about previous use of steroids or stimulants, hypnotics or hypotensives.

I have already touched upon the possible effect of diet, in relation to cheese and monoamine oxidase inhibitors, and it may well be that some adverse effects of some of the drugs we use can be attributed to unknown components in the diet with which there is interaction, or perhaps they may, in some instances, be due to effects of deprivation of some dietary factor, which previously exerted a protective influence. West (1964) has recently shown that, when testing for the teratogenic activity of drugs in rats, the toxicity of aspirin was greatly increased in rats fed on a high carbohydrate, as against a high protein diet; furthermore, dietary magnesium deficiency produced a greatly increased fœtal mortality rate in the aspirin-treated animals, particularly those on the carbohydrate diet.

However, not all interactions are undesirable. They may be beneficial if the incidence of adverse reactions is appreciable with larger doses of the single drug. Or, again, an interaction may be the basis for the desired pharmacological activity, such as the enhanced oxidation of ethyl alcohol to acetaldehyde which occurs when alcohol is taken after disulfiram. The consequent discomfort and manifestations of toxicity are said to be helpful in the management of alcoholism.

Much research will continue to be conducted on the interactions in man of the drugs that we use, and no doubt, as in the past, we shall tell each other, with the wisdom given us by hindsight, that the reactions we discover could all have been predicted if we had adequately assimilated and collated and appreciated the mass of data already at our disposal. This may well be true, but the imperfections of our systems of research and communication of scientific information may well mean that we shall always depend on our retrospectroscopes to understand them.

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Displacement of One Drug by Another from Carrier or Receptor Sites

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Abstract

The medium of drug transfer is the water of plasma and extracellular fluid. Without complicating factors, the level of drug at a receptor site would be equal to that in the tissues and in plasma, and in dynamic equilibrium. Actually, almost all drugs are reversibly bound to proteins in plasma or tissue. The bound drug, often a high proportion of the total, acts as a reservoir, preventing wild fluctuations between ineffective and toxic levels of the biologically active unbound fraction.

Displacement from a receptor site diminishes drug activity, but displacement from plasma or tissue proteins augments the effect by making more unbound drug available at the receptor site.

Atropine has no intrinsic activity, but displaces acetylcholine or pilocarpine from receptors at parasympathetic nerve endings. Similarly guanethidine competes with noradrenaline at sympathetic nerve endings, but in turn is displaced by amphetamine-like drugs.

Many acidic drugs (phenylbutazone, sulfonamides, coumarin anticoagulants, salicylates, &c.) are highly bound to one or two sites on albumin molecules. When the limited carrying capacity of the plasma proteins is filled, any unbound surplus is usually soon metabolized or excreted, so the plasma level becomes restabilized. Meanwhile, however, there may be dramatic effects such as hypoglycemia, when sulfonamides are given to patients on tolbutamide, or bleeding when phenylbutazone is given to patients on warfarin.

Although hormones, like thyroxine, insulin and cortisol, are carried by specific proteins, they too can be displaced. All the antirheumatic drugs so far examined have displaced cortisol and presumably driven it into tissues. This may be one mechanism of action. Possibly the sulfonylurea drugs act by displacing insulin from proteins in the pancreas, plasma or elsewhere.

In 1924 Storm van Leeuwen wrote:

> 'It may be assumed that every drug, before acting must be absorbed by dominant receptors, present at the sites of action of the drug. The question rises as to what happens when two drugs are introduced in the body at the same time. If the two drugs have the same dominant receptors, it is very likely that one drug will be more easily absorbed than the other. The second drug may be replaced by the first one and by this procedure the action of the second drug may be antagonized. . . . replacement of one drug by another on

secondary receptors will *per se* be an important feature in drug action, as it will certainly influence the general action of both drugs.'

At that time there was no experimental evidence to support these perceptive beliefs and for many years their significance went unrecognized. Perhaps it is still unrecognized.

A fundamental premise in pharmacology is that the response to a drug is determined by the concentration of unbound drug in plasma water (Brodie 1964*a*). This fluid is the common medium through which all exchanges of drugs are made in the body. It is the fluid through which the drug is transported to sites of action, excretion and metabolism. Assuming that a drug has no difficulty in penetrating various body membranes, its concentration in plasma water will depend on how much of the agent is adsorbed on to nonaqueous components of tissues. Certain chemicals such as alcohol and antipyrine are hardly adsorbed at all, except for the few molecules taken up by the drug receptors. For all practical purposes these substances remain confined to body water as unbound drug in direct equilibration with drug receptors (Fig 1).

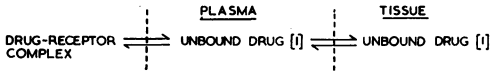

Fig 1

Few drugs are distributed in this simple way; usually there are other kinds of attachments – gross attachments I might say – to various components of body tissues, which have nothing to do with the primary drug action, and which consist of complexes of the drug with plasma or tissue proteins. These attachments may withdraw a large amount of drug from plasma water and decrease the intensity of response by lowering the concentration of unbound drug in direct equilibrium with receptor sites (Fig 2).

