

# HANDBOOK OF PRE- CLINICAL CONTINUOUS INTRAVENOUS INFUSION

## Chapter 21 – The Use of Mini-Osmotic Pumps in Continuous Infusion Studies

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## **Chapter 21: The Use of Mini-Osmotic Pumps in Continuous Infusion Studies**

Lorri Perkins, BA, MBA, Clarisa Peer, BS, MSN, Peg Murphy-Hackley, BS, MS, DVM, ALZA Corporation.

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### **Introduction: The Advantage of Using ALZET® Pumps for Continuous Infusion Studies**

When evaluating a compound for clinical development, it is essential to obtain predictive information on efficacy and toxicity. While bolus injections can generate usable data for some drugs, continuous infusion is critical for others. After injection, plasma levels rise markedly, only to fall, more or less, very quickly. This allows the possibility that drug levels will become negligible in the intervals between injections. Fluctuating plasma levels, which depend on the half-life of the agent relative to the length of the intervals between injections, can suggest intermittent efficacy, and high peaks in concentration may trigger unwanted side effects. Continuous drug exposure can obviate these problems. Additional key information on pharmacodynamics and pharmacokinetics can be obtained by comparing differences between the infusion and injection of a drug. Also, data from continuous infusion experiments can be used to hone drug selectivity, and may be helpful when choosing the mode of administration and dosing frequency in a clinical setting.

Compounds with short half-lives *in vivo* are notoriously difficult to evaluate, in that drug presence may not be maintained for a period long enough to assess efficacy. Differences in half-life between species can also create marked discrepancies between data generated from animals and humans. Small animals frequently metabolize and clear drugs very quickly, as their metabolism is accelerated relative to humans. If a drug with likely clinical value has a short half-life in the chosen animal model, and it is administered by injection, it may produce disappointing results. Continued drug presence in an animal model can help to diminish these complications by allowing the generation of key information regarding the full pharmacodynamic profile of a drug. Altering the route of delivery or schedule of administration can widen the therapeutic index of a drug, painting a more definitive picture of its clinical efficacy.

Osmotic pumps have been used to deliver agents continuously, as cited in over 6,000 published research papers (references from which can be found in the bibliography section of [www.alzet.com](http://www.alzet.com)). These references include articles on many candidates for clinical development, in addition to citations on proteins and peptides such as growth factors, cytokines, and hormones.

The pumps are powered by the osmotic difference between the pump and the body fluid of an animal, and thus require no external power source.

The ability to implant these pumps under the skin with or without a catheter connection can minimize the chance of animal interference and infection, and allows unrestrained movement. The stress that an animal undergoes when subjected to repeated injections or connected to an external infusion pump can be avoided when osmotic pumps are used.

ALZET pumps can be implanted subcutaneously or intraperitoneally, or used with a catheter to infuse a vein, artery, or other target tissue such as the brain. The ability to target flow from the osmotic pump has allowed researchers to localize drug delivery, avoiding potentially high levels of systemic toxicity or untoward side effects.

For many compounds, pharmacokinetics are very similar for agents administered continuously via intravenous or subcutaneous infusion. Subcutaneous pump implantation is quicker and less stressful to the animal than intravenous catheterization, and obviates potential problems with preservation of catheter and vein patency. Additionally, osmotic pumps provide long-term infusion for up to 28 days (or longer if implanted serially), which can contribute to a more complete pharmacokinetic profile than data obtained from a short infusion period.

This chapter explores many key uses of osmotic pumps in toxicology and preclinical experiments. After initial summaries of the principle of operation and range of products available, techniques for optimizing the drug administration regimen will be discussed. Applications follow, which are related to intravenous and subcutaneous infusion studies for toxicologic and pharmacologic purposes, teratology, and cell proliferation. The chapter ends with an appendix describing very specific surgical and animal care techniques related to pump implantation.

## **Principle of Operation**

ALZET osmotic pumps consist of three concentric cylinders. The outermost layer is a semipermeable membrane constructed of cellulose materials. Within this cylinder is a supersaturated salt solution, which surrounds the innermost impermeable drug reservoir. (Please see Figure 21.1). The pump is filled by introducing a 25 or 27 gauge filling tube connected to a drug-filled syringe into this reservoir, and the drug is delivered from the same port.

When implanted, interstitial fluid enters the pump via the semipermeable membrane because of the osmotic difference between this fluid and the salt solution in the pump. This fluid causes expansion of the salt layer, which compresses the flexible drug reservoir, and forces solution out the delivery portal. Since the outer membrane is rigid and cannot expand, the rate at which fluid enters the pump is the same as the rate the solution is delivered from the pump, which provides constant and predictable delivery. The drug solution used to fill the pump does not itself need to pass through any membrane, which allows the delivery of compounds of any molecular weight, including proteins and peptides.

## **Range of products available**

ALZET pumps have been designed in a range of sizes to account for differences in agent solubility, potency, and desired experimental duration. Ten models of pumps are available currently. These pumps provide continuous delivery for one to 28 days at infusion rates of 0.25 to 10 microliters per hour. The model of pump chosen depends on the size of the research animal, the solubility of the agent, the desired experimental duration, and the route of administration. Lower flow rates may be desired for intracerebral or solid tissue microperfusion, while higher flow rates may be advantageous when infusing agents intra-arterially. For longer duration studies, pumps can be implanted serially. Figure 21.2 shows the range of pumps available. Table 21.1 lists the recommended animal size for each pump model.

ALZA also offers two brain infusion kits that can be used to infuse agents intracerebrally via the ventricles or to a specific site in the brain. These kits consist of 28 gauge stainless steel cannulae which can penetrate 3 to 5 millimeters below the surface of the skull, and polyvinyl tubing to connect the cannula to the osmotic pump. These kits enable the researcher to keep all infusion instrumentation under the skin, minimizing the chance for infection or animal interference.

## **Surgical Implantation Techniques**

ALZET pumps can be implanted subcutaneously or intraperitoneally, and can easily be used with a catheter. Further information on implanting these pumps can be attained from ALZET Technical Services, 800-692-2990, or by e-mail at [alzet@alza.com](mailto:alzet@alza.com). A free videotape is available which shows how to implant the pumps for subcutaneous, intravenous, intraperitoneal, intragastric, and cerebral infusion. Intravenous infusion will be described more comprehensively in Appendix One, with specific recommendations on the selection of pump model, route of delivery, anesthesia, and surgical instruments and equipment, as well as jugular vessel cannulation procedures and post-operative considerations.

## **Optimizing a Drug Delivery Regimen**

A full evaluation of the therapeutic potential of a compound includes investigation of the influence of dosing schedule on efficacy and side effects. Characterization of the dosing schedule can help to optimize drug effects and facilitate selection of the recommended dosing regimen early in preclinical development, allowing clinical studies to be conducted at lower cost and with better results. Research conducted over the last 30 years has demonstrated that the schedule of administration of a compound can be integral to both its efficacy and toxicity. The development of rate-controlled delivery systems during this time, for both research and clinical use, has facilitated the study and clinical application of different dosing regimens.

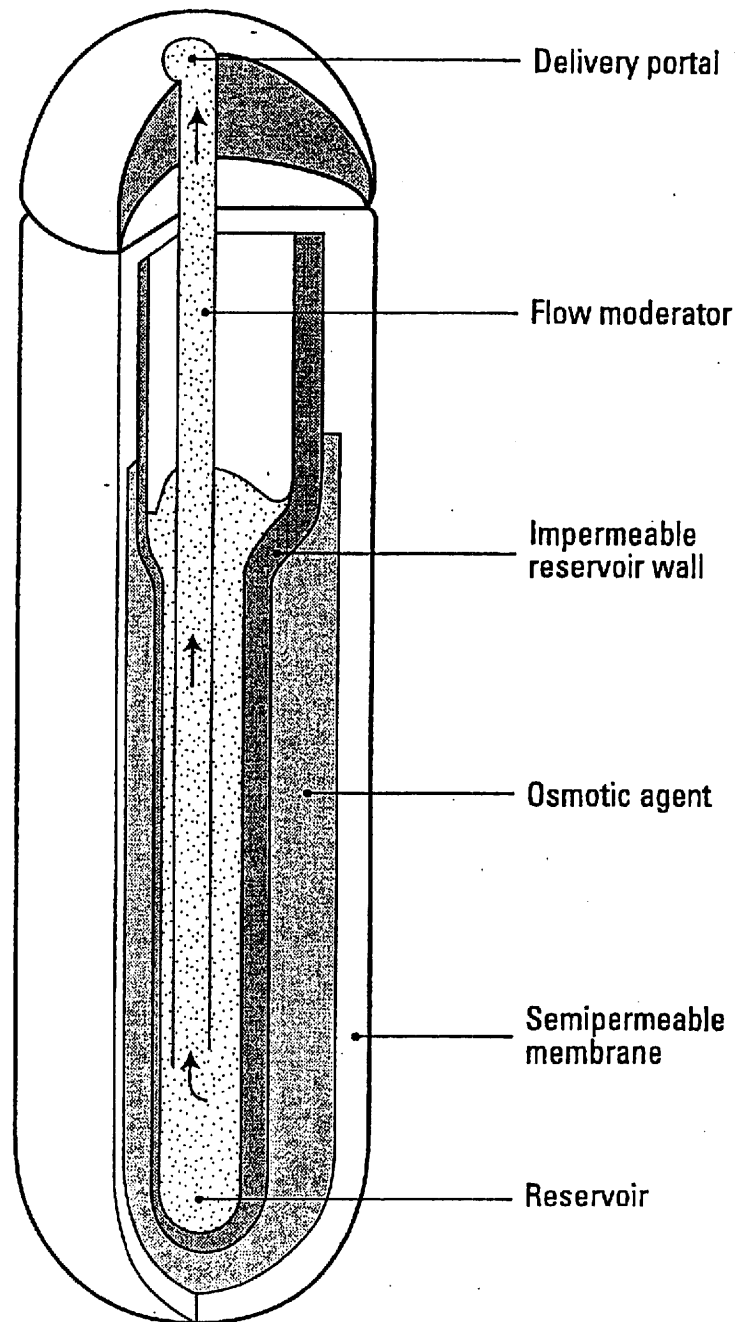


Figure 21.1: Cross section of an ALZET® osmotic pump showing its design, components, and mechanism of operation. (Copyright ALZA Corporation).

