

Routes of Administration

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General

Substances such as chemical elements, compounds, drugs, antibodies, cells or other agents may be administered by different routes. Numerous routes have been well documented in the literature. As every route has both advantages as well as disadvantages and as, for instance, the absorption, bioavailability and metabolism of the substance are factors which should be considered carefully, a knowledge of available methods and techniques of administration and of the disposition and fate of the administered substance will aid the scientist in choosing the most suitable route for his/her purpose. The exact details of any administration must be checked prior to any experiment.

A complete review of all methods used for administration would go far beyond the scope of this chapter. For a full discussion of all administration routes available, the reader is referred to the periodical literature. Only some administration routes, techniques and guidelines for safe injection volumes,

sites of administration, preparation of sites, injection techniques etc. will be described here. As a number of questions frequently arise concerning the suitability of solutions for injection and precise answers are often not forthcoming (Waynforth and Flecknell, 1992), some remarks are made with respect to volume and rate of injection, absorption of the administered substance, bioavailability and distribution of substances in the body, as well as factors that may modify the dosage etc.

An introduction to this topic would be incomplete without a reference to the humane treatment of laboratory rats which are, beside mice, the most frequently used animals in experimental studies. Nearly every route of administration can be performed relatively painlessly if attention is given to the proper restraint of the rat and adequate technical skill is employed. The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purpose states that persons carrying out such procedures should be well trained in handling and restraining experimental animals, should have the foundation for responsible

use of the animals and should have a scientifically high standard, in order to protect the animals used in those procedures which may possibly cause pain, suffering or distress and to ensure that, where unavoidable, they shall be kept to a minimum. (ETS 123, 1986).

Principles of Administration

Handling and Restraining

Rats can be trained to accept handling and restraining and can become familiar with their handlers. Though time-consuming, it is essential to minimize distress. All procedures should therefore be carried out only by persons well known to the animal. Sedation or general anaesthesia is generally only required if the technique involved is more than a pinprick or the administered substances are known to cause pain etc.

Site of Administration

Numerous sites have been described in the literature for the administration of substances to rats, but some of these sites are unacceptable nowadays (e.g. foot-pad injection of Freund's Complete Adjuvant) (CCAC, 1989).

Preparation of the Site

Sometimes the area must be clipped or cleaned with warm water. Afterwards the skin should be swabbed with disinfectant or alcohol. In some cases it may be necessary to apply local analgesics to the site before administration to prevent pain.

Safety and Solubility of Substances

All parenteral administration must be done using an aseptic technique and the substances or injection solutions must be sterile and free from pyrogenous material. In order to administer an accurate dosage and to avoid causing tissue damage the toxicity of

the substance, the volume and the way of administration has to be considered. If the substance has to be diluted, the diluent selected must be safe. Physiological saline (0.9% sodium chloride) or other physiological solvents like phosphate buffered saline (PBS) or various culture media are suitable vehicles. Although distilled water can under certain conditions be used, saline should be preferred because water *ad injectionem* injected subcutaneously causes pain and intravenous injection produces haemolysis.

For reasons of solubility or rate of absorption some substances require a more complex solvent to render them suitable for administration. Many solvents, for example water, water with 0.85% sodium chloride, 60% polyethylene glycol, 10% Tween 80, 0.5% methylcellulose, have been found suitable in most instances and do not greatly affect the activity of interest of the substances to be investigated due to their own inherent properties (Woodard, 1965). All of these vehicles can be administered by any of the injection routes available, but the concentrations mentioned are the maximum practicable, and in many cases it is possible and indeed desirable that lower concentrations should be used (Waynforth and Flecknell, 1992). When administering drugs, the solvent should ideally be the same as the one in which the drug is normally formulated.

Lipid-soluble substances can only be dissolved in oil, but their absorption is delayed when administered. As oil cannot be injected intravenously, lipophilic substances must be injected in a 15% oil-water emulsion. Oil-based adjuvants or oil-water emulsions given intraperitoneally may cause acute peritonitis. They should only be administered by this mode when all other routes have proved ineffective.

Substances can be injected in the form of a suspension. Since suspended particles have the tendency to sediment, these particles should be evenly distributed before the suspension is injected intravenously. If injected intravenously it should be noted that the particles will be filtered out in the capillary beds of the extremities and the lung, modifying the distribution of the injected material and sometimes causing pulmonary distress to the animal (Waynforth and Flecknell, 1992).

pH of the Injected Solution

Rats, like humans, tolerate the injection of solutions within a fairly wide range of pH. For all routes of administration, a working range is in the range of

pH 4.5–8.0 (Woodard, 1965). The widest tolerance to pH is shown by the intravenous route because of the buffering capacity of the blood and of the very quick dilution through the side-on flow of venous blood, followed by the intramuscular and then the subcutaneous route. Nevertheless, the rate of intravenous injection must be kept slow and precautions taken to avoid irritating solutions getting outside the vein.

Volume and Frequency of Administration

Though volume and frequency of administration are mainly determined by the requirements of the experiment, the animal should not be strained or stressed. The volume of substances given is limited by their toxicity and by the size of the rat and should be as small as possible. Likewise the frequency of administration should be restricted to a minimum, to avoid unnecessary stress. The volume and rate of injection have to be considered, particularly if solutions are given intravenously, because haemodynamic changes and pulmonary oedema may occur and very rapid injections can produce cardiovascular failure and be lethal.

The Rate of Absorption and Distribution of Administered Substances

The rate of absorption is influenced by the blood flow to the site of administration, the nature of the substances and the manner and concentration in which they are presented (Wolfensohn and Lloyd, 1994). The rate influences the time-course of the effect of the substance and is an important factor in determining substance dosage (Waynforth and Flecknell, 1992). Only in a few cases is it possible to inject substances at the place where they should be effective. Normally they must be absorbed from the site of administration into the blood. Therefore, the size of the absorbing surface, the blood flow to the site of administration and the solubility of the substance in the tissue fluids are important factors that will determine the rate of absorption. Whilst endogenous compounds normally pass through biological membranes by special transport mechanisms, the penetration of xenobiotics follows physical

principles, such as passive diffusion according to the concentration gradient (Frimmer and Lämmle, 1977). Lipid solubility, physicochemical properties, degree of ionization and molecular size of substances are important factors in respect to the rate of absorption. Compounds which are highly soluble in the body fluids will be absorbed quickly. Substances which are ionized and are not lipid soluble will only be absorbed if a specific carrier exists (Wolfensohn and Lloyd, 1994). As absorption of administered substances mostly happens by diffusion down a concentration gradient, the absorption of substances is dependent on the dosage.

Enteral Administration

Enteral administration involves the introduction of substances into the gastrointestinal tract via the mouth or through the anus using a suppository. The latter method is not very practical when working with rats (Baumans *et al.*, 1993). The advantage of enteral administration is the fact that it is possible to give comparatively large amounts of nonsterile substances or solutions. For oral preparations, a pH as low as 3 can be tolerated for a solution of fairly high buffer capacity. On the other hand, alkaline solutions are very poorly tolerated by the mouth (Woodard, 1965).

In principle, gastrointestinal absorption takes place over the whole length of the digestive tract. Absorption of most orally given substances occurs by diffusion of their nonionized forms, since the mucosal lining of the gastrointestinal tract is almost impermeable to ionized molecules. Consequently, absorption of substances will be enhanced in the acid stomach or in the nearly neutral intestine depending on the ionic character of the compound. The far larger surface area of the intestinal villi, however, makes intestinal absorption dominant (Claassen, 1994a).

Buccal or sublingual administration avoids the destructive effects that may be encountered in the stomach following oral administration (Woodard, 1965). Consequently this route is sometimes required in animal experimentation. The absorption rate of substances has not been investigated to any considerable extent and is probably insignificant in rats. It is

known that the mucosa of the mouth cavity can only absorb hydrophobic, nonionized substances.

The gastric juice of rats is highly acidic (pH 2.0–4.0) (Dittmer, 1961). For substances given orally the stomach is a significant site of absorption for many acidic or neutral compounds, whereas only the weakest bases are absorbed to any appreciable extent at normal gastric pH values (Baggot, 1977). All other substances are absorbed only at a very low rate.

Using the oral administration route it should be understood that substances can be destroyed by the gastric juice, that the food content of the stomach influences both the rate and order of gastric emptying and that the rate of substance absorption is markedly influenced by its residence time in the stomach and is directly related to the rate at which substances are passed from the stomach into the intestine (Levine, 1970).

