13

Biologics

The inclusive term **"biologic"** may encompass any product derived from a living plant or animal source. However, strictly interpreted, biologics are substances defined by the Center for Drugs and Biologics of the Federal Food and Drug Administration under the Public Health Service Act of 1944, as amended. The law refers to "any virus, therapeutic serum, toxin, antitoxin or analogous product," and it has been interpreted to include a lengthy list of such products as vaccines of bacterial, rickettsial, and viral origin, immune serums for the prevention or treatment of disease, various miscellaneous and diagnostic products, human blood, and products derived from human blood. Such substances as insulin, liver extract, and antibiotic products are not classified as biologics. Much of this reasoning depends on legal definitions and considerations.

The broad term "biologics" thus includes the immunizing biologics that are derivatives of animals (serums, antitoxins, globulins) or of microscopic plant organisms (vaccines, toxins, toxoids, tuberculins), which either directly or indirectly confer a state of protection against pathogenic microorganisms. Because these products do not affect the microorganisms directly, they cannot be considered chemotherapeutic agents; nor can they be classified with the antibiotics.

Biologics can be classified into 2 general categories, antigens and antibodies. An antigen is the material that provokes the immune response, and it can be defined under 3 categories: biologic, chemical, and physical.

Biologically, an antigen is a substance that, when introduced into the tissue of humans or other vertebrates, causes the formation of antibodies. These antibodies then react specifically with the antigen that stimulated their production. Therefore, an antigen possesses 2 biologic properties: (1) immunogenicity, the capacity to induce antibody formation, and (2) specificity, governed by small chemical sites on the antigen molecule called the antigenic determinants. The antibody combines with one or more of these sites. Another important biologic concept of the antigen is that it must be considered foreign by the antibody-forming host.

Chemically, antigens are usually protein; however, some high-molecular-weight polysaccharides are antigenic.

Physically, antigens must possess a high molecular weight. A weight of more than 10,000 daltons is required. The high molecular weight is associated with the biologic property of immunogenicity.

Examples of antigens that are directly concerned in infectious disease are exotoxins, proteins and polysaccharides on the cell surface and capsules of bacteria, and the protein coat of virus particles. Microorganisms contain not one but many an-

tigens, which, in turn, may contain many antigenic determinants.

The number of distinct determinants on an antigen molecule usually varies with its size and chemical complexity. Aromatic amino acids contribute more to immunogenicity than nonaromatic residues. The simplest form of an antigenic determinant present on a complex antigenic molecule is called an epitope. Studies have shown that the antibody recognizes the overall 3-dimensional shape of the epitope rather than any specific chemical property, such as ionic charge. The epitope and antibody combining sites are structurally complementary and fit together in a lock-and-key arrangement.

Compounds with a molecular weight lower than 10,000 daltons can be partial antigens. They are called haptens. Because of their low molecular weight, they cannot induce the formation of antibodies by themselves. They lack the property of immunogenicity. However, they can attach to host proteins to form a complete antigen that will induce the formation of antibodies specific for the particular hapten. Drugs, or their breakdown products, may act as haptens, and this action is the basis of many drug allergies, e.g., penicillin allergy (see page 422). In these cases, the drug molecule becomes the epitope.

Antibodies are found predominately in the serum fraction of the blood, although they also exist in other body fluids and in association with other tissues, such as lymph nodes and mucous membranes. When serum proteins are separated by electrophoresis, the 4 predominant fractions obtained are serum albumin and alpha, beta, and gamma globulins. The antibodies occur predominately in the gamma globulin fraction and are called immunoglobulins. On the basis of their physical, chemical, and immunologic properties, the immunoglobulins can be separated into 5 subclasses: IgA, IgD, IgE, IgG, and IgM. IgG is the most abundant of the serum immunoglobulins, and the major part (up to 80%) of the serum antibody found after bacterial and viral infections belongs to this class of antibodies. Immunoglobulin G has a molecular weight of approximately 150,000 and contains about 1400 amino acids. These acids are not linked in 1 continuous chain but are arranged in 4 polypeptide chains—2 heavy and 2 light. Each pair has identical structures. The chains are connected by disulfide bonds, which help impart a tertiary structure to the molecule (Figure 13–1). With papain, the peptides can be cleaved into 2 antigen-binding fragments (Fab) and a third fragment, which cannot combine with antigen and crystallizes in neutral salt solutions (Fc). The 2 antigen-binding fragments are identical and arise from the amino-terminal ends of the 4 peptide chains. Each fragment contains the amino-terminal portion of one light chain and one heavy chain, and studies have shown that the amino acid composition of these portions of the peptide chains is variable from one antibody to another. The amino acid composition of the portion of the peptide chains representing the carboxy-terminal ends that make up the Fc fragment is relatively constant among different antibodies. The variability in the Fab region of the molecule may reflect the unique structure of each specific antibody against a specific antigen.

Because of the 2 combining sites on the IgG molecule, these antibodies are particularly well adapted to form macromolecular lattices with antigens and are usually good precipitating antibodies. The Fc fragments of human IgG contain various sites that are important in specialized functions of the immunoglobulin. One site facilitates placental transmission, one site fixes the antibody to macrophages, which allows them to function in a cytotoxic fashion, and one site fixes the complement. Complement is a complex of serum proteins and is required for the completion of certain antigen-antibody reactions, including the

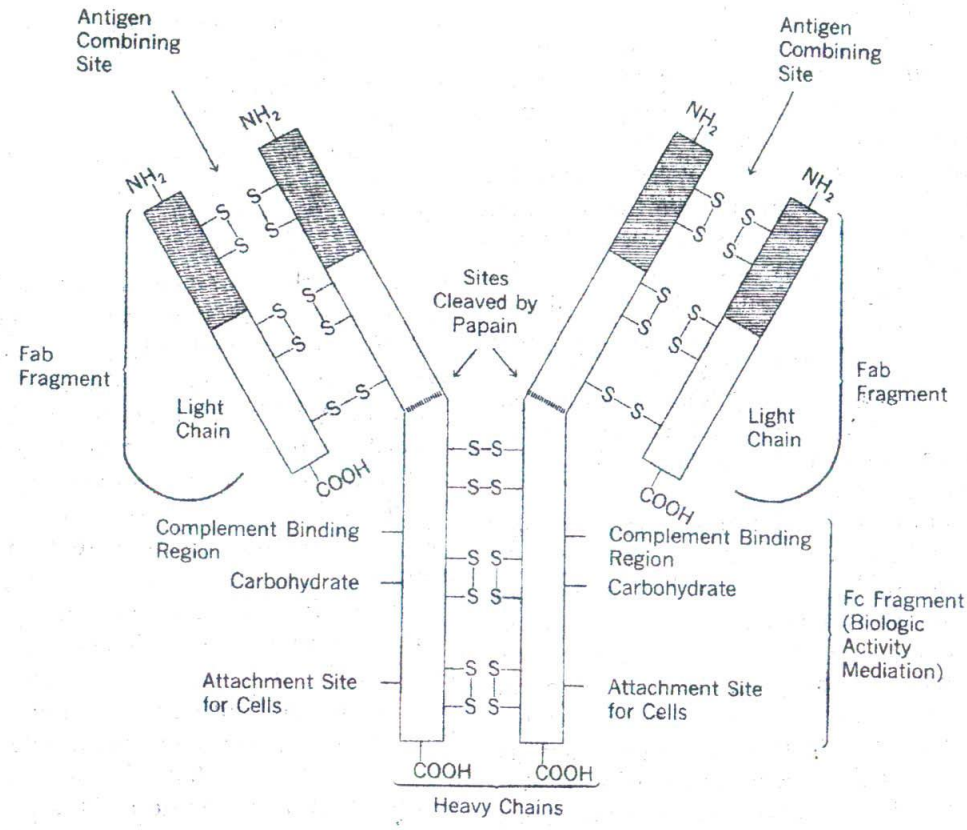

Fig. 13–1. A schematic representation of the structure of immunoglobulin G (IgG); ▒ designates amino acid variable region; ▭ designates amino acid constant region.

lysis of bacterial cells or erythrocytes by antibody.

When the newborn infant begins its own antibody production, the first immunoglobulin to appear is IgM. Molecules of IgM are pentamers of the basic 4-chain immunoglobulin unit such as that found in IgG. The 4-chain units are linked by disulfide bonds like a 5-pointed star. The antigen-binding sites point outward. Because of the increased number of possible binding sites on a single antibody molecule, IgM can react with closely spaced antigenic determinants on the surface of cells, thereby making this antibody efficient in agglutinating or clumping erythrocytes and bacteria. For example, the ABO blood group antibodies are of the IgM type. IgM, with IgD, is the major immunoglobulin expressed on the surface of B cells. It is also the most efficient complement-fixing immunoglobulin.

IgA, IgD, and IgE are found in relatively low concentrations in the blood serum. IgA is the predominate immunoglobulin in external secretions, such as saliva and secretions of the respiratory and gastrointestinal tracts. These antibodies probably form a specific defense mechanism in these areas of the body. Each secretory IgA molecule consists of two 4-chain basic units and 1 molecule each of secretory component and J chain, both of which are polypeptides with a molecular weight of approximately 70,000 and 15,000 daltons, respectively.

IgE molecules also known as reagins constitute only 0.004% of the total serum immunoglobulins but bind with high affinity to mast cells, which may be mediated by a cell attachment site on the Fc frag-

ments in the molecule. Upon combination with certain specific antigens called allergens, IgE molecules trigger the release from mast cells of chemical mediators such as histamine, which are responsible for the symptoms of immediate hypersensitivity, such as asthma, hay fever, anaphylaxis, and skin eruptions (see Chapter 14). Immunoglobulin D is a monomer, and its main function has not been determined. It is found on the surface of B lymphocytes, suggesting that it may be involved in the differentiation of these cells. IgD's specific role may be as an antigen receptor on antibody-producing cells that are designed for triggering the production of antibody.

Immunity is classified into 2 major types: **natural (innate) immunity** and **acquired immunity.** The term natural or innate means the defense mechanisms that are present in the body because of race, species specificity, and a multitude of other factors not easily defined, but it does *not* include any mechanisms especially developed during the lifetime of the individual. Thus, natural immunity is endowed at birth and is retained because of an individual's constitution.

On the other hand, acquired immunity is quite specific and generally is subdivided into 2 classes: **active immunity** and **passive immunity,** each of which is further subdivided as follows:

Acquired immunity
1. Active immunity
   a. Naturally acquired active
   b. Artificially acquired active
2. Passive immunity
   a. Naturally acquired passive
   b. Artificially acquired passive

Active immunity means the specific immunity developed by an individual in response to the introduction of antigenic substances into the body. In this type of immunity, the antigenic substances may be received by the body in a natural manner (naturally acquired active immunity) or they may be received by the body through

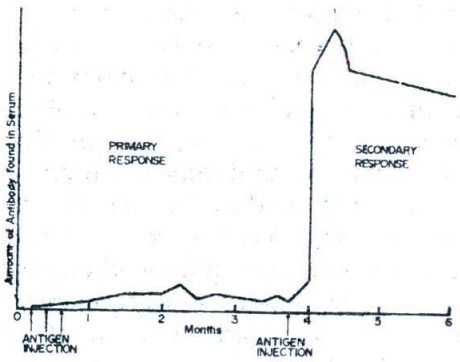

Fig. 13–2. An illustration of the recall or anamnestic phenomenon in antibody production.

the administration of a vaccine or toxoid (artificially acquired active immunity). In the first instance, recovery from an infection, such as measles or scarlet fever, produces an immunity that is acquired naturally, is developed rather slowly, and is usually long-lasting. In the second case, the immunity may be produced as the response to a series of injections (of typhoid or pertussis vaccine, for example), thus stimulating the body cells to make their own antibodies and producing an immunity that is acquired artificially, is developed gradually, and is usually long-lasting.

Depending on the nature of the antigen and the site of injection, antibody can be detected in the serum several days after the first injection of antigen. The antibody titer rises gradually to a low peak after the first and immediately subsequent injections and then falls slowly over a period of months (Fig. 13–2). A second injection of antigen, administered while antibodies from the first stimulus are still present, results in a rapid rise to a much higher peak than with the first injection. The second injection should not be too close in time to the first injection. If so, there is no additional effect on antibody production. The

antibodies disappear much more slowly after the second stimulus than after the first. The rapid rise of antibody titer following a second administration of the antigen (the booster shot) presumably indicates that the antibody-producing cells have been primed by the first contact with antigen and, therefore, respond more effectively and more quickly when they encounter the antigen a second time. This phenomenon is termed the recall or anamnestic phenomenon and has great practical significance in immunization against infectious disease.

The major cellular components of the immune system are the macrophages and the lymphocytes. The origin of serum antibodies is now believed to be certain lymphocytes called B cells (so named because they were first described as originating from the bursa of Fabricius of chickens), which arise from the bone marrow in humans (Figure 13–3). This is known as the humoral system of immunity because the B cells circulate in the body fluids, primarily in blood. The B-cell system handles most of the infectious organisms that are bacteria.

Companion to the B-cell system is another lymphocyte population, the T cells. These cells originate in the bone marrow but depend on the thymus gland for their differentiation (Fig. 13–3). The T cells are the agents of cellular immunity, more stationary than the B cells, and seldom found circulating in the blood. Cellular immunity resists infections by fungi, acid-fast bacilli such as *Mycobacterium tuberculosis*, and viruses. T cells are also responsible for delayed hypersensitivity, e.g., tuberculin reactions and poison ivy dermatitis, and serve as the sentinels of immune surveillance against cancer and the mediators of graft rejection.

