungs of all neonates were fixed in Bouins solution for 12 hr, and all lobes from each lung were embedded together in paraffin at +53°C after a 6-hr processing cycle. The lobes were arranged such that near-sagittal sections were obtained (one per lobe) which would yield representative portions of all airway types both proximal and distal to the hilus. Sections of 5 μm thickness were stained with the Grimelius argyrophil method (Grimelius, 1968). To achieve optimal results the argyrophil reaction was compared with control slides, known to stain well, of which 1 was included in each batch. If the control slide did not demonstrate optimal reaction, the staining procedure was repeated on a new batch of slides. The Grimelius stain specifically reacts with endocrine cells (Figs. 1, 2) and nerve tissue. The argyrophil substance has not been identified, but acid sialoglycopeptides (Vasallo et al., 1971) have been suggested.

In addition, adjacent sections were stained with an elastin stain (Miller, 1971) for section surface area tracing and vascular media identification (see below), and with hematoxylin and eosin for tissue fraction analysis.

Morphometric Methods

The numbers of individually occurring neuroendocrine cells (NEC) and clusters of cells (neuroepithelial bodies, NEB) that displayed argyrophilia were counted on the sections of all lobes from each hamster, and the surface area (mm^2) of these sections was determined. NEC and NEB numbers were then expressed relative to unit surface area and this value was divided by the lung tissue volume fraction for that individual. In addition, the mean numbers of argyrophil cells per NEB section were recorded by counting cells seen in near-sagittal NEB sections. Only cells displaying a nucleus surrounded by stained cytoplasm were counted. These calculations provided neuroendocrine (NE) cell indices, reflecting relative densities of NEC, NEB, or cells per NEB.

Further, the width and height of argyrophil NEB were measured in half of the hamsters equally representing each treatment group (n = 57 hamsters, 4 NEB per hamster). This was done using an eyepiece filar micrometer, on near-sagittally sectioned NEB, all similarly oriented in a longitudinal fashion in bronchi and bronchioles (Fig. 1, insert). The width was measured at the widest point from one side to the other and paralllel to the basal lamina. The height was measured at the highest point and from the basal lamina to the lumen. Again, only NEB with discernible nuclei were considered. From these data, the area of each NEB section was calculated assuming an elliptical shape, using the formula width/2 × height/2 × π.

To determine whether transplacental exposure to maternal smoking affected pulmonary arteriolar smooth muscle, or altered lung tissue fraction relative to airway lumen, the following 2 morphometric techniques were used.

The thickness of the muscular media of cross sectional arterioles, ranging between 50 and 100 μm in outer diameter (OD), was measured at 4 equidistant points on 10 transversely sectioned vessels per lung. The media, outlined by the inner and outer elastic laminae, was measured with a calibrated eyepiece filar micrometer, and the OD was similarly measured at 2 perpendicular sites per vessel. The double medial thickness (DMT) was calculated relative to the mean OD of each vessel (DMT/OD) in μm, constituting the medial thickness index.

Lung tissue fraction relative to airway lumen (bronchi through alveoli) was determined by the use of a Weibel grid superimposed on projected tissue sections, and an Intelligent Systems computerized morphometry system. A total of 126 points were analyzed in 3 random fields per hamster using a linear magnification of 230x. The tissue fraction was expressed as percent of total points, constituting an index reflecting change of lung tissue volume relative to change in luminal (airway) volume.

Statistical Methods

Within each of the three age groups there were four treatment groups: namely, no smoking controls, sham controls, and hamsters smoking the weak or the strong cigarette. Since the same litter was represented at all three sampling times, the time specific responses are not independent of each other, and are here considered to be a type of repeated measures. The experimental design (Table 1) contains 13 litters, 32 littermate groups, and 108 individual neonates. Because of relatively small variations within litters and large variations between litters, the littermate group was chosen to be the sampling unit. Thus, littermate group averages were calculated from individual means, and used in the final analysis. Statistical analysis utilized repeated measures analysis with a single grouping factor. Statistically significant differences were identified with 1-way analysis of variance for each variable within each age and Duncan's Multiple Range Test was used to describe where the significant difference occurred. All differences described in text are significant at the $P \leqslant .05$ level unless otherwise indicated.

