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Infrared identification of lysergide (LSD)

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Infrared spectra of LSD and its tartrate salts, and factors affecting their reproducibility, have been investigated. Spectra corresponding to amorphous and crystalline forms of LSD base, the neutral tartrate and two forms of the hydrogen tartrate were obtained. The neutral tartrate was found to undergo conversion to the hydrogen tartrate on long standing. Comparison with spectra of related compounds shows that all the LSD spectra are distinctive and can be used for identification purposes, but the use of potassium bromide discs was found to cause spectral changes in the salts.

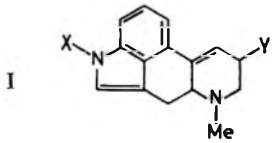
The increasingly widespread abuse of hallucinogenic drugs has led to their being controlled by legislation in many countries, which in turn has necessitated the development of simple and unambiguous methods for their identification. Attention has been concentrated mainly on lysergic acid diethylamide (lysergide; *NN*-diethyl-D-lysergamide; LSD) and its derivatives, for which methods requiring sub-milligram amounts are essential. Thin-layer or paper chromatography has been widely accepted as a means of identification, supplemented by spectrophotometry or spectrofluorimetry for quantitative estimation (Genest & Farmilo, 1964; Dal Cortivo, Broich & others, 1966; Martin & Alexander, 1967, 1968; Look, 1968). Gas chromatography has been used for the identification of other hallucinogenic amines (Genest & Hughes, 1968) but is not suitable for direct use with lysergic acid derivatives (Radecka & Nigam, 1966), although Lerner (1967) has obtained satisfactory results with the trimethylsilyl derivative of LSD. These methods, however, are not completely specific, and where evidence is liable to be contested in a court of law, a more positive identification is desirable. Mass spectrometry has been recommended for this purpose (Bellman, 1968), but is not available to all laboratories engaged in pharmacological or forensic work, and in general infrared spectrophotometry is the most widely used technique (Hale & Taylor, 1967; Cromp & Turney, 1967). It is therefore surprising to note how little attention has been paid in published methods to means of ensuring consistent spectra and to the factors affecting reproducibility.

Seizures of illicit LSD may range between substantially pure samples of LSD tartrate and a variety of dosage forms including tablets, capsules, impregnated sugar cubes or blotting paper, from which the active material must be extracted, usually as the free base. Any infrared identification procedure must therefore be capable of dealing with either the salt or the base. Previous work on the infrared identification of steroids (Mesley & Johnson, 1965), sulphonamides (Mesley & Houghton, 1967) and barbiturates (Mesley & Clements, 1968) has demonstrated the necessity, whenever possible, to compare the spectrum of the sample with that of an authentic specimen recorded under the same conditions, and solvent treatments have been recommended as a means of overcoming polymorphism in these compounds. For LSD, authentic specimens are not readily available, and limitations of sample size may prevent any further treatment of the sample if the first recorded spectrum is not identifiable.

In examining samples of illicit origin it is therefore desirable to have reference spectra already available and to have ascertained in advance any factors which are likely to affect the reproducibility of such spectra. In the work here described the variability of LSD spectra has been investigated together with the spectra of some other lysergic acid derivatives with which confusion is possible.

EXPERIMENTAL

Materials



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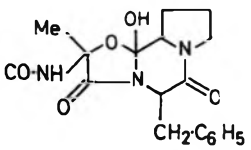

II

I. LSD	X = H	Y = CO.NEt$_2$
1-Acetyl-LSD	X = Me.CO	Y = CO.NEt$_2$
Lysergic acid	X = H	Y = CO.OH
Ergometrine	X = H	Y = CO.NH.CH(Me).CH$_2$OH
Methylergometrine	X = H	Y = CO.NH.CH(Et).CH$_2$OH
Methysergide	X = Me	Y = CO.NH.CH(Et).CH$_2$OH
Ergotamine	X = H	Y = II

Materials were obtained from the following sources: LSD (*NN*-diethyl-D-lysergamide): neutral tartrate (Delysid, LSD-25) from Sandoz Products Ltd.; synthesized sample of neutral tartrate (see discussion); also illicit samples of hydrogen tartrate and free base extracted from dosage forms. 1-Acetyl-LSD: tartrate from Sandoz Products Ltd. (ALD-52). Ergometrine (Ergonovine; *N*-[1-(hydroxymethyl)ethyl]-D-lysergamide): hydrogen maleate from Burroughs Wellcome and Co. Methylergometrine (Methylergonovine; *N*-[1-(hydroxymethyl)propyl]-D-lysergamide): hydrogen maleate from Sandoz Products Ltd. Methysergide (*N*-[1-(hydroxymethyl)-propyl]-1-methyl-D-lysergamide): hydrogen maleate from Sandoz Products Ltd. Ergotamine: tartrate from Burroughs Wellcome and Co. D-Lysergic acid: from Chemical Defence Experimental Establishment.

Spectra

Infrared absorption spectra were recorded using a Grubb Parsons GS2 grating spectrometer. Samples were prepared as mulls in Nujol (liquid paraffin) or as pressed alkali halide discs prepared from potassium bromide (Spectroscopic grade, E. Merck, A.G., Darmstadt) or potassium chloride (Hopkin and Williams Analar grade).

DISCUSSION

LSD. The reference sample of Delysid was stated by the manufacturers to be a methanol solvate of the neutral tartrate, with the formula $(C_{20}H_{25}N_3O)_2.C_4H_6O_6.CH_3OH$. Spectra of this material recorded as a Nujol mull and as a potassium bromide disc showed detail differences, but were both characterized by the absence of absorptions attributable to free carboxyl groups (Figs 1 and 2). The limited quantity available precluded investigation of the possibility of polymorphism in the neutral tartrate. The sample of synthetic material, which when first prepared gave the same spectrum as the Delysid sample, was found after two years to have undergone conversion to the hydrogen tartrate, shown by the appearance in the Nujol mull spectrum of a carbonyl absorption at 1730 cm^{-1} and by the fact that the potassium bromide disc spectrum shows bands characteristic of potassium hydrogen tartrate (Figs 3 and 4).

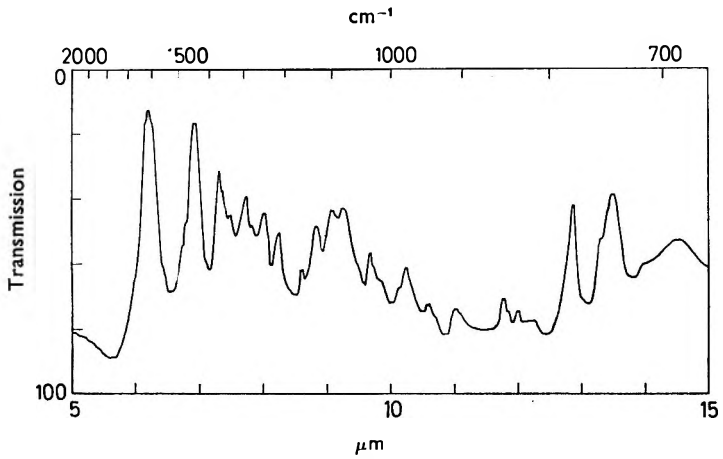

FIG. 1. Nujol mull spectrum of LSD tartrate (Delysid).

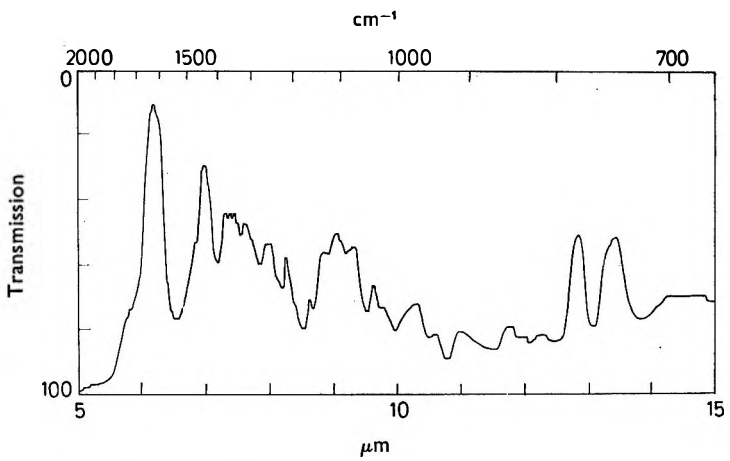

FIG. 2. Potassium bromide disc spectrum of LSD tartrate (Delysid).

A very similar potassium bromide disc spectrum (described in error as the base) appears in the Sadtler Standard Spectra (No. 30580), and evidence of partial conversion to the hydrogen tartrate has also been found in spectra from other sources of allegedly authentic LSD tartrate. It must therefore be concluded that the neutral tartrate is not stable indefinitely.

The fate of the second molecule of LSD is not clearly established, but there is some evidence to suggest that it may be present in the form of the free base. This is supported by the detection in some capsules of illicit origin of LSD in both free base and salt form. It is also uncertain whether the remaining hydrogen tartrate is still solvated with methanol. The spectrum of this material is certainly quite different from that of another illicit sample (Fig. 5) which also showed the characteristics of a hydrogen tartrate, i.e. the presence of a carbonyl absorption and conversion in a potassium bromide disc to potassium hydrogen tartrate. The latter sample was apparently unsolvated as it could be recovered unchanged from a variety of solvents. One of the patents concerning LSD (Pioch, 1956) specifically refers to crystallization of the hydrogen tartrate in the absence of methanol, and the illicit material may

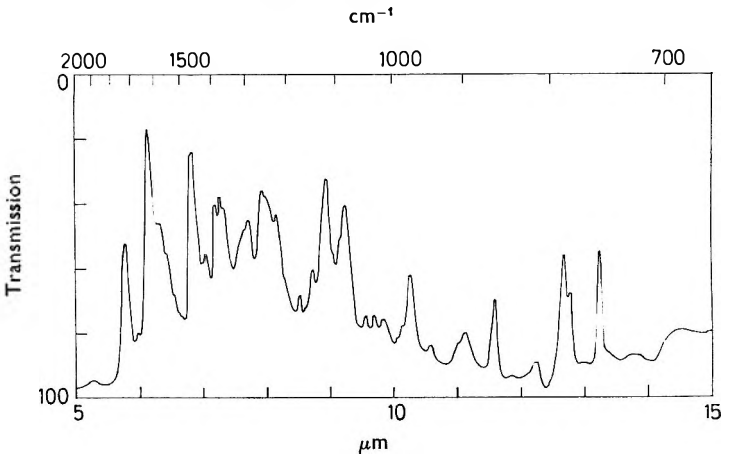

FIG. 3. Nujol mull spectrum of LSD tartrate after two years.

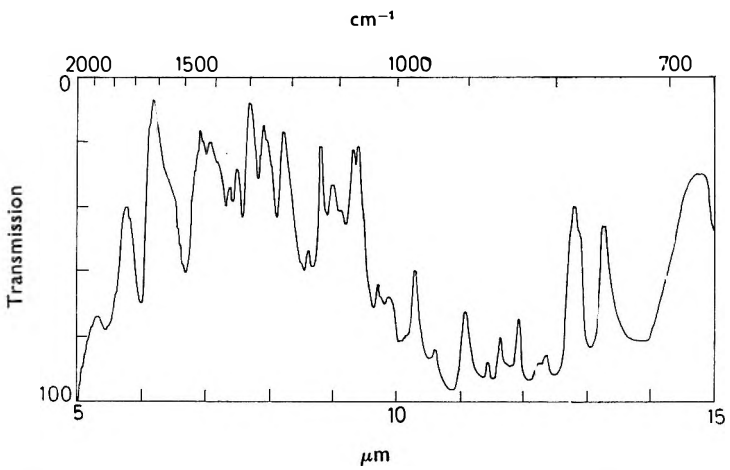

FIG. 4. Potassium bromide disc spectrum of LSD tartrate after two years.

well correspond to this product. No method of interconversion of the two forms of the hydrogen tartrate has been discovered, but both yielded LSD base when made alkaline and both showed evidence of conversion to potassium hydrogen tartrate when prepared as potassium bromide discs. The existence of two crystalline forms could thus be due either to polymorphism or to solvation of one form.

LSD base has been encountered in two forms. Evaporation of a chloroform solution directly on to a rock salt plate yielded an amorphous smear, the spectrum of which (Fig. 6) agrees with those published by Troxler & Hofmann (1957), Hayden, Brannen & Yaciw (1966), Lerner (1967) and Cromp & Turney (1967). On the other hand, precipitation of the base from an aqueous solution of the hydrogen tartrate by cautious addition of alkali gave a crystalline product, which was also obtained by crystallization of the base from aqueous acetone; a second crystalline form is also known but was not encountered in this work. The spectrum of the crystalline product (Fig. 7) is undoubtedly more distinctive than that of the amorphous form, but in practice is unlikely to be achieved from the average seizure sample of LSD, where

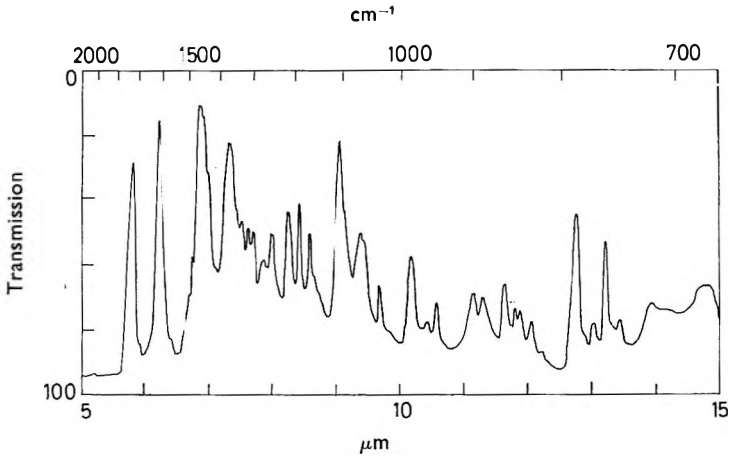

FIG. 5. Nujol mull spectrum of LSD hydrogen tartate (of illicit origin).

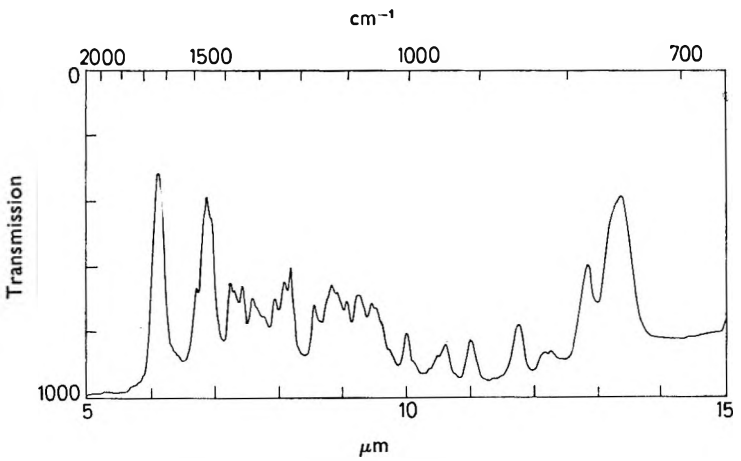

FIG. 6. Infrared spectrum of amorphous LSD base (smear from chloroform solution).

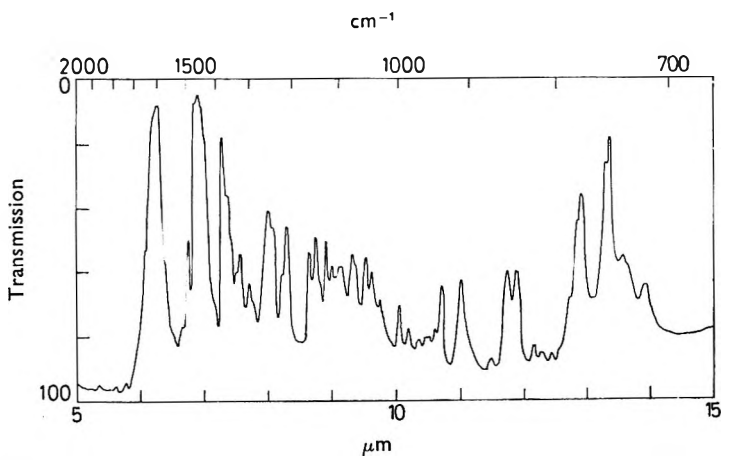

FIG. 7. Nujol mull spectrum of crystalline LSD base.

the small quantity generally precludes both recrystallization and the use of a Nujol mull. For handling sub-milligram quantities the most practicable technique is the potassium bromide disc method using cardboard or metal foil formers with a small aperture, together with a beam condenser or ordinate scale expansion, and this will normally give the spectrum of amorphous LSD.

The spectra obtained by Hale & Taylor (1967), using this technique, in fact correspond to the neutral tartrate. These workers claimed to have obtained the base from an aqueous solution of the tartrate, using sodium bicarbonate to make the solution basic before extracting with chloroform. This was presumably intended to avoid conversion to the isolysergamide derivative, but they were obviously too cautious. There appears to be no objection to the use of sodium hydroxide for this purpose, provided that the alkaline solution is immediately extracted with chloroform before epimerization can occur.

1-*Acetyl-LSD.* The small sample described as ALD-52 was a salt, identified as a hydrogen tartrate by the similarity of its infrared spectrum with that of LSD hydrogen tartrate. As with the latter the potassium bromide disc showed spectral differences from the Nujol mull consistent with ion exchange. The free base was isolated and examined as an amorphous smear deposited on a rock salt plate, giving a spectrum which agreed with that published by Troxler & Hofmann (1957) for a Nujol mull.

Other lysergamide derivatives. Ergotamine was obtained as the neutral tartrate and converted to the free base. The salt and the base had rather similar spectra, but were both readily distinguishable from all the other compounds examined. Ergometrine, methylergometrine and methysergide were all obtained as hydrogen maleates, the spectra of which, as Nujol mulls, were similar to each other but readily distinguishable. When prepared as potassium bromide discs, however, all three underwent ion exchange to give potassium hydrogen maleate and the hydrobromides of the bases. Under these conditions the methylergometrine and methysergide salts were not clearly distinguishable. The free bases all showed a tendency to darken on standing, and recovery of ergometrine and methylergometrine from a variety of solvents yielded either amorphous products or solvated crystalline forms from which the solvent could not be removed without decomposition.

Lysergic acid. The original material was probably a hydrate; recoveries from solvents yielded two additional crystalline forms and an amorphous form. A reproducible product was usually obtained by evaporation of acetone or chloroform solution on a water bath. Acidification of an aqueous solution with hydrochloric acid, followed by concentration at room temperature, yielded the hydrochloride which separated out as lustrous plates. Attempts to recover this material from organic solvents gave amorphous products which were brilliantly coloured, red from acetone, green from ethanol and sky blue from chloroform.

DISCUSSION OF INFRARED SPECTRA

All the lysergic acid amides, when examined as free bases, show prominent absorption bands near 775 cm^{-1} (medium intensity) and 745 cm^{-1} (strong), which may be ascribed to C—H out-of-plane deformation vibrations in the indole part of the molecule. The tertiary amides (LSD and 1-acetyl-LSD) have a single strong C=O band at 1600–1630 cm^{-1}; in addition, acetyl-LSD has a second band at 1700 cm^{-1},

the high frequency of which was attributed by Troxler & Hofmann (1957) to the lack of basic character of the indole nitrogen, causing the group to behave like a ketone rather than an amide. The four secondary amides examined all show the characteristic amide I and II bands at about 1640 and 1550 cm^{-1}; ergometrine, methylergometrine and methysergide also have a strong band in the 1030–1050 cm^{-1} region due to the primary hydroxyl group, and the two former compounds are distinguished from methysergide by the presence of a strong band of uncertain origin at 1200–1220 cm^{-1}. The presence of the bands at 1550 and 1040 cm^{-1} in these three compounds and at 1540 and 1725 cm^{-1} in ergotamine serves to distinguish these lysergamide derivatives from LSD.

The high frequency region is not particularly helpful, as amorphous LSD and the secondary amides all have bands centred at about 3270 cm^{-1}, which must include both N—H and O—H stretching absorptions; however, acetyl-LSD is noteworthy as having no absorptions above 3100 cm^{-1}.

The spectra of the salts show features characteristic of the anion as well as those due to the base, and as some of these are unusual they are mentioned here. All carboxylic acid salts normally show two absorptions due to the carboxylate ion at approximately 1570 and 1400 cm^{-1}. With a dicarboxylic acid in which only one hydrogen atom has been replaced, the free carboxyl group should give rise to a C=O stretching absorption near 1710 cm^{-1}. The spectra of the two forms of LSD hydrogen tartrate (Figs 3 and 5) both show a sharp absorption near 1730 cm^{-1}, the rather high frequency of which may be due to the influence of the α-hydroxyl group. Fig. 3 also shows absorptions at 1600 and 1418 cm^{-1} attributable to the carboxylate ion, but these are obscured by other bands in the spectrum of Fig. 5. The potassium bromide disc spectrum (Fig. 4), corresponding to the formation of potassium hydrogen tartrate, has bands at 1570 and 1410 cm^{-1} due to the carboxylate ion, but the 1730 cm^{-1} band is broadened and reduced in intensity and is accompanied by a weaker broad band at about 1860 cm^{-1}; the latter may be an indication of strong hydrogen bond formation in the anion.

Other tartrate absorptions occur at approximately 1105, 1075 and 690 cm^{-1} in the neutral LSD salt (the latter band being very broad), shifting to 1110, 1070 and 680 cm^{-1} in the potassium bromide discs. Corresponding absorptions in the acid tartrates are at about 1255, 1110, 1075 and 690 cm^{-1} for the mulls, whilst in the potassium bromide disc the prominent bands at 1305, 1260, 1215, 1135, 1075/1067 (doublet) and 675 cm^{-1} are all associated with the hydrogen tartrate ion, the last-mentioned band being particularly intensified. All of these bands are also present in the potassium bromide disc spectrum of ALD-52, confirming that this also is a hydrogen tartrate, although the band due to the free carboxyl group is largely obscured by the C=O absorption of the 1-acetyl group.

The maleate salts, all of which are known to be hydrogen maleates, show no free carboxyl absorption apart from a weak shoulder at about 1680 cm^{-1} in the mull spectra, which disappears altogether when they are examined as potassium bromide discs. The absence of the carbonyl absorption in the spectrum of potassium hydrogen maleate has been ascribed by Cardwell, Dunitz & Orgel (1953) to the symmetry of the anion, in which the single proton is almost centrally placed between the two carboxylate groups. Other characteristic bands in the maleates occur at 887 and 866 cm^{-1} in the mull spectra, converging in the potassium bromide discs to give a double peak at 873 and 862 cm^{-1}.

The spectra of the various forms of lysergic acid all show absorptions at approximately 1580 and 1360 cm^{-1} attributable to COO^- ions and at 2300 cm^{-1} due to NH^+, indicating internal ionization. On conversion to the hydrochloride the 2300 cm^{-1} band remains, but the carboxylate absorptions are replaced by a band at 1700 cm^{-1} consistent with the free carboxyl group.

CONCLUSIONS

The infrared spectra of LSD base and its tartrate salts are quite distinctive and can therefore be used for identification purposes. With the salts, the use of alkali halide discs can cause marked changes in the spectrum, and in the case of the maleates of ergometrine and related compounds can prevent identification of the parent base. This could conceivably also occur with tartrates of compounds closely related to LSD, so it would be wise to record a mull spectrum if quantities permit. Similarly, the mull spectrum of crystalline LSD is more distinctive than that of the potassium bromide disc, in which partial conversion to the amorphous form takes place. Nevertheless, in dealing with sub-milligram quantities of extracted bases, the pressed disc method is likely to be the only practicable way of obtaining a spectrum.

For the identification of hallucinogens infrared spectroscopy will most frequently be used in conjunction with thin-layer chromatography, and in many instances it may be necessary to use the same material for both purposes. Nothing has been said here concerning the practical technique of recovering material from thin-layer plates for infrared examination, as this has been adequately covered elsewhere (e.g., Cromp & Turney, 1967; Hale & Taylor, 1967). However, it should be emphasized that both the substrates and the solvents generally used contain impurities which may well obscure the sample spectrum when working at the 50 μg level or below. It is therefore essential to use the highest purity solvents (preferably redistilled), to wash the substrate with the eluting solvent before running the chromatogram, and whenever possible to take an authentic sample through the same procedure and use its spectrum for reference purposes.

REFERENCES

Bellman, S. W. (1968). *J. Ass. off. analyt. Chem.*, **51**, 164–175.

Cardwell, H. M. E., Dunitz, J. D. & Orgel, L. E. (1953). *J. chem. Soc.*, 3740–3742.

Cromp, C. C & Turney, F. G. (1967). *J. forens. Sci.*, **12**, 538–546.

Dal Cortivo, L. A., Broich, J. R., Dihrberg, A. & Newman, B. (1966). *Analyt. Chem.*, **38**, 1959–1960.

Genest, K. & Farmilo, C. G. (1964). *J. Pharm. Pharmac.*, **16**, 250–257.

Genest, K. & Hughes, D. W. (1968). *Analyst, Lond.*, **93**, 485–489.

Hale, D. & Taylor, C. (1967). *Perkin-Elmer Instrum. News*, **18** (1), 6

Hayden, A. L., Brannon, W. L. & Yaciw, C. A. (1966). *J. Ass. off. analyt. Chem.*, **49**, 1109–1153.

Lerner, M. (1967). *Bull. Narcot.*, **19** (3), 39–45.

Look, J. (1968). *J. Ass. off. analyt. Chem.*, **51**, 1318–1323.

Martin, R. J. & Alexander, T. G. (1967). *Ibid.*, **50**, 1362–1366.

Martin, R. J. & Alexander, T. G. (1968). *Ibid.*, **51**, 159–163.

Mesley, R. J. & Clements R. L. (1968). *J. Pharm. Pharmac.*, **20**, 341–347.

Mesley, R. J. & Houghton, E. E. (1967). *Ibid.*, **19**, 295–304.

Mesley, R. J. & Johnson, C. A. (1965). *Ibid.*, **17**, 329–340.

Pioch, R P. (1956). U.S. Patent 2,736,728.

Radecka C. & Nigam, I. C. (1966). *J. pharm. Sci.*, **55**, 861–862.

Sadtler Standard Spectra, published by Sadtler Research Laboratories Inc., 3316, Spring Garden Street, Philadelphia, Pa.

Troxler, F. & Hofmann, A. (1957). *Helv. chim. Acta*, **40**, 1706–1720.

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The flow of granular solids through circular orifices

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It has been shown that the use of the bulk density term in place of the particle density, in the equation of flow for granular solids passing through a circular orifice, very largely eliminates differences due to the shape, rugosity, density, porosity and friction of the particles.

The equation

$$Do = (1{\cdot}136 + 0{\cdot}000173Dp)\left(\frac{4W}{60\pi\rho B\sqrt{g}}\right)^{\frac{1}{0{\cdot}903+0{\cdot}675\log Dp}}$$

has been tested on seven different materials and has been found to predict the flow of single and binary systems with an overall accuracy of $\pm 5\%$ and $\pm 10\%$ respectively.

The flow of granular materials through circular orifices has been studied previously and equations have been derived which allow predictions of the flow rate to be made. Most of these equations have been either empirical or based on dimensional analysis (Deming & Mehring, 1929; Bingham & Wikoff, 1931; Rose & Tanaka, 1959; Fowler & Glastonbury, 1959; Brown & Richards, 1959). Relatively few have been based on theoretical considerations (Brown, 1961; Zenz, 1962; McDougall & Evans, 1965; Shinohara, Demitsu & others, 1968).

Brown & Richards (1959) proposed a dimensionally balanced equation* of the form

$$(Do - k) = A\left(\frac{4W}{60\pi\rho p\sqrt{g}}\right)^{0{\cdot}4} \quad .. \quad .. \quad .. \quad (1)$$

where k was a measure of the width of the empty annulus observed at the periphery of the orifice. This equation was found to apply to materials such as coal, glass beads, tapioca and sand flowing through a wide range of orifice sizes (Brown & Richards, 1959). Variation in the parameters A and k were shown to be due, *inter alia*, to variations in particle size and shape of the materials.

Jones and Pilpel (1966) considered one material, magnesia, which was available in a large range of particle sizes, and were able to study the effect of particle size without the complicating effects of variation in shape, rugosity and density, etc.

By writing equation (1) in the form

$$Do = A\left(\frac{4W}{60\pi\rho p\sqrt{g}}\right)^{\frac{1}{n}} \quad .. \quad .. \quad .. \quad (2)$$

they were able to show that the parameters A and n were functions of particle size. Although difficulties then arise in regard to the dimensions of the terms A and n, which in equation (1) were dimensionless, having established a numerical relation between A and n and particle size, predictions could then be made of the flow rate of any other given size fraction.

* For rotation see p. 729.

For equation (2) to have practical importance it would be necessary to include a term or terms which would account for variations in shape and rugosity of the particles of different materials. Previous equations have involved angular properties (Takahashi, 1935; Franklin & Johanson, 1955), others have included a shape factor (Rose & Tanaka, 1959; Ahmad & Pilpel, 1969). Many workers have used a bulk density term (Fowler & Glastonbury, 1959; McDougall & Evans, 1965; Beverloo, Leniger & Van der Velde, 1961) since this embodies the shape, rugosity and frictional characteristics of the materials.

The high correlation of bulk density and flow rate has been pointed out in a recent paper (Sumner, Thompson & others, 1966) and Delaplaine (1956) has shown that the bulk density of a flowing bed is only 0·02 units lower than that of the static bed.

An advantage of bulk density is that it compensates for differences between the apparent and effective particle densities: differences which may be very large (up to 40%) in the case of granulated cohesive materials (Harwood & Pilpel, 1968).

This study is a test of the use of the bulk density term instead of the particle density term to establish an equation of the same form as equation (2), which can be applied to materials that differ considerably in the shape, rugosity, density and frictional characteristics of their particles.

EXPERIMENTAL

Materials

The materials tested were smooth and irregular griseofulvin granules, silica sand and glass beads. The reported results for magnesia (Jones & Pilpel, 1966) and for smooth and irregular lactose granules (Ahmad & Pilpel, 1969), obtained using the same apparatus, have been included to extend the generality of the results obtained.

The materials were separated into sieve fractions on British Standard sieves and surface fines were removed by sieving 20–40 g portions on an Alpine Airjet sieve for 3 min. The samples were dried in an air oven and stored in stoppered glass jars.

The tap and bulk densities were measured using a standard apparatus (British Standard, 1948). The particle densities were measured using the specific gravity bottle method.

Some of the physical properties of the materials are given in Table 1.