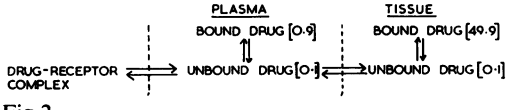

Fig 2

At one time it was thought that the site of a drug's action could be defined from its location in tissues. This view is no longer tenable since the amount of drug bound by specific sites is usually negligible compared to the large quantity localized by nonspecific bindings. Only by using tracer doses of highly labeled drugs is it possible to show that some agents are selectively fixed to specific sites.

One drug may affect the action of another by altering its concentration at receptor sites. It may act directly by displacing the other drug from its specific site of action. Or it may act indirectly by displacing the second drug from proteins in plasma or tissues, thereby increasing its concentration at specific receptors. In one case the biological activity of the first drug is diminished; in the other it is enhanced.

DRUG INTERACTION AT RECEPTOR SITES

Drugs, Enzymes and Receptors

Before discussing what happens when a drug is displaced from its receptor by another drug, I shall briefly discuss the nature of the drug-receptor complex.

Drugs, as well as the biological systems on which they act, consist of molecules, and molecules of drug presumably exert their peculiar effects by interacting with molecular complexes. We call these sites 'drug receptors', but the use of this term does not mean that we understand their nature.[1] However, a number of drug receptors are known to be enzymes and it is now suspected that receptors, even in the field of general pharmacology, are enzyme processes or are closely integrated with such processes. For this reason, a better appreciation of drug action is gained by discussing the action of drugs in molecular terms, using the language of biochemistry (Ariëns 1964).

In biochemistry the substrate (S) reacts reversibly with the active site on the enzyme (E) to yield a transition state. This enzyme-substrate complex may be represented by the law of mass action[2]: $E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES$ where ES is the enzyme-substrate complex; and k_1 and k_2 are the rate constants of the forward and reverse reactions. At equilibrium, the rate of formation of the complex, k_1 [E][S], becomes equal to its rate of dissociation, k_2 [ES]. Hence $\frac{[ES]}{[E]\,[S]} = \frac{k_1}{k_2} = K$, where K is the association or affinity constant, which describes the probability of an interaction between substrate and enzyme.

[1] In this paper terms such as receptor, drug receptor, and reactive site are used synonymously to mean *site of action* according to the meaning of Paul Erlich who considered a receptor as the place on which a drug is anchored in eliciting its effect. In pharmacology, the term receptor is more restrictive and means the precise site where a neurohormone acts. This restricted meaning is confusing to scientists in other disciplines, and raises the question whether communication among the disciplines is not more important than retaining the traditional semantics of a particular discipline

[2] In some instances the binding of a drug to the receptor is irreversible. For the purpose of this paper, only reversible interactions are discussed

The enzyme-substrate complex now breaks down to form a product, P, and the enzyme is said to have catalyzed the conversion of S to P. Thus the transition state, ES, may be visualized as a conveyor on which is fixed the raw material and from which flows the finished product P. The *intrinsic activity* is a measure of the tendency of the complex ES to form the product P. The total reaction sequence now becomes

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

How does a competitive inhibitor act? Suppose that a substance B also reacts with the active sites on the enzyme $E + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EB$ but in this case the complex EB does not form a product. The conveyor is now clogged. In other words, B is an inhibitor since it occupies enzyme sites unproductively and reduces the opportunity for substrate S to react with the enzyme. The more sites occupied by the inhibitor, the less will be available for S to form productive complexes. The degree to which the inhibitor blocks the enzyme will depend not only on the concentration of the inhibitor but on its affinity for the enzyme. Whether a substance acts as a substrate or an inhibitor in this game of musical chairs depends mainly on whether the combination of drug with enzyme can decompose to yield a product.

Certain substances lie between 'substrate' and 'inhibitor'. For example, a compound may have a high affinity for the enzyme and the complex may form a product, but its performance rating may be poor and yield only a small amount of product. Because of its high affinity for the enzyme, however, low concentrations of the substance may take possession of so many sites that the metabolism of the normal substrate is inhibited.

In pharmacology the substrate becomes the drug (D) and the active site on the enzyme becomes the receptor site (R). Mass action relationships may be applied in the same way. A drug (often a neurohormone) is reversibly attached by the receptor to form a transition state (complex):

$$D + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} = DR$$

where DR is the receptor-drug complex and k_1 and k_2 the rate constants of forward and reverse reactions. At equilibrium $\frac{[DR]}{[D]\,[R]} = \frac{k_1}{k_2} = K.$

K, the affinity constant, is a measure of the tendency of the drug to react with the receptor.

This admittedly oversimplified account of the interaction between drug and receptor[1] affords a picture of the complex having properties quite different from those of the individual parts. In order to exert an effect, not only must a drug have a high affinity for the receptor, but its combination with the receptor must be able to initiate a biological response. The tendency of the complex to react is called the intrinsic activity. Thus $D + R \rightleftharpoons DR \longrightarrow$ response.

The interaction of drug and receptor is represented therefore by two stages, the *affinity* of the drug for the receptor and *intrinsic activity* or effectiveness of the drug-receptor complex.