Results

The density index of NEB detected with the argyrophil method was increased at birth with the strong cigarette compared to no smoking and the weak cigarette (Table 2). However, there was no difference between the weak or the strong cigarette and sham smoking and no differences were noted between any groups at 3 and 6 days.

The number of cells per sectioned NEB that were positive with the argyrophil stain tended to be increased with the strong cigarette compared to all other groups at birth (Table 3). This number was also increased with the strong cigarette at 3 days compared to the no smoking group, but did not differ from sham smoking or the weak cigarette at that time. The number of argyrophil NEB cells was likewise higher compared to no smoking and the weak cigarette at 6 days, but was not different from the sham smoking group.

The argyrophil NEB size measurement data (Table 4) indicate that the NEB area tended to be larger ($p = 0.064$) with the strong cigarette at birth, whereas no size changes were noted at 3 and 6 days. The density of NEC did not show any significant trend and is thus not reported here.

The lung tissue volume fraction was elevated with the weak cigarette at birth and 3 days only (Table 5), and there was no difference between the other groups at any time. The medial thickness of pulmonary arterioles (DMT/OD) was not affected by maternal smoking at any age and did not differ between ages. The pooled average for all groups was 0.16 ± 0.04 SE.

In addition, body weights did not differ between groups at birth and 3 days (Table 6), but were lower compared to non-smoking controls among 6-day-old neonates whose mothers smoked the strong cigarette.

Maternal body weights were similar between groups at the beginning of the experiment, and did not differ between tests and controls at the end of the experiment (125.6 grams ± 10.0 SE and 123.9 ± SE 8.6 respectively).

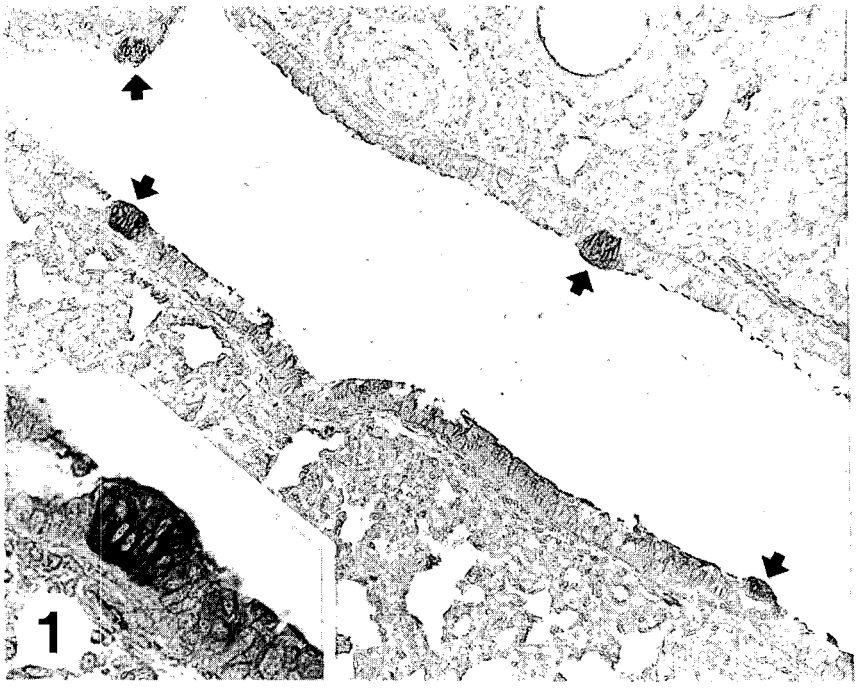

Fig. 1. Grouped neuroendocrine cells (NEB, arrows) in bronchial epithelium, stained with the Grimelius argyrophil method (× 155). Insert: enlargement of the left lower NEB which is sectioned in a near-saggital plane, × 410

Fig. 2. Solitary neuroendocrine cell (NEC) in bronchiolar epithelium, stained with the Grimelius argyrophil method, × 410

Table 1. Experimental outline: number of neonates and number of litters represented in each group.