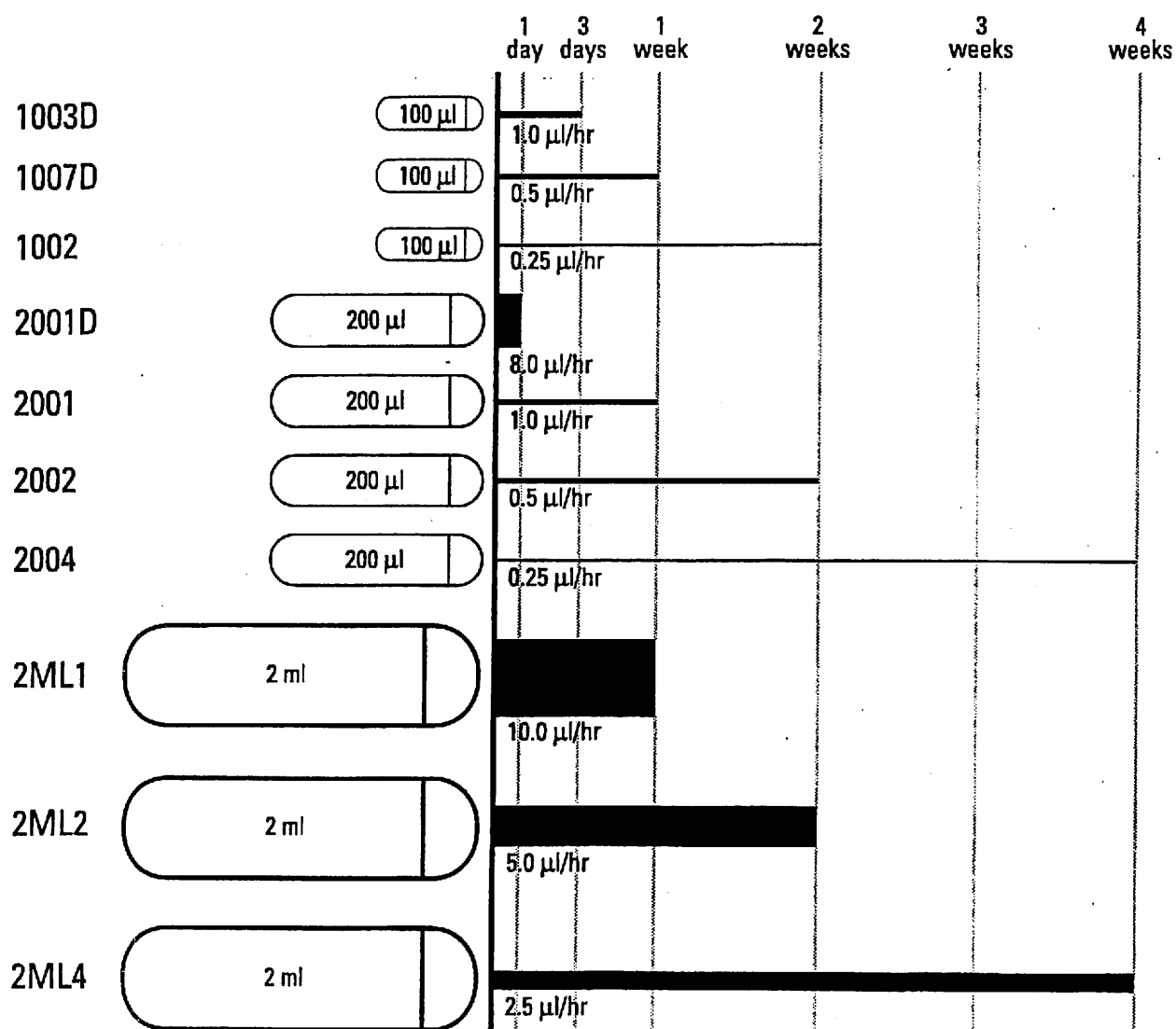


Figure 21.2: Rates and durations of ALZET® osmotic pumps. (Copyright ALZA Corporation).

**Table 22.1****Estimated Minimum Animal Size for Implantation of ALZET Pumps**

<b>Model</b>	<b>Mice</b>		<b>Rats</b>	
	<b>Subcutaneous</b>	<b>Intraperitoneal</b>	<b>Subcutaneous</b>	<b>Intraperitoneal</b>
1003D 1007D 1002	10 g	20 g	10 g	20 g
2001D 2001 2002 2004	20 g	Not recommended	20 g	150 g
2ML1 2ML2 2ML4	Not applicable	Not applicable	150 g	300 g

Note: The minimum animal size estimates are based on experience with male Sprague Dawley rats and Swiss Webster mice. When using the pumps with other types or genders of rats and mice, or with animals other than rats and mice, these guidelines should be modified accordingly.

Compounds whose efficacy depends upon their administration schedule demonstrate a shift of the dose-response curve to the right or left according to the time-pattern of drug administration. This means that different effects can be achieved with the same dose of drug by altering the administration pattern. For some compounds, administration by daily injections produces the desired biological effect. However, this regimen can produce widely fluctuating plasma levels which are inappropriate for many other compounds, either leading to a lack of apparent efficacy or to side effects. Lack of efficacy due to serum peaks and troughs may occur simply because the drug is not maintained long enough at a therapeutic concentration, or because a critical event during the treatment period occurs when the serum level of the experimental compound is outside its effective range.

ALZET pumps provide continuous infusion, a regimen that can be conceptualized as injections administered with the smallest possible intervals. Injections typically produce a high plasma level which rapidly declines, as can be seen in Figure 21.3 A. Injections can be administered with intervals of various lengths based upon the experimental design (Huber 1993; please refer to Figure 21.3 B and C). The fluctuation in serum levels which occurs during an injection regimen depends upon the half-life of the agent relative to the length of the intervals between injections. Figure 21.4 shows a simulation of serum peaks and troughs following injections at regular intervals. In this example, the peaks greatly exceed the therapeutic range, which is represented by the shaded horizontal band. In addition, since the half-life of the compound is much shorter than the injection interval, the serum level falls to insignificant levels between injections (Horton 1989). One approach is to assume that if the interval between injections exceeds 4 times the half-life of the drug, plasma concentrations will fall rapidly to zero between doses (Fara 1984). This suggests that compounds with short half-lives may not achieve sufficient concentrations unless administered by continuous infusion.

Slate *et al.* compared the effects of injection versus infusion when studying the chemotherapeutic combination of doxorubicin and verapamil. ALZET pumps were used "in order to mimic prolonged i.v. co-administration in the clinic, and to compare the efficacy and toxicity of this delivery scheme with more traditional bolus IP regimens" (Slate 1993). These researchers suggested that "continuous delivery may produce a greater cell kill for doxorubicin and verapamil by eliminating variants that are resistant to repeated dosing" (Slate 1993). Prior work on the regimen-dependence of verapamil had demonstrated that while ALZET pump infusion produced steady-state plasma levels, intraperitoneal injection achieved a comparable level for less than one hour, and was limited by lethal side effects to half of the daily infusion dose (Horton 1989).

The ideal administration regimen maintains the drug at a therapeutic concentration for the time period required to elicit the desired effect, which can be challenging for proteins and peptides with short half-lives *in vivo*. In addition, the ideal regimen maximizes the drug's therapeutic index, which is the ratio of therapeutic to adverse effects. However, in a preclinical situation, the precise half-life and optimal therapeutic concentrations may not be well understood.