Many important drugs act as competitive inhibitors or antagonists. For example, an inhibitory substance, B, may have a high affinity for a nerve ending receptor. The formation of this drug-receptor complex is expressed by

$$B + R \rightleftharpoons BR$$

In this case the complex BR is biologically inert; despite the high affinity for the reactive site, the intrinsic activity is zero. An inhibitor may occupy sites usually reserved for a neurohormone and, in sufficient concentration, occupy all the sites. However, if the concentration of the hormone is raised sufficiently, it will displace the inhibitor and once more assume its normal role. Thus the ability of a competitive inhibitor to displace the natural substrate from receptors will depend on the relative concentrations and affinities of substrate and inhibitor.

A drug may have a biphasic action if it has a high affinity for the receptor, but the complex so formed has a low intrinsic activity. In small doses the drug may inhibit the action of a neurohormone, but in high doses weakly mimic it.

Displacement from Drug Receptors

We tend to forget that a drug like atropine is really an inert substance, exerting no pharmacological action of its own. But its molecular structure is sufficiently like that of acetylcholine to deceive the receptor at the parasympathetic nerve ending. Atropine is avidly taken up and acetylcholine is crowded out and prevented from discharging its normal function. Both atropine and acetylcholine have a high affinity for the receptor but the complex of the receptor with atropine differs sufficiently from that with acetylcholine not to elicit a biological response. Atropine also displaces a number of drugs, such as

[1] Forces such as electrostatic attraction, hydrogen bonding, Van der Waals' forces and charge transfer phenomena are insufficient to explain the unique nature of the transition state or complex formed by an organic compound with the reactive site on an enzyme or receptor site. It is possible that the structure will only be disclosed by isolation and examination of the crystalline complex between receptor and enzyme

pilocarpine and methacholine, that have an affinity for the receptors and mimic the action of acetylcholine.

At the motor end-plate atropine does not work very well but tubocurarine does the same thing. Tubocurarine has no action of its own, but produces paralysis by occupying receptors normally reserved for acetylcholine. This effect of the drug may be reduced competitively by acetylcholine or by neostigmine, which inhibits cholinesterase thereby increasing the level of acetylcholine. In addition it is antagonized by 3-hydroxyphenyldimethylethylammonium (edrophonium chloride) which acts like acetylcholine. I might point out here that a curare-like paralysis is also induced by decamethonium and succinylcholine, but these drugs exert more than 'squatter's rights'; they could almost be called subversive agents since they not only displace acetylcholine from receptor sites, but produce persistent depolarization of their own. It is important to keep such actions in mind, for acetylcholine or acetylcholine-like substances that would be expected to overcome the effects of curare would only aggravate the effects of decamethonium and succinylcholine by 'adding fuel to the fire'.

Similarly there are substances like tetraethylammonium and hexamethonium that displace acetylcholine and act as blocking agents at autonomic ganglia. Nicotine and tetramethylammonium not only displace acetylcholine but mimic its depolarizing ability.

An example of a displacing agent with a biphasic action is dichloroisopropylnoradrenaline. This has a high affinity for the adrenergic receptor site but the complex has only a low intrinsic activity. In large doses, therefore, the agent exerts a sympathomimetic effect, but in small doses can antagonize the action of noradrenaline (NA) and adrenaline at certain adrenergic sites. A number of agents which evoke weak ganglionic blocking effects are quite potent in antagonizing more powerful blocking agents by displacing them from sites of action. In our laboratory we have demonstrated that tetrabenazine (Nitoman), a benzoquinolizine drug, which by itself elicits relatively weak responses characteristic of reserpine, is able to prevent the action of the more potent and long-lasting reserpine (Quinn *et al.* 1959).

So far I have discussed relatively simple examples of competitive displacement. The antihypertensive drug, guanethidine, can be counteracted by amphetamine in a peculiar way that is related to its interesting mechanism of action. Guanethidine is taken up not only by nonspecific binding sites, but is also taken up and stored in adrenergic neurones by the same process that stores NA. Thus reserpine releases the drug from these sites as though it were NA (Chang *et al.* 1964).

The specific sites of uptake are saturable, accumulating a maximum of about 3 molecules of guanethidine for each molecule of endogenous NA (Chang *et al.* 1965). Kinetic studies with heart slices indicate that the drug is taken up by an active transport system (Schanker & Morrison 1965). The sympatholytic effect is directly proportional to the quantity of guanethidine that accumulates in these specific sites. The results are compatible with the view that guanethidine depolarizes the presynaptic membrane and that the sympatholytic effect results from this action. The uptake of guanethidine into these specific sites can be prevented or displaced by amphetamine.

One cannot help speculating on the possibility of developing 'silent' antagonists for various classes of drug; that is to say, agents that would not have any action of their own but which would simply displace drugs from receptor sites. For example, benzquinamide, a tetrabenazine analogue, in a dose that exerts little or no sedation by itself, prevents the action of reserpine, presumably by blocking its access to the drug receptor (Sulser *et al.* 1964). Again, 3-hydroxyphenyltriethylammonium, an analogue of edrophonium chloride, is reported to be a potent anticurare drug that elicits little excitatory effect of its own on skeletal muscle (Randall 1950). Silent antagonists would be extremely useful in studies of drug action; and of course they might serve as the perfect antidote in cases of poisoning or overdosage. I believe that this is more than a theoretical possibility and might be worth some thought.

