Learning Objectives

After studying this chapter you should be able to:

- List the A-B-O antigens and antibodies found in the blood for each of the four blood types: A, B, AB, and O
- Understand and describe how whole blood is typed
- List and describe forensic tests used to characterize a stain as blood
- Understand the concept of antigen–antibody interactions and how it is applied to species identification and drug identification
- Contrast chromosomes and genes
- Learn how the Punnett square is used to determine the genotypes and phenotypes of offspring
- List the laboratory tests necessary to characterize seminal stains
- Explain how suspect blood and semen stains are properly preserved for laboratory examination
- Describe the proper collection of physical evidence in a rape investigation

In 1901, Karl Landsteiner announced one of the most significant discoveries of the twentieth century—the typing of blood—a finding that twenty nine years later earned him a Nobel Prize. For years physicians had attempted to transfuse blood from one individual to another. Their efforts often ended in failure because the transfused blood tended to coagulate, or clot, in the body of the recipient, causing instantaneous death. Landsteiner was the first to recognize that all human blood was not the same; instead, he found that blood is distinguishable by its group or type.

Out of Landsteiner's work came the classification system that we call the *A-B-O system*. Now physicians had the key for properly matching the blood of a donor to that of a recipient. One blood type cannot be mixed with a different blood type without disastrous consequences. This discovery, of course, had important implications for blood transfusion and the millions of lives it has since saved.

Meanwhile, Landsteiner's findings opened a new field of research in the biological sciences. Others began to pursue the identification of additional characteristics that could further differentiate blood. By 1937, the Rh factor in blood had been demonstrated and, shortly thereafter, numerous blood factors or groups were discovered. More than 100 different blood factors have been identified. However, the ones in the A-B-O system are still the most important for properly matching a donor and recipient for a transfusion.

Until the early 1990s, forensic scientists focused on blood factors, such as A-B-O, as offering the best means for linking blood to an individual. What made these factors so attractive was that in theory no two individuals, except for identical twins, could be expected to have the same combination of blood factors. In other words, blood factors are controlled genetically and have the potential of being a highly distinctive feature for personal identification. What makes this observation so relevant is the great frequency of bloodstains at crime scenes, especially crimes of the most serious nature—homicides, assaults, and rapes. Consider, for example, a transfer of blood between the victim and assailant during a struggle; that is, the victim's blood is transferred to the suspect's garment or vice versa. If the criminalist could individualize human blood by identifying all of its known factors, the result would be strong evidence for linking the suspect to the crime.

The advent of DNA technology has dramatically altered the approach of forensic scientists toward individualization of bloodstains and other biological evidence. The search for genetically controlled blood factors in bloodstains has been abandoned in favor of characterizing biological evidence by select regions of our **deoxyribonucleic acid** (DNA), which carries the body's genetic information. As a result, the individualization of dried blood and other biological evidence has become a reality and has significantly altered the role that crime laboratories play in criminal investigations. In fact, as we will learn in the next chapter, the high sensitivity of DNA analysis has even altered the types of materials collected from crime scenes in the search for DNA.

deoxyribonucleic acid (DNA)
The molecules that carry the body's genetic information.

The next chapter is devoted to discussing recent breakthroughs in associating blood and semen stains with a single individual through characterization of DNA. This chapter focuses on underlying biological concepts that forensic scientists historically relied on as they sought to characterize and individualize biological evidence prior to the dawning of the age of DNA.

The Nature of Blood

The word *blood* refers to a highly complex mixture of cells, enzymes, proteins, and inorganic substances. The fluid portion of blood is called **plasma**; it is composed principally of water and accounts for 55 percent of blood content. Suspended in the plasma are solid materials consisting chiefly of several types of cells—red blood cells (**erythrocytes**), white blood cells (leukocytes), and platelets. The solid portion of blood accounts for 45 percent of its content. Blood clots when a protein in the plasma known as *fibrin* traps and enmeshes the red blood cells. If the clotted material were removed, a pale yellowish liquid known as **serum** would be left.

plasma
The fluid portion of unclotted blood.

erythrocyte
A red blood cell.

serum
The liquid that separates from the blood when a clot is formed.

Obviously, considering the complexity of blood, any discussion of its function and chemistry would have to be extensive, extending beyond the scope of this text. It is certainly far more relevant at this point to concentrate our discussion on the blood components that are directly pertinent to the forensic aspects of blood identification—the red blood cells and the blood serum.

