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Infrared identification of some hallucinogenic derivatives of tryptamine and amphetamine

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The use of infrared spectroscopy for the identification of psychotomimetic derivatives of amphetamine and tryptamine is discussed. Numerous characteristic absorptions are assigned on the basis of spectra recorded from 123 bases and salts. These permit the recognition of compounds of these types even when no reference materials or spectra are available. Distinction between the spectra of optically active and racemic forms of some amphetamine derivatives is also possible.

The identification of hallucinogenic drugs can present difficulties, particularly when no reference data are available. The most important substance in this category is undoubtedly lysergide (LSD), which has been considered previously (Mesley & Evans, 1969), but there are potentially many other compounds having similar properties. Some of these are not recognized drugs, but have been synthesized with the deliberate intention of evading existing legislation. When a hitherto unknown compound is encountered, its identification may require the combined use of several analytical techniques, as in the case of the illicit drug known as "STP" (Martin & Alexander, 1968; Phillips & Mesley, 1969).

Of the individual methods available, mass spectrometry is especially suitable for handling small samples and can give valuable structural information (Bellman, 1968), while thin-layer chromatography is useful for distinguishing related substances, provided that the material has been previously examined (Phillips & Gardiner, 1969). Infrared spectroscopy, however, has the widest applicability since on the one hand it provides a fingerprint for a given substance which can be compared with reference standards, while on the other it can yield valuable information concerning functional groups present in an unknown compound, which should permit at least partial identification. It can also be used to examine both free bases and their salts in the form of solids, liquids or solutions. Spectra of crystalline solids are generally more complex, and therefore more characteristic, than those of the liquid phase, and can sometimes be used additionally to identify optical isomers.

Apart from lysergide, the psychotomimetic drugs fall mainly into two classes: (i) those derived from tryptamine [3-(2-aminoethyl)indole], of which LSD is perhaps an extreme case; and (ii) ring-substituted derivatives of phenethylamine, though with the exception of mescaline these are all amphetamine derivatives. The present study is intended to establish characteristic absorption frequencies which will enable these compounds to be identified from their spectra. For this purpose it has proved necessary to consider also the simple amphetamines having no substitution in the benzene ring, and also a number of substances related to the hallucinogens and with which they might be confused.

Substances examined and their legal status

In the United Kingdom the important hallucinogens and amphetamines are controlled under the Drugs (Prevention of Misuse) Act 1964 and its subsequent modification (S.I. 1966, No. 1001). Apart from lysergide and its salts the only tryptamine derivatives included are hydroxy-*NN*-dimethyltryptamines, their esters or ethers, and salts of any of these substances. The intention is thus to control the naturally occurring hallucinogens psilocin and bufotenine (4- and 5-hydroxy-*NN*-dimethyltryptamine) and psilocybin (the phosphate ester of psilocin). It may be noted that the 6- and 7-hydroxy-compounds, which are not known to be hallucinogenic, are also included whereas *NN*-dimethyltryptamine and its homologues, many of which have hallucinogenic properties, are not covered.* Table 1 lists the tryptamines and related substances, including some based on gramine (3-dimethylaminomethylindole), which were examined; in addition to the simple compounds, the naturally occurring substances harmine, harmaline and ibogaine were included on account of their reported psychotomimetic properties (Downing, 1962).

The legal status of the amphetamines is more complex, and has been summarized by Phillips (1967). The 1964 Act covered amphetamines substituted in the side-chain but not in the ring, these compounds being liable to abuse as stimulants, but specifically excluded certain compounds of the ephedrine type. With the identification of the illicit material known as "STP" as 2,5-dimethoxy-4-methylamphetamine (Martin & Alexander, 1968; Phillips & Mesley, 1969), following reports of similar properties for other related compounds (Shulgin, 1964), it was thought necessary to control ring-substituted amphetamines and these are included in the Poisons List (No. 2) Order 1968 (S.I. 1968 No. 1682). Similarly the entry for mescaline has been extended to include other derivatives of phenethylamine formed by substitution in the aromatic ring. The choice of ring-substituted amphetamines for inclusion in the present study was limited by availability; those without ring-substitution were selected to show the effects of most simple substituents. The complete list is given in Table 2, optical isomers being indicated only where more than one was examined (structural formulae of these compounds are given by Beckett, Tucker & Moffat, 1967).

EXPERIMENTAL

Materials used were mostly commercial samples of the salts, apart from a series of *N*-substituted tryptamine salts which were supplied by the Chemical Defence Establishment. Most of the samples were examined by thin-layer chromatography (Phillips & Gardiner, 1969) and found to be substantially pure. Free bases were generally obtained by solvent extraction from an alkaline solution of the salt. In some instances, where the original material was either the free base or a salt of an organic acid, the hydrochloride was prepared by careful treatment of the base with dilute hydrochloric acid and evaporation to dryness. Many of the materials, both bases and salts, were treated with a variety of solvents and the solutions evaporated under varying conditions as a check for the incidence of polymorphism.

* This position is likely to be reversed by the Misuse of Drugs Bill currently before Parliament. In Part I of Schedule 2 to that Bill *NN*-dimethyl- and *NN*-diethyltryptamine are explicitly cited, whereas the 6- and 7-hydroxy-derivatives are not specified. In the same Bill the control of amphetamine-like substances will be modified to make explicit reference to a limited number of those drugs for which there is evidence of misuse.

Infrared spectra were recorded using a Grubb Parsons GS2 grating spectrometer. Solid samples were examined as mulls in Nujol (liquid paraffin B.P.) or as pressed discs using potassium chloride (A.R. quality) or potassium bromide (E. Merck A.G.,

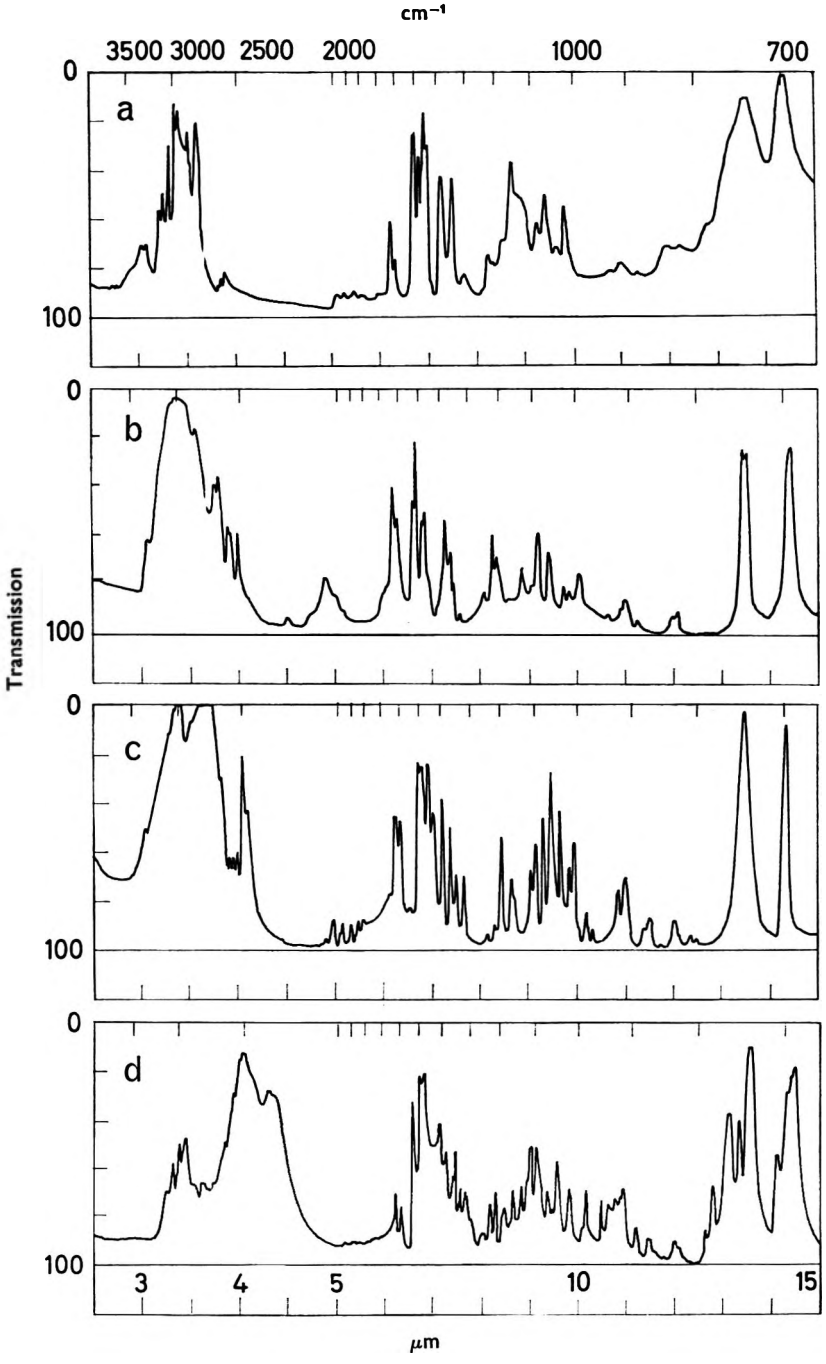

FIG. 1. Typical infrared spectra of amphetamines: (a) (+)-Methylamphetamine (liquid film). (b) (+)-Amphetamine hydrochloride (KCl disc). (c) (+)-Methylamphetamine hydrochloride (KCl disc). (d) Benzphetamine hydrochloride (KCl disc).

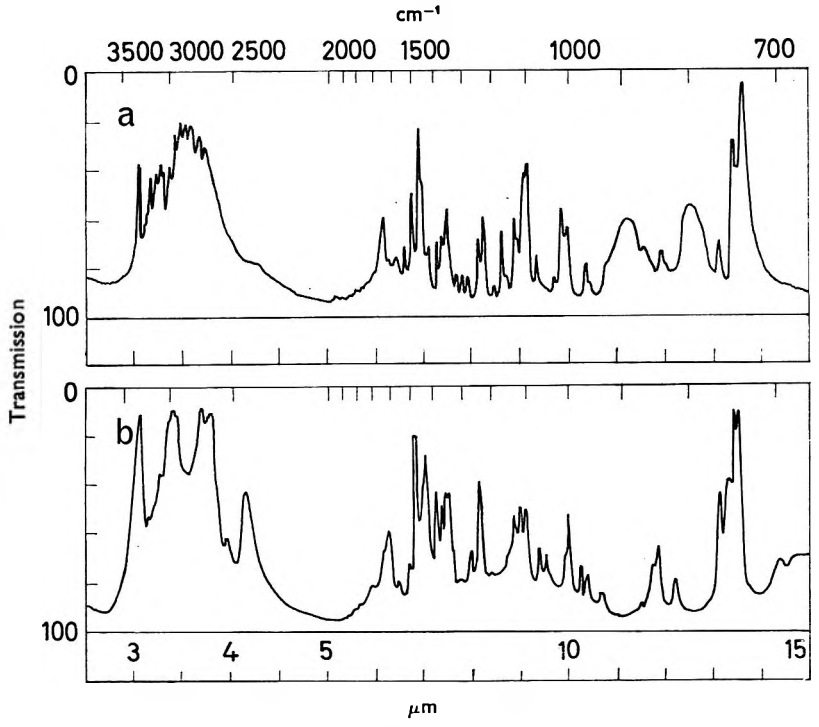

FIG. 2. Typical infrared spectra of tryptamines: (a) *N*-Methyltryptamine (KBr disc). (b) *N*-Methyltryptamine hydrochloride (KCl disc).

spectroscopic grade). Some typical spectra are shown in Figs 1 and 2. Spectra of many of the substances have been published elsewhere, details of which are given in Tables 1 and 2. Unless otherwise stated the spectra now obtained are in agreement with these published spectra.

RESULTS AND DISCUSSION

Characteristic absorptions of amphetamines

Aromatic absorptions (no ring substituent). The most prominent absorptions in the spectra of most of the simple amphetamines and their hydrochlorides are two strong bands at approximately 740 and 700 cm^{-1}, both of which may be slightly split in crystalline solids. These absorptions are characteristic of monosubstituted benzenes in general and are therefore not specific, but the amphetamines are the only significant group of drugs in which these are the strongest bands. From the examination of a large number of monoalkylbenzenes, all of which were liquid, Hawkes & Neale (1960) showed that the frequency of the 700 cm^{-1} band was not affected by the nature of the substituent, but that the higher frequency band (assigned to out-of-plane deformation of five adjacent C–H bonds) was sensitive to branching in the α-position of the alkyl chain; average frequencies were found to be 743, 761 and 764 cm^{-1} for CH_2R, CHR_1R_2 and $CR_1R_2R_3$ substituents respectively. In the present work, where the substituents are not simple alkyl groups, this effect is less predictable; all the compounds with a CH_2 group adjacent to the ring, with the exception of those mentioned below, absorb at 746–735 cm^{-1}, but the effect of hydroxyl substitution

Table 1. *Tryptamine derivatives examined*

Base	Salts	Published spectra: Base	Published spectra: Salt
Tryptamine	hydrochloride		a (21381)
5-Methyltryptamine	hydrochloride		
7-Methyltryptamine			
α-Methyltryptamine	hydrochloride		
N-Methyltryptamine	hydrochloride, hydrogen oxalate	a (31613)	
5-Hydroxytryptamine (serotonin, 5-HT)	hydrogen maleate	b	
5-Methoxytryptamine	hydrochloride	a (21683)	
5-Benzyloxytryptamine	hydrochloride		a (21272)
5-Hydroxy-*N*-methyltryptamine	hydrogen oxalate		
NN-Dimethyltryptamine	hydrogen oxalate	c, a (21676)	
5-Hydroxy-*NN*-dimethyltryptamine (Bufotenine)	hydrogen oxalate	d	a (10781)*
5-Methoxy-*NN*-dimethyltryptamine	hydrochloride		
5-Benzyloxy-*NN*-dimethyltryptamine	hydrochloride		a (29034)
4-Phosphoryloxy-*NN*-dimethyltryptamine (Psilocybin)			
Gramine		a (3824, 10608)	
5-Ethylgramine		a (33647)	
5-Methoxygramine	hydrochloride		
5-Benzyloxygramine		a (18488)	
N-Ethyltryptamine	hydrochloride		
N-n-Propyltryptamine	hydrochloride		
N-Isopropyltryptamine	hydrochloride		
N-n-Butyltryptamine	hydrochloride		
N-Benzyltryptamine	hydrochloride		
NN-Diethyltryptamine	hydrochloride	a (33656)	
NN-Di-n-propyltryptamine	hydrobromide		
NN-Di-isopropyltryptamine	hydrochloride		
NN-Di-n-butyltryptamine	hydrochloride		
NN-Pyrrolidinotryptamine	hydrochloride		
5-Benzyloxy-*NN*-pyrrolidinotryptamine	hydrochloride		a (29038)
Harmine	hydrochloride	c, a (3358)	
Harmaline	hydrochloride	a (2969)	a (2974)
Ibogaine	hydrochloride	a (909)*	a (923)

Notes (Tables 1 and 2):

* Published spectrum shows changes associated with preparation of KBr disc.

† Published spectrum is of a different optical isomer.

‡ Published spectrum not in agreement with that obtained in this work.

§ Base not isolated.

References: a—Sadtler Standard Spectra (serial number in parentheses); b—Clarke (1969); c—Cromp & Turney (1967); d—Stoll & others (1955); e—Chatten & Levi (1959); f—Sammul, Brannon & Hayden (1964); g—Hayden, Brannon & Yaciw (1966); h—Fazzari & others (1968); i—Phillips & Mesley (1969).

on the α-carbon is variable, giving frequencies in the range 761–738 cm^{-1}. Thus a band at 750 cm^{-1} or above indicates branching, but a frequency near 740 cm^{-1} does not necessarily imply an absence of branching.

Phentermine and mephentermine and their salts, which have two methyl groups on the second carbon from the ring, are anomalous in showing two bands at approximately 770 and 730 cm^{-1} in place of the expected 740 cm^{-1} band. The same effect was noted by Hawkes & Neale (1960) for hydrocarbons with two methyl groups on the β-carbon, and they concluded that the 770 cm^{-1} band was the aromatic C–H absorption. Whatever their origin, it appears that both bands are characteristic of

Table 2. *Phenethylamines examined*

Base	Salts	Published spectra: Base	Published spectra: Salt
(+)-Amphetamine	hydrochloride, sulphate		e, f, a (14721)*
(—)-Amphetamine	sulphate	a (34382)	a (34383)*
(±)-Amphetamine	hydrochloride, sulphate	a (134)	e, g, a (14722)*
(+)-Methylamphetamine	hydrochloride	g†	e, f, a (14723)
(±)-Methylamphetamine	hydrochloride		a (14724)‡
Benzphetamine	hydrochloride		f
Phentermine	hydrochloride	a (28933)	a (28934)
Mephentermine	hydrochloride, sulphate		
Phenylpropanolamine ((±)-norephedrine)	hydrochloride	g	f, a (27968)
(—)-Norephedrine	hydrochloride, sulphate		
Norisoephedrine	hydrochloride		
Ephedrine	hydrochloride	a (520)	e, a (7550)
Pseudoephedrine	hydrochloride		g
(—)-*N*-Methylephedrine	hydrochloride	a (15536)	
(±)-*N*-Methylephedrine	hydrochloride		
Phenylephrine	hydrochloride	g	f, a (15582)*
Noradrenaline	hydrogen tartrate	a (21296)†	a (1027)*
Adrenaline		b, a (7393)	
Isoprenaline§	sulphate		
Tranylcypromine	sulphate		
Phenmetrazine (*trans*)	hydrochloride	g	f
Phenmetrazine (*cis*)	hydrochloride		
Chlorphentermine	hydrochloride		h
Methoxyphenamine	hydrochloride		a (20485)
Methoxamine	hydrochloride		
3,4-Methylenedioxyamphetamine (MDA)	hydrochloride, sulphate	a (20160)	
3-Methoxy-4,5-methylenedioxy-amphetamine (MMDA)	hydrochloride		
2,5-Dimethoxy-4-methyl-amphetamine (DOM, "STP")	hydrochloride	i	i
Mescaline	hydrochloride, sulphate	c	f

benzenes with a single $CH_2 \cdot CMe_2 \cdot R$ substituent, and together with the 700 cm^{-1} band they are the most noticeable spectral features of such compounds.

Apart from these low-frequency absorptions, all the amphetamines have bands at approximately 1600, 1580, 1490 and 1450 cm^{-1}, due to ring stretching, and at least five sharp bands in the 1290–1015 cm^{-1} region due to C–H in-plane deformation.

Aromatic absorptions (ring-substituted). In place of the 740 and 700 cm^{-1} bands of the monosubstituted benzenes the following are found (values quoted are for individual compounds examined and are not necessarily typical).

1,2-Disubstitution (methoxyphenamine): very strong band near 750 cm^{-1} (four adjacent hydrogens).

1,3-Disubstitution (phenylephrine): strong band 780 cm^{-1} (three adjacent hydrogens), medium bands at 868 cm^{-1} (base) or 900 cm^{-1} (hydrochloride) due to isolated hydrogen, and at 700 cm^{-1} (ring deformation).

1,4-Disubstitution (chlorphentermine): strong band 810 cm^{-1} (pairs of adjacent hydrogens), also bands at 842 and 750 cm^{-1} (latter may correspond to 730 cm^{-1} band in phentermine).

1,2,4-Trisubstitution (adrenaline, isoprenaline, methoxamine, MDA): strong band 823–798 cm^{-1} (two adjacent hydrogens), other bands at 884–863 cm^{-1} (isolated hydrogen) and 782–772 cm^{-1} (latter replaced by 710 cm^{-1} in methoxamine).

1,2,3,5-Tetrasubstitution (mescaline, MMDA): Only isolated hydrogens, doublet at 813/803 cm^{-1} in MMDA, strong band 830 cm^{-1} in mescaline (two hydrogens equivalent in mescaline, not in MMDA).

1,2,4,5-Tetrasubstitution (DOM): Only isolated hydrogens, several bands around 870–840 cm^{-1}.

Absorptions associated with amine group. The nature of the bases is not readily determined from their spectra. The liquid amines, both primary and secondary, have broad N–H stretching bands near 3300 cm^{-1} in which NH and NH_2 absorptions are not distinguishable. In the solid phase the bands are generally sharper and as a rule the secondary amines show only one band, but the primary amines can have one, two or three bands. The liquid primary amines show a broad band near 850 cm^{-1}, but OH groups can also give similar bands; the liquid secondary amines have a broad band near 745 cm^{-1}, but this is generally obscured by the 740 cm^{-1} aromatic band. In the solid phase these bands cease to be recognizable, whilst tertiary amines have no characteristic absorptions at all, apart from those due to *N*-methyl groups (see below).

The amine salts are more clearly differentiated. All the primary amine hydrochlorides have a very strong broad band centred near 2950 cm^{-1} with several weaker bands between 2800 and 2450 cm^{-1} and frequently a band of medium intensity at 2060–1945 cm^{-1}. Secondary salts usually have two very strong bands near 2950 and 2730 cm^{-1}, a medium, sharp band at 2450 cm^{-1} and weaker bands at 2500 and 2030 cm^{-1}. The tertiary amine salts generally have a single strong, broad band in the range 2700–2450 cm^{-1}, sometimes accompanied by other weaker bands.

Methyl group absorptions. Methyl groups attached to carbon normally absorb at approximately 2960, 2870, 1460 and 1370 cm^{-1}, but the first three of these are liable to be obscured by CH_2 absorptions. However, the presence of the 1370 cm^{-1} band serves to distinguish amphetamines from the corresponding phenethylamines, provided there are no other *C*-methyl groups in the molecule (not applicable if examined as liquid paraffin mull). Thus mescaline and adrenaline, which contain *O*-methyl and *N*-methyl groups respectively but no *C*-methyl, have no band at 1370 cm^{-1}.

The presence of two methyl groups on the same carbon atom, as in the phentermine series, gives rise to two bands of equal intensity at about 1375 and 1360 cm^{-1}. There are also additional absorptions at 1180 cm^{-1} (liquids) or near 1170 and 1160 cm^{-1} (solids), but these are not very distinctive.

N-Methyl groups are clearly distinguishable by a fairly strong band at 2785–2770 cm^{-1}, absent in all other compounds (except MDA which has a weak band at 2780 cm^{-1} due to the $O–CH_2–O$ group). *O*-Methyl groups are not clearly distinguishable, the bands at 2825 and 1450 cm^{-1} being close to the normal CH_2 and CH_3 absorptions. The C–O absorptions, on the other hand, are very pronounced (see below).

Absorptions due to OH groups in side-chain. All the ephedrine-type compounds have a secondary hydroxyl group which gives characteristic absorptions in the salts. The O–H stretching absorption is a fairly strong band near 3300 cm^{-1}, and there is a second fairly strong band at 1055–1030 cm^{-1} (1080 cm^{-1} in phenylephrine hydrochloride) which is noticeably broader than the aromatic bands already present in this region.

In the free bases there is obviously strong intermolecular hydrogen bonding between

the OH group and the nitrogen atom, causing the O–H stretching band to appear as a very broad background extending from about 3300 to 2500 cm^{-1}, though phenylpropanolamine is exceptional in having two maxima at 3040 and 2725 cm^{-1}. There is another strong band, usually near 1080–1060 cm^{-1}, and the O–H out-of-plane deformation now appears as a broad band between 975 and 800 cm^{-1}, but there is often confusion between this and the corresponding N–H band.