DRUG INTERACTION AT NONSPECIFIC BINDING SITES

Nature of Drug-protein Binding

Because there has been considerable misunderstanding about the binding of drug to plasma proteins, I shall review what is known of the nature of these attachments as a prelude to discussing drug displacement. Tissue and plasma proteins are remarkably catholic in their ability to form reversible complexes with drugs. An important difference between these interactions and those with receptor sites is the large number, often the bulk, of the drug molecules which are involved. Research on the nature of the binding on to tissue proteins has been rather scanty.[1] In

[1] Binding of drugs to tissue components is a relatively unexplored area. Our own work indicates that acridines, phenothiazines, procaine amide, barbiturates, cinchona alkaloids and phenylbutazone are bound to tissues by different mechanisms. Despite the potential importance of tissue binding to pharmacology, toxicology and drug development, in no instance is the protein component known

contrast the nature of binding on to plasma proteins has withstood the challenge of perennial investigation. About the only important generalizations that have emerged are that the binding is readily reversible, is confined mainly to the albumin fraction, and is remarkably unspecific.

At present it is doubtful if the binding of a single drug to plasma protein is fully understood. One still hears the argument about whether drugs are bound to proteins by physical or chemical forces. This seems somewhat like the problem of how many angels can dance on the head of a pin. As a convenient oversimplification, the interaction between drug and protein is described as physical adsorption, but all sorts of forces are involved and it is a brave chemist who would dare to say when a binding force leaves the physical realm to enter the chemical.

At one time binding to plasma protein was attributed to electrostatic attraction between the ionic form of the drug and a charged group on the albumin. This would explain why alcohol and antipyrine, relatively neutral substances, show little affinity for plasma proteins or, for that matter, for other proteins. But forces other than electrostatic attraction are needed to explain the relation between degree of binding and chemical structure. For example, in a series of barbiturates, all of which have the same acid strength (pKa 7·6), barbitone is hardly bound to plasma albumin, but as the chain is lengthened, the binding increases and reaches 55% with pentobarbitone.

In such cases, the primary bond may be electrostatic but the resulting complex is stabilized by forces such as hydrogen bonding and dipole-dipole interactions. But these forces fail to account for the increase in binding caused by lipid-solubility. Thus thiopentone is more lipid-soluble than its oxygen analogue pentobarbitone and is more highly bound to plasma albumin; N-methylthiopentone is much more lipid-soluble than thiopentone, and correspondingly is bound more tightly to plasma proteins (Marks *et al.* 1950). To explain this, Van der Waals' forces are invoked. These forces are described as a short-range mutual attraction of molecules which causes them to cluster together. Unfortunately these forces are not measured directly, but are calculated from what is left over after all the other forces are measured. The use of this term to describe drug binding should not delude us into thinking we understand the nature of these binding forces. All we can say for certain is that there is an empirical relationship between the lipid-solubility of drugs and their degree of binding to plasma proteins, and that many highly lipid-soluble compounds such as cyclopropane and methylcholanthrene do not even require an ionic bond. Perhaps a descriptive term like lipophilic bond should be used to describe the binding of lipid-soluble substances; at least such a term would serve as a constant reminder that we do not understand the mechanism of binding.

Kinetics of Drug Binding

Although the nature of the forces is still unclear, binding may be calculated in terms of the capacity of the protein and the tightness of the attachment. The attachment of drugs to proteins may also be described by the law of mass action:

$$D + P \underset{k_2}{\overset{k_1}{\rightleftharpoons}} DP \quad \text{and}$$

$$\frac{[DP]}{[D]\,[P]} = \frac{k_1}{k_2} = K$$

where D is drug; P is protein; DP is drug-protein complex; k_1 and k_2 are rates of forward and reverse reactions and K is the association or affinity constant.

Equilibrium is a dynamic state where there is no net change but the forward and reverse reactions are equal. If the drug has a high affinity for the protein, almost all the drug will be bound before the concentration of the complex is high enough for k_2 [DP], the rate of the reverse reaction, to become equal to k_1 [D] [P], the rate of the forward reaction. If the affinity is low, the forward reaction will be barely under way before equilibrium is established.

The effects of protein binding on the biological activity of a drug depends on the relative proportion of bound and unbound molecules. Thus $\frac{1}{F} = 1 + \frac{1}{KnP} + \frac{D}{nP}$ where F is the fraction $\frac{\text{bound drug}}{\text{total drug}}$; K is the association or affinity constant; D the concentration of unbound drug; P the concentration of protein and n the number of binding sites for each protein molecule (Goldstein 1949). From this equation it is evident that:

(1) The fraction of bound drug is increased with an increase of protein concentration. Conversely, dilution of the system with water will lower the bound fraction by causing dissociation of the complex.
(2) The bound fraction decreases with an increase in drug concentration; conversely it increases with a decline in drug concentration.
(3) The higher the affinity constant, the tighter will be the binding and the greater the bound fraction.

