SITE-SPECIFIC DRUG DELIVERY

EDITED BY
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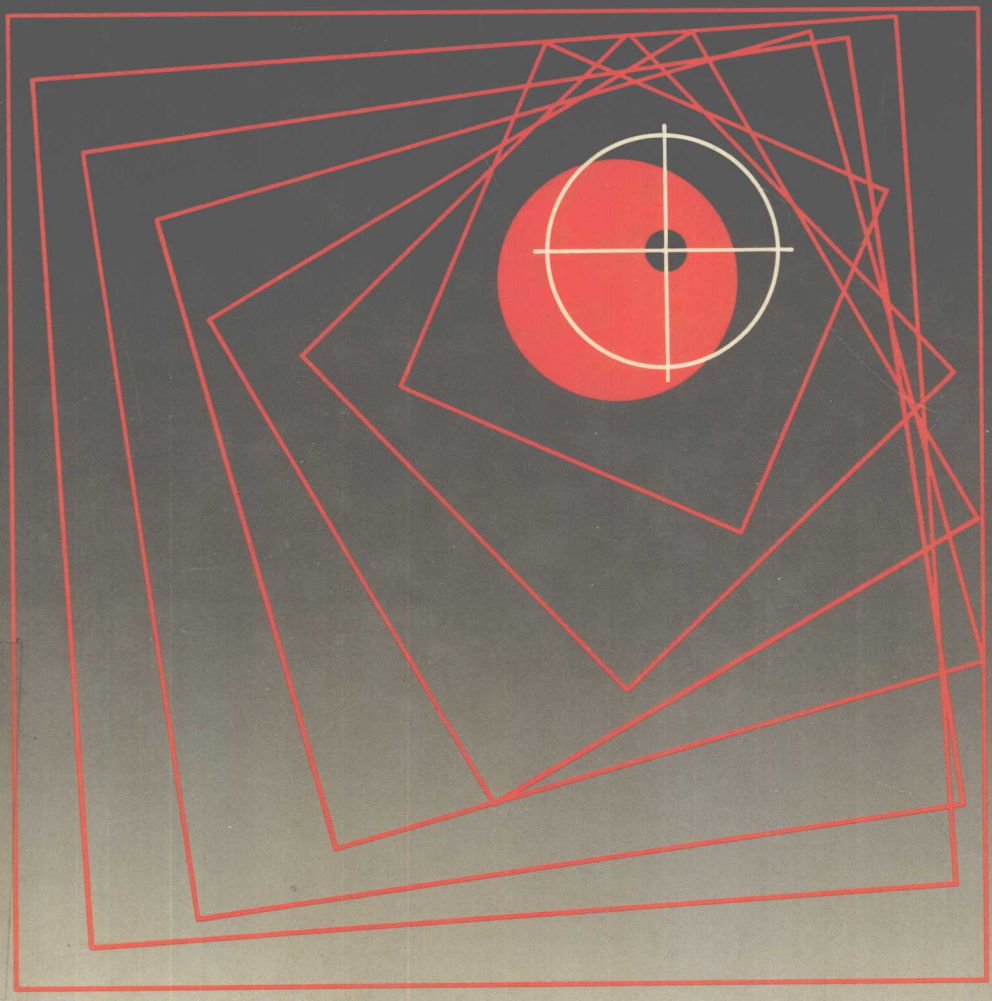

