largest magnitude in 1968 was 3.2, recorded in November. The total number of Denver earthquakes recorded since the Bergen Park Observatory began operations in 1962 is 1698 of magnitude $\gtrsim$ 1.0.

Rangely, Colorado, a seismically active region during the years studied, is near the southeastern edge of a large oil field. An earthquake of magnitude 4.5, located by the U.S. Coast and Geodetic Survey, occurred there in 1966. Munson tried to correlate numbers of earthquakes near Rangely with millions of gallons of water pumped into the field as oil was withdrawn (*9*). He found no change in numbers of earthquakes whether pumping rate increased or decreased in the period of time considered.

Colorado seismicity has been virtually unchanged in the last 100 years. The same areas that were seismically active in the historical record were seismically active in the years 1966, 1967, and 1968; those areas that were seismically inactive 100 years ago were inactive in 1966, 1967, and 1968. The intervening years have seen a large population growth in the Rocky Mountain region, along with increased interest in seismic activity and the earthquake hazard problem for construction projects.

The possibility of a devastating earthquake of greater than magnitude 6, which might cause loss of life and extensive damage to property, always exists. On the basis of this seismicity study, however, the possibility that Colorado will experience a larger magnitude shock than has yet occurred, and its location and time, cannot be predicted.

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Obstructive Lung Disease and α_1-Antitrypsin Deficiency Gene Heterozygosity

Abstract. *The phenotypes of serum α_1-antitrypsin were determined by antigen-antibody crossed electrophoresis. There were five homozygotes and 25 heterozygotes for the deficiency gene found in a group of 103 patients with obstructive lung disease. The frequency of heterozygotes was 14 and 9 percent in two control groups with different mean ages of 36 and 80. There was only one heterozygote among 39 healthy males over 70 years of age. Heterozygosity may be a predisposing factor in chronic obstructive lung disease, especially in the male population.*

Inherited deficiency of serum α_1-antitrypsin in man is transmitted by an autosomal codominant gene (*1*). The serums of homozygotes for the deficiency gene have persistently low levels of α_1-antitrypsin (mean, 25 ± 6 mg per 100 ml), approximately 10 percent that of normals (212 ± 32 mg) (*2*). Such a very low level of proteinase inhibitor in the serum predisposes an individual to lung disease, as judged by the fact that more than 90 percent of such individuals who have reached the age of 50 years have chronic obstructive lung disease and often well-defined emphysema.

The serums of heterozygotes have intermediate levels of α_1-antitrypsin (125 ± 46 mg per 100 ml); previously heterozygotes were thought not to have a higher incidence of obstructive lung disease than normal individuals (*3*). However, heterozygotes may develop a similar but less severe form of chronic obstructive lung disease (*4*). Lieberman has reported a higher than expected incidence of heterozygosity in patients with emphysema (*5*).

Our data suggest that heterozygosity as well as homozygosity for the α_1-antitrypsin deficiency gene may be a predisposing factor in as many as 30 percent of patients with chronic obstructive lung disease or emphysema. Our working hypothesis is that lung tissue is destroyed in individuals whose serums are deficient in α_1-antitrypsin because of their inability to inactivate proteolytic enzymes released during inflammatory processes in the lung (*6*). Accordingly, we tested for an association between heterozygosity and obstructive lung disease.

We determined the α_1-antitrypsin phenotypes of a group of patients with chronic obstructive lung disease and two control groups. By means of antigen-antibody crossed electrophoresis (*7*), α_1-antitrypsin from homozygotes can be distinguished from that of heterozygotes (Fig. 1). The difference between the two minor α_1-antitrypsin components in serum from homozygotes for the normal gene and heterozygotes for the deficiency gene is small but consistent in all known heterozygotes, that is, offspring from matings between homozygotes for the deficiency gene and homozygotes for the normal gene. The pattern (Fig. 1B) for the heterozygotes can best be explained by the presence of two electrophoretically distinct but antigenically identical species of α_1-antitrypsin in the heterozygote. A mixture of equal parts of serum of the two homozygotes yields the same pattern as that of a heterozygote (*8*). The antigen antibody crossed electrophoresis is a more reliable method for determining the α_1-antitrypsin genotype, as it is based on a qualitative electrophoretic difference and not merely on the serum concentration of α_1-antitrypsin; the concentration can be raised in heterozygotes to the normal range by a variety of pathologic and physiologic conditions such as infection and pregnancy (*9*).