Antigens and Antibodies

Red blood cells transport oxygen from the lungs to the body tissues and remove carbon dioxide from tissues by transporting it back to the lungs, where it is exhaled. However, for reasons unrelated to the red blood cell's transporting mission, on the surface of each cell are millions of characteristic chemical structures called **antigens**. Antigens impart specific characteristics to the red blood cells. Blood antigens are grouped into systems depending on their relationship to one another. More than fifteen blood antigen systems have been identified to date; of these, the A-B-O and Rh systems are the most important.

antigen
A substance, usually a protein, that stimulates the body to produce antibodies against it.

If an individual is type A, this simply indicates that each red blood cell has A antigens on its surface; similarly, all type B individuals have B antigens, and the red blood cells of type AB individuals contain both A and B antigens. Type O individuals have neither A nor B antigens on their cells. Hence, the presence or absence of A and B antigens on the red blood cells determines a person's blood type in the A-B-O system.

Another important blood antigen has been designated as the *Rh factor*, or D antigen. Those people having the D antigen are said to be *Rh positive*;

those without this antigen are *Rh negative*. In routine blood banking, the presence or absence of the three antigens—A, B, and D—must be determined in testing compatibility of the donor and recipient.

antibody
A protein in the blood serum that destroys or inactivates a specific antigen.

antiserum
Blood serum that contains specific antibodies.

agglutination
The clumping together of red blood cells by the action of an antibody.

Serum is important because it contains proteins known as **antibodies**. The fundamental principle of blood typing is that for every antigen, there exists a specific antibody. Each antibody symbol contains the prefix *anti-*, followed by the name of the antigen for which it is specific. Hence, anti-A is specific only for A antigen, anti-B for B antigen, and anti-D for D antigen. The serum-containing antibody is referred to as the **antiserum**, meaning a serum that reacts against something (antigens).

An antibody reacts only with its specific antigen and no other. Thus, if serum containing anti-B is added to red blood cells carrying the B antigen, the two will combine, causing the antibody to attach itself to the cell. Antibodies are normally *bivalent*—that is, they have two reactive sites. This means that each antibody can simultaneously be attached to antigens located on two different red blood cells. This creates a vast network of cross-linked cells usually seen as clumping or **agglutination** (see Figure 8–1).

