REVIEW ARTICLE

BIOLOGICAL ASSESSMENT OF TRANQUILLISERS. PART I*

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No review on tranquillising drugs would be complete without definitions of the novel jargon to be used. We shall almost entirely avoid the use of some titles recently coined, including psychosedative, hypnosedative, neuroleptic, neuroplegic and ataraxic. We think that at present these postulated types of drug are insufficiently distinguished from each other to allow their description other than by the general terms psychotropic drug and tranquillising drug. We define a psychotropic drug as one affecting the mind in some manner, and we define a tranquillising drug as a non-hypnotic drug that has a sedative or calming effect, including an action of this kind in psychoses or psychoneuroses. A tranquillising drug or tranquilliser falls last in the following series of central depressants: anaesthetic, hypnotic, sedative, tranquilliser. There is overlap between adjacent members of this series, but none between members not adjacent.

Most tranquillising drugs have only recently been introduced and the methods available for their assessment, though numerous, are as yet imprecise. This situation is widely recognised and frequently attacked. Thus the following statement appeared[1] in July, 1957: "Dr. D. R. Laurence expressed the astonishment of a pharmacologist at the flimsy evidence which launched new drugs on the tranquilliser market and apparently persuaded clinicians to prescribe them for their patients". No pharmacologist who has worked in this difficult field would deny that this statement can to some extent be justified, but many would consider it overemphatic. The major problem of replicating in the laboratory the actions of potential tranquillisers in human psychoneuroses and psychoses, remains almost completely unsolved, but very many experimental techniques have been proposed for the evaluation of tranquillisers and do, we believe, provide a secure foundation on which more specific methods will be elaborated and on which clinical investigations may be based. Our object is to survey these known techniques and to give our personal opinions of their usefulness.

We shall concentrate mainly on methods suitable for the pharmacological evaluation of tranquillisers. These drugs can certainly be sought by examining the ability of novel compounds to affect enzymes concerned in the physiological disposition of presumed transmitters such as noradrenaline or 5-hydroxytryptamine (which we shall call serotonin throughout) or by examining their ability to block the arousal reaction of Magoun,

* This review is being published in two parts. Part II will follow on page 721, in the December 1958 number of this *Journal*.

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but we prefer to regard such biochemical and neurophysiological techniques as essentially investigative at present and shall consider them only briefly.

The methods we shall survey are grouped under the following rather arbitrary headings:

(1) Methods of measuring general sedative action.

(2) Behavioural methods (not involving conditioning).

(3) Neurophysiological techniques.

(4) Antagonism to psychotomimetic drugs.

(5) Conditioning methods.

We have attempted to cover the literature available to us before April, 1958, and apologise to authors whose papers have been missed.

1. Methods of Measuring Sedation

Many methods of measuring sedation have been proposed and some of them have been proved valuable by continuous use since long before the introduction of tranquillising drugs. Their value, of course, depends on the extent to which one expects or wishes sedation to be a property of the drug one is looking for. Thus most methods in this section will detect reserpine or chlorpromazine, both powerful sedatives, but many will miss benactyzine, which is not a general sedative.

A. *Potentiated Narcosis*

The potentiated narcosis test measures the influence of experimental drugs on the duration of sleep induced by a standard hypnotic. This technique was first introduced as a test for sedative action by Winter in 1948[2], though there had been many earlier investigations on the combined actions of two central depressant drugs, including several on the effect of alcohol on barbiturate sleep. When the combined effect of two drugs is to be determined there are many possible procedures[3]: the most informative are relatively complex. However, Winter[2] found that if the potential sedatives were given to groups of mice at a definite interval before a fixed dose of the hypnotic, comparisons of the geometric means of the sleeping times provided a simple but adequate measure of sedative action. This is the method generally used, but Winter's criterion of recovery from sleep, the ability of mice to walk normally with their eyes open, has been abandoned in favour of the sharper end point of recovery of the righting reflex. The hypnotic most frequently used is hexobarbitone. We find that precision is much increased by carrying out the test at a constant temperature between 34 and 36°.

Tranquillisers that prolong sleep after barbiturates or other hypnotics include the phenothiazines, chlorpromazine[4–6], promethazine[4,5], promazine[7], and chlorpiprozine (perphenazine)[8], the rauwolfia alkaloids Rauwiloid[9], reserpine[10,11], and rescinnamine[10] and a mixed group: benactyzine[12], hydroxyzine[13], methylpentynol[14], methylpentynol carbamate[15] and meprobamate[16]. This is probably the only test that will accept every type of drug

for which tranquillising actions have been claimed. Unfortunately a very large variety of other substances are accepted too. Such central depressants as sedatives, hypnotics and anaesthetics are obviously effective. But so are adrenaline, analgesics[17], antihistamines[2], histamine[18], serotonin[19,20], lysergic acid diethylamide[21], iproniazid[22], thiamine[23], cholesterol[24], glucose and its metabolic products[25], sucrose[26], glycerin[26], inorganic nitrates and nitrites[27], iodides[26], sodium chloride[28], various solutions[29], and water[28]. The activity of this wide range of substances is doubtless due to the existence of several possible ways in which drugs may prolong barbiturate action. Hypnotics, sedatives and tranquillisers may give true addition of effect, even if a tranquilliser is of a type which by itself does not cause marked sedation. Thus, tranquillisers diminishing awareness or blocking the arousal reaction should be active. Some other substances may make the brain more susceptible to the action of barbiturates. Brain tissue respiration is inhibited by barbiturates[30], and it has been suggested that iodides, which are known to decrease the oxygen uptake of tissues, might thus potentiate barbiturate narcosis. Phenothiazines[31–33] and serotonin[20] also depress brain metabolism *in vitro*, and might partly act in a similar way. On the other hand, it has been claimed that the relatively feeble depression of brain metabolism caused by barbiturates may not be associated with their hypnotic effects[34]. Also, a drug given in toxic dose might be expected to prolong apparent sleep: some so-called depressants of brain metabolism might well be systemically toxic in the doses necessary to depress brain metabolism.

The hypnotic would act more effectively if its access to the brain were facilitated, and one way in which this might occur is through increased permeability of the brain capillaries. Histamine and possibly serotonin could act in this way. Nitrates cause dilatation of capillaries and might also facilitate the passage of hypnotics into the brain. It has been suggested that substances with a high osmotic pressure when injected intraperitoneally draw water from the tissues and blood and so raise the hypnotic concentration[26].

The hypnotic will also act longer if its absorption is delayed and prolonged, e.g., by vasoconstriction such as adrenaline or serotonin might cause, or if its metabolism or excretion is blocked. Iproniazid[22], SKF525A[35–37] and Lilly 18,947[38] all block the metabolism of barbiturates and so potentiate their action. Fouts and Brodie[22] put forward a method of rejecting such "false" potentiators. In their view the true potentiator will reinduce sleep if administered during awakening. Drugs that act by interfering with the metabolism of the hypnotic have no effect under these conditions. This reverse test can be used for screening purposes though we find that high doses even of potent drugs, e.g., 20 mg./kg. of, chlorpromazine i.p., may have to be used.

The advantages of the potentiated narcosis test are that it is easy to use as a routine procedure, and that potentially useful tranquillisers are unlikely to be missed. However, its gross lack of specificity means that it must be supplemented with other more selective tests. In our experience 20 to 30 per cent of randomly selected compounds are able to prolong hexobarbitone

sleep when given (by pretreatment) in the relatively modest dose of 100 mg./kg., and their subsequent examination is a formidable task.

B. *Hypothermia and Reduced Metabolic Rate*

The sedative effects of chlorpromazine and reserpine as measured by potentiation of barbiturate sleep or reduction of activity have been found to be proportional to the accompanying fall in body temperature[39] and it has consequently been suggested that sedation is caused by interference with the mechanism of temperature regulation[40]. We do not support this view, though we agree that hypothermia may contribute to sedation measured by some non-specific methods, including potentiated narcosis.

Tranquillisers have also been tested for their action on the metabolic rate. The oxygen consumption of the whole animal may be measured, e.g., by the methods of Maclagan and Sheahan[41], or Capraro[42]. Chlorpromazine[4], pecazine (mepazine)[43], reserpine[44] and other central depressants[44] reduce oxygen consumption as does serotonin[44,45]. The respiration of isolated brain slices is depressed by chlorpromazine[46,47] and pecazine[47], but not by reserpine even when the tissues are made more sensitive by electrical stimulation[47]. However, the oxygen uptake of the whole brain is reported not to be affected by chlorpromazine[48].

We do not consider these non-specific methods of estimating tranquillising activity very useful as screening methods, though they are useful in enlarging knowledge of the drug's type and site of action and of its side effects.

C. *"Fall-time" Methods*

The "fall-time" tests assess the agility of control and treated animals, usually mice. The methods fall into two groups, those using inclined planes or fixed horizontal rods, and those using rotating rods and cylinders. The angle of the slope or the rate of revolution of the cylinder is adjusted so that normal mice remain on the apparatus and mice dosed with the type of drug to be studied fall off. The results are expressed in terms of the time they stay on, or as the percentage falling off.

Of the first group, the earliest was Thompson's[49] sloping wire-mesh, designed for the assay of insulin. This method has also been used for the assay of curare[50], and, more recently, for investigating the combined effects of alcohol and tranquillisers in rats[51]. Some workers replaced the wire-mesh by a smooth metal plate and used it for measuring sedation[52,53]: the controls ran down the plate and sedated mice slid down. Sedation can also be studied by putting mice on a narrow horizontal rod and observing how long they stay on.

The second group of tests forces the animal to move if it is to stay on the apparatus. The first apparatus of this type was the hollow rotating cylinder inclined at an angle, designed by Young and Lewis[54] for the assay of insulin, and later used for the assay of curare[55], and the measurement of sedation[56–58]. Horizontal rotating rods have also been used for evaluating sedatives[59–61].

The classes of substances that are active in these tests are those like curare[50,55] which cause paralysis, convulsants such as strychnine[57] and insulin[49,54], hypnotics such as pentobarbitone[57], alcohol[51,57] and methylpentynol carbamate[62], and tranquillisers such as the phenothiazines promethazine[52], chlorpromazine[51,52,63,64] and pecazine[52], reserpine[57,58,63,64] and deserpidine[58], and also meprobamate[63,64], but not benactyzine[63,64]. The effect measured is clearly neurotoxicity since it is not, at least hypothetically, an essential concomitant of tranquillisation, and since its counterpart in man must be loss of ability to perform adroit movements, including those of automobile-driving. The tests might be most valuable if used to discard rather than select potential tranquillisers.

D. *Reduction of Spontaneous Activity*

One of the most obvious signs of sedation in animals is a reduction in their so-called spontaneous activity. Methods of measuring activity have been in use since the end of the last century. Pedometers were used on dogs as early as 1896[65] and they have since been used for sheep and pigs[66]. But for small laboratory animals other, more convenient, methods were devised, and these methods fall into three main groups involving three different types of activity cage, those which revolve, those which move up and down ("jiggle" cages), and those which are fixed.

The revolving drum activity cage that rotates about a horizontal axis as the animal runs in it was described by Stewart in 1898[67]. The revolutions of such a drum may be registered kymographically[67–70], or by means of a mechanical[39–74] or electrical[75] revolution counter. Methods of estimating the reliability of revolving drums and of calibrating them have been suggested by Shirley[76] and Lacey[77]. The experimental animal can be forced to take all its exercise in the drum by allowing it no external living cage[67,68,72,74,78–80] or only a very small one[69,71,73]. Voluntary running activity may be recorded by allowing the animal a larger living cage so that it may enter the drum at will[70,75]. A variation of the revolving drum is the horizontal turntable described by Farris and Engvall[81], but records of activity from this apparatus will vary according to whether the rat has been running round the periphery of the turntable or nearer the centre.

The revolving wheel records only the running activity of an animal and not small movements. The second type of activity cage enables total activity to be measured. Szymanski[82] in 1914 devised a cage supported by an air tambour so that movements of the animal caused pressure changes which were recorded on a kymograph by means of another tambour. It was originally designed for salamanders and mice but has
nd monkeys[84,85]. Spring-mounted activity
zymanski[86] and these have been more widely
ecorded kymographically by means of an
attached lever[87–93], or by a pneumatic system[94–97], but kymographic recording makes quantitative treatment of results difficult. Quantitative records have been obtained using a Harvard work adder[98,99] or even a device of sealskin[100] to convert the vertical cage movements into the rotation

Addendum

A SIMPLE METHOD FOR THE PRODUCTION OF HIGH TITRE PENICILLINASE

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This Journal, 1957, **9**, 609

P. 610, line 16. For "One-tenth ml." read "One ml."

of a wheel which inscribes a cumulative record on the drum[98] or works a revolution counter[99,100]. A numerical measure of activity can also be obtained by using electrical contacts to operate either a lever making vertical marks on a kymograph[101] or, most conveniently, a pulse counter[102,103]. If the cage is suspended by a strip metal spring instead of a coiled spring the strip itself can provide one of the contacts[103]. Photoelectric recording, using a flag attached to the cage to break a light beam, has also been tried[104].

Szymanski[82] introduced another type of activity cage constructed like a lever balance, with one arm supporting the cage and the other arm recording the movements of the animal towards and away from the pivot. The recording arm writes directly on a kymograph[82] or operates a work adder[105]. Other cages have been designed in which movement of the animal causes tilting of the cage in any direction[106] or in one plane[107,108], the recording being mechanical or electrical.

Waterman[109] attempted to improve the "jiggle" cage by an arrangement that reduces cage movements to a minimum compatible with mechanical recording. Work adders and microswitches respond to very slight cage movements[110]. Even less movement of the cage is necessary when the vibrations are transmitted to a gramophone pickup[111,112], or to the diaphragm of a permanent magnet loudspeaker[113]. The output of either is amplified and a numerical count[113] or an ink recording[111–113] obtained. Another electrical means of recording very slight cage movement is provided by the change in resistance of carbon granules by which the cage is supported[114].

The third main group of methods measures the activity of the animal more directly. An attached thread or chain has been used to measure the activity of fish[82], mice[115,116] and monkeys[117]. Direct observation of the number of squares an animal enters on a squared floor has also been used[118]. An animal moving on smoked paper will record its own activity[119] and the results can be made quantitative by measuring light reflected from the paper[120]. A mouse may be placed on dry sand on a gauze so that as the mouse moves sand comes through the gauze and is collected and measured[121]. The activity of the animal within a cage can be used to produce changes in capacitance between a vertical metal antenna in the centre of the cage and the cage itself[122]. A similar method employs metal foil squares in the insulated roof of the cage instead of the antenna: movements of the animal induce changes in capacitance between roof and floor, this capacitance forming part of that of a tuned circuit[123].

The photoelectric method of recording activity was first introduced by Siegel[124], who used a rectangular cage across which a light beam shone on to a photoelectric cell. When the animal interrupted the light beam an impulse counter was activated. Winter and Flataker[125] used a similar method but reflected the light beam twice off the sides of the cage. Dews[126] used a single direct light beam and found that results showed less variation when mice were tested in groups of five. Modifications include the use of circular cages[61] and of several separate light beams[127]. Infra-red rays can be used to record activity in total darkness[124].

These devices are very numerous and their design has involved much time and inventive ingenuity but differences between them are probably relatively unimportant when the gross effects of drugs are studied. The most important distinction is that the wheel, tilting cage and photo-beam methods measure mainly running and walking activity, whereas the "jiggle" cage also measures small cleaning, and other localised movements, and tremors. The best methods are probably those which cause the least possible disturbance to the animal, allowing it to move freely on a stable flooring. Another disadvantage of the moving cages is that they are difficult to calibrate. Our own preference is for the light-beam type.

Spontaneous activity of rats and mice is depressed by chlorpromazine[105,128], reserpine[129], meprobamate[105] and by small doses of azacyclonol[130,131]. Benactyzine[111] and large doses of azacyclonol[130] increase activity. There are some species differences because benactyzine reduces activity in the monkey[132], whereas azacyclonol does not affect monkey activity, and has mainly a stimulant effect on cats and dogs[131]. The results may also differ according to the conditions of the test. Brown[133] has used differences in action on the spontaneous activity of grouped and single mice to distinguish hypnotics from tranquillisers, and it has also been shown that phenobarbitone depresses the nocturnal activity of rats but has little effect on diurnal activity[134].

Such differences merit more attention than they have received. It is possible that more careful study of animal movement under a variety of different conditions by precise photo-beam methods would much increase the specificity of such methods towards different drugs. It might also considerably increase their convenience. Thus, when spontaneous daytime activity of mice is studied it is often the brief exploratory activity displayed by these nocturnal rodents when they are placed in a new cage. After 15 to 30 minutes such activity rapidly declines and the control animals subsequently appear quite tranquil. Nocturnal activity, though intense and prolonged, is less conveniently recorded, and because it occurs in bursts, more variable.

For these reasons and because it has other theoretical advantages, many authors study the effects of tranquillisers on hyperactivity rather than on spontaneous diurnal or nocturnal activity. Hyperactivity can be induced in monkeys by frontal lobe lesions, and this type has been shown to be reduced by chlorpromazine and reserpine[127]. More usually it is induced by stimulant drugs such as amphetamine, pipradrol, methyl phenidate, or caffeine.

E. *Antagonism of Drug-induced Hyperactivity*

Chlorpromazine, rauwolfia alkaloids and azacyclonol antagonise amphetamine[9,128,130,131], pipradrol[131,135] and methyl phenidate[63,64,136,137], though in monkeys the combination of reserpine and methyl phenidate causes alternation of depression with violent biting and jumping activity[136]. Small doses of meprobamate antagonise methyl phenidate stimulation but larger doses and benactyzine enhance the effects[63,64]. Other stimulants such as caffeine, cocaine[56] and mescaline[63,64] have been used. The

effects of most stimulants decline quickly, but four injections of β:β-iminodipropionitrile in mice produce a hyperactive state which lasts for months[138,139]. Mice so treated show circling activity similar to that described as a genetical abnormality[140,141]. Other chemical agents also produce a similar "waltzing syndrome"[142–144] but the mice treated with iminodipropionitrile (IDPN mice, "souris tournantes") have been investigated most thoroughly and used for testing sedatives and tranquillisers. Chlorpromazine[138] and reserpine[138,139] both reduce the activity of IDPN mice. Thuillier and Nakajima[145] divide psychotropic drugs on the basis of their action on IDPN mice into four classes; neuroleptics, tranquillising sedatives, hypnotics, and autonomic excitants. Delay coined the term neuroleptic for drugs that have powerful sedative actions but are not narcotic, that antagonise aggressiveness, agitation and psychotic states, that act predominantly on subcortical regions and that have important autonomic effects[146]. The neuroleptics, which include chlorpromazine, reserpine and hydroxyzine, are said to stop the agitation and circling of IDPN mice and to normalise their response to noxious stimuli, whereas the tranquillising sedatives, which include benactyzine, mephenesin, meprobamate, methylpentynol and analgesics, reduce the hyperactivity and circling but produce ataxia and ataxic responses to noxious stimuli[145]. Hypnotics, including barbiturates, arrest circling activity only at narcotic doses, and autonomic excitants, including methamphetamine and lysergic acid diethylamide, diminish activity and produce trembling but no ataxia[145]. This is an interesting method: it is to be hoped that further study may confirm its usefulness.

F. *Anticonvulsant Tests*

Central depressants, including tranquillisers, may antagonise convulsions produced by passing an electric current through the brain, or by injecting convulsant drugs such as leptazol.

Drugs (other than specific anti-epileptic drugs) effective against electroshock include barbiturates[105,147], alcohol[147] and stimulants such as mescaline and dexamphetamine[147]. Of the tranquillisers, meprobamate[105,148] and hydroxyzine[13] and some phenothiazines[149] are effective, chlorpromazine being variously reported to be effective[105] or to have only slight activity[149]. Azacyclonol has little or no activity[130], whereas reserpine[150,151] and benactyzine[132] enhance the susceptibility to convulsions, though again reports differ, and reserpine is said to have no effect and benactyzine even a very slight protective action[63]. We find that reserpine enhances the susceptibility of rats and that chlorpromazine is relatively ineffective, though large doses lower the threshold.