Absorptions due to ether groups. Purely aliphatic ethers have a single very strong absorption near 1110 cm^{-1} (this is also true of cyclic ethers such as phenmetrazine), but when one of the two alkyl groups is replaced by benzene there are usually two bands at approximately 1250 and 1140 cm^{-1}, and these are largely due to stretching of the aromatic and aliphatic C–O bonds respectively. From the spectra of the various methoxy-substituted amphetamines it is apparent that the positions and intensities of these bands and of those in this region due to C–H deformation are affected by other substituents in the ring, and this has been confirmed by comparison with a large number of published spectra of aromatic ethers.

Thus methoxyphenamine, with a strong band at 1240 cm^{-1} and medium bands at about 1125, 1045 and 1025 cm^{-1}, is typical of *o*-substituted anisoles. Addition of a second methoxy group in the *para*-position relative to the alkyl group generally gives a strong band at 1210 cm^{-1} and a medium band at 1040 cm^{-1}, and of the intermediate bands that at 1150 cm^{-1} appears to be characteristic; methoxamine conforms to this pattern, though in the base the 1040 cm^{-1} band is enhanced by a contribution from the hydroxyl group, while the hydrochloride has two bands at 1048 and 1020 cm^{-1}. 2,5-Dimethoxy-4-methylamphetamine also has strong bands at 1210 and 1045 cm^{-1}, but with no intervening bands of appreciable intensity (for spectra see Phillips & Mesley, 1969).

Published spectra show that 1,3-dimethoxybenzene, with or without an additional 5-substituent, has a very strong absorption at about 1150 cm^{-1}, which is unusual for an aromatic ether. Mescaline follows this pattern with a very strong band at 1125 cm^{-1} attributable to the two methoxy groups which are *meta* to each other, but with an additional fairly strong band at 1240 cm^{-1} which may be due to the third methoxy group between the other two. MMDA, in which two of the methoxy groups are replaced by a methylenedioxy ring, also has its strongest band at 1130 cm^{-1} but without the 1240 cm^{-1} band (there are in addition medium bands at 1195, 1090 and 1040 cm^{-1}). MDA has the typical aromatic ether pattern of strong bands at 1240 and 1040 cm^{-1}, and is thereby distinguishable from 1,2-dimethoxy compounds which give two strong bands at 1265 and 1235 cm^{-1}. This is worth noting, as the presence or absence of *O*-methyl groups is difficult to establish directly.

General conclusions. The distinctive features noted above permit the identification of (i) the benzene ring, and the position of any other ring substituents; (ii) primary, secondary and tertiary amines (from spectra of the salts); (iii) methyl substitution on nitrogen, and on a side-chain carbon (distinction between phenethylamines and amphetamines); (iv) hydroxyl groups in the side-chain (distinction of ephedrines from amphetamines); (v) ether groups on the ring, and their position.

The only structural features not established with certainty are the length of the side-chain and the position of the amino-group. Thus the spectrum of norisoephedrine, in which the positions of the OH and NH_2 groups are interchanged, shows no anomalous features which might distinguish it from the ephedrine series.

Nevertheless, all the spectra examined (apart from those of optical isomers mentioned in the final section) are quite distinctive and could be used to identify any of these compounds by comparison with an authentic specimen.

Characteristic absorptions of tryptamines

Absorptions due to indole nucleus. The simple compounds with no substitution in the benzene ring all have a very strong band near 740 cm^{-1} (1,2-disubstituted benzene) and in most cases a prominent, fairly sharp band near 810 cm^{-1}. The latter is shown in the range 825–800 cm^{-1} by 18 of the 23 solid compounds examined, whilst three of the remainder have a similar peak at 789–783 cm^{-1}; the liquid bases all show a broad band near 800 cm^{-1}. Kanaoka, Ban & others (1960) have ascribed a band at 810–760 cm^{-1} in 3-substituted indoles to out-of-plane C–H bending in the 2-position. The 5-substituted compounds are less predictable, as the band due to two adjacent ring hydrogens also occurs near 810 cm^{-1}, giving either a single strong absorption or multiple bands. Elsewhere in the spectrum the indole nucleus is characterized by a multiplicity of bands between 1620 and 1300 cm^{-1}, particularly between 1400 and 1300 cm^{-1}.

NH absorptions. All the crystalline bases (except bufotenine, psilocybin and ibogaine), and also several of the liquids, are characterized by a remarkable broad absorption extending between approximately 3300 and 2500 cm^{-1}, on which may be superimposed as many as fifteen sharp peaks. This feature disappears in spectra of dilute solutions, being replaced by a single sharp peak at 3470 cm^{-1}, and is ascribed to the formation of a strong hydrogen bond linking the indole NH group with the side chain nitrogen of another molecule. The broad absorption thus represents the N–H stretching, the superimposed peaks being mainly overtones and combination bands intensified by Fermi resonance. This feature, though particularly noticeable in the tryptamine series, is also found in the gramines and in some compounds in which the substituent is on the benzene ring. It is absent in the salts, in which the lone pair of electrons on the side-chain nitrogen is no longer available as a proton-acceptor site, and these compounds show instead a single peak between 3400 and 3100 cm^{-1}. The actual position of this band depends upon the degree of substitution of the side-chain nitrogen: thus the primary amine salts absorb at 3290–3250 cm^{-1}, the secondary salts very close to 3400 cm^{-1} and the tertiary salts within the range 3320–3125 cm^{-1}. The reason for this dependence is not apparent.

The tryptamine bases containing NH or NH_2 in the side-chain show respectively one or two sharp peaks near 3300 cm^{-1}, which are well separated from the feature described above and are thus quite diagnostic. The hydrochlorides show the characteristic absorptions of primary, secondary and tertiary amine salts, as described for the amphetamine derivatives.

Other characteristic tryptamine absorptions. The spectra of the tryptamines are more complex than those of the amphetamines and it is therefore more difficult to assign individual absorptions, but certain bands may nevertheless be regarded as characteristic of the basic structure. Bands at 1330 (stronger than most neighbouring peaks), 1225, 1100 and 1010 cm^{-1} are prominent in the tryptamines with no ring substitution and in their salts. In the 5-substituted tryptamines the 1330 cm^{-1} band, though still present, ceases to be prominent, the 1225 cm^{-1} band is usually obscured by the strong ether absorption (see below) and the 1010 cm^{-1} band either disappears or becomes less prominent. The gramines, whether ring-substituted or not, all show

a prominent band near 990 cm^{-1} which is not found in the tryptamines, and this serves as a distinguishing feature.

Bands due to ring substituents. The methyl and benzyl ethers all have a prominent absorption near 1210 cm^{-1}, but the second ether band is not clearly defined. The benzyl group also gives additional absorptions at lower frequencies, particularly near 740 and 690 cm^{-1}. The corresponding phenols (5-hydroxytryptamine, bufotenine), also absorb near 1200 cm^{-1} in the amorphous forms usually obtained by extraction from neutralized solutions, though in crystalline bufotenine the strong band is at 1239 cm^{-1}. Bufotenine is readily distinguished from its ethers by a strong O–H stretching band at 3390 cm^{-1}, and in place of the complex band usually centred at 2900–2800 cm^{-1} there is a broad hump at 2560 cm^{-1}, presumably due to N–H . . . O bonds.

Psilocybin is exceptional as most of the strong absorptions are due to the phosphate group, and the spectrum is barely recognizable as that of a tryptamine derivative.

Bands characteristic of N-*alkyl groups.* In the series of *N*-alkyl and *NN*-dialkyltryptamines no correlations have been found which can be used to identify individual compounds, though all the spectra are clearly distinguishable from each other. The only identifiable alkyl substituent is the isopropyl group, which gives a band near 1165 cm^{-1} in a region usually free from absorption, though the anticipated double peak near 1370 cm^{-1} due to the CMe_2 group is obscured by other absorptions.

General conclusions. The features described permit the identification of an indole derivative containing a basic side-chain, and also indicate whether this base is primary, secondary or tertiary. The 810 cm^{-1} band appears to be specific for 3-substitution and the particular bands quoted above serve to distinguish the aminoethyl side-chain from others closely related. 5-Substituted derivatives can be distinguished without difficulty, so that the only groups not readily identifiable are the individual *N*-alkyl substituents. It should be noted that the correlations quoted here apply to the bases and their hydrochlorides, but the characteristic bands may be obscured in salts of organic acids, such as oxalates.

Polymorphism

Polymorphism can be a disturbing factor in the infrared identification of drugs (Mesley & Johnson, 1965; Mesley & Houghton, 1967; Mesley & Clements, 1968), but fortunately it seems to be rare amongst these compounds, the incidence in each class being greater in the salts than in the bases. 2,5-Dimethoxy-4-methylamphetamine was the only amphetamine base to give two forms (Phillips & Mesley, 1969), whilst phenylpropanolamine hydrochloride, tranylcypromine sulphate and phenmetrazine hydrochloride also exhibited the phenomenon. Potassium bromide discs of mescaline sulphate apparently show evidence of polymorphism: when prepared with a moderate amount of grinding the disc gives a spectrum consistent with mescaline hydrobromide and potassium sulphate, but on prolonged grinding further changes occur suggesting conversion to a second form of mescaline hydrobromide. This effect was not observed with potassium chloride discs.

Of the tryptamine bases only *NN*-dimethyltryptamine and 5-methoxy-*NN*-dimethyltryptamine yielded two forms. The hydrochlorides of 5-benzyloxytryptamine, 5-benzyloxy-*NN*-dimethyltryptamine and ibogaine all gave two forms, 5-methoxytryptamine hydrochloride and 5-hydroxytryptamine bimaleate three each and harmaline hydrochloride no less than five different forms.

Distinction between optical isomers

Samples of the salts of amphetamine, methylamphetamine, norephedrine and *N*-methylephedrine were available both in the optically active form and as the racemic mixture. As is to be expected, the (+)- and (−)-forms of amphetamine sulphate have identical spectra, but the (±)-form is significantly different. Obviously the (+)- and (−)-forms crystallize in lattices which have identical unit cell dimensions but which are mirror images of each other, and these cannot be distinguished by infrared spectroscopy. If these unit cells are not superimposable then the lattice structure of the racemic mixture is bound to be different, and this will cause differences in the infrared spectrum analogous to those caused by polymorphism. The (+)- and (±)-amphetamine hydrochlorides show similar differences, but on conversion to the respective bases, which are liquids, the differences disappear as the spectrum is no longer affected by any lattice constraints.

The hydrochlorides of (−)- and (±)-norephedrine and (−)- and (±)-*N*-methylephedrine are similarly distinguishable, as are the *N*-methylephedrine bases, which are both crystalline solids. In the case of the norephedrine bases the (±)-form (phenylpropanolamine) is crystalline but (−)-norephedrine remained liquid.

Methylamphetamine proved to be exceptional in that the (+)- and (±)-hydrochlorides gave the same infrared spectrum. Polarimetry confirmed that the two samples differed in optical activity and x-ray diffraction showed that they were crystallographically identical. It was therefore inferred that in this case the unit cells of the (+)- and (−)-forms can be superimposed, allowing the racemic form to crystallize with the same lattice structure, a conclusion which implies that the crystal symmetry is orthorhombic or higher.

The spectral differences between the (+)- and (±)-amphetamine salts are sufficient to allow them to be distinguished using as little as one milligram of material. Thus it should be possible to identify a single 5 mg dexamphetamine tablet, whereas the present B.P. test requires 20 tablets.

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Inhibition of enzymes by alkylsalicylic acids

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5-n-Alkylsalicylates inhibited a variety of enzymes that transform acidic substances, viz. glucose-6-phosphate dehydrogenase, glyoxalase, xanthine oxidase, carbonic anhydrase and D-amino-acid oxidase. Inhibitory potency rose to a peak at the n-nonyl derivative. None of the tests were of value, either singly or in combination, as screening processes for anti-inflammatory activity. The comparable results with a trinitrobenzaldehyde reagent suggest that the various inhibitions arise by non-specific association of the drugs with arginine and lysine residues in proteins.

Acidic anti-inflammatory agents at 10^{-3}M concentration will partially inhibit several enzymes *in vitro*. Skidmore & Whitehouse (1965) considered that inhibition of, for example, histidine decarboxylase, could arise by drug molecules associating with free amino-groups in the enzymes, so reducing their availability to pyridoxal co-enzyme. On this basis they devised a simple chemical test that attempted to relate anti-inflammatory activity to the degree of inhibition of a reddening when 2,4,6-trinitrobenzaldehyde reacted, presumably, with lysyl amino-groups in serum albumen. The test gave too many false positives to be of value for even rough screening purposes (cf. Phillips, Sancilio & Kurchacova, 1967).

Since the test gave positive results with well-known anti-inflammatory compounds, an attempt was made to eliminate at least some of the false positives by supplementary tests *in vitro*. Chemical tests will rarely be a measure of biological activity within a group of substances since they are usually a measure for a limited group of atoms within a molecule, whereas, biological activity is quantitatively dependent on the whole structure of the drug molecule. Nevertheless, a sequence of tests for different partial chemical structures within a molecule could give a degree of qualitative specificity by elimination. It was found that a combination of the trinitrobenzaldehyde test with enzymic inhibitory tests, notably that of glyoxalase, did not fulfil the expectation. This is illustrated by results with a homologous series of 5-n-alkylsalicylates in which there was a parallel between inhibitory potency in various tests with alkyl chain length. The compounds had no anti-inflammatory effect in animals.

EXPERIMENTAL

Methods

Glucose-6-phosphate dehydrogenase of rat adrenal was assayed in a Thunberg tube using an azine dye as H-acceptor (Doxiadis, Fessas & Valaes, 1961) or manometrically with ferricyanide as H-acceptor (Quastel & Wheatley, 1938). Other enzymes were prepared and assayed as described in the following references: glyoxalase of rabbit kidney (Platt & Schroeder, 1934), xanthine oxidase of milk (Dhungat & Sreenivasan, 1954), carbonic anhydrase of rat erythrocytes (Roughton & Booth, 1946), D-amino-acid oxidase of pig kidney (Bartlett, 1948), glutamic decarboxylase of guinea-pig brain (Roberts & Frankel, 1951) and succinic dehydrogenase of rat liver (Bernath & Singer, 1962).

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RESULTS AND DISCUSSION

The 2,4,6-trinitrobenzaldehyde (TNB) colour reaction with serum albumen. TNB, colourless when pure, soon turns red. It gives red products (maxima at 425, 525 nm) with various compounds but the chemistry can be obscure and complex. The products from TNB and albumen are unlikely to be simple aldimines. In a broad superficial survey of the reaction, none of a variety of amino-acids and their derivatives and polymers exceeded the tinctorial capacity of bovine serum albumen. There was large variation in albumens from different sources (Table 1). Egg albumen gave no

Table 1. *Chromogenicity of serum albumens with 2,4,6-trinitrobenzaldehyde.* Albumen (16 mg = 0·24 μmol bovine albumen), TNB (0·3 μmol in dimethylformamide), 0·1M sodium phosphate pH 7·5 (3 ml) at room temperature in the dark for 2 h. *E* taken at 425 nm. Bovine albumen = 100 (*E* =0·358–0·404). TNB control, *E*, 0·006.

Species	Colour intensity (% bovine)
Rabbit	74
Human	20
Guinea-pig	79
Sheep	109
Horse	32

colouration. The following comments are based on a large number of simple experiments, that need no detailed description. The red colour with albumen is probably due to a compound rather than a polynitro-complex. Absorption spectra in the visible range varied very little for the products of different reactants. No stoichiometric relation could be devised even for simple chemical reactants. (The test described in the literature specifies 1 mol TNB per mol albumen though the latter contains about 60 lysyl residues.) Contrary to hypothesis, TNB reacted more readily with guanidine (arginine) compounds than with lysine or its derivatives. Nevertheless, thermal polylysines and polyarginines had equivalent tinctorial capacity when prepared under similar conditions, the colour obtained being greater as the degree of polymerization increased. Clupeine gave a red colour. Conversion of lysyl amino-groups in egg white lysozyme to either guanidine or acetamidine (McCoubrey & Smith, 1966) had no effect on the chromogenicity of lysozyme. Other proteins, including various blood fractions, gave but weak colour development. Destruction of the guanidine groups of bovine serum albumen by malondialdehyde (King, 1966) or cyclohexan-1,2-dione (Toi, Byrum & others, 1967) virtually abolished colour formation and also the ability of albumen to associate with phenylbutazone in equilibrium dialysis experiments. Indomethacin would not inhibit colour development in the test by more than 60% at concentration up to 10^{-3} M.

The above results made it difficult to place the TNB test on a quantitative basis and there was no evidence to support the hypothesis that TNB reacted preferentially with lysyl amino-groups as opposed to arginyl residues.

Inhibition of glucose-6-phosphate dehydrogenase by anti-inflammatory agents. 5-n-Alkylsalicylates, among other substances, inhibited rat adrenal glucose-6-phosphate dehydrogenase when tested in a Thunberg tube assay using brilliant cresyl

blue as the final H-acceptor, and either glucose-6-phosphate or 6-phosphogluconate as substrate. The peak inhibitory activity was at the n-decyl derivative, (Table 2).

Table 2. *Inhibition of enzymes by 5-n-alkylsalicylates.* Drugs are at 10^{-3}M unless shown otherwise. Glucose-6-phosphate dehydrogenase assay with brilliant cresyl blue (Thunberg) or ferricyanide (manometric) as H-acceptor. Values for the Thunberg method are ratios of test to control times for dye bleaching. Values elsewhere are % inhibition. TNB = 2,4,6-trinitrobenzaldehyde test.

5-n-alkyl group	Glucose-6-phosphate dehydrogenase: Thunberg 10^{-3}M	Thunberg 10^{-4}M	Thunberg 10^{-5}M	Manometric	Glyoxalase	Xanthine oxidase	D-Amino-acid oxidase	Carbonic anhydrate	TNB
Methyl	1·0	—	—	—	—	—	—	—	23
Ethyl	1·0	—	—	—	7	17	11	18	45
Propyl	1·4	—	—	0	14	10	—	24	52
Butyl	>4	1·0	—	—	23	0	11	40	67
Pentyl	>4	1·1	—	0	54	13	46	49	64
Hexyl	>4	1·1	—	—	57	8	71	57	75
Heptyl	—	>4	1·0	14	73	12	100	63	72
Octyl	—	>4	1·0	23	78	42	100	73	83
Nonyl	—	>4	1·1	62	85	84	100	90	76
Decyl	—	>4	1·5	77	80	51	100	52	84
Dodecyl	—	>4	1·3	77	19	10	92	62	80
Tetradecyl	>4	1·1	1·0	0	0	12	96	36	67
Hexadecyl	>4	1·6	1·2	0	0	10	86	—	42
Octadecyl	>4	1·1	—	0	0	—	83	—	28
Myristate	>4	1·3	—	—	14	0	53	58	60

Confirmation by direct spectrophotometric measurement of the rate of NADPH formation was not possible due to strong absorption by the drugs at 340 nm, but since they roughly doubled the time needed for bleaching of brilliant cresyl blue (0·1 μmol) by NADPH (0·5 μmol) in the enzyme free system at 10^{-4}M, and preserved the colour for long periods at 10^{-3}M, it was inferred that the inhibition was an artefact due to formation of drug-dye complexes. In support of this conclusion, malic dehydrogenase was also apparently inhibited to the same degree by the decyl derivative in a Thunberg system. Solutions of excess drug with brilliant cresyl blue slowly turned purple and deposited a black amorphous powder during several days. The supernatant remained pale blue but this residual colour was attributed to impurities since the dye could readily be separated into at least six components on a cellulose column. Lauric, myristic and palmitic acids, and lauryl sulphate (10^{-3}M) were weak inhibitors of the system, though inactive by spectrophotometric assay.

Colour change with or without precipitation, or failure to function as H-acceptor in association with the salicylates, or both, were noted for seven other commercial dyes of the dibenz-1,4-oxazine, -thiazine and diazine type but not with celestine blue B, pyronin G or 2,6-dichlorophenolindophenol. Salicylate (10^{-3}M) did not interfere with the assay of rat liver succinic dehydrogenase using the diazine, phenazine methosulphate, as H-acceptor. The inhibition of xanthine oxidase by the alkylsalicylates was not reversed by added flavin adenine dinucleotide.

Peak inhibitory activity was found at the decyl derivative when glucose-6-phosphate dehydrogenase was assayed manometrically using ferricyanide as H-acceptor (Table 2).

It was concluded that there was a weak inhibition of glucose-6-phosphate dehydrogenase by 5-n-alkylsalicylates but this was increased in the anaerobic system by the formation of drug-dye complexes. Potent anti-inflammatory agents also inhibited glucose-6-phosphate dehydrogenase at 10^{-3}M in the Thunberg assay (Table 3).

Table 3. *Inhibition of enyzmes by anti-inflammatory drugs.* TNB = 2,4,6-trinitrobenzaldehyde test. Drugs are at 10^{-3}M unless indicated otherwise. Values for the Thunberg method are ratios of test to control times for dye bleaching. (1 = inactive). Values elsewhere are % inhibition.

Drug	Glucose-6-phosphate dehydrogenase: Thunberg 10^{-3}M	Glucose-6-phosphate dehydrogenase: Thunberg 10^{-4}M	Glucose-6-phosphate dehydrogenase: Manometric	Glyoxalase	Xanthine oxidase	Carbonic anhydrase	D-Amino-acid oxidase	TNB
Indomethacin	>4	1·8	12	65	30	15	48	56
Flufenamic acid	>4	2·2	27	65	75	0	60	61
Phenylbutazone	1·8	1·2	61	18	7	15	76	37
Ibufenac	1·4	—	0	0	0	0	0	27
Salicylate	0[3]	—	0	0[1]	0[2]	10	18	18
Glycyrrhetinic acid	1·7	—	0	43	0	18	21	10
Phenazone	1·4	—	0	19	0	0	10	7

[1] 0 at 10^{-2}M. [2] 37 at 10^{-2}M. [3] with preincubation 30 min/37°, 2·0.

Inhibition of glyoxalase by anti-inflammatory agents. In a different approach to anti-inflammatory testing, Whitehouse (1967) used methylglyoxal as a reagent. Table 3 shows that potent anti-inflammatory agents inhibited the conversion of methylglyoxal to lactate by rabbit liver glyoxalase. Milk xanthine oxidase, rat erythrocyte carbonic anhydrase and pig kidney D-amino-acid oxidase were also inhibited. By contrast with specific histidine decarboxylase, the pyridoxal dependent glutamic decarboxylase of guinea-pig brain was not inhibited up to 10^{-3}M concentration.

In contrast to xanthine oxidase and carbonic anhydrase, glyoxalase was completely inhibited by TNB at 10^{-3}M (80% at 10^{-4}M, 0 at 10^{-5}M). Bovine albumen tended to reverse the inhibition of glyoxalase by indomethacin but further investigation of this finding was not informative since the protein inhibited the enzyme at high concentrations.

The anti-glyoxalase test, like the TNB test, was found to give too many false positives when seeking anti-inflammatory activity in new compounds. The test and the TNB test were not complementary in the manner sought for. There was indeed a striking parallel between inhibitory potency in four *in vitro* tests and the chain lengths of 5-n-alkylsalicylates (Table 2). It could be reasonably concluded that the salicylates, acidic anti-inflammatory agents, and TNB can occlude from other reagents, the basic side chains of lysyl and arginyl residues in proteins, whether these be albumen or enzymes. This could occur in a non-specific manner and so include those special instances where a particular arginine or lysine residue is essential to biological activity. All the enzymes examined are concerned with transformation of acids. By inference, arginine or lysine residues, or both, may be involved in anti-inflammatory activity or inflammation. It is notable, however, that bradykinin (2 μg), a α,ω-*bis*-guanidine gave no trace of colour with TNB. It is interesting that while association with blood

proteins is usually considered to be detrimental to drug activity, the TNB test, a measure of drug-protein association, was proposed as a measure of pharmacological activity. This factor is a possible reason why the 5-n-alkylsalicylates had no anti-inflammatory activity. They are known to form stronger associations with albumen than does salicylate (Davison & Smith, 1961) and this was confirmed in preliminary experiments with the 5-n-decyl derivative.