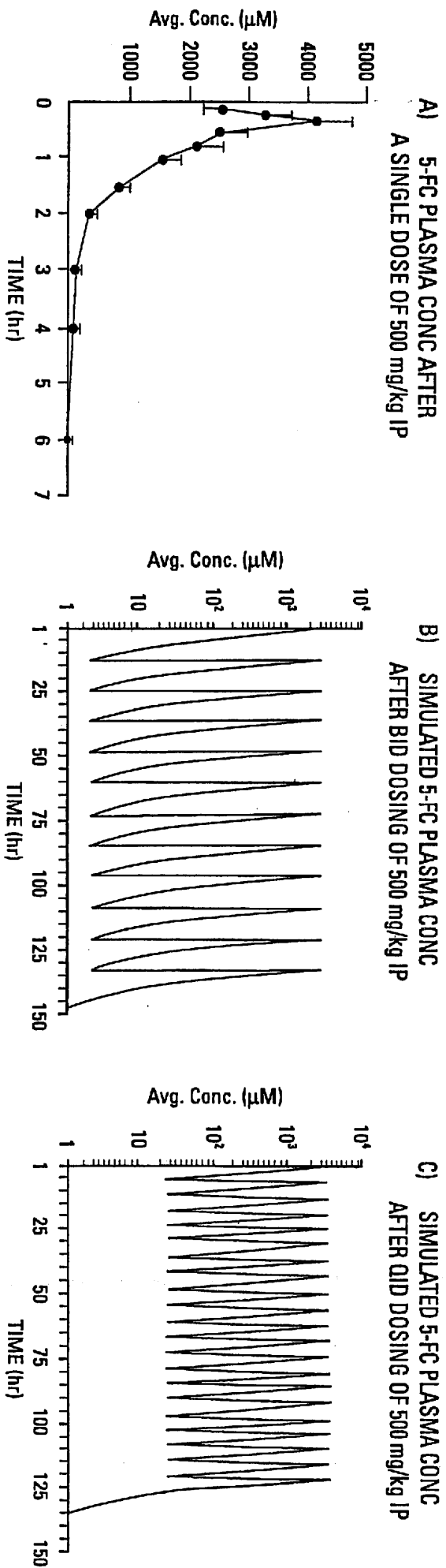
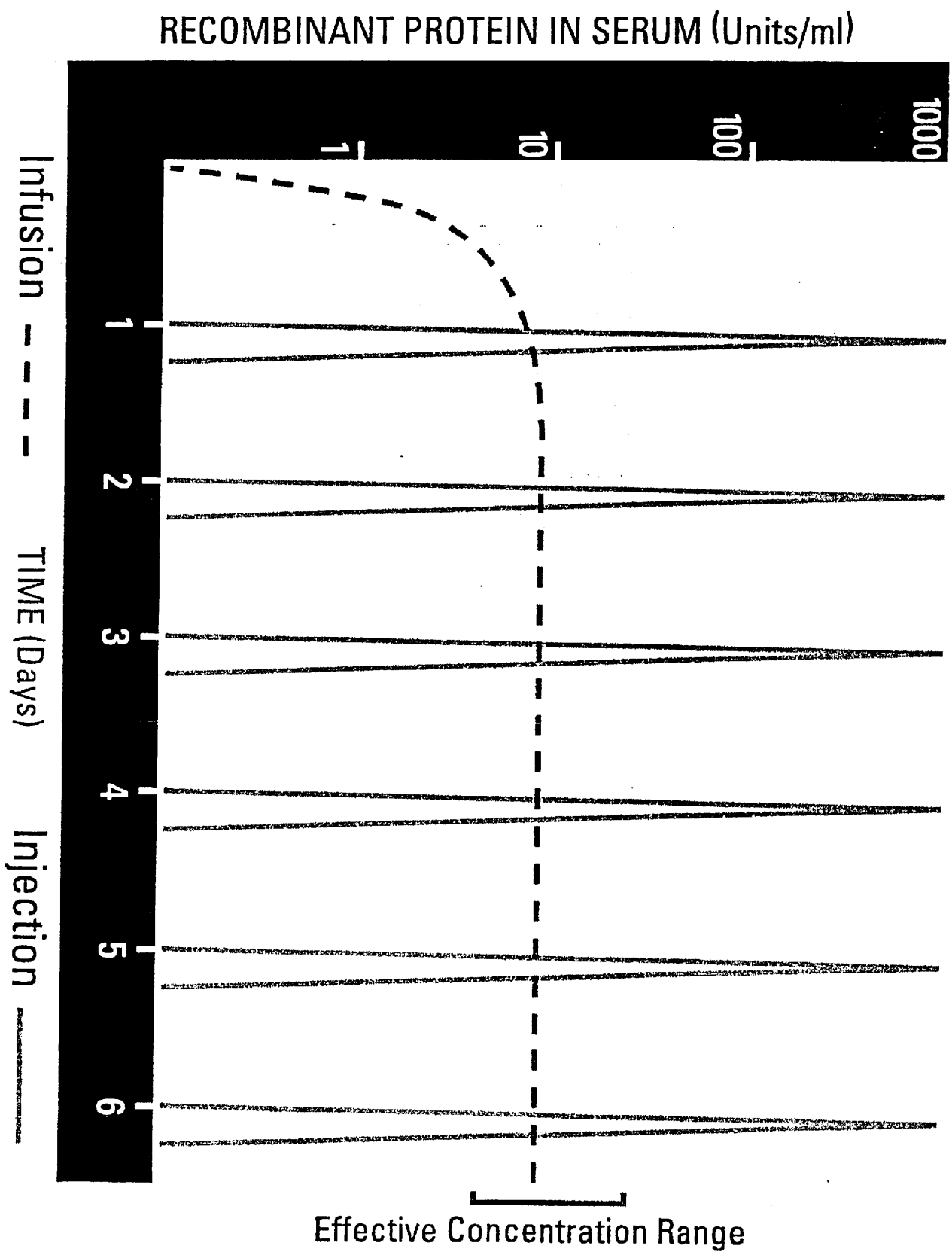


Figure 21.3 A: Plasma levels following a single IP injection of 500 mg/kg 5-Fluorocytosine to nude mice. (Reproduced with permission from Huber).

Figure 21.3 B and C: The pharmacokinetic profile of 5-Fluorocytosine administered to nude mice was determined following a single IP injection of 500 mg/kg to nude mice. These data were used to simulate plasma concentrations which might result from injections of 5-Fluorocytosine given twice daily (BID) or four times daily (QID) to nude mice. (Reproduced with permission from Huber).

Figure 21.4: Injections of short half-life proteins can result in great variations in protein concentration in serum and tissues. Immediately after injection, serum concentrations commonly exceed effective levels. Rapid elimination of the injected protein results in long periods between injections when the protein is absent from serum and tissues, hence is unable to exert an effect. (Copyright ALZA Corporation).



A useful approach compares the dose-response relationship when the same total dose of drug is administered over several days via once or twice daily injections versus multiday, constant-rate infusion. As Urquhart noted in his review of pharmacology for drug development and preclinical research, "this injection-infusion comparison (IIC) protocol may well be the logical starting point in the systematic pharmacodynamic study of many classes of drug, and a step toward basing dosage form and regimen design on pharmacodynamic data" (Urquhart 1985).

The method of drug delivery must be considered when predicting a drug's effects in humans based upon results obtained from an animal model. Many drugs are metabolized and excreted in small animals more quickly than in humans, as seen in Figure 21.5 (Nau 1985a; Prevo 1993). By way of estimation, the ratio of the half-lives of a compound in two different species is generally proportional to the one-fourth power of the ratio of the average weights of the two species (Fara 1986; Fara 1984). Using this rule, the plasma half-life of a drug in a 10 g mouse would be  $1/10^{\text{th}}$  the half-life in a 100 kg human:

$$100 \text{ kg human} \div 0.01 \text{ kg mouse} = 10,000$$

$$10,000^{\frac{1}{4}} = 1/10$$

A compound may fail to demonstrate efficacy when administered via an injection regimen simply because of its short half-life in the chosen animal model. In addition, a drug delivery regimen which produces long periods of low or no plasma levels between doses may not elicit toxicity, making it difficult to predict adverse effects in humans (Fara 1986). It is therefore critical that infusion be considered in the study design, especially for a compound with a short half-life in the preclinical animal model.

A pioneering study of schedule-dependence in chemotherapeutics was conducted by Sikic *et al.* This group compared the effectiveness of the injection versus the infusion of bleomycin, which has a short half-life in humans (< 2 hours) and mice. Tumor-bearing mice received one of three bleomycin regimens for 5 days: (1) injection every 12 hours, (2) injections on days one and four, or (3) continuous subcutaneous infusion via ALZET pumps. The same total dose was used in each group. Lung toxicity was measured using lung hydroxyproline content as a marker for fibrosis. Although survival was improved for the frequent injection regimen, both injection regimens were pneumotoxic. Continuous infusion significantly reduced tumor growth compared with the injection groups ( $P < 0.05$ ; see Figure 21.6), and did not result in pulmonary fibrosis. These results show that continuous infusion improved the therapeutic index, as the dose-response curve for bleomycin toxicity shifted to the right, while the dose-response curve for efficacy shifted left. Sikic *et al.* concluded that "the decreased pulmonary toxicity associated with continuous infusion clearly indicates that the therapeutic effects of bleomycin may be selectively improved by dosage schedule" (Sikic 1978).

