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SCOR Director: John Butler, M.D. Division Code: D

Responsible Investigator: R. Frank, Professor (F_1) and Edwin S. Boatman, Associate Professor (F_2)

Project Number and Title: F_1 and F_2. Studies of the lungs of animals exposed to ozone.

Participating Investigators: SCOR: D. Luchtel, C.J. Martin; outside SCOR: R. Holub, P. Meyer (A.E.C.)

PROGRESS REPORT: March 1, 1976

A. Rationale:

The purpose of these experiments is to describe the acute and chronic effects of ozone on the structure and function of the lung. Ozone, as a pollutant intimately associated with automotive exhaust, is a hazard to public health.

B. Summary of Accomplishments:

Acute studies: Mr. Richard Holub and members of the Lawrence Livermore Laboratory of California continued their studies of the effects of O_3 on overall pulmonary function and regional ventilation and perfusion in dogs. The computer programs for assessing regional function were completed the past year. Preliminary analysis shows that significant changes in overall function and regional ventilation occurred in dogs during 3 hour's exposure to 0.5 ppm of O_3. Possible effects on regional perfusion have not yet been assessed.

Chronic studies: The pilot study involving the exposure of SPF rats to O_3 (0.45 ppm, 6 hours/day, 5 days/week) for a maximum of 10 weeks was completed. The reduction and analyses of data are still underway.

Results: 1. Function (R. Holub): R_L decreased progressively with growth as measured by body weight (Fig. 1), while C_L increased (Fig. 2). During exposure to O_3, the rate at which R_L decreased with growth was slowed. This feature appeared to be a more sensitive index of response than was the absolute value for R_L. Also, during the exposure to O_3, the rate at which C_L increased was depressed. Unfortunately, the original control values for the two groups of animals differed, being higher in the control group. In future studies, our analyses will be improved by spacing the measurements more widely.

Analysis of D_{LCO}, slow volume/pressure curves, and nitrogen washout curves is continuing.

2. Alveolar wall length-tension characteristics (C.J. Martin): Four to six alveolar walls were dissected from each lung for measurement. Data were collected before exposure began and after five and ten weeks of exposure on maximum extensibility yield strain, breaking strain, yield force, breaking force, stress relaxation, tissue compliance or slope, and force at $\lambda 1.5$. There were no statistically significant differences between control and exposed animals for any measurement. However, large differences in mean value were noted between the two groups of animals for: yield force and tissue compliance or slope. Yield force was lower in the exposed animals suggesting that permanent deformation of the scleroproteins took place with less stress The increase in slope suggests that the lungs were less compliant (see Function), and that alveolar diameters are reduced at any fixed pressure (see Morphometry).

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REPOSITORY LLNL B361 Rm B940A
COLLECTION Institutional Review Board
BOX No. IRB Protocol File
ACTIVE GRANTS - Collaborative Univ of WA
FOLDER Ozone Effects on Overall Lung Function

3. Morphology (D. Luchtel): The lungs were prepared by ruthenium red staining to show the thickness and staining characteristics of the mucous layer. Particular attention was given to the mucous layer in the terminal and respiratory bronchioles. In control animals, the mucous layer appeared as a densely staining film ranging in thickness from 0.1 - 0.2μ in terminal bronchioles, down to 0.02μ in respiratory bronchioles. Flocculent patches of mucus (up to 10μ in thickness) were occasionally seen. There was little or no staining of the surface of the alveolar epithelium. In animals exposed for 10 weeks, the flocculent-like mucus formed almost a continuous layer in the terminal and respiratory bronchioles. The alveolar layer also appeared somewhat thicker than in the controls.

Dr. Luchtel continued his collaboration with other SCOR investigators in the following projects:

H_1 and H_2 a) Ultrastructural examination of rabbit lungs exposed to high concentrations of O_2. Observations were made on control lungs, and after 48-hour and 72-hour exposures to O_2.

b) Development of the monkey lung. A sequence of normal lung development in the primate was studied by examining lungs fixed at the following gestational times: 88, 107, 115, 126, 135, 142, 149, 155, 160, and 168 days.

M_5 Alveolar macrophage cultures were examined at 0, 2 and 10 days (control and conditioned medium) by transmission and scanning electron microscopy.

4. Morphometric analysis (E. Boatman); Lungs were fixed at a constant distending pressure of 25 cm of water. Transverse sections from both left and right lungs were cut at 1μ for light microscopy point and intersection counting.

