in healthy skeletal muscle, rapidly appeared in increasing amounts following muscle injury. The significance of this finding was further investigated by administration of exogenous LIF, or phosphate buffered saline (PBS) as a control, directly into the vastus lateralis muscle at the site of an inflicted crush trauma. Delivery was accomplished by connecting a subcutaneously implanted ALZET pump, Model 2001, to a polyethylene catheter, the tapered end of which was inserted into the injured region of the muscle. After 24 hours of LIF infusion, the diameter of regenerating fibers increased by 27% versus PBS controls (p < 0.005), leading (continued on page 6)

Muscle Regeneration

The stimulation of myoblast proliferation induced by LIF in cell culture led researchers to examine whether it conferred any beneficial effect on muscle regeneration in vivo. Barnard et al., of Monash University, Australia, observed that LIF mRNA, undetectable biological responses. Rapid increases in plasma or tissue LIF concentration following a physical insult, or chemical challenge to the body, have led researchers to question whether the various actions of LIF are beneficial or detrimental in nature. Unfortunately, the very short half-life exhibited by LIF in vivo has complicated assessment of its biological effects in animals. Recent studies have overcome this difficulty by utilizing the ALZET® Osmotic Pump for the constant, controlled delivery of LIF, both systemically and directly into target tissues. The ability to maintain effective levels of this cytokine has greatly facilitated evaluation of its actions, and such studies have indicated they are predominantly favorable in nature.
A crucial component in the local invasion of tissues by growing cancers is their ability to present a proteolytic interface to surrounding tissue matrices, which can be achieved through proteolytic enzymes such as urokinase plasminogen activator (uPA). This serine protease binds to receptors (uPAR) in the plasma membrane, then interacts with serum plasminogen to generate plasmin, which activates collagenases and metalloproteases, and stimulates production of uPA in a cyclic cascade reaction (Figure 2). The presence of plasmin at the cell surface provides the proteolytic interface essential for tumor invasion and metastasis.

As the uPA/uPAR system has been implicated in the progression of many malignancies, it presents a potential therapeutic target for controlling or preventing the spread of cancer cells. Antiproteolytic agents, specifically targeted at the plasmin proteolytic cascade, are currently being assessed for their antiproliferative and antimetastatic effects in animals. ALZET pumps have proved valuable in such investigations, including those presented here, enabling researchers to maintain effective concentrations of test agents, thereby facilitating evaluation of their anti-invasive capability.

**uPA Inhibitors**

A study conducted by Evans and Lin, of Akron Medical Center, Ohio, examined the ability of the uPA inhibitor PAI-2 to prevent the metastasis of breast cancer cells into the lung. Rats received an IV bolus of MatB rat mammary cancer cells, followed by an infusion of PAI-2 or saline, via an ALZET pump attached to an intravenous catheter, for 7 days. The control group, and eight of the ten receiving PAI-2, exhibited lung metastases, however there were significantly fewer metastases in animals receiving the uPA inhibitor (p < 0.033).

In a study conducted by Xing et al., of Royal Victoria Hospital, Montreal, MatB-III cells, or MatB-III cells engineered to overexpress uPA receptors (MatB-III-uPAR cells), were injected into the mammary fat pads of rats. Animals received the urokinase inhibitor B-428, or vehicle, via intraperitoneally implanted ALZET pumps for up to two weeks, with or without daily injections of tamoxifen (TAM), and were subsequently assessed for tumor volume and the development of macroscopic metastases. Tumor volume decreased in all rats treated with either B-428 or TAM compared to untreated controls. Rats inoculated with the modified MatB-III-uPAR cells and infused with B-428 demonstrated the greatest reduction in tumor volume. The magnitude of the effect elicited by TAM was comparable in animals inoculated with either MatB-III or MatB-III-uPAR cells, and similar to that produced by B-428 following MatB-III cell injection.

