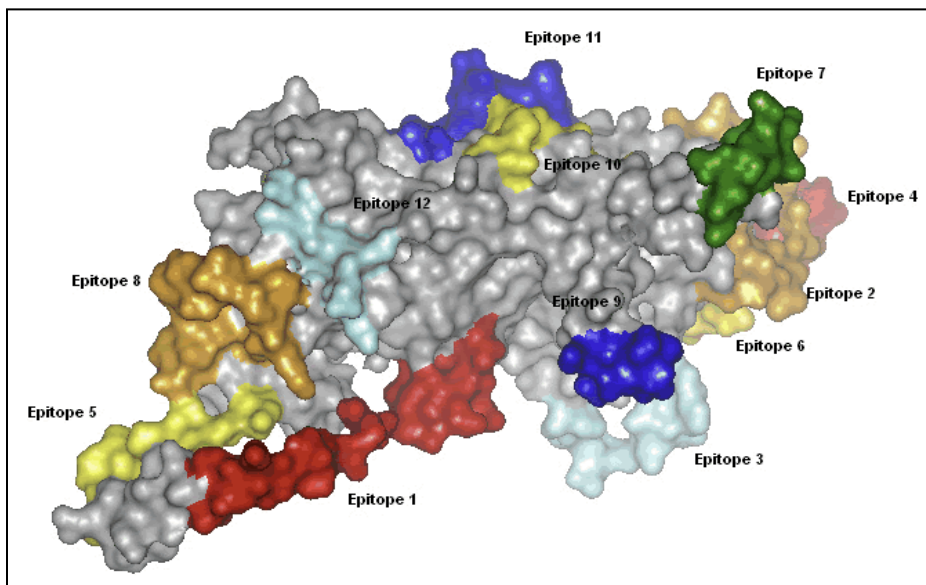


ANTIBODY FUNCTION AND COMPLEMENT

ANTIBODY FUNCTION. We've considered the structure and genetics of antibodies. Now it's time to look again at what antibodies *do*. They are important; if you can't make antibodies, you will have a very severe problem with infections, and may not survive very long (*see* Immunodeficiency). Based on a very old concept and word, antibody-mediated immunity is often called 'humoral¹.' (T cell mediated immunity is 'cellular.')

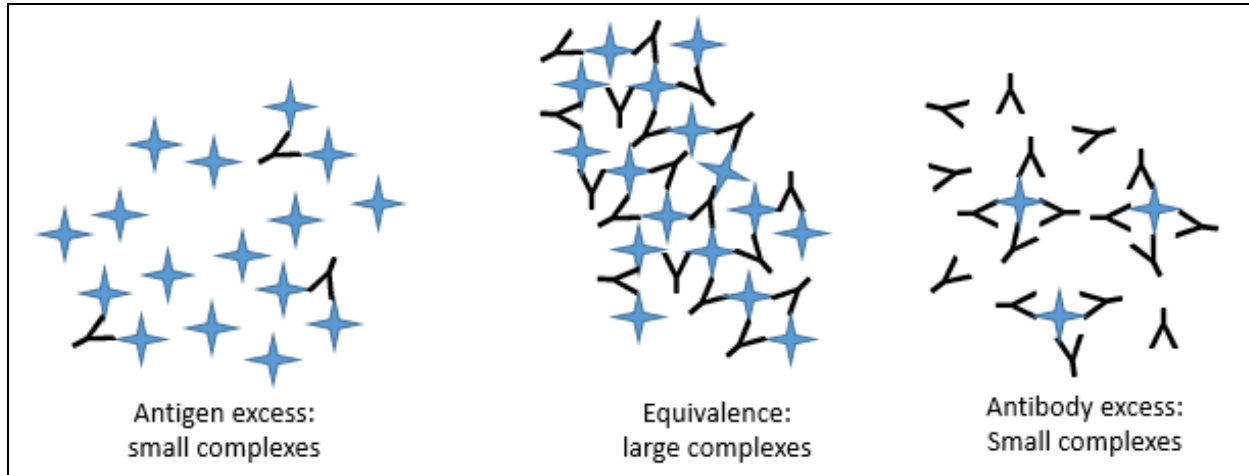
We'll first look at some properties of antibodies in general, and then deal with the individual classes. ► First, a reminder: Not all of an antigen binds specifically to an antibody; the part that actually interacts is usually 10 to 20 amino acids long, and is, as you know, called an **epitope** or **antigenic determinant**. Typical proteins have several epitopes which elicit and bind to different antibodies; this single protein from the human papilloma virus (HPV) has at least a dozen identified:



ANTIGEN BINDING. The basic structure of antibodies, 2 L and 2 H chains, is rotationally symmetrical, so each antibody can bind, in theory, two *identical* antigenic determinants; we say that such an antibody is *divalent*. A complete IgM molecule, then, would be predicted to be decavalent. This has been demonstrated experimentally: If we prepare soluble isolated antigenic determinants—in this form they are sometimes referred to as **haptens**—and mix them with the appropriate antibody, some will be bound. Bound and unbound haptens can readily be separated and, if they are suitably labeled (radioactive or fluorescent), the number of bound haptens/antibody molecule can be counted. (This simple property, incidentally, is the basis of many important laboratory and diagnostic tests. More on this later.)

PRECIPITATION. ► Most real-life antigens, and all immunogens, are bigger than haptens, and in fact have multiple, usually different, antigenic determinants or epitopes (a bacterium or a cell may have hundreds). These antigens are therefore *multivalent*. If we mix such antigens and antibody in solution, not only do the epitopes bind the appropriate antibody's binding sites but the antibody, being at least divalent, has a very good chance of cross-linking two antigens; a **lattice** will begin to grow (the lattice is commonly called an **immune complex**).

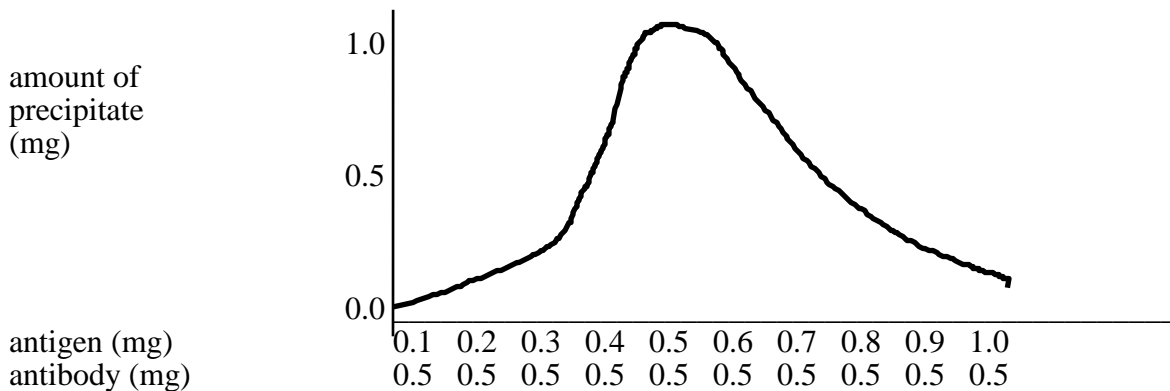
¹ To the ancient Greek physicians, the four humors (liquids) were phlegm, bile, black bile, and blood. They controlled your personality—phlegmatic, choleric, melancholic, or sanguine—as well as your health.



► The large immune complexes that are formed at or near equivalence (where ratios of antigen to antibody are optimal) tend to become insoluble and fall out of solution. When the antigen is a molecule, the phenomenon is called **precipitation**; when it's a cell or cell-sized particle, it is called **agglutination**². Agglutination is more readily detected than precipitation, and so, for the same amount of antibody, an agglutination test is more sensitive.

ASK YOURSELF: Do you see why?

Note that this simple model predicts that the lattice will be smaller if there is a relative excess of either antigen or antibody. Is this prediction true? We can find out, because the larger the complex is, the more likely it is to be insoluble. So, let's mix antigen and antibody in different ratios, and see how much precipitate is obtained (this is a "quantitative precipitin test"):

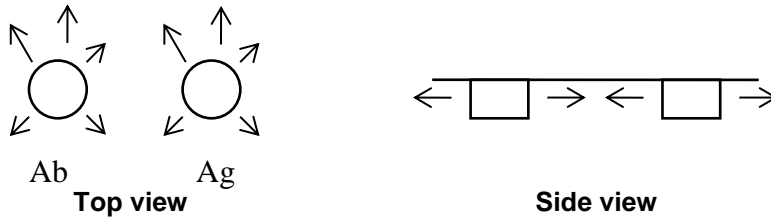


► You can see from the top diagram that in relative **antigen or antibody excess** the amount of precipitate is diminished because the complexes are smaller and not every molecule may get bound.