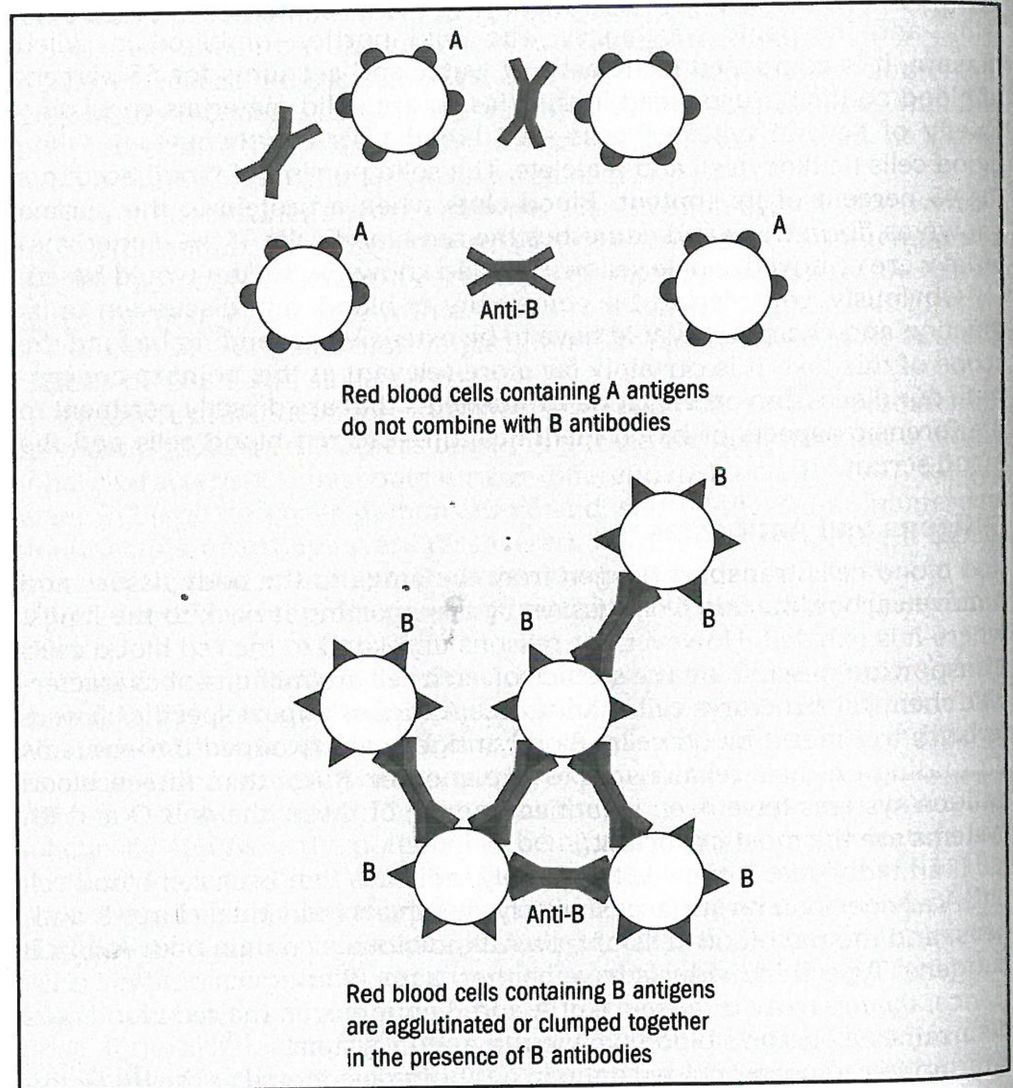

FIGURE 8–1

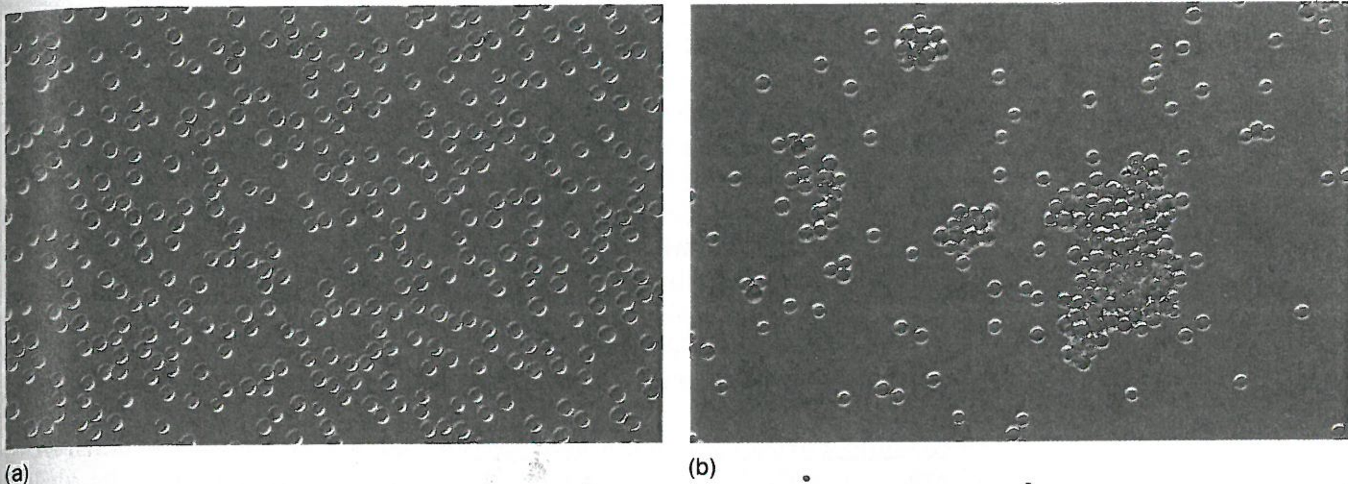

FIGURE 8–2 (a) Microscopic view of normal and red blood cells (500×). (b) Microscopic view of agglutinated red blood cells (500×). *Courtesy J. C. Revy, Phototake NYC*

Let's look a little more closely at this phenomenon. In normal blood, shown in Figure 8–2(a), antigens on red blood cells and antibodies coexist without destroying each other because the antibodies present are not specific toward any of the antigens. However, suppose a foreign serum added to the blood introduces a new antibody. This results in a specific antigen–antibody reaction that immediately causes the red blood cells to link together, or agglutinate, as shown in Figure 8–2(b).