Neonate Age	Sample Unit	Control hamsters		Test hamsters		Total All Groups
		No Smoking	Sham Smoking	Weak (3A1)	Strong (2R1)	
Birth	neonates	8	15	8	10	41
	litters	2	4	3	3	12
3 days	neonates	5	7	9	9	30
	litters	2	2	3	3	10
6 days	neonates	3	15	8	11	37
	litters	1	3	3	3	10
Total	neonates	16	37	25	30	108
	litters	5	9	9	9	32

Table 2. Density indices of argyrophil pulmonary NEB[1]

Age Group	Treatment Groups				Duncan's Multiple Range Test[2]	P
	No Smoking (No)	Sham, Smoking (Sh)	Weak (We)	Strong (St)		
Birth	1.24 ± 0.82 (2)	3.36 ± 0.85 (4)	2.24 ± 0.17 (3)	3.78 ± 0.76 (3)	No We Sh St	0.015
3 days	3.64 ± 2.79 (2)	2.01 ± 0.42 (2)	3.58 ± 0.46 (3)	1.15 ± 0.61 (3)		NS
6 days	0.76 (1)	1.45 ± 0.60 (3)	1.51 ± 0.27 (3)	0.88 ± 0.49 (3)		NS

[1] Means and standard deviations. (N) = number of litters represented. NS = not significantly different.
[2] Means arranged in ascendig order. Groups not significantly different are underscored with the same line whereas groups significantly different at the P level indicated are not underscored by the same line.

Table 3. Number of argyrophil cells per sectioned NEB[1]

Age Group	Treatment Groups				Duncan's Multiple Range Test[2]	P
	No Smoking (No)	Sham, Smoking (Sh)	Weak (We)	Strong (St)		
Birth	4.04 ± 1.08 (2)	4.51 ± 1.45 (4)	4.63 ± 0.40 (3)	6.70 ± 0.86 (3)	No Sh We St	0.073
3 days	3.52 ± 0.67 (2)	5.04 ± 0.94 (2)	4.88 ± 0.29 (3)	5.96 ± 0.48 (3)	No We Sh St	0.020
6 days	3.40 (1)	4.92 ± 1.06 (3)	3.49 ± 0.30 (3)	5.43 ± 0.19 (3)	We No Sh St	0.034

[1] Means and standard deviations. (N) = number of litters represented.
[2] Means arranged in ascendig order. Groups not significantly different are underscored with the same line whereas groups significantly different at the P level indicated are not underscored by the same line.

Table 4. Area of argyrophil clustered neuroendocrine cells (NEB) as measured on near-sagittally sectioned NEB among test and control hamsters at three ages[1].

Age Group	Treatment Groups				Duncan's Multiple Range Test[2]	P
	No Smoking (No)	Sham, Smoking (Sh)	Weak (We)	Strong (St)		
Birth	0.75[3]	0.77 ± 0.06	0.55 ± 0.23	1.18 ± 0.02	We No Sh St	0.064
3 days	0.71 ± 0.00	0.67 ± 0.24	0.87 ± 0.33	1.04 ± 0.37		NS
6 days	0.42[3]	0.63 ± 0.11	0.80 ± 0.25	0.58 ± 0.24		NS

[1] Means and standard errors based upon the average of 4 NEB from each of 57 hamsters equally representing treatment groups. NS = not significantly different.
[2] Means arranged in ascendig order. Groups not significantly different are underscored with the same line whereas groups significantly different at the P level indicated are not underscored by the same line.
[3] N = 1 littergroup.

