Volume One

PROGRESS IN MEDICINAL CHEMISTRY

Edited by
G. P. ELLIS, B.Sc., Ph.D., F.R.I.C.
and
G. B. WEST, B.Pharm., D.Sc., Ph.D.

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1

Edited by

G. P. ELLIS, B.Sc., Ph.D., F.R.I.C.

Research Department, Benger Laboratories Limited
Holmes Chapel, Cheshire

and

G. B. WEST, B.Pharm., D.Sc., Ph.D.

School of Pharmacy, University of London

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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.

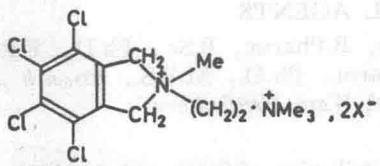
We wish to thank the authors, societies and publishers for permission to use illustrations and tables which have appeared in previous publications.

G. P. ELLIS
G. B. WEST

October 1960

ERRATA

p. 46: Formula (*XI*) should read:



(XI) Chlorisondamine

p. 88, line 4: For ' tissues [37].' read ' tissues [137].'
p. 98, column 5, last line: For ' Vetactil ' read ' Veractil '
p. 113, line 7 from bottom of page: For ' brain lesions [116].' read ' brain lesions [166].'
p. 159: After legends of both *Tables 4.2 and 4.3* insert reference [154b]
p. 261: For ' 1,3,5-Thiadiazole derivatives ' read ' 1,3,4-Thiadiazole derivatives '

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E. P. TAYLOR, B.Pharm., B.Sc., Ph.D., F.R.I.C., and P. F. D'ARCY, B.Pharm., Ph.D., M.P.S., *Research Division, Allen and Hanburys Limited, Ware, Hertfordshire*

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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

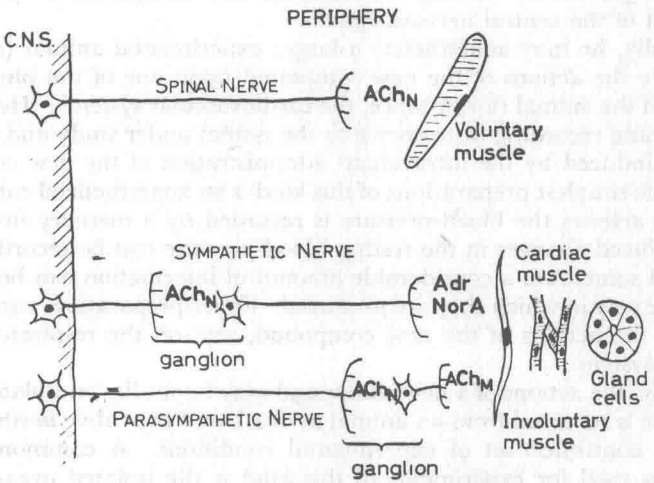

Figure 1.1. Diagram of the motor nerves of the mammalian body to indicate the peripheral structures which they control
Chemical transmitters in the synapses are shown thus: ACh_M for acetylcholine with a muscarinic action, ACh_N for acetylcholine with a nicotinic action, Adr for adrenaline, and NorA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

