A MOLECULAR APPROACH TO THE MODULATION OF PHARMACOKINETICS: MODIFICATION OF METABOLIC CONVERSION BY MOLECULAR MANIPULATION

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INTRODUCTION

In pharmacology the interest in the question: "what are the effects of drugs?" has shifted more and more to the basic question: "how do drugs act?" Whatever the pharmacodynamic effect of a drug may be, it can only be due to an interaction between the molecules of the drug and the molecules constituting the biological object. Taking into account the usually small doses of drugs required to obtain responses, even in large animals, only an extremely small fraction of the molecules in the biological object can be primarily involved in the drug action. An insight in the various types of molecular interactions in which the drug molecules take part in the body are an essential requisite in the rational approach to the development of new drugs. From a functional point of view the interactions of the drug molecules with all kinds of molecules in the biological object can be differentiated in different types, especially according to the molecular sites of action and the consequences of the interaction:

1. Those molecular sites of interaction which are directly and essentially involved in the induction of the effect. They usually are indicated as the sites of action for the drug. They are characteristic for the type of response, e.g. cholinergic, curariform, convulsant, antihistaminic, etc.

2. The molecular sites of interaction which are neither directly nor essentially involved in the induction of the effect, but which mainly influence the distribution of the drug over the various compartments. They are not related to the type of response involved. These sites may interact with drugs with totally different types of action.

Examples are the sites of binding for drugs on plasma proteins, often indicated as silent receptors or indifferent sites of binding, and the sites of biochemical conversion on drug degrading enzymes. They are not related to the type of response involved. Drugs with totally different types of action may interact with these sites.

PHARMACOKINETICS

The interaction with the sites of action, or specific receptors, is as mentioned determinant for the type of pharmacodynamic response obtained. The interaction with the silent receptors or indifferent sites of binding and the bioinactivation and bioactivation by enzymatic conversion is of special

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importance for the distribution of the drugs over the various compartments in the body (*Figure 1*) and the time-relationship therein. These aspects of drug distribution are generally indicated as *pharmacokinetics*, of which two types can be differentiated.
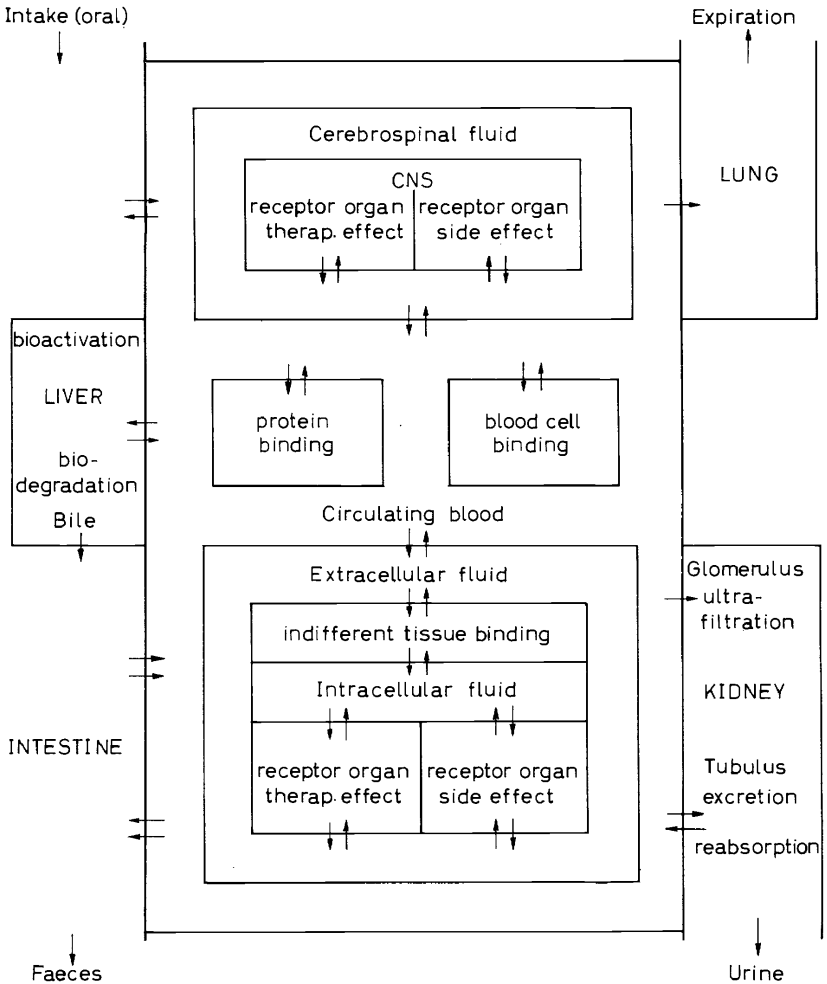
Figure 1. Schematic representation of pharmacokinetics.

In the classical sense pharmacokinetics take man or animals as objects of study. Main emphasis is put on plasma concentrations, levels of accumulation in plasma, half-life time of drugs in plasma and therewith the rate of degradation and excretion in man or animal species in general. Since the distribution of drugs or bioactive compounds in individuals is involved one might talk about individual pharmacokinetics, in contrast to the environmental pharmacokinetics. This concerns the distribution of drugs and bioactive agents in general, not restricted to man or animals as biological

objects, but it covers the whole complex of interactions between living matter, man, animals and plants and the bioactive compounds in our man-made environment.

The study of environmental pharmacokinetics does not deal with individuals, but with populations and complexes of populations in their environment. The main compartments in the multi-compartment system are the air, soil, ground water, surface water and the various populations of plant and
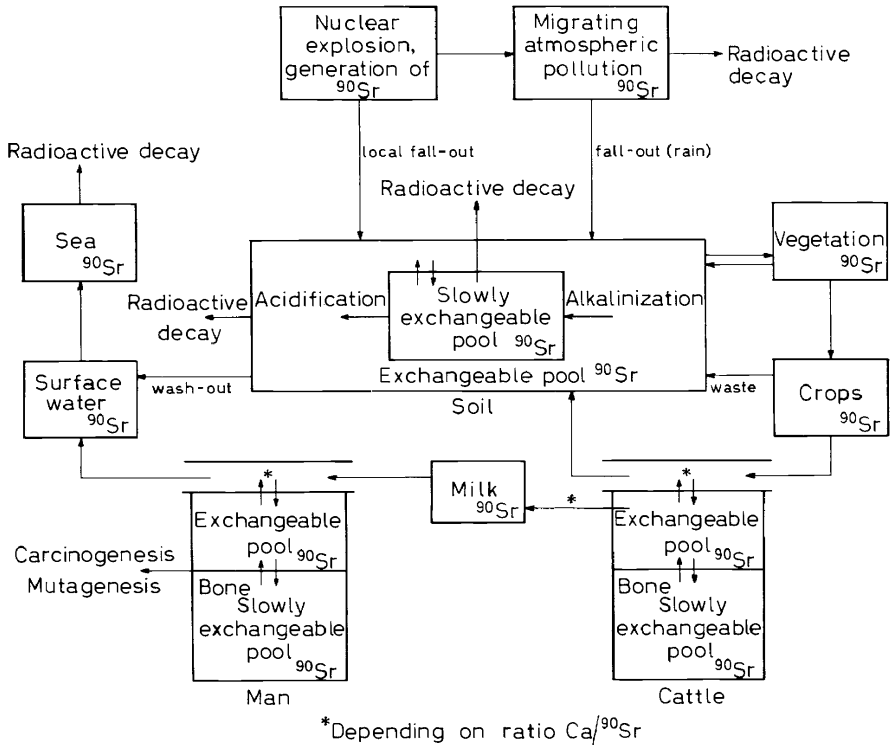

Figure 2. Schematic representation of environmental pharmacokinetics of ^{90}Sr.

animal species, the biomasses therein. The input of the bioactive or potentially bioactive compounds is mainly based on the world-wide highly intensified chemical approach to all kinds of daily problems from fighting insects or pests, adding taste, flavour and colour to our foods and cleaning our houses. The elimination of the bioactive compounds is mainly based on the degradation, which in a number of cases is performed by the biomasses themselves, a process indicated as biological self-clearance.

Among the various problems arising in environmental pharmacokinetics, long-term exposure to low concentrations, long-term accumulation, multiplicity of exposures, and the complexity of the flows and fluxes of the compounds in the multi-compartment system can be mentioned. *Figure 2* gives in a schematic way some of the main features of the environmental pharmacokinetics of radioactive fall-out. As in individual pharmacokinetics

so in environmental pharmacokinetics the significance of the quantity of contaminant, introduced per unit of time, and the half-life time, which as a matter of fact is dependent on the rate of elimination, are determinant for the level of accumulation finally reached. So, for example, the radioactive fall-out over long periods, the quantity that comes down per unit of time in relation to the half-life time of the radionuclides, and the rate of elimination by uptake in crops and wash-out through rain, are determinant for the level of accumulation reached in the soil[22,53,65,67,78,93]. In the case of water pollution again the input of contaminant per unit of time and the half-life time, mainly dependent on biological self-clearance determine whether the level of accumulation finally reached will or will not be detrimental to the biomasses involved in the biological clearance and when this level is reached. If the critical value is surpassed, the half-life will be greatly prolonged because of suppression of biological self-clearance. Then the level of accumulation of the pollution further rises and finally persistent pollution will be the result.

An insight in environmental pharmacokinetics can be of great help in our efforts to prevent persistent environmental pollution and to develop more selectively acting pesticides. This may be of more and farther reaching significance for the health of mankind than the efforts to control individual pharmacokinetics in drug therapy.