As each B cell matures in the bone marrow, it becomes committed to the synthesis of antibodies that recognize a specific antigen. All the progeny of each such cell retain the same specificity and thus form a clone of immunologically identical cells. The antibodies produced by B cells remain bound to the cell membrane, and when an antigen binds to an antibody in the membrane, the cell is stimulated to proliferate; this is the clonal selection process. In ad-

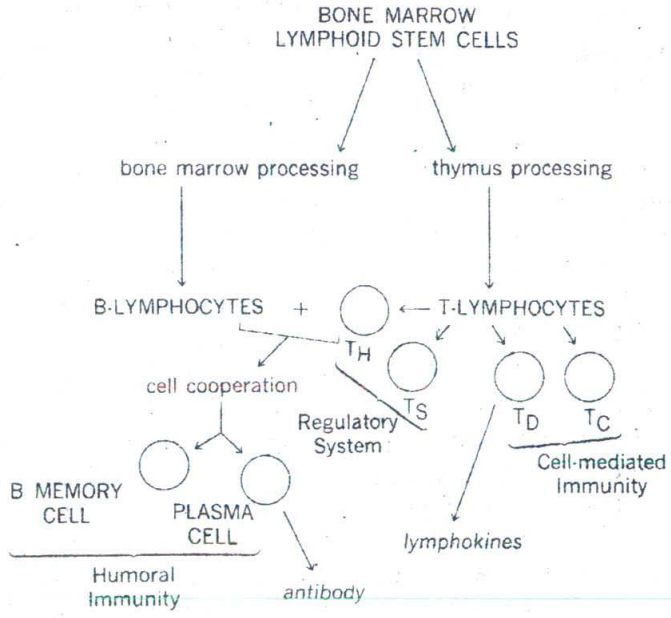

Fig. 13–3. Pathways for maturation of lymphocytes involved in the human immune system: T_H, helper cells; T_S, suppressor cells; T_D, delayed hypersensitivity cells; and T_C, cytotoxic cells.

dition, the origin of this specialization involves complex gene rearrangement.

Some of the progeny of the selected clones remain as circulating B lymphocytes. They serve as the immune system's memory, providing a faster response to any subsequent exposure to the same antigen; hence, they are called B memory cells. Other members of the selected B-cell clones, called plasma cells, grow larger, stop reproduction, and continuously secrete large quantities of antibody. They have a short life of only 2 to 3 days.

There are 4 known subsets of T cells. They are identical in appearance but can be distinguished by function. Cell-mediated immunity is a function of 2 subsets of T cells: T_C cells, which are cytotoxic and attack cell membranes bearing their specific antigen, and T_D cells, delayed hypersensitivity cells. During clonal expansion of sensitized T_D cells, a number of biologically active protein factors called lymphokines are secreted, and these recruit and activate macrophages. Because several hours are required for synthesis of lymphokines, their effect is delayed.

The other population of T cells, T_H (helper) cells and T_S (suppressor) cells, have an immunoregulatory role over the entire specific immune system. Cellular interaction between T_H and B cells is essential for optimal humoral immune response to most antigens. When the T_H cells recognize an antigen, they stimulate B cells and other T cells specific for the same antigen, which results in their proliferation and differentiation. The T_S cells have the opposite effect and diminish the activity of the same group of cells.

Passive immunity is the type developed by the introduction of preformed antibodies (not antigens) into the body. In this type, the body cells are not stimulated to produce their own antibodies. Because the immunity acquired by the individual is not self-developed but is passed from one individual (or animal) to another, the term passive immunity is applied. The immunity developed in a newborn infant through transmission of the antibodies from the blood of the mother is an example of naturally acquired passive immunity; it is produced quickly but is not long-lasting. The injection of immunizing biologics containing preformed antibodies in forms such as diphtheria antitoxin or gamma globulin produces artificially acquired passive immunity, which again is produced quickly but is not long-lasting.

Obviously, certain biologics are intended for **prophylactic or preventive therapy,** whereas others are serviceable as **therapeutic or curative measures.** Vaccines and toxoids in their preventive capacities do not offer immediate protection to the patient; antitoxins, serums, and globulins give instant protection to the patient.

The importance of vaccination cannot be stressed too highly; its value has been proved beyond question. As a result of the judicious use of smallpox vaccine, the World Health Organization declared the world free of smallpox in May, 1980. Typhoid fever and epidemic typhus fever were nonexistent among the armed personnel during the recent wars, an outstanding medical accomplishment related primarily to rigid vaccination schedules maintained by military and naval medical staffs. Conceivably, any disease could be eradicated anywhere in the world if such proper preventive measures as sanitation, vaccination, and education were instituted. At the present time, many childhood diseases can be effectively prevented by utilizing the recommended immunization schedule illustrated in Table 13–1.

Antibiotics are currently available for controlling many infectious diseases, but antitoxins and related passive immunologic agents are still useful in treating infections caused by viruses and other pathogens that fail to respond to antibiotics. Also, the unique utility of antibody-containing biologics for prophylactic purposes must be emphasized. It is infeasible and undesirable to use antibiotics for prophy-

Table 13–1. Recommended Immunization Schedule for Children

Immunizing Agent	Preferred Age for Initial Dose	Dosage for Primary Immunization	Booster
Adsorbed Diphtheria and Tetanus Toxoids and Pertussis Vaccine[1] (DTP)	2 to 3 months	0.5 ml, intramuscularly, repeated twice at 4- to 6-week intervals.	0.5 ml 1 year after primary and 4 to 5 years later. Td every 10 years thereafter.
Adsorbed Tetanus and Diphtheria Toxoids for Adult Use (Td)	7 years and over	0.5 ml, intramuscularly, repeated once after 4 to 6 weeks.	0.5 ml 1 year after primary and every 10 years thereafter.
Live Oral Poliovirus Vaccine, Trivalent	2 to 3 months	Two doses given at not less than 8-week intervals (in the volume indicated in the labeling), and a third, reinforcing dose 8 to 12 months later.	One dose at entry into school.
Live Attenuated Measles Virus Vaccine[2]	15 months of age or older	1000 $TCID_{50}$ subcutaneously[3]	None recommended.
Live Rubella Virus Vaccine[2]	Between age 15 months and puberty	1000 $TCID_{50}$ subcutaneously[3]	None recommended
Live Mumps Virus Vaccine[2]	15 months of age or older	1000 $TCID_{50}$ subcutaneously[3]	None recommended.

[1]For primary immunization or boosters over age 6, use Td.
[2]May be given in bivalent or trivalent vaccine.
[3]Quantity of virus estimated to infect 50% of inoculated tissue cultures × 1000.

laxis. These reasons suggest that biologics must be accorded a special place among medicinally useful materials obtained from natural sources.

All biologics are "dated," i.e., carry an expiration date on the label of the package, because they do not retain their potency for an indefinite period. Specific regulations govern the determination of the expiration date for given biologic formulations. For example, diphtheria antitoxin can have a 5-year expiration date provided that the preparation has a 20% excess of potency. Whether the potency of the biologic still exists near the end of the expiration time depends on the methods of storage.

The nature of biologic products requires that they be refrigerated during storage. They represent either living or dead microorganisms or their metabolic products as well as the active components of the blood of animals. To ensure their activity as immunogenic materials, they should be stored at a temperature ranging from 2 to 8° C. In certain instances, lower temperatures are indicated. Yellow fever vaccine should be stored at a temperature no higher than 5° C and preferably lower than 0° C; live poliomyelitis vaccine should be preserved at a temperature below −10° C. Because biologics are usually stored in mechanically operated refrigerators, they may occasionally become frozen. Provided the container is not broken, such freezing does not affect the potency of the product unless the label states otherwise.

All immunizing biologics must comply with the identity, safety, sterility, and potency tests and other requirements for the individual product in accordance with the Food and Drug Regulations—Code of Federal Regulations, as administered by the Bureau of Biologics of the FDA. Each lot of the product must be released individually before its distribution. The labeling must correspond to certain specifications. It must bear the name of the product, the lot

number, and expiration date; the manufacturer's name, license number, and address; and a statement regarding storage and refrigeration. Biologics are to be dispensed in the unopened container in which they were placed by the manufacturer.

In addition to the commercially available biologics, the Centers for Disease Control (CDC), U.S. Public Health Service, can supply various rare immunologic agents in emergency situations. These products are available through the Drug Immunobiologic and Vaccine Service, Center for Infectious Diseases, Building 1, Room 1259, CDC, Atlanta, Georgia, 30333.

VACCINES

Vaccines may contain living, attenuated, or killed viruses, killed rickettsiae, or attenuated or killed bacteria, and they are used as inoculations to stimulate the production of antibodies.

Primary active immunity from vaccination develops more slowly than the incubation period of most infections and must be induced prior to exposure to the infectious agent; therefore, the general action of vaccines should be considered prophylactic. One exception is the rabies vaccination. Because the rabies virus has a median incubation period of 35 days in humans, there is usually sufficient time for protective antibodies to develop when the vaccine is administered after exposure.

Nonliving vaccines provide protection for only a limited time, and repeated vaccination is required to maintain protection against typhoid fever, cholera, plague, and typhus. Active immunization with living agents is generally preferable to immunization with killed vaccines because of a superior and more long-lived immune response. For example, a single vaccination of measles, rubella, or mumps vaccine is sufficient to produce a long-lasting if not permanent immunity. Multiple immunizations are recommended for polio because interference among the 3 simultaneously administered virus types present in the trivalent vaccine could prevent completely successful primary immunization.

The benefits of active immunization far outweigh the dangers associated with the use of vaccines; however, precautionary measures should be followed to ensure optimum effectiveness with a minimum of adverse reactions. Use of vaccines is contraindicated under conditions in which the immune response may be depressed, such as during therapy involving corticosteroids, antineoplastic agents, immunosuppressive agents, or radiation; in patients with immunoglobulin deficiency (agammaglobulinemia and dysgammaglobulinemia); and in patients with latent or active infections.

Active immunization may cause fever, malaise, and soreness at injection sites. Some reactions are relatively specific for a particular vaccine, such as arthralgia and arthritis following rubella vaccine or convulsions following pertussis vaccine. During the 1976 "swine flu" immunization program in the United States, there was an 8-fold increase in postimmunization Guillain-Barré syndrome (acute febrile polyneuritis) in comparison with unvaccinated controls. This complication arises within 8 weeks of immunization and has resulted in a 5% mortality rate among patients who developed the syndrome.

Allergic reactions may result either from the organism constituting the vaccine or from a protein incorporated into the vaccine during manufacture, e.g., egg protein from chick embryo tissue cultures. Consequently, a careful history of the patient should be taken before vaccination to detect possible hypersensitivity to the protein to be injected.

VIRAL VACCINES

Viral vaccines for prophylaxis against mumps, rubella, rubeola, smallpox, and yellow fever contain living viruses. Inactivated or killed viruses are used in influ-

Table 13–2. Recommended Immunization Schedule for Adults

Immunizing Agent	Indications for Use	Dosage
Adsorbed Tetanus and Diphtheria Toxoids for Adult Use (Td)	Every adult.	Primary immunization: 0.5 ml, intramuscularly, repeated once after 4–8 weeks, then once 6–12 months later. Booster every 10 years.
Live Attenuated Measles Virus Vaccine[1]	Unimmunized born after 1956 and recipients of inactivated vaccine given 1963–1967.	1000 $TCID_{50}$[2] subcutaneously
Live Rubella Virus Vaccine[1]	Unimmunized young women.	1000 $TCID_{50}$[2] subcutaneously
Influenza Virus Vaccine	Patients with diabetes or other metabolic diseases, severe anemia, or chronic pulmonary, cardiovascular, or renal disease, immunocompromised patients, those in chronic care facilities, and everyone over 65 years of age.	0.5 ml intramuscularly annually
Polyvalent Pneumococcal Vaccine	Patients with chronic cardiac or pulmonary disease, alcoholism, cirrhosis, diabetes, Hodgkin's disease, nephrotic syndrome, renal failure, cerebrospinal fluid leaks, immunosuppression, and everyone over 65 years of age.	0.5 ml subcutaneously or intramuscularly
Hepatitis B Vaccine	Medical and laboratory workers with frequent exposure to blood or blood products, intravenous drug abusers, male homosexuals, dialysis patients, recipients of clotting factors VIII or IX, mortuary workers, residents and staff of institutions for the mentally retarded, and immunocompromised patients.	1 ml intramuscularly in deltoid muscle, repeated after 4 weeks and again 6 months after first dose.

[1]May be given in bivalent vaccine.
[2]Quantity of virus estimated to infect 50% of inoculated tissue cultures × 1000.

enza and rabies vaccines. Preparations containing live attenuated or killed viruses are available for immunization against poliomyelitis.

The cultivation of viruses poses a problem because they are completely dependent on living cells for their sustenance. No method of growing viruses in artificial culture media is known. Viruses for smallpox vaccine and for rabies vaccine were obtained for years from vesicular tissues of vaccinated calves and brain tissues of infected rabbits, respectively, but this approach has limited utility for many viruses. The use of living chick or duck embryos for viral culture offers advantages in some cases, but the development of techniques for tissue culture of mammalian cells provided the major basic advancement necessary for significant expansion in the practical use of many viral vaccines. A number of viruses currently employed in viral vaccines are grown on tissue cultures prepared from chick embryo, monkey kidney, or human diploid cells. Primary tissue cultures have created some problems, espe-

cially because of the need for large numbers of monkeys and because of the continuous need for extensive tests, with resulting expense and delays, to ensure the absence of undesirable simian viruses in each monkey kidney donor. It appears that advancements will be forthcoming and will permit indefinite propagation of suitable cell lines. The use of tissue cultures of human cells has now become a reality (see Rabies Vaccine).

Smallpox Vaccine

Smallpox vaccine is the living virus of vaccinia (cowpox) that has been grown in the skin of a vaccinated bovine calf. It is available in dried and in liquid form; the latter consists of a smooth, aqueous suspension of infected tissue that contains 40 to 60% of glycerin or of sorbitol and may contain not more than 0.5% of phenol as a preservative.

The pioneering work of Dr. Edward Jenner in England in 1796 established that when a mild case of cowpox (vaccinia) is developed by a person, the same person is immune to smallpox. Using this information, he inoculated a young boy with pus from a milkmaid infected with cowpox. Two months later, the boy was inoculated with pus from a patient infected with smallpox, but no disease developed. Immunity had been established.

In producing the vaccine, a calf is prepared by washing and shaving its belly, then scarifying the epidermis so that serum oozes through the cuts. The "seed virus" is inoculated into the scarifications merely by hand rubbing (the workers are protected by rubber surgical gloves). The calf is maintained in an aseptic stall and given food and water during the growth of the virus. The vesicles that develop are removed at the time of maximum potency (Fig. 13–4), thoroughly triturated, and either made into a smooth suspension with an aqueous solution of glycerin or sorbitol or reduced to a dried pellet.

The animal must be in good health prior to inoculation. After the virus is harvested, the animal is killed and a necropsy is performed. If the organs show no effects of disease from other causes, the virus is deemed satisfactory for manufacture.

Smallpox vaccine should be dispensed in the containers in which it was placed by the manufacturer. Liquid vaccine should be kept below 0° C during storage and in shipment because it loses potency rapidly at higher temperatures. Dried vaccine should be kept at a temperature between 2 and 8° C.

