REVIEW ARTICLE

SOME PHYSICO-CHEMICAL FACTORS IN DRUG ACTION

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THERE are two facets to the problem of the biochemical and physical aspects of drug action: the influence of drugs on the body and the influence of the body on drugs. Although the present article is concerned mainly with the latter a few thoughts on the influence of drugs on biochemical mechanisms may not be out of place. This aspect of drug action is truly an enigma, which attempts to solve have yielded little success thus far. The mechanism of action of drugs is explainable at a gross physiological level only. This permits us to say, for example, that dibenamine blocks the action of noradrenaline at hypothetical receptor sites; or that quinidine acts by slowing the conduction time or by increasing the refractory period of heart muscle. But the question of their precise biochemical mechanisms is unanswered*. The orthodox approach, repeated faithfully with almost every new therapeutic agent of importance, has been to determine its effect on one known enzyme system after another on the assumption that the response to the drug is caused by an interference with a known biochemical process. The almost constant failure to relate the effect of a drug on an enzyme system *in vitro* with its response in the body arouses suspicion that the present approach may be aimed at the wrong target.

Physiologists have also experienced little success in connecting known biochemical events with the specialised functions of organs—the beating of the heart, the response of a nerve cell, or the gastric secretion of acid are good examples. Perhaps the present failure to relate biochemical reactions to physiological function or to drug action is an inevitable consequence of thinking in terms of the "universality" of tissue catalysts, itself a useful concept in biochemical speculation, but one which may have been carried too far. This idea implies that functional differences between organs like brain and kidney arise mainly from differences in the organisation and control of the same tissue catalysts. This is like saying that nature plays all her combinations with a pack of only fifty-two cards and that the difference between various kinds of cells is a reflection merely of the difference in the way the cards are dealt. It seems more plausible to explain the specialised functions of the brain and kidney in terms of biochemical reactions that have an unique role in each organ and that these are unlikely to be present in the unicellular organism. The

* This statement is not meant to apply to chemotherapeutic agents which selectively destroy invading micro-organisms presumably by affecting biochemical mechanisms qualitatively different from those of mammals; nor to those anti-cancer drugs which are designed to interact with biochemical mechanisms common to normal and cancer tissues, thus walking the tightrope between death of the diseased and normal cell.

contemporary advances in biochemistry have been concerned mainly with the reactions that supply the energy for, and build the housing of the cellular machinery but have not helped us to understand the nature of the machinery nor how it is harnessed to the energy produced by the cell. It is possible that drugs exert their rather specific effects by modulating organ-specific enzyme systems and that these systems must be known in order to understand the action of drugs*.

Even though we are far from understanding the intimate action of a drug, it is useful to think of a therapeutic agent interacting with some receptor site to produce its pharmacological response. To be effective, a substance must not only possess the intrinsic capacity of impinging on a particular receptor site, but it must also have characteristics which allow it to reach its objective in an adequate concentration. The latter aspect of drug action is fortunately a simpler story since it mainly involves known physico-chemical principles.

It is usually not possible to measure the drug at its site of action but it is possible to measure the level of the drug in plasma. Since plama is the physiological medium of exchange between tissues, the level of a drug in plasma may be considered to be in physico-chemical equilibrium with the concentration at the locale of action.

In studying what happens to drugs in the body it is therefore important to consider those factors that affect the plasma level of the drug†. These are depicted in a schematic fashion in Figure 1.

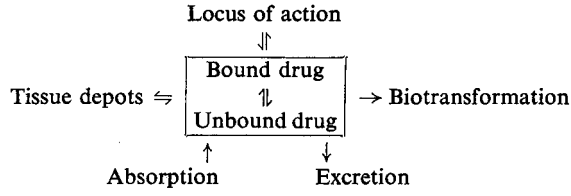


FIG. 1

After oral administration, a drug to act systemically must be absorbed from the gastrointestinal tract. It is then carried by the blood stream to the various tissues, but before it gains access to its locus of action it must cross various hurdles like the blood-brain barrier, cross the boundaries of various tissue cells and even intracellular barriers. Having reached

* It is pertinent that with the few drugs whose action can be reasonably explained at a chemical level, the interaction is not with a "universal" enzyme system, but with one involved in the biochemistry of function—for instance, physostigmine acts by inhibiting acetylcholinesterase, an enzyme which has an integral role in the function of nervous tissue. In addition, there is knowledge of the mechanism of action of a number of general poisons, but these are generally toxic to the organism by interfering with biochemical reactions that are required for the maintenance of all cells. In this category is cyanide which acts on iron porphyrin enzymes, and dinitrophenol which affects oxidative phosphorylation.

† Some drugs have been shown to act irreversibly. For example, the effects of substances such as dicoumarol, difluorophosphate and reserpine persist long after the active drugs have disappeared from the body. But the response to these drugs still depends on the agents reaching their locus of action in suitable concentration and remaining there for a certain period of time.

its objective the duration of action of a drug will be determined to a considerable degree by localisation in various tissue depots, by metabolic transformation and by the interplay of the actions of absorption and excretion. The study of drug disposition should be of considerable value not only in understanding drug action, but in defining those physico-chemical properties that are important in the formulation of useful therapeutic agents.

The first article of this series considered the various mechanisms by which the body inactivates drugs and the importance of these in drug action[1]. The present review will describe some of the vicissitudes a drug must experience before arriving at its site of action.

As drugs must penetrate a number of cell barriers before reaching their site of action, the behaviour of these membranes towards foreign compounds should be understood. Almost sixty years have elapsed since Overton presented his speculations on the nature of cellular membranes[2]. He described the boundary between living cells and their environment in terms of penetration by organic compounds. His classical experiments showed that a number of compounds permeated cells by passive diffusion at rates determined by their solubility in fat-like solvents, and he concluded that the membrane of living cells is essentially fat-like in nature.

As study of cell permeability progressed, it soon became clear that very small molecules even though lipid-insoluble, could passively penetrate cells. This led Collander and Bärlund to propose that the face of the cell membrane was not a continuum of lipid but was interspersed with tiny holes through which certain molecules could *leak*, in spite of their lipid insolubility[3].

As time went by this architectural scheme for the cell boundary became inadequate, for while the lipoid barrier might ensure living matter against the loss of its proteins and most of its water soluble organic substrates, it could not explain the passage of inorganic ions and many endogenous organic compounds. Lipid-insoluble organic substrates required by the cell obviously have to have some way of entering other than through the pores. Furthermore, something more than a passive barrier was needed to explain the preferential uptake of potassium over sodium. Accordingly, the presence of specialised transport systems was advanced to explain the transfer of materials between the environment and the cell. In these systems a cellular element or carrier is presumed to interact with a substrate at the cell surface and "carry" it as a carrier-complex into the cell where the substrate is "unloaded". The carrier by returning again and again to "carry" more substrate may be considered to act as a catalyst in the same sense as haemoglobin is a catalyst for the transport of oxygen.

The nature of these transport mechanisms is one of the challenges of biology and is under intensive study. But preoccupation with these mechanisms has relegated consideration of the essentially lipoid nature of the boundary between the cell and its environment to the background. Much of the difficulty in applying physico-chemical criteria to the diffusion of drugs through cellular membranes stems from the fact that most agents

of pharmacological interest are weak organic electrolytes. At physiological hydrogen ion concentration these weak acids or bases are present partly in the dissociated and partly in the undissociated form. This complicates the problem of characterising the passage of drugs across membranes since usually only the undissociated molecules are lipid-soluble, and can be expected to penetrate readily. The concentration of the non-ionised drug depends on both the dissociation constant of the organic electrolyte and the pH of the solution in which it is dissolved. Consequently, in order to apply the thesis of Overton to mammalian membranes not only the oil to water partition ratio of the undissociated drug must be known but also the ionisation constant.

The literature contains fragmentary evidence that cellular membranes are penetrated by the uncharged moiety of organic electrolytes, but not by the ionic form. For example, Clowes[4] studied a number of barbiturates and concluded that only the undissociated form permeated Arbacia eggs. But the available experimental evidence is too meagre to provide a discernible pattern of the nature of mammalian membranes especially those determining the passage of drugs from the gastrointestinal tract to the blood stream, and from the blood stream to the brain and to other tissues.

We have become interested in the penetration of drugs through various cellular membranes as an important aspect of drug action, beginning with the premise that their passage is governed mainly by physical processes and is predictable from the dissociation constant and the lipid solubility of their undissociated forms. To what extent the premise of a lipoid membrane explains the gastrointestinal absorption of drugs, their excretion by the kidney and their penetration of the central nervous system will be discussed first.

Gastrointestinal Absorption of Drugs

The medicinal chemist and the pharmacologist have had few signposts to aid in predicting the absorption of a new drug after oral administration. The difficulty has been in part psychological—a feeling that mechanisms of absorption are complicated—and partly experimental, the lack of simple, accurate methods of drug assay. The development in recent years of simple chemical and physical methods of analysis has altered this and the time is now near when the absorption of a drug may be predicted with reasonable accuracy from its physicochemical properties and often merely by perusal of its structure.

Our interest in absorption from the gastrointestinal tract began with the curious observation by Dr. Shore that when levorphanol (Dromoran), a synthetic analgesic, was intravenously administered to dogs it appeared in the gastric juice in a concentration about 40 times that in the plasma. This unexpected observation stimulated us to study whether other drugs of basic reaction would also be concentrated in the gastric juice. Preliminary results soon indicated that we were really studying the characteristics of the membrane separating plasma from the lumen of the stomach. In our experiments, dogs with Heidenhain pouches were infused intravenously with drugs at rates ensuring constant plasma levels, together with

histamine to stimulate the secretion of an acid gastric juice. After one hour, samples of gastric juice and blood were collected and the concentration ratio, R, was calculated by dividing the level of the drug in the gastric juice by that in the plasma.

Only the basic drugs appeared in gastric juice at a higher concentration than in plasma (Table I). The concentration ratio increased with the

TABLE I

DISTRIBUTION OF DRUGS BETWEEN GASTRIC JUICE AND PLASMA OF DOGS

$$R = \frac{\text{Concentration in gastric juice}}{\text{Concentration in plasma}}$$

Drug	pKa	Experimental R	Experimental R (corrected for plasma binding)	Theoretical R
BASES				
Acetanilide	0·3	1·0	1·0	1·0
Theophylline	0·7	1·5	1·3	1·5
Antipyrine	1·4	4·2	4·2	4·2
Aniline	5·0	40		10^{4}
Amidopyrine	5·0	42		10^{4}
Quinine	8·4	38		10^{6}
Dextrorphan	9·2	40		10^{8}
ACIDS				
Salicylic acid	3·0	0	0	10^{-4}
Probenecid	3·4	0	0	10^{-4}
Phenylbutazone	4·4	0	0	10^{-3}
p-Hydroxypropiophenone	7·8	0·13	0·5	0·6
Thiopentone	7·6	0·12	0·5	0·6
Barbitone	7·8	0·6	0·6	0·6

basicity of the drugs until it reached a limiting value of 40 for compounds with a pKa* of 4 or higher. In contrast acidic drugs appeared in the gastric juice in low concentration, and only the very weak acids were found in detectable amounts. The weaker the acid, or the higher its pKa, the higher the concentration ratio; but for none of the acids did it reach a value of one.

