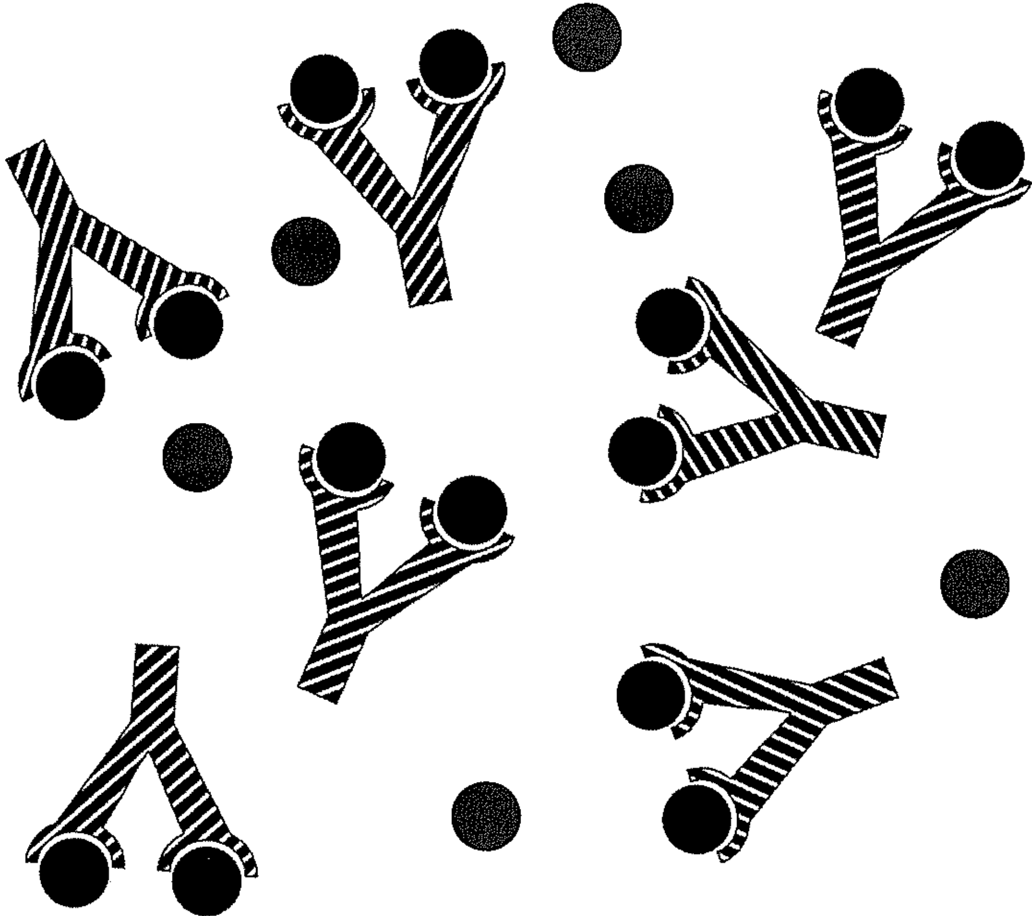
Understanding Immunology



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Understanding Immunology

Introduction

Immunology, the study of how the body resists disease and invasion by foreign substances, is an exciting and fast-moving field. Immunologists have eradicated or dramatically decreased the frequency of many contagious diseases such as smallpox and polio, and have provided us with a number of useful laboratory tests based on the principles of immunology. In addition, more recent advances in the field of immunology, have made successful organ transplants and new, more effective treatments for autoimmune diseases possible. A more detailed understanding of immunity may also provide a key to the treatment or prevention of cancer, and may be critical in figuring out more effective ways to control or prevent infection by destructive viruses such as HIV and hepatitis C. We can expect that immunology is going to play an ever expanding and increasingly important role in our lives.

This booklet is an introduction to the body's defense mechanisms, how scientists have discovered some of these mechanisms, and some of the modern medical tests that evolved from these discoveries.

The Beginnings of Immunology

People who recover from certain infectious diseases often become resistant to subsequent attack by the same or similar pathogenic agents. This fact has been known at least since the bubonic plague in Athens in 430 B.C. Thucydides noted that only those who had previously survived the plague could safely assist the sick. Certain infections, such as measles, mumps, and chicken pox occur primarily during childhood because a person can rarely get these diseases a second time. Fortunately, the frequency of these diseases in the United States has dramatically decreased because of the use of vaccines. As another example, a person with pneumococcal pneumonia, if not treated, suffers with high fever and discomfort for 7 to 14 days until suddenly a turning point is reached and he recovers rapidly. Subsequently, the patient resists or is immune to the particular bacterium that caused the disease. It is clear that after the first contact with an infectious organism the body retains an imprint or "memory" of this organism, allowing the body to resist further infections.

In the past, some people mistakenly believed that recovery from bubonic plague produced immunity to all diseases; however, the body's immune system is very specific and able to recognize differences between pathogens. A good example of the specificity of the immune system is the well established observation that someone immune to one strain of influenza may not be immune to a second strain of influenza. The degree of immunity a person has to the second strain depends upon how similar the second strain is to the first.

Humans learned how to exploit the immune system for their protection a hundred years before they began to understand how the system works. For centuries, smallpox had been one of humankind's most devastating diseases. In the 17th and 18th centuries in Europe, virtually all children became infected—one out of six died, many were blinded, and most were scarred with pockmarks for life.

In 1798, Edward Jenner showed that he could give people a disease called cowpox by taking a small amount of pus from a cow or a person infected with cowpox and injecting this into the skin of a healthy person. The patient would become slightly sick but would subsequently be immune to smallpox. We now know that the cowpox virus is very similar to the smallpox virus. In fact, they are so similar that the body's immune system does not distinguish between the two. The milder infection caused by the cowpox virus provides immunity to smallpox. This important discovery has saved many lives. The application of Jenner's discovery has enabled humans to eliminate smallpox.

How does the body recognize and resist foreign organisms? Louis Pasteur believed that each pathogen requires a specific nutrient and lives in the body until it has exhausted that nutrient. This would mean that immunity is produced by the lack of something (i.e., that nutrient). In 1889, Emil von Behring immunized rabbits with