Similar results are reported against leptazol-induced convulsions. Barbiturates[63,64,105,152] and meprobamate[63,64,105,148] have protective actions, azacyclonol has only slight activity[63], chlorpromazine has no effect[63,105,144], and reserpine is ineffective[63] or enhances susceptibility[150]. Hydroxyzine[13] and benactyzine[63] are ineffective.

It has been suggested that tranquillisers should be tested against but should not antagonise strychnine-convulsions[153]. Phenothiazines[63,154],

reserpine[63,150,153], benactyzine[63] and azacyclonol[63] have no effect on strychnine convulsions, and hydroxyzine potentiates them[13], but meprobamate[64,148], like phenobarbitone[63,152], does have an antagonistic effect. Other convulsants that have been used include picrotoxin, nicotine, cocaine and amphetamine.

We consider that these anticonvulsant methods may be helpful in defining the pattern of a drug's central actions; moreover, the frequent association of psychoneuroses or psychoses with epilepsy may sometimes allow them to have direct clinical application. They are, nevertheless, useless as primary screening methods for novel tranquillisers.

G. *Amphetamine Toxicity*

In 1940 Gunn and Gurd[155] noticed that the symptoms of excitement caused by injection of amphetamine or related compounds in mice, were much more pronounced if the mice were kept together in one cage, rather than singly. Chance[156] reported that the increased stimulation that occurs with grouped mice led to a marked increase in the toxicity of stimulants. The toxicity of amphetamine was increased nearly ten times by keeping the injected mice in groups of ten instead of in individual cages. Protection against a lethal dose of amphetamine has been used as a test for tranquillisers[4,15], but the effect of tranquillisers on amphetamine toxicity to grouped mice particularly, has only been investigated more recently by Lasagna and McCann[157] and by Burn and Hobbs[158]. Pentobarbitone did not affect toxicity for grouped or individual mice, but phenobarbitone raised the LD50 for grouped mice to that of individual mice, but only at doses that produced prolonged sedation and ataxia[157]. Chlorpromazine and reserpine protected grouped mice at doses that hardly affected the toxicity of amphetamine for individual mice[157,158] and had no prolonged after-effects[157]. Promazine had a similar action but was less potent[157]. Meprobamate, methylpentynol and benactyzine were inactive[158]. Burn and Hobbs[158] conclude that the difference between drug effects on grouped and single mice shows that the test is more than an anti-amphetamine test: they claim that it is a test against fright and therefore a valid test for tranquillising agents. We think that the specificity of the method needs further study, and that its advantage over the methods described in sections D and E has not been fully demonstrated.

H. *Audiogenic Seizures*

Donaldson in 1924[159] was the first to describe running seizures in rats precipitated by the sound of jingling keys. Since then there has been an enormous amount of work on these seizures. Reviews of the literature have been published by Finger[160] up to 1947 and by Bevan[161] from 1947 to 1954. For some time there was a controversy as to whether such seizures were a reflex response to auditory stimulation, or were caused indirectly by conflict between the need to escape and the inability to do so. The evidence for each view has been brought together by Munn[162] who concludes that it is rare to find seizures produced by conflict alone without auditory stimulation. But audiogenic seizures are not simply reflex

behaviour, since providing a shelter[163] or allowing the animal to make a well established instrumental response[164] often has a protective effect.

The seizure in the rat consists of an initial startle response followed by violent running and jumping activity which usually leads to tonic and clonic convulsions followed by coma, but which may pass straight into the comatose state[165] sometimes described as "catatonia"[166] or "catalepsy"[167]. Much work has been done in an attempt to define the essential characteristics of the seizure-inducing stimulus. Sounds are usually of high frequency, but high intensity is a more important factor. Interrupted tones are less effective than steady tones[168], but a short priming sound before the test stimulation enhances susceptibility[169]. In spite of all this work, the essential stimulus characteristics have not been clearly defined, and most investigators, like those last mentioned[169], use an electric doorbell as the sound source.

Not all rats or mice are susceptible to audiogenic seizures. The percentage in any colony depends on the genetics of the strain. By selective breeding it is possible to produce strains with very differing susceptibility, but though a correspondence between susceptibility and emotionality as otherwise measured has been reported, it would appear to be due only to chance combination[170]. Rats can be made more or less susceptible by change in diet or by administration of drugs. The dietary factors are very varied. Deficiencies of magnesium[171,172], amino acids[173] or even a reduced food intake[173] increases susceptibility, whereas excess thiamine decreases seizure-incidence and injection of L-glutamic acid reduces severity[174]. It is interesting that hydration protects against audiogenic seizure although it increases susceptibility to electrical and leptazol-induced convulsions[175].

But although seizure incidence is influenced by so many factors, the use of a single strain of animals fed on a standard diet allows the incidence to be used as a criterion of drug activity. The clinical anticonvulsants bromide[176,177], phenytoin[178–181], phenobarbitone[176,181,182] and troxidone[181] are effective against audiogenic seizures, the last three more effective than against comparable electrically-induced convulsions[181].

Alcohol is very effective in preventing audiogenic seizures in rats[183,184] at non-ataxic doses[183]. Reserpine protects[56,167,185] and so do chlorpromazine[167,185], pecazine[167] and meprobamate[167,185]. Benactyzine gives a maximum of 50 per cent protection, higher doses enhancing the convulsions[167].

The audiogenic seizure is probably one of the most useful techniques available for assessing tranquillisers. This view appears justified both by the nature of the seizure and by the reported activities of known drugs. Its chief fault, in this context, is its susceptibility to non-tranquillising anticonvulsants, which must be separately eliminated.

I. *Stress and Adrenocortical Function*

Audiogenic seizures and other behavioural responses to alarming stimuli are stressful responses and stress is known to activate the adrenal cortex[186] by causing release of corticotrophic hormone from the pituitary [187,188]. Mason and Brady[189], in an experiment on the conditioned

emotional suppression of lever-pressing in monkeys (see section Q) showed that disruption of lever-pressing was associated with high plasma concentrations of 17-hydroxycorticosteroids. Daily intramuscular doses of reserpine protected against the disturbance of behaviour and at the same time the amount of plasma steroid remained normal However, the behavioural and endocrine responses are not absolutely linked because on withdrawal of reserpine the conditioned anxiety response reappeared within a week whereas the steroid concentrations took about three weeks to rise again. Reserpine given intravenously, like chlorpromazine and azacyclonol, itself produces moderate rises in the amount of circulating corticosteroids[190] though pentobarbitone reduces the amount. The effects of chlorpromazine[191] and reserpine[192] at least are mediated through the pituitary.

Besides influencing the amount of circulating corticosteroids, experimental stress causes depletion of adrenal ascorbic acid[193]. Usually the ascorbic acid concentration is expressed in weight per hundred grams of gland, but Olling[194], investigating reported sex differences in sensitivity to the ascorbic acid depletion test, concluded that a better index would be the total quantity of ascorbic acid in the gland, to allow for differences in adrenal weight. Although rauwolfia alkaloids reduce adrenal ascorbic acid[195], both reserpine and chlorpromazine given to rats before submitting them to stress, prevent the depletion of ascorbic acid that would otherwise have occurred[196]. We have been unable to demonstrate a comparable action of meprobamate, using aversive conditioning as a stressful procedure.

The anti-stress activity of a series of barbiturates measured by the ascorbic acid method appears only at doses that produce deep sleep[197]. Most non-barbiturate hypnotics can also depress the stress reaction and some of these, including alcohol, do so at non-hypnotic doses[197].

Instead of using experimental stress, drugs can be tested for their action against known pituitary-adrenal activating agents. Various substances can cause depletion of adrenal ascorbic acid, including adrenaline, histamine and morphine[198]. Chlorpromazine and reserpine block this effect of adrenaline, and, to a lesser extent, that of morphine, whereas pentobarbitone blocks the depletion due to aspirin and morphine but not that due to adrenaline or histamine[199]. The difficulty of interpreting much of this work arises from the uncertainty whether effects of such drugs as chlorpromazine and reserpine arise from central or peripheral actions: this is unfortunate because the assessment of stress is in principle one of the best approaches to tranquilliser assessment, and there is much to be said for attempts to make this assessment of stress one of objective measurement rather than observation. Such alternatives to adrenal cortical studies as evaluation of defaecation, micturition and muscle tension suffer from similar disadvantages because of side-effects of tranquillisers, and emotional elimination in any case is not a good measure of fearfulness[200,201]. Kreezer[202] has listed methods of measuring emotionality, including the "startle" response to disturbing stimuli which Tripod has used for testing tranquillisers[63,64] and which was earlier used for testing sedatives[87]. These methods deserve further study.

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RESEARCH PAPERS

PHARMACOLOGY OF TREMOR-PRODUCING AMINO ALCOHOLS

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Received July 14, 1958

The pharmacological properties of a series of amino alcohols of the general formula $RCH(NH_2)\cdot(CH_2)_n\cdot C(OH)R_2$ have been studied The most striking action of these compounds is their ability to produce a sustained tremor, which is compared with those produced by other tremorogenic agents. The activities of various drugs as antagonists of the amino alcohol tremor are described.

IN recent years considerable attention has been directed to tremor-producing compounds because of their unusual central excitant activity, their usefulness in studying the phenomenon of tremor and for screening anti-Parkinsonian agents. Nicotine and eserine produce transient tremor often associated with clonic convulsion and anoxia. Tremor of short duration is also produced by aminothiols such as β-mercaptoethylamine[1], but drugs which evoke sustained tremor in experimental animals are rare. Harmine and harmaline produce tremor which lasts for 15–30 minutes in mice, while Tremorine, 1 : 4-di-(1-pyrrolidino)-2-butyne, produces severe tremor lasting for 1–3 hours in mice, and for 24 hours or more in dogs and monkeys[2]. Besides causing tremor, Tremorine also gives rise to salivation, lachrymation, diarrhoea and muscular weakness with rigidity.

The present paper reports the production of sustained tremor and ataxia in experimental animals by some amino alcohols recently synthesized in this department. The paper also presents a detailed pharmacological study of one typical representative of the tremor-producing amino alcohols.

In those amino alcohols containing an asymmetric carbon atom the tests described were made with the racemic compounds.

METHODS

Tremor producing activity. Albino mice weighing 18 to 30 g. were used. Doses were given on a mg./kg. basis. Solutions of the compounds in 0·1 N HCl were diluted with 0·9 per cent saline so that the required amount could be given intraperitoneally in a volume of 0·5 ml./25 g. Groups of 5 or 10 mice were used for each dose. As control 5 or 10 animals were injected with saline (0·5 ml./25 g.). The percentage of animals in each group showing tremor within a period of 3 hours after injection was noted. Only those animals which showed sustained tremor of head, body and limbs, and rigid erection of tail were assessed as positive responses. The Median Effective Dose (ED50) and its standard error were calculated for

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each compound by Irwin and Cheesman's modification[3] of Kärber's method[4]. Tests were repeated on the same group of animals after an interval of 7 to 10 days.

Influence of various substances on amino alcohol-induced tremor. Aqueous solutions of various substances were given subcutaneously to groups of five or ten albino mice at three or more dose levels. The drug concentration was adjusted to enable the volume injected in each case to be 0·25 ml. per 25 g. body weight. Thirty minutes later the animals were given an intraperitoneal injection of a tremor-producing dose (ED80) of an amino alcohol. In each group the fraction of the animals which showed tremor of the head, body and limbs within 3 hours was noted. As controls 5 or 10 animals were given the tremor-producing agent intraperitoneally.

Local anaesthetic activity. The intracutaneous weal test of Bülbring and Wajda[5] was employed. Each substance was tested in at least four guinea pigs. The dose of each compound corresponding to half the total number of stimuli was taken as the measure (ED50) of local anaesthetic activity. The relative potencies were derived from the dose-response curves.

Spasmolytic acitivity. Sections of ileum from freshly killed guinea pigs were suspended in a 2·5 ml. bath of Tyrode solution at 36°. After equilibration sufficient spasmogen (acetylcholine, histamine or nicotine) was added to the bath to evoke a submaximal response. The effect of amino alcohols on contractions produced by the above spasmogens was studied qualitatively.

The potency of two of the compounds as acetylcholine antagonists was quantitatively measured by determining pA_2 and pA_{10} values by Schild's method[6].

Isolated heart. An isolated frog heart attached to a Straub cannula was perfused with Ringer solution of the following composition: 0·65 NaCl, 0·01 KCl, 0·01 $CaCl_2$, 0·02 $NaHCO_3$ per cent (w/v) in distilled water. In most instances drug dilutions of 1×10^{-7}, 1×10^{-6}, 1×10^{-5} were tested. The pH of the drug solutions was adjusted to approximately 7. Between tests the perfusion was exchanged at least 3 times and a minimum of 10 minutes was allowed for recovery of the tissue.

Isolated rabbit auricles. The effect of amino alcohols on isolated rabbit auricles suspended in a 50 ml. bath of oxygenated Ringer solution at 29° was studied.

Isolated frog rectus abdominis muscle. The muscle was suspended in a 5 ml. bath of aerated frog Ringer solution. The stimulant effect of acetylcholine was recorded for 90 seconds every 5 minutes till consistent responses were obtained. The amino alcohol was then added to the bath 90 seconds before the next dose of acetylcholine. Its own effect on the muscle was also recorded for 90 seconds. The action of tubocurarine in modifying the stimulant effect of acetylcholine and amino alcohols was observed in the same way.

Blood pressure. The carotid blood pressure was recorded in cats anaesthetized with ether followed by chloralose 50 mg./kg., intravenously.

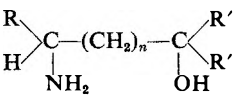
The amino alcohol was given in 0·9 per cent saline through a cannula in the femoral vein.

RESULTS

The chemical formulae of the twelve amino alcohols studied are shown in Table I together with their tremor-producing activity. Of these, nine compounds produced symptoms of central excitation which, at least in the dose range employed (15 to 30 mg./kg. i.p.), was the same qualitatively. As the first sign of excitation, the mouse showed motor unrest and frequent spasmodic forward movement. Some mice, particularly those treated

TABLE I

TREMOR PRODUCING ACTIVITY OF AMINO ALCOHOLS



Compound No.	Chemical formula			ED50 (Tremor) mg./kg. i.p.	Limits of ED50 mg./kg. i.p. P = 0·95
	R	R′	*n*		
1	C_6H_5	C_6H_5	0	45·24	42.77–48·26
2	C_6H_5	$C_6H_5CH_2$	0	49·64	41·85–58·7
3	C_6H_5	*o*-$MeOC_6H_4$	0	41·32	36·48–46·84
4	H	C_6H_5	0	30·32	26·16–35·17
5	CH_3	C_6H_5	0	36·18	30·5 –40·92
6	C_6H_5	C_6H_5	1	23·7	20·25–27·73
7	C_6H_5	$C_6H_5CH_2$	1	23·8	20·6 –27·38
8	C_6H_5	*p*-MeC_6H_4	1	—	—
9	C_6H_5	*m*-MeC_6H_4	1	28·64	24·43–32·7
10	C_6H_5	*o*-$MeOC_6H_4$	1	—	—
11	H	C_6H_5	1	>100	—
12	2-Furoyl	C_6H_5	1	35·36	30·4 –41·1

with aminoethanols, or the furyl-substituted aminopropanol (Compound 12) showed jumping and squeaking fits. These animals recovered from the fit within an hour, but showed marked reflex hyperexcitability.

Compounds No. 6, 7 and 9 produced a characteristic syndrome within the dose range of 20 to 40 mg./kg. intraperitoneally. The animals showed tremor of the head, body and limbs with rigid erection of the tail within 6 to 15 minutes after the injection. The tremor was continuous, severe, and sustained, lasting from 1 to 3 hours. With the tremor there was continuous struggling forward movement and frequent retropulsion or circling movement. At times the animal exhibited forward extensor spasm particularly when stimulated by sound or touch. Tremor was present at rest, but became more marked on movement. All four limbs, especially the forelimbs showed continuous irregular movements. These movements resembled neither the normal clonic convulsion nor the running movements provoked by nicotine. After 1 to 3 hours, the animals became more or less quiet showing occasional bursts of transient tremor. With higher doses (40–100 mg./kg. i.p.), the animals showed tremor which soon developed into clonic convulsion. Recovery followed or death occurred from respiratory paralysis.

The animals showed no salivation or lachrymation, but sometimes passed faeces and urine frequently.

TABLE II

INFLUENCE OF VARIOUS DRUGS ON TREMOR INDUCED BY COMPOUND 6 (1 : 1 : 3-TRIPHENYL-3-AMINOPROPAN-1-OL)

Drug	Control: No. showing tremor/No. injected	Treated: Dose mg./kg. s.c.	Treated: No. showing tremor/No. injected
Atropine sulphate	7/10	5	9/10
		10	9/10
		20	9/10
Hyoscine hydrobromide	4/5	5	4/5
		10	4/5
		20	4/5
	4/5	5	5/5
		10	5/5
		20	5/5
	16/20	2·5	8/10
		5	7/10
		10	10/10
		20	9/10
Benzhexol	8/10	1·25	7/10
		2·5	8/10
		10	8/10
		20	9/10
Caramiphen	9/10	2·5	9/10
		5	10/10
		10	9/10
		20	8/10
Chlorpromazine	17/20	2·5	8/10
		5	4/10
		10	2/10
Morphine Sulphate	4/5	2·5	4/5
		5	5/5
		10	4/5
		20	4/5
Apomorphine HCl	4/5	5	5/5
		10	3/5
Mephenesin	16/20	20	8/10
		100	4/5
		200	2/5
		300	2/6
S.K.F. 525A	3/5	200	5/5
		400	5/5
Meprobamate	4/5	50	5/5
		100	5/5
		200	5/5
Pentobarbitone sodium	5/5	60	9/10
		100	0/5
Phenytoin sodium	8/10	10	10/10
		20	9/10
Trimethadione	4/5	500	3/5
		1000	3/5
Tubocurarine chloride	4/5	2·5	4/5
Magnesium sulphate	9/10	1000	4/7
Calcium gluconate	4/5	2500	5/5
5-HT	3/4	10	4/5
		20	4/5
		40	3/5
Reserpine	3/5	10	4/5
		20	5/5
		40	3/5
Bulbocapnine	4/5	10	5/5
		20	5/5
		40	5/5
LSD	4/5	2	4/5
Hexamethonium bromide	4/5	10	5/5
1 : 1-Di-(*p*-tolyl)-3-phenyl-3-amino-propan-1-ol (Compound 8)	8/10	25 50	8/10 4/5
1 : 1-Di-(o-anisyl)-3-phenyl-3-amino-propan-1-ol (Compound 10)	8/10	40 80	10/10 6/6
1 : 2-diphenyl-2-aminoethanol	4/5	25	4/5
		50	5/5
		100	4/5

In higher doses compounds 1 to 5 produced tremor and convulsion, but ataxia was not evident.

Compounds 8 and 10 caused no tremor or convulsion even in doses of 100 mg./kg. intraperitoneally. This dose, however, killed the animals.

TABLE III

LOCAL ANAESTHETIC ACTIVITY OF THE AMINO ALCOHOLS ASSAYED BY GUINEA PIG WEAL METHOD

Compound	Median effective dose (ED50) mg.	Local anaesthetic activity (Procaine = 1)
1	0·125	5·9
2	0·089	8·3
3	0·1	7·4
4	0·162	4·5
5	0·125	5·9
6	0·081	9·1
7	0·085	8·7
8	0·055	13·4
9	0·062	12·0
10	0·05	14·8
11	0·199	3·7
12	0·14	5·3
Procaine	0·74	1
Cocaine	0·1	7·4
Cinchocaine	0·066	11·2

Compound 11 caused slight tremor in mice after injection of 100 mg./kg. intraperitoneally.