USE AND DOSE. Smallpox vaccine is a specific immunizing agent and is used as a prophylactic before the infection occurs. It creates active immunity that usually lasts for about 7 years. The usual dose is, percutaneously, the contents of 1 capillary tube, by the multiple-puncture method. In the United States, military personnel are routinely vaccinated against smallpox; however, because adverse reactions to primary vaccinations can cause death (less than 1 per 100,000 vaccinations) and because the disease has undergone complete worldwide eradication, smallpox vaccination of civilians is now indicated only for laboratory workers directly involved with variola virus or closely related orthopox viruses. The vaccine is not available commercially but can be obtained from the CDC in Atlanta, Georgia (see page 387).

Rabies Vaccine

Rabies vaccine, also known as **human diploid cell rabies vaccine (HDCV),** is a sterile lyophilized preparation of either the whole virion or subvirion rabies virus. The whole virion vaccine is prepared from Wistar rabies virus grown in cultures of human diploid embryo lung tissue and inactivated with tri-*N*-butyl phosphate and β-propiolactone. The subvirion vaccine is prepared from the Pasteur-derived Pitman-Moore virus grown on human diploid cell cultures developed in Europe and inactivated with β-propiolactone. Both vaccines are supplied as 1.0 ml, single-dose vials of

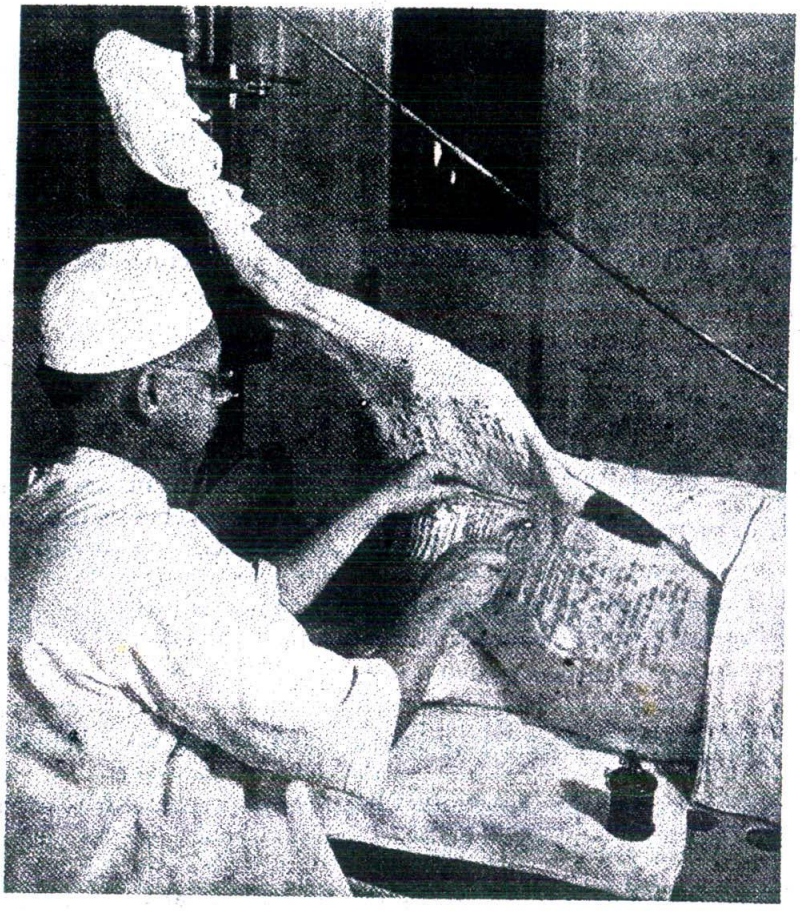

Fig. 13–4. Harvesting smallpox virus from vesicles on belly of calf. (Photo courtesy of Eli Lilly & Company.)

lyophilized vaccine with accompanying diluent.

In addition to his other famous accomplishments, Louis Pasteur is associated with rabies. Pasteur was able to "fix" the virus of rabies by passing it from an infected dog to the brain of a rabbit and then from one rabbit to another until a uniformity was established that resulted in attenuated virulence for humans. In the first immunization against rabies in 1885, Pasteur used such a fixed virus to achieve active immunity.

The Pasteur treatment is not a curative treatment, but it actually accomplishes the same result because immunization of a patient bitten by a rabid animal proceeds more quickly than the incubation period of the disease. Because the treatment consists of a series of injections for 28 days, the development of antibodies probably inhibits the growth of the virus.

Brain tissue, formerly used in the preparation of the vaccine, contains a significant amount of myelin, which is thought to be the "paralytic factor" that causes rabies treatment paralysis. With the advent of HDCV, problems with paralysis have been greatly reduced. Rare cases of Guillain-Barré syndrome have been reported; however, these patients recovered completely from the paralysis.

USE AND DOSE. The vaccine is an active immunizing agent and is recommended primarily for the prevention of rabies in persons bitten by an animal supposed or

known to be rabid. However, the vaccine may be used for pre-exposure immunization for veterinarians or other high-risk individuals. The usual pre-exposure dose is 3 injections of 1.0 ml of reconstituted vaccine on each of days 0, 7, and 21. Post-exposure immunization should be started as quickly as possible after the wound has been inflicted; the usual administration schedule is 5 injections of 1.0 ml of reconstituted vaccine on each of days 0, 3, 7, 14, and 28. Rabies immune globulin should be administered at the time of the first dose of vaccine for additional protection, particularly in the case of a bite from a wild animal.

PRESCRIPTION PRODUCTS. Imovax Rabies Vaccine®, WYVAC Rabies Vaccine®.

Yellow Fever Vaccine

Yellow fever vaccine is an attenuated strain of living yellow fever virus, selected for high antigenic activity and safety. It is prepared by culturing the virus in the living embryo of the domestic fowl *(Gallus domesticus)* (Fig. 13–5). The virus-infected, chick-embyro pulp is suspended in water and, after appropriate aseptic processing, is distributed in suitable quantities into ampuls and dried from the frozen state. Afterward, the ampuls are filled with dry nitrogen and flame-sealed. The expiration date of this vaccine is not longer than 1 year from the date of issue, and it must be stored at a temperature preferably below 0° C but never above 5° C. Yellow fever vaccine should be hydrated immediately before use. It does not contain human serum.

Yellow fever or "yellow jack" was considered an endemic disease in certain tropical regions, including the Caribbean Islands and Central America. Work on the Panama Canal was abandoned by the French because of the terrific death toll caused by yellow fever. Through the heroism of Walter Reed, Carlos Finlay, and numerous volunteers among the American troops stationed in Cuba during the Spanish-American war, the *Aëdes* mosquito was finally proved to be the vector of the disease. Further investigation was necessary to determine that the cause of yellow fever was a noncultivatable, filter-passing virus.

USE AND DOSE. Yellow fever vaccine is an active immunizing agent that is used to develop active immunity against the disease. The usual dose, given subcutaneously, is 0.5 ml. The use of yellow fever vaccine in the United States is limited largely to persons planning to travel through parts of the world where yellow fever is endemic.

PRESCRIPTION PRODUCT. YF-VAX®.

Influenza Virus Vaccine

Influenza virus vaccine is a sterile, aqueous suspension of suitably inactivated influenza virus types A and B, either individually or combined, or virus subunits prepared from the extra-embryonic fluid of influenza virus-infected chick embryo. The strains of influenza virus used in the preparation of this vaccine are those designated for the particular season by the Center for Drugs and Biologics of the Federal FDA. It contains a suitable preservative and may contain an adsorbent, such as aluminum phosphate or protamine. During the commercial preparation of the vaccine, the virus growths are collected, concentrated, refined by ultracentrifugation, and inactivated by ultraviolet irradiation.

Each lot of influenza virus vaccine must be tested to determine its potency; its power to stimulate the formation of specific virus-neutralizing antibodies in mice is correlated with the potency. Each ml is labeled according to the number of CCA units it contains; the unit refers to the chicken red-cell agglutination titer. This vaccine must be stored at a temperature between 2 and 8° C, and the expiration date is not longer than 18 months from the date of issue.

USE AND DOSE. Influenza virus vaccine is an active immunizing agent. Its usual dose is 0.5 ml intramuscularly, preferably in the deltoid muscle. Annual vaccination is rec-

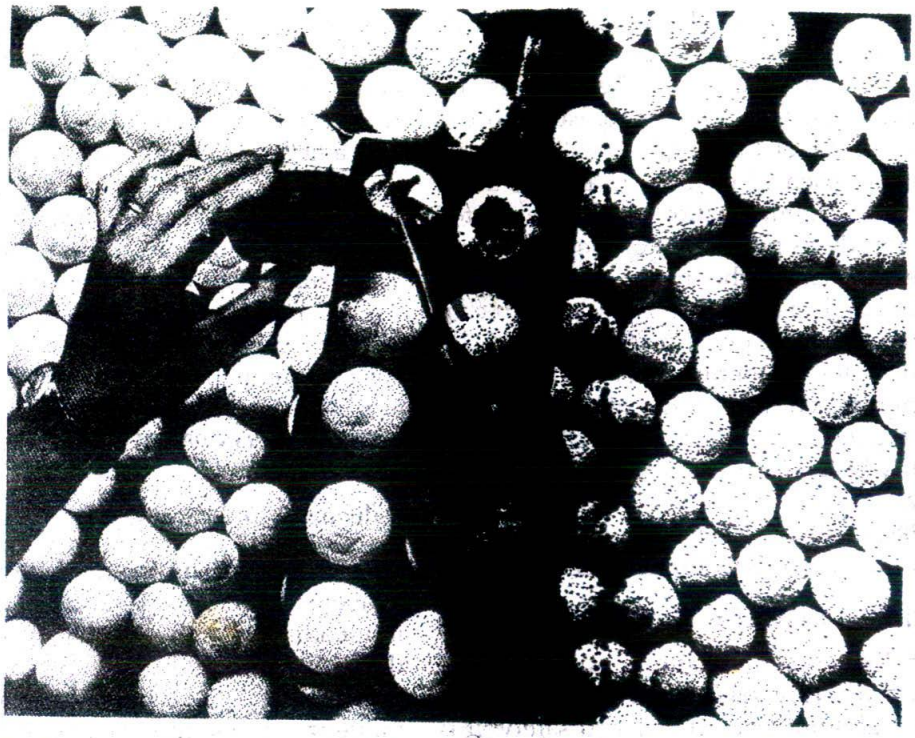

Fig. 13–5. Preparation of vaccine from virus-infected chick embryo. (Photo courtesy of Eli Lilly & Company.)

ommended for individuals in high-risk categories (see Table 13–2).

Influenza viruses have a high degree of strain specificity and of genetic instability. These factors require a continual reevaluation of the components of influenza virus vaccine and result in periodic infections of epidemic proportions even among immunized persons. Most available vaccines are bivalent and contain types A and B virus strains.

PRESCRIPTION PRODUCTS. Fluzone®, Fluogen®.

Poliomyelitis Vaccines

Poliovirus vaccine inactivated (IPV; Salk) is a sterile suspension of inactivated poliomyelitis virus of types 1, 2, and 3. The virus strains are grown separately in primary cultures of Rhesus monkey kidney tissues bathed by a complex nutrient fluid containing more than 60 ingredients (Fig. 13–6). After incubation, the virus is harvested by decanting the nutrient fluid that is clarified by filtration; then, formaldehyde in a concentration of 1:4000 is added. The formaldehyde-treated virus is maintained at 37° C at pH 7 until all viruses are killed. A series of elaborate tests is performed to ascertain that all viruses are inactivated. Following these quality control tests, the formaldehyde is neutralized and a preservative is added (Fig. 13–7). The 3 types of virus are then pooled, and the resultant mixture is the trivalent vaccine (Fig. 13–8).

In addition to the 3 types of poliomyelitis virus that have been cultured and identified, other paralysis-producing strains undoubtedly exist. In general, however, poliomyelitis epidemics of major proportions have been caused by type 1 (Brunhilde). Type 3 (Leon) has proved to be the etiologic agent in less frequent epidemics, and type 2 (Lansing) has been concerned only in sporadic cases. Immunization with one type of virus does not offer protection against the other types; thus, the current

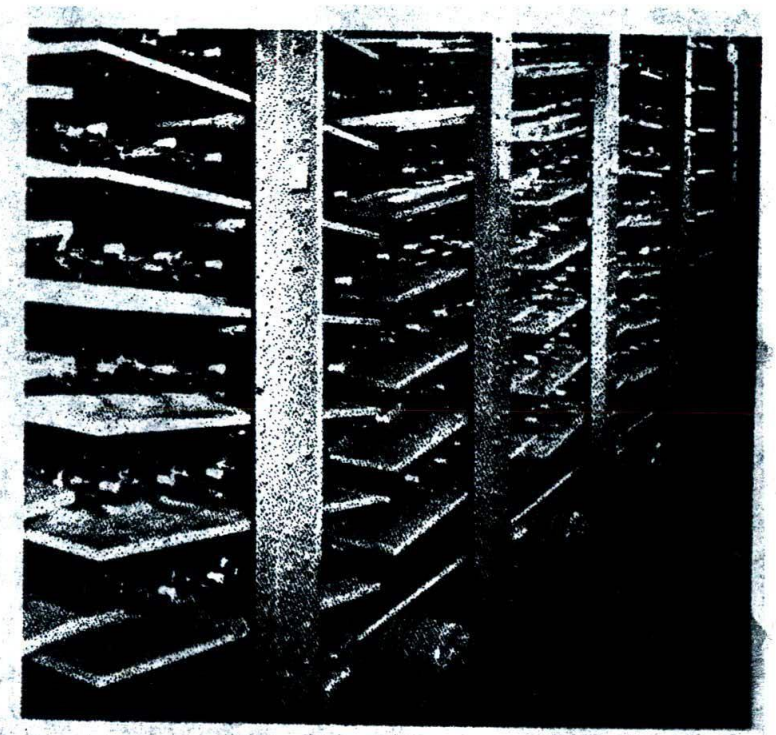

Fig. 13–6. Polio vaccine. The virus-inoculated tissue cultures are gently rocked for 6 days at 35° C (approximately body temperature) in a special incubator room. (Tile and Till, courtesy of Eli Lilly & Company.)

vaccine is a trivalent preparation. Improved strains of the various viral types are the object of continuous selection studies; the type 1 Mahoney strain, type 2 MEF-1 strain, and type 3 Saukett strain are now used in preparing poliomyelitis vaccines.

Landsteiner and Popper, in 1908, first transmitted and isolated poliomyelitis virus experimentally in monkeys. It was subsequently ascertained that monkeys that had survived one attack of poliomyelitis were resistant to further attacks; furthermore, blood serum from such monkeys neutralized the virus in vitro. Still later, this observation resulted in the successful attempt to induce passive immunity through the use of serum obtained from immune donors.

