PROGRESS IN MEDICINAL CHEMISTRY

Volume 1

G. P. Ellis &
G. B. West

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PROGRESS IN MEDICINAL CHEMISTRY

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PREFACE

THE rapidly increasing volume of work now published annually in medicinal chemistry and fields allied to it makes heavy claims on the reader's time if he is to be familiar even with the main trends of development in any one field. It seems that this increase will be maintained in the foreseeable future and hence the need for periodic reviews of the various fields of medicinal chemistry becomes greater. The main purpose of the present volume is to present surveys, written by specialists, of selected topics. These reviews should not only be of interest to those who are working in the fields selected but also present a summary of the present position to those who may be approaching it for the first time.

This collection of reviews is written for the chemist, biochemist, pharmacologist, and to a smaller extent, the clinician. The treatment of the subjects is not identical in every case, and the individual emphasis reflects the author's special interest and experience. It is rarely possible, however, to cover each field exhaustively as the literature is so extensive. A discussion of pharmacological screening tests is believed not to have been published before in such detail. It is not put forward as an infallible or invariable approach to this complex subject but it should help chemists and others to understand the pharmacologist's aims and difficulties in such work. The subjects of the other chapters were chosen as it was considered that a critical evaluation of the literature might be a valuable guide for future work. The inevitable delay between the completion of the reviews (January–March 1960) and their publication will have to be allowed for; so rapid is the progress made in some fields dealt with in the present volume that the reader is asked to be mindful of this.

We wish to thank the authors, societies and publishers for permission to use illustrations and tables which have appeared in previous publications.

G. P. ELLIS
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October 1960

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PHARMACOLOGICAL SCREENING TESTS

W. G. SMITH

INTRODUCTION

WHEN pharmacologists meet organic chemists to discuss the biological action of organic compounds, the terms 'screening test' or 'pharmacological screening' frequently enter the conversation. Most organic chemists have only vague ideas as to the exact meaning of these terms, and any attempt on their part to obtain true definitions is usually confused if the explanations require the liberal use of other pharmacological terms such as 'parasympathomimetic' or 'ganglionic blocking'.

It is highly desirable that the medicinal chemist should have some understanding of the tests to which the end products of his labours are subjected, and some guidance from the pharmacologist whereby he can assess the validity of the results. This is impossible without some knowledge of basic pharmacological principles. This paper attempts to explain how a number of established pharmacological test procedures are used for screening purposes, and also contains a discussion of both the value and limitations of the results so obtained.

There are many avenues which must be explored while developing a new therapeutic agent. The chemist must first explore the various synthetic pathways which are available for producing the required compound. The biological actions of the new compound must then be explored by the pharmacologist. Both of these procedures are costly. For example, each of the three thousand compounds synthesized annually in the Research Laboratories of the Pharmaceutical Division of Imperial Chemical Industries in Great Britain costs on the average about £50 to produce and £150 to test[1]. Moreover, only about one compound in a thousand gets as far as a clinical trial in man, and even then it may not be successful. The industrial pharmacologist thus spends a great deal of his time rejecting the end products of his chemical colleagues' work. Since most new therapeutic agents originate in a chemical laboratory and a great deal of research effort is often spent on their development before they reach the pharmacologist, the medicinal chemist is frequently disappointed with the results of a biological assessment of the compounds. This is to be all the more so if he cannot appreciate how and why they were tested in a particular way. It is to be hoped that the present account, which is written mainly for the non-pharmacologist, will help to dispel some of the lack of understanding on which such disappointments are based.

DEFINITION OF A SCREENING TEST

In any pharmacological laboratory where new organic chemicals are to be subjected to a series of experimental assessments, several guiding principles

must operate. The screening operations which are carried out must supply answers to the following three important questions:

(*a*) What is the main pharmacological activity of the compound?
(*b*) What other biological properties does it possess?
(*c*) Has the compound sufficient activity to justify further study?

In order to provide answers to these questions, each compound must be subjected to a number of established pharmacological test procedures. These must involve a minimum expenditure of time, effort, and material, yet provide results of maximum reliability. Since only a small percentage of the compounds entering the screening laboratory will reach clinical trial in man, the object of screening operations is to find as early as possible those compounds which are going to be successful. The pharmacologist must reach a decision after using no more material than can be provided by a chemist's first successful synthesis (often not more than 200 mg). The pharmacological screening tests about to be described are not specialized tests of novel design; they are well established experimental procedures which can be used within the imposed conditions just considered.

When screening operations of this kind have been completed and answers are available to the three key questions listed earlier, pharmacological research in the true meaning of the term may then begin. Both the main and subsidiary pharmacological actions of the compound must be scrutinized in detail. A number of tests must be conducted with the object of reaching some understanding of those actions known to be present. The compound must be compared in a number of pharmacological tests with other substances known to possess the same or similar activity. As this work proceeds, information must be collected about the absorption, distribution, and metabolism of the new compound within the mammalian body. Data must be collected on the circulating blood levels after various routes of administration. The excretion of the material must be studied. These studies will then prompt toxicological studies of the material, and from a new series of animal experiments, the relative safety of the potential new drug in man must be anticipated.

The completion of screening operations thus constitutes only a beginning to the pharmacological assessment of a potential new drug. Since all work subsequent to screening is detailed and consequently time-consuming and costly, the reasons why screening operations are performed with the object of eliminating unsuitable compounds should now be obvious. It must not be thought that screening operations reject all unsuitable compounds and pass only suitable ones. The problem of designing new therapeutic agents is so complex that the inherent disadvantages attached to the use of a given compound may only become apparent when its actions are studied in detail. A compound may be rejected, therefore, at any stage of the pharmacological assessment, but the initial screening operations should be wide enough in scope and so well integrated that any major disadvantages inherent in the intended use of a compound are quickly brought to light. It is in this context that the tests may be considered to operate as a screen. Full-scale pharmacological research can then be concentrated on compounds whose expectancy of success is high.

TYPES OF PHARMACOLOGICAL TESTS

A pharmacologist who is studying the biological activity of a new organic compound collects his information in one of three ways. Firstly, he may administer the new compound to an intact animal and then observe any changes in its normal behaviour. In practice, small rodents are used for such investigations. If the animals become hypnotized or lose consciousness, probably the compound is a depressant of the central nervous system. If they become agitated or develop convulsions, the compound is probably a stimulant of the central nervous system.

Secondly, he may anaesthetize a larger experimental animal (*e.g.* a cat) and study the actions of the new compound upon one of the physiological systems of the animal (for instance, the cardiovascular system). He does this by attaching recording instruments to the system under study and recording changes induced by the intravenous administration of the new compound. One of the simplest preparations of this kind is an anaesthetized cat from one of whose arteries the blood-pressure is recorded by a mercury manometer. Drug-induced changes in the resting blood-pressure can be recorded in this way, and sometimes a considerable amount of information can be obtained about the way in which they are produced. Other preparations can be set up to study the actions of the new compound, say, on the respiratory or the urinary system.

Thirdly, the actions of a new compound may be studies on isolated tissues. The tissue is removed from an animal at death and kept alive *in vitro* under a carefully controlled set of experimental conditions. A common piece of apparatus used for experiments of this kind is the isolated organ bath in which a study may be made of the actions of the new compound upon isolated smooth muscle. This preparation is an important tool in some fields of pharmacological research, although the results obtained sometimes have a narrow application due to the artificiality of the test system. As will be seen later, examples of all three types of pharmacological test are used in an integrated programme of pharmacological screening.

THE ORGANIZATION OF PHARMACOLOGICAL SCREENING TESTS

Classified Pharmacological Actions

Before considering in detail how pharmacological screening may be organized and carried out, it is necessary to understand a little about the nervous control of the activity of peripheral tissues. *Figure 1.1* shows the three different types of motor nerve which convey impulses from the brain and spinal cord (central nervous system) to other organs in the periphery of the mammalian body.

The spinal nerve controls the activities of striated muscle which is sometimes called voluntary muscle since the control exercised is one permitting voluntary intervention. A spinal nerve has only one synapse. This is the gap between the controlling nerve cell (neurone) and the muscle cell whose activity it controls. A nerve impulse leaves the central nervous system and passes towards the periphery along the axon process of the neurone. It

eventually reaches the synapse, where it causes the release of a small quantity of a chemical substance which is said to function as a chemical transmitter or neurohormone. It is the molecules of this chemical transmitter which diffuse across the synapse and cause the muscle cell to contract. The chemical transmitter of nerve impulses in the synapse of a spinal nerve is acetylcholine (ACh_N).

Tissues other than voluntary muscle also have their activities controlled by motor nerves. Since these nerves are never under the control of the will, they

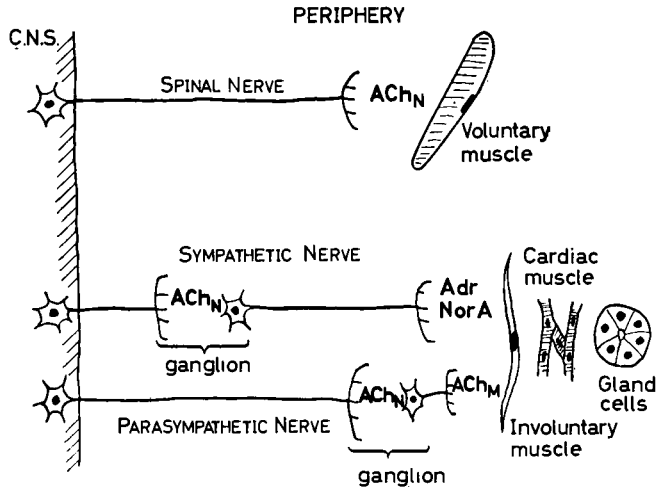

Figure 1.1. Diagram of the motor nerves of the mammalian body to indicate the peripheral structures which they control
Chemical transmitters in the synapses are shown thus: ACh_M for acetylcholine with a muscarinic action, ACh_N for acetylcholine with a nicotinic action, Adr for adrenaline, and NorA for noradrenaline

are said to constitute the autonomic nervous system. The second nerve shown in *Figure 1.1* is a sympathetic nerve supplying involuntary muscle, cardiac muscle or gland cells. Unlike the spinal nerve we have just considered, the sympathetic nerve has two synapses. It has a terminal synapse at the point in the periphery where the nerve ends, and in addition, a synapse near the central nervous system, called the ganglionic synapse. In the ganglionic synapse, acetylcholine is the chemical transmitter, but in the terminal synapse, the chemical transmitter is usually a mixture of adrenaline and noradrenaline.

The third motor nerve shown in *Figure 1.1* is also an autonomic nerve, called a parasympathetic nerve. Like the sympathetic nerve but unlike the spinal nerve, it has two synapses. Both are near the peripheral end of the nerve. One is the terminal synapse and the other a ganglionic synapse. The chemical transmitter in both synapses is acetylcholine. Acetylcholine is thus a chemical transmitter of nerve impulses in three different anatomical situations: the terminal synapses of spinal nerves, the ganglionic synapses of all autonomic nerves (both sympathetic and parasympathetic) and also the terminal synapses of parasympathetic nerves.

A drug may stimulate the activity of voluntary muscle, imitating the action of the chemical transmitter (acetylcholine) through which normal nervous control is exercised. This is described as a cholinergic drug (see *Figure 1.2*). Alternatively, a drug may block the effects of acetylcholine liberated as a result of normal nervous activity, in which case it is described as a neuromuscular blocking drug to imply that it has a blocking action in a nerve–muscle synapse. An example of this kind of drug is the alkaloid tubocurarine. Since there are few substances that modify voluntary muscular activity by a direct action on the muscle cells themselves, the above two drug actions are the more important ones concerning the spinal nerve.

The situation in the autonomic nervous system is a little more complicated since autonomic nerves have both a ganglionic and a terminal synapse. The chemical transmitter in a sympathetic ganglionic synapse is acetylcholine, and any substance capable of imitating the actions of acetylcholine in such a situation is called a ganglionic stimulant drug. Conversely, a substance which is capable of blocking the actions of acetylcholine in the same locality possesses ganglionic blocking action. DMPP (dimethylphenylpiperidinium) is a ganglionic stimulant drug and hexamethonium is an example of a ganglionic blocking compound. The alkaloid nicotine has ganglionic actions and is frequently used in the experimental pharmacology laboratory. In small doses it stimulates the ganglion, but in larger doses it is a ganglionic blocking drug.

Some compounds have biological actions similar to those resulting from stimulation of sympathetic nerves. These are known as sympathomimetic drugs. Such substances, *e.g.* phenylephrine, exert their characteristic effects by acting at the terminal synapses of sympathetic nerves on the smooth muscle, cardiac or gland cells normally stimulated by adrenaline or noradrenaline. Drugs which antagonize the actions of adrenaline or noradrenaline in this situation (the terminal sympathetic synapse) are known as adrenergic blocking drugs. Dibenamine is an example.

Other substances imitate the actions of acetylcholine in the terminal synapses of parasympathetic nerves. They are said to have a parasympathomimetic or muscarinic action. Many chemists find this last term confusing. Perhaps the reasons for using it may remove some of the confusion. Long before acetylcholine was found to be a chemical transmitter of nervous impulses (a discovery made in 1921) parasympathomimetic agents were known. The most outstanding of these was muscarine, an alkaloid found in a species of red and white spotted toadstool called *Amanita muscaria.* Thus acetylcholine was once regarded as an imitator of muscarine and was said to possess a muscarinic action. Not all actions of acetylcholine however are muscarinic. Besides its action at terminal parasympathetic synapses, it has, as stated earlier, a stimulant action in ganglionic synapses (both sympathetic and parasympathetic). These actions were also known before its function as a chemical transmitter. Since they imitated the stimulant actions of small doses of nicotine, these actions became known as the nicotinic actions of acetylcholine, to distinguish them from the muscarinic actions. This classification of acetylcholine effects was made by Dale[2]. We have already noted that the nicotinic effects of acetylcholine are antagonized by ganglionic blocking drugs such as hexamethonium. The muscarinic actions in terminal

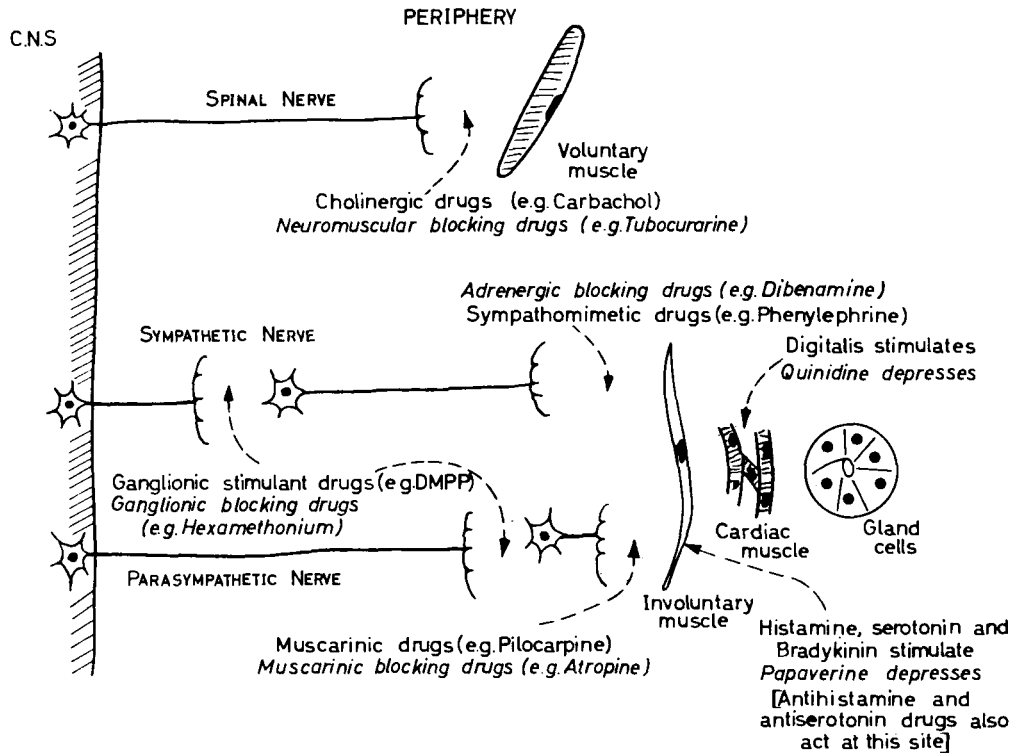

Figure 1.2. Diagram of the motor nerves of the mammalian body indicating the points at which some drugs exert an action

parasympathetic synapses are blocked by muscarinic blocking drugs, of which the alkaloid atropine is an example.

Involuntary muscle, heart muscle and glands are usually under the control of both the sympathetic and the parasympathetic nervous systems and this is illustrated in *Figure 1.2.* One system stimulates activity and the other depresses activity. This arrangement is spoken of as reciprocal innervation. With cardiac muscle, the stimulant nerve is the sympathetic and the depressant nerve is the parasympathetic. With involuntary muscle, *e.g.* that of the intestines, the roles are reversed and the parasympathetic is the stimulant nerve while the sympathetic is the depressant nerve.

Before leaving the subject of drug actions and autonomic nervous control, we must refer to an observation which many chemists find perplexing. How can a drug like atropine, which is described as a muscarinic blocking drug, have an action increasing the heart-rate? Is this not an indication that it possesses stimulant actions? If a drug imitates the action of a chemical transmitter in a nerve synapse, it will produce an effect equivalent to stimulating the nerve in question. This effect may be a depressant one, as is the case with the parasympathetic nerve (the vagus) controlling the heart. A muscarinic action on the heart is equivalent to increased vagal activity and is exhibited as a decrease in the heart-rate. A muscarinic blocking drug has effects equivalent to decreased vagal activity. Atropine, which possesses a muscarinic blocking action, may increase the heart-rate, not by stimulating at the terminal synapse of the sympathetic nerve but by blocking the terminal synapse of an inhibitor nerve. Since these nerves are continuously discharging impulses, those travelling down the sympathetic nerve will be free to exert a stimulant action.

We have now to consider those drugs which act directly on involuntary muscle, cardiac muscle and glands, without interfering with the normal mechanism of autonomic nervous control. There are four different types of drug which exercise a direct action on involuntary muscle. These types are represented by the compounds—histamine, serotonin, bradykinin, and papaverine. The first three stimulate while the fourth depresses involuntary muscular activity. Specific inhibitors of histamine are known as antihistamine drugs. Specific inhibitors of serotonin are known as antiserotonin drugs. There is no specific inhibitor of bradykinin. Direct stimulants of cardiac muscle are represented by the digitalis glycosides. An example of a direct acting cardiac depressant is the alkaloid, quinidine. Direct stimulants of glandular activity are alimentary hormones with physiological roles connected with the control of digestive processes. Their actions are specific for one type of gland. Gastrin, for example, stimulates the gastric glands of the stomach to produce gastric juice and it is relatively inactive as a stimulant of other glands. Secretin is just as specific a stimulator of the cells in the pancreas that produce pancreatic juice. Such highly specific actions are not included in screening operations and for that reason are not shown on *Figure 1.2.* Reference to that figure will show that a consideration of motor nerve innervation has enabled us to classify eighteen different pharmacological activities which must be differentiated when a new compound is screened for peripheral pharmacological actions.

Turning our attention to those drugs which have actions on the central

nervous system (C.N.S.) itself, we must first understand the division of this system. *Figure 1.3* is a diagrammatic drawing of the central nervous system of man.

The brain itself consists of four parts, and these are joined to the spinal cord. The cerebrum or forebrain is the largest part. It contains the so-called higher centres, the possession of which differentiates man from lower

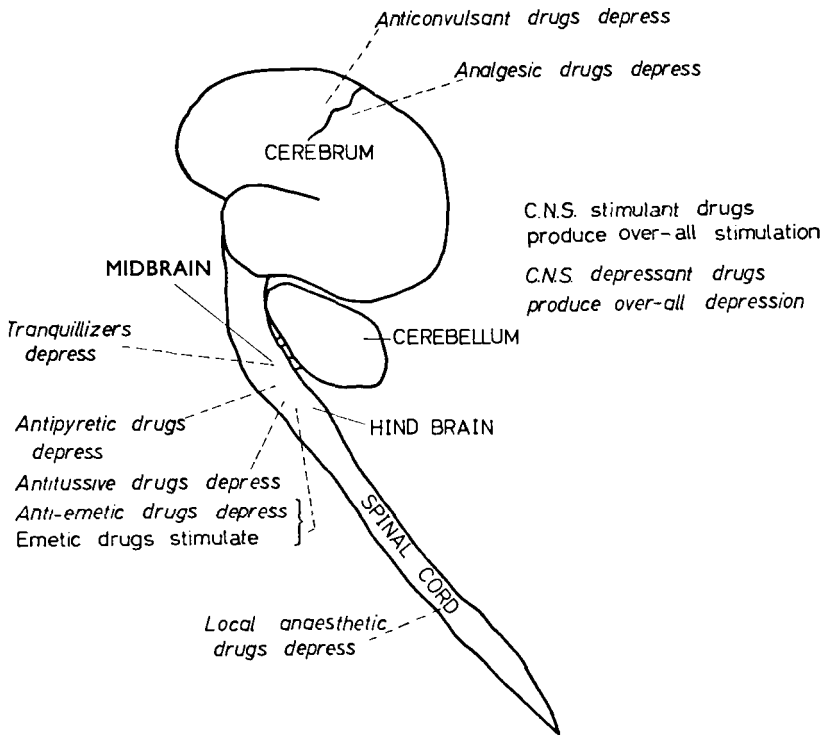

Figure 1.3. Diagram of the central nervous system of the mammalian body indicating the areas in which some types of drugs are believed to exert an action

mammals. As far as drug actions are concerned, it has two specialized areas on its outer surface or cortex. One is the motor area which controls muscular movement and which is selectively depressed by anticonvulsant drugs. Near it is the sensory area where sensations are interpreted. Analgesic drugs selectively depress pain perception in this area.

In the midbrain, tranquillizers are believed to exert their characteristic form of C.N.S. depression. Antipyretic drugs which lower the elevated body-temperature of fever, do so by selectively depressing the activity of the heat regulatory centre situated in this area of the brain.

The hind brain or medulla oblongata contains a number of centres which control essential body activities. Among these are centres controlling respiration and blood circulation. Many drugs owe their lethal actions to

their ability to depress, and finally to paralyse, these centres. Situated also in this part of the brain are the cough centre, which can be selectively depressed by antitussive drugs, and the vomiting centre, which is stimulated by emetic and depressed by anti-emetic drugs.

The cerebellum is a co-ordinating centre. Like all other parts of the central nervous system, it is affected by C.N.S. depressant and stimulant drugs. It is generally not the site of any one specific drug action.

There remain for consideration three types of drug with actions on the nervous system: local anaesthetic drugs, C.N.S. depressant and C.N.S. stimulant drugs. Local anaesthetic drugs have the property of depressing all nerve cells. They can be used to depress the activity of the spinal cord, when they may be called spinal anaesthetics. They can also depress the activity of motor nerves, such as those shown in *Figure 1.1.* Lastly, they depress activity in sensory nerves, so far unmentioned, which convey impulses from the periphery to the central nervous system, *i.e.* in the reverse direction to motor nerves which, as we have seen, convey impulses from parts of the central nervous system to the periphery. Local anaesthetic drugs are thus used, as their name implies, to produce local blockade of nervous activity.

C.N.S. depressant drugs are capable of depressing all parts of the central nervous system. Clinical medicine distinguishes several types according to the severity of the depression produced. In decreasing order of potency, we have anaesthetic, hypnotic and sedative drugs. This classification is too vaguely defined for use in the pharmacology laboratory. C.N.S. depressant drugs usually work on the higher centres in low doses, and then, as the dose is increased, exhibit midbrain, cerebellar and medullary depression. The ability to stimulate central nervous activity is often manifested in the laboratory by a convulsant action, caused by excessive stimulation of the motor area of the cerebrum. Thus ten central actions of drugs have been named and these form the basis of screening tests when central, as distinct from peripheral, pharmacological activities are under study.

The foregoing account of classified pharmacological actions will no doubt have left a distinct general impression in the mind of the non-biological reader that, whereas peripheral actions are systematically and exactly defined, central actions have a much less definite form. The central nervous system is composed of cells of one type (neurones) and these are interconnected in a most elaborate manner. Anatomically, it is possible to differentiate those areas rich in cell bodies from those areas which consist predominantly of connecting processes (dendrites and axons). Synapses within the central nervous system are however difficult to identify, and although chemical transmitters are believed to operate in central synapses, none have as yet been identified.

Toxicity Tests

Toxicity tests on mice are usually performed first in any screening programme. Time must be spent in observing the behaviour of the animals after injection, for, by so doing, valuable indications of the mode of action of the drug may be obtained. Such observations are easier to make during small-scale pilot experiments of the kind about to be described than they are in full-scale

toxicity tests. In deciding how toxicity tests are to be performed, due regard must be given to the following four factors which may influence the results.

Solubility of the new compound—If the material under study has a low solubility in water, the amount of material that can be injected into an animal is limited by the volume of solvent it will tolerate. Up to 50 ml./kg body-weight of an aqueous solution may be administered by intravenous injection without introducing a solvent toxicity factor. By intraperitoneal injection the limit is raised to 100 ml./kg body-weight. Since most drugs are less toxic by the latter route, the additional information available from experiments using this route of administration may be small.

Solvent used—Whenever possible the solvent should be 0·9 per cent sodium chloride in pyrogen-free distilled water. Although some water-miscible solvents such as glycerol or propylene glycol can be administered intravenously, such measures are best avoided. Even if control experiments are performed to establish the tolerance of the test animals to the solvent, the results obtained when subtoxic doses of solvent are given with toxic doses of test material are always subject to unknown errors.

pH of the injection fluid—It is possible to administer intravenously a comparatively large volume of either 0·1N hydrochloric acid or 0·1N sodium hydroxide. When such solutions enter the circulation they are rapidly buffered to the pH of the blood-plasma (7·3) but any previously dissolved drug may precipitate out and cause toxic effects due to the mechanical effects of a circulating precipitate rather than a pharmacological action of the drug. Solutions with pH values much removed from 7·3 must, therefore, be administered slowly and with care.

Speed of injection—Water-miscible organic solvents suitable for injection are usually more viscous than water, and hence must be given extremely slowly otherwise effects are produced by a high concentration of solvent in local areas of the blood-stream. As with acidic or alkaline solutions, there is a danger of intravascular precipitation when such solvents mix with blood-plasma. If it is known or suspected that the material under study has an action on the central nervous system or on the heart, slow injections are also necessary. Death may otherwise result with a sublethal dose of the new substance because the total amount of drug administered was not equally distributed throughout the circulating blood. Thus pentobarbitone is a safe intravenous anaesthetic when given slowly, but small doses given rapidly are lethal.

Observations on the injected animals

Four records must be made immediately. These are:

(*a*) The source of the mice, their sex, age, and body-weight.

(*b*) The time, the route, and rate of injection.

(*c*) The dosage in both milligrammes per kilogramme and millilitres per kilogramme.

(*d*) The absence or presence of an immediate reaction if the route of administration is intravenous.

Convulsions during injection can usually be felt as a tremor in the tail, or observed as running movements of the feet. Respiratory arrest, when immediate, is almost invariably accompanied by a raising of the head.

Whenever such effects are observed, the injections should be repeated using different animals and a slower rate of injection to minimize the effects.

Following injection, the investigator should try to obtain answers to the following questions:

(*a*) When do symptoms develop, *i.e.* how long after injection?
(*b*) How long do symptoms persist?
(*c*) Does the animal walk normally or crawl?
(*d*) Are the animal's movements co-ordinated?
(*e*) Does the animal show convulsions? If so, what kind?
(*f*) Does the animal remain on its back when turned over?
(*g*) What kind of respiration does the animal display?
(*h*) Is the animal sweating or cold with its hair standing?
(*i*) Is the animal salivating?
(*j*) Are the ears pale or flushed?
(*k*) Are the eyes normal, protruding or closed?
(*l*) Is the cornea of the eye cloudy or opaque?
(*m*) Is the pupil of the eye dilated or constricted?
(*n*) Are tears present? If so, are they normal or blood-stained?
(*o*) Does the animal urinate? If so, is the urine either excessive or blood-stained?
(*p*) Does the animal develop diarrhoea?
(*q*) Is the animal hypersensitive to noise?
(*r*) Are tremors present when the animal is still or when it moves?
(*s*) Does the animal show any other peculiarities?

Any small deviation from that which the experimenter considers to be normal is carefully noted. It may be thought unimportant at the time of its occurrence, but there is no way of knowing how important it may become in the light of further knowledge. Once the observation is on record it is always available.

Details of the tests

Having due regard for what has been outlined above, the compound under investigation (test material) is administered intravenously to pairs of mice in ascending doses. A good dose range for general consideration is 12·5, 25, 50, 100, 200, 400, and 800 mg/kg. The injected mice should be observed continuously for 2 hours, and then intermittently for a further 4 hours. Finally the overnight mortality should be recorded. The LD_{50} is the dose which causes death in 50 per cent of the group of animals receiving it. In the experiment just described there are two animals per dose level so that the dose killing one out of two is a very approximate estimate of the LD_{50}.

When the test material is too insoluble for lethal effects to be observed at a saline dose level of 50 ml./kg, the toxicity determination may be carried out using intraperitoneal instead of intravenous injections. If necessary, the test material can be given in aqueous suspension by this route. When this is done, however, all the injected animals that survive overnight are killed and their abdominal cavities examined with a hand-lens (magnification $\times 10$) to establish whether or not the test material has been absorbed.

The intravenous toxicity study at the dose levels suggested uses about 75 mg of test material. If both routes of administration are studied, about

150 mg of test material is required. Only about 50 mg are needed for the later tests for central action. The tests for peripheral action require even less, about 30 mg.

Tests for Peripheral Pharmacological Actions

The isolated guinea-pig ileum

The isolated guinea-pig ileum preparation consists of a short (2 to 3 cm) length of guinea-pig ileum, which, after removal from the animal at death, is kept alive in a saline solution thermostatically controlled at 37°C. The saline solution is Tyrode fluid which is buffered 0·9 per cent sodium chloride with added potassium, calcium, magnesium and glucose. It is oxygenated by bubbling either oxygen or laboratory air through it. The piece of ileum is so arranged inside a glass vessel of about 2 ml. capacity that contractions of its smooth muscle can be recorded by a lever. Readers unfamiliar with the appearance or use of isolated organ baths should consult a standard text on pharmacology[3a].

Having set up the ileum satisfactorily, an experiment with a compound having unknown actions usually begins with the administration of a dose of acetylcholine to give a bath concentration of 0·01 to 0·1 μg/ml. The ileum generally responds to this concentration of acetylcholine with a well marked contraction, but if it does not, the preparation is washed with fresh Tyrode solution and the dose of acetylcholine is increased ten-fold. Having obtained a satisfactory response to acetylcholine, the preparation is washed with Tyrode solution and the test material is added at a bath concentration of 1·0 μg/ml. Any response that occurs in the 60 seconds after administration is recorded, after which the preparation is washed and the first dose of acetylcholine is repeated. These three responses represent a first clue to the actions of the test material.

If the material has caused a contraction of the ileum, it has smooth muscle-stimulant activity at a concentration of 1·0 μg/ml. Alternatively, the test material may have elicited no response by itself but the response to the dose of acetylcholine given after the dose of test material may have been smaller than that produced by the first acetylcholine exposure. Such evidence indicates that the test material is an acetylcholine antagonist. The third possible result is the observation that the test material produces no response and that there is no difference in the two acetylcholine responses. This indicates absence of pharmacological activity at the dose level used. The experiment should then be repeated using higher concentrations of the test material until either an effect is obtained or it can be concluded that the test material is inactive on this preparation. A concentration of 1·0 mg/ml. is the highest that is usually used.

So far we have been able to decide whether the test material is a smooth muscle stimulant, an acetylcholine antagonist or simply inactive on the guinea-pig ileum. We must now expand our study of any antagonist activity that has been observed. Three other substances should be studied in conjunction with the test material. These are nicotine at a ganglion stimulating dose (about 2·0 μg/ml.) histamine (about 0·2 μg/ml.) and serotonin (about 0·2 μg/ml.). As with acetylcholine, each of these three substances

should be studied in a three-dose sequence, *i.e.* nicotine, wash, test material, wash, nicotine, wash. As before, the concentration of the test material should be increased progressively from 1·0 μg/ml. up to 1·0 mg/ml., when either nicotine antagonism will have been established or the test material will be inactive as a nicotine antagonist at that concentration. In this way, any antagonistic action which the test material possesses against the four standard stimulants can be detected. Furthermore, it will be found whether a given

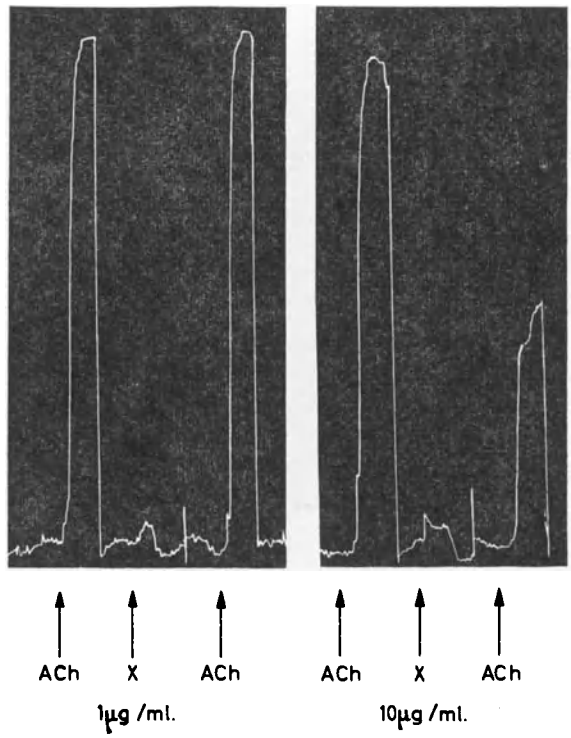

Figure 1.4. The action of a new organic compound on the isolated guinea-pig ileum preparation illustrating the way in which an antagonist action is detected
Compound X has no stimulant action at either 1 μg/ml. or 10 μg/ml. but at 10 μg/ml. it reduces the response to a fixed dose of acetylcholine (ACh)

concentration of test material, *e.g.* 10 μg/ml., antagonizes acetylcholine, nicotine, histamine and serotonin to an equal extent or whether antagonism against one particular stimulant is preferential. For example, antagonism of histamine at low concentrations when the responses to acetylcholine, nicotine and serotonin are unaltered, would be the pattern of activity observed with an antihistamine drug.

If the test material causes contraction of the ileum, steps must be taken to establish the exact site of this stimulant action.

To determine if the new compound stimulates parasympathetic ganglia,

the stimulant dose of the test material is first administered, then washed out, and a dose of nicotine causing a contraction of equal amplitude (a match-dose) is then given. Having obtained two matched responses, hexamethonium (a ganglionic blocking drug) is introduced in a concentration of 10 μg/ml. After 60 seconds, the hexamethonium is left in the bath and the match-dose of nicotine is added. Generally, a much reduced response to nicotine is recorded and the ileum is then washed. The dose of hexamethonium is repeated, left in the bath for 60 seconds, and then the stimulant dose of test material is administered. The response is recorded and the preparation is then washed.

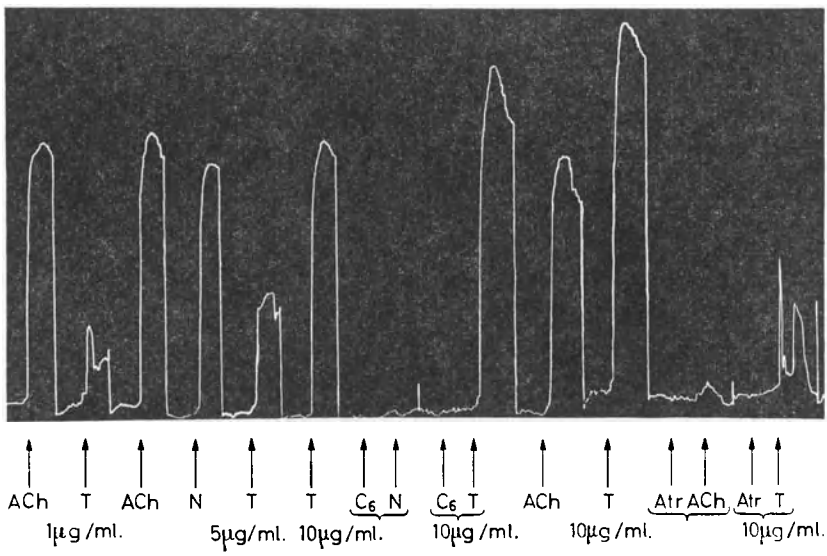

Figure 1.5. The muscarinic action of compound T on the isolated guinea-pig ileum preparation
The compound at a concentration of 10 μg/ml. produced a contraction comparable with those produced by acetylcholine (ACh) and nicotine (N). Its action was unaffected by a dose of hexamethonium (C_6) which abolished the response to nicotine. Atropine (Atr) reduced the responses to both acetylcholine and compound T.

As in the acetylcholine experiments previously described, a dose of smooth muscle stimulant is added before and after an antagonist to observe any reduction in response which would indicate an antagonist action. In the last experiment, the antagonist was hexamethonium which will antagonize or greatly reduce the ganglionic stimulant action of the match-dose of nicotine. The match-dose of nicotine functioned as a control whereby the expected action of hexamethonium was recorded. Having established that hexamethonium antagonizes nicotine, it should be observed whether or not it antagonizes an equi-active dose of the test material. If it does, it is established that the test material, like nicotine, is a ganglionic stimulant. If hexamethonium under these conditions does not reduce the response of the test material, the parasympathetic ganglion as the site of its action can be excluded.

Next, a stimulant dose of test material is given and the response is observed over 60 seconds. The preparation is washed and the response to a match-dose of acetylcholine is observed. As before, there is a response to the new compound and a control dose, this time, of acetylcholine. Atropine is now introduced in a concentration of 0·01 μg/ml. After 60 seconds, it is left in the bath and the match-dose of acetylcholine is administered. This should give no response. The preparation is washed and the dose of atropine is repeated. After 60 seconds, it is left in the bath and the dose of test material is given. The response of the test material either will have been abolished, like the response to acetylcholine, or will have been unaffected. If the response is abolished, it has been established that the test material has an action in the terminal synapses of parasympathetic nerves. Like acetylcholine, it is a muscarinic agent. If the test material produces contractions in a preparation after treatment with both hexamethonium and atropine, it must be working independently of motor nerve synapses and owes its effect to a direct action on the smooth muscle cells. It may, in this case, imitate either histamine or serotonin. If the response is due to a histamine-like action, it will be abolished by mepyramine at a concentration of 0·01 μg/ml., as will be the response of a match-dose of histamine. By a similar argument, if the test material has a serotonin-like action, its response will be reduced after the preparation has been stimulated by a high concentration (20 μg/ml.) of serotonin itself. A match-dose of serotonin will be inhibited to the same degree.

If the response of the test material is resistant to all the above attempts to antagonize it, it is a direct-acting smooth muscle stimulant with an unknown site of action. Naturally occurring polypeptides belong to this category.

The functional components of the isolated guinea-pig ileum preparation are shown in *Figure 1.6.* In setting up the preparation in an isolated organ bath, we have surgically excised a quantity of intestinal smooth muscle. With this muscle we have removed the terminal synapse of a sympathetic nerve, although the sympathetic ganglion belonging to that nerve remained in the animal. Parasympathetic ganglia, it may be remembered, occur near the termination of parasympathetic nerves. In the case of a parasympathetic nerve controlling intestinal smooth muscle the ganglion is actually to be found in the wall of the intestine. Our excised length of intestine thus contains a parasympathetic ganglionic synapse as well as a terminal parasympathetic synapse. We must now examine this collection of structures and note how various types of drug action manifest themselves in the kind of experiments that we have been considering.

First let us consider a compound which exerts no action on the ileum at any concentration investigated, but which nevertheless reduces the responses to acetylcholine, nicotine, histamine and serotonin. If its action is selective against acetylcholine, it has a muscarinic blocking action like that displayed by atropine. If its action is selective against histamine, it is an antihistamine drug and is behaving like mepyramine. Finally, if all four standard stimulants are blocked to about the same extent by one concentration of the test material, it has a direct depressant action on the smooth muscle cells like that of papaverine. Caution is necessary here, however. As can be seen from *Figure 1.6,* the preparation contains a functional terminal sympathetic

synapse. Adrenaline and noradrenaline, the chemical transmitters of such nerves, and also other compounds with sympathomimetic actions, can therefore be expected to exert an action on the isolated guinea-pig ileum. Adrenaline (or noradrenaline) does not produce a detectable relaxation of the smooth muscle fibres in the preparation, but its inhibitor action manifests itself in the form of a lowered sensitivity to smooth muscle stimulants of all types. After a dose of adrenaline (or noradrenaline) an isolated guinea-pig ileum exhibits smaller contractions when standard doses of smooth muscle

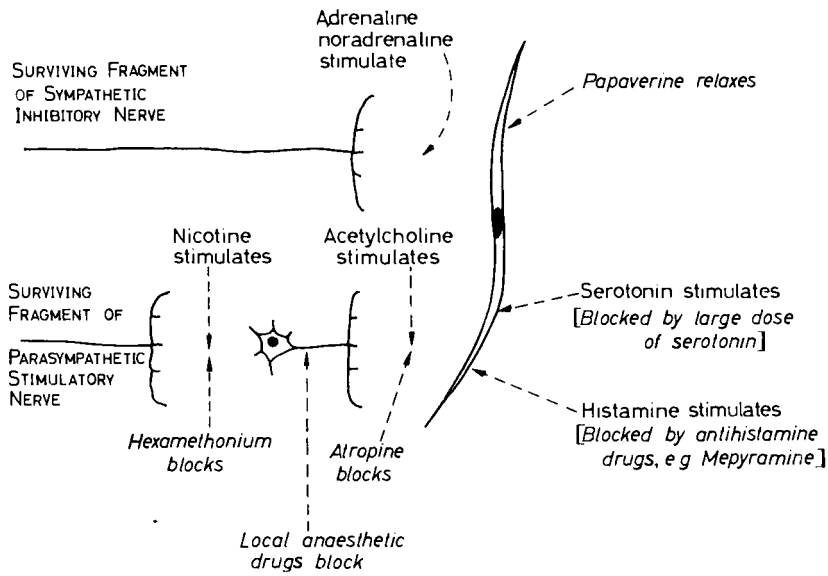

Figure 1.6. Diagram (which is a fragment of *Figure 1.2*) indicating the different types of drug action which can be detected on an isolated guinea-pig ileum preparation

stimulant drugs are administered. Adrenaline, noradrenaline and other sympathomimetic drugs thus exhibit a picture of antagonism indistinguishable from that given by papaverine. Further experiments are needed before the mode of action of the substance can be correctly and indisputably established.

We may have discovered that our test material selectively antagonizes nicotine at concentrations which do not influence the responses to other drugs. Since nicotine works in the isolated guinea-pig ileum as a stimulant of parasympathetic ganglia (sympathetic ganglia susceptible to its action are absent), a compound selectively antagonizing nicotine appears to be a ganglionic blocking drug. The use of the words 'appears to be' implies that there is an alternative explanation. Examination of *Figure 1.6* shows that this is the case.

Nicotine exerts a stimulant action in the ganglionic synapse of a parasympathetic nerve. It causes excitation of the ganglion cell which fires off impulses along its axon process to the terminal synapse. There, acetylcholine is liberated from the axon terminations, the nerve–muscle junction becomes stimulated, and the muscle contracts. This sequence of events may be

interrupted by hexamethonium, which, by virtue of its ganglionic blocking action prevents nicotine from exciting the ganglion cell. The action of nicotine however is also antagonized by a local anaesthetic drug such as procaine which is capable of slowing down, and, in high concentration, of blocking impulse conduction in the axon process of the ganglion cell. When it is established that a test substance has preferential anti-nicotine activity, the observed activity is due to one of these two actions. The material under test is either a ganglionic blocking drug or a local anaesthetic drug. Experiments on the cat nictitating membrane preparation and tests for local anaesthetic activity in guinea-pigs are necessary to reach a final decision.

One interesting fact should be remembered about serotonin. Earlier, its action was said to be a direct stimulant one on smooth muscle cells. It is shown to be working in this way in *Figure 1.6*. Nevertheless, serotonin responses are noticeably reduced by both hexamethonium and procaine, suggesting that some fraction of the total activity of serotonin on guinea-pig ileum preparations is due to an action at the parasympathetic ganglion like the action of nicotine. This interesting and, as yet, unexplained finding must be borne in mind when interpreting the actions of new synthetic materials on guinea-pig ileum. The method of interpreting the evidence obtained with this preparation is given in *Table 1.1*.

Table 1.1. The interpretation of data obtained in guinea-pig ileum experiments

Compound stimulates ileum		*Compound antagonizes stimulant drugs*	
Antagonized by	*Inference*	*Stimulant drug antagonized*	*Inference*
Hexamethonium (10^{-4} g/ml.)	Ganglionic stimulant drug	Acetylcholine	Muscarinic blocking drug
Atropine (10^{-7} g/ml.)	Muscarinic drug	Histamine	Antihistamine drug
Hexamethonium and Atropine	Ganglionic stimulant drug	Serotonin	Antiserotonin drug
Mepyramine (10^{-8} g/ml.)	Histamine-like compound	Nicotine	Ganglionic blocking drug or local anaesthetic drug
Serotonin (10^{-5} g/ml.)	Serotonin-like compound	Nicotine and Serotonin	Ganglionic blocking drug or local anaesthetic drug
None of the above	Smooth muscle stimulant drug of unknown action	Acetylcholine, Histamine Serotonin and Nicotine	Papaverine-like drug or sympathomimetic drug

The isolated guinea-pig vas deferens

This preparation is similar to the isolated guinea-pig ileum preparation in that its functional motor component is smooth muscle, and that it is maintained in aerated Tyrode solution in an isolated organ bath. Unlike intestinal

smooth muscle, vas deferens smooth muscle contracts in response to adrenaline or noradrenaline. The preparation and its normal responses to these drugs were described by Leach[4]. The vasa deferentia are dissected out from the genital tract of a male guinea pig immediately after death, and placed in cold aerated Tyrode solution. A single vas deferens is then transferred to a 10 ml. isolated organ bath maintained at 35°C. As with ileum, contractions are recorded with a light lever.

Since this preparation contracts after the administration of adrenaline, it will distinguish between sympathomimetic substances and smooth muscle relaxants such as papaverine. Substances acting like papaverine have the same effects on this preparation as they have on guinea-pig ileum, *i.e.* an antagonistic one to all stimulant drugs.

Sympathomimetic activity can be distinguished from papaverine-like activity as follows. If the test material on this preparation has no stimulant action but reduces the response to acetylcholine, it may have papaverine-like action. This may be confirmed by administering matched doses of histamine and acetylcholine before and after an active dose of the test material; equal antagonism of both stimulants will result, and the non-specific nature of the effect will be confirmed.

If the test material has a stimulant action on this preparation, it may have sympathomimetic action. This may be confirmed as follows. After administering an active dose of test material and matching the contraction with a dose of adrenaline, piperoxan (an adrenergic blocking drug) may be given in a concentration of 0·1 μg/ml. If the test material has sympathomimetic activity, its response, like that of a matched dose of adrenaline, will be reduced by piperoxan. The reduction in these responses may not, however, be equal, since under the experimental conditions used, adrenaline is more readily antagonized than noradrenaline.

Local anaesthetic tests in guinea-pigs

If the results of experiments on isolated guinea-pig ileum indicate that the test material has either local anaesthetic or ganglionic blocking activity, tests for local anaesthesia should next be carried out. The technique by which this can be done, in guinea-pigs, was described by Bülbring and Wajda[5]. The test material is injected into the skin (intradermally) in 0·25 ml. of saline, the animals having been shaved with electric clippers on the previous day. The area of the injection is marked with Indian ink and the time of injection noted. A test for local anaesthesia is then applied to the injected area at regular intervals of 5 minutes for 30 minutes. The test consists of applying six pricks with a needle to the injection area, and noting how many of these pricks fail to produce a response from the animal. The normal response is a contraction of the skin surrounding the injection area, which may or may not be accompanied by a squeak from the animal. The total number of failures observed is a measure of the degree of local anaesthesia developed.

In practice it is usual to test several different concentrations of the test material. Some results obtained in this way are given in *Table 1.2.* The concentration producing 50 per cent local anaesthesia over a period of 30 minutes, *i.e.* 18 failures out of the 36 pricks, has been calculated for each substance. A compound which has been found to antagonize

nicotine and serotonin on the isolated guinea-pig ileum at a concentration of 10 μg/ml. may be expected to produce 50 per cent local anaesthesia in guinea-pig skin at a concentration of about 0·5 per cent. If the test material is active at 1 μg/ml. on the guinea-pig ileum, it should produce 50 per cent local anaesthesia at about 0·1 per cent. Similarly a compound active at 0·1 μg/ml. on the guinea-pig ileum should produce 50 per cent local anaesthesia in guinea-pig skin at about 0·01 per cent. In some laboratories this test is carried out in the presence of adrenaline, which is added to the injection solution in a concentration of 1 mg/100 ml. It constricts the blood-vessels in the injection site, and by so delaying the absorption of a local anaesthetic prolongs its action. This effect on the local anaesthesia produced by cinchocaine and by procaine is shown in *Table 1.2.*

Table 1.2. Local anaesthesia (calculated on a percentage basis) after intradermal injection in guinea-pigs

Compound	*Concentration of test material used* (g/100 ml.)										*Concentration for* 50% *anaesthesia*
	0·003	0·007	0·015	0·03	0·06	0·12	0·25	0·5	1·0	2·0	
Cinchocaine hydrochloride			35	50	76	94					0·03
Cinchocaine hydrochloride with adrenaline		16	40	95							0·02
Procaine hydrochloride							40	66	86	99	0·37
Procaine hydrochloride with adrenaline					38	59	90	97			0·08

If the test material has no local anaesthetic activity in guinea-pig skin, or has much less activity than the results of guinea-pig ileum experiments lead one to expect, it should be concluded that the test material owes its activity on guinea-pig ileum to a ganglionic blocking action. Such a conclusion may be confirmed on the cat nictitating membrane preparation.

The cat nictitating membrane preparation

Those unfamiliar with this preparation should consult a standard text[3b,c]. The nictitating membrane of the cat is a third eyelid which closes laterally across the surface of the cornea. It is composed of smooth muscle cells, and is normally controlled by a sympathetic nerve which originates from the spinal cord in the chest region. This nerve runs up the neck of the animal to the head and has a ganglion along its length. To set up this preparation, the cat is anaesthetized, and its head is rigidly clamped on to the operating table, a lever being attached to the nictitating membrane of one eye through a length of thread running over a system of pulleys. The sympathetic nerve is dissected in the neck region, and a pair of electrodes attached to it at a point between its origin and its ganglion. The functional components of this test system are shown in *Figure 1.7.* Usually blood-pressure recordings are made at the same time as contractions of the nictitating membrane. Drug solutions are given intravenously into one of the leg veins of the animal.

The sympathetic chain is stimulated so as to obtain a maintained and steady contraction of the nictitating membrane as shown in *Figure 1.8*, and then hexamethonium (0·25 mg/kg) is administered into the leg vein. A reduction in the height of the nictitating membrane response occurs, followed by a fairly rapid recovery, taking about 10 minutes. The response of the nictitating membrane is generally accompanied by a fall in blood-pressure, and recovery is a little slower than the recovery of the nictitating membrane. If these effects do not occur the dose of hexamethonium should

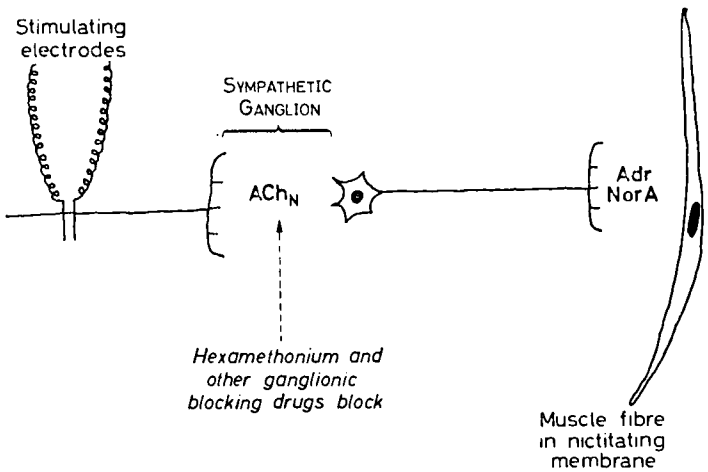

Figure 1.7. Diagram indicating the site of action of ganglionic blocking agents on the cat nictitating membrane preparation

be doubled. Knowing that hexamethonium is active at 10 μg/ml. as a nicotine antagonist on isolated guinea-pig ileum, the dose of test material which should be equi-active to the hexamethonium response is calculated and injected intravenously. A decrease in the height of contraction of the nictitating membrane, accompanied by a fall in blood-pressure, indicates that the test material is a ganglionic blocking drug. If the test material has no effect on the preparation, the dose should be progressively increased until some activity is observed. Procaine has anti-nicotine activity of the same order as hexamethonium on the isolated guinea-pig ileum, but it has only about one-fortieth of the activity of hexamethonium on the nictitating membrane preparation. It also has a different effect on the blood-pressure (*Figure 1.8*). Differentiation of true ganglionic blocking action from local anaesthetic action is thus possible with the help of this preparation.

Tests on the Cardiovascular System of the Cat

The blood-pressure of a cat under surgical anaesthesia is recorded continuously from a main artery, usually the carotid artery in the neck. Any disturbance in the normal functioning of the cardiovascular system following the intravenous administration of the test material is detected as a change in

the blood-pressure. The preparation is sensitive to effects produced by: (*a*) interference in the normal autonomic control of the heart or blood-vessels, (*b*) direct stimulant or depressant actions on the heart, (*c*) direct stimulant or depressant actions on the blood-vessels, or (*d*) central actions of the test material if these influence the central origins of the autonomic nerves controlling the heart and blood-vessels. The preparation is described by Burn[3c]. Its use as a screening test is based upon a design for tests on the cardiovascular system of the dog, described by Nieschulz, Popendiker and Hoffmann[6].

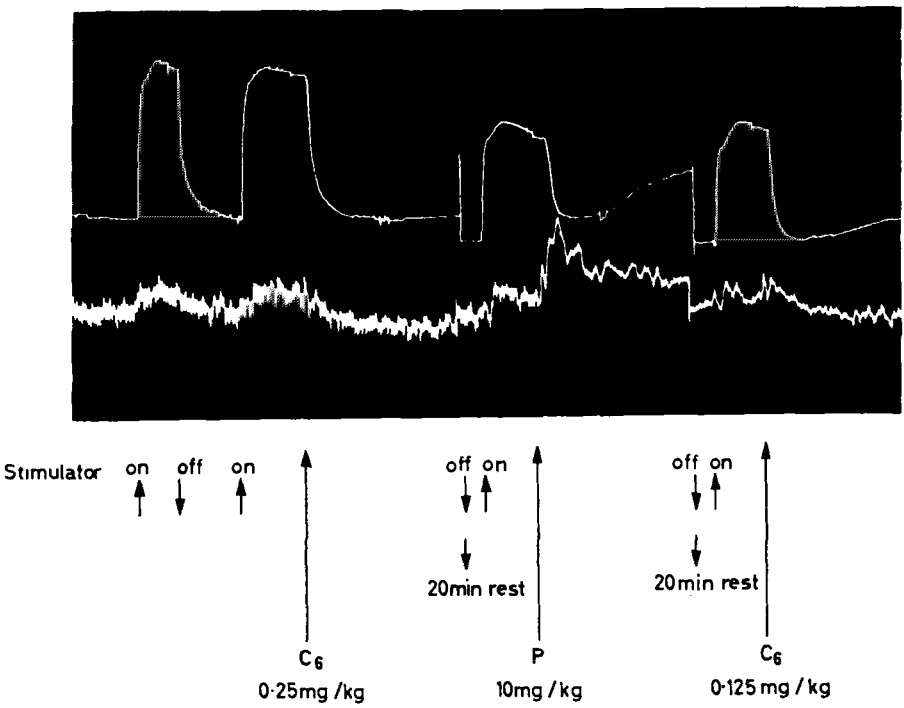

Figure 1.8. The actions of hexamethonium (C_6) and procaine (P) on the cat nictitating membrane preparation
Upper record is that of the nictitating membrane. Lower record is that of the blood-pressure. Note that procaine has a more transient effect than hexamethonium on the nictitating membrane.

The investigation of a test material is started by examining the responses to five reference compounds. These responses which constitute the 'normal response bracket', are those produced by: (*i*) electrical stimulation of the vagus nerve for 2 seconds, (*ii*) injecting acetylcholine at a dose of 5 μg/kg body-weight, (*iii*) occluding both carotid arteries for 45 seconds, (*iv*) injecting adrenaline at a dose of 5 μg/kg body-weight, and (*v*) injecting noradrenaline at a dose level of 5 μg/kg body-weight. The material under investigation is then injected at a dose level of 5 μg/kg. Any effect on the blood-pressure is recorded, and after 5 minutes the normal response bracket is repeated. Vagal stimulation and the injection of acetylcholine produce a transient reduction

in the blood-pressure. Injections of adrenaline and noradrenaline produce a transient rise in the blood-pressure. Occlusion of both carotid arteries invokes a reflex rise in blood-pressure. Those who are unfamiliar with this response may consult the paper by Procknik, Maison and Stutzman[7]. Any one or more of these responses may be altered by prior treatment with the test material. The effect which atropine has on the 'normal response bracket' is shown in *Figure 1.9.*

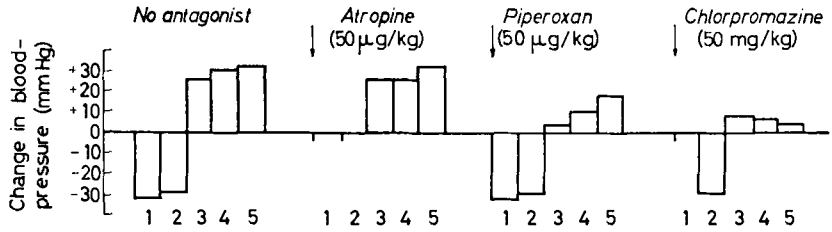

Figure 1.9. The actions of atropine, piperoxan and chlorpromazine on the blood-pressure changes in the cat induced by 1. vagal stimulation, 2. acetylcholine, 3. adrenaline, 4. noradrenaline, and 5. carotid occlusion

There is no response either to vagal stimulation or to the injection of acetylcholine if atropine has been previously injected. The other three responses remain unaltered. The functional components of this preparation are given in *Figure 1.10.* Examination of this diagram explains why atropine

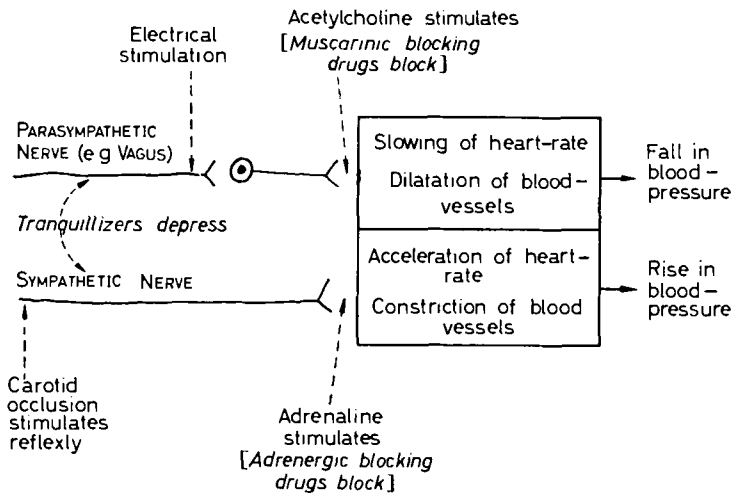

Figure 1.10. Diagram showing the sites of action of a number of drugs and experimental procedures which alter the blood-pressure of the anaesthetized cat

has the effects we have just observed. Being a muscarinic blocking drug, it exerts a blocking action in the terminal synapses of parasympathetic nerves.

Piperoxan has different actions on the normal response bracket. *Figure 1.9* shows that it markedly reduces the response to adrenaline. It reduces the noradrenaline response, too, but not to such an extent, and it also slightly

reduces the response to carotid arterial occlusion. Since it is an adrenergic blocking drug, it does not alter the effects of either vagal stimulation or the injection of acetylcholine.

Chlorpromazine is a tranquillizer and its actions are most noticeable on the responses to vagal stimulation and carotid arterial occlusion. It is also an adrenergic blocking drug and so it reduces the responses both to adrenaline and to noradrenaline. The response to injections of acetylcholine is unaltered.

When investigating a new compound, doses of the test material given after the first dose of 5 μg/kg depend upon the effects observed. If no alteration in the normal response bracket is observed, the dose of the test material may be increased four-fold to 200 μg/kg and then if need be to 800, 3,200, 12,800 *etc.* If, as with atropine, the effects of the first dose are most marked, lower doses may be investigated on a fresh preparation.

Besides any effects on the normal response bracket, these experiments disclose any activity which the test material itself has on the blood-pressure. The test material may produce a fall or a rise in the blood-pressure. Such blood-pressure changes may be of short, intermediate, or long duration.

The preparation will detect, by alterations in the normal response bracket, adrenergic blocking action, muscarinic blocking action, and some types of C.N.S. depressant action. It will also reveal muscarinic activity (behaviour like acetylcholine), sympathomimetic activity (behaviour like adrenaline) and ganglionic blocking activity. It will detect depressant or stimulant actions on the heart or blood-vessels. Depressant actions of this direct type

Table 1.3. The interpretation of drug actions observed on the cardiovascular system of the cat

A. Actions on the 'normal response bracket'

Changes in blood-pressure produced by					*Interpretation*
Acetylcholine	*Vagal stimulation*	*Adrenaline*	*Noradrenaline*	*Carotid occlusion*	
Lowered	Lowered	—	—	—	Muscarinic blocking action
—	—	Lowered	Lowered	Lowered	Adrenergic blocking action
—	Lowered	—	—	Lowered	Ganglionic blocking action or Tranquillizer action
—	Lowered	Lowered	Lowered	Lowered	Tranquillizer action
Raised	Raised	—	—	—	Anticholinesterase action
—	—	Raised	Raised	Raised	Antiaminoxidase action

B. Direct action of compound on blood-pressure of cat

Fall in blood-pressure		*Rise in blood-pressure*	
Less than 2 min duration	*2 to 15 min duration*	*Less than 5 min duration*	*More than 5 min duration*
Muscarinic action *or* Vasodilator action *or* Cardiac depressant action	Ganglionic blocking action *or* Tranquillizer action *or* Cardiac depressant action	Sympathomimetic action *or* Vasoconstrictor action	Antiaminoxidase action

will produce a fall in the blood-pressure and stimulant actions will produce a rise.

Besides confirming many pharmacological actions which will have been observed in earlier tests, this preparation also detects some new ones, for example, actions against two important enzymes, cholinesterase and monoaminoxidase. The former inactivates acetylcholine in the mammalian body, the latter inactivates adrenaline and noradrenaline. *Table 1.3* shows interpretation of the responses obtained with this preparation. Unless the material is to be used in cardiovascular disease, any cardiovascular effects represent major side reactions.

Tests for Central Pharmacological Actions

Analgesic triple tests

Each test requires 60 mice of the same sex and similar body-weight. They are divided into six groups of ten. Each group then receives the test material intraperitoneally at one of the following dose levels: 1·875, 3·75, 7·5, 15, 30, 60, 120, 240, 480, 960 mg/kg. The dose volume is fixed at 0·5 ml. The individual mice are injected with a thirty second interval between doses so that 60 mice are injected in a period of 30 minutes. After injection, the individual mice are placed singly in compartments of a large partitioned box where they remain for 30 minutes before being tested for the absence or presence of analgesia. Each mouse is subjected to three tests in which its reaction to thermal, mechanical, and electrical pain stimuli are observed. Any signs of hyperactivity or sedation are also noted at that time. Six consecutive dose levels (out of the ten given above) are selected after reference to the intravenous and/or intraperitoneal toxicity of the test material.

The first pain stimulus is a mechanical one described by Bianchi and Franceschini[8]. A small Dieffenbach artery clip with its end sheathed in rubber tubing is applied to the base of the tail for 15 seconds. A mouse which responds to this pain stimulus by making continuous attempts to dislodge the clip is considered to be showing a positive response. A mouse which appears to be feeling pain but not showing a positive response by trying to remove the pain source is considered to be showing a 'false positive' response. Some types of tranquillizer induce this kind of response.

Immediately after the mechanical stimulus, a thermal stimulus is applied by placing the mouse on a copper bath through which hot water is circulated and whose surface is thermostatically controlled at a temperature of 55°C. A positive response is shown by active signs of discomfort, *e.g.* sitting on the hind legs with licking of the front paws, followed by the raising or kicking of the hind legs. A mouse failing to show this reaction in 15 seconds is considered to be showing a negative response. This test is essentially that of Woolfe and Macdonald[9].

At this point in the testing procedure the mouse is handed to an assistant who subjects it to a third pain stimulus of an electrical kind. The animal is transferred to a Perspex mouse holder from which its tail is left projecting. The tail is wiped with cotton wool soaked in 70 per cent ethyl alcohol and then secured to the base of the mouse holder with two single strip electrodes. A drop of saline is placed between the tail and the point of contact with each

electrode, and a smear of silicone grease placed on the Perspex base between the electrodes to prevent the added saline from coalescing to form a continuous film. The pain stimulus consisting of a single 15 V shock of 100 milliseconds duration is then applied from a square wave stimulator. This stimulus produces as a positive response an 'escape reaction' whereby the animal jerks its tail and attempts to move forward up the mouse holder away from the electrodes. It may or may not be accompanied by squeaking. A mouse failing to respond to three single shocks of this kind is considered to be showing a negative response. The whole procedure takes 30 seconds.

The number of mice showing negative reactions to each stimulus at each dose level is then converted to a percentage response described as percentage analgesia. Each mouse in each group of ten thus contributes 10 per cent or 0 per cent to each group total. The responses obtained in this type of triple test are independent of the order in which the pain stimuli are applied.

Table 1.4. Analgesic triple tests
Analgesia estimated as a percentage of 10 mice 30 minutes after injection

Drug and pain stimulus	*Intraperitoneal dose* (mg/kg)										LD_{50} (mg/kg)
	1·875	3·75	7·5	15	30	60	120	240	480	960	
Morphine sulphate											
Thermal	60	70	80	100	100						
Mechanical	0	10	50	80	100						500
Electrical	30	40	80	100	100						
Pethidine hydrochloride											
Thermal	0	30	60	70	100						
Mechanical	0	20	30	60	100						150
Electrical	0	20	40	80	100						
Acetylsalicylic acid											
Thermal						10	20	30	50	90	
Mechanical						0	0	10	20	50	800
Electrical						0	10	20	40	60	
Acetanilide											
Thermal					0	30	90*	100*			
Mechanical					0	20	50*	100*			1,500
Electrical					0	0	20*	80*			
Phenobarbitone											
Thermal			0*	0*	0*	0*	(anaesthetized)				
Mechanical			0*	0*	0*	0*	(anaesthetized)				200
Electrical			0*	0*	0*	0*	(anaesthetized)				
Chlorpromazine											
Thermal	70*	100*	100*								
Mechanical	0*	0*	0*								30
Electrical	50*	70*	100*								
Phenytoin sodium											
Thermal					0	0	60	100			
Mechanical					20	60	80	100			200
Electrical					0	0	30	70			

* Indicates mice drowsy or sleeping.

Some responses obtained with this test using seven well-known drugs are given in *Table 1.4*. Morphine and pethidine which are analgesics are readily detected as such; they are slightly more potent against thermal pain than mechanical and electrical pain. Acetylsalicylic acid is also active against all three types of pain stimulus, but only at doses approaching lethal levels. Acetanilide is active at about one-tenth of its LD_{50}, but, at dose levels where analgesia is detected, non-specific C.N.S. depression is well marked. Phenobarbitone shows no analgesia at any dose level; its characteristic non-specific C.N.S. depressant activity can be independently observed. Chlorpromazine is an interesting compound in that it is active against thermal and electrical pain stimuli, but totally inactive against mechanical pain stimuli.

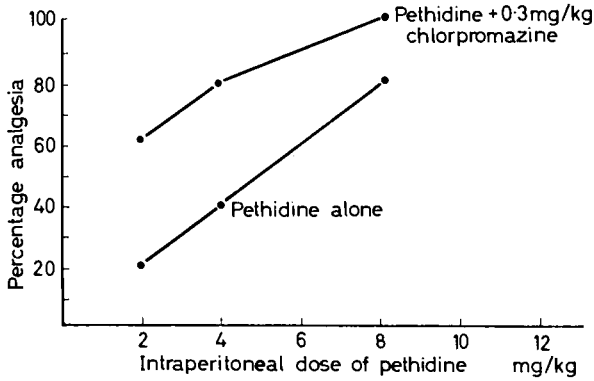

Figure 1.11. The potentiating effect of chlorpromazine on the analgesic activity of pethidine (The pain stimulus used was the thermal stimulus as described in the text)

This is also true of other phenothiazine tranquillizers, but not true of reserpine, benactyzine or meprobamate which all behave like phenobarbitone. Phenytoin sodium is active at dose levels approaching lethal ones; it shows, however, much higher activity against mechanical pain stimuli than against other types.

These triple analgesic tests indicate whether the test material possesses the following activities: (*1*) analgesic, behaving like morphine or pethidine; (*2*) analgesic, behaving like acetylsalicylic acid or acetanilide; (*3*) tranquillizer, behaving like chlorpromazine; (*4*) non-specific C.N.S. depressant or a tranquillizer, behaving like phenobarbitone; or (*5*) anticonvulsant, behaving like phenytoin sodium. If the results indicate tranquillizer or C.N.S. depressant activity, an analgesic potentiation test should be performed. If the results indicate anticonvulsant activity, leptazol antagonism experiments should be performed.

Analgesic potentiation tests

This test is performed with 60 mice divided into six groups of ten. Two groups receive 2 mg/kg pethidine intraperitoneally, two receive 4 mg/kg, and two receive 8 mg/kg. One group on each dose of pethidine also receives the test material (one quarter of the LD_{50} dose). The percentage analgesia is

then calculated and the results are plotted graphically. All tranquillizers show potentiation under these conditions. Any one of the pain stimuli described in the analgesic triple tests may be used in this experiment. In *Figure 1.11* a thermal pain stimulus was used. In practice both thermal and mechanical stimuli are equally convenient.

Leptazol antagonism tests

This test is conducted as described by Bianchi[10]. Sixty mice of the same sex and similar body-weight are divided into six groups of ten. Each group receives the test material intraperitoneally at one of the six consecutive dose levels used in the analgesic tests. Two hours later the mice in all the groups are given a maximal convulsant dose of leptazol intraperitoneally (110 mg/kg). Leptazol at that dose level induces convulsions, one characteristic of which is extension of the limbs due to contraction of the extensor muscles (so-called tonic contractions). Following injection of leptazol at the dose level suggested, all the mice can be expected to die in convulsions. The number of mice in which tonic convulsions are absent is recorded for each of the six experimental groups. Inhibition of the tonic phase of leptazol convulsions is indicative of anticonvulsant activity. The responses obtained in one experiment with phenytoin sodium, phenobarbitone, chlorpromazine, reserpine, meprobamate and benactyzine are given in *Table 1.5.*

Table 1.5. Leptazol antagonism tests. Inhibition of tonic hind limb extension (estimated as a percentage) in groups of 10 mice receiving leptazol (110 mg/kg) intraperitoneally.

Drug	*Dose* (mg/kg)								LD_{50}
	1·875	3·75	7·5	15	30	60	120	240	
Phenytoin sodium	20	40	70	90	100	100	100	100	200
Chlorpromazine	0	0	0	0	0	0	0	0	30
Reserpine	0	0	0	0	0	0	0	0	200
Meprobamate	0	0	0	0	10	40	70	100	400
Benactyzine	0	0	0	0	0	0	0	0	120

THE USE OF PHARMACOLOGICAL SCREENING TESTS

Scope and Limitations

The major pharmacological actions which are known today are given in *Table 1.6* (p. 30). The table also indicates those pharmacological actions which are detected by the screening tests that have been considered in this paper. Out of the 34 activities listed, the absence or presence of 22 can be established. The total amount of material used in these tests varies according to the degree of activity displayed. Weak activity makes it necessary to use high doses to observe a particular effect. In general, therefore, the expenditure of material is inversely proportional to the degree of activity discovered. As suggested earlier, screening tests must provide answers to certain key questions, and use no more material than can be provided by an organic chemist's first successful synthesis. The tests described require up to 200 mg of material, most of which is used in the toxicity tests.