Site-Specific Drug Delivery

Cell Biology, Medical and Pharmaceutical Aspects

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Preface

Recent developments in cell and molecular biology are providing new and exciting opportunities for the rational design of novel types of highly specific and effective therapeutic agents. These opportunities will often only be turned into clinical success through strategies which ensure the delivery of such agents to their desired site(s) of action via events that prevent loss of drug activity, avoid toxicity, and which are both cost-effective and convenient to use. This Book addresses some of the biological opportunities and other scientific challenges for the site-specific delivery of drugs, and indicates the formidable challenges which face both the pharmaceutical industry and academe alike in bringing these new therapeutic systems into general usage. Topics include an examination of the cellular and pathological opportunities which present themselves for site-specific delivery. In addition, the receptor-dependent targeting of various conventional and newer drug types are examined, including the targeting of lipoproteins (for treating atherosclerosis), and toxins (cancer chemotherapy). An important feature of some of the Chapters is that for targeting to be successful not only is site-selection and access important but so is the persistence of adequate drug levels at the target, and methods for examining and solving this question are covered. Various carrier systems are examined, including the study of opportunities and problems which exist in the use of particulate drug carriers. Recent advances in understanding the tissue tropisms present in viruses are examined, as these are of undoubted potential benefit in designing carriers which may penetrate both epi- and endothelial barriers and seek out their target organ. Some examinations of the possibilities and pitfalls for gene therapy are made.

The content of the book is designed to indicate that site-specific drug delivery is as important a specification in drug design as is drug interaction with its

pharmacological receptor. The rapidly accelerating advances in numerous biological sciences are constantly opening up new opportunities both in terms of understanding normal and pathological processes as well as in providing new types of molecules to combat disease. Clearly, the potential provided by site-specific drug delivery, as shown in this Book, will only be realised through a multidisciplinary and integrated innovative research effort

E. Tomlinson
S. S. Davis October 1986

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This Book is based largely on the Proceedings of a Symposium held to mark the opening of the new Advanced Drug Delivery Research (ADDR) building at Ciba-Geigy Pharmaceuticals, Horsham, West Sussex, United Kingdom.

The Symposium was held at the Pharmaceutical Society of Great Britain, London, UK during October 1986, and was attended by more than 250 academe- and industry-based scientists representing the areas of cell and molecular biology, biochemistry, immunology, colloid, polymer and surface chemistry, clinical pharmacology and pharmaceutics.

E. Tomlinson.

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(Patho)physiology and the Temporal and Spatial Aspects of Drug Delivery

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> '[Ehrlich's] achievements are like those of a marksman who is intimately familiar with his weapon and the capabilities of his missiles and the path they must take, and whose eyes are blind to all else but the specific target which is his aim. At first his missiles may land wide but, as his aim improves, the number of hits increases until finally, fired by the marksman's hand, and guided by observation and experience, the perfect shot lands in the bull's-eye.
>
> Ehrlich's ... success is the result of painstaking work directed at predefined goals and based on scientific hypotheses. The motto "We must learn to aim" which he has given to the science of chemotherapy also applies in the wider, more general sense to his entire work.'
>
> *Paul Ehrlich, Eine Darstellung seines wissenschaftlichen Wirkens (Paul Ehrlich — a review of his scientific work).* (1914, Abstract from Chapter by Professor Albert Neisser).

1. INTRODUCTION

In the fields of parasitology, bacteriology, cancer research and cell morphology, the seminal views of Ehrlich are often with us today. His *Corpora non agunt nisi fixata* theory (substances act only when they are linked), the side-chain theory and his concepts of tropisms of toxins to host cells and to infectious cells — organotropy and parasitotropy (sic) — began an understanding of drug selectivity and disposition that laid the basis for much of modern chemotherapy research. Ehrlich's efforts were designed to bring chemistry and biology together (Baumler, 1984) with the purpose being to 'aim drugs and to aim in the chemical sense.' His views on toxin and later drug interactions with cells, his prediction of

the existence of antibodies and, after their discovery, his discussion on their specific binding, led him to talk of 'magic bullets', to Alexander Fleming to later eulogise about Ehrlich's 'wonder weapons', and Edward G. Robinson as Ehrlich, in the less than factual film 'The Magic Bullet' (Warner Brothers, director William Dieterle, U.K. released September 23rd 1940), to create public interest in the specificity of drug action. The impact of the receptor theory of Ehrlich (and of Langley) was limited in their lifetimes, and it was not until the 1950s that this became a major area of research (Parascandola, 1981).

