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Presentations given at this conference, held in December 1987 in London, UK, are reviewed in detail. Topics include development of new dosage forms; controlled release (including oral dosage forms, implants, and transdermal systems); the possibilities and limitations of greater selectivity in targeted delivery of currently available systems such as liposomes, macromolecules, monoclonal antibodies, and prodrugs; and delivery of peptides and proteins via the gastrointestinal and nasal routes.

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BIOTECHNOLOGY CONFERENCE: DRUG DELIVERY AND DRUG TARGETING SYSTEMS

Introduction

This focused and informative conference took place in London, UK, on 14 and 15 December 1987. The meeting was sponsored by IBC Technical Services Ltd., UK. However, the program format had been arranged by leading UK scientists. The delegates numbered 155, 76 percent representing industrial organizations and the balance from academic institutions. Although the majority of attendees were from the UK, 11 West European countries were also represented as well as the US and Israel.

In recent years a whole new range of possibilities has become available for delivering constant therapeutic drug levels, or even for targeting the drug to its site of action for a more specific effect. At this conference, an international panel of speakers from academia and the pharmaceutical industry described both the state-of-the-art and the advances at present in development.

The topics covered at this conference were:

- Impact of new formulations in drug delivery
- Hydrogels and drug delivery
- Mucoadhesives for delivery across mucous membranes
- Nasal delivery of protein/peptide therapeutics
- Polymers in controlled-release systems
- Design of transdermal systems
- Clinical development of drug delivery systems
- Design of novel drug delivery systems
- Site-specific delivery and optimal drug action
- Liposomes as drug delivery devices
- Liposomes as drug carriers for antimicrobials and antineoplastics
- Drug targeting using monoclonal antibodies
- The future of drug delivery – new concepts and perspectives.

A fairly detailed account of most of the various presentations is given in this report.

Impact of New Formulations in Drug Delivery

This topic was discussed by S.S. Davis (Department of Pharmacy, University of Nottingham, UK), who said that drug delivery has now come of age and today there is active interest in drug delivery research both in academia and industry. A great diversity of systems is available for attempting to achieve objectives in drug delivery or drug targeting. Davis said that a simple way of classifying these systems is to categorize them as being physical, chemical, or biological in approach. Examples of each taken from the recent literature are as follows: (1) the oral administration of controlled-release systems based upon membrane technology and osmosis, (2) chemical delivery systems for overcoming the blood-brain barrier based on the soft (pro) drug concept, and (3) targeting of cytotoxics to cells using cross-linked antibody heteroaggregates. A reflection of this upsurge of interest in drug delivery is the large number of books dealing with different approaches and systems, and an increasing number of specialist meetings and workshops.

According to Davis there are three main reasons for considering a new drug delivery system:

1. Controlled-Release. A popular approach is to develop a controlled-release system for an existing drug that may have been on the market for a considerable number of years. Such a controlled-release system may lead to better bioavailability and improved pharmokinetics as well as extension of the patent and commercial life of the drug, according to Davis.

2. Drug Targeting. The site-specific delivery of drugs (drug targeting) can be exploited in order to reduce adverse reactions and side effects, according to Davis. The obvious example here is cancer chemotherapy, but in other situations, it would be advantageous to deliver the drug to an organ or even to a cell or intracellular structure. In doing so, not only would it be possible to administer far less drug substance but it might even allow substances not presently used because of their high general toxicity.

3. Delivery of Peptides and Proteins. Davis said that there is an increasing interest in delivery systems for the products of biotechnology, namely, peptides and proteins. These are complex, high-molecular-weight materials that are usually unstable, active in very low concentration, and do not cross biological barriers very effectively. Consequently, there are considerable problems in formulating effective delivery systems. This can be coupled, Davis said, with the fact that many of these molecules are not covered by strong patents and therefore are available to a large number of competing companies. Thus, a successful delivery system (by a noninjectable route) could lead to large commercial success.

Some of the successes that have been achieved in drug delivery are:

- Controlled-release systems – oral theophylline, transdermal nitroglycerine, the colonic delivery of 5-amino salicylic acid. Drug targeting – liposome formulations for Pentostam, colloidal delivery systems for Amphotericin
- Peptides and proteins – implants for LHRH analogues, nasal delivery of DDAVP, and other low-molecular-weight peptides.

Impact of New Delivery Systems.

Davis said that as well as achieving commercial success, a new formulation in drug delivery can impact a number of different areas: academia, industry, medical practice, regulatory authorities, and the patient. Exactly how and where a new delivery system may achieve its impact can differ widely. Davis then gave an example using ALZA Corporation, US, which was set up almost 20 years ago. The first products from ALZA's research were based upon membrane technology (in the form of the ophthalmic product, Ocusert), and the contraceptive agent, Progestasert. Both had a considerable impact in redirecting thinking in the pharmaceutical sciences, according to Davis. At that time, the idea that drugs should be delivered according to well-defined time profiles was novel, as was the exclusive development of new delivery systems rather than the prevalent emphasis on new chemical entities. Unfortunately, the original products from ALZA made little impact upon the medical profession and the patient. However, according to Davis, the single-minded attention of ALZA to delivery systems awakened the attention of the pharmaceutical industry as a whole, and the origin of many of the successful drug delivery groups within industry today can be traced back to the pioneering effects of the research scientists at ALZA.

Possibilities and Pitfalls

Davis said that an important, but obvious point in developing drug delivery systems, is that they should be developed with full attention given to the disease state to be treated and the drug to be delivered. However, he said also that in many cases one sees individuals and companies who are committed to the exclusive exploitation of their particular delivery system. Consequently, excellent delivery systems are being used inappropriately for the wrong drug material. Davis said that it is also important to bear in mind the various pitfalls that may be awaiting those who are attempting to bring products to the marketplace. These pitfalls are largely biological in nature. For example, for transdermal systems, one can cite problems associated with metabolism, irritation, sensitization, and tolerance. For monoclonal antibody-drug conjugates and liposomes, the biological problem comprises recognition and capture of the carrier by the reticuloendothelial system. This largely prevents targeting to desired sites. Even with controlled-release systems, biological problems can occur, according to Davis. Some examples are tolerance, dose dumping, and toxic manifestations (such as that apparently occurring with Osmosin).

In order to illustrate in more detail the impact of certain new formulations in drug delivery, Davis presented some selected examples, detailed in the following.

Controlled-Release System For Existing Drugs

The success of transdermal nitroglycerine and similar products is an excellent example of how the application of physicochemical principles can lead to the development of elegant membrane systems. As mentioned above, transdermal delivery is not without its problems and it is to be expected that only a small number of suitable drugs can be delivered in this way. Davis said that claims that 80 percent or more of available chemical entities will be delivered through the skin in the future are totally naive. With peptides and proteins, the problem of effective transdermal delivery will be enormous even with the application of techniques such as iontophoresis. Oral controlled-release dosage forms are well known and are quite widely prescribed. In some situations, controlled-release systems have changed the utilization of some drugs: examples include theophylline and morphine. The oral route, Davis said, will always be one of the most popular and acceptable ways of administering drugs. For this reason, many groups are actively exploring ways of using the gastrointestinal tract in a more selective way. It would be an advantage if the normally rapid gastrointestinal transit of controlled-release systems could be altered through the use of strategies like bioadhesives, density, etc., according to Davis. The targeting of colloids to M-cells in the small intestine and the delivery of drugs to the colon are two relevant objectives.

Drug Targeting And Site Specificity

The targeting of drugs to specific sites is a more glamorous but difficult task to achieve, Davis said. Here, chemical, physical, and biological approaches have all been pursued with moderate degrees of success. Prodrugs, polymers, and colloidal carriers – and more recently, monoclonal antibody-conjugates – have all been championed. Davis said that perhaps too much has been made of the use of liposomes as delivery vehicles because their promise has not been as great as had been hoped. He also pointed out that the liposomal systems that will reach the market place in the near future seem to achieve their beneficial activity more by protecting the host from the drug than by active delivery to a specific site unless the site happened to be the reticuloendothelial system.

Apparently, recent work has shown that liposomes are often little different in their delivery capabilities from emulsions and microspheres. As for the future, Davis said that a greater understanding of biological recognition processes and the role of surface chemistry in the detemination of targeting with colloidal carriers would be of great advantage. It is now known that by exploitation of different surface coatings, it is possible to direct colloidal systems to the bone marrow or to sites of inflammation, or to retain them in the systemic circulation. The development of carriers with attached homing devices should allow targeting to sites within the vascular compartment, Davis said. In his opinion, the concept that colloidal particles can escape from the vascular compartment and reach tumors is probably untenable.

Delivery of Peptides and Proteins

According to Davis, the number of products that are now available through recombinant DNA technology or by the increased sophistication of peptide synthesis, provide great challenges to those in the field of drug delivery. The easiest way to deliver many of these materials is by simple injection, but even here, problems can arise in terms of stability and inappropriate delivery patterns. In nature, biological mediators usually act over short distances and exist for short time periods. Furthermore, toxicological manifestations can arise if large doses are administered, especially if these are delivered inadvertently to inappropriate sites.

Peptides and proteins are not normally well absorbed from the gastrointestinal tract although there are some exceptions such a Cyclosporin. Davis said that the attempts to increase the transport of peptides and proteins across mucosal barriers are centered on the use of so-called absorption-enhancing agents that may or may not cause toxicological problems. In the short term, the nasal route of administration seems to have many advantages since the mucosa here is far more permeable than the corresponding buccal, vaginal, or rectal barriers. Delivery systems for nasal products are already on the market. Implant systems – for example, those developed for LHRH and analogues thereof – are also commercially available. Davis thinks that future success in the field of peptide and protein delivery will be concentrated upon a greater knowledge of the relevant aspects of cell biology and processes such as signal recognition and receptor-mediated transport systems. A better understanding of how nature achieves similar delivery objectives, such as the sorting and selective trafficking of proteins or the selective tropisms of viruses, may provide clues, Davis said, for future development of novel delivery systems. He thinks that additional research in the field of epithelial and endothelial transport would be a reasonable starting point.

Davis' Conclusion

In conclusion, Davis said that the field of drug delivery and drug targeting offers many exciting challenges and possibilities for a whole range of scientific disciplines. Successful drug delivery development groups normally comprise individuals from a wide variety of backgrounds, including cell biology, pharmacology, physiology, medicinal chemistry, colloid science, polymer science, and others. According to Davis, novel delivery systems could have a very important role to play in the future, especially for cancer chemotherapy and for the biological mediators in the form of peptides and proteins.

Hydrogels and Drug Delivery

An excellent review of this area as well as recent studies were presented by A.T. Florence (Department of Pharmacy, School of Pharmacy and Pharmacology, University of Strathclyde, UK). Florence said that Wichterle and Lim (1960) must be credited with the introduction of hydrogels as biomaterials following their report of the synthesis of gels of poly (hydroxyethylmethylacrylate), called polyHEMA. Hydrogels can be defined as three-dimensional networks of hydrophilic polymers, generally covalently or physically cross-linked, which interact with aqueous solutions swelling to some equilibrium value, but not dissolving. They are sometimes known as Type I gels to distinguish them from reversible, Type II gels formed from polymers such as poly (vinyl alcohol); see Table 1. Hydrogels form a diverse range of materials whose physical, chemical, mechanical, and therefore biological properties depend on the physicochemical nature of the polymers used in their synthesis, on the nature of the cross-linking molecules, and on the degree of cross-linking. Florence said that the principal interest in hydrogels as drug delivery vehicles lies in the fact that they have a high degree of compatability with biological surfaces and tissues.

Table 1. Some definitions of the gel state.

Type I: irreversible systems with a three-dimensional network formed by covalent bonds between the macromolecules; also known as hydrogels.

Type II: heat-reversible gels formed by intermolecular bonds such as hydrogen bonds or mediated by additive molecules. Solutions of type II systems gel at a temperature known as the gel point.

Heterogels: gels of either type I or type II which form heterogeneous gel structures due to differential solubilities of segments of the macromolecule, often a block copolymer of the type AAAABBBBAAAA.

Xerogel: the material left after removing most or all of the water from a hydrogel.

The hydrophilicity of these systems and the fact that they can be designed to form a range of materials in various configurations, from monolithic implants to medical dressings, porous "sponges," nonporous gels, and films or coatings on non-hydrogel substrates suggests, according to Florence, that they have a future in enhancing quantitative approaches to drug delivery in novel ways, particularly as their structural and mechanized parameters can be to some extent predetermined and their permeability predicted. Emulsions of the w/o/w type have been stabilized by hydrogel formation of poloxamers in the continuous phase by Florence and coworkers. Florence said that grafting of hydrogels onto other surfaces is sometimes necessary because of their poor mechanical strength, particularly those hydrogels with high water content. Both bulk and surface properties have importance in relation to drug release rate, biocompatability, and, in certain systems, biodegradation.

Terminology

The term "hydrogel" (Table 1) can be applied more generally than to those systems described above which have stable cross-links, although Florence, in his presentation, focused on cross-linked systems. Many hydrophilic polymers will themselves form gels (Type II gels) without cross-linkers, and some of these are of interest in medicine and pharmacy, according to Florence.

The principal difference is the stability of the union between the main polymer chains in the three-dimensional network. In non-crosslinked systems, the molecules can dissolve, although slowly, whereas dissolution of hydrogels does not occur, except when they contain biodegradable elements.

Cross-linked hydrogels are irreversible gel systems, whereas Type II gels are heat reversible. Poly (vinyl alcohol [PVA]) solutions, for example, gel on cooling, the gel point in water being 14°C. PVA can be induced to gel by the addition of borax to the system, but in spite of the utility of Type II gels, drug release from them cannot be controlled as accurately as that from hydrogels, Florence said, because the dissolution rate of the polymer, and subsequent formation of viscous solutions, which largely controls drug transport, cannot be engineered to the same tolerance as the swelling and pore size of hydrogels. On the drying of hydrogels, a xerogel is obtained (Table 1). These drug systems can be implanted *in vivo* to swell in contact with body fluids, according to Florence.

Uses Of Hydrogels

Some potential and actual uses of hydrogels as coatings, homogenous systems and devices are given in Table 2. This wide applicability suggests, according to Florence, that many of these systems, when loaded with drug, can be transformed into delivery systems for a variety of routes of administration. Some possible applications of drug delivery systems are listed in Table 3; hydrogels can be applied as reservoirs or rate-limiting membranes to any of these routes. For such uses, hydrogels have special advantages over other synthetic polymers, partcularly when the hydrogel and biological surfaces are in close contact. They resemble biological tissue in many properties, according to Florence, having generally a high water content, being porous, and being usually flexible – this last reducing mechanical injury to tissue. The ability of the hydrogel matrix to swell also means that initiators and other materials used in their synthesis can be readily removed by appropriate solvent washing procedures. Their permeability also allows ions and nutrient molecules and metabolites to move to and from tissues surrounding implanted hydrogel materials. Their permeability to oxygen has led to some being employed as soft contact lenses – tolerance to the hydrogel being greater than to the less hydrophilic materials. Florence said that the nature of the hydrogel/water interface is such that the interfacial tension is low and adsorption onto the gel surface is minimized.

Table 2. Biomedical Applications of Synthetic Hydrogel.

Coatings	Homogeneous materials	Devices
Sutures	Electrophoresis gels	Artificial organs
Catheters	Contact lenses	Drug delivery systems
IUD's	Artificial corneas	Photoresponsive membranes
Blood detoxicants	Vitreous humor replacement	
Vascular grafts	Soft-tissue substitutes	
Cell-culture substrates	Burn dressings	
	Bone ingrowth sponges	
	Dentures	
	Ear drum plugs	
	Synthetic cartilages	
	Haemodialysis membranes	
	Carriers of cytotoxics	
	Wound dressings	
	Encapsulation of cells	

Monomers And Cross-Linkers

Some common monomers used in the preparation of hydrogels are given in Table 4. The several monomers listed can be cross-linked by different molecules, according to Florence, which expands the possibilities and leads to the potential of a range of materials tailored by the choice of monomer, monomer concentration, and cross-linker and cross-linker concentration. PolyHEMA alone has been used in a variety of ways (Table 5), its proper-

Table 3. Characteristics of sites for application of drug delivery systems.

