Vinyl Acetate Casts of Emphysematous Rat Lungs

CARMELO COLOMBO, SALVATORE COLOMBO, and BERNARD STEINETZ ˣ

Abstract □ Emphysema was induced in rats by chronic exposure to a papain aerosol according to established methods. The emphysematous changes were evaluated by previously described histological and biochemical parameters and by a new method employing infiltration of the lungs with vinyl acetate to make casts of the air spaces of the pulmonary tree. The weights and gross and microscopic appearance of the casts demonstrated the emphysematous changes in lungs of the papain-treated rats. Vinyl acetate casting may offer a new and useful tool in evaluating experimental induction of emphysema in rats.

Keyphrases □ Vinyl acetate—lung infiltration, casts formed, papain-induced emphysematous changes evaluated, rats □ Lung infiltration—vinyl acetate, casts formed, papain induced emphysematous changes evaluated, rats □ Emphysema, papain induced—lung changes evaluated by infiltration with vinyl acetate to form casts, rats

Pulmonary emphysema is a chronic respiratory disease characterized by a gradual destruction of alveolar walls, resulting in the formation of enlarged air spaces distal to the terminal bronchioles. Various substances have been used to induce an emphysema-like condition in laboratory animals. These substances include aerosols of papain, polymorphonuclear leukocyte homogenates, and other noxious substances (1–4) and intratracheally administered papain, phytohemagglutinin, and silica dust (5, 6).

The severity of the lung disease produced may be evaluated histologically, biochemically, or physiologically, but none of these parameters is entirely satisfactory (1, 3). Latex casts were used to study acinar changes in human emphysema (7). In the present investigation, the pulmonary trees of rats were infiltrated with vinyl acetate and the resulting casts were freed from tissue by digestion in mineral acid. The technique revealed distinct differences between control and emphysematous lungs.

EXPERIMENTAL

Male Charles River CD albino rats, ~100 g, were used in two major groups of 18 rats each. Each group was divided into three subgroups of six animals each. The subgroups were used for: (*a*) lung histology, (*b*) lung β-glucuronidase activity, and (*c*) vinyl acetate casts of lungs.

One major group of rats served as the control (water aerosol), and the other group was exposed to a 10% papain[1] aerosol in a Laskin chamber as previously described (3). The animals were exposed to the aerosol twice a week for 3 weeks, after which they were sacrificed and used for the various determinations. The lungs of six rats from each group were infused with 10% neutral buffered formalin by means of a tracheal cannula attached to a reservoir; the cannula delivered the fixative at a pressure of 10 cm water. When the flow of fixative ceased, the trachea was ligated and the thoracic cavity was opened. The entire chest contents were carefully removed and placed in additional formalin.

Following fixation, the lungs were routinely sectioned and stained with hematoxylin and eosin. The slides of right and left lungs of each rat were examined at 100× magnification with an ocular micrometer, and the alveoli falling on a line 0.2 mm long were counted. Ten fields per lung were randomly chosen, and 10 counts were made in each field. The average number of alveoli per linear millimeter was computed.

The lungs of six rats from each group were used for β-glucuronidase determination as previously described (3). The tracheas of the remaining six rats from each group were exposed and cannulated with 0.6-cm (0.25-in.) (i.d.) dimethicone tubing[2]. Vinyl acetate[3] (12% stock solution) was mixed 2:1 (v/v) with acetone and delivered into the lungs *via* the tracheal cannula from a calibrated reservoir at a height of 40 cm. The volume of vinyl acetate infused was recorded. The apparatus was washed with acetone immediately after infusion to prevent permanent clogging by the vinyl acetate.

After 1 hr, the lungs were carefully removed and immersed in 100 ml of concentrated hydrochloric acid in screw-capped jars. Twenty-four hours later, the remaining bits of tissue were removed by gentle washing. Then the casts were air dried and weighed prior to examination under a dissecting microscope at 40× magnification. By means of an ocular micrometer, the long and short axes of 20 alveoli were measured for each lung and the cross-sectional areas were calculated. The alveoli to be measured were picked at random in areas that could be clearly seen.