We selected two control groups. Group 1 consisted of 100 healthy individuals (62 males, 38 females); their mean age was 36 years (range 18 to 67 years). Group 2 consisted of 88 older individuals (49 females, 39 males); their mean age was 80 years (range 70 to 97 years). All of group 2 were residents of a home for old people; none had a history of obstructive lung disease.

Group 3 consisted of 76 males and 22 females who had obstructive lung

Table 1. Distribution of the α_1-antitrypsin deficiency gene. Control group 1 consisted of 100 healthy individuals whose mean age was 36 years (range, 18 to 67). Control group 2 consisted of 88 individuals with no respiratory disease, whose mean age was 80 years, the range being from 70 to 97. Group 3 consisted of 98 patients with chronic obstructive pulmonary disease. The mean age was 59 years, the range was 40 to 81 years.

Cases	Homozygotes for the normal gene (No.)	Heterozygotes for the deficiency gene	
		No.	Percent
	Control group 1		
Males	53	9	14.5
Females	33	5	13.2
Total	86	14	14
	Control group 2		
Males	38	1	2.6
Females	42	7	14.3
Total	80	8	9.1
	Group 3		
Males	58	18	23.7
Females	15	7	31.8
Total	73	25	25.5

disease; their mean age was 59 years (range 40 to 81 years). These patients had at least two of the following abnormalities of pulmonary function that persisted after they had inhaled isoproterenol aerosol. (i) The volume of air forcibly expired after maximum inspiration was less than 60 percent of the vital capacity in 1 second, or less than 85 percent of the vital capacity in 3 seconds. (ii) The maximum expiratory flow rate was less than 35 percent of that predicted. (iii) Airway resistance, as determined by constant volume body plethysmography, was greater than the normal range, as defined by Briscoe and DuBois (*10*). (iv) The ratio of residual volume to the total lung capacity was greater than the upper limits of normal for their

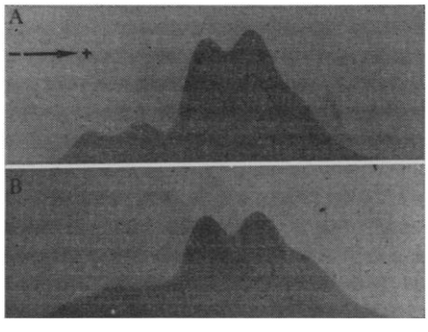

Fig. 1. Antigen-antibody crossed electrophoresis of α_1-antitrypsin in serum of homozygote for normal gene (A) and heterozygote for deficiency gene (B). Normal individuals typically have two small cathodal peaks. Heterozygotes have broader cathodal peaks with maxima in different positions. The faint pointed peak toward the anode in (A) is not α_1-antitrypsin because it differs antigenically.

age. (v) Diffusing capacity measured by the single-breath carbon monoxide test was less than 60 percent of that predicted (*11*). Patients were excluded if there was a known cause for the obstructive lung disease such as tuberculosis, silicosis, sarcoidosis, or well-defined allergic asthma (onset before age 20).

Of the 103 individuals who met these criteria, 5 (4.9 percent) were homozygotes. This incidence is far greater than that expected in a normal population and confirms the reported association between the virtual absence of serum α_1-antitrypsin and the presence of obstructive airway disease. Since the purpose of this study was to examine the association between heterozygosity and obstructive lung disease, these 5 patients were excluded from the following statistical analysis.

Table 1 shows that the percentage of heterozygotes in control groups 1 and 2 (14 and 9.1 percent, respectively) is greater than the previously reported estimates of 2 to 5 percent (*1, 3*) in the general population; the latter figures, however, were based on serum concentrations of antitrypsin rather than the more sensitive qualitative electrophoretic method that we used. We also measured the concentration of α_1-antitrypsin and found normal amounts in 2 of the 14 heterozygotes in control group 1, in 5 of the 8 heterozygotes in control group 2, and in 12 of the 25 heterozygotes with known lung disease, group 3. The normal concentration found in some of the heterozygotes with lung disease could be explained by the known presence of bronchopulmonary infection at the time of blood sampling. These individuals with normal serum concentrations would have been incorrectly classified as homozygotes for the normal gene if only quantitative criteria had been used; they would account, at least in part, for the higher incidence of heterozygosity in this report, particularly in the group with chronic obstructive lung disease.