Thus, it appears that drugs of widely diverse chemical structure pass from plasma to gastric juice to a degree that is determined by a physical characteristic, the dissociation constant. The higher the proportion of drug present in the plasma as the unionised form, the more it is to be found in the gastric juice. This become comprehensible on the assumption that the barrier between the blood and the gastric juice behaves towards foreign compounds as an oily layer.

Figure 2 illustrates the consequences of a barrier like this separating gastric juice from plasma. A weak base is present in plasma in two

* The term "weak electrolyte" will be applied to those acids and bases which exist, in part, as undissociated molecules at physiological pH. The dissociation constant of both acids and bases, is expressed as a pKa which is the negative logarithm of the acidic dissociation constant. The pKa is defined by the Henderson-Hasselbalch equations:

$$\text{For acids} \quad pKa = pH + \log \frac{\text{Unionised acid}}{\text{Ionised acid}}$$

$$\text{For bases} \quad pKa = pH + \log \frac{\text{Ionised base}}{\text{Unionised base}}$$

An acidic drug with a small pKa is a very strong acid and one with a large pKa a very weak acid. A base with a small pKa is a very weak base.

forms, the ionised and the non-ionised. Only the lipid-soluble undissociated form would pass the boundary, while the penetration by the ionised form would be barred. At equilibrium, the concentration of the undissociated drug will be the same on both sides of the membrane irrespective of the pH. But on each side of the barrier the uncharged moiety will also be in equilibrium with its charged counterpart and consequently the relative concentration of the latter will depend on the pH. For example, aniline (see Fig. 2), a relatively weak base, is present in plasma largely as the undissociated form but in the highly acidic gastric juice the amine is almost entirely in the ionised form. Since the level of the non-ionised moiety is equal on both sides of the lipoid barrier, the total aniline (ionised plus unionised) is much higher in gastric juice than in plasma.

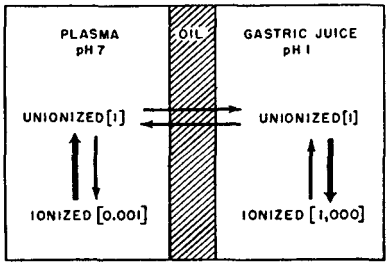


FIG. 2. Partition of an organic base pKa 4 e.g. aniline. The barrier between the blood and the gastric juice behaves towards drugs like an oil layer. Only the lipid-soluble undissociated form passes the boundary.

The equations given in Figure 3 (p. 355) were derived by mathematical transformation of the Henderson-Hasselbalch expression and show the concentration ratios that should theoretically obtain at equilibrium. In these equations R is the theoretical concentration ratio and pKa is the negative logarithm of the acid dissociation constant for each drug.

Theoretical and experimentally determined concentration ratios were in excellent agreement for all the drugs in Table I except for those of the relatively strong bases, that is, those with a pKa greater than 4. These ought to have been present in gastric juice in concentrations as great as a million times those in plasma, but instead they reached a limiting concentration ratio of about 40*. The explanation for this limiting value was found by comparing the concentration of aniline in blood entering and leaving the stomach. So much aniline was removed that it was apparent that virtually all was cleared from the blood in a single passage through the gastric mucosa. Thus, this limiting ratio of 40 for various drugs is no more than an expression of the fact that the gastric juice cannot remove more drug than is delivered to it by the blood reaching the gastric mucosa. And incidentally, we find ourselves with a potentially useful physiological tool, a method of measuring the blood flow through the gastric mucosa.

Thus the general pattern of drug secretion from blood to gastric juice conforms to the concept that the barrier between plasma and gastric juice has the characteristics of a lipoid membrane. As such it allows the

* The high concentration of a basic drug which can appear in the gastric juice even though it is given parenterally should be a matter of some concern to the toxicologist and to the writer of mystery novels.

passage of drugs in their undissociated form while restricting the entry of the dissociated form.

A logical consequence is that acidic drugs, but not basic drugs, should be rapidly absorbed directly from the stomach. This represents a departure from the usual picture of the stomach, viewed as unimportant in drug absorption. While there are isolated reports of the absorption from the stomach of substances like ethanol and acetylsalicylic acid, there has been no comprehensive study of this organ as a site of absorption. With Dr. Lewis Schanker, we examined gastric absorption of drugs in the rat. In these experiments the stomach was ligated at its cardiac and pyloric ends, solutions of various drugs in 0·1N HCl were introduced and the amount of absorption measured after one hour. Ready absorption was demonstrated for most of the acids which were present in the stomach in their undissociated forms (Table II). Exceptions were 5-sulpho-

TABLE II

ABSORPTION OF DRUGS FROM STOMACH OF RATS

Acids	pKa	Absorption per cent	Bases	pKa	Absorption per cent
5-Sulphosalicylic	strong	0	Acetanilide	0·3	36
Phenol red	strong	2	Caffeine	0·8	24
5-Nitrosalicylic	2·3	52	Antipyrine	1·4	14
Salicylic	3·0	61	Aniline	4·6	6
Acetylsalicylic	3·5	35	Amidopyrine	5·0	2
Benzoic	4·2	55	*p*-Toluidine	5·3	0
Thiopentone	7·6	46	Quinine	8·4	0
p-Hydroxypropiophenone	7·8	55	Dextrorphan	9·2	0
Barbitone	7·8	4	Mecamylamine	11·2	0
Quinalbarbitone	7·9	30	Darstine	strong	0
Phenol	9·9	40	Tetraethylammonium	strong	0

1 hour absorption of 1 mg. of drug in 5 ml. of 0·1N HCl.

salicylic acid and phenol red, which are such strong acids that they were present in the acidic medium almost entirely as their lipid-insoluble ionic forms. Another notable exception that proved the rule was barbitone, which though almost unionised in the acid of the stomach, was not well absorbed. Barbitone, however, even in its uncharged form is only poorly lipid soluble and would not be expected to cross a lipoid barrier rapidly. As will be seen later this barbiturate constantly reappears in this review as a poorly lipid-soluble substance which penetrates cell membranes only sluggishly.

As expected, most organic bases were poorly absorbed since usually they are ionised almost completely in acid solution. However, certain drugs like acetanilide, caffeine and antipyrine (phenazone) were absorbed because they are so weakly basic ($<$ pKa 2·5) that they are partially unionised even in 0·1N HCl.

More direct evidence for a barrier that is permeable only to the uncharged form of organic electrolytes was obtained by changing the hydrogen ion concentration of the gastric contents. Making the gastric contents alkaline with sodium bicarbonate, would depress the absorption of an acidic drug since the concentration of the lipid soluble undissociated form would be decreased. Conversely, a basic drug would be absorbed

more readily. This is seen in Table III. It may seem strange that salicylic and nitrosalicylic acid still showed some absorption from bicarbonate solution (about one-quarter of that from 0·1N HCl) since these compounds are virtually completely ionised at pH 8. But irrespective of the large change in the concentration of hydrogen ion and consequently of unionised salicylic acid within the bulk solution, it should not be expected that these changes would be faithfully reflected within the gastric tubules. The continuous secretion of acid into the narrow confines of these tubules would necessarily maintain a more acid environment at the site of absorption, thus permitting the absorption of some salicylic acid.

TABLE III

COMPARISON OF GASTRIC ABSORPTION AT pH 1 AND pH 8 IN THE RAT

	pKa	Absorption at pH 1 per cent	Absorption at pH 8 per cent
ACIDS			
5-Sulphosalicylic ..	strong	0	0
5-Nitrosalicylic ..	2·3	52	16
Salicylic	3·0	61	13
Thiopentone	7·6	46	34
BASES			
Aniline	4·6	6	56
p-Toluidine	5·3	0	47
Quinine	8·4	0	18
Dextrorphan	9·2	0	16

1 hour absorption of 1 mg. of drug in 5 ml. of solution

These results are consistent not only with the concept of the gastric mucosa being selectively permeable to the undissociated form of drugs, as previously suggested by Travel[5], but they indicate that the stomach could have an important role in the absorption of drugs. The important question for the pharmacologist and clinician is the practical significance of this site of absorption in man.

The course of absorption from the stomach in man was followed by administering by stomach tube a solution containing both a drug and phenol red. The dye, since it was not absorbed, permitted the correction both for the disappearance of drug solution into the intestines and for dilution of the drug solution by salivary and gastric secretions. Three scientists volunteered for the experiments summarised in Figure 4 (p. 356). The similarity between the rat and the scientist is inescapable. Three acidic drugs, salicylic acid, acetylsalicylic acid and thiopentone, were absorbed from the stomach more rapidly than was ethanol, while quinalbarbitone penetrated the gastric mucosa less rapidly. The almost neutral base, antipyrine, was absorbed to some extent and there was no detectable absorption of the stronger basic drugs, amidopyrine, quinine, and ephedrine.

Factors other than the intrinsic absorbing ability of the human stomach may influence the proportion of an administered drug absorbed. For example, the bulk contents of the stomach may empty within a few minutes or remain for several hours. In the experiments that we have

just considered about one-half of the 200 ml. of drug solution emptied into the intestine during the 40 minutes of observation. Taken on top of a meal, the contents of the stomach would empty much more slowly. But this factor favouring absorption directly from the stomach would be offset by the larger volume from which the drug would have to be absorbed. Of perhaps more practical importance is the solubility of a drug in acid solution. Many acidic drugs, for instance, dicoumarol, are so insoluble in the acid medium of the stomach that they would not be absorbed by the gastric mucosa. However, a number of drugs may be absorbed from the stomach at a faster rate than ethanol which is described usually as the exceptional drug acting rapidly because of its direct absorption from the gastric lumen[6].

The predictable manner in which the stomach absorbs drugs warrants asking whether intestinal absorption may be simply also a matter of passive diffusion across a mucosa which is selectively permeable to the non-ionised form of the organic electrolyte. Dr. Schanker and Mr. Tocco have employed a simple technique to study quantitatively the nature of the intestinal barrier. The rat intestine was cannulated at the duodenal and ileal ends, replaced in the abdomen and the operative incision closed. Solutions of drugs in isotonic saline were perfused through the intestine at a constant rate, and the relative rate of absorption of each drug was estimated by measuring the difference in the concentration entering and leaving the intestine.

TABLE IV

INTESTINAL ABSORPTION BY THE RAT: EFFECT OF CONCENTRATION

	Initial concentration of perfusion solution mM/l.	Absorption per cent
Salicylic acid	0·5	52
	1·0	58
	10·0	53
Aniline	0·1	58
	1·0	53
	10·0	59

In looking for evidence that drugs might be absorbed by transport mechanisms, the absorption of a typical base and acid were measured at various concentrations (Table IV). The proportion of aniline and salicylic acid absorbed was found to be constant over a wide concentration range. If the absorption of the compounds occurred by a transfer mechanism, there might be evidence of saturation at the high concentrations. That this was not observed suggests, but does not prove, that these drugs are absorbed by a physical process.

The relative rates of absorption of a considerable number of acidic and basic drugs were then determined. Before interpreting the results summarised in Table V, it must be made clear that the experiments were designed to delineate the general characteristics of the boundary between the intestinal lumen and plasma and not to yield definitive criteria for the absorption of drugs as administered therapeutically. The results do indicate that many drugs would be rapidly absorbed, but they do not indicate whether those substances which were absorbed relatively poorly would also be absorbed poorly in the intact animal. In these experiments, the drug solution raced through the intestine in seven minutes, compared with the several hours that a drug might remain in the lumen when used

therapeutically. Consequently, even the most rapidly absorbed compound attained the upper limit of about only 60 per cent absorption. This could be increased by slowing the flow of solution through the gut or decreased by quickening the flow. Some of the compounds such as quinine or ephedrine showed a relatively slow rate of absorption under the conditions of the experiments, but are known to be adequately absorbed in therapeutics.