Site	Therapy: Locus of therapy	Therapy: Duration	Therapy: Examples	Duration of delivery system	Biomaterials limitations
Skin	Local	Acute (A)	Dermatology	1-7 days	Local reaction
Skin	Systemic	Acute	Antiemetics	1-7 days	Adhesion
Skin	Systemic	Chronic (C)	Hypertension	1 week (?)	Adhesion
Subcutaneous or Intra-muscular space	Systemic	Chronic	Infectious diseases/ endocrinology	1-7 days 3 months (?)	Insertion migration and encapsulation
Mucosa:					
(1) Ocular	Local	Chronic	Glaucoma	1 week	Size
	Local	Acute	Infection	1-7 days	Size
	Systemic	Acute	(?)	(?)	Size
(2) Vaginal	Local	Chronic	Contraception	1 month	Biomechanics
	Local	Acute	Infection	1-7 days	Biomechanics
	Systemic	Chronic	Contraception	3-12 months	Biomechanics
	Systemic	Acute	(?)	(?)	Biomechanics
(3) Buccal	Local	C/A	(?)	1 day	Adhesion
	Systemic	C/A	Cardiovascular	1 day	Adhesion
(4) Rectal	Systemic	C/A	(?)	1 day	Expulsion
(5) Uterine	Local	Chronic	Contraception	1 year +	Insertion
Intravascular	Systemic	Acute	Hospital diseases	1-21 days	Thrombosis

ties controlled, to some extent, by the cross-linker used and by solvent additives present during the process.

Preparation. Three-dimensional networks can be synthesized by:

1. Bulk polymerization of monomer with cross-linking reagent
2. Cross-linking of the polymer in solution
3. Simultaneous copolymerization and cross-linking of monomer with a cross-linker in solution
4. Gamma-irradiating a hydroxylic polymer such as high-molecular-weight poly (oxyethylene) glycols or the poloxamer or polyamine surfactants
5. Chemically initiating reactions between bifunctional monomeric polymers.

If method 3 is adopted, polymerization can be achieved close to ambient conditions and the gel can be formed into the shape of the container (mold). According to Florence, choice of solvent during polymerization is important, as the solubility of the growing polymer in the solvent can determine the degree of homogeneity of the system. When water exceeds certain concentrations (40 to 60 percent) opaque, heterogeneous, macroporous gels can be formed. The presence of water-soluble additions such as glycerol, AVP, or diacetin alters the mechanical and physical properties of polyHEMA hydrogels. Florence and coworkers have clearly shown the morphological changes on increasing the concentration of monomer (poloxamer surfactant) in the starting solution before gamma irradiation, the porosity decreasing with increasing concentration in these systems which are self cross-linked. Florence and coworkers have shown that markedly different structures could also be formed by altering the ratio of hydrophobic and hydrophilic polaxomer monomer, cross-linked in the liquid state by initiator (Law et al., 1984; and Law et al., 1986).

Florence said that techniques for grafting hydrogel polymers onto other surfaces have been reported (Hoffman, 1984). The polymer surface is prepared for interaction with the hydrogel monomer by exposure to ionizing radiation, electric discharge, or other means of produc-

Table 4. Monomers used in the synthesis of hydrogels.

Nonionic
Hydroxyethyl methacrylates [HEMA]
2,4, pentadiene-1-o1
acrylamide derivatives
N-vinylpyrrolidone [NVP]
vinyl alcohol
block copolymeric surfactants (e.g., polyoxamers)

Anionic
acrylic acid derivatives
sodium styrene sulphonate

Cationic
aminoethyl methacrylate
vinylpyridine

Cross-linkers
ethylene glycol dimethacrylate [EGDMA] derivatives
methylene-bis-acrylamide

Table 5. Biomedical uses of polyHEMA.

Antibiotic delivery (from coated sutures and urethral catheters and in otolarygology)
Antitumor drug delivery device
Coated intrauterine device
Coated sutures
Soft contact lenses
Ureter prosthesis
Breast augmentaiton

ing surface reactive species. The reactive sites will be the focus of polymerization when monomer is present. Homogeneous and interpenetrating networks of hydrogel polymer in polymeric substances can also be achieved, according to Florence. Copolymerized HEMA and NVP have been, for example, incorporated into silicone rubber by irradiation-induced polymerization, producing systems whose surface characteristics were altered from hydrophobic to hydrophilic, thus decreasing the deposition of fibrin and cellular elements when used as an artery-vein shunt. Florence said that order, roughness, coatings, and heterogeneity all play a role in biointeractions. PolyHEMA devices have been treated by plasma discharge to control the release of pilocarpine.

Swelling

Florence said that the swellability of hydrogels is controlled by the hydrophobicity of the polymer backbone, the hydrophilic characteristics of the cross-linker and the degree of cross-linking. The extent of swelling decreases as the degree of cross-linking increases; swelling can be related to drug release rate. Additives in the solvent which influence water structure will affect the degree of swelling. Swelling-controlled release systems have been studied in particular by Peppas (1987). Peppas defines solvent-activated controlled-release systems that include products where the drug release is triggered or controlled by water or biological fluids and includes swellable systems, swelling-controlled systems, and osmotically controlled systems. Swellable systems are based on polymers which swell rapidly without dissolution and which have release kinetics controlled by the pore network rather than the polymer, whereas in the swelling-controlled systems the swelling process controls release. According to Florence, swelling – and hence permeability – can be controlled by external factors such as pH, UV radiation, and enzymatic activity in systems designed as responsive drug delivery vectors.

Drug Delivery

Controlled drug release from hydrogel matrices have been considered from a theoretical standpoint by several investigators (Gander et al., 1987) as well as by Florence. Applications, according to Florence, include such varied targets as periodontal disease, wound repair (with sustained release of epidermal growth factor), vaginal and cervical (release of prostaglandins), rectal (administration of morphine), and delivery of anticancer drugs and antibiotics. Hydrogel contact lenses have been impregnated with pilocarpine and other drugs for sustained delivery to the eye.

Membranes and Control of Permeability

Florence said that hydrogels can be fabricated as membranes. In advanced delivery systems this offers the ability to control drug permeation rates by taking advantage of the extent to which hydrogel properties can be controlled by enzymatic reactions, heat, pH, or UV irradiation. Among the many literature reports, according to Florence, are ones on the glucose-sensitive membranes containing glucose oxidase, macroporous membranes for an artificial pancreas, and photosensitive systems. Photostimulation of dilation and contraction of polymer gels has also been achieved, allowing the possibility of control over permeability of the membranes.

According to Florence, the pH sensitivity of some hydrogels is quite remarkable. PolyHEMA systems containing small amounts of MMA swell dramatically in dilute urea, not by a direct effect but on decomposition of the urea to ammonium ions at pH greater than 6.

Florence said that incorporation of enzymes into hydrogel matrices can control their permeability by: (1) affecting the protonation of basic side chains, thus causing swelling (for example, the glucose-gluconic acid transition catalyzed by glucose oxidase); (2) by affecting the oxidation state of redox polymers; and (3) by affecting the pH of the medium and causing breakdown of pH-sensitive polymers (hydrolytically unstable polymers such as the n-hexyl half ester of methyl vinyl ether-maleic anhydride copolymer) inside an albumin-urease system cross-linked with glutaraldehyde (Heller et al., 1987).

Thermosensitive polymers have been suggested as "on-off" switches for drug release (Bae et al., 1987). A cross-linked poly (N-isopropylacrylamide)-butyl methacrylate system was used which responds to change in temperature over a 10 degree range. Kumar et al. (1987) have recently exploited low-voltage electrophoretic techniques to control the passage of insulin across a hydrogel membrane.

Florence said that a more passive use of hydrogels has been the fabrication from polyacrylonitrile hydrogels of "soft pliable pouches" for the topical delivery of beta-aminopropionitrile (BAPN), a collagen polymerization inhibitor, for acute spinal cord injury in dogs. Perforated, this hydrogel of high mechanical strength was used as a spreader of BAPN following its delivery from an osmotic pump.

Wound Dressings

Contact between hydrogel and biological tissue is at its most intimate in wounds, according to Florence. Table 6, from a review by M. Chvapil (1982), shows a list of the characteristics of the optimal wound dressing material. Florence said that xerogels applied to wounds absorb moisture and aid wound healing. Dextranomer, for example, is a cross-linked dextran containing sorption

Table 6. Characteristics of the optimal dressing material

Functional Characteristics	Biological Characteristics	Technical Characteristics
Controls evaporation, loss of exudate, and thermoregulation	Nontoxic	Available in any size and thickness
Protects against secondary bacterial contamination	Nonantigenic	Easy sterilization and resterilization
Debrides the wound under redressing	Insoluble matrix	Stability of properties under storing
Protects against mechanical insults	Pliable and resilient	Versatility of uses on various types of wounds
Binds and immobilizes fluid		
Adheres to wet substrate		
Serves as drug delivery for any medication		

modifiers, available as beads 100 to 300 μm in diameter. *In vitro* 5 g dry weight of dexatranomer beads absorb 16 g of water or 11 g of blood, according to Florence. Iodosorb is a cross-linked dextranomer containing 0.1-percent iodine to act as an antiseptic. Florence said that antibiotic-impregnated dressings are also available. Other materials used in wound care include Vigilon, Scerisorb, and Geliperm.

Florence concluded by saying that hydrogels form an almost infinite class of polymeric materials, some of which may be biodegradable as well as highly biocompatible. He believes that in the future their application will be restricted only by the ingenuity of those who design delivery systems.

Mucoadhesives for Delivery Across Mucous Membranes

J.R. Robinson (School of Pharmacy, University of Wisconsin, Madison), who addressed this subject, said that mucoadhesive drug delivery systems are therapeutic systems that have incorporated into them materials, typically polymers, capable of binding to the glycoprotein components of the mucous overlying mucosal epithelia. They can be used on a variety of mucosal epethelia that have demonstrated capability for or show characteristics amenable to drug absorption. According to Robinson, mucoadhesive systems have two primary goals. The first is to "localize" the delivery of the therapeutic agent from a well-defined region of the selected epithelial surface. In this way, it functions as a "platform" for drug delivery. This goal is achieved through the use of a mucoadhesive polymer – with well-defined binding characteristics to the desired epithelium – incorporated into the system in such a way that it maximizes its intimacy and duration of contact with the tissue surface. According to Robinson, the selection and characterization of such a polymer, and the accompanying fabrication of a suitable delivery system that effectively utilizes its mucoadhesive properties, require a substantial amount of research effort. Robinson said that several studies currently being conducted in his laboratory as well as others are aimed at elucidating the structural requirements and mechanisms for mucoadhesion in order to optimize the affinity and specificity of binding.

The second goal of mucoadhesive systems, according to Robinson, is to allow control over the rate of absorption of the therapeutic agent into the circulation. This can be accomplished through design characteristics that modify either the rate of release of drug from the system, or the permeability characteristics of the epithelium, or both. Robinson said that much effort over the past decade has been devoted to the issue of physically controlling the release rate of drug out of a delivery system and that while this is hardly a trivial problem, many techniques are available in the literature. On the other hand, according to Robinson, it remains an extremely challenging task to modify the permeability characteristics of the tissue without damaging it, due to the lack of a clear understanding of how drug molecules traverse epithelial membranes.

Advantages of Mucoadhesive Drug Delivery

According to Robinson, mucoadhesive delivery systems offer several potential advantages over conventional systems. Localization enables selection of a specified region or type of epithelium from which absorption is desired to occur. It confers a degree of immobility to the system, which circumvents many physiological clearance processes that occur on mucosal epithelia and thereby allows less frequent dosing. Robinson said that the most obvious example of one of these processes is gastrointestinal motility, a powerful force that propels material along the entire length of the gastrointestinal (GI) tract. Thus, Robinson said, one might envisage oral mucoadhesive dosage forms requiring infrequent administration, designed for placement in selected regions of the GI tract. Immobilization, coupled with absorption rate control, would also be expected to reduce fluctuations in blood drug levels commonly observed with conventional systems, thus reducing intra- and intersubject variability in

therpeutic response. According to Robinson, increased intimacy and duration of contact with the absorbing tissue should result in less nonabsorptive drug loss, and if univectoral systems are fabricated – from which drug release occurs only in the direction of the absorbing surface – this benefit will be even more pronounced. Localization also affords the opportunity to manipulate factors affecting drug absorption in a circumscribed area of epithelium through the use of penetration enhancers or metabolic regulators. Robinson also said that the design of controlled release systems would also be greatly facilitated since a number of variables can be more easily isolated and studied independently.

Peptide Drug Delivery and Mucoadhesives

Robinson said that over the past years, a great deal of interest has been generated in the use of peptides for drug therapy, and the accompanying issue of peptide drug delivery adds another important dimension to the argument that supports the development of mucoadhesive systems. They have the potential to address many of the problems presented by the transport of peptides across epithelial tissues. One example is the incorporation of peptidase inhibitors into the system to quench inactivation of the peptide by tissue peptidases. Such an application requires an understanding of the mechanisms of the movement of peptides through epithelial tissues, Robinson said. He and his group have begun studies aimed at achieving this understanding, and some of the results obtained thus far were presented as follows.

Comparative Aspects of Various Routes of Administration for Peptide Delivery from Muco-adhesive Systems. Robinson said that the success, in terms of patient acceptance, of peptides as drugs, will ultimately be determined by the convenience of the route and the method of delivery. The most convenient route is undoubtedly oral, but because of the presence of a wide variety of digestive enzymes, oral delivery does not appear promising. He said that, in addition, it would be desirable to have the ability to exert control over the location and removal of a mucoadhesive system from a particular region of the GI tract after it has been swallowed, and existing technology does not yet allow this. Thus, we are left with choosing between various other non-parenteral routes of administration. Robinson and coworkers selected the buccal route for two primary reasons: the region is accessible for placement and removal of a mucoadhesive system, and hepatic first-pass metabolism is bypassed.

Robinson said that the problem with buccal epithelia with respect to drug delivery is twofold: (1) it is not well characterized with respect to absorptive properties, especially those relevant to peptide transport, and (2) it is difficult to employ *in vivo* experimental approaches with this tissue. Robinson said that many previous researchers have used excised tissue for *in vitro* permeability studies, and the state of the tissue in these experiments (and those utilizing epithelia from regions other than buccal) has been an issue of concern. While several investigations have been carried out on the permeability of various compounds through a number of different regions of oral mucosa from several animal species, there have been few studies that have attempted a systematic investigation of the *in vitro* permeability characteristics of this tissue, and of how *in vitro* results may relate to *in vivo* performance, according to Robinson.

Criteria for Evaluating Permeability of Epithelia. Robinson said that the task of pharmaceutical scientists engaged in research on epithelial drug transport is to characterize as precisely as possible the pathways through which water and solute movements occur, the driving forces responsible for these movements, and how they are regulated at the cellular and junctional level to maintain epithelial functioning. It is becoming customary for physiologists, he said, to refer to epithelia as "leaky" or "tight," based upon several experimentally observed properties that appear to correlate with the tightness of intercelluar junctions. The magnitude of these properties may be used by pharmaceutical scientists to estimate potential pathways and mechanisms for movement of drugs, especially peptides. These properties have not been previously examined in buccal epithelia, and Robinson and his group have initiated studies directed at the determination of several of them. To date, the hydraulic conductivity and spontaneous transepithelial potential difference have been measured. Robinson said that these values indicate that the buccal epithelium falls into the "leaky" category, similar to the small intestine.

Significance of Water Permeation Across Epithelia. According to Robinson, many of the problems associated with water permeation across epithelia are essentially the same as those for nonelectrolytes in general. Thus, knowledge of the permeability of water across a selected epitheium (such as buccal) may aid in understanding the route and mechanism of other compounds. He said that comparison of the value of diffusional water permeability for buccal epithelia to previously published values for other epithelia and single cells reveals some interesting results. For the cornea, which has been characterized as a "tight" epithelium, the value of diffusional water permeability is about a factor of four times greater than in buccal epithelia. Single cells, devoid of intercellular junctions, typically show a range of diffusional permeabilities that are from one to two orders of magnitude greater. On the other hand, the values for both frog and dog gastric mucosae, also tight epithelia, are very similar to buccal epithelia, according to Robinson. He said that these comparisons point out the fact that diffusional flux cannot be

confused with hydraulic flux as a criterion to judge relative "tightness" or "leakiness." In addition, Robinson said that the fact that single cells have greater permeabilities does not imply that the intercellular route is insignificant, but more likely that both the apical plasma membranes and intercellular junctions in these epithelia are substantial barriers to diffusion as compared to the single cells' membranes.

