ADVANCES IN
DRUG RESEARCH

Edited by

BERNARD TESTA

VOLUME 13

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PREFACE: "SAILING AGAIN"

Advances in Drug Research, under the able captaincy of Drs. N. J. Harper and Alma B. Simmonds, has sailed twelve fruitful voyages, twelve volumes of exploration in the unlimited realm of drug research. Now, after a number of years spent in dry dock, the ship is out at sea again, a fresh captain at the helm.

In recent years, drug research has progressed enormously in several directions. Established therapeutic classes have yielded better analogs, and a number of entirely novel classes of drugs have been discovered. These aspects will of course be given due attention in the series, and specific classes of drugs will be critically reviewed in all future volumes. This is also the case in comparable series, and the point does not need to be emphasized further.

But drug research has also progressed in fields of general significance such as drug metabolism, molecular pharmacology, and drug design. These topics are important, even more in my opinion than specific classes of drugs. Indeed, these general fields are the ones that offer the best promises of understanding how drugs work and of discovering novel and better therapeutic agents. As a result of what may be a personal bias—but it is the captain who sets the course—general topics will be given constant attention in the series. Constant, but not overwhelming: all efforts will be made to offer a good balance between specific and general topics, bearing in mind that this discrimination is not always meaningful. In the first chapter, which is in fact an oversized introduction to the series, some considerations are given on the structure of drug research and on a number of general fields in which impressive advances have been witnessed.

Schematically, and again being aware of the dichotomic trap, drug research has two goals—scientific goals, that is! The first that comes to mind is the discovery of new, more specific, and more active drugs. The second goal, first recognized by clinicians, is to improve the activity of existing drugs by increasing their beneficial actions and by decreasing their unwanted effects. This can be achieved by optimizing the modes and routes of administration, in other words by taking into account the patient's characteristics (age, state of health, etc.), drug interactions, bioavailability, chronopharmacological and pharmacokinetic factors, and many other influences. These two goals are far from being mutually exclusive. Rather, they proceed from approaches that have much in common. To these goals we adjust our compass. But a feedback regulation is needed, which you, the reader, should provide. Comments, suggestions, criticisms, all reactions will be gratefully welcome.

BERNARD TESTA

CONTENTS

Central and Peripheral α-Adrenoceptors. Pharmacological Aspects and Clinical Potential

P. A. VAN ZWIETEN AND
P. B. M. W. M. TIMMERMANS

Novel Approaches to the Design of Safer Drugs: Soft Drugs and Site-Specific Chemical Delivery Systems

NICHOLAS BODOR

Drugs? Drug Research? Advances in Drug Research? Musings of a Medicinal Chemist

BERNARD TESTA

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> Knowledge is one. Its division into subjects is a concession to human weakness.
>
> Halford J. Mackinder

What are drugs? How complex and fuzzy a network of theories, concepts, models, findings, assumptions, fictions and errors is hiding behind the simple term "drug research"? Where and how is drug research advancing? Such questions defy complete and explicit answers, but even very fragmentary ones may draw attention to interesting perspectives. In the following pages, a number of rationalizations and of more intuitive views will be offered. Findings and discoveries are not mere data, they also provide the incentive and input for intellectual creations such as recepts, concepts and intuitions.

ADVANCES IN DRUG RESEARCH VOL. 13
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These thoughts have inspired the present chapter, which is meant as a general introduction to this and future volumes.

1 Drugs?

Many years ago, workers in drug metabolism realized that they were dealing not only with drugs, but also with many other compounds foreign to the organism. The word "xenobiotics" was thus coined to describe such foreign compounds, i.e. exogenous chemicals of no physiological benefit. A list of xenobiotics is given in Table 1. Drugs make up an important group of xenobiotics, as do other categories in this list. Some cosmetics do find their way into the body, e.g. lipstick constituents.

TABLE 1

Compounds classified as xenobiotics

Drugs
Food constituents devoid of physiological roles
Food additives (preservatives, colouring and flavouring agents, antioxidants, etc.)
Drugs of "pleasure" and of abuse (ethanol, coffee and tobacco constituents, hallucinogens, etc.)
Constituents of cosmetics
Various chemicals (insecticides, herbicides, etc.)
Polluting agents

The difference between xenobiotics and chemicals (of endogenous or exogenous origin) fulfilling a physiological role is far from sharp and well defined (Testa *et al.*, 1981). Thus, where should we categorize the nitrogen gas we inhale? Many examples could be given, but to little avail in the present context. However, to stress the point further, it must be remembered that not all drugs are xenobiotics, no more than all xenobiotics are drugs. This statement is trivial when one considers the therapeutic use of such physiological compounds as vitamins, amino acids, complex lipids, hormones, common salts, and others. From the above, we conclude that it is its use rather than its origin or nature which tells us if a given compound must be considered a drug or not, in close analogy with the well-known fact that the dose makes the poison.