It is of some importance that the screening tests themselves are essentially qualitative tests whereby one type of pharmacological activity is distinguished from another. Such quantitative considerations as do enter into these tests do so for one reason. Absence of a particular pharmacological action can be established only by showing that large doses are inactive. It is for this reason that the test material is investigated over a range of dose levels. The necessity for doing this may be turned to some advantage when interpreting the results. The main pharmacological effect is obviously the one which occurs with the lowest dose level. The relative supremacy of this one activity over all others will also be indicated to some degree by the ratio of the dose levels invoking the main, as distinct from the remaining, pharmacological actions. For example a test material showing antihistamine activity at 0·01 μg/ml. in a guinea-pig ileum test, will also show antagonism of serotonin, nicotine and acetylcholine at higher dose levels. If these other activities occur at ten times the dose, they will obviously occur as side effects if the compound is used therapeutically as an antihistamine drug. If, on the other hand, the antihistamine level is 0·01 μg/ml. and no other effects occur until the concentration is raised to 100 μg/ml., the chances of using the antihistamine activity without side effects are very good.

A selection of one or more reference standards has next to be made to try to decide whether the test material has advantages over other substances currently used in medical practice.

The Use of Reference Standards

With the aid of a reference standard, one is trying to decide how the activity of a test material compares with the activity of its possible therapeutic rivals. The ability to make such a decision at an early stage of a compound's test history is dependent upon certain conditions being fulfilled.

Firstly, the existence of a set of test data for each reference standard is essential. These test data may be obtained in the screening laboratory as and when they are required by applying the same tests to known drugs and to the compounds under investigation. The design of such experimentation is important. Alternatively the data may be abstracted from the pharmacological literature. Ideally, both internal laboratory data and literature data should be available. Secondly, the test data should be available in such a form that the therapeutic disadvantages can easily be deduced. The fact that the data are in the form of experimental observations is a potential source of difficulty. Obviously all the features of a drug's behaviour which a clinician considers to be disadvantages cannot be referred back to the experimental pharmacological laboratory and dealt with at that level. Diphenhydramine, for instance, is an antihistamine drug which also produces dryness of the mouth in patients. This is due to a muscarinic blocking action on the nerves which control salivary secretion. This fact will be found while investigating diphenhydramine as a reference standard in the screening laboratory, when its anti-acetylcholine activity is observed to be almost as great as its antihistamine activity. Khellin is a substance of plant origin with papaverine-like action. Unfortunately it is a centrally-acting emetic drug producing vomiting by a direct stimulant action on the vomiting centre in

the hind brain. Such a clinical disadvantage would not be apparent in the screening data on khellin. Emetic activity is not covered by the screening tests. Furthermore, central emetic actions can only be studied in cats and dogs as many other laboratory animals do not vomit. Studying all compounds for central emetic action would thus require substantial numbers of costly experimental animals and be both time-consuming and costly. Since the inclusion of central emetic action in screening tests is not economically sound, it must be dealt with at a stage later than the initial screening, as and when interest is provoked in this particular action.

The intelligent use of data obtained with reference standards, nevertheless, permits the disposal of many potential disadvantages. Those not accounted for in this way must be noted as potential features of future difficulty. The following is a hypothetical but illustrative screening test conclusion, based upon reference standard evaluation: 'This material is a ganglionic blocking drug with an apparent activity midway between mecamylamine and hexamethonium. Its duration of action is greater than that of hexamethonium. Its intravenous toxicity is of the same order as hexamethonium. It has no actions on the central nervous system except a depressant effect at near-lethal dose levels. Since it is a quaternary ammonium compound, its oral absorption is likely to be erratic and incomplete. The compound justifies further study but this difficulty should be borne in mind.'

Blind Testing Compared with Planned Testing

So far the scope and limitations of screening tests and the necessity for using reference standards have been discussed. The medicinal chemist usually produces new synthetic organic chemicals, believing generally that one particular activity of potential clinical usefulness is present in one particular chemical structure. The structural formula of the compound is then the target of his research. He sets out to make the compound and a series of closely related compounds for evaluation in a pharmacology laboratory. Since the activity of interest is defined beforehand, this type of attack on a problem calls for planned screening by the pharmacologist. Alternatively, the medicinal chemist may become interested in a group of chemically related compounds because of their accessibility. This interest frequently arises if his own research opens up a route to some hitherto inaccessible structures. Usually the expected pharmacological activity is only vaguely known. To the pharmacologist, the problem is thus one of blind testing.

The problems of planned testing and the value of screening tests in such a programme can be illustrated as follows. Suppose that interest is concentrated on materials having a pharmacological activity in connective tissue. The relevant pharmacological activities are described in *Table 1.6* as anti-anaphylactic, anti-inflammatory, and anti-oedema. Using 150 mg of material, a series of project tests are carried out for the purpose of answering the question: 'Does this material have a better anti-anaphylactic activity than other known anti-anaphylactic agents?'

If a compound is tested in this way and its anti-anaphylactic activity is considered to be worthy of further study, attention is automatically turned

towards its potential clinical value. What side reactions will it have? This question is answered by using 200 mg for screening tests. Any activity brought to light by such tests constitutes a potential side reaction if the material is used as an anti-anaphylactic agent in man. Screening tests, however, cover 22 out of 34 major pharmacological actions. What about the remaining 12? Each one of these 12 activities must be investigated separately. The nature of these activities is such that it is not possible to obtain answers to questions of wide scope such as: 'Does this material have

Table 1.6. Major pharmacological actions of compounds

Central actions	*Peripheral actions*	
1. C.N.S. DEPRESSANT	9. GANGLIONIC BLOCKING	22. CARDIOVASCULAR STIMULANT
2. C.N.S. STIMULANT	10. GANGLIONIC STIMULANT	23. CARDIOVASCULAR DEPRESSANT
3. TRANQUILLIZER	11. SEROTONIN-LIKE	24. ANTICHOLINESTERASE
4. ANALGESIC	12. ANTISEROTONIN	25. ANTIAMINOXIDASE
5. ANTICONVULSANT	13. HISTAMINE-LIKE	26. Neuromuscular stimulant
6. Antipyretic	14. ANTIHISTAMINE	27. Neuromuscular blocking
7. Antitussive	15. MUSCARINIC	28. Anti-oedema
8. Anti-emetic	16. MUSCARINIC BLOCKING	29. Anti-inflammatory
	17. SMOOTH MUSCLE STIMULANT	30. Anti-anaphylactic
	18. PAPAVERINE-LIKE	31. Antifibrillatory
	19. LOCAL ANAESTHETIC	32. Diuretic
	20. ADRENERGIC	33. Expectorant
	21. ADRENERGIC BLOCKING	34. Purgative

ACTIONS SHOWN IN CAPITALS are detected by the tests described in this paper.

any other central actions?' The questions which can be answered are all limited to one specific pharmacological action. Is this compound an antipyretic drug? This question may be answered by setting up a test to provide the answer. If antipyretic action is absent, the results will not indicate either the absence or presence of antitussive activity.

Obviously the cost of testing prevents a pharmacologist from running a further twelve specialized tests to cover all the activities in *Table 1.6*. Even if that were done, the possible endocrinological effects of the test material will not be known since they are not included in *Table 1.6*, but in practice the project test results, together with those of the screening tests, offer a very valuable guide to a compound's potential clinical future.

A slight variant of the practice described above is necessary if the activity of interest is one of the 22 included in the screening tests. Theoretically, the screening test results should make any project tests unnecessary. In practice, this is not so. Let us consider for a moment ganglionic blocking activity. While the screening tests disclose the number and types of side reactions present, the main activity of interest is known only as a drug concentration antagonizing nicotine on the guinea-pig ileum. Since the advantages and disadvantages of the test material relative to a number of reference standards (hexamethonium, mecamylamine, pempidine) should be known as soon as possible, more data about the ganglionic blocking activity should be available. These may be obtained by running a series of project tests permitting a more extensive study of ganglionic blockade. A good over-all assessment of any single test material can then be made by examining the project test results side by side with the results of the screening tests.

With blind testing, the results of screening test procedures carry with them important limitations. It has already been pointed out that screening tests are essentially qualitative tests, whereby one type of pharmacological activity can be distinguished from another. When blind testing is practised these results constitute our only knowledge of a test material's pharmacological actions. How useful is this information?

By the very nature of current pharmacological knowledge, the peripheral activities have been differentiated with a much higher degree of precision than the central ones. As each different test for peripheral activity has been described, reference has been made to the functional components of the test preparation in use. Peripheral pharmacological actions can thus be localized with accuracy to certain anatomical situations. Hexamethonium is known to exert its actions on autonomic ganglia which can readily be distinguished from other tissue in the histology laboratory. When describing tests for central activities, reference cannot be made to such accurately defined sites of action. The differentiation of one type of central action from another is imperfect. For this reason, central actions detected in screening tests constitute little more than indicators of the directions in which further studies should be conducted. This is especially true when the results come from blind testing.

Blind testing should be used to find useful avenues for further research. These avenues should then be covered by project tests as soon as it becomes apparent that the level of interest is likely to justify the work involved. While blind testing is in operation the chemist must be prepared for the pharmacologist to change his mind sometimes about the potential value of a test material. Blind screening test results, when written on paper, look just as reliable as planned screening results, but they sometimes convey quantitative implications which are not supported by later evidence. When a pharmacologist alters his opinion, the chemist should remember that the first decision was equivalent to deciding which of two synthetic routes produces the best yield of compound when the evidence was based on only a few qualitative tests. Blind screening starts as intelligent guesswork and remains as such until leads have been established.

A further application of blind screening is in the testing of antibiotics and other chemotherapeutic materials used for the control of infections. Completely blind screening of the material will establish whether or not it has clinical limitations. With such materials, the pharmacological activity disclosed by the screening tests should be negligible or non-existent. The same application exists for the screening of endocrinological materials, *e.g.* synthetic oestrogens. The tests can also be used to limit impurities in the manufacture of materials like protein hydrolysates.

Efficiency and Cost

The costing of single research operations is a difficult procedure. Nevertheless, in screening operations like the tests that have been described, the relative cost of the individual component tests can be calculated. *Table 1.7* shows an analysis of screening test cost based upon the cost of labour and animals. The weekly output levels given in this table are as valuable as the

cost figures, but are applicable only when the tests are operated continuously. When one individual performs one test on one day and a different test the following day, the output figures fall. The extent of the fall is dependent upon the versatility of the individual concerned. It is also influenced by the strain which the varying animal demands places on the running of the animal units from which the test animals are drawn.

Table 1.7. Weekly cost analysis of screening tests

Test procedure	*Animals*		*Labour*		*Output of compounds*	*Estimated cost per compound* (£)
	No. used	*Estimated cost* (£)	*No. of workers*	*Estimated cost* (£)		
Intravenous toxicity tests	240 mice	12	1	24	24	1·5
Intravenous and intraperitoneal toxicity tests	240 mice	12	1	24	12	3·0
Analgesic tests	1,530 mice	76	2	48	30	4·0
Analgesic potentiation tests	1,530 mice	76	2	48	30	4·0
Leptazol antagonism tests	1,530 mice	76	2	48	30	4·0
Guinea-pig ileum	10 guinea-pigs	5	1	24	5	6·0
Guinea-pig vas deferens	10 guinea-pigs	5	1	24	5	6·0
Cat nictitating membrane	5 cats	5	1	24	5	6·0
Cat cardiovascular system	5 cats	5	1	24	5	6·0
Local anaesthetic tests	50 guinea-pigs	25	2	48	8	9·0

This table is based upon several assumptions: (*1*) that the operation of any single test is continuous; (*2*) that labour and animal costs form an adequate basis for costing one type of test relative to another; (*3*) that animals are continuously available in the required numbers.

Reference to the footnotes to *Table 1.7* will indicate its limitations for calculating actual cost. Screening for central actions is less costly than screening for peripheral ones in spite of the larger numbers of animals used in the central tests. The most expensive test is the test for local anaesthetic activity. Whereas central actions can be studied at the rate of 30 compounds per week, peripheral actions can only be studied at the rate of five a week.

Screening test data are required in their entirety before any decisions can be made about rejecting a compound or using it for further study. *Table 1.7* offers information which can be used to ensure maximum co-ordination between the different phases of screening. It indicates how to employ a given labour force to achieve the maximum number of complete data reports per

week. It also provides a base-line level of operation from which to calculate the cost of factors such as fluctuations in animal supplies.

CONCLUSIONS

It has been demonstrated how basic pharmacological principles can be used to evolve a highly efficient system for screening new organic compounds for pharamacological activity. The scope and limitations of the tests have been discussed, and the best uses for the information obtained have been indicated. This thesis is based upon personal experience but has been evolved as a result of numerous discussions with past and present colleagues in both pharmacology and medicinal chemistry laboratories. It is now presented in its entirety in the hope that it will contribute towards a more wide-spread understanding of those problems met with in the conduct of any search for new and better drugs. As such understanding grows, it may become practicable to establish a universally accepted set of standard screening procedures. One day, perhaps, it may even be possible to describe the 'spectrum of pharmacological activity' of any new organic compound in terms as precise as its structural formula.

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2

HYPOTENSIVE AGENTS

R. WIEN

INTRODUCTION

THE ever increasing number of new drugs being introduced into medicine makes it important that those interested in their use can learn something about them without having to delve too deeply into reams of literature. This chapter on hypotensive agents is not intended to give *all* the references on *all* the chemical substances which have been claimed to have the property of lowering the blood-pressure. It is an attempt, rather, to give an over-all picture, looking at the whole from the point of view of a pharmacologist. As the entire field of hypotensive agents is a vast one, it is hoped that those who feel that some particular aspect of the subject with which they are especially concerned has been inadequately considered or even omitted, will appreciate that in order to gain sufficient perspective one must lose detail. Moreover, in order to make the subject matter digestible to those of various tastes, certain sections of this chapter may appear elementary; again the reader is asked to be patient. If the comment is made that more attention has been paid to those substances which paralyse transmission in autonomic ganglia than to others, then the reply offered is that the author may be excused on the grounds that his interests and work have been mainly in that sphere. In any case he is of the opinion, rightly or wrongly, that most progress has been made in this sphere, not only by the discovery of several useful therapeutic substances, but also by a more precise analysis of their pharmacological actions than has been accorded to other hypotensive agents. Ganglion-blocking substances have also been of great value as a research tool to the physiologist and pharmacologist in the wider study of the autonomic nervous system. Much of this work has taken place only recently, starting some twelve years ago when Paton and Zaimis[1] first advocated the use of hexamethonium in medicine.

The literature is bedevilled with cryptic statements about the hypotensive properties of all kinds of compounds, with little or no attempt to analyse their effects. Frequently one hears no more of them because they have failed in initial clinical trials, or because clinicians may not even have been persuaded to try them as the pharmacological information available has been scanty. What is required today, more than anything else in this expanding field of hypotensive drugs, is a fuller understanding of the drugs available to the clinician. Hypotensive drugs have been introduced into medicine only comparatively recently, and it will take time for both patient and physician to gain confidence in their use, and to arrive at a proper understanding of what can be achieved with them.

BLOOD-PRESSURE

The pressure of the blood in the arteries results from the pumping action of the heart and the resistance to the flow of blood in the peripheral vessels. The general arrangement of the circulation may be considered as originating from the heart. This consists of two pumps, the right for the pulmonary circulation to the lungs to oxygenate the blood, and the left for the general systemic circulation. The output of the heart can be measured directly in the laboratory using the heart–lung preparation of the dog, or indirectly in man by measuring the volume of blood which flows through the lungs in a given time. The right and left pumps of the heart are each divided into two chambers, the more muscular ventricle and the less muscular auricle. When at rest the output of the heart is mainly dependent upon the pressure at which blood enters the right auricle.

The pressure in the arterial system is considerable. During the contraction of the heart or the phase of ventricular systole, the rapid distension of the aorta produces a high pressure wave (pulse) which is then followed by a return to a basal pressure. Two pressures can be recorded with each beat of the heart, a minimum or diastolic pressure and a maximum or systolic. Diastole corresponds to the pause between the heart beats and normally occupies a longer time than systole. The clinician measures the systolic and diastolic values by means of a mercury manometer, and the commonly accepted average values for normal young adults are about 120 mm systolic and 80 mm diastolic. The pharmacologist usually measures the mean arterial pressure in animals by connecting a cannula inserted into an artery directly to a mercury manometer, whereas the physician measures the blood-pressure in man indirectly using the well-known auscultatory method which involves a sphygmomanometer. Direct methods of measuring the arterial pressure in man involve intricate apparatus, *e.g.* capacitance and photo-electric manometers, but these are usually reserved for the research worker.

The blood-pressure in normal individuals is labile and is altered by several factors. Exercise, posture or exposure to heat and cold, for example, may alter the blood-pressure, but it remains fairly constant from day to day. The nervous system performs this function, the heart-rate being controlled by the vasomotor centre in the brain through the autonomic nerves. Autonomic motor nerves are termed cholinergic, if they liberate acetylcholine at their nerve endings, and adrenergic, if they liberate a mixture of noradrenaline and adrenaline. Cholinergic nerves consist of pre- and postganglionic parasympathetic and preganglionic sympathetic fibres and liberate acetylcholine close to the effector cell, whereas adrenergic nerves consist of sympathetic postganglionic fibres. The injection of noradrenaline produces effects which are similar to those resulting from excitation of the adrenergic nerves.

When dilatation of the blood-vessels in one area of the body occurs, there is usually a compensatory constriction of the vessels in an area elsewhere in the body to maintain the output of the heart. This change is regulated by the vasomotor centre which is influenced by impulses it receives from presso-receptors in the aortic arch and carotid sinus. Thus, the blood-pressure may be influenced by changes in the activity of the vasomotor centre in the brain

thereby affecting both heart and blood vessels, as well as by the endogenous liberation of adrenaline and noradrenaline from the adrenal glands which are also under the control of the nervous system.

HYPERTENSION AND ITS TREATMENT

An abnormally raised blood-pressure (*hypertension*) is a clinically observed phenomenon, the cause of which is not fully understood except in those cases where the raised blood-pressure is secondary to a disease, often of renal origin. Where no primary cause is known, the term *essential hypertension* is used.

At the outset it should be stressed that no sharp cleavage can be made between individuals with a normal blood-pressure and those with a raised blood-pressure[2]. This view is based on the concept that essential hypertension represents a quantitative deviation from the norm. The view held by some[3,4], however, is that the deviation is qualitative, and that essential hypertension is a specific disease. Biologists are only too familiar with the variation or normal distribution that occurs in a group of animals in response to a given drug. Clinicians are equally well aware of this phenomenon, and so design their trials to distribute the variation amongst individuals as fairly as possible. The position is readily accepted that individuals differ extensively in height but still lie within a normal range. There is a mean height, but the exceptional individual who is six feet six inches can still be a normal individual in the physiological sense. So it is with blood-pressure, a range of different pressures being normally distributed throughout the population. For statistical purposes pressure readings of 140 mm systolic and 90 mm diastolic (140/90) can be regarded as the upper limit of the normal range[5], whereas blood-pressures of 160/95 may be considered in the hypertensive range. This does not imply that these figures are of particular significance for any given individual. It is more important to know if the blood-pressure has been rising recently, or if it has always been at a slightly elevated level without causing any symptoms. The latter condition is called *benign hypertension.*

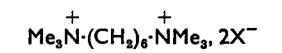
A distinction is made between *essential hypertension* for which no specific cause can be found and *malignant hypertension,* a severe and rapidly progressive disease. In this latter condition, the heart becomes enlarged as the disease progresses to compensate for the increased resistance in the vessels, with consequent deterioration in the general circulation. Changes also occur in other organs, especially in the eyes and kidneys.

Renal disorders, such as chronic nephritis, can be a cause of hypertension. The kidney liberates a pressor substance, renin. This is an enzyme which splits hypertensinogen, a constituent of plasma globulins, to form hypertensin. Hypertensin is a vasoconstrictor substance and causes a rise of blood-pressure without affecting the cardiac output. The release of renin can be demonstrated in experimental animals (rats and rabbits) when the renal artery is constricted[6,7,8]. Floyer[9] believes that there is also an extra renal mechanism for maintaining the raised blood-pressure in rats whose renal arteries have been clipped.

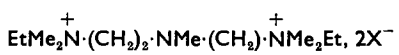
Other factors involved in hypertension include: (*a*) the liberation of noradrenaline, (in a small proportion of cases[10] there is a significant increase

in the excretion of catechol amines); (*b*) an alteration of electrolytes and extracellular volume[11]; and (*c*) proprioceptive reflexes arising from the carotid sinus and aortic arch[12].

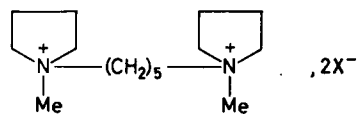
Apart from giving the patient rest, sedatives and a restricted diet (usually a reduction of sodium intake), treatment consists chiefly of the administration of ganglion-blocking substances (for example, hexamethonium (*I*), azamethonium (*II*), pentolinium (*III*), mecamylamine (*IV*) or pempidine (*V*), and *Rauwolfia* alkaloids. These are given either alone or in combination with



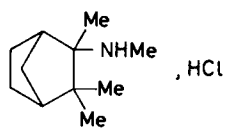
(*I*) Hexamethonium



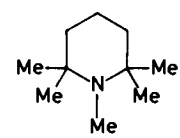
(*II*) Azamethonium



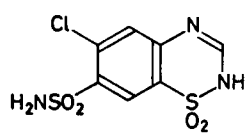
(*III*) Pentolinium



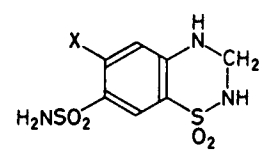
(*IV*) Mecamylamine hydrochloride



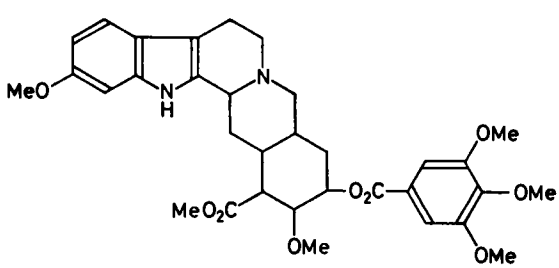
(*V*) Pempidine



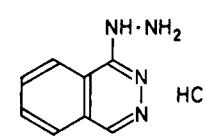
(*VI*) Chlorothiazide



(*VII*) Hydrochlorothiazide, X = Cl
Hydroflumethiazide, X = CF_3



(*VIII*) Reserpine

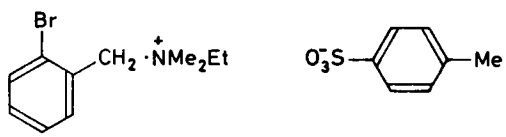


(*IX*) Hydrallazine

the diuretic compound, chlorothiazide (*VI*), and its near chemical relatives, hydrochlorothiazide and hydroflumethiazide (*VII*). Hydrallazine (*IX*), the *Veratrum* alkaloids and sometimes iproniazid are used to a much smaller extent. The *Veratrum* alkaloids are limited in their use as their emetic properties may prevent the administration of an effective dose. For instance, Smirk and Chapman[13] found *Veratum* alkaloids to be unsuitable, compared with hexamethonium, for the treatment of acute cardiac complications of hypertension as there was frequently vomiting, retching and retrosternal discomfort.

The prognosis in hypertension and the selection of hypertensive patients for treatment have been studied by Leishman[14], and Newman and Robertson[15]. (Perry and Schroeder[16] have also studied the effect of treatment on mortality rates in severe hypertension, especially with a view to comparing medicinal and surgical regimens.) Leishman made several interesting observations. He found that in untreated hypertension the diastolic pressure did not increase much over 5 years. The progress of 211 untreated hypertensive patients was followed from 1946, and compared with 118 patients treated with ganglion-blocking drugs. Half of the untreated patients died, while the mortality rate in the treated cases was only about one-third of that among those not treated. The average duration of treatment in the 86 surviving patients was 3·7 years. When treatment was first started hexamethonium bromide was the only drug employed and 16 patients who were well stabilized on it continued to use it. The remainder received pentolinium. Both drugs were given parenterally, the patients injecting themselves twice daily. Since the patients received reserpine by mouth in addition, the results cannot be attributed solely to the ganglion-blocking substances. Leishman thought there was no doubt that with this kind of treatment results could be obtained which were at least the equivalent of a successful sympathectomy operation. Since this favourable position was attained when the blood-pressure was effectively lowered for only a part of the day, even better results might be expected with substances such as mecamylamine and pempidine, which maintain a lowered blood-pressure for longer periods. Newman and Robertson[15] were more cautious in their conclusions. They suggested that the use of ganglion-blocking drugs in symptomless hypertension without demonstrable disease in the retina, heart or kidney should be restricted to the young male patient and that more controlled clinical trials were necessary to make a proper evaluation. In 1950 they commenced the treatment of 89 patients with ganglion-blocking drugs. Four patients were treated with reserpine or hydrallazine, and eight were given no specific therapy. The most important group of cases comprised 53 patients with neuroretinopathy. Of these 28 survived, 24 of whom were well or substantially better than when first seen. All the untreated patients died within 8 months. Although, in their experience, retinopathy shows little tendency to relapse when once cleared, Newman and Robertson advocated treatment to be continued indefinitely to diminish the risk of cerebrovascular accidents or heart failure. An excellent survey has been made by Harington, Kincaid-Smith and McMichael[17] of the results of treating 82 malignant hypertensive patients with ganglion-blocking drugs (hexamethonium, pentolinium and mecamylamine) and reserpine. They found that the expectation of life of the treated patients was increased by a factor of about seven times that expected from the control series.

The organo-mercurial compounds have been used for many years for their diuretic action in heart diseases, where relief of cardiac oedema or pulmonary congestion is required. Chlorothiazide is comparable with mercurials in its effect, and its chemical congeners, hydrochlorothiazide and hydroflumethiazide, are even more potent. Chlorothiazide inhibits renal tubular reabsorption of sodium and produces only a slight increase in bicarbonate excretion. It causes a considerable increase in the excretion of chloride and, to a lesser extent, of potassium. Chlorothiazide may therefore be given in the treatment of congestive heart failure with or without ganglion-blocking drugs. Potentiation or synergism of the hypotensive effect is obtained by combination of these different compounds[18,19], and indeed, Freis, Wanko, Wilson and Parrish[20], have found that chlorothiazide lowers the blood-pressure by itself. However, Dollery and his colleagues[19] found that single intravenous doses of chlorothiazide alone did not lower the blood-pressure appreciably and did not modify the hypotensive response to a standard intravenous dose (2·5 mg) of pentolinium. When chlorothiazide was given by mouth for 3 days, they then found an increased sensitivity to pentolinium. The action of chlorothiazide, therefore, is probably cumulative, and the increased sensitivity to pentolinium might be accounted for by the fall in plasma volume rather than by the direct result of sodium depletion, although if the sodium depletion has been large it also may play a part. The excretion of the secondary and tertiary amines, mecamylamine and pempidine, is, however, influenced by the pH of the urine[21,22]. When chlorothiazide is given concurrently with these amines, there is a slight shift in urinary pH towards the alkaline side so that less of these amines is excreted and their hypotensive effects are consequently increased[23,24]. Wilkins[25] and Freis *et al.*[20] have also used chlorothiazide, alone and combined with other hypotensive agents.



(X) Bretylium tosylate

Reserpine, unlike the ganglion-blocking drugs, lowers the blood-pressure more gradually. Vakil[26] found that, after 4 weeks' treatment with reserpine in a daily dose of 1 mg, there was a mean fall in pressure of 12–24 mm. Similar results have been obtained by other workers[27–31]. Smirk, Doyle and McQueen[32] treated 37 cases of severe hypertension with reserpine and pentolinium, and found that together these drugs reduced the variations in the blood-pressure levels which are generally observed when ganglion-blocking drugs are given alone. Freis[33] increased the action of reserpine by combining it with hydrallazine, hexamethonium or pentolinium. Orgain, Munroe and Donnelly[34] have also used reserpine in combination with hydrallazine and pentolinium. Darvill[35], in a clinical trial of the carbethoxysyringate ester of methyl reserpate, found that it exerted a significant hypotensive action with minimal side effects, 1 mg being equivalent to 0·25 mg of reserpine.

It is too early yet to say what will be the future of bretylium tosylate (*X*),

a drug which effectively blocks the peripheral sympathetic system by its action on adrenergic nerves. Boura, Green, McCoubrey, Laurence, Moulton and Rosenheim[36] showed in 36 patients (with moderate to severe hypertension) that there was a slight depression of the supine blood-pressure, and that the drug caused postural hypotension. Since it is a quaternary salt, the hypotensive response after oral administration is variable. By virtue of its selective effect on the sympathetic system, bretylium tosylate is a drug of considerable interest. The absence of any inhibitory effect on the parasympathetic system benefits the patient, but the postural hypotension and the variability of absorption by mouth are still factors to be considered.

EXPERIMENTAL PROCEDURES IN TESTING HYPOTENSIVE AGENTS

Hypotensive drugs have, for the most part, been tested on animals with normal blood-pressure, and not on animals in which hypertension has been experimentally induced. This is a sound principle since many drugs also exert their effect in normotensive states. There is, however, a new hypotensive drug[37], [2-(octahydro-1-azocinyl)ethyl]-guanidine sulphate or *N*-(2-guanidinoethyl)octahydroazocine sulphate, called guanethidine (*XX*), which lowers the blood-pressure of dogs with renal and neurogenic hypertension, but has little effect in dogs with normal blood-pressures.

Methods of producing hypertension experimentally[6,8] by constriction of a renal artery and/or removal of a kidney require an operative procedure, and are not readily suitable for the testing of a series of new chemical compounds. This is one reason why comparatively little attention has been paid to studying their effects in experimental hypertension. Another reason is that simple measurement of the blood-pressure is not always a good indication of the usefulness of a hypotensive agent. Although by definition a hypotensive agent is a substance which lowers the blood-pressure, the pharmacologist can test for this property in several indirect ways.

Transmission in Sympathetic Ganglia

Hexamethonium, pentolinium and related compounds can be tested for their ability to block the effect of preganglionic excitation of the cervical sympathetic nerve of the anaesthetized cat. The intravenous injection of these substances prevents transmission through the superior cervical ganglion, the indicator for this response being the contraction of the nictitating membrane in the eye. On electrical stimulation of the nerve[38], a sustained contraction of the membrane is obtained, and this relaxes on the intravenous injection of a ganglion-blocking compound, as the result of paralysis of transmission at the ganglion synapse[39]. The fact that a drug produces this effect does not mean that it is a ganglion-blocking drug of the competitive type. Transmission at the ganglion synapse involves the liberation of acetylcholine, and competitive substances are those which compete with acetylcholine for the receptor cells in the ganglion, so interfering with the normal process of transmission of autonomic impulses. Other tests which may be used to identify the type of block that has been obtained[40] include those where:

(*a*) The effect of the injection of adrenaline is unaltered, showing that the

test drug does not interfere with the ability of the nictitating membrane to contract; (adrenaline antagonists however modify this response).

(*b*) Postganglionic excitation remains fully effective in the presence of a completely paralysing dose of the compound being examined; (the anti-adrenergic compound, bretylium tosylate, inhibits the effect of both pre- and postganglionic excitation of the nerve-trunk).

(*c*) The release of acetylcholine from the preganglionic nerve terminals is unaltered. This can be determined by perfusing the ganglion and estimating the amount of acetylcholine released during excitation of the preganglionic nerve in the presence of the test compound; (procaine and procainamide modify the release of acetylcholine, and their mode of action is consequently different from competitive ganglion-blocking drugs[41].)

(*d*) The block is not preceded by an excitatory phase, for this type of action (*e.g.* nicotine-like) is undesirable in a hypotensive drug.

Experimental Hypertension in Animals

Drugs can decrease the blood-pressure in manifold ways, and the reader is warned to be wary of the numerous claims made in the literature for hypotensive compounds without any indication of their mechanism of action.

The blood-pressure may be lowered when the output of the heart is reduced by a harmful effect of the drug or when histamine is released; neither of these effects is desirable in a therapeutic compound. Lowering of the pressure may also be obtained by central as well as peripheral actions of drugs, by inhibition of the effects of adrenaline and by stimulation of the sensory receptors in the heart[42]. One or several of these factors may be involved when a fall in blood-pressure is observed, so that measurement of the blood-pressure alone gives no indication of whether the effect is a useful or harmful property of the drug.

Experimental hypertension in the rat, rabbit, monkey and dog has been produced by renal artery constriction[6,43,44]. Hypertension may also be produced experimentally by excision of the kidneys, by section of the carotid sinus and depressor nerves, and by cerebral ischaemia. Smirk and Hall[45] developed a colony of hypertensive rats by breeding from those with excessively high blood-pressures. Most attention however has been given to hypertension induced by renal artery constriction.

A knowledge of the action of a drug in hypertensive animals may be informative. Grollman[46], for example, has studied the effect of some hypotensive drugs in rats and dogs which were made hypertensive by ligating the renal artery of one kidney and removing the contralateral kidney after an interval of some weeks. The drugs studied were reserpine, hexamethonium, hydrallazine, pentolinium, protoveratrine and dibenyline. He found that the doses of these compounds needed to produce a moderate fall in pressure were much higher than those used in man.

Blood-pressure Determinations in Conscious Animals

Determinations of the blood-pressure in conscious rats, rabbits and dogs are possible without much difficulty. Although the methods are more laborious

than measurement of the blood-pressure in the anaesthetized animal, it would be rewarding if more attention were given to estimating the effects of well-known hypotensive agents in the conscious animal, if only for comparative purposes with other methods.

In the conscious rat, blood-pressure can be measured using a cuff for compression on a distal organ (foot, tail or ear) and a plethysmograph sufficiently sensitive to detect the pulse on decompression[47–49].

In the rabbit, blood-pressure can be measured with a capsule on the ear[50]. The animal is placed in an electrically heated box maintained at body-temperature[51], and a capsule is slipped on to the ear so that the central artery is enclosed. The pressure is raised in the capsule until the pulsation just fails to penetrate to the distal end of the artery, and then lowered until it first returns. The mean of these two values is taken as the reading of the blood-pressure.

In the conscious dog, blood-pressure determinations can be made by direct puncture of the femoral artery. A cannula containing a sterile solution of an anticoagulant is then connected to a mercury manometer or other device for measuring the pressure. A more elegant way of doing this is with an exteriorized carotid loop; measurement of the blood-pressure can then be made either by a method similar to that used in man, where a cuff is placed on the artery which compresses the artery until the pulse disappears, or by direct puncture of the artery and connecting the needle to a system for recording the pressure. Hall[52], at the School of Veterinary Medicine, Cambridge, has kindly allowed me to give the following unpublished description of his method. He uses normotensive dogs with exteriorized carotid loops for measuring the blood-pressure and the heart-rate. One of the carotid arteries in the neck is brought externally through the muscle layer and tied into a fold of skin. The operation is easily performed and after some weeks to allow the skin suture to heal, the loop is ready for use. The animal must be trained to sit quietly through the experiment. Using heparin solution to prevent clotting, the carotid loop is punctured and connected to a photo-electric cell and Cambridge recording instrument, a record being made of the pressure with a pen writer. At the same time, plate electrodes can be fastened to the limbs and connected to an electrocardiograph apparatus to measure the heart-rate and observe any arrhythmias. This procedure is necessary as it is difficult to measure the blood-pressure in the leg with the usual cuff and sphygmomanometer. The carotid loop method offers a means of studying the effect of drugs on the blood-pressure not only in normotensive, but also in hypertensive, animals.

Antagonists to Adrenaline and Adrenergic Nerve Stimulation

Adrenaline

One of the simplest methods of testing adrenaline antagonists is that using vessels of the perfused rabbit's ear[53]. In a freshly killed rabbit, a glass or polythene cannula is tied into the central artery of the ear after it has been severed from the head. The ear is perfused at room temperature with Ringer–Locke solution, saturated with a 95 per cent oxygen–5 per cent carbon dioxide mixture. The vessels of the ear are sensitive to very small doses of

adrenaline (0·001 to 0·05 μg), which produce a constriction of the vessels easily recordable with an outflow recorder. On obtaining a constant response to a test dose of adrenaline, the effect of an adrenaline antagonist is found by adding it to the perfusion fluid, and observing the decrease in the response. Fleckenstein[53] determined the concentration of the antagonist which reduced the adrenaline effect to one-tenth of the original value (*Figure 2.1*). The effects can be reversed by washing through the cannula

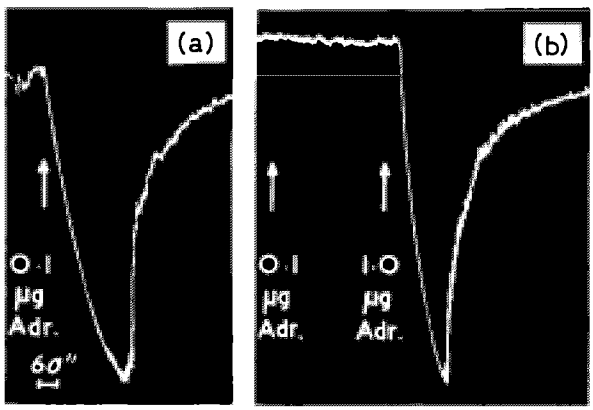


Figure 2.1. The anti-adrenaline activity of ergotoxine ethane-sulphonate in the perfused rabbit-ear preparation[53]
(*a*) Control constrictor effect of 0·1 μg adrenaline
(*b*) A dose of adrenaline (1 μg) 10 times greater than the initial test dose produces the same degree of constriction when ergotoxine ethane-sulphonate (0·02 μg/ml.) has been perfused for 30 minutes

with perfusion fluid, the time of recovery depending on the particular antagonist. Since the isolated ear can be preserved for several days by keeping it overnight in the refrigerator, several experiments can be made with the one preparation.

A method widely used for testing adrenaline antagonists depends on their ability to diminish, suppress or even reverse the rise of blood-pressure produced by adrenaline in the anaesthetized cat or dog. Dale[54], in 1913, first showed that ergotoxine inhibited and reversed the effect of adrenaline in the pithed cat (that is, a cat with its central nervous system destroyed). The effects of adrenergic nerve stimulation, resulting in the release of chemical transmitters, noradrenaline and adrenaline, can be divided into two types[55], α and β. Ergotoxine abolishes the α or excitatory action more readily than the β or weakly inhibitory effect. Adrenaline injected intravenously after an effective dose of ergotoxine produces a fall in blood-pressure as the vasodilator receptors in the vessels are not paralysed whereas the constrictor ones are blocked. The rabbit is not a suitable animal for demonstrating this effect as the vasodilator fibres in adrenergic nerves are not prominent in this species. Stimulation of the splanchnic nerve produces a rise of blood-pressure, resulting partly from a direct constrictor action of the arterioles and

partly from the liberation of adrenaline and noradrenaline by the adrenal medulla. The effect of an adrenaline antagonist on splanchnic stimulation as well as on the adrenaline pressor response may thus be determined. Usually anti-adrenaline compounds reduce the effects of splanchnic stimulation on the blood-pressure much less than the pressor response to injected adrenaline.

Adrenergic nerve stimulation

It is possible to find in the anaesthetized cat whether a test substance is a ganglion-blocking drug or a specific anti-adrenergic compound. The response of the nictitating membrane is recorded by attaching a thread from the membrane to a frontal writing lever. The cervical sympathetic nerve is

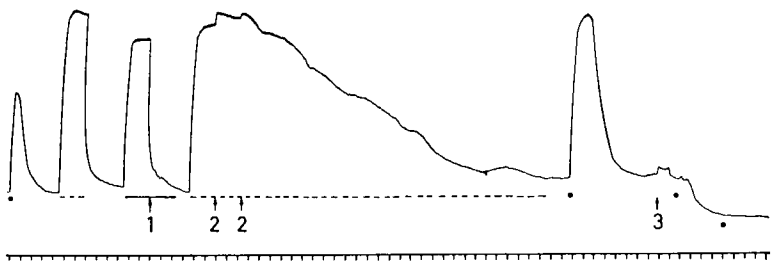


Figure 2.2. Tracing showing contractions of nictitating membrane in a cat under chloralose anaesthesia
At●, 50 mg adrenaline, injected intravenously. At broken line (– – –) postganglionic stimulation of cervical sympathetic nerve at 10 stimuli/second. (0·5 millisecond pulse width, supramaximal, 10 V.) At continuous line (——) preganglionic stimulation at 10/second. Intravenous injections at *1* of 0·5 mg/kg pempidine hydrochloride; at *2*, of 5 mg/kg bretylium toluene-*p*-sulphonate; and at *3* of 10 mg/kg dibenyline. Bottom tracing, time intervals of 60 seconds.
(N. D. Edge, personal communication)

dissected sufficiently high in the neck to expose the nerve-trunk beyond the superior cervical ganglion, and electrodes are placed both central and distal to the ganglion. On electrical stimulation of the nerve-trunk with 'square wave' stimuli either *pre-* or *post*ganglionically, a sustained contraction of the membrane is obtained (*Figure 2.2*)[56]. A brief contraction of the membrane is also obtained by the intravenous injection of adrenaline. It is then possible to distinguish between the effects of: (*a*) a ganglion-blocking compound, *e.g.* hexamethonium or pempidine, (*b*) an anti-adrenergic drug, *e.g.* bretylium tosylate, and (*c*) an adrenaline antagonist, *e.g.* dibenyline (phenoxybenzamine). Type (*a*) blocks the effects of *pre*ganglionic stimulation of the sympathetic nerve-trunk, but leaves the contractions of the membrane to *post*ganglionic stimulation and adrenaline unaltered; type (*b*) blocks the effects of *post*ganglionic stimulation, and type (*c*) prevents the response of the membrane to injected adrenaline.

Rauwolfia Alkaloids

There are many alkaloids of the numerous *Rauwolfia* plant species, but reserpine is the one which has been most extensively studied. Since its pharmacological properties are complex[57], its mode of action has not been completely worked out. Reserpine relaxes the nictitating membrane in cats,

which indicates a diminution in sympathetic tone; it has a slow onset of action in lowering the blood-pressure in both normotensive and hypertensive animals, it depletes the stores of 5-hydroxytryptamine and noradrenaline from the tissues, and it blocks the pressor response to occlusion of the carotid sinus, but is without effect on the fall in blood-pressure produced by severing the vagus and depressor nerves.

However, what is required is a property of reserpine which characterizes it from other hypotensive agents. Since tissues innervated by sympathetic nerves contain noradrenaline[58] and since treatment with reserpine depletes the tissues (heart, aorta) of noradrenaline[59,60], a test for reserpine-like action may be carried out by administering the compound to a rabbit and determining whether the aorta contains noradrenaline. Another method which is used depends on the action of tyramine, the pressor action of which is lost in the cat previously treated with reserpine. This experiment can be carried out in the spinal cat or anaesthetized rat, and involves recording the arterial blood-pressure. Although the tyramine response is at first inhibited or reduced in the reserpinized animal, it is augmented after the infusion of noradrenaline.

Veratrum Alkaloids

Several methods of bioassay of *Veratrum* preparations have been described. Toxicity tests have been used, but the amount required to produce a toxic

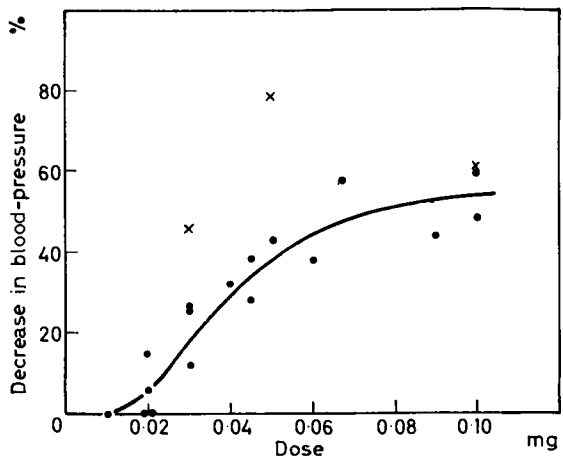


Figure 2.3. Relation of blood-pressure response to dosage of veratridine[61]
Solid circles represent individual responses in 4 dogs, to all of whom were given repeated doses beginning with 0·01 mg. Points indicated by × represent, from left to right, the average responses to 0·03 mg given as an *initial* dose in 4 experiments, to 0·05 mg in 1 experiment, and to 0·1 mg in 9 experiments.

effect is a measure of toxicity not necessarily correlated with hypotensive action or therapeutic usefulness. Methods used for measuring the fall of blood-pressure have been described by Moe, Basset and Krayer[61], Maison and Stutzman[62] and Rubin and Burke[63]. Moe, Bassett and Krayer[61] injected veratridine intravenously (via the right atrium through a tube passed down

the right external jugular vein) in dogs anaesthetized with pentobarbital sodium. The doses used were 0·01–0·10 mg (*Figure 2.3*). A rapid decrease in pressure was obtained, reaching a minimum level in 30 seconds and returning to normal in 2–3 minutes. The fall in blood-pressure resulted partly from a slowing of the heart and partly from vasodilatation of reflex nature. There was a relationship between the dose of veratridine and the fall of blood-pressure produced. For protoveratrine, the range of dosage was 0·01–0·05 mg[64]. The fall of pressure was less abrupt with this compound, a minimum level being reached in 1–3 minutes. The initial level was not regained until 10–30 minutes. The respiration, as well as the heart-rate and blood-pressure, could also be determined. After cutting the vagi, the effects of veratridine in lowering the blood-pressure, slowing the heart-rate and inhibiting the respiration, are abolished.

DRUGS ACTING ON AUTONOMIC GANGLIA

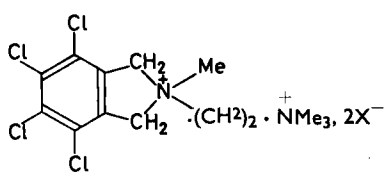
Quaternary Ammonium Compounds

The autonomic nervous system is divided into sympathetic and parasympathetic divisions, and the postganglionic nerve fibres when stimulated release either noradrenaline and adrenaline (adrenergic fibres) or acetylcholine (cholinergic fibres). All preganglionic nerve fibres, however, when stimulated release acetylcholine[65] in the ganglia.

Several excellent reviews have already appeared[66–71], concerning hypotensive substances which affect the autonomic nervous system. Although many substances have been described as ganglion-blocking substances, relatively few of them have had their pharmacological properties sufficiently analysed to be sure that they act like the parent compound, hexamethonium. Hexamethonium has the property of selectively blocking transmission at the preganglionic nerve ending, leaving postganglionic transmission unaffected. It does not inhibit or modify the release of the chemical mediator, acetylcholine, at the ganglionic synapse.

Azamethonium[72] and Oxaditon[73,74], are structurally related, Oxaditon having the —NMe— group in the chain replaced by oxygen. Neither of these compounds, however, differs significantly in activity from hexamethonium and offers no advantage over it. Replacement of a methylene group by —NMe— in the chain in hexamethonium and in pentolinium[75] likewise does not alter the relative blocking activities of the parent compounds.

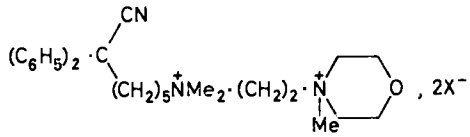
Chlorisondamine (*XI*)[76], is a potent ganglion-blocking agent with a long



(XI) Chlorisondamine

duration of action and is readily absorbed by mouth. There is insufficient evidence, however, that this compound is better absorbed orally than other quaternary ammonium compounds. Smirk and Hamilton[77] found in man

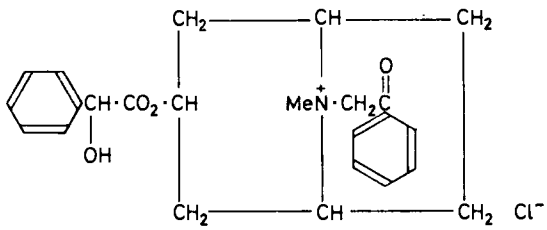
that it was only one-fifteenth as potent orally as when given parenterally. Patients showed drug toleration so that daily doses had to be increased during the early stages of treatment. Another unsymmetrical bisquaternary salt, pentacynium (*XII*)[78] gave promising results in early clinical trials



(XII) Pentacynium

causing postural syncope less frequently than hexamethonium. It is of interest that the polymethylene chain between the quaternary nitrogen atoms in pentacynium is shorter than in hexamethonium yet adequate ganglion-blocking activity is retained.

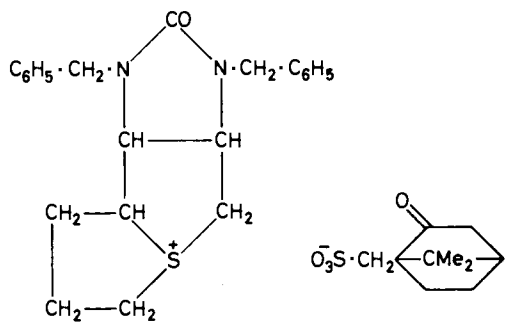
Phenactropinium chloride (*XIII*)[79,80], has been used to produce controlled hypotension in anaesthesia for bloodless field surgery. It is ten times as



(XIII) Phenactropinium chloride

potent as hexamethonium in the cat nictitating membrane preparation, with about one-half the duration of action. The hypotensive action of trophenium is attributed to block of transmission across the sympathetic ganglia, but the evidence for this is inconclusive.

The pharmacological properties of some aralkyl quaternary tropeines have been reviewed by Gyermek and Nádor[81], and there are interesting structure–activity relationships in this series. Trimetaphan *d*-camphor sulphonate (*XIV*) is a thiophanium compound and has been widely



(XIV) Trimetaphan *d*-camphor sulphonate

used in anaesthesia for the production of bloodless field surgery[82]. The pharmacology of this compound has been described[83], and there are several reports on its use in anaesthesia[84,85]. It has a direct vasodilator action and liberates histamine and is also a potent ganglion-blocking agent. These mixed properties make it difficult to unravel the precise method whereby it lowers blood-pressure. In this respect, it is an effective hypotensive drug as its action is rapid, transient and quickly reversed when given by intravenous infusion[86].

The ganglion-blocking compounds most widely used so far in the United Kingdom for treating hypertensive patients are hexamethonium, pentolinium, mecamylamine and pempidine. Although hexamethonium and pentolinium are quaternary ammonium compounds, they have been found to be sufficiently well absorbed by the oral route to be of value. The other two compounds, which are secondary and tertiary amines respectively, are better absorbed orally. All of them, however, suffer from the disadvantage that they affect the parasympathetic as well as the sympathetic system, producing dry mouth, paralysis of eye accommodation, and constipation. These side-effects are not likely to be encountered with the anti-adrenergic compound. bretylium tosylate, as it has a more selective effect on the sympathetic division of the autonomic system. Experience with this compound, however, is not sufficiently well advanced to forecast how it will fare in clinical practice.

A study of the structural features required for ganglion-blocking activity in various bisquaternary ammonium series has produced some interesting results[39,87,88]. It was first shown by Paton and Zaimis[1,38], in $R_3\overset{+}{N}(CH_2)_n\overset{+}{N}R_3$ that maximum activity occurred when $n = 6$. Wien, Mason, Edge and Langston[87] found that on varying the alkyl radicals (R) attached to the nitrogen atoms[89] maximum activity resulted ($n = 5$ or 6) when the terminal groups were $EtMe_2$. Ganglion-blocking activity was related not only to chain length but also to the type of terminal group. When a phenyl radical[90] was introduced on to one end of the chain, or heterocyclic nuclei were present[91], activity was still dependent on chain length and the most active members had approximately the same interquaternary distance between the cationic centres as the most active member of the polymethylene series[92] (*Figure 2.4*). It has been postulated that the pattern of activity in any particular series depends not only on the nature of the chain system but also on the substituents on the nitrogen. This implies that similar compounds with the same maximum interquaternary distance should have the same activity. However, Wien and Mason[88] found that two geometrical isomers of a bisquaternary cyclohexylethyl compound had a ten-fold difference in activity. Furthermore, Gyermek and Nádor[93] found that a bisquaternary *p*-xylylene compound was inactive, whereas an isomeric phenylethyl compound examined by Wien and Mason[88] was one of the most active compounds (four times the potency of hexamethonium) they had encountered. Interquaternary distance, therefore, may be less important than the spatial configuration of the molecule. The assumption that chain length is a measure of inter-receptor distance is complicated by the fact that chains are flexible and their 'lengths' cannot precisely be defined. Gill[94] has considerably extended these views by calculating interquaternary distance/probability distributions for homologous polymethylene and phenylalkane

compounds. He found that the distributions so obtained are correlated with ganglion-blocking activities in terms of the extent of overlap between them and an assumed distance between two receptors, varying between 6 and 7·8 Å. Acetylcholine may act on ganglia by combining reversibly with an anionic group in a protein constituent of the synaptic membrane. This combination may then interfere with the interactions of neighbouring ionic groups in the protein producing a reversible rearrangement of the protein structure and so allowing depolarization of the membrane to occur.

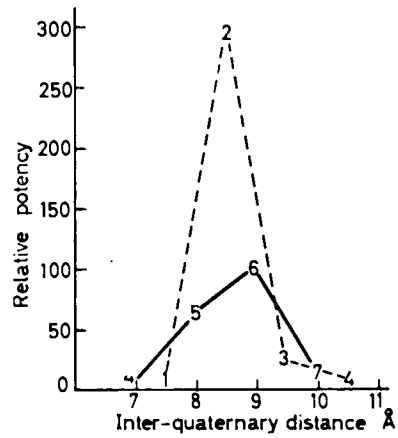


Figure 2.4. Graph relating potency (paralysis of superior cervical ganglion) and interquaternary distance in two homologous series[88]

Solid line, $Me_3\overset{+}{N}\cdot(CH_2)_n\cdot\overset{+}{N}Me_3$. Broken line, $p\text{-}Me_3\overset{+}{N}\cdot C_6H_4\cdot(CH_2)_n\cdot\overset{+}{N}Me_3$. Figures in the curves show the values for *n*.

In 1954, Perry and Reinert[95] studied the effects of preganglionic denervation on the reactivity of ganglion cells in the cat. In experiments on the perfused superior cervical ganglion which had been previously denervated, they found that hexamethonium failed to block the stimulating effect of injected acetylcholine. A similar effect was also observed in innervated preparations perfused with Locke's solution containing a reduced amount of potassium, although the effect of preganglionic stimulation was blocked. The explanation of these results is difficult. More recently, Ricker and Szreniawski[96], using close intra-arterial injections to evoke potentials in pre- and postsynaptic nerves, found that hexamethonium has a presynaptic site of action. These experiments seem to raise more questions than they answer. Their findings imply that the classification of ganglion-blocking drugs may have to be broadened to include actions at pre- and postsynaptic sites. In another direction, Payne[97] studied the influence of carbon dioxide on the blood-pressure response of cats to hypotensive drugs and found that the effect on the blood-pressure was governed by the type of drug used. On the one hand, after doses of hexamethonium the blood-pressure rose when carbon dioxide was given. On the other hand, the hypotensive effect of mecamylamine was enhanced. Payne suggested that the results were influenced by the ability of the compounds to block the sympathetic ganglia. This was limited for hexamethonium as it is unable to penetrate the sub-arachnoid space where some of the ganglia are located, but not limited for mecamylamine, which might be expected therefore to block all the ganglia.

Secondary and Tertiary Amines

The advent of mecamylamine (*IV*) showed that high ganglion-blocking activity was not confined to compounds possessing a quaternary nitrogen atom. Mecamylamine, a secondary amine, is well absorbed from the gastro-intestinal tract, and has made an important contribution to the treatment of hypertension[98,99]. It has a longer-lasting effect than pentolinium on both sympathetic and parasympathetic ganglia, and suffers from the same disadvantages, producing such effects as constipation, dry mouth and blurring of vision. Baer, Panison, Russo and Beyer[21] have shown that the pH of the urine has a profound effect on the renal clearance of mecamylamine in dogs. The drug was freely secreted by the renal tubules when the urine was acid, but reabsorption occurred if the urine became alkaline. Allanby and Trounce[100] found that in man the excretion of mecamylamine was extremely low in an alkaline urine, but that over 50 per cent of an oral dose was recovered the first day if the urine was acid. The production of an alkaline urine was associated with a prolonged hypotensive effect as a result of the retention of the compound in the body. Since mecamylamine penetrates into cells more readily than quaternary ammonium compounds and therefore enters the central nervous system, it is not surprising to find that central nervous effects (such as tremor symptoms) have been reported[23] in patients.

One of the surprising features of the pharmacology of mecamylamine is that, although its structure is entirely different from that of hexamethonium, its mode of action and specificity on autonomic ganglia were reported by Stone, Torchiana, Navarro and Beyer[101] to be similar. Bennet, Tyler and Zaimis[102], however, concluded that the mode of action of mecamylamine was completely different from that of hexamethonium and pentolinium. Using the cat nictitating membrane, the tibialis anterior muscle of the cat, and the isolated mammalian heart and intestine, they deduced that the effects of mecamylamine on autonomic ganglia and effects at the neuromuscular junction were not produced by competition with acetylcholine. They also showed that mecamylamine has a direct action on the intestine and heart, and an action on the central nervous system. It was proposed that the compound alters the physiological state of the ganglion cell and of the muscle fibre, resulting in a modified response of the structures to acetylcholine. The fall in blood-pressure in man was therefore the result of actions of mecamylamine at several sites in the body.

The introduction of mecamylamine naturally stimulated wide investigation and resulted in the production of another useful compound, pempidine (*V*), for the treatment of hypertension. This discovery was made independently by two groups of workers[103–106]. Pempidine acts at the ganglion, since it inhibits preganglionic excitation of the sympathetic nerve without influencing postganglionic stimulation. Like other ganglion-blocking drugs, it inhibits transmission in *both* sympathetic and parasympathetic ganglia. Its action is slow in onset and of prolonged duration. It is well absorbed from the gastro-intestinal tract, and has a neuromuscular blocking effect only when large doses are injected intravenously.

It is of interest that Spinks, Young, Farrington and Dunlop[106] proceeded

to the development of pempidine from weakly active tertiary alkylamines on the hypothesis that high activity was conferred by the presence in the molecule of a sterically hindered secondary or tertiary nitrogen atom, closely surrounded by alkyl groups. Slight activity (one-tenth that of mecamylamine) was first observed in *NN*-dimethyl-t-octylamine. Then greater activity was found in *N*-ethyl-t-butylamine (one-third that of mecamylamine). Finally they prepared 1,2,2,6,6-pentamethylpiperidine (pempidine) which was twice as active as mecamylamine. The results of the examination of a wide variety of related heterocyclic compounds supported their hypothesis that the essential feature in the molecule conferring activity was the close juxtaposition of several alkyl groups to a secondary or tertiary nitrogen atom. By contrast, Lee, Wragg, Corne, Edge and Reading[104] arrived at the same compound, pempidine, by studying structure–action relationships amongst bridged cycloalkylamines. They found that the amino bicycloheptane structure in which the nitrogen is exocyclic, and the isomeric azabicyclo-octane structure in which the nitrogen is endocyclic, possessed similar high ganglion-blocking activity.

The experimental findings of Spinks, Young, Farrington and Dunlop[106] and of Corne and Edge[105] lend support to the hypothesis advanced by Bennet, Tyler and Zaimis[102] regarding the mode of action for mecamylamine, and it is likely that the actions of both pempidine and mecamylamine are intracellular. On the other hand, competitive interference with synaptic transmission, as found with hexamethonium and pentolinium, is probably extracellular. The prolonged duration of action of pempidine and mecamylamine prompted Corne and Edge[105] to draw attention to several mechanisms which might be responsible for the ganglion-blocking activity. Although the opinion that these amines act intracellularly was not rejected, these authors suggested that, by virtue of the slow release of the amines from the ganglion cell body, the acetylcholine membrane receptors might be blocked in the same way as has been postulated for the quaternary ammonium compounds. It is also possible that their prolonged effects may even be due to stronger binding at the membrane receptors themselves. Since mecamylamine is excreted into the stomach and absorbed by the small intestine[107], it undergoes a phase of excretion and absorption which may also contribute to the prolonged action of this compound.

Milne, Rowe, Somers, Muehrcke and Crawford[108] have shown that certain organs selectively store mecamylamine, and the distribution between erythrocytes and plasma (partition ratio about 1·15) was a function of the pH of the extracellular fluid. Muggleton and Reading[22] did not consider this was the result of protein binding. The partition ratios of mecamylamine and pempidine are similar yet their protein-binding properties are different. Other evidence on the action of mecamylamine has been provided by the experiments of Payne and Rowe[109]. They showed that carbon dioxide inhalation increased the plasma concentration of mecamylamine, and enhanced its hypotensive effect. One explanation advanced was that carbon dioxide, by lowering the plasma pH, brought about the transfer of mecamylamine from the cells into the extracellular spaces resulting in greater activity. This is evidence against an intracellular site of action. Another possibility is that carbon dioxide has a direct vasodilator action during sympathetic block,

and that the hypotensive response to mecamylamine is then produced by myocardial depression.

Recent work has shown that mecamylamine and pempidine do not alter the rate of formation of acetylcholine. Gardiner[110] found that neither amine had any effect on acetylcholine formation when choline acetylase preparations from guinea-pig brain were used. Parkinson[111] likewise came to the same conclusion using choline acetylase from rabbit brain.

Selective Action on Sympathetic and Parasympathetic Ganglia

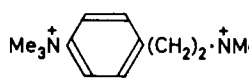
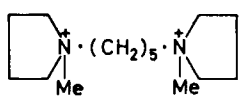
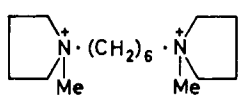
The chemist hopes, that in devising new ganglion-blocking compounds, one will be discovered which has a selective action on the sympathetic ganglia. One method of searching for this effect is for the pharmacologist to screen compounds on a few selected ganglia of both the sympathetic and parasympathetic systems. Mason[112] has studied this problem in the following way. Noting that the ganglion-blocking activity of compounds previously examined had been compared on both the nictitating membrane preparation in the cat and the peristaltic reflex in isolated guinea-pig intestine, he considered that comparisons of this kind suffered from two objections. Firstly, one was an *in vivo* preparation and the other was *in vitro*, and secondly the comparison was drawn from the results of experiments on different species. He therefore developed two preparations in the cat, one using the mydriatic response in the sympathetically denervated eye and the other dependent on the inhibition of the salivary flow. In the first preparation, both superior cervical ganglia and one ciliary ganglion were removed under anaesthesia. Consequently, one eye was completely denervated and the other had only its parasympathetic supply intact. In the presence of light, the pupil of the innervated eye was reduced to a slit and the iris of the denervated eye was immobile, with the pupil about three-quarters dilated. Injection of ganglion-blocking drugs produced a dilatation of the innervated pupil, which was measured on the ground glass screen of a camera and found to be proportional to the degree of block of the ciliary ganglion. (When it had been shown that the compounds tested had no direct action on the denervated iris, the operation of removing the ciliary ganglion was omitted.) For the second preparation, Wharton's duct was cannulated. The combined lingual nerve and chorda tympani were divided centrally as far as possible, and the distal portion laid on platinum electrodes. Stimuli at the rate of 5 per second and of 0·4 millisecond duration were applied continuously. The flow of saliva displaced a weak saline solution from a reservoir and this was recorded using a Gaddum drop-timer. Results on four compounds are shown in *Table 2.1*, activities relative to hexamethonium being recorded. Even in one species, these ganglion-blocking drugs show a lack of selective action, both sympathetic and parasympathetic ganglia being equally affected.

Perry and Wilson[113] have described a useful method for studying the action of drugs concurrently on the sympathetic and parasympathetic ganglia supplying the heart. The vagus and sympathetic nerves in the cat were exposed throughout their course to the heart. Blood-pressure was recorded by inserting a cannula in the femoral artery; pulse-rate and pressure were also recorded in the carotid artery by transmission through a

cannula to a rubber diaphragm carrying a light balsa-wood lever which wrote on a kymograph tracing. Parasympathetic and sympathetic fibres to the heart were stimulated presynaptically in the cervical vagus and in the thoracic sympathetic trunk respectively. Another stimulating electrode was placed on postsynaptic sympathetic fibres (the accelerator nerve). Square-wave pulses of 0·5 millisecond duration at a frequency of 17 per second were

Table 2.1. Comparison of activity of four ganglion-blocking drugs in the cat using (*a*) the nictitating membrane preparation (sympathetic ganglia), (*b*) the ciliary ganglion preparation (parasympathetic ganglia), and (*c*) the salivary flow rate (parasympathetic ganglia)[112]

Activities are determined relative to that of hexamethonium (taken as unity)

Compound	*Relative activities* a	b	c
$Me_3\overset{+}{N}\cdot(CH_2)_6\cdot\overset{+}{N}Me_3$, $2Br^-$	1·0	1·0	1·0
$EtMe_2\overset{+}{N}\cdot(CH_2)_6\cdot\overset{+}{N}Me_2Et$, $2Br^-$	1·5	1·8	1·7
	3·0	3·5	3·5
	5·0	6·0	4·0
	3·0	2·9	2·8

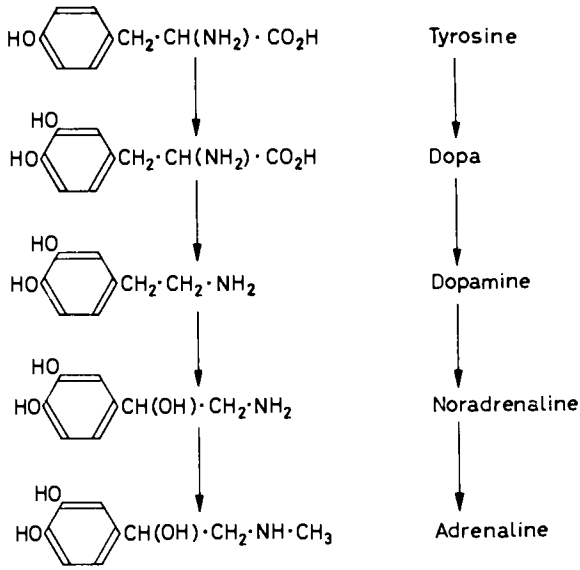
used for stimulation. The presynaptic nerves were stimulated alternately at 2-minute intervals for periods of 5 and 10 seconds respectively, and occasional periods of postsynaptic sympathetic stimulation for 10 seconds were interpolated. Hexamethonium had no selective action on either sympathetic or parasympathetic ganglia, but pentamethonium was ten times more effective in blocking parasympathetic than sympathetic ganglia. Relatively large doses were required to produce this result, but this differentiation has not been substantiated in patients.

Zaimis[67] has recently discussed the tolerance which develops in practice with ganglion-blocking compounds. She suggests that they sensitize the receptors to adrenaline and noradrenaline, (an effect shown in *Figure 2.2*), and that this action masks the block of transmission in the ganglia, when repeated doses are given. Morrison[114] has also commented on tolerance after noting marked differences in the speed with which it develops in patients.

ADRENALINE ANTAGONISTS AND ANTI-ADRENERGIC COMPOUNDS

Since compounds under this heading may antagonize the effects of adrenaline, noradrenaline, or adrenergic nerve stimulation, it is necessary to trace how the pressor amines may be formed biologically. A scheme proposed by Blaschko[115] is shown in *Table 2.2.*

Table 2.2. Biosynthesis of noradrenaline and adrenaline[115]

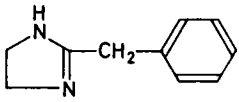


Blaschko considers that dopamine dehydrogenase is a rate-limiting step in this sequence of reactions and it might be profitable to search for compounds which inhibit the action of this enzyme. The significance of the catechol amines (adrenaline and noradrenaline) in hypertension, however, is still open to question. For example, von Euler, Hellner and Purkold[10] in a study of 500 cases of essential hypertension found that there was a significant increase in the urinary excretion of noradrenaline above the normal daily excretion rate in only about 16 per cent of the cases.

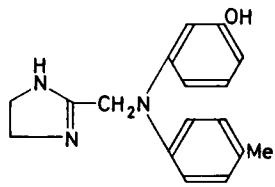
There are two comprehensive reviews by Nickerson[116,117], on the subject of adrenergic blocking drugs. These drugs inhibit the responses of tissues to sympathetic nerve stimulation and to the chemical transmitters, noradrenaline and adrenaline, although they are usually more effective against the effects of circulating adrenaline than they are against those of sympathetic nerve stimulation. Further, these drugs inhibit the excitor effects of adrenaline more readily than the inhibitory effects such as relaxation of the intestines and bronchi.

The ergot alkaloids were among the first adrenaline antagonists to be used, and the partially hydrogenated alkaloids, dihydroergocornine, dihydroergocristine and dihydroergokryptine have been found useful as hypotensive agents by some clinicians[118,119] but not by others[120,121].

The two imidazolines (dihydroglyoxalines), tolazoline and phentolamine, have also not been used extensively as hypotensive agents. The pharmacology of tolazoline (*XV*) was studied by Hartman and Isler[122] who found it

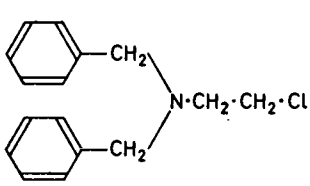


(XV) Tolazoline

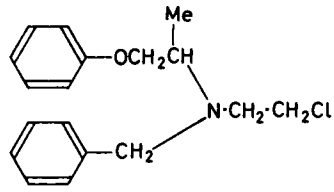


(XVI) Phentolamine

to be the most active of a series of 2-substituted imidazolines. Both tolazoline and phentolamine (*XVI*) are antagonists of adrenaline; phentolamine[123] has the more powerful action but the effects of both drugs are relatively transient. Both drugs also have parasympathomimetic effects, such as cardiac slowing in rabbits, stimulation of the gastro-intestinal tract, and stimulation of gastric secretion.



(XVII) N,N-Dibenzyl-β-chloroethylamine or Dibenamine



(XVIII) Dibenyline

These two β-haloalkylamines are potent adrenaline antagonists and their effects may last for several days. Nickerson[116] describes these compounds as non-equilibrium antagonists suggesting that they react with the adrenaline receptors in two steps, the first being a reversible adsorption and the second a more stable chemical reaction.

Three types of adrenaline antagonists may be listed:

(*a*) Competitive antagonists—drugs which modify the activation of adrenaline or noradrenaline with specific receptors. The drugs are in mass-action equilibrium with the receptors and the block produced is a measure of competition between the antagonist and the agonists (noradrenaline and adrenaline) for occupation of the receptors. Ergotamine is a competitive antagonist of adrenaline.

(*b*) Non-equilibrium antagonists—drugs which combine with the same receptors or some adjacent group to form a stable chemical bond. Dibenyline is such an example.

(*c*) Non-competitive antagonists—drugs which act at a point between the receptors and the ultimate effector site.

The subject of various types of antagonism of drugs has provided a great deal of interesting speculation and has been reviewed by Gaddum[124]. It is possible[117] that the β-haloalkylamines exert their effects through a highly reactive ethyleneimmonium intermediate resulting from intramolecular

alkylation, and this is then able to form covalent derivatives with nearby nucleophilic radicals. The persistence of their effects might also depend on lipoid solubility as there is a correlation between the amount found in the fatty tissues and their duration of action[125]. The blood-pressure is lowered in both the supine and erect positions (thereby differing from the effect of ganglion-blocking drugs). Although the β-haloalkylamines have been used in the treatment of those peripheral vascular disorders which are characterized by excessive spasm of the vessels, these drugs are rarely used alone in hypertension.

In a quantitative study of adrenaline antagonists on the vessels of the rabbit's ear[53], it was found that some antihistamine compounds were as active as the adrenaline antagonists, tolazoline and phentolamine. The latter two compounds inhibited the constrictor action of histamine as well as that of adrenaline, but when they were washed out from the perfusion fluid recovery of the response to adrenaline was much longer than that to histamine. On the other hand, antihistamine compounds blocked the effects of histamine for a longer time than those of adrenaline.

Burn and Rand[60] explained the depressor action recorded after an injection of dopamine on the conception of a 'partial agonist'. Dopamine is a compound which is capable of combining with adrenergic receptors, but possesses only a feeble action by itself. In the presence of a more powerful agonist (such as noradrenaline) which combines with the same receptors, dopamine reduces the vasoconstrictor effect of noradrenaline, and the resultant effect may be a decrease in blood-pressure. Can the reversal of the action of adrenaline by ergotamine or by dibenyline be similarly explained? Burn and Rand state that ergotamine is a powerful vasoconstrictor drug and it is thus possible that the ergotamine reversal of adrenaline described by Dale[126] is similar in nature to the apparent noradrenaline reversal of dopamine. Dale's original hypothesis that ergotamine blocked the vasoconstrictor receptors leaving the vasodilator receptors free, might also apply to a substance such as dibenyline.