Significance of Tissue Viability in In Vitro Permeability Studies. Robinson said that previous researchers conducting *in vitro* permeability studies have used a variety of techniques to assess the maintenance of morphologic and biophysical integrity of the tissue. Among these are maintenance of electrical potential or transport of glucose across the epithelium, and duration of steady-state flux of some passively transported substance. Robinson and his group have measured the concentration of ATP in the buccal epithelium as an indicator of tissue viability. Results showed a nearly exponential drop in ATP concentration over a 24-hour period for buccal epithelium stored in Krebs-Ringer buffer at 37°C; this was accompanied by a fourfold increase in diffusional water permeability. These investigators are currently examining the relationship between ATP decreases and accompanying changes in permeability for other compounds in order to decide upon the utility of ATP determinations for assessing *in vitro* experimental approaches to both permeability and subsequent mucoadhesive studies.

Nasal Delivery of Protein/Peptide Therapeutics

In his discussion of this topic, J.P. Longnecker (California Biotechnology Inc., Mountain View) said that the biotechnology industry continues to introduce large numbers of peptide and protein molecules with therapeutic potential. According to him the availability of these bioactive molecules, made possible by advances in peptide sysnthesis methods and recombinant DNA technology, make this one of the fastest growing and most exciting areas in the history of pharmaceutical development. These drugs, which include hormones for replacement therapy as well as superagonists and antagonists of natural biological molecules, offer the potential advantages of high biological specificity, low dose levels, and few side effects. However, the production of peptides or proteins with specific therapeutic bioactivities is only part of the process required to produce a drug. Longnecker said that many classical pharmaceutical/pharmacological problems remain, including formulation, stability, toxicity, etc. Of particular interest to Longnecker are the problems associated with systemic delivery of peptide/protein drugs.

As therapeutic agents, peptides and proteins present unique problems with respect to drug delivery. The body is designed to keep out foreign proteins. The most favored method of drug delivery, the oral route, is not available for these drugs since these compounds are digested in the gastrointestinal tract. Longnecker said that the present solution to the use of peptide/protein therapeutics is subcutaneous or intramuscular injection. This form of delivery has several obvious disadvantages, he said, including patient compliance and less than optimal pharmokinetics. According to him, the widespread, patient-controlled use of peptides and proteins as drugs depends in large part on the development of reproducible, reliable, and noninvasive means for systemic delivery of these compounds.

Longnecker said that although peptides and proteins cannot be delivered via the oral route, other mucosal surfaces such as nasal, rectal, vaginal, and conjunctival membranes represent possible routes of delivery. He thinks that the nasal cavity is a particularly attractive site for transmucosal delivery of drugs. It is readily accessible and has a high level of acceptance as a route for drug administration. The vascular bed of the respiratory mucosa within the nose is well suited for rapid passage of fluid and dissolved materials from the blood to the tissues and vice versa. The rich submucosal blood supply and epithelial cells are particularly well suited for absorption of drugs into the systemic circulation, according to Longnecker. He also said that the fact that materials absorbed via the nasal route reach the systemic circulation before passing through the hepatic circulation offers advantages for many drugs which are subject to "first pass" metabolism.

Over the past several decades, a wide variety of pharmaceuticals have been administered intranasally. However, Longnecker said that the nasal administration of peptides and proteins is made more difficult than the administration of traditional, small-molecular-weight drugs (i.e., steroid hormones, nitroglycerin, etc.) since by themselves they do not cross the nasal mucosa to an appreciable degreee. He said that a number of approaches to enhance absorption of proteins and peptides have been attempted and that the most successful of these approaches has been the use of absorption enhancers.

According to Longnecker, the history of transnasal insulin delivery serves as a paradigm for nasal drug delivery of proteins. Attempts to utilize non-parenteral routes of administration are nearly as old as insulin therapy itself. As early as 1922, studies were reported which examined the efficacy of insulin delivery by intranasal, oral, rectal, and vaginal routes. This early work and that of many others through the 1960's met with very limited success. According to Longnecker, these early studies highlighted three major difficulties of intranasal delivery which were : (1) low bioavailability, (2) unreli-

able or low reproducibility of dosing, and (3) acute local toxicity associated with the use of absorption enhancers.

Longnecker said that recent work has focused on the use of a variety of surfactants as permeation enhancers. These include ionic polyoxyethylene ethers and bile salts. Although these studies indicated that formulations of insulin with bile salts or other surfactants could provide reasonable efficacy of transport and reproducible dosing, a number of concerns remained regarding the local toxicity and irritation of these compounds. Longnecker said that the potential toxicity of these surfactants led to the search for molecules that were equally or more potent, but which had an excellent safety record in humans. Attention was turned to derivatives of the steroidal antibiotic, fusidic acid. This antistaphylococcal antibiotic has been used widely in intravenous, oral, and topical formulations for the treatment of *Staphylococcus aureus* infections. According to Longnecker, these derivatives of the sodium fusidates are the subject of an issued patent and are the basis of the Nazdel^{R} (trademark of California Biotechnology Inc.) drug delivery systems. The preferred enhancer used in nasal insulin formulations, according to Longnecker, is the taurine conjugate of sodium 24, 25 dihydrofusidate (STDHF). This derivative is devoid of antibiotic properties and is highly effective as an enhancer of insulin absorption.

According to Longnecker, the use of Nazdel technology with insulin has proceeded through formulation, efficacy testing, and safety and toxicity studies in rat, dog and sheep models, and, most recently, a Phase I clinical trial. Longnecker said that the main purpose of this clinical study was to assesss the safety, reproducibility, dose response characteristics, and relative efficacy of the nasal route of administration. To analyze these factors, levels of serum insulin and blood glucose were measured over time following intranasal or intravenous insulin delivery. Multiple administrations of a nasal insulin formulation at two doses were given to 14 normal healthy individuals. The insulin was administered as a nasal spray using a metered spray valve. Each subject received three intranasal applications at a dose of 0.35 U/kg, three intranasal applications at a dose of 0.70 U/kg, and two bolus intravenous (IV) doses at 0.05 U/kg.

Longnecker said that measurement of plasma insulin showed that the insulin is very rapidly absorbed following nasal administration. Insulin delivery by subcutaneous injection (SC) is known to result in a sustained delivery of insulin (over hours) rather than a spike or pulse of insulin (over minutes). Insulin secretion in a healthy nondiabetic individual is pulsatile and on demand as determined by blood glucose levels. This pulsatile secretion is generally true of hormones and illustrates a significant advantage, according to Longnecker, of nasal delivery over SC or intramuscular injection of protein therapeutics. He said that the nasal route is not only more convenient and less painful, but allows for the design of therapies which more closely mimic protein plasma levels and responses seen in healthy individuals.

The data from this study, he said, also show that nasal dosing is more reproducible – as assessed by a number of criteria – than SC dosing. Some of these parameters are the time-to-maximum insulin concentration – i.e., lowering of blood glucose – and the maximal insulin levels (C max). He said that the C max values were also shown to be insulin dose-dependent following intranasal administration. Analysis of subject to subject variability for six dosings per subject showed that for any given individual the bioavailability was very consistent. The bioavailability following SC dosing is approximately 40 to 50 percent, according to Longnecker. The average bioavailability from serum insulin measurements was 11.4 percent and from blood glucose measurements 10.9 percent, indicating that essentially all insulin absorbed via the nasal route was biologically active.

Longnecker said that these data show that a nasal insulin formulation based on the use of the Nazdel permeation enhancer STDHF can provide a much needed form of insulin administration which is practical, acceptable, reproducible, and economically practical. In addition, he considers it important that the pharmokinetics of delivery of insulin via this route – i.e., the pulsatile administration – will allow for design of a therapeutic regimen which may alleviate many of the side effects of current insulin use.

Longnecker said that the possibilities of the Nazdel technology for a number of therapeutic proteins is being actively explored by his company. They have developed stable pharmaceutically acceptable formulations of a number of proteins, including human growth hormone and various forms of calcitonin, and peptides including LHRH and GHRH. Longnecker said that clinical studies have been very encouraging for these products and show both high bioavailability and bioactivity of intranasal forms. He also said that they have greatly expanded their toxicology data-base on the enhancer STDHF used in nasal formulations and are increasingly confident in its safety profile. It is intended to move quickly into further Phase I clinical trials with these formulations.

Polymers in Controlled-Release Systems

R. Langer (Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge) reviewed this area as well as presenting research from his laboratory. Langer said that over the past decade there has been increasing attention devoted to the development of controlled-release systems for drugs, pesticides, nutrients, agricultural products, and fragran-

ces. However, nearly all of the systems that have been developed have not been capable of slowly releasing drugs of large molecular weight (greater than 600). He said that up until 1976 it was commonly accepted in the field of controlled-release that effective systems could not be developed for macromolecules. However, Langer said that after several years of effort he and his group discovered an approach that permitted the continuous release of biologically active molecules as large as 2 million daltons from normally impermeable, yet biocompatible polymers for more than 100 days. Two different Monte Carlo techniques were developed for predicting transport in constricted pores of these polymer systems. Microstructural analysis techniques were developed and used to evaluate the mechanism of release. Based on these studies, systems were designed to release insulin for 100 days *in vitro* and *in vivo*, as well as anti-calcification agents and other molecules. More recently, Langer and his group have focused on the development of novel biodegradable polymer systems as well as modulated controlled-release systems which were discussed by Langer in his presentation.

Biodegradable Polymers

Langer said that biodegradable controlled-release systems have an advantage over other systems in obviating the need to surgically remove the drug-depleted device. In many cases, however, the release is augmented by diffusion through the matrix, rendering the process difficult to control, particularly if the matrix is hydrophilic and thereby absorbs water, and thus promotes degradation in the interior of the matrix. Langer said that in order to maximize control over the release process, it is desirable to have a polymeric system which degrades only from the surface and deters the permeation of the drug molecules. Achieving such a heterogeneous degradation requires the rate of hydrolytic degradation on the surface to be much faster than the rate of water penetration into the bulk. With this in mind, Langer and his group proposed that an ideal polymer would contain hydrophobic monomers, connected by water-labile linkages. In designing a biodegradable system that would erode in a controlled heterogeneous manner without requiring any additives, these investigators thought that polyanhydrides might be promising candidates due to the high hydrolytic lability of the anhydride linkage. Langer said that he and his coworkers have now examined several approaches for synthesizing polyanhydrides: melt polycondensation, dehydrochlorination, and dehydrative coupling.

He said that one major drawback of all previous work on polyanhydrides, was their low molecular weight (less than 40,000), which made them impractical for many applications. Therefore, he and his group conducted a systematic study to determine the mechanism of polymerization and the factors affecting the polymer molecular weight. The highest molecular weight polymers were obtained by operating under conditions which optimize the polymerization process while at the same time minimizing the depolymerization process. They were finally able to obtain exceeedingly high molecular weights of polyanhydrides by carefully taking into account the factors affecting both these mechanisms. For example, by reacting pure individually prepared prepolymers to produce P (CPP:SA) – a copolymer of carboxyphenoxy propane – and sebacic acid (1:4), a molecular weight of 116,800 was achieved in comparison to 12,030 when an unisolated prepolymer mixture was used. Langer and his group also studied the use of catalysts in polyanhydride synthesis. Since the reaction is an anhydride interchange which involves a nucleophilic attack on a carbonyl carbon, a catalyst which will increase the electron deficiency under the carbonyl carbon will affect the polymerization, according to Langer. He and his coworkers examined over 20 coordination catalysts in the synthesis of 1:4 (CPP-SA) copolymer. He said that significantly higher molecular weights in shorter times were achieved by utilizing cadmium acetate, earth metal oxides, and $ZnET_2$-H_2O. The molecular weights ranged from 140,935 to 245,010 with catalysts in comparison to 116,800 without catalysts. When acidic p-Toluene sulphonic acid (p-TSA) or basic 4-Dimethyl amino pyridine (4-DMAP) catalysts were tested, the acid catalyst p-TSA did not show any effect while the basic catalyst (4-DMAP) caused a decrease in molecular weight. Langer said that since the polymerization and the depolymerization reactions involve anhydride interchange which leads to a high-molecular-weight polymer with the removal of acetic anhydride as the condensation product (polymerization) and internal ring formation (depolymerization), catalysts affect both reactions, and optimizing the reaction time in the presence of catalysts is critical.

Langer said that he and his group have also developed two approaches for one-step solution polymerization of polyanydrides at ambient temperature. In the first approach highly pure polymers (> 99.7 percent) were obtained by the use of sebacoyl chloride, phosgene, or diphosgene as coupling agents and poly 4-vinyl-pyridine) or K_2CO_3 as insoluble acid acceptors. The second approach for one-step synthesis of pure polyanydrides was the use of an appropriate solvent where the polymer is exclusively soluble but the corresponding polymerization byproduct (for example, $Et_3N.HCl$) is insoluble.

Degradation Characteristics

The more hydrophobic polymers, PCPP and PCPP-SA 85:15, displayed constant erosion kinetics over 8

months, according to Langer. By extrapolation, 8-mm-thick discs of PCPP would completely degrade in over 3 years. The degradation rates were enhanced by copolymerization with sebacic acid. An increase of 800 times was observed when the sebacic acid concentration reached 80 percent. Langer said that by altering the CPP/SA ratio nearly any degradation rate between 1 day and 3 years can be achieved.

The effect of different backbones on erosion rates was demonstrated in a study of the homologous poly (p-carboxyphenoxy alkane) series. As the number of methylene groups in the backbone increased from one to six, thus decreasing the reactivity of the anhydride linkage and rendering the polymer more hydrophobic, the erosion rates underwent a decrease of three orders of magnitude, according to Langer.

Release Characteristics. Langer said that the release behavior depended on the formulation procedure. The best results were obtained with injection-molded samples. The drug release pattern followed closely that of the polymer degradation over a period of 8 months for PCPP. The correlation between release and degradation was still maintained in the more hydrophilic PCPP-SA 21:79, where both processes were completed in 2 weeks. Langer said that compression molding can also be used but the correlation between drug release and polymer degradation is not as good.

Microspheres. Langer and coworkers have also developed two approaches for producing polyanhydride microspheres. The first is a hot melt technique. Langer said that this process yields spherical microspheres, and the capsule yield is almost quantitative. The release of acid orange and insulin from PCPP-SA was studied. This approach, Langer said, is most useful for molecules that are exposed to the polymer melting temperature (76°C) without inactivation.

A second method to prepare polyanhydride microspheres – via solvent removal – has also been developed by Langer and his group. Polyanhydrides composed of the following diacids were used: sebacic acid (SA), bis (p-carboxyphenoxy) propane (CPP), and dodecanedioic acid (DD). Drug release was affected by polymer composition, physical properties of the microspheres, and type of drug. The potential for injectable microspheres (size range 1-300 μm) made of CPP-SA (50:50) for the controlled release of insulin was assessed. Both 5-percent and 10-percent w/w-insulin-loaded microspheres were prepared. Ten-percent-loaded microspheres produced the best clinical response, according to Langer, demonstrating 5 days of urine glucose control and 4 days of serum glucose control in diabetic rats.

Stability. Langer and his group studied the stability of poly(anhydrides), (PA) composed of the diacids: sebacic acid (SA), bis (p-carboxyphenoxy) methane (CPM), 1,3-bis(p-carboxyphenoxy) propane (CPP), 1,6-bis (p-carboxyphenoxy) hexane (CPH), and phenylenedipropionic acid (PDP), in solid-state and in organic solutions for a period of over a year. Aromatic PA – (poly(CPH) and poly(CPM) – were found to maintain their original molecular weight in both solid-state and solution while aliphatic PA – (poly(SA) and poly(PDP) – decreased in molecular weight over time. Langer said that the decrease in molecular weight showed first-order kinetics with activation energies of 7.5 Kcal/mol °K. The decrease in molecular weight was explained by an internal anhydride interchange mechanism, as shown from elemental and spectral analysis. Langer said that the depolymerization in solution can be catalyzed by metals. Among several metals tested, copper and zinc were the most effective. Studies on the stability of aliphatic poly(esters) in chloroform showed that poly (orthoesters) depolymerized over time whereas poly (α-esters) and poly (ethylenesuccinate) remained intact. Langer said that the solid-state stability of the polymers did not correlate with their hydrophobic stability.