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Some observations on the use of fatty alcohols and fatty acids to increase the consistency of oil-in-water emulsions

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A range of fatty alcohols and fatty acids of different chain lengths and chemical configuration has been examined to determine which of these may be usefully employed to increase the consistency of liquid paraffin-in-water emulsions stabilized by condensed complex films. Of the alcohols, a straight chain of 16 carbon atoms (cetyl alcohol) or a physical mixture of straight chains of 16 and 18 carbon atoms (cetostearyl alcohol) gave maximum bodying action. To a lesser extent a straight chain of 14 carbon atoms (myristyl alcohol) and in some cases a straight chain of 12 carbon atoms (lauryl alcohol) also increased the consistency of emulsions. Other homologues (C_8, C_{10} and C_{18}), unsaturated or branched chain alcohols and fatty acids were, in general, found to be of little value for this purpose.

Martin (1960) made the broad statement that fatty alcohols and fatty acids could be used to increase the consistency of oil-in-water emulsions. Both the pharmaceutical and cosmetics industries employ a wide range of these materials but only oleic acid, stearic acid and cetostearyl alcohol are the subjects of official monographs. The latter is well known as a consistency improver and is used in conjunction with sodium lauryl sulphate, cetrimide or cetomacrogol 1000 to form self-bodying waxes. This paper is concerned with investigations into which of the fatty alcohols and acids could usefully be employed to increase the consistency of oil-in-water emulsions stabilized by condensed complex films.

EXPERIMENTAL

Materials. Octyl, decyl, lauryl, myristyl, cetyl and stearyl alcohols (Laurex 8–18) (from Cyclochemicals Ltd., London), oleyl alcohol (BDH, Poole), octyldodecyl alcohol (Henkel International Gmbh., Dusseldorf), octoic and decoic acids (BDH, Poole), lauric, myristic, palmitic, stearic, behenic and erucic acids (Univol U. 314B, 320, 332, 334, 344 and 342) (Universal Oil Co. Ltd., Hull) were all commercial grades and were used without purification. All other materials conformed to the requirements of the B.P. or B.P.C.

Methods. These are as previously described (Talman, Davies & Rowan, 1967, 1968).

RESULTS AND DISCUSSION

The apparent viscosities and static yield values (as defined by Talman & others, 1967) of emulsions containing a range of fatty alcohols together with cetomacrogol 1000 or cetrimide at 0·5 or 5·0% w/w concentrations are shown in Table 1. It will be

Table 1. *Apparent viscosities (η_{100} in centipoises) and static yield values* [SYV in mNm^{-2} (dynes/cm^2)] *of 50% w/w liquid paraffin emulsions containing varying concentrations of fatty alcohols (% w/w) with 0·5 and 5·0% w/w cetomacrogol 1000 or cetrimide*

Fatty alcohol	Cetomacrogol 1000 (0·5%)												Cetrimide 0·5%											
	Concentration of fatty alcohol in emulsion												Concentration of fatty alcohol in emulsion											
	1%		2%		4%		6%		8%		10%		1%		2%		4%		6%		8%		10%	
	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV
Oleyl alcohol	11	0	16	0	18	0	21	0	25	0	27	0	13	0	11	0	13	0	14	0	20	0	21	0
Octyldodecyl alcohol	14	0	14	0	19	0	20	0	25	0	29	0	10	0	14	0	10	0	17	0	25	0	30	0
Octyl alcohol	14	0	14	0	17	0	18	0	22	0	24	0	14	0	14	0	16	0	16	0	24	0	34	0
Decyl alcohol	16	0	16	0	15	0	17	0	21	0	24	0	14	0	14	0	19	0	20	0	24	0	30	0
Lauryl alcohol	15	0	15	0	16	0	18	0	21	0	28	0	39	151	64	352	58	477	74	503	79	691	94	754
	0·25%		2%		4%		6%		8%		10%		0·25%		2%		4%		6%		8%		10%	
Myristyl alcohol	11	0	23	0	74	302	128	1131	166	2512	229	3392	12	0	67	553	87	628	190	1583	300	3517	334	6030
	0·25%		0·75%		1·5%		2·5%		4·0%		7·0%		0·25%		0·75%		1·5%		2·5%		4·0%		7·0%	
Cetyl alcohol	13	0	18	0	57	226	156	854	316	2456	447	3581	14	0	21	0	61	377	139	1005	265	2362	361	6287
Cetostearyl alcohol	11	0	15	0	51	276	126	905	275	2337	468	3895	11	0	15	0	46	251	130	1005	269	2412	638	6659
	0·15%		0·25%		0·75%		1·5%		2·5%		4·0%		0·15%		0·25%		0·75%		1·5%		2·5%		4·0%	
Stearyl alcohol	14	0	11	0	18	0	88	452	120	704	/		13	0	15	0	20	0	49	x	125	≈829	177	≈1508
	Cetomacrogol 1000 (5·0%)												Cetrimide (5·0%)											
Oleyl alcohol	33	0	37	x	44	x	53	x	64	x	76	x	17	0	19	0	82	x	139	x	186	≈1382	228	≈1809
Octyldodecyl alcohol	32	0	36	x	44	x	51	x	54	x	70	x	20	0	24	0	28	0	32	x	38	x	121	≈377
Octyl alcohol	35	0	40	x	40	x	48	x	54	x	65	x	23	0	37	0	144	3618	X	X	X	X	X	X
Decyl alcohol	31	0	33	x	38	x	47	x	50	x	61	x	21	0	37	0	182	904	X	X	X	X	X	X
Lauryl alcohol	28	0	22	0	46	x	55	x	65	x	83	x	14	0	14	x	183	930	X	X	X	X	X	X
	0·25%		2%		4%		6%		8%		10%		0·25%		2%		4%		6%		8%		10%	
Myristyl alcohol	30	0	50	x	94	226	133	578	320	2375	324	5528	22	0	34	x	177	879	329	4184	X	X	X	X
	0·25%		0·75%		1·5%		2·5%		4·0%		7·0%		0·25%		0·75%		1·5%		2·5%		4·0%		7·0%	
Cetyl alcohol	35	0	40	0	66	x	84	201	128	377	433	3882	21	0	24	0	30	0	44	x	94	376	363	2940
Cetostearyl alcohol	33	0	36	0	63	x	94	151	141	276	393	3015	16	0	20	0	28	0	44	101	87	126	343	2952
	0·15%		0·25%		0·75%		1·5%		2·5%		4·0%		0·15%		0·25%		0·75%		1·5%		2·5%		4·0%	
Stearyl alcohol	30	0	35	0	40	0	64	x	95	≈201	133	≈251	18	0	20	0	22	0	26	x	41	x	69	≈188

≈ = Mean static yield value. x = Pseudoplastic. X = Traces uninterpretable. / = Sample contained waxy particles.

noted that although the type and concentration of the water-soluble surfactant affected the absolute magnitude of the flow parameters, the only alcohols that produced a marked increase in the consistency of preparations were cetyl and cetostearyl alcohols and to a lesser extent myristyl alcohol.

Emulsions containing more than 4·0% w/w stearyl alcohol were difficult to prepare and as they contained waxy particles of the alcohol their apparent viscosities and static yield values could not be determined. Preparation of these samples at 70°, i.e. 10° above that used in the standard procedure, did not reduce the incidence of waxy particles. As noted previously (Talman & others, 1967) products containing lauryl alcohol and cetrimide were thicker than corresponding emulsions prepared with lauryl alcohol and cetomacrogol 1000. Increasing amounts of oleyl, octyldodecyl, octyl and decyl alcohols with either surfactant at 0·5% w/w gave products of slightly increased viscosity but all were Newtonian fluids. Comparison of these results with those where the oleyl alcohol content was kept constant but the quantity of liquid paraffin altered, suggested that the rise in viscosity could be attributed to an increase in total disperse phase (Fig. 1). With all four alcohols, cetomacrogol 1000

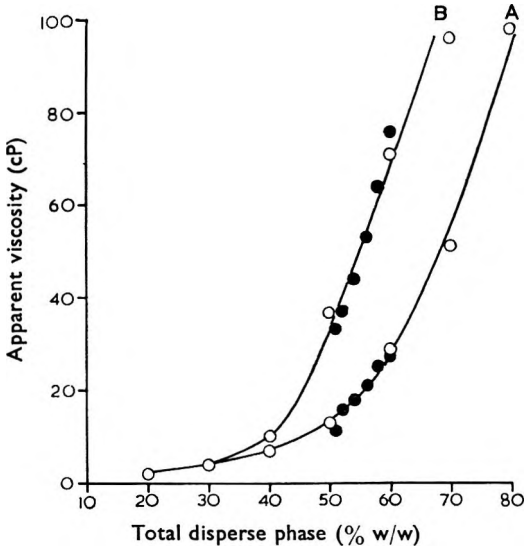

FIG. 1. Effect of varying total disperse phase (% w/w) on apparent viscosity of emulsions containing 0·5 (A) or 5·0% w/w (B) cetomacrogol 1000. ○, 2% w/w oleyl alcohol with varying amounts of liquid paraffin. ●, 50% w/w liquid paraffin and varying amounts of oleyl alcohol.

(5·0% w/w) produced emulsions of greater apparent viscosity than those containing 0·5% w/w; some were pseudoplastic and the results were again comparable with those from preparations of constant fatty alcohol content but varying disperse phase. This too has been illustrated in Fig. 1. As reported previously (Talman & others, 1968), the data for emulsions containing oleyl alcohol could not be correlated with emulsifier concentration, phase volume ratio or globule size distribution by expressions of the type proposed by Sherman (1959, 1963). Octyl and decyl alcohols were included to extend the range of chain lengths investigated. In addition to their having little value as consistency improvers, both have unpleasant odours and are therefore of little interest in practice.

The preparation of products containing high concentrations of $C_8 - C_{14}$ straight chain alcohols together with 5·0% w/w cetrimide gave emulsions so viscous during the initial stages of preparation that they could not be passed four times through the Q.P. homogenizer as was the normal procedure. The rheograms obtained with the Ferranti-Shirley viscometer were irregular, neither a reliable apparent viscosity nor static yield value could be determined from the traces and visual examination of the samples suggested that this irregularity might be due to marked visco-elastic properties. Visco-elasticity has been observed and measured in related systems by Barry (1968) and Davis (1969). Continuous shear methods are not applicable to the measurement of the fundamental rheological parameters of visco-elastic systems (Warburton & Barry, 1968). Nevertheless, as noted by Barry (1969), instruments employing such methods, e.g. the Rotovisko and Ferranti-Shirley viscometers, are valid tools for investigating trends in the behaviour of a series of samples. As equipment for examining elastic properties was not available this latter aspect of the flow behaviour of our samples could not be studied.

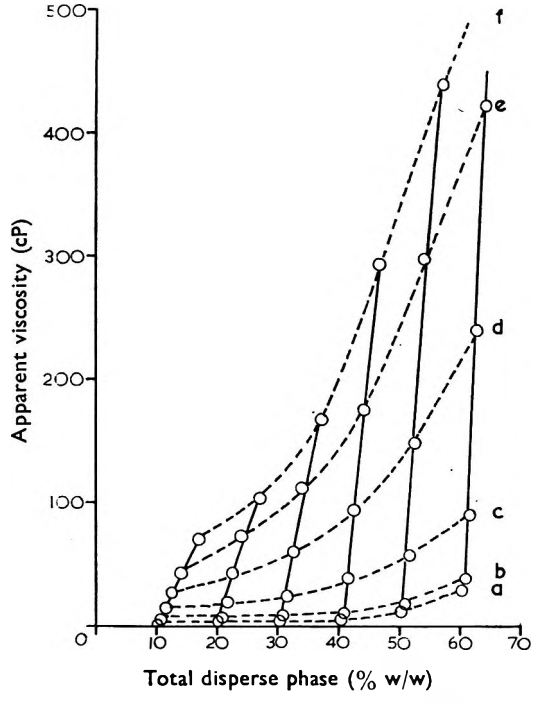

FIG. 2. Effect of varying total disperse phase on apparent viscosity of emulsions prepared with cetostearyl alcohol and 0·5% w/w cetomacrogol 1000. ○—○, Constant liquid paraffin content. ○- - - ○, Constant cetostearyl alcohol content. a, b, c, d, e and f are 0·25, 0·75, 1·5, 2·5, 4·0 and 7·0% w/w cetostearyl alcohol in the emulsion.

The effects of varying the amounts of both cetostearyl alcohol and liquid paraffin on the apparent viscosities and static yield values of a range of emulsions are shown in Figs 2 and 3 respectively. They clearly show that increasing the total disperse phase with this alcohol had a greater effect on the flow parameters than the addition of a comparable amount of liquid paraffin. This may be contrasted with the behaviour of emulsions containing oleyl alcohol (Fig. 1). The latter amphiphile does not form a gel in the continuous phase (Talman & others, 1967).

Table 2. *Apparent viscosities (η_{100} in centipioses) and static yield values* [SYV in mNm^{-2} (dynes/cm^2)] *of* 50% *liquid paraffin emulsions containing varying concentrations of fatty acids (% w/w) with* 0·5 *and* 5·0% *w/w cetomacrogol* 1000 *or cetrimide*

Fatty acid	Cetomacrogol 1000 (0·5%)												Cetrimide (0·5%)									
	Concentration of fatty acid in emulsion												Concentration of fatty acid in emulsion									
	0·25%		1·0%		2·0%		3·0%		5·0%		7·0%		0·25%		1·0%		2·0%		3·0%		5·0%		7·0%	
	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV
Oleic acid	12	0	10	0	12	0	13	0	16	0	20	0	12	0	7	0	10	0	10	0	12	0	15	0
Erucic acid	10	0	12	0	14	0	17	0	19	0	19	0	11	0	11	0	13	0	15	0	16	0	19	0
Octoic acid	12	0	14	0	12	0	12	0	15	0	18	0	12	0	13	0	9	0	9	0	12	0	13	0
Decoic acid	11	0	12	0	10	0	13	0	13	0	16	0	12	0	12	0	12	0	14	0	17	0	17	0
Lauric acid	10	0	14	0	9	0	13	0	15	0	19	0	10	0	12	0	12	0	12	0	16	0	20	0
Myristic acid	10	0	11	0	13	0	/		/		/		11	0	11	0	13	0	12	0	16	0	/	
Palmitic acid	11	0	13	0	14	0	/		/		/		10	0	17	0	44	x	/		/		/	
Stearic acid	10	0	14	0	15	0	/		/		/		8	0	23	0	/		/		/		/	
Behenic acid	/		/		/		/		/		/		7	0	/		/		/		/		/	
	Cetomacrogol 1000 (5·0%)												Cetrimide (5·0%)									
Oleic acid	27	0	29	0	27	0	30	0	37	x	50	x	20	0	22	0	20	0	23	0	26	0	78	x
Erucic acid	26	0	25	0	27	0	27	0	37	0	44	0	20	0	19	0	22	0	28	0	45	0	185	x
Octoic acid	30	0	31	0	31	0	34	0	43	0	45	0	14	0	19	0	29	0	31	0	107	x	X	X
Decoic acid	9	0	27	0	30	0	32	0	35	0	46	0	15	0	20	0	25	0	38	x	137	879	X	X
Lauric acid	27	0	29	0	32	0	33	0	35	0	41	0	16	0	13	0	46	0	84	314	X	X	X	X
Myristic acid	26	0	27	0	32	0	36	0	/		/		14	0	24	0	27	0	46	x	152	603	250	4020
Palmitic acid	25	0	29	0	40	0	42	0	91	x	113	x	11	0	17	0	37	x	53	x	286	1809	293	4146
Stearic acid	27	0	42	x	72	x	104	x	/		/		16	0	24	0	29	0	52	x	92	603	200	2198
Behenic acid	27	0	30	0	40	x	49	x	/		/		19	0	26	0	29	0	54	x	/		/	

/ = Samples unstable and contained waxy particles. x = Pseudoplastic. X = Traces uninterpretable.

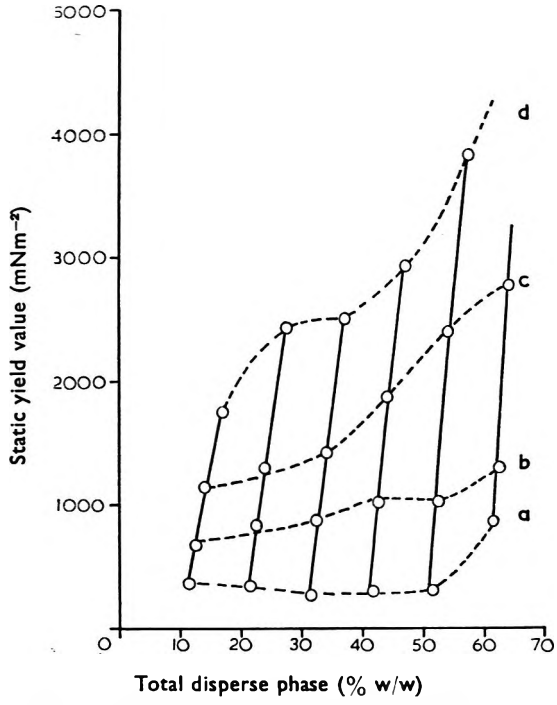

FIG. 3. Effect of varying total disperse phase on static yield value of emulsions prepared with cetostearyl alcohol and 0·5% w/w cetomacrogol 1000. ○—○, Constant liquid paraffin content. ○ – – – ○, Constant cetostearyl alcohol content. a, b, c and d are 1·5, 2·5, 4·0 and 7·0% w/w cetostearyl alcohol in the emulsion.

Table 2 shows data for comparable emulsions containing a range of fatty acids. The flow properties of preparations containing 5·0% w/w cetrimide were similar to those made with the alcohols; high concentrations of myristic, palmitic or stearic acids produced the most marked increases in consistency. However, such preparations are of little use for topical application as high concentrations of cetrimide can cause drying and sensitization of the skin (Martindale, 1967). It was only with 5·0% w/w cetrimide that the acids could be induced to form gels (Table 3) comparable in strength to those produced by the alcohols and which were reported elsewhere (Talman & others, 1967, 1968).

Table 3. *Apparent viscosities* (η_{100} *in centipoises*) *and static yield values* [SYV in mNm^{-2} (dynes/cm²)] *of gels containing* 10% *w/w fatty acids and* 0·5 *or* 5·0% *w/w cetomacrogol* 1000 *or cetrimide*

Fatty acid	Cetomacrogol 1000 (% w/w) 0·5		5·0		Cetrimide (% w/w) 0·5		5·0	
	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV	η_{100}	SYV
Oleic acid	1	0	4	0	1	0	7	0
Erucic acid	1	0	3	0	1	0	41	x
Lauric acid	/		/		17	239	165	1897
Myristic acid	/		/		14	x	102	1168
Palmitic acid	/		/		/		36	415
Stearic acid	/		/		/		18	176
Behenic acid	/		/		/		/	

/ = Samples contained waxy particles.
x = Pseudoplastic.

Samples with high concentrations of octoic, decoic or lauric acids and 5·0% w/w cetrimide were difficult to prepare on account of the high viscosity of the mixture of phases before homogenization and the rheograms could not be interpreted due to visco-elastic effects. Waxy particles were present in emulsions with the 5·0% w/w concentration of cetrimide and more than 5·0% w/w behenic acid. The phases employed for the preparation of emulsions containing 0·5% w/w cetrimide or 0·5 or 5·0% w/w cetomacrogol 1000 together with the acids did not readily emulsify whilst the final products were either thin fluids or contained waxy particles. Here too, preparation at 70° did not yield products free from particles of fatty acid. It should be remembered that stearic and some other acids can be used in a partially neutralized form to produce bodied emulsions. Spalton (1953) gives a number of examples but such products were not included in the present investigation.

These results demonstrate that contrary to Martin's general statement on the ability of fatty acids and alcohols to increase the consistency of emulsions, only a limited number of these are useful for this purpose. The fluid amphiphiles have little or no bodying action whilst some solid amphiphiles give rise to waxy particles in the aqueous phase of the emulsion. The actual self-bodying mechanism involved has not been investigated for the preparations discussed here, but it is reasonable to suppose that transfer of fatty acid or alcohol from the oil to aqueous phase took place as was postulated by Talman & others (1967) for cetostearyl alcohol and, in some cases, lauryl alcohol. If transfer was followed by interaction with the surfactant in the aqueous phase a semi-solid preparation was obtained, but where this interaction did not occur waxy particles of amphiphile were formed. The requirements for self-bodying action, based on the concepts of amphiphile transfer to and interaction in the aqueous phase of an emulsion, have been discussed by Barry (1969). Further studies (Talman & Rowan, 1970) suggest that self-bodying action is related to the penetrability of the fatty amphiphile by the surfactant. Palmitic acid (m.p. 63–64°) is not penetrated by a 0·5% w/w cetrimide solution nor does it interact with that solution at temperatures above its melting point. This explains the occurrence of waxy particles in corresponding emulsions even when a temperature of 70° is used for preparation. Generally, penetration is more likely to occur and is more extensive with the low molecular weight ionic surfactants at high concentration and this is reflected in the properties of the emulsions.

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The effect of granule properties on the pore structure of tablets of sucrose and lactose

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Air permeability and liquid penetration of tablets of sucrose and lactose have been measured. The tablets had been compressed over a wide pressure range from granules which varied in bulk density, size and strength. The degree to which inter- and intra-granular pore structure within the tablet was sustained varied with these properties; low pressure, high density, high strength and large size promoting a more open but less uniform structure. Such structures allowed rapid penetration of liquid through a coarse pore network which isolated a large fraction of the total pore space. Thus tablets of high permeability gave low final degrees of saturation whereas less permeable tablets became fully saturated.

The initial stage in the dissolution of a compressed tablet is the penetration of the structure by the dissolution medium by way of a network of pores. To ensure the effective disruption which is necessary for fast dissolution, penetration should be even and quick.

The pore network of a tablet is exceedingly complex. Capillaries run through in a tortuous random manner, their cross-section varying in both area and shape. Interconnection is profuse and some capillaries may be blind. The network originates in two ways. The first is from the voids present within the granules themselves. These will be referred to as intragranular pores and their size and shape will depend primarily on the size and shape of the particles composing the granules, and the method of granulation. A network of larger, intergranular pores is derived during compression from the spaces between the granules, the size and friability of the granules mainly determining its structure.

The duality of pore structure will be lost, in part at least, during compression when, by the processes of fragmentation and consolidation, granule integrity is progressively lost. These processes will be least effective in tablets prepared from granules which are both large and strong, when resistance to deformation will partly sustain a coarse, intergranular pore network. If, on the other hand, granules are easily deformed, this effect will be absent, the distribution of pore sizes will become more uniform and the properties of the tablet determined by pore structure will not depend upon the properties of the granules.

In this study some aspects of the pore structure of tablets have been examined using air-permeability and liquid-penetration techniques on tablets prepared from sucrose and lactose granules.

Theoretical considerations

One analysis of flow in complex pore systems originates with the work of Kozeny (1927) and Carman (1937). Viscous flow in a capillary of non-circular cross-section was evaluated by means of an hydraulic radius, m, this radius being the ratio of the

cross-sectional area of the capillary to its perimeter. The equation describing flow is then

$$u = \frac{m^2}{k_0 \eta} \frac{dP}{dL} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where u is the flow velocity, η is the viscosity of the fluid and dP/dL is the pressure gradient, k_0 is a constant depending on the shape of the section. If it is circular, for example, $k_0 = 2$ and equation (1) becomes Poiseuille's Law.

