REVIEW ARTICLE

THE PHARMACOLOGICAL CLASSIFICATION OF CENTRAL NERVOUS DEPRESSANTS

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Not a few of the antihistamine drugs which appeared during the last half of the nineteen-forties made the patients so drowsy that they wondered whether perhaps the sneeze was the less disturbing. This fact became a challenge to theoretical and practical pharmacology. It was met in two ways. Some workers made antihistamines without this disturbing side-effect. Others found that it could perhaps be turned to good account, studied the effect, and tried to develop compounds without antihistamine activity but which possessed the special effect on the central nervous system of the antihistamines. The phenothiazine derivative promethazine was originated as an antihistamine and first made by Halpern in 1947[1]. Its pronounced sedative properties caused further investigations which led to chlorpromazine[2], the first and still one of the most important of the new psycho-sedative drugs. From the antihistamine substance diphenhydramine came captodiame[3], and from chlorcyclizine, hydroxyzine[4]. The anti-acetylcholine properties of some of the sedative antihistamines inspired the search which discovered benactyzine[5,6]. Two other routes also lead to this new field of pharmacology. One was the introduction of *Rauwolfia serpentina* and its alkaloids in Western medicine[7], and another was the synthesis of mephenesin by Berger[8]. The further search for compounds with the same effect as mephenesin eventually produced meprobamate[9], so popular in America. However, the initial clinical—and financial—success has had the effect that new compounds with sedative properties are still being synthesized and new drugs are appearing like mushrooms in an October forest.

The chemical formulae of the compounds arranged according to their effects and chemical constitution are given in the Tables I to VII. Many of these new sedatives act on the central nervous system in different ways and in many respects differ from the sedatives which were used before 1950. Some of them have properties in common with old and well-known compounds, others have properties hitherto undescribed. The whole field is becoming confusing for many pharmacologists and bewildering for many clinicians. Therefore, it might be useful to compare the actions of all these compounds, try to find out how they are mutually related, and make an attempt to classify them. This I have attempted to do.

The Pharmacological Basis of Classification

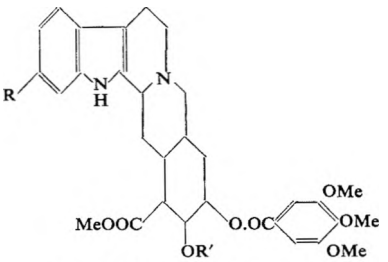
The Effect on Gross Behaviour

Most of the compounds decrease the spontaneous motor activity in quite small doses, but the differences in action become clearer when the doses are increased. After reserpine (Table I) and chlorpromazine

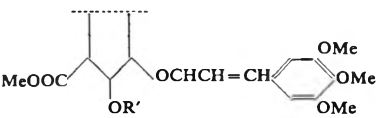
(Table II), the decreased spontaneous activity passes over into an immobility. The animals do not move spontaneously and if they are placed in bizarre and unusual positions they remain so for some time. But the righting reflexes are maintained even after the largest tolerated dose, and

TABLE I

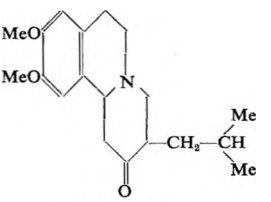
SOME INDIRECTLY ACTING MAJOR TRANQUILLISERS



R = —H; R' = —Me = dereserpine
R = —OMe; R' = —Me = reserpine



R = —OMe; R' = —Me = rescinnamine



Tetrabenazine

it is impossible to place mice or rats on their backs even if they are given near toxic doses of the compounds in question. An anaesthesia is never seen. For lack of a better word this reaction may be described, using an expression borrowed from psychiatry, as a cataleptic reaction.

Meprobamate and mephenesin (Table IV) give another type of reaction —flaccid paralysis. This is of central origin and distinguished from a curare effect by the fact that the monosynaptic reflexes are preserved. Some species of animal may show a complete flaccid paralysis without being anaesthetised.

The anaesthetic reaction is generally preceded by an increasing ataxia. This reaction is found after all hypnotics and most of the older sedatives.

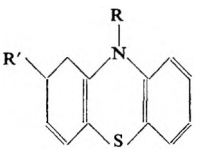
However, the mere observation of the gross behaviour is not sufficient to characterise the compounds. The effects on the more specialised functions of the central nervous system must also be considered. Many methods from neurophysiology and from psychology have been used in order to analyse and characterise the compounds.

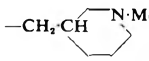
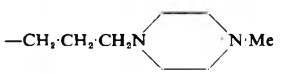
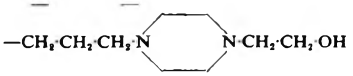
The Spinal Level

Some compounds can abolish the spinal and medullar polysynaptic reflexes, for example the flexor reflex of the hindlimbs. With the same doses the monosynaptic reflexes, such as the knee jerk reflex, are unchanged. Also some medullar polysynaptic reflexes are inhibited in the same way, but it seems as if the intraneuronal processes in the forebrain

TABLE II

SOME MAJOR TRANQUILLISERS OF THE PHENOTHIAZINE GROUP



R	R'	Generic name
$-CH_2\cdot CH_2\cdot CH_2\cdot NMe_2$	$-H$	promazine
— —	$-O\cdot Me$	methopromazine
— —	$-O\cdot CON\cdot Me_2$	acetopromazine
— —	$-Cl$	chlorpromazine
— —	$-CF_3$	triflorpromazine
	$-H$	mepazine
	$-Cl$	prochlorperazine
— —	$-CF_3$	triflorperazine
	$-Cl$	chlorpiprozine, perphenazine

and in the midbrain are comparatively uninfluenced. This effect was first discovered and described as the main action of mephenesin[10] (Table IV). Since then, a long series of compounds have been synthesised which all have the same characteristic action of which meprobamate[11] and phenaglycodol[12] (Table IV) will be discussed as examples, later on. Other compounds with different chemical constitution, aminobenzoxazoles and aminobenzothiazoles[13] (e.g., zoxazolamine[14]) (Table V), also abolish the polysynaptic reflexes in the same way. Other spinal functions as well as the reflexes are abolished by these agents acting like mephenesin, for example the facilitation and inhibition of the knee jerk reflex seen after stimulation of higher centres[15].

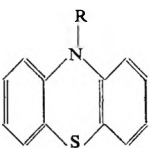
All compounds which show this inhibition on the spinal and medullar polysynaptic reflexes antagonise the effect of strychnine. They increase the threshold for the tonic seizures and considerably increase the lethal doses[10].

The Medullary Level

Many centrally acting compounds depress the medullary centres. Morphine is one good example among several which depress the respiratory centre. Others, like the barbiturates in high anaesthetic doses, have a paralyzing effect on the cardiovascular centre. The depression can be antagonised by a series of stimulating compounds like leptazol, picrotoxin, camphor, and the amphetamines. But these antagonisms are

TABLE III

SOME PHENOTHIAZINE DERIVATIVES



R = $-CH_2 \cdot CH_2 \cdot CH_2 \cdot NMe_2$ = promazine, (minor tranquilliser)
R = $-CH_2 \cdot CHMeNMe_2$ = promethazine (antihistamine, anti-emetic and minor tranquilliser)
R = $-CH_2CHMeNEt_2$ = ethopropazine (anti-Parkinsonism).

those of classical pharmacology and will not be described here. The compounds dealt with here have generally little influence on the medullary level. Exceptions are seen with chlorpromazine and its congeners which depress the trigger zone of the vomiting centre and in this way act anti-emetically[16,17]. Experimentally, this effect is demonstrated, by the antagonism of the compound to apomorphine. Most of the other compounds have little or no anti-emetic effect except hydroxyzine[18] (Table IIIA) and barbiturates in anaesthetic or subanaesthetic doses[19].

The Meso-diencephalic Level

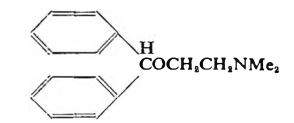
Many functions which have centres in the meso- and diencephalon are influenced. Firstly may be mentioned the dysfunction which results in motion-sickness. This state is presumably due to the disturbing influence of repeated vestibular impulses. The connection between the vestibular nucleus and the vomiting centre is not precisely known, but some compounds, for example diphenhydramine[20] (Table IIIA), are able to regulate the disturbance. In man, chlorpromazine does not act in this way in spite of its effect on the trigger zone of the vomiting centre[21].

A long series of other functions regulated by meso-diencephalic centres are inhibited by reserpine, chlorpromazine and related compounds. One of the most important is the centre or centres of the sympathetic nervous system. After administration of reserpine the tonus of this system is depressed showing myosis, ptosis, and bradycardia[22]. The effect of chlorpromazine on the sympathetic system is also depressing, but perhaps less pronounced. Here it is difficult to distinguish between the drug's peripheral anti-adrenergic action and a central depression of the sympathetic centres.

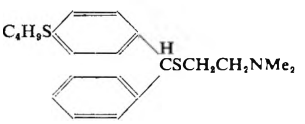
The autonomic centres of the cardiovascular system and those of heat regulation are also depressed. It has been clearly shown that the fall of blood pressure after reserpine is due to an action on meso-diencephalic centres[22–24]. But there has been some discussion whether the fall after chlorpromazine is caused by a central or a peripheral action since chlorpromazine has a strong peripheral sympatholytic action. However, an effect is seen in monkeys after intracisternal application of small doses

TABLE IIIA

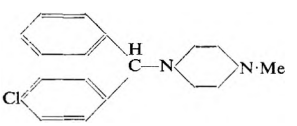
SOME MINOR TRANQUILLISERS AND CHEMICALLY RELATED COMPOUNDS
(Benzhydrol- and benzhydryl derivatives)



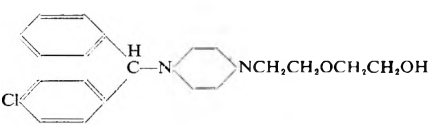
Diphenhydramine (anti-motion sickness)



Captodiame (tranquilliser)



Chlorcyclizine (antihistamine)



Hydroxyzine (tranquilliser)

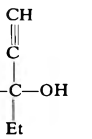
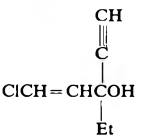
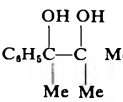
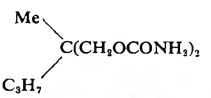
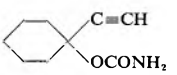
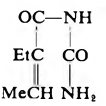
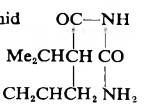
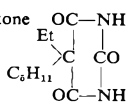
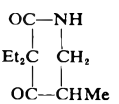
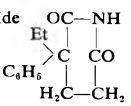
of chlorpromazine, which suggests at least a partial central action[25]. Reserpine and chlorpromazine also inhibit the centre for heat regulation causing a decrease of the body temperature when the external temperature is below the body temperature[16,26] and an increase when it is above. Hydroxyzine has a similar effect[18], but most other psycho-sedatives have little or no influence on the heat regulation.

The so-called sham rage is a state which causes violent outburst of all sympathetic functions. It can *inter alia* be provoked in decorticated cats. This state is suppressed by chlorpromazine[27] and reserpine[28], actions which might be connected with the general effect on the sympathetic system.

Many hormonal functions are also disturbed after chlorpromazine and reserpine, functions which are not easy to study in animal experiments, and our knowledge rests on clinical observations only. Patients treated with chlorpromazine or reserpine often show a formidable increase in appetite and gain considerably in weight. Disturbances of the menstruation, and even lactation in female patients who are not or never have been

TABLE IV

SOME TRANQUILLO-SEDATIVES AND HYPNO-SEDATIVES

No.	Generic name	Hypnotic	Sedative	Inhibiting polysynaptic reflexes
I	Ethanol EtOH	+	+	O
II	Methylpentynol 	+	+	O
III	Ethchlorvynol 	+	+	O
IV	Phenaglycodol 	+	+	+
V	Mephenesin *o*-$MeOC_6H_4OCH_2CHOHCH_2OH$	O	O	+
VI	Reorganin *o*-$MeC_6H_4OCH_2CHOHCH_2OH$	O	O	+
VII	Urethane $MeCH_2OCONH_2$	(+)	O	O
VIII	Meprobamate 	(+)	+	+
IX	Ethinamate 	+	+	O
X	Ectylurea 	(+)	+	O
XI	Sedormid 	+	+	O
XII	Pentobarbitone 	+	+	
XIII	Methyprylone 	+	+	O
XIV	Glutethimide 	+	+	O

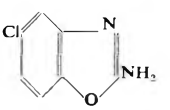
pregnant, have also been described after reserpine. All these observations may be interpreted as an influence of the drugs on the hypothalamic centres regulating the hormonal system.

The Extrapyramidal System

The extrapyramidal system is the name for all motor functions not belonging to the pyramidal tracts. In man, disturbances of the extrapyramidal system are manifested through Parkinson's syndrome with muscular rigidity and a characteristic tremor. The functions of clinical importance regulating the extrapyramidal system are located in di- and mesencephalon, but neurophysiological experiments in animals have shown that centres influencing the motor system are found in the cortex,

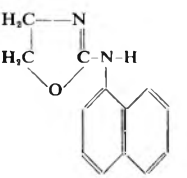
TABLE V

TWO COMPOUNDS NOT CHEMICALLY RELATED TO MEPHENESIN, BUT INHIBITING POLYSYNAPTIC MEDULLAR REFLEXES



(I)

(I, zoxazolamine, no sedative effect, no hypnotic effect)



(II)

(II, 2-(1-naphthylamino)2-oxazoline, tranquillising effect)

cerebellum and many other parts of the central nervous system. Parkinson symptoms are the most common side-effects seen during the clinical use of chlorpromazine and reserpine. The symptoms are very similar to those described by von Economo during the epidemic encephalitis after the first World War and shown to be due to pathological changes in the meso- and diencephalic centres. There is a slight difference between the effect of chlorpromazine and reserpine, the Parkinsonism after the first is more characterised by rigidity, after the latter by tremor and restlessness[29]. Extrapyramidal symptoms are also provoked in animals, especially monkeys by, for example, reserpine[30]. The symptoms in man and animals disappear when the drug is discontinued.

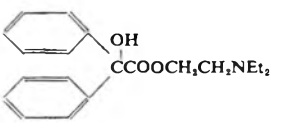
The agents used in the clinic for Parkinsonism are able to ameliorate the extrapyramidal symptoms provoked by reserpine and chlorpromazine. Some of these compounds are chemically and pharmacologically closely related to chlorpromazine, for example, ethopropazine and diethazine. Only slight changes in the molecule are necessary to change a compound from one provoking Parkinsonism to one able to depress the symptoms of Parkinsonism (Table III). It would seem that these compounds are bound to the same or nearly the same elements in the central nervous system, and that their capacity to provoke or improve Parkinsonism depends upon their capacity to inhibit or stimulate these elements. Diphenhydramine[31] and some derivatives, mephenamine[32] (with a tolyl-group replacing a

phenyl-group) and Rigidyl[33] (with a diethylamino-group) are also clinical effective anti-Parkinsonism agents.

The Meso-diencephalic Reticular System (Arousal Syndrome)

The reticular system is an anatomically poorly defined structure described as "all grey masses of the tegmentum of the medulla, pons and midbrain which do not belong either to the cranial nerves, to the relay nuclei of the cerebellar system or to the relay nuclei of the lemniscal systems"[34]. To a part of this reticular system is ascribed a function in the extrapyramidal system and this is called the descending reticular system.

TABLE VI

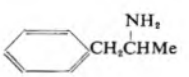


Benactyzine

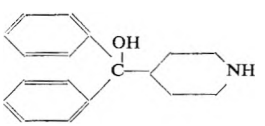
Another part, the ascending reticular system, belongs to the sensory pathways of the central nervous system[35]. The impulses received through the sensory apparatus are not only carried directly to the specific localised areas in the sensory cerebral cortex, but they also pass through side branches into the reticular system. Here they are transformed and transmitted diffusely over the whole cortex. The electric activity of the cortex of, for example, the cat or the rabbit not receiving these diffuse

TABLE VII

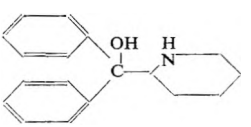
THE AMPHETAMINE–PIPRADROL GROUP



Amphetamine



Azacyclonol



Pipradol

impulses is characterised by slow waves with large amplitudes. This resting pattern is changed to one with fast waves of considerably smaller amplitudes when the reticular system is stimulated by a sensory impulse. This effect is called the EEG arousal reaction. The constant inflow of diffusely spread impulses from the reticular system seems to be connected with the state of consciousness so that a cortex which does not receive these impulses is incapable of perception.

Some of the compounds have no influence on the EEG arousal reaction, mephenesin is an example. The mere suppression of the polysynaptic reflexes has thus no relation to the function of the reticular system[36,37].

Chlorpromazine and its congeners incompletely inhibit the arousal reaction. After these drugs a stronger stimulation is necessary to provoke a reaction and its duration is shorter. But still some effect can be seen

even when the doses are increased so much that other factors influence the EEG[38,39]. A complete inhibition of the arousal syndrome is found after barbiturates[36,40] and other hypnotics and anaesthetics. This inhibition is closely connected with the loss of consciousness. Similarly meprobamate depresses the reaction[19].

Other compounds completely block the EEG arousal effect so that it cannot be provoked even by the strongest stimulation. This effect is seen after the anti-acetylcholines with a central effect; hyoscine, atropine[41] and benactyzine[42,43] (Table VI). Diphenhydramine and similar compounds also show the same blocking effect which is attributed to their anti-acetylcholine effect[41]. The abolition of the arousal syndrome after these compounds is seen without any of the loss of consciousness observed after the anaesthetics. In spite of a constant resting EEG pattern, the animals behave normally and react normally to various impulses. The blocking of the EEG arousal is not linked to any special central effect characteristic of the different compounds of this group[44]. Atropine and benactyzine have the same influence on the EEG, but their psychic effects differ considerably. Some authors suggest that this EEG effect is connected with the anti-Parkinsonism effect of the compounds[41].

Some substances provoke the EEG arousal syndrome. Acetylcholine itself and agents acting like acetylcholine are examples, and the centrally acting anti-acetylcholines are able to abolish this effect[41]. Adrenaline and other sympathomimetics including the amphetamines and pipradrol are also able to provoke the EEG arousal syndrome[43]. All these compounds give a hyperexcitability and restlessness corresponding to a super-awake state. Lysergic acid diethylamide (LSD) facilitates the formation of the arousal pattern[45]. This effect might be connected with the general stimulation of the sympathetic centres by LSD, but other mechanisms are presumably involved.

The effect of reserpine is paradoxical. Administration gives an arousal EEG pattern[46,47], in spite of the fact that the animals are heavily sedated.

Azacyclonol (Table VII) has no effect except in large doses where it provokes arousal[41], but it is able to restore the normal arousal pattern enhanced by lysergic acid diethylamide and mescaline[48]. It has no effect on the arousal syndrome after amphetamines or its progenitor pipradrol. The antagonising effect of azacyclonol on the LSD-arousal is not specific. The same antagonism is also seen after many other sedatives, including chlorpromazine.

Other EEG Effects

The other effects on the electrical activity of the brain are not all thoroughly investigated, but some light has been thrown on the difference between one group and another.

In addition to the already mentioned arousal effect, what is called the primary localised response, and the recruiting response also belong to the sensory system.

The primary localised response is the short brain potential found localised in the area of the sensory cortex corresponding to the region

which has been stimulated. The impulse follows the classical lemniscal pathways described in every textbook of physiology. This response is remarkably uninfluenced by any of the drugs; barbiturates, even in anaesthetic doses[39,40,49,50], benactyzine[51], mephenesin[52], chlorpromazine[39,53] or reserpine[53].