TABLE V

ABSORPTION OF DRUGS FROM SMALL INTESTINE OF RATS

Acids	pKa	Absorption per cent	Bases	pKa	Absorption per cent
5-Sulphosalicylic	strong	0	Theophylline	0·7	27
Phenol red	strong	0	*p*-Nitroaniline	1·0	67
Bromophenol blue	strong	0	Antipyrine	1·4	32
o-Nitrobenzoic	2·2	0	*m*-Nitroaniline	2·5	76
5-Nitrosalicylic	2·3	6	Aniline	4·6	53
Salicylic	3·0	59	Amidopyrine	5·0	33
m-Nitrobenzoic	3·4	52	*p*-Toluidine	5·3	58
Acetylsalicylic	3·5	18	Quinine	8·4	13
Benzoic	4·2	50	Ephedrine	9·6	4
Phenylbutazone	4·4	64	Tolazoline	10·3	7
Acetic	4·7	40	Mecamylamine	11·4	0
Thiopentone	7·6	54	Darstine	strong	0
p-Hydroxypropiophenone	7·8	59	Procaine amide ethobromide	strong	0
Barbitone	7·8	28	Tetraethylammonium	strong	0
Phenol	9·9	50	Tensilon	strong	0

Per cent change of concentration of 1 mM of drug in saline perfused at 1·5 ml./minute through the entire small intestine.
Zero absorption indictes that absorption is too slow to be measured by this method.

A relation between the dissociation constant and the degree of absorption of drugs is evident from the data in Table V. Most of the drugs were readily absorbed except for the stronger acids and bases. A drastic reduction in the extent of absorption occurred for acids with a pKa 2·5 and for bases with a pKa 8·5. This suggests that the intestinal mucosa, like the gastric mucosa, is preferentially permeable to the undissociated form of drugs. This conclusion was buttressed by the rates of absorption of representative drugs from solutions of different pH. If the concept is valid, the penetration of weak electrolytes should be favoured by increasing the concentration of the undissociated form. Accordingly, when the perfusion solution was made more acid the absorption of acids was favoured, while that of bases decreased; if made more alkaline the opposite changes were observed (Table VI).

TABLE VI

COMPARISON OF INTESTINAL ABSORPTION IN THE RAT AT SEVERAL pH VALUES

	pKa	Absorption per cent at pH 4	pH 5	pH 7	pH 8
ACIDS					
5-Nitrosalicylic	2·3	40	27	0	0
Salicylic	3·0	64	35	30	10
Acetylsalicylic	3·5	41	27	—	—
Benzoic	4·2	62	36	35	5
BASES					
Aniline	4·6	40	48	58	61
Amidopyrine	5·0	21	35	48	52
p-Toluidine	5·3	30	42	65	64
Quinine	8·4	9	11	41	54

One point that still remained to be settled was the pH at the actual absorbing surface, since this would determine the proportion of undissociated drug and therefore the rate of absorption. Let us consider the distribution of a base, aniline between plasma and an intestinal fluid

separated by an oil layer as shown in Figure 5 (p. 356). The picture will be the same as for the stomach (Fig. 2), except that the acidity in the intestine will be less than that in gastric juice. At equilibrium, the concentration of undissociated drug will be the same on both sides of the boundary irrespective of the pH. But on both sides, the concentration of undissociated molecules will also be in equilibrium with the ionised molecules, and the relative concentration of the ionic species will depend on the pH. The concentration ratio intestinal level : plasma level will depend on the pH of the absorptive surface. This pH can be calculated from the observed equilibrium ratio by using the equation similar to that given in Figure 3.

The equilibrium concentration for aniline was found by injecting the drug intravenously while simultaneously perfusing it through the intestine at a concentration ensuring that there would be no net absorption of the drug. The equilibrium concentration ratio of aniline was found to be 1·2, a ratio that would be expected if the mucosa were selectively permeable to the unionised drug and if the intestinal lumen had a pH of about 5·3.

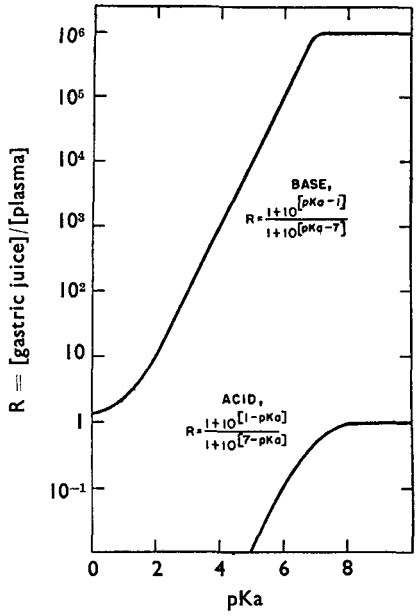


FIG. 3. Theoretical partition ratios of organic electrolytes between gastric juice and plasma.

This calculated pH which we shall speak of as a "virtual" pH was lower than the pH 6·5 of the saline leaving the intestine. The equilibrium distribution of several other drugs also gave a "virtual" pH that was lower than that of the saline. While not yet proven by direct experiment, the low pH of the absorptive surface is qualitatively consistent with the demonstrated ability of the intestinal mucosa to establish an acid pH[7].

A pH of 5·3 at the absorbing surface would explain why the lowest pKa of an acidic drug compatible with rapid absorption is 3 (Table V) while the corresponding pKa for bases is about 8. Given a "virtual" pH of 5·3 the proportion of unionised to ionised drug necessary for rapid absorption is 1 : 300 for an acid of pKa 2·8 and a base of pKa 7·8. On the other hand, if the pH is accepted as 6·5 it can be calculated that the necessary proportion of non-ionised to ionised molecules is 1 : 5000 for acids and 1 : 15 for bases—an improbable circumstance if the lipoid boundary concept is valid.

Whatever the precise nature of the absorption of foreign compounds from the intestine, it is possible to make a number of tentative predictions. Organic electrolytes should be readily absorbed from solution if the undissociated form has a favourable oil to water partition ratio, and if the pKa for an acid is greater than 2, and for a base is less than 10. The poor absorption of certain water-soluble drugs formerly considered somewhat of a mystery now becomes logical because they are completely ionised in the gut or because their uncharged forms are not lipid-soluble. For example, quaternary compounds, streptomycin and sulphaguanidine, are poorly absorbed because of their virtually complete ionisation at all pH's. This property is exploited in the use of sulphaguanidine for bacillary dysentery, but limits the value of orally administered quaternary amines as ganglion-blocking agents. Succinylsulphathiazole, also used for intestinal infections, is poorly absorbed presumably because even its uncharged form is too lipid-insoluble for rapid absorption. Similarly very lipid-insoluble non-electrolytes like xylose are poorly absorbed.

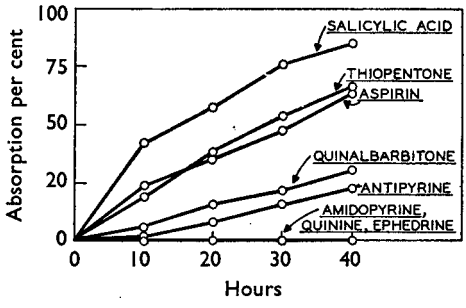


FIG. 4. Relative rates of drug absorption from the human stomach. Rate of absorption of ethanol falls between that of aspirin and quinalbarbitone.

Factors other than the dissociation contstant of a drug may limit its gastrointestinal absorption. Some drugs are poorly absorbed because they are unstable in the gastrointestinal tract; for instance, dibenamine and its congeners would be much more useful agents except for their chemical interaction with material in the gut. It is difficult to control therapy with these agents since the fraction of a given dose that is changed in the gut is quite variable.

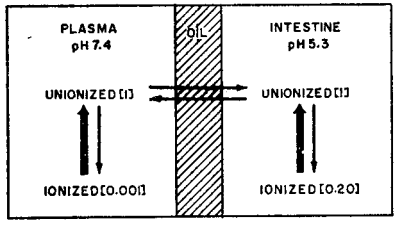


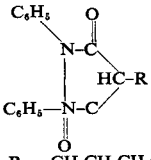
FIG. 5. The distribution of aniline, pKa 4·6, between plasma and intestinal fluid separated by an oil layer.

A most important factor in limiting drug absorption appears to be the absolute solubility at the pH of the gut. Thus, one of the difficulties in the therapeutic use of dicoumarol lies in its extremely slow solubility at pH 7, which results in slow and erratic absorption. The importance of solubility in limiting the rate of drug absorption was brought to our attention in a rather embarrassing as well as expensive manner. Dr. Burns, who is interested in the development of non-steroidal antirheumatic agents, screened a number of analogues of phenylbutazone.

The compounds when given parenterally to rats were highly active in protecting against formaldehyde induced oedema and we looked forward to finding among this group of drugs an orally active compound for the treatment of rheumatoid arthritis. To our disappointment, the drugs were virtually devoid of action when given by mouth to man. Poor absorption was evident from plasma levels which were very low in spite of the relatively slow biotransformation of the drugs when given intravenously.

TABLE VII

COMPARISON OF SOLUBILITIES OF PHENYLBUTAZONE ANALOGUES AT pH 7·0 WITH THEIR ORAL ABSORPTION

Compound	Structure	Solubility mg./ml. at pH 7·0	Absorption after oral administration to man
			
Phenylbutazone ..	R = $CH_2CH_2CH_2CH_3$	2·2	Rapid and complete
Metabolite 1* ..	R = $CH_2CH_2CH_2CH_3$	10	"
G-1	R = $CH_2CH_2SC_6H_5$	1·6	"
G-3	R = $CH_2CH_2CH_2C_6H_5$	0·14	Slow and incomplete
G-8	R = $CH_2CH_2SC_6H_3op(CH_3)_2$ ..	0·13	"
G-20*	R = $CH_2CH_2CH_2CH_3$	0·09	"
G-21*	R = $CH_2CH_2CH_2CH_3$	0·12	"
G-23	R = $C(=O)-CH_2CH_2CH_3$	0·12	"

* Metabolite 1 is *p*-hydroxyphenylbutazone.
G-20 is *pp*-dichlorophenylbutazone.
G-21 is *pp*-dimethylphenylbutazone.

In Table VII is shown the relative solubility of a number of phenylbutazone analogues in aqueous solution at pH 7·0. The importance of solubility is evident. Compounds with solubilities greater than 1·5 mg./ml. were well absorbed, while those soluble to the extent of only about 0·1 mg./ml. were poorly absorbed. These results show not only how important the absolute solubility of drugs can be in defining the rate of absorption, but that for a given series of compounds, a simple solubility test might be helpful in a screening programme to indicate whether a compound is soluble enough for oral administration.

It is traditional to assume that drugs given intramuscularly are rapidly absorbed. But this is not so with at least one drug. Phenylbutazone is absorbed much more slowly on intramuscular injection than after oral administration, because of extensive tissue binding or precipitation at the site of injection (Fig. 6). Yet, a number of authors have claimed that phenylbutazone given intramuscularly several times weekly has a more rapid and certain action than when given orally. There would seem to be little justification for not giving phenylbutazone orally, when possible.

