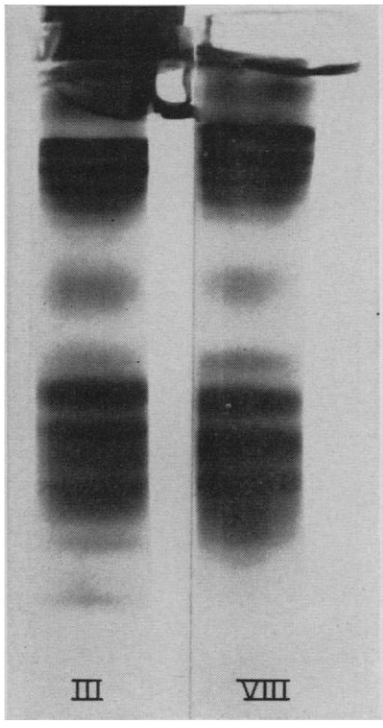

Fig. 2. Disc-gel electrophoresis of heavy and light chains of type III and type VIII antibodies. Each sample in the gel was equivalent to the heavy and light chains obtained from 200 μg of antibody.

order to exclude the possibility of appreciable contamination of these precipitates with nonspecific γ-globulin, ^{125}I-labeled γ-globulin was added to the serum before precipitation. There was less than 4 percent contamination of the precipitate.

The antigen-antibody precipitate was suspended in 1*M* tris chloride, *p*H 8.2, and sufficient solid guanidine hydrochloride was added to give a final concentration of 7*M*, whereupon the precipitate dissolved. Reduction and alkylation was carried out (*12*), the reaction mixture was dialyzed overnight against 1000 volumes of 8*M* urea, and, after electrophoresis (*3*), the gels were stained with Coomassie brilliant blue (*13*). Both pneumococcal polysaccharides, when treated in the same manner as the antigen-antibody precipitate and subjected to electrophoresis, did not show any staining with the dye.

In the electrophoretic pattern of heavy and light chains of the antibodies, for which the binding analysis was described above, the upper cluster of bands represents the heavy chains and the lower cluster represents the light chains (Fig. 2). Considerable electrophoretic heterogeneity is evident throughout. The background stain between the bands probably represents unresolved components of heavy and light chains since this background stain was also observed when one-fifth the quantity of reduced and alkylated antibody was applied.

Whereas the binding data indicate functional homogeneity of the binding site, the electrophoretic data show substantial structural heterogeneity of these antibodies. This apparent discrepancy may be reconciled in two ways. There may be amino acid sequence homogeneity in the region of the binding site, the electrophoretic heterogeneity observed being the result of amino acid sequence heterogeneity in parts of the molecule not essential to the structure of the binding site. The alternative possibility is that the binding homogeneity is only apparent because of a lack of sensitivity in the equilibrium dialysis method. For example, if one or two residues of the oligosaccharide ligand were immunodominant, these residues could make a major contribution to the binding energy (*14*). Heterogeneity of binding energy attributable to the other residues would not be detected.

A choice between these two and other possible explanations cannot be made until the amino acid sequence in the combining site region of an antibody is identified and the relation to the sequence of the remainder of the molecule is determined. It is, however, apparent that binding homogeneity does not necessarily indicate structural homogeneity.

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References and Notes

1. H. N. Eisen and F. Karush, *J. Amer. Chem. Soc.* **71**, 363 (1949); F. Karush, *ibid.* **78**, 5519 (1956); A. Nissonoff and D. Pressman, *J. Immunol.* **80**, 417 (1958); H. N. Eisen and G. W. Siskind, *Biochemistry* **3**, 996 (1964).
2. G. M. Edelman, B. Benacerraf, Z. Ovary, M. D. Poulik, *Proc. Nat. Acad. Sci. U.S.* **46**, 1751 (1961).
3. R. A. Reisfeld and P. A. Small, Jr., *Science* **152**, 1253 (1966).
4. M. D. Mamet-Bratley, *Immunochemistry* **3**, 155 (1966); A. Nissonoff, S. Zappacosta, R. Jureziz, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 89 (1967); E. Haber, F. F. Richards, J. Spragg, K. F. Austen, M. Vallotton, L. B. Page, *ibid.*, p. 299.
5. A. M. Pappenheimer, Jr., W. P. Reed, R. J. Brown, *J. Immunol.* **100**, 1237 (1968).
6. B. A. Askonas, C. P. Farthing, J. H. Humphrey, *Immunochemistry* **3**, 336 (1966).
7. F. Kauffmann, M. Bjorneboe, B. Vammen, *Z. Hyg. Infektionskrankh.* **121**, 36 (1938).
8. J. H. Campbell and A. M. Pappenhéimer, Jr., *Immunochemistry* **3**, 195 (1966).
9. The structures of the ligands (before reduction) were as follows. M. Heidelberger [*Annu. Rev. Biochem.* **36**, 1 (1967)] gives structure of the repeating units of the polysaccharide.