Pharmacological Significance of Drug-protein Interactions: Role of Bound Drug

Since most therapeutic agents are extensively bound to tissue or plasma proteins, the possible influence of such binding in drug screening and in therapy must be considered.[1] It seems almost self-evident that protein-bound drug molecules are unable to exert a biological effect, and are hindered from gaining access to sites of metabolism and excretion. However, the relationship of bound to unbound drug is still a source of considerable confusion. Many textbooks lump together nonspecific binding, excretion and metabolism as *sites of loss*. Let us see how such terminology can lead the pharmacologist and medicinal chemist astray.

Suppose that two penicillins (A and B) exert equal antibacterial action in a protein-free medium, but 90% of B is taken up by tissue and plasma proteins, while all of A remains in plasma water. After equal doses of the drugs, the concentration of A in plasma water will be 10 times that of B. Dose for dose, therefore, penicillin B will have only one-tenth the activity of A since 10 times as big a dose is needed to elicit the same antibacterial activity as A.

But let us pursue the problem one step further. All things being equal, the systemic effects of A and B will also be dependent on the concentration of unbound drug in plasma water. It will take 10 times more drug B than drug A to elicit the same side-effects. Thus the extra amount of B needed to achieve the same antibacterial activity as A is not wasted – it is stored. Moreover, assuming that rates of inactivation or excretion of the two drugs are also related to the unbound level, the duration of action of drug B will be 10 times that of drug A. In practice then, if protein binding were ignored, drug B might be discarded as an inactive agent. Actually the intrinsic activities of the two drugs are equal and the prolonged duration of action of B provides a possibly important therapeutic advantage.

These results show that the bound drug is not 'lost' but merely held in reserve. Binding to proteins is not usually a disadvantage, in fact without such binding most drugs would elicit too transient an effect to be of much use; they would have to be administered with such frequency that the plasma concentration would oscillate between toxic and ineffective levels (Brodie 1964*b*). Therapy is easier to control when a drug is stable in the body and the plasma concentration does not fluctuate widely.

How Changes in Plasma Concentration are 'Buffered' by Bound Drug

At true equilibrium the rate at which DP is formed (k_1 [D] [P]) would equal the rate at which it is dissociated (k_2 [DP]). However, equilibrium between drug and protein is never really established since, between doses, the unbound drug is excreted or metabolized and the plasma level falls continuously. Consequently the rate of dissociation of DP is greater than its rate of formation. Because of the ready reversibility of the drug-protein complex, there is a continuous shift of bound to unbound drug. The greater the amount of bound drug, the less rapidly will the plasma level of unbound drug decline, since it is continuously being replenished through dissociation of the complex. In this way the concentration of unbound drug is buffered and stabilized.

Drug Excretion

Protein-bound drug is not filtered through the glomeruli of the kidney and, since filtration removes unbound drug and water in equal proportions, the equilibrium between bound and unbound drug is not disturbed.

Secretion of drugs by the kidney tubules is another matter. Why is it that despite a considerable degree of binding to plasma proteins, a drug may be completely cleared by kidney tubules? In the equation showing the binding of drug to protein

$$D + P \underset{k_2}{\overset{k_1}{\rightleftharpoons}} DP \quad \text{and} \quad \frac{k_1}{k_2} = K$$

the fact that K, the affinity constant, is determined at equilibrium conditions should not obscure the fact that this state represents a hypothetical balance of opposing processes, with equal rates of formation and dissociation of the drug complex. A high affinity merely signifies that the rate constant, k_1, of the forward reaction is high *relative* to the rate constant of the reverse reaction, k_2. This does not mean, however, that the rate constant k_2 is low. For example, substances like phenol red and penicillin are respectively 80% and 50% bound to proteins, but they are completely cleared from plasma in a single passage through the kidney. The unbound fraction is secreted almost instantaneously; the amount of protein-bound substance that is also secreted will then depend on k_2, the rate constant of the dissociation of drug-protein complex. In the case of phenol red and penicillin, k_2 is sufficiently high that the rate of dissociation does not limit the rate of secretion. However, the

[1] Extrapolation of drug effects from animals to man is made on the assumption that the binding to plasma protein is the same in all species. In general this is true but, for sulfonamides and perhaps other drugs, binding is much less in the mouse than in man, monkey, and cow. Since the mouse is commonly used to screen sulfonamides and antibiotics, comparisons of the antibacterial effects, based on the total concentration of drug (bound plus unbound) in plasma, can be misleading

secretion of a drug may be hindered if it is bound very extensively and, of course, if the drug binding were 100% there would be no way in which the kidney could secrete it.

Drug Distribution

When a lipoid membrane separates two fluid compartments, plasma and CSF for example, the plasma protein and its attached drug cannot diffuse across the membrane; hence only unbound drug in plasma will equilibrate with the drug in the protein-free fluid on the other side of the membrane. When a drug is lipid-soluble, the concentration of drug in the CSF will be identical to that of the unbound drug in plasma. With some drugs, in fact, a comparison of the concentrations in CSF and plasma is a simple and precise way of measuring protein binding (Brodie 1964*a*). Keeping these relationships in mind, let us follow the distribution of a drug, such as chlorpromazine (Largactil), between plasma, CSF, and brain tissue (Fig 2).

The total concentration of chlorpromazine in plasma will be taken as 1 μg/ml, of which 90% is bound. Only the unbound drug penetrates into CSF and brain water, hence at equilibrium the level of unbound drug in these fluids will be 0·1 μg/ml. However, the total concentration of drug in brain is about 50 μg/g since the drug is highly bound to brain lipoproteins.