If the pore space of a tablet is regarded as a bundle of capillaries, it too may be characterized by an hydraulic radius which will be determined by the total cross-section of the pores, their total perimeter and their size distribution. If the pore structure is reasonably uniform, this radius is equal to the ratio of the porosity and the specific surface. If, on the other hand, a wide distribution of pore sizes is present, the contribution of the fine pores, which give much of the internal surface, to flow within the tablet is negligible. A quite disproportionate amount of fluid passes through the coarse pores and experimental values of the mean hydraulic radius derived from equation (1) greatly exceed those calculated from porosity and specific surface.

The permeability of tablets. The volumetric flow rate Q of a fluid passing through a tablet of area A and thickness L is given by the equation

$$Q = \frac{V}{t} = -\frac{B_0 A}{\eta} \frac{dP}{dL} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where η is the viscosity of the fluid and dP/dL is the pressure gradient within the tablet. The constant B_0 is the permeability coefficient. Since Q is related to the velocity of flow by $Q = uA\epsilon$, A being the area of the tablet and ϵ its porosity, equation (2) can be written

$$u = -\frac{B_0}{\eta \epsilon} \frac{dP}{dL}$$

Comparison with equation (1) shows that

$$B_0 = \frac{\epsilon m^2}{k_0} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

In measuring this coefficient by gas permeation, the flow rate varies from point to point within the tablet due to expansion of the gas as it moves from high to low pressure. Since the volume V of a gas is inversely proportional to the pressure, P:

$$Q = \frac{V}{t} = \frac{k}{Pt}$$

Substitution and rearrangement of equation (1) gives

$$\int_{P_1}^{P_2} P dP = -\frac{k\eta}{B_0 t A} \int_{O}^{L} dL$$

from which we derive the equation:

$$Q = \frac{B_0 A}{2L\eta} \cdot \frac{(P_1^2 - P_2^2)}{P_1} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where Q is the flow rate measured at the upstream pressure P_1, and P_2 is the downstream pressure.

Penetration of liquids into a tablet. The force driving a liquid into a tablet is derived from the pressure differential ΔP associated with the curved liquid meniscus formed as the liquid enters the capillary. This force is determined by the capillary size, the contact angle θ between liquid and solid and the surface tension of the liquid, γ. The rate of penetration is dictated by the balance of this force and the opposing viscous resistance as the liquid moves through the capillary. Since the latter increases with penetration whereas the former remains fairly constant, penetration rate will fall as saturation proceeds. The capillary pressure developed in the tablet is given by Carman (1941)

$$\Delta P = \frac{\gamma \cos \theta}{m} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5)$$

At some time t, the liquid has penetrated a distance L. The velocity of penetration u, is given by equation (1).

$$u = \frac{dL}{dt} = \frac{m^2}{k_0 \eta} \frac{\Delta P}{L}$$

Substituting for ΔP by means of equation (5)

$$\frac{dL}{dt} = \frac{m\gamma \cos \theta.}{k_0 \eta L} \quad \text{At } t = 0,\ L = 0: \int_0^L L dL = \frac{m\gamma \cos \theta}{k_0 \eta} \int_o^t dt$$

Therefore

$$L^2 = \frac{2m\gamma \cos \theta}{k_0 \eta} t \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

Since the total cross-sectional area of the capillaries does not vary with L, the volume of liquid taken up will be proportional to the length of penetration. Hence, as first shown by Washburn (1921), there is a linear relation between the square of the volumetric uptake and the time.

EXPERIMENTAL

Granulation

Lactose and sucrose, both as fine powders, were massed with water in a Z-blade mixer and then forced through coarse screens. The granules were dried to constant weight at 70° and rescreened. The amount of water used during massing was varied. Four lactose batches were prepared with the water content varying in four equal steps from 13 to 25% of the dry powder weight. With sucrose, the water content was 5, 7 or 9%. The granules were sieved and the mesh fractions: $-8 + 16$, $-16 + 22$, $-30 + 44$ and $-60 + 85$ selected for study.

Compression

The tapped bulk density of each fraction was determined in a 50 ml cylinder, the internal diameter of which corresponded approximately to the diameter of the punch and die (19·17 mm) used for compression. A weighed quantity of granules was

placed in the lubricated die, sealed at one end by a spigot. The upper punch was inserted and the assembly compressed over a pressure range 9–105 MN m^{-2} between the platens of a hydraulic press. The compaction force was measured in a manner described by Shotton & Ganderton (1960) by means of strain gauges affixed to the shank of the punch. The porosity achieved at any pressure was calculated from the weight and volume of the tablet whilst still retained in the die. Measurements were therefore taken when the tablet was under some radial constraint. These conditions were reproduced in the permeability and penetration studies by testing the tablet in the die. This ensured a good seal between tablet and die in these tests and also allowed very friable tablets to be studied, advantages which outweighed the account of the very slight expansion suffered by the tablet during ejection.

Permeability and penetration tests

The permeability of the tablets was measured with an apparatus similar to that designed by Lea & Nurse (1939). A capillary flowmeter was used to measure the rate at which dry air was drawn through the tablet. Pressures promoting flow varied from 270 N m^{-2} to 65 kN m^{-2} and were measured by manometers containing kerosene or mercury. All manometers were fitted with one wide limb to facilitate reading and the other sloped to increase accuracy. The permeability coefficient was calculated from equation (4).

The rate at which a liquid penetrated the tablet was measured by moving the tablet to a position at which its lower surface was flush with the bottom of the die. This was placed in a cup which formed one arm of liquid-filled U-tube and the rate of uptake measured as the withdrawal from the other arm. Cyclohexane was used in all experiments to avoid the dissolution or disruption of the tablets.

RESULTS

From simple crushing tests with a spatula, it was observed that increase in the amount of the massing liquid increased the strength of the granules. This effect was most marked with the stronger sucrose granules.

The tapped bulk density of the granules varied little with their size, increasing only slightly as the size decreased. On the other hand, increase in the water content during massing markedly increased the bulk density, the porosity changing by up to 6% in the sucrose series and up to 10% in the lactose series.

With lactose, neither granule size nor massing water concentration influenced the relation between porosity and compaction force. The porosity of sucrose tablets produced at any pressure level increased as the size and massing concentration decreased. For example, at 27 MN m^{-2}, the porosity of sucrose tablets prepared from fine granules massed with 9% water was 4% less than the corresponding tablet massed with 5% water.

The relation between permeability and porosity for lactose massed with 13% water is shown in Fig. 1A. A permeability independent of granule size was also found with 17% massed lactose. With 21% massing, however, coarse granules gave more permeable tablets at very low pressures, the effect extending to higher pressures for the lactose massed with 25% water (Fig. 1B).

Data are presented for the coarsest granules in Fig. 2A which shows the extent to which granule strength affected permeability. This factor became less important as the size decreased until, at finest size, no effect could be found.

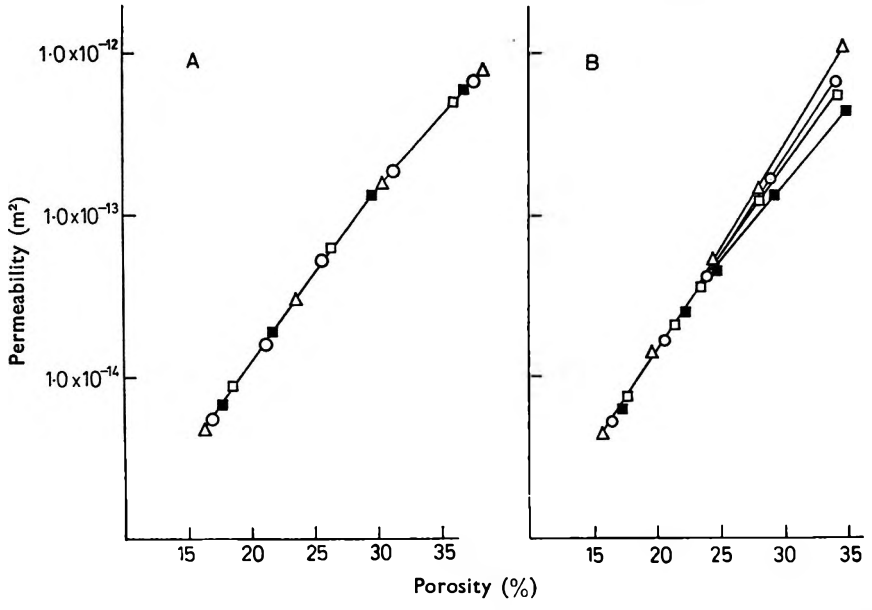

FIG. 1. The effect of granule size on the permeability of lactose tablets massed with A, 13% and B, 25% water. △ 1000–2000 μm. ○ 710–1000 μm. □ 355–500 μm. ■ 180–250 μm.

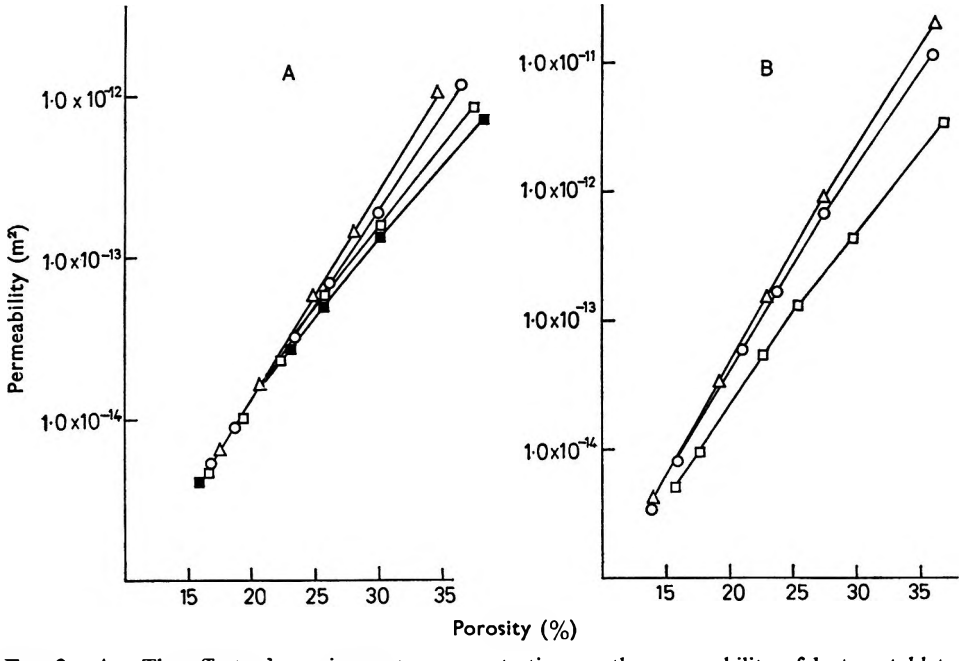

FIG. 2. A. The effect of massing water concentration on the permeability of lactose tablets. ■ 13%. □ 17%. ○ 21%. △ 25%.

B. The effect of massing water concentration on the permeability of sucrose tablets. □ 5%. ○ 7%. △ 9%.

The effects shown by the larger, stronger lactose granules were exhibited to a much higher degree by sucrose. As shown in Fig. 2B, the ability of large granules to maintain an open, permeable structure extended to high compaction pressures as the massing water concentration went up.

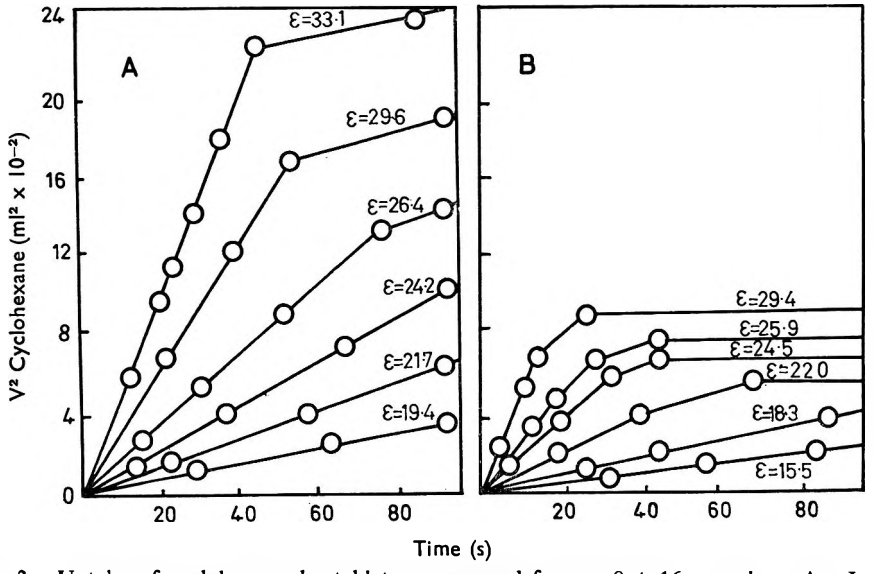

FIG. 3. Uptake of cyclohexane by tablets compressed from −8 + 16 granules. A. Lactose massed with 13% water. B. Sucrose massed with 9% water. Porosity values (ε%) are shown on the curves.

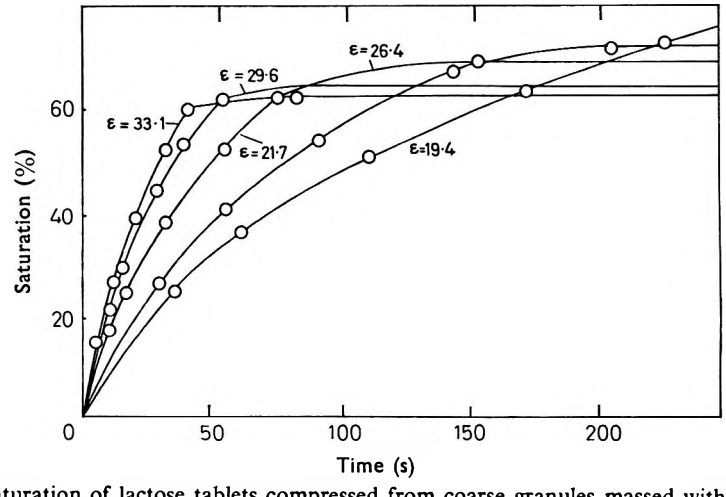

FIG. 4. Saturation of lactose tablets compressed from coarse granules massed with 13% water. Porosity values (ε%) are shown on the curves.

The permeability coefficient is related to a hydraulic radius by equation (3). The effect of granule properties on the latter are summarized in Table 1. Definition of the shape constant, k_0, is avoided by expressing values relatively, the hydraulic radius of the least permeable tablet being given the value 1.

Data from some penetration tests are given in Fig. 3. From the origin, the relation between the square of volume taken up and the time was linear as dictated by equation

(6). Before saturation was complete, however, penetration slowed, sometimes very abruptly, an effect most marked with stronger or larger granules.

The percentage of pores saturated when penetration ceased altogether increased with lower porosity (Fig. 4). At any given porosity, for the coarser granules, increase in granular strength reduced the final percentage saturation, although this became less effective at high pressure.

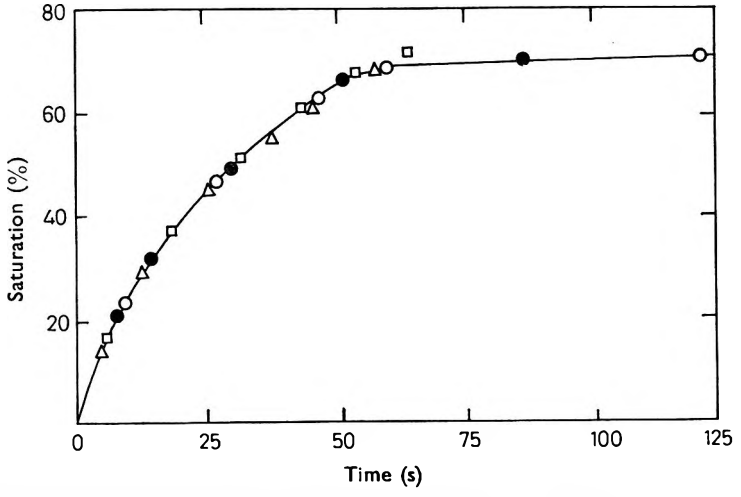

FIG. 5. Saturation of lactose tablets compressed to a porosity of 26% from fine granules massed with varying quantities of water. △ 13%. ○ 17%. ● 21%. □ 25%.

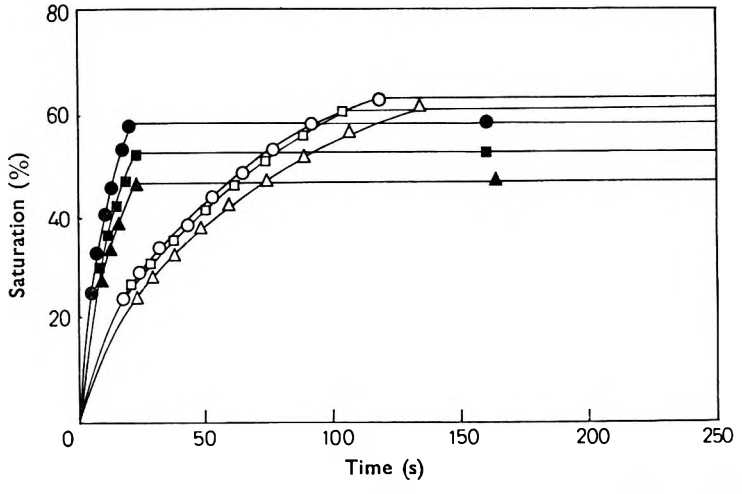

FIG. 6. Saturation of sucrose tablets prepared from fine granules massed with varying quantities of water. Triangles 5%. Squares 7%. Circles 9%. Open symbols represent data from low porosity tablets and closed symbols from high porosity tablets.

For the small lactose granules studied in Fig. 5 no effect of granule strength can be seen, while with the fine sucrose, the final percent saturation increased as the strength of the granules increased, although this effect was only noted at high porosities (Fig. 6).

DISCUSSION

The results described show that granule properties can greatly influence the pore structure of tablets. It is probable that the duality of this structure, as described earlier, is sustained in part even when compaction pressures are very high.

Some information on the size of the intragranular pores may be inferred from the bulk density of the uncompacted materials. Increase in the massing water concentration progressively increased bulk density. If we assume that the former has little effect on the shape and surface of the granules, packing to define the intergranular pore space will be much the same. Increase in the bulk density must then be ascribed to a decrease in the intragranular pore space. Compaction to a given overall porosity therefore requires less deformation in those granules massed with the most water.

A coarser intergranular pore structure characterized by a high permeability is more easily sustained in these cases. This is not necessarily related to the strength of the granules but rather to the particle packing within the granules before compression. Thus comparison, at any granule size, of the permeability of the lactose massed with 13% water to that massed with 17% shows that although both granules are very friable, the latter are more permeable. If, on the other hand, granule size is compared at the same particular massing concentration, when the intragranular pore structure before compaction is the same, no effect on permeability can be seen. The coarse intergranular pore structure of the large granules is not sustained in these materials and the overall pore structure, at any porosity level, is the same. Granule size does not, therefore, influence pore structure. This is not true of the lactose massed with higher concentrations of water. Here large granules are able to maintain a coarser intergranular pore structure as shown by their higher permeability, although this effect is limited to lower pressures when deformation is small. With the more robust sucrose granules summarized in Table 1, increase in granule size and strength simultaneously operate to maintain a coarse intergranular pore network which, in the case of the strongest granules, remains influential until almost the lowest porosities.

Inherent in the maintenance of an intergranular pore system is a wide and perhaps discontinuous distribution in the size of the pores. Such a distribution is characteristic of tablets produced from sucrose granules, especially at low pressure. The

Table 1. *Experimental values of relative mean hydraulic radius for sucrose and lactose tablets*

	$\epsilon = 30\%$			$\epsilon = 20\%$		
	Sucrose massing concentration					
Mesh size	5	7	9	5	7	9
8–16	1·34	2·37	2·92	1·14	1·45	1·64
16–22	1·18	2·00	2·28	1·05	1·38	1·52
30–44	1·14	1·70	1·97	1·05	1·34	1·45
60–85	1·00	1·41	1·67	1·0	1·26	1·41

	Lactose massing concentration							
	13	17	21	25	13	17	21	25
8–16	1·00	1·03	1·16	1·35	1·00	1·04	1·04	1·08
16–22	1·00	1·03	1·11	1·25	1·00	1·04	1·04	1·08
30–44	1·00	1·03	1·09	1·17	1·00	1·04	1·04	1·08
60–85	1·00	1·02	1·02	1·02	1·00	1·04	1·04	1·08

large pores will carry a disproportionate amount of fluid and the permeability will be high. Expressing this system in terms of a channel, a threefold increase in hydraulic radius occurred when size, strength and bulk density of the granules were varied in tablets compressed to a porosity of 30%. This factor was still over 1·6 at a porosity of 20%.

With the weaker lactose granules, the pore structure will be relatively uniform and the effect of granule properties on mean hydraulic radius are quite small, as shown in Table 1.

The inferences on pore structure drawn from permeability are supported by liquid penetration. A coarse intergranular pore structure permits a more rapid movement of liquid through the tablet than is found in tablets of the same porosity but with more even pore structure. However, in the former case, rapid penetration isolates other pore areas which cannot then be penetrated because the trapped air cannot escape. The fractional pore space of sucrose tablets which was saturated diminished as the size or strength, or both, of the granules increased. Similarly, destruction of the intergranular pores was shown by increased fractional saturation as the tablet was compressed to lower porosity.

The more even pore structure of the lactose tablets was reflected in the total saturation found with this material. Only at high porosities and with larger, stronger granules was the effect of an intergranular pore structure found.

Early stages of penetration with all materials obeyed a relation of the form given in equation (6). The linearity of this relation and its passage through the origin precludes any constriction at the surface of the tablet. The pore structure at the surface is not, therefore, made atypical by contact with a smooth metal surface during compression.

Acknowledgement

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A comparison of the effects of 6-hydroxydopamine immunosympathectomy and reserpine on the cardiovascular reactivity in the rat

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In the conscious rat, 6-hydroxydopamine, or reserpine (5 mg/kg) pretreatment produced a marked fall in the mean systolic blood pressure whilst immunosympathectomized rats had resting blood pressures just below that of control animals. In pithed preparations, 6-hydroxydopamine treatment or immunosympathectomy potentiated the pressor responses to injected noradrenaline; reserpine pretreatment did not potentiate the noradrenaline response to the same degree. Tyramine responses were abolished after 6-hydroxydopamine or reserpine pretreatment but were unaffected by immunosympathectomy. Stimulation of the sympathetic outflow by the Gillespie & Muir (1967) preparation was abolished after 6-hydroxydopamine and reserpine pretreatment, and reduced after immunosympathectomy. It is concluded that 6-hydroxydopamine produces a destruction of the sympathetic nerve endings, abolishing the physiological uptake process and, therefore, producing supersensitivity to injected noradrenaline. Immunosympathectomy, although showing a marked reduction in sympathetic nerve supply leaves a functional uptake process. Reserpine (5 mg/kg), given 6 h previously, depletes endogenous catecholamines without significantly altering the sensitivity to injected noradrenaline, the uptake process remaining functional.

6-Hydroxydopamine has been shown to deplete adrenergic nerves of their endogenous noradrenaline (Porter, Totaro & Stone, 1963; Laverty, Sharman & Vogt, 1965). Recently, electron microscope studies have shown that pretreatment with 6-hydroxydopamine leads to destruction of the adrenergic nerve endings and a consequent depletion of their amine stores (Thoenen & Tranzer, 1968).

Immunosympathectomy (IS) produced by nerve growth factor anti-serum has been shown to destroy sympathetic ganglia of the rat (Levi-Montalcini & Booker, 1960; Zaimis, 1967). Biochemical studies have further shown that the noradrenaline contents of some sympathetically innervated tissues are markedly reduced in IS animals (Zaimis, 1966; Iversen, Glowinski & Axelrod, 1966).