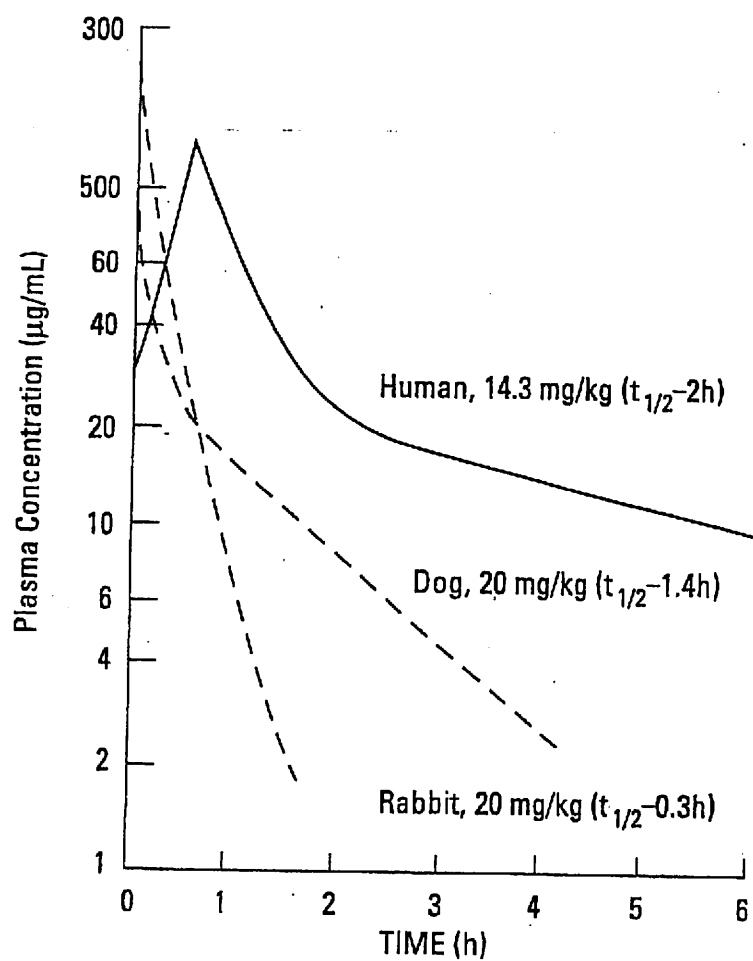


Figure 21.5: Inter-species differences in half-lives of a cephalosporin: (Reproduced with approval from Bristol-Myer-Squibb).

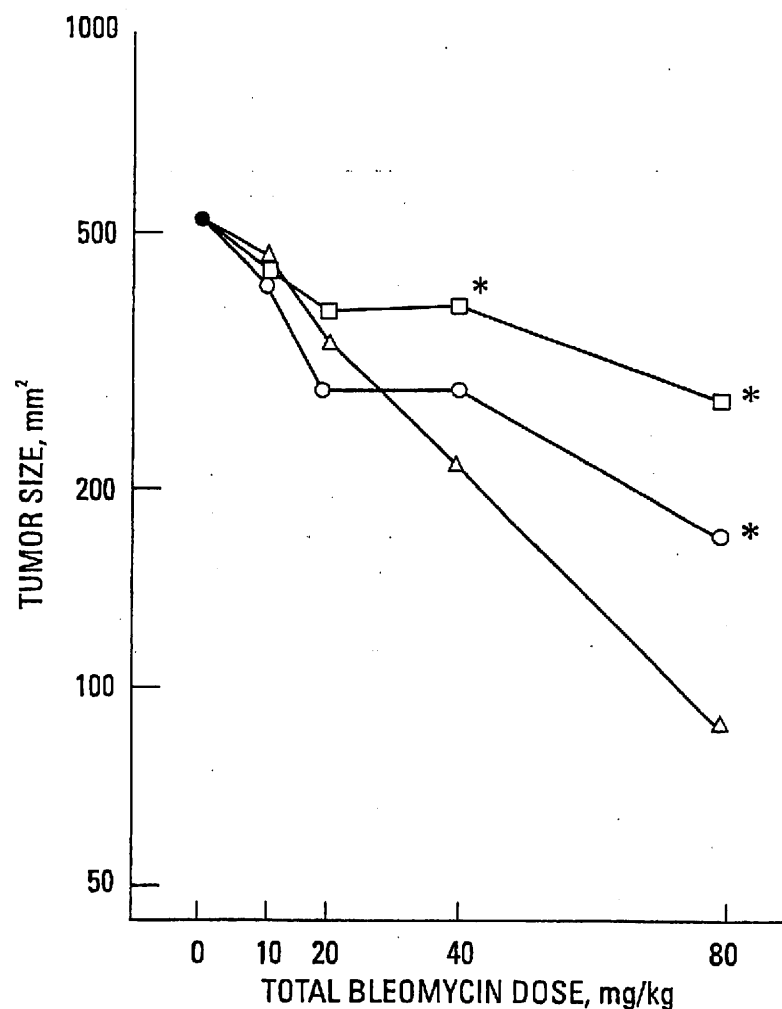


Figure 21.6: Dose-response curve of the antitumoral effect of bleomycin against Lewis lung carcinoma. Three schedules of administration were compared. Measurements were made on day 15 after osmotic pump implantation, but are representative of differences which existed throughout the course of tumor growth. Open squares represent subcutaneous (SC) injections given twice weekly; open circles represent SC injections 10 times weekly; open triangles represent continuous SC infusion. \*Significantly different from continuous infusion,  $P < 0.05$ . (Reproduced with permission from Sikic.)

Many peptides, such as insulin-like growth factor-I (IGF-I), also have short half-lives *in vivo*. Tomas *et al.* compared the efficacy of IGF-I and its analog LR<sup>3</sup>IGF-I when administered to normal and catabolic rats for 7 days via once or twice daily injections or constant delivery by subcutaneous osmotic pump. The catabolic state was induced by dexamethasone, delivered from a second osmotic pump implanted simultaneously. Continuous infusion of both IGFs was more effective than dosing by injection, as measured by changes in tissue growth, such as reduction in dexamethasone-induced weight loss ( $P < 0.001$ ), and changes in metabolic functions. The authors demonstrated a clear advantage for this mode of administration over injections (Tomas 1996).

These results with bleomycin and IGF-I join many other studies which show improved efficacy for compounds when administered by continuous infusion rather than injection. These include heparin (Edelman 1994), growth hormone and IGF-I (Gargosky 1994), interferon  $\gamma$  (Flynn 1993), and morphine (Yoshimura 1993).

In some instances, manipulation of the administration schedule elicits effects which cannot be demonstrated at all by injections. For example, Albiston *et al.* administered growth hormone either twice daily by injections or continuously by osmotic pumps for 4 days to hypophysectomized female rats. This group studied the extent to which hepatic levels of the 11 $\beta$ HSD2 enzyme are modulated by growth hormone. Injections of growth hormone had no effect on hepatic expression of the enzyme, while infusion significantly decreased both enzyme mRNA levels and enzyme activity compared with untreated, hypophysectomized controls. An administration regimen comprised only of injections might have led these authors to conclude that growth hormone had no effect on 11 $\beta$ HSD2 enzyme expression in the liver. Instead, these results emphasize that the schedule of administration can be integral to demonstrating the efficacy of a compound (Albiston 1995).

O'Neill *et al.* also uncovered the therapeutic activity of an agent in a gerbil model of cerebral ischemia. These authors compared continuous infusion of the nitric oxide synthase inhibitor 7-nitroindazole sodium salt by frequent injection versus continuous intraperitoneal infusion using ALZET pumps. The injection regimen was ineffective; however, continuous infusion elicited significant neuroprotection ( $P < 0.01$ ). This effect would not have been identified without an experimental design that included continuous administration (O'Neill 1996).

Characterizing the rate dependence of drug action is one means by which its therapeutic index can be optimized. For many compounds, continuous infusion should be considered integral to the preclinical development effort.

### **Preclinical Pharmacology and Toxicity Applications: Intravenous Infusion**

Continuous infusion can be invaluable in toxicology experiments when the effects of the compound are area-under-the-curve-dependent. The use of intravenous infusion studies can provide useful data for those who wish to mimic the clinical dosing of drugs.

The lack of data from continuous infusion studies has hampered the clinical testing of some drugs, as the relevant toxicity information that elucidated side effects was not generated during standard toxicology testing.

Milacemide, or 2-n-pentylamineacetamide HCl, was originally used as an antiepileptic treatment. It was also found to deliver glycine to the brain, which interacted with N-methyl-D-aspartate receptors to cause anti-anoxic and memory-enhancing effects. When tested in patients suffering from severe depression and Alzheimer's disease, it caused an unexpected elevation in plasma transaminases, an indication of drug-induced liver toxicity. Rogiers and her group designed an experiment which combined *in vivo* and *in vitro* approaches to detect early alterations in key markers of metabolic and liver function (Rogiers 1997).

Before clinical testing on milacemide started, two years of preclinical research had been conducted. In rats and cynomolgus monkeys, milacemide was given via gavage at multiples of the expected therapeutic dose by the route intended for man. There were no signs of liver toxicity in these experiments. Rogiers *et al.* conducted their experiments by infusing 250 and 500 mg/kg/day of milacemide to rats via IV jugular infusion by osmotic pump for 7 days. When 500 mg/kg/day of milacemide was infused, hepatocyte triglyceride levels increased 3.1-fold. Electron and light microscopy on both total liver and isolated hepatocytes showed a concentration-dependent accumulation of lipid droplets, numerous vacuoles in the cytoplasm, and other structural abnormalities. These findings demonstrated the potential hepatotoxic properties of milacemide. The authors of this study deduced that the short half-life of this drug of 0.5 to 1 hour in rats, combined with the gavage used in the preliminary toxicologic assessment, maintained systemic plasma levels at the appropriate level for only a few hours per day. They also stated that "the continuous intravenous infusion of 2-n-pentylamineacetamide HCl used here and the associated exposure levels reflect better the therapeutic situation in man and could therefore be a determining factor to explain the difference of effects recorded in regular toxicity studies" (Rogiers 1997).

