REVIEW ARTICLE

SOME PHYSICO-CHEMICAL FACTORS IN DRUG ACTION

By Bernard B. Brodie and C. Adrian M. Hogben

National Heart Institute, National Institutes of Health, Bethesda, Maryland

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.

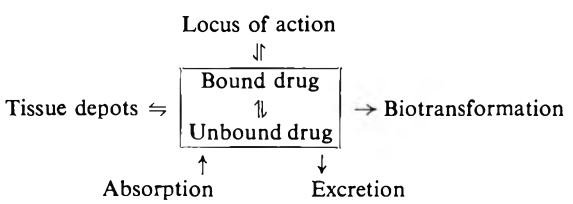


FIG. 1

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^{1}
Amidopyrine	5·0	42		10^{1}
Quinine	8·4	38		10^{2}
Dextrorphan	9·2	40		10^{2}
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:

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

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

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.

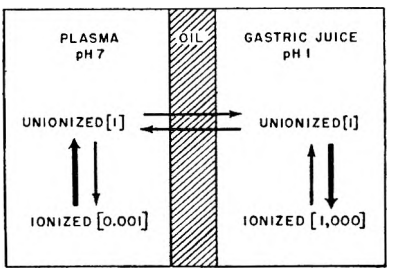


FIG. 2. Partition of an organic base pKa 4 e.g. aniline. The barrier between the blood and the gastric juice behaves towards drugs like an oil layer. Only the lipid-soluble undissociated form passes the boundary.

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.

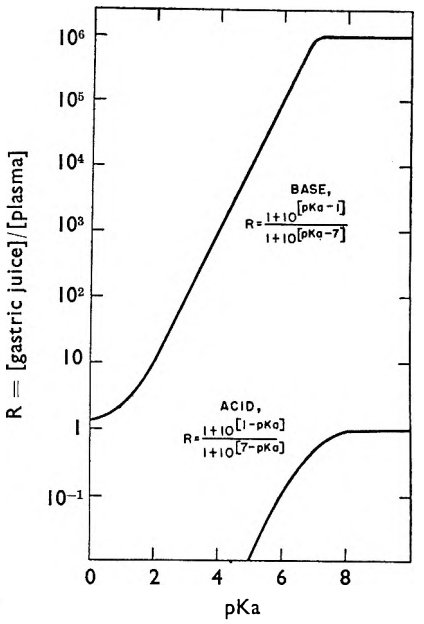


FIG. 3. Theoretical partition ratios of organic electrolytes between gastric juice and plasma.

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.

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.

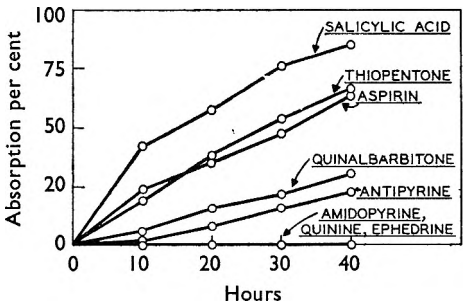


FIG. 4. Relative rates of drug absorption from the human stomach. Rate of absorption of ethanol falls between that of aspirin and quinalbarbitone.

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.

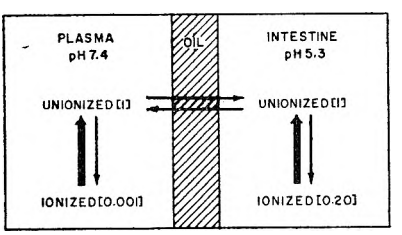


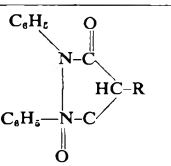
FIG. 5. The distribution of aniline, pKa 4·6, between plasma and intestinal fluid separated by an oil layer.

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.

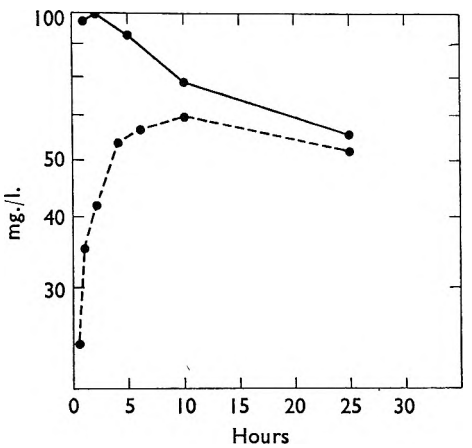


FIG. 6. Comparison in man of the plasma levels of phenylbutazone after oral and intramuscular administration.

●—● 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.

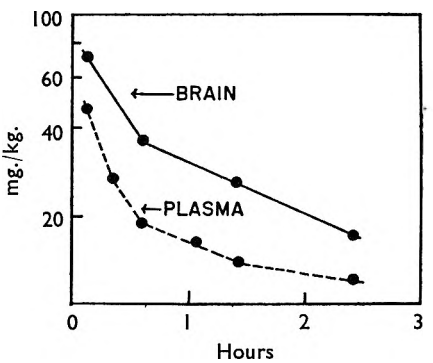


FIG. 7. Thiopentone levels in a dog after intravenous injection of 40 mg./kg.

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.

SOME PHYSICO-CHEMICAL FACTORS IN DRUG ACTION

LIPID SOLUBILITY OF DRUGS AS A LIMITING FACTOR IN URINARY EXCRETION

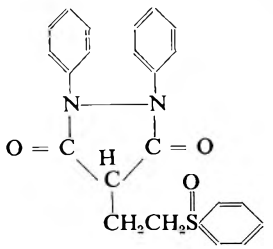
The action of a drug has been generally considered to be limited by the combined effects of urinary excretion and enzymatic inactivation. In recent years it has become increasingly evident that disposal of drugs by the kidney is of relatively minor importance in limiting their duration of action. Most 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.

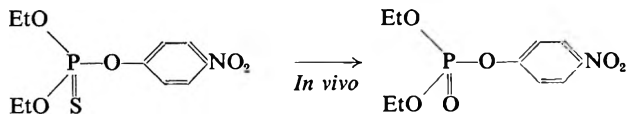
The non-selectivity of the biochemical mechanisms for the oxidation of foreign compounds reveals an ancillary problem. How are normally-ocurring substrates protected from destruction by the action of the non-selective enzymatic scavengers? Nature appears to have solved this problem. Normal substrates 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

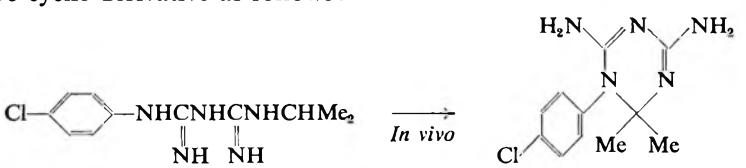
that the parent compound does not. The acquisition of a new kind of pharmacological activity is most likely to occur when modification of the parent drug unmasks or produces a radically different functional group. One of the most interesting examples of unmasking a chemical grouping arose from studies 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:



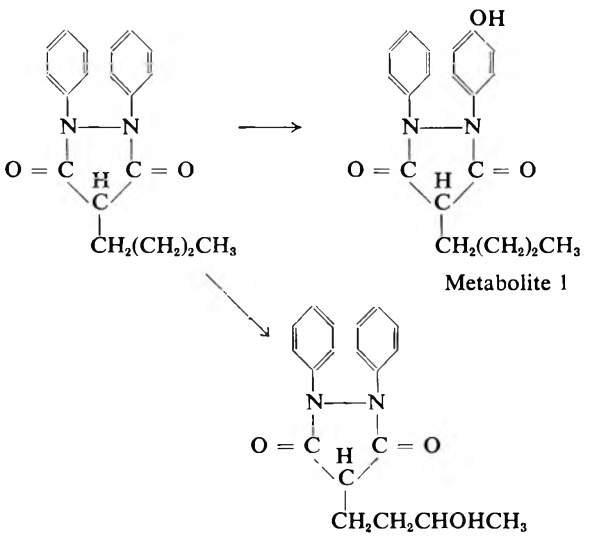
Proguanil Metabolic product

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

Metabolite 2

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.

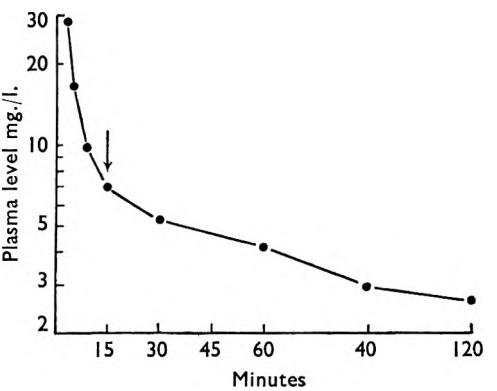


FIG. 8. Plasma levels of thiopentone in man after intravenous injection of 0·4 g.

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.

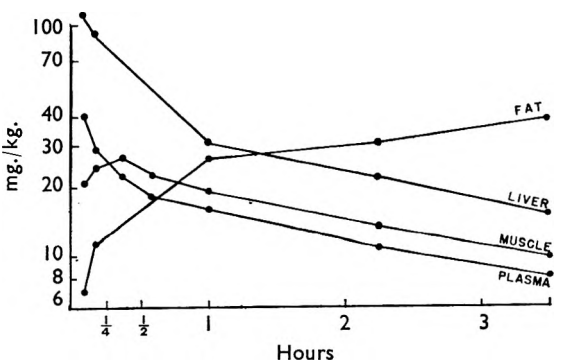


FIG. 9. Thiopentone levels in various tissues after intravenous administration of 25 mg./kg. to a dog.

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.

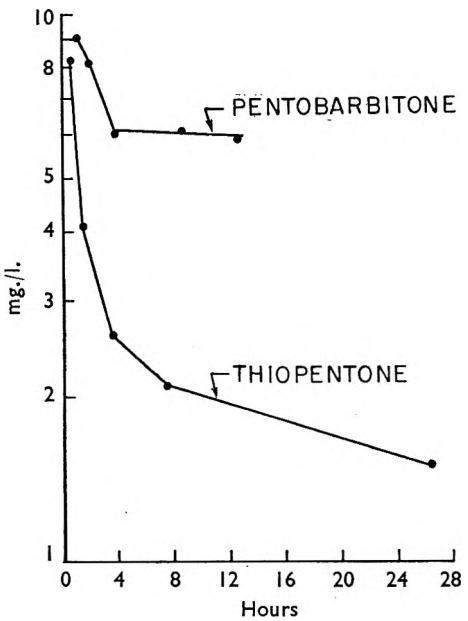


FIG. 10. Plasma levels of thiopentone and pentobarbitone after intravenous administration of 0·75 g. to man.

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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RESEARCH PAPERS

A NEW SERIES OF POTENT ANALGESICS:

DEXTRO 2: 2-DIPHENYL-3-METHYL-4-MORPHOLINO-BUTYRYLPYRROLIDINE AND RELATED AMIDES

PART I. CHEMICAL STRUCTURE AND PHARMACOLOGICAL ACTIVITY

BY PAUL A. J. JANSSEN AND ANTON H. JAGENEAU

From the Pharmacology Department, Laboratoria Pharmaceutica Dr. C. Janssen Turnhout, Belgium

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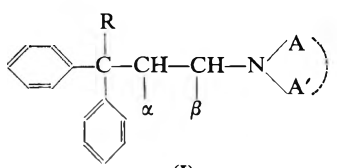
SUBSTITUTED diphenylpropylamines of general structure I, in which R is a radical and NAA′ an open or closed tertiary amino group, have been shown by different workers to possess pronounced analgesic or atropine-like activity[1–8,14,16–22,24,25]. No known compound is both a



(I)

(I *a*, $\alpha = H$, $\beta = H$; I *b*, $\alpha = CH_3$, $\beta = H$; I *c*, $\alpha = H$, $\beta = CH_3$)

potent analgesic and a potent atropine-like substance[1,5]. Which sort of activity predominates seems to depend, in the first place, upon the nature of substituent R of structure I[2].

Analgesics have been found among compounds of the methadone type, for example I, $R = COC_2H_5$, methadols and acylated methadols, I, $R = CHOR' \cdot C_2H_5$; sulfones, I, $R = SO_2C_2H_5$; esters, I, $R = COOC_2H_5$; ketimines and acylated ketimines, I, $R = C:NR' \cdot C_2H_5$[2,5,16,17–24].

Atropine-like activity has been found among primary amides like R 79 ($R = CONH_2$), "reversed" amides like R 79, for example $R = NH-COR'$, nitriles, $R = CN$, tertiary alcohols, $R = OH$, and unsubstituted amines $R = H$[1–8]. Within these groups of compounds the relative potency depends not only upon the nature of R, but also upon the configuration of the basic side chain, $CH\alpha \cdot CH\beta \cdot NAA'$.

Potent analgesics of type I are usually dimethylamino-, morpholino-, piperidino- or pyrrolidino-derivatives.

Open dialkylamines with alkyl groups other than methyl have generally little activity, the analgesic activity decreasing with increasing size of the alkyl groups.

Only a few heterocyclic amino analogues of the pyrrolidino-, piperidino-, and morpholino-derivatives are known; they were found to be less active than the parent compounds.

Quaternisation of tertiary amines of type I decreases the analgesic activity, but increases the atropine-like activity[6].

The presence of the methyl group on the basic side-chain (*Ia* → *Ib* or *Ic*) generally increases the analgesic activity and lessens the atropine-like activity. Ketones, R = COC_2H_5, primary amides, R = $CONH_2$, and nitriles, R = CN of type *Ic* are more active than their isomers of type *Ib*. Some acetylmethadols of type *Ib* however are more active than their isomers of type *Ic*.

Some racemates of type *Ib* and *Ic* have been resolved, and in all, the analgesic or atropine-like activity is found with only one of the optical isomers.

The spatial configuration of the analgesically active isomers of type *Ic*, NAA′ = $N(CH_3)_2$, is identical and related to that of D-(-)-alanine[11–13].

A large number of modifications of structure I have been made by attacking the molecule at all points[2].

In general, reduction or complete loss of analgesic activity occurs when one or both phenyl groups are substituted or replaced by other groups, or when the side chain is lengthened, shortened, or branched with groups other than methyl.

A number of new chemical modifications of structure I have not yet been examined, and the available information on the pharmacological properties of many of the known derivatives is poor and often conflicting[2]. We therefore decided to continue our research program[1–8] and to investigate a series of secondary and tertiary amides of type I[8], R = CONR′(R′ = secondary or tertiary amide group). The synthesis and physico-chemical properties of the new compounds, listed in Table IV, will be described elsewhere.

Pharmacological Methods

Analgesic Activity in Mice

The analgesic activity in mice was measured with an adaptation of the "hot plate" method[17–21]. Male albino mice of 20–30 g. and of a mixed inbred strain were used.

The hot plate was a restraining cylinder on a copper bath containing equal parts of boiling (55°–55·5°) acetone and ethyl formate[18,21].

The reaction time is the interval, measured in intervals of 2×10^{-1} seconds, between the moment the mouse reaches the hot plate and the moment the animal either licks its feet or jumps out of the cylinder. All other signs of discomfort, such as kicking of the hind legs, dancing around the cylinder are disregarded.

Using groups of five mice, the reaction time is measured 10 and 5 minutes before and 10, 20, 30, 45, 60, 90, 120, 180 and 240 minutes after subcutaneous injection or oral administration of 0·1 ml./10 g. body weight of an aqueous solution. The "normal reaction time" is defined as the average of both reaction times, estimated 10 and 5 minutes before giving the drug. The response was considered to be positive when the reaction time after injection was longer than 30 seconds at least once, or when three

or more readings exceeded the normal reaction time by a factor of three or more. All responses were negative in 300 mice, injected with saline or water over a period of one year.

The results were statistically evaluated using the graphical method of Litchfield and Wilcoxon[9], and expressed using the following symbols: ED50: median effective dose (mg./kg,); L.L. and U.L.: lower and upper fiducial (confidence) limits; S = slope; P.R. = potency ratio; f_{ED50}, f_S and $f_{P.R}$ = factors for computing confidence limits (P = 0·05) of ED50, S and P.R.; s.c. = subcutaneous injection; and or = oral administration.

The distribution of the differences between the first and second estimation of the "normal reaction time" on 1000 untreated mice is shown in Table I.

TABLE I

DISTRIBUTION OF DIFFERENCES BETWEEN THE FIRST AND SECOND ESTIMATION OF NORMAL REACTION TIME ON 1000 MICE

Seconds	Frequency		Total	Eddy[10,21]
	Positive	Negative		
			per cent	per cent
0		108	10·8	19·6
0·2–1·0	298	384	68·2	42·6
1·2–2·0	72	99	17·1	27·7
2·2–3·0	12	20	3·2	8·1
3·2–4·0	3	4	0·7	2·0
Total	385	507		

Statistical analysis of the frequency distributions of the normal reaction time, with 20 successive groups of 1000 mice during about one year, showed insignificant differences between these populations. The average normal reaction time for a group of 10,000 successively examined mice was 4·96 seconds, compared with the significantly different 9·51 seconds figure, reported by Eddy (Fig. 1). Ninety per cent of our values fall within the range of 3·3 to 7·0 seconds (Eddy: 6 to 13 seconds). There was no significant correlation of body weight and normal reaction time. The difference between the lowest and the highest of five ED50 values for morphine hydrochloride, estimated during a period of one year, was also statistically insignificant (subcutaneous injection). Rank correlation analysis failed to show a significant correlation of body weight (15 to 35 g.) and frequence of positive analgesic response after subcutaneous injection of 10 mg./kg. morphine hydrochloride.

For 150 substances with an ED50 value of 100 mg./kg. (subcutaneous injection) or less, the average slope value S was 1·58. The highest value of S was 2·74 and the lowest was 1·10; 90 per cent of these 150 values fall within the range of 1·20 to 2·20. The average f_S-value for this whole group was 1·255. In some instances therefore the dose-effect curves of two substances deviated significantly from parallelism (S.R. exceeds $f_{S.R.}$). A few examples are given in Table II.

Mydriatic Activity in Mice

The mydriatic activity in mice after subcutaneous or oral administration was estimated as described previously[1–3,5,7]. The same animals were used

for the simultaneous measurement of the analgesic and mydriatic effects.

The mydriatic effect was considered positive when the pupil diameter equalled or exceeded 30/25 mm. at 10, 20, 30, 60, 90, 120, 180, 240, 300, 360 or 420 minutes after giving a drug. In a series of 300 mice, injected with water or saline, all mydriatic responses were negative. The results were statistically evaluated and expressed as before.

TABLE II

ANALGESIC ACTIVITY IN MICE AND RATS BY SUBCUTANEOUS INJECTION
POTENCY RATIO, P.R.: MORPHINE HYDROCHLORIDE = 1·00

		P.R.†	L.L.†	U.L.†	$f_{P.R.}$†	S.R.†	$f_{S.R.}$†
R875	M	18·5	16·7	20·5	1·11	1·06	1·40
	R	40·5	33·0	49·9	1·23	1·31	1·20*
R610	M	9·60	8·65	10·7	1·11	1·06	1·10
	R	23·4	17·1	32·1	1·37	1·01	1·47
R660	M	8·70	7·91	9·57	1·10	1·01	1·23
	R	30·6	21·3	44·1	1·44	1·14	1·45
R888	M	1·54	1·38	1·72	1·12	1·01	1·27
	R	1·20	0·97	1·49	1·24	1·14	1·22
R530	M	0·88	0·80	0·96	1·10	1·03	1·10
	R	0·48	0·34	0·68	1·41	1·18	1·45
Heroin	M	6·00	5·46	6·66	1·11	1·11	1·16
	R	15·0	11·7	19·2	1·28	1·22	1·20*
Phenadoxone HBr ..	M	4·90	4·29	5·59	1·14	1·06	1·34
	R	6·52	5·05	8·41	1·29	1·07	1·20
Methadone HCl ..	M	2·32	2·11	2·55	1·10	1·10	1·20
	R	3·06	2·28	4·10	1·34	1·05	1·44
N-morpholino-ethyl-nor-pethidine 2 HCl	M	0·65	0·52	0·81	1·25	1·27	1·47
	R	0·65	0·47	0·90	1·38	1·03	1·35
Pethidine HCl	M	0·43	0·39	0·47	1·10	1·19	1·13*
	R	0·28	0·21	0·37	1·31	1·01	1·26
Codeine phosphate ..	M	0·23	0·21	0·26	1·11	1·11	1·23
	R	0·11	0·08	0·14	1·30	1·01	1·25

* The curves deviate significantly, 19/20 probability from parallelism (S.R. exceeds $f_{S.R.}$).
† For definition see page 383.