Table 1. *Sieve fractions and densities of granular materials*

Material	B.S.S. size	Arithmetic mean size (μm)	Density (g cm^{-3})		
			Particle	Bulk	Tap
Irregular griseofulvin granules	60–85	215	1·430	0·507	0·551
	44–60	300	1·431	0·463	0·500
	25–44	430	1·433	0·407	0·456
	22–25	655	1·428	0·393	0·438
	16–22	855	1·435	0·385	0·435
	10–16	1340	1·435	0·378	0·419
	8–10	1866	1·421	0·374	0·419
Smooth griseofulvin granules	16–22	855	1·448	0·556	0·609
	12–16	1200	1·443	0·538	0·579
	10–12	1540	1·432	0·522	0·560
	8–10	1866	1·421	0·507	0·556
	6–8	2435	1·403	0·500	0·551

Table 1—*continued*

Material	B.S.S. size	Arithmetic mean size (μm)	Density (g cm^{-3}) Particle	Bulk	Tap
	<85	<180	2·653	1·212	1·437
	60–85	215	2·625	1·317	1·543
Sand	44–60	300	2·642	1·371	1·594
	25–44	475	2·698	1·434	1·661
	16–25	800	2·823	1·464	1·661
	—	<53	2·965	1·289	1·409
	—	113	2·962	1·716	1·876
	—	150	2·970	1·737	1·765
Glass beads	60–85	213	2·973	1·746	1·886
	36–52	368	2·978	1·818	1·848
	25–44	486	2·981	1·717	1·855
	—	605	2·979	1·774	1·896
	300–350	48	3·439	1·000	1·095
	150–200	90	3·458	0·920	0·988
	72–150	158	3·431	0·903	0·985
	36–52	358	3·456	0·856	0·938
Magnesia	22–36	561	3·458	0·870	0·930
	16–22	851	3·445	0·860	0·930
	10–16	1340	3·460	0·860	0·941
	8–10	1866	3·456	0·856	0·933
	72–150	160	1·535	0·672	0·738
	52–72	252	1·526	0·556	0·608
	36–52	358	1·535	0·511	0·563
Rounded	22–36	560	1·536	0·505	0·542
lactose	16–22	851	1·500	0·495	0·529
granules	12–16	1201	1·541	0·484	0·529
	10–12	1538	1·535	0·483	0·542
	8–10	1866	1·536	0·501	0·536
	6–8	2435	1·550	0·489	0·535
	72–150	160	1·544	0·555	0·645
	52–72	252	1·551	0·519	0·588
Irregular	36–52	358	1·523	0·512	0·575
lactose	22–36	560	1·501	0·488	0·555
granules	16–22	851	1·563	0·481	0·548
	12–16	1201	1·544	0·475	0·535
	10–12	1538	1·502	0·476	0·525

Apparatus

The apparatus was as used by Jones & Pilpel (1966). It consisted of a vertical copper tube 30 cm long and 3·82 cm internal diameter. A Perspex base plate held a shutter and a sliding orifice plate into which six circular orifices with mean diameters of 0·6–1·7 cm had been cut.

Precautions

In measuring the flow rate from a vertical copper tube, certain restrictions have been well established by previous authors in order to avoid the complicating effects of apparatus geometry (for review see Jones, 1966). End effects arising during the flow measurements are eliminated by measuring the flow rate only when steady conditions are obtained, that is, over the central 3/5ths (approximately) of the flowing column.

Blocking of the orifice will occur when the particle size, Dp, is approximately 1/6th of the orifice size, Do. The conditions must be such that $Do \geq 6Dp$. Finally, the column diameter Dc must be such that $Dc \geq 2{\cdot}5\ Do$ to eliminate wall effects (Beverloo, Leniger & Van der Velde, 1961; Brown & Richards, 1959; Rose & Tanaka, 1959).

Procedure

With the above reservations in mind the column was filled with the material. The mass emerging from the various sized orifices was then measured in time intervals ranging from 5–60 s. Each measurement was made in triplicate and it was found that the maximum variation between separate determinations was ±5%.

RESULTS

The measured flow rates for the 42 size fractions of the seven different materials when flowing through six orifice sizes are given in Table 2.

The effect of particle size on the flow rate is shown in Fig. 1. The curves follow the anticipated form (Rose & Tanaka, 1959; Jones & Pilpel, 1966) where the flow rate increases with decrease of particle size to a maximum at approximately 200 μm and then falls rapidly as the cohesive forces become increasingly effective.

The present study has been concerned only with free flowing materials and for this reason only those size fractions above 200 μm have been considered in the analysis to follow.

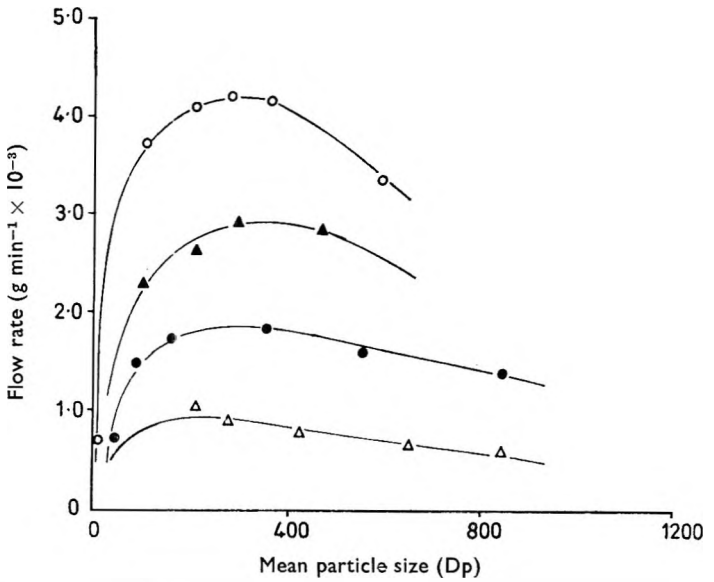

FIG. 1. Flow rate versus particle size for different materials passing through a 1·30 cm orifice. O, Glass beads; ▲, sand; ●, magnesia and △, irregular griseofulvin.

DISCUSSION

From equation (2) it is seen that a plot of the logarithm of Do against the logarithm of $\left(\frac{4W}{60\pi\rho_p\sqrt{g}}\right)$ should give a straight line, the slope of which is 1/n and the intercept log A. The values of 1/n and A have been shown to depend on particle size, and, since in the present study 42 size fractions were available and the results were required using both particle density and also the bulk density, the readings were subjected to regressional analysis using a digital computer (Elliott 803) and the correlation coefficients were in all cases better than 0·93. The calculated values of both n and A are given in Table 3.

Table 2. *Flow rates for griseofulvin, sand, glass beads, magnesia and lactose* (g min^{-1})

Mean particle size (μm)	Orifice diameter (cm) 0·605	0·707	0·900	1·130	1·330	1·650
Griseofulvin						
Irregular						
1866	B	B	B	207–221	383–398	836–846
1340	B	82–86	124–125	275–281	456–484	907–964
855	47–49	109–112	160–165	347–349	565–575	1075–1109
655	59–61	129–130	185–188	399–400	659–662	1175–1198
430	75–76	157–159	220–226	462–475	753–768	1411–1441
300	99–100	206–207	283–287	579–584	879–897	B
215	120–121	235–243	302–331	604–618	1044–1082	B
Smooth						
2435	B	B	B	230–254	490–510	950–971
1866	B	B	150–163	352–354	592–605	1105–1132
1540	B	140–142	199–204	446–451	727–746	1310–1380
1200	81–85	180–182	255–260	549–560	923–938	1542–1692
851	105–105	224–229	313–320	651–667	1063–1075	1732–1847
Sand						
800	—	583–614	780–792	1541–1549	2470–2600	4750–4772
475	—	589–611	912–949	1682–1702	2841–2849	5023–5066
300	—	698–726	1045–1075	1722–1744	2911–2949	4595–4696
215	—	624–640	941–959	1573–1617	2637–2661	4680–4692
Glass beads						
605	—	514–545	1256–1284	2320–2340	3657–3735	—
485	—	515–554	1210–1216	2138–2190	3500–3510	—
368	—	715–742	1501–1525	2572–2625	4154–4194	—
284	—	728–762	1526–1546	2709–2729	4203–4217	—
213	—	701–711	1505–1515	2562–2580	4087–4120	—

Mean particle size (μm)	Orifice diameter (cm) 0·603	0·740	0·898	1·140	1·353	1·686
Magnesia						
1866	B	B	B	524–560	936–953	1788–1836
1340	B	B	322–388	484–692	1128–1158	2129–2148
851	135–140	241–249	428–453	860–870	1401–1413	2598–2650
560	171–175	288–305	501–538	1015–1020	1610–1630	2798–2860
358	208–212	352–358	610–621	1158–1175	1835–1905	3010–3062
252	245–255	397–404	670–684	1218–1262	1916–1958	3034–3148
160	248–260	398–410	638–678	1180–1210	1760–1784	2684–2840

Mean particle size (μm)	Orifice diameter (cm) 0·58	0·75	0·86	1·10	1·31	1·60
Lactose						
Irregular						
1540	B	B	B	318–331	547–568	1005–1035
1201	B	B	149–164	345–358	590–612	1090–1140
851	B	123–129	188–199	390–408	664–675	1221–1242
560	74–82	154–162	230–246	474–495	806–834	1427–1476
358	95–103	192–216	293–314	550–592	972–1044	—
252	109–118	216–230	318–342	626–648	982–1038	—
160	121–130	226–244	325–347	615–654	1054–1096	—
Smooth						
1540	B	B	156–165	336–358	585–614	1095–1148
1201	B	120–128	180–192	401–408	664–690	1208–1251
851	66–69	140–161	224–236	468–486	780–798	1356–1392
560	78–92	180–192	260–282	548–574	880–912	1500–1564
358	106–114	225–234	320–335	638–652	1000–1022	1622–1672
252	126–130	261–272	368–378	743–756	1190–1215	1756–1804
160	160–170	314–330	456–484	878–892	1404–1436	2092–2140

B denotes orifice blocked.

Table 3. *Values of constants A and n*

Mean particle size Dp (μm)	Log Dp	n	A	
			Using ρB	Using ρp
Griseofulvin				
Irregular				
1840	3·265	3·447	1·477	2·190
1340	3·127	2·929	1·409	2·224
855	2·932	2·965	1·324	2·152
655	2·816	2·858	1·280	2·016
430	2·633	2·801	1·222	1·919
300	2·477	2·629	1·198	1·840
215	2·332	2·547	1·188	1·784
Smooth				
2435	3·387	3·536	1·509	2·032
1866	3·271	3·291	1·442	2·007
1540	3·188	2·793	1·363	1·961
1200	3·079	2·865	1·274	1·802
851	2·930	2·733	1·222	1·736
Magnesia				
1866	3·271	3·080	1·496	1·782
1340	3·127	2·930	1·416	1·690
851	2·930	2·874	1·305	1·573
560	2·748	2·746	1·251	1·515
358	2·554	2·639	1·186	1·455
252	2·401	2·489	1·174	1·438
160	2·204	2·360	1·214	1·492
Sand				
800	2·903	2·535	1·246	1·248
475	2·677	2·564	1·197	1·208
300	2·477	2·271	1·159	1·195
215	2·332	2·395	1·175	1·329
Glass beads				
505	2·782	2·731	1·175	1·096
485	2·686	2·706	1·189	1·120
368	2·566	2·584	1·129	1·041
284	2·453	2·577	1·119	1·030
213	2·328	2·546	1·116	1·043
Lactose				
Irregular				
1540	3·188	3·056	1·416	2·046
1201	3·079	3·160	1·377	1·967
851	2·930	2·995	1·332	1·935
560	2·748	2·878	1·257	1·839
358	2·554	2·815	1·194	1·735
252	2·401	2·693	1·180	1·736
160	2·204	2·625	1·198	1·734
Smooth				
1540	3·188	3·032	1·405	1·995
1201	3·079	3·039	1·335	1·923
851	2·930	2·972	1·279	1·844
560	2·748	2·852	1·229	1·785
358	2·554	2·671	1·179	1·750
252	2·401	2·632	1·157	1·673
160	2·204	2·552	1·161	1·576

In Figs 2 and 3 the values of A, using ρp and ρB respectively, are plotted against particle size. It can be seen immediately that by using ρB a good correlation is found between A and the particle size for all of the materials examined. Applying regressional analysis to obtain the best straight line through the points in Fig. 3 gives the relation $A = 1{\cdot}1356 + 0{\cdot}000173$ Dp. The correlation coefficient for this line is 0·940 which represents an excellent fit for all the points.

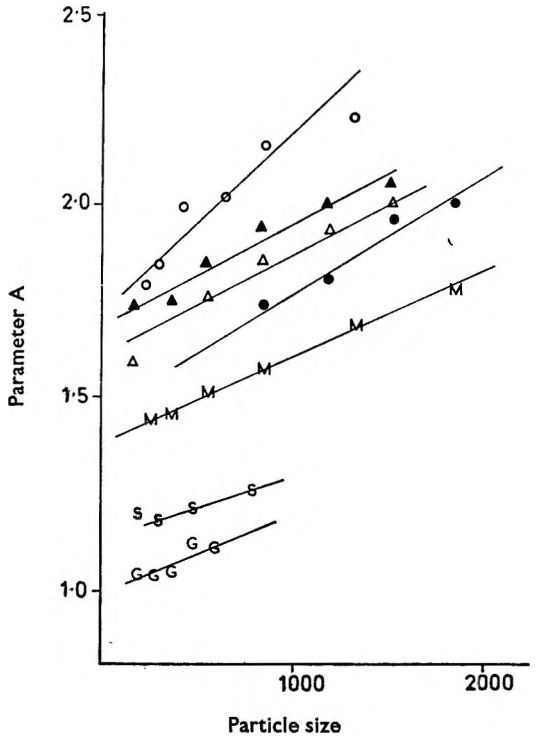

FIG. 2. Variation of parameter A with particle size using ρp. ○—○, Irregular griseofulvin; △—△, irregular lactose; ▲—▲, smooth lactose; ●—●, smooth griseofulvin; M—M, magnesia; S—S, sand and G—G, glass beads.

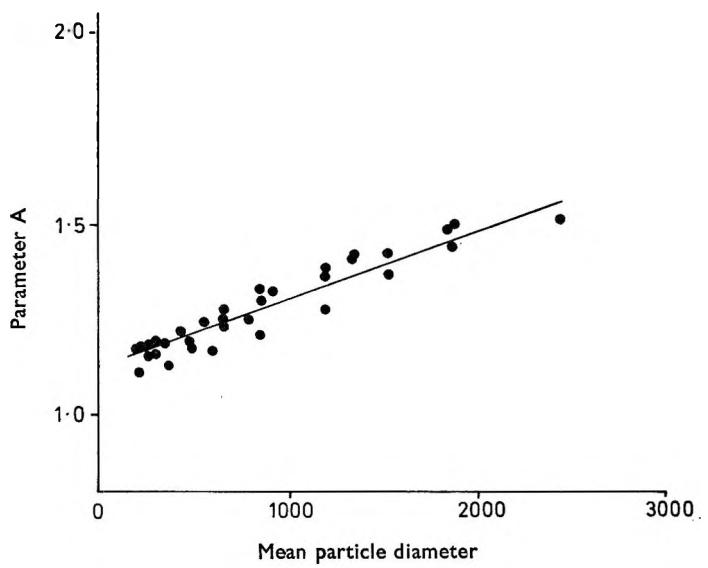

FIG. 3. Variation of parameter A with particle size using ρB (all materials).

Table 4. *Comparison of predicted and measured flow rates*

Material	Orifice size Do (cm)	Mean particle size Dp (μm)	Flow rate (g min⁻¹) Found	Flow rate (g min⁻¹) Calculated	Error %
Griseofulvin					
Irregular	0·707	855	109–112	101	−7
	1·130	300	579–584	595	+2
	1·330	1340	456–484	520	+7
Smooth	0·900	1540	199–204	204	0
	1·650	1200	1542–1692	1480	−4
	1·650	851	1731–1847	1720	−1
Magnesia	0·740	252	397–404	397	0
	1·140	560	1015–1020	1030	+1
	1·686	1866	1788–1836	2030	+11
Sand	0·707	215	624–640	547	−12
	1·140	475	1682–1702	1763	+3
	1·650	800	4750–4772	4620	−3
Glass beads	0·707	284	728–762	689	−5
	1·140	485	2138–2190	2190	+1
	1·330	605	3657–3735	3250	−11
Lactose					
Irregular	0·580	252	109–118	127	+7
	0·860	560	230–246	265	+8
	1·600	1540	1005–1035	1066	+3
Smooth	0·750	252	261–272	259	−1
	1·310	560	880–912	890	0
	1·600	1201	1208–1251	1210	0

Table 5. *Comparison of measured and predicted flow rates for binary mixtures of magnesia*

Dp (μm)	Mixture Concn (% w/w)	Mixture Dp_2 (μm)	Mixture Concn (% w/w)	ρB g/cm³	Do (cm)	W calc. (g/min)	W obs. (g/min)	Error %
	10		90	0·910	0·898	420	321–357	+17·6
				0·910	1·686	2761	2184–2431	+13·6
253	50	1340	50	0·965	0·898	588	489–533	+10·3
				0·965	1·686	3410	698–706	+11·4
	90		10	0·954	0·898	682	698–706	− 2·3
				0·954	1·686	3525	3168–3268	+ 7·9
	10		90	0·900	0·898	392	328–334	+17·0
				0·900	1·686	2627	2256–2292	+14·6
561	50	1340	50	0·888	0·898	458	431–435	+ 8·5
				0·888	1·686	2988	2686–2754	+ 5·3
	90		10	0·875	0·898	522	507–517	+ 5·2
				0·875	1·686	3065	2837–2914	+ 1·0
	10		90	0·877	1·140	1035	1036–1040	0
				0·877	1·686	3095	3040–3092	0
851	50	561	50	0·878	1·140	997	963–988	+ 1·0
				0·878	1·686	3048	2842–2911	+ 4·6
	90		10	0·867	1·140	928	914–921	+ 1·0
				0·867	1·686	2895	2753–2830	+ 2·3
	20		80	0·930	0·603	173	149–151	+14·6
				0·930	1·686	3215	2758–2880	+11·6
253	40	851	60	0·958	0·603	198	176–190	+ 4·2
				0·958	1·686	3398	3074–3160	+ 7·5
	60		40	0·955	0·603	218	213–242	0
				0·955	1·686	3485	3168–3223	+ 8·1

The relation between n and log Dp is shown in Fig. 4 for all of the materials tested. The regression line for all these points was found to be $n = 0{\cdot}9034 + 0{\cdot}6748 \log Dp$. The correlation coefficient was 0·844 which signifies a good fit for all the materials.

Thus using the bulk density in equation (2) leads to a general equation of the form

$$Do = (1{\cdot}136 + 0{\cdot}000173Dp)\left(\frac{4W}{60\pi\rho B\sqrt{g}}\right)^{\frac{1}{0{\cdot}903 + 0{\cdot}675 \log Dp}} \quad .. \quad .. \quad (3)$$

for relating the flow rate to the orifice size and the particle size of the material.

This equation has been tested by comparing the calculated values with the experimental values of flow rates and the results are shown in Table 4. It can be seen that the average agreement is $\pm 5\%$ which, considering the wide range of materials tested, is regarded as very satisfactory.

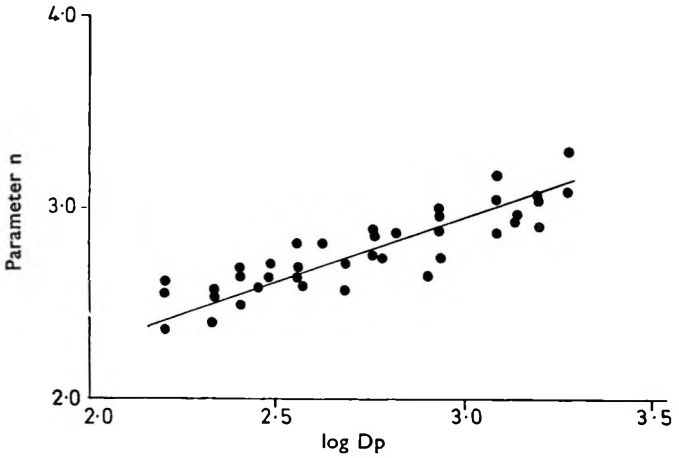

FIG. 4. Variation of n with log Dp.

To further test the validity of equation (3), it has been used to predict the flow rates of some binary mixtures of two different size fractions. These mixtures were prepared by a standard procedure (Jones & Pilpel, 1966), the values of Dp for substituting into equation (3) being taken as geometric means.

Table 5 shows that the agreement between the observed and predicted flow rates for the mixtures was about $\pm 10\%$, which was again very satisfactory.

The remaining errors are probably due to the use of sieving as a method for classifying and measuring particle size, to segregation of particles in mixtures of sizes and to the use of the B.S. method for measuring bulk density. It is possible that a better method would be to measure the bulk density after fluidizing the sample and then allowing it to settle by slowly reducing the air flow. This value should be closer to that of the flowing material, which was shown by Delaplaine (1956) to be 0·02 units lower than the static bulk density.

In conclusion it should be noted that further work on a variety of materials containing a range of particle sizes will be desirable to establish the generality of the present findings for predicting the flow rates of granular pharmaceuticals.

Notation

A: An empirical function of Dp; Dc: Tube diameter, cm; Do: Orifice diameter, cm; Dp: Particle diameter, μm; g: Acceleration due to gravity, cm s^{-2}; k: A function of the empty annulus dependent on Dp; n: An empirical function of Dp; ρp: Apparent particle density, g cm^{-3}; ρB: Bulk density, g cm^{-3}; W: Flow rate, g min^{-1}.

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REFERENCES

AHMAD, M. & PILPEL, N. (1969). *Rheol. Acta*, in the press.

BEVERLOO, W. A., LENIGER, H. A. & VAN DER VELDE, J. (1961). *Chem. Engng Sci.*, **15**, 260–269.

BINGHAM, E. C. & WIKOFF, R. W. (1931). *J. Rheol.*, **2**, 395–400.

British Standard (1948), 1460.

BROWN, R. L. (1961). *Nature, Lond.*, **191**, 458–461.

BROWN, R. L. & RICHARDS, J. C. (1959). *Trans. Instn chem. Engrs*, **37**, 108–119.

DELAPLAINE, J. W. (1956). A.I.Ch.E.J., **2**, 127.

DEMING, W. E. & MEHRING, A. L. (1929). *Ind. Engng Chem. ind. Edn*, **21**, 661–665.

FOWLER, R. I. & GLASTONBURY, J. R. (1959). *Chem. Engng Sci.*, **10**, 150–156.

FRANKLIN, F. C. & JOHANSON, L. N. (1955). *Ibid.*, **4**, 119–129.

HARWOOD, C. F. & PILPEL, N. (1968). *J. pharm. Sci.*, **57**, 478–481.

JONES, T. M. (1966). *Flow Properties of Magnesia*, Ph.D. Thesis, University of London.

JONES, T. M. & PILPEL, N. (1966). *J. Pharm. Pharmac.*, **18**, 81–93.

MCDOUGALL, I. R. & EVANS, A. C. (1965). *Rheol. Acta*, **4**, 218–225.

ROSE, H. E. & TANAKA, T. (1959). *The Engineer*, **208** (2), 465–469.

SHINOHARA, K., DEMITSU, Y., GOTOH, K. & TANAKA, T. (1968). *I & EC Process Des. and Dev.*, **7**, 378–383.

SUMNER, E. D., THOMPSON, H. O., POOLE, W. K. & GRIZZLE, J. E. (1966). *J. pharm. Sci.*, **55**, 1441–1445.

TAKAHASHI, H. (1935). *Bull. Inst. phys. chem. Res. Japan*, **12**, 984.

ZENZ, F. A. (1962). *Petrol. Refiner*, **41**, 159–168.

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The effect of dimensions on the compaction properties of sodium chloride*

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Using a tablet press instrumented with strain gauges, anhydrous particulate sodium chloride was compressed to form compacts of different lengths in three dies of different diameters. For the limited range of dimensions applicable to most pharmaceutical tablets, there was a common linear relation between the applied compaction pressure and the force lost to the die wall per unit area of apparent die wall contact, during compression. Ejection forces were correlated using a similar expression. The mechanical strength of compacts was determined by diametrical compression. A relation was proposed to express the strength (F_c), of the compacts of different sizes in terms of the diametrical cross-sectional area at zero porosity ($D.L_o$), the relative volume (V_r) and the mean compaction pressure (P_m):

$$\left(\frac{F_c}{D.L_o}\right) . V_r = k.P_m - c, \text{ where k and c are constants.}$$

Previous investigators of the compaction behaviour of powders have prepared compacts of various lengths in dies of different diameters, and it is often difficult to compare the published results for compression forces, and physical and mechanical properties of the final compacts. The objective of the present investigation was to establish relations between these variables and the dimensions of the compacts produced.

As early as 1882, Forscheimer was aware that the pressure in a column of sand decreases with depth, due to friction between the particulate material and the container wall.

Shaxby & Evans (1923) derived an expression to describe the vertical pressure, P_z, at depth z in a system of non-coherent, particulate material. If gravitational effects are considered negligible in comparison with the applied pressure P_a, the relation becomes:

$$\frac{P_z}{P_a} = e^{-\left(\frac{2cz}{r}\right)} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where r is the radius of the cylindrical container, and c is a constant.

Numerous equations have been proposed which are modifications of the original expression quoted by Shaxby & Evans. For example, Unckel (1945) developed a relation in which the constant c, was equal to the product of the coefficient of friction at the container wall μ, and the stress ratio η (radial/axial stress). In Unckel's equation, P_z became the pressure transmitted through a compact, and the term "z" was equal to the *initial* length of the compact.

* This work forms part of a thesis by J. E. R., accepted for the degree of Ph.D. in the University of London.

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In 1954, Duffield derived the first relation to account for changes in the bulk density of a compact during pressing,

$$\frac{P_b}{P_a} = V_r \cdot e^{-\left(\frac{2\mu\eta L}{r}\right)} \qquad (2)$$

in which P_b is the pressure transmitted through a compact of *actual* length L, and the relative volume V_r, is the ratio of the actual volume of the compact to the volume of solid material present.

The differences between these expressions are characteristic of the disagreements between results obtained for various materials by investigators such as Spencer, Gilmore & Wiley (1950), Ballhausen (1951), Sheinhartz, McCullough & Zambrow (1954) and Toor & Eagleton (1956).

Experimental results obtained by Squire (1947) for metal powders demonstrated that, for rectangular and cylindrical compacts, the density was indirectly proportional to the ratio of wall area to pressing area. Consequently, either a reduction in length for compacts of a certain diameter, or an increase in diameter for a constant length, produced an increase in density.

The mechanical properties of a powder compact may also be affected since they are determined to a great extent by the density. Unfortunately, Squire (1947) made no measurements of frictional effects, or of mechanical properties in this aspect of his investigations.

EXPERIMENTAL

Plane faced punches of 0·8, 1·0 and 1·2 cm diameter, were used in conjunction with a single-punch eccentric tablet press (Lehman). Each upper punch and the lower punch holder were instrumented in a manner similar to that of Shotton & Ganderton (1960), using Saunders Roe $\frac{1}{8}$-inch, linear foil resistance strain gauges.

Sodium chloride was selected as a pure, cubic-crystalline material which forms a coherent compact by direct compression. A batch of sodium chloride (B.P. quality) was screened to obtain a 30–40 mesh fraction, and fine particles were removed using an Alpine Air-Jet Sieve. The density of the characterized material (2·17 g cm^{-3}) was determined pycnometrically at 25° using *m*-xylene.

The weights of material for compression were selected to facilitate comparison of the present results with those of previous workers, including Shotton & Ganderton (1960), who employed a $\frac{1}{2}$-inch (diameter) die. Sufficient material was compressed in each die (Table 1) to produce compacts of the following length, L_o, at zero porosity:

1. $L_o = 0{\cdot}4$ cm
2. $L_o = 0{\cdot}4 \times \left(\frac{\text{diameter of respective die}}{\text{diameter of } \frac{1}{2}\text{-inch die}}\right)$ cm
3. $L_o = 0{\cdot}4 \times \left(\frac{\text{cross-sectional area of respective die}}{\text{cross-sectional area of } \frac{1}{2}\text{-inch die}}\right)$ cm

Compression samples were weighed to $\pm 0{\cdot}5$ mg, dried for 1 h at 110° and stored over silica gel for 24 h before use. The ambient relative humidity was maintained at less than 20%, during the pressing operation, and at least five pressure levels were investigated for each diameter-length combination. Five compacts were prepared at each pressure and the die was cleaned before the preparation of each compact.

To evaluate the influence of die wall "contamination", the experiments with a 1·2 cm (diameter) die were repeated. At each pressure level, the die wall was conditioned by compaction of two samples of material before the compression forces were measured for five subsequent samples.

Resulting compacts were weighed to $\pm 0{\cdot}1$ mg and the dimensions were measured to $\pm 0{\cdot}005$ mm. The diametrical crushing strength was then determined using the apparatus of Shotton & Ganderton (1960). A strict time schedule of experimentation ensured that a constant time interval of 10 min elapsed between the compression and testing of each compact.

RESULTS

The data presented in this section relate the forces of compression to the properties of the compacts produced. In the subsequent discussion, these results are correlated and expressions are derived from them.

The results in Figs 1 and 2 show that both the force lost to the die wall, F_d, and the force required to initiate movement of the compact during ejection, F_e, were increased by a reduction in die diameter, and in most cases by an increase in the

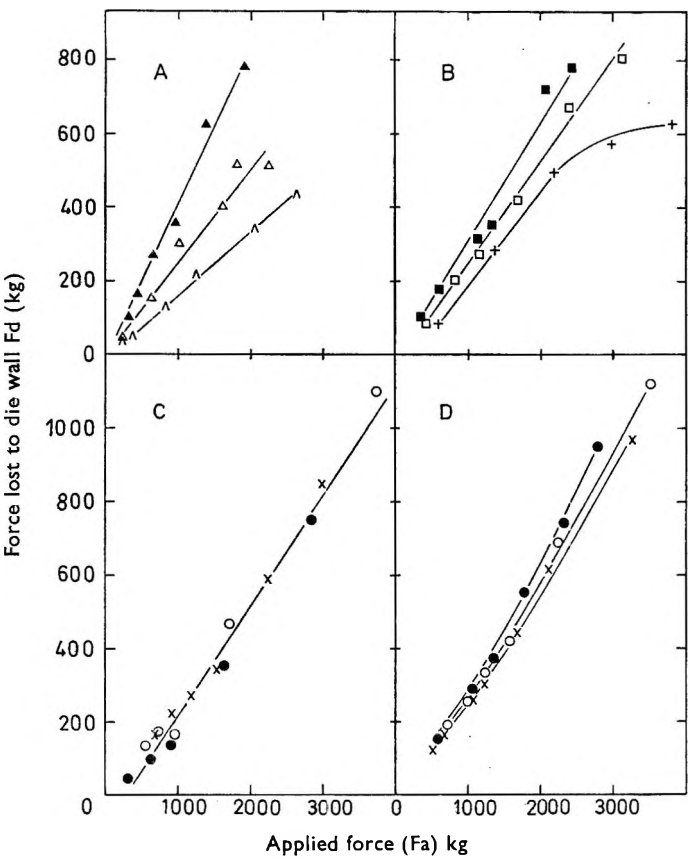

FIG. 1. Effect of die diameter and compact length on the relation between force lost to the die wall and applied force. Length at zero porosity, L_0: ▲, 0·40 cm; △, 0·25 cm; ∧, 0·16 cm; ■, 0·40 cm; □, 0·32 cm; +, 0·25 cm; ●, 0·40 cm; ○, 0·38 cm; ×, 0·36 cm. A. 0·8 cm (diam.) clean die.; B, 1·0 cm (diam.) clean die; C, 1·2 cm (diam.) clean die; D, 1·2 cm (diam.) conditioned die.

length of a compact. A linear relation between F_d and the applied force was observed for all compacts prepared in a clean 0·8 cm and 1·2 cm (diameter) die, and for all except the shortest compact pressed in a clean 1·0 cm die (Fig. 1). In the latter case, F_c approached a constant value above 2000 kg applied force. However, for the conditioned 1·2 cm die, an increase in slope occurred with increasing applied force over the range of conditions investigated (Fig. 1D).