Because of its extensive surface area (total length 1020–1385 mm, average diameter 9.5–13 mm) (Hebel, 1969) and rich blood supply, the upper small intestine of the rat is the major site of absorption for all substances after oral administration. Absorption in the intestine is dependent on: (1) the physicochemical state of the substances, (2) the nonabsorptive physiological function and state of the intestine, (3) the metabolic activity and function of the absorbing cells, and (4) the structure of the absorbing surface (Levine, 1970). Through the efflux of intestinal juice, pancreatic juice and bile the intestinal content becomes nearly neutral. This will reduce the degree of ionization and the absorption rate of substances will increase. Lipid-soluble substances of the digestive content are rapidly absorbed, whereas, dependent on size, the absorption of solids is moderate.

The absorbing surface of the large intestine (length 220–270 mm, average diameter 23 mm) (Hebel, 1969) is much smaller than the surface of the small intestine; the large bowel is therefore not so important for absorption.

Using the oral administration routes it has to be kept in mind that enzymes of the microflora of the digestive tract can metabolize substances. Under physiological conditions, microorganisms are only to be found in the large intestine and normally such enzymic metabolism applies only to those substances not yet absorbed in the upper tract. This means that oral administration has the disadvantage that the enzymic activity of microorganisms may alter substances before they are absorbed in the bowel. If the metabolites are not biologically active,

the administration of these substances may be without any effect. On the other hand, some insoluble substances become soluble as the result of enzymic activity during their passage through the stomach and the small intestine and hence their absorption then becomes possible in the large intestine.

Excluding those absorbed in the mouth and rectum, all substances given and absorbed through the gastrointestinal tract are transported by the portal vein to the liver. Some substances can be metabolized there to a large extent, before reaching the systemic circulation and/or the site of action. This phenomenon is called the 'first-pass effect' and has to be considered in selecting routes of administration. Note that similar, but slightly different lengths of the small and large intestine are quoted in Chapter 15.

Oral Administration (per os)

The simplest method for administering substances is to mix them with food or drinking water. However, this is not practicable with substances which are unpalatable, insoluble or chemically unstable in drinking water or when they irritate the mucosa of the gastrointestinal tract.

Giving substances with food or drink is an easy method of administration. It is not necessary to train the rat for the procedure because no handling or restraining is required. The animal is not disturbed, the administration takes place without any stress, and eating, drinking or digestion happens under normal physiological conditions and only the substances are effective. With compounds that are rapidly metabolized, it is possible to administer in the diet quantities of some chemicals over 24 hours that would be lethal in a single intubation dosage (Woodard, 1965).

Mixing substances into food must be done carefully and decomposition must be prevented otherwise the animal might refuse to eat the substances. Some technical equipment for preparing an accurate mixture is necessary. In most cases it is best to involve a foodstuff company to produce an exact mixture pressed in pellets. When feeding pellets decomposition of the food is prevented.

The daily food and water intake of the rat must be known before starting so that the quantity of substance to be mixed with the food or drinking water can be calculated (Table 24.1). Other factors may also come into play. If, for instance, the circadian rhythm influences the effect of a substance then the feeding behaviour of the rat should be

Table 24.1 Food intake of rats and specification of common diets

	Intake (g/rat/day)	ME content of standard diets ^a (kJ ME/g diet)
Growing rat	8.0–20.0	12.6–14.6
Fully grown rat	15.0–20.0	11.7
Nursing or pregnant rat	up to 65	12.6–14.6

known. It is well known that rats eat only a small part of their food during the day itself and consume 80% of their total daily food intake at night, taking 5–8 meals during this period (Claassen, 1994a) (see also Chapter 4 about food intake).

Some substances are better absorbed when given orally on an empty stomach (Woodard, 1965). In consideration of this fact, it must be known how long the animal can be starved without any harm to its welfare. For instance, Jeffrey *et al.* (1987) observed variable amounts of food in the stomachs of male rats following an overnight fast and noted that the diet type and diet regimen can result in variable quantities of food being retained in the stomach after fasting overnight. However, Schlingmann *et al.* (1997) found that the stomach of rats fasting 6, 12 and 18 hours was almost empty and that only rats fasted for 6 hours did not show any distress. Prolonged food deprivation with water available *ad libitum* leads to a gradual increase of the haematocrit value, with a corresponding decrease in plasma volume. A decrease of the plasma and interstitial volume may result in a significant decrease of substance distribution volume (Claassen, 1994b). Therefore, the duration of food deprivation should be as short as possible.

Oral administration by diet or drinking water is not suitable when an exact amount of substance intake is required. Because food and water wastage happens all the time, it is, for instance, impossible to determine the exact amount of diet or water intake of rats. Therefore, neither the precise food or water intake nor the correct intake of substances with food or water is normally feasible. The only way this can be done is by keeping the animals in metabolic cages and recording the wastage.

The feeding or drinking intake is influenced by

the conditions of housing and especially by the environment. Normally the temperature in rooms for laboratory animals is well controlled by air conditioning. It should be noted that high temperatures will decrease the intake of food and increase the consumption of water. For instance, the water intake at 22°C is about 20% higher than the food intake, while at 30°C water intake is more than double (Weihe, 1987). For this reason the temperature and humidity must be recorded during the experiment.

The substances in food or drinking water can cause the rat to refuse to eat or drink and the animal may lose weight or become dehydrated. If the substances increase the metabolic rate, overeating must be prevented to avoid an overdose. For these reasons the animal and its food and water intake must be observed carefully.

Another disadvantage is that rats must be caged singly for observing or measuring food or water intake. This housing condition may cause stress to rats and will reduce the success of this route of administration.

Intragastric Administration by Gavage

Gavage makes it possible to administer substances directly into the stomach with accurate dosages and reliable timing. When a liquid volume is administered by gavage a substantial amount of the dose passes rapidly through the stomach to the small intestine. This occurs both in fasted and in fed animals (Claassen, 1994a) and results in the substances being absorbed very rapidly. For example, within one minute after quinine solution administration to the fasting rat, 24% is absorbed (Watanabe *et al.*,

Table 24.2 Recommended sizes of bulbs for oral dosing needle and administration volume for rats of different body weights

Body weight (g)	Diameter of bulb (mm)	Volume (mL)
30	1.0	1.0
50	1.0	2.0
100	1.5	3.0
200	2.0	4.0
300	2.0	5.0

1977). Absorption is much faster than that with dietary administration, leading to a peak rather than a slow and prolonged gradient in the blood. Using this technique there is a risk of introducing trauma or a fatal accident; only trained and experienced personnel should carry out intragastric administration. Training can be done with dead animals or with a rat model (e.g. Koken Rat, Koken Co. Ltd, Tokyo, Japan). When training with anaesthetized rats it is important to choose an anaesthetic that allows the animal to retain its swallowing reflex (e.g. ketamine/xylazine).

Knowledge of the rat's drinking or feeding habits will also help to determine whether or not a substance should be given on a full stomach or an empty one. When fed *ad libitum*, rats never have an empty stomach at any time of the day. The stomach has its maximum content at the end of the dark period and a minimum is found at the end of the light period. Therefore, only very small volumes should be administered and rats must not be starved

prior to gavage in the afternoon. In the morning, rats should not receive any food before intragastric administration, because it is not possible to determine how full the stomach is if the rats are getting food and water *ad libitum*.

Numerous methods have been devised to administer materials into or through the oral cavity and ultimately into the stomach of rodents (Kraus, 1980). Ferrill and Hill (1943) published a simplified method for feeding. Although described many years ago and only minimally modified over the years, this method for administration of liquid substances is probably even nowadays the mostly frequently used technique as it is both easy to learn and to perform.

Intragastric administration of solutions

This route of administration is carried out using a special blunt-ended, curved or straight needle with a ball tip. The ball tip helps to prevent the needle from damaging the oesophagus and from passing through the glottal opening into the trachea. The diameter of the bulb and the length of the needle will depend on the size of the rat to be gavaged (Table 24.2) Before starting administration, it is checked that the probe is of a suitable length, i.e. reaching from the mouth to the caudal end of the breastbone (outside the rat) and this should be noted on the tube (Figure 24.1). If much more than this length protrudes during administration then it indicates that the trachea has been entered and the needle must be removed for a fresh try (Waynforth and Flecknell, 1992).