Analgesic Activity in Rats

The analgesic activity in rats was estimated by the hot plate method described for mice, except that the bath was bigger.

Male albino rats of an inbred Wistar-strain, weighing from 100 to 250 g., were used.

The copper bath (diameter: 40 cm., height: 15 cm.) contains 4 litres of a boiling mixture of equal parts of acetone and ethyl formate (55° to 55·5°). A restraining glass cylinder had the following dimensions; height 25 cm., internal diameter 26·5 cm., and external diameter 27·5 cm.

About 95 per cent of all animals eventually lick their feet after being dropped on to the hot plate and about one rat in 20 learns to jump out of the cylinder.

The frequency distribution of 1500 successively determined "normal reaction times" is shown in Figure 1. The adopted experimental design was practically identical with the one described for mice. The same

definitions of "reaction time", "normal reaction time" and "positive response" were adopted. Groups of ten rats were used and subcutaneously injected with 0·2 ml./100 g. weight of an aqueous solution containing various amounts of the drugs. The pupil diameter was not measured. The results were statistically evaluated and expressed as before. No significant rank correlation could be detected with body weight and normal reaction time, although heavy rats jump out of the cylinder oftener than light ones.

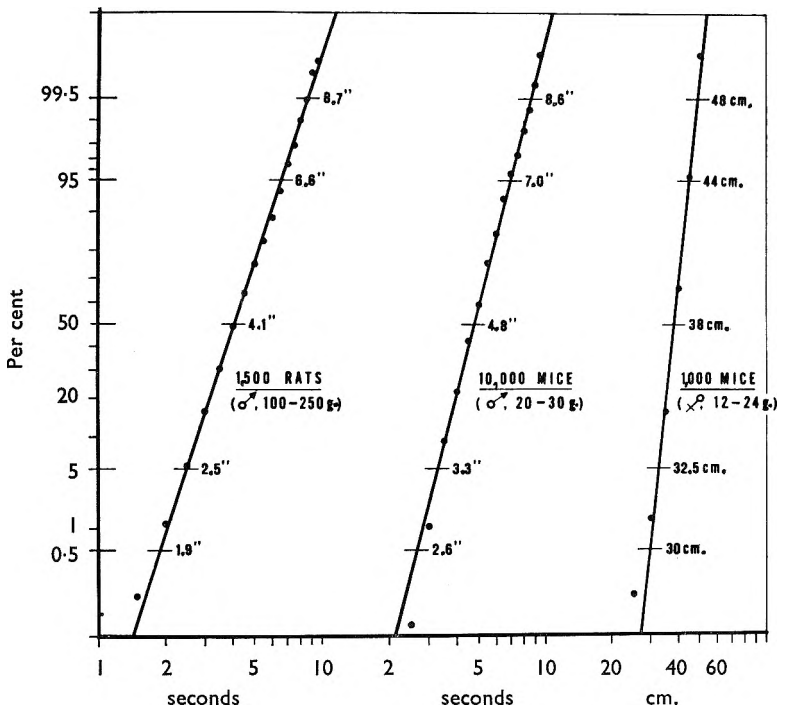


FIG. 1. Frequency distribution of the "normal reaction time" in seconds on 1500 rats and 10,000 mice, and of the distance from pylorus to appendix in cm. of 1000 mice.

Relative Atropine-like Activity In Vitro

The atropine-like activity was evaluated as previously described[1–4,6,7], using the inhibition of acetylcholine-induced spasms on the isolated intestine of rabbits as the criterium of activity, and atropine sulphate as the standard. All potency ratios (P.R.), calculated on an equimolar basis, are based on the results obtained with 3 × 12 doses of atropine and 3 × 12 doses of the substance investigated.

Inhibition of the Gastrointestinal Propulsion of a Charcoal Suspension in Mice

Groups of ten young female albino mice, 12 to 24 g., 2 to 4 months old of a mixed inbred strain, fasted overnight, were injected intraperitoneally

with 0·1 ml./10 g. weight of an aqueous solution containing varying amounts of the drug. One hour later the animals were given by stomach tube 0·3 ml. of an aqueous suspension containing 10 per cent charcoal and 5 per cent gum acacia. Two hours after the charcoal meal, the mice were killed, the intestines immediately excised from cardia to anus, and carefully laid out on clean white glass or stainless steel for inspection and measurement of the distances "pylorus to anus" and "pylorus to appendix". In 300 control mice, which were injected with saline only and given charcoal by stomach tube in the course of one year, the appendices of all were filled with charcoal (black appendix). Pretreatment with increasing doses of substances like analgesics or antispasmodics, which are known to depress gastrointestinal motility, increased the proportion of "white appendices" (no charcoal detected inside the appendix) in these experimental conditions. An all-or-none criterium was therefore adopted, the effect being considered "positive" when the appendix was "white", and "negative" when the appendix was "black" (contained some charcoal).

TABLE III

RELATION OF BODY WEIGHT AND DISTANCE FROM PYLORUS TO APPENDIX

Distance from pylorus to appendix cm.	Weight in g.			Total
	10–15	15·5–20	20·5–25	
20·5–25	0	0	1	1
25·5–30	5	6	1	12
30·5–35	56	78	11	145
35·5–40	116	309	78	503
40·5–45	21	172	101	294
45·5–50	1	24	19	44
50·5–55	0	0	1	1
Total	199	589	212	1000

In our experience the frequently used quantitative criterium, the distance traversed by the charcoal meal, expressed as the proportion of the total length of the intestine is unsatisfactory, because of the inaccuracy with which these measurements can be made and of the very flat dose-effect curves thus obtained. The results were statistically evaluated using the graphical method of Litchfield and Wilcoxon[9]. Observations on 1000 female albino mice, weighing from 10 to 25 g. and 2 to 4 months of age, showed that the distance from pylorus to appendix slowly increases with increasing weight. (Table III.)

As shown in Figure 1, 99 per cent of these values fall between 30 and 48 cm. (average: 38·6 cm.). In spite of these variable intestinal lengths, we were unable to detect a significant rank correlation of body weight or intestinal length and percentage positive effects in large groups of mice treated with ED40 to ED60 doses of R 875, R 79 (Priamide), morphine hydrochloride, atropine sulphate and chlorpromazine hydrochloride.

RESULTS

Analgesic Activity in Mice and Chemical Structure

The analgesic activity in mice by subcutaneous injection of 21 secondary and 58 tertiary amides of type I are listed in Table IV. Some 38 primary

amides, ketones of the methadone type, esters, alcohols, ketimines and acetylketimines of structure I, as well as 12 miscellaneous analgesics are included in Table IV for comparison. The relation between analgesic activity in mice and chemical structure of secondary and tertiary amides of structure I, can be described in the following way.

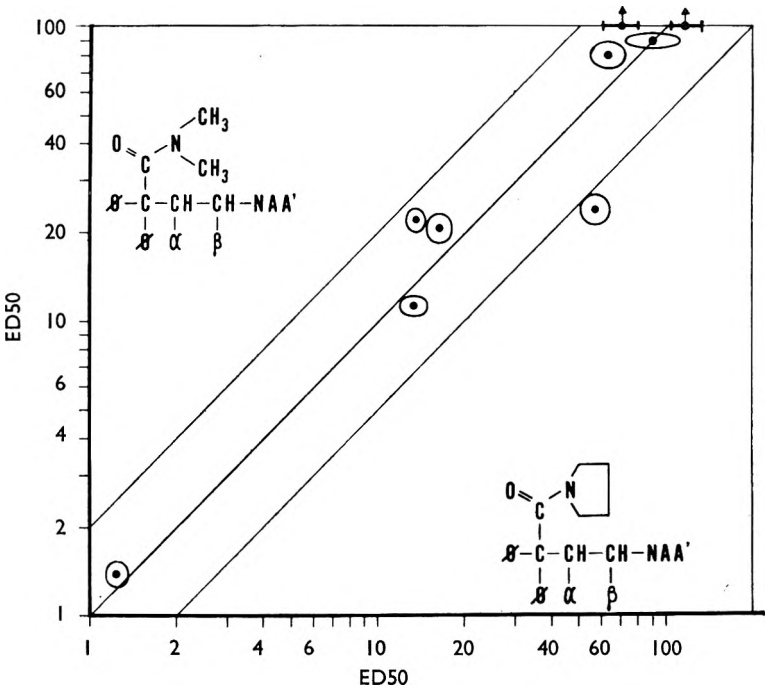


FIG. 2. The analgesic activity in mice by subcutaneous injection of 9 basic butyryl-pyrrolidines and of 9 corresponding butyryl-dimethylamines. The confidence limits, P 0·05 are shown graphically.

The Amide Group (R = CONHR′ or CONR′R″)

Highest analgesic activity was found among *N*-pyrrolidine- and *NN′*-dimethylamides. As shown in Figure 2, the corresponding derivatives of these two amides show about the same activity. Considerable loss of activity occurs when the tertiary amide group is derived from an open amine having alkyl-substituents other than methyl or from a cyclic amine larger than pyrrolidine. The most active secondary amides are *N*-ethyl-derivates, a few of them being about as active as pethidine.

α and β

A methyl group in the α-position of the side chain (type I*b*), increases the analgesic activity; inactive unbranched substances (I*a*) may even become as active as morphine when branched with a methyl-group in the α-position. This advantageous effect is more pronounced among tertiary- than among secondary amides. A methyl group in the β-position of the

side chain gives less active compounds than their α-methyl isomers of type I*b*, and as a rule even less active than their unbranched analogues. Most of them are completely inactive. Lengthening, shortening or branching the chain joining the tertiary nitrogen atom and the quaternary carbon atom with groups other than methyl causes reduction of activity.

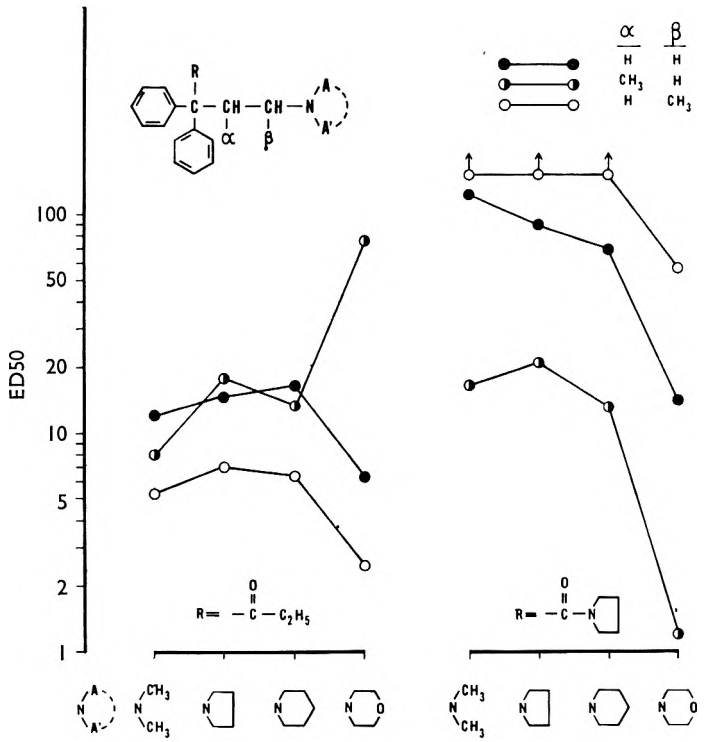


FIG. 3. The analgesic activity in mice by subcutaneous injection of 12 basic ketones of the methadone-type (R = COC_2H_5) and of the 12 corresponding basic pyrrolidino-amides of the R 610-type. The substances with a methyl-group in α- or β-position are the racemic mixtures; one of the two optical isomers is about twice as active as the racemate; the other optical isomer is inactive.

The various effects of methyl-branching in the α- and β-position on the analgesic activity of representative members of the 2:2-diphenyl-4-amino-butyryl-pyrrolidino-, and of the 4:4-diphenyl-6-amino-hexan-3-one-, (methadone) type are graphically represented in Figure 3.

These effects are more pronounced among basic amides than among ketones of type I.

Whereas isomethadone-like aminoketones may be more or less active than the unbranched parent compounds, the introduction of a methyl group in β-position increases the analgesic activity by about two and a half times. The fact that the β-methyl substituted amides are nearly inactive might therefore be more surprising than the high activity of the α-methyl analogues.

The Basic Group NAA'

The most active analgesics in mice and rats are basic amides with a morpholino group in the NAA' position. Among tertiary amides with a methyl-group in the α-position, the dimethylamine-, pyrrolidine- and piperdine analogues were also found to cause analgesia. They are about as active as morphine, but their β-methyl-isomers as well as the unbranched

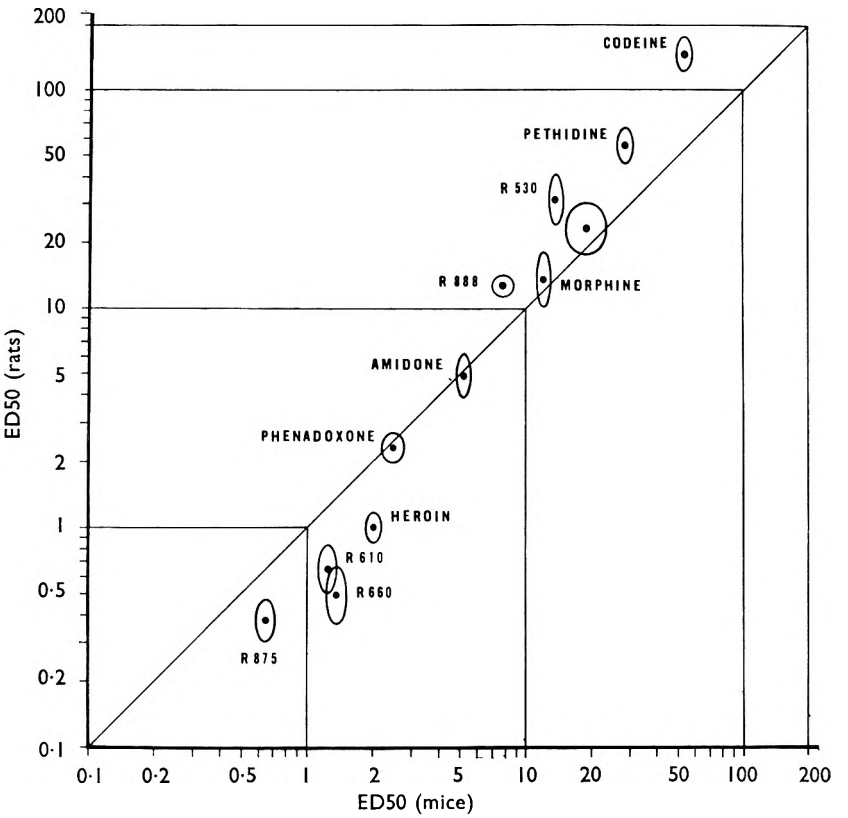


FIG. 4. Analgesic activity in mice versus analgesic activity in rats of 12 compounds listed in Table IV by subcutaneous injection. The confidence limits (P 0·05) of the ED50 values are shown graphically.

parent compounds are nearly inactive. All known analgesically active dimethylamino-derivatives of type I (NAA' = $N(CH_3)_2$) are proportionally inactivated by replacement of one or both methyl groups by alkyl groups of increasing size. Ring-substitution with alkyl groups in derivatives of heterocyclic amines also results in reduction of activity.

All known quaternary amines of type I are much less active than the tertiary amines from which they are derived.

Optical Isomers

The presence of the methyl-group in the α- or β-position introduces an asymmetric carbon atom. In the α-methyl series, one of the optical

isomers of each enantiomorphic pair is about twice as active as the racemic mixture, while the other optical isomer is devoid of significant analgesic activity. The spatial configurations of active optical isomers of type I will be discussed elsewhere.

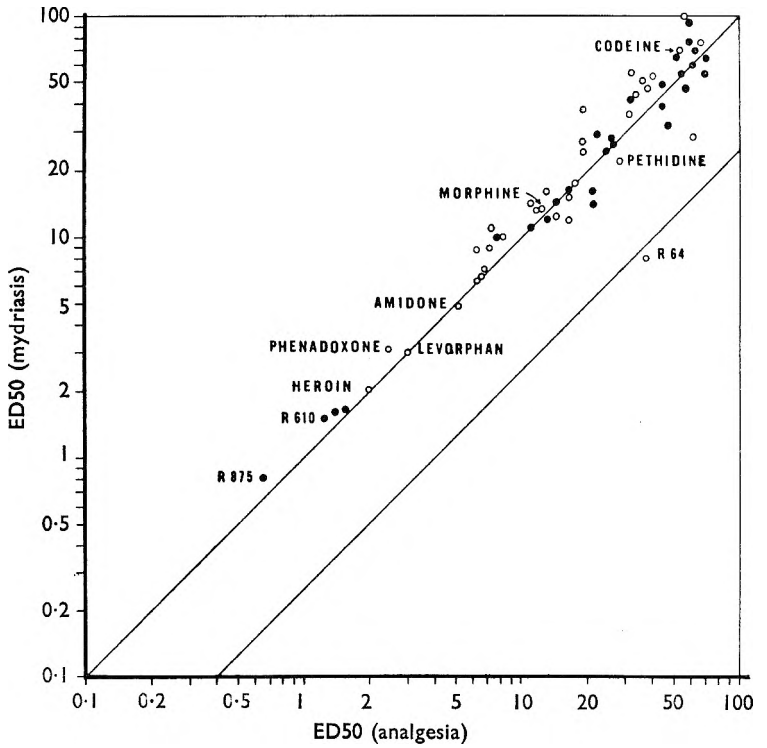


FIG. 5. Mydriatic versus analgesic activity in mice by subcutaneous injection of 29 basic amides related to R 875 (black circles) and of 33 other analgesically active substances (white circles), listed in Table IV.

Modification of the Diphenylmethane-group

The unpublished data from tests which we have made with this group show that replacement of one or both phenyl groups by hydrogen, various alkyl groups or other aryl-groups as well as substitution by an alkyl group, an alkoxy group or a halogen atom, invariably leads to less active compounds.

Analgesic Activity in Rats

As shown in Figure 4, significant correlation is observed with the analgesic activities in mice and rats by subcutaneous injection. Six compounds with an ED50 in mice of less than 10, also show an ED50 rat : mouse ratio of less than one; the other six compounds, shown in Figure 4, having ED50 in mice values of 10 or more, also have ED50 rat : mouse ratios greater than one. In four instances, however, this ratio was not significantly different from one.

Atropine-like Activity In Vitro and *Mydriatic Activity in Mice*

The compounds listed in Table IV have been tested for relative atropine-like activity *in vitro*. All secondary and tertiary amides, ketones, esters, methadols, acetylmethadols, ketimines, and acetylketimines of type I are devoid of significant activity (P.R. $< 0{\cdot}02$ atropine). As shown in Figure 5, a significant correlation is found in mice with the analgesic and the mydriatic action of the analgesically active compounds listed in Table IV.