Penetration of Drugs into Tissue Cells

After a drug reaches the blood stream, it must cross other cell boundaries before it gains access to its site of action. The capillary wall offers little or no hindrance to the passage of substances and though the boundary has some lipoid characteristics[8], it is sufficiently porous to permit the ready penetration of water-soluble substances of relatively large size. Even albumin molecules can escape from capillaries to some extent.

The cells of organ tissues present a real barrier to the passage of foreign substances. Lipid-insoluble substances like sucrose do not penetrate tissue cells, and special mechanisms are needed to transport glucose, and also to maintain potassium at a high concentration.

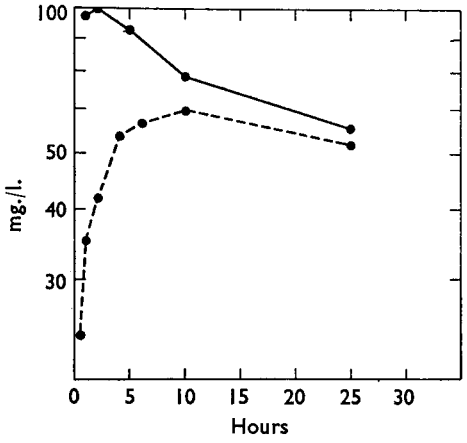


Fig. 6. Comparison in man of the plasma levels of phenylbutazone after oral and intramuscular administration.

●—● 800 mg. oral; ● - - ● 800 mg. i.m.

Preliminary studies in this laboratory indicate that the boundaries of tissue cells have the characteristics of a lipoid membrane. Highly lipid-soluble compounds penetrate tissue cells more rapidly than lipid-insoluble substances, but drugs having relatively slight lipid solubility still penetrate tissue cells fairly rapidly because of the large interfacial area in contact with extracellular fluid. The essentially lipoid character of the tissue cell membrane is emphasised by the distribution of a strong organic base like hexamethonium, a compound which, completely ionised at physiological pH, does not enter tissue cells[9].

Of late, it is becoming apparent that there may be barriers to organic compounds within the cell itself. The cell contents cannot be regarded as a disorganised pool of enzymes and substrates. Rather the interior of the cell is architecturally a structure of great complexity, whose real form is still unknown; usually studied are the ruins—the nuclei, mitochondria, microsomes and cytoplasm, left over when the cell is demolished by our homogenisation procedures.

An intracellular barrier may explain how it is possible for a neurohumoral agent to be stored in tissues in the presence of its highly active inactivating enzyme. For example, serotonin appears to be present in brain in a "bound" form, that is, in a form that is protected from the enzyme, monoamine oxidase. Dr. Shore in some recent work has evidence that serotonin may not be physically bound, but instead may be maintained as the free form in considerable amounts in spite of the

presence of the enzyme that destroys it. The results of others suggest that histamine and acetylcholine may also be present in the cell in a free form[10,11].

An apparent intracellular barrier to a drug can be demonstrated with iproniazid, a potent inhibitor of monoamine oxidase. In the intact animal, while the drug enters various body cells, it is relatively ineffective in blocking monoamine oxidase and in protecting administered serotonin from metabolic transformation. In contrast, when iproniazid is added to homogenised tissues inhibition of monoamine oxidase is complete, indicating that there are compartments within the cell which isolate an enzyme system from ready contact with a drug. It is possible that other "inactive" drugs actually penetrate cells that contain the receptor site, but do not reach their objective.

On the Nature of the Blood-brain Barrier

At first glance the brain seems to be in a class by itself where the penetration of drugs is concerned. Many therapeutic agents, while penetrating other organs with ease, pass into the central nervous system slowly or hardly at all. This presents a practical problem to the medicinal chemist and to the pharmacologist, who would like to anticipate which chemical structures are likely to enter the brain.

The boundary between the brain and the blood is generally called the blood-brain barrier; but it should be recognised that the barrier does not lie between brain cells and the fluid surrounding them, since drugs are known to penetrate the cells of brain as readily as those of any other tissue. Rather the obstacle seems to lie between plasma and the extracellular fluid of the central nervous system. In this article we shall take the attitude that cerebrospinal fluid and the extracellular fluid of the central nervous system constitute to all intents and purposes a continuum, a hypothesis for which there exists considerable evidence[12].

Two ways are used to describe the penetration of substances into the central nervous system. The first compares the brain to plasma*, or CSF to plasma concentration ratios, after diffusion equilibrium between blood and nervous tissue has been established. The value of these ratios is limited since they do not indicate the actual rate at which the agent crosses the barrier and for many kinds of drug action, such as, for example, in anaesthesia, rate is a factor of the greatest practical importance. Thus, barbitone and thiobarbitone achieve the same brain to plasma ratios, but the latter compound does so much faster than the former.

The second method of expressing the passage of drugs into the central nervous system compares the time required for the CSF to plasma* concentration ratios to become unity. Many therapeutic agents ultimately reach the same concentration in cerebrospinal fluid as in plasma, but may take vastly different periods of time to do so. Still other drugs pass into the cerebrospinal fluid, but regardless of time intervals fail to reach a concentration equal to that in plasma or may even fail to enter at all.

* Plasma concentrations corrected for protein binding.

The nature of the blood-brain barrier has been the subject of innumerable studies with perhaps a tendency on the whole to overcomplicate the picture. There is little doubt that a real barrier exists and that special mechanisms are required to transport inorganic electrolytes and numerous endogenous substrates. But for foreign compounds there may be no need to consider that this barrier is essentially different from that which separates plasma from the stomach or from the intestines, that is a lipoid membrane which drugs will cross or not, according to the extent of their dissociation and the lipid solubility of the undissociated molecules.

A number of isolated reports hint at the lipoid nature of the blood-brain barrier*. Water-soluble organic compounds like sucrose penetrate the central nervous system very slowly while lipid-soluble substances penetrate it rapidly; and it is well recognised that highly ionised bases like tetraethylammonium and tubocurarine penetrate the central nervous system with great difficulty. But there have not been enough quantitative data to lead to an overall formulation of the behaviour of the blood-brain barrier toward foreign compounds.

TABLE VIII

CORRELATION OF PENETRATION OF DRUGS INTO CEREBROSPINAL FLUID OF RABBITS AND CHLOROFORM TO WATER PARTITION RATIO OF THE DRUGS

Compound	pKa	Partition ratio at pH 7·4	Time to attain C.S.F. to plasma ratio of 1 (minutes)
Thiopentone ..	7·6	102	>2
Amidopyrine ..	5·0	73	>2
Antipyrine ..	1·4	28	>2
Aniline	4·6	19	>2
4-Aminoantipyrine	4·1	15	2
Barbitone	7·6	4·8	40
Acetanilide ..	0·3	3·7	120
N-Acetyl-4-amino-antipyrine ..	0·5	1·4	>180
Salicylic acid ..	3·0	0·02	>360

The concentration ratio of the *N*-acetyl-4-aminoantipyrine and salicylate reached only 0·6 by 3 and 6 hours respectively.

Dr. Mayer and Mr. Maickel have correlated the rates of penetration of a number of drugs into the central nervous system of rabbits, and the chloroform to water partition ratios of the drugs at pH 7·4. As seen in Table VIII, compounds may be listed in the order of descending partition ratios: drugs like thiopentone which reached a CSF to plasma ratio of one within two minutes; compounds like acetanilide which took considerably longer to achieve a CSF to plasma ratio of one; and substances such as *N*-acetyl-4-aminoantipyrine and salicylic acid that did not achieve a CSF: plasma ratio of one within three to six hours. Quaternary ammonium compounds like tetraethylammonium, not included in Table VIII, barely enter the central nervous system at all.

These results would make it appear that the rate of penetration of drugs into the central nervous system is dependent on the lipid solubility of the uncharged molecule and that rapid penetration is assured provided there is a sufficient proportion of the lipid-soluble uncharged moiety in

* Since nerve tissue contains lipids it is often stated that drugs gain access to the central nervous system by virtue of their solubility in brain lipids. There is no indication that lipid-soluble drugs have any predeliction for brain tissue, suggesting that the structural lipids do not have the solubility characteristics of neutral fat. It is presumably the lipid characteristics of the blood-brain barrier and not of the individual tissue cells which regulate the entrance of drugs into the brain.

plasma. Turning again to Table VIII, the compounds that rapidly penetrated the central nervous system were present in plasma largely or in part as the lipid-soluble uncharged molecules. Barbitone is undissociated to about the same extent as thiopentone, but its undissociated species is much less lipid-soluble, hence its slower penetration into the central nervous system. *N*-Acetyl-4-amino-antipyrine is almost entirely undissociated in plasma, but even in this form is poorly lipid-soluble and therefore it did not penetrate rapidly. Sodium salicylate slowly passed the barrier because of its high degree of ionisation at pH 7·4.

The same general rules that applied to the entrance of drugs into the central nervous system seemed to apply to their exit as well. Those drugs like thiopentone which entered the central nervous system rapidly also left it rapidly when injected intrathecally. In contrast, drugs which slowly entered the central nervous system left it slowly.

TABLE IX

UNIFORM DISTRIBUTION OF TWO DRUGS IN VARIOUS PARTS OF THE RABBIT BRAIN 10 MINUTES AFTER INTRAVENOUS ADMINISTRATION OF 50 MG./KG.

Brain area	Amido pyrine mg./kg.	*N*-Acetyl-4-amino-antipyrine mg./kg.
Left cortex	23	20
Right cortex	23	18
Cerebellum	22	18
Medulla	22	18
Brain stem	21	18
Cerebrospinal fluid ..	25	18
Plasma	22	42

While considerably more data must be accumulated to establish firmly the concept that the blood-brain barrier is a lipoid membrane to foreign compounds, one factor seems clear even with the limited information at hand: to ensure the ready penetration of the central nervous system by drugs, the medicinal chemist should synthesise compounds with a high oil to water partition coefficient.

The question arises about the locus of the barrier. The highly lipid-soluble amidopyrine penetrated the brain so rapidly that it must have entered from blood capillaries in all parts of the brain. Indeed, the concentration of amidopyrine was uniform in various parts of the brain at any given time from two minutes to one hour after administration and *N*-acetyl-4-aminoantipyrine which entered the central nervous system more slowly, was also evenly distributed throughout the brain after ten minutes (Table IX). This means that the barrier exists throughout the central nervous system. Since highly ionised compounds like quaternary amines are almost completely absent from extracellular fluid as well as from the brain cells, the barrier must be at the brain capillaries or some structure close to the capillaries.

The importance of lipid solubility in barbiturate anaesthesia is emphasised by some investigations of Drs. Burns and Mark. Thiopentone which has a high-partition ratio between oil and water penetrated the central nervous system of dogs so rapidly that after intravenous injection it appeared in maximal concentration in brain within one minute, that is, within one or two circulation times. Its passage into brain was so rapid that it must be unhindered by the blood-brain barrier and the rate was presumably limited only by the rate of cerebral blood flow. Similarly,

the decline in plasma concentrations of this barbiturate was mirrored closely by the decline in the cerebrospinal fluid and brain levels (Fig. 7). This rapid establishment of diffusion equilbrium between blood and brain has important clinical implications as it is essential to the precise control of the depth of anaesthesia.