During 1948, Dr. John F. Enders and his associates at Harvard University originated a method of cultivating polio virus in vitro on animal tissues other than nervous tissue. Then, in 1953, Dr. Jonas Salk and his coworkers at the University of Pittsburgh perfected the roller-tissue method of polio virus culture, as well as the final detoxified form of polio vaccine. The Nobel Prize was awarded to Dr. Enders for his achievement in virus cultivation; international acclaim was bestowed on Dr. Salk for his development of the vaccine and its success in the extensive inoculation tests.

Field trials using polio vaccine were conducted during 1954 on a total of 1.83 million schoolchildren, of whom 440,000 received 1 or more injections of the vaccine; 210,000 received placebo injections consisting of a nutrient medium similar to but not used for actual growth of the virus organisms; and the remaining 1.18 million were observed as controls. Vaccination consisted of an initial intramuscular injection of 1 ml of vaccine followed by a second 1-ml injection 1 week later and a third 1-ml injection about 4 weeks after the second.

Fig. 13–7. Salk polio vaccine. After inactivation of the virus, the formaldehyde is neutralized. Special tissue culture technique is used to prove that the virus is entirely inactivated. (Tile and Till, courtesy of Eli Lilly & Company.)

(NOTE: *The time intervals for active immunization have changed.*) The mass inoculations covered a total of 217 selected areas in 44 states of the United States and 48 areas of Canada and Finland.

The results of this carefully controlled study encouraged the National Foundation for Infantile Paralysis to purchase sufficient vaccine to inoculate approximately 9 million schoolchildren during 1955. The success of the mass polio vaccinations in both 1954 and 1955 received international prominence, not only because of the enthusiastic response from the children's parents and for the cooperative spirit of the biologic manufacturers but also because of the ingenuity and tireless efforts of Dr. Jonas Salk. (Poliovirus vaccine inactivated is more commonly referred to as Salk polio vaccine.)

USE AND DOSE. Poliovirus vaccine inactivated is an active immunizing agent that has definite value in creating active immunity to the disease. The usual dose, given subcutaneously, is 3 injections of 1 ml, 4 or more weeks apart, and a fourth reinforcing dose of 1 ml, 6 to 12 months later.

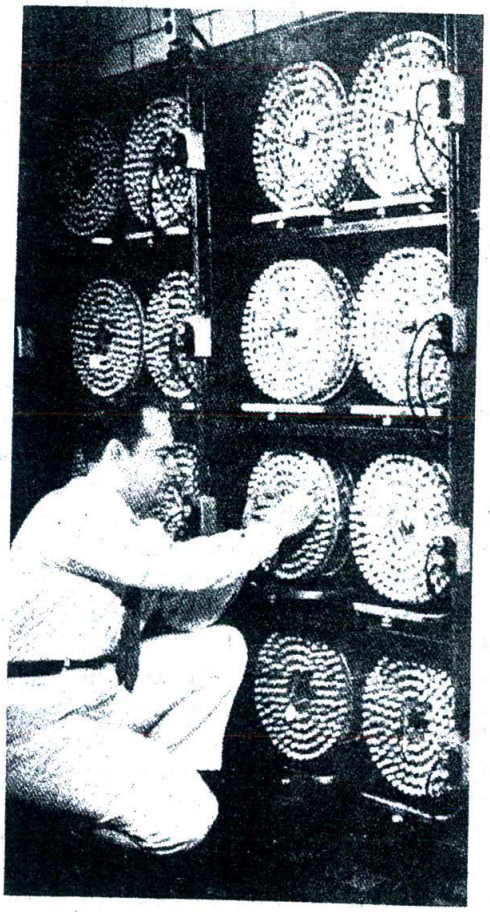

Fig. 13–8. Polio vaccine. All 3 types of vaccine are mixed together to make the final product. This mixture is again tested to be sure no active viruses are present. (Courtesy of Eli Lilly & Company.)

Poliovirus vaccine live oral is a preparation of one or a combination of the 3 types of live, attenuated polioviruses. The virus strains are grown separately in primary cultures of monkey kidney tissue. It has been manufactured and tested in a manner suited only for oral administration and is free from any known microbial agent other than the attenuated poliovirus or polioviruses intended to be present. This vaccine is commonly called **trivalent oral polio vaccine (TOPV)** and contains one or all of types 1, 2, or 3 Sabin poliovirus strains.

Three scientists working independently developed procedures for the manufacture of this vaccine: Dr. Albert Sabin of the University of Cincinnati, Dr. Harold Cox of Lederle Laboratories, and Dr. Hilary Koprowski of the Wistar Institute of Philadelphia. The results of large-scale trials extending over a period of several years indicated that oral polio vaccine has longer-lasting immunity, greater ease of administration, and presumably lower costs of production than the Salk polio vaccine. The tests involved more than 13 million people in countries outside of the United States. Extensive domestic trials were performed in the Dade Country, Florida; Minneapolis; Cincinnati; and Rochester, New York areas. In the Dade County field trials, the vaccine was incorporated into a cherry-flavored oral preparation that was designed to immunize against all 3 strains of poliomyelitis.

Safety tests conducted by the U.S. Department of Public Health resulted in the announcement of August, 1960, that the Sabin formula was the most "suitable for use in the United States." Licensed manufacture of this trivalent vaccine began early in 1963; quantity production was reached later that year.

The manufacture of poliovirus vaccine live oral is similar to that of poliovirus vaccine inactivated (Salk) because the virus strains are grown separately in monkey tissue cultures. However, the viruses are not killed by treatment with formaldehyde, as is done with Salk vaccine; instead, the viruses are attenuated. Therefore, the Sabin oral vaccine should never be administered parenterally.

Live poliovirus vaccines offer protection against strains of poliomyelitis virus that cause paralysis. The attenuated live virus, when present in the intestinal tract, multiplies and produces a localized resistance to reinfection by the same type of virus, thus stimulating the production of type-specific serum antibodies. The development of such localized resistance to the growth of the virus affords a protection that is independent of specific, circulating

antibodies. Salk vaccine provides protection against paralytic poliomyelitis through the stimulation of serum antibodies specific for types 1, 2, and 3 poliovirus but does not cause the inhibition of viral growth in the intestine that characterizes the Sabin vaccine.

Poliovirus vaccine live oral is generally frozen. When stored at a temperature of −10° C, the expiration date is not later than 1 year after date of manufacture or date of issue. It may be thawed and refrozen not more than 10 times, provided that the thawed material is kept refrigerated and the total cumulative duration of the thaw is not more than 24 hours.

USE AND DOSE. Poliovirus vaccine live oral is an active immunizing agent. When administered orally, it effectively develops immunity to the poliovirus, Sabin strains types 1, 2, and 3. The usual administration schedule involves an initial administration of 2 doses at not less than 8-week intervals. A third, reinforcing dose is administered 8 to 12 months later. The volume of vaccine indicated on the label as representing one dose is generally placed on a cube of sugar, which is eaten by the individual to be immunized. The immunization schedule should be carried out in the winter and spring to avoid the summer peak of other intestinal enteroviruses that may interfere with the desired immunologic response.

PRESCRIPTION PRODUCT. Orimune®.

Measles Vaccines

Vaccines containing live attenuated rubeola (measles) and rubella (German measles) viruses are available for active immunization. Viruses for production of these vaccines are grown on cultures of either avian embryo tissue or human diploid cell tissue. The vaccines are available in a lyophilized form. They should be stored at a temperature of between 2 and 8° C and have a 1-year expiration date.

Measles virus vaccine live or rubeola vaccine is prepared from attenuated viruses derived from the original Edmonston B strain. The Enders strain is a modified Edmonston strain, and it is claimed to have a high degree of antigenicity with a low incidence of adverse reactions; coadministration of immune globulin may not be necessary with vaccines employing this strain. The rubeola virus is grown on cultures of chicken embryo tissue.

Rubeola vaccine is recommended for active immunization of children 15 months of age or older. Use in infants under 15 months of age is not recommended. Good immunity is obtained with a single subcutaneous injection of not less than 1000 $TCID_{50}$ (tissue culture infectious doses) of the reconstituted vaccine. The $TCID_{50}$ is the quantity of virus estimated to infect 50% of inoculated tissue cultures.

PRESCRIPTION PRODUCT. Attenuvax®.

Rubella virus vaccine live is prepared from the Wistar Institute RA 27/3 strain grown on human diploid cell tissue. Rubella vaccine is recommended for active immunization against German measles for children aged 1 to puberty and for certain other individuals. This vaccine should not be administered to pregnant or immediate postpartum women, and special caution must be exercised if it is given to sexually active females. Precautions must be taken to eliminate the possibility of pregnancy in women of child-bearing age for at least 3 months following immunization. Immunity is obtained with a single subcutaneous injection of not less than 1000 $TCID_{50}$ (tissue culture infectious doses) of the reconstituted vaccine. Use in infants under 1 year of age is not recommended.

PRESCRIPTION PRODUCT. Meruvax II®.

Mumps Vaccine

Mumps virus vaccine live is prepared with the B-level Jeryl Lynn strain of the virus, which is grown in cell cultures of chicken embryo tissue. It provides active immunity for at least 10 years after immunization and is particularly valuable to susceptible individuals approaching puberty and to adults. It is not recommended

for infants less than 1 year old because they may retain maternal mumps antibodies that may interfere with the immune response. The vaccine is available in a lyophilized form; immunization involves a single subcutaneous injection of not less than 5000 $TCID_{50}$ of mumps virus vaccine.

PRESCRIPTION PRODUCT. Mumpsvax®.

Hepatitis Vaccine

Hepatitis B vaccine is composed of chemically inactivated hepatitis B surface antigen (HBsAg) particles obtained from the plasma of healthy chronic HBsAg carriers by plasmapheresis, separated from the infectious Dane particle by density gradient centrifugation (Fig. 13–9), and absorbed on aluminum hydroxide. Specific antibody (anti-HBs) develops in 75 to 90% of healthy adults after the first 2 doses of vaccine and in 85 to 90% after the third dose. Vaccine-induced antibody has persisted for at least 3 years; longer periods of observation may indicate that booster doses will be required.

The hepatitis B virus causes hepatitis in hundreds of thousands of people each year in the United States. The initial infection is fatal only in a few cases, but others go on to develop chronic active hepatitis, cirrhosis, and hepatocellular cancer. In less-developed areas of the world, the incidence of all of these disorders is much higher; it has been estimated that 200 million are chronically infected with the hepatitis B virus.

USE AND DOSE. Vaccination is recommended for individuals in high-risk categories (see Table 13–2). The vaccine is given intramuscularly as 3 doses of 1.0 ml (20 μg), with the first 2 doses one month apart and a booster dose administered 6 months after the first dose. For patients on dialysis and others who are immunocompromised, 3 doses of 2.0 ml (40 μg) should be used. For children under 10 years old, 3 doses of 0.5 ml (10 μg) are recommended.

PRESCRIPTION PRODUCT. Heptavax-B®.

Combination Virus Vaccines

Combination live virus vaccines containing either measles virus and rubella virus, rubella virus and mumps virus, or measles virus, rubella virus, and mumps virus are available. These combination vaccines are administered subcutaneously to children 15 months of age or older. Use in infants under 15 months of age is not recommended.

PRESCRIPTION PRODUCTS. Live measles and rubella virus vaccine: M-R-Vax II®; live rubella and mumps virus vaccine: Biavax II®; live measles, mumps, and rubella virus vaccine: M-M-R II®.

RICKETTSIAL VACCINES

Rickettsiae are cultured in chick embryos or in monkey kidney tissue cultures in a manner similar to that for viruses. They cannot be grown in artificial culture media and must be subjected to the same precautions as viruses. At the present time, rickettsial vaccines are not produced commercially in the United States. Murine typhus, tsutsugamushi fever, and rickettsial diseases as well as epidemic typhus are of considerable importance in other parts of the world. Vaccines are available in these problem areas for all of these rickettsial diseases.

BACTERIAL VACCINES

Bacterial vaccines consist of suspensions of attenuated or, more commonly, killed pathogenic bacteria in isotonic sodium chloride solution or other suitable diluents. The strains of bacteria employed in preparation of the vaccines must be selected for high antigenicity, and a measure of the potency of a vaccine may be expressed as the number of organisms per unit volume or as biologic reference units. Suspensions of young, living organisms grown in standard culture media are killed chemically, by application of moist heat at a tempera-

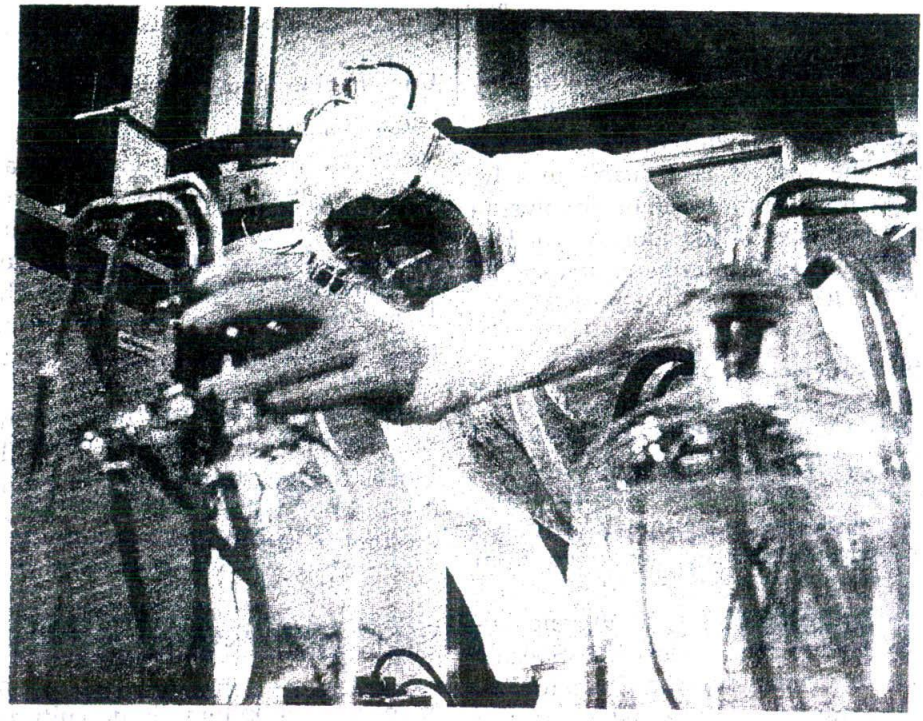

Fig. 13–9. Hepatitis B vaccine. Separation of the hepatitis B surface antigen (HBsAg) from the infectious Dane particle is accomplished by density gradient centrifugation. A technician prepares bottles containing carrier plasma for centrifugation. (Courtesy of Merck Sharp & Dohme.)

ture slightly above the thermal deathpoint, or by exposure to ultraviolet light.