Had this chapter been written only a year ago a description of adrenergic blocking drugs would have been confined to those substances typified by ergotamine, dibenyline, tolazoline and their relatives. Mention might have been made of the interesting properties of choline 2,6-xylyl ether bromide[127] as it suppresses conduction in the postganglionic fibres which arise in the superior cervical ganglion. It has other effects by virtue of its muscarinic and neuromuscular-blocking properties[128], and these make it unsuitable as a therapeutic agent. The search for a *selective* ganglion-blocking drug with a *preganglionic* site of action was not fruitful, and all compounds hitherto available affected parasympathetic as well as sympathetic ganglia. However, since *postganglionic* sympathetic transmission is adrenergic in nature, a *specific* block of adrenergic transmission might be expected to be free from any inhibition of the parasympathetic system.

It has now been found[36] that bretylium tosylate (*X*) is a drug which produces a block of the sympathetic system peripheral to the ganglionic synapses leaving the adrenal medulla and the parasympathetic nervous system unaffected. The blood-pressure falls gradually after an intravenous injection, and the contraction of the nictitating membrane on stimulation of

the cervical sympathetic nerve is inhibited, when the stimulus is applied either distally (postganglionic) or proximally (preganglionic) to the superior cervical ganglion. The drug is not an adrenaline antagonist as the adrenaline response is potentiated, just as it is after sympathectomy.

Choline phenyl ether can be regarded historically as one of the parents of this series. Its properties were described by Hunt and Renshaw[129] in 1929, although Hunt and Taveau[130] in 1911 had made the generalization that maximum activity ('muscarinic' and 'nicotinic') was to be found in compounds containing the grouping —$OCH_2CH_2\overset{+}{N}Me_3$. Choline phenyl ether is a powerful ganglion stimulant and thus formed the basis of the valuable researches of Hey[131] which led to the important observation of the adrenergic blocking properties of choline 2,6-xylyl ether. This compound produces an adrenergic block which is fundamentally different from that of previously so-called adrenergic blocking drugs. It reduces the amount of adrenergic transmitter[132] released, probably as a result of interference with the biosynthesis of the chemical mediator at adrenergic nerve-endings[133,134]. Choline 2,6-xylyl ether abolishes the effects of adrenergic nerve stimulation in cats leaving the reactions of the effector organs to adrenaline unimpaired. Its site of action has been deduced to be close to the nerve terminals since it does not impair conduction along adrenergic nerves and its adrenergic blocking effect is not associated with its local anaesthetic action.

Although bretylium resembles choline 2,6-xylyl ether in many of its properties (for example, relaxation of nictitating membrane and local anaesthetic action) there are important differences. Bretylium does not produce salivation (muscarinic action) nor does it reduce the stores of catechol amines in the tissues. The effects of stimulation of the cardiac accelerans nerve (a postganglionic sympathetic nerve) are unaffected. The injection of dimethylphenylpiperazinium releases adrenaline and noradrenaline from the medulla and this response is also not inhibited by bretylium. Studies with labelled bretylium showed that the high specificity of the compound may be associated with a selective accumulation in adrenergic nerves. It is intriguing to note[135] that the substitution of one methyl for an ethyl group on the cationic head in bretylium renders the compound inactive; this is but another illustration[92] of the fine balance of structural features required for optimal pharmacological activity. Ing[68] has drawn an elegant picture of structure–action relationships, and has compared choline phenyl ether (a ganglion-stimulating drug) with (*m*-bromophenoxyethyl)triethylammonium (a ganglion-blocking drug). This led him to postulate that in any ganglionic stimulating drug which contains one methylated onium atom the replacement of all the methyl groups by heavier groups will convert the compound into a ganglionic blocking agent.

RAUWOLFIA ALKALOIDS

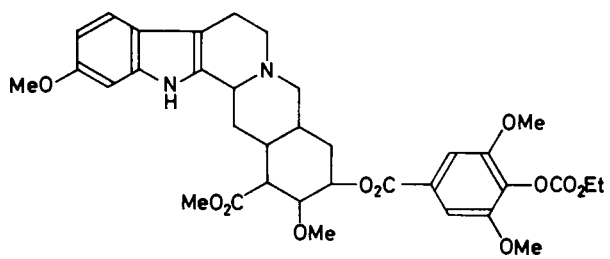
Reserpine (*VIII*) is the most important of many alkaloids found in *Rauwolfia serpentina*, which have been used in India for many years for medicinal purposes. The alkaloid was isolated[136] in 1952 and several excellent reviews are available on the chemistry and pharmacological properties[57] of alkaloids

of *Rauwolfia* species[137,138]. Reserpine is effective when given by mouth, and possesses both sedative and hypotensive properties. Although much is known of its pharmacological properties, its mode of action has not yet been clearly established. It releases 5-hydroxytryptamine[139] and noradrenaline from the central nervous system, and noradrenaline from adrenergic nerves. The fall in blood-pressure which it produces is not affected by atropine or by sectioning of the vagus.

Burn[140] has shown by using reserpine-treated animals that two classes of sympathomimetic amines may be distinguished: (*a*) those which act like adrenaline and noradrenaline; and (*b*) those which act by the release of noradrenaline. In the first class are adrenaline, noradrenaline and dopamine (3,4-dihydroxyphenylethylamine) which exert an enhanced pressor effect. Tyramine, phenylethylamine, amphetamine and ephedrine are in the second class, as their effect is prevented or greatly reduced. Reserpine treatment results in a loss of noradrenaline from adrenergic nerves, and those amines which act by the release of noradrenaline will thus be ineffective in reserpine-treated animals. Nicotine, besides having ganglionic stimulant effects, also has a direct peripheral action on sympathetically-innervated organs such as the blood-vessels, and its vasoconstrictor effect after pretreatment with reserpine is similarly abolished. This 'direct' peripheral action of nicotine may therefore be mediated by the release of noradrenaline.

A characteristic feature of the action of reserpine is its delay in onset. Other actions include inhibition of the pressor reflex of the carotid sinus and the pressor response to stimulation of the sciatic nerve, and vasodilatation with reduction in the cardiac output which is said to be secondary to a central inhibition of sympathetic activity. There is no reduction of the pressor response to splanchnic stimulation, and no peripheral ganglion-blocking effect.

The tranquillizing and hypotensive actions of reserpine may be separated, and derivatives incorporating separate fractions have been prepared. The ethoxycarbonylsyringate ester of methyl reserpate[141] (*XIX*) possesses the



(*XIX*) Ethoxycarbonylsyringate ester of methyl reserpate (Syrosingopine)

hypotensive action of reserpine with little sedative effect. Decaserpyl (10-methoxydeserpidine), an isomer of reserpine, also has the hypotensive action of reserpine without its central effects, and does not produce drowsiness. Rescinnamine has a hypotensive action in animals[142] and in man[143] similar to that of reserpine. In some individuals rescinnamine and deserpidine may produce the desired hypotensive action without producing the depression and drowsiness caused by reserpine, but other effects such as tenseness and

anorexia may occur. The structure of reserpine (*VIII*) is closely related to that of yohimbine and it is of interest that alkoxybenzoic mono- and diesters of yohimbyl alcohol have been reported to have a hypotensive action[144].

Prolonged treatment with reserpine leads to the disappearance of noradrenaline from adrenergic neurones, which in turn leads to a failure of sympathetic activity[145]. The depletion may produce varying degrees of 'functional sympathectomy,' resulting in a reduced urinary excretion of noradrenaline. The disappearance of noradrenaline from all sympathetic ganglia[59,146,147], may result in blocking the effects of electrical stimulation of preganglionic fibres. This action is peculiar to the *Rauwolfia* alkaloids as other substances which diminish noradrenaline in the hypothalamus do not reduce it in the superior cervical ganglion.

The concurrent use of reserpine and a ganglion-blocking drug permits a reduction in the amount of ganglion-blocking compound required. Reserpine has also been used in conjunction with chlorothiazide, hydrallazine, or the *Veratrum* alkaloids.

VERATRUM ALKALOIDS

Veratrum alkaloids are obtained from the plants of *Veratrum album*, (European species); *Veratrum viride*, indigenous to the U.S.A. and Canada, and *Veratrum sabadilla*, the Mexican or West Indian sabadilla. Two of the preparations available are Veriloid, which is a purified mixture of alkaloids obtained from *Veratrum viride*, and protoveratrine from *Veratrum album*. There are about a dozen *Veratrum* alkaloids each possessing different pharmacological effects, and Krayer and Acheson[148] have made an exhaustive study of the pharmacology of several of the pure compounds. For example, protoveratrine has been found[149] to produce flaccid paralysis, veratridine respiratory depression, jervine motor excitation and convulsive seizures, and protoverine brief periods of tremor and convulsive movements. The actions of these alkaloids are therefore very different. Heymans[150], too, has drawn attention to important differences in pharmacological action between veratridine and protoveratrine. In the dog, intravenous doses of veratridine produce a slight slowing of the heart-rate and a fall of blood-pressure, which are abolished on cutting the vagi. Protoveratrine has a similar effect on the blood-pressure but this is abolished only when both vagus and carotid sinus nerves are severed.

When *Veratrum* alkaloids are administered intravenously, they usually slow the rate of the heart[151], reduce cardiac output and produce a sharp fall in blood-pressure[149]. The alkaloids initiate a repetitive discharge in the afferent nerves concerned in the reflex arc. The coronary chemoreflex is excited as a result of the sensitization of vagal afferent end-organs, the alkaloids having no action on efferent nervous pathways. This complex effect is called the Bezold reflex, named after its first description by Bezold and von Hirt in 1867. Dawes and Comroe[42] suggest that more attention might be paid to compounds which lower blood-pressure by this mechanism, as this would obviate the blocking of sympathetic vasoconstrictor pathways and so eliminate the postural hypotension that accompanies the use of ganglion-blocking drugs. Nevertheless, *Veratrum* alkaloids have not been

widely used in the treatment of hypertension, as the dose lowering the blood-pressure is too near to the dose producing nausea and vomiting. The emetic effect is thought to be due to a direct action on the vomiting centre[152,153]. Borison and Fairbanks[154], for example, performed experiments in cats using Veriloid, and found that ablation of the chemoreceptor trigger zone did not prevent emesis, whereas section of the vagus above the nodose ganglion inhibited vomiting. Preparations of *Veratrum* alkaloids are of value in eclampsia and in hypertensive crises, and they have the advantage that they can be given either orally or by intravenous infusion.

Veratrine (a name applied to the total alkaloids from *Schoenocaulon officinale* or *Veratrum sabadilla*) has a characteristic effect on nerve and muscle. When injected into a frog, the voluntary muscles remain contracted for a long time. A resting muscle shows no effect on treatment with the drug, but, when a brief stimulus is given, contraction is followed by a phase of slow relaxation. The effect is a tetanus and not a contracture[155]. It is exerted directly on the muscle, for it occurs after denervation or after curare, a drug which blocks the effects of stimulation of spinal nerves supplying voluntary muscle. Kuffler[156], in experiments on single fibres from the frog's sartorius muscle, showed that a brief stimulus delivered to the veratrinized muscle-fibre resulted in a series of action potentials from the stimulated spot, but only a few millimetres away there were few action potentials. Thus, a tetanus recorded in a single fibre may not be fully recorded as a tetanus from the whole muscle.

MISCELLANEOUS COMPOUNDS

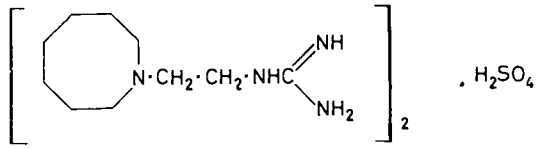
Apart from the hypotensive drugs already mentioned, hydrallazine or 1-hydrazinophthalazine hydrochloride (*IX*) has useful properties as a renal vasodilator drug. It is usually employed in conjunction with reserpine and ganglion-blocking compounds. It has an anti-adrenaline action but it also has a central action[157]. After an intravenous injection in man, the blood-pressure falls in about 10 minutes and remains low for several hours; there is an increase in renal blood-flow, but the glomerular filtration rate is unchanged. When given by mouth, it has a remarkably long action and may persist for days, but on repeated administration the effect gradually disappears[158]. An excellent review of structure–activity relationships among hydrazinophthalazines and related compounds has been published[159].

Other compounds which contain an unsubstituted amidine grouping have been described by Dawes and Mott[160]. These compounds lower blood-pressure by an action on afferent nerve endings in the heart and lungs. Their activity may depend on an intact amidine group, since substitution markedly reduces or abolishes the depressor action.

Raventos[161] suggested that the fall in blood-pressure which the anaesthetic halothane produces is caused by partial block of conduction in the sympathetic nervous system. From other experiments[162] with the isolated heart–lung preparation, it is likely that the fall in blood-pressure is also associated with peripheral vasodilation.

A compound which has recently been claimed[37] to have interesting hypotensive properties is guanethidine, [2-(octahydro-1-azocinyl)-ethyl]guanidine sulphate, or *N*-(2-guanidinoethyl)octahydroazocine sulphate (*XX*).

Given intravenously, guanethidine lowered the blood-pressure of conscious dogs which had been made hypertensive but it had little effect in normotensive animals. It relaxed the nictitating membrane, an effect which the



(XX) Guanethidine

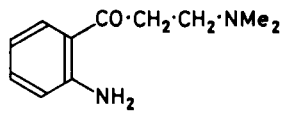
authors claimed was associated 'with a blockade of transmission somewhere in the cervical sympathetic trunk–smooth-muscle complex, such that the nictitating membrane could not be retracted by preganglionic faradization'. Transmission across the ganglion and conduction along the nerve-fibres was unmodified. The effects were remarkably long-lasting, being still present 5 to 20 days after a single intravenous injection. The deduction made was that guanethidine 'inhibited the release and/or the distribution of the transmitter substances from sympathetic nerve terminals'. In other experiments[163] it has been confirmed that no fall of pressure occurred in the normotensive conscious dog, but in the anaesthetized dog there was a sharp fall in pressure on intravenous injection. The effect of noradrenaline was increased whereas the effects of amphetamine and ephedrine were decreased. Given orally to 18 hypertensive patients, a fall in both supine and standing blood-pressure was not noted until after a week or more.

Penbutamine, *N,N*-2,2,3-pentamethylbutylamine-(3), *(XXI)* is claimed to

(XXI) Penbutamine

be 1·5 to 2 times as potent a ganglion-blocking drug as mecamylamine in cats anaesthetized with chloralose-pentobarbitone. This conclusion is based on the results obtained from experiments using preganglionic stimulation of the cervical sympathetic nerve, and measuring the fall in blood-pressure[164].

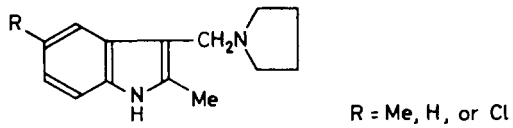
Dimethyl kynurinamine[165], or *o*-amino-*β*-dimethylaminopropiophenone, *(XXII)* lowers the blood-pressure in rabbits under urethane anaesthesia, whereas the 5-hydroxy derivative has an even greater effect.



(XXII) Dimethyl kynurinamine

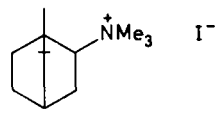
Capsaicin[166], a pungent principle present in various *Capsaicin* sp., is a decylenic acid amide of vanillylamine. Intravenous injection produces a fall in blood-pressure which is abolished by cutting the vagus nerves.

Some analogues of 5-hydroxytryptamine have also been described as hypotensives[167] (see formula (*XXIII*)).



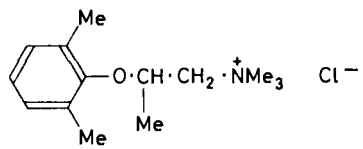
(XXIII) 5-Substituted 2-methyl-3-(N-pyrrolidinomethyl) tryptamine

The compound β-(4-dimethylaminobutyl)-*N*-methylpiperidine dimethiodide[168], is as active as hexamethonium, and blocks the effect of postganglionic excitation of the cervical sympathetic nerve. Also, trimethyl-*d*-bornylammonium iodide[169] (*XXIV*) in small doses lowers the blood-pressure in anaesthetized cats.



(XXIV) 2-Bornyltrimethylammonium iodide

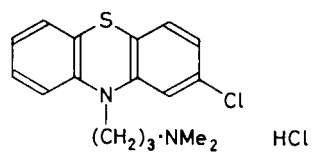
A relative[170] of choline 2,6-xylyl ether (*XXV*) has been tried in hypertension in man. It lowered the blood-pressure and reduced the heart-rate in anaesthe-



(XXV) Trimethyl-[2-(2,6-xylyloxy)-propyl]-ammonium chloride, monohydrate

tized cats and dogs. Its blocking action may be selective for sympathetic nerve endings.

Chlorpromazine, 2-chloro-10-(3-dimethylaminopropyl) phenothiazine hydrochloride, (*XXVI*) is mainly used in psychiatry. It produces a calming

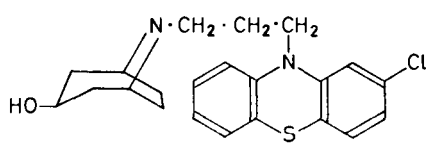


(XXVI) Chlorpromazine

effect and sedation without hypnosis. It also has peripheral actions, and produces a fall in blood-pressure resulting partly from its inhibition of the effects of stimulation of the sympathetic system. There is no clear evidence of the ganglion-blocking activity of chlorpromazine. Mason (who has kindly allowed me to describe these unpublished observations) found that, in the cat under chloralose, chlorpromazine blocked the nictitating membrane response

to preganglionic excitation; its potency was similar to that of hexamethonium, but its type of action was different. Postganglionic excitation and the response to adrenaline were both reduced. The effect observed might be partly accounted for by a peripheral action beyond the ganglion. On perfusing the ganglion, acetylcholine was released showing that the effect was different from that of local anaesthetic drugs. On intra-arterial injection, the effects of adrenaline and acetylcholine on the nictitating membrane were abolished; this result again failed to indicate a true ganglion-blocking effect. Due to the manifold properties of chlorpromazine, it is not possible from these experiments to define its type of action on ganglia. There are several reports[171–173] of hypotensive effects being encountered during treatment of mentally-ill patients with chlorpromazine. For instance, in one series of 1,400 cases[171], a reduction of recumbent blood-pressure occurred in more than half the cases. After intramuscular injection, the blood-pressure decreased by about 30 mm within half an hour. This fall in blood-pressure was most marked in hypertensive patients. The effect depends on posture, as it is more noticeable when the patient is standing than when lying down. Chlorpromazine has been used in eclampsia[174], but generally not for the treatment of hypertension, as its effects are variable and there are other drugs whose mode of action is better known.

Bovet[175] and his colleagues[176] have found that some phenothiazines possess both ganglion-blocking and central depressant actions, which they call 'ganglionplégiques centraux'. Phenothiazine derivatives other than chlorpromazine have been tried with the aim of producing a fall in blood-pressure by a central, instead of a peripheral action. Rochelle and Ford[177] gave 8-{-[10-(2-chlorophenothiazinyl)]propyl}pseudo-3-hydroxynortropane or 8-[3-(2-chlorophenothiazin-10-yl)propyl]norpseudotropine (*XXVII*) to hypertensive patients in a daily dose of 50–400 mg. They found the compound to



(XXVII) 8-[3-(2-Chlorophenothiazin-10-yl)propyl] norpseudotropine

be as effective as *Rauwolfia* alkaloids when given with a ganglion-blocking drug, but it had sedative properties and has consequently not been widely used.

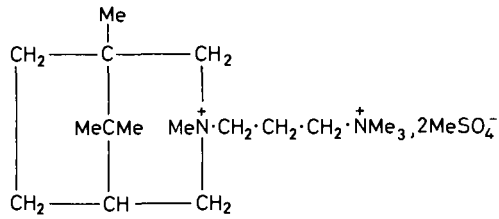
Inhibitors of monoamine oxidase have been used with some benefit in the treatment of hypertension[178,179]. Hallwright[180] found that iproniazid, given in a dose of 50 mg twice daily, produced a maximum fall in diastolic blood-pressure after about 4 weeks' treatment. His impression was that the results obtained were better than those found with reserpine. Side-effects consisted of dry mouth, constipation, sleeplessness and a decreased sexual capacity. Iproniazid is used mainly in psychiatric medicine for its central stimulant action. Its effect in lowering blood-pressure is partly explained by Gertner, Paasonen and Giarman[181] who showed that when the superior cervical

ganglion was perfused with Ringers' solution containing iproniazid in a concentration of 400 μg/ml. it completely blocked transmission. Gertner[182] also found that two other monoamine oxidase inhibitors, pheniprazine (2-hydrazino-1-phenylpropane), and the alkaloid harmine, blocked transmission in concentrations ranging from 20 to 50 μg/ml. These observations are very interesting, and a wider study of the peripheral effects of iproniazid and other monoamine oxidase inhibitors should be rewarding. One recalls, of course, that choline *p*-tolyl ether, which has some of the properties of bretylium, is also a potent inhibitor of monoamine oxidase.

A diuretic compound, quite different in structure from chlorothiazide, has been tried in hypertensive patients. This compound, aspirolactone, 3-(3-oxo-17β-hydroxy-4-androstene-17α-yl)propionic acid-γ-lactone, given orally in a daily dose of 500 mg, reduced blood-pressure, increased excretion of sodium and decreased the urinary excretion of conjugated 17-hydroxy-corticosteroids[183]. Spirolactone antagonizes the action of aldosterone on sodium reabsorption by the renal tubules[184,185].

An imidazoline compound, (2-*o*-chlorobenzyl)imidazoline hydrochloride, closely related to tolazoline, has been tried clinically[186], but tolerance to its depressor action prevented an extensive clinical study.

Trimethidinium[187–189] (*XXVIII*) has a ganglion-blocking action, slow in onset and long in duration (about 6–10 hours). Useful depressor effects were obtained in patients when the compound was given either intravenously or



(*XXVIII*) Trimethidinium

orally. Side-effects on the bowel were less pronounced than those with mecamylamine, but visual disturbances were greater.

RELATIVE POTENCIES OF COMPOUNDS IN ANIMALS AND IN MAN

A satisfactory feature of the testing of compounds for their ability to block transmission in the superior cervical ganglion of the cat is the close parallelism of their relative activities in the cat and in man. This is illustrated by the relative activities of the following compounds:

1. Hexamethonium dibromide
2. Hexamethylene-1,6-bis(ethyldimethylammonium)dibromide
3. Tetramethylene-1,4-bis(diethylmethylammonium)dibromide
4. Pentolinium bitartrate
5. Hexamethylene-1,6-bis[*N*-(*N*-methylpyrrolidinium)]dibromide

On the cat nictitating membrane, their relative activities were 1·0, 1·5, 1·0, 5·0, and 3·0, respectively[39,87,88], whereas in man[190] their relative activities

were 1, 2, 1, 5, and 2. In cross-over experiments on unanaesthetized rabbits[51], the depressor activity of pentolinium was 10 times that of hexamethonium. The secondary amine corresponding to pempidine has recently been found[191] to be about twice as active as pempidine in man, and on the cat nictitating membrane it was 1·4 times as active[192]. These results are very helpful to the chemist and pharmacologist, for they give confidence that the experimental findings can be reproduced clinically.

CONCLUSIONS

Hypotensive agents may be conveniently grouped into (*a*) ganglion-blocking compounds; (*b*) *Rauwolfia* alkaloids; (*c*) *Veratrum* alkaloids; (*d*) hydrallazine, and (*e*) bretylium and guanethidine, which selectively inhibit sympathetic impulses at nerve-endings. The diuretic compound, chlorothiazide, and its congeners, given in conjunction with hypotensive agents, enhance their effects. The principal actions of these compounds have been succintly described by Harington[193].

Ganglion-blocking compounds may be sub-divided into quaternary ammonium compounds and amines. The former are not so well absorbed on oral administration as the latter, and their distribution in the body is confined to the extracellular body fluids. Hexamethonium, pentolinium and chlorisondamine block transmission in both sympathetic and parasympathetic ganglia, their side-effects consisting mainly of parasympathetic block.

The secondary and tertiary amines, mecamylamine and pempidine, are much better absorbed from the gastro-intestinal tract, and freely diffuse across cell membranes, being more concentrated inside the cell. They block impulses in parasympathetic as well as in sympathetic ganglia, and consequently produce side-effects similar to those of the bisquaternary ammonium compounds. Excretion of these compounds and of the amines is by the renal tubules and the excretory process probably involves an active transport mechanism; the rate of excretion of the amines by the kidneys varies with the pH of the urine, and when the urine is acid the rate of renal clearance may exceed the glomerular filtration rate[194]. The hypotensive response to pempidine is related to the plasma concentration rather than to the total amount in the body[195]. In a study of structure–activity relationships it was found that the successive introduction of *C*-methyl groups surrounding the nitrogen atom in congeners of both pempidine and mecamylamine resulted in a progressive increase in activity[196].

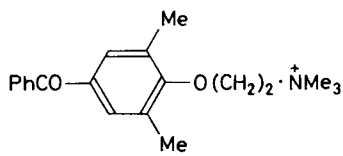
The fall in blood-pressure resulting from the administration of reserpine has been attributed partly to a central action and partly to a peripheral effect on the sympathetic system. Reserpine produces a loss of noradrenaline from both the brain and the peripheral adrenergic neurones. Much of the cerebral noradrenaline is localized in sympathetic centres and, if the stores of this amine have an essential role in sympathetic activity, this activity might be impaired after reserpine. Iggo and Vogt[197] were interested to see whether the brain depleted of noradrenaline was still discharging normally into preganglionic sympathetic fibres by comparing preganglionic activity in normal and reserpine-treated cats. They found that reserpine did not inhibit this discharge of impulses and deduced that the noradrenaline content of the

brain bore no relationship to the sympathetic outflow. Noradrenaline, therefore, was not essential, or was only required in minute amounts, for normal sympathetic activity. Preganglionic sympathetic activity was not fundamentally altered by doses of reserpine which abolished all peripheral sympathetic activity and caused severe depletion of stores of noradrenaline (and of 5-hydroxytryptamine) in the brain.

Hydrallazine has the property of dilating the renal vessels as well as lowering the blood-pressure, but it has side-effects which limit its usefulness. The *Veratrum* alkaloids act on sensory receptors in the heart, mediated by the vagus nerves to the brain. They do not produce postural hypotension like the ganglion and adrenergic neurone-blocking compounds, and were it not for their emetic action they might be used more widely.

Chlorothiazide and its close relatives, flumethiazide and benzthiazide (3-benzylthiomethyl-6-chloro-7-sulphonamide-1, 2, 4-benzothiadiazine-1,-1-dioxide) are essentially diuretic agents. Flumethiazide produces a significant fall in blood-pressure which is paralleled by a fall in body-weight[198]. Chlorothiazide and hydrochlorothiazide also reduce blood-pressure as a result of a diuretic action and a reduction in plasma volume[199]. Chlorothiazide alters the cell/plasma distribution of pempidine, and the enhanced blood-pressure lowering action when the two drugs are given together may be explained mainly by a reduction of blood volume[196].

A recently-introduced adrenergic neurone-blocking agent (*XXIX*) is even more powerful than bretylium in animal tests, but in a few patients it has been less active as a hypotensive agent[200].



(XXIX)

Bretylium is not well absorbed by mouth and it may produce tolerance[201–2]. The glomerular filtration rate and renal plasma flow decrease as the blood-pressure falls, but subsequently they may exceed the resting level[203]. It is of interest that bretylium has recently been reported[204] to have a muscarinic action on the salivary glands.

Guanethidine, like bretylium, blocks sympathetic impulses at nerve-endings and many of its effects are similar to those of bretylium, but there is evidence that it also has some action in temporarily suppressing the effect of peripheral vagal stimulation[205–6]. It has been suggested that guanethidine lowers blood-pressure by depletion of noradrenaline at the peripheral nerve-endings[207]. In this event there may be a similarity in the mechanisms of action of reserpine and guanethidine. Guanethidine produces satisfactory control of blood-pressure, a maximum fall in pressure being obtained when the patient is standing and during exercise[208]. It does not cause such a large fall in pressure on exercise as does bretylium, and guanethidine does not appreciably change

the pulmonary vascular resistance[209]. Since guanethidine suppresses sympathetic nervous activity and its hypotensive action is influenced by the posture of the patient, more experience is required to determine its place in the treatment of hypertensive disease. Tolerance to some of these hypotensive agents is still a problem in the treatment of hypertensive patients. Subsequent doses of hexamethonium or pentolinium, for example, produce less effect, probably as the result of a peripheral sensitization of the blood-vessels to adrenaline and noradrenaline[210–11].

Table 2.3

Approved name	*Other names*	*Approved name*	*Other names*
Alkavervir	Veriloid	Phenactropinium	Trophenium
Azamethonium	Pendiomide	Phenoxybenzamine	Dibenyline
Bretylium	Darenthin		Dibenzyline
Chlorisondamine	Ecolid	Protoveratrine	Puroverine
Deserpidine	Harmonyl		Provell
Guanethidine	Ismelin	Rescinnamine	Moderil
Hexamethonium	Vegolysen	Reserpine	Reserpex
Hydrallazine	Apresoline		Serpasil
Mecamylamine	Inversine	Syrosingopine	Singoserp
Pempidine	Perolysen	Trimetaphan	Arfonad
	Tenormal	Trimethidinium	Ostensin
Pentacynium	Presidal		Camphidonium
Pentolinium	Ansolysen	Veratrum	Veratrone

The *Table 2.3* lists the approved names and some of the proprietary names of the most important hypotensitive drugs.

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3

TRANQUILLIZERS

M. W. PARKES

INTRODUCTION

THE rapid development, within the last five or six years, of drugs principally affecting behaviour has given rise to many new terms which have been used freely and with much resulting confusion. Since their principal field of medical application is in psychiatry, they have been fittingly called *psychotropic* drugs or drugs affecting the mind, and a study of their actions has been termed *psychopharmacology*. Those drugs which are the subject of this chapter belong to the larger class of central depressant drugs and have been variously termed *ataractics* (from Greek '*ataractos*'—cool, calm and collected), *psychosedatives*, *neuroleptics*, *neuroplegics* and *antipsychotics*, as well as the term *tranquillizers* employed here. It is clear that the actions of these agents are not satisfactorily denoted by any of these terms, and it is doubtful if the situation can now be remedied by introduction of yet others.

The term *tranquillizer* has been used so freely in connection with possible uses for these drugs that it is necessary to make clear the standpoint taken here in judging their utility. Their value in psychiatric medicine is immense and it is not an overstatement to say that their use in hospitals has completely transformed the mental ward. Their actions are to reduce the over-activity of agitated psychotics, stabilizing the mood and promoting calm and emotional detachment in place of anxiety and tension. They may not abolish delusions and hallucinations, but they reduce the extent to which these affect the patient. This pacifying action, which is generally less marked in non-psychotic conditions, has been claimed in some quarters to extend to all forms of anxiety and agitation, and has led to the conception of tranquillizing drugs as a resort for any who wish to allay their everyday worries and frustrations, 'the thousand natural shocks that flesh is heir to'.

This view has been given some respectability by reasoning such as that expressed by Aldous Huxley in his opening address to a symposium on meprobamate held in 1957[1]. Huxley traced the history of tension and its social causes and enumerated the means that man has adopted to reduce or dissipate tension; among these means he included alcohol, tobacco and natural drugs, as well as social phenomena such as herd behaviour and religion. On this argument, he visualized a future for psychopharmacology in fashioning new dopes for the masses. Views such as this have found echoes in the Press and elsewhere, and have caused responsible medical opinion to react somewhat suspiciously to the name 'tranquillizer'.

Present developments suggest that these conceptions are fading for want of support and the realization is growing that, although tranquillizers have a definite place in medicine, they are unlikely to furnish remedies for man's ordinary worries.

Tranquillizers are a somewhat indistinct class of central depressant drugs, in that they invariably show some properties of other classes. Riley and Spinks[2] placed them last in the series—anaesthetic, hypnotic, sedative, tranquillizer—and stated that overlap existed between the properties of adjacent members but not between those of non-adjacent members. In general, this is true and tranquillizers are frequently sedative. Indeed, many of the laboratory tests for tranquillizing properties actually reveal sedative properties, but to some extent this depends on definition of the terms used. All tranquillizers produce some degree of drowsiness or lethargy which, however, differs from that produced by hypnotics in that clear consciousness and responsiveness are retained[3]. In large doses, hypnotics are anaesthetic, whereas in small doses some hypnotic agents such as methylpentynol may be tranquillizing rather than sedative.

CLINICAL ASPECTS

The present position of tranquillizers in the therapy of mental disease has been the subject of several recent reviews[4–6] and the basis for their therapeutic use has been examined by a World Health Organization study group[7]. The use of sedatives, such as barbiturates, to calm violent psychotics is a practice of long standing, but these drugs have the disadvantage that difficulties then arise in the management of stuporous patients. The advantage of the use of tranquillizers is that 'they quieten the over-activity of agitated psychotics without producing this disabling somnolence, inducing emotional detachment which allows the arousal of the patient to care for his needs'[8]. Although remissions may follow the use of tranquillizers, their action seems to be solely symptomatic; the basic pathology is not affected but they reduce the violent emotional expressions of the disorder and allow the patient to re-establish contact with reality, so rendering his condition amenable to other forms of treatment.

Tranquillizing drugs have been classified by a variety of criteria. The most practical is that of their clinical action although the resulting classification is not unrelated to one based on chemical structure[4,7,9–11]. The *major tranquillizers*, or antipsychotics, which include some phenothiazine derivatives and the *Rauwolfia* alkaloids, are clearly effective in psychotic states, particularly when acute or of recent origin, although not in disorders of a depressive character. They are of less certain value in psychoneurotic conditions, where the emotional disturbance less profoundly affects the personality. The *minor tranquillizers* are less clearly effective in psychotics and, equally with the major group, are variously reported in the treatment of psychoneurotic anxiety and tension. This group includes the less active phenothiazine derivatives and a number of other chemical types. The inclusion in each group of chemically related agents such as phenothiazines suggests that the distinction between them may be one of degree only.

The uncertainty of the effectiveness of these agents in a number of conditions, in spite of numerous trials, is due to several causes. Firstly, there is the frequent uncertainty of the disorders suffered by the patients on whom the drug has been tried. To a large extent this is due to the present lack of knowledge of the nature of the conditions which make for proper

classification. Secondly, there is the difficulty of assessing objectively the initial condition of the patients and the degree of improvement resulting from treatment. Complete remissions of long-standing conditions occur rarely, even with the most effective tranquillizers. Lesser degrees of improvement require something more than a subjective opinion by observer or patient on whether the condition is better, worse or unchanged; many reports are of this nature. Sometimes, strictly objective measures are possible: 'on a closed psychiatric ward, where noisy, aggressive patients require potent sedatives with antipsychotic actions, one can determine the noise level in decibels or the average life-span of water pitchers and flower vases'[4]. A large variety of psychological tests is also available and it is sometimes possible to wrest from the results of these the basis for a statistical comparison of groups of patients receiving different treatments.

The technique of the double-blind trial and comparison of treatment with the drug and with an inert dummy or placebo, is widely used in clinical assessment of the efficacy of many types of drug[12]. Such trials have been usefully discussed by Modell[13] and there is perhaps no field where the considerations he raises apply with greater force than in the treatment of mental disorders. The treatment of one group of patients with the trial drug and an equivalent group with an inert dummy substance, without the knowledge of either patients or observers as to which is drug and which dummy, has the object of ensuring that any improvement shown by the treated patients is compared, not with the lack of improvement resulting from no treatment at all, but with any improvement which may result from treatment with a dummy, for improvement on dummy treatment is a commonly observed phenomenon, and may, indeed, be an inevitable consequence of treatment in many conditions[14,15]. It frequently amounts to as much as 30–40 per cent of complete effectiveness[12]. Dummy effects may have many features in common with drug effects[16]; and they are often accompanied by undesirable side-effects of a character commonly occurring with active drugs[12].

Such dummy effects may be suspected to be psychosomatic in character, and, on that account, may be expected to play a large part in the treatment of mental disorders. In trials of tranquillizers reviewed by Haas, Fink and Härtfelder[12], improvements on dummies ranged up to 61 per cent in psychoneuroses and up to 75 per cent in psychoses; in groups of less than 40 patients the mean was 30 per cent, falling to 15 per cent in large groups. In comparing the effects of trial drugs with such dummy effects it is commonly assumed that the difference is due to the desired action of the drug[13]. This is not necessarily so, for the drug may be inactive in the desired manner and yet cause some other pharmacological effects; and a pharmacologically-active dummy may well be more effective than an inert dummy. An inert dummy is, to the unknowing patient, a treatment to the extent to which his belief leads to the dummy effect. But perception of a pharmacological effect, whatever it may be, is likely to strengthen the belief and the effect.

This possibility represents one of the difficulties in the way of conducting truly blind trials with inert dummies. Any side-effects occurring with the drug, for example, may suggest to both the patient and the observer that

the treatment is an active one and may result in spurious impressions of improvement[17].

This might suggest that trials of drugs should be controlled with dummies exerting an appropriately neutral pharmacological effect, but the difficulties in the way of this may well be too great. It may therefore not be possible to correct the suspicion that many of the agents regarded as tranquillizers on the basis of trials in mental disorders, particularly in psychoneuroses, are in fact pharmacologically active dummies, or placebos. This gives rise to the thought: 'Are they, nevertheless, inappropriate for the treatment of mental disorders?' A placebo which improves 40 per cent of those treated with it is well on the way to being an effective therapeutic agent.

THE DEVELOPMENT OF TRANQUILLIZERS

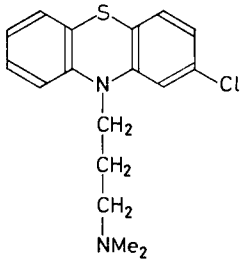
The drugs now available as tranquillizers have been developed from a variety of chemical types in which central depressant activity was observed in addition to their other pharmacological properties. These developments have been reviewed by Bovet[11].

The compounds in which tranquillizing properties have been found may be placed in the following classes:

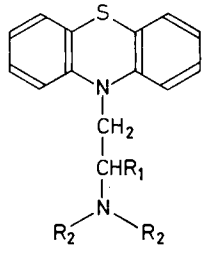
1. Phenothiazine derivatives
2. Diphenylmethane derivatives
3. Propanediol derivatives
4. The *Rauwolfia* alkaloids

together with a variety of compounds of less related structure.

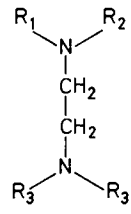
The first *phenothiazine derivative* to be used as a tranquillizer was chlorpromazine (*I*)[18–20] which still finds a wide use for this purpose in addition to its use in anaesthesia and as an anti-emetic. The history of its development is related by Viaud[21] in a review of the properties of the earlier phenothiazine derivatives. The first members of this Rhône–Poulenc series of derivatives (*II*) were made as variants on the ethylenediamine structure (*III*) which was associated with antihistamine activity[22].



(I) Chlorpromazine



(II)



(III)

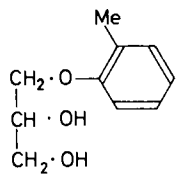
Promethazine ((*II*); $R_1 = R_2 = Me$) has proved to be a powerful histamine antagonist and, in common with other antihistaminic drugs of diverse structure, causes drowsiness and sedation. Another derivative, diethazine ((*II*); $R_1 = H$; $R_2 = Et$) is only weakly antihistaminic but has some action in suppressing the symptoms of paralysis agitans (Parkinsonism), as well as

producing drowsiness. Both these drugs have been tried in anaesthesia for their effect in increasing the hypnotic effect of barbiturates, a property found by Winter[23] for many antihistamine agents. Further development produced chlorpromazine[24], which is superior in this respect; this compound also produces profound hypothermia which has been used in the 'artificial hibernation' method of anaesthesia[25]. Moreover, chlorpromazine was found to exhibit strong anti-emetic properties[26], possessed to a lesser extent by promethazine, and has proved useful against nausea and vomiting due to numerous causes[27]. Nevertheless, the antihistamine activity of chlorpromazine is less than 1 per cent of that of promethazine. Subsequently, a large number of phenothiazine derivatives have been made and these show varying degrees of tranquillizing and anti-emetic activity (see *Table 3.2*).

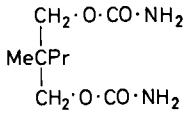
The success of these developments in exploiting the sedative properties of antihistaminic drugs has also led to the use of others as tranquillizers, including some *diphenylmethane derivatives* such as captodiamine, hydroxyzine and phenyltoloxamine (see *Table 3.5*). These, however, are of less proven value as tranquillizers than the phenothiazines and it is doubtful whether antihistamine properties, as such, play any part in tranquillizing action. It remains, nevertheless, intriguing that sedative properties, and anti-emetic properties also, should be associated with antihistamine activity in the same types of chemical structure.

Other derivatives of diphenylmethane, such as adiphenine, show anticholinergic properties resembling those of atropine[22] and have been used as spasmolytics, although with little success. One member of this series, benactyzine (see *Table 3.4*), has been marketed as a tranquillizer, although its clinical value cannot be regarded as established.

Interest in *propanediol derivatives* derives from introduction of mephenesin (*IV*) as a skeletal muscle relaxant in anaesthesia by Berger and Bradley in 1946[28]. This action was shown to be due to the specific depression of spinal



(IV) Mephenesin

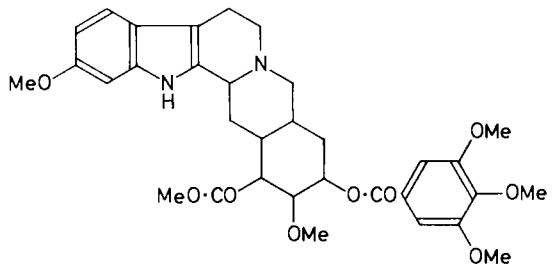


(V) Meprobamate

interneurones[29]. The possibility that the drug might exert similar depression at higher levels in the central nervous system was suggested by reports of effectiveness in relieving anxiety states, producing drowsiness and mild euphoria[30], but the compound was too toxic for general use[31]. Berger examined a large number of derivatives of propanediol for depressant activity and in 1954 selected meprobamate (*V*) as a possible tranquillizer[32]. As a mild agent for anxiety states this compound has enjoyed a tremendous vogue, particularly in the U.S.A. Related compounds claimed as tranquillizers include promoxolane, phenaglycodol and oxanamide (see *Table 3.4*).

The root of *Rauwolfia serpentina* has a long history in the treatment of mental disorders and other conditions in Indian traditional medicine. An

account of its introduction to the West in 1953 is given in an early paper by Kline[33] on the use of *Rauwolfia* alkaloids in neuropsychiatric conditions. Reports of successful treatment of schizophrenia and other conditions with *Rauwolfia* in India prompted the extraction and separation of the active alkaloid, reserpine, in 1952[34], and its examination in a variety of psychiatric and other disorders, including hypertension. Reserpine (*VI*) the constitution



(VI) Reserpine

of which was established by Schlittler and his co-workers in 1954[34a] and confirmed by the total synthesis by Woodward in 1956[34b], has a structure related to that of the adrenolytic alkaloid yohimbine, but its properties are unique. It has undoubted value in psychotic states[4], although its use may be limited by too persistent a depressive effect[6], and by its action in lowering blood-pressure, for which it is of value in hypertension. Related alkaloids occur in other *Rauwolfia* species, and derivatives and synthetic analogues have been studied.

THE PHYSIOLOGICAL BASIS FOR TRANQUILLIZING ACTION

Studies of the pharmacological effects of the various types of tranquillizer have yielded a large body of information in which relevance may be sought for the mechanisms by which the clinical effects may be produced. The evidence has, however, been obtained in several separate fields of inquiry and it is not possible at present to integrate these findings into coherent explanations, nor to demonstrate their pertinence for the clinical effects of the drugs. The evidence will be considered under the following aspects:

1. Effects upon the electrical activity of the brain
2. Effects upon emotional behaviour
3. Interaction with naturally-occurring effector substances
4. Effects upon conditioned behaviour

Effects upon the Electrical Activity of the Brain

This section concerns evidence of the effects of tranquillizers upon the activity of various parts of the brain, as detected by recordings of their electrical activity. Since the original recording by Bremer of the electrical activity of the brain surface, a great deal of study has been made of this phenomenon and much progress made in its interpretation. In animals, it has been possible to place electrodes anywhere in the brain substance and record the

pattern of potential changes (the electroencephalogram (EEG)) associated with observed behaviour or consequent upon stimulation elsewhere or upon treatment with drugs. These effects may be studied in the conscious animal, with chronically implanted electrodes, or acutely in immobilized, conscious animals. Use may also be made of the *encéphale isolé* preparation, in which the spinal cord is severed and the behavior of the head studied in nervous isolation from the body.

The feature of EEG patterns which concerns our evidence relates to activity or inactivity of the area of brain studied. When a body of nerve-cells is inactive, their activity tends to fall in step and the potential changes recorded show slow, regular fluctuations. When the subject is asleep, this kind of synchronous pattern is recorded from the cortex, together with occasional spindles of faster waves. Wakening is associated with irregularity of activity or desynchronization, as smaller, faster waves are superimposed. A sleeping cortex is desynchronized by stimulation of various kinds, *e.g.* a noise, or electrical stimulation of a sensory nerve or several areas of the brain. In an unanaesthetized animal, these procedures may also be followed by awakening. This effect is termed 'arousal'. Thus we have the EEG arousal of the cortex and behavioural arousal of the animal.

The regions of the brain to which we need to refer constitute systems subserving the transmission of sensory information to, and effector outflow from the cortex, by amplifying, attenuating, modifying and co-ordinating these operations. They are thus functionally, as well as anatomically, subcortical. Reference will be made to the *reticular formation*, an anatomically diffuse region of the midbrain rich in neuronal interconnections. In its ascending functions this system transmits sensory inflow via the *thalamus* to the cortex, and also to the *hypothalamus*, a region which controls many autonomic functions. Motor impulses from the cortex which do not proceed by uninterrupted (pyramidal) fibres to the spinal tracts are conveyed by the *extrapyramidal system* which projects via the caudate nucleus to the descending reticular formation (*Figure 3.1*).

The ascending reticular system, by the activity promoted within it by sensory inflow, exerts an activating influence on the cortex, and is probably the operating factor in consciousness and attention[35]. Moruzzi and Magoun showed in 1949[35a] that electrical stimulation of the reticular formation desynchronizes the cortex, and a study of this arousal reaction has yielded evidence of effects of psychotropic drugs upon this system. Stimulation of the diffuse thalamic projection, on the other hand, results in synchronization in the cortex, the 'recruiting response', and high-voltage, slow waves appear; this effect may be blocked by reticular activation. The motor functions of the extrapyramidal system are to exert controlling influences upon skeletal muscle-tone and spinal reflex activity by the operation, at various regions within the reticular formation, of suppressor and facilitatory actions.

Activity appearing in any of these systems may generally be considered as the result of activating and inhibitory influences operating at various levels in the system and it is often only possible to speculate whether any observed effect is the result of reduced activation or intensified inhibition, or vice versa. For this reason, the observed effects of tranquillizers have not yet

led to a clear idea of just where in the central nervous system their actions are exerted.

Thus, anaesthetics, hypnotics, sedatives and many tranquillizers cause sleep patterns to appear in the cortical EEG, and this we may regard as the counterpart of the loss of consciousness, sleep or drowsiness they are observed

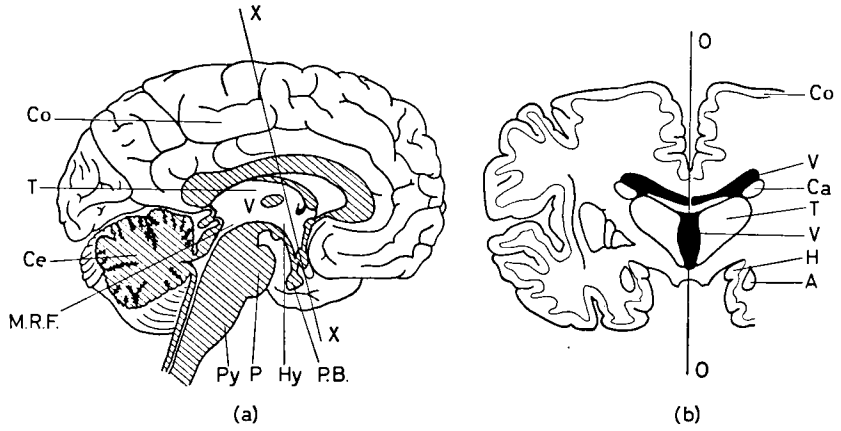


Figure 3.1. Human brain (*a*) in longitudinal section (after Gray) (*b*) in transverse section (after Cunningham)
O—O Plane of section of (*a*)
X—X Plane of section of (*b*)
V—Ventricles; Co—Cerebral cortex; Ce—Cerebellum; T—Thalamus; Hy—Hypothalamus; P.B.—Pituitary body; M.R.F.—Region of midbrain reticular formation; P—Pons; Py—Pyramids; Ca—Caudate nucleus; H—Hippocampus; A—Amygdala.

to produce. However, these agents may be differentiated in other respects and some of these are summarized in *Table 3.1*[36–40].

Chlorpromazine slows the EEG pattern and inhibits the arousal response to sensory stimulation[41]. Reserpine, however, although causing sedation and tranquillization like that of chlorpromazine, differs in not synchronizing the cortical EEG and even, in high doses, causing a continuously alert pattern although the animal is asleep. Both reserpine and chlorpromazine differ from hypnotics and anaesthetics in not blocking completely the arousal response due to sensory or reticular stimulation (*Figure 3.2*). It is probably for this reason that they produce only reduced alertness and not unconsciousness, since reticular activity, rather than cortical, seems to determine the level of consciousness[35]. Again, whereas barbiturate hypnotics enhance thalamic recruitment, chlorpromazine is only slightly effective in this respect and reserpine is without effect. While meprobamate causes synchrony of the cortical EEG, some inhibitory action is exerted on both reticular arousal and thalamic recruitment; it has little effect, if any, upon the level of consciousness.

The anaesthetics are considered to depress the cells of the reticular formation specifically[35], and chlorpromazine, which shows many depressant properties (see below), may also exert its less profound action on this region. Equally, however, it may act by depriving the reticular formation of sensory inflow[36]. The extrapyramidal motor stimulation, which is a frequent

Table 3.1

Effect on	*Barbiturates Volatile anaesthetics*	*Chlorpromazine and other phenothiazines*	*Reserpine*	*Scopolamine Benactyzine*	*Meprobamate*
Behaviour	Unconscious	Drowsy	Drowsy	Awake	Relaxed
Cortical EEG	Sleep pattern	Sleep pattern	Alert pattern, particularly high doses	Some synchronization	Sleep pattern
Arousal response to sensory stimulation	Blocked	Raised threshold (EEG arousal less affected than behavioural)	Slight inhibition of EEG. Behavioural arousal blocked.	No effect	Some inhibition
Arousal response to stimulation of reticular formation	Blocked	Slight inhibition	No effect	Depressed	Some inhibition
Thalamic recruitment	Enhanced (barbiturates) Blocked (volatile anaesthetics)	Slightly enhanced	No effect	No effect	Depressed

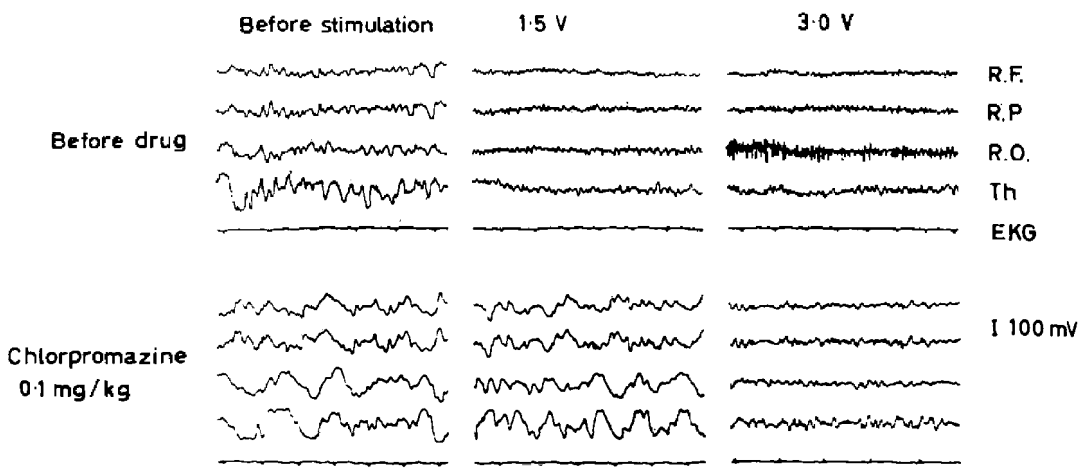


Figure 3.2. Portions of the electroencephalogram trace of a cat, showing cortical effects due to stimulation of the midbrain reticular formation at two voltage levels, before and after injection of chlorpromazine, 0·1 mg/kg intravenously[165]

R.F.—Right frontal lead; R.P.—Right parietal lead; R.O.—Right occipital lead; Th—Thalamic lead; EKG—electrocardiogram lead II.

Chlorpromazine is seen to slow the cortical activity and the response to stimulation.

side-effect of treatment with phenothiazine tranquillizers, may also be ascribed to effects upon the reticular formation, but whether by stimulation of facilitatory or inhibition of suppressor activity, or again, by release from control by higher levels, is a matter for speculation[37]. It is noteworthy that reserpine treatment may also cause extrapyramidal side-effects, in this case possibly by stimulation of the reticular formation. This has been suggested as the basis of the tranquillizing action of reserpine, reduced alertness being ascribed to block by overstimulation[42], the principle implied being the familiar one of the lack of effectiveness of an unchanging level of stimulus. Alternatively, the block may be at higher subcortical levels[43], or cortical inhibition of subcortical processes may be promoted[36].

Effects upon Emotional Behaviour

The above considerations bear principally upon the actions of the tranquillizers on the level of alertness and do not provide an explanation of their antipsychotic actions. This is exerted primarily upon the emotional factors involved in the clinical condition. The effect which is produced by tranquillizers in animals and which perhaps most closely resembles that seen clinically, is that of 'taming' aggressive or wild monkeys. This is a characteristic action of reserpine[44], chlorpromazine[45] and meprobamate[32] but not of benactyzine[46]. Emotional expression is mediated by the hypothalamus to the extent to which autonomic activity is concerned, particularly in relation to stress situations[7], and the activity of this region is depressed by tranquillizers[37]. The over-all picture of the effects produced by reserpine has been compared with that following hypothalamic stimulation and with the effects of certain tumours in this region[47].

In cats in which the brain has been severed immediately above the hypothalamus, thus excluding the cerebrum, a 'sham rage' reaction may be produced on mild stimulation; the animal shows all the signs of feline fury in response to a touch. This reaction is suppressed in cats treated with reserpine[48] or chlorpromazine[49]. These findings suggest that the characteristic taming effect which these tranquillizers exert may be evidence of a depression of these areas, either directly or secondarily as a result of effect on higher regions normally modifying their activity[48].

Other evidence implicates a region of the cerebral cortex, the *limbic system*, comprising, among other structures, the hippocampus and the amygdala (*Figure 3.1*) which may exert balanced, opposing influences[7]. This system has connections particularly with the thalamus, hypothalamus and reticular formation. Its inflow appears to convey principally visceral sensations and those of pain, while its activity controls basic behaviour patterns of smelling, searching, eating and sexual function, as well as escape, or acceptance, or attack of objects in the environment[7]. Whereas the hypothalamus may be concerned with the expression of emotion, the limbic system may be more concerned with its experience. On this conception, the activity of the limbic system may 'set' the emotional level at which the functions of arousal and emotional expression are brought into play and thus determine the attitude towards the environment[7].

Abnormal activity has been shown in this system in studies on schizophrenic subjects[50], taking the form of large synchronous electroencephalogram spikes in the septal region, hippocampus and amygdala, particularly during disturbed periods. Lesions in the amygdala cause animals to become docile, the effects resembling those of reserpine treatment[51], while stimulation of the hippocampus produces similar effects[52]; stimulation of the amygdala, on the other hand, produces rage reactions[53]. Chlorpromazine and reserpine depress the EEG arousal response in the limbic system and provoke electrical seizures of the amygdala[54,55]. Rats in which lesions have been produced in the septal region also show savage behaviour, which is suppressed by meprobamate, although not by chlorpromazine[56]. Tranquillizers may interfere with afferent–efferent connections in this region, resulting in modified hypothalamic activity[51].

Interaction with Naturally-Occurring Effector Substances

This section concerns the evidence bearing on a possible mode of tranquillizing action, namely, that of antagonizing the function of certain substances as transmitters of nervous impulses in the brain. The substances considered are acetylcholine, serotonin and the catecholamines.

The establishment of acetylcholine as an effector neurotransmitter substance, appearing at motor nerve-endings and ganglionic synapses, led to the postulation of a similar role for this substance within the central nervous system. This has been rendered probable by demonstration of the appearance of acetylcholine in the spinal cord during reflex activity and by the presence of choline acetylase activity in the brain; moreover, inhibitors of cholinesterase may exert central effects, typically convulsions and EEG seizures or arousal patterns[57,58] while competitive antagonists of acetylcholine, such as atropine, suppress these effects and produce an EEG sleep pattern, although without impairing alertness[36]. However, the identification of cholinergic transmission at any particular central synapse has not so far been possible, and the only tranquillizers which may be suspected to act by affecting such transmission are benactyzine and scopolamine. These resemble atropine in antagonizing the peripheral effects of acetylcholine[46,59] and in their effects on EEG[36] and, as shown in *Table 3.1*, they differ markedly in these respects from other tranquillizers.

The identification of the catecholamines, adrenaline and noradrenaline, as mediators of transmission at adrenergic nerve-endings has been followed by the demonstration of the occurrence of noradrenaline within the brain, principally in the hypothalamus and those regions of the midbrain and medulla associated with sympathetic activity[60]. A related catecholamine, dopamine (3-hydroxytyramine) is also found in numerous tissues, its distribution in the brain differing from that of noradrenaline, since it is found particularly in the caudate nucleus and not in the hypothalamus[61,62]. Serotonin (5-hydroxytryptamine) occurs widely in tissues, including the brain, and its distribution in this organ follows closely that of noradrenaline[63]. The evidence that these amines function in the brain as synaptic transmitters is of similar circumstantial nature as that for acetylcholine, although, in the

case of dopamine or serotonin, there is no support derived from its identification as a peripheral transmitter substance.

Evidence for these functions derived from the actions following the administration of serotonin is of doubtful significance in this connection, since this amine does not readily enter the brain[64,65]. The effect of injected serotonin may therefore be the result of its vascular and other peripheral effects. Evidence regarding the penetration of catecholamines is less certain and significant amounts of these may enter the brain after injection[66,67]. Direct application of these amines into the ventricles of the brain, by chronically implanted needles, is a means of circumventing the blood–brain barrier, but the small repertoire of effects obtained with numerous drugs suggests that only the structures lining the ventricles may be affected by this technique[35].

In all probability the amines occur in the brain as the result of local formation by decarboxylation of their amino acid precursors, 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (HTP). Enzymes performing this decarboxylating reaction are present in the brain and, in the case of HTP, the distribution of the decarboxylase parallels that of serotonin[68]. On injection, HTP enters the brain sufficiently well to result in a marked increase in the level of serotonin[69], although DOPA causes no increase in noradrenaline[70]. Under appropriate circumstances, both amino acids may produce signs of stimulation, increased motor activity, excitement and elevated sympathetic activity[64,69,71]. These observations suggest that the central role of these amines can be classed as stimulant, although, as with many central actions, it is not possible to decide if direct excitation or diminution of inhibitory activity occurs.

The appropriate circumstances just referred to concern the inactivation of the enzyme monoamine oxidase, responsible, at least in part, for oxidative destruction of these amines[72]. The distribution of monoamine oxidase in the brain also parallels that of noradrenaline and serotonin[73] and its irreversible inhibition by agents such as iproniazid results in a rise in brain levels of serotonin[74], total catecholamines[75] and dopamine[71], although the effect upon noradrenaline level varies in different species[70,76]. After the inhibition of monoamine oxidase the increase in brain amine levels resulting from the administration of DOPA and HTP is greatly enhanced[69,71].

Drugs are classified as antagonists of the catecholamines or serotonin if they block the peripheral effects of these amines. However, caution should always be exercised in relating evidence of drug interactions at, for example, smooth muscle, to hypotheses dealing with central sites. Nevertheless, the adrenolytic properties found for chlorpromazine and other tranquillizers have prompted the suggestion that their tranquillizing action is the result of this antagonism exerted at adrenergic synapses in the brain[36,77–81]. This suggestion receives some support from the depressant properties observed in certain adrenolytic agents[76,77,79]. The hypothesis has so far led to compounds which are related to adrenolytics[79–81] and which behave as tranquillizers in some laboratory tests. There has not so far been clinical confirmation of their tranquillizing effects in man. A similar attempt to account for the actions of reserpine by an adrenolytic mechanism is more difficult, since this drug, although reducing sympathetic activity[47], potentiates the pressor effects of adrenaline and noradrenaline.

Deductions from the action of substances known to antagonize serotonin is hampered by the small number of effective agents so far available. Only two are worthy of consideration: lysergic acid diethylamide (LSD)[82] and its 2-bromo- derivative (BOL)[83]. LSD has marked central stimulant activity, provoking hallucinations and states resembling acute psychoses[84]; these effects can be decreased by chlorpromazine, but are intensified by reserpine[85]. Antagonism to the psychotomimetic action of LSD is the principal claim for recognition of azacyclonol (*Table 3.5*) as a tranquillizer[86] but this effect is disputed[85]. At one time, the association between the psychotomimetic actions and antiserotonin properties of LSD was invoked to support an hypothesis for a central depressant role of serotonin[87].

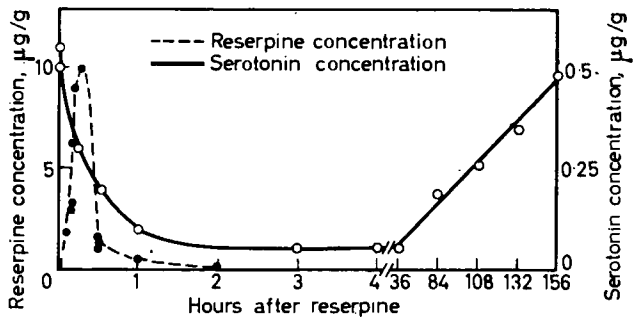


Figure 3.3. Concentration of reserpine and serotonin in the brains of rabbits at various intervals after intravenous injection of reserpine, 5 mg/kg[114]

However, the even more powerful serotonin antagonist BOL shows a complete lack of central stimulant properties[83,88] and, moreover, will prevent the psychic effects of LSD[89]. This suggests that the central stimulant properties of LSD are not a result of any action as a serotonin antagonist.

Reserpine has been shown to produce the unique effect of depleting many tissues of amines and a great deal of work has been done in investigating this effect, since it is attractive to suppose that the tranquillizing actions of the drug may be the result of depletion of the brain of its neuro-effector amines. The amines affected by reserpine include serotonin[90,91], adrenaline and noradrenaline[92,93], and dopamine[61,62,94], although not histamine[95]. This depletion has been shown in animals to occur in the intestinal tract[90,96], skin[95], blood-platelets[97] and heart[98,98a], as well as the brain[90,91,93] (*Figure 3.3*). In man, small doses have been shown to deplete blood-platelets of serotonin[99,100]. This amine-depleting effect is produced only by those *Rauwolfia* alkaloids effective as tranquillizers[101] and by some recent synthetic analogues[102,103] but not by other tranquillizers[101].

The primary effect, from a study of the effect on serotonin uptake in platelets[104,105], appears to be the impairment of the ability of the cell membranes to retain the amine. The serotonin thus mobilized is oxidized by monoamine oxidase to 5-hydroxyindolylacetic acid and the output of the acid in the urine increases soon after a dose of reserpine[106]. The intimate mechanism of this action of reserpine is not known; since uptake and

storage of the amines appears to be an active process[107], depletion may well be the consequence of inhibition of enzyme activity, some instances of which have been reported for reserpine[108].

Reserpine does not interfere with synthesis of serotonin[109] and, after the initial rise, excretion of 5-hydroxyindolylacetic acid returns to the pre-treatment level[110–112]. The tissues appear incapable of taking up serotonin[99,113] and this has been interpreted as loss of the normal ability to 'bind' the amine[113,114]. It is likely that 'bound' serotonin may be identified with the fraction contained in granules within the cell, since the ratio of serotonin in the particulate and non-particulate fractions of rat brain homogenate was found to be changed from 3·5 in favour of the former in untreated animals, to 1·4 after treatment with reserpine[115].

The relation between amine depletion and the pharmacological properties of reserpine is not clear. The evidence for a relationship comprises that for the central stimulant role of the amines, quoted above, and that of the effect of monoamine oxidase inhibition upon the action of reserpine. After treatment with iproniazid, reserpine causes far less amine depletion[116–118] and is no longer followed by many of its characteristic actions[118,119]. Some of these may even be reversed; in iproniazid-treated rabbits, reserpine causes excitement and signs of sympathetic overactivity[119–121]. This might be attributed to the amines which, although still liberated by the reserpine, are not destroyed but remain free to exert a stimulant effect.

However, comparison of changes in amine levels and appearance of the effects of reserpine shows that much remains to be explained. Depletion of amines in experimental animals commences soon after reserpine administration and proceeds rapidly[90] its maximum rate corresponding to the period of peak concentration of reserpine in the tissues[122,123] (*Figure 3.3*), although the picture presented by studies using radioactively-labelled reserpine is conflicting on this point[123,124,124a]. On the other hand, the sedative and hypotensive effects of a dose of reserpine given by any route[44] appear only after a delay of an hour or so by which time amine levels have reached a low value and oxidation products are appearing in the urine[106]. During the period of delay, adrenaline and noradrenaline discharged from the adrenal glands may be found in the blood-stream[125]; this is a process initiated centrally, since it does not occur if the adrenal glands have previously been denervated[93]. This is evidence that some central effects of reserpine are occurring during the period of delay but for some reason do not manifest themselves as sedation.

The persistence of the effect of reserpine upon amine levels is of the order of duration of irreversible enzyme inactivation, such as poisoning of cholinesterase by organo-phosphorus compounds[58] or of monoamine oxidase by iproniazid[126], and suggests that recovery depends on a regeneration process. It may account for the cumulative effect of repeated reserpine dosage[96,127] and for the delay in disappearance of effects such as depression following cessation of therapy. This delay is the principal clinical disadvantage of the drug[6].

Much attention has so far been paid to events concerning serotonin in particular, although it is also possible that changes in catecholamine levels may have significance for sedative effects[128,129] (see pp. 107, 108).

Claims that sedation due to reserpine may be reversed by administration of HTP or DOPA are disputed[130,131]. Other amines may be affected by reserpine. Tryptamine, for example, has been recently implicated[132], but its effect in reversing reserpine sedation is more uncertain than those of other amines[131]. It is not known whether the doses employed clinically affect brain amine levels as they do in animals, but the amine content of the platelets is reduced by clinical doses[99,100,127]. It is also possible that changes in amine levels may be the consequence of effects upon behaviour rather than the cause. Changes in brain serotonin levels, for example, are said not to occur with reserpine in mice kept at a temperature of 38°C, while the animals are not sedated[133]. The same is reported for reserpine in animals which have been stressed[133a].

Brodie[76,121] has suggested a unifying conception for the actions of psychotropic drugs, based on Hess's idea that the homeostatic control of the relation between an animal and its environment is determined by the balance between two opposing systems: an *ergotropic* system, which makes for arousal activity and raised sympathetic tone, and a *trophotropic* system which is regenerative, effecting reduced activity and raised parasympathetic tone. The balance postulated here is reminiscent of that adduced for the function of the limbic system of the cortex (see p. 82) and the two conceptions may be complementary. Brodie further supposes that the neuronal basis of the ergotropic system is adrenergic while that of the trophotropic system is serotoninergic; chlorpromazine blocks transmission in the former system while reserpine activates the latter, both producing similar effects by different mechanisms[76,121].

It should be pointed out that Brodie reported in his earlier papers[106,110,134,135] upon the depressant effects following injection of serotonin in animals, and then postulated that the depressant effects of reserpine were mediated through the serotonin released. However, later findings appear to make this view untenable and it seems at present more reasonable to regard serotonin as exerting a stimulant function centrally.

An example of the readiness with which central events may be interpreted according to opposite conceptions is provided by two further possibilities. Both Brodie[121] and Woolley[87] have invoked a property for serotonin already established for other agents such as acetylcholine and nicotine; this is the property of producing the reverse of its normal effect when present in high concentration, an action known for serotonin on isolated smooth muscle[136]. On this principle, the stimulant action of HTP or of reserpine after monoamine oxidase inhibition may be due to reversal of the inhibitory function which these authors ascribe to serotonin, by the larger amount present.

The studies of Burn and Rand relate to effects of reserpine treatment upon the actions of catecholamines on the blood-pressure, but they illustrate an important pharmacological principle which may be of wider application. Following depletion of the artery walls of noradrenaline by reserpine, the blood-pressure responses to injections of the amine are greater than normal and can be restored to normal by an infusion of noradrenaline[137]. This suggests that reactivity depends, in an inverse manner, upon the amine content of the tissue. A similar hypothesis has been suggested for the effect of serotonin upon uterine muscle[64] and for the action of monoamine oxidase inhibitors on the effect of noradrenaline on heart muscle[138]. Further,

pressor amines can be distinguished according to whether their action is potentiated after injections of reserpine and reduced by a noradrenaline infusion, or reduced by reserpine and increased by noradrenaline (the latter being pressor amines which release noradrenaline from the tissues)[37].

So far, these principles have not been shown to apply to the central actions of amines, although they may well do so, in that the effects of reserpine may be the manifestation of enhanced sensitivity of central sites to endogenous amines.

Effects upon Conditioned Behaviour

The concept of the conditioned response originated from Pavlov and his school, and the term denotes the elicitation of a response appropriate to one stimulus by an unrelated stimulus after repeated association with the first. The conditioning process, which is a form of learning, and factors affecting its operation, are studied by experimental psychologists and their methods have been applied to the psychopharmacological examination of tranquillizers.

An experimental animal has to have some incentive to perform in a meaningful manner for the psychopharmacologist; the experimental situation must be planned to take advantage of motivated behaviour. Frequently, animals are given the prospect of obtaining a natural satisfaction (reward) or of avoiding a painful experience (punishment). In both cases the situations employed differ from those established by Pavlov, in that the response studied is not the natural consequence of the primary stimulus, but precedes it, either to obtain it (reward) or to avoid it (punishment). Experiments of the latter type require the animal to learn to escape an unpleasant experience, for instance, an electric shock delivered through the floor of the cage, by moving when a buzzer sounds prior to the shock; this is the 'conditioned' avoidance response.

The differentiation of the central actions of drugs which is possible with these methods may be illustrated by comparing chlorpromazine and reserpine with a barbiturate in this situation. The two tranquillizers first affect the conditioned response, the animals failing to move at the warning although fleeing when shocked (unconditioned response) (*Figure 3.4*). At higher doses this response may also be suppressed although they still appear to feel the shock. Barbiturates affect the conditioned response only in doses at which the unconditioned response is also impaired[139–141]; methylpentynol and meprobamate also affect the responses non-specifically, the latter being required in ataxic doses[139]. Benactyzine, however, is said to facilitate the conditioned response[142].

The property of specifically inhibiting conditioned responses was first described for chlorpromazine in the original report on the pharmacology of this drug by Courvoisier and her colleagues in 1953[24]. It is the expression of a central depressant action characteristic of the antipsychotic tranquillizers, although other classes are not without effects on conditioned behaviour. The results of investigations of this effect of chlorpromazine and reserpine suggest that it reflects an action upon the associative process. Thus, chlorpromazine has been shown to increase the number of repetitions required for acquisition of the response and to decrease the number of 'false alarms'

required to extinguish it[143,144]. Reserpine has been shown to inhibit the conditioned response to an auditory warning less than that to a visual one, which needed more repetition to establish[145]. Escape in anticipation of the

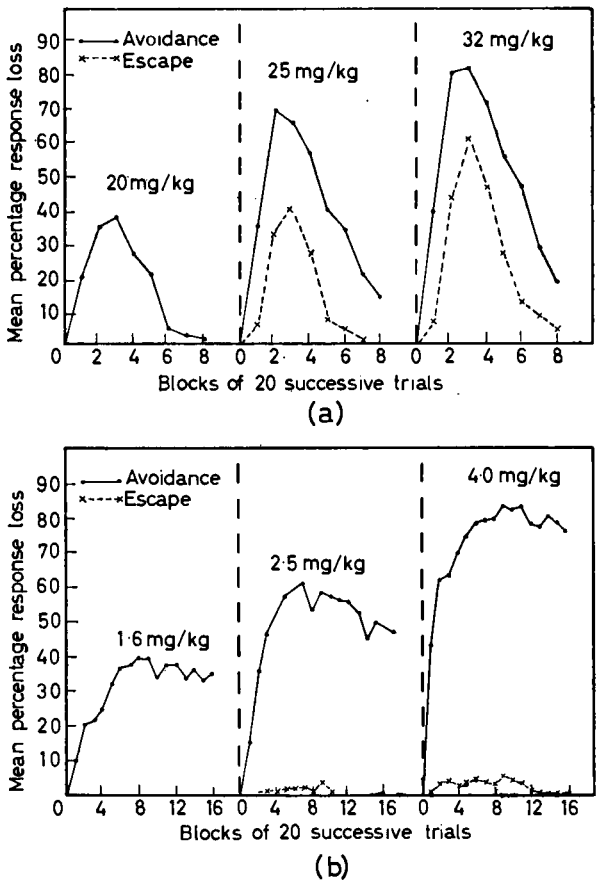


Figure 3.4. Effects of (*a*) secobarbital and (*b*) chlorpromazine, injected subcutaneously, upon the conditioned avoidance responses of rats (closing a switch at the sound of a buzzer, thus avoiding an ensuing electric shock) and upon a learned escape response (switching off the shock on receiving it)
Unlike secobarbital, chlorpromazine selectively depresses avoidance, having little effect on escape[141]

warning, termed the 'secondary conditioned response' is blocked by chlorpromazine and reserpine in doses blocking conditioned avoidance, whereas meprobamate and other mild tranquillizers block the anticipated response preferentially[146].