The recruiting response of the cortex is seen when the anterior thalamic part of the reticular formation is stimulated electrically with frequencies similar to those of the spontaneous rhythms of the cortex. It consists of a successive increase in amplitude of the cortical response found diffusely over great areas of the cortex, and is called recruiting, because more and more neurones become involved. The recruiting response cannot be provoked in a cortex which gives the arousal syndrome, and therefore the effect of compounds giving such a reaction cannot be studied. Some true anaesthetics depress the recruitment, for example, ether[52], but the barbiturates enhance it considerably[39,40,49,50]. Benactyzine has no effect[51] nor has reserpine[53]. A slight inhibition is found after mephenesin[52]. No statement can be found for meprobamate. Finally, small doses of chlorpromazine facilitates the recruiting response while larger doses inhibit it[53].

The spontaneous electrical activity (EEG). The spontaneous electrical activity pattern after ether or barbiturate anaesthesia, or sleep, and the suppression of the α-waves after stimulating drugs has been frequently described. After moderate doses of reserpine, chlorpromazine, the minor tranquillizers, meprobamate, mephenesin, and azacyclonol no effect is seen on the human EEG. Benactyzine forms an exception in being able to suppress the α-waves[55].

In animals the spontaneous electric activity is mainly regulated from the reticular system and the effects of the compounds giving resting or arousal syndromes have been described already. With larger doses other electrical activities may dominate. Such activities start at different parts of the central nervous system according to the type of agent, and may perhaps help to characterise the mode of action of the compound. A regular continuous activity starts in the hippocampus after reserpine[53,56,57]. After chlorpromazine in fairly large doses, seizure-like discharges begin in the amygdala and spread to the rest of the brain with increasing doses[39]. The depressing effect of the barbiturates is first seen in cortex and the caudate nucleus in doses which leave the thalamus and hypothalamus unaffected[52]. Meprobamate gives a synchronisation of the electric activity showing 10–20 c/s waves beginning in the thalamic centres and spreading from there to the cortex[58].

The two neuron intercortical system. An electric stimulation applied to a point in, for example, the optic cortex evokes a response in the connecting symmetrical point in the contralateral cortex. This response is inhibited by a series of stimulating agents; adrenaline, noradrenaline, mescaline, LSD, bulbocapnine and 5-hydroxytryptamine. The inhibiting effect of mescaline is abolished after azacyclonol, reserpine and chlorpromazine[59].

Nicotine convulsions. Intravenous administration of nicotine to rabbits give EEG changes characteristic of a grand mal seizure. A series of

compounds, caraminiphen, ethopropazine, chlorpromazine, trihexyphenidyl, promethiazine, diphenhydramine, chlorcyclizine and pyrilamine, most of these being anti-Parkinsonism drugs (except, as we have seen, chlorpromazine), are able to antagonise this effect[60,61].

Electric stimulation of the brain with liminal voltage. Electric stimulation of this kind gives a discharge occurring during the stimulation and an after-discharge outlasting the stimulus. Meprobamate increases the threshold for the first reaction and most antiepileptics the threshold for the second[57,62].

The effects of the differing types of the psychotropic compounds on the electric activity of the brain may help to characterise them. However these effects give little information on the understanding of the mode of action of the compounds. So far, it has been difficult to correlate the changes of the EEG and the neurophysiological and behavioural changes after administration of the compounds, but it is to be hoped that the intense research being made in this field will throw more light on the problems.

The Psychic Level

The psychic effect of the compounds on animals is studied by assessing their reactions to standardised impulses. These reactions may be classified in groups; the unlearned reactivity, the learned reactivity, the stress induced behaviour, and the capacity for solving problems. The influence of the psychotropic agents on the last named capacity has been little examined and is of limited interest in this context. It will therefore not be discussed.

Unlearned Reactivity

The unlearned reactivity is the innate response to certain impulses which is characteristic for each species. Some of these reactions are simple and may perhaps be compared with mere reflexes. Others, in the form of instincts may be extremely complicated "chain-reactions". A few examples will be mentioned.

The calming effect of the compounds on the natural aggressive-defensive behaviour of monkeys is frequently described under the name of the "taming" effect. This taming is seen after reserpine[22], meprobamate[11], and to a minor degree after chlorpromazine[63]. Other compounds, such as barbiturates and benactyzine[51], have no effect. The same compounds seem to have a similar effect on the fighting instinct of the male Siamese fighting fish (*Betta splendens*)[64].

Another form of unlearned reactivity is the so-called orientating reflex. The somatic behaviour of a dog when it is exposed to an unknown and unexpected signal is well known to every dog-owner. Less well known is the fact that autonomic reactions also occur, for example, in the form of a temporary increase in the pulse rate. Chlorpromazine and reserpine are able to abolish the somatic and autonomic orientating reflexes[65]. Barbiturates[66] and alcohol[67] never completely abolish the orientating reflexes unless the animals are fully anaesthetised.

Some further examples of more complicated innate behaviour have been investigated, but only the effect of one or two types of agents have been examined. For this reason the results are difficult to compare. However, it seems that if an agent has an inhibiting influence on one type of innate behaviour, it also inhibits other types of innate behaviour. Naturally, quantitative variations from test to test are found, and reservations must be made for new discoveries in this field.

Learned Behaviour

The learned behaviour includes all conditioned reactions and other forms of trained behaviour. The methods applied have varied from classical Pavlovian experiments on conditioned reflexes to the study of complicated reactions learned by the animals in order to avoid a disagreeable stimulus or to obtain a reward. The influence of the compounds on the learned activity seems to be so constant from method to method that it is unnecessary to distinguish between the different methods here.

The learned activity is inhibited by reserpine[65,68–71], chlorpromazine[65, 70–72], and barbiturates[66]. After reserpine and chlorpromazine only the somatic, but not the autonomic reaction disappears[65]. The author, together with Sonne, has observed that meprobamate seems to have no effect in the few experiments made. In contrast to the other compounds, benactyzine increases the number of conditioned responses[73] and decreases the latency time of the somatic conditioned reaction.

Some drugs have a stronger influence on the unlearned activity than on the learned activity. The orientating reflex disappears after doses of chlorpromazine and reserpine which preserve the conditioned reflexes[65]. The autonomic response in the form of the increased heart rate disappears during the orientating reflex, but can never be brought to disappear completely in the conditioned response. On the other hand, other drugs have a stronger influence on the unlearned reactivity. After alcohol and barbiturates the orientating reflexes may be detected at stages where the conditioned reflexes have disappeared completely[66,67]. Unfortunately not all compounds have been examined with the same technique so that a complete comparison cannot be made.

Stress-induced Behaviour

Animals exposed to a mental stress will show a characteristic behaviour varying with the species and the experimental situation. The classical mental stress-induced behaviour is the so-called experimental neurosis. Animals exposed to situations to which they do not know how to react show a well-defined behavioural pattern for which Pavlov coined the word "experimental neurosis". For example, cats placed before a box, which might contain food or might have fear-inducing properties, react to the situation with a characteristic somatic behaviour and might also show some autonomic symptoms, like vomiting and diarrhoea[74,75]. Administration of barbiturates[76], alcohol[74], meprobamate (author, unpublished results)

and especially benactyzine[77] has a normalising influence on these conflict-induced behavioural patterns in cats or monkeys.

The stiffened, tense behaviour of rats expecting the sound signal to which they have to react in order to avoid an electric shock is abolished and normalised by benactyzine[78] and meprobamate (author, unpublished results).

The general depressive effect of chlorpromazine and reserpine are such that a different technique must be applied to demonstrate the effect of these compounds. In such experiments reserpine has an improving effect on a psychic stress situation although the normal performance is considerably inhibited[79].

Self-stimulation

Until recently there has been a wide gap between the pharmacological investigations based on neurophysiological methods and those based on the methods of animal and human psychology. This gap is now beginning to be closed. Electric stimulation applied to specific hypothalamic and palaeocortical structures of the rat brain has the same effect as an anticipated effective reward[80]. Bipolar electrodes are chronically implanted in the brains of rats who rapidly learn to stimulate themselves by means of a lever-contact placed within the cage. The frequency of lever pressing is an expression of the force of the drive with which the animal seeks this peculiar form of a reward. Rates of as much as 80 responses per minute are found. This lever pressing rate is influenced by some of the psychotropic agents. Thus the rate is decreased after reserpine, chlorpromazine and pentobarbitone, but the different drugs have different effects according to the site of the implanted electrodes[81,82]. The further development of this technique seems to give promise of localising the site of these drug effects and thus provide a further basis for their classification.

Antagonisms, Synergisms and Potentiation

When given together, two psychotropic agents may act antagonistically or synergistically. As a rule the depressing agents antagonise the stimulating agents and *vice versa*, but this rule is not without exceptions.

The property of mephenesin and similar agents to antagonise strychnine has already been mentioned. However, if a compound antagonises strychnine it does not necessarily antagonise the effect of other convulsants. In comparison with mephenesin, meprobamate antagonises leptazol more than it does strychnine[11], and some oxazoline derivatives have been found which antagonise strychnine without affecting leptazol convulsions. The same compounds which antagonise leptazol generally also increase the threshold for electro-convulsive seizures (the liminal voltage, mentioned earlier), but the two effects do not run absolutely parallel[83]. It is well known that the anti-epileptics also are capable of antagonising leptazol and electro-convulsions, but neither meprobamate or mephenesin have an effect on epilepsy similar to that of the true anti-epileptics. Chlorpromazine has no effect on the strychnine, picrotoxin, cocaine[84] and leptazol convulsions[85], nor has reserpine[86–83]. The

TABLE VIII

		Reserpine	Chlorpromazine	Diphenhydramine	Hydroxyzine	Pentobarbitone	Phenaglycodol	Meprobamate	Mephenesin	Benactyzine	Azacyclonol	Pipradol	Amphetamine	Mescaline	Lysergic acid diethylamide
General effects	Polysynaptic reflexes	O	O	O?	O?	O	–	–	–	O	?	?	O	O	O
	Convulsions after electrostimulation	+	+	?	?	–	–	–	–	±	?	?	?	?	?
	" " strychine	O	O	O?	O	i	?	–	–	?	?	?	?	?	?
	" " leptazol	–	O	?	?	–	–	–	–	?	?	?	?	?	?
	" " nicotine	?	–	–	–	O	?	O?	O?	?	?	?	?	?	?
Gross behaviour	Spontaneous activity	–	–	+	–	–	–	–	–	+	–	+	+	+	+
	Catalepsia	+	+	O	O	O	O	O	O	O	O	O	O	+	O
	Paralysis	O	O	O	(+)	O	+	+	+	O	(–)	O	O	O	O
	Anaesthesia	O	O	O	O	+	+	e	O	O	O	O	O	O	O
	Convulsions	O	e	+	O	O	O	O	O	e	e	O	O	O	O
Synergism or antagonism	Anaesthetics and hypnotics	+	+	+	+		+	+	(+)	+	+	–	–	–	–
	Amphetamine	–	–	?	–	–	– ?	– ?	?	O	–			+ ?	+ ?
Lower centres	Vomiting	O	–	(–)	–	i	O	O	O	O	O	O?	O	(+)	(+)
	Motion sickness	O	O	–	– ?	i	O	O	O	O	O	O?	O	O	O
	Blood pressure	–	–	O?	O	O	O?	O	O	O	O	O	+	±	+
	General sympathetic tonus	–	–	O?	O	O	O?	O	O	O	O	O	+	(+)	+
	Body temperature	–	–	O?	–	i	O?	O	O	O	O	?	+	+	+
	Appetite	+	+	O?	O	O	O?	O	O	O	O	O	–	–	(–)
	Endocrine centres	±	±	?	O	O	O	O	O	O	O	?	?	?	?
Extra pyramidal system	+ Disturbing equilibrium – Regulating	+	+	–	– ?	O	O?	O	O	(–)	O?	O?	O?	O?	+

TABLE VIII—*continued*

EEG	Recruiting	O	(±)	?	?	+	+	– ?	–	O	?	?	?	?	?
	Arousal	+	(–)	–	– ?	—	(–)	–	(–)	–	O +	+	+	+	+
	Two neuron intercortical	normal-ising	normal-ising	?	?	–	?	?	?	?	normal-ising	(–)?	–	–	–
	α Waves in normal EEG	O	O	O	O	spind-les	?	O	O	–	O	O	(–)	(–)	(±)
Higher psychic functions in animals	Unlearned reactivity	–*	–*	O	O?	–	–	–	O?	O	O	?	+	O	?
	Avoidance reaction: motor	–	–	O	–	–*	?	O	O?	+	O	?	±	O –	–
	" " : autonomic	O	O	O	O	–*	?	?	O?	?	O?	?	?	(+)* –	?
	Reward reaction	–*	–*	O	O	–*	?	?	O?	±	O?	?	+	?	?
	Stress or conflict-induced behaviour	O –	O	O	O	O –	?	–	O?	–	O?	?	?	?	?
	Fatigue	?	?	?	?	?	?	?	?	?	?	–	–	–	–
Higher psychic functions in man	Emotional impression of disturbing stimulus	–	–	O – ?	–	(– ?)	–	–	?	–	(–)?	O	O	(–)	(–)
	Euphoria	O	O	O	?	O(+)	O	O(+)	O	O	O	O	+	±	±
	Thought blockade	O	O	(e)	?	O	O?	O	O	+	O	O	O	(+)	(+)
	Distorted sensual perception	O	O	O	O –	O	O	O	O	O	O	O	O	+*	+*
Basic actions of possible importance	Acetylcholine	O	–	–	–	O	O	O	O	–	O	O	O	?	O
	Adrenaline	(–)	–*	O	(–)	O	O	O	O	O	O	O	+	+(?)	–
	5-Hydroxytryptamine	O	O	O	?	O	O	O	O	–	O	O	?	?	–*
	Local anaesthetic effect	O	+	+	+	O	O	O	O	+	O	O	O	O	O
	Release of 5-hydroxytryptamine	+*	O	O	O?	O	O	O	O	O	O	O	O	O	O

Key.—O, no effect; +, enhancement, lowering of threshold, synergism; –, inhibition, increase of threshold, antagonism; ( ), incomplete effect; i, inhibition in large doses; e, enhancement in large doses; ?, lack of information; *, dominating effect; ±, O, etc., effect depending on the experimental conditions.

hyperactivity induced by amphetamine—which never ends in convulsions —is antagonised by barbiturates, chlorpromazine, and azacyclonol, but not by reserpine.

Very characteristic for all the recent sedatives is their property to potentiate the effect of anaesthetics of which hexobarbitone and alcohol are the most commonly used in such experiments. This effect was discovered by Winter with diphenhydramine[89]. As mentioned, most of the compounds are not anaesthetics *per se*, and some give even hyperactivity in moderate—and convulsions in larger—doses. This anaesthesia-prolonging property is common to almost all the more recent sedatives[90,91], but it is not found with every depressant of the central nervous system, for example, not with morphine[91].

Some Typical Groups

From this analysis it is seen that the depressants differ in their action upon the single functions of the central nervous system. When all these actions are tabulated as in Table VIII, it is possible to distinguish a "profile of action" which is characteristic for each agent. Drugs with similar profiles of action may be collected into groups.

The Major Tranquillizers

This group consists of reserpine (and some other active rauwolfia alkaloids) and chlorpromazine with its more recent congeners. The major tranquillizers are mainly characterised by their depressive effect on the lower centres, including the extrapyramidal system. They give a cataleptic reaction in gross behaviour. Psychically, they have a greater influence on the unlearned behaviour than on the learned behaviour.

Although reserpine and the reserpine-like alkaloids on one side and chlorpromazine and its congeners on the other side have many properties in common, one fundamental difference is found in their mode of action. Reserpine does not act directly on the cells of the central nervous system. After administration of reserpine, 5-hydroxytryptamine[92–94] and the catecholamines[95] disappear from the central nervous system. The symptoms observed follow closely the changes of the amine-concentration, not the concentration of reserpine in the brain or the tissues[96]. It is therefore reasonable to assume that the effect of reserpine is closely connected with the deprivation of the amines, although their precise function in the central nervous system is not clear. A further proof of this conception is the effect of reserpine on animals pretreated with iproniazid (Marsilid, 2-*iso*propyl-1-*iso*nicotinyl hydrazide) an antituberculosis agent. The compound is a potent and irreversible inhibitor of monoamino oxidase, an enzyme which *inter alia* deaminates noradrenaline, adrenaline, and 5-hydroxytryptamine. When animals are pretreated with iproniazid and given reserpine, they are not sedated, but become hyperactive and show symptoms similar to those seen after LSD or amphetamines with a general stimulation of the sympathetic system instead of an inhibition[93,97]. Furthermore, the content of 5-hydroxytryptamine and presumably also that of catecholamines shows very little

decrease after reserpine when the animals are pretreated with iproniazid[98]. Apart from reserpine and two other rauwolfia alkaloids, dereserpine and recinnamine[99], other compounds, chemically unrelated to reserpine, are found to have the same effect. The best known is tetrabenazine (Table I)[98]. It has also the same pharmacodynamic and biochemical effect as reserpine but is shorter acting.

Chlorpromazine has no effect on the amines, and its pharmacodynamic effects cannot be modified by iproniazid. Hence, it must have a mode of action different from that of reserpine. Some examples of differences between reserpine and chlorpromazine have been mentioned previously, but the similarity in the action of reserpine and chlorpromazine is more striking than the differences. This fact suggests that both exert their effect on the same parts of the brain. The aminocatechols[100] and 5-hydroxytryptamine[98] are found in the highest concentrations within the central nervous system in the thalamic-hypothalamic areas, in those regions where it would be expected from the symptoms that these tranquillizers would have their main site of action.

So far, only phenothiazine-derivatives having a similar action to chlorpromazine have been described. All have a side chain in the 10-position, and the 3-position is as a rule substituted. A number of examples are given in Table II. There is some variation in the effect from compound to compound. The potency increases with the following substitutions: H,$CONH{\cdot}NH_2$,OCH_3, $COOCH_3$, Cl,CF_3[102]. The variation is not only quantitative, thus e.g., perphenazine is 7 to 10 times as potent as is chlorpromazine but also qualitative. Comparison between chlorpromazine and triflorperazine shows that the latter is about 3 times stronger when measured by the inhibition of conditioned responses in rats, 5 times as effective in tranquillising monkeys, and 10 times as effective as an anti-emetic[102]. Some clinicians state that the compounds differ in their ability to provoke symptoms of Parkinsonism compared with their clinical effect on psychoses[103,104].

The Minor Tranquillisers

The effects of these compounds are qualitatively or quantitatively less pronounced than those of the major tranquillisers. Nevertheless, they act as tranquillisers; they potentiate the anaesthetic effect of barbiturates and alcohol, and in the clinic they have sedative properties. They also act on functions which have centres in the midbrain and diencephalon, but these actions are weaker or more specialised than those of the major tranquillisers. Some are potent against motion sickness, others have an anti-Parkinsonism effect. Most of them are antihistamines or have been developed from antihistamines. Tables III and IIIA give some examples.

The Hypno-sedatives and the Tranquillo-sedatives

The members of this group are distinguished from the tranquillisers at least in two ways. They have not the specific effect on the same functions with centres in the meso- and diencephalon as have the tranquillisers, and they antagonise convulsants. Some of the products are true hypnotics in

the old sense of the word, giving anaesthesia in sufficiently large doses. The barbiturates, and alcohol, are described in every textbook of pharmacology. Others inhibit the polysynaptic reflexes, and still others have both properties. Table IV gives examples of compounds of this type.