Toxicity. Langer said that he and his group have conducted a large number of toxicity studies. and that the polymers were found to be extremely biocompatible *in vivo.*

In 1985, Langer and his group began a collaboration with a neurosurgery group headed by Dr. Henry Brem at Johns Hopkins University, Baltimore, Maryland, to explore the possibility of implanting polyanhydride discs containing the nitrosoureas BCNU and CCNU for brain cancer following surgery. Langer said that surface erosion would be critical for such drugs for if bulk erosion occurred, uncontrolled amounts of this potentially toxic drug could be released during breakup of the matrix. The Johns Hopkins group in conjunction with Nova Pharmaceuticals (US division) extended the safety studies and received Institutional Review Board approval to conduct human clinical trials with polyanhydrides. Langer said that the US FDA has now approved these polyanyhydrides for human clinical trials and at this time, five patients have been treated with the polyanhydride-BCNU combination. It is too early to know the efficacy of the treatment, Langer said, but all patients have been discharged from the hospital.

Pseudopolyamino Acids and Other Biopolymers

Langer said that he and his group have also developed several other novel biodegradable polymer systems. The most significant of these stems from the observation that many polymers used in medicine today were not initially designed for that purpose (for example, ethylene vinyl acetate was orginally used as a coating and is now used in controlled-release systems). Langer thought that it would be particularly useful to have avail-

able biodegradable biopolymers that are composed of – and would break down into – naturally occurring substances. Therefore, he and his group proposed and developed initial synthesis procedures for structurally new poly (amino) acids in which α-L-amino acids or dipeptides are polymerized by non-amide bonds (for example, ester, iminocarbonate) involving the functional groups located on the amino acid side chains, rather than the approach used extensively by others involving the amino acid termini. Langer and his group attempted the direct polymerization of suitably protected amino acids or dipeptides by polymerization reactions involving functional groups located on the amino acid side chains. He said that they considered this approach since it would permit the synthesis of biomaterials (for drug delivery systems, sutures, artificial organs, etc.), while also having other desirable properties – for example, the incorporation of an anhydride linkage into the polymer backbone could result in rapid biodegradability, an iminocarbonate bond may provide mechanical strength, and an ester bond may result in better film and fiber formation.

Langer and his group synthesized several polymers to initiate the development of this concept. In one case (single amino acid), Poly (palmitoyl-hydroxyproline (Pal-Hpr)-ester was obtained by melt transesterification of N-Pal-Hpr-Me in the presence of aluminum isopropoxide as catalyst. In a second case (diepeptide), the tyrosine (Tyr) dipeptide Carbobenzoxy-Tyr-Tyr-Hex was cyanylated at the tyrosine side chain hydroxyl groups to yield Carbobenzoxy-Tyr-Tyr-Hex-dicyanate. By solution polymerization of equimolar quantities of carbobenzoxy-Tyr-Tyr-Hex and carbobenzoxy-Tyr-Tyr-Hex-dicyanate in tetrahydrofuran, poly (carbobenzoxy-Tyr-Tyr-Hex-iminocarbonate) (poly CTTH) was obtained with a molecular weight of 19,500. Langer said that this latter study was aided by new analytical techniques which he and his group had developed and which enabled for the first time the identification and determination of organic cyanates in nanomolar quantities as well as a new polymerization technology they developed to synthesize polyiminocarbonates.

Considering the known adjuvanticity of L-tyrosine and its derivatives, Langer and coworkers thought that a polymeric antigen delivery system which could degrade into tyrosine or a tyrosine derivative could provide sustained adjuvanticity while simultaneously serving as a repository for antigen. In order to test this hypothesis, these investigators selected poly (CTTH-iminocarbonate) as a candidate material for a polymeric antigen delivery system. Poly-(CTTH-iminocarbonate) is a structurally new biodegradable polymer in which tyrosine dipeptide units are linked together by hydrolytically labile bonds via the tyrosine side chain hydroxyl groups. Their study showed that the release of antigen from poly(CTTH-iminocarbonate) gave rise, in mice, to higher antibody titers than release of the same dose of antigen from a comparable control polyiminocarbonate or complete Freund's adjuvant over a 1-year period even though a single injection was used. In addition to these studies, Langer and his group also evaluated polyiminocarbonates and polyphosphazenes as new biodegradable biomaterials and drug delivery systems.

Modulated Release Systems. Langer said that several polymeric systems capable of delivering drugs at increased rates on demand were studied and developed. The first system consists of drug powder dispersed within a polymeric matrix (generally ethylene vinyl acetate copolymer, EVAc), together with magnetic beads. Recently these investigators developed new apparatus for evaluating this system and systematically studied parameters affecting release rates *in vitro* and *in vivo*.

Release rates were controlled by an oscillating external magnetic field, which is generated by a device that rotates permanent magnets beneath the vials. By placing small plastic cages containing animals on the top disc, it can also be used for *in vivo* studies. Langer said that polymer matrices containing drug and magnets can release up to 30 times more drug when exposed to the magnetic field and that release rates return to normal when the magnetic field is discontinued. Langer said that the magnetically controlled implant does not cause inflammation *in vivo*. This was confirmed by the lack of edema, cellular infiltrate, or neovascularization as judged by gross histologic examination in the animals.

Magnetic Field Characteristics. The amplitude of the magnetic field was varied by increasing the distance between the external and embedded magnets or by changing the embedded magnet's strength. The extent of release enhancement was found to increase as the field amplitude rose. When the frequency of the applied field was increased from 5.0 Hz to 11.0 Hz the rate of release rose linearly.

To study magnet orientation, samples containing a single 1100-G magnet were used. In half the cases, the magnet was placed perpendicular to the applied field and in the other half it was oriented parallel to the field. The mean release rate enhancement was 2.1 times in the parallel cases, and 12.4 times in the perpendicular cases. Langer said that the difference between the two is presumably due to rotational torque. When placed in parallel, the magnet rotates in an attempt to align its pole vector with the field; therefore the displacement is smaller.

Polymer Properties. Langer said that the mechanical properties of the polymeric matrix also affect the extent of magnetic enhancement. For example, the modulus of elasticity of the polymeric matrix can be altered by changing the vinyl acetate content of the

polymer. The release rate of enhancement induced by the magnetic field increases as the modulus of elasticity of EVAc decreases.

Mechanism. Langer and coworkers have studied the release of macromolecules from EVAc systems not containing magnetic beads. They found that although molecules with molecular weights greater than 300 cannot permeate through the polymer, the direct incorporation of macromolecules in the polymer-macromolecule casting procedure caused a tortuous and complex series of pores to form within the matrix. Langer said that factors affecting permeation of water into the polymer and drug out of these pores determine the release rates. Video recordings of the polymer matrix surface showed that the beads actually move within the matrix in response to the external magnetic field and move adjacent material containing polymer and drug with it, "squeezing" out the dissolved drug through the pores. A model, proposed for the enhanced release, suggested that the major effect stems from the alternate compression and expansion deformation of the pores, causing the fluid within to undergo a pulsatile flow which alone (no net convection) is able to greatly improve diffusive mass transfer. A mathematical model was developed based on ideal axial diffusion in a cylinder with pulsed flow which describes the observed trend of increasing release rate with increasing frequency. This provides a preliminary estimate of drug release rate enhancement as a function of frequency of the oscillating magnetic field.

Release Dynamics. A flow-through spectrophotometer provided a continuous recording reflecting bovine serum albumin (BSA) release. Release rates were very stable at baseline. When a matrix was exposed to a 390-G magnetic field for 20 minutes the absorbence of BSA rose after the magnetic field was applied, plateaued at this elevated level, and then returned to baseline after the field was withdrawn. Within the time constraints of the system the rise and fall were instantaneous, and the increased release remained at a constant elevated level for the duration of field exposure.

Refractory Time. Langer said that the modulation (release rate during magnetic exposure compared to release rate without magnetic exposure) was independent of the duration of the interval between repeated pulses. The same modulation was elicited for the same strength magnetic fields regardless of the time between application of the pulses.

Field Amplitude. When the strength of the magnetic field was altered by adjusting the input voltage to an electromagnet, modulation changed accordingly. Langer said that the relationship between modulation and the strength of the applied magnetic field ranging from 200 to 700 G is linear.

Controls. Heat had little effect on the rate of BSA release. The rate of BSA release was nearly constant from polymer matrices transferred every 2 hours from 25 to 37°C. Also, the heat generated by the electromagnet did not alter release rates from polymer matrices. The temperature rose at the electromagnet surface at a rate of 8°C/hr for the first 30 minutes after the magnet was turned on. The rate of BSA release from a polymer matrix without an embedded magnet adjacent to the electromagnet face did not change over the time period studied and for 7 hours afterward.

In Vivo Studies. Langer and his group used a (3_H) insulin model for monitoring *in vivo* release kinetics (insulin is a polysaccharide of 5200 daltons that is not metabolized, or reabsorbed, or secreted by the kidney). Using this as a marker, the above effects observed *in vitro* (response time, response duration, amplitude) were also observed *in vivo*.

Furthermore, implants composed of EVAc-embedded magnets and bovine zinc insulin were placed SC in diabetic rats for 2 months. After implantation the blood glucose level decreased due to diffusion of insulin from the polymer. When the diabetic rats were exposed to an oscillating magnetic field, the blood glucose levels were further lowered below the basal level depending on the magnetic field conditions. Langer said that these results were confirmed by radioimmunoassays. He said that this phenomenon was not observed in: (1) diabetic rats receiving EVAc implants with insulin without a magnet, (2) control rats receiving implants with a magnet but without insulin, and (3) diabetic rats not containing any implant. All these animals were exposed to the same manipulations as the experimental groups of animals.

Ultrasound Modulation. Langer said that he and his coworkers also discovered that ultrasound could affect the release of substances from polymers. The ultrasound system has a potential advantage over many other systems, according to Langer, in that no additional substance (for example, magnetic bead) is required in the polymeric matrix. Furthermore, in the case of ultrasound the polymer can be injected, and since it can be erodible there is no need for surgical removal. Langer said that the application of ultrasound in humans, both for diagnostic and therapeutic purposes has been studied extensively and is considered a safe practice. Enhanced (up to 20 times baseline) polymer erosion and drug release were observed when the biocrodible samples were exposed to ultrasound. The system's response to the ultrasonic triggering was also rapid (within 2 minutes) and reversible. This was determined by an on-line UV spectrophotometric response in a closed-loop detection system where the concentration of the releasing agent was continuously monitored.

Langer said that the enhanced release was also observed in nonerodible systems exposed to ultrasound where the release is diffusion-dependent. This was tested on EVAc copolymers loaded with BSA or insulin. The

released insulin was also evaluated by high-pressure liquid chromatography (HPLC). No significant difference was detected between insulin samples exposed to ultrasound and unexposed samples, suggesting, according to Langer, that the ultrasound is not degrading the releasing molecules.

The effect of ultrasound on nonerodible polymers suggests that ultrasound also affects the diffusion of the releasing molecules in addition to polymer degradation. Support for this can be found, according to Langer, when comparing release rates of erodible polymers, where the increase in the rate of drug release is more pronounced than that of the polymer degradation. As the drug release is both matrix-degradation- and diffusion-dependent, the study suggests, Langer said, that ultrasound affects both processes.

Experiments were also performed to evaluate if the extent of enhancement could be regulated externally. By varying the intensity of the ultrasound, the degree of enhancement for both polymer degradation and drug release for the bioerodible and nonerodible systems could be altered tenfold, according to Langer.

In vivo experiments were performed using para aminohippuric acid (PAH) as a marker inside the polymers. Data of rat's PAH concentration in the urine after implantation showed that the PAH concentration in the urine is pronounced during exposure and mainly in the timespan just after the exposure. Langer said that the delay is presumably the diffusion time from the implantation site to the bloodstream and then the removal by the kidneys. When control animals were treated by the same procedure – i.e., placing the ultrasonic applicator head over the treated area with the power level of the ultrasound set to zero – no effect of ultrasound could be detected. Also, there was no difference in the rat skin histopathology of ultrasound-treated areas after an exposure of 1 hour at 5 W/cm and untreated areas.

Langer said that he and his group proposed that cavitation and acoustic streaming are responsible for this augmented degradation and release. In experiments conducted in a degassed buffer, where cavitation was minimized, the observed enhancement in degradation and release rates was much smaller. It was also considered that several other parameters (temperature and mixing effects) might be responsible for the augmented release due to ultrasound. However, experiments were conducted which suggested that these parameters were not significant. A temperature rise of only 2.5°C was recorded in samples during the triggering period. A separate release experiment done at 40°C instead of 37°C, however, showed that the rate increase was below 10 percent. Langer said that this suggests that the enhancement phenomena cannot be attributed to heat generated. To evaluate the ultrasound effect on the diffusion boundary layer, release experiments were performed under vigorous shaking. The increase of the release rates due to shaking were always below 20 percent. Therefore the effect of the ultrasound on the augmented release cannot be due to mixing only, according to Langer. Even though the ultrasound may eliminate or diminish the diffusion boundary layer, that effect cannot be responsible for the 10- to 30-fold increase in release rates.

Enzyme-Mediated Release. Langer and coworkers have also developed an approach for feedback control of polypeptides incorporated within polymeric drug delivery systems. This approach is based on the observation that changes in pH can cause dramatic shifts in the solubility of polypeptide drugs; solubility is one of the prime determinants of release rate in any diffusion, dissolution, or osmotic controlled-release system, according to Langer. The system components involve an external trigger molecule and a polymer-bound enzyme that, in the presence of the trigger molecule, will cause acid or base to form. To test this concept, Langer and his group used insulin as a drug and diabetic rats as the animal model. This model has been used for several previous systems such as lectin-bound insulin and glucose sensitive membranes. These investigators chose to adapt a biocompatible ethylene vinyl acetate (EVAc) polymeric insulin delivery system capable of treating diabetic rats for over 100 days. To establish feedback, they utilized the fact that insulin solubility is pH dependent and that, in the presence of glucose oxidase, glucose is converted to gluconic acid. Thus, when this enzyme is incorporated within a controlled-release polymer matrix, external glucose should theoretically reduce the pH in the polymer microenvironment. Langer said that since the isoelectric point of insulin is 5.3, when the polymer is exposed to the physiological pH of 7.4, a decrease in insulin solubility and release rate is expected. This undesired effect is overcome by using a modified insulin which contains more basic groups and thus has a higher isoelectric point. Tri-lysyl insulin with an isoelectric point of 7.4 was synthesized for this purpose. The feasibility of this enzyme-mediated feedback mechanism was investigated by three sets of experiments: (1) the effect of glucose on the pH in the microenvironment of the polymer, (2) the effect of glucose on insulin release *in vitro*, and (3) the effect of glucose on insulin release *in vivo*.

When polymer matrices containing insulin and glucose oxidase were exposed to buffer solutions containing 1000 mg/dl glucose, a decrease of nearly 0.5 pH units inside the matrix was recorded after 4 minutes; the pH then stabilized. The effect was reversible. The pH returned to its original value when the solution was replaced with fresh buffer containing no glucose. Control matrices containing no enzyme did not show any changes in pH after exposure to glucose solutions.

The results of insulin release from different groups of polymer matrices before, during, and after glucose ex-

posure over a 1-month period were analyzed by the method of analysis of variances with repeated measurements. When polymer matrices containing enzyme and tri-lysyl or regular insulin were exposed to glucose, an average increase in release rate of tri-lysyl insulin of 19 percent and an average decrease in release rate of regular insulin of 29 percent were observed. Control polymers containing either regular or tri-lysyl insulin with no enzyme showed no response to glucose.

The results of serum insulin levels in different groups of diabetic rats before and during the infusion of glucose showed a significant increase in serum insulin level when the diabetic rats, implanted with polymer matrices containing tri-lysyl insulin and enzyme were infused with glucose, compared to the insulin levels before glucose infusion. Similar to the previous results *in vitro*, a decrease in insulin release rate was observed when the diabetic rats implanted with polymer matrices containing regular insulin and enzyme were infused with glucose. The control diabetic rats implanted with polymers containing no enzyme or with no polymer implanted showed no insulin response to glucose infusion in both cases.

