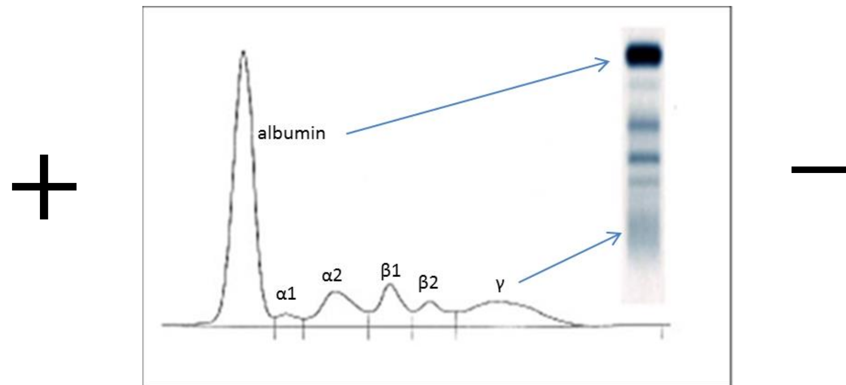
ANTIBODY STRUCTURE

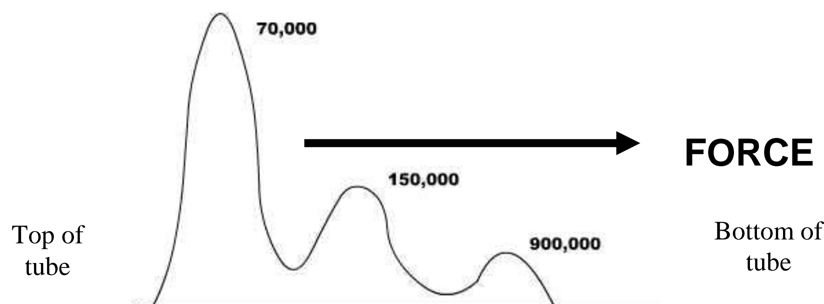
ANTIBODY IS IMMUNOGLOBULIN. Almost 130 years ago it was observed that a new activity appeared in the blood plasma of animals or humans who had been immunized with a bacterial toxoid (an inactivated but still immunogenic form of a toxin). These plasmas could neutralize the related toxin so that it was no longer harmful to experimental animals, and they also ►yielded a precipitate when mixed with a solution of toxoid or toxin. The new activity was called **antibody**. At the time no one had any idea about its nature—not even that it was composed of protein.

With time, new techniques for characterizing large molecules like proteins were developed. Among these was **electrophoresis**, which in its simplest form separates proteins on the basis of net electrical charge. At pH 8.2 or so, most –COOH groups are $–COO^-$ (ionized,) and most NH_3^+ are NH_2 (not ionized), so many proteins have a net negative charge (are anionic) and will migrate towards an anode. If serum is separated in an electrical field, the proteins segregate into an **albumin** and α/β/γ **globulin** bands:



Antibody activity was found mostly in the gamma (γ) globulin zone; antibodies were called, and still sometimes are, *gamma globulin* or *immune globulin*. Since some activity was also located in the beta region, it was decided to come up with a better generic term: **immunoglobulin**. It had also become clear that 'antibody' was not a single substance. The antibodies to one toxin did not react with a different toxin, so that antibodies differed in **specificity**.

