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Ventilatory Functions in Germfree and Conventional Rats Exposed to Coal Dusts (39821)

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Introduction. For many years there has been speculation concerning the role of infective processes in the development of respiratory disease in workers exposed to industrial dusts. In particular, miners exposed to coal dust have demonstrated pulmonary reactions similar to those found resulting from chronic respiratory infections in nonminers. Although it is commonly accepted that coal miners as a group have lower ventilatory capacities than nonminers, it is not currently known whether chronic obstructive ventilatory disease develops as a result of coal dust exposure alone (1). The uncontrollable factors in human populations make it evident that another experimental model would be desirable to demonstrate the pure toxic responses to coal dust.

The germfree rat was selected as the experimental animal because of its low profile of endogenous (spontaneous) disease manifestations. These rats also show clearly defined susceptibilities to many disease-inducing agents. Therefore, the results from germfree studies may reveal more clearly the unique characteristics of disease processes in the absence of complications from incidental microbial infections.

The germfree rat has been used as a tool in inhalation toxicology in this laboratory and others. Buckley and Loosli studied structural and metabolic responses of germfree mice lungs to inhaled nitrogen dioxide (2). They found that NO_2 exposure produced alterations in the bronchioles without an inflammatory response. There was also a significantly higher rate of oxygen consumption in the NO_2-exposed lung slices.

Wagner (3) and Wagner and Groth (4) presented pathological findings in a quartz dust inhalation study utilizing germfree and conventional rats. The most outstanding differences between the two types of rats were that the germfree animals had few or no plasma cells in the lung, tracheobronchial lymph nodes, spleen, mesenteric lymph nodes, and small and large intestines.

No significant differences were detected in the number, size, or composition of the quartz-containing nodules in the lung. The lymph nodes were larger in the germfree rats. It was also of interest that the germfree animals had signs of greater quartz dust retention than the conventional rats, possibly indicative of a decrease in pulmonary clearance activity. The purpose of this report is threefold: first to add data useful in the delineation of the role of the infective processes in the development of pneumoconioses; second, to present new physiological data for the rat, both conventional and germfree; and third, to present methods which demonstrate the advantages of the germfree rat as a model for respiratory research.

Materials and methods. Experimental design. Four groups of germfree Wistar rats (200–300 g) were divided as follows: Group (A), 16 rats exposed to sterile Pennsylvania coal dust at a concentration of 10 mg of dust/m^3 of air (geometric mean < 5 μm) for 120 days (8 hr/day); Group (B), 15 rats conventionalized (removed from germfree conditions for 2 months) and then exposed at 10 mg/m^3 of the same Pennsylvania coal (not sterile) also for 120 days (8 hr/day); Group (C), 19 rats maintained in germfree isolators with filtered clean air and used as germfree controls; Group (D), 15 rats conventionalized and maintained without dust exposure serving as conventional controls. This regimen is presented in Fig. 1.

Pulmonary function testing apparatus. Pulmonary function testing was accomplished by the use of a custom-designed, variable-pressure plethysmograph. The de-

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sign and use of a larger unit for subhuman primate testing has been reported by Moorman *et al.* elsewhere (5). The basic method employed is similar to that used in an external tank respirator; however, a hydraulically powered displacement mechanism enables the operator to completely control inspiration, expiration, breath holding, and breathing rate within the anatomical and physiological limits of the animal.

Figure 2 is a diagram of the pulmonary function testing apparatus. The plethysmograph is an airtight rectangular chamber 25 × 22 × 42 cm fitted with a rubber diaphragm attached to a hydraulic cylinder. As the diaphragm is moved, the plethysmographic pressure changes, simulating the function of the diaphragm and thoracic chamber in animals. The hydraulic system is powered by an Oildyne 2000-psi pump.[1] The total system is controlled by a four-stage Rex Hanna limit switch assembly attached to a hydraulic flow valve.

The important design aspect regarding the large diaphragm is that its displacement is in direct communication with the chamber, resulting in instantaneous pressure reflection throughout the chamber. Two external ports were installed in one side of the chamber. One port houses a Fleisch pneumotach and the other port is attached to a large bore valve for the purpose of equilibrating chamber pressure with ambient pressure.

The differential pressures generated in the pneumotach during inspiratory and expiratory airflow are electrically transduced by a Statham PM 5 strain guage connected to an SGM-2 Electronics for Medicine pressure amplifier. A 12-channel photographic recorder (also Electronics for Medicine) is used to record flow and volume. Volume is obtained by electrical integration of airflow with a variable time constant.