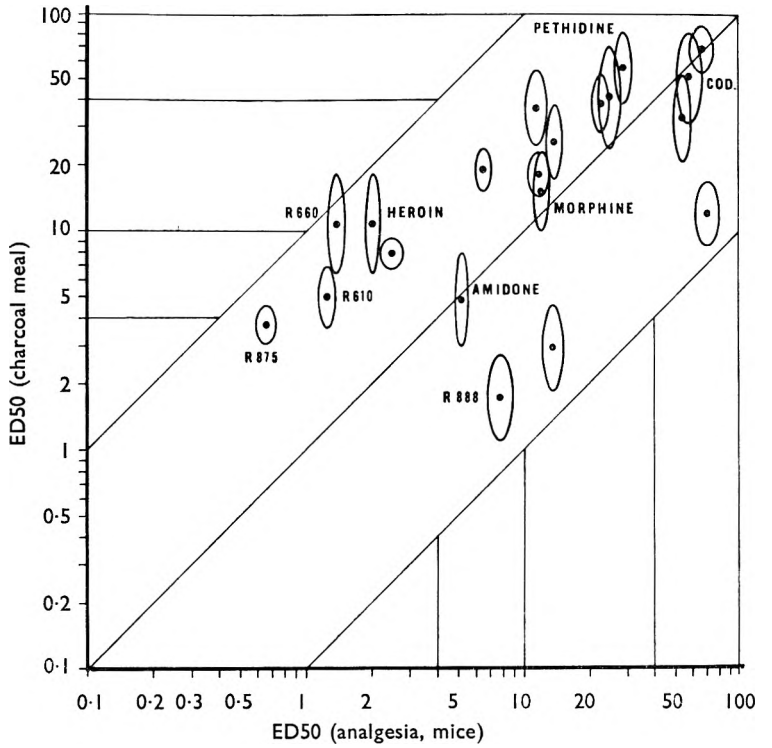


FIG. 6. The relation between the analgesic activity by subcutaneous injection in mice and the activity in the charcoal test in mice of twenty analgesically active compounds listed in Table IV. The confidence limits (P 0·05) of the ED50-values are graphically represented.

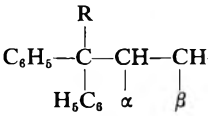
This typical relationship has been discussed previously[5]. A number of analgesically inactive secondary and tertiary amides of type I however are quite active mydriatics in mice. Since these substances are devoid of atropine-like action *in vitro* and the mydriatic activity after subcutaneous injection develops slowly—the maximal effect being seen after 2 to 3 hours, it seems likely that metabolic transformation into active metabolites occurs, possibly into the corresponding primary amides.

Inhibition of the Gastrointestinal Propulsion of a Charcoal Meal in Mice

All known analgesics significantly inhibit the gastrointestinal propulsion of a charcoal meal in mice, but we find no obvious quantitative correlation

(*continued on p. 398*)

TABLE IV



Serial number	R	α	β	NAA'	Salt	Animals*	ED50	L.L.†	U.L.†	S†	f_s
R802	–C(=O)–NH–CH_3	CH_3	H	$N(CH_3)_2$	HCl	A	146	108	197	1·81	1·45
						M	>150	—	—	—	—
R637	"	H	H		base	A	>100	—	—	—	—
						M	>10	—	—	—	—
R608	"	H	H		"	A	>100	—	—	—	—
						M	3·19	2·64	3·86	1·61	1·10
R588	"	H	H		"	A	43·8	35·0	54·8	1·75	1·60
						M	39·0	32·0	47·6	1·63	1·44
R766	"	CH_3	H	"	HCl	A	44·1	40·1	48·5	1·18	1·06
						M	48·6	43·4	54·4	1·23	1·09
R727	"	H	CH_3	"	base	A	53·9	48·1	60·4	1·31	1·07
						M	53·9	48·6	59·8	1·28	1·06
R750	–C(=O)–NH–C_2H_5	H	H	$N(CH_3)_2$	"	A	>100	—	—	—	—
						M	>25	—	—	—	—
R847	"	CH_3	H	"	$C_2H_2O_4$	A	145	116	181	1·55	1·16
						M	98·0	74·2	129	1·47	1·17
R646	"	H	H		base	A	>100	—	—	—	—
						M	>50	—	—	—	—
R605	"	H	H		"	A	>50	—	—	—	—
						M	1·60	1·38	1·86	1·64	1·12
						CH	1·50	0·88	2·55	10·2	2·20
R1123	"	CH_3	H	"	"	A	54·0	45·4	64·3	1·58	1·27
						M	27·2	23·5	31·6	1·48	1·19
						CH	33·9	27·6	41·7	1·49	1·37
R590	"	H	H		"	A	47·0	42·3	52·2	1·28	1·11
						M	32·0	28·6	35·8	1·20	1·07
R685	"	CH_3	H	"	"	A	25·9	23·9	28·1	1·43	1·20
						M	26·0	22·4	30·2	1·91	1·85
R680	–C(=O)–NH–C_2H_5	H	CH_3		base	A	62·0	53·0	72·5	1·50	1·22
						M	65·5	59·0	72·7	1·31	1·09
R979	"	H	H	$N(iC_5H_7)_2$	"	A	>50	—	—	—	—
						M	25·0	22·5	27·8	1·26	1·06
R600	–C(=O)–NH–$CH(CH_3)_2$	H	H		"	A	>100	—	—	—	—
						M	1·99	1·46	2·71	1·54	1·22
R580	"	H	H		"	A	65·5	59·5	72·1	1·17	1·07
						M	16·0	12·1	21·1	1·80	1·24
R760	"	CH_3	H	"	"	A	82·0	72·6	92·7	1·19	1·10
						M	73·8	65·3	83·4	1·30	1·15
R591	–C(=O)–NH–nC_4H_9	H	H	"	"	A	>100	—	—	—	—
						M	>100	—	—	—	—

* Analgesic activity:
A: in mice (S.C.)
AO: in mice (oral)
AR: in rats (S.C.)

Mydriatic activity:
M: in mice (S.C.)
MO: in mice (oral)

Charcoal meal:
CH: in mice (I.P.)

† For definition see page 383

TABLE IV—*continued*

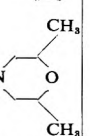
Serial number	R	α	β	NAA′	Salt	Animals*	ED50	L.L.	U.L.	S	f_s
R765	"	CH_3	H	"	"	A	>100	—	—	—	—
						M	>100	—	—	—	—
R909	$-\overset{O}{\overset{\|}{C}}-NH-C(CH_3)_3$	CH_3	H	"	"	A	>100	—	—	—	—
						M	>100	—	—	—	—
R910	$-\overset{O}{\overset{\|}{C}}-NH-CH_2-C_6H_5$	CH_3	H	"	"	A	>100	—	—	—	—
						M	>100	—	—	—	—
R731	$-\overset{O}{\overset{\|}{C}}-N(CH_3)_2$	H	H	$N(CH_3)_2$	"	A	>100	—	—	—	—
						M	>50	—	—	—	—
R777	"	CH_3	H	"	"	A	21·0	18·6	23·7	1·71	1·42
						M	14·3	12·7	16·2	1·51	1·30
R711	"	H	H		"	A	91·0	85·0	97·4	1·12	1·06
						M	8·25	6·76	10·1	1·44	1·09
R566 (ref. 15)	"	H	H		"	A	>100	—	—	—	—
						M	9·4	7·58	11·7	1·35	1·10
R700	"	CH_3	H	"	"	A	11·4	10·4	12·5	1·59	1·12
						M	11·0	10·2	11·9	1·49	1·09
R555	"	H	H		"	A	22·3	20·5	24·3	1·72	1·11
						M	29·1	26·9	31·4	1·56	1·07
						AO	44·6	39·1	50·8	1·34	1·17
						MO	63·0	50·4	78·8	1·79	1·74
						CH	38·3	28·0	52·5	1·67	1·35
R660	"	CH_3	H	"	"	A	1·38	1·25	1·52	1·71	1·22
						M	1·59	1·45	1·74	1·53	1·16
						AO	7·90	6·58	9·48	1·78	1·35
						MO	9·75	8·71	10·9	1·36	1·09
						AR	0·49	0·36	0·67	1·67	1·41
						CH	10·9	6·42	18·3	2·77	2·12
R630	"	CH_3	H	"	HCl	A	1·54	1·38	1·72	1·48	1·09
						M	1·65	1·42	1·91	1·77	1·18
						AO	15·0	12·8	17·6	1·87	1·33
						MO	19·0	17·8	20·3	1·28	1·06
R676	$-\overset{O}{\overset{\|}{C}}-N(CH_3)$	H	CH_3		base	A	24·5	21·7	27·7	1·60	1·13
						M	24·2	22·8	25·7	1·16	1·04
						CH	40·2	23·4	69·1	2·43	2·40
R876	"	H	H		HCl	A	79·9	71·3	89·5	1·45	1·18
						M	77·0	68·1	87·0	1·55	1·23
R881	$-\overset{O}{\overset{\|}{C}}-\overset{CH_3}{\overset{\|}{N}}-C_2H_5$	H	H	$N(CH_3)_2$	base	A	>100	—	—	—	—
						M	>25	—	—	—	—
R883	"	H	H		"	A	>100	—	—	—	—
						M	3·30	2·70	4·03	1·43	1·17
R869	"	H	H		"	A	>100	—	—	—	—
						M	4·01	3·29	4·89	1·44	1·17
R868	"	H	H		HCl	A	70·0	57·9	84·7	1·30	1·19
						M	53·8	45·3	63·1	1·26	1·16
R850	"	CH_3	H	"	base	A	26·0	23·2	29·1	1·50	1·34
						M	27·5	25·0	30·3	1·35	1·22
R732	$-\overset{O}{\overset{\|}{C}}-N(C_2H_5)_2$	H	H	$N(CH_3)_2$	base	A	>50	—	—	—	—
						M	>50	—	—	—	—
R567	"	H	H		"	A	>75	—	—	—	—
						M	6·35	5·08	7·94	1·57	1·24

TABLE IV—*continued*

Serial number	R	α	β	NAA'	Salt	Animals*	ED50	L.L.	U.L.	S	f_s
R945	"	CH_3	H	"	"	A	>50	—	—	—	—
						M	19·0	16·7	21·7	1·35	1·10
R974	"	H	CH_3	"	"	A	>25	—	—	—	—
						M	4·08	3·11	5·34	1·46	1·22
R558	"	H	H		"	A	62·0	57·1	67·3	1·18	1·08
						M	60·0	55·1	65·4	1·24	1·06
R775	"	CH_3	H	"	"	A	32·1	29·2	35·2	1·34	1·05
						M	42·2	37·7	47·3	1·35	1·06
R545		H	H	$N(CH_3)_2$	"	A	118·0	95·2	146	1·59	1·32
						M	77·0	65·3	90·7	1·37	1·15
R554	"	CH_3	H	"	"	A	16·3	14·8	17·9	2·16	1·33
						M	16·5	14·6	18·7	2·35	1·42
R616	"	H	CH_3	"	HCl	A	>100	—	—	—	—
						M	>20	—	—	—	—
R561	"	H	H	$N(C_2H_5)_2$	"	A	>100	—	—	—	—
						M	91·0	69·5	19·2	1·55	1·55
R535	"	H	H		base	A	89·5	71·2	111·3	1·58	1·57
						M	>20	—	—	—	—
R695	"	CH_3	H	"	"	A	20·9	18·8	23·2	1·41	1·11
						M	16·0	13·8	18·6	1·60	1·22
R720		H	CH_3		base	A	>50	—	—	—	—
						M	>50	—	—	—	—
R540	"	H	H		"	A	70·0	61·4	79·8	1·30	1·10
						M	15·0	12·6	17·9	1·40	1·28
						CH	12·0	8·51	16·9	1·99	1·40
R675	"	CH_3	H	"	"	A	13·2	11·9	14·7	2·14	1·28
						M	12·3	11·1	13·5	1·52	1·11
						AO	33·5	25·6	43·9	2·67	1·84
						MO	43·9	36·6	52·7	1·90	1·30
						AR	>25	—	—	—	—
						CH	2·90	1·84	4·58	3·25	1·65
R888	"	CH_3	H	"	dextro-base	A	7·80	6·96	8·74	1·70	1·26
						M	10·1	8·56	11·9	2·15	1·60
						AO	15·7	13·4	18·4	2·06	1·46
						MO	19·7	17·6	22·1	1·60	1·19
						AR	12·5	11·2	14·0	1·68	1·16
						CH	1·71	1·08	2·70	3·25	1·65
R982	"	H	CH_3	"	HCl	A	>50	—	—	—	—
						M	13·0	10·7	15·9	1·17	1·15
						CH	33·0	19·6	55·4	1·96	1·89
R530	"	H	H		base	A	13·6	12·7	14·6	1·64	1·09
						M	14·6	14·0	15·2	1·29	1·03
						AO	63·0	55·3	71·8	1·30	1·22
						MO	60·8	55·8	66·3	1·18	1·08
						AR	31·5	23·7	41·9	1·74	1·41
						CH	25·3	16·9	38·0	2·25	1·88
R610	"	CH_3	H	"	"	A	1·25	1·13	1·39	1·79	1·09
						M	1·50	1·38	1·64	1·58	1·05
						AO	4·75	4·32	5·23	1·58	1·20
						MO	7·00	6·19	7·91	1·49	1·24
						AR	0·64	0·50	0·82	1·49	1·44
						CH	5·00	3·60	6·95	2·11	1·31
R875	"	CH_3	H	"	dextro-base	A	0·645	0·58	0·72	1·64	1·07
						M	0·725	0·64	0·82	1·78	1·10
						AO	3·20	2·83	3·62	1·89	1·23
						MO	5·20	4·33	6·24	2·28	1·46
						AR	0·37	0·33	0·40	1·21	1·06
						CH	3·72	3·05	4·54	1·68	1·17
R898	"	CH_3	H	"	laevo-base	A	>150	—	—	—	—
						M	>150	—	—	—	—
						CH	>100	—	—	—	—

TABLE IV—*continued*

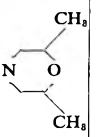
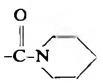
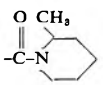
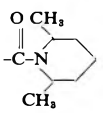
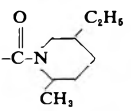
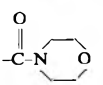
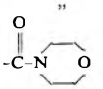
Serial number	R	α	β	NAA′	Salt	Animals*	ED50	L.L.	U.L.	S	f_s
R652	〃	H	CH_3	〃	base	A M CH	57·0 46·9 50·0	50·4 40·4 31·3	64·4 54·4 80·0	1·46 1·53 2·26	1·07 1·09 2·10
R822	〃	H	H		HCl	A M	63·0 69·8	53·8 60·7	73·7 80·3	1·50 1·43	1·15 1·12
R733		H	H	$N(CH_3)_2$	base	A M	>100 >100	—	—	—	—
R155	〃	H	H	$N(iC_3H_7)_2$	HCl	A M	>100 >25	—	—	—	—
R57 (ref. 15)	〃	H	H		base	A M	>100 16·4	— 12·5	— 21·5	— 1·94	— 1·30
R550	〃	H	H		〃	A M	73·0 87·5	62·9 79·6	84·7 96·3	1·40 1·23	1·24 1·05
R641	〃	CH_3	H	〃	〃	A M	59·0 77·0	53·9 68·1	64·6 87·0	1·33 1·37	1·13 1·20
R579		H	H	〃	HCl H_2O	A M	>200 >200	—	—	—	—
R687		H	H	〃	base	A M	>100 >100	—	—	—	—
R601		H	H	〃	〃	A M	>100 87·5	— 74·2	— 103	— 1·31	— 1·18
R66		H	H	$N(CH_3)_2$	〃	A M	>200 >25	—	—	—	—
R74	〃	H	H	〃	CH_3I	A	>100	—	—	—	—
R73		H	H	$N(C_2H_5)_2$	HCl	A M	140 164	114 143	172 189	1·29 1·25	1·24 1·12
R149	〃	H	H	$N(iC_3H_7)_2$	base	A M	>100 >75	—	—	—	—
R147	〃	H	H		〃	A M	>100 12·1	— 9·31	— 15·7	— 1·67	— 1·16
R152	〃	H	H	〃	CH_3I	A M	>100 >25	—	—	—	—
R151	〃	H	H	〃	C_2H_5Br	A M	>100 >25	—	—	—	—
R56	〃	H	H		base	A M	>100 12·5	— 9·12	— 17·1	— 1·55	— 1·19
R144	〃	H	H	〃	CH_3I	A M	>100 >25	—	—	—	—
R67	〃	H	H		base	A M	95·0 >25	89·6 —	100·7 —	1·12 —	1·10 —

TABLE IV—*continued*

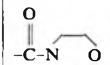
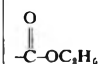
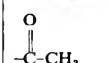
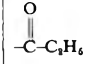
Serial number	R	α	β	NAA′	Salt	Animals*	ED50	L.L.	U.L.	S	r_g
R628		CH_3	H		base	A M	58·8 95·0	55·0 79·2	62·9 114	1·28 1·47	1·03 1·08
R642	"	H	CH_3	"	"	A M	70·0 65·0	64·2 58·0	76·3 72·8	1·10 1·37	1·07 1·18
R64		H	H		$C_4H_4O_4$	A M	37·0 8·10	31·4 6·75	63·7 9·72	1·52 1·40	1·13 1·09
R609	"	H	H		base	A M	19·2 23·7	17·8 19·9	20·7 28·2	1·16 1·65	1·06 1·36
R934	"	H	H		"	A M	32·5 43·2	28·8 34·8	36·7 53·6	1·69 1·70	1·30 1·60
R618		H	H		HCl	A M	35·8 50·5	31·1 43·2	41·2 59·1	1·47 1·60	1·17 1·23
R743		H	H	$N(CH_3)_2$	HCl	A M CH	11·6 13·2 18·3	10·3 12·3 14·4	13·1 14·1 23·2	1·55 1·22 1·31	1·20 1·04 1·27
Iso-methadone	"	CH_3	H	"	HCl H_2O	A M	7·90 10·0	7·12 9·00	8·77 11·1	1·52 1·50	1·10 1·10
Methadone	"	H	CH_3	"	HCl	A M AO MO AR CH	5·18 4·75 26·5 34·1 4·90 4·85	4·80 4·48 21·5 27·5 3·92 2·98	5·59 5·04 32·6 42·3 6·13 7·91	1·53 1·23 1·79 1·92 1·55 2·23	1·19 1·04 1·42 1·49 1·40 2·08
R738	"	H	H		base	A M	14·3 12·2	13·0 11·0	15·7 13·5	1·35 1·44	1·11 1·15
R892	"	CH_3	H	"	HBr ½ H_2O	A M	17·2 17·2	15·2 15·2	19·4 19·4	1·73 1·43	1·34 1·16
R833	"	H	CH_3	"	HCl	A M	6·82 7·09	6·14 6·39	7·57 7·87	1·58 1·53	1·20 1·18
R288	"	H	H		HCl	A M CH	16·2 14·5 7·82	14·5 12·6 5·32	18·1 16·7 11·5	1·51 1·87 1·87	1·20 1·44 1·30
R836	"	CH_3	H	"	HCl	A M	12·8 15·9	10·9 14·2	15·1 17·8	1·80 1·38	1·36 1·12
R831	"	H	CH_3	"	HBr	A M	6·20 6·30	5·49 5·73	7·01 6·93	1·55 1·43	1·21 1·13
R607	"	H	H		base	A M	6·15 8·75	5·77 7·74	6·55 9·89	1·17 1·27	1·07 1·12
R783	"	CH_3	H	"	"	A M	77·5 90·5	53·8 65·6	112 125	2·47 2·00	1·89 1·54
Phenadoxone	"	H	CH_3	"	HBr	A M AO MO AR CH	2·45 3·08 15·2 22·8 2·30 7·95	2·15 2·77 13·9 20·2 1·97 6·97	2·79 3·42 16·6 25·8 2·69 9·06	1·60 1·33 1·58 1·48 1·37 1·16	1·32 1·13 1·18 1·24 1·13 1·11
R770	"	H	H	$N(C_2H_5)_2$	base	A M	30·9 35·8	24·1 29·1	39·6 44·0	1·88 1·33	1·27 1·11
R744	"	H	H	$N(nC_3H_7)_2$	"	A M	>100 >100	—	—	—	—
R294	"	H	H	$N(iC_3H_7)_2$	HCl	A M	>200 >25	—	—	—	—