The administration of reserpine produces a long lasting depletion of catecholamines from the brain and peripheral tissues (Holzbauer & Vogt, 1956; Carlsson, Rosengren & others, 1957; Shore, 1962), although it is not thought to interfere with the uptake of catecholamines into the sympathetic nerves (Iversen, 1967). Supersensitivity develops slowly in reserpinized tissues and does not resemble that seen after cocaine (Trendelenburg, 1963). After chronic post-ganglionic denervation, supersensitivity to noradrenaline has been demonstrated on the nictitating membrane (Trendelenburg & Weiner,

1962). Zaimis (1967) showed that in the IS rat the pressor and inotropic responses to noradrenaline and adrenaline were prolonged when compared with control animals. More recently workers have shown that supersensitivity to noradrenaline occurs in the nictitating membrane after pretreatment with 6-hydroxydopamine (Häusler, Thoenen & Haefely, 1968). We have investigated the effects of 6-hydroxydopamine reserpine and IS on the cardiovascular system of the rat.

EXPERIMENTAL

Methods

All experiments were made using male C.S.E. rats (Scientific Products), of 180–200 g.

Mean systolic blood pressure in conscious animals was measured, using the tail cuff method, and a semi-conductor strain gauge (Ether Ltd.), mounted on a tail clip for detection of the pulse and visually displayed on an oscilloscope (Telequipment). Measurements were made with animals held in Bowman restraining cages and placed inside a warming cabinet (33° ± 1°) for 15 min. Each determination was the mean of three readings.

Anaesthetized and pithed preparations were set up as described previously (Finch & Leach, 1969). In some experiments the pithed preparation was used for stimulation of the entire sympathetic outflow (Gillespie & Muir, 1967); submaximal stimulation from a Multitone stimulator at strengths of 15–30 V, 0·03 ms duration and a frequency of 3 pulses/s was applied for periods of 18 s. Stimulations were repeated at not less than 10 min intervals. Atropine (0·5 mg/kg) and tubocurarine (1 mg/kg) were given before beginning stimulation.

Slices of atria and ventricles were prepared for histochemical fluorescence microscopy according to the method of Spriggs, Lever & others (1966), with additional details described by Clarke, Jones & Linley (1969).

Pretreatment

Chemical sympathectomy was carried out in rats of 160–175 g. 6-Hydroxydopamine hydrobromide was given 2 × 50 mg/kg intravenously on day 1, followed by 2 × 100 mg/kg on day 7 and experiments were made on day 8–10 (Thoenen & Tranzer, 1968). Reserpine 5 mg/kg was given intraperitoneally 6 h before recordings were taken. Immunosympathectomized rats were obtained using double strength cow nerve growth anti-serum, injected subcutaneoulsy on the day of birth and on the following four days with doses of 0·1, 0·1, 0·2, 0·2, 0·4 ml.

Drugs and solutions

All stock solutions of drugs were diluted in 0·9% w/v NaCl solution before use. Noradrenaline acid tartrate (Hoechst), calculated as base, was stored in 0·01N HCl. The following drugs were calculated as salt; atropine sulphate (Northern Pharmaceuticals); desipramine (Geigy); dimethylphenylpiperazinium iodide (Aldrich); mecamylamine hydrochloride (Merck, Sharp & Dohme); reserpine phosphate (CIBA) dissolved in 20% ascorbic acid; tubocurarine chloride (Burroughs Wellcome); and tyramine hydrochloride (BDH). 6-Hydroxydopamine HBr, generously donated by Dr's Thoenen and Hürlimann (Hoffmann-La Roche, Basle) was dissolved in 0·001 N

hydrochloric acid previously bubbled with nitrogen. Cow nerve growth anti-serum (Batch Ex 4945/46/47) was generously donated by Dr. C. Edwards (Wellcome Research Laboratories, Beckenham, Kent).

RESULTS

Effect of 6-hydroxydopamine reserpine and IS on the mean resting blood pressure of rats

In the conscious untreated rat the mean resting systolic blood pressure was found to be 115 mm of Hg whilst 6-hydroxydopamine treated rats or rats pretreated with reserpine 6 h previously exhibited much lower pressures of 94 and 84 mm of Hg respectively (Table 1). IS rats had slightly lower blood pressures (103 mm of Hg) than the control group. After anaesthesia (sodium pentobarbitone, 60 mg/kg), the mean resting blood pressures of 6-hydroxydopamine- and reserpine-treated rats were markedly lower than those of the corresponding control animals (Table 1). After pretreatment with mecamylamine (10 mg/kg), or pithing, the blood pressures of IS and 6-hydroxydopamine rats did not significantly differ from their respective control groups. However, rats pretreated with reserpine showed significantly elevated pressures in both cases when compared with control preparations.

Effect of 6-hydroxydopamine, reserpine and IS on noradrenaline, tyramine and dimethylphenylpiperazinium (DMPP) sensitivity in pithed rat preparations

In the pithed rat preparation the mean pressor response to a range of noradrenaline doses (0·125–1 μg/kg) was found to be potentiated in both magnitude and duration after pretreatment with 6-hydroxydopamine (Fig. 1). IS rats also showed potentiated

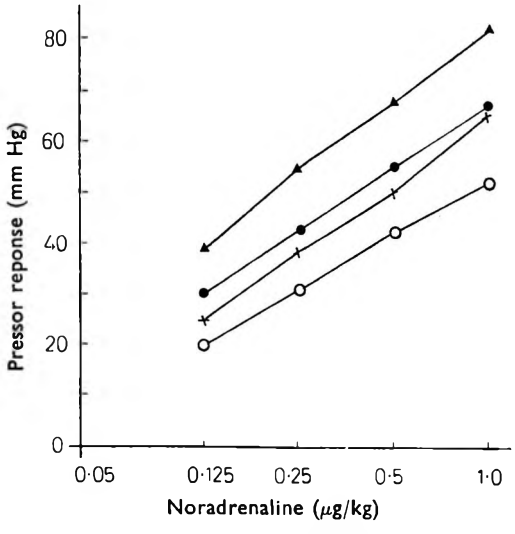

FIG. 1. Noradrenaline log dose-response curves obtained using pithed rat preparations. Each line represents the mean of four experiments. (○) control; (×) reserpine 5 mg/kg, 6 h previously; (●) Immunosympathectomized rats; and (▲) 6-hydroxydopamine pretreatment.

responses but the effect was not as marked as after 6-hydroxydopamine pretreatment. Reserpinized preparations showed only a slight increase in sensitivity to noradrenaline when compared with control preparations. However, reserpine pretreatment (5 mg/kg, 6 h previously) caused a marked slowing of the heart. Desipramine (0·5 mg/kg),

Table 1. *Effect of 6-hydroxydopamine, reserpine and immunosympathectomy on the resting blood pressure of rats.* The treatments significantly reduced the blood pressure in conscious and pentobarbitone treated animals.

Pretreatment	Conscious†	Pentobarbitone (60 mg/kg, i.p.)‡	Pentobarbitone and mecamylamine (10 mg/kg, i.v.)	Pithed preparations‡
Untreated	115±3 (12)	119±2 (17)	73±2 (17)	50±1·5 (18)
6-Hydroxydopamine	94±1·9 (13)*	89±3·3 (5)*	65±3·5 (5)	52±5·8 (6)
Reserpine (5 mg/kg, 6 h previously)	84±3 (7)*	80±4·5 (7)*	80±2·3 (8)*	64±3 (9)*
Immunosympathectomy	103±3 (7)*	101±2·7 (8)*	69±2·8 (9)	51±2·2 (6)

* $P < 0{\cdot}05$ compared with untreated groups.
† Indirect measurement (mean systolic blood pressure in mm of Hg ± standard error of mean).
‡ Direct measurement (mean blood pressure in mm of Hg ± standard error of mean).
( ) indicates number of experiments.

which is known to block the uptake of noradrenaline into adrenergic nerve terminals, was seen to potentiate the noradrenaline pressor responses in both magnitude and duration, in rats pretreated with reserpine, IS and the untreated control preparations (Fig. 2). In 6-hydroxydopamine rats, desipramine produced only a slight potentiation of noradrenaline responses, suggesting that 6-hydroxydopamine almost completely destroys the physiological uptake mechanism. It can also be seen that noradrenaline sensitivity after desipramine is approximately equal for the control and all the pretreated preparations.

6-Hydroxydopamine and reserpine pretreatment abolished the response to tyramine in pithed rat preparations (Table 2), whereas, IS rats gave similar responses to those

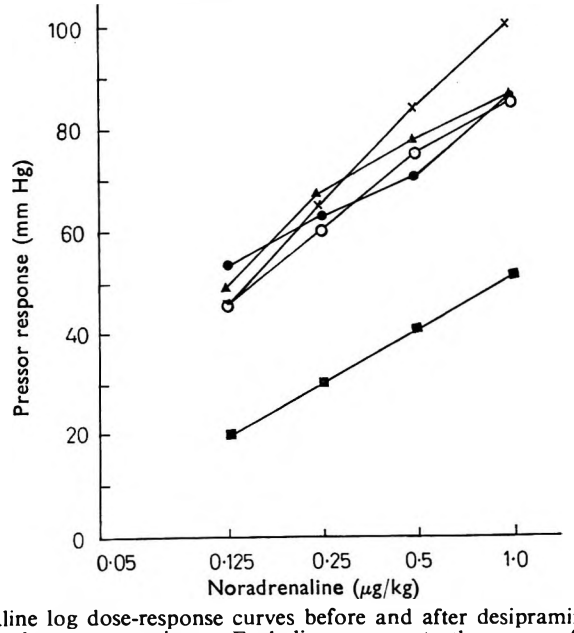

FIG. 2. Noradrenaline log dose-response curves before and after desipramine (0·5 mg/kg, i.v.) obtained using pithed rat preparations. Each line represents the mean of four experiments. (■) Untreated controls. After desipramine (○) control; (×) reserpine 5 mg/kg, 6 h previously: (●) immunosympathectomized rats; and (▲) 6-hydroxydopamine pretreatment.

obtained in control preparations. The pressor responses to DMPP, a ganglion stimulating agent, were markedly reduced after reserpine pretreatment whilst responses with 6-hydroxydopamine rats were potentiated. The responses to DMPP in IS rats were unaltered from those seen in the control pithed preparations.

Table 2. *The effect of 6-hydroxydopamine, reserpine and immunosympathectomy on tyramine and dimethylphenylpiperazinium responses in the pithed rat.* 6-Hydroxydopamine and reserpine abolished the response to tyramine. The response to DMPP was reduced after reserpine but potentiated after 6-hydroxydopamine.

Pretreatment	Mean blood pressure rise (Expressed in mm of Hg ± standard error of mean)			
	Tyramine		Dimethylphenylpiperazinium	
	125 μg/kg	250 μg/kg	50 μg/kg	100 μg/kg
Untreated	28±2·9 (8)	49±4·4 (8)	12±2·1 (6)	31±3·1 (6)
6-Hydroxydopamine	6±0·9 (8)*	8±1·2 (8)*	21±2·2 (7)*	41±2·6 (8)*
Reserpine (5 mg/kg, 6 h previously)	3±0·8 (8)*	6·5±1·5 (8)*	4±1·0 (6)*	11±1·2 (6)*
Immunosympathectomy	29±3·8 (8)	39±4·2 (8)	11·5±2 (6)	33·5±2·2 (6)

* $P < 0{\cdot}05$ compared with untreated groups.
( ) indicates number of experiments.

Effect of 6-hydroxydopamine, reserpine, IS on the Gillespie and Muir preparation

Using the stimulation parameters described under methods, reproducible submaximal pressor responses were obtained in all preparations. Pretreatment with reserpine or 6-hydroxydopamine produced a marked reduction in the magnitude of the pressor responses, together with a latency of 8–12 s in onset of the effect (Fig. 3). IS rats showed an overall reduction in the size of the pressor response, which was not as marked as after reserpine or 6-hydroxydopamine pretreatments; the onset of the response was normal in comparison with control preparations.

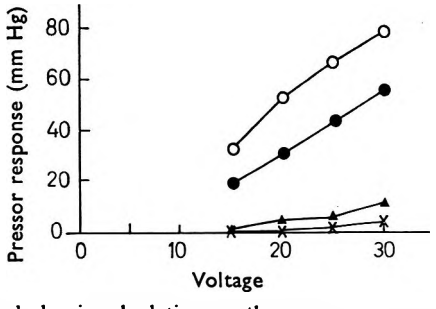

FIG. 3. The effect of catecholamine depletion on the pressor responses obtained from Gillespie and Muir preparations. Sympathetic outflow stimulated for 18 s at a pulse duration of 0·03 ms, a frequency of 3/s and repeated every 10 min at varying voltage. Each line represents the mean of four experiments. (○) control; (×) reserpine 5 mg/kg, 6 h previously; (●) immunosympathectomized rats and (▲) 6-hydroxydopamine pretreatment.

Histochemical fluorescence studies

Pretreatment with 6-hydroxydopamine or reserpine completely abolished the specific fluorescence attributable to adrenergic nerves in both atria and ventricle slices.

This is in agreement with catecholamine analysis of these pretreatments (Thoenen & Tranzer, 1968; Clarke & Jones, 1969; Carlsson & others, 1957).

IS rats showed a marked reduction in fluorescence intensity attributable to adrenergic nerves. However, in two preparations, isolated coronary vessels with adrenergic nerve attachments were observed.

DISCUSSION

Pretreatment with reserpine or 6-hydroxydopamine produced a significant fall in the resting systolic blood pressure in the conscious rat, although IS rats showed only a slight fall when compared with control animals. These results with IS rats confirm the findings of Zaimis (1967). The difference in resting blood pressures may be attributable to several factors. Firstly reserpine or 6-hydroxydopamine pretreatments, at the dose levels used, are more efficient at depleting endogenous catecholamines (Shore, 1962; Thoenen & Tranzer, 1968; Clarke & Jones, 1969) compared with IS procedures Iversen & others, 1966; Zaimis, 1967). Also the increased turnover of adrenal catecholamines in IS animals may be the reason for their near normal resting blood pressures (Iversen & others, 1966). Recently Thoenen, Mueller & Axelrod (1969) have demonstrated an increased adrenal tyrosine hydroxylase activity in rats pretreated with reserpine or 6-hydroxydopamine. However, in our experiments compensation of the blood pressure may not have taken place in the time interval of the pretreatment procedures.

Most sensitivity experiments concerned with denervation and blockade of sympathetic function have been made on cats and, therefore, results described in this paper cannot be fully compared with those described by Trendelenburg (1963). However, 6-hydroxydopamine pretreatment potentiated the pressor responses to noradrenaline in the pithed rat preparation suggesting that this pretreatment produces supersensitivity similar to denervation. These conclusions are in accord with the findings of Thoenen & Tranzer (1968) in which 6-hydroxydopamine produced a depletion of noradrenaline in sympathetically innervated nerve endings to an extent comparable with that seen after surgical denervation or IS procedures. Recently these workers also showed potentiation of the noradrenaline response on the nictitating membrane in cats pretreated with 6-hydroxydopamine (Häusler & others, 1968). The tyramine responses were almost completely abolished and desipramine failed to further potentiate injected noradrenaline to any marked degree in the pithed rat preparation pretreated with 6-hydroxydopamine. These results suggest that destruction of the sympathetic nerves supplying the cardiovascular system was virtually complete together with the elimination of their physiological uptake mechanism. Further evidence in support of these findings is the fact that sympathetic stimulation (Gillespie & Muir preparation) produces a pressor response which in terms of latency and magnitude is most likely attributable to stimulation of the adrenal medulla. Also Malmfors & Sachs (1968) showed pretreatment with 6-hydroxydopamine prevented the retention of endogenous noradrenaline and α-methyldopa in the mouse iris.

IS rats showed increased responsiveness to noradrenaline in pithed preparations but this was less marked than that seen in 6-hydroxydopamine-pretreated animals. Pressor responses to tyramine and DMPP were found to be normal yet responses after sympathetic stimulation (Gillespie & Muir preparation) were markedly reduced compared with normal preparations. These results suggest that although IS rats have a

severely reduced sympathetic supply to the cardiovascular system, near normal physiological function is capable of being maintained. It is also supported by the results of histochemical fluorescence showing that some sympathetic nerves were still present in the heart. Although the uptake process may be reduced after IS, the results suggest that a sufficient proportion still remains functional as shown by the fact that injected noradrenaline responses were capable of substantial potentiation after desipramine in IS pithed preparations. These conclusions are in accordance with the findings of Iversen & others (1966) who found that noradrenaline uptake was reduced in various sympathetically innervated tissues of IS rats, but in none of these experiments did they find the uptake process completely abolished.

Reserpine has been used throughout these experiments as a known depletor of adrenergic catecholamines. The fact that tyramine responses and sympathetic nerve stimulation were abolished suggests a complete depletion of sympathetic neuronal stores of catecholamines supplying the cardiovascular system. Desipramine markedly potentiated the noradrenaline responses and strongly suggests that the uptake processes still remain functional, although the responses to injected noradrenaline showed a slight increase when compared with control groups. This confirms the work of Zaimis (1966) who suggested that potentiated responses to noradrenaline in reserpinized rats may be due to increased cardiac output and not due to catecholamine depletion.

Acknowledgement

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Potentiation of oxotremorine lethality by antihistamines

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Antihistamines, representing the major chemical classes, administered intraperitoneally to mice in non-toxic doses potentiated the lethal effects of an LD10 dose (3·5 mg/kg) of oxotremorine in a dose related manner. Atropine and methylatropine were highly effective in blocking oxotremorine lethality alone and when it was potentiated by antihistamines. The antagonism by methylatropine suggests a peripheral site of toxicity. Antihistamines might enhance lethality by interfering with the inactivation of oxotremorine by liver microsomal drugs enzymes in a manner similar to SKF 525A.

Tremorine and its active metabolite oxotremorine [1-(2-oxopyrrolidino)-4-pyrrolindino-2-butyne] have proved useful in the evaluation of potential anti-Parkinsonian agents (Everett, 1964; Friedman & Everett, 1964) as well as in the study of chemical transmission in the central nervous system (Holmstedt, 1968). Both compounds produce peripheral and central cholinergic stimulation, resulting in tremors, muscle rigidity, akinesia, hypothermia, diarrhoea, salivation and lacrimation, all of which can be antagonized by tertiary cholinergic blocking agents like atropine (Everett, Blockus & Shepperd, 1956).

Several antihistamines, notably diphenhydramine, have been used successfully in the treatment of Parkinsonism, suggesting that an aberration in brain histamine may be involved in the aetiology of this disease (Barbeau, 1962; Friedman & Everett, 1964). Ungar & Witten (1963) found many antihistamines and atropine to antagonize the tremors and elevation in brain histamine induced by tremorine in the dog.

We have examined the interactions of antihistamines with oxotremorine, because it has a more rapid onset and intensive action than tremorine, also Leslie & Maxwell (1964) have suggested that compounds such as SKF 525A, which antagonize tremorine-induced tremors, inhibit the formation of oxotremorine, the agent responsible for the cholinergic stimulation observed (Cho, Haslett & Jenden, 1961).

EXPERIMENTAL

Materials and methods

Male Swiss albino mice, 20–25 g, were pretreated with antihistamine or atropine, or both, 10 min before oxotremorine.

All drugs were dissolved in distilled water in a concentration such that the volume injected (intraperitoneally) was 0·10 ml/10 g weight. Doses of atropine and the antihistamines were calculated as the salt; oxotremorine sesquifumarate as the base.

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† This work formed part of a thesis submitted by M. C. Gerald to the Graduate School of Indiana University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Pharmacology.

Optimal dosages were determined in preliminary experiments, so too was the feasibility of terminating the experiment at 90 min, after which time we found there was no significant increase in the number of animals that died. All studies were made at a room temperature of 22 ± 1°.

RESULTS

A single dose of oxotremorine (3·5 mg/kg) in mice is approximately an LD10 value (Table 1). Pretreating the mice with SKF S25A had the expected effect of increasing the potency of the oxotremorine dose to an LD90. All of the other compounds tested (at two or more dose levels) also potentiated the lethal effects of oxotremorine. At the lowest doses tested, benztropine and cyproheptadine both appeared to have some slight protective effect, although at higher doses both drugs clearly potentiated the oxotremorine lethality. The survival time was also decreased as the potentiation increased. Before death, the animals showed clear signs of excessive cholinergic stimulation, i.e. tremors, salivation, lacrimation.

The apparent ability of benztropine, cyproheptadine and diphenhydramine to afford some protection was further explored by pretreating mice with the various antihistamines, then giving an LD50 dose of oxotremorine (5·0 mg/kg). Table 2 shows the results. Benztropine significantly reduced the number of deaths of animals given this higher dose of oxotremorine while diphenhydramine and cyproheptadine conferred partial protection. All other compounds tested were either without protective effect, or produced enhanced lethality.

The results obtained when atropine or methylatropine was given before the antihistamine-oxotremorine combination are shown in Table 3. These cholinergic blocking agents effectively prevented the potentiation of oxotremorine lethality by the antihistamine. In this, the quaternary methylatropine was as effective as its tertiary analogue, suggesting that the lethal effects of oxotremorine have strong peripheral components.

DISCUSSION

Similarities between the actions of histamine and acetylcholine, and between their respective antagonists have been discussed by Barlow (1955), Loew (1947), Marshall (1955) and Rocha e Silva (1955). Histamine and acetylcholine both produce vasodilatation of capillaries, stimulation of intestinal smooth muscle, release of adrenaline from the adrenal medulla and stimulation of gastric acid secretion (Douglas, 1965; Koelle, 1965). Administration of either of these compounds into the lateral hypothalamus stimulates water intake in water-satiated rats (Gerald & Stern, 1968).

Structural similarities exist between antihistamine and anticholinergic agents with a transition from the predominance of one action to the other following chemical modifications (Barlow, 1955; Rocha e Silva, 1955). Marshall (1955) has shown indirectly the attraction of certain antihistamines to both histamine and acetylcholine receptors. Atropine, and antihistamines representative of the major chemical classes, decrease thirst-induced water consumption in a dose-related manner and have mutually parallel log dose-response curves (Gerald, 1968; Gerald & Stern, 1968). Ungar & Witten (1963) reported antagonism of tremorine-induced tremors and a rise in brain histamine in the dog with many antihistamines and atropine.

It was originally thought that the relative anticholinergic potency of the antihistamines tested would be found to be related to their effectiveness in blocking oxotremorine-induced lethality in mice; Leslie (1969) had related central anticholinergic

Table 1. *Antihistamine-potentiation of oxotremorine (OTMN) lethality.* Mice were pretreated with the antihistamine 10 min before oxotremorine (OTMN), i.p. Doses of antihistamines were calculated as the salt; oxotremorine as the base. Values are expressed as the mean survival time (min) ± s.e. after oxotremorine administration. Extreme values are given in parentheses. Number dead were counted at 90 min. Statistical comparisons were determined by the "u"-test (Li, 1964).