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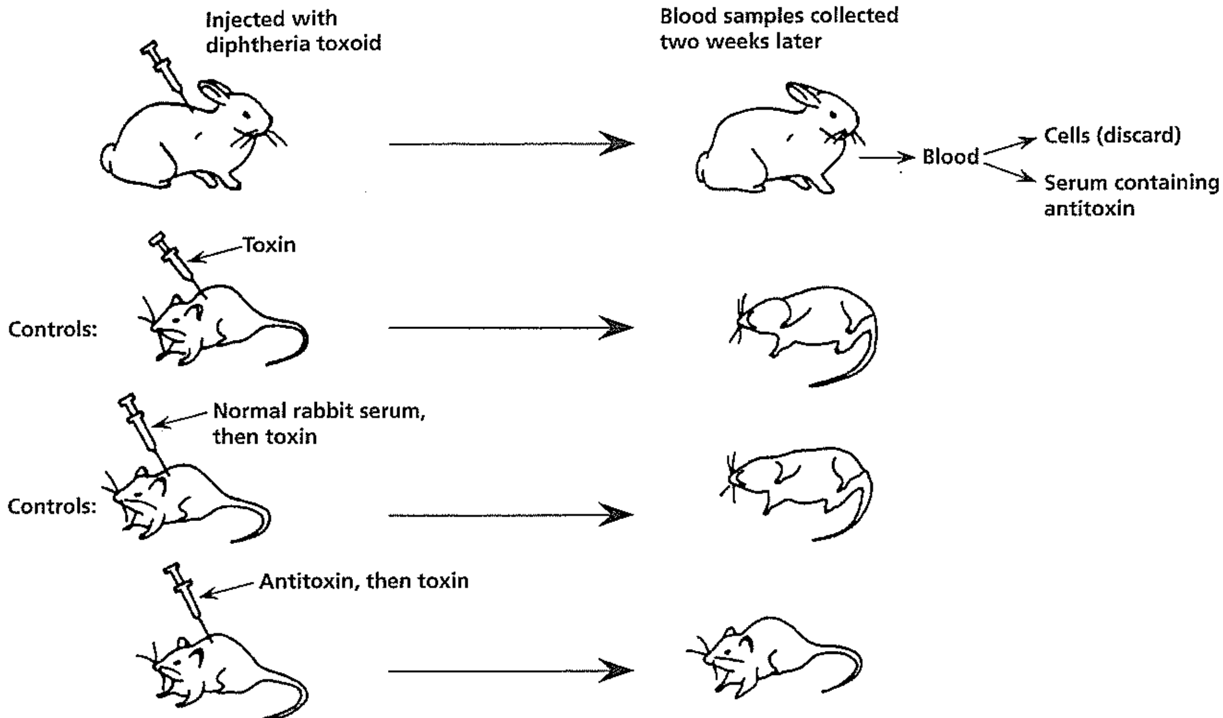

Figure 1. Diphtheria toxin is the poison produced by the diphtheria bacteria. If heated or chemically treated, it is rendered nontoxic but still stimulates production of antibody (it is referred to as a toxoid). The control experiments show that the toxin kills mice and that normal rabbit serum does not prevent death. The last experiment shows that rabbit antiserum to diphtheria toxin prevents death in the infected mice.

diphtheria toxoid (Fig. 1). He later injected serum (blood with the cells removed) from these rabbits into mice. When the mice were subsequently injected with diphtheria toxin, they did not become sick. The transferred serum had provided the mice with immunity to diphtheria toxin.

If immunity were a lack of something, as Pasteur believed, the transfer of serum would have no effect. This experiment proved instead that there was something in the serum that neutralized the toxin; it was called antitoxin. (Later work identified this antitoxin as antibody.)

A year after von Behring's experiment, a girl dying of diphtheria was injected with diphtheria antitoxin (antibody) as a last resort. Within hours, she began to recover. For this work, von Behring received the first Nobel prize for medicine, and the modern science of immunology was well on its way.

Immunity

There are two basic types of immunity—innate and adaptive. Adaptive immunity can involve multiple pathways.

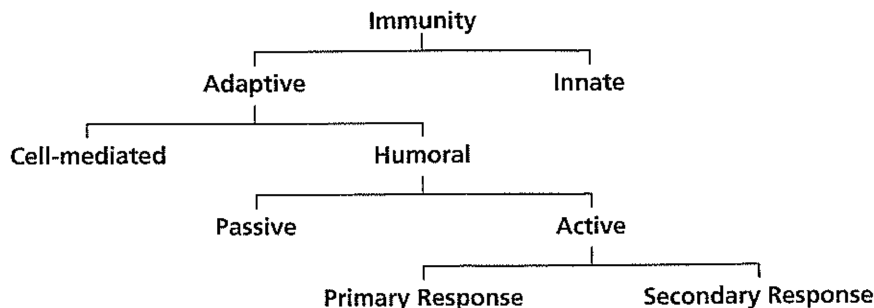

Figure 2.

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Innate Immunity (also called natural or native immunity)

Innate immunity, the body's general defense against infections, exists without previous contact with a particular pathogen. Innate immunity is provided first by physical barriers—the skin and the mucosal lining of the gut and respiratory tract—and by some of their secretions, such as stomach acid, lysozyme, or certain skin peptides, all of which have some bactericidal properties. Microorganisms that breach these defenses are attacked by other components of the innate immune system including natural killer cells, phagocytes, and several proteins in the plasma. The innate immune system also interacts with and takes cues from the adaptive immune system.

Adaptive Immunity (also called specific or acquired immunity)

Adaptive immunity develops as a result of infection or exposure to something, either through a natural route or an unnatural one, such as a vaccine. The adaptive immune system is composed of cells called B and T lymphocytes and of lymphocyte products including antibodies, proteins that will be described later in detail. Through vaccines, the body's adaptive immunity can be manipulated to prevent disease.

Adaptive immunity is divided into humoral immunity and cell-mediated immunity. Humoral immunity defends against extracellular organisms (especially those that enter the bloodstream). Humoral immunity is carried out by antibodies, Y-shaped proteins produced by B lymphocytes. Antibodies are present in the blood, lymph, and mucosal fluid. The B lymphocytes that produce antibodies originate in the bone marrow and migrate to the spleen and lymph nodes. Some remain circulating in lymph and blood.

Humoral immunity may be active or passive. In passive immunity, antibodies produced outside an infected individual are introduced to fight a disease. Von Behring was the first to utilize passive immunity, in order to fight disease in a patient who was already ill. Passive immunization takes effect quickly (within hours), but the immunity lasts only for weeks.

Active immunity occurs when an individual's own B cells produce antibodies as a result of contact with an antigen (a molecule that stimulates the adaptive immune system). After exposure to the antigen, B cells require 1 to 2 weeks to produce antibodies. Once it is established, active immunity lasts for years or even for a lifetime.

In contrast to humoral immunity, cell-mediated immunity, is effective in fighting intracellular invaders such as viruses. Cell-mediated immunity is also responsible for contact sensitivity to chemicals (e.g., poison ivy), rejection of transplanted tissues or organs, and for protection against some diseases such as tuberculosis, whose pathogens can survive within the host's cells. Cell-mediated immunity is carried out by T lymphocytes. T lymphocytes are so-named because they mature in the thymus, a gland behind the breastbone. Mature T cells circulate through the spleen and lymph nodes. They also migrate to places in the body where foreign molecules (such as bacterial toxins or viruses) are present.