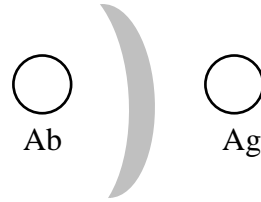
ASK YOURSELF: The above curve has three "zones": antigen excess, equivalence, and antibody excess. Label them, and try to sketch what the complex would look like in each zone. At equivalence, in theory all the antigen and antibody is together in one huge complex. Is that true in the above diagram?

² Before we knew antibody structure, they were named based on what they did: agglutinins, precipitins, opsonins, reagins, etc.

A USEFUL ADAPTATION: PRECIPITATION IN GELS. If you take a layer of agar gel made up in physiological buffer, cut two holes in it, and put antibody in one and antigen in the other, they will begin diffusing radially out of their wells:



In the area between the two wells, the situation is similar to a quantitative precipitin test; there is antigen excess near the antigen well, and antibody excess near the antibody well, so somewhere in between, eventually as they diffuse towards each other, equivalence must be reached. But: what happens at equivalence? The complex precipitates. In a day or two we look at our agar gel, and see...



...a line of precipitate between the wells! This technique is called **immunodiffusion**.

ASK YOURSELF: If you set up two such plates, both with the same antigen but in one plate put IgG antibody and in the other put IgM antibody to the antigen, will both give you a precipitation line? If so, where will the lines be relative to the wells in each plate?



ASK YOURSELF: I set up a plate as above, with antigen A in one well and antibody to A in the other. But this time the gel itself contains soluble isolated epitopes of antigen A. What do you predict I will see?



ASK YOURSELF: You dissolve rabbit antibody to the common laboratory antigen BSA (Bovine serum albumin) in warm agar, pour it into a plate and let it gel. You then cut two round wells in the gel and put 1% BSA in one and 2% BSA in the other. Put the whole thing in the fridge and look at it tomorrow. What do you see? This technique is used diagnostically.



INDIVIDUAL CLASSES OF ANTIBODIES. They all bind antigen, of course, since their light chains and V_H domains are the same (as we learned in the Antibody Genes unit). ► But **inherent in the structure of the C_H domains that make up their Fc regions** are different biological properties which give them interesting functions. Some of these involve **complement**, which is discussed next.

IgM. This is the oldest antibody phylogenetically. It is also ► the first seen in blood after immunization (sensitive tests reveal an increase by day 2). You would think it is the best possible antibody, being decavalent, but in practice its shape rarely allows more than two of its binding sites to interact with antigenic determinants. ► What does make it outstanding is its great capacity to activate complement; two adjacent Fcs are needed to get the complement cascade started, and IgM always has 5 adjacent. It can be as much as 500 times more efficient than IgG at activating complement. Why, then, even bother to have IgG? One reason is that IgM, being so large, has trouble getting from the blood into the tissues. It is also viscous in solution; if we had only IgM at the same molarity as we have IgG we would scarcely be able to pump our blood. And there are no useful IgM receptors on phagocytes. IgM is the only antibody to be made by the fetus. We make a little secretory IgM, which perhaps helps out people who lack secretory IgA (a common condition, *see* Immunodeficiency).

IgG. ► The most abundant immunoglobulin in the blood. It is the only class that passes the human placenta from mother to fetus (active transport required). It comes up a little later than IgM after primary immunization, but the antibody levels go higher and last longer. The plasma half-life of IgG is about ► **3 weeks**. If antigens (pathogens) get into the blood stream IgG antibodies are very important; phagocytic cells have receptors for the Fc of bound IgG, and so IgG is **opsonizing**;³ vital for clearance of most extracellular bacteria. It takes two IgGs close together to activate the first component of complement, and this will only happen if the density of epitopes on the antigen is high enough for this to occur (remember, binding a single epitope should be enough to allow an IgM molecule to activate complement).