It is easy to see the trap into which the unwary scientist might fall in extrapolating results obtained *in vitro* to the living animal. Suppose that the biochemist discovers that chlorpromazine elicits sedation in animals at a concentration in brain of 4 μg/g. He might now say: 'I shall study the mechanism of action of chlorpromazine from effects on tissue slices *in vitro*. But I shall not make the usual mistake of using the drug in non-pharmacological concentrations, but shall add it to the preparation in the same concentration as that which I found *in vivo*. Accordingly, whatever results I find *in vitro* will apply *in vivo*.'

He then incubates brain tissue (200 mg) with 10 ml of a solution containing 4 μg/ml of chlorpromazine, and finds that a large number of enzymes in brain tissue are almost completely inhibited. The chemist might conclude that chlorpromazine in the living animal elicits its characteristic sedation by inhibiting these enzymes; or if he really considered the implications, he might conclude that the drug is a deadly poison since it blocks so many enzymes of intermediary metabolism.

Of course his conclusions are wrong because the experiment is all wrong. He has not realized that 99·8% of the drug in intact brain is reversibly bound. Accordingly he has used a concentration of drug for the *in vitro* studies that is about 500 times greater than the unbound concentration *in vivo*.

This error is commonly found in studies of the effects of drugs *in vitro*. I cannot resist telling our goldfish story since it shows how a similar error may sometimes be made in experiments *in vivo*. We placed goldfish, each weighing about 20 grams, in fishbowls of various sizes containing chlorpromazine solution (4 μg/ml). After two hours the following results were observed:

Fishbowl 1 (150 ml) No effect
Fishbowl 2 (500 ml) Slight sedation
Fishbowl 3 (1,000 ml) Deep depression
Fishbowl 4 (3,000 ml) Fish is dead

When I discussed this problem with a bright young psychiatrist he pointed out to us that this was a clear-cut example of the dangers of *lebensraum* for fish. Unfortunately, the answer was far more mundane. The drug had diffused through the gills and had become highly localized by various tissues. This lowered the concentration in the smallest bowl and the unbound drug in the fish to a nontoxic level (0·2 μg/ml). The quantity of drug in brain increased with the volume of solution and, in the brain of the fish in the largest bowl, reached a concentration of 360 μg/g. From the results in the large fishbowl it might be concluded that chlorpromazine is an extremely potent drug in fish. After taking binding into account, however, it is evident that chlorpromazine is not particularly active in this species. Thus despite a concentration of 80 μg/g of drug in the brain, the fish in the smallest bowl was not sedated.

Displacement of Drugs from Nonspecific Binding Sites

A number of acidic drugs are attached to only one or two sites on the albumin molecule; for these drugs the protein has a limited carrying capacity. An acidic drug with a molecular weight of 300 has a binding capacity of about 100–200 μg of drug per ml of plasma. Beyond this concentration there is a rapid increase in the fraction of unbound drug which now becomes available for diffusion into tissues and into sites of metabolism and excretion. Such a drug finally reaches a limiting level regardless of dosage. Interesting therapeutic consequences will occur if the binding of a drug approaches saturation at therapeutic plasma levels (Brodie 1964*b*). An example is phenylbutazone which is highly bound, about 98%, to albumin. This incidentally protects the drug from rapid inactivation, which in man is only about 15% a day. Subjects receiving 1,600

mg of drug daily attain plasma concentrations of phenylbutazone only slightly higher than do subjects receiving 400 mg daily. Using each patient as his own control, dosages of 600 and 1,600 mg per day were found to give rise to plasma levels that differed by only about 10%.

On investigating the fate of the extra 1,000 mg of drug, each patient was shown to have a limiting plasma level of about 100 µg/ml. Raising this level causes an acceleration in drug metabolism until the level returns to 100 µg/ml and the former decline of 15% daily. This phenomenon at first seemed at variance with the laws of kinetics since the apparent velocity constant for the biotransformation of the drug is greater at high than at low concentrations.

This seeming paradox can be explained by the peculiar binding of phenylbutazone to plasma proteins. At levels of 100 µg/ml only about 2% of the drug is unbound. In contrast, at a plasma level of 250 µg/ml, about 12% is unbound; hence biotransformation proceeds at six times the normal pace until the plasma level declines to about 100 µg/ml, and the drug is only about 2% in the free form. Thus the tendency of phenylbutazone to remain at a constant level regardless of the dose, results from the peculiarity of its plasma binding.

This phenomenon might account for the early toxicity reports when the drug was given in doses up to 1,600 mg a day. It is possible that the toxicity was disproportionately high relative to the dose because of the disproportionate rise in unbound drug for a few hours each day. As an aside, one wonders to what extent the adverse effects of phenylbutazone could be averted if the physician established for each patient a dose that would not saturate the protein binding sites.

