CHAPTER 32

Routes of Administration

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General

Mice are the most widely used animals for a range of experiments including medical, chemical, pharmacological, toxicological, biological, and genetic. The administration of test substances, such as chemical elements, compounds, drugs, antibodies, cells or other agents, to mice is one of the major methods for evaluating their biological activity.

The route of administration is largely dependent on the property of the test substance and the objective of the experiment. All administration should be performed with knowledge of the chemical and physical characteristics of the substance. All routes have both demerit and merit, such as the absorption, bioavailability and metabolism of the substance. Consideration should be paid to the pH, viscosity, concentration, sterility, pyrogenicity, toxicity as well as the existence of hazardous substances. A knowledge of available methods and techniques of administration as well as knowledge of the deposition and fate of the administered substance will help the scientist/investigator to select the most appropriate route for her/his purpose. This route must be selected before the start of any experiment (Nebendahl, 2000).

Proper restraint is the most important technique when mice were treated as this decreases stress and increases successful treatment. Personnel using experimental animals should be well trained in handling and restraint, should obtain authentication for responsible use of experimental animals and attain a scientifically high standard (ETS 123, 1986; Nebendahl, 2000). Further experience will lead to repeatable and reliable results (see Chapter 31 on Handling and Restraint).

During administration mice should be protected from pain, suffering, distress or lasting harm or at least pain and distress shall be kept to a minimum (ETS 123, 1986). Some injections (such as footpad injection) are strongly discouraged and if required must be justified on a case by case basis (CCAC, 2002).

Principles of administration

Handling and restraint

Good handling and restraint is the most important technique for correct administration. Proper restraining leads to successful administration and varies with the routes of administration. Disposable gloves must be worn as manual restraint is frequently used for injections.

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There are two styles of manual restraint, one uses both hands and the other is single handed. (Chapter 31 on Handling and Restraint is helpful; Donovan and Brown, 1991; Suckow *et al.*, 2000).

Double handed manual restraint

The mouse is lifted by the base of the tail and placed on the cage lid or other solid surface with one hand and then its tail is pulled gently back (Figure 32.1a). It is quickly and firmly picked up by the scruff of the neck behind the ears with the thumb and index finger of the other hand (Figure 32.1b). The tail is transferred from the first hand to between the palm and little or ring finger of the other hand, then fixed (Figure 32.1c). The mouse is restrained (Figure 32.1d).

Single handed restraint

The tail is picked up using thumb and fore finger of the chosen hand (Figure 32.2a), then the mouse is placed on the cage lid or other solid surface (Figure 32.2b). The tail is immediately grasped by the palm and middle finger, ring finger and/or little finger, and the thumb and forefinger released (Figure 32.2c). The fold of skin from the scruff of neck down the back is immediately gripped using the thumb and forefinger (Figure 32.2d and e). The mouse is then restrained (Figure 32.2f).

To prevent kicking by the hind legs, the tail is fixed using the palm and forefinger and then the left hind leg is held firmly between the ring and little finger (where the mouse is restrained by the left hand) (Figure 32.3).

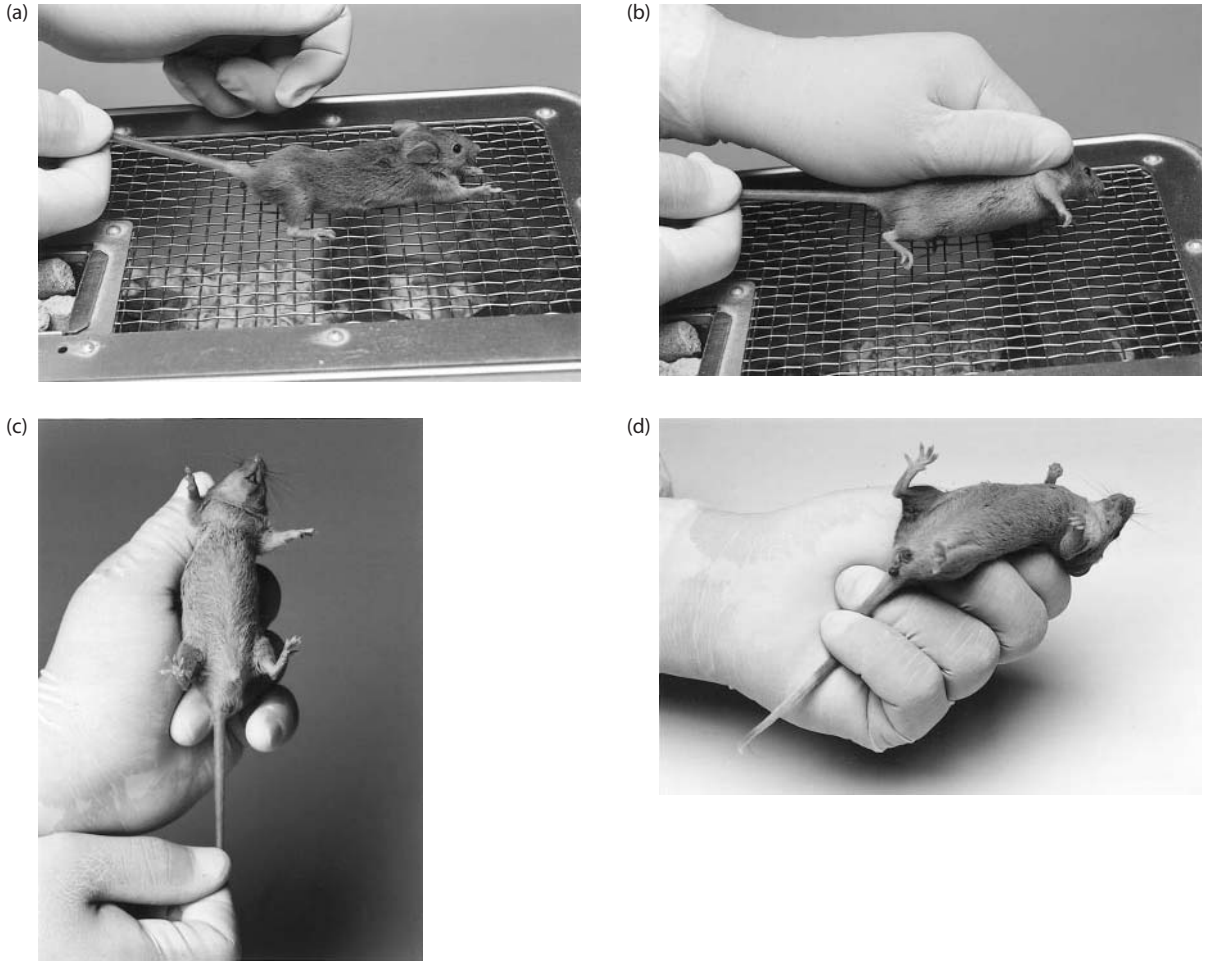

Figure 32.1 Manual restraint of a mouse using both hands. (a) The mouse is placed on the cage lid with the preferred hand. The tail is pulled gently back by the hand. (b) The mouse is quickly and firmly picked up by the scruff of the neck behind the ears with thumb and index finger of other hand. (c) The tail is transferred from the preferred hand to between palm and little or ring finger of the other hand, then held firmly. (d) The mouse is restrained.