Combination therapy had a synergistic effect, and was more pronounced in those animals inoculated with the MatB-III-uPAR cells. TAM exerted no effect upon the development
These studies confirm that inhibition of the proteolytic cascade and the associated decrease in growth factor levels and proteolytic interface, provide a means of inhibiting local tissue invasion and metastasis. The development of such compounds, to be used alone or in combination with other agents, provides another possibility in the search for novel anticancer treatments.

Role of the ALZET Pump

Wide fluctuations in plasma levels, often associated with conventional dosing regimens, can result in cytotoxic or ineffective drug concentrations, complicating evaluation of potential anticancer compounds. The steady-state conditions achieved using ALZET pumps offer researchers a distinct advantage in overcoming the difficulties associated with the administration of short half-life agents. A complete listing of leptin references is available on request (see page 7).

ALZET pumps have led to their use in these, and many other studies, investigating the efficacy and mechanistic actions of a diverse range of anticancer agents.

If you would like more information on pump applications in this or other areas, please contact us as detailed on page 7.
University of North Carolina, theorized a role in OME for nitric oxide, which has been implicated as a regulator of mucin production. Rose et al. created a rat model of LPS-induced chronic OME by surgically obstructing the eustachian tube and then transtympanically injecting either LPS or vehicle (Krebs-Ringer solution) into the middle ear. After one week, middle ear fluid was removed and its volume and mucin content measured. Rats instilled with LPS had greater effusion volumes and mucin concentrations than those receiving vehicle alone. Further validating the animal model, immunostaining revealed inflammatory infiltration of both middle ear epithelia and lumina, and increased goblet cells and submucosal glands in LPS-exposed ears versus controls.

After LPS or vehicle instillation, experimental animals also received a transtympanic catheter which continuously infused N-nitro-L-arginine methyl ester (L-NAME) from a subcutaneously implanted ALZET pump, Model 2001. L-NAME is a competitive inhibitor of nitric oxide synthase, the enzyme which synthesizes the free radical, nitric oxide. L-NAME infusion lowered effusion volume, although not to a statistically significant extent, but significantly decreased mucin content (p < 0.05) as compared with infusion of vehicle alone (see Figure 3).

The work by Rose et al. demonstrates a role for LPS in inducing both OME and mucin production.
Role of NMDA Receptor in Aminoglycoside Toxicity

Aminoglycoside (AG) antibiotics have been in use, beginning with streptomycin, for more than 50 years. They remain the drugs of choice in numerous circumstances relevant to pediatrics, including septicemia and other serious infections due to Gram negative bacilli. Increasingly sophisticated dosing strategies have not eliminated the main adverse effects of nephro- and ototoxicity. Although the molecular mechanisms are poorly understood, the hearing loss results from degeneration of the cochlear hair cells and can occur suddenly and unpredictably.

Hypothesizing a role for NMDA receptors in AG-induced excitotoxicity at the hair cell-afferent nerve synapse, Basile et al., at the National Institutes of Health and Louisiana State University, created an animal model of AG-induced ototoxicity by chronically administering neomycin or kanamycin to separate groups of guinea pigs daily injection for two or three weeks. The experimental group also received either dizocilpine or ifenprodil, both NMDA antagonists, infused from a subcutaneously implanted ALZET pump, Model 2002. To achieve continuous infusion for three weeks, the pumps were surgically replaced with fresh pumps after two weeks.

After four weeks, animals were stimulated with trains of 5-10 kHz tones at specific intervals and sound pressures. The number of pinna flicks, a known reflex in guinea pigs to such sounds, were recorded. The sound levels required to elicit a 50% pinna flick rate in experimental animals treated with both AGs and an NMDA antagonist did not differ significantly from control animals at 5 kHz (Figure 4), and were only modestly increased at 7 and 10 kHz. In addition, measurement of hair cell function in the organ of Corti via distortion product otoacoustic emissions revealed that concurrent administration of NMDA antagonists with AGs preserved outer hair cell function to nearly that of control animals, whereas AG-treated animals showed emissions indistinguishable from the noise floor, signifying severe loss of hair cell function. The authors propose that overstimulation of NMDA receptors at afferent synapses may be responsible for the AG-induced auditory deficit, although they also speculate that direct activation of NMDA receptors on the hair cells themselves could be causative. Continuous infusion was key to maintaining stable receptor occupancy in this model.