A comparison of the pharmacological effects of barbitone and thiobarbitone is particularly interesting since these barbiturates have a marked difference in the lipid solubility of their uncharged molecules. When thiobarbitone was given to a dog in a dose of 100 mg./kg. intravenously, the drug rapidly penetrated the brain and the animals rapidly lost consciousness. When the same dose of the poorly lipid-soluble oxygen analogue was given, the animals became progressively more depressed, but did not lose consciousness for almost one hour. Analysis of the brain showed that it required one hour for the same concentration of barbitone to appear in brain as appeared in a few minutes after thiobarbitone administration.

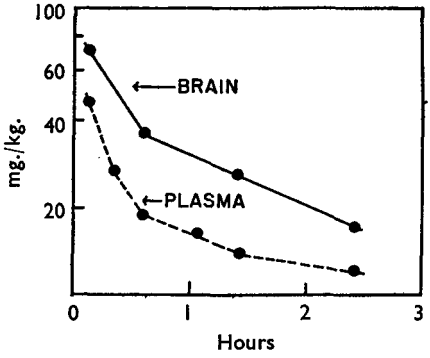


FIG. 7. Thiopentone levels in a dog after intravenous injection of 40 mg./kg.

Serotonin is a good example of a normally-occurring weak electrolyte that crosses into the brain with difficulty because of the low fat solubility of its neutral moiety. There has been considerable confusion in attempts to learn about the central actions of serotonin from its parenteral administration, since it crosses the blood-brain barrier in measurable amounts only after the administration of huge doses[13]. The current interest in this indole has created a flurry of interest in serotonin analogues with possible central activity. Of the not inconsiderable number that have been synthesised in the past few years, it is doubtful if many could possibly have passed the blood-brain barrier in significant amounts, in view of their low lipid solubility.

There is another membrane in connection with the brain which should be considered in drug action, that separating the ventricles from the brain itself. Few, if any studies of the characteristics of this boundary have been made. It is usual to assume that putting a drug into the cerebral ventricles is tantamount to putting it into brain tissue, but this has not been proved analytically. It is probable that if a substance is lipid-soluble it will easily pass the boundary separating the ventricle from the brain, but this may not be so for lipid-insoluble substances. Consequently, the physiological effects obtained from intraventricular injections of lipid-insoluble neurohumoral agents such as acetylcholine and noradrenaline must be interpreted with caution until the extent of their penetration into brain is established.

Lipid Solubility of Drugs as a Limiting Factor in Urinary Excretion

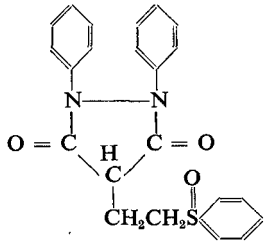
The action of a drug has been generally considered to be limited by the combined effects of urinary excretion and enzymatic inactivation. In recent years it has become increasingly evident that disposal of drugs by the kidney is of relatively minor importance in limiting their duration of action. Most drugs must undergo chemical modification before they can be excreted in more than minor amounts and why this is so becomes clear when the anatomy of the kidney is considered.

Plasma is filtered through the glomerulus, a membrane which like that of the capillary wall permits the passage of practically all solutes. The filtrate flows down tubules lined with epithelial cells, the walls of which may be considered to form a continuous membrane with lipoid characteristics. Normally occurring substances in the body are generally not lipid-soluble and therefore are not reabsorbed through the tubular wall unless they are small enough to pass through its pores or become involved in its transport mechanisms. Drugs, on the other hand, are usually weak organic electrolytes and will be passively reabsorbed as the lipid-soluble non-ionised moiety. Tubular reabsorption is virtually complete for most drugs. These drugs, trapped by their lipid solubility would be fated to wander about throughout the body fluids if the organism did not have ways of allowing them to escape into the urine as less-lipid-soluble derivatives.

There is considerable evidence for the concept that the tubular epithelium is permeable only to the undissociated molecules of weak organic electrolytes. If the absorption of weak electrolytes through the tubular membrane is dependent on the passive diffusion of undissociated molecules, it would be anticipated that the amount of compound excreted would be markedly affected by the pH of the tubular contents. Orloff and Berliner[14] concluded that a change in tubular pH influenced the excretion of various weak organic electrolytes. They showed that the administration of sodium bicarbonate decreased the urinary excretion of the weakly basic drugs presumably as a result of the increase in concentration of the unionised molecules in the more alkaline tubular lumen. Conversely, lowering the pH with ammonium chloride increased the concentration of the charged form of bases and accordingly increased their excretion. The excretion of organic weak acids has also been found to be affected by changes in pH, but in the opposite direction, that is, a decrease in the pH of the tubules decreased the excretion of acidic drugs[15]. A strong electrolyte, such as hexamethonium[16], is rapidly excreted at any pH without undergoing tubular reabsorption, as it is present only in ionic form.

There are notable exceptions to this simple pattern. Certain of the relatively stronger bases such as tolazoline, tetraethylammonium and methylnicotinamide are eliminated not only by glomerular filtration but are secreted in considerable amounts by special tubular mechanisms. In addition, a different mechanism transfers a number of the stronger acids, for instance phenol red, penicillin and *p*-aminohippuric acid.

These compounds seem to become enmeshed in transport mechanisms which are utilised for the rapid disposal of certain normally occurring, but as yet unknown, substances. Of considerable pharmacological interest is the observation that a number of compounds, also relatively strong acids, block the tubular secretory mechanism for acids. The most effective of these which is used clinically to depress the tubular secretion of penicillin has been probenecid. Dr. Burns has shown that a series of phenylbutazone analogues are considerably more effective, with one of the best being the sulphoxide shown.



Curiously enough, substances that reduce the secretion of penicillin, *p*-aminohippuric acid and phenol red also reduce the reabsorption of uric acid and are therefore uricosuric agents. This sulphoxide seems to be the most potent uricosuric agent available[17].

Conversion of Foreign Compounds to Polar Derivatives

The difficulty of excreting lipid-soluble compounds presents the so-called "detoxication" mechanisms in a different light; they may better be regarded as mechanisms for the transformation of lipid-soluble foreign substances into more polar derivatives which are readily excretable. The term "detoxication" has proved a useful one since drugs are generally metabolised to compounds with less pharmacological activity and less toxicity than the parent compound. As a logical description of a body function, however, the term leaves something to be desired since it implies that enzyme systems can make intelligent decisions. A new synthetic drug never before "seen" by a living organism is introduced into the body and undergoes "detoxication". Are we to consider that in the emergency the biochemical clans gather hurriedly together, form an *ad hoc* council of war and decide whether the intruder is undesirable, to be inactivated by concerted chemical assault? Occasionally a metabolic product is more toxic than the parent compound—have the enzymes erred and made the wrong decision?

It is worth considering the possibility that a number of unusually nonspecific enzyme systems have been developed to enable the body to excrete lipid-soluble foreign compounds by converting them to more polar derivatives. Generally, the resulting metabolic products would be less toxic than the parent compounds for at least two reasons—they are excreted more rapidly and their decreased lipid solubility prevents them from passing cellular barriers and reaching a potential site of action.

It may be argued that in the process of evolution, animals developed mechanisms which metabolize foreign compounds in response to a need to defend themselves against these materials. These substances which are ingested with food would accumulate and upset normal body functions unless some way were achieved of increasing their urinary excretion by decreasing their lipid solubility. According to this concept a drug is

the paradigm of a class of foreign compounds to which the organism has been exposed over the ages.

Dr. Gaudette[18] has shown that lower orders of animals, such as fish and amphibia, have liver microsomes which unlike the microsomes of mammals cannot oxidise the profusion of foreign substances which mammals so successfully oxidise. Accordingly, these aquatic animals cannot oxidatively demethylate amidopyrine, hydroxylate antipyrine, oxidise barbiturates, or split the ether linkage of phenacetin; but there is no need for them to do so for these lipid-soluble foreign substances permeate the lipoidal membrane of the gills or of the skin and so are excreted unchanged. It is probable that these oxidative disposal systems had to be developed before emancipation of life from the sea was possible. Before they could become land-bound, animals had to be able to conserve water and so perforce had to give up the damp semi-permeable skin of frogs and salamanders for the scaly skin of the reptile; but in so doing, another way of disposing of non-polar foreign compounds had to be developed. And so with reptiles the problem was solved by metabolising foreign compounds to derivatives that are more polar and therefore readily excretable in the urine. Reptilian microsomes contain enzymes that can oxidise foreign compounds in the presence of reduced triphosphopyridine nucleotide and oxygen. Enzyme systems with the same requirements are also present in the liver microsomes of birds, marsupials and other mammals which presumably inherited them from their ancestral reptiles.

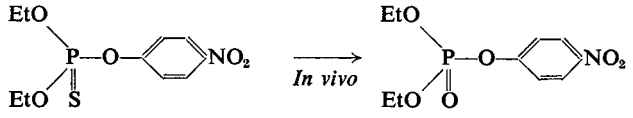
The non-selectivity of the biochemical mechanisms for the oxidation of foreign compounds reveals an ancillary problem. How are normally-ocurring substrates protected from destruction by the action of the non-selective enzymatic scavengers? Nature appears to have solved this problem. Normal substrates with chemical structures similar to those of foreign compounds somehow manage to remain unmolested by drug enzymes that have an extraordinary lack of specificity. A study of the metabolism of a variety of foreign substances has shown that only those that have a high oil to water partition ratio are metabolised by microsomes *in vitro*. This suggests that the microsomal enzyme systems are protected by some sort of a lipoid barrier which only fat-soluble substances can penetrate. A protective boundary would explain why the microsomal enzymes do not catalyse the hydroxylation of L-phenylalanine, L-tryptophane, kynurenine, anthranilic acid, and phenylacetic acid[19], all of which are lipid-insoluble and are hydroxylated by quite specific enzyme systems in other parts of the liver cell. Similarly, sarcosine and dimethylglycine are not demethylated by liver microsomes but are dealkylated by other enzyme systems[20].

Pharmacologically Active "Detoxication" Products

Although drug metabolites are usually less active or less toxic than the parent compounds, there are a number of important exceptions. In spite of its decreased lipid solubility, a biotransformation product may be more active than the administered compound or may exert an action

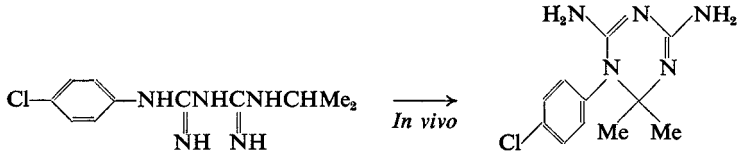
that the parent compound does not. The acquisition of a new kind of pharmacological activity is most likely to occur when modification of the parent drug unmasks or produces a radically different functional group. One of the most interesting examples of unmasking a chemical grouping arose from studies with prontosil. This compound, active against micro-organisms *in vivo* has no activity *in vitro*. A group of workers in Fourneau's laboratory at the Pasteur Institute demonstrated that prontosil is metabolised in the body to yield sulphanilamide, the active antibacterial substance[21]. Reductive splitting of the azo linkage unmasks the *p*-amino group, so important for true sulphonamide activity. From this discovery sprang the extraordinary spectrum of sulphonamides we know to-day.