Rodriguez-Martin *et al.* assessed the unexpectedly toxic effects of anilide by using continuous intravenous delivery. This group predicted that total absorption would be attained if anilide were infused at a constant and predictable rate, and that possible toxic and cumulative effects would not be affected by luminal hydrolysis or intestinal absorption (Rodriguez-Martin 1993). In 1981, a toxic oil syndrome was epidemic in Spain, and was traced to rapeseed oil contaminated with aniline. The rapeseed oil had been treated with 2% aniline for industrial purposes, was reprocessed to remove the aniline, and was then sold as cheap 'olive oil'. With refinery processing, large amounts of unsaturated fatty acid anilides formed, the amount of which correlated with the risk of illness. A host of symptoms were seen with this illness, including weight loss (Bell 1992).

Most of the studies conducted on anilide toxicity in rodents prior to this time relied upon acute injection or chronic administration in the diet, neither of which produced side effects in animals.

Rodriguez-Martin *et al.* infused  $^{14}$ carbon-labeled oleylanilide into the vena cava of rats by introducing catheters attached to ALZET pumps. The group of animals which received anilide rather than vehicle consumed more food and lost more weight than control animals, and their energy balance showed high inefficiency. These findings suggested that anilides could produce an emaciating effect in rats which was similar to that seen in humans afflicted with toxic oil syndrome (Rodriguez-Martin 1993).

Continuous IV delivery was also used by Houghton *et al.* when this group wished to maintain tightly-controlled plasma concentrations of gentamicin. Houghton *et al.* investigated whether a constant infusion of gentamicin for up to 6 months would cause chronic tubulointerstitial nephropathy. Patients who suffer from cystic fibrosis or osteomyelitis often require long-term treatment with gentamicin or other aminoglycosides, though the toxicity of long-term gentamicin treatment has limited its usage in other than extreme cases of infection. When clinicians prescribe this class of drug, it is given within very narrow therapeutic levels, and serum creatinine and creatinine clearance levels are monitored carefully to avoid nephrotoxicity (Houghton 1988).

To evaluate acceptable treatment ranges in rats, Houghton administered 20 mg/kg/day gentamycin via an IV jugular catheter connected to an ALZET pump. Pumps were changed every 28 days. The chosen dose was low enough to avoid acute tubular necrosis and renal failure. During treatment, serum creatinine and creatinine clearance overestimated glomerular filtration rate, and no differences were seen between treated animals and controls. However, in the month after the 6 month treatment regimen ended, tubular microcystic changes and active tubulointerstitial nephritis developed, with a continued fall in inulin clearance. This group concluded that careful maintenance of gentamicin serum levels did not preclude nephrotoxicity. By using continuous infusion, this group was able to answer key questions about clinical drug treatment modality and side effects (Houghton 1988).

Continuous IV infusion with ALZET pumps was also used to assess the effects of a gonadotropin releasing hormone (GnRH) antagonist. Previous compounds of this class have had clinical potential which has been limited by allergic side effects. Gordon *et al.* assessed the preclinical potential of A-75998, a fourth generation GnRH antagonist. This agent was promising because of its enhanced water solubility, high affinity for the GnRH receptor, and resistance to endopeptidase action (as compared with other such antagonists). It was also thought to have less than one-tenth of the histamine stimulation seen with other such antagonists. This group tested the effects of A-75998 when given via injection and infusion to female Cynomolgus monkeys. When given for 7 days via ALZET pumps connected to a catheter in the jugular vein, 0.025 and 0.05 mg/kg/day of A-75998 was fully effective in suppressing serum estradiol levels. The suppression of serum estradiol levels to below 30 pg/ml indicated that A-75998 had clinical potential as a safe and potent GnRH antagonist (Gordon 1994a).

Gordon and his group conducted additional studies on intact male Cynomolgus monkeys.



When this antagonist was administered via ALZET pumps attached to a jugular catheter, concentrations of 0.1 and 0.2 mg/kg/day A-75998 suppressed serum testosterone levels fully. In addition, no overt toxicity was seen in any monkey treated with A-75998, indicating that it merited clinical evaluation (Gordon 1994b). Finally, Leal *et al.* tested A-75998 and a series of GnRH antagonists to assess their effects on testosterone suppression in male dogs. This group examined AUC compared to peak concentrations, and also regimen-dependent toxicity, and concluded that a lower dose of A-75998 was needed when this compound was infused subcutaneously (Leal 1994).

Intravenous delivery using ALZET pumps allowed these researchers to see effects that were not apparent when the selected compounds were administered in a different fashion preclinically. In the case of milacemide, highly detrimental clinical effects were examined using long-term infusion studies that provided more information than standard long-term toxicity tests. Tightly controlled plasma levels of anilides and gentamicin yielded critical information about clinical exposure and side effects. A series of studies using GnRH antagonist A-75998 provided information about its clinical potential.

## **Preclinical Pharmacology and Toxicity Applications: Alternative Infusion Routes**

### **Intra-Arterial Infusion**

Using a similar though technically more advanced method, implantable pumps have been used for the intra-arterial administration of compounds. The constant delivery provided by the pump achieves and maintains a constant level of the test agent in the target organ. For most compounds, this method greatly minimizes systemic exposure, with the two-pronged advantage of reduced toxicity and lower effective dose. In addition, selective exposure of the chosen site can serve to isolate the effect of the compound on the tissue without the confounding responses which may arise during systemic administration.

If a compound is efficiently cleared when administered systemically, the amount of drug available to the target tissue is reduced markedly. For such a compound, targeted delivery may prove more effective than systemic administration. Ruers *et al.* achieved better efficacy with intra-arterial infusion of prednisolone as compared with intravenous jugular infusion, using ALZET pumps for both routes. Selective administration to a kidney allograft via the suprarenal artery prolonged graft survival when compared with systemic administration, and required a dose that was 50% lower than the systemic dose (Ruers 1986).

Intra-arterial infusion is pharmacokinetically advantageous if the overall rate of plasma clearance of the compound is greater than the blood flow through the target organ (Eksborg 1991). For example, Daemen *et al.* compared the pharmacokinetics of intrarenal versus intratesticular infusion of acenocoumarol, an anticoagulant. Rats received an ALZET pump, filled with acenocoumarol, which was attached to a catheter inserted either directly into a testis capsule or into one renal artery. Contralateral organs served as internal controls.

Tissue distribution was analyzed after 5 days of infusion. Since the testes receive little blood flow, direct intratesticular administration achieved a concentration of 370 ng/gram of testis in the target testis, but only 26 ng/gram in the control testis. Comparatively, the concentration of drug in the target kidney (30 ng/gram of kidney) did not differ appreciably from the contralateral organ (33 ng/gram). Because the kidney is an organ which receives high blood flow, and renal extraction of plasma acenocoumarol was low, a high concentration of drug was presented to the systemic circulation and in turn the contralateral kidney (Daemen 1988).

Higher target tissue concentrations via direct administration were also demonstrated by Gallo *et al.* These authors administered azidothymidine to rats by continuous intracarotid infusion using ALZET pumps, or by hourly intravenous injections. Intra-arterial infusion, even at the lower total dose of 40 mg, increased the percentage uptake into the brain 4.5 fold (28% vs. 6.2%), compared with 115 mg given by multiple intravenous injections. This compound clearly crossed from plasma to brain, although compounds which are polar and lipid-insoluble often do not, but instead require the use of a drug delivery method to bypass this diffusional barrier (Gallo 1989). These results highlight the fact that for many compounds, targeted infusion to a select organ or tissue site may be key to obtaining the most useful data on efficacy.

#### Preclinical Pharmacology Studies Using Subcutaneous Infusion

When evaluating compounds for clinical potential, continuous infusion studies have also been done using subcutaneously implanted osmotic pumps. When a compound is infused subcutaneously, its kinetics are usually closely similar to those seen with intravenous infusion.

Macromolecules such as globulins and other large proteins may have a substantially delayed or slower rise to steady state levels when given by subcutaneous as opposed to intravenous infusion. This is a result of the fact that macromolecules tend to be absorbed mainly by lymphatics rather than by capillaries (Urquhart 1999). It is thought that protein molecules up to the size of insulin (6 kD) are absorbed equally well by capillaries and lymphatics, while proteins over 20 kD are absorbed preferentially by lymphatics (Kompella 1991). Exemplary studies have been done with deoxyspergualin (with a molecular weight of 387.53), which was found to have similar steady-state plasma levels when infused intravenously or given via a subcutaneous ALZET pump (Plowman 1987).

Deoxyspergualin is an antibiotic with potential as an antitumor treatment. Plowman *et al.* evaluated its pharmacokinetics and effects against L1210 leukemia in mice. Initial dose ranging studies showed that the optimal therapeutic dose when given by a single IP injection was 31.5 mg/kg, and that higher doses caused death within 30 minutes. Plowman *et al.* found that this same dose could be injected every 3 hours without lethality, and that there was no cumulative toxicity. This suggested that the compound was rapidly cleared from plasma (Plowman 1987).