Treatment	Dose of antihistamine (mg/kg)	No. dead/ total	% Dead	"u" Test *P* values	Survival time (min ± s.e.)
Saline + OTMN 3·5	—	6/70	8·6		18·5 ± 0·7
Benztropine	16	0/10	0		—
+ OTMN 3·5	4	0/10	0		—
	8	3/10	30	0·5	18·0 ± 2·2
	16	10/10	100	0·01	3·9 ± 0·5
Bromodiphenhydramine	50	0/10	0		—
+ OTMN 3·5	12·5	3/10	30	0·5	23·0 ± 1·9
	25	4/10	40	0·1	22·8 ± 2·7
	50	9/10	90	0·01	9·0 ± 1·4
Chlorcyclizine	50	0/10	0		—
+ OTMN 3·5	12·5	2/10	20		(16, 34)
	25	8/10	80	0·01	11·4 ± 0·7
	50	10/10	100	0·01	5·2 ± 0·3
Chlorpheniramine	64	0/10	0		—
+ OTMN 3·5	4	3/10	30	0·5	17·0 ± 0·3
	8	6/10	60	0·01	12·8 ± 0·7
	16	8/10	80	0·01	9·6 ± 0·5
Chlorpromazine	40	0/10	0		—
+ PTMN 3·5	10	2/10	20		(11, 31)
	20	4/10	40	0·1	20·0 ± 3·9
	40	10/10	100	0·01	12·7 ± 0·9
Cyproheptadine	64	0/10	0		—
+ OTMN 3·5	4	0/10	0		—
	8	3/10	30	0·5	13·3 ± 1·4
	16	10/10	100	0·01	8·8 ± 1·9
Diphenhydramine	50	0/10	0		—
+ OTMN 3·5	12·5	1/10	10		16
	25	4/10	40	0·1	18·8 ± 2·3
	50	10/10	100	0·01	7·7 ± 0·6
Methapyrilene	40	0/10	0		—
+ OTMN 3·5	10	3/10	30	0·5	31·0 ± 1·5
	20	5/10	50	0·01	16·4 ± 2·5
	40	9/10	90	0·01	15·4 ± 1·9
Promazine	40	0/10	0		—
+ OTMN 3·5	20	4/10	40	0·1	13·3 ± 2·3
	30	7/10	70	0·01	13·7 ± 2·4
	40	10/10	100	0·01	8·5 ± 0·9
SKF 525A	40	0/10	0		—
+ OTMN 3·5	40	9/10	90	0·01	5·7 ± 0·9
Tripelennamine	50	3/13	23		(5, 7, 69)
+ OTMN 3	12·5	4/10	40		24·3 ± 2·9
	25	6/10	60	0·01	12·5 ± 2·2
	50	9/9	100	0·01	4·0 ± 0·8

activity to antagonism of oxotremorine-induced analgesia. Although certain antihistamines and anticholinergic agents are effective in the treatment of motion sickness, Brand & Perry (1966) were unable to correlate *in vitro* antihistamine and cholinergic blocking potency with efficacy as anti-Parkinsonian agents or in motion sickness.

Table 2. *Reduction in oxotremorine lethality by some antihistamines*

Treatment	Dose of antihistamine (mg/kg)	No. dead/ total	% Dead	Survival time (min ± s.e.)
OTMN 5·0	—	30/50	60	18·8 ± 1·3
OTMN 5·0 +				
Benztropine	4·0	1/10	10	13
Bromodiphenhydramine	12·5	5/5	100	11·2 ± 1·9
Chlorcyclizine	12·5	4/5	80	14·5 ± 3·6
Chlorpheniramine	4·0	3/5	60	13·6 ± 1·2
Cyproheptadine	4·0	4/10	40	15·3 ± 0·7
Diphenhydramine	12·5	3/10	30	17·0 ± 1·1
Methapyrilene	10·0	3/5	60	12·3 ± 0·7
Tripelennamine	12·5	4/5	80	13·3 ± 1·7

Mice were treated as described in Table 1.

Table 3. *Cholinergic blockade and potentiation of oxotremorine lethality by antihistamines.* Atropine or methylatropine, 10 mg/kg, was administered i.p. with the antihistamine 10 min before oxotremorine (OTMN), given i.p. Values are expressed as number of mice dead at 90 min.

Treatment	Dose anti-histamine mg/kg	Controls	Anti-histamine + OTMN 3·5	OTMN 3·5 + antihistamine with: Atropine 10 mg/kg	Me-atropine 10 mg/kg
Saline +					
OTMN 3·5		6/70			
Atropine 10		0/10			
Methylatropine 10		0/10			
Antihistamine:					
Chlorcyclizine	25·0		8/10	0/5	1/5
Chlorpheniramine	8·0		6/10	0/5	0/5
Chlorpromazine	20·0		4/10	0/5	0/5
Cyproheptadine	8·0		3/10	0/5	0/5
Diphenhydramine	25·0		4/10	0/5	0/5
Methapyrilene	20·0		5/10	0/5	1/5
Tripelennamine	25·0		6/10	1/5	2/5

A comparison of the results from those groups of mice that were given atropine with the pooled results from the non-atropine treated animals, indicates that atropine yields significant protection with $\chi^2 < 0{\cdot}001$.

We found that there was no apparent relation between protection against oxotremorine lethality and the relative anticholinergic potency of the antihistamines. Rather, whereas all higher doses of antihistamines tested potentiated oxotremorine lethality, atropine and methylatropine were effective in antagonizing this effect, presumably through their cholinergic blocking actions. The results with methylatropine suggest a strong peripheral toxicity component, since this quaternary compound does not cross the blood-brain barrier appreciably (Herz, Teschemacher & others, 1965; Khavari & Maickel, 1967).

The potentiation of oxotremorine by antihistamines can be reconciled with the antihistamine antagonism of tremorine observed by Ungar & Witten (1963), if it is assumed that the antihistamines are acting as liver microsomal enzyme inhibitors. Thus Ungar & Witten (1963) found these drugs reduce the conversion of tremorine to oxotremorine, whereas we found the metabolism of oxotremorine to be inhibited.

SKF 525A possesses antihistamine activity and is an effective microsomal enzyme inhibitor (Sjöqvist, Hammer & others, 1968). These workers demonstrated enhanced

hypothermia when SKF 525A was administered before oxotremorine in rats. We too found potentiation of oxotremorine by SKF 525A. The similarity of survival times of the SKF 525A + oxotremorine combination with the antihistamines + oxotremorine combinations supports the likelihood of enzyme inhibition.

No satisfactory explanation is available for the enhanced toxicity seen when benztropine is combined with a low dose of oxotremorine; with a higher dose of oxotremorine, benztropine, as expected, proved to be an effective antagonist. This clinically well-established anti-Parkinsonian agent possesses both anticholinergic and antihistamine activity.

The antihistamines tested possess anticholinergic, sympatholytic, antiserotonergic and local anaesthetic properties (Barlow, 1955; Douglas, 1965); antagonism of histamine is the only action common to all these agents. It is suggested that these antihistamines enhanced oxotremorine lethality by interference with the inactivation of oxotremorine by liver microsomal enzymes.

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Action of rare earth metal complexes on neurogenic as well as on bradykinin-induced inflammation

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Both neurogenically evoked inflammation and that induced by bradykinin are not satisfactorily inhibited by the usual anti-inflammatory agents. It has been found that anticoagulant rare earth metal compounds inhibit these types of inflammation. In rats, neurogenic inflammation induced either by antidromic electrical stimulation of the saphenous nerve, or by orthodromic stimulation of sensory nerve endings with capsaicin could almost totally be prevented by a neodymium complex of pyrocatechol sodium disulphonate. In rats and rabbits a dose-dependent inhibition of the permeability increase was observed at the site of the injection of bradykinin, kallikrein or bothrops venom. Similarly, there was marked inhibition of "thermic oedema." The results are in accord with the hypothesis that the blood clotting system plays an important role in the mechanism of inflammation.

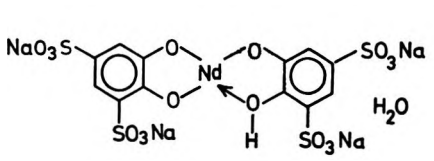
Jancsó & Jancsó-Gábor (1960) and Jancsó (1961) put forward the hypothesis that the blood clotting system plays an important role in the mechanism of inflammation, and the inhibition of blood coagulation prevents or greatly reduces the inflammatory responses. This hypothesis was recently confirmed by Barnhart (1968) and supported by several other authors. For instance Fontaine, Grand & others (1967) demonstrated with many coumarin and indandione derivatives that the anti-inflammatory and anticoagulant actions are inseparable. Wiseman & Yi-Han Chang (1968) reported a marked inhibition of the carrageenan oedema by heparin. In addition, several authors emphasize the inhibitory role of fibrinolysis and that of decrease of the fibrinogen level in the development of inflammation (Aschheim, Tsuluca & Copley, 1963; Gryglewski, 1966; Glenn, 1969).

In view of these considerations it was tempting to investigate whether anticoagulant agents are effective also in those types of inflammation which could scarcely be influenced by the commonly used anti-inflammatory drugs. Previous experiments revealed that rare earth metal complexes with anticoagulant action are effective antiphlogostic agents in many types of inflammation (Jancsó & Jancsó-Gábor, 1960; Jancsó, 1961; Oyvin, Baluda & others, 1964; Oyvin, Uklonskaya & Romanovskaya, 1966; Oyvin, Volodin & others, 1966). In the present experiments the effect of these compounds was tested on neurogenic and bradykinin-induced inflammation.

EXPERIMENTAL

Materials

The neodymium complex of pyrocatechol sodium disulphonate (I) synthesized by Jancsó (1961) was used for intravenous injections in form of a 5% solution (Phlogodym). For local treatment an ointment containing 3% samarium sulphosalicylate (II) (Phlogosam, Gedeon Richter) was used.



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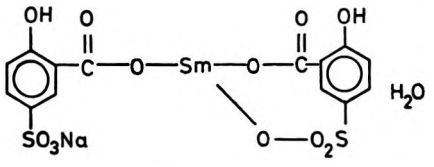

II

Other drugs used were: indomethacin, flufenamic acid, sodium salicylate, amidopyrine, phenylbutazone, cyproheptadine, bradykinin (Parke, Davies), *Bothrops jararaca* venom (Light), kallikrein (Depot Padutin, Bayer), azovan blue, capsaicin. Capsaicin was dissolved by means of ethanol and polysorbate 80 as described earlier (Jancsó, Jancsó-Gábor & Szolcsányi, 1967). Indomethacin was used as a suspension, the other drugs were dissolved in isotonic saline.

Methods

Neurogenic inflammation. In rats under pentobarbitone anaesthesia (40 mg/kg) an inflammatory reaction was induced in the skin area supplied by the saphenous nerve by antidromic electrical stimulation of the nerve for 10 min using rectangular pulses of 8 V, 8 ms at 25/s (Jancsó & others, 1967).

Neurogenic inflammation was also induced in the eye of the anaesthetized rat by instilling a drop of a 1% solution of capsaicin. In both experiments, 10 min before the stimulus, 50 mg/kg azovan blue dye was injected into the tail vein. The animals were killed by bleeding after the completion of nerve stimulation or 10 min after the instillation of capsaicin into the eye. The saphenous skin area or the eye lids and conjunctivae were excised and the amount of the exuded dye quantitatively determined by the suramin extraction method (Jancsó-Gábor, Szolcsányi & Jancsó, 1967).

Inflammation induced by bradykinin, bothrops venom and kallikrein

The inflammatory agents were injected intradermally, in rats in 0·05 ml into the dorsal skin of the hind paws, in rabbits in 0·1 ml into the shaved skin of the back. 10 min before the application of the agents, 50 mg/kg azovan blue was injected intravenously. The degree of the permeability increase was measured as in neurogenic inflammation.

Thermic oedema

Under pentobarbitone anaesthesia one rat hind paw was immersed in water at 46·5° for 30 min, the other paw serving as control. After the experiment the animals were killed, the paws amputated and weighed. The weight increase of the paw immersed at 46·5° was calculated by subtracting the weight of the control paw.

RESULTS AND DISCUSSION

Inhibition of the neurogenic inflammation

Previously it was reported that by antidromic electrical stimulation of a sensory nerve true inflammatory symptoms can be evoked and that these symptoms cannot be inhibited by anticholinergic, adrenergic blocking or ganglionic blocking agents, or by antagonists of histamine and 5-hydroxytryptamine (5-HT) (Jancsó & others, 1967).

The present experiments revealed that non-steroid anti-inflammatory agents and even prednisolone, in non-toxic doses, have no effect on this type of inflammation, or at best exert only slight inhibition. In sharp contrast the rare earth metal complex, Phlogodym, almost abolished the inflammation induced by nerve stimulation.

Table 1. *Effect of different agents on the azovan blue dye accumulation induced in the skin of the paw of rats by antidromic electrical stimulation of the saphenous nerve.* The excess dye values were obtained by subtracting the dye content of control sides from those of stimulated sides. Azovan blue dose: 50 mg/kg.

	No. exp.	Dose mg/kg	Interval min	Dye content (μg) stimulated side	Dye content (μg) control side	Excess dye (μg)	Inhibition %
Phlogodym ..	6	250 i.v.	30	2·5	1·8	0·7	97
	8	100 i.v.	30	4·5	1·8	2·7	88
	6	50 i.v.	30	6·4	1·1	5·3	77
Sodium salicylate	6	250 i.v.	30	16·4	1·3	15·1	35
Amidopyrine ..	6	200 i.v.	30	19·2	1·1	18·1	22
Phenylbutazone ..	6	100 i.v.	30	22·6	1·1	21·5	7
Indomethacin ..	7	10 oral	60	18·4	1·3	17·1	26
Flufenamic acid ..	8	20 i.v.	30	21·7	1·6	20·1	13
Cyproheptadine ..	4	5 i.v.	30	24·7	1·7	23·0	0
Control	33	—	—	24·6	1·5	23·1	—

Table 1 shows the effect of different agents on the permeability increase resulting from antidromic electrical stimulation of the saphenous nerve. In untreated rats the mean value of the exuded dye was 23·1 μg. This inflammatory response was slightly inhibited by non-steroid anti-inflammatory agents, but the histamine- and 5-HT-antagonist, cyproheptadine was ineffective. Phlogodym exerted a marked, dose-dependent inhibition which was very long-lasting: the dose of 100 mg/kg exerted 70% inhibition even 6 h after the injection. In rats pretreated with prednisolone (2 × 10 mg/kg) 24 and 1 h before the nerve stimulation, no inhibition was seen.

Phlogodym also prevented the inflammation evoked by orthodromic stimulation of sensory nerve endings with chemical irritants.

Table 2 shows marked inhibition of the inflammatory effect of the neurogenically acting capsaicin (Jancsó & others, 1967) on the eye of rats pretreated with Phlogodym.

Inhibition of the inflammation induced by bradykinin, bothrops venom and kallikrein

Some of the non-steroid anti-inflammatory agents are able to counteract the actions of bradykinin on musculature of the guinea-pig lung (Collier & Shorley, 1960, 1963), or on the vascular smooth muscle (Starr & West, 1966; Northover, 1967a). But the findings concerning its permeability increasing effect are contradictory, and in most cases inhibition could only be achieved with very high doses of anti-inflammatory agents (Collier & Shorley, 1960; Walters & Willoughby, 1965; Greaves & Shuster, 1967; Giordano & Scapagnini, 1967; Martelli, 1967; Starr & West, 1967; Northover, 1967b).

Table 2. *Azovan blue dye accumulation in the eye lids and conjuctivae of Phlogodym-pretreated and control anaesthetized rats after instillation of a drop of a* 1% *solution of capsaicin into the eye.* Phlogodym was injected intravenously 30 min before the instillation of the irritant. The excess dye values were obtained by subtracting the dye content of control sides from those of capsaicin-treated sides. Azovan blue dose: 50 mg/kg.

	No. exp.	Capsaicin 1%	Azovan blue (μg)	Excess dye (μg)	Inhibition %
Phlogodym, 250 mg/kg ..	14	+	4	1·7	87
	5	—	2·3		
Control	14	+	15·5	13·1	
	4	—	2·4		

With our quantitative method for measuring the amount of the leaked dye, the permeability increase induced by 0·5 μg of bradykinin could not be inhibited at all with 250 mg/kg of sodium salicylate or 100 mg/kg of phenylbutazone intravenously. In contrast, rare earth metal complexes parenterally or percutaneously greatly inhibited the permeability increase induced by bradykinin. Moreover, the effect of kallikrein and that of bothrops venom which releases bradykinin and potentiates its effect (Rocha e Silva, Beraldo & Rosenfeld, 1949; Ferreira, 1965), were also strongly inhibited by the rare earth complexes.

Fig. 1 shows the log dose-response relation of the mean value of dye exudation induced by 1, 2 and 10 μg bradykinin in the skin of 5 rabbits pretreated with 250 mg/kg Phlogodym and that of 5 controls. The degree of inhibition in the case of different doses of bradykinin was almost the same, between 50 and 63%. Comparable results were achieved if, instead of bradykinin, 1 IU of kallikrein was injected into the skin of the rabbit.

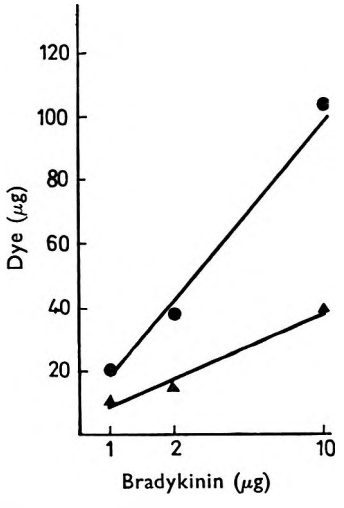

FIG. 1. Dose-response curve of the dye exudation induced by intradermal injection of bradykinin in the rabbit. ▲—▲ = rabbits pretreated with 230 mg/kg Phlogodym 30 min before the experiment; ●—● = controls. Azovan blue dose: 50 mg/kg.

Fig. 2 demonstrates the inhibitory effect of Phlogodym on the local dye exudation induced in the paw of the rat by the intradermal injection of bradykinin, bothrops venom or kallikrein. A marked dose-dependent inhibition (33 to 73%) was observed. Local application of an ointment containing 3% samarium sulphosalicylate (Phlogosam) inhibited (36%) the inflammation induced by bradykinin and (49%) that evoked by bothrops venom.

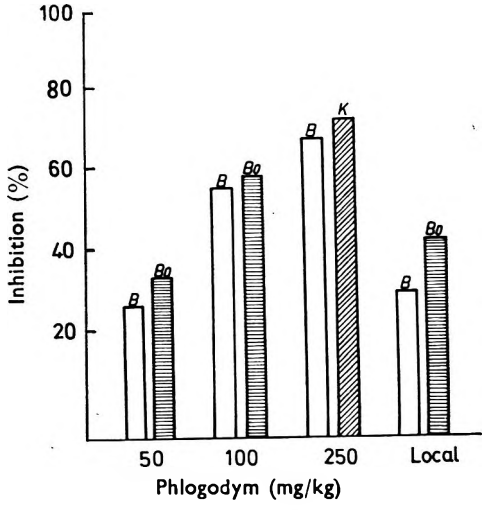

FIG. 2. Effect of 50, 100 and 250 mg/kg Phlogodym intravenously on the local dye exudation induced in the skin of the paw of rats. Phlogodym was given 30 min before the intradermal injection of 0·5 μg bradykinin (B), 2 μg bothrops venom (Bo) and 0·25 IU of kallikrein (K), respectively. The columns show the mean inhibition compared with the control values, on the basis of the amount of the leaked dye. The two last columns show the local effect on the dye exudation when the paws were painted with an ointment containing 3% samarium sulphosalicylate 1 and 3 h before the intradermal injection of 0·5 μg bradykinin or 2 μg bothrops venom. Each column represents the mean value of 5–10 experiments. Azovan blue dose: 50 mg/kg.

The fact that the rare earth metal complex produced approximately the same inhibition on the inflammation produced by kallikrein or bothrops venom and that induced by bradykinin suggests that the inhibition of kinin formation does not play a decisive role in the antiphlogistic effect of these compounds.

Inhibition of the thermic oedema

The thermic oedema of 46·5° is mediated (Rocha e Silva & Antonio, 1960), partly mediated (Starr & West, 1967) or at least accompanied (Urbanitz, Wiegand & Habermann, 1969) by release of bradykinin. This type of oedema can only be inhibited with very high doses of anti-inflammatory agents (Starr & West, 1967).

The weight increase of 6 paws immersed for 30 min in water at 46·5° was 703 mg (53%) over the control while the 6 paws of animals given Phlogodym (250 mg/kg, i.v.) 20 min before the experiment showed a weight increase of 342 mg, a 26% increase over the control. Thus the agent inhibited the oedema formation by 51%. This finding parallels the previous studies on the experimental burn of the rabbit ear at 54° (Oyvin & others, 1964) and provides experimental support for the claim that the rare earth containing ointment (Phlogosam) is of value in the treatment of superficial burns in man (Dömötör, 1969).

The marked effect of rare earth complexes with anticoagulant action in inflammations resistant to other agents support the hypothesis of Jancsó that the blood clotting system plays an important role in the development of inflammation.

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The effect of isoniazid and some anticonvulsant drugs on the γ-aminobutyric acid content of mouse brain in insulin hypoglycaemia

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Phenobarbitone, prominal and primidone protect mice from insulin convulsions and raise their lowered cerebral hemisphere GABA content. Phenobarbitone and primidone are superior to prominal and produce a significant increase in the insulin depleted GABA content. Isoniazid potentiates insulin convulsions and significantly lowers the insulin depleted GABA content. It is probable that insulin depletion of cerebral hemisphere GABA content is a rationale of hypolgycaemic convulsions.

The mechanism by which a high dose of insulin produces convulsions is vague. According to Sollmann (1957) it is not due to hypoglycaemia directly but to some secondary process. Insulin convulsions may be suppressed by narcotics, such as barbiturates, even with very low blood sugar.

Insulin produces a decrease in brain γ-aminobutyric acid (GABA) content (Cravioto, Massieu & Izquierdo, 1951; Okumura, Otsuki & Nasu, 1959; De Ropp & Snedeker, 1961; Maynert & Kaji, 1962). Saad (1969) found that the lowest level of GABA in the cerebral hemispheres of mice occurred 1 h after insulin administration. The hyperexcitability and convulsions in insulin-treated mice correlates with the significantly lowered levels of GABA. Woodbury & Esplin (1959) suggested that there is a striking and highly significant correlation of GABA content in brain and the electroshock seizure threshold. Decrease in GABA content was accompanied by an increase in brain excitability.

Isoniazid produces a decrease in brain GABA content (Sugawara, 1958; Bukin, 1959). Saad, El Masry & Scott (1969) found that its intraperitoneal administration to mice produces a sharp lowering of the cerebral hemispheric GABA content with a maximal effect after 1 h. The degree of convulsive activity increased as the GABA content fell.

Certain barbiturates which are effective against grand mal epilepsy increase the GABA content in the cerebral hemispheres of mice. Saad & others (1969) found that the GABA content increased to a peak 1 h after the intraperitoneal administration of the sodium salts of prominal or primidone, and 2 h after administration of phenobarbitone sodium. The barbiturates returned to normal the isoniazid depleted GABA contents and protected animals from convulsions.

The synergism and antagonism of the effects of isoniazid and certain barbiturates on insulin convulsions in mice has been examined. The effects have been related to the levels of GABA in their cerebral hemispheres.

EXPERIMENTAL

Determination of GABA

GABA was quantitatively determined using a chromatographic and colorimetric method previously described by Saad (1970) using the cerebral hemispheres from the brains of 3 animals for each analysis.

Experimental design

Adult male mice, 20 to 30 g, were kept on a bread diet several days before the experiment as suggested by Rowlinson & Lesford (1948) to give a more accurate response to insulin. The mice were fasted 1·5 h before the experiment. They were divided into 7 groups, each was subdivided into 3 sub-groups of 3 mice from which the cerebral hemispheres were pooled. The mice were killed at the time corresponding to maximal effect of the drugs on their cerebral hemispheric GABA content.

One group of animals was used as control. A second group was killed 1 h after the subcutaneous injection of insulin (2 u/kg). A third group was injected with phenobarbitone sodium (50 mg/kg i.p.) 2 h, and with insulin (2 u/kg, s.c.) 1 h before death. Prominal sodium, primidone sodium and isoniazid (50 mg/kg) were administered intraperitoneally separately into groups of mice. Insulin (2 u/kg) was administered subcutaneously at the same time to each group. The animals were killed 1 h later. The seventh group of mice was killed 1 h after the intraperitoneal administration of isoniazid alone (50 mg/kg).