Both Basile et al. and Rose et al. capitalized on the control over drug delivery afforded by ALZET pumps. In numerous other published studies, researchers have overcome obstacles to drug delivery presented by tissues which are difficult to penetrate or compounds with short half-lives. To request references on the delivery of agents to the ear, on NMDA antagonists, or other compounds relevant to your area of research, please contact us as outlined on page 7.

Figure 4. NMDA antagonists attenuate decreases in the pinna flick reflex due to aminoglycoside treatment in guinea pigs. Animals exposed to neomycin alone () showed minimal response to 5-kHz tones, even at high sound pressures. Concurrent treatment with an NMDA antagonist, either dizocilpine (■) or ifenprodil (▲), maintained response rates which did not significantly differ from animals with no aminoglycoside exposure (○).

fiber diameter over saline controls, which was still apparent seven days after cessation of treatment, together with a two-fold increase in the number of regenerating fibers. These findings led Kurek et al. to conclude that LIF did indeed stimulate muscle regeneration and may be of value in the treatment of neuromuscular diseases.

Experimental Glomerulonephritis

The observation that glomerular levels of LIF mRNA rapidly increased after treatment with anti-glomerular basement membrane antibody (anti-GBM Ab), prompted Tang et al., of Amgen Inc., to investigate the possible role of LIF in the pathogenesis of the ensuing glomerulonephritis. Animals implanted intraperitoneally with an ALZET pump, Model 1003D, were continuously treated with LIF for 24 hours prior to and following induction of anti-GBM Ab glomerulonephritis. LIF treatment attenuated the resultant glomerular inflammation, as evidenced by reduced proteinuria (85%) and glomerular macrophage infiltration (60%), together with a decreased expression of the inflammatory cytokines IL-1β and TNF-α, and the chemokine MCP-1. These protective effects of LIF led the researchers to suggest a potential role for this cytokine in the treatment of glomerular inflammation.

Cholesterol Lowering

Additional investigations by Moran et al., of the University of Queensland, Australia, have indicated that beneficial cholesterol-lowering and antiatherosclerotic effects may be attributable to LIF. These studies involved the continuous administration of LIF, via intraperitoneally implanted ALZET pumps, Model 2ML4, to rabbits over a four week period, during which time the animals were maintained on either a normal or cholesterol-rich diet. Plasma cholesterol levels and the formation of fatty streaks on the thoracic aortas of the animals were recorded throughout, or on completion of the treatment.

As expected, rabbits maintained on the cholesterol-rich diet had considerably higher plasma cholesterol levels and an increased incidence of aortic fatty streak formation compared to those on the normal diet. Administration of LIF to rabbits on the cholesterol-rich diet significantly reduced this elevation in plasma cholesterol levels and fatty streak formation compared to their non-treated counterparts (Figures 1 and 5). Moran et al. have suggested that

Figure 5. Continuous administration of LIF by ALZET pump over four weeks resulted in decreased fatty streak formation in the thoracic aorta. (Reproduced with permission from Moran CS et al. Arterioscler Thromb Vasc Biol 1997;17:1267-1273.)
these beneficial effects of LIF are, in part, related to an upregulation of hepatic LDL receptors giving rise to improved clearance of lipoprotein-associated cholesterol from the circulation.

Delivery Dilemma Resolved

Conventional delivery methods, such as injection, can result in wide variations in the level of LIF achieved in the plasma and tissues, due to the very rapid rate at which it is eliminated. The use of ALZET pumps in the above studies enabled effective systemic or local tissue levels of LIF to be maintained. This additional control over experimental variables greatly facilitated evaluation of the beneficial effects conferred by this cytokine in times of adversity.

Many other cytokines and growth factors have similar drawbacks associated with short half-life, poor bioavailability and the high costs of material. For information on how the osmotic pump has been successfully used for the delivery of a diverse range of such agents, please contact us as outlined below.