Scientists became aware of cell-mediated immunity after they learned of humoral immunity. Von Behring had shown that the passive transfer of antibodies could prevent diphtheria and tetanus. Others showed that the transfer of the appropriate antibody to a sick individual could prevent or cure many additional diseases. Transfer of antibodies did not, however, protect against some viral diseases or cause the rejection of skin or other transplants. It was known that some people who lacked a thymus produced antibodies but still frequently suffered from recurrent diseases, especially viral infections. In addition, in experimental situations, certain diseases could be cured or prevented only by the administration of lymphocytes (not antibodies) from immune individuals. Clearly, antibodies were not responsible for all immunity. Some additional mechanism must be at work. Further studies led to the discovery of cell-mediated immunity, a system that we still do not completely understand.

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Immunization, Antibodies, and Antibody Specificity

After injection of an antigen (active immunization), a patient begins to produce antibody after approximately 7 to 14 days (Fig. 3). The serum concentration of antibody reaches a peak and then begins to decrease; this is a primary response. If a patient is reimmunized, the antibody response to the second injection occurs more rapidly and reaches higher levels; this is a secondary response. Booster shots (subsequent injections of antigen) are often given to increase antibody production and to obtain greater immunity for a longer time. Similarly, when a person is naturally exposed to an antigen the second time, the antibody response occurs more rapidly and reaches higher levels.

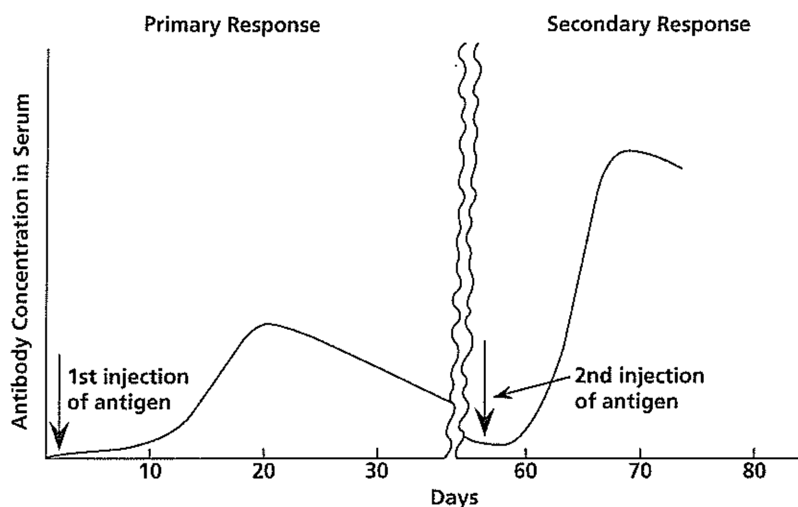

Figure 3. Comparison of a primary response and a secondary response to injection with an antigen.

Antibodies are Y-shaped protein molecules made up of four amino acid chains: two longer chains called heavy chains, and two shorter chains called light chains. Looped regions called "immunoglobulin domains" contribute to an antibody's function. Antigens bind to the ends of the arms of the Y; consequently, each antibody molecule can bind two of its specific antigens. Some antibodies are linked together as multimers and thus are capable of binding more than two specific antigens.

Antigens are the substances that stimulate the adaptive immune system. Pathogens such as pneumococcal (pneumonia) bacteria, the chicken pox virus, influenza viruses, and bacterial toxins are antigens. Substances other than pathogens may also stimulate antibody production. Most macromolecules (e.g., proteins, carbohydrates, and even synthetic polymers) can stimulate the immune system to produce antibody. Each type of antigenic molecule, whether existing freely or as part of an organism, has a specific shape. It is this shape, or a part of it, that an antibody "recognizes." The antibody binds to the complementary part of the antigen. Antibody–antigen binding is reversible and depends on the cumulative effects of weak forces such as charge interactions and hydrogen bonds.

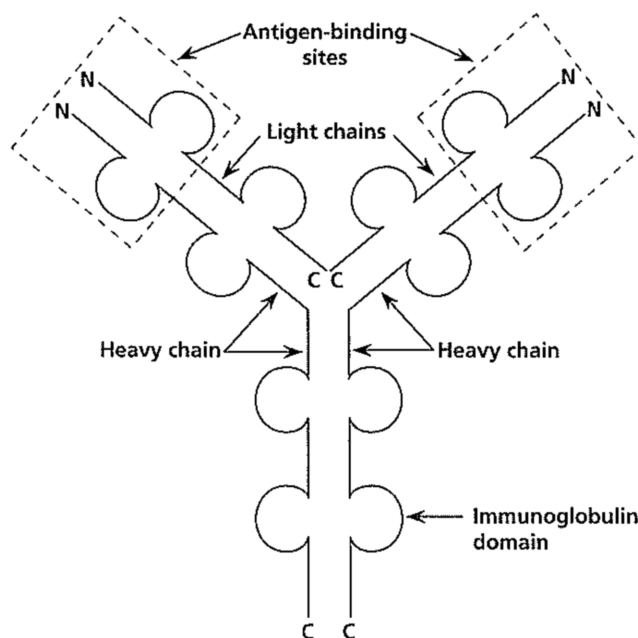

Figure 4. The basic structure of an antibody. The antigen-binding sites are highly variable and determine the specificity of the antibody.

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If there is enough similarity between two pathogens, antibodies produced to one pathogen may cross-react to the other one. For example, different strains of influenza virus often contain the same gene for the surface proteins neuraminidase or hemagglutinin. If a patient is infected with one virus strain, the antibodies produced in response to that infection help the patient fight an infection from a different strain if the two viral strains have the same form of one or both of these surface proteins. Another example involves the cowpox and smallpox viruses mentioned earlier. Antibodies people generated against the cowpox virus cross-reacted to the smallpox virus, because some part or parts of the two viruses were very similar.

Laboratory Applications of Immunology

For medical and research purposes, scientists use both purified antibodies and sera that contain antibodies (antisera). The antisera are produced in animals that the researchers have immunized with specific antigens. For example, scientists purify bovine albumin from cow serum. Albumin is a protein that makes up a large portion of the blood and functions as a carrier for smaller molecules. The purified cow albumin is used to immunize any animal other than a cow, e.g., a goat (Fig. 5). Since the cow albumin is different from goat albumin, the goat recognizes the cow albumin as "foreign" and produces antibodies to it. The goat is then given booster injections of bovine albumin to increase the concentration of anti-bovine albumin antibodies in its serum. One or 2 weeks after the booster injection, the goat is bled. The cells are removed from the extracted blood. The remaining fluid is an antiserum containing antibodies to cow albumin. This antiserum is called goat anti-bovine albumin (Fig. 6).