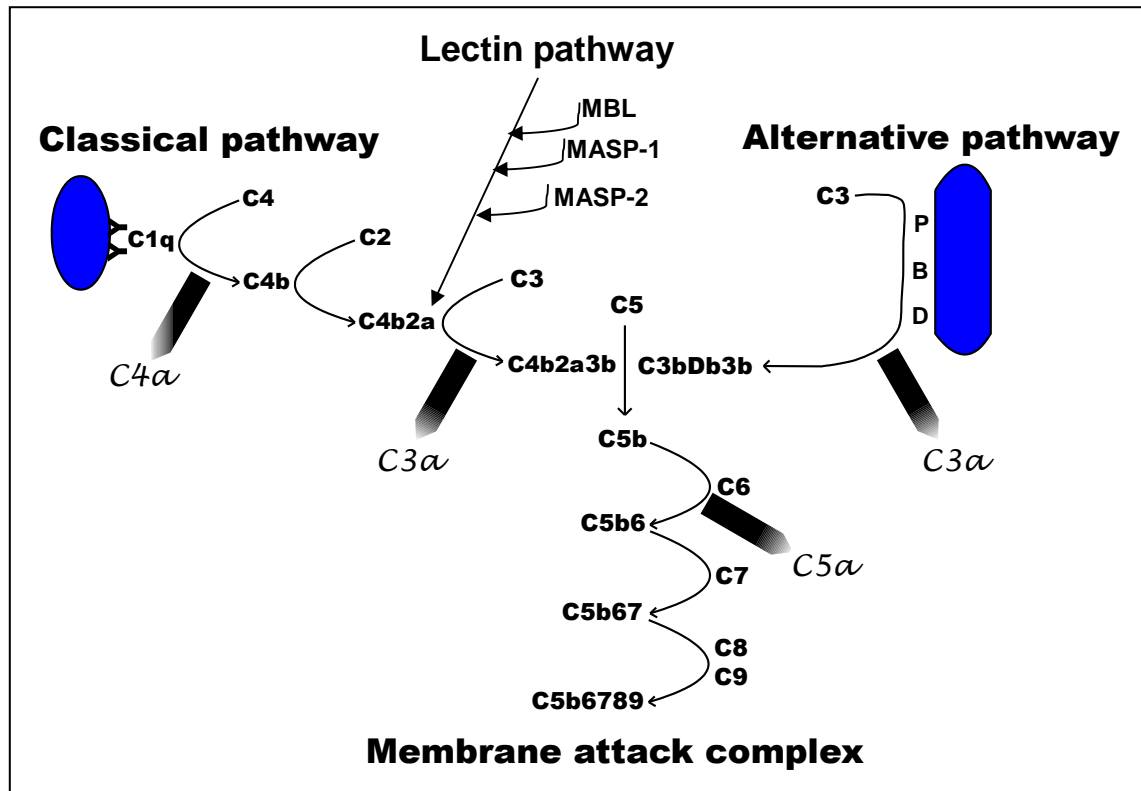
IgA. This antibody is preferentially made by plasma cells in lymphoid tissues near mucous membranes. It is assembled into a dimer by the addition of the J chain while in the plasma cell, and then secreted into the interstitial space. Adjacent epithelial cells have receptors for IgA which binds to them, is taken up and moves through the epithelial cell to the luminal (mucous membrane) side. There the IgA is exocytosed, still bound to the receptor, which is now called **Secretory Component**. Secretory Component protects the IgA from digestion in the gut, and makes it work well as our first line of immunological defense against invading organisms. There is some monomer and dimer IgA in the plasma, where it can bind pathogens and activate complement by the alternative pathway.

IgD. We will discuss its role in the activation of B cells soon. Although there is some IgD in the plasma, it is believed that the only important role for IgD is as a B cell receptor.

IgE. Because it adheres to histamine-containing mast cells and basophils, this antibody is the cause of immediate hypersensitivity or allergy (*see* Type I Immunopathology). Why does it exist? It is important for resistance to parasites, because it also triggers the mast cells to release **eosinophil chemotactic factor**. Eosinophils are uniquely effective at killing parasites.

³ “Opsonin is what you butter the germs with to make your white blood corpuscles eat them.” G.B. Shaw, (1908) *The Doctor's Dilemma*.