Evidently, nature has taken this situation into account, for when we examine the serum of type A blood, we find anti-B and no anti-A. Similarly, type B blood contains only anti-A, type O blood has both anti-A and anti-B, and type AB blood contains neither anti-A nor anti-B. The antigen and antibody components of normal blood are summarized in the following table:

Blood Type	Antigens on Red Blood Cells	Antibodies in Serum
A	A	Anti-B
B	B	Anti-A
AB	AB	Neither anti-A nor anti-B
O	Neither A nor B	Both anti-A and anti-B

The reasons for the fatal consequences of mixing incompatible blood during a transfusion should now be quite obvious. For example, the transfusion of type A blood into a type B patient will cause the natural anti-A in the blood of the type B patient to react promptly with the incoming A antigens, resulting in agglutination. In addition, the incoming anti-B of the donor will react with the B antigens of the patient.

Blood Typing

The term serology describes a broad scope of laboratory tests that use specific antigen and serum antibody reactions. The most widespread application of serology is the typing of whole blood for its A-B-O identity. In determining the A-B-O blood type, only two antiserums are needed—anti-A and anti-B. For routine blood typing, both of these antiserums are commercially available.

serology
The study of antigen-antibody reactions.

Table 8–1 summarizes how the identity of each of the four blood groups is established when the blood is tested with anti-A and anti-B serum. Type A blood is agglutinated by anti-A serum; type B blood is agglutinated

by anti-B serum; type AB blood is agglutinated by both anti-A and anti-B; and type O blood is not agglutinated by either the anti-A or anti-B serum.

The identification of natural antibodies present in blood offers another way to determine blood type. Testing blood for the presence of anti-A and anti-B requires using red blood cells that have known antigens. Again, these cells are commercially available. Hence, when A cells are added to a blood specimen, agglutination occurs only in the presence of anti-A. Similarly, B cells agglutinate only in the presence of anti-B. All four A-B-O types can be identified in this manner by testing blood with known A and B cells, as summarized in Table 8–2.

The population distribution of blood types varies with location and race throughout the world. In the United States, a typical distribution is as follows:

O	A	B	AB
43%	42%	12%	3%

Key Points

- Serology involves a broad scope of laboratory tests that use specific antigen and serum antibody reactions.
- An antibody reacts or agglutinates only with its specific antigen. The concept of specific antigen–antibody reactions has been applied to techniques for detecting abused drugs in blood and urine.
- Every red blood cell contains either an A antigen, a B antigen, or no antigen (this is called type O). The type of antigen on one's red blood cells determines one's A-B-O blood type. People with type A blood have A antigens on their red blood cells, those with type B blood have B antigens, and those with type O blood have no antigens on their red blood cells.

Table 8–1 Identification of Blood with Known Antiserum

Anti-A Serum + Whole Blood	Anti-B Serum + Whole Blood	Antigen Present	Blood Type
+	–	A	A
–	+	B	B
+	+	A and B	AB
–	–	Neither A nor B	O

Note: + shows agglutination; – shows absence of agglutination.

Table 8–2 Identification of Blood with Known Cells

A Cells + Blood	B Cells + Blood	Antibody Present	Blood Type
+	–	Anti-A	B
–	+	Anti-B	A
+	+	Both anti-A and anti-B	O
–	–	Neither anti-A nor anti-B	AB

Note: + shows agglutination; – shows absence of agglutination.

monoclonal antibodies
A collection of identical antibodies that interact with a single antigen site.

hybridoma cells
Fused spleen and tumor cells. Used to produce identical monoclonal antibodies in a limitless supply.

skip

Closer Analysis

Polyclonal and Monoclonal Antibodies (*continued*)

vary in composition over time. As a result, different batches of polyclonals may vary in their specificity and their ability to bind to a particular antigen site.

Modern forensic technologies occasionally require antibodies that are more uniform in their composition and attack power than the traditional polyclonals. Forensic scientists thus need a way to produce antibodies designed to attack one and only one site on an antigen. Such antibodies are known as **monoclonal antibodies.** How can such monoclonals be produced?