Table 5. Lung tissue volume fractions in neonate hamsters[1]

Age Group	Treatment Groups				Duncan's Multiple Range Test[2]	P
	No Smoking (No)	Sham, Smoking (Sh)	Weak (We)	Strong (St)		
Birth	0.50 ± 0.01 (2)	0.46 ± 0.02 (4)	0.58 ± 0.04 (3)	0.48 ± 0.06 (3)	Sh St No We	0.026
3 days	0.52 ± 0.02 (2)	0.56 ± 0.02 (2)	0.62 ± 0.02 (3)	0.56 ± 0.03 (3)	No Sh St We	0.014
6 days	0.63 (1)	0.62 ± 0.05 (3)	0.61 ± 0.08 (3)	0.58 ± 0.04 (3)		NS

[1] Means and standard deviations. (N) = number of litters represented. NS = not significantly.
[2] Means arranged in ascendig order. Groups not significantly different are underscored with the same line whereas groups significantly different at the P level indicated are not underscored by the same line.

Table 6. Body weights of neonate hamsters[1]

Age Group	Treatment Groups				Duncan's Multiple Range Test[2]	P
	No Smoking (No)	Sham, Smoking (Sh)	Weak (We)	Strong (St)		
Birth	2.71 ± 0.72 (2)	2.49 ± 0.19 (4)	2.58 ± 0.07 (3)	2.38 ± 0.10 (3)		NS
3 days	4.12 ± 0.46 (2)	3.90 ± 0.28 (2)	4.15 ± 0.31 (3)	3.53 ± 0.20 (3)		NS
6 days	9.77 (1)	6.85 ± 0.52 (3)	7.17 ± 0.21 (3)	6.44 ± 0.26 (3)	St Sh We No	0.001

[1] Means and standard deviations. (N) = number of litters represented.
[2] Means arranged in ascendig order. Groups not significantly different are underscored with the same line whereas groups significantly different at the P level indicated are not underscored by the same line.

Discussion

NE cell density indices and other parameters

Our results indicate that maternal smoking during pregnancy affects pulmonary NE cells in the progeny. For example, argyrophil NEB's appeared to be more numerous, contain more cells and tended to be larger at birth among neonates whose mothers smoked the strong cigarette during pregnancy. This suggests a dose-related effect as the weak cigarette group did not show such changes.

These results are supported by a recent study by Wang and coworkers (Wang et al., 1984) who revealed increased NEB numbers in baby mice at 5 days of age by maternal oral nicotine during pregnancy using quantitation by scanning electron microscopy. This increase was enhanced and remained at least throughout 30 days of age, if nicotine was also administered during lactation. Among mice only exposed *in utero*, values had returned to normal at 15 days and remained normal at 30 days.

Our observation that maternal restraint alone (sham smoking controls) may cause significant NE cell changes that usually do not differ from those of the strong cigarette group suggests that pulmonary NE cell physiology can be influenced by stress, and that the direct influence of nicotine in itself might not be as strong as suggested by others (Wang et al., 1984).

Our results further indicate that lung tissue volume fraction can be affected by maternal smoking. Specifically, the weak cigarette group had the highest values suggesting that low doses of nicotine had the strongest pharmacological effect on lung tissue growth. Mothers who smoked that cigarette had blood nicotine levels (ng/ml) of 22.0 ± 8.4 SE between 5 and 10 min. after smoking whereas the strong cigarette yielded 56.0 ± 9.6 ng nicotine per ml of blood (Keith, 1988). However, individual neonate doses could not be calculated. In the study by Wang et al., (1984), pregnant mice received 1 mg/l nicotine in their drinking water, but neonate doses were again unknown. It is thus difficult to compare effects between different studies. In humans, umbilical cord vein serum contained 1.12 ± 0.30 times more nicotine at birth than did maternal vein serum (Luck et al., 1985). The same figure, applied to our hamsters, would imply neonate levels of approximately 26 ng/ml with the weak cigarette which falls at the upper level in the range of 0.5-25 ng/ml measured in human babies of smoking mothers, whereas our strong cigarette would produce abnormally high neonate nicotine levels.