The question arises how the anaesthetic effect and the effect on the polysynaptic reflexes are connected with the sedative properties as measured by the inhibition of motor activity, the synergism with the effect of anaesthetics and the clinical experience. A small correlation of the effect on the polysynaptic reflexes and the sedative action exists. Meprobamate with its well-known depression of the polysynaptic reflexes has also a pronounced and very popular sedative action, but some benzoazoles exert quantitatively a higher inhibition on the polysynaptic reflexes than does meprobamate, and are deprived of a sedative effect[13]. We are perhaps not entitled to include compounds like mephenesin and zoxazolamine among the true psychotropic drugs. Thus it seems indeed as if a red herring rather than a precise knowledge of structure–action relations has led us to compounds like meprobamate.

It is more difficult to decide to what extent the hypnotic action and the sedative action is correlated. It is generally believed that they must be, but the two properties do not follow each other closely. With pentobarbitone, sedation begins at 4 per cent of the LD50, and the anaesthesia is complete at 35 per cent. Phenobarbitone starts sedation at 4 per cent and has a complete anaesthesia at 50 per cent. Finally, with ectylurea a sedation at 1 per cent and full anaesthesia at 90 per cent of the LD50 is observed[105].

Compounds like pentobarbitone and meprobamate differ in their effect. Psychically, alcohol and barbiturates influence the unlearned reactivity less than the learned reactivity while the opposite seems to be true with meprobamate.

As perhaps the psychic action of meprobamate is more like that of the tranquillisers than is the psychic action of the hypnotics, we may distinguish between tranquillo-sedatives for sedatives like meprobamate and hypno-sedatives for sedatives of the barbiturate-alcohol type. However, no sharp line can be drawn between the two types of sedation. Phenaglycodol and presumably many other compounds have traits from both groups[12].

Central Acting Anti-acetylcholines

Benactyzine differs in some respects from the compounds just discussed. It has no sedative action. On the contrary, it increases the spontaneous activity. It has no effect on the unlearned reactivity, as judged for example from the lack of the taming effect on monkeys. It has a stimulating effect on some learned reactions and differs in this way from the tranquillisers. It has also an ability to normalise stress-induced behaviour in some experimental situations. This ability is also found after alcohol, barbiturates and especially after meprobamate. Like all other anti-acetylcholines, for example atropine and hyoscine, benactyzine is a strong inhibitor of the EEG arousal syndrome. The same effect is

also found with the minor tranquillisers which have rather pronounced anti-acetylcholine properties. It is possible that the central effect of benactyzine is connected in some way with its strong anti-acetylcholine effect, but the interrelation is not yet clearly defined. In rats hyoscine, but not atropine, has effects comparable to those of benactyzine[69].

The Older Compounds

The bromides, the lithium salts, and the morphine-like acting drugs are examples of compounds which have not been considered. The reason is that they have not been examined so comprehensively with the same methods as the more recent ones. However, from what we know about them they cannot all be classified in the same groups shown in Table VIII.

Transitional Compounds

The groups presented here seem well defined and differ clearly from each other in their mode of action. Yet, it is easy to find compounds which have properties in common with members of two or more of the here described classes. Thus, as mentioned, phenaglycodol forms a link between the barbiturates and meprobamate.

However, we know of many examples of such "transitional compounds" between groups with little similarity. A recently described compound, 2-(1-*naphthylamino*)-2-*oxazoline* (Table V) is stated to depress the polysynaptic reflexes. In this respect, it belongs to the mephenesin-meprobamate group. It has quieting properties and a taming effect on monkeys. It potentiates the effect of anaesthetics. These properties are common for meprobamate and the major tranquillisers. Finally, it increases the convulsions after leptazol, a property found among the tranquillisers and definitely not characteristic of meprobamate[106]. In this way, naphthylamino-oxazoline forms a link between the sedatives and the tranquillisers as I have classified them in this review.

In addition, at least three examples can be found of compounds which in some respects belong to the sedatives and in others to the stimulants. Apart from its analgesic effect, morphine has some psychic effects similar to those of the major tranquillisers, for example, it inhibits the learned reactivity. On the other hand, it stimulates some lower centres, which generally are inhibited by the major tranquillisers, for example, the trigger zone of the vomiting centre and some of the sympathetic centres.

Azacyclonol[107] is developed from, and chemically closely related to, the stimulating agent pipradrol[108] (Table VII), which has central effects in common with the amphetamines. Azacyclonol is a mild sedative[109], and under experimental conditions its most clearly demonstrated effect is to regulate and normalise the effect of other active compounds. It has been mentioned how it is capable of restoring the constant EEG arousal syndrome provoked by LSD and how it normalises the inhibition of the two neuron intercortical system provoked, for example, by mescaline. It inhibits the motor hyperactivity after amphetamine and pipradrol, but it has no influence on the constant EEG arousal syndrome provoked by

these compounds. In man it is claimed to antagonise the hallucinogenic effect of SD[107,109]. Finally, we may remind ourselves once again how the sedative effect of reserpine and the other indirectly acting tranquillisers may be modified by means of iproniazid to produce the opposite effect; this is now a highly stimulating effect, not unlike that of LSD or the amphetamines. The background of this mechanism of action has already been discussed.

Conclusion

The groups given in Table VIII are merely thought of as a gross characterisation which makes it possible to describe briefly the effect of a well-known or a newly discovered compound. The gradual transition does not allow water-tight compartments into which every compound may be fitted precisely. For this reason it is necessary to determine the "profile of action" covering a long series of effects, similar to those given in Table VIII, if a compound has to be comprehensively described.

In Table VIII, the drugs are roughly arranged from the most depressing to the most stimulating. However, this arrangement does not give a true picture of the mutual relation between the compounds. If attempts must be made to place chemically and pharmacologically related compounds in strict relation to each other, then a multidimensional system is necessary.

Much more important is the interrelation between the pharmacodynamic action and the clinical effect of the compounds. Here our knowledge rests on very rough empirical observations. Only the major tranquillisers are able to ameliorate the major psychoses, the others have an effect only in psychic disturbances of minor, presumably functional, origin, such as the neuroses. In neither the major psychoses nor the neuroses can we expect an effect in every instance. It is not always possible to predict from the symptoms of a psychiatric patient which compound, if any, will have the best prospect of helping. In the development of new drugs we do not even know which property is most important for the required clinical effect. For example, if we want to make a new compound for treatment of schizophrenia—must it be a potent anti-adrenaline, a potent depressor of the extrapyramidal system, a potent depressor of the meso-diencephalic autonomic centres or must it have quite other hitherto unnoticed properties?

Here, the only way to make progress is to correlate as many as possible of the relevant biochemical, physiological, pharmacological, pathophysiological and clinical facts and then hope that we or those who come after us may make something out of them.

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RESEARCH PAPERS

THE EFFECT OF VARIOUS CHOLINE ESTERS ON THE ADRENAL GLAND OF THE CAT

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A comparison has been made between the abilities of various choline esters to cause a release of catechol amines from the adrenal gland of the atropinised cat, anaesthetised with chloralose. It was found that repeated intravenous doses of acetylcholine, carbamylcholine or benzoylcholine, but not acetyl-β-methylcholine, caused the liberation of similar proportions of adrenaline and of noradrenaline. This was irrespective of the overall degree of depletion of the glands, which ranged from 7·1 per cent to 86·5 per cent. Acetylcholine and benzoylcholine were more effective than carbamylcholine in causing a release of amines.

It is well known that the administration of large doses of acetylcholine to an atropinised animal causes the release of catechol amines from the adrenal medulla. A number of workers[1–4] have shown that other choline esters also cause a release. However few have studied the relative proportions of adrenaline and noradrenaline liberated by such substances. Outschoorn[5] has found that acetylcholine causes the liberation, in the adrenal venous effluent, of similar percentages of adrenaline and noradrenaline. Other substances, notably insulin[6] and nicotine[7] cause the preferential release of adrenaline and noradrenaline respectively. Thus it seemed of interest to study the effects of different choline esters, and in this paper a comparison is made of the ability of acetylcholine, carbamylcholine, benzoylcholine and acetyl-β-methylcholine to deplete the adrenal glands of cats of their catechol amines. Also the modification of the acetylcholine response by physostigmine is studied.

Method

Healthy, adult cats of both sexes were used. The animal was given 6 mg./kg. of atropine sulphate, intraperitoneally and anaesthetised 10 minutes later with ether, following by 60 mg./kg. of chloralose, intravenously. Doses of choline ester, as the chloride, were given by femoral vein and the blood pressure recorded from the carotid artery, the usual dose range being 0·2–3·0 mg./kg. Having obtained one pressor response of suitable magnitude, one adrenal gland was removed as the control gland and an extract prepared (see later). Subsequent to the unilateral adrenalectomy, repeated doses of the choline ester were given, the dose being sufficiently large to obtain a definite pressor response. In order to obtain a range of depletion, the number of doses of choline ester was

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varied in each experiment. The minimum number of doses given in any one experiment was 10 and the maximum was 166. In those experiments in which physostigmine was given, subsequent to the removal of one gland, a constant submaximal response to acetylcholine was obtained and then 0·2 mg./kg. of physostigmine salicylate was given intravenously. The main depletion of the gland was then produced by giving further doses of acetylcholine. In certain experiments where carbamylcholine was used as the depleting agent, one definite pressor response to acetylcholine

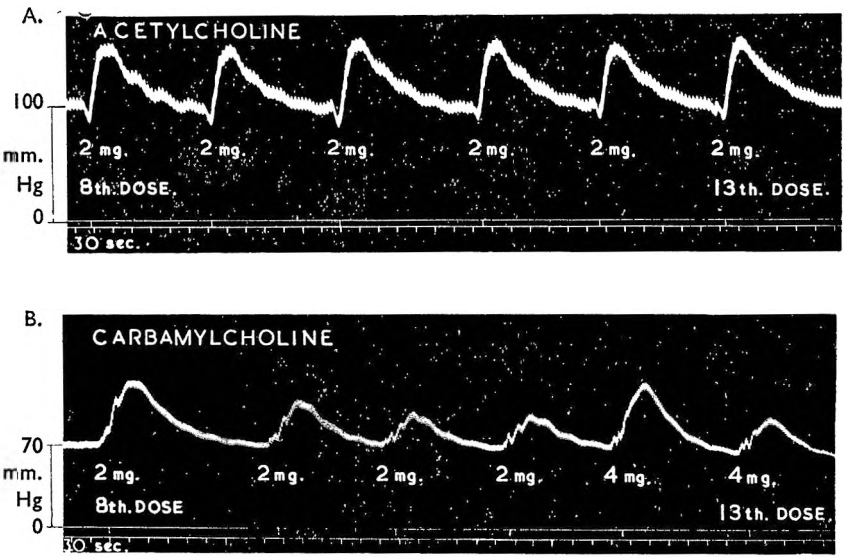

FIG. 1. A. Cat 2·1 kg. Atropine sulphate 6 mg./kg., followed by ether, and chloralose 60 mg./kg. The right adrenal gland was removed. Blood pressure tracing to show the similarity of successive responses to intravenous doses of acetylcholine. B. Cat 3·4 kg. Atropine and chloralose as above. The right adrenal gland was removed. Blood pressure tracing to show the rapid diminution in the response to consecutive intravenous doses of carbamylcholine.

and to electrical stimulation of the splanchnic nerve was obtained after the removal of one gland and before giving repeated doses of carbamylcholine. When the gland was no longer sensitive to carbamylcholine an attempt was made to obtain a rise in blood pressure by the injection of acetylcholine and by stimulation of the nerve. In all other experiments only one choline ester was employed throughout the experiment. After the depletion, the second gland was removed and an extract prepared as for the first gland.

Preparation of the extracts of the adrenal glands. Immediately after excision, the gland was dissected free from connective tissue and weighed on a micro-torsion balance. A 100 mg./ml. extract was prepared by grinding the gland in 0·1N hydrochloric acid with 300 mg. of acid-washed sand per 100 mg. of gland. After centrifugation at 5000 r.p.m. for 2 minutes the supernatant fluid was withdrawn and stored in an airtight container at 4° until assay.

Methods of assay. All the adrenal gland extracts were assayed chromatographically and some were also assayed biologically.

The chromatographic method was similar to that employed by Shepherd and West[8], using *n*-butanol—acetic acid—water as the solvent and potassium iodate to locate the catechol amines. The standard solution used was a mixture of 50 μg. (−)-adrenaline and 50 μg. (−)-noradrenaline, as base, per ml. The standard solution and each extract of

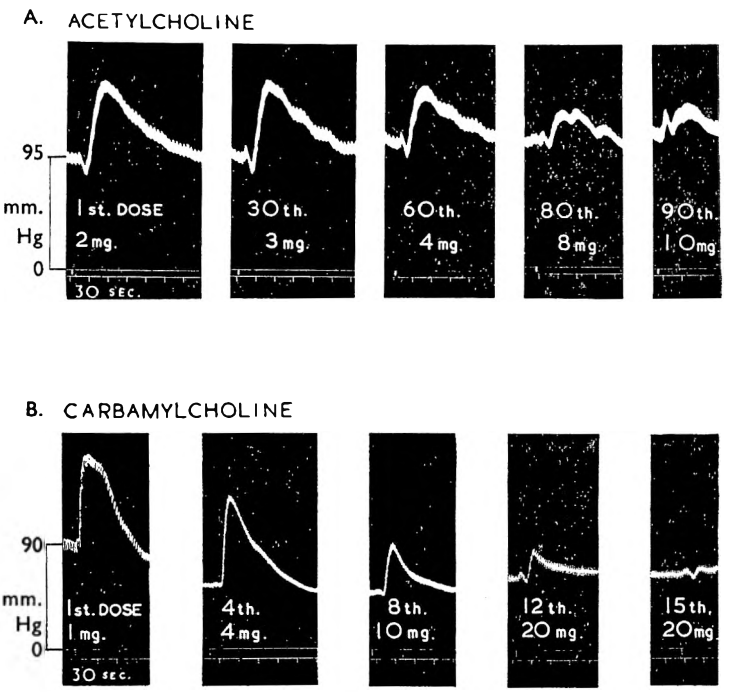

FIG. 2. A. Cat 3·6 kg. B. Cat 3·1 kg. Atropine, ether and chloralose as in Fig. 1. In both cats the left adrenal gland was removed. Fig. 2A shows the slow diminution in the response to acetylcholine. In comparison, Fig. 2B shows the rapid diminution in the response to carbamylcholine.

the glands were applied to the paper from Agla micrometer syringes. A range of spots was applied to each paper. The minimum volume used was 0·0025 ml. The maximum volume was 0·05 ml., applied in replicate drops of 0·01 ml., each spot being allowed to dry before the next spot was applied. When all the material had been applied, the papers were suspended in a glass tank and the solvent allowed to ascend for at least 15 hours. The papers were dried at 30°–40° for 10–15 minutes and then sprayed with 1 per cent (w/v) potassium iodate solution. The spots were located by placing the papers in an oven at 100°–110° for 2 minutes. Adrenaline and noradrenaline were rendered visible as pink and violet spots, having R_F values of 0·36 and 0·28 respectively. A direct comparison between the standard spots and those of the extract was made. At

least two separate assays of each gland were performed. The minimum amount of adrenaline and noradrenaline detectable varied slightly from experiment to experiment, ranging from 0·25 μg. to 1·0 μg.

The biological assays were made firstly, on the acutely denervated nictitating membrane of the cat and secondly, on the blood pressure of the same animal given hexamethonium bromide in a dose sufficient to

TABLE I

THE DEGREE OF DEPLETION OF CAT ADRENAL GLANDS, PRODUCED BY VARIOUS CHOLINE ESTERS

Choline ester	Number of experiments	Mean per cent loss		Range per cent loss	
		Adrenaline	Noradrenaline	Minimum	Maximum
Acetylcholine	15	50·4	50·1	7·1	86·5
Acetylcholine + physostigmine	12	60·1	62·8	35·7	83·5
Carbamylcholine	6	25·5	23·7	1·6	50·0
Benzoylcholine	5	46·7	47·2	26·1	67·6

lower the blood pressure to about 60 mm. Hg. The results were calculated by the formula of Bülbring[9] and were expressed, in terms of the laevo isomers of the base, as μg./gland, since Butterworth and Mann[10] have shown that this is the better method of calculation for cat adrenal glands.

RESULTS

Each dose of acetylcholine caused a marked pressor response. At first, repeated doses caused rises in blood pressure of a similar magnitude (Fig. 1A) but gradually, as the gland became depleted, the responses became smaller. If then a larger dose of acetylcholine was given the response returned to its initial magnitude (Fig. 2A). Eventually if enough doses of acetylcholine were given no further pressor response could be elicited. From the 15 experiments performed the mean depletion of adrenaline was 50·4 per cent and of noradrenaline was 50·1 per cent; there being no significant difference ($P > 0·9$) between the adrenaline depletion values and those of the noradrenaline. The range of depletion was wide; the minimum depletion obtained was 7·1 per cent and the maximum 86·5 per cent (Table I). The administration of physostigmine increased the sensitivity to acetylcholine 3 to 5 fold but again there was no significant difference ($0·7 > P > 0·6$) between the adrenaline and the noradrenaline depletion values (Table I).

Carbamylcholine, like acetylcholine, caused a pressor response. At the beginning of most experiments carbamylcholine was as active as acetylcholine in causing a release from the adrenal gland (Fig. 1B), but whereas repeated doses of acetylcholine gave rises in blood pressure of a similar magnitude, those to carbamylcholine rapidly diminished (Fig. 2B). Again there was no significant difference ($0·9 > P > 0·8$) between the adrenaline and the noradrenaline depletion values. However, due to the rapid decrease in the response to carbamylcholine, both the minimum and the maximum values were smaller than those for acetylcholine (Table I). Because of this rapid diminution in the carbamylcholine responses very

large doses (up to 1 g.) were eventually given, in some experiments, in an attempt to obtain a pressor response, but without success. This is in marked contrast to acetylcholine where there was never more than a 10-fold difference between the initial and the final doses. When carbamylcholine was no longer able to cause a pressor response, acetylcholine was

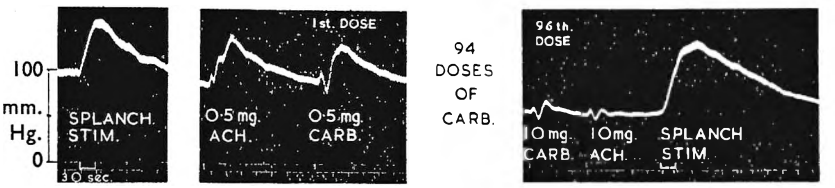

FIG. 3. Cat 3·4 kg. Atropine, ether and chloralose as in Fig. 1. The left adrenal gland was removed. A comparison of the vasopressor effects of acetylcholine (ACH.) and electrical stimulation of the greater splanchnic nerve (SPLANCH. STIM.) before and after the loss of response to carbamylcholine (CARB.).

ineffective also, but electrical stimulation of the splanchnic nerve caused as great a rise in blood pressure as it did initially (Fig. 3).

Benzoylcholine also caused a pressor response. Again there was no significant difference ($0{\cdot}6 > P > 0{\cdot}5$) between the adrenaline and the noradrenaline depletion values. The mean degree of depletion was similar to that for acetylcholine (Table I).