A number of acidic drugs appear to compete for the same limited number of protein binding sites. Hence one acidic drug (A) may be displaced by another, thereby increasing the concentration of unbound drug (A) at target sites. Thus highly bound acidic agents such as phenylbutazone, oxyphenbutazone, ethyl biscoumacetate (Tromexan), dicumarol, sulfinpyrazole and salicylic acid are able to displace the long-lasting, albumin-bound sulfonamides from plasma proteins (Anton 1960). Since these sulfonamides are not rapidly metabolized or excreted, the displaced molecules diffuse from plasma into tissues. As a result, the total concentrations of the sulfonamides decline in plasma but rise in skeletal muscle, CSF and brain, and their antibacterial activities are enhanced.

Anton (1960) has shown that phenylbutazone (100 µg/ml) added to plasma, containing the sulfonamide sulfaethylthiadiazole (100 µg/ml), increases the proportion of unbound sulfonamide by 3·5 times. In addition, the antibacterial activity is enhanced indicating that the unbound, rather than the bound, sulfonamide is the active moiety. Similarly, phenylbutazone increases the antibacterial activity of acidic antibiotics such as penicillin.

Displacement of a drug can be dangerous if it is bound so extensively to plasma proteins that the unbound moiety is only a small fraction of the total. Displacement of only a small proportion of the drug from protein may then double or treble its unbound concentration at the target site. Thus a highly bound sulfonamide, such as sulfaphenazole, can induce hypoglycemic coma by displacing tolbutamide from protein. Similar results have been elicited by phenylbutazone and salicylates (Christensen *et al.* 1963).

Other potentially hazardous drugs are anticoagulants of the coumarin type, which are highly bound to plasma albumin. A number of cases have been reported in which phenylbutazone, or oxyphenbutazone, causes profound bleeding in patients under treatment with warfarin (Eisen 1964).

Of particular interest are the recent studies of Dixon *et al.* (1965), showing that the antifolic agent methotrexate is bound in part to plasma albumin and is displaced by a number of drugs, including sulfonamides and salicylic acid. Since methotrexate is often used therapeutically in doses that are nearly toxic, the clinical implications of these findings are obvious.[1]

Drugs can also be displaced from proteins in organ tissues. A particularly dramatic example of this occurs in the treatment of malaria. When pamaquine is given to patients previously treated with mepacrine (Atabrine), the pamaquine is displaced from organ tissues and its plasma concentration is increased five- to ten-fold (Zubrod *et al.* 1948). Since pamaquine is a dangerous drug by itself, the increased concentration makes it much more so. The displacement of pamaquine is not surprising in view of the extraordinary capacity of tissues to localize mepacrine. After mepacrine is given daily for fourteen days, the concentration in liver is about twenty-two thousand times that in plasma and about two hundred thousand times that in plasma water. High concentrations of pamaquine are obtained even when the drug is given as long as two months after the last dose of mepacrine. Needless to say, these two drugs should not be administered together.[2]

[1] The fact that barbiturates, probenecid and suramin do not displace sulfonamides from binding sites suggests that these acids are bound by sites different from those which take up tolbutamide, sulfonamides, phenylbutazone analogues, coumarins and salicylates

[2] Almost no studies have been made of the displacement of other basic organic compounds from plasma or tissue proteins

Bilirubin

Premature babies have relatively low quantities of albumin and the acidic binding sites are readily saturated with bilirubin. In premature babies, sulfonamides can produce toxic effects by displacing bilirubin from albumin (Odell 1959). The unbound bilirubin is then free to diffuse into the brain and produce harmful effects. At one time, premature babies were routinely treated with sulfisoxazole (Gantrisin) and penicillin to protect against infection. In a well-known study, the antibacterial effects of tetracycline or a combination of penicillin and sulfisoxazole were compared in premature children. Death that occurred in a considerable percentage of the premature babies treated with the sulfonamide combination was not associated with infection, but with kernicterus (Silverman *et al.* 1956). It is now recognized that neonates should not be given sulfonamides, salicylates or other agents that can displace bilirubin from albumin.

Displacement of Hormones by Drugs

Various hormones such as thyroxine, corticosteroids and perhaps insulin are transported in plasma by specific proteins. In an attempt to find drugs that displace hormones from their protein carriers, we have studied the interaction of non-steroidal antirheumatic agents with protein-bound corticosteroids. Our interest in this problem was stimulated on reading Hench (1952), who reported that rheumatic patients who developed obstructive jaundice become free of rheumatic symptoms.[1] We thought it possible that, if antirheumatic effects had been elicited by corticosteroids displaced by bilirubin from sites of protein binding, other organic acids might act in a similar way. Such a concept might seem naïve, but no more so than the belief that acids as diverse in structure as salicylate, phenylbutazone, flufenamic acid and indomethacin should all act directly on the disease process, and have ulcerogenic and sodium-retaining properties as well.

Recent studies indicate that corticosteroids are transported in plasma by a special globulin, transcortin, for which the affinity constant of the hormone binding is very high (Daughaday 1956). Normally the amount of this protein appears to be closely adjusted to the concentration of corticosteroid, just enough being present in plasma to bind 95% of the basal concentrations of these hormones (Daughaday 1958). The physiological role of this protein is not yet clear. The high binding might give the hormones some measure of specificity since it would limit their transfer to cellular proteins which also have a high affinity for them.