Innumerable drugs exist which are used in a considerable variety of therapeutic indications, not to mention diagnostic and prophylactic agents. In a very schematic manner, these therapeutic indications, and the many pharmacodynamic actions of drugs can be classified into three large therapeutic

classes, namely chemotherapeutic agents, neuropharmacological agents, and a less well-defined category of agents acting on regulatory mechanisms (metabolic, hormonal and immunological). Of course all drugs can be considered as metabolic agents in the broadest sense, since in one way or another they interfere with biological processes. This, however, is hardly a classification. On the other hand, too many categories scatter a global view of drug action and therapeutic uses.

Chemotherapeutic agents are meant to inhibit or destroy a parasite while being as harmless as possible for the host—a problem of selectivity. "Parasite" is taken here in the broadest sense, to mean viruses, bacteria, fungi, protozoa, parasitic worms, and also tumour cells. Neuropharmacological agents have various impacts on the central and/or peripheral nervous system, acting directly on receptors or indirectly through neurotransmitters, or by less specific mechanisms as in the case for local or general anaesthetics. The third class includes those drugs acting on various enzyme systems, e.g. several groups of diuretics, or on immunological mechanisms; further, all agents with hormonal or antihormonal activities belong to this class.

Such a general classification cannot be absolute, and overlap exists. For example, there are enzyme inhibitors (metabolic agents) that are chemotherapeutic drugs (e.g. dihydrofolate reductase inhibitors) or neuropharmacological agents (e.g. inhibitors of some amino acid decarboxylases). Overlap between chemotherapeutic and neuropharmacological agents is seen for example with anthelmintic drugs blocking neuromuscular transmission. The above discussion is graphically summarized in Fig. 1.

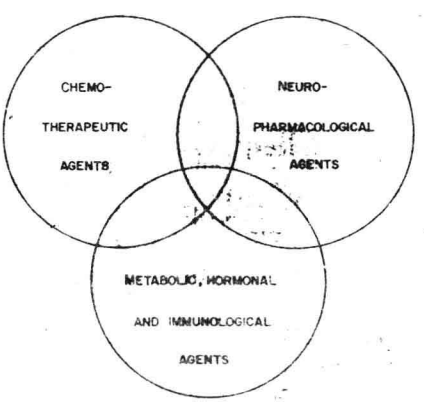

FIG. 1. A broad classification of therapeutic drug classes.

2 Drug research?

Drug research is such a multi-faceted and intricate domain of human activity that any description or discussion of it is bound to remain incomplete. However, a number of salient features exist in the structure of drug research which can usefully be considered.

A scheme summarizing the essence of drug research is presented in Fig. 2; it derives, with a number of additions and modifications, from a simpler scheme published by Kier and Hall (1977). The present section will be devoted to a discussion of various steps in this scheme, each of which corresponds to an important aspect of drug research. The starting point involves examining, in turn, biological and molecular systems. Indeed, essential to drug research is the deepest possible understanding of all relevant properties of drug molecules as well as of the biological systems with which they interact. To "understand" these properties not only means to have unravelled them, but also to be able to determine or measure them, and to express them in a manner suitable for the next steps in drug research.

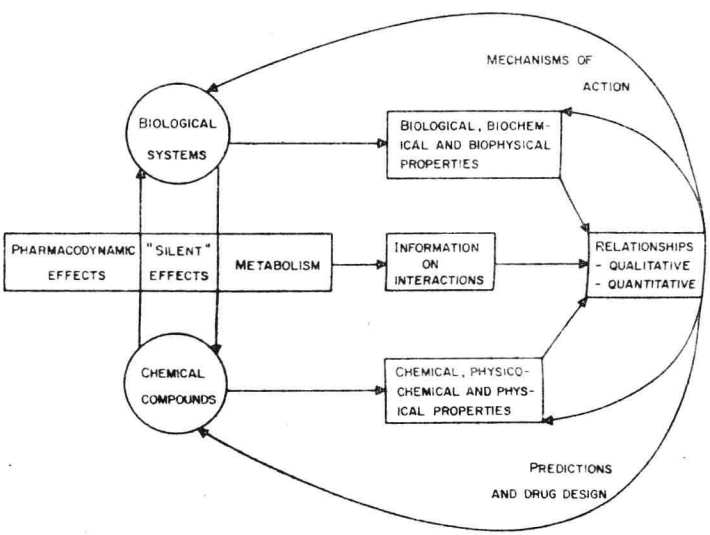

FIG. 2. A schematic view of drug research.

