The Production of an Emphysema-Like Condition in Rats by the Administration of Papain Aerosol (34750)

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Since Brown-Sequard (1) first discussed the production of experimental emphysema in the late nineteenth century, reports of various experimental procedures to induce this condition have appeared in the literature. In recent studies, emphysema has been produced experimentally by phosgene (2), cigarette smoke (3), tracheal ligation and phytohemagglutinin (4), and intratracheally administered papain (5).

While the latter route of papain administration has caused an emphysema-like condition in rats, and a single exposure to papain aerosol has produced emphysematous changes in hamster lungs examined 59 days after treatment (6), little is known of the effects of this agent when administered as an aerosol to rats.

The ability of any agent to produce a rapid and reliable emphysema-like state in a laboratory animal with easily measured pathologic test parameters may be of great importance in the evaluation of potential agents for clinical use. Utilization of the proteolytic enzyme papain to produce an experimental emphysema-like state may be of pertinent value in light of recent evidence that a deficiency of α_1-antitrypsin factor is associated with primary panlobular emphysema in man (7). The following experiments evaluated the usefulness of papain administered by aerosol and intratracheal techniques.

Materials and Methods. A. Protocol. Sprague-Dawley rats (male and female, 5–6 weeks old) were subjected to various treatment schedules.

Papain aerosol challenge. A 3% solution of papain[1] in water was delivered by four nebulizers attached to the back of a closed Plexiglas chamber (34 × 21 × 19 in.) containing the rats. The nebulizers were operated at a pressure of 10 lb/in.^{2} Rats were exposed to the aerosol in groups of 20. This procedure was performed three times a week for 1-hr durations over a 4-week period. Certain controls received identical treatment with solvent alone. Food and water were not allowed in the aerosol chamber during papain administration, but at all other times were allowed *ad libitum*.

Intratracheal (IT) administration. A miniature laryngoscope similar to the one described by Gross (8) was utilized to give an injection of either papain (0.5 mg in 1 ml of saline) or saline (1 ml) once a week for 4 weeks. A modified 22-gauge, 4-in. needle was inserted into the larynx with the animal under light ether anesthesia. A miniature bulb was mounted to the tip of the larngoscope to give direct illumination of the injection area.

B. Methods. At the end of the 4-week treatment period, the functional residual capacity (FRC) was determined using a modification of the method of King (9). Rats were anesthetized with Dial-urethane[2] (.5 ml/kg ip), tracheotomized, and connected by a short tracheotomy tube to a 10-ml syringe containing pure oxygen. Such attachment was made at the end of expiration and the animals then respired in this closed system for 7 min. Carbon dioxide was removed by an 11% solution of potassium hydroxide and oxygen was bubbled into the syringe to replace the carbon dioxide. The rat was disconnected from the syringe at the end of expira-

[1] Supplied by Warner-Chilcott Laboratories.

[2] 100 mg of allobarbital, 400 mg of urethane, and 400 mg of monoethylurea in each ml.

tion and the syringe was closed to atmosphere. The percentage nitrogen in the syringe was determined by use of a Scholander gas analyzer. Calibration was by means of a calibration curve obtained by equilibrating known volumes of air with the apparatus. With this calibration curve, it was possible to convert nitrogen concentration in the "oxygen syringe" to FRC (ml). Duplicate measurements which showed a difference of 0.4 ml or greater were repeated.

Following FRC determinations, the thorax of the anesthetized rat was opened and the lungs were distended with buffered neutral formalin fixation fluid at a pressure of 10 cm H_2O and removed intact. Lungs were left in the fixative until sectioning. After fixation, sections were cut at 5 μ from paraffin blocks and stained with hematoxylin and eosin.

For objective determinations of lung damage, a grid was fashioned with 30 cross marks 12 mm apart (6 $\times$ 5 rows) and placed over the viewing screen of a Unitron microscope. Slides were objectively examined at a magnification of 150$\times$. Eight fields were randomly chosen from a single lung slide from the right lower lobe and counted in the following manner: if the cross marks fell on alveolar septa, blood vessels, connective tissue or any large air vessel wall, no score was given; if the cross mark fell on an empty space, a plus one rating was given. Thus, a maximum of 240 points was possible. An increase in air spaces would indicate either a loss of tissue, enlarged air spaces or both. This counting system was adapted from the work of Dunnill (10) and Palecek *et al.* (11).