Table I—Comparison of Various Parameters in the Evaluation of Papain-Induced Emphysema

Parameter	Treatment: Control	Treatment: Papain Aerosol
Lung histology, alveoli/linear mm	92 ± 4	59 ± 4[a]
Lung β-glucuronidase, *E* at 450 nm	0.37 ± 0.02	0.73 ± 0.04[a]
Lung weight, g/100 g body weight	0.51 ± 0.02	0.87 ± 0.06
Vinyl acetate cast of lung		
Cross-sectional area, mm²	0.29 ± 0.02	0.48 ± 0.02[a]
Weight of cast, mg	420 ± 20	320 ± 30†
Lung capacity, vinyl acetate infused, ml	2.90 ± 0.14	2.03 ± 0.16[b]

[a] $p < 0.01$ *versus* control. [b] $p < 0.05$ *versus* control.

RESULTS AND DISCUSSION

The rats exposed to the papain aerosol developed unmistakable signs of emphysema as determined histologically and biochemically. The alveolar spaces were enlarged, leading to a 36% decrease in their number per linear millimeter (Table I). In agreement with previous findings (3), lung β-glucuronidase activity increased by 97% in the papain-treated rats (Table I). These results correlated well with observations made on the vinyl acetate casts.

Gross inspection of the casts revealed distinct differences between control and emphysematous lungs (Fig. 1). The terminal buds of lungs of exposed rats were coarser and sparser, and the bronchioles were more distended than those of the controls (Figs. 2 and 3). Fused and broken alveoli also were observed. The casts of alveolar spaces of the emphysematous lungs averaged 66% larger in cross-sectional area than those of controls (Table I). The casts of the emphysematous lungs also weighed less than the control casts. On the other hand, lung tissue weight increased by 71%.

Previous results showed that lungs of rats exposed chronically to papain demonstrated changes resembling those seen in lungs of human patients with diagnosed emphysema (1, 3, 6). In the present

[1] Papain–High Test, S. B. Penick and Co., New York, N.Y.

[2] Silastic tubing, Dow Corning Medical Products Division, Midland, Mich.

[3] Ward's Natural Science Establishment, Rochester, N.Y.

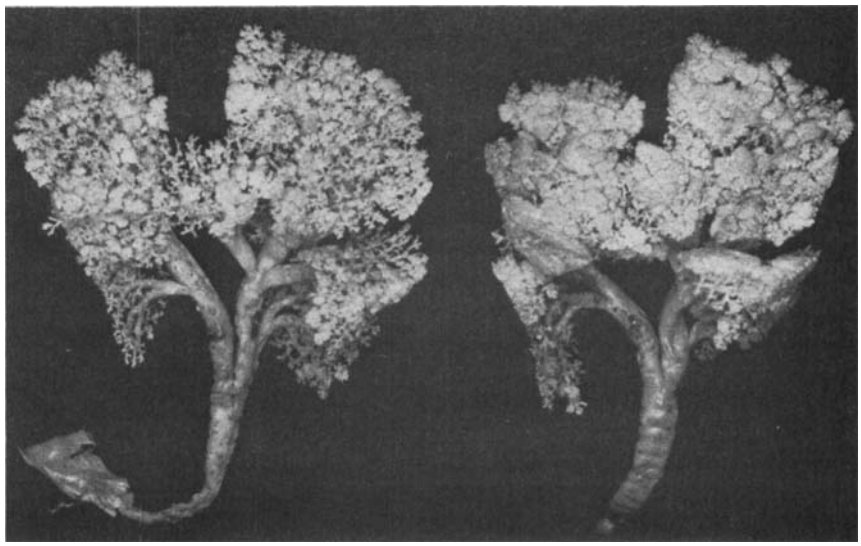

Figure 1—*Vinyl acetate casts of control (right) and emphysematous (left) rat lungs (approximately 2× natural size). Note "solid" appearance of control lungs (due to close spacing of alveoli) in comparison with lacy appearance of emphysematous lungs.*