The process begins by injecting a mouse with the antigen of interest. In response, the mouse's spleen cells produce antibodies to fight off the invading antigen. The spleen cells are removed from the animal and are fused to fast-growing blood cancer cells to produce **hybridoma cells.** The hybridoma cells are then allowed to multiply and are screened for their specific antibody activity. Hybridoma cells bearing the antibody activity of interest are then selected and cultured. The rapidly multiplying cancer cells linked to the selected antibody cells produce identical monoclonal antibodies in a limitless supply (see figure).

Monoclonal antibodies are being incorporated into commercial forensic test kits with increasing frequency. Many immunoassay test kits for abused drugs are being formulated with monoclonal antibodies. Also, a recently introduced test for semen that incorporates a monoclonal antibody has found wide popularity in crime laboratories.

Key Points

- To produce antibodies capable of reacting with drugs, the analyst combines a specific drug with a protein and injects this combination into an animal such as a rabbit. This drug–protein complex acts as an antigen, stimulating the animal to produce antibodies. The recovered blood serum of the animal now contains antibodies that are specific or nearly specific to the drug.
- When an animal is injected with an antigen, its body produces a series of different antibodies, all of which are designed to attack some particular site on the antigen of interest. These antibodies are known as polyclonal antibodies.
- A more uniform and specific collection of antibodies designed to combine with a single antigen site can be manufactured. Such antibodies are known as monoclonal antibodies.

Forensic Characterization of Bloodstains

The criminalist must answer the following questions when examining dried blood: (1) Is it blood? (2) From what species did the blood originate? (3) If the blood is human, how closely can it be associated with a particular individual?

Color Tests

The determination of blood is best made by means of a preliminary color test. For many years, the most common test was the *benzidine color test.*

However, because benzidine has been identified as a known carcinogen, its use has generally been discontinued, and the chemical phenolphthalein is usually substituted in its place (this test is also known as the *Kastle-Meyer color test*).[1]

Both the benzidine and Kastle-Meyer color tests are based on the observation that blood **hemoglobin** possesses peroxidase-like activity. Peroxidases are enzymes that accelerate the oxidation of several classes of organic compounds when combined with peroxides. For example, when a bloodstain, phenolphthalein reagent, and hydrogen peroxide are mixed together, oxidation of the hemoglobin in the blood produces a deep pink color.

hemoglobin
A red blood cell protein that transports oxygen in the bloodstream; it is responsible for the red color of blood.

The Kastle-Meyer test is not a specific test for blood; some vegetable materials, for instance, may turn Kastle-Meyer pink. These substances include potatoes and horseradish. However, such materials will probably not be encountered in criminal situations, and thus from a practical point of view, a positive Kastle-Meyer test is highly indicative of blood. Field investigators have found Hemastix strips a useful presumptive field test for blood. Designed as a urine dipstick test for blood, the strip can be moistened with distilled water and placed in contact with a suspect bloodstain. The appearance of a green color indicates blood.

Luminol

Another important presumptive identification test for blood is the **luminol** test.[2] Unlike the benzidine and Kastle-Meyer tests, the reaction of luminol with blood produces light rather than color. After spraying luminol reagent onto suspect items, agents darken the room; any bloodstains produce a faint blue glow, known as *luminescence*. Using luminol, investigators can quickly screen large areas for bloodstains.

luminol
The most sensitive chemical test that is capable of presumptively detecting bloodstains diluted up to 300,000 times. Its reaction with blood emits light and thus requires the result to be observed in a darkened area.

The luminol test is extremely sensitive, capable of detecting bloodstains diluted up to 300,000 times. For this reason, spraying large areas such as carpets, walls, flooring, or the interior of a vehicle may reveal blood traces or patterns that would have gone unnoticed under normal lighting conditions (see Figure 8–4). Luminol does not interfere with any subsequent DNA testing.[3]

Microcrystalline Tests

The identification of blood can be made more specific if microcrystalline tests are performed on the material. Several tests are available; the two most popular ones are the *Takayama* and *Teichmann tests*. Both depend on the addition of specific chemicals to the blood to form characteristic crystals containing hemoglobin derivatives. Crystal tests are far less sensitive than color tests for blood identification and are more susceptible to interference from contaminants that may be present in the stain.