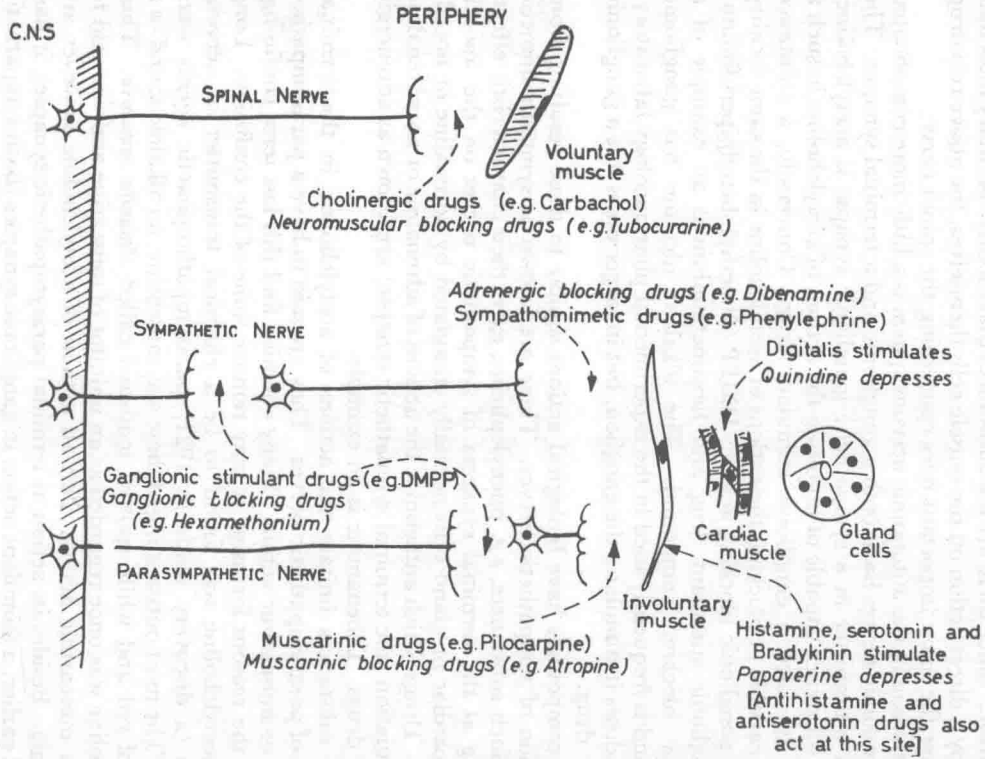

Figure 1.2. Diagram of the motor nerves of the mammalian body indicating the points at which some drugs exert an action

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

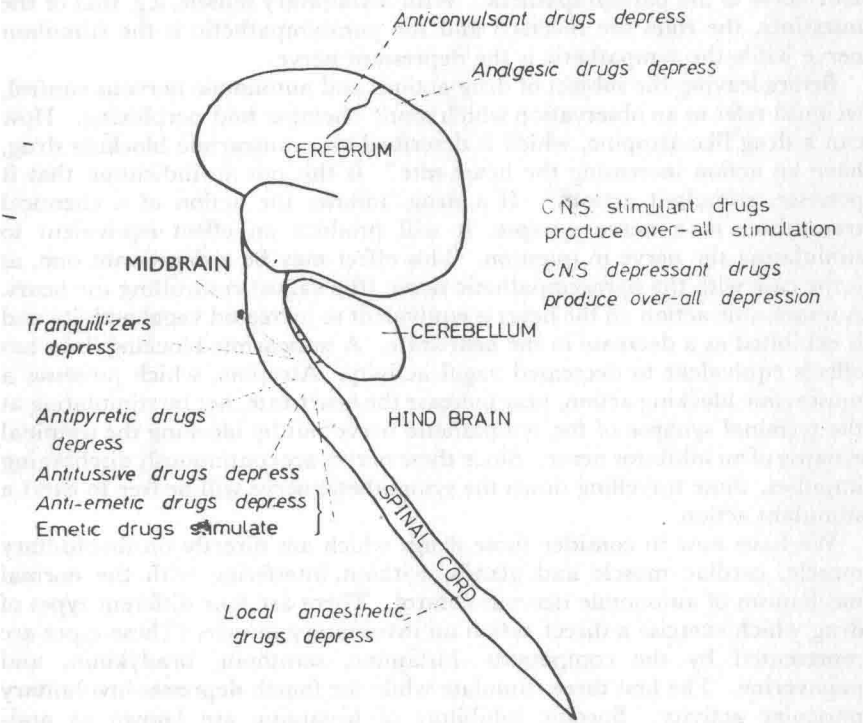

Figure 1.3. Diagram of the central nervous system of the mammalian body indicating the areas in which some types of drugs are believed to exert an action

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