value for the area in Fig. 2, just 5 km from Mount San Salvatore, we obtain the following probability for a strike 60 m from the observation point within the 35° camera field of view.

$$(1.1 \text{ flashes/yr } 10^6 \text{ m}^2)\ (\pi\ 60^2 \text{ m}^2/10) = 1.2 \times 10^{-3} \text{ flashes per year}$$

Thus, the probability of obtaining the close lightning photograph in Fig. 1 is about 10^{-3}, or once in 1000 years, at this site.

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1 August 1968

Antibodies to Pneumococcal Polysaccharides: Relation between Binding and Electrophoretic Heterogeneity

Abstract. *Antibodies to type III and type VIII pneumococcal polysaccharides were examined with respect to ligand binding and electrophoretic heterogeneity. Both antibodies showed apparent binding homogeneity, although multiple light chain and heavy chain electrophoretic species were demonstrated.*

Antibody heterogeneity is evidenced by the diversity of functional and structural properties in a given antibody population. The former is exemplified by the spectrum of binding energies observed in antibodies to haptens (*1*), and the latter in the electrophoretic heterogeneity of antibody heavy and light chains (*2, 3*).

Although several antibodies bind a hapten homogeneously (*4, 5*), criteria of structural homogeneity have not been applied to these. While it is reasonable to assume that structurally homogeneous antibodies should exhibit binding homogeneity, the reverse need not be true. Thus, a comparison of a criterion of structural homogeneity, the electrophoretic pattern of antibody heavy and light chains with the dispersion of hapten-binding constants, was made.

Rabbit antibodies to type III and type VIII pneumococcal polysaccharides were studied for the following reasons. (i) The polysaccharides are composed of linear repeating subunits and are similar in composition to one another; (ii) antibodies specific for one of these polysaccharides will not precipitate with the other; (iii) antibodies are produced in high titers (*6*); and (iv) labeled oligosaccharide ligands are available.

New Zealand white rabbits, homozygous for allotypes a_1 and b_4, were injected three times weekly with formalinized pneumococci (*7*) of either type III or type VIII. The initial dose of 2×10^9 organisms was increased each week, the final dose of 1.6×10^{10} organisms being given the 3rd week. Rabbits were bled on the 24th day. Precipitation analysis, carried out on selected serums from individual rabbits with purified polysaccharides (*8*), yielded 11.2 mg of precipitable antibody per milliliter of type III serum, and 12.3 mg per milliliter of type VIII serum.

Binding analysis was carried out as described (*5*). The ligands were two hexasaccharides that were derived from type III polysaccharide (*9*) and contained either a terminal reducing glucuronic acid or glucose residue that had been labeled by reduction to the alcohol with tritiated sodium borohydride (*10*), and an octasaccharide that was derived from type VIII polysaccharide (*9*) and contained tritiated deoxyglucuronic acid at its nonreducing end (*5*). The octasaccharide has already been used as a ligand to demonstrate homogeneous binding by horse and rabbit antibodies to type VIII pneumococcal polysaccharide (*5*). The results were analyzed according to the Sips equation (*11*)

$$\log \frac{r}{n-r} = a \log c + a \log K$$

where r is the number of moles of hapten bound per mole of antibody, n is the total number of binding sites (two for antibodies), c is the free hapten concentration, K is the average association constant, and a is the heterogeneity index. When a is 1 there is binding homogeneity; when a is less than 1 there is binding heterogeneity. Both preparations exhibit homogeneity of binding within the limits of experimental error (Fig. 1).

For disc-gel electrophoresis, antibodies were specifically precipitated at equivalence from serum with the appropriate polysaccharide. The precipitates were washed twice with a solution containing 0.15*M* sodium chloride and 0.01*M* sodium phosphate, *p*H 7.4. In

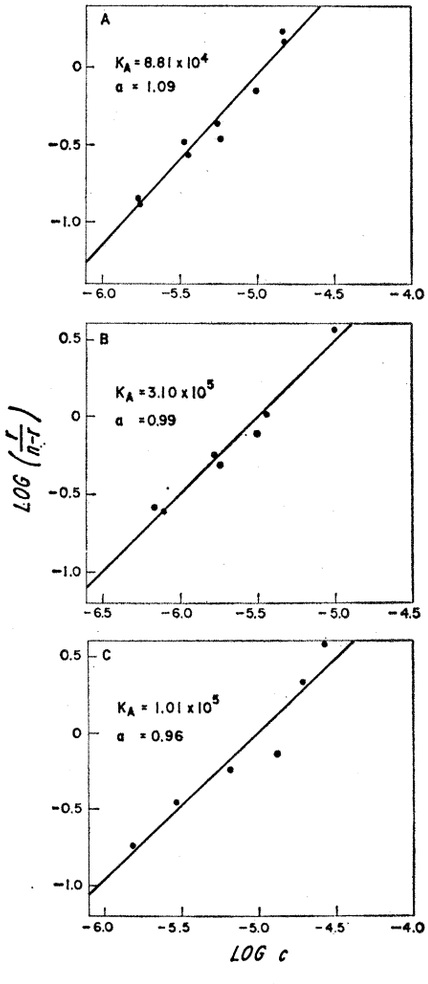

Fig. 1. Sips plots of antibodies to type III and type VIII pneumococcal polysaccharides. The ligands used were tritiated oligosaccharides. (A) Type VIII; (B) type III determined with a ligand containing a nonreducing glucuronic acid end group; (C) type III determined with a ligand containing a nonreducing glucose end group (*9*). The antibody concentration was determined by quantitative precipitation. The data was calculated with the aid of an SDS 940 computer, and the lines were drawn by the method of least squares; K_A is the average association constant in liters per mole.

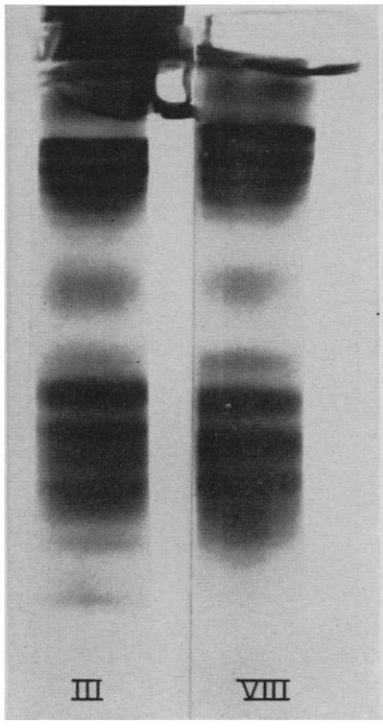

Fig. 2. Disc-gel electrophoresis of heavy and light chains of type III and type VIII antibodies. Each sample in the gel was equivalent to the heavy and light chains obtained from 200 μg of antibody.

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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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.
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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-