Both for individual and environmental pharmacokinetics metabolic conversion of the bioactive compounds plays an important role. One of the procedures in the development of new drugs or bioactive compounds implies the modulation of the pharmacokinetics of existing compounds. In this respect adaptation to specific requirements of those chemical properties of the compounds which are determinant for the metabolic conversion offers a possibility for a rational approach and therewith for the design of the new compounds wanted.

Biofunctional moieties

Various moieties in a drug molecule play, to a certain degree, an independent role with respect to its various chemical properties and therefore with respect to the various part processes involved in drug action, such as absorption, distribution, induction of the effect, degradation and excretion, since the various chemical properties are determinant for the interaction of the drug with the various sites of action, sites of binding, and enzymes involved. This is of great importance for drug design which means molecular manipulation on, as much as possible, a rational base aimed at specific changes in particular steps or aspects of the biological activity.

In the dissection of drug molecules into chemical groups or moieties, and the discussion of the possible contribution of the various groups to the biological activity two approaches are possible. One can dissect the drug molecule into chemical groups on the basis of their contribution to the forces in action between drug and environment and more particularly between drug and receptor, such as the ionic groups, dipoles, inducible dipoles, groups able to contribute to hydrogen bonds, moieties which contribute through van der Waal's forces, and moieties that contribute by means of hydrophobic bonds[35,49,76]. These may be called chemo-functional moieties.

On the other hand, one can dissect the drug molecule on a more biological basis, namely by the distinction between various moieties on the basis of their significance for particular aspects of the biological activity; they may be called biofunctional moieties.

The conception that particular moieties of a biologically active compound are of special significance for particular aspects of its action is already old. Ehrlich (1909)[29] differentiated between a haptophoric and a toxophoric group or a haptophoric and a pharmacophoric group in biologically active compounds. The differentiation of a 'Haftgruppe' and a 'Wirkgruppe' for vitamins[69,70] and of attachment sites and action sites in polypeptide hormones[51,52,90] indicates the same idea. This also holds for the different-iation between a supporting moiety conferring affinity upon the drug and a radical moiety determining the type of action[20]. The differentiation between moieties of particular significance for the fixation at the site of action and moieties of particular significance for the induction of the effect is reminiscent of the differentiation between affinity and intrinsic activity as parameters in drug action[1,2].

In the case of biologically alkylating agents used as cytostatics the terms carrier group and cytotoxic group or 'warhead' have been used[76]. Other terms to indicate carrier moieties primarily regulating the distribution of the drugs are 'selectophore' conferring selectivity to the action of, for instance, pesticides[72] and 'contactophore' for a group enhancing the penetration of the insecticide moiety into the insects[79].

Biofunctional moieties involved in pharmacokinetics

In this respect those moieties in the compounds of special significance for transport processes—free diffusion or carrier mediated transport—and those moieties involved in enzymic conversion —bioactivation or bioinactivation—require special attention. The presence of anionic or cationic groups com-bined with a suitable more hydrophobic group is required for membrane passage by carrier mediated—as a rule active—transport such as that involved in the excretion of certain drugs in bile and in the urine. Lipophilic and hydrophilic character and the degree of ionization of the bioactive compounds determine the lipid/water partition coefficients and therewith the capacity to penetrate the membranes between the various compartments.

Transport of drugs is strongly dependent therefore on certain moieties of the drug molecule such as anionic groups, cationic groups, undissociated carboxyl groups, amino groups or alkyl, aryl and aralkyl groups. The latter especially increase the lipophility and enhance the formation of hydrophobic bonds to binding sites. Those moieties which control transport serve as carrier moieties or *conducting moieties*[6]. Such moieties may be an intrinsic part of the drug in its active form, or only a part of a transport form of the drug, being split off in the bioactivation of the drug. The terms *fixed conducting moieties* and *disposable conducting moieties* are applicable then. Moieties, which confer to the drug molecule strong hydrophility, e.g. ionized groups, or strong lipophilic properties, and therefore restrict the passage through lipid membranes or via hydrophilic compartments, act as *restricting conducting moieties*. Moieties, which adapt the chemical properties to active transport

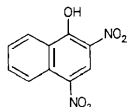*Table 1.* Fixed restricting conducting moieties
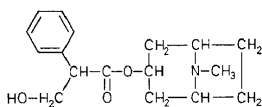
Atropine
anticholinergic
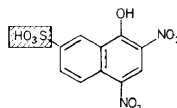penetrating blood-brain barrier
acting on CNS
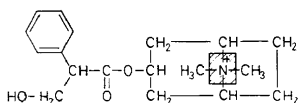
Methylatropine
anticholinergic
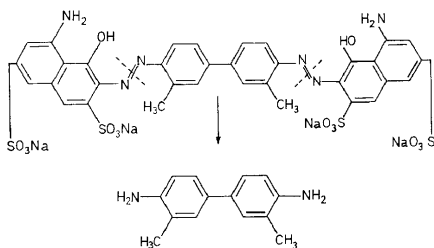because of hydrophilic onium group
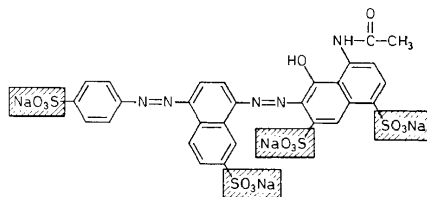devoid of action on CNS

Martius yellow
highly toxic

Naphthol-S-yellow
much less toxic because of strong
hydrophilic sulfonate group, restricting
the compound to extracellular
compartment

Trypan blue
toxic because of formation
of non-sulfonated benzidine derivative

Brilliant black
non-toxic because of sulfonation of
three moieties

processes and therefore result in selective concentration in certain compart-
ments act as *selecting conducting moieties.* There also is the possibility of moieties
conferring to the drug a balanced hydro-lipophility, making it suitable to
penetrate easily lipophilic as well as hydrophilic phases, thus facilitating the
distribution over the extra- as well as the intracellular compartments. They
act as *facilitating conducting moieties*[3-6]. Each of these moieties can be differen-
tiated in fixed and disposable ones (*Tables 1–3*). Changes in the solubility of
the compound by temporary binding to certain groups, *disposable solubilizing*
or *disposable desolubilizing groups,* are practised to modulate the rate of uptake.
Mostly application forms gradually releasing the compound in its active form
are aimed at (*Tables 1–3*)[3-6].

Table 2. Fixed selecting, fixed facilitating, disposable restricting, and disposable selecting conducting moieties
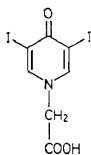
Fixed selecting conducting moieties
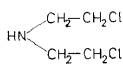
Nitrogen mustard
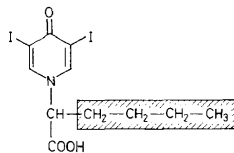indifferent alkylating agent
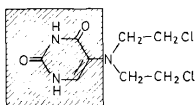
Uracil mustard
the uracil moiety confers high
affinity to the cell nuclei

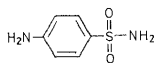*Fixed facilitating conducting moieties*

Diodrone
renal radiopaque agent
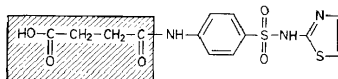mainly restricted to extracellular
fluid and urine

Alkylated diodrone derivative
(radiopaque agent)
the facilitating (lipophilic) moiety
enhances excretion via liver into bile

Disposable restricting conducting moieties
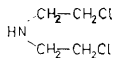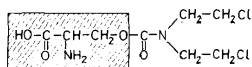
Sulphanilamide

Succinylsulphathiazole
the succinic moiety restricts
distribution to gut lumen; disjunction
is required for action

*Disposable selecting conducting moieties*

Nitrogen mustard

Mercasin
the amino acid moiety is meant to be
picked by the amino acid transport
system and therefore to enhance uptake
of the alkylating moiety into the cells;
disjunction is required for action

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Table 3. Disposable facilitating conducting and solubilizing and desolubilizing moieties

Disposable facilitating conducting moieties
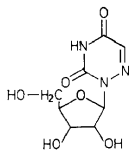
6-Azauridine
antimetabolite
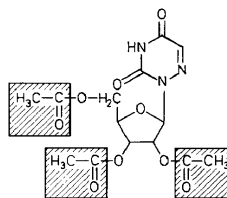
Triacetyl-6-azauridine
antimetabolite
by esterification the penetration into tissues
and cells is facilitated; disjunction is
required for action

Disposable solubilizing moieties
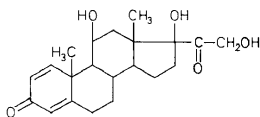
Prednisolone
glucocorticoid
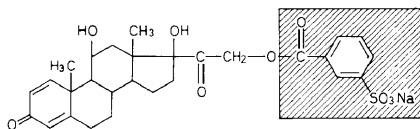
Prednisolone derivative
the disposable moiety confers water-
solubility to the steroid; disjunction is
required for action

Disposable desolubilizing moieties
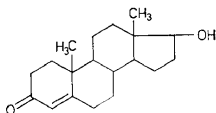
Testosterone
androgen
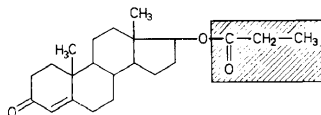
Testosterone propionate
androgen; depot preparation
the disposable moiety deprives the
steroid of its water-solubility;
disjunction is required for action

It will be clear that introduction of or changes in these various moieties in a drug molecule may essentially change its pharmacokinetics. If fixed moieties are involved the degree of freedom in molecular manipulation is restricted since also the pharmacodynamic action as such may change then. The distribution pattern of bioactive compounds also depends on those chemical properties of the drug which are determinant for the metabolic conversions, i.e. the chemical groups in the compounds primarily involved in e.g. hydrolytic or oxidative attack. These groups can be indicated as *vulnerable moieties*[3-6]. They are not necessarily related to or dependent on the

pharmacodynamic type of action of the drug. Determinant is the occurrence of specific moieties in the drug molecule. If the drug contains an ester group, it may be hydrolyzed by plasma esterases or other esterases in the body independent of the type of drug to which the compound belongs.