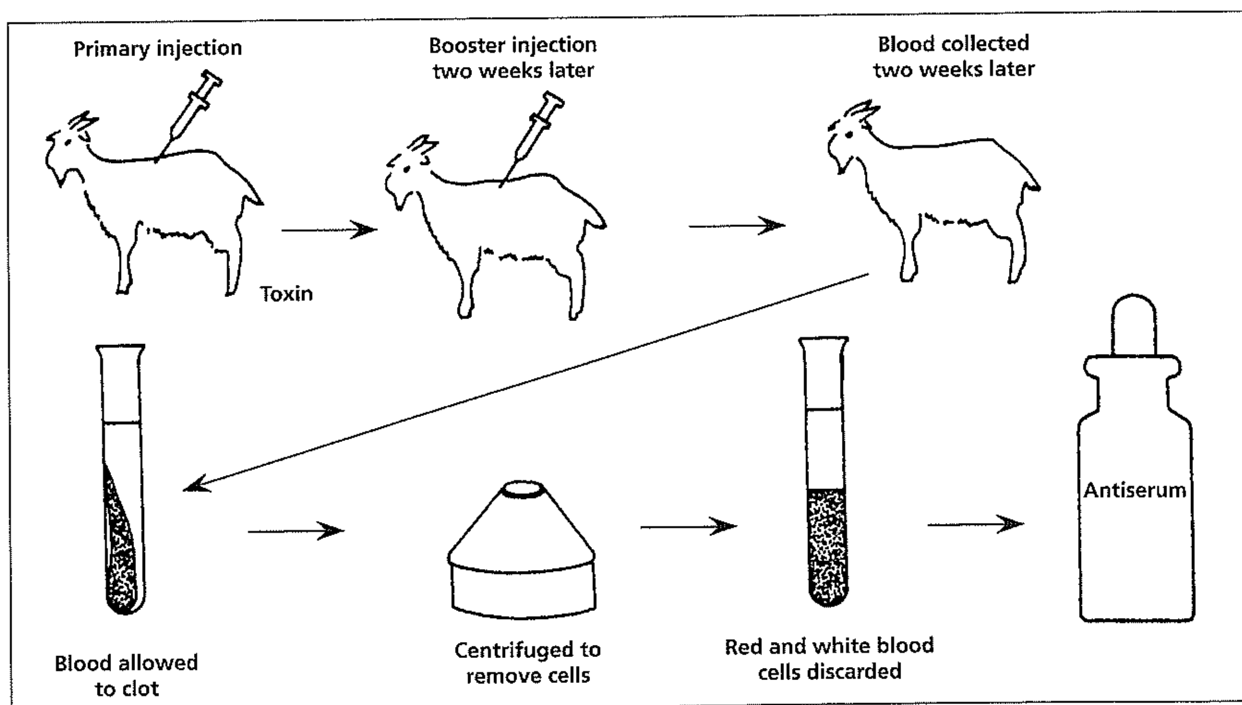

Figure 5. Production of an antiserum.

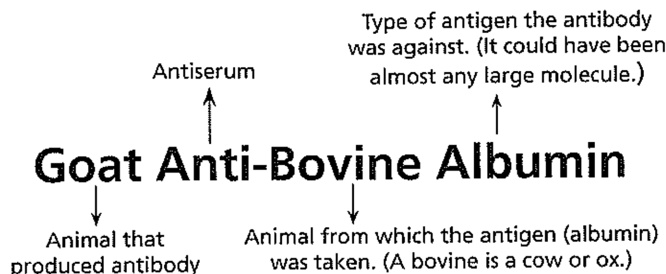

Figure 6. Explanation of antiserum nomenclature.

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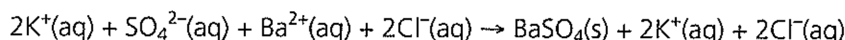
A specific type of antibody called a monoclonal antibody is also widely used in research and medicine. A monoclonal antibody binds specifically to one site on an antigen. These antibodies are made by taking B cells from an animal that has been immunized with an antigen and then fusing those cells with tumor cells that can be cultured longterm in the lab. B cells from an animal are normally short-lived, but the fused cells live indefinitely and can produce antibodies to the desired antigen. The fused cells are called hybridomas. From the population of hybridomas, it is possible to select one cell that produces the antibody with the desired binding characteristics and to expand that cell into a cell line that can be cultured. As the cell line produces the desired antibodies, they are collected and used for research or clinical purposes.

The first hybridoma cell lines to be produced were from mice. Although mouse antibodies can be used in many diagnostic tests, they have limited medical use because the human body tends to recognize them as foreign. More recent techniques have produced monoclonal antibodies suitable for use in people. These antibodies are now widely used in medical therapies. In research, monoclonal antibodies are regularly used in a variety of indispensible procedures.

Scientists have devised many techniques based on antibody–antigen reactions. When antibody and antigen combine in large quantities, they may form an insoluble substance called a precipitate. This is somewhat similar to the formation of a precipitate in an ordinary chemical reaction. For example, barium chloride and potassium sulfate both dissolve readily in water and form clear solutions. However, when solutions of the two are mixed, a chemical reaction occurs:



$$2K^+(aq) + SO_4^{2-}(aq) + Ba^{2+}(aq) + 2Cl^-(aq) \rightarrow BaSO_4(s) + 2K^+(aq) + 2Cl^-(aq)$$

The barium sulfate ($BaSO_4$) formed in the reaction is insoluble in water and forms a precipitate.

Figure 7 depicts an experiment using this reaction. In this experiment, barium chloride and potassium sulfate are each added to seven test tubes such that the concentration of barium chloride is kept constant while the concentration of potassium sulfate increases from tube 1 to tube 7. The amount of precipitate formed in each test tube is measured and plotted (Fig. 7).

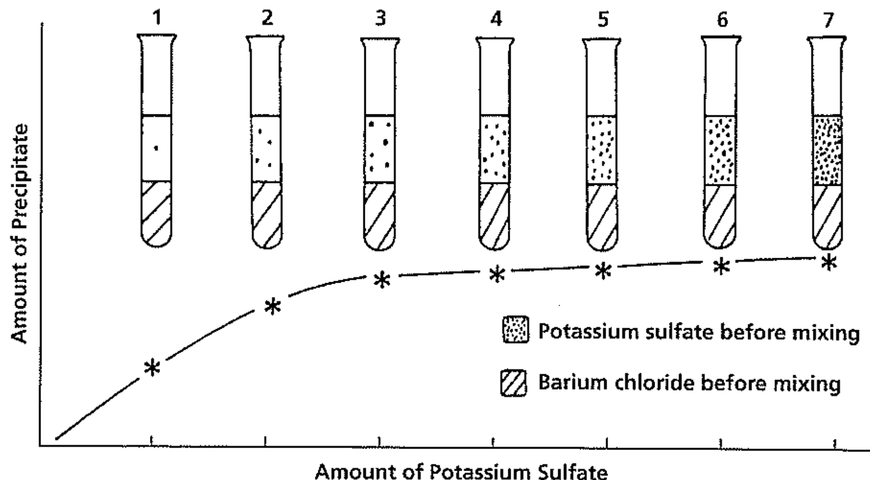


Figure 7. The amount of precipitate resulting from the addition of increasing amounts of potassium sulfate to a constant amount of barium chloride.

When a similar experiment is performed using increasing amounts of an antigen such as bovine serum albumin (BSA) and constant amounts of an antibody (Ab) directed to BSA, a different curve results (Fig. 8).