Great as Ehrlich's achievements were, translation of his ideas into the context of modern molecular and cell biology approaches to understanding (patho)physiology and drug action are like comparing the first airplane to the latest Stealth bomber. Modern cell and molecular biology is providing an understanding of (patho)physiology at a level of definition that suggests that the molecular basis of many diseases will be known before this Century is out. In parallel, control of gene expression in both eukaryotes and prokaryotes is providing a plethora of homologous and heterologous polypeptides in a pure and abundant form — seemingly adding dramatically to the armamentarium available to combat disease. It is quite apparent that many of these advances will become of significance clinically if both the newer bioengineered molecules or their synthetic counterparts could be delivered to their site(s) of action in an efficient, safe, convenient and cost-effective manner.

This Chapter (and indeed the remainder of this Book) attempt to show that although today we are guided by the dogma that often carriers are required to transport drugs to specific site(s) within the body in an exclusive and protected manner, this cannot be regarded as an 'add-on' activity in drug research, that drug arrival at [its] pharmacological site(s) of action(s) — as constrained by the properties of the disease and the temporal and spatial aspects of drug/pharmacological receptor interaction — is a feature of drug design as essential as drug action. As many different science disciplines begin to merge through the medium of molecular approaches to cell (patho)physiology these two technical aspects will become regarded as parts of the whole.

2. DRUG ACTION

In general, after the administration of drugs, their efficacy and safety are maintained by selective interaction with the pharmacological site of action coupled with a reliance on the body's normal detoxification and excretion processes to rid it of both unwanted active principle and its metabolites. These (dose-related) events define the drug's therapeutic index, dose regimen, and, often, administration route and dosage form. Apart from most interactions with the pharmacological receptor, all of the processes involved are passive for the majority of low molecular weight species, and although they may or may not be

linear they are generally diffusion-controlled. The contempory use of pharmacokinetics in describing and managing drug treatment, and the use of pharmacodynamics in undertanding drug action at the receptor, largely reflect this.

It is quite clear though that the new understanding of, in particular, (patho)physiology, (including receptor-mediated processes), indicates that *active* processes are probably the medium through which drug site-specific delivery could be effected.

3. BIOLOGICAL OPPORTUNITIES FOR SITE-SPECIFIC DELIVERY

Introduction of drug into the body immediately lays it open to a multitude of temptations and dangers, including degradation, unwanted disposition (either intravascular, e.g. protein binding, or extravascular, e.g. non-target tissues), and the initiation of toxic events (e.g. immune system activation or depression). To achieve appropriate drug action, site-specific systems serve to *protect* the body and the drug from these events, and to achieve exclusive site-specific action of the drug through (variously) *site-specific selection, access, release and adequate retention*, (Figure 1).

The biological opportunities which present themselves for site-specific delivery using carriers are *anatomical, (patho)physiological and biochemical*. Figure 2 gives the biological routes which a site-specific system may need to follow to