The fact that the medial thickness index of pulmonary arterioles was not changed by any treatment provides morphometric evidence that chronic pulmonary hypertension was not present in the neonates.

Because of the small sample sizes in this study, significant differences are difficult to identify. It is thus warranted to consider trends indicated by significance levels of up to $P = 0.1$.

The argyrophil stain developed by Grimelius (1968) is the histochemical stain most frequently used for pulmonary NE cells. In the present study, this method was more consistent in demonstrating NEC and NEB than anti-serotonin immunocytochemistry with the peroxidase-antiperoxidase method which was also tried. One drawback with the Grimelius stain is its sensitivity to reduction of reactive substance brought on by physiologic changes, for example, acute increases in airway oxygen tension (Keith and Will, 1981). Because detectability depends upon the quantity of cytoplasmic material available to react with the chosen stain, cells with subminimal quantities will go undetected.

Palisano and Kleinerman (1980) demonstrated that optimal detectability of NEB's and NEC's with the formaldehyde-vapor-induced fluorescence techique may be obtained by priming animals with the serotonin precursor 5-hydroxytryptophan (5-HTP) and with L-DOPA prior to killing. The administration of 5-HTP would likely also enhance detection by anti-serotonin immunocytochemistry. However, this approach might falsely increase cell counts due to uptake by non-endocrine cells. The optimal immunocytochemical method for central and peripheral neuroendocrine cell detection in humans, rhesus monkeys and rats has been considered to be the immunoperoxidase technique using antibodies to the glycolytic enzyme neuron specific enolase (NSE), specific for endocrine cells and neuronal elements (Wharton, et al., 1981). However, Lundquist and coworkers (1985) found that only 8 of 13 carcinoid liver metastases and 4 of 10 samples of normal human intestinal mucosa were immuno-reactive to anti-NSE, whereas all stained positively with the Grimelius technique and the argentaffin method by Fontana. In this study neither of 2 different batches of polyclonal antibodies to NSE gave consistent staining results.

Causative factors

The changes in pulmonary NE density indices demonstrated here may be looked upon as changes in NE cell development. Elevated NE cell indices, for example, could result from increased cortisol secretion and release which can be induced by nicotine (Seyler et al., 1984) and is common in stress. This view is supported by recent reports of rapidly increased NEB numbers in rhesus monkey fetuses from mothers treated with dexamethasone (Dayer et al., 1985), and in neonate rabbits and mice exposed *in utero* to maternal nicotine injections (Chen et al., 1984) and oral nicotine (Wang et al., 1984) respectively. Such rapid responses are most likely caused by accumulation of stainable substance by, for example, accelerated synthetic activity thus promoting detection. Other causes of NE cell increases might be differentiation and maturation of resident reserve cells, or actual mitosis. Because cell numbers are here related to section surface area, decreased numbers might have resulted from a relative increase in the amount of extra-epithelial tissue, devoid of NE cells as observed with the weak cigarette at birth and 3 days, or reduced airway lumen, thus «concentrating» cell

numbers. However, this was compensated for by dividing the number of cells per area with the lung tissue fraction.