The central process affected by the drugs producing these effects cannot be identified, nor can it be related to processes dealt with in the previous sections, except in one or two respects. The effect of reserpine in suppressing

conditioned avoidance is, in common with many of the effects of this drug, abolished by pretreatment with monoamine oxidase inhibitors[147]. Lesions in various parts of the brain, particularly in the temporal region, reduced the effect of reserpine upon conditioned avoidance in monkeys, without affecting the ability to respond[140].

Still less can these actions be related to the antipsychotic properties of the drugs. Animals in which reserpine has suppressed conditioned avoidance behaviour still show signs of disliking the situation[145]. The behaviour of rats anticipating a conditioning buzzer may readily be interpreted as anxiety. Jacobsen and his colleagues have studied this aspect of the situation and reported that although chlorpromazine and reserpine did not reduce the signs of anxiety, benactyzine and scopolamine would do so[148]; the author of this review has not been able to agree with this interpretation of the effects of these two drugs.

The action of tranquillizers upon the behaviour resulting from conflicting drives has also been studied. Jacobsen trained cats to press a lever for food at a signal and subjected them to an air blast on responding. Their subsequent uncertainty of response to the signal was dispelled by benactyzine, slightly by alcohol, but not by chlorpromazine or scopolamine[149], although meprobamate was effective in ataxic doses[150]. A simpler situation employed cats, which were allowed to catch mice, but were readily discouraged by receiving an electric shock when seizing their prey. Tranquillizers restored their willingness to pounce, even though shocked; as in Jacobsen's test, benactyzine was active, but here, chlorpromazine was also effective and meprobamate less so[151]. In these and other conflict situations depending on discouragement from feeding or drinking, tranquillizers suppress the signs of indecision and permit satisfaction despite punishment[152,153].

Studies have been made of the emotional component of the reaction to frightening situations, the expressions of which are suppressed by tranquillizers. Mice, exposed to a bright light, ran along a path more slowly. Although chlorpromazine reduced their running speed, it prevented the further reduction due to the light stimulus[154]. Similarly, rats when brightly illuminated in the open, show fear by defaecation and other signs which are reduced by chlorpromazine and reserpine, albeit in doses causing sedation. In this situation, meprobamate, methylpentynol and barbiturate affected the responses only in ataxic doses[155]. The reduction in emotional expression by reserpine is reflected in a reduced response to stress, as shown by 17-ketosteroid excretion[156] or adrenal ascorbic acid level[157]. Chlorpromazine has been shown to prevent the loss in weight which occurs in rats following their removal to unfamiliar surroundings[158].

More recent methods are based on strictly objective principles and have recently been reviewed by Sidman, one of the initiators of this discipline[159]. It is based on study of the animal as a 'free operant', in which it learns to perform a simple operation for a reward or to avoid punishment. Rats, cats or monkeys commonly press levers, while pigeons peck illuminated buttons; these operations close electrical circuits, thus permitting recording. In addition, cyclic programmes of events may be arranged to take place automatically. The effects of drugs on the behaviour of the animal are judged entirely from the record of its performance and subjective interpretation of its

feelings are not necessary; indeed, the observer is excluded from the environment.

Most simply, the operation is arranged to present a food pellet, or a drop of water, on completion of a fixed number of repetitions. When this is learnt, a conditioned warning can be superimposed at intervals, the effect of this upon performance giving an objective measure of disruption. This disruption is eliminated by reserpine, the animals working on undeterred, although their basic rate is reduced by the drug[160], but not all workers find that the fear response is suppressed[161].

Alternatively, a reward may be arranged to follow only a response occurring after a fixed interval[162], or maintenance of a stationary position for a fixed interval[163]. Chlorpromazine appears to decrease impatience under this situation, while barbiturates, curiously enough, reduce the time for which the animal is able to restrain itself[163,164].

There is evidence that tranquillizers are more effective in modifying behaviour motivated by punishment than that by reward[145]. Avoidance behaviour can be studied by a corresponding technique to that described for the reward situation. 'Sidman avoidance' is the term given to a situation in which a shock is delivered unless a lever is pressed within a fixed interval. This is rapidly learnt and the rate of operation settles to one in which nearly all shocks are avoided in time, but not quite all. Reserpine reduces the rate of responding, and increases the number of shocks received, although the animals still perceive the shocks[51]; chlorpromazine is also very effective[165]. Barbiturates will also depress this behaviour to a lesser extent in doses impairing co-ordination[51], while meprobamate is inactive in this situation[166].

These free operant techniques may be combined into a multiple schedule programme in which the actions of drugs upon different situations may be examined in the same animal. They represent a form of inquiry which is only beginning to yield objective information on drug modification of behaviour[159,167].

An ingenious technique which may achieve considerable significance for the study of psychotropic drugs is termed *self-stimulation* and has been reviewed by Olds[168]. Electrodes are chronically implanted in various parts of a rat's brain and stimulation initiated by a lever which the rat can press. When the electrodes are placed in many areas of the mid- and forebrain the rate of pressing is high. Chlorpromazine and reserpine depress the rate of stimulation when this is applied to the ventral hypothalamus or the amygdala but less so when placed in the septal region[169,170]. Chlorpromazine is effective in doses lower than those affecting other behaviour, but meprobamate is not[170a].

MODE OF ACTION OF TRANQUILLIZERS

Although a great deal of information has been obtained in the last five years from investigations of the actions of tranquillizers, it is clearly too early to hope to see an explanation of their mode of action. It is, indeed, probable that they have no common mode of action and it is interesting to consider the similarities between the effects of chlorpromazine and reserpine in view of the differences known to exist between them.

Both these drugs are undoubtedly effective in a similar range of psychiatric

disorders; both reduce emotional expression and excitability. On maintained high doses both cause extrapyramidal motor disturbance[4]. Experimentally, both 'tame' animals, reduce activity and suppress 'sham rage' and behavioural arousal, conditioned avoidance and the conditioned emotional response. Both impair thermo-regulation and antagonize emetics and certain stimulant drugs (see below). However the evidence suggests that these common effects are the result of different actions. In many of their effects on the EEG, chlorpromazine is a depressant, reserpine a stimulant; reserpine causes profound effects on the amine content of the brain, which chlorpromazine does not; most effects of reserpine can be abolished, or even reversed, by pretreatment with inhibitors of monoamine oxidase, while the actions of chlorpromazine are quite unaffected.

A single thread may link all these differences and account for the similarities, if the primary effect of reserpine be amine depletion and the operative property of chlorpromazine be that of antagonizing effector amines. If the amines in certain areas of the brain regulate the activity of these regions, depletion and antagonism might result in similar end effects. Yet there is little direct evidence at present to justify these speculations.

Attempts have been made to account for schizophrenia in terms of biochemical disturbance[171]. Various lines of evidence, including the production of states resembling psychoses by treating normal subjects with mescaline, which has chemical affinities with adrenaline, or with adrenochrome, an oxidation product of adrenaline, have led to one suggestion that the basic disorder in schizophrenia may be that of catecholamine metabolism. This hypothesis provides yet another pointer towards the possibility that these amines are concerned in the antipsychotic actions of tranquillizers.

LABORATORY TESTING FOR TRANQUILLIZERS

Selection of compounds for clinical trial from the results of tests in laboratory animals always carries the risk that activity in man may require different properties in a compound from those making for activity in the test animal. This is sometimes the case even when the test and clinical situations appear to have much in common. In the search for new tranquillizers, particular difficulties are met in devising animal test situations which may have some relevance for the ill-defined psychiatric conditions for which the drugs are intended. With increasing experience in evaluating the clinical value of existing tranquillizers, such as chlorpromazine and reserpine, these drugs can more reliably be used as standards of comparison for new compounds in a wide variety of promising test situations.

The charge has occasionally been brought that the pharmaceutical industry attempts to persuade clinicians to test tranquillizers in their patients without adequate preliminary research. Although it is true that some compounds have been described in the literature, and even marketed, as tranquillizers with insufficient evidence to justify these claims, the increasing battery of tests at the disposal of the pharmacologists has resulted in a number of recent studies of prospective drugs which are admirably comprehensive[166,172–174].

The tests at present in use have recently been reviewed by Riley and

Spinks[2]. These authors recount the origin and development of the methods employed and this detail is omitted from the present review, which will evaluate the various tests for their relevance, and discuss their limitations in this respect.

Among the tests most widely used are those for several forms of depressant activity, including 'taming', locomotor depression, reflex depression and muscular relaxation. It is often difficult to distinguish these in animals, since each action named may contribute to the effects preceding it in this series. The 'taming' of aggressive animals may be presumed the most relevant of these actions for tranquillizing properties, but the drug which appears to tame by causing muscular weakness may not necessarily prove to be a tranquillizer. A distinction may be attempted in this situation by quantitative comparison of the doses required to cause, for instance, taming and ataxia. Such quantitative determinations have been made in a number of studies, although many effects on behaviour are difficult to express quantitatively. It is generally appreciated that relative activity in a number of different tests may be necessary for characterization[175].

Other tests frequently used, particularly for screening, concern effects upon the actions of other drugs. They include augmentation of the hypnotic action of barbiturates, ethanol and other agents, and antagonism of the stimulant effects of amphetamine, mescaline, LSD, morphine and many other drugs. The adoption of these tests rests upon their convenience and upon an empirical argument for their relevance, namely, that known tranquillizers produce these effects. Caution must always be used in considering the results of such tests, however, since interactions of this kind may depend upon factors which have little relevance for tranquillizing properties. If, however, the most obvious of these factors can be excluded by further investigations, the results of these tests can serve as indications for wider examination, which is the function of screening tests.

The tests which have been used to characterize the actions of tranquillizers and for the examination of prospective drugs are listed below. They all depend upon some form of central depressant effect; broadly, tests (*a*) to (*g*) may be more specifically tranquillizing properties, (*h*) to (*k*) comprise sedative effects, (*l*) to (*o*) effects upon motor activity and co-ordination, and the remainder various related depressant properties. These distinctions are by no means clear, however, and interpretation is frequently difficult. This account excludes reference to any wider pharmacological examination and to tests for toxicity, which form an essential part of any complete preclinical study. Pharmacological tests applied to tranquillizers include those for:

(*a*) 'Taming'—reduction of natural aggressiveness, timidity or anxiety.

(*b*) Reduction of the responses of animals rendered reactive by operation—'sham rage', 'septal' animals.

(*c*) Suppression of behavioural arousal response to noise (startle) or to electrical stimulation of the reticular formation or hypothalamus.

(*d*) Suppression of EEG arousal response to sensory stimulation, electrical stimulation or injection of drugs (adrenaline, *etc.*).

(*e*) Suppression of responsiveness of animals sensitized by solitary confinement or treatment with LSD, mescaline or harmine.

(*f*) Suppression of conditioned responsiveness, including conditioned

avoidance response (C.A.R.), classical and free-operant (Sidman); conditioned emotional response (C.E.R.); free-operant reward performance and conditioned emotional disturbance of this; free-operant discrimination and conflict behaviour.

(*g*) Reduction of the hormonal effects of stress, including adrenal ascorbic acid depletion, 17-ketosteroid excretion and various endocrine effects.

(*h*) Reduction of spontaneous locomotor activity.

(*i*) Reduction of body-temperature of small animals.

(*j*) Potentiation of depressant drugs; hypnotics, analgesics.

(*k*) Antagonism of stimulant drugs; including toxicity (amphetamine, mescaline); increased locomotor activity (amphetamine, caffeine, *etc.*); excitement (morphine, LSD, mescaline).

(*l*) Impairment of muscular co-ordination, including performance on inclined plane, rotating rod or ladder; ataxia; catatonia.

(*m*) Unconditioned avoidance of noxious stimulus (electric shock).

(*n*) Reduction of reflex response (pinna reflex, corneal reflex).

(*o*) Abolition of righting reflex; paralysis; narcosis.

(*p*) Anticonvulsant action; suppression of convulsions due to noise (audiogenic), electric shock or drugs (leptazol, strychnine, nicotine, *etc.*).

(*q*) Anti-emetic action; suppression of apomorphine emesis in dogs.

(*r*) Analgesic action; delay in response to painful stimulus.

Not all these actions conveniently form the basis of screening tests for tranquillizing properties. Those which do are principally (*e*) and (*h*) to (*l*); with the application of automation, free-operant conditioned behaviour (*f*) may now also be used in this way. Observational behaviour methods are not generally particularly suitable for this purpose, and examination for taming activity in monkeys, for instance, is likely to be made only in selected cases, although such tests should form an essential part of the study of any compound intended for trial as a tranquillizer. However, the suppression of various forms of aggressive or defensive behaviour in other animals has been made the subject of screening tests. The adoption of a defence posture by the golden hamster when abruptly disturbed, has been shown to be suppressed by chlorpromazine and other phenothiazine derivatives[176]. The fighting ensuing between pairs of mice subjected to electric shocks through the floor of the cage was suppressed by chlorpromazine and reserpine only in sedative doses, but meprobamate appeared to affect fighting more than motor activity[177]. Aggressiveness is also said to develop in mice after solitary confinement for three weeks. Many tranquillizers appear to suppress this behaviour, although, again, in sedative doses[177a].

When rats or mice are frightened by an excessively loud noise, they run violently and some may go into convulsion (audiogenic seizure) and particularly susceptible strains may be used to test drugs for effectiveness in protecting against this response. Riley and Spinks[2] consider that this test may give results relevant for tranquillizing action. Chlorpromazine, reserpine and meprobamate will all protect, although large doses may be required[178].

The fighting response of the Siamese fighting fish is suppressed by many tranquillizers, although other depressants, such as barbiturates, are required in doses which reduce activity in order to affect fighting[176,179]. A response of

this fish to anoxic conditions has also been described as the basis for a test method[180]. Although there may be relevance for tranquillizing action in the aggressive and defensive behaviour of mammals, it is doubtful if similar relevance can be assumed for corresponding behaviour in lower vertebrates.

Perhaps the most widely used test is that measuring the prolongation of the time for which animals sleep when given a barbiturate or ethanol after pretreatment with the test compound. This prolongation was found by Winter to occur with antihistaminic drugs which caused drowsiness clinically[181]; it occurs with chlorpromazine clinically and is one of the purposes for which this drug is used in anaesthesia[182]. A drug may prolong the action of a hypnotic in a number of ways, however, and not all of these are of significance for central depression. Inhibitors of enzyme systems inactivating the hypnotic may prolong its action considerably[183], and so may agents affecting absorption and distribution. It has been suggested that potentiation by sensitization of the central nervous system to depression may be distinguished from effects of these alternative mechanisms by administering the test compound during awakening from the hypnotic, when a true potentiator will re-induce sleep[183]; there is, however, doubt of the reliability of this distinction. The prolongation of the sleeping time with barely effective doses of ethanol in mice may be more sensitive than tests employing barbiturates[103].

This test is usually performed in small animals, in which disturbance of temperature regulation readily results in a fall in body-temperature under normal laboratory conditions. It has been shown that many agents which lower the body-temperature of mice will prolong the effect of hypnotics[184], many of these agents acting by peripheral mechanisms[185]. These may be distinguished from centrally acting agents by their causing hypothermia at elevated ambient temperatures, at which the animals may maintain normal body-temperature without the operation of regulatory activity. Drugs which interfere only with temperature regulation do not reduce body-temperature under these conditions, nor do they cause other signs of sedation, such as reduction of locomotor activity, although they may still potentiate hypnotics[184]. It is not suggested that sedation is a by-product of hypothermia in larger animals, or clinically, although it does appear to be so in small animals[184]. The reason for this may be that, in small animals, the consequences of depression of this vital function are particularly readily detected, whereas, in larger animals, temperature regulation is of less importance than other central activities which are more readily depressed.

Measurement of spontaneous locomotor activity of small animals may give quantitative expression to their degree of excitement or sedation, and means of doing this are reviewed by Riley and Spinks[2]. A distinction bearing on the interpretation of such measurements may be mentioned here, between methods detecting all types of movement ('jiggle cage') and those recording only movements from place to place. Here, too, it may be shown that changes in body-temperature will affect the degree of locomotor activity exhibited[184]. Indeed, in view of the dependence of so many expressions of a small animal's activity upon its body-temperature, it is wise to bear this factor in mind when interpreting any observations on the behaviour of such creatures, particularly at laboratory temperatures common in this country.

These tests may detect sedative, rather than tranquillizing activity, and so, too, may those tests in which reduction is sought of enhanced locomotor activity, as a result of treatment with stimulant drugs, such as amphetamine, caffeine, and methyl phenidate[2]. A permanent state of frantic locomotor activity, characteristically in circles, may also be produced for this purpose by treatment of mice with several doses of 3,3′-iminodipropionitrile[186]. Use has been made of a stimulant property of amphetamine which may, however, depend upon a relevant factor. This drug is some ten times as toxic to mice if grouped in a cage than if housed singly, and it may be presumed that this difference derives from additive effects of the stimulation due to interaction between the mice. Chlorpromazine and reserpine reduce this toxicity in very small doses which do not affect the toxicity of amphetamine to single mice[187,188] and, consequently, may act by reducing the effect of grouping. Meprobamate and benactyzine are inactive[187], even although a characteristic of the effect of benactyzine on mice in which it enhances locomotor activity, is that of hurried movements about the cage, oblivious of each other even to the point of passing over each other as though over inanimate objects. Chlorpromazine, other phenothiazines and reserpine can abolish locomotor activity and induce catatonia in rats and monkeys; meprobamate does not cause this effect[189]. In common with anti-emetic activity, this property appears to be associated particularly with the ability of drugs to provoke extrapyramidal motor stimulation (see pp. 79, 103).

Antagonism towards the stimulant effects of LSD or mescaline has been regarded as relevant for tranquillizing properties, mainly because of the psychotomimetic actions of these agents in man. There is, however, no guarantee that their stimulant effects in mice are expressions of the properties which render them hallucinogenic in man, although Woolley can discern signs of hallucinations in mice[87]. A recognizable sign of stimulation with these agents is a characteristic shaking of the head, particularly in response to a light touch[190]. This also occurs with harmine[191] and some other, although not all, stimulant agents. If mice are kept in solitary confinement for three weeks, a proportion develop this characteristic response also[190]. This sign may be suppressed by chlorpromazine or reserpine but not by benactyzine[191,192], although this drug is reported to suppress aggressiveness induced by solitary confinement[177a].

Another effect observed in mice treated with mescaline is an increased incidence of scratching, which may be reduced by chlorpromazine or reserpine, but only incompletely by barbiturates and not at all by meprobamate[193]. LSD will also produce characteristic responses in the Siamese fighting fish[194] and in the guppy[195], which are influenced by tranquillizers. The relevance of such test subjects has already been questioned.

A number of tests assess the degree of impairment of motor co-ordination in mice, by the difficulty they have, after treatment, in remaining on an inclined plane or rotating horizontal rod[2]. However, many agents, including neuromuscular blocking agents, convulsants, insulin and hypnotics, will reduce this ability. As Riley and Spinks[2] comment, this property would seem to be related rather to undesirable features in a tranquillizer, and might result in the impairment of ability to perform tasks of co-ordination, such as driving a car. A recent test employed a ladder, which mice were presented

with the opportunity, but not the necessity, of climbing. A number of phenothiazine derivatives reduced the proportion of mice so doing and the relative activity of the drugs in this test differed from those calculated from depression of locomotor activity recorded in a 'jiggle-cage'[196]. It is not clear whether this test depends only upon the level of activity and co-ordination, or whether other factors are involved which may have a different relevance for behavioural depression.

REVIEW OF CLASSES OF TRANQUILLIZER

Previous reviews of tranquillizers include those by Jacobsen[9], Vogt[197], Kless[197a] and Bovet[11]. The properties of many of these drugs are listed in Volume IV of a *Handbook of Toxicology* prepared for the committee on the Handbook of Biological Data[198]. It is not proposed here to deal exhaustively with all the pharmacological properties of tranquillizers but only with those relevant either to their clinical uses, or to comparisons of structure with activity, where these are possible.

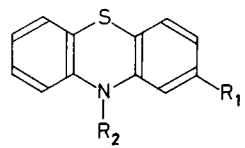
Antipsychotic Agents

Phenothiazine derivatives

This group, of which chlorpromazine is the best-known member, contains a number of related compounds with many properties in common, some of which are more potent than chlorpromazine. *Table 3.2* shows the formulae and lists the approved and trade names and applications of representatives of this group.

The principal pharmacological characteristics of *chlorpromazine* (*VII*–7), include sedation, reduction of locomotor activity and taming[45,189,199]; the doses required to tame monkeys are greater than those for sedation[200]. Large doses cause catatonia in rats[174]. The drug is very effective against conditioned responses (see p. 88) in doses which leave unconditioned activity unaffected[139,141]. It is reported to show analgesic activity[201,202] but the nature of analgesic tests in animals dictates that it is difficult to be sure whether a weakened response to pain is due to decreased perception or reduced ability to respond. Chlorpromazine is powerfully anti-emetic, acting centrally by selectively depressing the chemoreceptive emetic trigger zone[203,204]; it is hypothermic[24,184,205] and antipyretic, suppressing thermoregulation[24]. The effects of general anaesthetics, hypnotics, analgesics, muscle relaxants and local anaesthetics are potentiated[24,184,202]. The mania produced by morphine in cats is antagonized by chlorpromazine[206,207]. It lowers blood-pressure, reduces the pressor action of noradrenaline and reverses that of adrenaline[24,208,209]. Many other general depressant effects are produced by chlorpromazine, such as the prevention of inflammation, oedema and shock due to various procedures, while it protects from mortality due to irradiation[24,210,211]. Because of this, the drug has been considered as a general cell depressant, a 'narcobiotic[212], and the suggestion has been made that its central effects may result from sensitivity of nerve-cells to this influence. Chlorpromazine is metabolized to the corresponding sulphoxide, which is less active as a depressant[213,214].

Table 3.2. Phenothiazine derivatives and analogues



(VII)

Derivative number	R_1	R_2	*Approved name*	*Trade names*	*Principal uses*
1	H	$(CH_2)_2 \cdot Me_2$	Phenethazine	Anergan	Antihistamine
2	H	$(CH_2)_2 \cdot Et_2$	Diethazine	Diparcol	Antiparkinsonism
3	H	$(CH_2)_2 \cdot N$ 	Pyrathiazine	Pyrrolazote	Antihistamine
4	H	$CH_2 \cdot CHMe \cdot NMe_2$	Promethazine	Phenergan Lergigan	Antihistamine
5	H	$CH_2 \cdot CHMe \cdot NEt_2$	Ethopropazine (Prophenamine)	Parsidol Lysivane	Antiparkinsonism
6	H	$(CH_2)_3 \cdot NMe_2$	Promazine	Sparine Verophen	Antipsychotic
7	Cl	$(CH_2)_3 \cdot NMe_2$	Chlorpromazine	Largactil Megaphen Thorazine	Hypothermic Anti-emetic Antipsychotic
8	OMe	$(CH_2)_3 \cdot NMe_2$	Methoxypromazine	Mopazine Tentone	Antipsychotic
9	Ac	$(CH_2)_3 \cdot NMe_2$	Acepromazine	Plegicil Notensil	Antipsychotic
10	CF_3	$(CH_2)_3 \cdot NMe_2$	Trifluopromazine (Triphentizine)	Vesprin	Antipsychotic Anti-emetic
11	Me	$(CH_2)_3 \cdot NMe_2$	Alimemazine	Theralene	Antipsychotic
12	H	$CH_2 \cdot CHMe \cdot CH_2 \cdot NMe_2$	Trimeprazine	Temaril Vallergan	Antipruritic
13	OMe	$CH_2 \cdot CHMe \cdot CH_2 \cdot NMe_2$	Methotrimeprazine (Levomepromazine)	Nozinan Vetactil	Antipsychotic
14	O·CO·Et	$CH_2 \cdot CHMe \cdot CH_2 \cdot NMe_2$	Propiomazine		

Table 3.2—continued

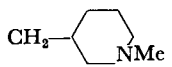
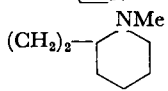
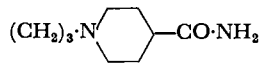
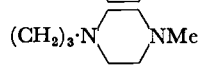
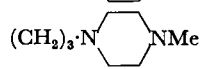
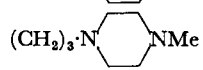
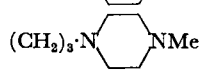
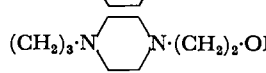
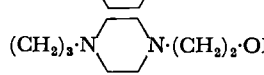
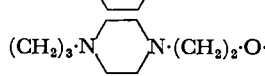
Derivative number	R_1	R_2	*Approved Name*	*Trade names*	*Principal uses*
15	H		Mepazine (Pecazine)	Pacatal	Antipsychotic
16	SMe		Thioridazine	Mellaril Melleril	Antipsychotic
17	Cl		Pipamazine	Mornidine	Anti-emetic (Non-tranquillizing)
18	H		Perazine	Taxilan	Antipsychotic
19	Cl		Prochlorperazine	Compazine Stemetil'	Antipsychotic Anti-emetic
20	CF_3		Trifluoperazine	Stelazine	Antipsychotic Anti-emetic
21	$SO_2 \cdot NMe_2$		Thioperazine		Antipsychotic Anti-emetic
22	Cl		Perphenazine (Chlorpiprozine)	Trilafon Decentan Fentazin	Antipsychotic Anti-emtic
23	CF_3		Fluphenazine	Prolixin	Antipsychotic
24	Cl		Thiopropazate	Dartal Dartalan	Antipsychotic

Table 3.3. Relative activities of phenothiazine derivatives (Chlorpromazine = 1·0)

	Action	*Promethazine* (*VII*–4)	*Promazine* (*VII*–6)	*Acepromazine* (*VII*–9)	*Trifluo-promazine* (*VII*–10)	*Alimema-zine*[225] (*VII*–11)
1	Reduction of spontaneous activity	0·045[218] 0·05[193] <0·01[196]	0.25[196] 0·5[196,220]	0·5[196] >1[223]	—	<1
2	Reduction of ladder climbing[196]	0·25	0·25	2.0	—	—
3	Reduction of conditioned response	0·2[193]	0·5[193]	—	2·5[224]	>1
4	Reduction of conditioned avoidance response	—	—	—	—	—
5	Reduction of conditioned emotional response	—	—	—	—	—
6	Impairment of postural reflexes and co-ordination (rotating rod)[202]	0·07	0·5	4·6	2·3	—
7	Abolition of righting reflex[202]	<0·015	0·2	2·7	1·2	—
8	Antagonism of apomorphine emesis	0·05[193]	0·05[193] <0·1[220a]	>1[21] 1·7[220a]	10·0[224] 2·5[220a]	0·33
9	Reduction of body-temperature	<1[21]	0·5[221]	<1[21]	—	<1
10	Potentiation of narcosis	0·08[202]	0·33[221] 0·22[202] ⩽1[196]	1·0[21,196] 1·7[202]	1·1[202]	—
11	Antagonism of mescaline toxicity	0·33[219]	0·25[193] 0·5[219]	—	—	—
12	*In vitro* antihistamine	~100[24]	1·0[222]	⩾1[223]	—	~100
13	Acute toxicity (mouse, oral route)[198]	—	0·3	—	0·2	0·15

Table 3.3—continued

Levomepromazine (*VII*–13)	*Mepazine* (*VII*–15)	*Thioridazine* (*VII*–16)	*Prochlorperazine* (*VII*–19)	*Trifluoperazine*[174] (*VII*–20)	*Perphenazine* (*VII*–22)	*Thiopropazate* (*VII*–24)	*Chlorprothixene*[235] (*X*)
4·0[226]	0·025[196] 0·03[218]	0·25[229] <1[230] 1·0[231]	0·8[218] <1[192,232] 1·0[226] 1·9[229] 2·5[196]	(mice) 6.0 (rats) 11.0	6·3 (p.o.)[172] 8·4 (inj.) 9·0[229] 21·0[196] 25·0[220]	1·5[202]	6·0 (i.p.) 2·0 (p.o.)
—	0·1	—	0·25	—	2·0	—	—
4·0[226]	—	1·0[231]	1·0[226] 1·6[232]	—	7·4 (p.o.)[172] 13·7 (inj.) 10·0[234]	—	—
—	—	0.14[229]	2·4[229]	10·0	11·0[229]	—	~1
—	—	0·6[229]	3·0[229]	—	4·0[229]	—	—
—	0·03	<1[231]	0·95	—	5·5	2·5	—
—	0·016	—	0·19	—	0·55	0·5	—
<1[226]	<0·05[228]	—	2·1[220a] 3·0[233] 4·0[226] 6·0[232]	—	24·2[233] 31·0[220a]	18·0[220a]	~1
>1[227]	—	—	0·33[226]	—	—	—	1·0
>1[227]	0·01[202] <1[196]	<1[231]	0·5[202] 1·0[196]	—	1·0[173,196,202]	1·5[202]	1·0
—	—	—	0·5[219]	—	1·0[219]	1·3[219]	—
~200[227]	1·0[222]	—	—	1·0	<0·5[173] 1·0[222]	—	0.33
—	—	—	0·06	0·05	0·6	0·25	1·1

Clinically, chlorpromazine is effective in psychiatric disorders, including manic and over-active psychoses, schizophrenia, senile agitation and anxiety reactions[4,18–20], but not in psychoneuroses[19,215]. In addition it is considered of value in intractable pain, particularly as an adjunct to analgesics[216]; it is widely used as an anti-emetic[27,198] and has been very successful in the treatment of tetanus[217]. Side-effects are principally drowsiness, dryness of the mouth, orthostatic hypotension (in 1·5 per cent of cases) and the rigidity and tremor of extrapyramidal motor disorder (40 per cent), particularly with higher doses. These motor effects disappear on stopping treatment or reducing dose, and, like those occurring in Parkinsonism, may be relieved with drugs such as diethazine or trihexyphenidyl. Allergic reactions can occur, and jaundice (0·4 to 1·4 per cent) and agranulocytosis (0·3 per cent) are reported[4,198].

The properties and uses of chlorpromazine are shared by those phenothiazine derivatives in *Table 3.2* which bear an *N*-alkylamine side-chain of not less than three carbon atoms. Members with a 2-substituted ethyl or 2-substituted propyl side-chain lack tranquillizing properties (*VII*–1 to 5); of these, the possession of a terminal diethylamino group (*VII*–2 and 5) confers antagonism towards the symptoms of Parkinsonism, whereas antihistamine properties are associated with a dimethylamino group (*VII*–1, 3 and 4).

Table 3.3 shows the relative activities of a number of phenothiazine derivatives, calculated from the figures given by the original authors, in a number of pharmacological tests in which suitable comparisons with chlorpromazine have been reported. Reference is made to these values in the discussion which follows.

Of the derivatives with an *N*-propylamine side-chain, those in which this chain is branched are also powerful antihistamine drugs, yet trimeprazine, (*VII*–12), with an isobutyl chain does not show marked tranquillizing activity. Where the chain is incorporated in a 2-piperidylethyl group as in thioridazine (*VII*–16) or a 3-piperidylmethyl group as in mepazine (*VII*–15), antihistamine potency is not outstanding and tranquillizing activity is of the order of chlorpromazine[236–238]. A number of derivatives of this type have been studied[239–243]. Mepazine and thioridazine are in clinical use; the former shares the side-effects of chlorpromazine, while the latter is so far claimed to cause but few[231,238].

Substitution of the phenothiazine nucleus in the 2-position (*VII*; R_1) numbered according to the Ring Index, but otherwise referred to as the 3-position, according to Beilstein generally increases activity. Thus, promazine (*VII*–6), is somewhat less active than chlorpromazine, both in pharmacological tests and clinically[5]; the incidence of side-effects may be similarly milder[5,198].

Compounds with a variety of substituents in this position have been studied. For suppression of conditioned responses in rats and taming action in monkeys, activity was found to increase in the following order with 2-substitution in the promazine series[244]:

$$H = CONHNH_2 = OMe < Ac < Cl < CF_3$$

These 2-substituents influence properties somewhat similarly throughout

the phenothiazine group. The methoxy derivative, levomepromazine, (*VII*–13), is considerably more active than trimeprazine and is reported to have the clinical effectiveness of chlorpromazine with similar side-effects[245,246]. In many of the actions listed in *Table 3.3* acepromazine (*VII*–9), is more active than chlorpromazine, although it is reported to be inactive in chronic schizophrenia[247]. Trifluopromazine, (*VII*–10), is also more active, particularly in antagonizing apomorphine emesis, but appears to be of similar effectiveness to chlorpromazine clinically[248], although it may cause somewhat fewer side-effects[248,249]. The trifluoromethyl group also confers high activity in the perazine series and is associated with similar potentiation in other classes of drugs, such as inhalation anaesthetics and diuretics[250] (see also the butyrophenone derivatives, p. 104).

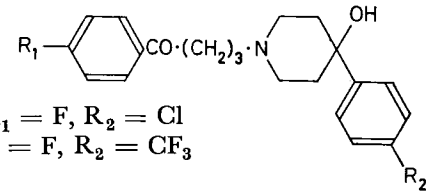
Alteration of the terminal dialkylamino group is followed by differences in activity, and those members with the piperazinopropyl side-chain are more active than the corresponding promazines (*Table 3.3*). In a comparative study of 2-acyloxy-substituted phenothiazines, qualitative differences were associated with different basic groups, in that whereas the compounds with the *N*-dimethylaminopropyl side-chain were predominantly sedative, those with a methylpiperazinopropyl chain were particularly anti-emetic and catatonic, while the morpholinopropyl derivatives were more active in tranquillizing fighting fish[176]. The effects of varying the length of the intervening carbon chain and of altering the *N*-alkyl group, in *N*-alkyl-piperazinoalkylphenothiazines has been studied[251]. Optimum tranquillizing properties occur in the piperazinopropyl derivatives.

Prochlorperazine, (*VII*–19), is somewhat more active than chlorpromazine in pharmacological tests, particularly as an anti-emetic[232] and may be twice as effective clinically[198,248], while extrapyramidal motor side-effects, in particular, are more marked[232,252]. Like chlorpromazine, this drug is relatively ineffective in psychoneuroses[253]. Trifluoperazine, (*VII*–20), is more active still and may be 10 times as active as chlorpromazine[238,254–256]; side-effects may be severe[238,257]. In perphenazine, (*VII*–22), the piperazine bears a *N*-hydroxyethyl substituent and this derivative is very much more active than the corresponding *N*-methyl compound, prochlorperazine, particularly as an anti-emetic; also, it is 12 times as active as chlorpromazine in producing catatonia in rats[172]. This drug is 5 to 10 times as effective as chlorpromazine clinically[198,258], but side-effects are so far reported as mild[198,248]. The substitution of 2-trifluoromethyl for 2-chloro-, to form fluphenazine (*VII*–23) further increases activity to about 25 times that of chlorpromazine. Side-effects readily occur[259,260]. When the *N*-hydroxy-ethyl group is acetylated, as in thiopropazate, (*VII*–24), pharmacological activity is reduced. Clinically, this compound is about 3 times as active as chlorpromazine[261] and extrapyramidal side-effects are common[198].

With the possible exception of perphenazine, increase in clinical effectiveness and the incidence of these side-effects tend to go hand in hand, and are particularly associated with the pharmacological properties of depressing locomotor activity and producing catatonia in rats and in antagonizing apomorphine emesis in dogs[226,229]. Further support for this association is found in the properties of the highly active sulphonamide derivative of perphenazine described by Delay and co-workers[262], namely thioperazine,

(*VII*–21). This compound is 150 times as active as chlorpromazine in causing catalepsy in rats and as an anti-emetic. It produces clinically a severe neurological syndrome, with hyperkinesis, tremor, rigor, sweating, salivation, anxiety and depression; all are reversible on withdrawal. Delay terms this action *neurodysleptic*.

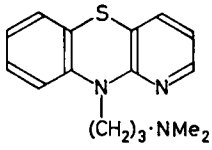
It is interesting that the unrelated butyrophenone derivative haloperidol (*VIII*–1) which is some 6 times as active as chlorpromazine in potentiating barbiturate narcosis and reducing motor co-ordination on the rotating rod[202] and 50 times as active as an anti-emetic[220a], is very effective in psychiatry and also produces a syndrome closely resembling that seen with thioperazine[263]. The trifluoromethyl member of this series, triperidol (*VIII*–2) is more active in this respect[263a].



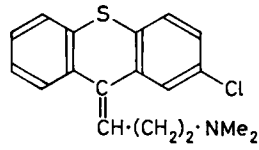
1. Haloperidol; $R_1 = F$, $R_2 = Cl$
2. Triperidol; $R_1 = F$, $R_2 = CF_3$

(*VIII*)

Prothipendyl (Dominal, *IX*) is an aza-analogue of promazine[264]. Its sedative properties, judged by reduction of locomotor activity and co-ordination, production of catalepsy, calming of fighting fish and potentiation



(*IX*) Prothipendyl



(*X*) Chlorprothixene

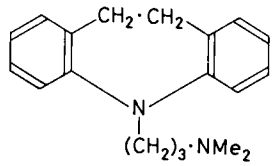
of narcosis, are claimed to be greater than those of promazine[265]; it is stated to be clinically effective[266,267]. A series of derivatives of this type has been studied[265].

The effects of substituents in the 2- and 10-positions have been studied in compounds with several ring-systems analogous to phenothiazine[268]. It is shown that tranquillizing activity varies with the nature of the substituents similarly in all these types of compound. Chlorprothixene (Truxal, Taractan, *X*) is an analogue of chlorpromazine based on thioxanthene instead of phenothiazine; the drug is the *trans*-isomer. It resembles chlorpromazine in many respects[235,268a] being equivalent in reducing body-temperature and locomotor activity and in potentiating barbiturate narcosis. It is more active in impairing postural reflexes and motor co-ordination; 5 times as active against harmine stereotypy[191] (see p. 96). Its effects upon the EEG are similar to those of chlorpromazine. The *cis*-isomer of (*X*) is considerably less active than the *trans*-isomer, except in antihistamine activity, where the *cis*-form is 6 times as active as chlorpromazine[235].

There are, however, differences from chlorpromazine. The phenothiazine

derivatives are rarely of benefit in any psychiatric disorder involving depression or obsession, whereas chlorprothixene is effective in depressive psychoses, phobias and obsessions, together with agitated types of disorder benefited by phenothiazines[269,269a]. No pharmacological evidence has yet been obtained, however, which demonstrates an antidepressant effect in animals.

In its effectiveness in depressions, chlorprothixene resembles another analogue of the phenothiazine derivatives, imipramine (Tofranil, *XI*) which is an iminodibenzyl compound.



(XI) Imipramine

This drug is particularly effective against endogenous depression and less against reactive depression[270,271]; it is said to be superior to iproniazid in these conditions and to produce similar side-effects to this drug[272]. It is not a stimulant in animals, however, and behaves like a weak form of promazine[273,274]; its effects on the EEG resemble those of benactyzine and scopolamine[36].

Reserpine and related compounds

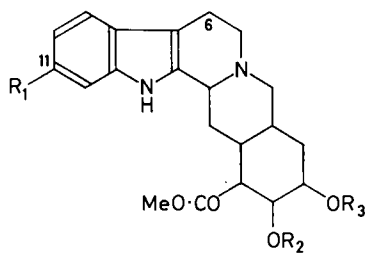
The pharmacology of reserpine and other *Rauwolfia* alkaloids has been reviewed by Bein[275] and Lewis[276]. The characteristics of the pharmacological actions of these agents are principally those of a depressant; in experimental animals reserpine promotes, typically after considerable delay, reduction in locomotor activity, sedation and sleep from which the animals may readily be briefly aroused when disturbed. The typical posture of sleep is adopted, and ptosis of the eyelids occurs as an early sign and has been used in bioassay of the drug. Aggressive animals, such as monkeys, become tame and apathetic. With large doses, catatonia may be produced and, after repeated administration, extrapyramidal motor stimulation appears[278]. Reserpine only slightly reduces reflex sensitivity, and conditioned responses are more susceptible to depression by the drug than is unconditioned behaviour. Body-temperature is reduced and the effects of pyretic agents diminished, temperature regulation being impaired. The emetic action of apomorphine is antagonized, reserpine acting by selectively depressing the emetic chemoreceptor trigger zone[279]. Endocrine function may be depressed as a consequence of reduced pituitary activity[280,281]. In pigeons, however, the drug causes emesis[282], in common with scopolamine and benactyzine. Respiration is depressed by large doses.

Many effects may be the result of reduced sympathetic tone, or consequent parasympathetic over-activity, including hypotension, bradycardia and vasodilatation, relaxation of the nictitating membrane in cats and dogs, and miosis. Gastric secretion is increased, in volume and acidity, and haemorrhage and erosion may result[283,284]. Intestinal motility is augmented and diarrhoea is an early sign, and these effects may be due to the serotonin released in the

initial period after reserpine administration[285], as other signs of initial stimulation, such as transient piloerection[286] and hypertension[287] may be the consequence of adrenaline and noradrenaline released from the adrenal medulla[125]

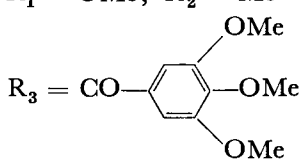
Reserpine potentiates the depressant effects of hypnotics and analgesics[288] and antagonizes the stimulant actions of morphine, caffeine and cocaine[288,289] although the convulsant action of leptazol is facilitated[290]. The threshold of electric shock stimulation is reduced[291]. Pretreatment of animals with iproniazid reduces or abolishes many effects of reserpine, including: sedation[119]; hypothermia[292]; hypotension[119]; potentiation of hypnotics[293], analgesics[294] and leptazol[295]; effects on gastric secretion and haemorrhage[283]; and in many respects, reserpine now exerts a stimulant action[119,120,296]. In parallel with these effects, iproniazid pretreatment reduces the effectiveness of reserpine in lowering brain amine levels (see p. 86).

Clinically, in addition to its use as a hypotensive, reserpine is effective in the treatment of a similar range of psychiatric disorders to those for which the phenothiazines are useful[4,5,297,298]. Opinions differ on its value, depending upon the relative importance attached to effectiveness[4] or the accompanying side-effects[6,299]. Chief of these is its depressant action, which renders it useless in any condition of a depressive nature[5,300]; the slow action of the drug and its persistence make it difficult to control such depression, which may become suicidal in tendency[4,6]. Extrapyramidal motor disturbance may also occur, as with the phenothiazines[4,252]. Other effects include hypotension, diarrhoea and gastro-intestinal hyperacidity and bleeding, drowsiness, nasal stuffiness and nightmares[4,198].

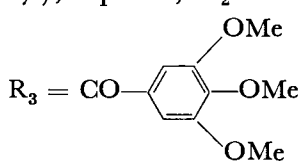


(XII)

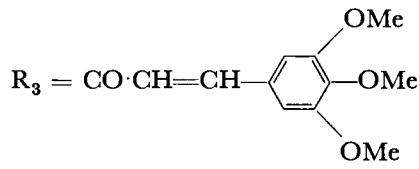
1. Reserpine (Serpasil, Raudixin); $R_1 = OMe$; $R_2 = Me$



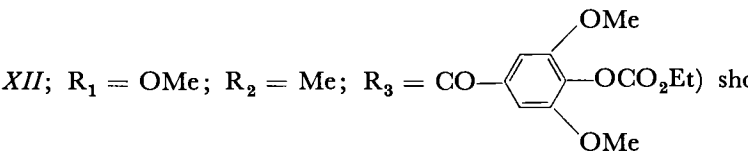
2. Deserpidine, recanescine (Harmonyl); $R_1 = H$; $R_2 = Me$;



3. Rescinnamine (Moderil); R_1 = OMe; R_2 = Me;

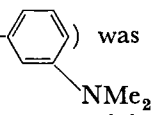


Few variants of the structure of reserpine, (*XII*–1), have so far proved to share the properties of this drug. Other *Rauwolfia* alkaloids differing in minor respects, such as deserpidine, lacking the 11-methoxy group of reserpine and rescinnamine, in which the trimethoxybenzoyl group of reserpine is replaced by trimethoxycinnamoyl (*XII*–2 and 3) have properties almost identical with reserpine[301,302], although rescinnamine appears relatively more potent in dogs and less so in mice[302]. Methyl reserpate (*XII*; R_1 = OMe; R_2 = Me; R_3 = H) is quite inactive, and few esters beside those represented by the natural alkaloids show activity. In the course of examining over 100 such compounds, two were found to show some separation of the two principal activities of reserpine[303]; Syrosingopine (Singoserp,



XII; R_1 = OMe; R_2 = Me; R_3 = CO— —OCO_2Et) showed two-

thirds the hypotensive activity of reserpine, but only 3 per cent of the sedative effect, while another ester, designated SU-5171 (*XII*; R_1 = OMe; R_2 = Me; R_3 = CO—) was 5 times as active as reserpine as a



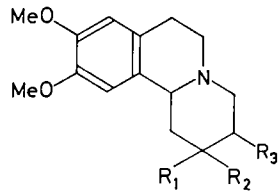
sedative, with only 2 per cent activity as a hypotensive agent[304,305]. Other derivatives of reserpine which have been studied include 6-alkoxy compounds, in which increased activity is claimed[306]. A separation of properties is also described for derivatives with substituents in the 10-position. 10-Methoxydeserpidine is said to be hypotensive but not sedative, while 10-chlorodeserpidine shows 10 per cent of the central depressant activity of reserpine, although is equally active in antagonizing amphetamine toxicity[307].

Only the pharmacologically effective alkaloids of *Rauwolfia* share with reserpine the property of depleting tissues of amines[97,101]. Raunescine (*XII*; R_1 = R_2 = H; R_3 = 3,4,5-trimethoxybenzoyl) which is sedative, reduces both the serotonin and catecholamine level in rat brain; its isomer, isoraunescine, which is not a sedative, reduces the catecholamine level[128] but increases that of serotonin[308]. A separation of amine-depleting properties is also shown by the two derivatives exhibiting pharmacological separation. Syrosingopine depletes peripheral tissues of catecholamines in hypotensive doses, although these have no effect on the brain amines, while SU-5171 depletes brain catecholamines in non-sedative doses, but serotonin only slightly. From these and other observations, Brodie concludes that sedation is more likely to be associated with changes in the level of serotonin

than that of catecholamines, while hypotension may be associated with peripheral depletion of catecholamines[133a,309,309a].

A number of similarities to reserpine have been reported for an oil extracted from the root and rhizome of *Acorus calamus*. Like reserpine, the extract induces sedation, hypotension, potentiation of hypnotics and leptazol facilitation, which are also prevented by pretreatment with iproniazid[310–313]. This plant also figured in Indian herbal remedies in which *Rauwolfia* was used[33]; the active principle has not been isolated.

Properties similar to those of reserpine have been described for the benzoquinolizine derivative, tetrabenazine (Nitoman, *XIII*; $R_1R_2 = O$; $R_3 = Bu^i$), in which some degree of analogy with reserpine structure may be seen.



(*XIII*)

The principal pharmacological difference between this drug and reserpine lies in the greater immediacy and shorter duration of its effects[314]; its action is limited to the period during which appreciable amounts remain in the body[314]. Also, its sedative effects are not accompanied by hypotension[314]. The drug is a non-hypnotic sedative, reducing locomotor activity and causing ptosis of the eyelids as reserpine does[315], although considerably larger doses are necessary. It potentiates hypnotics[103,315], facilitates leptazol convulsions[295] and depresses conditioned responses selectively[316,317]. Effects on gastric secretion and ulceration are reported as similar to those of reserpine[283], although this has been denied[284].

As with reserpine, many of the effects of tetrabenazine are greatly reduced by pretreatment with iproniazid, and may be replaced by signs of stimulation[103,315]. A further similarity is that tetrabenazine, in common with related benzoquinolizine derivatives, also depletes tissues, including the brain, of catecholamines and serotonin[102,103]. The duration of this effect is also short and related to the presence of the drug; recovery of normal levels occurs within 24 hours[103,314] (*Figure 3.5*). Reserpine fails to produce its characteristically prolonged action if given to animals soon after treatment with tetrabenazine, and recovery occurs in the period common with the latter drug. This suggests interaction with common receptors, the tetrabenazine temporarily blocking access during the critical period for reserpine action[314].

Other benzoquinolizine derivatives are active in these respects and some are more strongly hypotensive and less sedative than tetrabenazine[103]. One compound, (*XIII*; $R_1 = OH$; $R_2 = Et$; $R_3 = Bu^i$) is sedative and depletes both serotonin and catecholamines from the brain, as tetrabenazine does, while another (*XIII*; $R_1 = OH$; $R_2 = CH_2{\cdot}CH_2{\cdot}CHMe{\cdot}OMe$; $R_3 = Bu^i$) which is not sedative affects the levels of catecholamines less than those of serotonin[129]. This may suggest that sedation depends upon catecholamine level rather than the level of serotonin, although Brodie disputes that the

difference warrants this conclusion[133a]. The second compound facilitates leptazol convulsions, however, suggesting that this property may be related to effects on the serotonin level[318]; the effect of HTP in reducing leptazol toxicity further supports this view[295].

Clinically, tetrabenazine is reported useful in acute and subacute psychoses and in schizophrenia[319,319a], particularly in reducing thought disorders[319b].

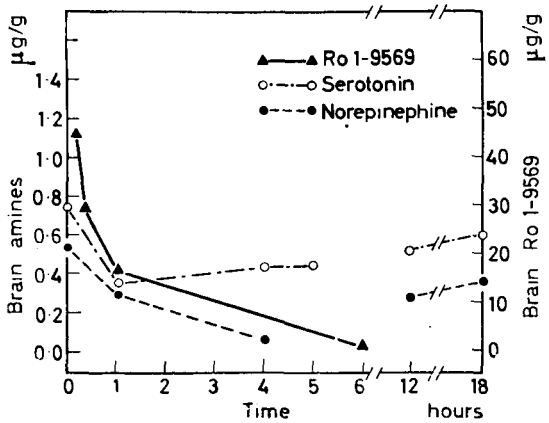


Figure 3.5. Concentration of tetrabenazine (Ro 1–9569), serotonin and noradrenaline in the brains of rabbits at various intervals after intravenous injection of tetrabenazine, 50 mg/kg[314]
Compare with similar data for reserpine, *Figure 3.3*

Its action is shorter and less cumulative than that of reserpine and, consequently, may be easier to control. Side-effects are of a similar nature, although hypotension does not occur and there is no complication from electro-convulsive therapy[319].

Mild Tranquillizers

The agents included in this group have not been shown to exert a clear effect in the treatment of psychoses.

Derivatives of propanediol and related compounds

The compounds in this group are typically muscle relaxants, reflex depressants and antagonists of convulsant drugs. Their formulae, names and applications are shown in *Table 3.4.* The principal representative is *meprobamate* (*XVI*–1) the properties of which were discussed at a symposium held in New York in 1957[320]. Many of its sedative effects in animals are produced only with doses which cause some degree of ataxia and motor co-ordination[321] and it is consequently difficult to exclude the possibility of muscular weakness as contributing to the effects of meprobamate. This applies to its undoubted effectiveness in taming monkeys[32,322] or septal rats[56,166] even when the doses required do not cause obvious paralysis.

In common with related compounds, meprobamate interferes with polysynaptic reflexes, whereas monosynaptic reflexes, such as the knee-jerk, are generally unaffected[32], although it has been stated that some are depressed[323].

Table 3.4. Propanediol derivatives and related compounds

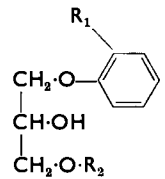
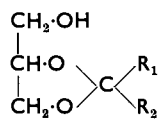
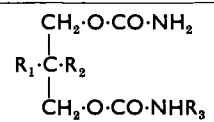
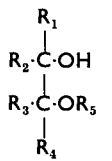
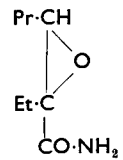
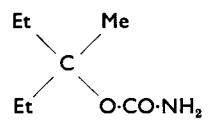
Formula	*Approved name*	*Trade names*	*Principal uses*
 (XIV)			
1. R_1 = Me; R_2 = H	Mephenesin	Myanesin	Muscle relaxant
2. R_1 = Me; R_2 = $CO{\cdot}NH_2$	Mephenesin carbamate	Tolseram	Muscle relaxant
3. R_1 = OMe; R_2 = $CO{\cdot}NH_2$	Methocarbamol	Neuraxin Robaxin	Muscle relaxant
 (XV)			
1. R_1 = Me; R_2 = C_5H_{11}	Glyketal		Muscle relaxant
2. R_1 = R_2 = Pr^i	Promoxolan	Dimethylane	Tranquillizer
 (XVI)			
1. R_1 = Me; R_2 = Pr; R_3 = H	Meprobamate	Miltown Equanil	Tranquillizer
2. R_1 = Me; R_2 = Pr; R_3 = Pr^i	Carisoprodol	Soma	Muscle relaxant
3. R_1 = Me; R_2 = CHMeEt; R_3 = H			Hypnotic

Table 3.4—(continued)

Formula	*Approved name*	*Trade names*	*Principal uses*
 (*XVII*) 1. R_1 =  Cl; $R_2 = R_3 = R_4 = Me$; $R_5 = H$ 2. R_1 =  ; $R_2 = R_3 = R_4 = H$; $R_5 = CO{\cdot}NH_2$	Phenaglycodol Styramate	Ultran Sinaxar	Tranquillizer Muscle relaxant
 (*XVIII*)	Oxanamide	Quiactin	Tranquillizer
 (*XIX*)	Emylcamate	Nuncital Striatran	Tranquillizer

In sufficient doses it heavily sedates animals, depressing the righting reflex[32,166] a property it shares with hypnotics; it has been regarded as exerting an action like that of the barbiturates[324]. Unlike barbiturates, however, a subhypnotic dose is not stimulant[32]. Conditioned responses are not affected in doses below those depressing unconditioned behaviour[139] and conditioned emotion is also unaffected[56].

The hypnotic effect of barbiturates is potentiated only with high doses[325]. Convulsant drugs, such as strychnine and leptazol, are antagonized by meprobamate[323] and it is also effective against convulsions due to electric shock[326,327]. All these effects are consistent with its property of depressing interneuronal activity, particularly at a spinal level[328]. There is evidence that similar effects are exerted at higher levels, such as the thalamus[329–331]. The effects of meprobamate upon the EEG resemble those produced by the barbiturates[324,330] but the cortex is affected less than the thalamus by meprobamate[328,330], and this may be the reason that this drug does not produce anaesthesia[322].

The reports of clinical experience with meprobamate present a confused picture. Its effect in psychotics appears to be slight[5,332,333] or absent[334], although a quietening action has been described[335]. There are many reports of the treatment of anxiety and tension states, some favourable[332–334,336] and a similar number unfavourable[215,337,338]. In general, the improvement rate is reported to be about 40 per cent and it is probable that the difference between this and the effect which can be produced with a placebo is marginal. In addition, there are reports of its use in alcoholism[334,339], dermatology[340], and a number of other conditions, including headaches[334,341] and stuttering[342] with variable success. Side-effects are minor[198], but it has been shown that meprobamate can impair performance of a skilled task[343,344], although doses larger than those used clinically may be needed[345]. However, even this has been reported little different from the effects of a dummy[346]. It is conceivable that, if this agent has any effect in lessening the feeling of tension in the anxious, it may be associated with the degree of reduction in muscular tone which the drug can undoubtedly produce[347].

Related derivatives of 1,3-propanediol show variation in anticonvulsant activity with alteration of the alkyl substituents in the 2-position (*XVI*; R_1, R_2) maximum activity and activity–toxicity ratio occurring with meprobamate[325,348]. This compound showed advantage in abolishing the righting reflex over the homologues, ebubamate (*XVI*; R_1 = Et; R_2 = Bu; R_3 = H) and dimebamate (*XVI*; $R_1 = R_2$ = Et; R_3 = H)[349]. Throughout this series, the carbamates have greater anticonvulsant activity and duration of action than the parent diols[348]. The mono-*N*-isopropyl derivative of meprobamate, carisprodol (*XVI*–2) shows greater activity as a muscle relaxant and less against strychnine convulsions; it lacks sedative and tranquillizing properties, which may be correlated with its failure to synchronize the thalamic EEG or affect behavioural arousal[350].

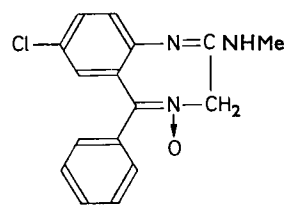
A series of compounds related to mephenesin (*XIV*–1), consists of derivatives of 4-hydroxymethyl-1,3-dioxolan (*XV*). These are muscle relaxants and anticonvulsants, for which activity is maximal if alkyl groups with a total of 6 to 8 carbon atoms are substituted in the 2-position (*XV*–R_1, R_2)[351]. In these compounds, the free —OH group was required for activity[351]. The

compound promoxolane (*XV*–2) is described as a tranquillizer[198,352]. Related hemiacetals are reported to be effective against audiogenic seizures, although in large doses[353].

Properties similar to those of meprobamate are reported for a derivative of 1,2-propanediol, namely phenaglycodol[354,355] (*XVII*–1). In general, compounds of this class are less active as anticonvulsants than 1,3-diol derivatives[356] although phenaglycodol is said to be relatively more effective against electric shock[327]. The effects of phenaglycodol on the EEG are reported to resemble those of chlorpromazine rather than those of meprobamate[330]. The drug appears to show anti-epileptic properties and is reported to relieve anxiety[4]. A related compound, styramate (*XVII*–2), is without effect on the EEG[327].

The short-acting hypnotic, oxanamide (*XVIII*)[357] may be considered as related to these derivatives of 1,2-diols. It has anticonvulsant properties and is said to improve the behaviour of irritable psychotics[358,359]. Emylcamate (*XIX*) is described as the most active of a series of primary aliphatic alcohols and their esters[359a]. It resembles meprobamate in its action and is more active in reducing spontaneous activity in mice, in antagonizing hyperactivity due to pipradrol, in weakening muscular activity and in antagonizing convulsions due to leptazol or electric shock. It is reported to impair skill less than does meprobamate and to be superior to this drug in tranquillizing alcoholics. Tranquillizing properties have also been claimed for other simple compounds, including 2-methyl-l-phenylpropyl carbamate[360] and γ-phenylpropyl carbamate, which is described as sharing the pharmacological properties of meprobamate[361].

Although a compound of an unrelated type, the similarity in its properties to those of meprobamate warrants mention at this point of methaminodiazepoxide (Librium), a recently introduced diazepine derivative (*XX*) in

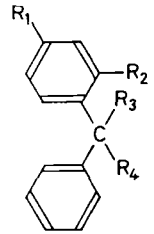


(XX) Methaminodiazepoxide

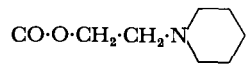
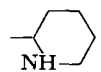
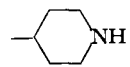
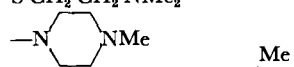
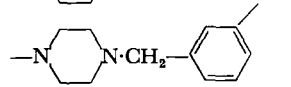
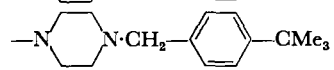
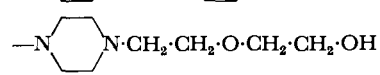
which structural similarities, although no pharmacological resemblance, may be seen to the diphenylmethane derivatives.

This compound reduces spontaneous activity in animals and, on increasing the dose, becomes paralysing and, finally, hypnotic. It tames monkeys in doses below those causing ataxia and is more effective than meprobamate in taming rats made aggressive by brain lesions[116]. Dramatic taming of wild animals has been reported[361a]. Conditioned avoidance is depressed, spinal reflexes are inhibited and EEG arousal by stimulation of the reticular formation is suppressed. The compound shows an anticonvulsant activity greater than that of meprobamate and is antipyretic, anti-inflammatory and, like meprobamate, stimulates appetite[166]. The drug has been reported to be of benefit in psychoneuroses, anxiety and tension, obsessional states,

Table 3.5. Diphenylmethane derivatives



(XXI)

Derivative number	R_1	R_2	R_3	R_4	*Approved name*	*Trade names*	*Principal uses*
1	H	H	H	$CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$	Adiphenine	Trasentin	Spasmolytic
2	H	H	OH	$CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$	Benactyzine	Suavitil Parasan	Tranquillizer
3	H	H	OH	$CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}N$ 		Sycotrol	Tranquillizer
4	H	H	OH		Pipradrol	Meratran	Antidepressant
5	H	H	OH		Azacyclonol	Frenquel	Tranquillizer
6	H	H	H	$O{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_2$	Diphenhydramine	Benadryl	Antihistamine
7	C_4H_9S	H	H	$S{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_2$	Captodiamine	Covatin	Tranquillizer
8	H	H	H		Cyclizine	Marzine	Antihistamine Anti-travel sickness
9	Cl	H	H		Meclizine	Bonamine	Antihistamine
10	Cl	H	H		Buclizine	Vibazine Softran	Antihistamine Tranquillizer
11	Cl	H	H		Hydroxyzine	Atarax	Tranquillizer

alcoholism and psychosomatic disorders, and to relieve agitation in psychotic conditions[361b,361c,361d], although no controlled trials are yet reported.

Diphenylmethane derivatives

The formulae, names and applications of the drugs to be discussed in this series are shown in *Table 3.5.* Esters of diphenylacetic acid, such as adiphenine (*XXI*–1), resemble atropine in antagonizing the peripheral actions of acetylcholine and have been employed clinically as spasmolytics. Similar properties occur in the benzilic acid esters, and benactyzine (*XXI*–2) had been included in many series of studies of spasmolytic activity before it was selected as a tranquillizer as the result of Jacobsen's study of such compounds in conflict situations in animals[142,362]. Jacobsen reported that, from its effect upon the performance of a conditioned avoidance response and upon the state of rats exposed to this situation judged by appearance and behaviour, benactyzine facilitated the response[363] and reduced the anxiety and tension shown by the animals[142]. It has been remarked that this interpretation may be disputed. Other investigations have suggested that the drug is less effective on conditioned reward behaviour[364] and on firmly established conditioned responses[365]. In this test[148] other benzilic esters were active; activity was retained on exchanging the *N*-alkyl groups in R_4 (*XXI*) for methyl or isopropyl, although the propyl member was inactive. On lengthening the carbon chain in R_4 to propyl or isopropyl, compounds were active if the *N*-alkyl group remained ethyl, although not where it was isopropyl. In position R_3 of (*XXI*) only —OH and chloride allowed activity. Diphenylacetic esters had little or no activity, and benzhydryl ethers of the type of diphenhydramine were also inactive. Quaternization of the amino group abolished activity, although this procedure strengthens spasmolytic activity, but this is likely to be due to the poor penetration to the brain of strongly ionized groups.

Many other classes of drug were inactive in this test, including barbiturates and chlorpromazine; reserpine was weakly active. Atropine was about one-fifth and scopolamine about 20 times as active as benactyzine. This hundred-fold ratio for the activities of the two *Belladonna* alkaloids has also been reported for their effect in suppressing EEG arousal following stimulation of the reticular formation[366]. Benactyzine is also effective in this respect, and further resembles them in not suppressing behavioural arousal and, moreover, in synchronizing the cortical EEG without inducing sleep[36,367,368]. These considerations suggest that all the effects concerned may be central manifestations of antiacetylcholine activity, in which benactyzine approaches the potency of atropine[46,59].

Jacobsen also found benactyzine effective in abolishing the anxiety and hesitation of cats faced with a conflict situation imposed on conditioned reward behaviour[362]. The activities of homologous compounds in this series were related in a similar manner to those found in the rat, but here scopolamine could not be shown active, possibly because ataxia occurs with low doses. Benactyzine prolongs barbiturate narcosis[46,369] but has no taming action[46]; it promotes increased locomotor activity and facilitates electric shock[46] and, like scopolamine, causes emesis in pigeons[318].

The clinical effects of benactyzine, in small doses, are described as a

blocking of thoughts and a relief of anxiety[370] although it may produce confusion and clumsiness[368,371]. Although there have been reports of favourable results in the treatment of anxiety states[370,372,373], the preponderance of adverse reports suggests that this drug may not be a useful tranquillizer[4,215,374,375]. A closely related benzilic ester (*XXI*–3) has also been reported to be effective in anxiety neuroses[375a].

Azacyclonol (*XXI*–5) is an isomer of the central nervous stimulant, pipradrol (*XXI*–4)[376] and in many respects the pharmacological properties of the two drugs are opposite and mutually antagonistic[377]. In common with all other drugs reviewed here, azacyclonol potentiates barbiturate narcosis in animals; it also reduces locomotor activity[377]. The compound has no effect on conditioned responses[155,378] or on EEG arousal[36], although it restores the EEG to normal after desynchronization by LSD or mescaline[379] and reduces morphine mania in cats[377]. Azacyclonol was first described as a suppressant of the hallucinations induced in man by LSD or mescaline[86,377], although a later report has questioned this action of the drug[85]. It has consequently been tried in conditions where hallucinations are a distressing feature: schizophrenia, acute psychoses, alcoholism, senile and toxic hallucinosis. The reports, some of which are favourable and some unfavourable[380–385], leave room for doubt on its status as a useful therapeutic agent[4].

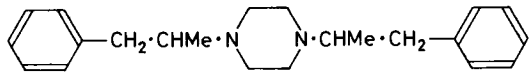
As the result of a survey of a series of compounds related to diphenhydramine (*XXI*–6) for sedative effects as judged by locomotor activity in mice, Weidmann and Petersen[386] found that captodiamine (*XXI*–7) was more than twice as active as promethazine in this respect and showed to lesser degree the stimulant action of diphenhydramine in higher doses[387]. Activity was reduced by higher *N*-alkyl groups and was rather higher in *S*-ethers than in *O*-ethers[386]. Increasing the length of the alkyl chain in the alkylthio substituent of one of the phenyl groups also increased activity[386].

Captodiamine prolongs barbiturate narcosis[388] and this has been shown to be due to inhibition of metabolism of the hypnotic[389]. It also potentiates analgesics and protects against electric shock but not convulsions due to leptazol or strychnine[388]; it does not antagonize harmine stimulation[191]. The drug has been claimed to sedate schizophrenics and mild psychotics, although it has no effect on severe cases[198,390,391].

Hydroxyzine (*XXI*–11) is a member of a series of piperazine derivatives of diphenylmethane which includes the antihistamines cyclizine, meclizine and buclizine (*XXI*–8 to 10). Hydroxyzine shares their antihistamine activity[392] and, like them, is sedative in man[393]; it causes chlorpromazine-like sedation in monkeys[394] and calms mice in low doses, although higher doses excite[394,395]. The drug prolongs barbiturate narcosis and raises the electric shock threshold, but does not antagonize the convulsant action of leptazol and potentiates that of strychnine[394]. It is a muscle relaxant and inhibits conditioned avoidance responses in the rat[396], and shows anti-emetic[396], analgesic and local anaesthetic activity[392]. Effects of adrenaline and serotonin are antagonized[397].

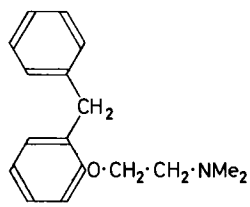
Hydroxyzine is claimed to benefit cases of psychosomatic disorder and oon-psychotic anxiety and tension, although not in schizophrenia; side-effects are minimal[4,5,393,398].

Other piperazine derivatives related to diphenazine (*XXII*) have been shown to possess adrenolytic activity and cause reduction of sympathetic tone, to antagonize amphetamine and to prolong barbiturate hypnosis. They are anticonvulsant and depress reflexes and conditioned behaviour[399,400].

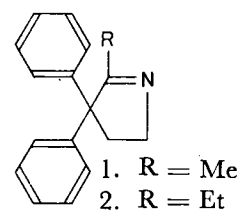


(XXII) Diphenazine

The antihistamine compound phenyltoloxamine (Bristamine, *XXIII*)[401] also shows sedative properties which may be clinically useful[198]. Other diphenylmethane derivatives for which sedative properties are described are the diphenylpyrroline compounds (*XXIV*–1,2). These are sedative in



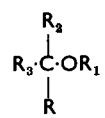
(XXIII) Phenyltoloxamine



(XXIV)

animals and potentiate barbiturate narcosis and analgesics. They are reported as tranquillizing in man but clinical use is precluded by the incidence of urethritis[402].

Table 3.6. Tertiary carbinols and derivatives



(*XXV*)

Derivative number	R	R_1	R_2	R_3	*Approved name*	*Trade name*
1	C≡CH	H	Me	Et	Methylpentynol (Methylparafynol)	Oblivon
2	C≡CH	CO·NH_2	Me	Et	Methylpentynol carbamate	N-Oblivon Oblivon C
3	C≡CH	H	ClCH=CH	Et	Ethychlorvynol	Placidyl
4	C≡CH	H	—$(CH_2)_5$—		Ethynylcyclohexanol	
5	CH_2·C≡CH	CO·NH_2	—$(CH_2)_5$—		Propynylcyclohexanol carbamate	Merinax

Miscellaneous classes of compounds

A number of hypnotics related to methylpentynol (*XXV*–1) and listed in *Table 3.6* show mild tranquillizing properties rather than sedation in subhypnotic doses. Pharmacologically, these are characterized by non-anaesthetic hypnosis, powerful antagonism of convulsants, protection against

audiogenic seizure and depression of reflexes[403–406]. Methylpentynol, unlike ethanol, increased the exploratory activity of rats, which may suggest reduction of fear,[407] and reduced fear-motivated learning[408]. It had no effect on the toxicity of amphetamine to grouped mice[187].

Methylpentynol and its carbamate (*XXV*–2) have been used, in sub-hypnotic doses, to reduce apprehension in dentistry[409,410] and in labour[411]; the carbamate has a stronger and more delayed action[410,412]. Compared with ethanol, less over-confidence and signs of intoxication occur[409]. Related alcohols include ethchlorvynol (*XXV*–3) which shows similar properties to methylpentynol[413] and has been described as an effective tranquillizer without side-effects[414], and propynylcyclohexanol carbamate (*XXV*–5) which is sedative and anticonvulsant, and ineffective against amphetamine toxicity[415].

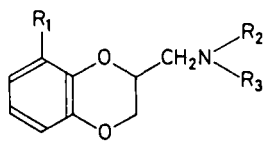
Ethylcrotonylurea (*XXVI*) occurs in two forms, both of which are sedative, but while the *cis*-form causes hypnosis in animals near the toxic dose,

$$Me{\cdot}CH{:}CEt{\cdot}CO{\cdot}NH{\cdot}CO{\cdot}NH_2$$

(XXVI)

the *trans*-form is stimulant and convulsant[416]. The *cis*-form, ectylurea (Nostyn, Levanil) is described as a mild tranquillizer, better than meprobamate in anxiety and tension, and free from side-effects[4,417].

The sedative properties occurring with adrenolytic compounds, of which piperoxan (*XXVII*–1) is an example of one type, have prompted the search for similar properties in other benzodioxan derivatives[79]. One such compound, ethoxybutamoxane (*XXVII*–2) is described as strongly sedative,



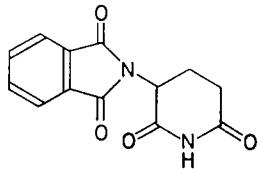
(XXVII)

1. $R_1 = H$; $R_2 = R_3 = Me$
2. $R_1 = OEt$; $R_2 = H$; $R_3 = Bu$
3. $R_1 = R_2 = H$; $R_3 = (CH_2)_3{\cdot}OMe$

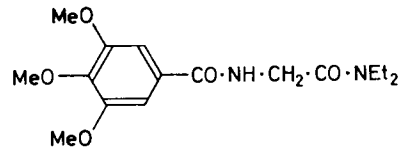
decreasing spontaneous activity and aggressiveness and causing catatonia. It antagonizes apomorphine emesis, depresses conditioned avoidance, reduces thalamic recruiting and blocks the arousal response to adrenaline, besides showing other adrenolytic actions[81,418]. The related compound Quiloflex (*XXVII*–3) while sharing the sedative properties of this group, is a more pronounced muscular relaxant and reflex depressant, resembling mephenesin[419]; it is found useful in the treatment of spasticity[420].