After mice were inoculated intraperitoneally with L1210 leukemia cells, deoxyspergualin was given via subcutaneous injection or infusion. The presence or absence of leukemia was assessed, with leukemia defined as the presence of ascites and/or splenomegaly at necropsy. Pharmacokinetic studies demonstrated rapid plasma clearance of the parent drug (20.8 ml/min/kg), and a  $\beta$  half-life of approximately 12 minutes. The greatest level of antitumoral activity was seen when plasma levels were maintained. As a result of these experiments, deoxyspergualin was developed to clinical trial. Initial Phase I protocols involved a 120 hour infusion schedule (Plowman 1987). Nishikawa also compared the antitumoral effects of deoxyspergualin given by injection versus infusion, and found that by infusing the drug, the total dose could be reduced (Nishikawa 1991).

Weckbecker and colleagues used a rat model with 7,12-dimethylbenzanthracene-induced mammary tumors to evaluate the potential of octreotide as a treatment for breast cancer. The continuous infusion of 10 ug/kg/h octreotide over 6 weeks reduced the number of tumors arising in the mammary gland by approximately 50%. Rats with 1-2 initial tumors showed significant reduction in further tumor development with the infusion of octreotide, suggesting its potential in the clinical treatment of breast cancer (Weckbecker 1992).

There are many other examples of preclinical toxicity studies in which schedule dependence data from infusion studies proved valuable in altering the antitumoral activity or toxicity of anticancer agents (Collins 1987).

#### Subcutaneous Infusion in Teratology Studies

It can be important to assess the long-term effects of an agent on the offspring of a pregnant animal. While injection and other modes of delivery can be useful, continuous infusion and its concomitant steady-state plasma levels can provide pertinent information on controlled agent exposure during select periods of organogenesis.

Heinz Nau and his colleagues used the 'injection-infusion comparison' protocol (Fara 1984) to compare differences in response to drugs administered to pregnant animals. This protocol was chosen due to the shorter half-lives of drugs in animals than man, which can be more pronounced in teratology studies. As a result, the pharmacokinetics obtained from once or twice daily injections given to animals throughout organogenesis, have often differed grossly from those seen in human therapy (Nau 1985b).

Since the 1960's, neural tube defects were thought to be caused by teratogens such as the antiepileptic drug valproic acid (VPA), especially if they were given in early pregnancy. Nau and his group used ALZET pumps to create a realistic model for testing the embryotoxicity of this drug, as the steady state plasma levels attainable with these infusion pumps could be held comparable to human therapeutic drug levels. VPA has a half-life of 8-16 hours in humans, compared to 0.8 hours in mice. This high plasma clearance rate in mice diminished plasma levels after one daily injection to approximately 10% of the original dose after 3-4 hours.

In humans, VPA was typically administered 2-3 times per day, maintaining a more consistent plasma level within the therapeutic range. During gestational days 7-15, pregnant mice received either one subcutaneous injection daily, or sodium valproate or water via subcutaneous osmotic pumps (Nau 1981). Significant growth retardation and increased resorption rates were seen in infused animals, but not exencephaly. Additional studies using a different injection-infusion regimen were undertaken (Nau 1985c). Nau *et al.* concluded that the doses or area-under-the curve (AUC) values did not correlate with the teratogenic response of the different regimens of administration, as doses 10-fold higher were needed to produce exencephaly formation when the drug was infused rather than injected. Steady-state concentrations of drug produced embryolethality and fetal weight retardation, primarily, while intermittent injections produced a high incidence of exencephaly (Nau 1985c).

In later experiments, this group saw that the effects of the teratogen cyclophosphamide were related to the maternal AUC values, and not to peak drug levels. Pregnant mice were given a single subcutaneous injection or a 24-hour infusion (during gestational days 9.5 to 10.5) via a subcutaneous pump. Dose-response curves for embryolethality, fetal weight retardation, and the incidence of malformations were shifted dramatically to the left by a factor of 70-140 when compared to data obtained from an injection regimen. Very low and sustained steady-state concentrations of cyclophosphamide were as teratogenic as more than 100X higher peak concentrations given by injection. Equivalent AUC values were achieved by high, short-lived peaks, or by low, sustained concentrations (Reiners 1987). This marked contrast to the situation with VPA probably relates to the fact that cyclophosphamide is an alkylating agent, whose actions occur independently of how the drug is administered (Urquhart 1999).

Nau *et al.* concluded "we believe that the injection-infusion comparison protocol is a valuable technique to study if the peak drug levels (such as the case of VPA) or the AUC values (such as the case with cyclophosphamide) will predominantly determine the toxicity of a drug" (Nau 1985b). This group intuited that if peak levels were of primary importance, toxicity could be related to absorption and distribution. If AUC values were the determining factor, toxicity could be related to drug clearance (Nau 1985b).

In an effort to protect against neural tube defects, several groups investigated the protective effects of infused folinic acid against valproic acid. Beneficial and time-dependent results were found (Trotz 1987, Wegner 1991).

Much teratology work has also been done to investigate the effects of drugs of abuse such as nicotine, cocaine, and morphine. Slotkin and his colleagues at Duke University have published widely on the effects of continuous nicotine exposure. Slotkin chose continuous infusion over injection because steady-state plasma levels more akin to human exposure are attainable using ALZET pumps. The placenta provides fetal protection by metabolizing a portion of drugs such as nicotine, and by introducing a phase delay between the maternal and fetal circulation. As a result, intermittent drug delivery produces less penetration into the fetal compartment than does continuous infusion.

The protective role of the placenta is further compromised because steady-state maternal plasma drug levels cause equilibration of all fluid compartments to the same final concentration. Nicotine targets specific neurotransmitter receptors in the fetal brain, and elicits abnormalities in cell proliferation and differentiation. These effects lead eventually to altered synaptic activity. Slotkin concluded that "nicotine infusion paradigms...produce drug exposure without the confounds of other components of tobacco or of episodic hypoxic-ischemic insult, [and] have enabled a mechanistic dissection of the role played by nicotine in fetal brain damage" (Slotkin 1998).

Since small animals eliminate chemicals much more rapidly than humans, Clarke and others have argued that constant rate infusion compensates for this difference more effectively than bolus delivery. The effect of low, steady-state plasma levels on toxicity during development, and the importance of total area-under-the curve exposure, may be examined and compared to the high plasma levels attained by bolus dosing (Clarke 1993). For some chemicals, the total exposure of the embryo over time can be of critical importance in inducing specific malformations. For example, experiments using infusion indicate that area-under-the curve is the prominent pharmacokinetic factor involved in the teratogenesis of retinoids, cyclophosphamide, and 2-methoxyacetic acid-induced digit malformations (Clarke 1993; O'Flaherty 1994).

#### Subcutaneous Infusion in Cell Proliferation Studies

Preclinical drug development requires consideration of cancer risk assessment, especially for agents likely to be dosed chronically. The timing of this evaluation varies based on laboratory protocol and when toxicity is identified. For example, hepatotoxicity identified early in development might trigger earlier carcinogenicity screening. Cell proliferation is a key factor in both the initiation and promotion seen in the clonal growth of cancerous cells. Currently, carcinogenesis cannot be fully evaluated using any single toxicological technique, but rather requires a coordinated approach which includes the measurement of induced cell proliferation (Butterworth 1992).

Measurement of cell proliferation via a labeling index involves administering a base analogue, such as  $^3\text{H}$ -thymidine or bromodeoxyuridine (BRDU), after or during exposure to the experimental agent. Cells in the replicative DNA synthesis phase, or S-phase, incorporate the base analogue into their chromosomes. Label incorporation is demonstrated using autoradiographic or immunochemical methods. Traditionally, the label was administered by injection, but this method only allows the detection of cells in S-phase at the time of label injection. Predicting when cell proliferation might occur can be difficult, because environmental factors can also alter the timing of this occurrence. Critical cell proliferation may be overlooked if the timing of label administration is not appropriate (Wilson 1989).

Results from early studies on cell labeling proved difficult to compare due to variations in animal size, species, label dose and timing of administration, among many other factors.

A team of researchers at the Chemical Industry Institute of Toxicology (CIIT) optimized and standardized cell labeling methods through extensive experimentation (Butterworth 1992; Wilson 1989; Goldsworthy 1991; Eldridge 1990; Wehorst 1991). In protocols developed at CIIT, subcutaneously implanted ALZET pumps containing either  $^3\text{H}$ -thymidine or BRDU achieved more sensitive labeling indices than did injections, even when injections were administered at 2-hour intervals (Eldridge 1990). This improved sensitivity occurred because continuous infusion maintained constant tissue levels of the base analogues, thereby labeling cells cumulatively over a longer time period.

ALZET pumps have been used for cell labeling in the study of a variety of compounds. In a recent study, Li and Helander at Astra Hassle used osmotic pumps filled with  $^3\text{H}$ -thymidine to label parietal cells as a way of comparing the effects of the antisecretory agents omeprazole and ranitidine on gastric mucosa (Li 1995). For 5 days, rats were given omeprazole by gavage or ranitidine by subcutaneous ALZET pumps. In addition, all rats received an ALZET pump infusing  $^3\text{H}$ -thymidine for 1, 2, 4, or 8 weeks. In animals receiving the label for more than one week, a fresh pump containing  $^3\text{H}$ -thymidine was implanted weekly and the spent pump was removed. The authors concluded that neither omeprazole nor ranitidine affected turnover rates of parietal cells or mucosal thickness. Authors of a prior study had observed morphological changes in parietal cells following gastric acid suppression, and claimed that this effect resulted in cell death. However, Li and Helander did not see an increase in cell turnover rates, suggesting that the changes seen previously were a functional, rather than pathological, effect (Li 1995).