Another possible influence is the effect of CO, inhaled by the mother and passed to the fetus. Although there may be synergism between CO and nicotine, the CO effect appears to be of less importance, as maternal nicotine alone increased NEB numbers in the mouse study (Wang et al., 1984). Furthermore, in our study, the weak and strong cigarettes had different effects although the generated CO can be assumed to be similar. Might neonate or maternal nutritional status, as reflected in reduced neonate body weights, influence NE-cell parameters?. One could speculate that lungs of lighter neonates are less developed and thus have the high NE-cell numbers found in near-term fetuses (Hernandez-Vasquez et al., 1977, 1978, Redick and Hung, 1984). However, all litters were born at full term and only one group (6-day-olds with the strong cigarette) showed a trend toward lower weights ($P \leqslant 0.09$). Furthermore, nicotine during pregnancy did not significantly change the number of alveolar intercepts in baby mice (Wang et al., 1983), and Miller and coworkers (1976) found that infants of smoking mothers showed symmetrical growth retardation. Maternal body weights did not seem to be coupled here with changes in NEB indices of the offspring, as maternal weights did not differ between tests and controls at the end of the experiment. This is supported by findings of others that the effect of cigarette smoking on birth weight is not mediated through decreased maternal appetite or weight gain (Surgeon General, 1983; Editorial, Lancet, 1979).

It is also possible that mutagenic cell transformation during fetal life might contribute to actual NE cell proliferation. Candidates in cigarette smoke are tar components such as benzo-a-pyrene, urethane, anthracenes, and also hydrazines, and nitrosamines, the latter having a documented proliferative effect on lung NE cells (Rezinck-Schüller, 1976; Linnoila, 1982). In the present study only mainstream smoke was inhaled, whereas the sidestream smoke containing approximately 50 times more nitrosamine (Stock, 1980) was evacuated by the smoking machine. Although tar concentration did not differ significantly between the weak and strong cigarette in our study, different effects were noted with these two strengths, suggesting that nicotine was directly or indirectly responsible. Radioactivity from polonium-214 and lead-210, both α-emitters found in cigarette smoke, also have a potential mutagenic effect on the sensitive fetus.

Particularly relevant to the present study is the rapid degranulation of pulmonary NE cells shown to be evoked by nicotine alone (Lauweryns, 1977), and the humoral releasing effects by nicotine on endocrine cells (Andersson et al., 1982; Pomerlau et al., 1983; Seyler et al., 1984). Because nicotine is known to cross the placenta (Wilson 1942; Suzuki et al., 1974; Guerin et al., 1979; Luck et al., 1985) one may speculate that chronic stimulation by nicotine during fetal life, at a critical time of cell differentiation, causes functional changes that might affect NE cell numbers and response to stimuli or *in utero* initation of NE cell transformation. To determine if NE cell numbers might have increased by mitosis, a «mitotic index» approach would have to be employed, e.g. the autoradiographic method based upon incorporation of radiolabeled thymidine in nuclei of dividing cells. Such a study is underway.

Acknowledgements. We thank Patrick Goeckner for skillful technical assistance and Dr. Barry Bavister, Dept. of Veterinary Science, for providing the time-bred hamsters. Environmental chambers at the Biotron, University of Wisconsin-Madison were used to maintain constant rearing conditions. This study was supported by the College of Agricultural and Life Sciences, and The Council for Tobacco Research, Inc. USA (grant number 1437).

References

Andersson K., Fuxe K., Eneroth P. and Agnati L.F. (1982). Effects of acute central and peripheral administration of nicotine on hypothalamic catecholamine nerve terminal systems and on the secretion of adenohypophyseal hormones in the male rat. Medical Biology 60, 98-111.

Auerback O., Garfinkel L. and Parks V.R. (1975). Histologic type of lung cancer in relation to smoking habits, year of diagnosis, and sites of metastases. Chest 67, 382-387.

Becker K.L. (1984). The endocrine lung. In: The endocrine lung in health and disease. Eds. Becker KL, Gazdar AF. W.B. Saunders Company, pp. 3-46.

Bensch K.B., Corrin B., Pariente R. and Spencer H. (1968). Oat-cell carcinoma of the lung. Its origin and relationship to bronchial carcinoid. Cancer 22, 1163-1172.

Chen M.F., Diotavelli M.J., Kimizuka G., King M. and Wang N.S. (1984). A scanning electron microscopy (SEM) and transmission electron miscroscopy (TEM) study of baby rabbit lungs following maternal injection of nicotine. Fed. Proc. 43; Abstr. 3478.