If too much antigen is added, no precipitate will form; in contrast $BaCl_2$ and K_2SO_4 react to form a precipitate even if there is an excess of K_2SO_4. This fundamental difference perplexed early immunochemists. The best explanation for the lack of precipitation in the presence of excess antigen and the general shape of the precipitate curve is the lattice hypothesis.

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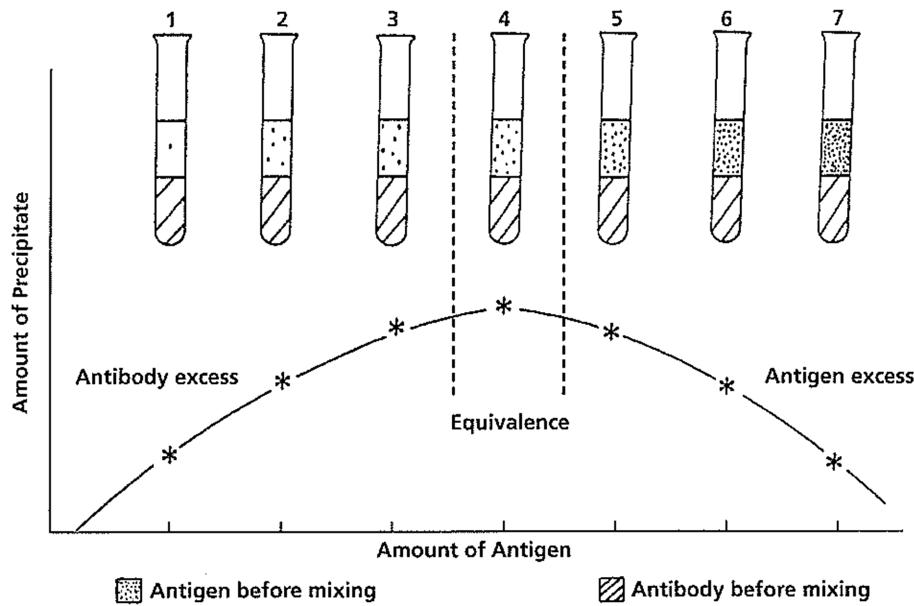

Figure 8. The amount of precipitate resulting from the addition of increasing the amount of antigen to antibody.

The lattice hypothesis is based on the fact that most antibody molecules are bivalent (can combine with two antigens) and that most antigens are polyvalent (e.g., bovine serum albumin can combine with several antibody molecules at the same time; another antigen, the tobacco mosaic virus, can combine with hundreds of antibody molecules simultaneously). Thus, an antigen and an antibody can combine in a number of different ratios. When the antibody is present in excess, a single bovine serum albumin molecule may combine with five or six antibody molecules to form a $BSA–Ab_5$ complex that is insoluble and will precipitate.

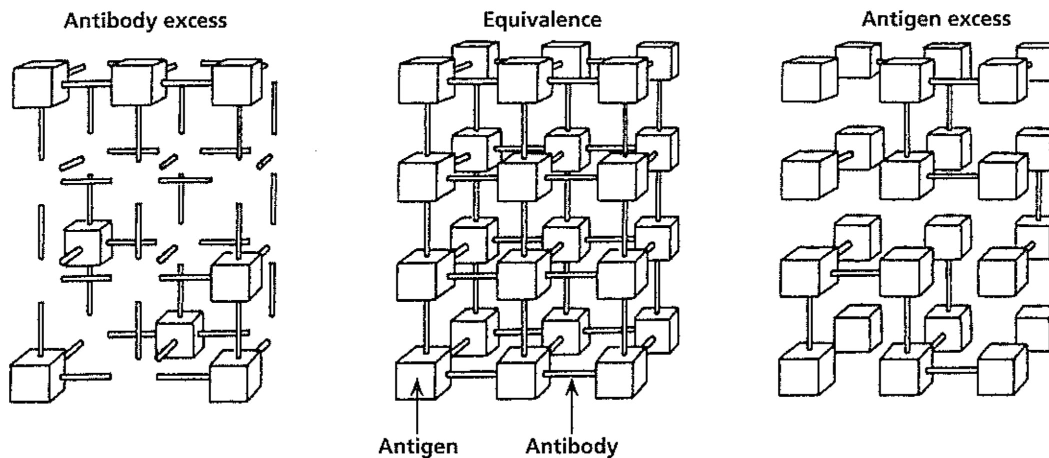

Figure 9. Representations of bonding between antibody and antigen (bovine serum albumin) at three different concentrations of antibody. (Modified from J.A. Bellanti, M.D., *Immunology*, W.B. Saunders Co., Philadelphia, 1971)

As more antibodies are added, more of the antigen and antibodies are able to form a lattice. At equivalence, the antigen-to-antibody ratio is such that all of both types of molecules have combined into one lattice, which is insoluble (neither free antigen nor free antibody is left in solution).

In contrast, if the amount of bovine serum albumin far exceeds the amount of antibody, $BSA_2–Ab$ complexes are formed with the many free BSA molecules. Since the $BSA_2–Ab$ complex is soluble, no precipitate forms. This accounts for the difference in the shape between the curves representing the amount of precipitate formed during the antibody–antigen interactions (Fig. 8) and during the inorganic precipitate reaction (Fig. 7). In addition, the bonding between antigen and antibody is noncovalent and reversible, so an antibody–antigen precipitate can be dissolved with excess antigen.

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Qualitative Immunologic Analysis

The formation of an antigen–antibody precipitate may be used to indicate a number of things. For example, if a physician or research scientist is looking for the presence of a specific virus in a patient's fluids or tissue, the formation of a precipitate may indicate that an antibody against the specific antigen (in this case a viral protein) is present in the serum or other fluid being tested. However, as discussed previously, in order for the immunoprecipitate to form, the antigen and antibody must be present in the correct ratio, a condition not always easy to create.

The Ouchterlony Procedure

To allow antibody and antigen to meet and react at proper concentrations, O. Ouchterlony and S.D. Elek developed the Ouchterlony double-diffusion technique. In this procedure, antibody is placed into a well cut in an agar plate, and antigen is placed in another well on the plate. As the antigen and antibody diffuse through the agar, a concentration gradient of each is formed, with the highest concentration near the well (Fig. 10).

This assures that the antibody and antigen will meet at a proper concentration somewhere between the wells. When this happens, a fine white line of antibody–antigen precipitate appears in the agar (Fig. 11).