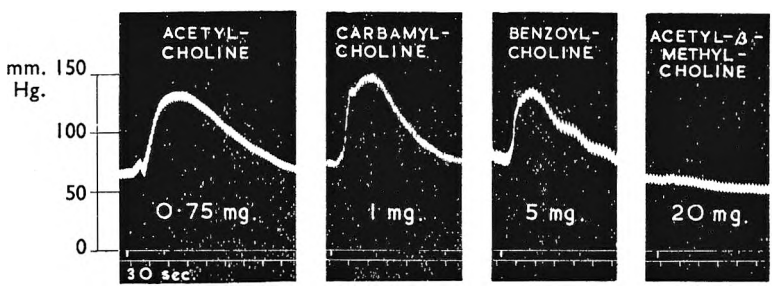

FIG. 4. Typical vasopressor responses to various choline esters given intravenously, at the beginning of each experiment, to atropinised, unilaterally adrenalectomised cats.

The fact that acetyl-β-methylcholine is incapable of producing a rise in blood pressure[11] was confirmed. Doses of up to 20 mg. had no effect. Figure 4 shows a comparison of the 4 choline esters studied.

DISCUSSION

In all experiments there was a similar depletion of adrenaline and noradrenaline. Thus, neither acetylcholine, carbamylcholine nor benzoylcholine caused a preferential loss of either amine. This similar percentage loss of amines was irrespective of the overall degree of depletion and of the actual amount (per cent) of noradrenaline present in the glands.

Although the chromatographic method of estimating the concentration of adrenaline and noradrenaline was suitable for this work, it proved to have certain disadvantages. The main disadvantage is that it is not possible to determine the accuracy of the result. Thus in some of the work biological assay methods were employed. An attempt to increase the clarity of the spots was made by previously soaking the chromatographic paper either in a 1 mg./ml. solution of ascorbic acid or in 0·01N hydrochloric acid. But little improvement was obtained. One advantage of the chromatographic method is that it is possible to detect the presence of other catechol amines; but in no experiment were any detected. Assays performed biologically gave results similar to those performed chromatographically.

There was no precise relation between the number of doses of choline ester and the degree of depletion obtained. Obviously there cannot be, since the amount of amine liberated by each dose of choline ester varied from cat to cat and also as the experiment proceeded. As might be expected, the rises in blood pressure produced by the choline esters became smaller as the doses were repeated. This was due to the liberation of less amine from the gland by each successive dose and not to a reduction in the sensitivity to adrenaline or noradrenaline. With acetylcholine and benzoylcholine this effect developed very gradually and the initial size of the response could be maintained if the dose was increased. With carbamylcholine the decrease in the response was much more rapid. This rapid reduction in the response made it impossible to deplete the glands to the same extent as with acetylcholine. This decrease in response to carbamylcholine was not due to deterioration of the drug in solution, to incomplete atropinisation of the cat or to tachyphylaxis but was due presumably to some "blocking" action of the drug. When the gland was no longer sensitive to carbamylcholine it was found to be insensitive to acetylcholine but splanchnic nerve stimulation caused as great a response as it did at the beginning of the experiment. Possible explanations of this difference could be either that the electrical stimulation is a more powerful stimulus or that the acetylcholine is liberated intracellularly on nerve stimulation as opposed to an extracellular effect when it is given intravenously. It is of interest that Eade[12] has found carbamylcholine to be ineffective as a releaser of catechol amines from chromaffin granules. He suggests that the action of choline esters on the chromaffin cell is an action on the cell membrane; possibly causing an increase in the permeability of the membrane. This would allow the amines present in the cytoplasm to leave the cell. He found no direct effect of the choline esters on the storage granules. It is hoped that further work will explain this difference between carbamylcholine and the other choline esters.

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FUNGAL GROWTH IN SYRUP OF TOLU

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A sample of Syrup of Tolu showing fungal growth had a distinct odour resembling toluene and yielded colonies of a species of *Penicillium* on subculture. The isolated organisms were grown on media containing sub-inhibitory concentrations of either benzoic or cinnamic acids as sole carbon source, and in the presence of cinnamic acid the toluene-like odour was apparent. Cultures of *P. nigricans* and of five recently isolated species or strains within species of *Penicillium* behaved similarly. Attempts to characterise the product with the toluene-like odour failed because of the presence of interfering substances. Samples of tolu syrups prepared with different sucrose contents, and adjusted to different pH values, were inoculated with the isolated fungus: preparations adjusted to pH 4·0–4·2 appeared to support growth irrespective of sucrose concentrations within limits of 50–67 per cent w/w, provided the inoculum was large.

Syrup of Tolu B.P. is a preparation in which spoilage due to microbial growth would appear to be unlikely. High sucrose concentrations are considered to be inimical to yeast and mould growth and the extracted benzoic acid which this preparation contains is a recognised fungistat and bacteriostat.

Experimental and Results

An amber 80 fl. oz. bottle of Syrup of Tolu of commercial origin was found to contain a submerged fungal growth and to have a pronounced odour which resembled toluene. The bottle closed with a bakelite cap fitted with a cork wad was opened after storage for several months at room temperature. There was no evidence of extensive production of carbon dioxide and the syrup was pH 3·96. Portions were plated on malt agar and Czapek-Dox agar and incubated at 24°. Colonies of only one type developed and these showed microscopical characters typical of the genus *Penicillium*.

It appeared that the substance of toluene-like odour in the infected syrup was produced by the fungus from one or both of the constituent aromatic acids: benzoic or cinnamic acids, or their esters. The possible utilisation of these acids raised the question of whether, as the sole nutritional source of carbon, they would support growth of the fungus. These points were investigated by the following fungistatic evaluation of the acids. A series of solutions containing graded concentrations of benzoic or cinnamic acids in Czapek-Dox medium at pH 5·0 were prepared. The media contained either no added sugar or 5 per cent of glucose or sucrose. The media were distributed in 10 ml. volumes in sterile, capped test tubes and were inoculated with spore suspensions, which were prepared by weighing small quantities of air-dry fungal spores,

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adding sufficient sterile water to provide 0·2 mg. spores per ml., and shaking vigorously to disperse the spores. An even distribution resulted if the suspensions were stored for two hours at room temperature, with intermittent shaking, before use. The tubes of media were inoculated with three drops of suspension delivered from the Cook and Yousef[1] pipette, so that the inoculum comprised about 10 μg. of spores. The

TABLE I

GROWTH OF *Penicillium* SPECIES IN MEDIA CONTAINING BENZOIC ACID IN VARYING CONCENTRATIONS AT pH 5·0

Concentration (per cent w/v)		2·0	1·0	0·5	0·25	0·10	0·05	0·005	Minimal fungistatic concn
Medium	Test organism								
Basal	T	—	—	+s	+++	+++	++	+	1·0
	N	—	+	+++	+++	+++	++	+	2·0
	A	—	—	—	—	+++	++	+	0·25
	B	—	—	+	+++	++	++	+	1·0
	C	—	+s	+	++	+++	++	+	2·0
	D	—	—	++	+++	+++	+++	+	1·0
	E	—	—	+	++	+++	++	+	1·0
With 5 per cent glucose	T	—	—	+s	++	+++	+++	+++	1·0
	N	—	+s	+++	+++	+++	+++	+++	2·0
	A	—	—	—	++	+++	+++	+++	0·5
	B	—	—	+	++	+++	+++	+++	1·0
	C	—	+s	++	+++	+++	+++	+++	2·0
	D	—	—	++	+++	+++	+++	+++	1·0
	E	—	—	+s	++	+++	+++	+++	1·0
With 5 per cent sucrose	T	—	—	+s	++	+++	+++	+++	1·0
	N	—	—	+	++	+++	+++	+++	1·0
	A	—	—	+	++	+++	+++	+++	1·0
	B	—	—	+s	++	+++	+++	+++	1·0
	C	—	—	++	+++	+++	+++	+++	1·0
	D	—	—	+	++	+++	+++	+++	1·0
	E	—	—	+	++	+++	+++	+++	1·0

The strains referred to in the table are T: isolated from the original infected syrup; N: *Penicillium nigricans*; A, B, C, D, E: freshly isolated *Penicillium* species.

— no growth visible;
+s very slight mycelial growth;
+ submerged mycelial growth;
++ heavier growth with prescence of spores, covering part of the surface of the medium;
+++ heavy sporing growth forming a complete layer at the surface of the medium and with production of pigment in the medium.

tubes were incubated at 24° for seven days, after which they were examined for evidence of growth and detectable toluene-like odour. Each experiment was in duplicate for both acids in three media and for seven different organisms: that isolated from the infected syrup, a verified strain of *P. nigricans*, and five recently isolated species, or strains within species, of *Penicillium*.

The results are shown in Tables I and II. Growth of all the test organisms was inhibited by either benzoic or cinnamic acids when present in sufficient concentration. In the absence of a sugar in the medium, growth was supported by sub-inhibitory concentrations of either of the acids, but the toluene-like odour was detected only when cinnamic acid was used. That the amount of growth increased as the concentrations of the acids were increased in sugar-free media was taken as evidence of the utilisation of the acids. In contrast, the amount of growth in media

containing sucrose or glucose was independent of aromatic acid concentration except when the inhibitory concentration was approached. The organism isolated from the infected syrup had a tolerance to benzoic acid approximately equal to the other organisms studied, but the tolerance to cinnamic acid was decidedly lower than that of the other species examined. It appears that the presence of sucrose or glucose, at the

TABLE II

GROWTH OF *Penicillium* SPECIES IN MEDIA CONTAINING CINNAMIC ACID IN VARYING CONCENTRATIONS AT pH 5·0

Concentration of cinnamic acid (per cent w/v)		1·0	0·6	0·4	0·3	0·2	0·1	0·05	0·03	0·005	Minimal fungistatic concn. (per cent)
Medium	Test organism										
Basal	T	—	—	—	—	O+	o+ +	o+ + +	+	+s	0·3
	N	—	O+s	O+	O+	O+ +	o+ + +	+ +	+	+s	1·0
	A	—	—	—	O+	o+ +	o+ + +	+ +	+	+s	0·4
	B	—	—	O+s	O+	O+ +	o+ +	+ +	+	+s	0·6
	C	—	—	O+	O+	O+	o+ +	+ +	+	+s	0·6
	D	—	—	O+s	O+	O+ +	o+ + +	+ +	+	+s	0·6
	E	—	O+s	O+	O+	O+	o+ + +	+ +	+	+	1·0
With 5 per cent glucose	T	—	—	—	O+	O+	o+ +	+ + +	+ + +	+ + +	0·4
	N	—	O+s	O+	O+	O+	o+ +	o+ + +	+ + +	+ + +	1·0
	A	—	O+s	O+	O+	O+	+ + +	+ + +	+ + +	+ + +	1·0
	B	—	O+s	O+	O+	O+	+ + +	+ + +	+ + +	+ + +	1·0
	C	—	O+	O+	O+	O+	o+ + +	+ + +	+ + +	+ + +	1·0
	D	—	O+	O+	O+	o+ +	o+ +	+ + +	+ + +	+ + +	1·0
	E	—	O+	O+	O+	o+ +	o+ + +	+ + +	+ + +	+ + +	1·0
With 5 per cent sucrose	T	—	—	—	O+	O+	o+ +	+ + +	+ + +	+ + +	0·4
	N	—	O+s	O+	O+	o+	o+ +	+ + +	+ + +	+ + +	1·0
	A	—	O+s	O+	O+	O+	+ + +	+ + +	+ + +	+ + +	1·0
	B	—	O+s	O+	O+	O+	o+ + +	+ + +	+ + +	+ + +	1·0
	C	—	—	O+s	O+s	O+	o+ +	+ + +	+ + +	+ + +	0·6
	D	—	O+s	O+	O+	O+	o+ +	+ + +	+ + +	+ + +	1·0
	E	—	—	O+	O+	o+ +	o+ +	+ + +	+ + +	+ + +	0·6

The strains used are denoted by symbols, a key to which appears below Table I.

O strong smell of toluene-like substance;
o just detectable toluene-like odour;
— no visible growth;
+s very slight mycelial growth;
+ submerged mycelial growth;
+ + heavier growth with presence of spores, the growth covering part of the surface of the medium;
+ + + heavy sporing growth forming a complete layer at the surface of the medium and with the production of pigment in the medium.

concentrations which were employed, raises the tolerance of *Penicillium* species to cinnamic acid, but has a doubtful influence on resistance to benzoic acid.

An attempt was made to isolate and identify the substance of toluene-like odour. One litre volumes of Czapek-Dox basal medium containing 0·2 per cent w/v cinnamic acid at pH 5·0 were inoculated with spores obtained from subculture of the original infected syrup, and the media were kept aerated and agitated by a stream of filtered, compressed air which was saturated with water vapour at the incubation temperature of 24°. The air-stream passing from the media was passed through a cold trap surrounded with ice. After incubation for five days, the condensate

in the cold trap was found to possess a strong odour resembling toluene and it appeared to be an aqueous solution of the metabolic product. Examined spectrophotometrically, the solution was found to show a maximal optical density at 242 mμ, compared with which a saturated aqueous solution of toluene showed peaks of absorption at 262 and 268 mμ. When equal portions of the condensate were treated with an equal volume of either 0·05N HCl or 0·05N NaOH, a difference in optical density was found between the acid and alkaline solutions, the maximal difference occurring at 257 mμ. It was considered that the true λ_{max} of the toluene-like substance was obscured by other metabolic products whose ultra-violet absorption was affected by change in pH.

Possible conditions for growth of *Penicillium* species in Syrup of Tolu were investigated as follows. Samples of the syrup were prepared to contain varying sucrose concentrations: 50, 55, 60, 62, 63, 64, 65, and 67 per cent w/w, in addition to a sample containing the Pharmacopoeial concentration of 66 per cent w/w. The pH of the preparations lay within the range of 2·8–2·9, compared with the value of 3·96 for the sample of infected syrup. A second series of samples were prepared in which the above sucrose concentrations were maintained, but the pH adjusted to 4·0–4·2 by the addition of sodium hydroxide. A 10 ml. portion of each of the samples prepared was placed in each of two sterile, stoppered test tubes, thus giving two series of tubes containing all of the samples which were prepared. One series was infected with one drop of the original infected syrup, and the other series was inoculated with approximately 1 mg. of air-dry spores obtained by subculture of the infected syrup. After incubation for three months at 24°, it was found that none of the samples infected with the smaller inoculum showed evidence of growth. Of the samples adjusted to pH 4·0–4·2 and subjected to a heavy inoculum of spores, a number showed well-defined growth and had developed the toluene-like odour. Growth occurred in this series of samples at sucrose concentrations of 55, 60, 62, 64, 65 and 67 per cent w/w, so that there appeared to be no relation between sucrose content, over the concentration range examined, and suitability for growth at the pH studied. None of the heavily inoculated syrups of pH 2·8–2·9 showed growth.

Discussion and Conclusions

It seems that benzoic or cinnamic acids can be used by several species of *Penicillium* as a sole nutritional source of carbon, provided the concentration of the acid lies below inhibitory levels. Similar behaviour was found with seven species, or strains within species, or *Penicillium*, these being morphologically or culturally distinct, and it is therefore possible that the capacity to utilise these acids is widespread in this genus. Growth in near-inhibitory concentrations of cinnamic acid was accompanied by the production of a substance of toluene-like odour. Toluene would appear to be a reasonable product of breakdown of cinnamic acid by a rupture at the unsaturated linkage, leaving a 2C compound, for example acetate, which could be metabolised.

From the attempts to establish the conditions required for growth of species of *Penicillium* in Syrup of Tolu, it is suggested that:

(i) variation in sucrose concentration within the limits of 55–67 per cent w/w had no effect in permitting growth;

(ii) growth would occur in samples adjusted to a less acid reaction (pH 4·0–4·2); and

(iii) growth in less acid samples took place only when the inoculum was large (1 mg. spores), but not when the small inoculum (0·016 ml. of original infected syrup) was used.

Dependence of microbial growth on the pH of solutions of aromatic acids is in accordance with the findings of Hoffman, Schweitzer and Dalby[2,3], Rahn and Conn[4], and Goshorn and Degering[5] that inhibition of fungi, yeasts and bacteria respectively by benzoic acid and certain of its derivatives is largely dependent upon the concentration of undissociated acid, the benzoate ion having a much lower inhibitory activity. It will be observed that growth did not take place in all samples adjusted to a less acid reaction and heavily inoculated with spores, from which it appears that factors other than those which have been considered here may operate to decide initiation of fungal growth.

It is concluded that fungal growth can occur in Syrup of Tolu when the concentration of aromatic acids is deficient, when the reaction of the product is less acid than that expected, and when the product is exposed to very large fungal inocula.

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ANTAGONISM TO THE ACTIONS OF HYDRALLAZINE, RESERPINE, POTASSIUM CYANIDE, SODIUM AZIDE AND ANOXIA ON ARTERIAL SMOOTH MUSCLE

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Many intermediates of carbohydrate, fat and protein metabolism give protection against the hydrallazine depression of acetylcholine, (−)-adrenaline, (−)-noradrenaline, histamine and 5-hydroxytryptamine-induced contractions of spirally cut strips of horse carotid arteries. Strips made anoxic behave in a manner similar to hydrallazine-treated strips. Inhibition of drug-induced contractions by potassium cyanide, iodoacetate and azide was of a different character from that caused by hydrallazine and anoxia. Few intermediates gave significant protection. Hydrallazine probably exerts its effect by a non-specific depression of metabolism rather than upon specific receptors. Reserpine depression of drug-induced contractions in artery strips was so persistent that experiments using intermediary metabolites could not be made.

Hydrallazine and reserpine antagonise contractions induced by acetylcholine, (−)-adrenaline, (−)-noradrenaline, histamine, 5-hydroxytryptamine and potassium chloride on spirally cut strips of horse, cat and rabbit arteries[1,2]. Antagonism to barium chloride has been observed only on strips of horse carotid arteries. These observations demonstrated that both reserpine and hydrallazine lacked specificity of action[2–6]. Reserpine appears to have a strong affinity for arterial smooth muscle and it is very difficult to reverse its effects. These observations and those of Gillis and Lewis[3–6] have indicated that we may be dealing with an effect upon cellular mechanisms connected with the production and utilisation of energy necessary for drug induced contractions rather than with effects upon specific receptors[2]. We have attempted to antagonise the depressant effects of hydrallazine and reserpine upon drug induced contractions and to imitate their effects by using potassium cyanide, sodium azide and sodium iodoacetate, or by rendering the tissue anoxic.

Materials and Methods

The bath fluid was oxygenated Tyrode's solution at 36° of the following composition in g./l., NaCl 8·0, KCl 0·198, $CaCl_2$ 0·2, NaH_2PO_4 0·05, $NaHCO_3$ 1·0, glucose 1·0.