When biological systems and molecular entities interact, it can be in a number of ways: biological effects (pharmacodynamic effects) are elicited by the drug molecules acting on the biological systems, which in turn can handle the former (metabolism) to absorb, distribute, store and excrete them

(disposition), as well as to chemically transform them (biotransformation). These two modes of interaction are not independent of each other, but rather are interdependent. Nor are they the only ones which can be characterized: witness "silent" interactions such as non-covalent binding to plasma or tissue proteins. To be useful in terms of drug research, all these types of interactions must be expressed as biological information in the form of qualitative or quantitative data. The discovery of relationships between the latter and some properties of the biological systems and/or the drug molecules is a crucial step in drug research. Indeed, such relationships, often interesting *per se*, are particularly valuable when they deepen our understanding of biological mechanisms and allow improved drugs to be designed.

2.1 BIOLOGICAL SYSTEMS

Biological systems not only interact with drug molecules, but also provide the environment in which these interactions take place. Such a fragmented approach is quite a common one, e.g. in molecular pharmacology, and has often been proven to be of immense value. But taken alone, it provides only a partial understanding of phenomena because any biological system is highly integrated and must also be apprehended holistically. Obviously such topics as biological complexity and levels of organization, biological environment, structure and information, are of capital significance in drug research.

2.1.1 Biological levels of complexity and organization

The targets of drugs, when these are used for therapy and not for research purposes, are always organisms or even populations of organisms (e.g. populations of bacteria in chemotherapy). An organism must be understood as defined by Yates (1982), namely a "complete living system . . . characterized by autonomous morphogenesis, nearly invariant reproduction, and teleonomic behaviour".

In contrast to therapy, drug research deals with biological systems of various levels of complexity, from the simple biological levels of molecules and macromolecules to the higher ones of organisms and populations. The main levels thus encountered are presented in Table 2. Biological complexity, as illustrated in Table 2, is intuitively understandable, but is difficult to explain rationally, as stressed by Yates (1982). Complexity is related to the degree of organization and information content, but also to size, inasmuch as the former depend on the latter. These problems of biological complexity, levels of organization, and biological information are receiving considerable interest (e.g. Cramer, 1979; Pattee, 1979; Ryan, 1980; Arenas *et al.*, 1981; Bolender, 1981; Garfinkel, 1982) and should not fail to interest every worker

in drug research. Indeed, it is always recognized in theory, but often forgotten in practice and when assessing results, that in research each biological level of complexity (Table 2) is at best a model of the higher level(s). What is lacking in an isolated system belonging to a given level is the coordination existing in the higher level(s), namely the constraints which regulate the functioning of this system as an integrative part of a larger system.

TABLE 2

Biological levels of complexity encountered in drug research (expanded from Testa, 1982a)

Entities	Examples
Small molecules	Monosaccharides, amino acids, fatty acids
Medium-sized molecular systems	Oligosaccharides, nucleotides, oligopeptides, lipids
Macromolecules	Polysaccharides, nucleic acids, proteins, enzymes
Supramolecular structures	Multienzymatic systems, membranes, chromosomes, "receptors"
Organelles	Mitochondria, nuclei
Cells	Neurons, hepatocytes, unicellular organisms
Tissues	Epidermis, renal cortex
Organs	Heart, brain
Functional systems	Digestive system
Organisms (pluricellular)	Parasitic worms, human beings
Populations	Intestinal microflora, human populations

Biological models used as tools in drug research take a considerable risk of being irrelevant when non-physiological conditions are applied, or because they bear insufficient relationship to the system on which the model is based. The permanent problem is thus: to what extent are models relevant to the ultimate object of study? There is no doubt that working with membrane preparations containing xenobiotic-metabolizing enzymes or pharmacological receptors has allowed explosive progress in drug metabolism and molecular pharmacology, respectively. But extrapolation to animal or human is far from straightforward as seen in the well-known problem of *in vitro–in vivo* correlations.

In many fields of biological research, the current approach is clearly a reductionistic one, with triumphant successes in great number. In drug research also, the reductionistic approach has been particularly fruitful as often shown in the following pages. However, as stated above, the reduction to models is bound to neglect major aspects of the reality we are investigating. Indeed, biological information is of a completely different nature in the lower

(molecular) and in higher levels of organization. This is illustrated by Pattee (1979) in a particularly striking manner:

> . . . there is no question that the structure of nucleic acids . . . obeys quantum mechanical laws. However, a complete detailed quantum mechanical description of these structures would give no more clue to the meaning of a DNA sequence as biological information than the chemistry of this ink and paper would give a clue to the meaning of these words.