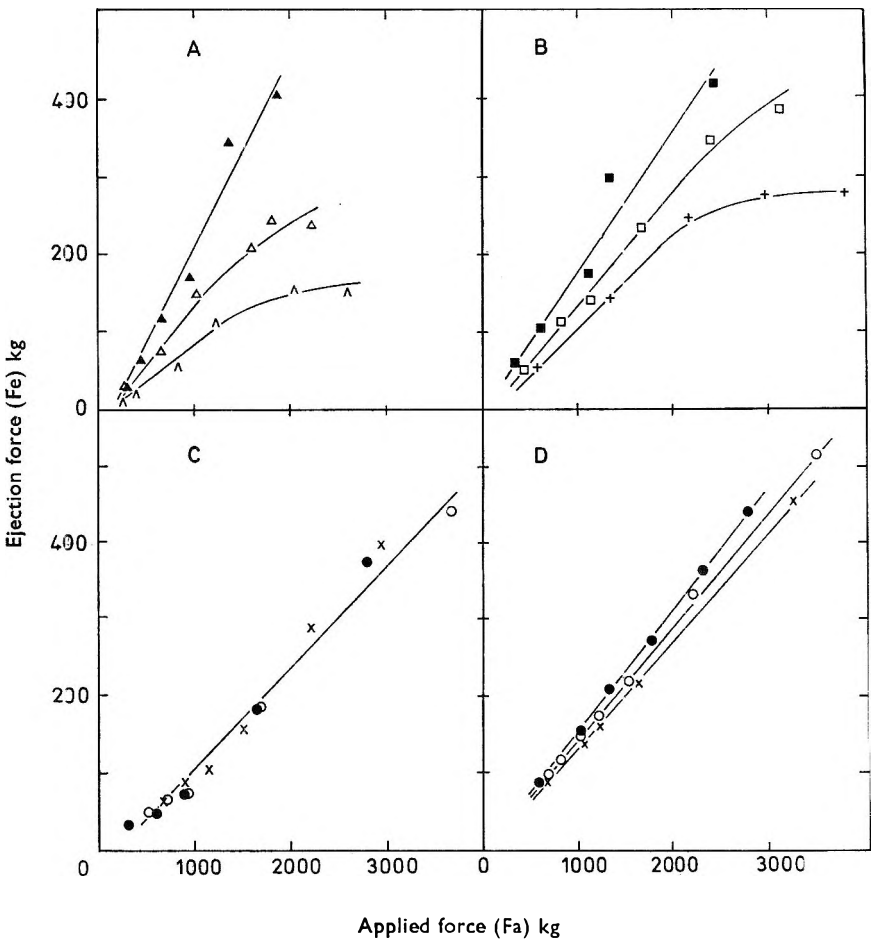

FIG. 2. Effect of die diameter and compact length on the relation between ejection force and applied force. Length at zero porosity, L_0: ▲, 0·40 cm; △, 0·25 cm; ∧, 0·16 cm; ■, 0·40 cm; □, 0·32 cm; +, 0·25 cm; ●, 0·40 cm; ○, 0·38 cm; ×, 0·36 cm. A–D as Fig. 1.

When the shorter compacts were pressed in the 0·8 and 1·0 cm dies, the ejection force approached a maximum value as the applied force increased (Fig. 2A and B).

Conditioning of the die wall produced an increase in the magnitude of F_d and F_e (Figs 1D and 2D), and the deviation of replicate values of F_d from a mean value was shown to be less than for samples pressed in a clean die (Table 2).

Table 1. *The weight, and length at zero porosity, L_o, of samples compressed to evaluate the effect of dimensions on the properties of a compact*

Die diameter (cm)	L_o (cm)	Compression weight (g)
1·2	1. 0·400	0·982
	2. 0·378	0·928
	3. 0·357	0·876
1·0	1. 0·400	0·682
	2. 0·315	0·537
	3. 0·248	0·423
0·8	1. 0·400	0·436
	2. 0·252	0·275
	3. 0·159	0·173

Compression of material in a die of large diameter produced a lower relative volume, V_r, than in a smaller diameter die (Fig. 3A). An increase in length apparently did not affect the relative volume of compacts pressed in the 0·8 and 1·0 cm clean dies, or in a 1·2 cm conditioned die (Fig. 3B). However, the longest compact prepared in a 1·2 cm clean die at low applied pressure possessed a low relative volume compared with the shorter compacts.

At each mean compaction pressure, P_m, an increase in the length of a compact produced an increase in the diametrical crushing strength (Fig. 4). Similarly, for compacts of equal length at zero porosity, an increase in diameter was associated with an increase in strength. Compacts pressed in a clean die were stronger than those prepared in a conditioned die.

Table 2. *Variation in force lost to the die wall for a "clean" and a "conditioned" 1·2 cm (diameter) die*

Weight of compact (g)	Clean die			Conditioned die		
	F_a	F_d	σ	F_a	F_d	σ
0·982	326	45	12·2	584	156	1·5
	611	94	16·2	1043	289	3·2
	883	133	25·7	1340	371	2·1
	1655	355	7·1	1789	555	3·0
	2852	752	11·2	2326	745	1·1
	—	—	—	2796	952	1·5
0·928	538	134	3·6	716	190	4·3
	725	155	14·3	1043	258	4·9
	945	164	14·7	1250	330	2·8
	1716	468	11·8	1562	424	3·4
	3734	1102	9·3	2227	690	3·3
	—	—	—	3533	1123	2·5
0·876	693	153	9·9	520	121	5·5
	916	215	9·9	1063	260	3·1
	1176	273	15·4	1232	310	2·7
	1533	342	15·1	1668	446	2·8
	2244	589	12·5	2125	615	0·8
	2999	857	6·8	3258	967	5·2

σ = standard deviation as % of mean of five replicate values of F_d.

A linear relation between crushing strength and mean compaction pressure was valid for every compact at low pressures. At approximately 2,000 kg cm^{-2} P_m, a deviation from the linear relation occurred, and the strength of compacts tended to a maximum value, or even decreased for the shortest compacts in the smaller dies.

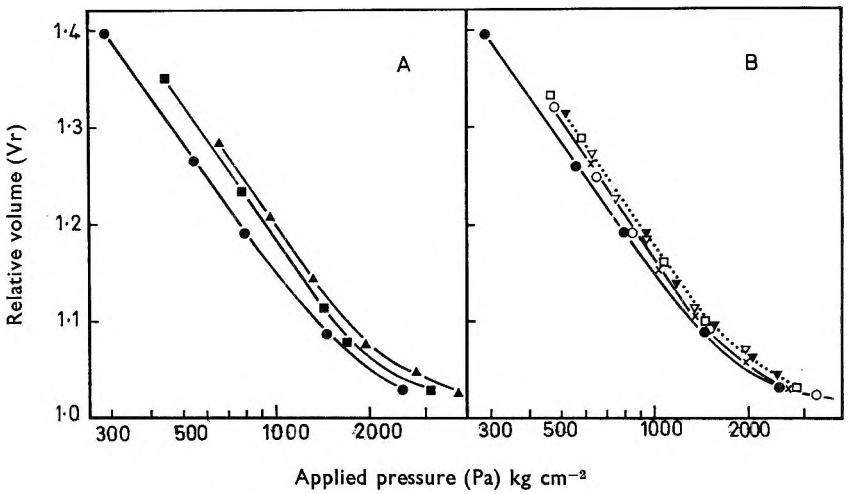

FIG. 3. Relation between relative volume and the applied pressure. A. Effect of diameter on compacts of 0·4 cm length (L_0) at zero porosity. Diameter: *clean die*: ●, 1·2 cm; ■, 1·0 cm; ▲, 0·8 cm.

B. Effect of length on compacts of 1·2 cm diameter. Length at zero porosity (L_0); *clean die*: ●, 0·40 cm; ○, 0·38 cm; ×, 0·36 cm; and *conditioned die* (dotted line): ▼, 0·40 cm; ▽, 0·38 cm; □, 0·36 cm;

DISCUSSION

Compression

In Fig. 5, the experimental results are plotted according to the relation (2) proposed by Duffield (1954), which may be written:

$$\ln\left(\frac{V_r}{R}\right) = 4\mu\eta\left(\frac{L}{D}\right) \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where R is the punch force ratio (F_b/F_a), and D is the diameter of the die.

The unsatisfactory correlation of the results in terms of this expression (correlation coefficient: 0·87) may be explained as follows. Contrary to the assumptions of previous authors, including Spencer & others (1950) and Sheinhartz & others (1954), the product $\mu\eta$ is not necessarily constant. From results obtained by compaction of granular polymers, Train & Hersey (1962) concluded that as the applied force increases, the stress ratio, η, also increases, whereas the coefficient of friction at the die wall, μ, decreases as indicated by the friction results of Pascoe & Tabor (1956). In addition, the exponential equation, originally proposed by Shaxby & Evans (1923), assumes that the vertical pressure at a certain depth is uniform across a horizontal section of a column of powder. It has been demonstrated more recently that compaction produces a radial, as well as axial, pressure distribution (Kamm, Steinberg & Wulff, 1947; Train, 1956).

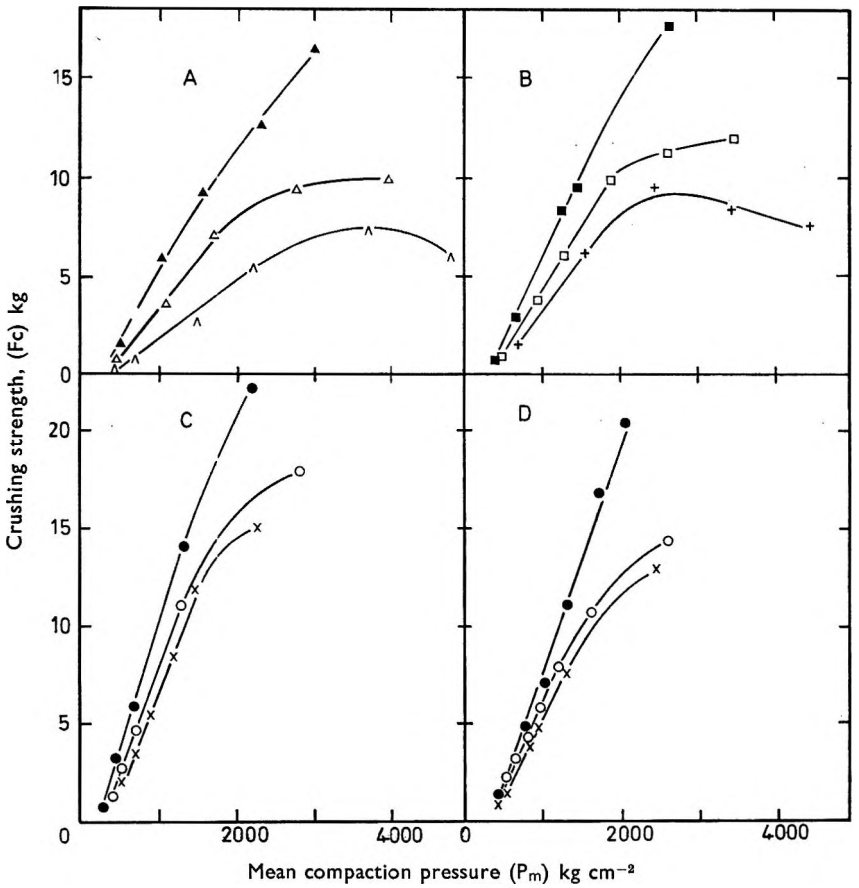

FIG. 4. Effect of diameter and length on the relation between crushing strength and mean compaction pressure. Length at zero porosity, L_0: ▲, 0·40 cm; △, 0·25 cm; ∧, 0·16 cm; ■, 0·40 cm; □, 0·32 cm; +, 0·25 cm; ●, 0·40 cm; ○, 0·38 cm; ×, 0·36 cm. A–D as Fig. 1.

In accordance with the friction theory of Bowden & Tabor (1954), Hersey (1960) proposed that the force lost to the die wall is proportional to the true area of contact, A_t, between the compact and the die wall, provided the shear strength of the compressed material remains constant:

$$F_d = S \cdot A_t \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

where the shear strength, S, of the junction between the material and the die wall is normally considered to be the shear strength of the weakest material, viz. the compressed material.

Hersey's equation explains the increase in F_d with an increase in the length of a compact pressed in the clean 0·8 and 1·0 cm dies (Fig. 1A and B) and in the conditioned 1·2 cm die (Fig. 1D). However, during the compression of material in a clean 1·2 cm die, the variation in replicate values of F_d was greater than the variation for a conditioned die at the same applied force (Table 2). Despite the precautions taken to standardize techniques, removal of the die for cleaning after each compression will cause a variation in the relative position of the punches and die, and differences in the surface condition of the die wall. Since there are only small differences in

the lengths, L_o, of the compacts prepared in the 1·2 cm die, a lack of precision in the values of F_d may obscure a relation between F_d and the length of a compact (Fig. 1C).

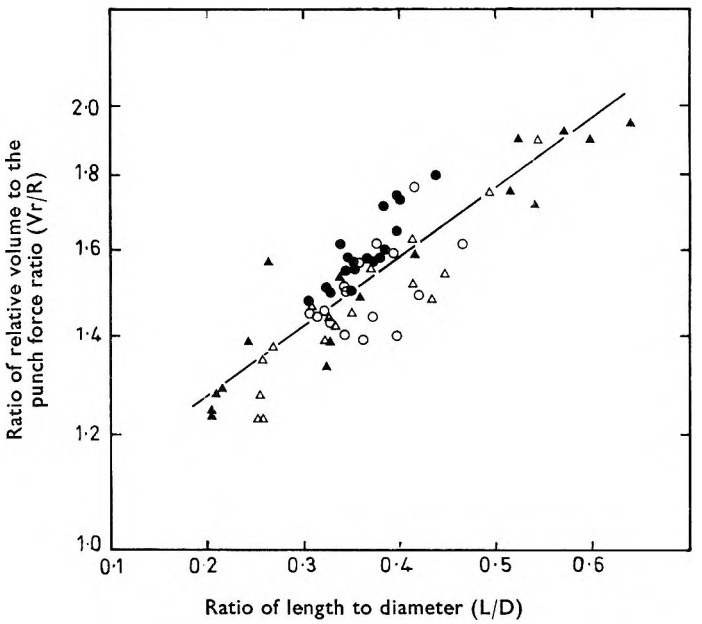

FIG. 5. Experimental results plotted according to the relation proposed by Duffield (1954). ▲, 0·8 cm (diameter) clean die; △, 1·0 cm (diameter) clean die; ○, 1·2 cm (diameter) clean die; ●, 1·2 cm (diameter) conditioned die.

The graph shown in Fig. 6 was obtained by regression analysis of sixty-six co-ordinates, and a correlation coefficient of 0·99 was determined. Thus, for compacts of different lengths compressed in the three dies at applied pressures of up to 4,000 kg cm^{-2}, good correlation of the results was provided by an expression of the form:

$$\left(\frac{F_d}{A}\right) = k_1 \left(\frac{F_a}{A_p}\right) - c_1 \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (5)$$

where $k_1 = 0{\cdot}224 \pm 0{\cdot}009$ (95% confidence limits), $c_1 = 51{\cdot}5$, A is the apparent area of contact at the die wall, and A_p is the punch face area.

The validity of this relation indicates that, for the range of dimensions studied, the total force lost to the die wall, F_d, may be considered to increase linearly with increasing distance from the plane of applied force. It is important to note that as the transmitted axial force, F_b, approaches zero in a compact of sufficient length, this linear approximation will become invalid, and equation (5) will not be applicable. However, during the compaction of pharmaceutical tablets it is essential that the force transmitted through the compact should be sufficient to produce adequate consolidation and bonding in regions of the compact remote from the plane of applied force. If the total force lost to the die wall, F_d, is of sufficient magnitude that the transmitted force approaches zero, the powder compact will be unsuitable since pharmaceutical tablets are not subjected to further treatment such as the sintering

of metal powder compacts or the firing of ceramics. Accordingly, within the range of conditions appropriate to pharmaceutical powder compaction the type of relation quoted in equation (5) is apparently valid.

Although the force lost to the die wall is greater in a conditioned die, consolidation of the compact is inhibited by the increased frictional resistance. Consequently, equation (5) was also valid for compression in a conditioned die. However, the equation did not correlate results obtained for material which had been exposed to ambient conditions of greater than 66% relative humidity. This effect may be explained by the lubricant effect of liquid films which reduces the friction at inter-particulate junctions and at the die wall boundary (Shotton & Rees, 1966).

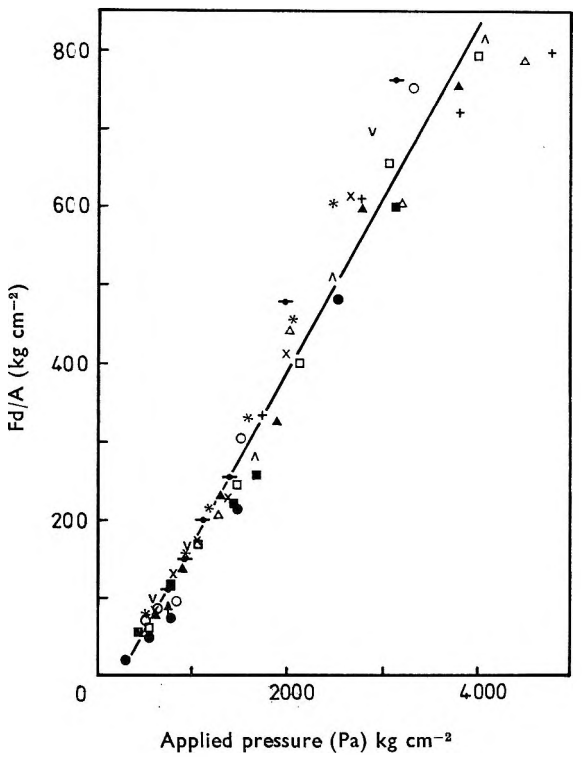

FIG. 6. The force lost per unit area of apparent die wall contact as a function of applied pressure. Length at zero porosity, L_0 (cm):

▲, 0·40; △, 0·25; ∧, 0·16 } 0·8 cm (diameter) clean die;
●, 0·40; ○, 0·38; ×, 0·36 } 1·2 cm (diameter) clean die;
■, 0·40; □, 0·32; +, 0·25 } 1·0 cm (diameter) clean die
*, 0·40; –●–, 0·38; ∨, 0·36 } 1·2 cm (diameter) conditioned die

Ejection

At a high compaction pressure, compression of the molecular lattice of crystalline material will occur, and subsequent removal of the applied load may permit elastic recovery of the crystal lattice. The residual force on the lower punch due to the die wall friction, and also the force required to eject the compact, will then approach constant values as the applied force increases (Fig. 2A and B).

As shown in Fig. 7, a reasonable correlation of the present results for compacts of different sizes was provided by the expression:

$$\left(\frac{F_e}{A}\right) = k_2\left(\frac{F_a}{A_p}\right) - c_2 \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (6)$$

where $k_2 = 0{\cdot}102 \pm 0{\cdot}007$ (95% confidence limits), and $c_2 = 12{\cdot}5$. For experimental values of F_e at applied pressures of less than 4,000 kg cm^{-2}, the significance of the regression line is defined by a correlation coefficient of 0·97.

A comparison of equations (5) and (6) shows that for anhydrous sodium chloride, the relation between F_d and F_e was linear to 4,000 kg cm^{-2} P_a, and the force lost to the die wall was almost exactly equal to twice the ejection force.

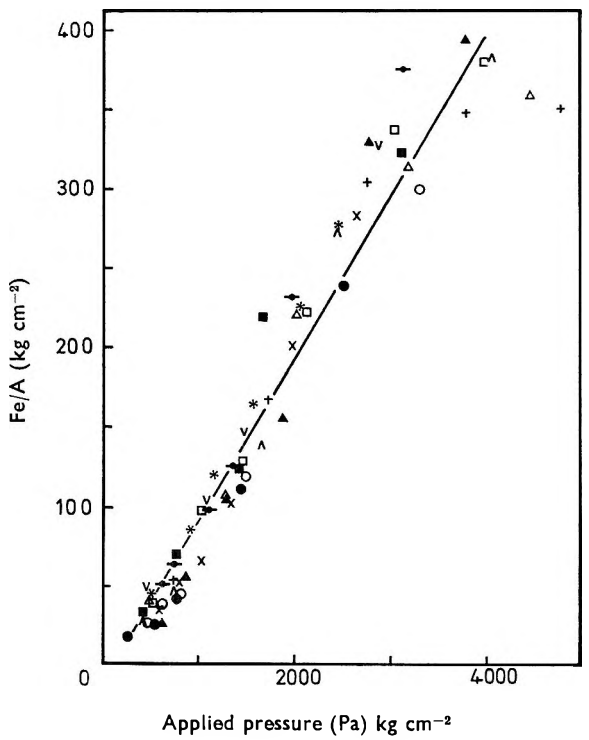

FIG. 7. The ejection force per unit area of apparent die wall contact as a function of applied pressure. Symbols for length at zero porosity as Fig. 6.

Relative volume

At low pressures, there was an exponential relation between the relative volume and the applied pressure (Fig. 3) as reported by Walker (1923) and Bal'shin (1938). This relation cannot be valid when the relative volume approaches unity, and the experimental results suggest that at high pressure, the relative volume decreases with applied pressure according to a power relation.

For compacts of equal length at zero porosity, L_o, a reduction in diameter produced an increase in the relative volume (Fig. 3A). Kamm, Steinberg & Wulff (1947) have shown that die wall friction exerts a retarding effect on the flow of powder during compaction and, as a result, greater consolidation occurs near the axis of

a powder compact than at the periphery. It is apparent that the retarding effect of the die wall will influence a larger proportion of the total volume of a small compact than one of larger diameter. Shear failure and consolidation of material in contact with the die wall produces a dense "skin" at the periphery of a compact. However, owing to the small thickness, this boundary layer will have little effect on the relative volume of the total compact.

For the limited range of dimensions studied, the present results showed no evidence of an increase in relative volume with the length of a compact. The unexpected low relative volume recorded for the longest compacts in a 1·2 cm diameter clean die appears to substantiate a similar observation by Bal'shin (1938). He reported that as the length of a tungsten powder compact was increased, there was an initial decrease in the relative volume, before subsequent increase. These observations appear to contradict the results of Walker (1923) and Seelig & Wulff (1946), who demonstrated an increase in the relative volume of a compact with increasing length, but the present results may be rationalized as follows. Boundary effects at the lower punch may restrict local consolidation at the base of a powder compact, and this would significantly increase the relative volume of a short compact. As the depth of material increases, the restraining effect of the plane punch surface may become insignificant, thus reducing the relative volume in a longer compact. A similar effect would occur if the diagonal stress components described by Train (1956) "converge" below the face of the lower punch during compaction of short compacts. As the length of the compact increases, the high density zone observed by Train in the mid-lower-centre of a compact would develop. On the basis of this assumption, an increase in the length of a compact would produce an initial decrease in the relative volume to a minimum value as the high density zone was formed. However, as shown by Seelig & Wulff (1946), a further increase in the length would be expected to increase the relative volume, as the axial stress decreases through the compact.

Strength

The increase in crushing strength with an increase in the dimensions of a compact (Fig. 4) suggested that an expression of the strength in terms of the diametrical cross-sectional area of a compact might permit correlation of the results.

For all compacts prepared in the clean dies at a mean compaction pressure P_m, not exceeding 2,000 kg cm^{-2}, a common linear relation was obtained between P_m and the crushing strength per unit diametrical cross-sectional area at zero porosity (F_c/DL_c). However, due to the higher die wall friction, the compacts pressed in a conditioned die were less dense, and therefore possessed a lower crushing strength than those prepared in a clean die at the same pressure. For this reason, the values of F_c/DL_o for compacts prepared in a conditioned die failed to correlate with the results for clean dies, but the introduction of a term to account for the differences in density provided a common relation of the form:

$$\left(\frac{F_c}{DL_o}\right) \cdot V_r = k_3 \cdot P_m - c_3 \qquad (7)$$

where $k_3 = 0{\cdot}021 \pm 0{\cdot}001$ (95% confidence limits), and $c_3 = 4{\cdot}9$. This expression was valid to 2,000 kg cm^{-2}, P_m (correlation coefficient: 0·97), as shown in Fig. 8.

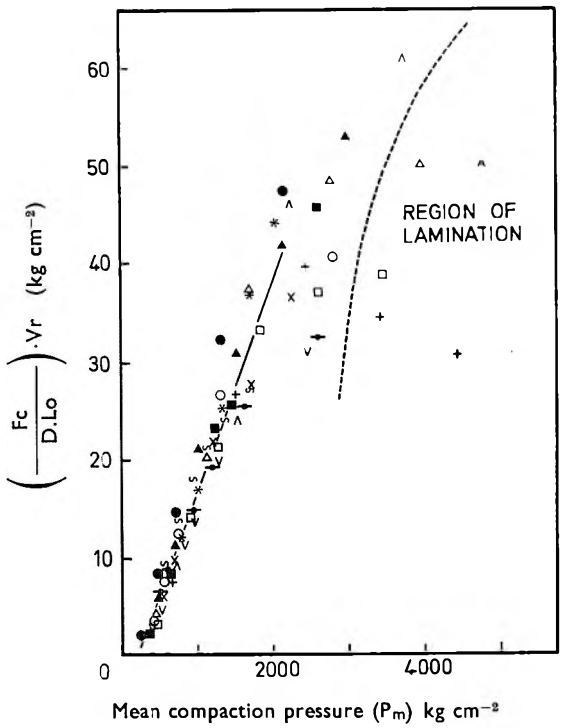

FIG. 8. The product of crushing "stress" and relative volume as a function of mean compaction pressure. Symbols for length at zero porosity as Fig. 6, ~: values derived from experimental results of Shotton & Ganderton (1960).

The correlation of results by the term F_c/DL_o suggests that the diametrical cross-sectional area at zero porosity, (DL_o), is proportional to the true area of inter-particulate bonding in the diametrical plane of the compact. For the sodium chloride compacts prepared in this investigation, the term F_c/DL_o may therefore be considered as a "stress". However, in the presence of a lubricant film or any other factor which inhibits bonding between contiguous crystals, the term F_c/DL_o will not represent a true stress, and may not provide correlation of the results for compacts of different sizes.

The results for crushing strength obtained by Shotton & Ganderton (1960), for compacts of sodium chloride (30–40 mesh size) prepared in a $\frac{1}{2}$ inch diameter die, were converted to values of $(F_c/DL_o)V_r$. As shown in Fig. 8, the derived values were in close agreement with the proposed relation.

As the void space in a compact is reduced at high pressure, the crushing "stress" will theoretically approach a maximum value which is a property of the solid material. However, the co-ordinates in Fig. 8 enclosed by the interrupted line represent those compacts which showed visual evidence of lamination and "capping" following ejection from the die. At high pressures, the deviation from a linear relation was relatively large for short compacts, and for compacts in which the die wall contact area was large in relation to the volume of the compact. It is reasonable to conclude that the decrease in strength of these compacts was due to flaws resulting from the large frictional resistance at the die wall during compaction.

For compacts pressed in clean dies, the dependence of crushing strength on the length at zero porosity, L_0, and not the actual length, L, implies that shear failure and subsequent consolidation of an annulus at the die wall does not produce a significant increase in strength. This evidence confirms that the shearing effect at the die wall boundary was extremely localized and produced only a thin layer of densely packed material.

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REFERENCES

BALLHAUSEN, C. (1951). *Arch. EisenhüttWes.*, **22**, 185–196.
BAL'SHIN, M. YU. (1938). *Vest. Metalloprom.*, **18**, 124–137.
BOWDEN, F. P. & TABOR, D. (1954). *The Friction and Lubrication of Solids*, Vol. 1. Oxford: Clarendon Press.
DUFFIELD, A. (1954). Ph.D. Thesis, University of London.
FORSCHEIMER, P. (1882). *Z. ost. Ing.-u. ArchitVer.*, **34**, 111–126.
HERSEY, J. A. (1960). Ph.D. Thesis, University of London.
KAMM, R., STEINBERG, M. & WULFF, J. (1947). *Trans. Am. Inst. Min. metall. Engrs*, **171**, 439–456.
PASCOE, M. W. & TABOR, D. (1956). *Proc. Roy. Soc.*, **A235**, 210–224.
SEELIG, R. P. & WULFF, J. (1946). *Trans. Am. Inst. Min. metall. Engrs*, **166**, 492–505.
SHAXBY, J. H. & EVANS, J. C. (1923). *Trans. Faraday Soc.*, **19**, 60–72.
SHEINHARTZ, I., MCCULLOUGH, H. M. & ZAMBROW, J. L. (1954). *J. Metals*, **6**, 515–518.
SHOTTON, E. & GANDERTON, D. (1960). *J. Pharm. Pharmac.*, **12**, *Suppl.*, 87*T*–92*T*.
SHOTTON, E. & REES, J. E. (1966). *Ibid.*, **18**, *Suppl.*, 160*S*–167*S*.
SPENCER, R. S., GILMORE, G. D. & WILEY, R. M. (1950). *J. appl. Phys.*, **21**, 527–535.
SQUIRE, A. (1947). *Trans. Am. Inst. Min. metall. Engrs*, **171**, 485–504.
TOOR, H. L. & EAGLETON, S. D. (1956). *Ind. Engng Chem.*, **48**, 1825–1830.
TRAIN, D. (1956). *J. Pharm. Pharmac.*, **8**, 745–759.
TRAIN, D. & HERSEY, J. A. (1962). *Ind. Chemist*, **38**, 113–116.
UNCKEL, H. (1945). *Arch. EisenhüttWes.*, **18**, 161–167.
WALKER, E. E. (1923). *Trans. Faraday Soc.*, **19**, 73–89.