The chosen needle and the appropriate syringe filled with the desired amount of solution are linked together and the needle can be moistened with water or oil to make it slippery. For administration, the conscious rat must be restrained very firmly by

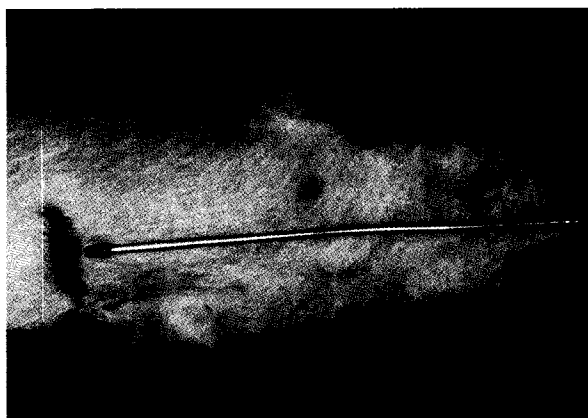


Figure 24.1 Measuring the suitable length of a probe for intragastric administration, i.e. reaching from the mouth to the end of the breastbone, and noting the length of the probe.

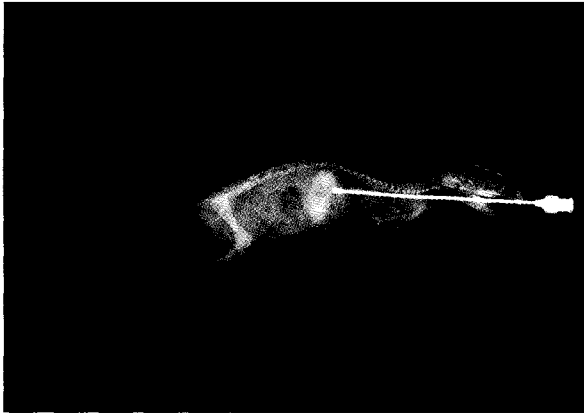


Figure 24.2 Radiograph of gavage of a rat with a straight, blunt-ended stainless steel needle. The stomach is filled with X-ray contrast medium.

gripping a fold of skin from the scruff of the neck down the back so that the head of the rat is kept immobile. Its position is vertical with the head tipped slightly forward. No mouth gag is necessary. To force the jaw open, pressure can be applied to the mandible. The ball tip is inserted behind the incisors into the back of the mouth. Using the tube as a lever, the head of the rat is tipped back. When the needle and the rat are in a straight line the probe is pushed gently along the groove of the hard palate through the very short pharynx (Hebel, 1969) into the oesophagus. Swallowing movements can help so that the probe slips through the oesophageal opening. If any obstruction is felt, no force must be exerted, but another try must be made to find the oesophageal opening (D'Amour *et al.*, 1965). As it is important to prevent the tube from entering the trachea, the rat must be watched carefully. The needle can usually be seen passing down the oesophagus on the left side of the neck (Wolfensohn and Lloyd, 1994). If it is in the trachea, the animal will cough and it is possible to feel the tube touching the cartilage rings of the trachea (Baumans *et al.*, 1993). Once in the oesophagus, the tube is gently pushed down into the stomach. The passage may be obstructed at the sphincter to the entrance of the stomach. Manipulation of the syringe to produce a gentle thrusting movement combined with a gentle backward and forward movement will often overcome this difficulty.

When the tube is in position the solution must be given slowly at first to ascertain that the needle is truly intragastric. If the rat struggles severely, coughs or the injected solution appears in the mouth or at the

nose, then the injection is being made erroneously into the lungs. If this happens administration must stop immediately, and the animal must be observed very carefully. If there is any sign of lung damage, it should be killed humanely. Once it has been ascertained that the needle is in the right position, the solution can be given fairly rapidly (Figure 24.2). As soon as administration is finished, the tube must be withdrawn. When administering a corrosive solution the needle must be flushed with saline before pulling back to make sure that the mucosa of the upper gastric tract is not irritated or even damaged.

Intragastric administration by this procedure can be performed very rapidly. One person can feed as many as 120 animals per hour after very little training (Ferrill and Hill, 1943). When properly performed, accidental tracheal infusion is rare (Kraus, 1980).

Intragastric administration of solid materials in capsules

The administration tube for capsules is very similar to the stainless steel needles used for soluble or liquid substances but has a cup for the capsule in place of the bulb. The internal diameter of the cup is in accordance with the external diameter of commercially available capsules. Further details about the needle and the gelatine miniature capsules are described by Lax *et al.* (1983). The filled capsule is placed firmly into the cup with the capsule cover facing the interior. The rat is held and the prepared tube is inserted in the same way and with the same care and caution as in the previously described intragastric method. Normally the tube is only inserted at the distal end of the oesophagus. The capsule is either ejected by air, which is forced through the cannula and the cup by depressing the plunger of the syringe, which was partly withdrawn before being attached to the tube (Waynforth and Flecknell, 1992) or by water (using 0.3 mL) (Lax *et al.*, 1983) or by pushing a steel rod with a plate on one side. The length of the rod is designed in such a way that when introduced into the tube the free end of the rod does not reach the distal end of the tube (Stanislaus *et al.*, 1979). After ejection of the capsule, the feeder is quickly pulled out. The rat's mouth may have to be held shut until it swallows. Regardless of the location of the capsule in the oesophagus or the method of ejection the capsule will reach the stomach providing normal peristaltic action occurs, and will discharge its contents within a few minutes.

Parenteral Administration

Parenteral administration involves application of a substance to the body in a manner that passes the gastrointestinal tract. Giving small amounts of solutions is called an injection, while administration of larger quantities of solutions is named an infusion. In both cases the skin must be penetrated by a needle. Other methods of parenteral administration include subcutaneous or intraperitoneal implantation of an osmotic pump and, without penetrating the skin, inhalation or topical application. Substances are transported by the blood from the site of administration to the target tissues. The rate of absorption is dependent on the route of administration.

For parenteral application all animals must be properly handled and restrained in order that injection procedures can be carried out without endangering either the rat or the operator. Various proper restraining techniques and many commercially available mechanical restraining devices have been described in the literature (see Chapter 3). The duration and extent of handling or restraining will depend, among other factors, on the method chosen for vascular access and the requirements of the experiment. Over the years, many routes and methods of administration have been used and documented in the literature. Some of the recommended techniques for injection are the same as for venous blood collection or bleeding procedures. To avoid repetition they are not described here but in Chapter 25.

Although injections should normally be given without anaesthesia, this is not a general rule. For instance, if the person carrying out the injection is not well trained, or if the substances are irritating, it is sometimes better to anaesthetize the rat with a short-acting narcotic.

When using injection or infusion techniques, several points deserve special attention. Parenteral administrations require strict asepsis to be maintained. Sharp needles of a size appropriate to the size of the rat must always be used. As a general rule, the smallest possible gauge of needle should be selected. It should be kept in mind that a thin needle prevents leakage of fluids and, as an injection may cause pain, helps to minimize discomfort to the rat. The viscosity of the solution must be considered relative to the size of needle used and the thickness of the cannula must be compatible with the viscosity

of the solution to be injected. On the other hand, using too thin needles may risk snapping the needle from the syringe. Because of the risk of embolism, air bubbles in fluids must be avoided. The injection of cold fluids is painful (Baumans *et al.*, 1993), so the fluid must be brought at least to room temperature or better still up to body temperature before use. Substances injected into the circulation must be soluble in suitable solvents. The solution should always be injected slowly and aspiration must be done to ensure that the tip of the needle is in the right place.

The physiological principles of fluid administration are not discussed here and only some recommendations are given with respect to volume.

Intradermal Injection (i.d.)

The most usual sites are the skin covering the back or the abdomen. When not using hairless or nude rats the hair must be removed. This can be done either with a depilatory or with an electric clipper or wet shaver. Depilatories are chemical substances and they could interfere with the study. Their use should therefore be considered carefully (Waynforth and Flecknell, 1992). If possible, the animal should be prepared several days in advance so as not to affect the experiment. Shaving should be done at least one day ahead because of the risk of microinjuries. Before starting the injection the site must be clean and swabbed with an antiseptic.

Only very small quantities of solutions (0.05–0.1 mL per injection site) can be deposited intradermally. For injection, a special short-bevelled hypodermic needle (Shick needle) or an ordinary 26-G needle is held bevel down almost parallel to the skin and is pierced into the skin only for 2–3 mm (Figure 24.3). An intradermal injection results in a small bleb at the injection site; no bleb is formed when the injection is done subcutaneously by mistake.

Topical Application

The skin is also a convenient site for the administration of drugs. The absorption of substances through the skin is an area of research that has been extensively studied for a number of years (Hughes and Hall, 1997). Dermal absorption represents a pathway for substances to enter the body, particularly in cases of occupational and environmental exposure.



Figure 24.3 Intradermal injection using a 26-G needle.