TABLE IV—*continued*

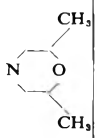
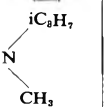
Serial number	R	α	β	NAA'	Salt	Animals*	ED50	L.L.	U.L.	S	f_s
R769	"	H	H	$N(iC_4H_9)_2$	base	A M	>100 >100	— —	— —	— —	— —
R1052	"	H	H	$N(CH_2)_6$	HCl	A M	16·8 11·6	13·9 9·83	20·3 13·7	1·85 1·45	1·46 1·22
R1093	"	H	H	$N(CH_2)_7$	$C_2H_2O_4$	A M	>25 >25	— —	— —	— —	— —
R863	"	H	H		base	A M	140 176	94·0 135	209 229	2·20 1·45	1·67 1·18
R919		CH_3	H	$N(CH_3)_2$	α-HCl	A M	32·1 55·0	28·2 43·3	36·6 70·0	1·49 2·12	1·22 1·99
R925	"	H	CH_3	"	"	A M	37·0 33·0	31·4 38·0	43·7 38·9	1·58 1·31	1·24 1·13
R895	"	H	CH_3	"	β-HCl	A M	40·0 53·2	35·1 45·5	45·6 62·2	1·56 1·68	1·27 1·37
R1078		H	CH_3	"	α-(−)-HCl	A M	7·20 10·8	5·81 9·07	8·93 12·9	1·94 1·68	1·45 1·28
R1080	"	H	CH_3	"	α-(+)-HCl	A M	1·50 2·0	1·29 —	1·74 —	1·55 —	1·22 —
R1079	"	H	CH_3	"	β-(−)-HCl	A M	1·23 >1·5	1·02 —	1·49 —	1·80 —	1·60 —
R950		CH_3	H	$N(CH_3)_2$	2 HCl	A M	55·0 39·5	47·4 32·9	63·8 47·4	1·42 1·73	1·15 1·28
R878	"	CH_3	H	$N(C_2H_5)_2$	2 HCl 3/2 H_2O	A M	>100 >100	— —	— —	— —	— —
R662	"	CH_3	H		base	A M	19·0 26·6	16·1 24·2	22·4 29·3	2·15 1·39	1·34 1·07
R832	"	CH_3	H		2 HCl	A M	22·2 >25	17·0 —	29·1 —	2·33 —	1·48 —
R877	"	CH_3	H		2 HCl H_2O	A M	61·0 37·3	41·5 27·8	89·7 50·0	2·23 1·60	1·90 1·34
R290		H	H		HCl	A	>200	—	—	—	—
R293	"	H	H	$N(iC_3H_7)_2$	"	A	>200	—	—	—	—
R247	"	H	H		"	A	>200	—	—	—	—
R916	"	CH_3	H	$N(C_2H_5)_2$	"	A M	>12·5 >12·5	— —	— —	— —	— —
R904	"	CH_3	H		"	A M	>25 >25	— —	— —	— —	— —
R835	"	CH_3	H		"	A M	37·9 47·3	33·0 43·2	43·6 53·0	1·55 1·43	1·27 1·16
R713	"	CH_3	H		"	A M	7·10 8·90	6·17 7·95	8·17 9·97	1·97 1·60	1·35 1·19

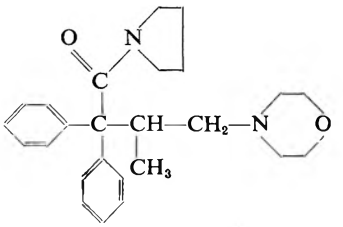
TABLE IV—*continued*

	Animals*	ED50	L.L.	U.L.	S	f_s
Morphine hydrochloride	A	12·0	11·2	12·8	1·69	1·08
	M	13·1	11·5	14·9	2·35	1·41
	AO	68·0	60·7	76·2	1·49	1·10
	MO	92·0	75·4	112·2	2·08	1·38
	AR	15·0	12·4	18·2	1·47	1·15
	CH	15·0	9·87	22·8	2·14	1·52
Morphine sulphate	A	10·5	9·38	11·8	1·66	1·33
Diacetylmorphine (heroin)	A	2·00	1·84	2·18	1·52	1·15
	M	2·03	1·85	2·24	1·59	1·17
	AR	1·00	0·85	1·17	1·20	1·12
	CH	10·8	6·39	18·3	2·79	2·03
Codeine phosphate	A	53·0	48·2	58·3	1·52	1·22
	M	70·0	61·4	79·8	1·58	1·32
	AR	142	118	170	1·45	1·20
	CH	32·5	20·3	52·0	2·16	1·98
Ethylmorphine HCl (Dionine)	A	55·8	45·7	68·1	1·75	1·26
	M	100	82·0	122	1·67	1·24
Levorphan	A	3·00	2·56	3·51	1·84	1·45
	M	2·94	2·21	3·91	2·98	2·25
Nalorphine HBr	A	>100	—	—	—	—
	M	>100	—	—	—	—
	AR	>75	—	—	—	—
Pethidine HCl	A	28·0	25·7	30·5	1·42	1·12
	M	21·5	19·5	23·7	1·27	1·07
	AO	65·5	59·5	72·1	1·35	1·14
	MO	69·5	62·1	77·8	1·41	1·18
	AR	54·5	45·0	66·0	1·45	1·21
	CH	56·0	38·1	82·3	1·56	1·40
N-Morpholino-ethyl-norpethidine 2 HCl (ref. 22)	A	18·6	15·0	23·1	2·14	1·46
	M	36·5	29·7	44·9	1·83	1·35
	AR	23·0	17·7	29·9	1·51	1·31
	CH	109·0	90·8	131	1·64	1·21
4-Carbethoxy-1-(2-hydroxy-2-phenethyl)-4-phenyl-piperidine HCl (ref. 27)	A	11·4	10·2	12·8	1·41	1·18
	M	14·4	12·6	16·4	1·59	1·30
	CH	36·0	24·2	53·6	1·92	1·64
1:1-(Di-2-(thienyl)-3-(*N*-piperidino)-buten-1-ylamine HCl (ref. 22)	A	6·50	5·99	7·05	1·28	1·05
	M	6·40	5·82	7·04	1·42	1·10
	CH	18·9	15·2	23·4	1·41	1·32
Propoxyphene HCl (ref. 26)	A	65·8	57·7	75·0	1·34	1·09
	M	76·0	64·4	89·7	1·43	1·25
	CH	67·0	52·3	85·8	1·63	1·26
Atropine sulphate	A	>100	—	—	—	—
	M	0·096	0·084	0·109	1·40	1·10
	CH	16·5	11·8	23·1	6·14	1·99
Papaverine HCl	A	>100	—	—	—	—
	M	>100	—	—	—	—
	CH	95·0	73·6	123	2·19	1·33
Adiphenine HCl	A	>100	—	—	—	—
	M	47·8	41·6	55·0	1·43	1·22
	CH	>100	—	—	—	—

of this property and the analgesic activity in mice or rats (Fig. 6). The ED50 (charcoal) to ED50 (analgesia) ratio for methadone, morphine and codeine is not significantly different from one. Heroin, R 875 and its *N*-morpholino-analogues have a ratio of 5 to 10, whereas the *N*-piperidino-analogues of R 875 are surprisingly active in the charcoal-test (ratio of 0·2 to 0·6). Pethidine has a ratio of about 2.

It is reasonable to assume an inverse relation between ED50 (charcoal) to ED50 (analgesia) ratio and the constipating effect of analgesics.

R 875, the dextrorotatory isomer of (2:2-diphenyl-3-methyl-4 morpholino-butyryl-pyrrolidine (II), appears to be worth more investigation. It has twice the analgesic activity in mice and rats as the racemic mixture R 610 (the laevorotatory isomer, R 898, is inactive), and is more active than any other analgesic we have tested (Tables II and IV).



(II)

(±)—R610. (+)—R875. (−)—R898.

Summary

1. Some pharmacological properties of a new series of secondary and tertiary basic amides are described.

2. The relation between the analgesic activity in mice and rats, and the chemical structure of these amides is discussed.

3. The *dextro*-rotatory isomer of 2:2-diphenyl-3-methyl-4-morpholino-butyrylpyrrolidine, R 875, is more active as an analgesic in mice and rats than any other compound tested.

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THE ESTIMATION OF THE COMPONENT CARDIAC GLYCOSIDES IN DIGITALIS PLANT SAMPLES

PART III. THE SEPARATION AND ESTIMATION OF THE GENINS AND ANHYDROGENINS

BY H. SILBERMAN* AND R. H. THORP

From the Department of Pharmacology, University of Sydney, Sydney, Australia

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IN two previous communications[1,2] methods were described for the separation of the "genuine" and desglucoglycosides of plants of *Digitalis* species using chromatography on unimpregnated paper sheets. It seemed possible to use these methods to separate the much less polar steroid nucleus of the cardiac glycosides. The genins of the digitalis glycosides, digitoxi-, gitoxi- and digoxigenin, are only very slightly soluble in water and it has been considered by Kaiser[3] and others that separation by partition between two phases one of which is water or mostly water could hardly provide an adequate driving force for their separation.

These considerations have been responsible for the wide and successful application of formamide impregnated paper in the chromatographic separation of the cardiac glycosides by Reichstein and Schindler[4], Jensen[5] and many others. On the other hand Bush[6] had shown conclusively that with an appropriate choice of solvents and careful control of the conditions it is possible to use unimpregnated paper for the separation of many steroids especially of the adrenocortical and sex hormone series which are also almost insoluble in water. The resolution in these instances is sometimes poor owing to a marked tailing, resulting from the strong adsorption of the steroid on the paper. But, with carefully controlled conditions, the genins and anhydrogenins of digitalis can be completely separated; the spots are well defined and separated by colourless intervals so that they can be cut out for elution.

This completes the study of an alternative procedure for the separation of these glycosides and offers an additional system on which their identity and purity may be confirmed. That such non-polar substances can be separated so well is a further indication of the complexity of the factors influencing separation by partition paper chromatography.

For the genins the mobile phase was a mixture of 80 parts of chloroform and 20 parts of benzene equilibrated with 50 parts of water; for the anhydrogenins a mixture of 10 to 20 parts of chloroform with 90 or 80 parts of benzene similarly equilibrated with water was used. The ascending method of irrigation is used most often as described previously.

The separation of the genins of the A series from those of the B and C series did not present difficulty as the R_F value of digitoxigenin is very much greater than that for gitoxigenin or digoxigenin.

* Commonwealth Drug Analyst.

The separation of the last two however is more difficult, especially when digoxigenin is present in high concentration and the use of the descending method of irrigation is necessary to achieve complete separation. If the ascending method is used the spots are more nearly circular whereas in the descending method the length of the spot in the direction of solvent flow may be $1\frac{1}{2}$ to 3 times the width.

This is parallel to the results found in the separation of the desglucoglycosides described previously. It is to be noted that the sequence of the two positional isomers of the aglycones and desglucoglycosides is reversed: whereas gitoxigenin travels faster than digoxigenin and parallels lanatosides B and C, the opposite is the case when comparing the faster moving digoxin which precedes gitoxin.

TABLE I

R_F VALUES OF GENINS AND ANHYDROGENINS OF DIGITALIS GLYCOSIDES

A series	R_F	B series	R_F	C series	R_F
Solvent: Chloroform 80, benzene 20, water 50					
Digitoxigenin	0·82	Gitoxigenin	0·42	Digoxigenin	0·33
Solvent: Chloroform 20, benzene 80, water 50					
Digitoxigenin	0·66	Gitoxigenin	0·15	Digoxigenin	0·09
Digitoxigenin acetate	0·82				
"α + β" Anhydrodigitoxigenin	0·96	Dianhydrogitoxigenin	0·88	"α + β" Anhydrodigoxigenin	0·60
"β" Anhydrodigitoxigenin	0·96			"β" Anhydrodigoxigenin	0·60
"β" Anhydrodigitoxigenin acetate	S.F.				

The anhydrogenins separate much more readily since the R_F values of anhydrodigitoxigenin and anhydrodigoxigenin are different, as can be seen from Table I, and the compound of the β series is dianhydrogitoxigenin (Δ 14,16) which shows an R_F value more remote from the other two than with the genins.

In the formation of the anhydrogenins the two possible isomers corresponding to the (Δ 8 : 14) and (Δ 14 : 15) position of the double bond are usually obtained and their chromatographic behaviour has been examined.

The "β" ("β" = Δ 14:15; "α" = Δ 8:14)[7] anhydrodigitoxigenin was prepared by the fractional crystallisation described by Smith[8]. When this substance was compared with a mixture of "α" and "β" anhydrogenins obtained by the usual treatment of digitoxigenin, separately or mixed with the latter, no separation could be achieved by these paper chromatographic methods. It is obvious that the resolution is inadequate to differentiate between compounds of this type differing only in the position of the double bond around the same carbon atom C(14) in the molecule.

Similar results were obtained by comparing "β" anhydrodigoxigenin with a mixture of the "α" and "β" isomers and again no separation could be achieved.

As an example of the efficiency of this chromatographic procedure for analytical work a sample of digoxin (m.p. 265° $[\alpha]_D$ + 12°) was hydrolysed by the method of Smith[9] and the genin thus obtained was twice recrystallised from ethanol-water. The correct melting point (221°) was obtained and the normal specific rotation ($[\alpha]_D$ + 18° ethanol). Upon chromatography using the solvent mixture described herein some anhydrogenin was clearly indicated by the presence of a second spot which was identified by comparison with an authentic sample of anhydrodigoxigenin. Two further recrystallisations reduced the amount of anhydrogenin very considerably although a trace still remained. To achieve complete purity of the genin, alumina column chromatography was necessary. The genin prepared from lanatoside C by the method of Stoll and Kreis[10] behaved in exactly the same way.

The hydrolysis of commercially available digitoxin, by the method of Windaus and Stein[11] resulted in 3 or sometimes 4 spots on the developed chromatogram, two of which corresponded to digitoxigenin and gitoxigenin respectively and the remainder to anhydrodigitoxigenin and dianhydrogitoxigenin when a fourth spot was observed.

When digitoxin was previously purified by partition chromatography as described by Silberman and Thorp[12] and the gitoxin-free material thus obtained was hydrolysed, only the genin and anhydrogenin of digitoxin was detected.

Harrison and Wright[13] of these laboratories have correlated the chromatographic placement and chemical structure of a considerable number of digitalis glycosides and have shown that in "reversed phase" chromatography proposed by Tschesche, Grimmer and Seehofer[14], using paper impregnated with medium molecular weight alcohols (amyl to octyl alcohol) as the stationary phase, changes in the aglycone part of the molecule have a decisive influence in determining the R_F value. These workers found that there was very little difference between the R_F values of the desglucoglycosides and the corresponding aglycones of the A, B and C series. With formamide impregnated paper, although the range of R_F values is much wider, here again the desglucoglycosides were found to run next to the corresponding aglycones.

With the use of unimpregnated paper, saturated with the vapour of the solvent mixture to form the stationary phase, the separation of the glycosides is conditioned strictly by the presence and number of sugar molecules in the compound so that the two systems make use of quite different chemical features of these compounds.

The system at present described is therefore complementary to those previously described and is of particular value in the examination of plant extracts where both "genuine" and desglucoglycosides occur and has also proved very useful in a study of the degradation of cardiac glycosides by ultra-violet irradiation where the progressive disappearance of the starting compound and corresponding formation of secondary products could be followed[15].

The authors regard the chromatographic separation on unimpregnated paper, on formamide paper and on the "reversed phase" system as

complementary in the sense that many compounds which are adjacent or closely placed on one system are widely separated on another and the use of the several methods provides valuable corroborative evidence of their nature or identity.

It has frequently been pointed out that in this field the determination of the R_F value is not a reliable method for the identification of cardenolides since these values are variable and influenced by the composition of the mixture and external conditions; but the application of two or more systems to the problem renders the determination of a definite R_F value much less important.

One disadvantage of the use of unimpregnated paper is the small quantity of material which can be applied to the spot compared with the much greater capacity of the formamide system, but this can be partly overcome by the use of thicker paper such as Whatman 3 MM which gives good resolution with 20 to 30 μg. of each component in a mixture compared with 3 to 5 μg. for Whatman N1 paper. Larger quantities cause streaking and tailing.

Satisfactory fluorescent chromatographic spots are produced by trichloroacetic acid which gives a strong reaction with the genins and anhydrogenins and the colours follow closely those found with the corresponding desglucoglycosides. No colour or fluorescence could be produced with trichloroacetic acid and 14-desoxydigitoxigenin or its acetate or with 14-desoxydigoxigenin although variations in temperature and heating time were tried. Dihydrodigoxin gives the reaction although not as strongly as digoxin itself. The reduced sensitivity in this instance where the lactone ring is hydrogenated and the loss of the reaction in the 14-desoxy-compounds may be of value in future rationalisation of the mechanism of this reaction.

Experimental

The solvents and apparatus were those described in previous communications[1,2]. The spots were applied to the papers and allowed to equilibrate overnight. They were then irrigated for $1\frac{1}{2}$ to 2 hours at a temperature of $23° \pm 3°$. The completeness of equilibration is not as important with the genins as it is with the genuine glycosides neither is the control of temperature. There is similarity in this case with the desglucoglycosides which also do not require such critical conditions. For the quantitative separation of digoxigenin from gitoxigenin paper sheets 4 in. wide by 14 in. long were used in the descending method and the time of irrigation was extended to 8 to 9 hours (23°).

The R_F values for the two solvent mixtures are given in Table I. The compounds used were prepared by the following methods. The genins and dianhydrogitoxigenin were prepared by known methods (*loc. cit.*) and purified by alumina column chromatography.

The mixed "α" and "β" and pure "β" anhydrodigoxigenin was kindly prepared and supplied by Mr. B. T. Brown of this Department. The mixed "α" and "β" anhydrodigitoxigenin was prepared by the method of Cloetta[16], and an attempt to separate the mixture on an alumina column

was unsuccessful, as the different fractions did not show progressive enrichment of one isomer.

A strongly enriched but not completely pure sample of "β" anhydrodigitoxigenin was prepared by fractional crystallisation of the mixture of the isomers. After six alternate recrystallisations from ethyl acetate and acetone a material with a m.p. 197 to 198° (suit 195) and $[\alpha]_D$ — 11° (methanol) was obtained in 10 per cent yield. Smith gives m.p. 202 $[\alpha]_D$ — 13° (methanol).

Pure "β" anhydrodigitoxigenin-acetate was prepared after Hunsiker and Reichstein[17].

A solution of 1·0 g. of digitoxigenin acetate in 12 ml. of pure dry pyridine was prepared, cooled in ice and 3·0 ml. of freshly double distilled phosphorus oxychloride was added dropwise with shaking and the mixture left in ice for a further ½ hour. The mixture was then orange brown and was kept at room temperature for 20 hours and then poured slowly on to 50 g. of ice. The solid white precipitate was repeatedly extracted with a mixture of 3 parts of chloroform and 1 part of ether. The collected extract was then washed free of pyridine with 0·5N hydrochloric acid followed by a 5 per cent solution of sodium bicarbonate and water. The mixture was dried and the solvent removed under reduced pressure. The solid residue was recrystallised twice from ether containing some chloroform. 0·55 g. of "β" anhydrodigitoxigenin acetate was obtained m.p. 187 to 188·5° (lit. 183°) and a further 0·31 g. was separated from the mother liquor showing an extended melting range 160 to 195°.

Summary

1. A chromatographic study of the genins and anhydrogenins of digitalis is described.

2. Complete separation of the anhydrogenins is readily achieved but the separation of gitoxigenin and digoxigenin is less satisfactory although both of these are readily separable from digitoxigenin.

3. The R_F values for these derivatives from the A, B and C series of glycosides are tabulated for several solvent mixtures used for chromatography on unimpregnated paper.

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THE ASSAY OF OXYTOCIN IN THE PRESENCE OF VASOPRESSIN ON THE DIOESTRUS UTERUS OF THE RAT

By Mary F. Lockett and G. E. Owen

From the Department of Physiology and Pharmacology, Chelsea Polytechnic, London, S.W.3

Received December 28, 1956

Extracts of whole posterior pituitary lobes of cattle are known to contain oxytocin and vasopressin in nearly equal proportions. Since oxytocin is assayed in these extracts, the circumstances under which, and the extent to which, vasopressin may interfere with these estimates should be known. Oxytocin is now assayed either by its depressor action on the fowl's blood pressure[1] or by the graded contractions which it induces in the rat's dioestrus uterus[2]. Whereas the possible interference by vasopressin in the assay of oxytocin by the fowl depressor method has been investigated[3], no such study has been made of the rat uterus method. The purpose of this investigation has therefore been to define, if possible, the conditions under which the dioestrus uterus of the rat can be used reliably to assay oxytocin contained in extracts of powders made from whole posterior lobes of the pituitary body.