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The effect of sodium salicylate on the binding of L-tryptophan to serum proteins

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In human serum, L-tryptophan is the only amino-acid bound to protein. Salicylate causes a release of tryptophan from its binding sites on human serum proteins and bovine albumin. Some implications of this finding are discussed.

Salicylate binds to circulating proteins, in particular to the serum albumin, in many species including man (Reynolds & Cluff, 1960; Davison & Smith, 1961). The drug also competes with some other substances for binding sites on proteins. Although the suggestion (Brodie, 1965) that they liberate corticosteroids from transcortin has not been confirmed experimentally (Stenlake, Davidson & others, 1968) it has been established that salicylates displace thyroxine and tri-iodothyronine from their binding sites on serum proteins (Christensen, 1959; Osorio, 1963). There is also some evidence that salicylate releases penicillin (Kunin, 1964) and sulphinpyrazone (Yu, Dayton & others, 1962) from their combination with circulating proteins.

Hormones and drugs comprise only a very small fraction of the small molecules which may be transported by the plasma in bound forms. Any interference by salicylate with such binding could cause a greater availability of a variety of diffusible molecules to enter body cells. The possibility exists that one or more of the metabolic or pharmacological actions of salicylate may be mediated by the increased release of such substances. The separation and measurement of all known plasma constituents is a formidable task but suitable methods are available for certain groups. These include the amino-acids, which may be analysed by automated systems. Only L-tryptophan appears to be bound to serum albumin to an appreciable extent (McMenamy & Orcley, 1958) and this has been confirmed in the present work, which also shows that the bound amino-acid is displaced by salicylate.

EXPERIMENTAL

Materials

Pooled human serum was obtained from the National Transfusion Service, Sutton. Bovine serum albumin (fraction V) and L-tryptophan (Sigma grade) were obtained from the Sigma Chemical Company, St. Louis, and Visking dialysis tubing ($\frac{8}{32}$ inch inflated diameter) from the Scientific Instrument Centre. All other chemicals were of analytical grade and distilled water was used throughout.

Dialysis of serum and measurement of amino-acids

Sacs of the Visking tubing, containing 5 ml of water, were immersed in 50 ml quantities of either the pooled human serum or the serum in which 10 mg of sodium salicylate had previously been dissolved. Each dialysis was allowed to proceed for 24 h at room temperature (22°) and the resulting dialysate stored at −15° for

subsequent analysis. Aliquots (1 ml) of a mixture of 1 ml of the dialysate plus 0·2 ml 0·6 mM norleucine in 0·6N HCl were analysed, using a Technicon amino-acid auto-analyser system. The total amino-acid composition of the undialysed serum was also determined after deproteinization as follows: 6 ml of serum were mixed with 0·5 ml 60% (w/v) trichloroacetic acid and centrifuged. The pellet was washed three times with portions (5 ml) of ice-cold 0·6% (w/v) trichloroacetic acid. The original supernatant and washings were combined with 1 ml of 2·5 mM norleucine in 0·01N HCl and made up to 25 ml with 0·01N HCl. One ml of this solution, after filtration, was analysed as described above. Amino-acids and other ninhydrin-positive substances were identified by comparing the elution times with those of authentic compounds and by co-chromatographing standards with the test sample in the standard Technicon system.

Tryptophan binding experiments

All dialysis tubing was presoaked in two changes of distilled water for 20 min before use. Bovine albumin was made up as a 3% (w/v) solution in 0·1M phosphate buffer, pH 7·4, and exhaustively dialysed against five changes of the same buffer to remove any diffusible ninhydrin-positive substances. Two ml of this solution was dialysed against 4 ml of the phosphate buffer containing all combinations of L-tryptophan (0·075, 0·25, 0·375, 0·75 and 1·13 mM) with salicylate (0·0, 0·45, 1·9 and 4·5 mM). The levels of salicylate were chosen to approximate the blood levels observed in patients receiving salicylates either as analgesics or as therapy for rheumatism and those found in acute intoxication with the drug. The dialysis vessels were shaken at 100 cycles/min on a Luckman rotary shaker at room temperature (22°) for 20 h. The final concentration of L-tryptophan remaining outside the dialysis tubing in the buffer was determined by reaction with ninhydrin, using a Technicon auto-analyser. The free concentration is determined by the concentration of L-tryptophan in the dialysate outside the dialysis tubing. The total amount of the amino-acid inside the dialysis tubing with the protein was calculated by subtracting the total amount outside the dialysis tubing from the amount originally added. The concentration of bound tryptophan is then the total concentration inside the dialysis sac minus the free concentration. The results of preliminary experiments showed that the presence of bovine albumin did not affect the determination of tryptophan, the equilibrium was reached in the experimental time and that the tryptophan was not absorbed on either the dialysis tubing or on the glassware. Extra analyses of the final tryptophan concentrations, in the absence of salicylate, were made on an Aminco Bowman spectrophotofluorimeter at an excitation wavelength of 278 nm and an emission wavelength of 366 nm, and the results were in good agreement with those obtained with the auto-analyser system.

RESULTS

Effect of sodium salicylate on the levels of free amino-acids in human serum

Table 1 gives the concentrations of amino-acids in the undialysed sera and in the dialysates. In this, and subsequent tables, the results have been analysed by the *t*-test; the minimal acceptable level of significance being taken as $P = 0{\cdot}05$. With the exception of tryptophan, the values for the levels of each amino-acid obtained by either procedure did not differ significantly. However, the level of tryptophan determined by the protein precipitation method was approximately five times higher

than that by equilibrium dialysis. These results show that, whereas most amino-acids are present in the serum as free molecules not bound to non-dialysable macromolecules, tryptophan occurs normally both as the free amino-acid and bound to protein.

In the presence of salicylate, the concentration of tryptophan in the experimental dialysate rose from 0·01 to 0·046 mM, showing a considerable release of tryptophan from its binding sites. Sodium salicylate had no significant effect on the free concentrations of any other amino-acids. When L-tryptophan was added to serum before dialysis, a proportion became bound, but when salicylate was present a far greater proportion remained free. For example, when the free tryptophan concentration of a control solution was increased to 0·045 mM, 20 mg sodium salicylate per 100 ml serum caused this level to rise to 0·132 mM.

Table 1. *Concentrations of amino-acids in pooled human serum.* The concentrations of amino-acids measured in the control dialysates (free amino-acids) have been multiplied by 1·1 to correct for the dilution during dialysis and to allow a comparison to be made with the total amino-acid concentrations determined in the deproteinized serum supernatants. Each value represents the mean of four determinations, ± standard deviation

Amino-acid	Total amino-acid concn (mM)	Free amino-acid concn (mM)	*P* value
Aspartate	0·062 ± 0·002	0·075 ± 0·025	N.S.*
Threonine	0·184 ± 0·005	0·181 ± 0·009	"
Serine	0·253 ± 0·009	0·246 ± 0·009	"
Glutamine	0·330 ± 0·003	0·380 ± 0·028	"
Proline	0·314 ± 0·027	0·325 ± 0·020	"
Glutamate	0·445 ± 0·023	0·457 ± 0·025	"
Citrulline	0·040 ± 0·006	0·042 ± 0·003	"
Glycine	0·529 ± 0·019	0·508 ± 0·061	"
Alanine	0·591 ± 0·032	0·581 ± 0·039	"
Valine	0·243 ± 0·014	0·259 ± 0·026	"
Methionine	0·039 ± 0·002	0·040 ± 0·001	"
Isoleucine	0·090 ± 0·003	0·097 ± 0·008	"
Leucine	0·218 ± 0·011	0·240 ± 0·017	"
Tyrosine	0·092 ± 0·002	0·087 ± 0·007	"
Phenylalanine ..	0·142 ± 0·004	0·145 ± 0·008	"
Ethanolamine ..	0·025 ± 0·007	0·022 ± 0·002	"
Tryptophan	0·059 ± 0·003	0·011 ± 0·002	<0·001
Ornithine	0·287 ± 0·011	0·266 ± 0·020	N.S.
Lysine..	0·297 ± 0·008	0·281 ± 0·013	"
Histidine	0·163 ± 0·011	0·162 ± 0·010	"
Arginine	0·083 ± 0·010	0·088 ± 0·004	"

* Not significant.

Albumin binding of tryptophan and effect of salicylate

Fig. 1 shows that bovine albumin (0·5 mM) binds about 80% of L-tryptophan when the free concentration is low and that saturation of the binding sites occurs when 0·4 mM L-tryptophan is bound. As the salicylate concentration is increased from 0 through to 4·5 mM, the amount of tryptophan bound for any given level of free tryptophan is decreased progressively. For example, the amount bound when 0·1 mM is free is reduced from 0·3 mM to 0·07 mM by 1·9 mM salicylate.

McMenamy & Oncley (1958) have shown that the ratio of moles of bound tryptophan to moles of protein is approximately 1. This allows the calculation of the

association constant K_A. Table 2 shows that the association constant for tryptophan and albumin decreases significantly as the level of salicylate increases relative to the albumin concentration.

Table 2. *Variation in the association constant K_A for L-tryptophan and 3% bovine albumin with increasing concentrations of salicylate*

$$K_A = \frac{\text{[Bound tryptophan]}}{\text{[Free tryptophan] [Free binding sites on albumin]}}.$$

The value of 60,000 has been used for the molecular weight of bovine serum albumin

Salicylate Concentration (mM)	0·0	0·45	1·9	4·5
K_A (M^{-1})	0·0143	0·0054	0·0017	0·0007
s.d.	±0·0019	±0·0004	±0·0002	±0·0002
P		<0·001	<0·001	<0·001

DISCUSSION

The results presented confirm the findings of McMenamy & Orcley (1958) that the only circulating amino-acid to be significantly bound to protein is L-tryptophan and that less than 20% is in a freely diffusable form. However, in the presence of sodium salicylate at a concentration of 20 mg per 100 ml serum, the proportion of free tryptophan rose to 85%. A proportion of L-tryptophan added to pooled human serum was partly bound to protein in a manner readily reversed by the presence of salicylate.

The dialysis experiments (Fig. 1) show that L-tryptophan is reversibly bound to purified bovine serum albumin and in the presence of increasing concentrations of sodium salicylate proportionally more of the amino-acid remains in a free unbound form.

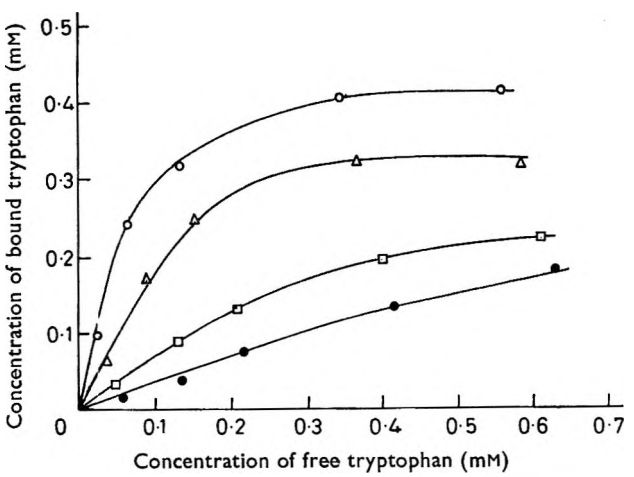

FIG. 1. The effect of salicylate on the relative concentrations of free and bound L-tryptophan in the presence of 3% bovine serum albumin. The experimental conditions were as described in the experimental section. Two ml portions of 3% bovine serum albumin were dialysed against 4 ml of buffer containing various concentrations of L-tryptophan and the following concentrations of sodium salicylate: ○ none. △ 0·45 M. □ 1·9 mM. ● 4·5 mM.

The results suggest that after ingestion of a therapeutic dose of aspirin the resulting serum concentration of salicylate ion could cause an increased level of free tryptophan in the blood. An increased rate of entry of L-tryptophan into the tissues, followed by an increased rate of its metabolism, would tend to restore the free level of L-tryptophan in the blood to that present before ingestion of the salicylate. However, after elimination of the salicylate by conjugation and excretion, more L-tryptophan would bind again to protein and the level of free L-tryptophan would drop. In cases of prolonged salicylate therapy, the concentration of bound L-tryptophan would be much lower and the free concentration would become established at a level similar to that present before salicylate therapy. The overall results would be a lowered reserve of bound L-tryptophan and a reduced capacity to bind free L-tryptophan when the amino-acid was added to the circulation by any route.

It has been shown that the urine of patients suffering from rheumatoid arthritis contains abnormally high concentrations of kynurenine and 3-hydroxyanthranilic acid (McMillan, 1960; Bett, 1962; Spiera, 1966). A tryptophan load given to these patients caused a rise in the urinary kynurenine levels much greater than that produced in normal individuals, whereas the urinary excretion of 3-hydroxyanthranilic acid was increased by similar amounts in both groups. Bett (1962) stated that previous salicylate therapy or continued therapy during a tryptophan loading test did not affect the quantitative pattern of tryptophan metabolites in the urine. Spiera (1966) studied the levels of various intermediates and end products of the metabolism of tryptophan *via* the kynurenine and 5-hydroxytryptamine (5-HT) pathways, in a group of control patients suffering from a variety of diseases and in a group of patients suffering from rheumatoid arthritis. He found that there were significant increases in the levels of kynurenine, 3-hydroxyanthranilic acid and xanthurenic acid, but not of *N*-methyl nicotinamide, total indoles, 5-hydroxyindole acetic acid, indole acetic acid or tryptamine. However, twelve of the thirty-one patients in the control group were receiving aspirin. If the control group is subdivided into two sub-groups with respect to aspirin administration (Table 3), it can be calculated, using the data of Spiera, that there is a significant difference in the urinary excretions of xanthurenic acid and of this metabolite plus kynurenine and 3-hydroxyanthranilic acid between these two sub-groups. There is also a significant difference in the values between

Table 3. *Excretion of tryptophan metabolites (kynurenine plus 3-hydroxyanthranilic acid plus xanthurenic acid) expressed as mg/24 h.* The original results of Spiera (1966) and the regrouped controls show that there is a significant difference due to aspirin and not rheumatism. Each value is the mean ± the standard deviation

Control				Rheumatoid
26·4 ± 19·6				63·4 ± 42·6
		$P < 0{\cdot}001$		
Control		Control		
No aspirin		Aspirin		Rheumatoid
20·4 ± 10·0		35·8 ± 26·9		63·4 ± 42·6
	$P < 0{\cdot}05$		$P < 0{\cdot}1$	

the non-aspirin treated controls and the rheumatoid patients, but there is no significant difference between the control sub-group receiving aspirin and the rheumatoid group, all of whom were treated with aspirin.

The levels of tryptamine, indoleacetic acid and 5-hydroxyindoleacetic acid in the three groups of patients were not found to be significantly different, and since aspirin was being prescribed to two of these groups, there is no indication that the tryptamine and 5-HT pathways are being affected by long-term salicylate therapy. However, these metabolites have not been measured in individuals following an acute dose of aspirin, and it may well be that the levels of tryptophan and its metabolites in blood and urine are raised following such a treatment.

The liver enzyme, tryptophan pyrrolase, which converts tryptophan to *N*-formyl kynurenine is an inducible enzyme. The activity of this enzyme has been increased as much as tenfold by feeding tryptophan (Knox & Mehler, 1950). The release of more free tryptophan into the tissues as a consequence of the presence of salicylate in the blood may result in an increased activity of this enzyme and may be a factor directing the metabolism of more tryptophan *via* the kynurenine pathway, thereby restoring the level of free tryptophan to normal. The activity of tryptophan pyrrolase and other enzymes involved in the metabolism of tryptophan has not been studied during salicylate therapy but it is known that 3-hydroxyanthranilic acid oxidase is strongly inhibited by salicylate *in vitro* (Vescia & di Prisco, 1962).

A high proportion of tryptophan is metabolized *via* acetate to carbon dioxide and water, and only a small proportion of ingested tryptophan can be accounted for in the urinary end-products of the kynurenine and 5-HT pathways (Jepson, 1966). If the oxidation of tryptophan to carbon dioxide and water is operating maximally at a stage after 3-hydroxyanthranilic acid formation, then increased levels of tryptophan would either accumulate as such or as one of its intermediates before the rate-limiting step of the metabolic sequence. If fluctuations in the level of tryptophan occur normally, such as would be expected following a protein meal, then in the presence of salicylate there will be fewer binding sites available to accommodate much of the tryptophan and there will be even greater fluctuations in the free level. This may cause at various times much more tryptophan to be metabolized *via* the kynurenine pathway, giving rise to higher levels of the intermediates in the tissues and blood, and if the renal threshold for these compounds is low, much more will appear in the urine.

It has been observed that, whereas most aminotransferases are inhibited by salicylate, the tryptophan-α-oxoglutarate aminotransferases from the liver, kidney and heart of the rat are activated by salicylate (Gould & Smith, 1965). A possible explanation for this activation could be envisaged if tryptophan were bound either to the enzyme protein itself at a site not involved in its activity, or to protein associated with the enzyme. In the presence of salicylate, more of the tryptophan might be released to give a higher effective concentration of free substrate which could have been responsible for the observed increased activity. If the enzyme were inhibited by salicylate, then the stimulation caused by the released substrate might have been sufficiently great to mask this.

Davis, Fisher & McGowan (1968) have shown that 0·1 ml of 0·01% solution of tryptophan inhibits the infiltration of leucocytes into an area of local inflammation of rats. There is, therefore, the possibility that part of the anti-inflammatory action of salicylate is mediated *via* tryptophan.

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REFERENCES

Bett, I. M. (1962). *Ann. Rheum. Dis.*, **21** (1), 63–69.

Brodie, B. B. (1965). *Proc. Roy. Soc. Med.*, **58**, 946–955.

Christensen, L. L. (1959) *Nature, Lond.*, **183**, 1189–1190.

Davis, R. M., Fisher, J. S. & McGowan, L. (1968). *J. Endocr.*, **41** (4), 603–604.

Davison, C. & Smith, P. K. (1951). *J. Pharmac. exp. Ther.*, **133**, 161–170.

Gould, B. J. & Smith, M. J. H. (1965). *J. Pharm. Pharmac.*, **17**, 83–88.

Jepson, J. B. (1966). *The metabolic basis of inherited disease*, p. 1286, Editors: Stanbury, J. B., Wyngaarden, J. B. & Frederickson, D. S., London: McGraw Hill.

Knox, W. E. & Mehler, A. H. (1950). *J. biol. Chem.*, **187**, 419–430.

Kunin, C. M. (1964). *Proc. Soc. exp. Biol., N.Y.*, **117**, 69–73.

McMenamy, R. H. & Oncley, J. L. (1958). *J. biol. Chem.*, **233**, 1436–1447.

McMillan, M. (1960). *J. clin. Path.*, **13**, 140–148.

Osorio, C. (1963). *Salicylates: An International Symposium*, Editors: Dixon, A. St. J., Martin, B. K., Smith, M. J. H. & Wood, P. H. N., 82–87. London: Churchill.

Reynolds, R. C. & Cluff, L. E. (1960). *Bull. Johns Hopkins Hosp.*, **107**, 278–290.

Spiera, H. (1966). *Arthritis Rheum.*, **9**, 318–324.

Stenlake, J. B., Davidson, A. G., Jasani, M. K. & Williams, W. D. (1968). *J. Pharm. Pharmac.*, **20**, *Suppl.*, 248S–253S.

Vescia, A. & di Prisco, G. (1962). *J. biol. Chem.*, **237**, 2318–2324.

Yu, T. F., Dayton, P. G., Berger, L. & Gutman, A. B. (1962). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **21**, 175.

J. Pharm Pharmac., 1969, **21**, 751–762 *Received May* 15, 1969

The inhibition of human platelet 5-hydroxytryptamine uptake by tricyclic antidepressive drugs. The relation between structure and potency

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Thirty-five compounds related to the antidepressive drug imipramine in chemical structure have been examined for their capacity to inhibit the uptake of 5-hydroxytryptamine by human platelets *in vitro*. Substitution by small-sized electropositive groups in positions 2 or 3 of a benzene ring gave compounds more active than the prototype, 3-chloroimipramine being five times as potent on this test. Alteration of the characteristic seven-membered ring of the antidepressive drugs reduced the activity while substitution in the basic side-chain destroyed it. The tertiary amines were more potent inhibitors than their demethylated derivatives. In this and other ways the active structure for the inhibition of 5-HT uptake by human blood platelets differs from that for the inhibition of noradrenaline uptake by the rat heart.

The antidepressive drug, imipramine, [*N*-(dimethylaminopropyl) iminodibenzyl hydrochloride], is a potent inhibitor of the active transport of 5-hydroxytryptamine (5-HT) into human blood platelets (Marshall, Stirling & others, 1960). Stacey (1961) found it to be much the most active in this respect of forty compounds comprising drugs of widely different pharmacological properties, together with compounds related to 5-HT and the endogenous catecholamines. Imipramine was some fifty times more active than the two next most potent compounds, cocaine and chlorpromazine. Long & Lessin (1961) failed to observe this superiority of imipramine over chlorpromazine when studying ox platelets, but confirmed it when they subsequently employed human platelets (1962).

The *N*-desmethyl derivative of imipramine (desipramine) and the chemically closely related ("isosteric") compounds amitripyline and *N*-desmethylamitriptyline (nortriptyline), all of which are used clinically in the treatment of depression, also inhibit the uptake of 5-HT by platelets (Yates, Todrick & Tait, 1964).

This *in vitro* activity is paralleled by the observation that patients receiving therapy with imipramine and desipramine gradually lose up to 80% of the original 5-HT content of their platelets (Marshall & others, 1960; Yates, Todrick & Tait, 1963).

Haefely, Hurlimann & Thoenen (1964b) demonstrated a similar inhibition by tricyclic antidepressive drugs of the re-uptake of noradrenaline at sympathetic nerve endings. Since there is much evidence that this re-uptake phenomenon is a physiological process of major significance in the disposal of the adrenergic transmitter (Brown & Gillespie, 1957; Kirpekar, Cervoni & Furchgott, 1962; Haefely, Hurlimann & Thoenen, 1964a; Thoenen, Hurlimann & Haefely, 1964; Iversen, 1966), the hypothesis has been put forward that imipramine exerts its antidepressive action by

inhibiting the uptake of noradrenaline by nerve endings in the central nervous system, thereby potentiating and prolonging the action of the transmitter (Bunney & Davies, 1965; Klerman & Cole, 1965; Schildkraut, 1965).

The present paper reports the investigation of the inhibition of platelet 5-HT uptake by an extended series of derivatives of imipramine, further derivatives of amitriptyline and some other antidepressive drugs containing modifications to the central seven-membered ring.

The work was carried out in the hope firstly, of throwing light on the spatial configuration of the amine uptake mechanism, and secondly, of establishing some correlation between inhibitory potency against amine uptake in a human test system and clinically assessed antidepressive activity. The latter issue will be discussed subsequently.

EXPERIMENTAL

Methods

The method described in a previous paper (Yates & others, 1964) was used with two modifications: (i) the platelet counting was omitted since it did not appear to be essential to a study of comparative drug activity; (ii) the volume of platelet-rich plasma taken for each test was the original 1·5 ml. Incubations were carried out using final drug concentrations in the series 10^{-n}M and 3×10^{-n}M where n is an integer, this gives an almost uniform logarithmic decrement. For any one compound, after preliminary experimentation to find the correct concentration range, tests were made at either three or four concentrations.

Thirteen compounds in the following categories received more intensive investigation than the rest, viz. about twelve incubations in all, and the data were subjected to a statistical analysis (see Appendix). (*a*) Drugs in clinical use. (*b*) Compounds which were highly active. (*c*) Compounds of particular interest for structure-activity relations.

For the remaining 22 of the total of 35 compounds tested, the inhibitory potency was determined graphically from about eight estimates (Finney, 1964). The data from an earlier paper (Yates & others, 1964) have been included in the results and in the statistical analysis where appropriate. Most of the data were obtained in the course of four separate periods of work, each lasting a few months, spread over four years. The prototype compound, imipramine, was tested during each phase as a reference standard. The absolute inhibitory potency of imipramine in the four phases differed slightly but significantly. Allowance has been made for this in the statistical analysis (see Appendix).

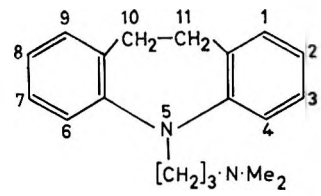
Since a wide variation in inhibitory potency was observed in the series of compounds the absolute measured potency has been expressed in terms of the negative logarithm of the concentration of the drug which causes 50% inhibition of 5-HT uptake (pI 50). This scale is recognized as having advantages for pharmacological studies; on it, the most potent compound has the highest value, a difference of 1 unit indicates a ten-fold difference in potency and a difference of 0·3 unit a two-fold difference in potency.

RESULTS

Table 1 lists the compounds possessing the imipramine nucleus which have been tested, their structure and their experimentally determined pI 50 values. The final column gives their activity as a percentage of that of the imipramine standard of

Table 1. *Inhibition of human platelet 5-HT uptake by compounds possessing the imipramine* (I) *nucleus*

I



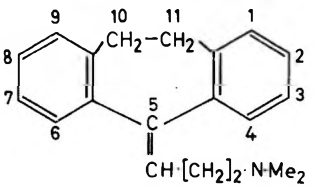
Compound	Nuclear substituents in positions 2	3	10	Side-chain substituents (in β-position)	Terminal amino-group	Salt	pI50	Experimental phase	Potency relative to imipramine (= 100) (comparison within phase)
Imipramine[1]	H	H	H	H	NMe_2	Hydrochloride	5·54	A	
							5·78	B	100
							5·62	C	
							5·73	D	
Desipramine[2]	H	H	H	H	NHMe	Hydrochloride	5·02	A	30
Desdimethylimipramine	H	H	H	H	NH_2	Hydrochloride	5·13	D	25
2-Hydroxyimipramine	OH	H	H	H	NMe_2	Hydrochloride	5·41	D	48
2-Hydroxydesipramine	OH	H	H	H	NHMe	Acid fumarate	5·13	A	39
2-Hydroxydesipramine glucuronide	O $C_6H_{10}O_6$	H	H	H	NHMe	Internal salt	3·61	B	0·7
10-Hydroxyimipramine	H	H	OH	H	NMe_2	Base	5·22	B	28
10-Hydroxydesipramine	H	H	OH	H	NHMe	½ Neutral fumarate, H_2O	4·37	B	3·9
10-Methyldesipramine	H	H	Me	H	NHMe	Hydrochloride	4·79	D	11
Imipramine *N*-oxide	H	H	H	H	NMe_2O	Base	4·11	B	2·1
3-Chloroimipramine[3]	H	Cl	H	H	NMe_2	Hydrochloride	6·50	B	520
2-Methoxyimipramine	OMe	H	H	H	NMe_2	Hydrochloride	6·03	C	260
3-Methoxyimipramine	H	OMe	H	H	NMe_2	Hydrochloride, ¼ H_2O	6·09	C	290
3-Methoxydesipramine	H	OMe	H	H	NHMe	Hydrochloride	5·22	D	31
3-Methylthioimipramine	H	SMe	H	H	NMe_2	Hydrochloride	5·72	C	125
3-Methylsulphinylimipramine	H	S(O)Me	H	H	NMe_2	Acid oxalate	5·57	C	89
3-Isopropylthiodesipramine	H	$SCHMe_2$	H	H	NHMe	Hydrochloride	4·74	C	13
3-Isopropylsulphinylimipramine	H	$S(O)CHMe_2$	H	H	NMe_2	Acid oxalate	4·89	C	19
3-Dimethylsulphamoxylimipramine	H	SO_2NMe_2	H	H	NMe_2	Hydrochloride	4·73	C	13
3-Dimethylsulphamoxyldesipramine	H	SO_2NMe_2	H	H	NHMe	Hydrochloride	4·71	C	12
Imipramine methochloride	H	H	H	H	NMe_3	Chloride	6·12	C	320
N-Hydroxydesipramine	H	H	H	H	N(OH)Me	Base	<3·5	B	<0·5
10-Oxoimipramine	H	H	O	H	NMe_2	Acid fumarate	4·64	B	7·3
Trimipramine[4]	H	H	H	Me	NMe_2	Hydrochloride	3·92	C	2·0
3-Chloro-β-methylimipramine	H	Cl	H	Me	NMe_2	Hydrochloride	3·85	C	1·7
Opipramol[5]	H	H	10,11Δ	H		Dihydrochloride	4·30	D	3·7

[1] In clinical use as an antidepressive drug under the name of Tofranil (Geigy).
[2] In clinical use as an antidepressive drug under the name of Pertofran (Geigy).
[3] On clinical trial under the name of Anafranil (Geigy).
[4] In clinical use as an antidepressive drug under the name of Surmontil (May and Baker).
[5] In clinical use as an antidepressive drug under the name of Insidon (Geigy).

the phase in which they were tested (this causes certain minor inconsistencies in rank-order between the last two columns). Table 2 lists similar data for compounds in which the nitrogen atom in position 5 is replaced by a carbon atom; included also are chlorpromazine and two drugs that have received clinical use in the treatment of depression, orphenadrine (Robinson, 1961) and thiazesim (Freeman, Oktem & others, 1965).

Table 2. *Inhibition of human platelet 5-HT uptake by compounds possessing the amitriptyline* (II) *nucleus and certain other compounds*

II



Compound	Nuclear substituents in positions 2	3	Bridge at 10–11	5	Terminal amino-group	Salt	pI50	Experimental phase	Potency relative to imipramine (=100) (comparison within phase)
Amitriptyline[1]	H	H	$-CH_2-CH_2-$	C⁼	NMe_2	Hydrochloride	5·28	A	54
Nortriptyline[2]	H	H	$-CH_2-CH_2-$	C⁼	NHMe	Hydrochloride	5·00	A	29
Desdimethylamitriptyline	H	H	$-CH_2-CH_2$	C⁼	NH_2	Hydrochloride	5·04	B	18
10,11Δ-Amitriptyline ..	H	H	$-CH^{=}CH-$	C⁼	NMe_2	Hydrochloride	4·83	B	11
10,11Δ-Nortriptyline ..	H	H	$-CH^{=}CH-$	C⁼	NHMe	Hydrochloride	4·89	B	13
Protriptyline[3]	H	H	$-CH^{=}CH-$	C–	NHMe	Hydrochloride	5·05	C	27
Thiazenone[4]	—	—	See note 5	—	NMe_2	Hydrochloride	3·77	C	1·4
Chlorpromazine	H	Cl	–S–	N–	NMe_2	Hydrochloride	4·41	A	7·4
Orphenadrine	H	H	No bridge	C–[6]	NMe_2	Hydrochloride	3·90	D	1·5

[1] In clinical use as an antidepressive drug under the names of Laroxyl (Roche), Sarcten (Warner) and Tryptizol (Merck).
[2] In clinical use as an antidepressive drug under the names of Aventyl and Allegron (Eli Lilly).
[3] In clinical use as an antidepressive drug under the name of Concordin (Merck).
[4] On clinical trial as an antidepressive drug under the name of Thiazesim (Squibb).
[5] 5-(Dimethyl aminoethyl)-2,3-dihydro-2-phenyl-1,5-benzothiazepin-4 (5H)-one hydrochloride.
[6] Orphenadrine has a methyl group at C–4 and the side chain at C–5 is $-O-(CH_2)_2-$. It is in clinical use under the name of Disipal (Brocades).