Drugs used were acetylcholine chloride (ACh), (−)-adrenaline hydrochloride (Ad), (−)-noradrenaline bitartrate (NA), 5-hydroxytryptamine creatinine sulphate (5-HT), histamine acid phosphate (Hm), potassium cyanide (KCN), sodium azide, sodium iodoacetate, sodium monofluoroacetate, glutathione, *p*-chloromercuribenzoate, 1-hydrazinophthalazine hydrochloride (hydrallazine), reserpine (as a buffered solution in

ascorbic acid-sodium ascorbate) and sodium thiocyanate. Substances used to antagonise hydrallazine and reserpine inhibition of drug-induced contractions are detailed in Tables I to III. Except for maleic acid (pH 1·8) and (±)-leucine (pH 1·0) they were added to the bath as neutral

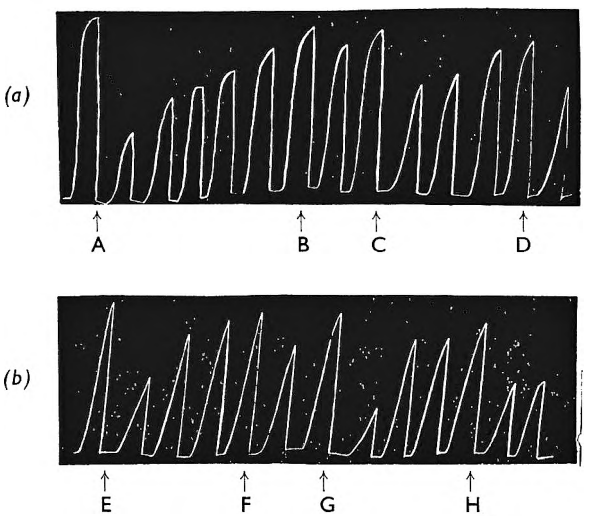

FIG. 1. The effects of pyruvate, succinate, *cis*-aconitate, fumarate, citrate and oxaloacetate on hydrallazine inhibition of ACh-induced contractions of strips of horse carotid artery. (*a*) All contractions due to 0·13 μg. ACh. At A, 0·13 mg. hydrallazine. At B, 1 mg. pyruvate and 0·13 mg. hydrallazine. At C, 1 mg. succinate and 0·13 mg. hydrallazine. At D, 1 mg. fumarate and 0·13 mg. hydrallazine. (*b*) All contractions due to 0·02 μg. ACh. At E, 0·066 mg. hydrallazine. At F, 1 mg. oxaloacetate and 0·066 mg. hydrallazine. At G, 1 mg. *cis*-aconitate and 0·066 mg. hydrallazine. At H, 1 mg. citrate and 0·066 mg. hydrallazine.

solutions of the pure substance to avoid pH effects. All drug concentrations refer to the final bath concentrations per ml. With compounds shown in Tables I to III this was one mg.

Spirally cut strips of common carotid artery, from horses just killed were set up in 10 to 75 ml. organ baths. The experimental procedure has already been described[2]. In testing for antagonism to the actions of reserpine and hydrallazine, the antagonists (Table I) were added to the bath 10 minutes before the addition of reserpine or hydrallazine and remained in contact with the tissue for 30 minutes.

Changes of pH were minimised by using neutral solutions as far as possible. Since the contents of the bath were a complex mixture of salts and drugs and the possibility of chemical inactivation was present, some experiments were made in which hydrallazine did not come into contact with the added compound. The chemical was allowed to remain in the bath for 10 minutes and was then washed out. Hydrallazine was

now added and the experiment continued. Release of Hm from the tissue might also result in an apparent antagonism to hydrallazine. We therefore repeated some experiments using Tyrode's solution containing 100 μg./l. of mepyramine maleate.

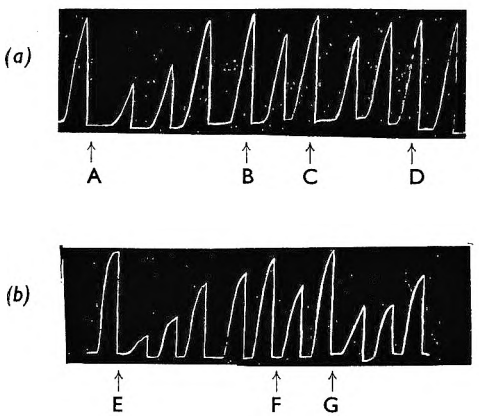

FIG. 2. The effects of 3-phosphoglycerate, (±)-alanine, butyrate, pyruvate and succinate on hydrallazine inhibition of Ad and NA induced contractions of strips of horse carotid artery. (*a*) All contractions due to 0·2 μg. Ad. At A, 0·26 mg. hydrallazine. At B, 1 mg. 3-phosphoglycerate and 0·26 mg. hydrallazine. At C, 1 mg. (±)-alanine and 0·26 mg. hydrallazine. At D, 1 mg. butyrate and 0·26 mg. hydrallazine. (*b*) All contractions due to 0·13 μg. NA. At E, 26 μg. hydrallazine. At F, 1 mg. pyruvate and 26 μg. hydrallazine. At G, 1 mg. succinate and 26 μg. hydrallazine.

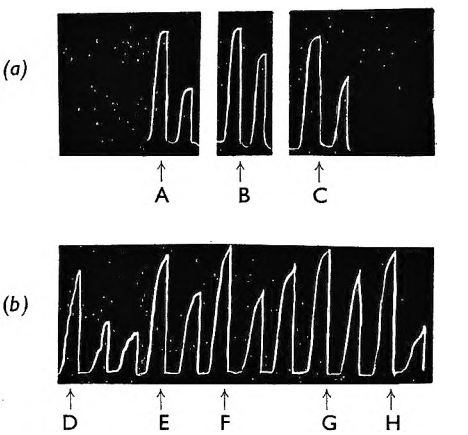

FIG. 3. The effects of pyruvate, succinate, (±)-alanine, glutamate, α-ketoglutarate and fumarate upon KCN inhibition of ACh-induced contractions of strips of horse carotid artery. (*a*) All contractions due to 0·2 μg. ACh. At A, 0·1 mg. KCN. At B, 1 mg. pyruvate and 0·1 mg. KCN. At C, 1 mg. succinate and 0·1 mg. KCN. (*b*) All contractions due to 0·1 μg. ACh. At D, 0·15 KCN. At E, 1 mg. (±)-alanine and 0·15 mg. KCN. At F, 1 mg. glutamate and 0·15 mg. KCN. At G, 1 mg. α-ketoglutarate and 0·15 mg. KCN. At H, 1 mg. fumarate and 0·15 mg. KCN.

Potassium cyanide, sodium azide, sodium iodoacetate, *p*-chloromercuribenzoate, sodium monofluoroacetate, sodium thiocyanate were added to the bath in place of reserpine or hydrallazine using the same time cycle[2].

To render the tissue anoxic, the bicarbonate-free Tyrode's solution was first of all boiled to drive off dissolved gases. It was cooled under a mixture of 95 per cent N_2/5 per cent CO_2, and the bicarbonate was added to the cooled solution. The final pH of the Tyrode's solution was 7·6 to 7·8. During the experiment the same gas mixture replaced oxygen in the bath fluid. The tissue was kept under these conditions for 30 minutes before any drugs were added.

RESULTS

The results of tests made on the activity of certain intermediates of carbohydrate, fat and protein metabolism on hydrallazine and reserpine antagonism to contractions induced by ACh (0·1 ng. to 2·0 μg.), Ad

(10·0 ng. to 5·0 μg.), NA (10·0 ng. to 5·0 μg.) 5-HT (40·0 ng. to 3·0 μg.), Hm (0·1 to 5·0 μg.) are shown in Tables I and II. The ability of the added compound to antagonise hydrallazine depression of contractile responses is expressed as the average inhibition per cent of the contractions induced by the stimulant drug. This approximate figure is calculated as follows:

TABLE I

ACTIVITY OF COMPOUNDS TESTED FOR ANTAGONISM TO THE ACTIONS OF HYDRALLAZINE AND RESERPINE ON HORSE CAROTID ARTERY STRIPS

Stimulant drug	Hydrallazine				Reserpine	
	ACh		Ad		ACh	
		Protection per cent		Protection per cent		Protection per cent
Intermediates of—						
(*a*) *Carbohydrate metabolism:*						
Glucose-1-phosphate	0	0	+	+28	*	
Fructose-1:6-diphosphate	0	0	0	−15		
Glucose-6-phosphate	0	0	++	+75		
Fructose-6-phosphate	+	+24	++	+43		
3-Phosphoglycerate	+	+29	++	+54		
Pyruvate	++	+78	++	+72	+	+12
Succinate	++	+58	++	+42	+	+35
Fumarate	++	+60	0	+10	*	
Citrate	0	+5	0	0		
*cis*Aconitate	0	−40	++	+50		
*iso*Citrate	0	0	+	+18		
α-Ketoglutarate	++	+51	++	+55		
Maleic acid	0	−50	0	−100		
Oxaloacetate	+	+26	++	+60		
Oxalosuccinate	+	+27	+	+19		
(*b*) *Fat metabolism:*						
3-Hydroxybutyrate	+	+17	++	+57	*	
Propionate	++	+37	++	+58		
(*c*) *Protein metabolism:*						
Glutamate	++	+35	++	+58	*	
(±)-Alanine	++	+38	++	+53		
(±)-Leucine	0	0	0	0		
(*d*) *Sulphydryl compound:*						
Glutathione	0	0	0	0	*	
(*e*) *Other compound:*						
Malate	+	+23	0	0	*	

0 = No activity or antagonism. + = Some activity. ++ = Marked activity.
* Experiments could not be done.

The height of the control contraction (A) is measured and also that of the contraction after addition of the antagonist (B). When there is complete recovery to the control level, or to a reproducible level, which may be slightly greater or less than the original control, the height of the contraction after the addition of antagonist with intermediary metabolite is measured (C). The protection per cent was then calculated,

$$\frac{C - B}{A - B} \times 100 = \text{protection per cent.}$$

Values from all experiments using the same combination of spasmogen, antagonist and the intermediary metabolite have been averaged to obtain these figures. Typical experiments are shown in Figures 1 and 2.

There is some parallelism between results obtained with ACh and Ad (Table I). The most active compounds when selected for use with NA,

5-HT and Hm showed a similar parallelism (Table II) (Fig. 2). Very few experiments could be done with reserpine since it was unusual to get a satisfactory recovery.

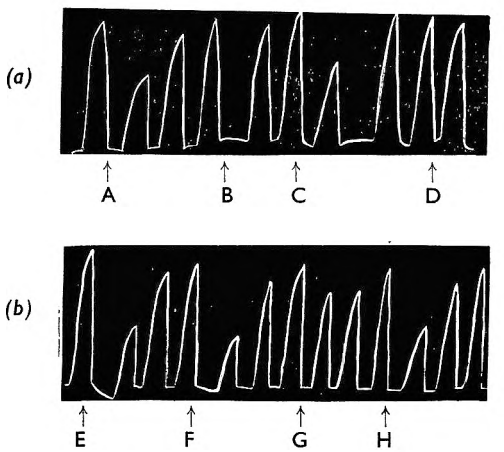

FIG. 4. The effects of succinate, fumarate, pyruvate, butyrate, oxalosuccinate and citrate upon anoxic inhibition of ACh-induced contractions of strips of horse carotid artery. (*a*) All contractions due to 0·04 μg. ACh. At A, anoxia. At B, 1 mg. succinate and anoxia. At C, 1 mg. fumarate and anoxia. At D, 1 mg. pyruvate and anoxia. (*b*) All contractions due to 0·2 μg. ACh. At E, anoxia. At F, 1 mg. butyrate and anoxia. At G, 1 mg. oxalosuccinate and anoxia. At H, 1 mg. citrate and anoxia.

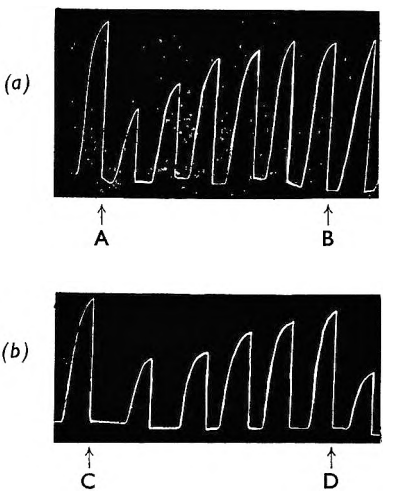

FIG. 5. The inconsistent effects of pyruvate in antagonising azide inhibition of ACh-induced contractions of strips of horse carotid artery. (*a*) All contractions due to 0·1 μg. ACh. At A, 0·4 mg. sodium azide. At B, 1 mg. pyruvate and 0·4 mg. sodium azide. (*b*) All contractions due to 0·4 μg. ACh. At C, 0·8 mg. sodium azide. At D, 1 mg. pyruvate and 0·8 mg. sodium azide.

KCN (0·04 mg. to 0·2 mg.) inhibited ACh-induced contractions of the artery strips. Recovery was generally prolonged or did not take place at all. Table III shows some of the results obtained. There is no close parallelism between the results shown in Table III and those shown in Tables I and II. Some typical experiments are shown in Figure 3.

Anoxia caused loss of smooth muscle tone and diminution of the contractile response to ACh. Recovery of the tissue after supplying oxygen was quite rapid. Table III shows some of the results obtained. A typical experiment is shown in Figure 4.

The results obtained using sodium azide (0·1 to 1·0 mg.) to inhibit ACh-induced contractions of artery strips were too inconsistent for us to be able to draw conclusions. Sodium azide had a prolonged effect and the recovery of response was often very slow (Fig. 5). The effects appeared to be similar to those of KCN.

DISCUSSION

It seems unlikely that hydrallazine and reserpine are acting on specific receptors in arterial smooth muscle. The experiments described were,

therefore, carried out to see whether the effects of hydrallazine and reserpine could be antagonised by supplying a series of known intermediates of carbohydrate, fat and protein metabolism and also to see whether the effects of hydrallazine or reserpine resembled those of anoxia or of known enzyme inhibitors such as azide, cyanide and iodoacetate.

TABLE II

ACTIVITY OF SOME INTERMEDIATES OF CARBOHYDRATE AND PROTEIN METABOLISM IN ANTAGONISING THE ACTIONS OF HYDRALLAZINE ON HORSE CAROTID ARTERY STRIPS

Stimulant drug	Hydrallazine		
	NA	Hm	5-HT
	Protection	Protection	Protection
Intermediates of—			
(*a*) *Carbohydrate metabolism:*			
Pyruvate	++	++	++
Succinate	++	++	++
α-Ketoglutarate	++	++	+
3-Phosphoglycerate	+	+	+
Fructose-6-phosphate	++	++	++
(*b*) *Protein metabolism:*			
Glutamate	+	+	+
(±)-Alanine	0	0	0

0 = No activity or antagonism. + = Some activity. ++ = Marked activity.

The following possibilities come to mind: the compounds may combine chemically with hydrallazine and so partly inactivate it; they may cause liberation of Hm or ACh and hence increase the magnitude of contraction; they may remove calcium ions and thus render the tissue more irritable; or they may act as sources of energy for the tissue. None of the compounds tested caused a direct contraction of the artery strip when added alone to the bath, nor did they potentiate the contractile responses to ACh and Ad. In addition, experiments made in Tyrode's solution containing mepyramine appeared to exclude the possibility of Hm-release. It has already been shown that removal of calcium ions by these compounds is not significant[6]. Citrate is inactive yet this compound is known to remove ionic calcium.

The results shown in Table I indicate that most of the compounds can antagonise hydrallazine depression of ACh and Ad-induced contractions. Why some of them should act and others not, is not clear. It is possible that the inactive compounds do not penetrate the cells or that they are not needed. Most of the active compounds are intermediates of the tricarboxylic acid cycle (although this has not been shown to exist in arterial smooth muscle). 3-Phosphoglycerate, pyruvate, succinate, fumarate, α-ketoglutarate and oxaloacetate are all potent antagonists of hydrallazine-induced inhibition. Citrate, *iso*citrate, *cis*aconitate and maleic acid are inactive or of lower potency. In general, intermediates of glycolysis are less potent, yet when Ad is used both glucose-6-phosphate and fructose-6-phosphate antagonise the actions of hydrallazine. Propionate, glutamate, (±)-alanine, and to some extent 3-hydroxybutyrate also antagonise Ad, whereas (±)-leucine and malate are inactive. The

ability of an amino acid like (±)-alanine to protect against hydrallazine inhibition may be explained by assuming that the arterial smooth muscle can deaminate alanine to its corresponding keto acid, which is pyruvic acid. In the same way the behaviour of oxaloacetate suggests that it may have exerted its effect after decarboxylation to pyruvic acid. It is,

TABLE III

ACTIVITY OF COMPOUNDS TESTED FOR ANTAGONISM TO THE ACTIONS OF KCN AND ANOXIA ON HORSE CAROTID ARTERY STRIPS

Stimulant drug	KCN		Anoxia	
	ACh		ACh	
		Protection per cent		Protection per cent
Intermediates of—				
(*a*) *Carbohydrate metabolism:*				
Glucose-1-phosphate	0	+10	0	0
Fructose-1 : 6-disphosphate	0	0	0	0
Glucose-6-phosphate	0	0	++	+40
Fructose-6-phosphate	+	+30	++	+45
3-Phosphoglycerate	0	0	+	+25
Pyruvate	++	+68	++	+74
Succinate	+	+16	++	+68
Fumarate	0	0	++	+54
Citrate	0	−70	0	−50
*cis*Aconitate	0	−50	0	−80
*iso*Citrate	0	−50	0	0
α-Ketoglutarate	++	+70	+	+25
Oxaloacetate	0	−55	0	0
Oxalosuccinate	+	+22	++	+44
(*a*) *Fat metabolism:*				
3-Hydroxybutyrate	0	0	0	0
Propionate	+	+20	0	0
(*c*) *Protein metabolism:*				
Glutamate	++	+50	+	+11
(±)-Alanine	++	+85	++	+76
(±)-Leucine	0	−36	0	+6
(*d*) *Other compound:*				
Malate	0	−50	0	+10

0 = No activity or antagonism. + = Some activity. ++ = Marked activity.

therefore, possible to speculate that hydrallazine is interfering with cellular metabolism—possibly in the tricarboxylic acid cycle and that the more potent compounds are supplying energy requirements or replacing a missing metabolite. Hydrallazine is reported to be an histaminase inhibitor[7,8] but it may also inhibit other enzymes.

The results obtained on anoxic tissues using ACh as the stimulant showed some similarity to experiments in which the antagonist was hydrallazine. Anoxic tissues lost their tone and were less sensitive to ACh but sensitivity was never completely lost. Moreover the recoveries of the anoxic and the hydrallazine-treated tissues were strikingly similar. The type of recovery was quite different from that seen after KCN, sodium azide, sodium iodoacetate or reserpine. In these instances the recovery was very slow or absent. When the results obtained with hydrallazine and those obtained with anoxic tissues are compared a close, but not complete resemblance is seen. Thus hydrallazine may alter or interfere with cell metabolism in a somewhat similar manner to oxygen lack.

KCN and sodium azide yielded quite a different picture. Sodium azide caused a pronounced and prolonged inhibition of ACh-induced

contractions but the compounds added had irregular effects and reproducible results could not be obtained. KCN also caused prolonged inhibition of ACh-induced contractions. The effects were antagonised by fructose-6-phosphate, pyruvate, α-ketoglutarate, glutamate, (±)-alanine and less strongly by succinate and propionate. A number of compounds known to combine with sulphydryl groups were tested to see whether their effects were similar to those of hydrallazine. None of these caused inhibition of ACh or Ad-induced contractions. Glutathione added before hydrallazine did not show a protecting effect.

Reserpine caused what was virtually an irreversible effect showing a remarkable affinity for the tissue. This may partly explain the prolonged hypotensive effects of this drug in man.

A few experiments were made with sodium thiocyanate instead of hydrallazine but this compound did not antagonise ACh-induced contractions of artery strips.