Numerous factors can affect the extent of percutaneous absorption of a substance. These include the physicochemical properties of the substance, the attributes of the vehicle and the permeability of the skin (Wester and Maibach, 1986; Franklin *et al.*, 1989). Important factors affecting dermal absorption are the water and lipid solubility properties of the substances used for topical application. The outermost layer of the skin is essentially a lipid barrier, whereas the viable epidermis, which lies below the stratum corneum, is basically an aqueous environment (Guy and Hadgraft, 1991). Thus, the ability of a substance to be absorbed through the skin and enter the systemic circulation is determined by the ability to partition into both lipid and water phases.

The usual sites and the preparation of the skin are the same as for intradermal injection. After clipping hair for topical administration, the hairless area should be cleaned of any fat and grease and other debris. The substances 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.

As active hair growth or the age of the skin will influence absorption of topically applied substances, the state of the hair cycle should be taken into consideration.

Subcutaneous Injection (s.c.)

In comparison with other routes of administration, the subcutaneous injection has several advantages. The subcutaneous area is well supplied with capillaries but their number may differ at various sites of injection and this may lead to differences in the rate of substance absorption. Nevertheless, as this method of administration will produce a substance depot,

subcutaneous injection is the best option when a relatively long period of absorption from a repository injection site is desired. Sensitivity of the tissues to irritant substances limits the application of this route. As stated by Woodard (1965), this route is less tolerant of nonphysiological pH and of chemical irritation than the intravenous or intramuscular routes. Substances or solutions which have such adverse characteristics should be diluted with suitable fluids before being administered subcutaneously. On the other hand, oil suspensions can be given subcutaneously. An increased rate of absorption can be achieved by the injection of hyaluronidase into the same site (Woodard, 1965).

Subcutaneous injection is the preferred method for the administration of substances into rats. This is due to the simplicity of the injection technique, greater choice of injection sites and the possibility of depositing large volumes. The maximum volume at each site should be 1 mL per 100 g body weight (Iwarsson *et al.*, 1994).

Recommended injection volumes and suggested hypodermic needle sizes for aqueous solutions are given in Table 24.3. Viscous solutions or suspensions require a needle of 2–4 wire gauge number.

In general, the dorsolateral areas of the neck and shoulder are the preferred sites (CCAC, 1980). Other recommended sites are the back or the flank. As subcutaneous injections are rarely painful (Wolfensohn and Lloyd, 1994), a conscious rat can usually be used.

A fold of loose skin is lifted between the thumb and the forefinger and at the base of the fold the needle, attached to the syringe, is passed in an anterior direction through the skin parallel to the body of the rat, to avoid penetrating deeper tissues. Ideally, the whole of the needle shaft should lie subcutaneously as this prevents leakage of the injected fluid. When in position, the tip of the needle should be moved up and down to reveal its whereabouts and also to ascertain that the needle is truly subcutaneous. If the tip cannot be discerned then the needle could be in an intraperitoneal or intramuscular position and must be slightly withdrawn to lie subcutaneously (Figure 24.4). Before injecting the substance, aspiration has to be done to ensure that the needle has not entered a blood vessel or has moved out of the skin again. When injecting a large volume subcutaneously (e.g. more than 2 mL), leakback and hence loss of fluid can be minimized further by changing the needle path after the needle has been pushed in half way (Waynforth and Flecknell, 1992).

Table 24.3 Recommended administration volumes and suggested hypodermic needle sizes

	Intravenous		Intraperitoneal		Intramuscular		Subcutaneous	
	Volume (mL)	G	Volume (mL)	G	Volume (mL/site) ^b	G	Volume (mL/site) ^b	G
Baumans <i>et al.</i> (1993)	0.5	23–25	5.0	24	0.1	25	1–5	24
Bauck and Bihun (1997)	slowly 0.5–3	≤22	max. 10	≤22	0.2–0.3	≤22	5–10	≤21
Waynforth and Flecknell (1972)	2 ^a	–	up to 10	–	0.2	–	up to 5	–
Weiss <i>et al.</i> (1996)	max. 2	–	max. 5	–	max 0.5	–	max. 5	–
Wolfensohn and Lloyd (1994)	1	25–27	5–10	23–25	0.1	25	1–2 ^b	25

^a Injected over 1–2 min.

^b Maximum of 2–4 sites.

Intramuscular Injection (i.m.)

This route usually results in more rapid absorption than from the subcutaneous route (Woodard, 1965). Absorption usually takes 45–60 minutes for most fluids. Repository forms are available which remain for days and weeks (Moreland, 1965). Small muscle masses of the rat restrict the number of practical injection sites and consequently the volume that can safely be given by the intramuscular route (Bauck and Bihun, 1997). Intramuscular injections are frequently painful due to the distension of muscle fibres which occurs, and therefore good technique and restraint are required (Wolfensohn and Lloyd, 1994).

Recommended injection volumes and suggested hypodermic needle sizes are given in Table 24.3.



Figure 24.4 Subcutaneous injection at the base of a fold of loose skin (area at the neck) using a 26-G needle.

Intramuscular injections for rats are usually given into the muscles of the thigh. Large volumes and potentially irritant compounds should be injected into the quadriceps muscle group, which covers the anterior aspect of the thigh. In rats, the quadriceps feels like a small peanut on the front of the thigh, and can be immobilized with the thumb and forefinger of one hand whilst injecting with the other (Wolfensohn and Lloyd, 1994). The muscles of the posterior thigh area should be avoided because the sciatic nerve runs along the back of the femur. Irritant substances that are inadvertently injected in close proximity to this nerve may result in lameness or in the animal's self-mutilation of the affected limb (Bauck and Bihun, 1997). Intramuscular injections can also be given into the area of the gluteal muscles of the hind leg.

Penetration by the needle of 5 mm is sufficient for a deep intramuscular injection and also avoids the risk of damaging the periosteum of the femur. It is not easy to be sure that the needle is truly intramuscular. However, it should not be possible to feel the tip of the needle through the skin if it is indeed in the muscle and not subcutaneous. Sometimes an intramuscular injection will fail, even though the needle is felt to be in the muscle mass, because it actually lies in one of the fascial planes (Waynforth and Flecknell, 1992).

Before injecting, aspiration must be done to rule out accidental injection into a blood vessel. After the injection the site should be massaged to disperse the dose (Wolfensohn and Lloyd, 1994).

Because of the difficulties described, intramuscular administration should only be used if

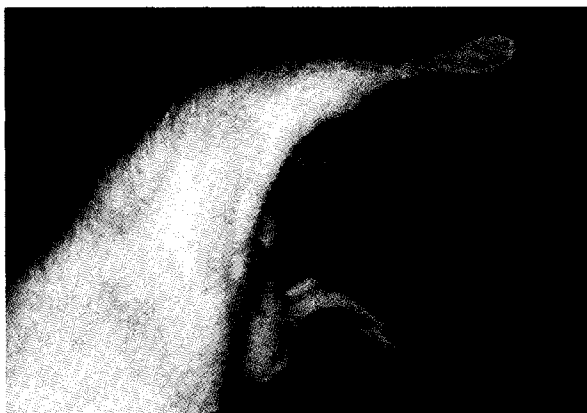


Figure 24.5 Intramuscular injection into the quadriceps muscle group using a 26-G needle.

there is no alternative and should only be performed by well-trained persons (Figure 24.5).

Intraperitoneal Injection (i.p.)

This is the most frequently used parenteral route of administration in rats. The large surface area of the abdominal cavity and its abundant blood supply facilitate rapid absorption. Absorption from this route is usually one-half to one-quarter as rapid as that from the intravenous route (Woodard, 1965). However, for long-term studies, repeated injections may lead to tissue reaction and adhesions. As relatively large volumes can be given intraperitoneally, potentially irritant substances can be generously diluted. When using this method it has to be kept in mind that substances given intraperitoneally are first absorbed into the portal circulation. Biotransformation of the injected substances may take place in the liver before they reach the general circulation, so that their bioavailability is quite different to that of an intravenous injection.

Intraperitoneal injections are generally undertaken without anaesthesia (CCAC, 1980). Recommended injection volumes and suggested hypodermic needle sizes are given in Table 24.3.

The abdomen can be divided into four quadrants by the midline and a line perpendicular to it passing through the umbilicus. Intraperitoneal injections should be given into the lower left quadrant of the abdomen (Figure 24.6). In this area of the rat there are no vital organs except for the small intestine. In contrast, the lower right quadrant contains much of the large bowel, and the upper abdomen is a hazardous area to inject because the liver, stomach and spleen are situated here.

At the start of an intraperitoneal injection, some workers tilt the rat so that the head is lower than the abdomen in an attempt to slide the viscera cranially and away from the needle. However, the viscera are quite immobile because of the slight vacuum in the abdomen and this manipulation is of questionable value (Fallon, 1996). Small rats can be properly restrained and injected by one person. When injecting larger animals, it is advisable to have an assistant. The hindquarters and tail are restrained by the assistant, and the operator extends one of the animal's hind legs and carries out the injection (Waynforth and Flecknell, 1992).