After the tremor induced by the first injection of 30 mg./kg. intraperitoneally had subsided, a second injection of Compound 6 produced more severe tremor within a few minutes. This indicated that no tachyphylaxis occurred.

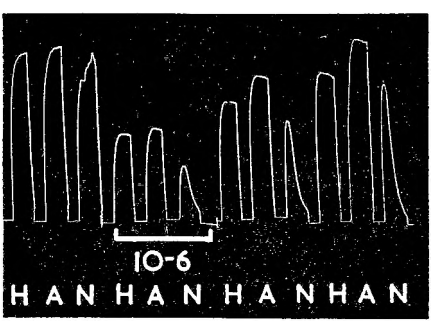


FIG. 1. *Guinea pig ileum.* 2·5 ml. bath. Interval 3 min. H, histamine 0·05 μg.; A, acetylcholine chloride 0·1 μg.; N, nicotine 5 μg. The white line indicates the period in which the bath contained Compound 6 (1×10^{-6}).

Influence of various substances on amino alcohol-induced tremor. To determine whether pretreatment with various substances could protect animals against the tremorogenic action, Compound 6, the most potent of the amino alcohols shown in Table I, was used to elicit the tremor. The results, which are presented in Table II, indicated that parasympatholytic drugs, anti-Parkinson agents, ganglion-blocking agents, neuromuscular blocking agents (curare), analgesics, anticonvulsants, and sedatives could not protect the animal from the tremor induced by Compound 6. Serpasil, 5-hydroxytryptamine (5-HT) and bulbocapnine also had no antagonistic effect in the doses used. The amino alcohols which did not themselves cause tremor (Compounds 8 and 10) were examined as possible antagonists of the tremor produced by Compound 6, but these

proved to be ineffective. The amino alcohol 1 : 2-diphenyl-2-aminoethanol, the pharmacology of which has been studied by Downman[7], neither evoked tremor itself nor did it antagonise tremor due to Compound 6.

The agents which significantly reduced the tremor were chlorpromazine, mephenesin and pentobarbitone sodium. Animals under ether anaesthesia showed no tremor, but during recovery tremor re-appeared.

S.K.F. 525 A (β-diethylaminoethyl diphenylpropylacetate[8]) and bulbocapnine in the doses used seemed to augment the tremor, as the animals pretreated with these substances showed marked tremor and clonic convulsion, while the control animals showed only moderate tremor without convulsions.

Local anaesthetic activity. Table III shows the activity of the twelve amino alcohols relative to procaine, cocaine, and cinchocaine as assessed

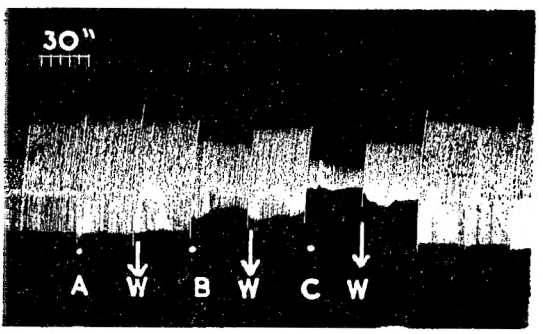


FIG. 2. *Isolated frog heart perfused through a Straub cannula.* At A, B and C the frog Ringer solution contained 1×10^{-7}, 1×10^{-6} and 1×10^{-5} of Compound 6 respectively. At W the perfusion fluid was exchanged 4 times. Note the marked depression in the presence of 1×10^{-5}, from which the heart recovered after 1 hour.

by the guinea pig intracutaneous weal method. All the compounds proved to be more potent local anaesthetics than procaine. The potency of three compounds was found to be greater than that of cinchocaine. In general 1 : 3-amino alcohols showed higher local anaesthetic activity than 1 : 2-amino alcohols.

Spasmolytic action. All the compounds showed a spasmolytic effect on guinea pig ileum. Figure 1 shows the effect of Compound 6 on histamine, acetylcholine and nicotine contractions. No attempt was made to assess the relative anticholinergic, antihistaminic and antinicotinic activity of all these compounds on guinea pig ileum. Estimation of anticholinergic activity of Compounds 6 and 10, by Schild's method, after 14 minutes' contact gave the following results:

Compound 6 $pA_2 = 5{\cdot}43$; $pA_{10} = 5{\cdot}09$; $(pA_2 - pA_{10}) = 0{\cdot}34$

Compound 10 $pA_2 = 5{\cdot}60$; $pA_{10} = 5{\cdot}08$; $(pA_2 - pA_{10}) = 0{\cdot}52$

According to the criterion of Marshall[9] these compounds are non-competitive antagonists of acetylcholine, as the $(pA_2 - pA_{10})$ difference is significantly less than 0·96. This was confirmed by determining

concentration-action curves for acetylcholine in the presence and absence of the antagonists. In the presence of either Compound 6 or 10, the curve was not parallel to that for acetylcholine alone.

Isolated frog heart. Compound 6 was the only amino alcohol tested on this and the following tissues. In a concentration of 1×10^{-7} it had no

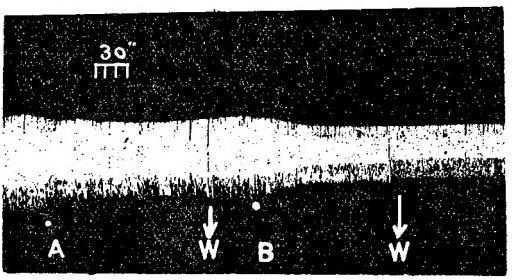


FIG. 3. *Isolated rabbit auricle.* 50 ml. bath. At A 50 μg. and at B 500 μg. of Compound 6 was added to the bath. At W the solution was changed.

appreciable effect on frog heart. Higher concentrations, 1×10^{-6} or 1×10^{-5}, progressively depressed the amplitude and rate of contraction, the depression gradually worsening with the time of contact. The heart, however, recovered after repeated washing (Fig. 2).

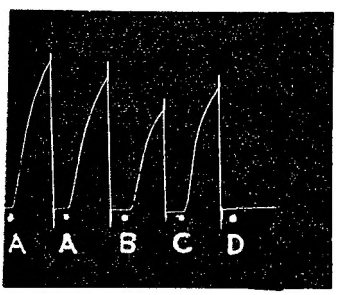


FIG. 4. *Isolated frog rectus abdominis muscle.* 5 ml. bath.

A, contraction due to 0·5 μg. of ACh for 90 seconds.

B, contraction due to 300 μg. of Compound 6 for 90 seconds.

C, contraction due to 300 μg. of Compound 6 in the presence of 50 μg. of tubocurarine chloride.

D, contraction due to 0·5 μg. of ACh in the presence of 50 μg. of tubocurarine chloride.

Isolated rabbit auricle. A dose of 0·5 mg. of Compound 6 in a 50 ml. bath depressed the amplitude of contraction without changing the rate. Smaller doses had little or no effect on the amplitude or rate (Fig. 3). The preparation required about 30 minutes to recover from the depression produced by Compound 6.

Isolated frog rectus muscle. 50 to 100 μg. of Compound 6 added to the 5 ml. bath produced no effect itself on the muscle, but depressed the stimulant action of acetylcholine. Higher doses (300–500 μg.) produced contracture which was not antagonized by curare (Fig. 4). When the dose was increased to 1 mg., the muscle showed an irreversible contracture.

Action on blood pressure. Doses from 0·5 to 2 mg./kg. of Compound 6 intravenously caused a fall in blood pressure.

DISCUSSION

Nine of the twelve amino alcohols reported here have the common property of producing symptoms of central excitation characterised by restlessness, tremor, ataxia, and reflex hyperexcitability. Sustained

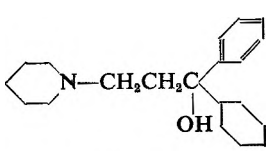
tremor without convulsion and death is a rare phenomenon. The compounds produce a profound and sustained tremor of the head, body and limbs accompanied by continuous struggling forward movements, and retropulsion or circling movements. Retropulsion is not seen during harmine or harmaline tremor, although the tremor produced by these alkaloids is sometimes associated with difficulty in walking. The amino alcohols do not cause parasympathetic stimulation, and in larger doses they produce convulsion. In these respects they resemble harmine.

Bulbocapnine distinctly inhibits the harmine tremor[10] while Zetler[11] has reported that lysergic acid diethylamide (LSD) and 5-HT are effective antagonists of harmine tremor. However, bulbocapnine, LSD and 5-HT did not antagonize the amino alcohol induced tremor. It is of interest that 1:1-di-(*p*-tolyl)-3-aminopropan-1-ol (Compound 8) and 1:1-di-(ortho-anisyl)-3-aminopropan-1-ol (Compound 10), which were not themselves tremorogenic, were incapable of antagonizing the tremor produced by Compound 6. Tremorine tremor is accompanied by marked parasympathetic stimulation and lack of movement. The animals do not exhibit the spasmodic forward extensor movement or retropulsion observed during the amino alcohol tremor. Tremorine tremor is specifically antagonized by anti-Parkinson drugs which are ineffective against the amino alcohol-induced tremor.

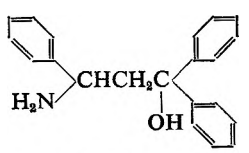
From the above facts it appears that the mechanism of production of tremor by the amino alcohols is quite different from that of harmine or Tremorine.

The tremor is not due to nicotinic-like action, as there is no evidence of tachyphylaxis, and it is not antagonized by hexamethonium which antagonizes nicotine convulsions[12]. Chlorpromazine, however, in 10 mg./kg. dosage, significantly reduced the tremor. In large doses pentobarbitone sodium and mephenesin also antagonized the amino alcohol tremor.

Tremor may be evoked by drugs having a variety of chemical structures. Although nicotine, harmaline and Tremorine all contain a five-membered ring of the pyrrole type, it is unlikely that this structure is responsible for the production of tremor, since so many pyrrole derivatives are known which do not cause tremor. The amino alcohols themselves neither possess a pyrrole ring, nor do they appear likely to give rise to one under physiological conditions. The failure of benzhexol to antagonize amino alcohol tremors is interesting in view of its close structural relationship to the amino alcohols in general and to Compound 6 in particular; it is in fact itself an amino alcohol:



Benzhexol



Compound 6

The principal difference between benzhexol and Compound 6 is in the environment of the nitrogen atom which is free in the latter, but is part of

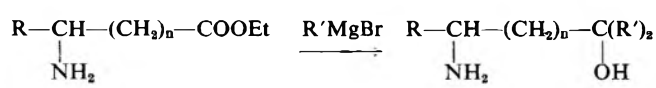
a piperidine ring in the former. Thus it would not be surprising if the extra bulk around the nitrogen atom of benzhexol prevented it from combining with the tremor receptor. If this were true, then, taken in conjunction with the fact that benzhexol antagonizes nicotine and Tremorine tremors, it would indicate that the amino alcohols act on a different receptor from nicotine or Tremorine. The fact that benzhexol is a satisfactory anti-Parkinson drug also supports our view that the tremor produced by amino alcohols is essentially different from the tremor of Parkinson's disease, and that amino alcohols of the type described here are unsuitable for eliciting tremor in the testing of anti-Parkinson drugs. They may, however, be useful tools for further study of the mechanism of tremor production.

The effects of the compounds on heart, intestine and blood pressure are probably caused by direct depression of muscles resulting from local anaesthetic actions. The muscular contracture which occurs in the frog rectus abdominis when exposed to a high concentration of the drug may be due to a toxic action on the muscle proteins.

Synthesis of Amino Alcohols

M.ps. are uncorrected.

The amino alcohols were prepared from esters of suitable α- or β-amino acids according to the general equation



Ethyl phenylamino-acetate was prepared as described by Marvel and Noyes[13].

Ethyl β-phenyl-β-aminopropionate was prepared as described by McKenzie and Richardson[14].

Ethyl β-(2-furyl)-β-aminopropionate

β-(2-furyl)-β-aminopropionic acid was prepared by a modification of the method described by Posner[15]. To a cold solution of sodium ethoxide (30 g. sodium dissolved in 1000 ml. absolute ethanol) was added a solution of 93 g. hydroxylamine hydrochloride in 65 ml. water. The precipitated sodium chloride was filtered off, and 90 g. furylacrylic acid was added to the filtrate. The clear solution was refluxed for 17 hours, solid beginning to separate after 15 hours. Filtration of the cold reaction mixture gave β-(2-furyl)-β-aminopropionic acid (19 g.), m.p. 202° to 206°.

Esterification of the acid with alcoholic hydrogen chloride gave the ethyl ester (12·5 g.), b.p. 60° to 64°/0·1 mm. Found C, 59·0; H, 7·3; N, 7·6. $C_9H_{13}O_3N$ requires C, 59·0; H, 7·1; N, 7·7.

The following example illustrates the method of applying the Grignard reaction to the preparation of amino alcohols from the above esters.

Preparation of 1 : 1-*di*-(m-*tolyl*)-3-*phenyl*-3-*aminopropan*-1-*ol*

An ethereal solution of ethyl β-phenyl-β-aminopropionate (9·6 g.; 0·05 mol.) was added to a cooled ethereal solution of the Grignard reagent

prepared from *m*-bromotoluene (51 g.; 0·3 mol.). After standing for 30 minutes at room temperature the reaction mixture was refluxed for 30 minutes. The resulting solution was cooled in a freezing mixture, and the organo-metallic complex was decomposed by addition of saturated ammonium chloride solution (1 litre) together with solid ammonium

TABLE IV

AMINO ALCOHOLS

No.		M.pt.°C	Formula	Found per cent			Required per cent			Ref.
				C	H	N	C	H	N	
1	1 : 1 : 2-Triphenyl-2-amino-ethanol	154	$C_{20}H_{19}NO$							14
2	1 : 3-Diphenyl-2-benzyl-3-amino-propan-2-ol	125	$C_{22}H_{23}NO$							16
3	1 : 1-Di-(*o*-anisyl)-2-phenyl-2-aminoethanol	160	$C_{29}H_{29}NO_3$	75·5	6·5	3·8	75·7	6·6	4·0	
4	1 : 1-Diphenyl-2-aminoethanol	110	$C_{14}H_{15}NO$							17
5	1 : 1-Diphenyl-2-aminopropan-1-ol	103	$C_{15}H_{17}NO$							18
6	1 : 1 : 3-Triphenyl-3-amino-propan-1-ol	149	$C_{21}H_{21}NO$							14
7	1 : 4-Diphenyl-2-benzyl-4-amino-butan-2-ol	125	$C_{23}H_{25}NO$	83·6	7·6	3·9	83·4	7·6	4·2	
8	1 : 1-Di-(*p*-tolyl)-3-phenyl-3-aminopropan-1-ol	147	$C_{29}H_{29}NO$	83·4	7·8	4·1	83·4	7·6	4·2	
9	1 : 1-Di-(*m*-tolyl)-3-phenyl-3-aminopropan-1-ol	139	$C_{29}H_{29}NO$	83·2	7·8	4·1	83·4	7·6	4·2	
10	1 : 1-Di-(*o*-anisyl)-3-phenyl-3-aminopropan-1-ol	160	$C_{29}H_{29}NO_3$	75·2	6·8	3·6	76·0	6·9	3·9	
11	1 : 1-Diphenyl-3-amino-propan-1-ol	143	$C_{15}H_{17}NO$							19
12	1 : 1-Diphenyl-3-(2-furyl)-3-aminopropan-1-ol	123	$C_{19}H_{19}NO_2$	78·0	6·5	4·9	77·8	6·5	4·8	

chloride (50 g.) to maintain saturation. The aqueous layer was extracted several times with chloroform. The chloroform extracts, combined with the original ether layer, were washed with water, dried and distilled. As the residual oil did not crystallize on trituration with ether, it was steam distilled to remove by-products of the diphenyl type; after isolation by chloroform extraction the oil gradually solidified on treatment with ether. The amino alcohol crystallized from ethanol as colourless prisms m.p. 139° to 140°. (3·2 g).

Acknowledgements. The authors wish to express their thanks to Dr. G. M. Everett of Abbott Laboratories for a generous supply of Tremorine; to Messrs. May and Baker for diethazine; to Geigy Pharmaceutical Company Ltd. for caramiphen; to Lederle Laboratories for benzhexol and meprobamate; to Messrs. Smith, Kline & French for S.K.F. 525 A; and to Abbott Laboratories Ltd. for trimethadione.

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A SPECTROPHOTOMETRIC DETERMINATION OF TRACES OF PHENOLIC STEROIDS IN 3-KETOSTEROIDS

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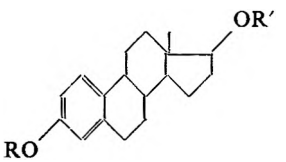
Received July 10, 1958

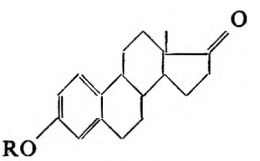
As little as 0·02 per cent of phenolic steroid may be determined in the presence of an excess of 3-ketosteroid. The interfering ketonic absorption is eliminated by selective reduction of ketone by potassium borohydride. The slight absorption of the reduction products may be corrected graphically.

In the presence of large amounts of ketosteroids the determination of oestradiol type steroids (I, II) is not possible by direct ultra-violet spectrometry since the phenolic band about 280 mμ is completely masked by the weak ketonic absorption (maximum about 300 mμ) (Fig. 1).



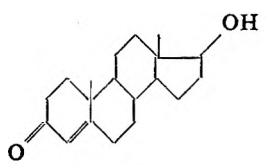
(I)

(a, R = R′ = H; b, R = Me, R′ = H; c, R = H, R′ = MeCO

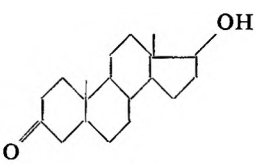


(II)

(a, R = H; b, R = Me)

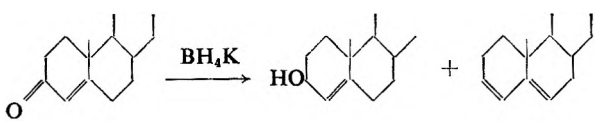


(III)



(IV)

Working with testosterone (III) or androstanolone (IV) the ketonic absorption can be eliminated by selective reduction using potassium borohydride in alkaline methanolic medium as follows.



(III)

Satisfactory results can be obtained with 4 moles BH_4K per mole of ketosteroid, the reaction being allowed to continue for 6½ hours at room temperature. When the reduction is complete and the solution re-acidified, the phenolic band becomes apparent. It is necessary to correct for the weak absorption arising from the reduction products of the ketosteroid. This can be most easily done by a graphical construction. The

residual absorption is nearly linear in the range 272 mμ–300 mμ. In the spectrum of the pure phenol, four wavelengths are selected within this range such that, $\epsilon_{\lambda 1} = \epsilon_{\lambda 3}$, $\epsilon_{\lambda 4}$ equals or is nearly 0, and $\frac{\epsilon_{\lambda 2}}{\epsilon_{\lambda 1}}$ is as large as possible, where λ_2 is the wavelength of the maximum of the phenol.

In the spectrum of a mixture after reduction, the difference in optical density at wavelengths λ_1 and λ_3 is due solely to parasitic absorption and this difference determines the slope s of a straight line △ which represents this absorption to the first approximation. The line △ intercepts the spectrum of the mixture at wavelength λ_4 since by hypothesis the optical density at this point is due solely to the parasitic absorption. The phenol concentration is obtained from the absorbance $DD' = d$ using an apparent E (1 per cent, 1 cm.) for compensating for the weak phenolic absorption at wavelength λ_4.