MODULATION OF PHARMACOKINETICS BY MODIFICATION OF THE VULNERABLE MOIETIES INVOLVED IN BIOCHEMICAL CONVERSION

As mentioned, in the biochemical conversion of drugs leading to bioactivation or bioinactivation, certain moieties called vulnerable moieties, suitable for the enzymic or, in general, chemical attack are primarily involved. Amongst these can be mentioned hydrolyzable ester, acid amide or glycoside bonds, oxidizable alkyl groups, hydroxy-alkyl groups and reducible azo groups. Changes in or introduction of vulnerable moieties can influence the distribution of the active compound in different ways:

1. There is the possibility to modulate the rate of bioinactivation or bioactivation and therewith of modulation of the half-life time and pharmacokinetics in general of the active compound. There is the possibility of the introduction of vulnerable moieties, of destabilization of vulnerable moieties, stabilization of vulnerable moieties and elimination of vulnerable moieties. The first two cases will result in a shortening of the half-life time of the drug, the last two procedures in a prolongation of the half-life time. Temporary protection of vulnerable moieties in the drug molecule by disposable protecting moieties is also aimed at a prolonged action although the half-life time of the drug in its active form strictly taken is not changed in this case.

2. The second possibility is the modulation in the distribution over the various compartments. This may imply a selective bioactivation in the target tissue or a selective bioinactivation outside the target tissue resulting in a high ratio for the concentrations of the active drug inside and outside the target tissue which may be of advantage to avoid toxic side-effects. Also in this case vulnerable groups are involved.

MODULATION OF THE RATE OF BIOCHEMICAL CONVERSION

Shortening of the action of compounds by introduction of vulnerable groups

One of the factors determining the time–concentration relationship for the drug in its active form is the rate of bioinactivation. A variety of biochemical processes may be involved. The most common ones are hydrolysis of ester and amide bonds and oxidation of alkyl groups possibly bearing certain substituents such as hydroxyl or amino groups. If a group essential for the action, for instance, an amino group, is eliminated by oxidative de-amination or if polar groups are introduced such as carboxyl groups, which are incompatible with the activity of the drug, a bioinactivation will be the consequence. Introduction into drugs or bioactive compounds in general of vulnerable groups, e.g. ester groups or destabilization of particular

groups in the drug so that metabolic degradation and inactivation are enhanced, will result in a shortening of the action by shortening of the half-life time. As a rule the aim of such preparations is a short or ultra-short and therefore controllable action and the avoidance of persistent environmental pollution.

Decamethonium

Succinyl choline [(a)]
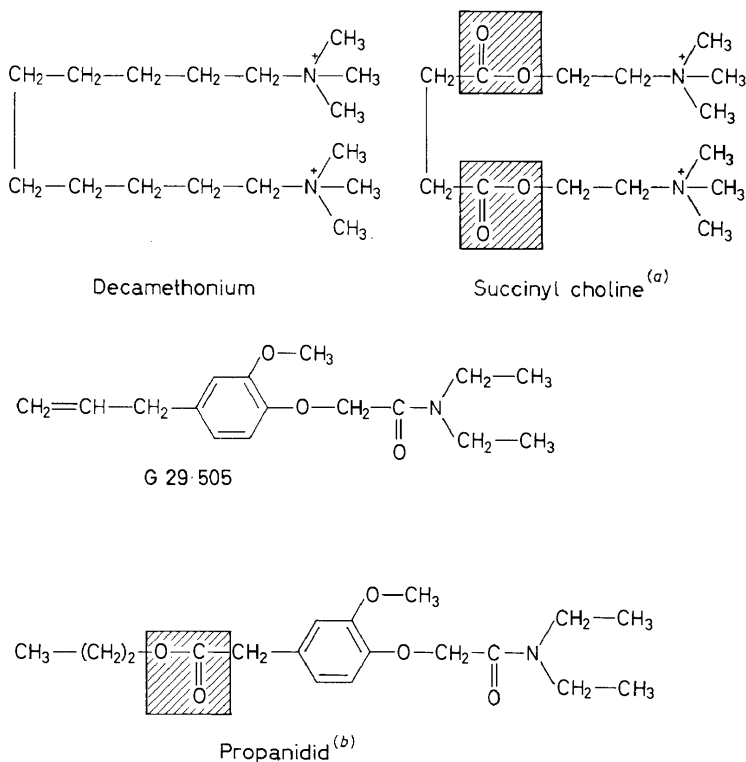
G 29·505

Propanidid [(b)]

Figure 3. Development of short acting forms by introduction of vulnerable groups [(a), The vulnerable moieties imply a rapid hydrolysis by plasma esterase and therefore a short action. (b), The vulnerable moiety implies a rapid hydrolysis and therefore a short action].

Short acting forms are developed in this way for a variety of drugs such as muscle relaxants, intravenous anaesthetics, local anaesthetics, etc. One of the best known examples of the introduction of vulnerable groups resulting in a shortening of the action is the synthesis of the muscle relaxant succinylcholine which as compared to the related compound decamethonium can be considered as a short acting compound (Figure 3). Hydrolysis of the ester groups by plasma esterases and esterases in the liver cells results in a rapid inactivation of the drug. A comparable procedure is followed in the curariform drug prodeconium where the vulnerable group is introduced into one of the substituents on the onium group[38,82]. Again hydrolysis results in a rapid loss of activity. The ultra-short intravenous general anaesthetic propanidid is a derivative of the intravenous anaesthetic G 29505[97]. Again

the introduction of a vulnerable group, the ester group which is readily hydrolyzed, results in a compound with an ultra-short action[55,77].

A special aspect of rapid inactivation is the avoidance of pesticide residues in food products and the avoidance of accumulation of pesticides in the body or environment. The avoidance of pesticide residues implies that the compound must be eliminated or degraded to non-toxic products by the time that the agricultural products are used for consumption. Also here the incorporation in the insecticide of a vulnerable moiety can be considered. An example is GS 13005 (supracid), an organic phosphate with a labile heterocyclic ring, a substituted thiadiazole ring, easily cleaved under formation of carbon dioxide and a metabolite that is inactive (*Figure 4*)[30]. This degradation is performed very readily in mammals, but also in the plant tissues. Of the insecticide taken up by the plant 95 per cent is inactivated within a period of 14 days.
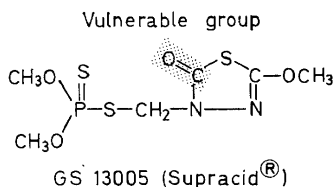
Figure 4. Introduction of a vulnerable group to obtain an insecticide quickly bioinactivated in plant and mammalian tissue to reduce the risk of pesticide residues [after H. O. Esser and P. W. Müller, *Experientia* **22**, 36 (1966)].

A particular problem is the tendency of the highly lipophilic chlorinated hydrocarbons such as DDT, which are very resistant against metabolic degradation, to accumulate in body fat[62,95]. This accumulation may lead to disastrous results for the animals at the ends of the various food chains, such as birds living on fish (*Figure 5*) and birds preying on other birds[19]. Introduction in these chlorinated hydrocarbons of vulnerable groups suitable for degradation and thus resulting in an increase in the hydrophility decreases the tendency for accumulation. In this respect the introduction in DDT of alkoxy and alkyl groups in the rings in the *meta*- or *para*-position has been performed (*Figure 6*). The compounds methoxychlor and perthane are examples. They still have a reasonable insecticidal activity while the oxidation of the alkyl groups has as a consequence the introduction of more hydrophilic phenolic OH or carboxyl groups suitable for conjugation and thus a reduction of the tendency to accumulate. With these insecticides the degree of accumulation is indeed found to be decreased. The objection that the dicarbonic acid obtained is inactive as an insecticide and that therefore the insects will bioinactivate the insecticide only holds if the insects do this quick enough. This seems not to be the case since both derivatives mentioned still have a reasonable insecticidal activity[25,79].

As a rule straight chain alkyl groups are more liable to biological degradation by oxidation than branched alkyl groups. Introduction of methyl groups such that vulnerable groups, primary OH-groups, NH₂-groups etc. are changed to secondary or tertiary groups results in a protection against
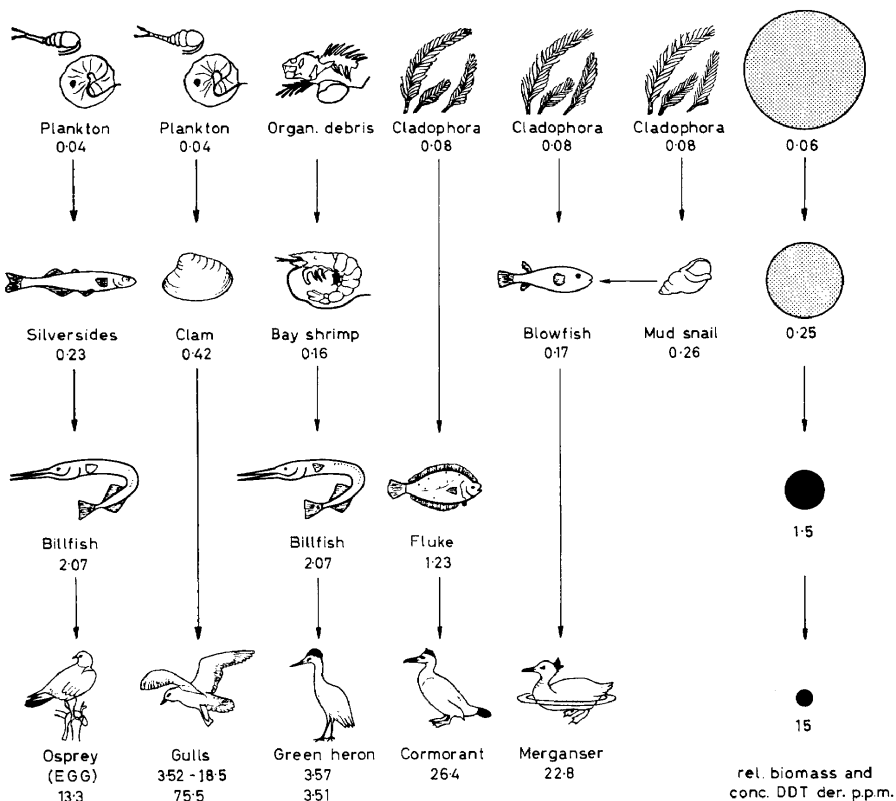
Figure 5. Schematic representation of the accumulation of DDT and its degradation product DDE (ppm) along various food chains. The surface of the circle represents the size of the biomasses involved; the degree of shadowing represents the concentration of DDT and its products [Modified after G. M. Woodwell, *Scient. Amer.* **216,** 24 (1967)].