The rats underwent pulmonary function testing following anesthesia (pentobarbital sodium 30 mg/kg) and tracheotomy. The trachea was attached to a cannula which joined the pneumotachograph. The rats were attached to the pneumotachograph only during the performance of the breathing maneuvers so the potential effect of the additional dead space (1.7 cm^3) was not a factor. The following pulmonary function tests were selected to assess ventilatory capacities: inspiratory capacity (IC), forced vital capacity (FVC), expiratory reserve volume (ERV), forced expiratory volume in 0.5 and 1 sec ($FVC_{0.5,\ 1.0}$), peak expiratory flow rate (PF), and the maximum expiratory flow at 25 and 10% of FVC (V_{max} 25, 10% VC). The latter parameters were taken from the maximum expiratory flow–volume curves (MEFV).

GROUP A (GERMFREE) COAL EXPOSED (10 mg/m^3)	GROUP C (CONVENTIONALIZED) COAL EXPOSED (10 mg/m^3)
GROUP B (GERMFREE) CONTROLS	GROUP D (CONVENTIONALIZED) CONTROLS

FIG. 1. Diagram of experimental design.

Inspection of flow–volume curves at increasing driving pressures indicated that flow limitation characteristics had been reached at volumes below 25% of the vital capacity when the plethysmograph pressure was greater than 75 cm H_2O.

Statistical methods. The study was designed as a 2 × 2 factorial experiment with two treatments (exposed and control) and two types of rats (conventional and germfree). An analysis of variance was used to examine the main effects of the two factors and their interaction upon the response variable. The Student's *t* test was used to make individual comparisons between pairs of mean responses for selected groups. An important assumption underlying this type of analysis is that intragroup responses conform to a normal distribution. Consequently each variable was tested to determine whether it could be considered normally distributed. Tests for outliers were performed and observations outside the group population were eliminated. Any variable which did not follow a normal distribution was examined using nonparametric techniques of statistical analysis. These consisted of the Wilcoxon rank-sum test and χ^2 contingency-table analysis.

[1] Mention of product or trade names does not constitute endorsement by the National Institute for Occupational Safety and Health.

Results. Significant ventilatory reduction was detected between the coal-exposed groups when compared to the control groups; however, the differences between the germfree and conventionalized rats were much greater. For most variables the variance associated with conventional rats was several orders of magnitude larger than the variance obtained from germfree rats. The pulmonary function which appeared to be most sensitive to exposure to coal dust was the flow maxima at 10% of the FVC.

Tables I and II present a summary of the data classified by response variable and by group. Table I is a list of means and their 95% confidence intervals for the response variables IC, FVC, ERV, PF, and $\dot{V}_{max}$ 25% VC which were considered to follow the normal distribution. Table II gives the frequency distribution (the number of responses falling within given intervals) and the range in each of the four groups for the three variables $FEV_{0.5}$, $FEV_{1.0}$, and $\dot{V}_{max}$ 10% VC. These three parameters yielded responses that tended to be categorical and therefore made a parametric analysis inappropriate.

Table III presents the results of the statistical tests which were made in order to detect differences among group means (from

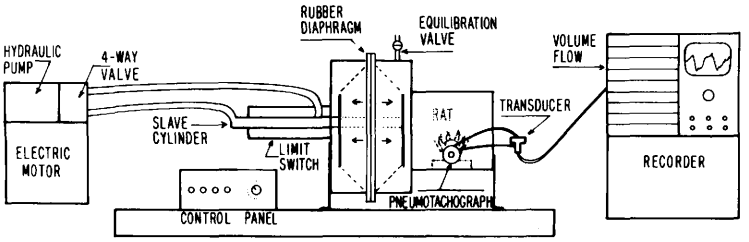

FIG. 2. Diagram of plethysmograph-respirator.

TABLE I. MEANS AND 95% CONFIDENCE LIMITS FOR NORMALLY DISTRIBUTED VARIABLES.

Group	IC (cm^3)	FVC (cm^3)	ERV (cm^3)	PF (cm^3/sec)	V_{max} 25%/VC (cm^3/sec)
(A) Germfree exposed	7.59 ± 0.42	9.17 ± 0.42	1.58 ± 0.26	40.4 ± 2.5	3.87 ± 0.33
(B) Conventional exposed	9.21 ± 1.21	11.06 ± 1.35	1.85 ± 0.28	57.3 ± 6.6	3.06 ± 0.83
(C) Germfree control	7.96 ± 0.38	9.48 ± 0.38	1.50 ± 0.24	36.3 ± 2.2	3.52 ± 0.30
(D) Conventional control	9.84 ± 1.26	11.70 ± 1.42	1.86 ± 0.29	62.2 ± 6.6	4.43 ± 0.83

TABLE II. FREQUENCY DISTRIBUTIONS AND RANGES OF NON-NORMAL VARIABLES.