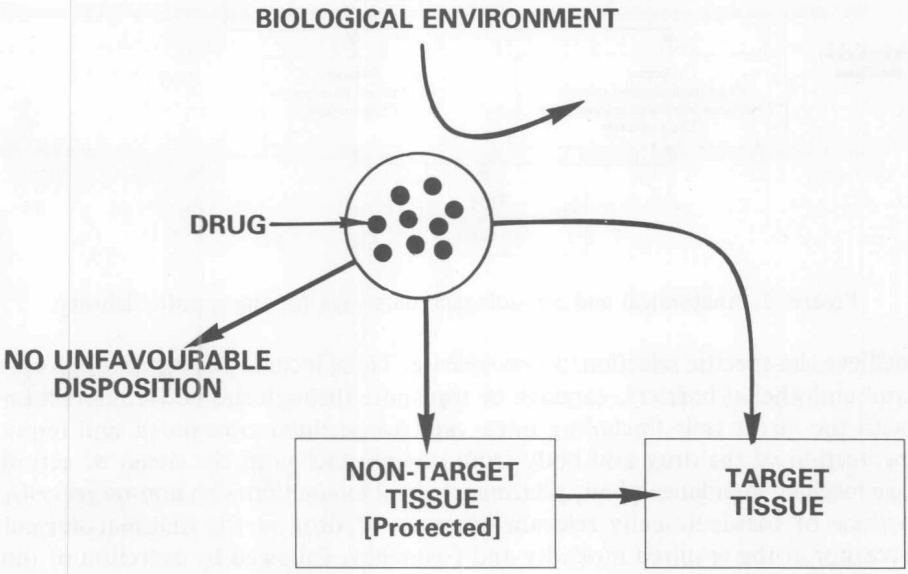

Figure 1. Aims of site-specific drug delivery.

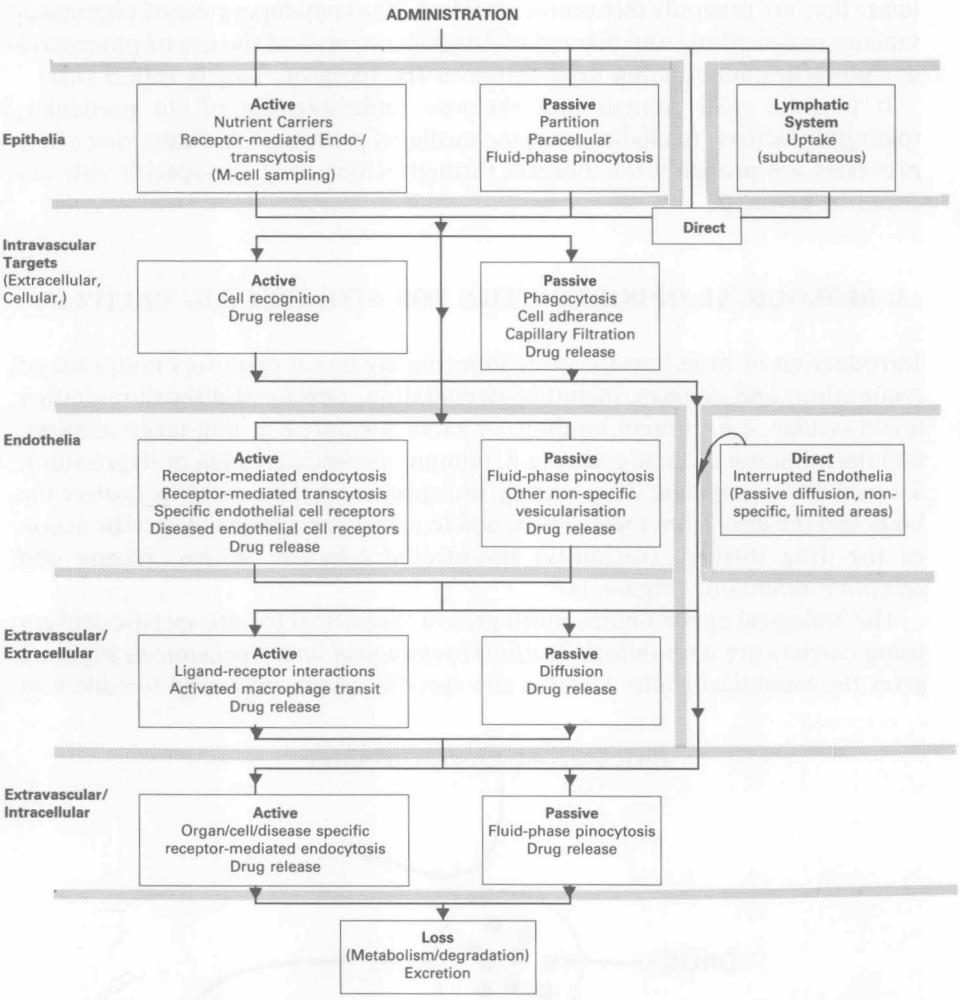

Figure 2. Anatomical and physiological pathways for site-specific delivery.

achieve site-specific selection/access/release. These include passage through epi- and endothelial barriers, carriage or transport through the body, interaction with the target cells (including intra- and transcellular transport), and imply protection of the drug and 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 pharmacological receptor 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 of carrier. Carriers should be either biologically degradable or readily excreted, and should not interact with either biochemical or immune systems of the body — unless desired).

