

THE INTERACTION BETWEEN PROTEIN DEFICIENCY AND NICOTINE: EFFECTS ON GROSS, HISTOLOGICAL, AND HISTOCHEMICAL FEATURES OF THE LUNG.

By:

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INTRODUCTION

Smoking hazards has been an international problem for several years. Experimentally, it has been proved that nicotine is the most effective extract of tobacco. It is absorbed into the blood circulation with each cigarette smoked, thus producing different toxicological effects. These effects might reach most; if not all of the body systems (Hug , 1970).

The vital role of dietary protein in the production, prevention, and repair of organ injury has been widely appreciated and extensively studied. Although the nutritional status in most of the developing communities is usually a protein deficient diet, yet smoking habit dominates, (Van Proosdij, 1960). Efforts are being done by the developed countries which are not governed by malnutrition to control smoking. It is worthwhile that developing countries governed by malnutrition should follow the same trend.

The present investigation deals with the interactive effect of protein deficiency and nicotine on the lung.

MATERIAL AND METHODS

A total number of 144 male weanling Albino rats, six weeks of age, and ranging from 50 : 60 grams of body weight were divided into four groups as shown in Table (1):

Table (1)
Experimental Design and Group Distribution

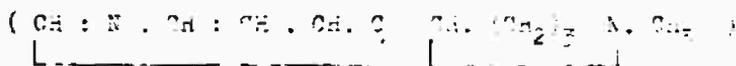
Group No.	Treatment	No. of Rats
Control (1)	Well balanced diet	32
Control (2)	Well balanced diet + Nicotine	36
Control (3)	Protein deficient diet	36
Experimental group	Protein deficient diet + Nicotine	40

The composition of the experimental diets used are shown in Table (2) :

Table (2)
Composition of the Experimental Diets

Dietary Component	Percentage Composition	
	Well balanced diet	Protein deficient diet
Casein	20%	5%
Corn starch	66%	81%
Cotton seed oil	7%	7%
Mineral mix. (Jones & Foster, 1942)	5%	5%
Vitamin mix. (Woodruff, 1961)	2%	2%

Pure nicotine (BDI Chemicals Ltd., England) of the formula :



was mixed with diet at a dose rate of 30 mg. / kg. body wt. / day. Offered amounts were adjusted weekly according to fluctuations in body weights and amounts of food consumed.

Autopsies were performed at weekly intervals for five weeks. Gross structure of lungs was observed and lung weights were determined to the nearest 0.1 mg. using a Meopta torsion balance.

For histological and histochemical investigations, representative samples from each lung were subjected to the following fixation and staining techniques:

- 1- Bouin's fluid for routine Haematoxylin and Eosin.
 - 2- 10% buffered formalin followed by staining in:
 Foulgen reaction for D.N.A.
 Pyronin / Methyl green stain for R.N.A.
 - 3- Rossman's picro alcohol fixative and staining by P.A.S. reaction for polysaccharides.
- The techniques used are those recommended by Pearse (1959).

RESULTS AND DISCUSSION

Lung Weights :

Relative lung weights, calculated as percentages per body weights are demonstrated by Table (3):

Table (3)
Average of Relative Lung Weights

Duration (weeks)	Control Groups			Experimental group	
	Well balanced diet	Well balanced diet + Nicotine	Low protein diet	L.P.D.	+ Nic.
1	0.610 ± 0.040	0.720 ± 0.014	0.710 ± 0.020	0.825 ± 0.007	
2	0.615 ± 0.049	0.715 ± 0.007	0.695 ± 0.091	0.785 ± 0.120	
3	0.723 ± 0.090	0.905 ± 0.106	0.820 ± 0.400	0.970 ± 0.110	
4	0.750 ± 0.127	0.920 ± 0.110	0.950 ± 0.032	0.935 ± 0.007	
5	0.837 ± 0.029	0.890 ± 0.110	0.940 ± 0.100	0.910 ± 0.042	

Data of the experimental group reveal a slight increase in the relative lung weights with respect to body weights. This increase is more clearly identified from the third week of the experiment and thereafter. Such finding was confirmed by Alexandrov and Raitchev (1963) in rats inhaling cigarette smoke and attributed this increase to the appearance of glandular tumours. Nevertheless, starving rats injected by nicotine showed a significant decrease in their relative lung weights, (Simkovich, 1973).