Because of the risk that the injection is made between the skin and the abdomen muscles or the risk of damage to the kidney, the needle should be inserted neither horizontally nor vertically (Baumans *et al.*, 1993). The needle should enter the skin at an angle of 20–45°. To avoid intestine or urinary bladder injection, it is essential to insert only the tip of the cannula into the peritoneal cavity. No resistance should be encountered to the passage of the needle (Wolfensohn and Lloyd, 1994).

It is often assumed that an intraperitoneal injection always delivers the substances to the peritoneal cavity. There are only a few references to incorrect intraperitoneal injections and it is probable that the error is not always noticed. A significant number of injections are actually made intragastrically, intraintestinally, subcutaneously, retroperitoneally or intracystically (Claassen, 1994c). The frequency of erroneous injections by skilled investigators has been reported to be from 11% to 20% (Lewis *et al.*, 1966). But even the well-controlled use of a standardized injection technique can only reduce the number of erroneous injections, for example as



Figure 24.6 Intraperitoneal injection into the lower left quadrant using a 26-G needle.

reported by Schneider and Schneider (1970) to 5.5%. Sometimes it is possible to recognize the error; for instance, when the injection is made into the intestine, fluid will often be seen issuing from the rectum immediately after the injection (Waynforth and Flecknell, 1992), or the rat will defecate.

For these reasons it is essential that aspiration is done before the injection to ensure that neither the intestine nor the urinary bladder nor a blood vessel has been entered.

If an injected fluid needs to be diluted quickly in the blood, then the intravenous rather than the intraperitoneal route should be given preference, thus also avoiding the risk of peritonitis.

Intravenous Injection (i.v.)

Intravenous administration offers various advantages over the other routes of injection. For example, it gives control over the rate of introduction into the general circulation, rapid response, etc. and it provides the most complete availability of substances with minimal delay. By controlling the administration rate, constant plasma concentrations can be obtained at the required level. Unexpected side-effects during administration can be halted by stopping the injection. Compounds that are poorly absorbed by the digestive tract or are unacceptably painful when given intramuscularly or subcutaneously may be administered intravenously when given carefully into the vein without leaks into the surrounding tissues.

Several general points on intravenous injection or infusion deserve special attention. Except in terminal experiments, reasonable aseptic techniques with sterile equipment must be employed, particularly when rats are being used in long-term studies and frequent injections are required. The syringe plus needle or the catheter must first be filled with the liquid so that no air bubbles are injected. When using large veins it should be easy to aspirate blood if the cannula lies correctly but it is not always possible to do so with small veins. After injecting a small amount of the solution into a small vein the injected fluid should be washed away by the blood in the vessel. If this does not happen the position of the needle is doubtful. If a bleb should arise, the position of the needle is certainly not in the vein but in the surrounding tissue. A fresh attempt must be made or the needle should be moved in the surrounding tissue in such a way that it then enters the

vein. When finishing the intravenous injection, a swab must always be pressed on to the injection site while pulling out the cannula to prevent backflow of administered fluid and/or blood.

If the vessel has to be used several times the first injection should be made as distal as possible in relation to the heart and subsequent administrations should be placed progressively more proximal. This procedure is necessary because venipuncture and the injection of substances can damage and/or block the vein and the distal part of the vessel may no longer be used for subsequent administrations.

When selecting the site of injection the consequences of a possible intravascular thrombosis, or a possible extravascular administration of substances etc. have to be considered. As injected substances can be metabolized by the liver it is important to know whether the selected vein is part of the portal or general circulation. While the intravenous route has many advantages, it is potentially the most dangerous route of substance administration, for instance with anaesthetics, and great care must be exercised in calculation of the total dose to be administered (Baggot, 1977).

The rat, although an extremely useful and widely used experimental animal, has no readily accessible veins of sufficient size for venipuncture. Therefore many methods for vascular access have been described in the literature (Moreland, 1965; Kraus, 1980; Petty, 1982; Cocchetto and Bjornsson, 1983; Waynforth and Flecknell, 1992). Percutaneous injections are made with conscious rats into the lateral tail vein, lateral marginal vein (v. saphena) or dorsal metatarsal vein, and with anaesthetized animals into the sublingual vein or penile vein. Some techniques are difficult to perform so many methods have been developed to make this task easier, including the use of a wide range of hypodermic needle sizes, improving visibility of the injection site by magnification, transillumination, shaving, surgical incision and the application of heat, tourniquets and chemicals for vasodilatation. After making a skin incision and surgical exposition administrations can be made into the external jugular vein or femoral vein. Both routes require the use of anaesthesia.

Different restraint devices and other equipment have been recommended for immobilizing the rat and thus facilitating injection (Waynforth and Flecknell, 1992). Anaesthetizing the animal is considered helpful, but may be contraindicated for some experiments. Use of these methods or equipment cannot guarantee a successful injection. For instance the

vein can be deflected by the needle, an inserted needle can dislodge or perforate the vein whenever the syringe is manipulated or the restrained rat flinches (Nachtman *et al.*, 1988).

The same vein can be used for intravenous administration or for blood collection, and intravenous injections, in general, can be performed using one of the numerous techniques as for bleeding. These are described in Chapter 25. For some routes a certain amount of technical skill is required and these are therefore not advisable for people who rarely use them.

In order to avoid pain and shock, injections must always be given slowly, especially when administering large volumes. Recommended intravenous injection volumes and the suggested hypodermic cannula size (gauges) are given in Table 24.3.

Lateral tail vein

If anaesthesia is not used, a restraint device is usually necessary because the tail is sensitive. Several types of clear plastic restraint devices that allow tail access are commercially available for rats (Waynforth and Flecknell, 1992; Fallon, 1996).

From the tip to the root of the tail the lateral vein lies immediately beneath the skin but the vein narrows from the root to the tip. Because of very small vessels at the tip of the tail the whole length of the vein cannot be used for intravenous injection. Videm (1980) presented a simple and relatively rapid method using the lateral tail vein at the root of the tail where the skin, after being shaved, is thin and smooth and where the vessels are superficial and accessible for intravenous injection.

Young rats are easier to inject into the tail vein than older ones whose tail skin is exceedingly tough and covered by scales, making it quite difficult to pierce and enter the vessels.

Since the tail of the rat is a major thermoregulatory organ with a large surface available for heat loss, an enhanced blood flow in the dilated veins and hence a successful venipuncture can be ensured by warming prior to injection. Warming the whole rat to a temperature around 40°C by placing the animal into a thermostatically warmed 'hot-box' (Conybeare *et al.*, 1988), warming the tail under a heating lamp (Waynforth and Flecknell, 1992) or holding the tail in warm water for 1–2 minutes (Fallon, 1996) can induce tail vein dilation. When placing rats in a warmed box, it is essential that the animal should be kept under constant observation

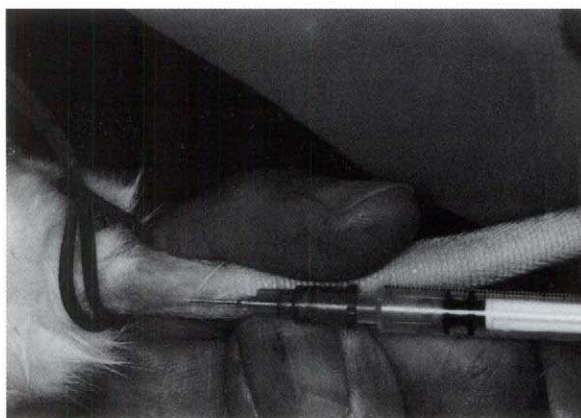


Figure 24.7 Lateral tail vein injection using a 24-G 'over the needle' catheter.

in order to prevent hyperthermia as indicated by rapid breathing, panting or salivating (Joint Working Group, 1993). Another method for obtaining good venous filling is constriction. There are several methods described using finger pressure (Barrow, 1968) and/or a tourniquet (Videm, 1980; Petty, 1982; Waynforth and Flecknell, 1992). The pressure or the tourniquet must be released just before the injection is made.

For injection, the tail should be bent down with one hand while the vein is punctured at the angle of the bend with the needle and syringe held in the other hand (Figure 24.7). The vessel must be entered at a very small angle almost parallel to the vein. After injection is started, failure of insertion is identified by swelling of the tail or blanching of the skin. A useful aid is to fit a tube between the needle and the syringe, so that the needle can be controlled better and not pulled out or allowed to penetrate through the vessel during infusions.

The injection is carried out using a 23–25-G needle.