The information derived from the statistical analysis of the thirteen most important compounds (given in detail in Table A of the Appendix), may be conveniently summarized in the following ranking list; in this, compounds or groups of compounds having potencies different at the 5% significance level are separated by solid lines:

3-Chloroimipramine 520,/imipramine methochloride 320, 3-methoxyimipramine 290, 2-methoxyimipramine 260,/3-methylthioimipramine 125, imipramine 100, 3-methylsulphinylimipramine 89, amitriptyline* 54,/desipramine 30, nortriptyline 29, protriptyline 27, desdimethylimipramine 25, desdimethylamitriptyline 18.

The compounds are almost completely separated into groups by the significance test. This non-random distribution is to some extent a fortuitous result of the choice of significance level, but it also reflects the selection of compounds; the more active 3-substituted compounds came wholly from the imipramine series while the less active 10,11Δ-compounds came from the amitriptyline series.

Although these thirteen compounds were regarded as being of particular interest and were given both more extended experimental investigation and arithmetical analysis, the pI50 values obtained graphically for the remaining compounds may be

* Amitriptyline is significantly less potent than 3-methylthioimipramine and imipramine, but not significantly different from 3-methylsulphinylimipramine.

assumed to be subject to the same error variance; a working figure for the difference in pI50 which indicates a potency difference at the 5% significance level can be calculated. In round numbers, it will be 0·2 for in-phase comparisons. For interphase comparisons the correct difference to take is (pI50 Drug A − pI50 standard A) − (pI50 Drug B − pI50 standard B); to be significant, it must not be less than 0·25.

General structure—activity relations

The series of compounds listed in Tables 1 and 2 provide examples of modification of the basic structure of the prototype compound, imipramine, as follows:

1. On the terminal nitrogen atom. 2. By substitution in the aliphatic side-chain. 3. By substitution in the 2 position of a benzene ring. 4. By substitution in the 3 position of a benzene ring. 5. By substitution in the 10 position (ethylene bridge). 6. By alteration of the seven-membered central ring.

1. Potency is maximal for the quaternary salt of imipramine and falls off through imipramine to desipramine, but there is no further loss of potency with the removal of the last methyl group: the same pattern holds for the amitriptyline series as far as it goes (the quaternary salt was not tested). The tertiary and secondary amines can be compared in four other instances: the highly active 3-methoxy- and the fairly potent 10-hydroxyimipramine are markedly more effective than their desmethyl analogues; the comparatively inactive pairs 10,11Δ-amitriptyline and 10,11Δ-nortriptyline and 3-dimethylsulphamoxylimipramine and 3-dimethylsulphamoxyldesipramine do not differ significantly.

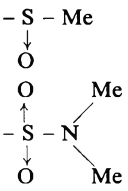
There is only one compound with an alkyl substituent other than the methyl group, viz. opipramol, which carries a substituted piperazine ring; this has very little activity. Finally, addition of → O or substitution of –Me by –OH on the terminal *N*-atom causes almost complete loss of inhibitory activity.

2. Two compounds substituted in the β-position of the side-chain, trimipramine and 3-chlorotrimipramine, are practically without activity.

3. Only four compounds substituted in the 2-position of a benzene ring have been tested, three of which are natural metabolites: 2-hydroxyimipramine is half as active as the parent substance while 2-hydroxydesipramine has the same activity as its less potent parent; 2-hydroxydesipramine glucuronide, which is the main excretion product from both drugs, is practically inactive. 2-Methoxyimipramine is, however, more potent than imipramine and not significantly less active than its 3-methoxy isomer.

4. Substitution in the 3-position of the benzene ring has been examined in some detail in an attempt to assess the influence of electromeric effects. The synthesis of compounds substituted with electronegative groups has not been possible, but electropositive substituents in the 3-position appear to increase potency; 3-chloroimipramine is five times as potent as the parent substance and 3-methoxyimipramine three times as potent. A series with 3-substituents of graded electropositivity was then examined. The results (Tables 1 and 3) show no correlation with degree of electropositivity but can be interpreted as indicating a steric factor since all compounds containing substituent groups larger than –S(O)Me are uniformly low in activity (12–19, imipramine = 100).

Table 3. *Effect of size of substituent group at position* 3 *on inhibitory potency of imipramine derivatives*

3-Substituent group	Relative potency (Imipramine = 100)	Molecular radius (Å)
– Cl	520	1·8
– O – Me	290	2·8
– S – Me	125	3·6
	89	—
	13	—

5. Since the possession of an ethylene bridge in the 10,11-position is the characteristic of the antidepressive drug molecule, as distinct from the tranquillizer molecule, it might have been thought that substitution in the 10-position would have had a marked adverse effect on the activity. However, 10-hydroxyimipramine possesses a quarter of the activity of the parent substance though its desmethyl derivative is much less active; 10-oxoimipramine is also only weakly active.

6. Substitution at the 5-position of the central ring of N– by C= gives amitriptyline which is slightly but significantly less potent than imipramine, though the singly and doubly demethylated compounds in the two series do not differ significantly. Dehydrogenation of the 10,11-bridge of amitriptyline results in a five-fold loss of potency (from 54 to 10). The activity of nortriptyline (29) also falls (to 13) on dehydrogenation: a shift of the double bond from the 5 γ-position of nortriptyline to the 10,11-position of protriptyline does not alter the activity. Replacement of the 10,11-bridge of 3-chloroimipramine by a sulphur atom (chlorpromazine) results in a marked loss of potency, from 520 to 8·5; an unbridged compound (orphenadrine) which was tested, though still less active (1·5), is not strictly comparable as the side-chain contains an ether linkage.

DISCUSSION

The tricyclic antidepressive drug molecule, which includes both the imipramine and amitriptyline series of compounds, can be modified or substituted in many positions of differing potentiality. The present series of compounds includes only a small number of modifications or substitutions at each position; any generalizations must be regarded with caution, but three provisional rules appear to emerge:

(*a*) Activity is practically destroyed (<2%) either by substitution of a methyl group in the β-position of the three-carbon side-chain or by substitution of other groups for the methyl groups or hydrogen atoms attached to the terminal N-atom of the side-chain, or both.

(*b*) Alteration of the central ring, either by replacing the N-atom in position 5 with C= or C– or by doubling the 10,11-bond or by monovalent substitution on the 10,11-bridge reduces the activity much less, broadly to one quarter to one half of that of the parent compound.

(*c*) Substitution in position 2 and 3 of a benzene ring can in certain instances increase the activity above that of imipramine but two conditions may require to be fulfilled, as (i) the increase in activity has only occurred with electropositive substituents (but few neutral and no electronegative substituents have been tested), and (ii) the substituent group must not be more than a certain size or the activity drops to one tenth of that of imipramine.

Since 2-methoxyimipramine is as active as 3-methoxyimipramine, the increase in activity on substitution can hardly be purely an electromeric induction effect on the N-atom in position 5.

As the mode of action of imipramine has been postulated to consist of interference with the uptake of noradrenaline, its molecule might be supposed to fit closely with the system responsible for this in the membrane of the nerve ending; it should also fit, but not quite so closely, with the adrenergic receptor. Hypotheses have been developed concerning the chemical structure of the adrenergic receptor based on the relative specificities of a wide range of noradrenergic agonists and antagonists. The primary point of attachment is anionic; Belleau (1960) and Bloom & Goldman (1966) have put forward evidence to support the view that the phosphate ion of ATP is involved but the results of Triggle (1965) and Graham & Al Katib (1966) favour the carboxylate ion of protein.

It has been argued that the agonist (noradrenaline) must possess a more specific structure than an antagonist since it has to perform a more specialized function following attachment; the same may reasonably be expected to hold good for the uptake mechanisms which appear to be basically similar whether the amine involved is noradrenaline or 5-HT. This lower specificity requirement may account for the wide range of chemical structures found among compounds interfering with amine uptake, e.g. imipramine, cocaine, metaraminol, prenylamine, guanethidine.

Iversen (1966) has analysed the specificity as "Uptake$_1$" inhibitors of a group of amines structurally related to noradrenaline. This in no way resembles the structure activity relationship found for the imipramine series in respect of 5-HT uptake inhibition, since methylation of hydroxy-groups and *N*-alkylation decrease inhibitory potency while side-chain methylation increases it.

Uptake inhibitors might be expected to be similar in structure to α-adrenergic blocking drugs and the latter do in fact inhibit the platelet 5-HT uptake system though they are less active than the tricyclic antidepressive drugs (Todrick, unpublished results). Bloom & Goldman (1966) suggest that the steric hindrance of the bulky cationic head is the primary factor with the α-blocking drugs; if there is any parallelism between the two systems, this may account for the increase in activity with increasing numbers of methyl groups on the terminal N-atom in the imipramine series.

In considering basic drugs, the effect of the degree of ionization on the activity must be taken into account. The imipramine series being comparatively insoluble in water, the pK_a is normally obtained by extrapolation. The available data are given in Table 4 (this includes only extrapolated figures or estimates by methods where extrapolation was considered unnecessary).

The pK_a for the imipramine quaternary salt may be assumed to be >13; these figures therefore provide no explanation for the increase in activity with increasing methylation of the terminal N-atom in the unsubstituted imipramine; the lower pK_a might partially account for the inactivity of trimipramine.

Table 4. pK_a *Values of imipramine and derivatives*

Compound	Method	pK_a in water	Experimenters
Imipramine	A	10·0	Schmidt (personal communication)
	B	9·57	Moody (unpublished results)
Desipramine	A	10·6	Schmidt (personal communication)
	B	10·43	Moody (unpublished results)
Desdimethylimipramine ..	B	10·19	Moody (unpublished results)
Trimipramine	C	8·0	Rhone Poulenc Research Laboratories, (personal communication)

Methods: A Titration in aqueous methyl cellosolve with extended extrapolation.
B Titration in aqueous solution with measurement of fluorescence change.
C Titration in 10% ethanol in water; not extrapolated.

There are in current use numerous laboratory tests characterizing compounds with a clinical antidepressive action; this of itself is perhaps the best evidence that no one is wholly satisfactory and that "in the meantime pharmacological characterization of antidepressant agents should be based on the use of a battery of tests outlined in several comparative studies" (Gyermek, 1966).

Table 5 compares the results obtained with eleven antidepressive drugs and two tranquillizers of similar chemical structure in some current tests.

Table 5. *Comparison of six tests for the assessment of antidepressive action*

Compound	Ro 4–1284 antagonism[1]	Noradrenaline potention of cat nictitating membrane[2] A	Noradrenaline potention of cat nictitating membrane[2] B	Potentiation of amphetamine hyperthermia[3] (increase in area under curve)	Inhibition of noradrenaline uptake by rat heart[4] pI50	Inhibition of 5-HT uptake by human platelets[5] pI50	5-HT potentiation of cat nictitating membrane[3]
Imipramine	—	++	++	+6·5	7·4	5·7	+++
Desipramine	++	+++	+++	+6·3	8·15	5·0	++
Desdimethylimipramine	++			+6·5	7·0	5·1	
2-Hydroxyimipramine ..		++		+3·8		5·4	+
3-Chloroimipramine ..	O	+				6·5	++
Amitriptyline	O	+	+		7·0	5·3	+
Nortriptyline	++	+++	+		7·6	5·0	++
10,11Δ-Imipramine ..	O	+++			7·5		++
10,11Δ-Desipramine ..	++	+++			8·2		++
10,11Δ-Amitriptyline ..	O	O				4·8	O
10,11Δ-Nortriptyline ..	O	++				4·9	+
Promazine (10–11 bridge replaced by S)	O	—			7·4		—
Desmethylpromazine ..	+	—			7·1		—

[1] Bickel & Brodie (1964). [2] A. Sigg & others (1965) as quoted in Gyermek (1966). B. Haefely & others (1964b). [3] Theobald, Buch & others (1966). [4] Callingham (1967). [5] Present work.
— = Blockage. O = No effect. + = Active/moderate potentiation. ++ = Very active/marked potentiation. +++ = Very marked potentiation.

The tests listed possess certain individual characteristics:

(*a*) The RO–4–1284 variant of the reserpine antagonism test (Bickel & Brodie, 1964) has consistently shown secondary amines (e.g. desipramine) to be more potent than tertiary amines (e.g. imipramine); only 2 out of 40 tertiary amines tested were found to possess any activity at all. Hjelte & Richter (1967), who used a different form of reserpine antagonism test, did not, however, confirm the inactivity of the tertiary amine structure.

(*b*) In the cat nictitating membrane test (Sigg, Soffer & Gyermek, 1963) the secondary amines consistently potentiated noradrenaline more effectively, except for the iminostilbene pair; in a later investigation, Haefely & others (1964b) did not find nortriptyline superior to amitriptyline.

(*c*) The other test in which noradrenaline is specifically involved, namely the study of "Uptake$_1$" by the perfused rat heart (Iversen, 1965; Callingham, 1967) again demonstrated a consistent superiority of the desmethyl series of antidepressive drugs. The difference disappeared in pairs of compounds of lower activity.

(*d*) The effect of 5-HT on the cat nictitating membrane can hardly be regarded as other than "pharmacological," whereas that of noradrenaline may be claimed to be "physiological." The potentiation of the action of 5-HT may therefore be a more artificial test than noradrenaline potentiation; of four pairs tested, the desmethyl compound is superior in two but imipramine is superior to desipramine and the iminostilbenes are equipotent.

(*e*) In the platelet 5-HT uptake test the active tertiary compounds were found to be superior to their desmethyl derivatives though the difference disappeared in less active pairs.

(*f*) Data for the amphetamine hyperthermia potentiation test are scarce but imipramine and desipramine were essentially equipotent.

We hope later to assess the relative value of different laboratory tests of antidepressive action in comparison with the findings of clinical trials. The problems in such a comparison are great, reflecting not only the complexity of clinical assessment in depression, but also the fact that some compounds have an additional (directly sedative) cerebral action. As a preliminary comment, however, our laboratory ranking of drug activity is at least in broad general concordance with clinical reports.

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REFERENCES

BELLEAU, B. (1960). In *Ciba Foundation Symposium on Adrenergic Mechanisms*, pp. 223–246, Editors: Vane, J. R., Wolstenholme, G. E. W. & O'Connor, M., London: Churchill.

BICKEL, M. H. & BRODIE, B. B. (1964). *Int. J. Neuropharm.*, **3**, 611–621.

BLOOM, B. M. & GOLDMAN, I. M. (1966). In *Advances in Drug Research*, Vol. **3**, pp. 121–169, Editors: Harper, N. J. & Simmonds, A. B., London: Academic Press.

BROWN, G. L. & GILLESPIE, J. S. (1957). *J. Physiol., Lond.*, **138**, 81–102.

BUNNEY, W. E. Jr. & DAVIES, J. M. (1965). *Arch. Gen. Psychiat.*, **13**, 483–494.

CALLINGHAM, B. A. (1967). In *Proc. of the First International Symposium on Anti-depressant Drugs*, pp. 35–43, Editors: Garattini, S. & Dukes, M. N. G., Amsterdam: Excerpta Medica Foundation.

FINNEY, D. J. (1964). *Statistical Methods in Biological Assay*, 2nd edn, London: Griffin.

FREEMAN, H., OKTEM, M. R., KRISTOFFERSON, M. W. & GORBY, C. K. (1965). *Current Ther. Res.*, **1**, 655–660.

GRAHAM, J. D. P. & AL KATIB, H. (1966). *Br. J. Pharmac. Chemother.*, **28**, 1–14.

GYERMEK, L. (1966). *Int. Rev. Neurobiol.*, **9**, 95–143.

HAEFELY, W., HURLIMANN, A. & THOENEN, H. (1964a). *Br. J. Pharmac. Chemother.*, **22**, 5–21.

HAEFELY, W., HURLIMANN, A. & THOENEN, H. (1964b). *Helv. physiol. pharmac. Acta*, **22**, 15–33.

HJELTE, N. & RICHTER, W. (1967). In *Proc. of the First International Symposium on Anti-depressant Drugs*, pp. 23–27, Editors: Garattini, S. & Dukes, M. N. G., Amsterdam, Excerpta Medica Foundation.

IVERSEN, L. L. (1965). *Br. J. Pharmac. Chemother.*, **24**, 387–394.

IVERSEN, L. L. (1966). In *Advances in Drug Research*, Vol. 2, pp. 1–46, Editors: Harper, N. J. & Simmonds, A. B., London: Academic Press.

KIRPEKAR, S. M., CERVONI, P. & FURCHGOTT, R. F. (1962). *J. Pharmac. exp. Ther.*, **135**, 180–190.

KLERMAN, G. L. & COLE, J. O. (1965). *Pharmac. Rev.*, **17**, 101–141.

LONG, R. F. & LESSIN, A. W. (1961). Communication to British Pharmac. Soc., Edinburgh Meeting.

LONG, R. F. & LESSIN, A. W. (1962). *Biochem. J.*, **82**, 4P–5P.

MARSHALL, E. F., STIRLING, G. S., TAIT, A. C. & TODRICK, A. (1960). *Br. J. Pharmac. Chemother.*, **15**, 35–41.

ROBINSON, R. A. (1961). *Acta Neurol. Psychiat. Belg.*, **61**, 34–40.

SCHILDKRAUT, J. J. (1965). *Am. J. Psychiat.*, **122**, 509–522.

SIGG, E. B., SOFFER, L. & GYERMEK, L. (1963). *J. Pharmac. exp. Ther.*, **142**, 13–20.

STACEY, R. S. (1961). *Br. J. Pharmac. Chemother.*, **16**, 284–295.

THEOBALD, W., BUCH, O., KUNZ, A. & MORPURGO, C. (1966). *Medna Pharmac. exp.*, **15**, 187–197.

THOENEN, H., HURLIMANN, A. & HAEFELY, W. (1964). *J. Pharmac. exp. Ther.*, **143**, 57–63.

TRIGGLE, D. J. (1965). In *Advances in Drug Research*, Vol. 2, pp. 173–189, Editors: Harper, N. J. & Simmonds, A. B., London: Academic Press.

YATES, C. M., TODRICK, A. & TAIT, A. C. (1963). *J. Pharm. Pharmac.*, **15**, 432–439.

YATES, C. M., TODRICK, A. & TAIT, A. C. (1964). *Ibid.*, **16**, 460–463.

APPENDIX

STATISTICAL EVALUATION OF RELATIVE POTENCIES OF SELECTED ANTIDEPRESSIVE COMPOUNDS

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One of the purposes of the investigation was to determine whether there was any empirical correlation of the clinical efficacies of the drugs and their potencies as inhibitors of platelet 5-HT uptake, that is, the external validity of the assay. We must first, however, establish the internal validity of the assay. In this section we therefore discuss the validity of the assumptions made in the analysis of the data and the estimation of the relative potencies.

Previous experience behind the assay has until recently been limited to this and one other laboratory (Cockrill, Somerville & Whittle, 1968) where, in terms of actual work done, it has been fairly extensive. The investigation arose from a chance observation that imipramine causes a fall in blood 5-HT level during therapy. It was extended to structurally related compounds synthesized in the research departments of pharmaceutical firms in the hope that the results might assist in the laboratory assessment of their therapeutic potential. These compounds inevitably became available at irregular intervals and, since their investigation was not the only problem in hand, the data here reported were collected in four phases of concentrated activity lasting 2–3 months and spaced out over a period of 4 years. Towards the middle of each phase the prototype drug, imipramine, was tested to give a standard for comparison. Modifications to the technique during the course of the investigation have been minimal and, of course, have affected the standard as well as the other compounds.

The investigation therefore developed in an unplanned manner and the fundamental condition for validity of any statistical analysis, that of randomization of treatments to experimental units, is absent. Although we proceed with the statistical analysis as if randomization had been used it must be remembered that the estimates of relative potency may be biased.

The data appeared on inspection to possess the properties of parallelism and linearity necessary for the application of the parallel line assay model (Finney, 1964, Chapter 4). The response, that is, the percentage inhibition of uptake of 5-HT by the platelets, appeared to be linearly related to log drug concentration over the ranges of concentration used. The slopes of these lines did not vary greatly from one drug to another.

In testing the assumptions of linearity and parallelism in an analysis of variance we must further assume that the observations are homoscedastic. A plot of the standard deviation of the variation between replicates against the mean response for each treatment suggested that the variation might be greater with treatments which cause less inhibition. This was confirmed by Bartlett's test, which gave a χ^2 of 63·7 with 45 degrees of freedom compared with the tabulated 5% level of 61·7. There is therefore evidence for heteroscedasticity, but the actual magnitude of the slope of the standard deviation/response line is low (0·066/1% change). Since quite a significant degree of heteroscedasticity does not materially affect the validity of the analysis it has been ignored.

The analysis of variance table was calculated; this confirmed that the percentage inhibition/log concentration regressions for the compounds could be assumed linear and parallel ($F = 1{\cdot}0$ and 0·8 respectively): hence the relative potencies of pairs of compounds may be estimated using the usual formulae (Finney, 1964, § 4.11 and 4.12).

The estimate of the common slope of the regression is 45·85 with standard error $\pm 1{\cdot}58$. With an error root mean square of 8·320 and $\Sigma Sxx = 27{\cdot}805$, the value of g for 117 degrees of freedom is negligible (0·0046). The fiducial limits may therefore be determined from the standard errors of the relative potency estimates.

We should next enquire whether the relative potencies can be estimated in the above manner irrespective of the phases in which the compounds were investigated. Our only check on this comes from the pI50 estimates of the imipramine standard which was assayed in each of the four phases. It is found that there are small but nonetheless significant differences between these estimates. No explanation of this in terms of experimental procedure, which would suggest grounds for discarding any estimate or estimates, can be given. The differences must therefore be regarded as real differences between the phases and taken into account in the inter-drug comparisons.

Comparisons between drugs investigated in different phases are therefore made via the standard, assuming that the difference between the pI50 values of any pair of drugs would be the same in all phases. Although this is the simplest model we can assume in calibrating phase differences it is not one that can be tested from our data.

For two compounds P and Q investigated in different phases A and B the relative potency R is then given by

$$M = \log R = m_{P(A)} - m_{Q(B)} - (m_{S(A)} - m_{S(B)})$$

where

$m_{P(A)}$ is pI50 of drug P in phase A
$m_{Q(B)}$,, ,, Q ,, B
$m_{S(A)}$,, standard ,, A
$m_{S(B)}$,, ,, ,, B.

Using the notation of Finney, 1964, § 4.11 and 4.12,

$$M = \bar{x}_{P(A)} - \bar{x}_{Q(B)} - (\bar{x}_{S(A)} - \bar{x}_{S(B)}) - \frac{\bar{y}_{P(A)} - \bar{y}_{Q(B)} - (\bar{y}_{S(A)} - \bar{y}_{S(B)})}{b}$$

and Standard Error of M

$$= \frac{s}{b}\left\{\frac{1}{N_{P(A)}} + \frac{1}{N_{Q(B)}} + \frac{1}{N_{S(A)}} + \frac{1}{N_{S(B)}} + \frac{[M - \bar{x}_{P(A)} + \bar{x}_{Q(B)} + (\bar{x}_{S(A)} - \bar{x}_{S(B)})]^2}{\Sigma S_{xx}}\right\}^{\frac{1}{2}}$$

The maximum likelihood estimates and standard errors of the log Relative Potencies have been calculated for all pairings of the thirteen compounds handled in this analysis and are given in Table A. The ranking list given in the results section of the main paper was prepared from this data.

Table A. *Relative potencies of tricyclic antidepressive drugs*
Values tabled are M (= log R) ± s.e.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
3-Chloroimipramine I		0·21 ±0·10 S	0·25 ±0·11 S	0·31 ±0·11 S	0·62 ±0·11 S	0·72 ±0·07 S	0·76 ±0·11 S	0·98 ±0·13 S	1·24 ±0·13 S	1·25 ±0·13 S	1·29 ±0·11 S	1·32 ±0·11 S	1·45 ±0·08 S
Imipramine methochloride II			0·04 ±0·07 NS	0·09 ±0·07 NS	0·41 ±0·07 S	0·50 ±0·07 S	0·55 ±0·07 S	0·77 ±0·13 S	1·03 ±0·13 S	1·04 ±0·13 S	1·07 ±0·07 S	1·10 ±0·11 S	1·24 ±0·10 S
3-Methoxyimipramine III				0·06 ±0·07 NS	0·37 ±0·08 S	0·47 ±0·08 S	0·51 ±0·07 S	0·73 ±0·13 S	0·99 ±0·13 S	1·01 ±0·13 S	1·04 ±0·07 S	1·07 ±0·11 S	1·20 ±0·10 S
2-Methoxyimipramine IV					0·31 ±0·07 S	0·41 ±0·08 S	0·46 ±0·07 S	0·67 ±0·13 S	0·93 ±0·13 S	0·95 ±0·13 S	0·98 ±0·07 S	1·01 ±0·11 S	1·05 ±0·10 S
3-Methylthioimipramine V						0·10 ±0·08 NS	0·14 ±0·07 NS	0·36 ±0·13 S	0·62 ±0·13 S	0·64 ±0·13 S	0·67 ±0·07 S	0·70 ±0·11 S	0·84 ±0·10 S
Imipramine VI							0·05 ±0·08 NS	0·26 ±0·11 S	0·52 ±0·11 S	0·54 ±0·11 S	0·57 ±0·08 S	0·60 ±0·09 S	0·74 ±0·07 S
3-Methylsulphinylimipramine VII								0·22 ±0·13 NS	0·48 ±0·13 S	0·49 ±0·13 S	0·52 ±0·07 S	0·58 ±0·11 S	0·69 ±0·10 S
Amitriptyline VIII									0·26 ±0·11 S	0·28 ±0·11 S	0·31 ±0·13 S	0·34 ±0·14 S	0·48 ±0·13 S
Desipramine IX										0·02 ±0·10 NS	0·05 ±0·13 NS	0·08 ±0·14 NS	0·22 ±0·13 NS
Nortriptyline X											0·03 ±0·13 NS	0·06 ±0·14 NS	0·20 ±0·13 NS
Protriptyline XI												0·03 ±0·11 NS	0·17 ±0·10 NS
Desdimethylimipramine XII													0·14 ±0·11 NS
Desdimethylamitriptyline XIII													

S = significantly
NS = not significantly
} different at the 5% level of probability (t_{111} = 1·9805).
(These were calculated on values correct to 3 significant figures.)

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REFERENCES

COCKRILL, S., SOMERVILLE, A. R. & WHITTLE, B. A. (1968). *Arch. Pharmak. exp. Path.*, **259**, 159.
FINNEY, D. J. (1964). *Statistical Methods in Biological Assay*, 2nd edn, London: Charles Griffin.

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On the local anaesthetic effect of barbiturates

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Controversy exists about whether the free acid (non-ionized) or the anionic (ionized) form of barbiturates abolish the excitation of nerves. The experiments made showed that sodium pentobarbitone and sodium phenobarbitone are more effective at pH 6·8 than 8·8 in blocking the action potential in the desheathed frog nerve. The experimental procedure employed excludes the possibility that this difference in activity is due only to a more effective rate of penetration of the non-ionized form across the nerve membrane. In the same preparation these barbiturates at both pH 6·8 and 8·8 did not interfere with the uptake and release of radiocalcium. These data suggest that barbiturates block the action potential by increasing the surface pressure of the lipid layer of the excitable membrane and do not interfere with the calcium binding to sites which govern the increased membrane conductance during excitation.

Barbiturates are known to block excitation in nerves (Heinbecker & Bartley, 1940). On the basis of extrapolated data from studies with *Arbacia* eggs (Clowes, Keltch & Krahl, 1940) and cardiac muscle (Hardman, Moore & Lum, 1959), it has been assumed that in contrast to tertiary amine local anaesthetics the unionized barbiturate is the most active form (Maynert, 1965; Sharpless 1968). Recently, however, Blaustein (1968) reported that sodium pentobarbitone blocks the action potential in the voltage-clamped lobster axon more effectively at pH 8·5 than at pH 6·7. From these results he concluded that the anionic form of these drugs appears to be more potent, and proposed that the ionized form increases the calcium binding to the excitable membrane.

In view of these contradictory results, we decided to re-evaluate the influence of pH on the local anaesthetic activity of phenobarbitone and pentobarbitone and to investigate the effect these barbiturates have on calcium binding to the nerve.

EXPERIMENTAL

Methods

Desheathed sciatic nerves of *Rana pipiens* were used. The effects of sodium pentobarbitone and sodium phenobarbitone on the action potential were examined using the sucrose gap method (Stämpfli, 1954). The nerves were mounted on a bipolar electrode and single supramaximal stimuli were applied every 2·5 min. However, when the bathing medium was changed, the nerves were stimulated every 5 s for 2 min. The evoked action potentials were recorded with an oscilloscope camera. The following schedule was observed in these experiments. The nerves were allowed to equilibrate in Ringer solution at pH 7·2 for 60 min. They were then exposed to 5 mM of either barbiturate in Ringer solution, first at pH 6·8 for 10 min, then at pH 8·8 for 10 min and finally again at pH 6·8 for 10 min. In control experiments the same schedule was followed, but no drug was present in the Ringer solution.

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The experiments on the effects of sodium pentobarbitone and sodium phenobarbitone on the uptake and kinetics of ^{45}Ca release were made on paired desheathed sciatic nerves of the same frog. One nerve was treated with either barbiturate and the other served as a control. The two procedures followed in these experiments are illustrated in Table 1. The technique for the collection of samples during the 1 h washout period was similar to that described by Bianchi & Bolton (1966). The samples were taken at 5- or 10-min intervals. The amount of ^{45}Ca remaining in the nerve after the washout period was determined in the nerve ash. The radioactivity of the collections and the remaining radioactivity of the ashed preparations were determined after adding Bray solution. The samples were counted in a Packard-Tri-Carb liquid scintillation counter.