Examples can be cited where biotransformation creates a functional group which changes an inactive substance to an active one. The insecticide parathion is relatively inactive *per se*, but is changed *in vivo* to a powerful anticholinesterase inhibitor by conversion of P=S to P=O[22]:



Parathion Paraoxon

The antimalarial proguanil has been shown to have no action *in vitro* on certain malarial parasites, but is transformed in the body[23] to an active cyclic derivative as follows:



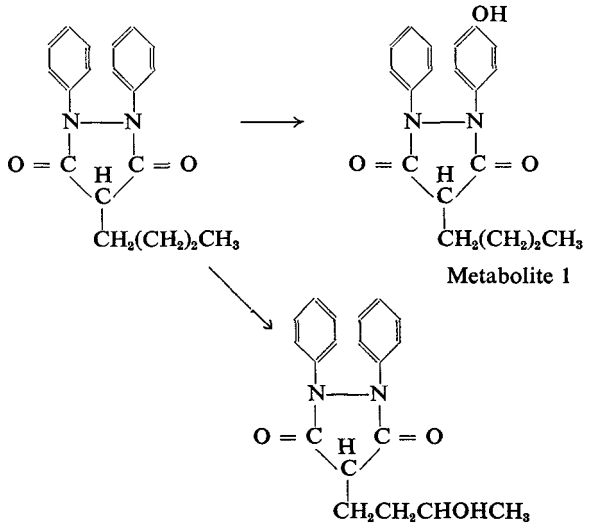
Proguanil Metabolic product

The drug G-25671, which is a mild antirheumatic agent, is oxidixed in the body to form the sulphoxide derivative[17] (p. 364). This metabolite has no antirheumatic activity, but appears to be a potent uricosuric agent. Finally, chloral hydrate is rapidly reduced in the body to trichlorethanol[24] $CCl_3CHO \longrightarrow CCl_3CH_2OH$. The hypnotic effects of chloral hydrate are attributable in large part to the alcohol.

A number of drug metabolites retain the same type of activity as the parent drug when they differ from it only very slightly in lipid solubility. Typical examples lie among the methyl-, or ethylamines, many of which are dealkylated to primary amines which are quite active. For example, ephedrine, amidopyrine, methamphetamine, and methylphenobarbitone all yield active metabolites that exert the same sort of pharmacological activity as the parent compound. Ephedrine in fact is so rapidly dealkylated in the dog to the nor-compound that its actions may be considered to be due to the metabolite[25]. Similarly certain alkyl ethers yield

active products. For instance, phenacetin is so rapidly dealkylated in man to yield an active analgesic *N*-acetyl-*p*-aminophenol that it is difficult to determine levels of the parent compound in the plasma[26].

Sometimes the addition of a phenolic hydroxyl group produces relatively little change in activity. For example, phenylbutazone, a non-steroidal antirheumatic agent, undergoes an interesting biotransformation in man as follows:



Metabolite 1 is formed by the introduction of a phenolic group in the *para* position of a benzene ring; metabolite 2 by the introduction of an alcohol group in the butyl side chain. The phenolic metabolite is an extremely active antirheumatic agent, possibly as active as the parent compound although unfortunately it also causes retention of sodium. The alcohol metabolite has little if any antirheumatic effect, but exerts a pronounced uricosuric action. In fact, the uricosuric action of phenylbutazone may be mediated entirely through this substance[27].

Storage of Drugs in Body Depots

After a drug permeates the cell it does not ordinarily remain at the same concentration on both sides of the cellular membrane, but forms a reversible attachment to one or more of the intracellular components. At one time there was a belief that demonstration of a high concentration of drug in a particular locale might pinpoint the dominant receptor responsible for the action of the drug. Since many drugs including barbiturates, reserpine and trimethadione have been found to be distributed almost equally in all parts of the brain this simplification is not necessarily true. In fact, it is doubtful whether the cell constituent responsible for the action of a drug will be found by gross measurements of drug distribution. The great bulk of drug molecules enter into secondary combinations

with a number of kinds of cell constituents, combinations that have nothing to do with the primary action of the drug.

Without these secondary bindings it is doubtful whether many drugs would remain in the body long enough to exert pharmacological effects. Most drugs with a long duration of action are characterised by extensive and reversible tissue localisation which acts as a brake on metabolic transformation and urinary excretion. These tissue depots provide reservoirs of drug which serve to damp otherwise rapid fluctuations of plasma level.

It is important that we learn something of the nature of the various body depots, and the physico-chemical laws that govern the interaction with drugs. By so doing we shall be in a better position to design drugs which have a desirable duration of action. Also important is a knowledge of the pattern of distribution of drugs before they are tried clinically. The physiological distribution may define the pharmacological response so sharply that unless the information is available a drug may not be used to the best advantage and may even be mistakenly discarded.

On the Importance of Fat in Drug Action

One way in which a drug may be stored in the body is by physical solution in neutral fat. Fat as a drug storage depot can be quite important as even the lean and muscular athlete embodies about 20 per cent of neutral fat and some hearty eaters consist of about 50 per cent of triglyceride. Even in starvation, neutral fat does not fall much below a seemingly irreducible minimum of 10 per cent. With so much fat in the body, most of a drug which has a high fat to plasma partition ratio may finally arrive there. The consequences of this may be seen in the behaviour of the well-known intravenous anaesthetic thiopentone.

When "very-short-acting" barbiturates, such as thiopentone, first became available, their fleeting action was naturally attributed to rapid metabolic degradation. This logical assumption did not entirely explain their behaviour. It did not explain the cumulative effect observed in animals and the persistent somnolence seen in some persons after receiving large doses. This was ascribed by some to a saturation of the biochemical mechanism which inactivated the drug and by others to the formation and accumulation of a longer acting transformation product.

Study of the disappearance of thiopentone from plasma disclosed an obvious reason for the very short action[28]. After a single intravenous administration of 0·4 g. of the barbiturate to man, the plasma levels fell abruptly and subjects awoke in about ten minutes. Subsequently, after the patients had awakened, thiopentone did not disappear rapidly from plasma. The relatively slow disappearance of only 10 to 15 per cent per hour (Figure 8) which followed the rapid initial disappearance must reflect the true rate of metabolism as it is hardly likely that the rate of metabolism should decline as the plasma level fell.

Clearly the transient action of thiopentone should be traced to its rapid early disappearance. Analysis of organs did not reveal any unusual localisation and in fact the total thiopentone in these tissues after three

hours accounted for only 20 per cent of the drug. Finally, body fat was analyzed and surprisingly an enormous concentration of drug was found in this tissue. About 70 per cent of the anaesthetic agent remaining in the body after 3 hours was localised in fat.

The movement of thiopentone between tissues of the dog is illustrated in Figure 9. Most tissues, including brain and cerebrospinal fluid, rapidly acquired a high concentration of thiopentone which then declined progressively, being parallel to the plasma concentration. In contrast, the level in fat at first low, rose rapidly and approached a peak in about 3 hours when it was ten times higher than in plasma.

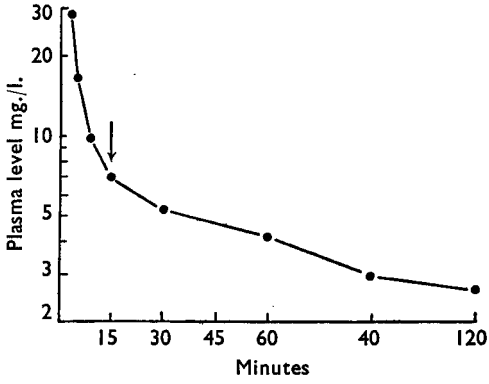


FIG. 8. Plasma levels of thiopentone in man after intravenous injection of 0·4 g.

The partition of thiopentone between the two phases was demonstrated by changing the pH of the aqueous phase. Thiopentone has a dissociation constant (pKa 7·6) in such a range that its degree of ionisation is markedly influenced by a small change in the pH of plasma. Accordingly, when the pH of the plasma was lowered in dogs to 6·8 by the inhalation of carbon dioxide, the proportion of the unionised form was markedly increased. As a result the concentration of drug in plasma fell by about 40 per cent, owing to the fat-solubility of the unionised form. Stopping the CO_2 inhalation brought the pH back to normal and the plasma level rose.

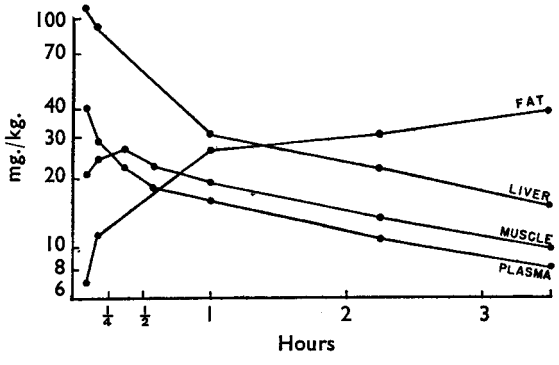


FIG. 9. Thiopentone levels in various tissues after intravenous administration of 25 mg./kg. to a dog.

During the inhalation of carbon dioxide there was no sign of lightened anaesthesia though the thiopentone levels in some animals were near to or below the anaesthetic level. Though the total barbiturate level in plasma and presumably in cerebrospinal fluid declined 40 per cent, it can be calculated that at pH 6·8 the barbiturate is almost entirely in the

undissociated form and that the concentration of the undissociated molecules was about the same as it had been at pH 7·4. This suggests that barbiturates are pharmacologically active in the undissociated rather than in the ionic form.

The importance of fat-solubility in defining the pharmacological activity of thiopentone is emphasised by a comparison of the disposition of thiopentone with that of its oxygen analogue pentobarbitone. Both compounds were distributed in all tissues, except fat, to about the same amount. The oxygen in the ring, however, makes the uncharged moiety of pentobarbitone considerably less fat-soluble than that of thiopentone. As a result, the concentration of pentobarbitone was no higher in fat than in other tissues. On intravenous administration of pentobarbitone to man, the plasma levels at diffusion equilibrium were about three times higher than those resulting from the same dose of the sulphur analogue (Fig. 10). This difference in concentration was too great to be explained by the somewhat faster metabolic transformation of thiopentone, and is explainable by the failure of pentobarbitone to localise extensively in fat.

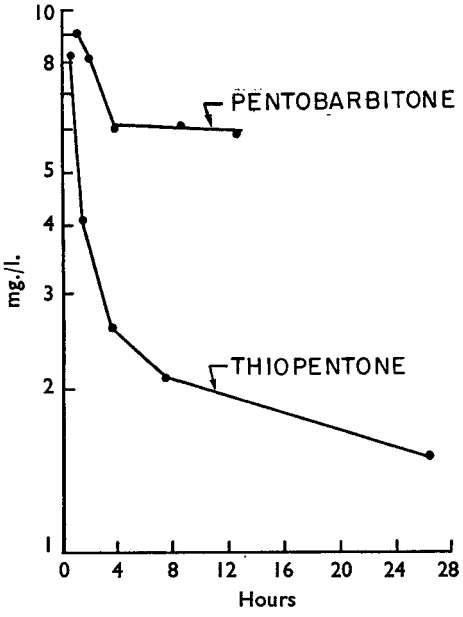


FIG. 10. Plasma levels of thiopentone and pentobarbitone after intravenous administration of 0·75 g. to man.

Examination of a number of other barbiturates showed that the plasma level curves of thiobarbiturates, including thialbarbitone (Kemithal) and thioquinalbarbitone (Surital) were similar to that of thiopentone[29]. There was an initial rapid decline, as the drugs were distributed throughout the body and then deposited in fat, followed by a more gradual decline reflecting the slow rate of biotransformation of the drugs. These barbiturates were also shown to be metabolised in man at the slow rate of about 10 per cent per hour.