Precipitin Test

Once the stain has been characterized as blood, the serologist determines whether the blood is of human or animal origin. The standard test is the **precipitin** test. Precipitin tests are based on the fact that when animals (usually rabbits) are injected with human blood, antibodies form that react with the invading human blood to neutralize its presence. The investigator can recover these antibodies by bleeding the animal and isolating the blood serum, which contains antibodies that specifically react with human

precipitin
An antibody that reacts with its corresponding antigen to form a precipitate.

skip
Replaced by HemaTrace Test

Bloodstain Patterns

The location, distribution, and appearance of bloodstains and spatters may be useful for interpreting and reconstructing the events that produced the bleeding. A thorough analysis of the significance of the position and shape of blood patterns with respect to their origin and trajectory is exceedingly complex and requires the services of an examiner who is experienced in such determinations. Most important, the interpretation of bloodstain patterns necessitates carefully planned control experiments using surface materials comparable to those found at the crime scene.

A number of observations and conclusions have important implications for any investigator who seeks to trace the direction, dropping distance, and angle of impact of a bloodstain. Some of them can be summarized as follows:

1. Surface texture is very important in the interpretation of bloodstain patterns, and correlations between standards and unknowns are valid only if identical surfaces are used. In general, the harder and less porous the surface, the less spatter. The effect of surface is shown in Figure 8–8.
2. The direction of travel of blood striking an object may be discerned by the stain's shape. The pointed end of a bloodstain always faces its direction of travel. In Figure 8–9, the bloodstain pattern was produced by several droplets of blood that were traveling from left to right before striking a flat level surface.
3. It is possible to determine the impact angle of blood on a flat surface by measuring the degree of circular distortion of the stain. A drop of blood striking a surface at right angles produces a nearly circular stain; as the angle decreases, the stain elongates. This progressive elongation is evident in Figure 8–10.

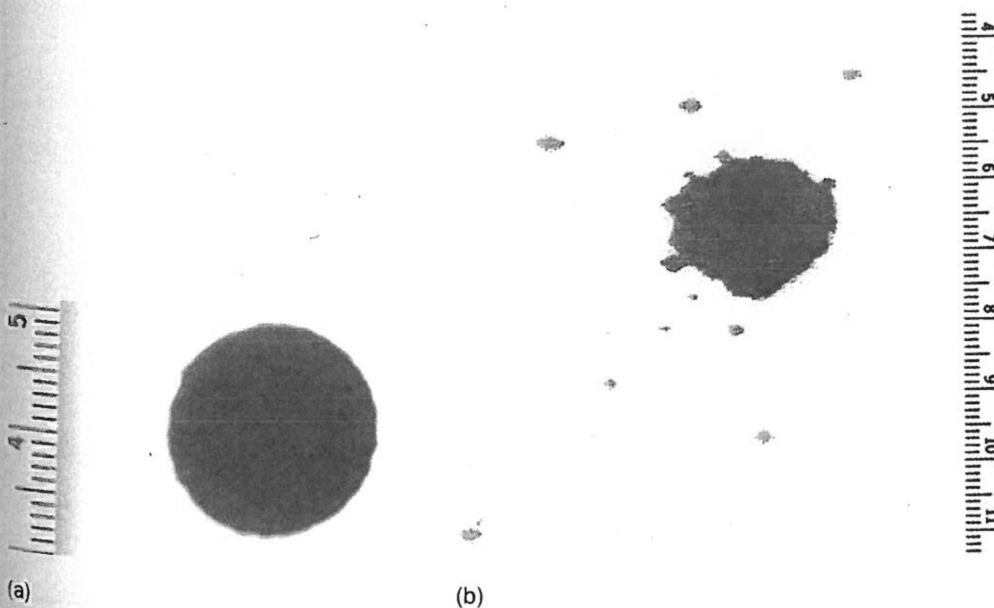

FIGURE 8–8 (a) Bloodstain from a single drop of blood that struck a glass surface after falling 24 inches. (b) Bloodstain from a single drop of blood that struck a cotton muslin sheet after falling 24 inches. *Courtesy A. Y. Wonder*

FIGURE 8–9 Bloodstain pattern produced by droplets of blood that were traveling from left to right. *Courtesy A. Y. Wonder*

FIGURE 8–10 The higher pattern is of a single drop of human blood that fell 24 inches and struck hard, smooth cardboard at 50 degrees. The lower pattern is of a single drop of human blood that fell 24 inches and struck hard, smooth cardboard at 15 degrees. *Courtesy A. Y. Wonder*

4. The origin of a blood spatter in a two-dimensional configuration can be established by drawing straight lines through the long axis of several individual bloodstains. The intersection or area of convergence of the lines represents the area from which the blood emanated (see Figure 8–11).