[1] The possibility that bilirubin might have displaced corticosteroids was suggested by Dr Terry Binns

Table 1

Effect of various compounds on binding of corticosterone to proteins in rat plasma

Compound	*Unbound corticosterone %*	*Antirheumatic activity*
Normal control	9·6	–
Saline	9·8	0
Phenylbutazone	18·3	+++
Oxyphenbutazone	19·1	++++
Sodium salicylate	18·8	+++
Flufenamic acid	16·6	++++
Sulfinpyrazone	18	+++
Indomethacin	40	++++
G 27463●	9·9	0
G 29665●	8·8	0
G 34905●	10·1	0
G 35202●	10·4	0

●Phenylbutazone analogues

In our studies, preliminary results (Table 1) show that the antirheumatic activity of a number of acidic drugs is correlated with their ability to displace corticosterone (the main glucocorticoid in the rat) (Maickel *et al.* 1965). For example, in a series of phenylbutazone analogues only those that exert an antirheumatic effect are able to displace corticosteroids. Large doses of salicylate are required to displace the corticosteroid; in contrast, flufenamic acid, a potent drug clinically, is needed in much smaller doses. The most dramatic effects are obtained with the new drug indomethacin (Indocid). This drug, which exerts potent, antirheumatic effects in the clinic and correspondingly potent adverse reactions in high doses, raises the unbound corticosterone from about 10% to 40%.

The possible mechanism of action of these drugs was investigated from the effects of phenylbutazone on the concentration and turnover time of plasma corticosterone. After treatment with the drug, the plasma level of the hormone is almost unchanged. In addition, its turnover time, determined from the decline in H^3-corticosterone injected in tracer amounts, is also unchanged. However, the specific activity of the plasma corticosterone in the animals given phenylbutazone is only 50% of that in the controls. Calculation of the volume of distribution of the labeled hormone (estimated from the plasma level extrapolated to zero time) shows that a considerable amount of endogenous corticosterone is normally present in tissues and that this amount is doubled after treatment with phenylbutazone.

These findings suggest the following events: phenylbutazone displaces corticosterone from transcortin; as a result the hormone is dispersed much more readily into tissues. Since the specific activity of plasma corticosterone is decreased by 50%, it may be inferred that the amount of

endogenous corticosterone in tissues has doubled, and that the hormone has been displaced from cellular as well as plasma protein. With twice as large a pool of tissue corticosterone turning over at the same fractional rate, it follows that the rate of secretion from the adrenal gland must be doubled.

These interpretations are only conjectural. A definite cause and effect relationship between displacement of corticosteroids and antirheumatic action has not yet been established. It will be necessary to carry out kinetic studies with pure transcortin as well as with tissue proteins. However, the results do suggest that the action of some drugs may be mediated in part through a physical displacement of endogenous hormone.

An enormous amount of work has been done to elucidate the mechanism of action of the sulfonylurea drugs in diabetes. It is postulated that their primary action may be the freeing of endogenous insulin from protein-bound complexes in the pancreatic β cells, the plasma and the tissues. This could account for the proven increased release of insulin, the duration of which is uncertain, and for the increased insulin-like action on the liver and peripheral tissues. Much more evidence, however, is required to substantiate or refute this hypothesis (Duncan & Clarke 1965).

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Enzyme Stimulation and Inhibition in the Metabolism of Drugs

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Abstract
Studies in recent years have disclosed two types of drug interaction which may be important in drug therapy:

(1) Administration of one drug can speed up the metabolism of another drug. Animal experiments show that this results from the ability of drugs to induce the synthesis of drug-metabolizing enzymes in liver microsomes. This effect has considerable importance in pharmacologic and toxicologic studies carried out in animals, and recent work indicates that it may explain altered therapeutic responses observed in some patients when they receive several drugs at the same time. Substances present in the environment, such as the insecticides chlordane and DDT, have been shown in animals to stimulate drug-metabolizing enzymes in liver, but the significance of this observation for man is not known. Drugs which stimulate drug metabolism also enhance the hydroxylation of testosterone, estradiol, progesterone and cortisol by enzymes in liver microsomes. Further research is required to establish the physiological importance of this interaction of drugs in steroid metabolism.

(2) One drug may inhibit the metabolism of another drug and thus intensify and prolong its pharmacologic action. Although this effect is well documented in animals, recent reports suggest that this may also be important in man. For instance, the action of coumarin anticoagulants can be potentiated by administration of certain drugs which inhibit their metabolism. Monoamine oxidase inhibitors block the metabolism of certain sympathomimetic amines and this can lead to serious side-effects. Thus, hypertensive crises have been observed in patients receiving monoamine oxidase inhibitors who have eaten cheese with a high tyramine content.

It is a common practice for patients to be given several drugs at the same time, but sometimes one drug may reduce or intensify the pharmacological efficacy of another or the combination may result in an unexpected adverse effect. Our laboratory has been particularly interested in the ability of drugs to stimulate or inhibit the metabolism of other drugs and thereby alter their duration of action. These effects have been well studied in experimental animals and they now appear to have importance in understanding certain drug interactions in man (Conney & Burns 1962, Remmer 1962, Burns 1964, Fouts 1964).

Enzymes in liver microsomes, which metabolize many clinically useful drugs, are associated