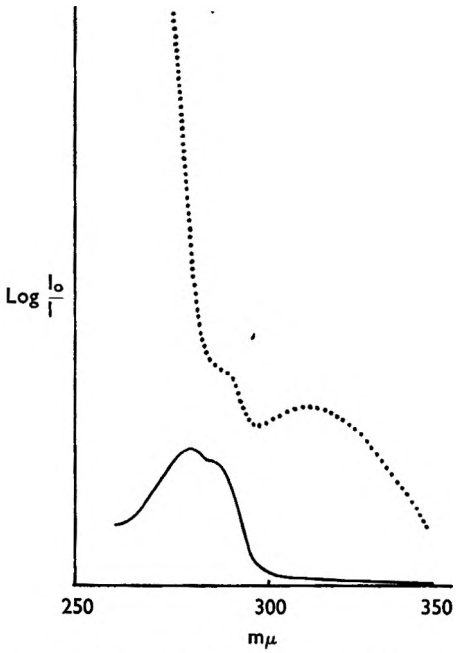


FIG. 1. Mixtures of testosterone and oestradiol.

. . . . . Mixture containing 2 per cent oestradiol before reduction.

——— Mixture containing 1 per cent oestradiol once reduced.

For the smallest concentrations in phenol one can no longer neglect the slight curvature of the true background and it is necessary to add to d an empirical correction $\delta = -a(s + b)$ where a and b are empirical factors, and s is the slope of △ expressed in absorbance variation for an increase in wavelength of 10 mμ.

EXPERIMENTAL METHOD

The steroids are pharmaceutical grade products. Methanol is refluxed with potassium borohydride for 4 hours: methanol 5000 ml., BH_4K 8g., NaOH N 40 ml. and then distilled. The potassium borohydride is a commercial product containing about 90 per cent pure BH_4K. The spectra have been recorded on a model 11 or 14 Cary spectrophotometer.

Recommended Procedure

In a 50 ml. calibrated flask dissolve P mg. (see Table I) of mixture in about 35 ml. of methanol. Add $\frac{4}{6}$ P mg. of potassium borohydride previously dissolved in 4 ml. of 0·1N aqueous sodium hydroxide. Prepare simultaneously a blank containing the same amounts of methanol, borohydride and sodium hydroxide. Allow the reaction to proceed for $6\frac{1}{2}$ hours at room temperature (20–25°) then add to both solutions 4 ml. of

normal aqueous hydrochloric acid. Eliminate dissolved gases by shaking and make up to 50 ml. with methanol. Record the spectrum of the steroid

TABLE I

QUANTITY OF SAMPLE AND CELL LENGTH USED

Phenol content of the sample (per cent)	P mg.	l cm.
About 1	200	5
0·5–0·05	200	10
0·1–0·02	500	10

solution with the blank solution in the reference cell. The weight p′ in mg. of the phenolic steroid per gram of mixture is

$$p' = \frac{5 \cdot 10^5 \, (d + \delta)}{l \, . \, E \, (1 \text{ per cent, } 1 \text{ cm.}) \, . \, P}$$

where l is the cell-length in cm.

Determination of the Numerical Values used in the Graph Correction

Reduce, as previously, known amounts of pure phenolic steroid. Select correct values for λ_1, λ_2, λ_3, λ_4; draw △ and determine with respect to △

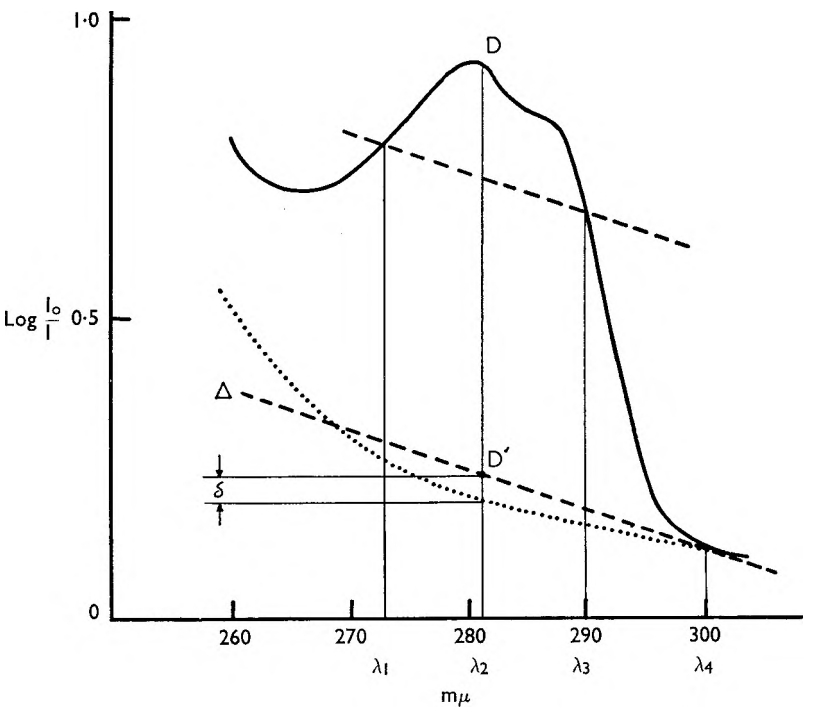


FIG. 2. Graphical correction.

. . . . . Pure testosterone after reduction by BH_4K.
——— Testosterone + 0·1 per cent oestradiol mixture after reduction by BH_4K.

taken as background the apparent E (1 per cent, 1 cm.) for the pure phenol. The values of a and b are determined under similar conditions on several reductions of pure ketosteroid. The slope s usually ranges from zero to $-0{\cdot}1$. The selected numerical values are collected in Table II. They are the same for mixtures containing either testosterone or androstanolone except that in the last case $\delta = 0$.

TABLE II

FIGURES USED FOR THE GRAPHICAL CORRECTION

Phenol determined	λ_1	$\lambda_{2\ max.}$	λ_3	λ_4	Apparent E (1 per cent, 1 cm.)	a	b	Standard deviation on a
Ia	273	281	290	300	71·4	1·15	0·01	0·43
Ib	273	279	288·5	300	67·8	0·97	0·01	0·35
Ic	272	281	290	300	62·0	1·04	0·01	0·26
IIa	273	281	290	300	73·2	1·15	0·01	0·43
IIb	272	278·5	289	300	68·0	1·01	0·01	0·27

TABLE III

RESULTS ON KNOWN MIXTURES

Phenol content	Number of measurements (n)	$\sqrt{\frac{\Sigma e^2}{n}}$
Testosterone mixtures		
0·5 per cent	14	2 per cent
0·2	12	2·5
0·1	18	5
0·05	13	6
0·02	9	12·5
Androstanolone mixtures		
0·5 per cent	4	2·5 per cent
0·2	4	3·5
0·1	2	7·5
0·05	2	10
0·02	2	18

RESULTS

The results obtained with synthetic mixtures are tabulated (Table III) where e is the relative error expressed in per cent. For very small amounts of phenol the determining error is that calculated with reference to δ, i.e., $\Delta\delta = (s + b)\,\Delta a + a\Delta\,(s + b) \simeq (s + b)\,\Delta a + a\Delta s$.

Δa is indicated in Table II and Δs can be evaluated from the graph. The method is considered to be applicable to mixtures containing other types of borohydride reducible ketones so long as the reduction products present no chromophore absorbing below 300 mμ.

THE PHARMACOLOGICAL PROPERTIES OF GLYCYRRHETINIC ACID—A NEW ANTI-INFLAMMATORY DRUG

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Received June 9, 1958

A study of the pharmacological properties of glycyrrhetinic acid, or glycyrrhetic acid, a new anti-inflammatory drug from liquorice, shows it has an extremely low toxicity and is non-irritant to the skin. It has no adverse effects on the heart, circulation or respiration and shows no glucocorticoid-like activity. In large doses in animals it produces water retention, slight sodium retention and an increased excretion of potassium. These effects are not seen with smaller doses used in man.

Glycyrrhetinic or glycyrrhetic acid, a triterpenoid obtained from liquorice, has been proved to be an anti-inflammatory agent[1]. The toxicological properties and pharmacodynamics of this drug are now described. Previously there have been no detailed reports on the pharmacology of this compound, most of the published literature referring to liquorice extract and glycyrrhizin. Molhuysen and others[2] found that a liquorice extract had a deoxycortone-like action, promoting the retention of sodium and water and increasing the excretion of potassium in normal persons. Groen and others[3] reported that liquorice and glycyrrhizinic acid maintained two patients with Addison's disease in correct electrolyte balance and a similar result was obtained by Calvert[4]. Pelser and others[5] found that glycyrrhetinic acid was more effective than glycyrrhizinic acid in this condition.

Liquorice extract was ineffective in one severe case of Addison's disease which had previously shown no response to ACTH[2]. While glycyrrhetinic acid potentiated the action of cortisone[6], alone, it was unable to maintain adrenalectomised patients. Glycyrrhizin also was unable to effect adequate maintenance of the patient with bilateral adrenalectomy[7].

Although glycyrrhetinic acid was thought to have deoxycortone-like actions, Galal[8] has shown that its antidiuretic action differs from that of deoxycortone in rats. Glycyrrhetinic acid has no glucocorticoid-like activity and Hems[9] showed it to be inactive in the mouse liver glycogen test. Recently Atherden[10] found glycyrrhetinic acid to inhibit the metabolism of progesterone and 11-deoxycorticosterone by rat-liver homogenates.

Materials. The glycyrrhetinic acid (Fraction "S") used in these investigations was supplied by Biorex Laboratories Ltd. It was used in the form of tablets and as a saline suspension.

Methods

Acute Toxicity

The acute toxicity of glycyrrhetinic acid was determined on albino mice of both sexes. Injections were made on a weight basis into animals

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weighing between 18 and 22 g. which had fasted overnight. For all routes of administration, where possible, the regression of mortality per cent as probits on the logarithm of the dose was found and the LD50 and limits of error (P = 0·95) calculated by the method of Finney[11].

Subacute Toxicity

The subacute toxicity of glycyrrhetinic acid was determined on young rats, which were injected intramuscularly three times a week for 4 weeks. Their weights were determined twice weekly and finally the rats were killed and examined pathologically. Histological sections of the major organs were prepared. The adrenal glands were weighed and frozen sections stained for lipid with Sudan III.

Dermal Toxicity

This was determined in rabbits as described under the "Procedures for the Appraisal of the Toxicity of Chemicals in Foods, Drugs and Cosmetics"[12]. The primary irritation of the skin was measured by an examination of the skin of an albino rabbit after treatment with glycyrrhetinic acid as follows. Small pellets of cotton-wool were saturated with 0·5 ml. of a suspension of glycyrrhetinic acid containing 100 mg./ml. Three of these saturated pellets were then fixed with adhesive plaster to the previously shaven skin of a rabbit's back. The trunk of the animal was then wrapped in a plastic film to minimise evaporation. The skin underneath the pellets was examined after 24 and 72 hours. In another rabbit the skin was abraded before the pellets were applied.

Pharmacodynamics

The pharmacological effects of glycyrrhetinic acid on the cardiovascular system, and on the central and autonomic nervous systems, were studied in mice, rats and anaesthetised cats. The cats were anaesthetised with chloralose (80 mg./kg.), the blood pressure was recorded from the carotid artery and the respiration was recorded from a tracheal cannula by the method described by Paton[13]. Glycyrrhetinic acid, having a low water solubility, could not be injected intravenously, therefore it was injected intraperitoneally or directly into the duodenum. The effects on gastrointestinal motility were studied *in vitro* on the isolated duodenum of the rabbit, and *in vivo* by the transport of a charcoal meal in mice as described by Bryant and others[14].

Urinary System

The effects of glycyrrhetinic acid on the secretion of urine and the excretion of sodium and potassium were studied in rats and anaesthetised cats. An experiment was also made in student volunteers. After an injection of glycyrrhetinic acid, rats were given 10 ml. of water orally per 100 g. body weight and randomly distributed into groups of three or six and placed into metabolism cages. Control groups were given saline. The urine was collected and measured hourly over 5 hours and the sodium and potassium estimated by flame photometry. Cats were anaesthetised

with chloralose and the bladder cannulated through the urethra for collection of the urine. After a 75 minute control period 100 mg./kg. of glycyrrhetinic acid was injected intraperitoneally. The urine was collected over another 60 minutes. The volumes of urine excreted were measured at 15 minute intervals and taken for estimation of the sodium and potassium present. Samples of blood were also taken at these times for estimation of sodium, potassium, total chloride and glycyrrhetinic acid in the serum.

Glycyrrhetinic acid was estimated in the serum by a modification of the method described by Van Katwijk and Huis in't Veld[15] for the determination of glycyrrhetinic acid in urine. Serum or plasma (0·2 ml.) was added to 0·1N sulphuric acid (1 ml.) and the mixture extracted three times with ether (3 × 2 ml.). The combined ethereal solution was then extracted with 0·5N ammonium hydroxide solution (2 ml.) and the ether layer discarded. The alkaline layer was acidified with 0·7 ml. of 2N sulphuric acid and extracted three times with ether. The combined ether extract was evaporated in a current of air and the residue dried over silica gel at 20° and 3 mm. The residue was dissolved in 3 ml. 95 per cent spectroscopically pure ethanol and the optical density measured at 248 mμ in a spectrophotometer. Serum from the same animal collected immediately before the administration of glycyrrhetinic acid was similarly extracted and the ethanolic solution of the final residue was used as a reference blank. A calibration curve was prepared by measuring the optical density of solutions of glycyrrhetinic acid in 95 per cent ethanol containing 1 to 40 μg./3 ml. The curve was linear throughout this range. The accuracy of the method was checked by adding known concentrations of glycyrrhetinic acid to a control sample of the serum. In a typical experiment in which 5·0 μg. and 10·0 μg. were added to 0·2 ml. serum samples, recoveries of 4·4 μg. (88 per cent) and 10·1 μg. (101 per cent) respectively were obtained. To ensure that the optical density at 248 mμ was specific for glycyrrhetinic acid, measurements were made over the wavelengths 230 to 270 mμ. Maximum absorption at 248 mμ was found unless the serum specimen was haemolysed, in which case non-specific absorption was observed.

The effects of an oral dose of glycyrrhetinic acid was determined in eight healthy male student volunteers in a blind cross over trial. Each student was given 0·2 g. or 0·5 g. of glycyrrhetinic acid or a dummy tablet and 30 minutes later drank 1500 ml. of water. The urine was collected at 30-minute intervals over 2½ hours and the volume was recorded.

Glucocorticoid Action

This was tested in adrenalectomised mice submitted to a cold stress[16]. Groups of 10 mice were adrenalectomised under ether anaesthesia. The following day one group was injected intraperitoneally with 170 mg./kg. of glycyrrhetinic acid and the other group with saline as the controls. Their survival times in a refrigerator at 4° were then recorded to the nearest half hour.

RESULTS

Acute Toxicity

Glycyrrhetinic acid had a low toxicity. Given orally to mice no deaths occurred following single doses as high as 610 mg./kg., which was the maximal dose that could be administered. Similarly by the subcutaneous route it was not possible to kill any mice at this dose level. By the intraperitoneal route deaths did occur over a period of 48 hours and the LD50 was 308 mg./kg. with fiducial limits of error (P = 0·95) from 279 to 340 mg./kg. (Table I). High doses caused sedation, palor of the extremities, and respiratory depression. Death was usually delayed, the mice generally dying on the second day after the administration. Pathologically there was evidence of peritonitis, probably caused by the presence of the insoluble glycyrrhetinic acid in the peritoneal cavity. Glycyrrhetinic acid could not be given intravenously because of its low solubility in water.

TABLE I

ACUTE INTRAPERITONEAL TOXICITY OF GLYCYRRHETINIC ACID IN ALBINO MICE

Dose mg./kg.	No. of mice	Deaths (after 2 days)
216	20	3
263	20	6
320	20	10
390	20	16

LD50 = 308 mg./kg. Fiducial limits (P = 0·95) 279 to 340 mg./kg.

Subacute Toxicity

The growth of young rats was not depressed by intramuscular injections of 10 and 20 mg. of glycyrrhetinic acid three times a week. The treated rats maintained good health, ate well and grew as well as the untreated controls. When killed after 4 weeks there was no evidence at post-mortem of any gross pathological effects and histological sections of the major organs showed no abnormalities. There was no adrenal atrophy, as occurs with cortisone, and sections of the glands were normal except for a slight thinning of the lipid in the zona glomerulosa. This was by no means as severe as occurs with deoxycortone.

TABLE II

THE EFFECT OF GLYCYRRHETINIC ACID ON THE EXCRETION OF WATER IN RATS

Treatment	Volume of urine excreted in ml. Hours after water administration				
	1	2	3	4	5
1. Saline controls	3·0*	7·0	8·3	8·7	9·3
2. Glycyrrhetinic acid 125 mg./kg. Water 30 min. after injection	0·8	4·2	7·2	7·3	8·0
3. Glycyrrhetinic acid 125 mg./kg. Water 2 hours after injection	3·7	7·8	8·3	8·6	9·5
4. Glycyrrhetinic acid 125 mg./kg. Water 4 hours after injection	2·5	9·2	9·5	9·8	10·0

* Each figure represents the mean volume from three rats.

Dermal Toxicity

There was no evidence of oedema or erythema of the normal or abraded skin, proving that glycyrrhetinic acid has no primary irritant action on the skin of the rabbit.

Pharmacodynamics

Glycyrrhetinic acid had no untoward effects on the central or autonomic nervous systems, nor on the heart and circulation.

The central nervous system. This was affected only by extremely large doses of glycyrrhetinic acid. In the mouse a dose of 25 mg. (1250 mg./kg.)

TABLE III

THE EFFECT OF GLYCYRRHETINIC ACID ON THE URINARY EXCRETION OF SODIUM AND POTASSIUM IN RATS

Treatment	Sodium excretion		Potassium excretion	
	mg./5 hr.	Per cent controls	mg./5 hr.	Per cent controls
1. Saline controls	3·2*	—	3·8	—
2. Glycyrrhetinic acid 125 mg./kg. Water 30 min. after injection	0·9	29	4·8	125
3. Glycyrrhetinic acid 125 mg./kg. Water 2 hours after injection	3·8	117	7·7	200
4. Glycyrrhetinic acid 125 mg./kg. Water 4 hours after injection	2·8	86	6·0	157

* Each figure represents the mean from three rats.

intraperitoneally caused sedation, hypnosis, hypothermia and respiratory depression.

The autonomic nervous system. In the mouse intraperitoneal and oral doses of 25 mg. or 12 mg. subcutaneously did not stimulate or depress

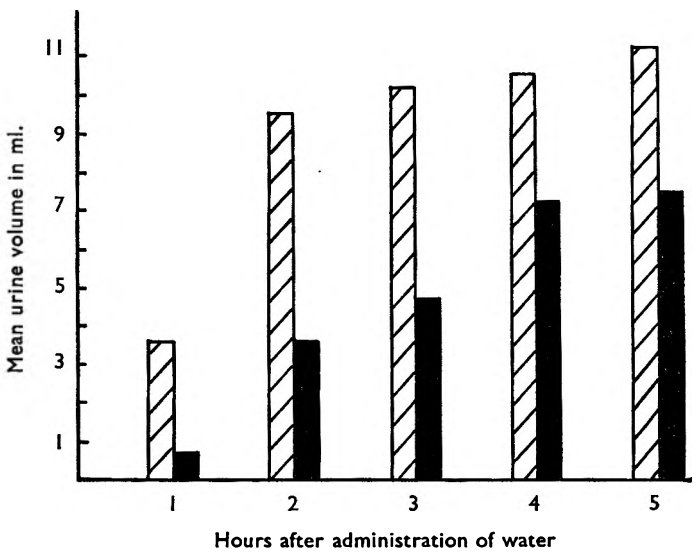

FIG. 1. The antidiuretic action of glycyrrhetinic acid (125 mg./kg. i.p.) in rats given 100 ml./kg. of water orally. Each value is the mean from six rats. Shaded area, controls; black area glycyrrhetinic acid.

either the sympathetic or parasympathetic branches of the autonomic nervous system. Similarly in the cat, an intraperitoneal dose of 125 mg./kg. did not alter the blood pressure or affect the normal responses to stimulation of the sympathetic or parasympathetic nerves. The responses

to an intravenous injection of acetylcholine, nicotine or adrenaline were normal.