Morphometric analyses were made by photographing 10 random fields from each section with a 35 mm camera and the film strips projected onto point-counting grids by a Weibel type projection apparatus. The volume proportions of air space (V_{va}), alveolar tissue V_{vt}), alveolar ducts (V_{vad}), air-space density (S_{va}) and mean chord length (L_m) were estimated for each pair of lungs.

The results of the volume proportion estimates are shown in Table 1 and the relationship of lung volume (V_L) to body weight and mean air-space dimensions (L_m) to body weight are shown in Figures 3 and 4 respectively.

Although the numbers of animals examined so far are small, there is a significant difference ($P = <.005$) in air-space dimensions (alveoli + alveolar ducts) between control lungs and those from animals exposed to ozone (57.67μ vs. 42.71μ) - a difference of 25%. This finding is interpreted to suggest that lungs exposed to ozone under our conditions are "stiffer" and therefore less expandable at a standard pressure than lungs exposed to air only.

Electron microscopy of these tissues at low magnification shows few if any structural abnormalities. It remains to be seen if this is also the case for the fibrous proteins of the lung parenchyma after a critical examination at high magnification.

C. Plans for the Coming Year:

These are described in Projects 1 and 2 of the Pulmonary SCOR Proposal: Chronic Diseases of Airways, submitted by Dr. C.J. Martin.

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D. Publications:

Kaplan, D., McJilton, C., and Luchtel, D.: Bisulfite induced lipid peroxidation. Arch. Environ. Health 30:507-509, 1975.

Watanabe, S. and Frank, R.: Lung volumes, mechanics, and single-breath diffusing capacity in anesthetized cats. J. Appl. Physiol. 38:1148-1152, 1975.

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E. New Grants or Awards:

1. Investigator: Robert Frank
   Source of funds: Unsolicited award from Ford Motor Company
   Amount per year: $5,000
   Number of years recommended: Up to 3
   Total funds for award period: $15,000

2. Investigator: Robert Frank
   Source of funds: National Heart and Lung Institute
   Amount per year: $42,670
   Number of years recommended: 6/30/75 through 6/30/78
   Total funds for award period: $124,915