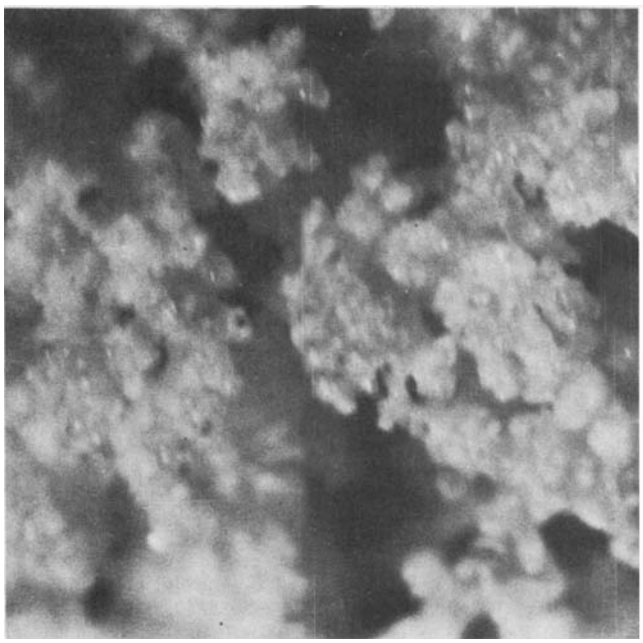

Figure 2—*Vinyl acetate cast of control lung (56× magnification). Note detail of alveoli.*

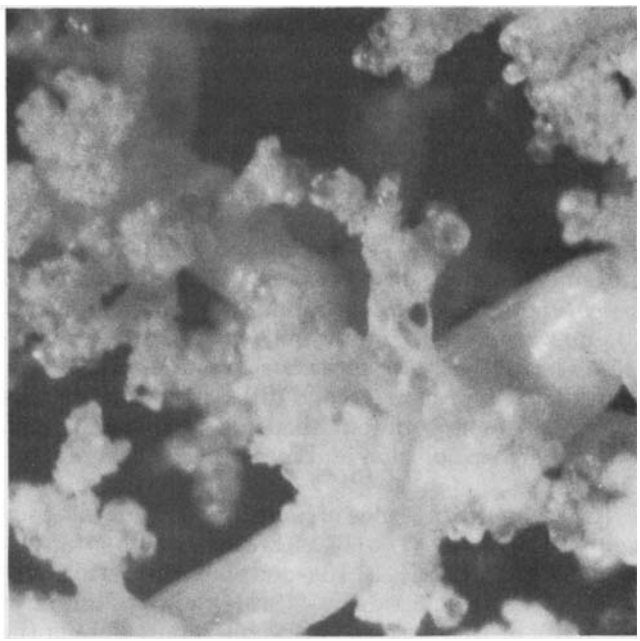

Figure 3—*Vinyl acetate cast of emphysematous lung (56× magnification). Note relative sparseness and coarseness of alveoli in comparison with Fig. 2.*

studies, the typical histological (dilation and depletion of alveoli) and biochemical (increased β-glucuronidase activity) changes were observed in papain-treated rats. The vinyl acetate casts accurately reflected the emphysematous changes. The terminal buds were grossly enlarged, presumably reflecting the breakdown and coalescence of smaller alveoli.

The distension of the bronchioles may have been due to a loss of elasticity together with a hyperplasia of nonfunctional parenchymal tissue, leading to obstruction of air passageways and enlargement and eventual destruction of alveoli. Emphysema has been reported to be due to loss of alveoli, resulting in enlargement of respiratory bronchioles, alveolar ducts, and lung distension (5). In corrosion models of emphysematous human lungs, a notable abnormality was a loss of alveoli on the respiratory bronchioles which appeared as smooth and bulging structures (7), similar to what was observed in the present study.

These phenomena were observable by gross inspection of the casts or by measurement of the alveolar cross-sectional area, which was increased by 66%. The decrease in functional alveolar capacity was also reflected in the 24% reduction in the weight of the lung casts and the decreased volume of vinyl acetate required to inflate the lungs of emphysematous rats. The reduction in these parameters probably was due to nonfunctional connective tissue hyperplasia, which resulted in obstruction of the respiratory bronchioles and broken or absent alveoli. The nonfunctional tissue was, of course, later removed during hydrochloric acid digestion.

Vinyl acetate casts of lungs, therefore, offer an effective, rapid, and relatively simple adjunct to histological and biochemical parameters in evaluating the severity of experimental emphysema in laboratory rats.

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[x] To whom inquiries should be directed.

Isolation of Cannabisativine, an Alkaloid, from *Cannabis sativa* L. Root

CARLTON E. TURNER [*], MEI-FENG H. HSU [§], JOSEPH E. KNAPP [‡], PAUL L. SCHIFF, Jr. [‡], and DAVID J. SLATKIN [‡x]

Abstract □ An ethanol extract of the root of a Mexican variant of *Cannabis sativa* L. (marijuana) afforded, after partitioning and chromatography, the new spermidine alkaloid cannabisativine.