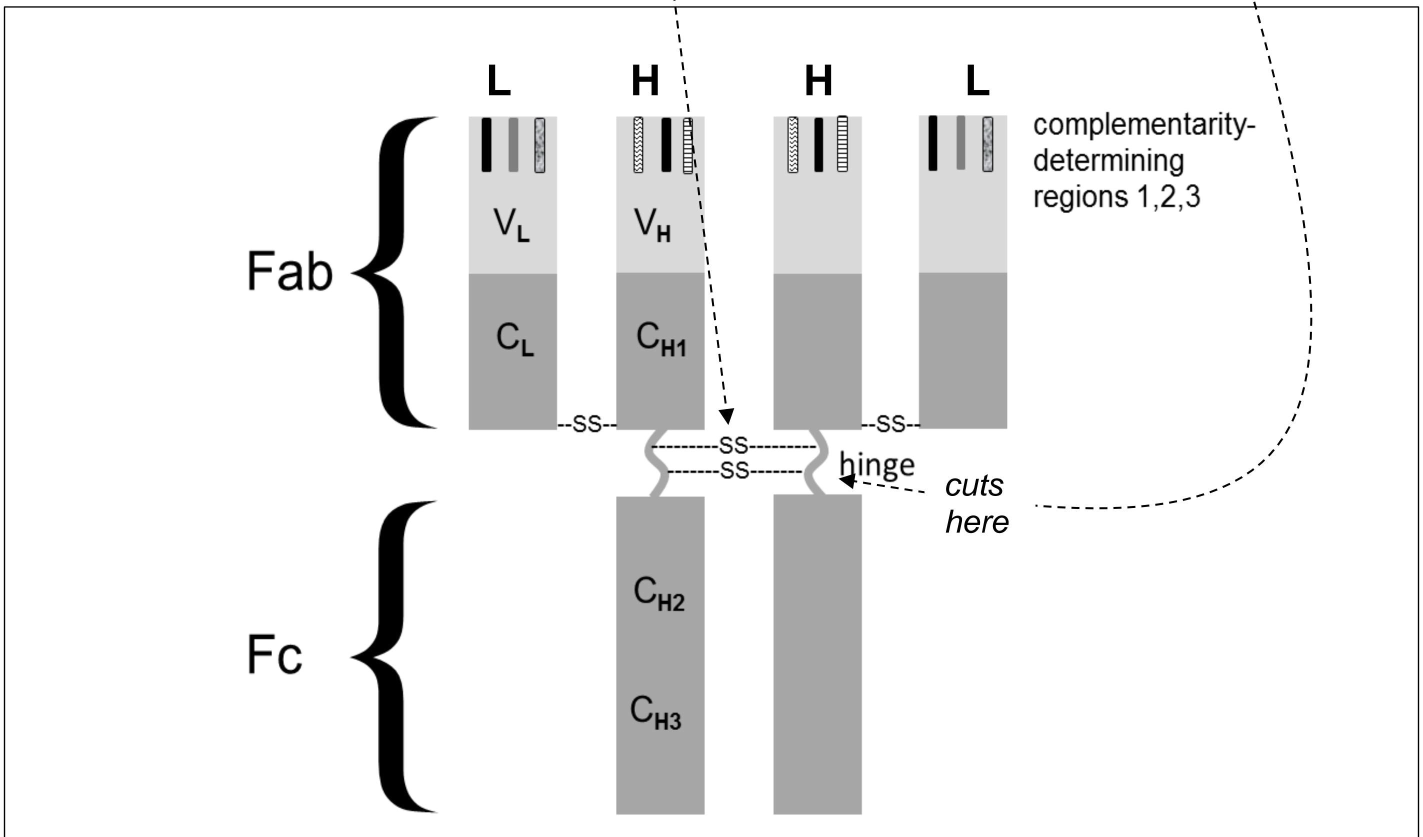
In addition, there were classes of antibody that could be separated physically, for example by size in the ultracentrifuge, which can actually spin molecules out of solution. This is a typical snapshot of serum proteins spinning down during a run in an analytical ultracentrifuge:



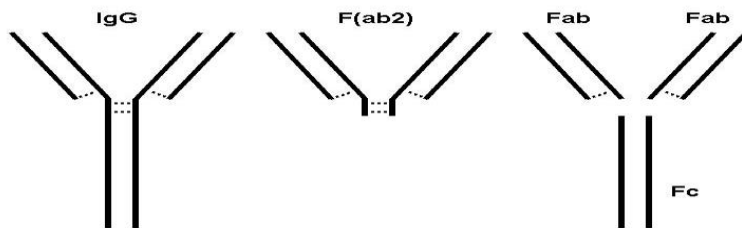
Antibody to a particular toxoid could be found in both the 150,000 MW and 900,000 MW peaks (the 70,000 MW peak was mostly albumin). The 150,000 MW was gamma globulin, so the new heavy antibody was named *macroglobulin*.