Each group of mice was left after the treatment at 29° before being killed for the determination of their cerebral hemisphere GABA content. The state of activity of all the mice just before death was noted.

RESULTS

The mice injected with phenobarbitone and insulin were markedly depressed before death and 78% were hypnotized. Phenobarbitone sodium completely protected the animals from insulin convulsions; primidone sodium protected 78% and prominal sodium 56% of the mice. The other animals showed mild convulsions just before death. The mice given isoniazid and insulin showed severe clonic convulsions, three died.

The GABA content in the cerebral hemispheres of mice was determined for each group and the results are in Table 1.

Table 1. *GABA content in the cerebral hemispheres of adult male mice after the treatment with insulin, phenobarbitone, prominal, primidone or isoniazid.* Insulin was administered subcutaneously in a dose of 2 u/kg 1 h before death. Drugs were administered intraperitoneally in a dose of 50 mg/kg 1 h before death except phenobarbitone (2 h).

	GABA content (mg/100 g wet tissue)						
	Controls	Insulin	Pheno-barbitone + insulin	Prominal + insulin	Primidone + insulin	Isoniazid	Isoniazid + insulin
1	32·00	18·30	26·30	18·10	23·30	14·20	10·20
2	34·40	18·90	28·30	20·50	24·30	15·10	13·50
3	37·90	19·90	29·90	22·40	26·70	17·20	17·10
x	37·76	19·03	28·16	20·30	24·76	15·50	13·60
s.e.	1·7132	0·4667	1·0413	1·2443	1·0088	0·8892	1·9925
P*			<0·05		<0·05		<0·05
P†		<0·05	<0·05	<0·05	<0·05	<0·05	<0·05

* The difference between the mean GABA content and that after insulin treatment.
† The difference between the mean GABA content and the controls.

DISCUSSION

The protection of mice from insulin convulsions by barbiturates was accompanied by an increase in the cerebral hemisphere GABA level reduced by insulin administration. The effect was significant after the administration of phenobarbitone and primidone. Both the degree of protection of the mice from insulin convulsions and effect on the level of cerebral hemisphere GABA was greatest with phenobarbitone, then primidone, finally prominal. However none of these barbiturates returned the insulin lowered level of GABA to normal.

The potentiation of insulin convulsions by isoniazid was accompanied by a small but significant decrease in GABA content from the already lowered level of GABA induced by insulin. However, the difference in terms of convulsive activity was marked.

Since there is a striking and highly significant correlation of GABA content in brain and excitability (Woodbury & Esplin, 1959), and since there is a tendency for a change in GABA content in protection from or potentiation of insulin convulsions it is possible that insulin depletion of cerebral hemisphere GABA content is a rationale of hypoglycaemic convulsions.

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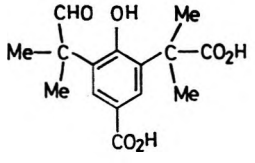
LETTERS TO THE EDITOR

The metabolism of butylated hydroxytoluene, (3,5-di-t-butyl-4-hydroxytoluene) in man

The urinary metabolites of [^{14}C]-3,5-di-t-butyl-4-hydroxytoluene (BHT) in man have been estimated by Daniel, Gage & others (1967), who found that over 50% of a 40 mg oral dose is excreted in the urine during the 24 h after dosing. The major metabolite (present to the extent of 35% of the dose) was later identified as a glucuronide of 4-carboxy-2-(1-carboxy-1-methylethyl)-6-(1-formyl-1-methylethyl)-



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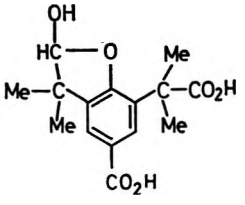

II

phenol (I) (Daniel, Gage & others, 1968). The agylcone was isolated as a hydrate of the dimethyl ester. Only 3% of the dose was eliminated as 3,5-di-t-butyl-4-hydroxybenzoic acid (BHT–COOH) and its glucuronide.

The oxidation of the t-butyl group has also been reported in rabbits given t-butylbenzene (Robinson & Williams, 1955) or BHT (Dacre, 1961). However, no t-butyl oxidation occurs with the t-butylcyclohexanones in the rat (Cheo, Elliot & Tao, 1967). Also Ladomery, Ryan & Wright (1967) were unable to demonstrate it with BHT in the rat and rabbit. It appears that when metabolic pathways other than oxidation of a t-butyl group are available such pathways predominate. It was therefore considered surprising that (I) was produced in such high yield in human urine. Investigations were made to verify the identity of this metabolite and determine the nature of the hydrate.

A group of 8 men each received 100 mg of BHT on two occasions with a 4 day interval. Urine was collected for 24 h after BHT administration and subjected to a work-up procedure identical to that employed by Daniel & others (1968), except that the DEAE cellulose column was omitted. The crude glucuronide gum was hydrolysed with 2M sodium hydroxide for 0·5 h and then continuously extracted with ether twice; once at pH 6 and again at pH 3. The metabolites present in the ether extracts were separated and identified using thin-layer chromatography (Holder, Ryan & others, 1970). Only BHT–COOH was detected in the first ether extract while the only component in the second extract to give a positive reaction with Gibb's reagent was identified as benzoylglycine (identified by infrared and nuclear magnetic resonance spectroscopy and melting point).

Subsequent urinary analyses were made in duplicate on the pooled 24 h urine of two adults each given 1·0 g of BHT. The urine was adjusted to pH 6 and extracted continuously with ether. A second extraction was made after further adjustment of the acidity to pH 2. Thin-layer chromatography of each extract showed the presence of a very polar compound which gave a positive test with naphthoresorcinol, and disappeared after β-glucuronidase or acid hydrolysis. Since the only compound present in significant amounts in the hydrolysed extract was BHT–COOH,

the polar component of the second extract was most probably BHT–COOH ester glucuronide. The carboxylic acid and its ester glucuronide were the only major metabolites detected in human urine.

It is rather surprising that these were the only metabolites present. It was expected that if the metabolite (I) were the major metabolite in urine, it would have been easily detected and isolated. However three separate attempts, using doses comparable and greater than those used by Daniel & others (1968) failed. The evidence for structure (I) is incomplete as it is based almost entirely on accurate mass measurements of a molecular ion peak at m/e 322 for the dimethyl ester. The specific radioactivity of the metabolite (a radiolabelled compound had been fed) and details of its infrared spectrum were not given. Furthermore, although some nmr data are given no signals were assigned to the aldehydic and phenolic protons, and D_2O exchange was not carried out. The absence of the aldehydic proton was ascribed to the formation of a stable hydrate, presumably the *gem*-diol of the aldehydic carbonyl. In general, diols obtained from aldehydes are unstable compounds only obtainable when the diol is stabilized by hydrogen bonding of the hydroxyl protons as with glyoxyllic acid, or by relief of dipole interactions as with trichloroacetaldehyde. For (I) there is no obvious driving force for such a reaction.

The cyclic hemiacetal (II) was considered as an alternative structure for Daniel's metabolite. However, the evidence presented is not sufficient since the nmr spectrum should show signals for one exchangeable hydroxyl proton and one methine proton. The signal at 4·6 τ (Daniel & others, 1968) could be assigned to the latter, but the absence of the hydroxyl signal remains. Furthermore since hemiacetals are usually too unstable to be isolated as such we consider structure (II) as unlikely.

In view of the foregoing, the occurrence of (I) as a BHT metabolite in human urine requires further verification. The postulated metabolite is unusual in that two alkyl groups have been completely oxidized to the carboxylic acid while the third is oxidized to the aldehyde. That oxidation of the t-butyl group proceeds beyond the alcohol is surprising, particularly in view of the results of Robinson & Williams (1955) who showed that t-butylbenzene is oxidized to 2-phenyl-2-methylpropanol only.

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On the dopaminergic nature of the gnawing compulsion induced by apomorphine in mice

The gnawing compulsion elicited by apomorphine is a consequence of the stimulation of the dopaminergic neurons in the corpus striatum (Ernst & Smelik, 1966; Ungerstedt, Butcher & others, 1969) but the inhibition of the synthesis of dopamine did not alter the response to the drug in rats (Ernst, 1967). Andén, Rubenson & others (1967) demonstrated a diminished turnover rate of dopamine in apomorphine treated rats. The authors concluded that apomorphine acted directly on dopaminergic receptors. Our results show the possibility of an indirect effect of apomorphine in eliciting the gnawing compulsion.

The gnawing was measured in white mice of our Institute's breeding, of either sex, 18–22 g (Ther & Schramm, 1962) in groups of 6, in a cage with a corrugated paper covering the floor. The holes in the paper caused by gnawing, were counted for 10% of the total surface, and the means and s.e. of at least 6 groups of animals were measured.

Reserpine, 0·3–3 mg/kg inhibited the gnawing compulsion caused by 5 mg/kg of apomorphine. The inhibition was dose dependent and statistically significant above the dose of 1 mg/kg ($P < 0{\cdot}02$, Table 1).

Monoamine oxidase inhibitors increased the number of holes caused by gnawing (Table 2). This effect was greatest for AB–15 [1-*m*-aminophenyl-(2-cyclopropylamino)ethanol 2HCl; Huszti, Fekete & Hajós, 1969]. The effect of tranylcypromine was less at 2 mg/kg, but augmenting the dose to 4 mg/kg produced an inhibition of the gnawing compulsion. Nialamide was almost ineffective at 8 mg/kg, while the effect of the dose of 16 mg/kg was equal to that of 4 mg/kg of AB–15, which also caused a reversal of the reserpine induced inhibition (Table 3). The interaction of the monoamine oxidase blocking agent and reserpine elicited an increased sensitivity to apomorphine.

Similar experiments were made in animals treated with dopa and 5-hydroxytryptophan (5-HTP). Neither AB–15, dopa, 5-HTP, nor reserpine caused gnawing

Table 1. *The effect of reserpine on the apomorphine-induced gnawing compulsion of mice*

Treatment 2 h before apomorphine	Treatment at 0 time	No. of groups	Number of gnawed holes ± s.e.
—	Apomorphine 5 mg/kg s.c.	23	193 ± 25
Reserpine 0·3 mg/kg i.p.	Apomorphine 5 mg/kg s.c.	12	162 ± 27
Reserpine 1·0 mg/kg i.p.	Apomorphine 5 mg/kg s.c.	12	78 ± 29
Reserpine 3·0 mg/kg i.p.	Apomorphine 5 mg/kg s.c.	12	66 ± 13

Table 2. *The apomorphine-induced gnawing behaviour as affected by monoamine oxidase inhibitors*

Treatment	Pretreatment time (h)	Number of gnawed holes after apomorphine (number of groups used) at: 2·5 mg/kg	5·0 mg/kg
Tranylcypromine 2 mg/kg i.p.	4	155 ± 23 (12)	297 ± 41 (11)
AB-15 4 mg/kg i.p.	18	174 ± 28 (17)	350 ± 31 (18)
Nialamide 16 mg/kg i.p.	18	256 ± 16 (10)	322 ± 7(10)
Dist. water 20 ml/kg i.p.	—	139 ± 15 (16)	193 ± 25(23)

Table 3. *The effect of dopa, 5-HTP, reserpine and AB–15 on the apomorphine-induced gnawing behaviour*

Pretreatment	Treatment	Number of groups	Number of holes gnawed
AB-15 4 mg/kg —18 h	Apomorphine 5 mg/kg s.c.	18	350 ± 31
Reserpine 1 mg/kg — 1 h	Apomorphine 5 mg/kg s.c.	12	147 ± 32
AB-15 4 mg/kg + Reserpine 1 mg/kg	Apomorphine 5 mg/kg s.c.	6	325 ± 75
Dopa 25 mg/kg i.p. — 1 h	Apomorphine 5 mg/kg s.c.	12	266 ± 70
Dopa 25 mg/kg	Apomorphine 5 mg/kg s.c.	7	408 ± 53
+ AB-15 4 mg/kg		23	193 ± 25
—	Apomorphine 2.5 mg/kg s.c.	16	139 ± 15
AB-15 4 mg/kg	Apomorphine 2.5 mg/kg s.c.	17	174 ± 28
5-HTP 12 mg/kg — 1 h	Apomorphine 2.5 mg/kg s.c.	8	97 ± 27
AB-15 4 mg/kg + 5-HTP 12 mg/kg	Apomorphine 2.5 mg/kg s.c.	7	258 ± 35

compulsion in the doses used. The precursor amino-acids alone did not change significantly the gnawing behaviour caused by apomorphine. In animals given AB–15, dopa elicited a marked hypersensitivity to apomorphine. Similar effect was seen in AB–15 treated animals when 5-HTP was injected before apomorphine administration, the dose of 2·5 mg/kg of apomorphine caused an effect greater than that of 5 mg/kg in control animals (Table 3).

These results show that the apomorphine-induced stimulation of the corpus striatum may be indirect in nature. Making more dopamine or 5-hydroxytryptamine available equally leads to a greater apomorphine effect; in contrast, the depletion of these amines caused by reserpine leads to an inhibition of the gnawing. Ther & Schramm (1962) and Andén & others (1967) could not show the inhibition of apomorphine-gnawing by reserpine, possibly because of the adverse pharmacologic effects of the high doses used. Similarly Ther & Schramm (1962) demonstrated an inhibition of the apomorphine-induced gnawing by a high dose of iproniazid, while in our experiments the enzyme inhibitors we used increased it.

It is not easy to relate our answers to those which show that apomorphine retards the utilization of dopamine in the brain (Andén & others, 1967; Roos, 1969; Butcher & Andén, 1969). Bearing in mind the two compartmental system of the storage and metabolism of catecholamines suggested by Sedvall, Weise & Kopin (1968), there are many ways of changing the metabolism of transmitter amines, in addition to the positive or negative feed back regulation of the synthesis. A delayed depletion after α-methyltyrosine may not be the obligatory consequence of a diminished utilization.

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with the technical assistance of
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February 10, 1970

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Pharmacological actions of pralidoxime in relation to therapeutic doses

Several years ago we referred (Berry, Davies & Rutland, 1966) to speculations that pyridinium aldoximes, used as antidotes to organophosphorus anticholinesterase poisoning, might exert a biphasic action at neuromuscular junctions, but pointed out that none of these speculations were accompanied by measurements of the concentration of oxime reached in end-plates after the administration of therapeutic amounts of the drugs. Berry & others (1966) found that the maximum concentration of TMB-4 [1,3-di(4-hydroxyiminomethylpyridinium)propane dichloride] in diaphragm muscle did not exceed 0·1 mmol/kg with therapeutic doses, but higher concentrations were produced by toxic doses. Goyer (1970) has recently revived these speculations about a biphasic action by showing that concentrations of PAM (pyridine-2-aldoxime methiodide) around 1 mM caused optimal stimulation of the release of acetylcholine from the rat phrenic-diaphragm preparation. Concentrations of drug reached *in vivo* are too low to stimulate the release of acetylcholine, and hence oxime-induced release of this substance could have no influence on the therapeutic action of the drug (Table 1).

Table 1. *Concentration of P2S in the diaphragm after intramuscular injection of* 30 *mg/kg*. Values, in μg/g fresh weight, are the mean of six observations (i.e. 36 animals of each species)

	Time after injection, min					
	5	10	20	40	60	90
Guinea-pig ..	10·2	15·0	17·7	13·2	11·0	8·6
Rat	12·2	16·8	20·3	21·0	25·7	12·2

A dose of 30 mg/kg of P2S (pyridine-2-aldoxime methylmethanesulphonate) affords excellent protection against organophosphates when used in conjunction with atropine (Davies & Willey, 1959). Rats or guinea-pigs were given this dose intramuscularly, and groups of six animals were killed at intervals thereafter. The concentration of P2S in the diaphragm was measured by a modification of the method of Creasey & Green (1959). The Table shows that the peak concentration reached in rat diaphragm was 26 μg/g, or about 0·11 mmol/kg calculated as pyridine-aldoxime methylmethane sulphonate. According to Goyer's (1970) data this concentration would not significantly alter the release of acetylcholine from the muscle.

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Cardiac effects of strychnine and their mechanism

Several reports indicate that strychnine affects various neural functions, including not only the post-synaptic inhibitory system but also neuromuscular and ganglionic transmission, as well as the cholinergic and adrenergic post-ganglionic conduction (Alving, 1961; Chieppa & Siro Brigiani, 1965; Chieppa, 1966; Lanari & Luco, 1939).

In view of these pharmacological properties, it seems conceivable that the cardiac effects of strychnine might be related to an interference with the regulation of the autonomic intrinsic system of the heart. However, although myocardial activities of strychnine were described many years ago (Burridge, 1928; Kakovsky, 1905; Mezey & Staub, 1936), present knowledge on their mechanisms appears unsatisfactory; an attempt to correlate the cardiac to the neural actions of the drug appears to be the only recent report (Paoletti, 1966).

In this paper are reported experiments *in vivo* and *in vitro* on the effects of strychnine on heart rate, made to evaluate the degree of activity of the drug and to elucidate the basic mechanisms involved in its actions.

The *in vitro* experiments were made on frog isolated heart and guinea-pig isolated atria. The effects *in vivo* were examined according to James & Nadau (1963), on the open-chest dog under pentobarbitone narcosis (30 mg/kg, i.v.), by injecting the drug into the artery supplying the sino-auricular node. Myocardial isometric contractile force was recorded by means of a calibrated strain gauge arch, according to Boniface, Brodie & Walton (1953).

The results of the experiments on frog heart indicate that strychnine exerts a clear, dose-related negative chronotropic activity; the decrease of heart rate ranges from 20 to 60%, depending on the drug concentration (5–10–20 μg/ml). Heart contractility was slightly enhanced by the lowest concentrations and transiently depressed by the highest one. The negative chronotropic effects of strychnine proved to be unaffected by atropine (2×10^{-6} w/v), prostaglandin E_1 (10^{-8}–10^{-7}), $CaCl_2$ (4×10^{-4}), GABA (10^{-4}) and glycine (10^{-4}). Only adrenaline exerted (at high concentration, 10^{-6}) a weak, transient antagonism, without restoring the normal heart rate. The degree of activity of strychnine was much greater on guinea-pig atria, where the chronotropic effect was clearly detectable at concentrations as low as 10^{-8}–10^{-7}: this last concentration currently induced in 20–30 min a 40–50% decrease of the frequency, without significantly affecting the amplitude of the contraction. As observed on the frog heart, this effect was not prevented by atropine (2×10^{-6}). Furthermore, unlike quinidine, the action of strychnine remained unaffected by a reduction of potassium concentration in the bathing medium to 25% of normal value. At high concentration (5×10^{-6}), strychnine proved to prevent the chronotropic effect of adrenaline (10^{-7}), but not the inotropic one. Both on guinea-pig atria and on frog heart, eserine failed to enhance the negative chronotropic activity of strychnine. The experiments on the open-chest dog demonstrated that strychnine retained its activity *in vivo* also. After intracoronary injection (5–50–100 μg), the drug elicited a sinusal bradycardia, without any impairment of conduction or contraction. The degree of activity appeared high, since the lowest dose employed induced an evident decrease of the rate, reaching the maximum value (−20·5%, mean of 4 experiments) 30–60 s after the injection and lasting 2–3 min.

The results of present investigations indicate that strychnine exerts a remarkable negative chronotropic activity, not only on isolated heart preparations but also *in vivo*. The mechanism of action appears independent of a cholinergic or a quinidine-like activity and deserves further research.

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The action of desipramine on noradrenaline depletion by reserpine in the vas deferens of the rat *in vivo*

The interaction between reserpine and desipramine at the level of adrenergic neurons (both in the central and in the peripheral nervous system) has been investigated by many authors. Although desipramine does not block the reserpine-induced depletion of endogenous noradrenaline in brain or heart (Brodie, Bickel & Sulser, 1961; Garattini, Giachetti & others, 1962; Pletscher & Gay, 1962; Sulser, Watts & Brodie, 1962; Stone, Porter & others, 1964), it does significantly reduce the rate at which reserpine releases noradrenaline from these tissues (Manara, Sestini & others, 1966; Manara, Algeri & Sestini, 1967; Sulser, Owens & others, 1969). Desipramine was also shown to inhibit the release of tritiated noradrenaline by small doses of reserpine from prelabelled mice hearts (Titus, Matussek & others, 1966).

In the present study, another peripheral organ, the vas deferens, was used because of its rich adrenergic innervation and its high noradrenaline content (Sjöstrand, 1965). Sprague Dawley rats, 200 g, were given desipramine 15 mg/kg, i.p. 1 h before reserpine and the animals were killed at selected times after reserpine. Noradrenaline measurements were made in the heart and in the vas deferens (Shore & Olin, 1958).

Four vasa deferentia were pooled for each sample. The releasing action of reserpine is not very much affected by the pretreatment with desipramine (Table 1). However, at some times and doses, desipramine pretreated animals show a lower concentration of noradrenaline in the vas deferens compared with controls given only reserpine. Fig. 1 shows noradrenaline levels determined simultaneously in the heart and in the vas deferens of the same animals. It is evident that while in the heart, as previously reported (Manara & others, 1966), desipramine counteracts the noradrenaline releasing action of reserpine, in the vas deferens desipramine rather facilitates the depletion of noradrenaline.

The reason for the discrepancy observed in these two organs may lie in differences in their pattern of innervation, (Sjöstrand, 1965), blood flow (Kopin, Gordon & Horst, 1965), or enzymatic activity (Svihovec & Weiner, 1967). In fact, it has been shown that in organs which have short adrenergic neurons, such as the vas deferens, the rate of noradrenaline release induced by reserpine is different from that of organs innervated by long adrenergic neurons such as the heart (Owman & Sjöberg, 1967; Sjöstrand & Swedin, 1968).

Table 1. *Effect of desipramine* (DMI) *on the release of noradrenaline induced by reserpine in the rat vas deferens*

Treatment	Dose mg/kg i.v.	Vas deferens noradrenaline (μg/g) ± s.e. determined at various times after reserpine			
		30′	60′	120′	240′
Reserpine	1	7·6 ± 0·3	6·4 ± 0·1	4·5 ± 0·5	2·3 ± 0·3
§DMI + Reserpine	1	7·5 ± 0·3	6·3 ± 0·4	4·2 ± 0·4	1·5 ± 0·1
Reserpine	2·5	8·0 ± 0·4	5·3 ± 0·6	2·6 ± 0·1	1·3 ± 0·2
§DMI + Reserpine	2·5	6·6 ± 0·5*	5·5 ± 0·8	2·5 ± 0·6	1·0 ± 0·1
Reserpine	5	6·3 ± 0·2	4·2 ± 0·5	1·7 ± 0·3,	0·9 ± 0·09
§DMI + Reserpine	5	5·2 ± 0·1†	4·2 ± 0·4	1·9 ± 0·3	0·6 ± 0·1
Reserpine	10	4·3 ± 0·3	3·2 ± 0·2	1·1 ± 0·07	
§DMI + Reserpine	10	4·5 ± 0·4	2·5 ± 0·1*	1·0 ± 0·02	
—		8·3 ± 0·2‡			
§DMI —			8·6 ± 0·3		

* $P < 0.05$.

† $P < 0.01$.

‡ Control values are the average of 29 experiments.

§ DMI (Desipramine) was injected i.p. 1 h before reserpine (Serpasil) at the dose of 15mg/kg. Numbers of experiments varied between 4 and 6 in each group.