Design of Transdermal Systems

Transdermal systems were addressed by J. Hadgraft (Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff, UK). He said that over the past decade there has been a large increase in the interest in transdermal drug delivery. A number of system designs have emerged but certain limitations common to all can be identified. Hadgraft then summarized the advantages and disadvantages of transdermal delivery.

On the positive side, this route of administration reduces the problems associated with first-pass metabolism. If the first-pass effect is avoided, low daily doses are feasible. Delivery into the circulation should be controlled by the device which will minimize inter- intrapatient delivery. Since some systems deliver drug with a zero order rate for periods of up to 1 week, constant sustained levels of drug in the plasma can be achieved. If unwanted side effects are produced, drug input is easily terminated with an instantaneous drop in plasma levels.

On the negative side, this route of delivery is limited to potent drugs. The skin provides a very effective barrier to drug ingress, which creates a limitation on the total daily dose that can be delivered. Typically this cannot exceed a few milligrams unless permeation enhancers are employed or the delivery system covers an excessively large body area. Due to the lipophilic nature of the skin, ionized drugs are difficult to deliver although recent developments have indicated the feasibility of employing iontophoresis to improve absorption. This technological development is important for the new generation of peptide drugs. The structure of the epidermis also precludes the delivery of extremely lipophilic drugs, and optimal log (octanol-water partition coefficients) appear to be in the range of one to two. Once the active substance has penetrated through the outer layers of the skin it reaches living tissue where it can create an irritant or allergic response. Components of the delivery system can also be a potential problem; for example, plasticizers from the polymers and adhesives and formulation components in the drug reservoir. This problem may be exacerbated by the presence of penetration enhancers. Although transdermal delivery minimizes first-pass metabolism the drug can encounter other enzyme systems. Microflora on the skin surface are capable of metabolizing some drugs (for example, nitroglycerin and steroid esters). Within the skin itself, a number of enzymes exist which are capable of Phase I oxidation, reduction, and hydrolysis reactions and Phase II conjugation reactions. Nonspecific esterase activity is probably the most significant of these, according to Hadgraft.

In some disease conditions, sustained constant blood levels are not desirable since tolerance can develop. According to Hadgraft, the subject is complex and, in the case of nitroglycerin, is currently being investigated in a multicenter clinical trial. It is possible to design systems in which the drug input is pulsed, which provides the body with resting periods. Another area that is often disregarded in controlled drug delivery is the biological half-life of the substance, according to Hadgraft. He said that it is pointless to produce a sophisticated transdermal delivery system for a drug which possesses a long half- life of elimination. Despite the problems involved, Hadgraft thinks it is possible to foresee a continuing development in transdermal drug delivery which will be optimized by appropriate design strategies.

According to Hadgraft, the feasibility of delivering a drug candidate via the transdermal route can be established using a kinetic model of the release and absorption processes. The *in vitro* release from the device will be a function of the design of the system and the physicochemical properties of the drug. Absorption and subsequent elimination of the drug will be related to both its physicochemical and pharmokinetic parameters.

Basic Design Strategies. Hadgraft said that three major design types can be identified for transdermal drug delivery with variants within these. They are membrane-moderated systems, matrix systems, and conventional "ointment" dosage forms:

1. Membrane-moderated. In these systems a reservoir of the drug (usually a saturated solution) is sandwiched between an inert backing membrane and a rate-controlling membrane (Figure 1). Drug transfer through this membrane should be slower than diffusion across the skin. A saturated solution is employed in the

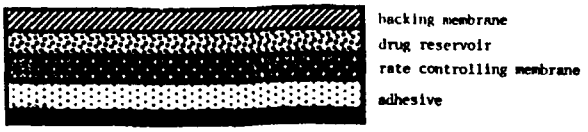

Figure 1.
Schematic representation of a membrane-moderated system.

reservoir in order to maintain a constant thermodynamic activity of the drug throughout the time course of application. This will ensure that there is a zero order flux through the membrane and hence to the skin surface. There is an adhesive layer of drug between the rate-controlling membrane and the skin which also contains the active drug. This serves as a loading dose to the skin. Hadgraft said that *in vitro* studies have shown that the adhesive layer releases drug rapidly with first-order kinetics. He also said that several transdermal systems which operate using this geometry have approval in the US. They contain clonidine, nitroglycerin, estradiol, and hyoscine (scopolamine). The first such membrane to be marketed contained scopolamine, and its design features are typical of the class. The reservoir contains the drug dispersed in a mineral oil-polyisobutylene mixture. A microporous polypropylene film forms the rate-controlling membrane which is designed to liberate drug at a rate of 3/μg/cm/h. This is contacted to the skin with a polymeric adhesive which contains 0.15 mg scopolamine. The surface area of the device is 2.5 cm. Steady release of the drug is maintained over the 3-day lifetime of the system. Figure 2 shows a comparison of transdermally and intravenously (IV) administered scopolamine. The results indicate the utility of the transdermal route of administration. A variant of this type of device can be produced by using the adhesive layer as the rate-controlling membrane (Figure 3). Frandol tape (Toseiyo, Yamanouchi; Japan) controls the transdermal delivery of isosorbide dinitrate using this approach.

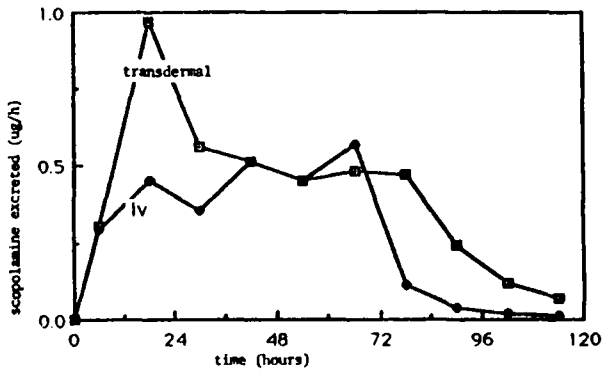

Figure 2.
Comparison of IV and transdermal scopolamine.

2. Matrix systems. In these devices the active drug is dispersed in a polymeric matrix. Diffusion through the polymer controls the rate of release. Various design

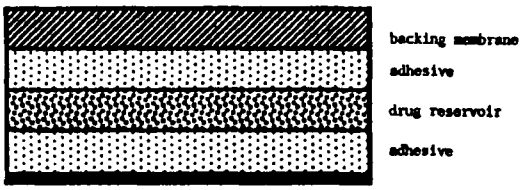

Figure 3.
Schematic representation of an adhesive controlled system.

strategies have been adopted. The adhesive may be positioned between the matrix and the skin. Alternatively it may be provided as a nonmedicated annulus around the periphery of the matrix. In some instances the adhesive itself can constitute the matrix. The major developments in matrix devices, according to Hadgraft, have been in the delivery of nitroglycerin; three major design strategies are as follows:

- Niro Dur. In the original system, nitroglycerin adsorbed onto lactose is distributed homogeneously in a polymeric matrix containing polyvinyl pyrrolidone and poly (vinyl) alcohol (Figure 4). A more recent formulation contains the GTN dispersed in acrylic-based polymer adhesives with a resinous cross-linking agent (Guy and Hadgraft, 1987). The former patch releases GTN linearly as a function of the square root of time. Of the 51 mg of drug contained in the 10-cm^2 system, 40 to 45 mg are released in a 24-hour period. The later development has reduced dimensions: 2.5 mg are released from a 5-cm^2 system in 24 hours. Hadgraft said that a larger, 30-cm device is also available.

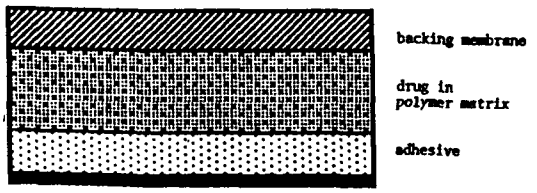

Figure 4. Schematic representation of a matrix system.

- Nitrodisc. Small drug reservoirs in the form of microcompartments are distributed within a silicone polymer (Figure 5). These devices are consequently referred to as "microsealed" drug delivery systems. The adhesive holding the device in place does not con-

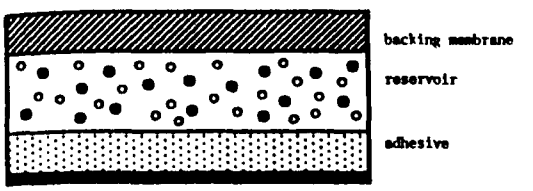

Figure 5.
Schematic representation of a microsealed drug delivery system.

tribute to the control of drug release. Typical delivery rates from a nitrodisc (8 cm) containing 16 mg GTN are approximately 15 mg over 24 hours. The amount released is a linear function of the square root of time.

- Deponit. GTN adsorbed onto lactose is dispersed in a nonhomogeneous manner into a 350-μm-thick adhesive film (Figure 6). The highest concentration is furthest from the skin. The system releases at approximately 10 $\mu g/cm^2/h$ after an initial burst phase of 3 hours' duration.

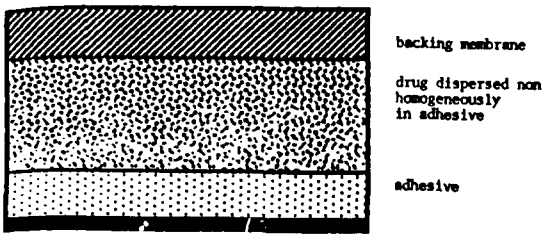

Figure 6.
Schematic representation of a matrix system, drug nonhomogeneously dispersed.

3. Conventional. Simple topical formulations can be used to deliver drugs transdermally. Ointments containing nitroglycerin have been available for many years, and a recent study has demonstrated the feasibility of delivering timolol from a polyethylene in mineral oil base. The vehicle containing 30 or 60 mg of the drug was spread over a 25 cm^2 area of skin. Despite some mild irritation, this route was considered satisfactory. Systolic blood pressure was reduced in all subjects. According to Hadgraft, the major problems in using conventional topical dosage forms are the lack of control of the area of application and the rate of release.

Kinetic Modeling of Transdermal Drug Delivery. Since the process of percutaneous absorption is a series of diffusion and partitioning steps, Hadgraft said that it is reasonable that drug levels can be predicted from a knowledge of the physicochemical properties of the drug. Diffusion processes will be dominated by molecular size, and partitioning within the skin may be estimated from the octanol-water partition cefficient. The overall scheme describing the release and elimination processes is shown in Figure 7. If the input and elimination rates are known together with the physicochemical properties of the drug, it is possible, Hadgraft said, to predict the plasma levels. He added that through use of this approach a number of drug entities have been examined and good correlation found between the theoretical and experimental levels. Thus, it is possible to use such modeling techniques in the evaluation of the transdermal drug delivery of novel substances.

Hadgraft said that for certain drugs the transdermal route of administration is very attractive. He thinks that it will be expanded with the use of penetration enhancers

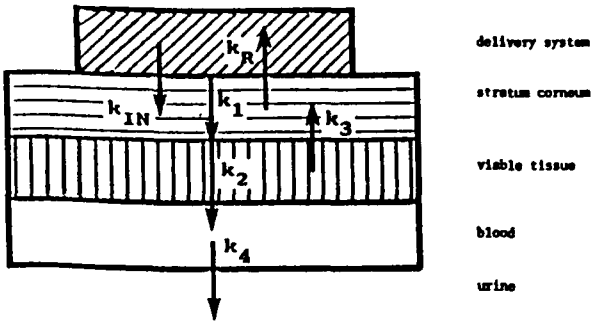

Figure 7.
Schematic representation of the kinetic model for transdermal drug delivery.

and the possible development of iontophoresis. Coadministration of drugs from more complex devices also provides new potential. However, the disadvantages of transdermal delivery as mentioned previously are manifold and cannot be ignored, according to Hadgraft. He believes that when the whole phenomenon of skin absorption is understood more thoroughly, perhaps even to a molecular level, the development of this delivery means will be facilitated.

Design of Novel Ocular Drug Delivery Systems

This topic was discussed by R. Gurny (School of Pharmacy, University of Geneva, Switzerland). Gurny said that, traditionally, ophthalmic dosage forms have been virtually limited to solutions, ointments, suspensions, and emulsions. There have been nevertheless a few successful efforts with ocular inserts – such inserts have been described in the pharmaceutical literature for more than 50 years. With the use of Ocusert, a diffusion-controlled system developed by ALZA Corp. in the 1970's, the excessively rapid drug exchange between the insert and the tear fluid was overcome. Even with this advance, the inserts in general still have the disadvantages of compliance. In recent years, several new formulations such as pH-sensitive dispersions, temperature setting gels, liposomes and nanoparticles have been developed; these were discussed by Gurny. Viscosity enhancers, like polysaccharides and mucopolysaccharides, have also been recently considered. Some of these systems have already been evaluated in man, and Gurny presented some of the results obtained.

In order to increase the activity of ocular drug delivery, an extremely large number of possibilities are offered, according to Gurny. These are summarized in Figure 8. In recent years, several colloidal preparations for ophthalmic use, based on nanoparticles or liposomes, have been investigated. However, little evidence could be found in most cases of their considerable advantage

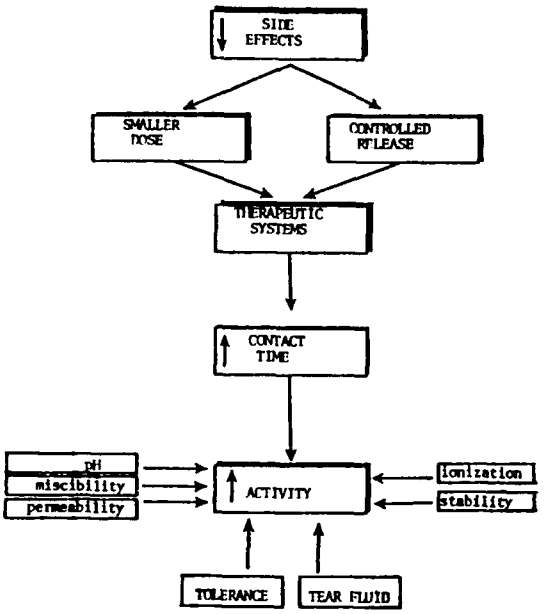

Figure 8. Concepts for the development of new systems.

over the conventional formulation. In contrast, Gurny said, introduction of the concept of *in situ* gel formation in the early 1980's by means of highly concentrated latex systems has demonstrated that a considerable prolongation in duration of action could be obtained. This had previously been obtained only with inserts. Gurny said that in the past 15 years, the coating technology developed in the paint industry has been the driving force for the very rapid evolution in the field of aqueous dispersions of polymers. Lattices of polymers with different solubility properties depending on the pH, such as cellulose derivatives, cannot be prepared by emulsion polymerization techniques. An alternative method for the preparation of polymeric dispersions in the nanometer-size range is the emulsification of the polymers, their solutions, or melts with water, using the conventional emulsifiers, stabilizers, and emulsification techniques. According to Gurny, different approaches for the preparation of these so-called "latex formulations" are possible; for example, solution emulsification, phase inversion, and self-emulsification. Gurny said that recently, *in situ* gel-forming products have been made as drug-carrying systems for the ocular route. These systems are based on the mechanism of drug adsorption onto the surface of colloidal particles (0.3 μm average particle size) which show good biocompatability.

Another *in situ* gel formation by thermogelation for the ocular route has been described, using thermosetting gels obtained from poloxamers. A classification of soluble therapeutic units for sustained drug delivery to the eye is shown in Figure 9. Gurny then went on to discuss preformed gels, thermosetting, and pH-setting gels.

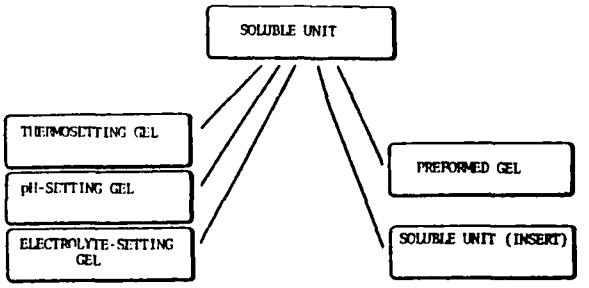

Figure 9.
Classification of soluble therapeutic units for sustained drug delivery to the eye.