The smooth or "S" strains of bacteria are uniformly more antigenic than the rough or "R" strains. Occasionally, stock cultures lose their antigenic qualities, and care must be exercised in a biologic manufacturer's laboratory to ensure the use of suitable strains.

Good immunologic responses are obtained with the following bacterial vaccines: cholera, pertussis, plague, and typhoid. The effectiveness of BCG vaccine, meningitis vaccines, and pneumococcal vaccine is still being evaluated.

Typhoid Vaccine

Typhoid vaccine is a sterile suspension or solid containing killed typhoid bacilli *(Salmonella typhi)* of the Ty 2 strain selected for high antigenic efficiency.

Typhoid vaccine has been called enteric vaccine because it prevents the effect of the disease on the intestinal tract.

USE AND DOSE. Typhoid vaccine is an active immunizing agent for producing immunization against typhoid fever. It is recommended for persons who have had household contact with a known typhoid carrier or for travelers going to areas of the world where typhoid fever is endemic. Typhoid vaccination is not recommended in the United States or in areas of natural disaster. The usual immunization schedule involves two 0.5-ml subcutaneous injections, at least 4 weeks apart, followed by 0.5 ml every 3 years thereafter. Booster injections are recommended when risk of typhoid fever occurs.

Cholera Vaccine

Cholera vaccine is a sterile suspension of killed cholera vibrios *(Vibrio cholerae)* in isotonic sodium chloride solution or other

suitable diluent. It is prepared from equal portions of suspensions of cholera vibrios of the Inaba and Ogawa strains. The Inaba strain possesses an antigenic value not less than that of N.I.H. Inaba strain 35-A-3, and the Ogawa strain possesses an antigenic value not less than that of N.I.H. Ogawa strain 41. This vaccine should be stored at a temperature between 2 and 8° C, and the expiration date is not longer than 18 months from the date of issue.

Statistically, the results reported on cholera vaccination in various parts of the world are sufficiently satisfactory to warrant its continued use in reducing morbidity and mortality from cholera.

USE AND DOSE. Cholera vaccine is an active immunizing agent in the development of immunity to the disease. The usual adult dose, given subcutaneously or intramuscularly, is 0.5 ml, and then 0.5 ml 1 to 4 weeks later; and a 0.5-ml dose repeated every 6 months, if necessary.

Plague Vaccine

Plague vaccine is a sterile suspension, in an isotonic sodium chloride solution or other suitable diluent, of killed plague bacilli *(Yersinia pestis)* of a strain selected for high antigenic efficiency.

The bacteria causing bubonic and pneumonic plague in humans are named *Yersinia* in honor of the Swiss bacteriologist Yersin, who was the first to isolate and identify the disease-causing organism. Rats serve as an animal reservoir for the organisms, but the disease is transmitted to humans through the bites of fleas that infest the rats. With rat control and large-scale vaccination, plague can be eliminated. In the United States, plague bacilli have been found in wild animals and their fleas in 15 western states.

USE AND DOSE. Plague vaccine is an active immunizing agent and is used to produce immunity to the disease. Its use is generally restricted to travelers to known plague areas, including Mongolia, southwestern Russia, central China, India, Pakistan, Nepal, Indonesia, Vietnam, South Africa, Saudi Arabia, Brazil, Bolivia, and Peru. It is also administered to persons who have frequent contact with wild rodents in plague enzootic areas in the United States, which include Arizona, California, Colorado, Idaho, Nevada, New Mexico, Oregon, and Utah. The usual immunization schedule involves 2 intramuscular injections, with the first dose of 1.0 ml followed in 1 to 3 months with a second dose of 0.2 ml. A third injection of 0.2 ml 3 to 6 months after the second injection is strongly recommended.

Pertussis Vaccine

Pertussis vaccine is a sterile bacterial fraction or suspension of killed pertussis bacilli *(Bordetella pertussis)* of a strain or strains selected for high antigenic efficiency. It has a potency of 12 protective units per individual immunizing dose, based on U.S. Standard Pertussis Vaccine. This vaccine should be stored at a temperature between 2 and 8° C and must be protected against freezing. The expiration date is not later than 18 months from the date of issue.

Bordetella pertussis is the organism that causes the disease known as whooping cough or pertussis. The cough is probably caused by a toxin in the bacterial body that also appears in filtrates of bacterial cultures. The organisms attach themselves to the cilia of epithelial cells in the trachea, and the irritation produced provokes the cough spasm.

Adsorbed pertussis vaccine consists of pertussis vaccine that has been precipitated or adsorbed by the addition of aluminum hydroxide or aluminum phosphate and resuspended.

Pertussis vaccine is combined with diphtheria and tetanus toxoids to give a multiple immunizing agent (Tri-Immunol®). Because the incidence rate and severity of pertussis decrease with age and because the vaccine may cause side effects and adverse reactions, pertussis immunization is

not recommended for children after the 7th birthday. See Table 13–3 for the recommended dosage schedule for primary immunization.

Tuberculosis Vaccines

Studies of the effectiveness of vaccines to produce immunity to tuberculosis are constantly in progress. The vaccine known as BCG (prepared from Bacillus Calmette-Guérin) is a freeze-dried preparation of the culture of an attenuated strain of bovine tuberculosis originally isolated by 2 bacteriologists, Calmette and Guérin. This vaccine has provided bacteriologists and immunologists with a subject of controversy for years. The chief point of difference concerned the relative safety of the vaccine, but refinements in the processing and improvements in the testing have now assured a safe, nontoxic product for human use.

BCG Vaccine

BCG vaccine is a dried, living culture of the bacillus Calmette-Guérin strain of *Mycobacterium tuberculosis* var. *bovis*. The culture is grown in a suitable medium from a seed strain of known history that has been maintained to preserve its capacity for conferring immunity.

The expiration date of BCG vaccine is up to 1 year if it is stored at 5° C. This vaccine should be used within 2 hours after reconstitution. BCG vaccine has been accepted by European physicians for a number of years, and endorsement by American investigators was forthcoming during the 1950s. Immunologic protection against tuberculosis is only relative and is not permanent or predictable. The vaccine is recommended primarily for use for people whose exposure to tuberculosis is unusually high or when other means of control are inadequate. It should be used only with individuals who have a negative tuberculin skin test.

USE AND DOSE. BCG vaccine is an active immunizing agent against tuberculosis. It is administered intradermally as the reconstituted vaccine in doses of 0.1 ml.

Meningitis Vaccines

Meningococcal polysaccharide vaccines contain the specific bacterial capsular polysaccharides for *Neisseria meningitidis* serogroups A, C, Y, and W-135 (Fig. 13–10). A bivalent vaccine with both serogroups A and C included is available, as is a vaccine with all 4 serogroups. The presence in human serum of antibodies to meningococcal polysaccharide antigens is strongly correlated with immunity to meningococcal meningitis. The use of meningococcal polysaccharide vaccine is indicated for children over 2 years of age and for military recruits and adult populations at risk in epidemic areas.

The immunizing dose is a single subcutaneous injection of 0.5 ml containing 50 μg of meningococcal polysaccharide.

PRESCRIPTION PRODUCTS. Menomune-A/C® and Menomune-A/C/Y/W-135®.

Pneumococcal Vaccine

Pneumococcal vaccine polyvalent affords protection against the 23 most prevalent capsular types of pneumococci, which account for at least 90% of pneumococcal disease. It is prepared by isolating and purifying the polysaccharide antigens from strains of *Streptococcus pneumoniae* that contain these serotypes (Fig. 13–11). Its use is indicated for those 2 years of age or older in whom there is an increased risk of morbidity and mortality from pneumococcal pneumonia (see Table 13–2). Even with current antibiotic therapy, the mortality rate in high-risk patients hospitalized with pneumococcal infection has remained higher than 25%.

The vaccine is administered as a single 0.5-ml dose given either subcutaneously or intramuscularly (preferably in the deltoid muscle or lateral mid-thigh). Severe local reactions have occurred after a second dose; therefore, more than one dose is not recommended, even for patients who re-

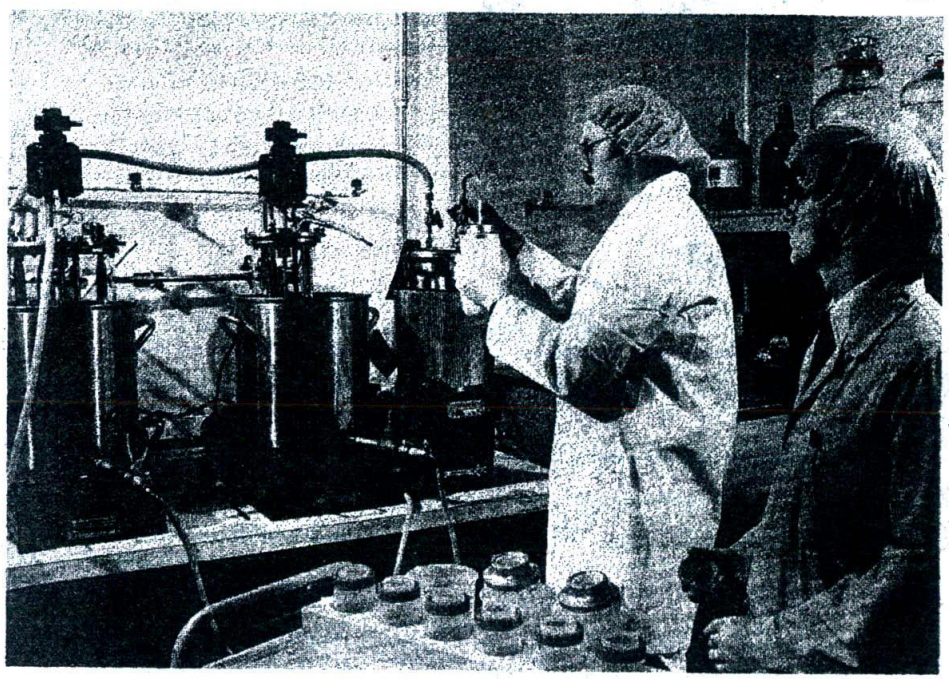

Fig. 13–10. Meningitis vaccine. Technicians are processing bacteria for extraction of the capsular polysaccharide antigen to be used in the manufacture of the vaccine. (Courtesy of Merck Sharp & Dohme.)

ceived an older vaccine that contained fewer pneumococcal types.

PRESCRIPTION PRODUCTS. Pneumovax 23®, Pnu-Imune 23®.

Haemophilus Vaccine

Haemophilus b polysaccharide vaccine is composed of the purified, capsular polysaccharide of *Haemophilus influenzae* type b (Hib). Antibodies to this antigen correlate with protection against invasive disease. Virtually all cases of *Haemophilus influenzae* meningitis among children are caused by strains of Hib. Despite effective antimicrobial therapy, the mortality rate from *Haemophilus* meningitis ranges from 5 to 10%, and about one third of the survivors have some form of permanent injury to the central nervous system. In addition, Hib can cause epiglottitis, osteomyelitis, arthritis, cellulitis, and pneumonia in children. Immunization is recommended for all children when they reach 2 years of age and possibly for children 2 to 5 years old who have not been previously immunized. Most unimmunized children over 5 years old and most adults have protective titers of naturally acquired antibodies. The vaccine is administered subcutaneously as a single 0.5-ml dose.

PRESCRIPTION PRODUCTS. b-Capsa.I® and Hib-Imune®.

DIAGNOSTIC ANTIGENS

A number of antigen-containing preparations are employed as diagnostic aids to determine whether an individual has developed hypersensitivity to certain types of organisms. Hypersensitivity is usually the result of a previous infection caused by the specific etiologic agent. Small quantities of the diagnostic preparations are usually injected intradermally, and the developing reaction is usually read at 48 hours, although observations at 24 hours and at 72 hours are often helpful. The usual type of

Fig. 13–11. Pneumococcal vaccine. A technician wearing sterile garments and using a biohazard safety cabinet inoculates cultures as the beginning step in the manufacture of the 23-valent pneumonia vaccine. (Courtesy of Merck Sharp & Dohme.)

positive response is a localized, well-defined wheal accompanied by erythema.

Antigen-containing diagnostic preparations that are commonly available include the tuberculins, histoplasmin, coccidioidin, diphtheria toxin, and mumps skin test antigen. Other preparations that are occasionally used for diagnostic purposes are formulated and employed according to the same basic principles.

Tuberculins

Tuberculins are preparations obtained in a number of ways from the human and bovine strains of the tubercle bacillus. The active substance of the tuberculin, which is apparently an albuminous derivative insoluble in alcohol, is elaborated by the organisms during their multiplication. In both human and veterinary practice, tuberculin may be applied as a diagnostic agent to determine whether the person or animal is or has been infected with *Mycobacterium*. The tuberculin may be applied by intracutaneous injection, by rubbing into the scarified skin, by dropping into the eye, or by other methods. In each case, a marked redness or inflammation indicates a positive reaction. A positive test result does not necessarily indicate the presence of an active infection but indicates that further evaluation should be done.

Old Tuberculin

Old tuberculin, concentrated tuberculin, crude tuberculin, or tuberculin-Koch is a sterile solution of the concentrated, soluble products of growth of the tubercle bacillus *(Mycobacterium tuberculosis)*. This solution is adjusted to the standard potency by addition of glycerin and isotonic sodium chloride solution. Its final glycerin content is approximately 50%. Old tuberculin, in diluted form suitable for injection, is prepared in a buffered diluent.

In the preparation of this product, tubercle bacilli are grown in broth medium for 8 weeks. Afterward, the culture is boiled, the organisms are filtered and discarded, and the filtrate is evaporated to one tenth its volume. The final product is a clear brownish liquid that is readily miscible with water and has a characteristic odor.

Old tuberculin should be stored at a temperature between 2 and 8° C. The expiration date of the undiluted old tuberculin is up to 5 years, but the expiration date of the diluted form is not more than 1 year after the date of manufacture or the date of issue.