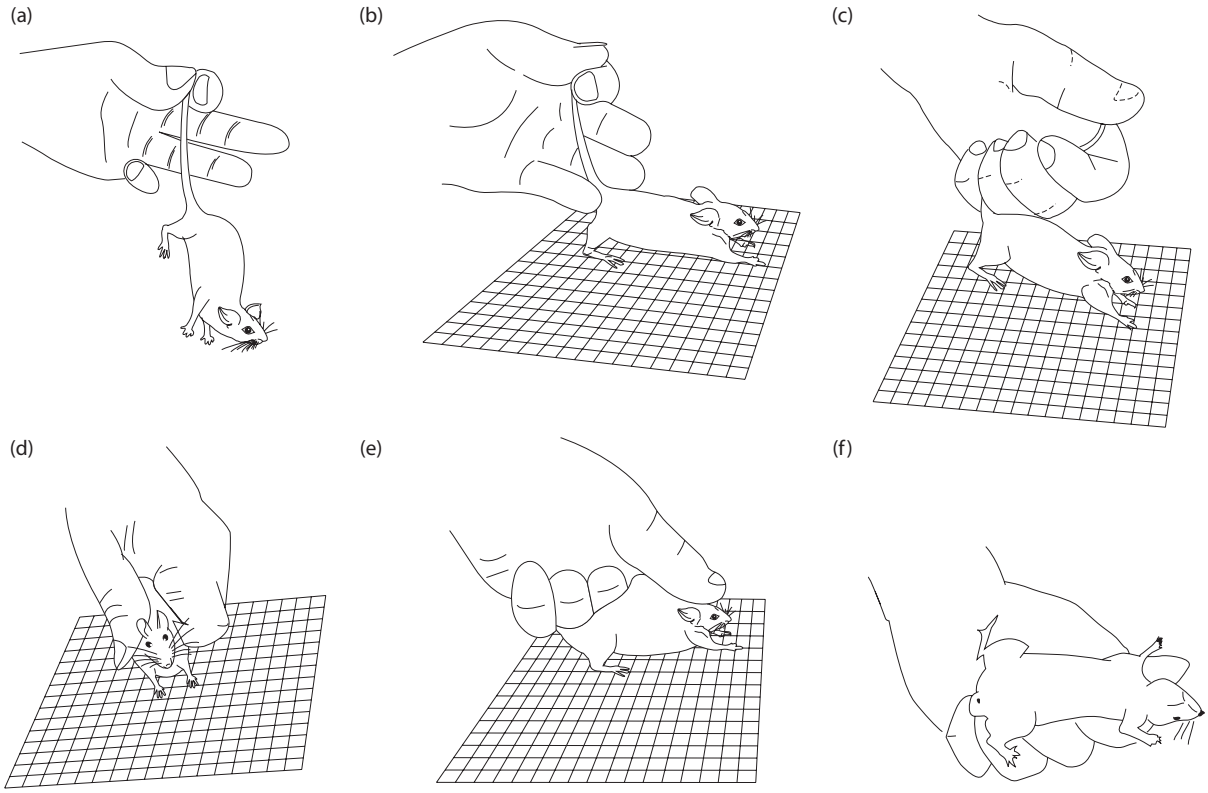

Figure 32.2 Single-handed restraint of the mouse. (a) The tail is picked up using thumb and forefinger of the preferred hand. (b) The mouse is placed on the cage lid or other solid surface pulling gently back by the hand. (c) The tail is immediately grasped by the palm and middle finger, ring finger and/or little finger and then, the tail held between thumb and forefinger is released. (d) and (e) The fold of skin from the scruff of the neck down the back is immediately gripped using the thumb and forefinger. (f) The mouse is restrained.

Site of administration

Among several possibilities for the administration of substances to mice, the most common routes are subcutaneous, intraperitoneal or intravenous injection. The intramuscular administration is not recommended, as the muscle of the mouse is too small. Some sites, such as footpad injection of Freund's complete adjuvant, intrasplenic injection and intra lymph node injection are unacceptable nowadays (CCAC, 2002), and should be restricted to cases where it is absolutely necessary.

Preparation of the site

The area for administration is clipped (Figure 32.4) or cleaned with warm water if necessary before cleaning the skin with alcohol- or disinfectant-moistened cotton. Where aseptic skin is necessary; the fur must be clipped followed by a three-stage surgical preparation: surgical soap, alcoholic rinse and surgical preparation solution. The skin is dried immediately before administration (CCAC, 2002). In some cases a local anesthetic may be applied first to prevent pain.

Preparation, solubility and safety of solutions

Test substances, solutions and equipment should be prepared aseptically and free from pyrogens, especially for parenteral injections. Solutions can be sterilised by filtration (0.22 μm). Living organisms or cells must be free from contaminants when administered. The toxicity of the substance, the volume and the way of administration should be considered to prevent tissue damage and to give precise dosage.

The following solvents or vehicles have been found suitable in most instances and do not greatly affect drug action because of their own inherent properties: water, water with 0.85% sodium chloride, water with up to 50% polyethylene glycol, water with not over 10% Tween 80, water with up to 0.25% methylcellulose or carboxymethylcellulose, corn oil; vegetable oil;

peanut oil (oral and intramuscular route only). A low percentage of the lower alcohols, glycols, and acetone can also be used, provided the volume administered is kept small (Woodard, 1965). Phosphate buffered saline (PBS) or various culture media are also suitable vehicles (Nebendahl, 2000). Lipid-soluble substances can only be dissolved in oil but this delays absorption. Oil soluble drugs have been successfully given intravenously in 15% oil–water emulsions using lecithin as an emulsifier (Woodard, 1965).

Figure 32.3 Manual restraint of a mouse to prevent kicks by hind leg. The tail is held using the palm and forefinger and then the left hind leg is fixed between the ring and little finger (when the mouse is restrained by the left hand).

Unless experience has indicated otherwise, solutions or suspensions should be prepared as near to the time of use as possible because some substances will deteriorate in solution within a few hours (Woodard, 1965). When administering drugs, the solvent should ideally be the same as the one in which the drug is normally formulated (Nebendahl, 2000).

Although distilled water can be used under certain conditions, saline is preferable because water *ad injectionem* injected subcutaneously causes pain and intravenous injection produces hemolysis. Oil and viscous fluids cannot be injected intravenously (Nebendahl, 2000). If suspended material is to be used for intravenous injection, the particles should be removed by filtration to prevent embolism (Woodard, 1965).

The temperature of fluids must be raised at least to room temperature or better still up to body temperature before use, because the injection of cold fluids is painful (Baumans *et al.*, 1993).

Concentration of substances

The concentration can vary over a fairly wide range without greatly influencing the end result of the experiment. Lower concentrations are clearly desirable (Waynforth and Flecknell, 1992). Factors limiting the use of aqueous solutions for parenteral administration are probably related to their osmotic pressure. Low concentrations can be corrected by the addition of sodium chloride but ought not to be so high as to materially exceed the osmotic pressure of 0.15 M sodium chloride (Woodard, 1965). Highly concentrated solutions can be administered intravenously provided the rate of injection is kept slow and precautions are taken to avoid getting the solution outside the vein.