Previous work on the uterus of the virgin guinea pig by Fraser[4] and by Stewart[5] showed that vasopressin exerted increasing oxytocic action on the uterus of this species as the magnesium content of the Ringer's solution was increased. Stewart[5] found, over all, that increase in magnesium concentration sensitised the guinea pig uterus more to vasopressin than to oxytocin, and that these uteri were almost equally sensitive to the two hormones at a concentration of 0·1 per cent (w/v) of magnesium chloride. Varying concentrations of calcium were also found to influence the sensitivity of the guinea pig uterus differently to oxytocin and to vasopressin. Concentrations of calcium chloride ranging from 0·029 to 0·034 per cent (w/v) predominantly potentiated the action of oxytocin on the guinea pig uterus, whereas higher concentrations of calcium chloride, ranging from 0·034 to 0·049 per cent (w/v), increased the responses of these uteri more to vasopressin than to oxytocin.

This earlier work on the guinea pig uterus has largely directed our study of the effect of variation in the concentration of individual ions on the responses of the dioestrus uterus of the rat to oxytocin and to vasopressin.

Methods

Single horns from the dioestrus uteri of adult white rats were suspended from frontal writing levers in 20 ml. baths of aerated Ringer's solution at 28°. Contractions of uteri were magnified from three to five times in different experiments.

Dose-effect curves. Selected doses of neurohypophyseal hormone, measured in milliunits (mU.), were given in a random order at 5 minute intervals. Each dose was repeated three times. The fluid was then

changed. Regular doses were continued in the new fluid until the responses of the tissue had become constant at a single dose level; then the dose-effect curve was re-established. The fluid was again changed. The whole process was repeated several times.

Assays of oxytocic activity were made according to the method described by Holton[2], except that the Ringer was modified, and the temperature used was 28°, not 32°.

Hormones. The preparations of oxytocin and vasopressin used were Pitocin (batch number LT803F) and Pitressin (batch number LT507F). Each of these commercially prepared hormones may be expected to contain 4 per cent of the other.

TABLE I

THE COMPOSITION OF RINGER SOLUTIONS. MEASURED VOLUMES OF PER CENT (W/V) SOLUTIONS OF A SINGLE SALT IN DISTILLED WATER WERE ADDED TO 800 ML. OF A BASIC SOLUTION (SEE TEXT) AND THE WHOLE WAS DILUTED TO 1 LITRE

Ringer Code No.	Basic solution	Additional compound	
		Added per litre	Final concentration
		magnesium chloride	mg./litre
I	A	0·5 ml. of 0·01 per cent	0·05
II	A	10·0 ml. of 10·0 "	1000
III	A	5·0 ml. of 1·0 "	50
IV	A	5·0 ml. of 10·0 "	500
		calcium chloride	g./litre
V	B	none	0·03
VI	B	7·5 ml. of 1·2 per cent	0·12
VII	B	2·5 ml. of 1·2 "	0·06
		potassium chloride	g./litre
VIII	C	none	none
IX	C	2·0 ml. of 10·0 per cent	0·2
X	C	4·2 ml. of 10·0 "	0·42
XI	C	0·7 ml. of 10·0 "	0·07

Ringer's solutions. Ringer's solution used for the study of the effect of change in magnesium concentration. A basic solution A, made freshly each day, contained the following substances in g./800 ml. of glass distilled water: NaCl, 9·0; KCl, 0·42; $CaCl_2$, 0·12; $NaHCO_3$, 0·5; glucose, 0·5. Ringer solutions of varied magnesium content were made by adding appropriate amounts of $MgCl_2$ in solution to 800 ml. of basic solution A, then diluting to 1 litre with distilled water (Table I).

Ringer's solution used for the study of the effect of change in calcium concentration. A basic solution B was freshly prepared each day to contain the following compounds in g./800 ml. of glass distilled water: NaCl, 9·0; KCl, 0·42; $MgCl_2$, 0·001; $CaCl_2$, 0·03; $NaHCO_3$, 0·5; glucose, 0·5. Ringer's solutions of varied calcium content were made by adding appropriate amounts of calcium chloride in solution to 800 ml. of the basic solution B, then diluting to 1 litre with distilled water (Table I).

Ringer's solution used for the study of the effect of change in potassium concentration. A basic solution C was prepared freshly each day, and contained the following compounds in g./800 ml. glass distilled water: NaCl, 9·0; $CaCl_2$, 0·12; $MgCl_2$, 0·001; $NaHCO_3$, 0·5; glucose, 0·5.

Ringer solutions of varied potassium content were made by adding potassium chloride solutions of known strength to 800 ml. of basic solution C, then diluting to 1 litre with glass distilled water (Table I).

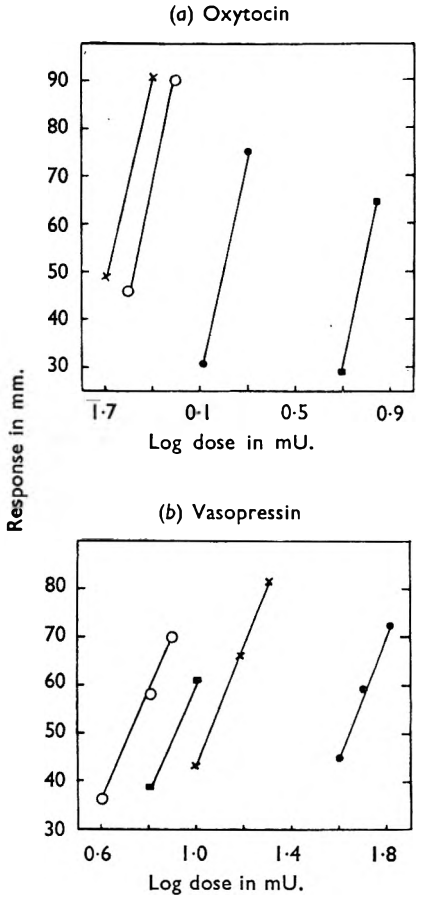

FIG. 1. The effect of variation in the magnesium content of the Ringer on the dose-response curves for the actions of oxytocin and vasopressin on horns from the dioestrus uterus of the rat.
Concentration of $MgCl_2$ mg./l.:—closed circles, 0·05; crosses, 50; open circles, 500; black rectangles, 1000.

RESULTS

Firstly, the changes in the sensitivity of the rat's dioestrus uterus to oxytocin and to vasopressin with alteration in the concentration of single ions have been measured separately for the two hormones. Secondly, a solution which contained both oxytocin and vasopressin in equal proportion has been assayed. Oxytocin was used as a standard. A series of Ringer's solutions was employed in which either the magnesium or the calcium concentration was varied.

The effect of variation in the concentration of magnesium chloride in the Ringer on the dose-response curve for the action of oxytocin on the dioestrus uterus of the rat was studied in five experiments. Four different concentrations of magnesium chloride were used (Table I). Typical results are shown in Figure 1*a*. In each of these five experiments maximum sensitivity of the uterus to oxytocin was found at a concentration of 50 mg. $MgCl_2$/l., and minimum sensitivity at a concentration of 1 g. $MgCl_2$/l. Uteri were only slightly less sensitive to oxytocin at a concentration of 500 mg. $MgCl_2$/l. than they were at 50 mg. $MgCl_2$/l. Figure 1*a* shows that these changes in sensitivity were brought about by shifts in the position of the dose-effect curves, without alteration in slope.

The changes induced in the dose-effect curve for vasopressin by variation in the magnesium concentration of the Ringer's solution were similarly examined in three experiments. Typical results are shown in Figure 1*b*. In each experiment, maximum sensitivity of the uterus to vasopressin was found with a concentration of 500 mg. $MgCl_2$/l., and minimum sensitivity

with a concentration of 0·05 mg. $MgCl_2$/l. A concentration of 1·0 g. $MgCl_2$/l. caused only slight depression in the responses of uteri to vasopressin. Again the changes in sensitivity were occasioned by alteration in the position of the dose-response curves, without change in the slopes of the curves.

The effects of these changes of the responses of the uteri to oxytocin and to vasopressin were reversible and reproducible.

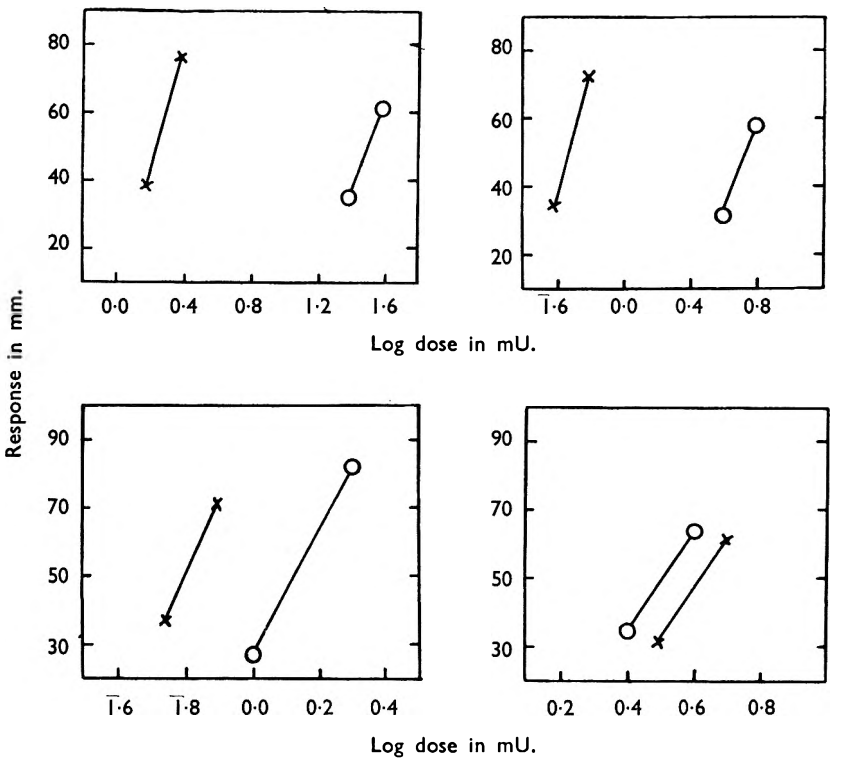

FIG. 2. The effect of variation in the magnesium concentration in the Ringer on the responses of a single rat uterine horn to oxytocin and to vasopressin.
Crosses, oxytocin; open circles, vasopressin.

The relative slopes of the dose-effect curves for the actions of vasopressin and oxytocin, and the effect of change in the magnesium concentrations of the Ringer's solution on the relative potency of these two hormones, were examined in two experiments. The results were in good agreement and are illustrated in Figure 2. First, there was no significant difference in the slopes of the dose-response curves for the actions of the two hormones. Secondly, the ratio of equally active doses, oxytocin to vasopressin decreased as the magnesium concentration in the Ringer's solution was raised from 0·05 mg./l. to 1·0 g./l. At the lower magnesium concentration the commercial oxytocin proved twenty times as effective

as commercial vasopressin; at the higher, the vasopressin was slightly more active than the oxytocin.

The effect of variation in the concentration of calcium chloride in the Ringer's solution on the dose-response curve for the action of oxytocin was examined in four experiments. Three different concentrations of calcium chloride were employed (Table I). Concentrations greater than 0·12 g. $CaCl_2$/l. were avoided, lest the uteri should develop spontaneous activity. Rat uteri did respond to oxytocin in the absence of calcium, but the contractions steadily weakened. By contrast, regular responses to a constant dose of oxytocin were obtained when the concentration of

TABLE II

THE RESULTS OF ASSAYS OF A MIXTURE OF 10 MU. OXYTOCIN AND 10 MU. VASOPRESSIN/ML. AGAINST 10 MU. OXYTOCIN/ML. ON THE RAT'S UTERUS IN RINGER OF VARIED MAGNESIUM CONTENT

Ringer		Oxytocic activity as mU. oxytocin per ml.			*b*	Assay
			Found			
Code	$MgCl_2$ mg./l.	Actual	Mean	Limits (P = 0·95)	(slope)	No.
I	0·05	10	10·1	9·3–11·0	196	11
		10	10·7	8·4–13·6	174	12
		10	11·3	9·4–13·5	70	13
		10	10·8	8·6–13·5	182	14
III	50·00	10	10·2	9·3–11·0	747	1
		10	12·2	12·1–12·3	580	5
		10	12·6	10·4–15·3	101	10
IV	500·00	10	12·5	12·4–12·6	2170	4
		10	15·5	15·3–15·7	944	7
		10	19·2	15·7–23·4	126	15
		10	18·3	13·8–24·3	112	16
		10	16·9	14·4–19·8	129	17
		10	14·6	13·0–16·3	1052	18
		10	12·9	11·6–14·4	353	19
II	1000·00	10	20·6	18·6–22·8	319	6
		10	28·5	26·4–31·5	350	9
		10	18·4	16·1–21·7	327	8

calcium chloride in the Ringer was only 0·03 g./l. Uteri became progressively more sensitive to oxytocin as the concentration of calcium chloride in the Ringer was increased from 0·03 to 0·12 g./l. Change in sensitivity took place without alteration in the slope of the dose effect curve, and was reversible and reproducible in each preparation (Fig. 3). Change in calcium concentration from 0·03 to 0·12 g. $CaCl_2$/l. had little effect on the sensitivity of the uterus to vasopressin.

The effects of variation in the potassium concentration of the Ringer's solution on the dose-response curve for the action of oxytocin were also examined. Four different concentrations of potassium chloride were used (Table I). Typical results are shown in Figure 4. Uteri proved insensitive to oxytocin in the absence of potassium. The addition of 0·07 g. KCl/l. increased the sensitivity approximately ten times. Further increase in potassium concentration, up to 0·42 g./l., caused a progressive small gain in sensitivity (Fig. 4). The changes in sensitivity with alteration in potassium concentration occurred without alteration in the slope of the dose effect curve and were reversible and reproducible.

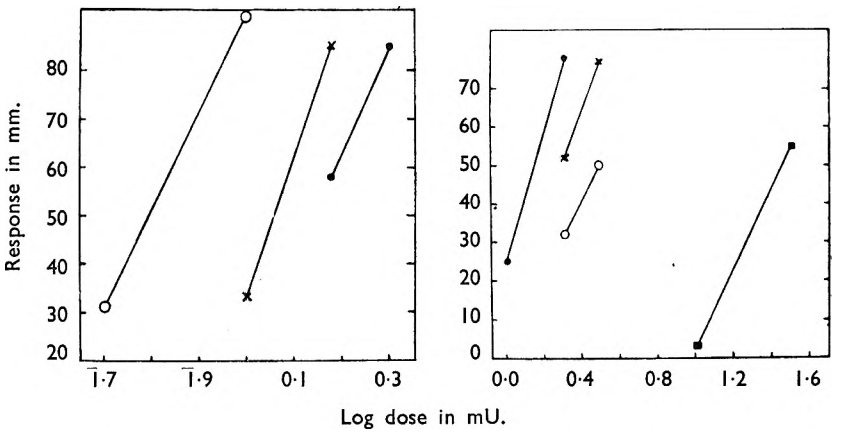

FIG. 3. The effect of variation in the calcium concentration of the Ringer on the dose-response curve for oxytocin on a horn of rat uterus.

Concentration of $CaCl_2$ g./l. :—closed circles, 0·03; crosses, 0·06; open circles, 0·12.

FIG. 4. The effect of variation in the potassium concentration of the Ringer on the dose-response curve for oxytocin on a horn of dioestrus uterus of the rat.

Concentration of KCl g./l. :—black rectangles, nil; open circles, 0·07; crosses, 0·2; closed circles, 0·42.

The assay of a mixture containing both oxytocin (10 *mU./ml.*) *and vasopressin* (10 *mU./ml.*) *against oxytocin* (10 *mU./ml.*)

The effect of variation in magnesium concentration. Assays were made in four Ringer's solutions which differed only in their content of magnesium chloride (Table I). The results of nineteen assays are given in Table II, and the information obtained from them has been summarised in Table III. These Tables show that oxytocin in a 1 : 1 mixture with

TABLE III

FURTHER ANALYSIS OF THE ASSAYS (TABLE II) OF THE MIXTURE OF OXYTOCIN AND VASOPRESSIN ON THE RAT'S UTERUS IN RINGER OF VARIED MAGNESIUM CONTENT

Ringer		Sensitivity mean dose oxytocin used mU.	Oxytocic activity in mU./ml.		
Code	$MgCl_2$ mg./l.		Actual	Found	Due to 10 mU. vasopressin/ml.
I	0·05	4·75 ± 0·44 (4)	10	10·73 ± 0·25 (4)	0·73
III	50·00	3·15 ± 1·57 (3)	10	11·63 ± 0·73 (3)	1·63
IV	500·00	3·70 ± 0·81 (7)	10	15·70 ± 1·07 (7)	5·70
II	1000·00	10·50 ± 1·55 (3)	10	22·50 ± 2·22 (3)	12·50

Values are entered as mean ±S.E. of mean (Number of Assays).

vasopressin can be reliably assayed on the dioestrus uterus of the rat in Ringer I. The concentration of magnesium chloride in this Ringer was 0·05 mg./l. Increase in the magnesium concentration to 50 mg. $MgCl_2$/l. (Ringer III) rendered uteri more sensitive to oxytocin (Fig. 1*a* and Table IV) but decreased the relative potency of oxytocin to vasopressin to 6 : 1 (Fig. 2). At 500 mg. $MgCl_2$/l. the vasopressin in the mixture had one half the activity of oxytocin, and at a concentration of 1 g. $MgCl_2$/l. the oxytocin and vasopressin were equiactive.

TABLE IV

THE RESULTS OF ASSAYS OF A MIXTURE OF 10 MU. OXYTOCIN AND 10 MU. VASOPRESSIN/ML. AGAINST 10 MU. OXYTOCIN/ML., ON THE RAT'S UTERUS, IN RINGER OF VARIED CALCIUM CONTENT

Ringer		Oxytocic activity as mU. oxytocin per ml.				
			Found			
Code	$CaCl_2$ g./l.	Actual	Mean	Limits (P = 0·95)	*b* (slope)	Assay No.
V	0·03	10	11·0	9·8–12·4	35	21*
		10	10·5	9·1–12·2	399	23
		10	10·2	9·3–11·1	389	27
		10	11·6	9·9–13·7	44	28
		10	10·0	9·3–10·8	128	32*
VII	0·06	10	10·8	9·8–11·9	37	22*
		10	11·3	9·8–13·0	241	26
		10	11·0	10·2–11·7	37	30
		10	10·1	9·2–11·0	214	31
VI	0·12	10	8·3	6·4–10·6	35	20
		10	9·5	7·7–11·8	284	24
		10	9·7	8·3–12·1	152	25

* The uterine horns used for these three assays were stored at 7° overnight. The dose levels required for these tissues were above normal; they have therefore been excluded from the estimation of sensitivity to oxytocin in the different Ringers in Table V.

The effect of variation in calcium concentration. Twelve assays were made. The three Ringer's solutions all contained 1 mg. $MgCl_2$/l., but varied in calcium concentration from 0·03 to 0·12 g. $CaCl_2$/l. The results of individual assays are shown in Table IV and the information gained from them is summarised in Table V. Oxytocin in 1 : 1 mixture with vasopressin appeared to have been reliably estimated in each of these three Ringer's solutions. The concentration of 1·0 mg. $MgCl_2$/l. was

TABLE V

FURTHER ANALYSIS OF THE ASSAYS (TABLE IV) OF THE MIXTURE OF OXYTOCIN AND VASOPRESSIN AGAINST OXYTOCIN AS STANDARD, ON THE UTERUS OF THE RAT, IN RINGER OF DIFFERING CALCIUM CONTENT

Ringer		Sensitivity mean dose oxytocin used mU.	Oxytocic activity in mU./ml.		
Code	$CaCl_2$ g./l.		Actual	Found	Due to 10 mU. vasopressin/ml.
V	0·03	0·59 ± 0·08 (3)	10	10·66 ± 0·29 (5)	+0·66
VII	0·06	0·72 ± 0·07 (3)	10	10·80 ± 0·51 (4)	+0·80
VI	0·12	0·53 ± 0·07 (3)	10	9·10 ± 0·44 (3)	−0·90

Values are entered as means ±S.E. of mean (number of assays).

preferred to 0·05 mg. $MgCl_2$/l. because the uteri developed less spontaneous activity and were more sensitive to oxytocin (cf. columns 3, Tables III and V). Of the three calcium concentrations examined, 0·12 g. $CaCl_2$/l. was preferred for assay work. Firstly, uteri gave constant responses to a fixed dose of oxytocin in Ringer VI after but short delay. Secondly, the latent period preceding the contractions was shorter in this Ringer's solution than in others. Thirdly, the uteri relaxed readily after contraction occurred.