DDT[a]

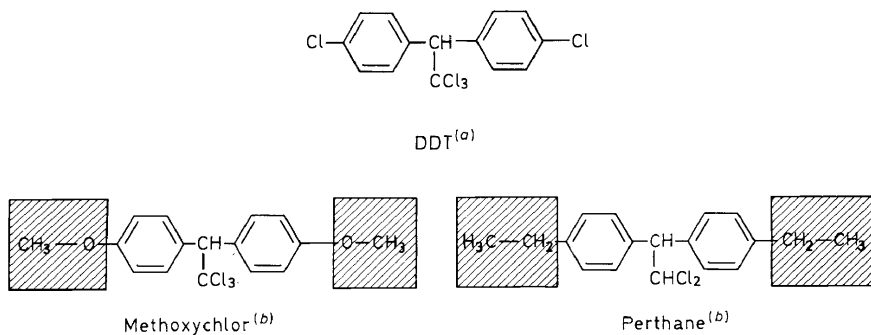Methoxychlor[b]   Perthane[b]

Figure 6. Introduction of alkoxy and alkyl groups in highly lipophilic chlorinated hydrocarbons [(a), highly resistant against oxidative degradation—strong accumulation; (b), converted by oxidation followed eventually by conjugation, into more water-soluble products, which implies a decrease in the tendency to accumulation].

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biodegradation processes attacking such groups. This type of chemical modification is known as packing of the vulnerable groups. The consequence of packing of the OH-group in alcohols for biological oxidation is demonstrated in *Table 4*. The packed alcohols are resistant to oxidative degradation and therefore appear to a higher degree as glucuronides in the urine. Branching of straight alkyl chains can be regarded too as packing of the

Table 4. Glucuronide conjugation of primary, secondary and tertiary alcohols in rabbits (After Williams[103]).

| Primary alcohol	Secondary alcohol	Tertiary alcohol
C—C$^\alpha$—OH	$\overset{\displaystyle C}{\underset{\displaystyle \vert}{C-C^\alpha-OH}}$	$\overset{\displaystyle C}{\underset{\displaystyle \vert}{C-\overset{\vert}{\underset{\vert}{C^\alpha}}-OH}}$ with C below
0·5	10	24
C—C—C$^\alpha$—OH	$\overset{\displaystyle C}{\underset{\displaystyle \vert}{C-C-C^\alpha-OH}}$	$\overset{\displaystyle C}{C-C-\overset{\vert}{\underset{\vert}{C^\alpha}}-OH}$ with C below
0·9	14	58
C—C—C—C$^\alpha$—OH	$\overset{\displaystyle C}{\underset{\displaystyle \vert}{C-C-C-C^\alpha-OH}}$	$\overset{\displaystyle C}{C-C-C-\overset{\vert}{\underset{\vert}{C^\alpha}}-OH}$ with C below
1·8	45	57
C—C—C—C—C$^\alpha$—OH	$\overset{\displaystyle C}{\underset{\displaystyle \vert}{C-C-C-C-C^\alpha-OH}}$	
6·7	54	

The numbers indicate the glucuronidation in percentage. The α-methyl and α-dimethyl substitution result in a stabilization of the alcohols with respect to oxidative degradation with as a consequence an increase in the fraction excreted as glucuronide conjugate.

vulnerable terminal of the chain. Destabilization of organic compounds therefore may be obtained by substituting branched alkyl chains by straight side chains which as a rule are more easily oxidized by, for instance, β-oxidation. An interesting example is found in the field of detergents. These surface-active compounds, originally mostly sulphonated branched alkyl or aralkyl derivatives, caused much trouble in the pollution of water, since they are resistant against biological attack by the microorganisms normally involved in the self-clearance of polluted water. These resistant detergents are called 'hard' detergents (*Figure 7*). Substitution of the branched alkyl chains by straight chains makes the detergents more vulnerable to biological attack with the result that for this type of detergents indicated as 'soft' detergents, biological self-clearance of polluted water can take place (*Figure 7*)[98]. The examples given in this section show that the same principles are applicable in the efforts to modulate individual as well as environmental pharmacokinetics.

A protraction of the action of compounds by stabilization of vulnerable groups

In the foregoing section shortening of drug action by introduction of vulnerable groups, resulting in an accelerated metabolic inactivation was described. Also the reversed situation occurs namely the aim to elongate the duration of action.

Drugs containing an ester group may be prone to degradation by enzymatic hydrolysis. The rapid inactivation of acetylcholine and procaine by esterases of plasma and cells, especially liver cells, is an example. Introduction
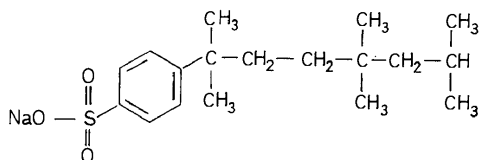
'hard' detergent
resistant against biological degradation
persistent water pollution
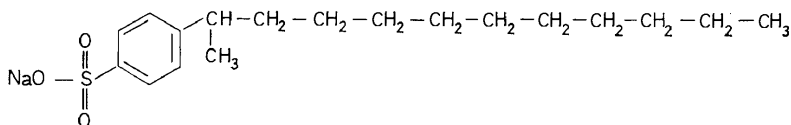
'soft' detergent
biologically degradated
non-persistent water pollution

Figure 7. Biological degradation of detergents and water pollution[98].

of suitable substituents, especially small alkyl groups next to the vulnerable group, in this case the ester group, often results in an increased resistance against metabolic attack. The introduction of a methyl group next to the ester group of acetylcholine gives acetyl-β-methylcholine, which is much more stable than acetylcholine against acetylcholine esterase. A clear-cut difference is found for the rate of hydrolysis of both isomers of acetyl-β-methylcholine. One of the isomers even acts as a competitive inhibitor of acetylcholinesterase (*Figure 8*)[7]. A variety of examples of the principle of stabilization of ester groups by alkyl substitution close to the vulnerable group, the so-called 'packing' of the vulnerable group, are reported by Levine and others[32,36,60,66,91] (see *Figure 8*). As a cause for the stabilization of vulnerable groups by this 'packing' procedure a sterical hindrance by the alkyl groups on the active site of the enzymes involved is feasible. In many cases, however, the protection against enzyme action also implies a protection against alkaline hydrolysis which means that also a stabilization of the ester bond as such may be involved. If esters of benzoic acid are involved, the ester group is linked directly to the system of conjugated double bonds in the

ring such that shifts in charge distribution resulting in stabilization or destabilization of the ester bond may be induced by introduction of suitable substituents. The type of substituent and the site of substitution in the ring will be determinant[37,40,68]. Small alkyl groups introduced in the *ortho*-position usually have a stabilizing effect[17,91].

Another type of vulnerable moieties are unbranched alkyl groups possibly bearing terminal amino or hydroxyl groups which may be involved in oxidative degradation. An example is phenylethylamine, a compound with a weak adrenergic activity and practically devoid of central nervous system stimulant activity. The introduction of methyl groups next to the amino
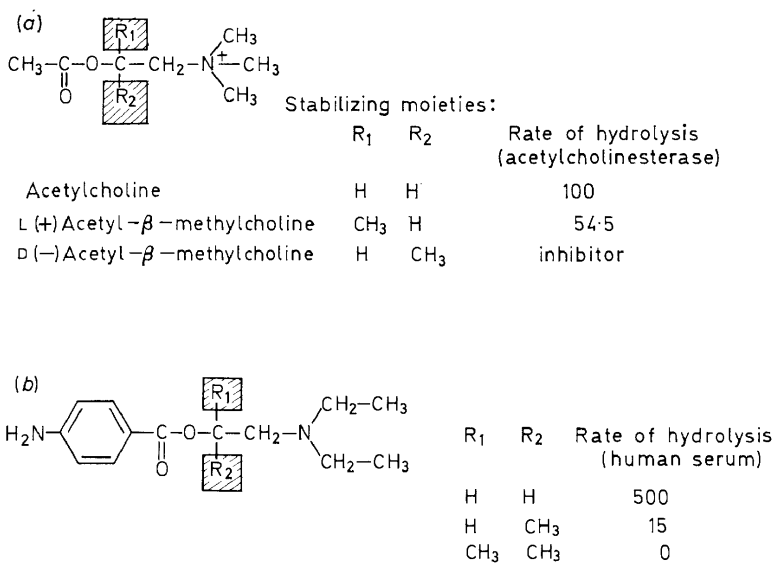
(a)

		R₁	R₂	Rate of hydrolysis (acetylcholinesterase)
			Stabilizing moieties:	
Acetylcholine		H	H	100
L (+) Acetyl −β −methylcholine		CH₃	H	54·5
D (−) Acetyl −β −methylcholine		H	CH₃	inhibitor