An example of the utility of blood spatter formations in performing crime-scene reconstruction is illustrated in Figures 8–12 through 8–14. This case relates to an elderly male who was found lying dead on his living room floor. He had been beaten about the face and head, then stabbed in the chest and robbed. The reconstruction of bloodstains found on the interior front door and the adjacent wall documented that the victim was beaten about the face with a fist and struck on the back of the head with his cane. A suspect was apprehended three days later, and he was found to have an acute fracture of the right hand. When he was confronted with the bloodstain evidence, the suspect admitted striking the victim, first with his fist, then with a cane, and finally stabbing him with a kitchen knife. The suspect pleaded guilty to three first-degree felonies.

WebExtra 8.1
See How Bloodstain Spatter Patterns Are Formed
www.prenhall.com/hsforensics

The Tail Tells the Tale!!

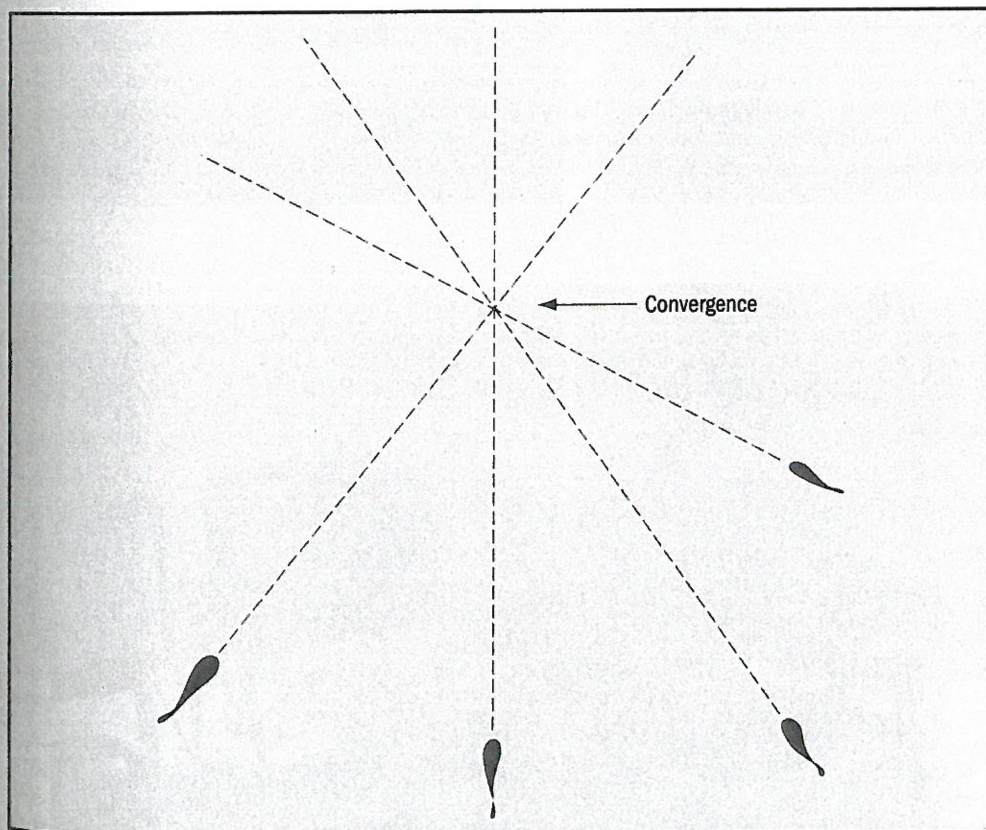

FIGURE 8–11 Illustration of stain convergence on a two-dimensional plane. Convergence represents the area from which the stains emanated. *Courtesy Judith Bunker, J. L. Bunker & Assoc., Ocoee, Fla.*