Type III =

$$\left[\rightarrow 3)\text{-}\beta\text{-D-glcA}\ (1\rightarrow 4)\text{-}\beta\text{-D-glc}\ (1\right]_3$$

or

$$\left[\rightarrow 4)\text{-}\beta\text{-D-glc}\ (1\rightarrow 3)\text{-}\beta\text{-D-glcA}\ (1\right]_3$$

Type VIII =

$$\left[\rightarrow 4)\text{-}\beta\text{-D-glcA-}(1\rightarrow 4)\text{-}\beta\text{-D-glc}\ (1\rightarrow\right.$$
$$\left.4)\text{-}\alpha\text{-D-glc-}(1\rightarrow 4)\text{-}\alpha\text{-D-gal-}(1\right]_2$$

where glc is glucose, glcA is glucuronic acid, and gal is galactose.
10. M. Katz, and A. M. Pappenheimer, Jr., in preparation.
11. F. Karush, *Advan. Immunol.* **2**, 1 (1962).
12. P. A. Small and M. E. Lamm, *Biochemistry* **5**, 29 (1966).
13. A. Chrambach, R. A. Reisfeld, M. Wyckoff, J. Zaccari, *Anal. Biochem.* **20**, 150 (1967).
14. W. F. Goebel, O. T. Avery, F. H. Babers, *J. Exp. Med.* **60**, 599 (1934).
15. R. G. Mage and E. A. Kabat, *Biochemistry* **2**, 1278 (1963).
16. Supported by PHS grants HE-5196-11, AI-04967, and HEP-06664 and NSF grant G-15836.

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Selective Venting of Cigarette Smoke in Dichotomous Ducts and Preserved Human Bronchi

Abstract. *Mechanically generated cigarette smoke and ambient air were injected into dichotomous ducts and geometrically preserved human bronchi in a fashion simulating typical smoking technique. When the air passages were at ambient temperature, the smoke settled into the lower branches. Smoke which was injected into passages warmed to body temperature rose to the upper branches. The latter selective distribution of the smoke resembled the distribution of centrilobular emphysema in the lungs.*

The chemistry of cigarette smoke has been studied extensively (*1*), but little is known of its physical characteristics. These characteristics could be linked to the pathogenesis of disease, for instance, emphysema.

In order to simulate the pathway of smoke in the human lungs, the following apparatus was used: (i) glass Y-tubular couplings, or (ii) normal human lungs which were fume-fixed, inflated, and hardened (*2*). A portion of the cortex of the lungs, 5 cm wide and 2 mm thick, was removed from the frontal projection. This caused the air passages, which were about 1 mm in diameter, to be exposed from the apices to the bases of the upper and lower lobes.

To simulate the typical pattern fol-

lowed by smoke from a cigarette, mechanically generated smoke was injected into the ducts and preserved bronchi in 2-second puffs. The smoke injection was followed by several hundred cubic centimeters of ambient air. The behavior of the smoke was visually assessed and recorded photographically (Figs. 1 and 2).

The temperature of the glass ductal systems and bronchi was crucial. At ambient temperature (approximately 26°C), smoke injected into passages would settle predominantly into lower routes (Figs. 1A and 2A). In contrast, smoke blown into passages heated to body temperature (37°C) would ascend into upper routes with considerable regularity (Figs. 1B and 2B).

The smoke bolus in the clear tubing tended to stay separate from surrounding or accompanying air even though it was changing in shape. In keeping with Liouville's theorem (*3*), inhaled cigarette smoke should act as a body until thermal transfer has brought it into temperature equilibrium with its attendant air.

With almost every rate of smoke injection into the warmed passages, the smoke went predominantly into the upper passages. This was most evident when the influx was very slow. However, with extremely vigorous injection, the impetus of the smoke overcame its relative buoyancy, and it was distributed fairly evenly to all channels. Since the total cross-sectional area of the air passages in the human lung increases through progressive branchings (*4*), flow rates decrease progressively toward the distal lung areas. This could enhance superior venting of smoke in man.

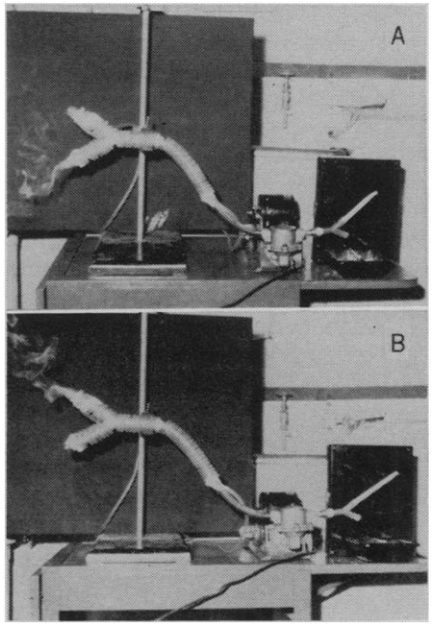

Fig. 1. Routing of mechanically generated smoke (via a fuel pump actuated by a cam driven off an electric motor) in dichotomous ducts. After a 2-second puff from the cigarette, ambient air was pumped through the unoccluded lower arm of Y branch holding cigarette. (A) With tubing at ambient temperature of 26°C, smoke sought the lower route; (B) in tubing heated to 37°C with a coil of circulating warm water, smoke traveled almost exclusively into upper arm.