The remaining compounds to be mentioned are chemically diverse and appear to possess no common feature; together with many of the compounds already mentioned, they illustrate the wide search for tranquillizing drugs taking place at present. Many hypnotics may be used as sedatives in low doses, but the advantage claimed for thalidomide (Distaval, Contergan)

or α-phthalimidoglutarimide (*XXVIII*) is safety due to low toxicity. This compound causes hypnosis, from which subjects may readily be aroused, and in animals, unlike glutethimide, to which it is chemically related, it causes no loss of righting reflex, no excitement and no loss of muscular co-ordination. It shows no analgesic or anticonvulsant properties[421,422].

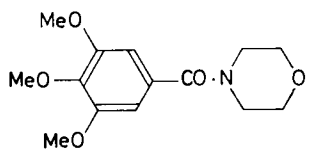


(*XXVIII*) Thalidomide

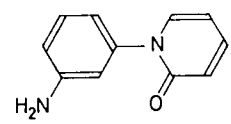


(*XXIX*) Trimeglamide

Clinically, it is claimed to be a valuable sedative in cases of anxiety, agitation, tension and irritability, and in psychosomatic disorders[423–425]. Similar properties are described for trimeglamide, 3,4,5-trimethoxybenzoylglycine diethylamide (*XXIX*)[426]. The trimethoxybenzoyl derivative of tetrahydro-1,4-oxazine (Trioxazin, *XXX*) has been claimed to be of benefit in psychomotor restlessness, anxiety and tension. It reduces locomotor activity and postural reflexes in animals but does not affect spinal reflexes and is not hypnotic[426a]. Amphenidone (Dornwal, *XXXI*) is described as a mild ataractic drug similar to meprobamate in relieving tension headaches[426b], although improvement has also been reported in schizophrenic patients[426c]. It is an anticonvulsant, depressing spinal polysynaptic reflexes, with mild analgesic properties[426d].

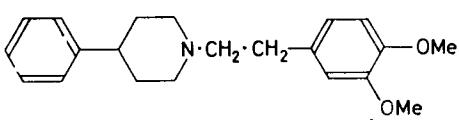


(*XXX*) Trioxazin

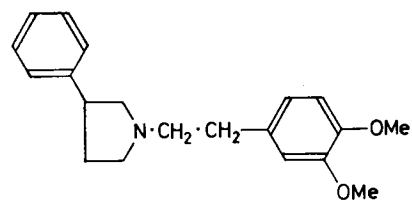


(*XXXI*) Amphenidone

The compounds 4-phenyl-*N*-homoveratrylpiperidine (*XXXII*) and the corresponding 3-phenylpyrrolidine (*XXXIII*)[427] are described as chlorpromazine-like tranquillizers, causing selective depression of conditioned



(*XXXII*)

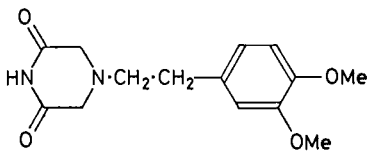


(*XXXIII*)

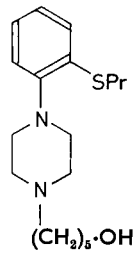
avoidance behaviour. Dehydrogenation of (*XXXII*) at the 4,5— bond in the piperidine ring enhanced activity and so did the introduction of *o*- and *m*-substituents in the 4-phenyl group. Many of these compounds showed adrenolytic properties[80].

Other compounds for which central depressant properties are described, although it is not clear that they merit use of the term tranquillizer, include

a group of diketopiperazine derivatives, of which (*XXXIV*) is the most active[428]; the compound referred to as piperazinepentanol (*XXXV*)[428a,428b], and some 2-aryl-4-metathiazanones, represented by chlormethazanone (Trancopal, *XXXVI*)[429,430].

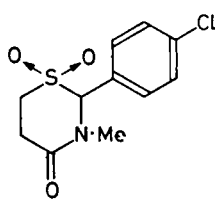


(XXXIV)

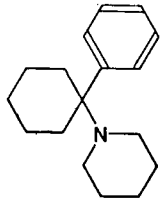


(XXXV) Piperazinepentanol

l-(l-Phenylcyclohexyl)piperidine or phencyclidine (Sernyl, *XXXVII*) is an anaesthetic, reported to cause interceptive sensory deprivation in man,

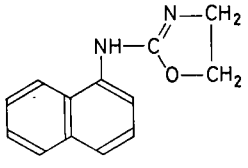


(XXXVI) Chlormethazone

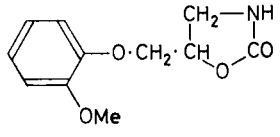


(XXXVII) Phencyclidine

in sub-anaesthetic doses. It calms many laboratory species in low doses, and produces catatonia, anaesthesia and convulsions with increased doses. In rats and mice it increases activity and reduces motor co-ordination; it prevents convulsions due to leptazol or electric shock but not those due to strychnine[431]. It has been reported of benefit in psychoneurosis[431a].



(XXXVIII) 2-(1-Naphthylamino)-2-oxazoline



(XXXIX) Metoxadone

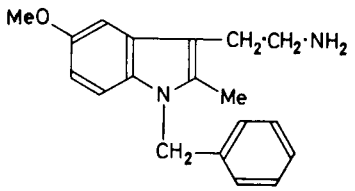
2-(l-Napthylamino)-2-oxazoline (*XXXVIII*) quietens animals and is an anaesthetic and spinal cord depressant, but unlike other drugs in this class, facilitates leptazol convulsions[432]. The oxazolidone derivative, metoxadone (*XXXIX*) is a muscle relaxant and anticonvulsant; it potentiates analgesics and inhibits conditioned avoidance[432a].

The compound BAS, l-benzyl-5-methoxy-2-methyltryptamine (*XL*) which Woolley describes as a serotonin antimetabolite[433] and which also displaces

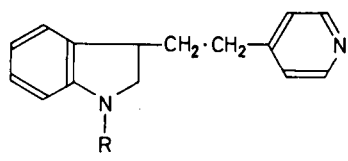
serotonin from tissues[434], is claimed to show tranquillizing properties in mice and in the treatment of psychotics[435].

Some indolylethylpyridine derivatives are also depressant, reducing locomotor activity and weakening polysynaptic reflexes in animals[435]. The compounds (*XLI*) where R = methyl or benzyl, showed clinical activity[436,437].

The older treatment of agitation and tension with bromide, which has long been in disrepute owing to its toxicity[6] has a modern equivalent in the use of lithium salts. This has been recently reviewed by Schou[438]; it appears equivalent to electroconvulsive therapy in mania. The side-effects of the antipsychotic tranquillizers are avoided, although here, too, toxicity is a disadvantage.



(*XL*) BAS



(*XLI*)

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4

DIURETIC DRUGS

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HISTORICAL INTRODUCTION

DIURETICS may be defined as drugs which increase urine flow or—in a stricter and therapeutic sense—as compounds which remove excess extracellular water in disease. Other measures such as purgation, diaphoresis and venesection may be used to decrease the water and electrolyte content of the body, but they are largely obsolete and a discussion of their value is beyond the scope of this article. Measures used to reduce 'local oedema' likewise will not be considered. Since there are many pathological conditions in which the water content of the tissues or the body cavities is increased, substances which raise urine output (or which at least were thought to do so) have long been in use.

The oldest diuretics are probably herbal preparations containing volatile oils, some of which are even mentioned in Egyptian medical papyri. Their use as diuretic preparations has only recently been abandoned: *Infusum Buchu*, for example, was retained in the B.P. up to 1932 and monographs on *Infusum Uvae Ursi* and *Spiritus Juniperi* are included in the B.P.C. 1934. Metallic mercury and inorganic mercury salts were introduced into diuretic therapy many centuries ago; Paracelsus (1493–1541) for example, was well aware of their diuretic action and recommended them for the cure of dropsy in his *Etliche Traktaten* (1520). However, it appears from the doses recommended that the water loss produced was mainly achieved by the laxative effect. Saline diuretics have likewise a long history; Thomas Willis[1] in his *Pharmaceutice Rationalis* (1724) mentioned the use of several salts, notably potassium nitrate, and potassium acetate was known to the old writers as *sal diureticus*. Urea, however, was only introduced by Friedrich[2] as late as in 1892.

It is difficult to say how long the cardiac glycosides have been employed as 'diuretics'. Digitalis was used as a herbal remedy long before William Withering introduced it into orthodox medicine (1785). The West Country physician John Blackall[3], for instance, writes in his famous book on *The Nature and Cure of Dropsies* (1813): 'It (digitalis) has been employed in this and other countries as a domestic drug, yet its exhibition was regulated by no sort of principle or distinction; and accuracy as to dose was wholly out of the question. Even lately, the common people of this neighbourhood have been in the habit of using very strong and copious infusions of it, made by throwing boiling water on the leaves, stem and root, without any measure or weight. The results have been some unexpected recoveries much talked of, and more failures, which tell no tales.' Withering himself used digitalis rather indiscriminately 'in the ascites, anasarca and hydrops pectoris' and only excluded 'ascites in female patients', believing that this condition was

due to 'dropsy of the ovary'. Even forty years later, Blackall's indications for the diuretic use of digitalis[4] were not much clearer although he recognized that it acted better in hydrothorax, *i.e.* presumably pulmonary embarrassment due to cardiac failure. A clear differentiation between cardiac and renal oedema—and hence a rational basis for the use of digitalis—was only achieved by Richard Bright[5] in his *Reports of Medical Cases* (1827).

During the rest of the nineteenth century little progress was made in diuretic therapy. Bouchardat[6] in 1859 and Koschlakoff[7] in 1864 used caffeine as a diuretic but the more potent xanthines, theobromine introduced by Schroeder[8] in 1889 and theophylline by Doering[9] in 1903, were followed in 1908 by the use of aminophylline. The diuretic effect of organic mercurials was accidentally discovered when Vogl[10] in 1919 used Novasurol (merbaphen) to treat a syphilitic patient. At about the same time[11], the increase of urine flow produced by acidifying salt was discovered as a result of laboratory experiments. Thereafter until the early fifties of this century the organic mercurials, given parenterally together with oral doses of ammonium chloride, ruled supreme. Although their efficiency was great there were several reasons why more satisfactory diuretic drugs were desirable: toxic effects were not rare and parenteral therapy over months and years was unpleasant and cumbersome. This led to the introduction of new mercury preparations which were effective in many patients when taken orally. Another development resulted in orally-active compounds not containing mercury, such as the aminouracils (which are chemically related to the methylxanthines) and the carbonic anhydrase inhibitors such as acetazolamide, and chlorothiazide and its congeners. Better insight into the mechanism of oedema formation and in the importance of endocrine influences on renal function led to the therapeutic use of certain adrenocorticosteroids, and quite recently substances which inhibit the secretion or interfere with the action of aldosterone have been tried.

RENAL MECHANISMS WHICH ARE AFFECTED BY DIURETIC DRUGS

Since diuretics act by altering the composition and volume of the urine, an account of the pharmacology of diuretics must be preceded by a brief, description of the relevant normal mechanisms in the kidney upon which they exert their actions.

The morphological unit of the kidney is the nephron, of which there are about one million in a human kidney. The essential features of a nephron are shown in *Figure 4.1.* The formation of urine starts at the glomerulus, a tuft of capillaries arising from the afferent arterioles of the renal artery. The capillaries are encapsulated in a double membrane called Bowman's capsule. An outlet from the space between the two layers of Bowman's capsule forms the commencement of the renal tubules. After extensive convolutions near the origin (proximal convolutions) the tubule descends into the kidney medulla and there completes a sharp hair-pin bend (Henle's loop) returning along a straight path to the region of the glomerulus where once again extensive convolutions occur (distal convolutions). In some nephrons, the loop is very short and the whole nephron may be confined to

the renal cortex. The outflows from the distal tubules are gathered together in collecting tubules which descend once more into the renal medulla and coalesce there, to form the collecting ducts in the renal pelvis. Blood emerging from the glomerular capillary bed is conveyed in the efferent arterioles to form a second bed, principally around the renal tubules. An outstanding feature of the nephron often obscured in diagrammatic representations is the great length of the tubules in comparison with the diameter of the lumen so that fluid flowing in the tubule comes in contact with a very large absorbing surface.

Urine formation starts with the ultrafiltration of plasma from the glomerular capillaries into the intracapsular space. The filtrate contains all the

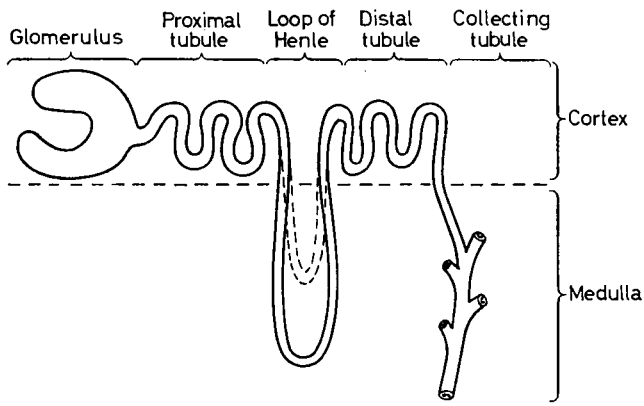


Figure 4.1. Schema of the structure of the nephron and its distribution between the cortex and medulla
(From de Wardener: *The Kidney* Churchill, London, 1959)

solute of the filtered plasma other than protein, lipid and other macromolecules. The driving force for glomerular filtration is the blood-pressure in the afferent arterioles which must exceed the sum of the pressures opposing ultrafiltration, the colloid osmotic pressure of the plasma and the intracapsular pressure. Normally, about 1/5th of the plasma-water circulating through the glomeruli is filtered, amounting to 150–200 l./day in man. During the passage of the filtered fluid through the tubules more than 99 per cent of the filtered water and salts and normally all the glucose and amino acids are reabsorbed. Although they may be partially reabsorbed, products of metabolism (*e.g.* urea) are concentrated in the final urine of which the volume, under normal conditions, is about 1·5 l./day.

There are three processes by which a constituent of the tubular urine may be reabsorbed—active reabsorption, passive reabsorption and exchange. Active reabsorption occurs when the transfer of a solute from the tubule proceeds even though the concentration gradient between the tubular fluid and blood opposes reabsorption. It must be assumed that metabolic energy is required but the biochemical reactions involved are not known. During active reabsorption of a single ionic species, electro-chemical equilibrium

between the tubular urine and tubular cells may be maintained in two ways: (*a*) by transfer of the equivalent amount of different ions of the same charge from tubular cell to tubular urine, the whole process being regarded as the exchange of one ion for another, and (*b*) by the reabsorption of equivalent amounts of ions of opposite charge; this is an example of passive reabsorption, the energy for the transfer coming from the electro-chemical potential created, in the first place, by the active reabsorption. After active reabsorption of solute, tubular urine tends to be hypotonic so that, where the tubule is permeable to water, osmotic forces will drive water from the tubule, *i.e.* cause passive reabsorption of water. The reabsorption of water creates a concentration gradient between the tubular fluid and the blood and interstitial space for those substances in the tubular urine which are not actively reabsorbed. This promotes passive reabsorption of such substances (*e.g.* urea) provided that they can diffuse through the tubular wall.

Reabsorption of glucose, phosphate and sulphate in the proximal convolutions is by independent active processes which remove them completely from the tubular urine, but there is a limiting rate for active reabsorption of each of these substances. If the filtered load (glomerular filtration rate multiplied by plasma concentration) of the substance is greater than the amount which is transferred from urine to tubular cell, reabsorption is incomplete and the substance appears in the final urine.

Throughout the proximal tubule, the osmotic pressure and sodium concentrations do not differ from those in the glomerular filtrate but the rising concentration in the tubular urine of substances which are not reabsorbed, shows that during the passage down the proximal tubule about 80 per cent of the sodium chloride and water are reabsorbed. Pitts[12] believes that the proximal reabsorption of sodium chloride arises from the active extrusion of sodium ion from the tubular cell into the peritubular fluid and that passive reabsorption of chloride ion follows the electro-chemical gradient.

Reabsorption of most of the bicarbonate also occurs in the proximal tubule and is associated with exchange of sodium ion from the urine with hydrogen ion from the tubular cells. Hydrogen ion is provided by the carbonic anhydrase catalysed reaction:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3; \qquad H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

The bicarbonate ion is transferred from the cell to the peritubular fluid while the hydrogen ion exchanges with sodium in the tubule, where it associates with bicarbonate ion from the glomerular filtrate, ultimately giving water and carbon dioxide. The latter is returned to the tubular cell by diffusion. The net effect of this operation is reabsorption of sodium bicarbonate without a significant change in urinary pH.

Reabsorption of water and salts in the proximal tubule is independent of changes in the composition and volume of body fluids except in so far as the filtered load may be altered. The part of the function of the kidneys concerned in maintaining the constancy of the internal environment is carried out in the distal system (Henle's loop, the distal tubule and the collecting tubules) where further reabsorption of water and electrolyte determines the ultimate concentration of the urine. Water and sodium are abstracted from the tubular urine in the distal system by processes which are

controlled respectively by the antidiuretic hormone (A.D.H.) of the neurohypophysis and by aldosterone, the sodium-retaining hormone of the adrenal cortex.

An ingenious explanation for the facilitation by A.D.H. of water reabsorption in the distal system has been put forward by Wirz[13] and by Gottschalk and Mylle[14]. They suggest that urine traversing the hair-pin loop of the nephron is subjected to a counter-current concentrating system, which requires water permeability in some parts of the nephron (and the collecting tubules in particular). The permeability of the collecting tubules is dependent on the presence of A.D.H. and in its absence, distal reabsorption of water is reduced and the urine flow rate is much increased. The secretion of A.D.H. from the neurohypophysis is determined by the osmotic pressure of the blood affecting osmoreceptors somewhere in the bed of the internal carotid artery[15,16] and by changes in the volume or distribution of circulating blood which are transmitted to the neurohypophysis through stretch receptors in the left auricle[17–19]. By these means A.D.H. secretion is geared to the need of the body for conservation or rejection of water.

The distal reabsorption of sodium ion which is controlled by aldosterone, involves the coupled exchange of sodium from the urine for hydrogen ion or potassium ion from the tubular cells[20,21]. Potassium ion filtered at the glomerulus is completely reabsorbed in the proximal tubule[22,23] and potassium appearing in the final urine arises from the exchange with sodium ion in the distal tubule.

The catalysed formation of carbonic acid by carbonic anhydrase is the source of the hydrogen ion, and the exchange of sodium for hydrogen ion in the distal tubule is responsible for the acidification of the urinary buffers. The excretion of ammonium ion, also, is associated with the distal exchange of sodium for hydrogen ion. Ammonia production in the tubular cells is a continuing process as a result of the deamination of glutamine by glutaminase. The transfer of the ammonia from the cell to the urine is facilitated when the ammonia can react with hydrogen ion in the tubular urine to form ammonium ion so that a concentration gradient is maintained. This, in its turn, maintains the concentration gradient for hydrogen ion between the cell and urine and so permits continued exchange of sodium for hydrogen ion. Thus, during acidosis, the effect of the two operations, excretion of ammonium and acidification of urinary buffers (*i.e.* $Na_2HPO_4 \rightarrow NaH_2PO_4$) frees some sodium ion from excretion as the necessary accompaniment to anion excretion, so that hydrogen ion can be eliminated while sodium ion is conserved[24].

OEDEMA

Oedema can be defined as the accumulation of excess fluid in tissues. The occurrence of severe oedema may be due to disease of the kidney (nephrotic oedema), liver (hepatic cirrhosis), or heart (congestive heart failure), endocrine disorders or dietary deficiencies. There are features common to all forms of generalized oedema, but others are peculiar to each type and these may determine the best form of therapy. It is necessary therefore to discuss the mechanism of oedema formation and the characteristics of the various conditions in which body water is pathologically increased.

Mechanism of Oedema Formation

The accumulation of oedema fluid is due to alteration of the balance between fluid in the blood capillaries and in the tissue spaces. This is determined, chiefly, by the hydrostatic pressure and the colloid osmotic pressure of the blood[25]. Fluid loss from the blood is favoured by capillary blood-pressure and the colloid osmotic pressure of the perivascular fluid and is opposed by the colloid osmotic pressure of the plasma and by tissue tension. Changes in two of these forces—increased intracapillary pressure and decreased plasma colloid osmotic pressure—occur in several forms of oedema. The protein content of the tissue fluid increases when the permeability of the capillary wall is altered, *i.e.* in inflammatory conditions such as acute nephritis and in allergic oedema. Lastly, changes in renal function (as distinct from renal lesions such as damage to the glomerular capillaries with the resultant increase in their permeability to proteins) may be also concerned in the origin of oedema and contribute to its maintenance. For example, oedema fluid may not be excreted by the kidneys because increased secretion of aldosterone causes absorption of excessive amounts of sodium by the kidney tubules.

The increased absorption of sodium ion (and water) may be the result of regulatory responses of the kidney, the adrenal cortex and the posterior pituitary to physiological stimuli which persist in the oedematous subject because factors arising from disease (such as increased venous pressure or a low level of plasma protein) continue to shift fluid from the circulation into the extracellular compartment.

Cardiac Oedema

Oedema in congestive heart failure has been attributed to the generalized increases in venous pressure which results in increased filtration pressure in the capillaries (backward-failure theory). The accompanying oliguria may be due to the immobilization of water and sodium in the tissues. Other results suggest that impairment of renal function due to inadequate cardiac output is the initiating event in oedema production and that expansion of the volume of extracellular fluid precedes the rise in venous pressure (forward-failure theory). Both theories imply changes in renal function of which the following have been observed: (*a*) renal venous pressure is increased; (*b*) renal blood flow is reduced to approximately half of the normal; and (*c*) glomerular filtration rate falls to about two-thirds of normal, partly due to intrarenal vasoconstriction. It has been shown experimentally[26,27] that a fall in renal perfusion pressure reduces renal blood flow, glomerular filtration rate and excretion rate of solutes. However, salt excretion in dogs may return to normal despite persistence of an experimentally reduced filtration rate[28]. Conversely, non-oedematous animals with lesions of the heart valves and normal filtration rates excrete salt injected into one renal artery less efficiently[29]. It seems therefore that reduction of the glomerular filtration rate affects sodium excretion but that other—perhaps endocrine—influences on the rate of tubular absorption of the electrolytes are more important in the maintenance of cardiac oedema.

Aldosterone is probably involved since its urinary excretion is increased in cardiac patients[30].

Treatment will be primarily directed at the cardiac disorder, *i.e.* digitalis will be given which may raise the urine volume in several ways—(*a*) by increasing cardiac output and renal blood-flow so that the rate of glomerular filtration will be raised and so more sodium filtered; (*b*) by lowering of renal vascular resistance; (*c*) by decreasing of intrarenal oedema and venous pressure and (*d*) by a direct inhibitory effect of the digitalis glucosides on sodium ion absorption by the renal tubules[31]. However, digitalis will not usually free the patient of the accumulated oedema fluid and diuretics which reduce the renal absorption of sodium and chloride more effectively are therefore given.

Hepatic Disease

Impairment of water metabolism in diseases of the liver is not dependent on the presence of ascites, *i.e.* on the accumulation of free fluid in the abdomen. Visible oedema and expansion of the extracellular fluid phase have been observed long before ascites occurred.

Liver cirrhosis may be defined as a combination of atrophy of liver cells and an increase in fibrous tissue. This increase produces a rise of intracapillary pressure within the portal venous system, *i.e.* it causes 'portal hypertension'. Ascites, however, is due not only to this but also to the decrease in the plasma protein concentration which is common in liver disease. If, for instance, a patient with portal hypertension has a gastrointestinal haemorrhage, ascites develops. As the plasma proteins recover the ascites disappears, even though the portal pressure remains increased[32].

As fluid passes into the peritoneal cavity, the circulating blood-volume decreases and provides a stimulus for the increased secretion of aldosterone[33]. Increased amounts of aldosterone have, in fact, been found in the urine of patients with cirrhosis and ascites[30,34], but this may be partly due to the inability of the diseased liver to inactivate the hormone[35]. In some patients increased sodium reabsorption is enhanced by a decrease in renal blood-flow. Increased pressure on the renal veins due to the accumulation of free fluid in the abdomen may be another contributory factor.

Little can be done to improve liver cell function. Measures which raise the plasma colloid osmotic pressure are only temporarily effective in reducing ascites. Lowering of the portal hypertension by surgery is occasionally successful. Diuretics are frequently unsuccessful[32]. Dietary sodium restriction usually prevents the re-accumulation of fluid.

Nephrotic Oedema

Chronic nephrosis is a disease of childhood and young adult life. It may be the result of infection with known organisms but usually is a stage of chronic glomerulo-nephritis of unknown origin. The essential defect is an increased permeability of the glomeruli to protein. The heavy loss of protein in the urine and the resulting decrease in plasma colloid osmotic pressure are important factors in the origin of the oedema. There is also a reduction in the effective filtering surface of the glomeruli which directly decreases

filtration rate. Renal sodium reabsorption is therefore decreased and is accompanied by extrarenal changes provoking the counter-regulatory secretion of aldosterone. Impairment in the excretion of an intravenously infused sodium load has been shown in nephrotic oedema[36] and increased excretion of aldosterone in the urine has been reported[30]. The metabolism of cellular protein is increased to replace plasma protein losses, and intracellular potassium is released into the circulation[36]. Moreover, so much water may be retained that the extracellular fluid becomes hypotonic[37,38] and 'intracellular potassium' enters the extracellular fluid compartments for osmotic reasons. When diuresis is induced in the oedematous child, potassium excretion rises considerably. These observations have an important bearing on diuretic therapy in nephrosis since they suggest that potassium depletion is easily produced.

Glucocorticoids and corticotrophin usually produce a diuresis and often decrease the loss of protein in the urine. Dietary treatment (high-protein diets) may be helpful. Infusions of concentrated human serum albumin or other high molecular substances raise the plasma colloid osmotic pressure and increase the urine volume. Mercurial diuretics are usually avoided for fear of further damaging the kidneys. Other diuretics with a renal tubular site of action are still in the process of evaluation.

Nutritional Oedema

There are at least two nutritional conditions which lead to an increase of the ratio of extracellular water to cellular tissue and to clinical oedema. The first is due to the prolonged intake of a low calorie diet deficient in most of the essential ingredients. This 'famine oedema' has been produced experimentally in men[39]. Its development is accompanied by a small decline in the plasma protein concentration. Its origin may therefore be ascribed to the decrease of tissue solids and to the arousal of those regulatory mechanisms which are activated by changes in the body fluid compartments.

The second type of nutritional oedema arises when the calorie supply of the diet is adequate but the diet markedly deficient in proteins. This type of 'protein deficiency oedema' or kwashiorkor is common in all parts of the tropics. It is usually found in weanling infants who are provided with vegetable diets poor in proteins. These children are often grossly oedematous and their urine output is markedly decreased. Plasma sodium concentrations are usually unchanged and so are plasma potassium levels, in the absence of diarrhoea[40]. The oedema of kwashiorkor resembles nephrotic oedema in occurring in patients with low plasma colloid osmotic pressure but differs from it by the absence of kidney damage. Hence, it can be regarded as being due mainly to the lowered plasma protein concentration. Increased blood concentrations of aldosterone resulting from regulatory responses due to changes of the body fluid compartments may also be expected.

The majority of children with 'protein deficiency oedema' recover rapidly when supplied with a high-protein diet. In those who do not recover, the oedema disappears slowly and the rise in the plasma protein level is delayed due probably to malabsorption of the therapeutic diet and to intercurrent diseases. The mercurial diuretics aimed at reducing the oedema

more rapidly have been tried[41–43] but have often been ineffective. Of other diuretic substances tried in the experimental oedema of protein deficiency glucocorticoids gave the best results[44].

Cyclical Oedema and Oedema in Pregnancy

Adult female patients frequently complain of gains in weight which are linked with their menstrual period. The increase of weight appears to be due mainly to retention of sodium and water[45,46]. Cyclical oedema differs from most other forms of water retention as it is probably caused by a

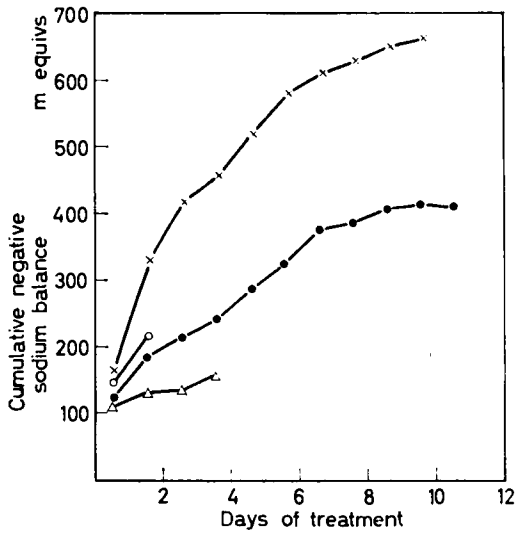


Figure 4.2. Sodium loss of four pregnant oedematous women whose only treatment was bed rest and dietary sodium restriction. Two of these patients lost 5·0 and 4·1 kg in body-weight during the first 6 days[55].

primary endocrine disturbance. It is well known that oestrogens and progesterone may impair sodium excretion[47] and phasic changes in the posterior pituitary during the oestrous cycle in rats have been observed[48]. Increased excretion of aldosterone has also been reported[49,50].

The treatment consists of dietary sodium restriction or the administration of 'mild' diuretics such as ammonium chloride or acetazolamide. Mercurial diuretics and chlorothiazide have been used in severe cases. Treatment with aldosterone inhibitors is still in the trial stage.

During pregnancy, water and solids are gained proportionately until the last weeks before delivery, when there appears to be a selective accumulation of water[51]. Thus, a certain degree of oedema in pregnancy is normal. Sometimes, however, oedema occurs in pregnancy accompanied by other 'toxaemic' symptoms. Sodium absorption by the toxaemic kidney is increased[53] although aldosterone excretion in pre-eclamptic toxaemia is not high and may in fact be lower than normal[52–54].

Patients with mild pre-eclampsia at rest in bed lose their oedema when put on a low sodium intake[55]. Their sodium balance becomes rapidly negative, *i.e.* they excrete more sodium than they ingest (*Figure 4.2*). The

response to this simple treatment has to be taken into account in this as in other forms of oedema, when the effects of diuretic drugs are evaluated.

In the more severe forms of pregnancy toxaemia, however, the more potent diuretic drugs are used. Intramuscular injections of hypertonic magnesium sulphate solutions (which have not only an anticonvulsive action but may also produce an osmotic diuresis) have also been recommended[53].

EVALUATION OF DIURETIC DRUGS

Like all potent drugs, diuretics have to be assayed before they are used clinically. One essential step is the determination of the over-all toxicity of the new compound both in acute and in 'chronic' experiments; another is the evaluation of its specific therapeutic effect, either in absolute terms or in comparison with a compound acting similarly. Toxicity tests of diuretics in animals are carried out according to well-established methods[56]. Gross toxicity only can be determined: the differences between any laboratory animal (or several species of animals) and man are such that many minor undesirable side-effects will only become manifest in clinical trials—or indeed with some drugs—after a lengthy period of therapeutic use.

A method devised by Burn[57] for the assay of antidiuretic hormone has also been applied to the evaluation of diuretics. Healthy rats are given 5 per cent of their body-weight of water by stomach tube and the time in which the rate of water excretion reaches the maximum is determined. The method can be improved by administering smaller volumes of water—say 2·5 per cent of the body-weight—and by charting the urine output for a given number of hours. A number of diuretic substances (aminophylline, azetazolamide, ammonium chloride, mersalyl, cortisone, prednisolone) have been tested by Heller and his co-workers[44] in this manner, and diuretic effects were observed with all these compounds except mersalyl. Mersalyl together with ammonium chloride or aminophylline failed to enhance urine output. Several doses of each diuretic were used but it was not possible to establish a linear log dose–effect relationship for any of the compounds tested. Lipschitz, Hadidian and Kerpcsar[58] fed small volumes of 0·9 per cent sodium chloride solution to their animals and this has the advantage of delaying the restitution of the extracellular fluid volume and the excretion of the administered water load. They tested urea, saline diuretics, xanthine derivatives and mersalyl and calculated the relative efficiency of drugs by plotting their dose–effect curves against that of urea. Difficulties were met in the evaluation of mersalyl, its diuretic effects being obtained over a narrow range of doses, for its toxic effects were pronounced. This is a common experience in tests of mercurial diuretics in rats. Some investigators[59,60], have in fact, failed to observe any diuretic effects. However, Lipschitz's results are remarkable in that the sequence of potencies in the rat was the same as that established in man. Herken, Senft and Wilutzky[61] used a similar method but refined it by infusing isotonic sodium chloride solution by vein or by mouth for 24 hours, and by assessing the effect of the diuretic from changes in the sodium excretion as well as the water balance. Recently, dogs (either conscious or anaesthetized) have been much used[61–64] for investigations of the mechanism of action of diuretics (including

that of the organic mercurials to which dogs appear to respond more reliably than rats).

Attempts have also been made to evaluate diuretics in animals with experimental oedema. Dogs can be made oedematous by giving them mineralocorticoids and sodium chloride, potassium chloride and water. Beyer[65] gave 6 mg of the very potent salt-retaining steroid, 9α-fluorohydrocortisone, together with 2 g sodium chloride, 150 mg potassium chloride and 1 l. of water daily. During this regime, the animals gained weight to such a degree that pitting oedema was demonstrable. However, conditions resembling the clinical syndromes are more difficult to produce experimentally. Ascites can be produced in dogs[66,67], and an experimental condition resembling protein deficiency oedema (kwashiorkor) in infants has been described[68] in weanling rats. Aminophylline, acetazolamide and aminometradine increase the urine flow in such animals although only cortisone raises the impaired water diuresis to the level of healthy controls; mersalyl is ineffective.

The difficulties inherent in animal experimentation have led to the proposal to assay diuretics clinically in patients with oedema. Such assay methods have been used by several groups of workers, all using patients with cardiac oedema for the main test, and cardiac patients without visible oedema and normal subjects for control studies. The essence of the technique used by Greiner and his co-workers[69,70] is as follows: Ambulant patients are selected. The patient is weighed, receives a dose of the diuretic, and is asked to return in 24 hours. The weight loss in the 24-hour interval is regarded as the measure of diuretic efficacy. The procedure is repeated weekly, each patient receiving, in random order, a dose of the preparation which serves as standard of comparison or a dose of the drug to be tested. Other treatment is so adjusted that the patient presents approximately the same amount of oedema at the time each dose is given throughout one assay period. Dose–response curves are constructed from the results and the potency of the new diuretic calculated in relation to that of the standard.

Two new organic mercurials were investigated in this manner, meralluride (Mercuhydrine) serving as the standard drug. The assay was based on tests on 37 patients each of whom received three doses of the three drugs. *Figure 4.3* shows the relative potency of the three drugs in terms of the adjusted mean responses. The three compounds were qualitatively similar but differed in potency. Men were more responsive than women to an increase in dosage but the potencies of drugs B and C relative to A were the same in both sexes. The validity of the relative potencies of the mercurials under test was determined by an analysis of variance. A somewhat simpler design, in which two-point dose–response curves were used, has been employed[69] for the comparison of orally-active pyrimidinedione compounds with intramuscular meralluride.

Ford and Moyer and their colleagues[71–74] employ a similar procedure and assess the diuretic potency of drugs from their effect on the sodium excretion. *Table 4.1* lists the relative potencies obtained for members of various groups of diuretics obtained by this method. Richterich, Spring and Thönen[75] used a combination of the procedures of Greiner and Ford.

To sum up, it may be said that animal experimentation has proved useful

for the screening of diuretics and for the study of their mode of action, but it has been rarely used for bio-assays. However, animal assays can be profitably applied, provided the species is carefully selected (it is inadvisable, for instance, to try to evaluate mercurial diuretics in rats), and the tests are restricted to comparisons of a standard drug with its chemical congeners, *i.e.* limited to the same group of drugs. At present most of the quantitative evaluations of diuretics are performed in man, usually on cardiac patients

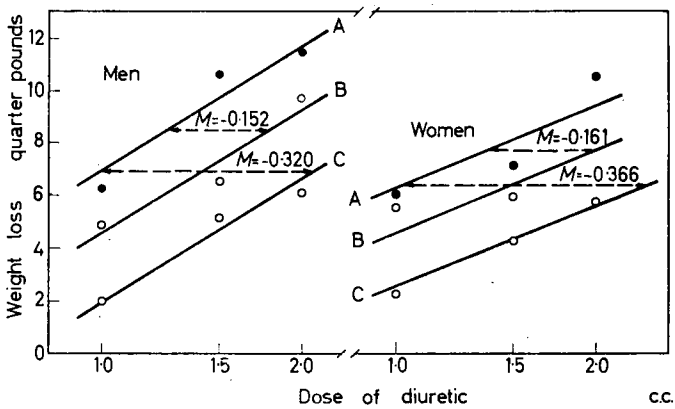


Figure 4.3. Evaluation of two new organic mercurial diuretics (B and C) in patients with cardiac oedema. Meralluride A was used as standard for dose–response relationship. The mean potency of B was 70 per cent of that of A and that of C 46 per cent of A[69].

with oedema. This is cumbersome in that special clinical facilities are usually necessary, but it permits the immediate therapeutic application of a potent new drug to the type of oedema studied in the assays.

Table 4.1. Potency of diuretics relative to meralluride in man as evaluated by the method of Ford, Moyer and Spurr[71]

Drug	*Route of administration*	*Potency estimation*
Hydrochlorothiazide	Oral	1·4
Meralluride	i.m.*	1·0
Chlorothiazide	Oral	0·8
Hydroflumethiazide	Oral	0·7
Mercaptomerin	i.m.*	0·5
Chlormerodrin	Oral	0·5
Acetazolamide	Oral	0·25

* i.m. = intramuscular.

(Modified from Ford[73])

CLASSIFICATION OF DIURETIC DRUGS

The classification of any group of drugs must, by necessity, be arbitrary. The scheme adopted here has taken into account the fact that individual diuretics may produce their effect in several ways. Moreover, diuretic drugs

may act differently in the healthy and in the diseased subject. Lastly, the mechanism of action of certain diuretics is still controversial.

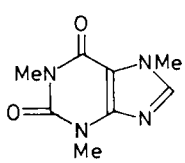
Diuretics fall into one of three main classes. Firstly, there are those which reduce sodium ion reabsorption by direct tubular action, *e.g.* xanthines, aminouracils, triazines, mercury compounds, carbonic anhydrase inhibitors, thiadiazines, benzenesulphonamides, sulphamoylbenzophenone derivatives, aldosterone antagonists and potassium salts. Secondly, there are compounds which affect tubular sodium ion reabsorption indirectly, such as osmotic diuretics and inhibitors of aldosterone secretion. Thirdly, there are drugs which increase the filtered load of sodium chloride, as for example acidifying salts, glucocorticoids, corticotrophin, and plasma expanders.

Xanthine, Aminouracil and Triazine Derivatives

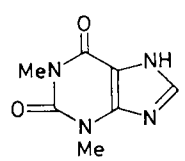
With the recent development of many new orally-active diuretics, the importance of the naturally-occurring xanthine derivatives, caffeine, theobromine and theophylline, has declined. Their diuretic action is relatively weak compared with mercurials or chlorothiazide and no new diuretics derived chemically from xanthines have been introduced. However, some useful diuretic drugs have been produced which contain a pyrimidine ring (*i.e.* one of the ring systems in the purine molecule) and some with the related *sym*-triazine ring.

Xanthines

Drugs in this group stimulate the brain, the heart and skeletal muscle, as well as exerting their diuretic action. The number and position of the methyl groups in the xanthine structure determines the dominant pharmacological properties of each substance. Thus, with caffeine, 1,3, 7-trimethylxanthine (*I*), central nervous system stimulation predominates; with



(I) Caffeine



(II) Theophylline

theophylline, 1,3-dimethylxanthine (*II*), cardiac and renal actions are more marked; and theobromine, 3,7-dimethylxanthine, has greater effects on skeletal muscle than the other xanthines. To improve their water solubility, the drugs are conjugated with other compounds as in caffeine sodium benzoate, theobromine sodium salicylate and theophylline ethylene diamine (aminophylline); this enhances their effectiveness by promoting absorption when given parenterally. The free bases are well absorbed from the gastrointestinal tract and only a small fraction of a dose of a xanthine is excreted unchanged in the urine, the bulk of the drug being metabolized rapidly.

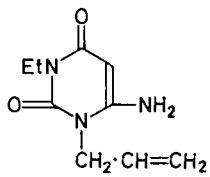
The analysis of the diuretic action of xanthines has not been given much attention recently. They produce their effect in two ways: (*a*) by circulatory

adjustment, both renal and extrarenal, and (*b*) by depressing renal tubular reabsorption. By their direct action on the heart, xanthines increase cardiac output, stroke volume and heart-rate and lower the filling pressure of the right heart. The resultant increase in renal blood-flow probably contributes to the diuresis although caffeine still increases urine output if the blood-flow is maintained constant in the perfused kidney. In frogs, caffeine increases the number of glomeruli in which the capillaries are patent[76], and this observation led to the suggestion that xanthines dilate the afferent arterioles to a greater degree than efferent arterioles, and so raise filtration pressure. The glomerular filtration rate may be increased by as much as 15 per cent, but diuresis may be produced with no evidence of an increase in tubular load[77], and an action on tubular reabsorption is probably involved. When xanthines are administered during water diuresis, the concentrations of sodium and chloride ion in the urine rise, strongly suggesting an effect on the tubular transport of these ions. This is supported by clearance studies with theophylline, although only small changes are observed in the clearance of sodium and chloride ion with caffeine and theobromine. There is no evidence that transport systems for other ions are affected by xanthines. Potassium excretion increases but this is probably non-specific as a similar increase occurs in other conditions where there is a rise in solute excretion.

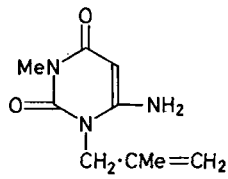
No systematic studies have been reported on the effects of xanthines on the composition of plasma or the effect of altered acid–base balance on the efficacy of these diuretics. Tolerance to the diuretic effects of xanthines develops with repeated administration and may be acquired by habitual tea or coffee drinking.

Aminouracils

Aminouracil derivatives are intermediates in the synthesis of xanthines. Papesch and Schroeder[78] showed that some of them are diuretics at least as potent as xanthines and better tolerated by man[79]. The 1,3-disubstituted derivatives of 6-aminouracil are diuretics while the monosubstituted compounds are not[80]. The 1-n-propyl-3-ethyl derivative, which is the most potent diuretic in this series, is unsuitable for clinical use owing to gastrointestinal effects. The compounds used clinically are the 1-allyl-3-ethyl, aminometradine, Mictine (*III*) and the 1-methylallyl-3-methyl, amisometradine, Rollicton (*IV*) derivatives.



(III) Aminometradine



(IV) Amisometradine

The diuretic action of aminouracils is attributable to inhibition of tubular reabsorption of sodium and chloride ions which are excreted in increased amounts although the glomerular filtration rate does not alter[81]. Potassium excretion also rises but to a smaller extent, and there is no change in

bicarbonate, phosphate or ammonia excretion. The actions of aminometradine and amisometradine are probably identical, the increase in urine volume being secondary to the augmented salt excretion. There have been no reports of studies on the location of the action in the nephron, on the changes in acid–base balance on the diuretic effect, or on enzyme inhibition by aminouracil derivatives.

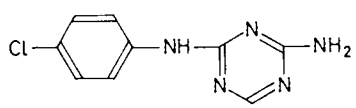
After daily administration of these drugs for five consecutive days, they lose their effectiveness. This is probably due to the decrease in plasma sodium, and Spencer and Lloyd-Thomas[82] have found that in sodium-depleted patients aminometradine is ineffective.

Aminouracils are adequately absorbed when given by mouth. The daily dose of aminometradine used by Spencer and Lloyd-Thomas[82] and by Kattus and others[81] was 1·2 g but Platts and Hanley[83] obtained adequate diuretic responses with doses of 400 mg/day. In all these studies, the incidence of side-effects such as nausea, vomiting and diarrhoea was about 20 per cent, even when the lower doses were used. The gastro-intestinal symptoms are usually mild and do not require the withdrawal of the drug. On a weight for weight basis, amisometradine is only half as potent as aminometradine[72] but gastro-intestinal disturbances occur less frequently and higher doses may be tolerated[84,85].

The effectiveness of the aminouracils as diuretics is about 50 per cent of that of intramuscular administration of mercurials and about equivalent to mercurials given orally[72].

Triazines

The diuretic properties of 2,4-diamino-*sym*-triazine (formoguanamine) and its derivatives have been known for some time[86,87] but until recently the compounds available were too toxic for use in man. From studies of a series of aliphatic derivatives of 2,4-diamino-*sym*-triazine, Shapiro, Parrino, Greiger, Kobrin and Freedman[88] concluded that a hydrogen atom on the substituent-bearing nitrogen is essential for diuretic activity. Where the substituent is branched at the α-carbon atom, no diuretic activity was found. The substitution of an aryl group on an amino group enhances the activity[89].



(V) Chlorazanil

4-Amino-2-*p*-chlorophenylamino-*sym*-triazine, chlorazanil (*V*) is one of the most active compounds of this group and is well tolerated by man. Most of the reports of the clinical use of this drug are from Hungary or Germany.

Chlorazanil may act by inhibiting tubular reabsorption since the diuresis is not associated with changes in glomerular filtration rate[90]. The main change in urinary composition is increased excretion of sodium chloride in man and dogs; potassium excretion is also increased slightly but in rats it is reduced. The diuretic effect of chlorazanil in rats is greater when a sodium chloride load is given, but it is antidiuretic when a water load is given; also the effects of deoxycorticosterone and chlorazanil on sodium and

potassium excretion are mutually antagonistic, suggesting that the natriuretic and diuretic effects of the drug are due to inhibition of sodium reabsorption in the distal segment[91].

In man, chlorazanil is about equal to acetazolamide in potency and tolerance to its diuretic effect does not develop[72]. Other triazine derivatives are toxic to the kidneys and it is recommended that chlorazanil should not be used in patients with renal disease.

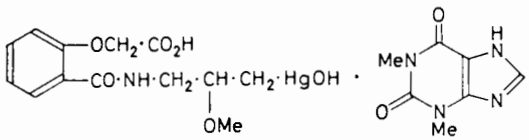
Mercurial Diuretic Drugs

Since the first account of their diuretic effect[10], organic mercury compounds have held the pre-eminent position among drugs in this field. Their diuretic action is powerful and dependable; they have been used extensively and successfully in the treatment of oedema, particularly when this is due to severe congestive heart failure; their limitations and hazards are well understood and documented.

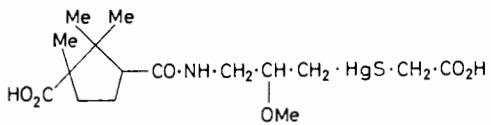
In terms of the amount of mercury in an effective dose, most organic mercurials are less potent than ionizable mercury salts. However, compared with inorganic compounds, organic mercurial drugs are usually less toxic, do not precipitate protein and so do not produce local irritation where injected. For these reasons, organic mercurial diuretics may produce a diuresis of greater magnitude and longer duration than that given by mercury salts.

All the mercurial diuretics in use today are basically derivatives of mercuripropanol: $R{\cdot}CH_2{\cdot}\underset{\displaystyle OY}{\underset{|}{CH}}{\cdot}CH_2{\cdot}HgX$. Kessler, Lozano and Pitts[92] suggest that for a high order of diuretic potency there must be: (*a*) a chain of at least three carbon atoms, (*b*) a mercury atom at one end of the chain, and (*c*) a hydrophilic group not less than three carbon atoms from the mercury. The Y substituent is determined by the solvent in which the mercuration is carried out, being —OH— where the solvent is water, and a methoxy or ethoxy group where the solvent is the corresponding alcohol; secondary amines also may be used as solvents in the mercuration reaction giving compounds with a substituted amino group in the Y position on the central atom of the propyl chain[93]. Within these limits, the potency and the toxicity of the compound are independent of the nature of the Y substituent.

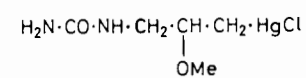
The nature of the R substituent has a marked effect on both toxicity and diuretic activity. In most of the drugs in clinical use, R contains a carboxyl group and is linked to the mercuripropyl chain through a carbamyl group[93]. The R may be derived from aromatic (mersalyl, *VI*), alicyclic (mercurophylline, *VII*; mercaptomerin, *VIII*) or heterocyclic (esidrone, *IX*) structures. These compounds are diuretics and, approximately, equal in potency, but where the R substituent is an amide of a lower fatty acid, the potency is reduced. Where R is part of a urea moiety, the compounds are diuretic, generally (but not invariably) decreasing in potency with the size of the groups attached to the urea nitrogen. Unsubstituted urea (chlormerodrin, *X*) methylurea and succinoylurea (meralluride, *XI*) derivatives are potent diuretics[64,94]. Where R is attached to the mercuripropyl chain by an



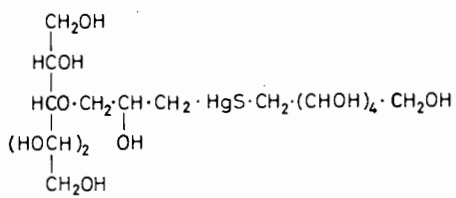
(VI) Mersalyl



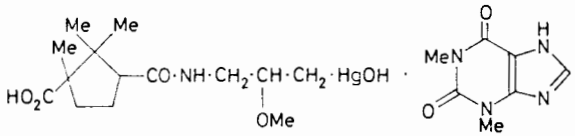
(VIII) Mercaptomerin



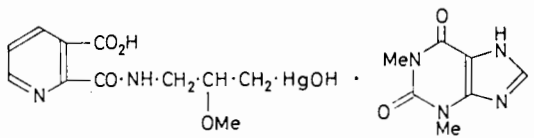
(X) Chlormerodrin



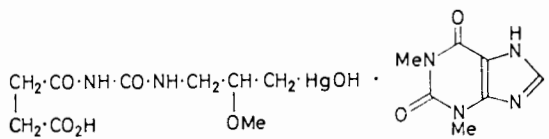
(XII) Diglucomethoxane



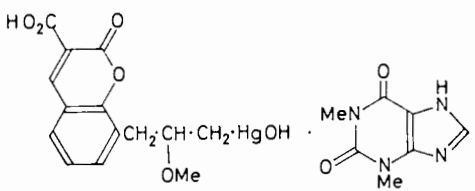
(VII) Mercurophylline



(IX) Esidrone



(XI) Meralluride



(XIII) Mercumatilin

ester or ether linkage, diuretic activity is weak except in the case of a mannitol derivative, diglucomethoxane (*XII*) which is one of the most potent mercurial diuretics in clinical use[95]. In the diuretic compound, mercumatilin (*XIII*) R is a coumarin structure linked by a carbon–carbon bond to the mercuripropyl chain, but where the heterocyclic ring contains nitrogen, the compounds are active only when the mercuripropyl chain is attached to a ring nitrogen[96].

The role of the X substituent, usually theophylline, halogen or a thiol derivative, is to reduce the toxicity of the mercurials and to increase their rates of absorption when given intramuscularly. After injection of mersalyl without theophylline, absorption of mercury from the injection site is not complete even after 24 hours, while when theophylline is present, absorption is nearly complete in 45 minutes[97]. Theophylline also diminishes the local irritant effects and pain of intramuscular injection, and increases the rate at which mercury appears in the urine, even after intravenous injection of an organic mercurial[98]. Farah and Maresh[99] showed that monothiols, cysteine and glutathione reduce the cardiac toxicity of mercurials without affecting the diuretic response, and following this, Lehman and King[100] placed mercaptoacetic acid as the X substituent as in mercaptomerin. This drug is absorbed as quickly as theophylline mercurials and, it is less toxic to the heart, is less irritant and may be given by subcutaneous injection[101].

Effect of mercurials on the composition of urine and extracellular fluid

For many years after the introduction of organic mercurial diuretics, there was some doubt as to whether their site of action was renal or extrarenal. It was argued that the primary effect was the change in plasma composition but it is now believed that this is secondary to the diuretic response. The unequivocal demonstration that the mercurials act upon the kidneys comes from the experiments of Govaerts[102] and Bartram[103]. Govaerts found that the diuresis continued when a kidney from a mercurialized dog was transplanted to the neck of an untreated dog. In Bartram's experiments, a small dose of a mercurial was injected into a renal artery and this provoked a diuresis only in the kidney on the injected side, while a large dose caused renal shut-down on the side injected and diuresis in the opposite kidney. These experiments clearly demonstrate three fundamental facts about mercurial diuretics: (*a*) that they act upon the kidneys, (*b*) that they are fixed in the kidneys and that some part of the fixed moiety is responsible for the diuretic effect, and (*c*) that in high dose they are toxic and cause renal damage. There is abundant evidence that organic mercurials in effective doses do not influence glomerular filtration rate or the renal haemodynamics so that the diuresis must be attributed to effects on the reabsorptive and secretory processes in the nephron (see *Figure 4.4*).

It has been established that the most prominent change in urinary excretion during mercurial-induced diuresis is an increased output of sodium ion and chloride ion. The extent of the increase in sodium excretion is not as great as that of chloride excretion. Effects on potassium ion excretion are variable; if plasma potassium is low, excretion of potassium may be increased; if it is high, potassium excretion may be reduced[104]. Excretion of ammonium ion, titratable acid and bicarbonate are not affected by mercurials. If

mercurials are given repeatedly for some time, unimpaired reabsorption of bicarbonate and loss of chloride in excess of sodium ions results in the depletion of extracellular chloride and its replacement by bicarbonate, *i.e.* a state of metabolic alkalosis.

The increase in sodium excretion occurs before the urine flow rate increases[105,106] and the magnitude of the mercurial-induced diuresis is determined by the amounts of osmotically active solute filtered at the glomeruli[107]. These observations suggest that the increase in urine volume is due to the osmotic pressure of solute retained in the tubular urine as tubular reabsorption mechanisms are inhibited. This situation is well exemplified by the experiments of Grossman, Gibbon, Weston, Bonn and Leiter[108] who found that when mersalyl was given to dogs which were secreting hypertonic urine, the greater the diuresis induced, the more closely did the osmotic pressure of the urine approach that of plasma.

While it is clear that the diuretic effect is due to impaired reabsorption of sodium chloride, there have been conflicting views as to whether the primary effect is on sodium or chloride ion reabsorption. Tubular reabsorption of sodium ion is the active process and, in its transfer from tubular urine to tubular cell, chloride ion passively follows the electro-chemical gradient created by the movement of sodium ion. The experimental observations leading to the conclusion that the primary action of mercurial diuretics is impairment of chloride ion reabsorption[109] are: (*a*) in mercurial diuresis the increase in the rate of excretion of chloride exceeds that of sodium ion; (*b*) during the alkalosis and depletion of extracellular chloride due to repeated administration of mercurials the diuretic action is impaired, and (*c*) under these conditions the diuretic effect may be restored by ammonium chloride. According to adherents of the view that mercurials primarily affect chloride ion reabsorption, the role of ammonium chloride is repletion of extracellular chloride. However, as Berliner[110] has pointed out, observations (*a*) and (*b*) may be explained by impaired sodium ion reabsorption in the proximal tubule and delivery of increased amounts of both sodium and chloride ions to the distal segment. If it is assumed that the exchange of sodium for potassium or hydrogen ion is inhibited to a lesser degree, this exchange process will reduce sodium but the chloride remains in the tubular urine. Thus chloride may be excreted in excess of sodium although the diuresis and the altered electrolyte excretion may be initiated by impaired sodium reabsorption in the distal tubule.

Site of action in the nephron

It was suggested by Duggan and Pitts[111] that mercurials act by inhibiting reabsorption of sodium chloride in the distal tubule rather than in the proximal tubule. While proximal reabsorption of sodium chloride accounted for 67–87 per cent of the amount filtered, the maximal increase in salt excretion due to mersalyl represented only 17–21 per cent of the filtered load. However, in later experiments, Farah, Cobbey and Mook[112] showed that in dogs given a sodium load, mersalyl decreased the sodium reabsorption by 35–40 per cent so that the effect is not attributable to inhibition of distal reabsorption only. Recent studies in dogs by Kessler, Hierholzer, Gurd and Pitts[113] using the 'stop flow' technique of Malvin, Wilde and Sullivan[114] have

established that the main site of action is proximal. Other functions attributed to the proximal tubule (such as glucose reabsorption and the secretion of weak organic acids) are also depressed by organic mercurials[115,116]. Pathological and biochemical observations are in agreement

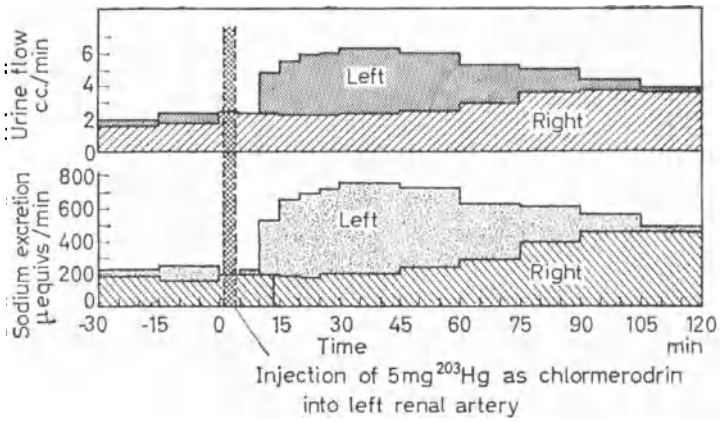


Figure 4.4. Unilateral diuresis in a dog produced by slow injection of chloromerodrin into the left renal artery[118]

with the functional studies. After minimal necrotizing doses of mercurials, only the proximal tubule shows evidence of pathological changes[117,118]; enzyme inhibition and depletion of protein-bound —SH groups may be detected only in proximal tubules.

Refractoriness to mercurials during alkalosis and potentiation during acidosis

It was shown as early as 1925 that ammonium chloride potentiates the action of organic mercurials[119]. This effect is a true synergism and cannot be attributed to summation of the diuretic effects of this salt and the mercurial[120]. Ammonium chloride also prevents the occurrence of refractoriness during repeated courses of treatment with mercurials and may restore sensitivity if a refractory state has developed[121]. There are two conflicting theories on the mode of action of ammonium chloride. The original hypothesis was that the organic mercurial decomposes in the body to liberate mercuric ion which is responsible for the diuretic action and that this process is pH-dependent[122]. This view was discredited by Axelrod and Pitts[109] who found that acidosis induced by ammonium chloride potentiated the effect of mersalyl, while that produced by inhalation of 12 per cent carbon dioxide did not. Their conclusion, which has been mentioned already (p. 150) was that ammonium chloride replenishes the depleted extracellular chloride and increases the chloride available for filtration. On the other hand: (*a*) organic mercurials are potentiated by ammonium nitrate[120]; (*b*) the diuretic effect is not reduced in the alkalosis produced in dogs by potassium depletion, which is characterized by low plasma chloride, extracellular alkalosis and intracellular acidosis[123]; (*c*) the action of inorganic mercury is affected only slightly by changes in acid-base balance[124,125]

(*Figure 4.5*); and (*d*) if a mercurial is given during the diuresis induced by acetazolamide, the effect is much reduced.

Inorganic versus organic mercury as the effector agent of diuresis

To explain the dependence of organic mercurial diuresis on acid–base balance, Mudge and Weiner[104] have revived the older hypothesis that mercurials act by liberation of mercuric ions and have supported it with additional evidence. They find that those organic mercurials which are diuretics are unstable at low pH. Also, mercuric cysteine is a diuretic, and its action is relatively independent of changes in acid–base balance.

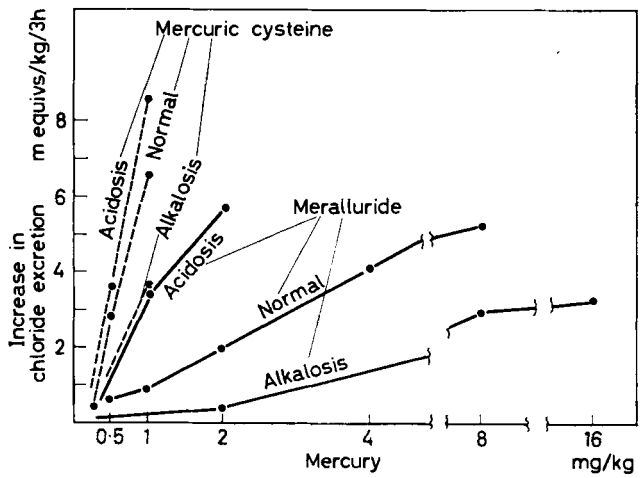


Figure 4.5. Dose–response relationship of an organic mercurial (meralluride) and an ionic mercury complex (mercuric cysteine) in normal, acidotic and alkalotic dogs[104]

However, the evidence that only a minute fraction of the mercury excreted in urine is in an inorganic form[126] does not support the view of Mudge and Weiner. In fact, Weiner and Muller[127] have found that the bulk of the mercury in urine following injection of mersalyl is a mersalyl–cysteine complex. The significance of this finding is difficult to assess because as much as 10–15 per cent of the mercury given as organic mercurial may be excreted before the diuresis starts[98].

Kessler, Lozano and Pitts[92] maintain that the steric configuration of the organic mercurials determines their diuretic property. They showed that the diuretic potency of chlormerodrin exceeds that of mercuric chloride per milligramme of mercury administered and therefore concluded that the intact organic mercurial molecule, and not mercuric ion is the diuretic agent. However, in a footnote, they acknowledge that the steric configuration for optimal diuretic activity may also determine the lability of the mercury–carbon bond.

A possible compromise between the views of Mudge and Weiner[104] and Kessler, Lozano and Pitts[92] which fits most of the experimental evidence is that when mercury is combined in a suitable organic structure its intracellular penetration is facilitated and that liberation of relatively small

amounts of mercuric ion in tubular cells, dependent on intracellular pH, impairs some part of the sodium reabsorption process.

Biochemical aspects of mercurial-induced diuresis

In common with many heavy metals, mercury (inorganic and organically combined) has a marked affinity for —SH groups and it is assumed that the mercurial diuretics act by forming inactive complexes with —SH enzymes. Mercurial diuretics inhibit adenosinetriphosphatase (ATPase) in kidney homogenates[128,129] but most of the enzyme work in this field has

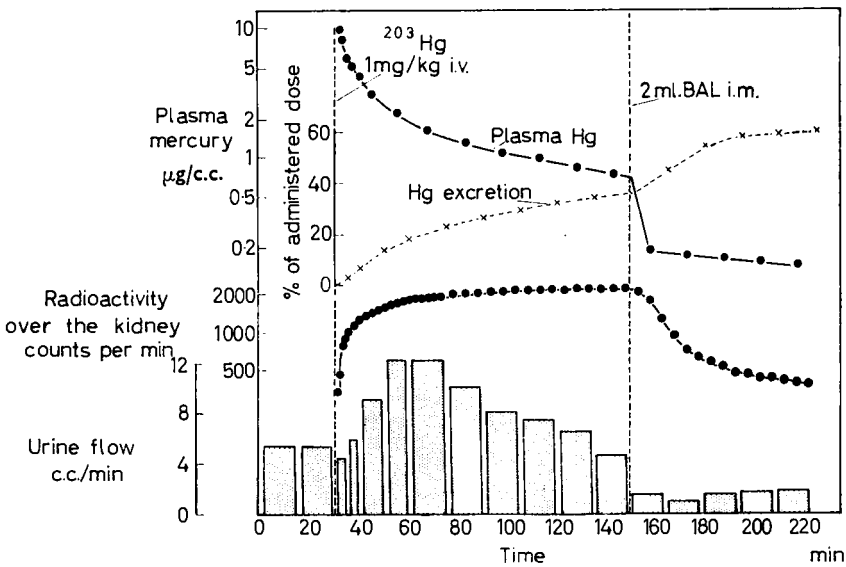


Figure 4.6. Disappearance from plasma and renal uptake of ^{203}Hg given intravenously as chlomerodrin, and the effect of dimercaprol (BAL) on the diuresis and urinary excretion of mercury[133]

been concerned with succinic dehydrogenase. Inhibition of this enzyme within the renal tubule has been demonstrated histochemically by Rennels and Ruskin[130] and Waschstein and Meisel[131], the inhibition being greater in the proximal tubule than in other parts of the nephron. Since no connection between succinic dehydrogenase and tubular reabsorption of sodium ion is known, the significance of these observations cannot be assessed. Moreover, some non-diuretic organic mercurials which are also concentrated in the kidneys are potent inhibitors of —SH enzymes *in vitro*[92]. Depletion of protein-bound —SH groups in the proximal tubule by organic and inorganic mercury has been reported by Farah, Cafruny and Di Stefano[132]. This effect does not occur in animals made alkalotic and given organic mercurial, but does occur when such animals receive inorganic mercury, so the change in the protein-bound —SH groups is related to the production of diuresis[125]. It would be interesting to know whether excretion of mersalyl as a complex with cysteine is also affected by changes in acid–base balance.

The dithiol, dimercaprol (BAL), prevents mercurial diuresis, or, if given during the course of the diuresis, instantly stops the response (see *Figure 4.6*).

Dimercaprol forms a stable chelate with mercury and organic mercurials and although its effect could be due to reactivation of inhibited —SH groups, such a conclusion does not necessarily follow. While dimercaprol antagonizes both the renal and cardiac effects of mercurials, monothiols such as cysteine and glutathione can protect only against the effect on the heart and have relatively little influence on the diuretic response. This difference between the effect of monothiols on the heart and kidney has been attributed to the high concentration of mercury in renal tubular cells, but it is difficult to see why this does not also restrict the action of dimercaprol.

Disposition of mercurials in the kidney

Most of the mercury of an organic mercurial diuretic injected intravenously in man may be recovered from urine within 24 hours. The fixation of mercurial by the kidney was indicated by the experiments of Govaerts[102] and substantiated more recently[133,134] in experiments with organic mercurials labelled with ^{203}Hg. Relatively small amounts of mercury are fixed in heart and skeletal muscle so that if the binding is by complex formation with —SH groups there must be some difference between the —SH groups in the heart and those in the kidney.

Most of the mercury bound in the kidneys is located in the renal cortex[135,136], where the concentration may be more than a hundred times than that in plasma. The binding precedes the onset of diuresis[133] and the increase in urinary excretion of mercury after treatment with dimercaprol is associated with the loss of bound mercury from the kidney. In homogenates of kidneys of dogs and rats treated with ^{203}Hg-labelled chlormerodrin, most of the mercury is found in the soluble fraction obtained after differential ultracentrifugation, although some mercury is bound to granules from which it cannot be removed by washing[136]. The proportion of mercurial extracted from renal arterial blood by the kidneys may exceed the filtration fraction[133]. Since the greater part of organic mercurial in plasma is protein bound[137] and therefore is unable to enter the tubules by filtration, those mercurial diuretics which are excreted rapidly must be secreted from the tubular cells into the tubular urine. Non-diuretic mercurials, although fixed in the kidneys, are not excreted rapidly[92] and in rats, in which mercurial diuretics are not very effective, the excretion of mercurial diuretics is much slower than in dogs[125]. These observations suggest a connection between the tubular secretion of a mercurial and its diuretic action.

Oral medication with organic mercurials

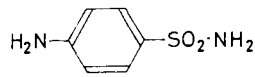
It is desirable that a drug which is to be used in chronic conditions should be suitable for self-administration. For many years, the best route of administration for the available organic mercurials was by intramuscular injection, most of them being poorly absorbed and causing nausea, vomiting, abdominal pain and diarrhoea when given orally. Chlormerodrin is better tolerated than other mercurials when given by mouth and has been used successfully to maintain an oedema-free state in patients with congestive heart failure, after the initial 'drying out' by intramuscular administration of the drug. Many patients complain of minor gastro-intestinal disturbances but they are rarely of sufficient severity to merit discontinuance of the drug[138].

Carbonic Anhydrase Inhibitors

Carbonic anhydrase is a metallo-protein enzyme containing zinc which accelerates the attainment of equilibrium in the reversible reaction:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

Mann and Keilin[139] found that the action of carbonic anhydrase was inhibited specifically by sulphanilamide (*XIV*) and that with substitution

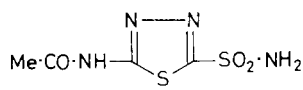


(XIV) Sulphanilamide

on the sulphonamido nitrogen (as in the commonly used bacteriostatic sulphonamides) the carbonic anhydrase inhibiting potency was lost. Shortly afterwards, Davenport and Wilhelmi[140] demonstrated the presence of high concentrations of carbonic anhydrase in the kidney. Sulphanilamide may produce metabolic acidosis, initiated by a rise in urinary pH and increased bicarbonate excretion, and the significance of this fact emerged in 1945 when Pitts and Alexander[141] postulated that acidification of the urine was due to secretion of hydrogen ions by tubular cells. They confirmed that the excretion of acid was reduced by sulphanilamide treatment. This they attributed to inhibition of carbonic anhydrase and consequent impairment of the supply of hydrogen ions for secretion.

The increase in bicarbonate excretion brought about by sulphanilamide involves also an increase in the volume of urine excreted and Schwartz[142] drew attention to the potentialities of sulphanilamide as a diuretic, effective by the oral route.

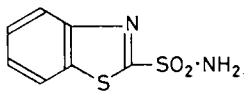
Later investigations showed that in some compounds where the sulphonamido group is attached to a heterocyclic ring the carbonic anhydrase inhibiting potencies are 300 to 800 times that of sulphanilamide[143]. The best-known carbonic anhydrase inhibitor, acetazolamide, 2-acetylamino-1,3,4-thiadiazole-5-sulphonamide (*XV*) is a compound in this series. It



(XV) Acetazolamide

was suggested that there is a correlation between increasing acidity of the heterocyclic group and carbonic anhydrase inhibiting potency, but in many cases steric effects and other important factors may overshadow the relationship between pK_a and biological activity[143]. In another series of thiadiazole sulphonamides, Young, Wood, Eichler and Vaughan[144] failed to find a correlation between pK_a and *in vitro* activity, and some compounds with considerable effect on the enzyme *in vitro* do not have corresponding diuretic potencies. In the case of the benzothiazole derivative (*XVI*), this is due to rapid metabolic degradation; the 6-ethoxy derivative (*XVII*) of this compound is more resistant to metabolic degradation but gives rise to side-effects. Blocking one of the two acidic centres in acetazolamide by

methylation (*XVIII, XIX*), enhances *in vitro* activity[144]. These compounds



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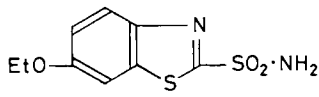
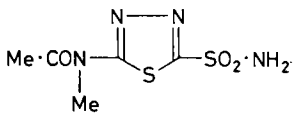
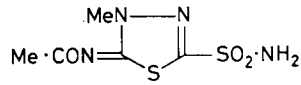


(XVII) Ethoxzolamide



(XVIII)



(XIX)

penetrate the eye and the brain better than acetazolamide and therefore may be useful as anticonvulsants and in treating glaucoma.

Mode of diuretic action of carbonic anhydrase inhibitors

Acetazolamide and its congeners are highly specific inhibitors of carbonic anhydrase and this action seems to be the sole basis for their diuretic effect. The processes in the kidney in which carbonic anhydrase participates have been described on p. 135 and involve the exchange of hydrogen ion from the tubular cell with sodium ion of the tubular urine. Inhibition of carbonic anhydrase will (*a*) depress the reabsorption of bicarbonate in the proximal tubule, (*b*) prevent acidification of the urine, (*c*) promote potassium excretion by virtue of the impaired supply of hydrogen ion for the coupled exchange of hydrogen and potassium for sodium, and (*d*) impede, by making the urine alkaline, the diffusion of ammonium ion from the tubular cell to the tubular urine.

The most important of these effects for diuretic action is the reduced bicarbonate reabsorption in the proximal tubule, and the increased bicarbonate excretion is accompanied by increased excretion of both sodium and potassium.