COMPLEMENT. Complement is the main inflammatory mediator of the humoral immune system. A long time ago it was noted that fresh serum from a person who had survived a bacterial infection would agglutinate *and lyse* the appropriate bacteria. Old stored antiserum could cause agglutination, but the bacteria were not lysed. If fresh serum, even from a non-immune donor, was then added, the agglutinated bacteria were rapidly lysed. Obviously, there was something in fresh serum that *complemented* the antibody's action: thus, complement. It was soon found that complement is, in fact, a large number of proteins, similar to the blood clotting system in that each exists in an inactive form, and when the first is activated the rest follow in a sort of cascade. There are at least three ways to activate the C cascade; the one that is most familiar is the *classical* pathway. Later, an *alternative* and a *lectin* pathway were described. Each pathway gets started differently ► but all represent different ways to activate C3.



The **classical pathway** is activated by complexes of IgG or IgM antibody with antigen. There is a change in the Fc portions of the antibodies after interaction with antigen, which allows the binding and activation portions of C1q. The C1q must interact with two Fcs simultaneously; it does so either by finding two IgGs close together, or a single IgM (this reinforces the point that IgM is a much more efficient a C activator than is IgG). C1 activates C4 and then C2, which together activate C3, which can then activate C5. ► Classical C counts: 1-4-2-3-5-6-7-8-9.

Note that for certain complement components, activation means splitting into 2 parts; the “b” component usually stays attached to the nearest membrane, and the other may float away (C3a, C4a, C5a) and have biological activity.

There always seems to be some C being activated, so the body also makes inhibitors of complement activity. The best known is C1INH, or **C1 esterase inhibitor**. If you don't have it you are at risk for (hereditary or acquired) angioedema, giant hives (urticaria) that can swell up your face like a balloon or, if you're very unlucky, can swell your larynx shut and kill you.⁴

The **alternative pathway** is activated by certain cell wall structures of microorganisms such as dextrans, levans, zymosan, and endotoxin; thus ► a bacterium might activate C this way even in the absence of antibody. So this pathway is considered part of the innate immune system. C is activated by a cascade that involves C3, which is always breaking down at a low rate to C3a and C3b, which are usually rapidly degraded. So if C3b could be stabilized, C5 could be activated. The cell wall structures provide a surface for the binding of C3b and factor B, properdin (P), and factor D; a stable C3bBbC3b complex (trimer of Bb and two C3b units) forms which can activate C5 (and thus 6-7-8-9). The alternative pathway seems to be a more primitive, early, less-specific sort of defense, since it can work even without waiting for antibody to be made. Recently it's been shown to accelerate the activation of C by the classical pathway, and it can make things worse in autoimmunity.

There is also the **lectin pathway** of complement activation, truly part of innate immunity. The lectin pathway is mediated by **mannose-binding protein**, a **lectin**, abbreviated **MBP** or **MBL**. Lectins are proteins that bind (usually foreign⁵) carbohydrates. MBP binds certain mannose-containing structures found in carbohydrates of bacteria but not humans. MBP is functionally similar to C1q in the classical complement pathway, so the lectin pathway goes MBP-4-2-3-5-6-7-8-9. There are several alleles of MBP in humans, and Caucasians have an allele that results in low levels of serum MBP; about 8% have very low levels. These people have marginal immunity—they may be fine except when the immune system is stressed (in infancy, in old age, in the presence of anything that compromises the immune system). Associating with MBP when it binds mannose are some serine proteases, the MASPs, which activate C2 and C4 and get the cascade rolling.

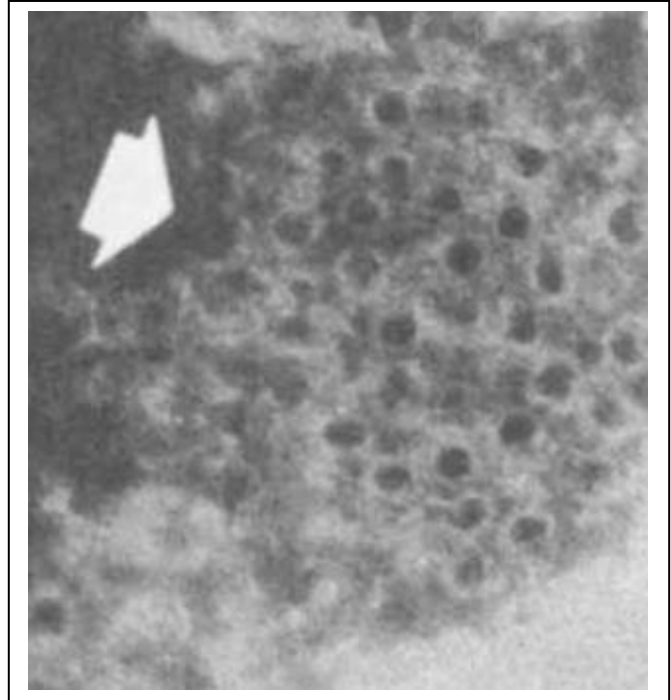
Activating C3 and C5 is a vital part of the complement-mediated inflammatory mechanism, responsible for 3 of the 4 actions listed below. The fourth is the formation of the **membrane attack (lytic) complex** or **MAC**: C5, activated by any of the three pathways described, activates C6, C7, C8, and C9. C8 and C9 form a lesion on the target cell membrane which, on electron microscopy, looks like a hole, which in fact it is; the cell loses its ability to regulate its osmotic pressure and lyses or pops.