ANTIBODY STRUCTURE. To get at the structure of these large molecules it was necessary to disassemble them into smaller, workable pieces. The first good attempt at this was published by R. R. Porter in 1959, who treated antibody (mostly gamma globulin) with the enzyme papain and an agent that reduced *some* disulfide bonds (-S---S- reduced to -SH HS-) and found that the antibody broke up into 3 fragments: two identical ones that are now each called **Fab**, and one **Fc**. Around the same time G. Edelman treated antibody with a strong reducing agent that broke *all* interchain disulfide bonds, and with a denaturant to straighten out and separate the polypeptides. He found that the molecule was composed of two identical **light** (MW about 25,000) and two identical **heavy** (MW about 50,000) chains. These studies led to the complete determination of the structure of immunoglobulins, and Porter and Edelman shared the Nobel Prize in 1972.

A TYPICAL IMMUNOGLOBULIN MOLECULE



If you adjust conditions during antibody digestion with proteolytic enzymes, you can get 2 Fab fragments (S—S bonds between the H chains fully reduced) or you can leave the 2 Fabs still joined; that's called $F(ab)_2$. Fab is univalent; like IgG, $F(ab)_2$ is divalent.



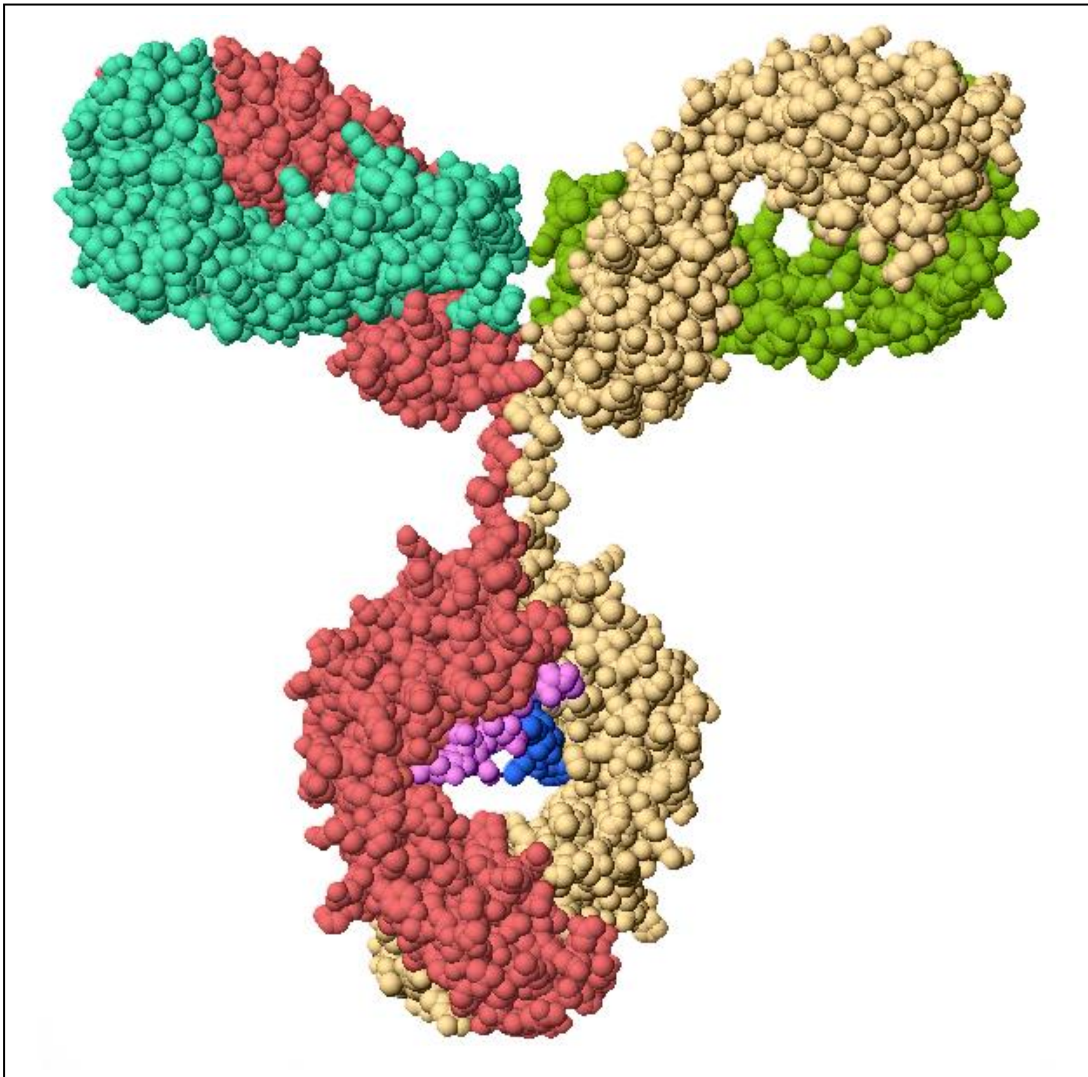
This is the structure of an IgG molecule as deduced from X-ray crystallography; each ball is an atom.