The response to acetazolamide is rapid in onset and of relatively short duration[145]. After administration of 250 mg (the usual therapeutic dose), bicarbonate excretion reaches a peak about 2 hours after ingestion and is restored to normal within 16 hours[146]. Thus, if the drug is given once per day there will be a period of only 8 hours during which the electrolyte imbalance produced by the drug may be adjusted from dietary sources. With continued administration of acetazolamide, urinary loss of bicarbonate and impairment of the renal mechanism for the conservation of 'fixed base' result in the development of metabolic acidosis. Under acidotic conditions[147], the diuretic effect of carbonic anhydrase-inhibition is much reduced or completely absent so that the effect of the drug is self-limiting. Since the diuresis is determined by increased bicarbonate excretion, one of the factors responsible for the development of refractoriness is the loss of bicarbonate from the body and the fall in the bicarbonate concentration in plasma[146] (*Figure 4.7*). However, this may not be the only or even the most important factor because (*a*) the diuretic effect of acetazolamide is unimpaired in respiratory alkalosis with reduced plasma bicarbonate[147], (*b*) refractoriness

to the diuretic effect occurs also in respiratory acidosis, when the bicarbonate concentration in plasma may be normal or even high[148], and (*c*) in metabolic acidosis carbonic anhydrase inhibitors have little effect on urinary pH[149]. It is possible that in acidosis the supply of hydrogen ion for secretion by the renal tubules is adequate at the uncatalyzed rate of hydration of carbonic acid and so the effect of carbonic anhydrase inhibition is reduced.

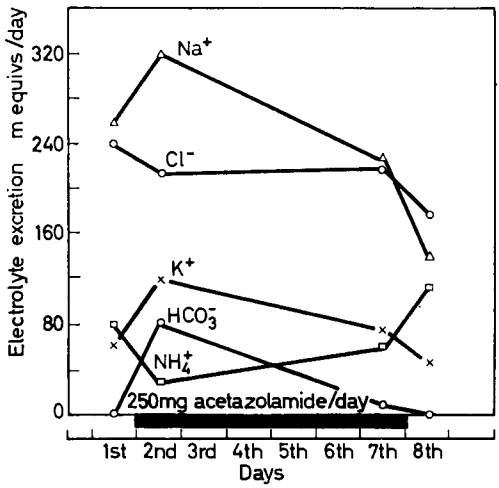
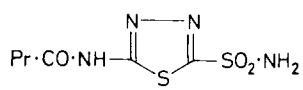


Figure 4.7. Effect of acetazolamide on urinary electrolyte excretion in man (redrawn from data given by Couniham, Evans and Milne[148])

Clinical use of carbonic anhydrase inhibitors

The extent to which the volume of extracellular fluid may be reduced by loss of bicarbonate is limited as bicarbonate and its accompanying cation represent only about 20 per cent of the extracellular electrolytes. Thus the efficacy of acetazolamide in patients with severe congestive heart failure is considerably less than that of mercurials[150]. Due to the self-limiting action,



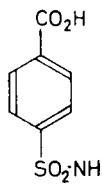
(XX) Butamide

the diuretic effect may not be sustained so that the use of carbonic anhydrase inhibitors is restricted to the treatment of mild oedema. They are particularly useful when the occurrence of the oedema is intermittent, as in premenstrual oedema. Acetazolamide has been used *between* doses of mercurials in the treatment of severe congestive heart failure[151] but the effect of a mercurial is suppressed if it is given during acetazolamide diuresis[152] presumably because of the alkalinity of the urine (see p. 151). Carbonic anhydrase inhibitors are not useful in the treatment of oedema resulting from the administration of adrenocortical steroids since both diuretic and steroid increase potassium excretion.

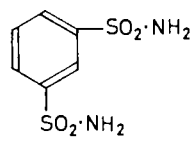
The three carbonic anhydrase inhibitors which have been used clinically (acetazolamide (*XV*) ethoxzolamide (*XVII*) and butamide (*XX*)) are approximately equal in potency[153]. Untoward reactions are not serious (nausea, lethargy, tingling of the face and extremities and muscular weakness) but occur less frequently with acetazolamide than with the other two drugs.

Benzothiadiazines, Benzenesulphonamide and Sulphamoylbenzophenone Derivatives

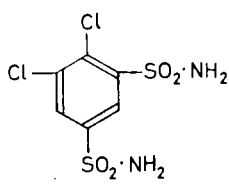
The discovery of chlorothiazide (like that of acetazolamide) stems from the observation that sulphonamides inhibit the activity of carbonic anhydrase. Although this action was first demonstrated in aromatic sulphonamides, the diuretic potency of most members of this group has proved to be too low to be of therapeutic value. The use of *p*-sulphamoylbenzoic acid, Dirnate



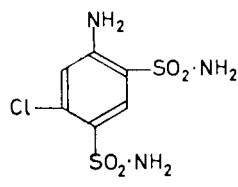
(XXI) *p*-Sulphamoylbenzoic acid



(XXII) Benzene-1,3-disulphonamide



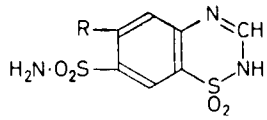
(XXIII)



(XXIV)

(*XXI*) for instance, has been abandoned. High diuretic potency was, however, found by Sprague, Beyer and their associates[154–157] in the benzenedisulphonamide series, particularly in derivatives of benzene-1,3-disulphonamide (*XXII*). The introduction of halogen or amino (or acylamino) groups (*XXIII, XXIV*) enhanced activity. Some of these compounds exhibited a degree of oral diuretic potency encountered previously only among the heterocyclic sulphonamides. Activity increases further with acylation of the amino group (*XXV*), the degree of enhancement increasing with the length of the aliphatic acyl group. In substance (*XXV*) ring closure occurs readily yielding the 1,2,4-benzothiadiazine-1,1-dioxides (*XXVI*). *Table 4.2* shows the action on electrolyte excretion in the dog of compounds of the type (*XXVII*) in which the 6-position is variously substituted. Introduction of an alkyl group or the phenyl group into position 3 of chlorothiazide depresses activity (*Table 4.3*) and so does a carbonyl oxygen at the 3-position (*XXVIII*). However, the dihydro derivative of chlorothiazide, hydrochlorothiazide (*XXIX*) is about 20 times as active as the parent compound[157a]. Substitution

Table 4.2. Effect on electrolyte excretion of substitutions in the 6-position of 7-sulphamoyl-1,2,4-benzothiadiazine-1,1-dioxide[1546]

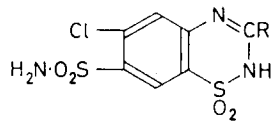


(XXVII)

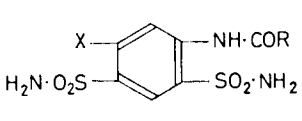
Derivative number	R	*Comparative effect upon electrolyte excretion (dog)* i.v. Na+	i.v. K+	i.v. Cl−	Oral Na+	Oral K+	Oral Cl−
1	H	+	+	+			
2*	Cl	++++	+	++++	++++	+	++++
3	Br	++++	+	++++	++++	+	++++
4	CH_3	+++	+	+++	+	±	+
5	OCH_3	++	+	++	++	±	++
6	NO_2	+++	+	+++	+++	±	+++
7	NH_2	+	+	+			
Chlormerodrin					+++	±	+++

* Chlorothiazide

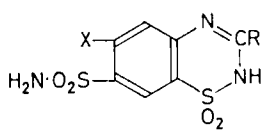
Table 4.3. Effect on electrolyte excretion of substitutions in the 3-position of chlorothiazide[1546]



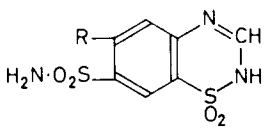
Derivative number	R	*Comparative effect upon electrolyte excretion (dog)* i.v. Na+	i.v. K+	i.v. Cl−	Oral Na+	Oral K+	Oral Cl−
1	H	++++	+	++++	++++	+	++++
2	CH_3	+++	+	+++	+++	±	+++
3	n-C_3H_7	+++	+	+++	+++	±	+++
4	n-C_5H_{11}	+++	+	+++	+++	±	+++
5	C_6H_5	+	+	+	±	±	±



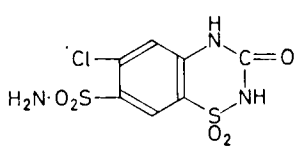
(XXV)



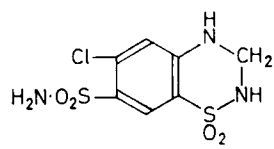
(XXVI)



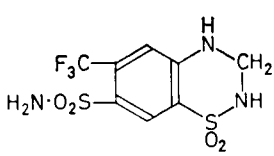
(XXVII)



(XXVIII)



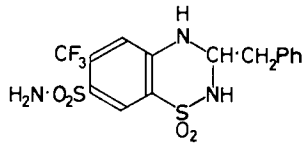
(XXIX) Hydrochlorothiazide



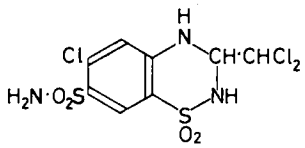
(XXX) Hydroflumethiazide

of the chlorine atom in hydrochlorothiazide by a trifluoromethyl group, (hydroflumethiazide, Hydrenox, Naclex, Rontyl, *XXX*) produces a compound which, in animal experiments[158] has also been shown to be 15–20 times as active as chlorothiazide. Its high activity has also been demonstrated in patients[158a].

More recent work has shown that substitution in the 3-position leads to highly active diuretic drugs[158b]. Thus, if the 3-substituent of hydroflumethiazide is an aralkyl, aryloxyalkyl, chloromethyl or bromomethyl group, the resulting compound may have activity equal to or greater than hydroflumethiazide[158c]. From this series, 3-benzyl-3,4-dihydro-7-sulphamoyl-6-trifluoromethyl-1,2,4-benzothiadiazine-1,1-dioxide (bendrofluazide, Aprinox, Centyl, Naturetin, Neo-Naclex, *XXXa*) was chosen for clinical trial[158d]. 3-Substituted derivatives of hydrochlorothiazide have also been described[158e],

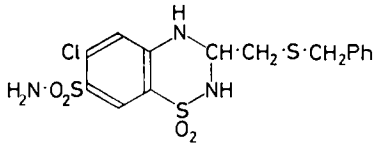


(XXX a)

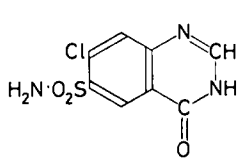


(XXX b)

some of which are very active, for example the 3-dichloromethyl[158f] (trichloromethiazide, Naqua, *XXXb*) and the 3-benzylthiomethyl[158g] (benzthiazide, Fovane, Urese, *XXXc*) derivatives.

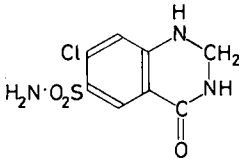


(XXX c)

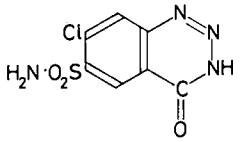


(XXX d)

Two interesting variations of this ring structure have been shown to retain the diuretic action of chlorothiazide. When the $—SO_2$ of this compound is replaced by a carbonyl group, a quinazolinone (*XXXd*) is obtained and this may be reduced to the corresponding tetrahydroquinazolinone (*XXXe*). The



(XXX e)

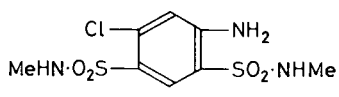


(XXX f)

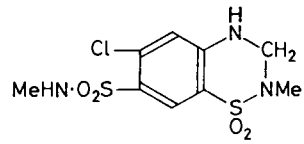
diuretic activities of these two compounds after oral administration to rats and dogs are reported to be about the same as those of chlorothiazide and hydrochlorothiazide respectively[158i]. In the second variation the heterocyclic ring has three nitrogen atoms forming a 1,2,3-triazin-4-one ring (*XXXf*).

This compound and some of its 3-substituted derivatives are reported to possess potent diuretic activity in animals[158].

Compounds in which the nitrogen atom of the sulphamoyl group is substituted have been reported not to be active[159], but diuretic activity has been found in some benzene-1,3-di(*N*-alkylsulphonamides)[159a], and 4-amino-6-chlorobenzene-1,3-di(*N*-methylsulphonamide) (*XXXI*) is a potent diuretic[160], thus showing that a free sulphonamide group—on which the inhibitory action on carbonic anhydrase depends—is not necessary for diuretic activity. Ring closure of compound (*XXXI*) produces 6-chloro-7-methylsulphamoyl-3,4-dihydro-2-methyl-1,2,4-benzothiadiazine-1,1-dioxide (*XXXII*). The activity



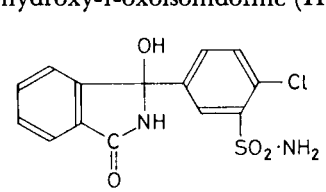
(*XXXI*)



(*XXXII*)

of this substance was much greater than that of compound (*XXXI*) and of a similar magnitude to that of hydrochlorothiazide[161] but its diuretic effect was more prolonged and its toxicity in rats was about half that of the latter compound.

Closely related to the thiadiazines is a new diuretic, 6-amino-4-chlorobenzene-1,3-disulphonamide (Salamid, *XXIV*) which according to Lund and Størling[162] is about three times as active as chlorothiazide but less potent than hydrochlorothiazide. It is rapidly absorbed and mostly excreted in the urine unchanged. Recently[163] a group of benzophenones which have a chlorine atom and a sulphamoyl group ortho to one another as in chlorothiazide have been studied for their diuretic activity. The most active member of this group so far investigated is the tautomeric 3-(4-chloro-3-sulphamoylphenyl)-3-hydroxy-1-oxoisoindoline (Hygroton, *XXXIII*). Two



(*XXXIII*)

related compounds, 4′-chloro-3′,4-disulphamoylbenzophenone and 4′-chloro-3′-sulphamoylbenzophenone-2-carboxylic acid were found to be more potent carbonic anhydrase inhibitors than (*XXXIII*) but they had a lower diuretic potency. Moreover, their diuretic effect in dogs ceased in 3½ hours while the action of the same dose of Hygroton lasted for more than 13 hours. The pronounced increase in urine flow was accompanied by increases in sodium and chloride excretion[163] but the excretion of potassium was only slightly raised. Renal blood-flow and glomerular filtration rate were not affected. The diuretic effect was not influenced by experimentally-induced acidosis or alkalosis. High doses of Hygroton given daily over a

period of several weeks did not alter the blood sodium, urea or uric acid concentration significantly although the serum potassium concentration slightly decreased. The mean lethal dose in several species of laboratory animal was more than 5 g/kg. No toxic effects were seen in rats when oral doses of 1·25 or 2·5 g/kg were given daily for 4 weeks. Given to dogs by mouth, the sodium salt of Hygroton was slowly but well absorbed. Ninety per cent of an intravenous dose was excreted unchanged in the urine within 24 hours[164]. Veyrat, Arnold and Duckert[165] studied Hygroton in over a hundred patients with cardiac and cirrhotic oedema and found that a single dose of 100–200 mg raised urine flow output and sodium and chloride excretion for 24–48 hours (*Figure 4.8*). Forty milliequivalents of potassium citrate were given daily to counteract the renal potassium loss.

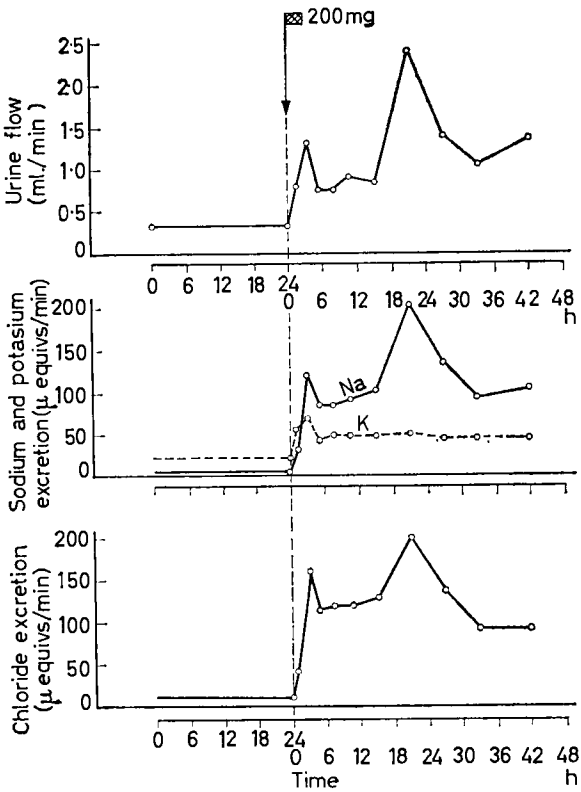
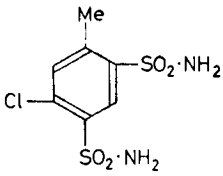


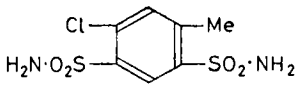
Figure 4.8. Effect of a single oral dose of Hygroton on urine flow and electrolyte excretion in a patient with cardiac oedema. Note long duration of action[165].

6-Chloro-4-methylbenzene-1,3-disulphonamide (disulphamide, Disamide, *XXXIV*) has been reported to be a potent diuretic[163a]. The dose recommended is 100-200 mg, which suggests that its potency is intermediate between chlorothiazide and hydrochlorothiazide. 5-Chloro-3-methylbenzene-2,6-disulphonamide (*XXXIVa*) was found[163b] to be one-tenth as active as hydrochlorothiazide when tested in dogs.

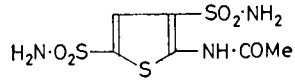
In order to correlate the aromatic and heterocyclic aspect of the diuretic activity of disulphonamides, a number of thiophen derivatives were tested by de Stevens, Halamandaris, Ricca and Werner[163b]. 5-Acetamido-2,4-thiophen-disulphonamide (*XXXIVb*) a compound closely related to acetazol-



(XXXIV)



(XXXIVa)



(XXXIVb)

amide (*XV*) was only slightly diuretic in rats but substitution of the acetamido group by a chlorine atom gave a compound which was as potent as chlorothiazide.

Mechanism of action of halogenated thiadiazines

Chlorothiazide—The first experiments with chlorothiazide on dogs[65] showed that its diuretic action is due to an action on the kidney and in particular—since glomerular filtration rate remained unaffected—on the renal tubules. *Figure 4.9* shows the effect of single doses of chlorothiazide on the urinary electrolyte excretion in a dog. After an intravenous dose of

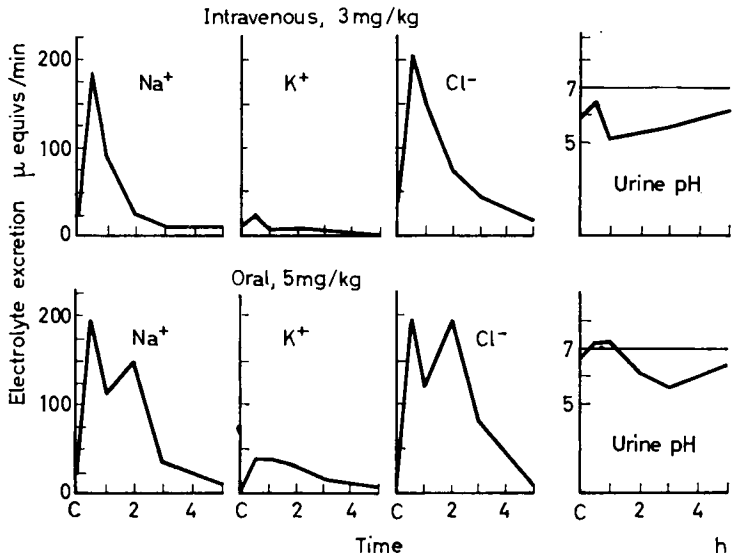


Figure 4.9. Excretion of electrolytes and changes in urinary pH after the administration of single doses of chlorothiazide orally or intravenously to a dog[65]

3 mg/kg or an oral dose of 5 mg/kg, urinary sodium and chloride excretion increased but there was a sufficiently greater chloruresis to depress urinary pH. (In other experiments the pH of the urine was often increased due to rise in bicarbonate excretion.) There was also some increase in potassium

excretion. Similar results were obtained in man (*Figure 4.10*). The urinary changes observed are reminiscent of those produced by potent carbonic

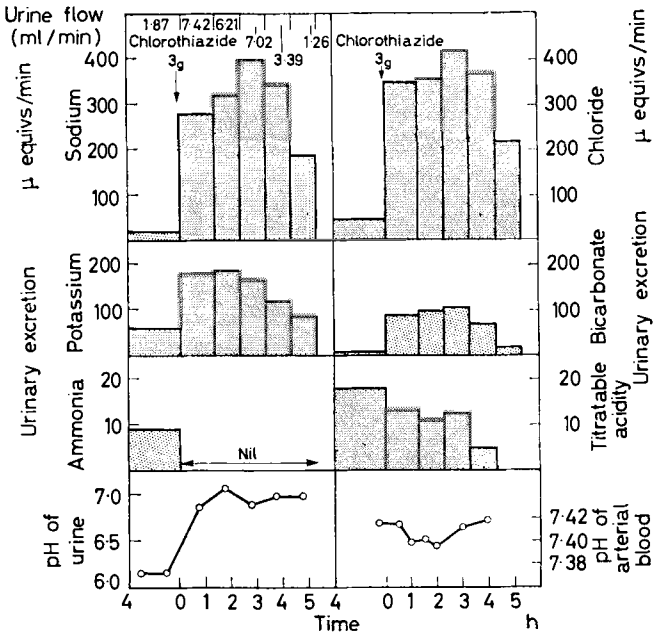


Figure 4.10. Patient with rheumatic heart disease and benign essential hypertension. Effects of a single oral dose of 3 g chlorothiazide on the pH of arterial blood, and on volume and composition of the urine[176].

anhydrase inhibitors such as acetazolamide. However, the carbonic anhydrase inhibitory action of chlorothiazide (and hydrochlorothiazide) is much weaker than that of acetazolamide (*Figure 4.11*) and bears no quantitative

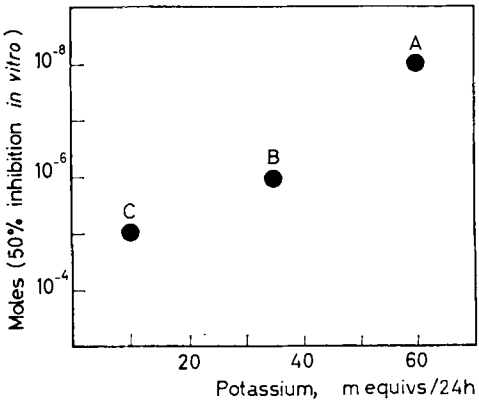


Figure 4.11. Carbonic anhydrase inhibition *in vitro* and increase in potassium excretion *in vivo* produced by acetazolamide A, chlorothiazide B and hydrochlorothiazide C[75].

relationship to the enhancement of electrolyte excretion. Moreover, the excretory pattern differs. Chlorothiazide may or may not increase bicarbonate excretion, and acetazolamide—while invariably raising sodium and

bicarbonate elimination—does not induce a notable chloruresis[166]. Also, in ammonium chloride acidosis, acetazolamide has been reported to be ineffective while chlorothiazide produced an increase in sodium excretion and a still greater enhancement in that of chloride.

The action of chlorothiazide can also be distinguished from that of organic mercurials. Chlorothiazide is not inhibited by dimercaprol and it is markedly active under the experimental condition of a bicarbonate alkalosis[65] when mercurial diuretics are ineffective[62,100]. These differences suggest that the site of tubular action is not the same, an assumption which

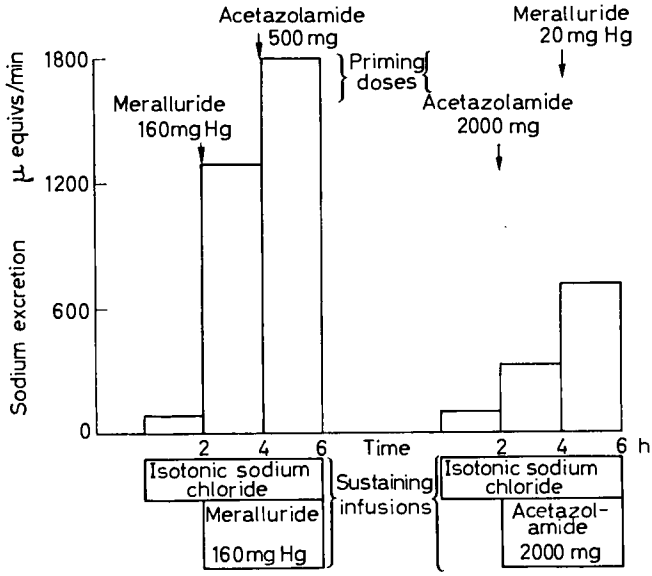


Figure 4.12. The effect of combinations of meralluride and acetazolamide on sodium excretion in a patient with chronic congestive heart failure. Even with a maximum response from either meralluride or acetazolamide, the administration of the other drug produced an additional increase in sodium output[169].

is substantiated by recent investigations of Pitts[12] in dogs and Ford and Rochelle[167] in man. Cardiac patients who had been on a standard diet received an intravenous infusion of 0·85 per cent sodium chloride solution until the urinary sodium excretion became stable. An injection of meralluride then raised the rate of sodium excretion from about 100 μ equivs./min to 1,360 ± 170 μ equivs./min which was stable at the end of 2 hours. At this point chlorothiazide produced a further increase to 1,732 ± 158 μ equivs./min (*Figure 4.12*). Similar results were obtained when the order of administration was reversed. The effects of combining chlorothiazide and acetazolamide were also studied and it was found that chlorothiazide decreased the bicarbonate response produced by acetazolamide but increased sodium and chloride excretion further (*Figure 4.13*).

Hydrochlorothiazide—Like chlorothiazide, hydrochlorothiazide produces a sodium and chloride diuresis but in much smaller doses. It is less inhibitory

to carbonic anhydrase (see *Figure 4.11*) and thus does not promote bicarbonate excretion. The loss of potassium after the administration of equiactive doses of the two drugs is similar[74,168–170].

Hydroflumethiazide—Tested in water-loaded rats[158], 0·625–10 mg hydroflumethiazide/kg given by mouth raised the excretion of sodium and chloride; potassium excretion was less affected. The diuretic action was at its maximum within 6 hours after the administration of the drug. Doses of chlorothiazide which were 15–20 times larger had to be given to the same animals to obtain similar increases in sodium and chloride excretion.

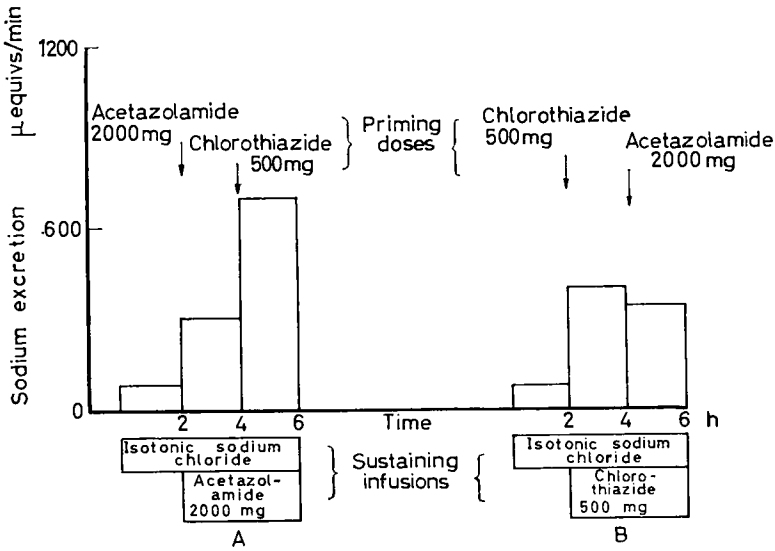


Figure 4.13. The effect of combinations of acetazolamide and chlorothiazide on sodium excretion illustrating a non-competitive A and a competitive B mechanism of action. Increased sodium excretion produced by chlorothiazide could not be inhibited by acetazolamide but chlorothiazide inhibited the response to acetazolamide[169].

Clinical reports on hydroflumethiazide are as yet scanty. The appropriate dosage (200 mg per patient per day) seems to be approximately 5 times smaller than that of chlorothiazide. The pattern of electrolyte excretion which it produces in man may be similar to that with chlorothiazide and hydrochlorothiazide[171,172]. Unlike chlorothiazide, however, it has been reported[173] to cause no increase in urinary pH or bicarbonate excretion (*Figure 4.14*). Like both its congeners it is liable to increase potassium loss (*Figure 4.15*).

Other pharmacological effects of chlorothiazide

Effects on the cardiovascular system have been reported[174] but only when large doses of chlorothiazide were used. The drug (*a*) slightly decreased the blood-pressure of anaesthetized dogs and diminished the responses to adrenaline, noradrenaline and hypertensin; (*b*) had a weak depressant effect on vascular smooth muscle but no atropine-like or ganglion-blocking properties, and (*c*) had a slight negative chronotropic effect on isolated hearts in high

doses. The electrocardiogram of normal animals was not affected. However, when cardiac arrhythmias were produced in dogs by prednisolone, digitalis or antihistamines, chlorothiazide tended to restore normal heart action[175].

Absorption, distribution and fate of halogenated thiadiazines

Although not very soluble in water, the halogenated thiadiazines are rapidly absorbed from the intestine and may produce their diuretic action within 30 minutes of oral administration. The volume of distribution approaches that of the extracellular fluid space, although when chlorothiazide was injected into nephrectomized dogs[65] none of the compound

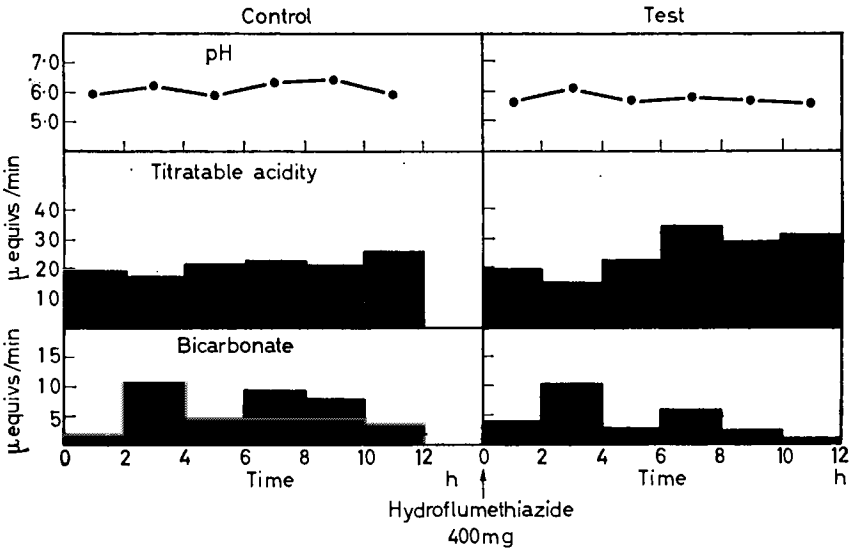


Figure 4.14. Comparison of mean urinary pH, titratable acidity and bicarbonate excretion in five oedematous patients on a control day and after administration of a single oral dose of 400 mg of hydroflumethiazide[173]

was found in the cerebrospinal fluid and little or none in the brain or aqueous humour.

Chlorothiazide is excreted in the urine and bile. The kidney eliminates it not only by glomerular filtration but also by tubular secretion.

Toxicity and side-effects of halogenated thiadiazines

Acute toxicity tests with the halogenated thiadiazines are difficult as their solubilities are relatively low. However, such estimations of the LD_{50} which have been made (chlorothiazide: mice (i.v.) = 8·5 g/kg, mice (p.o.) = 1·12 g/kg; hydrochlorothiazide: rats (p.o.) = >2·75 g/kg) showed them to be drugs of low toxicity. Long-term administration of large doses to dogs revealed no untoward effects beyond a depression of the serum potassium level.

Chlorothiazide and its congeners are well tolerated on oral administration to patients. Gastro-intestinal disturbances are rare[169,176]. No sensitivity

reactions have been reported. The danger of hypokalaemia may be the most important side-effect. Hydrochlorothiazide and hydroflumethiazide produce less potassium loss than chlorothiazide in patients with oedema of recent origin, but patients with chronic oedema on a low sodium diet may—unless supplied with potassium beyond the dietary intake—develop potassium depletion[168,169,171,177]. If so, cardiac patients may become increasingly more sensitive to digitalis and may in fact suffer from 'induced' digitalis intoxication.

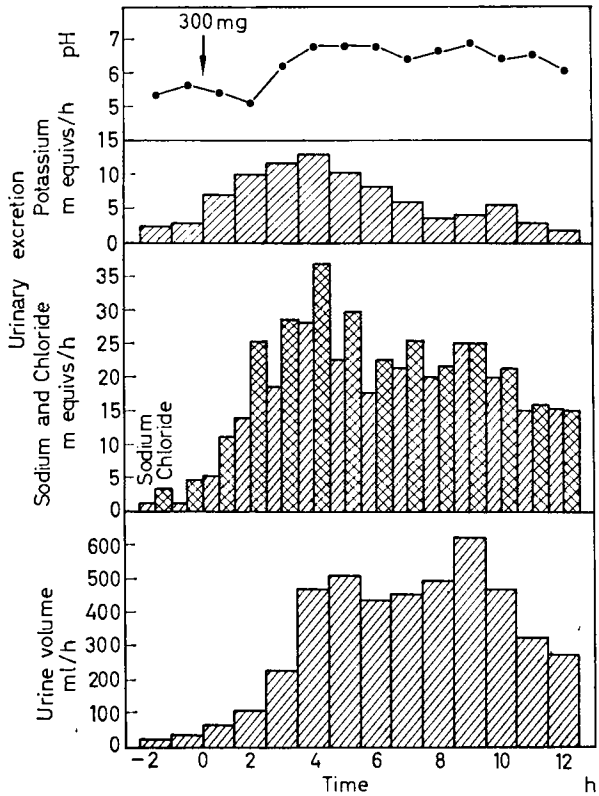


Figure 4.15. Effect of 300 mg of hydroflumethiazide on urine flow, electrolyte excretion and pH in a normal subject[171]

Clinical importance of the halogenated thiadiazines

The introduction of chlorothiazide and its congeners is undoubtedly the greatest advance in diuretic therapy since the discovery of the organic mercurials, as they are well tolerated and potent when given by mouth. They are effective in metabolic acidosis and alkalosis (when mercurial diuretics are essentially ineffective). They often act in patients who are resistant to mercurials. Refractoriness on prolonged use does not develop rapidly[178] and no adverse effects on damaged kidneys need to be expected. They are not only useful in cardiac oedema but also in other oedematous conditions. Schreiner[179] points out that in renal disease the efficacy of

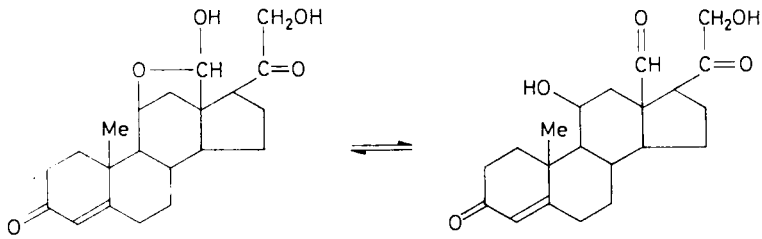
chlorothiazide depends on the absence of low glomerular filtration rates. In hepatic cirrhosis with ascites and oedema the diuretic response may not invariably be good or may not be maintained. Such patients appear particularly prone to severe losses of potassium.

Lastly, it was reported in 1957[180–182] that chlorothiazide potentiates the action of hypotensive drugs and this has been widely confirmed in hypertensive patients. The mechanism of this effect is not yet clear. The fact that chlorothiazide enhances the hypotensive action of ganglion-blocking drugs as well as of other hypotensive substances (reserpine, hydrallazine, *Veratrum* preparations) does not suggest a potentiating effect but rather that the antihypertensive action of chlorothiazide is connected with its diuretic effect. This is also suggested by the observation[183] that chlorothiazide may lower the blood-pressure of hypertensive patients (but not that of normotensives) and that organic mercurials also do so in the same patients, in doses sufficient to cause electrolyte and water losses similar to those produced by chlorothiazide.

Aldosterone Antagonists

In many oedematous states, there is increased tubular reabsorption of sodium which may be attributed to excessive secretion of aldosterone, the sodium-retaining hormone of the adrenal cortex.

The effects of excess aldosterone in the body can be attacked pharmacologically in two ways: (*a*) by blocking aldosterone action on the renal tubular cells, and (*b*) by inhibition of steroid synthesis in the adrenal gland. Drugs with the latter action are discussed in another section.

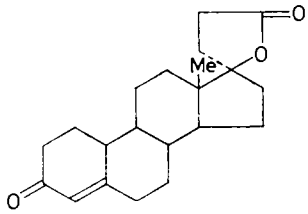


(XXXV) Aldosterone

Some steroid lactones have been shown by Kagawa, Cella and von Arman[184] to antagonize aldosterone (*XXXV*), and a spirolactone (SC-8109, *XXXVI*) has been employed in man. The effects of aldosterone on the kidney are blocked by SC-8109, so that sodium and chloride excretion is increased and excretion of potassium, hydrogen and ammonium ion is decreased. This is a unique effect upon the pattern of electrolyte excretion, since most other diuretics increase potassium excretion to a greater or lesser degree (*Figure 4.16*). The effect of a spirolactone is determined by the extent to which sodium reabsorption is controlled by aldosterone. For example, it is not effective in patients with untreated Addison's disease[185], nor in normal subjects receiving a low sodium diet[186].

When rats maintained on a low sodium diet are treated with SC-8109, the unrestrained loss of sodium ion through the kidney provokes a compensatory

increase in aldosterone secretion[187]. Likewise, in patients treated with this lactone, the sodium diuresis is accompanied by increased aldosterone excretion in the urine[188]. There are reports of the successful clinical use of the lactone in primary aldosteronism[189], in the nephrotic syndrome[190] and in hepatic cirrhosis[191]. Slater, Moxham, Hurter and Nabarro[192] obtained a satisfactory diuresis with SC-8109 in patients with nephrotic oedema and hepatic cirrhosis but patients with cardiac failure showed little response.



(XXXVI) SC-8109

Nabarro[193] suggests the use of a spirolactone in combination with chlorothiazide, thereby preventing excessive loss of potassium. More recently, spironolactone (SC-9420, Aldactone) which is the 7α-acetoxythio-10-methyl derivative of SC-8109 has undergone clinical trial[193a].

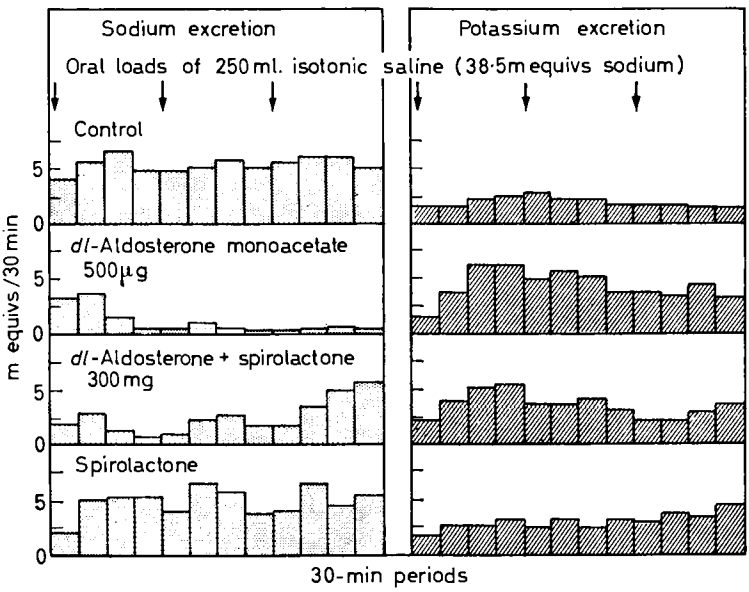


Figure 4.16. Effect of *dl*-aldosterone and spirolactone, alone and in combination, on the urinary excretion of sodium and potassium in an adrenalectomized dog[193].

Potassium Salts

Potassium salts, while acting as osmotic diuretics, produce their diuretic effect also by a more specific mechanism. Since potassium competes with hydrogen in the exchange of sodium in the distal renal tubules, the administration of potassium chloride is followed by the excretion of an alkaline urine

and a diuresis of sodium (*Figure 4.17*). However, Spencer[194] believes that the amount of sodium excreted is greater than can be produced by this mechanism alone and suggests that the administration of large quantities of potassium chloride may displace some of the excess intracellular sodium in oedematous patients.

It is possible that a vascular effect may have to be added to this tubular diuretic action of potassium salts. Quite recently, Scott, Emanuel and Haddy[195] have shown that potassium chloride infused directly into a renal artery in anaesthetized dogs will alter renal vascular resistance. The

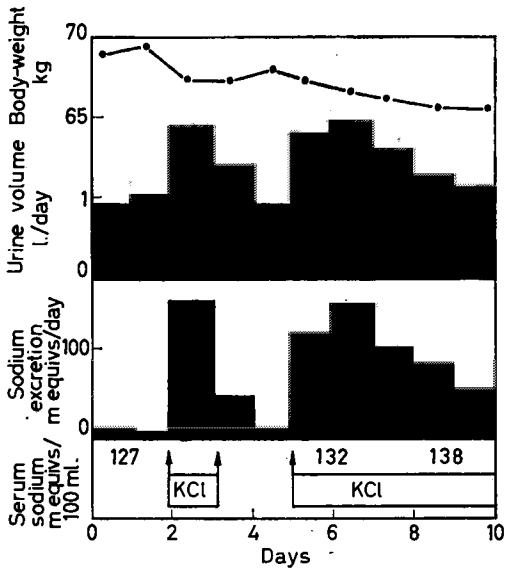


Figure 4.17. Effect of potassium chloride (15 g/day by mouth) on urine volume and sodium excretion in an oedematous patient[194]

resistance decreased in both intact and in denervated kidneys and the urine flow increased. It may be that a similar effect contributes to the diuretic action of potassium in the human kidney.

Osmotic Diuretic Drugs

Osmotic diuresis has been defined as the increased urine flow evoked by the intravenous administration of hypertonic solutions of compounds such as sodium chloride, sodium sulphate, urea, sucrose and mannitol, in conditions in which the urine is already loaded with osmotically-active constituents[196]. It also occurs when large amounts of such substances accumulate in the body (as, for instance, glucose in patients with diabetes mellitus) or when they reach the blood from the gastro-intestinal canal. With the progressive development of diuresis, the osmotic pressure of the urine decreases and approaches that of the plasma. The filtered 'osmotic' substance reduces water reabsorption in the proximal part of the tubule and retards the reabsorption of sodium which now adds its osmotic force to that of the

primary agent in opposing the reabsorption of water. Evidence has recently been presented[197] that the resulting high rate of flow then interferes with the distal mechanism which serves to concentrate the urine above the isosmotic level. Hence, the reabsorption of water from the distal system is also reduced and this, in turn, further increases the urine volume. The antidiuretic hormone, whose secretion is usually increased by a rise in plasma osmotic pressure, is unable to increase the concentration of the hypotonic or isotonic urine. Long ago[198] it was shown that it does not act during an osmotic diuresis, and according to present concepts[13,199,200], its role consists in increasing the permeability of the tubular epithelium. However, an

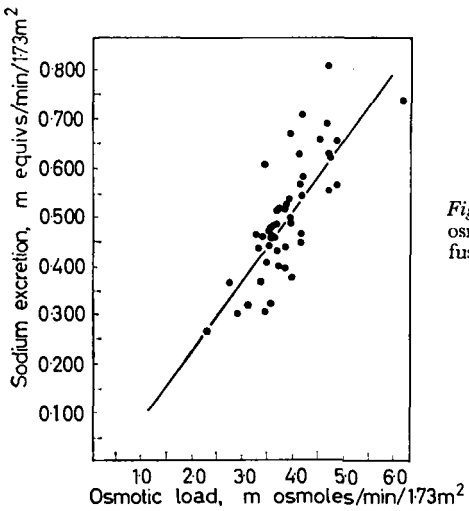


Figure 4.18. Relationship between osmotic load (mannitol solution infused i.v.) and urinary sodium excretion in healthy men[201].

increase in permeability does not matter if the absence of a concentration gradient fails to move water through the epithelial membrane.

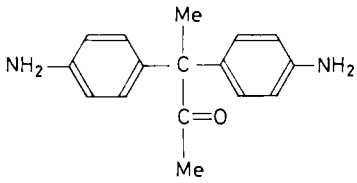
An increase in the excreted osmotic load, even when due to mannitol or urea, is thus associated with an increased excretion of sodium and chloride[201] (*Figure 4.18*). The oedematous patient treated with an osmotic diuretic therefore benefits from the water loss during the course of the enforced diuresis and the decrease in the body sodium store retards the re-accumulation of excess extracellular fluid. The excretion of potassium is largely independent of the excreted load in osmotic diuresis, *i.e.* potassium excretion may rise, fall or change irregularly[202].

In practice, osmotic diuresis induced by the oral or parenteral administration of such substances as urea or mannitol, is now rarely used in the therapy of oedematous states. Treatment with the more recently introduced oral diuretics is usually more effective.

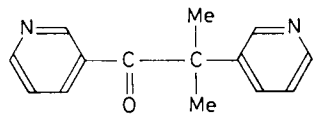
Organic mercurials and chlorothiazide ultimately act in a similar manner as osmotic diuretics by impairing the transfer mechanism for sodium ion and thus increasing the concentration of this ion (and chloride ion) in the tubular fluid. The same applies to the 'acidifying diuretics' (ammonium chloride, calcium chloride).

Inhibitors of Aldosterone Secretion

In 1949 Nelson and Woodward[203] showed that the insecticide DDD (2,2-di(*p*-chlorophenyl)-1,1-dichloroethane) caused adrenal atrophy in the dog. The effect on the adrenal cortex of the related compounds amphenone B (*XXXVII*)[204,205] and SU 4885 (*XXXVIII*)[206,207] has also been studied recently.



(XXXVII) Amphenone B



(XXXVIII)

Amphenone B interferes with a number of enzyme systems in the adrenal gland and thus causes generalized inhibition of steroid synthesis[208]. The output from the adrenal of both glucocorticoids and aldosterone are

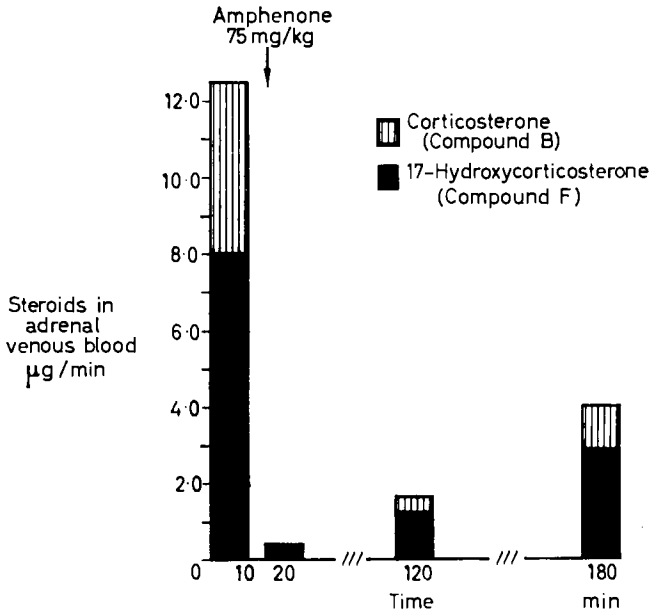


Figure 4.19. Effect of amphenone on the secretion of corticosteroids in a dog[207]

decreased[209,210] (*Figure 4.19*) and urinary excretion of sodium is increased[211]. Toxic effects of amphenone B, (drowsiness, gastric disturbances, methaemoglobinamia and possibly liver injury)[205] do not allow its general clinical use.

SU 4885 is less toxic[212] and its effect on the adrenal cortex is more selective than that of amphenone B[207,213]. Secretion of the major adrenal steroids, cortisol, corticosterone and aldosterone is reduced and the 11-deoxy derivatives of the cortical hormones are secreted at rates far beyond the normal

range. One of these, deoxycorticosterone has aldosterone-like actions and the effect of SU 4885 on sodium and potassium ion excretion depends upon whether reduced secretion of aldosterone or increased secretion of deoxycorticosterone predominates. A more consistent response to SU 4885 (increased sodium excretion and retention of potassium ion) is obtained by giving prednisolone to suppress the secretion of corticotrophin which controls the production of deoxycorticosterone[214].

Acidifying Salts

Acidifying salts which have been used clinically and experimentally as diuretics include ammonium chloride and nitrate, and calcium chloride and nitrate. The acidifying properties of ammonium salts are due to conversion of ammonium ion into urea, thus freeing the anion. Calcium salts are acidifying only when given orally, because their effect depends on the non-absorption of calcium ion which is precipitated as phosphate or carbonate in the gastro-intestinal tract, while the anion is readily absorbed.

The diuretic action of acidifying salts is greater than can be attributed to their osmotic effect or to their metabolites. For example, the combined osmotic effect of the urea and chloride ion from ammonium chloride is not sufficient to account for the diuresis produced, and, with continued administration of an acidifying substance, the diuretic effect ceases when the acidosis subsides. The changes in the electrolyte pattern of body fluids have therefore to be considered to explain the action of acidifying diuretics.

When an acidifying salt, for instance, ammonium chloride, is ingested, the immediate neutralization of excess anion is achieved by the conversion of bicarbonate to carbonic acid (and eventually carbon dioxide and water) freeing sodium ion to accompany the excess anion. The fall in plasma bicarbonate is equivalent to the increase in plasma chloride. Although there is no change in the total ion content, the proportion of chloride is increased in the extracellular fluid and, in particular, in the glomerular filtrate. The total amount of chloride ion reabsorbed in the tubules is increased but, as more chloride ion is filtered, an appreciably greater amount also escapes reabsorption, carrying with it its osmotic equivalent of water and, initially, equivalent amounts of sodium ion. The excess sodium ion is excreted in the urine, thus depleting the extracellular phase, and the mechanisms for the maintenance of acid–base balance and the electrolyte pattern of the extracellular fluid come into play.

Sodium loss is greatest during the first day of acidosis. During the second and third days, sodium loss is restricted by the removal of potassium from cells and calcium from bone, and these ions may replace the sodium in the urine. The renal excretion of ammonia and titrable acid rise, freeing more sodium for reabsorption and, after 5 or 6 days of administration of the acidifying agent the ammonium chloride excreted begins to equal the ammonium chloride intake, and sodium and water excretion are restored to their previous levels. The ammonia excreted is produced in the kidney while the ingested ammonia is converted to urea in the liver.

The diuretic effect of acidifying salts is manifest during the lag between the production of acidosis and the operation of the ultimate compensating

mechanism. When ammonia excretion produces complete compensation, the diuretic effect is lost so that acidifying diuretics should be used in cycles and discontinued when compensation is achieved. Acidifying agents potentiate the action of mercurial diuretics and this is perhaps their most important therapeutic application.

Glucocorticoids and Corticotrophin

Whenever a cortical hormone causes sodium retention, it also produces osmotic retention of extracellular water. However, the extent and duration of this effect is modified by many regulatory and pathological factors. The mineralocorticoids (deoxycortone, aldosterone)—so-called because their action on electrolyte excretion is much more pronounced than their general metabolic effect—may be expected to promote water retention most actively. However, in a normal subject, prolonged administration of deoxycortone does not produce continued sodium retention: after a week or 10 days, sodium output increases and the balance is restored, although the increased potassium output may continue[215,216]. In patients with adrenal deficiency, on the other hand, deoxycortone and aldosterone produce progressive sodium retention and oedema[217,218]. The glucocorticoids (cortisone, cortisol) may affect the excretion of sodium by raising glomerular filtration rate and by increasing its tubular reabsorption. If the former effect predominates, sodium loss increases; if the latter, sodium is retained. The sodium-retaining activity of cortisone has been estimated as being 1/40 or less of that of deoxycortone[219] but it may in practice be quite pronounced. There can, however, be no doubt that the glucocorticoids restore the normal response to water ingestion in patients with Addison's disease[220–222] and do likewise in oedema of protein deficiency[44]. They augment the diuretic rate in the healthy organism[223] and afford dramatic protection against water intoxication[224]. Corticotrophin causes initial sodium retention when renal and adrenal functions are normal but after a few days, sodium output increases and the patient comes into metabolic balance.

It is not clear how the glucocorticoids increase water output. The rise in glomerular filtration rate and renal blood-flow which they usually produce in healthy animals and men may be one important factor. A direct effect on tubular water reabsorption has been both suggested[225] and denied[226]. The possibility of an interaction between the posterior pituitary antidiuretic hormone (A.D.H.) and the glucocorticoids is controversial, mainly perhaps because of inadequate assays of A.D.H. on which much of the work on this problem is based. It is noteworthy, however, that there are some indications that the cortisol-like steroids (*a*) interfere with the release of A.D.H.[227], and (*b*) promote the disappearance of A.D.H. from the circulation[228].

The effect of cortisol on the water metabolism of patients with adrenal insufficiency has been analysed by Thorn, Laidlaw and Goldfein[229]. They showed that the over-all effect depends on the level of the circulating hormone. An intravenous infusion of cortisol at the rate of 10 mg/hour produced an initial sodium diuresis but raised glomerular filtration rate for the whole period of the infusion; tubular reabsorption of sodium took 8 hours to produce sodium retention. At the rate of 1 mg/hour the increase of

glomerular filtration rate was comparable but there was little increase in sodium reabsorption.

While restoration of normal water diuresis in Addison's disease by corticosteroids is part of a successful substitution therapy, patients suffering from nephrotic oedema are treated with corticosteroids for their 'pharmacological' effect in the presence of a normal anterior pituitary and adrenal. Corticotrophin, cortisone and its congeners produce a marked increase in

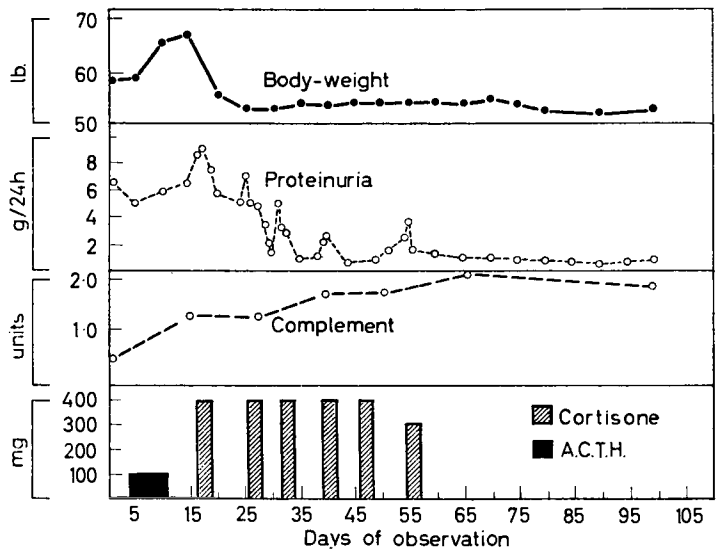


Figure 4.20. Child with nephrotic oedema. Effect of corticotrophin (A.C.T.H.) and cortisone on body-weight, protein excretion in the urine and serum complement[231].

urine output in many patients suffering from this condition. In addition, a decrease or even suppression of the renal protein loss often occurs[230]. The mechanism by which the corticosteroids augment urine flow in these patients appears to differ in some respects from that discussed in connection with healthy persons and adrenal deficiency. A rise in glomerular filtration rate may again be significant but the increase in plasma albumin which this form of therapy produces in nephrotic patients may be another important factor. Lange, Strang, Slobody and Wenk[231] have suggested that the nephrotic syndrome is the result of an antigen–antibody reaction in which large amounts of complement are used. They have shown that corticotrophin and cortisone depress the formation of certain antibodies in experimentally-produced nephrotic syndrome and that the diuresis in nephrotic oedema is preceded by a rise in complement (*Figure 4.20*). In this view, corticotrophin and the glucocorticoids act by interfering in some way with the antigen–antibody reaction.

Treatment of nephrotic oedema with cortisone or corticotrophin may have the following undesirable side-effects, mainly because the hormones have to be used in rather large doses: (*a*) sodium may be retained and the oedema may increase to an undesirable extent unless the sodium intake is kept very low; (*b*) too much potassium may be excreted in the urine (this

is usually prevented by giving the patient potassium chloride or citrate by mouth); (*c*) the protein and carbohydrate metabolism may be increased and thus the appetite stimulated with the result that the patient shows increased deposition of fat and develops a 'moon face'—the caloric intake

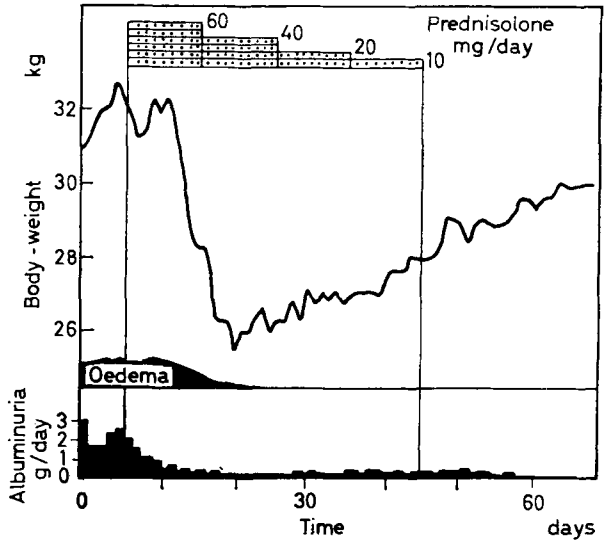


Figure 4.21. Effect of prednisolone on body-weight, albuminuria and oedema of a nephrotic patient. The precipitous fall in weight was due to increased urine output, the ensuing steady gain in weight followed the decreased loss of protein in the urine and was not due to the return of oedema[232].

has therefore to be kept at pretreatment level and (*d*) the patient will be more liable to infection and prophylactic treatment with antibiotics is indicated.

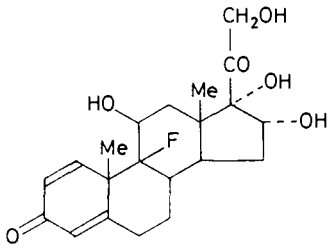
Synthetic analogues of cortisone and cortisol—Some of the synthetic analogues of the naturally-occurring glucocorticoids, particularly the Δ^1- compounds, prednisone (metacortandracin) and prednisolone (metacortandralone), are widely used in the treatment of nephrotic oedema (*Figure 4.21*).[232] The introduction of the double bond increases the glucocorticoid potencies of cortisone and cortisol about 4 to 5 times without increasing their mineralocorticoid activities. When, therefore, prednisone or prednisolone is used in the treatment of nephrotic oedema, sodium retention is not so marked and its restriction in the diet is not so important. Moreover, the control of the plasma potassium concentration is easier. Still more recently developed synthetic glucocorticoids, Triamcinolone (*XXXIX*) and dexamethasone, Decadron (*XL*) possess marked glucocorticoid properties, with little mineralocorticoid activity.

Plasma Expanders

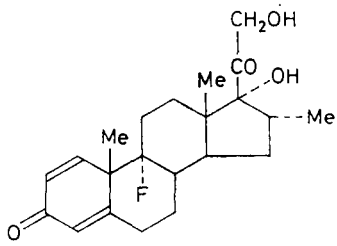
Plasma expanders are substances with a high molecular weight which act as substitutes for plasma proteins. They have been classified by Ravdin[233] as follows: (*a*) blood derivatives—albumin and other plasma protein fractions;

(*b*) modified proteins—heat-degraded gelatins and their chemical modifications (oxypolygelatin); (*c*) polymerized carbohydrates—acacia, pectin, dextran, and (*d*) plastics—methyl cellulose and polyvinylpyrrolidone.

Since a low plasma protein level is an important factor in the origin and maintenance of several forms of oedema, the introduction into the circulation of substances which increase plasma colloid osmotic pressure can be expected



(*XXXIX*)



(*XL*) Dexamethasone

to produce diuresis and increased loss of sodium. Several such substances have been evaluated in the treatment of hypoproteinaemic conditions such as liver cirrhosis and the nephrotic syndromes.

Human serum albumin—Properly-processed human albumin is not pyrogenic and probably does not cause sensitization[234]. Large amounts of concentrated albumin, however, have to be given to produce a rise of the plasma albumin concentration in patients with chronic hypoproteinaemia. In liver cirrhosis with ascites, albumin infusions do not invariably produce diuresis, but in patients with early ascites, albumin administration may be followed by a prompt and copious increase in urine flow and sodium excretion[235–239]. Albumin is given slowly to prevent sudden expansion of the blood-volume and pulmonary oedema. It may promote appetite and make the patient feel better, but lasting improvement cannot be expected although it has been claimed to occur in some patients with the nutritional type of cirrhosis. In nephrotic patients, Luetscher[240] found that a single injection of 25 g of concentrated serum albumin increased plasma volume by 25–30 per cent and colloid osmotic pressure by 10–20 per cent. There was a small increase in urine volume but no rise in chloride excretion. Due to the increased permeability of the glomerular capillaries in this condition, the injected albumin is largely excreted in the urine[234].

Dextran—Native dextran is produced by bacteria which polymerize glucose to a compound containing about 200,000 glucose units with a molecular weight of about 40 million. By partial hydrolysis and fractionation, the native dextran is reduced to a range of molecular sizes of the desired distribution. Treatment of nephrotic oedema with dextran has been tried[241,242]. Intravenous infusions for not less than 4 hours had to be given to produce a satisfactory loss of oedema fluid. The beneficial effect was only temporary in every instance but good responses were again obtained when the treatment was repeated. Intravenous dextran has also been used in the treatment of ascites but has proved of little value.

In summary, the 'plasma expanders' have not established themselves for routine treatment. In hepatic cirrhosis, the nephrotic syndromes, and in

kwashiorkor the cell protein stores are so low that large amounts of albumin are required to produce a sustained rise in the plasma protein level. In cirrhosis, much of the infused protein is lost into the ascitic fluid and in nephrosis much albumin passes through the damaged glomeruli. In all three conditions, treatment with the more recently introduced diuretic substances is more beneficial.

Inhibitors of Antidiuretic Hormone Secretion

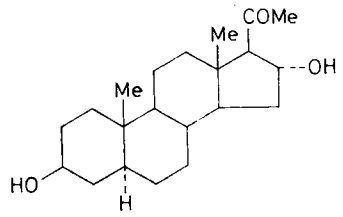
The diuresis which follows ingestion of water is due to the suppression of antidiuretic hormone secretion from the neurohypophysis, and this arises from the effect of the fall in blood osmotic pressure upon the osmoreceptors. The permeability of the collecting tubules to water is dependent on the presence of antidiuretic hormone so that in its absence the counter-current concentrating mechanism in the kidney is not effective. Water loss through the kidneys is increased until the secretion of antidiuretic hormone is resumed when the blood osmotic pressure rises to a level adequate for stimulation of the osmoreceptors. However, the excretion of electrolyte and other components of urine are not affected by water diuresis. In the healthy subject, there is little difference between the volume of extra water excreted and that ingested. Thus, from the point of view of the relief of oedema, water is not an effective diuretic.

The nature of the diuretic effect of ethanol is compatible with the view that it is due to suppression of antidiuretic hormone secretion when the concentration of alcohol in blood is increasing[243]. Stimulation of antidiuretic hormone release by acetylcholine and hypertonic sodium chloride is prevented by prior administration of ethanol[244], as also is the antidiuresis caused by positive pressure breathing[245] or circulatory disturbances[246]. The extra water excreted during alcohol diuresis is not accompanied by increased electrolyte excretion[247] and therefore ethanol is not useful, clinically, as a diuretic.

PRESENT STATUS AND PROSPECTS OF DIURETIC THERAPY

The outstanding development in diuretic therapy is the introduction during the last three years of drugs which are well tolerated when given by mouth and are at least as potent as organic mercury compounds given parenterally. These compounds, for example the thiadiazines, are less toxic than the mercurial diuretics in two respects: (*a*) their systemic toxicity is low, and (*b*) they may be used safely in renal disease when mercurial compounds would be hazardous. The new diuretics have the further advantage that the change in the composition of the urine produced, leads to a smaller disturbance of the electrolyte pattern of the extracellular fluid than with the older drugs. Nevertheless, some depletion of body potassium occurs and this is the only important drawback of these substances so far recognized. The most recently introduced members of this group (hydrochlorothiazide, hydroflumethiazide, Hygroton) are effective in such small doses that future development of drugs with the same type of action is not likely to be profitable.

Hitherto, diuretics have been used exclusively in the treatment of oedema but it has been realized recently that increased sodium and chloride excretion also benefits patients with hypertension. The new diuretics are the most suitable drugs for this purpose since—compared with the mercurial compounds—there is again less risk of further injury to diseased kidneys.



(XLI)

Since aldosterone plays an important role in many forms of oedema by increasing sodium absorption from the distal tubules, compounds which eliminate this effect are therapeutically desirable, particularly as chlorothiazide (and probably its congeners) act on the proximal tubules[248]. The spirolactones have been shown to antagonize the action of aldosterone on the kidney and to have the expected effect of decreasing sodium absorption without increasing potassium loss. Drugs of this type have a great future but quicker-acting compounds are required. Neher, Desaulles, Vischer, Wieland and Wettstein[249] have recently isolated from hog adrenal glands an adrenocortical steroid which promotes sodium excretion and this may be the prototype for new diuretics. This steroid, 3β,16α-dihydroxy-allopregnan-20-one (*XLI*) and a number of its derivatives, have been synthesized.

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5

ORAL HYPOGLYCAEMIC DRUGS

J. D. H. SLATER

INTRODUCTION

DIABETES mellitus is a chronic disease which derives its name from the fact that the urine is plentiful and sweet-tasting. About 1–2 per cent of the adult population of the United Kingdom suffer from the condition which develops either when the production of insulin (the internal secretion of the pancreas) is inadequate or, alternatively, when the action of insulin on the tissues is antagonized. Diabetes insipidus is a different condition due to a pituitary or renal lesion; in this condition, large quantities of a dilute, tasteless urine are produced.

von Mering and Minkowski in 1889 were the first to show that removal of the pancreas caused diabetes mellitus. In 1909 the name *insuline* was suggested by de Meyer for a hypothetical internal secretion of the pancreas as it seemed likely that the ductless Islets of Langerhans, which lie embedded in the body of the pancreas, were the source of this material. Despite suggestive results by other workers, it was not until 1922 that Banting and Best were able to obtain a preparation containing the antidiabetic hormone in a form which consistently alleviated all manifestations of diabetes in totally depancreatized dogs. Four years later crystals of insulin were isolated from pancreatic extracts[1].

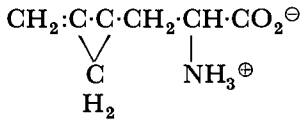
Insulin is a polypeptide which is rapidly destroyed by enzymic action in the gastro-intestinal tract. Although attempts have been made to protect insulin from being digested, preparations giving adequate and consistent absorption when administered by mouth have so far been unsuccessful[2]. Patients with severe diabetes mellitus therefore need daily injections in order to maintain good health.

The search for hypoglycaemic or antidiabetic drugs effective when given by mouth was stimulated by the isolation of insulin. In 1926, the hypoglycaemic effect of synthalin, a diguanide, was reported by Frank, Nothman and Wagner[3] and this drug was studied later by Graham and Linder[4]. It was soon abandoned, however, as it produced severe liver damage when given in therapeutic doses[5].

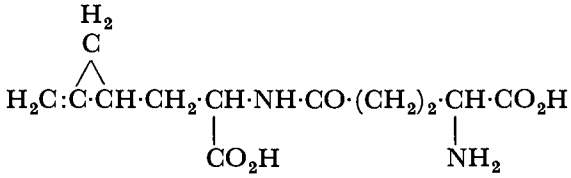
Extracts of plants have been used for a long time as traditional remedies for diabetes in many parts of the world. Blueberry leaf extracts (Myrtillin), for example, were investigated by Allen[6] in 1927, and extracts of periwinkle, mistletoe and the nicker berry (long popular as 'doctor' bush teas in Jamaica) were studied by Hugh-Jones[7] in 1955. Many other plant derivatives have been tested, and some have been found to possess marked hypoglycaemic properties. For example, the two alkaloids, galegine[8] (from the seeds of *Galega officinalis*) and lupanine[9] (from the seeds of *Lupinus albus*) reduce the blood-sugar of normal individuals and diabetic patients, and other material

with hypoglycaemic properties has been extracted from a wide variety of plant tissues ranging from cabbage and celery to yeasts[10]. Little, Levine and Best[11] found an insulin-like substance in the disintegration products of killed bacteria, while Collip[12] extracted from clams (and also many plant sources) a substance, glucokinin, which produced marked hypoglycaemia when injected subcutaneously into rabbits. These preparations were usually toxic to the liver and this action is probably the basis of the hypoglycaemic effect.

Recently, two polypeptides, hypoglycin A and B, have been isolated from the unripe 'ackee', the fruit of a plant, *Blighia sapida* which probably produces the vomiting-sickness sometimes seen among the poorer classes in Jamaica[13]. Both produce a marked hypoglycaemic action when given orally and although they are hepatotoxic, they are of considerable interest as insulin is a polypeptide which is *not* effective by mouth. The chemical structure of hypoglycin A has recently[14] been confirmed as the following:



Recent work[14a] has shown that hypoglycin B is a dipeptide with the following unit structure:

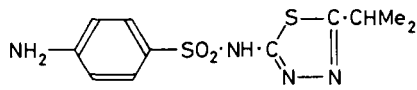


The structure and activity of related compounds has been reviewed recently[14b].

There are many other compounds which have hypoglycaemic properties. For example, oestradiol benzoate in large doses may reduce the hyperglycaemia of post-menopausal women suffering from diabetes[15]. Injections of dimercaprol sometimes produce a fall of blood-sugar concentration in diabetic patients who are receiving large doses of insulin[16]. Recently there has been renewed interest in the hypoglycaemic action of salicylates. Since the end of the last century, salicylates have been known to reduce the blood and urine sugar concentrations in diabetic patients[17] but adequate doses of acetylsalicylic acid produce troublesome side-effects and this limits their clinical usefulness for the treatment of diabetes[18]. They probably act by enhancing the peripheral utilization of glucose. A fall of blood-sugar concentration associated with depletion of liver glycogen may be produced by salicylates in alloxan-diabetic rats and in animals made hyperglycaemic with cortisone[19]. Further, they stimulate glucose uptake by the isolated rat diaphragm[20], probably by uncoupling oxidative phosphorylation.

The present era of renewed interest in oral hypoglycaemic agents began in 1942 when Janbon, Chaptal, Vedel and Schaap[21], while assessing the antibacterial properties of 5-isopropyl-2-sulphanilamido-1,3,4-thiadiazole (IPTD, RP 2254, *I*) in typhoid fever, discovered the dramatic hypoglycaemic action of the sulphonamide. A few of the patients died from hypoglycaemia.

Between 1942 and 1946, studies on the mode of action of IPTD and other thiadiazoles in dogs and rabbits were made by Loubatières[22] at Montpellier. He showed that IPTD (*a*) does not produce hypoglycaemia if the whole pancreas is removed but may do so if as little as one-sixth of the gland remains, (*b*) is most effective when injected directly into the pancreatic artery or through



(I)

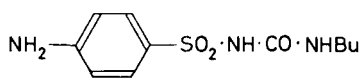
the duct of Wirsung under pressure, (*c*) acts independently of the nervous system, (*d*) lowers the blood-sugar in inverse proportion to the blood-sulphonamide level, and (*e*) raises the respiratory quotient after glucose administration. He postulated a pancreaticotropic mechanism to explain these actions of IPTD.

In 1946 Chen, Anderson and Maze[23] reported on the hypoglycaemic effect in intact rabbits of another thiadiazole, 5-cyclopropyl-2-sulphanilamido-1,3,4-thiadiazole. They showed that the compound was inactive or sometimes *hyper*glycaemic in animals with severe alloxan diabetes.

More recently the hypoglycaemic properties of many other sulphonamide derivatives have been discovered. This account is concerned chiefly with the new arylsulphonylurea compounds and the guanidine derivatives.

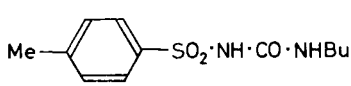
ARYLSULPHONYLUREA COMPOUNDS

Since 1955, when a group of German workers[24–26] published clinical observations on the potent hypoglycaemic action of a sulphanilamide derivative, *N*-butyl-*N'*-sulphanilylurea (carbutamide, *II*) many hundreds of chemically related compounds have been studied for their hypoglycaemic activity.[26a]

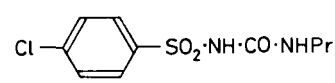


(II) Carbutamide

The synthesis of arylsulphonamides, *N*-arylsulphonylcarbamates, *N*-aryl-*N'*-alkylureas and of alkyl and other substituted sulphonylureas has been described[27–29]. The majority of these compounds are too toxic for human use but two analogues of carbutamide, namely tolbutamide, (*III*) and chlorpropamide (*IV*) have been subjected to extensive clinical and pharmacological studies in the past few years.