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CHEMISTRY AND PHARMACOLOGY OF ESTERS OF METHYLPENTYNOL AND RELATED COMPOUNDS

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The narcotic action of a range of methylpentynol esters has been investigated. In general, esterification reduces or abolishes narcotic activity, but the carbamate possesses increased and more prolonged activity and higher oral toxicity than the parent carbinol, though their therapeutic indices are similar. Contrary to previous claims, the *N*-methyl carbamate is inactive by our method of test. Carbamates of methylpentynol homologues and near relatives also possess increased narcotic properties compared with the parent carbinols. The anticonvulsant properties of methylpentynyl carbamate against leptazol in mice are superior to those of aloxidone. Chronic toxicity tests in mice over 4 to 5 months have shown no untoward effects, and the average weight gain follows a normal pattern: there is no effect on blood pressure or respiration in therapeutic doses. Methylpentynyl carbamate shows no analgesic activity on intravenous administration in mice compared with that of salicylamide. Bemegride acts as an antagonist. A new method[2] for the preparation of carbamates of tertiary alcohols is described.

In a search for an active solid ester of methylpentynol suitable for tabletting we found the acetate to be a liquid with reduced activity, and the benzoate, a liquid and inactive, as was the *N*-methyl carbamate[1]. Of the solid esters, the hydrogen phthalate and *N*-phenyl carbamate were inactive, the allophanate possessed slight activity, only the carbamate possessed high activity. Methylpentynyl carbamate was therefore investigated in detail, and the narcotic activity of the carbamates of certain homologues and related saturated carbinols was also determined. The difficulties in preparing carbamates of tertiary alcohols have been described by McLamore, P'an, and Bavley[3], who obtained methylpentynyl carbamate in 21 per cent yield by splitting the phenylcarbonate with ammonia.

The results of our attempts to prepare the intermediate chloroformates of methylpentynol and ethinyl*cyclo*hexanol, before reaction with ammonia, emphasised the instability of these esters and neither could be isolated. Thus, from the reaction of ethinyl*cyclo*hexanol with phosgene in pyridine at 0° 1-ethinyl*cyclo*hexene alone was obtained. Reaction of methylpentynol with phosgene, without a base, in a sealed tube at 150° yielded carbon dioxide and an unstable oil, b.p. 54° at 13 mm., which had the molecular formula $C_6H_{10}Cl_2$ and would result from the addition of two molecules of hydrogen chloride to the dehydration product of methylpentynol. The ready decomposition of tertiary butyl chloroformate to give *iso*butylene, carbon dioxide and hydrogen chloride has been described by Choppin and Rogers[4].

The preparation of methylpentynyl carbamate by the reaction of carbamyl chloride with methylpentynol in ether has been described in the patent literature[5], but a yield of only 16·5 per cent is claimed; we have confirmed this low yield. In the same specification a general method is described for the preparation of carbamates of tertiary acetylenic alcohols by the action of ammonia on the chloroformates, which were obtained (but not isolated) by the reaction of phosgene with the carbinol in ether or toluene and in the presence of a tertiary base, preferably trimethylamine. Using this method, with trimethylamine as base, we have obtained a yield of 50 per cent of methylpentynyl carbamate. Heterocyclic bases are less satisfactory, with both methylpentynol and ethinyl*cyclo*hexanol.

Attempted ester interchange between methylpentynol and ethyl carbamate, with both acidic and basic catalysts, was unsuccessful. Reaction of methylpentynol with cyanate in the presence of hydrogen chloride under a variety of conditions failed to yield the carbamate. Reaction of methylpentynol with sodium cyanate and trichloroacetic acid in an excess of methylpentynol as solvent gave the carbamate in about 65 per cent yield based on the sodium cyanate. This could be carried out at 20° for several days or at 50° for 24 hours. Carbon tetrachloride could be used as solvent instead of excess methylpentynol with only a small decrease in yield. Methylene dichloride, however, could only be used at 20° as at higher temperatures trichloroacetic acid and cyanate react in this solvent with the formation of carbon dioxide and trichloroacetamide. This reaction takes place in dioxan even at room temperature and gives a much lower yield of carbamate. A similar reaction takes place using acetic or monochloroacetic acids instead of trichloroacetic acid, and with these acids no carbamate is formed.

Preparation of Chemical Compounds

Methylpentynyl acetate was obtained by treating methylpentynol with acetic anhydride and acetic acid in the presence of boron trifluoride-acetic acid complex, as a liquid, b.p. 146 to 147° at 763 mm. (*cf.* Heilmann, Glénat, and Gaudemaris[6], 149° at 745 mm., and Keil, Muschawek and Rademacher[7], 148 to 150° at 760 mm.).

Methylpentynyl benzoate. Benzoyl chloride (35 g.) was added gradually to methylpentynol (19·6 g.) in pyridine (50 ml.) with cooling and stirring. The mixture was refluxed for 1 hour, then poured into water and extracted with ether. After drying, the ether was removed and the residue was distilled. Yield, 29·6 g. (73 per cent), b.p. 65 to 67° at 0·05 mm. Found: C, 77·2; H, 7·2. Calc. for $C_{13}H_{14}O_2$, C, 77·2; H, 7·0 per cent. Keil[7] gives b.p. 127·5 to 132·5° at 11 mm.

Methylpentynyl allophanate. Cyanuric acid (10 g.) was depolymerised by heating at *ca.* 400° in a slow stream of CO_2 in an electrically heated tube (*cf.* Blohm and Becker)[8], and the cyanic acid vapour passed into a solution of methylpentynol (9·8 g.) in anhydrous ether (20 ml.), protected from atmospheric moisture and cooled in ice. A loose cotton wool plug in the delivery-tube served to minimise sublimation of cyanuric

acid into the solution. The depolymerisation required approximately 6 hours, after which time the ether solution had becomed cloudy. The next day the separated crystals were recrystallised from ethanol (charcoal) giving needles, m.p. 135 to 137° decomp. (evolution of gas). Found: C, 52·5; H, 6·6; N, 15·0. Calc. for $C_8H_{12}O_3N_2$, C, 52·2; H, 6·6;N,l 5·2 per cent. Keil[7] gives m.p. 148° but no preparative details.

TABLE I

OTHER CARBAMATES OF ACETYLENIC CARBINOLS

Carbamates of	Yield per cent	m.p.	Analysis
3-Methylbut-1-yn-3-ol	49	106–107·5°	Found: C, 57·2 H, 7·2 N, 10·7
			$C_6H_9O_2N$ requires: C, 56·7 H, 7·1 N, 11·0
3-Methylhept-1-yn-3-ol	49	44–45° b.p. 93–94°/0·2 mm.	Found: C, 63·9 H, 8·9 N, 8·55
			$C_8H_{16}O_2N$ requires: C, 64·0 H, 8·9 N, 8·3
3-Methyloct-1-yn-3-ol	42	42·5–43·5°	Found: C, 65·9 H, 9·1 N, 7·95
			$C_{10}H_{17}O_2N$ requires: C, 65·6 H, 9·3 N, 7·65
3:5-Dimethylhex-1-yn-3-ol	37	28·5–29·5° b.p. 78–79°/0·3 mm.	Found: C, 63·7 H, 8·8 N, 8·55
			$C_8H_{15}O_2N$ requires: C, 64·0 H, 8·9 N, 8·3
3-Ethylpent-1-yn-3-ol	38	38·5–40°	Found: C, 62·3 H, 8·5 N, 9·5
			$C_8H_{13}O_2N$ requires: C, 62·0 H, 8·4 N, 9·05
3-Methylhex-1-yn-3-ol	43	53·5–54°	Found: C, 62·2 H, 8·5 N, 9·5
			$C_8H_{13}O_2N$ requires: C, 62·0 H, 8·4 N, 9·05
1-Ethinyl *cyclo*hexanol	22*	95–96°	Found: C, 64·7 H, 7·9 N, 8·1
			Calc. for $C_9H_{13}O_2N$: C, 64·7 H, 7·8 N, 8·4

* Reaction carried out with potassium cyanate and dioxan as solvent.

Methylpentynyl N-*phenylcarbamate*. Methylpentynol (4·9 g., 0·05 mole) was refluxed with phenyl *iso*cyanate (6.5 g., 0·055 mole) for 2 hours, and the product was dissolved in light petroleum (b.p. 40 to 60°), filtered from some *s*-diphenylurea, and the filtrate concentrated to crystallising point. Yield, 6·25 g. (57·5 per cent), m.p. 65 to 66° (*cf.* Young and Webb[9]).

Methylpentynyl N-*methylcarbamate*. Methylpentynol (9·8 g., 0·1 mole) was heated with methyl *iso*cyanate (5·7 g., 0·1 mole) in a sealed

tube at 170° for 4 hours, the contents added to water and extracted with ether. The ether was removed from the dried extract, and the residue was distilled. Yield, 5·2 g. (35 per cent), b.p. 100° at 11 mm. Found: C, 62·3; H, 8·5; N, 8·85; Calc. for $C_8H_{13}O_2N$, C, 61·9; H, 8·4; N, 9·0 per cent. The preparation of this ester at room temperature has been described[1], with the following constants: b.p. 103° at 11 mm., m.p. 54 to 55°, but no analysis was given.

*Reaction of ethinyl*cyclo*hexanol with phosgene in pyridine.* Ethinyl-*cyclo*hexanol (12·4 g.) in pyridine (20 ml.) was added with stirring to a suspension prepared by gradually adding phosgene (10 ml.) to pyridine

TABLE II

ACUTE ORAL TOXICITY OF METHYLPENTYNYL CARBAMATE IN FEMALE ALBINO MICE

Dose mg./kg.	No. of mice	Deaths (after 5 days)
400	27	25
333	27	9
277	26	3

LD 50 = 337 mg./kg.
Fiducial limits = 322–353 mg./kg.

(40 ml.) with cooling. After stirring at room temperature for 2 hours, the mixture was allowed to stand overnight, poured into water, and the product extracted with ether. After removal of the ether from the dried extract, the residue was distilled and 1-ethinyl*cyclo*hexene (6·3 g., 60 per cent) was obtained, b.p. 84° at 100 mm. Found: C, 90·5; H, 9·4. Calc. for C_8H_{10}, C, 90·5; H, 9·4 per cent.

Reaction of methylpentynol with phosgene. Methylpentynol (9·8 g.) and phosgene (8 ml.) were heated in a sealed tube at 150° for 1 hour. After cooling and releasing the carbon dioxide, the contents were poured

TABLE III

RED AND WHITE BLOOD CELL COUNTS OF ANIMALS IN SUB-ACUTE TOXICITY TEST

	Controls	Test
Average red blood cell count (millions/cu. mm.)	10·16	11·92
Average white blood cell count (thousands/cu. mm.) ..	16·63	16·97

into water, and the separated oil was extracted with ether. Working up in the usual manner yielded an *oil*, b.p. 54° at 13 mm. The structure was not elucidated. Found: C, 46·9; H, 6·5; Cl, 45·8. $C_6H_{10}Cl_2$ requires C, 47·1; H, 6·6; Cl, 46·3 per cent.

Methylpentynyl carbamate. To trichloroacetic acid (1634 g.), dried *in vacuo* in the reaction vessel, was added methylpentynol (2175 g., 2·5 l.) and sodium cyanate (650 g.), also thoroughly dried. The suspension, protected from moist air and mechanically stirred, was heated at 45 to 50° for 20 hours, and then neutralised to *ca.* pH 7 by the gradual addition

of anhydrous sodium carbonate. The mixture was cooled to 30°, filtered using a filter aid, and the vessel rinsed with carbon tetrachloride (200 ml.) which was then used to wash the filter cake (mainly sodium trichloroacetate). Carbon tetrachloride was then removed from the filtrate and washings. Excess of methylpentynol was then distilled off at *ca.* 6 mm. pressure whilst the temperature was gradually raised to 50°. Approximately 1240 g. of methylpentynol was recovered. Two volumes of water were then added to the residue with stirring. The oil which separated solidified on cooling to room temperature. The yellowish powder was collected and dried. Yield, 1138 g., m.p. *ca.* 40°. Recrystallisation

TABLE IV

NARCOTIC ACTIVITY OF METHYLPENTYNYL CARBAMATE

Expt.	Compound	No. of mice	Route	Dose g./kg.	Time after injection in hours: ½ N	½ D	1 N	1 D	2 N	2 D	3 N	3 D	4 N	4 D	5 N	5 D	6 N	6 D	24 N	24 D
1.	Methylpentynol	20	oral	1·140	20	–	20	–	19	1	15	5	7	13	5	15	5	15		
				0·760	16	–	19	–	19	–	19		19		20		19	1		
				0·506	12	–	13	–	16	–	10		9		9		4			
				0·338	2	–	1	–	0	–	0		0		0		0			
2.	Methylpentynyl carbamate	19	oral	0·506	19		19		19		18	1	16	3	15	4	14	5	13	5
				0·338	10		10		7		6	1	7	1	6	1	6	1	0	1
				0·225	0		0		0		0		0		0		0		0	

N = Narcotised
D = Dead (total)

from *cyclo*hexane gave an almost white product, the carbamate of 3-methylpent-1-yn-3-ol (940 g.), m.p. 53 to 55°. The aqueous medium from the solidified oil was cooled in ice for 24 hours and afforded a further 22 g. of pure material, giving a total yield of 962 g. (68 per cent). Found: C, 59·5; H, 7·9; N, 10·2. Calc. for $C_7H_{11}O_2N$, C, 59·5; H, 7·85; N, 9·9 per cent.

Other carbamates of acetylenic carbinols (Table I). The carbamates of tertiary acetylenic alcohols were prepared by the above method, except that the crude compounds were extracted with ether, and the extracts were washed with aqueous sodium bicarbonate and water, dried, and evaporated. Excess of the carbinol was removed at > 100° at 20 mm., and the residue was crystallised from light petroleum or *cyclo*hexane. Approximately 80 per cent of the unreacted carbinols was recovered. In addition to these in Table I *tert.*-butyl carbamate was prepared in low yield by an analogous method using potassium cyanate, and with dioxan as solvent. This is known to be less satisfactory than the method detailed above for methylpentynol. The product had m.p. 106 to 107° (*cf.* Choppin and Rogers[4] 108 to 108·5°).

BIOLOGICAL METHODS

Female mice of Schofield strain and weighing approximately 20 g. were used for all estimations involving this species.

Acute Toxicity

Food was witheld from mice 18 hours before use. Methylpentynyl carbamate was administered orally as a suspension in 5 per cent gum acacia solution, at a concentration such that 0·5 ml. was given per 20 g. of body weight. The animals were kept under observation for 5 days

TABLE V

NARCOTIC ACTIVITY OF ESTERS OF METHYLPENTYNOL AND RELATED COMPOUNDS
(5 mice in each case except where otherwise stated)

No.	Compound	Oral dose g./kg.	Time after injection in hours: ½	1	2	3	4	5	6	7	24
			N D	N D	N D	N D	N D	N D	N D	N D	N D
327	Methylpentynyl acetate ..		ED 50 approx. 1·17 g./kg. Later time of onset, shorter duration								
496	Methylpentynyl benzoate ..	1·17	0	0	0	0	0	0	0	–	–
502	Methylpentynyl *N*-phenyl carbamate	1·17	0	0	0	0	–	–	–	–	–
511	Methylpentynyl *N*-methyl carbamate	1·17	0	0	0	0					
521	Methylpentynyl allophanate ..	1·17	0	2	2 2	1 3	2 3	1 4			
		0·78	0	0	3	3	2 1	1 2			
		0·52	0	0	0	0	0	2			
	Methylpentynyl hydrogen phthalate	1·14		0 1	0 2	0 3	0 4	0 4	0 4		
533	Carbamate of 2-methylbutan-2-ol	1·14	0	0	0	0	0	0	0		
522	Carbamate of 3-methylhex-1-yn-3-ol	1·14	4 1	2 3	2 3	2 3	1 4	1 4	1 4		
		0·76	3 2	3 2	3 2	1 4	1 4	0 5	0 5		
		0·506	5	5	4 1	4 1	4 1	4 1	4 1		
		0·338	4	5	5	4	4	4	4		
		0·225	0	0	0	0	0	0	0		
555	Carbamate of 3-ethylpent-1-yn-3-ol 30/dose	0·506	28	29 1	29 1	28 2	27 2	23 5	23 5		
		0·338	28	29	29	28	26	14	4		
		0·225	5	4	2	0	0	0	0		
		0·150	0	0	0	0	0	0	0		
558	Carbamate of 3:5-dimethylhex-1-yn-3-ol	1·14	0	0	0	0	0	0	0		
559	Carbamate of 3-methyloct-1-yn-3-ol	0·506	0	0	0	0	0	0	0		
561	Carbamate of 3-methylhept-1-yn-3-ol	0·506	5	5	3	2	0	0	0		
		0·338	3	3	1	0	0	0	0		
		0·225	2	1	0	0	0	0	0		
		0·150	0	0	0	0	0	0	0		
566	Carbamate of 3-methylbut-1-yn-3-ol	1·14	5	5	5	5	5	5			1 4
		0·76	5	5	5	5	5	5			4 1
		0·506	5	5	5	5	5	5			5
		0·338	0	1	0	0	0	0			0
519	Carbamate of 1-ethinylcyclohexanol[13]	1·17	3 2	3 2	2 3	2 3	1 4	1 4			
		0·78	5	5	3 1	0 2	0 2	0 2			
		0·52	5	5	5	0	0	0			
		0·35	4	2	0	0	0	0			

N = Narcotised D = Dead

after administration of the drug, with free access to food (diet 41B) and water. At the end of this period LD 50 estimates were made according to the method of Bliss[10].

Subacute Toxicity

Twenty mice were given 170 mg. (approximately equivalent to half the LD 50) of methylpentynyl carbamate per kg. of body weight, orally as a suspension in 5 per cent gum acacia solution 5 days a week for 19 weeks. Ten mice were kept as controls. The animals had free access to their usual diet and water, and their weights were recorded at the beginning of the experiment and once every week thereafter. Any deaths or untoward symptoms during the experiment were noted. At the end of the experiment a sample of blood was taken from each of the

TABLE VI

ANTICONVULSANT ACTIVITY OF METHYLPENTYNYL CARBAMATE AND METHYLPENTYNOL

Compound	Dose mg./kg.	Number of mice	Number convulsing	Number protected	Per cent protection
Aloxidone	225	49	10	39	79·6
	150	47	16	13	66·0
	100	50	32	18	36·0
Methylpentynol	100	50	8	42	84·0
	67	50	15	35	70·0
	44	50	21	29	58·0
Methylpentynyl carbamate ..	100	50	1	49	98·0
	67	50	5	45	90·0
	44	50	20	30	60·0

survivors which were then killed and post-mortem examinations made. Histological preparations were made of the liver, kidney, and spleen of several animals in each group.

Narcotic Activity

Varying doses of methylpentynyl carbamate and other esters were administered orally to groups of mice, which were then kept at a temperature of 37° and examined 30 minutes, 1 hour and then hourly for 6 hours, after administration. The abolition of the righting reflex for at least 30 seconds was taken as the criterion of narcosis. The doses given were in accordance with one of two series of four geometric dose levels: 1·17, 0·78, 0·52 and 0·35 g./kg., or 1·14, 0·76, 0·56 and 0·34 g./kg. of body weight. Where a preliminary test indicated a very low order of activity, only the top dose was given, and occasionally also lower doses (e.g., 0·23 g./kg.) were given.

Anticonvulsant Activity

Comparative experiments were done with methylpentynyl carbamate and methylpentynol, 100, 67 and 44 mg./kg., and aloxidone, 225, 150 and 100 mg./kg. of body weight. All doses were administered orally as a suspension in 5 per cent gum acacia solution. Two hours later all the mice were given an intravenous injection (caudal vein) of leptazol (0·2 ml. of a 0·6 per cent solution per 20 g. of body weight). This dose of leptazol produced 100 per cent response in animals which received no premedication. The criterion of positive response was the characteristic tonic

extension of the hind leg. The median protective dose (ED 50) was estimated graphically.