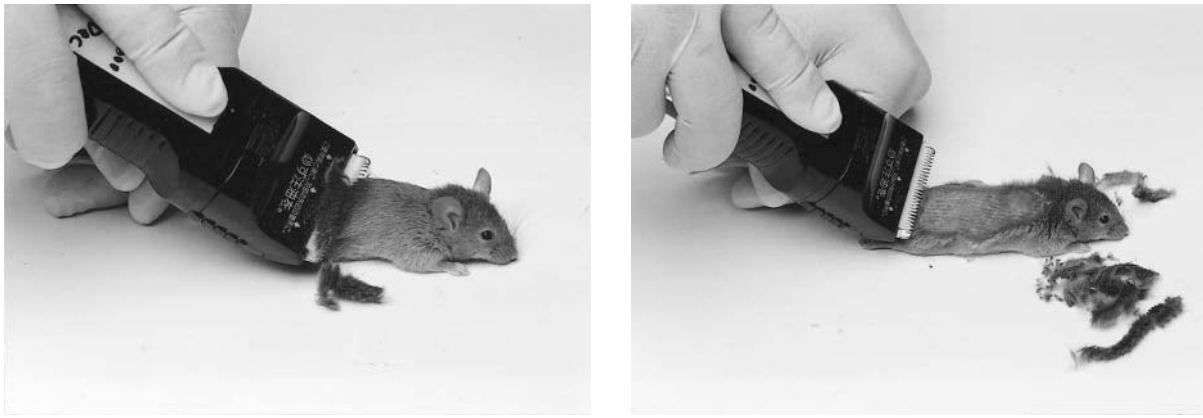

Figure 32.4 Clipping of hair on the back. Hair on the back is clipped by a cordless electric clipper.

pH of the injected solution

For most routes of administration, providing the solutions are not highly buffered, a pH range of 4.5–8.0 is satisfactory. For oral administration a pH as low as 3 can be tolerated, but alkaline solutions are very poorly tolerated. A rather wide range of pH is indicated for intravenous administration, because of the buffering effect of blood and dilution by blood flow, following use of the intramuscular and then subcutaneous routes. When low or high pH solutions are intravenously injected, the rate of injection is kept slow and again precautions taken to avoid getting solution outside the vein (Woodard, 1965).

Volume and frequency of administration

The injection volume is limited by any toxicity of the substance and by the size of the mouse. It should be kept as small as possible. Excess volumes of solution can startle the animal. The frequency of administration should be limited to a minimum, to avoid unnecessary stress. If solutions are administrated intravenously, hemodynamic changes and pulmonary oedema may occur while very rapid injections can produce cardiovascular failure and be lethal (Nebendahl, 2000). Maximum volumes are shown in Table 32.1. (Flecknell 1987; Reeves *et al.*, 1991; Wolfensohn and Lloyd, 1994). For immunization, the maximum is still lower, because of the mixing with adjuvant. Maximum volumes for injection of antigen with or without adjuvant per route are indicated in the section on Immunization of mice in this chapter.

The rate of absorption and distribution of administrated substances

The blood flow to the site of administration, the nature of the substance and its concentration influence the rate of absorption (Wolfensohn and Lloyd, 1994; Nebendahl, 2000). The time-course of the effect of the substance is an important factor in determining the dosage and is influenced by the rate of absorption (Waynforth and Flecknell, 1992). Normally, injected substances must be absorbed from the site of administration into the blood. Therefore, the rate of absorption will be determined by the size of the absorbing surface, the blood flow and the solubility of the substance in the tissue fluid. The rate of absorption is also influenced by lipid solubility, physicochemical properties, degree of ionization and molecular size of the substance (Nebendahl, 2000). Compounds, which are highly soluble in the body fluids, will be absorbed quickly. Substances that are ionized and are not lipid soluble can only be absorbed if a specific carrier exists. In general, the rate of absorption is arranged in the order iv > ip > im > sc > po (Wolfensohn and Lloyd, 1994).

Needles and syringes

Usually, 26–27-G, 1/2- to 5/8-in. (12.5–15.6-mm) needles are satisfactory for injection. The smallest gauge should be selected as a fine needle prevents leakage of fluids and will help to minimize discomfort to the animal (Nebendahl, 2000). 1–2 ml syringes size is enough for most injections. When a small volume (less than 1.0 ml) is administered, an insulin syringe plus needle is convenient (Figures 32.5 and 32.6; 27–30-G, 5/16- to 1/2-in. (8.0–12.5-mm)) these syringes can be obtained from the companies (Terumo, Tokyo, Japan; Becton Dickinson, Franklin Lakes, USA). Intradermal needles are practical for intracerebral injections (Figure 32.7; Top, Tokyo, Japan). Plastic syringes cannot be used with solvents such as acetone.

The withdrawal of hazardous substances from bottles requires great care. An alcohol-moistened cotton pledget can be kept at the point where the needle enters the stopper in order to minimize the inadvertent formation of aerosols (Silverman, 1987). Because of the risk of embolism, air bubbles in fluid and syringe and

TABLE 32.1: Guidelines for maximal administration volumes (in milliliters) and needle size

Oral	Subcutaneous	Intraperitoneal	Intravenous	Intradermal	Intramuscular	Intracerebral	Intranasal
0.2	2–3 (scruff) 0.2 (inguinal)	2–3	0.2	0.05	0.05	<0.03	<0.02
<22 G	<25 G	<23 G	<25 G	<26 G	25–27 G	<27 G	

Source: Flecknell, 1987; Reeves *et al.*, 1991; Wolfensohn and Lloyd, 1994.

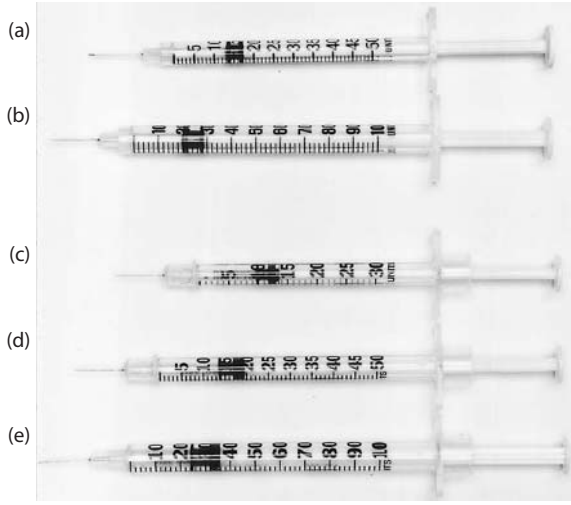

Figure 32.5 Insulin syringes. (a) 29 G × 1/2 in., 0.5 ml, Terumo; (b) 27 G × 1/2 in., 1.0 ml, Terumo; (c) 29 G × 1/2 in., 0.3 ml, Becton Dickinson; (d) 29 G × 1/2 in.,0.5 ml, Becton Dickinson; (e) 29 G × 1/2 in., 1.0 ml, Becton Dickinson.

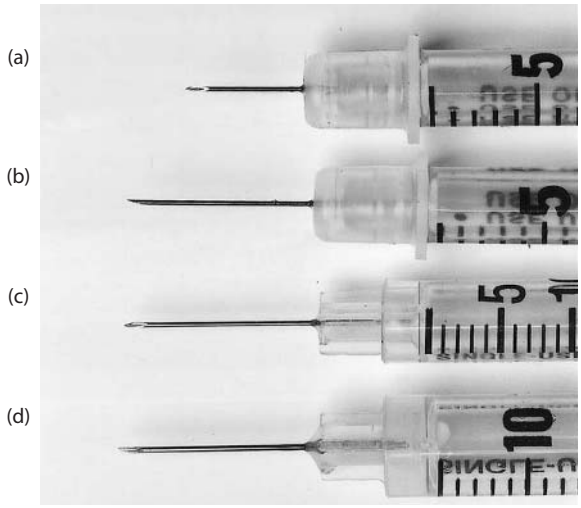

Figure 32.6 Needles for insulin syringes. (a) 30 G × 3/8 in., 0.3 ml, Becton Dickinson; (b) 29 G × 1/2 in., 0.5 ml, Becton Dickinson; (c) 29 G × 1/2 in., 0.5 ml, Terumo; (d) 27 G × 1/2 in., 1.0 ml, Terumo.

needle must be purged. Air bubbles can be purged by gently tapping the side of the syringe and slowly expelling the air into absorbent tissue to prevent any dispersion of the contents until fluid appears at the tip of the needle. The needle size will vary with the viscosity of the substance being used, the greater the viscosity, and the bigger the needle (Reeves *et al.*, 1991). If blood or body fluid flow back into the needle, it must be removed and a fresh attempt be made.