Table 1. *Experimental procedures.* In both procedures the experimental nerves were loaded with a barbiturate at pH 6·8. In procedure I the nerve was then equilibrated with ^{45}Ca Ringer solution at pH 6·8 so no further ionization could occur. In procedure II, however, the pH of the ^{45}Ca Ringer solution was 8·8 in order to increase the degree of ionization of the barbiturates bound to the nerve membranes

		Equilibration periods (min)			Washout period
Procedure	Barbiturate	0–60	60–70	70–75	75–135
I	Pentobarbitone	Ringer pH 7·2	Ringer pH 6·8 + Barbiturate (5 mM)	^{45}Ca Ringer pH 6·8	Ringer pH 6·8
	Phenobarbitone				
	Control	Ringer pH 7·2	Ringer pH 6·8	^{45}Ca Ringer pH 6·8	Ringer pH 6·8
II	Pentobarbitone	Ringer pH 7·2	Ringer pH 6·8 + Barbiturate (5 mM)	^{45}Ca Ringer pH 8·8	Ringer pH 8·8
	Phenobarbitone				
	Control	Ringer pH 7·2	Ringer pH 6·8	^{45}Ca Ringer pH 8·8	Ringer pH 8·8

The total amount of ^{45}Ca taken up by the nerve (total ^{45}Ca content), was calculated according to Bianchi (1965), and expressed in terms of μmol ^{45}Ca/g of nerve dry weight.

The Ringer solutions consisted of 111 mM NaCl, 1·6 mM KCl, 1·0 mM $CaCl_2$ and 10 mM tris(hydroxymethyl)aminomethane at pH 6·8, 7·2 and 8·8.

The means and standard errors of the results were calculated and the level of significance determined using Student's *t*-test. The experiments were made at 20° to 22° during the winter of 1968–69.

RESULTS

Effects of pentobarbitone and phenobarbitone on the action potential

Both pentobarbitone and phenobarbitone (5 mM) markedly decreased the amplitude of the action potential (Fig. 1). After 10 min exposure to the barbiturate at pH 6·8, the size of the action potential was reduced to 41·9 $\pm$ 0·91% by pentobarbitone and to 22 $\pm$ 5·1% by phenobarbitone of their original values. On replacing this barbiturate-Ringer solution at pH 6·8 by a Ringer solution containing the same concentration of the barbiturate at pH 8·8, an immediate increase in the amplitude of the action potential was observed. In the nerves treated with phenobarbitone this

recovery occurred at a faster rate and was more pronounced (77·1 ± 6·6% after 10 min) than in those treated with pentobarbitone (67·2 ± 8·9%), although phenobarbitone had originally produced a greater degree of blockade.

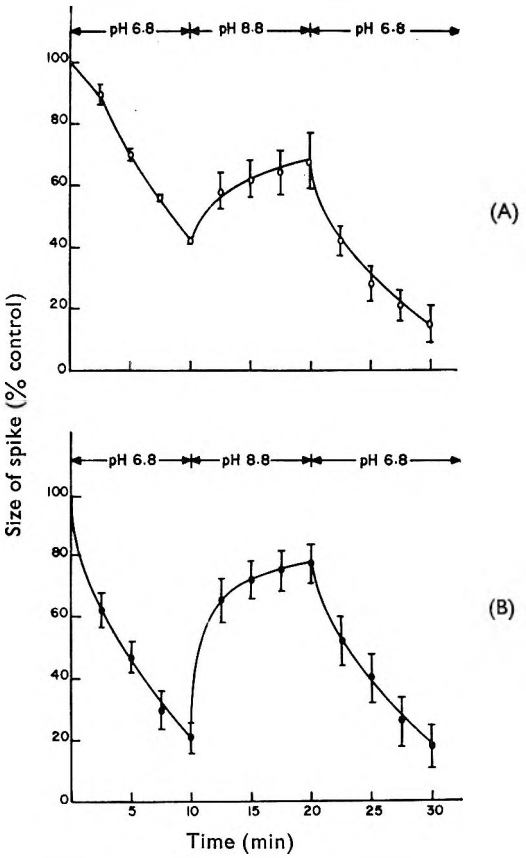

FIG. 1. Depression of the action potential of desheathed sciatic frog nerves by sodium pentobarbitone (A) and sodium phenobarbitone (B) at different pH. The preparations were successively exposed to a Ringer solution containing 5 mM of either barbiturate first at pH 6·8, then at pH 8·8 and then again at pH 6·8. The amplitude of the action potential is expressed as a percentage of its original value in barbiturate-free Ringer solution at pH 7·2 (pentobarbitone n = 8; phenobarbitone n = 6).

In no experiments, however, did the action potential return to its original size during the 10 min treatment with the barbiturate-Ringer solution at pH 8·8. When the barbiturate-Ringer solution at pH 6·8 was once more added, the size of the action potential decreased again markedly. After 10 min, the amplitudes were reduced to 14·7 ± 6·1% (pentobarbitone) and 18·0 ± 6·9% (phenobarbitone) of their original values.

Control experiments showed that the amplitude of the action potential was unaffected when the pH of the external bathing medium was varied between 6·8 and 8·8.

Effects of pentobarbitone and phenobarbitone on the uptake and kinetics of ^{45}Ca

The effects of the two barbiturates studied on the uptake of ^{45}Ca are described in Table 2. It can be seen that the total ^{45}Ca content was not significantly modified by any treatment with either pentobarbitone or phenobarbitone. Even in the nerves

Table 2. *Effects of pentobarbitone and phenobarbitone on the uptake of ^{45}Ca in paired nerves**

Experimental procedure	Total ^{45}Ca content (μmol/g)	^{45}Ca uptake in the slow component (μmol/g)	^{45}Ca uptake in the fast component (μmol/g)
Procedure I			
Pentobarbitone	0·77 ± 0·04	0·05 ± 0·004	0·72 ± 0·03
Control	0·74 ± 0·06	0·07 ± 0·005	0·67 ± 0·05
Procedure II			
Pentobarbitone	0·55 ± 0·02	0·06 ± 0·002	0·49 ± 0·02
Control	0·64 ± 0·05	0·06 ± 0·005	0·58 ± 0·04
Procedure I			
Phenobarbitone	0·67 ± 0·05	0·04 ± 0·003	0·63 ± 0·05
Control	0·65 ± 0·05	0·04 ± 0·004	0·61 ± 0·04
Procedure II			
Phenobarbitone	0·75 ± 0·05	0·05 ± 0·004	0·70 ± 0·05
Control	0·84 ± 0·04	0·07 ± 0·004	0·77 ± 0·04

* = mean and standard error (n = 5); in all experiments $P > 0·05$.

in which the uptake of radiocalcium occurred in the presence of the ionized form of the barbiturates (procedure II), in no experiment did the treatment lead to an increase in the uptake of ^{45}Ca.

The average desaturation curves obtained from nerves under control conditions and after treatment with pentobarbitone (procedure I) are shown in Fig. 2. The treatment of the nerves with pentobarbitone according to procedure II yielded almost identical desaturation curves. Analogous results were obtained with phenobarbitone (procedures I and II).

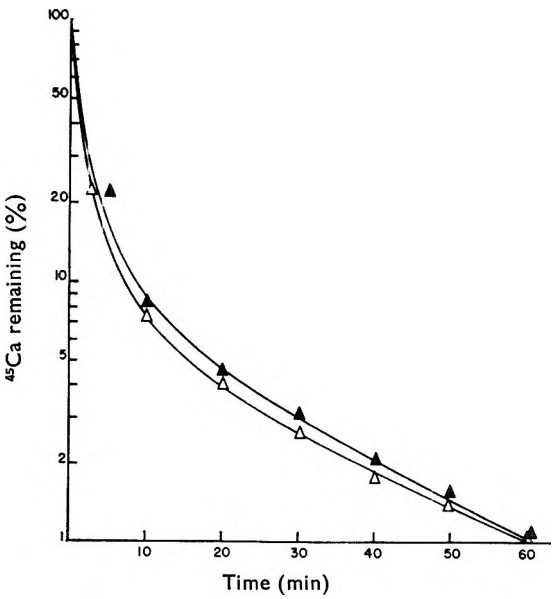

FIG. 2. ^{45}Ca desaturation curves for paired desheathed sciatic frog nerves (treatment according to procedure I). The experimental nerves were exposed first to 5 mM sodium pentobarbitone for 10 min and then soaked in [^{45}Ca] Ringer solution for 5 min. In the mate control nerves the same time schedule was observed; however, the nerves were exposed first to barbiturate-free Ringer solution and then to the [^{45}Ca]Ringer solution. All preparations were washed out in Ringer solution for 60 min. In this kind of procedure the pH of the solution was kept constant at 6·8. (Number of paired preparations = 5.) Open symbols control, closed symbols treated preparations.

All desaturation curves revealed at least two distinctive rates of ^{45}Ca release. After 30 min the curves showed a low rate of decline that appeared linear on a semi-logarithmic plot. The regression lines of this slow phase were calculated for each nerve and its intercept with the y-axis extrapolated. These intercepts correspond to the percentage of ^{45}Ca, taken up in a slowly exchanging compartment of the nerve, presumably of intracellular origin (Bianchi, 1968). On the other hand, the difference between the total ^{45}Ca content and ^{45}Ca uptake in this compartment of slow exchange is the amount of ^{45}Ca taken up in a compartment of the nerve characterized by a fast exchange. It can be assumed that this compartment represents the interstitial fluid, the interstitial connective tissue as well as the surface of the nerve membrane and the myelin sheath.

The treatment of the nerves with pentobarbitone or phenobarbitone had no effect on the shape of the desaturation curves. As shown in Table 2 the barbiturates studied did not interfere with the ^{45}Ca uptake in the two compartments of the nerves as revealed by the desaturation curves.

DISCUSSION

Our results indicate that the local anaesthetic activity of barbiturates is affected by their degree of ionization. In the same concentration the non-ionized forms of both pentobarbitone and phenobarbitone are more potent in blocking the action potential of desheathed nerves than the ionized form. The experimental procedure used in this investigation excludes the possibility that this difference in potency is only a consequence of the drug distribution across the excitable nerve membrane. Since barbiturates are acid, indeed, they penetrate biological membranes more rapidly at a low than at a high pH, it could, therefore, be argued that the higher blockade observed at pH 6·8 than at pH 8·8 is only the result of a higher drug concentration at the interphases of the excitable membrane. However, the sudden occurrence of a decrease in blockade, when the bathing solution is shifted from pH 6·8 to 8·8, demonstrates that the non-ionized form is more potent in affecting the action potential. It has been shown that such a shift in pH affects first the degree of ionization of tertiary amine local anaesthetics at the interphases of the excitable membrane; only thereafter a redistribution of these compounds occurs across the membrane (Bianchi & Strobel,1969). As a matter of fact the time course of the recovery of the action potential, when shifting the pH from 6·8 to 8·8 of the barbiturate-Ringer solution is of two phases. It is, therefore, tempting to assume that the first, fast phase of this recovery curve reflects the change in the degree of ionization, whereas the second, slow phase is the consequence of the redistribution of the barbiturates studied. Thus the initial rapid phase of recovery, when the pH is shifted from 6·8 to 8·8 would be a consequence of the ionization of the barbiturate trapped in the outer surface of the excitable membrane; the second slower phase of recovery would be attributed to the loss of barbiturate from the nerve fibres as the weak acid would tend to accumulate in the alkaline interstitial space.

On the other hand, if the ionized form of the barbiturates would be more potent, a transient increase of the blockage should have occurred immediately upon changing the pH from 6·8 to 8·8 (Bianchi & Strobel, 1968). However, the reverse, a diminution of the blockade, was observed in every experiment. This strongly supports the common concept that in contrast to tertiary amine local anaesthetics, barbiturates are more active in the same form that also diffuses more easy through biological

membranes (Sharpless, 1968). However our experimental data do not permit the exclusion of the possibility that the ionized form of these compounds may also diminish, but to a lesser extent, the excitability of the nerve membrane.

It has recently been shown that the ionized tertiary amine local anaesthetics interfere with the uptake of calcium in desheathed nerves (Suarez-Kurtz, Bianchi & Krupp, 1969). In contrast, the present results demonstrate that in neither form, the non-ionized or the ionized, do barbiturates modify the calcium binding and release in this preparation. These data, therefore, seem to indicate that the local anaesthetic effect of barbiturates is not related to the calcium binding to sites which govern the increased membrane conductance during nerve excitation.

The fact that barbiturates do not appear to interfere with the calcium binding in the nerve and more effectively block the action potential in the free acid form, suggests that these compounds affect the excitable membrane by increasing its surface pressure (Shanes, 1958). The lipid-soluble moiety of barbiturates may, indeed, decrease the conductance to sodium and potassium by dissolving in the lipid bilayer of the excitable membrane (Butler, 1950; Blaustein & Goldman, 1966). That the ionized form of these agents is less potent may reflect a repulsion of the anionic drug molecules by the negative charges in the membrane.

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REFERENCES

BIANCHI, C. P. (1965). *J. Pharmac. exp. Ther.*, **147**, 360–370.

BIANCHI, C. P. (1968). In: *Cell Calcium*, p. 23, Editor: Bittar, E. E., New York: Appleton-Century-Crofts.

BIANCHI, C. P. & BOLTON, T. (1966). *J. Pharmac. exp. Ther.*, **151**, 456–463.

BIANCHI, C. P. & STROBEL, G. E. (1968). *Trans. N.Y. Acad. Sci. Ser.* II, Vol. 30, 1082–1092.

BIANCHI, C. P. & STROBEL, G. E. (1969). In the press.

BLAUSTEIN, M. P. (1968). *J. gen. Physiol.*, **51**, 293–307.

BLAUSTEIN, M. P. & GOLDMAN, D. E. (1966). *Science, N.Y.*, **153**, 429–432.

BUTLER, T. C. (1950). *Pharmac. Rev.*, **2**, 121–160.

CLOWES, G. H. A., KELTCH, A. K. & KRAHL, M. E. (1940). *J. Pharmac. exp. Ther.*, **68**, 312–329.

HARDMAN, M. F., MOORE, J. I. & LUM, B. K. B. (1959). *Ibid.*, **126**, 136–142.

HEINBECKER, P. & BARTLEY, S. H. (1940). *J. Neurophysiol.*, **3**, 219–236.

MAYNERT, E. W. (1965). In: *Drill's Pharmacology in Medicine*, p. 121, Editor: DiPalma, J. P., New York: McGraw Hill.

SHANES, A. M. (1963). *Ann. Rev. Pharm.*, **3**, 185–204.

SHARPLESS, S. K. (1968). In: *The Pharmacological Basis of Therapeutics*, 3rd edn, p. 109, Editors: Goodman, L. S. & Gilman, A., New York: Macmillan.

STÄMPFLI, R. (1954). *Experientia*, **10**, 508–509.

SUAREZ-KURTZ, G., BIANCHI, C. P. & KRUPP, P. (1969). In the press.

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On the mechanism of 5-hydroxytryptamine release by thymoleptics

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Rabbits were treated with nialamide followed by chlorimipramine, imipramine, desipramine or amitriptyline. Cortical brain slices were prepared and incubated with Krebs-Henseleit solution. Release of 5-hydroxytryptamine (5-HT) into the incubation medium was measured. Chlorimipramine, imipramine and amitriptyline caused release of 5-HT, whereas desipramine was without effect. The ability of cortical brain slices to retain 5-HT was dependent on energy supply, involving both aerobic and anaerobic metabolism. The data support the view that the antidepressant drugs block the membrane pump of 5-HT neurons. The observations also indicate that 5-HT is involved in thermoregulation.

Previously we have shown that the amine uptake by the membrane group of 5-hydroxytryptamine (5-HT) neurons could be blocked by tertiary amines belonging to the group of tricyclic antidepressants, for example, imipramine, chlorimipramine and amitriptyline. Chlorimipramine proved particularly potent in this respect. Secondary amines, however, such as desipramine and protriptyline, were more efficient in blocking the membrane pump of noradrenaline than of 5-HT neurons (Carlsson, Corrodi & others, 1969a,b).

In a recent paper (Carlsson, Fuxe & others, 1969) we have reported that blockade of the membrane pump of 5-HT neurons leads to potentiation of the response to monoamine oxidase (MAO) inhibitors. If chlorimipramine was given, for example, to rabbits pretreated with nialamide, shivering-like tremors with marked increase in rectal temperature occurred. When cortical slices obtained from animals treated in this manner were incubated in a Krebs-Henseleit solution a highly significant release of brain 5-HT occurred, as compared to control animals treated with nialamide only.

In the present report we extend the earlier observations with chlorimipramine to include also imipramine, desipramine and amitriptyline. Furthermore, we have investigated the problem of whether the ability of the brain tissue to retain 5-HT is dependent on energy supply.

EXPERIMENTAL

White female rabbits (1·2–2·5 kg) were treated with nialamide (100 mg/kg, i.p.) about 16 h before the administration of chlorimipramine or other antidepressant drugs. The rectal temperatures were measured before the antidepressant drug was given and immediately before death. The animals were killed by air embolism 30 min after administration of the antidepressant drug. The chest cavity was then opened and the rabbits were exsanguinated before removing the brains. Cortical slices were prepared according to McIlwain & Rodnight (1962). The slices (about 1 g) were incubated at 37° in 10 ml of Krebs-Henseleit solution* equilibrated with 5%

* Each litre of Krebs-Henseleit solution contained: NaCl, 6·89 g; KCl, 0·35 g; $CaCl_2$, 0·28 g; $MgSO_4$, 0·14 g; EDTA.$2H_2O$, 0·015 g; KH_2PO_4, 0·16 g; $NaHCO_3$, 2·0 g; glucose, 2·0 g; ascorbic acid, 0·02 g.

carbon dioxide in oxygen. In some experiments 5% carbon dioxide in nitrogen was used instead, or the glucose in the Krebs-Henseleit solution was omitted, or both.

After 40 min incubation, tissue and incubation fluid were separated by centrifugation, and both fractions were analysed for 5-HT by the method of Andén & Magnusson (1967). The release was calculated as the ratio of the 5-HT in the incubation medium to that in the incubation medium plus the brain tissue.

RESULTS

Table 1 shows the effects of imipramine, desipramine, chlorimipramine and amitriptyline when given in different doses to rabbits pretreated with nialamide (100 mg/kg, i.p. 16 h beforehand) on the 5-HT content of slices and incubation fluid, and on rectal temperature and on gross behaviour. Chlorimipramine proved to be the most potent of the drugs tested in releasing 5-HT from cortical brain slices during incubation in Krebs-Henseleit solution. The release of 5-HT after 6·25, 12·5 and 25 mg/kg, i.p. was 24, 32 and 36%, respectively, compared to 9% in controls treated with nialamide only. Amitriptyline was less active than chlorimipramine, a release of 19% being observed after both 12·5 and 25 mg/kg. Imipramine, 12·5 mg/kg,

Table 1. *5-HT content of cortical brain slices and incubation medium after incubation for 40 min in Krebs-Henseleit solution.* Rabbits were treated with nialamide alone, 100 mg/kg, i.p., 16 h beforehand (controls) or in combination with various doses of antidepressant drugs. The animals were killed 30 min after the injection of the antidepressant drug. Values (μg/g tissue) are given as means ± s.e. Figures within brackets indicate number of experiments. Shown also are the average rectal temperatures immediately before the injection of the antidepressive drug and before killing the animals.

Treatment	Dose mg/kg i.p.	Incubation medium	Brain tissue	Release % total	Initial temperature	Final temperature
Control	—	0·06 (31) ±0·007	0·59 (30) ±0·027	8·9 (30) ±0·78	38·8 (24)	38·9 (18)
Imipramine HCl	12·5	0·14 (3) ±0·024	0·54 (3) ±0·035	21·2 (3) ±3·82	38·6 (3)	39·2 (3)
	25	0·05 (3) ±0·004	0·63 (3) ±0·133	7·5 (3) ±1·51	39·3 (3)	39·5 (3)
	50	0·04 (1)	0·74 (1)	5·1 (1)	39·1 (1)	39·3 (1)
Desipramine HCl	6·25	0·06 (1)	0·73 (1)	7·6 (1)	38·5 (1)	39·3 (1)
	12·5	0·05 (1)	0·60 (1)	7·7 (1)	38·2 (1)	38·1 (1)
	25	0·06 (2) ±0·015	0·71 (2) ±0·075	7·5 (2) ±2·55	39·4 (2)	39·5 (2)
	50	0·10 (2) ±0·025	0·67 (2) ±0·130	12·3 (2) ±0·75	39·0 (2)	39·5 (2)
Chlorimipramine HCl	6·25	0·18 (3) ±0·075	0·53 (3) ±0·081	23·6 (3) ±7·41	39·2 (3)	40·2 (3)
	12·5	0·26 (19) ±0·030	0·55 (20) ±0·033	31·5 (19) ±2·95	39·0 (17)	40·7 (17)
	25	0·31 (3) ±0·047	0·60 (3) ±0·168	35·5 (3) ±3·42	39·1 (3)	>41·7 (3)
Amitriptyline HCl	12·5	0·17 (3) ±0·024	0·71 (3) ±0·104	18·9 (3) ±0·54	39·3 (3)	39·5 (3)
	25	0·15 (3) ±0·050	0·62 (3) ±0·096	18·6 (3) ±3·35	38·8 (3)	38·9 (3)

appeared to be as efficient as the corresponding dose of amitriptyline but at higher dose levels (25 or 50 mg/kg) no effect was found. Desipramine was ineffective in all doses investigated.

In the experiments with chlorimipramine a correlation between the effect on gross behaviour (shivering-like tremors), rise in rectal temperature and 5-HT release appeared to exist. In the individual rabbits the response varied considerably.

Amitriptyline caused less marked tremor than chlorimipramine and almost no effect on temperature. The rabbits were heavily sedated, lying on their sides.

After imipramine and desipramine, following nialamide pretreatment, the rabbits were restless and tense, but no tremor and no rise in temperature were observed.

Combination of chlorimipramine with imipramine or desipramine was also tried. The doses of imipramine and desipramine were 25 mg/kg. When imipramine was given together with chlorimipramine, 25 mg/kg, the effects on behaviour, temperature and 5-HT release were about the same as after chlorimipramine alone. Combination of chlorimipramine, 12·5 mg/kg, with desipramine caused only a slight rise in temperature, and with imipramine the release of 5-HT seemed to be lower than that after chlorimipramine alone (Table 2).

Table 2. *Effect of combined treatment with chlorimipramine and imipramine or desipramine on 5-HT content of cortical brain slices and incubation medium after incubation for 40 min in Krebs-Hensleit solution.* Rabbits were treated with nialamide, 100 mg/kg 16 h beforehand, followed by chlorimipramine alone or in combination with imipramine or desipramine. The chlorimipramine plus imipramine or desipramine were injected simultaneously and the animals were killed after 30 min. For further explanations see Table 1.

	Incubation medium	Brain tissue	Release % total	Initial temperature	Final temperature
Chlorimipramine 12·5 mg/kg	0·26 (19) ±0·030	0·55 (20) ±0·033	31·5 (19) ±2·95	39·0 (17)	40·7 (17)
Chlorimipramine 25 mg/kg	0·31 (3) ±0·047	0·60 (3) ±0·168	35·5 (3) ±3·42	39·1 (3)	>41·7 (3)
Imipramine 25 mg/kg + chlorimipramine 12·5 mg/kg	0·13 (3) ±0·063	0·62 (3) ±0·080	17·2 (3) ±8·99	39·2 (3)	39·7 (3)
Imipramine 25 mg/kg + chlorimipramine 25 mg/kg	0·32 (3) ±0·075	0·46 (3) ±0·092	40·1 (3) ±1·33	39·5 (3)	40·9 (3)
Desipramine 25 mg/kg — chlorimipramine 12·5 mg/kg	0·17 (3) ±0·067	0·51 (3) ±0·049	22·9 (3) ±6·58	38·8 (3)	39·1 (3)

The ability of the brain tissue to retain 5-HT seemed to be dependent on energy supply. In experiments with rabbits treated with nialamide alone, cortical slices were incubated in glucose-free Krebs-Henseleit solution under an atmosphere of N_2 and CO_2. The release of 5-HT increased to about 70% compared to about 9% if the incubation was performed with glucose under O_2 and CO_2 (Table 3). If either glucose was omitted or oxygen replaced by nitrogen, intermediate release values were obtained.

Chlorimipramine given to rabbits pretreated with nialamide caused a release of about 35% in the presence of glucose and oxygen. If both glucose and oxygen were omitted (i.e. oxygen replaced by nitrogen) the release of 5-HT was about the same as after nialamide alone under these conditions. If either of the two was omitted intermediate values were obtained.

Table 3. *Effect of glucose deprivation and/or anoxia on the 5-HT content of cortical brain slices and incubation medium after incubation for 40 min in Krebs-Henseleit solution.* Rabbits were treated with nialamide alone or in combination with chlorimipramine. The animals were killed 30 min after injection of chlorimipramine. Single values are given in μg/g tissue. Shown also are rectal temperatures immediately before the injection of chlorimipramine and before killing the animals. The degrees of behavioural effect of chlorimipramine is indicated by an arbitrary scale ranging from O (no effect) to +++++ (very strong effect).

	0·2% glucose + oxygen			No glucose + oxygen			0·2% glucose + nitrogen			No glucose + nitrogen					
	Incubation medium	Brain tissue	Release % total	Incubation medium	Brain tissue	Release % total	Incubation medium	Brain tissue	Release % total	Incubation medium	Brain tissue	Release % total	Initial temperature	Final temperature	Effect on behaviour
Nialamide 100 mg/kg i.p. + Chlorimipramine 12·5 mg/kg i.p.	0·40	0·58	40·8	0·41	0·33	55·4	—	—	—	—	—	—	39·0	40·5	+++
	0·09	0·91	9·0	0·21	0·37	36·2	—	—	—	—	—	—	38·8	38·8	O
	0·23	0·32	41·8	0·33	0·35	48·5	—	—	—	—	—	—	38·6	41·0	+++
	0·37	0·54	40·7	—	—	—	0·33	0·39	45·8	—	—	—	38·8	42·0	++++
	0·41	0·57	41·8	—	—	—	0·40	0·37	51·9	—	—	—	38·9	40·5	++++
	0·25	0·42	37·3	—	—	—	0·23	0·39	37·1	—	—	—	38·7	40·6	+
	0·06	0·55	9·8	—	—	—	—	—	—	0·44	0·15	74·6	39·2	39·3	+
	0·47	0·45	51·1	—	—	—	—	—	—	0·72	0·29	71·3	38·8	41·3	++++
	—	0·45	—	—	—	—	—	—	—	0·32	0·10	76·2	39·7	38·5	+
Mean	0·29	0·53	34·0	0·32	0·35	46·7	0·32	0·38	44·9	0·49	0·18	74·0	38·9	40·3	
± s.e.	±0·054	±0·055	±5·55	±0·058	±0·012	±5·62	±0·049	±0·007	±4·29	±0·119	±0·056	±1·44			
Nialamide 100 mg/kg i.p.	0·02	0·64	3·0	0·18	0·50	26·5	—	—	—	—	—	—	39·0	38·8	
	0·06	0·51	10·5	0·15	0·29	34·1	—	—	—	—	—	—	39·0	39·0	
	0·03	0·68	4·2	0·04	0·57	6·6	—	—	—	—	—	—	38·7	38·7	
	0·05	0·59	7·8	0·11	0·47	19·0	—	—	—	—	—	—	38·4	—	
	0·06	0·62	8·8	—	—	—	0·19	0·53	26·4	—	—	—	38·8	38·8	
	0·18	0·81	18·2	—	—	—	0·45	0·57	44·1	—	—	—	39·3	39·6	
	0·05	0·54	8·5	—	—	—	0·17	0·37	31·5	—	—	—	—	—	
	0·05	0·61	7·6	—	—	—	—	—	—	0·58	0·18	76·3	38·8	38·8	
	0·08	—	—	—	—	—	—	—	—	—	0·20	—	38·5	38·5	
	0·06	0·52	10·3	—	—	—	—	—	—	0·54	0·22	71·1	39·2	39·2	
	0·06	0·64	8·6	—	—	—	—	—	—	0·44	0·21	67·7	39·0	39·0	
	0·08	0·71	10·1	—	—	—	—	—	—	0·56	0·26	68·3	38·6	—	
Mean	0·07	0·62	8·9	0·12	0·46	21·6	0·27	0·49	34·0	0·53	0·21	70·9	38·8	38·9	
± s.e.	±0·012	±0·027	±1·18	±0·030	±0·060	±5·86	±0·090	±0·061	±5·26	±0·031	±0·013	±1·96	—	—	

DISCUSSION

Imipramine and amitriptyline treatment of nialamide pretreated rabbits caused a moderate increase in the release of 5-HT from cortical brain slices *in vitro*. In this respect these drugs were less potent than chlorimipramine, whereas desipramine had no effect. This order of activity corresponds to our earlier observations on 5-hydroxytryptophan potentiation and the ability to block the membrane pumps of 5-HT neurons, using 5-HT depletion by 4-methyl-α-ethyl-*m*-tyramine (H75/12) as indicator. It therefore seems reasonable to assume that the 5-HT release induced by antidepressant drugs, as observed in the present investigation, is due to blockade of the membrane pump of 5-HT neurons, unmasking a rather pronounced leakage of 5-HT following inhibition of monoamine oxidase. In support of this assumption it was observed that the ability of cortical tissue to retain 5-HT was dependent on energy supply. Moreover, deprivation of energy supply eliminated the 5-HT releasing action of chlorimipramine.

To obtain a maximum 5-HT release it was necessary to deprive the tissue of both oxygen and glucose. The data indicate that the membrane pump of 5-HT neurons can derive its energy supply both from aerobic metabolism and anaerobic glycolysis.

The correlation between 5-HT release and rise in body temperature observed in the present investigation supports the hypothesis of Feldberg & Myers (Feldberg & Myers, 1963; Feldberg, 1968; cf. Corrodi, Fuxe & Hökfelt, 1967) that 5-HT is involved in thermoregulation.

Our results suggest a certain degree of antagonism between chlorimipramine and imipramine/desipramine as regards 5-HT release and rise in temperature. Further work is needed to establish the mechanism involved. It is tempting to speculate on an interaction between 5-HT and noradrenaline neurons. Such an interaction might also explain the apparently dual action of imipramine, indicated by an irregular dose-response relationship.

Acknowledgements

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REFERENCES

Andén, N.-E. & Magnusson, T. (1967). *Acta physiol. scand.*, **69**, 87–94.

Carlsson, A., Corrodi, H., Fuxe, K. & Hökfelt, T. (1969a). *Europ. J. Pharmac.*, **5**, 357–366.

Carlsson, A., Corrodi, H., Fuxe, K. & Hökfelt, T. (1969b). *Ibid.*, **5**, 367–373.

Carlsson, A., Fuxe, K., Jonason, J. & Lindqvist, M. (1969). *Brain Res.*, **12**, 456–460.

Corrodi, H., Fuxe, K. & Hökfelt, T. (1967). *Advances in Pharmacology*, Vol. 6B, pp. 49–54, New York: Academic Press Inc.

Feldberg, W. & Myers, R. D. (1963). *Nature, Lond.*, **200**, 1325.

Feldberg, W. (1968). *Recent Advances in Pharmacology*, pp. 349–397. London: Churchill.

McIlwain, H. & Rodnight, R. (1962). *Practical Neurochemistry*, pp. 109–133. London: Churchill.