No significance should be attached to variations in the slopes of the curves which were obtained in different assays, because neither the lever

weights nor the magnification given by the levers were standardised in these experiments. In none of these assays did the slopes of the curves given by oxytocin alone differ significantly from the slope of the curve given by the 1 : 1 mixture of oxytocin and vasopressin.

Discussion

The purpose of this investigation was to define conditions under which the oxytocin present in extracts of posterior lobes of the pituitary body could be reliably assayed on the rat's dioestrus uterus. This object has been achieved, for correct estimates of the oxytocin in 1 : 1 mixture with vasopressin were given in Ringer's solutions numbers I, V, VI, and VII (Table I). Solution VI was preferred for assay work for reasons given.

The effect of variation in magnesium concentration on the dose-response curves for the actions of oxytocin and vasopressin was similar whether these hormones were examined alone (Fig. 2) or in a mixture (Table III). The actions of the two hormones in 1 : 1 mixture was therefore additive. Moreover, their dose-effect curves were parallel; this has been shown both in Figure 2 and by the fact that there was no significant deviation from parallelism between the curves for oxytocin and for the 1 : 1 mixture of oxytocin and vasopressin in any of the assays recorded in Tables II and IV. The assumption that these two hormones, which are so closely related chemically, have the same mode of action on the rat's uterus therefore seems justifiable. A latent period of 45 seconds before the response may indicate, but does not prove, that the primary action of these hormones is exerted within the cell. Magnesium, potassium, and calcium ions are well known to produce effects by action at surfaces. The fact that these ions all proved capable of changing the position, but not the slope of the dose-effect curve for the action of vasopressin and oxytocin on the uterus demands further study.

Summary

1. The effect of $MgCl_2$ in concentrations of 0·05, 50, 500, and 1000 mg./l. on the dose-response curves given by oxytocin and vasopressin on the dioestrus uterus of the rat has been examined. Maximum sensitivity was found to oxytocin at 50 mg. $MgCl_2$/l., and to vasopressin at 500 mg. $MgCl_2$/l. A concentration of 1 g. $MgCl_2$/l. depressed sensitivity greatly toward oxytocin, much less toward vasopressin; the uterus was almost equally sensitive to the two hormones at this concentration. Changes in sensitivity were occasioned by change in position, but not in slope of dose-effect curves.

2. Increase in $CaCl_2$ concentration from 0·03 to 0·12 g./l. caused slight increase in sensitivity toward oxytocin, but no change toward vasopressin.

3. Conditions under which the rat's dioestrus uterus may be used to give reliable estimates of the oxytocin present in 1 : 1 mixtures with vasopressin have been defined. Concentrations of 1 mg. $MgCl_2$/l. and 0·12 g. $CaCl_2$/l. are recommended.

It is a pleasure to thank those honours students of Chelsea Polytechnic who have contributed assays to this work. The Pitocin and Pitressin used were given by Parke-Davis & Co. Ltd.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Alkaloid from Greek Belladonna Root. E. Steinegger and G. Phokas. (*Pharm., Acta Helvet.*, 1956, **31**, 330.) The alkaloids from the root were separated on a cellulose column by the formic-acetic acid method of the authors. The new alkaloid, "Hellaradine", gave a picrate of m.p. 117 to 118·5°. Pharmacological tests showed a mydriatic action, and a spasmolytic effect slower and weaker than that of atropine. The proportion of alkaloid in the root is about 0·002 per cent. G.M.

ANALYTICAL

Alkaloids, Buffered Chromatography of. A. Bettschart and H. Flück (*Pharm. Acta Helvet.*, 1956, **31**, 260.) For the buffered chromatography of alkaloids certain factors must be taken into consideration. A relatively high concentration of buffer (e.g., M/2) is often objectionable for paper chromatography since, if the components do not have the same solubility, then it is possible for a change in pH to occur when the paper is equilibrated with the solvent. The McIlvaine citrate-phosphate buffer is recommended. The buffer should not be soluble in the mobile phase, especially for column chromatography. Finally, the buffer should not be sensitive to carbon dioxide when used on paper. The authors report a considerable number of trials on the separation of morphine alkaloids, and show that morphine, thebaine, narceine and codeine can be separated on a phosphate-citric acid buffered paper at pH 6·8, while papaverine and narcotine can be separated at pH from 3·8 to 4·1, using ether as solvent. For detection, fluorescence and iodine-potassium iodide reagent are used. The authors give a review and bibliography of the chromatography of alkaloids. G. M.

Alkaloids of Hemlock (*Conium maculatum* L.), Separation, Micro-estimation and Distribution of. B. T. Cromwell. (*Biochem. J.*, 1956, **64**, 259.) Four of the five alkaloids of *Conium maculatum* have been isolated by extraction with ethanol and steam distillation, and separated by chromatography on paper (Whatman 3MM) using a single phase mixture of *tert.*-pentanol—*tert.*-butanol—N HCl. The following R_F values are recorded, coniine (0·74), *N*-methylconiine (0·64), conhydrine (0·50) and γ-coniceine (0·36). The relative amounts of the alkaloids present in the tissues varies with the stage of development and reproduction of the plant. An unknown steam-volatile base (R_F 0·33) was present in small amounts in the leaves and flowers, and in trace amounts in the root tissues of young plants. It was not identical with piperidine (R_F 0·34) and may be pseudoconhydrine. Root tissues of young plants had a low alkaloid content, mainly γ-coniceine. Sap exuding from stumps of decapitated plants also contained no alkaloids, but small quantities of a compound R_F 0·21 and giving a red colour with nitroprusside reagent was present. A method for the microdetermination of γ-coniceine in the presence of the other alkaloids, based on the nitroprusside reaction, is described. A method is also described for the

microdetermination of *N*-methylconiine, coniine and conhydrine after chromatographic separation by a colorimetric method, based on their reaction with bromothymol blue. Very young seedlings in the cotyledon stage show the presence of γ-coniceine, coniine and *N*-methylconine, whilst the two latter alkaloids were absent from older seedlings. γ-Coniceine was also the major alkaloid in the leaves of young plants, but coniine content increased towards the end of the first year. Actively growing plants had a low alkaloid content in their roots, but roots in the resting stage contained more γ-coniceine and coniine. Renewed vegetative growth was accompanied by concentration of much γ-coniceine in the leaves. During the reproductive phase flowers and developing fruits contained mainly coniine, the γ-coniceine content falling rapidly, with *N*-methylconiine as the predominant alkaloid in mature fruits.

J. B. S.

Caffeine, Aspirin and Phenacetin, Separation of, by Paper Electrophoresis. M. Vietti-Michelina. (*Pharm. Acta Helvet.*, 1956, **31**, 347.) A phosphate buffer solution of pH 6·2 is used for the separation. Phenacetin does not change its position after electrophoresis, while both caffeine and aspirin move, the latter at the most rapid rate. The aspirin is recognised by its fluorescence in ultra-violet light; phenacetin by nitration to yellow coloured *o*-nitrophenacetin; and caffeine by exposure to iodine vapour. G. M.

Narceine, Spectrophotometric Determination of. A. H. Witte. (*Pharm. Weekbl.*, 1956, **91**, 588.) To 10 ml. of a solution of narceine in 0·5N hydrochloric acid is added 5 ml. of chlorine water. After 5 minutes, 5 ml. of 10 per cent sodium sulphite solution is added, and the mixture is made up to 25 ml. 5 ml. of this solution is made up to 25 ml. with saturated solution of sodium acetate. After 1 hour the colour is determined at 510 mμ in a 1 cm. cell. A similar colour is given by narcotine, but not by morphine, codeine, thebaine or papaverine, although the latter interfere by combining with the chlorine and giving yellow solutions, and papaverine is precipitated on the addition of sodium acetate. G. M.

***Rauwolfia serpentina* Preparations, Assay of.** D. Banes, J. Wolff, H. O. Fallscheer and J. Carol. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 708.) The following method depends upon an extraction procedure which eliminates interfering alkaloids, followed by treatment with nitrite to produce a fluorescent green colour; it is stated to be more convenient and reproducible than the chromatographic procedure previously described (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 200; abstract, *J. Pharm. Pharmacol.*, 1956, **8**, 1174). An ethanolic extract is prepared in a Soxhlet apparatus, using powdered rauwolfia or tablets, and a quantity of the extract is mixed with sulphuric acid and extracted with trichloroethane to remove reserpinine and methyl reserpate. The weakly basic alkaloids are extracted with chloroform, and the chloroform solution is treated with sodium bicarbonate solution, diluted with ethanol and cautiously evaporated. The residue, redissolved in ethanol is allowed to react with sodium nitrite in the presence of sulphuric acid at 50° to 60° for 20 minutes, after which sulphamic acid is added and the light absorption measured at 390 mμ, against a blank. The quantity of alkaloids of the reserpine-rescinnamine group is calculated as reserpine, by comparison with the light absorption produced in a standard reserpine solution treated with acid nitrite in the same manner. The large blank readings encountered are probably due to substances formed naturally in the plant, by the oxidation of reserpine and rescinnamine. G. B.

Reserpine Preparations, Oxidative Degradation of. D. Banes, J. Wolff, H. O. Fallscheer and J. Carol. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 710.) Reserpine preparations may be subject to decomposition during manufacture and storage, 3-dehydroreserpates being produced by oxidative degradation. Since these substances have a fluorescent green colour, they may give rise to misleading results when the preparations are assayed by the nitrite colorimetric method. It is recommended that a control solution should be prepared and a correction applied for substances absorbing at 390 mμ prior to nitrous acid treatment. A comparison of corrected and uncorrected results indicates that elixirs and injections are susceptible to this type of oxidative degradation, and it also occurs occasionally in tablets. The corrected results of the nitrite method show reasonable agreement with those of the chromatographic assay method. G. B.

Sodium Tetraphenylboron for the Separation and Determination of Drugs with Basic Nitrogen Groups. L. Worrell and W. R. Ebert. (*Drug Standards*, 1956, **24**, 153.) A sample sufficient to react with 2 to 3 ml. of reagent is dissolved in water and the reaction of the solution adjusted to pH 5. A slight excess of a 3 per cent solution of sodium tetraphenylboron is added, while stirring. The solution is allowed to stand until the precipitate has settled, when a further drop of reagent is added to test for completeness of precipitation. The supernatant liquid is removed and the precipitate washed with the aid of an immersion filter. The precipitate is dissolved in acetone and 5 ml. of a saturated solution of mercuric chloride and 25 ml. of 0·02N sodium hydroxide are added and the solution is boiled. After adding 5 ml. of a 20 per cent solution of potassium iodide, the solution is cooled and excess of alkali titrated with a standard acid solution. The method is satisfactory for salts of amphetamine and methylamphetamine, and for cetyldimethylbenzylammonium chloride. Determinations may be performed in the presence of acetylsalicylic acid, caffeine, phenacetin, starch and talc; the method should therefore be useful for estimating amine salts, codeine, etc., in admixture with aspirin, phenacetin and caffeine. The method cannot be used if potassium or ammonium salts are present, because they give rise to insoluble tetraphenylboron derivatives. G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Histamine, Binding of, in Mammalian Tissues. R. K. Sanyal and G. B. West. (*Nature, Lond.*, 1956, **178**, 1293.) It is well known that tissue mast cells contain heparin and histamine. Experiments have been carried out to study the *in vitro* affinity of heparin and histamine for one another. When the two are mixed in solution and ethanol or acetone added, the precipitated heparin removes about 70 per cent of the histamine if the pH is about 5. This combination is dependent upon pH, since at a neutral or alkaline reaction much less histamine is removed by the heparin. The precipitated heparin-histamine complex can be washed with ethanol or acetone without loss of histamine but the complex is easily rendered soluble by saline, water or weak acid. If adenosine triphosphate is present in the original solution, the uptake of histamine by the heparin is increased to more than 90 per cent. The staining properties and chromatographic behaviour of the complex resemble those of the natural mast cell granules. The formation of this complex is fairly specific for histamine

since adrenaline, noradrenaline and 5-hydroxytryptamine are not removed under similar conditions. Thus the base histamine and the acid heparin can form a complex but so far it has not been possible to release the histamine from the synthetic histamine heparinate without solution of the heparin. M. M.

BIOCHEMICAL ANALYSIS

Fluothane, Estimation of, in Blood. R. R. Goodall. (*Brit. J. Pharmacol.*, 1956, **11**, 409.) Fluothane, ($CF_3CHClBr$) a new anaesthetic, is extracted into light petroleum and aliquots heated in sealed ampoules containing sodium amoxide. The bromide released is determined nephelometrically as silver bromide. For each test, place 4 ml. of light petroleum and 1 ml. of water in a 10 ml. tube. Place 4 ml. of the blood sample under the surface of the petroleum. Extract, centrifuge, and transfer 3·5 ml. aliquots to a 10 ml. ampoule containing 2 ml. of a solution of sodium amoxide (prepared by dissolving 1·1 g. of halide free sodium in 50 ml. of amyl alcohol). Seal the ampoules and heat in a pressure cooker at 15 lb./sq. inch for one and a half hours. Open each ampoule and transfer the contents into a test-tube. Rinse each ampoule with N sulphuric acid followed by sufficient water to make the total volume of the aqueous layer 8 ml. Mix well, allow to settle, remove the lower aqueous layer to a clean tube, add 2 ml. 0·01N silver nitrate, stir and transfer to a dark cupboard for 10 minutes. Read the optical density in a spectrophotometer. Compare with suitable standards, prepared from a standard solution of fluothane in water, treated in a similar way to the blood. Note: an improved method, using reduction by lithium aluminium hydride to release halide from fluothane, is being developed.

G. F. S.

Methanol in Blood and Biological Material, Rapid and Sensitive Test for. O. E. Skaug. (*Scand. J. clin. lab. Invest.*, 1956, **8**, 338.) To 5 ml. of blood (with or without anticoagulant) add 3 to 4 ml. of water and place in a suitable distilling apparatus. Place the flask in a boiling water bath and after 2 minutes collect the condensate. Transfer this with the aid of 0·5 to 1 ml. of water to a test tube and cool in ice. Add 0·1 ml. of 0·1N potassium permanganate followed by 0·5 ml. of sulphuric acid. Mix thoroughly, add 1 ml. of 2:7-naphthalenediol reagent (0·2 per cent with sodium sulphite 1 per cent), remix and add a further 2 ml. of sulphuric acid. Place the tube in a boiling water bath for 5 minutes. A reddish-violet colour indicates the presence of methanol. When a control experiment is carried out using water in place of blood, it is possible to detect a colour difference when 2·5 p.p.m. of methanol is present in the blood sample. The reaction depends upon the formation of formaldehyde; ethanol, diethyl ether, β-hydroxybutyric acid and acetone do not interfere. Two types of distilling apparatus are described, one for use with acetone and solid carbon dioxide as refrigerant, and the other for use with ice. G. B.

Tetraethyl-lead, Isolation from Liver, after its Inhalation. C. D. Stevens, C. L. Feldhake and R. A. Kehoe. (*J. Pharmacol.*, 1956, **117**, 420.) Tetraethyl-lead (TEL) was isolated from liver tissue of rats which had inhaled TEL vapour. The rats were exposed for four to seven hours to air nearly saturated with TEL (8 to 9 mg. per litre). They were then killed with carbon monoxide and their livers homogenized. The TEL was extracted by pentane, concentrated by low temperature vacuum distillation and identified by infra-red spectra and lead analysis. No homologues containing methyl groups were detected in the concentrates. G. P.

CHEMOTHERAPY

Novobiocin and Vancomycin; Antibacterial Activity. R. W. Fairbrother and B. L. Williams. (*Lancet*, 1956, **271**, 1177.) Tests to determine the sensitivity of the common pathogens to these two antibiotics were carried out by the dried disc technique and by serial dilution. 1350 organisms were tested against vancomycin by the disc method, the results showing that this antibiotic is active against Gram-positive and Gram-negative cocci but has little effect on Gram-negative bacilli. Of 540 strains of *Staph. aureus* tested all proved sensitive, though many of the strains were resistant to penicillin and other established antibiotics. These observations were confirmed in a limited number of tests by serial dilution; *Str. haemolyticus* (group A) proved the most sensitive organism by these tests. 1100 organisms were tested against novobiocin by the disc method, the results indicating considerable activity against Gram-positive cocci but only limited action against Gram-negative organisms. All of 470 strains of *Staph. aureus*, many of which were resistant to penicillin and other antibiotics, proved sensitive, as did strains of *Str. haemolyticus* and pneumococcus. All members of the coliform group proved resistant, and all strains of proteus. Strains of *Haemophilus influenzae* were sensitive; *Neisseria gonorrhoeae* and *Str. faecalis* gave irregular results. *Staph. aureus* proved the most sensitive organism by the serial dilution test. *Str. haemolyticus* proved relatively resistant and *Str. faecalis* even more resistant. The coliforms and pseudomonas showed marked resistance. The results indicate that vancomycin and novobiocin should prove useful additions to the list of routine antibiotics. The range of antibacterial activity of vancomycin is similar to that of penicillin, erythromycin and bacitracin, and it should be valuable in the treatment of infections caused by penicillin-resistant strains of *Staph. aureus*; novobiocin should also be of value in such infections, but carefully controlled clinical trials are necessary for a final assessment. Novobiocin-resistant strains of *Staph. aureus* have already been isolated in U.S.A. and this antibiotic should not be used indiscriminately. S. L. W.

Vancomycin; Laboratory and Clinical Experiences. G. E. Geraci, F. R. Heilman, D. R. Nichols, W. E. Wellman and G. T. Ross. (*Proc. Mayo Clin.*, 1956, **31**, 564.) Vancomycin is a bactericidal antibiotic obtained from *Streptomyces orientalis*. It is amphoteric and acts primarily against Gram-positive bacteria. Bacteria are slow to develop resistance to it. Vancomycin hydrochloride is a white solid, very soluble in water and relatively stable. It has a molecular weight of about 3300. Its activity is little affected by changes in the pH of the medium and by a variety of inorganic salts, amino acids, reducing agents and growth factors in the test medium. It acts only against multiplying bacteria. Injected parenterally into mice it protects them from experimental infections with *Micrococcus pyogenes*, *Streptococcus pyogenes*, *Diplococcus pneumoniae* and *Borrelia novyi*. Most micrococci are killed by 2 to 3 μg./ml. of medium; a concentration of 2·5 μg./ml. completely inhibited 110 of 112 strains of *M. pyogenes* tested. Of 12 strains of *Str. mitis* isolated from the blood of patients previously treated for bacterial endocarditis, half were killed by the same concentration. The therapeutic effectiveness of the drug was most convincingly demonstrated in a case of acute micrococcal endocarditis in a 71-year old man. 0·5 g. of vancomycin was given intravenously every 6 hours for 3 weeks, then every 8 hours for 4 days, and finally every 12 hours for 3 days, making a total of 51 g. in 28 days. The patient, who was almost comatose at

the beginning of treatment, began to improve almost immediately, and within a few hours much of the toxicity had vanished. The temperature was normal within 3 days. The patient has remained well for 5 months. Assays indicated that little or no vancomycin is present in the blood serum and only small amounts in the urine after 0·5 g. orally 6-hourly, but after a single intravenous injection of 0·5 g. an average value of 33 μg./ml. was obtained at 1 minute, and after 24 hours the value was still 0·7 μg./ml. Large quantities were excreted in the urine after intravenous administration, the average concentration after 24 hours being 100 μg./ml. After single and multiple intravenous injections adequate therapeutic levels were found in the pleural, pericardial, ascitic and synovial fluids; only small amounts appeared in the bile. With intravenous injections only small amounts appeared in the stools but with oral administration of 0·5 g. 6-hourly very much larger amounts appeared. Vancomycin does not appear to diffuse through the uninflamed meninges. After multiple intravenous doses of 0·5 g. 6-hourly it appeared to accumulate in the blood serum over the first 2 or 3 days. The only signs of toxicity were an occasional chill, dermatitis, and localised phlebitis. Preliminary clinical trials indicate that vancomycin offers promise in the treatment of micrococcal infections. Except in the treatment of micrococcal ileocolitis, in which it would appear to be the antibiotic of choice, it needs to be given parenterally. Nine detailed case reports are given. S. L. W.