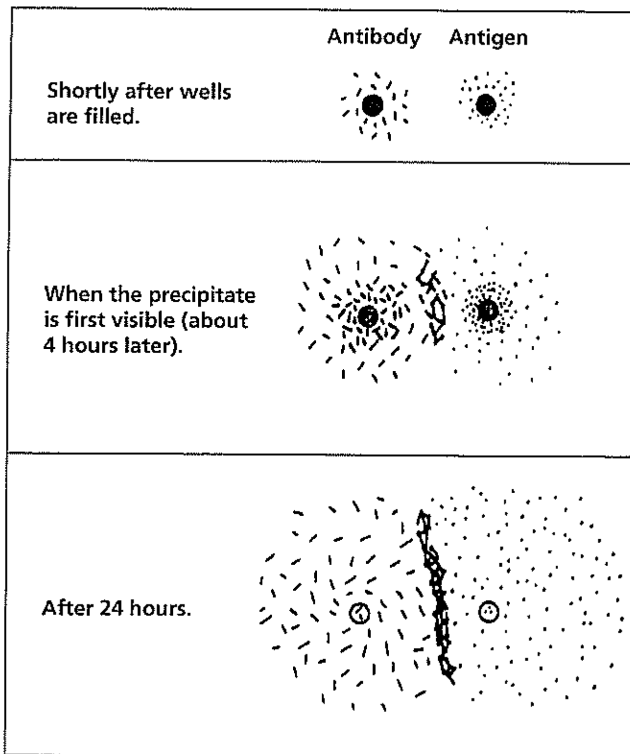

Figure 10. Diagram of Ouchterlony analysis.

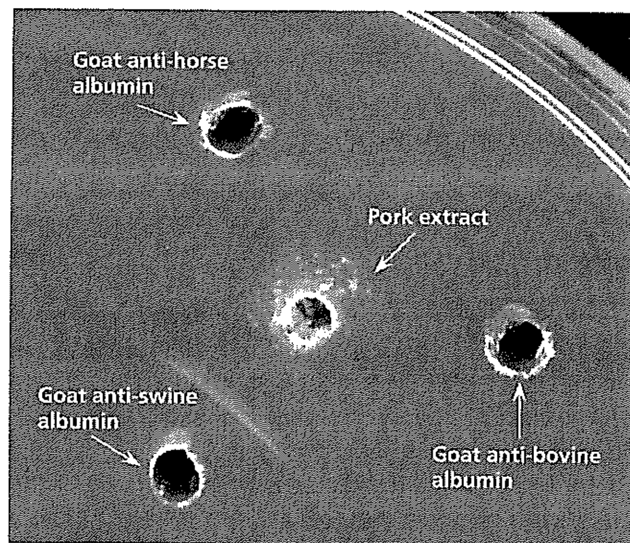

Figure 11. Results of an Ouchterlony analysis of ground pork. A precipitin line forms where goat anti-swine albumin antibody binds to the swine albumin (the antigen) in the pork sample in the optimal ratio. The presence of a precipitin line only in this location indicates that the meat sample does contain pork and does not contain any detectable amounts of beef or horse.

A common application of the Ouchterlony method has been to determine how closely antigens are related to each other. Recognition of two different antigens (proteins for example) by the same antibody indicates similarity between the two. Such similarities between proteins may help establish evolutionary relationships between organisms. In addition, the Ouchterlony analysis was once used in forensics to establish whether tissue or blood were of human origin. Some agricultural labs used the procedure to identify and test the purity of meat.

This technique works for raw meat but will not work if the meat has been cooked (hotdogs, for example, are cooked during processing). When proteins are heated, they are denatured. To understand denaturation, it is necessary to know that protein is made up of many amino acids in a long polypeptide chain, arranged precisely

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in a specific formation. Denaturing jumbles the shape. When a protein antigen loses its characteristic shape, the antibody cannot recognize the antigen and thus no precipitate will form.

In large part, the Ouchterlony technique has been replaced by faster, more informative methods; however, it remains a useful teaching tool and provides a vivid demonstration of antibody–antigen interactions.

Immunoprecipitation

The formation of antigen–antibody complexes in liquid is also a useful technique and is simply called immunoprecipitation. In this widely used technique, antigen–antibody complexes are allowed to form in a tube, which is then spun in a centrifuge. During centrifugation, the complexes sink to the bottom of the tube and form a pellet. The supernatant (the liquid above the pellet) is then removed, and the immunocomplexes are used for further analysis. The need for a correct antigen–antibody ratio is circumvented by adding a second antibody, attached to a very small bead. When this antibody binds to the first one, the weight of the bead causes the antigen–antibody complex to sink to the bottom of the tube.

Quantitative Immunologic Analysis

The Ouchterlony test is fairly sensitive but does not provide an accurate measure of how much of a specific antigen is present. For example, in a positive test identifying that a sample of meat had been contaminated by meat from a different animal, there is no way to identify whether 10 percent or 90 percent of the meat came from the wrong source; i.e., the Ouchterlony technique is a qualitative but not a quantitative assay. Some quantitative assays are discussed below.

Radial Diffusion

Radial diffusion is used when there is a relatively large concentration of a substance in the sample being analyzed (e.g., when a laboratory technician wants to determine the amount of albumin or other protein in a patient's serum). Like an Ouchterlony analysis, radial diffusion is performed using agar, but the antiserum is mixed into the agar rather than being placed in a well. For example, a person who wanted to quantitate the proportion of horsemeat in several ground meat samples would mix anti-horse albumin into the agar.

Extract from different meat samples is placed in one set of wells, and solutions containing known concentrations of horse albumin are placed in another set of wells as standards. During the next 24 to 36 hours, the albumin from the meat samples and the standards diffuses, and a precipitin ring forms around each well. Since the concentration of antigen decreases as the antigen diffuses from the well, and the heaviest precipitation occurs when the equivalence point is reached, the size of the ring is proportional to the concentration of the albumin in the sample. To quantitate the horse albumin in the meat samples, the diameter of the ring around each sample is compared with the diameter of the rings around the standards.

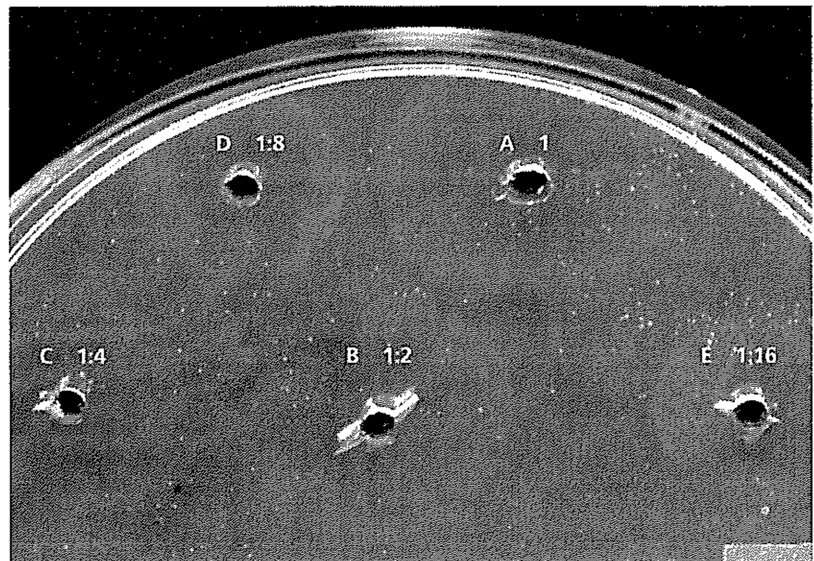

Figure 12. Demonstration of the proportional relationship between the concentration of antigen in a well and the diameter of the ring surrounding the well. The agar in this plate has been mixed with goat anti-bovine albumin. Serial dilutions of bovine albumin have been placed in the wells as indicated. Well A contains an undiluted sample.