Lateral marginal vein (saphenous vein)

In the opinion of Grice (1963) injection into this vein is the method of choice. The rat may be anaesthetized or placed in a restrainer, leaving one hind limb free. The posterior and lateral surface of the thigh and leg of the hind limb are shaved. The rat is held firmly by an assistant placing the right hand over the hips of the animal, with the free limb positioned between the first and second fingers and applying sufficient pressure to cause this vein to become quite prominent without the use of any

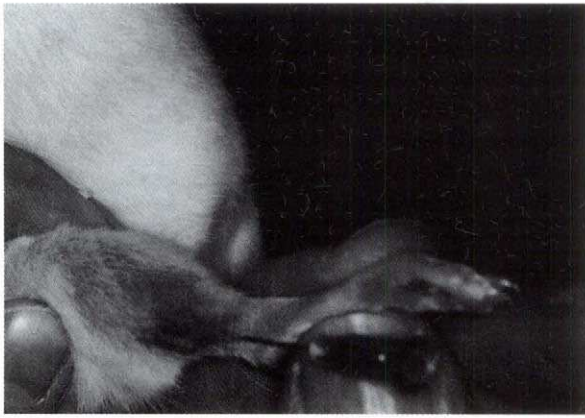


Figure 24.8 Insertion of a 26-G needle into the lateral marginal vein, and waiting for blood to ensure correct position.

form of tourniquet (Figure 24.8). The assistant's left hand holds the foot firmly (Pearce, 1957).

Pearce (1957) recommended a 26-G or 27-G hypodermic needle for injection.

Dorsal metatarsal vein (small saphenous vein)

Sawyer and Everett (1956) first described this injection technique (Figure 24.9). The vein is found on the dorsal surface of the foot. The skin over the lateral plantar surface is shaved and swabbed with antiseptic. The operator holds the foot by the toes during the procedure, and an assistant aids by holding the knee in such a manner that sudden flexion and withdrawal of the foot are prevented. A 26-G hypodermic needle kept almost horizontal to the surface and directed toward the ankle is inserted through the skin and into the vein at a point where it just starts to travel up the foot after first crossing the foot and supplying the toes with blood.

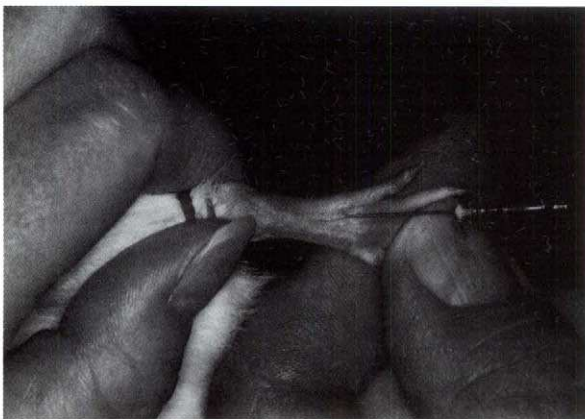


Figure 24.9 Dorsal metatarsal vein injection using a 26-G needle.

Sawyer and Everett (1956) recommended a 26-G hypodermic needle for injection.

Dorsal penis vein

Petty (1982) maintained that the dorsal penis vein injection for male rats is much simpler, more rapid, more reproducible and easier to accomplish than tail vein injection and the technique can be learned easily. Waynforth and Flecknell (1992), who described the method in detail, stated that this route should only be used under special circumstances, because of the consequences of damage to the vein.

Nightingale and Mouravieff (1973) investigated whether the penile vein is part of the portal or general circulation. Based on these experiments, they concluded that injection into this vein leads to the general circulation and that a first-pass effect on metabolism is not to be expected.

The rat is either restrained by an assistant or may be anaesthetized by a short-acting narcotic agent and its penis extruded by sliding the prepuce downwards while pressing at the base of the penis (Figure 24.10). The glans penis is held at the very tip. The large penile veins are seen along both sides of the penis. Once the vein is pierced, aspiration of blood is virtually impossible, therefore only a very small volume has to be injected first to see if it flows freely. After the injection, the injection site is pressed with a swab for a few seconds and the gland is encouraged to retract to prevent further bleeding.

For rats from weaning age and older, Waynforth and Flecknell, (1992) recommended a 24-G hypodermic needle and 30-G for smaller animals.

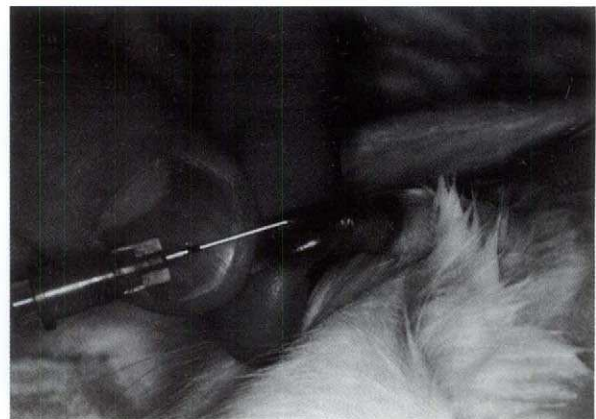


Figure 24.10 Dorsal penis vein injection using a 26-G needle.

Sublingual vein

As the entire procedure, namely, anaesthesia, suture and injection, can be done in less than 5 minutes, Petty (1982) recommended the sublingual vein which is frequently overlooked as an injection site (Figure 24.11). Any differences in the eating or drinking habits of the rat were noted after injection. As the injection technique is described in detail by Petty (1982) or Waynforth and Flecknell (1992) the reader is referred to these articles.

Waynforth and Flecknell (1992) recommended a 25-G or 30-G hypodermic needle for injection.

Without special experience, access to the jugular vein or the femoral vein is only possible after surgical exposure of these vessels. As injection via a needle and infusion via a catheter require similar preparation, more details in respect to these injection techniques are given later.

Intravenous Injection or Infusion by Catheter

Chronic venous cannulation or implantation of indwelling vascular catheters in rats are an accepted and extremely useful experimental technique for repeated injection or permanent infusion, since they reduce the stress of multiple injection associated with, for example, restraint and discomfort of repeated needle pricks. A number of authors have described techniques for chronic catheterization of different blood vessels of the rat. Each technique has its own individuality and the investigator must adapt to meet the needs of the experimental design (Petty, 1982). The procedures described differ also in the different protection devices employed to prevent the rat from manipulating the catheter through pushing, pulling away or biting, in the methods of maintaining proper catheter placement and of exteriorizing the catheter, in the use of different surgical techniques and in the techniques used to maintain the patency of the catheter.

The procedures used to fit rats with an indwelling vascular catheter fall into two general categories using either direct access or remote access.

Direct access is accomplished by attaching a syringe or a piece of tubing to the exteriorized distal end of the catheter just before injection or infusion. This requires some form of handling or restraint of the rat. The duration and extent of these

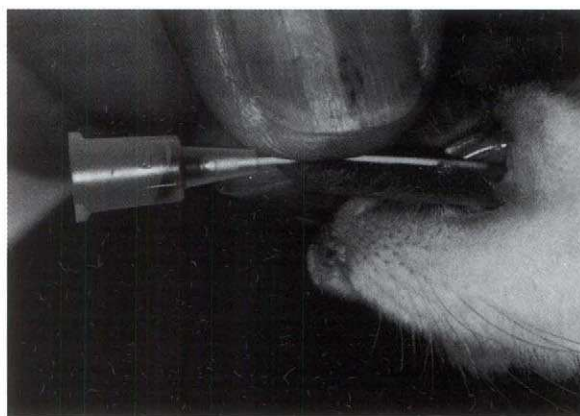


Figure 24.11 Sublingual vein injection using a 24-G 'over the needle' catheter.

manipulations will, of course, depend on the method chosen for implantation, on the type of catheter, on the requirement of the study and in particular on the experience of the operator.

Remote access involves tethering the rat, usually by a protecting device, as the catheter is extended beyond the rat's home cage. This method permits access to the vascular system of otherwise undisturbed, freely moving rats housed singly.

Tubing for catheters is available with various internal and external diameters. Advances in the polymer industry have led to the production of many synthetic materials with acceptable indices of biocompatibility. Vascular acceptance of extracorporeal devices is far more difficult to achieve than with other tissues *in vivo*. Two fundamental properties of the vascular system cause problems when foreign materials are placed in the bloodstream. First, rejection of intravascular implants is rapid due to the immediacy of thrombogenic reactions. Second, the surface properties of synthetic polymers differ from those of blood vessels, a feature favouring the accumulation of platelets and other thrombogenic agents. Hence, the physicochemical properties of intravascular materials are important factors influencing the long-term success of vascular implants (Desjardins, 1986). Intravascular catheters differ with respect to physical properties and biocompatibility and are available in a wide variety of synthetic materials. Silicon rubber (Silastic) tubing is adequate for many catheters used for rats, since it seems to cause little reaction, even after 18 months, whereas nonsilicon rubber materials tend to cause fibrotic reactions over time. The problem with Silastic is that it is too flexible and, therefore, predisposed to kinking, especially in smaller tubes (Joint Working Group, 1993).