Gross and Microscopical Studies :

Normal lungs of the first control group were soft in consistency, ~~high~~ pink in colour, and uniform spongy appearance (Figure 1-i). Regular increase in size with growth of rats was evident throughout experimentation. All studied specimens had normal microscopical features (Figure 2).

In the second control group ; where rats were given nicotine with the well balanced diet, a fraction of examined lungs showed congested lobes ; predominantly one of the left lobes. Congested lungs were slightly firm in consistency and dark red in colour. Lungs of rats autopsied at the end of experimentation showed areas of adhesions to the ribs. Such areas were markedly pale in colour and hard in the cut surface (Figure 1-ii). On microscopical examination, signs of internal haemorrhages were evident by the fourth week (Figures 3). Later, the alveolar walls became thickened and alveoli were filled with a firm network containing erythrocytes and polymorphs. Epithelial cells lining bronchioles were degenerative and distorted. The development of internal pulmonary haemorrhage during nicotine administration is also confirmed by Alexandrov and Raitchev (1963); while Mahrburg (1958) reported this lesion to be frequently transitory in subtoxic nicotine doses. Histochemically, lung tissue of this group showed gradual decline in the P. A. S. positive material. While D.N.A. gave intense reaction throughout experimentation, (Figure 4), R.N.A. stained faintly prior the fourth week; after which it acquired normal staining affinity. Thus while Rosenkranz and Sprague (1969) also reported similar elevations in lung D.N.A.

An explanation for this unexpected finding is offered by Oldelatt et al. (1975), to be due to the accumulation of D.N.A. in small clumps located in the reticulum and membrane of the nucleus during the process of cellular necrosis, therefore giving intense reaction.

In the third control group where rats were maintained on the protein deficient diet, lungs were grossly unaltered during the whole experimental period (Figure 1-iii). On microscopical examination, the organs showed some degenerative bronchioles. Slight fragmentation of tissue or zonal necrosis characterized specimens examined by the fifth week (Figure 5). Histochemically, a slight increase in the P. A. S. positive material occurred during the first two experimental weeks (Figure 6), followed by slight and gradual decline, while nucleic acids staining were not significantly faint.

In the experimental group, rats under the double stress of protein deficiency and nicotine showed severe grossly affected lungs. During the first two experimental weeks, the organ appeared wholly congested and dark red in colour. By the third week, multiple nodules of consolidation, predominantly restricted to the left lobe characterized most examined lungs. These white milky nodules were strongly adhered to the ribs where it was difficult to remove. Later, it invaded the whole lung (Figure 1-iv). Microscopical investigation showed degenerative bronchioles by the second week. During the fourth and fifth weeks, pulmonary tissue became wholly necrotic (Figure 7). No identifiable alveolar pattern could be distinguished besides the invasion of red blood cells and polymorphs (Figure 8). P. A. S. positive material appeared relatively dense during the first three weeks. Thereafter, it stained faintly (Figure 9). D.N.A. and R.N.A. were significantly faint and undifferentiated and therefore, finally depleted by the fifth week (Figure 10). Mortality of rats of this group after the fifth week ended the investigation.

ABSTRACT

Studies on the interactive effects of protein deficient diet and nicotine (30 mg./kg. body wt./day) on the lungs of male weanling Albino rats have indicated the following:

Relative lung weights showed slight increase. In the gross, lungs were wholly congested, dark red in colour, with multiple white nodules firstly restricted to left lobes and later extending to involve the whole organ. Microscopically, degenerative bronchioles, distorted alveolar pattern, zonal necrosis and internal haemorrhages were the main histological features. P. A. S. positive material gave an intense reaction during the early phase of investigation, followed by a gradual decline. D.N.A. and R.N.A. were finally depleted by the end of investigation.

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CAPTIONS OF FIGURES

Figure (1): Representative lungs from experimental and control groups:
i- Well balanced diet.
ii- Well balanced diet + nicotine.
iii- Low protein diet.
iv- Low protein diet + nicotine.