Keyphrases □ *Cannabis sativa*—cannabisativine isolated from ethanol extract of roots □ Alkaloids—cannabisativine isolated from ethanol extract of roots of *Cannabis sativa* □ Cannabisativine—isolated from roots of *Cannabis sativa*

The occurrence of several low molecular weight, nitrogen-containing substances in *Cannabis sativa* L. has been reported including choline (1–4), trigonelline (2, 3), muscarine (5), piperidine (6), *N*-(*p*-hydroxy-β-phenylethyl)-*p*-hydroxy-*trans*-cinnamamide (7), neurine (8), L-proline (8), and L-(+)-isoleucine betaine (9). Small amounts of four alkaloids were isolated (10), but the high-resolution and mass spectra were inconclusive.

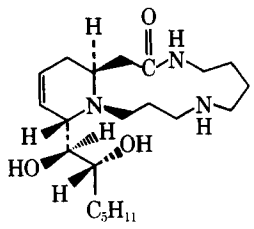
Recently, the presence of a new spermidine alkaloid, cannabisativine (I) [13-(1,2-dihydroxyheptanyl)-1,4,5,6,7,8,9,10,11,13,16,16a-dodecahydropyrido[2,1-*d*][1,5,9]triazacyclotridecin-2(3*H*)-one], was found in the roots of this species (11). This report details the procedure used in the isolation of this substance.

EXPERIMENTAL[1]

Plant Material—Roots of a Mexican variant of *C. sativa* L. were used[2].

Extraction—Air-dried ground roots of *C. sativa* (11.72 kg) were extracted by percolation with methanol (150 liters). The extract was evaporated *in vacuo* at 40° to leave a dark-brown syrup (323 g, 2.8%).

Isolation of Cannabisativine—The methanol extract (323 g) was partitioned between water (4 liters) and chloroform (4 liters) to give fractions of 266.0 g (2.3%) and 47.7 g (0.4%), respectively. The chloroform fraction was then partitioned between petroleum ether (bp 60–90°) (2 liters) and methanol–water (9:1) (2 liters) to yield fractions of 12.7 g (0.1%) and 33.4 g (0.3%), respectively.



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The aqueous methanol fraction was chromatographed on silicic acid[3] (450 g, 5 × 49 cm) packed in petroleum ether (bp 60–90°). Elution with 8% methanol–chloroform afforded a residue (3.0 g), which was dissolved in chloroform (25 ml) and partitioned with 1% hydrochloric acid (3 × 25 ml). The combined acid layers (75 ml) were basified to pH 8 with concentrated ammonium hydroxide and partitioned with chloroform (3 × 75 ml). The combined chloroform layers were dried over anhydrous sodium sulfate and evaporated, *in vacuo*, at 40° to yield a white residue (48 mg).

Crystallization from acetone afforded cannabisativine (29 mg), mp 167–168°; $[\alpha]_D^{25}$ + 55.1° (c 0.53, $CHCl_3$); UV: no maximum above 210 nm; IR: ν_{max} (KBr) 3300, 3020, 2960, 2920, 2850, 1628, 1580, 1470, 1250, 1130, 1045, and 707 cm^{-1}; NMR: δ 5.90 (2H, s, vinyl) and 9.6 (1H, s, broad, CONH); mass spectrum (M^+): *m/e* 381 (1%), 363 (1), 352 (1), 310 (2), 280 (3), 250 (64), 208 (100), 198 (6), 171 (12), 129 (5), 114 (5), 112 (6), 96 (4), 94 (6), 84 (8), 80 (6), 72 (7), 70 (8), and 55 (6). The structure of this compound was recently determined to be I (11).

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[1] Melting points were determined on a Thomas-Hoover Uni-Melt melting-point apparatus and are corrected. IR spectra were run in potassium bromide using a Perkin-Elmer 257 spectrophotometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. UV spectra were run on a Perkin-Elmer 202 spectrophotometer. NMR spectra were obtained in deuterated chloroform on a Hitachi Perkin-Elmer R-24 spectrometer, with tetramethylsilane as the internal standard. Mass spectra were recorded on a LKB-9000 spectrometer.

[2] Lot Me-A(2)-C-69 grown in 1971; voucher specimens were deposited in the herbarium of the School of Pharmacy, University of Mississippi.

[3] Mallinckrodt.