Differences between means of two treatment groups were determined using the Student's *t* test.

Results. The effects of both aerosol and intratracheal papain on lung parameters are shown in Tables I (male) and II (female). Both male and female rats receiving aerosol papain exhibited increases in FRC, FRC! kg, and air spaces which were significantly different ($p < .01$) from nontreated controls. Rats subjected to water aerosol treatment did not differ from untreated controls, and the latter served as the major control group in the experiment.

The histologic appearance of a normal lung is shown in Fig. 1. A lung from a rat treated with papain aerosol is shown in Fig. 2. Broken alveoli forming large confluent alveolar sacs appear in the lung treated with papain aerosol. Such an appearance is representative of all rats treated with either papain method, except for 1 papain aerosol male and 2 IT papain females.

Rats receiving papain IT showed a significant increase in air spaces when compared to either saline IT animals or untreated controls (males, $p<.01$, females, $p<.05$). While in-

TABLE I. The Effect of Various Treatments on FRC and Air Space Ratings of Male Rats.

Treatment	Wt (g)	FRC (ml)	FRC (ml/kg of body wt)	Air spaces[e]
None	247.3 ± 14.3[a] (11)[b]	2.69 ± 0.24 (11)	10.71 ± 0.52 (11)	47.1 ± 1.7 (10)
Papain aerosol	246.9 ± 9.2 (11)	3.64 ± 0.21[d] (11)	14.67 ± 0.76[d] (11)	64.3 ± 2.4[d] (11)
Water aerosol	258.0 ± 27.4 (5)	2.67 ± 0.33 (5)	11.48 ± 1.2 (5)	43.3 ± 2.4 (5)
Papain intratracheally	254.1 ± 27.9 (8)	3.94 ± 0.80 (8)	16.20 ± 4.1 (8)	76.3 ± 5.4[d] (7)
Saline intratracheally	303.3 ± 13.8[c] (6)	3.55 ± 0.38 (6)	11.81 ± 1.3 (6)	42.0 ± 4.8 (5)

[a] Mean ± SE.
[b] Number of rats in sample.
[c] Significantly different from no treatment group, $p < .05$; [d] $p < .01$.
[e] As described in Methods.

TABLE II. The Effect of Various Treatments on FRC and Air Space Ratings of Female Rats.

Treatment	Wt (g)	FRC (ml)	FRC (ml/kg of body wt)	Air spaces[e]
None	187.5 ± 7.8[a] (21)[b]	2.46 ± 0.13 (21)	13.09 ± 0.51 (21)	48.4 ± 2.9 (11)
Papain aerosol	201.3 ± 6.2 (23)	3.57 ± 0.18[d] (23)	17.92 ± 0.87[d] (23)	66.2 ± 2.8[d] (17)
Water aerosol	209.2 ± 15.0 (5)	2.22 ± 0.34 (5)	10.58 ± 1.40 (5)	52.4 ± 4.5 (5)
Papain intratracheally	227.5 ± 9.7[d] (11)	3.59 ± 0.50[d] (11)	15.97 ± 1.9 (11)	63.8 ± 4.7[c] (10)
Saline intratracheally	260.8 ± 15.2[d] (6)	2.77 ± 0.40 (6)	10.23 ± 1.08 (6)	49.9 ± 2.0 (6)

[a] Mean ± SE.
[b] Number of rats in sample.
[c] Significantly different from no treatment group, $p < .05$; [d] $p < .01$.
[e] As described in Methods.

creases in the mean values for FRC and FRC/kg parameters were observed in the papain IT group, only the FRC value in females was significantly different from untreated controls ($p < .01$). The mean weight of these animals was also significantly elevated ($p < .01$) from controls and the FRC/kg, while increased, showed no significant difference.