(b)

R₁	R₂	Rate of hydrolysis (human serum)
H	H	500
H	CH₃	15
CH₃	CH₃	0

Figure 8. Examples of the principle of stabilization of ester groups by alkyl substitution close to the vulnerable group [(*a*) After N. J. Harper, in *Progress in drug research*, Ed. E. Jucker, Vol. 4, pp. 221, 280; Academic Press, New York, 1962. (*b*) After R. M. Levine *et al.*[66]].

group, a packing procedure, results in a stabilization of the vulnerable moiety with the consequence that effective central nervous system stimulants such as amphetamine and mephentermine are obtained, compounds which also have prolonged vascular effects. Application of phenylethylamine to animals in which the amine oxidase is inhibited by suitable mono-amine oxidase inhibitors shows that under these circumstances phenylethylamine is about as active as amphetamine as far as central nervous system stimulant activity is concerned (*Figure 9*) and that the vascular effects are prolonged. The rapid oxidative de-amination of phenylethylamine and the stability in this respect of amphetamine and mephentermine in which the vulnerable amino group is protected appear to be the basis for the differences in the action of these compounds[88,89]. A similar type of substitution leading to protection of a vulnerable group is found in the field of herbicides. The herbicide 4-chloro-2-methylphenoxyacetic acid is inactive with respect to certain dicotyledonous weeds. This appears to be due to a rapid oxidation of
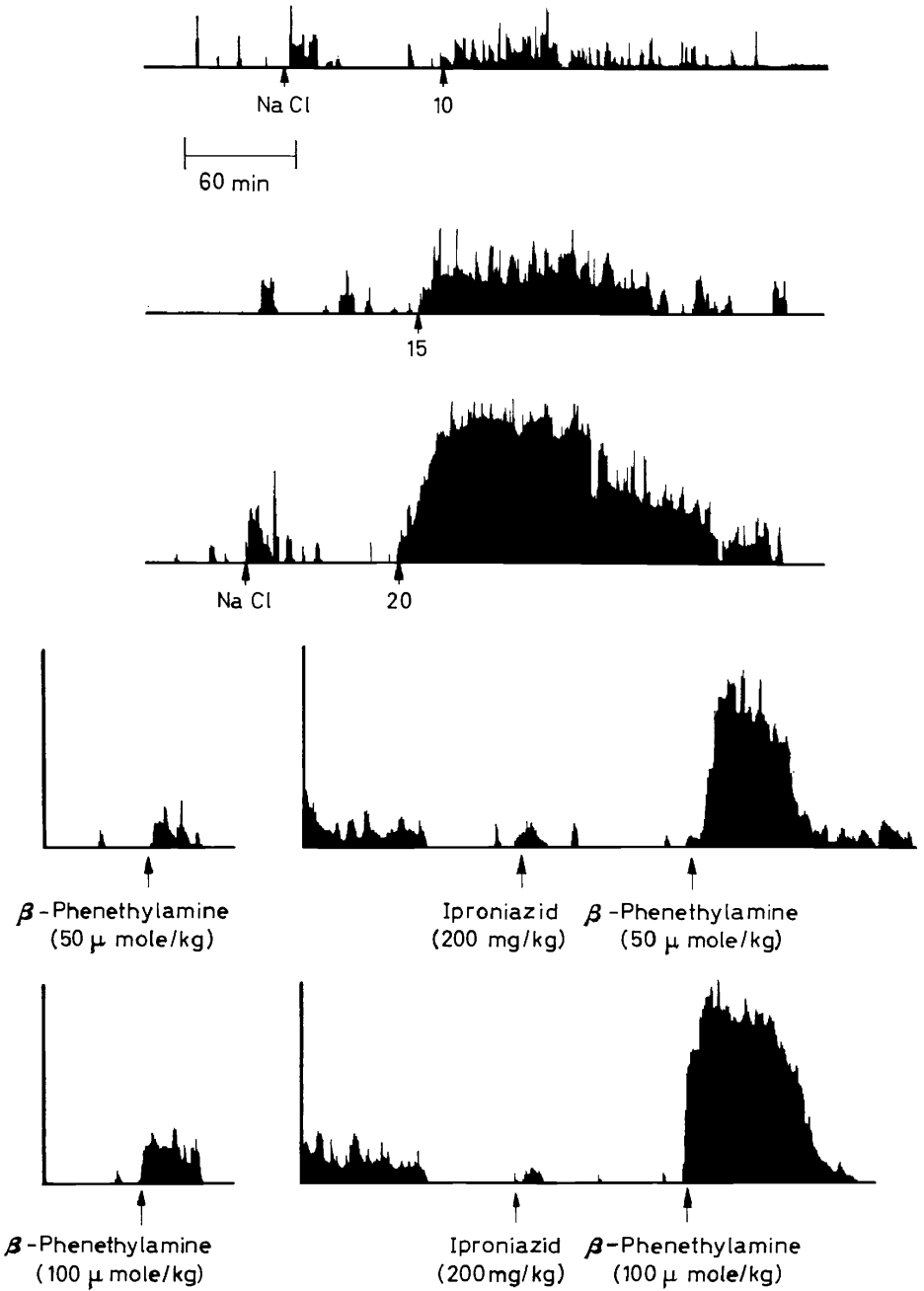
Figure 9. Action of various doses of amphetamine and phenylethylamine on the motility of mice. Note that phenylethylamine has only a slight activity. After iproniazid it becomes much more active[88].

the acetic group. Introduction of an α-methyl group in the acetic acid moiety of the compound blocks this degradation. The compound then is active in controlling the weed mentioned[64]. For the eradication of woody plants with weed killers α-methyl substituted phenylacetic acids are more suitable than the phenylacetic acid derivatives. This seems to be due to the greater stability of the α-methyl substituted compounds against oxidative degradation leading to inactive phenols[24] (*Figure 10*).

The principle of α-alkyl substitution in the case of terminal amino groups, carboxyl groups, etc. is comparable to the principle of terminal branching in

Weed–killer
quickly bioinactivated in plant tissue
(*Galium aparine*)
therefore ineffective

stabilizing group

Weed–killer
stabilized against bioinactivation
effective

Weed–killer
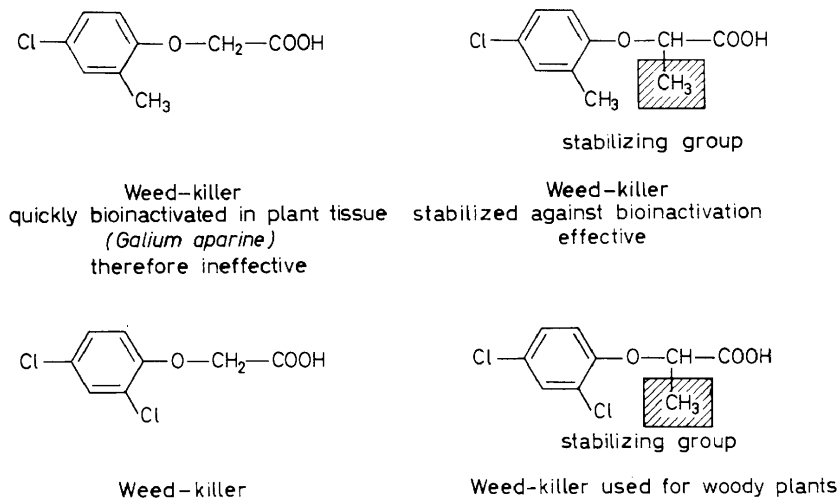
stabilizing group

Weed-killer used for woody plants

Figure 10. Stabilization of weed-killers against oxidative degradation[24,64].
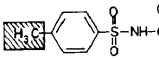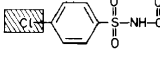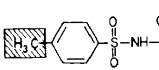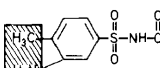
vulnerable alkyl groups. An increasing stability against oxidative dealkylation is observed for alkoxy and alkylamine groups of drugs in the series O-methyl, O-ethyl, O-isopropyl, O-tert. butyl and N-methyl, N-ethyl, N-isopropyl and N-tert. butyl[18,23]. The same holds true for alkyl substitution in meprobamate. Many other examples of an increased resistance of alkyl groups against oxidative degradation obtained by suitable branching in the alkyl groups are reported in the literature[1,28,34,57,103].

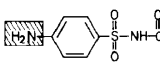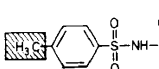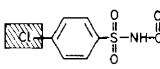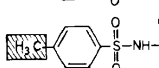## A protraction of the action of compounds by elimination of vulnerable moieties

Substitution of an amide link for the ester link often implies a stabilization of the drug with respect to hydrolysis. This change in the structure leads, however, to the introduction of a new opportunity for hydrogen bond formation, namely by the hydrogen on the amide nitrogen. Often this appears not to be compatible with respect to the action of the drug in the strict sense. The amide analogue of, for instance, acetylcholine is practically devoid of cholinergic action. In the case of procaine the switch to procainamide has been successful, at least as far as antiarrhythmic action is concerned. On the whole in local anaesthetics substitution of an amide link for

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an ester link is well tolerated[16,45,99]. An analogous procedure is followed in the development of meprobamate. 2,2-Diethyl-1,3-propanediol and related diols have an anti-convulsant and muscle-relaxing action which are of only a short duration due to their quick metabolic degradation. Conversion to esters resulted in a prolongation of the action. The carbamates had a still more prolonged action. Of the compounds thus obtained meprobamate was chosen as the best one[9,10,92]. With the carbamate formation besides the muscle-relaxant action also a sedative action, common to many carbamates and carbamides, was introduced.