USE AND DOSE. Old tuberculin is a diagnostic immunologic aid in testing patients suspected of having tuberculosis. Because of the glycerin, peptones, and mineral salts present in the final product, false-positive reactions may occur. The usual dose of old tuberculin is 5 tuberculin units, intradermally. A positive test consists of an area of inflammation and definitely palpable induration or edema at least 5 mm in diameter. It appears in 6 to 8 hours,

reaches its maximum in 24 to 48 hours, and usually disappears in 6 to 10 days.

PRESCRIPTION PRODUCTS. Tuberculin, Old, Mono-Vacc Test®; Tuberculin, Old, Tine Test®.

Purified Protein Derivative of Tuberculin

Purified protein derivative of tuberculin or tuberculin P.P.D. is a sterile, soluble, partially purified product of growth of the tubercle bacillus *(Mycobacterium tuberculosis)* prepared in a special liquid medium that is free from protein.

The tubercle bacilli are cultured in a synthetic medium until the desired growth is obtained. The filtered active tuberculin material is purified by precipitating with trichloracetic acid. It is composed of tuberculoprotein and is usually supplied as tablets in which lactose may be present.

The expiration date is not later than 2 years after date of manufacture or date of issue.

USE AND DOSE. Purified protein derivative of tuberculin is an immunologic diagnostic aid. It is used to test patients suspected of having tuberculosis; it is not used to treat the disease. The usual dose is 5 tuberculin units intradermally. The test is read 48 to 72 hours after administration, and a palpable induration measuring 10 mm or more is considered positive.

PRESCRIPTION PRODUCTS. Aplisol®, Tubersol®, Aplitest®, SclavoTest-PPD®, Tine Test PPD®.

Histoplasmin

Histoplasmin is a sterile, standardized liquid concentrate of the soluble growth products developed by the fungus *Histoplasma capsulatum* when grown in the mycelial phase on a synthetic medium. It is employed in skin tests to determine the presence of histoplasmosis, a disease that affects the reticuloendothelial system and usually results in enlargement of the liver, spleen, and lymph nodes.

Histoplasmin is of no value in treating this disease; it is used only to aid the physician in determining whether the patient is harboring the fungal organisms.

This biologic should be preserved at a temperature between 2 and 8° C. The expiration date is not later than 2 years after date of manufacture or date of issue.

USE AND DOSE. Histoplasmin is a diagnostic aid (dermal). The usual dose is 0.1 ml of a standardized sterile culture filtrate injected intradermally into the flexor surface of the forearm.

Persons who suffer from histoplasmosis always respond positively to the skin test; however, some individuals without any disease symptoms may also show a positive reaction, indicating a subclinical exposure or a cross-reaction of the fungal extract with other fungal organisms in the body. In addition, it is recommended that the tuberculin test be employed in conjunction with histoplasmin to exclude the possibility of tuberculosis. The histoplasmin skin test is seldom used because it may increase the complement fixation titer, which is the preferred method used to diagnose an active infection of histoplasmosis.

PRESCRIPTION PRODUCTS. Histoplasmin, Diluted®; Histolyn-CYL®.

Coccidioidin

Coccidioidin is a sterile solution containing the antigens obtained from the by-products of mycelial growth or from the spherules of the fungus *Coccidioides immitis*. Coccidioidin should be stored at a temperature between 2 and 8° C. The expiration date is not later than 3 years after the date of issue.

Coccidioidomycosis is a dust-borne disease that is caused by *C. immitis* and is indigenous to the arid regions of the southwestern United States. This disease may infect any part of the body. Approximately 60% of the cases are asymptomatic and identifiable only by a positive skin test; most of the remaining cases evidence moderate to severe symptoms of respiratory infection.

USE AND DOSE. Coccidioidin is a diagnostic aid in detecting cases of coccidioidomycosis. The usual dose, intradermally, is 0.1 ml of a 1:100 dilution.

PRESCRIPTION PRODUCT. Spherulin®.

Diphtheria Toxin

Diphtheria toxin or diphtheria toxin for the Schick test is a sterile solution of the diluted, standardized toxic products of growth of the diphtheria bacillus, *Corynebacterium diphtheriae*, of which the parent toxin contains not less than 400 MLD (minimum lethal doses) per ml or 400,000 MRD (minimum skin reaction doses) per ml in guinea pigs. The test method involves the intradermal injection of 0.1 ml of the control solution into the flexor surface of the left forearm with a 26- or 27-gauge one-half inch needle and an injection of 0.1 ml of the toxin into the right forearm. A positive reaction results in a circumscribed area of redness measuring 1 cm or more in diameter appearing in 24 to 36 hours on the right arm and reaching its greatest intensity on the 4th or 5th day. No reaction occurs on the control arm.

Mumps Skin Test Antigen

Mumps skin test antigen is a sterile suspension of formaldehyde-inactivated mumps virus prepared from the extra-embryonic fluids of the mumps virus-infected chicken embryo. It is concentrated and purified by differential centrifugation and diluted with isotonic sodium chloride solution so that each ml contains not less than 20 complement-fixing units. It should be stored at between 2 and 8° C and has an expiration date of 18 months.

The mumps intradermal skin test is utilized to define an individual's previous experience with mumps virus. In about 75% of the cases, infection with the mumps virus is followed by skin sensitivity to the organism. The mumps skin test antigen is particularly helpful during and after adolescence as an aid in identifying those who should be protected against the disease.

A control test is not necessary. An area of erythema at least 1.5 cm in diameter, with or without induration, developing 24 to 36 hours after injecting the antigen is indicative of probable immunity.

USE AND DOSE. Mumps skin test antigen is a diagnostic aid, and the usual dose, intradermally, is 0.1 ml.

TOXINS AND TOXOIDS

Toxins are bacterial waste products that are considered poisonous to the animal body. Notwithstanding, they act as antigens because of their power to stimulate certain cells of the body to produce antibodies called antitoxins. In practice, toxins are modified to inactivate the toxicophore group of the molecule, leaving the antigenic group unchanged.

When toxins are excreted from the bacterial cells producing them and are dissolved in the surrounding culture medium, they are referred to as **exotoxins.** In other cases, when they are retained within the bacterial body, they are called **endotoxins.**

To produce a solution of exotoxins commercially, the highly virulent organisms are cultured in beef broth medium and then killed by appropriate means. The organisms are removed by filtration through a bacterial filter, and the filtrate that contains the toxins and other products of growth is standardized on a suitable animal to determine the minimum lethal dose. This dose represents the smallest amount of the toxin that will kill a majority of a series of guinea pigs within 96 hours after subcutaneous administration. Commercial toxins serve as a starting point for the manufacture of antitoxins, as described below.

The source of "the most poisonous poison" is *Clostridium botulinum*, a microorganism generally unable to grow in the body of a warm-blooded animal but capable of causing death if its exotoxins are ingested. Thus, botulism is a matter of food poisoning. When the toxins produced by this bacterium are compared with other

types of protein poisons (diphtheria toxin and snake venom), their potencies range from 10 to 1000 times higher. Five kinds of neurotoxins have been determined; food poisoning in humans commonly is produced by types A, B, and E.

Treating exotoxins with formaldehyde reduces or eliminates the toxic properties without affecting the antigenic properties. These products, detoxified in this manner, are called **fluid toxoids,** and they are used to induce artificial active immunity in susceptible individuals. By precipitating or adsorbing the fluid toxoid with alum, aluminum hydroxide, or aluminum phosphate, an **adsorbed toxoid** is produced which, when administered, results in a slower release of the antigen from the site of injection and a subsequent production of higher and more prolonged antibody titers. However, the adsorbed toxoids are more prone to produce local reactions at the site of injection than are fluid toxoids. To avoid this, adsorbed toxoids should be administered by deep intramuscular injection, whereas the fluid toxoid may be administered subcutaneously.

Both fluid and adsorbed toxoids are used to produce active immunity against diphtheria and tetanus. They are used alone and in combination. In young children, diphtheria and tetanus toxoid combined with pertussis vaccine is often used, and the combination is commonly known as triple antigen or DTP.

Repeated immunization with diphtheria and tetanus toxoids may result in increasingly severe local reactions. Diphtheria antigen in absorbed diphtheria and tetanus toxoids for adult use (Td) is therefore 4- to 10-fold less than in adsorbed diphtheria and tetanus toxoids for pediatric use (DT) and in DTP. Also, a lower frequency of booster immunization for tetanus is now recommended (Table 13–3).

The toxoids alone and in combination with pertussis vaccine should be stored at a temperature of between 2 and 8° C. The expiration date is not later than 2 years after the date of issue.

ANTITOXINS

Antitoxins are prepared from the blood of animals, usually horses, that have been immunized by repeated injections of specific bacterial exotoxins. The toxin, in constantly increasing doses, induces the formation of antitoxin in the blood of the injected animal. After tests have been conducted to determine the antitoxin titer of the serum, the animal is bled, the clot is permitted to form, and the clear supernatant serum is separated for processing.

In the past, diphtheria antitoxin consisted of unprocessed serum which, when injected, often caused numerous cases of sensitivity to horse serum proteins. Today, depending on the manufacturer, either of 2 methods of processing is employed. The first involves a series of precipitations using varying concentrations of ammonium sulfate. During this process, the euglobulin and fibrinogen fractions are initially "salted" out, followed by the pseudoglobulin fraction, which contains the antitoxin. The latter fraction is redissolved, dialyzed, and filtered. The second method utilizes a pepsin solution to digest the plasma, thus removing up to 80% of the protein; however, a loss of about 20% in antitoxin content occurs also. The digested material is then treated with ammonium sulfate solution, redissolved, dialyzed, and filtered. Both of these methods aim to eliminate the proteins of horse serum and the resulting serum sickness.

Antitoxins are standardized in terms of "antitoxin units." The international unit of diphtheria antitoxin is the same as that of the American or National Institutes of Health unit: that amount of antitoxin that is contained in 1/6000 g of a certain dried, unconcentrated horse serum antitoxin that has been maintained since 1905 at the National Institutes of Health, Bethesda, Maryland. On the other hand, the international

Table 13–3. Therapeutically Important Toxoids

Agent	Indication for Use	Dosage Schedule	Products Available
Adsorbed Diphtheria Toxoid (Pediatric)	Active immunization against diphtheria in infants and children under 7 years of age.	For primary immunization, 2 injections of 0.5 ml given intramuscularly 6 to 8 weeks apart, and a third dose of 0.5 ml given 1 year later. Booster dose of 0.5 ml should be given at 5- to 10-year intervals.	generic
Tetanus Toxoid (Fluid)	Active immunization against tetanus.	For primary immunization, 3 injections of 0.5 ml given subcutaneously or intramuscularly 4 to 8 weeks apart, and a fourth dose of 0.5 ml given 6 to 12 months after the third injection. A booster dose of 0.5 ml should be given every 10 years.	generic
Adsorbed Tetanus Toxoid	Active immunization against tetanus and preferred agent over Tetanus Toxoid (Fluid) for primary immunization and booster doses.	For primary immunization, 2 injections of 0.5 ml given intramuscularly 4 to 8 weeks apart, and a third dose of 0.5 ml given 6 to 12 months later. Booster dose same as Tetanus Toxoid (Fluid).	generic
Adsorbed Diphtheria and Tetanus Toxoids for Pediatric Use (DT)	Indicated when it is inadvisable to give triple antigen containing pertussis vaccine to children less than 6 years of age.	For primary immunization, 2 injections of 0.5 ml given intramuscularly at intervals of 4 to 8 weeks. A reinforcing dose is given 6 to 12 months later. Booster dose of 0.5 ml at 5 years of age, then every 10 years thereafter.	generic
Adsorbed Diphtheria and Tetanus Toxoids for Adult Use (Td)	Primary active immunization in adults and for wound management. It contains the same amount of tetanus toxoid as in DT but only 10% to 25% of the diphtheria toxoid.	For primary immunization and booster dose, the same as for DT. For wound management of all wounds, whether tetanus-prone or not, and in cases in which the immunization status of the patient is unimmunized, uncertain, or incomplete, or last booster dose was administered longer than 10 years ago, a single 0.5-ml dose of either DT or Td should be administered intramuscularly.	generic
Adsorbed Diphtheria and Tetanus Toxoids and Pertussis Vaccine (DTP)	Active immunization of infants and children under 7 years of age against diphtheria, tetanus, and whooping cough.	For primary immunization, administer at the age of 2 to 3 months with three 0.5-ml doses injected intramuscularly at 4- to 8-week intervals with a reinforcing dose given 1 year after the third injection. Booster dose of 0.5 ml is administered when child is 4 to 6 years of age. For booster doses thereafter, Td should be used.	Tri-Immunol®

unit of tetanus antitoxin is equivalent to only one half the potency of the American or National Institutes of Health unit: that amount of antitoxin that is contained in 0.00015 g of a dried, unconcentrated horse serum antitoxin maintained since 1907, 3000 international units being equivalent to 1500 American units.

No antitoxin, antivenin, or antiserum prepared from horse serum should be given without carefully inquiring about prior exposure to horse serum or about allergic response upon exposure to horses. Whenever these products are administered, a syringe containing epinephrine injection (1:1000) and a tourniquet should be available to counter an anaphylactic reaction. Also, sensitivity testing should be performed before administration either by injecting intracutaneously 0.02 ml of a 1:100 dilution of the product to be administered or by instilling a drop of 1:100 dilution of the product into the conjunctival sac. A drop of sodium chloride injection, USP, placed in the opposite eye, provides a control.

The hypersensitivity reactions that can arise from the injection of biologics prepared from horse serum can range in severity from acute anaphylaxis and death occurring almost immediately after injection to serum sickness, which may arise hours to weeks following treatment. Typical manifestations of serum sickness include fever, urticaria, adenopathy, and arthritis.

Diphtheria Antitoxin

Diphtheria antitoxin is a sterile, nonpyrogenic solution of the refined and concentrated proteins, chiefly globulins, containing antitoxic antibodies obtained from the blood serum or plasma of healthy horses that have been immunized against diphtheria toxin or toxoid. It has a potency of not less than 500 antitoxin units per ml.

The expiration date with a 20% excess of potency is not later than 5 years after the date of manufacture or of issue. Diphtheria antitoxin should be stored at a temperature of between 2 and 8° C.

USES AND DOSE. Diphtheria antitoxin is a passive immunizing agent capable of inducing passive immunity against diphtheria. It is a valuable curative agent when used in sufficient amount to neutralize the pathogenic effects of the toxin formed in the patient. This is especially true when the antitoxin is used early in the disease and before the detrimental effects are too far advanced. Any person with clinical symptoms of diphtheria should receive the antitoxin at once without waiting for bacteriologic confirmation. The usual prophylactic dose, intramuscularly or intravenously, is 1000 to 10,000 units; the therapeutic dose, 20,000 units to 120,000 units.