This study is the most recent in a decade of work using ALZET pumps to compare the effects of these two drugs. Omeprazole, branded as Prilosec<sup>®</sup> or Losec<sup>®</sup> by Astra Hassle, and ranitidine, sold as Zantac<sup>®</sup> by Glaxo, are inhibitors of gastric acid secretion indicated for the treatment of peptic ulcers and other acid-related conditions. In the late 1980s, development of omeprazole was suspended when high-dose toxicology studies identified what appeared to be tumors of the gastric mucosa which on further investigation were identified as a physiological proliferative response to persistent hypersecretion of gastrin resulting from profound inhibition of acid secretion (SCRIP 1988). This effect initially appeared to be unique to omeprazole. To test this hypothesis, Astra researchers needed a method to achieve equivalent acid suppression with ranitidine, a short-acting drug and its long-acting competitor, omeprazole. ALZET pumps were employed to administer ranitidine continuously to rats for 28 days (Ryberg 1989). Both the group receiving infused ranitidine, and a group receiving oral omeprazole for 28 days, showed significant inhibition of gastric acid secretion, elevation of plasma gastrin, and increased numbers of enterochromaffinlike (ECL) cells in the oxyntic mucosa. These findings confirmed the hypothesis that profound inhibition of gastric acid secretion produced hypersecretion of gastrin.

In a subsequent paper, Ryberg *et al.* at AB Hassle and the Universities of Lund and Brussels investigated whether the hyperplasia seen with the inhibition of gastric acid secretion could be due to hypergastrinemia (Ryberg 1990).

For 12 days, animals received either ranitidine or gastrin infused subcutaneously by ALZET pump, or daily oral omeprazole. A single injection of  $^3\text{H}$ -thymidine was administered at the end of the treatment period. Hypergastrinemia was elicited in all treatment groups, accompanied by an increased proliferation rate for ECL cells. Having demonstrated that the hyperplasia observed following omeprazole treatment was a physiological effect of hypergastrinemia, the omeprazole development program was resumed, ultimately bringing it to market. Omeprazole went on to become the largest selling pharmaceutical in the history of the industry (Med Ad News 1997, 1998, 1999).

## **Conclusion**

Continuous infusion has proven helpful in scores of preclinical and toxicology experiments. Cell proliferation labeling using ALZET pumps filled with BRDU or  $^3\text{H}$ -thymidine has provided toxicologists and cancer researchers with a new tool which improves results over the injection of the labeling agent. In teratology studies, pronounced teratogenic effects were investigated by infusing pregnant animals during select periods of organogenesis. ALZET pumps are helpful in obviating differences in species clearance, and in generating data on the specific pharmacokinetics of compounds. The importance of teratology research can never be overemphasized.

With the long time frames necessary in the drug development process, tools which enable pharmaceutical and biotech companies to direct their development efforts more effectively are invaluable. When conducting pharmacokinetic and pharmacodynamic experiments by using bolus injections, data may be confounded by the lack of consistency in plasma levels throughout the dosing period. Data about likely toxicity or hyperplasia, in addition to the best mode of clinical administration, are helpful early in the discovery process. Protocols such as the injection-infusion comparison allow researchers to amass data on the pharmacokinetics of a series of compounds. Adjusting the route of administration can provide additional data, elucidating effects which would not be apparent with systemic administration. Research on dozens of peptides and proteins would not have been possible without a continuous infusion tool which circumvented problems with assessing the efficacy of agents with short half-lives.

When conducting preclinical and toxicology research, it is helpful to evaluate all of the tools which will direct the research most successfully. Continuous infusion is a tool that can help create a specific pharmacokinetic profile of a drug of interest.

## **Appendix One: Detailed Surgical Techniques for Intravenous Infusion Studies with Osmotic Pumps**

This section lists a variety of considerations to weigh when using osmotic pumps. This section contains information on the selection of the appropriately-sized pump, choice of implantation site and/or route of delivery, anesthetic, choice of surgical instruments and equipment, procedural recommendations for external jugular vessel cannulation, and post-operative care.

#### A. Selection of ALZET Pump Model (ALZA 1997).

- Choose the appropriate pump for the desired delivery rate and duration.
- Select the appropriate delivery route for the desired delivery target tissue and response.
- Use the smallest size pump allowable by agent solubility and experimental duration. Pumps can be implanted serially if longer delivery is required.
- If delivering to a target site via catheter tubing, choose the appropriate diameter, length, and rigidity of tubing (i.e., polyethylene tubing is more rigid than vinyl tubing). Decide whether to blunt or bevel the insertion end of the cannula, and on the appropriate technique for catheter sterilization.
- Fill and prime the pump maintaining aseptic conditions, and filter sterilize solution as needed. A sterile, particulate-free solution can obviate microbial contamination and resultant compound deterioration.
- Evaluate the stability of the solution at 37°C for the duration of the experiment.
- The functionality of the pump can be evaluated by weighing the pump before and after filling, and aspirating the residual volume at the end of the experiment. If an assay is available, serial plasma levels are the best technique for assessing constant drug delivery.

#### B. Implantation/Route of Delivery Considerations (ALZA 1997)

- Subcutaneous (SQ) implantation is technically the easiest and least invasive procedure.
- Intramuscular (IM) delivery is seldom utilized unless attempting to target delivery to a wound or tumor. In such cases, it may be appropriate to implant the pump SQ and use a cannula attachment to the target site.
- Intraperitoneal (IP) implantation is more invasive than SQ, but may be well tolerated. However, first pass effects must be evaluated. Some agents are metabolized differently when delivered IP because a large fraction of drug is absorbed into the hepatic portal circulation and passes through the liver (where some of the active drug may be inactivated) before entering the systemic circulation (Urquhart 1999).
- Intravenous (IV) delivery requires a catheter tubing attachment (such as polyethylene (PE) or other tubing) to a SQ-implanted pump. The external jugular vessel is usually chosen for cannulation. Technical skill is required for this type of surgery, but it can be done with the correct instruments, training, and practice.
- Other pump and cannula combinations can be used to target a variety of locations as indicated in the current literature.
- To deliver compounds that are incompatible with the pump reservoir (i.e., agents soluble only in oil or > 50% DMSO), one can utilize catheter tubing. This involves filling the appropriate length of PE or other tubing with the compound to be delivered, and then filling the pump with saline such that the pump displaces the compound out of the reservoir tubing.
- When working with catheter tubing, the stability and compatibility of the solution to the catheter material must also be considered. Additionally, tubing may contain plasticizers which may leach into the drug solution.



- SQ-implanted pumps (with or without cannula attachments) can be replaced with newly-primed pumps, preferably in a slightly different location (such as a new pocket on the alternate side of the midline) to extend the duration of infusion. If replacing a pump which is attached to a catheter, snip a small length of tubing off the existing catheter, and dispose of both the old pump and its flow moderator.

#### C. Anesthetic Considerations (ALZA 1997; ILAR 1996)

- Volatile inhalation anesthetics are best for most indications in most species as induction and recovery times are shorter and the surgical plane can be maintained for a short or long duration. Injectable anesthetics are an option in some instances.
- Delivery via a precision vaporizer is optimal, though one must assure adequate scavenging of waste gas for operator and surgeon safety (ILAR 1996). The chamber should be of the appropriate size for the species and should be scavenged, as this is one of the areas that causes greatest operator exposure to the gas.
- Chamber induction followed by intratracheal intubation is optimal, but an appropriately sized and placed nose cone is often more practical for rodent species. Active/suction scavenging systems which exhaust external to the operating room (OR) are recommended, although passive diffusion into fume or externally ducted appropriate level biosafety cabinets or trapping into charcoal filters can be acceptable alternatives in certain situations. Verify that scavenging methods are adequate by performing environmental monitoring, such as using commercially available diffusion badges.
- In all cases, the animal should be protected from hypothermia upon induction. Circulating water-heating pads are optimal because they are safe for the animal.
- Parenteral, physiological fluid administration is also recommended to guard against hypotension, especially when isolating and cannulating peripheral vessels. Deliver fluids SQ or IP at 1-3% of body weight on induction in a site that does not interfere with pump or cannula placement.
- Apply ophthalmic lubricant after induction to protect corneas from drying, chemical irritation from skin prep antiseptic solutions, or mechanical damage from the nose cone.
- Prepare the surgical site(s) aseptically according to standard veterinary practice. Clip hair with a fine blade over the entire SQ implantation area (not just the incision) to allow examination of the pump pocket site for signs of infection, irritation, or pressure necrosis. For rodents, a modified prep utilizing less fluid and alcohol may help minimize hypothermia from evaporative heat loss.
- If appropriate, administer post-op analgesics upon induction as a pre-emptive analgesic and part of a balanced anesthetic regimen which allows less inhalation anesthetic to maintain the surgical plane (ALZA 1996; ILAR 1996)