The cardiovascular system. In the anaesthetised cat very large doses (125 mg./kg.) administered intraperitoneally or injected directly into the duodenum did not affect the blood pressure or the heart beat. Intravenous administration of glycyrrhetinic acid was precluded because of its low solubility.

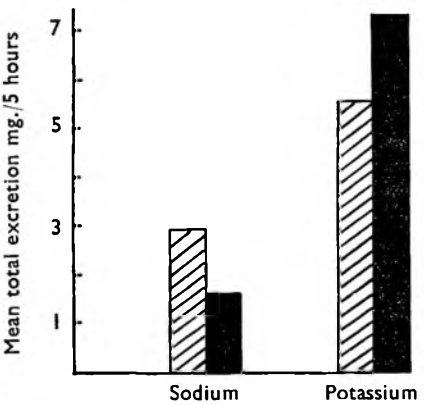

FIG. 2. The effect of glycyrrhetinic acid (125 mg./kg. i.p.) on the urinary excretion of sodium and potassium in rats. Each value is the mean from six rats. Shaded area, controls; black area, glycyrrhetinic acid.

The respiratory system. In the anaesthetised cat doses as high as 125 mg./kg., injected intraperitoneally, did not affect the depth or rate of respiration. In mice respiratory depression was only seen after toxic doses of glycyrrhetinic acid (610 mg./kg.) were given intraperitoneally.

The gastrointestinal tract. Glycyrrhetinic acid did not affect the motility of the gastrointestinal tract. *In vitro* the addition of 1 mg. of glycyrrhetinic acid to a 15 ml. bath did not affect the tone or contractions of the isolated duodenum of the rabbit. The normal responses to acetylcholine, adrenaline and barium chloride were unchanged, showing the absence of a spasmolytic action. *In vivo* the rate of transport of carbon through the stomach and intestine of the mouse was not affected when compared with untreated controls. The oral administration of glycyrrhetinic acid in rats and mice did not have a constipating action, or cause diarrhoea.

TABLE IV

THE EFFECTS OF GLYCYRRHETINIC ACID ON THE EXCRETION OF URINE, SODIUM AND POTASSIUM IN THE CAT

Urine sample (15 min. intervals)	Volume ml.	Sodium		Potassium	
		m. eq./l.*	m. eq./15 min.	m. eq./l.	m. eq./15 min.
1	4·8	210	—	30	—
2	3·2	205	0·66	26·5	0·085
3	2·6	205	0·53	26	0·068
4	2·3	195	0·45	25·5	0·059
5	3·4	185	0·63	27·5	0·063
	Injection of 200 mg./kg. of glycyrrhetinic acid				
6	3·5	170	0·60	31·5	0·110
7	2·0	180	0·36	32	0·064
8	1·7	175	0·30	28	0·048
9	1·2	185	0·22	28	0·038

* Milliequivalents per litre urine.

The urinary system. Glycyrrhetinic acid did have an effect on kidney function in the rat. This has been examined in some detail. There was a marked antidiuretic action, which confirms the work of Galal[8], a retention of sodium and an increase in the urinary potassium excretion.

Typical results are shown in Tables II and III. In this experiment group 1, the controls, were given normal saline, while groups 2, 3 and 4 were injected intraperitoneally with 125 mg./kg. of glycyrrhetinic acid. The corresponding groups of rats were given an oral dose of 10 ml./100 g. of water either half, 2 or 4 hours after the administration of glycyrrhetinic acid. The effects on sodium and water metabolism occurred in group 2,

TABLE V

THE EFFECTS OF GLYCYRRHETINIC ACID ON SODIUM, POTASSIUM AND CHLORIDES IN THE SERUM OF THE CAT

Treatment	Glycyrrhetinic acid mg./100 ml.	m. eq./l. serum		
		Sodium	Potassium	Chlorides
A. Control(70 min. before glycyrrhetinic acid)	—	150	3·4	124
B. Control (5 min. before glycyrrhetinic acid) ..	—	151	3·7	124
C. 35 min. after glycyrrhetinic acid	0·25	154	3·6	121
D. 75 min. after glycyrrhetinic acid	0·65	153	3·8	126

TABLE VI

EFFECTS OF GLYCYRRHETINIC ACID ON URINE SECRETION IN EIGHT MALE VOLUNTEERS AFTER DRINKING 1500 ML. OF WATER

Treatment	Time in hours after drinking water				
	½	1	1½	2	2½
Placebo controls	69*	294	609	782	904
Glycyrrhetinic acid 0·2 g.	65	286	608	828	936
Glycyrrhetinic acid 0·5 g.	124	406	731	931	1096

* Average cumulative total excretion in ml.

TABLE VII

GLUCOCORTICOID ACTIVITY OF GLYCYRRHETINIC ACID IN THE MOUSE SURVIVAL TEST

Treatment (dose 20 g. mouse)	Mean survival time in hours	Standard error
Controls saline	4·9*	± 0·45
Glycyrrhetinic acid 1·56 mg. ..	3·55	± 0·40
,, ,, 6·25 mg. ..	2·65	± 0·26
,, ,, 25 mg. ..	2·65	± 0·36

* Each value is the mean of ten mice.

where the administration of glycyrrhetinic acid preceded the water loading by 30 minutes, and not in groups 3 and 4 where the time interval was longer. Potassium excretion was increased in all groups.

These results were confirmed in a second experiment using two groups of six rats. The first group received 0·25 ml. of saline and the second group 125 mg./kg. of glycyrrhetinic acid intraperitoneally. 10 ml. of water per 100 g. rat was given orally 30 minutes later. The urine excretion is shown in Figure 1 and the excretion of sodium and potassium in Figure 2.

In the anaesthetised cat an intraperitoneal injection of 100 mg./kg. of glycyrrhetinic acid caused a reduction in the urine flow, a slight decrease in the urinary excretion of sodium and a slight increase in the excretion of potassium (Table IV). There were no significant changes in the serum concentrations of sodium, potassium and total chlorides (Table V).

Estimation of glycyrrhetinic acid in the serum showed that absorption occurred from the peritoneal cavity.

In eight student volunteers oral doses of 0·2 g. and 0·5 g. of glycyrrhetinic acid before drinking 1500 ml. of water did not produce an antidiuretic effect (Table VI).

Glucocorticoid action. Glycyrrhetinic acid did not increase the survival time of adrenalectomised mice submitted to a cold stress (Table VII). This confirms the observations of Wenzel and others[5-7] and D'Arcy and others[16]. Hems[9] was unable to find a glucocorticoid action with glycyrrhetinic acid when tested by the liver glycogen test.

Conclusions

Glycyrrhetinic acid is seen to have a remarkably low toxicity and therefore can be applied to the skin with complete safety in dermatological conditions. So far it has been little used internally, although liquorice extract has been taken orally for years. We have confirmed that exceptionally large doses of glycyrrhetinic acid in animals have an antidiuretic action associated with changes in the metabolism of sodium and potassium; but do not cause kidney damage. Water retention was not seen with small doses used in human volunteers. The low solubility of glycyrrhetinic acid in body fluids has so far precluded parenteral administration in man, but this will be possible with development of more soluble derivatives which may prove of value in rheumatic diseases. An important property of glycyrrhetinic acid is its complete freedom from glucocorticoid-like actions, a serious disadvantage with the corticosteroids. While it has been shown that little absorption of these steroids occurs through normal skin this cannot be assumed in dermatological conditions where the protective dermal layers may be broken. Much remains to be discovered about the mode of action of glycyrrhetinic acid, but it offers a new approach to the treatment of inflammatory conditions free from the disadvantages of corticoids which have claimed so much attention and disproves the concept that an anti-inflammatory agent must of necessity have a concomitant corticoid-like action.

Acknowledgements. The authors are indebted to Professor E. E. Turner, F.R.S., of Bedford College, University of London and Dr. S. Gottfried of Biorex Laboratories Ltd., London, E.C.1, for their help and generous supply of materials.

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SOME *IN VITRO* INHIBITORS OF CARBONIC ANHYDRASE

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Received July 3, 1958

Work on sulphonamides as *in vitro* inhibitors of carbonic anhydrase has been extended by the preparation and assay of novel types. Although diphenylsulphonamides, *p*-alkoxycarbonylbenzene sulphonamides and 5-alkoxycarbonylamino-1:3:4-thiadiazole-2-sulphonamides showed noteworthy activity *in vitro*, only the thiadiazole derivatives possessed diuretic activity in the rat.

IN 1940 Mann and Keilen[1] showed that certain aromatic sulphonamides could inhibit the enzyme "carbonic anhydrase" *in vitro*. This important discovery was confirmed shortly afterwards by Locke, Main and Mellor[2] and also by Höber[3] (who used an isolated frog kidney to determine enzyme inhibition) who likewise showed that whilst compounds possessing the free sulphonamide group were inhibitors of carbonic anhydrase, *N*-substituted sulphonamides were essentially inactive. Davenport[4] found that the five-membered heterocyclic ring structure thiophene-2-sulphonamide possessed up to forty times the activity of sulphanilamide. Krebs[5] carried out a systematic study of a series of aromatic sulphonamides and found that "Prontosil Red" (I) and "Prontosil Soluble" (II) were highly potent inhibitors of the enzyme. He also confirmed that substitution of the sulphonamide group, or its separation from the aromatic nucleus, as in ω-sulphonamidotoluene, were accompanied by virtual disappearance of activity.

Schwartz[6] pointed out in 1949 that as renal carbonic anhydrase catalysed the equilibrium reaction

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

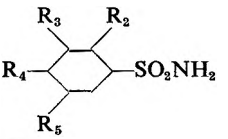
its inhibition would lead to a decrease in the rate of conversion of carbon dioxide into carbonic acid and consequently in the rate of production of hydrogen ions. The excretion of hydrogen ions was known to represent the normal mechanism for the conservation of sodium ions. Inhibition of renal carbonic anhydrase might consequently be expected to lead to increased excretion of sodium ions. Carbonic anhydrase inhibitors might consequently offer a new approach to the production of diuretics. In support thereof Schwartz reported on the use of sulphanilamide in the control of oedema associated with congestive heart failure. He also drew the attention of Roblin and his co-workers who were interested both in heterocyclic sulphonamides and in carbonic anhydrase inhibitors, to the implications of his work. In following up these developments, Roblin and others[7,8] found that, in general, increasing inhibitory activity was associated with increasing acidity of the sulphonamide, maximum activity being obtained by attachment of a sulphonamide group to a 5-membered heterocyclic ring (cf. Davenport)[4]. The 1:3:4-thiadiazoles proved particularly active, 5-acetamido-1:3:4-thiadiazole-2-sulphonamide being

selected for further study. Its introduction into clinical practice was subsequently reported[9].

Initially we directed attention to simple derivatives of benzene sulphonamide, but failed to find therein compounds of significant activity.

TABLE I

MISCELLANEOUS DERIVATIVES OF BENZENE SULPHONAMIDE



R_2	R_3	R_4	R_5	Activity
—	—	—	—	3
—	—	CH_3CONH-	—	17
—	Cl-	CH_3CONH-	Cl-	<1
—	—	 CONH-	—	9
—	—	*p*-Cl-C_6H_4CONH-	—	50
—	—	C_6H_5OCSNH-	—	55
—	—	C_6H_5OCSNH-	—	35
—	CH_3-	CH_3-	—	3
CH_3-	—	CH_3-	—	<1
CH_3-	—	—	CH_3-	<1
Cl-	—	CH_3-	—	105
—	Br-	CH_3-	—	105
—	Cl-	-COOH	—	5
—	Br-	-COOH	—	10
—	CH_3O-	-COOH	—	<1
—	CH_3O-	*n*-C_4H_9OCO-	—	30
—	Cl-	Cl-	—	240
—	CH_3-	Cl-	—	55
—	-COOH	Cl-	—	20
—	—	Br-	—	90
—	—	I-	—	200

Examination of the results (Table I) revealed that, in general, inhibitory activity is increased by electronegative *p*-substituents and in particular by halogens. More potent sulphonamides were found among dicyclic aromatic structures (Table II). Naphthalene 1- and 2-sulphonamides

TABLE II

COMPOUNDS CONTAINING 2 ARYL NUCLEI

	Activity
Naphthalene-1-sulphonamide	17
Naphthalene-2-sulphonamide	14
Diphenyl-4-sulphonamide	360
Diphenyl-4:4′-disulphonamide	690
2-Aminodiphenyl-4-sulphonamide	80
2-Acetamidodiphenyl-4-sulphonamide	40
2:2′-Diaminodiphenyl-4:4′-disulphonamide	135
Diphenylether-4-sulphonamide	50
Diphenylether-4:4′-disulphonamide	140
Diphenylsulphide-4-sulphonamide	80
Diphenylsulphide-4:4′-disulphonamide	515
Phenoxthin-3-sulphonamide	470
Phenoxthin-3:7-disulphonamide	245
N^4-*p*-Bromobenzenesulphonsulphanilamide	4
N^4-*p*-Tolylsulphonsulphanilamide	13

were of little interest (cf.[5]). Diphenyl derivatives, in contrast, were uniformly active. Thus diphenyl-4-sulphonamide was 360 times more potent than sulphanilamide itself, the activity being increased to nearly

700 times that of sulphanilamide by substituting a second sulphonamide group into the *p'*-position. Amino groups diminished potency, a result similar to that observed by Krebs[5] in the case of benzene sulphonamide. Phenoxthin-3 : 7-disulphonamide, in contrast, was less potent than the 3-monosulphonamide.

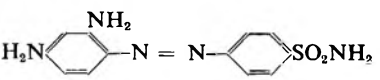
The high activity of Prontosil (I) led us to study the stilbene and anil derivative shown in Table III. The observed activities clearly demon-

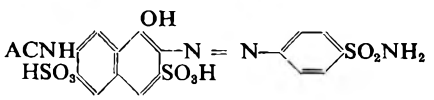
TABLE III

TWO RINGS LINKED BY UNSATURATED BOND(S)

	Activity
Prontosil	185
Stilbene-4-sulphonamide	155
Benzylidene sulphanilamide (III; R = H)	30
p-Chlorobenzylidene sulphanilamide (III; R = *p*-Cl)	55
2:4-Dichlorobenzylidene sulphanilamide	330
2-Hydroxybenzylidene sulphanilamide	35
3-Methoxy-4-hydroxy-5-iodobenzylidene sulphanilamide	120
p-Sulphamylbenzylidene *p*-chloroaniline	85
p-Sulphamylbenzylidene *p*-toluidine	230
p-Sulphamylbenzylidene aniline (IV; R = NH_2)	40

strate the superiority of the azo-linked structure (I) over the related anil types (III) and (IV). It is tempting to correlate this result with the


(I)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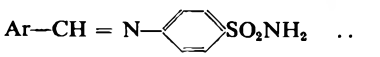
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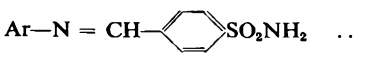

(IV)

observation that replacement of the =CH–CH= bridge in thiophene-2-sulphonamide by the diaza-group =N–N= is accompanied by impressive gain in inhibitory action (cf.[4,7,8]). Some derivatives of *p*-sulphamylphenyl urea and *p*-sulphamylphenylthiourea were also prepared, but none of these showed appreciable *in vitro* activity (Table IV) apart from products of unknown structure obtained by oxidation of *p*-sulphamylphenylthiourea with iodine or hydrogen peroxide.

Examination of *p*-carboxybenzenesulphonamide confirmed the earlier findings of Krebs[5] that this compound has relatively little activity. Its esters, in contrast, proved to be remarkably potent inhibitors of carbonic anhydrase *in vitro*. Butyl to dodecyl esters were particularly noteworthy, showing from 1000 to 2000 times the activity of sulphanilamide (Table V). Further increase in molecular weight was accompanied by rapid decrease in activity, possibly owing to the low solubility of the compounds in

aqueous solvents. Some substituted 2-amino-1 : 3 : 4-thiadiazoles[10] were also examined (Table VI) and proved moderately active.

Biological study of the above compounds by Dr. A. David and Mr. K. P. Fellowes, B.Sc. (Biological Laboratories, Godalming, Surrey) failed to

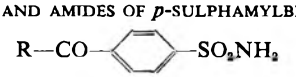
TABLE IV

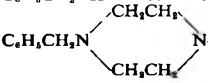
p-SULPHAMYLPHENYLUREA AND RELATED COMPOUNDS

	Activity
1-*p*-Sulphamylphenylurea	7
1 : 3-Bis-(*p*-sulphamylphenyl)-urea	210
1-*p*-Sulphamylphenylthiourea	4
1-Phenyl-3-(*p*-sulphamylphenyl)-thiourea	25
2-*p*-Sulphamylphenylaminothiazole	30
1 : 3-Bis-(*p*-sulphamylphenyl)-formamidine	135
p-Sulphamylphenylguanidine hydrochloride	3
2-Amino-4-(*p*-sulphamylphenylamino)-*sym*-triazine	18
5-Sulphamyl-1 : 3 : 4-thiazolo-1 : 2 : 1′ : 2′-quinoline	20
Unidentified oxidation products of *p*-sulphamylphenylthiourea:	
With iodine (i)	315
(ii)	335
With H_2O_2	220

TABLE V

ESTERS AND AMIDES OF *p*-SULPHAMYLBENZOIC ACID



R	Activity (Sulphanilamide = 1)
HO-	4
CH_3O-	100
C_2H_4O-	260
C_3H_7O-	505
C_4H_9O-	1140
$C_5H_{11}O$-	930
$C_6H_{13}O$-	2290
$C_7H_{15}O$-	1430
$C_8H_{17}O$-	2080
$C_9H_{19}O$-	2280
$C_{10}H_{21}O$-	1220
$C_{11}H_{23}O$-	2250
$C_{12}H_{25}O$-	1040
$C_{16}H_{33}O$-	1
$C_{18}H_{37}O$-	3
iso-C_3H_7O-	115
sec-C_4H_9O-	155
iso-C_4H_9O-	530
CH_2 : $CH.CH_2O$-	900
$C_6H_5CH_2O$-	2400
$C_6H_5.C_2H_4O$-	945
cyclo-$C_6H_{11}O$-	505
HOC_2H_4O-	135
NH_2-	40
NH_2NH-	15
$NH_2CSNHNH$-	18
$(HOC_2H_4)_2N$-	1
$C_6H_5CH_2NH$-	215
	3

reveal correlation between *in vitro* and *in vivo* activity. With the exception of the thiadiazole derivatives (Table VI), the more potent inhibitors of the enzyme *in vitro* were uniformly inactive *in vivo* in inducing diuresis in the rat on oral administration.

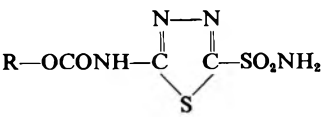
EXPERIMENTAL

N^4-(p-*Bromobenzenesulphonyl*)-*sulphanilamide.* *p*-Bromobenzenesulphonyl chloride (2·6 g.) was added with stirring at room temperature to a solution of sulphanilamide (1·7 g.) dissolved in the minimum volume of pyridine. The orange solution was allowed to stand at room temperature for 24 hours and was then diluted with water. The product was crystallised from aqueous ethanol in small needles of m.p. 210° to 212°. Found: C, 36·7; H, 2·8; N, 7·5; S, 16·1. $C_{12}H_{11}O_4N_2BrS_2$ requires C, 36·8; H, 2·8; N, 7·2; S, 16·4 per cent.

N^4-(p-*Toluenesulphonyl*)-*sulphanilamide* crystallised from acetic acid in shining needles of m.p. 188° to 189°. Found: C, 47·9; H, 4·6; N, 8·4; S, 19·3. $C_{13}H_{14}O_4N_2S_2$ requires C, 47·8; H, 4·3; N, 8·6; S, 19·6 per cent.