Although penicillin and other antibiotics kill the diphtheria organisms, they have no effect on the toxins.

Tetanus Antitoxin

Tetanus antitoxin is a sterile, nonpyrogenic solution of the refined and concentrated proteins, chiefly globulins, containing antitoxic antibodies obtained from the blood serum or plasma of healthy horses that have been immunized against tetanus toxin or toxoid. It has a potency of not less than 400 antitoxin units per ml.

Tetanus antitoxin should be stored at a temperature of between 2 and 8° C. The expiration date of the liquid antitoxin is not later than 5 years after the date of manufacture or issue with a 20% excess of potency.

USES AND DOSE. Tetanus antitoxin is employed in the treatment and prophylaxis of tetanus if tetanus immune globulin is not available. It creates passive immunity to tetanus. Like diphtheria antitoxin, it is a valuable therapeutic agent when used early in the disease. Prophylactic doses should be given to individuals who have had 2 or less injections of tetanus toxoid and who have tetanus-prone injuries that are more than 24 hours old. Tetanus toxoid

should also be administered at a different site on the patient. The usual prophylactic dose, intramuscularly or subcutaneously, is 1500 to 5000 units; the therapeutic dose is 50,000 to 100,000 units or more with at least part of the dose given intravenously.

Botulism Antitoxin

Botulism antitoxin is a sterile, nonpyrogenic solution of the refined and concentrated antitoxic antibodies, chiefly globulins, obtained from the blood serum or plasma of healthy horses that have been immunized against the toxins produced by both the type A and type B and/or type E strains of *Clostridium botulinum*. This antitoxin contains not more than 20% of solids and should be stored at a temperature of between 2 and 8° C. The expiration date is not later than 5 years after the date of issue.

This multivalent antitoxin is used to treat all cases of toxemia caused by the types of botulinus bacteria used in its preparation. A multivalent antitoxin is advantageous because the prescribing physician is not required to wait for a determination of the type of the causative organism.

USE AND DOSE. Botulism antitoxin is classed as a passive immunizing agent to be used in the treatment of botulism. The usual dose is, intravenously, 20,000 units, repeated at 2- to 4-hour intervals, as necessary. It is not available commercially but can be obtained from the Centers for Disease Control (see page 387).

VENOMS AND ANTIVENINS

Venoms are poisonous excretions produced by animals; they can be compared with the toxic waste products (exotoxins) of bacteria. The detrimental effects developed in humans and animals following the bite of poisonous snakes (rattlesnake, copperhead, moccasin, cobra, and others) have been known for many years. About 10,000 people are bitten by poisonous snakes every year in the United States. Poisonous snakebites often cause severe pain and can lead to tissue necrosis, amputation, and death. The venom of the rattlesnake is a complex mixture, chiefly of proteins, many of which have enzymatic activity and a nonenzymatic neurotoxic fraction. Similarly, the venoms of the tarantula, scorpion, black widow spider, honeybee, wasp, and other arthropods produce various deleterious effects, depending on the amount, time of year, and other conditions. Chemical examinations of the poisons of toads have revealed that both skin and glandular secretions possess toxic substances called bufotoxins. The chemical structures of the bufotoxins are somewhat similar to those of the aglycones of the cardiac glycosides; in fact, the bufotoxins appear to have a similar pharmacologic effect.

Snake venins or venoms are obtained by holding a poisonous snake over a conical glass container covered with a sheet of thin rubber (Fig. 13–12). The snake strikes the rubber and penetrates it with its fangs, whereupon the semiliquid venom is ejected into the container.

Mixtures of venins from the poisonous snakes of a locality, country, or continent are prepared and used in the preparation of polyvalent antivenins (antisnakebite serums).

Treatment of snakebite is controversial, but most authorities believe that early administration of antivenin is the therapy of choice. The location of antivenins for rare species and names and telephone numbers of experts on venomous bites can be obtained at any hour from the Oklahoma City Poison Control Center (405-271-5454).

Antivenin (Crotalidae) Polyvalent

Antivenin (Crotalidae) polyvalent or North and South American antisnakebite serum is a sterile, nonpyrogenic preparation derived by drying a frozen solution of specific venom-neutralizing globulins obtained from the serum of healthy horses immunized against venoms of 4 species of pit vipers. These are *Crotalus atrox* (Western

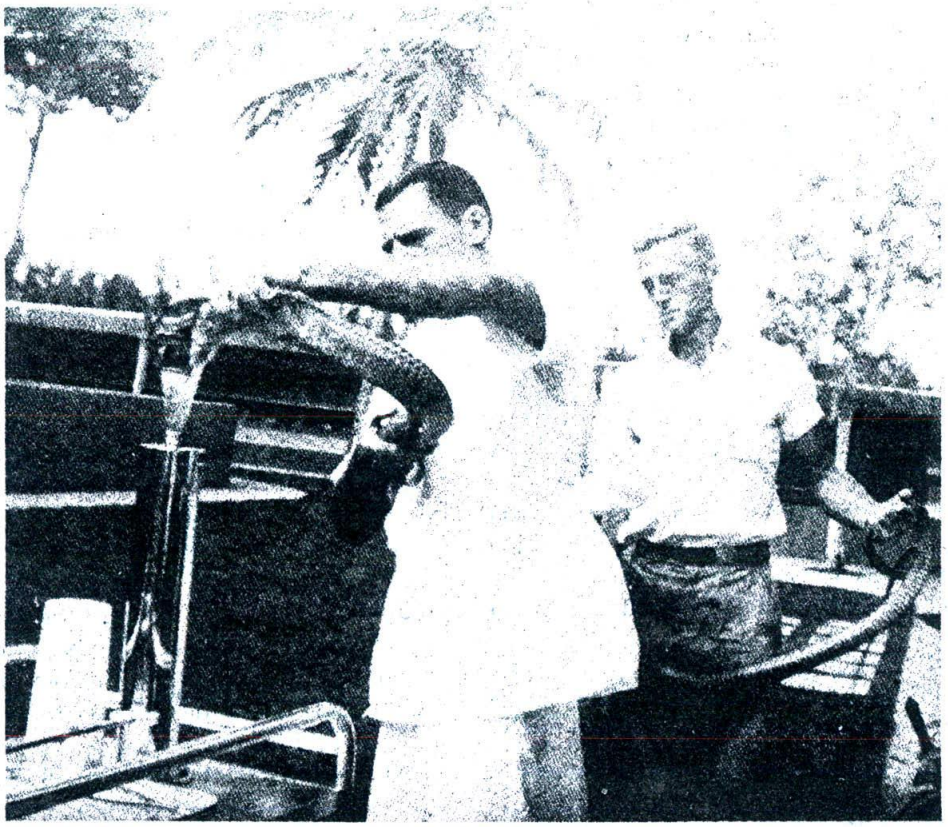

Fig. 13–12. Cobra being "milked" for its venom by Mr. Haast of the Miami Serpentarium. (Photo courtesy of Hynson, Wescott and Dunning.)

diamondback), *C. adamanteus* (Florida diamondback), *C. durissus terrificus* (South American rattlesnake) and *Bothrops atrox* (South American fer-de-lance) (Fam. Crotalidae).

This antivenin is standardized by biologic assay on mice in terms of venom neutralization. It should be protected against exposure to excessive heat. The expiration date for antivenin (Crotalidae) polyvalent with a 10% excess of potency is not more than 5 years after date of issue.

USE AND DOSE. Antivenin (Crotalidae) polyvalent is a passive immunizing agent used for treating snakebite of the species indicated. The preferred route of administration is intravenous infusion, as a 1:1 to 1:10 dilution of antivenin in sodium chloride injection or 5% dextrose injection, after testing for sensitivity to horse serum.

In general, antivenins are prepared in the same manner as antitoxins. The specific venin is injected into horses in gradually increasing doses until the blood titer reaches the desired strength. The animal is then bled, and the blood serum is subjected to the required processing. Antivenins have been prepared for use in many parts of the world. In addition to antivenin (Crotalidae) polyvalent, univalent or bivalent antivenins are available to protect against the copperhead *(Agkistrodon)* alone, or combined with antivenin against the rattlesnake *(Crotalus)* in the United States and other snakes in other countries, such as the bushmaster and palm vipers of tropical America and the boomslang, cobra, puff adder, and gaboon viper of Africa.

Antivenin *(Micrurus fulvius)* or North American coral snake antivenin is the ster-

ile, nonpyrogenic preparation derived by drying a frozen solution of specific venom-neutralizing globulins obtained from the serum of healthy horses that have been immunized with the venom of *Micrurus fulvius*, the eastern coral snake. This preparation also neutralizes the venom of *M. fulvius tenere* (Texas coral snake) but does not neutralize the venom of *Micruroides euryxanthus* (Arizona or Sonoran coral snake).

Spider-Bite Antivenin

Antivenin *(Latrodectus mactans)* or black widow spider antivenin is prepared from the serum obtained from horses immunized against the venom of the black widow spider *(Latrodectus mactans)*. It is available in a lyophilized form and is recommended as a specific treatment of the effects of venom from the bites of this spider. It may be given intramuscularly or intravenously over a 15-minute period when diluted in 10 to 50 ml of saline solution.

ANTISERUMS

Antiserums are biologics prepared in a manner similar to that for antitoxins and antivenins except that bacteria or viruses are used to stimulate the production of specific antibodies in a healthy animal such as the horse. Viral or bacterial cells, as found in vaccines, serve as the antigenic substances; these are introduced into the animal body in gradually increasing doses and are continued until the proper antibody titer of the blood serum is achieved. The destruction of the injected cells by phagocytes liberates antigenic materials with the subsequent development of corresponding antibodies. Antiserum against rabies is an example of this type of immunizing agent.

The therapeutic effectiveness of antiserums is based on their production of artificial passive immunity. Thus, each antiserum is a specific biologic employed to provide a supply of ready-made antibodies to combat the disease. Antiserum against rabies is useful in modern therapy, but many antiserums against bacteria that were formerly employed in therapy have been replaced by antibiotics.

Antirabies Serum

Antirabies serum is a sterile, nonpyrogenic solution containing antiviral substances obtained from the blood serum or plasma of a healthy horse that has been immunized against rabies by means of a vaccine.

In 1953, a new concept in the immunization treatment of rabies was introduced, consisting of the administration of antirabies serum in conjunction with rabies vaccine. Evidence that this method of treatment was superior to that of vaccine alone gradually was accumulated. At the present time, rabies immune globulin is preferred for this combined therapy; however, if it is not available, antirabies serum can be used after testing for sensitivity to horse serum.

USE AND DOSE. Injection of antirabies serum provides the patient with immediate protection against rabies. The biologic should bear an expiration date not later than 2 years after the date of manufacture. It is available in containers of 1000 units. The usual single dose is, intramuscularly, not less than 1000 units per 55 pounds of body weight. Part of the serum dose should be infiltrated into the tissue around the wound whenever feasible.

IMMUNE GLOBULINS

Immune globulins are immunizing biologics that contain specific antibodies derived from the blood of humans who have survived an attack of a specific disease or who have been immunized in some other manner. Chances of sensitization are less with human serum derivatives than with immune serums from animal sources.

Immune globulins may be obtained from the plasma or serum pool of a large number of random donors or from a limited num-

ber of individuals who have been hyperimmunized against a specific antigenic material. Preparations derived from a large, random source contain a general spectrum of antibodies and may be used for many diverse purposes. Standardization of the globulin fractions for specific antibodies provides specialty preparations of specific utility. Preparations such as pertussis immune globulin and tetanus immune globulin, which are obtained from hyperimmunized sources, contain high titers of specific antibodies and are intended for specific use.

This type of preparation should be stored at a temperature of between 2 and 8° C. The expiration date is usually not more than 3 years after the date of issue. Serum globulins offer rapid protection (artificial passive immunity) and are administered intramuscularly, except for immune globulin intravenous. Live virus vaccines should be administered 2 weeks before or 3 months after immune globulin administration because antibodies in the globulin preparation may interfere with the immune response to the vaccination.

Immune Globulin

Immune globulin, immune serum globulin (human), immune globulin intramuscular, or gamma globulin is a sterile, nonpyrogenic solution of globulins and contains many antibodies normally present in adult human blood. Each lot of immune globulin is prepared by pooling approximately equal amounts of material (source blood, plasma, serum, or placentas) from at least 1000 individuals.

Immune globulin has some prophylactic value in chicken pox, hepatitis A, rubella, and other diseases. In many instances, serum globulin offers no benefit after onset of disease symptoms. However, measles can be modified by using this preparation.

USE AND DOSE. Immune globulin is a passive immunizing agent. The dosage is based on body weight and varies with the intended use. The usual intramuscular dose is 0.2 ml per kg for measles prophylaxis and 0.02 ml per kg for prophylaxis against hepatitis A. It is also given to treat gamma globulin deficiency for the prevention of recurrent infections. It is injected intramuscularly in a dose of 1.3 ml per kg, followed in 3 or 4 weeks by 0.66 ml per kg, to be given every 3 to 4 weeks.

PRESCRIPTION PRODUCTS. Gamastan®, Immuglobin®, Gammar®.

Immune globulin intravenous (IGIV) provides immediate antibody levels, whereas intramuscular administration involves a 2- to 5-day delay before adequate serum levels are attained. It is used in the treatment of immunodeficiency syndrome, especially in patients who require an immediate increase in immunoglobulin blood levels. The usual dose is 100 to 200 mg per kg, administered once a month by intravenous infusion.

PRESCRIPTION PRODUCTS. Gamimune® Sandoglobulin®.

Pertussis Immune Globulin

Pertussis immune globulin or pertussis immune globulin (human) is a sterile, nonpyrogenic solution of globulins derived from the blood plasma of adult human donors who have previously been immunized with pertussis vaccine.

USE AND DOSE. Pertussis immune globulin is used in the prophylaxis and treatment of pertussis. The usual intramuscular prophylactic dose is 1.25 to 2.5 ml, repeated in 1 or 2 weeks as necessary. The therapeutic dose range is the same, but administration is repeated in 1 or 2 days, depending on the clinical response.

PRESCRIPTION PRODUCT. Hypertussis®.

Tetanus Immune Globulin

Tetanus immune globulin or tetanus immune globulin (human) is a sterile, nonpyrogenic solution of globulins derived from the blood plasma of adult human donors who have been immunized with tetanus toxoid.