Various methods have been used to minimize the incidence of thrombotic occlusion of the intravascular catheter and to remove existing thrombotic obstructions to prolong the patent lifetime of catheters. Schedules for routine catheter care of rats have not been compared or standardized. To prevent clotting in the catheter, it should be flushed with heparinized saline or another anticoagulant after placement and between infusions or injections at least twice a week, if not daily. The dead space in the cannula is then replaced by a carefully calculated amount of fresh anticoagulant. A concentration of 10–1000 IU heparin per mL saline is recommended (Joint Working Group, 1993). If the catheter still becomes blocked, it may be possible to dissolve the thrombotic occlusion by filling the catheter with a solution of urokinase or streptokinase (Hurtubise *et al.*, 1980). Cannulas without heparin form small clots at the tip, which, if dislodged, may cause pulmonary, renal or heart infarcts.

Numerous procedures for the insertion of catheters into different arteries and veins of rats by percutaneous or surgical techniques have been described.

Percutaneous insertion

Percutaneous methods involve the implantation of the catheter into a blood vessel by piercing the vessel with a needle and pushing the catheter through the needle into the vessel.

In a nonsurgical approach, catheters are inserted into the tail vein of the rat. For instance, Little *et al.* (1962) and Rhodes and Patterson (1972) inserted the needle through the intact skin into the tail vein. When blood flowed freely, the catheter was guided through the trough of the needle into the vein. Nachtman *et al.* (1988) and Waynforth and Flecknell (1992) used a commercially available over-the-needle catheter, comprising a short cannula fitted over the needle which is only a few millimetres longer than the cannula. This unit has two distinct advantages over hypodermic needles. It provides a visual check that the vein has been entered as blood fills the needle chamber, and once the cannula is established intravenously, any movement by the rat or the operator does not lead to penetration or laceration of the vessel wall as easily as a hypodermic needle because its cannula is pliable and blunt. After positioning the tip of the needle and cannula into the vein, the needle is withdrawn whilst holding the

cannula firmly in place. Once the cannula has been filled with blood, the needle can be withdrawn completely.

Surgical implantation

Detailed procedures for the preparation of catheter equipment and inserting the catheter have been described by Weeks and Davis (1964) and Harms and Ojeda (1974), who described an easy-to-prepare cannula and a simple procedure for cannulation of the jugular vein.

Numerous methods are available for implantation of catheters in different veins and/or arteries of rats (Petty, 1982). Cocchetto and Bjornsson (1983) reviewed many articles on arterial and venous implantations, gave methodological notes as well as procedural comments. The tail vein, right jugular vein and femoral vein or the left carotid artery and the aorta are mostly used for cannulation. Surgical techniques for the permanent catheterization of the jugular vein (Remie *et al.*, 1990a), femoral vein (D'Amour *et al.*, 1965), tail vein (Born and Moller, 1974), carotid artery (Waynforth and Flecknell, 1992) and femoral artery (Yoburn *et al.*, 1984) are comprehensively described in the literature. The reader is referred to these articles.

As it is not easy to get a tube into a surgically exposed, small vessel of a rat, a small vessel cannulator is sometimes recommended (Pope, 1968; Rezek and Havlicek, 1975). There are various techniques for fixing the extravascular or intravascular portion of a catheter with the vessel and the surrounding tissue or within the lumen of the blood vessel. Details can be found in the article by Cocchetto and Bjornsson (1983). With rats, intravascular catheters are commonly exteriorized by subcutaneous tunnelling from the vascular incision site to the back of the neck or between the shoulder blades (Cocchetto and Bjornsson, 1983), and sometimes also to the root of the tail (Jones and Hynd, 1981).

The various procedures for protecting the catheter differ in the protective device used to prevent the animal from manipulating the free end of the catheter and/or the connection to the infusion pump. Many authors (Kleinman *et al.*, 1965; Dalton *et al.*, 1969; Edmonds and Thompson, 1970; Cox and Beazley, 1975; Rhodes and Patterson, 1979; Jones and Hynd, 1981; Kanz *et al.*, 1989) have addressed the problem of the protection of the catheter from kinking and chewing by the animal.

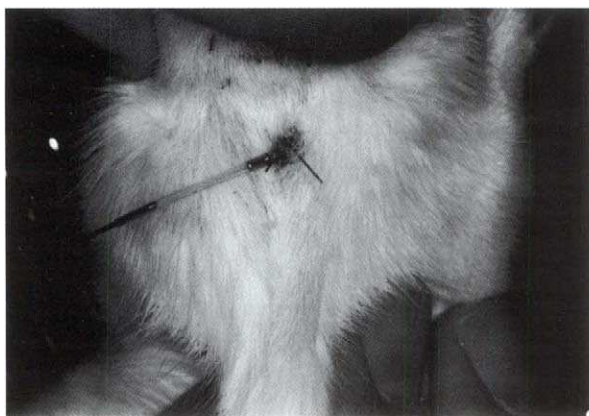


Figure 24.12 Free end of a jugular catheter exteriorized by subcutaneous tunnelling from the vascular incision site to the back of the neck. Catheter is sealed with a steel rod.

A number of authors (Little *et al.*, 1962; Popovic and Popovic, 1960; Engberg, 1969) have described techniques for proper catheter placement. Other writers (e.g. Wittgenstein and Rowe, 1965) have directed their attention towards minimal restraint or towards methods of compensation for rotational movement of the animal in the cage (Eve and Robinson, 1963).

If catheters are implanted for continuous infusion over long periods and the use of a portable pump is not practical, the tube must be connected with an infusion pump. Therefore, it is necessary to take special precautions to prevent twisting and kinking of the delivery tubing through movements of the rat. The freedom of the animal must be inhibited as little as possible by the protective device and must allow the animal to move in an unrestricted manner within its cage. If the implanted tube is to be used for multiple injection the free end of the tube must also be protected from biting or pulling out. For this purpose the same protective device as for long-term infusion can be used. If the catheter is exteriorized in the neck area and only a short piece is outside the body, it is not necessary to protect the catheter if the animal is housed singly (Figure 24.12).

Rats are not only stressed by the operative procedure but also by the fact that the animal has to wear a protecting device, is housed singly and is limited in movement (Birkhahn *et al.*, 1976; O'Neill and Kaufman, 1990). In general, rats require a period of several days to recover from the procedures for the implantation of chronic catheters. During the first 4 postoperative days the normal weight gain is disturbed and even weight loss can occur (Popovic and Popovic, 1960; Kleinman *et al.*, 1965; Claassen, 1994d).

As a general rule, infusion of about 1% of the blood volume per hour will not affect fluid disposition. Infusion of larger volumes should be based on preliminary studies designed to determine whether the cellular and ionic components of blood are maintained in a normal range (Desjardins, 1986). For example, for long-term feeding of rats (140–250 g) Steiger *et al.* (1972) infused a specially formulated solution intravenously at a rate of 30–60 mL/day. However, at low flow rates of less than 0.5 mL per hour, catheter occlusion by thrombosis may be observed (Cox and Beazley, 1975).

Other Methods for Parenteral Administration

Osmotic Minipump

Commercially available implantable osmotic minipumps are designed to deliver microlitre quantities of semisolid or liquid formulations of substances as a continuous infusion of precise volumes for a period of up to 6 weeks without the need for external connection or frequent animal handling (Waynforth and Flecknell, 1992). This small device has been used very successfully in laboratory rats and 3315 reports about the use of osmotic minipumps can be ordered from the Alzet[®] bibliography (ALZA Corp., Palo Alto, CA, USA).

The minipump system is composed of three concentric layers: the substance reservoir, the osmotic sleeve and the rate-controlling, semipermeable membrane. An additional component, called the 'flow moderator', is a 21-G stainless steel tube with a plastic end-cup (Figure 24.13). For more details and principle of operation see the technical information manual of the manufacturer.

Eleven different pumps are available, varying in size from 1.5 × 0.6 cm to 5.1 × 1.4 cm, with a nominal pumping rate from 0.25 µL/h to 10.0 µL/h, a nominal duration from 1 day to 6 weeks and a nominal reservoir volume from 100 µL to 2 mL (Figure 24.14).