	$FEV_{0.5}$ (%)			
Group	<95.6	95.8-97.7	97.8-100	Range
(A) Germfree exposed	7/16	6/16	3/16	92.8-98.3
(B) Conventional exposed	7/14	4/14	3/14	85.7-100
(C) Germfree control	0/19	7/19	12/19	96.0-99.2
(D) Conventional controls	7/14	4/14	3/14	92.8-100
	$FEV_{1.0}$ (%)			
	<98.4	98.4-99.5	99.6-100	Range
(A) Germfree exposed	9/16	4/16	3/16	95.3-100
(B) Conventional exposed	6/14	1/14	7/14	89.8-100
(C) Germfree controls	1/19	13/19	5/19	97.5-100
(D) Conventional controls	6/14	3/14	5/14	96.5-100
	V_{max} 10%/VC			
	0-1.50		>1.51	Range
(A) Germfree exposed	10/16		6/16	0.25-3.25
(B) Conventional exposed	10/14		4/14	0.05-2.60
(C) Germfree controls	0/19		19/19	1.71-3.48
(D) Conventional controls	3/13		10/13	0.83-4.01

TABLE III. PROBABILITIES (*P* VALUES) ASSOCIATED WITH GROUP COMPARISONS FOR ALL RESPONSE VARIABLES.

Response variable	Group A vs B[a]	Group A vs C	Group B vs D	Group C vs D
IC	0.012*	0.077	0.176	0.005**
FVC	0.008*	0.160	0.344	0.008**
ERV	0.124	0.281	0.354	0.122
PF	0.001*	0.993	0.133	<.001**
V_{max} 25%/VC	0.084	0.962	0.004**	0.011**
V_{max} 10%/VC	0.709	<0.001**	0.016**	0.050*
$FEV_{0.5}$ (%)	0.896	0.002**	0.999	0.001**
$FFV_{1.0}$ (%)	0.154	0.003**	0.685	0.009**

[a] Group A, germfree exposed; Group B, conventional exposed; Group C, germfree controls; Group D, conventional controls.

* Indicates significant difference between groups at the 0.05 probability level.

** Indicates significant difference between groups at the 0.01 probability level.

Table I) and among group distributions (from Table II). The numbers in this table are the probabilities that the observed differences between groups could have occurred due to purely random experimental variations. Any such probability less than 0.05 was designated as statistically significant.

As the table indicates, the most consistent and largest differences were found between the germfree control group and the conventional group. The germfree controls had significantly smaller lung volumes and flow rates than the conventional controls.

The germfree animals also displayed significantly lower values in the exposed group when compared to the control group for three variables: $\dot{V}_{max}$ 10% VC, $FEV_{0.5}$, and $FEV_{1.0}$, a result consistent with small airway obstruction. The conventional rats displayed such differences for only two variables: $\dot{V}_{max}$ 10% VC and $\dot{V}_{max}$ 25% VC.

Discussion. Exposure of germfree and conventional rats to Pennsylvania bituminous coal dust has produced significant ventilatory reduction. This reduction is primarily associated with obstruction of the smaller or peripheral airways, because no obstructive defects were detected in the maximum expiratory flow at large lung volumes. Limitations in peak flow relate to the resistance, primarily in the trachea and main bronchi. These airways are self-supported by cartilage rings and are not greatly influenced by volume change. The small airways however, lack self-supportive structures and depend on radial traction provided by the surrounding lung tissue; therefore, as the lungs deflate during expiration, they tend to collapse. This is the reason the flow rate rapidly decreases during expiration. Other invesigators have detected reduced $FEV_{1.0}$ and $\dot{V}_{max}$ at small lung volumes in coal miners (6–8).

To the authors' knowledge, this is the first study to demonstrate obstructive airway disease in rats. It is also the first to demonstrate airway obstruction in germfree animals. The issue of whether or not obstructive airway disease in coal miners develops from an increased incidence of respiratory infections is still not completely resolved. However, this work does present evidence that airway obstruction can occur as a result of coal dust exposure alone.

An interesting feature associated with the observed obstructive disease is that its development did not occur in all of the dust-exposed animals. The inference here is that some endogenous difference characterizing each individual predisposes it to the development of obstructive lung disease. In monkeys exposed to this same sample of coal dust at 2 mg/m^3 (respirable), we have associated the individual animals having chronic obstructive lung disease with increased smooth muscle of the small airways (8).

Summary. Ventilatory functions were studied in four groups of rats, two groups of conventional rats and two groups of germfree rats. A control and an exposed group were employed for each type. The exposures were 10 mg/m^3 of bituminous coal for 120 days. The pulmonary function parameters included: inspiratory capacity, vital capacity, forced expiratory volumes in 0.5 and 1.0 sec, peak expiratory flow rate, and flow maximas at 25 and 10% of the vital capac-

ity. The coal-exposed rats, both conventional and germfree, exhibited significantly lower responses for the flow maxima at 10% of the vital capacity when compared to the corresponding control groups. However, such differences in germfree rats were more clearly distinguishable because of the small intragroup variability. This work presents evidence that airway obstruction occurs in rats as a result of a coal dust exposure alone.

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