TABLE VI

2-ALKYLOXYCARBONYLAMINO-1 : 3 : 4-THIADIAZOLE-5-SULPHONAMIDES



R	Activity
CH_3-	230
C_2H_5-	105
C_3H_7- ..	280
C_4H_9- ..	195
C_5H_{11}- ..	410
C_6H_{13}- ..	330
$C_6H_5.CH_2$- ..	1020

2 : 2′-*Diaminodiphenyl*-4 : 4′-*disulphonamide.* 2 : 2′-Dinitrodiphenyl-4 : 4′-disulphonchloride prepared by the method of Feldmann[11] yielded the disulphonamide on treatment with liquid ammonia. The latter had m.p. 263° (softening 258°) after crystallisation from acetic acid. The disulphonamide (1 g.) was heated under reflux with 95 per cent ethanol (150 ml.), concentrated hydrochloric acid (1·5 ml.) and reduced iron powder (25 g.) for 8 hours. The suspension was filtered, the mother liquors neutralised and concentrated to dryness under reduced pressure. After addition of water to the residue the diamine (0·5 g.) was collected and crystallised from ethanol, m.p. 269°. Found: C, 42·2; H, 4·6; S, 18·5. $C_{12}H_{14}O_4N_4S_2$ requires C, 42·1; H, 4·1; S, 18·7 per cent.

Stilbene-4-*sulphonamide.* A mixture of sulphanilamide (8·6 g.) and sodium nitrite (3·8 g.) was dissolved in N sodium hydroxide solution (50 ml.), and this solution was added cautiously with stirring to ice cold sulphuric acid prepared from concentrated acid (9 ml.) and chopped ice (50 g.). After allowing to stand for 5 minutes a solution of cinnamic acid (7·4 g.) in acetone (270 ml.) was added, followed by a solution of cupric chloride dihydrate (2·1 g.) and sodium acetate (28·7 g.). The mixture was allowed to stand overnight, filtered and the product precipitated by dilution with water and crystallisation from ethanol. It had m.p. 249° to 250°[12]. Found: C, 64·4; H, 5·1; N, 5·4; S, 12·7. Calc. for $C_{14}H_{13}O_2NS$: C, 64·8; H, 5·0; N, 5·4; S, 12·4 per cent.

p-*Sulphamylbenzylidene* p-*toluidine*. A solution of benzaldehyde-4-sulphonamide was prepared by the method of Dakin[13]. Condensation with *p*-toluidine yielded the *product* which separated from ethanol in pale yellow needles, m.p. 218° to 219°. Found: N, 10·2. $C_{14}H_{14}O_2N_2S$ requires N, 10·2 per cent.

p-*Sulphamylbenzylidene* p-*chloroaniline* separated from aqueous ethanol in pale yellow needles, m.p. 194° to 196°. Found: C, 53·1; H, 3·7; N, 9·2. $C_{13}H_{11}O_2N_2ClS$ requires C, 53·0; H, 3·8; N, 9·5 per cent.

N^4-p-*Methylbenzylidene sulphanilamide*. A mixture of sulphanilamide (6·88 g.) and *p*-tolualdehyde (4·8 g.) in the minimum volume of ethanol was heated on the steam bath for 15 minutes. The *product* separated on cooling and was purified by crystallisation from a mixture of ethanol and acetone forming shining plates of m.p. 195°. Found: C, 61·5; H, 5·1; N, 10·3; S, 11·6. $C_{14}H_{14}O_2N_2S$ requires C, 61·3; H, 5·1; N, 10·2; S, 11·7 per cent.

N^1-p-*Chlorobenzylidene sulphanilamide*, after crystallisation from acetone, had m.p. 193° to 194°. Found: C, 52·9; H, 3·9; N, 9·5; Cl, 11·8. $C_{13}H_{11}O_2N_2ClS$ requires C, 53·0; H, 3·7; N, 9·5; Cl, 12·0 per cent.

N^4-(2′:4′-*Dichlorobenzylidene*)-*sulphanilamide*, after crystallisation from acetone had m.p. 205° to 206°. Found: C, 47·9; H, 2·9; N, 8·3; Cl, 21·4; S, 9·6. $C_{13}H_{10}O_2N_2Cl_2S$ requires C, 47·4; H, 3·0; N, 8·5; Cl, 21·5; S, 9·7 per cent.

N^4-(3′-*Iodo*-4′-*hydroxy*-5′-*methoxy*)*benzylidene sulphanilamide* crystallised from a mixture of ethanol and acetone, m.p. 208°. Found: C, 39·2; H, 2·9; N, 6·4; S, 7·4; I, 29·6. $C_{14}H_{13}O_4N_2IS$ requires C, 38·9; H, 3·0; N, 6·5; S, 7·4; I, 29·4 per cent.

1-(2′-*Quinolyl*)-*thiosemicarbazide*. To a solution of 2-hydrazinoquinoline hydrochloride (9·7 g.) in water (100 ml.) was added a solution of potassium thiocyanate (5·4 g.) dissolved in the minimum volume of water and the solution heated on the steam bath for 1 hour. The *product* which separated on cooling crystallised from ethanol in yellow needles m.p. 158°. Found: C, 55·1; H, 4·6; N, 25·4; S, 14·8. $C_{10}H_{10}N_4S$ requires C, 55·0; H, 4·6; N, 25·7; S, 14·7 per cent.

1:3:4-*Triazolo*-5-*mercapto*-1:2:1′:2′-*quinoline*. To a solution of 2-hydrazinoquinoline (1·59 g.) in ethanol (50 ml.) was added carbon disulphide (1·52 g.) followed by a solution of potassium hydroxide (0·56 g.) in water (5 ml.). The mixture was heated under reflux for 2 hours when evolution of hydrogen sulphide ceased. The solvent was largely distilled off, water added to dissolve the residual solid and the solution just acidified with hydrochloric acid. The *product* (1·8 g.) was collected, washed with water and crystallised from ethanol in needles, m.p. 258°. Found: C, 60·0; H, 3·5; N, 20·6; S, 15·6. Calc. for $C_{10}H_7N_3S$: C, 59·7; H, 3·5; N, 20·9; S, 15·9 per cent.

(Marchwald and Meyer[14], prepared this compound, m.p. 261°, by heating 4-phenyl-1-quinolyl(2)-thiosemicarbazide to 150°.)

1:3:4-*Triazolo*-5-*sulphamyl*-1:2:1′:2′-*quinoline*. The foregoing mercaptan (8 g.) was finely powdered, suspended in a mixture of glacial acetic acid (60 ml.) and water (30 ml.), cooled to 0° to 5° and treated with

a slow stream of chlorine gas for 3 hours. The product was collected, washed with cold water and added moist to concentrated ammonia solution (100 ml., d = 0·880). The sulphonamide crystallised and was purified by successive crystallisations from water, ethanol and glacial acetic acid, forming needles m.p. 255° to 256° (decomp.). Found: C, 48·4; H, 2·7; N, 22·3; S, 12·9. $C_{10}H_8O_2N_4S$ requires C, 48·4; H, 3·2; N, 22·6; S, 12·9 per cent.

n-*Propyl*-N-p-*sulphamyl phenyl thiocarbamate.* Powdered *p*-sulphamylphenyl *iso*-thiocyanate (4·3 g.) was suspended in *n*-propanol (100 ml.) and the mixture heated under reflux for 18 hours. The solid dissolved after several hours. Concentration to half-bulk followed by dilution with light petroleum (b.p. 60° to 80°) yielded the *product* which crystallised from a mixture of *n*-propanol and light petroleum (b.p. 60° to 80°) in white needles clusters, m.p. 165° to 167°. Found: C, 44·0; H, 5·3; N, 10·3. $C_{10}H_{14}O_3N_2S_2$ requires C, 43·8; H, 5·2; N, 10·2 per cent.

Ethyl-N-p-*sulphamylphenyl thiocarbamate* crystallised from aqueous ethanol in needles, m.p. 183° to 184° (decomp.). Found: C, 40·7; H, 4·7. $C_9H_{12}O_3N_2S_2$ requires C, 41·5; H, 4·7 per cent.

n-*Butyl*-N-p-*sulphamylphenyl thiocarbamate* crystallised from *n*-butanol in small prisms, m.p. 153° to 154°. Found: N, 9·7. $C_{11}H_{16}O_3N_2S_2$ requires N, 9·7 per cent.

Oxidation of p-*sulphamylphenyl thiourea.* To a solution of *p*-sulphamylphenylthiourea (9·2 g.) in water (500 ml.) containing concentrated hydrochloric acid (2 ml.) was added a solution of iodine in potassium iodide until the iodine colour just disappeared. After cooling, the solid was collected, dissolved in diluted sodium hydroxide solution and filtered to remove sulphur. The process was repeated. Acidification of the filtrate yielded the *product* (7·5 g.) which separated from water in small needles of m.p. 241° (decomp.). Found: C, 34·2; H, 4·0; N, 16·2; S, 22·1 per cent. A second experiment using hydrogen peroxide (13·8 g. of 30 per cent solution) yielded a product which separated from water in feathery needles of m.p. 220° (decomp.) (the m.p. was depressed on admixture with the above compound). Found: C, 32·1; H, 4·2; N, 16·9; S, 19·4 per cent.

Esters of p-*sulphamyl benzoic acid.* Esters of *p*-sulphamyl benzoic acid were prepared by three methods: (*a*) Direct esterification of *p*-sulphamyl benzoic acid by the Fischer-Speier method. (*b*) Trans-esterification from methyl *p*-sulphamyl benzoate. (*c*) A solution of the acid and alcohol in pyridine was treated with benzene sulphonyl chloride[15].

An example of each method of preparation is given and the properties of the various esters are summarised in Table VII.

Method (*a*) *Methyl* p-*sulphamyl benzoate.* A suspension of *p*-sulphamyl benzoic acid (56 g.) in methanol (500 ml.) containing hydrochloric acid gas (5 g.) was heated under reflux for 2 hours. All solid dissolved after *ca.* 30 minutes. The product separated on cooling and crystallised from methanol in prismatic needles of m.p. 185°.

Method (*b*) n-*Octyl* p-*sulphamyl benzoate.* Methyl *p*-sulphamyl benzoate (5 g.) was dissolved in *n*-octanol (50 ml.) containing hydrochloric acid gas (1 g.) and the solution heated under reflux for 5 hours.

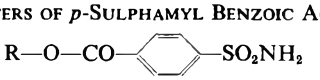
The product which separated on cooling crystallised from ethyl acetate in small shining plates of m.p. 106° to 107°.

Method (*c*) n-*Decyl* p-*sulphamyl benzoate*. A solution of *p*-sulphamyl benzoic acid (5 g.) in pyridine (150 ml.) was cooled in ice and treated with toluene *p*-sulphonyl chloride (9·5 g.). *n*-Decanol (4 g.) was then added with stirring. The mixture was allowed to warm up to room temperature over 1 hour and was then poured onto ice. The white solid was collected, washed with water and purified by crystallisation from a mixture of ethyl acetate and light petroleum (b.p. 60° to 80°) forming shining needles of m.p. 109° to 110°.

n-*Butyl*-2-*methoxy*-4-*sulphamyl benzoate* was prepared directly by esterification of 2-methoxy-4-sulphamyl benzoic acid. It crystallised from

TABLE VII

ESTERS OF *p*-SULPHAMYL BENZOIC ACID



R	Method	m.p. °C	Formula	Found				Required			
				C	H	N	S	C	H	N	S
Me	a	185	$C_8H_9O_4NS$	44·7	4·2	6·2		44·6	4·2	6·5	
n-Pr	a	109–110	$C_{10}H_{13}O_4NS$	49·6	5·4	5·9	13·6	49·4	5·4	5·8	13·2
iso-Pr.. ..	a	139–140	$C_{10}H_{13}O_4NS$	49·9	5·6	5·8	13·4	49·4	5·4	5·8	13·2
n-Bu ..	a	110	$C_{11}H_{15}O_4NS$	51·8	6·1	5·4	12·6	51·4	5·9	5·5	12·5
iso-Bu ..	a	136	$C_{11}H_{15}O_4NS$	51·8	6·0	5·3	12·2	51·4	5·9	5·5	12·5
n-Amyl ..	b	96	$C_{12}H_{17}O_4NS$	52·6	5·9	5·3	12·1	53·1	6·3	5·1	11·8
n-Hexyl ..	a	106–107	$C_{13}H_{19}O_4NS$	54·9	6·7	4·6	10·8	54·7	6·7	4·9	11·2
*cyclo*Hexyl ..	a	132–133	$C_{13}H_{17}O_4NS$	54·6	5·8	5·2	11·4	55·1	6·1	4·9	11·3
n-Heptyl ..	a	107–108	$C_{14}H_{21}O_4NS$	56·4	7·4	4·9	10·7	56·1	7·1	4·7	10·7
n-Octyl ..	b, c	107	$C_{15}H_{23}O_4NS$	57·6	7·3	4·5	10·3	57·5	7·4	4·5	10·2
n-Nonyl ..	b, c	107–108	$C_{16}H_{25}O_4NS$	58·6	7·5	3·9	9·4	58·7	7·7	4·3	9·8
n-Decyl ..	b, c	109	$C_{17}H_{27}O_4NS$	59·4	8·0	4·0	9·7	59·8	8·0	4·1	9·4
n-Undecyl ..	b, c	108–109	$C_{18}H_{29}O_4NS$	60·6	8·0	4·0	9·3	60·8	8·2	3·9	9·0
n-Dodecyl ..	b	107–108	$C_{19}H_{31}O_4NS$	61·3	8·2	4·3	9·2	61·8	8·5	3·8	8·7
n-Octadecyl	b	112–113	$C_{25}H_{43}O_4NS$	66·2	9·3	2·8	6·7	66·2	9·6	3·1	7·1
Allyl ..	a	108–109	$C_9H_9O_4NS$	49·4	4·7	5·8	13·4	49·8	4·6	5·8	13·3
Benzyl ..	a	162–163	$C_{14}H_{13}O_4NS$	57·9	4·5	4·8	11·4	57·7	4·5	4·8	11·0
β-Phenethyl ..	b	159	$C_{15}H_{15}O_4NS$	59·4	4·9	4·9	10·6	59·0	5·0	4·6	10·5
β-Hydroxy ethyl	b	126–128	$C_9H_{11}O_6NS$	44·1	4·5	5·4	12·7	44·1	4·5	5·7	13·1

water in silky needles of m.p. 98°. Found: C, 50·1; H, 5·8; N, 4·8; S, 11·3. $C_{12}H_{17}O_5NS$ requires C, 50·2; H, 6·0; N, 4·9; S, 11·2 per cent.

p-*Sulphamyl benzoic acid piperazine salt*, prepared to characterise the acid, crystallised from hot water in prisms, m.p. 280° (decomp.). Found: C, 44·5; H, 5·0; N, 11·2; S, 13·2. $C_{18}H_{24}O_8N_4S_2$ requires C, 44·3; H, 5·0; N, 11·5; S, 13·1 per cent.

p-*Sulphamyl benzamide* crystallised from water in needles, m.p. 242° to 244°. Found: N, 13·7. Calc. for $C_7H_8O_3N_2S$: N, 14·0 per cent.

N-(p-*Sulphamylbenzoyl*)di-β-*hydroxyethylamine* crystallised from a mixture of ethanol and ethyl acetate in needles, m.p. 155° to 156°. Found: C, 46·1; H, 5·2; N, 9·7. $C_{11}H_{16}O_5N_2S$ requires C, 45·8; H, 5·6; N, 9·7 per cent.

N-p-*Sulphamylbenzoyl benzylamine*. A mixture of methyl-4-sulphamyl benzoate (4 g.) and benzylamine (10 ml.) was heated on the steam bath for 8 hours. It was then cooled, stirred with dilute hydrochloric acid and the residual *solid* crystallised from ethanol in needles m.p. 186° to 188°.

The melt solidified rapidly and then remelted at 198° to 199°. Found: C, 57·5; H, 4·9; N, 9·7; S, 11·1. $C_{14}H_{14}O_3N_2S$ requires C, 57·9; H, 4·9; N, 9·7; S, 11·0 per cent.

p-*Sulphamylbenzhydrazide.* To a suspension of methyl *p*-sulphamyl benzoate (27 g.) in ethanol (50 ml.) was added hydrazine hydrate (24 g.). The solid dissolved rapidly and the solution was heated on the steam bath for 2 hours. The product separated on cooling and crystallised from ethanol in shining plates, m.p. 238° to 240° (decomp.). Found: N, 19·7. $C_7H_9O_3N_3S$ requires N, 19·5 per cent.

1-(p-*Sulphamylbenzoyl*)-*thiosemicarbazide.* The foregoing hydrazide (2·15 g.) was dissolved by warming in water (50 ml.) containing concentrated hydrochloric acid (1 ml.), treated with potassium thiocyanate (1·2 g.) and the solution heated on the steam bath for $2\frac{1}{2}$ hours with concentration to one-third volume. The *product* which separated on cooling crystallised from ethanol in shining plates, m.p. 233° (decomp.). Found: C, 35·4; H, 3·7; S, 22·6. $C_8H_{10}O_3N_4S_2$ requires C, 35·0; H, 3·7; S, 23·3 per cent.

Materials and Methods for Carbonic Anhydrase Assay

Carbonic Anhydrase. A freeze-dried preparation of crude "chloroform enzyme" was used, prepared by the method described by Roughton and Booth[16].

Assay procedure. The method is based on the colorimetric procedure of Roughton and Booth[16].

Three ml. of 0·05 M sodium veronal buffer of pH 8·2, 0·2 ml. of bromothymol blue (B.D.H. indicator solution), 1·8 ml. of water and 0·4 ml. of enzyme solution were pipetted into a 25 ml. weighing bottle, which was stoppered and equilibrated in ice for 20 minutes. Five ml. of ice-cold water saturated with carbon dioxide was then added from a chilled syringe, the nozzle of which was held below the liquid surface, and the mixture was rapidly mixed by rotation. The time taken for the indicator colour to match that of a standard of bromothymol blue made up in pH 6·3 phosphate buffer was timed with a stop-watch.

In the absence of enzyme, reaction times of 160–180 seconds were obtained. In the presence of enzyme (10 mg. in 100 ml. of water) reaction times were reduced to 40–50 seconds.

Assay of inhibitors. A solution of the inhibitor sample was made up in water, and 1·8 ml. of the solution was added to the weighing bottle in place of the 1·8 ml. of water. Where the sample was too insoluble in water the solution was made up in 50 per cent ethanol; 50 per cent ethanol was then also used for determination of the blank times.

About twelve readings were obtained in duplicate for serial dilutions of the inhibitor. The per cent inhibition was then plotted against $-\text{Log}$ (I) (where (I)=inhibitor concentration). A straight line was usually obtained over the concentration range giving 20–80 per cent inhibition, and the concentration causing 50 per cent inhibition could then be read off.

The molar concentration of substance causing 50 per cent inhibition was expressed relative to the concentration of sulphanilamide causing similar inhibition, this being given the arbitrary value of 1.

Results

In this system sulphanilamide was found to give 50 per cent inhibition at a concentration of $9{\cdot}5 \times 10^{-7}$M. Considerable variation was found in inhibitor activities from day to day, and all the figures quoted are subject to error of about $\pm$ 50 per cent. Similar variation was reported by Miller, Dessert and Roblin[8].

TABLE VIII

COMPARISON OF RESULTS WITH THOSE OBTAINED BY MILLER AND OTHERS[7,8] AND KREBS[5]

Inhibitor	Activity (Sulphanilamide = 1)		
	Observed	Miller and others	Krebs
Benzene sulphonamide	3	4	2
N^4-Acetylsulphanilamide	17	—	8
p-Sulphamylbenzoic acid	4	—	5
Naphthalene-1-sulphonamide	17	—	6
Naphthalene-2-sulphonamide	14	—	9
Prontosil	186	50	150
2-Acetamido-1 : 3 : 4-thiadiazole-5-sulphonamide (Diamox)	347	330	—
2-Amino-1 : 3 : 4-thiadiazole-5-sulphonamide	11	25	—

Results obtained appear to be roughly comparable with those quoted by previous workers. Table VIII gives comparative results obtained by Miller and others (*loc. cit.*), Krebs[5], and ourselves.