Overcoming Challenges in Neonatal Drug Delivery by Laura Whitman

A review by Sapolsky, at Stanford University, published in Science stated that as little as 15 minutes each day of human handling of newborn rats during the first few weeks of life can profoundly affect maternal interaction and, later, adult behavior. In order to study the effects of agents, while avoiding the physiological and behavioral changes associated with frequent handling required by repeated injections, researchers have used ALZET pumps. Thornton and Smith noted that "bolus injections require repeated handling and stress of neonatal rats and dams that could affect the development of tolerance… these concerns led us to begin using subcutaneously implanted ALZET osmotic minipumps to render neonatal rats tolerant and physically dependent on opioids."

The smallest ALZET pumps are designed for subcutaneous implantation in animals as small as 10 grams, the weight of a 6-day old rat pup. The surgical implantation takes about 5 minutes, following which the animals require no further handling for the duration of the infusion period.

While minimizing stress from frequent handling, these tiny implantable pumps also provide zero-order drug delivery in neonates for durations lasting from 3 to 14 days. Thornton and Smith found this aspect particularly beneficial: "mini-pumps have the advantage of delivering drug at a constant rate to provide stable plasma and tissue opioid concentrations for long periods of time.

The constant infusion of opioid by minipumps may also reduce the toxicity often associated with bolus drug administration. A neonatal information package, including surgical techniques and references on the use of ALZET pumps to administer agents to neonates, is available on request.

For technical information about ALZET osmotic pumps, or for a complimentary custom search of our extensive bibliography, please return the attached business reply card or contact us: 1-800-692-2990, 650-962-2251, Fax: 650-962-2488, E-mail: alzet@alza.com

www.alza.com/alzet/tb201
With great regret, we announce the departure of Nigel Ray from ALZA Corporation, as Nigel pursues his education at the Anderson School of Business, UCLA. Nigel joined ALZA in 1986 as a Technical Information Associate for ALZET Osmotic Pumps. He ascended the ranks to create, and then become director of, ALZA Scientific Products. Under his leadership there were numerous innovations, including introduction of the ALZET Brain Infusion Kit, the Technical Information Manual, and the ALZET Web Site. In addition, Nigel spearheaded the development of several new pump models.

We would like to thank Nigel for his enormous contributions to ALZA. ALZA Scientific Products exists, and is vital with activity, because of his efforts. We wish him all the best in his future endeavors at business school and thereafter.

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New Agents in the ALZET Literature

Including more than 5,200 publications from the scientific literature, the ALZET pump bibliography is a valuable source of information on the controlled delivery of a wide variety of agents. Updates are frequent, and the most recent includes references on the delivery of the agents listed here. References on these and other agents are available on request.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Therapeutic Category</th>
<th>Activity</th>
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<tbody>
<tr>
<td>1400W</td>
<td>Nitric oxide synthase inhibitor</td>
<td>Hepatocyte growth factor, recomb. human</td>
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<tr>
<td>A802715</td>
<td>Immunosupressant (methylxanthine derivative)</td>
<td>HR 720 Angiotensin II antagonist, Interleukin-8 Cytokine</td>
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<td>Vasodilator</td>
<td>Leptin, recomb. human Hormone</td>
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<td>Anticoagulant</td>
<td>Metyergoline Prolactin inhibitor, Metolazone Antihypertensive, diuretic</td>
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<td>Monocyte chemotactic protein-1 Chemoattractant, Oxyntomodulin Regulatory gut peptide</td>
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<td>Antibody, monoclonal CC49</td>
<td>Immunologic</td>
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<td>Antihypertensive</td>
<td>R-95288 Antiviral oligonucleotide derivative</td>
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<td>Antihypertensive</td>
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<td>Infertility agent (prostaglandin analog)</td>
<td>RO-23-7553 Vitamin D analog</td>
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<td>CP-98,113</td>
<td>NMDA antagonist</td>
<td>UCN-01 Protein kinase C inhibitor</td>
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<td>GR144053</td>
<td>Fibrinogen receptor antagonist</td>
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ALZET Expert Seeks Infusion of Academia

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