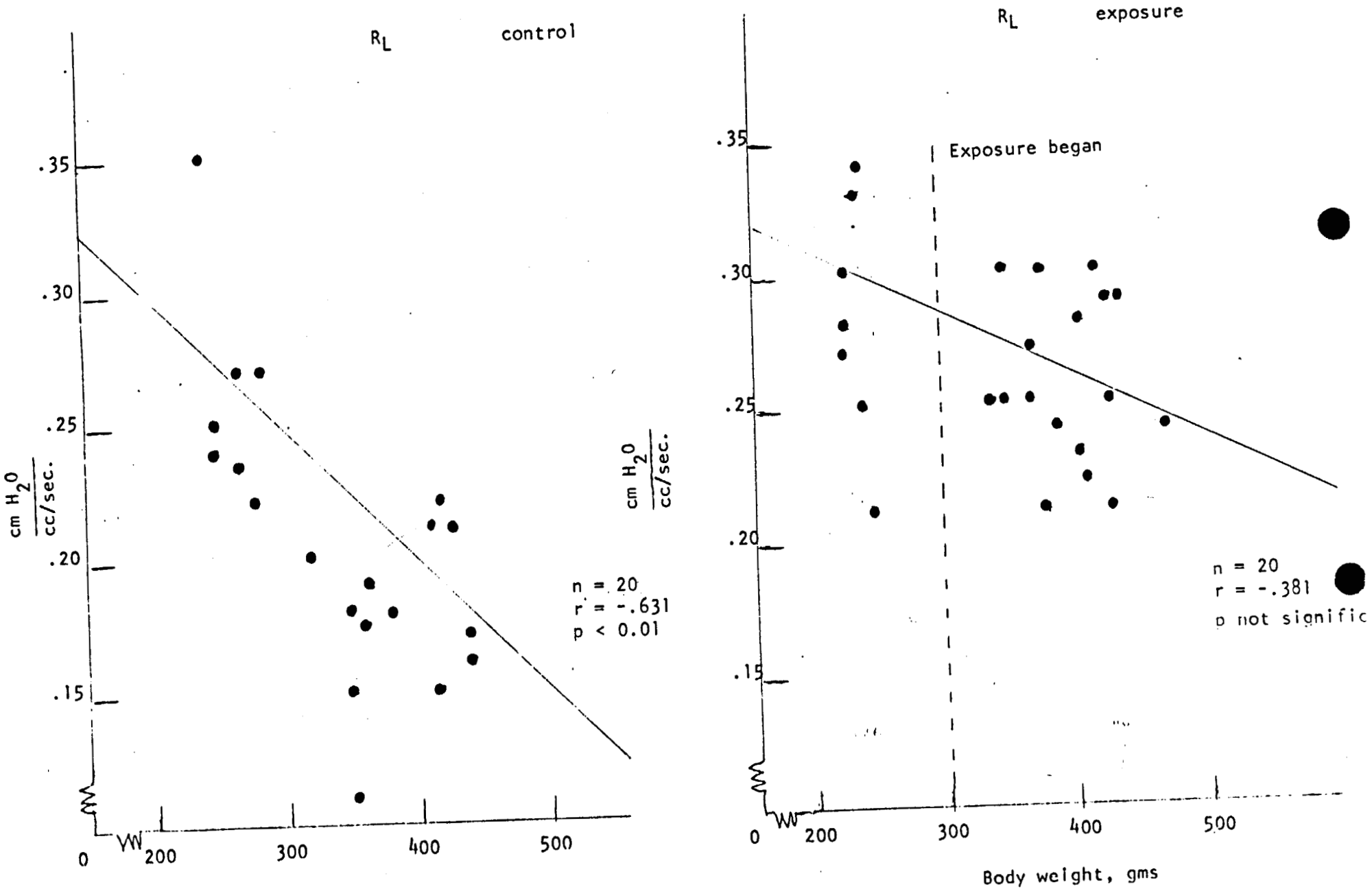

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Fig. 1

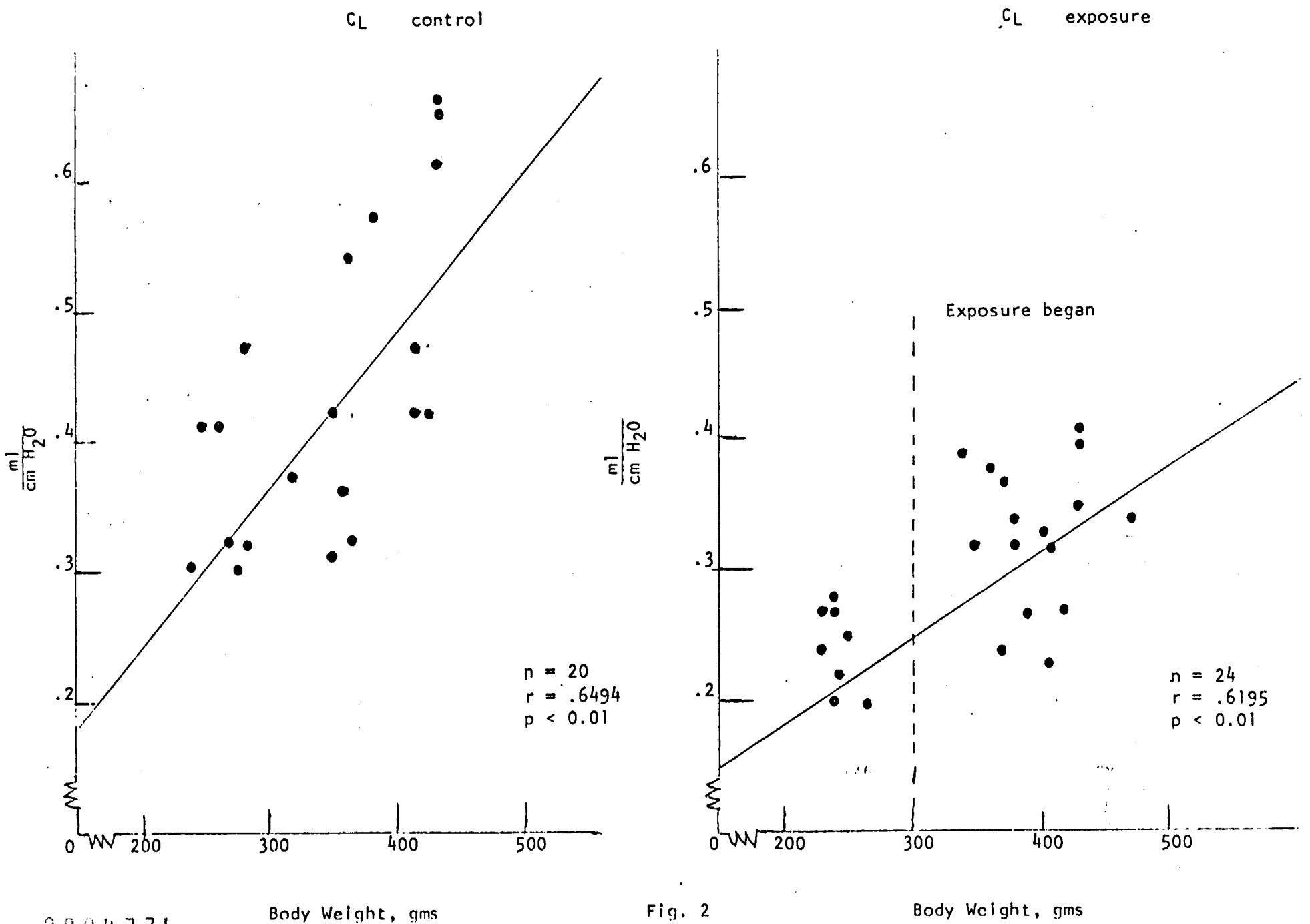