Each chain is composed of **domains**, compact areas held together by intrachain S---S bonds.

Light chains have one variable domain, V_L, and one constant domain, C_L.

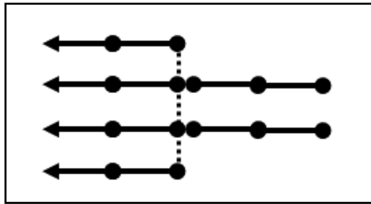
Heavy chains have one variable domain, V_H, and 3-4 constant domains, C_{H1}, C_{H2}, C_{H3}, (C_{H4}). There is considerable structural homology between different domains, which suggests that there was once an ancestral gene for one domain, which has duplicated many times.

There is a patch of carbohydrate (purple & blue in this picture) that pushes out the second constant domains (C_{H2},) of the heavy chains, allowing them to interact more easily with components of the **complement** system, which is how antibodies initiate inflammation (*see* Antibody Function).



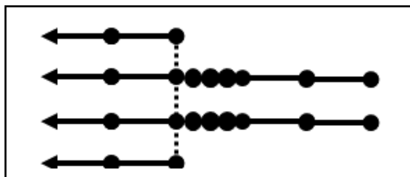
ASK YOURSELF: The above picture is a crystal structure of IgG. Color and/or label Heavy and Light chains, V_H, C_{H1}, C_{H2}, and C_{H3} domains, as well as V_L and C_L, the hinge, Fab, F(ab)2, Fc, and carbohydrate.

5 CLASSES OF IMMUNOGLOBULINS. Because of extensive gene duplication, we have 5 classes of immunoglobulins, all clearly derived from a common ancestor but each differentiated to carry out unique functions.



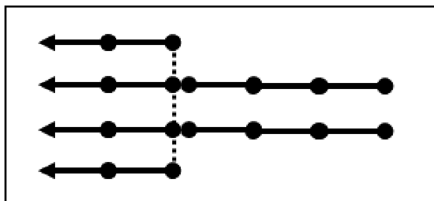
IgG. 2 light chains, and 2 gamma (γ) chains.

Molecular weight 150,000.