The frequency of heterozygotes in group 3 (patients with chronic obstructive lung disease) is 25.5 percent. This is significantly greater than in control group 2 ($P < .01$) and in both control groups combined ($P < .005$). The difference is less significant when the numbers of heterozygotes in group 3 and in group 1 are compared ($.05 < P < .1$). The difference in mean age between groups 1 and 3 may be an important factor. We know that homozygotes seldom develop obvious symptomatic lung disease before the age of 30 and we postulate from our recent studies that heterozygotes develop less severe lung disease at a later age (*4*). It is possible that some of the younger heterozygotes included in group 1 may go on to develop lung disease at a later age. This is supported by the fact that only 1 of 39 healthy males over the age of 70 (group 2) was a heterozygote. This low incidence (2.6 percent) differs from that in males in group 3 (24 percent, $P < .01$) and from that in the males in the younger control group 1 (14.5 percent, $.10 < P < .15$).

Such age-related differences were not seen in females; many factors influenced by sex may account for this difference. Cigarette smoking is two times more frequent in males than in females (*12*, p. 26) and contributes to chronic bronchopulmonary inflammation and to obstructive lung disease (*12*, pp. 260–313). Of 115 women in the old people's home only 12, or 9.6 percent, had smoked cigarettes; of 55 men, 36 percent were moderate to heavy cigarette smokers, and 84 percent had a history of smoking cigarettes, cigars, or pipes. Of the 8 older heterozygotes in group 2, only 2 were light cigarette smokers. Furthermore, of the 25 heterozygotes with obstructive lung disease in group 3 only 2 were over 70 years of age, both were female and both were nonsmokers; all of the remaining 23 were cigarette smokers. Therefore, it is possible that the very low incidence of healthy male heterozygotes over the age of 70 may be explained by their selective predisposition to obstructive lung disease related to cigarette smoking.

Should studies of larger populations confirm this high incidence of α_1-antitrypsin deficiency and indicate that such a deficiency renders an individual more susceptible to the harmful effects of cigarette smoke and predisposes him to obstructive lung disease, a large-scale detection program would be useful in preventing the onset of this largely irreversible and increasingly common disease.

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Ascorbic Acid: Cofactor in Rabbit Olfactory Preparations

Abstract. *A stimulant-induced decrease in absorbance at 267 nanometers in preparations from rabbit olfactory epithelium requires ascorbic acid as a cofactor. Vitamin C is bound to proteins in the olfactory mucosa in vivo. When the stimulant 3,7-dimethyl-6-octen-3-ol interacts with olfactory proteins, it triggers a change in protein conformation which renders ascorbic acid available for oxidation. The specific decrease in absorbance at 267 nanometers is produced when the "freed" ascorbic acid is oxidized.*

Ascorbic acid was identified as a cofactor required for the stimulant-induced changes observed in preparations from rabbit olfactory epithelium. The stimulant-induced changes were monitored with ultraviolet difference-spectroscopy techniques (*1*). Absorbance changes induced at 267 nm were described (*2*); linalool and linalyl isobutyrate were the stimulants. The decrease in specific absorbance at 267 nm was also induced by 3,7-dimethyl-6-octen-3-ol (DMO).

The olfactory preparations contained a cofactor of relatively low molecular weight; it was required for the stimulant-induced decreases in absorbance at 267 nm. Activity was lost when the cofactor was separated from protein fractions of high molecular weight. Several attempts (with dialysis and gel filtration) to isolate the active cofactor and add it to the protein fractions failed because the cofactor was inactivated during isolation. The cofactor was identified as ascorbic acid.