Because of the mechanism by which the pumps operate, their delivery profile is independent of the chemical and physical properties of the agent

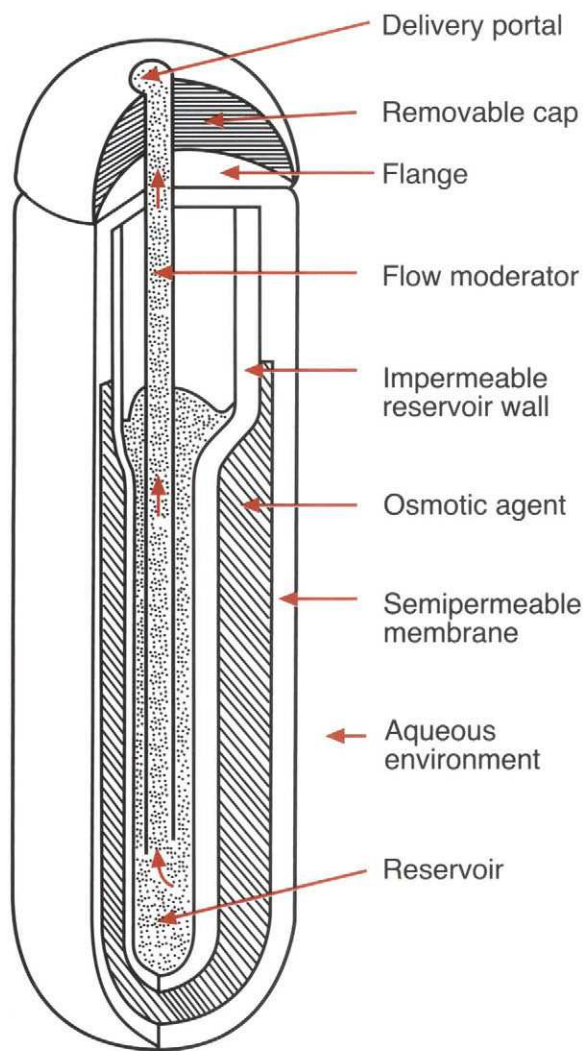


Figure 24.13 Cross-section of an Alzet[®] osmotic pump, demonstrating design components, working method and nominal performance.

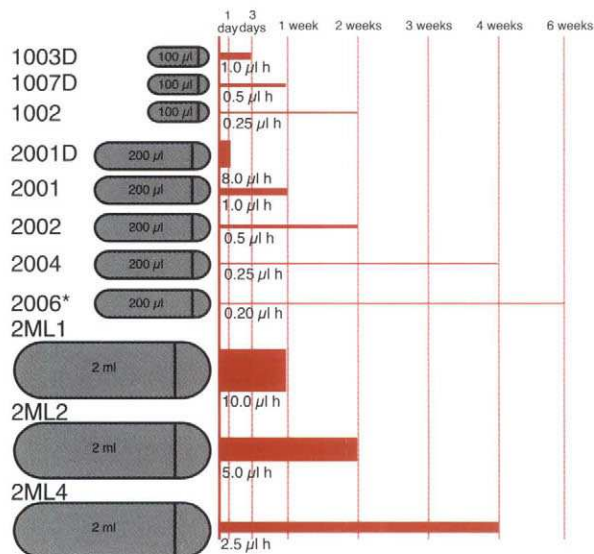


Figure 24.14 Available Alzet[®] osmotic pump models.

dispensed. Substances of various molecular configuration, including ionized substances and macromolecules, can be dispensed continuously in a variety of vehicles at constant rates. The average pumping rate should be calibrated and correct performance should be checked before implantation. In rats, pumps can be implanted subcutaneously or intraperitoneally following the animal size guidelines of the manufacturer. A kit for performing brain infusion is also available. As the attachment to a catheter does not alter the delivery rate of the pump, infusion into the venous or arterial circulation via a catheter is also possible. Full instructions for the correct use of the minipumps are given by the manufacturer.

The compelling advantage of these minipumps is that they can be placed *in situ* without further need for infusion equipment and that a large number of animals can be treated effectively with a uniform infusion rate. However, infusion is limited to specific volumes for restricted time periods (Desjardins, 1965). This disadvantage can perhaps be solved by implanting a new pump.

Oro-endotracheal Intubation

With the expanding use of rats in research involving surgery of increasing complexity, there is an increasing need for improvements in rat anaesthesia techniques. A free airway is useful in reducing mortality during and after operations. Inhalation anaesthetics provide excellent control over induction and maintenance of anaesthesia but are difficult to use because of the mode of delivery and miniaturizing the standard anaesthetic protocol. Numerous reports (Dudley *et al.*, 1975; McGarrick and Thexton, 1979; Levy *et al.*, 1980) using a mask for administration of volatile anaesthetics in spontaneously breathing rats have been published. However there is the problem of adequately fitting masks, lack of control of ventilation, waste of anaesthetic gases and hazardous pollution of the operating room. Tracheostomy is unacceptable for the recovery of the rat. Therefore, intubation has to be selected for further consideration. Acceptable techniques for endotracheal intubation of the rat are reported including blind intubation (Stark *et al.*, 1981), laryngoscopic techniques with specially designed (Proctor and Fernando, 1973; Nicholson and Kinkead, 1982; Costa *et al.*, 1986) or human laryngoscopes (Schaefer *et al.*, 1984) and direct tracheal visualization by a fibre-optic illuminator (Thet, 1983), a surgical microscope

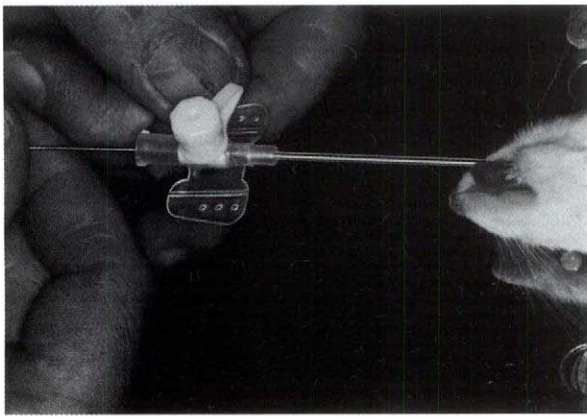


Figure 24.15 Insertion of an endotracheal tube ('over the needle' catheter) into the trachea using the Seldinger technique.

(Peña and Cabrera, 1980) or a head-mounted, mirror-reflected, adjustable-focus light (Alpert *et al.*, 1982).

For starting the intubation, an endotracheal cannula of suitable size (internal diameter of the trachea 2–3 mm) must be prepared and a careful examination of the pharynx and larynx carried out. The use of an endotracheal tube with an inflatable cuff is impossible because the lumen/cuff ratio is unrealistic and the deflated cuff makes insertion extremely difficult (Proctor and Fernando, 1973). For intubation a 16- or 12-G arterial cannula can be used. Some authors recommend modification of the introductory end of the tube to facilitate penetration of the tracheal lumen or to prevent unintentional intubation of a bronchus and the Luer fitting to provide connection to an anaesthetic circulation (Proctor and Fernando, 1973; Costa *et al.*, 1986; Flecknell, 1996).

Intubation of rats is possible using a purpose-made laryngoscope (Costa *et al.*, 1986) or an otoscope (Tran and Lawson, 1986; Remie *et al.*, 1990b). Prior to intubation, the animal is anaesthetized to a sufficient depth to abolish the cough and swallowing reflex. Atropine (0.01 mg) is useful in reducing mucus secretion and helps to prevent tube blockage. Laryngospasm may be prevented or alleviated by spraying a local anaesthetic solution on the cannula or on to the vocal cords before intubation. If the laryngeal region is covered with mucus, the area must be cleared with a cotton-tipped applicator to allow visualization. The technique of intubation using a laryngoscope and insertion of the tracheal tube between the vocal cords during the next inspiration into the lumen of the trachea is well described by Costa *et al.* (1986). By adopting the Seldinger vessel cannulation technique for intubation, Proctor and

Fernando (1973) inserted under direct vision a guidewire through the cords first, passed an endotracheal cannula over the wire and between the cords into the trachea without difficulty and then removed the wire (Figure 24.15). A guidewire from a Seldinger catheter is ideal as its tip is soft and flexible. Both techniques are also described in detail by Flecknell (1996).

After connecting the tube to the ventilator the rat must be ventilated and chest movement and air exchange must be checked for correct cannula placement. If the tube becomes plugged with mucus secretion during ventilation and causes respiratory difficulties, the cannula must be exchanged with a new one.

As some experimental protocols require that substances be given intratracheally, intubation techniques can also be used for these studies.

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