Fig. 2

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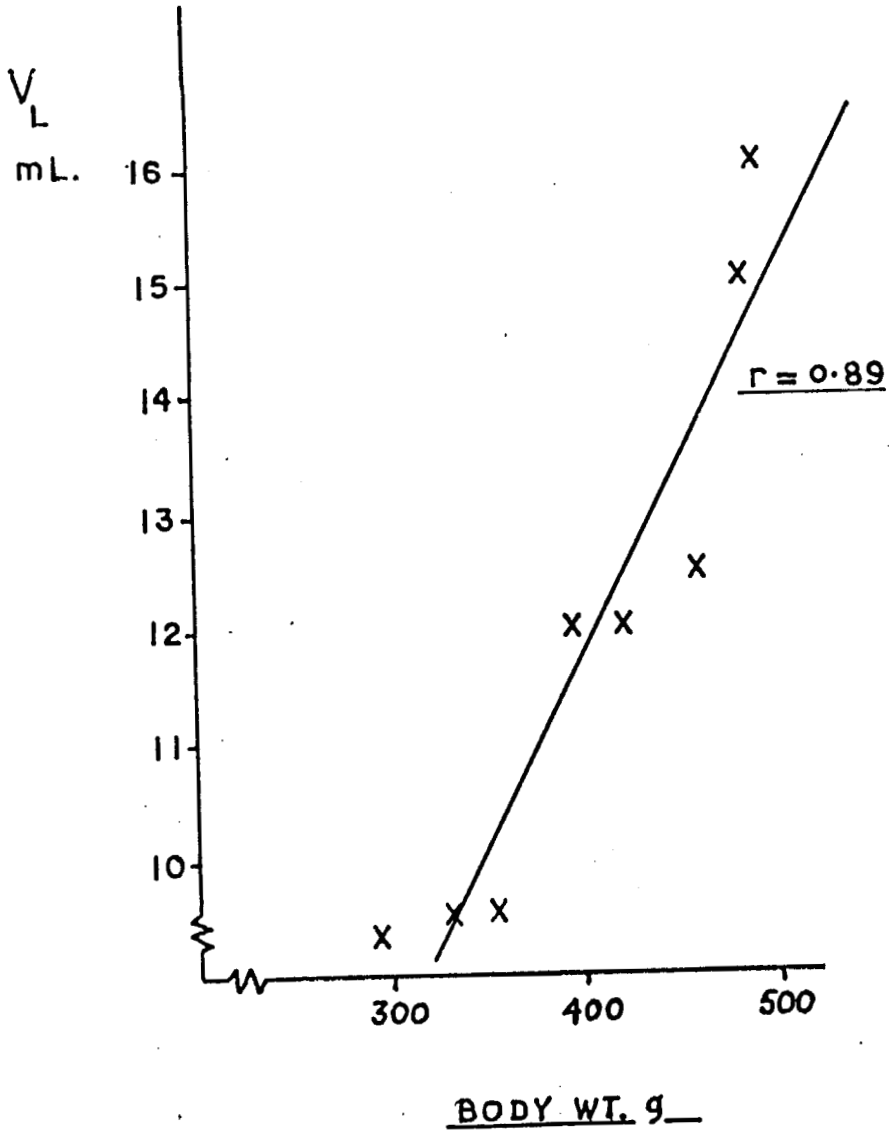
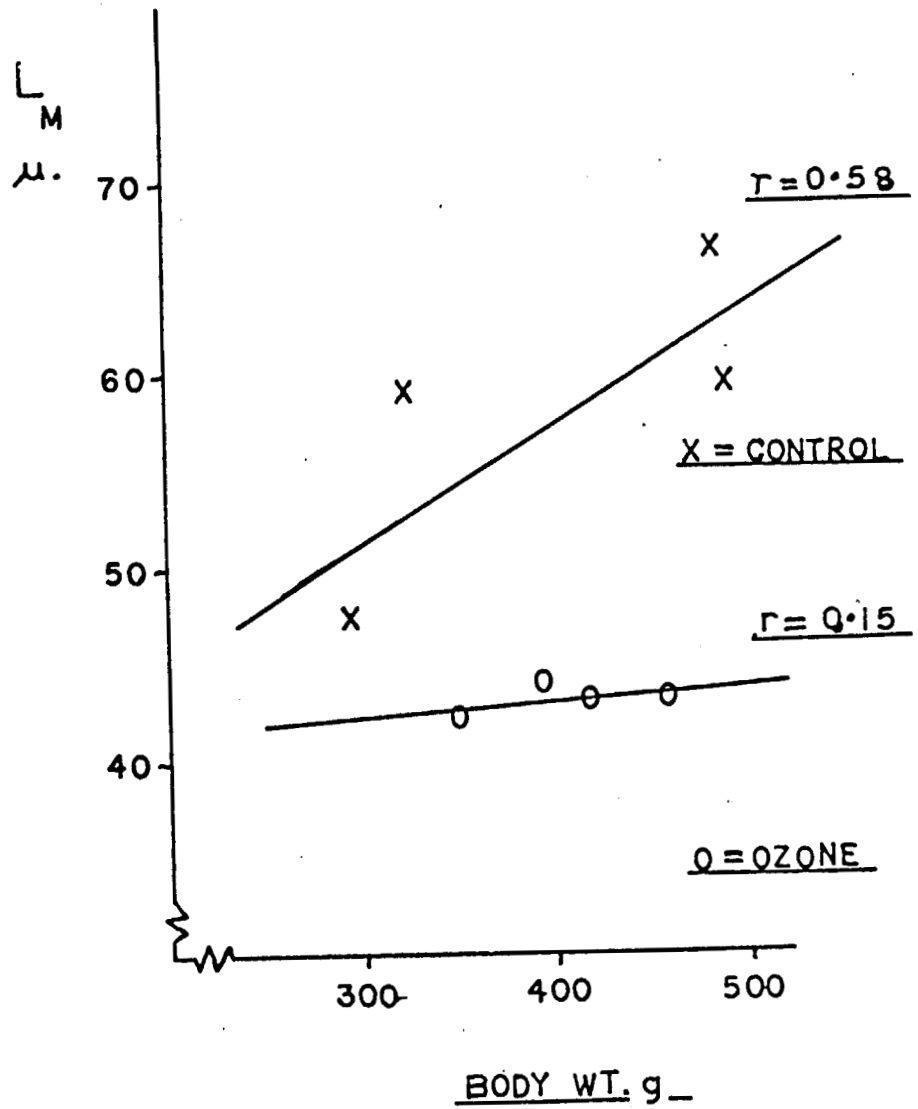
Fig. 3