The outcome is obvious; as pointed out by many epistemologists, biology is unique among all sciences in being currently unable to rationally define its object of study—life.

From the above, it should be clear that, while the reductionistic approach to drug research will continue to be followed with success for many years to come, the time now appears ripe for the synthetic, holistic approach to receive more attention. Only by giving comparable importance to these two complementary approaches, like walking on two legs, can drug research expect decisive therapeutic breakthroughs.

2.1.2 Water and hydration; lipophilic environments

A major characteristic of biological systems is the duality or plurality of many of their properties—certainly an important prerequisite of life. Let us consider here the hydrophilic/lipophilic duality of the biological milieu, or better, to avoid the dualistic trap, the plurality or even continuum of properties existing between highly hydrophilic and highly lipophilic ones.

Water is a compound with unique physical properties, particularly in the liquid state relevant to biology (e.g. Symons, 1981; Land and Lüdermann, 1982). But some interactions, particularly with biological constitutents, can modify its properties and behaviour. Conversely, interactions with water can modify some properties of molecules or macromolecules. Witness the fact that a molecule behaves quite differently by a number of criteria depending whether it is studied in isolation (in vacuum) or in solution.

Up to now, much effort has been spent assessing the influence of solvation in general and of hydration in particular on molecular properties. In the field of drug research, this is exemplified by solvent effects on such properties as acidity, basicity, and conformational behaviour (see section 2.2.2.). Much effort has also been devoted to understanding *hydration*, e.g. by defining hydration sites and calculating hydration energies (e.g. Scheraga, 1979; Edmonds, 1980, Mehrotra *et al.*, 1981). Amino acids and small peptides have been favoured objects of investigaton (e.g. Wolfenden *et al.*, 1981). Particularly noteworthy is an extraordinary research paper on the hydration of a dipeptide (Rossky and Karplus, 1979) which reports with exceptional detail the structure of the solute, the structure and dynamics of the solvent, and the

influencing factors. Such studies enlarge our understanding of biochemical properties, and as such afford major contributions to drug research. Thus, the thought-provoking book of Lewin (1974) stresses the importance of the displacement of water in the control of biochemical reactions, and this should have implications for the mechanism of action of some drugs. Unfortunately, this aspect of drug research remains essentially unexplored.

Another poorly understood topic concerns the changes in *water properties* caused by the hydration of solutes. A number of solutes have structure-promoting or structure-breaking properties towards liquid water, and this may affect processes occurring in an aqueous environment (Edmonds, 1980; Symons, 1981). Thus, we showed some years ago that, compared with controls, hydrolytic cleavage reactions are notably slowed down in buffered aqueous solutions rendered highly viscous by the addition of small concentrations of a very hydrophilic polymer (Testa and Etter, 1975). The understanding and modelling of the changes in water properties (e.g. Pullman, 1977; Tapia, 1980) have implications for drug research. Long-range ordering of water molecules adjacent to many interfaces, in particular polar macromolecules and various biological membranes, is supported by several lines of experimental evidence (Drost-Hansen, 1971). The role of "bound" water in biology is a challenging object of speculation (Drost-Hansen, 1971; Hazlewood, 1977), and the distinct possibility exists that long-range ordering, by even slightly modifying the thermodynamic properties of water, influences a drug's fate and action—an influence not present in too simple biological models.

The hydrophilic properties of biological aqueous phases are balanced by the *lipophilic properties* of membranes. Constituents such as phospholipids and cholesterol confer their lipid nature on membranes and do not display large variations in their properties. This contrasts with the very broad variations in lipophilicity and other properties existing between various proteins, and between various domains and/or various states of a given protein. The amazing plasticity of proteins in terms of structure and properties is perhaps the best model at the molecular level of the plasticity of organisms. Depending on the proportion of amino acids with polar or non-polar side-chains, a protein will be hydrophilic or lipophilic overall. These properties, however, will not be influenced only by the primary structure, but also by the secondary, tertiary, and quaternary structures, and hence depend on the state of the protein as determined by intrinsic and extrinsic factors. Particularly important is the presence of structural domains in proteins (Wodak and Janin, 1981). Thus, hydrophobic packing will create lipophilic micro-environments in, e.g. an aqueous phase (Ponnuswamy *et al.*, 1980). An illuminating and extensive presentation of the biochemical and biological roles of proteins has been given by Williams (1980).