Thermosetting Gels. The particular sol-gel behavior of poloxamers of the so-called temperature-sensitive polymeric gels has been investigated. Figure 10 shows the general structure of the poloxamer. This specific block polymer was chosen since it shows a reversible thermal

$$HO\text{-}(CH_2\text{-}CH_2\text{-}O)_n\text{-}(\underset{\displaystyle CH_2}{\underset{|}{CH}}\text{-}CH_2\text{-}O)_m\text{-}(CH_2\text{-}CH_2\text{-}O)_n\text{-}H$$

Figure 10. General structure of the poloxamer.

gelation phenomenon due to the very marked temperature dependence of its micellar size above a certain threshold temperature – i.e., about 25°C. The hydrophobic/hydrophilic ratio of the block polymers can be varied over a large range of total molecular weight, typically between 2,000 and 20,000. The enthalpy of gelation can be calculated according to Vadnere et al. (1984) by the following equation:

$$\ln c = \frac{\Delta H^\circ_{gel}}{RT_{gel \rightarrow Sol}} + \text{constant}$$

where c is the concentration of the polymer in solution, ΔH°_{gel} is the enthalpy of transition, and T is the temperature.

The enthalpy values of various poloxamers are listed in Table 7. It can be seen that there is no linear relation between the average molecular weight and the enthalpy of gelation and therefore, no criterion for the choice of a suitable polymer. In the present study by Gurny and his group, the poloxamer 407 was chosen because it has low toxicity and gelation enthalpy at low concentrations. It

Table 7. Enthalpy of gelation according to Vadnere et al. (1984).

Poloxamer	Mol.wt.	ΔH° (gel)
F108	14,000	8.0
F 98	13,000	8.1
F127	12,500	9.5
F 88	10,000	6.8
F 68	8,350	5.4
P105	6,500	8.6
P 85	4,600	6.5
P 84	4,200	6.2

also appears to be an apparent anomaly for poloxamers, according to Gurny, since the formation of a gel (which is a three-dimensional network more rigid than solution and infinite in extent) is expected to require a negative entropy change in order to produce an ordered system. However, if a ΔH° value is positive, the entropy change will be positive. The general investigation of the use of poloxamers as therapeutic systems was attempted with a poloxamer gel, 407 (Pluronic F 127, BASF) at a concentration of 25 percent wt/wt, using 4 percent wt/wt of pilocarpine hydrochloride for glaucoma treatment.

pH Setting Gels. The concept of producing a gel *in situ* (for example, in the conjunctival sac of the eye) from a nanoparticulate system has been developed over the past 7 years by Gurny and his group. The general method for the preparation of pilocarpine-containing nanoparticles was described by Gurny and Taylor (1980). This method involves the emulsification of an organic solvent solution of the polymer with an aqueous solution of the surfactant, followed by removal of the organic solvent and a fraction of the water. The bioactive material is then added to the dispersion where it is partially adsorbed onto the polymer. Gurny said that the active material can also be introduced at the beginning in one or the other phase before emulsification. The gel-forming polymers have to be carefully selected with respect to their physicochemical properties and biocompatability. Some possible gel-forming polymers for these dispersed systems are given in Heller et al. (1987). Solubility profiles in water of some selected polymers are shown in Figure 11, which explain clearly the potential of these macromolecules. Cellulose acetate hydrogen phthalate (CAP) has been investigated *in vivo* as the most promising candidate, according to Gurny. Indeed, only CAP shows the buffer capacity low

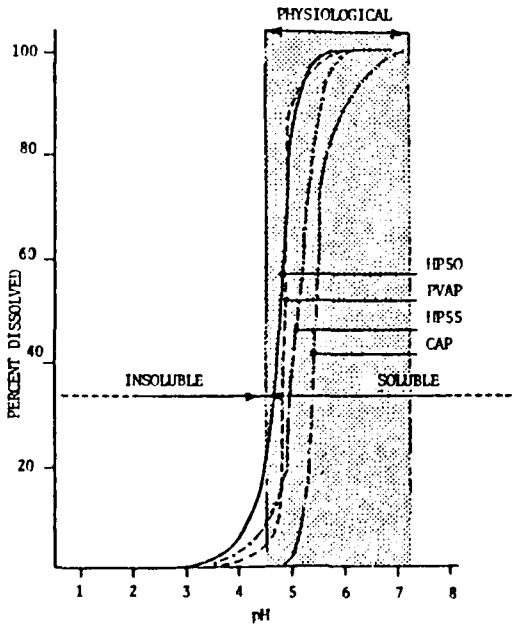

Figure 11. Solubility profiles of some polymers.

enough to gel effectively in the cul-de-sac of the eye. This cellulose derivative in dispersion starts to dissolve at a pH of about 5.0. The CAP latex contains the active compound (pilocarpine) adsorbed partially onto the surface of the polymer particles. The latex coagulates within a few seconds when placed in the cul-de-sac since the lacrymal fluid has a pH of 7.2. The pH of the ungelled formulation is 4.4 and is therefore sufficient to keep the dispersion in a stable form. The pH change of 2.8 units after installation – due to the surrounding tear fluid – leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel. *In vivo* results have been obtained by Gurny and his group with such *in situ* gel-forming systems. The long-acting latex, once coagulated, has no impact on vision. The latex formulation tested had a total polymer content (CAP) of 30 percent wt/wt, an average viscosity of 50 mPa's and an average particle size of 250 nm with a polydispersity index of 2. Its pharmokinetic analysis has been published recently by Gurny.

Scintigraphic Assessments Of Various New Formulations. Gurny and coworkers used six New Zealand white albino rabbits (weighing 2.0 to 3.0 kg) in all the experiments. Each formulation was tested in the right eye of the test animal. Precorneal clearance was measured with a gamma camera tuned to detect the 140-keV radiation of technetium-99m and fitted with a 3-mm pinhole collimator. The procedure used was the same as that described by C.G. Wilson (1987). The test rabbit was positioned on a table with its eye 5 cm from the collimator aperture. Twenty-five microliters of the various preparations or a saline solution containing approximately 1 MBq technetium-99m adsorbed on the sulfur colloid, were instilled by slightly pulling the eyelid. A total of 40 dynamic images (40×15 s) were recorded by the computer for later analysis. As mentioned earlier by Gurny, the purpose of such a study is to evaluate new formulations for controlled delivery to the eye and their relative contact time. Four formulations were investigated: a pH-sensitive dispersion, a temperature-setting gel, hyaluronic acid gel, and an aqueous solution of pilocarpine HCl. The results obtained were further analyzed and computed. The results showed that the improvement of the ocular residence time of some delivery systems was clearly visible. After 200 seconds, only about 24 percent of the applied amount of a normal solution was present on the ocular surface (cornea and inner canthus) as compared to 88 percent of the hyaluronic-acid-containing formulation.

Gurny said that any instilled solution causes reflex blinking with a considerable proportion splashing away in the first 100 seconds. Blinking is an important factor, especially in man. It is well established that rabbits blink much less frequently than man. Also, the turnover rate of lacrimal fluid is considerably faster in man, about 16

Table 8. Comparison of the pharmacokinetic values in rabbit and man (volume of instyillation: 25 µl containing 2-percent wt/wt of pilocarpine HCl).

Formulation	Man			Rabbit		
	AUC (%-min)	$_{1/2}\Delta$(min)	t_{max} (min)	AUC (%-min)	$_{1/2}\Delta$(min)	t_{max} (min)
Normal saline solution	8131(1399)*	218	35	2454(137)**	103	30
Hyaluronic acid solution 0.125% w/w	10233(1223)*	287	25	3967(210)**	143	30

* ( ) standard deviation, n=5
** ( ) standard deviation, n=6

percent/min compared to 7 percent/min in rabbit. Under these conditions, Gurny said it is interesting to analyze the comparative study of miosis in the presence of hyaluronic acid (0.125 percent wt/wt) in man and rabbit as seen in Table 8. In both cases pilocarpine HCl (2-percent wt/wt) was present.

Gurny said that for normal pilocarpine HCl solutions (2-percent wt/wt) the width of the miotic response peak at half the height ($\Delta_{1/2}$) is more than double in man compared to rabbit – 218 min and 103 min, respectively. This clearly shows, according to Gurny, that only rank order determination of various formulation should be made within one species, because, although precorneal clearance is faster in man, the pharmacological responses are more pronounced. It has been widely accepted, Gurny said, that factors increasing the precorneal contact time increase the ophthalmic bioavailability in most cases. However, Gurny showed that hyaluronic acid containing ocular formulations increase the miotic response in man even though the blinking and turnover rate are very high.

In conclusion, Gurny said that for ophthalmic use compared to polymeric dispersions with pH-sensitive behavior and thermosetting gels, solutions containing low concentrations of hyaluronic acid seem to have the unique capability of prolonging precorneal residence time. Hyaluronic acid interacts directly with the corneal tissue, but it appears that probably only a monomolecular layer of the cornea is effective since high concentrations of hyaluronic acid do not lead to better bioavailability.

Site Specific Delivery and Optimal Drug Action

Site-specific drug delivery serves to attain both the maximal potential intrinsic activity that a drug can have, and a reduction in any toxic events. E. Tomlinson (Advanced Drug Delivery Research Division, Ciba-Geigy Pharmaceutical, Horsham, UK) discussed the relationship between pharmacological action and the processes of drug availability at a receptor site in terms of the chronopharmacology of the drug and its anatomical site of action.

Tomlinson said that all drugs act on discrete pharmacological receptors; however, their clinical use relies on the appropriate combining of their pharmacodisposition and pharmacokinetics combining to give an appropriate pharmacological response, coupled with the ability of the body to detoxify itself of any drug that has been generally distributed. Although to date it has been possible to produce drugs having these properties, not only are the disease targets becoming more difficult to attain, but the probabilities are becoming low for the discovery of acceptable molecules using the various high-throughput screens often employed. The advent of the control of gene expression has given rise to a plethora of new classes of molecules as well as to an understanding of normal and pathological processes; this is leading to new approaches in both the design and clinical use of drugs. For both conventional drugs and the new classes of protein drugs, there are firm rationales for their site-specific delivery. It has been proposed that this can be achieved using carriers which travel along unique biological pathways and serve to guide drugs to pharmacological sites of action in a protected form. Tomlinson then went on to to examine the rationale for site-specific drug delivery in terms of the chronicity and position of disease and the properties of drug/carrier systems. He said that although site-specific drug delivery is still rather emperical in practice, there are examples from the literature to illustrate the mistakes and successes in the use of site-specific carrier systems. These include their use in macrophage activation, some other types of cancer chemotherapy, retroviral diseases including AIDS, gene therapy, enzyme storage diseases, inflammation, graft-versus-host rejection disease, and fungal manifestations.

Disease, Access, Retention, and Timing

Tomlinson said that it is evident that the basis for developing any one modality to fight a disease must be a knowledge of the disease itself (i.e., its pathophysiology, biochemistry, and temporal responsiveness). Hence, apart from the disease and the drug, the further essential components of site-specific drug delivery are access, retention, and timing.

Disease. Clinical medicine has the opportunity, Tomlinson said, to move rapidly away from being a descriptive

science into a mechanistic one. This is due to recent advances in molecular and cell biology, physiology, biophysics, materials science, and analytical methods.

In particular, the control of gene expression has many and varied consequences for the pharmaceutical community in their search for safe and effective medicines. These include, Tomlinson said, the evaluation of gene families and the markers of genetic originality, understanding the pathogenesis of disease and the molecular specificity of biological action, and also the ability to produce a plethora of pure amounts of new drug classes (i.e., both protein drugs and their low molecular weight analogues). Recombinant DNA technology has enabled some of the fundamental mechanisms which regulate the expression of genes at the level of transcription and translation to be elucidated. In particular, a dramatic increase in the availability of cloned DNA sequences and chromosomally restricted fragment length polymorphisms (RFLP's) has enabled many diseases to be studied at the genetic level.

Tomlinson said that declared intentions of mapping of the human genome lead to possibilities for showing predisposition to a particular disease. Although to date only approximately 0.3 percent of the human genome has been mapped, this means that 4200 genetic loci have been identified. Of these, 1360 have been mapped to their chromosome, 365 of which code for human genetic diseases, and a very small number have been sequenced; hence such techniques provide powerful methods for probing the molecular pathology of any disease. According to Tomlinson, their availability helps to ensure that clinical medicine is on the brink of an entirely new and different future.

Such tools are also being used to probe normal physiological functions, whether these are occurring intra- or extracellularly. This effort is being aided by sophisticated and powerful new imaging techniques such as fluorescence-activated cell sorting, confocal fluorescence microscopy, tunneling microscopy, etc. Cell processes being elucidated include simple secretory and receptor-mediated events, both of which require signal recognition and feedback control. These (intracellular) processes are often mediated through leader sequences which can act in a number of ways – i.e., either through their basicity/acidity properties or via some function of their primary and secondary structure. Tomlinson thinks that a knowledge of the minimum amount of structural information to cause a particular routing (or tropism) could be useful in the design of site-specific drugs.

New Classes Of Drugs. Tomlinson said that the ability to produce new classes of drugs also lies at the heart of modern clinical medicine. Proteins can be produced either as drugs per se, or may act as templates in the production of agonist or antagonist drugs. Little rational thought, has, he said, gone into the use of protein drugs in terms of their optimal arrival at various sites of action. The key feature is whether they are able to act systemically, or whether they are mimics of endogenous molecules that are produced to act locally. Tomlinson said that the current dogma is that protein drugs have an element of self-targeting in their structure, and hence no special provision needs to be made to ensure that they arrive at their site(s) of action; he questioned this dogma. Clearly, endocrine mediators are produced endogenously to act over long distances from their site of manufacture; also, they are stable in blood, and their size is no hindrance to their need to extravasate. Conversely, autocrine or paracrine-like mediators are endogenously produced to act locally. Also, these types of mediator are (1) unstable in blood (generally very low concentrations are to be found in the blood pool); (2) they act between neighboring cells and are rapidly catabolized; and (3) they have an inefficient and widespread disposition, an extremely variable and inefficient ability to extravasate, and need, therefore, a lymph-to-plasma ratio of greater than one. In addition, these mediators act on many different cell types (pleiotropic mediators), have a specificity which is due to their local release and action. Also, it is known that some of these types of molecules can produce different effects on the same cells when these are at different stages of their life cycle (Melchers and Andersson, 1986) and often have an extremely complex pharmacology (Talmadge, 1986). Thus, according to Tomlinson, the production and use of this latter class of molecules is contraindicated unless some means can be found (for example, using a carrier system) for adjusting their pharmacodisposition (and stability). Therefore, the key issue with such mediators is the differentiation between local action and the risk of action on on-target cell populations. Thus, according to Tomlinson, in the site-specific delivery of these mediators, it is necessary to consider the issues of chronicity in the activation of cells (including their temporal localization and responsiveness), and, since such agents may be acting as part of a polymediator cascade of events, also as the staging sequence in which they exist.

Tomlinson said that all of these features described above for endocrine- and auto/paracrine-like mediators serve to illustrate that as we begin to know more and more about disease, then the way in which we clinically apply drugs is often shown to be very naive. Therefore, Tomlinson believes that we often need to rethink our approach to drug application, both at the clinical stage but also, and perhaps even more importantly, at the drug discovery stage where, after designing these new classes of drugs we still administer them into inappropriate test models at inappropriate rates, amounts, stagings, and times.

Access. Tomlison said that numerous instances of disease can be evidenced where simply for a drug to attain a site of action located in a difficult-to-access region

would be beneficial – as, for example, with many intracellular infections, diseases of the central nervous system, diseases of the immune system, cancerous states, some cardiovascular diseases, and hemapoietic and arthritic diseases. Tomlison cited as an example, AIDS, where more and more attention is being placed on the viral regulatory genes of human immunodeficiency virus as being important targets for chemical intervention. The question of pinpoint access is clearly vital here, according to Tomlinson.