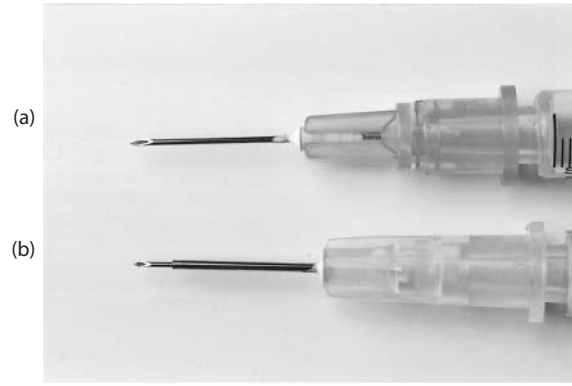

Figure 32.7 Intradermal needle. (a) 26 G × 1/2 in. needle, Terumo, Japan; (b) 1/2 in. intradermal needle, Top, Japan (tip is 27 G, base is 22 G).

Enteral administration

Enteral administration has the advantages that it is possible to give quite large amounts of non-sterile substances or solution and that a pH as low as 3 can be administrated by this route. On the other hand, alkaline solutions are very poorly tolerated by mouth (Woodard, 1965). When using the oral route it should be understood that substances can be destroyed by the gastric juices and that the food content of the stomach influences both rate and order of the gastric emptying. The rate of absorption is markedly influenced by its time of residence in the stomach and is also is directly related to the rate at which substances are passed from the stomach into the intestine (Levine, 1970). Enzymes of the host and microflora of the digestive tract can also metabolize the substance. On the other hand some insoluble substances become solubilized as the result of enzymatic activity during their passage through the stomach and intestine making absorption possible (Nebendahl, 2000). The two major methods for enteral administration are mixing the substance with food or water or direct administration using gavage. Rectal administration is also possible (Woodard, 1965).

Oral administration (per os, p.o.)

The simplest method for administration is to give the substance with food or drinking water. However, this is not practicable with those that are unpalatable, insoluble

or chemically unstable in drinking water or when they irritate the mucosa of the gastrointestinal tract (Nebendahl, 2000). The daily food and water intake of mouse should be known before the experiment, to calculate the quantity of substance to be mixed (see Part 5 on Animal Husbandry and Production).

Because food and water wastage happens all the time, it is difficult to determine the precise amount of food and water intake and therefore the precise intake of the substance. The only way this can be done is by keeping mice in metabolic cages and recording the wastage.

Intragastric administration

Direct administration by oral gavage is preferred to mixing substances with food or drinking water because the intake of the substances is precisely measured. A ball tip needle is used to prevent damaging the oesophagus and from passing through the glottal opening into the trachea (Figure 32.8). A 22 G ball tip needle is suitable for administration to adult mice and can be obtained from Popper and Sons, Inc. (New Hyde Park, USA). The conscious mouse is manually restrained firmly by gripping a fold of skin from the scruff of neck down the back (Figure 32.9a), immobilization of the head is essential for this procedure (Figure 32.9b). When the neck is extended the position is vertical. A straight line is formed between the mouth and the cardiac sphincter through the oesophageal orifice (Figure 32.9b). The needle is passed gently through the mouth and pharynx into the oesophagus (Figure 32.9c).

The mouse usually swallows as the feeding needle approaches the pharynx, these swallowing movements can help so that the probe slips through the oesophageal opening. The substance is then administered slowly. If any obstruction is felt, if the mouse coughs, chokes or begins to struggle vigorously after the gavage begins, or if fluid is seen coming out through the nose, these may indicate that the needle has entered the lungs. Any of these signs would necessitate immediate withdrawal of the needle, and the mouse must be observed very carefully. If there is any sign that fluid has got into the lungs, the mouse should be euthanized. As soon as administration is finished, the needle must be withdrawn (Cunliffe-Beamer and

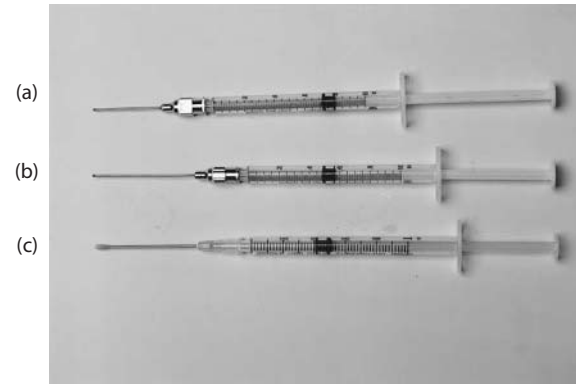

Figure 32.8 Syringes with a gavage needle. (a) 1.0 ml syringe with a 22 G × 1.0 in. feeding needle; (b) 1.0 ml syringe with a 20 G × 1½ in. feeding needle; (c) 1.0 ml syringe with a 20 G × 1½ in. disposable feeding needle.

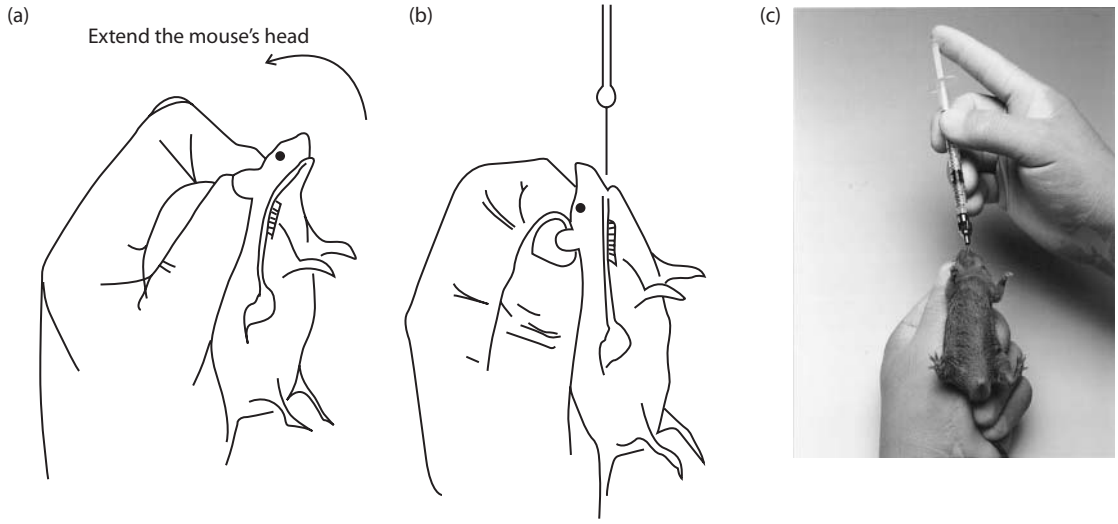

Figure 32.9 Procedure for intragastric administration using a ball tip needle. (a) First extend the neck; (b) A straight line is formed between the mouth and stomach; (c) Intragastric injection using 1.0 ml syringe with 22 G × 1.0 in. feeding needle is made.

Les, 1987; Suckow *et al.*, 2000). A volume of >2 ml is recommended.