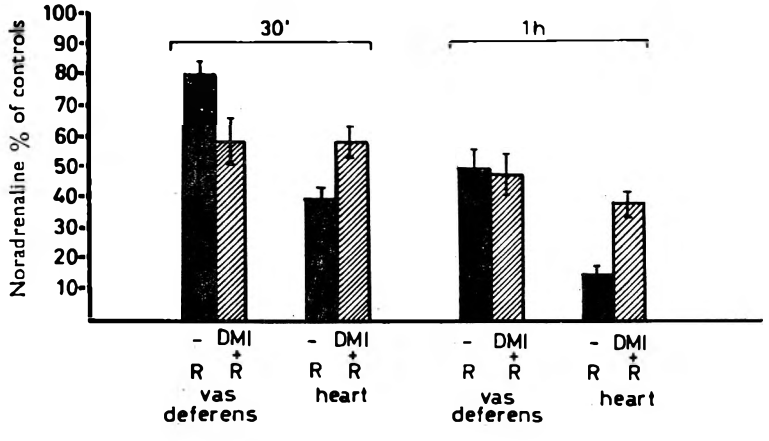

FIG. 1. Noradrenaline levels ± s.e. in heart and vas deferens after reserpine (R) and DMI + reserpine (DMI + R). Animals were killed 30 and 60 min after the i.v. injection of reserpine (2·5 mg/kg). Desipramine was given 60 min before reserpine at the dose of 15 mg/kg i.p. (Each column is the average of 4 determinations.)

Recently, Maître & Staehelin (1968), studying the effect of desipramine on the uptake of noradrenaline by various sympathetically innervated organs *in vivo*, showed that while the uptake of noradrenaline in the heart of the rat was inhibited by desipramine, it was "unexpectedly" enhanced in the vas deferens.

Taking into account these observations, it may be suggested that part of the noradrenaline released by reserpine from the vas deferens again enters into the nerve endings. Should the process of uptake be increased by desipramine, more noradrenaline would be available for MAO and therefore the concentration of noradrenaline would be reduced in animals given only reserpine.

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The effect of histamine releasers on the output of prostaglandins from rat diaphragms

Piper & Vane (1969) reported that antigen caused the release of prostaglandins E_2 and $F_2\alpha$ from appropriately sensitized perfused guinea-pig lungs. One of us has demonstrated that (+)-tubocurarine, a well-known releaser of histamine, causes the release of polar acidic lipids from the diaphragm of the rat (Laity, 1969). The same methods have revealed that in all three experiments with tolazoline hydrochloride (100 μg/ml) and morphine sulphate (50 μg/ml) and in duplicate experiments with pethidine hydrochloride (100 μg/ml) there was a release of polar acidic lipids from rat diaphragms. In these experiments, silicic acid column chromatography (Davies, Horton & Withrington, 1968), has separated the polar acidic lipids released by morphine, pethidine and tolazoline. This has shown that these polar acidic lipids consist of prostaglandins of the E and F series. The amount of PGF was larger than that of PGE (Fig. 1). Morphine, pethidine and tolazoline all produced a similar release of prostaglandins.

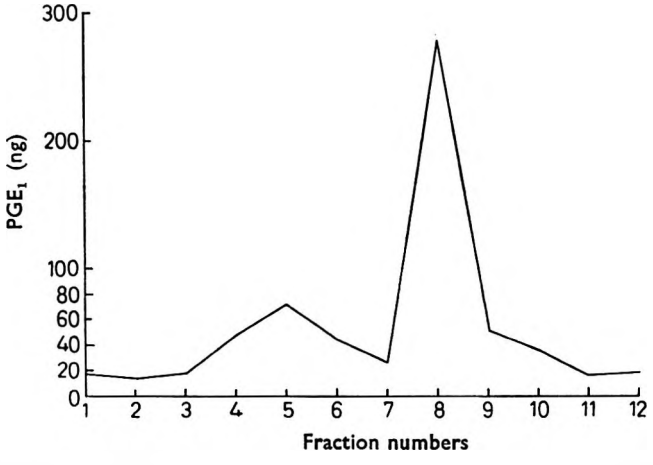

FIG. 1. Silicic acid column chromatogram of material extracted from bath fluid collected after exposure of rat diaphragms to Krebs solution containing pethidine (100 μg/ml) for 60 min at 37° gassed with 5% CO_2 in oxygen. Ordinate, biological activity in terms of PGE_1 assayed on the rat fundal strip. Abscissa, fraction numbers. Fractions were eluted with increasing concentrations of ethyl acetate in benzene: 1 and 2, 30%; 3 to 7, 40%; 8 and 9, 80% and 10, 100%. Fractions 11 and 12 were eluted with methanol.

It would thus appear that histamine releasers cause a similar release of prostaglandins from rat diaphragms to that produced by the antigen-antibody reaction in perfused guinea-pig lungs reported by Piper & Vane (1969).

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Oxotremorine: acute tolerence to it and its central "cholinolytic" effect in mice

Decsi, Várszegi & Méhes (1961a, b) reported that tremorine lost much of its analgesic, tremorigenic and narcosis-potentiating properties when given to mice. Keranen, Zaratzian & Coleman (1961) observed a progressive decline of the compound's tremorigenic effect after chronic treatment of unstated duration. Oelszner (1965) was unable to corroborate the observation on the analgesic effect of tremorine made by Decsi & others.

Doses of tremorine and oxotremorine prevent albino mice from clinging for more than 3 to 6 s to a rod operated at 7 rotations/min. Activity was regarded as negative in experiments in which the animal held fast to the rotarod for at least 180 s. The doses given are for oxotremorine oxalate, physostigmine salicylate, nicotine hydrogen tartrate, and tremorine dichlorhydrate.

Table 1 shows that a first injection of tremorine reduces the rotarod activity of a second injection given 16 h later, and totally prevents that of oxotremorine. The rotarod activity of oxotremorine (0·5–1·0 mg/kg, i.p.) wears off within 60–90 min.

Table 1. *Rotarod activity of tremorine and oxotremorine*

First i.p. injection at 0 h	Second i.p. injection at 16 h	n	% mice dropped off the rotarod at 16 h 30 min
0·9% NaCl	20 mg/kg tremorine	24	100
25 mg/kg tremorine	20 mg/kg tremorine	20	45
0·9% NaCl	0·5 mg/kg oxotremorine	10	100
25 mg/kg tremorine	0·5 mg/kg oxotremorine	10	0

Table 2. *Rotarod activity of 3 subsequent oxotremorine injections* (given at 0, at 60 or 90 min, and at 120 or 150 min)

Dose and route	n	% mice dropped off the rotarod 30 min after the: 1st injection	2nd injection	3rd injection
0·5 mg/kg all i.p.	56	89	29	5
1·0 mg/kg all i.p.	40	100	50	25
0·5 mg/kg i.p., i.p., i.v.	16	100	19	13
1·0 mg/kg i.p., i.p., i.v.	16	100	40	13

A second injection of the same dose is much less effective, and a third one almost ineffective (Table 2). Acute tolerance is not the result of decreased absorption, as even intravenous oxotremorine possesses negligible activity. No tremor was observed after the third injection.

A single injection of oxotremorine leaves nicotine lethality unaffected, but a second one given 30 min later inhibits it (Table 3). Similarly, a single dose of

Table 3. *The effect of a single and a repeated dose of oxotremorine on nicotine lethality*

Administration i.p. at 0 min	Administration i.p. at 30 min	n	Administration i.v. at 60 min	Lethality (%)
—	0·9% NaCl	12	2 or 2·5 mg/kg nicotine	100
—	1 mg/kg oxotremorine	12		92
0·9% Nacl	0·9% NaCl	26		92
1 mg/kg oxotremorine	1 mg/kg oxotremorine	26		19

Table 4. *LD50 values of physostigmine after oxotremorine and physostigmine*

Administration s.c.	Intervals between injections, min	LD50 values of physostigmine (95% confidence limit) mg/kg	(95% confidence limit) Relative activity
0·9% NaCl, once	30	approx. 1·2	>1·25
1 mg/kg oxotremorine, once	30	>1·5	
0·9% NaCl, twice	30	0·91 (0·8–1·04)	1·56 (1·34–1·8)
1 mg/kg oxotremorine, twice	30	1·42 (1·28–1·58)	
0·9% NaCl, 3 times	45	1·13 (0·89–1·44)	0·73 (0·52–1·03)
0·4 mg/kg Physostigmine, 3 times	45	0·83 (0·65–1·06)	

oxotremorine reduces physostigmine lethality (Table 4). A second dose applied 30 min later raises the LD50 significantly, by more than 50%. Thus, the development of the acute tolerance by oxotremorine is associated with the development of central anti-nicotine and anti-physostigmine effects.

The present experiments show that tremorine-tremorine, tremorine-oxotremorine, and oxotremorine-oxotremorine tolerances develop rapidly.

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Blockade of noradrenaline uptake and inhibition of gastric acid secretion by 2-[*p*-chlorophenyl-2-(pyridyl)-hydroxymethyl] imidazoline maleate (Sch-12650)

2-[*p*-Chlorophenyl-2 (pyridyl)hydroxymethyl] imidazoline maleate (Sch-12650) is as active in antidepressant tests in rats as the currently available antidepressants and possibly acts by interfering with catecholamine uptake mechanisms (Taber, Barnett & Roth, 1969). Imipramine is a blocker of noradrenaline uptake (Axelrod, Hertting & Potter, 1962) and also exhibits antigastric secretory activity (Bonfils, Dubrasquet & Lambling, 1962; Bass & Patterson, 1967; Lippmann, 1969). Similarly, 3,3-dimethyl-1-(3-methylaminopropyl)-1-phenylphthalan (Lu 3-010) has been shown to be effective both in blocking noradrenaline uptake (Waldeck, 1968; Lippmann, 1970b) and in inhibiting gastric acid secretion (Lippmann, 1970b). I have examined the effects of Sch-12650 on these activities.

The effects of intraperitoneally-administered Sch-12650 on the uptake and release of [^{3}H]noradrenaline (^{3}H–NA) in the heart of the rat are shown in Table 1. The animals (7–9 in each group) were injected with 2·5 μCi ^{3}H–NA (5·1 Ci/mM), intravenously, in 0·25 ml of 0·75% NaCl solution 45 min before or after the test compound and were killed 2 h after the test compound; ^{3}H–NA levels in the tissues were measured (Lippmann, 1969). Sch-12650 blocked the uptake, and did not cause an increased release, of ^{3}H–NA. The compound was similar in potency to imipramine as the ^{3}H–NA level was reduced to one-half by about 4 mg/kg of either compound.

Table 1. *Inhibition of uptake of* [^{3}*H*]*noradrenaline in the rat heart by Sch*-12650 *or imipramine given* 45 *min before or after the* ^{3}*H–NA*

Compound	Dose mg/kg, i.p.	Radioactivity content: counts/min g^{-1} ± s.e.	% Inhibition
Before ^{3}H–NA			
None	—	2761 ± 50 (P <0·001)	
Sch-12650 ..	10·0	775 ± 76 (P <0·001)	72
	5·0	1174 ± 57 (P <0·001)	57
	2·5	1871 ± 57 (P <0·001)	32
	1·25	2375 ± 109 (P <0·001)	14
Imipramine ..	5·0	781 ± 54 (P <0·001)	72
	2·5	1973 ± 162 (P <0·001)	29
After ^{3}H–NA			
None	—	2429 ± 48	
Sch-12650 ..	5·0	2554 ± 74	
	2·5	2622 ± 92	
Imipramine ..	5·0	2509 ± 115	

Table 2. *Inhibition of basal gastric acid secretion by Sch*-12650 *or imipramine*

Compound	Dose mg/kg, i.p.	Gastric acid secretion: m-equiv acid/ 3 h ± s.e. Exp. I	Exp. II	% Inhibition
None ..	—	0·33 ± 0·04	0·40 ± 0·04	
Sch-12650 ..	5·0	0·11 ± 0·03 (P <0·001)		65
	2·5	0·18 ± 0·01 (P <0·02)	0·22 ± 0·04 (P <0·01)	44, 46
	1·25		0·34 ± 0·07	
Imipramine ..	5·0	0·10 ± 0·03 (P <0·001)	0·13 ± 0·01 (P <0·001)	71, 69
	2·5		0·22 ± 0·07 (P <0·05)	46

Table 3. *Inhibition of pentagastrin-induced gastric acid secretion by Sch-12650 or imipramine*

Compound	Dose mg/kg	Gastric acid secretion: μ-equiv acid/ 2 h ± s.e.	Gastric acid secretion: % Inhibition
None	—	16 ± 1	
Pentagastrin (P)	0·001 (5×), s.c.	41 ± 8	
Sch-12650	5·0, i.p.	19 ± 2 (P <0·01)	88
+ P	0·001 (5×), s.c.		
Sch-12650	2·5, i.p.	30 ± 3	
+ P	0·001 (5×), s.c.		
Imipramine	10·0, i.p.	19 ± 3 (P <0·05)	88
+ P	0·001 (5×), s.c.		
Imipramine	5·0, i.p.	32 ± 2	
+ P	0·001 (5×), s.c.		

The effect of Sch-12650 on basal gastric acid secretion was measured using the Shay procedure (Shay, Sun & Gruenstein, 1954) as modified by Lippmann (1969). There were 8 animals in the control and 5–8 animals in each treated group. Sch-12650 inhibited the basal gastric acid secretion and at 2·5 mg/kg, intraperitoneally, decreased the gastric acid secretion to about one-half that of controls (Table 2). This activity was similar to that of imipramine.

In Table 3 are shown the effects of Sch-12650 on the induced increase in gastric acid secretion caused by pentagastrin (Lippmann, 1970a). There were 8 animals in the control and 6–8 in each treated group. Sch-12650 prevented the increase in gastric acid secretion at 5·0, but not at 2·5, mg/kg, intraperitoneally, while imipramine blocked the increase at 10, but not at 5, mg/kg. Thus, Sch-12650 was about twice as active as imipramine in preventing the pentagastrin-induced increase in gastric acid secretion.

Sch-12650 is similar to imipramine as it blocks uptake of noradrenaline and inhibits both basal and induced gastric acid secretion in the rat. The importance of the blockade of noradrenaline in relation to antisecretory activity of a drug has been previously discussed (Lippmann, 1970b). This compound has an advantage in that it does not antagonize acetylcholine (Taber & others, 1969), as does imipramine (Domenjoz & Theobald, 1959).

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Potentiation of the central actions of 5-hydroxytryptophan in rabbits by DL-α-hydrazino-α-methyldopa

DL-α-Hydrazino-α-methyldopa (HMD) (Porter, Watson & others, 1962) and RO 4–4602 [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine] (Burkard, Gey & Pletscher, 1964) are effective in potentiating both the central effects of dopa in animals (Bartholini, Blum & Pletscher, 1969; Lotti, 1969) and some of the anti-Parkinson actions in man (Cotzias, Papavasiliou & Gellene, 1969; Tissot, Bartholini & Pletscher, 1969). The mechanism of this potentiation is based on the ability of HMD to inhibit peripheral L-aromatic amino-acid decarboxylase, but presumably not of that in the central nervous system, thus permitting more of the dopa to reach the brain where decarboxylation to dopamine occurs. These findings suggested to us the possibility of using HMD in our experiments on the central actions of DL-5-hydroxytryptophan (5-HTP) in the rabbit, because this amino-acid is also decarboxylated by L-aromatic amino-acid decarboxylase to give 5-hydroxytryptamine (5-HT).

The behavioral and hyperthermic actions of 5-HTP in the rabbit were used as the end points in the experiments now described. 5-HTP, 35–50 mg/kg, intravenously, produced dose-dependent excitation and hyperthermia. We found previously that 75–100 mg/kg of 5-HTP caused extreme excitation, hyperthermia, and death of the animal (Horita & Gogerty, 1958). With the smaller doses of 5-HTP the hyperthermia reached a peak approximately 1 h after administration and gradually returned to normal over the succeeding 2–3 h. HMD alone (25 mg/kg, i.v.) had little effect on rectal temperature. The administration of 35 mg/kg of 5-HTP to 18 HMD-pretreated rabbits produced a mean increase of about 2·5° in rectal temperature in 13 animals, while five of the animals showed either no increase or a slight decrease in rectal temperature under these conditions. The temperature responses of these latter animals are plotted as a separate curve in Fig. 1. In those rabbits responding with hyperthermia, the peak increase occurred between 2 and 2½ h after 5-HTP injection, while in controls given only 5-HTP, responses averaging some 0·6°, occurred within the first hour after injection. The duration of action of the HMD + 5-HTP-treated animals also was consistently longer than in control animals. Five of the

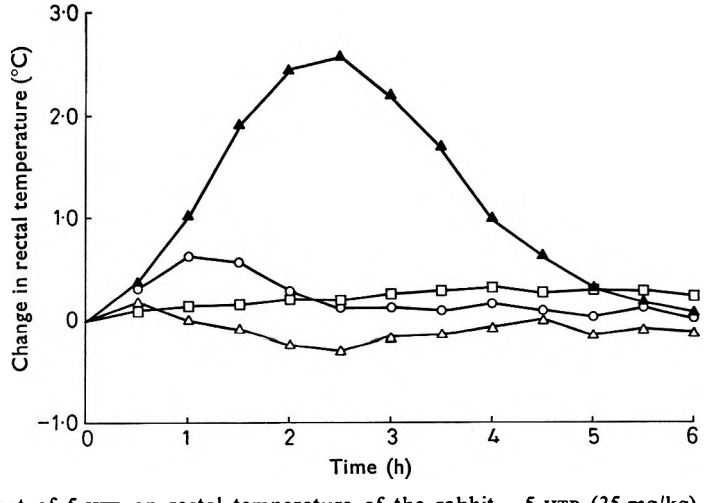

FIG. 1. Effect of 5-HTP on rectal temperature of the rabbit. 5-HTP (35 mg/kg), was administered at 0 h. HMD was given 30 min before 5-HTP administration. ○—control 5-HTP (n = 6); □—control HMD (n = 6); ▲—HMD + 5-HTP, responders (n = 13); △—HMD + 5-HTP, non-responders (n = 5).

Table 1. *Brain levels of* 5-HT *in control,* HMD-, 5-HTP-, *and* HMD + 5-HTP-*treated rabbits*

	Treatment	n	5-HT content (μg/g) ± s.d.
a.	Control	6	0·50 ± 0·02
b.	HMD	6	0·50 ± 0·06
c.	5-HTP	8	0·87 ± 0·15
d.	HMD + 5-HTP ..	8	1·47 ± 0·14

HMD was given i.v. in a dose of 25 mg/kg. 5-HTP dosage was 35 mg/kg, i.v., and in group d was administered 30 min after the HMD. Animals were killed 2 h after the 5-HTP injections.

13 responders exhibited temperatures above 43–44° and succumbed during the first 3 h after 5-HTP administration. A dose of 50 mg/kg of 5-HTP administered to HMD-pretreated rabbits produced marked excitation and hyperthermia, the latter rising to above 42°, and in most instances the animals succumbed at the peak of the temperature response.

Brain 5-HT levels were determined in control, HMD-treated, and HMD + 5-HTP-treated animals. Two h after 5-HTP was administered the animals were killed with an intravenous injection of air. The brains were rapidly removed, homogenized in 0·1N HCl, and 5-HT levels were assayed (Bogdanski, Pletscher & others, 1956). Care was taken to employ a double borate buffer wash of the butanol extract to completely remove any residual 5-HTP that might have been extracted. The results of these analyses are in Table 1. In control animals which had 35 mg/kg of 5-HTP 2 h before death, the brain 5-HT concentrations increased 74%, while in HMD-pretreated animals the same dose of 5-HTP produced a 194% increase. These data coincide with potentiation of the excitatory and hyperthermic actions of 5-HTP by HMD. HMD by itself did not affect brain 5-HT concentrations.

Thus HMD is effective in potentiating the central actions of 5-HTP and permits its use in smaller amounts to produce pharmacological responses. Also, the 5-HTP-induced hyperthermia in rabbits appears to be a centrally mediated phenomenon. This suggests the desirability of using HMD rather than a monoamine oxidase inhibitor to potentiate the central actions of 5-HTP, especially since the latter compounds may add further variables to the central nervous system pharmacology of 5-HTP and 5-HT (Green & Sawyer, 1964). Finally, these results point to a possible use for drugs such as HMD as an adjunct to 5-HTP therapy in Down's syndrome (Bazelon, Paine & others, 1967), much as HMD appears to be useful with dopa in alleviating symptoms of Parkinson's Disease.

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Identification of cannabis

The higher incidence of abuse of cannabis in recent years has necessitated identification of larger numbers of cannabis samples. This in turn has caused workers in the field (e.g. Turk, Dharir & Forney, 1969; de Faubert Maunder, 1969a, b, c) to search for more rapid tests with a view to reducing the time of analysis to the minimum that allows certainty of identification. We should like to describe a procedure for analysis that is advantageous in combining two independent techniques for the detection of three cannabis components and which offers positive identification in a reasonably short time (20 min). It consists of extraction of the suspected cannabis or cannabis resin sample with a stock solution of dibenzylphthalate in light petroleum, the extract then being analysed without further purification by gas chromatography and also by paper chromatography. Both chromatographic systems offer good resolution of the three cannabis components.

An extract is prepared by shaking the cannabis or cannabis resin vigorously for 1 min with sufficient stock solution of dibenzylphthalate (10 mg/ml) in light petroleum (40–60°) to produce a mixture containing approximately 20% w/v cannabis or 10% w/v of cannabis resin. The supernatant solution is used, without further purification, for chromatography.

In our experiments a Pye 104 Gas Chromatograph equipped with a flame ionization detector and a Kelvin Electronics servoscribe recorder has been used. The column is glass, 5 ft × 4 mm internal diameter, packed with 80–100 mesh acid-washed, siliconized Diatomite C which is coated with 1% cyclohexanedimethanol succinate (CDMS). A hydrogen pressure of 18 lb/inch^2, air 7 lb/inch^2, and a nitrogen flow rate of 50 ml/min is used throughout. The operating temperature is 220°. 1 μl of the extract is injected onto the column at an appropriate attenuation and the retention times of cannabidiol (CBD), Δ^1-3,4-*trans*-tetrahydrocannabinol (THC), and cannabinol (CBN) are calculated relative to dibenzylphthalate, (DBT) the internal standard. The total analysis time is approximately 15 min. Retention times of the cannabinols relative to dibenzylphthalate are: cannabidiol 0·26; THC 0·39; cannabinol 0·64.

For paper chromatography, Whatman SG81 paper (7 × 25 cm) is immersed in a 15% w/v solution of silver nitrate in distilled water, the excess solution is allowed to drain off, and the paper is then air dried. After applying spots of the extract of suspected cannabis or cannabis resin, and of Δ^1-3,4-*trans*-tetrahydrocannabinol, the paper is developed in chloroform using the ascending technique. Location of the cannabinols is by spraying successively with a 1% solution of Fast Blue Salt B in water and then 2N sodium hydroxide. Development time is 10 min for a 5 cm run. Rf values are: cannabidiol 0·3; THC 0·6; cannabinol 0·8.

A number of gas chromatographic systems for the analysis of cannabis samples have previously been reported, the most recent of which (Lerner, 1969) has described the use of OV.17 as the stationary phase and (±)-methadone hydrochloride as an

internal standard. This allows quantitation of cannabinols in samples should this be necessary. The method which we have described also allows quantitation of cannabinols but differs from that of Lerner in using an internal standard which has a longer retention time than that of any of the cannabis components. This, in our view, is advantageous in reducing the probability of one of the components of cannabis having the same retention time as the internal standard.

The use of silver nitrate impregnated media for separation of cannabinols has previously been reported by Caddy & Fish (1967), Hively, Mosher & Hoffman (1966) and by Turk & others (1969) and it is our experience that these systems offer satisfactory resolution of the cannabinols. However, the modification which we have described is ideally suited to routine analysis in that a large number of silver nitrate impregnated papers can be prepared in one batch and conveniently stored ready for use in an envelope. Papers may be stored thus, in the dark, for up to one month.

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