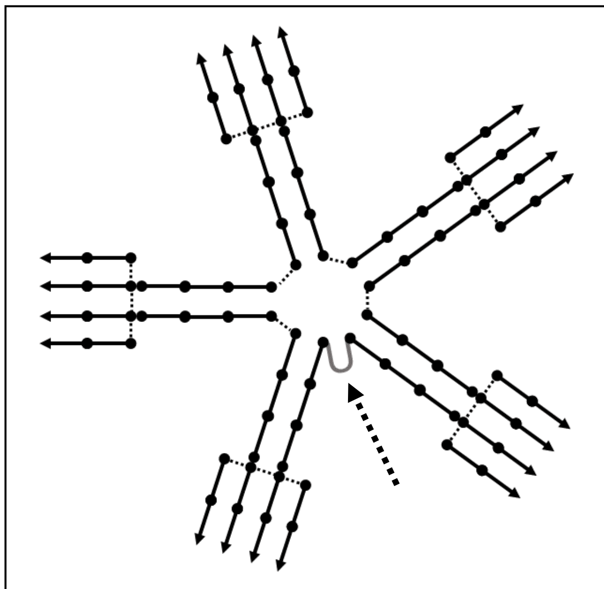
IgD. 2 light and 2 delta (δ) chains.

Extra-long hinge region; molecular weight 180,000.

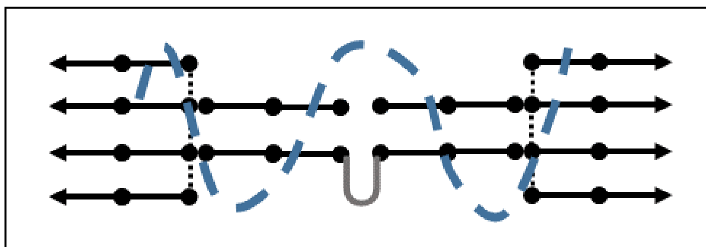


IgE. 2 light and 2 epsilon (ε) chains.
An extra constant domain, CHε4 and a lot of sugars.

Molecular weight 190,000.



IgM. In blood as a pentamer of a basic unit which is 2 light chains and 2 mu (μ) chains.
μ has an extra CHμ4 domain.
Linked by S-S bonds and closed by a J chain (arrow).
Molecular weight 900,000.

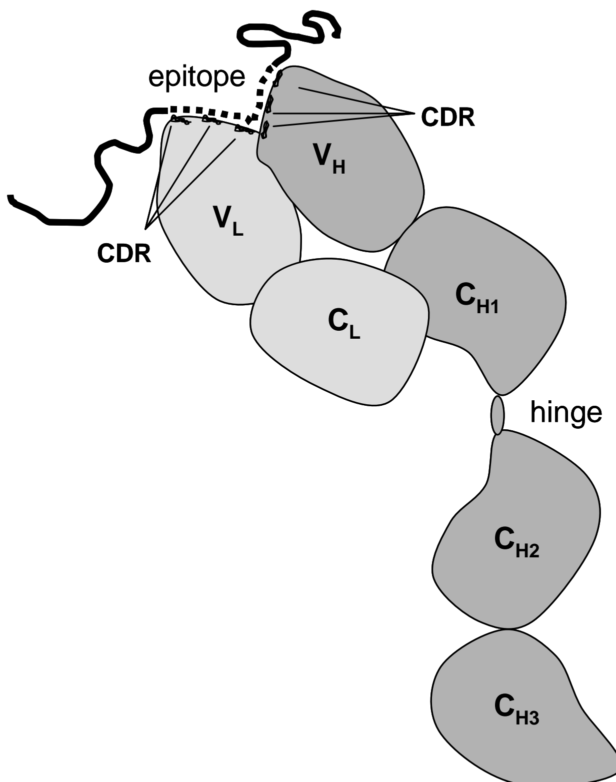


IgA. Secreted form is a dimer; basic unit is 2 light chains and 2 alpha (α) chains.
Joined by a J chain, and wrapped by Secretory Component.
Molecular weight 400,000.