Correa P., Pickle L.W., Fontham E., Lin Y. and Haenszel W. (1983). Passive smoking and lung cancer. Lancet ii, (8350), 595-597.

Curet L.B., Rao A.V., Zachman R.D., Morrison J., Burkett G. and Poole W.K. (1983). Maternal smoking and respiratory distress syndrome. Am. J. Obstet. Gynecol. 47(4), 446-450.

Cutz E. (1982). Neuroendocrine cells of the lung. An overview of morphologic characteristics and development. Exp. Lung Res. 3, 185-208.

Dayer A.M., Kapanci Y., Rademakers A., Rusy L.M., DeMey J. and Will J.A. (1985). Increased numbers of neuroepithelial bodies (NEB) in lungs of fetal Rhesus monkeys following maternal dexamethasone treatment. Cell Tissue Res. 239, 703-705.

Editorial (1979). Smoking and intrauterine growth retardation. Lancet i, (8115) 536-537.

Everson R.B. (1980). Individuals transplacentally exposed to maternal smoking may be at increased cancer risk in adult life. Lancet ii, (8186), 123-127.

Fergusson D.M., Horwood L.J., Shannon F.T. and Taylor B. (1981). Parental smoking and lower respiratory illness in the first three years of life. J. Epidemiol. Community Health 35, 180-184.

Fergusson D.M., Horwood L.J. and Shannon F.T. (1980). Parental smoking and respiratory illness in infancy. Arch. Dis. Child 55; 358-361.

Grimelius L. (1968). A silver nitrate stain for α^2 cells in human pancreatic islets. Acta Societatis Medicorum Upsaliensis 73, 243-270.

Guerin M.R., Stokely J.R., Higgins C.E., Monehun J.H. and Holmberg R.W. (1979). Inhalation bioassay chemistry - Walton horizontal smoking machine for inhalation exposure of rodents to cigarette smoke. J.N.C.I. 63, 441-448.

Harlap S. and Davies A.M. (1974). Infant admissions to hospital and maternal smoking. Lancet i,(7857), 30, 529-532.

Hernandez-Vasquez A., Will J.A. and Quay W.B. (1977). Quantitative characteristics of the Feyrter (APUD) cells of the neonatal rabbit lung in normoxia and chronic hypoxia. Thorax 32, 449-456.

Hernandez-Vasquez A., Will J.A. and Quay W.B. (1978). Quantitative characteristics of the Feyrter cells and neuroepithelial bodies of the fetal rabbit lung in normoxia and short term chronic hypoxia. Cell Tissue Res. 189, 179-186.

Isaak P.F. and Rand M.J. (1972). Cigarette smoking and plasma levels of nicotine. Nature 236, 308-310.

Keith I.M. and Will J.A. (1981). Hypoxia and the neonatal rabbit lung: neuroendocrine cell numbers, 5-HT fluorescence intensity and relationship to arterial medical thickness. Thorax 36, 767-773.

Keith I.M. (1988). Blood nicotine levels in hamsters after smoking and subcutaneous nicotine. J. Pharmacol. Methods 19, In Press.

Lauweryns, J.M., Cokelaere M., Deelersnyder M. and Liebens M. (1977). Intrapulmonary neuroepithelial bodies in newborn rabbits. Influence of hypoxia, hyperoxia, hypercapnia, nicotine, reserpine, L-dopa, and 5-HTP. Cell Tissue Res. 182, 425-440.

Linnoila I. (1982). Pulmonary endocrine cells in the normal and nitrosamine-treated hamster. Exp. Lung Res. 3,225-236.

Luck W., Nau H., Hansen R. and Steldinger R. (1985). Extent of nicotine and cotinine transfer to the human fetus, placenta and amniotic fluid of smoking mothers. Dev. Pharmacol. Ther. 8, 384-395.

Lundquist M., Wilander E., Esschert T. and Pahlman S. (1985). Neuron-specific enolase in mucosal endocrine cells and carcinoid tumors of the small intestine, a comparative study with neuron-specific enolase immunocytochemistry and silver stains. Histochem. J. 17, 323-331.