Figure (2): Normal lung tissue from rat offered the well balanced diet.
(H & E : X 150)

Figure (3): 4 weeks of well balanced diet + nicotine.
Internal pulmonary haemorrhage.
(H & E : X 400)

Figure (4): 3 weeks of well balanced diet + nicotine.
Intense staining for D.N.A.
(Feulgen reaction : X 300)

Figure (5): 5 weeks of protein deficiency.
Zonal necrosis of lung tissue.
(H & E : X 100)

Figure (6): 2 weeks of protein deficiency.
Increase in the P.A.S. +ve material.
(P.A.S. reaction : X 300)

Figure (7): 4 weeks of protein deficiency + nicotine.
Degenerative bronchioles invaded by fibroblasts.
(H & E : X 400)

Figure (8): 5 weeks of protein deficiency + nicotine.
Distortion of alveolar pattern, haemorrhage and infiltration.
(H & E : X 200)

Figure (9): 4 weeks of protein deficiency + nicotine.
Faint and undifferentiated P.A.S. +ve material.
(P.A.S. reaction : X300)

Figure (10): 5 weeks of protein deficiency + nicotine.
Depleted R.N.A.
(P.M.G. stain : X300)



(i)



(ii)



(iii)



(iv)

Fig. (1)

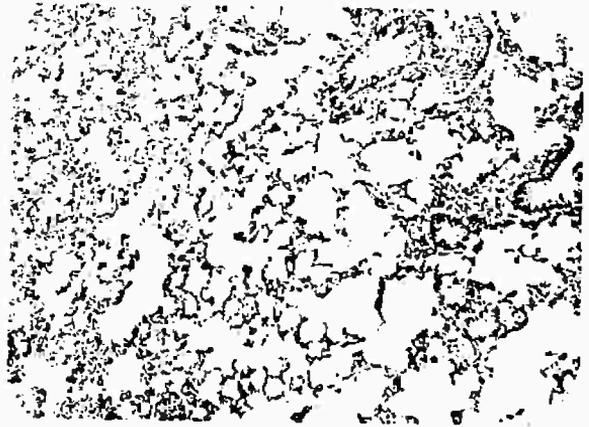


Fig. (2)

Fig. (3)



Fig. (4)

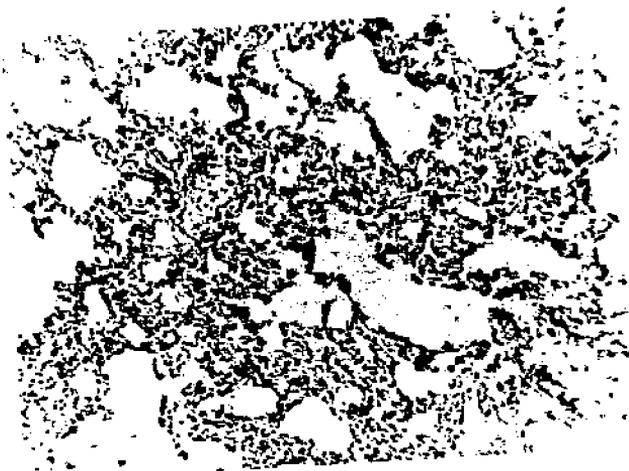


Fig. (5)

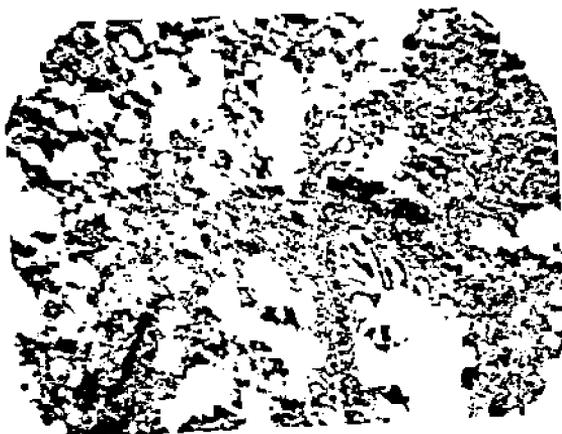


Fig. (6)



Fig. (7)



Fig. (8)



Fig. (9)



Fig. (10)