The behavior of residual smoke after injection into the systems warmed to body temperature was also of interest. During convection and gas phase diffusion (corresponding to the resting volume of human lungs during quiet tidal exchange), the smoke that had originally rested in the lower areas of the test apparatus promptly took a reverse upward drift. It may be mentioned here with pertinence that substantial amounts of smoke remain within the residual air of human lungs.

Centrilobular emphysema occurs predominantly in the upper lobes and the apices (*5*), and it has also been found most frequently in smokers (*6–8*). By contrast, panacinar emphysema is distributed unselectively and relatively diffusely (within lobules and lobes or lungs) (*5, 8*) and occurs with random frequency in both smokers and nonsmokers.

Selective distribution of a lesion (such as centrilobular pulmonary emphysema) in an organ, theoretically might result from (i) local tissue weakness or (ii) selective impact. The generally even distribution of panacinar emphysema is evidence against the former explanation of local tissue predisposition with respect to emphysema. Predilection of dependent lung areas to aspiration pneumonia is an uncontested example of the latter concept of disease developing from selective exposure. In aspiration pneumonia, pathogens heavier than inhaled air settle in the lungs. In centrilobular emphysema, injurious agents more buoyant than inhaled air, for example, warm cigarette smoke, might be selectively vented to upper lung reaches. Moreover, the relative density of cigarette smoke to inhaled air may be only one of the mechanisms in any presumed "clouding" phenomenon. Studies with radioactive xenon have shown selective flow of that agent to apical lung zones. This uneven flow could be a consequence of (i) regional differences in pleural pressure brought about by gravitational forces (*9*) and (ii) variations in elastic properties between apical and basilar areas (*10*). Other factors, for example, comparative bronchial diameters and angles, may also contribute.

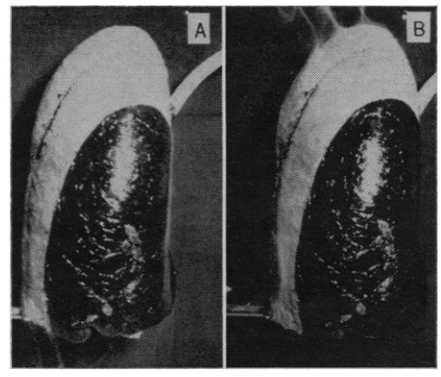

Fig. 2. Routing of mechanically generated cigarette smoke in geometrically preserved bronchi in hardened, expanded, fume-fixed lungs (outer 1 to 2 mm decorticated in 5-cm frontal projection; distance between cigarette butt and origin of mainstem bronchus in this preparation was 25 cm, with a turn of 90° about 10 cm from butt, corresponding roughly to circuit in man). (A) With bronchopulmonary structures at 26°C, smoke settled predominantly into lower air passages; (B) immediately after being warmed to 37°C, practically all of the smoke traveled upper routes.

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References and Notes

1. Advisory Committee to the Surgeon General of the Public Health Service, "Smoking and Health," *Public Health Serv. Publ.* No. 1103, (1964); "The Health Consequences of Smoking," *Public Health Serv. Publ.* No. 1696 (1967; Rev. January 1968).
2. B. J. Blumenthal and H. G. Boren, *Amer. Rev. Tuberc.* **79**, 764 (1959).
3. R. C. Tolman, *Principles of Statistical Mechanics* (Oxford Univ. Press, London, 1938).
4. H. von Hayek, *The Human Lung* (Hafner, New York, 1960).
5. J. A. Hernandez, A. E. Anderson, Jr., W. L. Holmes, A. G. Foraker, *Geriatrics* **21**, 155 (1966).
6. A. E. Anderson, Jr., J. A. Hernandez, P. Eckert, A. G. Foraker, *Science* **144**, 1025 (1964).
7. A. E. Anderson, Jr., J. A. Hernandez, W. L. Holmes, A. G. Foraker, *Arch. Environ. Health* **12**, 569 (1966).
8. J. Gough, in *The Lung*, A. A. Liebow and D. E. Smith, Eds. (Williams & Wilkins, Baltimore, 1968), p. 109.
9. J. Milic-Emili, J. A. M. Henderson, M. B. Dolovich, D. Trop, K. Kaneko, *J. Appl. Physiol.* **21**, 749 (1966).
10. B. Bake, J. Bjure, G. Grimby, J. Milic-Emili, N. J. Nilsson, *Scand. J. Resp. Dis.* **48**, 189 (1967).
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