Activity values obtained for various types of sulphonamide are summarised in Tables I to VI.

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THE SMOOTH MUSCLE CONTRACTING ACTION OF EFFLUENTS FROM THE ISOLATED GUINEA PIG ILEUM

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Received June 17, 1958

An analysis was made of the effect of effluents from both outside and inside of the isolated guinea pig ileum segments. The action of the outside effluents was directed towards nervous elements of the test segment of the isolated ileum. According to the effect of atropine, morphine and hexamethonium, the action of the effluent resembled that of 5-HT. Atropine and antazoline did not inhibit the effect of the effluent obtained from the inside of the donor gut upon the isolated rat fundus, but BOL and LSD did so in most experiments. The effect of this effluent upon the test segment of the guinea pig ileum was inhibited by morphine, or by a previous desensitation of the preparation by large doses of 5-HT. The smooth muscle stimulating effect of the effluent probably was due to its content in 5-HT and at least partly to some other as yet unidentified substance(s).

IT is known that if bath fluid, in which isolated segments of intestine have been suspended, is added to another bath, containing another segment of intestine, the latter will contract[1]. This activity was thought to be due to choline released by the gut[2]. Later, acetylcholine was identified as an active agent[3,4]. But Vogt found that some other smooth muscle stimulating substance also diffuses out, and ascribed the activity to "Darmstoff"[5] In the meantime other smooth muscle contracting substances have been isolated from the intestine, for example substance P[6] and 5-hydroxytryptamine[7].

An attempt was made in the present experiments to analyse the activity of effluents of segments of isolated guinea pig ileum by pharmacological antagonists.

METHODS

The drugs used were acetylcholine chloride (ACh), histamine dihydrochloride, atropine sulphate, nicotine hydrogen tartrate, hexamethonium bromide, morphine hydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), antazoline, lysergic acid diethylamide (LSD), bromlysergic acid diethylamide (BOL). All the doses and concentrations refer to the salts used.

Guinea pig ileum in a bath of 10 ml. capacity was the test tissue for effluents from a donor bath of 20 ml. capacity containing Tyrode solution gassed with O_2, and a segment 5–8 cm. long of guinea pig ileum incubated at 36°. The donor segment was suspended by Trendelenburg's method[8], and at the beginning of each experiment was suspended without extension. Later, it was extended by attaching it to a lever, or peristalsis was produced by raising the intraluminal pressure. During each assay all the bath fluid of the test segment was changed and the bath was refilled with the

effluent taken immediately from the donor bath. In some experiments only 5 of the 10 ml. was changed, because of the spontaneous contractions which appeared if the test tissue was exposed to the air. The contractions of the test segment were registered by an isotonic lever.

The analysis of the effluent obtained from the lumen of the gut was made on the preparation of the isolated rat fundus[9], and in a few experiments on the guinea pig ileum. The isolated rat fundus was suspended in Krebs fluid at 37°, with 95 per cent O_2 and 5 per cent CO_2. The effluent was obtained from the lumen as described by Beleslin and Varagic[10] and 0·1–0·3 ml. was added to a 10 ml. bath in which the test tissue was suspended. In a few experiments the donor gut was turned inside out and the bath fluid containing the substances which diffused from the mucous side of the gut was analysed on another segment of guinea pig ileum. The effluent from the donor gut were usually taken at 5 minute intervals.

RESULTS

The Response to Bath Fluid

During the incubation of the gut the bath fluid became biologically active; if added to another segment this contracted. The activity varied over a wide range. Thus, in some experiments the addition of a small volume of this fluid sufficed to induce a contraction of the recipient segment, while in others only an insignificant contraction was produced even if the undiluted bath fluid was allowed to act upon the test gut. In some experiments the activity of the bath fluid was increased if the donor gut was extended by the lever during incubation, or if the gut was stimulated to effect peristaltic movements. But these procedures did not influence the activity of the bath effluent in about half the experiments.

The activity of the bath fluid activated by the three ways described above corresponded to the activity of 5–50 ng. of ACh, or 50–100 ng. of 5-HT.

The influence of atropine. Atropine in a concentration of 5×10^{-9} to 10^{-8} g./ml. markedly depressed or abolished the effect of the bath fluid upon the guinea pig ileum. The effect of an equiactive dose of acetylcholine was inhibited by atropine to a similar degree. This fact suggested the possibility that the effect of the bath fluid upon the ileum was due to the acetylcholine released by the donor gut during its incubation, as shown by others[3,4].

The influence of nicotine and of hexamethonium. High inhibitory concentrations of nicotine and of hexamethonium were used in experiments designed to throw more light on the site of action of the bath fluid. Nicotine completely abolished the action of the bath fluid, depressing at the same time the effect of added ACh. Hexamethonium on the contrary did not depress the action of the bath fluid. Ganglionic blocking action of these drugs is exerted through different mechanisms and high doses of nicotine were found to exert some muscular inhibitory activity in addition. According to the concomitant depression of the action of ACh, it seems that the inhibitory action of nicotine upon the effect of the bath fluid was non-specific.

The influence of morphine. Morphine at a concentration of 10^{-8} markedly inhibited and in a few experiments abolished the effect of the bath fluid on the guinea pig ileum. Morphine also inhibited the effect of an equiactive dose of 5-HT, leaving intact the effect of ACh (Fig. 1).

The influence of high concentrations of 5-HT. It is known that morphine is a potent antagonist of the action of 5-HT upon the guinea pig ileum[11]. Therefore, the inhibition by morphine of the action of the bath fluid suggested that 5-HT might be released by the donor gut. It is known that 5-HT may be released by the mucous membrane of the gut[12,13].

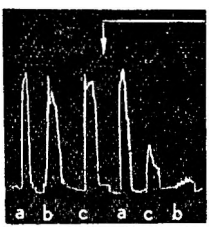

FIG. 1. Isolated guinea pig ileum. At *a*, acetylcholine 10 ng. was added; at *b*, bath fluid from the donor segment, after 5 min. of peristalsis; at *c*, 5-HT 100 ng. At arrow, morphine 100 ng. was added. White line indicates the presence of morphine in the bath of the test segment.

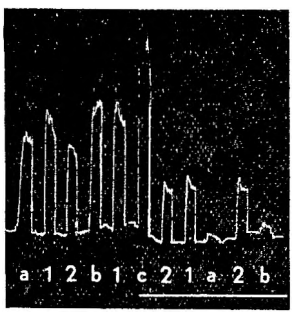

FIG. 2. Isolated guinea pig ileum. In its bath was added as follows: at *a*, bath fluid from the donor segment before exerting peristalsis, and at *b*, after 5 min. of peristalsis; at 1, acetylcholine 25 ng. and at 2, 20 ng. Starting at *c*, 100μg. 5-HT was added into the bath after each washing (white line).

Isolated guinea pig ileum can be desensitized to the action of 5-HT by a previous exposure of the preparation to high concentrations of the same substance[14]. In the present experiments, a preceding contact with 10^{-5} 5-HT completely desensitized the test tissue towards both 5-HT and the bath fluid. The effect of ACh was also depressed by this procedure, but always to a less degree than the effect of 5-HT and of the bath fluid (Fig. 2).

The Effect of Effluent from the Intestinal Lumen

In a series of experiments the influence of the distension of the gut, as well as of the peristaltic activity upon the potency of the effluent was tested. Some biologically active substances were found to be released from the mucous membrane of the gut, but distension of the donor preparation did not increase the activity of the effluent. The present experiments did not permit a definite conclusion about the production by peristaltic activity of increase in the quantity of the active substance(s) in the effluent.

The preparation of the isolated rat fundus has been described as being highly sensitive towards 5-HT. ACh and histamine also caused contractions, but only if higher concentrations were used[9]. The present

experiments confirmed these findings. Therefore, the antagonists of all three substances (5-HT, ACh and histamine) have been used in experiments which were designed to provide more data on the nature of the active substance(s) present in the effluent.

If compared with contractions caused by 5-HT, ACh or histamine, that caused by the effluent was slower. The relative activity of 0·2–0·3 ml. of

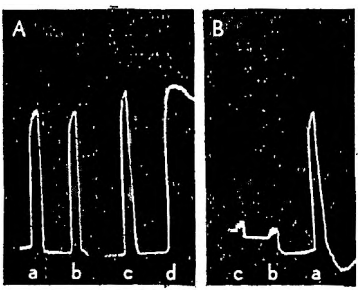

FIG. 3. Isolated rat fundus. At *a*, 50 ng. acetylcholine was added; at *b*, 0·3 ml. effluent from the lumen of the donor guinea pig ileum isolated segment; at *c*, 5 ng. 5-HT, and at *d*, 500 ng. LSD. Between A and B, LSD was washed out.

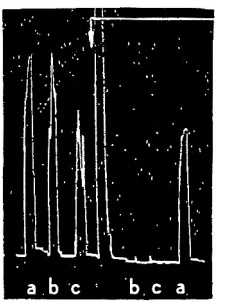

FIG. 4. Isolated guinea pig ileum. At *a*, acetylcholine 5 ng. was added, at *b*, 5-HT 100 ng., and at *c*, bath fluid from the bath in which the donor segment of ileum was suspended, but turned inside out previously (incubation time 5 min.). At arrow and after (white line) 200 ng. 5-HT was added.

the effluent (approximately one fifth of the whole content of the segment), corresponded to the activity of 1–5 ng. of 5-HT, 2–10 ng. of histamine, or 2–10 ng. of ACh.

The Influence of Atropine, Antazoline, LSD and BOL on the Effect of the Effluent

Atropine 10^{-8} caused in most experiments an increase of the tone of the isolated rat fundus. The same concentrations abolished the effect of ACh, but did not inhibit the action of the effluent.

Antazoline 10^{-8} similarly abolished the action of histamine, leaving intact the effect of the effluent. Both of these experiments showed that the effect of the effluent was not due to the released ACh or histamine.

Vane[9] has found that both LSD and BOL inhibited the effect of 5-HT upon the isolated rat fundus. This finding was confirmed in most of our experiments, although in a few the effect of 5-HT was only partially inhibited. But a marked increase in tone of the isolated rat fundus was produced both by LSD and BOL in almost all experiments. This side effect was an unexpected difficulty.

In half of our experiments LSD and BOL were found to inhibit the effect of the effluent and of 5-HT to a similar degree (Fig. 3). However, in the other half the effect of the effluent was more resistant to the inhibitory action of LSD and BOL than the effect of an equiactive dose of 5-HT.

Neither LSD nor BOL inhibited the effect of ACh on the isolated rat fundus, but the effect of histamine was usually depressed by both substances.

The effluent obtained from the intestinal lumen contracted another segment of the guinea pig ileum when it was added into the bath fluid. This effect was markedly depressed by morphine 10^{-8}, which antagonized, to a similar degree, the effect of 5-HT, while it was found to potentiate the effect of ACh. The previous saturation of tryptamine receptors by a high dose of 5-HT abolished the effect both of 5-HT and of effluent, but only slightly depressed the effect of ACh (Fig. 4). This inhibitory effect of high doses of 5-HT was obtained irrespectively of whether the effluent was obtained from the intestinal lumen, or from the bath containing a segment of the donor gut, turned inside out.

Discussion

The finding of Weiland[1], that during incubation of a segment of the intestine some biologically active substances are released into the surrounding fluid, has been confirmed in the present experiments. Surprisingly, in only 50 per cent of the present experiments the activity of the donor bath effluent was increased by distending the gut, or by stimulating it to perform peristalsis.

The action was abolished by nicotine, but not by hexamethonium, and was inhibited by atropine, morphine and by the previous saturation of the tryptamine receptors of the test segment by high doses of 5-HT. This analysis strongly suggested that the action of the active component(s) of the effluent was directed to the nervous elements of the test segment, presumably to the specific tryptamine receptors.

Morphine, in concentrations used in the present experiments, does not inhibit ACh contraction[15,16]. Thus the partial or even complete inhibition of the effect of the effluent by morphine indicated that the part played by ACh could not be significant; the same is true of histamine. 5-HT has been shown to be released by the gut into the lumen[12,13] and high doses of 5-HT abolished the action of the effluent. Also morphine, which has been shown to be a potent antagonist of 5-HT[11], inhibited the action of the effluent. These facts indicate that the effluent may act by its 5-HT content, or by some similar substance acting through tryptamine receptors.

BOL and LSD, substances known to be potent antagonists of 5-HT actions, were found to inhibit actions both of 5-HT and the effluent in most of our experiments which is in agreement with Bülbring and Lin[12,13], who found that 5-HT is released from the mucous membrane of the isolated intestine. In some experiments the inhibitory effect of 5-HT antagonists was more pronounced on the action of 5-HT than towards that of the effluent. In some experiments the action of 5-HT on the isolated rat fundus was inhibited only partially by BOL and LSD and in almost all experiments both drugs caused an increase in tone of the preparation.

On the guinea pig ileum the effect of the effluent from the intestinal lumen, or from the bath of the segment turned inside out, was inhibited

similarly to 5-HT by morphine and by high doses of 5-HT. The effect of ACh was not inhibited either by morphine or by 5-HT. These experiments confirm findings made by Bülbring and Lin[12,13], but the failure of 5-HT antagonists to inhibit the effect of the effluent in a few experiments might suggest that some other substance(s) might also be present in the effluent of the isolated intestine.

Acknowledgements. The 5-hydroxytryptamine was kindly supplied by Farmitalia (Milan) and the LSD and BOL by Sandoz (Basel).

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Barbiturates, Two-dimensional Paper Chromatographic Method for the Separation of. H. Möllerberg. (*Scand. J. clin. Lab. Invest.*, 1958, **10**, 59.) Paper chromatograms were first run with ammoniacal chloroform as solvent, by the descending technique. The second run, perpendicular to the first, was made using ammoniacal amyl acetate. The ammoniacal solvents were prepared by shaking 2 parts of solvent with one part of concentrated ammonia solution and separating. The chromatograms were dried at 100° and treated with ammonia vapour for 5 minutes, after which barbiturates were detected by examining the paper in ultra-violet radiation. On spraying the chromatogram with 0·02M potassium permanganate, barbiturates containing a reducing radical appeared as yellow spots on a red ground. Derivatives containing a *cyclo*hexenyl or *cyclo*heptenyl group showed delayed reduction and appeared as yellow spots about one minute after spraying. R_F values were determined for 18 derivatives, and it was shown that *N*-methylated derivatives have considerably higher R_F values than non-methylated ones. For the confirmation of the identity of unknown spots, solutions of authentic specimens should be chromatographed at the same time for comparison. G. B.

Cardiac Glycosides and Organic Nitrates, Chemical Determination of in Pharmaceutical Preparations. A. Kurkela. (*Pharm. Acta Helvet.*, 1958, **33**, 216.) The colorimetric reaction of cardiac glycosides with alkaline picrate (Baljet reaction) is suggested as a suitable basis for the assay of these in pharmaceuticals such as solutions, ampoules, tablets and suppositories containing more or less pure glycosides. The absorption maximum is at 492 mμ and this is non-specific. The standard curve must therefore be prepared from the same pure glycoside as that contained in the preparation. Most results fell within ± 5 per cent. The scillarins which do not give the Baljet reaction were assayed by the Lieberman reaction. Pharmaceutical organic nitrates such as glyceryl trinitrate and erythrityl tetranitrate were assayed either colorimetrically using phenoldisulphonic acid, or by reduction to ammonia and titration with sulphuric acid. Again, most results were within ± 5 per cent. D. B. C.

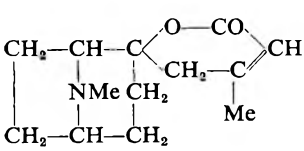
Colchicine, Chromatographic-Spectrophotometric Method for the Separation and Determination of. S. J. Smolenski, F. A. Crane and R. F. Voigt. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 359.) Samples of about 10 g. of colchicum seed were defatted by continuous extraction with light petroleum, and the colchicine extracted with ethyl acetate containing 1 per cent of ethanol. This solvent was used in preference to pure ethanol because it was found to extract less resin with the alkaloid. The extract was freed from resinous impurities by chromatography on an alumina column, colchicine being eluted with 10 per cent methanol in chloroform. Other impurities were then removed by chromatography on a silica column. The purified colchicine was treated with nitric acid

followed by sodium hydroxide to produce a red colour, and determined quantitatively by measuring the light absorption at 350 mμ, calculating the amount of colchicine by reference to a standard curve prepared using known amounts of pure colchicine. The colour reaction with nitric acid, which gives rise to a succession of colours, followed by red on the addition of alkali, was used for checking the identity and purity of the product, and testing for completeness of extraction. G. B.

Lysergic Acid Diethylamide and Ergonovine, The Fluorimetric Determination of. E. S. Boyd. (*Arch. int. Pharmacodyn*, 1958, **115**, 43.) A fluorimeter in which the sample was irradiated with mono-chromatic light and the fluorescence analysed monochromatically, was constructed and tested. The instrument was basically similar to the Bowman fluorimeter. The fading of fluorescence of lysergic acid diethylamide and ergonovine in this instrument was investigated and found to be influenced to some extent by pH, the least amount of fading occurring between pH 5 and 7. The author concludes that instruments of this type are useful for quantitative analysis of various pure materials which fluoresce, providing fading of fluorescence is avoided and for obtaining excitation and fluorescence maxima which are not necessarily true values but which may be useful for comparison purposes as long as the same instrument is used for the comparisons. W. C. B.

ORGANIC CHEMISTRY

Dioscorine, Constitution of. J. B. Jones and A. R. Pinder. (*Chem. Ind.*, 1958, 1000.) 5-Oxotropane has been synthesised from 6β-hydroxytropinone by Wolff-Kishner reduction to 6α-hydroxytropane, and chromic acid oxidation of the latter. 6-Oxotropane showed carbonyl bands in the infra-red at 1750 cm.$^{-1}$ (liquid film) and its methiodide at 1778 cm.$^{-1}$ (Nujol) in contrast to that of the oxotropane obtained by degradation of dioscorine (Büchi and others, XVIth International Congress of Pure and Applied Chemistry, Paris, July 1957) which absorbed at 1730 cm.$^{-1}$ (present work, 1737 cm.$^{-1}$). Comparison of the two oxotropane picrates confirmed their non-identity, which was supported by marked differences in the stability of the corresponding methiodides to aqueous sodium hydrogen carbonate at 30°. It is concluded, therefore, that the dioscorine degradation product is 2-oxotropane and that the alkaloid is formulated as shown (I). J. B. S.



(I)

6α-Methyl-17α-Hydroxyprogesterone 17-acylates; a New Class of Potent Progestins. J. C. Babcock, E. S. Gutsell, M. E. Herr, J. A. Hogg, J. C. Stucki, L. E. Barnes and W. E. Dulin. (*J. Amer. chem. Soc.*, 1958, **80**, 2904.) The synthesis of 6α-methyl-17α-hydroxyprogesterone and its acetate from the bisethylene acetal of 17α-hydroxyprogesterone is reported. The latter with peracetic acid gave a mixture of 5α, 6α-epoxy- and 5β, 6β-epoxy-17α-hydroxy pregnane-3, 20-dione bisethylene acetals. The α-epoxide with methylmagnesium bromide gave the bisethyleneacetal of 5α, 17α-dihydroxy-6β-methylpregnane-3 : 20-dione, which was hydrolysed with acidic acetone to 5α, 17α-dihydroxy-6β-methylpregnane-3 : 20-dione. This on dehydration by very

dilute sodium hydroxide in pyridine afforded 6β-methyl-17α-hydroxyprogesterone, which was epimerised with hydrogen chloride in chloroform to 6α-methyl-17α-hydroxyprogesterone. Acylation with the appropriate reagent gave the 17α-acetate, the 17α-(β-*cyclo*pentylpropionate), propionate, caproate and phenylacetate. The McPhain modification of the Clauberg assay showed 6α-methyl-17α-hydroxyprogesterone17-acetate to be 50–60 times more active than progesterone on subcutaneous administration and 100–300 times more active than ethisterone on oral administration. As an ovulation inhibitor it was 10–20 times more active parenterally than progesterone. J. B. S.