3.1. Cell processing

Frequently the pathway to a pharmacological site comprises passage into and through various cells. To survive and to maintain their function, both normal and diseased cells can take up and process numerous types of materials by various mechanisms. These have applicability for cell selection and access by site-specific systems. They are of both a passive and an active nature, and include fluid-phase pinocytosis (Lewis, 1931), phagocytosis (Griffin and Silverstein, 1974), and both constitutive and non-constitutive receptor-mediated endocytosis (Steinman *et al.*, 1983) (Figure 3). In addition, various other methods of gaining access to cells include membrane fusion and passive diffusion as well as surface binding to either specific or non-specific regions. Hopkins in this Book later describes vesicularisation in some detail, though for site-specific delivery it is relevant to note that cell processing includes trafficking of ligand and/or receptor to the lysosome, then into the cytoplasm and onward to discrete intracellular compartments, and thence through the cell to the opposite membrane (transcytosis), or to the original membrane of cell entry (diacytosis, retroendocytosis). It is highly encouraging to appreciate that today the molecular basis of numerous cell recognition and subsequent intracellular sorting events are being elucidated using molecular cell biology tools; for example the routing

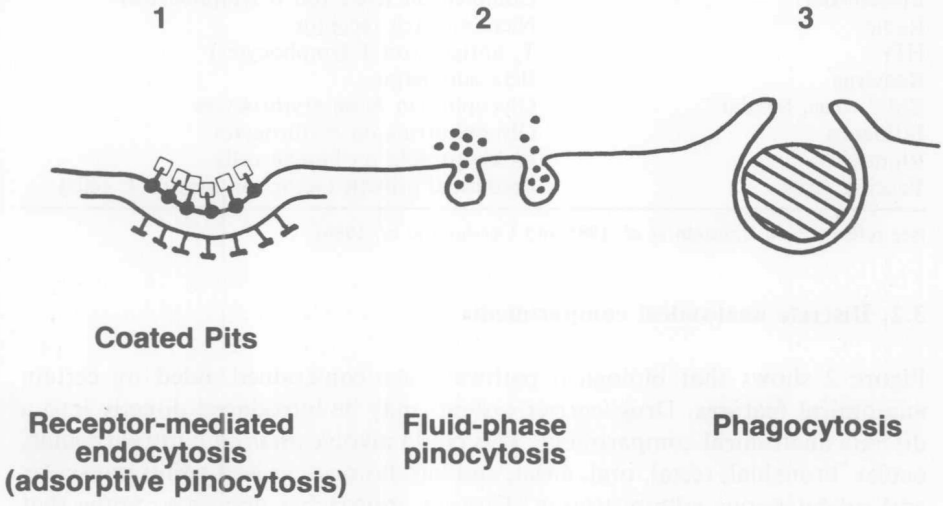

Figure 3. Endocytic vesicles at cell surface.

signal sequences for transporting (secreted) proteins to various subcellular compartments including the nucleus and the mitochondria are known (e.g. Schekman, 1985; Horwich *et al.*, 1986).

Fluid-phase pinocytosis is a non-specific process and because of its continuous nature and extent may be useful as a general process for transporting macromolecular constructs through epithelia, some endothelia and into various blood cells. In contrast, receptor-mediated endocytosis is a much more versatile process, encompassing an ability of specific cells and tissue to recognise or select and to transport into the cell (and sometimes through them), complex molecules in size from less than 100 daltons to more than one million daltons (e.g. virus particles). Phagocytosis on the other hand is a discontinuous process undertaken by macrophages responding to the presence of (opsonised) particles. Most cell processes involving membrane cycling appear to be able to function independently of one another.