Plot of lung volume (V_L) against body weight.

Fig. 4



Plot of mean air-space dimensions against body weight for control and ozone exposed rats.

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Table 1

Morphometric parameters of parenchyma tissue from lungs of control rats and from rats exposed to 0.5 ppm. of O_3 for 5 or 10 weeks.

	Control						Ozone exposed						
	V_{va}	V_{vt}	$V_{va/v}$	V_{vad}	L_m (μ)	S_{va}	V_{va}	V_{vt}	$V_{va/v}$	V_{vad}	L_m (μ)	S_{va}	O_3 weeks
1	36.61	9.7	1.00	52.86	58.8	.680	48.91	10.12	0.85	40.18	43.00	.930	10
2	38.99	7.64	0.60	52.78	59.16	.676	49.52	9.05	0.95	40.48	42.65	.938	10
3	32.04	6.45	0.79	60.52	66.16	.605	62.60	11.11	1.29	25.00	43.60	.917	10
4	45.93	12.22	3.27	37.50	46.54	.859	54.25	11.56	0.85	33.33	41.58	.962	5
x	38.39	9.0	1.42	50.92	57.67	.705	53.82	10.45	0.99	34.75	42.71	.936	

V_{va} = volume proportions % of air space.

V_{vt} = volume proportions % of alveolar tissue.

$V_{va/v}$ = volume proportions % of arterioles and venules.

V_{vad} = volume proportions % of alveolar ducts.

L_m (μ) = mean linear intersection (or mean chord length).

S_{va} = surface density of air space, $\frac{cm^2}{cm^3}$.

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