J. Pharm Pharmac., 1969, **21**, 774

LETTERS TO THE EDITOR

Some physico-chemical properties of amphetamine and related drugs

Pharmacokinetic studies of the amphetamine-like drugs have predominantly been made by using urinary excretion data (Beckett & others, 1968, 1969). The urinary excretion of amphetamine and related drugs depends largely on the lipid solubility of the undissociated form which amount is in turn governed by the degree of ionization of the drug at the urinary pH. The latter is measured by the pK_a value. From a search of the literature it appeared that only for some amphetamines have pK_a values been determined, whereas information on lipid solubility was even more scarce. The pK_a values of amphetamine, *N*-alkyl derivatives and related CNS stimulants were measured according to Leffler, Spencer & Burger (1954) using 0·1 mmol of the HCl salt of the drug.

The apparent partition coefficient (APC) in chloroform–water and heptane–water were measured in Teorell buffer of various pH values such that the concentration of drug in the organic and water layer was roughly the same. The concentrations of drug were measured by gas chromatography. The apparent partition coefficient at pH 7·4 and the true partition coefficient (TPC) being the partition coefficient of the

Table 1. *Partition coefficients (apparent, APC, true TPC) and* pK_a *values of a number of amphetamine-like drugs*

Drug		pK_a	% neutral at pH 7·4	APC at pH 7·4 $CHCl_3$–H_2O	TPC $CHCl_3$–H_2O	TPC Hept.–H_2O
Phenethylamine ..	b,c,e,f	9·88	0·33	0·075	20·8	0·277
Dexamphetamine ..	a,b,e	9·90	0·31	0·48	146	1·88
Methamphetamine ..	a,b	10·11	0·19	1·11	565	5·14
Ethylamphetamine ..	..	10·23	0·15	2·67	1790	38·6
Isopropylamphetamine ..	..	10·14	0·18	8·09	4460	117
Propylamphetamine ..	..	9·98	0·26	21·2	8080	312
Benzylamphetamine ..	..	7·50	44·1	1000	2250	110
Dimethylamphetamine ..	b	9·80	0·39	11·5	2890	108
Methylethylamphetamine ..		9·80	0·39	19·0	4760	166
Methylisopropylamphetamine ..		9·45	0·88	100	11300	200
Benzphetamine	..	6·55	87·2	1000	1400	74·8
Phentermine	..	10·11	0·19	1·00	514	63·2
Mephentermine	..	10·25	0·13	1·22	866	110
Chlorphentermine ..	..	9·60	0·62	4·00	797	17·5
Norephedrine	a	9·55	0·70	0·001	0·035	0·001
Ephedrine ..	a,b,d,e	9·60	0·62	0·015	2·42	0·001
Norpseudoephedrine ..	..	9·40	1·00	0·001	0·10	0·010
Pseudoephedrine	..	9·86	0·33	0·070	20·0	0·029
Methylephedrine	a,b	9·30	1·25	1·00	80·6	0·912
Phenmetrazine	..	8·45	8·20	15·60	191	2·15
Phendimetrazine	..	7·55	44·0	1000	2420	8·92
Propylhexedrine	..	10·74	0·043	1·11	2360	173
Fenfluramine	..	9·10	1·96	32·30	1640	678
4-Cl-Amphetamine ..	..	9·80	0·39	0·818	206	9·10
Fencamfamine	..	8·70	4·76	200	4200	110

For compounds for which pK_a values have been described the literature reference is indicated. a: Kisbye (1958); b: Leffler & others (1951); c: Tuckerman & others (1959); d: Brodie & Hogben (1957); e: Lewis (1954); f: Kappe & others (1965).

Since the error in the estimation of the pK_a and the APC is about 1%, the values are given to 3 decimal places.

neutral base were then computed. The following equation was used:

$$TPC = APC\ [1 + 10^{pK_a - pH}]$$

For a number of amphetamine-like drugs the pK_a values and partition coefficients, and also the fraction of neutral base at pH 7·4 are given in Table 1.

Ephedrine and related compounds are relatively strong bases but they have also an extremely low lipid solubility. It is therefore conceivable that these drugs are eliminated from the body mainly by urinary excretion. Dexamphetamine and a number of related drugs are relatively strong bases which implies that at the physiological pH they are more than 99% ionized. The *N*-alkyl substituted amphetamines are in general more lipid soluble than the parent compound (see Table 1).

The literature provides evidence that there is both glomerular filtration and tubular secretion but that tubular reabsorbtion of dexamphetamine under the conditions of acid urine hardly occurs (Rowland, 1969; Beckett, Salmon & Mitchard, 1969). With higher values of the urine pH the renal clearance is lower indicating that there is substantial reabsorbtion of dexamphetamine at pH 6–7.

The *N*-benzyl derivatives have less basic properties such that these drugs are to a large extent in the neutral form at the physiological pH. The renal clearance is rather low. Under slightly alkaline conditions such drugs therefore may not be detected in the urine. Illegal use of e.g. benzphetamine however can still be detected by urine analysis since these drugs are metabolized to amphetamine.

Other amphetamines such as phenmetrazine, fencamfamine and pipradrol are weak to moderately strong bases with relatively high lipid solubility. These drugs may not be excreted by the kidney under strong alkaline conditions. Indeed it has recently been shown that through the intake of sodium bicarbonate the renal excretion of fencamfamine can be suppressed completely (Vree & van Rossum, 1969). The findings have serious consequences from the standpoint of the control of doping. It is however not known what effect the level of alkalinity necessary to prevent excretion (pH 7–8 for some drugs) has on the performance of the subject taking the alkali. In general, athletes excrete acid urine so the presence of alkaline urine would certainly raise suspicion that suppression of excretion was being attempted.

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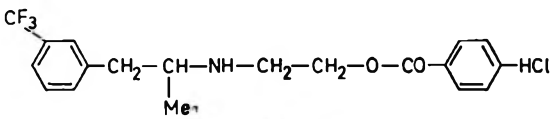
REFERENCES

BECKETT, A. H., BOYES, R. N. & TRIGGS, E. J. (1968). *J. Pharm. Pharmac.*, **20**, 92–97.
BECKETT, A. H., BOYES, R. N. & TUCKER, G. T. (1968). *Ibid.*, **20**, 269–276.
BECKETT, A. H., BOYES, R. N. & TUCKER, G. T. (1968). *Ibid.*, **20**, 277–282.
BECKETT, A. H., SALMON, J. A. & MITCHARD, M. (1969). *Ibid.*, **21**, 251–258.
BRODIE, B. B. & HOGBEN, A. M. (1957). *Ibid.*, **9**, 345.
KAPPE, T. & ARMSTRONG, M. D. (1965). *J. mednl Chem.*, **8**, 368–374.
KISBYE, J. (1958). *Pharm. Weekblad.*, **93**, 206–215.
LEFFLER, E. B., SPENCER, H. M. & BURGER, A. (1951). *J. Am. chem. Soc.*, **73**, 2611–2613.
LEWIS, G. P. (1954). *Br. J. Pharmac. Chemother.*, **9**, 488–493.
ROWLAND, M. (1969). *J. pharm. Sci.*, **58**, 508–509.
TUCKERMAN, M. M., MAYER, J. R. & NACHOD, F. C. (1959). *J. Am. chem. Soc.*, **81**, 92–94.
VREE, T. B. & ROSSUM, J. M. VAN (1969). *Europ. J. Pharmac.* In the press.

Anti-amphetamine activity of fenfluramine and S 992 in the isolated tail artery of the rat

Fenfluramine has been reported by several authors to reduce appetite in laboratory animals and in man in doses which do not influence the cardiovascular system (Franko, Houkomp & Ward, 1965; Le Douarec, Schmitt & Laubie, 1966; Colmore & Moore, 1966). S 992, a derivative of fenfluramine with the chemical structure I, elicits an anorexic activity of the same potency as that of fenfluramine, while the stimulant activity on the central nervous system is even less than that of fenfluramine (Le Douarec, personal communication). In a previous paper, fenfluramine was shown to decrease the toxicity of amphetamine in grouped mice while S 992 failed to show any protection in the same experimental conditions (Jespersen & Bonaccorsi, 1969). Because of the suggested interference between amphetamine and fenfluramine at the level of the receptor sites, we have studied the interaction in an isolated preparation, which is more suitable for the evaluation of this drug antagonism.



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Tail arteries from 300 g rats were isolated in an organ bath and perfused at constant flow (8 ml/mm) with Krebs-Hucovič solution saturated with 5% carbon dioxide in oxygen. The constrictor response of the artery was measured by recording the increase in perfusion pressure by means of a mercury manometer. Drugs were injected or infused through a rubber valve just before the artery.

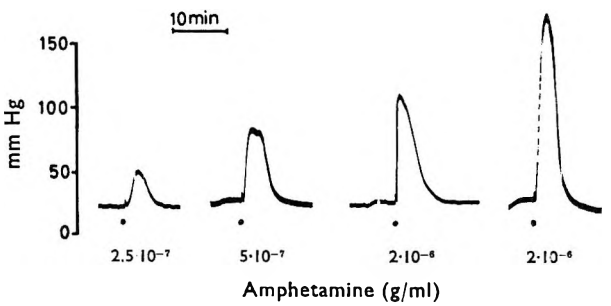

FIG. 1. The effect of tetrabenazine on the isolated tail artery of the rat after increasing amounts of amphetamine. At dots, 100 μg of tetrabenazine was injected. Amphetamine was perfused for 10 min before the tetrabenazine injection.

Reserpine and tetrabenazine elicit a long-lasting hypertensive effect when injected at a short interval after amphetamine (Bonaccorsi, 1968; Schmitt & Schmitt, 1969). The same kind of response can be reproduced in an isolated preparation. As can be seen from Fig. 1 the effect of tetrabenazine is dependent on the concentration of amphetamine. The onset of the constriction is slower compared with that of noradrenaline but it can reach high values. On the contrary tetrabenazine does not elicit any contraction when arteries are perfused with fenfluramine or S 992 at a concentration 10 or 100 times higher than that of amphetamine (Bizzi, Bonaccorsi &

others, 1968). To study the antagonism between amphetamine and fenfluramine, various concentrations of fenfluramine and S 992 were perfused for 10 min together with amphetamine. At the end of this period a constant dose of tetrabenazine was given which in control arteries, perfused with amphetamine, induced a nearly maximal contraction.

Table 1. *Inhibition by fenfluramine, S 992 or phentolamine (g/ml) of the constrictor response induced by amphetamine + tetrabenazine in the isolated tail artery of the rat*

	% inhibition after fenfluramine:				% inhibition after S 992:				% inhibition after phentolamine:	
	10^{-6}	$2{\cdot}5 \times 10^{-6}$	5×10^{-6}	$1{\cdot}25 \times 10^{-5}$	$2{\cdot}5 \times 10^{-8}$	5×10^{-8}	10^{-7}	$2{\cdot}5 \times 10^{-7}$	5×10^{-6}	10^{-5}
Amphetamine 10^{-6} g/ml + Tetrabenazine 100 μg Noradrenaline 0·05 μg ..	42	48	57	75	0	36	46	96	50	85
	0	0	15	40	0	10	20	60	35	50

Fenfluramine, S 992, or phentolamine were perfused for 15 min before adding amphetamine + tetrabenazine or noradrenaline.

As shown in Table 1 the antagonism is present for both substances tested but it is more evident for S 992, which, in fact, at a concentration of $2{\cdot}5 \times 10^{-7}$ g/ml abolished the contraction induced by amphetamine plus tetrabenazine, while fenfluramine was active only at the dose of 10^{-6} g/ml.

Since adrenolytics are potent inhibitors of the response induced by the combination amphetamine-tetrabenazine, we evaluated fenfluramine and S 992 for an adrenolytic effect. Fenfluramine hardly antagonized noradrenaline, while S 992 decreased the effect of noradrenaline at small concentrations. Its adrenolytic effect was promptly reversed by washing the preparation. Phentolamine, used as a control, was effective on the vasoconstrictor response induced by amphetamine, tetrabenazine or noradrenaline at a concentration at least 10 times lower than S 992.

The antagonism induced by S 992 is limited to the *in vitro* situation because *in vivo*, on the blood pressure, no adrenolytic activity can be shown.

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REFERENCES

BIZZI, A., BONACCORSI, A., JESPERSEN, S., JORI, A. & GARATTINI, S. (1969). In "*Amphetamine and related compounds*," Proc. International Symposium, Milan, 1969, in the press, New York: Raven Press.

BONACCORSI, A. (1968). *Europ. J. Pharmac.*, **3**, 97–105.

COLMORE, J. P. & MOORE, J. D. (1966). *J. new Drugs*, **6**, 123.

FRANKO, B. V., HOUKOMP, L. J. & WARD, J. W. (1965). *J. Pharm. Pharmac.*, **17**, 222–226.

JESPERSEN, S. & BONACCORSI, A. (1969). *Europ. J. Pharmac.* In the press.

LE DOUAREC, J. C., SCHMITT, H. & LAUBIE, M. (1966). *Archs int. Pharmacodyn. Thér.*, **161**, 206–232.

SCHMITT, H. & SCHMITT, H. (1969). In "*Amphetamine and related compounds*," Proc. International Symposium, Milan, 1969, in the press, New York: Raven Press.

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Concerning the histamine receptor (H_1)

A few years ago we proposed a model for the histamine receptor in the ileum of the guinea-pig (Rocha e Silva, 1960, 1966). This model was constructed on the basis of the following ideas then accepted: (*a*) the active form of histamine under physiological conditions was that in which hydrogen bonding was possible between the amine nitrogen (N^+) and the pyridine (N) nitrogen of the imidazole ring; (*b*) the secondary anchorage group of histamine to fit its receptor site, could be the imine (=NH) radical of the imidazole ring; (*c*) the pK of the receptor site was found to be around pH = 7·10–7·0, thus suggesting a histidine moiety at the receptor site. Having these facts in mind, a fit of the agonist to its receptor site might involve electrostatic attractions between the "pyridine" nitrogen of the imidazole of the agonist and the carbonyl oxygen (=O) of the peptide link in the receptor amino-acid chain. The function of histamine would be to protonate the "pyridine" nitrogen of the receptor histidine, shifting the double bond and releasing a high energy radical hypothetically bound to the "pyrrole" nitrogen of the histidine moiety, as shown in Fig. 1.

A reappraisal of this concept was made possible, after a recent publication by Kier (1968) in which the author reached the conclusion that the configuration of histamine acting upon H_1 receptors, would preclude any hydrogen bonding between the (N^+) amine nitrogen and the pyridine nitrogen of the imidazole ring. The distance between the two nitrogens is of the order of 4·55 Å, and therefore too large to allow for the occurrence of any hydrogen bonding, as shown in Fig. 2a.

On the basis of his calculations, Kier (1968) concludes that the nitrogen of the lateral chain of histamine forms a quaternary link and it is strongly positively charged. Furthermore, "if the quaternary nitrogen atom of the side-chain is regarded as a primary binding site to a receptor, it is quite likely . . . that the secondary site is the pyridine nitrogen (tr^2 tr tr π)N" and it is highly electron rich. The distribution of charges in the histamine molecule is indicated in Fig. 2b.

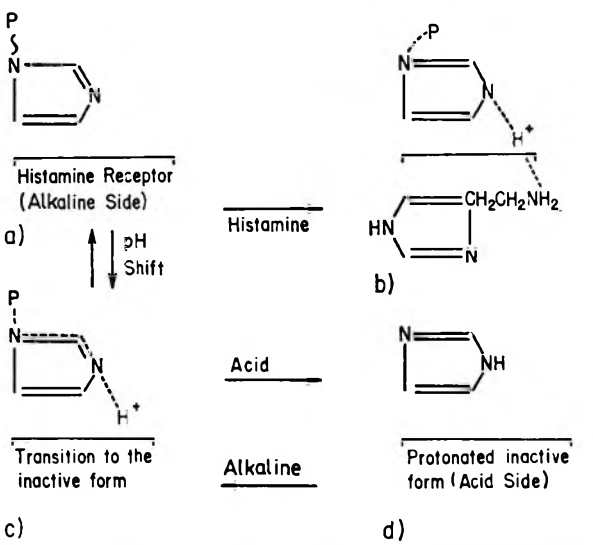

FIG. 1. The previous schematic representation of the receptor for histamine in different conditions of pH: (a) resting condition at pH about 7·0; (b) interaction of a histamine molecule to protonate the "pyridine" N of the imidazole ring of the receptor site; (c) activated form of the receptor, when the pH is shifted from a higher to lower values; (d) inactive form, in acid medium, when the receptor is "discharged" of its hypothetical metabolite ~P. (According to Rocha e Silva, 1961.)

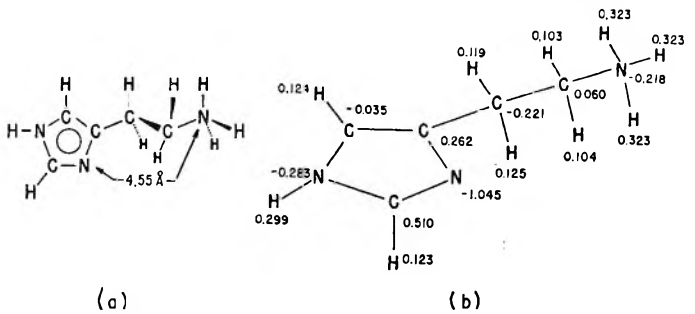

FIG. 2. (a) Configuration of the histamine cation under physiological conditions. The N^+-to-(tr^2 tr tr $\sigma + \pi$) N distance is too large to allow any hydrogen bonding to occur; (b) net charges ($\sigma + \pi$) distribution in the histamine monocation; note that the carbon flanking the two nitrogens in the imidazole ring is positively charged. (According to Kier, 1968.)

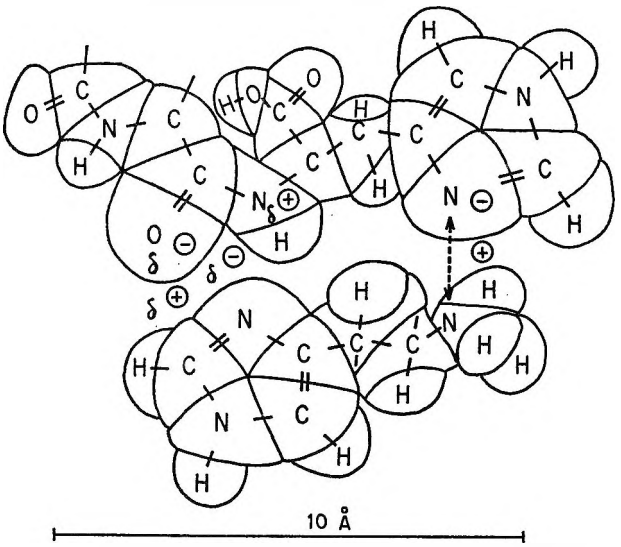

FIG. 3. A new version on the interaction of the histamine cation with the histidyl moiety supposedly existing in the H_1 receptor in the ileum of the guinea-pig.

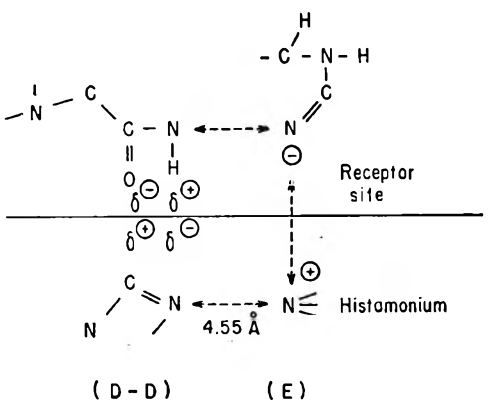

FIG. 4. Schematic representation of the forces involved in the interaction of histamine with its hypothetical receptor site. (E), electrostatic; (D–D) dipole-dipole interactions. Protonation of the pyridine (–N=) nitrogen of the histidyl moiety of the receptor would shift double bond to –C=N– position.

As indicated in Fig. 2b, a substantial positive charge is generated on the carbon flanked by the two nitrogen atoms, in the imidazole ring.

These findings contribute a great deal towards improving our previous model, since in this way a dipole appears in the imidazole ring that can fit the inverted dipole of the peptide link in the receptor model presented in Fig. 3.

We can therefore suggest that histamine is attracted to its specific receptor site (H_1) by: (*a*) strong electrostatic interaction between the pyridine (N^-) nitrogen of the histidine moiety and the strongly charged quaternary nitrogen (N^+) of the histamonium ion, and (*b*) the reciprocally inverted dipoles in the peptide link of the receptor and the carbon (C^+)–pyridine nitrogen (N^-) of the imidazole ring of the agonist (Fig. 4).

The other implications of the model are not changed, and rather are improved by the new scheme.

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REFERENCES

ROCHA E SILVA, M. (1960). *Archs int. Pharmacodyn. Thér.*, **128**, 355–374.
ROCHA E SILVA, M. (1961). *Chemotherapia*, **3**, 544–549.
ROCHA E SILVA, M. (1966). *Hab. exper. Pharmakol.*, XVIII/1, 225.
KIER, L. B. (1968). *J. mednl Chem.*, **11**, 441–445.

Diethyldithiocarbamate and structurally-related compounds and the uptake and release of noradrenaline in the heart of the rat

Disulfiram, tetraethylthiuram disulphide, and its reduction product diethyldithiocarbamate have been demonstrated to inhibit dopamine-β-hydroxylase [3,4-dihydroxyphenylethylamine, ascorbate: O_2 oxidoreductase (hydroxylating), E.C. 1.14.2.1] (Goldstein, Anagnoste & others, 1964; Green, 1964). Structurally-related compounds, such as phenylethyldithiocarbamate (Jonsson, 1967) and dimethyldithiocarbamate (Lippmann & Lloyd, 1969), also inhibit the *in vivo* conversion of exogenous dopamine to noradrenaline. The possibility exists that these compounds might cause an increased release of noradrenaline and that this might influence the activity observed. Whether compounds structurally-related to diethyldithiocarbamate and disulfiram affect the [^{3}H]noradrenaline content of the rat heart in animals injected with [^{3}H]noradrenaline is now reported.

Male albino rats (60–80 g) were injected intraperitoneally with the compounds which were in aqueous suspension of polysorbate (Tween) 80. Control animals were injected with an equal volume of the vehicle. After 45 min the animals were injected, in the tail vein, with 2·5 μCi(±)-[^{3}H]noradrenaline (Radiochemical Centre, Amersham, U.K.) in a 0·25 ml solution of 0·75% sodium chloride and 0·01N HCl. The animals were killed 15 min later, and the hearts removed, rinsed, blotted, weighed and placed on dry ice; they were then homogenized in ice-cold 0·4N perchloric acid

and centrifuged. The supernatant fluids from three hearts were combined and the [^{3}H]noradrenaline contents from the acetic acid eluates from alumina columns measured (Whitby, Axelrod & Weil-Malherbe, 1961).

Diethyldithiocarbamate, ethyldithiocarbamate, dimethyldithiocarbamate, disulfiram (tetraethylthiuram disulphide), tetramethylthiuram disulphide and tetramethylthiuram monosulphide at 125 mg/kg, intraperitoneally, neither prevented the uptake nor caused an increased release of [^{3}H]noradrenaline from the heart (Table 1). Although decreases in radioactivity were noted with some compounds, none was statistically significant.

Table 1. *The effect of diethyldithiocarbamate and structurally related compounds on the uptake of* [^{3}H]*noradrenaline in the rat heart*

Compound	[^{3}H]Noradrenaline counts/min g^{-1} ± s.e.	*P* value	% Control
Control	14,100 ± 600	—	100
Diethyldithiocarbamate	14,200 ± 1,000	<0·9	101
Ethyldithiocarbamate	12,500 ± 600	<0·2	89
Dimethyldithiocarbamate	13,300 ± 1,000	<0·7	95
Tetramethylthiuram disulphide	12,000 ± 1,000	<0·2	87
Tetramethylthiuram monosulphide	13,300 ± 1,000	<0·7	94
Disulfiram	13,700 ± 1,300	<0·9	97

Diethyldithiocarbamate and disulfiram are inhibitors of dopamine-β-hydroxylase in the rat heart (Goldstein, Anagnoste & others, 1964). Recently we have shown that dimethyldithiocarbamate (Lippmann & Lloyd, 1969), ethyldithiocarbamate (Lippmann & Lloyd, 1969), tetramethylthiuram disulphide and tetramethylthiuram monosulphide (Lippmann & Lloyd, unpublished observations) also inhibit the formation of [^{14}C]noradrenaline from [^{14}C]dopamine in the rat heart. Although it is known that disulfiram (400 mg/kg) does not interfere with the uptake, storage, metabolism or release of noradrenaline, nor the uptake, storage or release of dopamine in the rat heart (Goldstein & others, 1964; Musacchio, Goldstein & others, 1966; Musacchio, Kopin & Snyder, 1969), it was not known whether any of the active related compounds affected the ability of the heart to take up and store noradrenaline.

The experiments now reported indicate that these inhibitors of dopamine-β-hydroxylase which are structurally-related to disulfiram do not cause alterations in the uptake, short-term storage or release of exogenously administered noradrenaline in the rat heart.

The authors acknowledge the technical assistance of Miss Dorothy Mulrooney.

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REFERENCES

Goldstein, M., Anagnoste, B., Lauber, E. & McKereghan, M. R. (1964). *Life Sci.*, **3**, 763–767.

Green, A. L. (1964). *Biochim. Biophys. Acta*, **81**, 394–397.

Jonsson, J. (1967). *J. Pharm. Pharmac.*, **19**, 201–202.

Lippmann, W. & Lloyd, K. (1969). *Biochem. Pharmac.*, in the press.

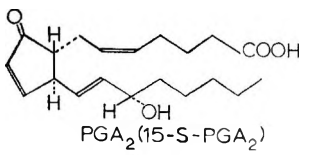
Musacchio, J. M., Goldstein, M., Anagnoste, B., Poch, G. & Kopin, I. J. (1966). *J. Pharmac. exp. Ther.*, **152**, 56–61.

Musacchio, J. M., Kopin, I. J. & Snyder, S. (1969). *Life Sci.*, **3**, 769–775.

Whitby, L. G., Axelrod, J. & Weil-Malherbe, W. (1961). *J. Pharmac. exp. Ther.*, **132**, 193–201.

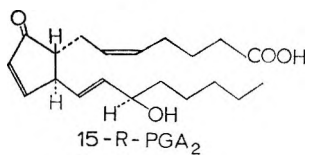
Cardiovascular effect of a prostaglandin isolated from a gorgonian *Plexaura homomalla*

Recently, Weinheimer & Spraggins (1969) isolated a prostaglandin (PG) A_2-like compound (II) from a sea animal, gorgonian, *Plexaura homomalla*. They found that the Sf value in thin-layer chromatography and the infrared and nuclear magnetic resonance spectra of this compound are identical with those of PGA_2 (I). The present study was undertaken to compare the cardiovascular effects of this compound with those of PGE_2 and PGA_2 in anaesthetized dogs.



PGA_2 (15-S-PGA_2)

I



15-R-PGA_2

II

Fifteen dogs weighing between 20 and 25 kg were anaesthetized by the intravenous administration of 30 mg/kg of sodium pentobarbitone. The technique to measure heart rate, systemic arterial pressure and myocardial contractile force were described previously (Nakano, 1967; Nakano & Kusakari, 1968). All haemodynamic parameters measured, except heart rate, were recorded simultaneously and continuously with an Electronics for Medicine recorder (DR8). PGE_2 and PGA_2 were obtained from Dr. J. Pike of the Upjohn Company, Kalamazoo, Michigan. The PGA_2-like compound was isolated from *Plexaura homomalla* and supplied from Dr. A. J. Weinheimer, Department of Chemistry, University of Oklahoma, Norman, Oklahoma. The purity of each PG compound was ascertained by thin-layer chromatography using the solvent systems described by Green & Samuelsson (1964).

The results of the cardiovascular effects of PGE_2, PGA_2 and PGA_2-like compound from the gorgonian are summarized in Fig. 1. The haemodynamic effects of both PGE_2 and PGA_2 were qualitatively similar to those of PGE_1 and PGA_1 (Nakano & McCurdy, 1967, 1968). The intravenous administration of 0·25–4·0 μg/kg of PGE_2 and PGA_2 increased heart rate and myocardial contractile force as mean systemic arterial pressure decreased. The haemodynamic changes induced by both PGE_2 and PGA_2 were essentially in proportion to the dose. In contrast, the intravenous administration of 2·25–256 μg/kg of the PGA_2-like compound isolated from the gorgonian caused no essential change in the three haemodynamic parameters.

Subsequently, further chemical and spectral analysis of this compound in the laboratory of Dr. A. J. Weinheimer showed it to be a 15-epimer of PGA_2 (15-*S*-PGA_2), 15-*R*-PGA_2 (II). According to the sequence rules formulated by Cahn, Ingold & Prelogg (1956), the priority sequence at the asymmetrical C-15 in this compound is directed in the *R* configuration instead of being *S* as with PGA_2. The sterochemical modification at C-15 in PGA_2 abolished completely its cardiovascular effects in dogs, although this structure activity relation observed in this present study is not a unique phenomenon in pharmacodynamic action of drugs. The gorgonian was

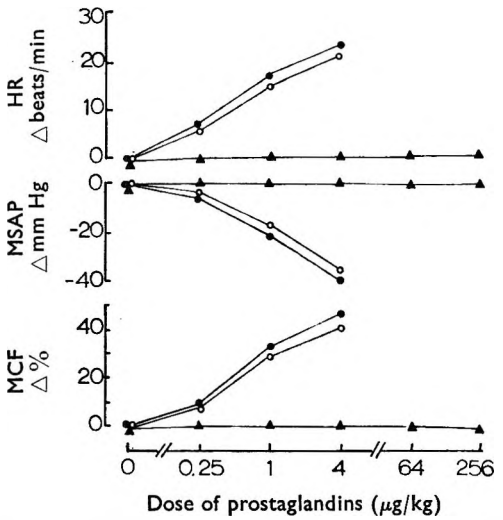


FIG. 1. Effects of the i.v. administration of graded doses (0·25–256 μg/kg) of PGE_2, PGA_2 and 15-*R*-PGA_2 on heart rate (HR), mean systemic arterial pressure (MSAP) and myocardial contractile force (MCF) in 15 dogs. Each value represents the mean of the maximal effects caused by each prostaglandin. ● PGE_2; ○ PGA_2; ▲ 15-*R*-PGA_2.

found to contain high concentrations of 15-*R*-PGA_2, amounting to 0·01–0·1% of its dry weight. Although this compound has no cardiovascular effects in dogs, it is tempting to speculate that 15-*R*-PGA_2 may play important physiological and biochemical roles. Further studies are indicated to elucidate this problem.

The author is indebted to Dr. J. E. Pike of the Upjohn Company, Kalamazoo, Michigan, and to Dr. A. J. Weinheimer for the generous supplies of PGE_2, PGA_2 and 15-*R*-PGA_2.