For site-specific drug delivery much should be learnt from understanding how pathogens such as toxins and virus particles are able to pass into and through epi- and endothelial barriers and to have unique tissue tropisms, (see Almond *et al.*, this Book). Table 1 illustrates, for example, the unique abilities that viruses have evolved to utilise specific normal physiological receptors to gain access to cells. The later Chapters by Vitetta *et al.*, Hopkins and Almond *et al.*, examine pathogen utilisation of various recognition systems.

Table 1. Viral utilities of receptors

Virus	Receptor
Epstein-Barr	Complement CR-2 (on B lymphocytes)
Rabies	Nicotinic Ach receptor
HIV	T_4 antigen (on T lymphocytes)
Reovirus	Beta-adrenergic
EMC virus, Sendai	Glycophorrin A on erythrocytes
Influenza	Glycophorrins on erythrocytes
Rhinovirus	80 kD protein on human cells
Vaccina virus	Epidermal growth factor (on murine L cells)

(see references in Eppstein *et al.* 1985 and Gershoni *et al.* 1986).

3.2. Discrete anatomical compartments

Figure 2 shows that biological pathways are constrained/aided by certain anatomical features. Drug/carrier systems may be introduced directly into a discrete anatomical compartment. This could involve *inter alia*: intraarticular, ocular, bronchial, rectal, oral, nasal, and intralesional, as well as intramuscular and subcutaneous administration. Current approaches appear to argue that simple drug retention at these sites would enable therapeutic effects to be

achieved (Dingle *et al.*, 1978) though the explicit disease would mitigate whether a further level of sophistication in terms of drug arrival at the pharmacological site is required.

Subcutaneous routes of administration for site-specific systems have an interesting appeal in that not only can targets within the lymph system be accessed by both particulate and soluble conjugate carrier systems, but there is also drainage into the general cardiovascular pool (via the thoracic duct). Access may be related to the presence of high density anionic sites within the intercellular clefts of mesothelial and lymphatic endothelial cells such that these clefts can provide channels between endothelial cells which permit movement of macromolecules and particles (Leak, 1986). Weinstein *et al.* discuss this route of administration further in this Book, though other references of interest are due to the important studies on lymphatic system disposition of liposomes (Parker *et al.*, 1981; Hirano *et al.*, 1985) and other colloidal systems (Bergqvist *et al.*, 1984).

3.3. Intravascular targets

Epithelial transport within the gut can occur through variously: fluid-phase pinocytosis, paracellular and transcellular passive diffusion, receptor-mediated endocytosis (and transcytosis?), and via nutrient carrier processes. Further access to the vasculature via either lymphatic translocations and/or direct parenteral administration enable both intravascular and extravascular targets to be reached. Important therapeutic targets exist within the vasculature, and as other Chapters in this book indicate it is possible to target (passively via phagocytosis) to fixed macrophages of the mononuclear phagocyte system (MPS) for either treating diseases of the MPS (e.g. neoplasms, parasitic, viral and bacterial infections — the latter reviewed by Baillie, 1985), or for stimulating these by use of macrophage activators and other lympho- and cytokines to treat abnormal cell dissemination (Poste and Fidler, 1982). Other blood cells may be targeted, presumably by either recognition of cell-specific markers (Singhal and Gupta, 1986), e.g. to subsets of lymphocytes, or by selective adherence to cell surfaces. In addition, the phenomenon of particle filtration by capillaries after intravenous and intraarterial administration is of potential use for the delivery of anti-emphysemics (Martodam, 1979), thrombolytics (Torchilin, 1983) and cytostatics (Ariel and Padula, 1978; Kato *et al.*, 1981; Tomlinson *et al.*, 1982).

Avoidance of the MPS is an interesting current problem which appears to be solvable. For particulate systems the later Chapter by Davis and Illum shows how surface modifications can help to resolve the problem; with soluble species the design specification is that immune complexes are not formed. Approaches to avoiding uptake by cells of the MPS include using mimics of natural carriers, (e.g. low density lipoprotein (Halbert *et al.*, 1985), erythrocytes (Utsumi *et al.*,