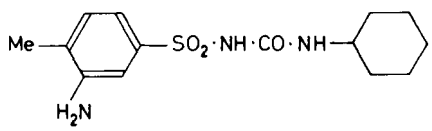
(III) Tolbutamide



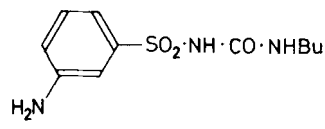
(IV) Chlorpropamide

After large-scale laboratory investigation and clinical trial in the United States of America, Canada and the United Kingdom, carbutamide was

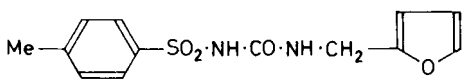
withdrawn from clinical use owing to the high incidence of serious toxic side-effects. About 5 per cent of 7,193 patients developed skin rashes, gastro-intestinal disorders, severe leucopenia, liver damage and generalized sulphonamide sensitivity reactions; eight patients died 10–30 days after commencing treatment[30]. However, tolbutamide (introduced in 1956) and more recently chlorpropamide, have been widely used without serious ill effects in the treatment of mild diabetes mellitus of the adult type. Doubtless other similar compounds will become available for clinical trial in the future, but their advantages and disadvantages may always have to be measured against the extensive data collected about tolbutamide and chlorpropamide. Already *N*-cyclohexyl-*N'*-(3-amino-4-toluenesulphonyl)-urea (metahexamide, Euglycin, *V*), *N*-(3-aminobenzenesulphonyl)-*N'*-*n*-butylurea (Sucrida Berna, SB 1, *VI*) and *N*-2-furfuryl-*N'*-*p*-toluenesulphonylurea, (furfuralurea, *VII*) have been used clinically although none of them offer any important new beneficial features.



(V)



(VI)



(VII) Furfuralurea

This account will therefore be chiefly concerned with the chemistry, metabolic features, mode of action and clinical applications of tolbutamide and chlorpropamide. Although carbutamide is not now used clinically, the data obtained from its use will be reviewed, as this compound was the first hypoglycaemic arylsulphonylurea to be discovered and many of the original investigations were carried out with it.

Chemistry and Metabolic Features

Carbutamide

Carbutamide (*N*-butyl-*N'*-sulphanilylurea, BZ55, U6987, Invenol or Nadisan, *II*) has physical properties similar to other *N*-monosubstituted sulphonamide derivatives, being a white crystalline substance which has weak acidic properties associated with the sulphamoyl group. It forms salts with alkalis and the sodium salts are easily soluble in water. Estimation of the free compound may be carried out in blood and urine by diazotization and coupling reactions[31].

In 1956, Ridolfo and Kirtley[32] showed that, as with the earlier sulphonamides, intestinal absorption of carbutamide after oral administration is rapid; the compound may be detected in the blood within 30 minutes

after taking 2·5 g by mouth. The peak blood-level (10–15 mg/100 ml. whole blood) is reached in 3–4 hours (at which time only 3–4 mg/100 ml. is acetylated) and after 6–7 hours the level begins to fall slowly. The decline of sulphonamide concentration in the blood behaves as a first-order exponential function, which gives a biological half-life of 30–60 hours[33]. Within 2–3 hours there is a definite lowering of blood-sugar concentration, the effective blood-level of carbutamide being 6–8 mg/100 ml.[34]. The liver partially detoxifies carbutamide by acetylation and then the drug is slowly eliminated via the kidneys. The urine contains about 66 per cent of the free sulphonamide and 33 per cent of the acetylated derivative.

As with other sulphonamides containing a *p*-amino group, carbutamide has some antibacterial action, and thyroid function is temporarily impaired. MacKensie and MacKensie[35], for example, showed that various sulphanilamide derivatives may produce goitre in small experimental animals, and Anderson, Worth and Harris[36] reported that this is also true of carbutamide. In human beings with mild diabetes mellitus, large doses of carbutamide (4 g daily) considerably depress thyroid ^{131}I uptake[37]. Longer term studies in 39 patients using therapeutic doses (2 g daily) for 47 weeks showed that the thyroid ^{131}I uptake is depressed to about 20 per cent of the pretreatment value by the end of the third week of therapy, and remains below normal for about 9 weeks. According to Brown and Solomon[38], the mechanism of the antithyroid effects of carbutamide in rats is inhibition of the organic binding of thyroidal iodide—an effect similar to that of propylthiouracil but only about 1/200th as strong.

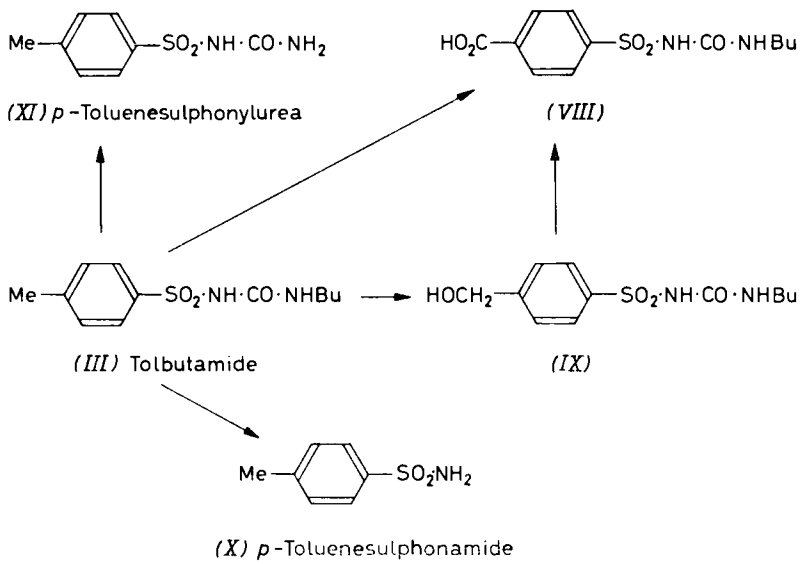
Tolbutamide

In tolbutamide (*N*-butyl-*N'*-*p*-toluenesulphonylurea, Rastinon, Orinase, D.860, U.2043, *III*) the *p*-amino group of carbutamide has been replaced by a methyl group and this change of chemical structure is responsible for many important biological differences. It is a white crystalline solid with a melting point of 128·5–129·5°C. It is practically insoluble in water although it forms soluble salts with alkalis. It dissolves readily in acetone, chloroform and alcohol.

Since tolbutamide lacks a *p*-amino group it cannot be estimated by the Bratton and Marshall procedure, but spectrophotometric methods have been developed[39,40] for the determination of total tolbutamide in blood and urine, based on its intense ultra-violet absorption at 228 mμ. After acidification with M/15phosphoric acid, the serum is extracted with chloroform (which must be completely removed subsequently as chloroform also absorbs strongly at 228 mμ). The solvent-free extract is then taken up in 95 per cent ethanol and shaken with charcoal before being estimated spectrophotometrically. This forms a useful and sensitive method for the range 1–25 mg/100 ml. but it lacks specificity; recoveries are good (85–90 per cent). Difficulties may be encountered with variations in the plasma blank[33] and even slight degrees of haemolysis lead to spuriously high readings. The method developed by Toolan and Wagner[41] for chlorpropamide (see below) has recently been found to be suitable for tolbutamide[42] and is unaffected by haemolysis. McDonald and Sawinski[43] and Spingler[44] have developed colorimetric procedures which are probably less satisfactory.

As with carbutamide, absorption of tolbutamide from the intestine is rapid and maximal blood levels are reached in 3–4 hours[33]. Based on the blood levels (extrapolated to zero time) after a given oral dose, the volume of distribution of tolbutamide is found to approximate to that of the extracellular fluid space in man[45] and in nephrectomized, eviserated rabbits[46]. In contrast to carbutamide, the tolbutamide concentration begins to fall according to a first-order exponential curve as soon as the maximal blood level is reached, giving a biological half-life of about 4 hours, both in normal and diabetic subjects[45,47]. There is considerable individual variation in the blood levels attained, but after 8–10 hours the concentration in most subjects has fallen below the minimum effective range of 6–10 mg/100 ml. after optimal therapeutic doses by mouth[33]. Increasing the dose does not increase the blood level comparably and even with doses of 6 g the blood level 24 hours later is still below the minimum required. Some serum protein-binding probably occurs as tolbutamide does not penetrate into the cerebrospinal[45] or oedema fluid[48].

In man, tolbutamide is converted into a freely soluble, non-toxic, carboxylic acid (*N*-butyl-*N'*-*p*-carboxyphenylsulphonylurea, *VIII*) by oxidation of the *p*-methyl group[49]. This acid has no hypoglycaemic action but is responsible for a variable proportion (10–28 per cent in 24 hours after a single oral dose) of the total blood tolbutamide level[45]. A small amount of *N*-butyl-*N'*-*p*-hydroxymethylphenylsulphonylurea (*IX*) has also been identified chromatographically in blood[50]. From the urine of dogs given tolbutamide, Mohnike and Wittenhagen[51] isolated a toxic metabolite, *p*-toluenesulphonamide (*X*) and also another compound which was later[52] shown to be *p*-toluenesulphonylurea (*XI*). Tolbutamide can therefore be metabolized in at least three ways:



However, there may be other species differences in the pathways of metabolism of the sulphonylurea compounds[53].

Tolbutamide is rapidly eliminated by the human kidneys, mainly as the carboxylic acid. This metabolite is freely water-soluble within the physiological urinary pH range[54] and the danger of crystalluria is negligible, but a white precipitate may develop on testing the urine with sulphosalicylic acid if large amounts of compound (*VIII*) are being excreted. Renal tubular transport is probably important in the excretion of the drug, as there is a much greater proportion of compound (*VIII*) in the urine compared with blood, and probenecid, which affects many other tubular transport mechanisms, delays the disappearance of tolbutamide from the serum[45].

The effective and optimal dose of tolbutamide for suitable diabetic patients is 1–3 g/day, but as it is rapidly excreted, the drug must be given in divided doses every 6–8 hours. The optimal blood level is variable and ranges from 8–18 mg/100 ml. Further increases in the dose are, paradoxically, associated with a diminishing hypoglycaemic effect[33].

In contrast to carbutamide, thyroid function as assessed by ^{131}I studies[37] is not significantly affected by tolbutamide. This agrees with the findings of MacKensie and MacKensie[35] that removal of the *p*-amino group from sulphamethyldiazine abolishes its goitre-producing properties. Tolbutamide has no antibacterial action.

Chlorpropamide

Chlorpropamide (*N*-propyl-*N'*-*p*-chlorobenzenesulphonylurea, Diabinese, P.607, *IV*) is a white crystalline compound melting at 127·5–128·5° C. In contrast to tolbutamide, there is a chlorine atom in the para position on the benzene ring and a propyl instead of a butyl group as the alkyl radical. It behaves as a monobasic acid in aqueous dioxan. Solubility in water is limited below pH 5 but it may readily be dissolved at room temperature in most organic solvents, particularly chloroform, acetone, ethanol and dioxan. Chlorpropamide absorbs strongly at 232·5 mμ in 0·01N hydrochloric acid, and this property forms the basis of its estimation in serum[41]. The serum is acidified with dilute phosphoric acid (0·067M), extracted with chloroform and then washed with 1 per cent sodium carbonate. Aliquots of the sodium carbonate layer are then neutralized with hydrochloric acid and the ultraviolet absorption measured in a spectrophotometer. The recovery, reproducibility and serum blank levels (about 1·3 mg/100 ml. of apparent chlorpropamide concentration) are good but the method lacks specifity. Toolan and Wagner[41] have shown that β-hydroxybutyric acid, *p*-chlorobenzenesulphonamide, acetylsalicylic acid and salicylic acid may seriously interfere with the estimation but haemolysis does not. The method is not suitable for detecting chlorpropamide in urine.

The metabolic fate of chlorpropamide after oral absorption has been studied using the radioactive ^{35}S-labelled drug[55]. Absorption from the intestine is more rapid than with carbutamide or tolbutamide[48], as maximum radioactivity and optical density is reached in 2 hours. The decay of the radioactivity (confirmed by measurement of chloroform-soluble optical density) in the blood-plasma showed that, unlike carbutamide or tolbutamide, chlorpropamide disappeared at two separate rates, a rapid one with an uncorrected biological half-life of 32 hours and a slower component with a half-time of approximately 16 days. In diabetic patients, a comparable

initial rapid decline was seen, but the second component was much slower. The diabetic patients received chlorpropamide or tolbutamide therapeutically for some time before the study was made, so that this observation does not provide good evidence for the suggestion that diabetic and normal subjects handle chlorpropamide differently. The two-phase disappearance rates from the blood may be explained by slow serum protein-binding or by chemical alteration of the compound. Chlorpropamide may be made partially non-dialysable by the plasma proteins, and the degree of binding increases as the chlorpropamide concentration rises[55]. Extrapolation of the slower component to zero time, however, suggests that about 20 per cent of chlorpropamide is represented as the slowly excreted component and this is three times greater than the values obtained from protein-binding experiments.

Urinary excretion of the radioactive chlorpropamide parallels its disappearance from the serum, but considerable variations are seen in diabetic patients pretreated with the drug. Nearly all the administered chlorpropamide is very slowly eliminated in the urine, 77 per cent being accounted for in 96 hours. The observation that salicylate increases the serum level of chlorpropamide and vice versa suggests that renal transport mechanisms are involved[56]. Faecal excretion of chlorpropamide is minimal.

In man, this compound in contrast to carbutamide or tolbutamide is probably not metabolized before excretion. No definite chromatographic differences have been found between the pure drug and that in serum or urine[55], and the ultra-violet absorption spectrum is identical in plasma and urine[56]. However, paper chromatography of the urine of dogs treated with chlorpropamide labelled with ^{35}S yields three different spots: the unchanged compound, *p*-chlorobenzenesulphonylurea and *p*-chlorobenzenesulphonamide, the three compounds accounting for about 30, 40 and 20 per cent respectively of the administered dose. In rabbits, 80 to 95 per cent of chlorpropamide is excreted unchanged[57].

On a weight for weight basis and using both duration of action and the degree of hypoglycaemia as criterion for evaluation, Root[58] showed that in rats and dogs chlorpropamide is more effective than tolbutamide. Similar comparisons were made in rhesus monkeys[59]; at dose levels comparable to those used in man (5–10 mg/kg), a definite and prolonged hypoglycaemic action was obtained, whereas a dose of tolbutamide (10 mg/kg) had only minimal effect and 5 mg/kg produced a rise of blood-sugar level. These animal studies, showing that chlorpropamide has a greater and more prolonged hypoglycaemic effect than tolbutamide, have been fully confirmed in man[56,60,61]. The optimal therapeutic dose is between 100 and 500 mg daily, the effective blood chlorpropamide level ranging from 3–17·5 mg/100 ml.[62,63]. With doses of 500 mg or more, blood levels of up to 40 mg/ml. are frequently seen. There is a fairly good relationship between the dose and blood level attained both in normal human beings[64] and in diabetic patients[63] but the relationship between serum level and hypoglycaemic effect varies considerably from patient to patient.

The increased hypoglycaemic potency of chlorpropamide as compared with tolbutamide is probably due to the higher initial and more prolonged blood levels attained, and not to any inherent increased 'potency'[42,65],

although a greater hypoglycaemic effect has been obtained with equivalent blood levels[56]. Studies of serum levels and blood-sugar concentration in human beings do not suggest a cumulative action in doses of 500 mg or less, either in normal[64] or diabetic subjects[63].

As with tolbutamide, thyroid function as assessed by plasma protein-bound iodine levels or the thyroid uptake of radioiodine, is not impaired by chlorpropamide[66] and the drug has no antibacterial action.

Metahexamide

Metahexamide (*N*-cyclohexyl-*N'*-(3-amino-4-toluenesulphonyl) urea, Euglycin, *V*) is a very effective hypoglycaemic agent with a duration of action comparable to that of carbutamide and a potency which is rather higher than that of chlorpropamide. In rats it is more active than either tolbutamide or chlorpropamide on a weight for weight basis[67]. In normal humans the hypoglycaemic potency of metahexamide is four times higher than chlorpropamide or tolbutamide when judged by the serum concentration attained or twice as active as chlorpropamide and fifteen times more potent than tolbutamide when assessed by weight[68]. Absorption from the intestine is rapid, being complete in 2–3 hours[48]. The biological half-time is 19–26 hours in normal people[68,69] but it may be longer in diabetic patients.[70] It is about half as effective as chloropropamide and about five times as effective as tolbutamide in producing sustained blood levels[68]. Dogs and rabbits excrete 30–35 per cent of the administered metahexamide dose unchanged in the urine and 45–50 per cent as 3-amino-4-benzenesulphonamide[57]. The aromatic amino group is resistant to acetylation even in rabbits, a species that acetylates carbutamide readily[53]. In appropiate diabetic patients, a single daily dose of 50–300 mg gives an adequate hypoglycaemic response and it compares favourably with tolbutamide and chlorpropamide[70–73]. Patients resistant to tolbutamide may respond to metahexamide but those resistant to chlorpropamide usually cannot be controlled with the drug[69]. Gastro-intestinal and allergic side-effects are probably as frequent as with other arylsulphonyl compounds, particularily when high doses are used. No information about the effect of metahexamide on the bone marrow is available at present, but jaundice of the obstructive variety occurs[74,74a], and for this reason it is no longer used clinically.

SB 1

This compound (*N*-(3-aminobenzenesulphonyl)-*N'*-*n*-butylurea,Sucrida Berna, *VI*) differs from carbutamide by having the aromatic amino group in the meta instead of the para position on the benzene ring. It has no antibacterial activity[75]. In divided doses of 0·5–1·5 g daily, SB 1 produces an adequate hypoglycaemic response in selected diabetic patients[76]. In rabbits it is rather less toxic and somewhat more potent than tolbutamide on a weight for weight basis[77]. Hypoglycaemia is maximal 6–9 hours after 250 mg/kg orally and lasts for 13–15 hours. Urinary excretion of an acetylated metabolite is rapid, 40–50 per cent being eliminated in 12 hours.

Furfuralurea

Furfuralurea (*N*-2′-furfuryl-*N*′-*p*-toluenesulphonylurea, *VII*) is mentioned by Danowski and Mateer[78]. Its hypoglycaemic activity is comparable to that of chlorpropamide.

The Toxicity of Tolbutamide and Chlorpropamide in Animals and in Man

Toxicity studies in animals with chlorpropamide and tolbutamide indicate that with both compounds the acute and chronic toxicity is low[59,67]. For tolbutamide, the oral LD_{50} (calculated according to the method of Litchfield and Wilcoxon[79]) in rats is 2·49 and in mice is 1·83 g/kg. By the intravenous route the corresponding figures are 0·77 and 0·70 g/kg respectively. Chlorpropamide is slightly more toxic, the oral and intravenous LD_{50} for rats being 2·39 and 0·59 and for mice, 1·67 and 0·50 g/kg respectively. Similar results are reported by Root, Sigal and Anderson[67]. Chronic toxicity experiments with chlorpropamide indicated that in dogs oral doses of 150 mg/kg (corresponding to more than 20 times the recommended clinical dose in man) may be tolerated, the only symptoms being ataxia and muscular weakness in some of the animals. In higher doses (up to 200 mg/kg) rhesus monkeys remained well, apparently, except for some intermittent diarrhoea. No histological or functional changes were observed in the liver[59].

This lack of hepatotoxic effect contrasts sharply with the effect of tolbutamide in depancreatized and partially depancreatized dogs and puppies[80]. In doses of 100–150 mg/kg, all the animals died in 3–6 weeks with severe liver damage; one out of three animals receiving 30 mg/kg (the amount usually recommended for humans) succumbed in a similar fashion. At all dose levels, the albumen and the protein-bound polysaccharide levels in the plasma declined, the serum alkaline phosphatase, glutamic-oxaloacetic and glutamic-pyruvic transaminase levels rose sharply, and in two animals the prothrombin time was prolonged terminally. This was unaffected by Vitamin K. The bromsulphalein excretion (often used as a more refined test of hepatic function in man) remained normal. These effects, which are seen in dogs, may be due to the formation of a toxic metabolite, possibly *p*-toluenesulphonamide[51]. Similar hepatotoxic effects in dogs have been described[81,82].

When used therapeutically in man, tolbutamide, however, does not damage the liver. Repeated liver function studies have shown that changes of the serum bilirubin, flocculation tests and bromsulphalein excretion have not been seen over a period of 12–16 months[83,84]. A slight rise of serum alkaline phosphatase level has been reported[85] but its significance is not clear. Dolger[86] has given tolbutamide to several patients who recently recovered from hepatitis or obstructive jaundice, without evidence of further liver damage.

Chlorpropamide, however, may cause an intrahepatic obstructive jaundice of the type seen sometimes following chlorpromazine or methyltestosterone. This develops most commonly in patients given over 500 mg daily but it has been reported after smaller doses[87,88]. The condition subsides rapidly on stopping the drug and evidence of permanent liver-cell damage has not, as

yet, been reported. Liver biopsy material which showed histological evidence of a healing pericholangiolitis was found in one out of seven patients on chlorpropamide, and four out of six patients taking metahexamide[89]. The hepatic histology was normal in all of six patients given tolbutamide.

With tolbutamide or chlorpropamide, most workers have been unable to detect any significant change in the quantity or appearance of the white blood-cells, but leucopenia has occasionally been reported. In contrast to the serious effects of carbutamide, the leucopenia is usually transient and no case of agranulocytosis has so far been recorded.

As far as important side-effects are concerned, tolbutamide and chlorpropamide are remarkably non-toxic when used in effective hypoglycaemic doses for appropriate diabetic patients. At the Joslin Clinic, since November 1957, only 1·1 per cent of 772 patients treated with tolbutamide have developed side-effects sufficiently severe to warrant stopping the drugs[85]. Dolger[86] has seen no serious toxic reactions among 500 private patients treated for 3–12 months. Chlorpropamide seems equally innocuous considering the large number of patients who do not suffer from any side-effects, but a curious ataxia with muscle weakness (not due to hypoglycaemia) has been described following large doses[90]. Minor side-effects are however more frequently encountered. Both drugs may cause gastro-intestinal symptoms such as epigastric pain, nausea and diarrhoea; and, in patients with peptic ulcer, their dyspepsia may be aggravated. These features appear to be rather more common with tolbutamide than with chlorpropamide. Urticarial skin rashes are not uncommon and a single case of purpuria has been reported by Sugar[91]. One patient nearly died of anaphylactic shock following chlorpropamide[92]. During treatment with tolbutamide, flushing of the face may occur if ethanol is taken, and an antabuse-like effect may occur with chlorpropamide[93]. Most clinical reports describe abdominal symptoms and skin rashes in 1–7 per cent of patients.

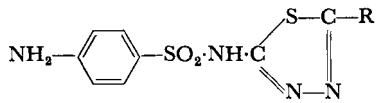
THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND ACTIVITY OF THE HYPOGLYCAEMIC SULPHONAMIDES

Sulphanilamidothiadiazoles

Between 1942 and 1946, Loubatières[22,94] and Bovet and Dubost[95] tested many thiadiazole derivatives in dogs and rabbits during studies of the relationship between structure and hypoglycaemic activity in this series. When the alkyl group of the original compound, 5-isopropyl-2-sulphanilamido-1,3,4-thiadiazole, (*I*) was replaced by either methyl or ethyl radicals, the hypoglycaemic effect was reduced or even lost. Maximum activity was obtained when the alkyl chain in the 5-position was either an iso or a tertiarybutyl group but longer hydrocarbon chains, *e.g.* hexyl or heptyl, produced compounds with little hypoglycaemic activity (*Table 5.I*). In a clinical trial, 5-t-butyl-2-sulphanilamido-1,3,4-thiadiazole (RP 2259, Glipasol) was shown to control the blood-sugar level in 65 per cent of the thirty-one patients but its use was not recommended because of its toxicity

to the liver[96]. Thiadiazole derivatives without a *p*-aminobenzenesulphonyl portion (*e.g.* 2-amino-5-propyl- or -5-isopropylthiadiazole, or 2-acetamido-5-isopropylthiadiazole) or without the *p*-amino group (2-benzenesulphonamido-5-isopropylthiadiazole) had no hypoglycaemic properties, but 2-(*p*-methoxybenzenesulphonamido)-5-isobutylthiadiazole (Stabinol) was recently reported to be active[96a]. Loubatières also found that alcohols

Table 5.1. Effect on the hypoglycaemic activity of changes in the 5-substituent (R) of the sulphanilamidothiadiazoles[94]:



5-Substituent (R)	*Change in blood-sugar concentration* %	*5-Substituent (R)*	*Change in blood-sugar concentration* %
Methyl	+21	t-Butyl	−38
Ethyl	−14	Ethylpropyl	−26
Propyl	−20	Pentyl	−30
Isopropyl	−21	Hexyl	−5
Butyl	−30	Heptyl	−14
Isobutyl	−38	Amino	−13

corresponding to the hydrocarbon chains, particularly isopropyl, butyl and pentyl alcohols, themselves have some hypoglycaemic activity, and he postulated that it was the hydrocarbon moiety that conferred hypoglycaemic activity, although the sulphanilamido portion was nevertheless essential as a 'reinforcing agent' to the rest of the molecule.

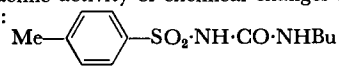
Arylsulphonylureas

Only a few of the many hundreds of sulphonylurea compounds which have now been synthesized possess sufficient hypoglycaemic potency and duration of action to justify more detailed investigation. Nevertheless, it is interesting to examine how variations in chemical structure modify hypoglycaemic activity. There is a close structural similarity between the early sulphanilamidothiadiazoles *e.g.* (*I*) and the later sulphonylureas *e.g.* (*II*). In view of this chemical resemblance, it is surprising that the hypoglycaemic effects of the sulphonylurea compounds were discovered so much later, and even then by chance[24]. The discovery of the hypoglycaemic properties of carbutamide was soon followed by the discovery that the aromatic *p*-amino group was not necessary for hypoglycaemic activity[97,98] and the potent *p*-methyl analogue, tolbutamide, was synthesized. This compound and all subsequent ones without a free aromatic *p*-amino group *e.g.* SB 1 (*VI*) have no antibacterial activity. Tolbutamide, however, is a short-lived drug within the body because of the metabolic oxidation of the methyl group, and a more potent, longer-acting drug was sought, with a similarly low degree of toxicity.

The effect of change in structure of a series of sulphonylureas, $ArSO_2{\cdot}NH{\cdot}CO{\cdot}NHR$, on hypoglycaemic activity was systematically studied by giving fasting rats a single oral dose of 100–300 mg/kg of the compound[99]. Modification of the urea portion led to loss of hypoglycaemic activity although

weak action was retained when the NH·CO·NHBu group was replaced by a NH·CS·NHBu, NH·CO·OBu or NH·CO·Ph group (*Table 5.2*). As with the

Table 5.2. Effect on hypoglycaemic activity of chemical changes in the urea part of the molecule[99]:



	Activity	*Duration*
Me—C6H4—SO2— NH·CS·NHBu	++	+
NH·CO·OBu	+	
NH·CO·CH2Me	±	
NH·CO·CH2Bu	±	
NH·CO—C6H5	+	+
NH·NH·CO·NHBu	±	
NH·NH·COPr^i	±	
NMe·CO·NHBu	±	

sulphanilamidothiadiazoles, changing the length and character of the alkyl radical has, in most of the active compounds, a definite but relatively small effect on hypoglycaemic activity (*Table 5.3*). Simple alkyl chains of three or four carbon atoms endow maximal activity and homologues having a

Table 5.3. Effect on hypoglycaemic activity of changes in the substituents on the terminal nitrogen atom of *N*-arylsulphonylurea compounds[99]: $ArSO_2{\cdot}NH{\cdot}CO{\cdot}NHR$

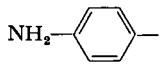
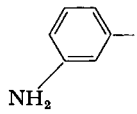
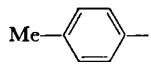
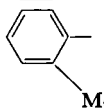
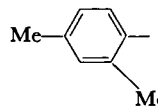
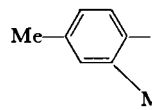
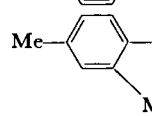
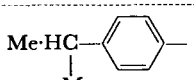
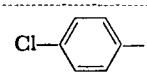
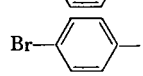
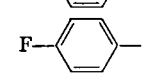
Radical, R	*Activity*
Normal alkyl, C_3H_7 or C_4H_9	+++ to ++++
Branched alkyl, C_3H_7 to C_5H_{11}	++ to ++++
Alicyclic, C_5H_9 or C_6H_{11}	+++ to ++++
Aryl, —C6H4—Cl	++ to +++
Dialkyl, N	++ to +++

branched alkyl chain (such as tertiary butyl) or a cyclic substituent have good activity; in the latter case, a five to seven carbon atom radical seems to give maximal effect *e.g.* metahexamide (*V*) and *N*-cyclohexyl-*N'*-*p*-toluenesulphonylurea which is reported to be about as active orally and intraperitoneally as tolbutamide but to have a lower toxicity[100]. Compounds in which the alkyl-carrying nitrogen atom is di-substituted are moderately active.

The greatest enhancement of hypoglycaemic effect is achieved, however, by substitution in the aromatic ring, Ar (*Table 5.4*). Para-substitution gives the most potent and longest-acting compounds, the halogens, especially chlorine, being particularly active. The *p*-methoxy analogue of tolbutamide has been reported to be about as active orally as tolbutamide in rabbits and to be less toxic[101]. But substitution of groups other than methyl, methoxy or halogen in the *p*-position gives compounds of low activity, for example,

p-isopropyl, *p*-carboxy, *p*-ethoxycarbonyl or *p*-hydrazinocarbonyl[28]. Unsubstituted phenyl derivatives are rather less active, and ortho-substituted and di-substituted (2,4-, 2,5-, 3,4-) phenyl compounds are even weaker[28].

Table 5.4. Effect on hypoglycaemic activity of substitution in the aryl radical of the *N*-alkyl-*N'*-arylsulphonylurea compounds[28,77,99]: $ArSO_2{\cdot}NH{\cdot}CO{\cdot}NHR$

Aryl radical, Ar	*Alkyl radical, R*	*Hypoglycaemic action*	
		Acute effect	*Duration*
	Butyl	++++	+++
	Butyl	+++	++
	Butyl	+++	++
	Butyl	±	—
	Butyl	+++	—
	Propyl	++	++
	Propyl	+++	++
	Isopropyl or ethyl	+++	—
	Butyl	+++	—
	Propyl	++++	++++
	Propyl	++++	+
	Propyl	++	++

Naphthylsulphonylureas are only feebly hypoglycaemic but compounds in which the aromatic ring is replaced by a heterocyclic group (for example,

2-thienyl, or 5-chloro-2-thienyl) are moderately active[99]. A detailed study of the effect of chemical modification on hypoglycaemic activity may throw considerable light on the mechanism of action of these compounds.

MODE OF ACTION OF THE ARYLSULPHONYLUREA COMPOUNDS

Sulphonylurea compounds are now widely used in selected cases of diabetes mellitus. Probably over a million patients all over the world have already been treated with tolbutamide or chlorpropamide, or, to a much lesser extent, with metahexamide. Responsive diabetics may take sulphonylurea compounds continuously for considerable periods of time. Many publications on the mode of action of the sulphonylurea drugs, particularly carbutamide, have appeared since 1955, but the question of how the drugs lower the blood-sugar concentration still remains partly unsolved.

It is generally agreed that in mammals the sulphonylurea compounds only produce hypoglycaemia when some functioning pancreatic beta-cells are present. Houssay and Penhos[102], for example, showed that the presence of some pancreatic tissue is essential for the hypoglycaemic effect of these compounds in much the same way that Loubatières[22] showed that this effect was true of the sulphanilamidothiadiazoles. The sulphonylureas reduce the fasting blood-sugar level of intact and partially pancreatectomized animals or animals made mildly diabetic with alloxan, but they do not do so after *total* pancreatectomy in dogs, rabbits, rats, cats, toads or man[46,102–7], nor in animals made *severely* diabetic with alloxan.

In human beings, the arylsulphonylurea compounds are effective only in individuals who have a pancreatic reserve of insulin. They reduce the fasting blood-sugar concentration in non-diabetics and mild diabetics whose illness started in adult life, but with few exceptions the drugs do not act in patients with severe diabetes of the juvenile type. This may be because the pancreas of diabetic adults contains over 30 per cent of the normal insulin content, while in juvenile diabetic patients little or no insulin may be extracted from the gland[108]. In a similar way, insulin-like activity may be readily detected in the plasma of many diabetic adults but generally is not found in plasma of patients with the juvenile form of the disease[109].

After evisceration in dogs[102,106], rabbits[46] and rats[110], the drugs cease to produce hypoglycaemia. This suggests that the lowering of blood-sugar concentration is not produced by a direct action on peripheral tissues, although some studies suggest that the sulphonylurea compounds stimulate isolated excised muscle to take up glucose (see below).

However, the dependence of the hypoglycaemic action of the sulphonylurea compounds on the presence of some islet-containing pancreatic tissue only applies to mammals. Mirsky and Gitelson[111], for example, showed that a hypoglycaemic response to tolbutamide may be obtained in alloxanized chickens and in depancreatized or enterectomized ducks. Hazelwood[112] has further shown that hepatectomy, with or without pancreatectomy does not prevent the hypoglycaemic response in domestic fowls. As the carbohydrate metabolism in avians is therefore complex, all further remarks in this review will be concerned with the mode of action of the sulphonylurea compounds in mammals.

The sulphonylurea drugs may produce hypoglycaemia in mammals by inhibition of the secretion of the pituitary or adrenal glands, but this is unlikely as removal of either or both of these glands does not prevent the hypoglycaemic effects. For example, hypophysectomy in dogs[102] increases the degree of hypoglycaemia (thus simulating the effects of insulin) yet hypophysectomized cats are not more sensitive to the sulphonylurea drugs than are normal animals[113]. Unlike insulin, however, adrenalectomy greatly enhances their hypoglycaemic effect in both cats and dogs. The drugs are effective in patients with pan-hypopituitarism and Addison's disease, both before and after replacement therapy with hydrocortisone[114], and the hyperglycaemic response to adrenaline is unaffected during the administration of these drugs.

The idea that the sulphonylurea compounds inhibit the release of glucagon from the alpha-cells of the pancreas was suggested in 1956 by Ferner and Runge[115] who found histological evidence of damage to the alpha-cells following carbutamide. Similar changes had previously been reported after IPTD (*I*)[116]. The sulphonylureas however are ineffective in severe alloxan diabetes. The disappearance of ^{131}I-labelled glucagon is unaffected by pretreatment with tolbutamide or carbutamide[117], and the hypoglycaemic response to injected glucagon is not inhibited in man[114]. The possibility of tissue antagonsim to glucagon is therefore unlikely. It appears that hypoglycaemia as such may alter the staining properties and decrease the granularity of the pancreatic alpha-cells[118].

From these results, the following conclusions may be drawn concerning the mechanism by which sulphonylurea compounds lower the blood-sugar level in mammals: (*a*) they do not possess a direct insulin-like action on peripheral tissues; (*b*) the presence of an insulin-containing pancreas is essential; (*c*) they do not act by reduction of glucagon secretion or by tissue antagonism to glucagon, and (*d*) inhibition of hormonal antagonists of insulin from the pituitary or adrenal glands is not involved.

Three ways of explaining the hypoglycaemic effect of the compounds therefore remain: (*a*) the pancreas is stimulated to release more insulin, (*b*) the rate of endogenous insulin destruction is diminished, and (*c*) hepatic glucose release and/or production is depressed.

Increased Release of Insulin from the Pancreas

This was first suggested by Loubatières[22] to explain the hypoglycaemic effects of IPTD and other thiadiazolylsulphonamides. In cross circulation experiments, he showed that when IPTD was injected into a dog whose pancreatico-duodenal vein was anastomosed to the jugular vein of a depancreatized dog, the blood-sugar level of the diabetic animal was depressed. In similar experiments with intact animals previously given intravenous carbutamide, the blood-sugar level of the recipient animal is lowered by injections of the pancreatic venous blood of the donor, but not by injections of blood from the donor's mesenteric vein[119]. Direct perfusion of the pancreas with tolbutamide via the right gastro-epiploic branch of the gastro-duodenal artery produced a greater reduction in the blood-sugar level than did injection into the femoral vein[120]. But hypoglycaemia was not produced by

pancreatic injection in doses which were too small to be effective when given peripherally. Other workers found that the hypoglycaemic effect when metahexamide[121] or chlorpropamide[122] was injected into the pancreatic artery was no greater than when either drug was given into the femoral vein.

The response to tolbutamide is roughly proportional to the insulin content of the pancreas of the mammal. Mirsky, Perisutti and Gitelson[123] showed that pretreatment of dogs with growth hormone from the pituitary gland, a hormone known to deplete the pancreas of insulin[124], reduces the acute hypoglycaemic effect of tolbutamide. Similarly, prolonged fasting, which also reduces the insulin content of the pancreas[125], diminishes the hypoglycaemic response. The onset of alloxan diabetes in cats is heralded by a phase of hypoglycaemia which is probably due to the release of preformed insulin from necrotic beta-cells in the pancreatic islets. Pretreatment with tolbutamide prevents this effect[126] so the drug may reduce the amount of insulin within the islets. Four weeks after hypophysectomy in rats, the amount of insulin in the pancreas falls to about half the pre-operative level and sensitivity to tolbutamide diminishes correspondingly[127]. In human beings, the rate at which the blood-sugar concentration is lowered following a single dose of tolbutamide (1 g intravenously) may be used as a diagnostic test for mild diabetes[128]; the decrease is more rapid and more profound in non-diabetic patients than in patients with mild diabetes. Wrenshall and Hamilton[129] have shown, using post-mortem material, that the levels of extractable pancreatic insulin are lower in diabetic men than women. Analysis of clinical data[130] also indicates that adult males with mild diabetes are considerably less likely to respond to the sulphonylurea drugs than are women, a result which adds more evidence to the hypothesis that the hypoglycaemic effect of the sulphonylurea compounds is related to the reserve of pancreatic insulin.

Morphological studies of the pancreatic islet tissue in animals show that tolbutamide or carbutamide produce degranulation of the beta-cells with swelling of their nuclei; the islets increase in size and number and mitoses are more frequent[117,131–3]. No significant morphological changes in the islet tissue have been reported as yet in diabetic patients who have died while receiving tolbutamide[115,134]. Histological changes in the beta-cells, however, have been correlated with changes of blood-sugar level and pancreatic insulin content[135]. In experiments with calves, oral tolbutamide was shown to produce a transient degranulation of the beta-cells, with increase in nuclear volume and a transient reduction of extractable insulin from the pancreas. The fall in blood-sugar concentration closely paralleled these changes. Carbutamide lowers the amount of insulin extractable from the pancreas of dogs but this does not persist[136]. The suggestion that beta-cell degranulation is a specific response to the hypoglycaemic sulphonylurea compounds has been made by Creutzfeldt, Detering and Welte[137], who found that large doses of two other inactive sulphonylureas (*N*-*p*-toluenesulphonyl-*N'*-methylurea and *N*-sulphanilyl-*N'*-ethylurea) failed to alter the histology of the beta-cells.

Perhaps, the most compelling evidence[138] that sulphonylureas produce hypoglycaemia by the release of endogenous insulin is that the insulin-like

activity of pancreatic venous blood of dogs increases 'many times' after the administration of hypoglycaemic sulphonylureas[139]. Goetz and Egdahl[140] have confirmed this by assaying the ability of pancreatic venous blood of dogs given tolbutamide to lower the blood-sugar level in fasting, intact mice. In rats, a group of German workers measured the incorporation of glycogen into rat diaphragm muscle as a test for insulin and reported that the insulin-like activity of peripheral venous blood is increased by carbutamide[141] and tolbutamide[142]. Evidence concerning the effect of sulphonylureas on the plasma insulin-like substances in human beings, however, is conflicting. Venous blood taken during maximal hypoglycaemia following intravenous or oral tolbutamide fails to stimulate the uptake of glucose by the rat diaphragm muscle[143,144]. But, using a more sensitive rat diaphragm technique[145], a two-fold rise of plasma insulin-like activity has recently been detected 2·5 hours after a single oral dose in normal subjects and tolbutamide-sensitive patients[146]. There was a correlation between the increase of glucose uptake by the rat diaphragm and the fall of blood-sugar concentration in the patients.

If the sulphonylurea compounds produce hypoglycaemia by stimulating insulin release from the pancreas, then it should be possible to demonstrate in man some of the metabolic effects of increased peripheral glucose utilization. After intra-arterial injections of insulin, the peripheral arterio-venous glucose differences of the injected limb increase[147], plasma potassium and phosphate concentrations decrease, lactate and pyruvate levels rise and the respiratory quotient increases. These changes have not generally been observed following tolbutamide, carbutamide or chlorpropamide[65,104,143,148,149,150]. Such negative findings lose much of their importance, however, when it is realized that these indices of peripheral glucose utilization are relatively insensitive and liable to considerable experimental error.

Measurement of peripheral arterio-venous glucose differences are difficult to interpret, and this is particularly so when the blood-flow through the tissues, known to be increased by insulin[151], is not measured simultaneously. The decrease in the blood-sugar concentration following intravenous insulin injections (or following the rise after a glucose load) is associated with a narrowing of peripheral arterio-venous glucose concentration differences[152] but the proportion of arterial glucose concentration taken up by the tissues $\left(\frac{A-V}{A}\right)$ nevertheless widens[153], where A and V are the glucose levels in artery and vein respectively. This parameter was measured following large doses of intravenous tolbutamide during 5 or 10 per cent glucose infusions[104]. In mildly diabetic and in normal subjects no change was detected in the ratio $\frac{A-V}{A}$ when the results were analysed statistically. However, simultaneous blood-flow measurements were not performed, and closer scrutiny of the data shows that when definite hypoglycaemia was produced the ratio $\frac{A-V}{A}$ increased considerably. A rise in the ratio $\frac{A-V}{A}$ in fasting normal subjects following intravenous doses of tolbutamide has been described[105]. Butterfield, Fry and Holling[154] developed a method for

the simultaneous measurement of forearm blood-flow and found that there is a critical blood-sugar concentration below which glucose fails to enter cells. This threshold value is raised in diabetes and may be lowered by oral tolbutamide.

When the changes in glucose uptake are slight, there is no correlation between the change of glucose assimilation and the maximal decrease in serum inorganic phosphorus concentration[155]. Likewise, alterations of serum pyruvate and lactate concentrations following the sulphonylureas are also insignificant[149,150,156,157].

Moreover, difficulties in finding consistent and unequivocal evidence of increased peripheral glucose utilization also apply to the changes following insulin. When insulin is given intravenously in doses which mimic the fall of blood-sugar level produced by tolbutamide, evidence of increased glucose uptake by the tissues is also often inconclusive, expecially if the injection is made slowly. Madison and Unger[158] claim that when insulin is injected into the portal vein (thus simulating endogenous insulin secretion) little effect on peripheral arterio-venous glucose differences may be demonstrated, although equivalent doses of insulin injected into a peripheral vein are followed by an increase which is easily measured. No qualitative differences have however been observed in the serum potassium, pyruvate and lactate levels after injections of insulin into the portal vein and into the femoral veins of dogs, although different rates of insulin administration (rapidly, or 1·0 and 0·1 units/5 minutes) were employed at two different dose levels (0·1 or 1·0 units/kg[150]. In intact animals, the sulphonylurea compounds increase the liver glycogen content[159] but do not affect significantly that of muscle glycogen, whereas single injections of insulin have the reverse effect. When an intravenous infusion of insulin is given to fasting intact rats[160], the blood-sugar decreases although the muscle glycogen remains unchanged. This again emphasizes that the metabolic response is greatly affected by the route and the rate of insulin administration.

Nevertheless, there are two effects other than the hypoglycaemic action in which the sulphonylureas may mimic the metabolic effects of insulin. Firstly, tolbutamide reduces the blood amino acid level in normal subjects and in stable diabetic patients[161], and, *in vitro*, an increased incorporation of ^{14}C-glycine into rat liver-slice protein has been observed[157]. Secondly, serum unesterified fatty acid levels are reduced by about 30 per cent following tolbutamide[162] and metahexamide[163]. These two findings are important as they show that the sulphonylureas do not merely produce hypoglycaemia but may also simulate some of the other effects of insulin.

Diminished Destruction of Insulin

This mechanism has been suggested since insulinase in rat liver-slices is inhibited by the sulphonylurea drugs[123]. It is of doubtful significance, however, since insulinase is not inhibited by the concentrations of tolbutamide reached in man following therapeutic doses[164]. The rate of degration of ^{131}I-labelled insulin in rabbits is not altered by tolbutamide[117,144] and the liver of rats pretreated with the drug destroys ^{131}I-labelled insulin at the normal rate[165]. Likewise most workers have not been able to show that the

sulphonylureas potentiate the effect of exogenous insulin either in severe diabetic patients or following pancreatectomy in man. However, there is considerable evidence in depancreatized dogs that large doses of carbutamide or tolbutamide[80–2,166,167] potentiate insulin. This effect, which is obtained only after prolonged administration, is probably related to the severe hepatic damage that these compounds produce in dogs. Diminished destruction of insulin therefore is not an important effect of the sulphonylureas, but, as Mirsky suggests[123], inactivation of insulinase may contribute to the slow rise in the blood-sugar level following tolbutamide-induced hypoglycaemia.

Reduction of Hepatic Glucose Output by a Direct Action on the Liver

There is little doubt that tolbutamide reduces the output of glucose from the liver in human beings and in dogs. This occurs both during fasting and after fructose administration[168,169] and may be measured directly by catheterization studies or indirectly by determining the specific activity of plasma glucose following injections of ^{14}C-labelled glucose[104,148,149,170,171].

Tolbutamide interferes with the *in vitro* conversion of liver glycogen to glucose. Large amounts added to the incubating medium prevent glucose release from both rat and rabbit liver-slices[172–4]. The increased glucose output induced by adrenalin is particularly affected[164]. Weber and Cantero[175] found that tolbutamide inhibits glucose-6-phosphatase predominantly, slightly reduces phosphohexosisomerase activity but does not alter liver phosphoglucomutase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. This suggests that tolbutamide *in vitro* has a specific effect on glucose-6-phosphatase but the concentrations necessary for this effect are 5–10 times higher than the peripheral blood levels which produce hypoglycaemia *in vivo*. Liver tissue removed from rats during tolbutamide hypoglycaemia, however, fails to show reduced glucose-6-phosphatase activity[176], and many other sulphonamides or sulphonamide derivatives which do not lower the blood-sugar level may also inhibit liver glucogenic enzymes. This has recently been confirmed by Jasmin and Johnson[177] who found that there is no relationship between the hypoglycaemic activity of 17 new 5-alkyl-2-benzenesulphonamido-1,3,4-thiadiazoles and their ability to inhibit glucose-6-phosphatase (using the method of Cori and Cori[178]) in mice liver homogenates. Tolbutamide produced a 50 per cent inhibition at 5×10^{-3}M concentration, and a comparable degree of inhibition was obtained with concentrations of sulphaethylthiadiazole which have antibacterial, but no hypoglycaemic, activity. The presence of the liver is not essential for the hypoglycaemic action of the sulphonylurea compounds, since hepatectomy does not prevent the hypoglycaemic response to tolbutamide. Further, moderate doses of tolbutamide produce a similar decrease in the blood-sugar concentration in hepatectomized and intact dogs[160,179].

These findings make it difficult to believe that, in therapeutic doses, the sulphonylurea compounds reduce hepatic glucose output by a *direct* action on the liver, and, as described above, the evidence points to the sulphonylurea compounds producing hypoglycaemia by reducing glucose release and/or

production from the liver. This apparent paradox can only be resolved if it is assumed that endogenous insulin, secreted into the portal vein, produces a diminution of hepatic glucose output. If this is not so, it is difficult to explain why the sulphonylureas are only effective in the presence of an insulin-containing pancreas, although it may be that the metabolic upset of alloxan diabetes, human juvenile diabetes and the diabetes following total pancreatectomy, masks in some way a primary hepatic effect of the drug. Our knowledge of the hepatic action of insulin is still inconclusive. An increase of hepatic glycogen under the influence of insulin can be demonstrated readily in intact animals provided the insulin is rigorously glucagon-free and no fall of blood-sugar is allowed to occur[180].

In man, the glucose output from the splanchnic area was shown by catheterization techniques to be reduced by insulin[181]. Later[182], it was suggested that insulin increased the glucose uptake by hepatic as well as by peripheral cells, but these experiments did not distinguish between the relative contributions of the liver and the rest of the splanchnic tissues, and the problem remains unsolved. Comparing the intraportal and peripheral venous routes of insulin administration in anaesthetized dogs, Madison and Unger[158] claim to have found that intraportal insulin produces a greater decrease of hepatic glucose production and a relatively smaller increase of peripheral glucose utilization than when insulin is injected into a foreleg vein. Their conclusions were based on glucose gradients without simultaneous blood-flow measurements. More recently, Shoemaker, Mahler and Ashmore[151] using direct simultaneous measurements of glucose concentration gradients across the liver, the total splanchnic bed, and the non-hepatic splanchnic tissue bed, were unable to detect any decrease of hepatic glucose output following various doses of insulin. These studies were performed in unanaesthetized dogs and were combined with simultaneous measurements of hepatic blood-flow. Tarding and Schambye[171] have also failed to show a reduction of hepatic glucose output following constant intraportal infusions of small amounts of insulin.

The rate of decay of the specific activity of plasma glucose after a single injection of ^{14}C-labelled glucose has been used as an index of hepatic glucose output[183]. After the intravenous administration of 10 units of insulin into unanaesthetized, intact dogs, a 'plateau' lasting 10–20 minutes was observed in the specific activity values. Similar effects have been reported in human beings[184]. These observations are claimed to indicate suppression of the entry of glucose into the circulation, but similar experiments using constant infusions of ^{14}C-labelled glucose to eliminate problems of equilibration[185] failed to confirm this interpretation. However, during a prolonged infusion of insulin, the increased hepatic glucose output in response to hypoglycaemia was held in abeyance until the infusion was stopped, and then it rose sharply.

Although the evidence from animal experiments that the sulphonylureas produce hypoglycaemia by releasing insulin from the pancreas is convincing, the only unequivocal evidence of such a mechanism in man is that juvenile diabetic and pancreatectomized patients do not respond to the drugs. Reduction of glucose production in the liver appears to be the reason for the decrease in the blood-sugar level after the administration of sulphonylurea

compounds. This may be produced pharmacologically by a direct, possibly enzymic, effect on the liver or physiologically by an increased output of endogenous insulin. The answer to this problem will probably not be found until our knowledge of the hepatic effect of insulin is more precise. Both mechanisms may operate, and a direct action on the liver-cells may allow endogenous insulin to reduce the hepatic glucose output.

Extrapancreatic Effects of Sulphonylurea Compounds

The *in vitro* effects of sulphonylureas on isolated tissues other than the liver are still largely unexplored. This type of study is important not so much to further our understanding of the mechanism of the fall in blood-sugar produced by the drugs, but to explore the possible ways in which insulin release from the pancreas is accomplished and to detect any secondary effects which may be harmful.

Most workers have been unable to detect any increase in the rate of glucose uptake by the rat hemidiaphragm or in the incorporation of glycogen into diaphragm muscle, when incubations are carried out in bicarbonate buffer with high concentrations of tolbutamide[186,187] or carbutamide[188]. It has been further shown that the stimulating effect of insulin on glucose uptake by the diaphragm is unaffected by tolbutamide[187].

However, Raphaelson[189] found that both carbutamide and tolbutamide considerably increase the glucose uptake by the diaphragm at a comparable sulphonylurea concentration. He found considerable variation at a given drug concentration and there was no suggestion of a dose–response relationship. Similar findings have also been reported elsewhere[190,191]. These different results are difficult to explain at the present time and further work is required.

Tolbutamide and chlorpropamide stimulate the rate of oxidation of ^{14}C-labelled glucose to carbon dioxide by the rat epididymal pad of fat and diminish the incorporation of glucose into lipid[192]. Further experiments with glucose-1-^{14}C and glucose-6-^{14}C show that most of the increased carbon dioxide production arises from carbon-1 of the glucose molecule, suggesting stimulation of the phosphogluconate oxidative pathway. Increased glucose oxidation is generally accompanied by increased synthesis of fatty acid from glucose carbon[193] so the significance of this finding is uncertain.

Slices of liver tissue removed from rats fasted for 48 hours show considerable inhibition of ketogenesis when incubated with chlorpropamide or tolbutamide at concentrations within the expected therapeutic range, and this effect is not altered by using liver tissue from pancreatectomized or alloxanized animals[192]. The relationship of this finding to the production of hypoglycaemia is obscure. Bornstein[194] has reported that hepatic alanine transaminase is inhibited *in vitro* by sulphonylureas.

These scattered, preliminary observations are compatible with the hypothesis that the sulphonylureas affect enzyme reactions which depend upon pyridine nucleotides as co-factors[195].

CLINICAL CONSIDERATIONS OF THE SULPHONYLUREA COMPOUNDS

The sulphonylurea compounds are only effective in patients with mild diabetes of the late-onset or adult type; 60–70 per cent of the diabetic population belong to this group. They are not insulin-deficient (as defined above) and they have no tendency to develop diabetic ketosis. In contrast, the sulphonylureas are useless in patients with severe diabetes of the juvenile type, who are insulin-deficient and need daily injections of insulin to maintain good health. Sulphonylurea compounds do not usually allow a reduction of the insulin dose in these patients, and a labile or 'brittle' diabetic patient cannot be made more stable.

The majority of patients with mild diabetes of the adult type are obese, and weight reduction with adequate restriction of carbohydrate in the diet will often control their symptoms and hyperglycaemia. There is, however, a small group of middle-aged or elderly diabetic patients, constituting perhaps 5–10 per cent of the total diabetic population whose diabetes has developed late in life; they have little or no tendency to ketosis, they are often under-weight, and their hyperglycaemia cannot be adequately controlled by diet alone. These patients would otherwise require insulin and will benefit most from treatment with the sulphonylurea compounds.

Patients suitable for treatment with the sulphonylurea compounds can usually be selected on clinical grounds alone although this may be difficult if the patient is already taking insulin. Objective tests, therefore, have been devised to assess the likelihood of a reasonable therapeutic response to tolbutamide. At the Joslin Clinic[83] a single 3-g oral dose of tolbutamide is given during fasting; after 4 hours, the blood-sugar in patients expected to respond to the drug falls to 100 mg/100 ml., or less (Somogyi–Nelson technique). Patients with a fasting blood-sugar of over 250 mg/100 ml. generally do not show the requisite reduction in the blood-sugar and are usually unsuitable for treatment with tolbutamide. Selection by this method is stringent and reasonably reliable, but some suitable patients may be missed. Duncan, Lee and Young[196] used the development of ketoacidosis as an index of response to tolbutamide; if ketones appear in the urine 8 hours after the last insulin dose, oral therapy is contra-indicated, but if no ketones have appeared within 24 hours, treatment with tolbutamide will usually be successful. There are, however, some patients unresponsive to tolbutamide who do not easily develop ketosis. Thus, the best method of selection is a therapeutic trial. This is best carried out by using placebo tablets[145,197] but great care must be taken with patients already receiving insulin, and the change-over is best performed in hospital if the patient is not of the maturity-onset type.

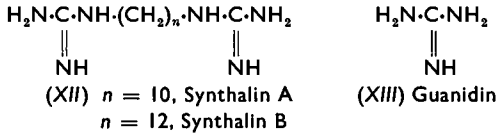
About 5–10 per cent of patients who initially respond well to tolbutamide cease to do so after some months of treatment. Dietary indiscretions may explain a few of these cases, but there are many who appear to develop a genuine resistance to the drug. When assessed in hospital, hyperglycaemia persists despite large doses and a rigid diet, and the drug may be stopped without any further rise of the blood-sugar level[198]. An 'exhaustion' of the pancreatic beta-cells may be produced due to repeated stimulation, although

Pfeiffer[199] has found that the dose of insulin needed afterwards is no higher than that needed before tolbutamide was begun, and chlorpropamide is often successful[90]. Nevertheless, following a period of insulin treatment, many of these patients may again respond to tolbutamide.

Sulphonylureas should only be used when frequent and detailed observation of the patient is possible. The long-term toxicity is unknown so that sulphonylurea treatment is only justified if hyperglycaemia is adequately controlled. A high renal threshold for glucose makes urine sugar-testing a poor index of blood-sugar concentration in many of the diabetic patients who are suitable for the sulphonylureas, and, therefore, frequent blood-sugar estimations should be performed. Ketoacidosis may occur at the time of changing over from insulin or it may appear rapidly during the course of an acute infection. Even without ketonuria, serious hyperglycaemia due to acquired resistance may develop insidiously. Hypoglycaemia is rare with tolbutamide but prolonged hypoglycaemia is an important danger with chlorpropamide. Like the hypoglycaemia of the long-acting insulin preparations, it may only respond slowly to glucose administration. Obesity with its attendant dangers develops easily and excessive weight gain can only be avoided by careful supervision of the patient's diet. It is important that the sulphonylurea compounds directly inhibit enzyme systems in the liver (and possibly elsewhere) as any drug which chronically affects hepatic function may ultimately damage the liver cells. It is too early to say whether the incidence of 'degenerative' complications of diabetes mellitus will be affected by treatment with tolbutamide. Better control of hyperglycaemia tends to reduce the risk of complications but any drug which increases the tendency to haemorrhage must be used with great caution, particularly in patients with retinopathy. Carbutamide increases capillary fragility[200] but careful studies have failed to reveal any difference in the incidence of haemostatic abnormalities between insulin-treated and tolbutamide-treated patients[201].

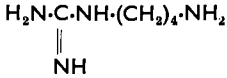
THE GUANIDINE DERIVATIVES

Synthalin (*XII*) was discovered in Minkowski's clinic in Breslau in 1926, after earlier scattered reports that administration of guanidine (*XIII*) lowers the blood-sugar concentration[201a]. Since guanidine is a highly toxic substance, particularly to the liver, attempts were made to produce com-



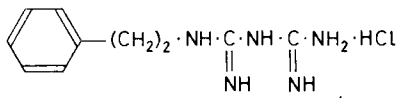
pounds which were less toxic but still exerted a hypoglycaemic action[3]. As the substituent side-chains were made longer, the compounds became less toxic and more effective as hypoglycaemic agents. For example, agmatine (*XIV*) is less toxic and more hypoglycaemic than guanidine and the pentamethylene and hexamethylene homologues are even better. The two synthalins, (*XII*) are equally efficacious and show high hypoglycaemic activity.

de Bodo and Marks[202] showed that synthalin inhibits tissue respiration but increases the glucose uptake into muscle with a concomitant increase of lactic acid production, thus simulating some of the effects of anaerobic glycolysis.



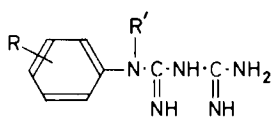
(*XIV*) Agmatine

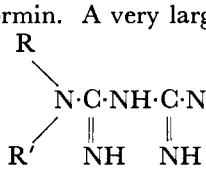
Synthalin was used extensively in diabetic patients during the late 1920's but following reports by Bertram[5] in 1927 that large doses may produce histological changes in the liver and kidney of animals within a few days, the drug fell into disfavour, although it was still used sporadically throughout the 1930's.



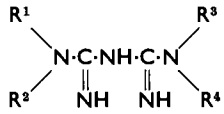
(*XV*) Phenethylguanylguanidine

In 1957 the hypoglycaemic effect of N^1-β-phenethylformamidinyliminourea hydrochloride or phenethylguanylguanidine (DBI, phenformin, Dibotin, *XV*) was described by Ungar, Freedman and Shapiro[203]. As will be seen from formulae (*XII*) and (*XV*), the drug has only a general structural resemblance to synthalin. The chemical properties of phenformin are given by Shapiro, Parrino and Freedman[204]. Hydrolyses suggested that it is stable in strongly acidic solutions and that it can be degraded in hot alkaline solutions to β-phenethylguanidine, β-phenethylurea and β-phenethylamine. Attempts at alkylation with alkyl halides yielded the corresponding hydrohalide salts. The same worker[205] synthesized a large number of arylbiguanides of the type:



where R represented mono-, di- or tri-substitution with alkyl, alkoxy, amino, arylamino, halogen, hydroxy, or R and R′ combined to give a heterocyclic ring, or where R′ was hydrogen, methyl or ethyl. None of these compounds, however, showed oral hypoglycaemic activity approaching that of phenformin. A very large number of N'-alkyl- and aralkylbiguanides of the type  were then prepared[206] and many of them showed good oral hypoglycaemic activity. In the series R = alkyl, the activity reached a peak with *n*-pentyl, then diminished through *n*-octyl and disappeared with *n*-decyl. In comparison, branched or cyclic structures showed less activity. The most effective variant of R′ was hydrogen. In

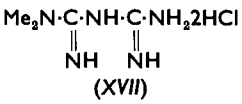
the aralkyl series, good activity occurred with R = benzyl and peak effects were obtained with the *p*-chlorobenzyl and *β*-phenethyl (phenformin, *XV*) compounds. Lengthening of or substitution on the alkylene chain diminished or abolished activity. In a series of N^1,N^5-substituted biguanides (*XVI*) good hypoglycaemic activity was attained, particularly when N^5-methyl or N^5-dimethyl substituents were introduced in physiologically active N^1-substituted biguanides[207]. However hypoglycaemic N^1,N^5-substituted biguanides were found to be less easily absorbed from the gastro-intestinal tract than the corresponding N^1-substituted compounds.



(*XVI*)

The most active compounds had R^2 = H, R^3 = R^4 = Me and R^1 = Bu or $PhCH_2$. Generally, the most active of these compounds also produced the most side-effects.

In 1929, Slotta and Tschesche[208] synthesized a series of biguanides and examined them for hypoglycaemic activity. The most active compound was *NN*-dimethylguanylguanidine, (*NN*-dimethylbiguanide, metformin, Glucophage, *XVII*) and this has recently reappeared for clinical trial. It produces a fall of blood-sugar concentration but gastro-intestinal side-effects were seen in five out of eight diabetic patients[70]. Other workers claim less gastro-intestinal disorder when it is compared with phenformin[208a].



(*XVII*)

Up to the time of writing, no means of estimating the biguanides in biological fluids have been developed, so that there is little or no information about their intestinal absorption or metabolic fate. Based on the doses necessary to lower the blood-sugar level, it seems that phenformin is absorbed to a greater extent than metformin. One study using ^{14}C-labelled phenformin has shown that radioactivity becomes concentrated in the stomach and liver with less in muscle[208b].

The mode of action of these compounds[208c] is different from that of the sulphonylureas as the drugs produce hypoglycaemia in pancreatectomized animals[209], in severe alloxan diabetes[203] and in patients with the juvenile or insulin-deficient type of diabetes[210]. In spite of the differences of detailed chemical structure, the recently synthesized diguanides affect biological tissues in much the same way as synthalin.

Phenformin increases the glucose uptake by the rat diaphragm *in vitro*, but the muscle glycogen content decreases[211]; there is also a marked increase in lactic acid production and a decrease in oxygen consumption. This suggests that phenformin stimulates anaerobic glycolysis by inhibiting oxidative enzyme systems, thereby mimicking the metabolic effect of severe muscular effort. Similar *in vitro* effects have been described with rat or guinea-pig liver slices[212]; glucose output is not reduced and there is no inhibition of glucose-6-phosphatase. Using adipose tissue, Wick, Larson and Serif[213]

have also shown that phenformin *in vitro* inhibits the oxidation of glucose, acetate and succinate by adipose tissue and also considerably reduces fat synthesis. Similar results have been obtained *in vivo*. Hepatic glycogen is reduced[211] and there is a decreased, hyperglycaemic response to glucagon and adrenaline[209]. Muscle glycogen is also reduced and blood lactate levels rise. Diaphragm muscle, adipose tissue and liver slices from phenformin-treated animals do not incorporate ^{14}C into protein or fat when incubated with ^{14}C-glucose[214]. In man, the blood pyruvate and lactate levels are raised[215] and the alkali reserve is lowered. In diabetic patients treated with phenformin, this may lead to a dangerous acidosis with ketonuria, although there is a normal blood-sugar concentration[216]. Hypoglycaemia cannot be obtained in normal humans[215] although with intact animals a decrease in the blood-sugar concentration is easily produced. In man, there is no change in hepatic vein glucose, urea, pyruvate or lactate nor in the oxygen consumption of the liver[217]. Lactate levels in the blood from the femoral artery also remain unchanged. These results confirm that the effects of phenformin are largely mediated on the peripheral tissues.

The evidence therefore suggests that tissue anoxia is produced but how this is brought about is unknown. It may be attributed to inhibition of cytochrome oxidase and succinc dehydrogenase[212,218], but a decrease in oxidative phosphorylation would have the same result. Many factors which inhibit oxidative phosphorylation increase the glucose uptake in the rat diaphragm[219]. Later work suggests that biguanides do not reduce oxygen uptake by liver mitochondria[220] but inhibit the transfer of energy-rich phosphate bonds to adenosine diphosphate[221].

Clinically, the biguanides are important because, if tolerated, they would be useful in controlling the hyperglycaemia of *all* types of diabetes. However, the therapeutic dose of phenformin is too near the toxic one, and produces severe anorexia, nausea and vomiting in about 40 to 50 per cent of patients. Weakness, lethargy and weight loss may develop later[222]. Despite the fact that toxic damage to the renal tubules has been reported in the rabbit[223] and in the guinea-pig[224] after large doses of phenformin, no serious toxic effects have been reported in humans during the past 2 years and repeated liver function studies have shown that phenformin, in contrast to synthalin, does not produce any hepatic abnormality[225,226]. The apparent lack of hepato-toxic effects may be related to the fact that biguanides are able to form stable chelate rings. Krall, White and Bradley[227] have shown, however, that phenformin combined with insulin may make a labile or 'brittle' diabetic patient more stable, and this may prove to be its main clinical application unless a less toxic derivative is discovered.

EVALUATION OF HYPOGLYCAEMIC ACTIVITY

The preliminary testing of compounds for hypoglycaemic activity may be carried out on rats, from which food has been withdrawn for 18 hours. For general screening purposes, the compound is given orally at a dose level of 100–300 mg/kg[99] and the blood glucose concentration is determined on samples taken from the tail vein at 1, 2, 3, 5, and 7 hours after administration. A measure of the degree and duration of the hypoglycaemia is then

calculated, and used to plot dose–response curves[57,67]. Rabbits, cats, dogs, guinea-pigs and monkeys have been used for extending the scope of these preliminary tests[203].

Compounds such as tolbutamide and chlorpropamide also exert their hypoglycaemic effect when given intravenously, after they have been dissolved in water containing sufficient 0·1N sodium hydroxide solution to bring the pH to 8–9[67]. Other compounds such as phenformin produce hypoglycaemia on subcutaneous injection into guinea-pigs but fail to lower the blood glucose concentration in dogs[203].

Animals in which diabetes is artificially induced are used in further studies. This may be achieved either by means of alloxan injections which destroy the insulin-producing beta-cells of the pancreas or by total pancreatectomy. Rats become diabetic when given alloxan monohydrate intravenously (40 mg/kg)[53] or intraperitoneally (250 mg/kg)[203], but rabbits and monkeys need a higher intravenous dose (150–200 mg/kg)[53,203]. Dogs may be made diabetic by an intravenous injection of 75 mg/kg of alloxan monohydrate or by removal of the pancreas[53]. After injection or pancreatectomy, the animals are kept in metabolism cages so that a regular check may be kept on urine volume, urine sugar excretion, and food consumption. It is of interest that injections of alloxan do not produce diabetes in the guinea-pig[228].

Variation in the effect of a compound within an animal species is often high and it is essential to have a sufficient number of animals for each dose level for the results to be analysed statistically[53,203]. Compounds such as the sulphonamides do not generally produce hypoglycaemia in severe alloxan-diabetic animals or after total removal of the pancreas, whereas other compounds such as phenformin do so in these conditions. When highly potent hypoglycaemic drugs are undergoing toxicity studies, the possibility that death is the result of a hypoglycaemic convulsion must be borne in mind.

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6

ANTIFUNGAL AGENTS

E. P. TAYLOR and P. F. D'ARCY

INTRODUCTION

OF ALL the microbial infections of man, the diseases caused by fungi are perhaps the most difficult to modify in their course or to prevent, and it is increasingly evident that the incidence of such diseases is mounting. The wartime experience of the armed forces with fungus infections was most discouraging but knowledge of suitable treatment is slowly accumulating and shows every sign of increasing importance in the future.

Ideally, a perfect antifungal agent should be a compound that is chemically, therapeutically and cosmetically acceptable, and active against a wide spectrum of pathogenic fungi without having any toxic effects to the host. This may seem to be a formidable target but the amount of research that has been carried out in this field has already resulted in considerable advances in the required direction; however, in many cases of surface fungal infections the treatments are generally round-about and non-specific. Methods of treatment are satisfactory in only two of the deep fungus infections, and even in these there is much room for improvement. Despite the advances that have been made in the treatment of many infections with chemotherapy and antibiotics, the natural defence mechanisms of the body remain the chief safeguard against fungal infection. As yet there are no practical methods of immunization. However, if there were a marked serological response to infection this might well produce a life-long immunity to subsequent re-infection. The physiological condition of the host may also influence fungal infection in man. For example, some fungal infections which are resistant in childhood clear spontaneously at puberty[1]. In addition, general moniliasis, frequently present in diabetics, has been claimed to be associated with a high glucose tolerance of the skin[2].

There have been many attempts to establish structure–activity relationships in the chemotherapy of fungal infections without any great success. A large number of compounds with quite unrelated chemical structure have been shown to possess marked but specific antifungal activity, as is indicated by the following random selection of medicaments at present commercially available: antibiotics, diamthazole, dequalinium chloride, hormones, chlorphenesin, salicylates, stilbamidine, hedaquinium chloride and undecylenic acid. In addition to these and other recent compounds, there are many well established older remedies that continue to be used with marked therapeutic success *e.g.*: iodine, Castellani's paint, Gentian Violet, Whitfield's ointment and formalin. Boric acid, although once widely used, particularly in monilia infections of the mouth, is now out of favour since there have been recent reports of its quite alarming toxicity.

ECOLOGY AND DISTRIBUTION OF FUNGI PATHOGENIC TO MAN

Probably all pathogenic fungi exist as saprophytes in soil, or on vegetation or humus; when these fungi become parasitic in man, the majority change their mode of growth and reproduction. Others which are unable to adapt themselves to a new environment by changing their growth habits fail to become pathogenic. Relatively few fungi are pathogenic to man and the higher animals, and probably none is an obligate parasite. The carefully controlled conditions of laboratory studies do not always reflect the cultural requirements of fungi in their natural habitat; this is well illustrated by the often widely diverging results obtained in the assessment of antifungal activity of compounds by *in vitro* and *in vivo* techniques. Moreover, in the *in vitro* tests there is only the relationship between fungus and antifungal agent to be considered, whereas in the *in vivo* tests there is a third factor, the host. It is difficult to generalize as to the ecology of pathogenic fungal infection but it has been well established that geographical features such as temperature, humidity, rainfall, and the nature of the substrate play an important role in the distribution of these fungi. A critical study of the nature of these environments may assist the planning of the chemotherapy of these infections. Furthermore, except for the dermatophytes which are transmissible from man to man or animal to man, there is little knowledge of how fungus diseases are spread.

Fungal diseases in man can be divided into two principal groups, the superficial and the systemic.

Superficial Infections

These diseases assume such a variety of form that many clinical states may be mimicked; indeed any classification of the superficial fungus infections on an etiologic basis is impracticable since similar symptoms may be produced by different organisms. A topographic classification must therefore be used.

The vast groups of infection resulting from tinea-causing organisms are widely distributed over the body. The causative organisms of these infections are *Trichophyton* species (*rubrum, verrucosum, mentagrophytes, tonsurans*), *Candida albicans, Epidermophyton floccosum,* and *Microsporum* species (*canis, audouini*) alone or together. The diseases are tinea pedis or athlete's foot; tinea manus, which affects the hands; tinea cruris or dhobi's itch, common in the groin, perineum and perianal organs; tinea corporis, probably responsible for the term ringworm and widely distributed in a variety of lesions over the body surface; tinea capitis, which attacks the scalp; tinea barbae, somewhat uncommon and easily confused with an allied bacterial infection, folliculitis barbae; tinea versicolor, a common very superficial fungus infection, usually asymptomatic, the complaint being of a cosmetic nature.

In addition, there are many fungal infections of the genito-urinary and anal regions, as for example mycotic vulvovaginitis and pruritus ani. The organism responsible for these monilial infections is usually *C. albicans.* Other superficial fungal infections include onchomycosis, which affects the nails and is caused by *Trichophyton* species (*rubrum* and *mentagrophytes*) and rarely by other dermatophytes; and otamycosis, an inflammatory disease involving the external ear and ear canal, is principally not a fungal infection,

since pathogenic fungi are only infrequently present. Certain saprophytic organisms found in association with this condition are also commonly observed in normal ears. Erythrasma, a benign fungal infection usually localized in the inguinal region and other intertriginous areas, is caused by *Nocardia minutissima.*

During recent years, particular attention has been paid to mycotic infections of the oral cavity. It has been demonstrated that fungi of many types can be isolated both from apparently normal mouths and from mouths showing various disorders. The main oral fungal infections[3] are by briefly described below.