Parenteral administration

Administration of substances other than via the alimentary canal to the body includes injection, infusion, topical application and inhalation, and implantation of an osmotic pump or a controlled-release drug delivery pellet. Small amounts of solution are injected, and large volumes are infused. In both cases the skin must be penetrated by a needle.

Subcutaneous, intraperitoneal and intravenous administration are the most common and major routes to inject substance solution or suspension in to the mouse. The rate of absorption is dependent on the route of administration. The substance will immediately disperse following intravenous injection; therefore the most rapid absorption is achieved by this route. The large surface area of the abdominal cavity and its abundant blood supply also facilitate rapid absorption, absorption from this route is usually one-half to one-quarter as rapid as that from the intravenous route (Woodard, 1965).

Subcutaneous administration (s.c.)

Subcutaneous administrations are easy. As they are rarely painful (Wolfensohn and Lloyd, 1994) a conscious mouse can usually be used. The rate of absorption is lower than from intraperitoneal or intramuscular injections (Simmons and Brick, 1970).

Subcutaneous administrations are made into the loose skin over the interscapular (Figure 32.10a) or inguinal area (Figure 32.10b). Subcutaneous administrations over the interscapular area are made as follows. The mouse is manually restrained and then placed on a clean towel or solid surface. The needle is inserted under the skin of the interscapular area tented by the thumb and forefinger and the substance then injected. A volume <3 ml is recommended. Subcutaneous administration over the inguinal area is made as follows. The mouse is restrained manually and the head tilted downwards. Holding the hind leg firmly helps this procedure (Figure 32.3). The needle is inserted into the lower left or right quadrant of abdomen avoiding the abdominal midline and the substance injected. A volume of <0.2 ml/site is recommended. To minimize leakage, the needle should be advanced several millimeters through the subcutaneous tissue (Cunliffe-Beamer and Les, 1987; Suckow *et al.*, 2000).

Intraperitoneal administration (i.p.)

This is the most common route being technically simple and easy. It allows quite long periods of absorption from the repository site. The rate of absorption by this route is usually one-half to one-fourth as rapid as from the intravenous one (Woodard, 1965). Limitations are the sensitivity of the tissue to irritating substances, less tolerance to solutions of non-physiological pH. These should be isotonic and quite large volume can be administered by this route.

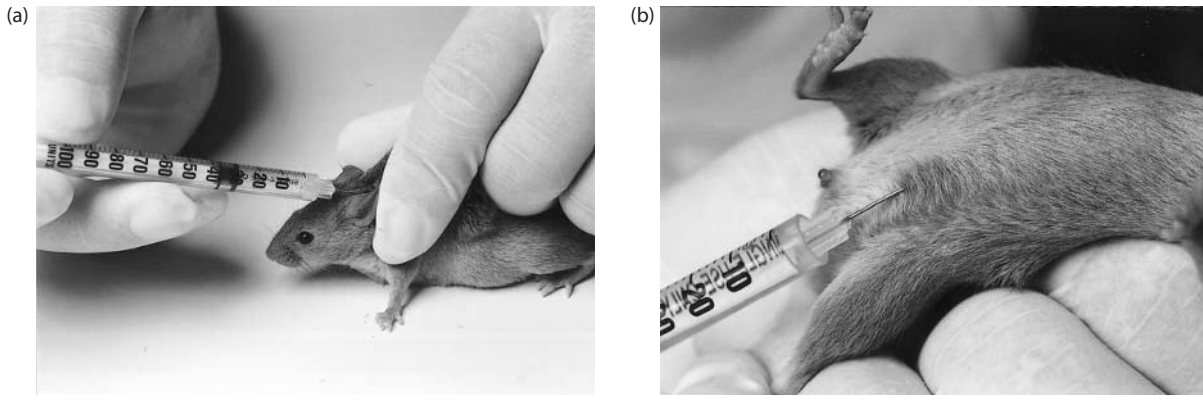

Figure 32.10 Subcutaneous injection. (a) Subcutaneous injection at the base of a fold of loose skin (area at the neck) using an Insulin syringe: 27 G × 1/2 in., 1.0 ml; (b) subcutaneous injection at the lower left quadrant using an Insulin syringe: 27 G × 1/2 in., 1.0 ml.

Figure 32.11 Intraperitoneal injection to lower left quadrant using an Insulin syringe: 27 G × 1/2 in., 1.0 ml.

The conscious mouse is manually restrained (Simmons and Brick, 1970) and is held in a supine position with its posterior end slightly elevated or the head can be tilted lower than the body (Figure 32.11). The needle and syringe should be kept almost parallel to its vertebral column in order to avoid accidental penetration of the viscera (Eldridge *et al.*, 1982). The needle is pushed in at an approximately 10° angle between the needle and the abdominal surface in the lower quadrant of the abdomen (Simmons and Brick, 1970). To avoid leakage from the puncture point, the needle is run through subcutaneous tissue in a cranial direction for 2–3 mm and then inserted through the abdominal wall (Cunliffe-Beamer and Les, 1987). The recommended volume is >3.0 ml.

Intravenous administration (i.v.)

Intravenous injection has advantages over other routes. Solutions at a high concentration, high or low pH or irritating can be administered intravenously provided that the rate of injection is kept slow and precautions are taken to avoid getting the solution outside the vein. Compounds that are poorly absorbed by the digestive tract may be given intravenously but intravenous administrations require technical expertise and skill. The syringe plus needle or the catheter must first be filled with the solution to remove air bubbles. Administrations are usually made into the lateral tail veins not into the dorsal tail vein (Figure 32.12a), as it is not straight.

The lateral veins are readily visualized, but have quite small diameters. If anaesthesia is not used, a restraining device is usually necessary (see Chapter 31 on Handling and Restraint; Reeves *et al.*, 1991; Suckow *et al.*, 2000; Weiss *et al.*, 2000).

The mouse is either placed in the restrainer or anesthetized and the tail is then warmed with a lamp or warm towel, or immersed in warm water (40–45°C) in order to dilate the vessels (Flecknell, 1987). The tail is swabbed with 70% alcohol on a gauze sponge or swab. Insert the needle parallel to the tail vein penetrating 2–4 mm into the lumen while keeping the bevel of the needle face upwards (Figure 32.12b). The solution is then injected slowly and no resistance should be felt if the solution is properly administered (Figure 32.12c). The injected solution temporarily replaces the blood but then should be washed away by the blood stream. If this does not happen the position of the needle is certainly not in the vein but in the surrounding tissue so it must be moved in the surrounding tissue in such a way that it then enters the vein or a new try must be made. When the intravenous administration is finished or the cannula is pulled out, the injection site must be pressed firmly with a swab or fingers to prevent backflow of the administered solution and/or blood (Nebendahl, 2000; Suckow *et al.*, 2000). If the same vein must be used several times the first administration should be made as distal as possible in relation to the heart and subsequent administrations should be placed progressively more proximally. Because venipuncture and the administration of substances can damage and/or block the vein, the distal part of vein may no longer be used (Nebendahl, 2000). The recommended volume is <0.2 ml.

The ophthalmic plexus route is also used for intravenous administration (Pinkerton and Webber, 1964). The technique resembles the blood collection by retro-orbital sinus puncture (see Chapter 33 on Collection of Body Fluids). The mouse is anesthetized, and then manually restrained on a solid surface being held gently but firmly by the nape of the neck. By pressing down with the thumb and forefinger in the occipital area and pulling back the skin, the point of the needle can be directed toward the back of the orbit at a 20–40° angle. The needle is inserted medially through the conjunctiva on the inner side of the ocular cavity. If entry is blocked by bone, the needle is withdrawn slightly (Figure 32.13a). Fluid is injected slowly loosening the skin slightly (Figure 32.13b). Also this route is useful for rescuing mice showing anaphylaxis by administration of an isotonic solution.