The extensive localisation of hexobarbitone in fat is of particular interest since this drug is not a thiobarbiturate[29]. It represents a particularly illuminating example of the relationship between the physical properties of a drug and its physiological activity. Hexobarbitone differs structurally from the other oxygen barbiturates in having a methyl group on one of the ring nitrogens. This presumably affects the enolisation of the barbiturate ring structure and consequently hexobarbitone and other *N*-methylated

barbituric acid analogues are weaker and have a pKa of about 8·4 compared with 7·6 for oxygen barbiturates. Accordingly, at physiological pH, hexobarbitone is present virtually entirely as the unionised form. In addition the lipid solubility of the undissociated form is increased since hydrogen bonding between nitrogen and an adjacent oxygen is eliminated. As a result of these two factors, hexobarbitone is highly lipid-soluble, and its very short anaesthetic action arises from its localisation in fat.

Recently there was made available to us a particularly interesting series of extremely short acting barbiturates which are fat-soluble not only because they are thiobarbiturates, but also because they have an alkyl group on one of the ring nitrogens. This series of *N*-alkyl thiobarbiturates has a high oil to water partition ratio as a result of which *N*-methylthiopentone achieves an extraordinary degree of localisation in body fat[30]. Almost all the injected drug was found in body fat within a period of 12 hours and only a minor amount metabolised. At this time the concentration in fat was about a hundred times that in plasma. Thus a dog could be anaesthetised with a given dose of the compound, allowed to recover, anaesthetised again with the same dose, again allowed to recover, and this procedure repeated a number of times without the dose being reduced to obtain the same depth of anaesthesia. Each time the recovery was due almost entirely to localisation in fat, with this tissue acting as a seemingly bottomless reservoir.

What are the clinical implications of the extraordinary localisation of short-acting barbiturates in body fat? Because the thiobarbiturates are metabolised slowly, a large dose of barbiturate will produce prolonged anaesthesia and depression out of all proportion to the increase in dosage over that necessary to induce anaesthesia. It is now generally appreciated that no matter how long the surgical procedure, the amount of barbiturate administered should not be more than that which can be adequately handled by chemical inactivation. Anaesthetists usually restrict the total amount of thiopentone to 1 to 1·5 g. and in major operations use it as a basal anaesthetic supplemented by some volatile agent to maintain anaesthesia.

In recent years a number of barbiturates have been put to clinical trial with the claim that they are metabolised rapidly as evidenced by their "very-short" action. Almost invariably this action results not from rapid metabolism but from their deposition in fat. Such compounds may produce prolonged postoperative depression if given in large amounts in surgical procedures of long duration. There may be a need for an intravenous anaesthetic which is short-acting because of rapid inactivation in the body, but it seems improbable that this compound will be found among the barbiturates.

Another example of how the solubility of a drug in fat can affect its action is shown by the adrenergic blocking agents of the haloethylamine type. It has been generally considered that dibenamine remains unchanged in the body only for a short time during which the drug irreversibly destroys the "receptor substance" necessary for the action of adrenaline and noradrenaline. The long duration of action was explained

on the basis that regeneration of the "receptor substance" must occur to restore the original sensitivity to the catechol amines[31].

This cannot be the whole story, for it should then follow that the duration of all effective doses of dibenamine should be the same. This is not so. Intravenous administration of 25 mg./kg. of dibenamine produced complete blockade in a dog for a period that was two to three times as long as the complete blockade produced by half the dose. Obviously some factor other than rapid irreversible inactivation of adrenergic sites must be considered.

The measurement of the concentration of drug in various tissues gave the clue to the missing factor[32]. After a single dose of dibenamine, plasma and tissue levels declined rapidly and in one hour the levels were barely detectable when blockade had just become complete. Simultaneously the level in fat increased, reaching a maximum in about three hours. At this time about 20 per cent of the administered drug was in fat and the rest was rapidly metabolised before it ever had a chance to localise. There are, accordingly, two stages of the physiological disposition of dibenamine. In the first stage, the drug rapidly disappears from plasma, mainly owing to its rapid metabolic transformation, but partly to its deposition in fat. In the second stage, the unmetabolised drug is virtually entirely present in fat and the level of drug in this depot declines very slowly as the dibenamine slowly diffuses into the blood stream.

The dibenamine in fat must contribute to the action of dibenamine since the duration of adrenergic effect was related to the cumulation of the drug in fat and complete blockade against adrenaline lasted until the concentration of drug in fat declined to a level of about 8 μg./g. It is probable that the dibenamine stored in the fat depots maintained a plasma level of drug for a considerable period of time and that this level though too small to initiate the action of adrenaline receptor sites, was sufficient to maintain it.

Dibenzyline, a dibenamine analogue, behaves in a similar way. Its duration of action is extended by its localisation in fat. Studies with ^{14}C labelled dibenzyline indicated that complete blockade was maintained in a dog until the plasma level had declined to a level of about 6 μg. of drug per litre of plasma[33].

Plasma Proteins as a Storehouse for Drugs

Another type of drug storage depot should now be discussed, the various proteins in the body. The problem of protein-substrate interactions has far reaching implications; for instance, this phenomenon is encountered in biochemistry with the interaction of enzyme and substrate, in pharmacology with the combination of drug and "receptor site", and in immunology with the union of protein and hapten. These interactions usually are highly specific in contrast to the binding of drugs to plasma proteins which is remarkedly non-specific. The nature of plasma binding has been the subject of perennial investigation and a few broad generalisations are pertinent.

First of all the binding is ordinarily readily reversible and the attachment

is mainly to the albumin fraction. For example, when dicoumarol was added to 0·5 per cent solutions of various protein fractions, beta globulin (fraction IV–I) and gamma globulin (fraction III–0) bound about 20 per cent of the drug, the alpha about 50 per cent and the albumin more than 99 per cent[34]. These results suggest that albumin is responsible for the major part of the interaction between plasma proteins and drug but the others may participate. The especial affinity of albumin for drugs may be more apparent than real, depending perhaps on its relatively small molecular weight and consequent large surface rather than on any structural peculiarity.

It is generally considered that protein binding resembles salt formation with only the ionic form of the drug interacting with albumin. This would explain why antipyrine, and *N*-acetyl-4-aminoantipyrine, relatively neutral substances, show little affinity for the proteins of plasma or for that matter of other tissues. In fact, these two compounds display so little interaction with cellular components that they may be used to measure total body water.

The positively charged cationic form of basic drugs, as expected, interacts with plasma proteins, by simple electrostatic attraction. Since the isoelectric point of albumin is about pH 5, it might be thought that acidic drugs in the anionic form would not become attached to albumin. It was once thought that only cations would interact, a supposition soon shown to be incorrect. It is now apparent that ionic combination occurs regardless of the net charge of the protein. Indeed acidic drugs are generally more avidly attracted, the relatively strong acid phenylbutazone (pKa 4) being bound to plasma albumin to the extent of 98 per cent at therapeutic plasma levels. The probable explanation for the binding of acids is that the albumin molecule contains a large number of ionic sites, both cationic and anionic, and at pH 7·4, there are still a number of sites sufficiently electropositive to bind organic anions.

Forces other than polar interaction must be considered when the relation between degree of binding and chemical structure is discussed. For example, in a homologous series of barbiturates, all of which have pKa's of about 7·6, barbitone is not appreciably bound to plasma albumin. But as the chain is lengthened, the binding increases and reaches 55 per cent with pentobarbitone[35]. Similarly, in a homologous series of fatty acids, the binding is enhanced by increasing the length of the carbon chain[36].

In these examples the primary bond may be considered to be electrostatic, but the resulting complex is stabilised by poorly understood forces through an intimate contact of the non-polar carbon chains with a non-polar portion of the adjacent protein surface. This might explain why in a homologous series of compounds, binding to albumin increases with increase in lipid solubility. Thus thiopentone, with about the same acid strength as its oxygen analogue pentobarbitone, has a greater fat solubility and degree of binding to plasma albumin; *N*-methyl thiopentone which is considerably more lipid-soluble than thiopentone also interacts more with plasma proteins[30].

That an organic compound need not be an electrolyte to combine with plasma proteins is shown by the examples of both cortisone and

hydrocortisone which are bound to plasma proteins to about 80 per cent[37]. These substances are also highly lipid-soluble. Even small symmetrical fat-soluble molecules like *cyclo*propane appear to be bound to plasma proteins since measurements indicate that they are more soluble in defatted plasma than in saline. An interaction of *cyclo*propane with albumin is shown by the striking increase in solubility of the gas in phosphate buffer on the addition of albumin[38].

Thus it appears that albumin can attract drugs by forces of association between ions, polar groups and non-polar groups and that more than one of these forces can be involved in a single combination. Any single explanation cannot satisfactorily explain the binding of all drugs, and a number of factors, either alone or in combination, including electrostatic forces, polarity and Van der Waals forces are involved. Irrespective of the mechanism, binding has important implications in the action of a drug since it hinders access to the sites of biotransformation, action, and excretion. Furthermore, information about binding of drugs is essential in understanding their mode of excretion and their passage into the central nervous system.

Certain acidic drugs are of particular interest because they are almost completely attached to plasma proteins. At therapeutic plasma levels 98–99 per cent of dicoumarol is bound to albumin and consequently more than one-third of the total drug in the body remains in plasma. This drug is extremely insoluble at physiological pH but extensive binding makes it about a hundred times more soluble in plasma than saline. If there were no plasma binding one wonders whether a drug as insoluble as this could be given intravenously without danger of precipitation in the blood stream and the possible clogging of small blood vessels. Particularly interesting is the finding by Dr. Burns that the de-esterified product of ethyl biscoumacetate is so firmly bound to proteins that it may be used after intravenous injection to measure the volume of plasma in the body.

A remarkable example of how a drug is protected from metabolic transformation and excretion by its binding to plasma proteins is the trypanicide suramin. A single intravenous dose remains in the body for several months and during this time, although highly protein bound, enough dissociates to prevent reinfection by trypanosomes of African sleeping sickness[39].

The high plasma binding of phenylbutazone explains certain extraordinary properties of the drug. Although structurally similar to antipyrine, phenylbutazone has an entirely different physiological distribution in the body. Antipyrine is not localised in any tissues, but is distributed fairly evenly throughout the water of the body. Phenylbutazone on the other hand, interacts to about 98 per cent with plasma protein at therapeutic levels.* Its binding to proteins protect it against rapid metabolic

* The plasma of different species vary considerably in the capacity to bind phenylbutazone. At levels of 120 mg./litre plasma binding is 98 per cent for man, 92 per cent for the dog and only 85 per cent for the rat. Whether the same differences would be shown by the purified albumin of the various species or whether the variability in plasma binding arises from competition with unknown substituents such as fatty acids is unknown.

alteration, so that the metabolism of phenylbutazone is slow in man, being about 15 per cent a day. This slow metabolic change is an important consideration in therapy since it makes it possible to treat a patient with single doses daily or every other day. This is a contrast to therapy with salicylate which must be administered several times daily because it is metabolised rapidly. Phenylbutazone, is by no means innocuous, having untoward effects that limit its usefulness, including the retention of salt and water, and undoubted gastrointestinal effects.