The FRC value for male rats receiving

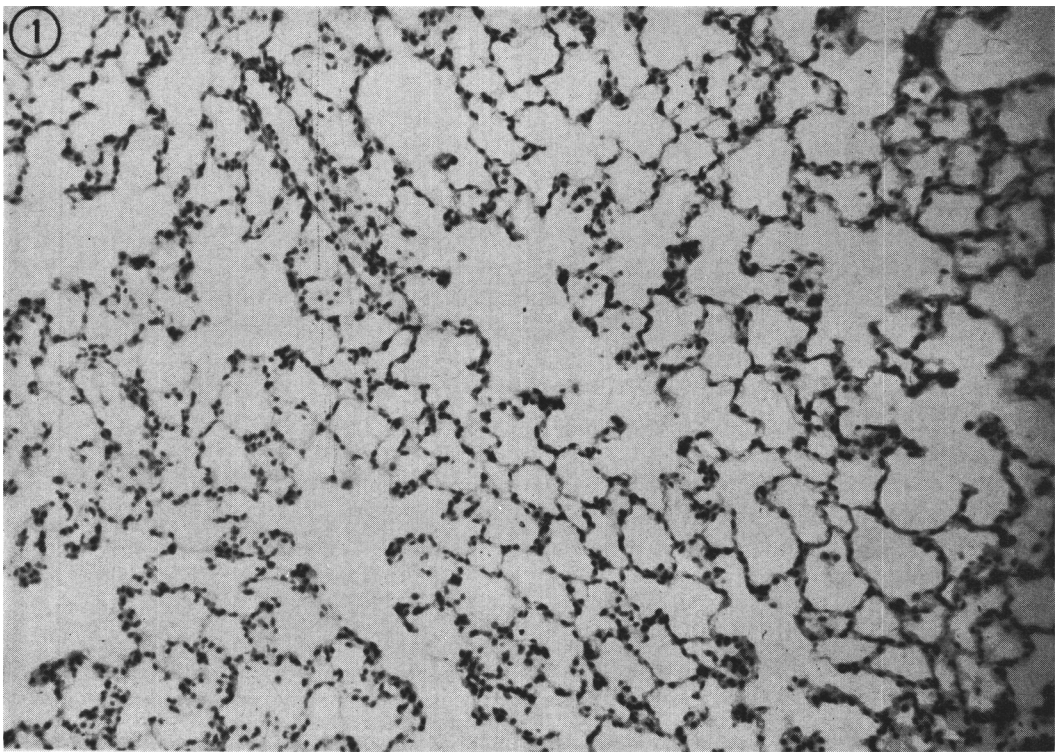

FIG. 1. Lung section of normal male rat: hematoxylin and eosin stain at 5-μ thickness; magnification 150×.

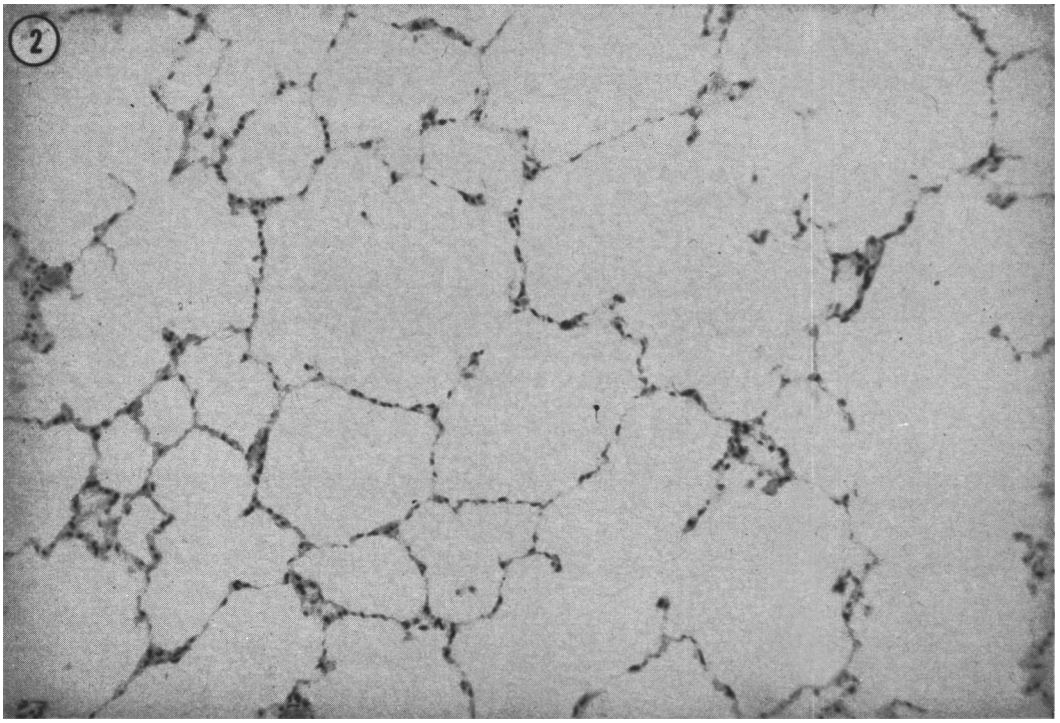

FIG. 2. Lung section of male rat treated with papain aerosol: hematoxylin and eosin stain at 5-μ thickness; magnification 150×.

saline IT was elevated, but was not significantly different from control values. The weight of the animals in this saline IT group was significantly elevated from control, ($p < .05$), as it was in the saline IT females $p < .01$).