⁴ This happens because of the anaphylatoxic property of activated complement (see below); also, the bradykinin system (powerful vasodilators) is overactive because C1INH controls its esterases, too.

⁵ Makes sense; if they bound your own carbohydrates you'd be kind of gummed up.

So in summary, ► complement has **four** distinct actions:

1. It is **LYTIC**, as just described, if the membrane attack complex is activated. *Neisseria* (gonorrhea, meningitis) are by far the most susceptible family of bacteria to C lysis. This electron micrograph shows MAC holes in a complement-treated bacterium.⁶



2. It is **OPSONIZING**. One split product of activated C3, namely C3b, adheres to membranes. Phagocytic cells (PMN, macrophages) have C3b receptors, and so can get a firm grip on an antigen if it is opsonized with C3b. As we said before, IgG is also opsonizing, because phagocytes have receptors for its Fc end called **FcR** (there are several different FcR). There are no FcR for IgM itself, but the complement it activates is, of course, opsonizing.

3. It is **CHEMOTACTIC**. The C5 activation product, C5a, is chemotactic for phagocytes, especially neutrophils. This explains much of the inflammation in an antibody-mediated reaction, and why PMN are the hallmarks of such a reaction.

4. It is **ANAPHYLATOXIC**. C3a, C4a and C5a are all capable of releasing histamine non-specifically from mast cells or basophils. This means that there will be increased blood flow in the area of antigen deposition, and a better chance for inflammatory cells to get out of the blood and into the tissues. Sometime, a person with a lot of complement activation will break out in hives, and you can confuse what's going on with an allergic reaction.

ASK YOURSELF: Could a person lacking C1 activate complement? Lacking C3? C5? C6? Every complement action, or just some?

⁶ Goldman, RC and Miller, MF. 1989. J. Immunol. 142: 185-194.

Learning Objectives for Antibody Function

1. Define: valence, affinity, precipitation, agglutination, hapten
2. Distinguish the five classes of immunoglobulins in terms of:
 - passage across the placenta
 - ability to activate complement by the classical pathway
 - ability to activate complement by the alternative pathway
 - involvement in allergic diseases
 - “first line of defense”
 - most resistant to enzymatic digestion
3. Describe a quantitative precipitin test where amount of antigen/tube is varied while antibody/tube is constant. Draw a graph which compares, on the ordinate, amount of precipitate obtained, with amount of antigen added/tube. Identify the zones of antigen and antibody excess, and equivalence.
4. Sketch the lattices obtained in antigen or antibody excess, and at equivalence, using **Y** as antibody and **✦** as antigen.
5. Discuss why a line of precipitate may form in agar gel when antigen and antibody diffuse towards each other.
6. Compare and contrast precipitation and agglutination in terms of the nature of the antigens involved, and sensitivity of the tests.
7. Discuss how complement plays roles in both innate and adaptive immunity.
8. List the components of complement in the order in which they become activated in the classical pathway. Name those that are also activated in the alternative pathway.
9. Discuss the lectin-mediated pathway of complement activation, and whether it is part of innate or adaptive immunity.
10. Discuss the different ways in which complement is activated by IgG and IgM.
11. Identify the complement components which are: opsonizing; lytic; anaphylatoxic; and chemotactic.
12. Discuss how complement is important in immunity to bacteria even if the bacteria are resistant to lysis by C9. Identify the family of bacteria most susceptible to lysis.