Table 5. Oral antidiabetics[71]

Short acting	Half-life time (h)	Long acting	Half-life time (h)
Tolbutamide	5·7	Chlorpropamide	33
Cycloheptolamide	4·7	Carbutamide	36
U-17,835	7·2	U-12,504	16·18
U-14,378	6·7	Metahexamide	22
Vulnerable moiety		Stable moiety	

PAC 19/1-2/11 (TB 21)

The conversion of the relatively short-acting oral antidiabetic tolbutamide to the longer acting chlorpropamide is an example of the avoidance of oxidative degradation by elimination of the vulnerable methyl-moiety. *Table 5* represents further examples[71]. In the case of metahexamide the methyl group in the ring is protected against oxidation by the presence of the amino group.

Approaches similar to those outlined before are followed in the efforts to protect biologically active polypeptides against bioinactivation. Smaller polypeptides such as angiotensin II and oxytocin are rapidly inactivated by specific polypeptidases in plasma such as angiotensinase and oxytocinase. The inactivating enzymes are polypeptidases attacking particular peptide links. As long as the amino acids constituting the vulnerable peptide link in the polypeptide allow changes in structure such as substitution by other amino acids, without a loss of the activity, the possibility for a stabilization is given[15,63,90]. An example is the substitution of the terminal L-amino acid in angiotensin by the unnatural D-amino acid resulting in a protection of the still active polypeptide obtained against angiotensinase[15,63].

Besides the specific degrading enzymes in plasma also enzymes in liver and kidney play a role in the inactivation of the polypeptide hormones. Polypeptidases breaking peptide links and hydrogenases breaking the —S—S—bonds which occur in the cyclic polypeptides such as oxytocin and ADH can be mentioned. Modification of the terminal hemicystine residue by methylation,
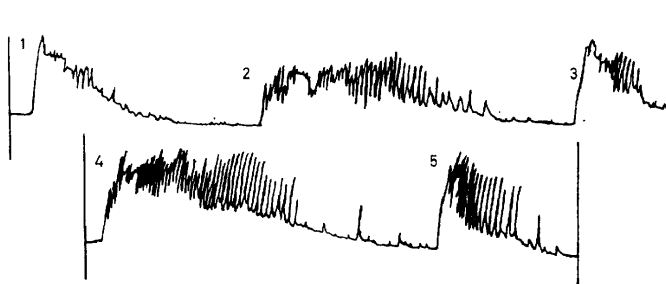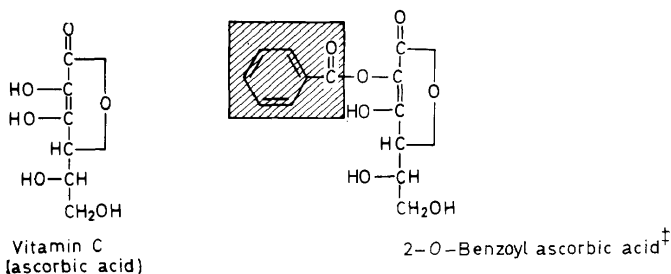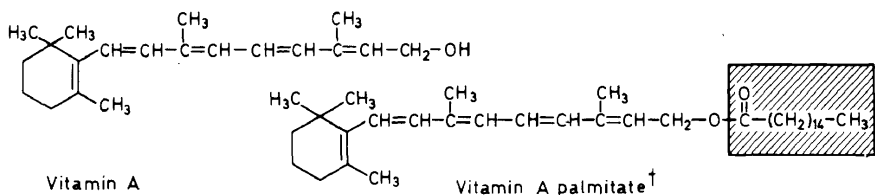
Figure 11. Record of the intra-uterine pressure measured on the rat uterus *in situ* after i.v. administration of oxytocin, 0–2, 0–1 and 0–1 I.U. (1, 3 and 5 respectively) and of Gly. Gly. Cys[1]-oxytocin, 10 and 20 μg (2 and 4 respectively) (After Z. Berankova-Ksandrova et al.[8]. Note the prolonged action of Gly. Gly. Cys[1]-oxytocin).

by acylation of the free amino group, by coupling of additional amino acids to the free amino group of the terminal hemicystine, or by replacing the terminal L-hemicystine group by its D-isomer, may lead to a stabilization of the compounds. On this basis more stable derivatives of oxytocin and vasopressin have been prepared[84,85]. The amino acids or other groups attached to the hormone often have to be split off in order to obtain the hormone free in its active form. The stabilized form which is resistant against inactivating degradation can be considered as a protected transport form and usually will be slightly or not active *in vitro*, but active *in vivo*. *Figure 11* and *Table 6* give examples for the stabilization of oxytocin on this basis[8,63]. The procedure just outlined was also applied to antidiuretic hormone, again resulting in derivatives with a protracted action[106].

The procedures mentioned imply a protection of vulnerable moieties by masking groups. Drugs which are unstable because of the presence of certain vulnerable groups, for instance easily oxidizable groups, can by a disposable protecting moiety be converted into transport forms, from which the active compound is gradually released. Examples of this procedure are the esterification of the terminal OH-group in vitamin A to an acetyl or palmityl ester and of the OH-group of tocopherol to the acetyl ester (*Figure 12*). Also the various conjugation products formed by the introduction of disposable moieties in other vitamins such as thiamine[59], riboflavin[94] and L-ascorbic acid[56] partially serve the purpose of increasing stability. The various

Table 6. Structure and activity of oxytocin analogues[8]

R-CyS-Tyr-iLeu-Glu(NH$_2$)-Asp(NH$_2$)-CyS-Pro-Leu-Gly-NH$_2$

Peptide R	Activity on rat uterus (IU/μmol)		Activity ratio in vitro/in vivo
	in vitro	*in vivo*	
H-	450	450	1
Leu-Leu-	9	80–100	10
Gly-Gly-	0·6	5–11	10–20
Phe-	2	46–57	25
Leu-Gly-Gly-	0·2	18–24	100

Figure 12. Protection of vulnerable moieties by masking groups, e.g. esterification of the terminal OH-group in vitamin A to a palmityl ester and the addition of a benzoyl group in ascorbic acid.

molecular manipulations described in the foregoing sections were aimed at a modulation of pharmacokinetics as far as time–concentration relationships, half-life times, and therefore duration of action and accumulation tendency of the compounds are concerned. The procedures outlined in the following sections were aimed at a modulation of pharmacokinetics as far as the distribution over the various compartments is concerned and well on basis of particular distribution patterns of enzymes involved in biochemical conversion. Again molecular manipulation on the vulnerable moieties in the compounds will be involved.

MODULATION OF THE SITES OF BIOCHEMICAL CONVERSION

Vulnerable moieties and selective bioinactivation

Local anaesthetics should be restricted in their action to the site of application. Once absorbed into general circulation the compounds should be degraded and thus inactivated as soon as possible. For this purpose introduction of vulnerable moieties can be considered and has proved to be effective. The short-acting local-anaesthetic baycaine® obtained by introduction of a vulnerable moiety in the long-acting compound lidocaine[47] is an example (Figure 13).

Besides the introduction of vulnerable groups in drugs in order to obtain short-acting compounds, also substitution of stable moieties by less stable moieties or destabilization of suitable moieties already present can be considered. The introduction of suitable substituents in benzoic acid esters

or anilides may result in destabilization of the ester or amide bond. An example of this procedure is the development of hostacaine®, which can be considered as a more quickly hydrolysed and therefore shorter acting analogue of lidocaine (*Figure 13*)[96]. In this area application of Hammett constants may be helpful in selecting the right substituents[6,87].

Lidocaine
(stable local anaesthetic)

Baycaine[†]

Hostacaine[‡]

Cytostatic

Cytostatic[§]
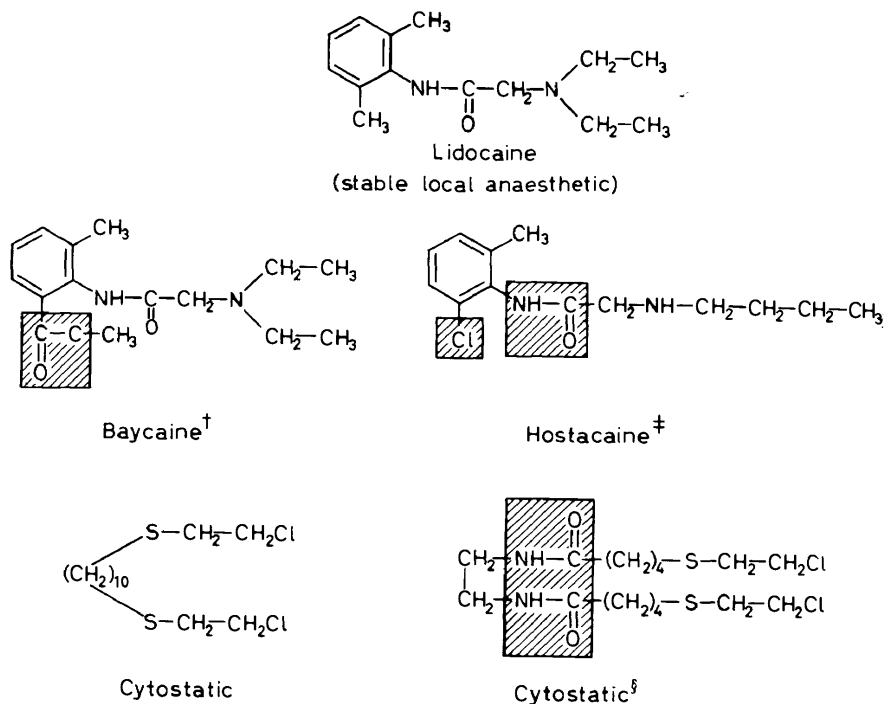
† Local anaesthetic, devoid of unwanted systemic action because of vulnerable moiety enhancing bioinactivation after the general circulation is reached.
‡ Local anaesthetic, devoid of unwanted systemic action because of destabilization by Cl of the acylamide group enhancing bioinactivation after the general circulation is reached.
§ Designed for regional perfusion; because of vulnerable moieties rapidly hydrolysed by liver amidase and therefore devoid of systemic toxicity.