Angular cheilosis

This condition is characterized by cracks, fissures and inflammatory changes at the angles of the mouth, and is mainly produced by various species of *Candida* (*C. albicans, C. krusei, C. parapsilosis*). In addition infection by *Torulopsis glabrata* has been recorded.

Gingivostomatitis

This term is used to describe all inflammatory changes of the gingivae and oral mucosa, and includes Vincent's disease. The causative organism is generally *C. albicans,* although the presence of *C. tropicalis* and *Aspergillus niger* has also been recorded.

Lingua nigra (*Black hairy tongue*)

This affects the dorsum of the tongue, generally anterior to the circumvallate papillae. The filiform papillae are markedly elongated and the affected area is usually black or dark brown. The elongated papillae may stimulate the soft palate and produce a feeling of nausea, but apart from this no other subjective symptoms occur. In addition to Gram-positive and Gram-negative cocci, *C. albicans* has been isolated from this condition. There is, however, some doubt as to whether this fungus is the causative agent or whether it is merely present as a saprophyte; indeed there is some discussion as to whether this condition is of mycotic origin.

Lingua geographica

The tongue is also infected in this condition, and shows a circumscribed circinate desquamation of the epithelium of the dorsum and of the edges, which apparently spreads to the periphery while healing at the centre. It is a chronic condition of unknown aetiology, continually changing in appearance; again *C. albicans* is present, although apparently not to an abnormal degree.

In all these oral conditions, it is obvious that other predisposing factors play important roles since *C. albicans* commonly occurs in the human mouth. It may be that the proliferating epithelia of the tongue provides a favourable habitat for the development of common saprophytic fungi.

A final example of superficial fungal diseases is the dermatophytid type caused by *Trichophyton* species. The so called 'ids' are secondary eruptions spreading from a primary focus in specially sensitized individuals. They commonly appear as vesicles on the ends of the fingers and on the hands, but

may in many instances be generalized. Criteria for 'ids' are, (*a*) demonstration of fungi in a primary focus (*b*) lack of fungi in the secondary 'id' lesion, and (*c*) disappearance of the 'id' when the primary focus is removed[4].

Systemic Infections

Until recently, the systemic fungal diseases were considered to be progressive and invariably fatal; however, recent advances in antifungal therapy have in many cases improved this dismal outlook. Examples of these infections are given below.

An excellent review on the systemic mycoses has recently been published by Kirk and Morgan[5].

Actinomycosis

Actinomycosis is one of the more common of the systemic mycoses, being distributed throughout the world. It is caused by *Actinomyces bovis* and is an infection of cattle communicable to man. Actinomycosis is thought to be acquired endogenously. The disease presents various forms, being most frequently characterized by the formation of tumours in the jaws and tongue; these tumours have characteristic hard woody lesions that subsequently break down and form multiple sinus tracts.

Blastomycosis

North American blastomycosis is a granulomatous disease caused by *Blastomyces dermatitidis* and is predominant in the central and eastern parts of the U.S.A. Dissemination by contagion has not been demonstrated, and the organism is therefore suspected of being a soil or plant saprophyte, although it has not yet been isolated from such sources. This disease shows both systemic and cutaneous forms. The systemic form is characterized by an initial pulmonary infection which is followed by a progressive dissemination to most organs. The cutaneous type, formerly thought to be the result of direct inoculation into the skin, is now believed to arrive as a secondary infection from pulmonary foci.

There is a second form of this disease endemic in many parts of south and central America. South American blastomycosis is a progressive granulomatous disease of the mucous membranes, lungs, lymph nodes, skin and viscera. The various symptom complexes are thought to depend on the portal of entry. One of the most common types combines lesions of the oral mucosa, cervical lymph nodes and lungs.

Nocardiosis

This granulomatous disease has, in the past, been confused with tuberculosis and actinomycosis. It is world wide in distribution, several species of *Nocardia* particularly *N. asteroides* and *N. brasiliensis* being responsible. These organisms have been frequently cultured from soil, and infection is thought to be acquired by inhalation of dust containing the organism or by direct inoculation of contaminated material into the skin. Pulmonary infections may closely simulate tuberculosis or actinomycosis, although neurological manifestation may be the first symptom. The incidence of nocardiosis as a

terminal condition of fatal diseases appears to be increasing. A localized form of the disease has been described as mycetoma or Madura foot; this is characterized by localized indurated areas, usually of the extremities, slowly involving underlying base tissue and eventually resulting in extensive deformity.

Cryptococcosis

Cryptococcosis, a disease world wide in distribution, was formerly thought to involve the central nervous system in every case and to cause death. However, exceptions are now being encountered with greater frequency. The disease is caused by *Cryptococcus neoformans*, an encapsulated budding fungus, recently found to lead a widespread saprophytic existence. Although once considered to be an aggressive invader, the organism is now recognized as an opportunist frequently taking advantage of defective defence mechanisms in the host. Cryptococcosis has been associated with Hodgkin's disease and other lymphophomas and has been found to co-exist with tuberculosis, histoplasmosis and coccidioidomycosis. It is thought that most instances of central nervous system involvement are dissemination from either clinical or subclinical pulmonary lesions. While the incidence of pulmonary involvement is unknown, it is becoming increasingly evident that transitory infections limited to the lungs occur more often than was formerly suspected.

Sporotrichosis

Sporotrichosis, which is world wide in distribution is frequently found in agricultural or horticultural workers. It is caused by *Sporotrichum schenckii*, which has been isolated from soil, wood and plants. The condition is characterized by multiple subcutaneous nodules along the course of the lymphatics. The organism is thought to invade the host by entry through damaged skin.

Histoplasmosis

Histoplasmosis, formerly considered rare, now appears to be the most common and one of the most widely distributed of the systemic mycoses. The causative organism is a small budding fungus, *Histoplasma capsulatum*, which is responsible for a variety of symptoms including anaemia, loss of weight, irregular fever and hepatosplenomegaly. Epidemiologic studies indicate that most infections produced by *H. capsulatum* are asymptomatic, or so slight in degree as not to require treatment. However, the serious disseminated form of this infection is most frequently found in infants and in middle-aged people. Patients with lymphomas, particularly Hodgkin's disease, tuberculosis and other crippling diseases appear to be susceptible to histoplasmosis.

Coccidioidomycosis

This infection is endemic in arid and semi-arid areas, the organism, *Coccidioides immitis*, having been repeatedly demonstrated in the soil of such areas. Infection is usually acquired by inhalation of the spores. The disease spectrum varies from slight non-specific pneumonitis to progressive, chronic,

disseminated granulomatous disease. Epidemiologic studies indicate that the disease is asymptomatic or slight in approximately 60 per cent of patients.

Aspergillosis

This is usually a secondary infection complicating an existing disease of the lungs. The fungus, *Aspergillus fumigatus*, is able to invade areas of devitalized lung tissue in diseases such as bronchiectasis, tuberculosis and carcinoma. The disease constitutes an occupational hazard for those who work with straw, grain and flour, which are liable to be heavily contaminated with the spores of the organism.

ANTIFUNGAL AGENTS IN CLINICAL USE

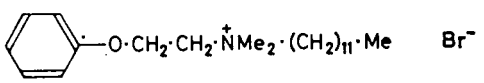
To be active as an antifungal agent, whether as a fungicide or a fungistat, a compound must possess two properties—first, the ability to penetrate to, and accumulate at, the site of action within or on the surface of the fungal cell, and second, the power to interfere with at least one process vital to the continued existence or growth of the cell. An antifungal agent may produce its action by inhibition of the enzyme system of the fungus, and/or by its disorganization of the structure or function of the cellular components. In addition, some agents do not exert any direct chemotherapeutic action against the fungus but act solely by stimulating the defence mechanism of the host.

There are no definite structure–activity relationships of general applicability in this field of chemotherapy, and therefore in this review it is proposed to group the antifungal agents in an arbitrary classification based solely upon their chemical structures. Where available commercial names are given.

Quaternary Ammonium Salts

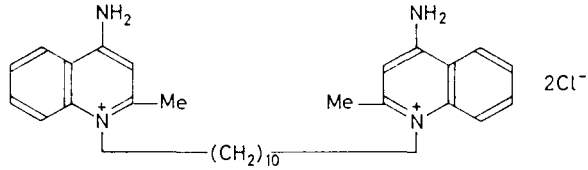
The chemotherapeutic application of these compounds has received a tremendous impetus since the end of the Second World War. Many members of this family have been shown to possess very marked antibacterial action, as well as other pronounced pharmacological and pharmacodynamic actions. More recent studies have also uncovered their potent antifungal activity. Although it has been demonstrated[6] that quaternaries possess powerful fungistatic activity against certain species, their fungicidal activity however is generally low. The most important members of this group are listed below.

Domiphen bromide (Bradosol) or dodecyldimethyl-β-phenoxyethylammonium bromide



This colourless powder (m.p. 112–113°C) is prepared by quaternizing phenoxyethyl dimethylamine with dodecylbromide for 2 hours at 100°C; it is readily soluble in water[7]. This agent is incorporated into antiseptic throat lozenges and is effective against *Candida* species *in vitro*[8,9].

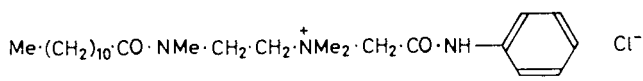
Dequalinium chloride (Dequadin) or decamethylenebis(4-aminoquinaldinium chloride)



This is an almost colourless powder (m.p. 326°C (d)) prepared by refluxing a solution of decamethylene di-iodide with excess 4-aminoquinaldine and converting the resulting quaternary iodide to chloride by treatment with silver chloride and methanol[10]. It is somewhat sparingly soluble in water. Although introduced primarily as an antibacterial agent[11], it is now finding use as a topical agent in the treatment of certain fungal diseases[12], notably lingua nigra[13], and the multiform vaginal conditions[14,15]. The clinical applications of dequalinium have recently been summarized[16]. Dequalinium is available as the active constituent of lozenges, pessaries, cream, paint, gelatin sponge and tulle. It has recently been marketed in combination with prednisolone in the form of a cream (Dequalone-P).

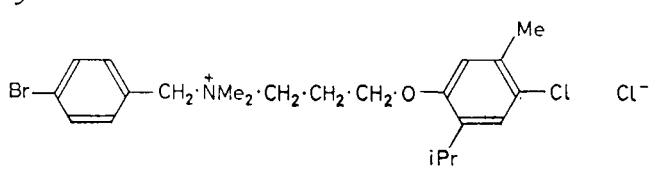
Recently it has been shown that monoquaternary derivations of 4-aminoquinaldine, particularly 4-aminoquinaldinium lauryl acetate (Laurodin) possess antifungal activity *in vitro* against *C. albicans* and *T. mentagrophytes* of approximately the same order as dequalinium[17].

(N'-Dodecanoyl-N'-methylaminoethyl)(phenylcarbamylmethyl)dimethylammonium chloride



This compound is a white crystalline solid (m.p. 124°C) completely soluble in water. It is prepared by refluxing an ethyl acetate solution of dodecanoyl-*N*,*N*-dimethylaminoethyl-*N'*-methylamide (obtained from lauric acid and trimethylethylenediamine) and chloroacetanilide for 6 hours, filtering off the product and recrystallizing from acetone[18]. This salt was primarily developed as a topical and general household disinfectant and detergent, but has also been shown to have high activity against monilial infections caused by *C. albicans*[19]. It is the active constituent of Desogen throat lozenges.

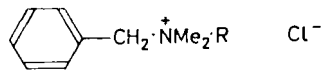
Halopenium or 4-bromobenzyl-3-(4-chloro-5-methyl-2-isopropylphenoxy)-propyldimethylammonium chloride



When *p*-chlorothymol (as its sodium salt) is treated with trimethylene bromhydrin, it gives an ether alcohol which, after conversion to the bromide

by phosphorus tribromide, is reacted with methanolic dimethylamine at 120–125°C. Quaternization of the resulting tertiary amine with benzyl-chloride yields halopenium (m.p. 191°C)[20]. Although originally prepared in 1942, it has only recently been marketed, as the antifungal constituent of Trillets throat lozenges which are active against *Candida* species.

Benzalkonium chloride (*Roccal*) *or alkylbenzyldimethylammonium chloride*

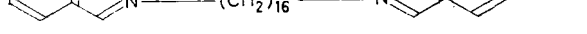


(R = alkyl from C_8H_{17} to $C_{18}H_{37}$)

This compound, which is not a single chemical entity, may be prepared by quaternizing benzyldimethylamine with a crude alkyl chloride. It is official in the U.S.A. as a white or yellow powder, but is only available as a solution in the United Kingdom (*British Pharmaceutical Codex* (B.P.C.)). Although primarily a general purpose and skin sterilizing agent a dilute solution has been recommended for the treatment of superficial fungal infections[21].

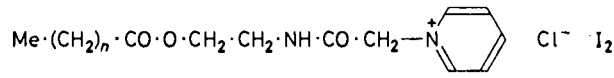
In an investigation into the antifungal activity of a number of commercial surface active quaternary compounds, which include Cetrimide, cetylpyridinium chloride, and laurylpyridinium chloride (which are closely related to Roccal), it has been shown that laurylpyridinium chloride is particularly active against *T. mentagrophytes* and has been recommended for clinical investigation in the therapy of tinea pedis[22]. More recently it has been suggested that the use of Cetrimide be investigated as an antifungal agent in swimming baths, since *in vitro* studies have shown this quaternary to be active against the growth of *T. interdigitale*, *T. rubrum* and *Epidermophyton floccosum*[23].

Hedaquinium chloride (*Teoquil*) *or hexadecamethylenebis*(*isoquinolinium chloride*)



This colourless powder (m.p. 128–129°C) is prepared by refluxing hexadecamethylene di-iodide with excess isoquinoline, and converting the resulting quaternary iodide to chloride by treatment with silver chloride and methanol; it is extremely water-soluble[24]. This compound has been shown to be markedly effective as a fungistatic agent *in vitro* against a wide spectrum of pathogenic fungi including *C. albicans*, *M. canis*, *Trichophyton* species (*mentagrophytes*, *rubrum* and *verrucosum*) and *Actinomyces bovis*[25]. Renzi, Garner and Burger[26] have used hedaquinium as their standard antifungal agent when investigating the effect on antifungal activity of opening the ring in certain phenothiazine-type compounds. Hedaquinium is used topically as a cream or dusting powder in the treatment of tinea infections of the skin, in particular tinea pedis[27].

Undecoylium chloride with iodine



(n = 6 to 12)

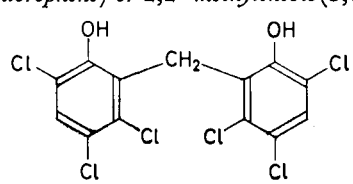
This is a complex of iodine with a quaternary salt, acylcholaminoformylmethylpyridinium chloride. This complex has been found to be effective as a vaginal douche in monilia infections, and is also used for the local treatment of fungal infections of the scalp and feet. It differs from other quaternary ammonium compounds in that the antimicrobial activity of the complex is derived almost wholly from the elemental iodine which is slowly released on contact with skin and mucous membranes. The wetting action of the quaternary detergents, however, facilitates contact of the iodine with surface areas. The complex, unlike tincture of iodine, does not cause stinging or irritation and in addition does not stain skin or clothing[28].

The antimicrobial activity of many of the quaternary ammonium compounds has been found to be markedly reduced in the presence of soaps[11]. It is of interest, therefore, to note a recent publication by Rebold, Bovi and Medici[29] in which it is stated that replacement of the halide ion by certain organic ions (*e.g.* dithiocarbamate, mercaptobenzothiazolate, mercaptobenzoate, naphthalene and naphthol sulphonates, phenol sulphonate, benzosulphimide and aminophthaleins) abolishes this soap antagonism. It is possible that this observation might be of value in the future development of new antifungal agents.

Phenols

Many phenolic substances have long been known to have antibacterial action, and some of them, for example, thymol and the halogenated phenols, have been used as antifungal agents. These halogenated compounds tend to be less toxic than phenol itself. Thymol has been used locally, mainly as a dusting powder in the treatment of superficial fungal infections. Chlorothymol (4-chloro-5-methyl-2-isopropylphenol) is also a potent antifungal but is intensely irritant to mucous membranes. Cross and his colleagues[30,31] have prepared the 4-halothymols and compared their antifungal activity in propylene glycol solution against several fungi including *Trychophyton* species, *C. albicans* and *Microsporum* species. They found that 4-iodo-5-methyl-2-isopropylphenol was the most effective agent, then came the corresponding bromo compound, and finally the chloro and fluoro compounds. Thymol itself appeared to be the least active. Some phenols in clinical use at the present time are given below.

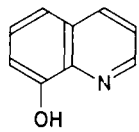
Hexachlorophane (*Hexachlorophene*) *or 2,2′-methylenebis(3,4,6-trichlorophenol)*



This crystalline compound is both colourless and odourless, and has melting

point of 161–167°C. It is prepared by condensing 2,4,5-trichlorophenol with formaldehyde in methanol in the presence of sulphuric acid at 0–5°C and recrystallizing the crude reaction product from benzene or ethylene dichloride[32]. It is insoluble in water. While the main use of hexachlorophane is as a non-irritant potent antibacterial agent, good results have been obtained in the treatment of certain dermatoses, in particular monilial intertrigo[33].

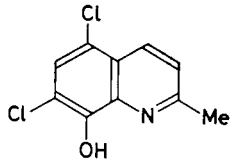
Oxine (8-hydroxyquinoline)



This is a white or faint yellow, pleasant smelling powder (m.p. 75–76°C) prepared by fusion of quinoline-8-sulphonic acid with 50 per cent caustic soda in an autoclave at 180°C. It is very sparingly soluble in water. Potassium hydroxyquinoline sulphate (B.P.C.) is an equimolecular mixture of potassium sulphate and 8-hydroxyquinoline sulphate, and is readily water soluble. Oxine has been shown to have an outstanding antifungal action against *T. mentagrophytes*[34] and although rarely used internally has been markedly successful as a skin lotion in superficial mycotic infections.

Vaidya and Cannon[35] have recently prepared some esters of 8-hydroxy-2-quinoline acrylic acid as antifungal agents, but no results of antifungal activity are as yet available.

Chlorquinaldol or 5,7-dichloro-8-hydroxy-2-methylquinoline



Yellowish needles (m.p. 111–112°C) of this compound are prepared by leading the calculated quantity of chlorine into a cooled solution of oxine in formic acid[36]. It is used locally as a cream or ointment (concentration 3–5 per cent) in the treatment of superficial mycotic infections and of perionychia[37]. This compound is the active constituent of Steroxin ointment and also of Siogen lozenges. Chlorquinaldol also possesses antibacterial and amoebicidal activity, the latter activity being shared by a variety of other halogenated 8-quinolinols, for example, iodochlorhydroxyquinoline (Vioform, Barquinol, 5-chloro-7-iodo-8-hydroxyquinoline) and di-iodohydroxyquinoline (Diodoquin, 5,7-di-iodo-8-hydroxyquinoline). In a recent publication, Das and Mukherji[38] have described a new method of preparing these iodinated derivatives in which oxine or related compounds are treated with iodine monochloride or trichloride. Both iodochlorhydroxyquinoline and di-iodohydroxyquinoline have been used in the topical treatment of certain pyogenic and fungal infections of the skin. In addition, di-iodohydroxyquinoline is the active principle of Floraquin, which has been used successfully in the treatment of a variety of vaginal infections.

It has recently been shown[39] that a new halogenated oxine derivative,

namely 5-chloro-8-acetoxyquinoline has increased efficiency against pathogenic fungi.

Derivatives of Hydroxybenzoic Acids

The parabens (esters of parahydroxybenzoic acid)

Alkyl esters of *p*-hydroxybenzoic acid have been known to possess antibacterial and antifungal activity for more than 30 years. These simple compounds can be readily obtained from the parent acid by conventional methods.

The commercial application of these esters, which have been widely used as preservatives for pharmaceuticals, cosmetics and foods, has been largely developed in this country. The methyl, ethyl, propyl and butyl esters have been studied most extensively, although benzyl *p*-hydroxybenzoate and other esters and salts have also been available commercially. A wide variety of applications has been reported for these colourless, neutral preservatives. They have the additional attributes of being essentially non-toxic, non-volatile, stable and effective in low concentrations in acid, neutral, and alkaline solutions, against diverse microorganisms. These esters have been the subject of several reviews, notably those by Neidig and Burrell[40], Sabalitschka[41], and Aalto, Firman and Rigler[42].

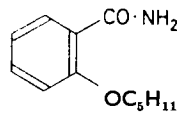
Methyl *p*-hydroxybenzoate (Nipagin M) and its soluble sodium derivative (Nipagin M Sodium), and the corresponding propyl derivatives (Nipasol M, and Nipasol M Sodium) are official in the United Kingdom (B.P.C.). The fungistatic activity of methyl and propyl paraben has been reported by Siegel[43], while Huppert[44] has recently described the antifungal activity of an homologous series of parabens, with up to 16 carbon atoms in the alkyl chain. Huppert reported maximum antifungal activity with the hexyl and heptyl esters, which were found to inhibit the growth of representative strains of most of the fungi known to cause disease in man, particularly those fungi producing systemic infections. Sabalitschka and her colleagues have also reported on the effect of methyl and other parabens on the growth of *C. albicans in vitro*[45,46].

One of the most disturbing features of therapy with some antibiotics (*e.g.* aureomycin, terramycin or chloramphenicol) is the overgrowth of monilia and other yeast-like organisms in the large intestine following the administration of the antibiotic[47]. Consequently particular interest was aroused when McVay and Sprunt[48] reported on their study of moniliasis during aureomycin therapy. These authors found that methyl and propyl paraben had an inhibitory effect *in vitro* on the growth of five strains of yeast and that these two parabens were of value in preventing the overgrowth of *C. albicans* in aureomycin therapy.

Salicylamides

Salicylamide and its derivatives stimulated the interest of workers in both this country and the U.S.A. during the past few years, particularly during the search for new analgesic compounds; in addition to analgesic properties many of these derivatives have shown marked antifungal activity.

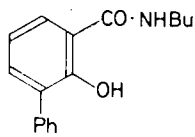
2-n-Amyloxybenzamide or 2-n-pentyloxybenzamide



This colourless crystalline solid (m.p. 186°C) is sparingly water-soluble. It is prepared by dissolving salicylamide in alcoholic sodium ethoxide, adding 1-bromopentane and refluxing the mixture for 6 hours. After filtration the solution is concentrated and the required material precipitated by the addition of water[49]. This substance is a slow acting fungicide of greater fungistatic potency than many of the common antifungal agents (*e.g.* undecylenic acid, salicylanilide, nystatin, and phenyl mercuric acetate); there is evidence that 2-*n*-pentyloxybenzamide has a specific action against dermatophytes commonly responsible for mycotic infections in man[50]. A paper presented to the 1959 British Pharmaceutical Conference reported data on the *in vivo* antifungal activity and toxicity of this and related compounds[51]. This drug is a constituent of Amoxal which is available as a gel, cream and dusting powder.

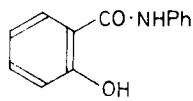
Faust, Jules and Sahyun[52] have also prepared a large number of derivatives of salicylamide as potential analgesics, the most potent being 2-allyloxybenzamide. Several of their compounds, notably 3,5-di-isopropylsalicylamide, exhibited marked antifungal activity. These workers have in addition prepared a large number of derivatives of 3-, 4-, and 5-phenylsalicylamides[53]; in addition to their analgesic activities, many of these were found to be highly active against dermatophytes[54,55]. The most potent member of this series is *N*-*n*-butyl-3-phenylsalicylamide.

Bynamid or N-n-butyl-3-phenylsalicylamide



This compound, which occurs as colourless needles (m.p. 70–72°C) is prepared by refluxing a mixture of methyl 3-phenylsalicylate (1 mole) with *n*-butylamine (2 moles) for 7 hours and then making alkaline with sodium bicarbonate[56]. The toxicity, absorption and skin irritant properties of this compound have been described by Seeberg, Hidalgo, Wilken, Beniams and Lundblad[57].

Salicylanilide or N-phenylsalicylamide



This colourless or slightly pink crystalline solid (m.p. 134–135°C) is slightly water-soluble. Although known for many years a new method of preparation has been recently described[58] where salicylic acid and aniline

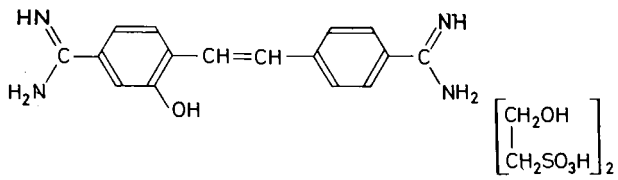
are heated in the presence of tetraethylpyrophosphite for 2 hours. In ointment form it has been used for the treatment of ringworm of the scalp. A mixture of salicylanilide with undecylenic acid and/or its salts has been patented[59].

Diamidines

In view of the interesting trypanocidal activity shown by certain diamidinoalkanes, notably diamidino-undecane dihydrochloride, Ashley and his colleagues[60,61] prepared a large number of aromatic diamidines. Later investigation showed that in addition to their anticipated trypanocidal activity many of these were found to have potent antibacterial and antifungal activity. These compounds may be prepared by the following general method: A suspension of the appropriate aromatic dinitrile in dry diluent with 2·5–3·0 moles of absolute ethanol is saturated with hydrogen chloride at 0–5°C and then kept at room temperature for 7–10 days. The resulting imino-ether hydrochloride is then filtered off and converted into the required diamidine by treatment with 10 per cent absolute ethanolic ammonia.

Although the original references mainly describe the dihydrochlorides, most commercial aromatic diamidines are now available in more readily soluble forms, particularly as the di-isothionates (di-β-hydroxyethanesulphonates). One of the earliest compounds marketed was stilbamidine (4,4'-diamidinostilbene) but owing to certain neurotoxic properties it has since been withdrawn. The commoner diamidines now available are described below.

Hydroxystilbamidine or 2-hydroxy-4,4'-diamidinostilbene di-isethionate

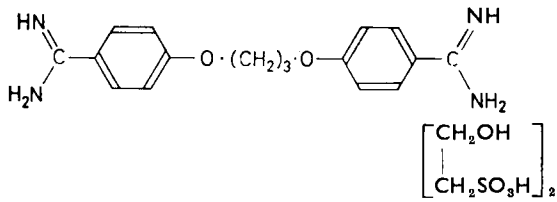


This is a yellow crystalline solid, readily soluble in water; the aqueous solution should be freshly prepared since solutions show some increase in toxicity on exposure to light. The material is therefore supplied in powder form. This compound is devoid of the neurotoxicity peculiar to stilbamidine, which it has replaced[62]. While the pharmacology has not been studied in such detail as that of stilbamidine, in general the toxicity, absorption, distribution and excretion of the two compounds are similar. Although its main clinical use is in the treatment of most systemic fungal and protozoal diseases[62], particularly against blastomycosis[63], hydroxystilbamidine has some activity against *C. albicans*[64] and against Gram-positive bacteria.

Propamidine or 1,3-bis(4-amidinophenoxy)propane di-isethionate

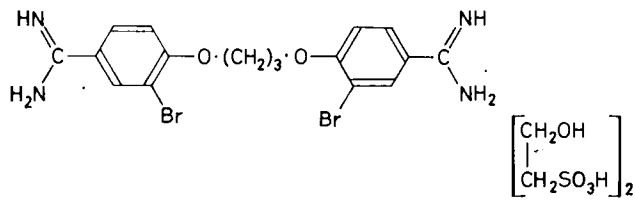
This white powder is hygroscopic and should be protected from air and moisture. It is readily water-soluble. It is available as a cream, a jelly and an ophthalmic solution. The pharmacology of propamidine has been investigated by Wien and his associates[65–67]. Although propamidine has

been shown to possess marked activity against a wide range of pathogenic bacteria and fungi[68], its main clinical use has been as an antibacterial agent



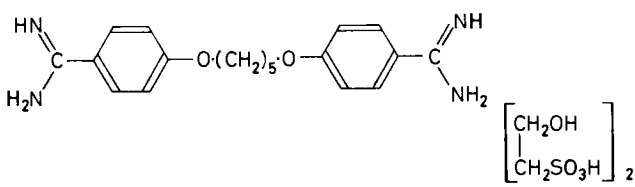
in the treatment of septic wounds and burns, and in the treatment of eye infections. There have been reports that local propamidine treatment has been effective in actinomycotic infections[69].

Dibromopropamidine or 1,3-bis(4-amidino-2-bromophenoxy)propane di-isethionate



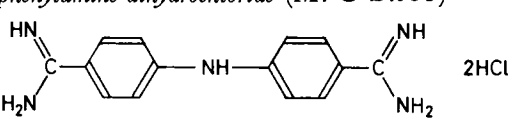
This is a white crystalline solid, which is readily soluble in water. It is the active constituent of Brulidine cream, Brolene eye ointment, and Otamidyl ear drops. Wien and his colleagues[67,70] have described the *in vitro* antibacterial and antifungal properties of dibromopropamidine. While largely used as an antibacterial agent in the treatment of septic wounds and burns[71,72], it has also been successfully used in the treatment of ringworm of the scalp, caused by *M. canis*[73].

Pentamidine or 1,5-bis(4-amidinophenoxy)pentane di-isethionate



This colourless, odourless hygroscopic powder is readily soluble in water. It is official in the B.P.C. by virtue of its value as a trypanocidal agent. However, the successful use of pentamidine in the treatment of moniliasis has been reported[74].

4,4'-Diamidinodiphenylamine dihydrochloride (M. & B.938)



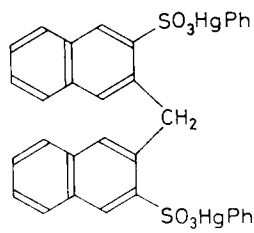
This is a yellowish crystalline solid (m.p. >300°C) which is readily soluble in water giving a stable solution which may be sterilized by autoclaving.

This compound is formulated as a powder and is also present as an antifungal agent in Otamidyl ear drops. Although prepared in 1942, it has only recently been shown to have marked antifungal activity and is still available for clinical trials. Andleigh[75] has shown that it is more active *in vitro* than hydroxystilbamidine isethionate against *Madurella mycetomi*, a causative agent of Madura foot; however, published data on preliminary clinical investigations does not appear very promising[76]. Mackinnon, Sanjines and Artagaveytia-Allende[77] have shown that M. & B.938 was the most active *in vitro* of a number of drugs (including other aromatic diamidines, sulphonamides and a sulphone) against *Paracoccidioides brasiliense*—the causative organism of South American blastomycosis. The results of clinical studies by Mackinnon[78], and Borelli and Rodriguez[79] are encouraging.

Organic Mercury Derivatives

Mercuric chloride has long been known as a wound disinfectant but can only be used in high dilution since it is very irritant; it has therefore proved to be more useful as a skin disinfectant. This disadvantage has been overcome to some extent by the introduction of organic mercury compounds, which have been found to be potent antiseptics, although their efficiency is often considerably reduced by serum. Among the earliest of such compounds were the simple phenylmercuric salts (*e.g.* nitrate, acetate, borate and chloride). Although once used for the treatment of bacterial, yeast and fungal infections of the skin, in general gynaecology and as non-irritant antiseptics for wounds, these compounds are now mainly used as preservatives in injection solutions. They are also used as plant fungicides and for the disinfection of skins and hides, leather, textiles, paper and timber[80,81]. The chief antifungal agent in this group at the present time is Penotrane.

Penotrane or phenylmercuric methylene-bis(3-naphthyl-2-sulphonate)



Methylene-bis(3-naphthyl-2-sulphonic acid) may be prepared by the condensation of naphthalene-2-sulphonic acid with formaldehyde, and the phenylmercuric salt precipitated as a white amorphous solid by the addition, for example, of a solution of phenylmercuric acetate[82]. It is insoluble in water but soluble in aqueous solutions of alkali metal methylene-bis(3-naphthyl-2-sulphonates). Its general properties have been described by Goldberg and his associates[80,82]. Penotrane is available in the form of a 0·1 per cent aqueous solution, a tincture, pessary, vaginal cream, jelly and dusting powder.

In a recent review by Murrell and Gray[83], on the treatment of trichomonal vaginitis, Penotrane was reported to have powerful antibacterial and

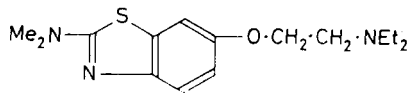
antifungal activity, and to be one of the more effective drugs available for the local treatment of multiform vaginal infection, in particular, in mixed trichomonas and monilia infestations. However, Catterall and Nicol[84] were disappointed with the results obtained when Penotrane pessaries were used concurrently with oral acinitrazole for the treatment of trichomonal infections. Its activity against *C. albicans* has been described by Horton-Smith and Long[85].

Other mercury compounds which possess powerful antibacterial and some antifungal activity include: Thiomersal (B.P.C.), sodium *o*-(ethylmercurithio)benzoate, Acetomeroctol (N.N.R.), 2-acetoxymercuri-4-(1,1,3,3-tetramethylbutyl)phenol, and Nitromersol (U.S.N.F.), anhydro-2-hydroxymercuri-6-methyl-3-nitrophenol.

Many discordant results have appeared in the literature concerning the antiseptic action of this group of organic mercurials; however, most investigators agree that the compounds are more active and less irritant than the inorganic mercurial salts. It should be emphasized that the organic mercurials are relatively ineffective in killing spores and that they are not as efficient for disinfecting instruments as is commonly believed[86].

Miscellaneous Compounds

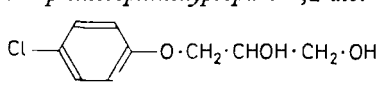
Diamthazole hydrochloride (Asterol) or 2-dimethylamino-6-(2-diethylaminoethoxy)-benzothiazole and its salts



2-Dimethylamino-6-alkoxybenzothiazole (prepared by a variety of standard methods) on treatment with aqueous hydrobromic acid or with aluminium chloride in chlorobenzene, is converted to the corresponding 6-hydroxy compound. Treatment of the sodio derivative of this with β-diethylaminoethyl chloride yields the required material, the dihydrochloride being a white crystalline solid (m.p. 240–243°C)[87]. Although originally marketed in this form, the dihydrochloride is rather acid (pH 2·6) and the monohydrochloride (m.p. 148–149°C) is now preferred[88]; it is readily water-soluble. Diamthazole is supplied as an ointment, a dusting powder and a tincture. It is a potent antifungal agent and is effective against a wide variety of superficial fungal infections of the skin, hair and nails[89].

Certain other benzothiazole derivatives, notably 2-mercaptobenzothiazole, have been reported as possessing antifungal activity, but they do not appear to have received any clinical application.

Chlorphenesin (Mycil) or 3-p-chlorophenoxypropane-1,2-diol



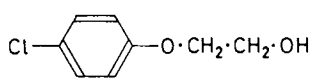
This is a crystalline solid (m.p. 79–81°C) which is sparingly soluble in water. When *p*-chlorophenol is heated with 3 moles of epichlorhydrin at about 100°C for 4–6 hours in the presence of a catalytic quantity of piperidine

acetate or hydrochloride, 3-*p*-chlorophenoxy-1-chloropropan-2-ol is formed[90]. This on refluxing with aqueous potassium carbonate yields the required 3-*p*-chlorophenoxypropane-1,2-diol[91]. Chlorphenesin is supplied as an ointment, a powder, pessary and an aerosol spray; its pharmacology has been described by Hartley[92]. It is a potent antifungal, antibacterial and trichomonicidal substance with remarkably low toxicity, being effective against the dermatophytes causing tinea pedis and other dermatomycoses, *Epidermophyton floccosum* and various *Trichophyton* species. The main use has been in the treatment of athlete's foot, dhobi itch, pruritus ani and pruritus vulvae.

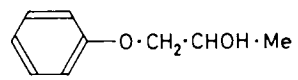
In a publication by Berger, Hubbard and Ludwig[93], the antimicrobial activity of several substituted phenolic ethers of glycerol, propylene glycol and trimethylene glycol has been described; the preparation and physical properties of the newer members of this series have been described by Ludwig, West and Currie[94]. In general all compounds examined showed greater antifungal than antibacterial activity. The effect of nuclear substitution was studied and it was shown that the antimicrobial activity was, to a certain extent, a function of the position of substitution; the methyl group and the chlorine atom were approximately equal in their effect. Trimethylene glycol ethers were the strongest antimicrobial agents but were irritant; propylene glycol ethers however, combined considerable antifungal activity with lack of irritant properties. These authors[93] have suggested that phenyl ethers of propylene glycol or glycerol might be used as stable, non-reactive and non-toxic fungicides and preservatives for pharmaceutical and other uses.

Derivatives of phenoxyethanol

Phenoxyethanol (Phenoxetol) has been known chemically since the 1890's. However, it was not until much later that it was shown to be valuable as an antibacterial agent when Berry and his associates demonstrated that it was highly effective against pyocyanea infections[95,96]. This led to the investigation of derivatives of phenoxyethanol, notably *p*-chlorophenoxyethanol (*I*) and propylenephenoxyethanol (*II*) both of which have been known for many years.



(I) *p* – Chlorophenoxyethanol



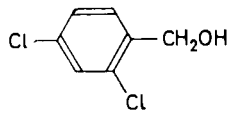
(II) Propylenephenoxyethanol

p-Chlorophenoxyethanol (*p*-chloro-phenoxetol) as colourless crystals (m.p. *ca.* 28°C) may be prepared by the action of ethylene oxide on *p*-chlorophenol in the presence of alcholic sodium ethoxide[97]. Propylene-phenoxyethanol (propylene-phenoxetol), which is 1-phenoxypropan-2-ol[98], may be prepared by the reaction of 1-chloropropan-2-ol with sodium phenoxide at 160°C[99], and boils at 134–135°C at 20 mm.

These two compounds have marked fungistatic and fungicidal activity *in vitro* against fungi commonly responsible for superficial mycotic infections, and have been successfully used in the treatment of athlete's foot[100]. Vanbreuseghem[101] has demonstrated that both these compounds possess

notable fungicidal and fungistatic properties against many of the pathogenic fungal species frequently encountered in the Congo.

Dybenal or 2,4-dichlorobenzyl alcohol



This crystalline solid (m.p. 56–57°C) may be prepared, for example, by the ammonolysis of 2,4-dichlorobenzaldehyde by treatment with ammonia and hydrogen in the presence of Raney nickel for 1 hour at 80°C under pressure[102], although several other methods are available. Dybenal is a potent antifungal agent with low toxicity[103] and is the antifungal constituent of the recently introduced antiseptic lozenges, Strepsils.

Undecenoic acid (undecylenic acid) or undec-10-enoic acid

$$CH_2{:}CH{\cdot}(CH_2)_8{\cdot}CO_2H$$

Undecenoic acid is a yellow liquid or pale yellow crystalline mass, which may be prepared by the destructive distillation of castor oil. It is almost insoluble in water. This substance is a powerful antifungal agent and is used as a local application in concentrations of 2–15 per cent in emulsions, ointments or dusting powders for the treatment of tinea pedis, capitis and cruris, moniliasis, mycotic vulvovaginitis and similar complaints. For application to mucous membranes, however, concentrations should not exceed 1 per cent, since higher levels may be irritant. To enhance its efficiency it is often combined with its zinc salt. As with other fatty acids its antifungal activity is greatest at acid pH.

Undecenoic acid and/or its zinc salt are the active constituents of Decilderm, Desenex, Fitoban, Mycota, and Tineafax. Mitchell-Heggs and Feiwel[104] have recorded the successful use of Monphytol, a preparation containing boric acid, salicylic acid, undecenoic acid and methyl salicylate, for the treatment of chronic perionychia. Certain other fatty acids *e.g.* octoic and propionic acids have also been used as antifungal agents.

Antihistamines

Mitchell, Arnold and Chinn[105] examined the antifungal activity of a large number of commercially available antihistaminic agents against a wide range of pathogenic fungi in order to investigate structure–activity relationships. Their most interesting observation was that chlorination seemed to increase the effectiveness as an antifungal agent; in every case examined, chlorinated substances were more active than their parent compounds.

Sulphonamides

The chemistry and antibacterial activity of the sulphonamides are so well known that reference will only be made to the application of these compounds in antifungal therapy. They are effective in many systemic fungal infections; the value of sulphonamide therapy in actinomycosis has been known for a number of years, particularly in combination with penicillin,

streptomycin and other antibiotics. Similarly, despite the recent development of certain antifungal agents for the treatment of nocardiosis (Madura foot), sulphonamide therapy, combined if necessary with surgical measures, is still one of the most satisfactory treatments for this condition.

The cutaneous form of infection by *Cryptococcus neoformans* (*Torula histolytica*) may respond rapidly to systemic sulphonamide therapy, which may also be successful in the pulmonary form of the infection. Results in central nervous system cryptococcosis are variable but cases of cryptococcus meningitis have recovered following this treatment.

In the localized forms of South American blastomycosis, rapid clinical cure can be achieved by sulphonamide treatment, although control of the disseminated infection is more difficult and relapse may occur after apparent cure. Da Rocha Passos[106] has reported the successful use of 6-sulphanilamido-2,4-dimethylpyrimidine (Elkosin) in this condition, although no fungistatic action was shown in *in vitro* tests. Local lesions may be treated by the topical application of a sulphonamide in association with systemic treatment.

Recently Louria and Feder[107] have shown that sulphadiazine has a marked fungistatic action *in vitro* against *Histoplasma capsulatum;* sulphadiazine was also shown to exert a marked protective effect against this infection in mice. These authors suggest that sulphadiazine treatment in histoplasmosis may be helpful both in direct antifungal action and in ameliorating the infection sufficiently to allow host defence mechanisms to prevail. The *in vivo* and *in vitro* activities of a number of sulphonamides against *H. capsulatum* have also been described by other workers[108].

Antibiotics

Since the Second World War, the development of antibiotics has completely revolutionized the treatment of bacterial infections; however, the majority of these agents have not been shown to possess comparable antifungal activity. The only antibiotics that are potentially interesting or have achieved reasonable therapeutic success in the treatment of fungal infections are: penicillin, streptomycin, the tetracyclines, erythromycin, eulicin, nystatin, Candicidin, amphotericin B, griseofulvin, and spiramycin.

With the exception of recent work, it is not intended to deal in detail with the chemistry of these antibiotics, since this aspect is not within the scope of this review.

Penicillin

Penicillin still remains the drug of choice in the treatment of actinomycosis, preferably in conjunction with a sulphonamide, or potassium iodide[109].

Streptomycin, tetracylines and erythromycin

Streptomycin has been found to be effective in the treatment of actinomycosis of the spine, where penicillin and potassium iodide had previously failed[110]. In addition, streptomycin and most of the broad-spectrum antibiotics in current use, including the tetracyclines[111] and erythromycin[112] are reported effective against actinomycosis and may be of definite value as

substitute treatment when the patient is sensitive to penicillin. Erythromycin has also been shown to possess therapeutic activity against acute and chronic brucellosis[113].

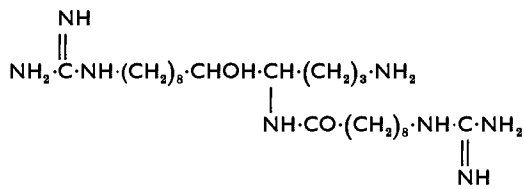
The influence of various antibiotics, including penicillin, streptomycin, chloramphenicol and viomycin on experimental moniliasis has been reported by Blyth[114].

Spiramycin (Rovamycin)

Spiramycin was first isolated in 1951 by Pinnert-Sindico, Ninet, Preud'homme and Cosar[115] from a *Streptomyces* species in soil. This species was later named *Streptomyces ambofaciens*. The exact chemical constitution of this antibiotic is as yet uncertain, but it is known to consist of a mixture of organic bases. Squires[116], and Freeman and Squires[116] have reported that while spiramycin is active against *Actinomyces* species *in vitro*, it is inactive against the majority of other pathogenic fungi.

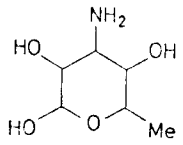
Eulicin

Eulicin is a new antifungal antibiotic recently isolated from a species of *Streptomyces* similar to *S. parvus*[117]. It has been shown to produce *in vitro* inhibition of various pathogenic fungi at relatively low concentrations; it is also fungicidal to a strain of *Candida neoformans* at a concentration only three times that of its fungistatic level[118]. Harman, Ham, Bolhofer, and Brink[119], as a result of degradative work, have proposed the following formula for eulicin:



Nystatin (Fungicidin, Mycostatin)

Nystatin, formerly called Fungicidin, was first reported by Hazen and Brown in 1950[120,121] and its preparation from a strain of *Streptomyces noursei* has been described by Dutcher, Boyack and Fox[122]. Knowledge of its chemistry is still incomplete, although Oroshnik, Vining, Mebane and Taber[123] have shown that a number of recently isolated antibiotics, including nystatin, Candidin and Candicidin, can be characterized as conjugated polyenes by their ultra-violet absorption spectra. Nystatin is known to contain a conjugated tetraene residue linked with an aminodeoxyhexose, mycosamine; the constitution of the latter compound[124] is represented below:



Nystatin is both fungistatic and fungicidal, being active against such pathogens as *Canäida albicans*, *Coccidioides immitis*, *Cryptococcus neoformans*,

Histoplasma capsulatum, and some *Blastomyces* and *Sporotrichium* species and yeasts, although resistance to the antibiotic may develop. Brown, Hazen and Mason[125] have reported on the ability of nystatin to protect mice from an otherwise lethal mixture of *C. albicans* and chlortetracycline; the development of toxicity to chlortetracycline in *Candida* infections had been previously reported by Seligmann[126].

Nystatin has proved of value in the treatment of superficial monilial infections, but is less effective in the treatment of dermatophyte infections. It has no antibacterial activity and does not interfere with the activity of antibacterial agents; however, when given prophylactically, nystatin is not completely successful in preventing mycotic superinfection in patients receiving antibacterial therapy[127]. Childs[128] showed that nystatin was effective in reducing the development of *Candida* infections in the rectum of patients undergoing tetracycline therapy, but was not so successful in the treatment of a *Candida* infection in the throat; he concluded, therefore, that nystatin might be ineffective in the treatment of systemic infections. Graham[129] has recently reported that nystatin is more effective than Gentian Violet treatment in the control of oral thrush in infancy, and Jennison and Llywelyn-Jones[130] have obtained encouraging results in the treatment of monilial vaginitis with nystatin. The recent publication of Kubista and Derse[131] on glycerol triacetate (triacetin) is therefore of particular interest. They reported that this compound, previously found to be equally as effective as nystatin in the treatment of monilial vaginitis, was, in fact, more stable, and less liable to produce resistance against *C. albicans in vitro* than was nystatin. Recently, Manning and Robertson[132] have recorded the successful treatment with nystatin, of a secondary infection due to *Aspergillus fumigatus* in a long-standing pyopneumothorax. Nystatin has been recommended as a useful therapeutic adjunct in order to prevent or minimize fungal proliferation during or after therapy with other antibiotics particularly the tetracyclines[133–135].

Nystatin, known in the U.S.A. as Mycostatin is available as tablets, ointment and vaginal tablets.

Candicidin and Candidin

It was shown by Lechevalier, Acker, Corke, Haenseler and Waksman[136] that a strain of *Streptomyces griseus* produced an antibiotic substance that was very active against yeasts and yeast-like fungi; it was not very active against filamentous fungi and had no activity against the bacteria tested. Owing to its marked fungistatic and fungicidal properties, especially against *Candida albicans*, it was named Candicidin. The following year Taber, Vining and Waksman[137] reported the isolation of Candidin from *Streptomyces viridoflavus*. This antibiotic, which also had marked *in vitro* activity against *C. albicans* and various dermatophytes, very closely resembled Candicidin, but the two were clearly distinguishable by comparison of biological activities, ultra-violet spectra and counter-current distribution. Later Vining, Taber and Gregory[138] confirmed by paper chromatography that Candidin and Candicidin were different. Preparations of both these antibiotics show evidence of inhomogeneity and contain two or more active fractions. These fractions have been separated in the case of Candidin, and

Candidin 'B' can be converted to Candidin 'A' by treatment with alcohol. Evidence indicates that the 'A' fraction is an artefact arising during the extraction process. Little is known of the chemistry of Candidin and Candicidin although both contain the heptaene chromophore[123].

Amphotericin B

Workers[139] have recently isolated two closely related crystalline antibiotics, amphotericin A and amphotericin B, from a *Streptomyces* species (M.4575). These were shown to be highly active *in vivo* against such fungi as: *Candida albicans*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Trichophyton mentagrophytes*, by both oral and subcutaneous routes[140]. The less soluble form, amphotericin B, is several times more active than amphotericin A against yeasts and yeast-like fungi, although amphotericin A has a broader antifungal spectrum; neither antibiotic is active against bacteria[141]. Sternberg, Wright and Oura[142] have also investigated the *in vivo* and the *in vitro* activity of amphotericin B. Its chemistry is still not fully established, although it is known to be a conjugated heptaene lactone linked with the aminodeoxyhexose, mycosamine[143]; the composition of the latter has been more recently described[124].

Amphotericin B has been shown[144] to decrease the degree of *C. albicans* infection in the gastro-intestinal tract when administered orally to man, alone or in combination with a tetracycline. Kozinn, Taschdjian, Dragutsky and Minsky[145] have shown that topical application of amphotericin B is practically as effective as nystatin in curing early lesions of cutaneous condidiasis in children. In a comparative trial of nystatin, amphotericin B and amphotericins A and B in children with oral moniliasis, all three agents were found to be reasonably effective, but in each case a high frequency of relapses occurred[146]. Amphotericin B, by oral or intravenous administration, is also effective in the treatment of certain systemic fungal diseases, including histoplasmosis, cryptococcosis, blastomycosis and coccidioidomycosis[147–150].

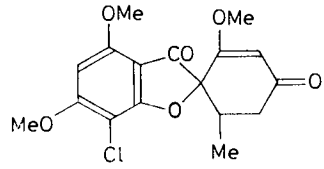
Mucormycosis, caused by various species of *Mucorales*, is becoming more frequently recognized as a complicating and frequently fatal fungus infection in diabetes, leukaemia and other conditions[151]. In two recent publications Chick, Evans and Baker[152,153] have shown that amphotericin B is effective in the treatment of experimental mucormycosis in rabbits and rats; these authors suggest that this drug may be of value in the treatment of human cases of mucormycosis. Amphotericin B has also been shown to have strong *in vivo* antifungal activity against *Aspergillus fumigatus* and it has been suggested that it should be effective in the treatment of selected cases of human aspergillosis[154].

An excellent review on the current status of amphotericin B in the treatment of systemic fungal infections has recently been published[155]. Amphotericin B is available commercially as a sterile powder under the name Fungizone.

Griseofulvin (Grisovin, Fulcin) or dd-7-chloro-4,6,2'-trimethoxy-6-'methylgris-2'-en-3,4'-dione

Although first reported 21 years ago[156] as a metabolic product of *Penicillium griseofulvum*, griseofulvin attracted comparatively little attention until after

the war when workers of Imperial Chemical Industries Limited began a series of papers on its constitution, degradation and reactions[157]. Scott[158] has reported the preparation of dehydrogriseofulvin by potassium ferricyanide



oxidation of the appropriate 2,4′-dihydroxybenzophenone, and has just described the conversion of this to griseofulvin[158a]. This antibiotic is now prepared on a large scale by fermentation and is of very considerable interest since it is effective against superficial fungal infections when administered orally. Its effect in the treatment of experimental ringworm in guinea-pigs has been described by Gentles[159]. Williams, Marten and Sarkany[160] have recorded the results of preliminary trials in humans suffering from ringworm of the skin or nails. According to recent reports by Cochrane and Tullett[161], and by Grant Peterkin[162], griseofulvin now seems to be the treatment of choice in acute cattle ringworm infections of the human skin.

Mycoses caused by various species of dermatophytes show a uniformly favourable response to oral therapy with griseofulvin; infections of long duration apparently respond as readily as those of recent occurrence[163]. However, Emmons and Piggott[164] have recently published evidence to show that griseofulvin, either by the oral or intravenous route, is ineffective in the treatment of mice experimentally infected with blastomycosis, histoplasmosis, cryptococcosis or coccidioidomycosis.

Recently the systemic fungicidal activity in plant species of a number of compounds closely related to griseofulvin has been reported by Crowdy, Grove and McCloskey[165]; they found no direct correlation between *in vitro* and systemic fungicidal activities.

The organization of an International Symposium on Griseofulvin and Dermatomycoses in Miami in October 1959 is evidence of the increasing interest attached to the antifungal activity of this antibiotic.

Other antibiotics

The list of antifungal antibiotics discussed in this review is by no means complete. Of necessity much material has had to be omitted and an attempt has been made to present only the most important of these agents. However, interest in the antifungal properties of the antibiotics continues to grow, as is illustrated by the following examples of other recently described substances.

Oligomycin

This is a crystalline solid isolated from a streptomycete resembling *Streptomyces diastatochromogenes;* knowledge of its chemistry is not yet complete. Oligomycin has no activity against representative bacteria and yeasts but is strongly inhibitory to the growth of filamentous fungi, being particularly active against the pathogen *Blastomyces dermatitidis*[166].

Fungichromin and Fungichromatin

These are two new polyene antifungal agents produced by *Streptomyces* species and isolated in crystalline form; *in vitro* they have some activity against fungal species including *Candida albicans*, *Blastomyces dermatitidis*, *Microsporum audouini* and others[167].

Flavofungin

This crystalline antibiotic has been recently isolated from *Streptomyces flavofungini*, and has an extremely wide antifungal spectrum *in vitro;* it appears to be effective against *C. albicans* infection in mice, but has no effect on the growth of common bacterial species[168].

Pimaricin

Pimaricin has recently been isolated from *Streptomyces natalensis* in crystalline form and has been shown to be active against a wide variety of fungi and yeasts *in vitro*[169]. The constitution of this tetraene antibiotic has been deduced by Patrick and his associates[170,171] as a result of studies on oxidation and hydrolysis products.

Tennecetin

This antibiotic, isolated from *Streptomyces chattanoogensis*, is another tetraene with a wide antifungal spectrum *in vitro*[172]. Barr[173], investigating the toxicity of this substance, reported that it was too toxic for systemic use but was likely to be acceptable for the topical treatment of fungal infections.

Sulfocidin

Sulfocidin is produced by a previously undescribed species of *Streptomyces*, which was discovered in a soil sample collected in the Punjab. This antibiotic is active against Gram-positive and Gram-negative bacteria, mycobacteria and a wide range of pathogenic fungi[174]. Since the infra-red spectrum of the purified antibiotic suggested the presence of the —C=C—CO—S— system, a series of compounds with related structures, already under investigation in another programme, was tested for antibacterial and antifungal activity[175]. Of these thiol esters, *p*-chlorophenyl thiol methacrylate and *p*-chlorophenyl thiol furoate were the most active against the bacterial and fungal species examined.

Hormones

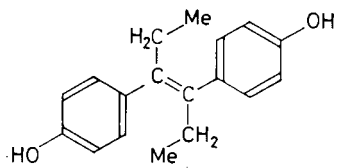
It is becoming evident from the literature that the pathogenic action of microorganisms depends not only upon the characteristics of the invading organism, but also on the condition of the host. The widespread use of hormones in the treatment of varied human pathological conditions has stimulated many investigators to evaluate hormonal influences on the course of infectious diseases.

As in the case of some of the antibiotics, it has been found that cortisone increased the severity of experimental moniliasis, induced by *C. albicans*[176,177]. Similarly Mankowski[178] showed that oestradiol shortened the survival time of mice infected with either *Candida albicans*, *Histoplasma capsulatum* or

Cryptococcus neoformans; progesterone did not have any specific effect on the development of *C. albicans* infection in mice, while testosterone showed a slight tendency to protect infected animals against experimental aspergillosis. However, various hormones, *e.g.* methyltestosterone, diethylstilboestrol and combined androgen and oestrogen have been reported as favourably influencing the course of coccidioidmycosis both in experimental animals and in man[109]. Cortisone and corticotrophin have been shown to be of little value in the treatment of mycosis fungoides, since the response they produce is only slight and temporary[179]. 3β-Methoxy-5-androsten-16β-ol has been shown to have a fungistatic effect *in vitro* against *Histoplasma capsulatum* equal to that of stilbamidine di-isethionate[180].

Of all the hormones, however, only stilboestrol appears to have achieved any reasonable success as an antifungal agent.

Stilboestrol or αα′-diethyl-4,4′-stilbenediol



This occurs as colourless crystals (m.p. 169–173°C) which are very slightly soluble in water. It was first prepared[181] in 1938 but much work has since been done in improving the synthesis[182]. One of the shortest methods is that of Kharasch and Kleiman[183]; anethole hydrobromide, on treatment with sodamide in liquid ammonia, gives a product isomeric with stilboestrol dimethylether. During demethylation with potassium hydroxide in ethylene glycol, re-arrangement occurs and stilboestrol is obtained in an over-all yield of 22 per cent.

Poth and Kaliski[184] successfully treated infections of tinea capitis with oestrone and stilboestrol. Law[185] observed the favourable effect of stilboestrol on ringworm of the scalp in humans. Fox, Carroll and Glacy[186] have compared the *in vitro* fungistatic action of 24 oestrogens or related compounds against eight species of pathogenic fungi. None of the natural oestrogens was active although ethinyl oestradiol showed slight activity; stilboestrol was the most active of the compounds tested. Replacing the phenolic hydroxyl groups of stilboestrol with methoxy groups decreased the activity. The reduced compound, hexoestrol, was on the whole less active than stilboestrol. Fox and his associates found that there was no relationship between fungistatic and oestrogenic activities in the compounds tested.

THE LABORATORY ASSESSMENT OF ANTIFUNGAL ACTIVITY

To examine the effect of agents reputed to be active against fungi, it is necessary to understand the behaviour of fungi. Since they are simple organisms, their metabolism is simple. Fungi can grow larger; they can take up oxygen or liberate carbon dioxide; they can reproduce and their spores can germinate. Any of these metabolic processes can conveniently be utilized in the assessment of the activity of antifungal agents.

It is advisable at this stage to define three terms often used indiscriminately. *Antifungal* is a general term used to describe any agent which is capable of either killing or inhibiting the growth of fungi; these agents can be further classified into two groups, *fungistatic* and *fungicidal.* A fungistatic agent inhibits the growth or reproduction of fungi but does not kill; the term fungicidal agent has a restricted meaning and should only be used to describe a compound which actually kills fungi or their spores. The distinction between a fungistatic and a fungicidal agent depends essentially on the difference between their mechanism of action.

In general, the criteria required for the tests to determine antifungal activity do not differ greatly from those required in the assessment of antibacterial activity. For the most part the techniques that have been developed closely parallel those for testing antibacterial agents, with suitable modifications for the comparatively slow growth rate and the more highly developed morphology of fungi. Three basic techniques are in common usage for the determination of antifungal activity:

(*1*) The test substance is incorporated into the nutrient medium of the growing fungus and activity is assessed by the retardation of growth of the fungus. In such a test, the compound may quite non-specifically interfere with any one or many of the numerous metabolic processess that are essential to the growth and survival of the intact fungus. A logical extension of these techniques is the development of methods to investigate the activity of compounds against fungal spore germination; many of the true fungicidal tests are based upon this latter technique.

(*2*) The manometric technique involves the measurement of the gaseous exchange between the fungus and its environment in the presence of the potential antifungal agent. In addition to gaseous exchange, fungi can produce other measurable effects, but the measurement may become rather difficult; for example, they can probably synthesize guanine and numerous other compounds, but the determination of the results of such syntheses becomes very tedious.

(*3*) A compound can be examined for its effect against a fungus infection maintained in the living animal. By no means the least of the problems encountered in this technique is the difficulty in simulating and maintaining human fungal infection in experimental animals. A further problem is the fact that many compounds, in addition to being highly toxic to the fungus, are in effective doses also toxic to the host. Absorption and penetration of a compound is also a major factor in *in vivo* experiments; thus, while a compound may be highly effective in *in vitro* studies, its poor absorption or absence of effect when applied topically or systemically may render it of doubtful value. In such cases a study and alteration of the physical properties of a compound may provide the lead by which an apparently worthless drug is made therapeutically useful. This may involve either solubilization of the compound, or, in cases where a depot effect is required, conversion to an insoluble form. In certain cases the toxicity of a compound to the host may be reduced without loss of antifungal activity by simple chemical means, such as changing the nature of the salt employed.

'In Vitro' Examination

In general, there is no standard method of testing compounds for antifungal activity *in vitro*, consequently methods used vary from laboratory to laboratory in accordance with the facilities available and the specific requirements of the test fungus and compound. A method that has been developed and used with success in the testing of quaternary ammonium compounds in the laboratories of Allen and Hanbury Limited[25] is described in brief detail below.

Candida albicans, *Trichophyton rubrum*, *T. mentagrophytes*, *T. verrucosum*, *Microsporum canis* and *M. audouini* are fungal species which have proved useful in a general *in vitro* antifungal screen. Tests of growth inhibition are carried out in Sabouraud's broth, composed of 1 per cent peptone (Eupeptone No. 2) and 4 per cent glucose in water. Drugs are serially diluted by 1 in 2 in 2 ml. volumes of broth and autoclaved at 10 lb./in.^{2} for 10 minutes. The dilutions are inoculated with a ground suspension of fungal culture in Sabouraud's broth. After inoculation and incubation at 27°C the tubes are read by eye after 3 days and subsequently. End-points are expressed as the minimum inhibitory concentration (M.I.C.) and final results as the geometric mean M.I.C. of several figures obtained independently. In tests of fungicidal activity, a suspension containing about 5,000,000 spores/ml. of saline is prepared from a 7–10 day culture of *T. mentagrophytes*. Tubes containing 2 ml. volumes of serially diluted drugs are inoculated with 0·04 ml. of spore suspension and incubated. After a measured time each tube is shaken and one loopful (4 mm diameter) is transferred into 4 ml. of 2 per cent dried bovine bile (Bacto-Oxgall) in Sabouraud's broth. The bile inhibits the antifungal activity of the quaternary transferred with the inoculum. When the test compound is other than a quaternary ammonium salt, an appropriate inhibitory agent has to be substituted in place of the bovine bile. Recent studies have shown that Lubrol W. is far more efficient than bile in inhibiting the effect of the transferred quaternary. Subcultures are examined for fungal growth after 14 days incubation at 27°C. The assessment of the antifungal activity of quaternary ammonium compounds is complicated by the fact that many of these substances are inactivated by agar; an agar-free medium is therefore necessary in order that a true evaluation of the activity of the quaternary may be achieved.

Other tests for antifungal activity *in vitro* have been described by many authors; the following have been selected from the literature as examples of different techniques. McPherson[187] used a paper disc technique to screen a variety of agents for antifungal activity against *Trichophyton verrucosum;* Gentles, Barnes and Fantes[188] extracted griseofulvin from the hair of guinea-pigs receiving the drug orally and estimated it by bio-assay against *Alternaria solani*, using a modification of the *Botrytis allii* hyphal curling assay[189]; and Hemphill, Herman and Young[190] determined the comparative antifungal activity of nystatin and amphotericin B in tissue culture, this effect being required to prevent fungal contamination in tissue culture fluid required for virus preparation. The use of the tissue-culture method in evaluating antifungal agents against systemic fungi has also been described by Larsh, Silberg and Hinton[191,192].

Walker, DeKay and Porter[193], commenting on the advisability of establishing a standard method for the *in vitro* testing of antifungal compounds, have pointed out that the solvent used plays a very important part in any *in vitro* experiment. Because of limited solubilities, it is not always possible to use water and many other solvents, such as alcohol, acetone and dioxan, have been employed. Varying results may, however, be obtained with different solvents, in many instances due to the specific solvent being toxic to fungi. After investigating this problem, Walker and his colleagues concluded that the question of solvents for fungistatic and fungicidal testing has yet to be answered satisfactorily.

'In Vivo' Examination

Techniques for the *in vivo* determination of antifungal activity vary widely, depending upon the selection of the animal species and the nature of the infecting fungus. Inherent in all these methods is the problem of simulating human fungal infections in animals, and it may also be difficult to maintain an infection, since many fungal conditions are self limiting and are liable to regress spontaneously without drug treatment. In contrast to *in vitro* examination, the *in vivo* techniques introduce a third factor into the fungus–antifungal agent relationship, namely the host. This results in a series of complex interactions, one of which is the possible toxic effect of the agent on the animal tissues. It is obviously impracticable to give a complete review of all available methods for *in vivo* examination of antifungal agents, but the following examples have been selected from the literature as being the most characteristic.

Baum, Rubel and Schwarz[194] have described a method of evaluating nystatin, amphotericin B and sulphadiazine, alone and in combination, against experimental histoplasmosis in hamsters. The animals were infected with an intraperitoneal injection of a broth suspension of a yeast phase culture of *H. capsulatum*, and the drugs administered subcutaneously.

In a publication describing both *in vitro* and *in vivo* examinations of a new series of aminobicycloheptanes, Chandler, Bridges and Gordon[195] infected mice with intraperitoneal injections of a saline suspension of arthrospores of *Coccidioides immitis* and examined their compounds for activity by subcutaneous injection. Mice infected intraperitoneally or intravenously with *Candida albicans* were treated by oral or intraperitoneal administration of the same compounds.

The course of a local closed monilial lesion in the thigh of the mouse has been described by O'Grady and Thompson[196] in an investigation of the comparative effects of chlortetracycline and cortisone. Infection of the thigh with 0·1 ml. of a saline suspension of *C. albicans* containing 150 million organisms/ml. was carried out by the intramuscular route, using the technique of Selbie and O'Grady[197]. This gave rise to a swelling which showed a peak size usually on the second day after inoculation, followed by a small decline, which was in turn succeeded by a second peak size usually about the fifth day (this second peak might be greater than the first); the size of the swelling then subsided to a plateau which might be maintained for a second week before resolution began. The medio-lateral diameters of the thighs

were measured daily with sliding calipers and compared with those of the control animals. These authors observed that treatment of the animals with subcutaneous chlortetracyline in near toxic doses led to a persistent suppression of the lesion indistinguishable from a curative effect, while subcutaneous cortisone merely suppressed the lesions during the course of the treatment.

In attempting to find an agent for the topical treatment of ringworm and other superficial mycoses, Gentles[159] established a technique by which guinea-pigs were infected experimentally with *Microsporum canis*, and the resulting lesions allowed to develop for 10 days, at which time they fluoresced under Wood's light. Gentles then administered oral griseofulvin daily and observed beneficial results within 4 days, the inflammatory reaction which developed in the control animals being prevented in those receiving treatment. These findings were subsequently confirmed by demonstrating the reduction of infection in the hair follicles of treated animals. Similar results were obtained by oral griseofulvin treatment of guinea-pigs experimentally infected with *Trichophyton mentagrophytes*.

Oral griseofulvin has also been found to prevent the establishment of artificial infection of calves with *T. verrucosum*[198]; similarly, McPherson[199] has assessed the value of numerous antifungal agents against *T. verrucosum* infections induced experimentally in calves; guinea-pigs were found unsuitable for this type of experiment.

A simple *in vivo* test that can be used to detect and evaluate drug activity against enteric *Candida albicans* infection in mice has recently been reported by Lindh[200]. In this test which is claimed to be reproduceable, mice are given a suspension of *C. albicans* in liquid Sabouraud's medium instead of drinking water for 18 hours; the test drugs are administered in the diet beginning 24 hours before and extending to 6 hours after infection. At the end of treatment a faecal pellet is collected from each mouse and examined for its fungal content. A reduction of 90 per cent or more of the average *C. albicans* colony count of the untreated mice is considered to indicate activity.

The Effect of Resistance on Antifungal Assessment

In carrying out *in vitro* evaluation tests it is important to take into account the possible development of resistance by the fungi against antifungal agents, since there is now accumulating evidence of the ability of fungi to become adapted to these agents. For example, contrary to prior reports, it has now been found possible to develop strains of various *Candida* species and of *Coccidioides immitis* that are resistant to amphotericin B and nystatin[201,202]. As yet there appears to be no references to the development of this resistance in *in vivo* studies.

DISCUSSION

This review does not contain any reference to the relative activity or toxicity of antifungal agents since the spectra of activity of these compounds differ widely. Moreover, there is a complete absence of published data on *in vitro* or *in vivo* comparisons of their activities in the same experimental or clinical conditions.

Many antifungal agents show reasonable to good activity, but nevertheless have very little similarity in chemical structure. Consequently, it would appear that there are no general structure–activity relationships within this field. This is perhaps not surprising since the study of the closely related antibacterial agents shows that there is a similar lack of any apparent relationships. There is, however, a developing tendency to compare the pharmacological activities of chemically unrelated compounds in the light of knowledge of their physico-chemical properties, rather than to lay too great an emphasis on some chance structural chemical resemblance. This approach to the problem might well explain the absence of any well-defined structure–activity relationship in the antifungal field; further work along these lines may provide an indication as to the precise nature of the relationship between biological activity and chemical structure. Classification of antifungal agents, therefore, presents a problem which seemingly is best solved by dividing the compounds into groups according to their chemical types; it is only within such groups that there is, at present, any indication of a limited structure–activity relationship.

A further point merits close attention; many of the most useful antifungal agents have been developed either empirically or by some fortuitous circumstance. In numerous cases antifungal activity has only been discovered by further investigation into the properties of known chemotherapeutic agents which have already achieved some measure of success in other fields, notably against bacteria. Rarely has a successful product been developed solely as the result of primary screening against fungal species; indeed it can be said that until fairly recently there has been a lack of direct attack in the search for antifungal agents. To some extent, this situation is now being rectified; fungal infections are more readily diagnosed and their importance recognized, providing a powerful stimulus to the search for effective compounds. An example of this interest is given by the organization of a Symposium of Fungus Diseases held in London in July 1957, papers presented at this meeting forming the basis of a recently published book, *Fungous Diseases and their Treatment*[203]. Other publications which will serve as a useful basis for further reading include *Therapy of Fungus Diseases*[204], *The Strategy of Chemotherapy*[205], and an extremely useful volume on the toxicity of fungicides[206].

The perfect antifungal agent is far from being a reality, and in our present state of knowledge it is far too early to predict or even to hazard a guess as to whether it will be a synthetic or a natural substance. Commercial interests could easily influence the line of investigation; while synthetic drugs often possess certain advantages, for example the ready availability and comparative cheapness of intermediates and the ease of analytical control of the final products, the recent rapid developments in the discovery and large-scale manufacture of many of the antibiotics may well have nullified the disadvantages of earlier procedures.

One of the criteria of the perfect antifungal agent has recently been achieved, namely, the introduction of antibiotics which are effective in the oral treatment of certain superficial and systemic fungal infections. It should not be overlooked in the search for, and formulation of, antifungal agents that therapy will always favour the use of oral medicaments rather than injections.

A point to be emphasized, however, is that even with a perfect antifungal agent, the chemotherapy of fungal infections is at best only a temporary substitute for the ideal form of therapy in which the natural defence mechanisms of the body play the deciding role. There is as yet but little evidence for the development, or artificial production of, immunity mechanisms within the host; however, there are some indications suggesting that innate and acquired immunity to fungal infections is a distinct possibility. For example *Candida albicans* can be frequently isolated from human skin and mucous membranes, and agglutinins can be demonstrated in the absence of clinical infection; occasionally resistance is lost and clinical symptoms result; a similar natural immunity can also be shown by laboratory animals after experimental infection. In the human subject, the majority of such agglutinins are probably acquired although the possibility of their being natural cannot be disregarded. Women show agglutinins more often than men, suggesting that these antibodies are, in fact, acquired since moniliasis occurs more frequently in women; as much cross immunity is associated with various species of *Candida* the agglutinins are by no means necessarily specific. Contrary to the above opinions there is evidence that the production of agglutinins does not necessarily coincide with the development of resistance[207].

An interesting problem which may well involve some form of immunity pattern is the fact that superficial ringworm fungi lack the capacity to invade living tissue. When these fungi invade the scalp and the hair shafts they progress downwards along the shaft only to the layer of parakeratotic cells and no further. There is indubitably something in the living cell which completely stops the growth of these organisms, but its nature is as yet completely unknown.

Before the production of therapeutically valuable artificial immunity can be achieved, research must be directed towards the elucidation of the cellular physiology and the biochemistry of the supportive enzyme mechanisms of the invading fungus. Many of the techniques required for such investigation are still only in their infancy and for the present, therefore, the only effective treatment of fungal infections in man lies in the development of new and more potent synthetic or natural antifungal agents.

Since this chapter was originally prepared several important books and review articles have been published or become available to us[208–214].

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