Analgesic Activity

The method of Woolfe and Macdonald[11] was used with minor modifications. Methylpentynyl carbamate was injected intravenously at 50 mg./kg. body weight.

TABLE VII

EFFECT OF BEMEGRIDE ON MICE NARCOTISED WITH METHYLPENTYNOL AND METHYLPENTYNYL CARBAMATE

Test No.	Drugs and Doses (Those in parentheses refer to bemegride) 10 mice/dose (intraperitoneal)		Number narcotised						
			½	1	1½	2	2½	3	3½ hrs after injection
3	Methylpentynol alone	700 mg./kg.	10	10	10	10	9	9	8
	Methylpentynyl carbamate alone	350 ,,	10	10	9	7	4	3	1
	Methylpentynol + bemegride	(40) ,,	10	↓10	9	7	5	5	3
	Methylpentynyl carbamate + bemegride	(40) ,,	10	↓ 0	0	0	0	0	0
4	Methylpentynol alone	600 ,,	10	10	10	10	10	—	6
	Methylpentynyl carbamate alone	400 ,,	10	10	10	10	10	—	10
	Methylpentynol + bemegride	(50) ,,	10	↓ 4	1	0	0	—	0
	Methylpentynyl carbamate + bemegride	(50) ,,	10	↓ 1	1	0	0	—	0

↓ = injection of bemegride

Effect on Blood Pressure and Respiration

The animals used were rabbits and cats, the former being anaesthetised with ether and pentobarbitone, and the latter with ether and chloralose. Methylpentynyl carbamate was injected into the cannulated femoral vein in doses of 0·1 to 10·0 mg. in aqueous solution.

Bemegride as Antagonist to Methylpentynol and its Carbamate

The method and experimental details of Frey, Hushahn and Soehring[12] were followed closely. Solutions were made up in Tyrode-Ringer solution with the exception of methylpentynyl carbamate which was administered as a suspension in 5 per cent gum acacia solution. The doses were kept as low as consistent with a high degree of narcosis, and paying due regard to toxicity. Two series of experiments were carried out, in which the drugs were given intraperitoneally and orally respectively. In all cases the bemegride was given intravenously in aqueous solution approximately 20 to 30 minutes after narcosis was complete, as judged by abolition of the righting reflex. Animals which were not narcotised were not given bemegride. In some cases the mice were examined after 24 and 72 hours. The doses of both methylpentynol and the carbamate were all greater than those normally used, the purpose being to establish whether any significant degree of antagonism could be demonstrated.

RESULTS

Acute Toxicity (Table II)

The median lethal dose of methylpentynyl carbamate was estimated at 337 mg./kg. body weight, the corresponding value for methylpentynol

itself being 810 mg./kg. The carbamate is therefore approximately 2·6 times as toxic as methylpentynol.

Subacute Toxicity

The graphical results of the weekly average weights of test and control animals are shown in Figure 1. There was no significant difference between the rate of growth per week in the two groups as shown by treating the results statistically using a '*t*' test. There were four deaths among the twenty test mice, and one death among the ten controls before the end of the experiment (20 and 10 per cent respectively). These deaths were almost exclusively due to subcutaneous abscesses from an unknown cause, generally in the thoracic area. Post-mortem examination of the remaining animals showed no gross pathological changes, and the histological preparations revealed no consistent abnormalities which could be related to the administration of the drug. The red blood corpuscles and the leucocytes were within normal limits, but the average haemoglobin level of the test group was 17·2 per cent greater than that of the controls (137·9 compared with 117·7 per cent—Sahli method). See Table III.

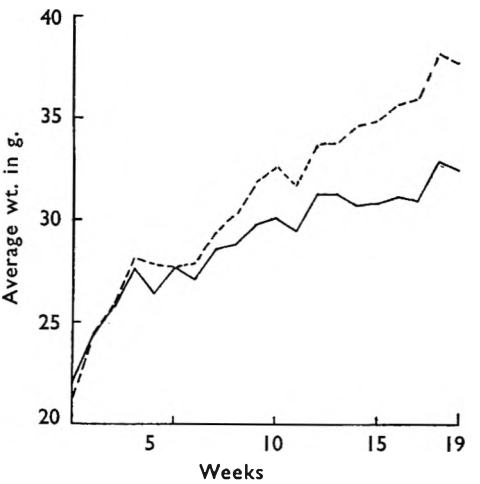

FIG. 1. Average growth curves for mice receiving 170 mg./kg. methylpentynyl carbamate per day (——) and controls (- - -).

Narcotic Activity

The results are shown in Table IV. The number dead and narcotised at each dose level is shown. The results show that methylpentynyl carbamate is at least twice as active as methylpentynol. Both substances act quickly, but the duration of action of the carbamate is greater, at equiactive doses. Table V shows that none of the other esters of methylpentynol possess any appreciable narcotic activity. The carbamates of most of the homologues of methylpentynol show significant activity.

Anticonvulsant Activity

Table VI shows the results of a comparison of the anticonvulsant activity of methylpentynyl carbamate with that of methylpentynol and aloxidone. The ED 50 estimates (obtained graphically) are as follows: aloxidone, 123, methylpentynol, 38, methylpentynyl carbamate, 39 mg./kg.

Analgesic Activity

Methylpentynyl carbamate had no activity.

Effect on Blood Pressure and Respiration

Methylpentynyl carbamate had no effect on the blood pressure or respiration of the animals at the doses used.

Antagonism of Bemegride (Table VII)

These results show that bemegride given intraperitoneally has a significant arousal action on mice narcotised with high doses of methylpentynol or its carbamate. There were no deaths in this series of experiments but the animals were only observed up to $3\frac{1}{2}$ hours after the first injection. Some preliminary experiments have been performed in which the drugs were administered orally. The results so far appear similar to those obtained in the first series, but they have not been so consistent.

It seems clear from the present results that bemegride cannot be regarded as a specific barbiturate antagonist.

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A CRITERION FOR OXYTOCIC ACTIVITY

Studies with Tochergamine

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NN-Diethyl-*N'*-(2-tetralyl)-glycinamide, a substance of high oxytocic activity in the isolated uterus preparation, has been tested on the intact human puerperal uterus and been found to be without demonstrable activity, in doses of 1–20 mg. parentally. The absolute necessity for testing oxytocic activity by an objective method on the intact human uterus is stressed.

Species difference in drug response may be the source of surprise or disappointment when a new compound fresh from the laboratory is given its first clinical trial. Some 30 years ago, for example, it was thought that ergotoxine and ergotamine were the essential substances in crude ergot[1]. Yet, despite their potency in the laboratory, the effect of these alkaloids on the uterus in everyday clinical practice fell far short of expectations. Ergotoxine and ergotamine were much slower in action than the crude Extractum Ergotae Liquidum, B.P.[2,3]

With the discovery of ergometrine[3,4] rather the reverse obtained. In the intact human uterus ergometrine was found to be the most rapid and powerful oxytocic of all[5], although its effect in the laboratory proved disappointing. The standard preparations of isolated guinea pig and rat uterus are not reliable and give many negative results[6]. From such uncertain evidence a compound may be thought to be oxytocic which, in fact, is not. Or, worse still, a compound inactive in the laboratory may have sharp oxytocic properties in practice[7,8]. A more stringent test than those used hitherto is therefore obligatory. There should be some definite criterion for oxytocic activity which must be satisfied before a new compound is used in clinical practice for its oxytocic properties. The need for such a test has recently been re-emphasised for us by our experience with the new synthetic ergot-like derivative, Tochergamine*.

Tochergamine, *NN*-diethyl-*N'*-(2-tetralyl)-glycinamide or 621 I.S., has been preprepared as the tartrate[9] and studied by Bovet-Nitti[6]. She has found that this compound exhibits oxytocic activity equal in potency to that of ergometrine when tested on the isolated guinea pig and rabbit uterus and that, like ergometrine, intraperitoneal injections of the drug induce abortion in pregnant guinea pigs. Toxicity tests have shown that Tochergamine has one great advantage in that it is 36 times less toxic than ergometrine.

Independent clinical trials by Bertini[10] in Turin and Rodriguez-Bravo[11] in Rome have suggested that Tochergamine, in parenteral doses of 2 to 6 mg., promotes easy and quick retraction of the uterine musculature after

* Trade name of Farmitalia, Milan.

delivery and that it can reduce the incidence of post-partum haemorrhage. These subjective clinical observations await confirmation by an objective method.

Method

Spontaneous human uterine motility was recorded in the first three days after delivery by the external tocograph described by one of us[12]. The method is convenient in that it entails placing only a small receptor unit

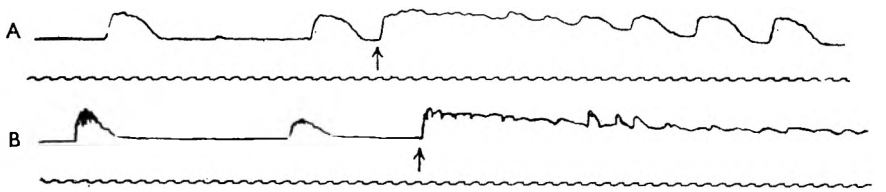

Fig. 1. Typical oxytocic response to pitocin and ergometrine (human puerperal uteri). A, at arrow, 1 unit pitocin i.v. B, at arrow, 0·25 mg. ergometrine i.v. Lower trace, time in minutes.

on the lower abdomen overlying the puerperal uterus, the contractions of which are relayed to a recording unit at the bedside. It is in effect a useful alternative to Moir's classical internal method[13] and one which is more acceptable to the patient.

After a suitable control period of recording, the drug to be studied is given to the patient and the uterine response observed graphically.

Results

The known oxytocics, pitocin and ergometrine, induced obvious uterine spasm in less than one minute by intravenous injection. This effect lasted a variable time (Fig. 1).

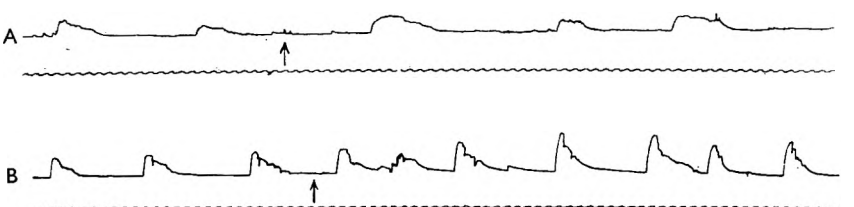

Fig. 2. Tochergamine has no significant effect on spontaneous uterine activity (human puerperal uteri). A, at arrow, 4 mg. drug i.v. B, at arrow, 20 mg. drug i.v. Lower trace, time in minutes.

Tochergamine was given similarly by intravenous injection to six volunteer patients in doses of 1 to 20 mg. The recommended dose is 2 to 6 mg. In none of these was an oxytocic response recorded, either immediate or delayed (Fig. 2, Table I), and no side effects were elicited.

Discussion and Conclusions

While Tochergamine has been found by others to act as a powerful oxytocic agent in laboratory animals, our experiments do not show it to

have a demonstrably useful effect on the human uterus in doses of 1 to 20 mg. The lesson is obvious, namely, that no substance can be considered suitable for clinical use as an oxytocic until it has been tested by an objective recording method on the intact human uterus, the only acceptable criterion.

TABLE I

THE EFFECT OF INTRAVENOUS DOSES OF TOCHERGAMINE, 1–20 MG., ON THE HUMAN PUERPERAL UTERUS

Patient	Day of Puerperium	Dose of Tochergamine mg. I.V.	Uterine response
1	3rd	1	No response in 28 minutes
2	3rd	4	No response in 80 minutes
3	2nd	4	No response in 10 minutes
4	3rd	10	No response in 41 minutes
5	1st	20	No response in 60 minutes
6	2nd	20	No significant response in 57 minutes

In the reports of Bertini and Rodriguez-Bravo, one may surmise that extraneous factors such as the stimulus of the observer's hand pressed on the fundus uteri initiated a uterine contraction which has been erroneously interpreted as a direct oxytocic effect of the drug. Favourable clinical impressions of an oxytocic agent may not always be borne out by tocographic experiment. Such objective methods remain the only safe way of determing oxytocic activity.

Acknowledgements. We are greatly indebted to Dr. Daniel Bovet of Rome for his suggestion that we should carry out this trial of Tochergamine and for making supplies of the drug available to us.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

***Dioscorea dumetorum*, a Convulsant Alkaloid of.** C. W. L. Bevan and J. Hirst. (*Chem. Ind.*, 1958, 103.) A convulsant alkaloid at first thought to be dioscorine was obtained from the fresh tubers of *D. dumetorum*, a wild plant common in West Africa, by extraction with alcoholic acetic acid. Chemically the alkaloid was found not to be identical with dioscorine, and a comparison with the infra-red spectrum of dihydrodioscorine synthesised by Pinder from dioscorine extracted from *D. hispida* Dennst left little doubt that the alkaloid was dihydrodioscorine. There are differences in the melting points of derivatives and in specific rotation between the natural and synthetic alkaloid, possibly due to the synthetic alkaloid being a mixture of stereoisomers. Pharmacologically the alkaloid showed considerable similarity to dioscorine, but is weaker in all its effects. J. R. F.

***Rauwolfia vomitoria* Afz. and Some Related African Species.** R. Paris and G. Dilleman. (*Ann. pharm. franç.*, 1957, **15**, 310.) In an investigation of the alkaloids of various species of *Rauwolfia*, a preliminary separation of the alkaloids provided two groups, a weakly basic group (including reserpine), and the remainder. These fractions were separated by paper electrophoresis under carefully controlled conditions. *R. caffra* and *R. inebrians* gave identical ionograms, which were, however, completely different from that of *R. vomitoria*. Samples of *R. vomitoria* root were similar to *R. caffra* and *R. macrophylla* in structure, but could be distinguished by certain microscopical characters, while the roots of *R. caffra* and *R. inebrians* showed only slight histological differences.

G. B.

ANALYTICAL

Ethinyloestradiol, Identification and Determination of. C. Heusghem and J.-M. Jehotte. (*J. Pharm. Belg.*, 1957, **12**, 418.) A simple method is given for the identification of ethinyloestradiol in quantities of a few mg. Tablets and other preparations are powdered, suspended in water and extracted with ether, the ethereal solution being dried over anhydrous sodium sulphate and evaporated. The residual ethinyloestradiol gives a pink colour with a green fluorescence on the addition of ethanol-sulphuric reagent (prepared by mixing 20 ml. of ethanol (95 per cent) with 80 ml. of sulphuric acid at 0°). Pink streaks appear on shaking, and after about 1 minute the ethinyloestradiol dissolves completely, imparting the maximum colour to the solution. No other steroid appears to give this reaction, but relatively large amounts of oestrone, oestradiol or methyltestosterone produce a yellow or green fluorescence which may partially mask the pink colour. Details are given of the quantitative determination of ethinyloestradiol, using the same reaction and measuring the colour at 535 mμ.

G. B.

Iproniazid and Related Compounds, Photometric Determination of. R. J. Colarusso, M. Schmall, E. G. Wollish and E. G. E. Shafer. (*Analyt. Chem.*, 1958, **30**, 62.) This method applies to drugs of general formula R′–CO–NH–NH–R″ where R′ = 4′-pyridyl and R″ = alkyl or aryl, and depends upon the formation of a red complex in acetone solution with molybdic acid. Peaks at 430 or 535 mμ are suitable for measurement, and the procedure is sensitive down to 10 μg. Since breakdown products will not react, it can be applied to stability studies. A method suitable for tablets and ampoules is described. No colour is produced when R″ = H or nicotoyl or when the terminal nitrogen is disubstituted. D. B. C.

Isoniazid, Photometric Determination of. B. Wesley-Hadžija and F. Abaffy. (*Acta Pharm. Jug.*, 1957, **7**, 137.) A simple and sensitive method for the determination of isoniazid in pharmaceutical preparations depends upon the development of a yellow colour with *p*-dimethylaminobenzaldehyde. A solution containing about 1 mg. of isoniazid in 50 ml. of water is used, and on the addition of 0·1 ml. of a 4 per cent solution of *p*-dimethylaminobenzaldehyde in 2N sulphuric acid, the maximum colour develops in 10 minutes. Measurements of the colour intensity are made at 425 mμ, the quantity of isoniazid being read from a calibration curve. Details are given for the assay of tablets and injections by this method. G. B.

Riboflavine in Pharmaceutical Specialities, Determination of. A. Maquinay and N. Brouhon. (*J. Pharm. Belg.*, 1957, **39**, 350.) For the determination of riboflavine in tablets by a polarographic method, a tablet is powdered, and sufficient sodium salicylate is added to make the polarographic solution contain 1 per cent. A phosphate buffer solution is added to make the final solution 0·1M, and the sample made up to volume. After shaking for 30 minutes (in the dark) the solution is placed in the cell and the polarographic curve recorded under suitable conditions. The height of the wave between −0·3 and −0·6 volt is read, and the result calculated by reference to a standard curve. The presence of salicylate aids solution of the riboflavine. The method has been applied successfully to a number of multivitamin preparations. Novalgin depresses the height of the wave, and should be removed by precipitation with cadmium sulphocyanide before making the polarogram. G. B.

Tyrothricin, Investigation of the Spectrophotometric Determination of. W. Oberzill. (*Sci. Pharm.*, 1957, **25**, 148.) The factors influencing the design of a direct spectrophotometric method for the determination of tyrothricin in solutions and pharmaceutical preparations are considered. Firstly it is shown that the extinction values for tyrothricin, which is a mixture of tyrocidin and gramicidin, are equal to the sum of those of the two components. Thus determinations can only be expressed in terms of a particular sample of tyrothricin since this is a mixture of at least two main components. The same applies to the colorimetric determination by means of the tryptophane reaction (treatment with *p*-dimethylaminobenzaldehyde in strong hydrochloric acid and sodium nitrite solution). Secondly it is shown that in preparations containing tyrothricin and cetyltrimethylammonium bromide, the extinction value is again equal to the sum of the individual components. In all cases the extinction is measured at 281 mμ where the curve for cetyltrimethylammonium bromide is almost horizontal. The concentrations used are of the order of 100 μg./ml. Measurements with known mixtures are quoted, and show that the results agree with those predicted to within a few per cent. D. B. C.

GLYCOSIDES

***Digitalis purpurea* Leaves, Presence of Gitoriside in.** D. Satoh, T. Wada, H. Ishii, Y. Oyama and T. Okumura. (*Pharm. Bull. Japan*, 1957, **5**, 253.) Gitoriside is the name given to a newly discovered glycoside present in very small quantity in dried digitalis leaves. This glycoside is gitoxigenin mono-(+)-digitoxoside. Some evidence is also produced to indicate that digitalonin (*Pharm. Bull. Japan*, 1956, **4**, 284) is diginigenin mono-(+)-digitaloside. Details for the production of crystalline gitoxin penta-acetate are also given.

J. W. F.

***Digitalis purpurea* Seeds, Presence of Glucodigifucoside in.** A. Okano. (*Pharm. Bull. Japan*, 1957, **5**, 272.) Further work on some of the 17 unknown glycosides of digitalis seeds, reported earlier (*ibid.*, 157) has led to the production of crystalline glucodigifucoside. This substance is a cardiotonic glycoside of empirical formula, $C_{35}H_{54}O_{13}.2H_2O$. On suitable hydrolysis it yields digitoxigenin, fucose and one molecule of glucose.