Figure 13. Vulnerable moieties and selective bioinactivation, e.g. the formation of short acting local anaesthetic Baycaine® and the development of Hostacaine®.

The introduction into cancer therapy of the technique of intra-arterial infusion and regional perfusion makes necessary the availability of cytostatics with a short action. The compound which leaves the region covered by the artery in which infusion takes place or leaks into the general circulation in the case of regional perfusion must be inactivated rapidly there such that no big harm is done to the healthy tissues by the cytostatic agent used. The fact that bifunctional alkylating agents as a rule are more active as cytostatics than monofunctional alkylating agents served as a basis for the development of short acting cytostatics. Two alkylating groups, for instance, sulphur mustard groups are conjugated by means of a vulnerable link such as a

hydrolysable amide or ester group. The disjunction caused by hydrolysis has as a result the formation of two monofunctional, only slightly active compounds (*Figure 13*). This implies a practically total loss of cytostatic activity. For detailed information on the thoughtful approach to this problem the reader is referred to the papers of Witten[104,105].

Analogous procedures are followed in the field of environmental pharmacokinetics. Here the need for selectivity in the toxic action of pesticides has been a main goal.
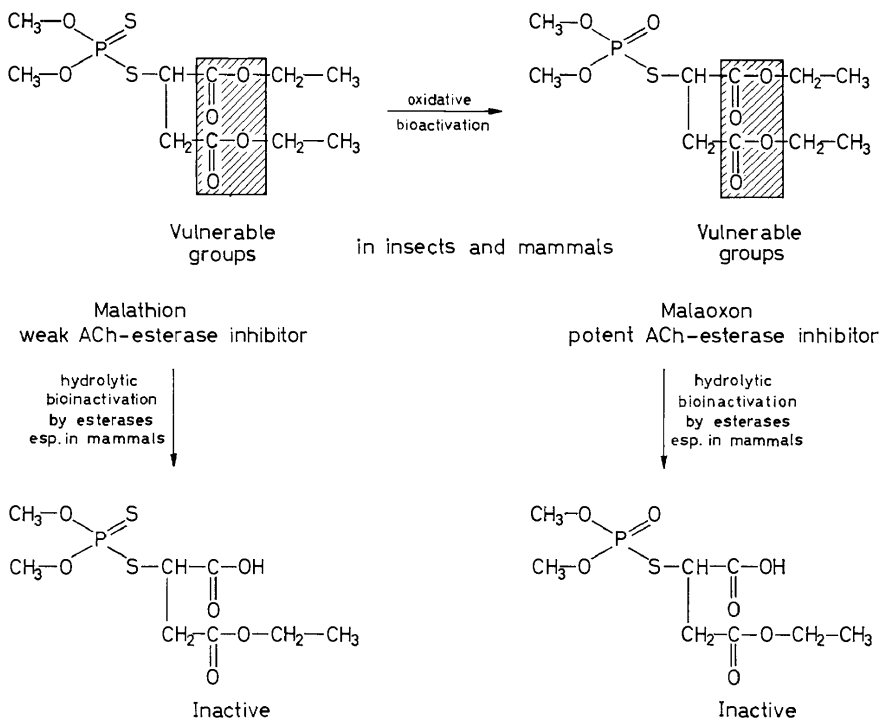
Figure 14. Malathion metabolism (Malathion is highly toxic to insects but less toxic to mammals).

The incorporation in insecticidal organic phosphates of an ester group which is easily hydrolysed by carboxy esterases, resulting in compounds with a free ionic carboxyl group close to the phosphate group, implies an inactivation. This opens possibilities for a selective bioinactivation. The insecticides concerned will be quickly inactivated by carboxy esterases present plentiful in plasma and liver especially of mammals and to a much lesser degree in the insect tissues. The insecticide malathion is an example. It shows a remarkable safety margin.

Radioisotope studies with insects and mammals showed that two competing processes, the oxidative bioactivation to malaoxon and the hydrolytic bioinactivation by splitting of the carboxy ester are involved (*Figure 14*)[31,61,72,73]. The species with a slow conversion into the active oxoderivatives but a rapid

conversion by hydrolysis will be less susceptible than species where the relation between bioactivation and bioinactivation is the reverse. The differential toxicity of malathion with respect to insects and mammals appears to be due to a difference in the relation between bioactivation and bioinactivation such that in insects high levels of malaoxon are reached, this is in contrast to mammals, where because of the high rate of hydrolysis, a quick inactivation takes place. The ester groups in malathion are the vulnerable moieties involved in selective bioinactivation.

Vulnerable groups and selective bioactivation

As indicated already, biochemical conversion not always means bioin-activation; in certain cases conversion of compounds inactive as such, to bioactive derivatives takes place. If the enzymes involved show a particular

Diethylstilbestrol
(estrogen)

Honvan†
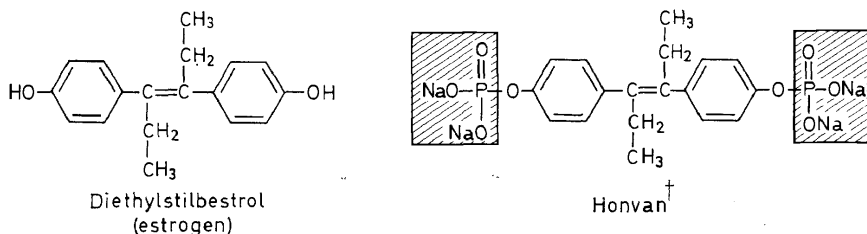
† Inactive transport form of estrogen; designed to be hydrolysed preferentially in the target tissue (prostate) rich in acid phosphatase.

Figure 15. Modulation of distribution on the basis of selective bioactivation by phosphor-ylation of diethylstilbestrol.

distribution over the various compartments or tissues, also the distribution of the drug in its active form will show such a particular distribution. One of the best-known examples of efforts to modulate the distribution on the basis of selective bioactivation is the phosphorylation of diethylstilbestrol (Figure 15), suggested by Druckrey[27]. As is well-known estrogens are used in the treatment of tumors of the prostate, a tissue rich in the enzyme acidic phosphatase. Stilbestrol in the phosphorylated form is an inactive transport form since the phenolic OH-groups essential for the estrogenic activity of the drug are masked. The ester is a substrate for acidic phosphatase. As a consequence one might expect a more or less selective bioactivation in the target-tissue, the prostate tissue. The formation of free diethylstilbestrol in the prostate tumor tissue of patients could be demonstrated; however, also other tissues such as bone and liver are rich in phosphatase able to split the ester, such that also in these tissues bioactivation takes place. This implies that there is no real restriction of the bioactivation to the target tissue.

Inactive transport forms from cytostatic biologically alkylating agents such as nitrogen mustard have also been prepared. The aim is an increase in the selectivity of these compounds with respect to the malignant tissue rich in the enzyme necessary for the bioactivation[12,46,50,81].

The occurrence of enzymes such as phosphamidase, carbamidase and phosphatases in certain cancer tissues led to the synthesis of transport forms

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of nitrogen mustard to be bioactivated by these enzymes. Cyclophosphamide is an example of such cytostatics[12,39]. Although the selective bioactivation in the target tissue could not be verified, the formation of this type of transport forms of the biologically alkylating nitrogen mustard has certain advantages. The bioactivation although not limited to the target tissue is limited to certain tissues, sparing those tissues which are poor in the enzymes involved. Also the risk of an interaction of the alkylating agent with, for instance, plasma proteins is reduced as far as the enzymes involved in the bioactivation are restricted to the intracellular compartment.

A special aspect of the development of transport forms is the elimination of unwanted side-effects. This procedure may be effective if the side-effects are due to local actions especially at the site of application. Transport forms bioactivated after leaving the site of application are needed. The transport forms should be especially inactive as far as the side-effects are concerned. The formation of, for instance, esters from which the active principle is set free by hydrolysis can be considered (*Figure 16*). Acetylsalicylic acid in which the phenolic OH-group is masked, with as a result a decrease in gastrointestinal irritation is an example. Salicylic acid is set free partly in the gut and further after absorption in plasma by plasma esterases. Avoidance of untolerable bitter taste is reached by formation of esters, e.g. the palmitate of chloramphenicol. In the gut and after absorption the active antibiotic is liberated by hydrolysis of the ester. Ethylmercaptan, a very evil smelling compound, used as tuberculostatic and for the treatment of leprosy is applied as an ester of phthalic acid, a non-volatile odourless product releasing the active ethylmercaptan gradually in the body[26] (*Figure 16*).

Selectivity in the action is also required for such biologically active compounds as insecticides and weed killers. Low hazard pesticides should be non-toxic for mammals and be specifically toxic to the insect of plant species that has to be eradicated. For compounds like the insecticide parathion which is bioactivated in the liver with the formation of the active compound paraoxon, toxicity after oral application is larger than the toxicity in the case of percutaneous absorption. There is a dependency of the activity of parathion and paraoxon on the way of application[54,102]. The consequence is that as long as the pesticide is not put into the farmers' coffee, there is a certain selective safety margin with respect to insects feeding on the plants sprayed, since in the insects bioactivation takes place more readily.

Another example of selective bioactivation in the field of insecticides is the use of non-toxic lipophilic compounds readily taken up by the plant leaves and converted in the plant to products with a high insecticidal activity. The aim is a restriction of the insecticide activity to certain plant tissues and therewith a selectivity in the action as far as phytophagous insects feeding on the plants treated are concerned, and a more sustained action of the insecticide taken up by the plants. This principle is known as endometatoxic action of insecticides. An example is Demeton (Cistox), a fat-soluble organic phosphate with a low insecticidal action which is bioactivated in the plant tissues under formation of products 1000 times more active than the parent product[48,80].