In addition, it is apparent that many of the intricate dosing regimens and the high doses of drug currently applied, are frequently needed because of a poor perfusion of the site of action coupled with an inappropriate pharmacodisposition of drug. Since the latter may lead to untoward metabolism and interaction with the host, all of these effects often combine to give rise to deleterious effects. Site-specific carriers serve to operate via use of their innate biological pathways and their ability to protect the drug. In addition, according to Tomlinson, they should also be able to make drug available at the site of action in the right amount, at the right rate, and at the right time (Gurny and Taylor, 1980). This may need to be effected by retention of the carrier at the site of action and via some type of trigger which enables the drug to be released either due to a normal or pathological event. Thus, according to Tomlinson, site-specific drug delivery may be defined as achieving the maximal potential intrinsic activity of drugs by optimizing their exclusive availability to their pharmacological receptors in a manner that affords protection to both the body and the drug alike (Tomlinson, 1987).

Carriers. Tomlinson defined site-specific carriers as soluble and particulate macromolecular carriers, which, although they behave in a variety of ways in the body, may need to have, variously, the properties of passage through epi- and endothelial barriers, carriage or transport through the body (including intracellular or transcellular transport), and then interaction with the target cells. Their use also implies protection of the drug and the body from one another until the site(s) of action are reached, avoidance of any pharmacological interaction with non-target cells, release of therapeutically relevant amounts of drug at the required modality and frequency, followed by excretion of the carrier and the drug. Drug availability can be due to simple passive events – such as diffusion from a carrier, or active processes including enzyme degradation.

Access of carriers to various sites is due to diffusion, convection, and participation in various receptor-mediated cell trafficking events (Tomlinson, 1987). However, because of their size, Tomlinson said that it is necessary to realize that particulate carriers greater than 20 to 50 nm diameter will not be able to extravasate through anything other than discontinuous or damaged endothelia. Table 9 shows that numerous soluble and particulate carriers have been suggested for effecting site-specific drug delivery.

Table 9. Types of carrier/ligand.

Soluble carriers
Synthetic polymers
Antibodies (fragments thereof)
Hybrid fusion proteins (genetically defined)
Particulate carriers
Lipid carriers
Proteinaceous carriers
Fusogenic carriers
Cell carriers
Viral/retroviral carriers
Synthetic particles

Many attempts have been made to control the biological dispersion of a carrier by linking it to some form of ligand which will be recognized by a particular normal or abnormal feature of the body. This ligand may be simple (for example, a sugar moiety) or more complicated, such as with a fragment of a natural ligand (for example, of interleukin-2, or of an antibody). Tomlison said that the latter is interesting and can include anti-idiotypic antibodies or even the synthetic antisense antibodies that have been discovered recently.

Tomlison stated that the three prime pharmacodynamic factors which have been shown to have a marked influence on drug action are: duration of drug-free interval, attainment of critical thresholds in plasma concentrations, and the rate of increase in drug plasma concentration. These findings have been arrived at by charting the behavior of conventional drugs using various routes and modes of administration. For site-specific systems, these observations will be altered due to altered kinetics and dynamics of drug distribution. Tomlinson said that as more becomes known about (patho)physiology and the local biochemistry of disease, and as molecular biology tools extend our present ability to manipulate and control gene expresssion, and protein engineering tools enable the *de novo* synthesis of therapeutic systems that have defined functions of transport, protection, spatial orientation, and temporal release to be made, then a revolution in the management of disease will occur. New classes of targeted drugs will appear (for example, hybrid fusion proteins). Gene therapy may become technically possible, according to Tomlinson. As a clearer understanding emerges of how targeting can be effected, a singular challenge for the pharmaceutical industry, Tomlinson said, will be how to assess the potency and toxicity of its existing store of candidate drugs in order to arrive at conclusions as to which molecules are suitable for drug targeting. It is important, therefore, that objective analytical methods are arrived at. Also, according to Tomlinson, there will be a need for a realization that site-specific drugs are new chemical en-

tities, and hence will require full-scale testing for their safety and their efficacy. The use of human-specific processes to effect site access, etc. will pose unique problems in this testing, according to Tomlinson.

Liposomes as Drug Delivery Devices

An overview of liposomes as drug deliverers was presented by I.W. Kellaway (The Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff, UK). Liposomes are structures composed of lipid bilayers entrapping aqueous volumes. Phospholipids are the most commonly employed lipid, forming a heterogeneous dispersion of multiple bilayers arranged as concentric closed shells. These multilamellar vesicles (MLV's) can be sonicated to yield small unilamellar vesicles (SUV's) in the typical size range of 25 to 100 nm. Large unilamellar vesicles (LUV's) of 100 to 500 nm may be formed by alternative preparation procedures. All such vesicles, according to Kellaway, are capable of entrapping drug molecules whether hydrophilic or hydrophobic and ranging in size from low molecular weight to macromolecules, and thereby forming one of the most widely researched colloidal drug-carrier systems. Vesciles formed from nonionic surfactants, although of similar structure, are generally considered as a separate drug carrier system (niosomes), according to Kellaway.

Liposomes as Drug-Carriers

Kellaway said that the clinical rationale for using drug carriers is to achieve greater selectivity than can be obtained by drug design alone. Liposomes can carry a relatively large drug payload which will be protected within the inner confines of the liposome structure from degradation while decreasing toxicity by control of drug and metabolite levels in blood and at organ sites. Cellular uptake can occur by various mechanisms without cytotoxic effects, and hence enhanced cellular drug levels can occur. Liposomal encapsulation will inevitably result in altered pharmacokinetics which lead to reduced toxicity and enhanced therapeutic effects, according to Kellaway. Such claims can be made on behalf of any putative carriers; however, some additional benefits may apply more specifically to liposomes. These include the use of endogenous, biodegradable, and general nontoxic constituents. A wide range of components leads to liposomes of different properties (both physical and biological) and hence, Kellaway said, it is possible to design a liposome for a specific therapeutic target. Chemical reactions are normally not used in liposome production which, together with the relatively mild conditions employed in the processing, ensures optimal drug stability. Most colloids enter cells by endocytosis; for liposomes, it is possible to achieve, albeit at a low efficiency, cell fusion leading to drug delivery to cells not accessible to other carrier systems. The surface properties of liposomes may be readily modified by selection of membrane-forming lipids, adsorption of macromolecules, or by covalent attachment of targeting moieties. Design of liposomes sensitive to *in vivo* triggering of drug release by temperature, by pH, and by photochemical modulation demonstrates the versatility possible from vesicular drug delivery systems, according to Kellaway.

Preparation Reproducibility And In Vitro Stability

Kellaway did not review the methodologies for liposome preparation but said that if such preparations are to progress beyond the stages of small-scale batches for clinical investigation into production batches, then it is critical that reproducible standard preparations can be obtained. MLV's made by whatever technique are heterogeneous and therefore require careful control and standardization of the production process in order to achieve acceptable batch-to-batch reproducibility. SUV's and REV's may be less problematic in this respect. Because in many applications, acute size-dependent properties have been reported, batch specifications would need to specify size distribution functions not merely a mean diameter. Other methods of preparation such as surfactant dialysis, membrane extrusion, and aerosol generation tend to yield more reproducible results.

Kellaway said that on storage it is possible to control, with sufficient care, any long-term degradative changes in lipids through the use of antioxidants, temperature control, and inert gases in a sealed container. However, the liposomal suspension is subject to many of the instability problems associated with colloids on storage such as floculation, and in addition may exhibit fusion or lysis. Alternative methods of storage include both frozen and freeze-dried preparations.

In Vivo Stability As Related To Drug Targeting

Following injection into the blood stream, various changes can occur to the drug-carrying vesicle. These may include aggregation, destruction, changes in vesicular membrane permeability, changes in size and surface charge, and adsorption of plasma components. The relative extent of these changes is dependent on liposome composition, according to Kellaway. Maximum plasma stability is often achieved by using phospholipids with a transition temperature (gel to liquid crystalline phase change temperature), well above 37°C together with equimolar cholesterol. The condensed state of these

liposome membranes prevents rapid interaction with plasma components. Proteins interact with liposomes, penetrating the bilayers which leads to an increase in the leakage rates of entrapped solute. However, Kellaway said, the major dismantling of liposomes is undertaken by both high- and low-density lipoproteins and although there is an exchange of lipids between the interacting assemblies, there is nevertheless a net transfer of lipid from liposomes to the lipoproteins.

Kellaway said that the adsorption of various plasma components including IgG, complement C_3D and fibronectin to the external membrane surface is the first step in the optimization of these particulates. This prepares the liposomes for "recognition" by the cells of the mononuclear phagocyte system (MPS). Although these cells are widely distributed throughout the body, the major regions for colloid removal from the plasma are in the liver (Kupffer cells) and spleen (free and fixed macrophages and sinusoidal cells). As saturation is approached, significant uptake will occur within the bone marrow.

According to Kellaway, reducing liposomal uptake by cells of the MPS can be achieved by altering liposome composition (for example, ganglioside and sphingomyelin incorporation) or by coating the external surface of the vesicles with a polyoxyethylene-polyoxypropylene copolymer. In the latter case, leakage of entrapped aqueous solute can occur, suggesting ingression of the polymer into the outer bilayer. An alternative to designing liposomes which avoid MPS cell uptake, according to Kellaway, is the suppression or blockade of Kupffer cell activity – for example, by pretreatment with drug-free liposomes. Blockade can have deleterious effects and hence the former approaches are to be preferred particularly if endocytosis is the mechanism of target cell uptake.

Kellaway said that SUV's are capable of penetrating the fenestrations of the liver sinusoids and hence reach the hepatocytes. Hepatic uptake can be increased by coating with lactosylceramide, which is recognized by the galactose-specific receptors. For most organs, however, extravasation of vesicles is not possible due to the tight junctions of the endothelial barrier – i.e., continuous type of endothelia overlying a continuous basement membrane. The endothelial barrier, Kellaway said, is undoubtedly the greatest obstacle to the successful therapeutic use of colloidal delivery systems to target many tissues or cell types.

Liposomal Drug Delivery To The Lungs

Kellaway then considered only one aspect of liposome drug delivery, namely, drug delivery to the lungs. He said that targeting the lung can be achieved by the intravenous route. The most simple mechanism involves the use of large vesicles which are filtered from the circulation by the lung capillaries. Smaller vesicles with appropriate surface characteristics may also be taken up by circulating blood monocytes with subsequent migration across tissues and into the alveolar space. Kellaway said that such a process could be utilized for delivering macrophage activating agents to eradicate pulmonary metastases.

Kellaway said that relatively few publications report on the delivery of vesicles to the lung by inhalation. The applications have been concerned with localized delivery of (1) cytotoxic agents for the destruction of pulmonary metastases and (2) bronchodilators and antiallergic agents. Macrophage-activating agents have also been examined. Early studies attempted to deliver phospholipids either as a powder or in a vesicular form for the treatment of respiratory distress syndrome.

Kellaway said that the direct administration to the lung ensures maximum retention of drug activity within the lung, with the liposome acting as a depot system and providing protection from enzymatic degradation. This is an example, he said, of what has been termed compartmental targeting. The drugs beta cytosine arabinoside and 5-fluorodeoxyuridine when liposome entrapped and administered to rats by intratracheal instillation, resulted in slow release and distribution within the lungs. Suppression of DNA synthesis occurred within the lung but with little effect in the gut and bone marrow. Intratracheal administration of free drug resulted in synthesis suppression at all three sites. Kellaway said that depot effects have also been demonstrated for liposome-entrapped 6-carboxyfluorescein with blood levels showing dependence on liposome charge and concentration. No evidence of liposome transport to the systemic circulation has been reported.

According to Kellaway, SUV's and MLV's are cleared from the lungs of healthy volunteers following nebulization. The extent of liposomal disruption following the nebulization process is dependent on the type and size distribution of the liposome suspension. Lung clearance was similar for both MLV's and SUV's with dependence on deposition site (and hence aerosol droplet size) and breathing pattern. Muco-ciliary clearance occurred within 6 hours for approximately 15 percent of the administered dose which was deposited in the conducting airways. The alveolar-deposited fraction (up to 85 percent) was cleared more rapidly than reported for insoluble particulates with up to 70 percent remaining in excess of 20 hours.

Kellaway said that an REV formulation of DPPC/cholesterol containing sodium cromoglycate (SCG) when administered to voluteers by nebulization resulted in a 33-fold increase in the absorption half-life compared with the free drug. SCG was detectable in plasma 24 hours after administration of the liposome for-

mulation. The bioavailability of the two preparations was comparable.

According to Kellaway, patent applications exist for a pressure-pack aerosol system for delivering phospholipid to the lungs for the *in situ* formation of vesicles. Other patents cover aerosol delivery of liposome encapsulated compounds including devices suitable for prolonged efficacy and reduced systemic effects from bronchodilators.

Drug Targeting Using Monoclonal Antibodies

Targeting with monoclonal antibodies was discussed by B.A. Rhodes (RhoMed Inc., Albuquerque, New Mexico). Rhodes said that in 1951, Nungesterm, Beierwaltes, and Knorpp (Merchers and Anderson, 1986) achieved the complete and prompt regression of widespread metastatic melanoblastoma in a patient which they treated with rabbit antisera labeled with Iodine-131. Although they were not able to repeat this result in other patients, the idea of using radiolabeled antibodies to target and concentrate a radioisotope specifically in a tumor to effect localized radiation was established. In recent times, this result has been repeated by several investigators.

Rhodes said that antibodies tagged with radioisotopes can also be used for diagnostic imaging. Iodine-131, Iodine-123, Iodine-125, Technetium-99m, and Indium-111 have been used diagnostically to localize tumors by gamma scintigraphy following injection of the radiolabeled antibodies. For therapeutic applications, Yttrium-90 has been used in addition to some of the foregoing radioisotopes; several other radioisotopes are being considered for this purpose.

According to Rhodes, tumors can also be treated by coupling toxins or chemotherapeutic agents to tumor-specific antibodies. Clinical trials on a variety of toxin and chemotherapeutic agents are being conducted, both in Europe and the United States.

Rhodes said that over the years, numerous clinical trials which test the concept that antibodies can be used to target drugs to solid tumors, either for diagnosis or therapy, have demonstrated that the targeting concept is valid. This concept has been shown to work with both polyclonal and monoclonal antibodies, in animal models, and in human clinical trials. However, with a roughly 30-year history of having established the principle of antibody targeting, antibody-based drugs are still not being used routinely in the management of patients with solid tumors.

Rhodes said that the efficacy of clinical trials, when measured in terms of the percentage of tumor lesions targeted relative to the total number of known tumor lesions, has usually ranged from slightly more than 50 percent to about 90 percent. When measured with therapeutic antibody-based drugs in terms of anticancer effect, such as tumor regression, the efficacy has been significantly lower. Clinically measured efficacy, while showing great promise for this method, has not been high enough to warrant the introduction of commercial products, according to Rhodes. In recent years, improvements in radiolabeling techniques have been developed, monoclonal antibodies (Mabs) have replaced polyclonal antibodies, milligram dosages of antibodies have been optimized, and other parameters, such as higher resolution imaging techniques, have improved. Yet even with these improvements, clinical efficacy by most measures has not increased to a level to support commercialization. Rhodes said that one major limitation to antibody targeting of cancer appears to be the heterogeneity of antigenic expression. When a series of clinical studies is evaluated the results usually fall into three categories: obvious targeting, equivocal targeting, and negative targeting. The frequency with which cases fall into the two latter categories appears to be related to the expression, both relative and absolute, of the antigenic determinants for which the antibody under study is specific. He said that for virtually all solid tumors which have been evaluated, this frequency – i.e., the equivocal and negative targeting – is high enough that overall efficacy is insufficient for routine clinical application. According to Rhodes, improvements in labeling, antibodies, and related technology have not addressed the very real problem caused by antigenic variations. This major problem, which appears to be the key to increasing low efficacy, remains to be solved. Thus Rhodes proposed one way to increase efficacy of targeting to solid tumors. The aim was to increase efficacy of targeting to a level that would be high enough to make use of antibody targeted drugs attractive for routine clinical use.