When freshly prepared ascorbic acid solutions were mixed with the olfactory preparations, the stimulant-induced activity at 267 nm was increased. Addition of ascorbic acid also restored the activity in preparations which were depleted of cofactor by dialysis or gel filtration. In the representative experiment ascorbic acid was added to the olfactory preparation to a final concentration of $5.0 \times 10^{-5}M$; the absorbance change induced by DMO was more than doubled (Fig. 1). The change after 30 minutes was 0.041 absorbance units compared to 0.100 units for the duplicate reaction mixture containing $5.0 \times 10^{-5}M$ additional ascorbic acid (Fig. 1a). After the reaction period the absorbance spectra of the reaction mixtures from 240 to 310 nm were determined approximately 40 minutes

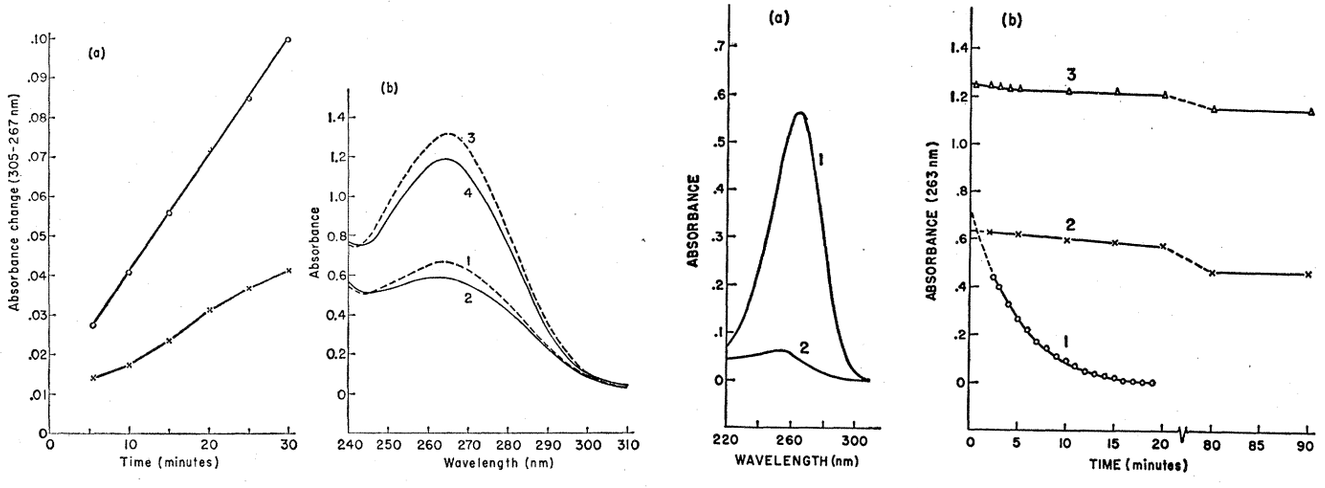

Fig. 1 (left). Effect of ascorbic acid on stimulant-induced absorbance change in olfactory preparations (a) and the absorbance spectra of the olfactory preparations (b). The reaction mixtures each contained 0.70 mg of protein in a final volume of 3 ml. Where added, the final concentration of ascorbate was $5 \times 10^{-5}M$, and final concentration of the stimulant (3,7-dimethyl-6-octen-3-ol) was $3.0 \times 10^{-3}M$. The *p*H was 7.0 and controlled by $0.07M$ phosphate buffer. For the lower curve of part (a) the reaction mixtures combined olfactory preparation, stimulant, and phosphate buffer. The only change for the upper curve was the addition of ascorbate prior to addition of the stimulant. For the absorbance spectra in part (b) the following components were compared after a reaction period of approximately 40 minutes: curve 1, olfactory preparation and buffer are plotted against buffer; curve 2, olfactory preparation, buffer, and DMO are plotted against buffer and DMO; curve 3, olfactory preparation, buffer, and ascorbate are plotted against buffer; curve 4, olfactory preparation, buffer, ascorbate, and DMO are plotted against buffer and DMO. Fig. 2 (right). Oxidation of ascorbate and the stabilizing of proteins. (a) The spectrum of $5 \times 10^{-5}M$ ascorbate in $0.09M$ phosphate buffer, *p*H 7.0, was determined approximately 1 minute after preparation; curve 1 of part (a); curve 2 is the same solution after having been shaken with air for 3 minutes. (b) The reaction mixture for curve 1 contained $5 \times 10^{-5}M$ ascorbate in phosphate buffer. For curve 2 the reaction mixture also contained 0.3 mg of bovine serum albumin per milliliter, and for curve 3 it contained olfactory preparation (0.43 mg of protein per milliliter) in addition to the ascorbate. The absorbance was followed on the Cary 14 spectrometer.