A statistical analysis of the measurements used to evaluate the emphysema condition was performed. An analysis of covariance of FRC on body weight yielded significant correlation for the untreated controls ($r = .666$, $p < .01$), the IT saline group ($r = .643$, ($p < .01$) and the papain aerosol rats ($r = .475$ $p < .05$). This is in agreement with the data of King (9). Rats receiving IT papain treatments had FRC results which were too variable to yield significant correlation values relative to body weight.

The survival rate of the animals was followed. While 84% of the original group of papain aerosol rats were alive after 4 weeks of treatment, only 42% of the IT papain group survived.

The number of animals having values greater than 2 standard deviations above the control mean is presented in Table III. This analysis presents a measure of the distribution of values from papain treated animals. From 35 to 85% of test measurements fell above the arbitrary demarcation lines except for the FRC values for aerosol papain males.

Discussion. These studies indicate that both nebulized and intratracheally administered papain induced lung damage resembling emphysema. The lesion appeared to be panlobular and was not necessarily associated with infection. Besides the increase in air trapping monitored by FRC determinations and a breakdown and enlargement of alveoli as determined by air space ratings, examination of lung sections revealed a thinning of alveolar walls characteristic of emphysema.

While there appeared to be no significant difference in the degree of lung damage observed after either method of papain administration, there was much less variation in the FRC and FRC/kg measurements used to determine lung damage and fewer deaths in

TABLE III. Quantal Respiratory Responses of Papain-Treated Rats.

Parameters	Sex	Mean control value (+2 SD)	n/N^a; papain treatments	
			Aerosol	Intratracheal
FRC (ml)	M	2.69 (4.29)	3/11	3/8
	F	2.46 (3.78)	10/23	4/11
FRC (ml/kg)	M	10.71 (14.17)	9/11	5/8
	F	13.09 (17.99)	10/23	4/11
Air spaces[b]	M	47.1 (58.2)	9/11	6/7
	F	48.4 (67.9)	8/17	4/10

[a] Number of rats above 2 standard deviations from untreated group mean value/total number of values in sample.

[b] As explained in Methods.

the aerosol treated group. The time required for aerosol treatment was less than that required for IT papain administration.

The elevated FRC rating in male rats receiving IT saline should not be taken as lung damage since both FRC/kg and air space ratings were normal. Since FRC is significantly correlated with body weight, it would appear necessary to use FRC/kg comparisons for groups of different weights. The reason for the significant weight increase of the IT saline rats is not known.

Arbitrary demarcation lines, two standard deviations above mean control values, were selected for presentation of data in a quantal fashion. Values above these points were taken as positive responses and represented lungs damaged sufficiently to place their test values in the upper portion of a distribution curve.

The ratios expresed in Table III should not be taken as expressing the total incidence of the emphysema-like condition, *i.e.*, animals with test values not exceeding this arbitrary limit still show pathologic signs of the disease. It would appear that the highest percentage of positive responders in this quantal procedure occurred in the air space ratings for male rats.

While 9 of 11 male rats treated with papain aerosol exhibited a positive quantal rating using FRC/kg analysis, only 3 of 11 rats from this group yielded similar findings using FRC data uncorrected for body weight differences. One of the reasons for this disparity may be related to the positive correlation between body weight and FRC. Pooling of FRC values not corrected for body weight introduces a significant variable to the sample. Female rats, however, did not show a similar disparity.

Summary. An emphysema-like change in rat lungs was produced in young animals by the chronic administration of papain for 4 weeks. Both aerosol and intratracheal administration produced lesions of equal severity. The aerosol method was quicker to administer, produced fewer fatalities and was more easily quantitated.

Aerosol papain treatment of rats may provide a means for studying the effect of drugs on an experimental emphysema condition.

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