Other routes for intravenous administration via the external jugular vein (Kassel and Levitan, 1953), the dorsal metatarsal vein (Nobunaga *et al.*, 1966) and the sublingual vein (Waynforth and Parkin, 1969) have been reported.

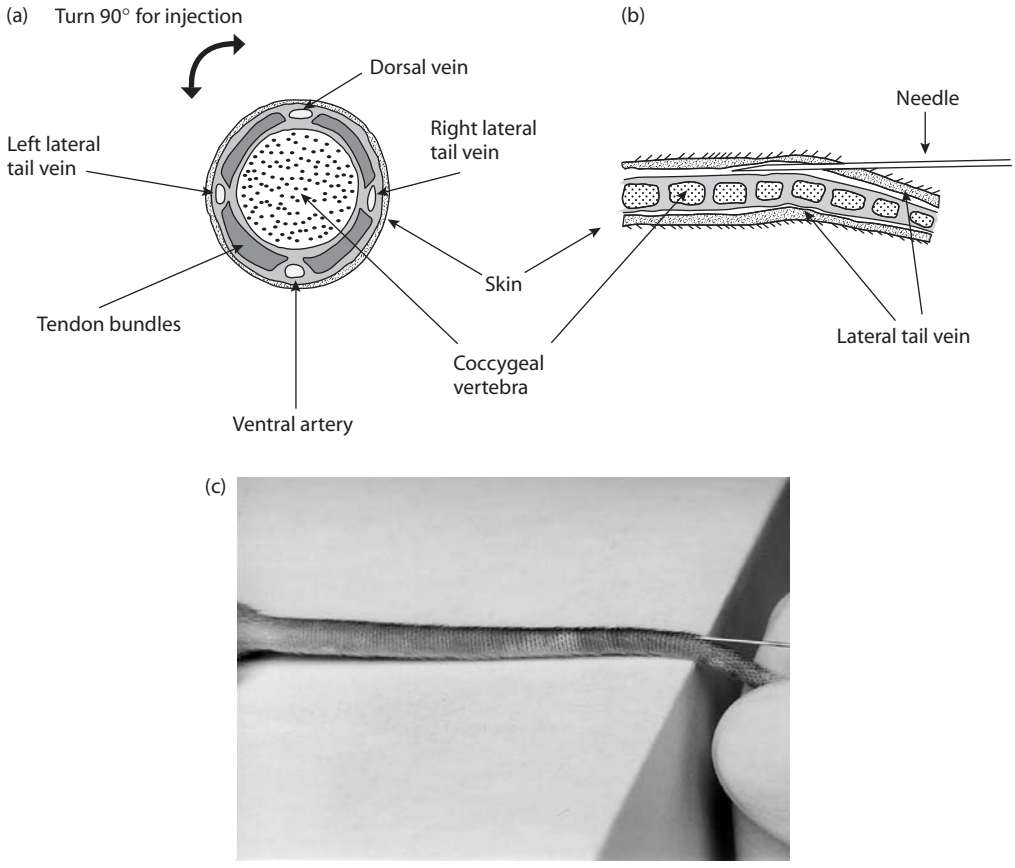

Figure 32.12 (a) Transverse section view of the mouse tail; (b) sagittal view of the mouse tail (the tail is turned 90°); (c) intravenous injection into the lateral tail vein of an anesthetized mouse using an Insulin syringe: 27 G × 1/2 in., 1.0 ml.

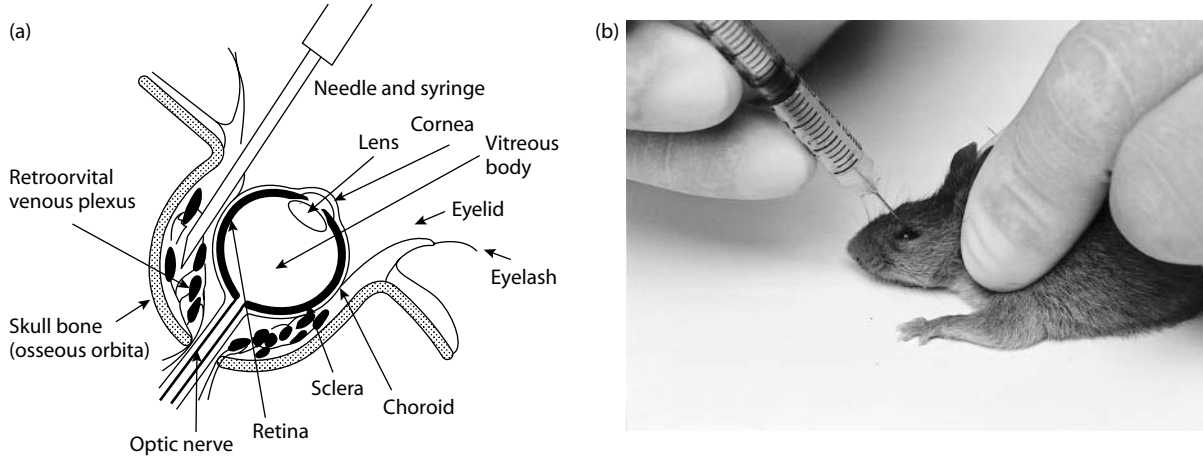

Figure 32.13 (a) Sagittal view of the mouse eyeball and retro-orbital injection; (b) intravenous injection into the retro-orbital sinus of an anesthetized mouse using an Insulin syringe: 27 G × 1/2 in., 1.0 ml.

Intramuscular administration (i.m.)

This should usually be avoided, as mouse muscles are small. If necessary, it may be given into the thigh muscle with injection volumes <0.05 ml. The tip of needle should be directed away from the femur and sciatic nerve (Figure 32.14). The mouse is anesthetized or is manually restrained by another person. The needle tip is inserted through the skin and into the muscle. Aspirate briefly with the syringe before injection. If blood or body fluid reverses, stop the procedure. The

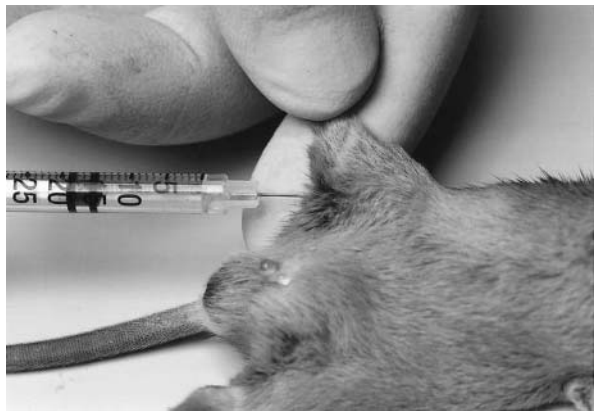

Figure 32.14 Intramuscular injection into the leg muscle.