Dr. Burns made the observation that patients receiving 400 mg. of the drug daily achieved concentrations that averaged 93 mg./litre while patients receiving twice the dose achieved levels that averaged only 15 per cent higher[40]. To confirm the observation that plasma levels of phenylbutazone did not increase commensurately with increasing doses, levels

TABLE X

PLATEAU PLASMA LEVELS OF PHENYLBUTAZONE IN THREE PATIENTS ON SUCCESSIVE DOSAGE REGIMENS OF THE DRUG

Daily dose of drug mg.	B. W. mg./l.	C. C. mg./l.	K. R. mg./l.
800	107	96	90
1200	108	108	100
1600	118	110	103

TABLE XI

UNBOUND DRUG AT DIFFERENT PLASMA LEVELS OF PHENYLBUTAZONE IN MAN

Total drug mg./l.	Unbound drug in plasma mg./l.
100	2
150	5
225	20
250	30

were measured in subjects receiving several dosage schedules. Each schedule lasted for a period of two weeks and plasma samples were taken for analysis each day before the morning dose. From Table X it is seen that increasing the doses of the drug did not proportionately increase the plasma levels which reached a limiting concentration. Three explanations for this self-regulating mechanism were considered: a disproportionate increase in the excretion of the drug in urine or faeces; an increased localisation in the tissues without a parallel elevation in plasma levels; a disproportionate enhancement in the rate of metabolic transformation of the drug immediately after its administration. The first possibility was ruled out since the analysis of urine and faeces failed to reveal that significant amounts of unchanged drugs were excreted with any of the doses. It is known that phenylbutazone is almost completely and rapidly absorbed at these doses. The second possibility was ruled out for reasons which need not be discussed here.

Direct confirmation of the last possibility was found. Subjects were given 1600 mg. of phenylbutazone daily, in divided doses, for a period of two weeks. Twelve hours after the last dose, the plasma level fell at the rate of about 20 per cent a day and until it was 180 mg./litre. A dose of 800 mg. of the drug was then administered intravenously. Fifteen minutes later the level was 205 mg./litre, but it rapidly declined and within four hours was again 170 mg./litre. Instead of disappearing at the rate of 15 per cent a day it disappeared at a rate of about 20 per cent in four hours. Thus, after a sudden acceleration, the biotransformation

returned to its original rate of 20 per cent daily. It must be concluded that the apparent velocity constant for the biotransformation of the drug is considerably greater at higher than at lower concentrations. Further evidence of a rapid metabolic transformation after intravenous injection of the drug was provided by demonstrating a disproportionate increase in the urinary excretion of metabolic products of the drug during the period in which the plasma level declined from 205 to 170 mg./litre.

At first these findings seemed paradoxical since it would be expected that with increasing concentrations of drug, the relative rate of metabolism might decrease as biotransformation mechanisms became saturated.

The excessive amount of biotransformation can be explained on a simple basis—the extent of plasma binding. If the degree of plasma binding is plotted against the concentration of a drug, the curve is best described by the Langmuir isotherm. When the concentration is high enough, the binding approaches saturation. Ordinarily, protein binding sites are not saturated by drugs at therapeutic levels. Phenylbutazone, however, though avidly bound at low levels, seems to be attached to only a few sites on the albumin molecule and saturation is approached at therapeutic levels. This is shown in Table XI which summarises the proportion of unbound drug at various plasma levels. At levels of 100 mg./litre only about 2 per cent of the drug was free while at a level of 250 mg./litre unbound drug increased to 12 per cent. The rate of biotransformation is probably proportional to the amount of unbound drug. Since this is disproportionately high at high plasma concentration, the rate of metabolic transformation proceeds at an accelerated pace until the amount of drug in plasma declines to the concentration at which it is about only 2 per cent unbound. Thus, the tendency of phenylbutazone to remain at a constant level, no matter how high the dose can be related to the peculiarity of its plasma binding.

What does this mean from the clinical point of view? Since patients given 1600 mg. daily achieve plasma levels that are not appreciably higher than those achieved with doses of 800 mg. daily, there is probably no advantage in administering the drug in high doses. If the desired therapeutic effect is not achieved, further benefit should not be expected with an increase in dose. Most subjects achieve, on a daily dosage of 400 to 600 mg. daily, plasma levels that are only slightly lower than those reached when 800 mg. are given. In general, these observations agree with clinical experience.

Drug Storage Depots in Tissues

Drugs are attached not only to proteins in plasma but also to proteins in various organs. This results in their storage in tissues, presumably reversible since all drugs finally disappear from the body. Few studies have been made on the interaction of drugs with tissue proteins, though knowledge of the binding of barbiturates in tissue homogenates indicates that it may be governed by the same principles involved in plasma binding[35].

The storage of mepacrine and other acridines in parenchymatous tissues is important in rational therapy since it is an example of cumulative

tissue storage. It is doubly important that therapeutic agents which are highly localised in tissues should not be mistakenly discarded through ignorance of their tissue distribution. This almost happened with mepacrine.

At first mepacrine was extensively used by the armed forces as a substitute for quinine, but after a few months it fell into disrepute. When used as a suppressive it produced considerable nausea and vomiting, especially in airmen; it was not particularly effective in preventing the outbreak of acute attacks; the slow onset of action of the drug made it necessary to hospitalise patients with acute malaria for long periods of time and a number with Falciparium malaria died who would have been saved with quinine.

As the result of a searching inquiry into its pharmacology, part of which was made at Goldwater Memorial Hospital, New York, under the direction of Dr. James A. Shannon with whom one of us had the privilege of working[41], mepacrine took its proper place as superior to quinine as a suppressive drug.

TABLE XII

DISTRIBUTION OF MEPACRINE IN DOG TISSUES

	Dog A 4 hrs after 10 mg./kg. daily for 14 days mg./kg.	Dog B 14 hrs after 20 mg./kg. daily for 14 days mg./kg.
Plasma ..	·04	0·06
Muscle ..	6·8	55
Lung ..	23	310
Spleen ..	16	570
Liver ..	70	1300

An unusual distribution of mepacrine was first observed in various organs of the dog (Table XII). Four hours after administration of the drug its concentration in liver was about 2000 times higher than in plasma. Muscle, which had the lowest concentration of any body organ, had almost 200 times the concentration of plasma. The tissue levels of mepacrine in a dog that had received the drug daily for fourteen days were then determined. Now, the tissue localisation of the drug was considerably more pronounced, the level in liver and in muscle being approximately 20,000 times and 1000 times respectively the level in plasma. We see here the extraordinary capacity of various tissues to take up mepacrine resulted in an extremely minute plasma concentration in equilibrium with large amounts of stored drug.

In the Pacific area where malaria was rampant, the Australians showed that a plasma concentration of about 30 μg./litre was required to abort an acute malarial attack[42]. It now became apparent that this concentration could be quickly achieved by giving large doses of mepacrine orally or parenterally and then maintaining the plasma level by the administration of small daily doses.

In its use as a suppressive agent, mepacrine had been used in a total dosage of 400 mg. a week given in doses of 200 mg. twice weekly. It was demonstrated also that the plasma level of mepacrine had to be approximately 15 μg./litre to prevent attacks of malaria. Because 200 mg. twice a week produced a concentration which averaged only about 10 μg./litre, it followed that there was insufficient protection.

This problem was solved by increasing the dose to raise the plasma level to 15 μg./litre by giving 600 mg. a week. Toxicity did not increase

with the higher dosage because it was largely due to local irritation of the gastrointestinal tract by the relatively large single dose of 200 mg. By limiting each single dose to 100 mg. the problem vanished. By making these simple changes in mepacrine therapy, malaria was no longer a tactical or strategic problem by January, 1944. Thus, a drug which acquired such a bad reputation was shown, only one year later, to be superior to quinine.

The peculiar behaviour of mepacrine intrigued us and after the war our curiosity led us to study the striking affinity of cellular components for the drug[43]. Dr. Tomkins gave the drug to rats intraperitoneally in a dose of 50 mg./kg. and subjected the homogenised livers to differential centrifugation in isotonic sucrose. The major portion of the drug was associated with material that had the sedimentation characteristics of nuclei. The same binding was observed after liver slices were incubated with the drug at 37° and then fractionated. When other acridines, including acridine itself, and 2:6-diaminoacridine were incubated with liver slices, the intracellular distribution was found to be essentially similar to that of mepacrine.

Since the nuclear fraction had such a high affinity for the drug the uptake of mepacrine by isolated nuclei was studied in detail. The reaction was rapid, equilibrium being achieved within 15 minutes. A change in temperature from 3° to 38° made little difference to the rate of binding. Furthermore, the uptake of mepacrine was about the same at the two temperatures.

The uptake of mepacrine did not depend on the integrity of the nuclear membrane as shown by experiments in which the nuclei were fragmented by sonic vibration. The binding of mepacrine by the fragments was about the same as that of the intact nuclei. The view had been expressed that the localisation of mepacrine is largely due to reaction with nucleic acid phosphorus. It is unlikely that this is a sufficient explanation since pretreatment of the nuclei with desoxyribonuclease or with ribonuclease did not diminish the ability to bind mepacrine. Even when the enzymatic hydrolysis of the desoxyribosenucleic acid was virtually complete there was no diminution in mepacrine binding. Accordingly, the affinity of nuclei for mepacrine cannot be based on their desoxyribosenucleic acid content, although Irvin and Irvin[44] have shown that interaction can occur between acridines and purified nucleic acid.

The question arose whether the process of the binding of mepacrine to nuclei involved electrostatic forces, coordinate bonding, or complex formation. It seems that the binding of mepacrine to nuclei is a "physical" rather than a "chemical" process. For example, a change in temperature of 0 to 37° failed to influence the rate of binding This is in contrast to a chemical reaction, of which the rate is ordinarily markedly decreased by a drop in temperature. The ready reversibility of the binding was further evidence for physical interaction. Heating the nuclei for one minute at 100° did not diminish their capacity to attach themselves to mepacrine. It is probable that the mepacrine interacts with the nuclei at

least partially by electrostatic forces since the binding process was dependent on pH, the maximum occurring at pH 5.

Perhaps the most striking aspect of the binding phenomenon was the apparently "limitless" capacity of nuclei to take up mepacrine. The amount of mepacrine taken up by nuclei increased as the concentration of drug increased with no suggestion that binding sites were saturated at concentrations of 60 mg./ml., the limit of solubility of the drug at pH 7·4. This concentration is, of course, an order of magnitude higher than that encountered pharmacologically. At the highest drug concentration more mepacrine molecules were bound than there were potential anionic binding sites (nucleic acid phosphorous and dicarboxylic amino acids) or even total amino acids in the proteins of the nucleus. At these high concentrations of mepacrine it is evident that another force is involved. It is probable that electrostatic ties to amino acid moieties are important for binding at low concentrations but at high concentrations there is perhaps polymerisation of the mepacrine molecules by hydrogen bonding. This laying down of one mepacrine molecule on another is reminiscent of the process of crystallisation. It would be of considerable interest to learn the nature of the cellular constituent that exerts this astonishing interaction with mepacrine.

The Intestines as a Storage Depot for Drugs

To show the variety of drug depots in the body, a mention will be made of a surprising type of localisation that Dr. Burns discovered for zoxazolamine, a new centrally acting muscle relaxant.

This drug is distinguished by its persistent action when given by mouth. Its long duration of action suggested that the drug is stable in the body. It was found, however, that the drug exerted only a fleeting action after intravenous injection and was metabolised with unusual rapidity. The long duration of action after oral dosage was found to be due to the low solubility of the drug in the gut. The drug precipitates in the intestines whence it is slowly absorbed during a period of many hours. It is only because of the relatively low toxicity of the drug that a large excess of the agent can be given orally to ensure a continuous absorption over a long period of time.

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