This immune globulin is especially useful for passive immunization against tetanus in individuals with wounds that may have been contaminated with tetanus microorganisms. It is intended particularly for persons who have not previously received tetanus toxoid for active immunization. Because it is derived from humans, tetanus immune globulin is much safer than tetanus antitoxin, which is also available (see page 407).

USE AND DOSE. Tetanus immune globulin is employed in the prophylaxis and treatment of tetanus. The usual intramuscular prophylactic dose is 250 units as a single injection. The therapeutic dose range is 3000 to 6000 units.

PRESCRIPTION PRODUCTS. Homo-Tet®, Hu-Tet®, Hyper-Tet®.

Rabies Immune Globulin

Rabies immune globulin is a sterile, nonpyrogenic solution of antirabies gamma globulin concentrated by cold alcohol fractionation from plasma of donors hyperimmunized with rabies vaccine.

Rabies immune globulin is indicated for passive protection against rabies in persons suspected of exposure to rabies, particularly in cases of severe exposure. After initiation of the vaccine series, it takes approximately 1 week to develop immunity to rabies; therefore, the value of immediate passive immunization is important for successful prevention of the disease. Its use is the same as antirabies serum (see page 410); however, because it is of human origin, it possesses the added advantage of removing the risk of serum sickness.

USE AND DOSE. It is recommended that rabies immune globulin be used in combination with rabies vaccine as the best postexposure prophylaxis. The usual dose is a single administration of 0.133 ml per kg of body weight at the time of the first vaccine dose. Up to half the dose should be used to infiltrate the wound and the rest administered intramuscularly. Repeating the dose may interfere with maximum active immunity expected from the vaccine.

PRESCRIPTION PRODUCT. Hyperab®, Imogam®.

Hepatitis B Immune Globulin

Hepatitis B immune globulin is a sterile, nonpyrogenic solution of immunoglobulin prepared from pooled plasma obtained from donors with high titers of antibody to hepatitis B surface (HBs) antigen. Administration is indicated for postexposure prophylaxis following accidental exposure to hepatitis B surface antigen. The exposure can be either parenteral, through direct mucous membrane contact, or through oral ingestion. The materials most often involved are blood, plasma, or serum that is positive for HBs antigen.

Injections should be given intramuscularly not later than 7 days after exposure, and the recommended dose is 0.06 ml per kg of body weight, repeated 28 to 30 days after the first dose.

PRESCRIPTION PRODUCTS. H-BIG®, Hep-B-Gammagee®, HyperHep®.

Varicella-Zoster Immune Globulin

Varicella-zoster immune globulin is the globulin fraction of human plasma, primarily immunoglobulin G, found in routine screening of normal volunteer blood donors. When absorbed into the circulation, the antibodies persist for 1 month or longer and are sufficient to mitigate or prevent varicella infection. It has its greatest effectiveness when administered within 96 hours of exposure to the varicella virus. Because supplies of the varicella-zoster immune globulin are limited, it is recommended that its use be restricted to susceptible immunodeficient individuals. The dose range is 125 units per 10 kg body weight, up to a maximum of 625 units administered by deep intramuscular injection in the gluteal muscle or in another large muscle mass.

RH_0 (D) Immune Globulin

Rh_0 (D) immune globulin is a sterile, nonpyrogenic concentrated solution of globulins derived from human blood plasma containing antibody to the erythrocyte factor Rh_0 (D). This antibody neutralizes the antigen in Rh-positive blood, which sensitizes Rh-negative women and results in Rh hemolytic disease of the newborn in subsequent pregnancies.

This preparation is recommended for administration to unsensitized Rh-negative women who give birth to Rh_0 (D)- or D^u-positive infants. It should be administered within 72 hours of Rh-incompatible delivery, miscarriage, abortion, or transfusion, and the usual dose is the entire content of 1 vial (containing 300 μg of antibody), given intramuscularly. The antibody neutralizes any antigen introduced into the mother as a result of mixing of fetal and maternal blood during childbirth and thus prevents sensitization.

PRESCRIPTION PRODUCTS. Gamulin Rh®, HypRho-D®, RhoGAM®, Rhesonativ®.

Lymphocyte Immune Globulin

Lymphocyte immune globulin or antithymocyte globulin (equine) is a lymphocyte selective immunosuppressant that is prepared by immunizing horses with human thymus cells and then isolating the equine gamma globulin. It is thought that it alters the function of the T-lymphocytes, which are responsible in part for cell-mediated immunity and, therefore, it is indicated for use in organ transplant.

When administered with conventional immunosuppressive therapy at the time of rejection in allograft renal transplant patients, it increases the frequency of resolution of the acute rejection episode. The usual adult dose is 10 to 30 mg per kg per day administered by intravenous infusion.

PRESCRIPTION PRODUCT. Atgam®.

BIOLOGICS RELATED TO HUMAN BLOOD

A number of human blood products that have no immunizing property or function are considered biologics. These products include whole blood, red blood cells, and various blood fractions. Such blood derivatives as various antihemophilic preparations have specialized application. Albumin human and plasma protein fraction serve as blood-volume supporters, and the radio-iodinated serum albumins are diagnostic aids. Fibrinolysin, which is obtained from human blood plasma, is used for its enzymatic action (see page 279). Recent years have been characterized by an increasing sophistication in the availability and use of blood products, and further developments, such as the common use of granulocyte and platelet fractions for treatment of granulocytopenia and thrombocytopenia, may be forthcoming.

A blood-related biologic from nonhuman sources is thrombin.

Whole Blood

Whole blood or whole blood (human) is blood that has been drawn from a selected donor under rigid aseptic conditions. It contains citrate ion or heparin as an anticoagulant. It should be stored at a constant temperature of between 1 and 6° C. The expiration date is 21 days after the date of bleeding if the anticoagulant is citrate dextrose solution or citrate phosphate dextrose solution, 35 days if the anticoagulant is citrate phosphate dextrose adenine solution, and 48 hours if the anticoagulant is heparin. It is used as a blood replenisher. It is administered intravenously, usually in a volume of 1 unit or 500 ml, as necessary.

Red Blood Cells

Red blood cells is whole blood from which plasma has been removed. Red blood cells may be prepared at any time during the dating period of the whole

blood from which it is derived by centrifugation or undisturbed sedimentation. It contains a portion of the plasma sufficient to ensure optimal cell preservation or contains a cryophylactic substance if it is used for extended manufacturers' storage at −65° C or colder. The expiration date for frozen red blood cells is not later than that of the whole human blood from which it was derived. The expiration date for frozen red blood cells, stored at −65° C or colder, is not later than 3 years after the date of collection of the source blood. This preparation is used as a blood replenisher. It is particularly useful in cases of anemia, when the additional volume of plasma is undesirable. The usual dose is the equivalent of 1 unit of whole blood.

Antihemophilic Derivatives

Concentrates of the antihemophilic factors in human plasma are available for control of 2 types of hemophilia. Details in the preparation of the various available products differ, but basically, human plasma is fractionated to eliminate many proteins that lack antihemophilic properties. The products are available in a lyophilized form and are standardized for antihemophilic activity. They are administered intravenously and offer the advantage of reducing the volume of fluid that must be injected.

Antihemophilic factor is a sterile, freeze-dried concentrate of human antihemophilic factor (prepared from the factor VIII-rich cryoprotein fraction of human venous plasma) for use in the therapy of hemophilia A (classic hemophilia) by accelerating the abnormally slow clotting time. Factor VIII is needed for the transformation of prothrombin to thrombin by the intrinsic pathway.

It should be stored at a temperature of between 2 and 8° C, and the expiration date is not later than 1 or 2 years from the date of manufacture or date of issue.

DOSE. Intravenous, 10 to 20 AHF units per kg of body weight, 1 or 2 times a day, or as necessary to maintain a proper blood level of factor VIII. One AHF unit is defined as the activity present in 1 ml of normal pooled human plasma less than 1 hour old.

PRESCRIPTION PRODUCTS. Factorate®, Hemofil®, Humafac®, Koate®, Profilate®.

Antihemophilic factor IX complex is a dried plasma fraction comprising coagulation factors IX (plasma thromboplastin component), II (prothrombin), VII (proconvertin), and X (Stuart-Prower factor). This preparation is indicated to prevent a dangerous bleeding episode or to perform surgery whenever one or more of these specific coagulation factors is absent in the blood of a patient.

DOSE. The dose depends on the patient and the circumstances.

PRESCRIPTION PRODUCTS. Konyne®, Proplex®, Profilnine®.

Albumin Human

Albumin human or normal serum albumin (human) is a sterile, nonpyrogenic preparation of serum albumin obtained by fractionating material (source blood, plasma, serum, or placentas) from healthy, human donors. This material is then tested for the absence of hepatitis B surface antigen. Not less than 96% of its total protein is albumin. It is a solution containing in each 100 ml, 25 g of serum albumin osmotically equivalent to 500 ml, or 5 g equivalent to 100 ml, of normal human plasma.

Albumin human is a blood-volume supporter. The usual dose, intravenously, is a volume equivalent to 25 g of albumin.

PRESCRIPTION PRODUCTS. Albutein®, Buminate®, Albuminar®, Plasbumin®.

Plasma Protein Fraction

Plasma protein fraction or plasma protein fraction (human) is a sterile solution of selected proteins derived by fractionating material (source blood, plasma, or serum) from healthy human donors and testing for the absence of hepatitis B surface antigen. It contains not less than 4.5 g and not more than 5.5 g of protein per 100 ml, of which not less than 83% is al-

bumin, and not more than 17% is alpha and beta globulins. This substance is a human blood fraction that is indicated for restoration of blood volume when the patient is in a state of shock caused by burns, crushing injuries, and any other causes, in which loss of plasma fluids, not loss of red blood cells, is predominant.

USE AND DOSE. Plasma protein fraction is a blood-volume supporter. The usual dose is 250 to 500 ml by intravenous infusion at a rate not exceeding 10 ml per minute.

PRESCRIPTION PRODUCTS. Plasmanate®, Protenate®, Plasma-Plex®, Plasmatein®.

Radio-Iodinated Serum Albumins

Preparations are available containing human serum albumin that has been iodinated using mild conditions with either ^{125}I or ^{131}I. The iodination is controlled to introduce not more than 1 gram-atom of iodine for each gram-molecule (60,000 g) of albumin.

Preparations of iodinated albumin are sterile, buffered, isotonic solutions prepared to contain not less than 10 mg of radio-iodinated normal human albumin per ml and adjusted to provide not more than 1 millicurie of radioactivity per ml. These solutions must be labeled to indicate the radioactivity, expressed in microcuries or millicuries per ml, at a specified time.

Iodinated ^{125}I albumin injection and iodinated ^{131}I albumin injection are diagnostic aids for determining blood volume and cardiac output. The usual dose is 5 microcuries intravenously. Correction for radioactive decay must be made in dosage calculations. The radioactive half-life of ^{125}I is 60 days, and the half-life of ^{131}I is 8.08 days. The expiration date for preparations of radio-iodinated serum albumin is 120 days after completion of iodination if ^{125}I is used and 30 days if ^{131}I is used. A preparation of **iodinated ^{131}I aggregated injection** is also used as a diagnostic aid for determination of pulmonary clearance.

Thrombin

Thrombin is a sterile protein substance prepared from prothrombin of bovine origin through interaction with added thromboplastin in the presence of calcium. It can, without the addition of other substances, cause the clotting of whole blood, plasma, or a solution of fibrinogen. Its activity is expressed in units on the basis of clotting a standard fibrinogen solution. Thrombin is available as a lyophilized solid and has an expiration date of not more than 3 years after the date of issue. Solutions should be used within a few hours after preparation.

Thrombin is a local hemostatic. It is applied topically to control blood oozing from capillaries and small venules when the area is accessible. It is useful in dental surgery, laryngeal and nasal surgery, plastic surgery, and skin grafting procedures. It may be applied as a powder or as a solution containing 100 to 2000 NIH units per ml in sodium chloride irrigations or sterile water for injection.

PRESCRIPTION PRODUCTS. Thrombinar®, Thrombostat®.

READING REFERENCES

Arnold, R.E.: *What to Do About Bites and Stings of Venomous Animals*, New York, Macmillan Publishing Co., Inc., 1973.

Bowry, T.R.: *Immunology Simplified*, 2nd ed., Oxford, Oxford University Press, 1984.

Bücherl, W., Buckley, E.E., and Deulofeu, V., eds.: *Venomous Animals and Their Venoms*, Vol. I, New York, Academic Press, Inc., 1968.

Bücherl, W., and Buckley, E.E., eds.: *Venomous Animals and Their Venoms*, Vols. II and III, New York, Academic Press, Inc., 1971.

Caras, R.A.: *Venomous Animals of the World*, Englewood Cliffs, New Jersey, Prentice-Hall, Inc., 1974.

Gadd, L.: *Deadly Beautiful: The World's Most Poisonous Animals and Plants*, New York, Macmillan Publishing Co., Inc., 1980.

Germanier, R.: *Bacterial Vaccines*, Orlando, Florida, Academic Press, Inc., 1984.

Hildemann, W.H.: *Essentials of Immunology*, New York, Elsevier Science Publishing Co., Inc., 1984.

Kimball, J.W.: *Introduction to Immunology*, New York, Macmillan Publishing Co., Inc., 1983.

Kirkwood, E.M., and Lewis, C.J.: *Understanding Medical Immunology*, Chichester, John Wiley & Sons Ltd., 1983.

McCollough, N.C., and Gennaro, J.F.: Treatment of Venomous Snakebite in the United States, Clin. Toxicol., 3 (3):483, 1970.

Minton, S.A., Jr.: *Venom Diseases*, Springfield, Illinois, Charles C Thomas, 1974.

Nisonoff, A.: *Introduction to Molecular Immunology*, 2nd ed., Sunderland, MA, Sinauer Associates, Inc., 1984.

Parrish, H.M., and Hayes, R.H.: Hospital Management of Pit Viper Venenations, Clin. Toxicol., 3 (3):501, 1970.

Paul, W.E., ed.: *Fundamental Immunology*, New York, Raven Press Books, Ltd., 1984.

Sela, M., ed.: *The Antigens*, Vols. I–V, New York, Academic Press, Inc., 1973–1979.

Steward, M.W.: *Antibodies: Their Structure and Function*, London, Chapman and Hall Ltd., 1984.

Stites, D.P., Stobo, J.D., Fudenberg, H.H., and Wells, J.V., eds.: *Basic and Clinical Immunology*, 5th ed., Los Altos, California, Lange Medical Publications, 1984.

Tu, A.T.: *Venoms: Chemistry and Molecular Biology*, New York, John Wiley & Sons, Inc., 1977.