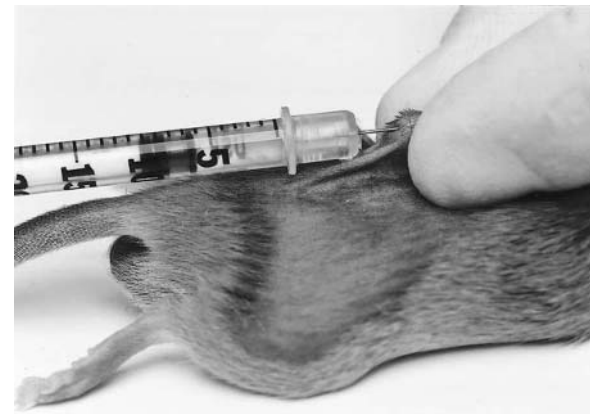

Figure 32.15 Intradermal injection into the back skin.

needle must be moved or a fresh attempt must be made. Good technique and restraint are necessary and intramuscular administration should only be performed by well-trained personnel (Woodard, 1965; Cunliffe-Beamer and Les, 1987; Donovan and Brown, 1991; Nebendahl, 2000).

Intradermal administration

This route is not recommended in general and should be restricted to cases of absolute necessity (Saloga *et al.*, 1993; CCAC, 2002). It is very difficult in the mouse due to the very thin skin. Using a fine needle (29 G or smaller) is recommended. The mouse is anesthetized, the fur clipped or hair removed from an area on the back, ventral abdomen, or hind footpad, which is wiped with 70% ethanol on a gauze sponge or swab. The skin is held tautly with thumb and index finger and the needle inserted, bevel up and at a shallow angle, just under the superficial layer of epidermis. The volume should be <0.05 ml per site. Resistance should be felt both as the needle is advanced and as the compound is injected. A hard bleb will be seen upon successful intradermal injection of even a small quantity of fluid (Figure 32.15; Suckow *et al.*, 2000). If multiple sites are injected, adequate separation is necessary to prevent coalescing of lesions.

Intracerebral administration

This is made as follows (Prier, 1966; Liu *et al.*, 1970). The mouse is anesthetized and then restrained manually on a solid surface. The site of injection is approximately half way between the eye and ear and just off the midline (Figure 32.16a). The recommended maximum volume per suckling mouse is 0.01 ml and that for weanling or older mice is up to 0.03 ml. The needle directly pierces the cranium (Figure 32.16b). An intradermal needle (Figure 32.7) is convenient in order to prevent the needle from extending too deeply into the brain.

Intrathoracic administration

Intrathoracic injection is restricted to special experiments. It can be made in mice with a slightly bent or curved needle, which should be inserted between the ribs at approximately the midpoint of the rib cage. Caution must be taken to insert it at an angle, thus preventing injection directly into lung tissue. The speed of absorption is similar to the intraperitoneal route (Simmons and Brick, 1970).

Intranasal administration (i.n.)

These are usually performed with the mouse lightly anesthetized. The mouse is manually restrained and the tail anchored between the small finger and the palm (Simmons and Brick, 1970). The mouse is held in a supine position with the head elevated. The end of the micropipette is placed at or in the external nares, and then the solution is poured in slowly (Figure 32.17; Prier, 1966; Shen *et al.*, 2000, 2001). The volume should be <0.02 ml, excess volume or rapid injection will induce suffocation and death.

Topical application

It is not often realized that the skin is the largest organ of the body and survival depends on its patency perhaps more than for most other organs. An animal or man can survive with only about one-seventh of its liver or

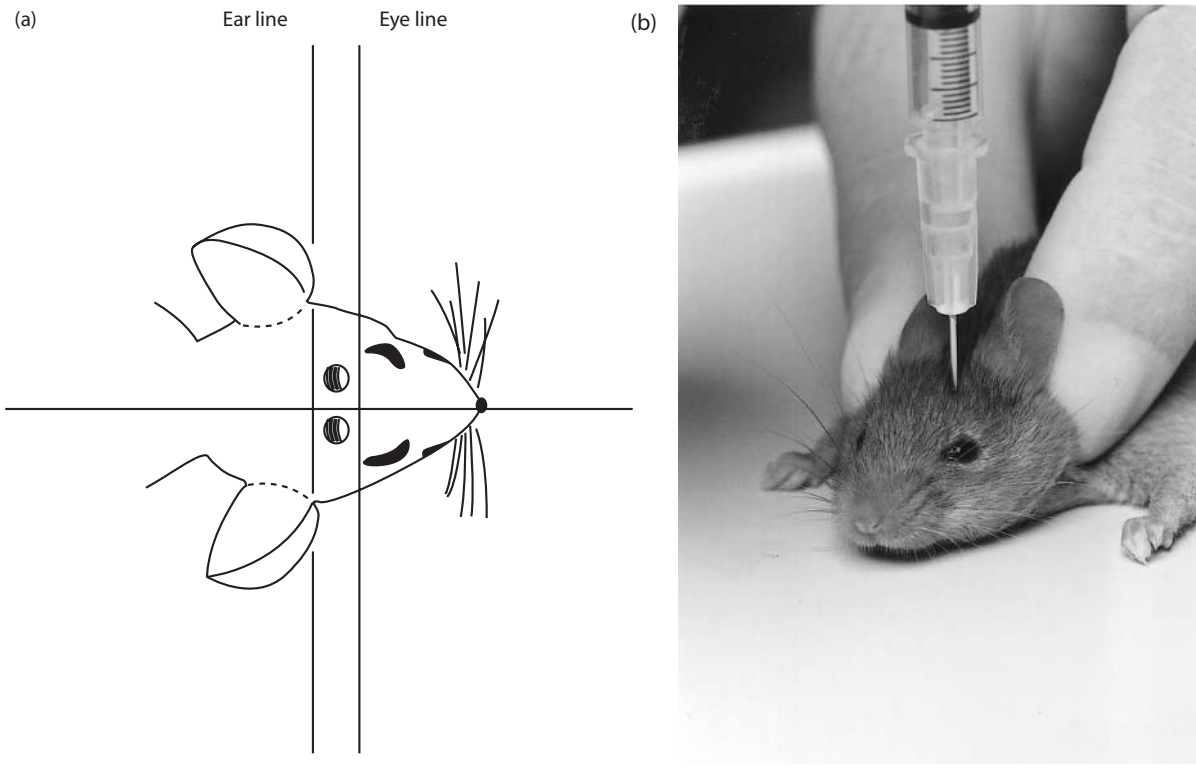

Figure 32.16 Intracerebral injection. (a) Injection site of head for intracerebral injection; (b) intracerebral injection into an anesthetized mouse using an intradermal needle.

Figure 32.17 Intranasal injection into an anesthetized mouse using a pipette (Gilson P-20).

one-fourth of its kidney functioning. In contrast, the destruction of more than 50% of the skin usually results in death (Woodard, 1965). The skin is also a convenient site for the administration of drugs. Numerous factors, such as the physicochemical properties of the substance, the attributes of the vehicle and the permeability of the skin, can affect the degree of percutaneous absorption. (Wester and Maibach, 1986; Franklin *et al.*, 1989). The ability of a substance to be absorbed through the skin and enter the systemic circulation is determined by its ability to partition into both lipid and water phases (Nebendahl, 2000).

The usual site is the skin covering the back or the abdomen. After clipping the hair for topical administration (Figure 32.4), the hairless area should be cleaned from any fat and grease and other debris. The substance should be dissolved in a volatile solvent or mixed in a suitable cream before application and then applied with a dropper or smeared onto the skin with a swab (Nebendahl, 2000). Some precautions are usually necessary to prevent the animal from licking or scratching the application sites (Woodard, 1965).