PHARMACY

Cyanocobalamin and its Analogues in Ascorbate Solution, Stability of. H. H. Hutchins, P. J. Cravioto and T. J. Macek. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 806.) When solutions containing 30 μg./ml. of vitamin B_{12} and 1 per cent of ascorbic acid in M acetate buffer, pH 4 were stored at 30° and assayed at intervals, the rate of decomposition of vitamin B_{12} was found to depend upon the proportion of cyanocobalamin present. Solutions prepared from pure cyanocobalamin or a commercial concentrate containing only cyanocobalamin lost about half their potency in 6 days, whereas solutions containing a proportion of other cobalamins decomposed more rapidly. In these experiments, vitamin B_{12} concentrates containing 50 per cent of cyanocobalamin lost about 95 per cent of their activity, and a concentrate containing 20 per cent of cyanocobalamin almost all of its activity in 6 days. Similar solutions prepared from chlorocobalamin, nitrocobalamin and thiocyanatocobalamin lost nearly all their activity on storage for 3 hours at 25°. G. B.

Vitamin B_{12} in B-Complex Injectable Solutions, Stability of. M. Blitz, E. Eigen and E. Gunsberg. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 803.) Solutions containing 5 μg. of vitamin B_{12} per ml. with varying amounts of aneurine and nicotinamide were stored at room temperature and assayed microbiologically at intervals. Solutions containing 50 mg. or more per ml. each of aneurine and nicotinamide lost most of their vitamin B_{12} content on storage for 6 months, whereas those containing smaller amounts of aneurine and nicotinamide were more stable, for example, 25 mg./ml. of aneurine and nicotinamide gave rise to a loss of 30 per cent of vitamin B_{12} in 1 year. Of 6 commercial B complex injections purchased at random 5 were below the stated potency and 2 had lost more than 95 per cent of their original potency. It was shown that a decomposition product of aneurine was responsible for the destruction of vitamin B_{12}. Heating a solution of 50 mg./ml. aneurine and nicotinamide at

100° for 4 hours at pH 4·5 gave a solution which accelerated the decomposition of vitamin B_{12}, as did a solution of aneurine which had been stored for 2 years. Further experiments indicated that it is the thiazole moiety of aneurine which accelerates the decomposition of vitamin B_{12}. G. B.

PHARMACOLOGY AND THERAPEUTICS

***N*-Acetyl-*p*-aminophenol as an Analgesic.** D. R. L. Newton and J. M. Tanner. (*Brit. med. J.*, 1956, **2**, 1096.) *N*-Acetyl-*p*-aminophenol (N.A.P.A.P.) is the non-toxic metabolite of acetanilide and phenacetin through which these drugs appear to exert their antipyretic and analgesic action. A controlled clinical trial of the analgesic effect of N.A.P.A.P. compared with that of Compound Codeine Tablet B.P. was carried out in 42 patients suffering from chronic painful rheumatoid conditions. Each patient received one drug for a period of a week followed by the other drug during the second week, and at the end of a fortnight was questioned concerning his symptoms; no suggestion was made that different preparations were being compared. During the next fortnight a second similar comparison was made. The drugs were given under a randomisation scheme which ensured that at no time did either the patient or the doctor know which drug was being taken. The statistical method known as sequential analysis was used, the principles and advantages of which are described. The analysis showed that Compound Codeine Tablet, 2 tablets 3 times a day, was in general superior to N.A.P.A.P. 1 g. (2 tablets) 3 times a day. At the same time a significant minority of patients considered that for them N.A.P.A.P. was a better analgesic, indicating that there is individual reaction to different types of analgesic. S. L. W.

Antilipaemic Agent without Anticoagulant Action. E. M. M. Besterman and J. Evans. (*Brit. med. J.*, 1957, **1**, 310.) Laminarin sulphates M and N, prepared by the sulphation of laminarin, a polysaccharide from *Laminaria cloustoni*, containing 0·62 and 0·37 sulphate groups per glucose unit, were examined for antilipaemic activity. In contrast to the more highly sulphated laminarins, they had very little anticoagulant activity. 12 patients with ischaemic heart disease were treated with heparin (10,000 units intravenously) and in 11 of these the electrophoretic pattern of the serum lipids was significantly altered. Of these 11 patients, 6 received a single intravenous injection of 100 mg. of laminarin sulphate M and 3 received the same dose of laminarin sulphate N. Samples of venous blood were submitted to paper electrophoresis, each paper being divided, half being stained for proteins and half for lipids. Laminarin sulphate M increased the electrophoretic mobility of lipoproteins and altered their distribution in the same way as heparin, whereas laminarin sulphate N had no demonstrable effect on electrophoretic mobility and distribtion of the lipoproteins. The effect of laminarin sulphate M was observed almost immediately after the intravenous injection of 100 mg. and persisted for 4 to 6 hours; this dose cleared the lipaemic serum of 2 patients who had had a fatty meal, but the serum clearing effect could not be demonstrated *in vitro*. No effect was observed when 100 mg. of laminarin sulphate M was injected intramuscularly. Since laminarin sulphate M has antilipaemic properties similar to heparin, without anticoagulant activity, it would seem to be a suitable substance to use in a long-term investigation of the effect of lipaemia-clearing substances on the course of atherosclerosis in man. G. B.

Barbiturate Poisoning Treated with Amiphenazole and Bemegride. A. Worlock. (*Brit. med. J.*, 1956, **2**, 1099.) Twelve cases of fairly severe barbiturate poisoning were treated with amiphenazole and bemegride. All the patients were unconscious on admission, and all regained consciousness within 24 hours (11 of them within 12 hours). No deaths occurred, there was no incidence of chest infection, and the stay of patients in hospital was reduced dramatically compared with former methods of treatment. On admission the patients were given a stomach wash-out with a dilute solution of sodium bicarbonate, 4 oz. of the solution being left in the stomach after the wash-out. The airway was kept clear and a laryngoscope passed to determine whether laryngeal reflexes were present. A 5 per cent dextrose-saline intravenous drip was given at the rate of 40 drops a minute, continued throughout the treatment. Procaine penicillin, 600,000 units, was given at once and then twice daily for 5 days. Amiphenazole and bemegride were injected intravenously at the rate of 1 ml. of amiphenazole and 10 ml. of bemegride every 3 minutes until a level of light anaesthesia was attained. The strengths of the solutions used were 0·5 per çent solution of bemegride (5 mg. per ml.) and a 1 per cent solution of amiphenazole (10 mg. per ml.), the latter being made up immediately before use. Postural drainage and breathing exercises were carried out daily. Case reports are given. S. L. W.

Bemegride as an Antagonist of Barbiturates. H. H. Frey, E. W. Hushahn and K. Soehring. (*Arzneimitt.-Forsch.*, 1956, **6**, 583.) The stimulating action of bemegride and amiphenazole were compared with that of leptazol, using mice which were under the influence of barbiturates. The first compound was twice as effective as leptazol; the second was inactive. With a combination of 3 parts of bemegride with one part of amiphenazole the effect of the former is increased to 3 times that of leptazol. The action is not that of a specific barbiturate antagonist but rather that of a stimulator with a satisfactory action against various hypnotics. G. M.

Bemegride, New Analeptics and Hypnotics Related to. T. C. Somers. (*Nature, Lond.*, 1956, **178**, 996.) A series of compounds based on the antibarbiturate β-methyl-β-ethylglutarimide (bemegride; Megimide) have been prepared and the results of preliminary pharmacological examinations are reported. The effects of test compounds on the sleeping times (elapsed time between loss and recovery of the righting reflex) induced by pentobarbitone sodium in mice were compared with that of bemegride. The test compounds were administered by intraperitoneal injection in aqueous solution or suspension. β-Methyl-β-ethylglutaramic acid and β-methyl-β-ethylglutaric acid were ineffective as barbiturate antagonists. The *N*-methyl-, *N*-ethyl- and *N*-phenyl-β-methyl-β-ethylglutarimides were also inactive. β-*spirocyclo*Pentaneglutarimide, β-methyl-β-*n*-propylglutarimide and ββ-diethylglutarimides had analeptic activities similar to that of bemegride, the first of these being almost as effective as the parent substance. The methyl *n*-propyl compound is the least toxic. These same compounds and also β-ethyl-, β-methyl-β-*iso*butyl-, β-*spirocyclo*hexane- and β-*spirocyclo*heptane-glutarimides all caused convulsions in mice when administered alone in doses varying from 15 to 50 mg./kg. β-Methyl-β-*n*-butylglutarimide, methyl-*n*-amylglutarimide and methyl *n*-hexylglutarimides have no analeptic activity, but on the other hand exert hypnotic effects in mice, the minimum hypnotic dose being about 150 to 200 mg./kg. Doses of 400 to 500 mg./kg. induced up to 5 hours of sleep. The approximate LD50 is 600 mg./kg., and the hypnotic effect is antagonized by bemegride. J. B. S.

Dextran Sulphate, Anticoagulation Effect and Urinary Excretion of. S. M. Jeavons, K. W. Walton and C. R. Ricketts. (*Brit. med. J.*, 1956, **2**, 1016.) When the effects of single equipotent intravenous doses of heparin and dextran sulphate are compared the latter substance shows a slightly longer anticoagulant activity. One factor which might cause this effect is a difference between the patterns of urinary excretion of the two drugs. Estimates of the urinary output were made in both man and rabbits over a period of 10 to 23 days. These results were related to the clotting time of the blood over that time. It was found that dextran sulphate, when administered intravenously to five patients suffering from thrombo-embolic disease, had a marked cumulative effect when the treatment was prolonged beyond three to five days. As a result, reduction in the dosage and/or lengthening of the spacing of the injections could be made without affecting the efficacy of the dextran. The cumulative effect in man could not be accounted for on the basis of the urinary excretion of a smaller percentage of the injected dose than that of heparin. A dosage schedule of dextran, similar to that used in man, was given to rabbits, but no cumulative effect was produced. It is suggested that in man dextran sulphate differs from heparin in duration of effect because of a slower rate of breakdown in the body and consequent accumulation, probably in the extravascular fluid. M. M.

Dextran Sulphate as an Anticoagulant, and Action in Lowering Serum Cholesterol. H. Cohen and G. R. Tudhope. (*Brit. med. J.*, 1956, **2**, 1023.) The effect on the clotting-time of single and repeated doses of dextran sulphate, given intravenously, and the use of it in the treatment of thrombotic disease are reported. The change in the serum total cholesterol is also measured. It was found that a single dose of 7500 units produced an increase in the clotting-time to more than twice normal for four to six hours. With repeated doses cumulation occurred so that the dose could be reduced progressively. During the treatment there was a fall in the serum cholesterol. It is suggested that dextran is an effective anticoagulant for clinical use and that it provides a possible alternative to the usual combination of heparin and oral anticoagulant. However toxic effects may occur, particularly if the dose is not carefully controlled. M. M.

Digitalis Glycosides and their Metabolites, The Distribution of. B. T. Brown, E. E. Shepheard and S. E. Wright. (*J. Pharmacol.*, 1956, **118**, 39.) An investigation has been made into the presence of cardioactive metabolites of the glycosides digoxin, lanatoside C and digitoxin in the blood and organs of the rat and compared with those found in the urine. The glycosides were given intraperitoneally or intravenously. After killing the rats the tissues were extracted with ethanol, the extract was shaken with carbon tetrachloride which was then rejected and the bulk of the ethanol evaporated off at 30°. The solution remaining was diluted with water and extracted continuously with chloroform for two hours. After evaporation to small bulk the cardioactive constituents were detected by paper chromatography. Cardioactive metabolites, as well as free glycosides, were found to be in the heart, liver, kidney and circulating blood of the rat immediately after the injection of digoxin and lanatoside C, and the metabolites were the same as those in the urine. Digitoxin and its metabolite, previously found in rat urine, were present in rat livers immediately after injection, but only the free glycoside could be detected in blood and kidney. The experiments indicate that although the major portion of the dose of all three glycosides is rapidly removed from the circulating blood, their rate of clearance and eventual disappearance differ. Digitoxin is removed more rapidly than lanatoside C and digoxin slowest of all. The rate of blood

clearance is believed to be conditioned by the relative ability of the liver to combine with each glycoside. Digitoxigenin or digoxigenin could not be detected in any tissue after administration of the glycosides. G. F. S.

1-Dimethylamino-3-cyano-3-phenyl-4-methylhexane HCl (Z-4) and 1-Dimethylamino-2-phenyl-3-methylpentane HCl (Z-134), Analgesic-potentiating and Diuretic Effects of. J. Y. P. Chen. (*J. Pharmacol.*, 1956, **117**, 451.) The above two compounds, which have a structural resemblance to methadone, had a much greater potentiating effect on the analgesic activity of morphine in rats than had chlorpromazine. Intramuscular Z-134 and oral Z-4 caused diuresis in rats which compared favourably with that produced by acetazolamide or mercurhydrin. In non-toxic doses in dogs the two compounds had prolonged respiratory stimulant and intestinal relaxant effects. Moderate local and topical anaesthetic actions were also demonstrated by the guinea pig weal and rabbit corneal methods. The duration of local anaesthesia in the guinea pig with 0·5 per cent solutions of the drugs was of the same order as with 0·5 per cent procaine. Only with higher concentrations did Z-4 produce any local irritation. Acute toxicity studies in mice and rats indicate low toxicity of the two drugs. The tests used for investigating analgesic and diuretic activity in these experiments are simple and give satisfactory results. G. P.

Fluothane, A New Volatile Anaesthetic, Action of. J. Raventós. (*Brit. J. Pharmacol.*, 1956, **11**, 394.) Fluothane ($CF_3CHClBr$) is a volatile, non-explosive inhalation anaesthetic. It is stable in contact with soda lime in a closed-circuit apparatus, but it is not stable in light. It should be stored in amber bottles with thymol as a stabilizer. It is more potent than ether or chloroform in experimental animals. Induction and recovery are both rapid and free from excitement, it produces good muscular relaxation and it does not cause salivation or vomiting. It is best used in a closed-circuit apparatus, as anaesthesia is difficult to control by the open mask method. Both amplitude and frequency of respiration are decreased at the level of surgical anaesthesia. High concentrations stop the respiration, but this apnoea is easily reversible, and the heart continues to beat for some time after respiration has ceased. Inhalation of fluothane causes hypotension roughly proportional to the vapour concentration, which appears to be due to reversible blocking of the sympathetic ganglia. It does not produce cardiac irregularities, but it increases the sensitivity of the heart to adrenaline. Hepatic function is not affected by anaesthesia with fluothane, but there is a mild dilatation of the proximal tubules of the kidney which is not associated with alteration of renal function. G. F. S.

Hemlock Water Dropwort, Pharmacological Studies on. H. F. Grundy and F. Howarth. (*Brit. J. Pharmacol.*, 1956, **11**, 225.) In unanaesthetized rabbits and mice crystalline oenanthotoxin and tinctures of *Oenanthe crocata* caused convulsions resembling those produced by picrotoxin. At the height of the attacks, respiration was impeded and the buccal mucosa was cyanosed. With lethal doses the heart was still beating feebly after respiratory arrest. Death occurred within 30 minutes after 1 mg./kg. of oenanthotoxin and within 60 minutes after 0·5 to 1 ml. of oenanthe tincture. In rabbits and cats the convulsions have their origin in the brain-stem. Oenanthotoxin was six times as toxic as picrotoxin in mice and was a more effective antidote for pentobarbitone poisoning. Respiratory stimulation in the rabbit was also greater with oenanthotoxin. The alkaloid had a biphasic effect on blood pressure in the rabbit: there was a transient fall, due to a direct depression of the myocardium, followed by a sustained rise. The rise was absent in spinal animals. G. P.

Hexamethylene Bis-dialkylsulphonium Salts, Ganglion-blocking Properties of. R. B. Barlow and J. R. Vane. (*Brit. J. Pharmacol.*, 1956, **11**, 198.) The pharmacological properties of tertiary sulphonium salts appear qualitatively similar to those of quaternary ammonium salts. Quantitatively, however, the sulphonium salts are much weaker than their ammonium analogues. This low activity may be due to the sulphonium salts only having three alkyl radicals in the cationic head where the quaternary ammonium has four. To test this hypothesis the ganglion-blocking properties of hexamethylene bis-dimethyl, bis-ethylmethyl and bis-diethyl sulphonium iodides were compared on the superior cervical ganglion of the cat. Activity was greatest in the bis-diethyl compound and least in the bis-dimethyl compound. The tertiary amine analogues, hexamethylene bis-dimethyl, bis-ethylmethyl and bis-diethyl amine dihydrobromides, also had ganglion-blocking properties. Again the bis-diethyl compound was the most active and the bis-dimethyl the least. On a molar basis the bis-diethyl sulphonium compound had about half the activity of hexamethonium on this preparation and over three times the activity of its bis-diethylamine analogue. This strengthens the argument that the low pharmacological activity of the sulphur analogue of acetylcholine, acetoxydimethylsulphonium, could be due to the presence of only two methyl groups at the cationic head. G. P.

5-Hydroxytryptamine, Analysis of the Actions of, on the Isolated Duodenum of the Rat. J. Lévy and E. Michel-Ber. (*C. R. Acad., Paris*, 1956, **242**, 3007.) In concentrations between $2{\cdot}5 \times 10^{-7}$ and 1×10^{-5}, 5-hydroxytryptamine (5-HT) caused contraction of the rat's isolated duodenum. However, in about 5 per cent of the preparations the drug caused a biphasic effect, depressing first before stimulating, an action similar to that of ganglion stimulants. The depressant action was encountered more often (in about 50 per cent) if before addition of 5-HT the duodenum was left in contact with (+)-lysergic acid diethylamide (LSD). Atropine, when used with the LSD, further increased the incidence of the depression to 90 per cent of the muscle preparations. This relaxation caused by 5-HT was blocked by yohimbine, in concentrations which abolished the action of adrenaline, and by high concentrations of nicotine or phenoxycholine, which had no effect on adrenaline. However, the relaxation was unaffected by concentrations of hexamethonium or tetraethylammonium sufficient to block ganglia. The relaxation would appear from the above results to be adrenergic in nature. An excitant action of 5-HT on post-ganglionic adrenergic nerve elements seems probable, either on the axons directly or on receptors in the ganglion cells other than those on which hexamethonium and tetraethylammonium act. G. P.

5-Hydroxytryptamine, and Certain Derivatives of Lysergic Acid, Antagonism Between. E. C. Savini. (*Brit. J. Pharmacol.*, 1956, **11**, 313.) When adrenaline and 5-hydroxytryptamine were injected together into the perfused isolated ear of a rabbit the combined vasoconstrictor activity was somewhat greater than would have been expected from the effects of the two drugs given alone. When 5-HT was combined with noradrenaline, tryptamine or pitressin, potentiation was absent or slight, the effects being simply additive. (+)-Lysergic acid diethylamide (LSD) and ergometrine, in low concentrations (1 μg./litre) both antagonized the vasoconstrictor action of 5-HT, but not that of adrenaline or noradrenaline. In higher concentrations both LSD and ergometrine had a direct vasoconstrictor action. 2-Bromo-(+)-lysergic acid diethylamide (BOL) also blocked in low concentrations the effects of 5-HT but in contrast to LSD and

ergometrine, BOL had no vasoconstrictor action of its own, it antagonized adrenaline and noradrenaline and its action developed quickly and was easily reversible. Also, the antagonism with BOL could be overcome with large doses of 5-HT. G. P.