2.1.3 Proteins and enzymes

The numerous functions of proteins such as bio-structural constituents, biochemical effectors (enzymes), and information carriers (hormones), make them privileged partners of drugs in many of the interactions to be considered later (section 2.3). As a consequence, the study of protein structure and function has much relevance to drug research be it to define binding sites, catalytic sites, or properties of active peptides, among others. These considerations of course do not apply only to simple proteins, but also to complex ones such as glycoproteins, the structures and roles of which have been excellently reviewed (Sharon and Lis, 1981)

Many *structural aspects of proteins* have been extensively investigated, e.g. the conformational aspects of backbone and amino acid side chains, the significance of flexibility, the various types of bends (β-bends, etc), the stabilization of the three-dimensional structure by H-bonds and other intramolecular interactions, the intermolecular interactions controlling the quaternary structure (e.g. tetrameric proteins), as well as description and representation problems (e.g. Hartley, 1979; Isogai *et al.*, 1980; Milner-White, 1980; Némethy and Scheraga, 1980; Pericolas and Kurtz, 1980; Schwyzer, 1980; Huber and Bennett, 1983). Such studies are important in order to help characterize the chemistry and topography of pharmacological receptors and active sites in enzymes.

The contributions of *enzyme research* to advances in drug research are particularly striking. Indeed, drugs interact with enzymes as substrates (in biotransformation reactions) or as inhibitors (a frequent mechanism of therapeutic action), not to mention inducers, activators, and uncouplers. As regards enzymatic reactions, they are characterized by stereochemical choices (Overton, 1979), electrostatic stabilizations (Warshel, 1981) and thermodynamic aspects (Page, 1977; Conrad, 1979; Warshel and Weiss, 1980; Warshel, 1981) which are not apparent in reactions in solution. The reasons for the macromolecular nature of enzymes are thus recognized in the necessity of creating a highly specific stereochemistry and microenvironment at the active site, the necessity of allosteric regulations and particular hydrodynamic properties, and in a number of other demands and justifications. These aspects have been discussed by Luisi (1979) and Williams (1980) and illustrate simpler levels of biological complexity and organization of living matter. From a higher viewpoint, chemical, osmotic and chemiosmotic enzyme catalysis have been formulated by Mitchell (e.g. 1979) in terms of a general ligand conduction principle. This appears as one of the most comprehensive attempts to unravel and model the basic mechanisms of life processes On a more theoretical plane, we find the conceptualization of cooperative phenomena and synergistic processes (Haken, 1980), or the theory of hypercycles

and hypercyclic regulations (Eigen and Schuster, 1979) with its vertiginous level of abstraction. Someday, it is hoped that fertile minds will take advantage of these or similar approaches in order to formulate new concepts of drug action.

2.2 DRUG MOLECULES

The search for new drugs by synthesis of a random collection and selection of the most active or least toxic compound is an approach which fell into obsolescence long ago. For many decades medicinal chemists have benefited from the powerful paradigm of structure–activity relationships, namely that biological activity varies qualitatively and/or quantitatively as a function of the molecular structure (see section 3.1). The study of molecular structure is thus an important field of medicinal chemistry.

2.2.1 Defining molecular structure

Innumerable scientists speak and write about "chemical structure", but what is understood by this term is anyone's guess and may vary considerably from case to case. More often than not, the term is taken as designating the *geometry of chemical entities*, be it simply the manner in which the constituent atoms are connected (atom connectivity, two-dimensional structure), or their arrangement in space (configuration). At these levels of model construction, molecules are considered as rigid geometrical objects. However, the concept of chemical structure extends far beyond this limited description, since to begin with molecules are more or less flexible. Their three-dimensional geometry will thus vary as a function of time (intramolecular motions, conformation).

The time dependence of molecular geometry is under the influence of *electronic properties*. Such properties are of paramount importance for a more realistic view of chemical structure since it can be stated that the geometric skeleton of a molecule is given flesh and shape in its electronic dimensions. This description, while simplistic, has deep meaning: witness the fact that the morphogenesis and definition of molecular structure and shape is a major problem in quantum mechanics (Wolley, 1978; Bader *et al.*, 1980; Trindle, 1980). In this respect, the theory of quantum topology, which appears as particularly promising, considers molecular structure as the generic property of the distribution of charges in a total system. As a consequence, molecular structure exists in spite of interactions with the environment and not as a result of them (Bader *et al.*, 1980).

Geometric and electronic properties are mutually interdependent. For example, and this is common knowledge for all chemists, the conformational