In summary: ►each molecule is made up of a basic unit consisting of four chains: 2 heavy (H) and 2 light (L) chains. Secreted IgA is a dimer of two of these basic units, and IgM is a pentamer. The basic units in IgA and IgM are held together by J chains, and IgA is wrapped by Secretory Component. ►*In any one antibody, the H chains are identical, and so are the L chains*, so the molecule has rotational symmetry. The 5 kinds of H chains (gamma, alpha, mu, epsilon, delta) define the class of antibody to which the molecule belongs, and therefore its biological properties.

KAPPA AND LAMBDA CHAINS. L chains come in 2 varieties: kappa or lambda (the original light chain gene family has duplicated long ago). Although each cell that makes an antibody has a choice of using kappa or lambda, it uses only one kind. So, for example, an IgA molecule will be kappa *or* lambda type, while another IgA might be the other. As we'll discuss soon, a single cell may switch from making, say, IgM to making IgA. ►When this happens, ***the heavy chain changes (mu replaced by alpha) but the L chain, either kappa or lambda, stays the same during the switch.***

Examine the diagram below. When the amino acids in many antibody molecules are sequenced, one finds that for each chain type there is a region that is essentially identical, no matter what the specificities of the antibodies were. This is the **constant region**, and it is made up of 1 (in L chains) to 4 (in epsilon and mu) compact, structurally-similar domains called C domains. Each chain also has, at its N-terminal, a domain that is different in sequence between antibodies of different specificities: the **variable domain** or V. The antibody's combining site, which binds antigen, is made up of the V domains of both the H and L chain (V_H and V_L.)



Amino acid sequence variability is not distributed uniformly along the V domain; most of the variability is in 3 areas called, therefore, **hypervariable** regions. It is more functionally significant to call them **►CDR**, **complementarity-determining regions**, because the amino acids in the hypervariable regions comprise the actual antigen-binding site.

ASK YOURSELF: Valence refers to the number of antigenic determinants an antibody molecule can theoretically bind. What is the valence of IgG? of secreted IgA? of IgM? of Fab? of $F(ab')_2$? How about of an isolated V_L or V_H?

SUBCLASSES OR ISOTYPES. On the basis of slight differences in the amino acid sequences of their H chain C regions, the 5 main classes of immunoglobulins are divided into subclasses:

IgG1, IgG2, IgG3, IgG4
IgA1, IgA2
IgM1, IgM2
IgD
IgE

So there are actually 10 subclasses or isotypes. It is as if very long ago there was a gene for one heavy chain, which was duplicated and reduplicated in early vertebrate genomes. Then each copy could evolve at its own rate, and the H chains they coded for would gradually acquire different functions. If this is so, then the genes for the 5 major classes evolved long ago, and the genes for the subclasses, which are still rather similar, evolved from them more recently. The copies are quite homologous and are close by each other; they are true duplicated genes, not alleles. There are differences between, say, the 4 IgG subclasses, which sometimes concern the more research-minded clinical immunologists and rheumatologists, but we won't say more about that.

ALLOTYPES. There are minor **allelic differences** in the sequence of immunoglobulins between individuals, just as blood types or eye color differ. These differences are called **allotypes**, and the allotypes you express are determined by the allotypes your parents had, in the usual Mendelian fashion. Allotypes are useful in genetics, for example in determining relatedness, and sometimes in forensic medicine. Occasionally, an immunodeficient patient getting immunoglobulin treatments will make antibodies to someone else's allotype; this could be awkward. If certain allotypes function more efficiently than others, it could explain why some people are more susceptible to some infections than other people; we don't know much about that yet, though there is some evidence for the idea.

IDIOTYPES. Each antibody will have its unique combining region, made up of the CDR amino acids of its L and H chains; we can call this unique structure an **idiotype** (*idio* means self). It might not surprise you that, under rather special circumstances, antibodies can be made (most easily in another species) that recognize the unique sequence of that combining site, and no other. Such an antibody is an **anti-idiotype**. ►In other words, it is almost completely correct to say that an idiotype is an antibody's (or T cell receptor's—we discuss these later) unique combining site **considered as an antigen**.