To assist in the discussion of the problem of efficacy of antibody targeting, Rhodes introduced and defined two terms: pharmacocentric drug testing and patient-centered drug testing. The traditional method for testing of a new drug is defined as pharmacocentric – i.e., a specific drug product is formulated and the drug is then tested for safety and efficacy in selected patients. If the criteria for safety and efficacy are met then the drug may be introduced into routine clinical use. When applied to monoclonal antibody-based drug products, a specific Mab is produced from a clone derived from a single cell. The processing of the antibody and formulation of it into a drug are specified and the product is then tested. A variation on this approach is to use more than one Mab in the formulation. This might be called a fixed-cocktail formulation. Cocktails of antibodies are proposed to increase the probability of achieving a match between expressed tumor antigens and at least one of the antibody components of the antibody-based drug.

The alternative approach, Rhodes said, is defined as patient-centered. In this approach, patients with a given type of medical problem, such as colorectal cancer, are considered as candidates for antibody targeted therapy. The antibodies used to prepare the drug are selected for each individual patient. This is in contrast to the pharmacocentric approach where the patients were selected because of a previously determined probability that the antibody-based drug reacts with a tumor marker frequently expressed by tumors of this general type. For example, antibodies reactive with Carcinoembryonic Antigen (CEA) are frequently used for the targeting of colorectal tumors because of the high association of this marker with tumors originating in this region of the body. Using the patient-centered approach, a drug based on anti-CEA would not be given unless there was evidence that the patient's tumor expressed this antigen and that the antigen was actually reactive with the anti-CEA Mab.

The Patient-Centered Monoclonal Antibody Approach to the Management of Cancer

Rhodes said that patient-centered approaches have traditionally been the preference of physicians whenever practical. For example, in the situations of cancer chemotherapy or antibiotic therapy, the physician can make a selection from a number of approved drugs for the treatment of individual patients. The drug or combinations of drugs which is believed to be best for each patient can be selected from lists of approved and available drugs.

With Mab-based drugs, this opportunity for choice may never be realized if the pharmacocentric approach is the only one taken. Rhodes said that it is unlikely that any single antibody will ever prove to be effective enough to receive regulatory approval. He added that to achieve the ability to select antibodies on a patient by patient basis, a novel clinical research program and regulatory approval process needs to be instituted from the start of clinical trials.

In the patient-centered approach, tailor-made cocktails of antibodies are used for each patient depending on which tumor markers are being expressed by the patient's tumor. The products for a patient-centered treatment will not be specific, fixed-formula drugs, according to Rhodes. The products will be kits for formulating antibody cocktails for the individual patient. Other products will be those needed to determine which antibodies react with antigens expressed by the patient's tumor and products which allow for the quality control testing of a drug formulation which is made for a specific patient.

Rhodes said that there are several key assumptions which underlie the development of the patient-centered approach to the clinical introduction of Mab-based drugs for use in the management of patients with solid tumors:

- No single antibody will be effective enough to be used alone in patients with a given type of cancer, such as colorectal cancer.
- For each type of cancer, there are several tumor markers which might be useful for antibody targeting.
- The best targeting will be achieved using a mixture or cocktail of antibodies, all of which bind to the tumor.
- Fixed cocktails of antibodies are not appropriate because some patients will have tumors which do not bind some of the antibodies in the cocktail.
- The primary purpose of targeting antibodies for a diagnostic goal is to determine if antibody-targeted therapy is likely to be successful – i.e., the clinical question should not be: Does this patient have cancer? rather it should be: Should this patient be treated with antibody targeted therapy?

Examples Of The Patient-Centered Approach

One example of the patient-centered approach mentioned by Rhodes is that established by R. Oldham (Tamlinson, 1987) which combines research laboratories and clinical activities so that cancer treatments can be tailored for individual patients. In this approach research and clinical services are marketed directly to the patient.

Another example of a patient-centered approach is under development by RhoMed. In this approach products and services will be marketed to clinicians who will provide the clinical services. This system of products and services will permit the clinician to use a decision tree to select antibodies for use with a specific patient and to determine if the patient should be treated with antibody-targeted drugs. The products include those needed to carry out each step in an overall process including selection of antibodies and formulation and quality control testing of the tailor-made drugs. The services will include computer analysis of the diagnostic images to calculate radiation dosimetry estimates to the tumor and to other tissues. These calculations will be used in deciding if antibody targeted drug therapy is indicated. Thus when an antibody cocktail formulation is administered for cancer therapy there will be a high level of confidence that adequate targeting can be achieved, according to Rhodes.

The scheme of the decision tree is shown in Figure 12. The process begins with the biopsy. The biopsy samples are tested against a panel of Mabs to tumor markers known to be frequently expressed by the general type of cancer which is under investigation. Only antibodies which show significant binding to the tumor specimen are selected, compounded, and radiolabeled to make up the specific diagnostic formulation. The radiolabeled formulation is then tested for reactivity with a sample from the original biopsy. This is done in the presence and absence of the patient's own sera. If bind-

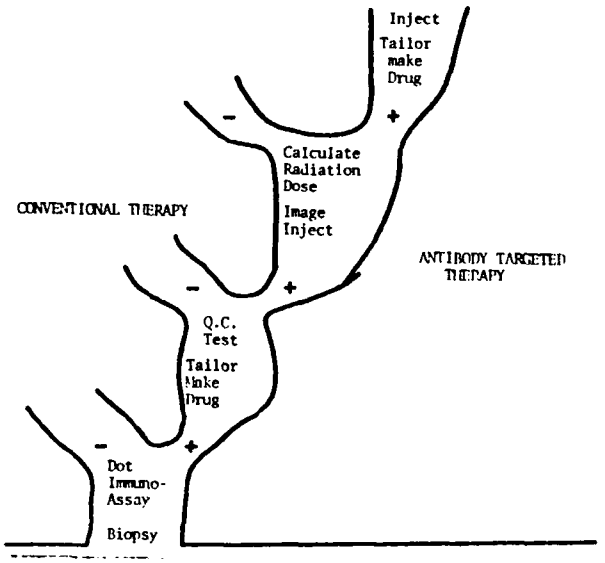

Figure 12. RhoMed's antibody delivery system.

ing of the radiolabeled formulation to the patient's tumor occurs and this binding is not inhibited by the patient's serum, then the formulation is administered to the patient.

The next stage of this process is to image the patient. The image, a nuclear medicine gamma scintigram, is interpreted to determine the regional distribution of radioactivity. If adequate targeting has occurred, the radioactivity will be concentrated in areas corresponding to the distribution of the cancer. Radiation doses can be derived from image analysis to determine if targeting is sufficient to predict the efficacy of antibody targeted therapy.

The final stage of the process is treatment. Using the cocktail of antibodies which were shown to target the cancer during the diagnostic stage of the process, therapeutic formulations are prepared and quality control tested. The therpeutic formulation would consist of antibodies coupled with a toxin, a chemotherapeutic agent, or a radioisotope.

According to Rhodes, the improvement of efficacy results from (1) using a cocktail of antibodies, all of which have been shown to react with the given patient's tumor samples; (2) eliminating antibodies from the cocktail when the antibody's binding is inhibited by the patient's serum; and (3) eliminating patients prior to therapy based on *in vitro* or *in vivo* evidence that targeting is unlikely to be adequate. Thus, the patients who are treated with the specifically selected Mabs are those most likely to have adequate antibody targeting. Rhodes said that if antibody targeted therapy is ever to be effective, it is most likely to be effective under these circumstances. Thus this system provides a way for maximizing the chance of therapeutic success.

Antibody/Antigen Matching

Rhodes said that the first decision in this antibody delivery system for patient-centered therapy is based on achieving a match of antibodies to the antigen expressed by the patient. The testing is done *in vitro* using samples obtained at biopsy. The test configuration which is under development utilizes dot immunoassay because several antibodies can be tested simultaneously using very small samples. Alternatively, the histological slides of the biopsy can be stained with each of the candidate antibodies.

According to Rhodes, one problem with this approach is sampling. The biopsy is small and may not be representative of the tumor. This problem is not unique to the use of the patient-centered approach but is a problem which is inherent in the use of biopsy samples in general. If the sample is nonrepresentative and a potential useful antibody is not utilized, then a patient who might have benefited from the therapy will go untreated. Rhodes said that the magnitude of the potential problem will have to be defined by clinical experience.

Patient-Centered Clinical Trials

Rhodes posed the question of how one can test for clinical efficacy of a Mab-based drug with an undefined product. He said that this could not be done. However, he stated that a change of definition is needed. Instead of a drug, a "kit" product is defined which allows for formula variations within defined limits. Rhodes said that this will work best using antibodies which have already undergone early phases of clinical evaluation so that safety is established, the appropriate milligram dosage is established and the targeting of the antibody in patients has been demonstrated.

One design which RhoMed is advocating utilizes the technique of immunoguided surgery. Patients scheduled for colon cancer resection are given an Iodine-125 labeled Mab reactive with a common colon tumor marker. After the antibody has cleared from the blood, the surgery is carried out using a hand-held gamma probe to assist the surgeon in locating and identifying the tissues which should be resected. The surgical specimens are available for measurements of the amount of radioactivity targeted to both cancerous and noncancerous tissues. Measures of target-to-nontarget ratios are determined. Also the tissues can be studied histologically to verify the presence of the antigenic marker with which the Mab is reactive. At least two different Mabs reactive with different colorectal tumor markers are needed to carry out a clinical test.

Rhodes said that the hypothesis which is tested is that efficacy is increased if the antibody-based drug is made from several antibodies, all of which are reactive with the biopsy in the presence of the patient's serum. In the first

phase of clinical testing, a biopsy specimen is tested against the panel of at least two antibodies. The results of the antibody matching test are not revealed to the investigator prior to surgery, rather the antibodies are administered randomly or serially to patients.The results of the antibody matching test are independently used to predict if targeting will occur in each patient. If successful, in a subsequent series of patients the antibody matching test will be used to determine which of the antibodies are to be administered to each patient. The mean target-to-nontarget ratios will be determined. The hypothesis is tested by determining whether the mean target-to-nontarget ratio of the selected antibodies is greater than that for the nonselected antibodies.

Quality Control Testing

Rhodes said that if a product to be sold is a kit for compounding a drug rather than a specific drug itself, then it becomes highly desirable to provide a means of testing each formulation prior to its administration to the patient. This can be done, again using a minute amount of the initial biopsy specimen for the test. The quality control test is a measure of how reactive the intended drug is with the patient's tumor. Rhodes said that at RhoMed they are developing tests for this purpose with the aim of making them rapid and easy to use in a clinical setting.

The principle of the test, according to Rhodes, is to make a solid phase dot from an extract of the biopsy specimen. A small amount of the patient's serum is also required. The degree of binding of the radiolabeled antibody cocktail to the solid phase is measured in the presence and absence of the patient's serum.

Rhodes said that an initial product has been produced which utilizes a solid phase representation of an unpurified mixture of several colorectal tumor markers. The antigen source is produced by growing large volumes of a human tumor cell line in nude animals. This product, according to Rhodes provides a general test of radioimmunoreactivity of a radiolabeled antibody to colorectal tumor markers.

According to Rhodes, because of the heterogeneity of tumor antigenic expression and the specificity of Mabs, a single Mab-based drug or a fixed-formula cocktail of Mabs may never prove to be efficacious for targeting of drugs or radionuclides to solid tumors for therapy. Therefore, a proposed solution to the problem of efficacy is the use of patient-centered therapies, described above, in which both the antibodies and the patients are selected using a series of tests and decisions such that when the therapeutic cocktail of antibody-targeted drug is administered to a given patient there is a high probability of adequate targeting. By assuring adequate targeting the chances for effective therapy are maximized; this is the approach being used at RhoMed.

Biosensors

J.C. Pickup (Division of Chemical Pathology, United Medical and Dental Schools, Guy's Hospital, London, UK) reviewed the topic of biosensors, which are probe-type devices that give a rapid and specific signal in response to an analyte of biological interest. Often a biological molecule like an enzyme or a tissue component is immobilized on the probe for recognition of the analyte, the signal being a change in potential, current, heat, light, etc. Pickup said that biosensors are being developed because they have the potential for being simple to operate, inexpensive, safe, portable, quick-responding, and allowing continuous *in vivo* monitoring.

In the context of drug delivery, biosensors may be useful, he said, for measuring drug levels or responses in the blood or other body fluids (such as urine) or in certain tissues and organs for detection of drug overdose (including accidental), and for feedback control of drug delivery systems such as infusion pumps.

Most biosensors are electrochemical and operate by potentiometry or amperometry. The former is based on the voltage change when the analyte or a product of analyte metabolism binds to the sensor. In potentiometric enzyme electrodes, enzyme is immobilized over a base sensor, such as a pH electrode. In the case of a glucose sensor, for example, the acid produced by oxidation of glucose can be detected by a pH change.

Potentiometric drug detectors include one for penicillin, Pickup said, in which the enzyme penicillinase is immobilized over a pH electrode. Amperometric sensors record current at a fixed potential, a well-known example being the oxygen electrode, where a platinum electrode set at about -600 mV reduces oxygen and produces a current change proportional to oxygen concentration. Amperometric enzyme electrodes include glucose sensors where H_2O production and O_2 consumption are monitored electrochemically. Drug sensors include a salicylate electrode with immobilized salicylate hydroxylase (with detection of O_2 consumption or catechol production) and a paracetomol electrode using aryl acylamidase to catalyze the production of p-aminophenol (measured at + 250 mV).

Optical sensors are also beginning to receive attention, according to Pickup. A bioaffinity probe for glucose, for example, uses fiber-optic technology to detect fluorescence changes when glucose displaces fluoresceinated dextram from immobilized concanavalin A.

Pickup believes that the future of biosensors in pharmacology is likely to involve development of dipsticks for one-shot analysis in *in vitro* samples, laboratory and bedside analyzers, and *in vivo* sensors for open-loop (direct read-out) or closed-loop (automatic coupling to a delivery system) use.

He said that these possible directions can be illustrated by recent sensor studies in diabetes. New enzyme-based reagent strips for home blood glucose monitoring are now appearing in which the reaction is detected electrochemically by a pen-sized meter. Handheld computers are becoming available for calculation of insulin dosages according to blood glucose values entered by the patient four times daily, and Pickup believes that these devices could be used with the new capillary blood meters for obtaining improved metabolic control.

Pickup said that insulin infusion pumps have been used for more than a decade for obtaining strict glycemic control in selected diabetic patients. Delivery of insulin is usually subcutaneous and selection of rates is by the patient (for example, activation from a meal-time boost). Closing the loop with an implanted glucose sensor to form an automatic artificial endocrine pancreas is a possible goal.

A new strategy for implanted sensors uses an organic mediator to relay electrons from the enzyme in the sensor to a base electrode. Ferrocene was one of the first of the immobilized mediators and has been incorporated in several devices now undergoing trial.

According to Pickup, one can expect in the future to see new enzymes, new mediators, and new technologies. For example, a recently discovered glucose dehydrogenase from *Acinetobacter calcoaceticus* is NAD-independent since it has a quinone prosthetic group. The organic metals such as the charge trans-fer complex tetrathiafulvanium tetracyanoquiodimethamide, TTF^+, $TCNQ^-$, is a good mediator (or electrode material) for glucose-based sensors.

New technologies which may be applicable to drug monitoring, Pickup said, include the several designs for immunosensors. The field effect transistor (FET) device uses an antibody attached to the gate of the FET. Field changes in the substrate of the transistor occur as the antigen binds to the antibody and causes current alterations as a signal of the analyte levels.

Conclusion

This intensive conference on drug delivery and drug targeting covered a variety of topics including developments that have taken place in producing new dosage forms for existing drugs, the area of controlled release including oral dosage forms, implants, and transdermal systems. In addition, the possibilities of greater selectivity in drug delivery were considered with a review of the possibilities and limitations of currently available systems of drug targeting such as liposomes, macromolecules, monoclonal antibodies, and prodrugs. Furthermore, the challenges now presented to drug formulators by the new generation of polypeptide drugs that cannot normally be delivered by conventional systems was explored. Opportunities for delivering peptides and proteins via the gastrointestinal tract and nasal routes were also presented.

It is evident that extensive research has been carried out in all the above areas both by academic scientists as well as scientists at industrial organizations in Europe as well as the US. However, further work is still needed before drug delivery and drug targeting products based on this research become available for routine clinical use.

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