5-Hydroxytryptamine and its Antagonists, Effects of, on Tidal Air. H. Konzett. (*Brit. J. Pharmacol.*, 1956, **11**, 289.) In anaesthetized or spinal cats and guinea pigs 5-hydroxytryptamine decreased tidal air during artificial respiration at constant stroke volume by a respiratory pump. The action was mainly a direct bronchoconstriction. (+)-Lysergic acid diethylamide, 1-acetyl-(+)-lysergic acid diethylamide and 2-brom-(+)-lysergic acid diethylamide specifically antagonized the bronchoconstrictor action. Atropine and the antihistamine 1-methyl-4-amino-*N'*-phenyl-*N'*-(2'-thenyl)-piperidine (Sandosten) also had some antagonistic effects, but in doses higher than were necessary to block the bronchoconstrictor actions of acetylcholine and histamine, respectively. Ergometrine had practically no blocking effect. G. P.

5-Hydroxytryptamine and Various Antagonists, Some Central Actions of. J. H. Gaddum and M. Vogt. (*Brit. J. Pharmacol.*, 1956, **11**, 175.) The distribution of 5-hydroxytryptamine (5-HT) in the central nervous system and the hallucinogenic actions of a powerful and specific antagonist of 5-HT, (+)-lysergic acid diethylamide (LSD), provoked the hypothesis that the actions of the LSD might be due to antagonism of naturally occurring 5-HT in the brain. To test this, the effects of LSD and other antagonists of 5-HT were studied on the actions of 5-HT injected, through a permanent cannula, into the lateral cerebral ventricle of the cat. The actions of 5-HT given in this way are: the cats become lethargic, hesitant and retiring, muscle tone is reduced and respiration is rapid; these effects last at least 6 hours. The depressant actions were antagonized by LSD, ergometrine, morphine, methadone and amphetamine, but not by 5-benzyloxygramine or methylmedmain. The sedative effects of intraventricular injections of reserpine, which were similar to those of 5-HT, were also antagonized by LSD, morphine and methadone. These antagonisms are probably not related to the specific antagonism between 5-HT and LSD on peripheral tissues. Some, but not all, of the central actions of LSD in man were inhibited by methylamphetamine. G. P.

5-Hydroxytryptamine, Identification of, in the Sting of the Nettle (*Urtica dioica*). H. O. J. Collier and G. B. Chesher. (*Brit. J. Pharmacol.*, 1956, **11**, 186.) In addition to histamine and acetylcholine, nettle stings contain a substance which resembles 5-hydroxytryptamine (5-HT) in the following respects: stimulation of the rat's isolated uterus; lysergic acid diethylamide antagonized the substance and 5-HT to the same extent; the rat's uterus desensitized to 5-HT was also insensitive to the substance; and paper chromatograms of nettle leaf and stem extracts gave a spot with the same colour reaction and R_F value as 5-HT. The estimates of 5-HT content per sting were: on the rat's uterus, 4·86 ± 1·03 ng.; guinea pig's ileum, 4·00 ± 0·38 ng.; rat's colon, 3·43 ± 0·53 ng. If the average sting contains 7 to 9 μg. fluid (Emmelin and Feldberg, *J. Physiol.*, 1947, **106**, 440), then this amount of 5-HT per sting (3·4 to 4·9 ng.) provides a concentration sufficient to cause pain in human skin. 5-HT and the third substance were inactivated at room temperature by nettle sting suspensions; this activity of nettle stings was lost after boiling and was presumably due to an enzyme. G. P.

Marplan; Clinical Studies. W. H. Bachrach. (*J. Lab. clin. Med.*, 1956, **48**, 603.) Marplan is a new atropine-like drug, 1-methyl-3-benziloyl-oxyquinuclidinium bromide. It has been shown by the balloon-kymographic method to inhibit both spontaneous and stimulated motility at all levels of the gut with the exception of post-Dromoran spasm of the choledochal sphincter. The onset of inhibition was immediate when the drug was administered intravenously in a dose of 0·5 to 1 mg., with similar doses the latent period was 5 minutes for the intramuscular and 15 minutes for the subcutaneous route. The inhibition extended for as long as 2½ hours after injection of the drug. The effective oral dose is 20 to 35 mg. Marplan was administered to 88 patients with various digestive disturbances; 62 of these were ulcer patients, 40 of whom had symptoms when treatment was started; 26 of these (65 per cent) obtained complete control of symptoms. The response of the patients with other digestive ailments (functional dyspepsia, functional bowel disturbance, etc.) was relatively poor. The drug was given in an average dose of one 5 mg. tablet orally at appropriate intervals during the day and 2 tablets at bedtime. Neither age nor body weight constitute any guide to the effective dose. The drug is well tolerated and has been given as long as 145 days without requiring an increase of the dose above 10 mg. Troublesome side effects with oral doses were infrequent, and with one exception occurred only in patients who had had similar reactions to other anticholinergic drugs; the principal side-effects were xerostomia, mydriasis and dysuria. In its physiological and clinical properties Marplan has been found comparable to atropine and to several synthetic anticholinergic preparations; while it has no particular advantage over the latter it is at least as good. S. L. W.

Mercury Absorption and Psoriasis. P. M. Inman, B. Gordon and P. Trinder. (*Brit. med. J.*, 1956, **2**, 1202.) The absorption of mercury when used in the form of an ointment containing ammoniated mercury or the yellow oxide in the treatment of psoriasis was studied in 2 groups of 12 patients, mostly women and children. The ointments were applied twice daily for six weeks; occlusive dressings were applied without cleaning or washing off the ointment, and many patients used 1 to 2 lb. of ointment weekly. A surprisingly high proportion became free from the eruption and it was thought the occlusive dressing increased the effectiveness of the treatment although increasing the risk of cutaneous absorption and clinical toxicity. One group was treated with an ointment containing per ounce 10 grains of ammoniated mercury and 30 minims of solution of coal tar in soft paraffin. An ointment containing yellow mercuric oxide 1 part, and prepared coal tar 2 parts, with hydrous wool fat and soft paraffin 10 parts of each was used for the other group. For the first of six weeks the mercury content of a 24-hour sample of urine was determined weekly, and thereafter from time to time in the patients with a high urinary mercury level. Only 2/24 failed to show at some time a mercury level in excess of 80 μg./l., regarded as the upper limit of normality. Absorption showed considerable individual variation and did not always depend on the area of skin covered. Even when 90 per cent of the skin surface was covered the mercury excretion was not as high as in some other cases. 13/24 had a level above 300 μg./l. One patient excreted 3300 μg./l. and 8 excreted more than 1000 μg./l. In no case were there any signs of clinical toxicity although often the level did not revert to normal for many months. In tentative explanation of the absence of such signs in spite of the causative connection of calomel with pink disease, it is suggested that perhaps some mercury compounds are more toxic than others. H. T. B.

Metal Chelates in Gas Gangrene. M. Moskowitz. (*Proc. Soc. exp. Biol. N.Y.*, 1956, **92**, 706.) The calcium chelate of ethylenediaminetetra-acetic acid calcium edetate, which is non-toxic, has been shown to protect mice against a lethal dose of *Cl. perfringens* toxin and guinea pigs against experimental gas gangrene. This may be due to an inhibition of one or more toxins produced by the organism. Mice innoculated intracutaneously with a 2 MLD dose of a toxic culture filtrate of *Cl. perfringens* Type A survived when injected intracutaneously in another area at the same time with a 5 or 2·5 per cent solution of calcium edetate. Guinea pigs were protected against infection with a culture of a human strain of *Cl. perfringens* by a subcutaneous injection of a 5 per cent solution of calcium edetate. G. F. S.

Noradrenaline, Effect of, on Urine and Renal Blood Flow. F. G. W. Marson. (*Brit. J. Pharmacol.*, 1956, **11**, 431.) An investigation has been made in anaesthetised dogs of the reduction in renal blood flow that occurs when noradrenaline is administered in doses which may be used to restore the blood pressure in shock. Urine flow was recorded from the catheterised ureters and blood flow from homotransplanted kidneys. Infusion of noradrenaline caused an abrupt fall in urine flow, proportional to the dose, anuria occurring with doses of 8·7 μg./kg./minute. The blood flow in transplanted kidneys was reduced and 4·6 to 13·8 μg./kg./minute completely arrested it, when the blood pressure was 190 to 250 mm. Following acute haemorrhage, with a fall in blood pressure, both urine and blood flow were reduced and further reduction occurred when the blood pressure was restored with noradrenaline. Renal function rapidly recovered on re-injection of the removed blood. G. F. S.

Oximes and Hydroxamic Acids as Antidotes in Anticholinesterase Poisoning. B. M. Askew. (*Brit. J. Pharmacol.*, 1956, **11**, 417.) Oximes and hydroxamic acids react with and reactivate certain anticholinesterases. In a study of the *in vivo* activity of all available oximes and hydroxamic acids, eight oximes have been found to be the most effective against twice the LD50 of sarin in rats. Four have the same general formula, namely mono*iso*nitrosoacetone (MINA), diacetylmonoxime (DAM), 2-oxo-3-oximinopentane, and 2-methyl-3-oximino-4-oxopentane and a fifth, *iso*nitrosodiethylketone is isomeric with 2-oxo-3-oximinopentane. All five oximes had a similar activity on an equimolar basis In other species DAM, because of its lower toxicity, was more effective than MINA. Both are effective in sarin poisoned rats even when given after the onset of anticholinesterase poisoning. Given before sarin, DAM afforded complete protection for a period of 55 to 95 minutes. G. F. S.

Parathion, Experimental Data on the Therapy of Poisoning by. Yu. C. Kagan. (*Farmakologiya i Toksikologiya*, 1956, **19**, No. 2, 49.) Tropatsin (hydrochloride of the tropine ester of diphenylacetic acid) and Pentafen (hydrochloride of the diethylaminoethyl ester of phenyl*cyclo*pentane carboxylic acid) were more effective than atropine in the treatment of parathion poisoning. Mice were given oral doses of 20 mg./kg. of parathion in 0·2 per cent oily solution; some were then treated by subcutaneous injections of atropine and some by Tropatsin, administered a few minutes after the parathion. After 4 days all the 36 control animals had died, but 10 of the 14 treated with Tropatsin survived; of 18 treated with atropine only one survived. Results obtained in experiments on rats also indicated the superiority of Tropatsin over atropine. Experiments on cats showed that Pentafen was an effective antidote in parathion poisoning. E. H.

APPLIED BACTERIOLOGY

Human Type Tubercle Bacilli, a New Chemical Method to Differentiate from other Mycobacteria. K. Konno. (*Science*, 1956, **124**, 985.) The author describes a simple chemical test by which strains of *Mycobacterium tuberculosis* var. *hominis*, whether virulent or attenuated, may be differentiated from other strains of the species and also other species of the genus *Mycobacterium*. Such a test was desirable because the only methods of differentiation in current use are based on cultural and pathogenic characteristics. Having previously shown a marked difference in nicotinamide production between human type and other mycobacteria (Konno, *Proc. Japan Acad.*, 1953, **29**, 289), this paper deals with a simplified method in which colonies of the organisms are directly tested. A total of 12 well-known strains of human, bovine and avian types were tested, along with 50 strains isolated from tuberculous patients, 10 strains of "atypical acid-fast bacteria," and such non-pathogens as *M. phlei* and *M. ranae*. The organisms were grown on Loewenstein-Jensen medium and allowed to grow for 1 to 3 months. A few colonies were transferred to 1 ml. 4 per cent alcoholic solution of aniline and 1 ml. of 10 per cent aqueous solution of cyanogen bromide was added. A positive test showed an intense yellow colour in the bacterial sediment and, after shaking, in the supernatant liquid. Controls with the medium gave only a slight green colour due to malachite green. A positive reaction was given only by human type strains, irrespective of virulence, no difference being noted between isoniazid sensitive and resistant strains. All other organisms, whether virulent, attenuated or non-pathogenic, gave a negative result. B. A. W.

Extinction Time Estimates, Further Studies on Reproducibility of. A. M. Cook and B. A. Wills. (*J. appl. Bact.*, 1956, **19**, 219.) The authors report variations in extinction times of *Escherichia coli* which result from alterations in the peptone concentration, pH and sterilising treatment of the medium used to detect growth of surviving organisms after exposure to aqueous phenol solutions. The effects of varying the composition and pH of media has received previous study, but the authors consider that these earlier findings were largely invalid because the experimental methods used attributed the within-estimate variability to the factor being studied. Conversely, the method used here enabled this within-estimate variability to be taken into account. A factorial experimental design was used in which each of the factors—peptone concentration, pH and duration of autoclaving—were tested at 2 levels. Experiments were performed on 3 occasions. An estimate of the "mean single survivor time" was obtained from each 15 replicate determination. Analysis of variance of the results showed that all 3 treatments were significant. It also appeared that the effects of increasing the concentration of peptone in the medium became more pronounced as the period of autoclaving was reduced, from which it was concluded that prolonged heating might destroy nutrient material, greater destruction occurring in more concentrated solutions. Provision of rigid conditions for sterilisation of media used in this type of estimation of bactericidal activity is recommended. Finally the authors point out the limitations of previous methods of determining extinction times and conclude that the method which they used provides estimates reproducible within such narrow limits as completely to invalidate all previously described techniques. B. A. W.

***Mycobacterium tuberculosis* in Mouse Tissues, Conversion of Infection to the Latent State by Pyrazinamide and a Companion Drug.** R. M. McCune, R. Tompsett and W. McDermott. (*J. exp. Med.*, 1956, **104**, 763.) Further to a report by McCune and Tompsett (*J. exp. Med.*, **104**, 737) that tubercle bacilli susceptible to various drugs *in vitro* were able to survive long exposure to the drugs when present in certain mouse organs, particularly the spleen, this paper deals with the capacity of pyrazinamide (pyrazine-2-carboxylic acid amide) and a second drug to reduce the numbers of surviving organisms in the mouse to below the level of detectability. The experimental procedure consisted of infecting mice intravenously with strains of *Mycobacterium tuberculosis* var. *hominis*. After suitable intervals from the start of infection the animals were killed. The lungs and spleens were homogenised and cultured quantitatively, colony counts being obtained by inoculation of oleic acid albumin plates. On treatment of the animals with pyrazinamide (2 per cent of daily diet) and isoniazid (0·0125 per cent) together, starting infection and therapy at the same time, no detectable organisms could be found in the lungs after 23 days or in the spleen after 37 days. Most animals receiving either drug alone showed detectable numbers in both organs (especially the spleen) for at least 90 days. For the first 16 days of treatment pyrazinamide appeared to antagonize the action of isoniazid. Animals treated with isoniazid, streptomycin and aminosalicylic acid together showed persistence of organisms in lungs and spleen after 118 days. Pyrazinamide with isoniazid still caused "vanishing" of organisms in the organs when treatment was commenced on the 21st day after infection. Attempts were made to establish presence of the bacteria in tissues which yielded no colonies on culture: increasing the inoculum for the plates to the total tissue homogenate; inoculating into guinea pigs for detection of tuberculin reaction; long-continued incubation of tissue homogenates; prolonged post-treatment observation of the animals. Only the last method detected presence of the organisms: in mice which had been treated with pyrazinamide with isoniazid for 90 days after infection, the organisms were cultured from spleens of 12 and lungs of one out of 30 animals examined after a consecutive 90 day treatment-free interval. The authors consider that the complete disappearance of the tubercle bacilli meets the definition of a truly latent infection—the infection is present but is hidden beyond the limits of diagnostic reach. All but one of the strains of organisms surviving in the animals and detectable in the post-treatment period were susceptible to pyrazinamide *in vitro*. A study was made of the factors essential for uniform disappearance of the organisms. It was found that pyrazinamide must be administered in high daily dosage for at least 8 of the total 12 weeks of treatment and that concurrent or prior treatment with isoniazid, or possibly other antituberculous drugs, was also essential. The authors consider that pyrazinamide susceptibility of *Mycobacterium tuberculosis* is closely related to the capacity of the organism to undergo a type of alteration in response to environmental influences including the presence of other antituberculous drugs.

B. A. W.

LETTERS TO THE EDITOR

Antimicrobial Activity of Certain Local Anaesthetics

SIR,—It is humiliating to find that the more successful a drug is in its own field, the more certain it is that any other activity it may possess will be overlooked.

While investigating and comparing a number of formulations for their properties, including antibacterial activities, certain of these formulae were found to have activity in excess of that of the known ingredients. The more usual experience is of course to find that the introduction of active bactericidal agents into lotions and ointments, reduces their activity. When, however, the opposite occurs it suggests either some synergism or unsuspected activity in the ingredients.

The samples being investigated contained local anaesthetics and it was found that these have an antiseptic action. A brief exploratory survey of other similar drugs showed that amethocaine and amylocaine had bactericidal and fungicidal activity of significance at the levels ordinarily used in topical applications, whereas procaine and lignocaine in equivalent concentrations were inactive.

The relation between some of the local anaesthetics and the sulphonamides, each being derivatives of *p*-aminobenzoic acid, suggested initially a possible explanation of this action. However, of the four local anaesthetics used, though procaine and amethocaine are so linked in structure, only the latter has antimicrobial activity. Further work is obviously needed to elucidate this problem.

This is another instance of the oversight due to narrowness of outlook in our approach to the action of drugs, particularly in the case of the older established drugs which have not been submitted to extensive screening. Classical examples of this disregard were cited by Professor M. Westergaard* (Copenhagen) at the recent Symposium on antibiotic resistance, when he pointed out that a number of antibacterial agents and other drugs were mutogenic in lower organisms.

We are continuing our investigation of this particular problem, and will be publishing our results later; meanwhile there would appear to be similar opportunities of beachcombing among the older drugs, even by workers in small departments with limited resources, using traditional methods!

W. H. HUGHES.
H. C. STEWART.

Departments of Bacteriology and Pharmacology,
St. Mary's Hospital Medical School,
London, W.2.

April 10, 1957.

* At the Public Meeting at the Royal Society of Medicine, following the Ciba Symposium on "The Emergence of antibiotic resistance", March 29, 1957.

The Presence of an Aloin-like substance in Cascara Bark

SIR,—At the British Pharmaceutical Conference in Dublin last September, one of us (J.W.F.)[1] stated that there was evidence for the presence of a "resistant glycoside" in cascara (*Rhamnus purshiana*) which was possibly based on aloe-emodin. We have now succeeded in isolating a small quantity of this substance and preliminary tests indicate that it shares several properties of barbaloin. Barbaloin has recently been shown[2] to consist of aloe-emodin anthrone linked to glucose by a –C–C–linkage and not by the normal glycosidal link. Our new compound possesses the following properties which are identical with those of barbaloin: (*a*) it is not hydrolysed by heating with 3 N HCl as is usual with anthracene glycosides; (*b*) heating with ferric chloride leads to the production of anthraquinones; (*c*) the ultra-violet light curve shows peaks at 266 to 268 mμ and 296 mμ. It differs, however, from barbaloin in the following respects: (*a*) it is sweet and is much more water soluble; (*b*) the ultra-violet light curve of the new compound has a small peak at 325 mμ but none at 355 mμ, barbaloin has no peak at 325 mμ but a pronounced one at 355 mμ; (*c*) ferric chloride oxidation leads to the production of two anthraquinones. The first one was identified as aloe-emodin by its position on a paper chromatogram, its ultra-violet light curve and the melting point of the few crystals obtained. The second one appeared to be chrysophanol judged by its position on the paper chromatogram; but its ultra-violet light curve differed markedly from that of pure chrysophanol. This may be due to interfering substances eluted from the paper along with the anthraquinone; the R_F value of chrysophanol is 0·95 and in this region a certain amount of fluorescent material accumulates even in washed paper. A few crystals of the substance gave a melting point consistent with chrysophanol containing a slight amount of impurity.

The new compound bears some resemblance to "Casanthranol" described by Lee and Berger[3]; but it differs from their compound in that it is stable in water and appears to contain chrysophanol. It seems to be present in comparatively large amounts in the crude drug and in extracts and we are preparing a quantity for biological experiments and for further work on the anthraquinones and sugars present.

J. W. FAIRBAIRN.
V. K. MITAL.

Department of Pharmacognosy,
School of Pharmacy,
17 Bloomsbury Square,
London, W.C.1.

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