D. Instrument and Equipment Considerations (ILAR 1996; Stepkowski 1994; Tu 1995; Brammer 1999)

- Anesthetic delivery equipment must be designed or modified and positioned to avoid interference with cannula placement. One can cut away a portion of the nose cone/scavenging tube to allow room for the surgeon to assess the ventral neck incision for jugular cannulation (Brammer 1999).
- Appropriately-sized and functional instruments are essential and should be sterilized before starting the implantation session (ILAR 1996).
- Instruments for rodents must be fine enough to handle small structures and delicate tissues.
- Specialized instruments such as vessel dilators and catheter introducers are recommended for rodent vessel cannulations (Stepkowski 1994; Tu 1995; Brammer 1999).
- Autoclaving is the preferred method for initial instrument preparation. The decision on how to sterilize or high-level disinfect instruments between animals, or batches of animals, depends on several factors such as the immune status of the animal, the skill of the surgeon, and the time and invasiveness of the implantation technique. When working with immunocompromised animals, instrument sterility is of vital importance. If possible, perform surgical and other techniques under a hood.
- Although the pumps are manufactured and packaged sterile, if they are not filled, primed, handled, and implanted with aseptic technique, they can act as a nidus for infection. Additionally, reservoir contamination can lead to the microbial contamination of the agent used to fill the pump.
- Other interim instrument preparation methods such as glass bead sterilizers and chemical disinfectants have limitations. Glass bead sterilizers used in conjunction with volatile agents and 100% oxygen could present a fire hazard. In addition, glass beads can dull fine instruments used for rodents, and can heat instruments to dangerously high temperatures if the instruments are left in for more than a minute. High-level disinfectants require a minimum contact time of approximately 30 minutes to be effective and should be rinsed with sterile water or saline before touching tissues. Prepare a disinfectant bath and a rinse bath and two sets of instruments, so that one is soaking while the other is in use.
- For rodent vascular cannulations (especially in mice) a high quality microsurgical apparatus and basic microsurgical skills are invaluable. Dissecting scopes, surgical loops, or magnifying lights are less acceptable alternatives, and for vascular cannulations in mice may not magnify the surgical field to the desired level (Brammer 1999).

E. Jugular Vessel Cannulation Procedures (ALZA 1997; Stepkowski 1994; Tu 1995; Brammer 1999; Popesko 1992)

- Position the animal in dorsal recumbency and secure its head and anesthetic delivery apparatus in place.
- In rats, it may be helpful to place a piece of rolled gauze beneath the dorsal surface of the neck to support the neck and aid in extending it to expose the jugular veins.

- Place traction on the upper incisors with a soft band or smooth bar to immobilize the head and neck region without inhibiting respiration or interfering with the surgical site. Incorporate a support bar for the upper incisors into the nose cone scavenging device (Brammer 1999).
- The skin incision should be made from the ramus of one side of the jaw to the tip of the sternum just lateral to the trachea/midline (Popesko 1992).
- Use a small, sharp scalpel blade in a single cut to facilitate closure and healing, as scissors generally do not make straight incisions and can crush tissues at wound edges, delaying healing.
- Gently dissect down through the salivary and lymphoid glands, adipose tissue, and fascia to the external jugular vein, which is superficial to most of the neck musculature. Take care not to stretch or traumatize the vessel as this may cause vasoconstriction (Popesko 1992).
- Electrocautery can be used if done carefully. Delicate hand-held units can be used on small branches off the vessel (Brammer 1999).
- Identify where the external jugular splits into two branches at the level of the mandibular and parotid salivary glands (maxillary and lingofacial branches). Place and tie off one branch with a ligature of fine (4-0 or 5-0) silk, and leave tails 4-5 inches long to use as handles to place gentle traction on the vessel while dissecting and inserting the cannula (Brammer 1999; Popesko 1992).
- Place two more loose ligatures, one at the rostral end, and one at the proximal end of the exposed external jugular vein.
- Apply a few drops of lidocaine or other vasodilatory substance (at body temperature), and allow time for vasodilation. When small vessels appear in the field, the lidocaine has had time to act, and will inhibit, but not eliminate, vasoconstriction as the vessel is punctured and the cannula is passed (Brammer 1999).
- Use a fine gauge needle (25 - 23 gauge for mice, and 22 - 20 gauge for rats) bent at an approximate 90-degree angle to pierce the vessel. Alternately, a small ellipsoidal piece can be cut from the ventral aspect of the vessel with very fine iris scissors. Do not cut so much tissue as to weaken the vessel such that it breaks when traction is applied via the rostral ligature ends while passing the cannula. (ALZA 1997; Brammer 1999).
- Once the vessel has been pierced, control hemorrhage with gentle traction on the rostral ligature ends (ALZA 1997; Brammer 1999).
- The cannula can then be passed directly or with the aid of a plastic catheter introducer or fine vessel dilator. Pass the cannula far enough into the vessel to assure stability and patency, but not so far as to enter the thorax or interfere with cardiac function. It may be helpful to do preliminary nonsurvival practice surgery to confirm cannula placement in the rodent model that is used (ALZA 1997; Brammer 1999).
- If it is acceptable to delay the initial compound delivery, the cannula can be attached to a 1cc syringe filled with physiological saline via a blunt needle adapter. Successful cannulation can then be confirmed, and any microclots can be dislodged by flushing a small amount of saline after the cannula is placed and before all ligatures are secured (Brammer 1999).

- After successful insertion of the cannula, secure the proximal and distal ligatures around the vessel and cannula. Trim the ends close to the knot so as not to interfere with closure or to delay healing, but not so short as to risk the integrity of the knot (ALZA 1997; Brammer 1999).
- Reattach the cannula to the pump and use fine blunt hemostats to make a subcutaneous tunnel from the neck incision to the dorsal scapular region, making sure to create a sufficient pocket for pump positioning (1 cm of space around each side of the pump is recommended). Progressive insertion of closed hemostats, followed by the opening of the hemostats, and their withdrawal while still open, results in blunt dissection through the fascial planes of least resistance (ALZA 1997; Brammer 1999).
- Pass the caudal end of the pump through this tunnel into the pocket.
- For jugular cannulation in mice, one may use a dab of sterile silicone glue to splice the polyethylene PE-50 or 60 size tubing that attaches the pump into the smaller PE-10 tubing used to cannulate the vessel. This small bleb of silicon can also be used as an anchor by tying one ligature on the pump side to the fascia or muscle, thus inhibiting traction on the cannulated portion as the animal heals and moves about. Take care not to tighten ligatures around the tubing so that they cause strictures and inhibit flow (Brammer 1999).
- Use a two-layer closure, with one layer of suture of the underlying fascial tissues, and one of the skin. The deep layer should be closed with 4-0 or 5-0 absorbable material in a simple continuous or interrupted stitch, but silk is acceptable for short-term survival studies of 2-4 weeks. The skin can be closed with the same material, nonabsorbable suture, or stainless steel wound clips. In mice, sutures are recommended for comfort. Wound clips or ligatures in the skin should be removed in 1-2 weeks if the animals are to survive longer than 2-4 weeks (ALZA 1997; Brammer 1999).

#### F. Post-Operative Considerations (ALZA 1997; ILAR 1996)

- Place anesthetized animals on a clean, smooth, and absorbent surface such as a paper towel or disposable diaper-type material. Conventional rodent bedding can obstruct airways and adhere to moist incisions.
- Reapply ophthalmic lubricant during recovery.
- Keep surgical patients warm, and observe them until they are fully ambulatory and exhibiting near-normal exploratory or grooming behaviors (ILAR 1996).
- After normal behavior is observed, return the animals to standard housing or as appropriate for the research protocol. Add a few moistened blocks of rodent chow on the bottom of the cage to facilitate feeding during the immediate post-operative period.
- Make sure that there are no rough or sharp edges on the caging or accessories that might abrade the skin over a subcutaneous implant.
- House the implanted animals individually to avoid excessive grooming damage to implants. After the incision has healed, utilize professional judgement as rodents are social animals and, except for adult male mice, tend to live in compatible groups.

- Animals should be observed by qualified individuals who are familiar with normal rodent behavior at least once a day for at least 3-7 days following any surgical procedure.
- Additional post-op analgesics are not usually required for SQ implantations and peripheral vein cannulations, but if animals appear dehydrated, guarded, aggressive, or have lost considerable weight, additional analgesics or other veterinary intervention is indicated (ILAR 1996).
- Fluid accumulation around the implantation site can indicate seroma formation and should be monitored closely for signs of infection.
- Redness of the skin or a shiny or dry appearance may indicate a devitalized area and should be monitored closely for necrosis or ulceration.
- Dehiscence of the incision or other loss of the implant should be noted and the study director, supervisor, or veterinary staff contacted immediately such that the area can be flushed and repaired, or euthanasia administered promptly.
- It should be noted that animal stress from osmotic pump implantation is rare. For more information, please consult the bibliography section of [www.alzet.com](http://www.alzet.com).

#### G. Summary of Surgical Techniques for Intravenous Infusion

As with any technique, osmotic pump implantation and vascular cannulations should be practiced initially on non- or short-term survival animals designated for training, under the direction of an individual experienced with the specific technique in the given species or strain indicated on an approved protocol. Depending on the level of previous experience and the manual dexterity of the personnel, the number of practice sessions required to establish proficiency will vary. Techniques related to anesthesia, aseptic protocols, pump, instrument, or animal preparation, can lead to the success or failure of the technique in a given situation.

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