Inhalation

This route is used for experiments on asthma, air pollution or respiration (Haddad el-B *et al.*, 2002; Hopfenspirger and Agrawal, 2002). The inhalation route incidentally is the nearest akin to an intravenous injection because of the relatively large area presented for absorption by a membrane that is separated from the blood by only one or two cell layers. Consequently, absorption of gases and aerosols that reach the alveoli is virtually complete. The greatest problems surrounding the use of the inhalation route are the generation of a suitable aerosol of the test substance, if it is not sufficiently

volatile, a constant and suitable air level of the material under study and the determination of the dosage given. Both small and large particle sizes are not adequate, it is generally believed that particle size of 0.5–2.0 μm in diameter are optimum (Woodard, 1965). Equipment is available to purchase from Omuron, Kyoto, Japan; Buxco Electronics, Inc., Sharon, USA among others.

Other routes

Other routes of administration have been reported such as intra-arterial administration using the femoral artery (Simmons and Brick, 1970) or the carotid artery (Sugano and Nomura, 1963), intrathymic injection (Donovan and Brown, 1991), or intraspinal injection (Habel and Li, 1951).

Dosing and treatment of new born mice provides special problems because of their size or that their mother is apt to reject or cannibalize neonates that have been handled. Subcutaneous injections can be made over the neck and shoulders using a less than 30 G × 5/16 needle. Up to 0.1 ml (depending on the age of the infant mice) may be administered orally using a piece of plastic tubing inserted over a needle (Ujiie and Kobari, 1970; Cunliffe-Beamer and Les, 1987). The direct injection in to the stomach of infant mice can be made through the abdominal wall (Dean *et al.*, 1972). Intravenous injection of infant mice has also been reported (Anderson *et al.*, 1959; Barnes *et al.*, 1963; Cunliffe-Beamer and Les 1987).

Implantable pump, controlled-release drug delivery pellet and cannulas

The delivery of substances at a slow, steady rate over a period of days, weeks or months without the need for external connection or frequent animal handling can be supplied by using an osmotic pump or controlled-release drug delivery pellets.

Osmotic pumps, ALZET pumps (Figure 32.18), can be used for systemic administration when implanted subcutaneously or intraperitoneally or can be attached to a catheter for intravenous, intracerebral or intra-arterial infusion. The pumps have been used to target delivery to a wide variety of sites including the spinal cord, spleen, liver, organ or tissue transplants and wound healing sites. ALZET pumps are supplied by DURECT Corporation (Cupertino, USA).

Controlled-release drug delivery pellets effectively and continuously release the active product in the animal. The pellets are intended for, although not limited to, simple subcutaneous implantation in laboratory animals. The pellets are available from Innovative Research of America (Sarasota, USA) and Southern BioSystems, Inc. (Birmingham, USA).

Implantable cannulas permit continuous access to the venous or arterial system for either intravenous substance administration or blood withdrawal. Using strict aseptic techniques, the cannula is inserted into a vein or artery (the femoral vessels, jugular vein, and

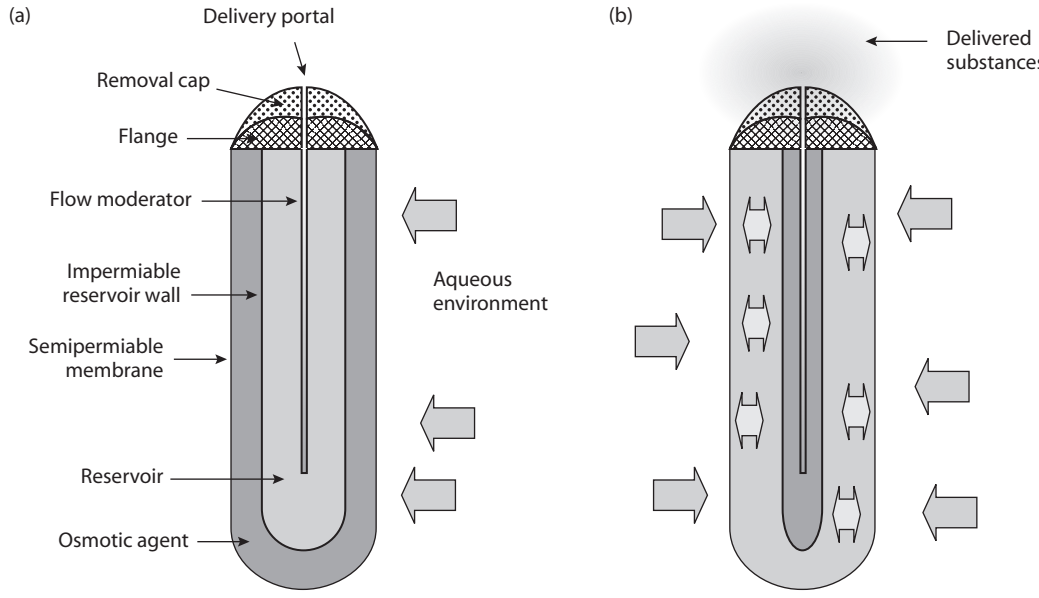

Figure 32.18 Cross section of an Alzet®-osmotic pump, demonstrating (a) the design and (b) the working method.

TABLE 32.2: Maximum volume (in milliliters) of administration of antigen with or without adjuvant per route

	Subcutaneous	Intramuscular	Intraperitoneal	Intravenous	Intradermal
with adjuvant	0.1	not recommended	0.2	no	not recommended
without adjuvant	0.5	0.05	1.0	0.2	0.05

ID route should be restricted to the cases where it is absolutely necessary (CCAC, 2002).

Source: van Zutphen *et al*., 1993; Iwarsson *et al*., 1994.

carotid artery are common sites) and secured in place. The other end of the cannula is attached to a small port that is secured in a subcutaneous location, most often over the shoulders (Suckow *et al.*, 2000). See Desjardins (1986) for more information on implantable cannulas.

Immunization

Mice are not used for the production of polyclonal antibodies because the small amounts produced. On the other hand, mice are a good source of antibody producing lymphoid cells or the production of hybridomas (Kohler and Milstein, 1975). In general, immunization consists of two stages; primary and booster. The primary antigen is usually injected with adjuvant. Boosters are injected once or more with/without adjuvant depending on the immunogen. The footpad, intrasplenic (Nilson and Larsson, 1992) or intra-lymph (Goudie *et al.*, 1966) node injection is not recommended in general. If required the scientist/investigators should provide scientific justification to ethical committees for such protocols (such as the need to use extremely unique and irreplaceable antigens, or extremely small quantities of antigen). The injection of immunogens at the base of the tail or in the popliteal area substitute for footpad injections with much less distress to the animal because immunogens injected into the footpad are processed by the popliteal node. (Leenars *et al.*, 1999; CCAC, 2002). The intraperitoneal route for injection of Freund's complete adjuvant (FCA) is permitted in small rodents only. FCA should be administered only once, and be limited to minimal volumes of up to 0.1 ml. In the mouse, up to 0.1 ml with adjuvant may be administered in the neck region subcutaneously. The injection of oil-based or viscous gel adjuvant should not be given by the intramuscular route (CCAC, 1991). The intravenous route should not be used for oil-based adjuvants, viscous gel adjuvants or large particle antigens due to the risk of pulmonary embolism (Herbert, 1978). Though FCA is the strongest adjuvant, use of other adjuvants can be recommended. Mice must be closely monitored immediately following injection for any anaphylactic reactions, both after the primary and any booster injections (CCAC, 2002). The recommended route and volumes are shown in Table 32.2. Mice showing anaphylaxis will recover by the administration of the isotonic solution by Opthalmic plexus route (see this chapter on intravenous administration).

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Additional information

Becton Dickinson, Franklin Lakes, USA: http://www.bd.com
Buxco Electronics, Inc., Sharon, USA: http://www.buxco.com/index.html