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Radioimmunoassay

To detect minute amounts of a substance present in a patient's serum or other sample, a more sensitive test called a radioimmunoassay can be used. The development of the radioimmunoassay was reported by Berson and Yalow in 1960. The assay detects even small numbers of specific molecules such as insulin or growth hormone (ACTH). A radioimmunoassay employs antibodies to the molecule that the assay is set up to detect.

For example, a patient's insulin level can be measured by mixing a sample of their serum with a known amount of radioactively labeled insulin. An insulin antibody is added to this mixture. (Alternatively, the mixture may be added to insulin antibodies that have been fixed to a solid surface.) The antiserum reacts both with the insulin in the patient's serum and with the radioactively labeled insulin. The antibody–insulin complexes are then separated from the free insulin, and the level of radioactivity associated with each is measured.

If the patient's serum contains no insulin and radioactive insulin is mixed with it, then all the insulin–antibody complexes formed will be radioactive. In contrast, if insulin is present in the patient's serum in an amount five times greater than the amount of radioactive insulin added, then only one of every five insulin–antibody complexes (20 percent) will be radioactive.

The exact amount of insulin in the patient's serum in micrograms per milliliter can be read using the standard curve as shown in Figure 13.

The standard curve is created by including in the assay samples that have various defined known amounts of nonradioactive insulin in place of patient antiserum. The amount of radioactive insulin bound to the antibody in the presence of those known amounts of insulin is then measured. The standard curve is created by plotting the known hormone concentration against the percentage of bound radioactive insulin [Bound/Free Antigen (%)]. When using the curve to determine the amount of insulin in the patient's serum, the point on the curved line corresponding to the percentage of bound radioactive insulin is determined from the y-axis, and the concentration of insulin in the patient's blood is read from the x-axis. For 20 percent bound radioactive insulin, the patient's level of insulin would be 15 micrograms per milliliter.

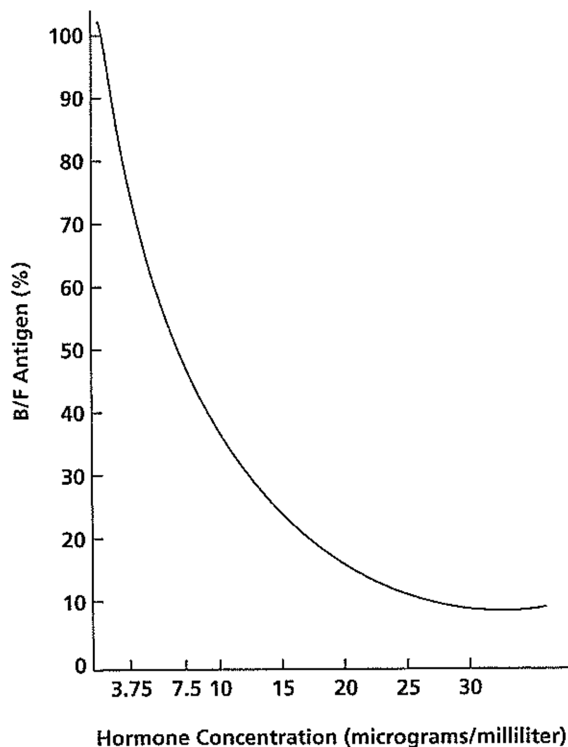

Figure 13.

ELISA

An enzyme-linked immunoabsorbent assay (ELISA) detects either a specific antigen or a specific antibody in a sample, such as fluid from a patient. A direct ELISA detects an antigen, and an indirect ELISA detects an antibody. The ELISA technique is also widely used in research and can be performed as a qualitative or quantitative assay. Like the radioimmunoassay, it is very sensitive.

For an indirect ELISA, the antigen known to bind to the antibody in question is affixed to a solid surface, usually the bottom of a well on a plastic plate. Then, a sequence of reagents is added. After each addition, the excess reagent is washed away. The first reagent added is the sample being tested. If that sample contains the antibody, it will bind to the antigen in the bottom of the well. The next reagent added is a second antibody with an enzyme attached. This antibody, called a secondary antibody, binds to the antibody being tested for. The substrate for the attached enzyme is a molecule that goes through a color change when it reacts with the enzyme. As the last step, this substrate is

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added, and any wells that contain the test antibody show a color change. The intensity of the color indicates the amount of antibody present (Fig. 14).

A direct ELISA is done in a similar manner but is set up to detect a specific antigen. The first step is to affix the antibody to that antigen to the bottom of the test well. Next, the test sample is added, and if any of the antigen being tested for is in the sample, it will be bound by the antibody and retained in the well. Next, a second antibody that recognizes the bound antigen (and has an enzyme attached) is added to the well. Finally, the substrate for the enzyme is added. If the antigen is present in a well, there will be a color change.

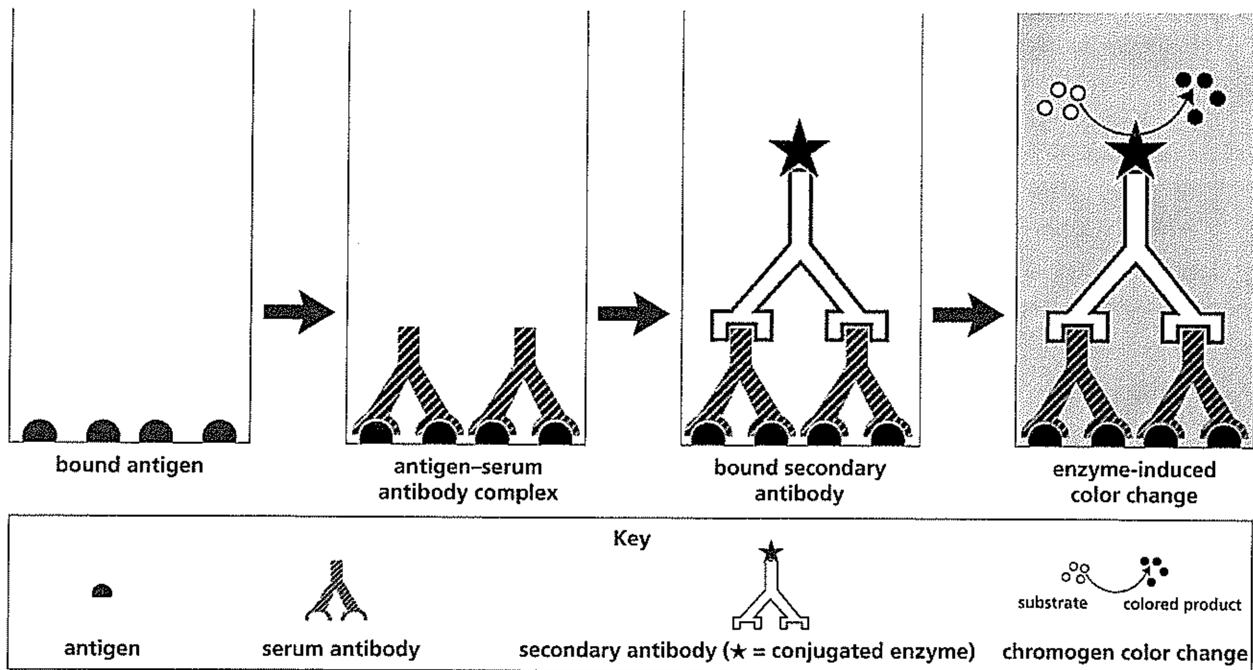

Figure 14. Steps of an indirect ELISA assay.