ASK YOURSELF: This is not an easy concept—do you get it? Can you think of a use for anti-idiotypes, supposing you could make any one you wanted at will?

ANTIBODIES IN HUMAN SERUM.

IgG:	1000	mg/deciliter (dL =100 mL)
IgA:	200	mg/dL
IgM:	100	mg/dL
IgD:	5	mg/dL
IgE:	0.02	mg/dL

A brief introduction to antibody class functions:

IgG is the main antibody in blood and tissue fluids. It neutralizes toxins and blood-borne viruses, binds bacteria and facilitates their destruction by activating complement and by binding them to phagocytic cells.

IgA can do similar things in the blood, but its real role is as the dimer form in secretions, where secretory component protects it from proteolysis.

IgM does much the same as IgG. It is the first antibody to appear in the serum after immunization, and it is very efficient at activating complement. It does not get into tissue fluids very efficiently, nor is it bound efficiently by phagocytic cells.

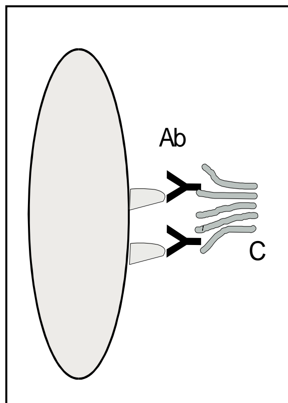
IgD's role in blood, if any, is uncertain; it seems to function mainly as a receptor on naïve B cells.

IgE is the antibody which causes Type I immunopathology, also called immediate hypersensitivity or allergy. Its true importance is in resistance to worms and other parasites.

ANTIGEN-ANTIBODY INTERACTION. When an IgG or IgM antibody binds antigen with at least one of its (two or ten) binding sites, there is a change in the angle between the Fab parts and the Fc. This "allosteric effect" is transmitted through the hinge, resulting in a bulging of the structure of the Fc part so that one or two very important biological activities can be initiated:

1. Binding to phagocytic cells, especially PMNs, eosinophils, and macrophages, which have receptors (**FcR**) for the altered Fc of IgG (but not of IgM), and

2. **C1q**, the first component of the complement system, now binds to ►two adjacent Fcs and is activated (*see* Antibody Function for a more complete discussion of complement.) Note: As shown in the figure below, 2 IgGs will have to be binding close together on the same (usually bacterial) surface, but one IgM can do it alone, because it carries 5 Fcs at all times. This makes IgM much better at activating complement.



It is always helpful to think about antigen-antibody interactions in steps: first there is **binding** (recognition), and then the antibody can do **something else**, like cross-link two antigen molecules, or activate complement (the 5-branched molecule marked C in the picture at left), or bind to a phagocyte (function). Some important defense mechanisms, and also useful tests, depend only on the first step, and others involve the secondary events as well.

ASK YOURSELF: Can you imagine a defense mechanism that might depend only on the first, binding, step?

Learning Objectives for Antibody Structure

1. Define:
   - H chain
   - L chain
   - kappa and lambda chains
   - hinge region
   - Fab, F(ab')2, Fc
   - complementarity-determining regions
   - hypervariable regions
   - variable (V) and constant (C) domains
   - V_L and C_L
   - V_H and C_H

2. Name the 5 antibody classes, and their characteristic heavy chains.

3. Draw a diagram of the structure of typical molecules of each class. Do not bother with the exact number of C_H domains. Label the heavy and light chains; Fc and Fab parts; J chains if any; antibody combining sites; main interchain disulfide bonds; secretory component if present.

4. Distinguish the immunoglobulin classes IgG, IgA, and IgM in terms of:
   - size
   - approximate concentration in serum

5. Describe the structure of antibody combining sites.

6. Explain why complementarity-determining regions are also called hypervariable regions.

7. Give an example of a class, a subclass, an allotype, an idiotype.

8. Diagram an electrophoretic separation of human serum. Label the anode and cathode. Identify the albumin, $alpha_1$, $alpha_2$, beta and gamma peaks.