

This Week's Citation Classic

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Fleischman J B, Porter R R & Press E M. The arrangement of the peptide chains in γ -globulin. *Biochemical J.* **88**:220-8, 1963.
[Department of Immunology, St. Mary's Hospital Medical School, London, England]

This paper describes the isolation and biochemical properties of the polypeptide chains of rabbit IgG antibodies. Based on these data, the paper proposed a structural model for the antibody molecule made up of four polypeptide chains (two heavy and two light). [The SC[®] indicates that this paper has been cited over 465 times since 1963.]

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"It is a pleasure to learn that this paper emerges as one of the most frequently cited according to the *Science Citation Index*[®]. The paper proposed a structure for the anti-body molecule; it was based on work in R.R. Porter's laboratory in the early 1960s. I think the main reason that this paper was the one most cited is that it unified the known structural features of antibodies into a consistent model.

"The background for the work was (a) Porter's 1959 discovery that rabbit antibody could be split by papain into three fragments (two Fab plus one Fc), with an intact antigen-combining site in each Fab fragment,¹ and (b) Edelman's simultaneous proof that the molecule was made up of more than one polypeptide chain.² I started a postdoctoral fellowship in Porter's laboratory at St. Mary's in London in the autumn of 1961. The objective at that stage was to separate and recover the antibody polypeptide chains in a soluble form so that they could be properly characterized. Porter had exploited Cecil and Wake's observation that interchain disulfide bonds were reducible under mild conditions.³ This proved the key to separating the heavy and

light chains of rabbit antibody and recovering them in soluble form. I had brought to the lab some goat antisera to the Fab and Fc fragments which I had helped prepare in Melvin Cohn's lab at Stanford. (Our most pungent scrub goats made by far the best antisera—the titers were so high that Boris, the biggest and ripest goat, keeled over in anaphylactic shock after his last boost.) The antisera proved most useful in demonstrating that both heavy and light chains were present in Fab, but only heavy chains were in Fc—an important clue to the architecture of the molecule. St. Mary's had no goats but propionic, butyric, and valeric acids (which we used to separate chains), laced with smog from Paddington Station, provided the olfactory stimulation.

"Figuring out the four-chain structure of the antibody molecule had a trace of the 'double-helix' element to it. We had to arrange the chains according to their distribution in Fab and Fc, and we also knew that similar efforts were under way at the Rockefeller Institute. We each promised to spend the weekend independently trying to work out a model. I had a couple of headscratching days in Hampstead, and we met in Porter's office on Monday.

"The model we came up with is the fourchain structure illustrated in our paper. I remember stressing Nisonoff's critical observation that pepsin digestion of the antibody molecule left the two Fabs intact and linked by a single disulfide bond. This forced us to line up the two Fabs, each with a single antigen-combining site, next to each other, giving us the now-familiar Y-shaped molecule. We reluctantly discarded the then-popular cigar-shape with combining sites at either end, a favorite among both immunologists and textbook illustrators at the time. I think that the main strength of our model was that it provided a basic pattern for the many different classes of vertebrate antibodies studied since that time."⁴

1. **Porter R R.** The hydrolysis of rabbit gamma-globulin and antibodies with crystalline papain. *Biochemical J.* **73**:119-27, 1959.
2. **Edelman G M.** Dissociation of gamma-globulin. *J. Amer. Chem. Soc.* **81**:3155-6, 1959.
3. **Cecil R & Wake R G.** The reactions of inter- and intra-chain disulfide bonds in proteins with sulphite. *Biochemical J.* **82**:401-6, 1962.
4. **Nisonoff A, Hopper J E & Spring S S.** *The antibody molecule.* New York: Academic Press, 1975. 542 p.