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REFERENCES

CAHN, R. S., INGOLD, C. K. & PRELOGG, V. (1966). *Experientia*, **12**, 81–124.
GREEN, K. & SAMUELSSON, B. (1964). *J. Lipid Res.*, **5**, 117–120.
NAKANO, J. (1967). *J. Pharmac. exp. Ther.*, **157**, 16–31.
NAKANO, J. & KUSAKARI, T. (1966). *Am. J. Physiol.*, **210**, 833–837.
NAKANO, J. & MCCURDY, J. R. (1967). *J. Pharmac. exp. Ther.*, **156**, 538–547.
NAKANO, J. & MCCURDY, J. R. (1968). *Proc. Soc. exp. Biol. Med.*, **128**, 39–42.
WEINHEIMER, A. J. & SPRAGGINS, R. L. (1969). The Abstracts of the Am. Chem. Soc. Meeting, New York, Aug. 1969.

Effect of diazepam on plasma corticosterone levels in the rat

The activity of steroid hormones may be altered by many drugs not necessarily chemically or pharmacologically related. Among sedatives and tranquillizers, the effect of barbiturates, morphine, ethanol, reserpine, chlorpromazine and meprobamate on the pituitary adrenal axis is well known (Gaunt, Chart & Renzi, 1965; Gaunt, Steinetz & Chart, 1968; Kakihana, Noble & Butte, 1968).

Little is known about the action of benzodiazepines on steroid activity and what is known seems contradictory. According to Dasgupta & Mukherjee (1967a) chlordiazepoxide in the rabbit shows an inhibitory action on the eosinopenia induced by stress or by ACTH. In addition it seems to have a protective action against stress-induced stomach ulcers (Dasgupta & Mukherjee, 1967b). Superstine & Sullman (1966) noticed an increased 17-ketosteroid excretion and an increased adrenal weight after chronic administration of chlordiazepoxide and diazepam in mice. Butler, Besser & Steinberg (1968) described a marked fall of plasma cortisol levels after chlordiazepoxide administration in man. Because the experimental evidence suggests a hypothalamic action (Shallek & Zabransky, 1966) we report the influence of small doses of diazepam on plasma corticosterone concentration.

Male Sprague-Dawley rats (150–200 g), housed in standardized conditions (22° and 60% humidity), were used. All the experiments were made in the morning. The rats, housed singly in cages and put in an acoustically isolated room 16 h before the experiment, received diazepam at 0·5; 1; 2·5; 5 and 10 mg/kg by intraperitoneal or oral route as indicated in Table 1.

Table 1. *Plasma concentrations of corticosterone* (μg/100 ml) *in the rat after a single treatment of diazepam*

Dose mg/kg	1 h (a)	2 h (b)
Solvent	6·7 ± 0·55	5·2 ± 0·4
Diazepam 0·5 i.p.	5·0 ± 0·4	5·0 ± 0·4
Diazepam 1 i.p.	7·6 ± 0·9	6·2 ± 0·4
Diazepam 2·5 i.p.	17·4 ± 1·6†	16·0 ± 1·5*
Diazepam 5 i.p.	49·6 ± 2·4†	12·1 ± 1·3*
Solvent, orally	9·4 ± 0·7	6·3 ± 0·6
Diazepam 5 orally	10·0 ± 1·7	11·0 ± 3·0
Diazepam 10 orally	12·0 ± 2·7	35·0 ± 7·2*

a = 6–10 animals, each group.
b = 6 animals, each group.
* $P < 0.01$ with respect to solvent group.
† $P < 0.001$ with respect to solvent group.

Blood samples were collected after 1 and 2 h and tested for corticosterone (Guillemin, Clayton & others, 1959). Both controls and diazepam-treated animals show an immediate increase of plasma corticosterone levels within 5–15 min from the injection. This effect, due to the handling, disappeared within 1 h. At 60 min the effect of the drug is clearly evident. As summarized in Table 1, plasma concentrations of corticosterone are significantly higher in the animals treated with 2·5 and 5 mg/kg intraperitoneally and remain so for 2 h, after which corticosterone concentrations approach the control values. The lower doses (0·5 and 1 mg/kg, i.p.) had no effect. After oral administration, the increase of corticosterone appears after 2 h but only with higher dosages (10 mg/kg). After repeated treatment the high corticosterone concentration is still present; either after the three days or after the eight day treatment, the corticosterone concentrations are higher than control values 60 min after the last administration of diazepam (Table 2).

Table 2. *Plasma levels of corticosterone* (μg/100 ml) *in the rat after repeated diazepam administration*

Dose mg/kg	1 h (a)	2 h (a)
Solvent, i.p. thrice daily for 3 days	7·59 ± 0·98	1·95 ± 0·28
Diazepam 1, i.p. thrice daily for 3 days	5·60 ± 0·76	2·70 ± 1·34
Diazepam 2·5, i.p. thrice daily for 3 days	15·75 ± 1·79†	4·19 ± 1·2*
Solvent, i.p. thrice daily for 8 days	9·73 ± 1·45	—
Diazepam 0·5, i.p. thrice daily for 8 days	6·75 ± 0·98	—
Diazepam 1, i.p. thrice daily for 8 days	6·36 ± 0·43	—
Diazepam 2·5, i.p. thrice daily for 8 days	31·48 ± 2·01†	—

a = 6 animals, each group.
* $P < 0.01$ with respect to solvent group.
† $P < 0.05$ with respect to solvent group.

The effect seems to be mediated, as for other drugs, through ACTH release via the hypothalamus and pituitary. A direct action on the adrenals does not appear to be present since after dexamethasone pretreatment (0·2 mg/kg 4 h before diazepam) there is no response to diazepam. Moreover, in our experimental conditions, diazepam seems to be differentiated from chlordiazepoxide. In fact the fall in corticosterone concentrations, as described for chlordiazepoxide (Dasgupta & Mukherjee, 1967a,b; Butler, Besser & Steinberg, 1968) was not present. In addition, the repeated treatment with diazepam resulted in a persistent increase of plasma corticosterone differing from that described for reserpine (Wells, Briggs & Munson, 1956; Kitay, Holub & Jailer, 1959; Maickel, Westermann & Brodie, 1961; Khazan, Sulman & Winnik, 1961; Gaunt, Chart & Renzi, 1965), chlorpromazine (Gaunt & others, 1968) and meprobamate (Gold, Di Raimondo & others, 1960; Mäkelä, Näätänen & Rinne, 1959). Recently Jori, Prestini & Pugliatti (1969) described an inducing effect of diazepam on liver metabolic activities, when given chronically at high dosages. The above reported increase in corticosterone plasma concentrations could partially be involved with this inducing effect of the drug. Metabolic activities are increased under stress (Driever, Bousquet & Miya, 1966) and on the other hand the inducing effect of steroids on hepatic microsomal enzymes has been reported (Gelboin & Conney, 1968). Recently a close relation between liver enzymatic activities and pituitary adrenal function has also been described (Orrenius & Ernster, 1967; Radzialowsky & Bousquet, 1968).

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REFERENCES

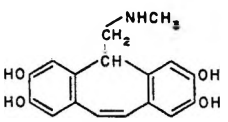
BUTLER, P. W. P., BESSER, G. M. & STEINBERG, H. (1968). *J. Endocr.*, **40**, 391–392.
DASGUPTA, S. R. & MUKHERJEE, B. P. (1967a). *Nature, Lond.*, **213**, 199–200.
DASGUPTA, S. R. & MUKHERJEE, B. P. (1967b). *Ibid.*, **215**, 1183.
DRIEVER, C., BOUSQUET, W. F. & MIYA, T. S. (1966). *J. Neuropharmac.*, **4**, 199–205.
GAUNT, R., CHART, J. J. & RENZI, A. A. (1965). *Ergebn. Physiol.*, **56**, 114–172.
GAUNT, R., STEINETZ, B. G. & CHART, J. J. (1968). *Clin. Pharmac. Ther.*, **9**, 657–681.
GELBOIN, H. V. & CONNEY, A. H. (1968). In "*Modern trends in toxicology*". Editors: Boyland, E. & Goulding, R., pp. 175–197. London: Butterworth.
GOLD, E. M., DI RAIMONDO, V. C., KENT, J. R. & FORSHAM, P. H. (1960). *Ann. N.Y. Acad. Sci.*, **86**, 178–190.
GUILLEMIN, R., CLAYTON, G. W., LIPSCOMB, H. S. & SMITH, J. D. (1959). *J. Lab. clin. Med.*, **53**, 830–832.

JORI, A., PRESTINI, P. E. & PUGLIATTI, C. (1969). *J. Pharm. Pharmac.*, **21**, 387–390.
KAKIHANA, R., NOBLE, E. P. & BUTTE, J. C. (1968). *Nature, Lond.*, **218**, 360–361.
KHAZAN, N., SULMAN, F. G. & WINNIK, H. Z. (1961). *Proc. Soc. exp. Biol. Med.*, **106**, 579–581.
KITAY, J. I., HOLUB, D. A. & JAILER, J. W. (1959). *Endocrinology*, **65**, 548–554.
MAICKEL, R. P., WESTERMANN, E. O. & BRODIE, B. B. (1961). *J. Pharmac. exp. Ther.*, **134**, 167–175.
MÄKELÄ, S., NÄÄTÄNEN, E. & RINNE, U. K. (1959). *Acta Endocr.*, **32**, 1–7.
ORRENIUS, S. & ERNSTER, L. (1967). *Life Sci.*, **4**, 1473–1482.
RADZIALOWSKI, F. M. & BOUSQUET, W. F. (1968). *J. Pharmac. exp. Ther.*, **163**, 229–238.
SHALLEK, W. & ZABRANSKY, F. (1966). *Archs int. Pharmacodyn. Ther.*, **161**, 126–131.
SUPERSTINE, E. & SULLMAN, F. G. (1966). *Ibid.*, **160**, 133–146.
WELLS, H., BRIGGS, F. N. & MUNSON, P. L. (1956). *Endocrinology*, **59**, 571–579.

An improved method for the preparation of adnamine

We have recently been interested in the preparation of some *N*-alkyl substituted homologues of noradnamine (5-aminomethyl-2,3,7,8-tetrahydroxydibenzo[*a*,*e*]cycloheptatriene) for pharmacological testing. The *N*-methyl derivative, adnamine (I), was originally obtained by Kawazu (1958) by boiling a solution of adrenaline (II) in 10% hydrochloric acid for 3·5 h. Recently Roberts & Broadley (1967) reported that the main crystalline product usually obtained in this manner was not adnamine (I) but was identical to the product isolated previously by Funk & Freedman (1923) and Öppinger & Vetter (1942) and described as diadrenaline ether (III). Roberts & Broadley (1967) further reported that reasonable yields of adnamine (I) could consistently be obtained with stronger acids and longer reaction times than those employed by Kawazu. The production of adnamine (I) by this latter procedure, is, however, often accompanied by the copious formation of tarry by-products which complicates the isolation and purification of the product.

Recent work in these laboratories has shown that the product previously described as diadrenaline ether (III) does not have structure III but is, in fact, 6-(3′,4′-dihydroxy-α-methylaminomethylbenzyl)adrenaline (IV). The trivial name adrepine was proposed for this substance (Forrest, Kašpárek & others, 1969). Preliminary paper chromatographic evidence suggested that IV was an intermediate in the conversion



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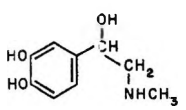

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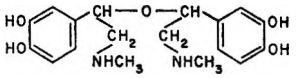
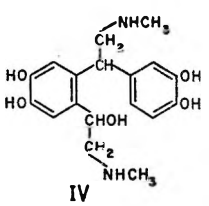


III



IV

of II into I. This conversion could be brought about by heating IV above its melting point or in solution in 10% hydrochloric acid. It has now been shown that good yields of pure adnamine can be consistently obtained by this latter procedure. The reaction parameters have been systematically varied and those giving the optimum yields of adnamine are described below.

Adrepine hydrochloride was prepared by the method used by Öppinger & Vetter (1942) to obtain "diadrenaline ether". A sample (500 mg) was dissolved in 10% hydrochloric acid [conc. HCl (2·85 ml) + water (7·15 ml)] and the solution heated, under reflux, on an oil bath at 95–100°. A colourless crystalline product began to crystallize out of the solution after about 2 h. After being maintained at this temperature for 5 h the reaction mixture was cooled and allowed to stand at 4° overnight and adnamine hydrochloride was obtained as a colourless crystalline solid. The product, obtained by filtration, was chromatographically homogeneous (n-butanol, saturated with 3N HCl) but was further purified by recrystallization from water or 70% ethanol. In this manner pure adnamine hydrochloride was obtained as colourless needles, m.p. 262–264°. λ_{max}^{EtOH}, nm (ϵ_{max}^{EtOH}): 215 (32,000); 235 (31,000); 312 (16,000). Found: C, 60·9; H, 5·4; N, 4·2; Cl, 10·65%, calc. for $C_{17}H_{18}O_4NCl$: C, 60·8; H, 5·4; N, 4·2; Cl, 10·6%. These values were obtained with a sample dried *in vacuo* at 120° for 1 h. Kawazu (1958) has reported that adnamine hydrochloride crystallizes from aqueous solution as a hemi-hydrate (m.p. 264–265°).

Adnamine exhibits an intense blue fluorescence in ultraviolet light. Solutions in dilute hydrochloric acid have excitation and fluorescence maxima at 310 and 395 nm respectively.

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REFERENCES

FORREST, J. E., KAŠPÁREK, S., HEACOCK, R. A. & FORREST, T. P. (1969). *Canad. J. Chem.*, **47**, 2118–2121.

FUNK, C. & FREEDMAN, L. (1923). *J. Am. chem. Soc.*, **45**, 1792–1795.

KAWAZU, M. (1958). *J. pharm. Soc. Japan*, **78**, 399–402.

ÖPPINGER, H. & VETTER, H. (1942). *Mednl. Chem.*, **4**, 343–367.

ROBERTS, D. J. & BROADLEY, K. J. (1967). *J. Chromat.*, **27**, 407–412.

Modification of the Randall-Selitto analgesic apparatus

In screening programs designed to detect analgesics, the initial testing procedure usually includes in rodents, some measurement of protection shown by a test compound against a noxious stimulus, such as chemicals (Siegmund, Cadmus & Lu, 1957), heat (Wolfe & MacDonald, 1944; Eddy & Leimbach, 1953), electricity (D'Amour & Smith, 1941) and air pressure (Randall & Selitto, 1957).

Because the air pressure method of Randall & Selitto (1957) detects antipyretic and anti-inflammatory analgesics and the more potent analgesics, its use is often preferred.

Our experience with the apparatus as modified by Winter & Flataker (1965) has been satisfactory except that, after repeated or prolonged use, the Teflon plunger sometimes becomes immobile. We now describe a simpler apparatus which has given reproducible results in our hands.

Two pieces of aluminum plate (*a*) are separated by a plexiglass tube (*b*) and "sandwiched" between the plates is a latex rubber diaphragm (*c*) which responds to externally applied low air pressure (*A*). To the diaphragm, a stainless steel rod (*e*) is attached, which in turn transmits pressure to the rat paw. The paw is placed over a positioning block similar to that described by Winter & Flataker (1965). The air pressure is regulated by a needle valve so that a rise of 10 mm/s is maintained and cut-off at 100 mm Hg. A sharp "squeak" or an escape response or both are used as an end point.

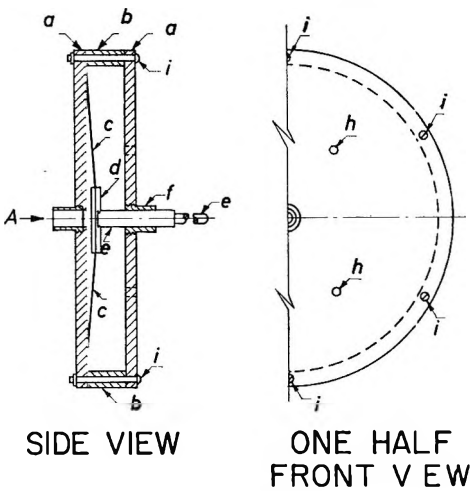

FIG. 1. *A*. Air supply. *a*. Aluminium plates—$\frac{1}{8}$ inch × 5 inch diameter. *b*. Plexiglass tube—$\frac{1}{4}$ inch × 1 inch × 5 in diameter. *c*. Latex rubber diaphragm (slightly inflated), 1/16 inch × 5 inch in diameter. *d*. Stainless steel plates—1/16 inch × 1 inch diameter. *e*. Stainless steel rod $\frac{1}{2}$ inch tapered to 2 mm at end. *f*. Guided sleeve. *h*. Air vents. *i*. Bolts.

Female weanling Sprague-Dawley rats 40–60 g weight were fasted overnight with water *ad lib*. After pre-testing the animals for threshold responses the procedure of Winter & Flataker (1965) was followed.

The oedema and hyperesthesia, ensuing after injection of 0·1 ml of 5% yeast into the right hind paw, were allowed to develop for 2 h before drugs were administered orally. The doses were given in a volume of 1 ml/kg. The suspending agent was 0·5% carboxymethylcellulose. The thresholds to pressure were measured 1 h later.

The minimum effective dose is calculated by the dose-response curve and represents the minimum dose that produces effects statistically significantly different ($P = 0{\cdot}05$) from control values based on Dunnett's "t" (Dunnett, 1955).

Typical antipyretic-anti-inflammatory analgesic compounds increase only the threshold of the inflamed foot, whereas the more potent analgesics will affect both inflamed and normal foot thresholds (see also Randall & Selitto, 1957). The effect of (+)-propoxyphene at 20 mg/kg on the normal foot is just beyond significant difference and it would seem probable that a dose of 25–30 mg/kg would show significance (Table 1).

Table 1. *Effect of various drugs upon air pressure thresholds in rats*

Drugs	Oral dose** (mg/kg)	No. of rats	Thresholds* (mm pressure) I.F.	Thresholds* (mm pressure) N.F.	Minimum effective dose mg/kg I.F.	Minimum effective dose mg/kg N.F.
Control	CMC	25	15 ± 2·6	48 ± 1·3	—	
Aspirin	75	10	20 ± 4·3	47 ± 0·8	100	—
	150	25	21 ± 4·3	48 ± 1·3		
	300	25	28 ± 3·9	48 ± 1·3		
Control	CMC	35	16 ± 2·2	49 ± 1·7		
Phenylbutazone	10	15	21 ± 2·1	49 ± 3·2	10	—
	20	35	29 ± 7·3	49 ± 0·9		
	40	35	32 ± 6·4	48 ± 1·3		
Control	CMC	20	14 ± 1·4	48 ± 1·7		
Indomethacin	0·4	20	16 ± 2·1	48 ± 1·3	1·4	—
	2	20	23 ± 2·6	49 ± 1·5		
	10	20	33 ± 2·8	50 ± 0·6		
Control		20	16 ± 2·4	50 ± 0·4		
Morphine..	10	15	27 ± 2·4	51 ± 4·1	<10	16
	20	15	36 ± 7·7	55 ± 2·4		
	40	15	47 ± 6·5	61 ± 4·1		
Control		25	15 ± 3·3	50 ± 1·6		
(+)-Propoxyphene ..	5	15	17 ± 1·6	50 ± 0·7	14	>20
	10	22	18 ± 1·5	50 ± 0·9		
	20	25	25 ± 4·5	54 ± 2·8		

CMC = carboxymethylcellulose. I.F.—inflamed foot– N.F.—normal foot.
* Figures represent mean ± standard deviation.
** Rats dosed 2 h after 0·1 ml of 5% yeast injected into subplantar tissue of hind paw. Thresholds measured 1 h after oral dosing.

The doses of compounds which show a significant difference from controls are considerably greater, especially for morphine, than those reported in the literature (Winter & Flataker, 1965, Randall & Selitto, 1957) but the animals were dosed orally rather than parenterally.

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REFERENCES

D'Amour, F. E. & Smith, D. L. (1941). *J. Pharmac. exp. Ther.*, **72**, 74–79.
Dunnett, C. W. (1955). *J. Am. Statist. Ass.*, **50**, 1096–1121.
Eddy, N. B. & Leimbach, D. (1953). *J. Pharmac. exp. Ther.*, **107**, 385–393.
Randall, L. O. & Selitto, J. J. (1957). *Archs int. Pharmacodyn. Thér.*, **111**, 409–419.
Siegmund, E., Cadmus, R. & Lu, G. (1944). *Proc. Soc. exp. Biol. Med.*, **95**, 729–731.
Winter, C. A., & Flataker, L. (1965). *J. Pharmac. exp. Ther.*, **148**, 373–379.
Wolfe, G. & MacDonald, A. D. (1944). *Ibid.*, **80**, 300–307.

A simplified dissolution rate apparatus

The rate at which the active ingredient of a solid dosage form dissolves into the fluid of the gastrointestinal tract may be the rate-determining step in the absorption sequence of the drug. This dissolution rate may show little correlation to the disintegration time of the formulation (Schroeter, Tingstad & others, 1962). These and other authors (Parrott, Wurster & Higuchi, 1955; Levy, 1961; Finholt, 1966) have suggested that a dissolution rate test should supplement the present official disintigration standards. Numerous methods have been devised to measure *in vitro* dissolution rates and have been reviewed by Hersey (1969). Several of these proposals referred to specialized techniques designed to determine intrinsic dissolution rates, others were concerned with one particular application or were unsuitable for evaluating commercial dosage forms. Many have disadvantages including, non-uniformity of agitation around the dose form, non-sink conditions, or over complexity of design which introduces other variable factors such as transport across membranes.

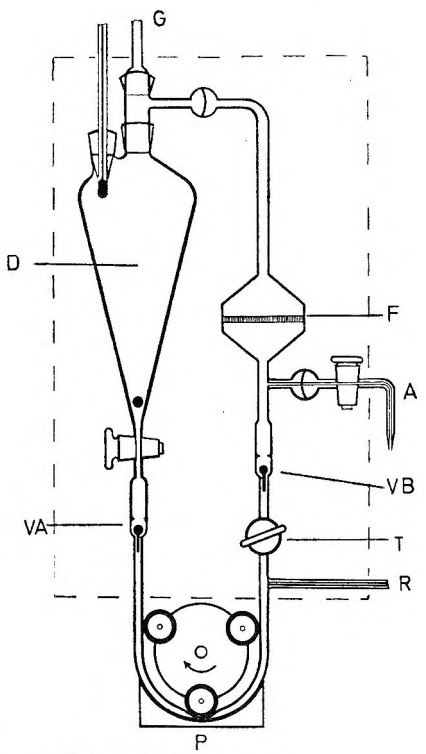

FIG. 1. Diagram of apparatus. For description see text.

The apparatus described here (Fig. 1) is designed to be simple whilst remaining versatile. Chapman, Crisafio & Campbell (1954) and Morrison & Campbell (1965) have pointed out that *in vitro* methods cannot simulate all the conditions encountered *in vivo*, hence only *in vivo* conditions which are easily contrived and reproducible have been included in the method. Agitation is provided by the flow of liquid in the apparatus which is maintained by a peristaltic pump (P) forcing liquid along a short length of lubricated nylon tubing. Except for this short length of tubing the rest of the apparatus is constructed entirely of borosilicate glass. The flow rate can be adjusted by altering the diameter of the tubing used or the speed of the rotor. Fluid leaving the pump passes through a one way valve (VA) to eliminate any back surge due to the peristaltic action. The resultant flow is a series of forward pulses with no

mixing of solutions once they have passed the sample under test in the dissolution vessel (D). The fluid enters this vessel, a standard 250 ml separating funnel, by lifting a simple glass ball valve which prevents any heavier particles from dropping out of the vessel at low pump rates. This valve and the narrow bore of the tap preceding it give rise to a small area of turbulence above the valve, the extent of which depends upon the pumping rate. Tablets, capsules or powders can thus be given any desired constant agitation by controlling the flow rate.

Above the turbulent region the flow is laminar with little mixing and therefore the actual amount of sample dissolving in the agitation zone at any given time is obtained by analysing the fluid leaving the dissolution vessel. X-ray studies have shown (Levy; 1963; Steinberg, Frey & others, 1965) that agitation in the stomach is usually very gentle with wide variations between subjects. These conditions can be reproduced in the apparatus by using very low flow rates or filling the base of the vessel with glass spheres which raise the sample into a zone of gentler laminar flow. The shape of the funnel causes the flow rate to decrease with height above the agitation zone and tests with solutions of dyes have indicated that no dead spots of static fluid are created i.e. it ascends as a perfectly horizontal plane. It can be argued that different sized particles will be subjected to a varying velocity of dissolution medium past them as smaller particles are carried up the vessel. Where this is thought to be significant the effect may be minimized by the inclusion of several large glass beads into the bottom of the dissolution vessel so that all particles, whether large or small, are retained in a region of more uniform fluid velocity.

Because of the very low velocity at the top of the vessel most drug particles are retained at lower levels and only very fine or light particles such as starch grains are carried over on to the glass sinter (F). In practice any drug particles leaving the vessel are so small that they dissolve completely before reaching the sinter or within a very short time after settling on it and do not affect the bulk dissolution rate significantly. However, to measure the degree of blocking of this filter a pressure gauge may be attached to the top of the dissolution vessel at G. Below the filter samples are removed for batchwise or on-stream analysis along capillary line (A). Replacement dissolution fluid is automatically drawn into the system from a reservoir (not shown), via the inlet tube R, by the pump, to compensate for the outflow. If the sampling rate equals the pump rate the ideal situation of a perfect sink is established with fresh fluid continually bathing the sample. As this situation is approached a one way valve VB prevents any tendency for fresh fluid to flow back and dilute the sampling line. For perfect sink conditions with rapidly absorbed drugs, the tap T is closed. The whole apparatus and the compensating reservoir, except the pump are immersed in a water bath (indicated by the broken line) maintained at 37·5°.

Fig. 2 shows typical concentration versus time histograms for the dissolution in water of three different commercial batches of 300 mg acetylsalicylic acid tablets B.P., the pumping rate being 50 ml/min. Histograms A1 and A2 are derived from one batch and give identical high initial concentration peaks which are characteristic of rapidly dissolving tablets. The rate of continuous sampling for histogram A1 was 13 ml/min, while that for histogram A2 was 10 ml/min. This resulted in a more rapid decrease in concentration with time in the former profile. Histogram B1 was from a different source but had the same B.P. disintegration time as tablets used for A1 and A2. The initial peak, however, was much lower and more delayed, indicating a slower dissolution rate. Histogram B2 represents a batch of tablets that disintegrated very slowly and the concentration profile illustrates the extremely slow release of drug under these circumstances. Continuous recording of concentration at point A provides a smooth concentration versus time plot, which is more desirable but requires more sophisticated analytical instrumentation.

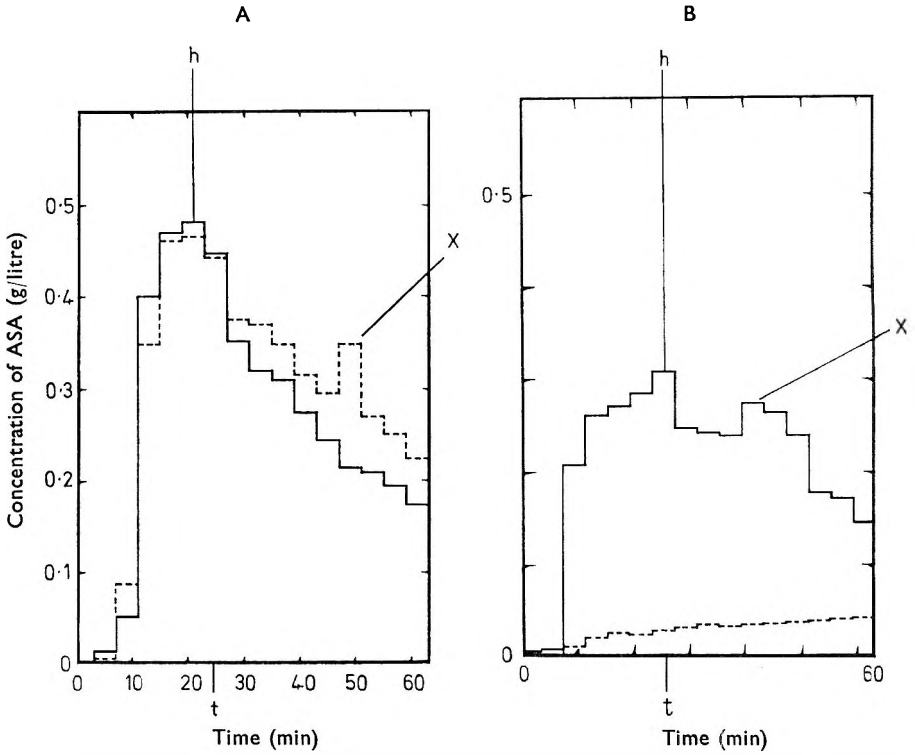

FIG. 2. Concentration versus time histograms of three batches of acetylsalicyclic acid tablets B.P. A1(—) sampled at 13 ml/min; A2(- - -), same batch sampled at 10 ml/min; B1(—), second batch with same B.P. disintegration time as A1 and A2 sampled at 13 ml/min; B2(- - -), from a batch of slow disintegrating tablets sampled at 13 ml/min.

It is tentatively suggested that a combination of the height of the concentration peak "h" together with the time "t" at which it occurs and possibly the time to reach some minimal residual concentration, might provide the numerical criteria necessary to characterize and compare solid dosage forms, although more work is necessary to establish their relative significance. The effect of only partial sink conditions can be clearly seen in histograms A2 and B1 where recycling causes the subsidiary peaks at X. Detailed studies of the variable parameters of the apparatus, different solid dosage forms and *in vivo* correlation are now proceeding.

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REFERENCES

CHAPMAN, D. G., CRISAFIO, R. & CAMPBELL, J. A. (1954). *J. pharm. Sci.*, **43**, 297–304.

FINHOLT, P., KRISTIANSEN, H., SCHMIDT, O. C. & WOLD, K. (1966). *Meddr norsk. farm. Selsk.*, **28**, 17–47.

HERSEY, J. A. (1969). *Mfg. Chem.*, Feb. 1969, 32–35.

LEVY, G. (1961). *J. pharm. Sci.*, **50**, 388–392.

LEVY, G. (1963). *Ibid.*, **52**, 1039–1046.

MORRISON, A. B. & CAMPBELL, J. A. (1965). *Ibid.*, **54**, 1–8.

PARROTT, E. L., WURSTER, D. E. & HIGUCHI, T. (1955). *Ibid.*, **44**, 269–273.

SCHROETER, L. C., TINGSTAD, J. E., KNOECHEL, E. L. & WAGNER, J. G. (1962). *Ibid.*, **51**, 865–874.

STEINBERG, W. H., FREY, G. H., MASCI, J. N. & HUTCHINS, H. H. (1965). *Ibid.*, **54**, 747–752.

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