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Glossary

Agar. An extract from certain seaweeds. Agar forms a semisolid transparent gel when heated with water and allowed to cool. The gel results from the reaction of agar molecules with each other to form a matrix that traps the water molecules. Because the matrix dissociates upon being heated, the agar gel can easily be converted back into a liquid. The agar supplied in your kit is produced by mixing agar with saline solution.

Albumin. The most prevalent protein in an animal's serum. It transports multiple molecules through the bloodstream.

Antibody. A protein in the blood produced when a foreign substance or antigen is introduced into the body. An antibody molecule can react with the antigen that stimulated the antibody's production.

Antigen. Any large molecule that causes the production of antibody when introduced into a living animal. An antigen can combine with an antibody that is specific for that antigen. In the laboratory, antibody–antigen binding is sometimes evidenced by the formation of a precipitate.

Antiserum (*pl.* **antisera**). Serum from an animal that has been immunized with an antigen and which therefore contains antibodies to that antigen.

Bovine Albumin. Albumin found in cow serum (see albumin).

Denature. To cause structural damage to a protein by shaking, heating, or chemical treatment. At the molecular level, the polypeptide chain that makes up the protein molecule becomes folded or arranged into a different shape. A familiar example of heat denaturation is illustrated by the change of egg white from a clear fluid to a white solid when cooked.

Goat Anti-bovine Albumin. An antiserum to bovine albumin produced in the body of a goat that has been injected with bovine albumin.

Goat Anti-horse Albumin. An antiserum to horse albumin produced in the body of a goat that has been injected with horse albumin.

Goat Anti-swine Albumin. An antiserum to swine albumin produced in the body of a goat that has been injected with swine albumin.

Horse Albumin. Albumin found in horse serum (see albumin).

Immunology. The science dealing with an organism's resistance to disease and invasion by foreign material.

Precipitate. An insoluble material that results from the interaction of two substances in solution.

Peptide. A short chain of amino acids linked together as they are in proteins.

Protein. One of the four major classes of organic compounds found in living tissue. Proteins consist of amino acids chemically linked to each other to form long chains or polypeptides.

Reagents. Substances or solutions used in chemical reactions.

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Serum (*pl.* **sera**). The yellow fluid that remains after blood has clotted and the red and white blood cells have been removed. Serum makes up approximately 50 percent of the volume of blood.

Wells. In an Ouchterlony procedure, wells are cylindrical holes cut in an agar plate into which reagents are placed.

Appendix

Diffusion

This description of diffusion goes beyond the interests and requirements of most teachers and classes. However, it is included because understanding diffusion is central to understanding how an Ouchterlony procedure works.

Diffusion is the process that by which molecules spread out to fill the space available to them. Albert Einstein described the process with the following model.

Start with a closed tube partially filled with an aqueous solution and filled the rest of the way with pure water. Imagine the tube to be divided into a large number of segments of equal size so that each segment on the left side contains 100 molecules of the substance, while each segment on the right side contains pure water (Fig. 15). The numbers in Figure 15 refer to the number of solute molecules. Molecules are in continual random motion (Brownian motion). Because of this, it is possible to pick a time when 10 percent of the molecules in each segment have moved across the boundary on each side. After one such time period, distribution of the solute in the tube would appear as shown in the "Result of change" in Step 1. The molecular arrangement depicted in Step 1 "Result of change" was arrived at by calculating 10 percent of the solute molecules in each segment (either 10 or 0 molecules) and then shifting this number of molecules as shown by the arrows. When the process is repeated, the distribution of solute in the tube appears as shown in Step 2 of Figure 15.

Diffusion is, of course, continuous and not stepwise as depicted in the figure; however, the model helps illustrate how continuous random movement results in the molecules' dispersion. After many intervals, there would be 50 molecules in each segment of the tube.

The model can be further manipulated to show the effects of two different phenomena: size of molecules and number of molecules. Larger molecules move more slowly; therefore, fewer than 10 percent would cross a boundary in the time required for dispersion of 10 percent of a "population" of smaller molecules. If more molecules are present (e.g., 1000 per segment), they diffuse faster.

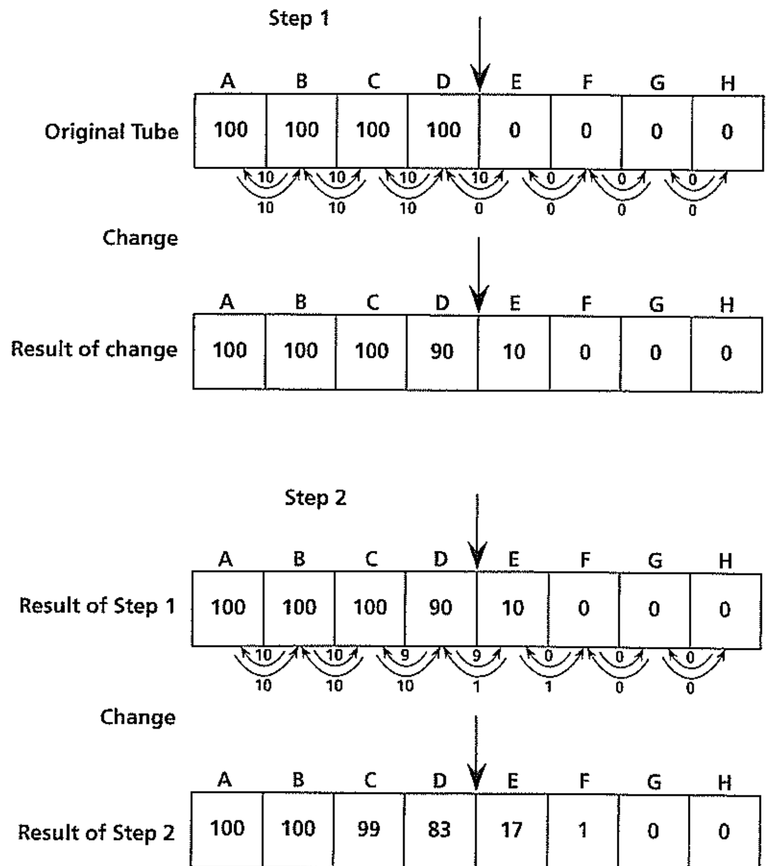

Figure 15. Einstein's diffusion model.

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