In the field of weed killers, too, the principle of selective bioactivation has been applied. Plants with an extensive foliage are highly vulnerable to

lipid-soluble weed killers because of the large surface available for the uptake. Highly water-soluble compounds, having a low lipid-solubility will be devoid of such an action since they cannot penetrate the lipophilic cuticula on the leaves. Hydrophilic compounds can be taken up easily by the plant roots,
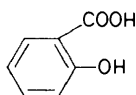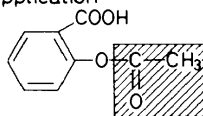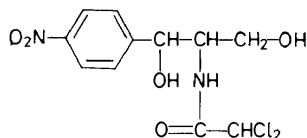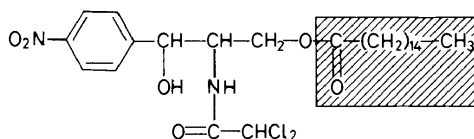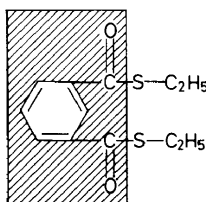

Figure 16. Development of transport forms, e.g. the formation of esters from which the active principle is set free by hydrolysis.

however. Highly water-soluble esters of compounds such as 2,4-dichloro-phenoxy-ethyl-alcohol, a precursor of the corresponding acid which acts as a weed killer have been prepared (*Figure 17*)[24]. These esters are not taken up by the plant leaves. After they reach the soil, where the esters are hydrolysed, the alcohol is oxidized to the corresponding acid, which is a potent weed killer readily taken up by plant roots. The result is a selectivity in the action. Plants with an extensive foliage and/or deep roots will be much less sensitive than weeds with extensive superficial root systems. The latter are exposed to

Figure 17. The principle of selective bioactivation as applied to weed-killers (After A. S. Crafts[24]).

the weed killer in its active form and therefore especially vulnerable to the treatment of the fields with such highly water-soluble transport forms.
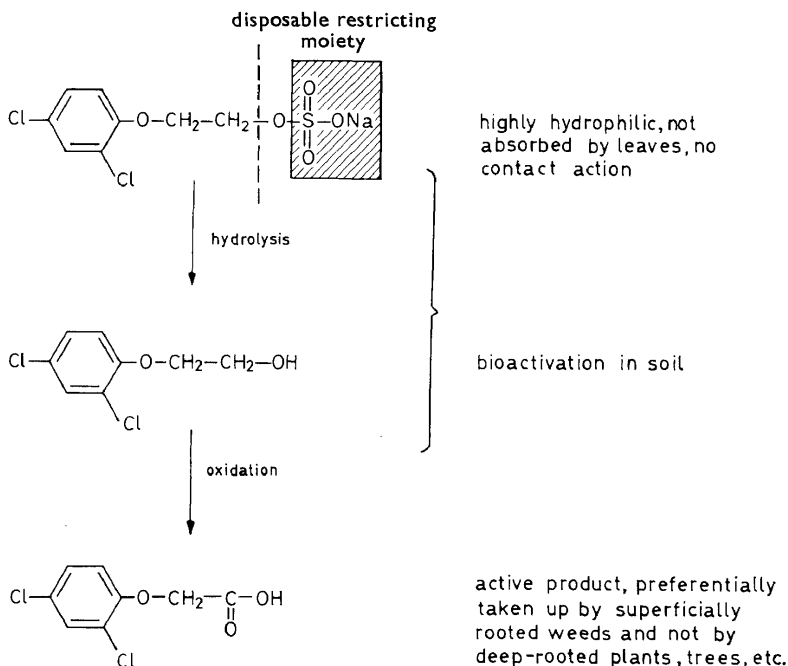
INTEGRAL APPROACH TO THE MODULATION OF PHARMACOKINETICS

For the induction of an effect, the presence of a certain concentration of the compound in its bioactive form in the compartment containing the sites of action or specific receptors is required. The activity of a compound in the strict sense—the capacity to induce a response once in the receptor compartment—differs from the potency of the bioactive compound—the dose that has to be applied to the individual or in the environmental system to obtain a certain response. The potency is strongly dependent on the pharmacokinetics of the compound, which is dependent on a large variety of part processes involved in its absorption and distribution. As such can be mentioned transport via the membranes or the barriers separating the various compartments, excretion by kidney or liver, in which active and often selective transport processes or passive transport by diffusion may be involved, partition over lipid compartments such as the body fat, and water compartments such as plasma, cerebrospinal fluid, interstitial fluid and intracellular fluid and binding of the drugs to plasma proteins and tissues. Further metabolic conversion by oxidation resulting in inactive or more active products and conjugation often leading to inactivation and an increase

in water-solubility are involved. The biological activity of a drug is, especially as far as dependent on its pharmacokinetics, *multiconditional*, because of the various processes involved. The lipid-water balance of the drug is of importance for all passive transport processes[13,21,100], for the partition over the various compartments, as well as for protein and tissue binding[11,58]. Beside lipophilic (hydrophobic) moieties and polar moieties the pK_a values of the ionizable groups in the molecule, too, are determinant in this respect. Also for drug metabolism and active transport besides certain special features such as the presence of moieties suitable for oxidative attack (*N*-alkyl groups, *O*-alkyl groups, etc.) or moieties required by the active transport systems such as anionic groups or cationic groups, the lipid-water balance, too, is of great influence[14,21,83,86]. Taking into account the differences in the chemical requirements for each of the part processes, the optimum in the chemical properties required for an optimal potency of the drug will always be a kind of compromise in which the requirements for the various part-processes are met to a degree, related to the significance of these various processes for the potency of the drug. An optimum in the pharmacokinetics will correlate with a certain value for the lipid–water balance, polarity, size and other physico-chemical properties of the drug. The relationship between certain physico-chemical constants and the potency will become manifest most clearly if homologous series of drugs—series of drugs with the same basic structure and differing only in one particular aspect, e.g. the length of an alkyl chain or the substituents in a phenyl ring—are tested under simple conditions.

A variety of physicochemical constants such as water solubility, surface activity, partition coefficient, vapour pressure, melting points, R_f values, critical concentrations for micelle formation, are expressions of the same basic properties of a compound and often are directly related to such simple parameters as the number of carbon atoms in the alkyl chain in the compound. Investigators have been looking for more basic physicochemical expressions or constants to be related to biological activity or potency. In general it can be said that if plotting of the potency against one of the parameters such as the number of carbon atoms in a chain, or the water–lipid partition coefficient, results in a regular pattern, this will be the case for any of the related physicochemical constants. Such a regular pattern implies that on basis of the values for a small number of well distributed drugs, by interpolation predictions can be made on the potency of compounds which fulfil certain basic physicochemical requirements.

This is not the place to discuss in detail the various theories relating physicochemical properties of drugs to action, from the point of view of the mechanisms of action of drugs. Here attention is focussed on the relationship between physicochemical overall properties or physicochemical constants and the potency of the members of certain groups of drugs.

As indicated for a homologous series of compounds based on gradual increase in the length of an alkyl chain or methylene chain, putting the compounds in a sequence based on the number of carbon atoms in the chain practically always will imply putting them in a sequence of a gradual increase or decrease of various physicochemical constants such as lipid solubility, partition coefficient, etc.

If a group of drugs with a variation in the site of substitution (o-, m-, p-) and of the substituents in a phenyl ring is involved, the situation is less self-evident. How to put the compounds of such a group in order of a particular physicochemical constant without measuring these constants for all the compounds in the particular group of drugs? Here the substituent constants as introduced by Hammett and Taft bring help[41].

The Hammett constants are an indicator for the charge distribution and therewith the reactivity in the carboxyl group in the substituted benzoic acids and in the corresponding groups of the other series of the substituted phenyl derivatives such as benzoic acid esters, benzoic acid amides, phenols, etc. As far as pK_a values are involved in such series the Hammett constants also give an indication for the water–lipid partition coefficients at a certain pH, since the unionized (fat-soluble) fraction depends on the pK_a values.

The basis for the substituent constants is the comparison of a particular physicochemical property or constant such as the dissociation constant, the water–lipid partition coefficient, the solvolysis in the case of esters, etc. of the unsubstituted derivative e.g. the phenyl derivative, with the corresponding constant for the substituted derivative. The various substituents differ in their contribution to the physicochemical constants concerned. Hansch[42,43,44] introduced π-constants, based on a comparison of the partition coefficients of substituted and unsubstituted derivatives[74].

The first efforts to apply the Hammett-Taft substituent constants to biological processes, or, better, biochemical activities are those from Ormerod (1953)[75] relating the rate of enzymatic hydrolysis of substituted benzoylcholine esters and Fukuto[33,72,74] relating acetylcholine-esterase inhibition by substituted phenyl-phosphates to Hammett's σ constants. It will be clear that the approach just outlined may also be helpful in designing the chemical modifications required in the adaptation of the biofunctional moieties, such as the conducting moieties and vulnerable moieties.

SUMMARY

After an introduction into some molecular aspects of drug action, the distribution of bioactive compounds, differentiated in individual and environmental pharmacokinetics, is discussed.

Certain moieties in bioactive compounds, such as drugs, called biofunctional moieties, are of special significance for particular aspects of drug action, namely for the various part processes involved in pharmacokinetics.

In a number of sections the possibilities modulating pharmacokinetics by modification of the rate of biochemical conversion and of the site of biochemical conversion, realized by molecular manipulation on the biofunctional moieties primarily involved in the metabolic degradation or bioactivation, called vulnerable moieties, and some practical consequences of this procedure are outlined. Finally the possible use of substituent constants in the efforts to modulate pharmacokinetics by molecular manipulation is indicated.

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