Miller P.J. (1971). An elastin stain. Med. Lab. Technol. 28, 148-149.

Miller H.C., Hassanein K. and Hensleigh P.A. (1976). Fetal growth retardation in relation to maternal smoking and weight gain in pregnancy. Am. J. Obstet. Gynecol. 125, 5560.

Nikolov J.G. and Chernozemsky I.N. (1979). Tumors and hyperplastic lesions in Syrian hamsters following transplacental and neonatal treatment with cigarette smoke condensate. J. Cancer Res. Clin. Oncol. 92, 249-256.

Nikonova T.V. (1977). Transplacental action of benzo-a-pyrene and pyrene. Bull Exp. Biol. Med. 84, 1025-1027.

Palisano J.R. and Kleinerman J. (1980). APUD cells and neuroepithelial bodies in hamster lung, methods, quantitiation, and response to injury. Thorax 35, 363-370.

Pearse A.G.E. (1969). The cytochemistry and ultrastructure of polypeptide-hormone producing cells of the APUD series and the embryonic, physiologic and pathologic implications of the concept. J. Histochem. Cytochem. 17, 303-313.

Polak J.M. and Bloom S.R. (1979). The diffuse neuroendocrine system. J. Histochem. Cytochem. 27, 1398-1400

Pomerlau O.F., Fertig J.B., Seyler L.E. and Jaffe J. (1983). Neuroendocrine reactivity to nicotine in smokers. Psychopharm. 81, 61-67.

Rantakallio P. (1978). Relationship of maternal smoking to morbidity and mortality of the child up the age of five. Acta Paediatr. Scand. 67, 621-631.

Redick M.L. and Hung K-S. (1984). Quantitation of pulmonary neuroepithelial bodies in pre- and postnatal rabbits. Cell Tissue Res. 238, 583-587.

Reznick-Schuller H. (1976). Proliferation of endocrine (APUD-type) cells during early N-diethylnitrosamine-induced lung carcinogenesis in hamsters. Cancer Lett. 1, 255-258.

Seyler L.E. Jr., Fertig J., Pomerlau O., Dent D. and Parleer K. (1984). The effects of smoking on ACTH and cortisol secretion. Life Sci. 34, 57-65.

Stock S. (1980). Risks the passive smoker runs, Lancet. ii, (8203), 1082.

Surgeon General Report. (1983). The health consequences of smoking for women. Publication 410-889/1284. Dept. of Health and Human Services, pp. 191-249.

Suzuki K., Horiguchi T., Comas-Urrutia A.C., Mueller-Heubach E., Morishima H.O. and Adamsons K. (1982). Placental transfer and distribution of nicotine in the pregnant rhesus monkey. Am. J. Obst. Gynecol. 119, 253-263.

Vasallo G., Capella C. and Solcia E. (1971). Grimelius silver stain for endocrine cell granules, as shown by electron microscopy. Stain Technol. 46, 7-13.

Wang N-S., Chen M-F., Schraufnagel D.E. and Yao Y-T. (1984). The cumulative scanning electron microscopic changes in baby mouse lungs following prenatal and postnatal exposure to nicotine. J. Path. 144, 89-100.

Wang N-S., Schraufnagel D.E. and Chen M.F. (1983). The effect of maternal oral intake of nicotine on the growth and maturation of fetal and baby mouse lungs. Lung 161, 27-38.

Wharton J., Polak J.M., Cole P.J., Marangos P.J. and Pearse A.G.E. (1981). Neuron-specific enolase as an immunocytochemical marker for the diffuse neuroendocrine system in human fetal lung. J. Histochem. Cytochem. 29, 1359-1364.

Wilson J.R. (1942). The effect of nicotine on lactation in white mice. Am. J. Obstet. Gynecol. 43, 839-844.

Accepted October 6, 1987