J. W. F.

***Digitalis purpurea* Seeds, Partition Chromatography of the Glycosides in.** K. Miyatake, A. Okano, K. Hoji and T. Mike. (*Pharm. Bull. Japan*, 1957, **5**, 157, 163, 167, 171.) A suitable extract of the seeds was subjected to adsorption chromatography on alumina using water saturated butanol as the developing solvent. Each fraction was subjected to ascending paper chromatography, using water saturated methyl ethyl ketone as the moving phase and water as the stationary phase. These paper chromatograms were done in triplicate and were treated separately by (*a*) $SbCl_3$-$CHCl_3$ solution, (*b*) 1 per cent HCl-MeOH solution and (*c*) 25 per cent *m*-dinitrobenzene-benzene solution. By this means, purpurea glycosides A and B, gitoxin, strospeside, digitalinum verum and 17 other substances (probably glycosides) were shown to be present. It was also shown that digitalinum verum was closely associated with a small quantity of one of these unknown glycosides. The presence of this impurity prevented crystallisation: when it was removed (by partition chromatography on Celite 535) crystals of pure digitalinum verum were readily obtained. This unknown glycoside is probably glucogitofucoside. Further work on one other of these unknown glycosides, which appeared to be present in comparatively large amount, was done. It was obtained in a crystalline form by using similar techniques employed previously for digitalinum verum. This new glycoside was named gitostin and further work showed that it was gitoxigenin-glucosido-glucosido-digitaloside. Partial decomposition by enzyme produces digitalinum verum and glucose.

J. W. F.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Adrenaline and Noradrenaline, Biosynthesis of, *In Vitro*. McC. Goodall and N. Kirshner. (*J. biol. Chem.*, 1957, **226**, 213.) Evidence for the conversion of tyrosine to adrenaline via dopa, hydroxytyramine and noradrenaline in bovine adrenal slices is presented. The tissue slices were incubated in a Krebs phosphate buffer solution with labelled L-tyrosine-^{14}C. At the end of the period of incubation the slices were homogenised and extracts of the homogenates were prepared for ion exchange chromatography by precipitating

the protein with trichloroacetic acid. The resin used was Amberlite IRC–50. The amounts of adrenaline, noradrenaline and hydroxytyramine were determined by measuring the optical density of the eluate at 279 mμ. An aliquot of each fraction was assayed for radioactivity. It was found that adrenal slices formed hydroxytyramine, noradrenaline and adrenaline from tyrosine and from 3:4-dihydroxyphenylalanine. Noradrenaline and adrenaline were formed from hydroxytyramine. In the presence of unlabelled dopa and unlabelled hydroxytyramine the amount of noradrenaline formed from ^{14}C-labelled tyrosine was decreased. Tyramine, under identical conditions, did not decrease the formation of noradrenaline from tyrosine. The data do not exclude the possibility that dihydroxyphenylserine may be involved in the pathway. Whether the sequence of the reactions is dopa to hydroxytyramine to noradrenaline or is dopa to dihydroxyphenylserine to noradrenaline has not been unequivocally established but the evidence presented supports the former sequence. M. M.

Digitoxin, C(12)-Hydroxylation of. B. T. Brown, S. E. Wright and G. T. Okita. (*Nature, Lond.*, 1957, **180**, 607.) When digitoxin was administered to rats or to humans, the urine was shown to contain, besides unchanged digitoxin, two cardioactive metabolites. One of these metabolites was shown to be digoxin (12-hydroxydigitoxin) by careful comparison with pure digoxin on paper chromatograms and by hydrolysis, and by the use of carbon-14 labelled digitoxin. J. W. F.

Mast Cells, Mechanism of the Disruption of. B. Högberg and B. Uvnäs. (*Acta physiol. scand.*, 1957, **41**, 345.) This paper presents the experimental work upon which is based the theory postulated by Uvnäs in his Review Article in the *J. Pharm. Pharmacol.*, 1958, **10**, 1. Using mast cells from the rat mesentery the authors found that disruption of the cells by compound 48/80 was inhibited by metal salts and other enzyme inhibitors. Of the enzymes investigated, lecithinase A was the only one that disrupted the cells, and its action was inhibited by the same agents that affected 48/80 activity. The action of 48/80 was temperature-dependent and inhibited by incubation with acetic anhydride and 1:3-diphosphoimidazole. Dephosphorylation caused disruption without the addition of a liberator. The disruptive action of both enzyme and 48/80 was blocked by high doses of a polyvalent serum (against snake venom) and a specific serum (against lecithinase A). The observations supported the hypothesis that 48/80 acts by activating a lytic enzyme attached to the mast cell membrane. The enzyme normally blocked by an inhibitor becomes active when the inhibitor is removed by liberators such as 48/80. J. R. F.

Polymyxin B, Structure of. G. Biserte and M. Dautrevaux. (*Bull. Soc. Chim. Biol.*, 1957, **39**, 795.) In order to study the peptide sequence of polymyxin B, partial acid hydrolysates were prepared, and the components separated by chromatography on a strongly acidic cation exchange resin, followed by electrophoresis and chromatography on paper. The peptides containing phenylalanine were isolated by chromatography on charcoal, followed by electrophoresis and chromatography on paper. It is suggested that polymyxin B consists of an octacyclopeptide or heptacyclopeptide structure with a side chain consisting of 2 or 3 amino acid residues connected at a $\alpha:\gamma$-diaminobutyric acid residue; the α-amino group of the $\alpha:\gamma$-diaminobutyric acid terminating this side-chain is joined to the carboxyl group of *iso*pelargonic acid. G. B.

BIOCHEMICAL ANALYSIS

Morphine, New Method for Determination of, in Urine. P. Paerregaard. (*Acta pharm. tox. Kbh.*, 1957, **14**, 38.) A new method is described for determining morphine in human urine. It is based on extraction of the bicarbonate-saturated urine with chloroform-*iso*propanol, paper chromatographic isolation of the morphine and finally quantitative determination by polarography. Pipette 10 ml. of the urine into an ampoule, add 1 ml. of 10 N hydrochloric acid, seal and autoclave at 120° for 30 minutes. Cool, saturate with sodium bicarbonate (pH = 8–9, the isoelectric point of morphine) and extract three times with equal volumes of a 3 : 1 mixture of chloroform and *iso*propanol. From the total solvent phase withdraw 15 ml. (corresponding to 5 ml. of urine) and evaporate to dryness in a flat-bottomed tube. Dissolve the residue in 100 μl. of methanol and apply 10 μl. to a strip of Whatman No. 1 filter-paper using a Carlsberg constriction pipette. Carry out descending paper chromatography with one of the two solvent mixtures (1) *n*-butanol formic acid and water 12 : 1 : 7 or (2) amylene hydrate di-*n*-butylether and water 80 : 7 : 13. Elute the morphine localised on the paper with N hydrochloric acid and determine the morphine present polarographically after transforming into 2-nitrosomorphine by means of nitrite in acid solution. G. F. S.

Oxytocin in Preparations of Vasopressin, Assay of. A. T. Nielsen. (*Dansk Tidsskr. Farm.*, 1958, **32**, 1.) While theoretically the oxytocin content of preparations of vasopressin can be calculated from the difference between the total oxytocin activity and the activity due to vasopressin, the conditions are such that a statistically significant result is hardly to be expected. To obtain a more accurate result the author describes a method for the assay of oxytocin in injection of vasopressin in which the oxytocic activity is assayed before and after inactivating the vasopressin enzymatically by a tryptic digest (trypsin 10 μg./ml. of injection) at pH 8–9 and 22° for 30 minutes. The oxytocic activity is assayed as described by Holton on the isolated uterus of the rat in oestrus, using a modified de Jalon Ringer. The intrinsic oxytocic activity of the pressor hormone was found to average about 3·8 units per 100 pressor units.

J. R. F.

PHARMACY

Adrenaline, Decomposition of, in the Presence of Copper. P. Varène. (*Bull. Soc. Chim. Biol.*, 1957, **39**, 1099.) Sodium chloride solution (0·9 per cent), buffered with 0·05M phosphate buffer was mixed with 1 ml. of solution containing a known concentration of copper sulphate, the total volume being 99 ml. This solution was heated to 37°, and 1 ml. of adrenaline solution added. The reaction was carried out in darkness, using pure materials and a high degree of cleanliness to avoid spurious results. The destruction of adrenaline in the presence of copper was measured in two ways; (1) by determination of the hypertensive action of the solution in the pithed dog treated with atropine and chloralose, and (2) photometrically using a blue-green filter having its maximum transmission at 490 mμ. The activity determined by method (1) decayed to zero at the end of 13 minutes contact with the copper sulphate solution. The blue colour reached a maximum at about that time, and on prolonged contact with the copper sulphate solution, faded to pale brown. The rate of destruction of adrenaline increased with increase in pH, copper concentration and temperature. G. B.

Dressings, Sterilization of. V. G. Adler and W. A. Gillespie. (*J. clin. Path.*, 1957, **10**, 299). An experimental study has been made of the factors affecting the sterilisation of surgical dressings. The experiments were carried out in cylindrical jacketed autoclaves. Two methods of sterilisation were used, (*a*) the double vacuum method, and (*b*) the downward displacement method. The drums were loaded with folded "huckaback" towels and the temperature inside measured with a thermistor. Bacteriological tests were carried out with filter papers impregnated with a spore suspension of *B. stearothermophilus* and chemical tests for heat penetration with Browne steriliser control tubes. The results showed that the double vacuum method was slightly better than the downward displacement method for removing air from the drum and the dressings, but this was never complete. Delay in heat penetration was due to entrapped air. Exposure to steam at 20 lb./sq.in. for 15 minutes was sufficient to sterilise all spore papers, but exposure for 10 minutes allowed some spores to survive at the centre of closely packed drums. Packing of the drums and incorrect positioning delayed sterilisation, and sterilisation was easier when the towels were wrapped in cloth instead of in drums. Browne's chemical tests were found to be good indications of safe sterilisation. G. F. S.

Silicone and Petrolatum Ointment Bases, Comparison of *In Vivo* and *In Vitro* for Absorption, Penetration and Diffusion of Medicinals from. J. B. Plein and E. M. Plein. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 705.) Six ointment bases were prepared, all of about the same consistency, including a simple paraffin ointment, an absorption base (containing about 10 per cent of wool fat), an emulsion base, and similar bases prepared with silicone fluids in place of paraffins. Sulphanilamide, iodine, sodium radio-iodide (^{131}I), salicylic acid and chlortetracycline hydrochloride were incorporated in the bases, which were subjected to *in vitro* diffusion tests. The ointments were also applied to the intact and abraded skin of white rats, the penetration into the inuncted skin being determined by analysis of a skin sample, and the absorption into the systemic circulation by analysis of the blood or a storage organ. Results of the skin penetration and intact skin absorption tests showed no correlation with the *in vitro* diffusion of the ointments. For 3 of the 5 drugs investigated, the absorption through the abraded skin was in accordance with the *in vitro* diffusion data. The significance of these results is discussed. G. B.

Sterile Ophthalmic Solutions, Preparation of. J. Schmid. (*Die Pharmazie*, 1957, **12**, 748.) Owing to the large number of cases of optical infection in recent years following cataract operations resulting in loss of sight traceable to infected opthalmic solutions, the use of a 1 : 50,000 solution of alkyldimethylbenzylammonium chloride was employed as solvent and the solutions prepared from previously sterilised materials in sterile containers with aseptic precautions. In no case during an 18-month trial were bacteria detected in the residues in bottles after one to two months use, and no case of irritation was reported. D. B. C.

Syringes, Sterilization of, by Infra-red Radiation. E. M. Darmady, K. E. A. Hughes and W. Tuke. (*J. clin. Path.*, 1957, **10**, 291.) An infra-red steriliser for syringes is described. It consists of a metal moving belt on which the assembled syringes are loaded on trays in their pre-sealed containers. They pass through an insulated tunnel over which infra-red projectors are placed at predetermined intervals. Experimental studies showed that the heating up time was rapid and a sterilisation time of 11 minutes at 180° ensured sterility. The apparatus can be used for the sterilisation of other glassware articles. G. F. S.

PHARMACOLOGY AND THERAPEUTICS

Anthelmintics, New Series of. F. C. Copp, O. D. Standen, J. Scarnell, D. A. Rawes and R. B. Burrows. (*Nature, Lond.*, 1958, **181**, 183.) A series of compounds of the general formula $R{\cdot}C_6H_4{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_2{\cdot}CH_2{\cdot}C_6H_4{\cdot}R'$ were examined for their activity against nematodes parasitic in the gastro-intestinal tract of animals. In all compounds R and R′ were in the *ortho* position, and those in which R = H, Me or Cl, R′ = H, Me, Cl or F were shown to be more active than others in which R = NO_2 or Br and R′ = Br. Some of the compounds examined were active against a wide range of species, particularly those nematodes which live in the mucosa rather than the lumen of the gut.

G. B.

Chlorothiazide, an Oral Diuretic. R. I. S. Bayliss, D. Marrack, J. Pirkis, J. R. Rees and J. F. Zilva. (*Lancet*, 1958, **1**, 120.) Chlorothiazide (6-chloro-7-sulphamyl-1 : 2 : 4-benzothiadiazine-1 : 1-dioxide) is a non-mercurial oral diuretic, with an action resembling that of mercurial diuretics. The value of chlorothiazide was assessed in 24 oedematous patients, of whom 17 had congestive heart failure and 11 had responded poorly to mersalyl. A daily dose of 2 g. (1 g. at 8.30 a.m. and 1 g. at 4.30 p.m.) produced good results, with clearing of the oedema, in 14 patients: in 7, the results were less satisfactory, and in 3 they were poor. Chlorothiazide may be effective in patients who do not respond to mersalyl, and it enhances the response to mersalyl even in patients who have become mersalyl-resistant. No toxic effects were observed except in 1 patient who developed malaise and anorexia. Chlorothiazide caused an approximately equimolar loss of chloride and sodium. It may cause potassium depletion, particularly if continuous treatment with 2 g. daily is given over a long period: in cases where this dosage is necessary the treatment should be supplemented with 2 to 6 g. of potassium chloride. In less severe cases, which respond quickly, a dose of 1·0 to 1·5 g. daily of chlorothiazide may suffice; for maintenance therapy the drug may be given intermittently on 3 or 4 days each week.

S. L. W.

Chlorothiazide, Clinical Experience with. J. D. H. Slater and J. D. N. Nabarro. (*Lancet*, 1958, **1**, 124.) Chlorothiazide was used successfully in 3 patients with the nephrotic syndrome, in 1 patient with ascites due to portal hypertension, and 1 patient with congestive heart failure. Two of the patients with nephrotic syndrome were satisfactorily controlled for 5 to 6 months. The chief action of chlorothiazide appears to be to cause a rapid increase in the rate of excretion of chloride by the kidneys, resembling closely the diuretic action of organic mercurial compounds. A subsidiary effect, suggesting inhibition of carbonic anhydrase, was observed in a short-term study. The drug is very well tolerated and seems to be free from side-effects, apart from the development of a hypokalaemia which is difficult to control with oral potassium supplements. If a patient has oedema persistent enough to need both severe restriction of sodium and chlorothiazide therapy (either continuous or intermittent), it is essential to have regular estimations of plasma-potassium concentrations. Initially, these should be made at weekly intervals and suitable supplements of potassium chloride given.

S. L. W.

BOOK REVIEW

DISINFECTANTS. THEIR VALUES AND USES, by W. E. Finch. Pp. 188 (including Index). Chapman & Hall, London, 1958. 30s.

To any pharmacist the values and uses of disinfectants should always be an interesting subject. He should not be disappointed, therefore, when he finds that this book deals primarily with the phenolic and quaternary ammonium type of disinfectants, together with some information given on the hypochlorites.

Its main concern is in the formulation of disinfectants and in the various methods available for assessing their activities; much of this information was hitherto unpublished. We are given an insight into the uses of different phenolic fractions, soaps and hydrocarbon carriers in the manufacture of Lysol and the black and white disinfectant fluids, and their influence on the physical characteristics and the germicidal properties of such preparations. There are also sections on the chloroxylenol disinfectants. The chapter on the quaternary ammonium compounds deals primarily with their adsorption on fibres and their efficacy in the disinfection of hospital blankets, and the chapter on the hypochlorites, which also mentions the chloramines, discusses briefly their use in various aspects of medicine and hygiene, including the treatment of wounds and burns.

Much attention is given to the methods of testing disinfectants and to the usefulness of the results so obtained. The Rideal-Walker and other phenol coefficient tests are criticised in this respect, the practicality of the Use-Dilution Confirmation test is discussed, and results from such tests are compared with those from a modified Chick-Martin test and an infected scalpel test.

The great difficulty with the book is that the information is presented in such a diffused manner that it makes it difficult to read and comprehend. There is a tendency to change the aspect of the topic under discussion and to insert bits of information which are really irrelevant in their context. For instance, there is a sentence on the photogenic properties of *Pseudomonas pyocyanea* inserted in a discussion on the activities of phenolic substances adsorbed on woollen fibres (p. 117). Some of the tables are difficult to understand, and the subject index could be improved; for example, skin disinfection is indexed on pp. 65, 74 and 75, but there is also a section headed "*Skin sterilization*" on p. 118. The glossary is trivial, and here and there are touches of unconscious humour; thus, "The water closet can be replaced by the chemical closet" (p. 150), and "other methods of sterilization . . . are filtration; exposure to ultra-violet light which is practised for the sterilization of air, sunlight and supersonic waves" (p. 152).

The emphasis is on a few specific disinfectant formulations, and others receive scant treatment; iodine is dismissed in eight lines, the mercurials in nine lines, heat sterilization (in any case out of context is such a book) in just over a page, and so on.

The print is clear, the book is easily handled, and there are very few typographical errors.

G. SYKES.

LETTER TO THE EDITOR

Histamine Release from Mast Cells by Lecithinases A and C

SIR,—In a letter on page 271 of the current volume of this Journal Dr. J. F. Riley comments upon a theory about the mechanism of the disrupting action of compound 48/80 on rat mast cells proposed by Högberg and Uvnäs[1]. According to this hypothesis compound 48/80 attacks the mast cell and liberates histamine by activating a lytic enzyme localized on the mast cell membrane. Högberg and Uvnäs[1] also describe some observations on the disrupting action of lecithinase A, prepared from various sources, on mast cells and they found a surprizingly good parallelism between the action of various enzyme blocking substances on lecithinase A, and on the disrupting action of compound 48/80. The observations were considered to support the hypothesis that compound 48/80 might act by activating a lytic enzyme. But which type of lytic enzyme was involved was left an open question. Dr. Riley has now gone a little further and has suggested that the lytic enzyme might be of the type lecithinase C. This might well be the case since as also pointed out by Riley lecithinase C disrupts mast cells. In fact, we have also observed that a lecithinase preparation from *Clostridium perfringens* (a strain pathogenic to man) which probably is a lecithinase C is very active in disrupting mast cells. And what is of special interest, the action of this lecithinase shows a pH optimum and a temperature sensitivity similar to those previously observed to be valid for the disrupting action of compound 48/80. Lecithinase A, on the other hand, lacks a distinct pH optimum above 7 and is very resistant to heat. More information about the influence of various factors on the disrupting action of the lecithinases A and C will be published shortly from our laboratories. But, since our knowledge about the chemistry of the mast cell membrane is so scanty, it is in my opinion very difficult to visualise the possible nature of a cell membrane phospholipase.

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REFERENCE

1. Högberg and Uvnäs, *Acta. physiol. scand.*, 1957, **41**, 345.