PHARMACY

Alginate Mucilages, the Influence of Different Physico-chemical Factors on the Viscosity of. R. Bolliger and K. Münzel. (*Pharm. Acta Helvet.*, 1958, **33**, 225.) The following factors were studied:—the degree of polymerisation of the alginate, its concentration, the pH of the solution, the influence of hydrophilic liquids such as ethanol when added before and after gelation and the influence of electrolytes when added before and after gelation. For the same concentration the viscosity rose with degree of polymerisation. Viscosity rose approximately logarithmically with concentration up to a few per cent, i.e., doubling the concentration would cause a 10-fold increase in viscosity, three times the concentration would cause a 100-fold increase etc. The maximum viscosity for a given concentration was found to be at pH 7, falling slightly—to about 85 to 90 per cent of this—at pH 4·1 and 10. At a pH lower than 4·1 alginic acid is precipitated. Ethanol causes an increase in viscosity up to a certain concentration due to incomplete flocculation or partial dehydration; above this concentration coarse flocculation occurs. If the alcohol is added to the gelled alginate, this critical concentration is about 25 per cent. If however the alginate is first suspended in the alcohol, and gelled by the addition of water, the concentration required to produce flocculation is lower—about 15 per cent, the concentration of the alginate being 1 per cent in each case. With glycerol however, flocculation only occurs with a content of 70 per cent or more. This difference was explained by postulating the formation of hydrogen bonds between glycerol and alginate molecules, and was said to be related to the gelation of pectin and alginates on the addition of sugar. It was observed that 10 per cent ethanol or 20 per cent glycerol had a stabilizing effect on viscosity especially when added after hydration. Electrolytes raised the viscosity up to a critical concentration when flocculation occurred. For a 1 per cent mucilage, if sodium chloride or sodium benzoate was dissolved in the water used for preparation, only 1 and 2 per cent respectively brought about flocculation. If the electrolytes were added to the prepared mucilage, higher concentrations were tolerated e.g., 4 per cent sodium chloride. D. B. C.

Physostigmine Eye-drops, Stability of. J. Mørch. (*Dansk Tidsskr. Farm.*, 1958, **32**, 93.) Specimens of eye-drops of the Danish Pharmacopoeia 1948, containing 1 per cent of physostigmine salicylate and 0·75 per cent of sodium chloride were assayed by a method involving extraction with ether after making alkaline with sodium carbonate, evaporation of the solution and titration. This method is specific for physostigmine in the presence of its degradation products, and showed that the eye-drops lost 1 per cent of their physostigmine content on storage for 3 months at 20°. The corresponding loss at 30° was 3 per cent.

Heating at 100° for 15 minutes resulted in a loss of 1 to 4 per cent, and the development of a red colour. Discoloration was prevented by the addition of 0·1 per cent of sodium metabisulphite, but this resulted in the eye-drops becoming too acid on storage. The inclusion of 2 per cent of disodium hydrogen citrate (sesquihydrate) was sufficient to buffer the solution to pH 5·0, and such solutions showed a loss of 1 to 2 per cent with no discoloration or change in pH on heating at 100° for 15 minutes. The loss on storage at 20° for 6 months was 10 per cent. The addition of disodium ethylenediaminetetra-acetate did not prevent discoloration. G. B.

Vitamin B_{12}, Stability of, in the Presence of Aneurine and Nicotinamide in Aqueous Combinations. A. S. Gambier and E. P. G. Rahn. *J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 356). In a continuation of a previous investigation (abstract *J. Pharm. Pharmacol.*, 1957, **9**, 637) it was shown that rapid inactivation of vitamin B_{12} occurs in the presence of 5000 times the quantity of aneurine, even when special precautions are observed in making the solutions. It was found possible to produce stable aqueous solutions containing aneurine, nicotinamide and vitamin B_{12} at pH 3·3, provided that the quantity of aneurine was not more than 120 times that of vitamin B_{12}. The tendency of the mixed vitamin solutions to darken was increased with rise in pH and temperature, and it was shown that the proportion of air in the ampoules is of importance. With a liquid to air volume ratio of 1·73, neither darkening nor precipitation occurred, but decreasing the ratio gave rise to darkening, and increasing it caused precipitation. Pyridoxine did not affect the stability of the mixed vitamin solutions. Stability tests at 37°, 40°, and 45° appeared to be more informative than those carried out at higher temperatures. G. B.

PHARMACOLOGY AND THERAPEUTICS

Anileridine and Pethidine in Man, Narcotic Potency and Side Effects of. F. F. C. Chang, P. Safar and L. Lasagna. (*J. Pharmacol.*, 1958, **122**, 370.) Anileridine is a new synthetic narcotic drug which is chemically related to pethidine. In animals this compound has been found to approach the analgesic potency of morphine, to be ten to twelve times the potency of pethidine, and to be relatively free of side effects such as respiratory depression, vomiting and sedation. Early clinical studies however suggested that it was only slightly more potent than pethidine. This work is therefore an attempt to evaluate the narcotic potency of anileridine and pethidine in surgical cases, and the side effects in both patients and healthy volunteers. The ability of the drugs to reinforce nitrous oxide analgesia under controlled experimental conditions was studied. It was found that while anileridine was a potent analgesic it was no more potent than pethidine. It produces respiratory depression and subjective side effects to as great an extent as pethidine, when given in equipotent doses. Thus the analgesic activity of anileridine in man compares unfavourably with its effect in animals. M. M.

Barbiturate, *N*-Methylated, and Acetylsalicylic Acid, Absorption of, from Different Suppository Bases. U. Samelius and A. Åström. (*Acta pharm. tox. Kbh.*, 1958, **14**, 240.) Suppositories were prepared with theobroma oil, Carbowax (polyethylene glycols), and Imhausen bases (glycerides of fatty acids) with and without the addition of Tweens. A preliminary series of experiments was carried out in rabbits, using hexobarbitone sodium as the medicament, absorption being assessed by depth of anaesthesia and death rates. In this

series the drug appeared to be rather better absorbed from theobroma oil than from the other bases. No absorption took place when hexobarbitone was used instead of the sodium derivative. A more extensive series of experiments was carried out using acetylsalicylic acid suppositories in human subjects, and determining the concentration of salicylic acid in the plasma. Maximum plasma concentrations of about 35 to 45 μg./ml. were observed 1–1½ hours after administration of 0·75 g. of acetylsalicylic acid in suppositories. In this series Imhausen bases permitted somewhat greater absorption than theobroma oil or Carbowax bases. The plasma levels obtained were comparable with those following the oral administration of acetylsalicylic acid following a light meal. Rectal administration of acetylsalicylic acid may therefore be of value in certain clinical cases, but attention is drawn to the occurrence of individual variations in absorption. Decreased absorption was observed in a person with an anal fissure.

G. B.

Bemegride, Analeptic Activity of, to Structurally Unrelated Hypnotics. A. Shulman and G. M. Laycock. (*Austral. J. exp. Biol. med. Sci.*, 1957, **35**, 559.) Previous work has shown that bemegride (β-methyl-β-ethylglutarimide, Megimide) can antagonise the hypnosis induced in mice by diverse but structurally related hypnotics such as monoureides, barbiturates, thiobarbiturates, glutarimides, diketo-piperidines, diketo-tetrahydropyridines, diketo-thiazanes and diketo-thiazolidines. It can also reverse the hypnotic activity of a wide range of structurally unrelated hypnotics such as saturated and unsaturated alcohols, aldehydes, carbamates, cyclic ethers and sterols. It was found that ether prevented or terminated convulsions induced by bemegride in mice, but the reverse form of antagonism could not be demonstrated. Bemegride antagonised morphine-induced respiratory depression in dogs but did not reverse the analgesia or hypnosis due to morphine in these animals. No obvious signs of antagonism or potentiation between these two substances were observed in mice. Thus bemegride is of possible value in the clinical management of respiratory depression caused by opiates as well as by barbiturates. Reference is made to the widespread safety associated with the administration of bemegride as an analeptic and to preliminary quantitative data which support the suggestion of a selective antagonism by bemegride to hypnotics structurally related to it.

M. M.

Carboxy Vinyl Polymer: a Bulk Laxative, Pharmacological Effects of. R. L. Cahen, E. Groskinsky and G. Leeson. (*Arch. int. Pharmacodyn*, 1958, **114**, 258.) A carboxy vinyl polymer (CP) the physical data of which is given has been found not to form a gel in the acid medium of the stomach but in an alkaline medium gel formation gradually occurs. This fact and other physical data warranted the pharmacological investigation of this compound as a possible laxative. Data obtained shows 1) the low toxicity of CP following oral administration to rats, mice, guinea pigs, and dogs and its high safety margin; 2) the hydrophilic laxative activity of CP in rats and dogs; 3) the absence of toxic effects by accumulation or sensitization following 14 months' repeated administration of a high dose of CP to parent generation rats and after 32 months' administration to dogs; 4) the absence of toxic effects on first filial and second filial generations of rats exposed to CP for 17 and 24 months respectively and 5) the absence of toxic effects on the first filial generation of dogs following 27 months' repeated oral administration. Comparison of the hydrophilic laxative potency with other bulk laxatives in the rat shows that CP is ten times more active than methylcellulose. In dogs CP produces a significant increase in the moisture of the faeces in contrast with the effects of bran and mineral oil. M. M.

Insulin Zinc Suspension: Clinical Experiences. W. M. Lancaster and I. Murray. (*Brit. med. J.*, 1958, **1**, 1331.) A review of 335 diabetic patients treated for at least 6 months with I.Z.S. showed that satisfactory control was obtained in 82·5 per cent of 134 new patients, and in 64·7 per cent of 201 patients previously receiving some other form of insulin. In 94 of the latter group, I.Z.S. produced a better degree of control. It proved unsatisfactory in 15 patients. For the 189 old patients shown as retaining equally good, or obtaining better, control the dose of I.Z.S. was greater than that of the former insulin in 86 cases, but in 40 a smaller dose sufficed. Where the dose had to be raised the increase was usually about 50 per cent, though some required as much as double the former dose. Neither the age of the patient nor the duration of the diabetes appeared to affect the nature of the response to transfer to I.Z.S. In the presence of intercurrent infection lapse from diabetic control with I.Z.S. appears to occur more readily than with some other insulins. It is suggested that in such conditions it is I.Z.S. crystalline which becomes relatively ineffective, since transfer temporarily to two injections a day of I.Z.S. amorphous has been shown to re-establish control. S. L. W.

Levonor (1-Phenyl-2-aminopropane Alginate) in Obesity. R. J. Gadek, H. S. Feldman and R. J. Lucariello (*J. Amer. med. Ass.*, 1958, **167**, 433.) Levonor was administered with diet in a dose of 5 mg. three times daily, half an hour before meals, to 80 overweight patients. Many of the patients received an additional dose at 8 or 9 p.m. The average weight loss was 2 lb. per week. There was a remarkable absence of side-effects. There were no adverse changes that affected either the blood pressure or the heart rate, and the drug can be used in the evening without causing insomnia. It has no effect on blood sugar levels nor on central vasomotor reflexes and can therefore be used in patients with diabetes and hypertension and in pregnant women. The drug is of no value for depressed obese patients or where psychic stimulation is indicated.

S. L. W.

Lysergic Acid Diethylamide, Comparison of Effect of, with Potassium Cyanide and other Respiratory Inhibitors on the Siamese Fighting Fish. H. A. Abramson B. Weiss and M. O. Baron. (*Nature, Lond.*, 1958, **181**, 1136.) Although it is known that lysergic acid diethylamine enters the brain, the mechanism by which it acts to produce the psychotic patterns in man is unknown. Experiments which are designed to investigate the brain process in the intact animal might lead to a concept that could be developed to study the chemical processes originating or connected with schizophrenia. This paper deals with the effect of potassium cyanide, sodium azide, hydrazine and lack of oxygen on the Siamese fighting fish. It has previously been shown that the behaviour of these fish changes markedly in the presence of small doses of lysergic acid diethylamide in the surrounding water. It is now found that potassium cyanide and sodium azide act similarly. Hydrazine sulphate was without effect. However anoxia and asphyxia also produced reactions similar to lysergic acid diethylamide. It has often been observed that human subjects under the influence of lysergic acid diethylamide suffer from confusion and other symptoms that are associated with anoxia. It may be therefore that lysergic acid diethylamide acts by poisoning some parts of the enzymatic processes connected with oxidation. It may also be that the schizophrenic process may be connected with a similar process where special respiratory enzymes of the brain are not functioning adequately.

M. M.

Meprobamate, Phenobarbitone and Dexamphetamine, effects of, on Reaction Time and Learning in Man. C. Kornetsky. (*J. Pharmacol.*, 1958, **123**, 216.) The effects of meprobamate (800 and 1600 mg.), dexamphetamine (5 and 15 mg.) and phenobarbitone (60 and 120 mg.) on simple motor behaviour, choice reaction time and learning rate were studied in a series of controlled experiments on normal human subjects. A multiple stimulus-response apparatus was employed which allows the measurement of a variety of types of behaviour while always evoking the same motor response on the part of the subject. Neither phenobarbitone nor dexamphetamine significantly affected motor co-ordination time, reaction time or learning. Meprobamate, at both dose levels, significantly affected the learning rate and at the higher dose level impaired motor co-ordination and reaction time. W. C. B.

Methocarbamol in Neuromuscular and Neurological Diseases. D. S. O'Doherty and C. D. Shields. (*J. Amer. med. Ass.*, 1958, **167**, 160.) Methocarbamol (3-o-methoxyphenoxy-2-hydroxypropyl-1-carbamate), a skeletal muscle relaxant, was evaluated in 75 trials in 70 patients with skeletal muscle hyperactivity secondary to neurological disorders. The drug was given in a dose of 1 to 4 g. daily by mouth in divided doses for a minimum period of 2 months in chronic conditions and for 2 weeks in acute conditions (unless recovery occurred earlier); the majority of patients with severe spasticity and contractures were treated for 6 months to a year. An excellent result was obtained in all patients with acute skeletal muscle spasm. A good result was obtained in 72 per cent of patients with both acute and chronic spasm. In the doses administered failures were noted in all patients with contractures, rigidity, spasticity and chronic myofibrositis. In six cases of herniated lumbosacral disc and eight cases of acute fibromyositis there was prompt alleviation of symptoms and the results were lasting with an average total dose of 4 g. An improvement in all patients (8) with incoordination was observed.

S. L. W.

Methocarbamol in Orthopaedic Conditions. H. F. Forsyth. (*J. Amer. med. Ass.*, 1958, **167**, 163.) Methocarbamol in an average daily dose of 6 g. in divided doses was given to 58 patients with acute orthopaedic conditions (especially herniated lumbar and cervical discs) causing painful muscular spasms, 7 patients with chronic conditions, and 18 patients recovering from orthopaedic surgery. A significant response was obtained in 94 per cent of the patients. Relief from pain was often prompt and striking, with resultant facilitation of treatment and hastening of recovery. No serious reactions to the drug were observed and very few unpleasant side-effects, apart from drowsiness, headache, slight lightheadedness, and nausea in a few patients. Methocarbamol was also used intravenously in some cases in a dose of 500–625 mg. three to four times daily, injected over 1 to 2 minutes in the form of a 2·5 per cent solution in saline.

S. L. W.

Morphine and Papaverine, Antipruritic effect of, in Experimental and Pathological Itch in Man. S. G. Macris, G. M. Smith and H. K. Beecher. (*J. Pharmacol.*, 1958, **123**, 220.) The effects of papaverine, morphine, pentobarbitone, aminophylline, tripelennamine and placebo were studied on experimental pruritis induced with cowhage. Only papaverine reduced experimental prutitis to a significant degree. In the case of pathological itch, however, morphine appeared to be effective while papaverine did not.

W. C. B.

LETTER TO THE EDITOR

A Note on the Irritant Properties of Sorbic Acid in Ointments and Creams

SIR,—During the last few years nonionic surfactants have been increasingly used as solubilising agents and emulsifiers. It soon became apparent that the preservation of products containing these emulsifiers offered special problems. Numerous reports have shown that the antimicrobial activity of different preservatives, e.g., *p*-hydroxybenzoic acid esters, is diminished by the presence of polyoxyethylene sorbitan esters (Tween) and other nonionic surfactants. This inactivation has been attributed on the one hand to the formation of a complex between phenolic preservatives and the polyether structure of Tween[1,2], on the other to a solubilisation of the preservative in the micelles of the emulsifier[3,4], which causes a decrease in its activity.

In some papers it has been demonstrated that sorbic acid has a comparatively good antimicrobial effect also in the presence of nonionic emulsifiers[2,4]. These observations have been confirmed at this laboratory, where sorbic acid has been employed with excellent results as a preservative for colloidal water dispersions of fat-soluble vitamins (A, D, E) as well as for ointments and creams of w/o and o/w types which previously often became contaminated by micro-organisms in spite of their being preserved by *p*-hydroxybenzoic acid esters in the usual concentrations.

Sorbic acid is reported to be non-toxic even in high concentrations, on oral administration[5–7]. No data seem to have been published on its use in dermatological or cosmetic products.

As mentioned, sorbic acid has been used in this laboratory for the preservation of ointments and creams. A test on 20 members of the staff showed, however, that application of these products to the face caused in one-half the number a more or less obvious erythema and slight itching, sometimes even slight oedema. The reaction appeared 5 to 15 minutes after the application and disappeared completely in 1 to 2 hours. In order to further investigate the irritative effect of sorbic acid, the following simple test was made.

0·7 g. of a saturated (0·15 per cent) solution of sorbic acid in water was absorbed by a piece of cotton (0·15 g.), about one sq. cm. in size and applied with adhesive tape on the forearms of the subject. Five different samples of sorbic acid from different manufacturers were tested. A control with water was applied at the same time. After one hour the reactions were read. All subjects showed a more or less intense reaction to all samples. Sorbic acid that had been recrystallised several times from water and chloroform induced similar reactions, from which it can be concluded that impurities are not the cause of the irritation. In three persons specially sensitive to the acid, the smallest concentration causing a positive reaction was determined as 0·01 to 0·02 per cent in a water solution and 0·025 to 0·05 per cent in cold cream (w/o).

The number of subjects in this investigation is too small to permit definite conclusions on the suitability of sorbic acid as a preservative for products for cutaneous application, but the reactions described have been so frequent that the laboratory has omitted sorbic acid from such products. A thorough dermatological investigation of sorbic acid seems to be required.

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September 16, 1958.

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BOOK REVIEW

TRACE ANALYSIS. Edited by J. H. Yoe and K. J. Koch. Pp. xiii + 672. Chapman & Hall, London, 1957. 96s.

Many current chemical and biochemical problems are concerned with the isolation and evaluation of small quantities of organic and inorganic materials, and a knowledge of suitably selective methods is often essential to their successful solution. The publication in this volume of a series of papers presented at a Symposium on Trace Analysis held at the New York Academy of Medicine in 1956 is therefore timely in that it provides a useful survey of such methods. It includes 24 separate papers, collected under three headings, and each dealing with an individual aspect of trace analysis, together with reports of the ensuing discussions. The first and by far the largest section, of methodology, cover such widely differing techniques as chromatography, electrophoresis, counter-current extraction, ion exchange techniques, chemical microscopy, colorimetry, fluorimetry, flame photometry, potentiometry, coulometry, polarography and voltammetry, amperometry, emission spectrochemical analysis, gamma-ray spectroscopy, mass spectrometry, X-ray spectroscopy, X-ray micrography, neutron activation analysis and microbiological techniques. This is followed by two shorter sections, Part II on instrumentation and the interaction of β-particles with matter, and Part III on sensitivity, and separation, concentration and contamination. The papers are not comprehensive, but provide a reasonable review of the use of the various techniques, and each section carries extensive references. The value of including verbatim records of the discussions is a little doubtful, and some editing of these sections might have reduced the cost of this otherwise excellent volume.

J. B. STENLAKE.